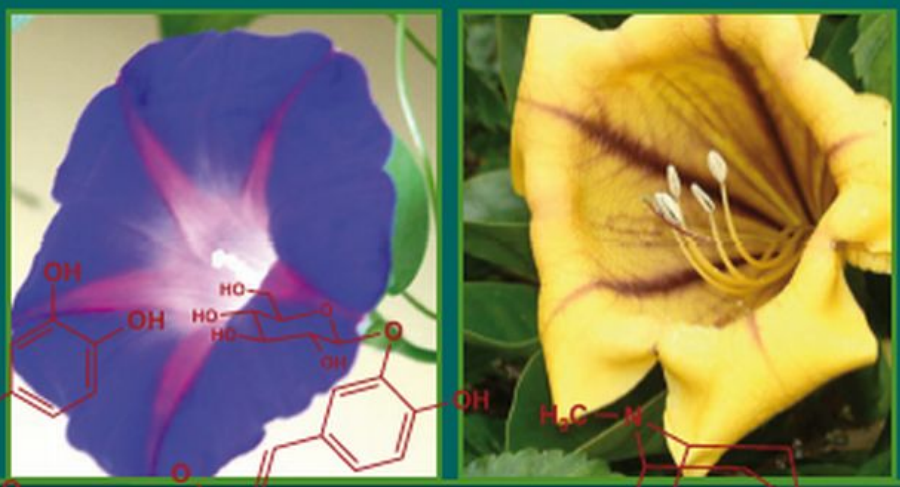


Eckart Eich

Solanaceae and Convolvulaceae: Secondary Metabolites

Biosynthesis, Chemotaxonomy, Biological and Economic Significance

A Handbook

Springer

Solanaceae and Convolvulaceae: Secondary Metabolites

Eckart Eich

Solanaceae and Convolvulaceae: Secondary Metabolites

Biosynthesis, Chemotaxonomy, Biological and Economic Significance (A Handbook)

Springer

Prof. Dr. Eckart Eich
Freie Universität Berlin
Institut für Pharmazie
- Pharmazeutische Biologie -
Königin-Luise-Str. 2 + 4
14195 Berlin
Germany
E-mail: eckeich@zedat.fu-berlin.de

Cover illustration: Flowers of *Ipomoea purpurea* (L.) ROTH [cultivar; Convolvulaceae] (*left*) and *Solandra maxima* (SESSÉ & MOCINO) P.S. GREEN [Solanaceae] (*right*). Plotted on the photographs are corresponding constituents: the major anthocyanin pigment and the major alkaloid hyoscyamine, respectively.

ISBN 978-3-540-74540-2 e-ISBN 978-3-540-74541-9

The Library of Congress Control Number: 2007933490

Cover design: WMXDesign GmbH, Heidelberg, Germany

Printed on acid-free paper SPIN 11003991 5 4 3 2 1 0

springer.com

„Was du ererbt von deinen Vätern hast,
Erwirb es, um es zu besitzen.“
(All that you have, bequeathed you by your fathers,
Earn it in order to possess it.)

Johann Wolfgang von Goethe (*Faust I*)

This book is based on a multitude of results obtained in botany, phytochemistry, and other biological sciences from all over the world since the beginning of modern research two hundred years ago. It is dedicated to all the scientists who have contributed to these results.

Acknowledgments

This interdisciplinary book is a result of my long-standing fascination for the botany of solanaceous and convolvulaceous plants as well as their ability to synthesize a multitude of chemical species with their biological activities. However, this book would not have been realized without the support of the following individuals to whom I express my sincere gratitude:

- For conscientious reading and competent commenting on the manuscript of the book: Priv.-Doz. Dr. Kristina Jenett-Siems and Prof. Dr. Heinz H. Pertz, Freie Universität Berlin, Germany (Chaps. 3–8), Priv.-Doz. Dr. Michael Stech, Universiteit Leiden, The Netherlands (Chap. 2), and my daughter, Dr. Susanne Eich-Greatorex, Universitet for miljø- og biovitenskap, Ås, Norway (Chap. 1)
- For introducing me, an ignoramus in computer affairs until 2003, to the secrets of the personal computer and its various functions with patience and unremitting efforts: Dr. Thomas Schimming, Berlin
- For rescue of my personal computer in severe cases of crashes: Dr. Colja Schubert, Heinrich Hertz Institut Berlin
- For long-term botanical advice and information: Daniel F. Austin, Ph.D., Emeritus Professor at Florida Atlantic University, Boca Raton, and Research Associate of the Arizona-Sonora Desert Museum, Tucson, USA
- For the excellent advice and guidance as well as for the generous acceptance of my requests: the life sciences editorial staff of Springer, especially Dr. Jutta Lindenborn and Dr. Dieter Czeschlik, Heidelberg, Germany and the copyeditor, John Kirby, Malvern, UK
- For the contribution of 53 photographs to the Colour Plates of this book taken during our common journeys through predominantly tropical countries, for the essential support in many different respects, e.g., by proof-reading, discussions, excellent organizing, and last but not least for the forbearance of my preoccupation with the book and long-term encouragement: my wife, Elisabeth Bäumel-Eich, Berlin

Berlin, August 2007 Eckart Eich

Contents

1
Introduction

1.1 Philosophy and Aims of this Book

1.1.1 The Large Solanales Families as a Topic

Solanales are from the Mid-Cretaceous (stem node age: 106 my; crown node age: 100 my) (Bremer et al. 2004). Solanaceae and Convolvulaceae are sisters representing the two large families of this order. Their last common ancestor lived about 70 my ago (Durbin et al. 2000). The main objective of the author is to focus on aspects of our extensive knowledge of secondary metabolites in the plant kingdom in order to account for the specific competitiveness and productivity of these two large Solanales families. To this end, it has been necessary to take a bird's-eye view of 200 years of phytochemical research on the Solanales, since first scientific reports with regard to both families were published in the early nineteenth century. Due to an almost complete lack of phytochemical reports (one single exception) on species of the three remaining, very small families of the order (see Chap. 2), they have not been considered.

1.1.2 General Role of the Secondary Metabolism for a Specific Characterization and Classification of Plant Taxa

While traditional systematics generally focused on morphologic-anatomical characters of plants, in some cases chemotaxonomic aspects with regard to low molecular secondary metabolites were also considered. However, plant biochemistry and chemotaxonomy normally played a minor role in classification. In contrast, phylogenetic approaches to plant systematics, based on (macro)molecular cladistic analyses, have received an extraordinary, increasing significance during the past 25 years (e.g., Judd et al. 1999). In the author's opinion, our extensive knowledge of secondary metabolites has not yet been integrated into the **characterization** of plant families, genera, and species to an extent appropriate to its significance. There are enormous numbers of flora from all over the world which document and maintain in

Eckart Eich, *Solanaceae and Convolvulaceae: Secondary Metabolites*

detail the morphologic-anatomical characters of the different taxa as well as their phytogeographic distribution. There are also extensive, daily growing databases available on specific DNA sequences of many plant species or even on their whole genome. These fields of botanical sciences have been used to characterize plants, especially on the species level.

On the other hand, plant biochemistry is still playing a minor role with regard to the specific characterization of plants. The primary metabolism of plants is more or less ubiquitously the same with few exceptions, e.g., C3 vs C4 plants. Therefore, it is of minor if any use for the characterization of the different taxa. The secondary metabolism as far as low-molecular constituents are concerned can add further important information to the characterization of taxa on different levels. Normal flora written for geographically restricted areas only occasionally included some information on secondary metabolites, e.g., "Flore de Madagascar et des Comores, famille 171, Convolvulaceae" on two pages (Deroin 2001). As a rare exception, the comprehensive monograph "Genera Solanacearum" (Hunziker 2001), based traditionally on morphologic/anatomical characters plus detailed information on chromosome numbers, also included aspects of the secondary metabolism – fortunately from the phytochemical point of view. As well as a short initial overview of the phytochemistry of the family, some limited but valuable information predominantly on groups/subgroups of secondary metabolites nested within the corresponding genera sections – exactly documented by references – were given. However, this information is more genus-orientated, i.e., not species-specific.

1.1.3 Bird's-Eye View of Two Centuries of Phytochemical Research on Solanaceae and Convolvulaceae

The author wants to place far greater emphasis on the integration of specific biochemistry into the individual characterization of taxa in the field of the two large Solanales families. In so doing, he is trying to contribute to a more interdisciplinary way of looking at things by combining botanical and chemical sciences. Chemical characters identified in single species in countless chemistry-oriented papers are not really noticed by botanists though they may be of additional value for the characterization of the respective species. It is of little use to note that a species or any other taxon contains, e.g., "alkaloids" or – slightly more specific – "tropane alkaloids". There are tens of thousands of alkaloids and hundreds of tropane alkaloids. The presence or absence of any single alkaloid or any other metabolite is a specific character and may contribute to the characterization of a taxon.

Similar to other characters, e.g., morphological ones, it must be taken into account that there may be qualitative and quantitative differences in any species due to intraspecific, ontogenetic, and morphogenetic variability, respectively.

Furthermore, from the ecological point of view, differentiation is necessary; it is usually not a class/group of metabolites which is associated with a certain activity. In contrast, two structurally closely related compounds may show clearly different effects.

Every single alkaloid as well as any other metabolite is more or less "bioactive" but in individually diverging qualities (effects, mechanisms of action). Its individual potency and its content (high or low accumulation or presence in traces) in the living plant species are of considerable significance. To come back to the example of tropane alkaloids, only a small subgroup of them (3α-tropoyloxytropanes and closely related congeners such as hyoscyamine/atropine) is highly poisonous due to an anticholinergic potency. The vast majority of tropane alkaloids do not possess this property.

Due to their relationship with botany and chemistry, other disciplines, such as pharmacognosy, pharmacology/toxicology, agricultural and food sciences, ecology etc., also play a part in this interdisciplinary approach.

Therefore, the author's objectives are to

- Document the secondary metabolites of the Solanaceae and Convolvulaceae discovered and structurally elucidated in 200 years of phytochemical research as far as low-molecular compounds ("small molecules") are concerned
- Describe the peculiarities of their secondary metabolism as compared to other plants in the plant kingdom, with main focus on angiosperms
- Show common features in secondary metabolism of both families, as well as their chemotaxonomic relationships
- Demonstrate the special qualities of the family Solanaceae vs the family Convolvulaceae, i.e., their differences
- Compare the special qualities on different intrafamilial taxonomic levels (subfamilies, tribes, genera, subgenera, species) of both families, in certain cases also on different intraspecific taxonomic levels (subspecies, sections) of both families
- Consider and integrate the ecological, pharmacological/toxicological, and economic significance of metabolites from both families

1.2 Secondary Metabolism of the Large Solanales Families

1.2.1 Historical Background

The discovery of morphine as the sleep-inducing principle ("principium somniferum") of the opium poppy, *Papaver somniferum* L. (Papaveraceae), by the German apothecary Friedrich Wilhelm Sertürner (1783–1841) in 1805 is of particular significance in the history of organic chemistry. It represented not only the first isolation of a basic plant metabolite, but also at least one of the first isolations of a secondary metabolite in the plant kingdom at all (Sertürner 1805; 1817). It was the initial and stimulating step in the search for further basic constituents. Particularly toxic plants have been screened for such compounds, which were named "alkaloids" (alkali-like) according to the proposal of Meissner (1819). It was therefore natural to look at poisonous species like the solanaceous herbs *Solanum nigrum* (black nightshade), *Nicotiana tabacum* L. (Virginian tobacco), and *Atropa belladonna* L. (deadly nightshade or poison black cherry). Thus, at the

beginning of scientific research on secondary plant metabolism the relationship between natural compounds and their toxicological/pharmacological/ecological significance led to the isolation and – many decades later – structure elucidation of these compounds. Over the past two centuries this principle has revealed the majority of secondary plant metabolites, i.e., discoveries by "bioassay-guided fractionation/isolation", the corresponding term in modern methodology of phytochemistry. Of course, this has been true not only for alkaloids – though they represent a huge class of secondary metabolites which are especially suitable for this method due to their particularly distinctive biological activities – but also for all other classes of secondary metabolites. The isolation of solanine (Desfosses 1820, 1821), nicotine (Posselt and Reimann 1828), and atropine (Mein 1833; Geiger and Hesse 1833a, b) from the three species mentioned above represented the first alkaloids from the Solanaceae and also their first secondary metabolites in general. A century later, the very first alkaloid from the Convolvulaceae, the 3α-acyloxytropane convolvine, was discovered as a constituent of *Convolvulus pseudocantabricus* SCHRENK. (Orechoff and Konowalowa 1933). Resin glycosides (glycoresins) belong to the early discoveries in this family, e.g., in *Ipomoea purga* (WENDER.) HAYNE, Mexican jalap (Cadet de Gassicourt 1817) and *I. orizabensis*, Mexican scammony (PELLET.) LED. ex STEUD. (Johnston 1840). Again, pharmacological reasons led to the isolation of products from plant material, in this case a complex mixture of structurally related resin glycosides as was found later (Mexican jalap resin and Mexican scammonium resin, respectively).

1.2.2 Common Ground and Differences of the Solanales Families

Solanaceae and Convolvulaceae share a number of obvious characters, properties, and other factors, most of them not very surprising due to the fact that they are sisters and a few perhaps only by pure chance:

- Common phylogeny
- Morphologic-anatomical similarities
- Similar phytogeographic distribution
- **Similar secondary metabolism**
- Similar order of magnitude of species in the family (~2500 vs ~1800 species)
- One genus dominating by far (*Solanum* ~1400 species vs *Ipomoea* ~650 species)
- One economically very important food plant containing starch in the tuber [*Solanum tuberosum* L. vs *Ipomoea batatas* (L.) LAM.]
- A remarkable number of popular ornamentals – though not suitable as cut flowers, e.g., from the genera *Brugmansia*, *Brunfelsia*, *Petunia*, *Solandra*, *Solanum* vs. *Argyreia*, *Convolvulus*, *Evolvulus*, *Ipomoea*, *Merremia*

Of course, the fourth point is the decisive one with respect to the objective of this book. Therefore, it shall be presented in more detail.

1.2.3 Similar Secondary Metabolism of the Large Solanales Families

Of course, classes/groups of metabolites which occur (almost) ubiquitously or at least frequently in the plant kingdom (**"general secondary metabolites"**) are also constituents of both large Solanales families: (i) phenolics such as simple cinnamic acid derivatives (Sect. 6.4), hydroxycoumarins (Sect. 6.6), hydroxycinnamate conjugates (Sect. 6.7), flavonoids (Sect. 6.8), lignans (Sect. 6.9), (ii) sterols (Sect. 7.6), (iii) carotenoids (Sect. 7.12), (iv) fats/oils and fatty acids (Sect. 8.1), (v) carbohydrates (Sect. 8.2) etc.

In addition, they share classes/groups of metabolites which occur rarely in the plant kingdom (**"specific secondary metabolites"**):

- The extensive class of ornithine-derived alkaloids such as hygrines, *N*-acylpyrrolidines, nicotinoids, tropanes, calystegines (Sects. 3.1–3.5)
- Sesquiterpenoid phytoalexins (Sect. 7.3)

On the other hand, they have in common the **absence** of the following classes although those are frequent constituents of closely related families also belonging to the lamiids (euasterids I):

- Iridoids (constituents of Apocynaceae, Gentianaceae, Lamiaceae, Rubiaceae)
- Volatile oils (essential oils), complex mixtures of lipophilic mono-/sesquiterpenes and/or phenylpropanoids secreted in oil cells, secretion ducts/cavities or glandular hairs (constituents of Lamiaceae)
- Condensed tannins (proanthocyanidins; constituents of Rubiaceae)

The following groups **lacking in Convolvulaceae** represent *specific secondary metabolites* of certain solanaceous taxa:

- Capsaicinoids (Sect. 6.5)
- Steroidal saponins (Sect. 7.7)
- Steroidal alkaloids (Sect. 7.8)
- Withasteroids (Sect. 7.10)

On the other hand, the following groups **lacking in Solanaceae** represent *specific secondary metabolites* of certain convolvulaceous taxa:

- Indolizidine alkaloids (Sect. 3.6)
- Pyrrolizidine alkaloids (Sect. 3.7)
- Ergolines (Sect. 4.2)
- Resin glycosides (glycoresins) (Sect. 8.3)

Erratically occurring specific secondary metabolites, e.g., (i) β-carbolines in both families (Sect. 4.1), (ii) cardenolides (Sect. 7.9.2), in the Solanaceae or (iii) 2-alkylpiperidines (animartinines; Sect. 5.2.2), serotonin-hydroxycinnamic acid amides (ipobscurines; Sect. 6.7.4) in the Convolvulaceae, are not taken into consideration here.

1.3 Strategy

Phytochemical studies, published in thousands upon thousands of original reports over two centuries, have produced detailed knowledge on such a huge amount of metabolites in both families that it would be impossible to integrate it all into this monograph. Therefore, it is obvious to attach great significance to those phytochemical characters identified above as specific secondary metabolites (definition: Sect. 1.2.3). Such chapters/sections are the centre of attention in this book resulting in a correspondingly comprehensive treatment. Consequently, limitations were necessary as far as general secondary metabolites (definition: Sect. 1.2.3) were concerned. It has to be added that the existence or lack of recent reviews on a class/group of metabolites in the literature has been another point of consideration in the present book, i.e., a lack of recent reviews has stimulated a more extensive treatment.

1.4 Criteria for Selection of Secondary Metabolites Based on their Specific Significance

The author is well aware of the subjectivity of his selection. Nevertheless, he hopes that it is comprehensible. Due to the frequency of their occurrence, general secondary metabolites are of limited value for the characterization of any taxon independent on its level (family, genus, species) – in contrast to specific secondary metabolites. General secondary metabolites may be interpreted as plesiomorphic characters, whereas derived secondary metabolites apparently represent apomorphic characters. Of course, this also implies that, from the evolutionary point of view, the former early products may have been lost in certain taxa during the evolution of the plant kingdom. Thus, e.g., simple lignans – low-molecular congeners of lignin macromolecules – are frequent though not ubiquitous constituents of living angiosperms. From the phylogenetic point of view, loss of a plesiomorphic characters may also be evaluated like an apomorphic character in cladistic analyses. This is – in a figurative way – also true for the phytochemical characterization of a taxon: Also absence of a general secondary metabolite or a class/group of such may contribute to the characterization of a certain taxon.

However, there is no doubt that the presence of specific secondary metabolites makes a much more valuable contribution. Consequently, the following criteria for the selection of secondary metabolites to receive a more detailed treatment in this book reflect this opinion. However, it is necessary to take into account additional aspects:

- Criteria of plant-characterizing and chemotaxonomic significance
  - Specific secondary metabolites or groups of such confined to certain taxa of one or both large Solanales families (“unique secondary metabolites”), e.g., 3α-tropoyloxytropanes (Sect. 3.4), resin glycosides (Sect. 8.3), sesquiterpenoid phytoalexins (Sect. 7.3)

  - Specific secondary metabolites or groups of such occurring in certain taxa of one or both large Solanales families but also in a restricted number of taxa outside the Solanales, e.g., steroidal alkaloids (Sect. 7.8), pyrrolizidine alkaloids (Sect. 3.7), calystegines (Sect. 3.6)
  - Specific secondary metabolites or groups of such **derived** from classes/groups of general secondary metabolites. Such specific secondary metabolites are confined to certain taxa of one or both Solanales families – in contrast to their generally/frequently occurring congeners, e.g., certain lignanamides (jacpaniculines) in *Jacquemontia paniculata* or certain sesquilignans (bonaspectins) in *Bonamia spectabilis* (both Convolvulaceae; Sect. 6.9.3) in contrast to members of the same class which belong to general secondary metabolites, i.e., frequently occurring in the plant kingdom, such as pinoresinol- or dibenzylbutyrolactone-type lignans
- The criterion of size of a special taxon (e.g., number of species of a certain genus) – though only if sufficient phytochemical data are available – e.g., genera *Solanum/Nicotiana* and *Ipomoea/Convolvulus*, respectively
- Criteria of ecological significance, e.g., nicotinoids (Sect. 3.3), calystegines (Sect. 3.5), ergolines (Sect. 4.2)
- Criteria of pharmacological/toxicological significance, e.g., hyoscyamine/atropine (Sect. 3.4), nicotine (Sect. 3.3)
- Criteria of economic significance (economically useful plants: food plants, safety of food plants, production of pure drugs, tobacco products etc.), e.g., capsaicinoids (Sect. 6.5), steroidal alkaloids (Sect. 7.8), hyoscyamine/atropine (Sect. 3.4), nicotine (Sect. 3.3)

1.5 Accumulation or Low-Level Occurrence of Secondary Metabolites

Another important point with regard to characterization is the question of whether a certain constitutive metabolite or a group of such is accumulated in a certain species or whether it is only present in a very low concentration. Accumulation of constitutive metabolites is advantageous, e.g., for defence against herbivores, microorganisms, viruses, or plant competitors. However, modern analytical methods such as GC/MS elucidated that many metabolites are present in very low concentrations in one taxon in contrast to their accumulation in another, closely related one. Such cases demonstrate that the principal ability to synthesize a certain compound is given for, e.g., an individual species though this metabolite is not really "used" due to differential expression of the corresponding genes. A species may be rendered poisonous only by accumulation of, e.g., a major alkaloid. However, both accumulation as well as the principal ability to synthesize a certain metabolite – even if only in low amounts – contributes to the phytochemical characterization of a taxon. Accumulation

is another character exceeding a simple low-level presence; therefore, it may be considered as an additional contribution to the characterization of a taxon.

1.6 Significance of Chemotaxonomy

According to Cronquist, cited by Spring and Buschmann (1998) "….. chemical characters are like other characters: they work when they work, and they don't work when they don't work. Like all taxonomic characters, they attain their value through correlation with other characters, and perfect correlations are the exception rather than the rule." Based on comprehensive studies with regard to Fabaceae, Solanaceae, and Lamiaceae, Wink (2003) concluded "The inconsistent secondary metabolite profiles mean that the systematic value of chemical characters become a matter of interpretation in the same way as traditional morphological markers. Thus, the distribution of secondary metabolites has some value for taxonomy but their occurrence apparently reflects adaptations and particular life strategies embedded in a given framework."

1.7 Principal Nomenclatural Points

1.7.1 Species Names

"Worldwide, not all authors of research papers use the currently accepted name, so caution is necessary and botanical sources should be checked" (Trease and Evans 2002). The author of the present book has tried to meet these requirements to the best of his knowledge. Apparently invalid names used in original reports have been supplied by valid ones, e.g., the species termed in the original reference *Lycopersicon esculentum* is cited as *Solanum lycopersicum* L. sub nom. *Lycopersicon esculentum* MILL. Unfortunately, species epithetons lack the respective authorities in the majority of phytochemical reports. This sometimes led to equivocal situations. As far as possible the corresponding authorities have been added to each species. If species are listed in tables, the authorities are added there but not in the corresponding part of the text.

1.7.2 Chemical Trivial Names

For practical reasons, trivial names for secondary metabolites rather than names according to systematic rules of the IUPAC or Chemical Abstracts have been widely accepted in plant biochemistry. This is also the case for Chemical Abstracts

and the corresponding SciFinder Scholar™ 2006. However, such systematic names can easily be taken from the latter. In very rare cases, original research reports avoided trivial names for novel secondary metabolites, thus forcing the author to use names according to the systematic rules.

References

Bremer K, Friis EM, Bremer B (2004) Molecular phylogenetic dating of asterid flowering plants shows Early Cretaceous diversification. Syst Biol 53:496–505

Cadet de Gassicourt L (1817) J Pharmacie 3:495; fide Shellard EJ (1961a)

Deroin T (2001) Flore de Madagascar et des Comores, famille 171, Convolvulaceae. Muséum National d'Histoire Naturelle, Paris

Desfosses M (1820) Extrait d'une lettre. J Pharmacie 6:374–376

Desfosses M (1821) Extrait d'une lettre. J Pharmacie 7:414–417

Durbin ML, McCaig B, Clegg MT (2000) Molecular evolution of the chalcone synthase multigene family in the morning glory genome. Plant Mol Biol 42:79–92

Geiger PL, Hesse (1833a) Darstellung des Atropins. Liebigs Ann Chem 5:43–81

Geiger PL, Hesse (1833b) Fortgesetzte Versuche ueber Atropin. Liebigs Ann Chem 6:44–65

Hunziker AT (2001) Genera Solanacearum – the genera of Solanaceae illustrated, arranged according to a new system. A.R.G.Gantner Verlag, Ruggell, Liechtenstein

Johnston JFW (1840) Philos Trans R Soc, London A 341; fide Noda et al. (1990)

Judd WS, Campbell CS, Kellogg EA, Stevens PF (1999) Plant Systematics – a phylogenetic approach. Sinauer Associates, Inc, Sunderland, MA, USA

Mein (1833) Ueber die Darstellung des Atropins in weißen Krystallen. Liebigs Ann Chem 6:67–72

Meissner CFW (1819) Ueber ein neues Pflanzenalkali (Alkaloid). J Chem Phys 25:381

Noda N, Kogetsu H, Kawasaki T, Miyahara K (1990) Resin glycosides. VI. Scammonins I and II, the resin glycosides of Radix Scammoniae from *Convolvulus scammonia*. Phytochemistry 29:3565–3569

Orechoff A, Konowalowa R (1933) Über die Alkaloide von *Convolvulus pseudocantabricus* Schrenk. (1.Mitt.) Arch Pharm 271:145–148

Posselt W, Reimann L (1828) Chemische Untersuchung des Tabaks und Darstellung eines eigenthümlich wirksamen Prinzips dieser Pflanze. Poggend Ann Phys Chem 8:399–410

Sertürner FW (1805) Darstellung der reinen Mohnsäure (Opiumsäure) nebst einer chemischen Untersuchung des Opiums mit vorzüglicher Hinsicht auf einen darin neu entdeckten Stoff und die dahin gehörigen Bemerkungen. J Pharmacie 14:47–93

Sertürner FW (1817) Über das Morphium, eine neue salzfähige Grundlage, und die Mekonsäure, als Hauptbestandteile des Opiums. Ann Phys 55 (neue Folge 25):56–89

Shellard EJ (1961a) The chemistry of some convolvulaceous resins. Part I. Vera Cruz Jalap. Planta Med 9:102–116

Spring O, Buschmann H (1998) Grundlagen und Methoden der Pflanzensystematik. Quelle & Meyer Verlag, Wiesbaden, Germany

Trease D, Evans WC (2002) Pharmacognosy, 15th edn. W.B. Saunders, Edinburgh, UK

Wink (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64:3–19

2
Classification and System in Solanales

2.1 Position of the Order Solanales and its Families

Traditional classifications predominantly based on morphological characters have already taken into account – with increasing effort – the significance of interrelationships reflecting the evolutionary history of taxa. However, only the exciting developments during the past two decades, resulting from huge advances in molecular biology, have achieved a phylogeny-based classification of broad scientific acceptance. Concerning the role of morphology in phylogeny reconstruction / systematics, a passionate summing-up has recently been published by Scotland et al. (2003): "We disagree that morphology offers any hope for the future to resolve phylogeny at lower or higher taxonomic levels. In other words, just because there are enough morphological synapomorphies for careful observers to recognize many monophyletic groups over the years in traditional taxonomic studies, further dissection of morphology by present or future scientists may still not be able to resolve the full branch structure of the tree of life. Molecular phylogenetics holds several orders of magnitude more hope for that end, even though an honest observer would have to agree that even whole genomes for all species will probably not yield a fully resolved, highly confident tree." An updated phylogenetic classification includes the order Solanales DUMORTIER comprising five families with altogether 165 genera and 4080 species (Table 2.1; Stevens 2001 onwards). An alternative estimate has taken 140 genera and 4800 species as a basis (Albach et al. 2001). The two large families Solanaceae and Convolvulaceae are both characterized by an almost world-wide distribution whereas the minor families Hydroleaceae, Montiniaceae, and Sphenocleaceae are limited to the tropics.

2.1.1 Traditional Systematics

Above the rank of order, Solanales have been nested traditionally within subclass Asteridae s.l. (e.g., Takhtajan 1964) which comprised all former Sympetalae Tetracyclicae or alternatively within subclass Lamiidae (e.g., Ehrendorfer 1991),

Table 2.1 Families of the order Solanales DUMORTIER according to the Angiosperm Phylogeny Website (Stevens 2001 onwards; version May 2006)

Family	Authority	Genera	Species	Distribution
Convolvulaceae (morning glory or bindweed family)	A.L. DE JUSSIEU	57 (55)[a]	1601 (1930)[a]	World-wide (most diverse in tropical and subtropical regions)
Hydroleaceae	BERCHTOLD & J. PRESL	1	12	Tropical
Montiniaceae	NAKAI	3	5	Africa / Madagascar
Solanaceae (nightshade or potato family)	A.L. DE JUSSIEU	102 (147)[a] (92)[b]	2460 (2930)[a] (2300)[b]	World-wide (overwhelmingly Neotropics)
Sphenocleaceae	(LINDLEY) BASKERVILLE	1	2	Pantropical

[a] Numbers in brackets show one alternative point of view: Judd et al. (1999)
[b] Numbers in brackets show another alternative point of view: Hunziker (2001)

a more restricted taxon with, e.g., Gentianales, Boraginales, Scrophulariales, Lamiales, although excluding Campanulales and Lamiales, i.e., Asteridae s.str. According to other views Solanaceae, Nolanaceae (nowadays included in Solanaceae), Convolvulaceae, Cuscutaceae, and Dichondraceae (both nowadays included in Convolvulaceae) have been ranked together with, e.g., Boraginaceae, Hydrophyllaceae, Polemoniaceae among the order Polemoniales (Takhtajan 1959, 1973; Heywood 1978), whereas Ehrendorfer has integrated Polemoniaceae into Solanales. Though many authors have placed Convolvulaceae in the order Solanales (e.g., Cronquist 1988; Dahlgren 1989; Thorne 1992) the family has been segregated alternatively into its own order, Convolvulales; this opinion was based on a number of characteristics not shared with other Solanales, e.g. presence of articulated latex canals and latex cells (Takhtajan 1997).

2.1.2 Phylogenetic Classification Based on Predominantly (Macro)molecular Data Sets

Macromolecular data have revolutionized the view of phylogenetic relationships not only in the plant kingdom. “The big difference is that there are simply many more molecular (DNA) characters available (than morphological) and their interpretation is generally easier – an adenine is an adenine, whereas compound leaves, for example, can form in quite different ways in different plants. As a result, molecular data are now widely used for generating phylogenetic hypotheses” (Judd et al. 1999).

2.1.2.1 Angiosperms, Asterids

A number of recent cladistic analyses predominantly based on molecular data have extraordinarily improved the understanding of phylogenetic relationships among angiosperms, e.g., studies inferred from chloroplast *rbcL* sequences (Chase et al. 1993), nuclear 18S rDNA sequences (Soltis et al. 1997), and a combination of chloroplast *atpB* and *rbcL* sequences (Savolainen et al. 2000). Moreover, a combined cladistic analysis including molecular, morphological, and even chemical characters, i.e., small molecules, was published (Nandi et al. 1998). Another study comprised a combined data set for 560 angiosperms and 7 outgroups based on sequences of three genes (18S rDNA, *rbcL*, *atpB*; Soltis et al. 2000). In this case stronger support could be received from the jackknife consensus tree than from bootstrap or jackknife analyses in previous studies based only on single genes. The asterid clade (99% support) was one of six subclades of the core eudicots (core tricolpates). Monophyly of the core eudicots and the subclades, respectively, was also strongly supported (100%). This asterid clade showed trichotomy comprising Ericales, Cornales, and euasterids I / II (core asterids), each also with strong support (89–98%). Basal interrelationships among Cornales, Ericales, and core asterids could be resolved with strong support in a more recent study (Bremer et al. 2002; see below). An ordinal classification for the families of flowering plants has been published (Angiosperm Phylogeny Group 1998).

2.1.2.2 Core Asterids, Euasterids I (Lamiids)

The core asterid clade includes the euasterid I clade (renamed as lamiids sensu Bremer et al. 2002; Fig. 2.1) and the euasterid II clade (renamed as campanulids sensu Bremer et al. 2002). In contrast to the euasterid II clade (88% support) the euasterid I clade was only weakly supported (56%) in the study of Soltis et al. (2000); it comprised a trichotomy: (1) Oncothecaceae, (2) Garryales, and (3) a large well-supported monophyletic group (99%) consisting primarily of three again well-supported orders, Lamiales (99%), Gentianales (100%), Solanales (100%). Moreover, two families, Vahliaceae and Boraginaceae (100%), were left unassigned to an order. This was in contrast to certain previous studies based on sequences of only single genes which placed Boraginaceae as part of Solanales (e.g., Chase et al. 1993). However, the third large subclade in the study of Soltis et al. still included polytomy.

One phylogenetic analysis focused on asterids only, with an expanded number of species, based on sequences of the three genes mentioned above combined with those of a fourth, chloroplast *ndhF*, and a high number of asterid-specific parsimony-informative characters led to further elucidation (Albach et al. 2001). The Solanales constitute a subclade including again Solanaceae, Convolvulaceae, Hydroleaceae, and Montiniaceae. Relationships within Solanaceae were congruent with results from a previous study (Olmstead and Sweere 1994; see below). Moreover, a recent phylogenetic study of asterids based on three coding and three

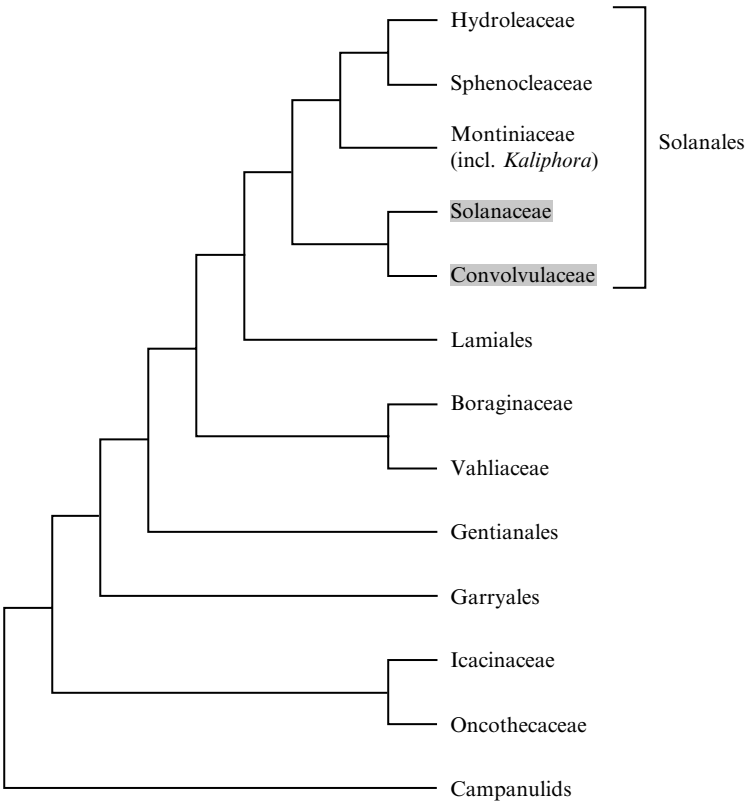


Fig. 2.1 Phylogenetic tree of the lamiids sensu Bremer et al. (2002) (syn.: Euasterids I) established by cladistic analyses based on molecular data sets; outgroup: Campanulids sensu Bremer et al. (2002) (syn.: Euasterids II); for details and further references see text

non-coding chloroplast DNA markers proved that the three noncoding markers are almost equally useful as the three coding genes in phylogenetic reconstruction at the high level of orders and families in asterids (Bremer et al. 2002). In general this study has provided increased support for resolution within the asterids. Thus, the strict consensus tree from the combined analysis of all of the six markers also shows strong support (100%) for the lamiids (new name replacing euasterids I) in contrast to the study of Soltis et al. (2000; see above). However, the nodes representing interrelationships among the orders of the lamiids are more or less supported by jackknife analysis: Garryales/Gentianales/Vahliaceae, Boraginaceae are successive sister groups to Solanales/Lamiales though mainly with low support (<50%). Only the resolution of Gentianales and the remaining lamiids (including Solanales) is strongly supported (100%).

Molecular phylogenetic dating – based on the data matrix from Bremer et al. (2002) – showed diversification of lamiids like other major subgroups of the

asterids during the Early Cretaceous; Solanales are from the Mid-Cretaceous (stem node age: 106 my; crown node age: 100 my) (Bremer et al. 2004).

2.1.2.3 Solanales and Families Belonging to this Order

In the study of Soltis et al. (2000) the Solanales genera *Solanum*, *Nolana*, *Nicotiana*, *Petunia*, *Schizanthus*, and *Duckeodendron* (Solanaceae), *Ipomoea* and *Convolvulus* (Convolvulaceae), *Montinia* (Montiniaceae), and *Hydrolea* (Hydroleaceae) were integrated into a jackknife consensus tree of the euasterid I clade (lamiids) with predominantly excellent support: Montiniaceae/Hydroleaceae were sister to Convolvulaceae/Solanaceae (96%); Montiniaceae were sister to Hydroleaceae (99%), Convolvulaceae was sister to Solanaceae (99%). Within the Solanaceae subclade (100%), there was a trichotomy comprising *Duckeodendron*, *Schizanthus*, and a clade with the remaining genera (97%); *Petunia* is sister to *Nicotiana/Nolana/Solanum* (99%). Only the *Nolana/Solanum* subclade showed low support (58%). The study of Bremer et al. (2002) included representatives of all five Solanales families, i.e., the four families mentioned above and in addition Sphenocleaceae. For the first time these families were supported as a monophyletic group (90%). Solanaceae/Convolvulaceae together formed the sister of the three remaining families (Montiniaceae, Sphenocleaceae, Hydroleaceae). The relationship between Solanaceae and Convolvulaceae again received 100% jackknife support. The time of divergence between this two families is – assuming molecular clock arguments – about 70 my ago (Durbin et al. 2000).

2.2 Solanaceae A.L. de Jussieu: Delimitation, Intrafamilial Circumscription and Relationships

Fossil palynomorphs belonging to the genus *Solanaceaepollenites* from the Eocene (55–38 my ago) were found in Taiwan (Shaw 1999).

2.2.1 Traditional Systematics

Already in the famous New Kreüterbuch (The New Herbal) by Leonhart Fuchs (1501–1566), a pioneer of modern Botany, eight solanaceous species from seven genera were described scientifically and depicted in excellent plant illustrations (coloured woodcuts; Fuchs 1543): *Atropa belladonna* L. (deadly nightshade); *Capsicum annuum* L. (paprika, red and green sweet pepper); *Datura metel* L. (downy thornapple); *Hyoscyamus niger* L. (henbane); *Mandragora officinarum* L. (mandrake); *Physalis alkekengi* L. (Chinese or Japanese lantern); *Solanum melongena* L. (aubergine, eggplant), *S. nigrum* L. (black nightshade). Linnaeus (1707–1778) recognized

13 genera still accepted nowadays in his Species Plantarum (1753) and Genera Plantarum (1754), respectively (Panzer 1788): *Atropa* (3 spp. including *A. mandragora* = *Mandragora officinarum*), *Browallia* (3), *Brunfelsia* (1), *Capsicum* (3), *Cestrum* (3), *Datura* (5), *Hyoscyamus* (7), *Lycium* (4), *Nicotiana* (7), *Nolana* (1), *Physalis* (12), *Schwenckia* (1), *Solanum* (34). The long history of the classification of the Solanaceae before and after Linnaeus has been summarized in a comprehensive paper by D'Arcy (1979).

Recently, Hunziker (2001) has published a synopsis of the family as "… the first attempt, in the last 110 years after Wettstein (1891), to coordinate the profusion of available information on every genus of Solanaceae, integrating it critically in a system of classification". He divided the nightshade family in 6 subfamilies composed of 92 genera and ca. 2300 species according to Table 2.2. Hunziker excluded the related genera *Duckeodendron, Goetzea*, and *Nolana* from Solanaceae as independent families.

2.2.2 Phylogenetic Classification Based on Predominantly (Macro)molecular Data Sets

In early attempts to reconstruct phylogeny certain prominent genera have been subjected to analyses by assessing chloroplast DNA restriction site variation: *Nicotiana* (Kung et al. 1982; Olmstead et al. 1990), *Lycopersicon* (Palmer and Zamir 1982), *Solanum* sect. *Tuberosum* (Hosaka et al. 1984), *Capsicum* (Mitchell et al. 1989). Meanwhile, a number of further studies on single genera, especially those which include economically important cultivated species, have been published integrating, e.g., a chloroplast *atpB-rbcL* noncoding spacer region, ITS nrDNA, and different loci of plastid DNA, respectively in case of, e.g., *Capsicum* (Walsh and Hoot 2001), *Nicotiana* (Chase et al. 2003; Knapp et al. 2004a; Clarkson et al. 2004). For other genera see certain chapters/sections, e.g., *Solanum* in connection with steroid alkaloids (Sect. 7.8). A first higher-level chloroplast DNA analysis including 42 species, representing 12 of the 14 tribes recognized by D'Arcy (1991), with *Ipomoea coccinea* L. (Convolvulaceae) as the outgroup representative had already been published a few years later (Olmstead and Palmer 1992). This phylogenetic study revealed the important insight that the Cestroideae are the ancestral subfamily and not the Solanoideae as considered traditionally.

As a matter of priority this book is intended to document the occurrence as well as the distribution of secondary metabolites within the Solanales and to demonstrate relationships of chemotaxonomic relevance resulting from this documentation. In this connection a **provisional** phylogenetic tree of the Solanaceae (Fig. 2.2) has been established *by theoretical combination of different cladistic analyses*. This has been based on (i) restriction site analysis of the entire chloroplast genome and (ii) on chloroplast *rbcL* and *ndhF* gene sequences, respectively (Olmstead et al. 1998, 1999; Garcia and Olmstead 2003; Santiago-Valentin and Olmstead 2003) or on chloroplast *rbcL* and *matK* gene sequences (Gemeinholzer and Wink 2001; Wink 2003).

Table 2.2 Classification and geographic distribution of the Solanaceae based on data of Hunziker (2001)

Genera in *bold* = significant for this book (studies on secondary metabolites published); genera in *standard* type = no phytochemical results available; *underlined* genera = classification different from the phylogenetic tree based on (DNA) molecular analysis (see Fig. 2.2)

Subfamily	Tribe	Subtribe	Genus	Species	Distribution[a]
Cestroideae	Cestreae		***Cestrum***	175	Neotropics
			Vestia	1	Chile
			Sessea	28	Neotropics
	Metternichieae		*Metternichia*	1	Brazil
	Latueae		***Latua***	1	Chile
	Nicotianeae	Nicotianinae	***Nicotiana***	66	AM/AUS
			Petunia	34	S-AM
			Fabiana	15	S-AM
		Nierembergiinae	***Nierembergia***	21	S-AM
			Bouchetia	3	AM
		Leptoglossinae	***Leptoglossis***	7	S-AM
			Hunzikeria	3	AM
			Plowmania	1	C-AM
	Benthamielleae		*Benthamiella*	12	Chile/Argent.
			Pantacantha	1	Argentina
			Combera	2	Chile/Argent.
	Francisceae		***Brunfelsia***	46	Neotropics
	Browallieae		***Browallia***	17?	AM
			Streptosolen	1	S-AM
	Schwenckieae		***Schwenckia***	25	AM
			Melananthus	5	AM
			Protoschwenckia	1	Boliv./Brazil
			Heteranthia	1	Brazil
Juanulloideae	Juanulloeae		*Juanulloa*	8	Neotropics
			Dyssochroma	2	Brazil
			Ectozoma	1	S-AM
			Hawkesiophyton	3	Neotropics
			Markea	17	S-AM
			Merinthopodium	2	Neotropics
			Rahowardiana	2	Panama/Col.
			Schultesianthus	5	Neotropics
			Trianaea	2	S-AM
Solanoideae	Nicandreae		***Nicandra***	1	Peru/Argent.
	Mandragoreae		***Mandragora***	2	E-Mediterr.
	Datureae		***Datura***	11	N/C-AM
			Brugmansia	6	S-AM
	Lycieae		***Lycium***	81	Cosmopol.
			Phrodus	1	Chile
			Grabowskia	4	Neotropics
	Solaneae	Witheringinae	***Witheringia***	28	Neotropics
			Brachistus	3	C-AM
			Cuatresia	11	Neotropics
			Deprea	7	Neotropics
			Discopodium	1	trop. Africa
			Exodeconus	6	S-AM
			Jaltomata	50	Neotropics
			Nothocestrum	6	Hawaii
			Acnistus	1	Neotropics

(continued)

Table 2.2 Classification and geographic distribution of the Solanaceae based on data of Hunziker (2001) (continued)

Genera in *bold* = significant for this book (studies on secondary metabolites published); genera in *standard* type = no phytochemical results available; *underlined* genera = classification different from the phylogenetic tree based on (DNA) molecular analysis (see Fig. 2.2)

Subfamily	Tribe	Subtribe	Genus	Species	Distribution[a]
		Physalinae	***Physalis***	90	AM
			Quincula	1	N-/C-AM
			Leucophysalis	3	AM
			Chaemasaracha	10	N-/C-AM
		Iochrominae	***Iochroma***	16	S-AM
			Saracha	2	S-AM
			Oryctes	1	N-AM
			Tubocapsicum	1	E-/SE-AS
		Capsicinae	***Capsicum***	20	C-/S-AM
			Aureliana	5	S-AM
			Athenaea	7	Brazil
			Darcyanthus	1	Peru/Boliv.
			Eriolarynx	3	Argent./Boliv.
			Vassobia	2	S-AM
			Larnax	12	S-AM
			Dunalia	5	S-AM
			Withania	20	Old World
		Solaninae	***Solanum***	1033	Cosmopol.
			Cyphomandra	41	Neotropics
			Lycopersicon	7	S-AM
			Lycianthes	150	Neotropics[b]
			Triguera	1	SW-Mediterr.
			Normanna	2	Macronesia
	Atropeae		***Atropa***	2	EUR/AS[c]
	Jaboroseae		***Jaborosa***	23	S-AM
			Salpichroa	15	S-AM
			Nectouxia	1	Mexico
	Solandreae		***Solandra***	10	Neotropics
	Hyoscyameae		***Hyoscyamus***	23	Old World
			Anisodus	4	China/Nepal
			Atropanthe	1	China
			Physochlaina	8	AS
			Przewalskia	1	China
			Scopolia	2	EUR/AS
Salpiglossoideae	Salpiglossideae		***Salpiglossis***	2	Chile/Argent.
			Reyesia	4	Chile/Argent.
Schizanthoideae	Schizantheae		***Schizanthus***	12	Chile/Argent.
Anthocercidoideae	Anthocercideae		***Anthocercis***	10	W/S-AUS
			Anthotroche	3	W-AUS
			Cyphanthera	9	W/S-AUS
			Crenidium	1	W-AUS
			Duboisia	4	AUS[d]
			Grammosolen	2	S-AUS
			Symonanthus	2	W-AUS

[a] Abbreviations: capital letters for the four points of the compass (C = Central) and continents (e.g.: AM = America), respectively; Medit. = Mediterranean

[b] 20 species native to South-East Asia

[c] Distribution of *A. belladonna* L.; *A. baetica* WILLK.: SE-Spain/N-Morocco

[d] *D. myoporoides* R.BR. additionally in New Caledonia

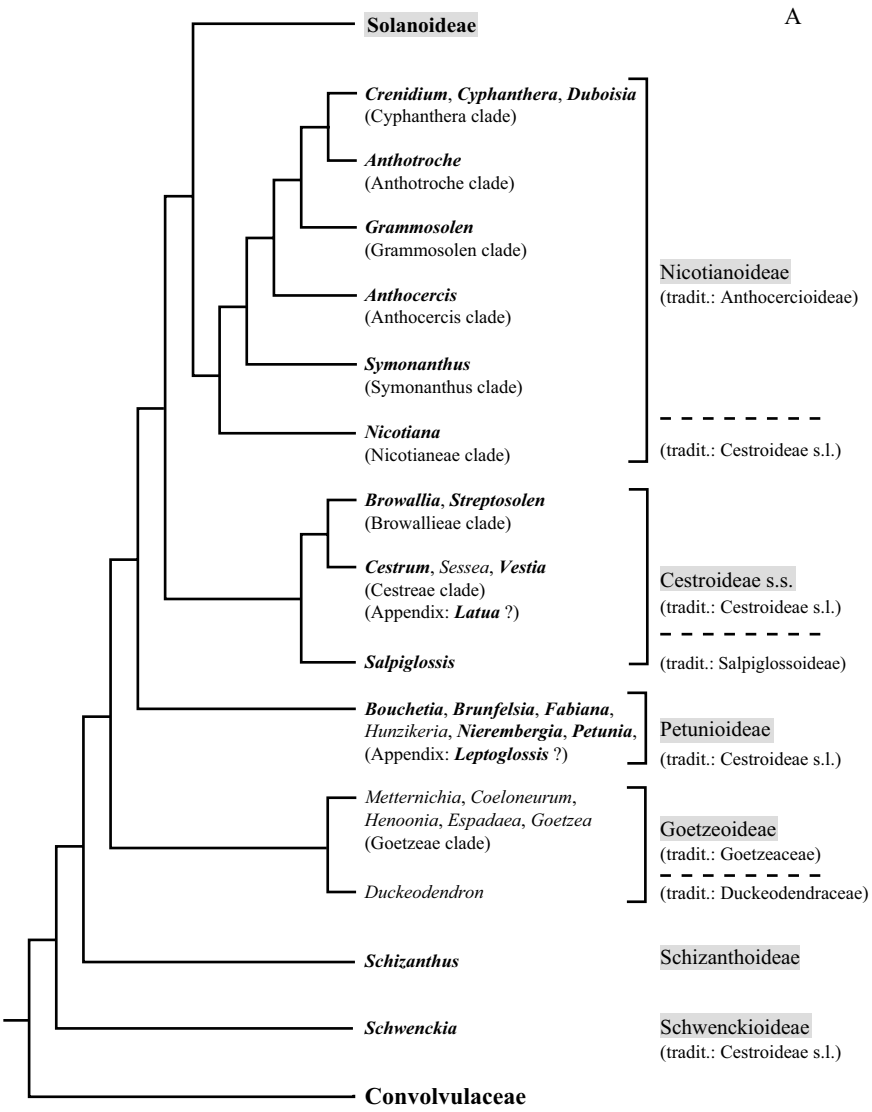


Fig. 2.2 Provisional phylogenetic tree of the Solanaceae established by theoretical combination of different cladistic analyses; outgroup: Convolvulaceae; for details and references see text. Genera *in bold* indicate the significance for this book (presence of phytochemical data in the literature for this specific genus); genera *in standard type* indicate that there are no data available. Genera *with a question mark* listed under "Appendix" have been nested within a clade according to the traditional classification of Hunziker (2001) due to the lack of molecular data for this specific genus. The genera *Schultesianthus* and *Markea* have to be added in bold as an "Appendix" to the Juanulloinae subclade with a question mark; this is also true for the genus *Eriolarynx* to the Iochrominae subclade. Genera without molecular data *and* without phytochemical reports are not listed

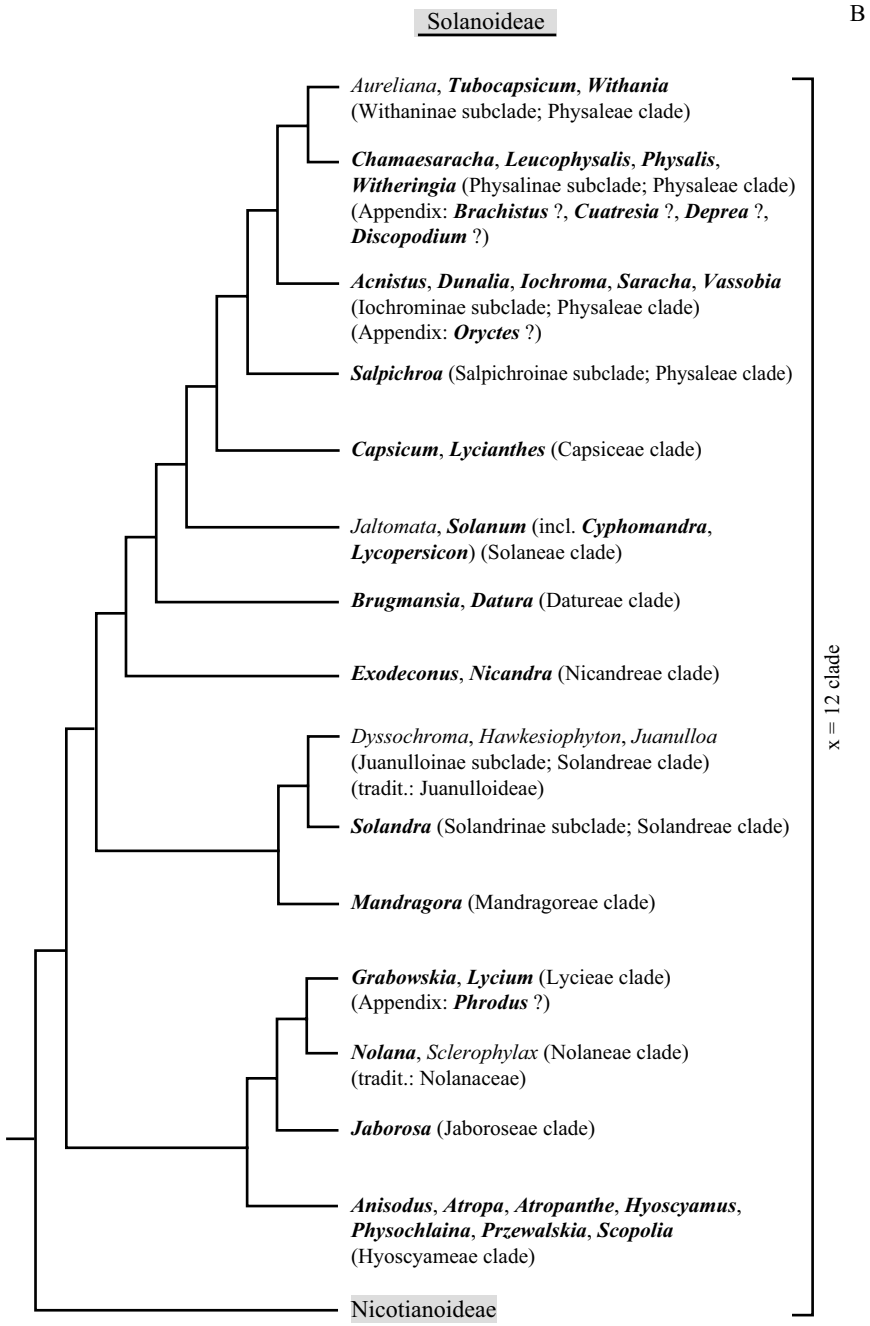


Fig. 2.2 (continued)

Genera in bold indicate the presence of phytochemical data in the literature for this specific genus (otherwise there are no data available). Genera with a question mark listed under "Appendix" have been nested within a clade according to the traditional classification of Hunziker (2001) due to the lack of molecular data for this specific genus.

Part A of Fig. 2.2 comprises six subfamilies according to Olmstead et al. (1999). Part B comprises only the subfamily Solanoideae. The strongly supported X = 12 clade, so called because of a base chromosome number synapomorphy and indicated by one huge square close bracket (right hand side) in the complete part B, represents a large monophyletic group comprising the subfamilies Nicotianoideae (i.e., the traditional Anthocercideae as well as *Nicotiana*, both resolved into branches of part A) and Solanoideae (Olmstead and Sweere 1994; Olmstead et al. 1999). Confidence in the branching order among progressively deeper branches [Cestroideae s.s., Petunioideae, Goetzeoideae, Schizanthoideae, Schwenkioideae (part A)] is low (e.g., low bootstrap values). In order to avoid non-monophyletic groups distinct monophyletic subfamilies were recognized (Olmstead et al. 1999). Thus, e.g., the Cestroideae s.s. were shown to be strongly supported (96–100%; Santiago-Valentin and Olmstead 2003). The inversion of the originally sequence Cestroideae s.s. (deeper branch) / Petunioideae (Olmstead et al. 1999) to Petunioideae / Cestroideae s.s. is based on the 2003 paper.

At present the "synthesized" cladogram of Fig. 2.2 is rather a *heuristic tool* which may be useful for chemotaxonomic considerations of different classes of metabolites than an unequivocal representation of the Solanaceae phylogeny. Its monophyletic character is advantageous over Hunziker's traditional classification (Hunziker 2001) (Table 2.2) which recognized many paraphyletic and some polyphyletic groups. This should make it possible to establish a more consistent framework for chemotaxonomic aspects. A similar approach, a diagram which they also called "a heuristic tool", has been made by Knapp et al. (2004b) in order to explore the distribution of certain morphological characters in the family. According to Knapp (2002), monophyletic groups are more informative for the examination of character evolution. This is also true for chemical characters.

Differences between classifications of Hunziker and Olmstead et al. are indicated in Table 2.2. Those interested in more details are referred to the corresponding synopsis in Table 2 (including comments) of the paper of Olmstead et al. (1999).

2.3 Convolvulaceae A.L. de Jussieu: Delimitation, Intrafamilial Circumscription and Relationships

Fossil pollen was found worldwide, with the oldest occurrence being *Calystegiapollis microechinatus* from the early Eocene (~55 my ago) of Africa (Martin 2001). The separation of *Ipomoea nil* (L.) Roth, imperial morning glory, and *I. purpurea* (L.) Roth, common morning glory, is assumed to be ~8 my ago (late Miocene) (Durbin et al. 2001).

2.3.1 Traditional Systematics

As in the case of Solanaceae the New Kreüterbuch (The New Herbal) by Leonhart Fuchs (Fuchs 1543) already contained convolvulaceous species: *Calystegia sepium* (L.) R. Br. (hedge bindweed) and *Convolvulus arvensis* L. (field bindweed). Linnaeus recognized six genera still accepted nowadays in his Species Plantarum (1753) and Genera Plantarum (1754), respectively (Panzer 1788): *Convolvulus* (52 spp.) *Cressa* (1), *Cuscuta* (3), *Evolvulus* (5), *Ipomoea* (21), *Porana* (1). However, the number for *Convolvulus* included several species which are nowadays nested within other genera, e.g., *Calystegia*, *Ipomoea*, and *Merremia*. In the beginning pre-cladistic classifications were based on very few characters which have been considered to be of high importance, e.g., pollen surface features, fruit and style characters. The historical development of such schemes from Choisy (1845) to Roberty (1964) has been reviewed in detail (Austin 1973; Manos et al. 2001; Stefanović et al. 2002, 2003 and references therein). In order to reconstruct the phylogeny of the Convolvulaceae a comprehensive cladistic analysis of the family including all genera and based on 128 traditional, i.e., non-macromolecular characters such as habit, vegetative morphology and anatomy, reproductive structures, embryo features, and chromosome numbers, was published by Austin (1998). Recently, a study on pollen types in South American Convolvulaceae and their taxonomic significance has been produced (Tellería and Daners 2003).

2.3.2 Phylogenetic Classification Based on Predominantly (Macro)molecular Data Sets

Attempts to reconstruct phylogeny of the Convolvulaceae based on (macro)molecular data sets started with certain prominent genera which have been subjected to cladistic analyses by assessing different data sets such as nuclear ITS or *waxy* sequences. The largest genus by far, *Ipomoea*, has been the studied most extensively, though in part only on a limited intraspecific level (subgenus *Quamoclit*; series *Batatas*) (McDonald and Mabry 1992; Huang and Sun 2000; Miller et al. 2002, 2004; Manos et al. 2001). Further reports are available on the second largest genus, *Convolvulus* (Carine et al. 2004) and the holoparasitic genus *Cuscuta* (Stefanović and Olmstead 2004, 2005).

A comprehensive higher-level analysis based on DNA sequences of four chloroplast loci including 112 species (106 green, 6 holoparasitic taxa) nested inside 46 out of 54 recognized genera, representing all nine traditionally recognized tribes (Austin 1973, 1998), with *Nicotiana tabacum* L. / *Schizanthus pinnatus* Ruiz & Pav. (Solanaceae) and *Montinia caryophyllacea* Thunb. (Montiniaceae) as the outgroup representatives has been published by Stefanović et al. 2002. The most important results of this study were:

- Convolvulaceae are monophyletic and sister to Solanaceae
- *Cuscuta* and Dichondreae, previously proposed as separate families, are nested within the Convolvulaceae
- Several distinct monophyletic groups have been identified, some of which correspond to traditional classifications
- Certain traditional tribes (Merremieae, Convolvuleae, Poraneae, Erycibeae) turned out to be polyphyletic
- *Humbertia*, a monotypic Madagascan genus, is the sister to all other members of the family
- *Convolvulus* and *Jacquemontia* are distantly related in contrast to traditional classifications, where they were considered closely related

Based on the numerous results of this comprehensive analysis a phylogenetic approach to a classification of the family has been published (Stefanović et al. 2003) which has been included in Table 2.3 (see also Fig. 2.3). The family is now circumscribed within 12 tribes; the corresponding differences to the traditional classification may be summarized as follows:

- New tribes: Aniseae, Cardiochlamyeae, Humbertieae, Jacquemontieae, Maripeae
- Expanded tribes: Cresseae s.l., Dichondreae s.l., Ipomoeeae s.l.
- Restricted tribes: Convolvuleae s.s., Erycibeae s.s.
- Tribe retained in the traditional sense: Cuscuteae
- Tribe tentatively retained though monophyly uncertain: "Merremieae"
- Abandoned tribes: Argyreieae, Hildebrandtieae, Poraneae

Additionally, two large well-supported clades, which are not assigned formal ranks, are recognized and their names defined: **/Convolvuloideae** and **/Dicranostyloideae**. This is also true for two subclades of the former clade, /Argyreiinae and /Astripomoeinae. The concept for these subclades was adopted by the one proposed by Manos et al. (2001). /Argyreiinae consists primarily of Old World genera such as *Argyreia*, *Lepistemon*, *Stictocardia*, and *Turbina* [the latter genus being only predominantly of Old World origin; exceptions: *T. abutiloides* (H.B.K.) O'Donell, *T. corymbosa* (L.) Raf.] and some Old World *Ipomoea* species, whereas /Astripomoeinae include the small African genus *Astripomoea* and, as its sister, a predominantly New World group of more than 500 *Ipomoea* species (Austin and Huáman 1996).

2.4 Uncharted Territory with Regard to Secondary Metabolites of the Solanales

There is at least one report on structurally elucidated secondary metabolites in 65% of altogether 95 solanaceous genera but only in 55% of altogether 55 convolvulaceous genera. Thus, one third and almost the half of the respective genera are absolutely unexplored from the phytochemical point of view.

Table 2.3 Synopsis of a revised tribal classification of the convolvulaceous genera according to a phylogenetic approach based on molecular data sets (Stefanović et al. 2003; left column) vs of a traditional classification based on non-molecular characters, e.g., morphology, chromosome numbers (Austin 1973, 1998; *right column*)

Left column: Two large well-supported clades (see Fig. 2.3) characterized by initial oblique strokes, enlarged letters in bold and underlining

Genera column: Genera in *bold* = significant for this book (studies on secondary metabolites published); genera in *standard* type = no phytochemical results available

Tribes, based on molecular data; /clade	**Genera**[a]	**Species**[b]	**Distribution**[c]	**Traditional Tribes**
/Convolvuloideae				
Ipomoeeae	***Argyreia***	95	SE-AS/N-AUS	Argyreieae
HALLIER f. (s.l.)	***Astripomoea***	12	Tropical AF/S-AF	Ipomoeeae
	Blinkworthia[d]	3	SE-AS (Burma)	Argyreieae
	Ipomoea[e]	500[f]	**Pantrop.**/temper. reg.	Ipomoeeae
	Lepistemon	10	Paleotropical	
	Lepistemonopsis[d]	1	E-AF	
	Paralepistemon	1	C-/S-AF	
	Rivea	6	SE-AS/AUS	Argyreieae
	Stictocardia	11	AF – SE-AS[g]	Ipomoeeae
	Turbina[e]	15	Pantropical	
"Merremieae" AUSTIN (s.s.)	***Merremia***[e]	70	Pantropical	Merremieae
	Hewittia	1	Paleotropical	
	Hyalocystis[d]	2	Tropical AF	
	Decalobanthus[d]	1	Sumatra	
	Xenostegia	2[h]	AF – Pacific islands	
	Operculina	20	Pantropical	
Convolvuleae (CHOISY) CHOISY (s.s.)	***Convolvulus*** (incl. ***Calystegia***)	250	Cosmopolitan	Convolvuleae
		25	Cosmopolitan	
	Polymeria	7	**AUS/**New Caledonia	
Aniseieae STEFANOVIĆ & AUSTIN	***Aniseia*** (incl. ***Iseia***)	5	**Neotrop.(orig.)/** pantr.	Merremieae
		1	Neotropical	
	Odonellia	2[i]	Neotropical	
	Tetralocularia	1	S-AM	
Cuscuteae CHOISY	***Cuscuta***	155	Cosmopolitan	Cuscutaceae
/Dicranostyloideae				
Jacquemontieae STEFANOVIĆ & AUSTIN	***Jacquemontia***	130	**AM/**AF/AS/AUS	Convolvuleae
	Dicranostyles	8	Neotropical	Erycibeae
Maripeae WEBB. & BERTH.	***Maripa***	19	Neotropical	
	Lysiostyles[d]	5	S-AM	

(continued)

Table 2.3 Synopsis of a revised tribal classification of the convolvulaceous genera according to a phylogenetic approach based on molecular data sets (Stefanović et al. 2003; left column) vs of a traditional classification based on non-molecular characters, e.g., morphology, chromosome numbers (Austin 1973, 1998; *right column*) (continued)

Left column: Two large well-supported clades (see Fig. 2.3) characterized by initial oblique strokes, enlarged letters in bold and underlining

Genera column: Genera in *bold* = significant for this book (studies on secondary metabolites published); genera in *standard* type = no phytochemical results available

Tribes, based on molecular data; /clade	**Genera**[a]	**Species**[b]	**Distribution**[c]	**Traditional Tribes**
Cresseae BENTH. & HOOK. (s.l.)	***Hildebrandtia*** (incl.	9	E-AF/Madag./ Arabia	Hildebrandtieae
	Cladostigma and	3	NE-AF	
	Sabaudiella)	1	AF	
	Seddera	25	**AF**/Mad./Arabia/ India	Cresseae
	Evolvulus	100	**Neotrop.** / 2 spp. pantr.	
	Cressa	1	Pantropical	
	Bonamia[e]	43	**Pantropical/** S-USA	
	Stylisma	10	E-USA	
	Wilsonia	3	AUS	
	Itzaea	1	C-AM	
	Neuropeltis	11	W-AF/S-/SE-AS	
	Neuropeltopsis[d]	1	Borneo	
Dichondreae (CHOISY) CHOISY (s.l.)	***Dichondra***	9	**Mex./SW-US/** pantr.	Dichondreae
	Falkia	3	S-/E-AF	
	Nephrophyllum[d]	1	Ethiopia	
	Petrogenia	1	Mexico/SW-USA	Not accepted ![j]
	Porana p.p.[k]	20?	SE-AS/AUS	Poraneae
	Metaporana	5	Trop.AF/ **Madagasc.**	
	Calycobolus[e]	41	Neotrop./trop. W-AF	
	Dipteropeltis	3	Tropical AF	
	Rapona	1	Madagascar	
Erycibeae (ENDL.) HALLIER f. (s.s.)	***Erycibe***	75	AS/N-AUS	Erycibeae
Cardiochlamyeae STEFANOVIĆ & AUSTIN	*Cordisepalum*	1	SE-AS	Poraneae
	Poranopsis	4	S-/SE-AS/China	
	Cardiochlamys	2	Madagascar	
	Tridynamia	4	NE-India/SE-AS	
	Porana p.p.[l]	1?	AUS	
	Dinetus	7	AS	
Humbertieae (PICHON) STEFANOVIĆ & AUSTIN	***Humbertia***	1	Madagascar	Erycibeae

(continued)

Table 2.3 Synopsis of a revised tribal classification of the convolvulaceous genera according to a phylogenetic approach based on molecular data sets (Stefanović et al. 2003; left column) vs of a traditional classification based on non-molecular characters, e.g., morphology, chromosome numbers (Austin 1973, 1998; *right column*) (continued)
Left column: Two large well-supported clades (see Fig. 2.3) characterized by initial oblique strokes, enlarged letters in bold and underlining

Genera column: Genera in *bold* = significant for this book (studies on secondary metabolites published); genera in *standard* type = no phytochemical results available

[a] According to Stefanović et al. (2003)
[b] Number of species according to Austin (1975), Deroin (2001), Johnson (1992), Meeuse and Welman (2000), van Ooststroom (1953); especially in case of large genera the numbers represent average values based on the estimates published by the different authors; the numbers of species of those genera which are not included in these publications are taken from the "electronic plant information centre", Royal Botanic Gardens, Kew/UK
[c] Mainly according to Stefanović et al. (2003); dominant areas (biodiversity) in bold; abbreviations: Capital letters for the four points of the compass (C = Central) and continents (e.g., AM = America), respectively
[d] Genus not sampled in any molecular study; reasons for integration: see text
[e] Genus not monophyletic as circumscribed traditionally
[f] According to Austin and Huáman (1996) *Ipomoea* is more likely to contain 600 - 700 species
[g] *S. tiliaefolia* (Desr.) Hallier f. introduced into the Neotropics
[h] According to Austin and Staples (1980)
[i] According to Robertson (1982)
[j] According to Austin and Staples (1985) *Petrogenia* should be a synonym of *Bonamia*
[k] *P. velutina* Hall. f., *P. volubilis* Burm f.
[l] *P. commixta* Staples

The number of the solanaceous genera is composed in the following manner: 92 genera recognized by Hunziker (2001) minus 4 genera meanwhile placed in *Solanum* (*Cyphomandra*, *Lycopersicon*, *Normanna*, *Triguera*) plus 7 genera (former Duckeodendraceae, Goetzeaceae, Nolanaceae) meanwhile transferred to Solanaceae.

	Number of genera with at least one report on identified secondary metabolites	Number of genera without any report on identified secondary metabolites
Solanaceae	61	34
Convolvulaceae	31	24

In the case of Solanaceae, no or almost no reports exist especially on the subfamilies Schwenckioidiae (traditional: Schwenckieae) and Goetzeoideae (traditional: Goetzeaceae/Duckeodendraceae), on the traditional subfamily Juanulloideae (Juanulloinae subclade of the Solandrinae clade), the small traditional tribe Benthamielleae, and the Nolaneae clade (traditional: Nolanaceae) (Fig. 2.2; Table 2.2). In the case of Convolvulaceae this is also true for the tribes Cardiochlamyeae and Dichondreae s.l. (Fig. 2.3; Table 2.3). Thus these taxa especially are still a field for the potential discovery of novel metabolites in both families. However, it should

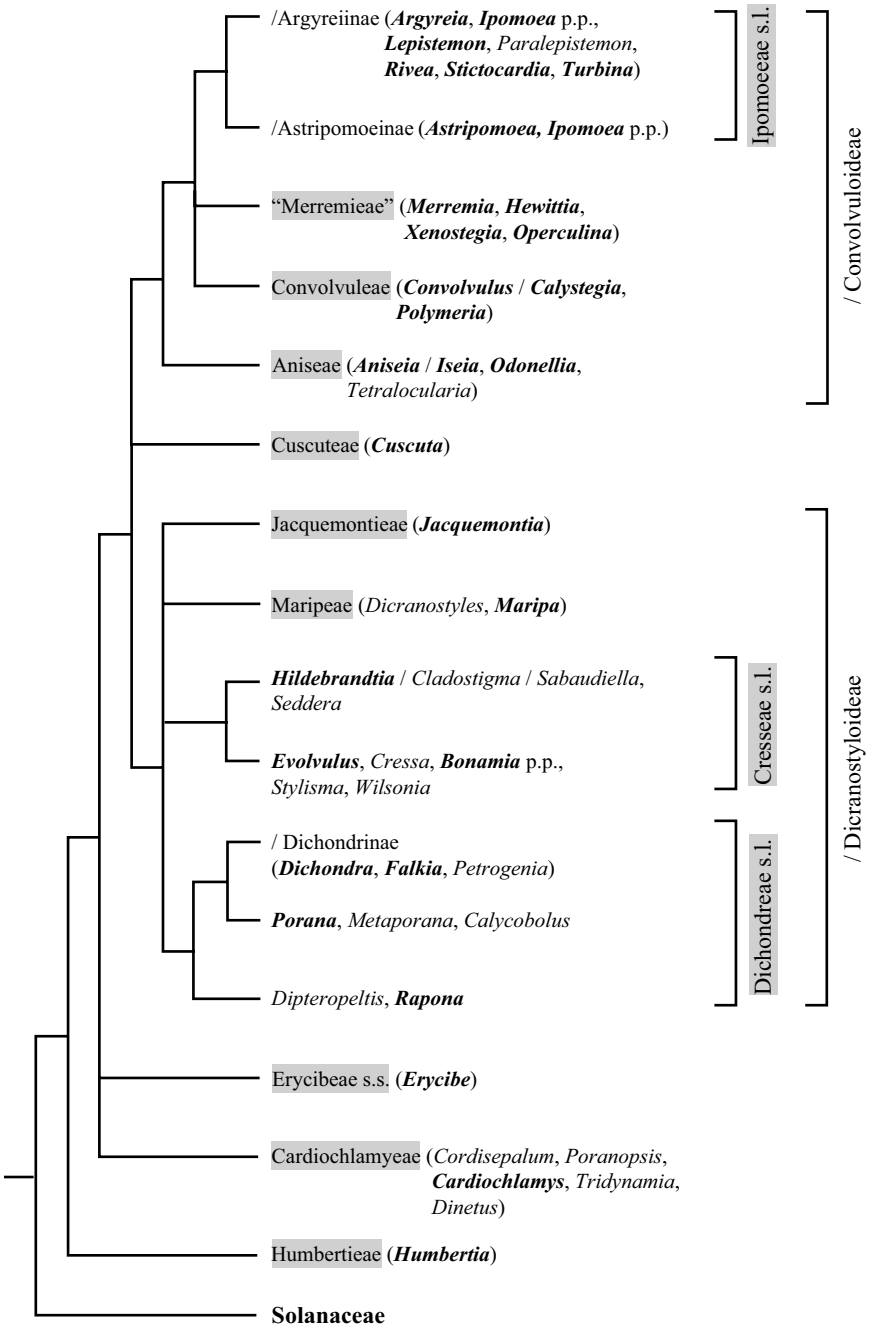


Fig. 2.3 Phylogenetic tree of the Convolvulaceae established by cladistic analyses based on molecular data sets (Stefanović et al. 2003); outgroup: Solanaceae; for details see text. Genera *in bold* indicate the significance for this book (presence of phytochemical data in the literature for this specific genus), in addition the genus *Cressa* ought to be printed in *bold-face;* genera *in standard type* indicate that there are no data available

be noticed that – with the exception of certain famous, often poisonous genera, e.g., *Atropa*, *Datura*, *Nicotiana*, *Solanum* – many species of other already phytochemically characterized genera are also still unexplored. Furthermore, it would be interesting to check the secondary metabolism of the small Solanales families (Hydroleaceae, Montiniaceae, Sphenocleaceae) and to compare the results with those of the large ones.

To date, there is only a single report on unique cyclic thiosulfinates, named zeylanoxides, and known secoiridoid glucosides, both groups discovered as constituents of the tropical weed *Sphenoclea zeylanica* GAERTN. (Sphenocleaceae), one of the most serious weeds of rice. All these metabolites are assumed to be potent allelochemicals since they completely inhibited the root growth of rice seedlings at 3.0 mM (Hirai et al. 2000).

References

Albach DC, Soltis PS, Soltis DE, Olmstead RG (2001) Phylogenetic analysis of asterids based on sequences of four genes. Ann Missouri Bot Gard 88:163–212

Angiosperm Phylogeny Group (1998) An ordinal classification for the families of flowering plants. Ann Missouri Bot Gard 85:531–553

Austin DF (1973) The American Erycibeae (Convolvulaceae): *Maripa*, *Dicranostyles*, and *Lysiostyles*. I. Systematics. Ann Missouri Bot Gard 60:306–412

Austin DF (1975) Family 164. Convolvulaceae. In: Woodson RE Jr, Schery RW and collaborators (eds) Flora of Panama, part IX. Ann Missouri Bot Gard 62:157–224

Austin DF (1998) Parallel and convergent evolution in the Convolvulaceae. In: Mathews P, Sivadasan M (eds) Diversity and taxonomy of tropical flowering plants. Mentor Books, Calicut, India, pp 201–234

Austin DF, Huáman Z (1996) A synopsis of *Ipomoea* (Convolvulaceae) in the Americas. Taxon 45:3–38

Austin DF, Staples GW (1980) *Xenostegia*, a new genus of Convolvulaceae. Brittonia 32:533–536

Austin DF, Staples GW (1985) *Petrogenia* as a synonym of *Bonamia* (Convolvulaceae), with comments on allied species. Brittonia 37:310–316

Bremer B, Bremer K, Heidari N, Erixon P, Olmstead RG, Anderberg AA, Källersjö M, Barkhordarian E (2002) Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. Mol Phylogenet Evol 24:274–301

Bremer K, Friis EM, Bremer B (2004) Molecular phylogenetic dating of asterid flowering plants shows early Cretaceous diversification. Syst Biol 53:496–505

Carine MA, Russell SJ, Santos-Guerra A, Francisco-Ortega J (2004) Relationships of the Macaronesian and Mediterranean floras: molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in *Convolvulus* (Convolvulaceae). Am J Bot 91:1070–1085

Chase MW, Soltis DE, Olmstead RG, Morgan D, Les DH, Mishler BD, Duvall MR, Price RA, Hills HG, Qiu YL, Kron KA, Rettig JH, Conti E, Palmer JD, Manhart JR, Sytsma KJ, Michael HJ, Kress WJ, Karol KG, Clark WD, Hedrén M, Gaut BS, Jansen RK, Kim KJ, Wimpee CF, Smith JF, Furnier GR, Strauss SH, Xiang QY, Plunkett GM, Soltis PS, Swensen SM, Williams SE, Gadek PA, Quinn CJ, Eguiarte LE, Golenberg E, Learn GH Jr, Graham SW, Barrett SCH, Dayanandan S, Albert VA (1993) Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. Ann Missouri Bot Gard 80:528–580

Chase MW, Knapp S, Cox AV, Clarkson JJ, Butsko Y, Joseph J, Savolainen V, Parokonny AS (2003) Molecular systematics, GISH and the origin of hybrid taxa in *Nicotiana* (Solanaceae). Ann Bot 92:107–127

Choisy JD (1845) Convolvulaceae. In: De Candolle A (ed.) Prodromus systematis naturalis regni vegetabiles 9:323–465

Clarkson JJ, Knapp S, Garcia VF, Olmstead RG, Leitch AR, Chase MW (2004) Phylogenetic relationships in *Nicotiana* (Solanaceae) inferred from multiple plastid DNA regions. Mol Phylogenet Evol 33:75–90

Cronquist A (1988) The evolution and classification of flowering plants. The New York Botanical Garden, Bronx

D'Arcy WG (1979) The classification of the Solanaceae. In: Hawkes JG, Lester RN, Skelding AD (eds) The biology and taxonomy of the Solanaceae. Linnean Society Symposium Series. 7. Academic Press, London, pp 3–47

D'Arcy WG (1991) The Solanaceae since 1976, with a review of its biogeography. In: Hawkes JG, Lester RN, Nee M, Estrada N (eds) Solanaceae III – taxonomy, chemistry, evolution. Royal Botanic Garden, Kew, UK, pp 75–137

Dahlgren G (1989) The last Dahlgrenogram: system of classification of the dicotyledons. In: Tann K, Mill RR, Elias TS (eds) Plant taxonomy, phytogeography, and related subjects. Edinburgh University Press, Edinburgh, UK, pp 249–260

Deroin T (2001) Famille 171. Convolvulaceae. In: Morat P (ed) Flore de Madagascar et des Comores. Muséum National d'Histoire Naturelle, Paris, pp 11–287

Durbin ML, McCaig B, Clegg MT (2000) Molecular evolution of the chalcone synthase multigene family in the morning glory genome

Durbin ML, Denton AL, Clegg MT (2001) Dynamics of mobile element activity in chalcone synthase loci in the common morning glory (*Ipomoea purpurea*). PNAS 98:5084–5089

Ehrendorfer F (1991) Allgemeine Grundlagen der Evolution und Systematik (Samenpflanzen). In: Strasburger – Lehrbuch der Botanik für Hochschulen, 33. Aufl, Gustav Fischer Verlag, Stuttgart, Germany, pp 798–807

Fuchs L (1543) New Kreüterbuch / The New Herbal of 1543. Complete coloured edition (facsimile) 2001. Taschen, Köln, Germany

Garcia VF, Olmstead RG (2003) Phylogenetics of tribe Anthocercideae (Solanaceae) based on *ndhF* and *trnL/F* sequence data. Syst Bot 28:609–615

Gemeinholzer B, Wink M (2001) Solanaceae: occurrence of secondary compounds versus molecular phylogeny. In: van den Berg RG, Barendse GWM, van der Weerden GM, Mariani C (eds) Solanaceae V – advances in taxonomy and utilization. Nijmegen University Press, Nijmegen, The Netherlands, pp 165–178

Heywood VH (ed) (1978) Flowering plants of the world. Elsevier, Oxford, UK

Hirai N, Sakashita S, Sano T, Inoue T, Ohigashi H, Premasthira C, Asakawa Y, Harada J, Fujii Y (2000) Allelochemicals of the tropical weed *Sphenoclea zeylanica*. Phytochemistry 55:131–140

Hosaka K, Ogihara Y, Matsubayashi M, Tsunewaki K (1984) Phylogenetic relationship between tuberous *Solanum* species as revealed by restriction endonuclease analysis of chloroplast DNA. Jap J Genet 59:349–369

Huang JC, Sun M (2000) Genetic diversity and relationships of sweetpotato and its wild relatives in *Ipomoea* series *Batatas* (Convolvulaceae) as revealed by inter-simple sequence repeat (ISSR) and restriction analysis of chloroplast DNA. Theor Appl Genet 100:1050–1060

Hunziker AT (2001) Genera Solanacearum – the genera of Solanaceae illustrated, arranged according to a new system. A.R.G. Gantner Verlag, Ruggell, Liechtenstein

Johnson RW (1992) Family 115. Convolvulaceae. In: Harden GJ (ed) Flora of New South Wales. New South Wales University Press, Kensington, Australia, pp 373–384

Judd WS, Campbell CS, Kellogg EA, Stevens PF (1999) Plant systematics – a phylogenetic approach. Sinauer Associates, Sunderland, MA, USA

Knapp S (2002) Tobacco to tomatoes: a phylogenetic perspective on fruit diversity in the Solanaceae. J Exp Bot 53:2001–2022

Knapp S, Chase MW, Clarkson JJ (2004a) Nomenclatural changes and a new sectional classification in *Nicotiana* (Solanaceae). Taxon 53:73–82

Knapp S, Bohs L, Nee M, Spooner DM (2004b) Solanaceae – a model for linking genomics with biodiversity. Comp Funct Genom 5:285–291

Kung SD, Zhu YS, Chen K (1982) *Nicotiana* chloroplast genome III. Chloroplast DNA evolution. Theor Appl Genet 61:73–79

Manos PS, Miller RE, Wilkin P (2001) Phylogenetic analysis of *Ipomoea*, *Argyreia*, *Stictocardia*, and *Turbina* suggests a generalized model of morphological evolution in morning glories. Syst Bot 26:585–602

Martin HA (2001) The family Convolvulaceae in the Tertiary of Australia: evidence from pollen. Austral J Bot 49:221–234

McDonald JA, Mabry TJ (1992) Phylogenetic systematics of New World *Ipomoea* (Convolvulaceae) based on chloroplast DNA restriction site variation. Plant Syst Evol 180:243–259

Meeuse ADJ, Welman WG (2000) Convolvulaceae. In: Germishuizen G (ed) Flora of Southern Africa, vol 28, part 1. National Botanical Institute, Pretoria, South Africa, pp 1–138

Miller RE, Buckley TR, Manos PS (2002) An examination of the monophyly of morning glory taxa using Bayesian phylogenetic inference. Syst Biol 51:740–753

Miller RE, McDonald JA, Manos PS (2004) Systematics of *Ipomoea* subgenus *Quamoclit* (Convolvulaceae) based on ITS sequence data and a Bayesian phylogenetic analysis. Am J Bot 91:1208–1218

Mitchell CD, Eshbaugh WH, Wilson KG, Pittman K (1989) Patterns of chloroplast DNA variation in *Capsicum* (Solanaceae). Int Organ Pl Biosyst Newslett 12:3–11

Nandi OI, Chase MW, Endress PK (1998) A combined cladistic analysis of angiosperms using *rbcL* and non-molecular data sets. Ann Missouri Bot Gard 85:137–212

Olmstead RG, Palmer JD (1992) A chloroplast DNA phylogeny of the Solanaceae: subfamilial relationships and character evolution. Ann Missouri Bot Gard 79:346–360

Olmstead RG, Sweere, JA (1994) Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. Syst Biol 43:467–481

Olmstead RG, Jansen RK, Michaels HJ, Downie SR, Palmer JD (1990) Chloroplast DNA and phylogenetic studies in the Asteridae. In: Kawano S (ed) Biological approaches and evolutionary trends in plants. Academic Press, London, pp 119–134

Olmstead RG, Reeves PA, Yen AC (1998) Patterns of sequence evolution and implications for parsimony analysis of chloroplast DNA. In: Soltis PS, Soltis DE, Doyle JJ (eds) Molecular systematics of plants II: DNA sequencing. Kluwer, Boston, USA, pp 164–187

Olmstead RG, Sweere JA, Spangler RE, Bohs L, Palmer JD (1999) Phylogeny and provisional classification of the Solanaceae based on chloroplast DNA. In: Nee M, Symon DE, Lester RN, Jessop JP (eds) Solanaceae IV: advances in biology and utilization. Royal Botanic Gardens, Kew, UK, pp 111–137

Palmer JD, Zamir D (1982) Chloroplast DNA evolution and phylogenetic relationships in *Lycopersicon*. Proc Natl Acad USA 79:5006–5010

Panzer GWF (1788) Allgemeines Register über die in den sämtlichen dreyzehn Theilen des Linneischen Pflanzensystems beschriebenen Gattungen und Arten nebst einem besondern die denselben eigenen Synonymen erläuternden. Vierzehnter Teil, Nürnberg, in der Raspischen Buchhandlung.

Robertson KR (1982) *Odonellia*, a new genus of Convolvulaceae from tropical America. Brittonia 34:417–423

Roberty (1964) Les genres des Convolvulacées (esquisse). Boissiera 10:129–156

Santiago-Valentin E, Olmstead RG (2003) Phylogenetics of the Antillean Goetzeoideae (Solanaceae) and their relationships within the Solanaceae based on chloroplast and ITS DNA sequence data. Syst Bot 28:452–460

Savolainen V, Chase MW, Hoot SB, Morton CM, Soltis DE, Bayer C, Fay MF, de Bruijn AY, Sullivan S, Qui YL (2000) Phylogenetics of flowering plants based upon a combined analysis of plastid *atpB* and *rbcL* gene sequences. Syst Biol 49:306–362

Scotland RW, Olmstead RG, Bennett JR (2003) Phylogeny reconstruction: the role of morphology. Syst Biol 52:539–548

Shaw CL (1999) Eocene angiospermous palynomorphs of Taiwan. Taiwania 44:423–478

Soltis DE, Soltis PS, Nickrent DL, Johnson LA, Hahn WJ, Hoot SB, Sweere JA, Kuzoff RK, Kron KA, Chase MW, Swensen SM, Zimmer EA, Chaw SM, Gillespie LJ, Kress WJ, Sytsma KJ (1997) Angiosperm phylogeny inferred from 18 S ribosomal DNA sequences. Ann Missouri Bot Gard 84:1–49

Soltis DE, Soltis PS, Chase MW, Mort ME, Albach DC, Zanis M, Savolainen V, Hahn WH, Hoot SB, Fay MF, Axtell M, Swensen SM, Prince LM, Kress WJ, Nixon KC, Farris JS (2000) Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. Bot J Linn Soc 133:381–461

Stefanović S, Olmstead RG (2004) Testing the phylogenetic position of a parasitic plant (*Cuscuta*, Convolvulaceae, Asteridae): Bayesian inference and the parametric bootstrap on data drawn from three genomes. Syst Biol 53:384–399

Stefanović S, Olmstead RG (2005) Down the slippery slope: plastid genome evolution in Convolvulaceae. J Mol Evol 61:292–305

Stefanović S, Krueger L, Olmstead RG (2002) Monophyly of the Convolvulaceae and circumscription of their major lineages based on DNA sequences of multiple chloroplast loci. Am J Bot 89:1510–1522

Stefanović S, Austin DF, Olmstead RG (2003) Classification of Convolvulaceae: a phylogenetic approach. Syst Bot 28:791–806

Stevens PF (2001 onwards) Angiosperm Phylogeny website. Version 7, May 2006

Takhtajan A (1959) Die Evolution der Angiospermen. Gustav Fischer Verlag, Jena, Germany

Takhtajan A (1964) The taxa of the higher plants above the rank of order. Taxon 13:160–164

Takhtajan A (1973) Evolution und Ausbreitung der Blütenpflanzen. Gustav Fischer Verlag, Stuttgart, Germany

Takhtajan A (1997) Diversity and classification of flowering plants. Columbia University Press, New York

Tellería MC, Daners G (2003) Pollen types in Southern New World Convolvulaceae and their taxonomic significance. Plant Syst Ecol. 243:99–118

Thorne RF (1992) An updated phylogenetic classification of flowering plants. Aliso 13:365–389

van Ooststroom SJ (1953) Convolvulaceae. In: van Steenis CGGJ (ed): Flora Malesiana, ser I, vol 4^4. Noordhoff-Kolff, Djakarta, Indonesia, pp 389–512

Walsh BM, Hoot SB (2001) Phylogenetic relationships of *Capsicum* (Solanaceae) using DNA sequences from two noncoding regions: the chloroplast *atpB-rbcL* spacer region and nuclear *waxy* introns. Intl J Plant Sci 162:1409–1418

Wettstein R v (1891) Solanaceae. In: Engler A, Prantl K (eds) Die natürlichen Pflanzenfamilien, vol 4 (3b), f 1–16. Engelmann, Leipzig, Germany, pp 4–38

Wink M (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64:3–19

3
Ornithine-Derived Alkaloids

The authorities of the scientific species names are only added in the text of Sect. 3.1–Sect. 3.5 if a species is not involved in Table 3.1 (Solanaceae) and Table 3.2 (Convolvulaceae), respectively, where the authorities of the corresponding other species may be found.

Biogenetic Outline. Six alkaloid groups occurring in both Solanales families, i.e., (i) simple pyrrolidines (hygrine type; Sect. 3.1), (ii) *N*-acylpyrrolidines (pyrrolidides, pyrrolidine amides; Sect. 3.2), (iii) pyrrolidine-type nicotinoids (tobacco alkaloids; Sect. 3.3), (iv) tropanes (Sect. 3.4), (v) calystegines (polyhydroxylated *nor*tropanes; Sect. 3.5), and presumably also (vi) indolizidines share a common biogenetic building block, the L-ornithine-derived *N*-methyl-Δ^1-pyrrolinium cation (Fig. 3.1). The corresponding pathway leads to the biogenic amine of L-ornithine, putrescine. However, there are two alternative routes from ornithine to putrescine in higher plants: the direct route via simple decarboxylation catalyzed by ornithine decarboxylase (ODC) and the indirect route via L-citrulline, L-arginine, its biogenic amine agmatine [catalyzed by arginine decarboxylase (ADC)], and *N*-carbamoylputrescine (Fig. 3.2). It has been shown that both routes occur in cell cultures of *Nicotiana tabacum*, Solanaceae. Surprisingly, if ODC was inhibited irreversibly, the putrescine pool was not reduced. This was interpreted as an indication that the cells compensated by increased formation of putrescine via the second route (Leete 1983 and references therein). However, ADC rather than ODC, turned out to be the more important enzyme in the biosynthesis of pyrrolidine-type alkaloids in two strains of *N. tabacum* callus (Tiburcio and Galston 1985).

The next step, the methylation of putrescine, already belongs to the secondary metabolism and is catalyzed by the first pathway-specific enzyme, putrescine *N*-methyltransferase (PMT) discovered in tobacco roots (Mizusaki et al. 1971). Its product, *N*-methylputrescine, represents the direct precursor of 4-methylaminobutanal, as was shown by a cell free preparation of tobacco roots. The oxidative deamination of the diamine is catalyzed by *N*-methylputrescine oxidase, an enzyme of a high substrate specificity (Mizusaki et al. 1972). 4-Methylaminobutanal is cyclized spontaneously forming the *N*-methyl-Δ^1-pyrrolinium cation (present as a salt) (Leete 1967). An alternative route has been proposed for the biosynthesis

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2

Key to abbreviations

Simple pyrrolidines: P1 = Hygrine; **P2** = Cuscohygrine; **P3-A** = Hygrolines; **P3-B** = Norhygrine; **P3-C** = Anahygrine; **P3-D** = *N*-Methylpyrrolidinylhygrines; **P3-E** = *N*-Methylpyrrolidinylcuscohygrines; **P3-F** = Oxohygrine; **P3-G** = Phygrine

Nicotinoids: **N1** = Pyridylpyrrolidines (nicotine, nornicotine); **N2** = Pyridylpiperidines (e.g., anabasine)

3α-Acyloxytropanes (3α-AOT): **T1** = Aliphatic esters of 3α-hydroxytropane; **T2/T3** = Esters of 3α,6ß-dihydroxytropane (**T2**) / 3α,7ß-dihydroxytropane (**T3**); **T4** = Esters of 3α,6ß,7ß-trihydroxytropane; **T5** = Esters of 3α-hydroxytropane/-*nor*tropane with Solanaceae-specific phenylpropanoid acids, e.g., hyoscyamine/atropine, anisodamine (6β-hydroxyhyoscyamine), littorine; **T6** = Esters of 6β,7β-epoxy-3α-hydroxytropane (scopine)/-*nor*scopine with Solanaceae-*specific* phenylpropanoid acids (e.g., scopolamine (hyoscine)); **T7** = Rare congeners: **T7-A** = Anisodine (daturamine); **T7-B** = *N*-Oxides of **T5**- and **T6**-type alkaloids; **T7-C** = Esters of 3α-hydroxytropane with Solanaceae-*unspecific* phenylpropanoid acids (e.g., 3α-cinnamoyloxytropane, 3α-phenylacetoxytropane); **T7-D** = "Dimeric" and "trimeric" tropanes (e.g., certain schizanthines, grahamine)

3β-Acyloxytropanes (3β-AOT) = T8

Symbols in the fields: + = Compound(s) detected; **?** = compound(s) not unequivocally detected due to very low concentrations; – = no compound(s) detected though looked for; **blank** = not checked or not reported though found to be negative

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
Schizanthoideae													
Schizanthus alpestris POEPP.							+				D?		
S. grahamii GILL.			A			+	+				D		San-Martin et al. 1987; Muñoz et al. 1991; Humam et al. 2005
S. hookeri GILL.	+		A			+	+				D?		San-Martin et al. 1980; Gambaro et al. 1983; Jordan et al. 2006
S. integrifolius PHIL.			A								D		Muñoz et al. 1994 (**P3** represents a glucoside)
S. litoralis PHIL.			A			+	+				D		Muñoz et al. 1996
S. pinnatus RUIZ & PAV.			A				+				D		Ripperger 1979; Gambaro et al. 1983; De la Fuente et al. 1988
S. porrigens GRAHAM							+				D		Muñoz and Cortés 1998

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
Petunioideae													
Brunfelsia bonodora (VELL.) J.F.MACBR.		+											Evans 1979
B. pauciflora (CHAM. & SCHLTDL.)BENTH. sub nom. *B. calycina* BENTH.		+											Evans 1979
B. hopeana BENTH.		+											Evans 1979
B. undulata SW.		+											Evans 1979
Nierembergia linariaefolia GRAHAM sub nom. *N. hippomanica* auct. non MIERS	+		B			+							Gonzalez et al. 1981: Pomilio et al. 1996
Petunia violacea LINDL. **or** *P. violacea* CHODAT & HASSLER, non LINDL. = *P. inflata* R.E.FRIES				+									Leete 1983
Cestroideae													
Salpiglossis sinuata RUIZ & PAV.				+	+								Schröter 1958, 1963
Cestreae clade													
Cestrum diurnum L.				+									Halim et al. 1971
C. nocturnum L.				+									Halim et al. 1971

(continued)

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
Latua pubiflora (GRISEB.) BAILL.									+	+	C		Silva and Mancinelli 1959; Plowman et al. 1971; Muñoz and Casale 2003
do. sub nom. *L. venenosa* PHIL.									+	+			Bodendorf and Kummer 1962
Browallieae clade													
Streptosolen jamesonii (BENTH.) MIERS				+	+								Schröter 1963
Nicotianoideae													
Nicotianeae clade													
Genus *Nicotiana*: see Table 3.4				+	+								
Symonanthus clade													
Symonanthus aromaticus (C.A.GARDN.) HAEGI				-	-	+	+	+	-	+	-	+	Evans and Ramsey 1983
Anthocercis clade													
Anthocercis angustifolia F.MUELL.				-	-	-	+	-	+	+	-	+	Evans and Ramsey 1983
A. anisantha ENDL. ssp. *anisantha*				-	-	-	+	+	+	+	-	-	Evans and Ramsey 1983
do. ssp. *collina* HAEGI				-	-	-	-	-	+	+	-	-	Evans and Ramsey 1983
A. fasciculata F.MUELL.						-	-	-	+	-	-	-	[a]
A. genistoides MIERS				-	-	-	+	+	+	+	-	-	Evans and Ramsey 1983; El Imam and Evans 1984
A. gracilis BENTH.				-	-	-	-	-	+	+	-	-	Evans and Ramsey 1983
A. ilicifolia HOOK. ssp. *ilicifolia*				-	-	-	+	+	+	+	-	+	Evans and Ramsey 1983; El Imam and Evans 1984
A. intricata F.MUELL.				-	-	-	+	+	+	+	-	-	Evans and Ramsey 1983

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
A. littorea LABILL.		+		-	-	+	+	+	+	+	-	+	Evans and Treagust 1973a; Evans and Ramsey 1979
A. viscosa R.BR.		+		-	-	+	-	+	+	+	-	+	Evans and Treagust 1973a; Evans and Ramsey 1979
do. ssp. *caudata*				-	-	+	+	+	+	+	-	-	Evans and Ramsey 1983
Grammosolen clade													
Grammosolen dixonii (F.MUELL. & R.TATE) HAEGI				-	-	+	-	-	+	+	-	-	Evans and Ramsey 1983
Anthotroche clade													
Anthotroche myoporoides C.A.GARDN.				-	-	+	-	-	+	+	-	-	Evans and Ramsey 1983
A. pannosa ENDL.				-	-	-	-	-	+	+	-	-	Evans and Ramsey 1983
A. walcottii F.MUELL.				-	-	+	-	-	+	+	-	-	Evans and Ramsey 1983
Cyphanthera clade													
Crenidium spinescens HAEGI				-	+	-	-	-	+	+	-	-	Evans and Ramsey 1983; El-Imam and Evans 1984
Cyphanthera albicans (A.CUNN.) MIERS ssp. *albicans*				-	-	+	+	-	+	+	-	-	Evans and Ramsey 1983
do. ssp. *notabilis* HAEGI				-	-	+	+	-	+	+	-	-	Evans and Ramsey 1983
C. anthocercidea (F.MUELL.) HAEGI				+	+	-	-	-	+	+	-	-	Evans and Ramsey 1983

(continued)

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
C. frondosa (MIERS) J.M.BLACK sub nom. *Anthocercis frondosa* = putative hybrid *Duboisia myoporoides* × *C. albicans*				+	+	-	-	-	+	+	-	-	Evans and Ramsey 1979
C. microphylla F.MUELL.				-	-	-	-	-	+	+	-	-	Evans and Ramsey 1983
C. myosotidea (F.MUELL.) HAEGI				-	-	+	-	+	+	+	-	-	Evans and Ramsey 1983; El-Imam and Evans 1984
C. odgersii (F.MUELL.) HAEGI ssp. *odgersii*				-	-	-	-	-	+	+	-	-	Evans and Ramsey 1983; El-Imam and Evans 1984
do. ssp. *occidentalis* HAEGI				-	-	-	-	-	+	+	-	-	Evans and Ramsey 1983
C. racemosa (F.MUELL.) HAEGI				+	+	-	-	-	-	-	-	-	Evans and Ramsey 1983
C. scabrella (BENT.) MIERS				-	-	-	+	-	+	+	-	-	Evans and Ramsey 1983
C. tasmanica MIERS				+	-	-	+	+	+	+	-	+	Evans and Ramsey 1983; El-Imam and Evans 1984
do. sub nom. *Anthocercis tasmanica* (MIERS) HOOK. f.				+						+			Bick et al. 1974
Duboisia arenitensis CRAVEN, LEPSCHI & L.A.R.HAEGI									+	+			Griffin and Lin 2000
D. hopwoodii F.MUELL.	+	+		+	+				+	+			Petit 1879; Petrie 1917b; Rothera 1911; Späth et al. 1935; Bottomley and White 1951; Kennedy 1971; Luanratana and Griffin 1982; Endo and Yamada 1985 (cell cultures)

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
D. leichhardtii F.MUELL.				+	+	+			+	+			Loftus Hills et al. 1954b; Griffin 1965; Kagai et al. 1980 (cell cultures); Endo and Yamada 1985 (cell cultures); Leete et al. 1990
D. myoporoides R.BR.	+			+	+	+			+	+		+	Gerrard 1880; Ladenburg 1880; Barger et al. 1937, 1938; Loftus Hills et al. 1953, 1954a; Mortimer and Wilkinson 1957; Coulson and Griffin 1967, 1968; Kitamura et al. 1980; Endo and Yamada 1985 (cell cultures); Gritsanapan and Griffin 1991
D. leichhardtii × *myoporoides*									+	+			
Solanoideae													
Hyoscyameae clade													
Anisodus acutangulus C.Y.WU & C.CHEN sub nom. *Scopolia acutangula*									+	+	A		
A. tanguticus (MAXIM.) PASCHER sub nom. *S. tangutica* MAXIM.		+					+		+	+	A		Hsiao et al. 1973; Wang and Wu 1979; Xiao et al. 1983; Ghani 1985
A. luridus LINK sub nom. *S. lurida* (LINK & OTTO) DUNAL		+							+	+[a]			Rabinovich and Konovalova 1946; Reynouts-van Haga 1954; Jovankovics 1966; Xiao et al. 1983; Ghani 1985
do. sub nom. *S. anomala* LINK & OTTO		+											Ghani 1985

(continued)

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
do. sub nom. *S. stramonifolia* (ROXB.) N.P.BALAKR.	+	+											Parr et al. 1990
Atropa baetica WILLK.	+	+							+	+			Heltmann 1979
A. belladonna L. similar results for *A. belladonna* sub nom. *A. acuminata* ROYLE, *A. caucasica* KREYER, *A. komarovii* BLIN & SCHAL, *A. pallidiflora* SCHÖNB.-TEM.	+	+	AD			+	+		+	+	B		Reynouts-van Haga 1954; Heltmann 1979; McGaw and Woolley 1979; Hartmann et al. 1986; Harborne and Khan 1993
Atropanthe sinensis (HEMSLEY) PASCHER sub nom. *Scopolia sinensis* HEMSLEY[b]		+							+	+			Reynouts-van Haga 1954; Ripperger 1995
Hyoscyamus albus L.[c]	+	+	BDEG			+	+	-	+	+	C	+	Ghani et al. 1972; Parr et al. 1990; Doerk-Schmitz et al. 1994; El-Shazly et al. 1997
H. aureus L.	+	+							+	+			Ghani et al. 1972; Ghani 1985
H. boveanus ASCH. & SCHWEINE.	+	+	DE			+	-	-	+	+	C	-	El-Shazly et al. 1997
H. desertorum (ASCH.) BOISS.	+	+	BG			+	-	-	+	+	C	-	Parr et al. 1990; El-Shazly et al. 1997
H. muticus L.	+	+	BG			+	-	-	+	+	C	+	Parr et al. 1990; El-Shazly et al. 1997
H. niger L.[d]		+							+	+	B		Reynouts-van Haga 1954; McGaw and Woolley 1979
do. var. *pallidus* RCHB.									+	+			
H. pusillus L.	+	+	B			+			+	+		+	Parr et al. 1990
H. reticulatus L.									+	+			Ionkova 2002

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
H. senecionis Willd.									+	+			
H. turcomanicus Pojark.									+	+			
H. × *gyoerffyi* (*H. niger* × *H. albus*)	+	+							+	+			Ionkova et al. 1994 (transformed roots)
Physochlaina alaica E.Korot.							+		+	+			
P. dubia Pascher									+				
P. infundibuliformis Kuang sub nom. *P. infundubularis* and *P. infundibulum*, respectively		+							+	+			Hsiao et al. 1973
P. orientalis G.Don	+	+							+	+			Reynouts-van Haga 1954; Gorinova et al. 1994 (cell cultures)
do. sub nom. *Hyoscyamus orientalis*									+	+			
P. physaloides (L.) G.Don		+							+	+			Reynouts-van Haga 1954; Hsiao et al. 1973
P. praealta (Decne) Miers		+							+	+			Xiao et al. 1983
Przewalskia tangutica Maxim.		+							+	+	A		Hsiao et al. 1973; Xiao et al. 1983
do. sub nom. *P. shebbearei* (C.E.C.Fisch.) Grubov		+							+	+	A		Hsiao et al. 1973
Scopolia carniolica Jacq.						+			+	+	B		
S. japonica Maxim.									+	+			
do. sub nom. *S. parviflora* (Dunn.) Nakai									+	+			

(continued)

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
Lycieae clade													
Lycium barbarum L.[e]									+?	+?			Harsh 1989
L. barbarum sub nom. *L. halimifolium* MILLER	-	-	-	-	-	-	-	-	-	-	-	-	Christen and Kapetanidis 1987
Mandragoreae clade													
Mandragora caulescens C.B.CLARKE[f]		+							+	+			Xiao et al. 1983
do. sub nom. *M.chinghaiensis* KUANG & A.M.LU		+							+	+			Xiao et al. 1983
M. officinarum L.[g]		+				+	+		+	+	B		Reynouts-van Haga 1954; Staub 1962
do. sub nom. *M. autumnalis* BERTOL.		+				+	+		+	+			Jackson and Berry 1973
do. sub nom. *M. turcomanica* MIZGIREVA									+	+			Razzakov et al. 1999
M. vernalis BERTOL.		+				+	+		+	+			Jackson and Berry 1973
Solandreae clade / Solandrinae subclade													
Solandra grandiflora SW.		+				+			+	+		+	Evans et al. 1972b
S. longiflora TUSSAC									+	-			Petrie 1917a
do. sub nom. *S. macrantha* DUNAL.		+				+			+	+		+	Evans et al. 1972b
S. guttata D. DON		+				+			+	+		+	Evans et al. 1972b
S. hirsuta DUNAL.		+				+			+	+		+	Evans et al. 1972b

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
S. maxima (SESSÉ & MOCINO) P.S.GREEN sub nom. *S. hartwegii* N.BR.[h]		+				+			+	+		+	Evans et al. 1972b
Nicandreae clade													
Nicandra physalodes (L.) GAERTNER	+	+	AB			(+)[l]							Romeike 1965a, b; McGaw and Woolley 1978b; Parr 1992 (transformed root cultures)
Datureae clade													
Brugmansia arborea (L.) LAGERH. sub nom. *Datura cornigera* HOOK.		+					+	+	+	+		+	Evans et al. 1972c
Brugmansia aurea LAGERH. sub nom. *D. aurea* (LAGERH.) SAFF.									+	+			El-Dabbas and Evans 1982; De Garcia et al. 1985; El-Imam and Evans 1990
B. candida PERS. sub nom. *D. candida* (PERS.) SAFF.[i]		+		+		+	+	+	+	+		+	Evans et al. 1972c; Gambaro and Roses 1989 (flowers)
do. sub nom. *D. candida* hybrid		+	D								A		Evans et al. 1972c; Christen et al. 1990 (hairy root cultures)
B. candida × *aurea*	+	+	F			+	+		+	+	A		Parr et al. 1990; Boswell et al. 1999 (root cultures)
do. sub nom. *D. candida* × *D. aurea*									+	+			El-Dabbas and Evans 1982

(continued)

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
B. sanguinea (Ruiz & Pav.) D.Don sub nom. *D. sanguinea* Ruiz & Pav.	+	+				+	+	+	+	+			Evans et al. 1972c; Parr et al. 1990
B. suaveolens (Willd.) Bercht. & Presl sub nom. *D. suaveolens*		+				+	+	+	+	+			Evans and Lampard 1972; Freitas et al. 1996
B. versicolor Lagerh.										+			Bhatt et al. 2004
Genus *Datura*[j]													
Section *Stramonium*													
Datura ferox L.	+	+		-		+	+	+	+	+	C		Reynouts-van Haga 1954; Evans et al. 1972c; Parr et al. 1990; Vitale et al. 1995
D. quercifolia H.B.K.	+	+				+	+	+	+	+			Parr et al. 1990
D. stramonium L.[k]	+	+		+		+	+	+	+	+	B	+	Wahl 1953; Reynouts-van Haga 1954; Evans et al. 1972c, Parr et al. 1990; Ford et al. 1994; Philipov and Berkov 2002; Berkov et al. 2003
Section *Dutra*													
D. discolor Bernh.		+				+	+	+	+	+			Evans and Somanabandhu 1974a
D. inoxia Mill.	+	+	D			+	+	+	+	+		+	Reynouts-van Haga 1954, Evans et al. 1969, 1972c; McGaw and Woolley 1978b; Witte et al. 1987; Parr et al. 1990; Berkov and Zayed 2004; Berkov et al. 2005
do. sub nom. *D. meteloides* DC.		+		+		+	+		+	+			Wahl 1953; Evans et al. 1972c
D. leichhardtii Benth.		+				+		+	+	+			Evans et al. 1972c

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
do. ssp. *pruinosa* (GREENM.) HAMMER		+				+	+	+	+	+		+	Evans and Treagust 1973b
D. metel L.		+		+			+	+	+	+			Wahl 1953; Reynouts-van Haga 1954
do. var. *fastuosa* (BERNH.) DANERT[m]	+	+		+		+	+	+	+	+		+	Wahl 1953; Evans et al. 1972c; Parr et al. 1990
D. wrightii REGEL	+	+				+	+	+	+	+		+	Parr et al. 1990
Section *Ceratocaulis*													
D. ceratocaula ORT.		+	D			+	+		+	+		+	Beresford and Woolley 1974; Berkov 2003
Solaneae clade													
Genus *Solanum*: 25 out of 52 species checked were cuscohygrine-positive (including 3 species sub nom. *Cyphomandra*)		+				-	-	-	-	-	-	-	Evans and Somanabandhu 1980
Solanum betaceum CAV. sub nom. *Cyphomandra betacea* (CAV.) SENDT. or sub nom. *C. crassifolia* (ORT.) KUNTZE		+		–		(+)[l]	-	-	?	-	-	?	Evans et al. 1972d; Evans and Somanabandhu 1980; Leete 1983
S. carolinense L.		+			+								Evans and Somanabandhu 1977
S. luteoalbum PERS. sub nom. C. *luteoalba* (PERS.) CHILD		?				-	-	-	-	-	-	-	Evans and Somanabandhu 1980
S. lycopersicum L. (syn.: *Lycopersicon esculentum* MILL.)				+								(+)[n]	Wahl 1952; Siegmund et al. 1999

(continued)

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
S. melongena L.				+								(+)[n]	Siegmund et al. 1999
S. tuberosum L.				+								(+)[n]	Leete 1983; Siegmund et al. 1999
Capsiceae clade													
Capsicum annuum L. var. *annuum*				+								(+)[n]	Siegmund et al. 1999
C. annuum L. var. *frutescens* (L.) KUNTZE				+								(+)[n]	Siegmund et al. 1999
Lycianthes rantonnetii (CARR.) BITT.		+?											Evans and Somanabandhu 1980
Physaleae clade / Salpichroinae subclade													
Salpichroa origanifolia (LAM.) BAILLON	+	+				(+)[l]			?				Evans et al. 1972a
Physaleae clade / Iochrominae subclade													
Iochroma australe Griseb. sub nom. *Acnistus australis* (Griseb.) Griseb.		+											Ghani 1985
Physaleae clade / Physalinae subclade													
Leucophysalis viscosa (SCHRAD.) HUNZ. sub nom. *Saracha viscosa* SCHRAD.		+											Evans 1979

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	
Physalis alkekengi L.	+	+	G			+	-		-	-		+	Basey and Woolley 1973c; Yamaguchi and Nishimoto 1965; Yamaguchi et al. 1965, 1974; McGaw and Woolley 1979; Evans 1979; Basey et al. 1992
P. angulata L.			G										Basey et al. 1992
P. minima L.			G										Basey et al. 1992
P. peruviana L.	+	+	DG			+	-		-	-	-	+	Romeike 1965a, b; Evans 1979; Basey et al. 1992; Kubwabo et al. 1993
P. philadelphica LAM.	+		G				-		-	-			Romeike 1965a, b; Evans 1979; Basey et al. 1992
do. sub nom. *P. ixocarpa* BROT.	+		G				-		-	-			Romeike 1965a, b; Evans 1979; Basey et al. 1992
P. pruinosa L.	+		G				-		-	-			Romeike 1965a, b; Evans 1979; Basey et al. 1992
P. pubescens L.	+		G				-		-	-			Romeike 1965a, b; Evans 1979; Basey et al. 1992
P. solanaceous (SCHLTDL.) AXELIUS sub nom. *Margaranthus solanaceous*		+					-		-	-			Evans 1979; Evans and Somanabandhu 1980
P. viscosa L.			G										Basey et al. 1992
Physaleae clade / Withaninae subclade													
Withania somnifera (L.) DUNAL	+	+	C	+		+							Majumdar 1952, 1955; Leary et al. 1963; Schwarting et al. 1963

(continued)

Table 3.1 Occurrence of the most frequent types of pyrrolidine, tobacco, and tropane alkaloids in the Solanaceae proved by isolation or chromatographic methods (PC, TLC, GC/MS); tropanes mainly based on data of Lounasmaa and Tamminen (1993 and references therein). Classification according to Fig. 2.2 (continued)

Subfamily tribe/clade *species*	**Simple pyrrolidines**			**Nicotinoids**		**3α-Acyloxytropanes (3α-AOT)**						**3β-AOT**	**References** (Tropanes only: For all species, especially those without reference, see (also) Lounasmaa and Tamminen 1993 and references therein)
	P1	**P2**	**P3**	**N1**	**N2**	**T1**	**T2/T3**	**T4**	**T5**	**T6**	**T7**	**T8**	

[a] Including *S*-(−)-norscopolamine (Ripperger 1995)
[b] Analogous results with "*S. himalayensis*", probably another but invalid term for "Himalayan Scopola", *A. luridus*
[c] Also sub nom. *H. canariensis* KER GAWL.
[d] Also sub nom. *H. bohemicus* F.W.SCHMIDT
[e] Data from Harsh (1989); no alkaloids found by Christen and Kapetanidis (1987) and others
[f] Probably also sub nom. *M. chinghaiensis* K.Z.KUANG & A.M.LU
[g] Also sub nom. *M. autumnalis* BERTOL.
[h] Another common synonym is *S. nitida*
[i] Probably a natural hybrid of *B. aurea* LAGERH. and *B. versicolor* LAGERH. (Lockwood 1973; Hunziker 2001)
[j] Classification of the sections according to Mace et al. (1999)
[k] Hyoscyamine as well as scopolamine in the varieties *inermis* (JACQ.) LUNDSTR., *stramonium*, and *tatula* TORR., respectively; var. *godronii* DANERT: 3α,7β-ditigloyloxy-6ß-hydroxytropane
[l] 3α-Hydroxytropane (not acylated)
[m] Hyoscyamine also in var. *metel*; scopolamine also in var. *metel* and var. *rubra*
[n] 3β-Hydroxytropane (not acylated)

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3

Key to abbreviations

Simple pyrrolidines:

P1 = Hygrine; **P2** = Cuscohygrine; **P3-A** = Hygrolines; **P3-B** = Norhygrine; **P3-C** = Propylhygrines; **P3-D** = *N*-Methylpyrrolidinylhygrines; **P3-E** = *N*-Methylpyrrolidinylcuscohygrines; **P3-F** = Oxohygrine; **P3-G** = Phygrine; **P3-H** = 2,6-Dehydrohygrine

Nicotinoids: N = Nicotine

Simple tropanes (T1): T1-A = Tropan-3-one (tropinone), 3α-hydroxytropane (tropine), 3β-hydroxytropane (pseudotropine), and/or their *nor* derivatives (for details see Table 3.9); **T1-B** = Calystegines (polyhydroxylated *nor*tropanes; for details see Table 3.9); **T1-C** = Dihydroxy*nor*tropanes; **T1-D** = *O*-Acylated dihydroxy*nor*tropane (baogongteng A); **T1-E** = 6β-Hydroxytropan-3-one; **T1-F** = Methylpseudoecgonine (2α-carbomethoxytropan-3β-ol)

3α-Acyloxytropanes:

T2 = Aliphatic esters [e.g., 3α-acetoxytropane, 3α-tigloyloxytropane, 3α-(2-methylbutyryloxy)tropane]

T3 = Simple aromatic esters and *nor* derivatives (e.g., convolamine/convolidine, datumetine, merresectine A; for details see Table 3.5)

T4 = Prenylated aromatic esters (merresectines B-G; for details see Table 3.6)

T5 = Phenylpropanoid esters (e.g., 3α-caffeoyloxytropane, 3α-feruloyloxytropane)

T6 = Rare congeners: **T6-A** = Bonabilines, **T6-B** = Consabatines, consiculine; **T6-C** = 3α-Nicotinoyloxytropane

T7 = Acylated 3α,6β- / 3α,7β-dihydroxytropanes

3β-Acyloxytropanes:

T8 = Aliphatic esters [e.g., 3β-acetoxytropane, 3β-tigloyloxytropane (tigloidine), astrimalvines]

T9 = Simple aromatic and/or prenylated aromatic esters, *nor* derivatives (e.g., concneorine/*nor*concneorine, 3β-merresectines B-F; for details see Table 3.6)

T10 = Phenylpropanoid esters (e.g., 3β-caffeoyloxytropane, 3β-feruloyloxytropane, 3β-sinapoyloxytropane)

Symbols in the fields: + = Compound(s) detected; **?** = compound(s) not unequivocally detected due to very low concentrations; − = no compound(s) detected though looked for; **no symbol** = not checked or not reported though found to be negative

(continued)

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
Erycibeae															
Erycibe elliptilimba Merr. & Chun					CD										Lu et al. 1986
E. hainanensis Merr.					CD										Wang et al. 1989
E. micrantha Hall. f.	+	-	-	+	AB	+	+	-	-	-	-	-	+	-	
E. obtusifolia Benth.					CD										Yao et al. 1981
E. rheedii Blume	+	-	-	+	B	-	-	-	-	-	-	-	-	-	[a]
E. schmidtii Craib.					C										Song et al. 1997
Dichondreae															
Dichondra micrantha Urb.					AB										
D. sericea Sw.	+	+	CD	+	AB	+	?	+	+	-	-	-	+	+	[a]
Falkia repens L. f.	+	+	CDEG	-	AB	+	?	-	+	-	-	+	+	+	Tofern, 1999; Ott et al. 2007
Cresseae															
Bonamia brevifolia (Benth.) Myint	-	-	B	-		-	+	-	-	-	-	-	-	+	
B. dietrichiana Hall. f.	+	+	-	-	-	-	-	-	-	-	-	-	-	-	
B. semidigyna (Roxb.) Hall. f. var. *semidigyna*	+	+	BCDH	+	ABE	+	+	+	+	-	-	+	+	+	Henrici 1996
B. spectabilis (Choisy) Hall. f.	+	+	CD	+	ABF	+	-	+	-	A	-	-	+	-	Tofern 1999; Schimming 2003
B. trichantha Hall. f.	+	+	BCG	+	A	+	-	-	-	-	-	+	-	-	
Evolvulus alsinoides (L.) L.	-	-	-	-	A	+	-	-	-	-	-	+	-	-	

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**					**3β-Acyloxytropanes**				**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
do. var. *decumbens* (R.Br.) Ooststr.	+	+	BCDF	-	A	+	-	+	-	B	+	+	-	-	
E. argyreus Choisy	-	+	D	-	AB	+	-	-	-	-	?	+	-	-	
E. glomeratus Choisy cv. 'Blue Days'	+	+	C	+	A	+	-	-	-	-	-	+	-	-	
E. nummularius L.	+	+	CD	+	AE	-	-	-	-	-	-	+	+	+	[a]
E. sericeus Sw. var. *holosericeus* (Kunth.) Ooststr.							?								Consuelo-Fonseca and Salive 1972[b]
Maripeae															
Maripa nicaraguensis Hemsl.	+	-	A	+		-	-	-	-	-	-	-	+	+	[a]
M. panamensis Hemsl.	+	-	C	+	AB	+	+	-	+	-	-	+	+	+	[a]
Jacquemontieae															
Jacquemontia corymbulosa Benth.	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Henrici 1996[c]
J. paniculata (Burm. f.) Hall. f.	+	-	C	+	-	-	-	-	-	-	-	-	-	-	Henrici 1996[c]
J. pentantha (Jacq.) G.Don	+	+	-	+	A	-	-	-	-	-	-	-	-	-	
J. tamnifolia (L.) Griseb.	+	-	CD	-	ABE	-	+	+	-	-	-	-	+	-	Henrici 1996; Schimming 2003
Cuscuteae															
C. australis R.Br.	+	+	-	+	A	-	-	-	-	-	-	-	-	-	[a]
Aniseieae															

(continued)

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
Aniseia martinicensis (JACQ.) CHOISY	+	+	A – E, G	?	AB	-	-	-	-	-	-	-	-	-	
Iseia luxuri-ans (MORIC.) O'DONELL	+	+	D	-	AB	-	-	-	-	-	-	-	-	-	
Odonellia hirtiflora (MART. & GAL.) K. ROB.	+	+	BCD	-	A	-	-	-	-	-	-	-	-	-	Tofern 1999
Convolvuleae															
Calystegia japonica CHOISY					AC										Asano et al. 2001
Calystegia macrostegia ssp. *cyclostegia* (HOUSE) BRUMMIT	+	+	DEG	-	A	-	-	-	-	-	-	-	-	-	Jenett-Siems 1996; Schimming 2003
C. sepium R.BR.	+	+	C	+	AB	-	-	-	-	-	-	-	-	-	Evans and Somanabandhu 1974b; Schimming 2003
C. silvatica (KIT.) GRISEB.	+	+	DEG	?	AB	-	+	-	-	-	-	-	-	-	Evans and Somanabandhu 1974b; Schimming 2003
C. soldanella (L.) ROEM. & SCHULT.		+			B										Evans and Somanabandhu 1974b; Asano et al. 2001
Convolvulus althae-oides L.	+	+	CDG	+	A	+	+	-	-	-	-	-	-	-	Jenett-Siems 1996; Schimming 2003
C. arvensis L.	+	+	DE	-	AB	-	-	-	-	-	-	-	-	-	Todd et al. 1995; Jenett-Siems 1996
C. canariensis L.	+	+	DEG	-	A	-	-	-	-	-	-	-	-	-	Jenett-Siems 1996; Jenett-Siems et al. 1998b
C. cantabrica L.	+	+	CDH	+	A	-	-	-	-	-	-	-	-	-	Jenett-Siems 1996; Jenett-Siems et al. 1998b

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
C. caput-medusae LOWE	+	+	A	+	AB	-	-	-	-	-	-	-	-	-	
C. chilensis PERS.	+	+	C – H	+	A	-	-	-	+	-	-	-	-	+	Jenett-Siems 1996; Schimming 2003
C. cneorum L.	+	+	CDEG	+	AB	+	+	-	-	-	-	-	+	-	Mann 1997
C. demissus CHOISY	+	+	D	+	A	-	-	-	-	-	-	+	-	-	Jenett-Siems 1996; Schimming 2003
C. dorycnium L.	+	+	-	-	A	-	+	-	+	-	-	-	-	+	[d]
C. elongatus WILLD.	+	-	C	+	AB	-	-	-	-	-	-	-	-	-	
C. erinaceus LEDEB.		+[e]					+[f]								Aripova et al. 1972
C. farinosus L.	+	+	B–EH	+	A	-	-	-	+	-	-	-	-	-	Jenett-Siems 1996[g]
C. floridus L. f.	+	+	CDG	+	A	-	+	-	-	-	-	-	+	-	Jenett-Siems 1996; Schimming 2003
C. glandulosus (WEBB.) HALL.	+	+	CDG	+	AB	-	-	-	+	-	-	-	-	+	Jenett-Siems 1996[g]
C. graminetinus (R.BR.) SPRENG.	+	+	CD	+	AB	+	-	-	+	-	-	-	+	-	[a]
C. hamadae (VVED.) PETROV	+[e]	+[e]													Lazurevskii 1939
C. hermanniae L'HÉRIT.	+	+	CDEG	+	AE	-	-	-	-	-	+	-	-	+	Jenett-Siems 1996; Schimming 2003
C. humilis JACQ.					B										
C. kilimandschari ENGL.	-	?	-	?	A	-	+	-	-	-	-	-	-	-	[a]

(continued)

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
C. krauseanus REGEL & SCHMALH.							+[f]			text					Aripova et al. 1977, 1983; Aripova and Yunusov 1979; Aripova 1985
C. lineatus L.							+[f]			text					Israilov et al. 1965
C. lopezsocasii SVENT.	+	+	CDE	+	A	-	-	-	-	-	-	-	-	-	Jenett-Siems 1996; Jenett-Siems et al. 1998b
C. pseudocantabricus SCHRENK							+[f]								Orechoff and Konowalowa 1933, 1934, 1935, 1937; Yunusov et al. 1958
C. sabatius VIV. ssp. *mauritanicus* (BOISS.) MURB.	+	+	CD	+	AB	-	+	+	-	B	-	-	+	-	Mann 1997; Jenett-Siems et al. 1998b
C. sagittatus THUNB.	+	+	CEG	-	A	+	-	-	+	-	+	+	-	+	
C. scoparius L. f.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	[a]
C. siculus L.	+	+	CD	+	A	-	+	+	+	B	-	-	-	-	Jenett-Siems 1996; Jenett-Siems et al. 1998b
C. subauriculatus (BURCH.) LINDING.	-	+	-	+	AB	-	-	-	-	-	-	-	-	-	[a]
C. subhirsutus REGEL & SCHMALH.		+					+[f]			text					Aripova et al. 1972; Sharova et al. 1980; Aripova and Yunusov 1986a, b; Razzakov et al. 2004
C. tricolor L. ssp. *tricolor*	+	+	CDEG	+	AB	-	-	-	-	-	-	-	-	-	Jenett-Siems 1996; Jenett-Siems et al. 1998b
Polymeria ambigua R.BR.	+	+	-	-	A	-	-	-	-	-	-	-	-	-	[a]
P. calycina R.BR.	+	-	-	-	A	-	-	-	-	-	-	-	-	-	[a]
P. longifolia LINDL.	-	-	-	-	A	+	-	-	-	-	-	-	-	-	[a]

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
P. marginata BENTH.	-	-	-	+	-	-	-	-	-	-	-	-	-	-	[a]
P. pusilla R.BR.	+	+	-	+	-	+	-	-	-	-	-	-	-	-	[a]
"Merremieae"															
Hewittia sublobata (L. f.) KUNTZE	+	-	-	+	A	-	-	-	-	-	-	-	-	-	Henrici 1996
Genus *Merremia*															
Sectio *Hailale* OOSTSTR.															
M. peltata (L.) MERR.	+	+	-	+	A	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005b[a]
Sectio *Merremia* D.F. AUSTIN															
M. emarginata (BURM. f.) HALL. f.	+	-	C	-	A	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005b[a]
M. gemella (BURM. f.) HALL. f. ssp. *gemella*	+	+	CD	+	AF	-	-	-	-	-	-	-	-	+	Jenett-Siems et al. 2005b
M. hederacea (BURM. f.) HALL. f.	+	+	CDG	+	A	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005b
Tuberosa allies ("section") D.F.AUSTIN															
M. aurea (KELL.) O'DONELL	+	+	C	+	AB	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005b
M. tuberosa (L.) RENDLE	+	+	ACD	+	A	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005b
Sectio *Cissoides* O'DONELL															

(continued)

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
M. cissoides (VAHL) HALL. f.	+	+	ADE	-	A	-	-	+	-	-	-	-	+	-	Jenett-Siems et al. 2005b
M. quinquefolia (L.) HALL. f.	+	+	A – D	+	A	-	+	+	-	C	-	-	+	-	Jenett-Siems et al. 2005b
Vitifolia allies ("section") D.F.AUSTIN															
M. aegyptia (L.) URBAN	+	+	CD	+	AEF	-	-	+	-	-	-	-	+	-	Jenett-Siems et al. 2005b
M. dissecta (JACQ.) HALL. f.	+	?	CE	+	ABE	+	+	+	-	-	+	+	+	-	Jenett-Siems et al. 2005b
M. vitifolia (BURM. f.) HALL. f.	+	+	CDGH	+	A	+	+	+	-	C	+	+	+	-	Jenett-Siems et al. 2005b
Sectio *Streptandra* HALL. f.															
M. guerichii A.MEEUSE	+	+	CDEG	+	AE	+	+	+	-	-	+	+	+	-	Jenett-Siems et al. 2005b
M. quinata (R.BR.) OOSTSTR.	+	+	-	+	-	-	-	+	-	B	-	-	-	-	Jenett-Siems et al. 2005b[a]
Sectio *Xanthips* OOSTSTR.															
M. umbellata (L.) HALL. f.	+	+	CD	+	AB	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005b
M. pterygocaulos (STEUD. ex CHOISY) HALL. f.	+	-	A	-	A	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005b[a]
Section not determined															

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
M. kentrocaulos (C.B.Clarke) Rendle	+	+	CDEGH	+	A	+	+	+	-	-	-	-	+	-	Jenett-Siems et al. 2005b
Operculina aequisepala (Domin) R.W.Johnson	+	-	B	-	A	-	-	-	-	-	-	+	-	-	
O. codonantha (Benth.) Hall. f.	+	-	A	+	-	-	-	-	-	-	-	-	-	-	Henrici 1996
O. pteripes (Don) O'Donell	-	-	-	-	-	-	-	-	-	-	-	-	-	-	[g]
O. riedeliana (Oliv.) Ooststr.	+	+	-	+	A	-	-	-	-	-	-	+	-	-	
Xenostegia medium (L.) D.F.Austin & G.Staples sub nom. *Merremia medium* (L.) Hall. f.	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005b
X. tridentata (L.) D.F.Austin & G.Staples sub nom. *M. tridentata* (L.) Hall. f.	+	-	-	+	-	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005b
Ipomoeeae															
Argyreia capitata (Vahl) Choisy	+	+	AD	+	AB	+	-	-	-	-	-	-	-	-	Tofern 1999

(continued)

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
A. hookeri CLARKE	+	+	CG	+	AB	-	-	-	-	-	-	-	-	-	Tofern 1999[a]
A. mollis (BURM. f.) CHOISY	+	+	D	+	AB	-	-	-	-	-	-	-	-	-	Tofern 1999
A. nervosa (BURM. f.) BOJ.	+	+	-	+	A	-	-	-	-	-	-	-	-	-	Tofern 1999
Astripomoea malvacea (G.KLOTZ) MEEUSE	+?	-	C	-	A	-	-	-	-	-	-	+[h]	-	-	Ott et al. 2007
Genus *Ipomoea*															
Section not determined															
I. hildebrandtii VATKE	+	+	C	+	-	-	-	-	-	-	-	-	-	-	[a]
I. jaegeri PILG.	-	-	C	-	-	-	-	-	-	-	-	-	-	-	[a]
I. velutina R.BR.	+	+	B	-	A	-	-	-	-	-	-	-	-	-	
Sectio *Calonyction*															
I. alba L.	+	+	A – E GH	+	AB	+	-	-	-	-	-	-	-	-	Tofern 1999[i]
I. turbinata LAG. [syn.: *I. muricata* (L.) JACQ.]	+	+	A – E, G	+	A	-	-	-	-	-	-	-	-	-	Tofern 1999[i]
Sectio *Eriospermum*															
I. anisomeres ROB. & BARTL.	+	+	ACDG	+	A	-	-	-	-	-	-	-	-	-	[i]
I. arborescens G.DON	?	?	-	-	-	-	+	+	-	-	-	-	+	-	
I. batatas (L.) LAM. (wild form)	+	+	D	+	AB	-	-	-	-	-	-	-	-	-	
do. (cultivar)	+	+	CDE	+	AB	+	-	-	-	-	+	-	-	-	Tofern 1999; Schimming 2003
I. batatoides CHOISY	+	+	-	+	A	-	-	-	-	-	-	-	-	-	

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
I. cairica (L.) SWEET	+	+	ACDEG	+	AB	+	-	-	-	-	-	-	-	-	
I. carnea JACQ. ssp. *carnea*	-	-	-	-	AB	-	-	-	-	-	-	-	-	-	
do. ssp. *fistulosa* (MART. ex CHOISY) D.F.AUSTIN	+			+	AB										[d]
I. eremnobrocha D.F.AUSTIN	?	+	-	+	A	-	-	-	-	-	-	-	-	-	Tofern 1999[g]
I. mauritiana JACQ. [syn.: *I. digitata* L.]	+	+	A	-	A	-	-	+	-	-	-	-	-	-	
I. ramosissima (POIR.) CHOISY	+	+	BD	+	A	-	-	-	-	-	-	-	-	-	
I. regnellii MEISN.	+	+	-	-	A	-	-	-	-	-	-	-	-	-	Mann 1997[i]
I. reticulata O'DONELL	+	-	A	+	A	-	-	-	-	-	-	-	-	-	Tofern 1999
I. rubens CHOISY	+	-	C	+	A	-	-	-	-	-	-	-	-	-	
I. squamosa CHOISY	+	+	B	-	AB	+	-	-	-	-	-	+	-	-	
I. tiliacea (WILLD.) CHOISY	-	+	C	-	A	-	-	-	-	-	-	-	-	-	
I. trifida (H.B.K.) G.DON	+	+	CD	+	AB	-	-	-	-	-	-	-	-	-	
I. triloba L.	+	+	D	+	A	-	-	-	-	-	-	-	-	-	[g]
I. tuxtlensis HOUSE	?	+	-	+	-	-	-	-	-	-	-	-	-	-	[a]
Sectio *Erpipomoea*															
I. abrupta R.BR.	+	+	C	+	-	+	-	-	-	-	+	-	-	-	

(continued)

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
I. aquatica FORSSK.	+	+	ACDE	+	AB	+	+	+	-	-	-	-	-	-	Tofern 1999; Schimming 2003
I. argillicola R.W.JOHNSON	+	+	CD	+	A	+	+	+	-	C	-	+	+	-	
I. asarifolia ROEM. & SCHULT.	+	+	CDEG	+	A	+	-	-	-	C	+	+	-	-	
I. coptica (L.) ROTH EX ROEHM & SCHULT.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	[a]
I. lonchophylla J.M.BLACK	+	+	-	+	A	+	+	-	-	C	-	-	+	+	
I. muelleri BENTH.	+	+	ACD	+	A	+	-	+	-	C	-	-	+	-	
I. obscura (L.) KER-GAWL.	+	+	A	+	A	-	-	-	-	-	-	-	-	-	
I. ochracea (LINDL.) G.DON	-	-	A	-	-	-	-	-	-	-	-	-	-	-	
I. pes-caprae (L.) R.BR.	+	+	D	+	AB	-	-	-	-	-	-	-	-	-	Henrici 1996; Schimming 2003[i]
I. sepiaria KOEN. ex ROXB.	+	+	CD	-	A	+	-	-	-	-	-	-	-	-	[g]
I. shirambensis BAKER	+	+	CD	+	A	-	-	-	-	-	-	-	-	-	[g]
I. trichosperma BLUME	+	-	CH	-	A	-	-	-	-	-	-	-	-	-	
I. violacea L. [syn.: *I. tuba* (SCHLECHT.) G.DON]	+	+	CD	+	AB	+	-	-	-	-	+	-	-	-	Tofern 1999; Schimming 2003
Sectio *Involucratae*															
I. involucrata BEAUV.	+	+	C	-	AB	+	-	-	-	-	-	-	-	-	
Sectio *Ipomoeae*															

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
I. pes-tigridis L.	+	-	-	+	A	-	-	-	-	-	-	-	-	-	[a]
I. wightii (WALL.) CHOISY	+	+	CDG	-	AB	+	-	-	-	-	-	-	-	-	
Sectio *Mina*															
I. cholulensis KUNTH.	+	+	CD	-	A	+	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005a
I. coccinea L.	+	+	-	-	-	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005a
I. cristulata HALL. f.	+	+	D	-	A	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005a
I. hederifolia L.	+	+	D	+	A	-	-	-	-	-	-	-	-	-	Jenett-Siems 1996; Jenett-Siems et al. 1998a, 2005a
I. lobata (CERV.) THELL. [syn.: *Mina lobata* CERV.]	+	+	CD	-	B	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 2005a
I. neei (SPRENG.) O'DONELL	+	+	B	+	-	-	-	-	-	-	-	-	-	-	Jenett-Siems et al. 1998a, 2005a
I. quamoclit L.	+	+	CD	+	A	-	-	+	-	-	+				Jenett-Siems et al. 1998a, 2005a
I. sloteri (HOUSE) OOSTSTR. cv. "Cardinal" sub nom. *I. perigrenium*	+	+	CDH	+	A	-	-	+	-	-	-	-	+	-	Jenett-Siems 1996; Jenett-Siems et al. 1998a, 2005a
Sectio *Orthipomoea*															
I. plebeia R.BR.	+	+	CD	+	A	+	+	-	+	-	-	+	-	+	
I. tenuirostris STEUD. ex CHOISY	+	+	CDEG	-	A	+	-	-	-	-	-	-	-	-	
Sectio *Pharbitis*															
I. eriocarpa R.BR.	+	+	-	+	AB	-	+	+	-	-	-	-	-	-	
I. hederacea JACQ.	+	+	CD	-	A	-	-	-	-	-	-	-	-	-	

(continued)

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
I. indica (BURM. f.) MERR. var. *acuminata*	+	+	-	+	A	+	-	-	-	-	-	-	-	-	
I. meyeri (SPRENG.) G.DON	+	-	AD	+	-	-	-	-	-	-	-	-	-	-	Tofern 1999[g]
I. nil (L.) ROTH (Zanzibar/Tanzania)	+	-	-	+	A	-	-	-	-	-	-	-	-	-	[i]
do. (Ecuador)	+	-	H	-	A	-	-	-	-	-	-	-	-	-	
I. pubescens LAM.	+	+	-	-	A	-	-	-	-	-	-	-	-	-	
I. purpurea (L.) ROTH	+	+	-	-	A	-	-	-	-	-	-	-	-	-	Henrici 1996[g]
I. setifera POIR.	+	+	AC	+	AB	+	-	-	-	-	-	-	-	-	
Sectio *Tricolores*															
I. chiriquiensis STANDL.	-	-	C	+	AB	-	-	-	-	-	-	-	-	-	[a]
I. tricolor CAV. cv. 'Heavenly Blue'	+	+	BC	+	AB	-	+	+	-	C	-	-	-	-	[g]
do. cv. "Flying Saucers"	+	+	D	+	A	-	+	-	?	C	-	-	-	-	
Lepistemon urceolatum (R.BR.) F.MUELL.	+	+	CDG	-	AB	+	-	-	-	-	-	-	-	-	
Stictocardia beraviensis (VATKE) HALL. f.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	[d]
S. laxiflora	-	-	-	+	-	+	-	-	-	-	-	-	-	-	[a]

Table 3.2 Occurrence of frequent types of pyrrolidine and tropane alkaloids as well as nicotine in the Convolvulaceae proved by isolation or GC/MS analysis. Classification according to Fig. 2.3 (continued)

Tribe clade *species*	**Simple pyrrolidines**			**Nico-tinoids**	**Simple tropanes**	**3α-Acyloxytropanes**						**3β-Acyloxytropanes**			**References** Schimming (2003) if no reference is indicated
	P1	**P2**	**P3**	**N**	**T1**	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	
S. tiliaefolia (DESR.) HALL. f. [syn.: *S. campanulata* (L.) MERR.]	+	+	DE	+	AB	-	-	-	-	-	-	-	-	-	
S. mojangensis (VATKE) D.F.AUSTIN & EICH	+	+	CD	+	AB	+	-	-	-	-	-	-	-	-	Mann 1997[i] (sub nom. "*S. madagascariensis*"); Austin and Eich 2001
Turbina abutiloides (H.B.K.) O'DONELL	+	-	C	+	A	-	-	-	-	-	-	-	-	-	Mann 1997[g]
T. corymbosa (L.) RAF.	+	-	-	+	A	+	-	-	-	-	-	+	-	-	

[a] Only epigeal vegetative parts available for GC/MS analysis, i.e., roots not checked
[b] Doubtful results (convincing identification of convolvine, convolvamine, and convolvidine is lacking)
[c] Only roots and seeds available for GC/MS analysis, i.e., epigeal vegetative parts not checked
[d] Only epigeal vegetative parts and seeds available for GC/MS analysis, i.e., roots not checked
[e] Alkaloid characterized by isolation
[f] Different isolated alkaloids; for details see Table 3.5
[g] Only roots available for GC/MS analysis, i.e., epigeal vegetative parts not checked
[h] Present as *N*-oxides
[i] Also seeds checked in addition to roots and epigeal vegetative parts

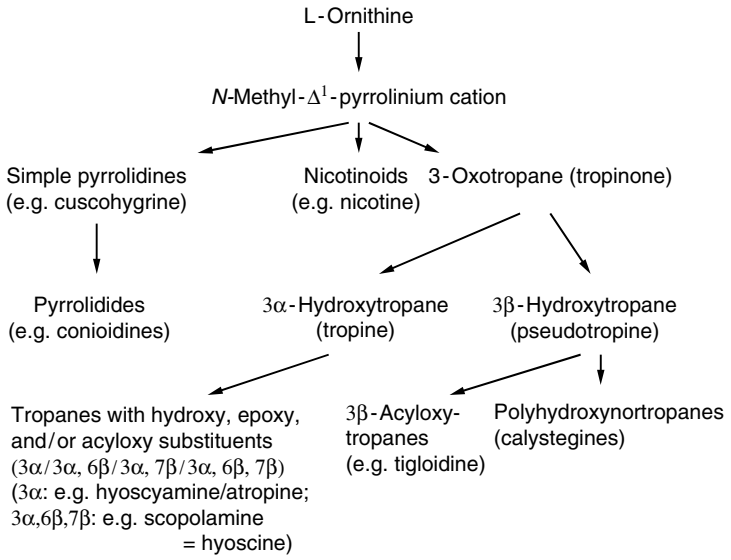


Fig. 3.1 The *N*-methyl-Δ^1-pyrrolinium cation as a specific precursor of certain types of ornithine-derived alkaloids

of simple pyrrolidine alkaloids and tropanes in *Atropa belladonna* and *Datura stramonium*, Solanaceae (Baralle and Gros 1969; Ahmad and Leete 1970). δ-*N*-Methylornithine fed to these plants served as a precursor, i.e., the methyl group might be incorporated already at an earlier stage in the biosynthesis followed by decarboxylation in contrast to *Nicotiana* and nicotine. However, PMT was also characterized in root cultures of *Hyoscyamus albus*, another solanaceous species forming simple pyrrolidines and tropanes. Furthermore, PMT activity was found in cultured roots of 17 further species of 7 solanaceous genera (*Atropa*, *Datura*, *Duboisia*, *Hyoscyamus*, *Physalis*, *Physochlaina, Solanum*) as well as one convolvulaceous species (*Calystegia sepium*) (Hibi et al. 1992; Stenzel et al. 2006). All these species are able to synthesize simple pyrrolidines and/or tropanes/calystegines. Since no PMT activity could be measured in the root cultures of several other species which are unable to form methylputrescine-derived alkaloids, e.g., *Browallia americana* L. (Solanaceae), it may be concluded that the pathway leading to the *N*-methyl-Δ^1-pyrrolinium-derived alkaloids is proceeding generally via *N*-methylputrescine rather than via δ-*N*-methylornithine.

Metabolites of Sect. 7 (pyrrolizidines) do not belong to the *N*-methyl-Δ^1-pyrrolinium-derived alkaloids; their structures are based biogenetically on *two* molecules of ornithine.

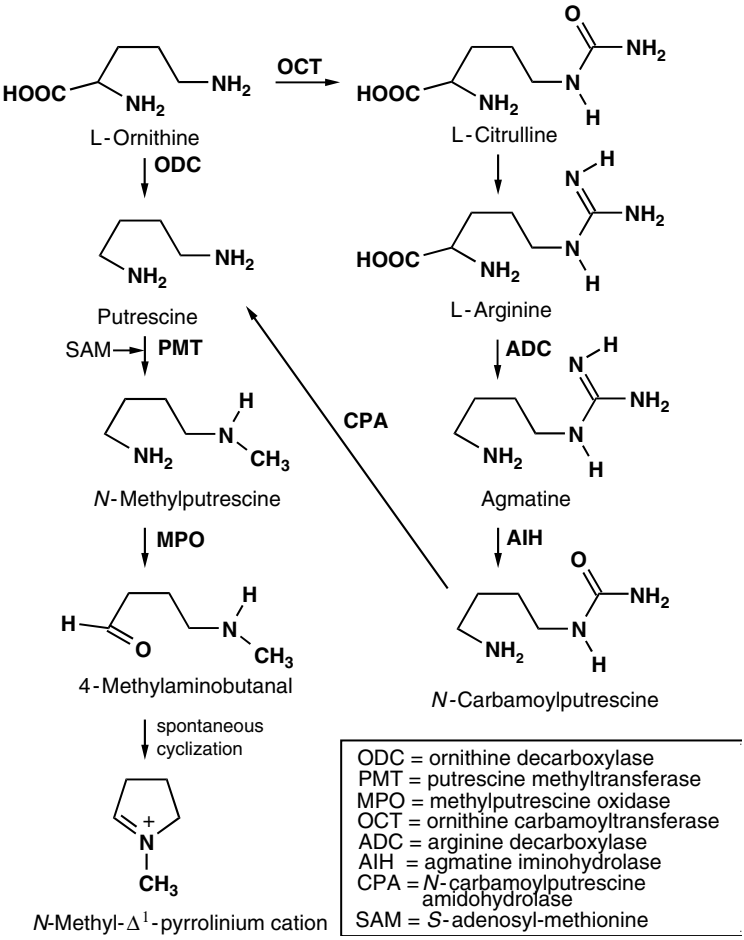


Fig. 3.2 Biosynthesis of the *N*-methyl-Δ^1-pyrrolinium cation, precursor of simple pyrrolidine alkaloids, pyrrolidides, nicotinoids, tropanes, calystegines, and (presumably) indolizidines

3.1 Simple Pyrrolidines

3.1.1 Discovery and Structural Elucidation

The term "simple pyrrolidines" is chosen here for those alkaloids whose structure is characterized by one or two isolated pyrrolidine rings without any other heterocyclic moiety. This restriction separates them from the biogenetically closely related nicotinoids and tropanes, respectively. Shortly after the discovery of the famous tropane alkaloid cocaine (Niemann 1860) a second alkaloid, "hygrine", was discovered in the leaves of *Erythroxylum coca* Lam., Erythroxylaceae (Wöhler and

Lossen 1862; Lossen 1865). However, this "hygrine" – named according to the classical Greek name for "liquid" due to its liquid consistency – represented a mixture of two components, one of which could be isolated and characterized as (the final) hygrine (Fig. 3.3), the first pure pyrrolidine alkaloid of all (Liebermann 1889). Later the second component turned out to be another alkaloid of this type, cuscohygrine which was named according to the provenance of the "Cusco leaves" (from the ancient Inca capital in Peru) being investigated in that study (Liebermann and Cybulski 1895). Its constitutional formula was established correctly already in this early report but it was confirmed only two decades later (Hess and Fink 1920). Cuscohygrine is the main alkaloid besides cocaine in coca leaves from Bolivia and Peru (Hegnauer and Fikenscher 1959). It was also found in the majority of those solanaceous and convolvulaceous plants, respectively, which contain tropane alkaloids formed as an offshoot of their biosynthetic pathway (Huang et al. 1996). Due to an early finding in the roots of *Atropa belladonna* an alkaloid was named "bellaradine" (King and Ware 1941; Reynouts van Haga 1954) before its constitution turned out to be identical to the already known cuscohygrine (Steinegger and Phokas 1955). The first finding of simple pyrrolidines in the Convolvulaceae was documented for hygrine and cuscohygrine, respectively, in *Convolvulus hamadae* (Lazur'evskii 1939).

Occurrence in Non-solanaceous/Non-convolvulaceous Taxa. Later it was reported that hygrine and/or closely related derivatives are also constituents of different unrelated families found throughout the plant kingdom. Such sporadic occurrence was discovered – besides the Erythroxylaceae (see above) – for certain *Sedum* spp. (Crassulaceae) (Stevens et al. 1992; Kim et al. 1996), *Carallia brachiata* (Lour.) Merr. (Rhizophoraceae), *Cochlearia arctica* Slecht (Brassicaceae), as well as for *Dendrobium chrysanthum* Wall. (Orchidaceae) (Massiot and Delaude 1986 and references therein) and *Picea breweriana* S.Watson, Pinaceae (Schneider et al. 1995).

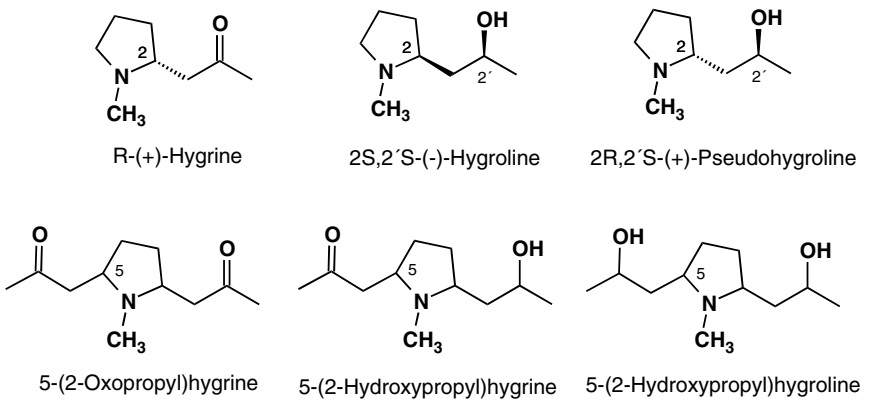


Fig. 3.3 Simple pyrrolidine alkaloids of the hygrine and hygroline type

Alkaloids (Figs. 3.3–3.6). Cuscohygrine and hygrine are not only the most frequent simple pyrrolidine alkaloids but also commonly found in both large Solanales families. The stereochemistry of these two compounds and their derivatives is rather complicated due to unusual structural elements. Consequently, the scientific literature is often unclear, incomplete or even contradictory. Natural hygrine, the basal pyrrolidine alkaloid with only one pyrrolidine moiety turned out to be *R*-(+) configurated (Massiot and Delaude 1986). However, optically active hygrine racemizes as a free base very fast in contrast to its salts which are stable (Lukes et al. 1960). Since basic alkaloids in plants are stored as salts the stability of R-(+)-hygrine in the living plant seems to be given. However, depending on the method chosen for the isolation from plant material racemization may take place. Furthermore, this may also happen during certain metabolic turnovers in the living plant including non-enzymatic, spontaneous reactions due to the chemical structure [for analogies see, e.g., hyoscyamine (Sect. 3.4), ergopeptines (Sect. 4.2)]. Enzymatic reductions of hygrine in certain species may lead to 2*R*,2′*R*-(+)- and/or 2*R*,2′*S*-(+)-hygroline. The former (syn.: hygroline A) was isolated from, e.g., an *Erythroxylum* species, Erythroxylaceae (Christen et al. 1993), the latter (syn.: (+)-pseudohygroline), e.g., from a *Schizanthus* species, Solanaceae, however together with its 2*S*,2′*S*-(−)- diastereomer (San-Martin et al. 1980).

Norhygrine, the *N*-demethylation product of hygrine, was identified in a *Nierembergia* species, Solanaceae (Pomilio et al. 1996). Hygrine derivatives with a second C_3 unit, i.e., 5-(2-oxopropyl)hygrine, 5-(2-hydroxypropyl)hygrine, and 5-(2-hydroxypropyl)hygroline, are further metabolites with only one pyrrolidine moiety. They have been discovered in certain *Merremia* spp., Convolvulaceae (Jenett-Siems et al. 1996, 2005b). The stereochemistry of these alkaloids is still unknown. Characteristically these three congeners, apparently consecutively

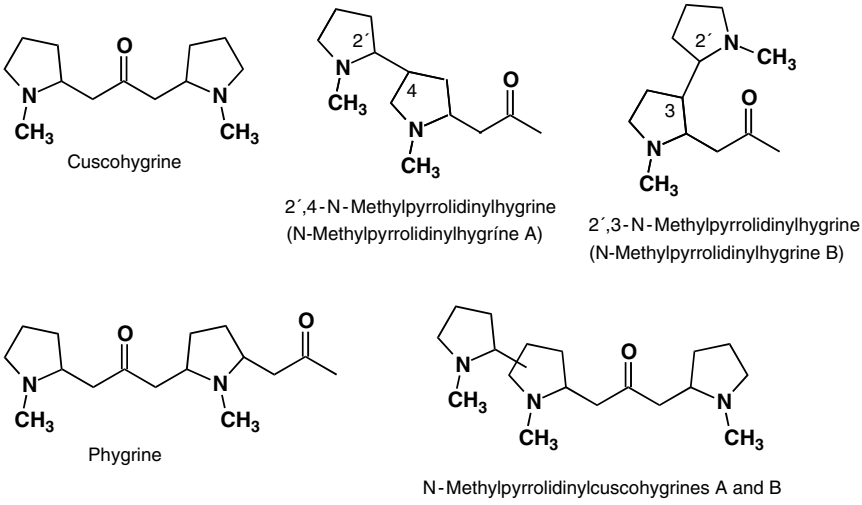


Fig. 3.4 Simple pyrrolidine alkaloids of the cuscohygrine and *N*-methylpyrrolidinylhygrine type

biosynthesized, show co-occurrence; the biosynthetically final 5-(2-hydroxypropyl) hygroline represents the major metabolite of this subgroup.

Moreover, alkaloids with one or even two additional pyrrolidinyl rings are known. (i) Two *N*-methylpyrrolidinylhygrines, originally named A and B as constituents of *Datura inoxia,* Solanaceae (Witte et al. 1987), have been proved as 2′,3 and 2′,4 position isomers, respectively (Jenett-Siems et al. 2005b). However, their stereochemistry remains to be elucidated. (ii) Natural cuscohygrine, optically inactive, turned out to be a mixture of *meso* (2*R*,2′*S*) and racemic forms (2*R*,2′*R* and 2*S*,2′*S*, respectively) apparently due to the easy racemization leading to an equilibrium of diastereoisomers (Fig. 3.5) (Massiot and Delaude 1986; Leete et al. 1988). Therefore the structure is given without stereochemical differentiation in Fig. 3.4. (iii) Finally, metabolites with three pyrrolidine moieties, *N*-methylpyrrolidinylcuscohygrines A and B, were discovered in *Hyoscyamus albus* (Doerk-Schmitz et al. 1994). Whether these alkaloids are also 2′,3 and 2′,4 position isomers, respectively, remains to be determined.

3.1.2 Occurrence in the Solanaceae (Table 3.1)

Our knowledge of the occurrence and distribution of alkaloids in this family is generally based on (i) traditional isolation and structure elucidation procedures and (ii) different chromatographic methods, predominantly TLC.

Schizanthoideae. Hygrolines A and B were detected in four *Schizanthus* spp.; in addition a hygroline glycoside was found in a fifth species, *S. integrifolius*. **Petunioideae.** Cuscohygrine turned out to be a constituent of four *Brunfelsia* spp. Hygrine and its nor congener were identified in *Nierembergia liniariaefolia*. There are no reports on species of the **Cestroideae**. This is also the case for most clades

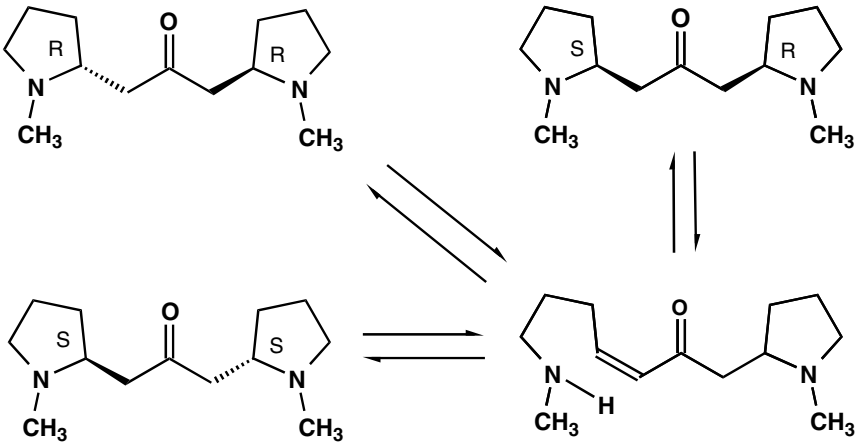


Fig. 3.5 Natural cuscohygrine representing an equilibrated mixture of diastereoisomers [*meso* (2*R*,2′*S*) form and *racemic* forms (2*R*,2′*R* and 2*S*,2′*S*), respectively]

of the **Nicotinoideae** though all are well-known producers of nicotinoids and/or tropanes. However, cuscohygrine was detected in two *Anthocercis* spp. [Anthocercis clade] and *Duboisia hopwoodii* [Cyphanthera clade], respectively, as well as hygrine in the latter species and *D. myoporoides*. In contrast, the occurrence of simple pyrrolidines has been reported from all clades and subclades of the large subfamily **Solanoideae**:

- Cuscohygrine in 75 species belonging to 18 genera
- Hygrine in 25 species belonging to 10 genera
- *Nor*hygrine: 4 *Hyoscyamus* spp. [Hyoscyameae clade]; *Nicandra physalodes* [Nicandreae clade]
- *N*-Methylpyrrolidinylhygrines: *H. albus*; *Brugmansia candida*, *Datura inoxia*, *D. ceratocaula* [Datureae clade]; *Physalis peruviana* [Physaleae clade/Physalinae subclade]
- Hygrolines A and B: *Atropa belladonna* [Hyoscyameae clade]; *N. physalodes* [Nicandreae clade]
- Oxohygrine: *B. candida* × *aurea* [Datureae clade]
- *N*-Methylpyrrolidinylcuscohygrines: *H. albus* [Hyoscyameae clade]
- Phygrine ("***Phy***salishy**grine**") had been believed to be a specific metabolite of the genus *Physalis* detected in nine species; attempts to discover this compound in other solanaceous genera including the closely related *Withania* had failed (Basey et al. 1992). However, in a more recent study this alkaloid could be identified also in four *Hyoscyamus* spp. (El-Shazly et al. 1997).

Quantitative information concerning simple pyrrolidine alkaloids is rather rare. The roots of *Nicandra physalodes* contained 0.1% hygrine whereas five *Physalis* spp. contained "considerably less" (Romeike 1965a,b). The content of the epigeal parts of *N. physalodes* was even extremely low. Surprisingly, cuscohygrine turned out to be the main alkaloid of *Datura discolor* roots with a concentration of 0.06% which amounts to 20% of the total alkaloid content (0.31%). This is in contrast with other *Datura* spp. where tropanes (hyoscyamine/scopolamine) represent the principal alkaloids (Evans and Somanabandhu 1974a).

Simple pyrrolidine alkaloids may be considered as plesiomorphic characters present in both large Solanales families. In the Solanaceae the occurrence of this type of alkaloids was reported especially for species which contain tropane alkaloids and/or calystegines; this is not surprising since both latter groups of alkaloids, which also may be considered as plesiomorphic characters, share basal parts of the biogenetic pathway with the pyrrolidines as already mentioned above (Huang et al. 1996 and references therein). On the other hand, this implicates that simple pyrrolidine alkaloids might be present also in those species which have been checked only for tropanes but not yet for pyrrolidines (missing data in Table 3.1). Tropanes are apparently more attractive for investigations due to their famous biological significance. For two genera (*Brunfelsia*, *Solanum*) reported to include pyrrolidine-positive species no occurrence of tropanes (sensu Table 3.1) was documented. However, calystegines whose biosynthetic pathway involves simple tropanes were found in certain species of these genera (see Sect. 3.4.3). Nevertheless, there may

also be solanaceous genera or species which have lost the ability to synthesize the tropane skeleton but still are able to produce pyrrolidines. Anyhow, such examples are existent in the Convolvulaceae (Table 3.2).

Chemotaxonomic Relevance. The existing data for an intrafamilial evaluation of this topic are full of gaps in case of the Solanaceae (Table 3.1). However, since simple pyrrolidines are plesiomorphic characters shared with the well-studied Convolvulaceae (Table 3.2) and at least the occurrence and distribution of cuscohygrine is also well-documented in the Solanaceae, it might be that there is a similar tendency in both Solanales families.

Appendix: Alkaloids from *Withania somnifera* (Fig. 3.6). The occurrence of two additional 1,3-disubstituted 2-propanones besides cuscohygrine, anahygrine (one methylpyrrolidinyl and one piperidyl substituent) and anaferine (two piperidyl substituents), both isomeric with cuscohygrine, was reported for the roots of *W. somnifera* (Rother et al. 1962; Schwarting et al. 1963, Leary et al. 1964; El-Olemy and Schwarting 1965). In addition to hygrine a lysine-derived analogue of norhygrine, isopelletierine, discovered in *Punica granatum* L. (pomegranate tree), Lythraceae (Hess 1919), was detected also in *W. somnifera*. This alkaloid is a precursor of anaferine (O'Donovan and Keogh 1968; Keogh and O'Donovan 1970). Isopelletierine is also a constituent of *Salpiglossis sinuata* (Schröter 1963), *Duboisia hopwoodii* (Kennedy 1971), and *D. myoporoides* (Mortimer and Wilkinson 1957). This metabolite had been the decisive component of *Punica* root/stem bark in its – meanwhile obsolete – utilization as a specific anthelmintic drug against *Taenia* (tapeworm) before more suitable synthetic remedies were developed (Steinegger 1972). Furthermore, a pyrazole alkaloid, withasomnine, could be isolated from the roots of *W. somnifera* (Schröter et al. 1966) which is synthesized in the plant by condensation of Δ^1-pyrroline (originated from L-ornithine) and phenethylamine (originated from L-phenylalanine (O'Donovan and Forde 1970). Later withasomnine was also identified as a constituent of *Newbouldia laevis* SEEM., Bignoniaceae (Adesanya et al. 1994) and *Elytraria acaulis* LINDAU, Acanthaceae (Ravikanth et al. 2001). This alkaloid was shown to be relatively non-toxic in mice, to induce narcosis; furthermore, it displayed slight analgesic, local anaesthetic, and spasmolytic effects and was depressive in the central nervous and circulatory system (Hueller et al. 1971).

3.1.3 Occurrence in the Convolvulaceae (Table 3.2)

Our knowledge of the presence of simple pyrrolidine alkaloids in solanaceous species is based mainly on reports about the isolation of these metabolites from certain species. Due to comprehensive GC/MS studies on 150 convolvulaceous species more detailed information and comparability is given concerning the presence or absence of single compounds or subtypes of pyrrolidines in the different taxa of this family. One outstanding advantage of the GC/MS analysis is that this method generally enables the unequivocal characterization of every compound

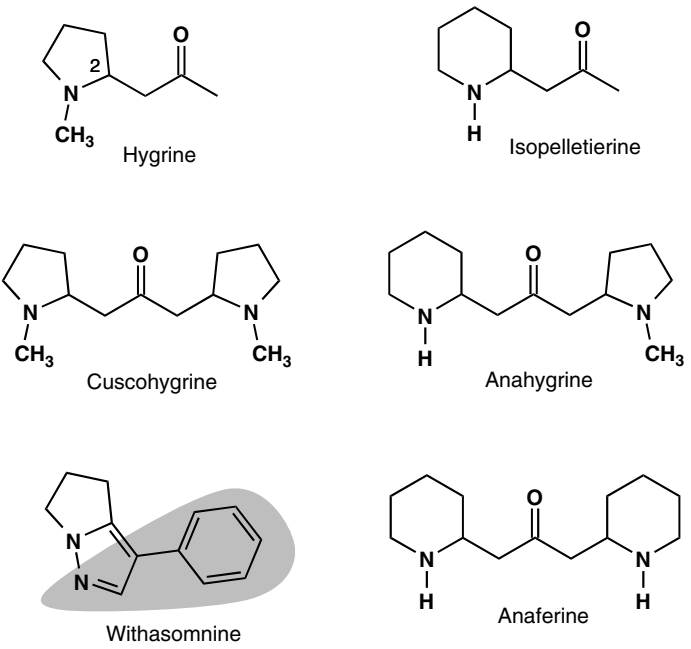


Fig. 3.6 Pyrrolidine and piperidine alkaloids from *Withania somnifera*; stereochemistry not given due to isomerizations (see Fig. 3.5). In the structure of the pyrazol alkaloid withasomnine, a masked pyrrolidine, the second biogenetic element, phenethylamine (derived from L-phenylalanine) is highlighted in *grey*

based on the combined specific data obtained from gas chromatography (retention time) and mass spectrometry (parent peak, base and fragmentation peaks). Furthermore, it must be pointed out that this method even allows the detection of very low concentrations due to its high analytical sensitivity which causes a very low detection limit.

Therefore, Table 3.2 (Convolvulaceae) indicates "plus" or "minus" for any of these characters instead of gaps in Table 3.1 (Solanaceae). Such gaps may represent either the absence of a character (perhaps just not reported in the literature though noticed) in the corresponding species or the lack of check (not determined).

The alkaloid fractions of roots and epigeal vegetative parts, respectively, have been analyzed in the dominating majority of the convolvulaceous species by GC-MS analysis. Simple pyrrolidines have been detected in 143 species out of 150 (95%). Characteristically the content of such alkaloids turned out to be much higher in the roots though they are still detectable in the leaves or stems more often than not. However, no alkaloids of this type have been found in the vegetative epigeal parts of the remaining seven species (*Convolvulus kilimandschari*, *C. scoparius*, *Polymeria longifolia*, *P. marginata*, *Ipomoea bonariensis*, *I. coptica*, *Stictocardia beraviensis*). Since no roots were available in the studies on these seven species it cannot be ruled out that pyrrolidines might be present there, perhaps only in low

concentrations. Anyhow, the occurrence of pyrrolidine alkaloids is a consistent trait within the Convolvulaceae, from basal to most advanced tribes.

Hygrine turned out to be the most frequent metabolite of this type of alkaloids (133 spp. = 89%), followed by cuscohygrine (114 spp. = 76%). Both major congeners have been detected in 105 spp. (= 70%). Propylhygrines (75 spp. = 50%) and *N*-methylpyrrolidinylhygrines (71 spp. = 47%) are further frequent metabolites. Compared with them, phygrine (26 spp. = 18%), *N*-methylpyrrolidinylcuscohygrines (25 spp. = 17%), hygrolines (21 spp. = 14%), norhygrine (12 spp. = 8%), and 2,6-dehydrohygrine (9 spp. = 6%) are rather rare constituents. Both hygrolines, i. e., A and B, are identical with those from an *Erythroxylum* sp. and four *Schizanthus* spp., respectively (see above), which means that the stereochemistry of hygroline A is known [2R,2′S-(+)-form], whereas the one of hygroline B is still undetermined. In contrast to the Solanaceae with a rare occurrence of phygrine in only two genera (*Physalis*, *Hyoscyamus*) and the absence in many others (see above) this alkaloid is distributed throughout the Convolvulaceae with an occurrence in 26 species (18%) from 9 basal to advanced genera. Certain species have shown a very broad profile of those pyrrolidines which are listed in column **P3** of Table 3.2 in addition to the common alkaloids hygrine (**P1**) and cuscohygrine (**P2**): *Ipomoea alba* (seven further pyrrolidines); *Aniseia martinicensis*, *Convolvulus chilensis*, *I. turbinata* (six each); *Convolvulus farinosus*, *Merremia kentrocaulos* (five each). Furthermore, there are 14 species from 6 genera with at least 4 of these "**P3**-alkaloids". The majority of the convolvulaceous species (89 = 59%) turned out to contain only one to three **P3**-type compounds.

On the other hand, there are 38 species (25%) from 15 genera which did not show any **P3**-type compound. However, only 3 genera were found to be exclusively "**P3**"-negative [P*olymeria* (5 spp. included in the GC-MS study), *Erycibe* (2 spp.), and *Xenostegia* (2 spp.)], whereas 10 genera showed also "**P3**"-positive species (*Bonamia*, *Evolvulus*, *Jacquemontia*, *Convolvulus*, *Merremia*, *Operculina*, *Argyreia*, *Ipomoea*, *Stictocardia*, *Turbina*) beside "**P3**"-negative ones (*Cuscuta* is only involved with 1 species, *Hewittia* is a monotypic genus).

In almost every species containing cuscohygrine it was the dominating congener in the fraction of the simple pyrrolidines, especially in the roots. This is true for all convolvulaceous genera checked (Eich, unpublished results). Only in a few such cases was hygrine the major alkaloid (*Argyreia capitata*, *A. nervosa, Merremia tuberosa*) or at least equal to cuscohygrine (*Bonamia semidigyna, Convolvulus althaeoides*). Propylhygrines turned out to be the main alkaloids in *M. aurea* and *M. aegyptia*, respectively, whereas it is evident that both *N*-methylpyrrolidinylhygrines are the principal alkaloidal metabolites in *M. hederacea* (Jenett-Siems et al. 2005b).

The content of cuscohygrine in dried roots is widely divergent and may vary from 0.00001% (*Merremia tuberosa*) to 0.15% (*Convolvulus floridus*) according to a study on 14 species [*Calystegia* (1), *Convolvulus* (9), *Merremia* (3), *Ipomoea* (1)] (Jenett-Siems 1996).

Chemotaxonomic Relevance. If specific, individual species are characterized by the *additional* presence of alkaloids from different biogenetic origin, e.g., ergolines

(Sect. 4.2), indolizidines (Sect. 3.6) or pyrrolizidines (Sect. 3.7), the content of simple pyrrolidine alkaloids turned out to be generally rather low. This may also be the case if alkaloids are synthesized by the same general pathway. The content of tropanes in *Merremia dissecta*, *M. quinata*, and *M. quinquefolia* outdoes the content of pyrrolidines by far. However, in contrast cuscohygrine is dominating in *M. cissoides*, *M. guerichii*, *M. kentrocaulos*, and *M. vitifolia* though these species are also producing tropanes (Jenett-Siems et al. 2005b). As may be concluded from the discussion on the number of **P3**-type pyrrolidines (see above) the occurrence of such constituents generally is not a characteristic of taxonomic value for the convolvulaceous genus level. Due to the almost ubiquitary occurrence and distribution of pyrrolidines (especially hygrine, cuscohygrine) in the Convolvulaceae, all these alkaloids are not suitable for intrafamilial chemotaxonomic discussions (Jenett-Siems et al. 2005b). However, as plesiomorphic characters which are shared with the Solanaceae they may serve as distinguishing feature to orders closely related to the Solanales.

3.1.4 Biosynthesis

Data on the biosynthesis of simple pyrrolidine alkaloids are rather poor. The following first part of the pathway leading to hygrine is supported by radiolabelled-precursor experiments: After decarboxylation of L-ornithine to putrescine this is methylated; the resulting *N*-methylputrescine is converted to 4-methylaminobutanal by subsequent oxidation of the primary amino group; spontaneous cyclization, i.e., without enzymatic catalyzation finally leads to a *N*-methyl-Δ^1-pyrrolinium salt (Leete 1985); for details see Fig. 3.2. There is no doubt that two acetate units are able to complete the biosynthesis of hygrine. Therefore, it has been proposed that condensation of the cation with acetoacetate and subsequent decarboxylation may yield hygrine. However, experimental support is lacking. At least in experiments which should yield support for its role in the related biosynthesis of tropanes (Sect. 3.4.4) acetoacetate has been metabolised back to acetate and not incorporated intact (Humphrey and O'Hagan 2001 and references therein).

Since the 1970s hygrine had been considered to be the precursor of cuscohygrine (O'Donovan and Keogh 1969; McGaw and Woolley 1978a). However, it was shown later that labelled ethyl (*R,S*)-4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate administered to *Erythroxylum coca*, Erythroxylaceae, resulted in a much more effective incorporation into cuscohygrine (Newquist et al. 1993). From these findings the authors concluded that this ß-keto acid in its natural thioester form, i.e., bound to coenzyme A is a more likely precursor than hygrine. The corresponding hypothesis for the biosynthesis of cuscohygrine includes the consecutive reaction of a first molecule *N*-methyl-Δ^1-pyrrolinium salt (Leete et al. 1988) with two acetyl-CoA. Afterwards this conjugate is condensed with a second *N*-methyl-Δ^1-pyrrolinium salt followed by decarboxylation of the resulting intermediate to yield cuscohygrine.

The above mentioned ß-keto acid in its thioester form was also established as a precursor to the tropane alkaloids, respectively (Sect. 3.4.4). It was speculated that there is a polyketide enzyme that accepts the *N*-methyl-Δ^1-pyrrolinium cation as the starter which is fully committed to two acetate/malonate condensations before 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate is released from the enzyme (Humphrey and O'Hagan 2001). This would explain why neither1-methylpyrrolidine-2-acetic acid (Huang et al. 1996) nor acetoacetate (see above) can serve as precursors. To date there are no experimental reports on the biosynthesis of further simple pyrrolidine alkaloids though their relationships to hygrine and cuscohygrine, respectively, are obvious (additional C_3 unit or *N*-methyl-Δ^1-pyrrolinium unit). Compounds like propylhygrines, *N*-methylpyrrolidinylhygrines or *N*-methylpyrrolidinylcuscohygrines have been detected only in certain species. This fact indicates that such compounds are natural metabolites rather than artefacts of hygrine/cuscohygrine.

3.1.5 Significance

There are almost no reports on the significance of simple pyrrolidine alkaloids neither from the ecological nor from the pharmacological point of view. However, in the latter field some derivatives were investigated. Thus, the twice-quaternary *N*-methyl cuscohygrinium salt ("cuscohygrine dimethiodide") led to a decreased arterial blood pressure for a short time and exhibited parasympathomimetic activity (Minina et al. 1976). Derivatives of hygrine and cuscohygrine, respectively, were reduced to the corresponding alcohols, e.g., hygroline, which were acylated. Some of the produced esters showed spasmolytic activity (Zhang and Xu 1995).

3.2 *N*-Acylpyrrolidines (Pyrrolidides, Pyrrolidine Amides)

These metabolites are not a topic of Tables 3.1 and 3.2.

Occurrence in Non-solanaceous/Non-convolvulaceous Taxa. Pyrrolidine amides in the plant kingdom are mainly restricted to the Asteraceae (e.g., Greger 1984; Greger et al. 1987) and the Piperaceae (e.g., Singh et al. 1971), two unrelated families. However, most of the compounds detected there show olefinic or acetylenic acyl moieties whereas the metabolites found in the Solanales are characterized by saturated acyl moieties.

There are only two reports on the occurrence of pyrrolidine amides in both large Solanales families, one each. They share their main structural skeleton including the amide group with one of the two moieties of pyrrolidine-type *N*-acylnornicotinoids (see Table 3.3). Therefore, they are also not basic [for details see Sect. 3.3.1 ("*N*-Acylated nicotinoids")].

3.2.1 Occurrence in the Solanaceae

Screening of extracts from the Texas plant *Chamaesaracha coniodes* (DUN.) BRITT. exhibited activity in a cytotoxicity assay; bioassay-guided fractionation led to the isolation and structure elucidation of two constituents, conioidines A and B (Chan et al. 1993). The "i" after the "o" ought to be unnecessary because the correct species epithet is *coniodes* (not "*conioides*" as has been reported). These compounds are *N*-*n*-decanoylhygroline esters with tiglic acid (conioidine A) and its 4-hydroxy congener (conioidine B), respectively (Fig. 3.7). Stereochemistry at C-2 and at C-2′, respectively, remains to be determined.

3.2.2 Occurrence in the Convolvulaceae

Aliphatic pyrrolidine amides with linear and branched saturated C_{15}–C_{19} acyl moieties were discovered by GC-MS analysis in vegetative plant organs of *Ipomoea aquatica* FORSK. (three compounds), whose leaves are used as vegetable in Southeast Asia, and *Merremia quinquefolia* (L.) HALL. f. (seven compounds), a pantropical twiner of neotropical origin (Tofern et al. 1999). The structure of *N*-*n*-hexadecanoyl- as well as *N*-*n*-octadecanoylpyrrolidine was confirmed by comparison of their GC/MS data with those of synthesized compounds. These two metabolites had been also characterized by this method in *Piper amalago* L., Piperaceae (Achenbach et al. 1986). The tentative GC-MS-identification of five compounds as congeners with branched acyl moiety (C_{15}–C_{19}) could be confirmed by the isolation and structure elucidation of *N*-(14-methylhexadecanoyl)pyrrolidine (Fig. 3.7) from both convolvulaceous species (Tofern et al. 1999). This metabolite, the first pyrrolidine amide with a branched saturated acyl moiety found in nature, was the main pyrrolidide and the only one present even in the seeds of *M. quinquefolia*. It has been determined

Table 3.3 *N*′-Acylnornicotines as constituents of leaf surface lipids of *Nicotiana repanda*, *N. stocktonii*, and *N. nesophila* (section *Repandae*)

N′-Acylnornicotine	Partial structure
n-Dodecanoyl-	$-CO(CH_2)_{10}CH_3$
n-Tridecanoyl-	$-CO(CH_2)_{11}CH_3$
iso-Dodecanoyl- to *iso*-tetradecanoyl-	$-CO(CH_2)_nCH(CH_3)_2$ (n = 8 – 10)
anteiso-Tridecanoyl-	$-CO(CH_2)_8CH(CH_3)CH_2CH_3$
3-Hydroxy-*n*-dodecanoyl- to 3-hydroxy-*n*-hexadecanoyl-	$-CO(CH_2)CH(OH)(CH_2)_nCH_3$ (n = 8 – 12)
3-Hydroxy-*iso*-dodecanoyl- to 3-hydroxy-*iso*-hexadecanoyl-	$-CO(CH_2)CH(OH)(CH_2)_nCH(CH_3)_2$ (n = 6 – 10)
3-Hydroxy-*anteiso*-tridecanoyl-[a]	$-CO(CH_2)CH(OH)(CH_2)_6CH(CH_3)CH_2CH_3$
3-Hydroxy-*anteiso*-pentadecanoyl-[a]	$-CO(CH_2)CH(OH)(CH_2)_8CH(CH_3)CH_2CH_3$

[a] The prefix *anteiso* indicates that this isomer shows its methyl branch at the position two carbon atoms before the final methyl group, thus resulting in two different final groups (methyl and ethyl) in contrast to the prefix *iso* indicating two final methyl groups

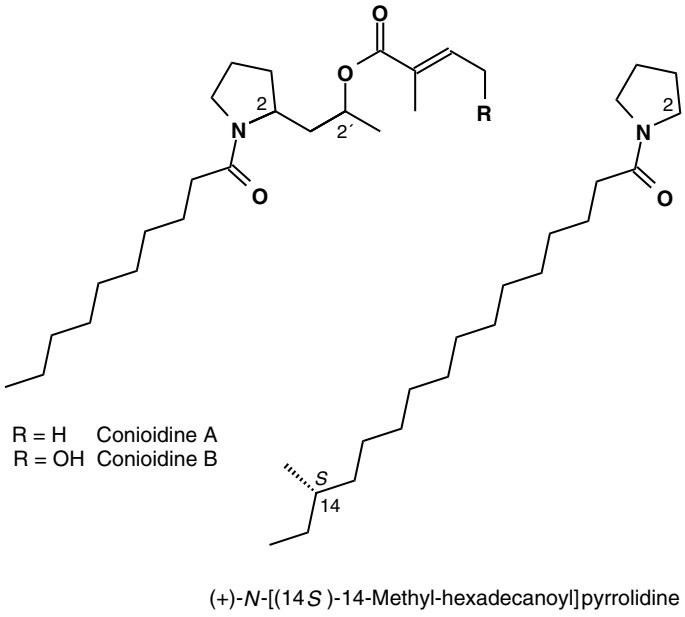


Fig. 3.7 Pyrrolidides (*N*-acylpyrrolidines) from *Chamaesaracha coniodes*, Solanaceae (*left*), and *Ipomoea aquatica / Merremia quinquefolia*, Convolvulaceae (*right*)

to represent the (+)-*S*-form (Yajima and Yabuta 2001). The occurrence of pyrrolidine amides does not seem to be a general feature in the family, since such compounds could not be detected in numerous genera including altogether 150 species (Mann 1997; Tofern 1999; Eich, unpublished results).

3.2.3 Biosynthesis

There are no data concerning the biosynthesis of pyrrolidides found in Solanales species. However, it may be assumed that it involves again the *N*-methyl-Δ^1-pyrrolinium unit since (i) the conioidines are hygroline derivatives and (ii) the convolvulaceous metabolites show strong structural similarity to pyrrolidine-type nicotinoids. The acyl moieties apparently come from the fatty acid pathway. The esterifying acids of the conioidines are derivatives of L-isoleucine.

3.2.4 Significance

The conioidines showed cytotoxicity to the transformed human cell line KB. However, this toxicity could be reduced or reversed by the addition of exogenous calf thymus DNA. This happened by interaction of the compounds with DNA in a

doxorubicin-like manner though they are lacking aromatic structural elements. The exact mechanism of their DNA-binding ability is still unknown. Anyhow, this binding affected the ability of the conioidines to enter the KB cells exerting their cytopathic effect (Chan et al. 1993). There is no information on the biological significance of the convolvulaceous congeners.

3.3 Nicotinoids (Tobacco Alkaloids)

The authorities of the scientific species names are only added in the text if a species is not involved in Table 3.1 (Solanaceae), Table 3.2 (Convolvulacae) or Table 3.4 (genus *Nicotiana*), respectively, where the authorities of the corresponding other species may be found.

3.3.1 Discovery and Structure Elucidation

Nicotiana tabacum, the main species used commercially for the production of tobacco, is one of the most, if not the most, studied species today in plant and biological science as well as in chemistry (Tso 1999). According to Leete (1983) tobacco was more thoroughly examined than any other plant product. The scientific history of the alkaloid which turned out to become one of the most (ab)used secondary metabolites of the plant kingdom, the active principle of tobacco leaves, started already in the beginning of the nineteenth century when Vauquelin (1809a) discovered its volatile property ("essence de tabac"). Two decades later it was isolated and named nicotine by Posselt and Reimann (1828). However, complete structure elucidation took up one century starting with the summation formula (Barral 1847), followed by the constitution (proposed: Pinner 1893, 1895; proved: Pictet and Genequand 1897; Pictet and Rotschy 1904) and the configuration which turned out to be the same as L-proline (Karrer and Widmer 1925).

Nicotine is also **accumulated in considerable proportions** in other *Nicotiana* species and in a few species of some other solanaceous genera, e.g., *Duboisia* (for details see below). Moreover, it was detected **in extremely small amounts** in species of several other genera of this family. Furthermore, it is also a common **minor** component in the Convolvulaceae beside pyrrolidine and tropane alkaloids (for details see below).

Occurrence in Non-solanaceous/Non-convolvulaceous Taxa. Like the Solanales the unrelated genus *Erythroxylum* P. BROWNE (Erythroxylaceae) also shows co-occurrence of nicotine (low concentrations) and pyrrolidines and tropanes (Steinegger 1972). Furthermore, due to its relatively simple biosynthesis close to the primary metabolism (Sect. 3.3.4), the sporadic occurrence of nicotine in several unrelated families found throughout the plant kingdom is not very surprising. Thus, nicotine

Table 3.4 Alkaloid profiles in the genus *Nicotiana* based on data from studies of Saitoh et al. (1985) as well as Sisson and Severson (1990), updated according to the classification of Knapp et al. (2004). However – for direct comparison with the original reports – sections and species in the order used by Saitoh et al. or by Sisson and Severson, i.e., not alphabetically like Knapp et al.
++ = Principal alkaloid detected by GC; + = minor alkaloid detected by GC; – not detected by GC

Subgenus (according to Goodspeed 1954, not monophyletic, see text) **Section** *Species*	**Pyridyl-pyrrolidines**				**Pyridyl-piperidines**	
	Nicotine	Nornicotine	Myosmine[a]	N-Acetylnornicotine[a]	Anabasine	Anatabine
Rustica						
***Paniculatae* Goodsp.**						
N. paniculata L.	++	+	+	+	+	+
N. knightiana Goodsp.	++	++[b]	–	–	+	+
N. solanifolia Walp.	+	++	–	+	++[b]	+
N. benavidesii Goodsp.	++	+	–	–	++[b]	+
N. cordifolia Phil.	++	+	–	–	++[b]	+
N. raimondii J.F.Macbr.	++	+	–	+	+	+
N. cutleri D'Arcy (no data)						
***Thyrsiflorae* Goodsp**. (former monotypic section, omitted by Knapp et al. 2004)						
***Rusticae* G.Don**						
N. rustica L.	++	+	–	++	+	+
Tabacum						
***Tomentosae* Goodsp**.						
N. tomentosa Ruiz & Pav.	++[b]	++	–	+	+	+
N. tomentosiformis Goodsp.	++[b]	++	–	+	+	+
N. otophora Griseb.	++[b]	++[c]	–	+	+	++[d]
N. setchellii Goodsp.	+	++	–	+	+	+
N. kawakamii Y.Ohashi	+	++	–	+	+	+
***Nicotiana* (syn.: *Tabacum* G.Don; *"Genuinae"* Goodsp.)**						
N. tabacum L.	++	+	–	–	+	+
Petunioides						
***Undulatae* Goodsp.**						
N. undulata Ruiz & Pav.	++	+	–	+	+	+[e]
N. arentsii Goodsp.	++	+	–	–	+	+
N. wigandioides Koch & Fintelm.	++	+	–	+	+	+
N. glutinosa L.	+	++	–	+	+	+
N. thyrsiflora Bitter ex Goodsp.[a]	+	++	–	+	+	+
***Trigonophyllae* Goodsp.**						
N. obtusifolia M.Martens & Galeotti sub nom. *N. trigonophylla* Donal	++[b]	++	–	+	+	+
N. palmeri A.Gray[b]	+	++	–	+	+	+
***Alatae* Goodsp.**						
N. langsdorffii Weinm.	++	+	–	–	+	+

(continued)

Table 3.4 Alkaloid profiles in the genus *Nicotiana* based on data from studies of Saitoh et al. (1985) as well as Sisson and Severson (1990), updated according to the classification of Knapp et al. (2004). However – for direct comparison with the original reports – sections and species in the order used by Saitoh et al. or by Sisson and Severson, i.e., not alphabetically like Knapp et al. (continued)

Subgenus (according to Goodspeed 1954, not monophyletic, see text) **Section** *Species*	**Pyridyl-pyrrolidines**				**Pyridyl-piperidines**	
	Nicotine	Nornicotine	Myosmine[a]	N-Acetylnormicotine[a]	Anabasine	Anatabine
N. alata Link & Otto	++	+	+	+	+	+
N. forgetiana Hemsl.	++	+	–	–	+	+[f]
N. bonariensis Lehm.	++	+	–	–	+	+[f]
N. longiflora Cav.	++	++[c]	–	+	+[f]	+
N. plumbaginifolia Viv.	++[b]	++	–	+	+	+
N. sanderae Hort. ex W.Watson[b,g]	++	+	–	–	+	–
N. azambujae L.B. Smith & Downs, *N. mutabilis* Stehmann & Samir (no data)						
***Sylvestres* S.Knapp**						
N. sylvestris Speg. & Comes	++	+	–	+	+	+
***Repandae* Goodsp.**						
N. repanda Willd.	++[b]	++	–	+	+	+
N. stocktonii Brandegee	++	+	–	+	+	+
N. nesophila I.M.Johnston	++[f]	++	–	+	+	+
N. nudicaulis S.Watson	++[b]	++	–	+	+	+
***Noctiflorae* Goodsp.**						
N. noctiflora Hook.	++[f]	++[f]	–	+	++[c]	+
N. petunioides (Griseb.) Millán[b]	+	+	–	+	++	+
N. glauca Graham	+	+	–	+	++	+
N. acaulis Spegg.[b]	+	++	–	+	++	+
N. ameghinoi Spegg., *N. paa* Mart. Crov. (no data)						
***Petunioides* G.Don (syn.: *Acuminatae* Goodsp.)**						
N. acuminata (Graham) Hook.	++	+	–	–	+	+[e]
N. pauciflora J.Rémy	++	+	–	–	+	+[e]
N. attenuata Torrey ex S.Watson	++	+	+	+	+	+
N. miersii J.Rémy	++	++	–	–	+	+[e]
N. corymbosa J.Rémy	++	+	+	+	+	+
N. linearis Phil.[b]	++	+	–	–	–	–
N. spegazzinii Millán	++	++	–	+	+	+
N. longibracteata Phil. (no data)						
***Polydicliae* G.Don (syn.: *Bigelovianae* Goodsp.)**						
N. quadrivalvis Pursh sub nom. *N. bigelovii* (Torrey) S.Watson	++	+	+	–	+	+
N. clevelandii A.Gray	++	+	+	–	+[e]	+
***Nudicaules* Goodsp.** (former monotypic section, omitted by Knapp et al. 2004)						

(continued)

Table 3.4 Alkaloid profiles in the genus *Nicotiana* based on data from studies of Saitoh et al. (1985) as well as Sisson and Severson (1990), updated according to the classification of Knapp et al. (2004). However – for direct comparison with the original reports – sections and species in the order used by Saitoh et al. or by Sisson and Severson, i.e., not alphabetically like Knapp et al. (continued)

Subgenus (according to Goodspeed 1954, not monophyletic, see text) **Section** *Species*	**Pyridyl-pyrrolidines**				**Pyridyl-piperidines**	
	Nicotine	Normicotine	Myosmine[a]	N-Acetylnormicotine[a]	Anabasine	Anatabine
***Suaveolentes* Goodsp.**						
N. benthamiana Domin	++	+	–	+	+	+
N. umbratica N.T.Burb.	++	++	–	–	+	+
N. cavicola N.T.Burb.	++[b]	++	–	–	+	+
N. debneyi Domin	+	+	–	–	++	+
N. gossei Domin	++	+	–	–	+	+
N. amplexicaulis N.T.Burb.	++	+	–	–	+	+
N. maritima H.-M.Wheeler	+	++	–	+	++[b]	+
N. velutina H.-M.Wheeler	++[b]	++	–	+	+	+
N. hesperis N.T.Burb.	++	+	–	–	++[f]	+
N. occidentalis H.-M.Wheeler	++	++	–	+	++	+
N. simulans N.T.Burb.	++	++	–	+	++[b]	+
N. megalosiphon Van Heurck & Müll. Arg.	++[b]	++	–	+	++	+
N. rotundifolia Lindl.	++	+	–	+	++[b]	+
N. excelsior J.M.Black	++	+	–	+	+	+
N. suaveolens Lehm.	++	+	–	+	+	+
N. ingulba J.M.Black	++	++[f]	–	+	++[f]	+
N. exigua H.-M.Wheeler	++	++[c]	–	+	+	+
N. goodspeedii H.-M.Wheeler	+	++	–	+	++[c]	+
N. rosulata (S.Moore) Domin	++[b]	++	–	+	+	+
N. fragrans Hook.	++	+	–	+	+	+
N. africana Merxm.	++[b]	++	–	+	+	+
N. stenocarpa H.-M.Wheeler[h]	++	+	n.d.	n.d.	+	–
N. burbidgeae Symon, *N. heterantha* Kenneally & Symon, *N. truncata* Symon, *N. wuttkei* Clarkson & Symon (no data)						

[a] Data only from the study of Sisson and Severson (1990)
[b] Detected as a major component only in the roots of the study of Saitoh et al. (1985)
[c] Detected as a major component only in the study of Sisson and Severson (1990)
[d] Detected as a major component only in the leaves of the study of Saitoh et al. (1985)
[e] Detected as a minor component only in the leaves and roots of the study of Saitoh et al. (1985)
[f] Detected as a major component only in the leaves and roots of the study of Saitoh et al. (1985)
[g] Not integrated in the classification of Knapp et al. (2004)
[h] Data from a study of Vasinev (1970)

was detected in, e.g., *Lycopodium* spp., Lycopodiaceae (Manske and Marion 1942; Marion and Manske 1948); *Equisetum* spp. (horsetail), Equisetaceae (Phillipson and Melville 1960); *Asclepias syriaca* L., Apocynaceae (Marion 1939); *Sedum* spp. (stonecrop), Crassulaceae (Marion 1945; Gill et al. 1979); *Zinnia elegans* JACQ., Asteraceae (nornicotine and anabasine in addition as minor congeners) (Schröter 1955); *Acacia* spp., Fabaceae (Fikenscher 1960; Clement et al. 1997); *Prunus cerasus* L. (cherry tree), Rosaceae; *Aesculus hippocastanum* L. (horse chestnut) Sapindaceae; *Juglans regia* L.(walnut), Juglandaceae; *Humulus lupulus* L., Cannabaceae; *Urtica dioica* L. (stinging nettle), *U. urens* L. (annual nettle), Urticaceae (Blaim 1962); *Herpestes monniera* KUNTH, Scrophulariaceae (Schulte et al. 1972); *Areca catechu* L. (betel nut palm), Arecaceae (Holdsworth et al. 1998). However, in many of these cases the accumulation of the alkaloid may be rather low compared with *Nicotiana tabacum* and other *Nicotiana* spp., e.g., 9.1 kg *Lycopodium cernuum* L. (dried plant) yielded just 1 mg nicotine (Marion and Manske 1948).

Nicotinoids with Basic Pyrrolidine or Piperidine Moiety. Nicotinoids are (i) congeners of nicotine, usually minor alkaloids but the term also includes (ii) the principal tobacco alkaloid, nicotine itself. From the structural point of view the nicotinoids may be divided into five groups (Figs. 3.8 and 3.9):

I. 2,3′-Pyridylpyrrolidines and their derivatives, e.g., nicotine, nornicotine, myosmine, nicotyrine, cotinine, *N*′-acylnornicotines, nicotine-*N*′-oxides
II. 2,3′-Pyridylpiperidines, e.g., anabasine, *N*′-methylanabasine, *N*′-formylanabasine
III. 2,3′-Pyridyl-Δ^4-piperideines, e.g., anatabine, *N*′-methylanatabine, *N*′-formylanatabine
IV. Dipyridyls (bipyridines), e.g., 2,3′-dipyridyl and its isomers (2,4′-, 3,3′-, 4,4′-), 5-methyl-2,3′-dipyridyl
V. Tripyridyls (terpyridyls) and partially hydrogenated derivatives, e.g., nicotelline, anatalline, anabasamine

It must be taken into account that only the first group is yielded by the biogenetic pathway starting with L-ornithine. However, there is a structural and biogenetic relationship to the other groups based on the second moiety of their molecules, the pyridine skeleton which is shared by all five groups. Thus, it makes sense to treat the tobacco alkaloids altogether (for details of the biosynthesis see Sects. 3.3.4 and 3.3.5).

The name *Nicotiana* for tobacco was created in 1582 by Adam Lonitzer (Lonicerus) for his "Kreüterbuch" (herbal) in honour of Jean Nicot de Villemain (1530–1600), a French ambassador in Portugal. There he became acquainted with tobacco plants – imported from the New World to Portugal – in 1560. Nicot de Villemain took plants with him to the court in Paris where they were cultivated; it is disputed whether these plants belonged to *N. rustica* (Wolters 1994) or to *N. tabacum* (Austin 2004). However, this was only one way for the rapid introduction of tobacco throughout Europe. Later *Nicotiana* was taken by Carl von Linné (Linnaeus) as the genus name (Linné 1788). The terms of the alkaloids of group (I) were apparently chosen following the genus name due to their discovery except myosmine, so called due to its strong smell of mice according to their Greek term

(Späth et al. 1936). However, those of group (II) were named according to the original detection of anabasine in *Anabasis aphylla* L., Chenopodiaceae (Orechoff 1929; structure elucidation: Orechoff and Menschikoff 1931, 1932). By the way, this species does not produce nicotine itself but if this alkaloid is fed to the plant it is transformed into anabasine (Lovkova et al. 1994). Curiously ana**tab**ine (group III) got its name "to indicate its origin from (the German word for tobacco) "**Tab**ak" (Späth and Kesztler 1937a) since anabasine had been already known as a *Nicotiana* alkaloid, too (Späth and Kesztler 1937b).

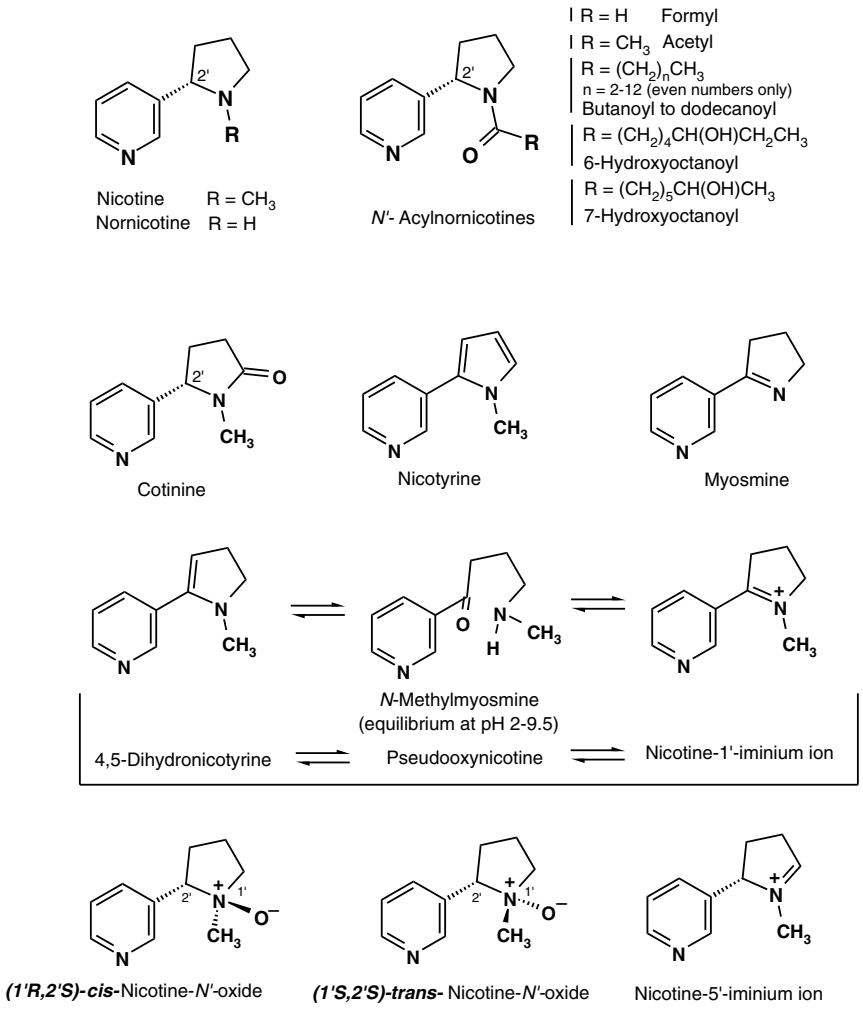


Fig. 3.8 Pyrrolidine-type nicotinoids; the structural changes of *N*-methylmyosmine based on pH are highlighted by the bracket (Maeda et al. 1980)

Nicotine is the principal alkaloid in commercial tobacco, *N. tabacum*, usually accounting for >90% of the alkaloid fraction whereas nornicotine, anabasine, and anatabine seldom accumulate to >5%. Further nicotinoids are present only in very small concentrations in tobacco (Bush et al. 1999). The unusually high proportion of the principal alkaloid had been the reason why it needed one century before the first pure minor alkaloid could be isolated though an early report of Pictet and Rotschy (1901) which had described some minor components as, e.g., "nicoteine", "nicotimine". However, these compounds later turned out to be mixtures. Thus, Ehrenstein (1931) reported that he had been able to resolve "nicoteine" into two fully individual alkaloids, *l*-nornicotine and "anabasine". According to Späth et al. (1935) this nornicotine probably contained only about 30% of the *l*-form together with 70% *dl*-nornicotine. This group could isolate pure *l*-nicotine [today *S*-(−)-nicotine] from tobacco for the first time (Späth and Zajic 1935). In this chapter, the natural form is referred to as nicotine (in traditional references: *l*-nicotine) unless otherwise noted. *l*-nornicotine [today *S*-(−)-nornicotine] could be detected as the predominating alkaloid in *N. sylvestris* with nicotine as a minor congener (Smith 1937). However, its enantiomeric form, *d*-nornicotine [today R-(+)-] as well as nicotine were reported to be constituents of leaves and twigs of *Duboisia hopwoodii,* a plant which is chewed as 'pituri' by the Aborigines in Australia (Hicks et al. 1935; Hicks and LeMessurier 1935; Späth et al. 1935; Hicks 1936). Späth and Zajic (1935) found out that the nornicotine of this species contained 43% of the active (*d*-) form and 57% of the racemic (*dl*-) form. The question if the active form is racemized by the isolation procedure or if the enantiomeric form is also present as such in the plant, was decided in favour of the latter (Späth and Kesztler 1937b). Plants are apparently able to synthesize both *d*-form and *l*-form of nornicotine, thus yielding in part racemates whereas nicotine present in tobacco is >95% *S*-(−)-nicotine (Bush et al. 1999). Interestingly, demethylation of the latter by cell cultures yielded only *S*-(−)-nornicotine (Hao and Yeoman 1996a). This was also true for *N. glauca* and *N. otophora* fed with *S*-(−)-nicotine. However, in longer term in vivo experiments a partial racemization of the nornicotine was measured in plants (Bush et al. 1999 and references therein).

"Anabasine-Ehrenstein" turned out to be *l*-anatabine [today: *S*-(−)-anatabine] (Späth and Kesztler 1937a) though *l*-anabasine [today: *S*-(−)-anabasine] is also a constituent of *N. tabacum* (Späth and Kesztler 1937b). Anabasine isolated from *N. alata* was also the almost pure *S*-form in contrast to *N. glauca* (both enantiomers of anabasine present) (Friesen et al. 1992); [*N. glauca* (tree tobacco) contains this alkaloid as the principal one (Dawson 1945)]. Regardless of the tobacco type, anabasine always had the highest relative percentage of the minor *R*-(+)-enantiomeric form (40–46%), i.e., the lowest enantiomeric excess, compared with nornicotine, anatabine, nicotine (Armstrong et al. 1999). The latter alkaloid had the highest enantiomeric excess. Furthermore, *l*-*N*-methylanabasine and *l*-*N*-methylanatabine (Späth and Kesztler 1937c) as well as 2,3′-dipyridyl (Späth and Zajic 1936) were discovered as minor tobacco alkaloids. Three further dipyridyl (= bipyridine) position isomers (2,4′-, 3,3′-, 3,4′-) are known as constituents of *N. tabacum* (Nytredy et al. 1986 and references therein). However, 2,3′-dipyridyl is the isomer

most frequently detected in the literature, probably due to its close relationship to anatabine (see Fig. 3.9). 5-Methyl-2,3′-dipyridyl was also proved as a genuine constituent of *N. tabacum* (Warfield et al. 1972).

Nicotelline, discovered very early and already assumed to be a terpyridyl at that time (Pictet and Rotschy 1901), indeed turned out to be 2,4-di-(ß-pyridyl)-pyridine (= 3″,4-pyridyl-2,3′-dipyridyl) (Kuffner and Faderl 1956). Congeners of similar, however partially hydrogenated structure, anatalline and anabasamine, respectively, could be isolated from the roots of *N. tabacum* (Kisaki et al. 1968; Warfield et al. 1972, respectively). Anatalline [2,4-di(3-pyridyl)piperidine] was shown to be accumulated in two isomeric forms, *cis* and *trans*, respectively, in *N. tabacum* cv. by-2 cell cultures (Haekkinen et al. 2004). Anabasamine is also a constituent of *Anabasis aphylla* (Chenopodiaceae) as can be assumed already from its name (Leete 1983).

It was not always clear whether a series of nicotinoids found in aged or cured tobacco leaves had already existed in the living plant or had been produced in the fermentation process (e.g., Wenusch 1935; Leete 1983). However, it could be proved that myosmine, *N*-methylmyosmine, nicotyrine (syn: β-nicotyrine), cotinine, and *N*-methylnicotinamide are already present in green freeze-dried leaves of Virginia tobacco (Wahlberg et al. 1977; Enzell et al. 1977). Myosmine was also isolated from *N. glutinosa* at the flowering stage (Kisaki and Tamaki 1966). Cotinine, famous as an important metabolite of nicotine in humans (Langone et al. 1999), was detected again in *N. glutinosa* (Leete 1983). Both nicotine-1′-*N*-oxides (*trans* and *cis* isomers due to the chiral nature of the oxidized *N*′) were identified in all vegetative organs of *N. affinis*, *N. sylvestris*, and *N. tabacum* (Phillipson and Handa 1975a).

Nicotinoid Content of Cured Tobacco Leaves and Tobacco Smoke. Of course there are differences concerning the qualitative alkaloid profile of green tobacco leaves, cured leaves, and tobacco smoke. This is also true from the quantitative point of view. Thus, due to enzymatic transformation during senescence and air-curing, e.g., the nicotine content may be reduced in favour of an increased amount of nornicotine. This may happen even to an extreme extent. Due to individual genetic conversion so-called "converters" are able to metabolize leaf nicotine to its nor congener up to 95% (Siminszky et al. 2005 and references therein). This happens more frequently in burley cultivars than in flue-cured tobaccos. Moreover, aging and flue-curing turned out to lead to a reduction on the concentrations of minor nicotinoid components. There are detailed reviews summarizing such chemical changes including probable degradation products like pyrrole and pyrrolidine derivatives (Enzell et al. 1977; Long and Weybrew 1981; Burton et al. 1983; Baker 1999). Due to pyrolysis smoke contains, e.g., much less nicotine and nornicotine, both degraded in part to substituted pyridines which contribute to smoke flavour; furthermore, nornicotine is converted to myosmine (Weeks 1999).

***N*′-Acylated Nicotinoids (Fig. 3.8, Table 3.3).** The usual nicotinoids are classified as (relatively) weak bases which nevertheless are still able to form stable salts with organic as well as mineral acids due to their pyrrolidine nitrogen (Crooks 1999).

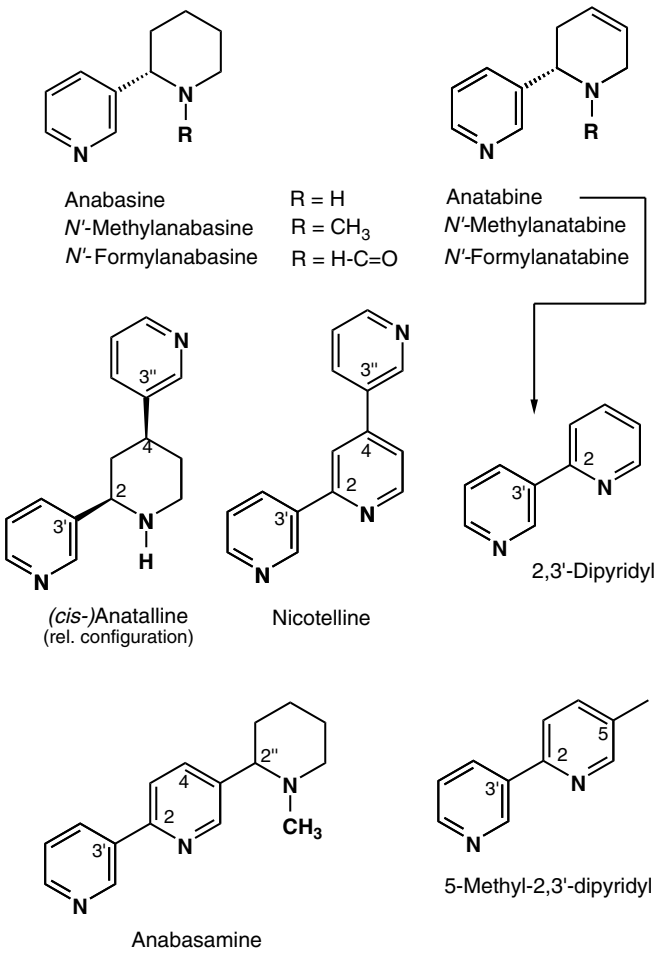


Fig. 3.9 Nicotinoids of the piperidine-, Δ^4-piperideine-, dipyridyl-, and tripyridyl-type as well as partially hydrogenated derivatives of the latter

N′-Acylnornicotines or -anabasines/-anatabines form a unique group of minor *Nicotiana* alkaloids whose pyrrolidine or piperidine/-piperideine nitrogen is not basic due to their *N*′-acyl moiety. They only share the less basic pyridine nitrogen with the latter group. Thus, the *N*′-acylated nicotinoids are still much weaker bases than, e.g., nicotine, nornicotine, anatabine. Therefore, they cannot be recovered from the so-called base-fraction, as can the classical nicotinoids (Zador and Jones 1986). This might have been one reason why the *N*′-acyl derivatives were discovered comparably late. The simple formyl and acetyl derivatives of nornicotine were detected in tobacco (Bolt 1972; Warfield et al. 1972); the latter later turned out to be a common trait in the genus *Nicotiana*. The *N*′-formyl derivatives of anabasine (0.0002%) and anatabine (0.01%), respectively, were detected in aged burley tobacco (Miyano et al. 1979). *N*′-Formylanatabine and its acetyl congener were proved also in green leaves

of *N. tabacum* though in very low concentrations (Burton et al. 1988; Andersen et al. 1989). This indicates that such compounds are normal products of plant metabolism during late growth.

N′-n-Butanoylnornicotine (Matsushita et al. 1979), its *n*-hexanoyl and *n*-octanoyl congeners (Bolt 1972) as well as the 6-hydroxy and 7-hydroxy derivatives of the latter (Miyano et al. 1981) were isolated from flue-cured leaves of *N. tabacum*; further homologues up to *N′-n*-dodecanoylnornicotine including certain mono-hydroxy derivatives were also identified in tobacco (Crooks 1999). Later such compounds turned out to be normal products of plant metabolism during late growth and post-harvest air-curing (Andersen et al. 1989). However, such alkaloids are present in the plants in very low concentrations, e.g., in mature green leaves of certain cultivar (NC 95) isolines the acylnornicotine fractions comprised only 0.04–0.09 mg/g dry weight; the amount increased during curing (0.06–0.33 mg/g) (Djordjevic et al. 1990). In alkaloid lines of burley tobacco they were found to occur in the following order of decreasing content: Formyl- > *n*-octanoyl-> *n*-hexanoyl- > acetyl- > *n*-butanoyl-nornicotine (Andersen et al. 1989). Furthermore, some exotic congeners were discovered in flue-cured tobacco: (i) *N′*-carbethoxynornicotine, later found as a metabolite of nornicotine in cell suspension cultures of *Nicotiana plumbaginifolia* (Bartholomeusz et al. 2005), and (ii) *N′*-[4-(dimethylamino)butanoyl]nornicotine (cv. BY-260-9: Matsushita et al. 1979) as well as (iii) *N′*-nitrosonornicotine (burley lines). The latter turned out to be a product present mainly during curing and could only be detected in trace quantities (<0.005 mg/g) during plant growth (Andersen et al. 1989). *N′*-Nitroso derivatives of natural nicotinoids and their degradation products formed during curing are reported to have significant tumorigenic activity (Bush et al. 1993).

Three wild species of the genus *Nicotiana*, section *Repandae* (*N. repanda*, *N. stocktonii*, *N. nesophila*) were found to produce *N′*-acyl- as well as *N′*-3-hydroxy-acylnornicotines with an even longer-chain acyl group (C_{12}–C_{16}) as part of the leaf surface lipids besides duvatrienediols (Sect. 7.4.1) and hydrocarbons (Zador and Jones 1986; Huesing and Jones 1987; Matsuzaki et al. 1988; Severson et al. 1988a, 1988b). The chain of these acylnornicotines showed – typical of fatty acids – normal and methyl-branched structure, respectively (Table 3.3). The hydroxylated compounds are characterized by different chain length and branching pattern than their congeners lacking a hydroxyl group. The main component was identified as *N′*-(3-hydroxy-12-methyltridecanoyl)nornicotine [syn.: *N′*-(3-hydroxy*iso*tetradecanoyl)nornicotine]. Moreover, the corresponding derivative of anatabine was detected as a minor component. Apparently there is specificity for accumulation of *N′*-acylnornicotines vs *N′*-acylanatabines at least in burley tobacco, even though the concentrations of nornicotine and anatabine are equivalent (Burton et al. 1988). Whether this is also true for the species of the section *Repandae* remains to be elucidated since they are reported to have significant concentrations of nornicotine and only small proportions of anatabine (Saitoh et al. 1985). It was found that the *N′*-acylnornicotines of Table 3.3 show 2*S′*-configuration like nornicotine itself; this is also true for their *iso* congeners. Stereochemical details concerning the configuration of their acyl chain (3-OH, methyl branch) are not available because the 3-hydroxy derivatives were characterized only by GC/MS analysis.

The acyl derivative profile in total was quite similar in the three *Repandae* species (Huesing et al. 1989). The occurrence of such metabolites is of chemotaxonomic relevance for this section, since they turned out to be absent from 65 other *Nicotiana* species (Huesing and Jones 1987).

3.3.2 Occurrence in the Solanaceae (Tables 3.1 and 3.4)

Our knowledge of the occurrence and distribution of nicotinoids is based on (i) traditional isolation and structure elucidation procedures until the 1960s and (ii) in more recent times on different chromatographic methods, predominantly GC/MS analysis.

3.3.2.1 Nicotianoideae

The *accumulation* of *large* amounts of nicotine and/or its congeners is confined to four solanaceous genera belonging to two clades of the subfamily **Nicotianoideae** (Nicotianeae clade: *Nicotiana*; Cyphanthera clade: *Crenidium*, *Cyphanthera*, *Duboisia*).

Nicotianeae clade. *Nicotiana* is the fifth largest solanaceous genus and one of the most comprehensively studied flowering plant genera at all. It comprises 77 naturally occurring species [geographic distribution: America 49 spp., Australia 25 spp., Pacific islands (S-Melanesia) 1 sp., Namibia (SW-Africa) 1 sp., cultivated almost worldwide 1 sp.] (Chase et al. 2003 and references therein). Inferred from extensive phylogenetic analyses with multiple plastid DNA regions including 75 naturally occurring species, the genus is assumed to have evolved in southern South America east of the Andes and later dispersed to Africa, Australia, and southwestern North America (Clarkson et al. 2004). According to Goodspeed (1954) who recognized 60 species (several new species have been described since) the genus is divided into three subgenera (*Rustica, Tabacum, Petunioides*) and subdivided into 14 sections (see Table 3.4). None of these subgenera turned out to be monophyletic in a comprehensive phylogenetic study of the ITS nrDNA involving 66 species (Chase et al. 2003). However, most of these sections were coherent, others clearly polyphyletic. The genus as a whole seems to be monophyletic though this is supported in the analysis only in a limited manner (bootstrap percentage: 71). However, the least diverged taxa from the *Nicotiana*, the genera of Anthocercidae are more divergent from any species of *Nicotiana* than any of the latter is from other congeneric species. This fact is interpreted by the authors as a further support that *Nicotiana* is monophyletic. One year later the same authors published a new sectional classification of the genus based on the data from the former study (Knapp et al. 2004). Most of Goodspeed's sections are upheld by molecular analysis. Several species have been transferred to other sections. Only naturally occurring species have been included. The new classification is integrated in Table 3.4.

As already mentioned, most of the commercial tobaccos produced in the world belong to *Nicotiana tabacum* L., which is assumed to be an allotetraploid, natural hybrid of two wild species, *N. sylvestris* (maternal genome) and *N. tomentosiformis* (paternal genome), also supported by phylogenetic molecular analysis based on ITS regions of nuclear ribosomal DNA (Chase et al. 2003). Alternatively, though less likely the latter is assumed to be *N. otophora*. However, a molecular analysis based on five genes encoding putrescine *N*-methyltransferase supported the hypothesis of Kenton et al. (1993) that the progenitors are *N. sylvestris* and an introgressed hybrid between *N. tomentosiformis* and *N. otophora* (Riechers and Timko 1999 and references therein). There are innumerable cultivars, e.g., over 1500 entries in a United States Department of Agriculture inventory. The only other species used on a limited scale is *N. rustica* (Tso 1999), which is assumed to be a hybrid of *N. undulata* and *N. paniculata* (Wolters 1994; Hänsel 2004) though *N. knightiana* instead of *N. paniculata* might be an alternative candidate; both belong to the section *Paniculatae* (Chase et al. 2003). Tobacco is the most widely grown commercial non-food crop in the world, produced in at least 117 countries. In 2004, total world production of tobacco leaves was estimated at 6.5 million metric tons (FAO, statistical data bases). The tremendous commercial importance of tobacco plants has led to an unusual knowledge about their secondary metabolites: About 3000 constituents were identified and characterized in tobacco leaf and some 4000 in smoke. The decisive constituent, nicotine, ranges in concentration from 0.5 to 8% (dry weight) in the major cultivated tobacco species, *N. tabacum* and *N. rustica* (Enzell et al. 1977; Leffingwell 1999). However, there are also reports on cultivars with even higher proportions, e.g., *N. tabacum* up to 10% and *N. rustica* up to 18% (Wolters 1994).

It seemed reasonable to suppose that other, wild species of the genus are also able to synthesize nicotinoids. Furthermore, such wild species might be useful for improving tobacco germplasm. Thus, early studies led to reports on the occurrence of nicotine and its congeners already since the forties of the past century (e.g., Jackson 1941; Shmuk and Borozdina 1941; Smith and Smith 1942). Two extensive studies on 60 and 64 species, respectively, based on capillary GC, clearly demonstrated that the occurrence of nicotinoids is a consistent trait in the genus *Nicotiana* (Saitoh et al. 1985; Sisson and Severson 1990). Both reports included the same 60 species with additional four in the second one (*N. thyrsiflora*, *N. palmeri*, *N. sanderae*, *N. linearis*). They represented all 14 sections of the genus recognized at that time. The first report was focused on separated samples from leaves and roots; the second one compared samples from greenhouse and field-grown plants. These two reports show remarkably corresponding results concerning the alkaloid profile. Nicotine and nornicotine have been detected in all 60 of the common species of both reports, anabasine and anatabine in almost all of them; these four metabolites are documented with their relative percentage composition in every species. This refutes the observations of Smith and Abashian (1963), who found nicotine and nornicotine to be completely absent in some *Nicotiana* species. The diverging results were achieved apparently due to the different sensitivity of the methods used (GC vs TLC). Anabasine could not be detected in samples of *N. sanderae* and *N. linearis*, possibly due to the very low total alkaloid content of both species (falling below the

analytic detection limit). This is also true for anatabine and *N. linearis*. Additionally the second report included data on the presence of two minor pyridylpyrrolidines: myosmine, detected in only six species (<1%), and *N′*-acetylnornicotine, detected in 45 species (70%).

Table 3.4 shows the combination of the data of both reports mentioned above. Nicotine turned out to be a major alkaloid in 54 *Nicotiana* species (84%) and the principal component in 28 species (44%). Nornicotine was a major component of 32 species (50%) but predominating, i.e., without nicotine or another compound as a second main alkaloid, in only eight species (12%). With the exception of *N. alata*, *N. maritima*, and *N. africana*, the concentrations of nicotine surmounted those of nornicotine in the roots of all species (95%). However, this was the case in only 36 out of 60 species (60%) for the leaves. (See also Sect. 3.3.4).

There were only three species (5%) with anabasine as the principal alkaloid (*N. debneyi*, *N. glauca*, *N. petunioides*) though it was at least one of two or three major alkaloids (nicotine and/or nornicotine) in another 13 species (20%). However, considering only the roots anabasine was found predominating in 7 species (*N. glauca*, *N. solanifolia*, *N. benavidesii*, *N. cordifolia*, *N. debneyi*, *N. maritima*, *N. hesperis*). Ontogenetic variation of the alkaloid profile was observed for *N. glauca* (Lovkova et al. 1976). Nicotine was the main nicotinoid in 15-day-old seedlings whereas anabasine prevailed in 48-day-old plants. Anatabine turned out to be always a minor component with the exception of *N. otophora* where it represented the principal alkaloid in the leaves (roots: nicotine).

The total-alkaloid content in leaves (dry weight) varied in a wide range from 0.003% (*N. alata*) to 2.96% (*N. sylvestris*); the corresponding values in roots varied from 0.027% (*N. langsdorffii*) to 2.48% (*N. velutina*) (Saitoh et al. 1985). The total-alkaloid content of the roots was higher than in the leaves for 43 out of 60 species (72%) but there were very interesting exceptions, e.g., *N. sylvestris* (leaves: 2.96% vs roots: 0.786%), *N. attenuata* (2.227% vs 0.248%), *N. tabacum* (1.146% vs 0.218%), *N. langsdorffii* (0.258% vs 0.027%). In the roots of most species the concentrations of the two pyridylpiperidine-type nicotinoids were surmounting those of the leaves. However, there were some exceptions again, e.g., *N. glauca* or *N. tabacum* with higher concentrations of anabasine in the leaves than in the roots as well as of anatabine in *N. hesperis*.

Field-grown plants were found to contain significantly higher total-alkaloid levels than greenhouse plants, e.g. *N. rustica* 2.56% vs 0.51%. The range for the former turned out to be between 0.07% (*N. forgetiana*) and 2.87% (*N. arentsii*), for the latter between 0.10% (*N. forgetiana*) and 1.91% (*N. excelsior*). Remarkably, nicotine accounted for nearly the entire alkaloid fractions in samples with the highest total-alkaloid levels. There was only one exception, *N. noctiflora* with anabasine as the predominant alkaloid (total alkaloids: 1.70%). In both large reports the nicotine content of *N. sylvestris* was found to be much higher than the one of *N. tomentosiformis*. This is interesting because they are supposed to be the parents of *N. tabacum* as already mentioned. In the case of the presumable parents of the second cultivated tobacco species, *N. rustica*, there were diverging results with regard to the content of nicotine: *N. undulata* was in the lead according to the results

of Sisson and Severson; however, Saitoh et al. had opposite results, i.e., in favour of *N. paniculata*. As has been shown for *N. tabacum* nicotine is present in all parts of the plants including the generative organs like flowers (all parts, e.g., stigma, stamen, ovary, petal), seeds, immature and mature capsules (Saitoh et al. 1985).

Another study on leaves from 40 *Nicotiana* species should be mentioned which provides details on the occurrence of further congeners in addition to the main alkaloids (Sarychev and Sherstyanykh 1985). Accordingly, 2,3′-dipyridyl could be detected in 29, nicotyrine in 19, and *N*′-methylanabasine in 10 species. This study also comprised information on the occurrence of simple bases like pyridine, 3-acetylpyridine, 3-cyanopyridine, α-picoline, ß-picoline, quinoline, and isoquinoline. However, since these simple bases are typical pyrolytic degradation products of nornicotine and myosmine, respectively (Balasubrahmanyam and Quin 1962), it can be assumed that they may also have been artefacts in the study mentioned above.

Cyphanthera clade. This clade comprises three genera (*Crenidium*, *Cyphanthera*, *Duboisia*) endemic to Australia. The members of this clade are unique in that they accumulate nicotinoids as well as tropane alkaloids and – at least some of them – also simple pyrrolidine alkaloids (for details see Sects. 3.1.2 and 3.4.2, respectively, as well as Table 3.1). It is remarkable that neither tropanes nor simple pyrrolidines were ever found in the well-studied genus *Nicotiana*. The genus ***Duboisia*** comprises four species. They accumulate the alkaloids differently in the plant: In a first study on their location in *D. hopwoodii* it was reported that the tropanes are more or less restricted to the roots whereas the nicotinoids turned out to be concentrated in its leaves (Kennedy 1971). This contrasts with *D. leichhardtii* which accumulated the nicotinoids predominantly in its roots and tropanes in its leaves. The situation concerning *D. myoporoides* is more complicated due to the existence of three natural chemical varieties (chemovars), one of them with nicotinoids as dominant alkaloids (Mortimer and Wilkinson 1957). Finally, there is only one report on the tropane alkaloid content of the fourth species, *D. arenitensis* (Griffin and Lin 2000); whether this species is also able to synthesize nicotinoids is unknown. All chemovars of *D. myoporoides* are characterized by the occurrence of (i) tropane alkaloids (scopolamine, hyoscyamine), (ii) simple pyrrolidine alkaloids (hygrine), and (iii) nicotinoids (Griffin and Lin 2000). However, they differ in their dominant alkaloid, first discovered for varieties with different tropane alkaloids (Loftus Hills et al. 1954a). Seven decades after the discovery of tropane alkaloids in *D. myoporoides* (Gerrard 1880; Ladenburg 1880), nicotine (0.7–0.9% dry weight) and nornicotine (0.2–0.3%) were identified in the leaves of this species (cultivated from seeds from New Caledonia) besides scopolamine [syn.: Hyoscine; (0.25–0.55%)] (Loftus Hills et al. 1953). The isolation of nicotine and anabasine from a sample collected at a certain locality (Acacia Plateau) in the wild of South Queensland was documented (Mortimer 1957; Mortimer and Wilkinson 1957); these nicotinoids represent the principal alkaloids of the sample, thus establishing a novel chemovar. In a more recent study four leaf collections from natural stands of differing locations in Queensland representing high and low altitudes in semitropical and tropical regions were analyzed (Gritsanapan and Griffin 1991). The findings of

1957 could be confirmed. In contrast to the remaining three samples (major alkaloid: Scopolamine) the one from the Acacia Plateau could be characterized as a distinct nicotinoid ('pyridine') variety again. Finally, anatabine was also isolated from *D. myoporoides* cultivated in Japan (Kitamura et al. 1980). In a study on ontogenetic variations during the first year of growth only traces of nornicotine and anabasine had been detected in cotyledons; after two months the leaves contained nicotine, nornicotine, and anabasine; maximum of nicotine content was measured after four months. Afterwards it decreased until a constant value has been reached (Kitamura et al. 1985).

Nicotine content in the leaves of *D. leichhardtii* was reported to be very low; however, the roots contained relatively large concentrations (Kennedy 1971; Endo and Yamada 1985). The production of the nicotinoid has also been observed in different studies with root and cell cultures of this species (Kagei et al. 1980; Yamada and Endo 1984; Endo and Yamada 1985; Leete et al. 1990).

According to early monographs (Husemann et al. 1884 and references therein; Remington and Wood 1918), Gerrard and Petit, independently from each other (1879), had succeeded in isolating minute quantities of an alkaloid from the leaves of *D. hopwoodii*, the 'pituri' plant (see Sect. 3.4.6, "Ethnobotany"), which was named 'piturine'. Gerrard as well as Petit thought 'piturine' to be identical with nicotine. Though Ladenburg pointed out that the boiling point of 'piturine' and nicotine, respectively, was identical and even similar results in comparative pharmacological studies were obtained by Ringer and Murrell (Husemann et al. 1884 and references therein), other authors claimed to be sure that their results distinguish 'piturine' from nicotine, e.g., Senft (1911). However, the assumption of Gerrard/Petit could be confirmed by Rothera (1911) and Petrie (1917b) as well as by Hicks and Späth in their studies on nicotine and its nor congener in the thirties of the past century as already mentioned above (Sect. 3.3.1). Both alkaloids were detected as constituents in the leaves of a large number of specimens of *D. hopwoodii* by Bottomley et al. (1945). A comprehensive study of leaf and root collections of this species led, e.g., for samples from central Australia being used for the isolation of nicotine, nornicotine, myosmine, and *N*′-formylnornicotine as well as to the identification of cotinine, *N*′-acetylnornicotine, anabasine, anatabine, anatalline and "bipyridyl" (2,3′-dipyridyl) by GC/MS analysis from the leaves (Luanratana and Griffin 1982). The roots contained nicotine, nornicotine, and *N*′-formylnornicotine besides tropane alkaloids. The leaves may show large concentrations of nicotine (up to 5.3%) (Bottomley et al. 1945) and nornicotine (up to 4.1%), respectively. Both are potential principal alkaloids. This indicates that the leaves of *D. hopwoodii* are qualitatively and quantitatively equivalent to those of *Nicotiana tabacum* with regard to the nicotinoid profile. Empirical experience had led Australian aborigines during the nineteenth century to use the cured leaves of this *Duboisia* species for the preparation of a narcotic stimulant ('pituri'), applied preferentially as "chewing tobacco" (Watson et al. 1983; see also Sect. 3.3.6 "Ethnobotany and Ethnomedicine").

Nicotinoids were also identified as constituents of three ***Cyphanthera*** species (out of eight). Nicotine turned out to be the principal alkaloid of *C. tasmanica* in leaves and roots though tropane alkaloids were also present. Furthermore, it was a

major alkaloid in the leaves of *C. anthocercidea* accompanied by nornicotine and anabasine as well as tropanes. Nornicotine and anabasine were the alkaloids which could be characterized in the aerial parts of *C. racemosa* (no tropanes). However, the alkaloid content was rather low in this species (aerial parts: 0.01% compared with 0.21% and 0.17%, respectively, for *C. anthocercidea* and *C. tasmanica*). A further species, *C. frondosa*, also capable of synthesizing nicotine and anabasine, is supposed to be a hybrid of *C. albicans* and *Duboisia myoporoides*. Since *C. albicans* itself had turned out to be a nicotinoid-negative species the capability of *C. frondosa* to synthesize these alkaloids might be a heritage of its second (nicotinoid-positive) parent. Anabasine was the sole nicotinoid (besides tropanes) of the monotypic genus ***Crenidium***, represented by *C. spinescens* (Evans and Ramsey 1983; El Imam and Evans 1984).

3.3.2.2 Other Subfamilies

There are some reports on the erratic occurrence of nicotine or certain congeners in the remaining solanaceous subfamilies. This is the case for *Petunia violacea*, **Petunioideae** (minute concentrations).

Cestroideae. Nornicotine (major alkaloid), nicotine, and anabasine were detected in *Salpiglossis sinuata* (Salpiglossis clade) (Schröter 1958, 1963). The same alkaloids were found as constituents of *Streptosolen jamesonii* (Browallieae clade) (Schröter 1963). Nicotine and its nor congener were isolated also from the leaves of *Cestrum diurnum* and *C. nocturnum* (Cestreae clade), in addition cotinine and myosmine from the latter species only. This was the first report on the occurrence of cotinine and myosmine outside of the genus *Nicotiana*. However, all these alkaloids were present in such small concentrations that the toxicity attributed to both *Cestrum* species should be based on different constituents (steroidal saponins, see Sect. 7.7) (Halim et al. 1971).

Solanoideae. Furthermore, there are some reports on the occurrence of nicotine in this subfamily in extremely low concentrations, e.g., 0.0005–0.002% in dried leaves of tomato, *Solanum lycopersicum* sub nom. *Lycopersicon esculentum* (Solaneae clade) (Wahl 1952), of deadly nightshade, *Atropa belladonna* (Hyoscyameae clade), of three *Datura* spp. (Datureae clade) (Wahl 1953). Alternatively, it has been discussed that these previous data might reflect a contamination of the samples (e.g., due to smokers in the laboratories) instead of genuine production in the plant or a wrong identification (Gemeinholzer and Wink 2001). Recently, the nicotine content of edible nightshades, fruits of peppers and pepperonis (*Capsicum annuum*, Capsiceae clade), tomatoes (*Solanum lycopersicum*), aubergines (*S. melongena*) and tubers of potatoes (*S. tuberosum*), Solaneae clade, has been determined (Siegmund et al. 1999). The authors had paid special attention to the avoidance of any contamination with environmental nicotine, e.g., tobacco smoking. Thus, their results may be interpreted as *genuine* occurrence of nicotine in all these species. The GC/MS-detected the presence of nicotine in flowers of

Brugmansia candida was supposed to be at least in part – beside tropanes – responsible for the addiction induced by consumption of flowers by humans (Gambaro and Roses 1989). Finally, *Withania somnifera* (Physaleae clade, Withaninae subclade) is reported to contain nicotine (Majumdar 1952, 1955).

The unequivocally nicotine-positive GC/MS results with numerous convolvulaceous species (Sect. 3.3.3) may be an indication for a *genuine* distribution of this plesiomorphic character also throughout the Solanaceae though only in minute concentrations. Another indication might be that all solanaceous taxa mentioned above to contain small amounts of nicotine have turned out to synthesize also calystegines (see Sect. 3.5). Nicotine and calystegines share the *N*-methyl-Δ^1-pyrrolinium cation as a precursor in their biosynthetic pathway. The fact that *Nicotiana tabacum* is lacking calystegines is not inconsistent with this indication; apparently tobacco has lost this plesiomorphic characters as it has lost the ability to synthesize simple pyrrolidines.

Chemotaxonomic Relevance. The monotypic Nicotianeae subclade and the Australian endemic traditionally recognized tribe Anthocercideae G.Don form a sister pair (Garcia and Olmstead 2003 and references therein; Clarkson et al. 2004). This tribe is identical with the traditionally recognized subfamily **Anthocercidoideae** (G.Don) Tétényi used by Hunziker (2001). The Cyphanthera subclade is the most advanced of this tribe/subfamily, thus showing a maximum distance to the Nicotianeae subclade within the **Nicotianoideae** clade (see Fig. 2.2). Nevertheless, the occurrence of nicotinoids in combination with their accumulation seems to be a consistent trait of these both subclades involving *Nicotiana* on one hand as well as *Crenidium*, *Cyphanthera*, and *Duboisia* on the other. In contrast the remaining monotypic subclades between the Nicotianeae subclade and the Cyphanthera subclade, i.e., those involving the phytochemically well-checked genera *Symonanthus*, *Anthocercis*, *Grammosolen*, and *Anthotroche*, apparently lack nicotinoids. This may be interpreted as a loss of the ability to synthesize them, since they may be considered unequivocally as plesiomorphic characters present also, e.g., in the sister family Convolvulaceae.

Based on the results of their study already reported above, Saitoh et al. (1985) concluded that no clear-cut correlation between alkaloid pattern and classification of the genus *Nicotiana* exist. In contrast to this conclusion Sisson and Severson (1990) interpreted their results, also already mentioned above, as evidence for the association between alkaloid characteristics and the phylogenetic classification of the species in the genus. Thus, in the section *Paniculatae* (data for six species) total alkaloid levels were in the medium range relative to the other sections. Five species in this section produced mainly nicotine. The species in the section *Tomentosae* (data for five species) turned out to be characterized by low and medium total alkaloid levels and nornicotine was the predominant alkaloid in all five species. The section *Alatae* (data for seven species) showed mainly very low total alkaloid levels and nicotine as the principal alkaloid. Section *Noctiflorae* (data for three species) was characterized by the predominance of anabasine. The largest section *Suaveolentes* (data for 22 spp.), almost exclusively of Australian origin, could be

divided in three groups of species according to their different distribution in (i) the arid central and northern regions with high total-alkaloid levels and nicotine as the principal alkaloid, (ii) the south-central and eastern regions with total-alkaloid levels in the medium range and nornicotine as the predominant alkaloid, and (iii) the western regions with low total-alkaloid levels and nicotine as well as nornicotine making up the largest proportion.

The occurrence and distribution of nicotinoids in the **remaining subfamilies of the Solanaceae** did not offer further chemotaxonomic results of significance. This is not very surprising due to the fact that their biosynthesis is very close to the primary metabolism. Consequently, the simple occurrence of nicotinoids is of no chemotaxonomic or phylogenetic relevance as has been documented by plotting their occurrence on a molecular phylogenetic framework of the solanaceous genera (Gemeinholzer and Wink 2001) in contrast to their *accumulation* in certain taxa.

3.3.3 Occurrence in the Convolvulaceae (Table 3.2)

Our knowledge of the occurrence and distribution of nicotine in the Convolvulaceae is strictly based on GC-MS results. It could be detected in 99 out of 150 convolvulaceous species (66%) throughout the family in almost all tribes, genera and even sections of large genera (*Ipomoea*, *Merremia*) included. However, in almost every case it was a minor component, present only in traces. There have been only a few exceptions. Nicotine has been reported to amount 8% to the total alkaloid content of *M. quinata* (epigeal vegetative parts) (Jenett-Siems et al. 2005b). It was the principal ornithine-derived alkaloid (minor components: hygrine, cuscohygrine) in the epigeal vegetative parts of *Argyreia nervosa*. This is also true for *Ipomoea alba*, *I. indica*, and *I. pes-tigridis*. However, the concentrations were comparably low. Congeners of nicotine could not be found apart from odd exceptions: nornicotine, myosmine (*A. nervosa*) and cotinine (*I. indica*). These nicotinoids co-occurring with nicotine itself were present only in even lower concentrations (Eich, unpublished results).

3.3.4 Biosynthesis of Pyrrolidine-Type Nicotinoids (Fig. 3.10)

Nicotine is synthesized mainly in the roots (>97%) as has been proved, e.g., for *N. tabacum* and *N. rustica* (Dawson 1941; Dawson and Solt 1959). Besides its accumulation in the roots it is transported to the shoots in the xylem stream (Baldwin 1989). There it is accumulated especially in young leaves, stems, and reproductive organs as a defence agent. One of its precursors, the *N*-methyl-Δ^1-pyrrolinium cation is also synthesized in the roots. The formation of this cation has been explained already before (Fig. 3.2).

The second precursor, nicotinic acid, necessary for the pyridine moiety is formed in the pyridine nucleotide cycle which is also yielding the coenzymes nicotinamide-adenine-dinucleotide (NAD) and NADP, respectively, in all organisms. This cycle is fed by quinolinic acid (pyridine-2,3-dicarboxylic acid) which is formed by cyclization of L-aspartic acid and 3-phosphoglyceraldehyde as has been discovered during research work with tobacco (Leete 1983 and references therein). This is of general significance since it turned out to be a new biosynthetic pathway to the ubiquitous nicotinic acid which is the route of plants in contrast to the route of mammals (degradation of L-tryptophan). Quinolinic acid is introduced into the pyridine nucleotide cycle by decarboxylation at C-2 and by the simultaneous formation of an *N*-glycosidic linkage yielding nicotinic acid mononucleotide. This reaction is catalyzed by quinolinic acid phosphoribosyltransferase (QPT) whose activity turned out to be very high in *Nicotiana* roots in contrast to leaves (Mann and Byerrum 1974), more evidence for the location of the biosynthesis of nicotine in the roots. QPT is considered to be the main regulatory enzyme for nicotinic acid production in nicotine biosynthesis (Bush et al. 1999 and references therein). Nicotinic acid can be considered as the aglycone of this *N*-glycoside; the free acid may be formed by an immediate hydrolysis (catalyzed by nicotinic acid mononucleotide glycohydrolase) as well as after going through the whole cycle via NAD (Bush et al. 1993, 1999 and references therein). Nicotinic acid had been first

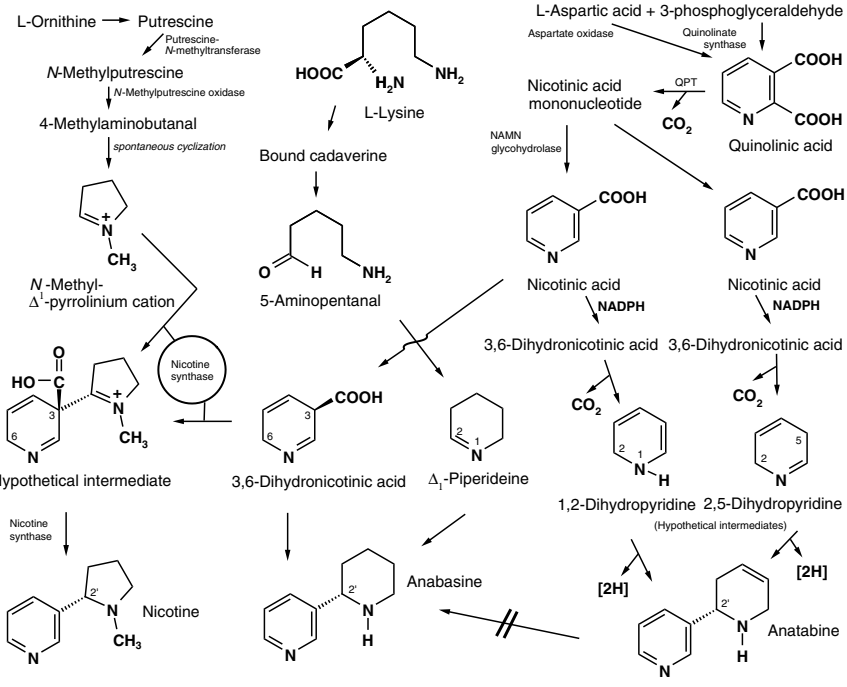


Fig. 3.10 Biosynthesis of the major tobacco alkaloids nicotine, anabasine, and anatabine

obtained in the nineteenth century by oxidation of nicotine during attempts to elucidate the structure of the alkaloid. It had been recognized already at that time as a pyridine carboxylic acid by Laiblin (1877, 1879). Based on the fact that this acid is also a natural product in plants discovered as a constituent of rice bran (Suzuki et al. 1912), it was proposed as a likely precursor of nicotine in *Nicotiana tabacum* (Winterstein and Trier 1931). Thirty years later it could be confirmed that nicotinic acid can function as a precursor; isotopic labelled nicotinic acid supplied to root cultures of *N. tabacum* (Dawson et al. 1960) as well as to those of *N. glauca* (Solt et al. 1960) yielded substantial incorporation. In a cell-free system obtained from *N. glutinosa* the reaction between the *N*-methyl-Δ^1-pyrrolinium cation and nicotinic acid forming nicotine could be proved (Friesen et al. 1992 and references therein). It is assumed that nicotinic acid is first decarboxylated (Leete 1983). An enzyme system which catalyzes the oxygen-dependent release of the carboxyl group from nicotinic acid was found in the roots of *N. rustica* (Chandler and Gholson 1972). The point of attachment of the pyrrolidine ring to the pyridine skeleton turned out to be at C-3 which had been the point of attachment of the carboxyl group before (Scott and Lynn 1967). It was proposed that the nicotinic acid is activated by reduction to 3,6-dihydronicotinic acid (Leete and Mueller 1982; Leete 1983) which may be a genuine precursor to react with the *N*-methyl-Δ^1-pyrrolinium cation. Both last steps in nicotine biosynthesis, the condensation with this cation as well as the decarboxylation, are catalyzed by nicotine synthase (Friesen and Leete 1990). These reactions may proceed via 1,2-dihydropyridine [for comparison see biosynthesis of anabasine (Fig. 3.10)].

Nornicotine in *N. glutinosa* and in *N. glauca* is formed only in the leaves and at the expense of nicotine translocated from the roots as could be proved by reciprocal graft combinations with tomato (*Solanum lycopersicum*) shoots/roots (Dawson 1945). Already in this early report it was speculated that nicotine is converted to nornicotine "probably by transmethylation". However, according to a proposal of Leete (1977), *N*′-formylnornicotine may be formed by oxidation of the *N*-methyl group of nicotine followed by oxidation to nornicotine. Later a partial characterisation of nicotine *N*-demethylase from microsomes of *N. otophora* was documented. Demethylation was interpreted to be associated with cytochrome P-450 rather than achieved by transmethylation (Bush et al. 1999 and references therein). The enzyme turned out to be NADPH-dependent in cell-free preparations from cell cultures of *N. tabacum* (Hao and Yeoman 1996b). Recently, it has been proved that CYP82E4 is involved in the metabolic conversion of nicotine to nornicotine in tobacco (Siminszky et al. 2005).

Alternatively, it was proposed that *N*-formylnornicotine is formed by formylation of nornicotine (Burton et al. 1988). Such an acylation of nornicotine might be supported by the fact that similarities with the profile of the formation for *N*′-acetylnornicotine could be observed. Recently, this alternative mechanism was supported additionally by a study with cell suspension cultures of *N. plumbaginifolia* fed with [^{13}C,$^{2}H_3$-methyl]nicotine or [1′-^{15}N]nornicotine. It could be demonstrated that *N*′-formylnornicotine is not an intermediate in nicotine demethylation: (i) nornicotine turned out to be derived directly from nicotine and (ii) it was evident that *N*′-formylnornicotine was one metabolite of nornicotine (Bartholomeusz et al. 2005). The authors concluded that the most

probable mechanism is an oxidative elimination of the *N*′-methyl group of nicotine, i.e., via a putative *N*′-hydroxymethylnornicotine which could be spontaneously decomposed into nornicotine and formaldehyde. Possibly different mechanisms may occur in different *Nicotiana* species.

N′-Acylated nornicotines discovered in *N. tabacum* are distributed within the leaf matrix (Bush et al.1993). However, their more complex congeners discovered in the *Repandae* species are synthesized in the trichomes on the leaf surface as reported for *N. stocktonii* and "the novel alkaloid *N*-hydroxyacylnornicotine". The fatty acid composition was not elucidated for the first; apparently "the novel alkaloid" had been a mixture of congeners (Zador and Jones 1986) which could be separated and resolved soon (see Sect. 3.3.1). Its synthesis had direct connection with the pool of nicotine produced in the root, transported to the aerial parts, and converted in the leaf to nornicotine. The latter alkaloid appeared to be the substrate of an acyltransferase which apparently is not very specific with respect to the length of the chain. After acylation the derivatives were secreted immediately into the exudate by the glandular cells of the trichomes. The long chain fatty acids needed for the acylation are products of the chloroplasts (Stumpf and Jones 1963).

The minor pyrrolidine-type nicotinoids, i.e., cotinine, nicotyrine, myosmine, *N*-methylmyosmine, and the nicotine-*N*′-oxides, are formed by simple chemical oxidation of nicotine with oxygen in solution. Therefore it was assumed that the presence of these alkaloids in the living plant is not due to a formation catalyzed by enzymes (Leete 1983). Primary carbon oxidation of nicotine in plants leads to (i) nicotine-1′-iminium ion (*N*-methylmyosmine), (ii) nicotine-5′-iminium ion which may be a precursor of cotinine [this is assumed for the mammalian metabolism (Gorrod 1993)], and (iii) nicotine-*N*-methyleniminium ion which may be a precursor of nornicotine via *N*-hydroxymethylnornicotine [this is also assumed for the mammalian metabolism (Gorrod 1993)] (Bush et al. 1999). Though cotinine is derived from the oxidation of nicotine (Burton et al. 1988 and references therein), very little conversion of nicotine to cotinine was found when administered to the living *N. glauca* plant (Leete and Chedekel 1974). Myosmine was formed via nornicotine (Leete and Chedekel 1974; Bush et al. 1993; Bartholomeusz et al. 2005).

3.3.5 Biosynthesis of Piperidine-/Piperideine-Type Nicotinoids (Fig. 3.10)

The biosynthesis of the piperidine-type nicotinoid anabasine proceeds in an analogous manner to nicotine, with Δ^1-piperideine (instead of the *N*-methyl-Δ^1-pyrrolinium cation) as the counterpart of nicotinic acid/3,6-dihydronicotinic acid (Leete 1969). Consequently, L-lysine is the source of the piperidine ring instead of L-ornithine (Solt et al. 1960). Unlike the conversion of the latter to the *N*-methyl-Δ^1-pyrrolinium cation via free putrescine, the conversion of lysine to Δ^1-piperideine is assumed to proceed via a bound cadaverine (Friesen et al. 1992).

According to a proposal of Leistner and Spenser (1973) the sequence proceeds via the Schiff base between lysine and pyridoxal-5-phosphate (phosphate of vitamin B_6) and similarly bound cadaverine. Anabasine isolated from 2′-^{14}C, ^{13}C-labeled anatabine-fed plants turned out to be unlabeled indicating that anabasine was *not* formed by the reduction of anatabine (Leete 1979).

In contrast to anabasine both rings of *S*-anatabine are derived from nicotinic acid (Leete 1977). It has been proposed that the latter nicotinoid could be formed by reaction of C-3 of the 3,6-dihydronicotinic acid with C-6 of the 2,5-dihydropyridine yielded from another molecule of the 3,6-dihydronicotinic acid due to facile decarboxylation. This decarboxylation is plausible since it is a β-imino acid. An alternative, also hypothetical route includes the isomerization of 2,5-dihydropyridine by a tautomeric shift to its 1,2-congener which then would condense with 3,6-dihydronicotinic acid (Leete 1983). 2,3′-Dipyridyl was supposed to be formed by aromatization of anatabine (Burton et al. 1988). However, this dipyridyl isolated from *N. glauca* and *N. glutinosa* fed with labelled anatabine was not radioactive (Leete 1979). Therefore it was concluded that the former is an artefact produced by the oxidation of the latter (Fig. 3.9); this might be supported by the fact that 2,3′-dipyridyl is formed increasingly in drying leaves. Leete and Chedekel (1972) reported on an aberrant formation of (−)-*N*-methylanabasine from *N*-methyl-Δ^1-piperideinium chloride in *Nicotiana tabacum* and *N. glauca*.

3.3.6 Significance

3.3.6.1 Pharmacology and Toxicology

Nicotine is a very potent poison for most animals from protozoa to humans. The acutely fatal peroral dose for an adult is probably 60 mg (Taylor 1995) which is equivalent to the nicotine content of five cigarettes or one cigar. However, smoking results in a considerable decomposition of this alkaloid due to pyrolysis; furthermore, much of the remaining volatile nicotine is not absorbed due to exhalation. Its toxicity was proved scientifically during the ninetenth century based on experiments with many species from different classes (Husemann et al. 1884). Posselt and Reimann (1828), who had isolated nicotine first, had already studied its toxicity in fish, snails, rabbits, dogs, and humans. Certain congeners (nornicotine, anabasine) turned out to be similarly or even more acutely toxic depending on the species (Neuwinger 1996 and references therein). However, since the symptoms of nicotine/nornicotine/anabasine poisoning are similar to those of tobacco poisoning the knowledge of its toxicity must have been present since ancient times. Tobacco is one of the oldest of the New World cultigens (Schultes 1979) used as a stimulating agent for hedonistic purposes. Furthermore, it is the most important and most used plant of the American Indian shamans (Rätsch 2005). Its ethnomedicinal significance was also given since many centuries. [For details see below (“Ethnobotany and Ethnomedicine”)].

Acetylcholine is the neurotransmitter (natural ligand) at the binding sites of all synapses of the cholinergic system. As postulated by Dale (1914), this neurotransmitter has dual actions, muscarinic as well as nicotinic. Therefore these binding sites are subdivided into muscarinic acetylcholine receptors and nicotinic acetylcholine receptors. They are named according to muscarine, an alkaloid of the mushroom *Amanita muscaria* (L. ex Fr.) Pers. (Agaricales), and to nicotine, respectively. Both have turned out to be **specific** agonists each at the corresponding receptors. This is not surprising since both alkaloids show distinctive structural similarities to acetylcholine; the following are given for nicotine (Fig. 3.11):

- The positive charge of the quaternary nitrogen of acetylcholine is comparable to the protonated pyrrolidine nitrogen of nicotine (which interacts with nicotinic acetylcholine receptors in its *N′*-protonated form at physiological pH).
- The pyridine nitrogen of nicotine is an electron donor like the carbonyl function of acetylcholine.
- Both structural elements may show the same molecular distance (42 nm) as the corresponding elements of acetylcholine (30–45 nm, variable due to higher flexibility of the molecule) may take up (values from Neuwinger 1996).

The nicotinic receptor mediates neurotransmission (i) at the neuromuscular junction, (ii) at the autonomic ganglia of both sympathicus and parasympathicus, and (iii) at some sites in the central nervous system. Accordingly, nicotinic receptors of the skeletal muscle type (i) and the neuronal type [(ii) and (iii), respectively] exist; all are ligand-gated ion channels (influx of Ca^{++} and Na^{+}) in contrast to muscarinic receptors (G-protein-coupled) (for details see Wink 2000). Nicotinic acetylcholine receptors are pentamers composed of nonidentical subunits with a large diversity depending on the location throughout the body and on the species, respectively (Domino 1999). Nicotine exhibits its major actions initially by transient stimulation of autonomic ganglia of both sympathetic and parasympathetic nervous system, thus causing multiple effects, e.g., on the cardiovascular system or the gastrointestinal tract. Since it can stimulate and desensitize receptors dose-dependently the response represents the summation of stimulatory and – due to a more persistent depression of these ganglia – to inhibitory effects of the alkaloid (Taylor 1995). It is beyond the scope of this chapter to treat the very complex pharmacology of nicotine, which may be found in numerous reviews and textbooks

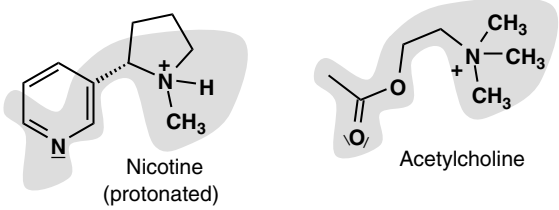


Fig. 3.11 Nicotine sharing structural similarities with acetylcholine, the physiological agonist of nicotinic acetylcholine receptors

(e.g., Taylor 1995; Domino 1999). Typical symptoms of acute intoxications with nicotine and tobacco are salivation and vomiting due to ganglionic stimulation, muscular weakness due to stimulation followed by depression at the neuromuscular junction, and, ultimately, clonic convulsions and cessation of respiration due to effects on the central nervous system (Klaassen 1995; Taylor 1995). Long-term poisoning by the abuse of nicotine promoting, e.g., arteriosclerosis, cardiovascular, and other diseases caused by the alkaloid itself (Dasgupta et al. 2006 and references therein), the carcinogenic potential of other tobacco smoke constituents left aside, leads to extremely important social problems of the nicotine/tobacco story all over the world. This is also true with respect to the addiction and the withdrawal syndrome (O'Brien 1995).

(+)-Nicotine, the synthetic, unnatural enantiomer was reported to be less potent and therefore also less toxic in almost all models checked than its natural antipode. It may possibly act at a site other than the nicotinic acetylcholine receptors, and induce desensitization (Brossi and Pei 1998 and references therein).

As already pointed out above (see Sect. 3.3.1) the pyrrolidine nitrogen is much more basic than the pyridine nitrogen with the consequence that only the former is protonated at physiological pH values. Nornicotine, anabasine, *N*′-methylanabasine, anatabine, and *N*′-methylanatabine also possess significant agonist activity at the nicotinic acetylcholine receptors in functional assays though with lower potency than nicotine (Crooks 1999 and references therein), e.g., *S*-(−)-nornicotine 4.5–73% of the effect of nicotine depending on the corresponding special receptor (different tissues, different species), anabasine 17–75% (Neuwinger 1996 and references therein). *R*-(+)-Nornicotine, the genuine alkaloid from *Duboisia hopwoodii*, was even reported to be 2.5 times more toxic than nicotine to rats (Hicks et al. 1935). These nicotine congeners exist also predominantly in their *N*′-protonated form at physiological pH values. Due to the oxidized C-2 position of cotinine forming a lactam moiety, thus neutralizing the originally basic pyrrolidine nitrogen, this alkaloid is not protonated and lacks agonist activity. This is of importance with the respect to the detoxification of nicotine in humans and many other species since cotinine is a major metabolite of nicotine (Gorrod and Schepers 1999; Langone et al. 1999). Cotinine can be excreted unchanged in most species though it may be oxidized in part (major urinary metabolite in humans: *trans*-3′-hydroxycotinine). Cotinine levels serve as useful objective biomarker of tobacco exposure, e.g., to distinguish smokers from non-smokers during screening for clinical trials (Bramer and Kallungal 2003) or to examine the reliability of self-reported smoking behaviour to avoid misclassifications of current smoking status in connection with disease risk (Lewis et al. 2003). It can be measured in urine, blood or saliva. The latter collection is favoured in nicotine treatment trials of smoking cessation because it is easy to obtain and non-invasive (Schneider et al. 1997). Anabasine and anatabine concentrations in urine turned out to be also useful for a validation of abstinence or measuring the extent of tobacco use (Jacob et al. 2002).

Numerous effects of nicotine, sometimes also including those of its congeners, on mammalian enzymes have been reported, e.g. competitive *inhibition* of certain enzymes of the glucocorticoid and sex steroid biosynthetic pathways, e.g., the

3α-hydroxysteroid dehydrogenase in canine prostate (Meikle et al. 1988), the human fetal adrenal mitochondrial 11β-hydroxylase as well as the microsomal 21-hydroxylase (Barbieri et al. 1989). The nicotine metabolite cotinine showed similar results with the exception of the inhibition of the 21-hydroxylase. Anabasine turned out to be the most potent inhibitor of these three nicotinoids in rat adrenal 11β-hydroxylase (Barbieri et al. 1987). *N′-n*-Octanoylnornicotine, a minor component of tobacco smoke, exhibited pronounced competitive inhibition of human placental microsomal aromatase, an enzyme responsible for the aromatization of androstenedione (converting this precursor to estrogens). Long-chain acylated nornicotines (optimum: C_{11}) showed much lower toxicity and significantly higher enzyme inhibition than nicotine and anabasine, respectively (Osawa et al. 1990; Bullion et al. 1991). Evaluation of nicotine and 13 of the most prevalent nicotine-related alkaloids and metabolites as inhibitors of human cDNA-expressed cytochrome P-450 2A6 (CYP2A6) mediated coumarin 7-hydroxylation indicated that β-nicotyrine inhibits this enzyme much more than nicotine, the second best inhibitor. β-Nicotyrine is also a constituent of tobacco smoke (Denton et al. 2004). An example for an *activation* of an enzyme by nicotine is the growth-promoting enzyme Janus kinase 2 in PC 12 cells (Shaw et al. 2003). Such results seem to be of significance with respect to the health-damaging aspects of tobacco smoke.

3.3.6.2 Therapeutics

From the modern medicinal and pharmaceutical point of view nicotine replacement therapy by patch [transdermal delivery systems (TDS) producing a steady blood level], chewing gum, lozenge (taken orally) or nasal spray is of significance in the treatment of the nicotine withdrawal syndrome for smokers wanting to achieve abstinence. Thus, the symptoms of nicotine withdrawal may be suppressed. However, this is only the pharmacological aspect of dependence. Therefore, additional psychological care is necessary (O'Brien 1995). Another indication for nicotine is the treatment of children suffering from the Tourette's syndrome, a neurological disorder characterized by persistent motor and phonic tics, combined with neuroleptics (Teuscher and Lindequist 1994 and references therein). However, it has been proposed that the anti-tic properties of nicotine might be due to antagonism of cortical 5-HT_{2A} receptors (Hayslett and Tizabi 2003, 2005). Furthermore, nicotine may have some beneficial effects as a neuroprotective agent in other disorders like Parkinson's disease and Alzheimer's disease (Lippiello et al. 1994; Rang et al. 1999). Nicotine improves cognitive functions like attention, concentration, and memory (Salin-Pascual et al. 2003). Further therapeutic potential of this alkaloid, perhaps as a lead structure for the development of new remedies is assumed for the treatment of ulcerative colitis as well as pain (Domino 1999). All these non-smoking related disorders are being evaluated. However, nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis (Heeschen et al. 2001). These effects are also mediated through nicotinic acetylcholine receptors. Such and other reasons, e.g. addiction, caution against the long-term use of nicotine. According to Jain (2001) "it is likely that the

balance between beneficial and detrimental effects of nicotine will depend upon the dose, schedule, duration of exposure, target tissue/cells and genetic profile of the host."

It is well-known that any drug might show certain more or less dangerous interactions with other drugs applied simultaneously to a patient. Recently, it has been reported that nicotine inhibits the welcome apoptosis (cell death) induced by the chemotherapeutic drugs gemcitabine, cisplatin, and taxol used to treat non-small cell lung cancer. This inhibition turned out to be caused by up-regulating XIAP (X chromosome-linked inhibitor of apoptosis) and survivin, two IAP (inhibitor of apoptosis) family proteins. The antiapoptotic effects were mediated by dihydro β-erythroidine-sensitive α3-containing nicotinic acetylcholine receptors (Dagupta et al. 2006 and references therein). This unwelcome protective effect of nicotine (simultaneously applied by smoking or as a therapeutic) against apoptosis induced by chemotherapeutic agents might be of considerable disadvantage for the treatment of tumour patients.

3.3.6.3 Ethnobotany and Ethnomedicine

Traditional **American Indian** rituals, in which psychoactive plants like "peyote" [*Lophophora williamsii* (Lemaire ex Salm-Dyck) Coulter, Cactaceae], "San Pedro cactus" (*Trichocereus pachanoi* Britton & Rose, Cactaceae), "ayahuasca" (*Banisteriopsis* C.B.Robinson & Small spp., Malpighiaceae), "ololiuqui" [*Turbina corymbosa* (L.) Raf., Convolvulaceae; see Sect. 4.2.] play an important role, almost always include smoking of large amounts of tobacco. It is assumed that the intensity of action of the sacred plants is maintained and/or increased by tobacco (Rätsch 1995 and references therein). It was and is still used in religious ceremonies to "travel to their respective master spirits on celestial bridges constructed by tobacco smoke" (Wilbert 1972). Furthermore, the use of tobacco is known as a traditional Indian remedy already in pre-Columbian times against all kinds of intoxications and bites (Rätsch 2005) as well as against various physical complaints and diseases, e. g., cold, fever, asthma, migraine, and different other kinds of pain (e.g., Wolters 1994 and references therein). "Tobacco was undoubtedly more widely used and for a greater variety of medicinal and magical purposes than any other plant" (Schultes 1979).

There are two main traditional tobacco product categories: (i) products that are smoked (cigarettes, cigars, cigarillos, pipes) and (ii) smokeless tobacco. Today the products of the former category are extremely used all over the world. The latter category includes products (snuff and chewing tobacco) that are sniffed in the nose, sucked or chewed in the mouth. Smokeless tobacco products are very popular still today in northern Europe (especially in Sweden), North America (especially in the United States), Africa, and Asia (especially in India and Pakistan) (Wahlberg 1999).

Nicotiana tabacum is assumed to have originated in north-western Argentina or nearby (Hunziker 2001), *N. rustica* in Peru (Wolters 1994). When the conquistadors arrived, cultivated tobacco was spread already throughout the New World, *N. tabacum*

primarily in South America and the West Indies and *N. rustica* in Mexico and North America (Schultes 1979 and references therein; Austin 2004 and references therein). In addition to these cultivated plants it must be pointed out that wild tobaccos were and are still also used since ancient pre-Columbian times by Central and South American Indian shamans during sacred magical ceremonies and also in medicinal contexts, e.g., leaves of *N. acuminata*, *N. alata*, *N. mangustifolia*, *N. glutinosa*, *N. paniculata*, *N. pusilla*, *N. repanda*. Often these species are more appreciated than cultivated tobacco. In North America only wild tobaccos have been smoked in prehistoric times. Seven *Nicotiana* species or so, e.g., *N. attenuata* (Coyote tobacco), *N. plumbaginifolia* dependent on the geographic distribution had been used by indigenous people before cultivation of *N. rustica* and *N. tabacum* arrived (Rätsch 2005 and references therein; Austin 2004 and references therein). Extracts of *Nicotiana* leaves were also observed as ingredients of curare blow-pipe dart poison of certain Indian tribes in Mexico (*N. obtusifolia*, *N. rustica*), Chile (*N. miersii*), and Brazil (*N. acutifolia*) (Neuwinger 1996 and references therein).

American ethnomedicinal applications have been adopted by **Europeans** in the seventeenth and eighteenth centuries though finally with low success. This is not surprising due to two main aspects: (i) the toxicity of tobacco is limiting every medicinal application and (ii) any traditional medicine is connected with its cultural roots and its potential success is not transferable easily to people with different cultural background. Tobacco arrived in **Africa** at the beginning of the seventeenth century where it served and still serves – besides for smoking as a stimulating agent for hedonistic purposes – as (i) a remedy in genuine African traditional medicine, often combined with other traditional medicinal plants, against different diseases (dependent on the country and/or tribe where it is applied), (ii) an insecticide, (iii) an ingredient of arrow poisons together with seeds of *Strophanthus* DC. spp. (Apocynaceae) for hunting (Cameroon, Nigeria), and (iv) especially in Nigeria as an additive to bait or pools in fishing in order to numb the fish (Neuwinger 1996 and references therein, 1998). Similar use as an ingredient of fishing or arrow poisons has been reported from traditional people in **Asian countries**, e.g., Cambodia, Indonesia, Japan, Vietnam (Lewin 1923; Neuwinger 1996, 1998 and references therein). The Portuguese introduced tobacco to India in the sixteenth century where it also spread rapidly. It is not held sacred by the Hindus, but aboriginal tribes ascribe to it a divine origin. Several myths built around tobacco have been reported (Mehra 1979). Aboriginal uses in **Australia** as chewing tobacco have been reported for the leaves, sometimes in addition flowers and flowering stalks of several *Nicotiana* spp., especially *N. benthamii*, *N. cavicola*, *N. excelsior*, *N. gossei*, *N. ingulba*, and *N. simulans* (Peterson 1979). Normally sun or fire dried leaves, sometimes, however also freshly collected plant material is rolled with wood ash to a quid. A certain tribe in south-eastern Queensland uses *Duboisia hopwoodii* as chewing tobacco ('pituri'). Apart from these locations 'pituri' is only used if *Nicotiana* spp. are unavailable. However, *D. hopwoodii* was widely used in Australia as hunting poison. Crushed leaves in natural pools of water used by kangaroos and emus caused uncoordinated movements and made them easier to kill (Peterson 1979 and references therein).

3.3.6.4 Ecological Significance

Insecticidal Activity. Nicotine is one of the best-studied putative plant resistance traits (Steppuhn et al. 2004 and references therein). Aqueous tobacco preparations had already been used in the Americas as an insecticide in pre-Columbian times (Domino 1999). Already in the nineteenth century there were scientific reports on tobacco preparations as insecticides (Leunis and Frank 1885). Tobacco suds as well as aqueous solutions of nicotine sulphate (40%) had been the main insecticide for crop protection in the first half of the past century (Casanova et al. 2005 and references therein). Methods for the recovery of nicotine from scraps of the tobacco manufacture with the aim to use its sulphate as an insecticide were already of importance in the 1930s (e.g., Bernardini 1931a, b). Nicotine and also its congeners turned out to be very efficient agents as contact insecticides against *Aphis rumicis* L. (Homoptera: Aphidina), an aphid colonizing on *Nasturtium* R.Br. (Brassicaceae) plants (Richardson and Shepard 1930). *S*-(−)-Nicotine, applied as aqueous spray to agamic females of *A. rumicis*, was more toxic than *R*-(+)-nicotine. In contrast, *S*-(−)-nornicotine showed almost the same toxicity as its enantiomer; furthermore, both were even more toxic than *S*-(−)-nicotine (Hansberry and Norton 1940). However, *S*-(−)-anabasine turned out to be the most toxic alkaloid of all these congeners to aphids (Richardson et al. 1936). Extensive structure-toxicity relationships have been established by Yamamoto et al. (1962) with the important result that the 3-pyridylmethylamine moiety, a common structural part of most natural nicotinoids including nicotine itself, is essential. Toxic nicotinoids were generally monoionic at physiological pH values, i.e., the stronger basic pyrrolidine nitrogen is protonated and the weaker basic pyridine nitrogen remains in a unionized form as already mentioned above. In an in-vitro-system of housefly head cholinesterase *N*-methylanabasine was shown to achieve the same inhibition as nicotine, whereas myosmine turned out to be less potent (36% of nicotine). Cotinine lacking basic pyrrolidine nitrogen, therefore unable to ionize was almost inactive (2.5% of nicotine). Cholinesterase inhibition was competitive and correlated to toxicity (Yamamoto et al. 1968). Studies at a certain binding site [later designated as ACh site (Matsuo et al. 1998)] of nicotinic acetylcholine receptors from housefly and honeybee head membranes revealed that nicotinoids with higher basic pyrrolidine nitrogens (nicotine, nornicotine, anabasine, dihydronicotyrine) had a strong binding affinity in contrast to those with lower basic or even not basic pyrrolidine nitrogens (myosmine, nicotyrine, cotinine; Tomizawa and Yamamoto 1992). *S*-(−)-Nicotine and its enantiomer were equally toxic against houseflies, American cockroaches, and rice weevils. Against mealy plum aphids and rice stern borers the natural *S*-(−)-form turned out to be twice as toxic. On the other hand *R*-(+)-nicotine was reported to be an 11-fold stronger cholinesterase inhibitor in the housefly head than its natural enantiomer (Soeda and Yamamoto 1969), an example for the observations that the natural form is usually, but not always, more active than its enantiomer (Domino 1999 and references therein). The outstanding acute toxicity of *S*-(−)-nicotine was demonstrated in a study with 25 alkaloids of very diverging structural types incorporated into artificial diet for deleterious effects towards neonate larvae of the generalist herbivore *Spodoptera*

littoralis BOISD. (Lepidoptera: Noctuidae). Only the famous spindle poison colchicine, the principal alkaloid of *Colchicum autumnale* L. (Colchicaceae), turned out to be equivalent to nicotine with respect to high larval mortality (Krug and Proksch 1993).

In the second half of the past century, synthetic insecticides became much more important mainly due to reduced acute mammalian toxicity. Nevertheless nicotine formulations are still nowadays used widely throughout the world as relatively cheap insecticides (Domino 1999). Recently, dispersions of nicotine oleate (oil phase-integrated) stabilized by sodium caseinate (aqueous solution) and similar emulsions of different nicotine salts with Tween 80 as an emulsifier have been proposed as formulations for botanical insecticides with reduced mammalian toxicity (Casanova et al. 2005 and references therein)

Neonicotinoid Insecticides. The important disadvantage for the use of nicotine as an insecticide in, e.g., crop cultivation, its high toxicity to mammals and other animals, led also to another development: the search for environmentally benign derivatives. This was enforced by the fact that nicotine exhibits a rather strong though limited insecticidal potency due to its poor penetration into the insect central nervous system [the nicotinic acetylcholine receptors in insects are located there, not in the peripheral system (Yamamoto et al. 1998)]. The penetration of nicotine is reduced because the ionization reduces hydrophobicity. Therefore synthetic "intellectual" derivatives with nicotine as a structural lead have been developed. The aim of environmentally benign insecticides could be achieved by so-called neonicotinoids, e.g., acetamiprid (Yamada et al. 1999), imidacloprid (Wollweber and Tietjen 1999) or CGA 293343 (Maienfisch et al. 1999) (Fig. 3.12). Such compounds share the same essential moieties with nicotine, but they show higher hydrophobicity. Therefore their penetration is higher. This is due to the fact that these compounds are characterized by a *partial* positive charge instead of a full one (like ionized nicotinoids) (Yamamoto et al. 1998). The unshared electron pair on the concerned nitrogen atom is delocalized by the presence of a strong electron-withdrawing group in the neonicotinoids which causes this partial positive nitrogen. The second advantage: This partial charge is enough for an interaction with insect nicotinic acetylcholine receptors in contrast to an interaction with vertebrate ones where neonicotinoids have been shown to possess poor binding affinities (Yamamoto et al. 1995; 1998). Thus, selectivity for the receptors of insects was achieved (Matsuo et al. 1998). The chlorine atom at C-6 of the 3-pyridinyl moiety (acetamiprid, imidacloprid) or at the C-2 of the 5-thiazolyl moiety (CGA 293343) contributes to increased binding affinity as well as hydrophobicity. Both aspects lead to increased insecticidal activity (Yamamoto et al. 1998). Certain neonicotinoids offer excellent control of a wide variety of commercially important pests in many crops. Such compounds are suitable for soil, foliar, and seed treatment exhibiting contact as well as systemic activity (e.g., Maienfisch et al. 1999).

Biochemical Ecology. Nicotine is supposed to be one of the defensive chemical weapons of the genus *Nicotiana* against herbivores besides sesquiterpenoid phytoalexins (Sect. 7.3.1), phenolics (Sect. 6.7.3), proteinase inhibitors, and pathogenesis response proteins (Baldwin 1988). Transgenic down-regulation of nicotine by transformation of *putrescine N-methyl transferase* (*pmt*) genes in inverted-repeat

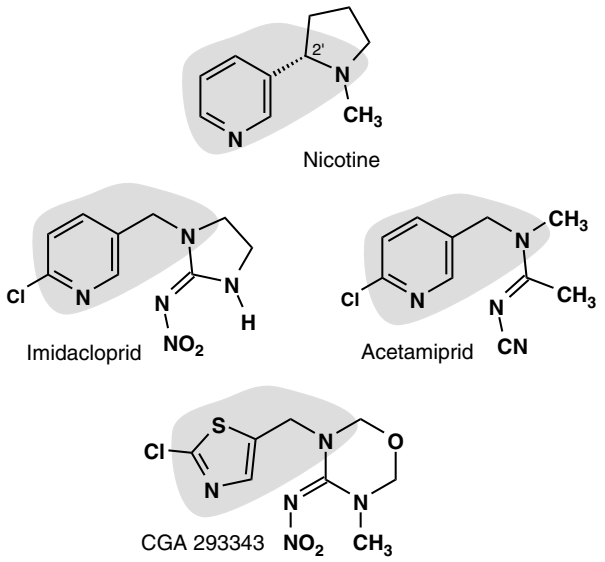


Fig. 3.12 Nicotine as lead structure for synthetic derivatives, so-called neonicotinoids, used as insecticides

(*IRpmt*) orientation demonstrated that *N. attenuata* was under relentless herbivore pressure. *IRpmt* plants when planted in their native habitat were preferred unequivocally by different native herbivores compared with wild-type plants (Steppuhn et al. 2004). These results provide strong evidence for the assumption that nicotine functions as an efficient defence in nature. A study on the survival and development of the tobacco budworm larvae, *Heliothis virescens* F. (Lepidoptera: Noctuidae), on isogenic tobacco lines with different levels of alkaloids revealed that even moderate increases in nicotinoids had negative effects (Jackson et al. 2002). In spite of the insecticidal capacity of *Nicotiana* spp. many insect as well as other pest problems (e.g., mites, nematodes) occur during tobacco production (Tso 1999; Blair 1999; Shepherd 1999 and references therein) apparently due to the reduction of the defensive value of nicotine in co-evolution of *Nicotiana* and its adapted herbivores (Steppuhn et al. 2004). A famous example for the tolerance of the defence chemistry of their host plant is the tobacco hornworm, *Manduca sexta* L. (Lepidoptera: Sphingidae), a common moth throughout the American continent (Self et al. 1964). Its larvae may live and feed on *Nicotiana* spp. Their nicotinic acetylcholine receptors are able to bind nicotine and do not show a substantial target site modification compared to other moths. Thus, the assumption that this tolerance is due to a change of the target has been disproved (Wink 1998). There are different mechanisms of protection. (i) The CNS of *Manduca* metabolizes/detoxifies nicotine to water-soluble derivatives which are different from mammalian metabolites (the latter are formed in the hepatic-portal system) (Morris 1983). (ii) Induction of midgut cytochrome P-450-related metabolism seems to be an adaptation to dietary nicotine, e.g., 9 of 12 enzyme activities were induced by 1.4- to 10-fold at a concentration

commonly found in *Nicotiana*; the alkaloid was metabolized to nicotine-*N*′-oxide and cotinine-*N*-oxide (Snyder et al. 1993). (iii) Nicotine may be eliminated rapidly without being metabolized (Self et al. 1964). (iv) The membranes of *Manduca* neurons are shown to be impermeable for nicotine (Blum 1983; Harborne 1993). Nevertheless, *M. sexta* as well as *M. quinquemaculata* HAWORTH responded with their gustatory organs to nicotine as a feeding deterrent at concentrations found in tobacco leaves. Both species selected feeding sites non-randomly but leaves in the middle region of tobacco where the concentration of nicotine is much lower than in the higher (younger) leaves. Thus, they avoid high nicotine proportions (Kester et al. 2002).

Damage to leaves by herbivores increased nicotine biosynthesis and nicotine accumulation 2- to 10-fold, e.g., in field-grown wild tobacco plants (*N. sylvestris*) up to 457% nicotine increase compared to same-age and same-positioned leaves on undamaged control plants (Baldwin 1988, 1999 and references therein). During reproductive growth nicotine was transported preferentially to attacked tissues (Baldwin 2001). Jasmonate was shown to be an essential signal compound for such wound-induced increases (Baldwin 1999 and references therein). Methyl jasmonate treatment elicited significant long-lasting increased in nicotine levels of *N. attenuata*, *N. bigelovii*, and *N. clevelandii*; the largest increase was observed in leaves at nodes (Lou and Baldwin 2003). Nicotine is an effective jasmonate-induced defense. The long-distance signal transduction cascade after wounding increases jasmonate pools in shoots which, in turn, increases jasmonate pools in roots. These stimulate nicotine synthesis and increase nicotine pools throughout the plant. Though nicotine is a constitutive metabolite of *Nicotiana* this immediate de novo synthesis as a result of an attack is of advantage for the plant from the economic point of view: high investments of nitrogen, i.e., large fitness costs are only necessary if the plant is attacked. An attack of *N. attenuata* by *M. sexta* or *M. quinquemaculata,* the most damaging lepidopterans of this native tobacco, suppresses the wound- and jasmonate-induced nicotine accumulation including its biosynthetic genes [especially *putrescine N-methyl trans*f*erase* (*pmt*) transcripts]. Such an attack causes a rapid ethylene burst which, in turn, leads to the down-regulation of the alkaloid by the nicotine-tolerant tobacco hornworm (Baldwin 2001). Fatty acid-amino acid conjugates as constituents of the regurgitant of many insect herbivores were shown to elicit herbivore-specific responses. Thus, during the attack of *N. attenuata* by *M. sexta* or *M quinquemaculata* *N*-linolenoyl-L-glutamine and *N*-linolenoyl-L-glutamic acid accounted for the majority of the regurgitant-specific alterations of the wound response in this plant (Halitschke et al. 2003). They also caused the ethylene burst (Baldwin 2001). However, the activity of an enzymatic elicitor, glucose oxidase with hydrogen peroxide as its product, was shown to inhibit wound-induced nicotine accumulation in *N. tabacum* (Halitschke et al. 2003; Musser et al. 2005). Interactions between plant allelochemicals and parasitoids should be considered with respect to insect herbivory and plant defense. Under laboratory conditions the wasps *Cotesia congregata* SAY (Hymenoptera: Braconidae), a parasitoid of *Manduca sexta*, and *Hyposoter annulipes* CRESSON (Hymenoptera: Ichneumonidae), a parasitoid of the fall armyworm, *Spodoptera frugiperda* J.E.SMITH (Lepidoptera: Noctuidae), (within their hosts) showed significant differences in development and survival

when their hosts were fed on nicotine-containing diets (Barbosa et al. 1986). A significantly increased mortality of *C. congregata* was observed in contrast to its host, *M. sexta* (Barbosa et al. 1991). More parasitoids failed to emerge from partially starved hosts when fed on nicotine diet. The authors concluded that nicotine may be directly toxic to the parasitoids. The alkaloid may act by mediating the availability of nutrients or by reducing assimilation of nutrients by developing parasitoids (Bentz and Barbosa 1992). Other insects, e.g., the housefly, *Musca domestica* L. (Diptera: Muscidae) are able to metabolize, i.e., in this case detoxify nicotine to cotinine like mammals (Harborne 1993). This had also been shown for male German cockroaches, *Blattella germanica* L., and female American cockroaches, *Periplaneta americana* L. (Dictyoptera: Blattellidae), respectively (Guthrie et al. 1957).

Nematodes also have cholinergic receptors the N-subtype of which is sensitive to nicotine (Martin et al. 2004). Root diffusates of *Nicotiana tabacum* inhibited hatching of cysts of potato root eelworm, *Heterodera rostochiensis* Woll. (Nematoda: Heteroderidae); nicotine showed a nematicidal effect on the young larvae (Schreiber and Sembdner 1960). Its nematicidal activity against pine wood nematode, *Bursaphelenchus xylophilus* Steiner & Buhrer (Nematoda: Aphelenchoididae) was about four times that of anagyrine, a tetracyclic quinolizidine alkaloid from the Fabaceae (Matsuda et al. 1989). Tobacco plants infested with root-knot nematodes like *Meloidogyne incognita* (Kofoid & White) Chitw. or *M. javanica* (Treub) Chitw. (Nematoda: Heteroderidae) both belonging to the most important phytoparasitic nematodes infecting almost all cultivated plants, turned out to contain increased levels of nicotine in the roots (Hanounik and Osborne 1977; Davis and Rich 1987; Siva Raju and Krishnamurthy 1996). The motility of *M. incognita* second stage juveniles and their ability to induce root galls in tomato, *Solanum lycopersicum*, were decreased by nicotine dependent on its concentration with EC_{50} values from 15 to 22 µg/ml (Davis and Rich 1987).

The development of certain apomorphic characters, long-chained *N*-acyl- and *N*-hydroxyacylnornicotines, found as constituents of the three *Nicotiana* spp. of the section *Repandae* (Zador and Jones, 1986; Huesing and Jones 1987; Matsuzaki et al. 1988; Severson et al. 1988a, b) turned out to be the successful evolutionary response of the plants to larvae of *Manduca sexta* or their ancestors: These compounds are about 1000-fold more toxic than nicotine (optimal chain length: C_{14} and C_{16}). These three *Nicotiana* species induced high levels of mortality and adverse effects on larval growth as well as on feeding (Huesing et al. 1989). Though being already constitutive metabolites the production of such compounds is rapidly induced on a trichome-based response as a consequence of herbivore attack; furthermore, it was observed that this induction preceded nicotine/nornicotine induction in *N. repanda* (Laue et al. 2000). The enzymatic ability to acylate nornicotine by long-chained fatty acids was inherited in interspecific hybrids (*N. repanda* × *N. tabacum*) in a dominant manner (Huesing et al. 1989).

Allelopathy. Nicotine was shown to affect adversely the germination, seedling vigour and other parameters of rice (*Oryza sativa* L., Poaceae) but favourably the growth of corn (*Zea mays* L., Poaceae). Since amylase activity was increased it was

suggested that this positive effect is partially due to increased solubilization of the stored starch (Rizvi et al. 1989a, b). *N′*-Acyl- and *N′*-hydroxyacylnornicotines had high germination inhibiting activity in an assay with seeds of *N. tabacum*; moreover, the growth of the cotyledons was inhibited, too (Matsuzaki et al. 1988). Furthermore, they decreased the growth of wheat coleoptiles and inhibited gram-positive bacteria to a moderate extent (Cutler et al. 1986; Severson et al. 1988b). Nicotine inhibited carotinoid cyclization, e.g., in the biosynthesis of ß-carotene, in *Mycobacterium marinum* (Mycobacteriaceae, Posibacteriota) (Batra et al. 1973), a microorganism causing rare aquarium-borne skin infections, and also in cotyledons of *Cucurbita ficifolia* C.D.Bouché (Cucurbitaceae) (Howes 1974), resulting in an accumulation of acyclic and monocyclic carotenes, e.g., lycopene. Furthermore, the biosynthesis of chlorophyll was inhibited in *C. ficifolia*.

Grafting. Grafting of *Zinnia elegans* (Asteraceae) on *Nicotiana* turned out to be successful because both species are able to synthesize nicotine in considerable amounts (*Z. elegans*: root content 0.1% freshly weighed) (Schröter 1955). A tip cutting from a seedling of *Nicotiana tabacum* grafted on a rootstock of *Duboisia myoporoides* had been also successful, apparently due to the same reason (Loftus Hills et al. 1946). In contrast, scions of solanaceous species not accustomed to increased concentrations of nicotine like *Solanum lycopersicum, Atropa belladonna,* and *Petunia* sp. showed characteristic damages (bleaching of the chloroplasts, resulting in a progressive variegation of the leaves) when grafted on stocks of *Nicotiana* (Mothes and Romeike 1954). The degree of the damages was proportional to the concentration of nicotine. However, the convolvulaceous holoparasites *Cuscuta reflexa* Roxb. and *C. platyloba* Prog. were cultivated successfully on *Nicotiana* host plants (*N. rustica*, *N. tabacum*) without damage though the parasites incorporated and accumulated all host plant alkaloids (Czygan et al. 1988). The assumption that the alkaloids might serve as protecting agents in favour of the parasite could not be confirmed. But the nicotinoids fed to callus cultures of *C. reflexa* as the only nitrogen source enabled good growth (Ehrenfeld 1999).

Potential Physiological Significance for Nicotinoid-producing Plants Themselves. Nicotine, nornicotine, anabasine, and anatabine were shown to produce a cumulative inhibition of the invertase activity of *Nicotiana glauca*. The alkaloids may have a role in the functional behaviour of this enzyme which cleaves sucrose (Rojo et al. 1998).

3.4 Tropanes

3.4.1 Discovery and Structure Elucidation

Tropane alkaloids are characterized by the bicyclic tropane skeleton (*N*-methyl-8-azabicyclo[3.2.1.]octane; Fig. 3.13). The scientific history of the tropanes started already in the beginning of the nineteenth century (see also above). The famous

solanaceous perennial herb *Atropa belladonna* L. (deadly nightshade or poison black cherry), native to European deciduous wood, has been analyzed phytochemically by Vauquelin (1809b) first. The following research work finally led to the isolation of the first tropane alkaloid, atropine, from *A. belladonna* (roots: Mein 1833; leaves: Geiger and Hesse 1833a, b). From the seeds of another poisonous solanaceous (annual) herb, *Datura stramonium* (thorn apple or jimson weed), distributed in temperate and tropical regions of all continents, also a tropane alkaloid, "daturine", was isolated very early (Geiger 1833) which later turned out to be identical to atropine. In the same paper Geiger reported the isolation of hyoscyamine from the seeds of *Hyoscyamus niger* (henbane), again a solanaceous annual herb. Later it was found that this alkaloid is "part of atropine". The summation formula of atropine was established by Liebig also in the 1830s (fide Ladenburg 1881a). Basic hydrolyzation of the ester atropine yielded tropine (nowadays: 3α-hydroxytropane or 3α-tropanol; Fig. 3.13) and atropic acid, an isomer of cinnamic acid (Kraut 1863, 1865, 1868). However, atropic acid turned out to be an artefact formed by dehydration of the genuine tropic acid which could be isolated after more moderate hydrolysis conditions (Lossen 1864, 1866). The reverse reaction, i.e., the synthesis of atropine was realized by Ladenburg (1879). Such esters were called tropeines.

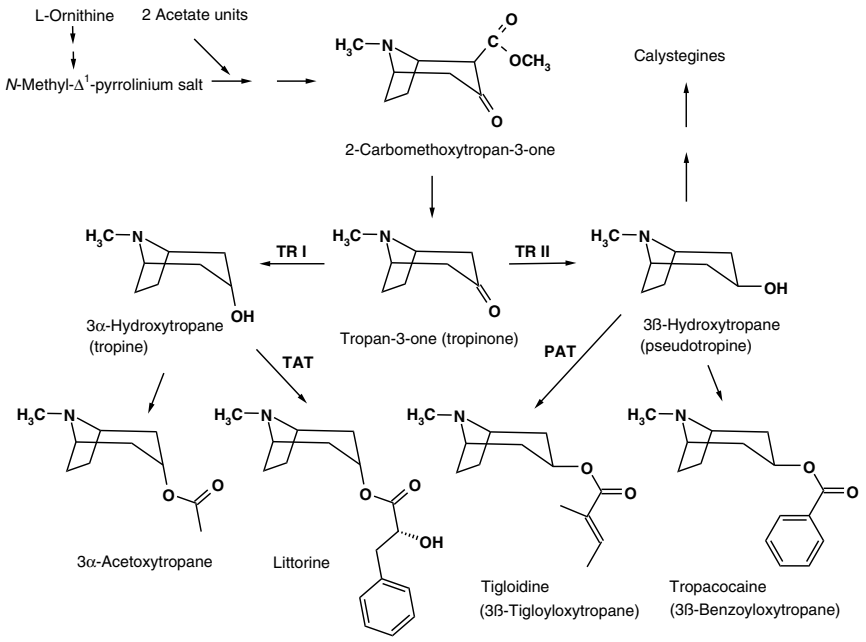


Fig. 3.13 Overview on the biosynthesis of different principal types of tropane alkaloids (aliphatic as well as arylalkyl and aryl esters of 3α- and 3β-hydroxytropane, respectively). **TR I** = tropinone reductase I; **TR II** = tropinone reductase II; **PAT** = pseudotropine acyltransferase; **TAT** = tropine acyltransferase

Ten years later it was elucidated that tropic acid exists in two optically active isomeric (enantiomer) forms (Ladenburg and Hundt 1889). This led to the discovery that hyoscyamine is the ester of 3α-hydroxytropane and (−)-tropic acid (Fig. 3.14). The absolute *S*-configuration of the latter could not be deduced until many decades later (Fodor and Csepreghy 1959); accordingly, hyoscyamine is also the *S*-(−)-isomer (3α-hydroxytropane is optically inactive, C-3 is pseudo-asymmetric). Hyoscyamine represents the dominating tropane alkaloid of all three species mentioned above whereas atropine is the corresponding racemate, i.e., the 50:50 mixture of (−)-hyoscyamine and its (+)-isomer. Gadamer (1901) was the first who recognized that the optically active form, hyoscyamine, is synthesized in the plants a priori and is submitted to autoracemization dependent on the time. Thus, young organs show almost pure hyoscyamine whereas older organs are characterized by considerable amounts of its *R*-(+)-isomer in addition to the genuine *S*-(−)-form. Accordingly, it is of course possible to *isolate* hyoscyamine as well as atropine from the same plant organ (particularly since the racemization may be strongly increased during the isolation procedure dependent on its method). On the other hand, it is not really correct to assume that both, hyoscyamine and atropine, are *constituents* of a plant. *S*-(−)- as well as *R*(+)-form are present in the organs in a certain ratio but almost never 50:50 (*stable* atropine). Normally the *unstable* ratio may be somewhere between 100:0 and 51:49 in favour of hyoscyamine (i.e., the *S*-(−)-form), with higher concentrations of it in younger organs. Thus, the plant constituents comprise an unbalanced mixture of the *S*-(−)- and the *R*-(+)-form **but no atropine**. Nevertheless, most textbooks and reference books ignore this fact still listing today both, atropine and hyoscyamine, as simultaneously occurring constituents of such solanaceous species. The principal structure elucidation of atropine (stereochemistry excluded) was realized by Willstätter (1898a, b).

Another important ester of tropic acid, with scopine (6β,7β-epoxy-3α-hydroxytropane) as an alkanolamine moiety (Fig. 3.14), was isolated from the roots of *Scopolia japonica* Maxim., a perennial herb of Eastern Asia (Schmidt and Henschke 1888). Like hyoscyamine/atropine this second tropeine is still today a remedy of remarkable significance (see Sect. 3.4.5). Already several years before, Ladenburg (1881a, b) had reported on the isolation of a novel alkaloid from *Hyoscyamus niger* which he named hyoscine. The structure of this compound has never been elucidated. For the first Schmidt and Henschke believed that their compound was identical with "hyoscine-Ladenburg". After having realized that this was not the case (e.g., diverging summation formulas) Schmidt changed the name for "hyoscine-Schmidt" into scopolamine in two reports on *Scopolia carniolica* Jacq. sub nom. *S. atropoides* (Schmidt 1892, 1894), a perennial herb of Central and South-East Europe. This change led to a scientific dispute (Hesse 1901a) and confusion. Thus, e.g., the scopolamine-identical alkaloid from the roots of *Mandragora officinarum* L. (mandrake), a famous herb of Mediterranean countries, was called hyoscine (Hesse 1901b). Unfortunately, both terms for the same tropane alkaloid, scopolamine and hyoscine, are still used nowadays; however, scopolamine should be preferred for two reasons. (i) Although it turned out later to be also a real constituent of *Hyoscyamus* spp. this compound had been discovered in *Scopolia* spp. (ii) Scopolamine is the common name used in

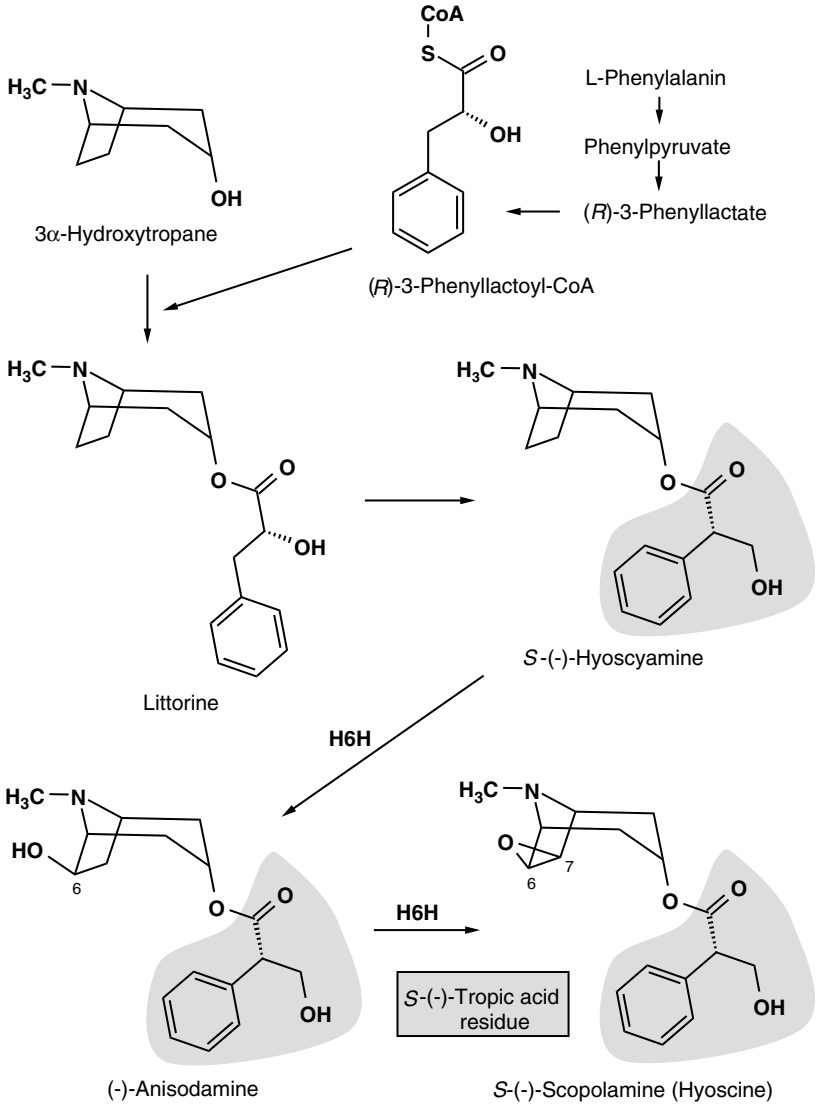


Fig. 3.14 Biosynthesis of tropane alkaloids of medicinal interest (hyoscyamine, anisodamine, scopolamine) confined to the Solanaceae; *S*-(−)-tropic acid is their common component. **H6H** = hyoscyamine 6-hydroxylase. For anisodine see text

pharmacology and pharmacy; also the official (WHO) International Nonproprietary Names (INN) of semisynthetic derivatives used in medicine include this name, e.g., butylscopolaminium bromide. However, in certain countries hyoscine butylbromide is preferred for this drug. Again the acyl moiety of scopolamine is represented by *S*-(−)-tropic acid (Fig. 3.14). Since C-3 of scopine is also pseudo-asymmetric and this alkanolamine is also optically inactive, accordingly the genuine (−)-scopolamine

shows *S*-configuration, too. Although the corresponding racemate, called atroscine, isolated first from *S. carniolica* sub nom. *S. atropoides* (Hesse 1901a), is known, *S*-(−)-scopolamine is much more stable than *S*-(−)-hyoscyamine. Only one century after the isolation of atropine the first tropane was discovered in the solanaceous sister family Convolvulaceae (Orechoff and Konowalova 1933, 1934). Almost 40 years after atropine the first pseudotropine (nowadays: 3β-hydroxytropane or 3β-tropanol; Fig. 3.13) derivative, cocaine was isolated from the leaves of *Erythroxylum coca* Lam. (Erythroxylaceae) by Niemann (1860) in the group of Wöhler indicating for the first time that tropane alkaloids are not confined to the Solanaceae.

Synoptic Outline: Types of Tropane Alkaloids. At least as far as both large Solanales families are concerned these metabolites *predominantly* represent esters of different mono-, di-, and trihydroxytropanes [sometimes in co-occurrence with their corresponding *nor* congeners (*N*-dimethyl derivatives)]. They may be divided into seven main structural types as far as 3α-hydroxytropane derivatives (sensu latiore) are concerned:

I. Esters of 3α-hydroxytropane ("tropine") with aliphatic acids [type **T1** in Table 3.1 (Solanaceae); type **T2** in Table 3.2 (Convolvulaceae)]
II. Esters of 3α-hydroxytropane ("tropine") with simple aromatic acids including *nor* derivatives [type **T3** in Table 3.2 (Convolvulaceae)]
III. Esters of 3α-hydroxytropane ("tropine") with prenylated aromatic acids [type **T4** in Table 3.2 (Convolvulaceae)]
IV. Esters of 3α,6β- or 3α,7β-dihydroxytropanes [types **T2/T3** and **T7-D** in Table 3.1 (Solanaceae); type **T7** in Table 3.2. (Convolvulaceae)]
V. Esters of 3α,6β,7β-trihydroxytropane (type **T4** in Table 3.1 (Solanaceae)
VI. Esters of 3α-hydroxytropane ("tropine") with phenylpropanoid acids, i.e.,
    a) Solanaceae: *S*-(−)-Tropic acid, 2′-hydroxytropic acid, *R*-phenyllactic acid, including *nor* derivatives (types **T5** and **T7-A** in Table 3.1)
    b) Convolvulaceae: Hydroxycinnamic acids (type **T5** in Table 3.2)
VII. Ester of 6β,7β-epoxy-3α-hydroxytropane ("scopine") with *S*-(−)-tropic acid (type **T6** in Table 3.1 (Solanaceae)

Alkaloids of the structural types (I), (IV), and (VI) occur in both large Solanales families whereas those of the types (V) and (VII) seem to be confined to the Solanaceae and those of the types (II) and (III) to the Convolvulaceae. However, in case of (II) which is the dominating tropane subtype in the latter family there is one exception in the Solanaceae (datumetine from *Datura metel*). Alkaloids of subtype (VI.a) including famous compounds like hyoscyamine/atropine and scopolamine (hyoscine) are unique solanaceous metabolites whereas certain convolvulaceous species are producers of subtype (VI.b) so far unknown from the Solanaceae.

Rare tropanes of unusual structure have been found in both families (for details see Table 3.1 / type **T7-A – T7-D;** Table 3.2 / type **T6-A – T6-C, T7**), e.g., schizanthines in the genus *Schizanthus* (Solanaceae) or consabatines in the genus *Convolvulus* (Convolvulaceae).

Furthermore, corresponding derivatives of 3β-hydroxytropane ("pseudotropine") may occur in both Solanales families (Table 3.1. / type **T8**; Table 3.2 /type **T8 – T10**).

3.4.2 Occurrence in the Solanaceae (Table 3.1)

Data of Table 3.1, as far as tropane alkaloids are concerned and if not taken directly from the original reports (cited in the last column of the table), are based on the extensive review of Lounasmaa and Tamminen (1993) comprising literature up to June 1992 and covering all known tropane-positive species throughout the plant kingdom. These authors had listed in detail the occurrence of all tropanes known at that time in their Table III in the systematic order of plant families according to the system of Dahlgren. The intrafamilial taxa of the Solanaceae had been classified mainly according to the chemotaxonomic system of Tétényi. However, this system is not acceptable against the setting of the present status of investigation (see Sect. 2.2). Therefore, Table 3.1 is listed according to the classification of Fig. 2.2.

It has to be pointed out that most references may be not cited directly in the following text but indirectly, i.e., by the last column of Table 3.1.

Our main knowledge of the occurrence and distribution of tropanes is based on (i) traditional isolation and structure elucidation procedures and (ii) different chromatographic methods, predominantly TLC. Results based on GC/MS analysis are – in contrast to the Convolvulaceae – rather rare since this method has been used only during the past two decades. They are confined to some species like *Atropa belladonna* (Hartmann et al. 1986), *Hyoscyamus albus* and *H.* × *györffyi*, respectively (Doerk-Schmitz et al. 1994, Ionkova et al. 1994), *Physalis peruviana* (Kubwabo et al. 1993), and above all to several species of the genus *Datura* (Witte et al. 1987; Ionkova et al. 1994; Philipov and Berkov 2002; Berkov 2003; Berkov et al. 2003, 2005; Berkov and Zayed 2004; Doncheva et al. 2004) The advantage of the GC/MS method lies in the possibility to detect even very low concentrations of a metabolite with high reliability. Thus, a large number of minor congeners can be identified in addition to those metabolites found by methods used in former decades. Thus, e.g., altogether 64 tropane alkaloids of almost all structural types could be detected in organs of different stages of development in *Datura stramonium*; 48 of them were structurally identified though not always with regard to the configuration at C-3 and/or C-6/C-7, respectively (Berkov et al. 2005).

Tropanes were detected in species of all five subfamilies, however with an extremely different extent of distribution. Thus, these alkaloids form consistent traits:

1. In the **Schizanthoideae** (all seven taxa checked were found positive)
2. With the exception of the Nicotianeae clade in the remaining five clades of the **Nicotinoideae** subfamily (all 33 taxa positive)
3. In four (out of nine) clades of the **Solanoideae** [Hyoscyameae (all 26 taxa positive), Mandragoreae (all 3 taxa positive), Solandreae/subclade Solandrinae (all five taxa positive), Datureae (all 18 taxa positive)]

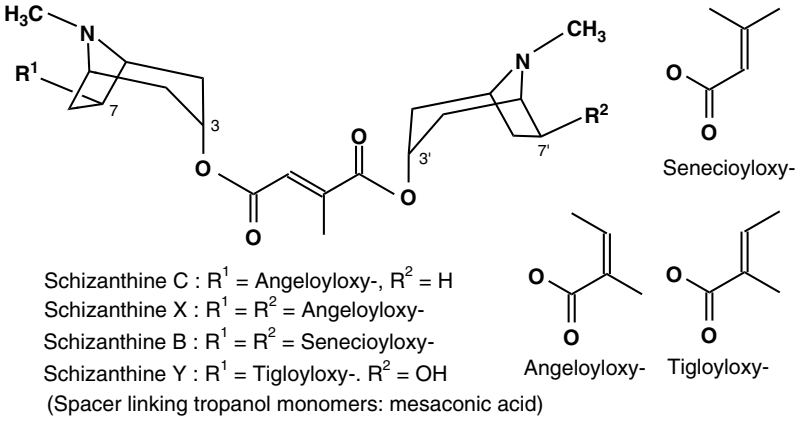
In contrast, to date only one species of the **Petunioideae**, *Nierembergia linariaefolia,* as well as one species of the **Cestroideae**, *Latua pubiflora*, were found to contain any tropane alkaloid. On the other hand, there are some reports on other taxa from both subfamilies from which one may conclude indirectly the absence of tropanes due to an alkaloidal screening with positive results for simple pyrrolidines and/or nicotinoids, e.g., *Brunfelsia / Petunia* and *Salpiglossis / Cestrum*. Finally, there exists a tentative report on the formation of tropanes in *Nicandra physalodes* without differentiation with respect to certain metabolites (McGaw and Woolley 1978b). Though it has never been confirmed in a final paper such an occurrence might be since 3-oxotropane ("tropinone"; Romeike 1966) and calystegines (Sect. 3.5) have also been detected in this species.

Furthermore, the frequent comment on the absence of tropane alkaloids in the largest solanaceous genus *Solanum* ought to be thought in relative terms: There is no doubt about the absence of acylated hydroxytropanes, e.g., valtropine, tigloidine, meteloidine, hyoscyamine. However, simple tropanes like 3-oxotropane, 3β-hydroxytropane, and calystegines, altogether plesiomorphic characters of the Solanales in total, could be detected unequivocally (Table 3.1, Sect. 3.5). This is also true for certain other genera like *Capsicum* and *Salpichroa.*

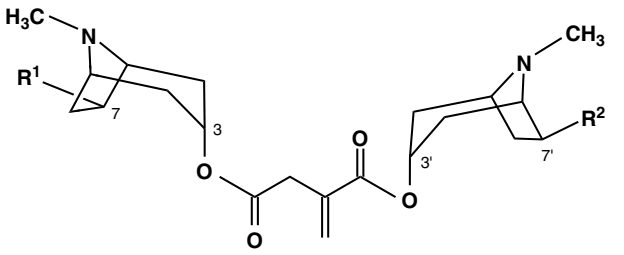
3.4.2.1 Aliphatic Esters of 3α-Hydroxytropane/-*nor*tropane (T1 in Table 3.1)

This type is found throughout those parts of the family which contain tropane alkaloids. If trivial names have been given to an alkaloid in the literature it is announced here in parentheses. The following saturated aliphatic acyl residues may occur: acetyl- (Fig. 3.13), propionyl-, *n*-butyryl-, isobutyryl- (butropine), 2-methylbutyryl- (3α-acyloxytropane = valtropine; 3α-acyloxy*nor*tropane = isoporoidine), isovaleryl- (3α-isovaleryloxy*nor*tropane = poroidine). Butropine and valtropine were discovered in the leaves of *Duboisia leichhardtii* (Deckers and Maier 1953) and named according to their acyl residues (Rosenblum 1954; Rosenblum and Taylor 1954). Poroidine and isoporoidine were identified as minor alkaloids in the leaves of *D. myoporoides* and therefore named after its species epithet (Barger et al. 1938). Furthermore, unsaturated acyl residues like tigloyl- and senecioyl- (Fig. 3.15) are possible. Especially the tigloyl residue is also very common in solanaceous tropane alkaloids of different types (see below). An unique metabolite, 3α-methylmesaconyloxytropane, was identified as a monomer of the schizanthines (see **T7-D**) in *Schizanthus hookeri* (Jordan et al. 2006).

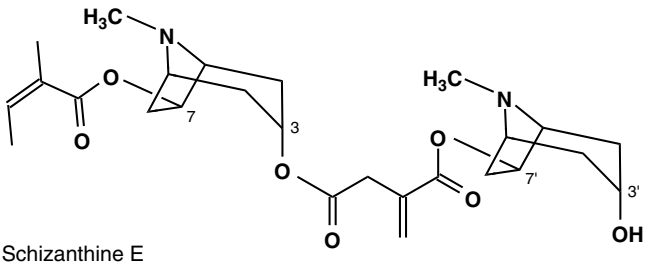
If any **T1**-type alkaloids occur in a specific taxon, there may be present between only one metabolite (e.g., *Anthocercis littorea*) and up to six congeners of this type [e.g., *Cyphanthera albicans*, *Hyoscyamus pusillus* (4); *Datura inoxia* (5); *Duboisia myoporoides* (6)]. Such alkaloids are normally accompanied by one or more different types of tropane alkaloids (**T3 – T6**; **Schizanthoideae**: **T7-D**), e.g., alkaloid extracts obtained from the leaves of *Duboisia leichhardtii* contained hyoscyamine as the principal component accompanied by minor congeners like scopolamine (hyoscine) as well as different **T1**-type esters (e.g., butropine, valtropine). Both latter



Schizanthine C : R^1 = Angeloyloxy-, R^2 = H
Schizanthine X : R^1 = R^2 = Angeloyloxy-
Schizanthine B : R^1 = R^2 = Senecioyloxy-
Schizanthine Y : R^1 = Tigloyloxy-. R^2 = OH
(Spacer linking tropanol monomers: mesaconic acid)



Schizanthine Z : R^1 = Tigloyloxy-, R^2 = OH
Schizanthine-C-isomer : R^1 = Angeloyloxy-, R^2 = H
(Spacer linking tropanol monomers: itaconic acid)



Schizanthine E
(Spacer linking tropanol monomers: itaconic acid)

Schizanthine D: mesaconic acid linked isomer of schizanthine E

Fig. 3.15 Schizanthines, unique dimeric tropane alkaloids of the genus *Schizanthus*

alkaloids could be separated already some 50 years ago from hyoscyamine/scopolamine by steam distillation since they turned out to be volatile in contrast to the tropoyl esters (Rosenblum 1954).

There are only a few species containing **T1**-type metabolites without any other tropane type, e.g., *Nierembergia linariaefolia* and *Withania somnifera*; in both

species only the common 3α-tigloyloxytropane has been detected. Though the occurrence of this metabolite in *Solanum betaceum* (syn.: *Cyphomandra betacea*, tree tomato) has been tentatively reported [TLC – comparison with an authentic sample, but ". … insufficient material prevented complete identification." (Evans et al. 1972d)], it is still equivocal since it has never been confirmed neither by these authors nor by others. A unique alkaloid, 3α-nonyloxytropane has been discovered in the leaves of *Duboisia myoporoides* besides butropine, valtropine, and 3α-tropoyl congeners (Shukla and Thakur 1992).

3.4.2.2 Acylated 3α,6β- and/or 3α,7β-Hydroxytropanes (T2/T3)

Acylated 3α,6β-Dihydroxytropanes (T2). As already mentioned the occurrence of tropane alkaloids covering the literature up to June 1992 is based mainly on the review of Lounasmaa and Tamminen (1993). These authors have used the generally accepted uniform numbering system of the tropane skeleton with the consequence that ". … most disubstituted tropane alkaloids designated as C-3,C-6 disubstituted in the literature become C-3,C-7 disubstituted". This has also been done ". … where the choice between the C-3,C-6 and C-3,C-7 notation in the literature has been arbitrary." However, if ". … the determination of absolute configuration has a solid basis, and where the structure is correctly presented by the C-3,C-6 notation also in the present numbering system", i.e., in the generally accepted one, "has the original C-3,C-6 notation been retained." With regard to the adjustment of Lounasmaa and Tamminen, only two monoesters of 3α,6β-dihydroxytropane are known. (i) An aliphatic ester, 3α-isovaleryloxy-6ß-hydroxytropane (valeroidine) discovered in *Duboisia myoporoides* has been found also in three *Cyphanthera* spp. and *Anthocercis ilicifolia* (Australian members of the **Nicotinoideae**) as well as in *Brugmansia sanguinea* and *B. candida* × *aurea* (members of the S-American Datureae clade, **Solanoideae**). (ii) The second well supported monoester of the 3,6-dihydroxytropane-type, anisodamine (6ß-hydroxyhyoscyamine) is described below (**T5/T6**), because it has to be considered in a special, biosynthetic connection. Therefore anisodamine has not been taken into account as a **T2**-type metabolite in Table 3.1.

Recent extensive studies by Berkov and his group on GC/MS analyses of *Datura stramonium*, *D. ceratocaula*, and *D. inoxia* (Philipov and Berkov 2002; Berkov et al. 2003; Berkov 2003; Berkov and Zayed 2004) apparently still have ignored the standards of Lounasmaa and Tamminen concerning the problematic of the stereochemistry at C-6 and C-7. Thus, all 6-substituted tropanes mentioned in these four reports are equivocal since the notation is based on GC/MS data only. Doerk-Schmitz et al. (1994) had already shown the necessary distance to their own C3,C6 notations in a comparable study (*Hyoscyamus albus*) with the following comment: "Whether it is (***3R***,***6R***)-6β-hydroxy-3α-acyloxytropane (= **7β**, if numbered clockwise proceeding from the 1*R* bridge carbon of 3α-hydroxytropane) or (***3S***,***6S***) (= **6β**) cannot be deduced from the GC/MS data, since both compounds are enantiomers and do not separate on an optically inactive GC-column" (bold face added by the author).

3α,6β-Dihydroxytropane itself has been detected in *Schizanthus hookeri* and *S. littoralis* in contrast to the corresponding esters. Unfortunately, there is again a confusion in the literature since *Schizanthus*-specific esters with a 3α,6β notation have been reported originally. This has not been accepted by Lounasmaa and Tamminen in contrast to 3α,6β-dihydroxytropane itself. Thus, these esters were assigned to the 3α,7β notation (Fig. 3.15).

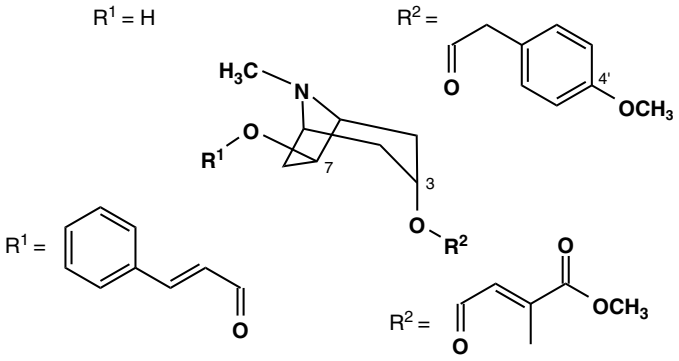
Esters of 3α,7β-Dihydroxytropane (T3). This type is characterized by the same saturated and unsaturated aliphatic acyl moieties like the esters of type **T1**; the only additional acyl moiety is formed by angelic acid, the *trans*-isomer of tiglic acid (structures of both acyl residues: Fig. 3.15). In contrast to **T1** four principal variations are given, two for mono- and diesters each. These variations include (I) 3α-acyloxy-7β-hydroxytropanes, (II) 3α-hydroxy-7β-acyloxytropanes, (IIIa) 3α,7β-diacyloxy congeners with identical acyl moieties, e.g., 3α,7β-ditigloyloxytropane, (IIIb) 3α,7β-diacyloxy congeners with different acyl moieties, e.g., 3α-tigloyloxy-7β-propionyloxytropane. Esters of all these types (I–IIIb) containing at least one tigloyl residue show a frequent occurrence.

Though occasionally also present in some other taxa (*Anisodus*, *Atropa*, *Hyoscyamus*, *Physochlaina*, *Mandragora*), such **T3**-type tropanes are frequent in the species of the Symonanthus / Anthocercis clades (**Nicotianoideae**) and the Datureae clade (**Solanoideae**). In certain species many individual metabolites of this type have been detected due to the variations mentioned above, in the lead *Brugmansia candida* × *aurea* (16 metabolites) and *Datura inoxia* (15). However, usually the number of such congeners ranges between two and five. In addition, those results with certain *Datura* spp. reported by the group of Berkov and mentioned already above (**T2**) should be placed here following Lounasmaa and Tamminen.

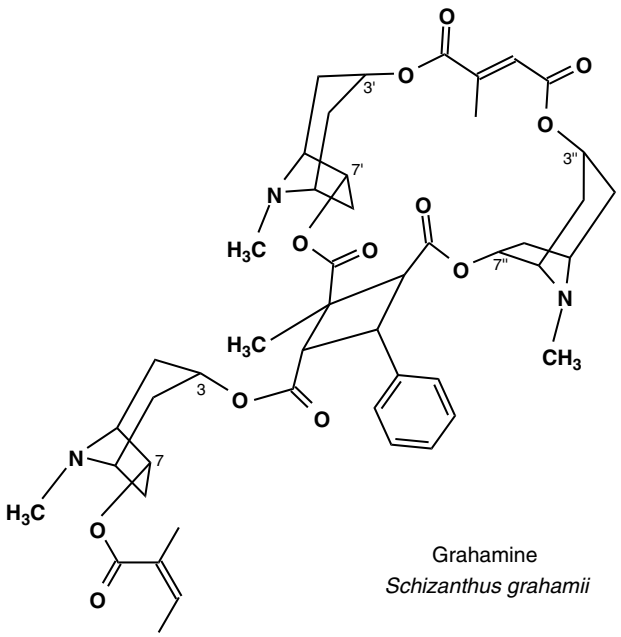
On the other hand, it should be appended to the adjustments of these Finnish authors their own saving clause: "The strict application of the system adopted here is certainly in several cases a simplification of the real situation and should be regarded as such." The frequently unsatisfactory situation with regard to this problem has given reason to the author of the present monograph to combine **T2**- and **T3**-type alkaloids in one column of Table 3.1.

A unique compound, physochlaine [3α-(4′-methoxyphenylacetoxy)-7β-hydroxytropane; Fig. 3.16] has been discovered in *Physochlaina alaica*. Another specific situation is given by the genus *Schizanthus* (**Schizanthoideae**). Its species also contain the variations (I) and (II), but not (IIIa) and (IIIb). Instead they are characterized by schizanthines, unique 3α,7β-diacyloxytropanes (schizanthines A, F–I, K–M) and their corresponding *dimeric* congeners, the monomers linked by dibasic unsaturated acids (mesaconic acid, itaconic acid; schizanthines B–E, Y, Z). These dimeric compounds (Fig. 3.15) are listed as type **T7-D** (see below). *Schizanthus pinnatus*, reported to contain 11 alkaloids of type **T3**, has shown a specific profile: Besides the *dimeric* schizanthine B tropane *monomers* with 7β-tigloyloxy, 7β-angeloyloxy or 7β-senecioyloxy substituents and derivatives of these dibasic acids mentioned above as substituents at C-3α (one of the following monoesters: 1-methyl- or 1-ethyl-mesaconyloxy, 1-methyl- or 1-ethyl-itaconyloxy, 3-ethoxycarbonylmethacryloyloxy).

Physochlaine [3α-(4'-methoxyphenylacetoxy)-7ß-hydroxytropane]
Physochlaina alaica



3α-Methylmesaconyloxy-7ß-cinnamoyloxytropane
Schizanthus litoralis



Grahamine
Schizanthus grahamii

Fig. 3.16 Unique tropane alkaloids detected in certain solanaceous species

Thus, instead of an esterification with a second hydroxytropane monomer (like in the dominating *dimeric* schizanthines of, e.g., *Schizanthus grahamii*) the second carboxyl group of the dibasic acids, the one geminal to the methyl group of mesaconic acid and the one geminal to the methylene group of itaconic acid, respectively, is esterified by

methanol or ethanol. In the case of a 3-ethoxycarbonylmethacryloyloxy substituent this esterification with ethanol has happened at the opposite second carboxyl group of mesaconic acid, i.e., the one with the vicinal hydrogen. A unique metabolite, 3α-methylmesaconyloxy-7β-cinnamoyloxytropane (reported as 6β-cinnamoyloxytropane-3α-methylmesaconate) (Fig. 3.16), was discovered in the leaves of *Schizanthus litoralis* (Muñoz et al. 1996).

3.4.2.3 Acylated 3α,6β,7β-Trihydroxytropanes (T4)

The principal adjustments of Lounasmaa and Tamminen have to be taken into account here, too. The theoretical number of possibilities for structural variations is enhanced compared with **T3**-type compounds due to the presence of *three* hydroxyls at the tropane skeleton. Nevertheless, the real number of **T4**-type compounds is very limited. Two alkaloids turned out to be the most frequent ones: (i) 3α-tigloyloxy-6β,7β-dihydroxytropane, named meteloidine because it has been discovered in *Datura meteloides* DC (valid synonym: *D. inoxia* MILL.; Pyman and Reynolds 1908), and (ii) 3α,7β-ditigloyloxy-6β-hydroxytropane, discovered in *Datura stramonium* (Evans and Wellendorf 1958). Both alkaloids are typical constituents of the taxa from the Symonanthus / Anthocercis clades (**Nicotianoideae**) and the Datureae clade (**Solanoideae**). Meteloidine turned out to be the major alkaloid of *Anthocercis genistoides* (Griffin and Lin 2000). Two rare **T4-di**esters with diverging acyl residues are 3α-tigloyloxy-6β-hydroxy-7β-isovaleryloxytropane, confined to *Brugmansia sanguinea*, *B. candida*, and *Datura ferox* (Vitale et al. 1995), and its 7-propionyl congener, identified in *D. stramonium* (Berkov et al. 2003). Two further **T4-mono**ester, 3α-(2′-hydroxy-3′-phenylpropionyloxy)-6β,7β-dihydroxytropane (6β,7β-dihydroxylittorine) and 3α,7β-dihydroxy-6β-tigloyloxytropane have been discovered in *B. candida* and *B. suaveolens,* respectively. No further congeners have been found in the Solanaceae. This implicates that no 3α,6β,7β-**triacyl**oxytropanes seem to be present. However, such a triacylated metabolite has been found in the family Erythroxylaceae (El-Imam et al. 1987). Finally, it should be pointed out that even mono- and diacylated **T4**-type compounds are rather rare in the family Solanaceae since they are confined to the few clades mentioned above.

3.4.2.4 Esters of 3α-Hydroxytropane/-*nor*tropane and 6β,7β-Epoxy-3α-hydroxytropane (Scopine/*Nor*scopine) with Solanaceae-specific Phenylpropanoid Acids (T5–T7-B)

Common Esters (T5, T6) (**Figs**. 3.13 and 3.14). The occurrence and distribution of hyoscyamine/atropine and scopolamine (hyoscine) are documented in an extraordinarily extensive manner in the literature due to their great significance in pharmacology and toxicology. First of all it must be considered that these alkaloids as well as their closely related congeners, i.e., esters with (i) tropic acid including

6β-hydroxy derivatives (anisodamine = 6β-hydroxyhyoscyamine), *nor* derivatives (*nor*hyoscyamine/*nor*atropine, *nor*scopolamine) and *N*-oxides (**T7-B**), (ii) (−)-anisodinic acid (2′-hydroxytropic acid) (anisodine; **T7-A**), (iii) phenyllactic acid (littorine), and (iv) 4-hydroxyphenyllactic acid (4′-hydroxylittorine) are confined to the Solanaceae. One report on the occurrence of scopolamine in *Heisteria olivae* STEYERM., Olacaceae (Cairo Valera et al. 1977) is very questionable; it is only based on an insufficient chromatographic detection and improbable that only one single tropane alkaloid without any congener is present in a species.

Second, it has to be pointed out that the co-occurrence with scopolamine (hyoscine) is a consistent trait in hyoscyamine-containing species. Furthermore, this fact involves that anisodamine (6β-hydroxyhyoscyamine) must also be synthesized in all these species because it is an intermediate representing the direct precursor in the biosynthesis of scopolamine (Fig. 3.14). Nevertheless, due to a certain accumulation this intermediate could be identified in many species, e.g., in the genera *Anthocercis*, *Cyphanthera*, *Duboisia*, *Datura*, *Hyoscyamus*, *Physochlaina*. The trivial name of this alkaloid has been given due to its occurrence in *Anisodus acutangulus*.

This biogenetic argument is also true in favour of hyoscyamine in one single case in which only scopolamine (but not hyoscyamine) has been detected (*Symonanthus aromaticus*). No occurrence of scopolamine has been reported to date for only two hyoscyamine-containing species (*Anthocercis fasciculata*, *Physochlaina dubia*). It may be assumed that these exceptions have been caused by very low concentrations of scopolamine in the corresponding sample. There are two arguments for this assumption. (i) All further *Anthocercis* spp. checked (10 taxa) as well as all further *Physochlaina* spp. checked (five taxa) have turned out to be scopolamine-positive. (ii) The sequence hyoscyamine → anisodamine → scopolamine is catalyzed just by one enzyme in two steps, hyoscyamine 6-hydroxylase (see Sect. 3.4.4), and at least anisodamine has been found in *P. dubia*.

T5/T6-type alkaloids are absent in the **Schizanthoideae**. They have been detected in the **Cestroideae** (only *Latua pubiflora*), in all clades of the **Nicotianoideae** except the Nicotianeae clade, and in the **Solanoideae**-clades Hyoscyameae, Mandragoreae, Solandreae (only Solandrinae subclade), Datureae. With the exception of the **Cestroideae** these alkaloids represent a consistent trait in all these taxa, i.e., they are their chemotaxonomic markers. Due to the fact that these alkaloids are potent poisons the discovery and distribution of the hyoscyamine/scopolamine-containing plant species has been facilitated. Therefore it is improbable that species of the **Schwenckioideae**, **Goetzeoideae,** and **Solanoideae**-clades Nolaneae as well as Solandreae (only Juanulloinae subclade) might contain such alkaloids in considerable amounts though these subfamilies represent chemical "terra incognita". Taxa which are phytochemically well-studied ought to be unequivocally **T5/T6**-negative; otherwise it would have been published (negative results are almost never reported). These taxa are **Petunioideae**, Nicotianeae clade (**Nicotianoideae)**, **Cestroideae** (except *Latua pubiflora*), and the **Solanoideae**-clades Jaboroseae, Lycieae, Nicandreae, Solaneae, Capsiceae, Physaleae. There are a few questionable reports: (i) Though the occurrence of hyoscyamine in *Solanum betaceum* (syn.: *Cyphomandra*

betacea, tree tomato) has been *tentatively* reported due to TLC-comparison with an authentic sample (Evans et al. 1972d) it is still equivocal since it has never been confirmed neither by these authors nor by others. It would be "the first reported example of a plant which produces both atropine-like alkaloids and edible fruits" (Evans et al. 1972d). (ii) The occurrence of hyoscyamine and scopolamine, reported for *Lycium barbarum* (Harsh 1989) could not be confirmed by other, more careful authors, e.g., Christen and Kapetanidis (1987). (iii) A tentative report on the occurrence of hyoscyamine on *Salpichroa origanifolia*, Physaleae clade/Salpichroinae subclade (Evans et al. 1972a) has never been confirmed. However, finally it has to be pointed out that hyoscyamine and scopolamine have been detected unequivocally in 75 species belonging to 19 genera (additional subspecies/varieties and hybrids not included). This is a very remarkable contribution to the reasons why the Solanaceae is called a poisonous family.

*Nor*hyoscyamine/*nor*atropine as well as *nor*scopolamine (*nor*hyoscine) were found in many species – though in low concentrations – in co-occurrence with the corresponding *N*-methyl congener. Interestingly, *nor*scopolamine was shown to be a *principal* alkaloid of the corollas of *Brugmansia suaveolens* (Evans and Lampard 1972). Almost a century ago it was reported that *nor*hyoscyamine ("solandrine") represented the major alkaloid *of Solandra longiflora* leaves (Petrie 1917a).

All hyoscyamine-containing species are able to synthesize littorine, since it is the direct biogenetic precursor of hyoscyamine. However, this does not mean that this intermediate is detectable in all these species. Its name was based on the occurrence in *Anthocercis littorea* (Cannon et al. 1969) though it had already been discovered shortly before in *Brugmansia sanguinea* sub nom. *Datura sanguinea* (Evans and Major 1968). Furthermore, it was detected in other species of *Anthocercis* and *Brugmansia* as well as in the genera *Duboisia*, *Datura*, and *Hyoscyamus*.

The unusual specific co-occurrence of nicotinoids and tropanes in *Duboisia* spp. has been already discussed in Sect. 3.3.2.

Rare Derivatives of Hyoscyamine and Littorine Not Included in Table 3.1. The principal adjustments of Lounasmaa and Tamminen (see **T2/T3**) have to be taken into account here again. 7β-Hydroxyhyoscyamine was identified – beside its 6β congener and scopolamine – in the leaves of a *Duboisia myoporoides* × *D. leichhardtii* hybrid, in the roots and root cultures of *Atropa belladonna* and *Hyoscyamus albus*, as well as in root cultures of *Datura inoxia, Brugmansia candida* × *aurea,* and *H. niger* (Ishimura and Shimomura 1989; Doerk-Schmitz et al. 1994).

The alkaloids traditionally named 6β-hydroxyhyoscyamine and 7β-hydroxyhyoscyamine represent two natural diastereoisomers, namely (+)-(**3*R*,*6R***,2′*S*)- and (−)-(**3*S*,*6S***,2′*S*)-6β-hydroxyhyoscyamine, respectively (Muñoz et al. 2006). This is again a consequence of the C-3 (pseudo)stereocenter of 3-hydroxytropane.

7β-Acyloxyhyoscyamines (acyl residues: isovaleryl-, 2-methylbutyryl-, tigloyl-) could be identified by GC/MS analysis in the roots and hairy root cultures of *Datura inoxia* (Witte et al. 1987; Ionkova et al. 1994). An ester of anisodamine, 6β-hydroxyhyoscyamine diacetate, turned to be a constituent of *Physochlaina dubia* (Mirzamatov et al. 1972). For 6β,7β-dihydroxylittorine see above (**T4**).

Another, 6,7-disubstituted derivative of hyoscyamine with the proposed structure 7β-hydroxy-6β-propenyloxy-3α-tropoyloxytropane was reported as a minor constituent of the seeds of *Datura ferox* (Vitale et al. 1995); however, due to the fact that the structure was determined only by GC/MS data the stereochemistry of this compound was not elucidated unequivocally; this also implicates that it is not in accordance with the adjustment of Lounasmaa and Tamminen (1993). Thus, it might be alternatively the 6β-hydroxy-7β-acyloxy isomer. By the way, the structural formula given by the authors is in accordance with this alternative if the usual numbering for tropanes would have been chosen by them.

Rare Derivatives of Scopolamine (Hyoscine) Not Included in Table 3.1. Another 3-acyloxyscopine derivative, 3-phenylacetoxy-6β,7β-epoxytropane was identified in the seeds of *Datura ferox* (Vitale et al. 1995). It may be assumed that this metabolite is 3α-substituted since only 3α-acyloxy congeners were found in this species and natural 3β-substituted scopines are unknown in general; however, the stereochemistry at C-3 has not yet been determined.

Rare Congeners of Type T7-A and T7-B Integrated in Table 3.1: Anisodine (T7-A). (−)-Anisodine (daturamine) is the rarely occurring 2′-hydroxy congener of scopolamine [acyl residue: (−)-anisodinic acid (2′-hydroxytropic acid)] (Xie et al. 1983). It was detected only in the **Solanoideae**; even in this large subfamily it is confined to two *Anisodus* spp. (the alkaloid is named after this genus), *Przewalskia tangutica* (Hyoscyameae clade), and two *Brugmansia* spp. (Datureae clade).

***N*-Oxides of T5- and T6-type Alkaloids (T7-B).** Two isomeric *N*-oxides of hyoscyamine (isomer 1 with equatorial N^+–O^-; isomer 2 with axial N^+–O^-) were isolated from five famous species of the **Solanoideae** which have been widely used as medicinal plants: *Atropa belladonna*, *Hyoscyamus niger*, *Scopolia carniolica* (Hyoscyameae clade), *Mandragora officinarum* (Mandragoreae clade), and *Datura stramonium* (Datureae clade) (Philippson and Handa 1975b). In addition, isomer 1 of the *N*-oxides of scopolamine (hyoscine) was identified in the same species with the exception of *M. officinarum*. It may be assumed that these compounds are also present in other species in minor concentrations; they might have been overlooked in other studies on tropane alkaloids of the classical tertiary amine type due to the fact that the identification of *N*-oxides needs different methods. It may be added that also anisodamine *N*-oxide was identified in one species (*Physochlaina alaica*).

3.4.2.5 Esters of 3α-Hydroxytropane with Solanaceae-unspecific Phenylpropanoid Acids (T7-C)

3α-Cinnamoyloxytropane and 3α-phenylacetoxytropane, two alkaloids already known from the Erythroxylaceae, were detected for the first time in the Solanaceae family in *Latua pubiflora* (Muñoz and Casale 2003) and *Atropa belladonna*

(Hartmann et al. 1986), respectively. To date, 3α-phenylacetoxytropane was found again only in transformed root cultures of a *Brugmansia candida* × *aurea* hybrid (Robins et al. 1990) as well as of *Hyoscyamus* × *györffyi* (Ionkova et al. 1994), whereas the occurrence of 3α-cinnamoyloxytropane is confined to *L. pubiflora*. A stereochemically not determined 3-phenylacetoxy-6,7-epoxytropane, found in the seeds of *Datura ferox*, has been mentioned already above (Vitale et al. 1995).

3.4.2.6 "Dimeric" and "Trimeric" Alkaloids Based on 3α-Hydroxytropanes (T7-D)

Apoatropine is an artefact spontaneously formed from genuine hyoscyamine or its racemate atropine by dehydration forming an atropic acid (2-phenylprop-2-enoic acid) residue (see also Sect. 3.4.1). Two molecules of apoatropine may be dimerized spontaneously yielding two isomeric derivatives, called α- and β-belladonnine, respectively. This reaction is favoured by acid or basic conditions as well as by increased temperatures. Though small amounts of such artefacts may be present in the living plant they are caused mainly by the procedures used for drying and/or extraction of the plant material. These dimerizations are due to the reactivity of the double bond in the atropic acid residue of apoatropine. The latter as well as belladonnine were found, e.g., in dried roots of *Mandragora* spp. but could not be detected in fresh roots (Jackson and Berry 1973). Thus, the belladonnines like their monomer, apoatropine, though often considered still today to be minor alkaloids of hyoscyamine-containing medicinal plants are no natural metabolites. This is also true for the analogous scopadonnines spontaneously formed from genuine scopolamine via aposcopolamine (apohyoscine). Consequently, such artefacts are not integrated in Table 3.1.

However, the basal genus *Schizanthus* which is not able to synthesize hyoscyamine and scopolamine, respectively, is characterized by the genuine, enzymatically catalyzed production of unique dimeric tropanols esterified with the dicarboxylic acids mesaconic acid or itaconic acid as spacers linking the two monomers (Fig. 3.15). One monomer may be 3α-hydroxytropane, the second 3α-hydroxy-7β-angeloyloxytropane, linked by a mesaconic acid moiety (schizanthine C; San-Martin et al. 1987). Another typical example is represented by schizanthine Z involving 3α,7β-dihydroxytropane and 3α-hydroxy-7β-tigloyloxytropane as monomers and itaconic acid as the linking spacer (Muñoz and Cortez 1998). As already discussed above, it should be taken into account that the application of the uniform numbering system of tropanes requires that in many cases compounds designated as C-3, C-6 disubstituted in the literature have to be designated as C-3, C-7 disubstituted. This is again true for the schizanthines (Lounasmaa and Tamminen 1993). The first dimeric congener, discovered in the epigeal vegetative parts of *Schizanthus pinnatus*, was schizanthine B (Ripperger 1979), followed by C–E (*S. grahamii*; San-Martin et al. 1987), X (*S. grahamii*, Muñoz et al. 1991), a C-isomer (*S. littoralis*, Muñoz et al. 1996), and finally Y and Z (*S. porrigens*; Muñoz and Cortes 1998). Unfortunately, the term

"schizanthines" does not include such spacer linked *dimeric* tropanes only, but also 3,7-disubstituted *monomeric* tropanes [schizanthines F–I, K–M; see above (**T3**)].

An unusual "trimeric" tropane alkaloid was isolated from *S. grahamii* (Hartmann et al. 1990). This congener of the schizanthines, named grahamine (Fig. 3.16), is characterized by three acylated 3α,7β-dihydroxytropane moieties thus forming altogether six ester groups with two mesaconic acid moieties (= four ester groups: at C-3α, C-7′β, C-3′α, C-3″α), one cinnamoyl residue (at C-7″β), and one angeloyl residue (at C-7β). Moreover, a cyclobutane ring is formed apparently caused by intramolecular [2+2] cycloaddition from (i) the mesaconic diester partial structure between the first and the second tropane skeleton and (ii) the cinnamic ester partial structure of the third one. Such cycloadditions between two unsaturated moieties are caused photochemically by the UV light of the sun inside the living plant as already shown for the intermolecular dimerization products of cinnamoylcocaine (truxillines), the cinnamic acid analogue of cocaine (Roth 2005).

Unfortunately, the authors caused a confusion in the literature because they named this compound "grahamine" ignoring the fact that a pyrrolizidine alkaloid from *Crotalaria grahamiana* Wight & Arn. (Fabaceae) had already been named grahamine two decades before (Atal et al. 1969). It is evident that it is not useful to create trivial names for natural compounds using only the epithet of the producing organism. Therefore, e.g., "schizangramine" would be more suitable for the trimeric alkaloid from *Schizanthus grahamii*.

The genus *Schizanthus* comprises 12 species, primarily from Chile, with one exception, *S. grahamii*, whose dispersal area reaches Argentina. Cladistic relationships in this genus, based primarily on morphology and chemical characters (tropane alkaloids), have been presented. According to the results of this study the chemical evolution within the genus runs, in parallel from the pyrrolidine to the tropane series, with subsequent unique dimerization or even trimerization (Peña and Muñóz 2002). It would be interesting to compare the results of this study with phylogenetic trees obtained by DNA-based cladistic analyses (see also Sect. 3.7).

3.4.2.7 3β-Acyloxytropanes (T8)

In contrast to the 3α congeners the total number of alkaloids of this structural type identified as solanaceous metabolites is very low. It is confined to 3β-acetoxytropane, 3β-tigloyloxytropane (tigloidine; Fig. 3.13), 3β-(2-methylbutyryloxy)tropane, and 3β-phenylacetoxytropane (Fig. 3.13). However, tigloidine is a characteristic and rather frequent metabolite in the tropane-synthesizing taxa of the family. This is not very surprising since tiglic acid has turned out to be a frequent acyl supplier also for an esterification of 3α-hydroxytropane (see **T1**), for mono- and diesters of 3α,7β-hydroxytropane (**T3**) as well as for mono- and diesters of 3α,6β,7β-trihydroxytropane (**T4**). Tigloidine was discovered as a minor alkaloid (0.1%) in the leaves of *Duboisia myoporoides* by Barger et al. (1937) and later on also identified in other genera of the **Nicotianoideae** [*Symonanthus* (1 species), *Anthocercis* (4), *Cyphanthera* (1)] as well as in five genera of the **Solanoideae** [*Hyoscyamus* (1),

Solandra (4), *Brugmansia* (3), *Datura* (5), *Physalis* (1)]. Surprisingly, 3β-acetoxytropane was identified only in a few species of the **Solanoideae** (*H. albus*, *H. pusillus*, *B. candida* × *aurea*, *D. wrightii*, *P. peruviana*). Finally, 3β-(2-methylbutyryloxy)tropane seems to be confined to *D. inoxia* (Witte et al. 1987).

3β-Tigloyloxy-6-hydroxytropane (Berkov et al. 2005) as well as 3β-tigloyloxy-6-propionyloxy-7-hydroxytropane could be characterized by GC/MS analysis as minor constituents of *D. stramonium* (Doncheva et al. 2004). As already explained repeatedly the substitution at C-6 and C-7, respectively, cannot be elucidated by GC/MS data. Thus, this assignment is questionable again in both cases. In the latter report the occurrence of the very first tropic acid ester with a 3β-configurated hydroxytropane derivative, "3β-tropoyloxy-6-hydroxytropane" has also been published. However, this result requires unequivocal stereochemical confirmation, because it is based on GC/MS analysis which would be only proving with a reference compound which was not available. Furthermore, not even a retention index is given in the report. Such a finding would represent a remarkable scientific discovery, if one takes into account that the Solanaceae in general, especially the genus *Datura,* and finally also this species itself represent taxa which were already well-studied phytochemically.

Rare Unacylated Hydroxytropanes/-*nor*tropanes not Included in Table 3.1. 3α,6β-Dihydroxytropane was detected in *Schizanthus hookeri* and *S. littoralis* only, its 3α,7β-isomer in *Anthocercis viscosa*, *Brugmansia arborea*, *Datura inoxia*, and *Physochlaina alaica*. 3α,7β-Dihydroxy*nor*tropane was identified as a constituent of *Duboisia leichhardtii* (Fig. 3.17); it was absent in *Cestrum nocturnum* and *Solanum dulcamara*. However, its 2α,7β-isomer, a metabolite of several convolvulaceous species (Fig. 3.17), could not be found in any of the three latter solanaceous species (Asano et al. 2001). A strange alkaloid named physoperuvine was discovered in the roots of *Physalis peruviana*, cape gooseberry/ground cherry (Ray et al. 1976). The free base turned out to be present as an equilibrium mixture of the 1β-hydroxytropane form (aminoketal; Fig. 3.17) and the corresponding monocyclic aminoketone form (4-*N*-methylaminocycloheptanone; Ray et al. 1982). This compound-immanent behaviour induced the term "secotropane" for this type of alkaloids. In addition, an (+)-*N*,*N*-dimethylphysoperuvinium salt could be isolated from the same species (Sahai and Ray 1980). The second *N*-methyl group of this derivative stabilizes the tropane skeleton.

In this connection it is necessary to mention already here that a novel group of natural tropane derivatives named calystegines (Sect. 3.5) was discovered a decade later. They are polyhydroxy*nor*tropanes characterized by the common presence of a 1β-hydroxy substituent, thus sharing the aminoketal partial structure with physoperuvine.

Finally, the discovery of methylpseudoecgonine (2α-carbomethoxytropan-3β-ol) in *Datura stramonium* should be mentioned; it represents an isomer of methylecgonine (2ß-carbomethoxytropan-3β-ol), the precursor of cocaine in *Erythroxylum* spp. (Erythroxylaceae). No further occurrence of methylpseudoecgonine in the Solanaceae family was reported to date; however, it was detected in three convolvulaceous species.

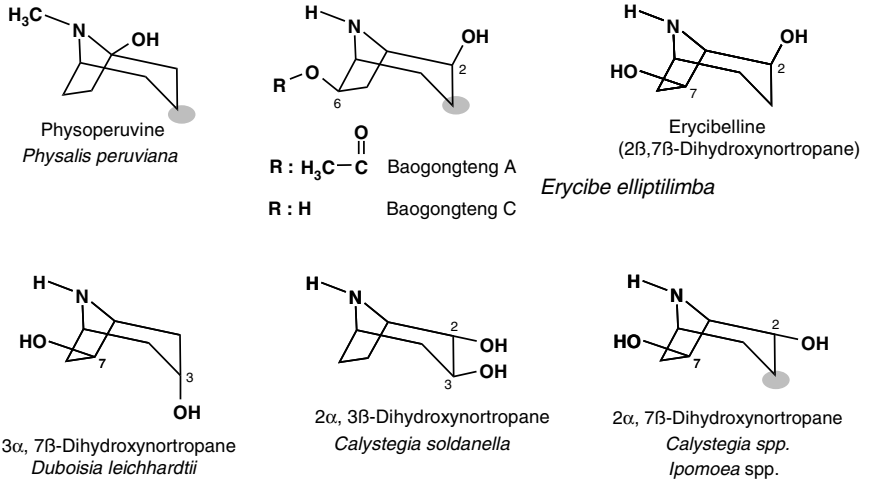


Fig. 3.17 Rare simple mono- and dihydroxylated tropanes / *nor*tropanes discovered in certain species of the Solanaceae (*Duboisia*, *Physalis*) and Convolvulaceae (*Calystegia*, *Erycibe*, *Ipomoea*); highlighted in *grey*: unusual lack of a hydroxyl group at C-3

3.4.2.8 Chemotaxonomic Conclusions

The explanations in this section and the details of Table 3.1 are summarized in Fig. 3.18 in which the provisional phylogenetic tree of Fig. 2.2 is combined with the occurrence of tropane alkaloids (two subclasses) and two other classes of metabolites by plotting four characters on this tree:

- Tropane alkaloids of the structural types **T5**, **T6**, and **T7-A – T7-B** (**subclass A**; very poisonous ester alkaloids with a tropic acid residue, e.g., hyoscyamine/atropine, scopolamine and their derivatives)
- Tropane alkaloids of the structural types **T1 – T4, T7-C**, and **T7-D** (**subclass B**; ester alkaloids of lower or unknown toxicity with acid residue other than tropic acid and its derivatives)
- Steroidal alkaloids/glycoalkaloids
- Withasteroids

Figure 3.18 shows that tropane alkaloids of **subclass A** are present in the genus *Latua*, in the complete subfamily **Nicotianoideae** except *Nicotiana*, the complete Hyoscyameae clade, the genera *Mandragora* and *Solandra*, and finally the complete Datureae clade. On the other hand, such tropane alkaloids are lacking in all of the most advanced as well as basal branches. Obviously, tropanes of subclass A represent synapomorphic characters of advanced solanaceous taxa. The capability to synthesize these characters was lost during the evolution of still more advanced branches starting with the Solaneae clade.

In contrast, tropane alkaloids of **subclass B** were already discovered in the basal **Schizanthoideae** and are still present in *Physalis*, one of the most advanced genera.

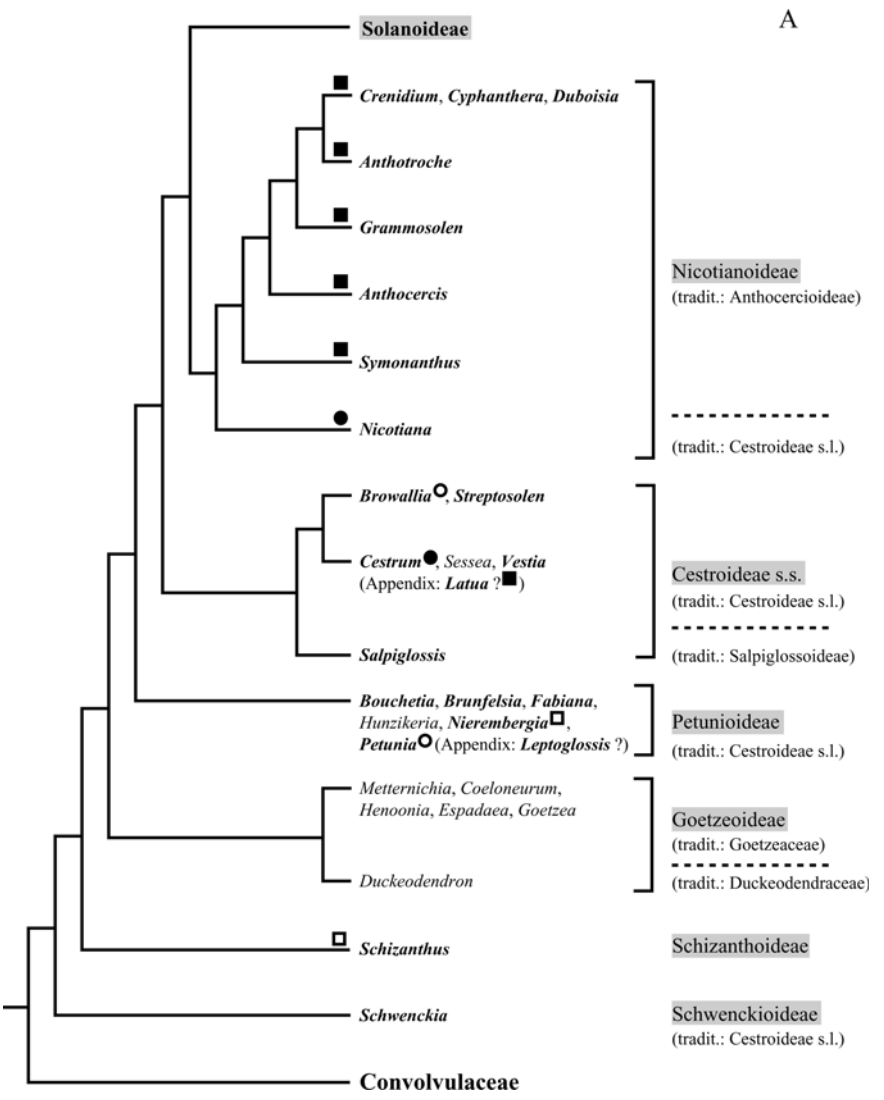


Fig. 3.18 Chemotaxonomy and phylogeny of the Solanaceae. The provisional phylogenetic tree of Fig. 2.2 is shown here again though without terms for the clades in order to have a clear structure in favour of metabolite symbols. Plotted on the tree is the occurrence of three dominant and characteristic classes of secondary metabolites of the Solanaceae family with two subclasses in one case: (I) Tropane alkaloids (two subclasses), (II) steroidal alkaloids/glycoalkaloids, (III) withanolides/withasteroids. These metabolites are indicated by the following symbols. Co-occurrence of different **classes** (rather rare) is also indicated, i.e., by two corresponding symbols. *Filled square*: tropane alkaloids of the structural types **T5**, **T6**, and **T7-A – T7-B** (very poisonous ester alkaloids with a tropic acid residue, e.g., hyoscyamine/atropine, scopolamine and their derivatives). Taxa highlighted by a *filled square* may also show co-occurrence with tropane alkaloids of the following subclass according to Table 3.1; however, this is not highlighted in such cases. *Open square*: tropane alkaloids of the structural types **T1 – T4, T7-C**, and **T7-D** (ester alkaloids of

(continued)

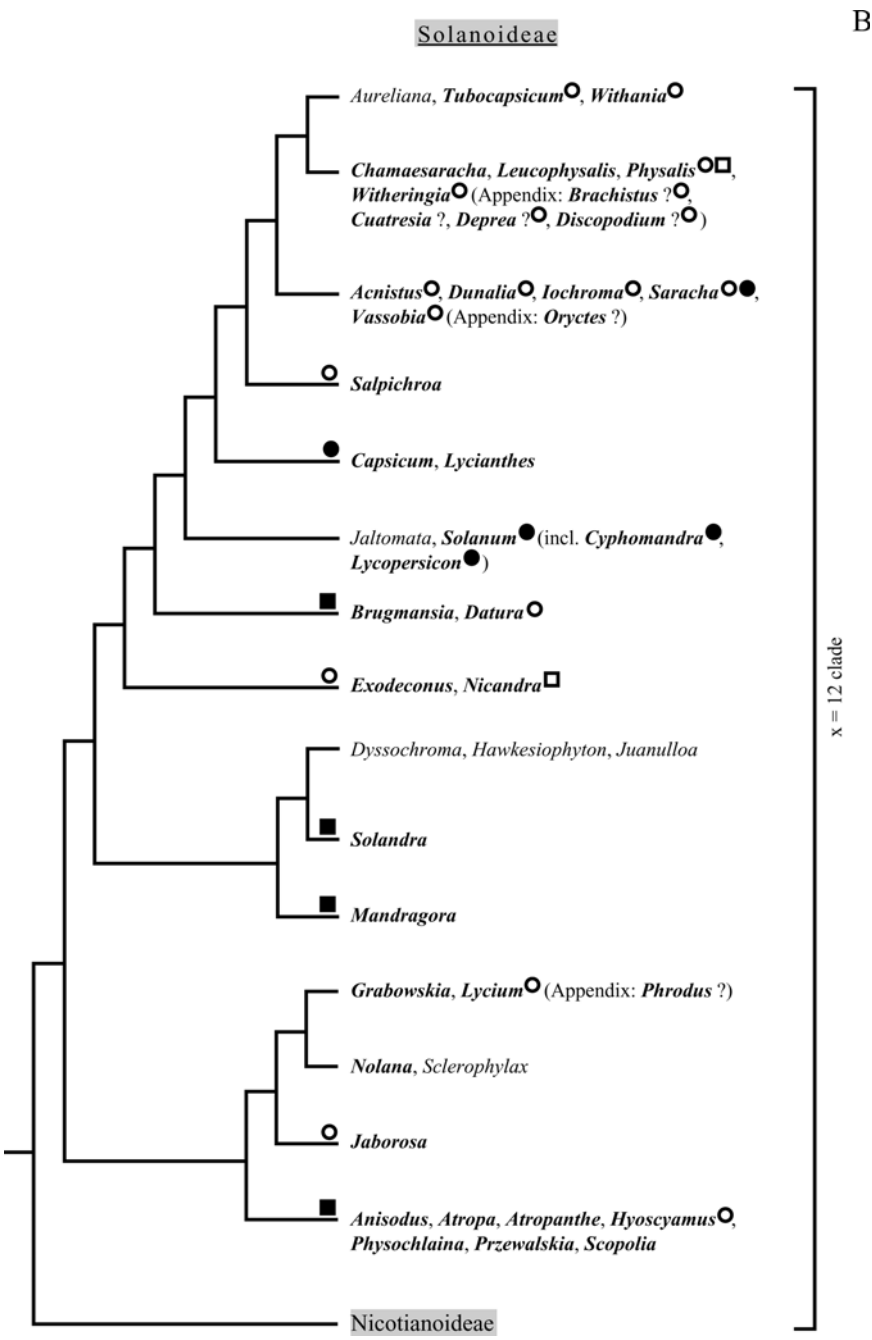


Fig 3.18 (continued) lower or unknown toxicity with acid residue other than tropic acid and its derivatives). Taxa highlighted by an open square lack tropane alkaloids of the preceding subclass; *Filled circle*: steroidal alkaloids/glycoalkaloids (see Sect. 7.8.2); *Open circle*: withanolides/withasteroids (see Sect. 7.10.3); in case of *Petunia*: petuniasteroids (Sect. 7.11). *Symbols above the branches* indicate that all of the corresponding genera are characterized by the occurrence of this class of metabolites; *symbols behind a genus* indicate that this is true only for the corresponding, individual genus

Nevertheless, it must be pointed out that the specific alkaloids of the South American genus *Schizanthus* are unique in the family. Though not indicated in Fig. 3.18, tropanes of subclass B are also frequently present in taxa which are characterized by tropanes of **subclass A** as demonstrated in Table 3.1. If not, this may be due to the lack of corresponding analyses. Subclass B alkaloids may be in part *apomorphic* characters, too. Thus, **T2-T4**-type tropanes show a similar distribution like **subclass A** congeners [present between **Nicotianoideae** (except *Nicotiana*) and Datureae clade, absent in basal and most advanced branches].

However, at least one group of **subclass B** type metabolites, namely **T1**-type tropane alkaloids as well as calystegines (see Sect. 3.5) represent *plesiomorphic* characters since both are also metabolites of the Convolvulaceae family. Such metabolites are found throughout the family. Obviously, they are the basis for *apomorphic* developments.

3.4.3 Occurrence in the Convolvulaceae (Table 3.2)

It has to be pointed out that most references may be not cited directly in the following text but indirectly, i.e., by the last column of Table 3.2. The type numbers, e.g., **T4**, of Table 3.1 (Solanaceae) and Table 3.2 (Convolvulaceae) are **not** compatible due to different requirements in each of the two families. For details see legends of the corresponding table.

Our knowledge of the occurrence and distribution of tropane alkaloids in the Convolvulaceae family is based on (i) the isolation and structure elucidation of Convolvulaceae-specific metabolites (**T3**, **T4**, **T6-A**, **T6-B**, **T9**) and (ii) extensive GC/MS data. This analytical method is of remarkable advantage due to its high resolution and sensitivity. Thus, even very low concentrations of a metabolite can be identified unequivocally.

Table 3.2 (tropanes) in combination with Table 3.9 [calystegines (polyhydroxy*nor*tropanes)] shows that tropanes could be detected in 152 out of 166 convolvulaceous species (92%) throughout the family in all 12 tribes, almost all genera checked (28 out of 29) and even in all traditional sections of large genera (*Ipomoea*, *Merremia*). Thus, these alkaloids can be considered as a consistent trait of the family. No tropanes at all could be identified only in the genus *Xenostegia* [*X. medium*, *X. tridentata* ("Merremieae")] and further 11 species from different tribes: *Bonamia dietrichiana* (Cresseae), *Jacquemontia corymbulosa*, *J. paniculata* (Jacquemontieae), *Convolvulus scoparius*, *P. marginata* (Convolvuleae), *Operculina codonantha*, *O. pteripes* ("Merremieae"), *I. tuxtlensis*, *I. coccinea*, *I. neei*, *I. meyeri* (Ipomoeeae). On the other hand, closely related species within a genus can have widely diverging tropane alkaloid pattern in contrast to the Solanaceae.

Only those species have been declared to be tropane-*negative* for which data of both organs, epigeal vegetative parts and roots have been available. Furthermore, due to the fact that the lists of species in Tables 3.2 and 3.9 are not identical (though many species are included in both tables), species found to be tropane-*negative* in

the calystegine analysis have only been declared to be tropane-*negative* if data of these species are also integrated in Table 3.2. Otherwise they have not been taken into account, e.g., *Hildebrandtia austinii* (Cresseae), because it can not be excluded that lipophilic tropanes might be present in such species though not determined due to the lack of sufficient plant material.

3.4.3.1 Simple Tropanes (T1 in Table 3.2)

This structural type includes biogenetically basal tropanes with an oxygen function at C-3 (=O or –OH), in certain cases with one or more additional hydroxyl function(s) at other positions, e.g., at C-6.

T1-A Type Metabolites. They involve tropan-3-one ("tropinone"), 3α-hydroxytropane ("tropine"), 3β-hydroxytropane ("pseudotropine") and/or their *nor* derivatives. These compounds can be assumed as plesiomorphic characters shared with the Solanaceae family; they represent biogenetically basal members for all of the other types of tropanes including the calystegines. Thus, it is not surprising that **T1-A** type metabolites could be detected in all tropane-positive species without exception. However, they are the only type of tropane alkaloids in the following taxa: *Jacquemontia pentantha* (Jacquemontieae), *Cuscuta australis* (Cuscuteae), *Odonellia hirtiflora* (Aniseieae), *Calystegia macrostegia* ssp. *cyclostegia*, *Convolvulus* (3 spp.), *Polymeria* (2 spp.) (Convolvuleae), *Hewittia sublobata*, *Merremia* (4 spp.), *Operculina aequisepala* ("Merremieae"), *Argyreia nervosa*, *Ipomoea* (20 spp.), *Turbina abutiloides* (Ipomoeeae). This early end of the tropane pathway in such cases is only "surpassed" by those taxa which even lost the ability to synthesize these plesiomorphic characters, i.e., the 13 taxa mentioned above to be tropane-*negative*. However, almost all of even these taxa, tropane-negative as well as simple tropanes producing, have kept the ability to synthesize simple pyrrolidines which share the first part of the pathway with tropanes (Table 3.2).

T1-B Type Metabolites. See calystegines (polyhydroxy*nor*tropanes; Sect. 3.5).

Dihydroxy*nor*tropanes (T1-C). These metabolites occupy a position between the classical lipophilic tropanes, e.g., 3-acyloxytropanes, and the hydrophilic calystegines. 2β,6β-Dihydroxy*nor*tropane (baogongteng C) and its 2β,7β isomer (erycibelline) (Fig. 3.17) were discovered in *Erycibe elliptilimba* (Lu et al. 1986). The corresponding 2α,7β isomer was identified in *Calystegia japonica, C. sepium*, C. *soldanella, Ipomoea batatas, I. carnea, I. hederifolia* sub nom. *Quamoclit angulata* (Asano et al. 2001; *C. sepium*: Scholl et al. 2001 2003). However, this alkaloid was no constituent of *I. nil*, *I. obscura*, and *I. pes-caprae*. Finally, 2α,3β-dihydroxy*nor*tropane could be detected only in *Calystegia soldanella* (Asano et al. 2001). Apparently, such dihydroxy*nor*tropanes and their closely related derivatives are rare metabolites. It is somehow obvious to assume that they may represent precursors of the calystegines (polyhydroxy*nor*tropanes); whether this is really the case remains to be elucidated. Alternatively, it may be that they are only formed occasionally as minor by-products. Anyhow, the main difference in comparison with the calystegines is

the lack of the calystegine-specific hydroxyl at C-1. Unique *N*-substituted derivatives, *N*-carbomethoxy- and *N*-carboethoxy-3β-hydroxy*nor*tropane, could be identified as minor constituents of the roots of *Bonamia trichantha*. Their presumable precursors, 3β-hydroxytropane and its *nor* congener, represented the principal tropane alkaloids in conspicuously high concentrations (Eich and Witte, unpublished results). With the exception of traces of 3-*nor*tropanone, the presumable precursor of 3β-hydroxy*nor*tropane, no further tropanes including calystegines could be detected. This tropane alkaloid pattern is very unusual. In addition simple pyrrolidines were detected (principal alkaloid: cuscohygrine).

***O*-Acylated Dihydroxy*nor*tropane (T1-D).** Baogongteng A (Fig. 3.17), the 6-acetyl derivative of baogongteng C, was identified first as a constituent of *Erycibe obtusifolia* roots (Yao et al. 1981); later it was also found as a constituent of *E. elliptilimba* (Lu et al. 1986) and *E. hainanensis* (Wang et al. 1989).

6β-Hydroxytropan-3-one (T1-E). This rare alkaloid was only detected in seven species: *Bonamia semidigyna*, *Evolvulus nummularius* (Cresseae), *Jacquemontia tamnifolia* (Jacquemontieae), *Convolvulus hermanniae* (Convolvuleae), *Merrremia aegyptia, M. dissecta, M. guerichii* ("Merremieae").

Methylpseudoecgonine (2α-Carbomethoxytropan-3β-ol, T1-F) (see Fig. 3.24). This metabolite was discovered in *Datura stramonium* (Solanaceae) and represents an isomer of methylecgonine (2ß-carbomethoxytropan-3β-ol), the precursor of cocaine in *Erythroxylum* spp. (Erythroxylaceae). Methylpseudoecgonine could be detected in *Bonamia spectabilis*, *Merremia aegyptia*, and *M. gemella*.

3.4.3.2 Esters of 3α-Hydroxytropane/-*nor*tropane (T2–T6)

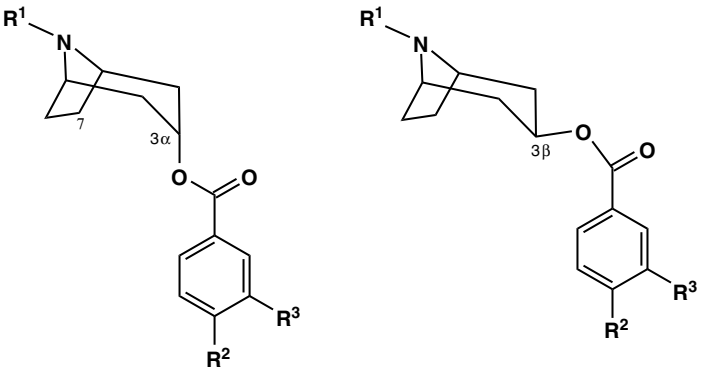
Any type of these esters could be detected in 75 convolvulaceous species. The number of 3α-acyloxytropane-negative species – as far as data are suitable to be evaluated – was almost the same (77 spp.). However, species which show all of the more frequent structural types (**T2**–**T5**), i.e., with the exception of the rarely occurring alkaloids of type **T6**, are confined to *Dichondra sericea* (Dichondreae) and *Bonamia semidigyna* (Cresseae). Even the co-occurrence of only three of these more frequent types is limited to *Ipomoea argillicola, I. aquatica* (both: **T2** – **T4**), *I. plebeia* (Ipomoeeae), and *Maripa panamensis* (Maripeae) (both: **T2**, **T3**, **T5**).

Aliphatic Esters of 3α-Hydroxytropane/-*nor*tropane (T2). These metabolites seem to be frequent in three tribes (Erycibeae, Dichondreae, Cresseae). Apparently, they do not occur in the Jacquemontieae. They turned out to be rather rare in the Convolvuleae (only 6 spp. out of 31) as well as in the "Merremieae" (4 spp. out of 23). In the largest tribe, Ipomoeeae, one third of the species (24 spp. out of 73) were **T2**-positive. However, the occurrence was commonly restricted to 3α-acetoxytropane and its *nor* congener, respectively. In contrast to the Solanaceae family there were only some convolvulaceous species which showed – in addition to the acetyl derivatives – other **T2**-type metabolites with different acyl residues. These residues were identical with

some of the sister family (propionyl, *n*-butyryl, isobutyryl, 2-methylbutyryl) and in addition 3-hydroxy-2-methylbutyryl. Corresponding alkaloids like 3α-propionyloxytropane, 3α-*n*-butyryloxytropane, butropine, or valtropine could be detected in *Evolvulus alsinoides* (Cresseae), *Merremia guerichii*, *M.vitifolia* ("Merremieae"), and *Falkia repens* (Dichondreae). *M. guerichii* (Jenett-Siems et al. 2005b) and *F. repens* (Ott et al. 2007) showed an especially rich pattern. In addition to the metabolites mentioned above a hexanoyl congener and several alkaloids with hydroxylated acyl residues could be identified in *M. guerichii*, e.g., 3-(hydroxy-*n*-butyryloxy)tropane, 3-(hydroxypentanoyloxy)tropane and their *nor* derivatives. Besides alkaloids with saturated aliphatic acyl residues a congener with an unsaturated acyl residue was characterized as a constituent of *F. repens*: 3α-tigloyloxytropane, a common constituent of tropane-positive solanaceous species. This metabolite is rather rare in the Convolvulaceae; it could be detected – besides *F. repens* – only in *Convolvulus cneorum* and *Ipomoea lonchophylla*. All in all, aliphatic esters of 3α-hydroxytropane/-*nor*tropane – with the exception of acetyl derivatives – are rather rare in the Convolvulaceae family compared with the Solanaceae.

Simple Aromatic Esters of 3α-Hydroxytropane/-*nor*tropane (T3). This structural type is named "simple" in order to separate it from the prenylated aromatic esters (**T4**-type); both types are Convolvulaceae-specific: Though there a few exceptions most of the individual **T3**-type compounds are confined to this family (Table 3.7). This is especially true of the alkaloids listed in Fig. 3.19 with trivial names already indicating their origin, e.g., convolamine. The common structural characteristic of the Convolvulaceae-specific **T3**-tropanes is a substituted benzoyl moiety with five variations concerning its substitution pattern: (i) no substituent, (ii) 4-hydroxy, (iii) 4-methoxy, (iv) 4-hydroxy-3-methoxy, resulting in a vanilloyl residue, and (v) 3,4-dimethoxy, resulting in a veratroyl residue. The groups with the substitution pattern (i)–(iv) are represented each by both, the corresponding acyloxytropane and its *nor* congener. The most diverging group of compounds turned out to be the one with the substitution pattern (v), i.e., the veratroyl moiety. Altogether 10 alkaloids show a unique differentiation with regard to their additional, rare *N*-substituents, e.g., formyl (confoline, discovered in *Convolvulus subhirsutus*), acetyl (convolicine, discovered in *C. krauseanus*), isopropyl (convosine; discovered in *C. subhirsutus*); furthermore, two of them represent *N*,*N*'-spacer-linked dimeric congeners bridged by a carbonyl link (subhirsine; discovered in *C. subhirsutus*, and a diethylene link (convolvidine; discovered in *C. pseudocantabricus*), respectively (structures: Fig. 3.19; occurrence in detail: Table 3.5). Convolvidine originally had been reported to be an artefact (Yunosov et al. 1958) but this could be disproved (Aripova and Yunosov 1986b).

T3-type metabolites could be detected in eight genera belonging to seven tribes, altogether 29 species (about 18% of all species checked): Dichondreae [*Dichondra* (1 genus out of 2 genera checked)], Cresseae [*Bonamia*, *Evolvulus* (2/2)], Maripeae [*Maripa* (1/1)], Jacquemontieae [*Jacquemontia* (1/1)], Convolvuleae [*Convolvulus* (1/3)], "Merremieae" [*Merremia* (1/3)], Ipomoeeae [*Ipomoea* (1/6)]. With the exception of the large genera *Convolvulus* and *Ipomoea* all **T3**-positive species are listed



Substituents			3α-Substituted tropane	3β-Substituted tropane
R^1	R^2	R^3	derivatives	derivatives
CH_3	H	H	3α-Benzoyloxytropane[a]	Tropacocaine[b]
H	H	H	3α-Benzoyloxynortropane[c]	Nortropacocaine[d]
CH_3	OH	H	3α-(4-Hydroxybenzoyloxy) tropane	3β-(4-Hydroxybenzoyloxy) tropane
H	OH	H	3α-(4-Hydroxybenzoyloxy) nortropane	
CH_3	OCH_3	H	Datumetine[e]	
H	OCH_3	H	Merresectine A	
CH_3	OH	OCH_3	Phyllalbine[f]	Concneorine
H	OH	OCH_3	Convolidine	Norconcneorine
H-C=O	OH	OCH_3	Confolidine	
CH_3	OCH_3	OCH_3	Convolamine	3β-Veratroyloxytropane
CH_3 + O	OCH_3	OCH_3	Convolamine N-oxide	
CH_3	OCH_3	OCH_3	Convolacine: **7-acetoxy-** (= 7-acetoxyconvolamine)	
H	OCH_3	OCH_3	Convolvine	3β-Veratroyloxynortropane
H-C=O	OCH_3	OCH_3	Confoline	
H_3C-C=O	OCH_3	OCH_3	Convolicine	
OH	OCH_3	OCH_3	Convoline	
$CH(CH_3)_2$	OCH_3	OCH_3	Convosine	
X-C=O[g]	OCH_3	OCH_3	Subhirsine [Fig. 3.24]	
X-CH_2-CH_2	OCH_3	OCH_3	Convolvidine [Fig. 3.24]	

[a] Discovered as "benzoyltropeine" in *Erythroxylum coca* LAM., Erythroxylaceae (Anonymous, about 1900, according to Wolfes and Hromatka 1933); this compound is sometimes also called "benztropine" in the literature (Caroll et al. 1992)
[b] Discovered in the leaves of *E. coca* from Java (Giesel 1891)
[c] Discovered in *E. macrocarpum* O.E. SCHULZ and *E. sideroxyloides* LAM. (Al-Said et al. 1986)
[d] Discovered in *E. mamacoca* MART. (El-Imam et al. 1985)
[e] Discovered in *Datura metel* L., Solanaceae (Siddiqui et al. 1986)
[f] Discovered in *Phyllanthus discoides* MÜLL. ARG., Euphorbiaceae (Parello et al. 1963)
[g] *N, N'*-Spacer-linked dimer, i. e., X in R_1 = a second convolvine moiety

Fig. 3.19 Simple (i.e., non-prenylated) aromatic 3α- and 3β-acyloxytropanes of the Convolvulaceae. *Blank spaces*: compound unknown from nature

Table 3.5 Occurrence of simple (i.e., unprenylated) aromatic 3α-acyloxytropanes in convolvulaceous species. All species with these metabolites as constituents are included except different additional species of the large genera *Convolvulus* and *Ipomoea*, respectively (see Table 3.2). For comparison: Occurrence of (3α-) merresectines

Species[a]	**Simple aromatic 3α-acyloxytropanes**										**References**
Genera arranged according to phylogenetic classification (basal taxa at the top), species alphabetically; authorities see Table 3.2	3α-Benzoyloxytropane	3α-(4-Hydroxybenzoyloxy)tropane	Datumetine[b] (4-methoxybenzoyl-)	Merresectine A (*nor*datumetine)	Phyllalbine (vanilloyl-)	Convolidine (*nor*phyllalbine)	Convolamine (veratroyl-)	Convolvine (*nor*convolamine)	Confoline (formyl*nor*convolamine)	**Merresectines** (details: Table 3.6)	
Dichondra sericea	-	-	-	-	+	-	-	-	-	+	(1)[c]
Erycibe micrantha	-	-	-	-	+	-	-	-	-	-	(1)[c]
Bonamia brevifolia	-	+	-	-	-	-	-	-	-	-	(1)
B. semidigyna	-	+	-	-	+	-	-	-	-	+	(2)
Evolvulus nummularius	+	-	-	-	-	-	-	-	-	-	(1)[c]
Maripa panamensis	-	-	-	-	+	-	-	-	-	-	(1)[c]
Jacquemontia tamnifolia	-	-	+	-	-	-	-	-	-	+	(1),(2)
Convolvulus cneorum	-	+	-	-	+	isol.	isol.	+	-	-	(3),(4)
C. dorycnium	-	-	+	+	-	-	+	+	+	-	(1)
C. floridus	-	-	-	-	+	+	+	+	-	-	(1)
C. krauseanus					isol.	isol.	isol.	isol.			(5),(6)
C. pseudocantabricus							isol.	isol.			(7),(8)
C. subhirsutus					isol.	isol.	isol.	isol.	isol.		(9) – (11)
Merremia dissecta	-	+	isol.	isol.	-	-	-	-	-	isol.	(4),(12)
M. guerichii	-	+	-	-	+	-	-	-	-	+	(4)
M. kentrocaulos	-	+	-	-	+	-	-	-	-	+	(4)
M. quinquefolia	+	-	+	isol.	-	-	-	-	-	isol.	(4)
M. vitifolia	+	-	-	-	-	-	-	-	-	+	(4)
Ipomoea argillicola	+	+	-	-	-	-	-	-	-	+	(1)
I. lonchophylla	-	+	-	-	+	-	-	-	-	-	(1)
I. tricolor cv. 'Heavenly Blue'	+	+	+	+	+	-	-	-	-	+	(1)

isol. = Compound isolated from this species; + = compound detected by GC-MS; - = compound not detected by GC-MS; **no symbol** = no data in the literature

[a] Combined data based on the GC/MS analysis of roots and epigeal vegetative parts if not indicated otherwise

[b] Discovered in *Datura metel*, Solanaceae (Siddiqui et al. 1986)

[c] Data based on the GC/MS analysis of epigeal vegetative parts only due to lack of root material

References: (1) Schimming 2003; (2) Henrici 1996; (3) Mann 1997; (4) Jenett-Siems et al. 2005b; (5) Aripova et al. 1977; (6) Aripova and Yunusov 1979; (7) Orechoff and Konowalowa 1933, 1934, 1935, 1937; (8) Yunusov et al. 1958; (9) Aripova et al. 1972; (10) Sharova et al. 1980; (11) Aripova and Yunusov 1986a; (12) Weigl et al. 1992.

in Table 3.5. Besides six *Convolvulus* spp. and three *Ipomoea* spp., respectively, already included in that table further seven species of the former genus and further three species of the latter one were found to be **T3**-positive (Table 3.4). Remarkably, two *Ipomoea* spp. [*I. argillicola, I. tricolor* (both cultivars checked)] well-known to contain ergoline alkaloids (see Sect. 4.2) showed co-occurrence with simple aromatic 3α-acyloxytropanes. However, the four remaining **T3**-positive *Ipomoea* spp. (*I. aquatica*, *I. lonchophylla*, *I. plebeia*, *I. eriocarpa*) were ergoline-negative.

Orechoff and Konowalowa (1933) reported in the first paper on tropanes from a convolvulaceous species at all, *Convolvulus pseudocantabricus*, a yield from the seeds of 0.52% with regard to the crude alkaloid fraction [principal compound: convolvine (90%)]. However, the epigeal vegetative parts contained convolvine and convolamine in equal shares (Orechoff and Konowalowa 1935). The total alkaloid content did not exceed 0.5% in the seeds and 0.4% in the epigeal vegetative parts of *C. subhirsutus* (adult plants; young plants: 2.1%). The roots of adult plants yielded 4.1% total alkaloids (Yunusov et al. 1958). Roots of *Convolvulus krauseanus* were reported to contain 0.62% total alkaloids (convolvine, convolamine, convolidine).The content of the corresponding epigeal parts were determined to be 0.82% total alkaloids (including convolicine and phyllalbine as well as the alkaloids of the roots) in the beginning of June and only 0.16% in the middle of August, when the plants were fructiferous already, respectively (Aripova et al. 1977; Aripova and Yunusov 1979).

Neither such alkaloids nor any other type of acyloxytropanes were present in the small tribe **Aniseae**, represented with three species checked (out of three genera). The absence of **T3**-type compounds in the remaining two tribes checked may be due to the fact that only epigeal vegetative parts were available (Erycibeae) or just only one species was checked (Cuscuteae).

Since simple aromatic esters of 3α-hydroxytropane/-*nor*tropane are absent in the Solanaceae family [the only exception (datumetine from *Datura metel*) can be interpreted as a convergence], these **T3**-type alkaloids occurring throughout many tribes of the Convolvulaceae can be considered as apomorphic characters of this family.

Prenylated Aromatic Esters of 3α-Hydroxytropane/-*nor*tropane ((3α-) Merresectines B-H; T4-type). This group of tropane alkaloids is unique in the plant kingdom; thus, they represent also convolvulaceous apomorphic characters. They were discovered as constituents of the roots of *Merremia dissecta* and therefore named merresectines (Weigl 1992; Weigl et al. 1992; Jenett-Siems et al. 1998b, 2005b). It became evident that the acyl moiety of merresectine B was kurameric acid (Fig. 3.20) which was already known as an acyl component conjugated with choline or a necine base, constituents of *Liparis kurameri* FRENCH & SAV. and *L. kumokiri* F. MAEKAWA (Orchidaceae), respectively (Nishikawa et al. 1967). Kurameric acid, the 4-β-D-glucoside of nervogenic acid [3,5-bis-(3-methylbut-2-enyl)-4-hydroxybenzoic acid], implicates that merresectine B turned out to be the first glycosidic tropane alkaloid at all. This compound is not detectable directly by GC/MS analysis due to its monosaccharide component; therefore it is not included in Table 3.6. However, it is detectable indirectly as its aglycone, merresectine C, after decomposition in the instrument. Nevertheless, the glycosidic congener B could be *isolated* – in addition to *M. dissecta* – from the roots of *M. cissoides*,

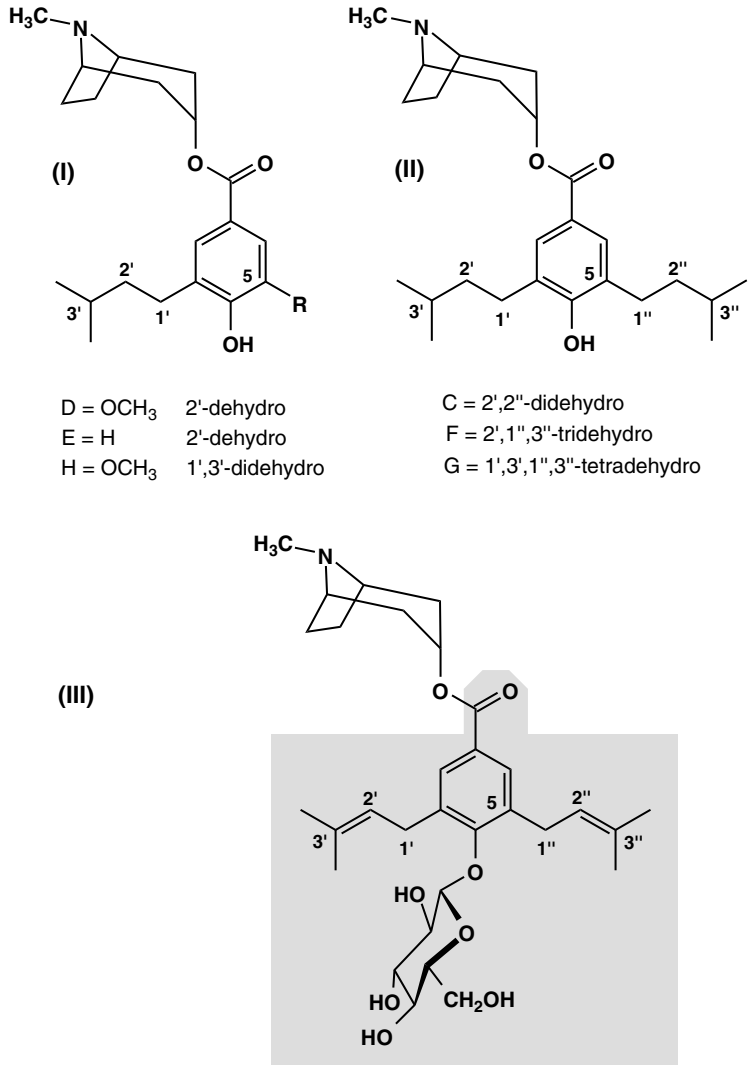


Fig. 3.20 Merresectines B–H, prenylated aromatic 3α-acyloxytropanes, confined to the Convolvulaceae; structural variations with regard to (i) the substitution pattern at C-5 and (ii) different numbers of double bonds in one or both 3-methylbutane residue(s): (I) Monoprenylated aglycones (merresectines D, H (R=OCH_3), E (R=H)); (II) Diprenylated aglycones (merresectines C, F, G); (III) Merresectine B = merresectine C β-D-glucoside; highlighted in *grey*: Kurameric acid residue

M. quinquefolia, *Convolvulus sabatius* ssp. *mauritanicus* (Mann 1997), and *C. siculus* (Jenett-Siems 1996). Since the aglycone, merresectine C, was also *isolated* besides the glycoside, merresectine B, from the roots of *M. dissecta* (Henrici 1996), a presence of the aglycone C documented by GC/MS analysis in further species (Table 3.6) may be due to a fragmentation of the glycoside in the instrument and/or a genuine occurrence of the aglycone. (Cave: merresectine A, the first alkaloid isolated from *M. dissecta*, is a *simple*, *non-prenylated* **T3**-type congener; Table 3.5)).

Table 3.6 Occurrence of simple and prenylated aromatic 3β-acyloxytropanes in convolvulaceous species. As far as reported in the literature all convolvulaceous species with simple aromatic 3β-acyloxytropanes and/or 3β-merresectines as constituents are included. Capital letters in the columns of both isomeric types of merresectines indicate the specific compound according the structures of Fig. 3.20 (C - H)

Isol = compound isolated from this species; + = compound detected by GC-MS; - = compound not detected by GC/MS

Species[a]	**Simple aromatic 3β-acyloxytropanes**							**References**
Genera arranged according to phylogenetic classification (basal taxa at the top), species alphabetically; authorities see Table 3.2	Tropacocaine (3β-benzoyloxytropane)	3β-(4-Hydroxybenzoyloxy)tropane	Concneorine (3β-vanilloyloxytropane)	3β-Veratroyloxytropane	**Simple aromatic 3α-acyloxytropanes**[b]	**3β-Merresectines**	**(3α-) Merresectines**	
Erycibe micrantha	-	-	-	-	+	C	-	(1)
Dichondra sericea	-	-	-	-	+	CE	CD	(1)[c]
Bonamia semidigyna	-	+	+	-	+	DF	CDF	(1),(2)
B. spectabilis	-	-	+	-	-	-	E	(1)
Evolvulus nummularius	+	-	-	-	+	-	-	(1)[c]
Maripa panamensis	-	+	+	-	+	-	-	(1)[c]
M. nicaraguensis	-	+	+	-	+	-	-	(1)[c]
Jacquemontia tamnifolia	-	-	-	-	-	C	CF	(1),(2)
Convolvulus cneorum	-	+	isol	+	+	-	-	(1),(3)
C. floridus	-	-	+[d]	+[d]	+	-	-	(1)
C. graminetinus	-	-	+	-	-	-	-	(1)
C. sabatius ssp. *mauritanicus*	-	+	-	-	-	E	C-F	(3),(4),(5)
Merremia aegyptia	-	-	-	-	-	F	CF	(1),(6)
M. cissoides	-	+	+	-	-	CEF	CEH	(1),(3),(6)
M. dissecta	-	-	-	-	+	CF	CFG	(1),(2),(6)
M. guerichii	-	+	+	-	+	CDF	C-F	(1),(6)
M. kentrocaulos	-	+	+	-	+	CDF	C-F	(1),(6)
M. quinquefolia	-	-	-	-	+	CE	C	(1),(3),(6)
M. vitifolia	-	-	-	-	-	C	CEF	(1),(5)
Ipomoea argillicola	+	-	-	-	+	-	CE	(1)
I. lonchophylla	-	+	+	-	+	-	-	(1)
I. muelleri	-	+	-	-	-	E	-	(1)
I. sloteri cv. 'Cardinal'	+[d]	-	-	-	-	-	CF	(1)

[a] Combined data based on the GC/MS analysis of roots and epigeal vegetative parts if not indicated otherwise (see [c])

[b] For details see Table 3.5

(continued)

Table 3.6 Occurrence of simple and prenylated aromatic 3β-acyloxytropanes in convolvulaceous species. As far as reported in the literature all convolvulaceous species with simple aromatic 3β-acyloxytropanes and/or 3β-merresectines as constituents are included. Capital letters in the columns of both isomeric types of merresectines indicate the specific compound according the structures of Fig. 3.20 (C - F) (continued)

[c] Data based on the GC/MS analysis of epigeal vegetative parts only due to lack of root material
[d] The corresponding *nor* (*N*-demethyl) congener has also been detected

References: (1) Schimming 2003; (2) Henrici 1996; (3) Mann 1997; (4) Jenett-Siems et al. 1998b; (5) Eich, unpublished results; (6) Jenett-Siems et al. 2005b

Besides the **di**prenylated merresectine B two **mono**prenylated congeners were discovered sharing a glycosidic character with B: Merresectine D β-D-glucoside was isolated from the roots of *M. guerichii* and merresectine E β-D-glucoside from the roots of *C. sabatius* ssp. *sabatius*; again both metabolites were structurally elucidated by spectral data (Jenett-Siems et al. 2005b). With regard to the occurrence in further species (Table 3.6) it has to be taken into account that again only the aglycones were detectable directly by GC/MS analysis due to the reasons already mentioned above. Based on the structural knowledge of the congeners B–E, three further aglycones F–H could be characterized structurally by GC/MS data (Fig. 3.20). It may be assumed that these three congeners occur also as glycosides in the corresponding plants. *Merremia guerichii*, a perennial suffrutex endemic to certain areas of Namibia (southern Africa), was found to contain even a disaccharide of merresectine C whose structure remains to be elucidated (Jenett-Siems, personal communication). This species is generally very remarkable: It showed the most extensive alkaloid pattern of all tropane-positive convolvulaceous species checked (Jenett-Siems et al. 2005b).

The co-occurrence of simple aromatic esters (**T3**) and prenylated aromatic esters (**T4**) of 3α-hydroxytropane could be documented in 14 species (Tables 3.2, 3.5 and 3.6): *Dichondra sericea* (Dichondreae), *Bonamia semidigyna* (Cresseae), *Jacquemontia tamnifolia* (Jacquemontieae) *Convolvulus sabatius*, *C. siculus* (Convolvuleae), five *Merremia* spp. ("Merremieae"), four *Ipomoea* spp. (Ipomoeeae). On the other hand, there are 15 species which are apparently unable to synthesize the **T4**-type though they turned out to contain **T3**-type congeners, e.g., *B. brevifolia*, *Evolvulus nummularius* (Cresseae), *Maripa panamensis* (Maripeae), *Calystegia silvatica*, nine *Convolvulus* spp. (Convolvuleae), *I. lonchophylla, I. plebeia* (Ipomoeeae). Finally, it should be mentioned that **T4**-type alkaloids could be detected in *B. spectabilis*, *E. alsinoides* var. *decumbens*, three other *Merremia* spp., and five other *Ipomoea* spp. without any **T3**-type congener. However, this is not very surprising since certain simple aromatic esters are assumed to be precursors of the merresectines B–H (Fig. 3.21). In these cases the concentration of the precursors might have been below the detection level. Thus, altogether 24 convolvulaceous species (14 **T3**-/**T4**-positive species and 10 **T4**-positive species) could be characterized by the occurrence of merresectines (merresectine A, belonging to **T3**, not included). This amounts to a percentage of about 15 with regard to all species checked. However, these Convolvulaceae-specific metabolites commonly do not

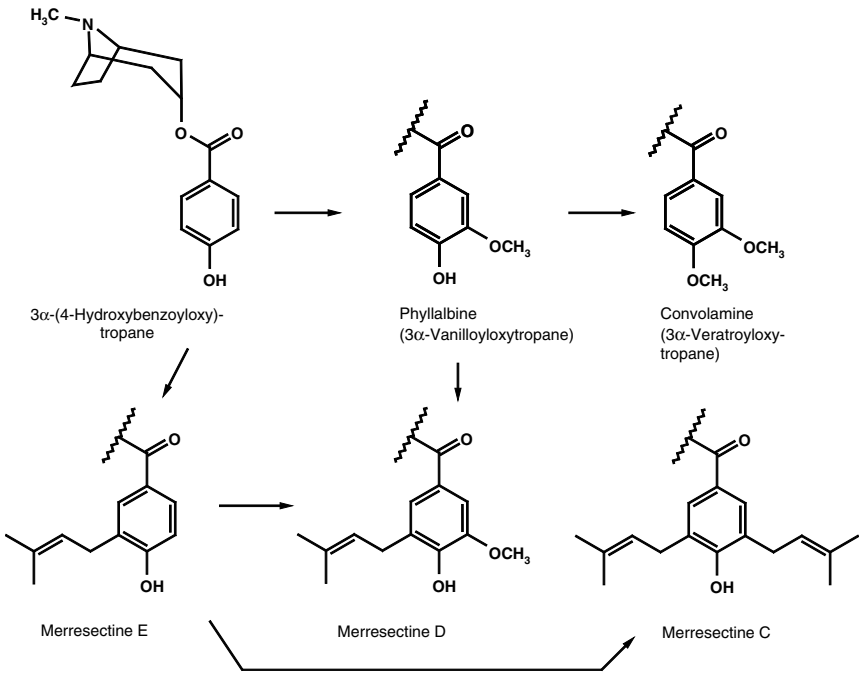


Fig. 3.21 Presumed biogenetic relationships between simple aromatic 3α-acyloxytropanes and prenylated congeners (merresectines), specific constituents of the Convolvulaceae

represent major alkaloids. There were a few exceptions (60% merresectine C of the total alkaloid fraction in the epigeal vegetative parts of *Merremia quinata* and 55% in the roots of *M. quinquefolia*, respectively; Jenett-Siems et al. 2005b). The content of the complete fraction of (3α-)merresectines in *M. guerichii* was determined to be only 20% in the vegetative epigeal parts vs 8% in the roots of the corresponding total alkaloid fraction with cuscohygrine as the principal alkaloid (50% vs 60%). This simple pyrrolidine (see Sect. 3.1) turned out to be dominating in most of the tropane-positive *Merremia* spp.

As already mentioned, (crude) cuscohygrine was the second alkaloid which could already be isolated from the leaves of *Erythroxylum coca* (Erythroxylaceae) in 1862. Taken into account the rather undeveloped methods in those days this indicates that cuscohygrine must have been present in considerable amounts besides the tropane alkaloid cocaine. In tropane-positive Solanaceae the content of tropane alkaloids outdoes the content of pyrrolidines including cuscohygrine by far. The contrary is true for the sister family Convolvulaceae with few exceptions. Obviously, this is one reason why the latter family is less poisonous compared with the former one.

Esters of 3α-Hydroxytropane/-*nor*tropane with Phenylpropanoid Acids (T5). This group of metabolites is a small one with hydroxycinnamic acids as acyl components, i.e., caffeic acid, ferulic acid, sinapic acid. It is also a rather rarely occurring group (six genera, 12 species = 7% of all species checked). Such alkaloids could be

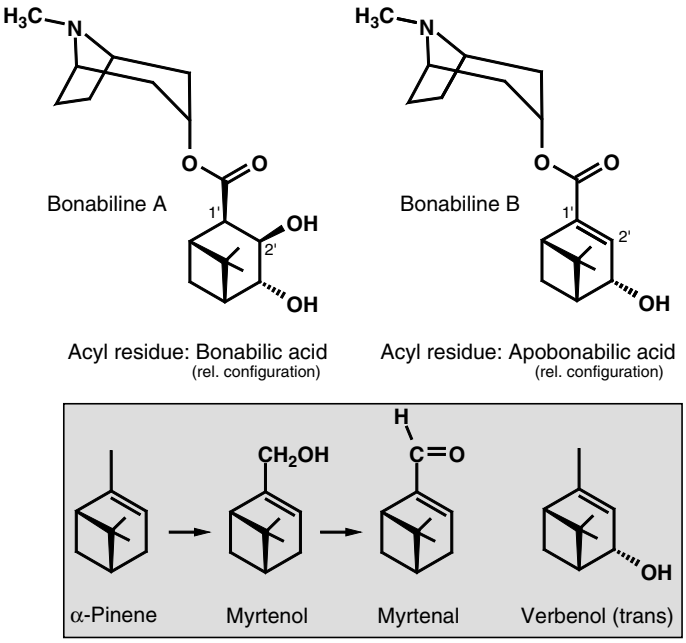


Fig. 3.22 Bonabilines, unique alkaloids from the roots of *Bonamia spectabilis* (Convolvulaceae); highlighted in *grey*: structurally related components of certain (non-convolvulaceous) essential oils for comparison. α-Pinene, myrtenol, and myrtenal are constituents of the essential oil of *Myrtus communis* L., Myrtaceae (Savikin-Fodulovic et al. 2000); the corresponding carboxylic acid, myrtenic acid, was detected as a constituent of the essential oil of *Cedronella canariensis* (L.) WEBB. & BERTHEL., Lamiaceae (Engel et al. 1995)

identified in *Dichondra sericea*, *Falkia repens* (Dichondreae), *Bonamia semidigyna* (Cresseae), *Maripa panamensis* (Maripeae), *Convolvulus chilensis*, *C. dorycnium*, *C. farinosus*, *C. glandulosus*, *C. graminetinus*, *C. sagittatus*, *C. siculus* (Convolvuleae), and *Ipomoea plebeia* (Ipomoeae). It is remarkable that there was only one species **T5**-positive in this latter large tribe and not even any species in the "Merremieae". Modified, rearranged phenylpropanoids like tropic acid or phenyllactic acid are confined to the Solanaceae.

3.4.3.3 Rare Esters of 3α-Hydroxytropane/-*nor*tropane (T6)

Bonabilines (T6-A). Besides rather common tropanes (**T2, T4**) two unique congeners, bonabiline A and B, were discovered in the roots of *Bonamia spectabilis*, a climbing shrub of tropical Africa including Madagascar (Ott et al. 2006). The acyl residues of the bonabilines are provided by monoterpenoic acids (Fig. 3.22), a novel combination of ester alkaloids of *any* type. The free acids, bonabilic acid and its

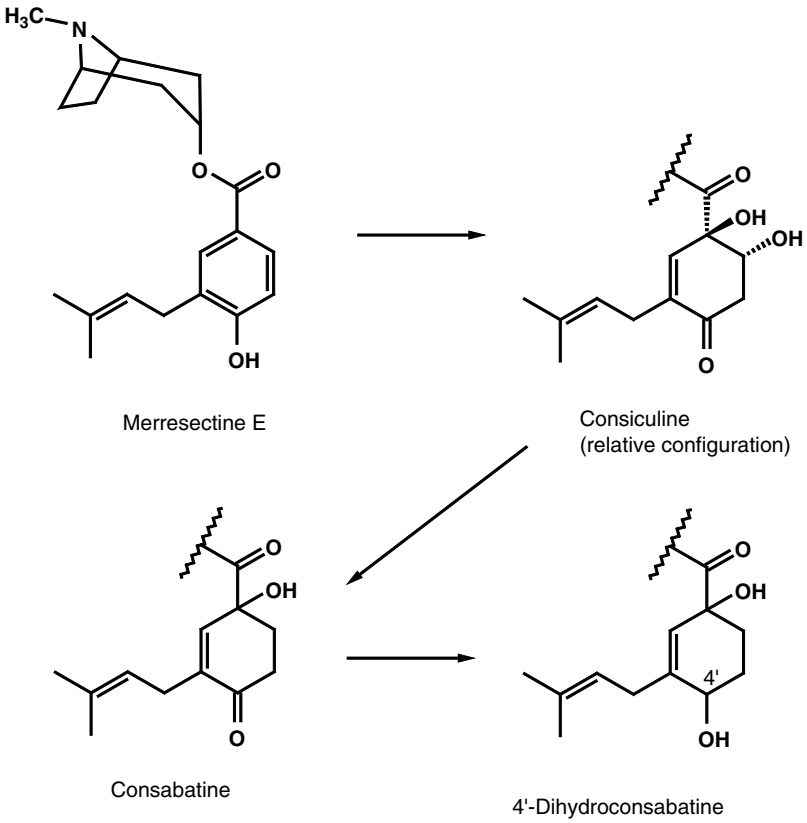


Fig. 3.23 Proposed biogenetic relationships between the prenylated aromatic 3α-acyloxytropane merresectine E and congeners confined to *Convolvulus sabatius*, *C. siculus*, and *Merremia quinata* (Convolvulaceae) whose structure has lost the aromatic character of the acyl moiety

dehydration product apobonabilic acid, were also unknown before. The latter represents an oxidation product of the bicyclic pinane-type monoterpene verbenol, a constituent of certain essential oils, e.g., the one of olibanum from the bark of *Boswellia* Roxb. ex Colebr. spp., Burseraceae (Guenther 1943). Such cyclic monoterpenoic acids in general seem to be very rare in the plant kingdom; myrtenic acid has been reported as trace compound in certain essential oils (Engel et al. 1995).

Consabatines, Consiculine (T6-B). Two tropane alkaloids with another unique type of acyl moiety were discovered in the roots of two *Convolvulus* species of Mediterranean origin, *C. siculus*, a small herbaceous twiner, and *C. sabatius* ssp. *mauritanicus*, a suffrutex used as a popular ornamental (Jenett-Siems et al. 1998b). These metabolites, consiculine and consabatine, are characterized by acyl residues, provided by consiculic acid and 2-deoxyconsiculic acid, respectively, which showed structural similarities to the one of merresectine E (Fig. 3.23). They share

(i) the prenylated six-membered ring, (ii) an oxygen function in the *ortho*-position of the prenyl residue, and (iii) the carboxyl in the *meta*-position of this prenyl. However, they differ in that (i) the six-membered ring represents a cyclohexene moiety instead of an aromatic one and (ii) there is a hydroxy substituent *geminal* to the carboxyl. Indeed, merresectine E and other merresectines occur together with consiculine/consabatine in the roots of *C. sabatius* and in the epigeal vegetative parts of *M. quinata* (Jenett-Siems et al. 2005b) suggesting a biogenetic relationship. It may be concluded that merresectine E has to be the precursor of consabatine/consiculine rather than vice versa. Otherwise it would not be plausible that merresectines are present in, e.g., seven *Merremia* spp. which did not even show traces of consabatine/consiculine. In conclusion the ring of the acyl residue of merresectine E seems to loose its aromatic character in certain convolvulaceous species. Such

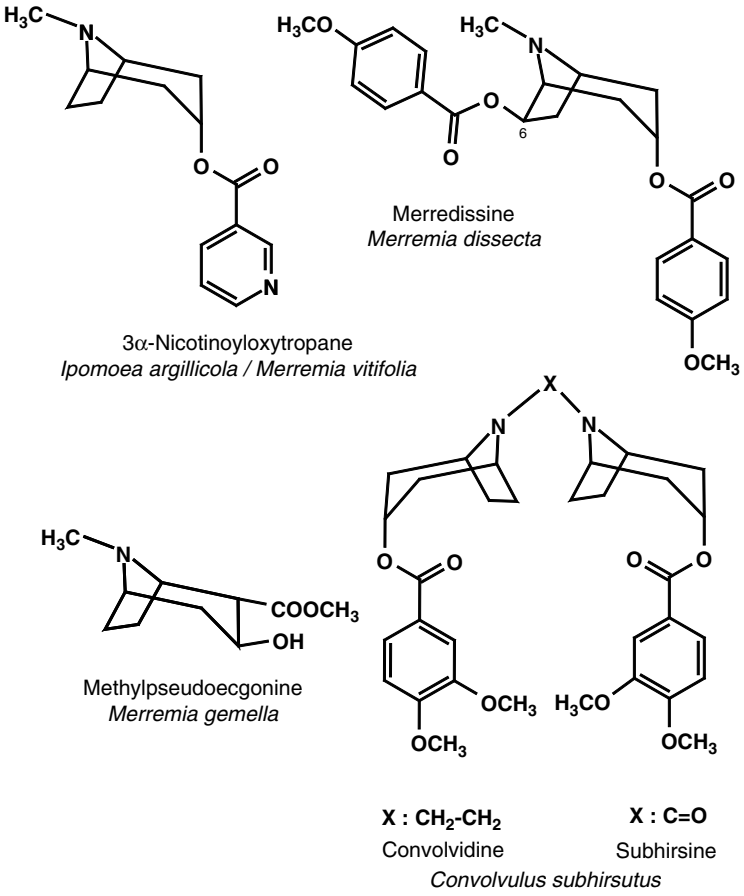


Fig. 3.24 Rare tropane alkaloids with unusual structure detected in certain convolvulaceous species; merredissine, convolvidine, and subhirsine are unique in the plant kingdom

dearomatizing reactions seem to be rare in the plant kingdom, e.g., ring A of flavonoids. They are well-known from the metabolization of aromatic compounds in mammalian livers (benzene, polycyclic aromatic hydrocarbons). From the second subspecies of *C. sabatius*, namely ssp. *sabatius*, a 4′-dihydro derivative of consabatine could be isolated. These **T6-B**-type metabolites seem to be rather rare being confined to the species already mentioned, i.e., both subspecies of *C. sabatius*, *C. siculus* (Convolvuleae), *M. quinata* ("Merremieae"), and in addition to *Evolvulus alsinoides* var. *decumbens* (Cresseae). Thus, also these compounds show (though rare) occurrence in both large subfamily clades, **/Convolvuloideae** and **/Dicranostyloideae**. This is remarkable, because consiculine/consabatine might be the most derived tropane alkaloids. Therefore a convergent development in a few species of both subfamilies seems to be reasonable.

3α-Nicotinoyloxytropane (T6-C) (Fig. 3.24). Nicotinic acid, the famous precursor of the biosynthesis of nicotinoids, is an unusual aromatic acyl component for tropanols. (i) All other known aromatic or phenylpropanoid acids esterified with these alkanolamines are biogenetic derivatives of phenylalanine. (ii) Though nicotine is also a minor component of the alkaloid profile of many convolvulaceous species, 3α-nicotinoyloxytropane could be detected only in very low concentrations in a few species: *Merremia quinquefolia, M. vitifolia*, *Ipomoea argillicola*, *I. asarifolia*, *I. lonchophylla*, *I. muelleri, I. tricolor*. Interestingly, the *Ipomoea* spp. except *I. tricolor* belong to the same section, *Erpipomoea*. On the other hand, there are also species of this section which were 3α-nicotinoyloxytropane-negative.

3.4.3.4 Esters of 3α,6β- and/or 3α,7β-Dihydroxytropane/-*nor*tropane (T7)

Our knowledge on the occurrence of these compounds is based mainly on GC/MS data. Unfortunately, this includes again problems with regard to the assignment to the 6β or 7β notation, respectively, as has been discussed already above in detail. There are only two reports on isolated **T7**-type alkaloids. (i) Convolacine (6ß-acetoxy-3α-veratroyloxytropane (Fig. 3.19) was identified as a constituent of *Convolvulus subhirsutus* (Aripova and Abdilalimov 1993). (ii) Merredissine from the roots of *M. dissecta*, which was structurally elucidated by spectral data as 3α,6β-di-(4-methoxybenzoyloxy)tropane (Fig. 3.24) (Jenett-Siems et al. 2005b). However, Lounasmaa and Tamminen (1993) would have assigned these compounds also to the 3α,7β notation as they did with, e.g., dibenzoyloxytropane from *Erythroxylum cuneatum* (WALL.) KURZ (Erythroxylaceae) originally published to be 3α,6β-configurated (El-Imam et al. 1988). A number of species from different genera were also found to contain **T7**-type alkaloids by GC/MS analysis (*Evolvulus alsinoides* var. *decumbens*, *M. guerichii*, *Ipomoea batatas* (cultivar), *I. abrupta*, *I. violacea*, *I. quamoclit*).

3.4.3.5 Esters of 3β-Hydroxytropane/-*nor*tropane (T8–T10)

A high percentage of tropane-positive convolvulaceous species (35 out of 67 analytically comparable species = 52%) turned out to contain both stereoisomers, 3α as well as 3β congeners, whereas in – at any rate – 26 spp. (39%) 3α esters only could be detected. However, the number of species showing 3β esters only was comparably low (6 spp. = 9%: *Evolvulus nummularius*, *Maripa nicaraguensis*, *Convolvulus demissus*, *Merremia gemella*, *Operculina riedeliana*, *Astripomoea malvacea*). The pattern of 3β-acyloxytropanes, very limited with regard to the number of specific, individual compounds in the Solanaceae, is generally more complex in the Convolvulaceae (Fig. 3.19; Table 3.6). All of the three structural types (**T8–T10**) could be detected only in *Falkia repens*, *Bonamia semidigyna*, *Evolvulus nummularius*, and *Maripa panamensis*.

Aliphatic Esters of 3β-Hydroxytropane/-*nor*tropane (T8). Such compounds were present in 20 species (12%) belonging to 10 genera from six tribes: *Falkia* (1 sp.), *Bonamia* (2), *Evolvulus* (4 spp. plus an additional var.), *Maripa* (1), *Convolvulus* (2), *Merremia* (3), *Operculina* (1), *Astripomoea* (1) *Ipomoea* (4), *Turbina* (1). *Astripomoea malvacea* (Ipomoeeae), a perennial subshrub widespread from western tropical Africa to eastern Africa and southwards, turned out to be phytochemically unique because the *dominant* alkaloid of its root bark is not a tertiary amine like in all other tropane-positive convolvulaceous species but an *N*-oxide, astrimalvine A *N*-oxide [3β-(3-tigloyloxy-2-methylbutyryloxy)tropane *N*-oxide] (Ott et al. 2006). *N*-oxides as principal constituents of the alkaloid fraction are well-known from pyrrolizidines, e.g., in plants such as *Senecio vulgaris* L. (Asteraceae) they are synthesized in the roots as *N*-oxides and translocated to the shoots where they are stored (Hartmann and Toppel 1987; Hartmann et al. 1989). Convolamine *N*-oxide had been characterized – as a *minor* component – from the epigeal parts of *Convolvulus krauseanus* (Aripova 1985). Some minor congeners of astrimalvine A in *A. malvacea* were also present presumably as *N*-oxides. After reduction of the crude alkaloid fraction with Zn/HCl further tertiary amines, e.g., 3β-(3-hydroxy-2-methylbutyryloxy)tropane (astrimalvine B), and the secondary amine *nor*astrimalvine A, could be identified by GC/MS analysis. The specific alkaloid profile of *A. malvacea* is characterized by the exclusive presence of *aliphatic* 3β-acyloxytropanes/-*nor*tropanes. Interestingly, the 3-acyloxy-2-methylbutyryl residue of astrimalvine A/*nor*astrimalvine A, unknown from other tropane-positive Convolvulaceae as well as from the Solanaceae, turned out to be structurally closely related to those of certain Convolvulaceae-specific pyrrolizidine alkaloids (ipangulines, minalobines; Sect. 3.7).

Falkia repens, a perennial herb endemic to certain coastal zones of southern Africa, was characterized by the content of a rather broad pattern of **T8**-type alkaloids (3β-acetoxytropane and its isobutyryl, 2-methylbutyryl, isovaleroyl, 3-hydroxy-2-methylbutyryl, and tigloyl congeners as well as astrimalvine B) besides the corresponding 3α isomers (**T2**-type) and furthermore**T5-,T9-,T10-**type tropanes (Tofern 1999; Ott et al. 2007). Remarkably, this co-occurrence of esters of both epimeric

forms is characterized by nearly equal concentrations of each. Other species showed a smaller **T8**-profile, e.g., *Evolvulus alsinoides* [3β-(2-methylbutyryloxy)tropane], *Bonamia semidigyna* (3β-acetoxytropane and astrimalvine B), *Merremia dissecta* (3β-acetoxytropane, 3β-acetoxy*nor*tropane). In a few cases acyloxytropanes in general are confined to aliphatic esters of 3β-hydroxytropane: *Convolvulus demissus*, *Operculina riedeliana*, *Ipomoea squamosa*. However, normally these **T8**-type compounds co-occur at least with their 3α congeners (**T2**-type).

Simple and Prenylated Aromatic Esters of 3β-Hydroxytropane/-*nor*tropane (T9). It is especially remarkable to notice that also alkaloids of this type often showed co-occurrence with the corresponding 3α derivatives (**T3**- and **T4**-type, respectively) and vice versa (Tables 3.5 and 3.6). In this connection it should be taken into account that simple and prenylated aromatic esters of 3α-hydroxytropane/-*nor*tropane are the convolvulaceous counterpart to the solanaceous (3α-configurated) hyoscyamine/scopolamine-type alkaloids from the family-specific point of view. However, the Solanaceae do not produce 3β analogues of their specific tropanes whereas the occurrence of aliphatic esters of 3β-hydroxytropane/-*nor*tropane besides the corresponding 3α metabolites is common also in this family.

Apparently, **T9**-type tropanes represent – like their 3α congeners (**T3/T4**) – apomorphic characters. The pattern of simple aromatic esters is reduced in the 3β series in contrast to the large number of specific, individual compounds in the 3α series (Fig. 3.19). Surprisingly, this is not the case with merresectines: The same 3β analogues like those of the 3α series may occur (Table 3.6). **T9**-type alkaloids were identified in almost the same genera like their **T8**-type congeners [exception: *Operculina*, *Astripomoea*, *Turbina* (**T8**-type only)]. However, the species differ in *Convolvulus* and *Ipomoea*. In case of *Merremia* three further species are to be added, whereas in *Evolvulus* the number of species is to be reduced from five to one. As an additional genus *Jacquemontia* is represented by one species.

Concneorine (Fig. 3.19), the first ester of 3β-hydroxytropane discovered in a convolvulaceous species, was isolated from the roots of the Mediterranean silverbush, *Convolvulus cneorum,* a bushy semi-climber and popular ornamental (Mann 1997; Jenett-Siems et al. 2005b). This alkaloid was identified also in five further genera though often only in very low concentrations. Its *nor* congener could be detected additionally as a constituent of *C. floridus*, an upright shrub, endemic to the Canary Islands and also used as an ornamental. Tropacocaine (3β-benzoyloxytropane) (Fig. 3.19), discovered in *Erythroxylum* spp. (Erythroxylaceae) (Lounasmaa and Tamminen 1993 and references therein), also turned out to be synthesized by *Evolvulus nummularius*, *Ipomoea argillicola* (in both cases in co-occurrence with its 3α analogue), and *I. sloteri* [in co-occurrence with its 3β-*nor* congener; discovered in *Erythroxylum mamacoca* MART. (El-Imam et al. 1985)].

Esters of 3β-Hydroxytropane/-*nor*tropane with Phenylpropanoid Acids (T10). Almost all species which contained the corresponding 3α isomers (**T5**-type) turned out to be also **T10**-positive. These esters were the only 3ß-acyloxytropanes of *Bonamia brevifolia*, four *Convolvulus* spp., and *Merremia gemella*.

3.4.3.6 Chemotaxonomic Conclusions

Recently, a first molecular phylogenetic analysis of the genus *Convolvulus* though confined to Macaronesian and Mediterranean species has been published (Carine et al. 2004). This analysis using data from the nuclear ribosomal ITS regions included 39 *Convolvulus* spp. and three *Calystegia* spp. which were nested within *Convolvulus*. According to Table 3.2, information on the occurrence of tropane alkaloids in altogether 16 out of these 42 species is documented. From the chemotaxonomic point of view it is interesting to see that the most characteristic convolvulaceous tropanes occur in species which are nested within two subclades in contrast to a remarkable number of further subclades:

- *C. floridus*, *C. cneorum*, and *C. dorycnium*, potent synthesizers of **aromatic** 3α- and 3β-acyloxytropanes (**T3**, **T9**) are nested within the same subclade. However, two further species (*C. caput-medusae*, *C. scoparius*) forming a **sub**-subclade which is sister to *C. floridus* do not synthesize such metabolites. These two species might have lost the ability to produce them during their evolution in contrast to their sisters (i.e., *C. floridus* etc.).
- *C. siculus* and *C.sabatius*, also potent synthesizers of **aromatic** 3α- / 3β-acyloxytropanes (**T3**, **T9**) and in addition of merresectines (**T4**) as well as of unique congeners named consabatine/consiculine (**T6-B**), are nested within in another subclade which is not closely related to the "*C. floridus / C. cneorum / C. dorycnium*" subclade.

Thus, occurrence of the most characteristic convolvulaceous tropanes is of chemotaxonomic meaningfulness for *Convolvulus*. Unfortunately, to date sufficient molecular phylogenetic data on the genus *Merremia,* another large genus in which characteristic convolvulaceous tropanes occur, are lacking. However, an extensive study on the occurrence of tropane alkaloids of 18 species of paleo-, neo-, and pantropical origin could contribute to the solution of intrageneric problems (Jenett-Siems et al. 2005b). Three groups of taxa have been found out (for details see Table 3.2):

- Taxa free of tropanes: *M. medium* (L.) HALL. f., *M. tridentata* (L.) HALL. f. This finding supported the transfer to the genus *Xenostegia* by Austin and Staples (1980).
- Taxa with simple tropanes (**T1**): two species belonging to the Tuberosa allies, three species of section *Merremia*, two species nested within section *Xanthips*, and one species of section *Hailale*.
- Taxa with **aromatic** 3α- / 3β-acyloxytropanes (**T3**, **T9**) plus merresectines (**T4**): three species belonging to the Vitifolia allies, two species of section *Cissoides*, two species of section *Streptandra* and one species not included in the present classification.

However, as can be concluded from Table 3.2 tropane alkaloids are no suitable chemotaxonomic indicators as far as **inter**generic relationships are concerned. Independent of this, tropanes in species from genera belonging to the advanced tribe Ipomoeeae (*Argyreia*, *Ipomoea* etc.) are rather rare with the exception of **Old** World (Australasian) *Ipomoea* spp. and the African *Astripomoea*. Furthermore,

Table 3.7 Occurrence of pyrrolidine, tobacco, and tropane alkaloids in both large Solanales families compared with the occurrence out of the Solanales. Data on tropanes based on Lounasmaa and Tamminen (1993), Griffin and Lin (2000) and references therein; data concerning the Convolvulaceae based on the literature given in Table 3.2

Key to the frequency of occurrence: ++++ = almost ubiquitous; +++ = frequent; ++ not rare; + = rare; — = not detected

Alkaloids		Solanales, euasterids		Malpighiales, eurosids I	Brassicales, eurosids II
Examples	**Type according two Table 3.2**	**Solanaceae**	**Convolvulaceae**	**Erythroxylaceae**	**Brassicaceae**[a]
Simple pyrrolidines					
Hygrine, cuscohygrine	**P1, P2**	++++	++++	++++	+
Nicotine	**N**	+++	+++	++	—
Simple tropanes					
3α-Hydroxytropane[b]	**T1-A**	+++	+++	+	+
3ß-Hydroxytropane	**T1-A**	+++	++++	+	+
3α-Acyloxytropanes					
3α-Acetoxytropane[b]	**T2**	+++	+++	—	—
3α-(2-Methylbutyryloxy)tropane[b]	**T2**	++	++	+[c]	—
3α-Tigloyloxytropane	**T2**	+++	+	—	—
3α-(4-Hydroxybenzoyloxy)tropane	**T3**	—	+	—	+
3α-(3-Hydroxybenzoyloxy)tropane (Cochlearine)	**T3**	—	—	—	+
Datumetine	**T3**	+[d]	+	—	—
3α-Vanilloyloxytropanes[e]	**T3**	—	++	—	—
3α-Veratroyloxytropanes	**T3**	—	++	+[f]	—
3α-(3,4,5-Trimethoxybenzoyloxy)tropanes	**T3**	—	—	+	—
Merresectines	**T4**	—	++	—	—
3α-Hydroxycinnamoyloxytropanes[b,g]	**T5**	?[h]	+	++	+
3α-Benzoyloxytropane[b]	**T3**	—	+	+++	+

Table 3.7 Occurrence of pyrrolidine, tobacco, and tropane alkaloids in both large Solanales families compared with the occurrence out of the Solanales. Data on tropanes based on Lounasmaa and Tamminen (1993), Griffin and Lin (2000) and references therein; data concerning the Convolvulaceae based on the literature given in Table 3.2 (continued)

Alkaloids		Solanales, euasterids		Malpighiales, eurosids I	Brassicales, eurosids II
Examples	**Type according to Table 3.2**	**Solanaceae**	**Convolvulaceae**	**Erythroxylaceae**	**Brassicaceae**[a]
3α-Nicotinoyloxytropane	**T6-C**	—	+	—	+
3α-Tropoyloxytropanes (e.g., hyoscyamine, scopolamine)		+++	—	—	—
Schizanthines		+	—	—	—
Esters of 3α,7β-dihydroxytropane[i]	**T7**	+++	+	+++	—
Esters of 3α,6β-dihydroxytropane	**T7**	+++	+	+[f]	—
Esters of 3α,6β,7β-trihydroxytropane		+	—	+++	—
3β-Acyloxytropanes					
Tigloidine	**T8**	+++	+	—	—
Tropacocaine	**T9**	—	+	+++	—
Cocaine	**T9**	—	—	++	—
3β-(4-Hydroxybenzoyloxy)tropane	**T9**	—	+	—	+
3β-Merresectines	**T9**	—	+	—	—
3β-Hydroxycinnamoyloxytropanes[j]	**T10**	?[h]	++	+	+
Calystegines[b] (see Sect. 3.5)	**T1-B**	+++	++++	+++[k]	++

[a] Data from two *Cochlearia* spp.: *C. arctica* (Platonova and Kuzovkov 1963), *C. officinalis* (Bachmann et al. 1997); results of further species: Brock et al. (2006)
[b] Also present in Rhizophoraceae
[c] *Nor* (*N*-demethyl) derivative
[d] Singular occurrence (*Datura metel*)
[e] Also present in Euphorbiaceae (Malpighiales, eurosids I)
[f] Christen et al. 1995
[g] e.g., 3α-Caffeoyl-, 3α-feruloyl-, 3α-sinapoyl-oxytropane; Erythroxylaceae: 3α-Cinnamoyl-, 3α-(3,4,5-trimethoxycinnamoyl-)oxytropane
[h] Detected by GC/MS analysis of hairy roots of *Hyoscyamus albus;* stereochemistry at C-3 undetermined (Doerk-Schmitz et al. 1994)
[i] Also present in Proteaceae (Proteales, "basal tricolpates")
[j] 3β-Caffeoyl-, 3β-feruloyl-, 3β-sinapoyl-oxytropane; Erythroxylaceae: 3β-Cinnamoyloxytropane
[k] Brock et al. (2005)

in contrast to the Solanaceae (i) occurrence of tropanes does not represent a consistent genus-typical trait and (ii) accumulation of higher amounts of tropanes is rather rare in the Convolvulaceae family.

Occurrence in Non-solanaceous/Non-convolvulaceous Taxa. Beside the Erythroxylaceae, tropane bases were detected in further plant families which are again not closely related with the Solanaceae [Brassicaceae, Euphorbiaceae, Proteaceae, Rhizophoraceae (Waterman 1998)]. For details on the occurrence and distribution of different types of tropane alkaloids inside the two Solanales family in comparison with the unrelated families mentioned above outside this order see Table 3.7.

3.4.4 Biosynthesis (Figs. 3.13 and 3.14)

In different feeding experiments with labelled hygrine it was shown that this compound could be a relevant precursor of the tropane skeleton. For a long time it was believed that dehydrohygrine (5-acetonyl-1-methyl-Δ^1-pyrrolinium salt, formed by oxidation of (2*R*)-hygrine, though never detected in plant extracts, represents the direct precursor of 3-oxotropane. More profound feeding experiments led to a very low or even no incorporation of hygrine into 3α-tropanol or hyoscyamine/scopolamine (Abraham and Leete 1995 and references therein; Robins et al. 1997). To date there is a consensus that hygrine is not involved in this pathway (Humphrey and O'Hagan 2001). For these purposes, the former hypothesis has been substituted by another one based on results for the formation of the tropane skeleton of cocaine in *Erythroxylum coca* LAM., Erythroxylaceae (Leete and Kim 1988). This involved the assumption that the *N*-methyl-Δ^1-pyrrolinium salt does not condense with the formerly supposed acetoacetyl CoA but successively with two molecules of malonyl CoA or acetyl CoA to yield finally the thio ester of pyrrolidine-2-acetoacetic acid which cyclizes via the corresponding Δ^1-pyrrolinium salt forming the thio ester of 2-carboxytropan-3-one (2-carboxy-3-oxotropane; 2-carboxytropinone). This intermediate was proposed to be converted to 2-carbomethoxytropan-3-one, a precursor of cocaine. On the other hand, this intermediate might also serve as a direct precursor of 3-oxotropane via hydrolyzation of the thio ester to the free acid, i.e., 2-carboxytropan-3-one itself, and decarboxylation (β-keto acids show a tendency to be easily decarboxylated). This hypothesis could be supported strongly by experiments with labelled ethyl (*R,S*)-4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate fed to *Datura inoxia* plants resulting in a high incorporation into scopolamine (Abraham and Leete 1995) as well as – fed to *D. stramonium* – into hyoscyamine (Robins et al. 1997). This 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate is the most advanced intermediate that has become successfully incorporated into the tropane skeleton of solanaceous tropanes. The occurrence of 2-carbomethoxytropan-3-one, the methyl ester of the putative final intermediate of tropan-3-one (3-oxotropane, tropinone), detected in *D. stramonium* root cultures is another support for the hypothesis (Humphrey and O'Hagan 2001 and references therein). Furthermore, it was

speculated that there is a polyketide enzyme that accepts the *N*-methyl-Δ^1-pyrrolinium cation as the starter which is fully committed to two acetate/malonate condensations before 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate is released from the enzyme (Humphrey and O'Hagan 2001). This would explain why neither 1-methylpyrrolidine-2-acetic acid (Huang et al. 1996) nor acetoacetate (see above) can serve as precursors. Anyhow, to a certain degree the title of the review published by Humphrey and O'Hagan, "Tropane alkaloid biosynthesis. A century old problem unresolved" is still true. Tropan-3-one (tropinone) is the common intermediate of all of the tropane alkaloids. It represents the branching point for two principal specific pathways. Two separate NADP-dependent tropinone reductases, TR I and TR II, catalyze the reduction to 3α-hydroxytropane (tropine) and 3β-hydroxytropane (pseudotropine), respectively (Dräger 2006 and references therein).

Biogenetic studies have been predominantly focused on hyoscyamine/atropine and their congeners rather than on any other type of tropane alkaloids. Tropic acid, already recognised as an isomer of the phenylpropanoid ("phenylpropionoid") skeleton by Robinson (1928), was proposed by the same author (Robinson 1955) to be formed by a rearrangement from L-phenylalanine. Indeed, in feeding experiments with *Datura inoxia* plants this amino acid turned out to be incorporated into the tropoyl moiety of hyoscyamine (O'Hagan and Robins 1998). It had been assumed for a long time that the conversion of phenylalanine to tropic acid proceeds on the stage of the corresponding free acids; tropic acid ought to be esterified in a final step with 3α-hydroxytropane to yield hyoscyamine (Robins and Walton 1993 and references therein). However, later it could be demonstrated in root cultures of *D. stramonium* that the well-known tropane alkaloid littorine, 3α-(*R*)-phenyllactoyloxytropane, represents the direct precursor of hyoscyamine. Free tropic acid turned out to be also a very poor precursor for hyoscyamine in root cultures of *Duboisia leichhardtii*. Extensive feeding experiments confirmed the role of littorine as the substrate for the isomerisation to hyoscyamine (O'Hagan and Robins 1998 and references therein). Hyoscyamine 6β-hydroxylase (H6H), a 2-oxoglutarate-dependent dioxygenase, catalyzes two consecutive oxidation steps: The first step involves hydroxylation of hyoscyamine at C-6β, the second one its dehydrogenation mediating an epoxide ring closure to generate scopolamine (Hashimoto et al. 1993). H6H could be localized at the pericycle of the root by means of monoclonal antibodies. This localization turned out to be a specific one and provided an anatomical explanation for the tissue-specific biosynthesis. In this connection the significance for translocation of the alkaloids from the root to the aerial parts was discussed (Hashimoto et al. 1991).

Interestingly, 3α-tigloyloxytropane and 3α-benzoyloxytropane were also hydroxylated by H6H from *Hyoscyamus niger* in vitro suggesting a hydrophobic site for binding the tropoyl moiety of hyoscyamine (Yamada and Hashimoto 1989).

In a few species scopolamine, normally the final member of the pathway, may be transformed to 7β-hydroxyhyoscyamine (presumably by cleavage of the 6β,7β-epoxide group of the aminoalkanol moiety) or anisodine (presumably by hydroxylation at C-2 of the tropic acid moiety) thus representing a branched

prolongation of the pathway. In this connection it may be mentioned again that the alkaloids traditionally named 6β-hydroxyhyoscyamine and 7β-hydroxyhyoscyamine represent two natural diastereoisomers, namely (***3R,6R,2′S***)- and (***3S,6S,2′S***)-6β-hydroxyhyoscyamine (Muñoz et al. 2006).

Next to tropic acid the unsaturated aliphatic tiglic acid is the most important agent for acylation of hydroxytropanes in the Solanaceae (Liebisch and Schütte 1985). Feeding experiments of labelled L-(+)-isoleucine with *Datura ceratocaula* plants showed that the radioactivity of this amino acid was located in the 2-methylbutyryl part of the corresponding 7β-acyloxy-3α-hydroxytropane (Beresford and Wooley 1974). The biosynthesis of the acyl moiety of different tigloyl diesters in *D. inoxia* plants had already turned out before to proceed from the same amino acid via *S*-(+)-2-methylbutyric acid (2-methylbutanoic acid; Basey and Woolley 1973a). Angelic acid, the isomer of tiglic acid, was not a precursor for tigloyl esters (Basey and Woolley 1973b). A number of acyl transferase activities was found to be present in root extracts of *Datura* and related genera such as acetyl-CoA:tropine, tigloyl-CoA:tropine, phenylacetyl-CoA:tropine forming the corresponding products acetyltropine, tigloyltropine, and phenylacetyltropine, respectively. This was also true for corresponding activities with regard to pseudotropine (Robins et al. 1994). However, the authors concluded "….. that the wide spectrum of minor bases observed in the alkaloidal extracts ….. is simply due to the low selectivity of the enzymes for acyl-CoA thioesters. Hence, the relative availability of different CoA thioesters may be an important factor in determining the extent to which each product accumulates." Tigloyl-CoA: pseudotropine acyl transferase from transformed root culture of *Datura stramonium*, the enzyme which catalyzes the formation of tigloidine could be characterized by Rabot et al. (1995). It turned out to possess a high substrate specificity for 3β-hydroxytropane (pseudotropine) though not for the corresponding acyl moiety: Acetyl-CoA and tigloyl-CoA competed for the same active site on the enzyme. However, 3α-hydroxytropane as well as 3β-hydroxy-*nor*tropane (*nor*pseudotropine) were not acylated.

It seemed reasonable to suppose that diesters such as 3α,7β-ditigloyloxytropane and 3α,7β-ditigloyloxy-6ß-hydroxytropane are formed by the progressive hydroxylation and esterification of 3α-tigloyloxytropane. However, this could not be verified. Experiments with labelled monoester fed to *D. inoxia* plants yielded unlabelled diesters. Therefore, it was supposed that the fed monoester might be hydrolyzed to 3α-hydroxytropane which subsequently might be hydroxylated to the corresponding 3,7-di- and 3,6,7-trihydroxytropanes, respectively. Finally, de novo acylation of the different hydroxytropane intermediates with *unlabelled* tiglic acid (synthesized by the plants themselves) might result in the formation of the monoester as well as of both diesters (Basey and Woolley 1975).

Several comprehensive reviews on the biosynthesis of tropane alkaloids have been published, e.g., by Leete (1990), Robins and Walton (1993), Humphrey and O'Hagan 2001. "Molecular aspects", i.e., corresponding enzymes and genes, were reviewed by Hashimoto and Yamada (1994).

3.4.5 Significance

3.4.5.1 Pharmacology and Toxicology

Tropane Alkaloids. Some 180 years ago an experiment showing mydriasis of a cat's eye due to the application of atropine *isolated* from *Atropa belladonna* was demonstrated to the famous German poet J.W. v. Goethe by the great chemist F.F. Runge (Runge 1824, 1825). Of course, such an effect had been known for centuries from the *crude drug* (leaves) providing this species with its scientific epithet ("bella donna" = beautiful lady) in Venice/Italy due to its application at that place in ancient times. However, Runge's experiment was the beginning of pharmacological research on 3α-tropoyloxytropanes. Meanwhile, pharmacological research on hyoscyamine/atropine and scopolamine represents a history of almost two centuries. Nowadays, accidental mydriasis of humans may cause neuroophthalmological disorders due to plant contact in horticultural connections. Thus, Wilhelm et al. (1991) reported that in Germany such accidents are caused in most cases with introduced solanaceous taxa such as neotropical *Brugmansia* spp. (angel's trumpet) sub nom. *Datura* spp. rather than by naturally growing indigenous plants like *Atropa belladonna* (deadly nightshade berry), *Datura stramonium* (thornapple/ jimson/Jamestown weed), and *Hyoscyamus niger* (henbane). These intoxications occur typically in autumn when (hobby) gardeners carelessly cut back such species (planted in tubs) to prepare them for inside protection during winter time.

Natural tropane type compounds causing such effects are hyoscyamine/atropine, 6β-hydroxyhyoscyamine (anisodamine), anisodine, and scopolamine. They are still today important drugs (Liu et al. 2005) though their semisynthetic derivatives are meanwhile even more important. Traditionally they were called parasympatholytics. Nowadays, they are termed competitive inhibitors (of all known types) of muscarinic acetylcholine receptors (for details see Sect. 3.3.6). As Schmeller et al. (2000) could demonstrate, such tropane alkaloids do not interfere only with these types of neuroceptors but also bind to the nicotinic ones albeit with much lower affinities. This was determined not only for atropine and scopolamine but also with similar affinities for their intermediates/congeners littorine, 6β- and 7β-hydroxyhyoscyamine. However, semisynthetic quaternary compounds like the *N*-methyl derivatives of atropine and scopolamine were found to show the highest binding.

6β-Hydroxyhyoscyamine (anisodamine) was tested first by Vakhabov et al. (1975). Due to their discovery in Chinese *Anisodus* spp. the latter alkaloid as well as anisodine have been predominantly investigated pharmacologically and used therapeutically in modern Chinese medicine. As examples for this research may be cited two reports entitled "Absolute configuration of (−)-anisodine (a new ganglio blocking agent) and (−)-anisodinic acid" (Xie et al. 1983) and "The effect of several memory-improving agents on memory impairment in mice by anisodine" (Shang et al. 2003).

A natural hyoscyamine analogue with structural similarities to atropine though reduced flexibility in the acyl moiety, bonabiline A, has been isolated from a convolvulaceous twining shrub, *Bonamia spectabilis* (Ott et al. 2006). This metabolite has shown remarkable muscarinic (M_3) receptor antagonist activity (pA_2 value: 6.65

vs 9.02 for atropine). Probably, the conformational restriction induced by the integration of C-2′ in a ring system was responsible for the lower activity of bonabiline A compared to atropine.

In contrast to hyoscyamine/atropine which shows central depressant as well as central excitatory properties, its congener scopolamine is only characterized by the former property. Thus, it is suitable as a prophylactic antiemetic applied by a certain patch before a surgery or a travel in order to avoid nausea. An advantage of such a so-called transdermal delivery system (TDS) is given in so far as a steady blood level for three days is produced which is high enough to act as an antiemetic but low enough to be free from severe side effects (Tolksdorf et al. 1985).

Poisoning with hyoscyamine/atropine, scopolamine, and solanaceous plant material containing this type of alkaloids is due to a parasympathetic blockade characterized by tachycardia, mydriasis, central excitement, strong motor restlessness, hallucinations,

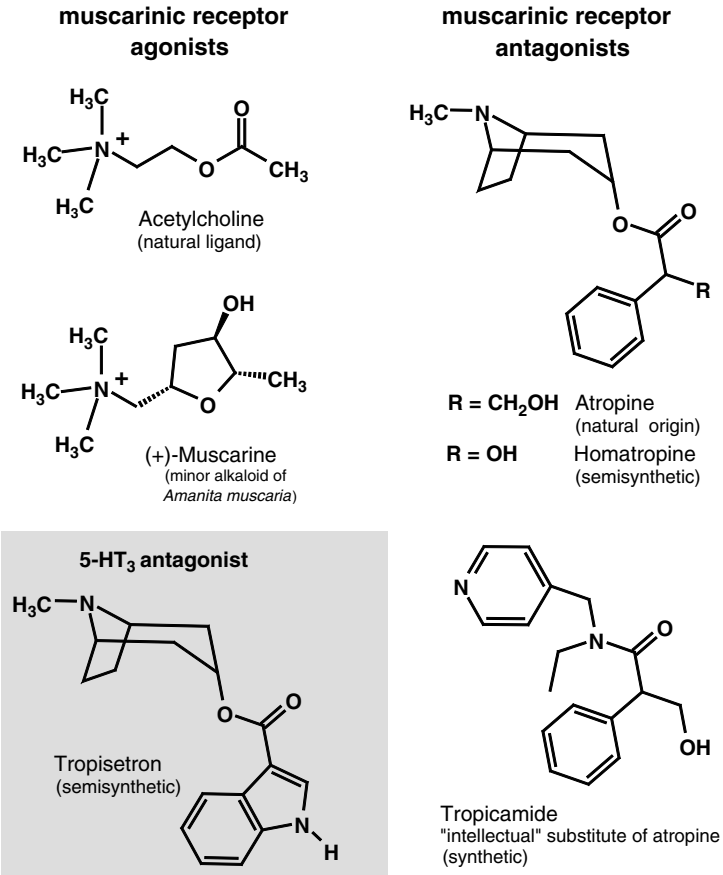
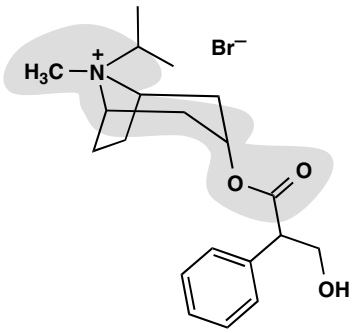
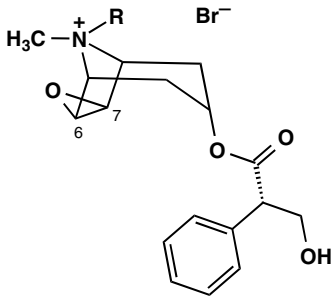


Fig. 3.25 Structural similarities between agonists at *m*-cholinoceptors and one selected natural, semisynthetic, as well as synthetic antagonist, respectively; in contrast tropisetron is a representative of 5-HT_3 antagonists sharing the 3α-acyloxytropane moiety with atropine



Semisynthetic quaternary ammonium substitute of atropine:

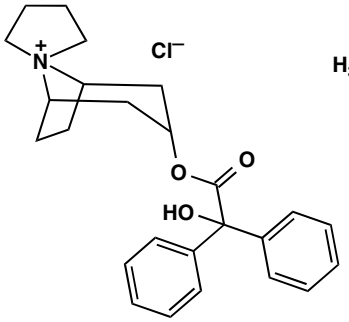
Ipratropium bromide



Semisynthetic quaternary ammonium substitutes of scopolamine (hyoscine):

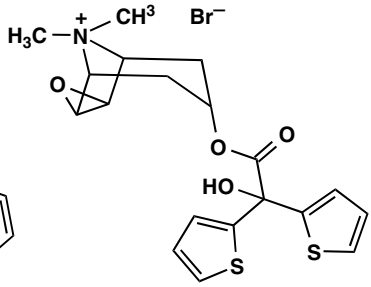
R = CH_2-CH_3 Oxitropium bromide

R = n-C_4H_9 *N*-Butylscopolaminium bromide



Trospium chloride

Semisynthetic quaternary ammonium substitute of atropine with synthetic acyl residue



Tiotropium bromide

Semisynthetic quaternary ammonium substitute of scopolamine (hyoscine) with synthetic acyl residue

Fig. 3.26 Semisynthetic derivatives of atropine and scopolamine (hyoscine) therapeutically used as *m*-cholinoceptor antagonists; highlighted in *grey*: common structural elements shared with the physiological agonist acetylcholine

coma, and respiratory paralysis (lethal dose for hyoscyamine: 100 mg/adult human). On the other hand, atropine is used as an antidote in poisoning by cholinesterase inhibitors such as physostigmine, its synthetic derivatives or alkyl phosphate/thiophosphate type insecticides. For further pharmacological and toxicological details the reader is referred to the textbooks of pharmacology and toxicology.

Apparently due to non-spectacular results, solanaceous tropane alkaloids of other structural types are not well-described with regard to biological/pharmacological properties. Aliphatic esters such as valeroidine, poroidine, isoporoidine, and valtropine had a depressant effect on the diphasic electric response of the Sartorius muscle of *Bufo marinus* L., cane toad (Bufonidae), characterized by a gradual fall of the action potential to a final abolition together with a 50–100% increase in the conduction time (Trautner and McCallum 1950). In contrast, atropine, hyoscine, and tigloidine had a delayed recovery effect.

Semisynthetic Derivatives of Tropane Alkaloids. Generally semisynthetic remedies are developed with the aim to improve the pharmacodynamic and/or pharmacokinetic properties of a natural agent. Homatropine (3α-mandeloyloxytropane) (Fig. 3.25) is a shorter acting mydriatic in contrast to atropine which is of advantage in diagnostic ophthalmology. It can be synthesized by acylation of 3α-hydroxytropane and represents one of the oldest semisynthetic drugs at all. A century ago, Jowett and Pyman (1909) continued with structure/activity relationship studies of tropanes by checking a series of semisynthetic 3α-acyloxytropanes ("tropeines"). They found out that certain derivatives possessed more or less marked mydriatic properties in contrast to others; most of them were less active than homatropine. Today, semisynthetic quaternary ammonium substitutes of atropine such as ipratropium bromide and of scopolamine such as oxitropium bromide and *N*-butylscopolaminium bromide (Fig. 3.26) play an important role as neurotrope spasmolytics. Their advantage is given by the fact that they are not able to pass the blood brain barrier. Thus, they act only peripherally, i.e., without the central adverse side effects of their natural starting products. These three semisynthetic compounds still share the acyl moiety with the latter ones. Moreover, there are also semisynthetic derivatives with (i) a quaternary centre and (ii) another, synthetic acyl residue such as trospium chloride and tiotropium bromide (Fig. 3.26). The significance of such remedies may be demonstrated, e.g., by the economic success of tiotropium chloride, an anticholinergic bronchodilator. This agent has become a very successful blockbuster representing the most prescribed COPD (chronic obstructive pulmonary disease) remedy at all (Spiriva®, Boehringer Ingelheim, sales: 1.2 billion US$ in 2005).

Intellectual, Synthetic Substitutes of Atropine. Atropine was used for many decades as a mydriatic in ophthalmologic diagnostics. The disadvantage of the alkaloid was the enduring irritation of the patients for hours (dilated pupils). Nowadays, ophthalmologists prefer, e.g., tropicamide whose name (INN) was created in memory of its "natural precursor"; it represents an intellectual derivative of atropine with decisive, retained structural characters of the alkaloid (Fig. 3.25).

Appendix: Tropisetron. This semisynthetic drug, 3α-hydroxytropane acylated by 3-carboxyindol (Fig. 3.25), turned out to be a potent 5-HT_3 antagonist. It is used as a remedy against emesis and queasiness induced by cytostatic agents and/or radiation during the therapy of tumor diseases.

3.4.5.2 Ethnobotany and Ethnomedicine

Compounds such as hyoscyamine/atropine belong to the delirantia, one famous group of hallucinogenic agents which differ from another one, the psychedelics by interfering with different neuroceptors. Solanaceous tropane alkaloids of the hyoscyamine type may induce hallucinations as a toxic effect in case of overdoses. This property led to a remarkable influence from the ethnobotanical point of view, e.g., in pre-conquest America or ancient Europe. Species from the South American genera *Brugmansia* and *Solandra* as well as *Latua pubiflora* in the New World and species from the genera *Atropa*, *Datura*, *Hyoscyamus*, and *Mandragora* (mandrake) in the Old World have played and still play a significant role in ethnomedicine and religious practices. Especially *Atropa belladonna*, *Datura stramonium*, *Hyoscyamus niger*, and *Mandragora officinarum* are of ethnobotanical relevance since ancient times ("witch's herbs"). Since the corresponding literature on such topics is overwhelming readers are referred to the existing literature, e.g., by Schultes (1979), Wolters (1994) [New World], Martinetz (1994), Rätsch (2005) [worldwide].

Special Misuse (Truth Drug). Tropane alkaloid containing plants have been misused since ancient times as agents which force people treated with overdoses to tell the truth in their special state of intoxication (lack of any self-control).

Recent Misuse. Unfortunately, in the past decade reports on the misuse of such crude magic drugs by youths and young adults in Europe increased with often fatal psychiatric complications after intoxication with, e.g., *Atropa* spp., *Brugmansia* spp., *Datura* spp., *Hyoscyamus* spp., *Mandragora* spp. (Göpel and Marcus 2000). Thus, e.g., self-made tea from leaf and flower of angel's trumpet caused psychotic and comatous condition of two patients (Dinkel and Bedner 2001).

3.4.5.3 Ecological Significance

Insects. Since muscarinic acetylcholine receptors are also present in insects, hyoscyamine/atropine and scopolamine (hyoscine) are neurotoxins for them, too (Kitamura et al. 2004). However, there are only a few reports on this topic and the ecological significance of these alkaloids is not well-described to date. Nevertheless, there is no doubt about the fact that plants synthesizing these alkaloids possess potent defending weapons also against insect herbivores in principle.

In contrast to primitive genera (3) more derived genera (35) of the neotropical butterfly subfamily Ithomiinae (Lepidoptera: Nymphalidae) feed as larvae on Solanaceae (Trigo et al. 1996 and references therein). However, there is little knowledge about the role of metabolites from this plant family with respect to these insects. In a detailed study larvae of *Placidula euryanassa* Felder & Felder were found to sequester tropane alkaloids, especially hyoscyamine, *nor*hyoscyamine, and scopolamine, from the host plant *Brugmansia suaveolens*. They were passed through the pupae to freshly emerged male and female adults (Freitas et al. 1996). GC/MS analysis revealed a number of tropanes which could not be detected in the plant but

in the insects, e.g., degradation products of scopolamine (scopine, scopoline) or transformation products of all three alkaloids (isomeric 6-hydroxyhyoscyamines and their *nor* congeners). Just the opposite was true for some "6-acyl-3-acyloxytropanes", minor alkaloids of the host plant, which were not present in the insects. Pooled individuals contained decreasing amounts of tropanes dependent on the stage of development: Larvae 63, pupae 31, and adults 0.4 µg/individuum. This storage apparently makes them unpalatable to vertebrate predators as was shown with chickens and monkeys. However, the neotropical spider *Nephila clavipes* L. (Araneae: Tetragnathidae) fed on tropane-containing adults and even on palatable prey coated with 200 µg atropine though it did not accept pyrrolizidine-containing prey. Interestingly, another ithomiine butterfly, *Miraleria cymothoe* HEWITSON feeding on the same host plant was found to lack even trace amounts of tropanes in all stages; they were detected in the larval faeces. However, pyrrolizidine alkaloids were identified in *wild-caught adults* of both species, in case of *P. euryanassa* together with tropanes (ratio tropanes/pyrrolizidines: 0.2–7.5:1 (see also Sect. 3.7). The larvae of *M. cymothoe* not sequestering/storing tropanes are cryptic in contrast to the larvae of *P. euryanassa* which are aposematic in colour and behaviour. In the opinion of the authors the capacity of storing these compounds is probably a primitive trait in the subfamily Ithomiinae.

Kitamura et al. (2004) observed that the winter cherry bug, *Acanthocoris sordidus* THUNBERG (Heteroptera: Coreidae) colonized the stems of *Duboisia leichhardtii* and fed on the phloem sap which contains scopolamine and hyoscyamine together with sucrose. Surprisingly, the ratio of scopolamine vs. hyoscyamine in the bugs turned out to be very low (0.46:1) in contrast to the one in the leaves of the plant (7.2:1). Laboratory feeding experiments showed that the bugs are able to take up both alkaloids and to de-epoxidize scopolamine to hyoscyamine, thus reversing the plant biosynthesis: Bugs reared with an artificial scopolamine/sucrose (hyoscyamine-/atropine-free) diet converted this alkaloid in total to hyoscyamine though the final alkaloid content of the bugs was a hundred times higher than those collected from the wild. The question remains: What is the advantage of this de-epoxidation for the bug? Both, scopolamine as well as hyoscyamine is a potent antagonist of the muscarinic acetylcholine receptors. The authors argued: "Scopolamine is more hydrophobic and hence able to pass the blood brain barrier that controls entry into the central nervous system." Winter cherry bugs may use accumulated tropanes for their own protection against potential predators.

Shonle and Bergelson (2000) found negative directional selection for scopolamine (natural selection acting to reduce scopolamine levels) and stabilizing selection for hyoscyamine (natural selection acting to maintain an intermediate level of hyoscyamine) in *Datura stramonium*.

Induced Plant Defence Against Herbivory. A dramatic increase of the alkaloid level doubling those in controls was found in a study concerning larval feeding of the tobacco budworm, *Heliothis virescens* F. (Lepidoptera: Noctuidae) on leaves of *Atropa belladonna* sub nom. *A. acuminata*. This change was achieved when 9% of the leaf tissue had been eaten whereas 5% or less caused only very little effect. The induction was limited to the leaves. However, comparable mechanical damage led to the same results (Khan and Harborne 1991).

3.4.5.4 Economic Significance

Domestication and crop development of solanaceous species producing 3α-tropoyloxytropanes such as hyoscyamine or scopolamine have been of remarkable economic significance. Nowadays, raw material is predominantly if not exclusively derived from cultivated plants. A number of species have been used for the production of hyoscyamine/atropine, especially *Atropa belladonna* (roots), *Hyoscyamus muticus*, Egyptian henbane (aerial parts), and *Scopolia carniolica* (root) as well as for scopolamine, e.g., *Duboisia* spp. (leaves) and *Datura metel* (seeds). A main source of raw material for tropane alkaloid production worldwide is represented by *Duboisia* hybrid clones from plantations in Queensland/Australia. These plants were obtained by cross-breeding from *D. leichhardtii* and *D. myoporoides*, two trees indigenous to Queensland. This hybrid can be easily propagated from cuttings. Most hybrid plantations yield leaf containing 1.5–2.5% scopolamine [for comparison: aerial parts of *Brugmansia aurea* 0.55% according to El-Dabbas and Evans (1982); *B. sanguinea* 0.8% (Griffin and Lin 2000 and references therein)]. The main advantage of the *Duboisia* hybrid from the phytochemical point of view is the fact that it does not contain nicotinoids in contrast to *D. myoporoides*. Content of nicotinoids would make the isolation of scopolamine less efficient (Griffin and Lin 2000). The review published by these two authors contains a section on commercial exploitation of tropane alkaloid containing species with two subsections: (i) cultivation of *Duboisia* spp. and (ii) cultivation of *Brugmansia* spp.

Ontogenetic Variations. The significance of certain solanaceous taxa as a commercial source of hyoscyamine and scopolamine, respectively, stimulated studies on ontogenetic variations. Thus, in an extensive study roots and stems of *Datura stramonium* were checked in four different stages of development, as well as leaves, pericarp, and seeds in two different stages each (Berkov et al. 2005). As expected the number and the structural types of tropanes turned out to depend on the stages of development. Due to the very complex alkaloid profile of this species a lot of interesting results could be obtained.

Tissue Cultures. It shall be pointed out that there are numerous reports on the production of tropane alkaloids by tissue cultures. This topic is of remarkable scientific interest. It was summarized with a lot of almost exclusively solanaceous species in Table I of a review written by Robins and Walton (1993). However, previous expectations to use tissue cultures for an economic production of such alkaloids did not come true.

Crude Drugs Containing Tropanes. Beside monographs of the pure tropane alkaloids many pharmacopoeias worldwide contain monographs of traditional *crude* drugs such as leaves of *Atropa belladonna* (Belladonnae folium), *Datura stramonium*, (Stramonii folium). However, these crude drugs are not suitable for the production of alkaloids due to their moderate content. Furthermore, the medicinal significance of such crude drugs in western industrial countries is limited meanwhile. Information on the content of such alkaloids in plant species and their crude drugs can be obtained from the corresponding *genus* monographs, e.g., *Atropa*, *Datura* (including *Brugmansia*), *Mandragora* (Hänsel et al. 1992).

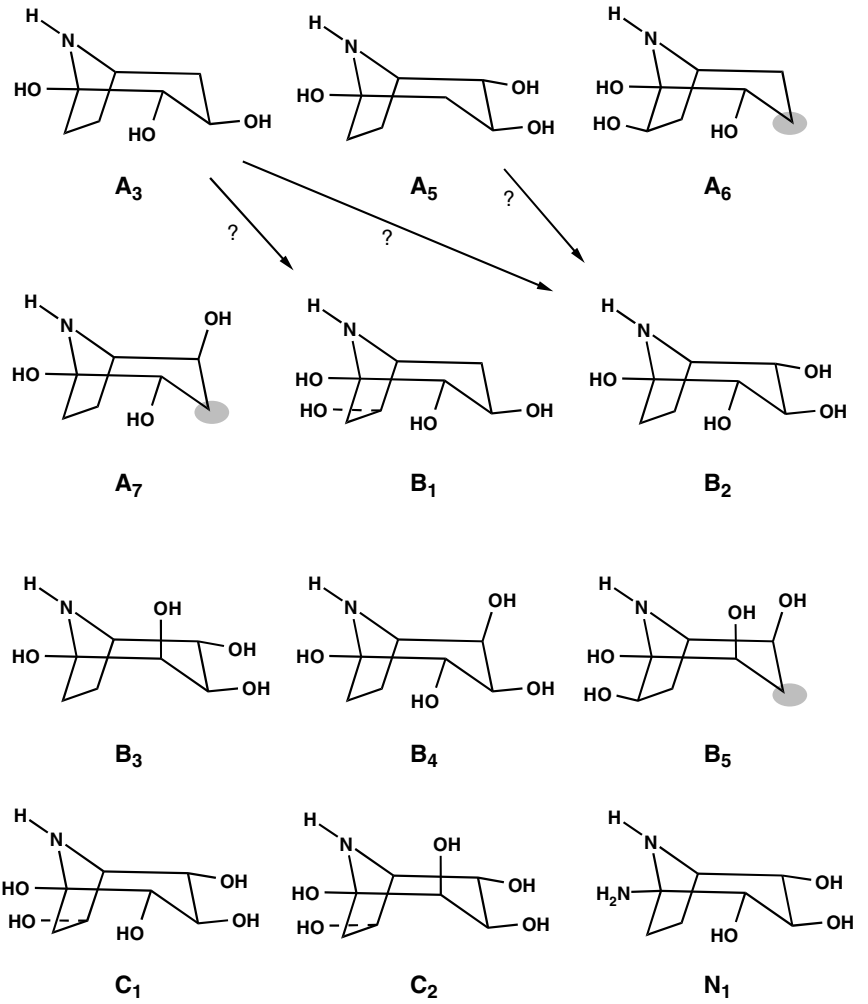


Fig. 3.27 Structures of calystegines (polyhydroxy*nor*tropanols); calystegines A_8 and B_6 (structure of both not shown) represent the 2-epimer of A_3 and the 4-epimer of B_3, respectively (numbering see Fig. 3.17). Highlighted in *grey*: lack of the usual oxygen functionality. *Arrows* indicate putative biogenetic relationships

3.5 Calystegines (Polyhydroxylated *Nor*tropanes)

3.5.1 Discovery and Structure Elucidation

In the late 1980s a French group interested in the identification of metabolites produced by plant roots that might act as nutritional mediators of specific plant-bacterium relationships discovered the first *nor*tropane-type polyhydroxy alkaloids

as constituents of the root exudates of *Calystegia sepium* (L.) R.Br., hedge bindweed, a convolvulaceous twining vine common in temperate zones of the world (Tepfer et al. 1988). Therefore these compounds have been named calystegines constituting a unique subgroup of the tropane alkaloids. Their late discovery, almost 160 years after that of the first tropane alkaloid atropine in 1833, is due to at least two reasons. In contrast to the classical tropane alkaloids (i) the calystegines occur in very low concentrations and (ii) they cannot be isolated by the classical procedures used for the usually lipophilic traditional alkaloids (e.g., extraction of the free bases by means of lipophilic organic solvents) due to their highly polar properties. Thus, they have been overlooked for such a long time. (For further types of polyhydroxy alkaloids see Sect. 3.5.5).

The calystegines have been detected in this first study also in another convolvulaceous species, *Convolvulus arvensis* L., field bindweed, a common trailing and twining herb of the temperate zones, as well as in the famous solanaceous perennial herb *Atropa belladonna* L., deadly nightshade/poison black cherry, native to European deciduous wood. The first three calystegine structures, the trihydroxy*nor*tropane A_3 and the tetrahydroxy-*nor*tropanes B_1 and B_2, respectively, have been published two years later (Goldmann et al. 1990; Ducrot and Lallemand 1990) (Fig. 3.27). These compounds turned out to be characterized by the unusual aminoketal functionality besides the high degree of hydroxylation and the absence of the *N*-methyl group thus forming a *nor*tropane skeleton.

Compounds. To date the structures of 15 calystegines have been elucidated in total (Asano et al. 2000) including five trihydroxy*nor*tropanes (A_3, A_5–A_8), six tetrahydroxy congeners (B_1–B_6), and two pentahydroxy derivatives (C_1, C_2) (Fig. 3.27). With the exception of A_3, B_1, B_2 (*Calystegia sepium*; Goldmann et al. 1990), and C_1 (*Morus alba* L., Moraceae; Asano et al. 1994b) they have been discovered in solanaceous species: A_5 and B_3 in *Physalis alkekengi* (Asano et al. 1995), A_6 and A_8 in *Hyoscyamus niger* (Asano et al. 1996a, 2000), A_7 and B_5 in *Lycium chinense* (Asano et al. 1997a), B_4 and B_6 in *Scopolia japonica* (Asano et al. 1996b, 2000). Besides free calystegines, several glycosides could be isolated and characterized, e.g., the 3-*O*-β-D-glucopyranoside of B_1 from *Nicandra physalodes* (Griffiths et al. 1996) and the same glycoside as well as the 4-*O*-α-D-galactopyranoside of B_2 from *Atropa belladonna* and *Solanum tuberosum*, respectively (Nash et al. 1998, Watson et al. 2000). Furthermore, another group of calystegine derivatives has been described: The *N*-methylated congeners of B_2 and C_1, respectively, again as constituents of *Lycium chinense* (Asano et al. 1997a).

An exceptional position is taken by calystegine N_1, a *nor*tropane with a bridgehead amino group instead of a hydroxyl group thus forming an amino analogue of B_2. This compound has been discovered as a constituent of *Hyoscyamus niger* (Asano et al. 1996a). However, there are doubts if it is really a natural product: It may be an artefact formed from B_2 during the isolation procedure using aqueous ammonia solution (Molyneux et al. 1996). Anyhow, as a matter of fact N_1 is not very stable: it has been converted in part to B_2 even on storage at 4 °C (Asano et al. 1996a).

Occurrence in Non-solanaceous/Non-convolvulaceous Taxa. To date, the calystegines seem to be confined to altogether six plant families. They have been detected

Table 3.8 Distribution of the most frequent calystegines in the Solanaceae including species found negative

Subfamily tribe/clade *species*	**Distribution of the most frequent calystegines**							**References (in bold: Ref. of isolation)**
	A_3	**A_5**	**B_1**	**B_2**	**B_3**	**B_4**	**C_1**	
Petunioideae								
Brunfelsia nitida BENTH.	+	–	+	+	+	–	+	(1)
Petunia × *hybrida* VILM.	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(2)
Cestroideae / Cestreae clade								
Cestrum nocturnum L.	–	–	–	–	–	–	–	(3)
Nicotianoideae								
Nicotianeae clade								
Nicotiana tabacum L.	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(2)
Cyphanthera clade								
Duboisia leichhardtii F.MUELL.[a]	–	isol.	isol.	isol.	–	isol.	isol.	**(3,4)**
Solanoideae								
Hyoscyameae clade								
Atropa belladonna L.[b]	+	+	+	+	+	–	–	(1,2,5–7)
Hyoscyamus albus L.[b]	+	–	+	+	+	–	–	(1,5)
H. aureus L.[c]	+	(–)	(–)	(–)	(–)	(–)	(–)	(5)
H. muticus L.[c]	+	(–)	+	+	(–)	(–)	(–)	(5,8)
H. niger L.[b,d]	isol.	isol.	isol.	isol.	isol.	–	–	(5,6,**9**)
H. pusillus L.[c]	+	(–)	(–)	(–)	(–)	(–)	(–)	(5)
Scopolia carniolica JACQ.[e]	+	(–)	+	+	(–)	(–)	(–)	(6)
S. japonica MAXIM.	isol.	isol.	isol.	isol.	isol.	isol.	isol.	(**10**)
Nolaneae clade								
Nolana humifusa (GOUAN) JOHNSTON	–	–	–	–	–	–	–	(11)
Lycieae clade								
Lycium chinense L.[a,b,f]	isol.	isol.	isol.	isol.	isol.	isol.	isol.	(4,**12**)
Mandragoreae clade								
Mandragora officinarum L.	+	(–)	+	+	(–)	(–)	(–)	(6)
do. sub nom. *M. autumnalis* BERTOL.	+	–	+	+	+	–	–	(1)
Nicandreae clade								
Nicandra physalodes (L.) GAERTN.[g]	(–)	(–)	isol.	(–)	(–)	(–)	(–)	(**13**)
Datureae clade								
Datura inoxia MILL.	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(2)
D. metel L.	–	–	–	–	–	–	–	(1)
D. stramonium L.	+	(–)	+	+	(–)	(–)	(–)	(2,6)
D. wrightii REGEL	(–)	(–)	(–)	+	(–)	(–)	(–)	(14)
Solaneae clade								
Solanum betaceum (CAV.) SENDT. sub nom. *Cyphomandra betacea*				+				(15)

(continued)

Table 3.8 Distribution of the most frequent calystegines in the Solanaceae including species found negative (continued)

Subfamily tribe/clade *species*	**Distribution of the most frequent calystegines**							**References (in bold: Ref. of isolation)**
	A_3	**A_5**	**B_1**	**B_2**	**B_3**	**B_4**	**C_1**	
S. capsicastrum LINK	(–)	(–)	(–)	(–)	(–)	(–)	(–)	(2)
S. dimidiatum RAF.[h]	–	(–)	(–)	+	(–)	(–)	(–)	(14)
S. dulcamara L.[b]	isol.	isol.	isol.	isol.	–	–	–	(**3**,14)
S. kwebense N.E.BR. ex C. WRIGHT	–	(–)	(–)	+	(–)	(–)	(–)	(14)
S. lycopersicum L. (syn.: *Lycopersicon esculentum* MILL.)	+			+				(15)
S. melongena L.	+		+	+				(15)
S. nigrum L.	–	–	–	–	–	–	–	(6)
S. scabrum MILL.[h]				+				(15)
S. sodomaeum L.[h]	–	–	–	+	+	–	–	(1)
S. tuberosum L.	+		+	+		+		(6,14–17)
Capsiceae clade								
Capsicum annuum L. var. *annuum* sub nom. *C. annuum*			+	+			+	(15)
C. annuum var. *frutescens* (L). KUNTZE sub nom. *C. frutescens*	+		+	+				(15)
Physaleae clade / Physalinae subclade								
Physalis alkekengi L. var. *francheti*	isol.	isol.	isol.	isol.	isol.	–	–	(**18**)
P. philadelphica LAM. sub nom. *P. ixocarpa* BROT.[i]				+				(15)
P. peruviana L.	+		+	+			+	(15)
Physaleae clade / Withaninae subclade								
Withania frutescens (L.) PAUQ.[b]	+	–	+	+	+	–	+	(1)
W. somnifera (L.) DUNAL	–	–	–	+	–	–	+	(1)

isol. = Compound isolated from this species; + = compound detected; – = compound not detected; (–) = compound assumed to be absent since no further differentiation is given in the literature

[a] In addition: C_2; [b] in addition: N_1; [c] cultured roots; [d] in addition: A_6, A_8; [e] in addition: B_6; [f] in addition: A_6, A_7, B_5, *N*-methyl-B_2, *N*-methyl-C_1; [g] in addition: B_1-β-D-glucoside; [h] supposed authority; no authority given in the corresponding literature though according to IPNI at least two possibilities exist; [i] named erroneously *P. exocarpa* in (15).

References: (1) Bekkouche et al. 2001; (2) Tepfer et al. 1988; (3) Asano et al. 2001; (4) Kato et al. 1997; (5) Dräger et al. 1994; (6) Dräger et al. 1995; (7) Rothe et al. 2001; (8) Sevón et al. 1997; (9) Asano et al. 1996a; (10) Asano et al. 1996b; (11) Schimming 2003; (12) Asano et al. 1997a; (13) Griffiths et al. 1996; (14) Nash et al. 1993; (15) Asano et al. 1997b; (16) Keiner et al. 2000; (17) Friedman et al. 2003; (18) Asano et al. 1995.

also in a four families unrelated to the Solanales: Moraceae (Asano et al. 1994a, b), Erythroxylaceae, Rhizophoraceae (Brock et al. 2005), and Brassicaceae (Brock et al. (2006). This is not very surprising because all of these are also known as producer of lipophilic tropanes, e.g., the 3β-tropanol derivative cocaine (*Erythroxylum coca* LAM.) and the 3α-tropanol derivative brugine [*Bruguiera sexangula* (LOUR.) POIR] (Rhizophoraceae) (see also Table 3.7). Samples of 102 further species from many families of the whole plant kingdom did not show the occurrence of calystegines (Tepfer et al. 1988).

3.5.2 Occurrence in the Solanaceae (Table 3.8)

To date, this family is still not screened systematically for calystegines. A few studies include several species each (Tepfer et al. 1988; Dräger et al. 1994; Asano et al. 2001; Bekkouche et al. 2001) but without taxonomic objective. Nevertheless, the addition of these results as well as of those obtained from species which have served as organisms for the discovery and first isolation of the calystegines (see above) justifies the assumption that these alkaloids are widespread in the family. The occurrence is documented in the literature in total for 13 genera (out of ~95) covering 31 species (out of ~2500). Due to common biogenetic precursors it is not very surprising that calystegines have been detected in solanaceous taxa which are well-known producers of classical lipophilic tropane alkaloids, e.g., hyoscyamine/scopolamine by genera like *Atropa*, *Duboisia*, *Hyoscyamus* and others. But it is really remarkable that genera which apparently are not capable to synthesize such esters of 3α-hydroxytropane like *Brunfelsia*, *Capsicum*, *Solanum* and others nevertheless do produce such 3β-hydroxy*nor*tropane derivatives (for references see Table 3.8). The isolation of a cDNA from potato (*Solanum tuberosum*) tuber sprout mRNA that encodes a 3β-hydroxytropane forming tropinone (= 3-oxotropane) reductase (TR II) has been reported. This enzyme showed high homology to the TR II from *Datura stramonium* and *Hyoscyamus niger*, respectively (Keiner et al. 2000, 2002).

On the other hand, to date, almost all calystegine-checked 3α-hydroxytropane-synthesizing solanaceous species are also calystegine-positive with the exception of *Datura inoxia* (Tepfer et al. 1988) and *D. metel* (Bekkouche et al. 2001). Furthermore, there are some reports on calystegine-negative non-3α-hydroxytropane-synthesizing species: *Cestrum nocturnum* (Asano et al. 2001), *Nicotiana tabacum* (Tepfer et al. 1988, Dräger et al. 1995), *Nolana humifusa* (Schimming 2003), *Petunia* × *hybrida*, *Solanum capsicastrum* (Tepfer et al., 1988); *S. nigrum* (Dräger et al. 1995).

Certain species turned out to contain a rich spectrum of calystegines, especially *Lycium chinense* (13 compounds, N_1 not counted) and *Scopolia japonica* (7). The majority of the species showed 3–5 compounds. The major components of solanaceous species are usually A_3 and B_2 (Keiner et al. 2000). These two metabolites and in addition B_1 are the most frequent ones followed by B_3, A_5, B_4, and C_1. The occur-

rence of the remaining congeners A_6–A_8, B_5, B_6, and C_2 as well as the *N*-methyl derivatives of B_2 and C_1, respectively, is limited to one or two species only. The exceptional role of N_1 found in some species has been discussed above.

Concentrations in µg/g fresh mass have been determined from 0.003 (*Physalis peruviana* fruit /A_3) to 450 (*Solanum tuberosum* tuber peel/B_2) or extraordinarily even to 2300 (*S. tuberosum* young tuber sprout/B_2) or rather dry mass from 4 (*Hyoscyamus muticus* leaf/A_3) to 350 (*Brunfelsia nitida* leaf/C_1) (Dräger 2004 and references therein). In principle calystegines may occur in any organ of a plant. They have been found in all vegetative and generative tissues though this must not be the case in every species or at every time of harvest. If present, the concentrations vary remarkably also within one single plant depending on the organs and their developmental stage. Young leaves of *Atropa belladonna* have shown concentrations of A_3, B_1, and B_2 which have been five times higher than those of adult leaves. In general it seems that especially the concentrations in young tissues surmount elder ones (Dräger et al. 1995).

The observation that half of A_3, the presumable precursor of certain calystegines of the B-series is not metabolized, in spite of the fact that the fourth hydroxyl group is introduced quickly, led to the assumption that calystegines are subjected to a compartimentation procedure, e.g., transport into and storage in the vacuole. Such a regulated hydroxylation and selective transport could explain how species may maintain their individual, constant, and characteristic calystegine pattern (Scholl et al. 2003).

Food Plants. Calystegines were detected in all the edible solanaceous fruits and vegetables tested (Asano et al. 1997b). They were found in over 70 varieties of potatoes (Nash et al. 1998; Watson et al. 2000). Friedman et al. (2003) reported on calystegine contents of eight potato cultivars. Particularly high levels of calystegines have been determined in *Solunum melongena*, eggplant/aubergine, and *Capsicum annuum* var. *annuum*, bell pepper/paprika (Andersson and Ahman 2004 and references therein).

3.5.3 Occurrence in the Convolvulaceae (Table 3.9)

In contrast to its sister family, the Convolvulaceae have been investigated systematically with regard to the calystegines. They turned out to be common metabolites: In two GC/MS studies comprising altogether 135 species (out of ~1850) from 29 genera (out of ~55) of all of the 12 tribes after all 69 species belonging to 22 genera and 11 tribes were found to be unequivocally calystegine-positive (Schimming et al. 1998, 2005). In the majority of the cases samples of epigeal vegetative parts and roots but sometimes also of flowers and fruits/seeds have been investigated. Otherwise, the situation is very similar to the Solanaceae: Like in their case again it is not very surprising that these calystegines have been detected in convolvulaceous taxa which synthesize lipophilic tropane alkaloids. However, it is remarkable

Table 3.9 Distribution of the most frequent calystegines in the Convolvulaceae including species found negative; data from Schimming et al. (1998, 2005) if not indicated otherwise

Tribe *species* (for certain genera: section)	**Distribution of the most frequent calystegines**							
	A_3	**A_5**	**B_1**	**B_2**	**B_3**	**B_4**	**C_1**	**Precursors[a]**
Humbertieae								
Humbertia madagascariensis LAM.	+	–	–	–	–	+	–	n.d.
Cardiochlamyeae								
Cardiochlamys madagascariensis OLIV.	–	–	+	+	–	+	–	n.d.
Erycibeae								
Erycibe macrophylla HALL. f.	–	–	+	+	+	–	?	n.d.
E. malaccensis C.B.CLARKE	–	–	–	+	–	–	–	n.d.
E. micrantha HALL. f.	+	–	+	+	–	–	+	1
E. parvifolia HALL. f.	–	–	–	+	–	–	–	n.d.
E. rheedii BLUME	–	–	–	–	–	–	+	n.d.
Dichondreae								
Dichondra micrantha URB.	+	–	–	+	+	+	–	1,2
D. sericea SW.	–	–	–	+	–	–	–	1,2
Falkia repens L. f.	–	–	+	+	–	–	–	1,2,4
Porana volubilis BURM. f.	?	–	+	+	+	–	+	n.d.
Rapona tiliifolia (BAK.) VERDC.	+	–	+	+	+	–	–	n.d.
Cresseae								
Bonamia dietrichiana HALL. f.	–	–	–	–	–	–	–	–[b]
B. semidigyna (ROXB.) HALL. f. var. *semidigyna*	–	–	–	+	–	–	–	1,2,4
B. spectabilis (CHOISY) HALL. f.	–	–	–	+	–	+	–	1,2,3,4
B. trichantha HALL. f.	–	–	–	–	–	–	–	2,3,4
Evolvulus argyreus CHOISY	–	–	–	+	–	–	–	2,4
E. glomeratus CHOISY cv. 'Blue Days'	–	–	–	–	–	–	–	1,2,4
E. nummularius L.	–	–	–	–	–	–	–	1,2
Hildebrandtia austinii STAPLES	–	–	–	–	–	–	–	n.d.
H. promontorii DEROIN	–	–	–	–	–	–	–	n.d.
H. valo DEROIN	+	–	–	+	+	–	–	n.d.
Maripeae								
Maripa panamensis HEMSL.	+	–	+	+	+	–	–	1,2
Jacquemontieae								
Jacquemontia corymbulosa BENTH.	–	–	–	–	–	–	–	–
J. paniculata (BURM. f.) HALL. f.	–	–	–	–	–	–	–	–
J. pentantha (JACQ.) G.DON	–	–	–	–	–	–	–	2
J. reclinata HOUSE	–	–	–	–	–	–	–	n.d.
J. tamnifolia (L.) GRISEB.	+	+	+	+	?	?	+	1,2
J. tomentella (MIQ.) HALL. f.	–	–	–	–	–	–	–	n.d.
Cuscuteae								
Cuscuta approximata BAB. ssp. *episonchum* WEBB. & BERTH.	–	–	–	–	–	–	–	n.d.

(continued)

Table 3.9 Distribution of the most frequent calystegines in the Convolvulaceae including species found negative; data from Schimming et al. (1998, 2005) if not indicated otherwise (continued)

Tribe *species* (for certain genera: section)	**Distribution of the most frequent calystegines**							
	A_3	**A_5**	**B_1**	**B_2**	**B_3**	**B_4**	**C_1**	**Precursors[a]**
C. australis R.Br.	–	–	–	–	–	–	–	2,4
C. europea L.	–	–	–	–	–	–	–	n.d.
C. palaestina Boiss.	–	–	–	–	–	–	–	n.d.
C. sp. (on *Launaea arborescens* (Batt.) Murb., Asteraceae)	–	–	–	–	–	–	–	n.d.
Aniseieae								
Aniseia martinicensis (Jacq.) Choisy	+	–	+	+	–	–	–	1,2,4
Iseia luxurians (Moric.) O'Donell	–	–	+	+	–	–	+	2,4
Odonellia hirtiflora (Mart. & Gal.) K. Rob.	–	–	–	–	–	–	–	1
Convolvuleae								
Calystegia japonica Choisy[b]	isol.	–	isol.	isol.	–	–	–	n.d.
C. macrostegia ssp. *cyclostegia* (House) Brummit	–	–	–	–	–	–	–	1,2
C. sepium R.Br.	+[c]	+	+[c]	+[c]	+	–	–	1,3,4
C. silvatica (Kit.) Griseb.	+	+	+	+	–	+	–	2,3,4
C. soldanella (L.) Roem. & Schult.[b]	isol.	–	isol.	isol.	isol.	–	–	n.d.
Convolvulus arvensis L.	+	+	+	+	+	+	?	1,2,3
C. caput-medusae Lowe	+	–	+	+	–	–	–	1,2
C. chilensis Pers.	–	–	–	–	–	–	–	1,2,3
C. clementii Domin	–	–	–	?	–	?	–	–
C. cneorum L.	+	+	+	+	–	–	–	1,2,3,4
C. demissus Choisy	–	–	–	–	–	–	–	1,2,4
C. elongatus Willd.	+	–	+	+	–	+	–	1,2,3,4
C. floridus L. f.	–	–	–	–	–	–	–	1,2,3,4
C. glandulosus (Webb.) Hall.	–	–	+	+	–	–	–	1,2,3
C. graminetinus (R.Br.) Spreng.	–	–	–	+	–	?	–	1,2
C. humilis Jacq.	+	–	+	+	–	–	–	n.d.
C. kilimandschari Engl.	–	–	–	–	–	–	–	2
C. lopezsocasii Svent.	–	–	–	–	–	–	–	1,2,3,4
C. sabatius Viv. ssp. *mauritanicus* (Boiss.) Murb.	+	–	+	+	?	–	+	1.2
C. sagittatus Thunb.	–	–	–	?	–	–	–	1,2,3
C. scoparius L. f.	–	–	–	–	–	–	–	–
C. subauriculatus (Burch.) Linding.	–	–	+	+	–	–	–	2
C. tricolor L. ssp. *tricolor*	–	+	+	+	–	–	?	1,2
Polymeria ambigua R.Br.	–	–	–	–	–	–	–	1
P. calycina R.Br.	–	–	–	–	–	–	–	1
P. longifolia Lindl.	–	–	–	–	–	–	–	2
P. marginata Benth.	–	–	–	–	–	–	–	–
P. pusilla R.Br.	–	–	–	–	–	–	–	–
"Merremieae"								

(continued)

Table 3.9 Distribution of the most frequent calystegines in the Convolvulaceae including species found negative; data from Schimming et al. (1998, 2005) if not indicated otherwise (continued)

Tribe *species* (for certain genera: section)	**Distribution of the most frequent calystegines**							
	A_3	**A_5**	**B_1**	**B_2**	**B_3**	**B_4**	**C_1**	**Precursors**[a]
Hewittia sublobata (L. f.) KUNTZE	–	–	–	–	–	–	–	1
Genus *Merremia*								
Tuberosa allies ("section")								
M. aurea (KELL.) O'DONELL	+	–	+	+	–	–	–	1
M. tuberosa (L.) RENDLE	–	–	–	–	–	–	–	1
Sectio *Cissoides*								
M. cissoides (VAHL) HALL. f.	?	–	?	–	–	–	–	1,2
M. quinquefolia (L.) HALL. f.	–	–	?	–	–	–	–	1,2,3
Vitifolia allies ("section")								
M. dissecta (JACQ.) HALL. f.	–	–	–	+	–	–	–	1,2,3
Sectio *Xanthips*								
M. umbellata (L.) HALL. f.	–	–	+	+	–	–	–	2,4
M. pterygocaulos (STEUD. ex CHOISY) HALL. f.	–	–	–	–	–	–	?	1,3
Operculina aequisepala (DOMIN) R.W.JOHNSON	–	–	–	–	–	–	–	1
O. pteripes (G.DON) O'DONELL	–	–	–	–	–	–	–	–
O. riedeliana (OLIV.) OOSTSTR.	–	–	–	–	–	–	–	1,2
O. triquetra (VAHL) HALL. f.	–	–	–	–	–	–	–	n.d.
O. turpethum (L.) SILVA MANSO	–	–	–	–	–	–	–	n.d.
Xenostegia medium (L.) D.F.AUSTIN & G.STAPLES	–	–	–	–	–	–	–	–
Ipomoeeae								
Argyreia androyensis DEROIN	–	–	+	+	–	+	+	n.d.
A. capitata (VAHL) CHOISY	–	–	+	+	–	–	–	1,2,3
A. hookeri CLARKE	–	–	+	–	–	+	+	1
A. mollis (BURM. f.) CHOISY	+	–	+	+	+	?	+	2
A. nervosa (BURM. f.) BOJ.	–	–	–	–	–	–	–	1,2
A. onilahiensis DEROIN	–	–	+	+	–	+	+	n.d.
A. vahibora DEROIN	–	–	+	+	–	+	+	n.d.
Astripomoea malvacea (G.KLOTZ) MEEUSE	–	–	–	–	–	–	–	1
Genus *Ipomoea*								
Sectio *Calonyction*								
I. alba L.	+	+	+	+	+	?	+	1,2,3,4
I. turbinata LAG.	–	–	–	–	–	–	–	1,2
Sectio *Eriospermum*								
I. anisomeres ROB. & BARTL.	–	–	–	–	–	–	–	1,2,3,4
I. batatas (L.) (LAM.) (wild form)	+	–	+	–	+	–	–	1,2,4
do. (cultivar)	–	+	+	+	+	–	?	1,2,3,4
do. var. *edulis* MAKINO[b]	–	–	isol.	isol.	–	–	–	n.d.
I. batatoides CHOISY	–	–	–	–	–	–	–	2
I. cairica (L.) SWEET	–	–	–	+	–	–	–	1,2,3,4
I. carnea JACQ.[b]	–	–	isol.	isol.	–	–	–	1
do. ssp. *fistulosa* (MART. ex CHOISY) D.F.AUSTIN	+	–	+	+	?	+	+	n.d.

(continued)

Table 3.9 Distribution of the most frequent calystegines in the Convolvulaceae including species found negative; data from Schimming et al. (1998, 2005) if not indicated otherwise (continued)

Tribe *species* (for certain genera: section)	**Distribution of the most frequent calystegines**							
	A_3	**A_5**	**B_1**	**B_2**	**B_3**	**B_4**	**C_1**	**Precursors**[a]
do.[d]	–	–	–	+	–	–	+	n.d.
I. eremnobrocha D.F.Austin	–	–	–	?	?	–	–	2,4
I. habeliana Oliv.	–	–	–	–	–	–	–	n.d.
I. horsfalliae Hook.	–	–	–	–	–	–	–	n.d.
I. mauritiana Jacq.	–	–	–	–	–	–	–	1
I. regnellii Meisn.	–	–	–	–	–	–	–	1,2
I. reticulata O'Donell	–	–	–	–	–	–	–	1
I. squamosa Choisy	–	–	+	+	–	–	–	1,2,3
I. trifida (H.B.K.) G.Don	–	–	+	+	–	–	–	1,2,3
I. tuxtlensis House	–	–	–	?	–	–	–	–
I. umbraticola House	–	–	–	–	–	–	–	n.d.
Sectio *Erpipomoea*								
I. aquatica Forssk.	+	–	+	+	?	+	–	1,2,3,4
I. asarifolia Roem. & Schult.	–	–	–	–	–	–	–	1,2,3,4
I. graminea R.Br.	–	–	–	–	–	–	–	n.d.
I. lonchophylla J.Black[e]	+	–	+	+	+	–	+	1,2
I. muelleri Benth.[e]	+	–	+	+	+	–	+	1,2,3,4
I. obscura (L.) Ker-Gawl.[b]	–	–	isol.	isol.	isol.	isol.	isol.	n.d.
I. pes-caprae (L.) R.Br.[b]	–	–	–	isol.	–	–	–	n.d.
do. ssp. *pes-caprae*	–	–	–	–	–	–	–	2
I. polpha R.W.Johnson[f]	–	–	–	+	–	–	+	n.d.
I. sp. Q6 [aff. *calobra*][e]	–	–	–	+	–	–	–	n.d.
I. stolonifera (Cyr.) Gmelin[g]	–	–	–	+	–	–	–	n.d.
I. trichosperma Blume	–	+	–	–	–	–	–	2,4
I. violacea L. [syn.: *I. tuba* (Schlecht.) G.Don]	–	–	+	+	?	–	+	1,2,3,4
Sectio *Involucratae*								
I. involucrata Beauv.	–	–	+	?	+	–	?	1
Sectio *Ipomoeae*								
I. wightii (Wall.) Choisy	–	–	+	–	–	–	–	1
Sectio *Leptocallis*								
I. capillacea G.Don	–	–	–	–	–	–	–	n.d.
Sectio *Mina*								
I. hederifolia L.	–	+	+	+	–	–	–	1
do. sub nom. *Quamoclit angulata* Boj.[b]	–	–	isol.	isol.	–	–	–	n.d.
I. lobata (Cerv.) Thell. [syn.: *Mina lobata* Cerv.]	–	–	+	+	–	–	+	–
I. neei (Spreng.) O'Donell	–	–	–	–	–	?	–	–
Sectio *Orthipomoea*								
I. plebeia R.Br.	–	–	–	–	–	–	–	1,2
I. tenuirostris Steud. ex Choisy	–	–	–	–	–	–	–	1
Sectio *Pharbitis*								
I. eriocarpa R.Br.	–	–	?	+	–	?	–	2,3,4
I. indica (Burm. f.) Merr.	–	–	–	–	–	–	–	1
I. nil (L.) Roth	–	–	–	–	–	–	–	1,2

(continued)

Table 3.9 Distribution of the most frequent calystegines in the Convolvulaceae including species found negative; data from Schimming et al. (1998, 2005) if not indicated otherwise (continued)

Tribe *species* (for certain genera: section)	**Distribution of the most frequent calystegines**							
	A_3	**A_5**	**B_1**	**B_2**	**B_3**	**B_4**	**C_1**	**Precursors**[a]
do.[b]	–	–	–	–	–	–	–	n.d.
I. purpurea (L.) ROTH	–	–	–	–	–	–	–	1,2,4
I. setifera POIR.	?	–	+	+	–	–	–	1,2
Sectio *Tricolores*								
I. chiriquiensis STANDL.	–	–	+	+	–	–	–	2,4
I. tricolor CAV. cv. 'Heavenly Blue'	–	–	–	+	–	–	–	1,2,4
Lepistemon binectariferum (WALL.) KUNTZE var. *borneense*	–	–	–	–	–	–	–	n.d.
L. urceolatum (R.BR.) F.MUELL.	–	–	–	?	–	–	+	1,3
Stictocardia tiliaefolia (DESR.) HALL. f. [syn.: S. *campanulata* (L.) MERR.]	+	–	–	+	–	–	–	1,2,4
S. mojangensis (VATKE) D.F.AUSTIN & EICH[h]	+	?	+	+	–	–	–	1,2
Turbina abutiloides (H.B.K.) O'DONELL	+	–	+	+	–	–	–	1
T. corymbosa (L.) RAF.	–	–	–	–	–	–	–	4

isol. = Compound isolated from this species; + = compound detected by GC-MS; **?** = compound not unequivocally detected due to very low concentrations; - = compound not detected by GC-MS

[a] Presumed biogenetic precursors: **1** = 3-oxotropane (tropinone), **2** = 3β-hydroxytropane (pseudotropine), **3** = 3-oxonortropane (nortropinone), **4** = 3β-hydroxynortropane;[b] data from Asano et al. (2001);[c] isolated by Goldmann et al. (1990) and also by Molyneux et al. (1993) from root cultures of this species; [d] data from de Balogh et al. (1998);[e] data from Dorling et al. 2004; [f] data from Molyneux et al. (1995);[g] Dräger, personal communication [syn.: *I. imperati* (VAHL) GRISEB.]; [h] in addition calystegine A_6

that taxa which apparently are synthesizing neither 3α-hydroxytropane esters and nor even 3α-hydroxytropane itself, e.g., *Argyreia mollis*, *Calystegia sepium*, *Convolvulus subauriculatus. C. tricolor*, *Ipomoea chiriquiensis, I. hederifolia*, *I. lobata, I. obscura, I. squamosa*, *Merremia aurea, Turbina abutiloides* turned out to be calystegine-positive. This leads to the assumption that TR I is lacking but TR II is present in such species analogous to *Solanum tuberosum* (Fig. 3.13). Vice versa, certain 3α-hydroxytropane-synthesizing convolvulaceous species did not show any calystegine, e.g., *I. plebeia*, *I. regnellii*, *I. turbinata*, *Jacquemontia pentantha*, *Evolvulus glomeratus*, *Odonellia hirtiflora*, *Polymeria calycina*, and *P. longifolia*. However, this does not demonstrate in every case that TR II may lack whereas TR I is present. The majority of the just mentioned exceptions have shown the additional presence of 3β-tropanol indicating that rather the lack of the enzymes catalyzing the presumably subsequent steps in the biosynthesis are the reason for the absence of calystegines.

Three genera with at least five species each showed a high percentage of positive species (*Argyreia* 6 out of 7, *Calystegia* 4/5, *Erycibe* 5/5). Other genera with at least

4 species included yielded 50/50 results: *Bonamia* (2/4), *Convolvulus* (10/18), *Ipomoea* (20/41), *Merremia* (3/7). *Jacquemontia* has been the strangest genus since the assay of 20 samples (different organs/provenances) taken from altogether six species resulted in 19 negative findings; only one sample turned out to be positive: The roots of *J. tamnifolia* with at least five calystegines. This is especially remarkable because the aerial parts from two provenances and the fruits of this species were also negative.

The number of compounds in calystegine-positive species varied between one and six. Only one compound each could be detected in the samples of 24% of these species in Table 3.9, followed by two (22%) and three (22%) compounds, four (20%), five (9%), and six compounds (3%). The tetrahydroxylated alkaloids B_2 and B_1 turned out to be the most frequent compounds (88% and 67% of the positive species, respectively) followed by the trihydroxy*nor*tropane A_3 (36%) and the pentahydroxylated congener C_1 (23%). Interestingly calystegine B_2 is also in the lead concerning the sister family Solanaceae (Dräger 2004). The calystegines A_5, B_3, and B_4 displayed a minor frequency in the convolvulaceous study ranging from 17% to 19%. Calystegine A_6 could only be detected in *Stictocardia mojangensis*. Neither its A_7 congener nor the *N*-methyl derivatives of B_2 and C_1, respectively, could be identified in any samples.

3-Oxotropane (tropinone), 3β-hydroxytropane (pseudotropine), and 3β-hydroxy*nor*tropane (*nor*pseudotropine) are consecutive candidates as precursors in the biosynthesis of calystegines (Scholl et al. 2001; Dräger 2004) (see Sect. 3.6.4). Therefore the results obtained with regard to these putative precursors (last column in Table 3.9) for as many species as possible are dependent on the availability of additional plant material (90 out of 129 species). In addition the occurrence of 3-oxo*nor*tropane, another potential precursor, was checked. In the vast majority of cases in which calystegines could be detected and also data for the putative precursors are available (39 out of 44 species = 89%) a co-occurrence of hydrophilic and lipophilic alkaloids was demonstrated. This finding strongly supports the assumption that such compounds are real precursors. Furthermore, though five species (11%) displayed the polyhydroxy alkaloids whereas no putative precursor could be found in any checked organ of the corresponding species also these cases are explainable: In principle the lipophilic putative precursors are present mainly in the roots. However, in four out of these five precursor-negative cases no roots have been available but only small amounts of aerial parts (*Argyreia hookeri*, *Ipomoea hederifolia*, *I. lobata*, *I. wightii*). This assumption has supported by the fact that 3-oxotropane had been found already in the roots of *Ipomoea hederifolia* in a previous study (Jenett-Siems 1996) as well as by the finding that another *Argyreia* sp., *A. capitata*, has shown calystegines in the aerial parts but not in the roots, however, their precursors vice versa.

There is also evidence that those calystegine-positive species for which no analysis of the precursors have been carried out nevertheless use them, too: *Maripa panamensis* unambiguously yielded esters of 3β-hydroxytropane (Table 3.6; thus, it may be assumed that this species is also able to synthesize 3β-hydroxytropane itself. *Ipomoea involucrata* as well as *I. squamosa* exhibited 3α-acetoxytropane

(Table 3.2); thus, these species must synthesize 3-oxotropane because this is a precursor of 3α- as well as of 3β-hydroxytropane and their derivatives.

On the other hand, there have been 34 species lacking calystegines though showing at least one precursor. This may be interpreted in different ways depending on the specific situation. (i) A certain species is able to synthesize more lipophilic, biogenetically basal tropanes but unable to transform it into the hydrophilic congeners due to the lack of corresponding enzymes, e.g., *Argyreia nervosa* (four organs / two provenances checked), *Astripomoea malvacea* (three organs checked), *Ipomoea plebeia* (two organs checked). (ii) An accidentally unfortunate selection of the stage of plant development has been carried out. (iii) Only one or two organs of this specific plant have been analyzed with calystegine-negative results whereas these hydrophilic metabolites might have been stored in another organ not checked. There are different examples in which one organ contained the precursors and another one the polyhydroxy alkaloids, e.g., *Argyreia capitata*, *Iseia luxurians*, *Merremia dissecta*. Thus, it might be also that this is the case in species found calystegine-negative, e.g., *Evolvulus nummularius*, *Hewittia sublobata*, *Merremia pterygocaulos*. Besides the large number of unequivocally calystegine-positive species there has been some evidence for the occurrence of calystegines in samples of further seven species. However, it has been impossible to reproduce their results due to the lack of sufficient amounts of the corresponding plant material. These ambiguous taxa have been two *Convolvulus* spp. (*C. clementii*, *C. sagittatus*), three *Merremia* spp. (*M. cissoides*, *M. pterygocaulos*; *M. quinquefolia*), and two *Ipomoea* spp. (*I. neei*, *I. tuxtlensis*), all of them genera with several other unequivocally calystegine-positive species. In such cases a question mark is set for the corresponding compound in Table 3.9.

Only the five species of the monotypic tribe Cuscuteae turned out to be generally calystegine-negative. Apart from *Cuscuta* only two further genera, *Operculina* (five species checked out of 20 spp. recognized; tribe "Merremieae") as well as *Polymeria* (five spp. checked out of seven spp. recognized; tribe Convolvuleae) might have lost the ability to synthesize these plesiomorphic metabolites since all samples from different organs of both genera (12 samples each) did not show any calystegine. Moreover, in the case of the genus *Polymeria* the samples yielded an almost total absence of the putative lipophilic precursors indicating that this genus or at least four out of the five species checked might have lost also the ability for the synthesis of biogenetically basal tropanes. Only one out of the 12 samples resulted in the detection of a very low concentration of 3β-hydroxytropane. Two other genera also found to be calystegine-negative comprise only two recognized species each (*Odonellia*, tribe Aniseieae; *Xenostegia*, tribe "Merremieae"). They were checked with only one species each and two or three samples, respectively. These limited data do not justify the assumption that these two genera are calystegine-free in principle. This is also true for the monotypic genus *Hewittia* (tribe "Merremieae") since only one sample was included as well as for *Astripomoea* (tribe Ipomoeeae) because only one out of 12 recognized species with three samples could be integrated. Moreover, species which did not show any calystegine may not be regarded necessarily as calystegine-negative. The stage of development of such "negative" species at the time of its harvest might have lead to concentrations below

the detection limit though this is very low. Even very small amounts of plant material may show unequivocally calystegine-positive results, e.g., leaves of *Cardiochlamys madagascariensis* (0.35 g dry weight), leaves of *Argyreia androyensis* (0.80 g dry weight), and fruits of *Porana volubilis* (1.5 g dry weight). However, in other cases much higher amounts of plant material might have been not sufficient.

Thus, e.g., 10 g aerial parts of *I. pes-caprae* did not show any calystegine in the study of Schimming et al. (2005) whereas the same organs from another provenance contained calystegine B_1 (Asano et al. 2001). Of course, this could also be a problem of chemotypes.

The concentrations of individual calystegines in the plant organs are often very low, e.g., the leaves were determined to contain calystegine B_2 2.7 mg/kg dry weight, B_3 0.3 mg/kg, C_1 0.25 mg/kg, and A_3 0.05 mg/kg in the case of *Ipomoea lonchophylla* (Dorling et al. 2004). From fresh flowers (590 g) of *I. carnea* ssp. *fistulosa* 27.7 mg B_2, 6.8 mg B_1, 5.2 mg C_1, and 0.8 mg B_3 could be isolated; 4.3 mg

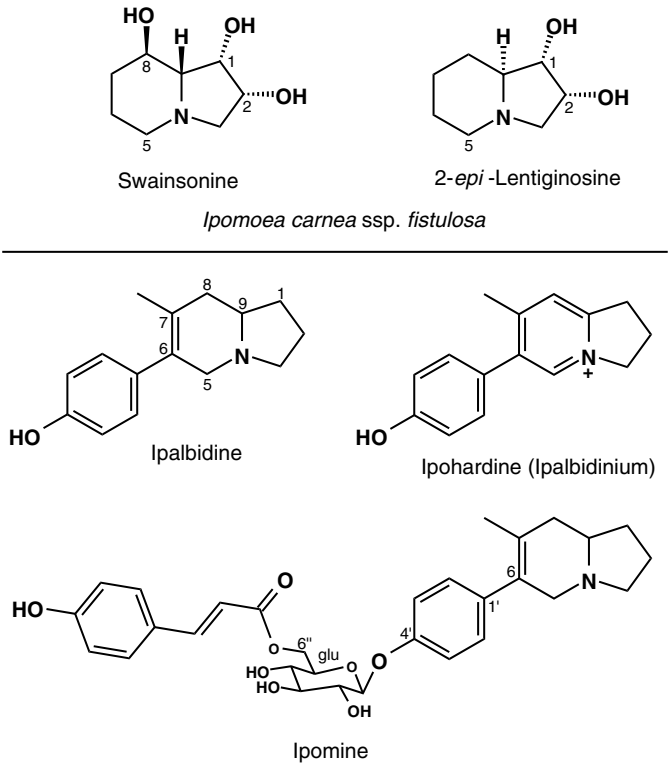


Fig. 3.28 Indolizidine alkaloids from the Convolvulaceae: (i) Hydrophilic polyhydroxy alkaloids (swainsonine, 2-*epi*-lentiginosine; see Sect. 3.5.3) and (ii) unique lipophilic indolizidine alkaloids occurring in *Ipomoea alba*, *I. hardwickii*, and *I. turbinata* (see Sect. 3.6)

B_2 and 2.1 mg B_1 were obtained from the seeds (130 g) (Haraguchi et al. 2003). On the other hand, calystegines are very stable metabolites: Nash et al. (1993) could identify by GC/MS analysis calystegines in dried specimens of the butterfly *Mechanitis polymnia* BATES, orange-spotted tiger clearwing (Lepidoptera: Lymphalidae), which feeds in the larval stage on *Solanum*; these specimens had been collected in 1907.

In conclusion, the results obtained clearly show that the occurrence of calystegines is an almost consistent trait in the Convolvulaceae in principle, from basal to most advanced tribes. It may be assumed that this is also the case in the sister family Solanaceae though its data are still limited. Anyhow, the broad occurrence of these plesiomorphic characters in the Convolvulaceae involves limitations concerning their significance for intra*familial* taxonomic relationships. However, in the case of certain intra*generic* relationships (*Cuscuta*, *Operculina*, *Polymeria*) the lack of these metabolites might be of relevance. Furthermore, it is obvious that the calystegines A_3 and A_5, respectively, could be detected rather frequently in the closely related genera *Convolvulus* and *Calystegia* (both together A_3: 9 out of 14 calystegine-positive species; both together A_5: 7) but turned out to be rather rare in the genus *Ipomoea*. (A_3: 4 out of 20 calystegine-positive species; A_5: 4 out of 20.)

Appendix: Indolizidine Type Polyhydroxy Alkaloids. There are reports on the co-occurrence of both, calystegines and swainsonine (Fig. 3.28), a trihydroxyindolizidine alkaloid well-known from the genus *Swainsonia* (Fabaceae), in the seeds of two Australian *Ipomoea* taxa, *I. polpha* and *I.* sp. Q6 (aff. *calobra*, Weir Vine), the latter supposed to be a subspecies of the former (Molyneux et al. 1995) and from the leaves of a pantropical *Ipomoea* species of American origin, *I. carnea* ssp. *fistulosa* (de Balogh et al. 1998; Asano et al. 2001). Neither swainsonine nor a second indolizidine type polyhydroxy alkaloid named castanospermine, also known from the Fabaceae, could be detected in any species of the studies reported by Schimming et al. (1998, 2005). Dorling et al. (2004) could find beside a number of calystegines "…. only a barely detectable trace of swainsonine" in their GC/MS analysis of extracts obtained from *I. muelleri* and *I. lonchophylla*, respectively. Thus, only a few *Ipomoea* spp. seem to represent unique exceptions in the Convolvulaceae family. This co-occurrence with calystegines is surprising since both types of polyhydroxy alkaloids are synthesized on different pathways. Recently, in two comprehensive studies on the toxicity of *I. carnea* ssp. *fistulosa,* the 8-deoxy derivative of swainsonine, a putative intermediate of its biosynthesis named 2-*epi*-lentiginosine (Fig. 3.28) has also been detected (Haraguchi et al. 2003; Ikeda et al. 2003).

In this connection the question arises whether swainsonine is a metabolite synthesized by such *Ipomoea* spp. themselves. This alkaloid was found as a L-lysine-derived metabolite in fungi, e.g., *Metarhizium anisopliae* (Sim and Perry 1997), *Rhizoctonia leguminicola* (Harris et al. 1988). Thus, it might be that the unusual occurrence of swainsonine in certain *Ipomoea* spp. is due to certain unknown symbiotic endophytes. Exactly this has already been proved in the case of ergoline alkaloids in a few species of this genus (see Sect. 4.2).

3.5.4 Biosynthesis

The early postulation that 3-oxotropane (tropinone, 3-tropanone) represents an intermediate in the biosynthetic pathway of the calystegines (Dräger et al. 1994) has been supported by feeding experiments using ^{15}N-labelled 3-oxotropane in root cultures of *Calystegia sepium* (Scholl et al. 2001, 2003). After two days 3β-hydroxytropane (pseudotropine, 3β-tropanol), the postulated next step of the pathway (Fig. 3.13) catalyzed by the specific tropinone reductase II (TR II), was completely labelled. During the first six days the ratio of labelled to non-labelled calystegines increased continuously. Calystegine A_3 was labelled faster than its congeners B_1 and B_2 thus supporting the assumption that A_3 could be a precursor of the latter (Fig. 3.27). Demethylation should be the intermediate step between 3β-hydroxytropane and the calystegines, i.e., the formation of 3β-hydroxy*nor*tropane (*nor*pseudotropine, 3β-*nor*tropanol). It has been speculated that (i) *N*-demethylation is an unlikely biosynthetic transformation and therefore (ii) the calystegines may be derived by a divergent pathway or from an entirely different precursor (Molyneux et al. 1996). However, *N*-demethylation is well-known from other alkaloids, e.g., nicotine, as well as from lipophilic tropane alkaloids in erythroxylaceous species (Dräger 2004 and references therein) and convolvulaceous species (Orechoff and Konowalowa 1935; Aripova et al. 1977; Fang et al. 1981), respectively. Meanwhile, *N*-demethylation is strongly supported by facts. 3ß-Hydroxy*nor*tropane could be detected in root cultures of *C. sepium* upon 3-oxotropane application (Dräger 2004). Furthermore, this metabolite has been proved in many calystegine-positive convolvulaceous species, e.g., *Anisea martinicensis*, *Evolvulus argyreus*, *Falkia repens* (Schimming et al. 2005) and also in *Morus alba* (Moraceae; Kusano et al. 2002). It has been postulated that this demethylation step may even be a rate-limiting step of the pathway since 3β-hydroxytropane accumulated in excess upon application of 3-oxotropane is acylated to the corresponding acetate and tiglate, respectively (Dräger et al. 1992). Anyhow, to date precise knowledge concerning any further hydroxylation step is missing (Dräger 2004).

An alternative biosynthetic pathway has been postulated by Asano et al. (1997a) due to doubts on the origin of the unusual aminoketal functionality at the bridgehead position and the high degree of hydroxylation as subsequent steps of simple tropane alkaloid biosynthesis. The postulate is based on the isolation of two trihydroxylated 1-aminocycloheptanes from *Physalis alkekengi* and *Lycium chinense*, respectively (Asano et al. 1996c, 1997a). The authors speculate that the aminocycloheptanes may be precursors to the calystegines which co-occur in these plants. This divergent pathway does not involve the formation of 3-oxotropane and 3β-hydroxytropane. However, it appears to be rather unlikely that these simple lipophilic tropanes and the structurally related calystegines are synthesized in the same species by two different pathways. This is especially true if one considers that both groups of metabolites represent plesiomorphic characters since both occur in the Solanaceae as well as in the Convolvulaceae. On the other hand it may be that A_5 is degraded, e.g., in *Physalis alkekengi* to its corresponding aminocycloheptanone which is

reduced subsequently to 1ß-amino-2α,3β,5β-trihydroxycycloheptane (Asano et al. 1996c). Furthermore, certain calystegines may be isomerized to others via corresponding aminocycloheptanones, e.g., A_3 to A_6 (Dräger 2004).

3.5.5 Significance

Polyhydroxy alkaloids in general are mimicking the structures of monosaccharides since the ring oxygen of the latter has been replaced by nitrogen (bioisosteric compounds). They **bind specifically to the active sites of certain glycosidases** thus inhibiting these enzymes in an antagonistic manner. Such properties have aroused increasing interest in pharmacological research as, e.g., potential antiviral, anticancer, and antidiabetic agents. Furthermore, such compounds may cause intoxications in cattle. Though they are believed to be widespread in the plant kingdom as well as in microorganisms their **significance for the producing species** is not yet clear (Dräger 2004). Polyhydroxy alkaloids are classified into five structural classes: polyhydroxylated piperidines, pyrrolidines, indolizidines, pyrrolizidines, and calystegines, respectively (Asano et al. 2000). A number of comprehensive reviews on polyhydroxy alkaloids (i) in general including calystegines, e.g., by Dräger 1996, Watson et al. 2001, as well as (ii) focused on calystegines alone, e.g., by Molyneux et al. 1996, Dräger 2004, has been published recently. Beside chemistry and occurrence main topics are biological activities including biochemistry (glycosidase inhibitory effects), therapeutic potential, and mammalian toxicology including livestock poisonings.

Prolonged ingestion of swainsonine, a potent inhibitor of rat lysosomal *α-mannosidase* (IC_{50} value: 0.02 µM), by animals leads to a phenocopy of the genetically induced lysosomal storage disease mannosidosis (α-mannosodosis is a human disease). Its congener 2-*epi*-lentiginosine is also such an inhibitor though with less potency (IC_{50}: 4.6 µM). Calystegines B_1, B_2, and C_1 turned out to be potent inhibitors of rat lysosomal *β-glucosidase* (IC_{50}: 2.1, 0.75, and 0.84 µM, respectively) whereas calystegine B_3 was a moderate inhibitor of α- and β-mannosidases. All of these polyhydroxy alkaloids co-occur in the flowers, leaves, and seeds of *I. carnea* ssp. *fistulosa* which causes outbreaks of natural poisoning in livestock in the tropics (Haraguchi et al. 2003). These authors have reported in another study (which is not yet included in the reviews mentioned above) on novel insights: They have checked the effects of the polyhydroxy alkaloids present in *I. carnea* on intracellular lysosomal glycosidase activities in human lymphoblast cultures. "Calystegines B_2 and C_1 seem to act as chemical chaperones, enhancing correct folding of rat lysosomal β-glucosidase and enabling smooth trafficking to the lysosome." This means that these calystegines were found to be enhancers rather than inhibitors of intracellular β-glucosidase in the cell culture system, i.e., they served as active-site-specific chaperones "to assist the correct folding of the unfolded or misfolded normal enzyme and its successful transport from the ER to the Golgi apparatus, resulting in correct

targeting to the lysosome" (Ikeda et al. 2003). The authors concluded that "calystegines – which are **in vitro** (i.e., in the enzyme assay) potent inhibitors of lysosomal β-glucosidase but non-toxic in the cell culture system – are expected to become candidates for the treatment of Gaucher's disease caused by deficient activity of lysosomal β-glucosidase, also known as β-glucocerebrosidase." Thus, the toxicity of *I. carnea* seems to be caused rather by swainsonine than by a combination of effects due to swainsonine and calystegines.

3.6 Indolizidines

The seeds of two closely related pantropical climbers of neotropical origin which in contrast to other convolvulaceous species open their flowers only at night, *Ipomoea alba* L. [syn.: *Calonyction bona-nox* (L.) Boj.], moonflower, and *I. turbinata* Lag. [syn.: *I. muricata* (L.) Jacq.], purple moonflower, turned out to contain unique indolizidine type alkaloids (Gourley et al. 1969; Dawidar et al. 1977; Ikhiri et al. 1987). Both species belong to the section *Calonyction* (see Fig. 3.32). Furthermore, such alkaloids were also found in the seeds of a third species, *I. hardwickii* (Spreng.) Hemsl. (Zhaolou et al. 1985). Whether this Chinese species belongs to the same section seems to be not determined (Austin, personal communication). Anyhow, the indolizidines are obviously confined to these three *Ipomoea* species: They were not detectable in 150 further convolvulaceous species, neither in other *Ipomoea* spp. nor in species from further 23 genera (Eich, unpublished results). These indolizidines were named ipalbidine, ipalbine (ipalbidine β-D-glucoside) (Gourley et al. 1969), ipomine (its 6″-*O*-p-coumaroyl derivative) (Dawidar et al. 1977; structure revision (ipalbidine 6″-*O*-*p*-coumaroyl-β-D-glucopyranoside, not 4″-*O*-): Chari et al. 1978), and the 4,5,8,9-tetradehydro derivative of ipalbidine, named ipohardine (ipalbidinium) (Zhaolou et al. 1985; Ikhiri et al. 1987) (Fig. 3.28). It was proposed that the biogenesis of ipalbidine proceeds via norhygrine as a key intermediate which might be conjugated with *p*-coumaric acid or the corresponding aldehyde (Hedges and Herbert 1979).

The occurrence of the indolizidines, with the exception of ipomine, turned out to be not limited to the seeds. Ipalbidine and ipohardine were detected in the roots as well as in the epigeal vegetative parts of *I. alba* and *I. turbinata*, ipalbine only in the roots of the latter species. However, the concentrations were considerably lower in these vegetative parts compared with the seeds. Furthermore, ipalbine and ipohardine, hitherto only known as constituents of the two other species, could also be detected in the seeds of *I. turbinata* (Tofern et al. 1996; Tofern 1999).

Interestingly, traces of ipalbidine could be identified by GC/MS analysis in the aerial parts of *Convolvulus caput-medusae* Lowe, *C. scoparius* L. f. (Schimming 2003), *Ipomoea batatas* (L.) Lam., *I. eremnobrocha* D.F.Austin, and *Odenellia hirtiflora* (Mart. & Gal.) K.Rob. (Tofern 1999). This indicates that the principal capability to synthesize this type of alkaloids might be widespread in the family.

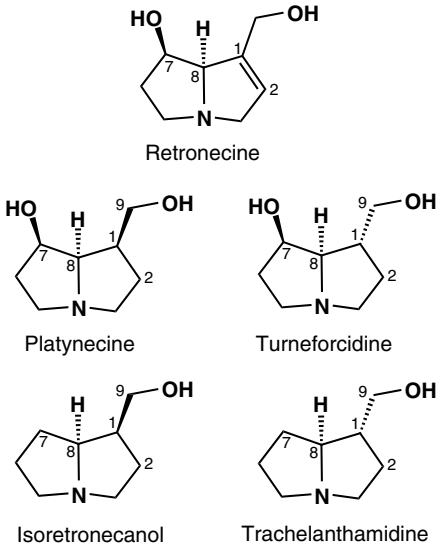


Fig. 3.29 Structure of necine bases occurring in pyrrolizidine alkaloids from the Convolvulaceae

3.7 Pyrrolizidines

From the chemical point of view pyrrolizidine represents a saturated *N*-heterobicyclic compound (4-azabicyclo[0,3,3]octane). However, the term "pyrrolizidine alkaloids" (PA) is normally used for ester alkaloids consisting of a so-called necine base and one or two necic acids. Thus, PAs may be represented by:

- A 1-hydroxymethylpyrrolizidine type necine base, e.g., trachelanthamidine, isoretronecanol (Fig. 3.29), conjugated with a necic acid forming a 9-*O*-monoester or
- A 7-hydroxy-1-hydroxymethylpyrrolizidine type necine base, e.g., retronecine, platynecine, turneforcidine (Fig. 3.29), conjugated with one or two necic acids forming a 7-*O*- and 9-*O*-monoester, respectively, or a 7-*O*,9-*O*-diester (instead of two acids one dibasic acid is also possible forming a handle-like macrocyclic diester).

PAs have been detected in 16 angiosperm families (Convolvulaceae included) though often only sporadically occurring. They were most studied in the Asteraceae, Fabaceae, Boraginaceae, and Orchidaceae where they are also most frequent (Robins 1995; Hartmann and Ober 2000). More than 95% of the PA-containing species belong to these four families (Hartmann 1999). Some 360 different metabolites were already known in the early 1990s (Hartmann and Witte 1995).

Two molecules of putrescine are incorporated into the necine bases, one of them via spermidine. Homospermidine synthase (HSS) catalyzes the first

pathway-specific step of the PA biosynthesis. HSS was recruited during angiosperm evolution from the ubiquitous enzyme deoxyhypusine synthase (DHS) (Ober and Hartmann 1999). Both enzymes catalyze analogous reactions: They transfer an aminobutyl moiety from spermidine to a primary amino group of putrescine (HSS) and a proteinous lysyl residue of DHS, respectively (Hartmann and Ober 2000). It could be demonstrated that different independent recruitments of HSS from DHS have happened, e.g., within the monocots, the Boraginaceae, and the Asteraceae (Reimann et al. 2004). Thus, it is perhaps not surprising that several unrelated families within the angiosperms are able to constitutively produce PAs as a defense against herbivores. Nevertheless, the fact of a polyphyletic origin of HSS and the structural identity of the final biosynthesis products in unrelated families is surprising. The necic acids are mainly derived from (i) branched aliphatic amino acids such as L-valine (e.g., trachelanthic acid), L-leucine, and L-isoleucine (e.g., senecic acid) or (ii) L-threonine (e.g., angelic acid, tiglic acid). Comprehensive reviews on the biosynthesis were published, e.g., by Robins (1995), Hartmann and Ober (2000). Lolines (Sect. 5.2.3) represent alkaloids which also contain a pyrrolizidine skeleton; however, they are synthesized by a diverging biosynthetic pathway.

Though evidence for a general occurrence of homospermidine in plants is obvious as has been proven for many species from numerous angiosperm families including *Datura stramonium* L. and *Nicotiana tabacum* L. (Ober et al. 2003), most families including the Solanaceae do not synthesize PAs.

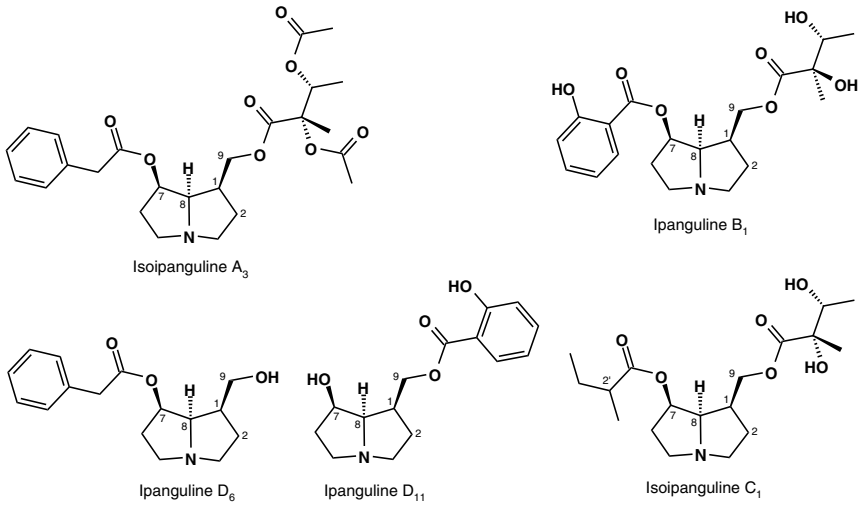


Fig. 3.30 Unique platynecine-based pyrrolizidine alkaloids occurring in species of the intrageneric *Ipomoea* taxon subgenus *Quamoclit*, section *Mina* (Convolvulaceae); necine base residues = absolute configuration, aliphatic necic acid residues = relative configuration

3.7.1 Occurrence in the Convolvulaceae

3.7.1.1 Platynecine and Trachelanthamidine Derivatives

Platynecine Esters. The occurrence of platynecine esters in the plant kingdom seemed to be restricted to certain *Senecio* spp. (Asteraceae), e.g., *S.* ***platy****phyllus* DC. (Hartmann and Witte 1995), before unique alkaloids were discovered as constituents of the neotropical twiner *Ipomoea hederifolia* L. (syn.: *I. angulata* LAM., *Quamoclit hederifolia* (L.) G.DON), scarlet morning-glory (Jenett-Siems et al. 1993). In order to avoid confusions with another *Ipomoea* sp. with a similar epithet, *I. hederacea* JACQ., these alkaloids were named ipangulines (instead of "iphederines") according to one of the synonymous names of this species. Five types of (−)-platynecine [1β-hydroxymethyl-7β-hydroxy-8α*H*-pyrrolizidine; Fig. 3.29] esters have been classified:

- A-series: 7-*O*- and 9-*O*-diacyl derivatives with one phenylacetic acid moiety (7-*O*) and one aliphatic necic acid residue (9-*O*); ipangulines/isoipangulines A_1–A_4, e.g., isoipanguline A_3 (Fig. 3.30)
- B-series: 7-*O*- and 9-*O*-diacyl derivatives with one salicylic acid moiety (7-*O*) and one aliphatic necic acid residue (9-*O*); ipangulines/isoipangulines B_1–B_3, e.g., ipanguline B_1 (Fig. 3.30)
- C-series: 7-*O*- and 9-*O*-diacyl derivatives with two aliphatic necic acid moieties; ipangulines/isoipangulines C_1 – C_7, e.g., isoipanguline C_1 (Fig. 3.30)
- D-series: either 7-*O*- or 9-*O*-monoacyl derivatives; ipangulines/isoipangulines D_1–D_{18}, e.g., ipangulines D_6 (7-*O*-phenylacetylplatynecine) and D_{11} (9-*O*-salicyloylplatynecine), respectively (Fig. 3.30); examples with aliphatic necic acid were also determined
- X-series: structures with still ambiguous necic acid moieties; ipangulines X_1–X_7

Thus, these metabolites are characterized by the novel combination of the saturated necine base platynecine with aliphatic and/or an aromatic moiety containing necic acids. Congeners with a 2,3-dihydroxy-2-methylbutyric acid moiety form epimeric pairs, i.e., an *erythro* form (ipangulines) and a *threo* form (isoipangulines) (Fig. 3.30). The corresponding specific free necic acids were named ipangulinic acid (*erythro* form) and isoipangulinic acid (*threo* form) by Roeder (1995) who assumed that they are generated from L-threonine via 3-hydroxy-2-oxo-butyric acid.

Almost all of the 48 ipangulines/isoipangulines were discovered as constituents of *I. hederifolia* (Jenett-Siems et al. 1998a; 2005a). Three alkaloids from the X-series, the only exceptions, were discovered in *I. neei* (SPRENG.) O'DONELL. Seven compounds were isolated from *I. hederifolia* and structurally elucidated by spectroscopic methods. With the exception of the metabolites belonging to the X-series the structure of the remaining alkaloids could be determined by GC/MS analysis based on the results with the isolated congeners. Further five species, *I. cholulensis* KUNTH., *I. coccinea* L. (redstar/orange morning glory), *I. cristulata* HALL. f. (scarlet

creeper/star glory), *I. quamoclit* L. (cypress-vine), and *I. sloteri* (HOUSE) VAN OOSTSTR. (cardinal climber) were also able to synthesize ipanguline type pyrrolizidines (Jenett-Siems et al. 2005a). However, *I. coccinea* and *I. quamoclit* were poor synthesizers with only a few compounds. In contrast, two other species showed a rich pattern though to a less extend compared with *I. hederifolia*: *I. cholulensis* (19 alkaloids; major compounds: Ipanguline A_1/isoipanguline A_1) and *I. neei* (15 alkaloids; major compounds: Ipangulines C_2 and X_6, isoipangulines C_1 and C_2):

- Ipanguline A_1/isoipanguline A_1 = *erythro-/threo*-7-*O*-phenylacetyl-9-*O*-(2,3-dihydroxy-2-methylbutyryl)-platynecine
- Ipanguline C_2 = *erythro*-7-*O*-2-methylbutyryl-9-*O*-(3-acetoxy-2-hydroxy-2-methylbutyryl)-platynecine
- Ipanguline X_6 = platynecine 7-*O*,9-*O*-diester, necic acid not elucidated
- Isoipanguline C_1 = *threo*-7-*O*-2-methylbutyryl-9-*O*-(2,3-dihydroxy-2-methylbutyryl)-platynecine (Fig. 3.30)
- Isoipanguline C_2 = *threo*-7-*O*-2-methylbutyryl-9-*O*-(3-acetoxy-2-hydroxy-2-methylbutyryl)-platynecine

The ipangulines/isoipangulines were stored in all of these species as tertiary alkaloids and not as *N*-oxides, generally in the shoots and roots. In case of *I. hederifolia* certain congeners also occurred in the seeds, but with the highest concentration in shoot tips and young leaves (up to 0.45% dry weight). In contrast, no pyrrolizidines were found in the seeds of *I. quamoclit* and *I. neei* (other species not checked) (Jenett-Siems et al. 1998a; 2005a).

Trachelanthamidine Esters. (−)-Trachelanthamidine is the specific precursor of retronecine (see below) (Hartmann and Ober 2000 and references therein). Esters

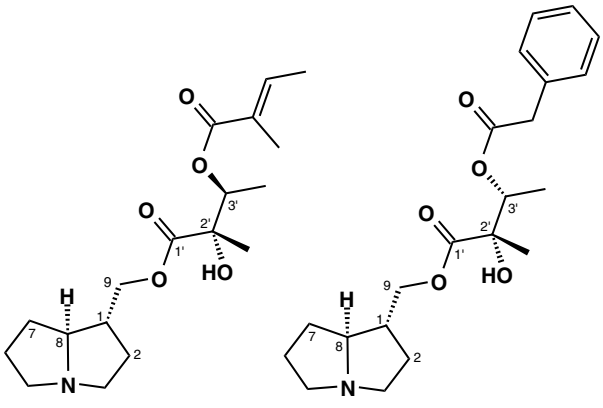


Fig. 3.31 Unique trachelanthamidine-based pyrrolizidine alkaloids occurring in species of the intrageneric *Ipomoea* taxon subgenus *Quamoclit*, section *Mina* (Convolvulaceae)

of trachelanthamidine, e.g., of the phalaenopsine type, were known from different genera of the Orchidaceae (Hartmann and Witte 1995). A corresponding novel structural type of PAs named minalobines has been discovered in *Ipomoea lobata* (CERV.) THELL. (syn.: *Mina lobata* CERV.), Spanish flag/firecracker vine. This is a neotropical twiner with worldwide horticultural use as a popular ornamental due to its unusual corolla form, stamens and style finally twice as long as the corolla, and unusual colours (red, later becoming whitish or pale yellow). Altogether 21 minalobines (congeners A–U) could be identified, 19 from *I. lobata* and additional two from *I. cristulata* (Jenett-Siems et al. 2005a). They turned out to be esters of (−)-trachelanthamidine (Fig. 3.29) with just the same necic acids already known from the ipangulines. Due to the lack of a hydroxyl group at C-7 only an esterification at 9-*O* is enabled. Furthermore, in contrast to (−)-platynecine the hydroxymethyl substituent at C-1 is α-configurated. The predominant alkaloid in leaves and roots of *I. lobata* was minalobine R (Fig. 3.31). Interestingly, it turned out to be confined to this species; moreover, it was the only minalobine with an aromatic residue-containing acyl moiety. The structural comparison with isoipanguline A_1 shows that just the same two necic acids are included in different combinations. Both alkaloids have in common isoipangulinic acid as the acid component at C-1. However, the second necic acid, phenyl acetic acid, is conjugated to (i) the C-3 hydroxyl of isoipangulinic acid in the case of minalobine R forming an acyl residue with a second acyl moiety whereas (ii) phenylacetic acid in case of isoipanguline A_1 is conjugated to the second hydroxyl (at C-7) of platynecine.

Both acids are very rare as necic acids: Isoipangulinic acid has been found only in one retronecine type PA of two *Cryptantha* spp. (Boraginaceae). Furthermore, this acid or its *erythro* isomer is conjugated with another retronecine type compound from *Senecio caudatus* DC. (Asteraceae) (Hartmann and Witte 1995 and references therein). Aromatic moieties containing necic acids were only known from the orchid alkaloids (phalaenopsine type).

One further congener in *I. lobata*, minalobine K, was also found in higher concentrations though only in the roots. Both alkaloids were only present in this species. Minalobine O (Fig. 3.31), absent in *I. lobata*, could be isolated as a major alkaloid of *I. cristulata* (beside only two minor minalobines); moreover, it was detected as a minor constituent of *I. sloteri* (beside only one further minor congener, minalobine E) which could not be detected in any other species. This was also true for minalobine Q and *I. cristulata*. The congeners L and M were identified in *I. cristulata* and *I. lobata*. The remaining 14 minalobines were only found as minor constituents in *I. lobata*.

All minalobines mentioned above are interpreted chemically in the following list:

- Minalobine E = 9-*O*-ester of trachelanthamidine with C_4H_9O–COOH (this necic acid moiety was not further characterized)
- Minalobine K = 9-*O*-[*threo*-2-hydroxy-2-methyl-3-(2-methylbutyryloxy)-butyryl]-trachelanthamidine

- Minalobine L = 9-*O*-[*erythro*-2-hydroxy-2-methyl-3-(2-methylbutyryloxy)-butyryl]-trachelanthamidine
- Minalobine M = 9-*O*-(*threo*-2-hydroxy-2-methyl-3-tigloyloxy-butyryl)-trachelanthamidine
- Minalobine O = 9-*O*-(*erythro*-2-hydroxy-2-methyl-3-tigloyloxy-butyryl)-trachelanthamidine
- Minalobine Q = 9-*O*-[2-hydroxy-2-methyl-3-(–OOC–C_4H_9O)-butyryl]-trachelanthamidine (the –OOC–C_4H_9O moiety was not further characterized)
- Minalobine R = 9-*O*-(*threo*-2-hydroxy-2-methyl-3-phenylacetoxy-butyryl)-trachelanthamidine

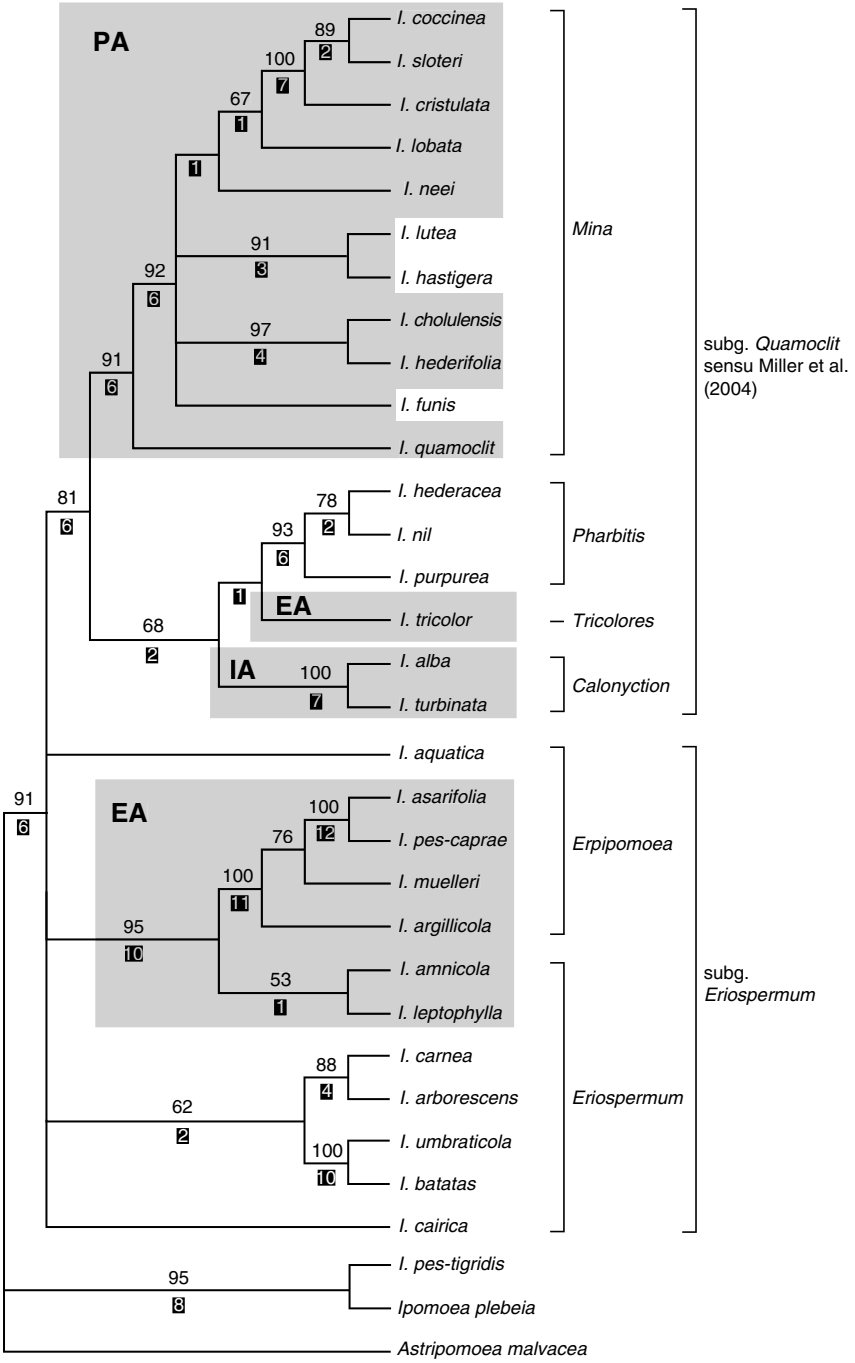
Some members of section *Mina* represent popular ornamentals, e.g., *I. hederifolia*, *I. lobata*, *I. quamoclit*, *I. sloteri*. Though of neotropical origin they have become popular pantropical and pansubtropical ornamentals. They are characterized by scarlet to red-yellow coloured (see Sect. 6.8.3), predominantly trumpet-shaped corollas; both characters are rather unusual for *Ipomoea* spp.

Chemotaxonomic Relevance for the Intrageneric *Ipomoea* Taxon Subgenus *Quamoclit*, Section *Mina*. From the phytochemical results it may be summarized that:

- All of the eight PA-synthesizing species mentioned above belong to the subgenus *Quamoclit*, section *Mina*
- Seven out of these eight species were able to synthesize ipangulines/isoipangulines (exception: *I. lobata*)
- Two of these seven species, *I. cristulata* and *I. sloteri*, were also capable of producing minalobines in addition to ipangulines/isoipangulines whereas five species are confined to synthesize the latter type of alkaloids
- One species, *I. lobata*, synthesized only minalobines though with the richest pattern

From the biosynthetic point of view, the co-occurrence of platynecine derivatives and trachelanthamidine derivatives is conspicuous. The difference between the two necine bases is not only caused by the presence or absence of an additional hydroxyl group at C-7 but also by the stereochemistry at C-1. It is unknown if the formation of platynecine in plants is based on isoretronecanol as an intermediate or on a configurative reversal at C-1 of trachelanthamidine (hydroxylation at C-7 left aside).

Figure 3.32 is based on not yet published results of Stech et al. (2007). It shall demonstrate the molecular relationships and the significance of phytochemical characters in certain sections of two *Ipomoea* subgenera (*Quamoclit*, *Eriospermum*) from the phylogenetic and chemotaxonomic points of view. The figure shows a strict consensus tree of 16 most parsimonious trees resulting from maximum parsimony analysis of ITS1 and ITS2 sequences of 29 species of *Ipomoea* "clade 2" sensu Manos et al. (2001) as well as *Astripomoea malvacea* (KLOTZSCH) A.MEEUSE, *I. plebeia* R.BR., and *I. pes-tigridis* L. as outgroup representatives. Details are given in the legend of Fig. 3.32.


PA
I. coccinea
I. sloteri
I. cristulata
I. lobata
I. neei
I. lutea
I. hastigera
I. cholulensis
I. hederifolia
I. funis
I. quamoclit
89
2
100
7
67
1
1
91
3
92
6
97
4
91
6
Mina
I. hederacea
I. nil
I. purpurea
78
2
93
6
1
EA
I. tricolor
68
2
Pharbitis
Tricolores
IA
I. alba
I. turbinata
100
7
Calonyction
81
6
subg. Quamoclit sensu Miller et al. (2004)
I. aquatica
91
6
EA
I. asarifolia
I. pes-caprae
I. muelleri
I. argillicola
100
12
76
100
11
95
10
Erpipomoea
I. amnicola
I. leptophylla
53
1
I. carnea
I. arborescens
I. umbraticola
I. batatas
I. cairica
88
4
62
2
100
10
Eriospermum
subg. Eriospermum
I. pes-tigridis
Ipomoea plebeia
95
8
Astripomoea malvacea

The molecular data are mainly based on those published by Manos et al. (2001) and Miller et al. (1999, 2004); they have been only extended with regard to three additional species belonging to sect. *Mina* (*I. cholulensis, I. cristulata, I. sloteri*).

As indicated in Fig. 3.32, different types of alkaloids can be considered as characteristic chemotaxonomic markers of certain intrageneric *Ipomoea* taxa. Within subg. *Quamoclit* unique pyrrolizidine alkaloids (ipangulines and minalobines) are exclusive for sect. *Mina* whereas unique indolizidine alkaloids seem to characterize sect. *Calonyction* (see Sect. 3.6). The occurrence of these different types of alkaloids can be regarded as synapomorphies for the respective sections, as they have so far neither been found in other *Ipomoea* spp. nor in any other plant group inside or outside the Convolvulaceae. This is especially true for 150 convolvulaceous species from 23 genera that were screened particularly for the occurrence of these alkaloids. In contrast, the well-supported clade of species formerly assigned to subg. *Ipomoea* sect. *Pharbitis* is rather characterized by the absence of relevant alkaloids. Ergoline alkaloids characterize a group of species of *Ipomoea* **subg**. *Eriospermum* (references see Sect. 4.2) that is molecularly well-supported. Vice versa, the phytochemical results support the molecular data (Miller et al. 1999; Manos et al. 2001). Thus, there is evidence from the molecular as well as from the chemical findings that the traditionally accepted **sections** *Erpipomoea* and *Eriospermum* based on morphologic/anatomical characters are not monophyletic. However, ergolines also occur in *I. tricolor* Cav. of sect. *Tricolores* and in further *Ipomoea* spp. not integrated in Fig. 3.32 (see Sect. 4.2).

As already mentioned, seven species of the sect. *Mina* were found to synthesize **ipangulines/isoipangulines**. The close relationship of *I. cholulensis* and *I. hederifolia* determined by the molecular data is strongly supported by phytochemical data: These species share, in addition to the highest alkaloid quantity, unusual acyl residues as partial structures of their ipangulines that can be regarded as synapomorphies, i.e., a phenylacetyl or a salicyloyl residue (A- and B-series, respectively). These findings support the assumption of *I. cholulensis* being a tropical highland relative of the tropical lowland *I. hederifolia* (Jenett-Siems et al. 2005a). All of the remaining five ipanguline-positive species lacked such A- or B-congeners. Even the third ipanguline-rich species, *I neei*, shared only alkaloids from C- and D-series with *I. cholulensis* and *I. hederifolia*.

Fig. 3.32 Strict consensus tree of 16 most parsimonious trees resulting from maximum parsimony analysis of ITS1 and ITS2 sequences of 29 species of *Ipomoea* "clade 2" sensu Manos et al. (2001) as well as *Astripomoea malvacea* (Klotzsch) A.Meeuse, *I. plebeia* R.Br., and *I. pestigridis* L. as outgroup representatives. Bootstrap values >50% and decay indices (*in black squares*) are above the branches. Plotted on the tree is the occurrence of three specialized types of alkaloids: PA = pyrrolizidines (ipangulines and minalobines) (Sect. 3.7.1), IA = indolizidines (Sect. 3.6), EA = ergolines (Sect. 4.2). *I. lutea* Hemsl., *I. hastigera* Kunth., and *I. funis* Schltdl. & Cham. were not checked for the presence of alkaloids (lack of plant material); however, all of the remaining species which were *not* highlighted in grey turned out to be negative with regard to the presence of any of the three alkaloid types. Ergoline alkaloids also occur in certain *Ipomoea* species which are not involved in this tree (for details see Sect. 4.2) [tree based on unpublished results of Stech et al. (2007)]

I. neei turned out to be sister to the species of the "minalobine clade". The molecular topology allows the hypothesis that the ability to synthesize **minalobines** evolved only once within sect. *Mina*, in the putative ancestor of *I. lobata, I. cristulata, I. sloteri* (minalobine-positive species) and *I. coccinea*, but that in the latter the synthesis pathway was either lost again or only very small, undetectable amounts are synthesized. As *I. coccinea* is one of the parental species of the hybrid *I.* × *multifida* (Raf.) Shinners from which the allotetraploid species *I. sloteri* arose (Eckenwalder 1986), the minalobine pathway must in principle be present in this species under this hypothesis, because the other parental species, *I. quamoclit*, is not part of this "minalobine clade".

Alternatively, it might be possible that all *Mina* species are principally able to synthesize minalobines, but for one reason or another, this specific pathway is not switched on. Following Wink (2003), the occurrence of secondary metabolites, which play a vital role as defence or signal compounds, reflects adaptations and particular life strategies embedded in a given phylogenetic framework rather than taxonomic relationships. This may also be true for both types of pyrrolizidine alkaloids of section *Mina*, ipangulines as well as minalobines, calling into question their **intra**sectional phylogenetic significance.

In fact, *I. cristulata* and *I. sloteri* closely related to each other showed co-occurrence of minalobines and ipangulines whereas *I. lobata*, sister of the two former species, exclusively synthesized minalobines but no ipangulines (Stech et al. 2007).

3.7.1.2 Turneforcidine Derivatives

Pyrrolizidine alkaloids with the 1-epimer of platynecine, turneforcidine, as the necine base are rather rare in the plant kingdom, e.g., *Crotalaria candicans* Wight & Arn., Fabaceae (Siddiqi et al. 1979). A novel natural compound, (−)-9-*O*-tigloylturneforcidine, was discovered as the major pyrrolizidine of *Ipomoea meyeri* (Spreng.) G.Don, a slender herbaceous neotropical twiner. It could be isolated from the roots. As minor congeners 7-*O*- as well as 9-*O*-monoesters of different necine bases were characterized by GC/MS analysis. The following unusual pattern of necine bases could be determined: (i) two 1-hydroxymethyl-7β-hydroxy-8α*H*-

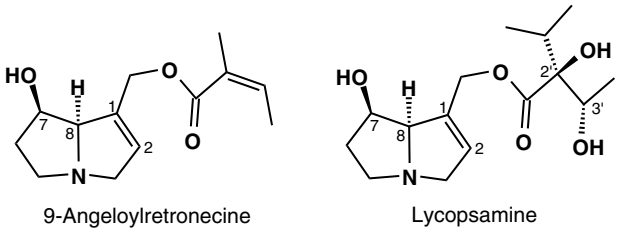


Fig. 3.33 Retronecine-based pyrrolizidine alkaloids occurring in *Merremia cissoides* and *M. quinquefolia* (Convolvulaceae)

pyrrolizidines, the 1β-form turneforcidine and its 1α-epimer platynecine (95:5), (ii) the corresponding 7-unsubstituted congeners, trachelanthamidine (1α) and isoretronecanol (1β) (90:10). However, the specific structures of the esters remained to be elucidated (Tofern 1999).

3.7.1.3 Retronecine Derivatives

Retronecine, by far the most widespread necine base of pyrrolizidine alkaloids in the plant kingdom (Hartmann and Ober 2000 and references therein), differs from the necine bases discussed previously by a 1,2-double bond. Retronecine alkaloids such as lycopsamine and intermedine were known as metabolites of the Boraginaceae, Asteraceae (tribe Eupatorieae), and Apocynaceae families (Hartmann and Witte 1995; Reimann et al. 2004). Retronecine monoesters were also detected as constituents of two closely related *Merremia* species, *M. quinquefolia* (L.) HALL. f. and *M. cissoides* (Mann et al. 1996, 1997; Mann 1997). The roots of the former species contained a complex mixture of pyrrolizidines with 9-angeloylretronecine as the main component and, e.g., (*erythro*-)lycopsamine (Fig. 3.33), its *threo* congener intermedine, and 7-angeloylretronecine as minor congeners. The pyrrolizidine pattern of *M. cissoides* was less complex; however, it also contained the compounds just mentioned. The occurrence of retronecine esters in these two species turned out to be unique in the family since they were absent in more than 150 further convolvulaceous species from 23 genera (Eich, unpublished results). On the other hand, no congeners with a saturated necine base could be detected in these two *Merremia* spp.

The biosynthesis of lycopsamine type alkaloids has been studied recently in three species of the Boraginaceae (Frölich et al. 2007).

3.7.2 Significance

"PA-containing plants are probably the most common poisonous plants affecting livestock, wildlife, and humans" (Frölich et al. 2006 and references therein). However, this is only true for such PAs which are characterized by *at least* two essential structural features: (i) a 1,2-double bond of the necine base which must be (ii) conjugated at position 9-*O* with a necic acid, e.g., 9-angeloylretronecine, lycopsamine (Fig. 3.33). Such PAs are not toxic per se; they require metabolic activation in the liver of vertebrates with the consequence of mutagenicity and carcinogenicity. Such PAs are also bioactivated by insects. There are several comprehensive reviews on this toxicological topic with regard to humans including acute and chronic diseases, e.g., Westendorf (1992); Roeder (1995). The latter review laid additional emphasis on structural aspects of the metabolism of PAs in the human organism. Furthermore, there are many comprehensive reviews with regard to the ecological role of such PAs, e.g., by Hartmann (1999; progress report 2004), Hartmann and Ober (2000). Central topics are storage of PAs as non-toxic *N*-oxides in the plant

as well as storage and use (sequestration, defense, transformation) of plant-acquired PAs in **specialized** insect herbivores [especially Lepidoptera (butterflies/moths), Chrysomelidae (leaf beetles)]. Thus, lycopsamine, also a constituent of the two *Merremia* spp., is a prominent example for such plant-derived PAs which are transformed to pheromones by Lepidoptera, e.g., danaidal (Arctiidae, Danainae).

N-Oxides of ipangulines and minalobines could not be detected. This may be related to the fact that the tertiary platynecine or trachelanthamidine derivatives do not necessarily need to be stored as non-toxic *N*-oxides, since they are not potentially mutagenic and cytotoxic like the 1,2-unsaturated necine derivatives, e.g., lycopsamine (Jenett-Siems et al. 1998a). However, the question arises whether such saturated non-toxic necine derivatives are nevertheless defense compounds. PAs with an 1,2-**saturated** necine base lack a pro-toxic (esterified) allylic hydroxyl group which is a precondition for the metabolic bioactivation. There is only a limited number of species which contain such PAs *without* co-occurrence of 1,2-unsaturated congeners (Orchidaceae, Convolvulaceae). Consequently, there is only a limited knowledge on the biological activity of such PAs. Recently, it could be demonstrated that also the largely neglected 1,2-**saturated** PAs must be of ecological importance: Highly polyphagous larvae of certain tiger moths (Lepidoptera: Arctiidae) (i) immediately recognize them as well as their unsaturated congeners by taste receptors, (ii) sequester and transmit them to the adult stages during metamorphosis after (iii) having converted also these saturated PAs into transmittable 'insect alkaloids' (Frölich et al. 2006 and references therein). Thus, the *Mina*-PAs (ipangulines, minalobines) as well as the turneforcidine esters of *Ipomoea meyeri* may also be of interest from the ecological point of view.

References

Abraham TW, Leete E (1995) New intermediate in the biosynthesis of the tropane alkaloids in *Datura innoxia*. J Am Chem Soc 117:8100–8105

Achenbach H, Fietz W, Wörth J, Waibel R, Portecop J (1986) Constituents of tropical medicinal plants: IXX. GC/MS-investigations of the constituents of *Piper amalago*: 30 new amides of the piperine-type. Planta Med. 52:12–18

Adesanya SA, Nia R, Fontaine C, Pais M (1994) Pyrazole alkaloids from *Newbouldia laevis*. Phytochemistry 35:1053–1055

Ahmad A, Leete E (1970) Biosynthesis of the tropine moiety of hyoscyamine from δ-*N*-methylornithine. Phytochemistry 9:2345–2347

Al-Said MS, Evans WC, Grout RJ (1986) Alkaloids of *Erythroxylum macrocarpum* and *E. sideroxyloides*. Phytochemistry 25:851–853

Andersen RA, Fleming PD, Burton HR, Hamilton-Kemp TR, Sutton TG (1989) *N*′-Acyl and *N*′-nitroso pyridine alkaloids in alkaloid lines of burley tobacco during growth and air-curing. J Agric Food Chem 37:44–50

Andersson C, Ahman A (2004) Calystegines – danger in potatoes? Vaar Foeda 56:24–27

Aripova SF (1985) Convolamine *N*-oxide from *Convolvulus krauseanus*. Khim Prir Soedin 275

Aripova SF, Abdilalimov O (1993) Convolacine – a new alkaloid from *Convolvulus subhirsutus*. Khim Prir Soedin:88–90

Aripova SF, Yunusov SY (1979) Alkaloids of the epigeal part of *Convolvulus krauseanus*. Khim Prir Soedin:527–529

Aripova SF, Yunusov SY (1986a) Structure of Convosine. Khim Prir Soedin:618–620

Aripova SF, Yunusov SY (1986b) Convolvidine, a native alkaloid of *Convolvulus subhirsutus*. Khim Prir Soedin:657–658

Aripova SF, Malikov VM, Yunusov SY (1972) Alkaloids of *Convolvulus*. Khim Prir Soedin:401–402

Aripova SF, Malikov VM, Yunusov SY (1977) Convolidine – a new alkaloid from *Convolvulus krauseanus*. Khim Prir Soedin:290–291

Aripova SF, Sharova EG, Abdullaev UA, Yunusov SY (1983) A new alkaloid from *Convolvulus krauseanus*. Khim Prir Soedin:749–751

Armstrong DW, Wang X, Lee JT, Liu YS (1999) Enantiomeric composition of nornicotine, anatabine, and anabasine in tobacco. Chirality 11:82–84

Asano N, Tomioka E, Kizu H, Matsui K (1994a) Sugars with nitrogen in the ring isolated from the leaves of *Morus bombycis*. Carbohydr Res 253:235–245

Asano N, Oseki K, Tomioka E, Kizu H, Matsui K (1994b) *N*-Containing sugars from *Morus alba* and their glycosidase inhibitory activities. Carbohydr Res 259:243–255

Asano N, Kato A, Oseki K, Kizu H, Matsui K (1995) Calystegines of *Physalis alkekengi* var. *francheti* (Solanaceae) – structure determination and their glycosidase inhibitory activities. Eur J Biochem 229:369–376

Asano N, Kato A, Yokoyama Y, Miyauchi M, Yamamoto M, Kizu H, Matsui K (1996a) Calystegine N_1, a novel *nor*tropane alkaloid with a bridgehead amino group from *Hyoscyamus niger*: structure determination and glycosidase inhibitory activities. Carbohydr Res 284:169–178

Asano N, Kato A, Kizu H, Matsui K, Watson AA, Nash RJ (1996b) Calystegine B_4, a novel trehelase inhibitor from *Scopolia japonica*. Carbohydr Res 293:195–204

Asano N, Kato A, Kizu H, Matsui K (1996c) 1β-Amino-2α,3β,5β-trihydroxycycloheptane from *Physalis alkekengi* var. *francheti*. Phytochemistry 42:719–721

Asano N, Kato A, Miyauchi M, Kizu H, Tomimori T, Matsui K, Nash RJ, Molyneux RJ (1997a) Specific α-galactosidase inhibitors, *N*-methylcalystegines – structure/activity relationships of calystegines from *Lycium chinense*. Eur J Biochem 248:296–303

Asano N, Kato A, Matsui K, Watson AA, Nash RJ, Molyneux RJ, Hackett L, Topping J, Winchester B (1997b) The effects of calystegines isolated from edible fruits and vegetables on mammalian liver glycosidases. Glycobiology 7:1085–1088

Asano N, Nash RJ, Molyneux RJ, Fleet GWJ, (2000) Sugar-mimic glycosidase inhibitors: natural occurrence, biological activity and properties for therapeutic application. Tetrahedron Asymm 11:1645–1680

Asano N, Yokoyama K, Sakurai M, Ikeda K, Kizu H, Kato A, Arisawa M, Höke D, Dräger B, Watson AA, Nash RJ (2001) Dihydroxy*nor*tropane alkaloids from calystegine-producing plants. Phytochemistry 57:721–726

Atal CK, Culvenor CC, Sawhney RS, Smith LW (1969) Alkaloids of *Crotalaria grahamiana*. Grahamine, the 3′-[(−)-2-methylbutyryl] ester of monocrotaline. Austral J Chem 22:1773–1777

Austin DF (2004) Florida ethnobotany. CRC Press, Boca Raton (FL), USA

Austin DF, Eich E (2001) Synopsis of *Stictocardia* with another Madagascan species, *S. mojangensis* (Convolvulaceae) Willdenowia 31:79–85

Austin DF, Staples GW (1980) *Xenostegia*, a new genus of Convolvulaceae. Brittonia 32:533–536

Bachmann P, Witte L., Wright A, Wray V (1997) Two new classes of tropane alkaloids: tropanol esters of 3-(1-carboxy-vinyloxy)-benzoic acid and nicotinic acid from plants of the genus *Cochlearia* (Brassicaceae). 45th Annual Congress of the Society for Medicinal Plant Research, Regensburg/Germany, Book of Abstracts, Abstracts of Posters:C03 (complete poster seen)

Baker RR (1999) Smoke chemistry. In: Davis DL, Nielsen MT (eds) Tobacco – production, chemistry and technology. Blackwell Science, Oxford, UK, pp 398–439

Balasubrahmanyam SN, Quin LD (1962) Pyrolytic degradation of nornicotine and myosmine. Tobacco International 6:133–136

Baldwin IT (1988) Damage-induced alkaloids in tobacco: pot-bound plants are not inducible. J Chem Ecol 14:1113–1120
Baldwin IT (1989) Mechanism of damage-induced alkaloid production in wild tobacco. J Chem Ecol 15:1661–1680
Baldwin IT (1999) Inducible nicotine production in native *Nicotiana* as an example of adaptive phenotypic plasticity. J Chem Ecol 25:3–30
Baldwin IT (2001) An ecologically motivated analysis of plant-herbivore interactions in native tobacco (Update on *Nicotiana attenuata*). Plant Physiol 127:1449–1458
Baralle FE, Gros EG (1969) Biosynthesis of cuscohygrine and hyoscyamine in *Atropa belladonna* from DL-α-*N*-methylornithine-methyl-^{3}H and DL-δ-*N*-methylornithine-methyl-^{3}H. J Chem Soc, Chem Commun 721
Barbieri RL, York CM, Cherry ML, Ryan KJ (1987) The effects of nicotine, cotinine and anabasine on rat adrenal 11β-hydroxylase and 21-hydroxylase. J Steroid Biochem 28:25–28
Barbieri RL, Friedman AJ, Osathanondh R (1989) Cotinine and nicotine inhibit human fetal adrenal 11β-hydroxylase. J Clin Endocrin Metabol 69:1221–1224
Barbosa P, Saunders JA, Kemper J, Trumbule R, Olechno J, Martinat P (1986) Plant allelochemicals and insect parasitoids. Effects of nicotine on *Cotesia congregata* SAY (Hymenoptera: Braconidae) and *Hyposoter annulipes* CRESSON (Hymenoptera: Ichneumonidae). J Chem Ecol 12:1319–1328
Barbosa P, Gross P, Kemper J (1991) Influence of plant allelochemicals on the tobacco hornworm and its parasitoid, *Cotesia congregata*. Ecology 72:1567–1575
Barger G, Martin WF, Mitchell W (1937) Minor alkaloids of *Duboisia myoporoides*. J Chem Soc 1820–1823
Barger G, Martin WF, Mitchell W (1938) Minor alkaloids of *Duboisia myoporoides*. II. Poridine and isoporoidine. J Chem Soc 1685–1690
Barral (1847) Empirical formula of nicotine. Ann Chim et Phys 20:345; fide Czapek (1925), p 277
Bartholomeusz TA, Bhogal RK, Molinié R, Felpin FX, Mathé-Allainmat M, Meier AC, Dräger B, Lebreton J, Roscher A, Robins RJ, Mesnard F (2005) Nicotine demethylation in *Nicotiana* cell suspension cultures: *N′*-formylnicotine is not involved. Phytochemistry 66:2432–2440
Basey K, Woolley JG (1973a) Biosynthesis of the tigloyl esters in *Datura*. Role of 2-methylbutyric acid. Phytochemistry 12:2197–2201
Basey K, Woolley JG (1973b) Biosynthesis of the tigloyl esters in *Datura. Cis-trans* isomerism. Phytochemistry 12:2883–2886
Basey K, Woolley JG (1973c) Alkaloids of *Physalis alkekengi*. Phytochemistry 12:2557–2559
Basey K, Woolley JG (1975) Biosynthesis of ditigloyl esters of di- and trihydroxytropanes in *Datura*. Phytochemistry 14:2201–2203
Basey K, McGaw BA, Woolley JG (1992) Phygrine, an alkaloid from *Physalis* species. Phytochemistry 31:4173–4176
Batra PP, Gleason RM Jr, Louda JW (1973) Cyclization of lycopene in the biosynthesis of ß-carotene. Phytochemistry 12:1309–1313
Bekkouche K, Daali Y, Cherkaoui S, Veuthey JL, Christen P (2001) Calystegine distribution in some solanaceous species. Phytochemistry 58:455–462
Bentz JA, Barbosa P (1992) Effects of dietary nicotine and partial starvation of tobacco hornworm, *Manduca sexta*, on the survival and development of the parasitoid *Cotesia congregata*. Entomol Exp Appl 65:241–245
Beresford PJ, Woolley JG (1974) 6β-(2-Methylbutanoyloxy)tropan-3α-ol, a new alkaloid from *Datura ceratocaula*. Structure and biosynthesis. Phytochemistry 13:2511–2513
Berkov S (2003) Alkaloids of *Datura ceratocaula*. Z Naturforsch 58c:455–458
Berkov S, Zayed R (2004) Comparison of tropane alkaloid spectra between *Datura inoxia* grown in Egypt and Bulgaria. Z Naturforsch 59c:184–186
Berkov S, Pavlov A, Kovatcheva P, Stanimirova P, Philipov S (2003) Alkaloid spectrum in diploid and tetraploid hairy root cultures of *Datura stramonium*. Z Naturforsch 58c:42–46
Berkov S, Doncheva T, Philipov S, Alexandrov K (2005) Ontogenetic variation of the tropane alkaloids in *Datura stramonium*. Biochem Syst Ecol 33:1017–1029

Bernardini L (1931a) The recovery of nicotine from leaves and other scrap in the manufacture of tobacco. Industria Chimica (Rome) 6:395–402
Bernardini L (1931b) The manufacture of nicotine sulphate. Industria Chimica (Rome) 6: 497–503
Bhatt ID, Chang JI, Hiraoka N (2004) In vitro propagation and storage of *Brugmansia versicolor* Lagerheim. Plant Biotechnol (Tokyo, Japan) 21:237–241
Bick IRC, Bremner JB, Gilllard JW, Winzenberg KN (1974) Alkaloids of *Anthocercis tasmanica*. Austral J Chem 27:2515–2518
Blaim K (1962) Zur Frage des Vorkommens von Nikotin in Pflanzen. Flora 152:171–172
Blair BW (1999) Insects and their management in tobacco production. In: Davis DL, Nielsen MT (eds) Tobacco – production, chemistry and technology. Blackwell Science, Oxford, pp 228–240
Blum MS (1983) Detoxification, deactivation and utilisation of plant compounds by insects. In: Hedin PA (ed) Plant resistance to insects. Am Chem Soc, Washington DC, pp 265–278
Bodendorf K, Kummer H (1962) Über die Alkaloide von *Latua venenosa*. Pharm Zentralhalle 101:620–622
Bolt AJN (1972) 1′-Hexanoylnornicotine and 1′-octanoylnornicotine from tobacco. Phytochemistry 11:2341–2343
Boswell HD, Dräger B, Eagles J, McClintock C, Parr A, Portsteffen A, Robins DJ, Robins RJ, Walton NJ, Wong C (1999) Metabolism of *N*-alkyldiamines and *N*-alkylnortropinones by transformed root cultures of *Nicotiana* and *Brugmansia*. Phytochemistry 52:855–869
Bottomley W, White DE (1951) The chemistry of western Australian plants. IV. *Duboisia hopwoodii*. Austral J Sci Res 4A:107–111
Bottomley W, Nottle RA, White DE (1945) The alkaloids of *Duboisia hopwoodii*. Austral J Sci 8:18–19
Bramer SL, Kallungal BA (2003) Clinical considerations in study designs that use cotitine as a biomarker. Biomarkers 8:187–203
Brock A, Bieri S, Christen P, Dräger B (2005) Calystegines in wild and cultivated *Erythroxylum* species. Phytochemistry 66:1231–1240
Brock A, Herzfeld T, Paschke R, Koch M, Dräger B (2006) Brassicaceae contain *nor*tropane alkaloids. Phytochemistry 67:2050–2057
Brossi A, Pei XF (1998) Biological activity of unnatural alkaloid enantiomers. In: Cordell GA (ed) The alkaloids – chemistry and biology, vol 50. Academic Press, San Diego CA, USA, pp 109–139
Bullion K, Ohnishi S, Osawa Y (1991) Competitive inhibition of human placental aromatase by N-n-octanoylnornicotine and other nornicotine derivatives. Endocrine Res 17:409–419
Burton HR, Bush LP, Hamilton JL (1983) Effect of curing in the chemical composition of burley tobacco. Recent Adv Tob Sci 9:61–153
Burton HR, Andersen RA, Fleming PD, Walton LR (1988) Changes in chemical composition of burley tobacco during senescence and curing. 2. Acylated pyridine alkaloids. J Agric Food Chem 36:579–584
Bush LP, Fannin FF, Chelvarajan RL, Burton HR (1993) Biosynthesis and metabolism of nicotine and related alkaloids. In: Gorrod JW, Wahren J (eds) Nicotine and related alkaloids – absorption, distribution, metabolism and excretion. Chapman & Hall, London, pp 1–30
Bush LP, Hempfling WP, Burton HR (1999). Biosynthesis of nicotine and related compounds. In: Gorrod JW, Jacob P III (eds) Analytical determination of nicotine and related compounds and their metabolites. Elsevier, Amsterdam, NL, pp 13–43
Cairo Valera G, De Budowski J, Delle Monache F, Marini-Bettolo GB (1977) A new psychoactive drug: *Heisteria olivae* (Olacaceae). Att Acad Naz Lincei, Classe Sci Fis Matem Nat Rendiconti 62:363–364
Cannon JR, Joshi KR, Meehan GV, Williams JR (1969) Tropane alkaloids from three western Australian *Anthocercis* species. Austral J Chem 22:221–227
Carine MA, Russell SJ, Santos-Guerra A, Francisco-Ortega J (2004) Relationships of the Macaronesian and Mediterranean floras: molecular evidence for multiple colonizations into

Macaronesia and back-colonization of the continent in *Convolvulus* (Convolvulaceae). Am J Bot 91:1070–1085
Carroll I, Lewin AH, Boja JW, Kuhar MJ (1992) Cocaine receptor: biochemical characterization and structure-activity relationships of cocaine analogues at the dopamine transporter. J Med Chem. 35:969–981
Casanova H, Araque P, Ortiz C (2005) Nicotine carboxylate insecticide emulsions: effect of the fatty acid chain length. J Agric Food Chem 53:9949–9953
Chan GW, Berry D, DeBrosse CW, Hemling ME, MacKenzie-LoCasto L, Offen PH, Westley JW (1993) Conioidines A and B, novel DNA-interacting pyrrolidines from *Chamaesaracha conioides*. J Nat Prod. 56:708–713
Chandler JLR, Gholson RK (1972) Nicotinic acid decarboxylation in tobacco roots. Phytochemistry 11:239–242
Chari VM, Jordan M, Wagner H (1978) Structure elucidation and synthesis of naturally occurring acylglycosides – II. Structures of tiliroside, tribuloside, and ipomine. Planta Med 34:93–96
Chase MW, Knapp S, Cox AV, Clarkson JJ, Butsko Y, Joseph J, Savolainen V, Parokonny AS (2003) Molecular systematics, GISH and the origin of hybrid taxa in *Nicotiana* (Solanaceae). Ann Bot 92:107–127
Christen P, Kapetanidis I (1987) Phytochemical study on the leaves of *Lycium halimifolium* Miller. Part 1. Studies on alkaloids. Pharmac Acta Helv 62:154–157
Christen P, Roberts MF, Phillipson JD, Evans WC (1990) Alkaloids of hairy root cultures of a *Datura candida* hybrid. Plant Cell Rep 9:101–104
Christen P, Roberts MF, Phillipson JD, Evans WC (1993) Alkaloids of *Erythroxylum zambesiacum* stem-bark. Phytochemistry 34:1147–1151
Christen P, Roberts MF, Phillipson JD, Evans WC (1995) Alkaloids of *Erythroxylum monogynum* root-bark. Phytochemistry 38:1053–1056
Clarkson JJ, Knapp S, Garcia VF, Olmstead RG, Leitch AR, Chase MW (2004). Phylogenetic relationships in *Nicotiana* (Solanaceae) inferred from multiple plastid DNA regions. Mol Phylogen Evol 33:75–90
Clement BA, Goff CM, Forbes TDA (1997) Toxic amines and alkaloids from *Acacia berlandieri*. Phytochemistry 46:249–254
Consuelo-Fonseca L, Salive GA (1972) Phytochemical study of *Evolvulus sericeus* var. *holosericereus*. Rev Colomb Cie Quim-Farm 2:27–50
Coulson JF, Griffin WJ (1967) The alkaloids of *Duboisia myoporoides*. 1. Aerial parts. Planta Med 15:459–466
Coulson JF, Griffin WJ (1968) The alkaloids of *Duboisia myoporoides*. 2. Roots. Planta Med 16:174–181
Crooks PA (1999) Chemical properties of nicotine and other tobacco-related compounds. In: Gorrod JW, Jacob P III (eds) Analytical determination of nicotine and related compounds and their metabolites. Elsevier, Amsterdam, pp 69–110
Cutler HG, Severson RF, Cole PD, Arrendale RF, Sisson VA (1986) Plant growth inhibitory and antimicrobial properties of hydroxyacylnornicotines from select *Nicotiana* species. Proceedings – 13th Meeting Plant Growth Regulation Society of America, pp 188–200
Czapek F (1925) Biochemie der Pflanzen, vol 3. Gustav Fischer, Jena/Germany
Czygan FC, Wessinger B, Warmuth K (1988) Cuscuta and its ability to take up and accumulate alkaloids of the host plant. Biochem Physiol Pflanz 183:495–501
Dale HH (1914) The action of certain esters and ethers of choline and their relation to muscarine. J Pharmacol Exp Ther 6:147–190
Dasgupta P, Kinkade R, Joshi B, DeCook C, Haura E, Chellappan S (2006) Nicotine inhibits apoptosis induced by chemotherapeutic drugs by up-regulating XIAP and surviving. PNAS 103:6332–6337
Davis EL, Rich JR (1987) Nicotine content of tobacco roots and toxicity to *Meloidogyne incognita*. J Nematol 19:23–29
Dawidar AM, Winternitz F, Johns SR (1977) Structure of ipomine, a new alkaloid of *Ipomoea muricata* Jacq. Tetrahedron 33:1733–1734

Dawson RF (1941) The localization of the nicotine synthetic mechanism in the tobacco plant. Science 94:396–397
Dawson RF (1945) On the biogenesis of nornicotine and anabasine. J Am Chem Soc 67:503–504
Dawson RF, Solt ML (1959) Estimated contributions of root and shoot to the nicotine content of the tobacco plant. Plant Physiol 34:656–661
Dawson RF, Christman DR, d'Adamo A, Solt ML, Wolf AP (1960) The biosynthesis of nicotine from isotopically labelled nicotinic acids. J Am Chem Soc 82:2628–2633
De Balogh KKIM, Dimande AP, van der Lugt JJ, Molyneux RJ, Naudé TW, Welman WG (1998) *Ipomoea carnea*: the cause of a lysosomal storage disease in goats in Mozambique. In: Garland T, Barr AC (eds) Toxic plants and other natural toxicants. CAB International, Wallingford, UK, pp 428–434
De Garcia LA, Rodriguez PH, Martinez M (1985) Alkaloid contents in some Colombian *Brugmansia* species. Rev Mex Cienc Farmac 16:11–13
De la Fuente G, Reina M, Muñoz O, San Martin A, Girault JP (1988) Tropane alkaloids from *Schizanthus pinnatus*. Heterocycles 27:1887–1897
Deckers W, Maier J (1953) Two new alkaloids from *Duboisia leichhardtii*. Chem Ber 86:1423–1428
Denton TT, Zhang X, Cashman JR (2004) Nicotine-related alkaloids and metabolites as inhibitors of human cytochrome P-450 2A6. Biochem Pharmacol 67:751–756
Dinkel M, Bedner M (2001) Der Biorausch – ein neuer Trend. Notarzt 17:105–107
Djordjevic MV, Bush LP, Gay SL, Burton HR (1990) Accumulation and distribution of acylated nornicotine derivatives in flue-cured tobacco alkaloid isolines. J Agric Food Chem 38:347–350
Doerk-Schmitz K, Witte L, Alfermann AW (1994) Tropane alkaloid patterns in plants and hairy roots of *Hyoscyamus albus*. Phytochemistry 35:107–110
Domino EF (1999) Pharmacological significance of nicotine. In: Gorrod JW, Jacob P III (eds) Analytical determination of nicotine and related compounds and their metabolites. Elsevier, Amsterdam, pp 1–11
Doncheva T, Philipov S, Kostova N (2004) Alkaloids from *Datura stramonium* L. Dokl Bulgarsk Akad Nauk (Compt Rend Acad Bulg) 57:41–44
Dorling PR, Colegate SM, Allen JG, Nickels R, Mitchell AA, Main DC, Madin B (2004) Calystegines isolated from *Ipomoea* spp. possibly associated with an ataxia syndrome in cattle in north Western Australia. In: Acamovic T, Stewart CS, Pennycott TW (eds) Poisonous plants and related toxins. CABI Publishing, Wallingford, UK, pp 140–145
Dräger B (1996) Glykosidasehemmstoffe – Biologische Aktivität und therapeutische Bedeutung. Dtsch Apoth Ztg 136:1199–1206
Dräger B (2004) Chemistry and biology of calystegines. Nat Prod Rep 21:211–223
Dräger B (2006) Tropinone reductases, enzymes at the branch point of tropane alkaloid metabolism. Phytochemistry 67:327–337
Dräger B, Portsteffen A, Schaal A, McCabe PH, Peerless ACJ, Robins RJ (1992) Levels of tropinone-reductase activities influence the spectrum of tropane esters found in transformed root cultures of *Datura stramonium* L. Planta 188:581–586
Dräger B, Funck C, Höhler A, Mrachatz G, Nahrstedt A, Portsteffen A, Schaal A, Schmidt R (1994) Calystegines as a new group of tropane alkaloids in Solanaceae. Plant Cell, Tissue Organ Cult 38:235–240
Dräger B, van Almsick A, Mrachatz G (1995) Distribution of calystegines in several Solanaceae. Planta Med 61:577–579
Ducrot PH, Lallemand JY (1990) Structure of the calystegines: new alkaloids of the *nor*tropane family. Tetrahedron Lett 31:3879–3882
Eckenwalder JE (1986) Nomenclature of the Cardinal Climber (Convolvulaceae) reconsidered. Taxon 35:169–170
Ehrenfeld K (1999) Alkaloide in pflanzlichen Parasiten. Dtsch Apoth Ztg 139:4277–4278
Ehrenstein M (1931) Tabakalkaloide. Arch Pharm 269:627–659

El-Dabbas SW, Evans WC (1982) Alkaloids of the genus *Datura*, section *Brugmansia*. X. Alkaloid content of *Datura* hybrids. Planta Med 44:184–185
El-Imam YMA, Evans WC (1984) Tropane alkaloids of species of *Anthocercis*, *Cyphanthera* and *Crenidium*. Planta Med 50:86–87
El-Imam YMA, Evans WC (1990) Alkaloids of a *Datura candida* cultivar, *D. aurea* and various hybrids. Fitoterapia 61:148–152
El-Imam YMA, Evans WC, Plowman T (1985) Alkaloids of some South American *Erythroxylum* species. Phytochemistry 24:2285–2289
El-Imam YMA, Evans WC, Grout RJ, Ramsey KPA (1987) Alkaloids of *Erythroxylum zambesiacum* root-bark. Phytochemistry 26:2385–2389
El-Imam YMA, Evans WC, Grout RJ (1988) Alkaloids of *Erythroxylum cuneatum*, *E. ecarnitum* and *E. australe*. Phytochemistry 27:2181–2184
El-Olemy MM, Schwarting AE (1965) Simulated biosynthesis of anahygrine. Experientia 21:249
El-Shazly A, Tei A, Witte L, El-Domiaty M, Wink M (1997) Tropane alkaloids of *Hyoscyamus boveanus*, *H. desertorum*, *H. muticus*, and *H. albus* from Egypt. Z Naturforsch 52c:729–739
Endo T, Yamada Y (1985) Alkaloid production in cultured roots of three species of *Duboisia*. Phytochemistry 24:1233–1236
Engel R, Nahrstedt A, Hammerschmidt F (1995) Composition of the essential oils of *Cedronella canariensis* (L.) WEBB. et BERTH. ssp. *canariensis* and ssp. *anisata* f. *glabra* and f. *pubescens*. J Essent Oil Res 7:473–487
Enzell CR, Wahlberg I, Aasen AJ (1977) Isoprenoids and alkaloids of tobacco. In: Herz W, Grisebach H, Kirby GW (eds) Progress in the chemistry of organic natural products. Springer, Wien, pp 44–79
Evans WC (1979) Tropane alkaloids of the Solanaceae. In: Hawkes, Lester, Skelding (eds) The biology and taxonomy of the Solanaceae. Linn Soc Symp Ser, vol 7. Linnean Soc & Academic Press, London, pp 241–254
Evans WC, Lampard JF (1972) Alkaloids of *Datura suaveolens*. Phytochemistry 11:3293–3298
Evans WC, Major VA (1968) Alkaloids of the genus *Datura*, section *Brugmansia*. V. Alkaloids of *D. sanguinea* and related esters of tropane-3α,6β,7β-triol. J Chem Soc C (Organic) 2775–2778
Evans WC, Ramsey KPA (1979) Alkaloids of *Anthocercis frondosa*. J Pharm Pharmacol 31, Suppl:9P
Evans WC, Ramsey KPA (1983) Alkaloids of the Solanaceae tribe Anthocercideae. Phytochemistry 22:2219–2225
Evans WC, Somanabandhu A (1974a) Alkaloids of *Datura discolor*. Phytochemistry 13: 304–305
Evans WC, Somanabandhu A (1974b) Cuscohygrine, a constituent of the roots of some British Convolvulaceae. Phytochemistry 13:519–520
Evans WC, Somanabandhu A (1977) Bases from roots of *Solanum carolinense*. Phytochemistry 16:1859–1860
Evans WC, Somanabandhu A (1980) Nitrogen-containing non-steroidal secondary metabolites of *Solanum*, *Cyphomandra*, *Lycianthes* and *Margaranthus*. Phytochemistry 19:2351–2356
Evans WC, Treagust PG (1973a) Distribution of alkaloids in *Anthocercis littorea* and *A. viscosa*. Phytochemistry 12:2505–2507
Evans WC, Treagust PG (1973b) Alkaloids of *Datura pruinosa*. Phytochem Rep 12:2077–2078
Evans WC, Wellendorf M (1958) l-3α,6β-Ditigloyloxytropane, a new alkaloid from *Datura* roots. J Chem Soc: 1991–1993
Evans WC, Stevenson NA, Timoney RF (1969) *Datura leichhardtii* MUELL. ex BENTH. V. Alkaloidal constituents of the cross *D. leichhardtii* × *D. inoxia*. Planta Med 17:120–126
Evans WC, Ghani A, Woolley VA (1972a) Alkaloids of *Salpichroa origanifolia*. Phytochemistry 11:469
Evans WC, Ghani A, Woolley VA (1972b) Alkaloids of *Solandra* species. Phytochemistry 11:470–472
Evans WC, Ghani A, Woolley VA (1972c) Distribution of littorine and other alkaloids in the roots of *Datura* species. Phytochemistry 11:2527–2529

Evans WC, Ghani A, Woolley VA (1972d) Alkaloids of *Cyphomandra betacea* SENDT. J Chem Soc Perkin 1:2017–2019
Fang YW, Zhao JJ, Bian ZL (1981) Determination of the structure of *Erycibe obtusifolia* BENTH.'s base II – a new medicine for glaucoma. Hua Hsuch Tung Pao:209–210
Fikenscher LH (1960) The occurrence of nicotine in the genus *Acacia*. Pharm Weekbl 95:233–235
Fodor G, Csepreghy G (1959) Configuration of (–)-tropic acid and its naturally occurring esters. Tetrahedron Lett 7:16–18
Ford YY, Fox GG, Ratcliffe G, Robins RJ (1994) In vivo ^{15}N NMR studies of secondary metabolism in transformed root cultures of *Datura stramonium* and *Nicotiana tabacum*. Phytochemistry 36:333–339
Freitas AVL, Trigo JR, Brown KS Jr, Witte L, Hartmann T, Barata LES (1996) Tropane and pyrrolizidine alkaloids in the ithomiines *Placidula euryanassa* and *Miraleria cymothoe* (Lepidoptera: Nymphalidae). Chemoecology 7:61–67
Friedman M, Roitman JN, Kozukue N (2003) Glycoalkaloid and calystegine contents of eight potato cultivars. J Agric Food Chem 51:2964–2973
Friesen JB, Leete E (1990) Nicotine synthase – an enzyme from *Nicotiana* species which catalyses the formation of (*S*)-nicotine from nicotinic acid and 1-methyl-Δ^1-pyrrolinium chloride. Tetrahedron Lett 31:6295–6298
Friesen JB, Burkhouse PC, Biesboer DD, Leete E (1992) Influence of alkaloid precursors on the alkaloid content of *Nicotiana alata* root cultures. Phytochemistry 31:3059–3063
Frölich C, Hartmann T, Ober D (2006) Tissue distribution and biosynthesis of 1,2-unsaturated pyrrolizidine alkaloids in *Phalaenopsis* hybrides (Orchidaceae). Phytochemistry 67:1493–1502
Frölich C, Ober D, Hartmann T (2007) Tissue distribution, core biosynthesis and diversification of pyrrolizidine alkaloids of the lycopsamine type in three Boraginaceae species. Phytochemistry 68:1026–1037
Gadamer J (1901) Die Beziehungen des Hyoscyamins zu Atropin und des Scopolamins zu i-Scopolamin. Arch Pharm 239:294–340
Gambaro VE, Roses OE (1989) The presence of nicotine in extracts and decoctions from flowers of *Brugmansia candida* PERS. Act Farmac Bonaerense 8:17–22
Gambaro V, Labbé C, Castillo M (1983) Angeloyl, tigloyl and senecioyloxytropane alkaloids from *Schizanthus hookerii*. Phytochemistry 22:1838–1839
Garcia VF, Olmstead RG (2003) Phylogenetics of tribe Anthocercideae (Solanaceae) based on *ndhF* and *trnL/F* sequence data. Syst Bot 28:609–615
Geiger PL (1833) Ueber einige neue giftige organische Alkalien. Liebigs Ann. Chem 7:269–280
Geiger PL, Hesse (1833a) Darstellung des Atropins. Liebigs Ann Chem 5:43–81
Geiger PL, Hesse (1833b) Fortgesetzte Versuche ueber Atropin. Liebigs Ann Chem 6:44–65
Gemeinholzer B, Wink M (2001) Solanaceae: occurrence of secondary compounds *versus* molecular phylogeny. In: van den Berg RG, Barendse GWM, van der Weerden GM, Mariani C (eds) Solanaceae V – advances in taxonomy and utilization. Nijmegen University Press, Nijmegen, The Netherlands, pp 165–178
Gerrard AW (1880) Tropane alkaloids of *Duboisia myoporoides*. Pharm J 11:383
Ghani A (1985) Cuscohygrine from some solanaceous plants. Ind J Pharm Sci 47:127–129
Ghani A, Evans WC, Woolley VA (1972) Alkaloids of *Hyoscyamus* species. Bangladesh Pharmac J 1:12–14
Giesel (1891) Pharm Ztg:419; fide Czapek (1925)
Gill S, Raszeja W, Szynkiewicz G (1979) Occurrence of nicotine in some species of the genus *Sedum*. Farmacja Polska 35:151–153
Goldmann A, Milat ML, Ducrot PH, Lallemand JY, Maille M, Lepingle A, Charpin I, Tepfer D (1990) Tropane derivatives from *Calystegia sepium*. Phytochemistry 29:2125–2127
Gonzalez MD, Pomilio AB, Gros EG (1981) Terpenoids and alkaloids from *Nierembergia hippomanica*. Anales de la Asociacion Quimica Argentina 69:297–299
Goodspeed TH (1954) The genus *Nicotiana*. Chron Bot 18:1–536

Göpel C, Marcus A (2000) Renaissance der "Hexenkräuter": Der Missbrauch alkaloidhaltiger Pflanzen. Krankenhauspsychiatrie 11:94–98

Gorinova NI, Velcheva MP, Dyulgerov AS, Atanassov AI (1994) Tropane alkaloids in cell cultures of *Physochlaina orientalis*. Fitoterapia 65:452–456

Gorrod JW (1993) The mammalian metabolism of nicotine: an overwiew. In: Gorrod JW, Wahren J (eds) Nicotine and related alkaloids – absorption, distribution, metabolism and excretion. Chapman & Hall, London, pp 31–44

Gorrod JW, Schepers G (1999) Biotransformation of nicotine in mammalian systems. In: Gorrod JW, Jacob P III (eds) Analytical determination of nicotine and related compounds and their metabolites. Elsevier, Amsterdam, pp 45–67

Gourley JM, Heacock RA, McInnes AG, Nikolin B, Smith DG (1969) The structure of ipalbine, a new hexahydroindolizidine alkaloid, isolated from *Ipomoea alba* L. J Chem Soc Chem Comm 709–710

Greger H (1984) Alkamides: structural relationships, distribution and biological activity. Planta Med 50:366–375

Greger H, Zdero C, Bohlmann F (1987) Pyrrole amides from *Achillea ageratifolia*. Phytochemistry 26:2289–2291

Griffin WJ (1965) The alkaloids of *Duboisia leichhardtii*. Australasian J Pharm 46:128–131

Griffin WJ, Lin GD (2000) Chemotaxonomy and geographical distribution of tropane alkaloids. Phytochemistry 53:623–637

Griffiths RC, Watson AA, Kizu H, Asano N, Sharp HJ, Jones MG, Wormald MR, Fleet GWJ, Nash RJ (1996) The isolation from *Nicandra physalodes* and identification of the 3-O-ß-D-glucopyranoside of 1α,2ß,3α,6α-tetrahydroxy-nor-tropane (calystegine B_1). Tetrahedron Lett 37:3207–3208

Gritsanapan W, Griffin WJ (1991) Alkaloid variation within *Duboisia myoporoides*. Phytochemistry 30:2667–2669

Guenther ES (1943) Characteristics and uses of oil of olibanum. Am Perfum Ess Oil Rev 45:41–43

Guthrie FE, Ringler RL, Bowery TG (1957) Chromatographic separation and identification of some alkaloid metabolites of nicotine in certain insects. J Econ Entomol 50:821–825

Haekkinen ST, Rischer H, Laakso I, Maaheimo H, Seppaenen-Laakso T (2004) Anatalline and other methyl jasmonate-inducible nicotine alkaloids from *Nicotiana tabacum* cv. By-2 cell cultures. Planta Med 70:936–941

Halim AF, Collins RP, Berigari MS (1971) Alkaloids produced by *Cestrum nocturnum* and *Cestrum diurnum*. Planta Med 20:44–49

Halitschke R, Gase K, Hui D, Schmidt DD, Baldwin IT (2003) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. VI. Microarray analysis reveals that most herbivore-specific transcriptional changes are mediated by fatty acid-amino acid conjugates. Plant Physiol 131:1894–1902

Hanounik SB, Osborne WW (1977) The relationships between density of *Meloidogyne incognita* and nicotine content of tobacco. Nematologica 23:147–152

Hansberry R, Norton LB (1940) Toxicities of optically active nicotines and nornicotines to *Aphis rumicis*. J Econ Entomol 33:734–735

Hänsel R (2004) Nicotianaalkaloide. In: Hänsel R, Sticher O (eds) Pharmakognosie – Phytopharmazie, 7. Aufl. Springer, Heidelberg, pp 936–941

Hänsel R, Keller K, Rimpler H, Schneider G (eds) (1992) Hagers Handbuch der Pharmazeutischen Praxis, Drogen A-Z, vol 4–6. Springer Verlag Berlin, Germany

Hao DY, Yeoman MM (1996a) Mechanism of nicotine *N*-demethylation in tobacco cell suspension cultures. Phytochemistry 41:477–482

Hao DY, Yeoman MM (1996b) Nicotine *N*-demethylase in cell-free preparations from tobacco cell cultures. Phytochemistry Phytochemistry 42:325–329

Haraguchi M, Gorniak SL, Ikeda K, Minami Y, Kato A, Watson AA, Nash RJ, Molyneux RJ, Asano N (2003) Alkaloidal components in the poisonous plant, *Ipomoea carnea* (Convolvulaceae). J Agric Food Chem 51:4995–5000

Harborne JB (1993) Introduction to ecological biochemistry, 4th edn. Academic Press, Oxford, UK
Harborne JB, Khan MB (1993) Variations in the alkaloidal and phenolic profiles in the genus *Atropa* (Solanaceae). Bot J Linn Soc 111:47–53
Harris CM, Schneider MJ, Ungemach FS, Hill JE, Harris TM (1998) Biosynthesis of the toxic alkaloids slaframine and swainsonine in *Rhizoctonia leguminicola*: Metabolism of 1-hydroxy-indolizidines. J Am Chem Soc 110:940–949
Harsh ML (1989) Tropane alkaloids from *Lycium barbarum* L., in vitro and in vivo. Curr Sci 58:817–818
Hartmann R, San-Martin A, Muñoz O, Breitmaier E (1990) Grahamine, an unusual alkaloid from *Schizanthus grahamii*. Angew Chem 102:441–443 (Int Ed Engl 29:385–387)
Hartmann T (1999) Chemical ecology of pyrrolizidine alkaloids. Planta 207:483–495
Hartmann T (2004) Plant-derived secondary metabolites as defensive chemicals in herbivorous insects: a case study in chemical ecology. Planta 219:1–4
Hartmann T, Ober D (2000) Biosynthesis and metabolism of pyrrolizidine alkaloids in plants and specialized insect herbivores. Topics Curr Chem 209:207–243
Hartmann T, Toppel G (1987) Senecionine *N*-oxide, the primary product of pyrrolizidine alkaloid biosynthesis in root culture of *Senecio vulgaris*. Phytochemistry 26:1639–1643
Hartmann T, Witte L (1995) Chemistry, biology and chemoecology of the pyrrolizidine alkaloids. In: Pelletier SW(ed) Alkaloids: chemical and biological perspectives, vol 9. Pergamon Press, Oxford, UK, pp 155–233
Hartmann T, Witte L, Oprach F, Toppel G (1986) Reinvestigation of the alkaloid composition of *Atropa belladonna* plants, root cultures, and cell suspension. Planta Med 52:390–395
Hartmann T, Ehmke A, Eilert U, v Borstel K, Theuring C (1989) Sites of synthesis, translocation and accumulation of pyrrolizidine alkaloid *N*-oxides in *Senecio vulgaris*. Planta 177:98–107
Hashimoto T, Yamada Y (1994) Alkaloid biogenesis: molecular aspects. Ann Rev Plant Mol Biol 45:257–285
Hashimoto T, Hayashi A, Amano Y, Kohno J, Iwanari H, Usuda S, Yamada Y (1991) Hyoscyamine 6β-hydroxylase, an enzyme involved in tropane alkaloid biosynthesis, is localized at the pericycle of the root. J Biol Chem 266:4648–4653
Hashimoto T, Matsuda J, Yamada Y (1993) Two-step epoxidation of hyoscyamine to scopolamine is catalyzed by bifunctional hyoscyamine 6β-hydroxylase. FEBS Lett 329:35–39
Hayslett RL, Tizabi Y (2003) Effects of donezipil on DOI-induced head twitch response in mice: implications for Tourette's syndrome. Pharmacol Biochem Behav 76:409–415
Hayslett RL, Tizabi Y (2005) Effects of donezipil, nicotine and haloperidol on the central serotonergic system in mice: implications for Tourette's syndrome. Pharmacol Biochem Behav 81:879–886
Hedges SH, Herbert RB (1979) An economical, biogenetically patterned synthesis of the alkaloid ipalbidine. J Chem Res Synopses 1
Heeschen C, Jang JJ, Weis M, Pathak A, Kaji S, Hu RS, Tsao PS, Johnson FL, Cooke JP (2001) Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. Nature Med. 7:833–839
Hegnauer R, Fikenscher LH (1959) Untersuchungen mit *Erythroxylum coca* Lam. Pharm Acta Helv 35:43–64
Heltmann H (1979) Morphologische und phytochemische Untersuchungen an Sippen der Gattung *Atropa*. Herba Hungarica 18:101–110
Henrici A (1996) Neuartige Sekundärstoffe unterschiedlichster Struktur aus tropischen Convolvulaceen. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany
Hess K (1919) Über die Alkaloide des Granatapfelbaums. VII. Das natürliche Vorkommen von Isopelletierin. Ber 52B:1005–1013
Hess K, Fink H (1920) Über die Alkaloide der Hygrin-Reihe. III. Die Aufklärung der Konstitution des Cuskhygrins. Umwandlung von Cuskhygrin in Hygrin. Ber 53B:781–809
Hesse O (1901a) Über Hyoscin und Atroscin. J Prakt Chem 172/64:353–386
Hesse O (1901b) Über die Alkaloide der Mandragorawurzel. J Prakt Chem 172/64:274–286

Hibi N, Fujita T, Hatano M, Hashimoto T, Yamada Y (1992) Putrescine *N*-methyltransferase in cultured roots of *Hyoscyamus albus*. Plant Physiol 100:826–835
Hicks CS (1936) Observations on the chemistry of *d*-nornicotine, an alkaloid of *Duboisia hopwoodii*. Austral J Exptl Biol Med Sci 14:39–43
Hicks CS, LeMessurier H (1935) Preliminary observations on the chemistry and pharmacology of the alkaloids of *Duboisia hopwoodii*. Austral J Exptl Biol Med Sci 13:175–188
Hicks CS, Brucke FT, Heuber EF (1935) Pharmacology of *Duboisia hopwoodii* (*d*-nornicotine). Arch Internat Pharmacodynam Ther 51:335–353
Holdsworth DK, Jones RA, Self R (1998) Volatile alkaloids from *Areca catechu*. Phytochemistry 48:581–582
Howes CD (1974) Nicotine inhibition of carotenoid cyclization in *Cucurbita ficifolia* cotyledons. Phytochemistry 13:1469–1471
Hsiao PK, Hsia KC, Ho LY (1973) Occurrence of important tropane alkaloids in Chinese solanaceous plants. Zhiwu Xuebao 15:187–194
Huang MN, Abraham TW, Kim SH, Leete E (1996) 1-Methylpyrrolidine-2-acetic acid is not a precursor of tropane alkaloids. Phytochemistry 41:767–773
Hueller H, Peters R, Scheler W, Schmidt D, Stremmel D (1971) Pharmacodynamics of withasomnine and two of its derivatives. Pharmazie 26:361–364
Huesing J, Jones D (1987) A new form of antibiosis in *Nicotiana*. Phytochemistry 26:1381–1384
Huesing J, Jones D, Deverna J, Myers J, Collins G, Severson R, Sisson V (1989) Biochemical investigations of antibiosis material in leaf exudate of wild *Nicotiana* species and interspecific hybrids. J Chem Ecol 15:1203–1217
Humam M, Bieri S, Geiser L, Muñoz O, Veuthey JL, Christen P (2005) Separation of four isomeric tropane alkaloids from *Schizanthus grahamii* by non-aqueous capillary electrophoresis. Phytochem Anal 16:349–356
Humphrey AJ, O'Hagan D (2001) Tropane alkaloid biosynthesis. A century old problem unresolved. Nat Prod Rep 18:494–502
Hunziker AT (2001) Genera Solanacearum – the Genera of Solanaceae illustrated, arranged according to a new system. A.R.G. Gantner Verlag, Ruggell, Lichtenstein
Husemann A, Hilger A, Husemann T (1884) Die Pflanzenstoffe in chemischer, physiologischer, pharmakologischer und toxikologischer Hinsicht, vol 2. Julius Springer, Berlin, pp. 1159–1180
Ikeda K, Kato A, Adachi I, Haraguchi M, Asano N (2003) Alkaloids from the poisonous plant *Ipomoea carnea*: effects on intracellular lysosomal glycosidase activities in human lymphoblast cultures. J Agric Food Chem 51:7642–7646
Ikhiri K, Koulodo DDD, Garba M, Mamane S, Ahond A, Poupat C, Potier P (1987) Nouveaux alcaloïdes indoliziniques isolés de *Ipomoea alba*. J Nat Prod 50:152–156
Ionkova I (2002) In vitro culture and the production of secondary metabolites in *Hyoscyamus reticulatus* L. In: Nagata T, Ebizuka Y (eds) Biotechnology in agriculture and forestry, vol 51. Springer, Berlin, Germany, pp 75–94
Ionkova I, Witte L, Alfermann AW (1994) Spectrum of tropane alkaloids in transformed roots of *Datura inoxia* and *Hyoscyamus* × *györffyi* cultivated *in vitro*. Planta Med 60:382–384
Ishimura K, Shimomura K (1989) 7ß-Hydroxyhyoscyamine from *Duboisia myoporoides-D. leichhardtii* hybrid and *Hyoscyamus albus*. Phytochemistry 28:3507–3509
Israilov I, Abduazimov KA, Yunusov SY (1965) Alkaloids of *Ungernica* and *Convolvulus lineatus*. Doklady Akad Nauk UzSSR 22:18–19
Jackson BP, Berry MI (1973) Hydroxytropane tiglates in the roots of *Mandragora* species. Phytochemistry 12:1165–1166
Jackson DM, Johnson AW, Stephenson MG (2002) Survival and development of *Heliothis virescens* (Lepidoptera: Noctuidae) larvae on isogenic tobacco lines with different levels of alkaloids. J Econ Entomol 95:1294–1302
Jackson KE (1941) Alkaloids of tobacco. Chem Rev 29:123–197
Jacob P III, Hatsukami D, Severson H, Hall S, Yu L, Benowitz NL (2002) Anabasine and anatabine as biomarkers for tobacco use during nicotine replacement therapy. Cancer Epidem Biomark Prevent 11:1668–1673

Jain RK (2001) Clearing the smoke on nicotine and angiogenesis. Nature Med 7:775–777
Jenett-Siems K (1996) Phytochemische Untersuchungen an Windengewächsen der Gattungen *Calystegia*, *Convolvulus*, *Ipomoea* und *Merremia* unter besonderer Berücksichtigung des Alkaloidvorkommens. Dissertation Fachbereich Pharmazie, Freie Universität Berlin/Germany
Jenett-Siems K, Kaloga M, Eich E (1993) Ipangulines, the first pyrrolizidine alkaloids from the Convolvulaceae. Phytochemistry 34:437–440
Jenett-Siems K, Henrici A, Tofern B, Bufacchi-Richter A, Kaloga M, Witte L, Hartmann T, Eich E (1996) Occurrence and distribution of hygrines and tropanes in the convolvulaceous genus *Merremia* including the report on merredissine, an new tropane alkaloid. Proceedings of the 44th Annual Congress of the Society of Medicinal Plant Research, Prague, p 128
Jenett-Siems K, Schimming T, Kaloga M, Eich E, Siems K, Gupta MP, Witte L, Hartmann T (1998a) Pyrrolizidine alkaloids of *Ipomoea hederifolia* and related species. Phytochemistry 47:1551–1560
Jenett-Siems K, Mann P, Kaloga M, Siems K, Jakupovic J, Eich E (1998b) Tropane alkaloids with a unique type of acyl moiety from two *Convolvulus* species. Phytochemistry 49:1449–1451
Jenett-Siems K, Ott SC, Schimming T, Siems K, Müller F, Hilker M, Witte L, Hartmann T, Austin DF, Eich E (2005a) Ipangulines and minalobines, chemotaxonomic markers of the infrageneric *Ipomoea* taxon subgenus *Quamoclit*, section *Mina*. Phytochemistry 66:223–231
Jenett-Siems K, Weigl R, Böhm A, Mann P, Tofern-Reblin B, Ott SC, Ghomian A, Kaloga M, Siems K, Witte L, Hilker M, Müller F, Eich E (2005b) Chemotaxonomy of the pantropical genus *Merremia* (Convolvulaceae) based on the distribution of tropane alkaloids. Phytochemistry 66:1448–1464
Jordan M, Humam M, Bieri S, Christen P, Poblete E, Muñoz O (2006) In vitro shoot and root organogenesis, plant regenaration and production of tropane alkaloids in some species of *Schizanthus*. Phytochemistry 67:570–578
Jovankovics K (1966) Himalayan scopola (*Anisodus luridus*) roots cultivated in Hungarian Research Institute of Medical Plants. Herba Hungarica 5:41–44
Jowett HAD, Pyman FL (1909) Relation between chemical constitution and physiological action in the tropeines. Part II. J Chem Soc 95:1020–1032
Kagei K, Ikeda M, Sato T, Ogata Y, Toyoshima S, Matsuura S (1980) Studies on *Duboisia* species. V. Alkaloids in cultured cells of *Duboisia leichhardtii*. Yakugaku Zasshi 100:574–575
Karrer P, Widmer R (1925) Konfiguration des Nikotins. Optisch aktive Hygrinsäure. Helv Chim Acta 8:364–368
Kato A, Asano N, Kizu H, Matsui K, Suzuki S, Arisawa M (1997) Calystegine alkaloids from *Duboisia leichhardtii*. Phytochemistry 45:425–429
Keiner R, Nakajami K, Hashimoto T, Dräger B (2000) Accumulation and biosynthesis of calystegines in potato. J Appl Bot/Angew Bot 74:122–125
Keiner R, Kaiser H, Nakajami K, Hashimoto T, Dräger B (2002) Molecular cloning, expression and characterization of tropinone reductase II, an enzyme of the SDR family in *Solanum tuberosum* L. Plant Mol Biol 48:299–308
Kennedy GS (1971) (−)-Hyoscyamine in *Duboisia hopwoodii*. Phytochemistry 10:1335–1337
Kenton A, Parakonny AS, Gleba YY, Bennett MD (1993) Characterization of the *Nicotiana tabacum* L. genome by molecular cytogenetics. Mol Gen Genet 240:159–169
Keogh MF, O'Donovan DG (1970) Biosynthesis of some alkaloids of *Punica granatum* and *Withania somnifera*. J Chem Soc C (Organic) 1792–1797
Kester KM, Peterson SC, Hanson F, Jackson DM, Severson RF (2002) The roles of nicotine and natural enemies in determining larval feeding site distributions of *Manduca sexta* L. and *Manduca quinquemaculata* Haworth on tobacco. Chemoecology 12:1–10
Khan MB, Harborne JB (1991) A comparison of the effect of mechanical and insect damage on alkaloid levels in *Atropa acuminata*. Biochem Syst Ecol 19:529–534
Kim JH, T'Hart H, Stevens JF (1996) Alkaloids of some Asian *Sedum* species. Phytochemistry 41:1319–1324
King H, Ware LL (1941) Alkaloids of Bulgarian belladonna root. J Chem Soc 331–337

Kisaki T, Tamaki E (1966) Phytochemical studies of the tobacco alkaloids. X. Degradation of the tobacco alkaloids and their optical rotatory changes in tobacco plants. Phytochemistry 5:293–300

Kisaki T, Mizusaki S, Tamaki E (1968) Phytochemical studies on tobacco alkaloids. XI. A new alkaloid in *Nicotiana tabacum* roots. Phytochemistry 7:323–327

Kitamura Y, Hasegawa S, Miura H, Sugii M (1980) On the pyridine alkaloids of *Duboisia myoporoides* R.Br. cultivated in Nagasaki Prefecture. Shoyakugaku Zasshi 34:117–121

Kitamura Y, Miura H, Sugii M (1985) Variations of alkaloids in the developing seedlings of *Duboisia myoporoides* R.Br. Shoyakugaku Zasshi 39:85–87

Kitamura Y, Tominaga Y, Ikenaga T (2004) Winter cherry bugs feed on plant tropane alkaloids and de-epoxidize scopolamine to atropine. J Chem Ecol 30:2085–2090

Klaassen CD (1995) Nonmetallic environmental toxicants. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Goodman Gilman A (eds) Goodman & Gilman's the pharmacological basis of therapeutics, 9th edn. McGraw-Hill, New York, pp 1673–1696

Knapp S, Chase MW, Clarkson JJ (2004) Nomenclatural changes and a new sectional classification in *Nicotiana*. Taxon 53:73–82

Kraut K (1863) Ueber das Atropin. Liebigs Ann Chem 128:280–285

Kraut K (1865) Ueber das Atropin. 2.Mitt. Liebigs Ann Chem 133:87–99

Kraut K (1868) Ueber das Atropin. 3.Mitt. Liebigs Ann Chem 148:236–241

Krug E, Proksch P (1993) Influence of dietary alkaloids on survival and growth of *Spodoptera littoralis*. Biochem Syst Ecol 21:749–756

Kubwabo C, Rollmann B, Tilquin B (1993) Analysis of alkaloids from *Physalis peruviana* by capillary GC, capillary GC-MS, and GC-FTIR. Planta Med 59:161–163

Kuffner F, Faderl N (1956) Die Konstitution des Nicotellins. Monatsh Chem 87:71–81

Kusano G, Orihara S, Tsukamoto D, Shibano M, Coskun M, Guvenc A, Erdurak CS (2002) Five new *nor*tropane alkaloids and six new amino acids from the fruit of *Morus alba* Linné growing in Turkey. Chem Pharm Bull 50:185–192

Ladenburg A (1879) Künstliches Atropin. Ber 12:941–944

Ladenburg A (1880) Ueber das Duboisin. Ber 13:257–258

Ladenburg A (1881a) Die natürlich vorkommenden mydriatisch wirkenden Alkaloide. Liebigs Ann Chem 206:274–307

Ladenburg A (1881b) Über das Hyoscin. Ber 14:1870–1872

Ladenburg A, Hundt C (1889) Ueber die Darstellung optisch activer Tropasäure und optisch activer Atropine. Ber 22:2590–2592

Laiblin R (1877) Zur Kenntnis des Nicotins. Ber 10:2136–2140

Laiblin R (1879) Ueber Nicotin und Nicotinsäure. Liebigs Ann Chem 196:129–182

Langone JJ, Gjika HB, van Vunakis H (1999) Use of immunoassay techniques fort he determination of nicotine and its metabolites. In: Gorrod JW, Jacob P III (eds) Analytical determination of nicotine and related compounds and their metabolites. Elsevier, Amsterdam, pp 265–283

Laue G, Preston CA, Baldwin IT (2000) Fast track to the trichome: induction of *N*-acylnornicotines precedes nicotine induction in *Nicotiana repanda*. Planta 210:510–514

Lazur'evskii GV (1939) Alkaloids from *Convolvulus hamadae*. Sbornik Rabot Khim 15:43–52

Leary JD, Khanna KL, Schwarting AE, Bobbitt JM (1963) Occurrence of cuscohygrine and 3α-tigloyloxytropane in *Withania somnifera*. Lloydia (J Nat Prod) 25:44–48

Leary JD, Bobbitt JM, Rother A, Schwarting AE (1964) Structure and synthesis of the alkaloid anahygrine. Chem & Ind (London, UK) 283–284

Leete E (1967) Biosynthesis of the *Nicotiana* alkaloids. XI. Investigation of tautomerism in *N*-methyl-Δ^1-pyrrolinium chloride and its incorporation into nicotine. J Am Chem Soc 89:7081–7084

Leete E (1969) Biosynthesis of the *Nicotiana* alkaloids. XIV. The incorporation of Δ^1-piperideine-6-^{14}C into the piperidine ring of anabasine. J Am Chem Soc 91:1697–1700

Leete E (1977) The incorporation of [5,6-$^{13}C_2$]-nicotinic acid into the tobacco alkaloids examined by the use of carbon-13 nuclear magnetic resonance. Biorg Chem 6:273–286

Leete E (1979) The metabolism of anatabine to α,ß-dipyridyl in *Nicotiana* species. Phytochemistry 18:75–78

Leete E (1983) Biosynthesis and metabolism of the tobacco alkaloids. In: Pelletier SW (ed) Alkaloids – chemical and biological perspectives, vol 1. Wiley, New York, pp 85–152
Leete E (1985) Biosynthesis of hygrine from [5-^{14}C]ornithine via a symmetrical intermediate in *Nicandra physaloides*. Phytochemistry 24:953–955
Leete E (1990) Recent developments in the biosynthesis of the tropane alkaloids. Planta Med 56:339–352
Leete E, Chedekel MR (1972) The aberrant formation of (–)-*N*-methylanabasine from N-methyl-Δ^1-piperideinium chloride in *Nicotiana tabacum* and *N. glauca*. Phytochemistry 11:2751–2756
Leete E, Chedekel MR (1974) Metabolism of nicotine in *Nicotiana glauca*. Phytochemistry 13:1853–1859
Leete E, Kim SH (1988) A revision of the generally accepted hypothesis for the biosynthesis of the tropane moiety of cocaine. J Am Chem Soc 110:2976–2978
Leete E, Mueller ME (1982) Biomimetic synthesis of anatabine from 2,5-dihydropyridine produced by the oxidative decarboxylation of baikiain. J Am Chem Soc 104:6440–6444
Leete E, Kim SH, Rana J (1988) The incorporation of [2-^{13}C, ^{14}C, ^{15}N]-1-methyl –Δ^1-pyrrolinium chloride into cuscohygrine in *Erythroxylum coca*. Phytochemistry 27:401–406
Leete E, Endo T, Yamada Y (1990) Biosynthesis of nicotine and scopolamine in a root culture of *Duboisia leichhardtii*. Phytochemistry 29:1847–1851
Leffingwell JC (1999) Leaf chemistry: basic chemical constituents of tobacco leaf and differences among tobacco types. In: Davis DL, Nielsen MT (eds) Tobacco – production, chemistry and technology. Blackwell Science, Oxford, UK, pp 265–284
Leistner E, Spenser D (1973) Biosynthesis of the piperidine nucleus. Incorporation of chirally labelled [1-^{3}H]cadaverine. J Am Chem Soc 95:4715–4725
Leunis J, Frank AB (1885) Synopsis der Pflanzenkunde, vol 2. Hahn'sche Buchhandlung, Hannover, pp 590–594
Lewin L (1923) Die Pfeilgifte: nach eigenen und ethnologischen Untersuchungen. Verlag JA Barth, Leipzig – Reprographischer Nachdruck Gerstenberg Verlag, Hildesheim/Germany, 2. Auflage (1984)
Lewis SJ, Cherry NM, Niven RM, Barber PV, Wilde K, Povey AC (2003) Cotinine levels and self-reported smoking status in patients attending a bronchoscopy clinic. Biomarkers 8:218–239
Liebermann C (1889) Über Hygrin. Ber 22:675–679
Liebermann C, Cybulski G (1895) Über Hygrin und Hygrinsäure. Ber 28:578–585
Liebisch HW, Schütte HR (1985) Alkaloids derived from ornithine. In: Mothes K, Schütte HR, Luckner M (eds) Biochemistry of alkaloids. VCH Verlagsgesellschaft, Weinheim/Germany, pp 106–127
Linné C von (1788) Allgemeines Register über die in den sämtlichen dreyzehn Theilen des Linneischen Pflanzensystems beschriebenen Gattungen und Arten nebst einem besondern die denselben eigenen Synonymen erläuternden. Vierzehnter Theil. Raspische Buchhandlung, Nürnberg, Germany
Lippiello PM, Caldwell WS, Marks MJ, Collins AC (1994) Development of nicotinic agonists for the treatment of Alzheimer's disease. Alzheimer Dis 186–190
Liu T, Zhu P, Cheng KD, Meng C, He HX (2005) Molecular cloning, expression and characterization of hyoscyamine 6β-hydroxylase from hairy roots of *Anisodus tanguticus*. Planta Med 71:249–253
Lockwood TE (1973) Generic recognition of *Brugmansia*. Bot Mus Leafl (Harvard Univ.) 23:273–283
Loftus Hills K, Trautner EM, Rodwell CN (1946) A tobacco-*Duboisia* graft. Austral J Sci 9:24–25
Loftus Hills K, Bottomley W, Mortimer PI (1953) Occurrence of nicotine together with hyoscine in *Duboisia myoporoides*. Nature (London) 171:435
Loftus Hills K, Bottomley W, Mortimer PI (1954a) Variation in the main alkaloids of *Duboisia myoporoides* and *Duboisia leichhardtii*. II. *Duboisia myoporoides*. Austral J Appl Sci 5:258–275
Loftus Hills K, Bottomley W, Mortimer PI (1954b) Variation in the main alkaloids of *Duboisia myoporoides* and *Duboisia leichhardtii*. III. *Duboisia leichhardtii*. Austral J Appl Sci 5:276–282

Long RC, Weybrew JA (1981) Major chemical changes during senescence and curing. Recent Adv Tob Sci 7:40–74
Lossen W (1864) Ueber das Atropin (2.Mitt.) Liebigs Ann Chem 131:43–49
Lossen W (1865) Ueber das Cocain. Liebig Ann Chem 133: 351–371
Lossen W (1866) Ueber das Atropin (3.Mitt.) Liebigs Ann Chem 138:230–241
Lou Y, Baldwin IT (2003) *Manduca sexta* recognition and resistance among allopolyploid *Nicotiana* host plants. Proc Natl Acad Sci USA 100:14581–14586
Lounasmaa M, Tamminen T (1993) The tropane alkaloids. In: Cordell GA (ed) The alkaloids – chemistry and pharmacology, vol 44. Academic Press, San Diego (CA)/USA, pp 1–114
Lovkova MY, Minozhedinova NS, Il'in GS (1976) Alkaloid spectrum at early stages of development of *Nicotiana glauca*. Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya:455–458
Lovkova MY, Kliment'eva NI, Sabirova NS, Moiseev RK, Buzuk GN (1994) Metabolism of alkaloids. Expansion of the nicotinic pyrrolidine heterocycle to piperidine anabasine. Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya:5–13
Lu Y, Yao T, Chen Z (1986) Constituents of *Erycibe elliptilimba*. Yaoxue Xuebao 21:829–835
Luanratana O, Griffin WJ (1982) Alkaloids of *Duboisia hopwoodii*. Phytochemistry 21:449–451
Lukes R, Kovar J, Kloubek J, Blaha K (1960) Configuration of nitrogen-containing compounds. VII. Absolute configuration of hygrine and hygroline. Collection of Czechoslovak Chemical Communications 25:483–491
Mace ES, Gebhardt CG, Lester EN (1999) AFLP analysis of genetic relationships in the tribe Datureae (Solanaceae) Theor Appl Genet 99:634–641
Maeda S, Matsushita H, Mikami Y, Kisaki T (1980) Structural changes of *N*-methylmyosmine based on pH. Agric Biol Chem 44:1643–1645
Maienfisch P, Brandl F, Kobel W, Rindlisbacher A, Senn R (1999). CGA 293′343: a novel, broad-spectrum neonicotinoid insecticide. In: Yamamoto I, Casida JE (eds) Nicotinoid insecticides and the nicotinic acetylcholine receptor. Springer, Tokyo, pp 177–209
Majumdar DN (1952) Alkaloidal constituents of *Withania somnifera*. Curr Sci 21:46
Majumdar DN (1955) *Withania somnifera*. II. Alkaloidal constituents and their chemical characterization. Ind J Pharm 17:158–61
Mann DF, Byerrum RU (1974) Activation of the *de novo* pathway for pyridine nucleotide biosynthesis prior to ricinine biosynthesis in castor beans. Plant Physiol 53:603–609
Mann P (1997) Zur Phytochemie und Chemotaxonomie tropischer und mediterraner Convolvulaceen unter besonderer Berücksichtigung des Alkaloidvorkommens. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin/Germany
Mann P, Eich E, Witte L, Hartmann T (1996) GC-MS study on the alkaloid pattern of *Merremia quinquefolia* (L.) H.HALL. f.: First occurrence of retronecine esters, simple phenylethylamine derivatives, and pyrrolidides in the Convolvulaceae. Book of Abstracts, 44th Annual Congress of the Society for Medicinal Plant Research and a Joint Meeting with the Czech Biotechnology Society, Prague/Czech Republic, p 148 (P 251)
Mann P, Kaloga M, Witte L, Hartmann T, Eich E (1997) Complex alkaloid type pattern of *Merremia quinquefolia* (L.) H.HALL.: first occurrence of pyrrolidides, retronecine type pyrrolizidine alkaloids, and simple phenylethylamine derivatives in the Convolvulaceae. Book of Abstracts, IOCD/CYTED International Joint Symposium "Chemistry, Biological and Pharmacological Properties of Medicinal Plants from the Americas", Panama City, A-12
Manos PS, Miller RE, Wilkin P (2001) Phylogenetic analysis of *Ipomoea, Argyreia, Stictocardia,* and *Turbina* suggests a generalized model of morphological evolution in morning glories. Syst Bot 26:585–602
Manske RHF, Marion L (1942) The alkaloids of *Lycopodium* species. I. *Lycopodium complanatum* L. Can J Res 20B:87–92
Marion L (1939) The occurrence of *l*-nicotine in *Asclepias syriaca*. Can J Res 17B:21-22
Marion L (1945) The alkaloids of *Sedum acre*. Can J Res 23B:165–166
Marion L, Manske RHF (1948) Alkaloids of *Lycopodium* species. X. *Lycopodium cernuum*. Can J Res 26B:1–2

Martin RJ, Clark CL, Trailovic SM, Robertson AP (2004) Oxantel is an N-type (methyridine and nicotine) agonist not an L-type (levamisole and pyrantel) agonist: classification of cholinergic anthelmintics in *Ascaris*. Int J Parasit 34:1083–1090

Martinetz D (1994) Rauschdrogen und Stimulantien. Urania-Verlag Leipzig/Germany

Massiot G, Delaude C (1986) Pyrrolidine alkaloids. In: Brossi A (ed) The alkaloids – chemistry and pharmacology, vol 27. Academic Press, San Diego, pp 270–321

Matsuda K, Kimura M, Komai K, Hamada M (1989) Nematicidal activities of (−)-*N*-methylcytisine and (−)-anagyrine from *Sophora flavescens* against pine wood nematodes. Agric Biol Chem 53:2287–2288

Matsuo H, Tomizawa M, Yamamoto I (1998) Structure-activity relationships of acyclic nicotinoids and neonicotinoids for insect nicotinic acetylcholine receptor/ion channel complex. Arch Insect Biochem Physiol 37:17–23

Matsushita H, Tsujino Y, Yoshida D, Saito A, Kisaki T, Kato K, Noguchi M (1979) New minor alkaloids in flue-cured tobacco leaf (*Nicotiana tabacum* cv. BY-260-9). Agric Biol Chem 43:193–194

Matsuzaki T, Miyano M, Yasumatsu N, Matsushita H, Koiwai A (1988) Germination and growth inhibition of acylnornicotines from Section Repandae of the genus *Nicotiana* and synthetic acylnornicotines. Agric Biol Chem 52:1899–1903

McGaw BA, Woolley JG (1978a) The biosynthesis of hygrine and tropane alkaloids. J Pharm Pharmacol 30:Suppl 83P

McGaw BA, Woolley JG (1978b) Stereochemistry of tropane alkaloid formation in *Datura*. Phytochemistry 17:257–259

McGaw BA, Woolley JG (1979) Metabolism of hygrine in *Atropa*, *Hyoscyamus* and *Physalis*. Phytochemistry 18:189–190

Mehra KL (1979) Ethnobotany of old world Solanaceae. In: The biology and taxonomy of the Solanaceae. Linnean Society Symposium Series No. 7. Academic Press, London, pp 161–170

Meikle AW, Liu XH, Taylor GN, Stringham JD (1988) Nicotine and cotinine effects on 3α-hydroxysteroid dehydrogenase in canine prostate. Life Sci 43:1845–1850

Mein (1833) Ueber die Darstellung des Atropins in weißen Krystallen. Liebigs Ann Chem 6:67–72

Miller RE, Rausher MD, Manos PS (1999) Phylogenetic systematics of *Ipomoea* (Convolvulaceae) based on ITS and *waxy* sequences. Syst Bot 24:209–227

Miller RE, McDonald JA, Manos PS (2004) Systematics of *Ipomoea* subgenus *Quamoclit* (Convolvulaceae) based on ITS sequence data and a Bayesian phylogenetic analysis. Am J Bot 91:1208–1218

Minina SA, Astakhova TV, Gromova EG, Ovchinnikova AA (1976) Production and pahrmacological study of cuscohygrine dimethiodide. Khimiko-Farmatsevticheskii Zhurnal 10:69–73

Mirzamatov RT, Malikov VM, Lutfullin KL, Yunusov SY (1972) Alkaloids of *Physochlaina dubia*. Khim Prir Soedin 8:493–495

Miyano M, Matsushita H, Yasumatsu N, Nishida K (1979) New minor alkaloids in burley tobacco (*Nicotiana tabacum*) Agric Biol Chem 43:1607–1608

Miyano M, Yasumatsu N, Matsushita H, Nishida K (1981) 1′-(6-Hydroxyoctanoyl)nornicotine and 1′-(7-hydroxyoctanoyl)nornicotine, two new alkaloids from Japanese domestic tobacco. Agric Biol Chem 45:1029–1032

Mizusaki S, Tanabe Y, Noguchi M, Tamaki E (1971) Phytochemical studies on tobacco alkaloids. XIV. Occurrence and properties of putrescine *N*-methyltransferase in tobacco roots. Plant Cell Physiol 12:633–640

Mizusaki S, Tanabe Y, Noguchi M, Tamaki E (1972) *N*-Methylputrescine oxidase from tobacco roots. Phytochemistry 11:2757–2762

Molyneux RJ, Pan YT, Goldmann A, Tepfer DA, Elbein AD (1993) Calystegines, a novel class of alkaloid glycosidase inhibitors. Arch Biochem Biophys 304:81–88

Molyneux RJ, McKenzie RA, O'Sullivan BM, Elbein AD (1995) Identification of the glycosidase inhibitors swainsonine and calystegine B_2 in Weir vine (*Ipomoea* sp. Q6 [aff. *calobra*]) and correlation with toxicity. J Nat Prod 58:878–886

Molyneux RJ, Nash RJ, Asano N (1996) The chemistry and biological activity of calystegines and related *nor*tropane alkaloids. In: Pelletier SW (ed) Alkaloids: chemical and biological perspectives, vol 11. Pergamon/Elsevier Science, London, pp 303–343

Morris CE (1983) Uptake and metabolism of nicotine by the CNS of a nicotine-resistant insect, the tobacco hornworm (*Manduca sexta*). J Insect Physiol 29:807–817

Mortimer PI (1957) A note on *Duboisia myoporoides* from the Acacia Plateau, near Killarney, Queensland. Austral J Sci 20:87–88

Mortimer PI, Wilkinson S (1957) The occurrence of nicotine, anabasine, and isopelletierine in *Duboisia myoporoides*. J Chem Soc (London):3967–3970

Mothes K, Romeike A (1954) Nicotin als Ursache der Unverträglichkeit von Pfropfungen. Flora 142:109–131

Muñóz MA, Muñóz O, Joseph-Nathan P (2006) Absolute configuration of natural diastereoisomers of 6β-hydroxyhyoscyamine by vibrational circular dichroism. J Nat Prod 69:1335–1340

Muñóz O, Casale JF (2003) Tropane alkaloids from *Latua pubiflora*. Z Naturforsch 58c:626–628

Muñóz O, Cortés S (1998) Tropane alkaloids from *Schizanthus porrigens*. Pharm Biol 36: 162–166

Muñóz O, Hartmann R, Breitmaier E (1991) Schizanthine X, a new alkaloid from *Schizanthus grahamii*. J Nat Prod 54:1094–1096

Muñóz O, Schneider C, Breitmaier E (1994) A new pyrrolidine alkaloid from *Schizanthus integrifolius*. Liebigs Ann Chem:521–522

Muñóz O, Piovano M, Garbarino J, Hellwig V, Breitmaier E (1996) Tropane alkaloids from *Schizanthus litoralis*. Phytochemistry 43:709–713

Musser RO, Cipollini DF, Hum-Mussser SM, Williams SA, Brown JK, Felton GW (2005) Evidence that the caterpillar salivary enzyme glucose oxidase provides herbivore offense in solanaceous plants. Arch Insect Biochem Physiol 58:128–137

Nash RJ, Rothschild M, Porter EA, Watson AA, Waigh RD, Waterman PG (1993) Calystegines in *Solanum* and *Datura* species and the death's-head hawk-moth (*Acherontia atropus*). Phytochemistry 34:1281–1283

Nash RJ, Watson AA, Winters AL, Fleet GWJ, Wormald MR, Dealer S, Lees E, Asano N, Molyneux RJ (1998) Glycosidase inhibitors in British plants as causes of livestock disorders. In: Garland T, Barr AC (eds) Toxic plants and other natural toxicants. CAB International, Wallingford, UK, pp 276–284

Neuwinger HD (1996) African ethnobotany – poisons and drugs. Chapman & Hall, London

Neuwinger HD (1998) Alkaloids in arrow poisons. In: Roberts MF, Wink M (eds) Alkaloids – biochemistry, ecology, and medicinal applications. Plenum Press, New York, pp 45–84

Newquist ML, Abraham TW, Leete E (1993) Biosynthetic incorporation of ethyl (*RS*) [2,3-$^{13}C_2$,3-^{14}C]-4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate into cuscohygrine in *Erythroxylum coca*. Phytochemistry 33:1437–1440

Niemann A (1860) Ueber eine organische Base in der Coca. Liebigs Ann Chem 114:213–217

Nishikawa K, Miyamura M, Hirata Y (1967) Chemotaxonomical alkaloid studies. II. Structures of kuramerine and kumokirine. Tetrahedron Lett 27:2597–2600

Nytredy S, Groß GA, Sticher O (1986) Minor alkaloids from *Nicotiana tabacum*. J Nat Prod 49:1156–1157

O'Brien CP (1995) Drug addiction and drug abuse. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Goodman Gilman A (eds) Goodman & Gilman's the pharmacological basis of therapeutics, 9th edn. McGraw-Hill, New York, pp 557–577

O'Donovan DG, Forde TJ (1970) Biosynthesis of withasominine, a unique pyrazole alkaloid. Tetrahedron Lett:3637–3638

O'Donovan DG, Keogh MF (1968) Biosynthesis of piperidine alkaloids. Tetrahedron Lett 265–267

O'Donovan DG, Keogh MF (1969) The role of hygrine in the biosynthesis of cuscohygrine and hyoscyamine. J Chem Soc C:223–226

O'Hagan D, Robins RJ (1998) Tropic acid ester biosynthesis in *Datura stramonium* and related species. Chem Soc Rev 27:207–212

Ober D, Hartmann T (1999) Homospermidine synthase, the first pathway-specific enzyme of pyrrolizidine alkaloid biosynthesis, evolved from deoxyhypusine synthase. Proc Natl Acad Sci USA 96:14777–14782

Ober D, Gibas L, Witte L, Hartmann T (2003) Evidence for general occurrence of homospermidine in plants and its supposed origin of deoxyhypusine synthase. Phytochemistry 62:339–344
Orechoff A (1929) The alkaloids of *Anabasis aphylla*. C R Acad Sci 189:945
Orechoff A, Konowalowa R (1933) Über die Alkaloide von *Convolvulus pseudocantabricus* Schrenk. (1.Mitt.) Arch Pharm 271:145–148
Orechoff A, Konowalowa R (1934) Über die Alkaloide von *Convolvulus pseudocantabricus* (2. Mitt.) Ber 67:1153–1156
Orechoff A, Konowalowa R (1935) Über die Alkaloide von *Convolvulus pseudo-cantabricus*, III. Mitteil.: Konstitution des Convolvins und Isolierung von zwei neuen Basen. Ber 68:814–819
Orechoff A, Konowalowa R (1937) Über die Alkaloide von *Convolvulus pseudocantabricus*. Zh Obscej Khimii 7:646–653
Orechoff A, Menschikoff G (1931) Über die Alkaloide von *Anabasis aphylla* L. I. Ber 64:266–274
Orechoff A, Menschikoff G (1932) Über die Alkaloide von *Anabasis aphylla* II. Zur Konstitution des Anabasins. Ber 65B:232–234
Osawa Y, Tochigi B, Tochigi M, Ohnishi S, Watanabe Y, Bullion K, Osawa G, Nakabayashi Y, Yarborough C (1990) Aromatase inhibitors in cigarette smoke, tobacco leaves and other plants. J Enzyme Inhibit 4:187–200
Ott SC, Jenett-Siems K, Pertz HH, Siems K, Witte L, Eich E (2006) Bonabiline A, a monoterpenoid 3α-acyloxytropane from the roots of *Bonamia spectabilis* showing M_3 receptor antagonis activity. Planta Med 72:1403–1406
Ott SC, Tofern-Reblin B, Jenett-Siems K, Siems K, Müller F, Hilker M, Onegi B, Witte L, Eich E (2007) Unusual tropane alkaloid pattern in two African convolvulaceous species. Z Naturforsch 62b:285–288
Parello J, Longevialle P, Vetter W, McCloskey, JA (1963) Structure of phyllalbine. Application of nuclear magnetic resonance and mass spectrometry to the study of tropane derivatives. Bull Soc Chim France, 2787–2793
Parr AJ (1992) Alternative metabolic fates of hygrine in transformed root cultures of *Nicandra physalodes*. Plant Cell Rep 11:270–273
Parr AJ, Payne J, Eagles J, Chapman BT, Robins RJ, Rhodes MJC (1990) Variation in tropane alkaloid accumulation within the Solanaceae and strategies for its exploitation. Phytochemistry 29:2545–2550
Pena RC, Muñóz O (2002) Cladistic relationship in the genus *Schizanthus* (Solanaceae). Biochem Syst Ecol 30:45–53
Peterson N (1979) Aboriginal uses of Australian Solanaceae. In: The biology and taxonomy of the Solanaceae. Linnean Society Symposium Series No. 7. Academic Press, London, pp 171–189
Petit (1879) Nicotine from *Duboisia hopwoodii*. J Pharm Chim 29:338–341
Petrie JM (1917a) The chemical investigation of some poisonous plants in the natural order Solanaceae.III. Occurrence of *nor*-hyoscyamine in *Solandra longiflora*. Proc Linnean Soc NSW 41:815–822
Petrie JM (1917b) The chemical investigation of some poisonous plants in the natural order Solanaceae. V. Proc Linnean Soc NSW 42:137–145
Philipov S, Berkov S (2002) GC-MS investigation of tropane alkaloids in *Datura stramonium*. Z Naturforsch 57c:559–561
Phillipson JD, Handa SS (1975a) Nicotine *N*-oxides. Phytochemistry 12:2683–2690
Phillipson JD, Handa SS (1975b) *N*-Oxides of hyoscyamine and hyoscine. Phytochemistry 14:999–1003
Phillipson JD, Melville C (1960) An investigation of the alkaloids of some British species of *Equisetum*. J Pharm Pharmacol 12:506–508
Pictet A, Genequand P (1897) Ueber die Jodmethylate des Nicotins. Ber 30:2117–2125
Pictet A, Rotschy A (1901) Über neue Alkaloide des Tabaks. Ber 34:696–708
Pictet A, Rotschy A (1904) Synthese des Nicotins. Ber 37:1225–1235
Pinner A (1893) Ueber Nicotin (5.Mitt.) Ber 26:292–305
Pinner A (1895) Ueber Nicotin (9.Mitt.) Ber 28:456–465

Platonova TF, Kuzovkov AD (1963) Alkaloids of *Cochlearia arctica*. Med Prom SSR (Med Ind UdSSR) 17:19–20
Plowman T, Gyllenhaal LO, Lindgren JE (1971) *Latua pubiflora* – magic plant from Southern Chile. Bot Mus Leafl Harvard Univ 23:61–92
Pomilio AB, Gonzalez MD, Eceizabarrena CC (1996) 7,8-Dihydroajugasterone C, norhygrine and other constituents of *Nierembergia hippomanica*. Phytochemistry 41:1393–1398
Posselt W, Reimann L (1828) Chemische Untersuchung des Tabaks und Darstellung eines eigenthümlich wirksamen Prinzips dieser Pflanze. Poggend Ann Phys Chem 8:399–410
Pyman FL, Reynolds WC (1908) Meteloidine. A new solanaceous alkaloid. J Chem Soc (Transact) 93:2077–2081
Rabinovich MS, Konovalova RA (1946) Alkaloids of the Himalayan scopola *Anisodus luridus* Link & Otto. Zhumal Obshchei Khimii 16:2121–2125
Rabot S, Peerless ACJ, Robins RJ (1995) Tigloyl-CoA:pseudotropine acyl transferase – an enzyme of tropane alkaloid biosynthesis. Phytochemistry 39:315–322
Rang HP, Dale MM, Ritter JM (1999). Pharmacology, 4th ed. Churchill Livingstone, Edinburgh, p 672
Rätsch C (1995) *äh kib lu'um* – "Das Licht der Erde" – Der Fliegenpilz bei den Lakandonen und in der Neuen Welt. Curare 18:67–93
Rätsch C (2005) The encyclopedia of psychoactive plants – ethnopharmacology and its applications. Inner Traditions, Vermont, USA
Ravikanth V, Ramesh P, Diwan PV, Venkateswarlu Y (2001) Pyrazole alkaloids from *Elytraria acaulis*. Biochem Syst Ecol 29:753–754
Ray AB, Sahai M, Sethi PD (1976) Physoperuvine, a new alkaloid of *Physalis peruviana* L. Chem Ind (London):454–455
Ray AB, Oshima Y, Hikino H, Kabuto C (1982) Revised structure of physoperuvine, an alkaloid of *Physalis peruviana* roots. Heterocycles 19:1233–1236
Razzakov NA, Aripova SF, Akhmedova E, Karimov A (1999) Alkaloids of *Mandragora turcomanica*. Chem Nat Comp (Khim Prir Soed) 34:741–742
Razzakov NA, Aripova SF, Yunusov SY (2004) Confolidine, a new alkaloid from the aerial part of *Convolvulus subhirsutus*. Chem Nat Comp 40:54–55
Reimann A, Nurhayati N, Backenköhler A, Ober D (2004) Repeated evolution of the pyrrolizidine alkaloid-mediated defense system in separate angiosperm lineages. Plant Cell 16:1772–2784
Remington JP, Wood HC (eds) (1918) The dispensatory of the United States of America, 20th edn. J.B. Lippincott, Philadelphia, USA
Reynouts-van Haga P (1954) Cuscohygrine, a normal alkaloid of *Atropa belladonna*. Nature (London) 174:833–834
Richardson CH, Shepard HH (1930) The insecticidal action of some derivatives of pyridine and pyrrolidine and some aliphatic amines. J Agric Res 40:1007–1015
Richardson CH, Craig LC, Hansberry TR (1936) Toxic action of nicotine, nornicotine and anabasine upon *Aphis rumicis*. J Econ Entomol 26:850–855
Riechers DE, Timko MP (1999) Structure and expression of the gene family encoding putrescine *N*-methyltransferase in *Nicotiana tabacum*: new clues to the evolutionary origin of cultivated tobacco. Plant Mol Biol 41:387–401
Ripperger H (1979) Schizanthin A und B, zwei neue Tropanalklaoide aus *Schizanthus pinnatus*. Phytochemistry 18:171–173
Ripperger H (1995) *S*-(−)-Scopolamine and *S*-(−)-norscopolamine from *Atropanthe sinensis*. Planta Med 61:292–293
Rizvi SJH, Mishra GP, Rizvi V (1989a) Allelopathic effects of nicotine on maize. I. Its possible importance in crop rotation. Plant and Soil 116:289–291
Rizvi SJH, Mishra GP, Rizvi V (1989b) Allelopathic effects of nicotine on maize. Some aspects of its mechanism of action. Plant Soil 116:292–293
Robins DJ (1995) Biosynthesis of pyrrolizidine and quinolizidine alkaloids. In: The alkaloids – chemistry and pharmacology, vol 46. Academic Press, San Diego, CA, USA, pp 1–62
Robins RJ, Walton NJ (1993) The biosynthesis of tropane alkaloids. In: Cordell GA (ed) The alkaloids – chemistry and pharmacology, vol 44. Academic Press, San Diego (CA), USA, pp 115–187

Robins RJ, Parr AJ, Payne J, Walton NJ, Rhodes MJC (1990) Factors regulating tropane-alkaloid production in a transformed root culture of a *Datura candida* × *D. aurea* hybrid. Planta 181:414–422

Robins RJ, Bachmann P, Peerless ACJ, Rabot S (1994) Esterification reactions in the biosynthesis of tropane alkaloids in transformed root cultures. Plant Cell Tissue Org Cult 38:241–247

Robins RJ, Abraham TW, Parr AJ, Eagles J, Walton NJ (1997) The biosynthesis of tropane alkaloids in *Datura stramonium*: the identity of intermediates between *N*-methylpyrrolinium salt and tropinone. J Am Chem Soc 119:10929–10934

Robinson R (1928) Proceedings of the University of Durham Philosophical Society, 1927–1932, vol 8, p 14; fide O'Hagan and Robins 1998

Robinson R (1955) The structural relations of natural products. Clarendon Press, Oxford, UK, p 59; fide O'Hagan and Robins 1998

Roeder E (1995) Medicinal plants in Europe containing pyrrolizidine alkaloids. Pharmazie 50:83–98

Rojo HP, Quiroga EN, Vattuone MA, Sampietro AR (1998) *Nicotiana glauca* invertase: characterization and effects of endogenous alkaloids. Phytochemistry 49:965–969

Romeike A (1965a) Über das Vorkommen von Hygrin in Wurzeln von *Nicandra physaloides* (L.) Gaertn. Pharmazie 20:738–739

Romeike A (1965b) Hygrin, das Hauptalkaloid der *Nicandra*-Wurzeln. Naturwissenschaften 52:619

Romeike A (1966) Presence of tropinone in *Nicandra* roots. Naturwissenschaften 53:82

Rosenblum EI (1954) Alkaloid variation in wild and cultivated *Duboisia leichhardtii*. Austral J Appl Sci 5:51–62

Rosenblum EI, Taylor WS (1954) The alkaloids of *Duboisia leichhardtii*: butropine and valtropine. J Pharm Pharmacol 6:410–415

Roth HJ (2005) Viergliedrige Ringe. Dtsch Apoth Ztg 145, 2036–2042

Rothe G, Garske U, Dräger B (2001) Calystegines in root cultures of *Atropa belladonna* respond to sucrose, not to elicitation. Plant Sci 160:1043–1053

Rother A, Bobbitt JM, Schwarting AE (1962) Structure and synthesis of the alkaloid anaferine. Chem Ind (London):654–655

Rothera ACH (1911) The alkaloid of pituri obtained from *Duboisia hopwoodii*. Biochem J 5:193–206

Runge F (1824) [Atropin-Nachweis durch Pupillenerweiterung] Ann Chim Phys (2), 27; fide Czapek (1925), p 280

Runge F (1825) [do.] Schweigg J 43:483 (1825) fide Czapek (1925), p 280

Sahai M, Ray AB (1980) Secotropane alkaloids of *Physalis peruviana*. J Org Chem 45:3265–3268

Saitoh F, Noma M, Kawashima N (1985) The alkaloid contents of sixty *Nicotiana* species. Phytochemistry 24:477–480

Salin-Pascual RJ, Alcocer-Castillejos NV, Alejo-Galarza G (2003) Nicotine dependence and psychiatric disorders. Rev Invest Clin 55:677–693

San-Martin A, Rovirosa J, Gambaro V, Castillo M (1980) Tropane alkaloids from *Schizanthus hookeri*. Phytochemistry 19:2007–2008

San-Martin A, Labbé C, Muñóz O, Castillo M, Reina M, de la Funte G, González A (1987) Tropane alkaloids from *Schizanthus grahamii*. Phytochemistry 26:819–822

Sarychev Y, Sherstyanykh NA (1985) Pyridine bases in the genus *Nicotiana*. Tabak (Moscow) 2:6–12

Savikin-Fodulovic KP, Bulatovic VM, Menkovic NR, Grubisic DV (2000) Comparison between the essential oil of *Myrtus communis* L. obtained from naturally grown and in vitro plants. J Ess Oil Res 12:75–78

Schimming T (2003) Beiträge zur Chemotaxonomie und Phylogenie der Convolvulaceen auf der Basis des Alkaloidvorkommens. Dissertation, Fachbereich Biologie, Chemie, Pharmazie, Freie Universität Berlin, Germany

Schimming T, Tofern B, Mann P, Richter A, Jenett-Siems K, Dräger B, Asano N, Gupta MP, Correa MD, Eich E (1998) Distribution and taxonomic significance of calystegines in the Convolvulaceae. Phytochemistry 49:1989–1995

Schimming T, Jenett-Siems K, Mann P, Tofern-Reblin B, Milson J, Johnson RW, Deroin T, Austin DF, Eich E (2005) Calystegines as chemotaxonomic markers in the Convolvulaceae. Phytochemistry 66:469–480

Schmeller T, Sporer F, Sauerwein M, Wink M (2000) Binding of tropane alkaloids to nicotinic and muscarinic acetylcholine receptors. Pharmazie 50:493–495
Schmidt E (1892) Über Scopolamin (Hyoscin) 1. Mitt. Arch Pharm 230:207–231
Schmidt E (1894) Über das Scopolamin. 2. Mitt. Arch Pharm 232:409–437
Schmidt E, Henschke H (1888) Über die Alkaloide der Wurzel von *Scopolia japonica*. Arch Pharm 226:185–199
Schneider MJ, Brendze S, Montali JA (1995) Alkaloids of *Picea breweriana*. Phytochemistry 39:1387–1390
Schneider NG, Jacob P III, Nilsson F, Leischow SJ, Benowitz NL, Olmstead RE (1997) Saliva cotinine levels as a function of collection method. Addiction 92:347–351
Scholl Y, Höke D, Dräger B (2001) Calystegines in *Calystegia sepium* derive from the tropane alkaloid pathway. Phytochemistry 58:883–889
Scholl Y, Schneider B, Dräger B (2003) Biosynthesis of calystegines: ^{15}N NMR and kinetics of formation in root cultures of *Calystegia sepium*. Phytochemistry 62:325–332
Schreiber K, Sembdner G (1960) Über die spezifische Wirkung einiger Solanaceen-Alkaloide auf den Kartoffelnematoden, *Heterodera rostochiensis* Woll. Planta Med. 8:107–113
Schröter HB (1955) Über den Nachweis von Nikotin in der Composite *Zinnia elegans* und die Bedeutung dieses Alkaloids für die interfamiliäre Propfung *Zinnia* auf *Nicotiana*. Arch Pharm 288:141–145
Schröter HB (1958) Ein Alkaloid aus *Salpiglossis sinuata*. Naturwissenschaften 45:338
Schröter HB (1963) Biosynthese von Pyridin-Alkaloiden. Abhandl Deut Akad Wiss Berlin, Kl Chem Geol Biol (4):99–101
Schröter HB, Neumann D, Katritzky AR, Swinbourne FJ (1966) Withasomnine. A pyrazole alkaloid from *Withania somnifera* Dun. Tetrahedron 22:2895–2897
Schulte KE, Rücker G, El-Kersch M (1972) Nicotin und 3-Formyl-4-hydroxy-2H-pyran aus *Herpestis monniera*. Phytochemistry 11:2649–2651
Schultes RE (1979) Solanaceous hallucinogens and their role in the development of New World cultures. In: Hawkes, Lester, Skelding (eds) The biology and taxonomy of the Solanaceae. Linn Soc Symp Ser, vol 7. Linnean Soc & Academic Press, London, pp 137–160
Schwarting AE, Bobbitt JM, Rother A, Atal CK, Khanna KL, Leary JD, Walter WG (1963) The alkaloids of *Withania somnifera*. Lloydia (J Nat Prod) 26:258–273
Scott TA, Lynn JP (1967) The incorporation of [2,3,7,-^{14}C]nicotinic acid into nicotine by *Nicotiana tabacum*. Phytochemistry 6:505–510
Self LS, Guthrie FE, Hodgson E (1964) Adaptation of tobacco hornworms to the ingestion of nicotine. J Insect Physiol 10:907–914
Senft E (1911) *Duboisia hopwoodii* F.Muell. (Pituri). Pharmaz Praxis 1
Severson RF, Huesing JE, Jones D, Arrendale RF, Sisson VA (1988a) Identification of tobacco hornworm antibiosis factor from cuticle of Repandae section of *Nicotiana* species. J Chem Ecol 14:1485–1494
Severson RF, Arrendale RF, Cutler HG, Jones D, Sisson VA, Stephenson MG (1988b) Chemistry and biological activity of acylnornicotines from *Nicotiana repanda*. In: Cutler (ed), Biologically active natural products: potential use in agriculture. ACS Symposium Series 380. American Chemical Society, Washington/Oxford University Press, pp 335–362
Sevón N, Dräger B, Hiltunen R, Oksman-Caldentey KM (1997) Characterization of transgenic plants derived from hairy roots of *Hyoscyamus muticus*. Plant Cell Rep 16:605–611
Shang Y, Wang YF, Liang YX, Cai NS (2003) The effect of several memory-improving agents on memory impairment in mice by anisodine. Zhongguo Xinyao Zazhi 12:821–823
Sharova EG, Aripova SF, Yunusov SY (1980) Alkaloids of *Convolvulus subhirsutus*. Khim Prir Soedin:672–676
Shaw S, Bencherif M, Marrero MB (2003) Angiotensin II blocks nicotine-mediated neuroprotection against ß-amyloid (1–42) via activation of the tyrosine phosphatase SHP-1. J Neurosci 23:11224–11228
Shepherd JA (1999) Nematode pests of tobacco. In: Davis DL, Nielsen MT (eds) Tobacco – production, chemistry and technology. Blackwell Science, Oxford, pp 216–227

Shmuk AA, Borozdina A (1941) Alkaloids of various plant species within the genus *Nicotiana*. C R (Doklady) Acad Sci URSS 32:62–65
Shonle I, Bergelson J (2000) Evolutionary ecology of the tropane alkaloids of *Datura stramonium* L. (Solanaceae). Evolution 54:778–788
Shukla YN, Thakur RS (1992) Tropane alkaloids from *Duboisia myoporoides*. Phytochemistry 31:4389–4390
Siddiqi MA, Suri KA, Suri OP, Atal CK (1979) New pyrrolizidine alkaloids from *Crotalaria candicans*. Phytochemistry 18:1413–1415
Siddiqui S, Sultana N, Ahmed SS, Haider SI (1986) Isolation and structure of a new alkaloid datumetine from the leaves of *Datura metel*. J Nat Prod 49:511–513
Siegmund B, Leitner E, Pfannhauser W (1999) Determination of the nicotine content of various edible nightshades (Solanaceae) and their products and estimation of the associated dietary nicotine intake. J Agric Food Chem 47:3113–3120
Silva M, Mancinelli P (1959) Atropina en *Latua pubiflora* (Griseb.) Phil Boletin Soc Chil Quim 9:49–50
Sim KL, Perry D (1997) Analysis of swainsonine and its early metabolic precursors in cultures of *Metarhizium anisopliae*. Glycoconj J 14:661–668
Siminszky B, Gavilano L, Bowen SW, Dewey RE (2005) Conversion of nicotine to nornicotine in *Nicotiana tabacum* is mediated by CYP82E4, a cytochrome P450 monooxygenase. PNAS 102:14919–14924
Singh J, Dhar KL, Atal CK (1971) Studies on the genus *Piper*: part XII. Structure of trichonine, a new *N*-pyrrolidinyl-eicosa-*trans*, *trans*-2,4-dienamide. Tetrahedron Lett:2119–2120
Sisson VA, Severson RF (1990) Alkaloid composition of the *Nicotiana* species. Beiträge zur Tabakforschung International 14:327–339
Siva Raju K, Krishnamurthy GVG (1996) Biochemical changes in tobacco plants infested with root-knot nematode *Meloidogyne javanica*. Tobacco Res 22:116–119
Smith CR (1937) Occurrence of *l*-nornicotine in *Nicotiana sylvestris*. J Econ Entomol 20:724–727
Smith HH, Abashian DV (1963) Chromatographic investigations on the alkaloid content of *Nicotiana* species and interspecific combinations. Am J Bot 50:435–447
Smith HH, Smith CR (1942) Alkaloids in certain species and interspecific hybrids of *Nicotiana*. J Agric Res 65:347–359
Snyder MJ, Hsu EL, Feyereisen R (1993) Induction of cytochrome P-450 activities by nicotine in the tobacco hornworm, *Manduca sexta*. J Chem Ecol 19:2903–2916
Soeda Y, Yamamoto I (1969) Nicotinoids as insecticides. VIII. Physiological activities of the optical isomers of nicotinoids. Bochu Kagaku 34:57–62
Solt ML, Dawson RF, Christman DR (1960) Biosynthesis of anabasine and of nicotine by excised root cultures of *Nicotiana glauca*. Plant Physiol 35:887–894
Song W, Liu J, Jin R (1997) Chemical constituents of the stems of *Erycibe schmidtii* Craib. Zhongguo Zhongyao Zazhi (China J Chin Mat Med) 22:359–360, 384
Späth E, Kesztler F (1937a) Tabak-Basen. XI. Mitteil. *l*-Anatabine, ein neues Tabakalkaloid. Ber. 70B:239–243
Späth E, Kesztler F (1937b) Tabak-Alkaloide. XII. Mitteil. Über das Vorkommen von *dl*-Nor-nicotin, *dl*-Anatabine und *l*-Anabasine im Tabak. Ber. 70B:704–709
Späth E, Kesztler F (1937c) Tabak-Alkaloide. XIII. Mitteil. Über neue Basen des Tabaks. Ber 70B:2450–2454
Späth E, Zajic E (1935) Tabak-Basen. III. *l*-Nor-nicotin. Ber 68B:1667–1670
Späth E, Zajic E (1936) Über neue Tabak-Alkaloide (VIII. Teil über Tabakbasen) und Bemerkungen zur Kenntnis des Rhoeadins, des *l*-Peganins und des Ammoresinols. Ber 69B:2448–2452
Späth E, Hicks CS, Zajic E (1935) Über *d*-Nor-nicotin, ein Alkaloid von *Duboisia hopwoodii* F. v.Muell. Ber 68B:1388–1393
Späth E, Wenusch A, Zajic E (1936) Tabak-Basen. V. Mitteil. Die Konstitution des Myosmins. Ber 69B:393–396

Staub H (1962) Über die chemischen Bestandteile der Mandragorawurzel. 2. Die Alkaloide. Helv Chim Acta 45:2297–2305
Stech M, Austin DF, Schimming T, Eich E (2007) Phylogenetic inference in *Ipomoea* section *Mina* (Convolvulaceae): Molecular relationships and the significance of phytochemical and morphological characters (to be published)
Steinegger E (1972) Alkaloiddrogen. In: Steinegger E, Hänsel R (eds) Lehrbuch der Pharmakognosie – Auf phytochemischer Grundlage, 3. Aufl. Springer Verlag, Berlin
Steinegger E, Phokas G (1955) Zur Konstitution von Bellaradin. Pharm Acta Helv 30:441–443
Stenzel O, Teuber M, Dräger B (2006) Putrescine *N*-methyltransferase in *Solanum tuberosum* L., a calystegine-forming plant. Planta 223:200–212
Steppuhn A, Gase K, Krock B, Halitschke R, Balwin IT (2004) Nicotine's defensive function in nature. PLoS Biol 2:1074–1080
Stevens JF, T'Hart H, Hendricks H, Malingre TM (1992) Alkaloids of some European and Macaronesian Sedoideae and Sempervivoideae (Crassulaceae). Phytochemistry 31:3917–3924
Stumpf PK, Jones AT (1963) The biosynthesis of long chain fatty acids by lettuce chloroplasts. Biochem Biophys Acta 70:20–32
Suzuki U, Shamimura T, Odake S (1912) Über Oryzanin, ein Bestandteil der Reiskleie, und seine physiologische Bedeutung. Biochem Z 43:89–153
Taylor P (1995) Agents acting at the neuromuscular junction and autonomic ganglia. In: Hardman JG, Limbird LE, Molinoff PB, Ruddon RW, Goodman Gilman A (eds) Goodman & Gilman's the pharmacological basis of therapeutics, 9th edn. McGraw-Hill, New York, pp 177–197
Tepfer D, Goldmann A, Pamboukdjian N, Maille M, Lepingle A, Chevalier D, Dénarié J, Rosenberg C (1988) A plasmid of *Rhizobium meliloti* 41 encodes catabolism of two compounds from root exudates of *Calystegia sepium*. J Bacteriol 170:1153–1161
Teuscher E, Lindequist U (1994) Biogene Gifte – Biologie, Chemie, Pharmakologie. Gustav Fischer Stuttgart, p 471
Tiburcio AF, Galston AW (1985) Arginine decarboxylase as the source of putrescine for tobacco alkaloids. Phytochemistry 25:107–110
Todd FG, Stermitz FR, Schultheis P, Knight AP, Traub-Dargatz J (1995) Tropane alkaloids and toxicity of *Convolvulus arvensis*. Phytochemistry 39:301–303
Tofern B (1999) Neue und seltene Sekundärstoffe des Phenylpropan-, Terpen- und Alkaloid-Stoffwechsels aus tropischen Convolvulaceen. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany
Tofern B, Kaloga M, Witte L, Hartmann T, Eich E (1996) Comparative study of two convolvulaceous species: complex pattern of alkaloids in *Ipomoea muricata* and *Ipomoea alba*. Book of Abstracts, 44th Annual Congress of the Society for Medicinal Plant Research and a Joint Meeting with the Czech Biotechnology Society, Prague, p 146 (P 247)
Tofern B, Mann P, Kaloga M, Jenett-Siems K, Witte L, Eich E (1999) Aliphatic pyrrolidine amides from two tropical convolvulaceous species. Phytochemistry 52:1437–1441
Tolksdorff W, Meisel R, Müller P, Bender HJ (1985) Transdermales Scopolamin (TTS-Scopolamin) zur Prophylaxe postoperativer Übelkeit und Erbrechen. Anaesthesist 34:656–663
Tomizawa M, Yamamoto I (1992) Binding of nicotinoids and the related compounds to the insect nicotinic acetylcholine receptor. J Pestic Sci (Internat Ed) 17:231–236
Trautner EM, McCallum IAN (1950) The action of tropine and heliotridine-alkaloids on the excitation, propagation, and recovery in muscle. Austral J Exp Biol Med Sci 28:343–360
Trigo JR, Brown KS Jr, Henriques SA, Barata LES (1996) Qualitative patterns of pyrrolizidine alkaloids in Ithomiinae butterflies. Biochem Syst Ecol 24:181–188
Tso TC (1999) Seed to smoke. In: Davis DL, Nielsen MT (eds) Tobacco – production, chemistry and technology. Blackwell Science, Oxford, pp 1–31
Vakhabov AA, Sultanov MB, Mirzamatov RT (1975) Pharmacology of 6-hydroxyhyoscyamine. Doklady Akademii Nauk USSR 26
Vasinev VS (1970) Composition of alkaloids in hybrids and wild species of tobacco. Studencheskie Nauchnye Raboty, Universitet Druzhby Narodov, No. 15, pp 108–112
Vauquelin LN (1809a) Tobacco. Ann Chim 71:139–157; fide Czapek (1925), p 277

Vauquelin LN (1809b) *Atropa belladonna*. Ann Chim 72: 53; fide Czapek (1925) p 280
Vitale AA, Acher A, Pomilio AB (1995) Alkaloids of *Datura ferox* from Argentina. J Ethnopharmacol 49:81–89
Wahl R (1952) Über das Vorkommen und den Nachweis kleinster Nikotinmengen in Tomatenblättern. Tabak-Forschung No 8:3
Wahl R (1953) Tabak-Forschung No 10:3-4 fide Hegnauer R (1973) Chemotaxonomie der Pflanzen vol. 6. Birkhäuser, Basel, p 407
Wahlberg I (1999) Smokeless tobacco. In: Davis DL, Nielsen MT (eds) Tobacco – production, chemistry and technology. Blackwell Science, Oxford, pp 452–460
Wahlberg I, Karlsson K, Austin DJ, Junker N, Roeraade J, Enzell C, Johnson WH (1977) Tobacco chemistry. Part 38. Effects of flue-curing and aging on the volatile, neutral and acidic constituents of Virginia tobacco. Phytochemistry 16:1217–1231
Wang P, Yao T, Chen Z (1989) Chemical constituents of *Erycibe hainensis*. Zhiwu Xuebao 31:616–619
Wang ZB, Wu XQ (1979) Variation of the contents of two alkaloids in *Anisodus tanguticus*. Zhiwu Xuebao 21:85–87
Warfield AH, Galloway WD, Kallianos AG (1972) New alkaloids from burley tobacco. Phytochemistry 11:3371–3375
Waterman PG (1998) Alkaloid chemosystematics. In: Cordell GA (ed) The alkaloids – chemistry and biology. Academic Press, San Diego, pp 537–567
Watson AA, Davies DR, Asano N, Winchester B, Kato A, Molyneux RJ, Stegelmeier BL, Nash RJ (2000) Calystegine alkaloids in the potato and other food plants. In: Natural and selected synthetic toxins: biological implications, ACS Symposium Series, vol 745. Oxford University Press, Washington DC, pp 129–139
Watson AA, Fleet GWJ, Asano N, Molyneux RJ, Nash RJ (2001) Polyhydroxylated alkaloids – natural occurrence and therapeutic applications. Phytochemistry 56:265–295
Watson PL, Luanratana O, Griffin WJ (1983) The ethnopharmacology of pituri. J Ethnopharmacol 8:303–311
Weeks WW (1999) Relationship between leaf chemistry and organoleptic properties of tobacco smoke. In: Davis DL, Nielsen MT (eds) Tobacco – production, chemistry and technology. Blackwell Science, Oxford, pp 304–312
Weigl R (1992) Entdeckung, Isolierung und Strukturaufklärung neuer Alkaloide im Rahmen chemotaxonomischer Untersuchungen an Convolvulaceen. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany
Weigl R, Kaloga M, Eich E (1992) Merresectines: novel tropane alkaloids from *Merremia dissecta* roots. Planta Med 58:A705
Wenusch A (1935) Über das Auftreten von Nicotyrin im Tabak. Biochem Z 275:361
Westendorf J (1992) Pyrrolizidine alkaloids – general discussion. In: De Smet PAG, Keller K, Hänsel R, Chandler RF (eds) Adverse effects of herbal frugs, vol 1. Springer, Berlin, Germany, pp 192–205
Wilbert J (1972) Tobacco and shamanistic ecstasy among the Warrao Indians of Venezuela. In: Furst PT (ed) Flesh of the gods. Praeger Publisher, New York, pp 55–83
Wilhelm H, Wilhelm B, Schiefer U (1991) Mydriasis caused by plant contact. Fortschr Opthalm 88:588–591
Willstätter R (1898a) Ueber die Constitution des Tropins. Ber 30:2679–2719
Willstätter R (1898b) Ueber die Constitution der Spaltungsproducte von Atropin und Cocain. Ber 31:1534–1553
Wink M (1998) Chemical ecology of alkaloids. In: Roberts MF, Wink M (eds) Alkaloids. Plenum, New York, pp 265–300
Wink M (2000) Interference of alkaloids with neuroreceptors and ion channels. In: Atta-ur-Rahman (ed) Studies in natural products chemistry, vol 21. Bioactive natural products (Part B). Elsevier, Amsterdam, pp 3–122
Wink M (2003) Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64:3–19

Winterstein E, Trier G (1931) Die Alkaloide, 2nd edn. Borntraeger, Berlin, p 1031
Witte L, Müller K, Arfmann HA (1987) Investigation of the alkaloid pattern of *Datura inoxia* plants by capillary gas-liquid-chromatography-mass spectrometry. Planta Med 53:192–197
Wöhler F, Lossen W (1862) Fortsetzung der Untersuchungen über die Coca und das Cocain. Liebigs Ann Chem 121:372–375
Wolfes O, Hromatka O (1933) Über ein Tropanderivat aus Cocablättern. Jahresberichte E. Merck, Darmstadt, pp 45–53
Wollweber D, Tietjen K (1999) Chloronicotinyl insecticides: a success of the new chemistry. In: Yamamoto I, Casida JE (eds) Nicotinoid insecticides and the nicotinic acetylcholine receptor. Springer, Tokyo, pp 109–125
Wolters B (1994) Drogen, Pfeilgift und Indianermedizin – Arzneipflanzen aus Südamerika. Urs Freund Verlag, Greifenberg, Germany
Xiao P, He L, Wang L (1983) Constituents in Tibetan traditional medicines. Zhongyao Tongbao 9:10–11
Xie JX, Yang JH, Zhao YX, Zhang YX, Zhang CZ (1983) Absolute configuration of (−)-anisodine (a new ganglio blocking agent) and (−)-anisodinic acid. Sci Sin Ser B (Chung-kuo K'o Hsueh Yuan, Chu Pan) 26:931–935
Yajima A, Yabuta G (2001) Synthesis and absolute configuration of MQ-A3 [1-(14′-methylhexad ecanoyl)pyrrolidine], a novel aliphatic pyrrolidine amide from the tropical convolvulaceous species. Biosci Biotech Biochem 65:463–465
Yamada T, Takahashi H, Hatano R (1999) A novel insecticide, acetamiprid. In: Yamamoto I, Casida JE (eds) Nicotinoid insecticides and the nicotinic acetylcholine receptor. Springer, Tokyo, pp 149–176
Yamada Y, Endo T (1984) Tropane alkaloid production in cultured cells of *Duboisia leichhardtii*. Plant Cell Rep 3:168–188
Yamada Y, Hashimoto T (1989) Substrate specifity of the hyoscyamine 6β-hydroxylase from cultured roots of *Hyoscyamus niger*. Proc Jpn Acad, Ser B Phys Biol Sci 65:156–159
Yamaguchi H, Nishimoto K (1965) Studies on the alkaloids of the root of *Physalis alkekengi* (I). Isolation of 3α-tigloyloxytropane. Chem Pharm Bull 13:217–220
Yamaguchi H, Numata A, Hokimoto (1974) Studies on the alkaloids of *Physalis alkekengi* (II). J Pharm Soc Jpn 94:1115–1123
Yamamoto I, Kamimura H, Yamamoto R, Sakai S, Goda M (1962) Studies on nicotinoids as an insecticide. I. Relation of structure to toxicity. Agric Biol Chem 26:709–716
Yamamoto I, Soeda Y, Kamimura H, Yamamoto R (1968) Nicotinoids as insecticides. VII. Cholinesterase inhibition by nicotinoids and pyridylalkylamines, its significance to mode of action. Agr Biol Chem 32:1341–1348
Yamamoto I, Yabuta G, Tomizawa M, Saito T, Miyamoto T, Kagabu S (1995) Molecular mechanism for selective toxicity of nicotinoids and neonicotinoids. Nippon Noyaku Gakkaishi 20:33–40
Yamamoto I, Tomizawa M, Saito T, Miyamoto T, Walcott EC, Sumikawa K (1998) Structural factors contributing to insecticidal and selective actions of neonicotinoids. Arch Insect Biochem Physiol 37:24–32
Yao T, Chen Z, Yi D, Xu G (1981) Chemical study on Bao Gong-teng (*Erycibe obtusifolia* Benth.). II. Structure of baogongteng A – a new myotic agent. Yaoxue Xuebao 16:582–588
Yunusov SY, Shakirov TT, Plekhanova NV (1958) Alkaloids of *Convolvulus subhirsutus*. Doklady Akademii Nauk USSR 10:17–20
Zador E, Jones D (1986) The biosynthesis of a novel nicotine alkaloid in the trichomes of *Nicotiana stocktonii*. Plant Physiol 82:479–484
Zhang J, Xu M (1995) Synthesis and antispasmodic activity of pyrrolidine alkaloid derivatives. Zhongguo Yaowu Huaxue Zazhi 5:109–112
Zhaolou Y, Naijue Z, Renrong L, Shaopei C, Xiafei L, Zhujin L (1985) Crystal and molecular structure of ipohardine picrate, $C_{15}H_{16}NOxC_6H_2N_3O_7$. Jiegou Huaxue 4:152–155

4
Tryptophan-derived Alkaloids

For ipobscurines, hydroxycinnamic acid conjugates of serotonin (5-hydroxytryptamine), see Sect. 6.6.4; for withanamides, fatty acid amides of serotonin, see Sect. 8.1.2.1.

4.1 β-Carbolines

Simple tricyclic β-carboline alkaloids are not rare in the plant kingdom since their biosynthesis is close to the primary metabolism (condensation of L-tryptophan/tryptamine with low molecular aldehydes or ketones). They occur in several families. A famous example is that of the harmala alkaloids, e.g., harman, harmine, named after their discovery in *Peganum harmala* L. (Zygophyllaceae; Geraniales).

4.1.1 Occurrence in the Solanaceae (Fig. 4.1)

The first report on the occurrence of a β-carboline alkaloid from the family Solanaceae was made when Faini (1978) et al. discovered 1-acetyl-3-carbomethoxy-β-carboline as a constituent of the leaves of *Vestia foetida* (RUIZ & PAV.) HOFFM. sub nom. *Vestia lycioides* WILLD. The corresponding 3-carboxylic acid derivative could also be identified (Faini et al. 1980). From *Solanum jabrense* AGRA & M.NEE 1,2,3,4-tetrahydro-2-methyl-β-carboline was isolated (Sarmento da Silva et al. 2002). Furthermore, 1,2,3,4-tetrahydro-β-carbolin-3β-carboxylic acid, a simple derivative of L-tryptophan, whose first detection in the plant kingdom was achieved in the leaves of *Aleurites fordii* HEMSL. (Euphorbiaceae) sub nom. 3-carboxy-1,2,3,4-tetrahydro-β-carboline (Okuda et al. 1975), was also found to be a constituent of tomato fruits, *Solanum lycopersicum* L. sub nom. *Lycopersicon esculentum* MILL. Accordingly it was named lycoperodine-1 (Yahara et al. 2004). Due to their fluorescing properties unique tetracyclic alkaloids discovered in the seeds of *Datura stramonium* L. have been named fluorodaturatine and homofluorodaturatine (Robien et al. 1988).

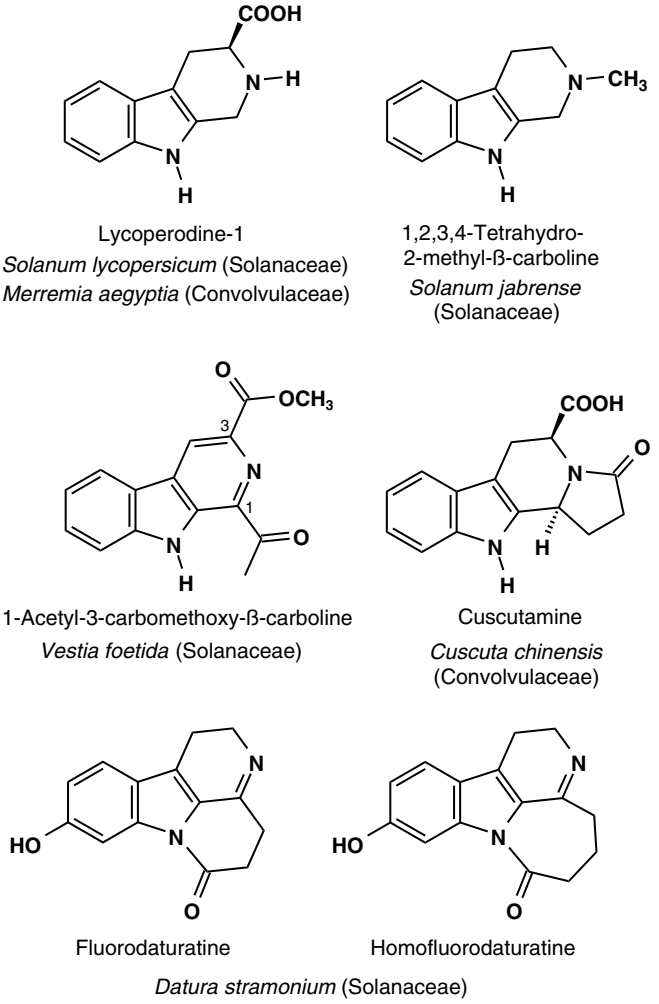


Fig. 4.1 β-Carboline alkaloids from both Solanales families

4.1.2 Occurrence in the Convolvulaceae (Fig. 4.1)

Minor amounts of lycoperodine-1 could be also isolated from the epigeal vegative parts of *Merremia aegyptia* (L.) Urb. (Henrici et al. 1995; Henrici 1996). Another β-carboline alkaloid named cuscutamine was discovered as a constituent of the fruits of *Cuscuta chinensis* Lam., a Chinese drug used as a tonic (Yahara et al. 1994). Finally, *nor*harman and harman were found by GC/MS analysis in the epigeal vegetative parts of *Merremia quinquefolia* (L.) Hall. f. (Mann 1997), *Argyreia hookeri* Clarke, *Ipomoea batatas* (L.) Lam. (Tofern 1999), *I. nil* (L.) Roth

(Schimming 2003), *Convolvulus altheoides* L., *C. subauriculatus* (BURCH.) LINDING., *Polymeria pusilla* R.BR., and *Lepistemon urceolatum* (R.BR.) F.MUELL. In the case of *I. cairica* (L.) SWEET these alkaloids have been detected not only in the aerial parts including the flower but also in the root and the rhizome (Eich and Witte, unpublished results). The structure of *nor*harman corresponds to 1-acetyl-3-carbomethoxy-β-carboline in Fig. 4.1 though without the substituents at C-1 and C-3 (harman is methylated at C-1).

4.2 Ergolines

4.2.1 Discovery and Structure

4.2.1.1 Occurrence in Fungi

Ergoline alkaloids (syn.: ergot alkaloids) are secondary natural metabolites which have been first identified as the toxic principle of ergot fungi (about 36 species of the genus *Claviceps*, Clavicipitaceae: Ascomycetes). They parasitize more than 600 monocotyledonous species of the families Poaceae, Juncaceae, and Cyperaceae (Tenberge 1999). The infection in nature is confined to host ovaries. The mature sclerotia of the fungi which develop epiphytically on the host within several weeks are characterized by the content of alkaloids. In 1917 ergotamine, the first crystalline compound of this type, had been isolated from the most important species, *Claviceps purpurea* (FR.) TUL., in the Sandoz laboratories/Basle, Switzerland (Stoll 1945). During the past nine decades ergotamine and its congeners as well as numerous part-synthetic derivatives have been developed as important pharmaceuticals (Hofmann 1964; Eich and Pertz 1994; Pertz and Eich 1999 and references therein).

Ergoline alkaloids are characterized by a tetracyclic skeleton; a minority, tricyclic analogs, however, can be considered as 6,7-*seco*ergolines. Meanwhile ergolines and *seco*ergolines, respectively, could be discovered in six additional *Claviceps* spp. and even in some other genera of the Clavicipitaceae (e.g., *Balansia, Epichloë*) (Pažoutová and Parbery 1999 and references therein). Beside these Hypocreales fungi some genera of the Eurotiales (e.g., *Aspergillus, Penicillium*) comprise many species producing ergoline alkaloids (Eich and Pertz 1994; Koslovsky1999 and references therein). Furthermore, fungi belonging to the Zygomycetes (e.g., *Cunninghamella blakesleana*) and even to the Basidiomycetes (e.g., *Corticium caeruleum*) turned out to be synthesizers of such compounds. Thus, ergoline alkaloids are typical products of the fungal secondary metabolism.

4.2.1.2 Discovery in the Convolvulaceae

In the early 1960s ergot alkaloids were also discovered in higher plants when Albert Hofmann was able to elucidate the hallucinogenic principle of the two

convolvulaceous Mexican Indio drugs "ololiuqui", the seeds of *Turbina corymbosa* (L.) RAF. sub nom. *Rivea corymbosa* (L.) H. HALL., and "badoh negro", the seeds of *Ipomoea tricolor* CAV. sub nom. *I. violacea* auct., non L. (Hofmann and Tscherter 1960; Hofmann 1961). This absolutely unexpected discovery received huge attention in the scientific community and consequently led to a lot of phytochemical studies with numerous different convolvulaceous species in order to find out if there are other ergoline alkaloid producing members of the family.

4.2.1.3 Structure

Ergot alkaloids are usually divided into three main structural types two of which include a lysergic acid amide moiety (Fig. 4.2):

(i) Ergopeptines with a rather complicated tricyclic tripeptidic cyclol joined to lysergic acid (e.g., ergotamine); compounds of this type are major alkaloids of *Claviceps purpurea*

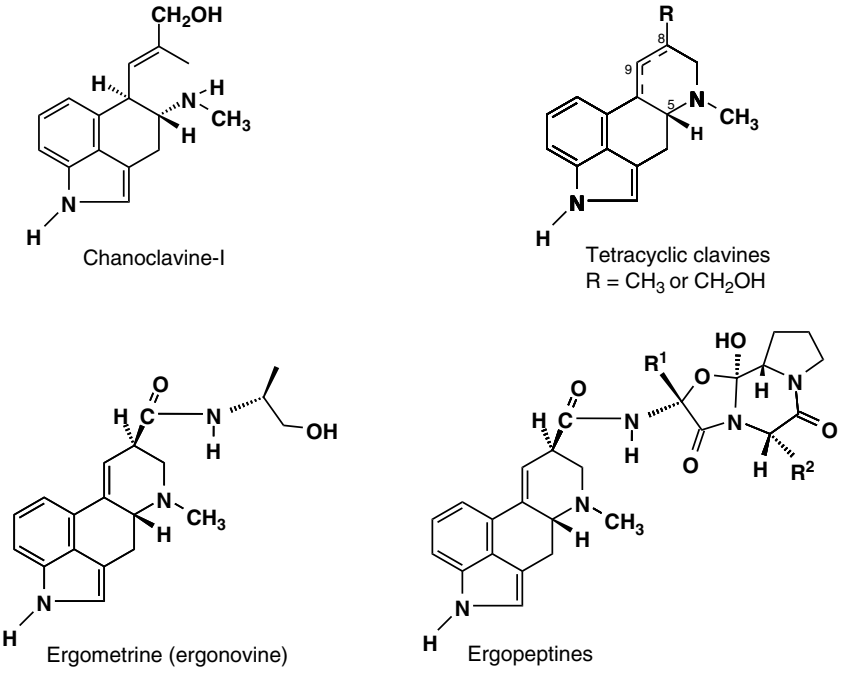


Fig. 4.2 Important basic structures of ergoline alkaloids (syn.: ergot alkaloids). Chanoclavine-I represents the tricyclic 6,7-*seco*ergolines; the three remaining structures show a common tetracyclic skeleton named ergoline. The clavines may possess alternatively a double bond between C-8 and C-9 or between C-9 and C-10; certain clavines may even lack such a double bond. Ergometrine (syn.: ergonovine, ergobasine) is an example for simple lysergic acid amides. Ergotamine as an example for the ergopeptines is the most famous ergot alkaloid (R^1 = methyl, R^2 = benzyl)

(ii) Simple lysergic acid amides with ammonia or low molecular amino alcohols joined to lysergic acid, e.g., ergine (lysergic acid amide), ergometrine; compounds of this type are minor alkaloids of *C. purpurea* but major alkaloids of most strains of *C. paspali* STEV. & HALL, a parasite of *Paspalum* grasses.

The names of those alkaloids which belong to these two types generally begin with "ergo. …" (with the exception of ergine). Since the hydrolysis of such lysergic amides, type (i) as well as type (ii), yields the free acid, this is called *lysergic* acid (named after "hydro*lys*is product of *erg*ot alkaloids") or, including its absolute configuration, 5*R*,8*R*-lysergic acid. While the configuration at C-5 is stable with the consequence that all natural ergoline compounds show a 5*R* configuration it is a common feature of all lysergic acid derivatives that they are unstable concerning the configuration at C-8. Thus, all these alkaloids are accompanied by their 5*R*, 8*S*-diastereomers already in the producing organism due to this chemical lability (keto-enol tautomerism) (Fig. 4.3). These C-8-epimeric ergopeptines, the derivatives of isolysergic acid (5*R*,8*S*-lysergic acid), are formed spontaneously and can be considered as artefacts. They are defined with the suffixal "-inine" instead of "-ine", e.g., ergotaminine vs ergotamine.

(iii) The third type of ergot alkaloids is characterized by a lower oxidation state of the substituent at *C*-8 (e.g., methyl, hydroxymethyl). Such compounds are called clavines. They can be divided into four sections:

- Compounds with a double bond in the 9,10 position (e.g., reduced derivatives of lysergic acid like lysergol)
- Compounds with a double bond in the 8,9 position (e.g., agroclavine, elymoclavine)
- Compounds without such a double bond (e.g., festuclavine, dihydrolysergol-I)
- Tricyclic 6,7-*seco*ergolines (e.g., chanoclavine-I)

Clavines are minor alkaloids of the mature sclerotium of *Claviceps purpurea*. However, agroclavine is the major alkaloid of a certain earlier developmental stage of this fungus; together with other clavines it is a biogenetic precursor of the

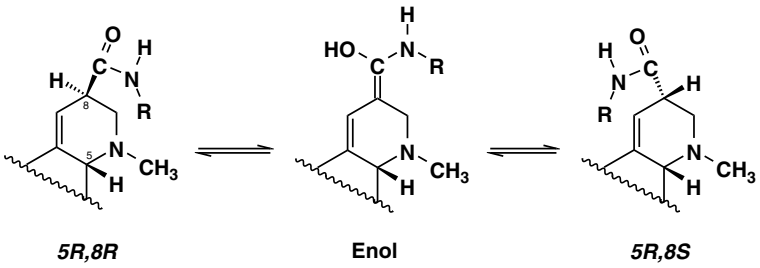


Fig. 4.3 *(5R,8R-)*Lysergic acid amide/*(5R,8S-)*isolysergic acid amide equilibrium in, e.g., aqueous solution due to keto-enol tautomerism

lysergic acid derivatives. Furthermore, clavines are accumulated as the major alkaloids of *C. fusiformis* LOVELESS, since this species is incapable of forming lysergic acid derivatives.

Surprisingly, members of all three structural types, i.e., ergopeptines, simple lysergic acid amides, and clavines turned out to be constituents of certain convolvulaceous species. Furthermore, this is also true for all four groups of clavines.

4.2.2 Biosynthesis

The complete knowledge about the biogenetic pathway of ergoline alkaloids is based on studies from the fungal genus *Claviceps* (Gröger and Floss 1998; Keller 1999 and references therein). There is only a single Convolvulaceae-specific report which is focussed on the question whether ergolines here are also derived from L-tryptophan and mevalonate, a precursor of dimethylallyldiphosphate. This could be proved by feeding experiments with radioactively-labelled compounds to *Ipomoea tricolor* CAV. sub nom. *I. rubro-caerulea* HOOK. (Gröger et al. 1963). The first pathway-specific step, catalyzed by the dimethylallyltryptophan synthase (substrates: L-tryptophan and dimethylallyldiphosphate), leads to 4-(γ,γ-dimethylallyl)tryptophan (DMAT) (Fig. 4.4). In a second step, DMAT is methylated at the basic *N*-6 catalyzed by DMAT *N*-methyltransferase. Changes of the oxidation states at one of the two geminal methyl groups as well as at C-5 and C-10 of the later ergoline nucleus, respectively, lead to decarboxylation and closure of the C ring. The product, chanoclavine-I, a 6,7-*seco*ergol-8-ene, is oxidized again yielding chanoclavine-I aldehyde.

4.2.2.1 Biogenetic Main Route

In the 1950s Rochelmeyer proposed the oxidative reaction sequence chanoclavine → agroclavine → elymoclavine → lysergic acid for the principal pathway in the biosynthesis of ergot alkaloids (Rochelmeyer 1958). However, some decades were needed to confirm this hypothesis and to elucidate the complete sequence in detail including the ergopeptines (Keller 1999 and references therein). Chanoclavine-I aldehyde, already mentioned above, is transformed to isochanoclavine-I aldehyde. Since chanoclavine-I aldehyde cyclase is able to convert both aldehydes into agroclavine, it can be assumed that this enzyme is bifunctional catalyzing the *cis-trans* isomerization as well as the D ring closure reaction. Agroclavine, the first tetracyclic ergoline alkaloid, is oxidized in a following pathway-specific step by agroclavine 17-hydroxylase to elymoclavine which is further oxidized by elymoclavine 17-monooxygenase to the corresponding carboxylic acid (Fig. 4.5). This alkaloid represents the $\Delta^{8,9}$-isomer of lysergic acid and is called paspalic acid because it has been isolated as a major constituent of a certain wild strain of *Claviceps paspali* which is unable to synthesize lysergic acid amides. It can be

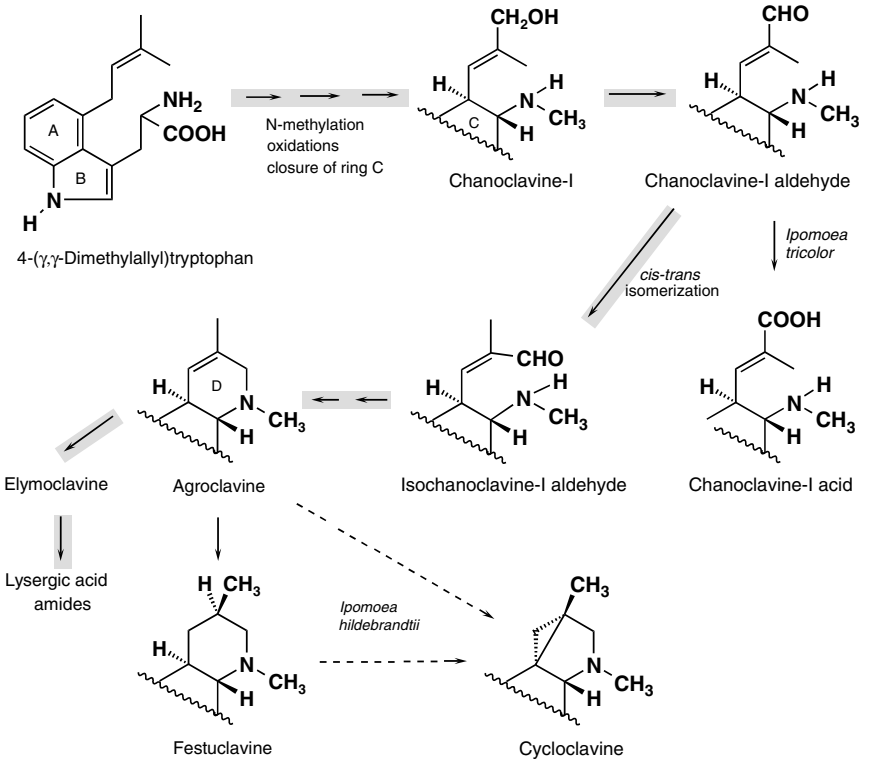


Fig. 4.4 Biosynthesis of ergoline alkaloids I. With the exception of chanoclavin-I acid and cycloclavine (specific convolvulaceous constituents not found in fungi) all alkaloids represent compounds occurring in both *Claviceps* spp. as well as in certain convolvulaceous species. *Grey arrows* indicate the principal pathway (main route), *regular arrows* side routes

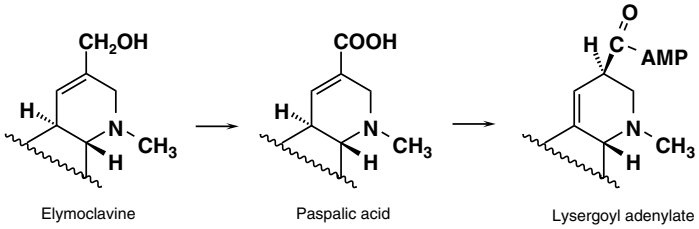


Fig. 4.5 Biosynthesis of ergoline alkaloids II. Paspalic acid as the key intermediate between clavine type and lysergic amide type ergoline alkaloids (oxidation of elymoclavine by elymoclavine 17-monooxygenase and isomerization of the resulting paspalic acid to activated lysergic acid)

considered as the last clavine-type ergoline in the pathway. The shift of the double bond from $\Delta^{8,9}$ to $\Delta^{9,10}$ is caused by the chemical property of paspalic acid to isomerize spontaneously forming 5*R*,8*R*-lysergic acid. Thus, elymoclavine 17-monooxygenase is a key enzyme in the biosynthesis of all lysergic acid amides,

since paspalic acid is necessary for supplying lysergic acid to the ergopeptine-synthesizing enzyme system including the simple lysergic acid amides. Paspalic acid is to be considered as a regular intermediate in the principal pathway. However, *Claviceps fusiformis*, the fungus with agroclavine and elymoclavine as major alkaloids and the incapability to synthesize any lysergic acid amide, is lacking elymoclavine 17-monooxygenase (Kim et al. 1983).

The ergopeptines like other oligopeptides are produced by non-ribosomal peptide synthesis well known as the *thiotemplate mechanism* (Keller 1999; Tudzynski et al. 2001; Haarmann et al. 2005 and references therein). The catalyzing agent is the multifunctional *d*-lysergyl peptide synthetase (LPS) which ought to be called correctly (5*R*,8*R*-)lyser*goyl* peptide synthetase since the prefix "*d*" is absolutely obsolete and lyser*gyl* is characterizing the 8-methylene-ergoline moiety ($R\text{-}CH_2$) and not the corresponding 8-carbonyl derivative (R-C=O) necessary here. The successive activation of lysergic acid by ATP to lysergoyl adenylate (Fig. 4.5) as well as of the prevailing three amino acids to their adenylates is followed by a covalent link to the SH group of the 4′-phosphopantetheine cofactors at different domains of the LPS for each of the four components. The stepwise attachment of the three amino acids to the lysergoyl residue proceeds to the corresponding linear lysergoyl tripeptide via the mono- and dipeptide (Fig. 4.6).

In vitro data have shown a lower substrate specificity of the LPS indicating that the spectrum of products formed in vivo is dependent on the actual concentrations of relevant amino acids in the cellular pool. In case of *Claviceps purpurea* usually L-alanine or L-valine are the dominating amino acids for the first amino acid associated intermediate whereas L-phenylalanine, L-leucine, L-isoleucine, or L-valine are candidates for the elongation step 1 leading to the lysergoyl dipeptide. The elongation step 2 joining the third amino acid to yield the linear lysergoyl tripeptidic intermediate always uses L-proline in case of *Claviceps purpurea* indicating a comparably high substrate specifity of the LPS for this step. The ensuing cyclization of the two final amino acids to the corresponding lactam leads to the product release from the LPS. A separate enzyme distinct from the LPS is converting the lysergoyl tripeptide lactam to the corresponding ergopeptine including the hydroxylation at the α-position of the first amino acid residue, e.g., L-alanine, and ensuing spontaneous cyclol formation. Ergopeptine-producing fungi contain multiple families of peptide synthetase genes. Though L-proline represents an invariable amino acid in the ergopeptines produced by *C. purpurea,* in case of certain *Balansia* spp. it is not (Powell et al. 1990), since an ergopeptine isolated from such fungi contains L-alanine instead of L-proline in the third position.

The biosynthesis of simple lysergic acid amides is not yet well understood. Ergometrine might be synthesized via lysergoyl-L-alanine (Fig. 4.7). In this case the amino acid is attached to lysergoyl adenylate by a specific peptide synthetase followed by an enzymatic reduction of the corresponding aldehyde released from the peptide synthetase. Lysergic acid α-hydroxy-ethylamide (syn.: lysergic acid methylcarbinolamide) could also be a product of lysergoyl-L-alanine converted by enzymatic modification after the release from the peptide synthetase. This alkaloid

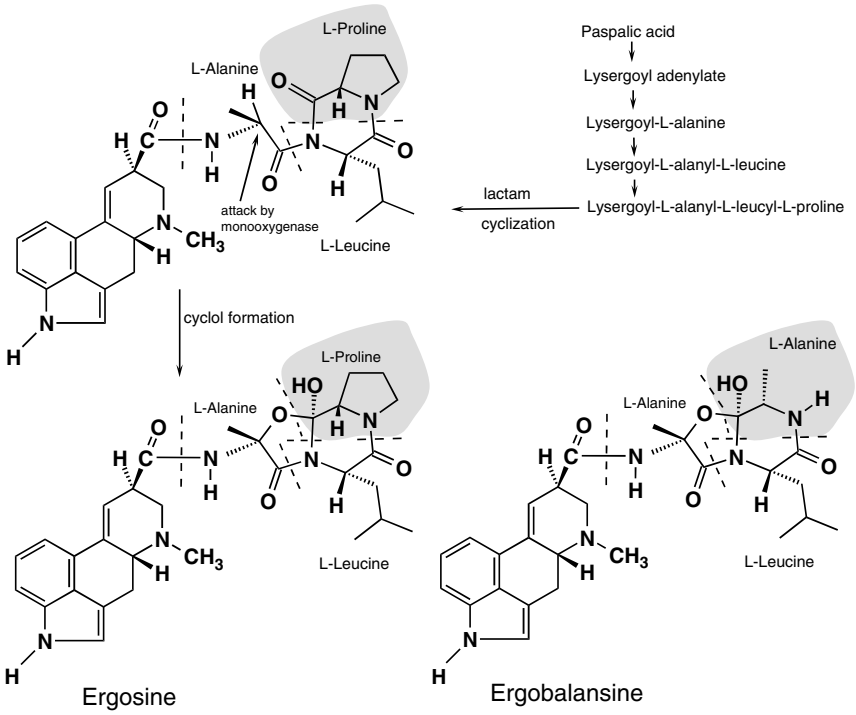


Fig. 4.6 Biosynthesis of ergoline alkaloids III. Formation of ergopeptines illustrated with the example of ergosine occurring in *Claviceps purpurea* and *Ipomoea argyrophylla*, respectively; ergobalansine, to date not found as a metabolite of *Claviceps* spp., is a proline-free (*highlighted in grey*) constituent of the fungal genus *Balansia* as well as of *Ipomoea asarifolia*

is not very stable showing a tendency to decompose spontaneously to ergine and acetaldehyde thus explaining the regular co-occurrence of both ergolines in biological material.

4.2.2.2 Other Interrelationships of Clavines

It is to be assumed that there are first side routes in the biogenetic pathway of ergolines already between the stage of *N*-methyl-DMAT and chanoclavine-I, since the biosynthesis of chanoclavine-II and *rac*. chanoclavine-II, respectively, should branch off here from the main route (Fig. 4.4). But this is not yet proved. Molliclavine is formed by hydroxylation of agroclavine at C-9 (Hofmann 1964), lysergol by shifting of the 8,9-double bond of elymoclavine into the 9,10-position. Apparently, festuclavine is a hydrogenation product of agroclavine, dihydrolysergol-I by analogy the corresponding one of elymoclavine and/or lysergol (Fig. 4.8). Hydroxylation of agroclavine leads to setoclavine and its 8-epimer isosetoclavine including a shift

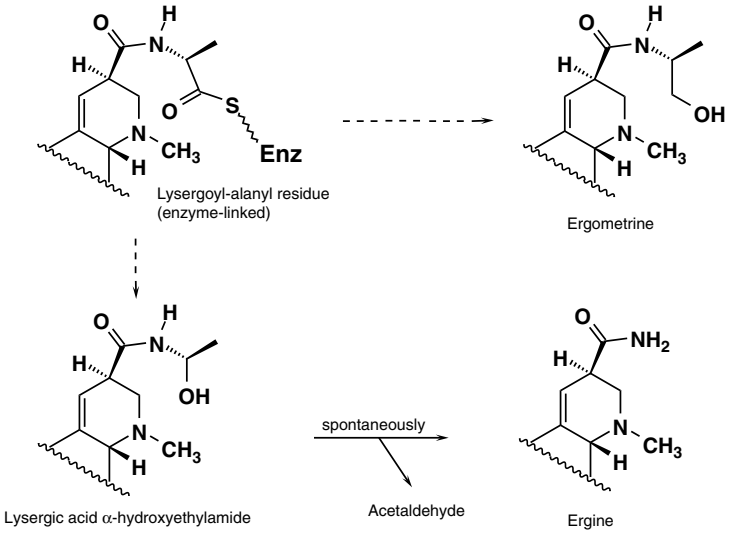


Fig. 4.7 Biosynthesis of ergoline alkaloids IV. Presumable formation of simple lysergic acid amides occurring in *Claviceps* spp. and certain convolvulaceous species; lysergoyl adenylate is transformed to an enzyme-linked lysergoyl-L-alanine by a peptide synthetase different to LPS; the low-molecular direct precursor of ergometrine released from the enzyme might be the corresponding aldehyde which in turn could be reduced in the last step; the homologous lysergic acid α-hydroxyethylamide might be produced in a similar manner

of the 8,9-position of the double bond into the 9,10-position catalyzed by peroxidases. The analogous reactions happen with elymoclavine yielding penniclavine and isopenniclavine. All these four 8-hydroxyergol-9-ene derivatives are not only formed by *Claviceps* spp. but after supplementation of agroclavine and elymoclavine, respectively, also by homogenates of other fungi or higher plants, e.g., tomato fruits, potato sprouts, morning glory seedlings (Tyler et al. 1965; Beliveau and Ramstad 1966), or by horseradish peroxidase (Taylor and Shough 1967; Chan Lin et al. 1967a, b; Shough and Taylor 1969).

4.2.3 Occurrence in the Convolvulaceae

The basic studies on the isolation of ergolines from the seeds of *Turbina corymbosa* and *Ipomoea tricolor*, respectively, already show that ergine (lysergic acid amide) including the 8-epimeric artefact erginine (isolysergic acid amide) represents the major alkaloid of both species with about 65% of the alkaloid fraction (Hofmann and Tscherter; 1960; Hofmann, 1961).

The spectacular discovery of the ergolines, typical fungal metabolites, in the seeds of two higher plants as well as the elucidation of these compounds as the

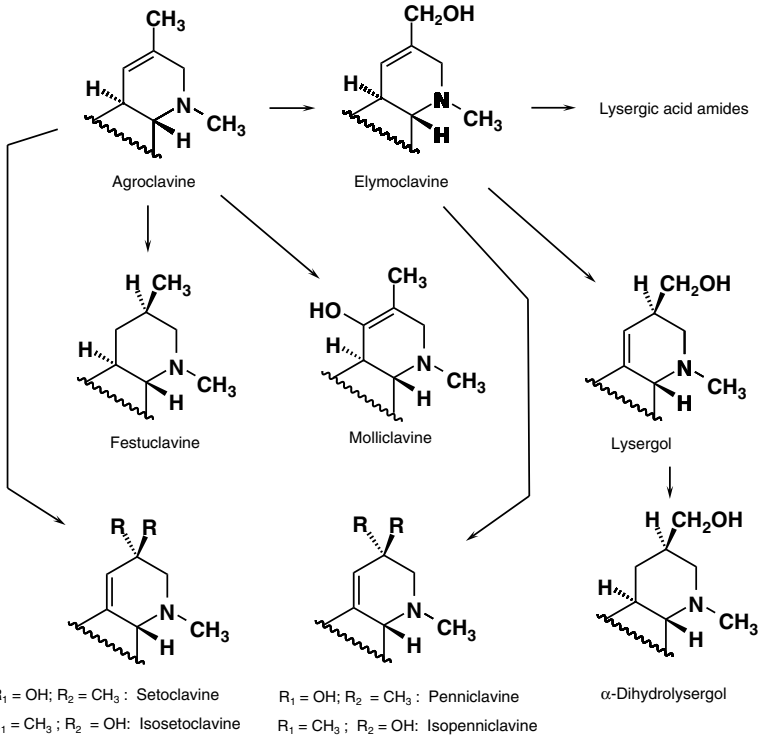


Fig. 4.8 Biosynthesis of ergoline alkaloids V. Relationships of those tetracyclic clavine alkaloids which could be detected in convolvulaceous species

psycho(to)mimetic principle of two Mexican magic drugs ("ololiuqui" and "badoh negro") from different genera led to the stimulation of numerous studies on further members of the Convolvulaceae family. Indeed, unambiguous evidence of ergoline alkaloids as constituents of further species could be provided. Unfortunately this large number of reports on ergolines in this family in general and in particular within the genus *Ipomoea* has caused considerable problems unique in phytochemistry. Beside excellent publications also studies of limited quality and confusing results have been published. This huge genus comprising about 650 species (Austin and Huáman 1996) with a comparably large availability in many parts of the world tempted also groups not experienced in ergoline analyses and with limited knowledge in botanic systematics to look for these spectacular compounds rather than smaller genera with much less available species. Thus, altogether 79 *Ipomoea* spp. have been analysed for the presence of these alkaloids. Beside 23 unambiguously ergoline-positive species there are apparently contradictory results for 15 other species at least causing considerable doubts on the occurrence of such alkaloids in those species for one reason or another. The remaining 41 species turned out to be apparently devoid of ergoline alkaloids.

What are the reasons for the contradictory results?

- The botanical identity of the plant material used has not always been properly verified causing misidentifications, e.g., *I. purpurea* (L.) ROTH (Table 4.2) which has been mistaken for *I. tricolor* CAV. (Amor-Prats and Harborne 1993a). Many reports are lacking sufficient and correct details concerning the species, e.g., the species authorities, or are leading to innocent misrepresentations. Such a confusing example resulting in numerous false repetitions in studies of other authors has happened already in the first ergoline paper on *Ipomoea tricolor* CAV. whose seeds are known as "badoh negro": Together with this correct synonym the species was incorrectly called *I. violacea* L. (Hofmann 1964) instead of *I. violacea* **auct., non L.** This is of importance since *I. violacea* L. is the currently accepted name of a different *Ipomoea* species, *I. tuba* (SCHLECHT.) G.DON (Austin and Huáman 1996). Unfortunately, in other cases the authority name following the species term has been omitted. Moreover, different misrepresentations apparently result from horticulturally used but scientifically invalid names of plants.
- The methods used in the extraction, detection, and quantification of the ergoline alkaloids have varied; thus, there is some uncertainty regarding the results obtained (Amor-Prats and Harborne 1993a). There are a lot of reports with insufficient analytical methods and details. For example, it is not acceptable that a species is already considered as ergoline-positive based only on a colour reaction of spots on the thin-layer chromatogram (TLC) (qualitative analysis) lacking comparison with any reference sample or by a colorimetric determination (quantitative analysis). Usually van Urk's reagent, a modified Ehrlich's reagent, is used for these purposes (Mayer and Eich 1975). The main component is 4-dimethylaminobenzaldehyde which is reacting under strong acidic conditions and the addition of $FeCl_3$ with ergolines (blue to blue-violet colour reactions, green in case of certain clavines). But this reagent is not a specific one. The reagent is indicating any compound with an 1,2-unsubstituted indole moiety showing colour reactions also with, e.g., tryptophan, tryptamine, indole acetic acid, or similar auxins. Moreover, many other false-positive reactions (with regard to ergolines) are possible caused by compounds of very different structures, e.g., pyrrolizidine alkaloids, benzofuranes, terpenoids. Thus, care must be taken in the application of this test and the interpretations of the results obtained.

Therefore, an up-date of the first critical view on the ergolines as constituents of the genus given some 20 years ago (Amor-Prats and Harborne 1993a) seems to be necessary.

4.2.3.1 Unambiguously Ergoline-positive *Ipomoea* Species (Table 4.1)

As already mentioned, at present only 23 *Ipomoea* spp. are to be considered as ergoline-positive without any doubt. Surprisingly, the alkaloid profiles of these species are apparently very similar. Seventeen species show a characteristic profile of

Table 4.1 Unambiguously ergoline-positive *Ipomoea* species

Ipomoea L.	**Alkaloids identified in the seeds** (TLC: comparison with authentic samples)[a]	**References**
I. amnicola MORONG.	Clavines: **7**	Amor-Prats and Harborne 1993a
I. argillicola R.W. JOHNSON	Clavines: **1**, **4**, **7** Lysergic acid amides: **14**, **16**	Amor-Prats and Harborne 1993a (TLC); Eich and Witte, unpublished results (GC/MS)
I. aristolochiifolia G.DON	Instead of TLC: HPLC Clavines: **1**, **7**, **8** Lysergic acid amides: **16**	McDonald 1982
I. argyrophylla VATKE (syn.: *I. jaegeri* PILGER)	Clavines: **4** Lysergic acid amides: **17** Epigeal vegetative parts (TLC and GC/MS): Clavines: **1**, **4** Lysergic acid amides: **14**	Isolation and structural elucidation of **4** and **17** from the seeds: Stauffacher et al. 1965. Epigeal vegetative parts: Eich and Witte, unpublished results
I. asarifolia (DESR). R. & SCH.	Clavines: **1, 7** Lysergic acid amides: **14–16**, ergobalansine All of these alkaloids also present in the epigeal vegetative parts	Isolation and structural elucidation of **1**, **14**, **15** and ergobalansine from the seeds: Jenett-Siems et al. 1994. Jirawongse et al. 1977; Kucht et al. 2004; Steiner et al. 2006
I. cardiophylla A. GRAY	Instead of TLC: HPLC Clavines: **1**, **7**, **8** Lysergic acid amides: **14**, **16**	McDonald 1982
I. costata F. MUELL. ex BENTH.	Clavines: **8**	Amor-Prats and Harborne 1993a
I. diamantinensis J.M. BLACK	Clavines: **7**, **8**	Amor-Prats and Harborne 1993a
I. dumetorum ROEM. & SCHULT.	Instead of TLC: HPLC Lysergic acid amides: **14**, **16**	McDonald 1982
I. hildebrandtii VATKE	Clavines: **5**, cycloclavine Epigeal vegetative parts (GC/MS): Clavines: **4**, **5, 7** Lysergic acid amides: **14**	Isolation and structural elucidation of **5** and cycloclavine from the the seeds: Stauffacher et al. 1969. Epigeal vegetative parts: Eich and Witte, unpublished results
I. imperati (VAHL) GRISEB. sub nom. *I. stolonifera* J.F. GMEL.	Clavines: **1**, **8** Lysergic acid amides: **14**	Jenett-Siems 1996 [b]

(continued)

Table 4.1 Unambiguously ergoline-positive *Ipomoea* species (continued)

Ipomoea L.	**Alkaloids identified in the seeds** (TLC: comparison with authentic samples)[a]	**References**
I. jujujensis O'Donell	Clavines: **1**, **4**, **7** Lysergic acid amides: **14**	Eich, unpublished results
I. leptophylla Torr.	Clavines: **1** Lysergic acid amides: **14**, **16**	DerMarderosian 1967a; Chao and DerMarderosian 1973b
I. marginisepala O'Donell	Instead of TLC: HPLC Clavines: **1**, **7**, **8** Lysergic acid amides: **14**, **16**	McDonald 1982
I. minutiflora (M.Martens & Galeotti) House	Instead of TLC: HPLC Clavines: **1**, **7**, **8** Lysergic acid amides: **14**	McDonald 1982
I. muelleri Benth.	Clavines: **1–13**, isopenniclavine Lysergic acid amides: **14–16**	Isolation and structural elucidation of **1** and **14**: DerMarderosian et al. 1974. Gardiner et al. 1965; Amor-Prats and Harborne 1993a; Eich and Witte, unpublished results
I. orizabensis (Pell.) Led. ex Steudl.	Clavines: **7**	Perez-Amador et al. 1980; Amor-Prats and Harborne 1993a
I. parasitica (H.B.K.) G.Don.	Clavines: **1**, **7**, **8** Lysergic acid amides: **14**, **16**	McDonald 1982 (HPLC), 1991; Amor-Prats and Harborne 1993a, 1993b
I. pedicellaris Benth.	Instead of TLC: HPLC Clavines: **1**, **7**, **8** Lysergic acid amides: **14**, **16**	McDonald 1982
I. pes-caprae (L.) R.Br.	Three unidentified ergolines	Banerjee and Bhatnagar 1974; Amor-Prats and Harborne 1993a
I. pes-caprae (L.) R.Br. ssp. *pes-caprae* Ooststr.	Clavines: **1** Lysergic acid amides: **14**, **16**	Mann 1997
I. pes-caprae (L.) R.Br. ssp. *brasiliensis* (L.) Ooststr.	Clavines: **1, 7** Lysergic acid amides: **14**, **16**, "ergotamine" (see text)	Isolation and structural elucidation of **1**: Kayser 1994. Jirawongse et al. 1977; Henrici 1996
I. phyllomega (Vell.) House	Clavines: **1**, **4**, **7** Lysergic acid amides: **14**	Eich, unpublished results
I. setifera Poir.	TLC and GC/MS: Clavines: **1**, **4**, **7** Lysergic acid amides: **14**	DerMarderosian 1967a; Schimming 2003 (GC/MS); Eich, unpublished results

(continued)

Table 4.1 Unambiguously ergoline-positive *Ipomoea* species (continued)

Ipomoea L.	**Alkaloids identified in the seeds** (TLC: comparison with authentic samples)[a]	**References**
	All of these alkaloids also present in the epigeal vegetative parts	
I. tricolor Cav. (syn.: *I. violacea* auct., non L.; *I. rubro-caerulea* Hook.) seeds: "badoh negro"	TLC and GC/MS: Clavines: **1–5, 7–9, 12,** chanoclavine-I acid Lysergic acid amides: **14–16**	Isolation and structural elucidation of **1, 7, 14,** and chanoclavine-I acid: Hofmann and Tscherter 1960; Hofmann 1961; Choong and Shough 1977. Gröger 1963; Taber et al. 1963a; DerMarderosian et al. 1964; Genest 1965; DerMarderosian and Youngken 1966; Chao and DerMarderosian 1973b; McDonald 1982; Hahn 1990; Eich and Witte, unpublished results (GC/MS)

[a] Since *5R,8S*-epimers of *5R,8R*-lysergic acid derivatives are artefacts their occurrence is not mentioned beside the natural alkaloid, e.g., only ergometrine, not ergometrinine

[b] *I. stolonifera* **Poirs**: no presence of ergolines in seeds and leaves (Jirawongse et al. 1977)

Key to the compounds

1 = chanoclavine-I
2 = chanoclavine-II
3 = *rac.* chanoclavine-II
4 = agroclavine
5 = festuclavine
6 = dihydrolysergol-I (α-dihydrolysergol)
7 = elymoclavine
8 = lysergol
9 = isolysergol
10 = setoclavine
11 = isosetoclavine
12 = penniclavine
13 = molliclavine
14 = ergine (lysergic acid amide)
15 = lysergic acid α-hydroxyethylamide
16 = ergometrine (ergonovine)
17 = ergosine

certain clavines and simple lysergic acid amides. The biogenetic route leading to lysergic acid derivatives suggests that these clavines in principal must be produced in every species in which lysergic acid amides are detectable. Thus, the detection of ergine and ergometrine in the case of *I. dumetorum* must include the production of these three true natural precursors although they were not detected in the respective analysis. On the other hand, elymoclavine (or its isomer lysergol) is the only alkaloid identified in the remaining four species suggesting that these species do not show the total main alkaloid profile known from the other 19 species. Indeed a more simple profile might be given genetically. However, it should be taken into

account that in these four species additional TLC spots of apparent ergoline alkaloids have been observed which could not be identified due to the lack of authentic samples (Amor-Prats and Harborne 1993a). Thus, the TLC analysis of *I. argillicola* had only shown elymoclavine beside two further unidentified ergolines in the study Amor-Prats and Harborne. The GC/MS analysis, a more sensitive method, used in a reinvestigation study led to the identification of chanoclavine-I, agroclavine, elymoclavine, ergine, and ergometrine (Eich and Witte, unpublished results). Thus, the "more simple chromatographic profile" (Amor-Prats and Harborne 1993a) of certain *Ipomoea* spp. might be only a question of the analytical sensitivities of the methods used, i.e., of a more or less excellent detection limit. Moreover, the most outstanding advantage of the GC/MS analysis is that this method includes the capability to characterize the identity of every compound without any doubts on the basis of gas chromatography (retention time) and mass spectrum (parent peak, characteristic base and fragmentation peaks).These properties cause the superiority of GC/MS over TLC or HPLC.

Beside the main route precursors the other clavines found in different species are products of side routes also derived from:

- Chanoclavine-I, e.g., chanoclavine-I acid (Fig. 4.4)
- Agroclavine, e.g., festuclavine, setoclavine, isosetoclavine (Fig. 4.8)
- Elymoclavine, e.g., lysergol, dihydrolysergol-I, penniclavine, isopenniclavine (Fig. 4.8)

These minor alkaloids have been detected especially in two species studied with particular intensity, i.e., *I. muelleri* Benth. and *I. tricolor* Cav. (Table 4.1).

Not more than two ergopeptines have been isolated and structurally elucidated unequivocally not only from an *Ipomoea* species but from a convolvulaceous species at all:

- Ergosine, which is also a minor constituent of *Claviceps purpurea* (Fig. 4.6), from the seeds of *Ipomoea argyrophylla* Vatke (Stauffacher et al. 1965)
- Ergobalansine/-inine (Fig. 4.6), a unique proline-free ergoline which is also a constituent of the fungal genus *Balansia* but has never been found in the genus *Claviceps,* from the seeds of *Ipomoea asarifolia* (Desr.) R. & Sch. (Jenett-Siems et al. 1994)

The surprising discovery of ergobalansine in a convolvulaceous species has consequences: in all those cases in which "ergosine" (Tables 4.1 and 4.4) or "ergotamine" (Table 4.1) have been characterized only by TLC comparison with an authentic sample (e.g., Chao and DerMarderosian 1973b; Banerjee and Bhatnagar 1974; Wilkinson et al. 1986, 1987, 1988), reinvestigations seem to be necessary because these two proline-containing ergopeptines and its proline-free congener ergobalansine show very similar chromatographic behaviour (R_f values) with the usual sorbents. Thus, many ergopeptine-positive results are not doubtful concerning the occurrence of this principal type of alkaloids at all but concerning the individual compounds. If ergopeptines are reported to be constituents of an

Ipomoea species or of any species of another convolvulaceous genus, especially *Argyreia* (see Table 4.4), these compounds are always minor metabolites. This might be the reason why they could not be detected in other ergoline containing species due to the lack of sufficient seed material. On the other hand, it might be that there are species which do not contain ergopeptines at all though being capable to synthesize clavines and simple lysergic acid amides. At least, this is the case within the fungal genus *Claviceps* since *C. paspali* in contrast to *C. purpurea* does not produce ergopeptines though being able to yield both other types of ergoline alkaloids.

As a matter of fact, the major alkaloids of mature ergot from *C. purpurea*, the ergopeptines ergotamine and the ergotoxine group (ergocristine, α-ergokryptine, β-ergokryptine, ergocornine), could not be detected in the Convolvulaceae. On the other hand, it is fascinating that almost all ergoline alkaloids ever detected in convolvulaceous species had been already known from fungal genera. Only two ergoline alkaloids are confined to the Convolvulaceae family both discovered in an *Ipomoea* species:

- Cycloclavine, an isomer of agroclavine as well as a dehydrogenated derivative of festuclavine (Fig. 4.4), a unique metabolite of *Ipomoea hildebrandtii* Vatke (Stauffacher et al. 1969)
- Chanoclavine-I acid, a unique metabolite of *Ipomoea tricolor* Cav. (Choong and Shough 1977)

It can be concluded that the qualitative profile of ergoline alkaloid containing *Ipomoea* spp. is more or less identical with simple lysergic acid amides as major components and clavines as minor congeners. The only outstanding exception is represented by *I. hildebrandtii*. This species shows in its epigeal vegetative parts – in addition to the normal profile of clavines and simple lysergic acid amides – several novel unidentified clavines which could be characterized concerning their GC/MS data (Eich and Witte, unpublished results). However, the exact chemical structure of these novel clavines remains to be elucidated.

4.2.3.2 Contradictory Reports on the Occurrence of Ergoline Alkaloids in the Seeds of *Ipomoea* species (Table 4.2)

Another group of *Ipomoea* species involves 15 species for which there are single positive reports concerning the occurrence of ergoline alkaloids. On the other hand, more convincing negative results have been reported for all of these species. The reasons for these doubtful cases have been already mentioned above. Moreover, several of these species meanwhile are characterized as synthesizers of two different alkaloid types: pyrrolizidines (*I. coccinea*, *I. hederifolia*, *I. lobata*, *I. quamoclit*, *I.* × *sloteri*; see Sect. 3.7) and indolizidines (*I. alba*, *I. turbinata*; see Sect. 3.6). These findings may explain additionally certain errors of the past.

4.2.3.3 *Ipomoea* Species Apparently Devoid of Ergoline Alkaloids in the Seeds (Table 4.3)

Table 4.3 summarizes 41 *Ipomoea* spp. for which only ergoline-negative reports have been published. Negative results are also important from the chemotaxonomic point of view. Tables 4.1–4.3 altogether include 79 out of about 650 species of this largest convolvulaceous genus. Twenty-three species only were found to be ergoline-positive (~30%).

4.2.3.4 Ergoline-positive *Argyreia* species (Table 4.4)

The ergoline pattern of positive *Argyreia* spp. is very similar to the one found for *Ipomoea* spp. (Chao and DerMarderosian 1973b). Again ergine and chanoclavine-I turned out to be the major alkaloids. It is remarkable that an ergopeptine could be detected in five out of 14 positive *Argyreia* spp. This has been characterized as ergosine by TLC comparison with an authentic sample. However, due to reasons already explained above (see *Ipomoea*) it remains doubtful, if this compound has been really ergosine rather than a similar ergopeptine such as ergobalansine.

The Hawaiian baby wood rose, *Argyreia nervosa* (Burm. f.) Bojer, is the species most studied in the genus. Chemical analyses showed that the seeds contain the highest percentage of ergoline constituents (0.5–0.9%) of all positive convolvulaceous species. Although the pericarp had shown the same alkaloid pattern as the seeds, the concentration was much lower (0.0015%). No alkaloids could be detected in the epigeal vegetative parts. The latter fact is also true for *A. mollis*. This is surprising since – in contrast to that – ergolines could be detected not only in the seeds but also in the epigeal vegetative parts of different *Ipomoea* spp. (Table 4.1) and *Stictocardia tiliaefolia* (Table 4.5), respectively. Another clavine-type alkaloid, lysergene (6-methyl-8-methylene-ergol-9-ene), not present in other convolvulaceous genera, could be identified in *A. cuneata* and *A. nervosa* (Table 4.4). Again this metabolite, a dehydration product of lysergol, was already known from *Claviceps* spp. (Hofmann 1964). The paleotropic genus *Argyreia* comprises about 100 spp. The percentage of species found to be ergoline-positive was higher than in the case of *Ipomoea* (14 out of 20 species checked).

4.2.3.5 Ergoline-positive *Stictocardia* and *Turbina* species (Table 4.5)

The paleotropic genus *Stictocardia* comprises only 11 species (Austin and Eich 2001). Four members, *S. beraviensis*, *S. tiliaefolia*, *S.* cf. *laxiflora*, and *S. mojangensis* have been screened for ergolines. With the exception of the latter species the analyses of the seeds yielded the typical clavines and simple lysergic acid amides of convolvulaceous species but no ergopeptines. The epigeal vegetative parts of the

Table 4.2 Contradictory reports on the occurrence of ergoline alkaloids in the seeds of *Ipomoea* species

***Ipomoea* L.**	**Ergoline-positive reports**	**Ergoline-negative reports**	**Evaluation by the author of this book**
I. aquatica FORSK.	Nair et al. 1987	Jirawongse et al. 1977; Amor-Prats and Harborne 1993a; Tofern 1999; Schimming 2003	Predominantly more convincing negative reports; in one of these reports: epigeal vegetative material also negative (TLC and GC/MS)
I. cairica (L.) SWEET (syn.: *I. palmata* FORSK.)[a]	Sharda and Kokate 1979; Nair et al. 1987	Jirawongse et al. 1977; Odebiyi and Sofowora 1978; Amor-Prats and Harborne 1993a; Kayser 1994; Schimming 2003	Like *I. aquatica*
I. carnea ssp. *fistulosa* (MART. ex CHOISY) D.F. AUSTIN	Banerjee and Bhatnagar 1974; Umar et al. 1980 (leaves)	Jirawongse et al. 1977; Perez-Amador et al. 1980; Amor-Prats and Harborne 1993a; Weigl 1992; Mann 1997 (epigeal vegetative parts); Schimming 2003	Though there is even a report on the isolation and unambiguous structural elucidation of festuclavine and dihydrolysergol-I (= α-dihydrolysergol) from the leaves (Umar et al. 1980), there are many negative reports including TLC and GC/MS of the leaves. A misidentification of the species analysed by Umar et al. might be an explanation[b]
I. coccinea L. (syn.: *Quamoclit coccinea* MOENCH)[a]	Gröger 1963; Perez-Amador et al. 1980; Wilkinson et al. 1987	Beyerman et al. 1963; DerMarderosian 1964; Genest and Sahasrabudhe 1966; Amor-Prats and Harborne 1993a; Jenett-Siems et al. 2005	Predominantly more convincing negative reports; epigeal vegetative material was also negative (TLC and GC/MS); real alkaloidal constituents: pyrrolizidines (see Sect. 3.7)
I. hederacea (L.) JACQ.	Wilkinson et al. 1986	Beyerman et al. 1963; DerMarderosian 1964; Amor-Prats and Harborne 1993a; Eich, unpublished results	Like *I. aquatica*

(continued)

Table 4.2 Contradictory reports on the occurrence of ergoline alkaloids in the seeds of *Ipomoea* species (continued)

***Ipomoea* L.**	**Ergoline-positive reports**	**Ergoline-negative reports**	**Evaluation by the author of this book**
I. hederifolia L. [syn.: *I. angulata* LAM.; *Quamoclit angulata* (LAM.) BOJER]	Nair et al. 1987; Wilkinson et al. 1987	DerMarderosian and Youngken 1966; DerMarderosian 1967b; Amor-Prats and Harborne 1993a; Jenett-Siems 1996; Jenett-Siems et al. 1993, 1998, 2005	Like *I. coccinea*
I. lacunosa L.	Wilkinson et al. 1986		No negative report available; nevertheless doubtful since the majority of the other species reported to be ergoline-positive in the papers of these authors (Wilkinson et al. 1986, 1987, 1988) are evaluated predominantly as ergoline-negative in this table
I. lobata (CERV.) THELL. [syn.: *Quamoclit lobata* (CERV.) HOUSE, *Mina lobata* CERV.]	Wilkinson et al. 1988	Jenett-Siems et al. 1999, 2005	Predominantly more convincing ergoline-negative reports also including epigeal vegetative material (TLC and GC/MS); real alkaloidal constituents: pyrrolizidines (see Sect. 3.7)
I. nil (L.) ROTH [syn.: *Pharbitis nil* (L.) CHOISY]	Nair et al. 1987;	DerMarderosian 1964, 1967b; DerMarderosian and Youngken 1966; Genest 1965; Genest and Sahasrabudhe 1966; Amor-Prats and Harborne 1993a; Schimming 2003	Predominantly more convincing ergoline-negative reports, sometimes even with more than one provenance or cultivar (e.g., 5 cultivars negative in one report); epigeal vegetative material also negative (TLC and GC/MS)
I. purpurea (L.) ROTH	Taber et al. 1963a; Hylin and Watson 1965; Nikolin and Nikolin 1971; Wilkinson et al. 1986	Beyerman and van de Linde 1963; DerMarderosian 1964, 1967b; DerMarderosian and Youngken 1966; Genest 1965; Genest and Sahasrabudhe 1966; Hahn 1990; Amor-Prats and Harborne 1993a; Schimming 2003	Like *I. nil*

Table 4.2 Contradictory reports on the occurrence of ergoline alkaloids in the seeds of *Ipomoea* species (continued)

***Ipomoea* L.**	**Ergoline-positive reports**	**Ergoline-negative reports**	**Evaluation by the author of this book**
I. quamoclit L (syn.: *Quamoclit vulgaris* CHOISY)[a]	Wilkinson et al. 1987	Beyerman and van de Linde 1963; DerMarderosian and Youngken 1966; DerMarderosian 1967b; Banerjee and Bhatnagar 1974; Amor-Prats and Harborne 1993a; Jenett-Siems et al. 2005	Like *I. coccinea*
I. × *sloteri* (HOUSE) OOSTSTR. (syn.: *Quamoclit sloteri* HOUSE)[a]	Wilkinson et al. 1988	DerMarderosian and Youngken 1966; DerMarderosian 1967b; Jenett-Siems et al. 2005	Like *I. coccinea*
I. trichocarpa ELL. var. *torreyana* (GRAY) SHINNERS	Wilkinson et al. 1986		Like *I. lacunosa*
I. turbinata LAG. [syn.: *Calonyction muricatum* (L.) G. DON][a]	Banerjee and Bhatnagar 1974; Nair et al. 1987; Wilkinson et al. 1988	DerMarderosian and Youngken 1966; DerMarderosian 1967b; Tofern 1999	Predominantly more convincing ergoline-negative reports, one of them includes different provenances and also epigeal vegetative material (TLC and GC/MS); real alkaloidal constituents: indolizidines (see Sect. 3.6)
I. wrightii GRAY	Wilkinson et al. 1987		Like *I. lacunosa*

[a] The species synonym was used in the corresponding ergoline-positive original report

[b] It cannot be ruled out that ergoline-positive and ergoline-negative forms of the same species exist but this is not yet proved for any convolvulaceous species (see Sect. 4.2.4)

Table 4.3 *Ipomoea* species apparently devoid of ergoline alkaloids

	Absence of ergolines[a]			
Ipomoea L.	**Seeds**	**EVP[b]**	**Roots**	**References**
I. alba L.	x	x	x	DerMarderosian and Youngken 1966; DerMarderosian 1967b; Tofern 1999
I. adenoides Schinz.	x	n.d.	n.d.	Amor-Prats and Harborne 1993a
I. albivenia (Lindl.) Sweet	x	n.d.	n.d.	Amor-Prats and Harborne 1993a
I. arborescens Sweet	x	x	x	DerMarderosian and Youngken 1966; Eich, unpublished results
I. batatas Lamk.	x	x	x	Jirawongse et al. 1977; Tofern 1999
I. batatoides Choisy	x	x	n.d.	Eich, unpublished results
I. bonariensis Hook.	x	x	n.d.	Eich, unpublished results
I. bracteata Cav.	x	n.d.	n.d.	Amor-Prats and Harborne 1993a
I. chloroneura Hall. f.	x	n.d.	n.d.	Amor-Prats and Harborne 1993a
I. coptica (L.) Roem. & Schult.	x	x	n.d.	Amor-Prats and Harborne 1993a; Eich and Witte, unpublished results
I. coscinosperma Hochst. ex Choisy	x	n.d.	n.d.	Amor-Prats and Harborne 1993a
I. cynanchifolia Meissn.	x	n.d.	n.d.	Amor-Prats and Harborne 1993a
I. eremnobrocha D.F. Austin	x	n.d.	x	Tofern 1999
I. eriocarpa R. Br.	x	n.d.	n.d.	Amor-Prats and Harborne 1993a; Eich, unpublished results
I. gracilisepala Rendle	x	n.d.	n.d.	Amor-Prats and Harborne 1993a
I. graminea R. Br.	x	n.d.	n.d.	Amor-Prats and Harborne 1993a
I. hochstetteri House	x	n.d.	n.d.	Amor-Prats and Harborne 1993a (2 provenances)
I. indica Burm.	x	x	n.d.	Amor-Prats and Harborne 1993a (2 provenances); Schimming 2003 (2 provenances)
I. involucrata Beauv.	x	x	n.d.	Weigl 1992; Schimming 2003
I. lindheimeri A. Gray	x	n.d.	n.d.	Der Marderosian 1964; DerMarderosian and Youngken 1966; Genest and Sahasrabudhe 1966; Amor-Prats and Harborne 1993a
I. mauritania Jacq. (syn.: *I. digitata* L.)	x	x	n.d.	Jirawongse et al. 1977; Schimming 2003
I. meyeri (Spreng.) G. Don	x	x	x	Tofern 1999
I. microsepala Benth.	x	n.d.	n.d.	McDonald 1982; Amor-Prats and Harborne 1993a

(continued)

Table 4.3 *Ipomoea* species apparently devoid of ergoline alkaloids (continued)

Ipomoea L.	Absence of ergolines[a]			References
	Seeds	EVP[b]	Roots	
I. mirandina (PITTIER) O'DONELL	x	n.d.	n.d.	Eich, unpublished results
I. murucoides ROEM. & SCHULT.	x	n.d.	n.d.	Amor-Prats and Harborne 1993a
I. obscura (L.) KER-GAWL.	x	x	x	Jirawongse et al. 1977; Weigl 1992; Amor-Prats and Harborne 1993a; Schimming 2003
I. pedatisecta MART. & GAWL.	x	n.d.	n.d.	Amor-Prats and Harborne 1993a
I. pilosa[c]	x	n.d.	n.d.	Banerjee and Bhatnagar 1974
I. plebeia R.BR.	x	x	x	Amor-Prats and Harborne 1993a (2 provenances); Schimming 2003
I. ramosissima CHOISY	x	x	x	Amor-Prats and Harborne, 1993a; Schimming 2003
I. regnellii MEISN.	x	x	x	Mann 1997
I. reptans POIRS	x	x	n.d.	Jirawongse et al. 1977
I. reticulata O'DONELL	x	x	x	Tofern 1999
I. rubens CHOISY	x	x	x	Weigl 1992
I. sepiaria KOEN. ex ROXB. (syn.: *I. maxima* G. DON)	x	x	x	DerMarderosain and Youngken 1966; Schimming 2003
I. setosa KER-GAWL.	x	n.d.	n.d.	Eich, unpublished results
I. shirambensis BAK.	x	x	n.d.	Amor-Prats and Harborne 1993a; Jenett-Siems and Eich, unpublished results
I. triloba L.	x	x	x	DerMarderosian and Youngken 1966; Kayser 1994; Eich, unpublished results
I. verbascoidea CHOISY	x	n.d.	n.d.	Amor-Prats and Harborne 1993a
I. violacea L.[d] [syn.: *I. macrantha* ROEM. & SCHULTES; *I. tuba* (SCHLTDL.) G.DON]	x	x	n.d.	Tofern 1999
I. wightii (WALL.) CHOISY	x	x	n.d.	Amor-Prats and Harborne 1993a; Schimming 2003

[a] Checked by comparison with authentic samples by means of thin-layer chromatography (TLC) and/or quantitative colorimetric analyses with van Urk's reagent; × = absent; n.d. = not determined

[b] EVP = epigeal vegetative parts

[c] No species authority in the original report; thus, a correct assignment is impossible since three authors are available: CAV. vs. HOUTT vs. SWEET

[d] Not to be mixed up with *I. violacea* auct., non L. (syn.: *I. tricolor* CAV.)

Table 4.4 Unambiguously ergoline-positive *Argyreia* species

***Argyreia* Lour.**	**Alkaloids identified in the seeds** (TLC: comparison with authentic samples)[a]	**References**
A. acuta Lour.[b]	Clavines: **1** Lysergic acid amides: **14**, **16**	DerMarderosian 1967a; Chao and DerMarderosian 1973b
A. barnesii (Merr.) Ooststr.	Clavines: **1 – 3**, **5**, **7**, **9** Lysergic acid amides: **14 – 16**	DerMarderosian 1967a; Chao and DerMarderosian 1973b
A. cuneata Ker-Gawl.	Clavines: **1 – 12**	Chao and DerMarderosian 1973b
A. hainanensis[c]	Clavines: **1** Lysergic acid amides: **14, 16**	DerMarderosian 1967a; Chao and DerMarderosian 1973b
A. hookeri C.B.Clarke	Clavines: **1**, **7** Lysergic acid amides: **14**	Eich, unpublished results
A. luzonensis (Hall. f.) Ooststr.	Clavines: **1 – 5**, **7 – 9**, **12** Lysergic acid amides: **17**	Chao and DerMarderosian 1973b
A. mollis (Burm. f.) Choisy	Clavines: **1 – 5**, **7**, **9**, **12** Lysergic acid amides: **17**	Chao and DerMarderosian 1973b; epigeal vegetative parts and roots: negative, Tofern et al. 1999
A. nervosa (Burm. f.) Bojer	Clavines: **1 – 13**; in addition to TLC also infrared spectra (IR) for **4 – 7**, **10**, **11** Lysergic acid amides: **14**, in addition to TLC also infrared spectrum (IR)	Isolation and structure elucidation of **14**: Miller 1970. Hylin and Watson 1965; DerMarderosian 1967a; McJunkins et al. 1968; Chao and DerMarderosian 1973a,b; epigeal vegetative parts and roots: negative, Tofern et al. 1999
A. obtusifolia Lour.	Clavines: **1 – 3**, **5**, **7**, **12** Lysergic acid amides: **14 – 17**	DerMarderosian 1967a; Chao and DerMarderosian 1973b
A. philippinensis (Merr.) Ooststr.	Clavines: **1**, **3**, **5**, **8**, **9**, **12** Lysergic acid amides: **14 – 17**	Chao and DerMarderosian 1973b
A. ridleyi (Prain) Prain ex Ooststr.	Lysergic acid amides: **17**	Chao and DerMarderosian 1973b
A. rubicunda Choisy	Clavines: **8**	Chao and DerMarderosian 1973b
A. splendens (Hornem.) Sweet	Clavines: **1**, **2**, **5**, **7**, **8** Lysergic acid amides: **17**	DerMarderosian 1967a; Chao and DerMarderosian 1973b
A. wallichii Choisy	Clavines: **1**, **5**, **9**, Lysergic acid amides: **14**, **16**	DerMarderosian 1967a; Chao and DerMarderosian 1973b

[a] Since *5R,8S*-epimers of *5R,8R*-lysergic acid derivatives are artefacts their occurrence is not mentioned beside the natural alkaloid, e.g., only ergometrine, not ergometrinine

[b] Species authority given in the original report is not correct.

(continued)

Table 4.4 Unambiguously ergoline-positive *Argyreia* species (continued)

[c] Species authority neither given in both original reports nor to be found in the data base of the International Plant Name Index (IPNI; Kew Botanical Gardens et al.) and the w3TROPICOS Nomenclatural Database (Missouri Botanical Gardens), respectively

Key to the compounds:

The key is identical to the one of Table 4.1 except compound **6** (dihydrolysergol-I has not been detected in the genus *Argyreia*; instead lysergene has got this number here)

1 = chanoclavine-I
2 = chanoclavine-II
3 = *rac*. chanoclavine-II
4 = agroclavine
5 = festuclavine
6 = lysergene
7 = elymoclavine
8 = lysergol
9 = isolysergol
10 = setoclavine
11 = isosetoclavine
12 = penniclavine
13 = molliclavine
14 = ergine (lysergic acid amide)
15 = lysergic acid α-hydroxyethylamide
16 = ergometrine (ergonovine)
17 = ergosine

first two species turned out to contain a similar pattern; whether this is also true for the third remains unknown. Only two species of the pantropical genus *Turbina* (15 spp.) have been checked for the presence of alkaloids in the seeds. Beside the famous, originally neotropical, woody liana *T. corymbosa* (seeds: ololiuqui) with meanwhile pantropical distribution the again neotropical *T. abutiloides* is also ergoline-positive. In both species the alkaloid profile is a typical convolvulaceous one: certain clavines and simple lysergic acid amides; no ergopeptines have been detected. Like in the case of those *Argyreia* spp. whose seeds have turned out to contain ergolines, these alkaloids were not found in the epigeal vegetative parts of *T. abutiloides*.

4.2.3.6 Intrafamilial Distribution and Chemotaxonomic Significance

The unambiguous occurrence of ergoline alkaloids in the Convolvulaceae family is confined to the traditional genera *Argyreia*, *Ipomoea*, *Stictocardia*, and *Turbina*, respectively. These four genera belong to the Ipomoeeae, the most advanced convolvulaceous tribe (Stefanovic et al. 2002, 2003). Since the other 11 tribes of the family as well as the sister family, the Solanaceae, are lacking such metabolites these apomorphic characters are of important chemotaxonomic significance though only on this higher taxon level.

The infrageneric distribution within *Ipomoea* is of a limited value from the chemotaxonomic point of view. There are ergoline-positive species in four out of seven traditionally accepted subgenera (Verdcourt 1963; Austin and Huáman 1996). In the case of **sub**genus *Ipomoea* they are confined to one section out of two, in the case of subgenus *Quamoclit* to three sections out of six. Within the subgenus *Eriospermum* comprising most of the known ergoline-positive species (12) these are distributed between two out of three traditional sections:

Table 4.5 Unambiguously ergoline-positive *Stictocardia* and *Turbina* species

	Alkaloids identified in the seeds (TLC: comparison with authentic samples)[a]	**References**
***Stictocardia* Hall. f.**		
S. beraviensis (Vatke) Hall. f.	Clavines: **1**, 7 Lysergic amides: **14**, **15**	Eich, unpublished results
S. tiliaefolia (Desr.) Hall. f. [syn.: *S. campanulata* (L.) Merr.]	Clavines: **1**, **2**, **6** – **9**, **12**, isopenniclavine Lysergic acid amides: **14**, **16**	Isolation and structural elucidation of **8** from epigeal vegetative parts: Schimming 2003. DerMarderosian 1967a; Chao and DerMarderosian 1973b; Lee et al. 1979 [b]
S. cf. *laxiflora* (Baker) Hall. f.	Clavines: **1**, **7** Lysergic acid amides: **14**, **15**	Eich, unpublished results
***Turbina* Raf.**		
T. abutiloides (H.B.K.) O'Donell	Clavines: **1**, **4**, **7** Lysergic acid amides **14** – **16**	Eich, unpublished results; epigeal vegetative parts and roots: negative, Mann 1997
T. corymbosa (L.) Raf. [syn.: *Rivea corymbosa* (L.) Hall. f.]	Clavines: **1**, **7**, **12** Lysergic acid amides: **14**, **16**	Isolation and structural elucidation of **1**, **7**, **14:** Hofmann and Tscherter 1960; Hofmann 1961.
seeds: "ololiuqui"		Taber et al. 1962**;** 1963b; DerMarderosian et al. 1964; Genest 1965; Genest and Sahasrabudhe 1966; DerMarderosian and Youngken 1966 (3 provenances); DerMarderosian 1967a and references therein

[a] Since *5R,8S*-epimers of *5R,8R*-lysergic acid derivatives are artefacts their occurrence is not mentioned beside the natural alkaloid, e.g., only ergometrine, not ergometrinine

[b] Identification of all ergolines by isolation and comparison with authentic samples by means of two dimensional thin-layer chromatography (2D-TLC)

Key to the compounds:

The key is identical to the one of Table 4.1; however, only compounds **in bold** have been detected in the genera *Stictocardia* and/or *Turbina*

1 = chanoclavine-I
2 = chanoclavine-II
3 = *rac*. chanoclavine-II
4 = agroclavine
5 = festuclavine
6 = dihydrolysergol-I (α-dihydrolysergol)
7 = elymoclavine
8 = lysergol
9 = isolysergol
10 = setoclavine
11 = isosetoclavine
12 = penniclavine
13 = molliclavine
14 = ergine (lysergic acid amide)
15 = lysergic acid α-hydroxyethylamide
16 = ergometrine (ergonovine)
17 = ergosine

- Subgenus *Ipomoea*
  - sect. *Pharbitis* (CHOISY) GRISEB.
    I. orizabensis, I. setifera
- Subgenus *Quamoclit* (MOENCH) HALL. f.
  - sect. *Exogonium*
    I. dumetorum
  - [sect.] *"Microsepalae"*
    I. minutiflora
  - sect. *Tricolores* D.F. AUSTIN
    I. aristolochiifolia, I. cardiophylla, I. marginisepala, I. parasitica, I. tricolor
- Subgenus *Eriospermum* HALL. f.
  - sect. *Eriospermum*
    I. amnicola, I. costata, I. jujujensis, I. leptophylla, I. pedicillaris, I. phyllomega
  - sect. *Erpipomoea* CHOISY
    I. argillicola, I. asarifolia, I. diamantinensis, I. imperati, I. muelleri, I. pes-caprae
- Subgenus *Poliothamnus* (HALL. f.) VERDC. (no sections)

  I. argyrophylla, I. hildebrandtii

However, there are also ergoline-*negative* species even within these sections. Moreover, it should be realized again that the vast majority of *Ipomoea* species are not yet checked for the presence of these alkaloids including all species of the two smaller African subgenera *Dasychaetia* HALL. f. and *Xerophyta* BAK. & RENDLE.

It should be added that the traditional sections *Erpipomoea* and *Eriospermum* turned out to be not monophyletic in molecular phylogenetic analyses (Miller et al. 1999; Manos et al. 2001). A well-supported monophyletic clade shown in Fig. 3.32 comprises four members of the traditional section *Erpipomoea* and two members of the traditional section *Eriospermum*. Exactly this clade is characterized by the occurrence of ergoline alkaloids thus supporting the molecular data (Stech et al. 2007).

Most species of the remaining monotypic to oligotypic genera of the tribe Ipomoeeae are also not checked for the presence of ergoline alkaloids, i.e., *Astripomoea* MEEUSE, *Paralepistemon* LEJOLY & LISOWSKI, *Lepistemon* BL., and *Rivea* CHOISY, with the exception of *A. malvacea* (KLOTZSCH) MEEUSE, *L. binectariferum* (WALL.) O.K., and *L. urceolatum* (R.BR.) F.v.M. These three species turned out to be ergoline-negative (Eich and Witte, unpublished results).

In this connection it is interesting to realize that a phylogenetic analysis of *Ipomoea*, *Argyreia*, *Stictocardia*, and *Turbina* based on certain DNA sequences has supported the supposed paraphyly of *Ipomoea* (Manos et al. 2001). Two major clades within *Ipomoea* s.l. were resolved: One clade (named clade 1 by the authors) with most of the smaller segregate genera (*Argyreia, Lepistemon, Rivea, Stictocardia,* and *Turbina*) interspersed with predominantly paleotropical species

of *Ipomoea* s.s., the other one (named clade 2) with neotropical species of *Ipomoea* s.s. and the small paleotropical genus *Astripomoea*. Furthermore, *Rivea* was nested within the *Argyreia* subclade and *Turbina* turned out to be polyphyletic. Interestingly, there are ergoline-positive species in both major clades:

(1) *Argyreia osyrensis, A. splendens, A. nervosa, I. imperati* sub nom. *I. jaegeri, Stictocardia tiliaefolia, Turbina corymbosa*
(2) *I. amnicola, I. leptophylla, I. pes-caprae, I. tricolor*

As already mentioned above, there is no unequivocal evidence that ergolines occur in other convolvulaceous species outside the tribe Ipomoeeae. There are different reports on the alleged discovery of ergolines by TLC comparison in certain species of the following genera:

- *Calystegia*, e.g., *C. sepium* (L.) R.Br. (Tuttobello et al. 1971)
- *Convolvulus*, e.g., *C. tricolor* L. (Taber et al. 1963a); *C. arvensis* L. (Tuttobello et al. 1971), *C. major* (Banerjee and Bhatnagar 1974)
- *Cuscuta*, e.g., *C. monogyna* Vahl (Ikan et al. 1968; see also below), *C. europea* L. (Tuttobello et al. 1971); *C. chinensis* Lamk. (Nair et al. 1987)
- *Jacquemontia*, e.g., *J. paniculata* (Burm. f.) Hall. f. (Nair et al. 1987); *J. tamnifolia* (L.) Griseb. (Wilkinson et al. 1988)
- *Merremia*, e.g., *M. aegyptia* (L.) Urban. sub nom. *Operculina aegyptia* (L.) House (Perez Amador et al. 1980), *M. quinquefolia* (L.) Hall. f. (Nair et al. 1987), *M. cissoides* (Lam.) Hall. f. (Perez Amador et al. 1988)

But this could never be verified in any case by corresponding evaluations (e.g., Beyerman et al. 1963; DerMarderosian and Youngken 1966; Genest and Sahasrabudhe 1966; DerMarderosian 1967b; Jirawongse et al. 1977; Hahn 1990). This is also true for *M. tuberosa* (L.) Rendle sub nom. *I. tuberosa* L. (Hylin and Watson 1965; McJunkins et al. 1968). Moreover, many years of phytochemical studies on some 160 species out of 29 genera did not reveal any ergoline-positive taxon outside the four genera mentioned above (Trumm 1990; Weigl 1992; Kayser 1994; Jenett-Siems 1996; Henrici 1996; Mann 1997; Tofern 1999; Schimming 2003; Eich and Witte, unpublished results). Even the only example for an isolation and structure elucidation of an ergoline alkaloid (agroclavine) from the seeds of such an alleged ergoline-producing species outside the Ipomoeeae, *Cuscuta monogyna* Vahl (Ikan et al. 1968), is more than doubtful. The authors have described their isolation procedure as follows: "After development (of TLC) was complete, the UV fluorescent band was scraped of the plate and extracted with chloroform, centrifuged and concentrated. The agroclavine content was 0.015%." However, it is a matter of fact that agroclavine – in contrast to lysergic acid amides – does not show any UV fluorescence. This doubtful report stimulated another author to repeat this investigation; the find could not be confirmed and led to a reappraisal (Mantle 1972).

On the other hand, a large number of **non**-convolvulaceous seeds showed negative results in a screening program (TLC, van Urk's reagent) (Taber et al. 1963a) supporting for the first the uniqueness of ergolines as metabolites in higher plants.

However, three decades later the presence of some alkaloids in the roots of the African tree *Securidaca longipedunculata* Fres (Polygalaceae) has been reported which were characterized as elymoclavine, "(5,10)-dehydroelymoclavine" and "a new ergoline alkaloid" with an additional aromatic E ring by mass spectrometry only (Costa et al. 1992; Scandola et al. 1994). Unfortunately, these exciting reports have never been supported by further structure elucidation procedures on the part of these authors. Thus, this occurrence remains doubtful. An independent confirmation of this finding with a complete structure elucidation would be extremely desirable (Eich and Pertz 1994; Gröger and Floss 1998).

4.2.4 Location and Origin of Ergoline Alkaloids

The sensational discovery that the seeds of certain higher plants show exactly the same constituents which were already known from fungi in view of the uniqueness of this find immediately led to the question, whether these ergoline alkaloids are actually produced by plant tissue or by fungi or bacteria present in the seeds (Taber and Heacock 1962). This was not inconceivable since seed-coat-borne fungi had been well-known and fungi can infect the ovary like *Claviceps* spp. in grasses. Indeed, fungi and bacteria were found to be present in the seed coat of *Turbina corymbosa* but not in the embryo of surface-sterilized seeds. However, on the other hand ergolines were found to be present in the embryo but not in the seed coat, in the resinous layer adjacent to the seed coat, or in the membranes located centrally in the seed. Both the hypocotyls and cotyledons contained ergolines. *Claviceps* spp. were not detected in the seeds. From all these data the authors concluded: "It was considered reasonably certain that the alkaloids are a true metabolic product of the plant and not of an invading microbial parasite or contaminant" (Taber et al. 1963a). Furthermore, it had been claimed that callus and cell suspension cultures of *Ipomoea tricolor*, *Turbina corymbosa*, and *Argyreia nervosa*, believed to be sterile concerning microorganisms, have shown production of ergolines (Dobberstein and Staba 1969). The results of these two reports have established for decades the scientifically accepted opinion that ergolines are original convolvulaceous metabolites.

Early studies again with *T. corymbosa* (Taber et al. 1963b) and *Ipomoea tricolor* Cav. sub nom. *I. rubro-caerulea* Hook. (Gröger 1963) had shown that these alkaloids are not only constituents of the seeds; they could also be detected in the epigeal vegetative parts of these species but not in the roots. This could be confirmed later for several other *Ipomoea* spp. (Table 4.1) and *Stictocardia tiliifolia* (Table 4.5) but remarkably not for *Argyreia* spp. (Table 4.4) and *T. abutiloides* (Table 4.5). Grafting experiments with, e.g., *I. tricolor* in 1973 showed that leaves are the principal sites of ergoline alkaloid biosynthesis which are translocated afterwards to the seeds where they are accumulated (Mockaitis et al. 1973). By this study it has been proved that neither roots nor stems and seeds are the site of alkaloid formation. Strangely enough, these results did not stimulate any experimental

study with the aim to find out if there are any fungal endophytes in ergoline-positive Convolvulaceae spp. in the following decades.

Nevertheless, the evolutionary relationship of ergoline biosynthesis in fungi and higher plants has been discussed over the years (e.g., Boyes-Korkis and Floss 1992). Theoretically there are three principal possibilities:

1. The genetic information has been developed twice independently in unrelated taxa during evolution
2. It evolved only once and then has been passed from fungi to higher plants in a horizontal gene transfer or – less probably – even vice versa ("biogenetic engineering")
3. Endophytic ergoline-producing fungi may be associated with higher plants

Taking into account the unusual and very complex biosynthesis of ergolines with its numerous enzymatically catalyzed steps resulting in almost congruent alkaloid patterns of such different types of organisms the first possibility seems to be rather unlikely. Genetic comparison studies between ergoline-positive and -negative convolvulaceous species, respectively, have not been published. Thus, there is no evidence for the second possibility. However, the third possibility was discovered in the 1970s for a non-convolvulaceous higher plant: Bacon et al. reported the first conclusive evidence of the presence of an endophytic fungus, *Epichloë typhina* (Fr.) Tul., in tall fescue, *Festuca arundinacea* Schreb. (Poaceae) (Bacon et al. 1977). This fungus has been later identified as *Acremonium coenophialum* Morgan-Jones & Gams (Morgan-Jones and Gams 1982), meanwhile re-classified to *Neotyphodium coenophialum* (Glenn et al. 1996). The new form genus *Neotyphodium* is the name for the anamorphic, asexual state of the genus *Epichloë*. Such *Acremonium/Neotyphodium* grass endophytes are taxonomically aligned with the family Clavicipitaceae and consequently with the genus *Claviceps* but they live or spend their entire life cycle within the aerial portion of their grass host (Bacon and DeBatista 1991). Thus, it was not very surprising that ergoline alkaloids belong to the major toxins associated with *Acremonium/Neotyphodium*-infected grasses. All types of ergoline alkaloids described above for certain Convolvulaceae spp. are also present here with lysergic acid α-hydroxyethylamide / ergine and the ergopeptine ergovaline as the major components (Porter 1995). The structure of ergovaline is very similar to ergosine (difference: R^2 in Figs. 4.2/4.6 = isopropyl instead of isobutyl). Such "ergoline-accompanied" infections with *Acremonium/Neotyphodium* endophytes are not limited to *Festuca* spp.: *Stipa robusta* Scribn., sleepygrass (Petrosky et al. 1992), and *Lolium perenne* L., perennial ryegrass (Porter 1995), are further examples among many others.

The so-called graminicolous Clavicipitaceae are obligate parasites of grasses and sedges, e.g., species of *Claviceps* Tul., *Balansia* Speg., *Epichloë* (Fr.) Tul. The grass endophytes are all members of the family Clavicipitaceae (White 1997), asymptomatic, systemic fungi that occur intercellularly within the leaves, stems, and reproductive organs of grasses. These microorganisms have dramatic effects on the physiology, ecology, and reproductive biology of their host plants. Through the production of toxic alkaloids, endophytic fungi defend their hosts

against a wide range of insect and mammalian herbivores (Clay 1990). Poisoning of domestic livestock had spurred a great deal of research on endophytic fungi in pasture grasses (see below). This led to the early discovery of the causal link between endophytes and toxins like ergolines in grasses. It could be confirmed that these alkaloids are also formed in saprophytic submerged cultures of the fungi in the absence of host tissues.

From *Cenchrus echinatus* L., sandbur grass, infected with the fungus *Balansia obtecta* DIEHL, the unusual ergopeptine alkaloid ergobalansine (Fig. 4.6) has been isolated (Powell et al. 1990) which could also be discovered in a non-poaceous higher plant. This surprising find prompted the authors to title their report "Ergobalansine, a proline-free peptide-type alkaloid of the fungal genus *Balansia*, is a constituent of *Ipomoea asarifolia*" (Jenett-Siems et al. 1994).

Taking into account all these results, the question arises if – in contrast to the traditional scientific view – Convolvulaceae of the tribe Ipomoeeae are playing perhaps the host role for ergoline-producing fungi within the tricolpate angiosperms which is given by the Poaceae within the monocots. This could be supported indirectly by the again very surprising discovery of loline alkaloids in the epigeal vegetative parts and roots of *Argyreia mollis* (BURM. f.) CHOISY (Tofern et al. 1999; see Sect. 5.2.3) which accumulates ergoline alkaloids in the seeds (Table 4.4). Fascinatingly sometimes these lolines even occur – just like in case of *A. mollis* – together with ergoline alkaloids in endophyte-infected grasses, e.g., *Festuca arundinacea* infected with *Acremonium/Neotyphodium coenophalium*. Therefore, the authors of the report on *Argyreia mollis* came to the cautious conclusion "Although it seems unlikely that endophytic fungi are involved in loline and ergoline formation in *Argyreia*, we cannot exclude this possibility by sure".

Amazingly enough, the first study in which it has been checked if there is any evidence for the presence of ergoline-producing endophytic fungi in the leaves, has not been published until 30 years after the discovery that the leaves are the site of alkaloid biosynthesis. Recently, it has been reported that the treatment of plants cultivated from seeds of exactly the same provenance of *Ipomoea asarifolia*, which had led to the surprising identification of ergobalansine (Jenett-Siems et al. 1994), with two fungicides eliminated the production of alkaloids (Kucht et al. 2004). This find strongly suggested that ergolines are metabolic products of an associated fungus rather than of the plant itself just like in certain grasses. Microscopic examination of the upper leaf surface after staining with aniline blue or wheat germ agglutinin revealed fungal hyphae closely associated with secretory glands in a very characteristic way. These hyphae were present when alkaloids were detectable; those plants treated with certain fungicides did not show hyphae and alkaloids, respectively. From these results the authors concluded that this particular, for the first not yet identified plant-associated fungus may be involved in the biosynthesis of ergoline alkaloids.

The results of this study contrasted starkly with those of another one already mentioned above, which had been published in the late 1960s (Dobberstein and Staba 1969). Therefore, Kucht et al. established again callus and cell suspension cultures of the three ergoline-positive convolvulaceous species applied in the study of 1969.

Even with the very sensitive analytical method they used (capillary electrophoresis) they were not able to detect any trace of ergoline alkaloids in such cultures. They criticized the analytical procedures of the former study (non-specifity of van Urk's reagent, problematics of TLC comparison) and reported that they also occasionally observed van-Urk-positive spots. But these have never been ergolines.

Further studies of the same group (Steiner et al. 2006) elucidated the following facts:

- Isolation of a new **epi**biotic fungus, designated *IasaF13*, from the upper leaf surface of *Ipomoea asarifolia* beside 12 **endo**phytic fungi
- Characterization of *IasaF13* as the only clavicipitaceous fungus out of all 13 fungi
- *IasaF13* groups together with known ergoline-producing clavicipitaceous fungi such as *Claviceps* spp. and *Balansia* spp. in phylogenetic trees based on 18S rDNA and ITS (internal transcribed spacer) data sets
- *IasaF13* turned out to be the only fungus responsible for the ergoline alkaloid accumulation in both in vitro and in vivo cultivation of *I. asarifolia*
- *IasaF13* was seed transmitted
- *IasaF13* carried the gene (*dmaW*) responsible for the prenylation of tryptophan, the first pathway-specific step of ergoline alkaloid biosynthesis; *dmaW* sequences showed very high similarity to a *Balansia obtecta* (Clavicipitaceae) homologue
- Attempts to grow *IasaF13* on 15 different agar media designed for fungal growth were even unsuccessful, when the media contained leaf homogenates of *I. asarifolia*
- The inability was demonstrated to establish *IasaF13* on *I. asarifolia* spraying onto or injecting into the leaves; even attachment of leaf surfaces from a normal plant and a plant devoid of fungi failed
- *IasaF13* was spread to the shoot of the plant during growth

An epibiotic fungus isolated from another convolvulaceous species, *Turbina corymbosa*, showed sequences of 18S rDNA and ITS which were 100% identical to those of the one from *I. asarifolia*. This was also roughly true for the alkaloid profile. There are no doubts that the nonculturable epibiotic clavicipitaceous fungus *IasaF13* is responsible for the production of ergoline alkaloids at least in these two convolvulaceous species. Future investigations involving further ergoline-positive convolvulaceous species are necessary to clarify whether it can be generalized that *IasaF13*-identical or -similar plant-associated fungi are responsible for the production of such alkaloids.

The results of Kucht et al. (2004) and Steiner et al. (2006) provide a possible explanation for an early study on the transformation of elymoclavine by *Ipomoea tricolor* Cav. sub nom. *I. rubro-caerulea* Hook. (Gröger 1963). This alkaloid, ^{14}C-labelled, has been applied to living leaves in feeding experiments. However, no ergolines of the main biosynthetic route (simple lysergic acid amides or ergopeptines) have been found. Elymoclavine was rapidly degraded to unknown non-ergoline products. Only small amounts of penniclavine, a product which is

formed from elymoclavine by unspecific peroxidases (see above), could be detected. The applied alkaloid probably did not meet the location of the fungal endophyte ergoline-producing system. Otherwise products of the main ergoline biosynthetic route should have been found.

Ergolines Accumulated "by" Convolvulaceae vs Poaceae. Though future studies are necessary on further ergoline-positive convolvulaceous species, there is a striking, apparent comparability concerning the origin of ergoline alkaloids as constituents of certain monocots (e.g., Poaceae) and certain tricolpate angiosperms (Convolvulaceae). In case of grasses the endophytic fungus is transmitted only through seed of an already infected mother plant and, thus, is an inherent, maternally transmitted component of a joint plant-fungus lineage (symbiotum) (Schardl 1996; Schardl et al. 2004 and references therein). During flowering endophytes grow into ovules and become incorporated into seeds. They do not occur in roots although they can be found in rhizomes and stolons of their hosts (Clay 1990). All this also seems to be true for infected Convolvulaceae species. In contrast to the Poaceae (endophytic fungi, e.g., certain *Balansia* spp. or epiphytic fungi, e.g., *Claviceps* spp.) the epiphytic fungus of *I. asarifolia* was not culturable without its host. This shows that the fungus (and with it the production of ergolines) "….. depends on the plant for growth and vegetative reproduction and that there is a highly specific interaction between both organisms. It is remarkable that the fungus never spread to *I. asarifolia* plants devoid of fungus and alkaloids although the plant carrying the fungus was kept in the immediate neighbourhood in the same green house." Though it might be assumed for both families that certain primary metabolites synthesized by the host may contribute to the fungal synthesis of ergoline alkaloids, two unique ergolines discovered in *I. hildebrandtii* (cycloclavine) and *I. tricolor* (chanoclavine-I acid), respectively, might be an evidence for contributions even in the secondary metabolism (assumed that these two species also have been fungus-associated). To the best of the author's knowledge such "exotic" ergolines are not known from the Poaceae.

From grasses it is well known that there are infected and non-infected populations, e.g., of *Festuca arundinacea*, which are defined scientifically in publications in abbreviated form as EI (= endophyte-infected) or E+ (= endophyte is present) vs EF (= endophyte free) or E- (= endophyte is not present), respectively. To date it is an unresolved issue whether there are also infected as well as uninfected species or populations of species in the ergoline-positive Ipomoeeae. If this would be the case this could explain perhaps – at least in some cases – the existence of contradictory reports on the occurrence of ergoline alkaloids in certain *Ipomoea* spp. (Table 4.2).

4.2.5 Significance

Ergoline alkaloids, natural products of remarkable therapeutical significance, are compounds with very distinctive and complex biological activities. The knowledge

of these activities is based on the results of the tremendous pharmacological research work about such alkaloids and their semisynthetic derivatives done in the course of the past century. Ergolines are very toxic. They caused epidemics of the so-called ergotism (ergot poisoning characterized by necrosis of the extremities) with frequently fatal consequences in former centuries due to a contamination of rye (*Secale cereale* L., Poaceae) by ergot sclerotia (Hofmann 1964; Berde and Schild 1978; Spano and Trabucchi 1978; Kraupp and Lembeck 1982; Eich 1992; Pertz and Eich 1994, 1999; Eich and Pertz 1999 and references therein).

4.2.5.1 Mechanisms of Action and Therapeutic Relevance

The pharmacodynamically complex profile of the ergolines as ligands for serotonin (5-HT) receptors, dopamine (D) receptors, and adrenoceptors, respectively, is explainable by the fact that these alkaloids include the essential structural features of the corresponding three monoamine neurotransmitters. This can be visualized by superimposition of the ergoline skeleton by these three natural agonists each (Fig. 4.9).

The advances in the knowledge of the receptor complexity and heterogeneity in the last three decades have uncovered a "receptor kingdom" with several hierarchic levels like superfamilies, families, subfamilies, types, and subtypes, respectively. This modern taxonomy for ligand binding sites is especially of increasing relevancy in the field of those targets which are involved in the pharmacodynamics of the

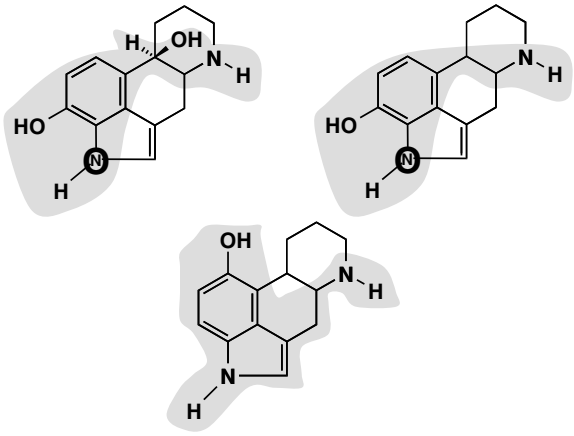


Fig. 4.9 Superimposition of the ergoline nucleus by monoamines whose receptors are involved in the pharmacodynamic profile of ergoline alkaloids thus demonstrating the structural similarities; natural ligands (*highlighted in grey*): noradrenaline (*above left*), dopamine (*above right*), serotonin (*below*). The (non-basic) indole nitrogen (*N*-1) and its hydrogen atom represent a group which is *bioisosteric* to a phenolic hydroxyl group in the *ortho*-position to the second phenolic hydroxyl of the so-called catecholamines; therefore, the oxygen atom drawn in an oversized manner is superimposing the indole nitrogen atom reduced in size in these two cases

ergoline pharmacophore. Thus, there are, e.g., at least 14 distinct subtypes of serotonin receptors, 5 subtypes of dopamine receptors, and 10 subtypes of adrenoceptors. Depending on (1) the mammalian species, (2) the kind and tone of the corresponding tissue, and (3) the specific structure of the ergoline, these compounds may act as agonists, partial agonists, or antagonists. Though there is evidence that the ergoline nucleus itself is responsible for high receptor affinities, e.g., in case of rat 5-HT_{2A} receptors (Pertz et al. 1999), the distinctness of the effects of a specific alkaloid is due to the individual combination of the ergoline nucleus with certain additional groups or moieties. Moreover, such structural differences are above all responsible for the pharmacokinetic properties of an individual ergoline rather than for the pharmacodynamic differences.

Ergoline alkaloids and their semisynthetic derivatives play an important role in different fields of pharmacotherapy of human diseases, e.g., migraine, postpartum haemorrhage, Parkinson's disease. Nevertheless, only a few show specificity with regard to the different monoamine receptor systems and selectivity concerning subtypes of each of these major groups. In particular, natural ergolines are so-called "dirty drugs" due to their multiple activity profile which may cause different side effects. Thus, e.g., ergotamine, for many decades the most important and potent drug against acute migraine until the nineties of the past century, has lost its leading position for two reasons. (i) The 5-$HT_{1B/1D}$ receptor, the target to which ergotamine shows affinity beside many other receptor subtypes, has been scientifically accepted as the deciding target concerning migraine; the stimulation of this target by an appropriate, i.e., more specific and selective agonist has been considered as a successful strategy against acute migraine. (ii) Therefore, the search for such agonists has been increased and led to the development of synthetic agonists with high 5-$HT_{1B/1D}$ receptor specificity and selectivity (triptans). These compounds show two advantages compared with ergotamine: (I) they lack the complex activity profile of ergotamine and therefore also most of the corresponding risks, (II) in contrast to the ergopeptine the triptans show different, positive kinetic properties. In the clinical situation, it is known that the effects of the ergopeptine-type alkaloid ergotamine sustain much longer than is to be expected from its plasma concentration profile (plasma elimination half-life) (Østergaard et al. 1981; Tfelt-Hansen and Paalzow 1985). These observations could be supported and, furthermore, explained by an in vitro study with human isolated coronary artery segments (MaassenVanDenBrink et al. 1998): the contractile responses to ergotamine persisted even after repeated washings, but those of triptans declined rapidly after washing. The sustained contraction by ergotamine seems to be an important disadvantage compared with the triptans. Therefore, to date ergotamine is clinically only used if triptans show insufficient therapeutic effects. This happens since patients are individual responders to any treatment with drugs due to, e.g., pharmacogenetic differences.

It is true that ergotamine has not yet been found in a convolvulaceous species. However, the structurally closely related congeners ergosine and ergovaline, which are not used in human therapy, presumably show a similar affinity profile compared with ergotamine to numerous monoamine receptor subtypes. This is proved for several receptor subtypes (Fig. 4.10), but not investigated for many others. Anyhow,

there is no doubt about the assumption that all these and other ergopeptines are "dirty drugs" (pharmacodynamic properties) with the above mentioned pharmacokinetic disadvantages in therapy. In contrast, the same properties provide important advantages for their producers, fungi or higher plants, in their struggle for existence: they defend them against a wide range of insect and mammalian herbivores (Clay 1990). Thus, the ergopeptines represent ecological multipurpose weapons. The slow washout from the receptor biophase observed for ergotamine and structurally related compounds should also be of importance concerning the in vivo effects as defending weapons in the wild. Since ergopeptines (ergosine, ergobalansine) are minor alkaloids in ergoline-positive Convolvulaceae spp., they seem to play only a minor role. In contrast, in the case endophyte-infected grasses, where ergovaline represents the dominating alkaloid, this ergopeptine plays a decisive role. However, the minor role of ergopeptines in ergoline-positive convolvulaceous species does not mean "a role which could be neglected" bearing in mind the sustaining effects as well as a contributing part to their total "ergoline cocktail".

Simple lysergic acid amides like ergine and ergometrine or clavines like agroclavine in principal show a similar profile concerning their targets: they are also ligands of the same monoamine receptor subtypes. For example, results of studies with isolated bovine dorsal metatarsal arteries and lateral saphenous veins confirmed that ergine has vasoconstrictor activity similar to that of ergometrine, and that response in arteries was minimal compared to that of veins. This effect is based on antagonism at 5-HT_2 and α_1 receptors, respectively (Oliver 1997). These "low molecular ergolines" are also ecological multipurpose weapons. The main

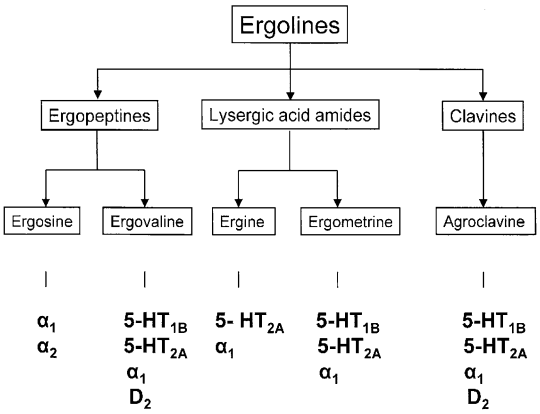


Fig. 4.10 Receptor subtypes as targets (as far as already investigated) for those members of the different groups of ergoline alkaloids which have been found in convolvulaceous species (exception: ergovaline, the dominating ergopeptine in endophyte-infected grasses). It should be taken into account that the receptor subtypes listed below the individual compounds are only given as targets, i.e., a certain alkaloid may act as a partial agonist or an antagonist in various monoamine receptor models (Hoyer and Boddeke 1993); α = adrenoceptor; 5-HT (= 5-hydroxytryptamine) = serotonin receptor; D = dopamine receptor

difference is not a pharmacodynamic but a pharmacokinetic one. The low molecular ergolines are lacking the complex peptide moiety, which is apparently responsible for the persistence of the ergopeptines at the receptor molecules. Thus, the effects of low molecular ergolines are more rapidly reversible by washout in pharmacological experiments with isolated tissues (Martin et al. 1995).

On the other hand, the pattern of biological activities of certain low molecular ergolines is enhanced by the discovery that agroclavine and festuclavine are agents with antimicrobial and potent cytostatic activities (Eich and Eichberg 1982; Schwarz and Eich 1983, 1984; Eich et al. 1984; Eich and Pertz 1999). Remarkably, ergopeptines and simple lysergic acid amides lack these activities. The antimicrobial and cytostatic effects of agroclavine and festuclavine are not associated with monoamine receptor interactions. The mechanism of action rather seems to be a fundamental novel one for ergolines in so far as these certain clavines interfere with DNA replication processes (Eich et al. 1984; Hibasami et al. 1990). Though it is unlikely that a genotoxic mechanism is involved in the cytostatic activity of agroclavine, it is remarkable that this alkaloid shows mutagenic properties in the AMES test with different pathogenic bacteria strains after addition of a subcellular rat liver preparation forming a xenobiotic metabolizing system (Glatt et al. 1987, 1992). As a consequence the cytotoxicity of agroclavine decreased due to the formation of one (or more) mutagenic metabolite(s) of unknown structure.

Of course, feeding deterrence may be caused simply by the bitter, unpleasant taste of many alkaloids. However, all mechanisms of action discussed above may contribute more or less to the protection of their fungal producers as well as of the ergoline-positive convolvulaceous or graminaceous species against herbivores due to intoxications – lethal or survived (avoidance in future). Even pathogenic/parasitic bacteria may be inhibited by certain clavines.

4.2.5.2 Ethnobotany

The fascinating history of the seeds of *Turbina corymbosa* (ololiuqui) and *Ipomoea tricolor* (badoh negro) used by the shamans of different Mexican Indio tribes since ancient times for ritual and medicinal purposes as well as their discovery and consequently the elucidation of their constituents by modern scientist has been written down by numerous authors, e.g., Hofmann 1961, 1979; Schultes 1965; Schultes and Hofmann 1979; Rätsch 2005. The reader is referred to such publications. The psychoactive ergoline alkaloids such as lysergic acid amide (ergine), structurally closely related to semisynthetic, most famous and most potent hallucinogenic agent lysergic acid diethyl amide (Hofmann 1979), led to a tremendous (mis)use of ergoline-containing convolvulaceous seeds, especially *Argyreia nervosa* due to their higher content, beside other drugs by young people all over the world during the psychodelic flower power era. However, such seeds are causing a hypnotic state rather than a hallucinogenic one. Therefore, it is assumed that in addition the specific cultural situation of the Indio shamans might play an important role in causing the strong visions reported by them (Rätsch 2005).

4.2.5.3 Protection Against Vertebrate Herbivores; Toxicoses in Livestock

As already mentioned, poisoning of domestic livestock has spurred a great deal of research on endophytic fungi in pasture grasses. The best-studied endophyte in the USA, *Neotyphodium coenophialum*, provides its host, tall fescue, *Festuca arundinacea* (Poaceae), with numerous benefits that help make tall fescue one of the most widely used plants for forage, turf, soil conservation, and land reclamation (Panaccione et al. 2001). Endophyte benefits include increases in drought tolerance, shoot growth, tillering, seed production, seed germination, phosphorus uptake, and resistance to insects and parasitic nematodes (Bacon and White 1994; Bacon and Hill 1997 and references therein). On the other hand, toxicosis observed in livestock grazing endophyte-infected tall fescue pastures causes extensive economic damages. The magnitude of tall fescue toxicosis, e.g., in the USA can be gauged by an estimated $600 million loss to the beef cattle industry during 1990. Considering inflation and losses to other livestock (e.g., dairy cattle, horses, and sheep), the economic cost associated with this problem are estimated in 2001 at $1 billion per year (Panaccione et al. 2001). Tall fescue toxicosis includes poor weight gain, hormonal imbalances leading to reduced fertility and lactation, and gangrene of the animals' limbs. The mechanistic base for such activities is known. The consistent observation of decreased serum prolactin levels in mammals receiving diet of infected tall fescue indicates the involvement of dopamine receptors in fescue toxicosis caused by D_2 agonistic activity of, e.g., ergovaline (Strickland et al. 1993, 1994). Moreover, it has been concluded that the powerful constrictor effect of ergovaline causing such gangrenes is mediated by activation of vascular 5-$HT_{1B/1D}$ and 5-HT_{2A} receptors (Schöning et al. 2001). A detailed review on physiological manifestations of endophyte toxicosis in ruminant and laboratory species has been published recently (Oliver 1997).

In contrast to numerous studies on toxicoses in livestock by ergoline-associated endophyte-infected grasses there are only a few reports on intoxications by these alkaloids due to Convolvulaceae spp. The reasons are apparent: *Argyreia*, *Ipomoea*, *Turbina*, and *Stictocardia* spp., respectively, are no pasture plants suitable for grazing by domestic livestock but predominantly climbing vines or even huge lianas. The only considerable report on a convolvulaceous species causing intoxications by ergolines has been published on *Ipomoea muelleri*. This completely prostrate vine, which often dominates the grazing of sheep in certain districts of Western Australia during wet years (Gardiner et al. 1965), develops under these climate conditions elongated lateral stems up to 1.5 m in length. The plant is associated with flood plains or low depressions or clay soil. In a normal year the individual plants are mostly short-stemmed, the whole plant frequently being no more than 30 cm in diameter, and the species does not provide the major part of the ground cover. In certain years, however, plentiful rains and/or the rise of river level which results in extensive flooding favor a vigorous growth, and large areas of clay country support an almost pure stand of this plant. Due to the lack of an alternative diet it is in these years that grazing in the area causes losses of huge numbers of sheep. Affected animals show locomotory difficulties, behavioural disturbances,

loss of weight, steady deterioration in condition with an apparent loss in the use of the hind limbs, and leucopenia. After many weeks the animals may die due to the combined action of nutritional stress and ergoline alkaloids. Of course, weaners are more severely affected than adult sheep. Thus, *Ipomoea muelleri* became one of the best-studied ergoline-positive convolvulaceous species.

It is obvious that ergoline alkaloids, known as powerful poisons for laboratory-raised murine and other mammals as well as for domestic livestock, must be protecting agents against all kinds of vertebrate herbivores in the wild. Predation of seeds by birds and small mammals may also be reduced by the presence of endophytes. Feeding trials with five species of passerines showed that these birds preferred endophyte-free poaceous seeds, and if they were forced to eat infected seeds, they lost weight and had difficulty walking and maintaining balance. Trapping of mice and moles in fields of endophyte-infected and endophyte-free tall fescue indicated that these animals were much more abundant in endophyte-free fields. Extracts from endophyte-infected tall fescue have been shown to affect rats and rabbits (Rowan and Latch 1994 and references therein).

4.2.5.4 Protection Against Invertebrate Herbivores

A large taxonomic diversity of insects and other pests are negatively affected by infected grasses (Siegel et al. 1990; Rowan and Latch 1994; Clement et al. 1994; Pestridge and Marshall 1997 and references therein). Unfortunately, there are only few reports which refer exactly to the metabolites involved, i.e., ergolines, indole diterpenes, peramine, or lolines. But there is no doubt that all these compounds in principal can be responsible for insect resistance. Ergopeptine-type alkaloids have been shown to affect the fall army worm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), and adults of the black beetle, *Heteronychus arator* Fabricius (Coleoptera: Scarabaeidae). Ergovaline was the most active alkaloid against adult stem weevil; ergotamine reduced feeding and growth of the armyworm. The latter effects were also observed for clavine-type alkaloids (agroclavine, elymoclavine). The ergopeptines turned out to be active at concentrations similar to those at which they occur in endophyte-infected ryegrass.

Serotonin receptors are an example for a potential target for ergolines also in invertebrates. These receptors are widely distributed throughout the animal kingdom. However, apparently little work has been undertaken in invertebrates in general and in insects in special. The classification of insect 5-HT receptors lags far behind that of the vertebrates. Nevertheless, various physiological and ligand binding studies indicate the presence of several distinct 5-HT receptor types in insects. Thus, the 5-HT receptors stimulating an increase in the number of contractions of the isolated gut of the caterpillar *S. frugiperda* have properties in common with mammalian 5-HT_2, 5-ht_6, and 5-HT_7 receptors as well as the *Drosophila* 5-HT_{dro1} and 5-$HT_{dro2a/2b}$. However, the primary amino acid sequence of these lepidopteran receptors will have to be elucidated before full comparisons can be made (Howarth et al. 2002).

In principle, the question arises if feeding deterrence, rather than insecticidal effects, appears to be the mechanism of endophyte-mediated resistance by ergolines against insect herbivores. Depending on the species both mechanisms are imaginable. Given the choice, polyphagous insects tend to select a diet with no or only a small dose of alkaloids in general. But if they have no choice or if they are very hungry, the deterrency threshold value is much reduced and they often feed on a diet containing alkaloids that they would normally avoid. In this case the toxicity of an ingested alkaloid can be assessed (Wink 1998). However, there are only a few experimental studies concerning insects and ergolines: ergometrine (ED_{50}: 1%) and ergotamine (ED_{50}: <0.1%) have shown feeding deterrent properties toward a polyphagous *Syntomis* sp. (Lepidoptera: Arctiidae). However, it is known that lolines (see Sect. 5.2.3) are implicated as a possible corn flea beetle feeding deterrent protecting endophyte-infected grasses (Ball et al. 1997). Some nematode species adversely affected by the presence of endophytes of perennial rye-grass and/or tall fescue are also known (Eerens et al. 1997).

References

Amor-Prats D, Harborne JB (1993a) New sources of ergoline alkaloids within the genus *Ipomoea*. Biochem Syst Ecol 21:455–462

Amor-Prats D, Harborne JB (1993b) Allelochemical effects of ergoline alkaloids from *Ipomoea parasitica* on *Heliothis virescens*. Chemoecology 4:55–61

Austin DF, Eich E (2001) Synopsis of *Stictocardia* with another Madagascan species, *S. mojangensis* (Convolvulaceae) Willdenowia 31:79–85

Austin DF, Huáman Z (1996) A synopsis of *Ipomoea* (Convolvulaceae) in the Americas. Taxon 45:3–38

Bacon CW, DeBattista J (1991) Endophytic fungi of grasses. In: Arora DK, Rai B, Mukerji KG, Knudsen GR (eds) Handbook of applied microbiology. Soils and plants, vol 1. Marcel Dekker, New York, p 231

Bacon CW, Hill NS (eds) (1997) *Neotyphodium*/Grass Interactions. Plenum Press, New York

Bacon CW, White JF Jr (eds) (1994) Biotechnology of endophytic fungi of grasses. CRC Press, Boca Raton

Bacon CW, Porter JK, Robbins JD, Lutrell ES (1977) *Epichloë typhina* from toxic tall fescue grasses. Appl Environ Microbiol 34:576–581

Ball OJP, Pless C, Gwinn KD (1997) Corn flea beetle (*Chaetocnema pulicaria*) responses to natural endophytes of tall fescue, meadow fescue, and perennial ryegrass. In: Bacon CW, Hill NS (eds) *Neotyphodium*/grass interactions. Plenum Press, New York, pp 243–245

Banerjee SK, Bhatnagar SP (1974) Indole bases of some seeds of *Ipomoea* species. Indian J Pharmacy 36:44–46

Beliveau J, Ramstad E (1966) 8-Hydroxylation of agroclavine and elymoclavine by fungi. Lloydia/J Nat Prod 29: 234–238

Berde B, Schild HO (eds) (1978) Ergot alkaloids and related compounds. Handbook of experimental pharmacology, vol. 49, Springer, Berlin Heidelberg New York

Beyerman HC, van de Linde A, Henning GJ (1963) Over ergot alkaloiden uit planten. Chem Weekbl 59:508–509

Boyes-Korkis JM, Floss HG (1992) Biosynthesis of ergot alkaloids: Some new results on an old problem. Prikl Biokhim Mikrobiol 28:843–857

Chan Lin WN, Ramstad E, Taylor EH (1976a) Enzymology of ergot alkaloid biosynthesis. Part III. 10-Hydroxyelymoclavine, an intermediate in the peroxidase conversion of elymoclavine to penniclavine and isopenniclavine. Lloydia/J Nat Prod 30:202–208

Chan Lin WN, Ramstad E, Shough HR, Taylor EH (1967b) Enzymology of ergot alkaloid biosynthesis. Part V. Multiple functions of peroxidase in the conversion of clavines. Lloydia/J Nat Prod 30:284P

Chao JM, DerMarderosian AH (1973a) Ergoline alkaloidal constituents of Hawaiian baby wood rose, *Argyreia nervosa* (BURM. f.) BOJER. J Pharm Sci 62:588–591

Chao JM, DerMarderosian AH (1973b) Identification of ergoline alkaloids in the genus *Argyreia* and related genera and their chemotaxonomic implications in the Convolvulaceae. Phytochemistry 12:2435–2440

Choong TC, Shough HR (1977) The isolation and synthesis of chanoclavine-I acid. Tetrahedron Lett 3137–3138

Clay K (1990) Fungal endophytes of grasses. Annu Rev Ecol Syst 21:275–297

Clement SL, Kaiser WJ, Eichenseer H (1994) *Acremonium* endophytes in germplasms of major grasses and their utilization for insect resistance. In: Bacon CW, White JF Jr (eds) Biotechnology of endophytic fungi in grasses. CRC Press, Boca Raton, pp 185–200

Costa C, Bertazzo A, Allegri G, Curcuruto O, Traldi P (1992) Indole alkaloids from the roots of an African plant *Securidaca longipedunculata*. I. Isolation by column chromatography and preliminary structural characterization by mass spectrometry. J Heterocycl Chem 29:1641–1646

DerMarderosian A (1964) The comparative morphology and indole alkaloid constituents of certain species and varieties of morning glories (Convolvulaceae). Ph.D. Thesis, University of Rhode Island, Health Sciences, Pharmacy, USA; see also: DerMarderosian et al. (1964)

DerMarderosian A (1967a) Hallucinogenic indole compounds from higher plants. Lloydia/J Nat Prod 30:23–38

DerMarderosian A (1967b) Psychotomimetic indoles in the Convolvulaceae. Am J Pharm. 139:19–26

DerMarderosian A, Youngken HW (1966) The distribution of indole alkaloids among certain species and varieties of *Ipomoea*, *Rivea* and *Convolvulus* (Convolvulaceae). Lloydia/J Nat Prod. 29:35–42

DerMarderosian A, Hauke RL, Youngken HW Jr (1964) Preliminary studies of the comparative morphology and certain indoles of *Ipomoea* seeds. Econ Bot 18:67–76

DerMarderosian A, Cho E, Chao JM (1974) The isolation and identification of the ergoline alkaloids from *Ipomoea muelleri*. Planta Med 25:6–16

Dobberstein RH, Staba EJ (1969) *Ipomoea*, *Rivea* and *Argyreia* tissue cultures: Influence of various chemical factors on indole alkaloid production and growth. Lloydia/J Nat Prod 32:141–147

Eerens JPJ, Visker MHPW, Lucas RJ, Easton HS, White GH (1997) Influence of the ryegrass endophyte on phyto-nematodes. In: Bacon CW, Hill NS (eds), *Neotyphodium*/grass interactions. Plenum Press, New York, pp 153–156

Eich E (1992) Ergolin-Derivate – "schmutzige", spezifische und selektive Arzneistoffe. Pharmaz Ztg 137:1601–1614

Eich E, Eichberg D (1982) Zur antibakteriellen Wirkung von Clavinalkaloiden und deren partialsynthetischen Derivaten. Planta Med 45:146

Eich E, Pertz H (1994) Ergot alkaloids as lead structures for differential receptor systems. Pharmazie 49:867–877

Eich E, Pertz H (1999) Antimicrobial and antitumor effects of ergot alkaloids and their derivatives. In: Křen V, Cvak L (eds) Ergot – the genus *Claviceps*. Harwood Academic Publishers, Amsterdam, The Netherlands, pp 441–449

Eich E, Eichberg D, Müller WEG (1984) Clavines – new antibiotics with cytostatic acitivity. Biochem Pharmacol 33:523–526

Faini F, Castillo M, Torres R (1978) A new β-carboline alkaloid from *Vestia lycioides*. Phytochemistry 17:338

Faini F, Torres R, Delle Monache F, Marini-Bettolo GB, Castillo M (1980) 1-Acetyl-3-carboxy-β-carboline, a new acid and other constituents of *Vestia lycioides*. Planta Med 38:128–132

Gardiner MR, Royce R, Oldroyd B (1965) *Ipomoea muelleri* intoxication of sheep in Western Australia. Brit Vet J 121:272–277

Genest K (1965) A direct densitometric method on thin-layer plates for determination of lysergic acid amide, isolysergic acid amide, and clavine alkaloids in morning glory seeds. J Chromat 19:531–539

Genest K, Sahasrabudhe MR (1966) Alkaloids and lipids of *Ipomoea*, *Rivea* and *Convolvulus* and their application to chemotaxonomy. Econ Bot 20:416–428

Glatt H, Eich E, Pertz H, Becker C, Oesch F (1987) Mutagenicity experiments on agroclavines, new natural antineoplastic compounds. Cancer Res 47:1811–1814

Glatt H, Pertz H, Kasper R, Eich E (1992) Clavine alkaloids and derivatives as mutagens detected in the AMES test. Anti-Cancer Drugs 3:609–614

Glenn AE, Bacon CW, Price R, Hanlin RT (1996) Molecular phylogeny of *Acremonium* and its taxonomic implications. Mycologia 88: 369–383

Gröger D (1963) Über das Vorkommen von Ergolinderivaten in *Ipomoea*-Arten. Flora 153:373–382

Gröger D, Floss HG (1998) Biochemistry of ergot alkaloids – achievements and challenges. In: Cordell GA (ed) The alkaloids, vol 50. Academic Press, San Diego, CA, USA, pp 171–218

Gröger D, Mothes K, Floss HG, Weygand F (1963) Zur Biogenese von Ergolin-Derivaten in *Ipomoea rubro-coerulea* Hook. Z Naturforsch 18b:1123–1124

Haarmann T, Machado C, Lübbe Y, Correia T, Schardl CL, Panaccione DG, Tudzynski P (2005) The ergot alkaloid gene cluster in *Claviceps purpurea*: extension of the cluster sequence and intra species evolution. Phytochemistry 66:1312–1320

Hahn E (1990) Qualitative and quantitative examination of lysergic acid derivatives in the species *Ipomoea*. Gyogyszereszet 34:349–358

Henrici A (1996) Neuartige Sekundärstoffe unterschiedlichster Struktur aus tropischen Convolvulaceen. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany

Henrici A, Kaloga M, Eich E (1995) 1,2,3,4-Tetrahydro-β-carboline-3β-carboxylic acid from *Merremia aegyptia* (L.) Urb. (Convolvulaceae). Book of abstracts, 43rd Annual Congress on Medicinal Plant Research, Halle, Saale, Germany, H 37

Hibasami H, Nakashima K, Pertz H, Kasper R, Eich E (1990) Inhibitory effects of novel festuclavine derivatives on nucleoside uptake and incorporation into DNA and RNA in human lymphoid leukaemia Molt 4B cells. Cancer Lett 50:161–164

Hofmann A (1961) Die Wirkstoffe der mexikanischen Zauberdroge "Ololiuqui". Planta Med 9:354–367

Hofmann A (1964) Die Mutterkornalkaloide. Ferdinand Enke Verlag, Stuttgart, Germany

Hofmann A (1979) LSD – Mein Sorgenkind. Klett-Cotta, Stuttgart, Germany, pp 137–150

Hofmann A, Tscherter H (1960) Isolierung von Lysergsäure-Alkaloiden aus der mexikanischen Zauberdroge Ololiuqui [*Rivea corymbosa* (L.) Hall. f.] Experientia 16:414

Howarth CJ, Prince RI, Dyker H, Lösel PM, Seinsche A, Osborne RH (2002) Pharmacological characterisation of 5-hydroxytryptamine-induced contractile effects in the isolated gut of the lepidopteran caterpillar *Spodoptera frugiperda*. J Insect Physiol 48:43–52

Hoyer D, Boddeke HWGM (1993) Partial agonists, full antagonists, antagonist: dilemmas of definition. Trends Pharmacol Sci 14:270–275

Hylin JW, Watson DP (1965) Ergoline alkaloids in tropical wood roses. Science 148:499–500

Ikan R, Rapaport E, Bergmann ED (1968) The presence of agroclavine in *Cuscuta monogyna* seeds. Israel J Chem 6:65–67

Jenett-Siems K (1996) Phytochemische Untersuchungen an Windengewächsen der Gattungen *Calystegia, Convolvulus, Ipomoea* und *Merremia* unter besonderer Berücksichtigung des Alkaloidvorkommens. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany

Jenett-Siems K, Kaloga M, Eich E (1993) Ipangulines, the first pyrrolizidine alkaloids from the Convolvulaceae. Phytochemistry 34:437–440

Jenett-Siems K, Kaloga M, Eich E (1994) Ergobalansine/ergobalansinine, a proline-free peptide-type alkaloid of the fungal genus *Balansia*, is a constituent of *Ipomoea piurensis*. J. Nat. Prod.57:1304–1306; Erratum: Correction of the species: *I. asarifolia* (DESR.) R. & SCH. (2004) J Nat Prod 67:2160

Jenett-Siems K, Schimming T, Kaloga M, Eich E, Siems K, Gupta MP, Witte L, Hartmann T (1998) Pyrrolizidine alkaloids of *Ipomoea hederifolia* and related species. Phytochemistry 47:1551–1560

Jenett-Siems K, Schimming T, Siems K, Gupta MP, Witte L, Eich E (1999) Unique pyrrolizidine alkaloids as potential chemotaxonomic markers of the infrageneric *Ipomoea* taxon subg. *Quamoclit*, sect. *Mina*. Book of abstracts, XVI International Botanical Congress, St. Louis, USA, p 383 (P542) and p 645 (P2111)

Jenett-Siems K, Ott SC, Schimming T, Siems K, Müller F, Hilker M, Witte L, Hartmann T, Austin DF, Eich E (2005) Ipangulines and minalobines, chemotaxonomic markers of the infrageneric *Ipomoea* taxon subgenus *Quamoclit*, section *Mina*. Phytochemistry 66:223–231

Jirawongse V, Pharadai T, Tantivatana P (1977) The distribution of indole alkaloids in certain genera of Convolvulaceae growing in Thailand. J Nat Res Counc 9:17–24

Kayser C (1994) Phytochemische Untersuchungen an pantropischen Arten der Gattung *Ipomoea* als Beitrag zur Chemotaxonomie der Convolvulaceen. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany

Keller U (1999) Biosynthesis of ergot alkaloids. In: Křen V, Cvak L (eds) Ergot – the genus *Claviceps*. Harwood Academic Publishers, Amsterdam, The Netherlands, pp 95–163

Kim SU, Cho YJ, Floss HG, Anderson JA (1983) Conversion of elymoclavine to paspalic acid by a particulate fraction from an ergotamine-producing strain of *Claviceps* sp. Planta Med 48:145–148

Koslovsky AG (1999) Producers of ergot alkaloids out of *Claviceps* genus. In: Křen V, Cvak L (eds), Ergot – the genus *Claviceps*. Harwood Academic Publishers, Amsterdam, The Netherlands, pp 479–499

Kraupp O, Lembeck, F (eds) (1982) Mutterkornalkaloide heute – Therapeutische Konsequenzen einer chemisch-pharmakologischen Differenzierung im Lichte neuer Forschungsergebnisse. Georg Thieme Verlag, Stuttgart, Germany, pp 1–119

Kucht S, Groß J, Hussein Y, Grothe T, Keller U, Basar S, König WA, Steiner U, Leistner E (2004) Elimination of ergoline alkaloids following treatment of *Ipomoea asarifolia* (Convolvulaceae) with fungicides. Planta 219:619–625

Lee TM, Chao JM, DerMarderosian A (1979) Isolation and identification of ergoline alkaloids from seeds of *Stictocardia campanulata* (L.) MERRILL. Planta Med 35:247–252

MaassenVanDenBrink A, Reekers M, Bax WA, Ferrari MD, Saxena PR (1998) Coronary side-effect potential of current and prospective antimigraine drugs. Circulation 98:25–30

Mann P (1997) Zur Phytochemie und Chemotaxonomie tropischer und mediterraner Convolvulaceen unter besonderer Berücksichtigung des Alkaloidvorkommens. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany

Manos PS, Miller RE, Wilkin P (2001) Phylogenetic analysis of *Ipomoea, Argyreia, Stictocardia,* and *Turbina* suggests a generalized model of morphological evolution in morning glories. Syst Bot 26:585–602

Mantle PG (1972) A reappraisal of the occurrence of ergoline alkaloids in seeds of *Cuscuta monogyna* VAHL. Planta Med 21:218–219

Martin GR, Martin RS, Wood J (1995) Long-acting 5-HT_{1D} receptor agonist effects of dihydroergotamine. In: Olesen J, Moskowitz MA (eds) Experimental Headache Models, Lippincott-Raven Publishers, Philadelphia, USA, pp 163–167

Mayer K, Eich E (1975) C-17-Oxydation von Clavinalkaloiden mit primärer alkoholischer Hydroxylgruppe. Arch Pharm 308:819–824

McDonald JA (1982) Biosystematics of the *Ipomoea tricolor* complex (Convolvulaceae) Ph.D. dissertation. University of Texas

McDonald JA (1991) Origin and diversity of Mexican Convolvulaceae. Anal Inst Biol Unives Nat Atonom Mex, Ser Bot 62:65–82; fide Amor-Prats D, Harborne JB (1973a)

McJunkins SP, Thornton JI, Dillon DJ (1968) Identification notes on the tropical wood rose. Forens Sci Soc J 8:121–124

Miller MD (1970) Isolation and identification of lysergic acid amide and isolysergic acid amide as the principal ergoline alkaloids in *Argyreia nervosa*, a tropical wood rose. J Ass Offic Agric Chem 53:123–127

Miller RE, Rausher MD, Manos PS (1999) Phylogenetic systematics of *Ipomoea* (Convolvulaceae) based on ITS and *waxy* sequences. Syst Bot 24:209–227

Mockaitis JM, Kivilaan A, Schulze A (1973) Studies of the loci of indole alkaloid biosynthesis and alkaloid translocation in *Ipomoea violacea* plants. Biochemie und Physiologie der Pflanzen 164:248–257

Morgan-Jones G, Gams W (1982) Notes on Hyphomycetes. XLI. An endophyte of *Festuca arundinacea* and the anamorph of *Epichloë typhina*, new taxa in one of two new sections of *Acremonium*. Mycotaxon 15:311–318

Nair GG, Daniel M, Sabnis SD (1987) Ergolines in the seeds of some Indian Convolvulaceae. Indian J Pharm Sci 100–102

Nikolin B, Nikolin A (1971) The presence of clavine and ergot alkaloids in seeds of various *Ipomoea* species. Acta Pharm Jug 21:109–112

Odebiyi OO, Sofowora EA (1978) Phytochemical screening of Nigerian medicinal plants. Lloydia/J Nat Prod 41:234–246

Okuda T, Yoshida T, Shiota N, Nobuhara J (1975) L-3-Carboxy-1,2,3,4-tetrahydro-β-carboline, a new amino acid from the seeds of *Aleurites fordii*. Phytochemistry 14:2304–2305

Oliver JW (1997) Physiological manifestations of endophyte toxicosis in ruminant and laboratory species. In: Bacon CW, Hill NS (eds) *Neotyphodium*/grass interactions. Plenum Press, New York, pp 311–345

Østergaard JR, Mikkelsen E, Voldby B (1981) Effects of 5-hydroxytryptamine and ergotamine on human superficial temporal artery. Cephalalgia 1:223–228

Panaccione DG, Johnson RD, Wang J, Young CA, Damrongkool P, Scott B, Schardl CL (2001) Elimination of ergovaline from a grass-*Neotyphodium* endophyte symbiosis by genetic modification of the endophyte. Proc Nat Acad Sci USA 98:12820–12825

Pažoutová S, Parbery DP (1999) The taxonomy and phylogeny of *Claviceps*. In: Křen V, Cvak L (eds), Ergot –the genus *Claviceps*. Harwood Academic Publishers, Amsterdam, The Netherlands, pp 57–77

Perez Amador MC, Gonzalez E. A Marquez J, Bailin J, Garcia Jimenez F, Collera O (1980) Perfiles cromatograficos de semillas de algunas especies de Convolvulaceas. Phyton 39:85–94

Perez Amador MC, Garcia Argaez A, Varala G, Garcia Jimenez F (1988) Perfiles cromatograficos de semillas de algunas especies de Convolvulaceas. II. Analisis de tres especies de *Merremia*. Phyton 48:97–99

Pertz H, Eich E (1999) Ergot alkaloids and their derivatives as ligands for serotoninergic, dopaminergic, and adrenergic receptors. In: Křen V, Cvak L (eds) Ergot – the genus *Claviceps*. Harwood Academic Publishers, Amsterdam, The Netherlands, pp 411–440

Pertz HH, Milhahn HC, Eich E (1999) Cycloalkanecarboxylic esters derived from lysergol and elymoclavine as partial agonists and antagonists at rat 5-HT_{2A} receptors: Pharmacological evidence that the indolo(4,3-fg)-quinoline system of the ergolines is responsible for high 5-HT_{2A} receptor affinity. J Med Chem 42:659–668

Pestridge RA, Marshall SL (1997) The effects of *Neotyphodium*-infected perennial ryegrass on the abundance of invertebrate predators. In: Bacon CW, Hill NS (eds), *Neotyphodium*/grass interactions, Plenum Press, New York, pp 195–197

Petroski RJ, Powell RG, Clay K (1992) Alkaloids of *Stipa robusta* (sleepygrass) infected with an *Acremonium* endophyte. Nat Tox 1:84–88

Porter JK (1995) Analysis of endophyte toxins: fescue and other grasses toxic to livestock. J Anim Sci 73:871–880

Powell RG, Plattner RD, Yates SG, Clay K, Leuchtmann A (1990) Ergobalansinine, a new ergot-type peptide alkaloid isolated from *Cenchrus echinatus* (sandbur grass) infected with *Balansia*

obtecta, and produced in liquid cultures of *B. obtecta* and *Balansia cyperi*. J Nat Prod 53:1272–1279

Rätsch C (2005) The encyclopedia of psychoactive plants – ethnopharmacology and its applications. Inner Traditions, Vermont, USA

Robien W, Poehm M, Jurenitsch J (1988) Fluorodaturatin – ein Canthin-6-on-Derivat (Revision der Struktur). Scientia Pharmac 56:133–136

Rochelmeyer H (1958) Problem der biologischen Synthese der Mutterkornalkaloide. Pharmaz Ztg 103:1269–1275

Rowan DD, Latch GCM (1994) Utilization of endophyte-infected perennial ryegrasses for increased insect resistance. In: Bacon CW, White JF Jr (eds), Biotechnology of endophytic fungi of grasses. CRC Press, Boca Raton, USA, pp 169–184

Sarmento da Silva TM, Braz-Filho R, de Carvalho MG, Agra MdF (2002) 1,2,3,4-Tetrahydro-2-methyl-β-carboline and solavetivone from *Solanum jabrense*. Biochem Syst Ecol 30:1083–1085

Scandola M, Games DE, Costa C, Allegri G, Bertazzo A, Curcuruto O, Traldi P (1994) Structural study of alkaloids from *Securidaca longipedunculata* roots. II. Isolation and characterization by supercritical fluid chromatography/maß spectrometry. J Heterocycl Chem 31:219–224

Schardl CL (1996) *Epichloë* species: fungal symbionts of grasses. Annu Rev Phytopathol 34:109–130

Schardl CL, Leuchtmann A, Spiering MJ (2004) Symbioses of grasses with seedborne fungal endophytes. Annu Rev Plant Biol 55:315–340

Schimming T (2003) Beiträge zur Chemotaxonomie und Phylogenie der Convolvulaceen auf der Basis des Alkaloidvorkommens. Dissertation, Fachbereich Biologie, Chemie, Pharmazie, Freie Universität Berlin, Germany

Schöning C, Flieger M, Pertz HH (2001) Complex interaction of ergovaline with 5-HT_{2A}, 5-$HT_{1B/1D}$, and $alpha_1$ receptors in isolated arteries of rat and guinea pig. J Animal Sci 79:2202–2209

Schultes RE (1965) Ein halbes Jahrhundert Ethnobotanik amerikanischer Halluzinogene. Planta Med 29:125–156

Schultes RE, Hofmann A (1979) Plants of the Gods. McGraw-Hill, Maidenhead, UK

Schwarz G, Eich E (1983) Influence of ergot alkaloids on growth of *Streptomyces purpurascens* and production of its secondary metabolites. Planta Med 47:212–214

Schwarz G, Eich E (1984) Einfluß von Agroclavin auf Wachstum und Sekundärstoffproduktion von *Saccharomyces uvarum* und *Blakeslea trispora*. Pharmazie 39:572

Sharda S, Kokate CK (1979) Indole alkaloids from the leaves of *Ipomoea palmata* Forsk. Indian Drugs:70–71

Shough HR, Taylor EH (1969) Enzymology of ergot alkaloid biosynthesis. Part IV. Additional studies on the oxidation of agroclavine by horseradish peroxidase. Lloydia/J Nat Prod 32:315–326

Siegel MR, Latch GCM, Bush LP, Fannin FF, Rowan DD, Tapper BA, Bacon CW, Johnson MC (1990) Fungal endophyte-infected grasses: Alkaloid accumulation and aphid response. J Chem Ecol 16:3301–3315

Spano PF, Trabucchi M (eds) (1978) Ergot alkaloids. Pharmacology 16 (Suppl 1):1–209

Stauffacher D, Tscherter H, Hofmann A (1965) Isolierung von Ergosin und Ergosinin neben Agroclavin aus den Samen von *Ipomoea argyrophylla* Vatke (Convolvulaceae). Helv Chim Acta 48:1379–1380

Stauffacher D, Niklaus P, Tscherter H, Hofmann A (1969) Cycloclavin, ein neues Alkaloid aus *Ipomoea hildebrandtii* Vatke. Tetrahedron 25:5879–5887

Stech M, Austin DF, Schimming T, Eich E (2007) Phylogenetic inference in *Ipomoea* section *Mina* (Convolvulaceae): molecular relationships and the significance of phytochemical and morphological characters (to be published)

Stefanovic S, Krueger L, Olmstead RG (2002) Monophyly of the Convolvulaceae and circumscription of their major lineages based on DNA sequences of multiple chloroplast loci. Am J Bot 89:1510–1522

Stefanović S, Austin DF, Olmstead RG (2003) Classification of Convolvulaceae: a phylogenetic approach. Syst Bot 28:791–806

Steiner U, Ahimsa-Müller MA, Markert A, Kucht S, Groß J, Kauf J, Kuzma M, Zych M, Lamshöft M, Furmanova M, Knoop V, Drewke C, Leistner E (2006) Molecular characterization of a seed transmitted clavicipitaceous fungus occurring on dicotyledoneous plants. Planta 224:533–544

Stoll A (1945) Über Ergotamin. Helv Chim Acta 28:1283–1308

Strickland JR, Oliver JW, Cross DL (1993) Fescue toxicosis and its impact on animal agriculture. Vet Hum Tox 35:454–464

Strickland JR, Cross DL, Birrenkott GP, Grimes LW (1994) Effect of ergovaline, loline, and dopamine antagonists on rat pituitary cell prolactin release in vitro. Am J Vet Res 55:716–721

Taber WA, Heacock RA (1962) Location of ergot alkaloid and fungi in the seed of *Rivea corymbosa* (L.) HALL. f., "Ololiuqui". Canad J Microbiol 8:137–143

Taber WA, Vining LC, Heacock RA (1963a) Clavine and lysergic acid alkaloids in varieties of morning glory. Phytochemistry 2:65–70

Taber WA, Heacock RA, Mahon ME (1963b) Ergot-type alkaloids in vegetative tissue of *Rivea corymbosa* (L.) HALL. f. Phytochemistry 2:99–101

Taylor EH, Shough HR (1967) Enzymology of ergot alkaloid biosynthesis. Part II. The oxidation of agroclavine by horseradish peroxidase. Lloydia/J Nat Prod 30:197–201

Tenberge KB (1999) Biology and life strategy of the ergot fungi. In: Křen V, Cvak L (eds) Ergot – the genus *Claviceps*. Harwood Academic Publishers, Amsterdam, The Netherlands, pp 25–56

Tfelt-Hansen P, Paalzow L (1985) Intramuscular ergotamine: plasma levels and dynamic activity. Clin Pharmacol Ther 37:29–35

Tofern B (1999) Neue und seltene Sekundärstoffe des Phenylpropan-, Terpen- und Alkaloid-Stoffwechsels aus tropischen Convolvulaceen. Dissertation, Fachbereich Biologie, Chemie, Pharmazie, Freie Universität Berlin, Germany

Tofern B, Kaloga M, Witte L, Hartmann T, Eich E (1999) Occurrence of loline alkaloids in *Argyreia mollis* (Convolvulaceae). Phytochemistry 51:1177–1180

Trumm SM (1990) Dem Shikimat-Weg entstammende niedermolekulare Sekundärstoffe der Convolvulaceen. Dissertation, Fachbereich Chemie und Pharmazie, Johannes-Gutenberg-Universität Mainz, Germany

Tudzynski P, Correia T, Keller U (2001) Biotechnology and genetics of ergot alkaloids. Appl Microbiol Biotechnol 57:593–605

Tuttobello L, Macri A, Valfre F (1971) Sulla presenza di alcaloidi clavinici in piante infestanti e foraggere. Atti Soc Ital Sci Vet 25:299–302

Tyler VE Jr, Erge D, Gröger D (1965) Biological conversions of agroclavine and elymoclavine. Planta Med 13:315–325

Umar S, Junior P, Wichtl M (1980) Isolierung und Identifizierung von Agroclavin und α-Dihydrolysergol aus den Blättern von *Ipomoea fistulosa*. Planta Med 40:328–332

Verdcourt B (1963) 110. Convolvulaceae. In: Hubbard CE, Milne-Redhead E (eds) Flora of East Africa. London, pp 1–163

Weigl R (1992) Entdeckung, Isolierung und Strukturaufklärung neuer Alkaloide im Rahmen chemotaxonomischer Untersuchungen an Convolvulaceen. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany

White JF Jr (1997) Systematics of the graminicolous Clavicipitaceae. In: Bacon CW, Hill SH (eds) *Neotyphodium*/grass interactions. Plenum Press, New York, pp 27–39

Wilkinson RE, Hardcastle WS, McCormick CS (1986) Ergot alkaloid content of *Ipomoea lacunosa*, *I. hederacea*, *I. trichocarpa*, and *I. purpurea* seed. Canad J Plant Sci 66:339–343

Wilkinson RE, Hardcastle WS, McCormick CS (1987) Seed ergot alkaloid content of *Ipomoea hederifolia*, *I. quamoclit*, *I. coccinea,* and *I. wrightii*. J Sci Food Agric 39:335–339

Wilkinson RE, Hardcastle WS, McCormick CS (1988) Psychomimetic ergot alkaloid contents of seed from *Calonyction muricatum*, *Jacquemontia tamnifolia*, *Quamoclit lobata*, and *Q. sloteri*. Bot Gaz 149:107–109

Wink M (1998) Chemical ecology of alkaloids. In: Roberts MF, Wink M (eds) Alkaloids – biochemistry, ecology, and medicinal applications, Plenum Press, New York, pp 265–300
Yahara S, Domoto H, Sugimura C, Nohara T, Niiho Y, Nakajima Y, Ito H (1994) An alkaloid and two lignans from *Cuscuta chinensis*. Phytochemistry 37:1755–1757
Yahara S, Uda N, Yoshio E, Yae E (2004) Steroidal alkaloid glycosides from tomato (*Lycopersicon esculentum*). J Nat Prod 67:500–502

5
Miscellaneous Alkaloids

A number of alkaloids whose biogenetic origin has not yet been identified have been discovered in one or a few species of both large Solanales families and some of them turned out to be unique. They are listed in the following in chronological order with regard to their discovery or first detection in the corresponding family.

5.1 Occurrence in the Solanaceae

5.1.1 Fabianine

A volatile tetrahydroquinoline alkaloid named fabianine (Fig. 5.1) was identified from the aerial part of *Fabiana imbricata* Ruiz & Pav. (Edwards and Elmore 1962). This base is a congener of bicyclic, cadinene/norcadinene type sesquiterpenes (see Sect. 7.3.1). Fabianine seems to share a common precursor with a monocyclic (*N*-free) diketo*nor*cadinene type metabolite. Thus, it may be assumed that the structure of fabianine is characterized by a sesquiterpenoid skeleton (Schmeda-Hirschmann and Papastergiou 1994).

5.1.2 2-Methoxy-3-isobutylpyrazine

As well as a number of monoterpenoids and compounds from other classes, one major component of the characteristic pleasant aroma of green bell peppers, *Capsicum annuum* L. var. *grossum*, was identified as 2-methoxy-3-isobutylpyrazine (Fig. 5.1) (Buttery et al. 1969). Afterwards, this metabolite also turned out to be a constituent of further vegetables from different plant families.

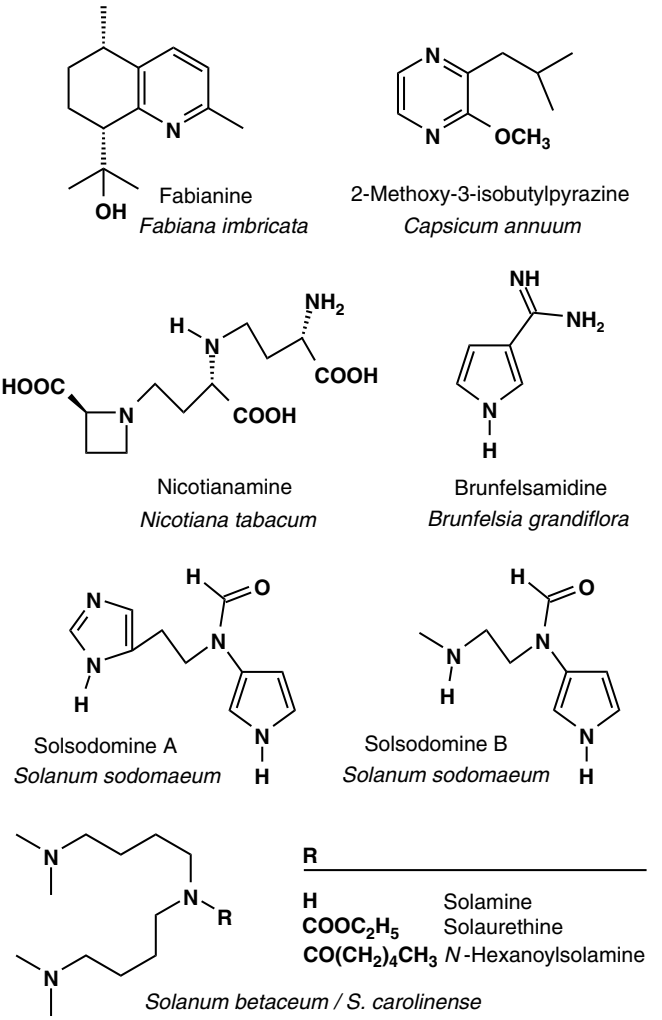


Fig. 5.1 Miscellaneous alkaloids discovered in certain species from the Solanaceae family

5.1.3 Nicotianamine

The discovery of nicotianamine, a new non-proteinogenic amino acid, in green tobacco leaves, *Nicotiana tabacum* L. (Noma et al. 1971) led to remarkable insights with regard to its general significance in plant physiology. Its structure was finally determined to be (2*S*:3′*S*:3″*S*)-*N*-[*N*-(3-amino-3-carboxypropyl)-3-amino-3-carboxypropyl]azetidine-2-carboxylic acid (Fig. 5.1) showing its structural relationship to azetidine-2-carboxylic acid (Kristensen and Larsen 1974). Furthermore, nicotianamine was detected not only in other solanaceous species

(five further *Nicotiana* spp., *Datura metel*, *Lycium chinense*, *Solanum lycopersicum*, *S. melongena*) but also in *Zea mays* L. (Poaceae), *Rohdea japonica* ROTH (Convallariaceae) (Noma and Noguchi 1976), and *Fagus silvatica* L. (Fagaceae) (Kristensen and Larsen 1974). Later, the ubiquitous occurrence of this alkaloid could be demonstrated (Scholz et al. 1992 and references therein). It turned out to possess an optimal structure for chelating iron ions. Therefore, it was considered a possible phytosiderophore with an essential function in cellular iron transport and/or metabolism (Buděšínsky et al. 1980). Meanwhile, there is no doubt about the fact that nicotianamine represents a significant plant-endogenous chelator not only for iron but also for other metal micronutrients. They are transported as nicotianamine complexes via phloem/xylem (e.g., Schmidke and Stephan 1995). Interestingly, nicotianamine has also been discovered as a component of soy sauce which may have health benefits: The compound is able to inhibit the angiotensin-converting enzyme (ACE) (Kinoshita et al. 1994 and references therein). Synthetic ACE inhibitors have been established as antihypertensive drugs.

5.1.4 Solamines

Solamine, 4*N*,4′*N*-bis(dimethylamino)-dibutylamine and its *N*-hexanoyl derivative solacaproine (Fig. 5.1) were discovered as constituents of the roots of *Solanum betaceum* CAV. sub nom. *Cyphomandra betacea* (CAV.) SENDT., tree tomato (Evans et al. 1972). Solamine and its urethane (carbamate) derivative solaurethine (Fig. 5.1) were identified in the roots of *S. carolinense* L., horse nettle (Evans and Somanabandhu 1977).

Addition. The root-wood of *Duboisia leichhardtii* was found to contain tetramethylputrescine (*N*,*N*′-tetramethyl-1,4-diaminobutane) (Griffin 1967).

5.1.5 Pyrrole Alkaloids

Brunfelsamidine was discovered in the root bark of *Brunfelsia grandiflora* D.DON ssp. *schultesii* PLOWMAN, an ethnomedicinal drug and narcotic of the indigenous people of the upper Amazon in Peru (Lloyd et al. 1985). It turned out to induce convulsions in mice. Its structure was determined to be pyrrole-3-carb(ox)amidine (Fig. 5.1). Two years later this alkaloid was identified as the decisive poisonous principle of the whole plant of *Nierembergia linearіaefolia* GRAH. sub nom. *N. hippomanica* auct. non MIERS (Buschi and Pomilio 1987). Though other constituents may contribute to its toxicity which causes severe consequences, brunfelsamidine turned out to account for the lethality observed after ingestion of this Argentinean plant by cattle, horses, sheep, goats etc. Two other pyrrole alkaloids, solsodomine A and B (Fig. 5.1), were discovered as constituents of fresh berries from *Solanum sodomaeum* L. (El Sayed et al. 1998).

5.1.6 Benzodiazepines

Surprisingly, sterile potato herb, *S. tuberosum* L., contained two established **synthetic** anxiolytic/sedative drugs, temazepam and diazepam, in a range of 70–450 ng/g cell tissue (Kavvadias et al. 2000). This was the first report on the endogenous formation of benzodiazepines by plant cells, as any interaction of microorganisms and environmental factors was excluded.

5.1.7 Catecholamines

Dopamine, norepinephrine, and epinephrine are neurotransmitters in animals; they have also been detected by GC-MS in *Solanum tuberosum* L., potato. Such catecholamines were demonstrated to be involved in plant responses towards biotic and abiotic stress (Świędrych et al. 2004 and references therein).

5.1.8 Betaines

Alkaloids like (glycine)betaine and trigonellin represent widely distributed minor constituents of angiosperms. They have also been detected in *Solanum wendlandii* Hook. f. (Blunden et al. 2005). Another non-proteinogenic amino acid, nicotianine, was discovered as a constituent of tobacco leaves; it turned out to be L-(+)-*N*-(3-amino-3-carboxypropyl)-β-carboxypyridinium betaine (Noguchi et al. 1964).

5.2 Occurrence in the Convolvulaceae

5.2.1 Benzylisoquinolines

A metabolite from the Chinese drug Chin-Kuo-Lan, *Calystegia hederacea* Wall., originally named "calystegin" or "calystigine" (Chu and Chian 1955) turned out to be the known benzophenanthridine type benzylisoquinoline alkaloid palmatine (Fig. 5.2) discovered in *Jateorhiza palmata* Miers (Menispermaceae) (Huang and Chen 1957). The detection of the closely related protopine (Fig. 5.2) as a minor constituent of the leaves of *C. sepium* (L.) R.Br., hedge bindweed, confirmed the erratic, unusual presence of benzylisoquinolines in this genus (Schimming 2003). This class of alkaloids is largely constricted to the order Ranunculales and the eumagnoliids, i.e., basal angiosperms.

Addition. Iseluxine. In this connection the discovery of an isoquinolinone named iseluxine (Fig. 5.2) as a constituent of the roots and vegetative epigeal parts of *Isea*

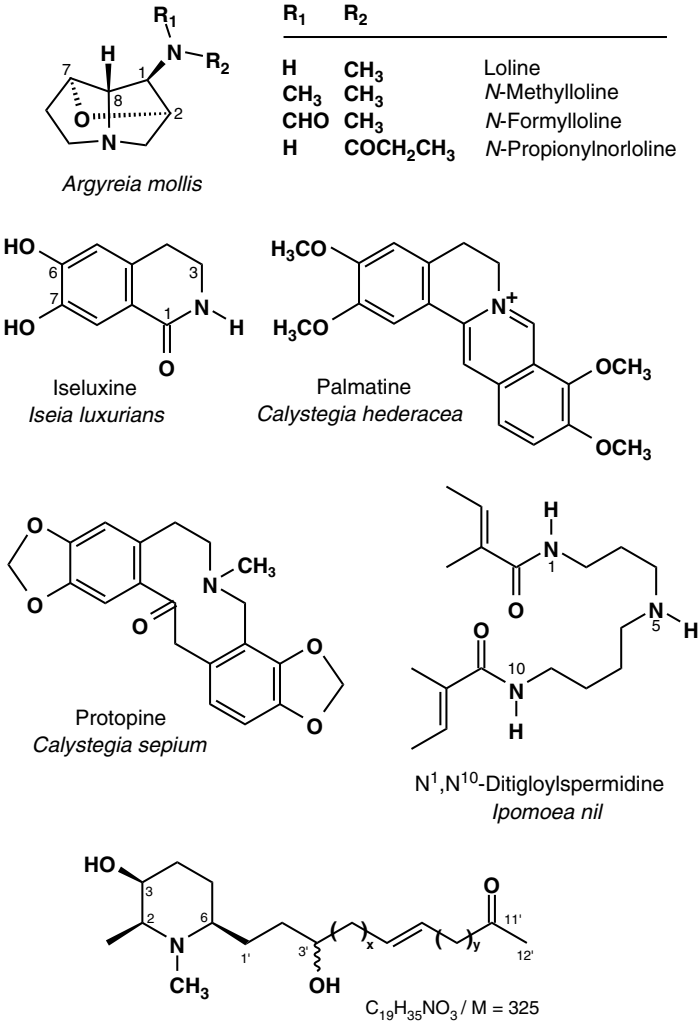


Fig. 5.2 Miscellaneous alkaloids discovered in the Convolvulaceae (iseluxine, ditigloylspermidine, animartinines) or detected as rare metabolites also in this family

luxurians (MORIC.) O'DONELL is of some interest (Schimming et al. 2000). This alkaloid could only be detected during the first year of cultivation in the greenhouse; afterwards it was no longer detectable. *N*- and/or *O*-methyl derivatives of iseluxine were already known from certain Magnoliidae families though not present in *I. luxurians*. Nevertheless, from the biogenetic point of view, iseluxine may be interpreted as the potential "missing link" in the biosynthesis of those

methyl derivatives. Iseluxine might be synthesized from dopamine retaining the catechol substructure of the latter.

This alkaloid has also been detected in the roots and stems of *Aristolochia elegans* MAST. (Aristolochiaceae) together with benzylisoquinoline/bisbenzylisoquinoline alkaloids (Wu et al. 2002; Shi et al. 2004). It was named pericampylinone-A by these authors who apparently did not know its former discovery as iseluxine.

5.2.2 Animartinines

Two series of altogether 75 novel piperidine alkaloids named animartinines were identified by GC/MS analysis as constituents of the aerial parts and roots of *Anisea martinicensis* (JACQ.) CHOISY, a slender climber of neotropical origin growing close to or even in fresh water biotopes (Schimming et al. 1999; Schimming 2003). Metabolites characterized by an *N*-methyl group were listed in an A-series, the corresponding *nor* compounds in a B-series. The number of compounds was almost equal in both series. The animartinines shared a 2-methyl-3-hydroxy-6-substituted piperidine skeleton. They differ in the length of the side chain at C-6 (C_7–C_{15}; M = 225–371). However, all compounds showed an oxygen functionality in the ω minus 1 position (=O or –OH), e.g., Fig. 5.2. Thus, their structures turned out to be very similar to those of cassine and carnavaline known from *Cassia excelsa* SCHARD. and *C. carnaval* SPEG. (Fabaceae), respectively (Highet 1964; Lythgoe and Vernengo 1967). This was also true for prosopine from *Prosopis africana* TAUB. (Mimosaceae) (Khuong-Huu-Qui et al. 1972) and cryptophorine from *Bathiorhamnus cryptophorus* CAPURON (Rhamnaceae). In contrast to these known piperidine alkaloids which are characterized by saturated side-chains (cassine, prosopine) or multiply unsaturated ones (cryptophorine) animartinines are usually characterized by only one double bond. However, the position of this double bond remains to be elucidated. Furthermore, there might be stereochemical differences since most structures proposed for the animartinines were only based on GC/MS data. From the biogenetic point of view, it should be added that all of such piperidine alkaloids independent of the plant family are obviously synthesized via the polyketide pathway.

5.2.3 Lolines

Lolines are pyrrolizidine alkaloids synthesized by seed-transmitted fungal endophytes living in poaceous species from certain genera, e.g., *Festuca arundinacea* SCHREB., tall fescue, infected with *Acremonium/Neotyphodium coenophialum* MORGAN, JONES & GAMS (Yates et al. 1990; Bush et al. 1993; TePaske et al. 1993; Porter 1995). These metabolites are characterized by unusual structural characters such as an amino substituent at C-1, a strained ether bridge between C-2 and C-7,

and the lack of a 1,2-double bond (Fig. 5.2). They have insect antifeedant and insecticidal activities comparable to nicotine, but little or no toxicity to mammals. Recently, Schardl et al. (2007) have published a comprehensive review on the history of loline discovery, methods of analysis, biological activities, distribution in nature, genetics and biosynthesis. A general review on seedborne fungal endophytes of grasses was given by Clay (1993) and also by Schardl et al. (2004).

The biosynthesis of the lolines has been almost completely elucidated though a few questions remain. The pathway is diverging from the one leading to "normal" pyrrolizidine alkaloids (see Sect. 3.7). A decisive intermediate is represented by the 1-(3-aminopropyl)pyrrolinium cation which might be generated from host metabolism of spermine catalyzed by plant polyamine oxidase as well as synthesized by the fungus from L-proline and *O*-acetylhomoserine (condensation of the 2-aminobutyric acid moiety from the latter intermediate with the *N* of L-proline). The strained ether bridge is formed after formation of the pyrrolizidine rings (Blankenship et al. 2005; Schardl et al. 2007 and references therein).

The same lolines which were discovered in endophyte-infected grasses (loline, *N*-methylloline, *N*-formylloline) could be identified as constituents of the epigeal vegetative parts and roots of *Argyreia mollis* (Burm.f.) Choisy (Tofern et al. 1999) which accumulates ergoline alkaloids in the seeds (see Sect. 4.2). Fascinatingly, sometimes these lolines even occur together with ergoline alkaloids in such grasses, just like in case of *A. mollis*. Therefore the authors of the report on *A. mollis* came to the cautious conclusion "Although it seems unlikely that endophytic fungi are involved in loline and ergoline formation in *Argyreia*, we cannot exclude this possibility by sure". Meanwhile, such a possibility has got more probability since seed-transmitted infection with clavicipiteous endophytes could be demonstrated at least for ergolines in certain convolvulaceous species (see Sect. 4.2): "An intriguing possibility is that lolines in *Adenocarpus* [this fabaceous genus is the only further source for lolines] and *Argyreia* species might be products of as yet undiscovered fungal symbionts." (Schardl et al. 2007). It should be added that *N*-propionylnorloline (decorticasine) (Fig. 5.2), also present in *Argyreia mollis*, was discovered as a constituent of *Adenocarpus decorticans* Boiss. (Fabaceae) (Petroski et al. 1989 and references therein).

Within the Convolvulaceae the occurrence of lolines is rather isolated. They turned out to be absent from other *Argyreia* spp. such as *A. capitata* (Vahl) Choisy, *A. hookeri* Clarke, and *A. nervosa* (Burm. f.) Boj. as well as from numerous other species belonging to 14 further genera (Tofern et al. 1999 and references therein).

5.2.4 Betaines

Alkaloids like (glycine)betaine and trigonellin represent widely distributed minor constituents of angiosperms (Blunden et al. 2005). In the Convolvulaceae family at least one of these two betaines has been detected in the vegetative epigeal parts

and/or roots of 13 out of 16 species checked [genera: *Argyreia* (4 spp.), *Bonamia* (1), *Falkia* (1), *Ipomoea* (7), *Merremia* (1), *Odonellia* (1), *Xenostegia* (1)]. Trigonellin and (glycine)betaine were found in three species, the former alkaloid alone in further six spp. and the latter one alone in further 10 spp. (Tofern 1999). Neither in the epigeal parts nor in the roots could any betaine be detected in *I. aquatica* Forsk., *I. batatas* (L.) Lam., and *Xenostegia medium* (L.) D.F.Austin & Staples.

5.2.5 N,N-Diacylspermidines

Amides of the polyamine spermidine bearing aliphatic or hydroxycinnamic acyl residues are widely distributed in the plant kingdom (Bienz et al. 2002). In most cases the acyl moieties represent the latter residues. For such conjugates see Sect. 6.6.4.3. A novel metabolite with an aliphatic acyl residue, N^1,N^{10}-ditigloylspermidine, has been isolated from the seeds of *Ipomoea nil* (L.) Choisy collected on Zanzibar/Tanzania (Schimming et al. 2005). It turned out to dominate to an unusual extent in the alkaloid fraction beside seven minor congeners. Most of them were isomers of the main metabolite. All these metabolites were also present in unripe fruits and stems. However, they were not detectable in such parts from another provenance (Ecuador) of this species (seeds not checked).

References

Bienz S, Detterbeck R, Ensch C, Guggisberg A, Häusermann U, Meisterhans C, Wendt B, Werner C, Hesse M (2002) Putrescine, spermidine, spermine, and related polyamine alkaloids. In: Cordell GA (ed) The alkaloids, vol 58. Academic Press, San Diego, CA, USA, pp 83–338

Blankenship JD, Houseknecht JB, Pal S, Bush LP, Grossman RB, Schardl CL (2005) Biosynthetic precursors of fungal pyrrolizidines, the loline alkaloids. Chembiochem 6:1016–1022

Blunden G, Patel AV, Armstrong N, Adrian Romero M, Melendez P (2005) Betaine distribution in Angiosperms. Biochem Syst Ecol 33:904–920

Buděšínsky M, Budzikiewicz H, Procházka Ž, Ripperger H, Römer A, Scholz G, Schreiber K (1980) Nicotianamine, a possible phytosiderophore of general occurrence. Phytochemistry 19:2295–2297

Buschi CA, Pomilio AB (1987) Pyrrole-3-carbamidine: a lethal principle from *Nierembergia hippomanica*. Phytochemistry 26:863–865

Bush LP, Fannin FF, Siegel MR, Dahlman DL, Burton HR (1993) Chemistry, occurrence and biological effects of saturated pyrrolizidine alkaloids associated with endophytic-grass interactions. Agric Ecosyst Envir 44:81–102

Buttery RG, Seifert RM, Guadagni DG, Ling LC (1969) Characterization of some volatile constituents of bell peppers. J Agric Food Chem 17:1322–1327

Chu JH, Chian YH (1955) Ein neues Alkaloid, Calystegin, und eine neutrale Stammverbindung aus der chinesischen Droge Chin-Kuo-Lan, *Calystigia hydraceae*. Acta Chim Sinica 21: 168–172; ref. in Chem Zentralbl 131:1859 (1960)

Clay K (1993) Fungal endophytes of grasses. Annu Rev Ecol Syst 21:275–297

Edwards JS, Elmore NF (1962) Fabianine. Canad J Chem 40:256–264
El Sayed KA, Hamann MT, Abd El-Rahman HA, Zaghloul AM (1998) New pyrrole alkaloids from *Solanum sodomaeum*. J Nat Prod 61:848–850
Evans WC, Somanabandhu A (1977) Bases from roots of *Solanum carolinense*. Phytochemistry 16:1859–1860
Evans WC, Ghani A, Woolley VA (1972) Alkaloids of *Cyphomandra betacea* SENDT. J Chem Soc Perkin I:2017–2019
Griffin WJ (1967) Alkaloids of *Duboisia leichhardtii*: tetramethylputrescine. Australas J Pharm 48:S20–S21
Highet RJ (1964) Alkaloids from *Cassia species*. I. Cassine. J Org Chem 29:471–474
Huang WY, Chen YC (1957) The identification of calystigine as palmatine. Huaxue Xuebao 23:230–233
Kavvadias D, Abou-Mandour AA, Czygan FC, Beckmann H, Sand P, Riederer P, Schreier P (2000) Identification of benzodiazepines in *Artemisia dracunculus* and *Solanum tuberosum*. Biochem Biophys Res Commun 269:290–295
Khuong-Huu-Qui, Ratle G, Monseur X, Goutarel R (1972) Structures of prosopine and prosopinine, alkaloids from *Prosopis africana*. Bull Soc Chim Belg 81:425–441
Kinoshita E, Yamakoshi J, Kikuchi M (1994) Antihypertensive substance in soy sauce. Nippon Jozo Kyokaishi 89:126–130
Kristensen I, Larsen PO (1974) Azetidine-2-carboxylic acid derivatives from seeds of *Fagus silvatica* L. and a revised structure for nicotianamine. Phytochemistry 13:2791–2798
Lloyd HA, Fales HM, Goldman ME, Jerina DM, Plowman T, Schultes RE (1985) Brunfelsamidine: a novel convulsant from the medicinal plant *Brunfelsia grandiflora*. Tetrahedron Lett 26: 2623–2624
Lythgoe D, Vernengo MJ (1967) Alkaloids from *Cassia carnival*. Cassine and carnavaline. Tetrahedron Lett:1133–1137
Noguchi M, Sakuma H, Tamaki E (1968) The isolation and identification of nicotianine: a new amino acid from tobacco leaves. Phytochemistry 7:1861–1866
Noma M, Noguchi M (1976) Occurrence of nicotianamine in higher plants. Phytochemistry 15:1701–1702
Noma M, Noguchi M, Tamaki E (1971) A new amino acid, nicotianamine, from tobacco leaves. Tetrahedron Lett 12:2017–2020
Petroski RJ, Yates SG, Weisleder D, Powell EG (1989) Isolation, semi-synthesis, and NMR spectral studies of loline alkaloids. J Nat Prod 52:810–817
Porter JK (1995) Analysis of endophytic toxins: fescue and other grasses toxic to livestock. J Anim Sci 73:871–880
Schardl CL, Leuchtmann A, Spiering (2004) Symbioses of grasses with seedborne fungal endophytes. Annu Rev Plant Biol 55:315–340
Schardl CL, Grossman RB, Nagabhyru P, Faulkner JR, Mallik UP (2007) Loline alkaloids: currencies of mutualism. Phytochemistry 68:980–996
Schimming T (2003) Beiträge zur Chemotaxonomie und Phylogenie der Convolvulaceen auf der Basis des Alkaloidvorkommens. Dissertation, Fachbereich Biologie, Chemie, Pharmazie, Freie Universität Berlin, Germany
Schimming T, Jenett-Siems K, Siems K, Witte L, Gupta MP, Eich E (1999) Animartinines, novel piperidine alkaloids from *Anisea martinicensis* (Convolvulaceae). Book of Abstracts, 5th Joint Meeting of the American Society of Pharmacognosy, Association Française pour l'Enseignement et la Recherche en Pharmacognosie, Gesellschaft für Arzneipflanzenforschung and the Phytochemical Society of Europe, Amsterdam, NL, P221
Schimming T, Jenett-Siems K, Siems K, Witte L, Gupta MP, Eich E (2000) Iseluxine: a novel isoquinolinone alkaloid from *Iseia luxurians*. Z Naturforsch 55c:1023–1025
Schimming T, Jenett-Siems K, Siems K, Witte L, Eich E (2005) N^{1},N^{10}-Ditigloylspermidine, a novel alkaloid from the seeds of *Ipomoea nil*. Pharmazie 60:958–959
Schmeda-Hirschmann G, Papastergiou F (1994) Sesquiterpenes from *Fabiana imbricata*. Phytochemistry 36:1439–1442

Schmidke I, Stephan UW (1995) Transport of metal micronutrients in the phloem of castor bean (*Ricinus communis*) seedlings. Physiol Plant 95:147–153

Scholz G, Becker R, Pich A, Stephan UW (1992) Nicotianamine – a common constituent of strategies I and II of iron acquisition by plants: a review. J Plant Nutr 15:1647–1665

Shi LS, Kuo PC, Tsai YL, Damu AG, Wu TS (2004) The alkaloids and other constituents from the root and stem of *Aristolochia elegans*. Bioorg Med Chem 12:439–446

Świędrych A, Lorenc-Kukula K, Skirycz A, Szopa J (2004) The catecholamine biosynthesis route in potato is affected by stress. Plant Physiol Biochem 42:593–600

TePaske MR, Powell RG, Clement SL (1993) Analyses of selected endophyte-infected grasses for the presence of loline-type and ergot-type alkaloids. J Agric Food Chem 41:2299–2303

Tofern B (1999) Neue und seltene Sekundärstoffe des Phenylpropan-, Terpen- und Alkaloid-Stoffwechsels aus tropischen Convolvulaceen. Dissertation, Fachbereich Biologie, Chemie, Pharmazie, Freie Universität Berlin, Germany

Tofern B, Kaloga M, Witte L, Hartmann T, Eich E (1999) Occurrence of loline alkaloids in *Argyreia mollis* (Convolvulaceae), Phytochemistry 51:1177–1180

Wu TS, Tsai YL, Damu AG, Kuo PC, Wu PL (2002) Constituents from the root and stem of *Aristolochia elegans*. J Nat Prod 65:1522–1525

Yates SG, Petroski RJ, Powell RG (1990) Analysis of loline alkaloids in endophyte-infected tall fescue by capillary gas chromatography. J Agric Food Chem 38:182–185

6
Phenylalanine-derived Metabolites/ Phenylpropanoids

Biogenetic Outline (Fig. 6.1). Two out of three aromatic amino acids, L-tyrosine and L-tryptophan, are precursors from the primary metabolism which retain – predominantly via the corresponding biogenic amine – their principal *C*- and *N*-skeleton in the pathway-specific biosynthesis of many classes of alkaloids in the plant kingdom. In contrast, significance of the third aromatic amino acid, L-phenylalanine, for this purpose is generally rather low. There are only a few structurally simple alkaloids in the whole plant kingdom whose carbon skeletons *as well as* their nitrogens are derived from phenylalanine, e.g., *N*-acylphenylethylamines (Sect. 6.1). Furthermore, cyanogenic glycosides, another class of secondary plant metabolites, are biogenetic derivatives of several amino acids, among them predominantly phenylalanine. During biosynthesis of these glycosides the majority of the carbon skeleton as well as the nitrogen of the amino acid is also retained (Conn 1979; phenylalanine-derived cyanogenic glycosides see Sect. 6.2).

On the other hand, this amino acid represents the common precursor of all groups of plant constituents summarized by the very popular – though not very meaningful – term "phenolics", preferable to be substituted by "phenylpropanoids". This is a ubiquitous and voluminous class of *N*-free secondary metabolites (Sects. 6.3–6.8). Nevertheless, these sections include a few *N*-containing groups of compounds, i.e., those of Sects. 6.4, 6.6.4, and 6.8. However, these groups have got their nitrogen by conjugation of an *N*-containing molecule from another origin with the *N*-free phenylpropanoid. Already the first pathway-specific reaction L-phenylalanine → cinnamic acid – catalyzed by phenylalanine ammonia lyase (PAL) – leads to loss of its nitrogen. A comprehensive review on natural phenolic compounds 1900–2000 was published by Whiting (2001).

Origin of Some Central Trivial Names. Some names were chosen according to the discovery of the corresponding compounds such as cinnamic acid [*Cinnamomum verum* PRESL., cinnamon tree (Lauraceae)], coumaric acids/ coumarin [*Dipterix odorata* (AUBL.) WILLD. sub nom. *Coumarouna odorata* AUBL. (Fabaceae)], caffeic acid [*Coffea arabica* L., coffee tree (Rubiaceae)], and ferulic acid (*Ferula* spp., Apiaceae). Quinic acid is named according to its discovery as a constituent of cinchona bark [*Cinchona* L. spp. (Rubiaceae)]; this compound was named according to the German "Chinarinde" (cinchona bark) – corrupted from "quina" (Spanish

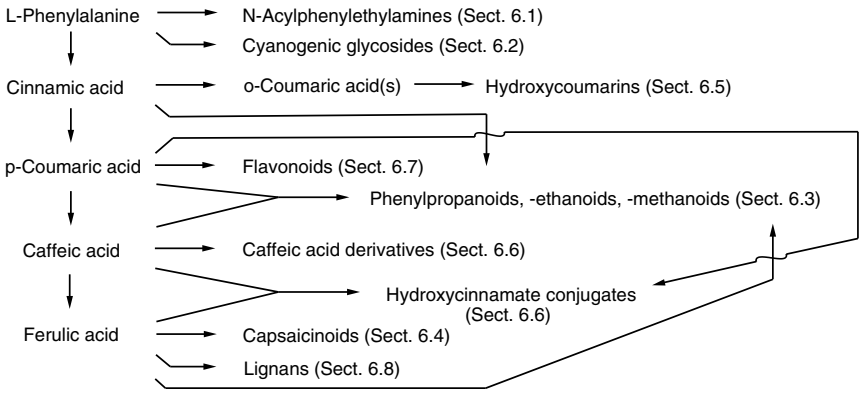


Fig. 6.1 Biogenetic outline on phenylalanine-derived metabolites (mentioning of cyanogenic glycosides refers to certain compounds only)

spelling of the South American indigenous "kina", i.e., bark) – which led to "Chinasäure", in turn translated from German into English as "quinic acid" [like "Chinin" (German) → "quinine" (English)]. Chlorogenic acid (derived from the Greek for "to become green") is named according to its change in aqueous solution from colourless to green on influence of ammonia.

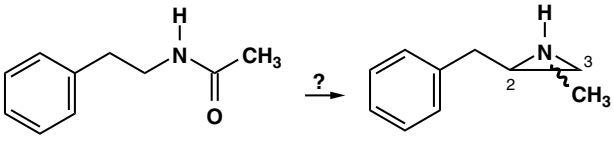
6.1 *N*-Acylphenylethylamines and Derivatives

6.1.1 Occurrence in the Solanaceae

β-Phenylethylamine is the biogenic amine of L-phenylalanine and therefore a frequently occurring metabolite in nature. Nevertheless, reports on it are rather rare in the Solanaceae family. It was detected, e.g., together with tyramine and its *N*-methyl derivatives in *Nierembergia linariaefolia* GRAHAM sub nom. *N. hippomanica* Auct. non MIERS (Gonzalez MD et al. 1981). However, *N*-acylphenylethylamines from this family are unknown.

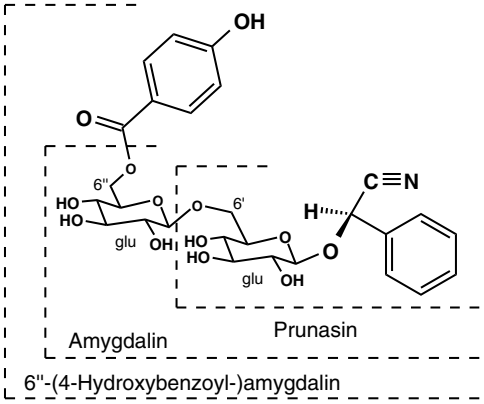
6.1.2 Occurrence in the Convolvulaceae

Phenylethylamine turned out to be rather rare as a constituent in the Convolvulaceae (Eich, unpublished results). It was detected in the epigeal vegetative parts of *Argyreia nervosa* (BURM. f.) BOJ., *Ipomoea alba* L., *I. turbinata* LAG., and *Xenostegia medium* (L.) D.F.AUSTIN & STAPLES. Tyramine was identified in *Bonamia spectabilis* (CHOISY) HALL. f., *I. eremnobrocha* D.F.AUSTIN, *I. turbinata*,



N-Acetyl-phenylethylamine (2-Benzyl)methylaziridine

Merremia quinquefolia



Merremia dissecta

Fig. 6.2 Phenylalanine-derived convolvulaceous metabolites with a retained *C*- **and** *N*-skeleton of the respective biogenic amine. The position of the methyl group of (2-benzyl) methylaziridine remains to be determined though it would be plausible to suppose C-3 **if** *N*-acetylphenylethylamine represents the precursor

M. umbellata (L.) HALL., and *Odonellia hirtiflora* (MART. & GAL.) K.ROB. (Tofern 1999). Together with three simple though unknown or rare *N*-acylated derivatives (formyl, acetyl, propionyl; Fig. 6.2) phenylethylamine could be determined by GC/MS in the basic extract of the epigeal vegetative parts of *Merremia quinquefolia* (L.) HALL. f. Only *N*-(2-phenylethyl)acetamide had been described once as a constituent of the volatile oil of *Robinia pseudacacia* L., Fabaceae (Li et al. 1993). In addition to these amides, three unusual components were characterized as 2-benzyl-, (2-benzyl)methyl-, and (2-benzyl)-hydroxymethyl-aziridine in the extract from *M. quinquefolia*. The position of the respective methyl and hydroxymethyl group of the latter two compounds could not be elucidated due to concentrations which were too low for any isolation. However, it might be assumed that 2-benzyl- and (2-benzyl)methylaziridine were formed by cyclization of corresponding *N*-acylphenylethylamines since formation of artefacts could be excluded (Fig. 6.2; Mann et al. 1996; Mann 1997). *N*-Formyl- and *N*-acetyl-phenylethylamines were also detected in epigeal vegetative parts of *Hewittia sublobata* (L. f.) O. KUNTZE, however only after infestation by mites, (Henrici 1996) and *Xenostegia medium* (Tofern 1999).

6.2 Cyanogenic Glycosides

6.2.1 Discovery, Distribution in the Plant Kingdom, Ecological Significance

Cyanogenic glycosides are predominantly (mono)glucosides. The most famous exception is represented by amygdalin (Fig. 6.2), the gentiobioside (1 → 6-diglucoside) of D(−)-mandelonitrile. It was isolated and crystallized from the seeds of the bitter variety of the almond tree, *Prunus dulcis* (MILL.) D.A. WEBB. var. *amara* (DC.) BUCHHEIM sub nom. *Amygdalus communis* L. var. *amara* (Rosaceae), already by Robiquet and Boutron-Charlard (1830). Wöhler and Liebig (1837) could prove that "emulsin", a mixture of enzymes (glycosidases, mandelonitrilase), – also a constituent of bitter almonds though separated by compartmentalization – in the presence of water was able to cleave amygdalin yielding benzaldehyde, hydrocyanic acid, and glucose. Thus, chewing of bitter almonds leads to disruption of the compartmentalization with the consequence that the nitrile group is released as toxic hydrocyanic acid. Amygdalin itself is not poisonous. The corresponding monoglucoside prunasin, an intermediate in amygdalin biosynthesis as well as in its catabolism, could be detected as a major component in different *Prunus* spp., e.g., *P. laurocerasus* L., cherry-laurel.

Cyanogenic glycosides are widely distributed in the plant kingdom, present in more than 2500 species including ferns, gymnosperms, and angiosperms involving about 110 families (Trease and Evans 2002; Zagrobelny et al. 2004). They play an important role in plant defence against herbivores as a two-edged sword: They are (i) characterized by an immediate bitter taste as intact metabolites (repellent poison) and (ii) toxic after cleavage forming HCN (respiratory poison). Hydrocyanic acid (hydrogen cyanide, prussic acid) is dangerous due to the affinity of CN^- for the terminal cytochrome oxidase in the mitochondrial respiratory pathway (lethal dose 35–150 µmol kg^{-1}; Zagrobelny et al. 2004 and references therein). However, certain insects feed on plants containing cyanogenic glycosides since they have acquired the ability to metabolize these glycosides. Others even sequester them for their predator defence. It may be concluded that cyanogenic glycosides are plesiomorphic characters since they were found throughout the plant kingdom though many taxa apparently lost the ability to synthesize them. For reviews on the different topics see Conn (1991), Lechtenberg and Nahrstedt (1999), and Zagrobelny et al. (2004).

6.2.2 Occurrence in the Convolvulaceae

In contrast to the Solanaceae, which seem to have lost the ability to synthesize cyanogenic glycosides, there are a number of reports on the occurrence of these compounds in the Convolvulaceae. These reports often documented only presence of

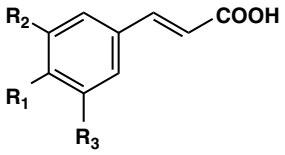
such metabolites in principle. Already indicated by a marzipan-like smell of freshly crushed plant material, this may be objectified by the proof of HCN in different assays, e.g., the so-called Feigl-Anger test (Tantisewie et al. 1969). Already at the end of the nineteenth and the beginning of the twentieth century a few convolvulaceous species were assumed to be cyanogenic (Hegnauer 1964 and references therein). Such early reports lacked any detailed structural elucidation with the exception of a meanwhile pantropical vine of American origin, *Merremia dissecta* (JACQ.) HALL. f. sub nom. *Ipomoea sinuata* ORTEGA for which benzaldehyde and hydrocyanic acid could be proven (Van Romburgh 1893). Indeed, 90 years later prunasin and its 6′-*O*-malonyl derivative could be isolated from the leaves of this species (Nahrstedt et al. 1989), whereas its seeds revealed amygdalin, its 6″-(4-hydroxy)benzoate (Fig. 6.2), and its 6″-(4-hydroxy)-*E*-cinnamate (Nahrstedt et al. 1990). The three cyanogenic glycosides characterized by acylation of a glucose unit at the hydroxyl of C-6 represented novel natural compounds. A paleotropical species, *M. vitifolia* (BURM. f.) HALL. f. (erroneously named "*M. ficifolia*"), was also found long ago to be cyanogenic (Weehuizen 1906). Again 90 years later, prunasin could be isolated from its *fresh* leaves, whereas *dried* leaves only revealed benzoic acid, presumably formed by oxidation of benzaldehyde after decomposition of prunasin. The seeds contained a cyanogenic compound which corresponded in its chromatographic behaviour (TLC, HPLC) to amygdalin (Jenett-Siems et al. 1995; Jenett-Siems 1996).

A number of *Ipomoea* spp. (*I. alba*, *I. batatas*, *I. littoralis*, *I. obscura*, *I. pes-caprae*, *I. triloba*, *I. tuba*; authorities see Table 3.2) were also found to be cyanogenic though without structural identification (Hegnauer 1964, 1989 and references therein; Kaplan et al. 1983). Furthermore, the seeds of *I. habeliana*, endemic to the Galapagos Islands, turned out to be cyanogenic (89 nmol HCN/g fresh wt; Adsersen et al. 1986). Finally, this was also true for the seeds and leaves of the Madagascan endemic species *Stictocardia mojangensis* (VATKE) D.F. AUSTIN & EICH (Austin and Eich 2001). However, numerous other convolvulaceous species belonging to many genera, e.g., *Calystegia*, *Convolvulus*, *Ipomoea*, *Jacquemontia*, *Merremia*, *Operculina*, *Turbina* grown in the greenhouse did not reveal the characteristic smell when their fresh leaves were crushed (Jenett-Siems 1996; Henrici 1996; Mann 1997; Eich, unpublished results). In this connection it should be added that the marzipan-like smell is a property of hydrocyanic acid itself as well as of benzaldehyde.

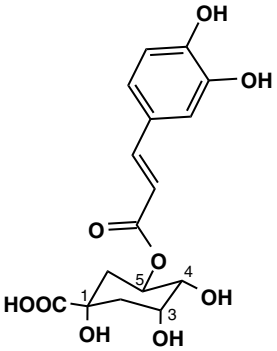
Thus, it may be concluded that cyanogenic glycosides are no frequent constituents in the family Convolvulaceae.

6.3 Cinnamate, Hydroxycinnamates and their Derivatives (Phenylpropanoids Sensu Latiore)

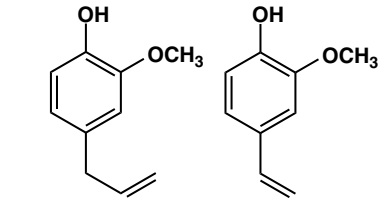
Cinnamic acid and Hydroxycinnamic acids. The biosynthetic sequence cinnamic acid → *p*-coumaric acid → caffeic acid → ferulic acid → 5-hydroxy-ferulic acid → sinapic acid (Fig. 6.3) includes progressive hydroxylation at the



R_1	R_2	R_3	
H	H	H	Cinnamic acid
OH	H	H	p-Coumaric acid
OH	OH	H	Caffeic acid
OH	OCH_3	H	Ferulic acid
OH	OCH_3	OH	5-Hydroxyferulic acid
OH	OCH_3	OCH_3	Sinapic acid
H	OH	OH	3,5-Dihydroxycinnamic acid

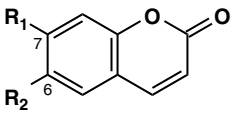


Chlorogenic acid
(5-O-caffeoylquinic acid)



Eugenol 4-Vinylguaiacol

Ipomoea pes-caprae (Convolvulaceae)



R_1	R_2	
OH	H	Umbelliferone
OH	OH	Aesculetin
OH	OCH_3	Scopoletin

Fig. 6.3 Central phenylpropanoids and certain derivatives

aromatic nucleus followed by *O*-methylation with the exception of the hydroxyl group at C-4. Thus, all these derivatives of cinnamic acid share the complete structural elements of *p*-coumaric acid which is of special significance with regard to their common chemical properties. They are summarized by the term

hydroxycinnamic acids (HCA) and occur predominantly as *trans* (*E*)-isomers. Their *cis* (*Z*) isomers may be formed spontaneously by light or by enzymatic isomerization (Strack 1997). Not only free hydroxycinnamates but also their *O*-glucosides are common metabolites of higher plants. The carboxylic group of free cinnamic acid and its hydroxy derivatives must be activated via synthesis of coenzyme-A thioesters before any derivatization or modification is possible. The resulting hydroxycinnamoyl-CoA derivatives are central intermediates in the biosynthesis of many phenylpropanoids (Bernards and Lewis 1998). Alternatively, hydroxycinnamate 1-*O*-acylglucosides (1-*O*-hydroxycinnamoylglucose) may serve as acyl donor molecules in the biosynthesis of *O*-esters (Strack 1997) e.g., 1-*O*-caffeoyl-β-glucose (see Sect. 6.6.3).

Hydroxycinnamates as well as their conjugates may act as powerful antioxidants. In edible plants they have received much attention as protecting agents against oxidative deterioration of food. Antioxidant mechanism studies on ferulic acid and its coupling products with linoleate on the molecular level have been conducted recently. A radical scavenging reaction occurred at the 3′-position of the ferulate radical with four types of peroxyl radicals of ethyl linoleate. The produced peroxides subsequently underwent intramolecular rearrangement to afford stable tricyclic peroxides (Masuda et al. 2006).

p-Coumaryl, coniferyl, and sinapyl alcohols are the monomeric constituents of lignin, the stabilizing polymeric structural elements of cell walls in plants. Dimerization of one of these monolignols, namely coniferyl alcohol, leads to lignans (Sect. 6.8). Formation of monolignols proceeds by stepwise reduction of the respective hydroxycinnamate, e.g., ferulic acid → feruloyl-CoA → coniferaldehyde → coniferyl alcohol.

6.3.1 Phenylpropanoids Sensu Stricto (C_6C_3 Skeleton)

6.3.1.1 Phenylpropanoid Acids (Phenylacrylic Acids)

Occurrence in the Solanaceae. *p*-Coumaric acid, caffeic acid, methyl caffeate, and methyl ferulate as well as certain of their 2,3-dihydro derivatives have been identified as constituents of the leaves of *Cestrum parqui* L'HERIT. with good phytotoxic activity against different species (D'Abrosca et al. 2004). Family-specific phenylpropanoid acids like tropic acid or 2-hydroxytropic acid as acyl moieties of tropane alkaloids are synthesized via phenylalanine → phenylpyruvic acid → (*R*)-3-phenyllactic acid (Fig. 3.14; Table 3.1 (**T5–T7-B**)]. Tropic acid may occur as a metabolite of, e.g., hyoscyamine, but the *free* acid is not synthesized as such (for details see Sect. 3.4).

Occurrence in the Convolvulaceae. In contrast to the Solanaceae common hydroxycinnamoyl residues form acyl moieties of ester type tropane alkaloids in this family (Table 3.2 (**T5**, **T10**)).

6.3.1.2 Phenylpropanoid Aldehydes (Phenylacryl Aldehydes), Phenylpropanoid Alcohols (Phenylallyl Alcohols), Phenylpropenes

Occurrence in the Solanaceae. In contrast to the corresponding HCA their reduced derivatives as well as HCA esters show volatile properties. For a number of these metabolites see Sect. 6.3.4.

Occurrence in the Convolvulaceae. The phenylpropene eugenol (Fig. 6.3), discovered as the dominating component of essential clove oil {obtained from the dried flower buds of *Syzygium aromaticum* (L.) MERR. & L.M. PERRY [syn.: *Eugenia caryophyllus* (SPRENG.) BULLOCK & S.G. HARRISON], Myrtaceae}, was isolated as a constituent of the leaves of *Ipomoea pes-caprae* (L.) BR., beach morning glory. It was detected by bioassay-guided fractionation (prostaglandin synthesis inhibiting properties) (Pongprayoon et al. 1991). This finding confirmed the anti-inflammatory activity of the crude extract of the leaves which previously was proven based on its use in traditional medicine of many tropical countries (see also Sect. 6.3.3).

Addition. Cesternosides. Two unusual glucosides, cesternosides A and B, were discovered in the leaves of *Cestrum nocturnum* L. Congener A turned out to be the 1*O*-β-D-glucopyranoside of 1,2,4-trihydroxy-6-(1-methylpropyl)benzene; B represented a derivative of A acetylated at C-6 of the glucosyl moiety (Sahai et al. 1994).

6.3.2 Phenylethanoids (C_6C_2 Skeleton)

6.3.2.1 Phenylethanoid Acids

Occurrence in the Solanaceae. Phenylacetic acid is integrated as an acyl moiety into the structure of certain ester type tropane alkaloids (Table 3.1 (**T7-C**)).

Occurrence in the Convolvulaceae. In this family phenylacetic acid is integrated as an acyl moiety into the structure of certain ester-type pyrrolizidine alkaloids (Sect. 3.7; Fig. 3.30).

6.3.2.2 Phenylethanoid Aldehydes, Phenylethanoid Alcohols, Phenylethenes

Occurrence in the Solanaceae. 2-*p*-Hydroxyphenylethanol and its 2-oxo congener (*p*-hydroxyphenyl-hydroxymethyl-ketone) have been identified as constituents of the leaves of *Cestrum parqui* L'HERIT. with good phytotoxic activity against different species (D'Abrosca et al. 2004). Aldehydes and alcohols of the C_6-C_2 subgroup also show volatile properties in contrast to the corresponding acids. For a number of these metabolites see Sect. 6.3.4.

Occurrence in the Convolvulaceae. The phenylethene 4-vinylguaiacol (Fig. 6.3) was characterized as another constituent of the leaves of *Ipomoea pes-caprae*. It was detected by the same procedure as described above for eugenol, thus including its prostaglandin synthesis inhibiting properties (Pongprayoon et al. 1991).

6.3.3 Phenylmethanoids (C_6C_1 Skeleton): Benzoates, Hydroxybenzoates, and their Derivatives

p-Hydroxybenzoic acid, protocatechuic acid (3,4-dihydroxybenzoic acid), vanillic acid (4-hydroxy-3-methoxybenzoic acid) are widespread in the plant kingdom. However, their concentration is rather low compared with the corresponding hydroxycinnamic acids (Klick and Herrmann 1988 and references therein). Salicylic acid (*o*-hydroxybenzoic acid) mediates plant defences against pathogens as a signal compound. It accumulates in infected and distal leaves in response to pathogen attack and is necessary for the biosynthesis of defensive metabolites associated with local and systemic acquired resistance (Wildermuth et al. 2001 and references therein).

Side-chain degradation of hydroxycinnamates by removal of acetate or acetyl-CoA is supposed to proceed in the biosynthesis of (hydroxy)benzoates. Whether this would reveal (i) the corresponding C_6C_1 aldehyde, e.g., benzaldehyde in case of cinnamic acid, vanillin (4-hydroxy-3-methoxybenzaldehyde) in case of ferulic acid, or alternatively (ii) the corresponding CoA-activated C_6C_1 carboxylic acid, e.g., benzoyl-CoA and vanilloyl-CoA, respectively, may depend on the specific plant species. The final substitution pattern may be determined not only by the C_6C_3 precursor, but alternatively caused by hydroxylation/methylation of an already formed C_6C_1 compound (Strack 1997). Recently, in vivo studies on benzenoid metabolism in *Petunia* × *hybrida* petal tissue revealed that both (i) the CoA-independent, non-β-oxidative pathway as well as (ii) the CoA-dependent, β-oxidative pathway contribute to the biosynthesis of volatile benzenoid metabolites in flowers of this species. The flux through pathway (i) turned out to be about two times higher than the one through pathway (ii) (Boatright et al. 2004). The non-oxidative pathway to benzoic acid does not function in *Nicotiana attenuata* Torr. ex S.Wats. The pathway from cinnamic acid to salicylic acid via benzoic acid was shown to be involved in stress-induced flowering of *Ipomoea nil* (L.) Roth sub nom. *Pharbitis nil* (L.) Choisy. Benzoic acid 2-hydroxylase, a soluble enzyme from tobacco, catalyzes salicylic acid biosynthesis (Mustafa and Verpoorte 2005 and references therein). However, an alternative pathway could be proven: Isochorismate synthase turned out to be required to synthesize salicylic acid for plant defence [*Arabidopsis thaliana* (L.) Heinh., thale cress (Brassicaceae)] (Wildermuth et al. 2001). This alternative pathway implicates that salicylic acid is not synthesized via chorismate → L-arogenate → L-phenylalanine.

The role of salicylic acid as a defense response (systemic acquired resistance) inducing signal molecule in plants was reviewed, e.g., by Klessig and Malamy (1994) and Vernooij et al. (1994).

6.3.3.1 Occurrence in the Solanaceae

A number of substituted benzoic acids, methyl benzoates, and benzaldehydes (substituents: 3-OH; 3-OCH_3; 4-OH; 4-OH + 3-OCH_3; 4-OH + 3,5-di-OCH_3) has been identified as constituents of the leaves of *Cestrum parqui* L'Herit. (D'Abrosca et al. 2004). These compounds showed a good phytotoxic activity against different species. *p*-Hydroxybenzoic acid 4-β-D-glucoside and vanillic acid 4-β-D-glucoside as well as 1-*O*-vanilloyl-D-glucose turned out to be constituents of *Capsicum annuum* L. fruits (commercial paprika powder; Klick and Herrmann 1988). Both glucosides together with salicylic acid 2-β-D-glucoside were also detected in fungus-infected leaves of *Solanum tuberosum* (Keller et al. 1996). After exposure to the cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), the leaves of *S. lycopersicum* L. sub nom. *Lycopersicum esculentum* Mill., tomato, rapidly accumulated a high level of salicylic acid accompanied by an increase of H_2O_2 (Peng et al. 2004). The significance of salicylic acid in the pathogen resistance of tobacco, *Nicotiana tabacum*, was studied, e.g., by Hennig et al. (1993), Silverman et al. (1993), and Yalpani et al. (1994).

Addition. Arbutin. 4-*O*-β-D-Glucopyranosylhydroquinone (arbutin), discovered as a major metabolite of the leaves of *Arctostaphylos uva-ursi* (L.) Spreng., bearberry (Ericaceae), was detected as one of the soluble phenolics in fungus-infected leaves of *S. tuberosum* (Keller et al. 1996). Its aglycone hydroquinone (1,4-dihydroxybenzene) represents a "C_6C_0 derivative" of the benzenoid metabolism (oxidative decarboxylation of *p*-hydroxybenzoic acid).

C_6C_1 / C_6C_2 / C_6C_3 Plant Volatiles (PVs). An update on biochemistry of plant volatiles with regard to all respective classes and groups has been published recently (Dudareva et al. 2004), followed by another review with a specific focus on enzymatic aspects of the biosynthesis of PVs (Pichersky et al. 2006). Volatile C_6C_1 / C_6C_2 / C_6C_3 compounds ("benzenoids") are common constituents of floral scent (Knudsen et al. 1993). Phenylmethanoids, phenylethanoids, and phenylpropanoids (sensu stricto) represent three groups of metabolites, beside mono- and sesquiterpenoids as well as *N*-containing volatiles, which play also a significant role in fragrance chemistry of flowers from a number of hawk moth-pollinated *Nicotiana* spp. as attractants. Metabolites characterized in seven species of this genus include:

(i) C_6C_1/C_6C_2/C_6C_3 aldehydes [e.g., benzaldehyde, salicylaldehyde, phenylacetaldehyde (= benzenacetaldehyde), cinnamic aldehyde]
(ii) C_6C_1 / C_6C_2 / C_6C_3 alcohols [e.g., benzyl alcohol, phenylethyl alcohol (= 2-phenylethanol), cinnamic alcohol]
(iii) Esters of C_6C_1/C_6C_2/C_6C_3 *alcohols* (e.g., benzyl acetate, 2-phenylethyl acetate, cinnamyl acetate)
(iv) Esters of C_6C_1/C_6C_3 *acids* (e.g., methyl benzoate, benzyl benzoate, methyl salicylate, methyl cinnamate)

Such metabolites were detected in the floral volatiles of *N. rustica* (sect. *Rusticae*), *N. suaveolens* (sect. *Suaveolentes*), *N. sylvestris* (sect. *Alatae* s.l.), *N. langsdorffii*,

N. bonariensis, *N. forgetiana*, and *N. alata* (sect. *Alatae* s.s.). On the other hand, no C_6C_1/C_6C_2/C_6C_3 compounds could be found as constituents of the floral volatiles in case of *N. longiflora* and *N. plumbaginifolia* (sect. *Alatae* s.l.; authorities see Table 3.4. Independent of the class/group of components all species emitted more fragrance at night, especially benzenoids (if at all produced) (Raguso et al. 2003). Timing of rhythmic odour emissions of a remarkable number of benzenoids by flowers of *Petunia axillaris* (Lam.) Britt., Sterns & Poggenb. was also shown to be in tune with nocturnal hawk moth activity. Flower-volatile composition turned out to be adapted to the antennal perception of the pollinators, *Manduca sexta* L. (Lepidoptera: Sphingidae), tobacco hornworm. The dominating compounds were benzaldehyde, benzyl alcohol, and methyl benzoate beside some minor components – diverging dependent on the accession – like benzyl acetate, benzyl benzoate, methyl salicylate, benzyl salicylate, vanillin, eugenol, and isoeugenol. Further minor components were characterized as phenylethanoids (phenylacetaldehyde, 2-phenylethanol) and the aliphatic metabolite *Z*-9,17-octadecadienal. In contrast, benzaldehyde was the only component obtained from flowers of *P. integrifolia* (Hook.) Schinz & Thell., a bee-pollinated wild species (Hoballah et al. 2005). Like already established for *Nicotiana*, moth-pollinated species produce qualitatively and quantitatively rich blends of volatiles for attraction, whereas bee or hummingbird pollinated species produce less scent (Raguso et al. 2003; Hoballah et al. 2005). Methyl salicylate was found to contribute also to the aroma of tomatoes and potatoes (Herrmann 1978).

A number of enzymes that catalyze the formation of floral volatiles were identified and characterized, e.g., benzoyl-CoA:benzyl alcohol/phenylethanol benzoyltransferase capable of catalyzing the formation of benzylbenzoate and phenyl benzoate in petunia. This latter identification was part of a report with the aim to establish a benzenoid network in *Petunia* × *hybrida* (Hook.) Vilm. (garden petunia) which represents a hybrid between the two wild *Petunia* spp. mentioned above. This paper shows in its Fig. 1 (legend: "Proposed biosynthetic pathways leading to some benzenoid compounds in petunia") a comprehensive overview with numerous chemical structures on biogenetic relationships of the phenylpropanoid metabolism (C_6C_1/C_6C_2/C_6C_3 compounds; Boatright et al. 2004 and references therein).

6.3.3.2 Occurrence in the Convolvulaceae

Benzoic acid was detected, e.g., in the leaves of *Merremia vitifolia* (Jenett-Siems 1996) as well as in the roots of *Ipomoea purpurea*, two *Jacquemontia* spp., and *Merremia aegyptia* (Henrici 1996). *p*-Hydroxybenzoic acid could be proven, e.g., in 14 out of 29 convolvulaceous species (Nair et al. 1986). Salicylic acid was detectable, e.g., in eight out of 10 species (genera: *Hewittia, Ipomoea, Jacquemontia, Merremia, Operculina*) (Henrici 1996).

p-Hydroxybenzoic acid, vanillic acid (4-hydroxy-3-methoxybenzoic acid), veratric acid (3,4-dimethoxybenzoic acid), 4-hydroxy-3-prenyl-, 4-hydroxy-3-methoxy-5-prenyl-, and 4-hydroxy-3,5-diprenyl-benzoic acid (nervogenic acid) are of

significance as moieties of ester-type tropane alkaloids (Figs. 3.19–3.22; Tables 3.2 (**T3**, **T4**, **T6-B**), 3.5, and 3.6). However, it is unknown, whether these acids are synthesized (i) as free metabolites in the plants conjugated afterwards to 3-hydroxytropanes or (ii) by progressive substitutions of a primarily synthesized alkaloid, e.g., 3-(4-hydroxybenzoyloxy)-tropane, with the final result of compounds like merresectine C (Fig. 3.21). Salicylic acid is integrated as an acyl moiety into the structure of certain pyrrolizidine alkaloids (Sect. 3.7; Fig. 3.30).

A rare glycoside, benzyl-[O-β-D-apiofuranosyl-(1→6)]-β-D-glucopyranoside, discovered as one of the major metabolites of benzoic acid added to cultures of *Lemna paucicostata* HEGELM., Lemnaceae (Suzuki et al. 1988) and also found as a constituent of *Pyrus bourgaeana* DECNE, Rosaceae (Bilia et al. 1994), could be isolated from the epigeal vegetative parts of *Merremia aegyptia* (L.) URB. (Henrici 1996).

Addition. Ellagic acid, a dimer of gallic acid and belonging to the gallotannin subclass ellagitannins, was isolated from the leaves of *Ipomoea batatas* LAMK., sweet potato (Terashima et al. 1991). This finding was surprising since such compounds are unusual for Solanales taxa. Gallic acid is synthesized by plants directly via shikimic acid, i.e., not via phenylalanine.

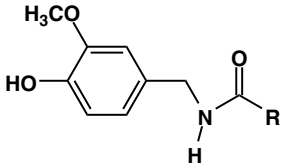
6.4 Capsaicinoids

6.4.1 Discovery and Structure Elucidation

Capsaicinoids are unique secondary metabolites; their occurrence is confined to the solanaceous genus *Capsicum*. The characteristic pungent taste of fruits from this genus is caused by a group of ~25 metabolites named capsaicinoids (Fig. 6.4). This term is based on capsaicin which frequently represents the dominating alkaloid. Capsaicinoids are among the most widely used secondary metabolites, since roughly a quarter of the world's population consume chillies each day (Tewksbury et al. 2006 and references therein). The genus *Capsicum* is of American origin (see Sect. 6.4.2). However, by the middle of the seventeenth century, *C. annuum* had become cultivated throughout southern and middle Europe to Asian and African tropical and subtropical regions as a spice and/or medicinal drug (Suzuki and Iwai 1984).

An extensive review entitled "Constituents of red pepper species: Chemistry, biochemistry, pharmacology, and food science of the pungent principle of *Capsicum* species" was published by Suzuki and Iwai (1984). A comprehensive monograph on *Capsicum* has been edited by De (2003).

The pungent principle of *Capsicum* was first isolated in 1876 by Tresh (1876), who assigned to it the name capsaicin. From Cayenne pepper obtained on the market Nelson (1919) isolated 12 g of crude capsaicin from 40 pounds of pepper. A vanilla like odour caused by treating an alcoholic solution of capsaicin with platinic



Trivial names	Acyl residues	R =
Capsaicin	8-Methylnon-6-enoyl	$(CH_2)_4$-CH=CH-CH(CH_3)-CH_3
(6,7-)Dihydrocapsaicin	8-Methylnonanoyl	$(CH_2)_6$-CH(CH_3)-CH_3
*Nor*capsaicin	7-Methyloct-5-enoyl	$(CH_2)_3$-CH=CH-CH(CH_3)-CH_3
*Nor*dihydrocapsaicin I	7-Methyloctanoyl	$(CH_2)_5$-CH(CH_3)-CH_3
*Nor*dihydrocapsaicin II (*anteiso*)	6-Methyloctanoyl	$(CH_2)_4$-CH(CH_3)-CH_2-CH_3
*Homo*capsaicin I	9-Methyldec-7-enoyl	$(CH_2)_5$-CH=CH-CH(CH_3)-CH_3
*Homo*capsaicin II (*anteiso*)	8-Methyldec-6-enoyl	$(CH_2)_4$-CH=CH-CH(CH_3)-CH_2-CH_3
*Homo*dihydrocapsaicin I	9-Methyldecanoyl	$(CH_2)_7$-CH(CH_3)-CH_3
*Homo*dihydrocapsaicin II (*anteiso*)	8-Methyldecanoyl	$(CH_2)_6$-CH(CH_3)-CH_2-CH_3
*Nornor*capsaicin	6-Methylhept-4-enoyl	$(CH_2)_2$-CH=CH-CH(CH_3)-CH_3

Fig. 6.4 Branched-chain capsaicinoids, acid amide type alkaloids from *Capsicum* spp.; all unsaturated acyl residues are *trans*-configurated (*E*-form). Straight-chain and further congeners see text

chloride and hydrochloric acid suggested the presence of a vanillin residue in capsaicin. However, Nelson could demonstrate that capsaicin was an acid amide of a decenic acid with vanillylamine. A few years later, the final constitution was determined to be 8-methylnon-6-enoyl-vanillylamide (Nelson and Dawson 1923). In the latter study it was also observed that hydrogenated capsaicin was as pungent as capsaicin itself showing that a double bond was not necessary for pungency. This 6,7-dihydrocapsaicin (8-methylnonanoyl-vanillylamide) could be discovered as a natural congener by Kosuge et al. (1958, 1961). The name "capsaicinoid" was assigned to the mixture of capsaicin and its dihydro derivative by these authors. However, later this term has been used for capsaicin and its congeners in general. The constitutions of both compounds including the *trans* configuration of capsaicin were re-confirmed by Bennett and Kirby (1968). Afterwards, the crystallized pungent principle was fractioned by TLC into four components adding to the already known two capsaicinoids two novel congeners which turned out to be homologues of dihydrocapsaicin: *Nor*dihydrocapsaicin [*N*-(vanillyl)-7-methyloctanamide] and *homo*dihydrocapsaicin [*N*-(vanillyl)-9-methyldecanamide] (Kosuge and Furuta 1970). Further branched-chain congeners are listed in Fig. 6.4 (Suzuki and Iwai 1984; Jurenitsch and Kastner 1994 and references therein). In addition to mono-*nor* capsaicinoids integrated there, also di-*nor* and even tri-*nor* derivatives, e.g., *nornornor*-dihydrocapsaicin, have been discovered (Zewdie and Bosland 2001 and references therein). Furthermore, capsaicinoids with straight-chain fatty acyl residues (C_8–C_{13})

have also been characterized as constituents of *Capsicum* fruits (Suzuki and Iwai 1984 and references therein). For details about the nonanoyl derivative nonivamide see Sect. 6.4.5.

Glycosides. Capsaicin β-D-glucopyranoside and dihydrocapsaicin β-D-glucopyranoside have been discovered recently in the fruits of various pungent cultivars of *C. annuum*, *C. chinense*, and *C. frutescens* (Higashiguchi et al. 2006). They represent the first capsaicinoid glucosides in nature. However, they constitute only a very small quantity of the total capsaicinoid content in the fruits.

6.4.2 Botanical Aspects

Based on classical morphological taxonomy, Bosland (1994) recognized 22 wild and five domesticated *Capsicum* species. According to Hunziker (2001) the genus represents a natural assemblage of ca. 20 species and a few varieties. He described four centres of diversity for these taxa in detail between southern USA and Central Argentina. Furthermore, he recognized three species for cultivated peppers:

- *C. annuum* L. with two or three varieties (var. *annuum*, var. *frutescens*), related to the wild species *C. chacoënse* HUNZ. and *C. annuum* var. *glabriusculum*. "The cultivars of peppers cultivated all over the world belong to *C. annuum* with a few exceptions in favour of *C. baccatum*" (Hunziker 2001). Apparently, this author nested *C. chinense* JACQ. and *C. frutescens* L., two cultivated species frequently recognized to be independent by other authors, within *C. annuum*
- *C. baccatum* L. with three varieties (var. *baccatum*, var. *praetermissum*, var. *umbilicatum*), two of them also known from the wild; a species with very hot fruits, common as "Ajis" in Bolivia, Peru, Paraguay, North Argentina, and Brazil; meanwhile extended to Mexico, Hawaii, and India (Thampi 2003)
- *C. pubescens* RUIZ & PAV.; very limited consumption (Thampi 2003)

More than 2000 cultivars derived from these 3 or 5 of the wild species have been developed (Tewksbury et al. 2006 and references therein). These authors estimated a total number of 23–27 wild species.

A study with 11 *Capsicum* species on phylogenetic relationships using DNA sequences from two noncoding regions (chloroplast *atpB-rbcL* spacer region and nuclear *waxy* introns) revealed monophyly of the genus though with moderate support (Walsh and Hoot 2001). *C. ciliatum* (H.B.K.) O.KUNTZE turned out to be sister of the remaining 10 *Capsicum* spp. Their monophyly was strongly supported; however, this core clade turned out to be largely unresolved. Based on previous enzyme studies, hybridization studies, and their DNA analysis Walsh and Hoot proposed an informal classification as follows:

- *Ciliatum* group (*C. ciliatum*)
- *Eximium* group (*C. eximium* HUNZ., *C. cardenasii* HEISER & SMITH)
- *Baccatum* group (*C. baccatum* L., *C. chacoënse* HUNZ.)

- *Annuum* group (*C. annuum* L., *C. chinense* JACQ., *C. frutescens* L., *C. galapagoense* HUNZ.)
- Unassigned group (*C. tovarii* ESHBAUGH, SMITH & NICKREN, *C. pubescens* RUIZ & PAV.)

From the economic-botanical point of view comprehensive descriptions of cultivated *Capsicum* species and varieties, discussions on hybrids as well as taxonomic aspects have been compiled and discussed recently by Basu and De (2003).

6.4.3 Occurrence

Capsaicinoids were detected in all *Capsicum* spp. with one exception. The basal species *C. ciliatum*, sister of all other, more derived *Capsicum* spp. (see Sect. 6.4.2), is never pungent (Walsh and Hoot 2001and references therein). Thus, production of capsaicinoids represents a monophyletic apomorphic character. However, pungency is not consistently present in more ancestral taxa (*Eximium* group, *Baccatum* group): Several *wild* non-pungent forms of *C. chacoënse* were also found (Walsh and Hoot 2001 and references therein). Recently, it has been demonstrated in a report entitled "Where did the chili get its spice? Biogeography of capsaicinoid production in ancestral wild species" that beside *C. chacoënse* two further wild species, *C. baccatum* and *C. eximium*, are polymorphic for production of capsaicinoids: completely pungent and completely non-pungent individuals of these three species co-occurred in some populations (Tewksbury et al. 2006). However, above an altitude of 900 m almost all individuals of *C. chacoënse* turned out to be pungent. Implications with regard to the ecology and evolution of capsaicinoids in ancestral *Capsicum* spp. exhibiting polymorphisms for its production have been discussed in detail by the authors.

Commercial fruits cultivated in Europe and northern America were usually obtained from different cultivars of *C. annuum* var. *annuum*, those in tropical Asia and Africa from *C. chinense*/*C. frutescens* and in Brazil from *C. baccatum* var. *pendulum* (Jurenitsch and Kastner 1994). Bell pepper is obtained from non-pungent cultivars of *C. annuum* var. *grossum*.

The fruits of *C. annuum* varied in their content of the major components capsaicin and dihydrocapsaicin in a range of 77–90%; the corresponding values for *C. frutescens* were determined in a range from 89 to 98% (Díaz et al. 2004 and references therein). An extensive study on the distribution of seven capsaicinoids within nearly 200 accessions of 6 *Capsicum* spp. (*C. annuum, C. baccatum, C. chacoënse, C. chinense, C. frutescens, C. pubescens*) revealed that the capsaicinoid profiles are not good chemotaxonomic indicators. The distribution and the percentage of the capsaicinoids turned out to be inconsistent within a species. Thus, it was not unequivocally possible to identify a species by a certain metabolite profile though this had been proposed previously by other authors (Zewdie and Bosland 2001 and references therein). Furthermore, it could be demonstrated

that capsaicin and dihydrocapsaicin are frequently but not always the major metabolites. On the opposite, major capsaicinoids of certain *C. pubescens* accessions were the isomer of dihydrocapsaicin and homodihydrocapsaicin.

6.4.4 Biosynthesis (Fig. 6.5)

Capsaicinoids are localized in the vacuole of the epidermal cells of placentas and septa of *Capsicum* spp. (adjacent to the seeds). In *C. annuum* their accumulation was found to reach the maximum, when the size of the fruits reached the maximum (Ishikawa 2003 and references therein). In contrast, the production in fruits of *C. frutescens* was reported to take place after the increase in fruit length had ceased (Sukrasno and Yeoman 1993). Biosynthesis of capsaicinoids has not been elucidated completely. Tritium-labelled L-phenylalanine and vanillylamine were found to be precursors in feeding experiments with ripening seed pods of *C. annuum* (Bennett and Kirby 1968). Vanillylamine is supposed to be generated from vanillin catalyzed by a putative aminotransferase. A high pungent *Capsicum* variety had 147 times more vanillylamine than a low pungent one in a study which has determined certain putative intermediates (Prasad et al. 2006a). This amine, representing the consistent moiety of all capsaicinoids, is one of the substrates of capsaicin(oid) synthase which

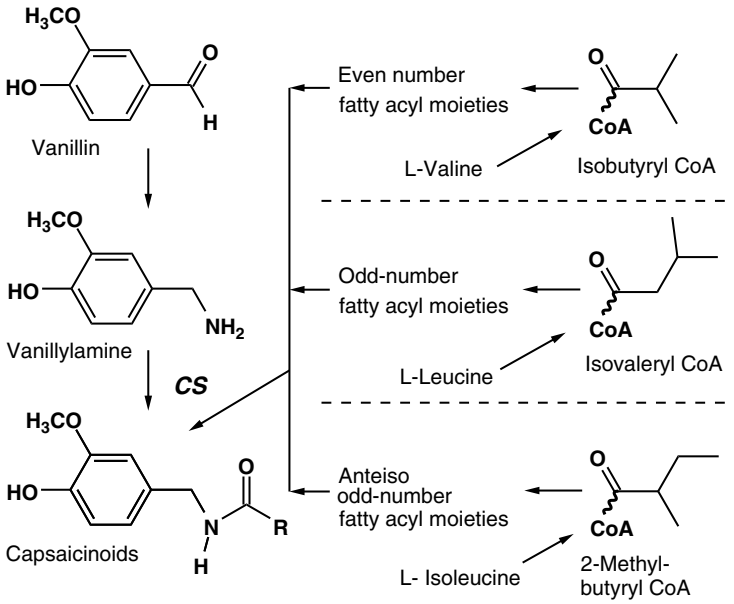


Fig. 6.5 Biosynthesis of branched-chain capsaicinoids starting with a branched amino acid, e.g., L-valine → 2-oxovalerate → isobutyryl CoA → elongation (+ 3 × malonyl CoA) → [− 3 × CO_2] → (activated) 8-methylnonanoic acid + vanillylamine → dihydrocapsaicin [R = $(CH_2)_6CH(CH_3)CH_3$]; ***CS*** = capsaicin(oid) synthase

catalyzes the conjugation with varying – predominantly branched-chain – fatty acids, e.g., 8-methyl-6-nonenoic acid, to form the respective amides. The second substrate of this enzyme is represented by one of several C_8–C_{11} branched-chain fatty acids. They may be even-numbered (C_8, C_{10}), derived from L-valine → 2-oxovalerate → isobutyryl CoA, and odd-numbered (C_9, C_{11}), derived from L-leucine → 2-oxoisocaproate→ isovaleroyl CoA (Ishikawa 2003 and references therein; Manirakiza et al. 2003 and references therein). Isobutyryl CoA and isovaleroyl CoA, respectively, act as starter molecules for successive elongation by 2–3 malonyl CoA according to fatty acid biosynthesis (Ravishankar et al. 2003 and references therein). Furthermore, 2-methylbutyryl CoA – derived from L-isoleucine via 2-oxo-3-methylvalerate – may lead to *anteiso* odd-numbered derivatives (Suzuki and Iwai 1984), i.e., congeners which possess their methyl branch in the ω −2 position instead of a terminal branch. Capsaicin synthase has been characterized recently. Functionality of the corresponding gene (csy1) has also been demonstrated through heterologous expression in recombinant *Escherichia coli* (Prasad et al. 2006b). 8-Methylnonanoic acid is assumed to be the precursor of 8-methylnon-6-enoic acid (catalyzed by 8-methylnonanoic acid dehydrogenase) (Prasad et al. 2006c). Further biosynthetic considerations, e.g., with regard to enzymes in the earlier stages of the pathway, have been summarized recently by Diaz et al. (2004 and references therein).

Metabolism of Capsaicinoids. Turnover and degradation during fruit development is possible. The role of peroxidases in capsaicinoid oxidation has been reviewed in detail recently (Diaz et al. 2004 and references therein). 5,5′-Dicapsaicin and 4′-*O*-5-dicapsaicin ether were obtained from the oxidation of capsaicin by pepper B_6 peroxidase/H_2O_2 (radical-radical coupling reactions). Indeed, a first capsaicinoid dimer, 6″,7″-dihydro-5′,5‴-dicapsaicin, has been discovered as a constituent of *C. annuum* fruits (Ochi et al. 2003).

Addition. Capsinoids. From the fruits of a non-pungent cultivar (CH-19 Sweet) of *C. annuum* Kobata et al. (1998, 1999) could isolate ***N*-free** analogues of three major capsaicinoids. They were characterized as esters of vanillyl alcohol and the same fatty acids, which represent the acyl residues in capsaicin, dihydrocapsaicin, and nordihydrocapsaicin, respectively. Consequently, these esters were named capsiate (vanillyl *trans*-8-methyl-non-6-enoate), dihydrocapsiate, and nordihydrocapsiate. Furthermore, the authors proposed the term *capsinoids* for this novel group of metabolites, which could be detected also in several further cultivars of the species.

6.4.5 Significance

6.4.5.1 Use in Diets

In English speaking countries small-fruited, pungent *Capsicum* commercial varieties are named “chillies”, while the larger-fruited but less pungent varieties are known as “capsicums” or “paprika”. Of the total world consumption of *Capsicum*

fruits chillies account for one third, whereas paprika comprises two thirds. World production of chillies is estimated to be 2.5 million tonnes with an increasing tendency every year due to the fact that they are meanwhile incorporated into cuisines worldwide (Thampi 2003). Fruits of *Capsicum* spp. are known by different common names (in addition to chillies/paprika) such as chili pepper, (hot) red pepper, Cayenne pepper, tabasco, bell pepper, pimento, aji, and many others (Basu and De 2003; Thampi 2003). Assigning of these popular names to certain taxa is not always unequivocal.

This led also to confusion in the nomenclature of oleoresins, a term used for desolventized total extracts by a specified solvent, from different origin. Therefore, the Essential Oils Association of USA issued guidelines according to certain colour values, colour descriptions (carotenoids; see Sect. 7.12), and pungency/bite intensity. Thus, e.g., oleoresin paprika is characterized by negligible pungency and deep red colour (colour value: 40,000–100,000) in contrast to oleoresin *Capsicum* [very high pungency, clear red/light amber colour (colour value: 4000 max.)] (Thampi 2003). The following oleoresins are of importance:

- Oleoresin *Capsicum* (origin: fruits of *C. frutescens*), the most pungent one, used in pharmaceutical preparations
- Oleoresin red pepper (*C. annuum*), used in products of food industries, e.g., in canned meats, sausages etc. due to its colour and pungency
- Oleoresin paprika (*C. annuum*), used as a food colouring agent, e.g., in processed meats, dairy products, soups, sauces etc. (Ravishankar et al. 2003); negligible pungency
- Oleoresin chilli (*C. annuum*), highly pungent
- Oleoresin bird's eye chilli ("bird chilli") (*C. annuum* var. *minimum*), highly pungent (Thampi 2003)

An organoleptic method for measurement of pungency was established by Scoville (1912). He expressed the greatest dilution at which pungency could be recognized as the reciprocal of the dilution in Scoville Heat Units (SHU: 0–5000 = mild; 5000–20,000 = medium; 20,000–70,000 = hot; 70,000–300,000 extremely hot). Pure capsaicin and dihydrocapsaicin were measured to possess 16×10^6 SHU; nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin possess only half the pungency. Chemical methods for the measurement of capsaicinoids have also been used, e.g., by HPLC. A linear regression due to a standardized procedure could be developed between SHU and capsaicin content allowing a conversion. Chilli cultivars vary from 0 to 300,000 SHU corresponding to a content of up to 2.0% capsaicinoids (Ravishankar et al. 2003 and references therein). Recently, Associated Press published in daily newspapers that the most pungent cultivar, Bhut Jolokia Chili, characterized by a SHU value of more than 1.0 million (corresponding to > 6.66% capsaicinoids) has been developed in India, thus exceeding the previous leader "Red Savina" by almost the double.

Domestication of *Capsicum* might have taken place 10,000–12,000 years ago in the Americas. "This places chilli among the oldest cultivated crops of the Americas" (Basu and De 2003 and references therein). Recently, starch microfossils (objects

of an archaeological study including seven sites dating from 6000 years ago to first European contacts and ranging from the Bahamas to Peru) could be assigned to domesticated chillies still used nowadays. "Neither microfossils typical of wild species nor transitional forms of *Capsicum* were recovered from any site" (Perry et al. 2007).

6.4.5.2 Therapeutic Use

Chillies (powdered crude drug/oleoresins and other extracts), capsaicin or capsaicinoids are used internally in atonic dyspepsia and flatulence. However, their use for external purposes is apparently more significant, e.g., for the relief of pains against rheumatic diseases, lumbago, post-herpetic neuralgia or diabetic polyneuropathy in the form of plasters, ointments, creams or liniments (Trease and Evans 2002 and references therein; Ravishankar et al. 2003).

The crude drug "Capsici fructus" is integrated into many pharmacopoeias as a monograph, e.g., according to the regulations of the European Pharmacopoeia (Ph. Eur.) the dried, ripe fruits of *C. annuum* var. *minimum* (Mill.) Heiser and/or small-fruited varieties of *C. frutescens* L. Furthermore, other monographs include different extracts, e.g., "Capsicum oleoresin, refined and quantified" ["Capsici oleoresina raffinata et quantificata" (Ph. Eur.)] and "Capsicum tincture, standardised" ["Capsici tinctura normata" (Ph. Eur.)]. A synthetic analogue, nonivamide INN [desmethyldihydrocapsaicin], already known since 1919, is used for the same indications. It has been and still is also added occasionally, in varying concentrations, to commercial Capsicum oleoresins as an adulterant; this is also true for spice industries. However, it could be confirmed that nonivamide is also a natural minor constituent of *Capsicum* fruits. The percentage compared to all capsaicinoids was 0.25% (Constant and Cordell 1996 and references therein).

A meta-analysis found that topically applied capsaicin reduced pains caused by diabetic neuropathy, osteoarthritis, and psoriasis, whereas it was less effective in reducing pain from postherpetic neuralgia (Zhang et al. 1994). Another meta-analysis with the similar objective to determine the efficacy of topically applied capsaicin for chronic pain from neuropathic or musculoskeletal disorders based on different blind placebo controlled and pooled trials led to the conclusion that it is better than placebo. It has been concluded that it may be useful as an adjunct or sole therapy for a limited number of patients who are unresponsive to, or intolerant of, other treatments (Mason et al. 2004).

6.4.5.3 Pharmacology and Toxicology

Pharmacological studies on gastrointestinal tract, cardiovascular and respiratory systems, thermoregulation, as well as nociception and anaesthesia were summarized by Suzuki and Iwai (1984). Relationship between pungency and chemical structure of capsaicin and its natural as well as synthetic analogues was also a topic

of that review. Some crucial aspects have been demonstrated recently by natural congeners. ω-Hydroxycapsaicin turned out to be a non-pungent natural capsaicinoid (Ochi et al. 2003). The pungency of capsaicin glucoside was only 1/100 that of capsaicin (Higashiguchi et al. 2006 and references therein), apparently due to the fact that an intact vanillyl residue is another stringent structural requirement for pungency (Szolcsányi 2003 and references therein). A third crucial point is the presence of the acid amide moiety; capsinoids, the ester analogues of capsaicinoids, are non-pungent (Kobata et al. 1998, 1999).

Mechanisms of Action and Capsaicin Receptor. The potent pungent, pain inducing effect of capsaicinoids is absent in non-mammalian species such as frogs or birds, where nociceptive protective reflexes were not evoked. This may elucidate, why pungent wild *Capsicum* fruits in southern Arizona seemed to be consumed exclusively by birds. Thus, capsaicin(oids) favour(s) birds in pepper seed dispersal (Díaz et al. 2004 and references therein). Recently, results of a field test with marked fruits have supported the *directed deterrence hypothesis* in two species of wild chillies, *C. chacoënse* and *C. annuum*, both containing capsaicinoids (Levey et al. 2006). Fruit removal occurred only during the day by birds, when (granivorous) rodents are inactive. The authors providing the first support for the directed deterrence hypothesis in naturally occurring fruits concluded "…. that fruiting plants distinguish between seed predators and seed disperser by producing fruits that repel the former and attract the latter".

In mammalians capsaicin "…. binds to nociceptors in the skin, causing an initial excitation of the neurons and a period of enhanced sensitivity, …. perceived as itching, pricking, or burning, with cutaneous vasodilatation" (Mason et al. 2004). Capsaicin was found to stimulate sensory nerve fibres (afferent C fibres) and release and subsequently deplete selectively substance P, a neuropeptide, from the primary afferent neurons and their terminals. The effect is mediated by agonism at the capsaicin receptor (VR1 = vanilloid receptor subtype 1) (Caterina et al. 1997; Szolcsányi 2003 and references therein). This is an excitatory cation channel expressed by sensory neurons of the pain pathway (Siemens et al. 2006). When activated, the channel opens leading to an influx of calcium and sodium ions. Consequently, pain fibres depolarize and initiate a nerve impulse through the dorsal root ganglion to the brain. "Noxious temperature uses the same elements, explaining why our mouths feel hot when we eat chilli peppers" (Clapham 1997). Therefore, VR1 was assumed to function as a transducer of painful thermal stimuli in vivo (Caterina et al. 1997). Rat (rVR 1) as well as human (hVR1) capsaicin receptors have been already cloned. Since it has turned out that VR1 belongs to the nociceptive membrane proteins of the TRP (transient receptor potential) superfamily (Caterina et al. 1997), it has been renamed TRPV1 recently. The present knowledge on TRPV1/VR1 and the future perspectives of capsaicin as a lead structure for the development of a new generation of analgesic–anti-inflammatory agents as well as new trends in the peptidergic neuroregulatory functions of capsaicin-sensitive afferents have been summarized comprehensively by Szolcsányi (2003 and references therein). Furthermore, the mechanism of sensory desensitization

induced by capsaicin and the role of VR1 receptors in brain has been discussed in detail. Desensitization of the nerve response may be the predominant mechanism by which capsaicin is acting (Clapham 1997).

Toxicology. Capsaicin turned out to be an excitatory neurotoxin that selectively destroys afferent nociceptors in vivo and in vitro. It could be proven that it killed cells that express TRPV1/VR1 (Caterina et al. 1997 and references therein). Capsaicinoids may show acute fatale toxicity when a large dose is given at one time (Suzuki and Iwai 1984 and references therein). Neurotoxicity, cytotoxicity, and genotoxicity have been reviewed recently by Manirakiza et al. (2003).

Capsaicin and Prostate Cancer. Recently, capsaicin has been shown to have profound antiproliferative effect on different prostate cancer cells due to novel mechanisms of action. This compound, when given orally, significantly slowed the growth of prostrate cancer xenografts as measured by size and weight (Mori A et al. 2006). The results of this study suggest that capsaicinoids may have a role in the treatment of prostate cancer.

Health Benefits. Dietary capsaicin has been suggested to be useful for the *prevention* of cancer, e.g., human colon cancers based on experimental results obtained with male rats (Yoshitani et al. 2001). Capsaicinoids increase the resistance of isolated LDL (low density lipoprotein) cholesterol and/or oils and fats to oxidation, when incubated together. This is caused by delaying the initiation and/or slowing the rate of oxidation. For the first time such effects have been checked on whole serum thus reflecting the in vivo situation more closely than isolated LDL. It has been shown that oxidation of serum lipids has been reduced also. A 50% increase of the lag time (time before initiation of oxidation) could be determined in a concentration of ~0.6 µM capsaicin and dihydrocapsaicin. Furthermore, the rate of oxidation (slope of propagation phase) decreased with increasing concentrations of the capsaicinoids (Ahuja et al. 2006).

6.4.5.4 Other Uses

There are many patents on pesticides, insecticides, insect control agents/insect repellents, and animal-repellents (e.g., dogs, cats from garbage bags, feed containers) containing capsaicinoids. Furthermore, this is true for formulations to sterilize and disinfect surfaces or to kill bacteria on contact with regard to food and meat stuffs as well as for food and meat processing equipment and facilities (Neumann 2001). It has been shown that capsaicin had excellent toxicity and control effect on *Myzus persicae* SULZER, green peach aphid (Homoptera: Aphididae); synergistic effects with synthetic insecticides could be observed (Liu and Lin 2003). In another study with the same aphid it was reported that capsaicinoid-containing extracts provided low levels of mortality alone, but acted synergistically in mixtures with other biorational insecticides, e.g., pyrethrins, leading to higher than expected levels of mortality using laboratory conditions.

However, under field conditions these insecticides did not provide significant levels of control of aphids (Edelson et al. 2002). Phytotoxicity of capsaicin used as an insecticide on greenhouse-grown herbs turned out to be less than a number of other insecticides (Cloyd and Cycholl 2002).

Furthermore, pepper spray is a lachrymatory agent used as a weapon for defence against human or animal attacks. This weapon is also called Oleoresin Capsicum (OC) spray or just Capsicum spray. In many countries there exist restrictions for personal self-defence, e.g., possession of pepper spray requires a license or is even not allowed. However, it is of considerable significance for authorities (police etc.).

6.5 Hydroxycoumarins

Simple hydroxycoumarins such as umbelliferone (7-hydroxycoumarin), aesculetin (6,7-dihydroxycoumarin), scopoletin (7-hydroxy-6-methoxycoumarin) are common constituents of plants (Fig. 6.3). Some rutaceous, apiaceous, and solanaceous species accumulate large amounts. Their substitution patterns derived probably from *p*-coumaric acid, caffeic acid, and ferulic acid, respectively, via (i) the corresponding 2-hydroxy derivatives, (ii) glucosidation of this hydroxyl group, (iii) isomerization of the *E*-form (*trans*) to its *Z*-isomer (*cis*), (iv) final enzymatic deglucosidation, and (v) spontaneous lactonization. Steps (iv) and (v) might happen after harvest as a consequence of tissue/cell disruption (wilted material following disruption of the compartimentation). At least, this is the proved pathway for unsubstituted coumarin itself. On the other hand, it is also possible that the hydroxy/methoxy substitution patterns are determined at the coumarin level (Strack 1997). Hydroxycoumarins are involved in plant defence reactions (e.g., Baumert et al. 2001 and references therein).

Umbelliferone (**U**) and aesculetin (**A**) were named after their discovery in species belonging to the family Umbelliferae (nowadays: Apiaceae) and in *Aesculus hippocastanum* L., horse chestnut (Sapindaceae), respectively. Scopoletin (**S**) and its glucoside scopolin were named after their discovery in the roots of *Scopolia japonica* (Solanaceae) (Eijkman 1884; Schmidt 1890).

6.5.1 Occurrence in the Solanaceae

Simple hydroxycoumarins, especially scopoletin and/or its glucoside scopolin, could be detected in many species, e.g., *Atropa belladonna* L. (**U**, **S**; Mothes and Kala 1955), *Brunfelsia* L. spp. (Hegnauer 1973 and references therein), *Fabiana imbricata* Ruiz & Pav. (**S**; Schmeda-Hirschmann et al. 2004), *Nicotiana tabacum* L. (**S**, **A**; Hegnauer 1973 and references therein), *Schulthesianthus leucanthus* (Donn. Sm.) Hunz. sub nom. *Markea megalandra* D'Arcy (syn.: *M. leucantha*

DONN. SM.) (**S**; Lopez 1980), *Solanum pinnatisectum* DUN. (**S**, **A**; Harborne 1960). Beside the glucoside scopolin the corresponding disaccharidic primveroside (xylosyl-1 → 6-glucoside), fabiatrin, was discovered in branches/leaves of *F. imbricata*. The latter metabolite was also detected in the roots of *Petunia* × *hybrida* (HOOK.) VILM. Furthermore, both position isomer glucosides of aesculetin were found, aesculin (6-*O*-glucoside) as well as cichoriin (7-*O*-glucoside) (Hegnauer 1973 and references therein). The latter metabolite, named after its discovery in *Cichorium intybus* L., chicory (Asteraceae), was detected in the flowers of *S. pinnatisectum* for the first time in this family (Harborne 1960). It was assumed that solanaceous species without hydroxycoumarins do not exist though the extent of accumulation can be widely divergent (Hegnauer 1973). Inoculation of tobacco leaves, *N. tabacum*, with tobacco mosaic virus as well as infection of potato tubers, *S. tuberosum*, by the fungus *Phytophthora infestans* (MONT.) DE BARY (Phytiaceae), causing late blight disease, induced scopolin accumulation (Baumert et al. 2001 and references therein).

6.5.2 Occurrence in the Convolvulaceae

Simple hydroxycoumarins, especially scopoletin and its glucoside scopolin, could be detected in many species, e.g., *Convolvulus puricaulis* CHOISY (**S**; Deshpande and Srivastava 1969), *C. arvensis* L. (**U**; Khalil et al. 1981), *C. lanatus* VAHL (**U**, **S**; El-Nasr 1982), *Cressa cretica* L. (**S**; Khalil et al. 1981), *Ipomoea batatas* (L.) LAM. (**U**, **S**, **E**; Minamikawa et al. 1964), *I. tricolor* CAV. cv. 'heavenly blue' (**S**; Shimizu et al. 2004).

Further detections of umbelliferone as well as of scopoletin/scopolin have been documented in different species of the following nine genera (number of species in brackets): *Calystegia* (2), *Convolvulus* (6), *Cuscuta* (1), *Ipomoea* (4) (Trumm 1991); *Convolvulus* (1), *Ipomoea* (3), *Merremia* (1) (Weigl 1992); *Ipomoea* (4) (Kayser 1994); *Calystegia* (1), *Convolvulus* (13), *Ipomoea* (5), *Merremia* (3) (Jenett-Siems 1996); *Bonamia* (1), *Hewittia* (1), *Ipomoea* (2), *Jacquemontia* (3), *Merremia* (2), *Operculina* (1) (Henrici 1996).

De novo synthesis of umbelliferone as well as its glucoside skimmin, scopoletin/scopolin, and aeculetin in sweet potato (*I. batatas*) roots/tubers attacked by the black rot fungus, *Ceratocystis fimbriata*, was reported by Minamikawa et al. (1964). Thus, these coumarins are not only constitutive components of this plant but also phytoalexins. However, they play a minor role in contrast to certain sesquiterpenoid phytoalexins which are also synthesized in such a situation (see Sect. 7.3). *I. tricolor* cv. 'heavenly blue' was found to accumulate rapidly scopoletin and scopolin after interaction with a non-pathogenic isolate of the fungus *Fusarium oxysporum* (Shimizu et al. 2004).

An unusual derivative, cresoside, the 7-*O*-β-D-glucoside of a "7,4′-dihydroxy-5-methoxy-coumaranochromone", was discovered as a constituent of the fruits of *Cressa cretica* L. (Ahmed 1998).

6.6 Hydroxycinnamate Conjugates/Caffeic Acid Derivatives

Hydroxycinnamate conjugates are defined as condensation products of hydroxycinnamates (preserving their C_6C_3 structure) with – predominantly – alkanols (Sect. 6.6.1) or alcoholic hydroxyl groups of other molecules e.g., quinic acid (Sect. 6.6.3), thus forming esters or with amines forming acid amides (Sect. 6.6.4). Furthermore, beside others hydroxycinnamates may acylate sugars (Sect. 6.6.2) and monosaccharide units in different types of glycosides (e.g., Sects. 6.7.1 and 6.7.3). A distinctive tendency to accumulate caffeic acid derivatives is another family character of the Convolvulaceae beside the accumulation of resin glycosides (Sect. 8.3) (Hegnauer 1964). They share the former tendency with the Solanaceae and other related families in contrast to the latter, family-specific one.

6.6.1 Long Chain Alkyl Esters of Hydroxycinnamic Acids

6.6.1.1 Occurrence in the Solanaceae

Long chain alkyl esters of ferulic acid are common constituents in the family. From the seeds of *Hyoscyamus niger* even a C_{24} diester, 1,24-tetracosanediol diferulate could be isolated (Ma et al. 2002). *Solanum tuberosum* started to accumulate long chain alkyl (mono)esters three to seven days after wound treatment. The alcohol components ranged from hexadecyl ($C_{16}H_{33}$) to octacosyl ($C_{28}H_{57}$) {all even numbers plus two esters of odd chain length alkanols [nonadecyl ($C_{19}H_{39}$), heneicosyl ($C_{21}H_{43}$)]}. The major metabolites were represented by hexadecyl and octadecyl ferulates. The authors suppose that the formation of all these ferulates is temporally and spatially correlated with suberin formation since they were restricted to the wound periderm (Bernards and Lewis 1992). For a coherent account of suberin chemistry interested readers are directed to a review on the macromolecular aromatic domain in suberized tissues (Bernards and Lewis 1998).

6.6.1.2 Occurrence in the Convolvulaceae

Long chain alkyl esters of *p*-coumaric acid and/or ferulic acid are common constituents in the family. From the fruits of *Argyreia populifolia* Choisy octadecyl *p*-coumarate (sub nom. stearyl 4-hydroxycinnamate) could be identified (Gunatilake and Sultanbawa 1973). Alkyl (C_{16}, C_{17}, C_{18}) ferulates were reported as constituents of the roots of *Ipomoea batatas*, sweet potato (Kawanishi et al. 1990). In co-occurrence to these ferulates the corresponding *p*-coumarates were determined in the epigeal vegetative parts of *Convolvulus canariensis* L., with straight-chain alcohol components (hexadecanol, heptadecanol, octadecanol) as well as their branched-chain isomers, probably 13-methyl-pentadecanol and 15-methylheptadecanol (in case of isoheptadecanol undetermined) (Trumm 1991). A similar pattern could be detected for *Ipomoea purpurea*. In this case branched-chain isomers were absent; however, in addition

straight-chain eicosanyl congeners could be characterized, though in much lower concentration than their C_{16} and C_{18} homologues (Jenett-Siems 1996). Octadecyl caffeate was isolated from the roots of *Merremia tuberosa* and a branched isomer, 6-methylheptadecyl caffeate, from the roots of *M. dissecta* (García-Argáez et al. 1999).

Further detections of such alkyl *p*-coumarates and ferulates have been documented in the following genera (number of species in brackets): *Convolvulus* (2), *Ipomoea* (1) (Weigl 1992); *Calystegia* (1), *Convolvulus* (13), *Ipomoea* (5), *Merremia* (3) (Jenett-Siems 1996); *Bonamia* (1), *Hewittia* (1; ferulates only), *Ipomoea* (1), *Jacquemontia* (3), *Merremia* (2), *Operculina* (1; *p*-coumarates only) (Henrici 1996). Trichanthins, esters with unsaturated acyl components have been isolated from the leaves of *Bonamia trichantha*: (2*E*,6*E*)-3,7,11-trimethyl-2,6,10-dodecatrienyl caffeate (trichanthin A) and *p*-coumarate (B) as well as (11*Z*)-11-hexadecenyl caffeate (C) and *p*-coumarate (D) (Hussein et al. 2005).

Cuscutin, a metabolite of the holoparasitic *Cuscuta lehmanniana* Bge. growing on elms (*Ulmus* L., Ulmaceae), turned out be an ester with an unusual substitution pattern in its acyl residue, namely hexadecyl 3,5-dihydroxycinnamate (Fig. 6.3). This metabolite could not be detected in the host (Kamilov and Nikonov 1977). Such a rare 3,5-dihydroxycinnamoyl moiety is also present in an anthocyanin of *Ipomoea asarifolia* (Table 6.1).

6.6.2 Hydroxycinnamoyl Glucose Esters and O-Glucosides

Monosaccharide (e.g., glucose) esters with hydroxycinnamic acids (HCA) are fairly common in all dicotyledons. Such esters with *p*-coumaric acid or ferulic acid were found in the Solanaceae, alternatively with caffeic acid in the Convolvulaceae (Mølgaard and Ravn 1988). 1-*O*-*p*-Coumaroyl-β-D-glucose and its feruloyl analogue were detected in leaves of *Nicotiana tabacum* L. and flowers of *Datura stramonium* L. Both metabolites and in addition their caffeoyl congener were found as constituents of flowers and/or leaves of *Brunfelsia pauciflora* var. *calycina* (Benth. ex DC.) J.A. Schmidt sub nom. *B. calycina* Benth. ex DC., *Petunia* × *hybrida* (Hook.) Vilm., and tuber-bearing *Solanum* spp. Caffeoylgentiobiose turned out to be another constituent of *P.* × *hybrida* (Herrmann 1978 and references therein). On the other hand, the 3-*O*-β-D-glucoside of caffeic acid was found in wild potato berries (Corner and Harborne 1960).

For acylation of monosaccharide (glucose) units in the carbohydrate chain of anthocyanins with HCA as well as glycosidic linkages to phenolic hydroxy groups of HCA see Sect. 6.7.3.

6.6.3 Chlorogenic Acid, Dicaffeoylquinic Acids, and Related Caffeic Acid Derivatives

Chlorogenic acid (Fig. 6.3) and similar derivatives, e.g., positional isomers or dicaffeoylquinic acids, were found in Asteraceae, Apiaceae, Brassicaceae, Malaceae, Rosaceae, Saxifragaceae, but they are also common in Solanaceae, Convolvulaceae,

and the genus *Coffea* (Rubiaceae) as an alternative distribution to disaccharide esters occurring mainly in Scrophulariaceae and Oleaceae (Mølgaard and Ravn 1988).

Trivial name	Partial trivial name
Chlorogenic acid	5-*O*-Caffeoylquinic acid
Cryptochlorogenic acid	4-*O*-Caffeoylquinic acid
Neochlorogenic acid	3-*O*-Caffeoylquinic acid
Isochlorogenic acid	3,5-di-*O*-Caffeoylquinic acid
	3,4-di-*O*-Caffeoylquinic acid
Cynarin	1,3-di-*O*-Caffeoylquinic acid

This numbering is according to IUPAC rules. It has to be taken into account that former reports might have used another numbering, e.g., chlorogenic acid had been 3-*O*-caffeoylquinic acid. Also in former times the term "isochlorogenic acid" was used for unseparated mixtures of isomeric dicaffeoylquinic acids. Finally, the question is still unresolved, whether all these isomers are naturally occurring metabolites or at least in part artefacts formed by transacylation during, e.g., isolation procedures (Friedman 1997 and references therein).

6.6.3.1 Occurrence in the Solanaceae

Chlorogenic acid was detected in altogether 37 species belonging to 14 genera (*Atropa, Brunfelsia, Capsicum, Cestrum, Datura, Hyoscyamus, Lycopersicum, Mandragora, Petunia, Physalis, Physochlaina, Scopolia, Solanum, Withania*) (Politis 1948). This report included detailed information on the distribution of the metabolite in *Capsicum annuum*, *Datura stramonium*, and *Solanum melongena*. The highest concentration was found in the fruits cells, whereas the seed embryo turned out to be devoid of it. This metabolite was also detected in many other solanaceous taxa, e.g., in cotyledons from tomato (*S. lycopersicum* sub nom. *Lycopersicon esculentum*) seedlings (Strack et al. 1987), stressed/wound healing potato (*S. tuberosum*) tubers (Bernards and Lewis 1992 and references therein), aerial parts of wild growing *Fabiana imbricata* Ruiz and Pav. plants (Schmeda-Hirschmann et al. 2004).

About 90% of the total phenolic compounds of potato tubers are constituted by chlorogenic acid (10–20 mg/100 g fresh weight; sprouts 750 mg/100 g) (Dao and Friedman 1992 and references therein). Different potato cultivars grown under the same conditions contained beside chlorogenic acid its isomers, cryptochlorogenic and neochlorogenic acids, as well as 3,4-dicaffeoylquinic and 3,5-dicaffeoylquinic acids. Antioxidative, antimutagenic, and anticarcinogenic effects of chlorogenic acids and other polyphenolic compounds in potato as well as further potential health beneficial effects were reviewed by Friedman (1997 and references therein). Chlorogenic, cryptochlorogenic, and neochlorogenic acid could be also identified in tobacco (*Nicotiana tabacum*) leaves (Baumert et al. 2001 and references therein). Even 3-feruloylquinic acid was found as a constituent of tobacco and tomato leaves (Herrmann 1978 and references therein).

A precursor-product relationship between chlorogenic acid and caffeoylglucaric acid, a constituent of tomato leaves, could be demonstrated. Glucaric acid represents the oxidation product of glucose at C-1 (CHO → COOH). Caffeoylglucaric acid could be characterized as 2-*O*- or 5-*O*-acylated product. In contrast, the biosynthesis of this metabolite in leaves of *Cestrum elegans* (BRONGN.) SCHLTDL. proceeds via 1-*O*-caffeoyl-β-glucose (Strack et al. 1987 and references therein).

6.6.3.2 Occurrence in the Convolvulaceae

A century ago chlorogenic acid was already established as a constituent of *Ipomoea batatas* LAMK., sweet potato, as well as of *Argyreia kurzei* BOERL., *Erycibe tomentosa* BL., *Lepistemon flavescens* BL., *Merremia dissecta* HALL. f., and *Porana paniculata* ROXB.

Ipomoea. Five decades after the first report on chlorogenic acid in *I. batatas* isochlorogenic and neochlorogenic acid could be found additionally in sweet potatoes (Hegnauer 1964 and references therein). Another four decades later, 3,5-dicaffeoylquinic acid was isolated for the first time from that species (Terashima et al. 1991). This compound was also characterized together with its 4,5-isomer in the seeds of *I. habeliana* OLIV., endemic to the Galapagos Islands (Trumm 1991). Chorogenic acid and 3,5-dicaffeoylquinic acid were detected in the seeds of *I. carnea* JACQ. ssp. *fistulosa* (CHOISY) D.F. AUSTIN sub nom. *I. fistulosa* MART. ex CHOISY (Sattar et al. 1995). Furthermore, chlorogenic acid and its dicaffeoyl congeners turned out to be the main components of *I. obscura* (L.) KER-GAWL. seeds beside minor amounts of macrolactam-type HCA amide alkaloids (see Sect. 6.6.4.2) (Jenett-Siems et al. 2003). Different novel metabolites have been discovered recently in two further *Ipomoea* species. 3,5-Di-*O*-caffeoyl-4-*O*-*p*-coumaroylquinic and 4,5-di-*O*-caffeoyl-1,3-di-*O*-*p*-coumaroylquinic acids turned out to be additional constituents of the leaves of *I. pes-caprae* L., goatsfoot convolvulus/beach or coastal morning glory, beside a number of known chlorogenic acids. These novel compounds were shown to inhibit the collagen decomposing enzyme collagenase (IC_{50} values: 14–19 μM). Thus, such compounds could be lead structures for the development of agents preventing aging of the skin (Teramachi et al. 2005). Another polyphenolic congener of dicaffeoylquinic acids, 4,5-di-*O*-caffeoyldaucic acid, has been discovered in the tubers of *I. batatas*. Interestingly, the antioxidative activity of this compound was higher than that of all standards, e.g., L-ascorbic acid, gallic acid, used at the same molar concentration (Dini et al. 2006). Recently, 3,4,5-tri-*O*-caffeoylquinic acid, another constituent of the leaves of this species, was demonstrated to possess potent antimutagenicity effects (Yoshimoto et al. 2002). Furthermore, it suppressed also the growth of certain human stomach and colon cancer cells by apoptosis (Kurata et al. 2007). These results indicated that this metabolite rather than other sweetpotato leaf polyphenolics may have potential for cancer prevention. The leaves are used as a vegetable in different Asian countries.

In case of *I. batatas*, the biosynthesis of chlorogenic acid proceeds via 1-*O*-caffeoyl-β-glucose in contrast to different solanaceous species e.g., *Nicotiana tabacum*, which used caffeoyl-CoA for that purpose (Strack et al. 1987 and references therein). Furthermore, it was shown that chlorogenic acid acted as the acyl donor in the biosynthesis of isochlorogenic acid in the roots of this species (Kojima and Kondo 1985) which was also shown to produce neochlorogenic acid.

Cuscuta. Nine species of the holoparasitic genus *Cuscuta* revealed characteristic patterns of soluble phenolic constituents that can be used as taxonomic markers. This is of special significance since many *Cuscuta* spp. are difficult to identify or distinguish from related ones (see also Sect. 6.7). Furthermore, the species-specific patterns proved to be stable independent of the host plant or different localities. *C. lupuliformis* KROCK, *C. pedicellata* LEDEB, and *C. reflexa* ROXB. accumulated significantly higher amounts of chlorogenic acid as well as 3,5- and 4,5-dicaffeoylquinic acid than of flavonoids. In contrast, *C. campestris* YUNCK., *C. chinensis* LAM., and *C. platyloba* PROGEL contained only small amounts of the former class of metabolites (flavonoids: ~90% of total phenolics). A third group of species, *C. europea* L., *C. odorata* RUIZ & PAV., and *C. gronovii* WILLD. revealed the ratio of both classes is approximately 1:1 (Löffler et al. 1997).

Other Genera. Chlorogenic acid was also isolated from the epigeal vegetative parts of *Convolvulus floridus* L.f. (Trumm 1991). This compound as well as 3,4- and 3,5-dicaffeoylquinic acids could be detected in all species investigated by Henrici (1996), i.e., *Bonamia semidigyna* var. *semidigyna* (ROXB.) HALL. f., *Hewittia sublobata* (L. f.) O. KUNTZE, *Ipomoea pes-caprae* (L.) R.BR. ssp. *brasiliensis* (L.) VAN OOSTSTR., *I. purpurea* (L.) ROTH, *Jacquemontia corymbulosa* BENTH., *J. paniculata* var. *paniculata* (BURM. f.), *J. tamnifolia* (L.) GRISEB., *Merremia aegyptia* (L.) URB., *M. dissecta* (JACQ.) HALL. f., *Operculina codonantha* (BENTH.) HALL. f. In another study this could be confirmed for further species (Jenett-Siems 1996): *Calystegia* (1 species), *Convolvulus* (13 spp.), *Ipomoea* (6 spp.), *Merremia* (4 spp.).

6.6.4 Hydroxycinnamic Acid Amides

Nowadays, hydroxycinnamic acid amides (HCAA) are considered as alkaloids like any natural amide though lacking basic properties. HCAA of tyramine, putrescine, and the polyamine spermidine were identified as the main phenolic constituents in the reproductive organs of a broad spectrum of angiosperms including *Solanum lycopersicum* sub nom. *Lycopersicum esculentum*, *Nicotiana tabacum*, and *Petunia* × *hybrida* (see below). It was suggested that they play a physiological role in flowering, since an increase in the amount of these amides in the apical part of *N. tabacum* plants at the time of floral induction was observed. Furthermore, they were shown to possess antiviral properties [tobacco mosaic virus (TMV)] in *N. tabacum*; they were formed after virus-infection as a protective mechanism (Martin-Tanguy

et al. 1978 and references therein). Antibiotic properties were also demonstrated (Sattar et al. 1990 and references therein).

6.6.4.1 *N*-Acyltyramines

Occurrence in the Solanaceae. Besides the examples mentioned already above there are reports on further occurrence in the family. *N-trans*-Feruloyltyramine (*E*-feruloyltyramine) and its octopamine [2-hydroxytyramine = 1-(*p*-hydroxyphenyl)-2-aminoethanol] congener as well as *N-trans-p*-coumaroyloctopamine were discovered in the roots of *S. melongena* L., eggplant/aubergine. Furthermore, the already known *N-trans-p*-coumaroyltyramine was detected in this sample (Yoshihara et al. 1978). All these compounds turned out to be also constituents of the roots of *Capsicum annuum* L. *var. grossum* SENDTN., bell pepper (Yoshihara et al. 1981). Both coumaroylamides were also found in *S. verbascifolium* L. (Zhou and Ding 2002). The biosynthesis of feruloyltyramine is catalyzed by feruloyl-CoA:tyramine *N*-feruloyl-CoA transferase. Its activity was increased in *N. tabacum* leaves five- to eightfold after infection by TMV (Negrel and Martin 1984). The level of *E*-feruloyltyramine and *E-p*-coumaroyltyramine increased 10-fold in tomato leaves in response to mechanical wounding and 25-fold in response to the oligosaccharide elicitor chitosan (Pearce et al. 1998). Apparently, they function as components of the chemical and physical defences (i.e., incorporation into polymers like suberin). Ether-linked feruloyltyramine and feruloyloctopamine were detected in suberin-enriched samples of natural and wound periderms of potato tubers. These ether-bridges were formed predominantly with the hydroxyl of the ferulic acid moiety but to a smaller extent also to the tyramine phenolic group (Negrel et al. 1996).

Addition. *N*-(*p*-Carboxymethylphenyl)-*p*-hydroxybenzamide. This unique metabolite has been discovered as a constituent of the leaves of *Cestrum parqui* L'HERIT. with good phytotoxic activity against different species (D'Abrosca et al. 2004).

Occurrence in the Convolvulaceae. *N-trans*-Feruloyltyramine and its *cis* isomer were detected, e.g., in the epigeal vegetative parts of *Ipomoea aquatica* (Tseng et al. 1986) and *I. purpurea* (Trumm 1991).

Further detections of *N-trans*-feruloyltyramine have been documented in the following species: *Convolvulus arvensis* L., *C. chilensis* PERS., *Ipomoea hederifolia* L., *I. littoralis* BOISS. sub nom. *I. stolonifera* (CYRILL.) J.F. GMEL., *Merremia tuberosa* (L.) RENDLE, *M. vitifolia* (BURM. f.) HALL. f. (Jenett-Siems 1996); *Bonamia semidigyna* var. *semidigyna* (ROXB.) HALL. f., *Hewittia sublobata* (L. f.) O. KUNTZE, *I. purpurea* (L.) ROTH, *M. aegyptia* (L.) URB., *M. dissecta* (JACQ.) HALL. f., *Operculina codonantha* (BENTH.) HALL. f. (Henrici 1996); *C. cneorum* L., *C. sabatius* VIV. ssp. *mauritanicus* (BOISS.) MURB., *I. regnellii* MEISN., *M. aurea* (KELL.) O'DONELL, *M. cissoides* (LAM.) HALL., *M. quinquefolia* (L.) HALL. f., *Stictocardia mojangensis* D.F.AUSTIN & EICH, *Turbina abutiloides* (H.B.K.) O'DONELL (Mann 1997).

6.6.4.2 *N*-Acyl-5-hydroxytryptamines

Occurrence in the Convolvulaceae. HCA conjugates of tryptamine as well as of its 5-hydroxy derivative serotonin are very rare in the plant kingdom. N_b-(*p*-Coumaroyl)- and N_b-(feruloyl)tryptamine were characterized as constituents of mature kernels of *Zea mays* L., sweet corn (Poaceae) (Ehmann 1974). Three indole alkaloids of a *unique* type, named ipobscurines B–D (Fig. 6.6), were discovered in the seeds of the common and widespread paleotropical perennial twining herb *Ipomoea obscura* (L.) Ker-Gawl. They were characterized as serotonin (5-hydroxytryptamine) HCA-type conjugates with a *second* phenylpropanoid moiety (*erythro*-7,8-dihydroxy-dihydroconiferyl alcohol) ether-linked to the 5-hydroxyl group of the indole nucleus. Due to an oxidative phenolic coupling

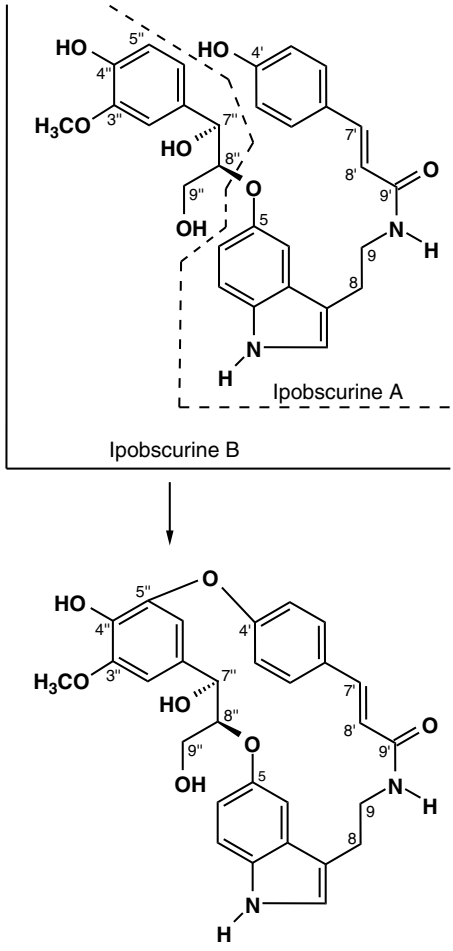


Fig. 6.6 Ipobscurine C, a macrolactam-type indole alkaloid from the seeds of *Ipomoea obscura* with a 4′.*O*.5″ neolignan moiety (biogenetic numbering), as well as its congeners A [N_b-(*p*-coumaroyl)serotonin] and B representing presumptive precursors; ipobscurine D = *cis* isomer of C

between the two phenylpropanoid moieties of the supposed precursor ipobscurine B two 21-membered macrolactams with a diaryl ether moiety are formed (4′.*O*.5″-type neolignan substructure), the *trans-cis* isomers ipobscurines C and D. All metabolites belonged to the *erythro* series (Eich et al. 1986, 1989; Weigl et al. 1991; Weigl 1992; Jenett-Siems et al. 2003). Ipobscurine A turned out to be N_b-(*p*-coumaroyl)serotonin, already known as constituent of safflower seeds [*Carthamus tinctorius* L. (Asteraceae)] (Sakamura et al. 1978, 1980). Its 5-*O*-β-D-glucopyranoside was also a constituent of both species.

The ipobscurines were only present in mature seeds. Neither flowers and undeveloped fruits nor epigeal vegetative parts and roots show any of these alkaloids. These compounds are synthesized apparently in the late phase of the seed development. Their biosynthesis probably proceeds as follows: tryptamine → serotonin → N_b-(*p*-coumaroyl)serotonin (ipobscurine A) → B → C/D (Jenett-Siems et al. 2003).

Ipobscurines could not be detected in 150 convolvulaceous species belonging to 23 genera with one exception: Seeds of *I. ochracea* (LINDL.) G.DON, another paleotropic perennial herb found throughout tropical Africa (Eich, unpublished results). This is interesting from the chemotaxonomic point of view, since *I. obscura* and *I. ochracea* are closely related species. They were shown to be sisters with 100% support in a DNA-based cladistic analysis (ITS and waxy sequences; Miller et al. 1999). In the traditional classification both are nested within sect. *Erpipomoea* CHOISY (Austin and Huáman 1996).

Compounds arising from the amide formation between serotonin and a HCA were observed – beside *C. tinctorius* – in two asteraceous species, *Centaurea moschata* L. (Sarker et al. 1997) and *C. cyanus* L., cornflower (Sarker et al. 2001). These metabolites showed significant toxicity in a brine shrimp lethality bioassay. A cyclization of such a conjugate between the side-chain of the HCA moiety and the indole nucleus at C-4 and C-5 of the latter could be observed in safflower seeds forming the phenol ether serotobenine (Sato et al. 1985). However, none of these asteraceous species contained serotonin HCA amides with a *second* phenylpropanoid moiety like the ipobscurines B–D. The neolignan substructure of compounds C and D is similar to that of another though spermine-based macrocyclic alkaloid, chaenorhine from *Chaenorhinum origanifolium* (L.) WILLK. & LGE (Scrophulariaceae) (Hesse 2000).

6.6.4.3 Polyamine-HCA Conjugates (Fig. 6.7)

HCAA of this group are frequent constituents in the plant kingdom. In a study which included *Nicotiana tabacum* L. they turned out to be significant radical scavengers; the HCA moiety might contribute to the role of polyamines as protectants against ozone damage (Bors et al. 1989). Such *N*-acylspermidines are known from pollen of the families, e.g., Acanthaceae, Amaryllidaceae, Betulaceae, Fagaceae, and Juglandaceae (Hesse 2000). They were also found in many other families (Bienz et al. 2002). With regard to the general role of these and similar HCA

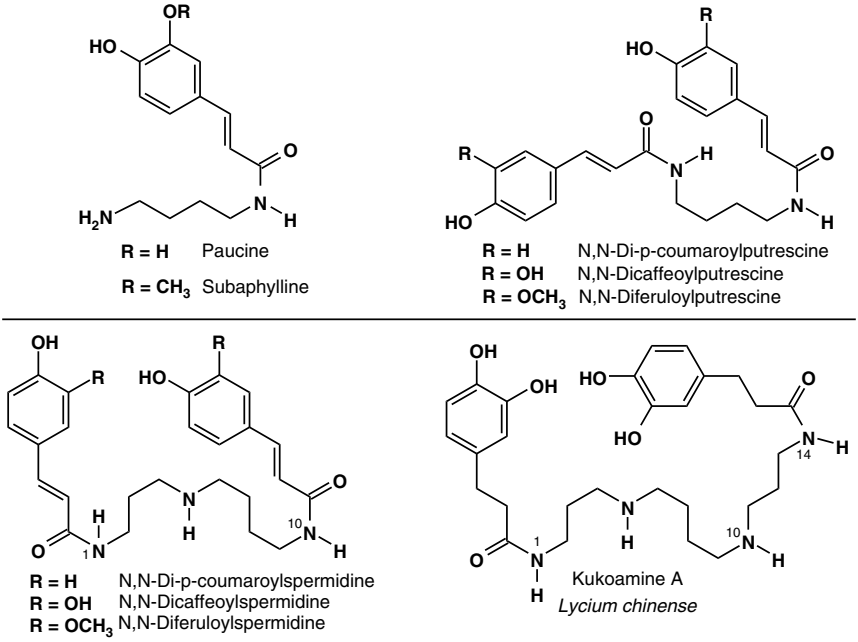


Fig. 6.7 Polyamine-HCA conjugates, constituents of *Nicotiana tabacum* and/or *Solanum lycopersicum* (*N*-acyl- and *N,N*-diacylputrescines; *N,N*-diacylspermidines), as well as of *Lycium chinense* (*N,N*-diacylspermine)

conjugated polyamines in plant defence and the generation of disease resistant plants readers are directed to a review of Walters (2003).

Occurrence in the Solanaceae. As already mentioned above, mono-acylated **HCA conjugates of putrescine** were detected as constituents of *Solanum lycopersicum* sub nom. *Lycopersicum esculentum*, *Nicotiana tabacum*, and *Petunia* × *hybrida* (Martin-Tanguy et al. 1978):

- *N-p*-Coumaroylputrescine in all of the three species
- *N*-Caffeoylputrescine [discovered in *Pentaclethra macrophylla* BENTH. (Fabaceae)] named paucine (Hesse 2000 and references therein), in *N. tabacum* and *P.* × *hybrida*
- *N*-Feruloylputrescine [discovery in *Salsola subaphylla* C.A.MEY (Chenopodiaceae)] named subaphylline (Ryabinin and Il'ina 1949), in all of the three species
- *N,N*-Di-*p*-coumaroylputrescine in *N. tabacum*
- *N,N*-Dicaffeoylputrescine not present in any of the three species
- *N,N*-Diferuloylputrescine in *S. lycopersicum* and *P.* × *hybrida*

HCA conjugates of spermidine were detected only in two of three species:

- *N,N*-Di-*p*-coumaroylspermidine in *S. lycopersicum* and *N. tabacum*
- *N*-Feruloyl- and *N,N*-diferuloylspermidine in *S. lycopersicum* (Martin-Tanguy et al. 1978).
- *N*-Caffeoyl- and *N,N*-dicaffeoyl-spermidine in the flower buds of *N. tabacum* (Herrmann 1978 and references therein)

There are reports on further occurrence in the family: paucine and subaphylline were found to be the most prominent phenolics of potato leaves infected with *Phytophthora infestans* (MONT.) DE BARY (Phytiaceae), responsible for the most destructive fungal disease (late blight) in *S. tuberosum* (Keller et al. 1996). Methyl jasmonate treatment of *N. attenuata* TORR. ex S.WATS. leaves elicited a 12.5-fold increase in case of paucine (Keinanen et al. 2001). Besides paucine and subaphylline the novel *N*1,*N*10-bis(dihydrocaffeoyl)spermidine was isolated from the epigeal vegetative parts of *Iochroma cyaneum* (LINDL.) M.L.GREEN (Sattar et al. 1990). *N*1,*N*14-Bis(dihydrocaffeoyl)spermine, named kukoamine A, was discovered in the root bark of *Lycium chinense* L. This metabolite turned out to contribute to the hypotensive activity of the crude drug which is used clinically in Oriental medicine ("jikoppi") for hypertension (Funayama et al. 1980 and references therein).

Addition. *N*-Cinnamoylhistamine and Derivatives in the Solanaceae. *Cis*- and *trans*-N_{α}-cinnamoylhistamine as well as their *N*1-methyl derivatives were isolated from the leaves of *Lycium cestroides* SCHLTDL. (Chiale et al. 1990).

6.7 Flavonoids

More than 9000 flavonoids have been identified up to now (Martens and Mithöfer 2005). The most comprehensive publication on this huge class of metabolites in general, a handbook of two volumes, was edited by Harborne and Baxter (1999). Harborne and Williams (2000) reviewed advances in flavonoid research since 1992, especially with regard of the topics "flavonoids and blue flower colour", "flavonoids and UV-B protection in plants", "antimicrobial flavonoids", "the role of flavonoids in animal-plant interactions", and in an extensive manner "medicinal properties of flavonoids". A biogenetic outline is given in Fig. 6.8. An extensive schematic-structural overview of the major flavonoid pathway in plants has been contributed by Schijlen et al. (2004). This review includes variations in the biosynthesis of flavonoids with regard to crop plants involving tobacco, tomato, potato and petunia, respectively. Our current knowledge on flavones and flavone synthases has been summarized by Martens and Mithöfer (2005). Another review on flavonoids and evolution in the dicotyledons includes a detailed overview on the general occurrence of certain metabolites and groups of such in the Solanaceae/Nolanaceae as well as in the Convolvulaceae/Cuscutaceae families (Giannasi 1988).

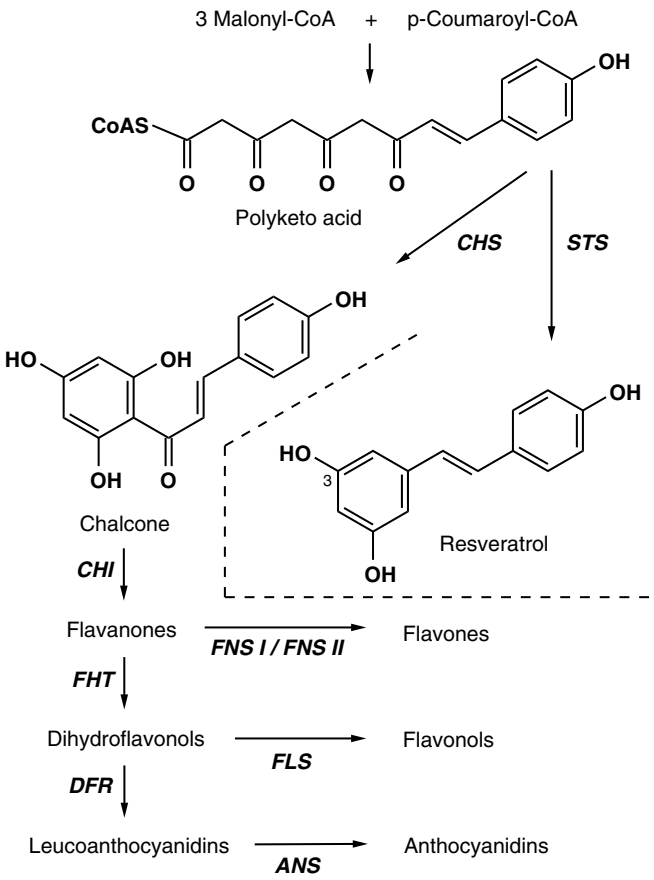


Fig. 6.8 Common polyketide pathway leading to flavonoids (***CHS*** = chalcone synthase) and to the stilbene resveratrol (***STS*** = stilbene synthase). ***CHI*** = chalcone isomerase; ***FNS I*** = flavone synthase I; ***FNS II*** = flavone synthase II; ***FHT*** = flavanone 3-hydroxylase; ***FLS*** = flavonol synthase; ***DFR*** = dihydroflavonol 4-reductase; ***ANS*** = anthocyanidin synthase (abbreviations of enzymes according to McArdle et al. 2006)

6.7.1 Flavones, Flavonols, and their Derivatives

6.7.1.1 Occurrence in the Solanaceae

Glycosides of the flavonols kaempferol (**K**) and quercetin (**Q**), especially 3-*O*-glucosides and 3-*O*-rutinosides, are characteristic constituents in the family. The distribution of twelve glycosides in 60 wild potato species and in 39 cultivated clones of the potato, altogether belonging to *Solanum* sect. *Petota* Dumort, was documented by Harborne (1962). Six glycosides were derived from **K**, four from **Q**, one from myricetin (5′-hydroxy-**Q**) and one from the flavone luteolin (3-deoxy-**Q**). 3-Glucosides, 3-rhamnosyl-glucosides, 3-diglucosides, and 3-glucosyl-rhamnosides

could be characterized. The richest sources for such metabolites were the petals and sepals as well as the seeds, whereas the leaves, sprouts, tubers, and berry flesh did not usually contain significant amounts. **K** 3-diglucoside 7-rhamnoside and **K** 3-triglucoside 7-rhamnoside were found in potato seeds, luteolin 7-glucoside in the flowers of *S. stoloniferum* SCHLTDL. A general scheme for a proposed biosynthetic pathway for flavonoid glycoside production in the potato was added. In a review published almost two decades later it was reported that in 70% of 32 spp. belonging to 24 solanaceous genera **Q** could be detected, whereas **K** was present in 50%. Two flavonols, **Q** 3-*O*-rutinoside and **Q** 3-*O*-rutinoside-7-*O*-glucoside, were detected in the leaves of the rare, pygmy, erect terophyte *Oryctes nevadensis* S. WATS. endemic to California/Nevada, USA (Averett and D'Arcy 1983). Furthermore, 95% of 100 *Solanum* spp. turned out to be **Q**-positive and 20% **K**-positive (Harborne and Swain 1979). Further economically useful plants such as *Nicotiana tabacum* L., tobacco, and *Petunia* × *hybrida* (HOOK.) VILM., petunia, were also investigated. Two acylated glycosides, **K** 3-*O*-(2‴-feruloyl)sophoroside, named petunoside, and its **Q** congener neopetunoside, were discovered in the flowers of *P.* × *hybrida* (Herrmann 1978 and references therein). Flavones were found as constituents of the genus *Capsicum*. Beside *O*-glycosides (e.g., luteolin 7-glucoside) "*C*-glycosides", i.e., *C*-glycosyl compounds of apigenin and luteolin (3′-hydroxyapigenin) such as vitexin (8-glucosyl-apigenin) and orientin (8-glucosyl-luteolin), respectively, were detected (Harborne and Swain 1979 and references therein).

In another study leaf flavonoids were isolated from more than 100 tuber-bearing *Solanum* spp., i.e., again altogether belonging to sect. *Petota* DUMORT. Novel **K** and **Q** glycosides were detected: 3-arabinosides, 3-xylosides, 3-galactosides [in addition isorhamnetin (3′-*O*-methyl-**Q**) as aglycone], 3-neohesperidosides [α-L-rhamnosyl-(1→2)-β-D-glucosides], 3-rhamnosylrutinosides, 3-sophorotrioside 7-rhamnosides. Isorhamnetin 3-galactoside and 3-rutinoside were also found. In certain glycosides sugar units may be *O*-acylated (*p*-coumaric acid or ferulic acid) (Reznick and Wietschel 1979). In two additional reports the flavonoid patterns of tuber-bearing species from ser. I–XVI (Wietschel and Reznik 1980a) and from ser. XVII (Wietschel and Reznik 1980b) were published in details. The authors discussed an occurrence of flavones as a derived character in comparison to flavonols.

Since the series numbers of that report, following the classification of Hawkes (1963), have been changed meanwhile according to the modern classification of Nee (1999) which in turn was based on a revised classification of Hawkes (1990) a corresponding translation is necessary. Thus, ser. I–XVI (Wietschel and Reznik 1980a) nowadays correspond to the following series of sect. 10. *Petota* DUMORT:

Subsect. *Estolonifera* HAWKES	ser. 1. and 2.
Subsect. *Potatoe* G.DON	ser. 1.–4., 6., 7., 10., 11., 14.– 19.

Ser. XVII (Wietschel and Reznik 1980b) corresponds to subsect. *Potatoe*, ser. 13 [*Tuberosa* (RYDB.) HAWKES]. For details with regard to the background of these series see Table 7.6 of the present monograph.

A further study on the phylogenetic relationship of *Solanum* flavonols involved sections *Androceras*, *Basarthrum*, and *Solanum* in addition to *Petota*. **K** and **Q**

glycosides were confirmed as characteristic metabolites of the genus. In addition methylated and acylated congeners were characterized. As a result sections *Petota* and *Basarthrum* appeared to be more "primitive" than sections *Solanum* and *Androceras* (Steinharter et al. 1986).

Recently, an updated review on the occurrence of flavones and flavonols as well as their glycosides in the genus *Solanum* has been published (Sarmento da Silva et al. 2003). Catechin and epicatechin represent the predominant flavonoids in the potato tuber (Brown 2005 and references therein). Chemodiversity of surface flavonoids in the family (glandular trichomes, resinous exudates) has been studied by Wollenweber et al. (2005). This comprehensive original report revealed detailed results on the occurrence of numerous flavonoid aglycones from *Nicotiana* (12 spp.), *Petunia* (3), *Solanum* (18), *Datura* (2), *Physalis* (2), *Chaemaesaracha* (2), *Iochroma* (2), *Atropa* (1), *Hyoscyamus* (1), and *Salpiglossis* (1). Numerous further species of these and additional genera (*Cestrum*, *Nicandra*, *Vestia*) are documented for which no exudate flavonoids could be detected. Taxonomic alignments were based on the classification of Hunziker (2001). Most of the aglycones were widespread flavonols. Flavones were found throughout the family, flavanones were rare.

Finally, a novel flavonol glycoside, kaempferol 7-methylether 3-*O*-[6-*O*-sinapoyl-glucosyl 1 → 2 rhamnosyl 1 → 6]-glucoside, isolated from the leaves of *Cestrum nocturnum* L., should be mentioned as an example for an HCA-acylated flavonol glycoside (Mimaki et al. 2001).

Addition. Resveratrol. The biosynthetic pathway to resveratrol diverges from the flavonoid pathway after the third malonyl-CoA condensation. Cyclization of the common polyketide intermediate catalyzed by resveratrol synthase yields the stilbene derivative resveratrol, whereas the same intermediate catalyzed by chalcone synthase yields the common flavonoid precursor chalcone (Fig. 6.8). Resveratrol, well known as a functional food ingredient (e.g., grape skin, red wine) with strong antioxidant properties (cardiovascular protection), and its 3-glucopyranoside piceid turned out to be also present in the skin of tomato fruits, *S. lycopersicum* (Ragab et al. 2006).

6.7.1.2 Occurrence in the Convolvulaceae

Glycosides of the flavonols kaempferol (**K**) and quercetin (**Q**), especially 3-*O*-glucosides and 3-*O*-rutinosides, are also characteristic constituents in the Convolvulaceae family. Numerous reports on flavonols, predominantly **Q** but also **K** though less frequent as well as their methyl ethers, and/or their glycosides were published with regard to 15 genera: *Argyreia, Bonamia, Calystegia, Convolvulus, Cressa, Cuscuta, Erycibe, Hewittia, Ipomoea, Jacquemontia, Merremia, Operculina, Porana, Stictocardia, Xenostegia* sub nom. *Merremia*. Flavones, predominantly apigenin and/or luteolin, and/or their glycosides were described as constituents of only 8 genera: *Argyreia, Evolvulus, Ipomoea, Jacquemontia, Merremia, Operculina, Xenostegia* sub nom. *Merremia*. These results were summarized comprehensively and in detail by Hegnauer (1964, 1989) and Tofern (1999).

Three known glycosides, **K** 3-*O*-(6-*O*-*p*-coumaroyl)-β-galactopyranoside and **K** as well as **Q** 3-*O*-β-glucopyranosyl-(1→2)-β-galactopyranoside could be isolated for the first time from a convolvulaceous species, *Argyreia capitata* (VAHL) CHOISY (Tofern 1999). Nine species of the holoparasitic genus *Cuscuta* revealed characteristic patterns of soluble phenolic constituents that can be used as taxonomic markers. This is of special significance since many *Cuscuta* spp. are difficult to identify or distinguish from related ones. This topic has been already discussed above including flavonoids (Sect. 6.6.3.2).

Several new flavone glycosides could be discovered in the family, e.g., acacetin-7-*O*-β-D-galactoside in the leaves of *I. carnea* JACQ. ssp. *fistulosa* (CHOISY) D.F.AUSTIN sub nom. *I. fistulosa* MART. ex CHOISY (Dubey et al. 1982), hispidulin 7-*O*-neohesperidoside [6-methoxy-5,7,4′-trihydroxyflavone α-L-rhamnosyl-(1→2)-β-D-glucoside] in the flowers of *I. purpurea* (L.) ROTH (Ragunathan and Sulochana 1994), 7-*O*-methylluteolin 3′-*O*,4′-*O*-diglucoside in the epigeal vegetative parts of *J. tamnifolia* (L.) GRISEB. The latter metabolite turned out to be also present in *J. corymbulosa* BENTH., *J. paniculata* (L.) HALL. f., and *M. aegyptia* (L.) URB. (Henrici 1996). A novel flavanone glycoside, eriodictyol [5,7,3′,4′-tetrahydroxyflavanone] 7-*O*-ß-D-xylopyranosyl-(1→4)-β-D-arabinopyranoside, was characterized as a constituent of *I. purpurea* seeds (Bhatt et al. 1981).

Recently, a novel flavanol and two novel flavans have been discovered in the stems of *Erycibe expansa* originating in Thailand (Morikawa et al. 2006): 7,4′-dihydroxy-3′-methoxy-2,3-dihydroflavonol (erycibenin D), 2′-hydroxy-5,7,4′-trimethoxyflavan (erycibenin E) and its 2*O*-methyl derivative (erycibenin F).

The *relative* infrequency of flavones in both sister families was found to be surprising, since most families related to the Solanaceae and Convolvulaceae predominantly contain flavones (Harborne and Swain 1979).

6.7.2 Flavonoid Sulfates

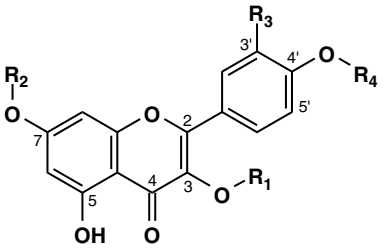
These metabolites represent a specific group of flavonoids which are linked covalently via hydroxyl groups to SO_3^-, thus forming ester moieties; mono-, di-, tri-, and even tetrasulfates were discovered. In 1975 a first review on flavonoid sulfates was published by Harborne (Harborne 1975) which documented the presence of such metabolites in over 160 species from nine dicotyledonous and seven monocotyledonous families. It was followed by an update of Barron et al. (1988) which listed in all known flavonoid sulfates, i.e., 56 flavanol sulfates as well as 45 flavone sulfates. They were usually isolated as their potassium salts. Such metabolites were found in at least 250 species belonging to 17 dicotyledonous and 15 monocotyledonous families. They were assumed to have no overall systematic significance, since many of these families are widely separated from each other. However, a general point was that they were found only in families which were herbaceous and/or morphologically advanced. Furthermore, this review included information on position-specific flavonol sulphotransferases.

6.7.2.1 Occurrence in the Convolvulaceae (Fig. 6.9)

In the first report on sulfated flavonoids from the Convolvulaceae family three novel derivatives of quercetin were described: Its 7-methyl ether-3,3′-disulfate was obtained from the roots of *Argyreia mollis* (Burm. F.) Choisy, as well as its 3,7-dimethyl ether-4′-sulfate and its 3′,4′,7-trimethyl ether-3-sulfate from the epigeal vegetative parts of *Ipomoea regnellii* Meisn. Further, though already known sulfates of quercetin and/or kaempferol could be characterized in both species as well as in *A. capitata* (Vahl) Choisy, *A. nervosa* (Burm. f.) Boj., and *I. reticulata* O'Donell., e.g., kaempferol 7-methyl ether-3-sulfate. These *Argyreia* spp. are *paleo*tropical woody twiners (up to 10–15 m), whereas both *Ipomoea* spp. are *neo*tropical vines. No flavonoid sulfates could be detected in *Ipomoea alba* L., *I. turbinata* Lag. [syn.: *I. muricata* (L.) Jacq.], *Merremia umbellata* (L.) Hall., and *Xenostegia medium* (L.) D.F.Austin & Staples [syn.: *M. medium* (L.) Hall. f.] (Mann et al. 1999). There are no reports on the occurrence of flavonoid sulfates in the Solanaceae or other related families.

6.7.3 Anthocyanins

Besides yellow and orange carotenoids anthocyanins are the most frequent pigments in the plant kingdom (chlorophylls left aside). Many common "normal" flavonoids and their glycosides are characterized by cream and more or less yellow colours (Saito et al. 1994b). However, anthocyanin pigments are flavonoid glycosides with anthocyanidins as aglycones. Due to a specific structural moiety of these aglycones, the flavylium cation (oxonium ion), they show colours with a spectrum shifted to



R_1	R_2	R_3	R_4	
H	H	H	H	Kaempferol **(K)**
H	H	OH	H	Quercetin **(Q)**
SO_3^- Na^+	CH_3	H	CH_3	**K** 4',7-dimethyl ether-3-sulfate
SO_3^- Na^+	CH_3	OCH_3	CH_3	**Q** 3',4',7-trimethyl ether-3-sulfate
SO_3^- Na^+	CH_3	OSO_3^- Na^+	H	**Q** 7-methyl ether-3,3'-disulfate
CH_3	CH_3	OH	SO_3^- Na^+	**Q** 3,7-dimethyl ether-4'-sulfate

Fig. 6.9 Flavonol sulfates as constituents of *Argyreia* spp. and *Ipomoea* spp.

red, purple or blue. There are three anthocyanidins characterized by a diverging substitution pattern of ring C: pelargonidin (4′-hydroxy) associated with pink and orange colours, cyanidin (3′,4′-dihydroxy) with magenta colour, delphinidin (3′,4′,5′-trihydroxy) with mauve and blue colours. Furthermore, there are some methylated derivatives such as peonidin (3′-methyl-cyanidin), petunidin (3′-methyl-delphinidin) or malvidin (3′,5′-dimethyl-delphinidin; mauve to purple) (Harborne and Williams 2000). However, it has to be taken into account that the structures of the aglycones and consequently also the colours may be transformed depending on the pH value. Thus, hydration including loss of the oxonium structure would take place in plant vacuoles leading to colourless structures. As mechanisms for protection of anthocyanins against hydration in vivo intermolecular copigmentation with, e.g., flavonoid glycosides or intramolecular copigmentation with hydroxycinnamoyl residues of the anthocyanin molecule itself could be elucidated (Strack 1997). The scientific history of the anthocyanins – starting with the basal studies of Willstätter in the second decade of the past century – and the development of this topic were reviewed by, e.g., Harborne and Grayer (1980), Goto and Kondo (1991), Harborne and Williams (2000). Recent advances in the biosynthesis and accumulation of anthocyanins have been reviewed by Springob et al. (2003).

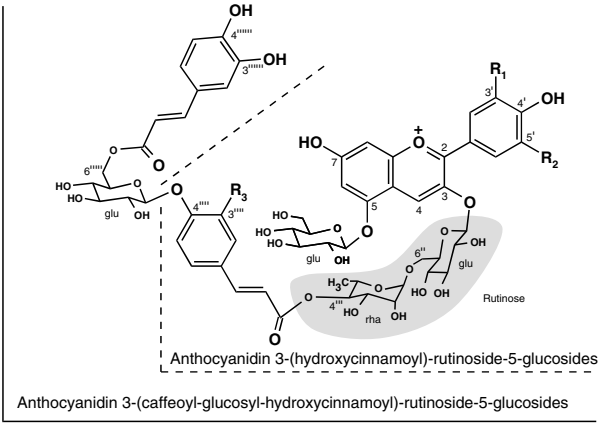
Biological Significance. Anthocyanins play a definite role in the attraction of animals for pollination and seed dispersal. They are of remarkable significance in the co-evolution of such plant-animal interactions. Furthermore, they may be of importance in the resistance of plants to insect attack. A review on these and further biological activities, especially their antioxidant and radical scavenging properties as well as their interaction with DNA has been published recently (Kong et al. 2003). Anthocyanins have also been reported as antimutagenic, antidiabetic, and anticarcinogenic agents as well as functional food colorants (Tian et al. 2005 and references therein).

Blue flower colour, the preferred attractant of bee pollinators, was found to be restricted to the more highly evolved angiosperm plant families (Gottlieb 1982). It is usually due to a delphinidin-based anthocyanin. More primitive families are characterized by cyanidin-based anthocyanins in the red to magenta range (Harborne and Williams 2000).

6.7.3.1 Occurrence in the Solanaceae (Fig. 6.10)

Anthocyanin biosynthesis has been extensively studied and well established in terms of genetics and molecular biology (Fukui et al. 1998 and references therein). A comprehensive general review on recent developments starting with chalcone synthase included information on pathway-specific enzymes from *Nicotiana tabacum*, *Petunia hybrida*, and *Solanum melongena* (Springob et al. 2003). It is obvious that commercially used species have been in the centre of flower pigment research; this is especially true for ornamental plants.

All of the anthocyanidins mentioned above were detected as aglycones of solanaceous pigments. The principal anthocyanin of the family – as far as known



R_1	R_2	Anthocyanidin
H	H	Pelargonidin
OH	H	Cyanidin
OCH_3	H	Peonidin
OH	OH	Delphinidin
OCH_3	OH	Petunidin
OCH_3	OCH_3	Malvidin

R_3	Residue
H	p-Coumaroyl
OH	Caffeoyl
OCH_3	Feruloyl

Fig. 6.10 General structures of anthocyanins in solanaceous genera such as *Capsicum*, *Petunia*, and *Solanum*; beside "normal" *trans*-hydroxycinnamoyl residues their *cis* isomers may co-occur

to date – is anthocyanidin 3-acylrutinoside-5-glucoside [rutinose = 6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranose]. Acyl substituents turned out to be *p*-coumaroyl, caffeoyl or feruloyl residues. Such HCA residues are integrated into the anthocyanin molecules by simple conjugation to C-6*O* of glucose units and/or used as spacers between two glucose units of a respective metabolite. The latter case – only found for caffeoyl substituents – involves a double covalent linkage of the acyl residue: (i) acylation like in the former case and (ii) glycosidic linkage between a glucose unit and the aromatic skeleton at C-3*O* or alternatively at C-4*O*.

Petunia. *Petunia* × *hybrida* (HOOK.) VILM., garden petunia, obtained by crossing of *P. axillaris* (LAM.) BRITTON, STERNS & POGGENB. with *P. integrifolia* (HOOK.) SCHINZ & THELL. already in the first third of the nineteenth century, is one of the world's most popular annual bedding plants characterized by a wide range of flower colours (Hunziker 2001). Peonanin, peonidin 3-*O*-(4-*O*-*p*-coumaroyl-rutinoside)-5-glucoside, was already isolated from the flowers of *Petunia* × *hybrida* in 1963 and later from *Solanum tuberosum* (Pomilio and Sproviero 1972b and references therein). The corresponding petunidin-, malvidin-, and cyanidin-based pigments were also discovered in garden petunia. Petanin represents the petunidin congener, later also found in *Solanum* spp. Furthermore, the acylation pattern of petunia anthocyanidin 3-acylrutinoside-5-glucosides was extended to include caffeic acid by a wide occurrence in a study with 11 cultivars. Delphinidin and pelargonidin could be only detected as 3-glucosides and 3-rutinosides in trace amounts (Griesbach et al. 1991 and references therein). Finally, also ferulic acid could be proven as acyl residue in a malvidin-based anthocyanin of petunia (Gonzalez E et al. 2001). The relative floral anthocyanidin contents of 195 commercial petunias with floral colours other than white and yellow have been published in a comprehensive HPLC study (Ando et al. 2004). Cyanidin, delphinidin (not a major component), malvidin, peonidin, and petunidin could be confirmed; pelargonidin

was not detected. The results led to a classification of three phenotype groups: (i) cyanidin group (69 cultivars), (ii) malvidin group (67 cultivars), and (iii) peonidin group (59 cultivars). Commercial petunias obviously failed to accumulate large amounts of delphinidin in their flowers. The authors hypothesized "…. that the accumulation of delphinidin 3-glucoside in flowers is associated with a specific floral character, a dull-coloured extremely crumpled corolla-limb, which impairs its ornamental value. Such inferior floral traits may be the driving force that has led breeders to remove the delphinidin group from commercial petunias." On the oppposite, wild *Petunia* spp. such as *P. exserta* STEHMANN, *P. reitzii* L.B.SM. & DOWNS, and *P. saxicola* L.B.SM. & DOWNS were found to accumulate delphinidin. Therefore, the authors proposed from a horticultural perspective, these wild *Petunia* spp. should be used as a genetic resource to create novel floral colours in commercial petunias thus establishing a fourth group.

On the other hand, this was also claimed for red-flowered *P. exserta* though based on a diverging anthocyanin pattern: cyanidin-3-glucoside and 3-rutinoside (together 87%) and the corresponding pelargonidin anthocyanins (13%) (Griesbach et al. 1999). Furthermore, a certain mutant of *Petunia* × *hybrida* was shown to contain pelargonidin (Cornu et al. 1972) in contrast to the results of Ando et al. mentioned above.

Floral anthocyanins in 16 wild species (20 taxa) of the genus *Petunia* were investigated in another comprehensive HPLC study (Ando et al. 1999). A total of 18 known and six novel anthocyanins were detected. The novel compounds, isolated from *P. occidentalis* R.E.FR., could be structurally elucidated. Cyanidin, delphinidin, malvidin, and petunidin were characterized as anthocyanidins. Like in garden petunia anthocyanidin 3-hydroxycinnamoyl-rutinoside-5-glucosides with *p*-coumaroyl or caffeoyl residues were proven. Furthermore, congeners with longer "chains" were discovered bearing an additional glucosyl or even caffeoyl-glucosyl residue, respectively, linked to the C4-*O* of the hydroxycinnamoyl (HCA) moiety (Fig. 6.10). Finally, *cis* isomers of this latter moiety may occur. Every species showed its more or less specific pattern. The taxa could be placed into four groups (A–D) and five D subgroups (D1–D5) with regard to (i) their constituents, (ii) level of major compounds, and (iii) their anthocyanin biosynthesis. For example, species of group A (three subspecies of *P. axillaris*) showed poor ability to synthesize anthocyanins. Group B, only formed by *P. exserta*, was characterized by the accumulation of the simplest anthocyanins in this genus (3-glucosides and 3-rutinosides); cyanidin-based pigments were only found in this group. On the opposite, group D showed strong ability for methylation of 3′- and 5′-OH, for 5-*O*-glucosylation, and for complete acylation with HCA of rhamnosyl and terminal glucosyl residues. Species belonging to this group had red-purple – purple flowers.

Brunfelsia. Some *Brunfelsia* spp. such as *B. australis* BENTH., *B. pauciflora* (CHAM. & SCHLTDL.) BENTH., *B. undulata* SW. are widely cultivated garden plants due to their showy or fragrant flowers (Hunziker 2001). The common name of *Brunfelsia*, yesterday-today-tomorrow, indicates that the flowers show an extreme and rapid decrease in pigment concentration from dark purple-violet via bright purple to white, i.e., a change in anthocyanin concentration during flower development. Recently, an

in planta study with *B. pauciflora* var. *calycina* (BENTH. ex DC.) J.A.SCHMIDT sub nom. *B. calycina* BENTH. ex DC. revealed active anthocyanin degradation requiring novel mRNA and protein synthesis (Vaknin et al. 2005). A significant increase in peroxidase activity was shown to correlate with anthocyanin degradation. This could be inhibited by treatment of the flowers with reducing agents indicating that oxidative enzymes might be involved. It was demonstrated that the degradation of anthocyanins was not part of the general senescence process of the flowers.

Eggplant and Violet Pepper. The fruit colour of the eggplant/aubergine, *Solanum melongena* L., is also caused by anthocyanins. The most common compound, delphinidin 3-*p*-coumaroylrutinoside-5-glucoside, was named nasunin after the Japanese common name for this species ("Nasu"), discovered by Kuroda and Wada (1933, 1936). Ichiyanagi et al. (2005) could isolate two major anthocyanins from the peel of eggplant which turned out to be the *cis-trans* isomers of nasunin. In vivo isomerization of *p*-coumaroyl residues and its significance for colour variance and stabilisation were also reported from the red-purple flowers of *Petunia integrifolia* (George et al. 2001). The authors concluded that such *cis*-hydroxycinnamic isomers may be more profilic in nature than has previously been thought. In another study a comparison of the anthocyanin pattern in the fruit peel of *S. melongena* and a violet variety of *Capsicum annuum* L., "violet pepper", revealed that the major component in the eggplant variety used in this study was delphinidin-3-rutinoside vs delphinidin-3-*p*-coumaroylrutinoside-5-glucoside in violet pepper. Nevertheless, the former compound is also a – though minor – constituent of violet pepper, whereas the latter one did not occur in the eggplant peel (Sadilova et al. 2006). Gastrointestinal uptake of both nasunin isomers in their original acylated forms could be confirmed. Both exhibited a bioavailability almost identical to that of nonacylated anthocyanins (Ichiyanagi et al. 2006). This is significant, since anthocyanins may play an important role in human health promotion.

Solanum. An early report on violet potato tubers, *S. tuberosum*, was given by Chmielewska (1935). The author was able to isolate two closely related novel anthocyanins, "tuberin" and "negretein"; the latter turned out to be a malvidin-based bioside (glucose and isorhodeose = rhamnose). Three decades later, peonanin [peonidin 3-*O*-(4-*O*-*p*-coumaroyl-rutinoside)-5-glucoside] and congeners based on other anthocyanidins such as delphanin (delphidin), negretein (malvidin), pelanin (pelargonidin), and petanin (petunidin) could be identified as constituents of potato *flowers* (Harborne 1960, 1964). Peonanin, petanin and their caffeoyl (instead of *p*-coumaroyl) congeners were proven as constituents of purple *sprouts* from a Norwegian potato cultivar. The same major anthocyanins were detected in the violet zone located in the flesh 0.5–1 cm from the surface of the *tubers* (Fossen et al. 2003). Skin alone of red and purple potatoes may be pigmented or in addition the flesh partially or even entirely. This topic including further anthocyanins in tubers of certain cultivars has been documented by recent reviews, e.g., Lachman and Hamouz (2005), Brown (2005).

There are only a few reports on anthocyanins in wild *Solanum* spp. Petanin and delphanin could be detected in the ripe berries of *S. americanum* MILL., *S. interandinum*, and *S. intrusum* as well as in the flowers of *S. auriculatum* AIT. (Briggs et al. 1961).

The dark bluish-violet berries of the Ethiopian *S. nigrum* L. var. *guineënse* L. (syn.: *S.guineënse* L. = *S. aggregatum* JACQ.), garden huckleberry, were found to contain again petanin and malvidin-based congeners, e.g., negretein (Saito et al. 1965; Francis and Harborne 1966). Anthocyanin contents of coloured cultivars of *S. tuberosum* (Lewis et al. 1998a) were compared with eight wild tuber-bearing *Solanum* spp. (Lewis et al. 1998b). Results revealed a strong association between the various cultivars with distinct differences from the wild relatives. The total anthocyanin concentration in the tuber skin of the latter species ranged from 0 to 300 µg g^{-1} FW, whereas the content of the cultivars was determined to be 0–7000 µg g^{-1} FW. The tuber flesh of the wild species was free of pigments. Anthocyanin concentrations in their flowers ranged from 100 to 2500 µg g^{-1} FW, in leaves from 0 to 50 µg g^{-1} FW. Petunidin 3-*p*-coumaroylrutinoside-5-glucoside was found to be the major pigment in all wild species with its malvidin congener as one of the minor components.

6.7.3.2 Occurrence in the Convolvulaceae

It is also true for the Convolvulaceae that commercially used species have been in the centre of flower pigment research, especially ornamental plants.

Ipomoea* (Fig. 6.11) and *Evolvulus. Anthocyanins found in these two convolvulaceous genera are family-specific. *Ipomoea* represents the most studied genus of this family as far as anthocyanins are concerned. It is noticeable that the occurrence of anthocyanidins is confined to pelargonidin, cyanidin and its 3′-methyl ether peonidin, whereas 3′,4′,5′-trisubstituted analogues, i.e., delphinidin as well as its methylated congeners petunidin and malvidin, are lacking (Table 6.1). However, delphinidin-based

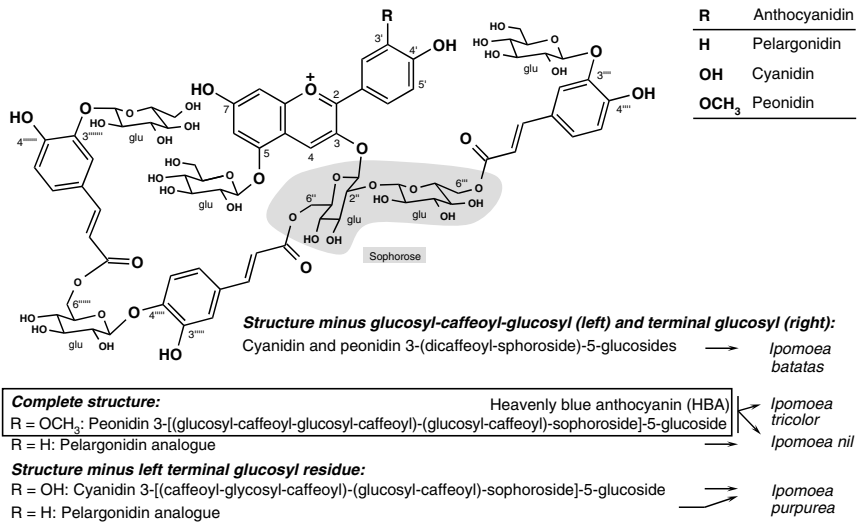


Fig. 6.11 Major anthocyanins of *Ipomoea* spp. characterized by common partial structures

Table 6.1. Anthocyanins isolated from the genus *Ipomoea*

***Ipomoea* species (ornamental species in bold)**	**Organ**	**Anthocyanin**[a] (anthocyanidins: **C** = cyanidin; **Pg** = pelargonidin; **Po** = peonidin)	**No. of additional congeners**	**References**
I. asarifolia (DESV.) ROEM. & SCHULT.	Flower	**C** 3-(dicaffeoyl-sophoroside)-5-glucoside	1	Pale et al. (1998)
do.	Flower	**C** 3-[(3,5-dihydroxycinnamoyl-glucosyl-**caffeoyl**)-caffeoyl-sophoroside]-5-glucoside	1	Pale et al. (2003)
***I. batatas* (L.) LAM.**[b] (sweet potato)	Stem	**C + Po** 3-(dicaffeoyl-sophoroside)-5-glucosides	1	Imbert et al. (1966)
do. cv. 'Yamagawamurasaki' (YGM)	Tuber	**C + Po** 3-(caffeoyl-feruloyl-sophoroside)-5-glucosides	5	Odake et al. (1992)
do. cv. 'Yamagawamurasaki' (YGM)	Tuber	**C + Po** 3-(caffeoyl-sophoroside)-5-glucosides	1	Goda et al. (1997)
do. cv. 'Yamagawamurasaki' (YGM)	Tuber	**C + Po** 3-(caffeoyl-*p*-hydroxybenzoyl-sophoroside)-5-glucosides (YGM-1a / YGM-5a)	7	Terahara et al. (1999)
***I. cairica* (L.) SWEET** (railway creeper)	Flower	**C** 3-(*p*-coumaroyl-caffeoyl-sophoroside)-5-glucoside	3	Pomilio and Sproviero (1972a)
***I. carnea* JACQ. ssp. *fistulosa* (CHOISY) D.F.AUSTIN sub nom. *I. fistulosa* MART. ex CHOISY**	Flower	**Po** 3-arabinosyl-glucoside	0	Gupta et al. (1980)
I. congesta R.BR.	Flower	**Po** 3-(*p*-coumaroyl-caffeoyl-sophoroside)-5-glucoside	5	Pomilio and Sproviero (1972b)
I. hederifolia L. sub nom. *Quamoclit angulata*	Flower	**Pg** (carbohydrate moieties undetermined)	?	Yoshitama et al. (1980)
***I. nil* (L.) ROTH sub nom. *Pharbitis nil* (L.) CHOISY** (Japanese morning glory)	Violet-blue fl.	**Po** 3-(4-glucosyl-**caffeoyl**-sophoroside)-5-glucoside	0	Lu et al. (1991)
do.	Violet-blue fl.	**C** 3-(4-glucosyl-**caffeoyl**-sophoroside)-5-glucoside	0	Saito et al. (1993)

Table 6.1. Anthocyanins isolated from the genus *Ipomoea* (continued)

***Ipomoea* species (ornamental species in bold)**	**Organ**	**Anthocyanin**[a] (anthocyanidins: **C** = cyanidin; **Pg** = pelargonidin; **Po** = peonidin)	**No. of additional congeners**	**References**
do.	Red-purple fl.	**Pg** 3-[(glucosyl-**caffeoyl**-glucosyl-**caffeoyl**)-(glucosyl-**caffeoyl**)-sophoroside]-5-glucoside / [Pg analogue to HBA (Fig. 6.11)]	4	Lu et al. (1992a)
do.	Violet-blue fl.	**Po**-containing 'heavenly blue anthocyanin' (HBA) (Fig. 6. 11)	4	Lu et al. (1992b)
do.	Maroon fl.	**Pg** 3-[6-(3-glucosyl-**caffeoyl**)glucoside]	5	Saito et al. (1994a)
do.	White to pale yellow fl.	No anthocyanins; instead flavonoids and caffeic acid derivatives	14	Saito et al. (1994b)
do.	Slate fl. (grey-purple-blue)[c]	**C + Po** 3-[6-(3-glucosyl-**caffeoyl**)glucoside]	3	Saito et al. (1996a)
do. cv. 'Danjuro'	dusky red-dish-brown ("kaki") fl.[c]	**Pg** 3-[6-(3-glucosyl-**caffeoyl**)glucoside]	2	Yoshida et al. (2003)
***I. purpurea* (L.) Roth (common morning glory)**	Flower	**Pg** 3,5-diglucoside	0	Kataoka (1936)
do.	Violet-blue fl.	**C** 3-[(caffeoyl-glucosyl-**caffeoyl**)-(glucosyl-**caffeoyl**)-sophoroside]-5-glucoside	5	Saito et al. (1995)
do.	Red-purple fl.	**Pg** 3-[(caffeoyl-glucosyl-**caffeoyl**)-(glucosyl-**caffeoyl**)-sophoroside]-5-glucoside	3	Saito et al. (1996b)
do.	Brownish-red fl.	**C** 3-caffeoyl-sophoroside	5	Saito et al. (1998)
***I. tricolor* Cav. cv. 'Heavenly Blue'**	Blue flower	**Po**-containing 'heavenly blue anthocyanin' (HBA) (Fig. 6.11)	0	Kondo et al. (1987)

[a] **"Caffeoyl" in bold characters** represents such residues which are linking two *isolated* glucose units by (i) acylation at C-6*O* of one unit and (ii) a glycosidic bond from another unit to C-4 or alternatively to C-3 (*p*- or *m*-position of the phenolic OH; positions left out here for reasons of clarity); "caffeoyl" in light typeface indicates that both phenolic OH are free (Fig. 6.10).

[b] There are cultivars of *I. batatas* which are only used for ornamental purposes (flowers, leaves) and not for tuber production

[c] This colour development is mostly caused by the free hydroxyl at C-5 of the anthocyanidin nucleus (Yoshida et al. 2003)

anthocyanins were discovered as flower pigments of an ornamental species belonging to the genus *Evolvulus* (see below). Four decades ago, the first acylated anthocyanins, caffeoylated glucosides of cyanidin and peonidin substituted at C-3*O* and C-5*O*, respectively, were discovered in the stems of *Ipomoea batatas* (L.) LAM., sweet potato (Imbert et al. 1966). This economically important species is known to contain anthocyanins in flowers, leaves, stems, and roots/tubers (periderm and flesh) showing many variations dependent on the cultivars (Terahara et al. 1999). Of course, due to the dominant economic role of the storage roots/tubers of *I. batatas* most reports on anthocyanins deal with this organ. The anthocyanin carbohydrate moieties of this species and also of almost all other *Ipomoea* spp. checked to date are based on sophorose [2-*O*-(β-D-glucopyranosyl)-β-D-glucopyranose] conjugated to C-3*O* of the aglycone as well as β-D-glucose (C-5*O*). Acyl substituents turned out to be predominantly caffeoyl residues; ferulic acid, 3,5-dihydroxycinnamic acid as well as *p*-hydroxybenzoic acid may also occur occasionally (details: Table 6.1). Like in the Solanaceae caffeoyl residues may be integrated into the anthocyanin molecules by simple conjugation to C-6*O* of glucose units and/or used as spacers between two glucose units of a respective metabolite. The latter case involves a double covalent linkage of the caffeoyl residue: (i) acylation like in the former case and (ii) glycosidic linkage between a glucose unit and the aromatic skeleton of the caffeoyl residue (hydroxyl group at C-3 or alternatively at C-4). Dependent on the length of the caffeoyl-glucose chains linked to the corresponding anthocyanidins they may fold over the chromophore and strongly influence its colour properties (Brouillard and Dangles 1994).

Extensive studies were carried out in case of the *tubers* of the Japanese variety *I. batatas* cv. 'Yamagawamurasaki' (YGM) (Teramara et al. 1999). Instead of trivial names its pigments got the abbreviation YGM added by a specific number, e.g., YGM-1a. However, from the economic point of view greens of this species are also of interest, since they are consumed, like *I. aquatica* FORSSK. (water spinach) in Southeast Asia, as a fresh vegetable in many parts of the world (Islam et al. 2002). Furthermore, there are even *I. batatas* cultivars which were bred only with regard to showy flowers or even leaves. Thus, there exist cultivars with purple-coloured leaf suggesting that these leaves contain high contents of anthocyanins. This could be proven for three varieties raised for use as leafy vegetables or even medicinal purpose.

Table 6.1 summarizes the knowledge of anthocyanins occurring in the genus *Ipomoea* which contains a number of very popular twining ornamentals with showy funnel-shaped flowers, often with diverging colour mutants. Furthermore, a few species from the wild have been investigated.

Examples for flower colour variation from optical and scientific points of view can be found in the literature, e.g., *Ipomoea purpurea* (L.) ROTH, common morning glory (Habu et al. 1998; Clegg and Durbin 2000). Such reports included pictures of pigmentation phenotypes including white flowers with coloured flakes and sectors as well as the corresponding genetic background. The study of flower colour polymorphism in the morning glory as a model for the analysis of adaptation was reviewed by the latter authors. At least 21 floral phenotypes were determined by five genetic loci in *I. purpurea*. Most of them showed analogous forms in the

Japanese morning glory, *I. nil* (L.) ROTH [syn.: *Pharbitis nil* (L.) CHOISY]. Almost all of the mutations responsible for the phenotypic differences were found to be the result of transposon insertions. "Thus, the flower color diversity seized on by early human domesticators of this plant is a consequence of the rich variety of mobile elements that reside in the morning glory genome" (Clegg and Durbin 2000). Another report on the genetic basis of a flower colour polymorphism in *I. purpurea* has been added by Zufall and Rausher (2003). As a result of a recent molecular phylogenetic study corolla colours/pigments of *I. purpurea* (bee-pollination, blue/purple flowers with inserted stigma and anthers like most *Ipomoea* spp.) and *I. quamoclit* [bird-pollination, (scarlet)red flowers with exserted stigma and anthers like all of the species of the sect. *Mina*] have been interpreted as an initial stage of degeneration in the anthocyanin pigment pathway associated with an adaptive change from blue (cyanidin-based) to red flowers (pelargonidin-based) (Zufall and Rausher 2004).

The common name of the popular ornamental twiner *I. nil*, Japanese morning glory, is due only to cultural reasons, since this species is a putative tropical American plant. Cultural and horticultural significance of this ornamental twiner in Japan ("Asagao") was documented recently (Austin et al. 2001). A natural-colour illustrated monograph of Japanese morning glory emphasizes this significance (Yoneda and Takenaka 1981). This species has been domesticated in Japan and its various spontaneous mutations exhibited a wide variety of different flower pigmentation (Table 6.1) (Saito et al. 2005 and references therein). Consequently, the anthocyanins of this species and several other ornamental morning glories were investigated almost exclusively by Japanese scientists. *I. nil* from the wild is *blue*-flowered; these flowers are characterized by peonidin glycosides. This species and moreover even the genus *Ipomoea* may be regarded as less effective in its production of blueness, as compared to usually delphinidin-based blue-flowered species. Delphinidin-based colour requires less flavone copigment to be present in order to shift the spectrum to blue. In case of *blue*-flowered *I. nil* a peonidin 3-(tricaffeoylpentaglucoside)-5-glucoside (identical to HBA; see below and Fig. 6.11) could be elucidated (Lu et al. 1992b). In contrast, the blue colour of the flowers from *Evolvulus pilosus* cv. 'Blue Daze', another popular ornamental, revealed a delphinidin 3-(dicaffeoyltriglucoside)-5-malonylglucoside as a major pigment (Toki et al. 1994). Recently, the structure of the major anthocyanin of this species has been revised insignificantly and found that it was identical to phacelianin, isolated from the blue petals of *Phacelia campanularia* A. GRAY (Hydrophyllaceae) (Mori M et al. 2006). However, it should be added that this ornamental *Evolvulus* species represents cultivars of *E. glomeratus* CHOISY, erraneously named *E. pilosus* by horticultural companies (Austin 2002).

Five delphinidin-based anthocyanins, isolated from the flowers of the above mentioned *Evolvulus* cultivar, were integrated in physicochemical studies on intramolecular copigmentation (Figueiredo et al. 1996) and complexation with aluminium and gallium ions (Elhabiri et al. 1997), respectively.

A major pigment elucidated from the corolla of a very popular ornamental twiner, *I. tricolor* CAV. cv. 'Heavenly Blue', named 'heavenly blue anthocyanin'

(HBA), turned out to be a peonidin-based glycoside with six glucose units acylated by three caffeoyl residues (Fig. 6.11) (Kondo et al. 1987). Two of the caffeoyl residues are thought to be stacked intramolecularly with the anthocyanidin nucleus thus stabilizing the pigment (Mistry et al. 1991). As already mentioned, HBA could be also identified in violet-blue-flowered cultivars of *I. nil* (Lu et al. 1992b). Furthermore, its pelargonidin congener was identified in the flowers of red-purple cultivars of this species. The red flower colour gradually changed into more bluish colour with increasing numbers of caffeoyl residues in acylated congeners; in addition, it could be observed that the stability of the pigments increased with them (Lu et al. 1991).

Calystegia. Besides *Ipomoea* spp. and this *Evolvulus* sp. there are only a few reports on anthocyanins from other convolvulaceous species. From the stems of *Calystegia silvatica* (WALDST.) GRISEB. a pigment could be isolated which was characterized as a cyanidin glycoside; partial hydrolysis revealed cyanidin 3-monoglucoside and rutinose (Imbert 1969). Corollas of four *Calystegia* spp. growing naturally in Japan, *C. hederacea* WALL., *C. japonica* CHOISY, *C. soldanella* (L.) ROEM. & SCHULT. (sea bindweed), *C. sepium* (L.) R.BR. (hedge bindweed), were analyzed (Tatsuzawa et al. 2004). The latter two species show a widespread distribution in temperate parts of the world. All of these *Calystegia* spp. do not show a rich variation in the colours of their flowers, since these are confined to pink or white. This study indicated that *Calystegia* showed a primitive feature of anthocyanin constitution in contrast to *Ipomoea* and *Evolvulus*. Cyanidin 3-[6-(malonyl)-glucoside] was determined to be the major component of the corollas beside cyanidin 3-glucoside and cyanidin 3-rutinoside as minor congeners in case of *C. hederacea* and *C. japonica. C. soldanella* showed low concentrations of cyanidin 3-[6-(malonyl)-glucoside]. White flowers of *C. sepium* were anthocyanin-negative as expected.

Surprisingly, to date there are no reports on such prominent convolvulaceous genera as *Argyreia*, *Astripomoea*, *Bonamia*, *Convolvulus*, *Maripa*, *Stictocardia*, or *Turbina* though the corollas – of at least certain species – apparently are characterized by anthocyanin-type flower pigments.

6.7.3.3 Health Benefit of Acylated Anthocyanins

Food-derived polyphenols are assumed to be beneficial for human health due to their antioxidant and anticarcinogenic properties (Matsubara et al. 2005 and references therein). This is also true for acylated anthocyanins from red-purple potato, eggplant, and red-purple sweet potato. The significance of pigmented potatoes has been documented by recent reviews, e.g., Lachman and Hamouz (2005), Brown (2005), Hayashi et al. (2006b). Acylated anthocyanins from different edible sources and their applications in food systems including potatoes and sweet potatoes in comparison with radishes, red cabbage etc. has been reviewed by Giusti and Wrolstad (2003).

Antiangiogenic Effects. In various angiogenesis models including human umbilical vein endothelial cells an antiangiogenic activity of nasunin in a dose-dependent manner (50–200 µM) could be demonstrated in addition to the known antioxidant effects. The authors concluded that this pigment might be useful to prevent angiogenesis-related diseases, e.g., solid malignant tumours, atherosclerosis, diabetic retinopathy, due to both activities (Matsubara et al. 2005).

Antitumor Effects. Anthocyanins obtained from coloured potatoes have been shown to induce apoptosis in cultured human stomach cancer cells. Growth of mouse stomach cancer was suppressed by 46% and 39% (feeding of steamed red potato vs purple potato) (Hayashi et al. 2006a).

Prevention of Diabetes. Anthocyanin extracts from 12 species of different plant families were found to have a potent α-glucosidase (AGH) inhibitory activity. The extract obtained from the roots of *I. batatas* cv. 'Yamagawamurasaki' (YGM) as well as from the flowers of *I. nil* sub nom. *Pharbitis nil* cv. 'Scarlet O'Hara' (SOA) revealed the strongest maltase inhibitory effects (IC_{50} values: 0.35 mg/mL). Both extracts also inhibited α-amylase action. This indicated that the anthocyanins of both extracts might suppress the increase of postprandial glucose level otherwise caused by ingestion of starch, a special problem for patients suffering from diabetes (Matsui et al. 2001a). Certain diacylated anthocyanidin 3-sophoroside-5-glucoside (SOA-4, SOA-6, YGM-3, YGM-6) isolated from those two *Ipomoea* extracts, showed strong maltase inhibitory activities (IC_{50} values: <200 µM). SOA-4 {a pelargonidin 3-[(3-glucosyl-**caffeoyl)**-caffeoyl-sophoroside]-5-glucoside} turned out to be the most potent compound (IC_{50} value: 60 µM). (For an explanation with regard to "**caffeoyl**" bold-type letters see footnote[a] of Table 6.1.) Deacylation led to a reduced maltase inhibition (Matsui et al. 2001b). From red-coloured vinegar produced via fermentation with the storage root paste of purple-fleshed sweet potato a cleavage product of YGM-6 could be isolated which turned out to be 6-*O*-caffeoylsophorose. This metabolite showed also α-glucosidase inhibiting and antihyperglycemic properties though to a lower extent than its precursor YGM-6 (Matsui et al. 2004).

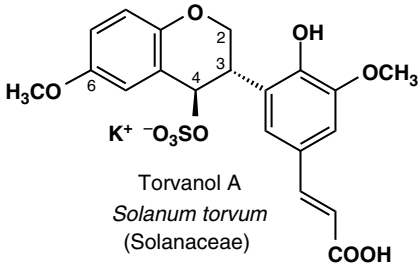
Pharmacokinetics. Recently, it has been shown that human urine of 87 volunteers after ingestion of a purple-fleshed sweet potato beverage contained peonidin 3-caffeoylsophoroside-5-glucoside. Absorption of this compound into the bloodstream of rats and presence as an intact acylated form in their plasma has been demonstrated previously (Oki et al. 2006 and references therein). Urinary excretion of anthocyanins is usually low due to metabolization.

6.7.4 Isoflavonoids

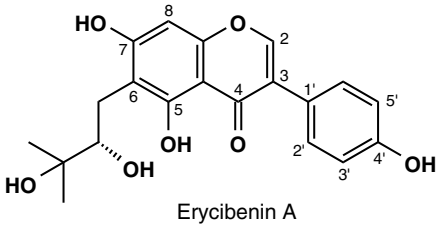
There are reports on the presence of isoflavonoids in numerous families of the plant kingdom inclusive 49 angiosperm families (Mackova et al. 2006). The most famous example is their occurrence in the Fabaceae. Isoflavones are formed by structural

rearrangement of the flavanone naringenin including a 2,3-aryl shift (Strack 1997). Isoflavonoids are classified as (i) simple isoflavones, e.g., genistein, (ii) prenylated isoflavones, (iii) pterocarpans, and (iv) rotenoids. Pterocarpans represent derivatives of (i) or (ii) formed by cyclization (ring B → ring C, ether-linked in *o*, *o′*-position) (Fig. 6.12). Rotenoids are pentacyclic isoflavone derivatives characterized by a dihydro-γ-pyrone ring and a dihydropyrane ring anellated to each other in the centre of the molecule which are flanked by one aromatic nucleus at both sides; the fifth ring, a tetrahydrofurane moiety, is formed by a hemiterpenoid anellated to one of the aromatic residues. The dihydropyrane formation is caused by integration of a C_1 unit into the isoflavonoid molecule.

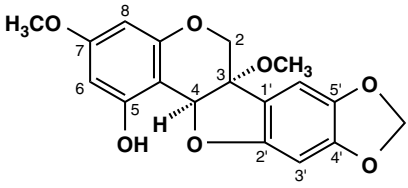
Isoflavonoids show fungicidal and bactericidal effects. They play an important role as constitutive constituents and also as phytoalexins. From the pharmacological point of view estrogenic activity of isoflavones such as genistein and daidzein is of increasing relevance (Harborne and Williams 2000). Rotenoids, especially rotenone, are potent insecticides.



Torvanol A
Solanum torvum
(Solanaceae)



Erycibenin A
Erycibe expansa (Convolvulaceae)



Erycibenin C
Erycibe expansa (Convolvulaceae)

Fig. 6.12 Examples for isoflavones (torvanol A, erycibenin A) and a pterocarpane (erycibenin C); the third novel metabolite of *Erycibe expansa*, erycibenin B, represents a positional isomer of congener A characterized by an identical dihydroxydimethylpropyl substituent (prenyl derivative) at C-8

6.7.4.1 Occurrence in the Solanaceae

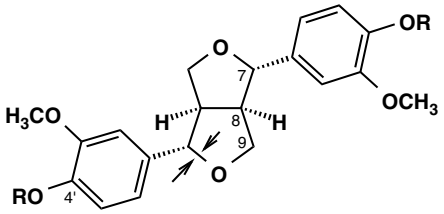
The only isoflavonoid *isolated* from a solanaceous species is torvanol A discovered in the fruits of *Solanum torvum* Sw. This metabolite turned out to be a potassium salt of an unusual 6-methoxyisoflavanol sulfate composed of a ferulic acid moiety C-5-linked to C-3 of 6-methoxychroman-4β-sulfate (Fig. 6.12). It exhibited moderate antiviral activity (herpes simplex virus type 1) (Arthan et al. 2002). However, low levels of genistein and daidzein, their methoxy derivatives, and not yet determined glycosides were regularly detected in leaves and flowers of several *Nicotiana* spp., *Solanum dulcamara* L., and *S. lycopersicum* L. sub nom. *Lycopersicum esculentum* Mill. (Mackova et al. 2006).

6.7.4.2 Occurrence in the Convolvulaceae

From the stems of *Erycibe expansa* Wall., a woody climber of wide distribution in Southeast Asia, altogether 20 isoflavonoid aglycones have been isolated and structurally elucidated. Two new prenylisoflavones, erycibenins A and B, and one new pterocarpane, erycibenin C (Fig. 6.12), could be characterized together with ten known isoflavones, e.g., genistein, formononetin, as well as seven known pterocarpans, e.g., pterocarpin, medicarpin. Some of these metabolites exhibited in vitro hepatoprotective activities (cultured mouse hepatocytes) equivalent to that of the potent hepatoprotective clinically used silybin. Thus, these results represent a certain support for the use of the stems for the treatment of hepatitis and other hepatic diseases in traditional Thai medicine (Matsuda et al. 2004). The already known isoflavones clyosin and erythrinin B as well as two rotenoids, deguelin and rotenone, altogether constituents of *E. expansa*, were found to show inhibitory activities on lipopolysaccharide nitric oxide (NO) production in mouse peritoneal macrophages (Morikawa et al. 2006). Together with two further congeners, tephrosin and 12a-hydroxyrotenone, these four rotenoids represent the first members of this group of metabolites found in a Solanales species.

6.8 Lignans and Neolignans

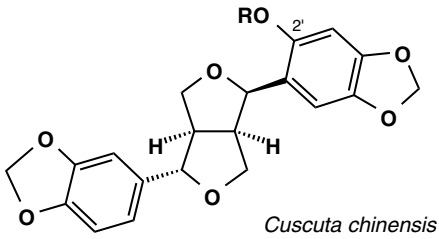
Lignans are *C-C* linked dimeric phenylpropanoids. This *C-C* linkage proceeds predominantly by the central carbon of the monomeric C_3-side chains ("tail-to tail") (Strack 1997). A corresponding biogenetic sequence is the following: coniferyl alcohol → (+)-pinoresinol (8,8′-furofuran type) → (+)-lariciresinol (do.) → (−)-secoisolariciresinol (8,8′-dibenzylbutanediol type) → (−)-matairesinol (dibenzylbutyrolactone type) → (−)-arctigenin (do.) → (−)-trachelogenin (do.) (Fig. 6.13). Diversity in lignan biosynthesis has been reviewed comprehensively by Umezawa (2003). Systematics and nomenclature of lignans were summarized by Freudenberg and Weinges (1961). A monograph on lignans was published by Ayres and Loike (1990).



R = H Pinoresinol

R = CH_3 Pinoresinol dimethyl ether

Humbertia madagascariensis

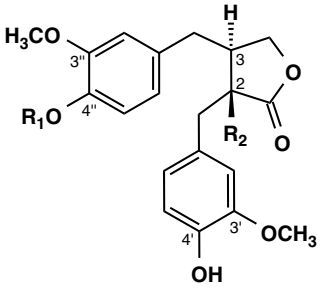


Cuscuta chinensis

R = glu-(6-1)-api Cuscutoside A

R = glu-(6-1)-xyl Cuscutoside B

[R = H 2'-Hydroxyasarinin]



R_1	R_2	
H	H	Matairesinol
CH_3	H	Arctigenin
CH_3	OH	Trachelogenin

Ipomoea cairica

Fig. 6.13 Furofuran and dibenzylbutyrolactone type lignans isolated from convolvulaceous species (glu = β-D-glucose; api = β-D-apiose; xyl = β-D-xylose). The arrows in the structure of pinoresinol indicate that this bond is not existing in the structure of lariciresinol, i.e., there is a free hydroxyl group which is glycosylated in case of its 9-glucoside (a constituent of *Solanum tuberosum*, Solanaceae)

Neolignans are "head-to-tail" linked dimeric phenylpropanoids, i.e., a C_3-side chain of one monolignol (coniferyl alcohol) is linked to the aromatic nucleus of a second one. This may proceed via *C-O-C* linkages, e.g., 8.*O*.4′-type. A comprehensive review on neolignans was published by Gottlieb (1978).

Lignans and neolignans are widely distributed in the plant kingdom and represent plesiomorphic characters. They vary substantially in oxidation level, substitution pattern, and the chemical structure of their principal carbon framework (Umezawa 2003). They may be antibiotic and antifeedant agents. Certain groups, e.g., lignanamides as well as sesquilignans and sesquineolignans are rather rare. Their occurrence is an erratical one in the whole plant kingdom as well as from the infrafamilial point of view in respective families. Sesquilignans are known from 15–20 families, the presence of sesquineolignans seems to be even more restricted.

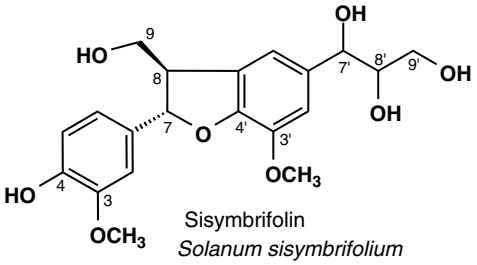
6.8.1 Occurrence in the Solanaceae

6.8.1.1 Miscellaneous Types of Lignans

Lariciresinol and 5′-methoxylariciresinol were isolated from *Nierembergia rigida* MIERS sub nom. *N. aristata* Auct. non MIERS (Gil et al. 1995). It could be demonstrated that roots and stolons of *Solanum tuberosum* produced lariciresinol 9-glucoside (Fig. 6.13) as a stress metabolite due to an infestation of the potato cyst nematode *Globodera rostochiensis* (WOLL.) BEHRENS (Hegnauer 1990 and references therein). A new benzofuran type neolignan, sisymbrifolin (Fig. 6.14), was isolated from the berries of *S. sisymbrifolium* LAM., a perennial suffrutex (Chakravarty et al. 1996). It represented the first lignan from a *Solanum* species. Recently, three new metabolites were isolated from the leaves of *Cestrum parqui* L'HÉRIT. (D'Abrosca et al. 2006). Unfortunately, the authors have missed to choose trivial names for their really unusual structures. It's about (i) a "head-to-head" linked 4.*O*.4′-type neolignan with two chain-terminal ester groups, (ii) a 2(3*H*)-furanone type *nor*lignan, and a bibenzofuran type sesquilignan. To avoid complicated nomenclature according to the rules of IUPAC the reader is referred to the structures of Fig. 6.14.

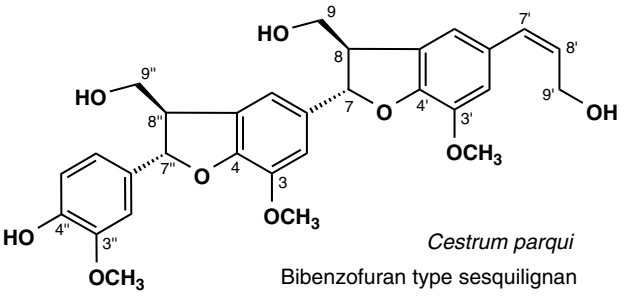
6.8.1.2 Lignanamides (Fig. 6.15)

A neolignandiamide, grossamide, could be discovered in roots of *Capsicum annuum* L. var. *grossum* SENDTN. It was assumed to be a product formed by oxidative coupling of two molecules of *N*-feruloyltyramine since it showed co-occurrence with this monomer (Yoshihara et al. 1981, 1983). Later, it could be detected also in *Cannabis sativa* L. (Cannabaceae) and *Annona crassiflora* MART. (Annonaceae) (Santos et al. 1996 and references therein). From the seeds of *Hyoscyamus niger* L., well-known for their content of tropane alkaloids, four closely related metabolites were isolated and structurally elucidated. Indeed, it is obvious to suppose that they were coniferyl alcohol dimers. Three of them represent lignandiamides: Cannabisin D ("tail to tail" as well as "head to tail" linked monomers, i.e., phenyldihydronaphthalene type) and cannabisin



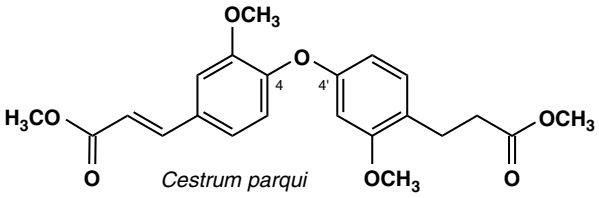
Sisymbrifolin

Solanum sisymbrifolium



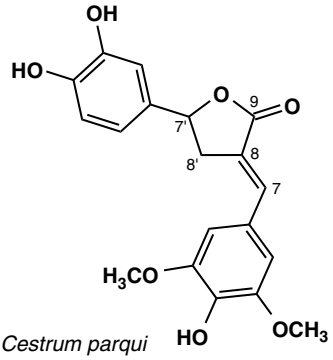
Cestrum parqui

Bibenzofuran type sesquilignan



Cestrum parqui

"Head-to-head" linked 4.O.4'-type neolignan



Cestrum parqui

2(3H)-furanone type norlignan

Fig. 6.14 Novel neolignan, sesquineolignan, and norlignan metabolites from the Solanaceae (numbering according to HCA monomers)

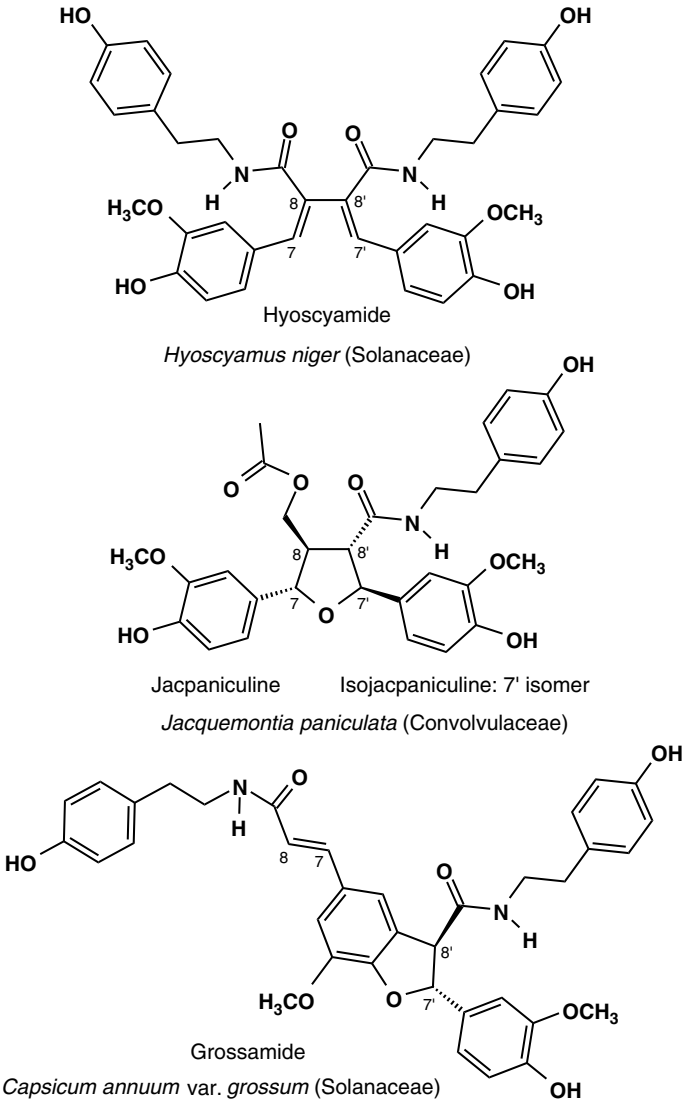


Fig. 6.15 Examples for a lignandiamide, two lignan(mono)amides, and a neolignandiamide discovered in the order Solanales. Hyoscyamide is a dimer of *cis*-*N*-feruloyltyramine; the corresponding dimeric *trans* isomer, cannabisin G, was also detected in the seeds of *Hyoscyamus niger*

G (only "tail-to-tail" linked monomers), discovered as constituents of *Cannabis sativa* L., and a novel congener named hyoscyamide. The fourth metabolite turned out to be the neolignandiamide grossamide already mentioned above (Ma et al. 2002 and references therein).

6.8.2 Occurrence in the Convolvulaceae

6.8.2.1 Furofuran-type Lignans (Fig. 6.13)

Pinoresinol dimethyl ether represented the first lignan characterized in a convolvulaceous species, isolated from the wood of the basal tree *Humbertia madagascariensis* Lam. (Humbertieae) (Combes et al. 1959). The popular ornamental twiner *Ipomoea nil* (L.) Roth sub nom. *Pharbitis nil* (L.) Choisy revealed pinoresinol 4-*O*-β-D-glucoside (Hirai et al. 1993). The corresponding 5,5′-dimethoxy derivative, the glucoside of syringaresinol, was identified as a constituent of *Cressa cretica* L. (Shahat et al. 2004). Cuscutosides A and B, two novel glycosides of 2′-hydroxyasarinin, were discovered in the seeds of *Cuscuta chinensis* Lam. along with three known lignans [(+)-pinoresinol, its 4-*O*-glucoside, (+)-epipinoresinol] (Yahara et al. 1994).

6.8.2.2 Dibenzylbutyrolactone-type Lignans (Lignanolides) (Fig. 6.13)

The lignanolide-type metabolites arctigenin and trachelogenin as well as their 4-*O*-β-gentiobiosides and the 4-*O*-β-D-glucoside tracheloside were isolated from the seeds of *Ipomoea cairica* (L.) Sweet, railway creeper/mile-a-minute, a pantropical and pansubtropical climbing shrub also used as an ornamental (Trumm and Eich 1989; Trumm 1991; Kayser 1994). Furthermore, matairesinol, the precursor of arctigenin/trachelogenin, could be detected. These lignanolides and their glycosides were confirmed as constituents of *I. cairica* by two other groups (Lin and Chou 1997; De A. Lima and Braz-Filho 1997). Furthermore, arctigenin and trachelogenin were obtained from callus cultures of this species (Páska et al. 1999).

(−)-Arctigenin and (−)-trachelogenin, isolated from *I. cairica*, exhibited remarkable cytostatic activity (L 5178y mouse lymphoma cell system) with ED_{50} values of 1.2 and 2.0 μM, respectively (Trumm and Eich 1989), probably due to their DNA topoisomerase II inhibiting activity (Eich et al. 1991). Furthermore, they turned out to inhibit strongly replication of human immunodeficiency virus type 1 (HIV-1) in infected human cell systems (Eich et al. 1990; Schröder et al. 1990; Pfeiffer et al. 1992). Arctigenin represented a lead structure for studies with a series of semisynthetic derivatives. As a first result it could be demonstrated that its *O*-demethylated congener characterized by a catechol substructure exhibited promising activities against HIV-1 integrase. This enzyme is responsible for the last step in HIV infection inserting the HIV proviral DNA into host DNA; therefore it represents a promising new target for anti-HIV drugs. The semisynthetic derivative of arctigenin with two catechol substructures (3,3′-*O,O*-didemethyl-matairesinol) revealed an increased potency (IC_{50} value of 5.4 μM) (Eich et al. 1993, 1995, 1996). It could be convincingly demonstrated that the inhibiting activity of lignanoid *bis*-catechols is a specific one with respect to HIV integrase (LaFemina et al. 1995). Thus, *O*-demethylated arctigenin derivatives, especially the bis-*O*,*O*-demethyl derivative

were the first specific potent HIV-1 integrase inhibitors in vitro. Later, different natural *bis*-catechols, e.g., (i) lignans like α-conidendrol (LaFemina et al. 1995) and globoidnan A (Ovenden et al. 2004), (ii) dicaffeoyl derivatives like dicaffeoylquinic acids, dicaffeoyltartaric acids, L-chicoric acid (Robinson et al. 1996; McDougall et al. 1998), rosmarinic acid as well as (iii) semisynthetic *bis*-catechols like dicaffeoylmethane (lead structure: curcumin) (Mazumder et al. 1997; Eich 1998 and references therein) were found to possess equal or even higher potency.

Arctigenin (**A**), matairesinol (**M**), and trachelogenin (**T**) and/or their glucosides, already known from other plant families, e.g., *Arctium lappa* L., greater burdock, and *Cnicus benedictus* L. (Asteraceae), were also detected in a few further convolvulaceous species, e.g., *Merremia gemella* (BURM. f.) HALL. f. ssp. *gemella* (roots: **A**, **M**, **T**) (Jenett-Siems 1996), *Jacquemontia corymbulosa* BENTH. (leaves: **A**, **M**); *J. paniculata* (L.) HALL. f. var. *paniculata* (roots: **A**, **T**), *Hewittia sublobata* (L. f.) O.KUNTZE (roots: **M**), *Operculina codonantha* (BENTH.) HALL. f. (leaves: **A**, **M**) (Henrici 1996), *I. alba* (roots: **A**, **M**) (Tofern 1999). These lignans could not be detected in numerous further convolvulaceous species of different genera.

6.8.2.3 Neolignans

Dehydrodiconiferyl alcohol 13-*O*-D-glucoside, a benzofuran type neolignan structurally closely related to sisymbrifolin (Fig. 6.14), discovered in *Catharanthus roseus* (L.) G. DON sub nom. *Vinca rosea* L. (Apocynaceae), was detected in the cotyledons of *Ipomoea nil* (L.) ROTH sub nom. *Pharbitis nil* (L.) CHOISY (Hirai et al. 1994). Virolongin A (Fig. 6.16), an 8.*O*.4′-type metabolite first isolated from *Virola elongata* WARB. (Myristicaceae), turned out to be a constituent of the epigeal vegetative parts of *Bonamia spectabilis* (CHOISY) HALL. f. (Kraft et al. 2002).

6.8.2.4 Lignanamides (Fig. 6.15)

A pair of tetrahydrofuran-type lignanamides, jacpaniculine and its 7′-isomer isojacpaniculine, was discovered in the very small seeds of *Jacquemontia paniculata* (L.) HALL. f. var. *paniculata*, a paleotropical herbaceous twiner. Other parts of this species turned out to lack these metabolites. Jacpaniculine could be characterized by trans-trans-*trans*-configuration concerning the substitution of the tetrahydrofuran moiety with trans-trans-*cis*-configuration found for its isomer. The unusual substitution pattern of the tetrahydrofuran moiety is characterized by an *O*-acetylated hydroxymethyl group at C-8 and the amide group at C-8′ formed by conjugation of the C-9′ carboxyl to tyramine (Henrici et al. 1994). These lignanamides represent unique metabolites, since they could not be detected in any other species checked, neither in the genus *Jacquemontia* [*J. corymbulosa* BENTH., *J. pentantha* (JACQ.) G.DON, *J. tamnifolia* (L.) GRISEB.] nor in numerous further convolvulaceous species belonging to many other genera (Henrici 1996; Eich, unpublished results).

Two lignanamides also formed by conjugation with tyramine – though of the arylnaphthalene-type and including two amide moieties (lignandiamide) – were discovered in the branches of *Porcelia macrocarpa* (WARM.) R.E.FRIES (Annonaceae) (Chaves and Roque 1997). These lignandiamides were closely related to the structure of cannabasin A, discovered in the fruits of *Cannabis sativa* L. (Cannabaceae) (Henrici et al. 1994 and references therein).

6.8.2.5 Sesquilignans and Sesquineolignans (Fig. 6.16)

Eight new tetrahydrofuran-type sesquilignans, bonaspectins A–H, as well as two new 8.*O*.4′-type sesquineolignans, neobonaspectins A and B, were isolated and characterized from the epigeal vegetative parts of *Bonamia spectabilis* (CHOISY) HALL. f., a climbing shrub endemic to tropical east Africa and Madagascar. Bonaspectins C and D were isolated as 4″-*O*-β-D-glucosides. Glycosidation of the remaining metabolites is impossible due to the lack of any hydroxyl group. Two lignans were additional constituents representing the basal skeleton of the sesquilignans: *rel*-(7*S*,8*R*,7′*R*,8′*R*)-3,3′,4,4′,5,5′-hexamethoxylignan, discovered in *Aglaia leptantha* MIQ. (Meliaceae), and its 8*S* isomer, known from *Aristolochia birostris* DUCH. (Aristolochiaceae). Virolongin A, already mentioned above, represented the basal skeleton of the sesquineolignans (Tofern et al. 2000; Kraft et al. 2002 and

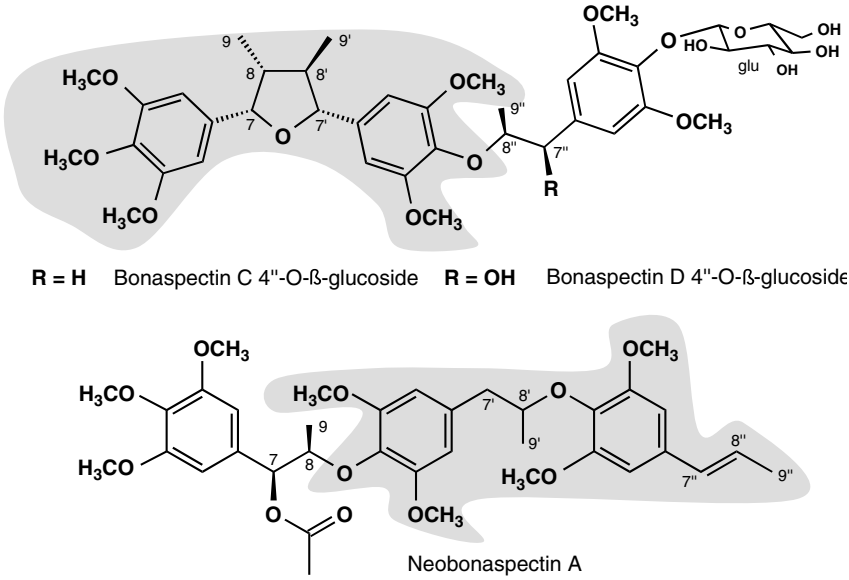


Fig. 6.16 Examples for sesquilignans (bonaspectins) and a neosesquilignan (neobonaspectin) from *Bonamia spectabilis* (Convolvulaceae). The co-occurring (mono)lignan, a hexamethoxylignan (above), and the neolignan virolongin A (below) are highlighted in grey; instead of the linkage to the remaining molecule a methyl group is substituted in both cases

references therein). All constituents were tested for their antiplasmodial activity against a chloroquine-resistant and a chloroquine-sensitive strain of *Plasmodium falciparum*, the most dangerous human malaria parasite. Both sesquilignan glucosides and the aglycone of one of them, bonaspectin C, revealed the highest activity (IC_{50} values: 1.3–6.5 µM). Bonaspectin C 4″-*O*-β-D-glucoside obviously acted mostly on the trophozoite-stage, inhibiting the formation of schizonts. A general cytotoxic mode of action could be excluded, as this sesquilignan exhibited no marked cytoxicity against endothelial ECV-304 cells [IC_{50} values: 52.8 µM (cytotoxicity) vs 1.3 µM (antiplasmodial)] (Kraft et al. 2002).

References

Adsersen A, Adsersen H, Brimer L (1986) A semiquantitative screening for the presence of cyanogenic constituents in plants from the Galápagos Islands. Book of Abstracts, 34th Annual Congress on Medicinal Plant Research, Hamburg, Germany, p 33 [complete poster (P29) seen]

Ahmed B (1998) Cresoside, a new coumaranochromone glycoside from the fruits of *Cressa cretica* L. Indian J Nat Prod 14:29–32

Ahuja KDK, Kunde DA, Ball MJ, Geraghty DP (2006) Effects of capsaicin, dihydrocapsaicin, and curcumin on copper-induced oxidation of human serum lipids. J Agric Food Chem 54:6436–6439

Ando T, Saito N, Tatsuzawa F, Kakefuda T, Yamakage K, Ohtani E, Koshi-ishi M, Matsusake Y, Kokubun H, Watanabe H, Tsukamoto T, Ueda Y, Hashimoto G, Marchesi E, Asakura K, Hara R, Seki H (1999) Floral anthocyanins in wild taxa of *Petunia* (Solanaceae). Biochem Syst Ecol 27:623–650

Ando T, Takahashi M, Nakajima T, Toya Y, Watanabe H, Kokubun H, Tatsuzawa F (2004) Delphinidin accumulation is associated with abnormal flower development in petunias. Phytochemistry 65:2219–2227

Arthan D, Svasti J, Kittakoop P, Pittayakhachonwut D, Tanticharoen M, Thebtaranonth Y (2002) Antiviral isoflavonoid sulfate and steroidal glycosides from the fruits of *Solanum torvum*. Phytochemistry 59:459–463

Austin DF (2002) Personal communication to Eich E

Austin DF, Eich E (2001) Synopsis of *Stictocardia* with another Madagascan species, *S. mojangensis* (Convolvulaceae) Willdenowia 31:79–85

Austin DF, Huáman Z (1996) A synopsis of *Ipomoea* (Convolvulaceae) in the Americas. Taxon 45:3–38

Austin DF, Kitajima K, Yoneda Y, Qian L (2001) A putative tropical American plant, *Ipomoea nil* (Convolvulaceae), in pre-Columbian Japanese art. Econ Bot 55:515–527

Averett JE, D'Arcy WG (1983) Flavonoids of *Oryctes* (Solanaceae). Phytochemistry 22:2325–2326

Ayres DC, Loike JD (1990) Lignans – chemical, biological and clinical properties. Cambridge University Press, Cambridge, UK

Barron D, Varin LV, Ibrahim RK, Harborne JB, Williams CA (1988) Sulphated flavonoids – an update. Phytochemistry 27:2375–2395

Basu SK, De AK (2003) *Capsicum*: Historical and botanical perspectives. In: De AK (ed) *Capsicum* – the genus *Capsicum*. Medicinal and aromatic plants – industrial profiles, vol 33 (Hardman R, ed) Taylor & Francis, London, pp 1–15

Baumert A, Mock HP, Schmidt J, Herbers K, Sonnewald U, Strack D (2001) Patterns of phenylpropanoids in non-inoculated and potato virus Y-inoculated leaves of transgenic tobacco plants expressing yeast-derived invertase. Phytochemistry 56:535–541

Bennett DJ, Kirby GW (1968) Constitution and biosynthesis of capsaicin. J Chem Soc (C) 442–446
Bernards MA, Lewis NG (1992) Alkyl ferulates in wound healing potato tubers. Phytochemistry 31:3409–3412
Bernards MA, Lewis NG (1998) The macromolecular aromatic domain in suberized tissue: a changing paradigm. Phytochemistry 47:915–933
Bhatt SK, Saxena VK, Singh KV (1981) A new eriodictyol glycoside from seeds of *Ipomoea purpurea*. Indian J Pharmaceut Sci 43:109–111
Bienz S, Detterbeck R, Ensch C, Guggisberg A, Häusermann U, Meisterhans C, Wendt B, Werner C, Hesse M (2002) Putrescine, spermidine, spermine, and related polyamine alkaloids. In: Cordell A (ed) The alkaloids, vol 58. Academic Press, San Diego, CA, USA, pp 83–338
Bilia AR, del Mar Escudero Rubio M, Ladero Alvarez M, Muñoz Gonzalez J (1994) New benzyl alcohol glycosides from *Pyrus bourgaeana*. Planta Med 60:569–571
Boatright J, Negre F, Chen X, Kish CM, Wood B, Peel G, Orlova I, Gang D, Rhodes D, Dudareva N (2004) Understanding in vivo benzenoid metabolism in Petunia petal tissue. Plant Physiol 135:1993–2011
Bors W, Langebartels C, Michel C, Sandermann H Jr (1989) Polyamines as radical scavengers and protectants against ozone damage. Phytochemistry 28:1589–1595
Bosland PW (1994) Chiles: history, cultivation, and uses. In: Charalambous G (ed) Spices, herbs and edible fungi (herbs). Elsevier Science Publishers, Amsterdam, NL, pp 347–366
Briggs LH, Cambie RC, Hoare JL (1961) Constituents of some *Solanum* species and a reassessment of solasodamine and solauricine. J Chem Soc 4645–4649
Brouillard R, Dangles O (1994) Flavonoids and flower colour. In: Harborne JB (ed) The flavonoids. Advances in research since 1986. Chapman & Hall, London, pp 565–588
Brown CR (2005) Antioxidants in potato. Am J Potato Res 82:163–172
Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824
Chakravarty AK, Mukhopadhyay S, Saha S, Pakrashi SC (1996) A neolignan and sterols in fruits of *Solanum sisymbrifolium*. Phytochemistry 41:935–939
Chaves MH, Roque NF (1997) Amides and lignanamides from *Porcelia macrocarpa* (Annonaceae). Phytochemsitry 46:879–881
Chiale CA, Cabrera JL, Juliani HR (1990) N_{α}-Cinnamoylhistamine derivatives from *Lycium cestroides*. Phytochemistry 29:688–689
Chmielewska I (1935) Pigments of violet potatoes. Roczniki Chem 15:491–505
Clapham DE (1997) Some like it hot: spicing up ion channels. Nature 389:783–784
Clegg MT, Durbin ML (2000) Flower color variation: a model for the experimental study of evolution. Proc Natl Acad Sci USA 97:7016–7023
Cloyd RA, Cycholl NL (2002) Phytotoxicity of selected insecticides on greenhouse-grown herbs. HortScience 37:671–672
Combes G, Billet D, Mentzer C (1959) On the isolation, structure and properties of a new lignan from *Humbertia madagascariensis*. Bull Soc Chim France 2014
Conn EE (1979) Biosynthesis of cyanogenic glycosides. Naturwissenschaften 66:28–34
Conn EE (1991) The metabolism of a natural product: Lessons learned from cyanogenic glycosides. Planta Med 57, Suppl Issue:S1–S9
Constant HI, Cordell GA (1996) Nonivamide, a constituent of *Capsicum* oleoresin. J Nat Prod 59:425–426
Corner JJ, Harborne JB (1960) Cinnamic acid derivatives of potato berries. Chem Ind (London) 76
Cornu A, Paynot M, Touvin H (1972) Pelargonidin in the flowers of a mutant of *Petunia hybrida*. Phytochemistry 13:2022
Czapek F (1925) Biochemie der Pflanzen, vol 3. Gustav Fischer, Jena
D'Abrosca B, DellaGreca M, Fiorentino A, Monaco P, Zarrelli A (2004) Low molecular weight phenols from the bioactive aqueous fraction of *Cestrum parqui*. J Agric Food Chem 52:4101–4108
D'Abrosca B, DellaGreca M, Fiorentino A, Golino A, Monaco P, Zarrelli A (2006) Isolation and characterization of new lignans from the leaves of *Cestrum parqui*. Nat Prod Res Part A Struct Synth 20:293–298

Dao L, Friedman M (1992) Chlorogenic acid content of fresh and processed potatoes determined by ultraviolet spectrophotometry. J Agric Food Chem 40:2152–2156

De A. Lima OO, Braz-Filho R (1997) Dibenzylbutyrolactone lignans and coumarins from *Ipomoea cairica*. J Braz Chem Soc 8:235–238

De AK (ed) (2003) *Capsicum* – the genus *Capsicum*. Medicinal and aromatic plants – industrial profiles, vol 33 (Hardman R, ed) Taylor & Francis, London

Deshpande SM, Srivastava DN (1969) Chemcial studies of *Convolvulus puricaulis* CHOIS. J Indian Chem Soc 46:759–760

Díaz J, Pomar F, Bernal A, Merino F (2004) Peroxidases and the metabolism of capsaicin in *Capsicum annuum* L. Phytochem Rev 3:141–157

Dini I, Tenore GC, Dini A (2006) New polyphenol derivative in *Ipomoea batatas* tubers and its antioxidant activity. J Agric Food Chem 54:8733–8737

Dubey P, Khare N, Gupta PC (1982) A new flavonoid glycoside from the leaves of *Ipomoea fistulosa*. Curr Sci 51:351–352

Dudareva N, Pichersky E, Gershenzon J (2004) Biochemistry of plant volatiles. Plant Physiol 135:1893–1902

Edelson JV, Duthie J, Roberts W (2002) Toxicity of biorational insecticides: activity against the green peach aphid, *Myzus persicae* (SULZER). Pest Managem Sci 58:255–260

Ehmann A (1974) *N*-(p-Coumaryl)-tryptamine and *N*-ferulyl-tryptamine in kernels of *Zea mays*. Phytochemistry 13:1979–1983

Eich E (1998) Secondary metabolites from plants as antiretroviral agents: promising lead structures for ant-HIV drugs of the future. In: Lawson LD, Bauer R (eds) Phytomedicines of Europe – Chemistry and Biological Activity, ACS Symposium Series 691. American Chemical Society, Washington/DC-USA, pp 83–96

Eich E, Sattler HJ, Henn E (1986) Ipobscurines, non-ergoline type indole compounds from the seeds of *Ipomoea obscura*. Planta Med 52:523–524

Eich E, Henn E, Kohlshorn H, Pertz H, Schulz J (1989) Ipobscurine B, a melatonin analogous novel indole alkaloid from the seeds of *Ipomoea obscura*. Planta Med 55:607

Eich E, Schulz J, Trumm S, Sarin PS, Maidhof A, Merz H, Schröder HC, Müller WEG (1990) Lignanolides: novel in vitro anti-HIV active agents. Planta Med 56:506

Eich E, Schulz J, Kaloga M, Merz H, Schröder HC, Müller WEG (1991) Interference of epipodophyllotoxins and natural lignanolides with topomisomerase II: a proposed molecular mechanism. Planta Med 57, Suppl Issue 2:7

Eich E, Pertz H, Schulz J, Fesen MR, Mazumder A, Pommier Y (1993) Lignanolides from herbal remedies as prodrugs and their presumptive mammalian metabolites as potent HIV-1 integrase inhibitors. Book of abstracts, PSE-Symposium on Phytochemistry of Plants Used in Traditional Medicine, Lausanne, Switzerland, P97

Eich E, Pertz H, Kaloga M, Schulz J, Fesen MR, Mazumder A, Pommier Y (1995) (–)-Arctigenin as lead structure for HIV-1 integrase inhibitors. Book of abstracts, NIH Conference on Retroviral Integrase, Bethesda, MD, USA, P2

Eich E, Pertz H, Kaloga M, Schulz J, Fesen MR, Mazumder A, Pommier Y (1996) (–)-Arctigenin as a lead structure for inhibitors of human immunodeficiency virus type-1 integrase. J Med Chem 39:86–95

Eijkman (1884) Discovery of scopolin/scolpoletin discovery in *Scopolia carniolica*. Ber 17:442; fide Czapek (1925)

Elhabiri M, Figueiredo P, Toki K, Saito N, Brouillard R (1997) Anthocyanin-aluminium and –gallium complexes in aqueous solution. J Chem Soc Perkin Transact 2:355–362

El-Nasr MMS (1982) Coumarins of *Convolvulus lanatus* and *C. arvensis*. Fitoterapia 53:189–190

Figueiredo P, Elhabiri M, Toki K, Saito N, Dangles O, Brouillard R (1996) New aspects of anthocyanin complexation. Intramolecular copigmentation as a means for color loss? Phytochemistry 41:301–308

Fossen T, Øvstedal DO, Slimestad R, Andersen ØM (2003) Anthocyanins from a Norwegian potato cultivar. Food Chem 81:433–437

Francis FJ, Harborne JB (1966) Anthocyanins of the garden huckleberry, *Solanum guineënse*. J Food Sci 31:524–528
Freudenberg K, Weinges K (1961) Systematik und Nomenklatur der Lignane. Tetrahedron 15:115–128
Friedman M (1997) Chemistry, biochemistry, and dietary role of potato polyphenols. J Agric Food Chem 45:1523–1540
Fukui Y, Kusumi T, Yoshida K, Kondo T, Matsuda C, Nomoto K (1998) Structure of two acylated anthocyanins from *Petunia hybrida* cv. Surfinia Violet Mini. Phytochemistry 47:1409–1416
Funayama S, Yoshida K, Konno C, Hikino H (1980) Structure of kukoamine A, a hypotensive principle of *Lycium chinense* root barks. Tetrahedron Lett 21:1355–1356
García-Argáez AN, Pérez-Amador MC, Aguirre-Hernández E, Martínez-Vázquez M (1999) Two new caffeate esters from roots of *Merremia tuberosa* and *M. dissecta*. Planta Med 65:678–679
George F, Figueiredo P, Toki K, Tatsuzawa F, Saito N, Brouillard R (2001) Influence of trans-cis isomerization of coumaric acid substituents on colour variance and stabilisation in anthocyanins. Phytochemistry 57:791–795
Giannasi DE (1988) Flavonoids and evolution in the dicotyledons. In: Harborne JB (ed) The flavonoids (advances in research since 1980). Chapman & Hall, London, pp 479–504
Gil RR, Lin LZ, Chai HB, Pezzuto JM, Cordell GA (1995) Cardenolides from *Nierembergia aristata*. J Nat Prod 58:848–856
Giusti MM, Wrolstad RE (2003) Acylated anthocyanins from edible sources and their applications in food systems. Biochem Engin J 14:217–225
Goda Y, Shimizu T, Kato Y, Nakamura M, Maitani T, Yamada T, Terahara N, Yamaguchi M (1997) Two acylated anthocyanins from purple sweet potato. Phytochemistry 44:183–186
Gonzalez E, Fougerousse A, Brouillard R (2001) Two diacylated malvidin glycosides from *Petunia hybrida* flowers. Phytochemistry 58:1257–1262
Gonzalez MD, Pomilio AB, Gros EG (1981) Terpenoids and alkaloids from *Nierembergia hippomanica*. Anal Asoc Quim Argent 69:297–299
Goto T, Kondo T (1991) Struktur und molekulare Stapelung von Anthocyanen – Variation der Blütenfarben. Angew Chem 103:17–33
Gottlieb OR (1978) Neolignans. In: Zechmeister L, Herz W, Grisebach H, Kirby GW (eds) Progress in the chemistry of organic natural products, vol 35. Springer, Wien, Austria, pp 1–72
Gottlieb OR (1982) Micromolecular evolution, systematics and ecology. Springer, Berlin Heidelberg New York, pp 142–148
Griesbach RJ, Asen S, Leonnarat BA (1991) *Petunia hybrida* anthocyanins acylated with caffeic acid. Phytochemistry 30:1729–1731
Griesbach RJ, Stehmann JR, Meyer F (1999). Anthocyanins in the "red" flowers of *Petunia exserta*. Phytochemistry 51:525–528
Gunatilake AAL, Sultanbawa MUS (1973) Chemical investigation of Ceylonese plants. III. Extractives of the fruits of *Argyreia populifolia* Choisy (Convolvulaceae). J Chem Soc Perkin Trans 1:1155–1157
Gupta OCD, Gupta R, Gupta PC (1980) Chemical examination of flowers of *Ipomoea fistulosa*. Planta Med 38:147–150
Habu Y, Hisatomi Y, Iida S (1998) Molecular characterization of the mutable *flaked* allele for flower variegation in the common morning glory. Plant J 16:371–376
Harborne JB (1960) The coumarins of *Solanum pinnatisectum*. Biochem J. 74:270–273
Harborne JB (1962) The flavonol glycosides of wild and cultivated potatoes. Biochem J 84:100–106
Harborne JB (1964) The structure of acylated anthocyanins. Phytochemistry 3:151–160
Harborne JB (1975) Flavonoid sulphates: a new class of sulphur compounds in higher plants. Phytochemistry 14:1147–1155
Harborne JB, Baxter H (eds) (1999) The handbook of natural flavonoids, 2 vols. Wiley, Chichester, UK

Harborne JB, Grayer RJ (1980) The anthocyanins. In: Harborne JB (ed) The flavonoids. Chapman and Hall, London, pp 1–20
Harborne JB, Swain T (1979) Flavonoids of the Solanaceae. In: Hawkes JG, Lester RN, Skleding AD (eds) The biology and taxonomy of the Solanaceae. Linn Soc Symposium Series No 7, Academic Press, London, pp 257–268
Harborne JB, Williams CA (2000) Advances in flavonoids research since 1992. Phytochemistry 55:481–504
Hawkes JG (1963) fide Nee (1999)
Hawkes JG (1990) fide Nee (1999)
Hayashi K, Hibasami H, Murakami T, Terahara N, Mori M, Tsukui A (2006a) Induction of apoptosis in cultured human stomach cancer cells by potato anthocyanins and its inhibitory effects on growth of stomach cancer in mice. Food Sci Technol Res 12:22–26
Hayashi K, Mori M, Tsukui A (2006b) Functional property of anthocyanin-containing colorful potato. New Food Ind 48:29–32
Hegnauer R (1964) Chemotaxonomie der Pflanzen, vol 3, Convolvulaceae. Birkhäuser Verlag Basel/Switzerland, pp 547–561, 661–662
Hegnauer R (1973) Chemotaxonomie der Pflanzen, vol 6, Solanaceae. Birkhäuser Verlag Basel, Switzerland, pp 403–452, 750–759
Hegnauer R (1989) Chemotaxonomie der Pflanzen, vol 8, Convolvulaceae. Birkhäuser Verlag Basel, Switzerland, pp 321–331, 709–710
Hegnauer R (1990) Chemotaxonomie der Pflanzen, vol 9, Solanaceae. Birkhäuser Verlag Basel/Switzerland, pp 567–602
Hennig J, Malamy J, Grynkiewicz G, Indulski J, Klessig DF (1993) Interconversion of the salicylic acid signal and its glucoside in tobacco. Plant J 4:593–600
Henrici A (1996) Neuartige Sekundärstoffe unterschiedlichster Struktur aus tropischen Convolvulaceen. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany
Henrici A, Kaloga M, Eich E (1994) Jacpaniculines, the first lignanamide alkaloids from the Convolvulaceae. Phytochemistry 37:1637–1640
Henrici A, Kaloga M, Eich E (1995) 1,2,3,4-Tetrahydro-β-carboline-3β-carboxylic acid from *Merremia aegyptia* L. (Convolvulaceae). Book of Abstracts, 43rd Annual Congress of the Society for Medicinal Plant Research, Halle (Saale), Germany, H37
Herrmann K (1978) Hydroxyzimtsäuren und Hydroxybenzoesäuren enthaltende Naturstoffe in Pflanzen. In: Zechmeister L, Herz W, Grisebach H, Kirby GW (eds) Progress in the chemistry of organic natural products, vol 35. Springer, Wien, Austria, pp 73–132
Hesse M (2000) Alkaloide – Fluch oder Segen der Natur? Verlag Helv Chim Act, Zürich, Switzerland/Wiley-VCH Weinheim, Germany, pp 82–83, 153
Higashiguchi F, Nakamura H, Hayashi H, Kometani T (2006) Purification and structure determination of glucosides of capsaicin and dihydrocapsaicin from various *Capsicum* fruits. J Agric Food Chem 54:5948–5953
Hirai N, Kojima Y, Koshimizu K, Shinozaki M, Takimoto A (1993) Accumulation of phenylpropanoids in the cotyledons of Morning Glory (*Pharbitis nil*) seedlings during the induction of flowering by poor nutrition. Plant Cell Physiol 34:1039–1044
Hirai N, Yamamuro M, Koshimizu K, Shinozaki M, Takimoto A (1994) Accumulation of phenylpropanoids in the cotyledons of Morning Glory (*Pharbitis nil*) seedlings during the induction of flowering by low temperature treatment, and the effect of precedent exposure to high-intensity light. Plant Cell Physiol 35:691–695
Hoballah ME, Stuurman J, Turlings TCJ, Guerin PM, Connétable S, Kuhlemeier C (2005) The composition and timing of flower odour emission by wild *Petunia axillaris* coincide with the antennal perception and nocturnal activity of the pollinator *Manduca sexta*. Planta 222:141–150
Hunziker AT (2001) Genera Solanacearum – the genera of Solanaceae illustrated, arranged according to a new system. A.R.G. Gantner Verlag, Ruggell, Liechtenstein

Hussein AA, Olmedo DA, Vasquez Y, Coley PD, Solis PN, Gupta MP (2005) New cytotoxic cinnamic acid derivatives from leaves of *Bonamia trichantha*. Rev Latinoameric Quim 33:90–95

Ichiyanagi T, Kashiwada Y, Shida Y, Ikeshiro Y, Kaneyuki T, Konishi T (2005) Nasunin from eggplant consists of cis-trans iosmers of delphinidin 3-[4-(*p*-coumaroyl)-L-rhamnosyl(1→6)glucopyranoside]-5-glucopyranoside. J Agric Food Chem 53:9472–9477

Ichiyanagi T, Terahara N, Rahman MM, Konishi T (2006) Gastrointestinal uptake of nasunin, acylated anthocyanin in eggplant. J Agric Food Chem 54:5306–5312

Imbert MP (1969) Anthocyanin pigment in *Calystegia silvatica*. Phytochemistry 8:937

Imbert MP, Seaforth CE, Williams DB (1966) Anthocyanin pigments of the sweet potato, *Ipomoea batatas*. J Am Soc Hort Sci 88:481–485

Ishikawa K (2003) Biosynthesis of capsaicinoids in *Capsicum*. De AK (ed) *Capsicum* – the genus *Capsicum*. Medicinal and aromatic plants – industrial profiles, vol 33 (Hardman R, ed) Taylor & Francis, London, pp 87–95

Islam S, Yoshimoto M, Terahara N, Yamakawa O (2002) Anthocyanin composition in sweetpotato (*Ipomoea batatas* L.) leaves. Biosci Biotechnol Biochem 66:2483–2486

Jenett-Siems K (1996) Phytochemische Untersuchungen an Windengewächsen der Gattungen *Calystegia*, *Convolvulus*, *Ipomoea* und *Merremia* unter besonderer Berücksichtigung des Alkaloidvorkommens. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany

Jenett-Siems K, Kaloga M, Eich E (1995) *N*-Containing secondary metabolites from the tropical bindweed *Merremia vitifolia* (Convolvulaceae). Book of abstracts, 43rd Annual Congress of the Society for Medicinal Plant Research, Halle (Saale), Germany, H38

Jenett-Siems K, Weigl R, Kaloga M, Schulz J, Eich E (2003) Ipobscurines C and D: macrolactam-type indole alkaloids from the seeds of *Ipomoea obscura*. Phytochemistry 62:1257–1263

Jurenitsch J, Kastner U (1994) Klassische Pharmakognosie – eine Wissenschaft mit Zukunft? Pharmazie in unserer Zeit 23:93–97

Kamilov KM, Nikonov GK (1977) Esters of *Cuscuta lehmanniana*. Khim Prirod Soed 112

Kaplan MAC, Figueiredo MR, Gottlieb OR (1983) Variation of cyanogenesis in plant with season and insect pressure. Biochem Syst Ecol 11:367–370

Kataoka T (1936) Acta Phytochim Jpn 9:35; fide Pomilio and Sproviero (1972a)

Kawanishi K, Yasufuku J, Ishikawa A, Hashimoto Y (1990) Long-chain alkyl ferulates in three varieties of *Ipomoea batatas* (L.) LAM. J Agric Food Chem 38:105–108

Kayser C (1994) Phytochemische Untersuchungen an pantropischen Arten der Gattung *Ipomoea* als Beitrag zur Chemotaxonomie der Convolvulaceen. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany

Keinanen M, Oldham NJ, Baldwin IT (2001) Rapid HPLC screening of jasmonate-induced increases in tobacco alkaloids, phenolics, and diterpene glycosides in *Nicotiana attenuata* J Agric Food Chem 49:3553–3558

Keller H, Hohlfeld H, Wray V, Hahlbrock K, Scheel D, Strack D (1996) Changes in the accumulation of soluble and cell wall-bound phenolics in elicitor-treated cell suspension cultures and fungus-infected leaves of *Solanum tuberosum*. Phytochemistry 42:389–396

Khalil AM, El-Tawil BAH, Ashy MA, El Beih FKA (1981) Distribution of some coumarins in plants of different plant families grown in Saudi Arabia. Pharmazie 36:569–571

Klessig DF, Malamy J (1994) The salicylic acid signal in plants. Plant Mol Biol 26:1439–1458

Klick S, Herrmann K (1988) Glucosides and glucose esters of hydroxybenzoic acids in plants. Phytochemistry 27:2177–2180

Knudsen JT, Tollsten L, Bergstrom G (1993) Floral scents – a checklist of volatile compounds isolated by headspace techniques. Phytochemistry 33:253–280

Kobata K, Todo T, Yazawa S, Iwai K, Watanabe T (1998) Novel capsaicinoid-like substances, capsiate and dihydrocapsiate, from the fruits of a nonpungent cultivar, CH-19 Sweet, of pepper (*Capsicum annuum* L.). J Agric Food Chem 46:1695–1697

Kobata K, Sutoh K, Todo T, Yazawa S, Iwai K, Watanabe T (1999) Nordihydrocapsiate, a new capsinoid from the fruits of a nonpungent pepper, *Capsicum annuum*. J Nat Prod 62:335–336

Kojima M, Kondo J (1985) An enzyme in sweet potato root which catalyzes the conversion of chlorogenic acid, 3-caffeoylquinic acid, to isochlorogenic acid, 3,5-dicaffeoylquinic acid. Agric Biol Chem 49:2467–2469

Kondo T, Kawai T, Tamura H, Goto T (1987) Structure determination of heavenly blue anthocyanin, a complex monomeric anthocyanin from the morning glory *Ipomoea tricolor*, by means of the negative NOE method. Tetrahedron Lett 28:2273–2276

Kong JM, Chia LS, Goh NK, Chia TF, Brouillard R (2003) Analysis and biological activities of anthocyanins. Phytochemistry 64:923–933

Kosuge S, Furuta M (1970) Pungent principle of *Capsicum*. XIV. Chemical constitution. Agric Biol Chem 34:248–256

Kosuge S, Inagaki Y, Uehara K (1958) Pungent principles of *Capsicum*. I. Chemical constitution of the pungent principles. 1. Isolation of the pungent principles. Nippon Nogei Kagaku Kaishi (J Agric Chem Soc Jpn) 32:578–581

Kosuge S, Inagaki Y, Okamura H (1961) Pungent principles of red pepper. VIII. Chemical constitutions of the pungent principles. 5. Chemical constitution of the pungent principle II. Nippon Nogei Kagaku Kaishi (J Agric Chem Soc Jpn) 35:923–927

Kraft C, Jenett-Siems K, Köhler I, Tofern-Reblin B, Siems K, Bienzle U, Eich E (2002) Antiplasmodial activity of sesquilignans and sesquineolignans from *Bonamia spectabilis*. Phytochemistry 60:167–173

Kurata R, Adachi M, Yamakawa O, Yoshimoto M (2007) Growth suppression of human cancer cells by polyphenolics from sweetpotato (*Ipomoea batatas* L.) leaves. J Agric Food Chem 55:185–190

Kuroda C, Wada M (1933) The coloring matter of eggplant (Nasu). Proc Imp Acad (Tokyo) 9:51–52

Kuroda C, Wada M (1936) Constitution of natural coloring matters, kuromamin, shisonin, and nasunin. Bull Chem Soc Jpn 11:272–287

Lachman J, Hamouz K (2005) Red and purple potatoes as a significant antioxidant source in human nutrition – a review. Plant Soil Envir 51:477–482

LaFemina RL, Graham PL, LeGrow K, Hastings J, Wolfe A, Young SD, Emini EA, Hazuda DJ (1995) Inhibtion of human immunodeficiency virus integrase by bis-catechols. Antimicrob Agents Chemother 39:320–324

Lechtenberg M, Nahrstedt A (1999) Cyanogenic glycosides. In: Ikan R (ed) Naturally occurring glycosides. Wiley, New York, pp 147–191

Levey DJ, Tewksbury JJ, Cipollini ML, Carlo TA (2006) A field test of the directed deterrence hypothesis in two species of wild chili. Oecologia 150:61–68

Lewis CE, Walker JRL, Lancaster JE, Sutton KV (1998a) Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: Coloured cultivars of *Solanum tuberosum* L. J Sci Food Agric 77:45–57

Lewis CE, Walker JRL, Lancaster JE, Sutton KV (1998b) Determination of anthocyanins, flavonoids and phenolic acids in potatoes. II: Wild, tuberous *Solanum* species. J Sci Food Agric 77:58–63

Li Z, Dunyuan X, Jinquan Z, Chen Y (1993) Study on chemical constituents of volatile oil of *Robinia pseudacacia* L. J Lanzhou Univ (Nat Sci) 29:125–126

Lin LC, Chou CJ (1997) Lignans and flavonoids of *Ipomoea cairica*. Chin Pharmac J 49:13–20

Liu X, Lin Y (2003) Biological activity of capsaicin and its joint action with other pesticides. Nongyaoxue Xuebao 5:94–96

Löffler C, Czygan FC, Proksch P (1997) Phenolic constituents as taxonomic markers in the genus Cuscuta (Cuscutaceae). Biochem Syst Ecol 25:297–303

Lopez JA (1980) Separation of scopoletin in *Markea megalandra* D'Arcy ("*M. leucantha*" Donn Smith). Ingen Cie Quim 4:154

Lu TS, Saito N, Yokoi M, Shigihara A, Honda T (1991) Acylated peonidin glycoside in the violet-blue flowers of *Pharbitis nil*. Phytochemistry 30:2387–2390

Lu TS, Saito N, Yokoi M, Shigihara A, Honda T (1992a) Acylated pelargonidin glycosides in the red-purple flowers of *Pharbitis nil*. Phytochemistry 31:289–295

Lu TS, Saito N, Yokoi M, Shigihara A, Honda T (1992b) Acylated peonidin glycosides in the violet-blue cultivars of *Pharbitis nil*. Phytochemistry 31:659–663

Ma CY, Liu WK, Che CT (2002) Lignanamides and nonalkaloidal components of *Hyoscyamus niger* seeds. J Nat Prod 65:206–209

Mackova Z, Koblovska R, Lapcik O (2006) Distribution of isoflavonoids in non-leguminous taxa – an update. Phytochemistry 67:849–855

Manirakiza P, Covaci A, Schepens P (2003) Pungency principles in *Capsicum* – analytical determinations and toxicology. In: De AK (ed) *Capsicum* – the genus *Capsicum*. Medicinal and aromatic plants – industrial profiles, vol 33 (Hardman R, ed) Taylor & Francis, London, pp 71–86

Mann P (1997) Zur Phytochemie und Chemotaxonomie tropischer und mediterraner Convolvulaceen unter besonderer Berücksichtigung des Alkaloidvorkommens. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany

Mann P, Eich E, Witte L, Hartmann T (1996) GC-MS study on the alkaloid pattern of *Merremia quinquefolia* (L.) H.HALL. f.: first occurrence of retronecine esters, simple phenylethylamine derivatives, and pyrrolidides in the Convolvulaceae. Book of abstracts, 44th Annual Congress of the Society for Medicinal Plant Research and a Joint Meeting with the Czech Biotechnology Society, Prague, Czech Republic, p 148 (P 251)

Mann P, Tofern B, Kaloga M, Eich E (1999) Flavonoid sulfates from the Convolvulaceae. Phytochemistry 50:267–271

Martens S, Mithöfer A (2005) Flavones and flavone synthases. Phytochemistry 66:2399–2407

Martin-Tanguy J, Cabanne F, Perdrizet E, Martin C (1978) The distribution of hydroxycinnamic acid amides in flowering plants. Phytochemistry 17:1927–1928

Mason L, Moore RA, Derry S, Edwards JE, McQuay HJ (2004) Systematic review of topical capsaicin for the treatment of chronic pain. Brit Med J 328:991–994

Masuda T, Yamada K, Maekawa T, Takeda Y, Yamagucchi H (2006) Antioxidant mechanism studies on ferulic acid: identification of oxidative coupling products from methyl ferulate and linoleate. J Agric Food Chem 54:6069–6074

Matsubara K, Kaneyuki T, Miyake T, Mori M (2005) Antiangiogenic activity of nasunin, an antioxidant anthocyanin, in eggplant peels. J Agric Food Chem 53:6272–6275

Matsuda H, Morikawa T, Xu F, Ninomiya K, Yoshikawa M (2004) New isoflavones and pterocarpane with hepatoprotective activity from the stems of *Erycibe expansa*. Planta Med 70:1201–1209

Matsui T, Ueda T, Oki T, Sugita K, Terahara N, Matsumoto K (2001a) α-Glucosidase inhibitory action of natural acylated anthocyanins. I. Survey of natural pigments with potent inhibitory activity. J Agric Food Chem 49:1948–1951

Matsui T, Ueda T, Oki T, Sugita K, Terahara N, Matsumoto K (2001b) α-Glucosidase inhibitory action of natural acylated anthocyanins. 2. α-Glucosidase inhibition by isolated acylated anthocyanins. J Agric Food Chem 49:1952–1956

Matsui T, Ebuchi S, Fukui K, Matsugano K, Terahara N, Matsumoto K (2004) Caffeoylsophorose, a new natural α-glucosidase inhibitor, from red vinegar by fermented purple-fleshed sweet potato. Biosci Biotechnol Biochem 68:2239–2246

Mazumder A, Neamati N, Sunder S, Schulz J, Pertz H, Eich E, Pommier Y (1997) Curcumin analogs with altered potencies against HIV-1 integrase as probes for biochemical mechanisms of drug action. J Med Chem 40:3057–3063

McArdle BM, Campitelli MR, Quinn RJ (2006) A common protein fold topology shared by flavonoid biosynthetic enzymes and therapeutic targets. J Nat Prod 69:14–17

McDougall B, King PJ, Wu BW, Hostomsky Z, Reinecke MG, Robinson WE Jr (1998) Dicaffeoylquinic and dicaffeoyltartaric acids are selective inhibitors of human immunodeficiency virus type 1 integrase. Antimicrob Agents Chemother 42:140–146

Miller RE, Rausher MD, Manos PS (1999) Phylogenetic systematics of *Ipomoea* (Convolvulaceae) based on ITS and waxy sequences. Syst Bot 24:209–227

Mimaki Y, Watanabe K, Ando Y, Sakuma C, Sashida Y, Furuya S, Sakagami H (2001) Flavonol glycosides and steroidal saponins from the leaves of *Cestrum nocturnum* and their cytoxicity. J Nat Prod 64:17–22

Minamikawa T, Akazawa T, Uritani I (1964) Two glucosides of coumarin derivatives in sweet potato roots infected by *Ceratocystis fimbriata*. Agr Biol Chem 28:230–233
Mistry TV, Cai Y, Lilley TH, Haslam E (1991) Polyphenol interactions. Part 5. Anthocyanin co-pimentation. J Chem Soc Perkin Trans 2:1287–1296
Mølgaard P, Ravn H (1988) Evolutionary aspects of caffeoyl ester distribution in dicotyledons. Phytochemistry 27:2411–2421
Mori A, Lehmann S, O'Kelly J, Kumagai T, Desmond JC, Pervan M, McBride WH, Kizaki M, Koeffler HP (2006) Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 prostate cancer cells. Cancer Res 66:3222–3229
Mori M, Kondo T, Toki K, Yoshida K (2006) Structure of anthocyanin from the blue petals of *Phacelia campanularia* and its blue flower color development. Phytochemistry 67:622–629
Morikawa T, Xu F, Matsuda H, Yoshikawa M (2006) Structures of new flavonoids, erycibenins D, E, and F, and NO production inhibitors from *Erycibe expansa* originating in Thailand. Chem Pharm Bull 54:1530–1534
Mothes K, Kala H (1955) Die Wurzel als Bildungsstätte für Cumarine. Naturwissenschaften 42:159
Mustafa NR, Verpoorte R (2005) Chorismate derived C6C1 compounds in plants. Planta 222:1–5
Nahrstedt A, Jensen PS, Wray V (1989) Prunasin-6′-malonate, a cyanogenic glucoside from *Merremia dissecta*. Phytochemistry 28:623–624
Nahrstedt A, Sattar EA, El-Zalabani SMH (1990) Amygdaline acyl derivatives, cyanogenic glycosides from the seeds of *Merremia dissecta*. Phytochemistry 29:1179–1181
Nair GG, Daniel M, Sabnis SD (1986) Chemosystematics of *Ipomoea* L. and some related taxa. Curr Sci 55:961–965
Nee M (1999) Synopsis of *Solanum* in the New World. In: Nee M, Symon D, Lester RN, Jessop JP (eds) Solanaceae IV – advances in taxonomy and utilization. Royal Botanic Gardens, Kew, UK, pp 285–333
Negrel J, Martin C (1984) The biosynthesis of feruloyltyramine in *Nicotiana tabacum*. Phytochemistry 23:2797–2801
Negrel J, Pollet B, Lapierre C (1996) Ether-linked ferulic acid amides in natural and wound periderms of potato tubers. Phytochemistry 43:1195–1199
Nelson EK (1919) The constitution of capsaicin, the pungent principle of *Capsicum*. J Am Chem Soc 41:1115–1121
Nelson EK, Dawson LE (1923) The constitution of capsaicin, the pungent principle of *Capsicum*. III. J Am Chem Soc 45:2179–2181
Neumann RH (2001) *Capsicum*-based disinfectant and sterilizant. US Pat Appl Publ, 12 pp, Cont.-in-part of US Ser No 747,225
Ochi T, Takaishi Y, Kogure, K Yamauti I (2003) Antioxidant activity of a new capsaicin derivative from *Capsicum annuum*. J Nat Prod 66:1094–1096
Odake K, Terahara N, Saito N, Toki K, Honda T (1992) Chemical structure of two anthocyanins from purple sweet potato, *Ipomoea batatas*. Phytochemistry 31:2127–2130
Oki T, Suda I, Terahara N, Sato M, Hatakeyama M (2006) Determination of acylated anthocyanin in human urine after ingesting a purple-fleshed sweet potato beverage with various contents of anthocyanin by LC-ESI-MS/MS. Biosci Biotechnol Biochem 70:2540–2543
Ovenden SPB, Yu J, Wan SS, Sberna G, Tait RM, Rhodes D, Cox S, Coates J, Walsh NG, Meurer-Grimes BM (2004) Globoidnan A: a lignan from *Eucalyptus globoidea* inhibits HIV integrase. Phytochemistry 65:3255–3259
Pale E, Nacro M, Vanhaelen, M, Vanhaelen-Fastré R, Ottinger R (1998) Acylated anthocyanins from the flowers of *Ipomoea asarifolia*. Phytochemistry 48:1433–1437
Pale E, Kouda-Bonafos M, Nacro M, Vanhaelen, M, Vanhaelen-Fastré R (2003) Two triacylated and tetraglucosylated anthocyanins from *Ipomoea asarifolia* flowers. Phytochemistry 64:1395–1399
Páska C, Innocenti G, Kunvári M, László, Szilágyi L (1999) Lignan production by *Ipomoea cairica* callus cultures. Phytochemistry 52:879–883

Pearce G, Marchand PA, Griswold J, Lewis NG, Ryan CA (1998) Accumulation of feruloyltyramine and *p*-coumaroyltyramine in tomato leaves in response to wounding. Phytochemistry 47:659–664

Peng J, Deng X, Huang J, Jia S, Miao X, Huang Y (2004) Role of salicylic acid in tomato defense against cotton bollworm, *Helicoverpa armigera* HÜBNER. Z Naturforsch 59c:856–862

Perry L, Dickau R, Zarrillo S, Holst I, Pearsall DM, Piperno DR, Berman MJ, Cooke RG, Rademaker K, Ranere AJ, Raymond JS, Sandweiss DH, Scaramelli F, Tarble K, Zeidler JA (2007) Starch fossils and the domestication and dispersal of chili peppers (*Capsicum* spp. L.) in the Americas. Science 315:986–988

Pfeiffer K, Merz H, Steffen R, Müller WEG, Trumm S, Schulz J, Eich E, Schröder HC (1992) In vitro anti-HIV activity of lignans – differential inhibition of HIV-1 integrase reaction, topoisomerase activity and cellular microtubules. J Pharm Med 2:75–97

Pichersky E, Noel JP, Dudareva N (2006) Biosynthesis of plant volatiles: nature's diversity and ingenuity. Science 311:808–811

Politis J (1948) Distribution of chlorogenic acid in Solanaceae and in the organs of these plants. Compt Rend 226:692–693

Pomilio AB, Sproviero JF (1972a) Acylated anthocyanins from *Ipomoea cairica*. Phytochemistry 11:1125–1128

Pomilio AB, Sproviero JF (1972b) Complex anthocyanins from *Ipomoea congesta*. Phytochemistry 11:2323–2326

Pongprayoon U, Baeckström P, Jacobsson U, Lindström M, Bohlin L (1991) Compounds inhibiting prostaglandin synthesis isolated from *Ipomoea pes-caprae*. Planta Med 57:515–518

Prasad BCN, Gururaj HB, Kumar V, Giridhar P, Parimalan R, Sharma A, Ravishankar GA (2006a) Influence of 8-methyl-nonenoic acid on capsaicin biosynthesis in in-vivo and in-vitro cell cultures of *Capsicum* spp. J Agric Food Chem 54:1854–1859

Prasad BCN, Kumar V, Gururaj HB, Parimalan R, Giridhar P, Ravishankar GA (2006b) Characterization of capsaicin synthase and identification of its gene (csy1) for pungency factor capsaicin in pepper (*Capsicum* sp.) Proc Nat Acad Sci USA 103:13315–13320

Prasad BCN, Gururaj HB, Kumar V, Giridhar P, Ravishankar GA (2006c) Valine pathway is more crucial than phenyl propanoid pathway in regulating capsaicin biosynthesis in *Capsicum frutescens* MILL. J Agric Food Chem 54:6660–6666

Ragab AS, van Fleet J, Jankowski B, Park JH, Bobzin SC (2006) Detection and quantitation of resveratrol in tomato fruit (*Lycopersicon esculentum* MILL.) J Agric Food Chem 54:7175–7179

Ragunathan V, Sulochana N (1994) A new flavone glycoside from the flowers of *Ipomoea purpurea* ROTH. Indian J Chem 33B:507–508

Raguso RA, Levin RA, Foose SE, Holmerg MW, McDade LA (2003) Fragrance chemistry, nocturnal rhythms and pollination "syndromes" in *Nicotiana*. Phytochemistry 63:265–284

Ravishankar GA, Suresh B, Giridhar P, Ramachandra S, Sudhakar Johnson T (2003) Biotechnological studies on *Capsicum* for metabolite production and plant improvement. In: De AK (ed) *Capsicum* – the genus *Capsicum*. Medicinal and aromatic plants – industrial profiles, vol 33 (Hardman R, ed) Taylor & Francis, London, pp 96–128

Reznik H, Wietschel G (1979) Die Flavonoid-Muster der knollentragenden *Solanum*-Arten. I. Charakterisierung der Flavonoid-Glycoside. Z Pflanzenphysiol 95:239–253

Robinson WE Jr, Reinecke MG, Abdel-Malek S, Jia Q, Chow SA (1996) Inhibitors of HIV-1 replication that inhibit HIV integrase. Proc Nat Acad Sci USA 93:6326–6331

Robiquet, Boutron-Charlard (1830) Ann Chim Phys 44:352; fide Czapek (1925)

Ryabinin AA, Il'ina EM (1949) The alkaloid of *Salsola subaphylla*. Dok Akad Nauk SSSR 67:513–516

Sadilova E, Stintzing FC, Carle R (2006) Anthocyanins, colour and antioxidant properties of eggplant (*Solanum melongena* L.) and violet pepper (*Capsicum annuum* L.) peel extracts. Z Naturforsch 61c:527–535

Sahai M, Singh M, Singh AK, Hara N, Fujimoto Y (1994) Cesternosides A and B, novel glucosides from the leaves of *Cestrum nocturnum*. J Chem Res Synopses 22–23

Saito N, Hotta R, Imai K, Hayashi K (1965) Petanin isolated from the berries of *Solanum nigrum* var. *guineënse*. Proc Jpn Acad 41:593–598

Saito N, Lu TS, Yokoi M, Shigihara A, Honda T (1993) An acylated cyanidin 3-sophoroside-5-glucoside in the violet-blue flowers of *Pharbitis nil*. Phytochemistry 33:245–247

Saito N, Lu TS, Akaizawa M, Yokoi M, Shigihara A, Honda T (1994a) Acylated pelargonidin glucosides in the maroon flowers of *Pharbitis nil*. Phytochemistry 35:407–411

Saito N, Cheng J, Ichimura M, Yokoi M, Abe Y, Honda T (1994b) Flavonoids in the acyanic flowers of *Pharbitis nil*. Phytochemistry 35:687–691

Saito N, Tatsuzawa F, Yoda K, Masato K, Kasahara K, Iida S, Shigihara A, Honda T (1995) Acylated cyanidin glycosides in the violet-blue flowers of *Ipomoea purpurea*. Phytochemistry 40:1283–1289

Saito N, Tatsuzawa F, Kasahara K, Yokoi M, Iida S, Shigihara A, Hondo T (1996a) Acylated peonidin glycosides in the slate flowers of *Pharbitis nil*. Phytochemistry 41:1607–1611

Saito N, Tatsuzawa F, Yokoi M, Kasahara K, Iida S, Shigihara A, Hondo T (1996b) Acylated pelargonidin glycosides in the red-purple flowers of *Ipomoea purpurea*. Phytochemistry 43:1365–1370

Saito N, Tatsuzawa F, Kasahara K, Iida S, Hondo T (1998) Acylated cyanidin 3-sophorosides in the brownish-red flowers of *Ipomoea purpurea*. Phytochemistry 49:875–880

Saito N, Toki K, Morita Y, Hoshino A, Iida S, Shigihara A, Honda T (2005) Acylated peonidin glycosides from *duskish* mutant flowers of *Ipomoea nil*. Phytochemistry 66:1852–1860

Sakamura S, Terayama Y, Kawakatsu S, Ichihara A, Saito H (1978) Conjugated serotonins related to cathartic activity in safflower seeds (*Carthamus tinctorius* L.). Agric Biol Chem 42:1805–1806

Sakamura S, Terayama Y, Kawakatsu S, Ichihara A, Saito H (1980) Conjugated serotonins and phenolic constituents in safflower seed (*Carthamus tinctorius* L.). Agric Biol Chem 44:2951–2954

Santos LP, Boaventura MA, Braga de Oliveira A, Cassady JM (1996) Grossamide and *N*-trans-caffeoyltyramine from *Annona crassiflora* seeds. Planta Med 62:76

Sarker SD, Savchenko T, Whiting P, Sik V, Dinan L (1997) Moschamine, *cis*-moschamine, moschamindole and moschamindolol: Four novel indole alkaloids from *Centaurea moschata*. Nat Prod Lett 9:189–194

Sarker SD, Laird A, Nahar L, Kumarasamy Y, Jaspars M (2001) Indole alkaloids from the seeds of *Centaurea cyanus*. Phytochemistry 57:1273–1276

Sarmento da Silva TM, de Carvalho MG, Braz-Filho R, Agra MdF (2003) Occurrence of flavones, flavonols and its glycosides in species of the genus *Solanum*. Quim Nova 26:517–522

Sato H, Kawagishi H, Nishimura T, Yoneyama S, Yoshimoto Y, Sakamura S, Furusaki A, Katsuragi S, Matsumoto T (1985) Serotobenine, a novel phenolic amide from safflower seeds. Agric Biol Chem 49:2969–2974

Sattar EA, Glasl H, Nahrstedt A, Hilal SH, Zaki AY, El-Zalabani SMH (1990) Hydroxycinnamic acid amides from *Iochroma cyaneum*. Phytochemistry 29:3931–3933

Sattar EA, Gala A, Rashwan O (1995) Caffeoyl derivatives from the seeds of *Ipomoea fistulosa*. Int J Pharmacog 33:155–158

Schijlen EGWM, Ric de Vos CH, van Tunen AJ, Bovy AG (2004) Modification of flavonoid biosynthesis in crop plants. Phytochemistry 65:2631–2648

Schmeda-Hirschmann G, Jordan M, Gerth A, Wilken D, Hormazabal E, Tapia AA (2004) Secondary metabolite content in *Fabiana imbricata* plants and in vitro cultures. Z Naturforsch 59c:48–54

Schmidt E (1890) Discovery of scopolin/scolpoletin discovery in *Scopolia carniolica*. Arch Pharm 228:435; fide Czapek 1925

Schröder HC, Merz H, Steffen R, Müller WEG, Sarin PS, Trumm S, Schulz J, Eich E (1990) Differential in vitro anti-HIV activity of natural lignans. Z Naturforsch 45c:1215–1221

Scoville WL (1912) Note on Capsicums. J Am Pharm Assoc 1:453–454

Shahat AA, Abdel-Azim NS, Pieters L, Vlietinck AJ (2004) Isolation and NMR spectra of syringaresinol-β-D-glucoside from *Cressa cretica*. Fitoterapia 75:771–773

Shimizu BI, Miyagawa H, Ueno T, Sakata K, Watanabe K, Ogawa K (2004) Morning glory systematically accumulates scopoletin and scopolin after interaction with *Fusarium oxysporum*. Z Naturforsch 60c:83–90

Siemens J, Zhou S, Piskorowski R, Nikai T, Lumpkin EA, Basbaum AI, King D, Julius D (2006) Spider toxins activate the capsaicin receptor to produce inflammatory pain. Nature 444:208–212

Silverman P, Nuckles E, Ye XS, Kuc J, Raskin I (1993) Salicylic acid, ethylene, and pathogen resistance in tobacco. Mol Plant-Microbe Interact 6:775–781

Springob K, Nakajima J, Yamazaki M, Saito K (2003) Recent advances in the biosynthesis and accumulation of anthocyanins. Nat Prod Rep 20:288–303

Steinharter TP, Cooper-Driver GA, Anderson GJ (1986) The phylogenetic relationship of *Solanum* flavonols. Biochem Syst Ecol 14:299–305

Strack D (1997) Phenolic metabolism. In: Dey PM, Harborne JB (eds) Plant biochemistry. Academic Press, San Diego, USA, pp 387–416

Strack D, Gross W, Wray V, Grotjahn L (1987) Enzymic synthesis of caffeoylglucaric acid from chlorogenic acid and glucaric acid by a protein preparation from tomato cotyledons. Plant Physiol 83:475–478

Sukrasno N, Yeoman MM (1993) Phenylpropanoid metabolism during growth and development of *Capsicum frutescens* fruits. Phytochemistry 32:839–844

Suzuki T, Iwai K (1984) Constituents of red pepper species: chemistry, biochemistry, pharmacology, and food science of the pungent principle of *Capsicum* species. In: Brossi A (ed) The alkaloids – chemistry and pharmacology, vol 23. Academic Press, Orlando, FL, USA, pp 227–299

Suzuki Y, Yamguchi I, Murofushi N, Takahashi N (1988) Biological conversion of benzoic acid in *Lemna paucicostata* 151 and its relation to flower induction. Plant Cell Physiol 29:439–444

Szolcsányi J (2003) Future perspectives of capsaicin. In: De AK (ed) *Capsicum* – the genus *Capsicum*. Medicinal and aromatic plants – industrial profiles, vol 33 (Hardman R, ed) Taylor & Francis, London, pp 248–269

Tantisewie B, Ruijgrok HWL, Hegnauer R (1969) Die Verbreitung der Blausäure bei den Cormophyten. Pharmac Weekbl 104:1341–1355

Tatsuzawa F, Mikanagi Y, Saito N (2004) Flower anthocyanins of *Calystegia* in Japan. Biochem Syst Ecol 32:1235–1238

Teramachi F, Koyano T, Kowithayakorn T, Hayashi M, Komiyama K, Ishibashi M (2005) Collagenase inhibitory quinic acid esters from *Ipomoea pes-caprae*. J Nat Prod 68:794–796

Terahara N, Shimizu T, Kato Y, Nakamura M, Maitani T, Yamaguchi M, Goda Y (1999) Six acylated anthocyanins from the storage roots of purple sweet potato, *Ipomoea batatas*. Biosci Biotechnol Biochem 63:1420–1424

Terashima S, Shimizu M, Horie S, Morita N (1991) Studies on aldose reductase inhibitors from natural products. IV. Constituents and aldose reductase inhibitory effect of *Chrysanthemum morifolium*, *Bixa orellana and Ipomoea batatas*. Chem Pharm Bull 39:3346–3347

Tewksbury JJ, Manchego C, Haak DC, Levey DJ (2006) Where did the chili get its spice? Biogeography of capsaicinoid production in ancestral wild chili species. J Chem Ecol 32:547–564

Thampi PSS (2003) A glimpse of the world trade in *Capsicum*. In: De AK (ed) *Capsicum* – the genus *Capsicum*. Medicinal and aromatic plants – industrial profiles, vol 33 (Hardman R, ed) Taylor & Francis, London, pp 16–24

Tian Q, Konczak I, Schwartz SJ (2005) Probing anthocyanin profiles in purple sweet potato cell line (*Ipomoea batatas* L. cv. Ayamurasaki) by high performance liquid chromatography and electrospray ionization tandem mass spectrometry. J Agric Food Chem 53:6503–6509

Tofern B (1999) Neue und seltene Sekundärstoffe des Phenylpropan-, Terpen- und Alkaloid-Stoffwechsels aus tropischen Convolvulaceen. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany

Tofern B, Jenett-Siems K, Siems K, Jakupovic J, Eich E (2000) Bonaspectins and neobonaspectins, first sesquilignans and sesquineolignans from a convolvulaceous species. Phytochemistry 53:119–128

Toki K, Saito N, Kawano K, Lu TS, Shigihara A, Honda T (1994) An acylated delphinidin glycoside in the blue flowers of *Evolvulus pilosus*. Phytochemistry 36:609–612

Trease D, Evans WC (2002) Pharmacognosy, 15th edn. W.B.Saunders, Edinburgh, UK

Tresh LT (1876) Isolation of capsaicin. Pharm J 1:941; (1877) 7:21, 259, 473; fide Czapek (1925)

Trumm S (1991) Dem Shikimat-Weg entstammende niedermolekulare Sekundärstoffe der Convolvulaceen. Dissertation, Fachbereich Chemie und Pharmazie, Johannes Gutenberg-Universität Mainz, Germany

Trumm S, Eich E (1989) Cytostatic activities of lignanolides from *Ipomoea cairica*. Planta Med. 55:658–659

Tseng CF, Mikajiri A, Shibuya M, Goda Y, Ebizuka Y, Padmawinata K, Sankawa U (1986) Effects of some phenolics on the prostaglandin synthesizing enzyme system. Chem Pharm Bull 34:1380–1383

Umezawa T (2003) Diversity in lignan biosynthesis. Phytochem Rev 2:371–390

Vaknin H, Bar-Akiva A, Ovadia R, Nissim-Levi A, Forer I, Weiss D, Oren-Shamir M (2005) Active anthocyanin degradation in *Brunfelsia calycina* (yesterday-today-tomorrow) flowers. Planta 222:19–26

Van Romburgh P (1893) fide Hegnauer (1989)

Vernooij B, Uknes S, Ward E, Ryals J (1994) Salicylic acid as a signal molecule in plant-pathogen interactions. Curr Opin Cell Biol 6:275–279

Walsh BM, Hoot SB (2001) Phylogenetic relationships of *Capsicum* (Solanacea) using DNA sequences from two noncoding regions: The chloroplast *atpB-rbcL* spacer region and nuclear *waxy* introns. Int J Plant Sci 162:1409–1418

Walters DR (2003) Polyamines and plant disease. Phytochemistry 64:97–107

Weehuizen F (1906) *Merremia ficifolia*, een blauwzuurplant. Pharmac Weekbl 43:907–908

Weigl R (1992) Entdeckung, Isolierung und Strukturaufklärung neuer Alkaloide im Rahmen chemotaxonomischer Untersuchungen an Convolvulaceen. Dissertation, Fachbereich Pharmazie, Freie Universität Berlin, Germany

Weigl R, Kaloga M, Eich E (1991) Iposcurine C: a macrocyclic novel serotonin alkaloid with neolignan substructure from the seeds of *Ipomoea obscura*. Planta Med 57, Suppl Issue 2: A135

Whiting DA (2001) Natural phenolic compounds 1900–2000: a bird's eye view of a century's chemistry. Nat Prod Rep 18:583–606

Wietschel G, Reznik H (1980a) Die Flavonoid-Muster der knollentragenden *Solanum*-Arten. II. Die Flavonoid-Glycoside der Arten aus Series I – XVI. Z Pflanzenphysiol 97:79–88

Wietschel G, Reznik H (1980b) Die Flavonoid-Muster der knollentragenden *Solanum*-Arten. III. Flavonoid-Glycoside der Arten aus Series XVII. Z Pflanzenphysiol 99:149–158

Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defense. Nature 414:562–565

Wöhler F, Liebig J (1837) Ann Chim Phys 64:185; fide Czapek (1925)

Wollenweber E, Dörsam M, Dörr, M, Roitman JN, Valant-Vetschera KM (2005) Chemodiversity of surface flavonoids in Solanaceae. Z Naturforsch 60c:661–670

Yahara S, Domoto H, Sugimura C, Nohara T, Niiho Y, Nakajima Y, Ito H (1994) An alkaloid and two lignans from *Cuscuta chinensis*. Phytochemistry 37:1755–1757

Yalpani N, Enyedi AJ, Leon J, Raskin I (1994) Ultraviolet light and ozone stimulate accumulation of salicylic acid, pathogenesis-related proteins and virus resistance. Planta 193:372–376

Yoneda Y, Takenaka Y (1981) Genshoku Asagao Kensaku Zukan (Natural-color illustrated monograph of Asagao (Japanese morning glory)), 2nd edn. Hokuryukan, Tokyo, Japan

Yoshida K, Osanai M, Kondo T (2003) Mechanism of dusky reddish-brown "kaki" color development of Japanese morning glory, *Ipomoea nil* cv. Danjuro. Phytochemistry 63:721–726

Yoshihara T, Takamatsu S, Sakamura S (1978) Three new phenolic amides from the roots of eggplant (*Solanum melongena* L.). Agric Biol Chem 42:623–627

Yoshihara T, Yamaguchi K, Takamatsu S, Sakamura S (1981) A new lignan amide, grossamide, from bell pepper (*Capsicum annuum* var. *grossum*). Agric Biol Chem 45:2593–2598

Yoshihara T, Yamaguchi K, Sakamura S (1983) The relative configuration of grossamide and hordatines. Agric Biol Chem 47:217–220

Yoshimoto M, Yahara S, Okuno S, Islam MS, Ishiguro K, Yanakawa O (2002) Antimutagenicity of mono-, di-, and tricaffeoylquinic acid derivatives isolated from sweetpotato (*Ipomoea batatas* L.) leaf. Biosci Biotechnol Biochem 66:2336–2341

Yoshitama K, Ishii K, Yasuda H (1980) A chromatographic survey of anthocyanins in the Flora of Japan, I. J Fac Sci, Shinshu Univ 15:20–25

Yoshitani SI, Tanaka T, Kohno H, Takashima S (2001) Chemoprevention of azoxymethane-induced rat colon carcinogenesis by dietary capsaicin and rotenone. Int J Oncol 19:929–939

Zagrobelny M, Bak S, Rasmussen AV, Jørgensen B, Naumann CM, Møller BL (2004) Cyanogenic glucosides and plant-insect interactions. Phytochemistry 65:293–306

Zewdie Y, Bosland PW (2001) Capsaicinoid profiles are not good chemotaxonomic indicators for *Capsicum* species. Biochem Syst Ecol 29:161–169

Zhang WY, Li Wan Po A (1994) The effectiveness of topically applied capsaicin. A meta-analysis. Eur J Clin Pharmacol 46:517–522

Zhou LX, Ding Y (2002) A cinnamide derivative from *Solanum verbascifolium* L. J Asian Nat Prod Res 4:185–187

Zufall RA, Rausher MD (2003) The genetic basis of a flower color polymorphism in the common morning glory (*Ipomoea purpurea*). J Hered 94:442–448

Zufall RA, Rausher MD (2004) Genetic changes associated with floral adaptation restrict future evolutionary potential. Nature 428:847–850

7
Terpenoids (Isoprenoids)

Biogenetic Outline. The basal, central building block for any biosynthesis of terpenoids (isoprenoids), isopentenyl diphosphate, may be generated in plants by two different pathways, the cytosolic mevalonate and the plastidic methylerythritol (deoxyxylulose) pathways. The cytosolic pathway was assumed to provide the precursors for sesquiterpenoids and triterpenoids/steroids, the plastidic one for mono-, di-, and tetraterpenoids. However, it has been proven recently that this strict separation of both pathways does not exist. Cross-talk between both pathways has been discovered (De-Eknamkul and Potduang 2003; Bartram et al. 2006 and references therein). The transformation of isopentenyl diphosphate to dimethylallyl diphosphate is catalyzed by a corresponding isomerase. A condensation of both isomers catalyzed by a plastidic prenyltransferase leads to the common precursor of all monoterpenoids (C_{10}), geranyl diphosphate ("head-to-tail" condensation). Condensation of geranyl diphosphate and another isopentenyl diphosphate generates farnesyl diphosphate (C_{15}), the common precursor of all sesquiterpenoids. Farnesyl diphosphate in turn acts as a prenyl donor for isopentenyl diphosphate to form geranylgeranyl diphosphate (C_{20}), the common precursor of all diterpenoids. In contrast to all these "head-to-tail" condensations, "head-to-head" condensation of two molecules of farnesyl diphosphate is responsible for the formation of squalene (C_{30}), the common precursor of all triterpenoids. The analogous reaction of two molecules of geranylgeranyl diphosphate yields phytoene (C_{40}), the common precursor of all tetraterpenoids. Almost all of these precursors are generated as *all-trans* isoprenoids (exception: phytoene is yielded predominantly in the 15-*cis* configurated form). Nevertheless, they may change their configuration in the course of specific pathways leading to certain secondary metabolites.

Solanesol, an *all-trans* nonaprenyl alcohol (C_{45}), meanwhile known as a ubiquitous plant metabolite, was discovered in flue-cured tobacco; later it was also detected in green tobacco. Its acetate as well as fatty acid esters, e.g., palmitate, were also identified (Enzell et al. 1977 and references therein). Plastoquinone A, fulfilling a role in photosynthesis, is the most abundant nonaprenylquinone in *N. tabacum* (Irvine et al. 1972). Furthermore, ubiquinone-10, characterized by a terpenoid portion composed of ten all head-to-tail linked isoprene units, was isolated from tobacco (Enzell et al. 1977 and references therein).

Eckart Eich, *Solanaceae and Convolvulaceae: Secondary Metabolites*

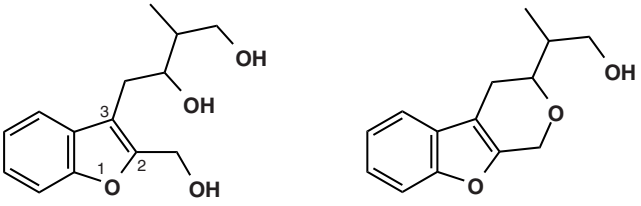
The use of high-solanesol tobacco for smoking should be avoided due to the possible formation of excessive levels of carcinogenic aromatic hydrocarbons such as benzo[α]pyrene from solanesol. This is also true for nonsmoking tobacco products when fire-cured (Chamberlain et al. 1988).

7.1 Hemiterpenoids (C_5 Isoprenoids)

Hemiterpenoid residues have been discovered frequently as part of, e.g., aromatic natural structures. They are integrated into such molecules by prenylation of the aromatic skeleton, i.e., substitution by a dimethylallyl group (or a biogenetic equivalent). Such groups may be also transformed secondarily, e.g., furanocoumarins (Apiaceae, Rutaceae).

7.1.1 Occurrence in the Convolvulaceae

Tropane alkaloids of the merresectine type (Fig. 3.20) as well as those of the derived consabatine type (Fig. 3.23) are characterized by prenylated benzoyl moieties or their derivatives. The first pathway-specific step in the biosynthesis of ergoline alkaloids also represents a prenylation: A prenyltransferase catalyzes the transfer of a dimethylallyl moiety from dimethylallyldiphosphate to C-4 of the tryptophan nucleus (Fig. 4.4). Furthermore, the structure of certain isoflavones, erycibenins A and B (Fig. 6.12), includes oxidized prenyl residues. Bonaseminols A and B (Fig. 7.1) representing a novel type of benzofurans were discovered as constituents of the epigeal vegetative parts of *Bonamia semidigyna* (Roxb.) Hall. f. var. *semidigyna* (Henrici 1996; Böhm et al. 1999). In contrast to common natural benzofurans the aromatic skeleton of these compounds is unsubstituted in its six-membered part. The benzofuran moiety might be formed by cyclization of *o*-hydroxycinnamic alcohol or an equivalent congener; alternatively, it might be



Bonaseminol A Bonaseminol B

Bonamia semidigyna

Fig. 7.1 Unique benzofurans substituted by a hemiterpenoid at C-3 (subsequent ring closure in case of congener B) from a paleotropical convolvulaceous species

synthesized by the polyketide pathway. Finally, this benzofuran nucleus is apparently substituted at C-3 by a prenyl unit or an equivalent with two hydroxyl groups.

7.2 Monoterpenoids (C_{10} Isoprenoids)

Geranyl diphosphate is the common biogenetic precursor of all monoterpenoids. They are common components of essential oils (volatile oils, aetherolea) stored in specific cells / organs (alternatively glandular hairs, oil cells, secretion ducts or cavities) of, e.g., Pinaceae, Apiaceae, Lamiaceae. However, Solanaceae and Convolvulaceae do not belong to the families characterized by the occurrence of essential oils stored in specific cells / organs. Nevertheless, monoterpenoids are probably ubiquitous in higher plants (Bramley 1997), e.g., as volatile constituents in the fragrance of flowers. Monoterpenoids represent one major class of the so-called volatile organic compounds (VOCs) or plant volatiles (PVs). Compounds characterized by alcoholic or phenolic hydroxyl groups may be stored also as glycosides. Iridoids, a large subclass of non-volatile monoterpenoids do not occur in the two large Solanales families.

7.2.1 Occurrence in the Solanaceae

7.2.1.1 Volatile Organic Compounds (VOCs) / Plant Volatiles (PVs)

The largest class of VOCs (PVs) is represented by lipophilic mono-, sesqui-, and diterpenoids with high vapour pressure. The vast majority consists of mono- and sesquiterpenoids. Such compounds serve various ecological roles. A recent review on the biosynthesis of VOCs including other classes beside terpenoids such as phenylpropanoids/benzenoids (see Sect. 6.3) and fatty acids derivatives (see Sect. 8.1) has been published by Pichersky et al. (2006).

***Cestrum*.** Among the odoriferous volatile floral constituents of C. *nocturnum* the monoterpenes *cis*- and *trans*-ocimene and the monoterpenoid linalool could be detected (Fig. 7.2) (Li et al. 1988).

***Nicotiana*.** In a comprehensive study on the floral and vegetative fragrance chemistry, nocturnal rhythms and pollination "syndromes" numerous monoterpenoids were identified by GC/MS analyses of nine *Nicotiana* spp. (*N. alata, N. bonariensis, N. forgetiana, N. langsdorffii, N. plumbaginifolia, N. longiflora, N. rustica, N. suaveolens, N. sylvestris*; authorities see Table 3.4) (Raguso et al. 2003). The following subclasses could be detected (Fig. 7.2):

- Acyclic monoterpenes, e.g., myrcene, and cyclohexanoid congeners, e.g., limonene, α- and β-pinenes, camphene
- Acyclic oxygenated monoterpenoids, e.g., nerol, geraniol, linalool, and cyclohexanoid congeners, e.g., α-terpineol, 1,8-cineole, camphor

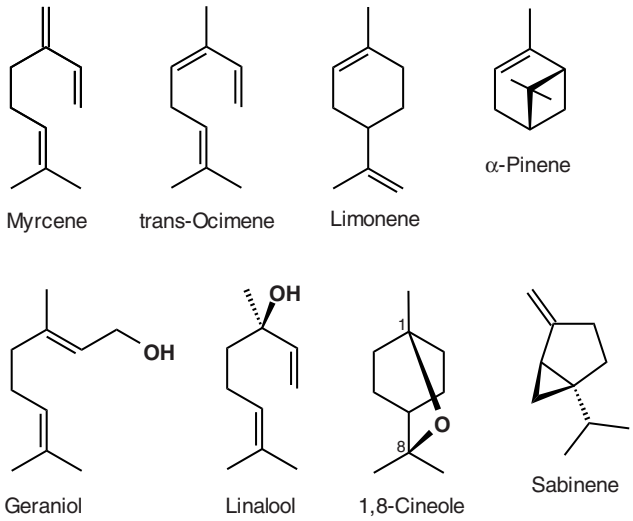


Fig. 7.2 Monoterpenes/monoterpenoids from floral and vegetative volatile emissions of *Nicotiana* spp. (Solanaceae)

- Cyclopentanoid monoterpenoids, e.g., sabinene
- Irregular monoterpenoids, e.g., 1,3,3-trimethyl-7-oxabicyclo[4.1.0]heptan-2,5-dione (derived from β-carotene; see also Sect. 7.12.1)

Four species belonging to sect. *Alatae* sensu strictu, *N. alata*, *N. bonariensis*, *N. forgetiana*, and *N. langsdorffii*, were found to emit large amounts of 1,8-cineole, known as the main component of the essential oil of certain *Eucalyptus* spp. (Myrtaceae). Together with 1,8-cineole smaller amounts of monoterpenes (myrcene, limonene, pinenes, sabinene) and α-terpineol were emitted on a nocturnal rhythm. Interestingly, subsets of these compounds were emitted by **other** *Nicotiana* spp., usually by vegetative sources such as calyx, leaf or stem tissues.

The study involved the detection of altogether 125 volatiles. Beside monoterpenoids the following classes of metabolites were also found (species-dependent): Sesquiterpenoids (Sect. 7.3), phenylpropanoids (Sect. 6.3.3.1), aliphatic alcohols/ketones/esters, and nitrogenous compounds such as nitriles or aldoximes (Raguso et al. 2003).

In a subsequent, again comprehensive study the same group of authors has extended the previous analyses especially to include two additional, newly discovered species from the American section *Alatae* (*N. mutabilis*; "Rastroensis") and three species of their sister group, the largely Australasian section *Suaveolentes* (*N. cavicola, N. ingulba*, *N. africana*). It has been confirmed that species of the former section emit large amounts of 1,8-cineole and smaller amounts of monoterpenes on a nocturnal rhythm, constituting a chemical synapomorphy for this lineage. Furthermore, it could be confirmed that species of the latter section emit fragrances dominated by benzenoids/phenylpropanoids ("aromatic-dominated floral scents"). Interestingly, the sole

sub-Saharan African species, *N. africana*, nested within the *Suaveolentes*, did not show any distinct floral scent; instead only methyl 5-methylhexanoate was emitted during day and night in small amounts (Raguso et al. 2006).

Brugmansia/Datura. Floral VOCs of two *Brugmansia* spp. [*B. arborea* (L.) LAGERH. sub nom. *D. arborea* (L.), *B. candida* PERS. sub nom. *D. candida* (PERS.) SAFF.] and three *Datura* spp. [*D. inoxia* MILL., *D. metel* L., *D. stramonium* L.] were analyzed by Kawashima (1996). The results showed rich blends of monoterpenes (e.g., ocimene, pinenes, limonene, sabinene) and oxidized congeners including esters (e.g., linalool, linalyl acetate, citronellol, nerol, neryl acetate, neral, geraniol, geranyl formate, camphor, carvone, 1,8-cineol, fenchol) (Fig. 7.2) beside members of different classes of metabolites (e.g., phenylpropanoids, fatty acid esters), but almost no sesquiterpenes.

Solanum lycopersicum. In response to insect feeding, tomato plants (*S. lycopersicum* L. sub nom. *Lycopersicon esculentum* MILL.) were found to release locally and systemically elevated levels of the monoterpenes α-pinene, β-pinene, 2-carene, and β-phellandrene as well as different sesquiterpenes (Sect. 7.3) (Farag and Paré 2002).

Capsicum frutescens. Volatiles of Cayenne pepper have been studied in a *comprehensive* GC-MS analysis (Cardeal et al. 2006). Its blend is composed of monoterpenes (e.g., limonene, β-phellandrene, sabinene, β-pinene, γ-terpinene), oxidized congeners (e.g., linalool, terpinen-4-ol, α-phellandrene epoxide), sesquiterpenes (see Sect. 7.3.1.1), and members of different other classes of metabolites.

Glycosidic Precursors. Monoterpenoid volatiles containing a hydroxyl group may be stored as non-volatile glycosides, e.g., (*R*)-linalyl-β-D-glucopyranoside or (R)-linalyl-α-*O*-arabinopyranosyl-(1→6)-β-D-glucopyranoside, found as an aroma precursor (linalool) in *Solanum vestissimum* DUN. (Osorio et al. 2003 and references therein).

7.2.2 Occurrence in the Convolvulaceae

Abundant monoterpene alcohols such as linalool, nerol, geraniol, citronellol, and α-terpineol were found in damaged sweet potatoes, the tubers of *Ipomoea batatas* LAM. In contrast, fresh sweet potatoes are free of such compounds. The so-called "Imo-Itamisyu" flavor, often detected with stressed storage roots of this species, was found to be mainly caused by nerol and citronellol. "Imo-Shochu", a Japanese spirit (sake), produced from such sweet potatoes is characterized by a flavor similar to muscat possibly caused by "Imo-Itamisyu" (Kamiwatari et al. 2005). Volatile extracts from storage roots, site of oviposition, were attractive to female sweetpotato weevil, *Cylas formicarius elegantulus* SUMMERS (Coleoptera: Curculionidae), the single most devastating pest of sweet potato. A large number of terpenes could be identified in the extracts including monoterpenoids such as nerol, *Z*-citral, and methyl geranate. These three metabolites were shown to be attractants, whereas the sesquiterpenoid fraction was repellent (see Sect. 7.3.2) (Wang and Kays 2002).

7.3 Sesquiterpenoids (C_{15} Isoprenoids)

all-trans-Farnesyl diphosphate represents the common precursor of all sesquiterpenoids. However, this has to be relativised for those which are formed by cleavage of carotenoids (see Sect. 7.12). Most sesquiterpenoids are volatile and therefore also components of essential oils (volatile oils, aetherolea) stored in specific cells / organs (alternatively glandular hairs, oil cells, secretion ducts or cavities), often in co-occurrence with monoterpenoids. Beside this occurrence in essential oils sesquiterpenoids have been discovered also as constitutive metabolites in other plant tissues. However, there exists a further aspect. As pointed out by Stoessl et al. (1976) injury to a plant may lead to the production of **stress compounds** which accumulate to substantially higher levels in the damaged than in healthy tissue. This may happen, e.g., by liberation of toxic aglycones from non-toxic constitutive glycosides or by de novo synthesis of secondary metabolites. A subgroup of the stress compounds consists of the **phytoalexins** which are formed due to the contact with a fungus. Such metabolites cause inhibition of this phytopathogenic organism (Stoessl et al. 1976; Kojima and Uritani 1981). Sesquiterpenoids represent one specific class of phytoalexins synthesized by certain species of both large Solanales families besides other characteristic classes in other families. The original work which led to the principal phytoalexin hypothesis was conducted with potatoes (Müller and Börger 1940). However, there is a fluent transition between constitutive metabolites and phytoalexins. E.g., in the case of two closely related compounds, one may belong to the former group and the other to the latter.

7.3.1 Occurrence in the Solanaceae

7.3.1.1 Constitutive Sesquiterpenoids Including Volatile Organic Compounds (VOCs)

***Fabiana*.** Fourteen sesquiterpenes with muurolane (10 congeners) and amorphane (4) skeletons were isolated from the aerial parts of *F. imbricata* Ruiz & Pav. (Brown 1994). The most abundant compound was the 15-malonate ester of 4-muurulen-7,15-diol (pernetyl malonate). An example for the amorphane derivatives is given by an isomer, the corresponding ester of 4-amorphen-11,15-diol (Fig. 7.3). In addition, three novel cadinane type metabolites (fabiaimbricatane derivatives) and one norcadinane congener (armarillone) were identified (Schmeda-Hirschmann and Papastergiou 1994).

***Petunia*.** During petal limb expansion germacrene D and cadina-3,9-diene were emitted by the flowers of the garden petunia, *P.* × *hybrida* (Hook.) Vilm., a hybrid between *P. integrifolia* (Hook.) Schinz & Thell. and P. axillaris (Lam.) Britton, Stern & Poggenburg (Verdonk et al. 2003). Structures of closely related derivatives of both sesquiterpenes are shown in Fig. 7.4.

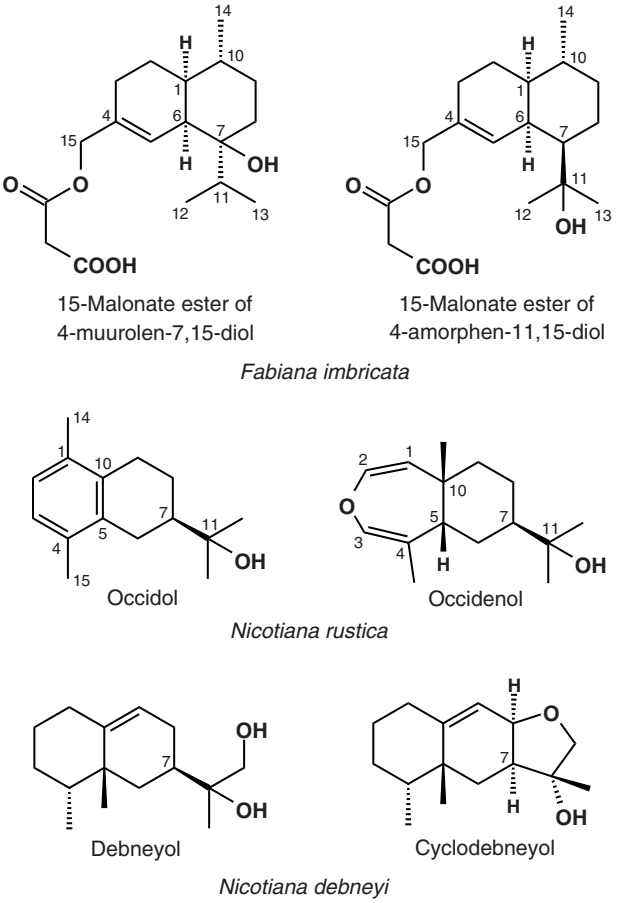


Fig. 7.3 Sesquiterpenoids from the Solanaceae: constitutive constituents of a *Fabiana* sp. and stress metabolites discovered in two *Nicotiana* spp. inoculated with TMV (*N. rustica*) and TNV (*N. debneyi*)

Cestrum. α-Farnesene, an isomer of β-farnesene (Fig. 7.4), turned out to be one of the major components of VOCs from fresh flowers of *C. nocturnum* L. beside the monoterpenoid linalool and benzenoids/phenylpropanoids (Li et al. 1988).

Nicotiana. The comprehensive study mentioned already above (see Sect. 7.2.1.1) demonstrated also the occurrence of C_{15} VOCs, i.e., sesquiterpenes {e.g., β-caryophyllene (Fig. 7.4), α-cedrene, different farnesenes (Fig. 7.4), α-humulene} and oxygenated sesquiterpenes (e.g., different *cis-trans* isomers of nerolidol, farnesal, and farnesol, as well as caryophyllene oxide) (Raguso et al. 2003). Further constitutive sesquiterpenoids from *Nicotiana* will be discussed below in connection with phytoalexins of this genus.

cis-α-Bergamotene, a carbobicyclic sesquiterpene named after its occurrence in the essential oil of *Citrus bergamia* Risso & Poit. (Rutaceae), represents the most prominent terpenoid VOC of *Nicotiana attenuata* Torrey ex S. Watson. Blends

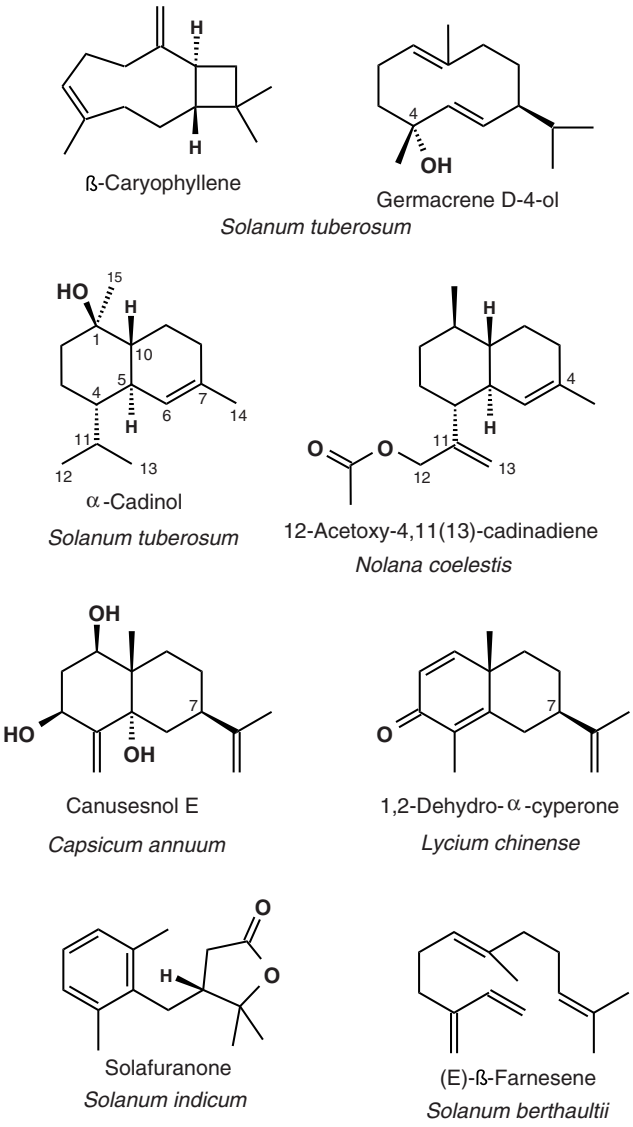


Fig. 7.4 Constitutive sesquiterpenoids from the Solanaceae

deficient in this sesquiterpene have been found to regulate numerous genes in "**receiver** plants" after emission from herbivory attack-caused damage of transformed (genetically silenced) "**emitter** plants" of neighboring conspecifics. This indicates a suppressive effect of terpenoids like *cis*-α-bergamotene (Paschold et al. 2006). Emission of bergamotene after herbivore attack was also demonstrated to increase lepidopteran egg predation rates by a generalist predator in nature (Kessler and Baldwin 2001).

Nolana. In the aerial parts of *N. coelestis* MIERS ex DUN. four 4,11(13)-cadinadienes were discovered, e.g., the 12-acetoxy derivative (Fig. 7.4). Its congeners showed structural differences with regard to the substituents at C-4 (CH_2OAc) and/or the lack of the acetyl group at 12*O* (OH). Unfortunately, the authors neither created trivial names nor published correct chemical nomenclature (Garbarino et al. 1993).

Lycium. Solavetivone (Fig. 7.5) and (−)-1,2-dehydro-α-cyperone (Fig. 7.4), were identified as constituents of the volatile components from the half-dried berries of *Lycium chinense* (Sannai et al. 1982).

Phrodus. A new acyclic sesquiterpenoid, 9-acetoxynerolidol, was isolated from the aerial parts of *P. bridgesii* MIERS (Gambaro et al. 1986) [nerolidol represents a position isomer of farnesol (terminal methylene group and hydroxyl at C-3 instead of a terminal primary hydroxyl group and 2,3-double bond)].

Brugmansia/Datura. Floral VOCs of two *Brugmansia* spp. [*B. arborea* (L.) LAGERH. sub nom. *D. arborea* (L.), *B. candida* PERS. sub nom. *D. candida* (PERS.) SAFF.] and three *Datura* spp. [*D. inoxia* MILL., *D. metel* L., *D. stramonium* L.] were analyzed by Kawashima (1996). The results showed rich blends of monoterpenes/monoterpenoids (see Sect. 7.2.1.1), but only a very small number of sesquiterpenes (farnesol, longifolene).

Solanum. Recently, 17 sesquiterpene hydrocarbons and two alcohols [germacrene D-4-ol, α-cadinol; Fig. 7.4], altogether known compounds, have been identified in the leaf surface of 10 *S. tuberosum* L. (potato) varieties. The distribution turned out

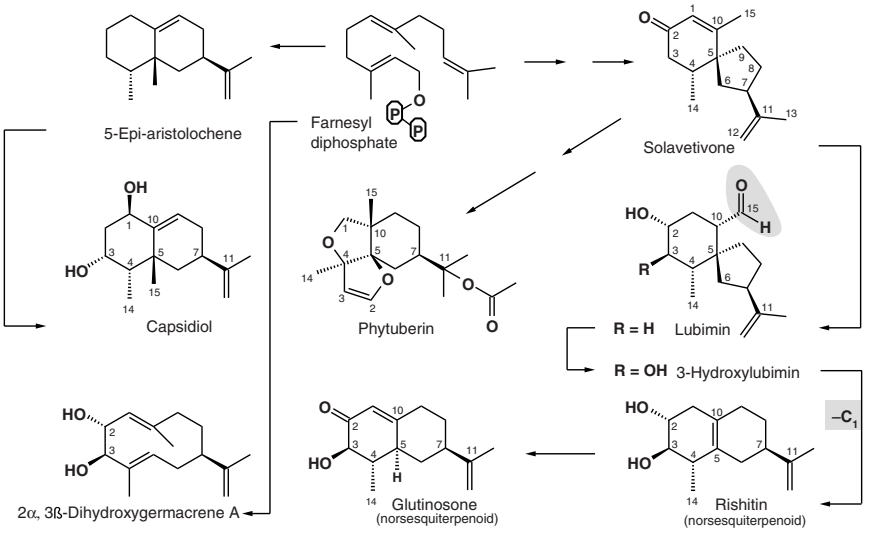


Fig. 7.5 Solanaceous sesquiterpenoidal/*nor*sesquiterpenoidal phytoalexins and their biogenetic relations. Formation of rishitin (C_{14}) by elimination of the exocyclic C-15 (*highlighted in grey*) presumably via oxidation of CHO to COOH followed by decarboxylation (the corresponding lubiminoic acid was identified as a constituent of *Solanum aethiopicum*)

to be variety-specific. β-Caryophyllene (Fig. 7.4), germacrene D, β-sesquiphellandrene, and germacrene D-4-ol (Fig. 7.4) have been the dominating metabolites although not in all varieties (Szafranek et al. 2005). Two further alcohols, the tricyclic aromadendrane type sesquiterpenoid ledol discovered as a constituent of *Ledum palustre* L. (Ericaceae) and kunzeaol [6α-hydroxygermacra-1(10),4-diene], have been characterized as constituents of a certain potato cultivar. Interestingly, the latter metabolite was found to act as a feeding attractant for the Colorado potato beetle, *Leptinotarsa decemlineata* SAY (Coleoptera: Chrysomelidae) (Szafranek et al. 2006). The presence of substantial quantities of (*E*)-β-farnesene (Fig. 7.4) in air around the foliage of the wild potato *S. berthaultii* HAWKES, probably released from glandular hairs, was shown to repel the green peach aphid, *Myzus persicae* SULZ. (Hemiptera: Aphididae). This sesquiterpene represents the aphid alarm pheromone which thus is used by the plant as an allomone (Gibson and Pickett 1983). Insect feeding on *S. lycopersicum* triggered the release (locally and systemically) of a blend of VOCs composed of the sesquiterpenes β-caryophyllene (Fig. 7.4), α-humulene, and δ-elemene as well as of the homo-sesquiterpene (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, different monoterpenes (see Sect. 7.2.1.1) and C_6-volatiles (see Sect. 8.1) (Farag and Paré 2002). Terpenoid hydrocarbons occur in the leaf but not the fruit (Buttery and Ling 1993). Zingiberene, the major constituent of the essential oil of ginger, *Zingiber officinale* Rosc. (Zingiberaceae), was found to be associated with resistance to the Colorado potato beetle, *Leptinotarsa decemlineata*, in *S. hirsutum* (DUN.) MACBRIDE sub nom. *Lycopersicon hirsutum* DUN. var. *hirsutum*. This monocyclic bisabolane type sesquiterpene was present only in a certain type of trichomes (VI) of the wild tomato species (Carter et al. 1989). The already reported function of zingiberene and its isomer curcumene (one double bond out of three is at a different position) as insecticides, repellents, and insect feeding deterrents led to an investigation on the content of these two sesquiterpenes in different species of wild tomatoes. Especially certain accessions of *S. hirsutum* sub nom. *L. hirsutum* f. *typicum* turned out to produce remarkable amounts of these two metabolites (up to 10 g/kg fresh leaves) (Antonious and Kochhar 2003). Other potentially constitutive sesquiterpenoids from *Solanum* will be discussed below in connection with phytoalexins of this genus.

Capsicum. From the stems and roots of *C. annuum* L. four sesquiterpenoids already known from the phytoalexin research with the fruits (see below), e.g., capsidiol (Fig. 7.5), 13-hydroxycapsidiol, have been isolated. However, the stems/roots turned out to be a rich source of novel constitutive congeners named canusesnols (Kawaguchi et al. 2004). Canusesnols A–E belong to eudesmane type compounds (for compound E see Fig. 7.4). The eremophilene type congeners F–H share their skeleton with capsidiol (compound F = 11-carboxy-11-demethyl-1-*epi*-capsidiol). Finally, the vetispirane type canusesnols I and J represent 4-epimers of 15-dihydrolubimin and 13,15-dihydroxysolavetivone (principal structures: Fig. 7.5). Volatiles of the fruits of Cayenne pepper, *C. frutescens* L., have been studied in a comprehensive GC/MS analysis (Cardeal et al. 2006). The VOC blend is composed of sesquiterpenes (e.g., δ-elemene, α-humulene, γ-himachalene, β-bisabolene), different monoterpenes/monoterpenoids (see Sect. 7.2.1.1), and members of different other classes of metabolites.

7.3.1.2 Phytoalexins

Sesquiterpenoid phytoalexins are of significant relevance in the Solanaceae family. Obviously, cultivated species have been in the centre of research on this topic. However, there are also reports on wild species. Comprehensive reviews on this topic were published by Stoessl et al. (1976), Gross (1977), and Kuc (1982). Figure 7.5 summarizes frequent solanaceous metabolites in a biogenetic context. Only 2α,3β-dihydroxygermacrene A is representing a rather rare phytoalexin detected in *Datura stramonium* L., jimson weed/thorn apple, induced by inoculation with different fungi (Stoessl et al. 1975). The following compounds turned out to represent central solanaceous types of sesquiterpenoid phytoalexins:

- Rishitin/glutinosone (*nor*-eudesmane type)
- Solavetivone/lubimin/3-hydroxylubimin (vetispirane type)
- Phytuberol/phytuberin (*seco*-eudesmane type)
- Capsidiol (eremophilane type)

As can be concluded from their names, these compounds were discovered in solanaceous species, e.g., capsidiol, a sesquiterpenoid induced in fruits of *Capsicum frutescens* L. by several phytopathogenic fungi (Stoessl et al. 1972). This metabolite turned out to inhibit spore germination and mycelial growth of fungi. For the origin of the name "rishitin" see below.

Biogenetic Relations (Fig. 7.5). Experimental results indicate that one major pathway is proceeding via (−)-solavetivone → (+)-lubimin → (+)-3-hydroxylubimin (C_{15})→ [3-hydroxylubiminoic acid] → (−)-rishitin (C_{14}) (Stoessl et al. 1976 and references therein; Murai et al. 1982a; Desjardins et al. 1995 and references therein). Glutinosone co-occurring with rishitin might be formed by oxidation of the latter metabolite. Feeding experiments in diseased potato tuber tissues indicated that phytotuberin, one of the most representative solanaceous phytoalexins, was synthesized also from solavetivone (Murai et al. 1987). This might occur by cleavage between C-1 and C-2 of solavetivone followed by cyclic rearrangements as indicated by the numbering in Fig. 7.5. Another pathway is leading to eremophilane-type sesquiterpenoids such as capsidiol and debneyol (Fig. 7.3) via 5-*epi*-aristolochene (Fig. 7.5) (Whitehead et al. 1989; Bohlmann et al. 2002).

***Solanum*.** More than 20 sesquiterpenes were isolated and characterized from potatoes (Desjardins et al. 1995 and references therein). The first solanaceous (nor)sesquiterpenoid phytoalexin, rishitin, was elucidated by Tomiyama et al. (1968). It was named after its discovery in sliced potato tubers from *S. tuberosum* × *S. demissum* cv. Rishiri which had been inoculated with *Phytophthora infestans*, a fungus causing the late blight of potato and other *Solanum* spp. Beside rishitin, tubers synthesize antifungal metabolites in response to corresponding infections such as lubimin and solavetivone. These major (nor)sesquiterpenoids have been detected in 46 cultivars and breeding selections including such with germplasm of *S. brevidens* PHIL., *S. chacoense* BITTER, *S. demissum* LINDL., *S. microdontum* BITTER (Desjardins et al. 1995). A number of biogenetically closely related congeners

such as 3-hydroxylubimin and phytuberin were also identified. Rishitin was also reported from *S. lycopersicum* L. sub nom. *Lycopersicon esculentum* MILL., tomato. Lubimin and three (acyclic) oxygenated farnesol derivatives were identified as stress metabolites of *S. melongena* L., eggplant/aubergine (Stoessl et al. 1976 and references therein). Furthermore, a eudesmane-type sesquiterpenoid, aubergenone, could be identified (Murai et al. 1982b).

The biosynthesis of such stress metabolites could be induced by certain bacteria and fungi or even by treatment with cell-free, boiled extracts and sonicates of virulent and avirulent races of *P. infestans* (Stoessl et al. 1976 and references therein). Arachidonic and eicosapentaenoic acids, two polyunsaturated fatty acids which were constituents of this fungus, also elicited the accumulation of rishitin and lubimin in potato as well as of capsidiol in fruits of *Capsicum* spp. Interestingly, these fatty acids did not elicit an accumulation of phytoalexins in the tubers of *Ipomoea batatas* LAM., sweet potato (Convolvulaceae) (Bloch et al. 1984). The elicitation caused by these fatty acids in potato could be enhanced by addition of oligosaccharides (branched chain β-linked glucanes) isolated from *P. infestans* (Maniara et al. 1984). Marked suppression of steroidal glycoalkaloid accumulation in potato tubers caused by inoculation with different fungi turned out to be associated with the accumulation of high levels of rishitin (Shih et al. 1973).

A number of novel sesquiterpenoids such as solavetivone, 3β-hydroxy- and 13-hydroxy-solavetivones, lubimin and its corresponding carboxylic acid (lubiminoic acid), *epi*lubimin (epimeric position of the aldehyde function) and its carboxylic acid (*epi*lubiminoic acid), and others were identified as constituents of the roots and/or root exudates of *S. aethiopicum* L. (Nagase et al. 2001; Nagaoka et al. 2001). However, these compounds were reported as **constitutive** metabolites. This was also true for solavetivone and a novel lactone-type sesquiterpenoid with an aromatic moiety, solafuranone (Fig. 7.4), from *S. indicum* L. (Syu et al. 2001), solavetivone from *S. jabrense* AGRA & M. NEE (Sarmento da Silva et al. 2002), and 3β-acetoxysolavetivone from *S. abutiloides* (GRISEB.) BITTER & LILLO (Yokose et al. 2004).

***Nicotiana*.** Though most reports were published on *N. tabacum* L. there are also such with regard to the less important cultivated *N. rustica* L. as well as to wild species, e.g., *N. clevelandii* A. GRAY, *N. glutinosa* L., *N. sylvestris* SPEG. & COMES (Fuchs et al. 1983 and references therein), *N. undulata* RUIZ & PAV. (Uegaki et al. 1988), *N. attenuata* TORREY ex S. WATSON (Bohlmann et al. 2002). These seven species belong to six sections (see Table 3.4) indicating that the ability for the production of phytoalexins might be a common trait of the genus. Almost all of the phytoalexins of Fig. 7.5 (except 2α,3β-dihydroxygermacrene A) and in addition their closely related congeners, e.g., phytuberol (deacetylphytuberin), could be detected in tobacco leaves, e.g., inoculated with tobacco mosaic virus (TMV) (Uegaki et al. 1983 and references therein; Fuchs et al. 1983 and references therein). Three novel stress compounds with a tetrahydronaphthalene skeleton, occidol (Fig. 7.3), its acetate, and two position isomers of occidol

(1,2- and 3,4-dimethyl-, respectively) were discovered as stress metabolites of *N. rustica* inoculated with TMV (Uegaki et al. 1983). A few years later three additional congeners were identified, e.g., occidenol (Fig. 7.3), a derivative apparently caused by an oxidative ring cleavage and subsequent novel ring closure forming an *O*-heterocyclic structure (Uegaki et al. 1985). Capsidiol was isolated from *N. tabacum* and *N. clevelandii* after infection with TMV (Stoessl et al. 1976 and references therein). Debneyol and cyclodebneyol (Fig. 7.3) were characterized from tobacco necrosis virus (TNV)-inoculated *N. debneyi* DOMIN (Burden et al. 1985, 1986). A novel norsesquiterpenoid, glutinosone (Fig. 7.5), was discovered as a phytoalexin from *N. glutinosa* infected with TMV (Stoessl et al. 1976 and references therein). Altogether 19 stress sesquiterpenoids – including seven congeners found in the genus for the first time, e.g., aubergenone, 1,2-dehydro-α-cyperone (Fig. 7.4) – were detected as stress compounds in the leaves of *N. undulata* (Uegaki et al. 1988).

Neither capsidiol nor glutinosone could be detected in healthy tissues. According to Bohlmann et al. (2002) "…. it is conceivable that capsidiol contributes not only to an inducible defense against pathogens, but also to a constitutive defense in an organ-specific manner in some species of *Nicotiana*": Constitutive expression of 5-epi-aristolochene synthase (EAS) and capsidiol accumulation could not be observed in **shoots** of *N. attenuata*, but enzyme transcripts were induced by feeding of *Manduca sexta*, the tobacco hornworm. However, EAS was expressed **constitutively in roots** of this *N.* species and *N. sylvestris* SPEG. & COMES.

Further Species. Interestingly, tropane alkaloid accumulating species like *Datura stramonium* L. or *Hyoscyamus albus* L. were also found to accumulate major solanaceous phytoalexins such as solavetivone, lubimin, and rishitin as a consequence of an inoculation with fungi (Stoessl et al. 1976; Miguel and Barroso 1994). Furthermore, 2α,3β-dihydroxygermacrene A (Fig. 7.5) was identified as another stress metabolite in the former species.

Metabolism and Detoxification of Phytoalexins by Phythopathogenic Fungi. Capsidiol was shown to be detoxified due to its oxidation to the corresponding ketone, capsenone (1-deoxy-1-oxo-capsidiol), by the fungi *Botrytis cinerea* and *Fusarium oxysporum* f. *vasinfectum* (Ward and Stoessl 1972). All highly virulent strains of *Gibberella pulicaris* (FR.) SACC. (anamorph. *Fusarium sambucinum* FUCKEL) causing worldwide dry rot in stored potato tubers turned out to be tolerant of and able to metabolize rishitin to 13-hydroxyrishitin and 11,12-epoxyrhishitin (Gardner et al. 1994). The latter compound was shown to lack fungitoxicity. Further reports have been summarized by Pedras and Ahiahonu (2005).

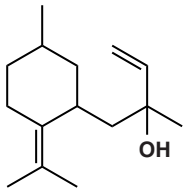
Toxicology Regarding Human Consumption. No limits have been set for the consumption of sesquiterpenoid phytoalexins in the human diet (e.g., damaged or infected potato tubers) due to the lack of corresponding literature (Matthews et al. 2005).

7.3.2 Occurrence in the Convolvulaceae

7.3.2.1 Constitutive Sesquiterpenoids

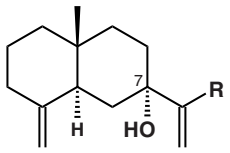
A sesquiterpene alcohol of novel structural type named humbertiol (Fig. 7.6) was isolated from the heartwood of *Humbertia madagascariensis* LAM., the basal dioecious tree endemic to Madagascar (Raulais and Billet 1970). According to this novel structure the corresponding skeleton was termed "humbertiane" for compounds of this type, e.g., from *Baccharis dracunculifolia* DC., Asteraceae (Weyerstahl et al. 1992). Sesquiterpenes such as α-gurjunene (an unsaturated tricyclic metabolite), α-humulene (an unsaturated monocyclic 11-membered congener), and ylangene (a different unsaturated tricyclic congener), constituents of the volatile extracts from storage roots and aerial plant parts of *Ipomoea batatas* LAM., turned out to be repellents to sweetpotato weevil, a devastating pest of the plant, whereas monoterpenoids showed attractant properties (see Sect. 7.2.2) (Wang and Kays 2002). A derivative of α-gurjunene, spathulenol, was isolated from the epigeal vegetative parts of *I. hederifolia* L. beside phytol (Jenett-Siems 1996).

Four furanosesquiterpenoids, named merrekentrones A–D, were discovered in the roots/rootstocks of *Merremia kentrocaulos* (C.B. CLARKE) RENDLE, a large woody climber from tropical Africa and India (Jenett-Siems et al. 2001). These compounds, especially merrekentrone C, are structurally related to the phytoalexins ipomeamarone and its congeners (Fig. 7.7), known from the tubers of *Ipomoea batatas* (L.) LAM., sweet potato, infected by phytopathogenic fungi (see below). Interestingly, the merrekentrones occurred in undamaged, uninfected roots, i.e., they seem to be constitutive metabolites in contrast to the phytoalexins of sweet potato. Merrekentrone A was also detected in the roots of *M. guerrichii* Meeuse, an herbaceous species endemic to Namibia/SE Africa, and *M. aurea* (KELL.) O'DONELL, an endemic plant from Baja California/Mexico cultivated as an ornamental due to its marvellous "golden" flowers.



Humbertiol

Humbertia madagascariensis



7-Hydroxycostal **R = CHO**

7-Hydroxycostol **R = CH_2OH**

Ipomoea batatas
infected by
Ceratocystis fimbriata

Fig. 7.6 Convolvulaceous sesquiterpenoids without a furanoid moiety [in contrast to the metabolites of Fig. 7.7]

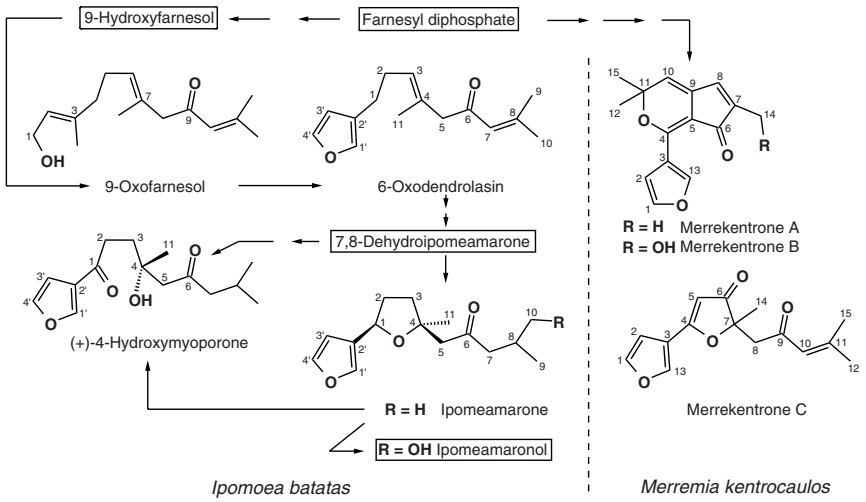


Fig. 7.7 Convolvulaceous furanosesquiterpenoids: stress metabolites from *Ipomoea batatas* infected by phytopathogenic fungi compared with constitutive metabolites from *Merremia kentrocaulos*; numbering for ipomeamarone and congeners according to chemical rules, for merrekentrones according to the biogenetic origin from farnesyl diphosphate (like in 9-oxofarnesol)

Merrekentrone D, a derivative of merrekentrone A cleaved between C-11 and the oxygen at C-4, can be regarded as 6-oxomyomontanone, a derivative of (+)-myomontanone, a hepatotoxic furanosesquiterpenoid from *Myoporum montanum* R.Br. (Myoporaceae). Further relations to this family will be discussed below.

7.3.2.2 Furanosesquiterpenoid Phytoalexins and Derivatives

Discovery. From the essential oil of *Myoporum laetum* Forst (Myoporaceae), a tree growing in New Zealand and named "ngaio" by the Maori, the major component was isolated by McDowall (1925). At that time this compound, named ngaione, could be characterized as an "oxygenated sesquiterpene ketone" ($C_{15}H_{22}O_3$). Ipomeamarone [at that time named (+)-ngaione], was isolated as the first phytoalexin at all by Hiura in 1941 from tubers of *Ipomoea batatas*, sweet potato, infected by the phytopathogenic black rot fungus *Ceratocystis fimbriata* Ell. & Halst (fide Kojima and Uritani 1981). Its chemical structure was determined in 1952 by Kubota and Matsuura (1956 and references therein) (Fig. 7.7). (−)-Ngaione turned out to be diastereomeric with (+)-ipomeamarone (Birch et al. 1954). The absolute configuration of the latter metabolite was determined by Schneider et al. (1983) to be 1*R*,4*S*. Another example for the close structural relationship between furanosesquiterpenes from *Myoporum* spp. (Myoporaceae) and certain convolvulaceous species has been given above, a further one will follow below. Interestingly, the family Myoporaceae belongs to the order Lamiales which are closely related to the Solanales.

The formation of such stress metabolites was shown to be inducible by (i) fungi such as *C. fimbriata*, *Fusarium javanicum*, *F. solani* (Schneider et al. 1984 and references therein), (ii) mycotoxins (Fujita and Yoshizawa 1989), and (iii) mercuric chloride ($HgCl_2$) (Schneider et al. 1984 and references therein).

Furanosesquiterpenoids. The discovery of the phytoalexin character of ipomeamarone led to many studies on volatile constituents of black-rotted sweet potato and related substances (e.g., Kubota 1958; Kato et al. 1971; Yang et al. 1971; Burka et al. 1974, 1977, 1981; Inoue et al. 1977; Schneider et al. 1984). They have been summarized in a biogenetic scheme comprising 25 sesquiterpenoid stress metabolites plus 4 decomposed derivatives by Schneider et al. (1984 and references therein). Beside others the following compounds listed in the biogenetic pathway of Fig. 7.7 could be isolated as stress metabolites of sweet potatoes in addition to ipomeamarone:

- 9-Hydroxyfarnesol, 9-oxofarnesol, 6-oxodendrolasin due to treatment with mercuric chloride ($HgCl_2$) (Burka et al. 1981); due to infection with *Ceratocystis fimbriata*: 9-Hydroxyfarnesol, 6-oxodendrolasin (Schneider et al. 1984 and references therein)
- 7,8-Dehydroipomeamarone due to infection with *C. fimbriata* (Schneider et al. 1984 and references therein)
- 4-Hydroxymyoporone due to infection with *C. fimbriata* or *Fusarium solani* as well as by treatment with $HgCl_2$ (Burka et al. 1974); its 7,8-dehydro derivative was also isolated due to infection with *C. fimbriata* (Inoue et al. 1977 and references therein)
- Ipomeamaronol due to infection with *C. fimbriata* (Kato et al. 1971; Yang et al. 1971)

4-Hydroxymyoporone [(+)-hydroxymyoporone] was determined to be *R*-configurated based on a total synthesis of the enantiopure (+)-form (Tietze et al. 1999). This corrected the configurational assignment (*S*) based on the absolute configuration of ipomeamarone at C-4 in a former publication (Dimitriades and Massy-Westkropp 1984). Thus, athanagrandione, isolated by Bohlmann and Zdero (1978) from *Athanasia grandiceps* HILLIARD & BURTT (Asteraceae), represents the S-(−)-form, i.e., *ent*-4-hydroxymyoporone (Tietze et al. 1999). (+)-4-Hydroxymyoporone is another example for the structural similarities of furanosesquiterpenoids from the Myoporaceae and Convolvulaceae families: Its 4-deoxy derivative, myoporone, was first isolated from *Myoporum bontioides* A. GRAY (Schneider et al. 1984 and references therein).

Degraded Furanosesquiterpenoids. A number of structurally related β-substituted furans (mainly C_9-compounds) which are formed by metabolization of furanosesquiterpenes in the infected tissues could be identified, e.g., batatic acid, ipomeanine [1-(3-furyl)-pentan-1,4-dione], β-furancarboxylic acid (Kubota 1958), ipomeanol [1-(3-furyl)-4-hydroxypentanone], ipomeadiol [1-(3-furyl)-1,4-dihydroxypentane] (Boyd and Wilson 1972; Burka et al. 1974). Interestingly, such compounds result from fungal metabolism (*Fusarium javanicum* KOORDERS) of furanosesquiterpenoid stress metabolites of the host (Burka et al. 1974). However, furanosesquiterpenoids were not converted to C_9-derivatives by *C. fimbriata* due to the lack of corresponding enzymes in this fungus (Kojima and Uritani 1981).

Toxicology. Damaged sweet potato tubers when fed to animals produce a characteristic and often lethal respiratory disease (Wilson et al. 1970). 4-Ipomeanol (4-IPO), one of the metabolites produced by degradation of sesquiterpenoid stress factors present in fungi-infected tubers, was the first metabolite which turned out to be a lung-toxic principle. This substance produces a disease in mice "similar to that seen in natural outbreaks of poisoning in cattle caused by mold-damaged sweet potatoes" (Boyd and Wilson 1972). Later, ipomeanine (IPN), 1-ipomeanol (1-IPO), and 1,4-ipomeadiol (DIOL) were also found to contribute to the pulmonary toxicity of fungi-infected sweet potatoes [oral LD_{50} values/mice: 26–104 mg/kg; IPN (26) and IPO (38) were the most toxic] (Chen et al. 2006 and references therein). However, intact furanosesquiterpenoids such as ipomeamarone and ipomeamaronol are hepatotoxic agents. "Perhaps the greater potency of the lung edema toxin may account for failure to note liver damage in many of the natural outbreaks of mold sweet potato poisoning in this species" (Boyd and Wilson 1972). 4-IPO-treated human lung cancer patients developed severe hepatotoxicity with little toxicity to the lungs indicating that this compound may exhibit dualistic toxicity (Chen et al. 2006 and references therein).

These furans require metabolic activation to elicit toxicity. Recently, extensive in vitro studies (rat liver microsomes) with IPN, 1-IPO, 4-IPO, and DIOL led to the observation that the oxidation of 4-IPO to IPN and reduction to DIOL occurred. Furthermore, it was found that more IPN was metabolized to a reactive species than 4-IPO or DIOL. Enedial metabolites of IPN and 4-IPO with thiols and/or amines turned out to be responsible for their toxicity. However, in vivo experiments are necessary for the determination of the occurrence of the IPN and 4-IPO interconversion and the predominant scavengers for these reactive enedials in vivo (Chen et al. 2006 and references therein).

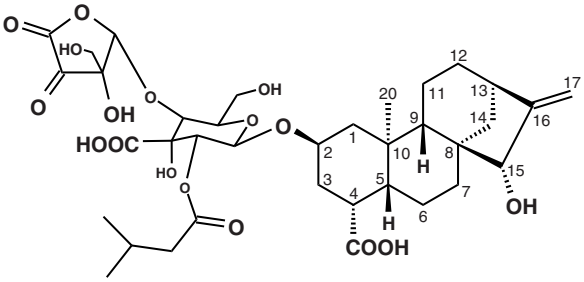
7.3.2.3 Selinene-type Phytoalexins

Five stress metabolites of another structural, namely selinene-type, 7-hydroxycostal and 7-hydroxycostol (Fig. 7.6) accompanied by their already known 7-deoxy congeners β-costal and β-costol as well as β-selinene, were isolated from *C. fimbriata*-infected sweet potato root tissue in addition to furanosesquiterpenoids and their derivatives (Schneider and Nakanishi 1983). These compounds are characterized by the bicyclic eudesmane (selinane) skeleton.

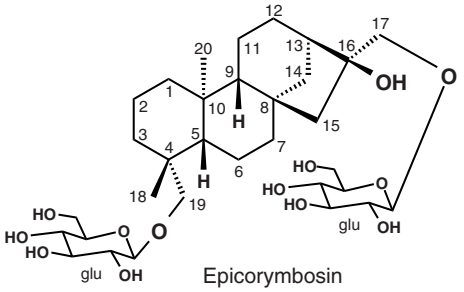
7.4 Diterpenoids (C_{20} Isoprenoids)

all-trans-Geranylgeranyl diphosphate represents the progenitor of all diterpenoids. Phytol [(2*E*)-3,7,11,15-tetramethyl-2-hexadecen-1-ol] is the ubiquitous acyclic diterpenoid residue of chlorophylls a and b as well as of α-phyllochinone (phytomenadione INN; vitamin K_1). Furthermore, it is a constituent of tocopherols (vitamin E) integrated in part into their chromane skeleton. Another ubiquitous

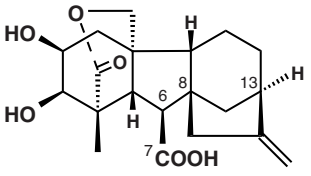
group of diterpenoids is represented by the gibberellins. These tetracyclic phytohormones "…. form a group of ~130 highly functionalized diterpenoids which are distributed widely throughout the plant kingdom where they play an important role in plant growth and development" (Mander 2003). They are synthesized in minute quantities (Bramley 1997 and references therein) via labdadienyl diphosphate and kaurane type diterpenoids. A ring cleavage between C-6 and C-7 of the kaurane-type intermediate is followed by a subsequent ring closure between C-6 and C-8 forming a gibberellane skeleton; C-7 represents an exocyclic carboxyl group in the gibberellins (for structural comparison see Fig. 7.8). The review of Mander is focused on the chemistry of these metabolites with no regard to their occurrence in plants.



Carboxyparquin
Cestrum parqui



Epicorymbosin
Turbina corymbosa



Gibberellin A_{27}
Ipomoea nil

Fig. 7.8 Examples for diterpenoids of the kaurane type (carboxyparquin; epicorymbosin) and the gibberellane type (*C. parqui*: Solanaceae; *T. corymbosa* and *I. nil*: Convolvulaceae)

7.4.1 Occurrence in the Solanaceae

7.4.1.1 Gibberellins, Kauranes

There are some reports on the identification of endogenous gibberellins, e.g., for shoot apices and flower buds of *Nicotiana tabacum* L. (A_1, A_3) (Sembdner and Schreiber 1965), corollas and anthers of *Petunia* × *hybrida* (A_1, A_4, A_9) (Weiss et al. 1995). Two unique kaurene glycosides, carboxyparquin (Fig. 7.8) and parquin, were discovered in the leaves of *Cestrum parqui* L'HERIT., a common shrub occurring in South America and Australia (Pearce et al. 1992). Though the only structural difference between both congeners is given by the lack of the carboxyl group at C-4 in case of parquin this metabolite appeared to be a relatively non-toxic metabolite. On the opposite, carboxyparquin is believed to be responsible for widespread poisoning of grazing animals in South America and Australia.

7.4.1.2 Acyclic Diterpenoids

9-Hydroxygeranyllinalool and (3*S*,6*E*,10*E*,14*Z*)-20-hydroxygeranyllinalool were isolated from *Nicotiana sylvestris* SPEG. & COMES (Wallin et al. 1980). *Capsicum annuum* has turned out to be a resistant species *on mature stage* against the American serpentine leafminer fly, *Liriomyza trifolii* BURGESS (Diptera: Agromyzidae), a well-known serious pest to many vegetables and crops in the world. Its resistance was found to be based on free phytol as the ovipositional deterrent in the leaves (Kashiwaga et al. 2005).

7.4.1.3 Acyclic Diterpenoid Glycosides

Yahara et al. (1988) reported a novel acyclic diterpenoid glycoside from polar fractions of the fresh fruits of *Capsicum annuum* L. var. *fasciculatum*. This metabolite was initially named capsianside A. However, in the following publications of these authors with a number of congeners the term "capsiansides" was substituted by capsianosides. These metabolites were classified into two groups:

- (i) Monomeric diterpene glycosides individualized by Roman numbers:
    - 3*O*/17*O*-Bisdesmosides of 3,17-dihydroxyditerpenoids, e.g., capsianosides II, III, XV (Fig. 7.9), and the majority of the congeners
    - 17*O*-Monodesmosides of 3,17-dihydroxyterpenoids, e.g., capsianoside VII
    - 3*O*/17*O*-Bisdesmosides of 15-carboxy-3-hydroxyditerpenoids, i.e., including an ester-type glycosidic linkage [COOH (= 17*O*) at C-15 linked glycosidically to the carbohydrate chain], e.g., capsianoside IV
    - 3*O*-Monodesmosides of 15-carboxy-3-hydroxyditerpenoids, e.g., capsianosides I, V
- (ii) Their dimeric esters individualized by capital letters, e.g., capsianoside L in which one monomer is closely related to the congener XV (Fig. 7.9) with the

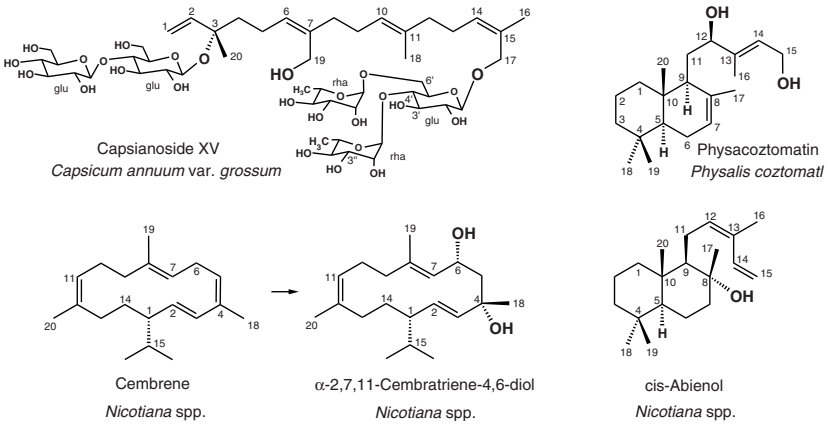


Fig. 7.9 Examples for acyclic diterpenoid glycosides (*above left*), cembrenoids (*below left*), and labdenoids (*above and below right*) from the Solanaceae

same trisaccharide chain at *O*-17 and the second monomer represents an acyclic diterpenoid carboxylic acid (terminal COOH at C-15′) which is esterified with the hydroxyl group at C-3′ of one rhamnose unit of the trisaccharide. Thus, the two diterpenoid moieties share this central trisaccharide residue; however, each diterpenoid monomer possesses a 3*O*-conjugated carbohydrate chain of its own.

Starting with capsianosides I–V and A–F, respectively, from the fruits of various cultivars (Izumitani et al. 1990), many congeners have been discovered in *C. annuum* varieties by these and other authors: Capsianosides VI, G, and H in the leaves and stems of *C. annuum* (Yahara et al. 1991); VII in ripe fruits of *C. annuum* var. *acuminatum* (Iorizzi et al. 2001); II, VIII–X, and XIII in fruits of *C. annuum* var. *annuum* (jalapeño) as well as II, IX, X, XIII, XV, and XVI in fruits of *C. annuum* var. *grossum* BAILEY (paprika/sweet pepper) (Lee et al. 2006); VIII, IX, and L as well as the methyl ester of capsianoside I in fresh sweet pepper fruits (De Marino et al. 2006). Capsianoside I is a 14*O*-linked sophoroside of 4,14-dihydroxy-2,6,10,14-tetramethyl-2,6,10,15-hexadeca-2,6,10,15-tetraenoic acid. Unfortunately, the last two papers were published independently at the same time with the consequence that "capsianosides VIII and IX / Lee et al." and "capsianosides VIII and IX / De Marino et al." are **different** though novel compounds each. Capsianosides, especially compound F, were shown to affect the cytoskeletal function by modulating the reorganization of actin filaments, by which the tight-junctional structure and permeability were changed (Hashimoto et al. 1997).

7.4.1.4 Cembranoids and Labdanoids

***Nicotiana*.** Most *Nicotiana* species have multicellular, glanded leaf trichomes which may produce chemical secretions containing diterpenes and/or sugar esters (see Sect. 8.2). The diterpenes are synthesized in the trichomes and secreted on the

leaf surface contributing to its gum. They may affect, e.g., aphids and moths. On the other hand, they may contribute as precursors to the aroma formed during curing and fermentation of tobaccos (Heemann et al. 1983; Severson et al. 1994). The terms cembranoids and labdanoids, proposed by Wahlberg and Eklund (1992) in order to get a more standard chemical nomenclature, are meanwhile accepted for tobacco exudate diterpenoids (Wagner 1999). Early reports on cembranoids used terms like duvanes/duvatrienes or later thunberganoids for such metabolites, e.g., Reid in his review (Reid 1979) according to the original reports in the 1960s and 1970s. Thus, e.g., the former duvatrienediols were named cembratrienediols. Cembranoids are 14-membered carbo**mono**cyclic diterpenoids with a three-membered branched side chain at C-1, whereas labdanoids are characterized by a 10-membered carbo**bi**cyclic ring system with a 6-membered branched side chain at C-9 (Fig. 7.9). These data refer to carbocycles; additional ring closures forming *O*-heterocyclic systems (tri- or tetracyclic ring systems) are known as derivatives of basal labdanoids (e.g., levantenolides, see below).

These two diterpenoid types, cembranoids and labdanoids, represent the most abundant of the three major groups of the green tobacco surface chemicals. The amounts of these diterpenoids are considerable in fresh leaf (0.5–10.0 g/100 g dry weight). The importance of such compounds for the tobacco aroma was clearly recognized already in the 1940s (Reid 1979 and references therein). The two other groups are sugar esters (Sect. 8.2) and surface waxes (*n*-alkanes and wax esters). Furthermore, there are two minor groups: (i) volatiles produced at the surface, e.g., the sesquiterpene β-caryophyllene (Fig. 7.4), and (ii) *N*′-acylnornicotines (Table 3.3).

Cembranoids and labdanoids are synthesized in trichome glands alone (Wagner 1999 and references therein). More than 50 **cembranoids** have been isolated from tobacco, predominantly α- and β-cembra-2,7,11-triene-4,6-diols (structure of the α isomer see Fig. 7.9; β isomer = 4-epimer) (Leffingwell 1999). They were discovered sub nom. α- and β-4,8,13-duvatriene-1,3-diols in very small amounts from cured tobacco by Roberts and Rowland (1962). Cembrene, an unsaturated hydrocarbon, i.e., a diterpene sensu stricto, represents the structural key compound lending its name to the corresponding diterpenoid type. It was discovered in *Pinus* spp. (Pinaceae) and later also detected in tobacco leaves after enzyme-blocking agents were administered (Enzell et al. 1977 and references therein). Labelled cembrene was found to be converted to cembra-2,7,11-triene-4,6-diol when fed to tobacco leaves. The first **labdanoids**, α- and β-levantenolides [tetracyclic di-*O*-heterocycles (see above) including one lactone residue], were isolated from Turkish tobacco (Giles and Schumacher 1961). A series of labdanoids such as *cis*-abienol [(12*Z*)-labda-12,14-diene-8α-ol; Fig. 7.9], labdadienediols, labdanediols, and congeners with only one hydroxyl group were unequivocally detected in green leaves of *N. tabacum* and other *Nicotiana* spp. The majority of labdanoids producing *Nicotiana* spp. were found to yield *cis*-abienol and (13*E*)-labda-12-en-8α,15-diol. There are *Nicotiana* spp. which produce labdanoids, e.g., *N. tomentosiformis* Goodsp., whereas others produce cembrenoids, e.g., *N. sylvestris* [thunbergol (= isocembrol); 4-*epi*-isocembrol] (Wahlberg et al. 1981; Heemann et al. 1983). Cultivars of *N. tabacum* were found to synthesize either labdanoids (commercial

Oriental tobaccos) or cembranoids (commercial Burley/Virginia tobaccos). Some varieties produce trace amounts of the other group, too (commercial Greek and Turkish tobaccos) (Enzell et al. 1977 and references therein; Heemann et al. 1983). Three further examples for the occurrence of labdanoids in wild *Nicotiana* spp. are *N. raimondii* J.F. MACBR. (raimonol; isoraimonol) (Noma et al. 1982), *N. setchellii* (labda-7,13*E*-dien-15-ol; labda-8,13*E*-dien-15-ol) (Suzuki et al. 1983), and *N. tomentosa* RUIZ & PAV. [12*Z*-abienol (Fig. 7.9) and oxidized congeners] (Heemann et al. 1983). However, occurrence of diterpenoids is restricted in the genus *Nicotiana* to only a few species.

Heemann et al. could not find any diterpenoids in the leaf surface gum neither in the cultivated *N. rustica* nor in 13 further wild *Nicotiana* spp. (*N. acuminata, N. africana, N. alata, N. debneyi, N. glauca, N. knightiana, N. langsdorffii, N. longiflora, N. megalosiphon, N. paniculata, N. plumbaginifolia; N. repanda, N. sanderae*; authorities see Table 3.4).

The genus *Nicotiana* has been a rich source of terpenoids which have been summarized in several reviews (e.g., Enzell et al. 1977; Wahlberg and Eklund 1992; Leffingwell 1999; Wagner 1999). In particular many diterpenoids were isolated only from *cured* tobacco. Therefore, it has to be taken into account that they might be artefacts of the curing and isolation processes (Stoessl et al. 1976; Weeks 1999; Wahlberg and Ringberger 1999): "During the curing process, which involves air, heat, fire or sun-drying and leads to the creation of typical tobacco aroma, the tobacco constituents are subjected to various enzymatic, microbial, photochemical and oxidative reactions" (Enzell et al. 1977).

Biochemical Ecology. Ovipositional response of *Heliothis virescens*, tobacco budworm moth (Lepidoptera: Noctuidae), to cuticular labdanes – isolated from the green leaves of *N. glutinosa* L. – was stimulated by spraying of these compounds onto a leaf avoid of them (Jackson et al. 1991). Analogous results were obtained with certain cembranoids (Jackson et al. 1986). *Nicotiana* species which did not produce observable trichome exudates did not receive as many eggs of tobacco budworm or tobacco horn worm, *Manduca sexta* L. (Lepidoptera: Sphingidae), as a flue-cured tobacco (Severson et al. 1991). Cembranoids and labdanoids affect tobacco aphids, *Myzus nicotianae* BLACKMAN (Hemiptera (Homoptera): Aphididae), in "…. influencing the acceptance or rejection of plants for colonization by alate migrants aphids and the survival and fecundity of alate and apterous aphids" (Severson et al. 1994). For comparable effects of sugar esters, the other group of metabolites from the trichomes, readers are referred to Sect. 8.2. The composition of leaf surface diterpenoids of 25 *Nicotiana* spp. was analyzed in another study in detail. They included a series of labdanoids and α- and β-cembra-2,7,11-triene-4,6-diols sub nom. α- and β-4,8,13-duvatriene-1,3-diols. The inhibitory effects of such metabolites on germination of *Peronospora tabacina* ADAM sporangia were investigated (Kennedy et al. 1992).

Nolana. A series of labdanoids could be discovered in this genus, distributed in the desert and semidesert zones of Peru and Chile. Six new metabolites were isolated from the aerial parts of *N. rostrata* (Garbarino et al. 1986). Remarkably,

all of them represent labda-8(17),13-dien-15-oic acids or methyl esters of such acids. They showed diverging substitutions at C-2/C-3 (2-oxo, 3-oxo, 2β,3β-dihydroxy) and at C-4α [CH_3, CH_2OH, CH_2O-C=O(CH_3)]. A decade later, the *E*- and *Z*-isomers of 2α,3α,9β-trihydroxy-9-*epi*-labd-13-ene-15-oic acid could be discovered as further constituents of the same source (Chamy et al. 1997). Two further labda-8(17),13-dien-15-oic acids (13*E*- and 13*Z*-isomers of the 2β-acetoxy derivative) were isolated from the aerial parts of *N. filifolia* (Garbarino et al. 1988). Finally, labdane-8α,15-diol, labd-8(17)-ene-15-ol, and 3β-acetoxy-labd-8(17)-ene-15-ol, have been identified in the aerial parts of *N. elegans* (Chamy et al. 2002).

***Physalis*.** A new labdane-type diterpene, physacoztomatin (Fig. 7.9), has been discovered as a constituent of the aerial parts of *Physalis coztomatl* Moc. & Sessé ex Dun. whose fruits are used in Mexican cuisine ("tomate agrio" or "tomate amarillo") (Pérez-Castorena et al. 2006).

7.4.2 Occurrence in the Convolvulaceae

7.4.2.1 Gibberellins, Kauranes

Results on the structure and biological activities of novel gibberellins and their glucosides from the immature seeds of *Ipomoea nil* (L.) Roth sub nom. *Pharbitis nil* Choisy, Japanese morning glory, were published by Yokota et al.: seven gibberellin glucosides (Yokota et al. 1969a, 1970; 1971b), gibberellins A_{26}, A_{27} (Fig. 7.8) (Takahashi et al. 1969; Yokota et al. 1969b; 1971a), biological activities (Yokota et al. 1971c). The identification and quantification of gibberellins (A_1, A_{19}, A_{20}) in phloem exudates and cotyledons of this species together with phytohormones of other classes were reported by Wijayanti et al. (1995). From immature seeds of *Ipomoea alba* L. sub nom. *Calonyction aculeatum* (L.) House, moonflower/evening glory, several new gibberellins (A_{30}, A_{31}, A_{33}, A_{34}) were isolated and structurally elucidated beside a number already known ones (Murofushi et al. 1970, 1971; Takahashi et al. 1972). From the same species *ent*-7α,16α,17-trihydroxykauran-19-oic acid and its 6α-tetrahydroxy derivative could be identified (Murofushi et al. 1973).

Several kauranetriol glycosides were discovered in two convolvulaceous species. The seeds of *Turbina corymbosa* (L.) Raf., famous for their content of ergoline alkaloids ("ololiuqui"; Sect. 4.2), were also found to contain a number of such diterpenoid glycosides such as turbicorytin, corymbosin, and *epi*corymbosin (Garcia Jiménez and Pérezamador 1967; Garcia Jiménez et al. 1979, 1993). In contrast to the two latter metabolites which represent 16-epimers of a bisdesmosidic 16,17,19-kauranetriol 17*O*,19*O*-diglucoside (Fig. 7.8) the former is a monodesmosidic 6β,16,17-kauranetriol 6β*O*-glucoside.

Unfortunately, two natural compounds discovered in the same year were named corymbosin: (i) the diterpenoid glycoside from *T. corymbosa* mentioned above

(Garcia Jiménez et al. 1967) and (ii) a flavone from *Webera corymbosa* WILLD., Rubiaceae (Joshi and Rane 1967). This led to unpleasant confusions, e.g., SciFinder Scholar™ 2006 (CAS®) presents the structure of the flavone if asked for the structure of corymbosin from the *Turbina* species. This shows again that thoughtlessly chosen trivial names ought to be avoided, e.g., by additional integration of parts of the genus name together with the species epithet.

The seeds of the second species, *Merremia aurea* (KELL.) O'DONELL sub nom. *Operculina aurea* KELL., were reported to contain a monodesmosidic 19*O*-gentiobioside of *ent*-16,17,19-kauranetriol, named aureoside (Canonica et al. 1976). Furthermore, bisdesmosidic glycosides of *ent*-3β,16β,17-kauranetriol, e.g., isoaureoside (3*O*,16*O*-diglucoside) and *ent*-3α,16α,17-kauranetriol, e.g., aniseoside (3*O*-gentiotriosyl-16*O*-glucoside), were structurally elucidated (Canonica et al. 1977a, b).

7.5 Triterpenoids (C_{30} Isoprenoids)

Squalene, the precursor of all triterpenoids sensu latiore, is synthesized by "head-to-head" condensation of two *all-trans*-farnesyl diphosphate molecules. In this subsection only triterpenoids sensu stricto, i.e., with a retained C_{30}-skeleton, will be discussed. Derivatives with reduced carbon skeleton, e.g., C_{27}-sterols, steroidal sapogenins/alkaloids, will follow in Sects. 7.6–7.11. A frequent type of triterpenoids sensu stricto is represented by pentacyclic structures of the β-amyrin type (oleanane-type). Such triterpenoids beside phytosterols are constituents of the unsaponifiable matter of fatty oils/fats from the seeds. They may also occur on leaf surfaces.

7.5.1 Occurrence in the Solanaceae

β-Amyrin (Fig. 7.10) was detected as a constituent of the seeds of several solanaceous species such as *Capsicum annuum* L., *Lycium chinense* L., *Nicotiana tabacum* L., *Datura stramonium* L., *Solanum esculentum* L., *S. melongena* L., *Physalis alkekengi* L. Furthermore, two novel oleanane-type triterpenoids, daturadiol (Fig. 7.10) and its 3β-deoxy-3-oxo congener daturaolone, were isolated from *D. stramonium*. The latter metabolite could be also detected in *Solanum arundo* MATTEI (Grace and Saleh 1996). Lupeol (Fig. 7.10), characterized by another pentacyclic structural type (lupeane type), could be identified, especially in *C. annuum* (Itoh et al. 1977). Oleanolic acid (Fig. 7.10) was found in the aerial parts of *Fabiana imbricata* RUIZ & PAV. (Petunioideae) (Schmeda-Hirschmann et al. 2004) and together with β-amyrin in *Vestia foetida* (RUIZ & PAV.) HOFFM. sub nom. *V. lycioides* WILLD. (Cestroideae s.s.) (Faini et al. 1984). β-Amyrin and friedelin were detected as metabolites of *Browallia grandiflora* GRAHAM (Cestroideae s.s.) (Rozkrutowa 1991). The occurrence of ursolic acid, an isomer of oleanolic acid characterized by two vicinal methyl groups at C-19β and C-20α instead of the two

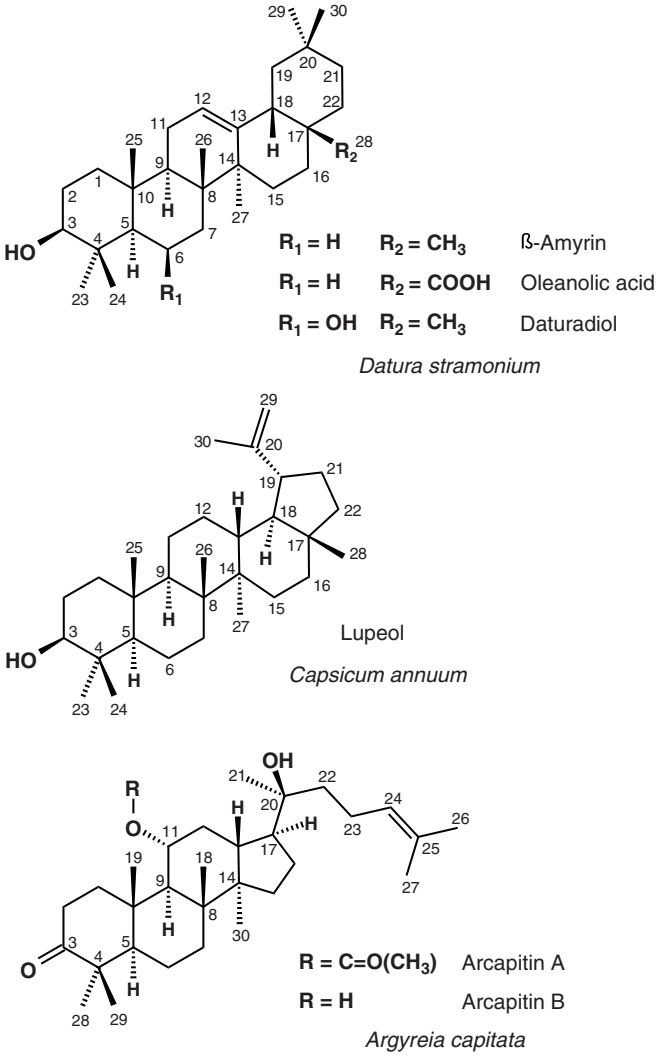


Fig. 7.10 Examples for different types of triterpenoids: oleanane- (*D. stramonium*), lupeane- (*C. annuum*, both Solanaceae), and dammarane-type (*A. capitata*, Convolvulaceae)

geminal CH_3 at C-20, was reported for many solanaceous species, e.g., *Nierembergia rigida* MIERS sub nom. *N. aristata* auct. non MIERS (Gil et al. 1995), *N. linariaefolia* GRAHAM sub nom. *N. hippomanica* auct. non MIERS (Pomilio et al. 1996 and references therein), *Leptoglossis texana* TORREY [syn.:*Hunzikeria texana* (TORREY) D'ARCY] (Dominguez et al. 1975) (Petunioideae), and *Anthocercis, Crenidium, Cyphanthera, Duboisia, Grammosolen* spp. [Nicotianoideae (trad.: Anthocercioideae)] (El Imam et al. 1984, 1991). In addition, oleanolic acid, α- and β-amyrins, and uvaol were found to be further pentacyclic terpenoid constituents of *N. linariaefolia*.

α-Amyrin [vicinal methyl groups at C-19/C-20; R_1 = H, R_2 = CH_3 in Fig. 7.10] as well as uvaol [do., but R_2 = CH_2OH] are closely related congeners of ursolic acid. An isomer of oleanolic acid, 3-*epi*-katonic acid (3β-hydroxy-olean-12-en-29-oic acid), was isolated from the leaves of *Salpichroa diffusa* MIERS (Solanoideae, Physaleae clade) (Moreno-Murillo et al. 2001). Two new highly oxygenated triterpenoids, 2α,3α,24-trihydroxyolean-12-ene-28,30-dioic acid and 2α,3α,24,28-tetrahydroxyolean-12-ene, were discovered in the roots of *Atropa belladonna* L. sub nom. *A. acuminata* ROYLE (Hyoscyameae clade) (Mehmood et al. 2002).

Triterpenoid Saponins. A unique occurrence of triterpenoid saponins in a species from the Solanales was reported on two constituents of *Cestrum parqui* L'HERIT. leaves (Abdel-Gwad et al. 1997). These compounds were characterized by the aglycone oleanolic acid 3β*O*-linked to a trioside composed of (i) two glucose units and one xylose and (ii) glucuronic acid, rhamnose, and glucose.

7.5.2 Occurrence in the Convolvulaceae

β-Amyrin, ursolic acid, and 24-*nor*-12-ursene were isolated from *Cressa cretica* L. (Hussain et al. 2005). The oleanane type triterpenoid taraxerol acetate was identified in the root tubers of *Ipomoea mauritiana* JACQ. sub nom. *I. digitata* (Bheemasankara Rao et al. 1984). Taraxerol, taraxerone, and α-amyrin were identified as constituents of the seeds of *I. quamoclit* (Das et al. 1999). The leaves of *I. batatas* LAM., sweet potato, were found to contain two other known oleanane-type metabolites, friedelin and acetyl-β-amyrin (Guishan et al. 1991), whereas boehmeryl acetate, a lupeane type compound, was identified as the dominant triterpenoid of the periderm of sweet potato storage roots. This compound induced significantly higher oviposition by female sweetpotato weevils, *Cylas formicarius elegantulus* SUMMERS (Coleoptera: Curculionidae) (Wilson et al. 1990). From the leaves of *Argyreia speciosa epi*-friedelinol and its acetate were isolated (Sahu and Chakravarti 1971). Three novel dammarane-type triterpenoids, named arcapitins A–C (Fig. 7.10), were discovered as constituents of the roots and leaves of *A. capitata* (VAHL) CHOISY (Tofern et al. 1999). They turned out to be 11α-acetoxy-20*S*-hydroxydammar-24-en-3-one (arcapitin A), its 11α-hydroxy congener (B), and the 3β,20*S*-diol derivative of A (C). These metabolites could also be obtained from the leaf surface.

7.6 Phytosterols (C_{27}–C_{29} Isoprenoids)

Phytosterols are ubiquitous metabolites involved in the stabilization of the cell membranes. They are characterized by a 3-hydroxysterane skeleton substituted by an 8–10-membered isoprenoid side chain at C-17. The cholesterol (C_{27}) pathway in plants proceeds by stepwise demethylations (two at C4, one at C-14) of the

so-called 4,4-dimethylsterol cycloartenol [C_{30}; Fig. 7.11] via 4-methylsterols (Heftmann 1983; Bramley 1997). Most plants synthesize typical phytosterols (sensu stricto, i.e., 4-demethylated compounds) such as β-sitosterol [24α(*R*)-ethylcholesterol; C_{29}; Fig. 7.11], stigmasterol [trans-Δ^{22}-24α(*S*)-ethylcholesterol; C_{29}] or similar congeners by two subsequent methylations at C-24 of their biogenetic precursor cholesterol (Fig. 7.11), i.e., via a C_{28} intermediate. Cholesterol itself is a minor component of the "phytosterol cocktails" though it represents the key intermediate in the biosynthesis of all C_{27}-steroids and their derivatives in plants (Sects.

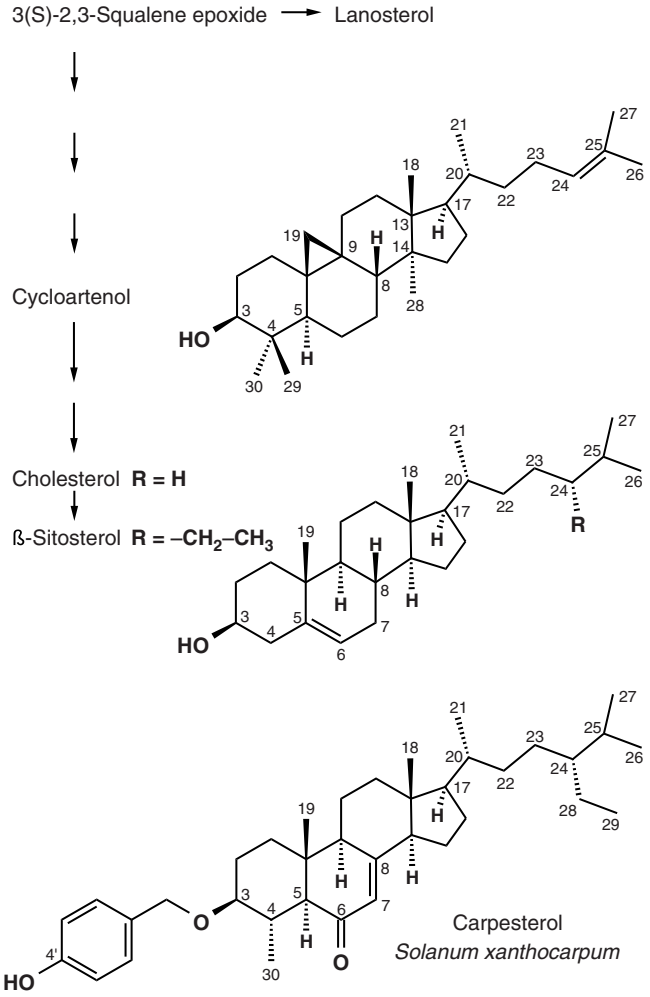


Fig. 7.11 Outline of the pathway from squalene epoxide, the common link between all triterpenoids sensu latiore, to cholesterol and β-sitosterol. Carpesterol might be synthesized via cycloartenol → 24-methylenecycloartenol → subsequent methylation at C-28 → 4-methylsterol congener → oxidation at C-6 → 3*O*-acylation

7.7–7.11). In higher plants β-sitosterol and stigmasterol often constitute more than 70% of the total sterols (Bergenstraahle et al. 1996).

Beside their free presence phytosterols may also co-occur as 3*O*-conjugates, i.e.:

- Esterified by a fatty acid (steryl esters), e.g., sitosteryl palmitate (Sticher 2007 and references therein)
- Glycosylated at the same position (steryl glycosides), e.g., sitosterol-ß-D-glucoside
- Acylated steryl glycosides, e.g., 6-*O*-palmitoyl-β-D-glucosyl-sitosterol (Sticher 2007 and references therein)

7.6.1 Occurrence in the Solanaceae

An extensive review on the biosynthesis of sterols (C_{27}–C_{29}) and the metabolism of cycloartenol including those results obtained from solanaceous species such as *Capsicum annuum* L., *Nicotiana tabacum* L., *Solanum demissum* LINDL., *S. dulcamara* L., *S. polyadenium* GREENM., *S. tuberosum* L., and *S. xanthocarpum* SCHRAD. & WENDL. was published by Heftmann (1983).

4,4-Dimethyl- and 4α-Methylsterols. 4,4-Dimethylsterols such as (i) lanost-8-en-3β-ol, lanosterol, and 24-methylenelanost-8-en-3β-ol as well as (ii) their 9β,19-cyclopropyl residue containing isomers cycloartanol (24,25-dihydrocycloartenol), cycloartenol (Fig. 7.11), and 24-methylenecycloartanol were identified in the same seven species as those mentioned above with regard to β-amyrin (see Sect. 7.5.1). Interestingly, the compounds of (i) were found in all of those species except *N. tabacum*. However, mainly the metabolites of (ii) were present in much higher concentrations. This suggested that the opening of the cyclopropane ring may occur at the 4,4-dimethylsterol level at least in these plants (Itoh et al. 1977). With the exception of *S. melongena*, cycloartenol was the dominating compound; in case of the eggplant its saturated congener cycloartanol turned out to be in the lead. Potato (*Solanum tuberosum* L.) tuber disks which normally accumulate steroidal glycoalkaloids were treated in a study with inhibitors of this accumulation. This led to the observation that in the 4,4-dimethylsterol and the 4α-methylsterol fractions only compounds with a non-alkylated side-chain were detected (Bergenstraahle et al. 1996). Cycloartenol, 24-methylenecycloartenol, the 4α-methylsterols cycloeucalenol, lophenol, 24-methylenelophenol, and 24-ethylenelophenol were isolated as free sterols and also as esters with fatty acids, as glycosides, and as acylated glycosides from the leaves of *S. dulcamara* L (Willuhn and Koestens 1974, 1975). The last four terpenoids were also identified as free sterols in *Nicotiana tabacum* (Enzell et al. 1977 and references therein).

3-*O*-Acylated 4α-Methyl-24α-ethyl-sterols. Saiyed and Kanga (1936) isolated a metabolite from the fruits of *Solanum xanthocarpum* SCHRAD. & WENDL. which they named carpesterol. Its structure (Fig. 7.11) could be elucidated only decades

later by Tsay et al. (1970). This unusual C_{30}-sterol is characterized by a *p*-hydroxybenzoyl residue at position 3β*O*. It was also detected, sometimes together with its 4′-deoxy congener (benzoyl residue), in other *Solanum* spp., e.g., the berries of *S. indicum* L. (Gan et al. 1993) and of *S. sisymbrifolium* LAM. (Chakravarty et al. 1996).

4-Demethylsterols and their Derivatives. β-Sitosterol and/or its 3-*O*-β-D-glucoside have been detected in, e.g., *Browallia grandiflora* GRAHAM (Rozkrutowa 1991) and *Solanum indicum* L. (Gan et al. 1993), stigmasterol and/or its glucoside in, e.g., *S. indicum* (Gan et al. 1993) and *Withania somnifera* (L.) DUN. (Lal et al. 2006). Cholesterol, β-sitosterol, stigmasterol, campesterol, brassicasterol, isofucosterol, and 24-methylenecholesterol were isolated from the leaves of *S. dulcamara* L. (Willuhn and Koestens 1974). Leaves from six *Solanum* spp. (*Solaneae* clade, subfamily **Solanoideae**) showed an unusual abundance of sterol glycosides compared with eight species from different other clades of this subfamily (*Hyoscyameae*, *Lycieae*, *Nicandreae*, *Datureae*, *Physaleae*) and other solanaceous subfamilies (**Schizanthoideae**, **Cestroideae**, **Nicotianoideae**) (Duperon et al. 1984). This abundance in *Solanum* spp. was mainly caused by an enhanced content of acylated steryl glycosides (ASG) {ASG: 39–56% of the total sterols; unacylated steryl glycosides (SG): 26–28% [one exception (9%)]; steryl esters (SE): 7–13%; free sterols (FS): 12–38%}. In contrast, all other solanaceous species checked showed the following percentages: 2–19% (ASG; one exception: 30%); 1–16% (SG); 17–34% (SE; two exceptions: 9%); 37–73% (FS). The corresponding values for *Convolvulus arvensis* L. (Convolvulaceae) were 4%:10%:12%:74%. Interestingly, these values were almost the same for the basal solanaceous *Schizanthus pinnatus* RUIZ & PAV. as well as for species from the families Scrophulariaceae, Boraginaceae, and Lamiaceae. This emphasizes the diverging results for *Solanum*. The total sterol content of leaves of all the plants involved in this study (collected at flowering time) was within a range of 0.1–0.2% (dry weight). The study also included specific results for *S. lycopersicum* L.: Ungerminated seeds showed only a low proportion of steryl glycosides, but during the course of germination their level was increased progressively. Such a development could also be observed for all other organs of this plant with a decreasing tendency when these parts became senescent.

Pollinastanol (9β,19-cyclopropane-/C_{27}-type), 5α-cholest-8-en-3β-ol, and isofucosterol (substituent at C-24: =C–CH_3; C_{29}) were accumulated in discs of potato tubers when treated with synthetic inhibitors of the steroidal alkaloid biosynthesis (Bergenstraahle et al. 1996).

Potential Health Benefits. Plant sterols may have potential health benefits, mainly based on their cholesterol-lowering effect. A comprehensive study on the composition and content of sterols in plant-based foods commonly consumed in Spain with regard to the intake of these metabolites in the Spanish diet has been published recently including solanaceous vegetables [*Capsicum annuum* var.

grossum (sweet green pepper/bell pepper/paprika), *Solanum lycopersicum* sub nom. *Lycopersicon esculentum* (tomato), *S. melongena* (eggplant, aubergine), *S. tuberosum* (potato)] (Jiménez-Escrig et al. 2006).

7.6.2 Occurrence in the Convolvulaceae

4-Demethylsterols and their Derivatives. From the leaves of *Argyreia speciosa* and of *Ipomoea batatas* LAM. β-sitosterol was isolated (Sahu and Chakravarti 1971; Guishan et al. 1991). β-Sitosterol and its 3-*O*-β-D-glucoside were identified in the aerial parts of *Ipomoea reptans* (L.) POIR. (Ishi 1933), in the root tubers of *I. mauritiana* JACQ. sub nom. *I. digitata* L., and in the seeds of *Merremia kentrocaulos* (C.B. CLARKE) RENDLE sub nom. *Ipomoea kentrocaulos* C.B. CLARKE (Bheemasankara Rao et al. 1984). Both metabolites and in addition 5α,8α-epoxyergosta-6,22-diene-3β-ol have been detected in the stems of *Erycibe expansa* WALL. (Morikawa et al. 2006). β-Sitosterol and stigmasterol were identified as constituents of the seeds of *I. quamoclit* (Das et al. 1999).

7.7 Steroidal Sapogenins/Saponins (C_{27} Isoprenoids)

Authorities. There will be no addition of the corresponding authorities to the species epithets in the text of Sect. 7.7, since they are added to the species names in the complete list of Tables 7.1 and 7.2.

Outline. Saponins, sometimes termed saponosides, are glycosidic derivatives of steroids or polycyclic triterpenes which – dissolved in water – behave like soaps (lat. sapo!), i.e., produce a stable foam, emulsify fatty oils, and stabilize suspensions. They are able to lower the surface tension of aqueous solutions and to cause haemolysis. This is due to their lyobipolar character which is based on the lipophilic aglycone and the highly hydrophilic carbohydrate moiety. There is only one report on *triterpenoid saponins* in the Solanaceae (*Cestrum parqui* L'HÉRIT., see Sect. 7.5.1.), whereas *steroidal saponins* are common metabolites of this family. Saponins do not occur in the Convolvulaceae.

Common steroidal sapogenins, sometimes termed sapogenols, occurring in the Solanaceae are

- Derivatives of spirostane (16β,22:22α,26-diepoxycholestane), i.e., a C_{27}-skeleton whose side chain is integrated into a spiroketal partial structure
- Derivatives of furostane (16β,22-epoxycholestane)

For examples of both types see Fig. 7.12. Since they were isolated from so many of *Solanum* species, steroidal *sapogenins* are assumed to be present in all species

Table 7.1 Examples for the occurrence of steroidal sapogenins in solanaceous species

Sapogenin	Species	References
Andesgenin	*Solanum hypomalacophyllum* Bitt.	(1)
Anosmagenin	*S. vespertilio* Ait.	(2)
Barogenin	*S. tuberosum* L.	(3)
Chlorogenin	*S. torvum* Sw., *S. wrightii* Benth.; *S. meridense* Bitt. ex Pittier; *S. scorpioideum* Rusby	(4); (5); (6)
15-Dehydro-14β-anosmagenin	*S. vespertilio* Ait.	(2)
Digalogenin	*Cestrum parqui* L'Hérit.	(7)
Digitogenin	*Cestrum parqui* L'Hérit.; C. *laevigatum* Schltdl.	(8)
Diosgenin	*S. auriculatum* Ait., *S. dulcamara* L., *S. macrocarpum* L., *S. sodomaeum* L., *S. xanthocarpum* Schrad. & Wendl.; *S. meridense* Bitt. ex Pittier; *S. scorpioideum* Rusby; *S. melongena* L.; *Vestia foetida* (Ruiz & Pav.) Hoffm. sub nom. *V. lycioides* Willd.; *Capsicum annuum* L.; *Cestrum pallidissimum* Backer	(4); (5); (6); (9); (10); (11); (12)
Gitogenin	*Schwenckia americana* L.; *Solanum sodomaeum* L.; *Capsicum annuum* L.; *Cestrum elegans* (Brongn.) Schltdl.	(13); (4); (11); (14)
Hecogenin	*S. hispidum* Pers.; *S. scorpioideum* Rusby	(4); (6)
Hispigenin	*S. hispidum* Pers.	(15)
Isocaelagenin/caelagenin	*S. jamaicense* Mill.	(16)
(25*R*)-Isonuatigenin/-nuatigenin	*Vestia foetida* (Ruiz & Pav.) Hoffm. sub nom. *V. lycioides* Willd.	(10)
(25*S*)-Isonuatigenin/-nuatigenin	*S. sisymbrifolium* Lam.; *S. aculeatissimum* Jacq.; *Vestia foetida* (Ruiz & Pav.) Hoffm. sub nom. *V. lycioides* Willd.	(4); (17); (10)
Neochlorogenin	*S. paniculatum* L., *S. torvum* Sw.	(4)
Neogitogenin	*Lycianthes biflora* (Lour.) Bitt.	(18)
Neosolaspigenin	*S. hispidum* Pers.; *S. torvum* Sw.	(19); (20)
Neotigogenin	*S. hirsutum* Dun. sub nom. *Lycopersicum hirsutum* (Dun.) Macbride, *S. lycopersicum* L. sub nom. *L. esculentum* Mill., *S. pimpinellifolium* Jussl. sub nom. *L. pimpinellifolium* (Jussl.) Mill.; *Nicotiana tabacum* L.	(4); (21)
Nuatigenin	*S. sisymbrifolium* Lam.; *S. aculeatissimum* Jacq.	(4); (17)
Paniculogenin	*S. paniculatum* L.; *S. torvum* Sw.	(22); (23)

(continued)

Table 7.1 Examples for the occurrence of steroidal sapogenins in solanaceous species (continued)

Sapogenin	Species	References
Parquigenin	*Cestrum parqui* L'HÉRIT.	(24)
Scopologenin	*Scopolia japonica* MAXIM.	(25)
Sisalagenin	*S. scorpioideum* RUSBY	(6)
Solagenin	*S. hispidum* PERS.	(26)
Solaspigenin	*S. hispidum* PERS.; *S. torvum* SW.	(19); (20)
(22*R*,23*S*,25*S*)-5α-Spirostan-3β,6α,23-triol	*S. torvum* SW.	(27)
(25*S*)-Spirost-5-en-3β,15α-diol	*S. laxum* STEUD.	(28)
(25*R*)-Spirost-5-en-2α,3β, 15β-triol	*Cestrum nocturnum* L.	(29)
(25*R*)-Spirost-5-en-2α,3β, 17α-triol	*C. nocturnum* L.	(29)
(25*R*)-Spirost-5-en-3β,23α, 26β-triol	*S. indicum* L.	(30)
(25*S*)-Spirost-5-en-3β,15α, 23α-triol [(25*R*) isomer: Scopologenin]	*S. laxum* STEUD.	(31)
Tigogenin	*Schwenckia americana* L.; *Solanum alatum* MOENCH, *S. curtipes* BITT., *S. dulcamara* L., *S. lycopersicum* L., *S. mandonis* VAN HEURCK & MÜLL. ARG., *S. quitoense* LAM.; *S. scorpioideum* RUSBY; *Cestrum laevigatum* SCHLTDL., *C. parqui* L'HÉRIT.; *Capsicum annuum* L.; *Cestrum diurnum* L.; *S. melongena* L.	(13); (4); (6); (8); (11); (32); (33)
Yamogenin	*S. dulcamara* L., *S. tuberosum* L.; *S. panduraeforme* E.MEY; *S. spirale* ROXB.	(4); (34); (35)

References: (1) González et al. 1975; (2) Gonzalez et al. 1974; (3) Kaneko et al. 1977b; (4) Schreiber 1968 and references therein; (5) De Valeri and Usubillaga 1989; (6) Cuervo et al. 1991; (7) Bianchi et al. 1963; (8) Canham and Warren 1950a,b; (9) Kintya and Shvets 1985a,b; (10) Faini et al. 1984; (11) Gutsu et al. 1987a, Yahara et al. 1994; (12) Mi et al. 2002; (13) Kapundu and Delaude 1988; (14) Kereselidze et al. 1970; (15) Chakravarty et al. 1978; (16) Döpke et al. 1976; (17) Saijo et al. 1983; (18) Ripperger 1990; (19) Chakravarty et al. 1980; (20) Mahmood et al. 1983; (21) Shvets et al. 1996b; (22) Ripperger et al. 1967b; (23) Schreiber and Ripperger 1968; (24) Baqai et al. 2001; (25) Okamura et al. 1992; (26) Chakravarty et al. 1979; (27) Iida et al. 2005; (28) Soulé et al. 2000; (29) Mimaki et al. 2001; (30) Yahara et al. 1996b; (31) Ferreira et al. 1996; (32) Ahmad et al. 1993; (33) Kintya and Shvets 1984, 1985a; (34) Döpke et al. 1987; (35) Quyen et al. 1987

belonging to this genus (Heftmann 1983 and references therein). However, this must not implicate that the corresponding glycosides, i.e., steroidal *saponins*, are also constituents of all *Solanum* species though they are frequent metabolites of this and another solanaceous genus, *Cestrum*.

Table 7.2 Examples for the occurrence of steroidal saponins in different organs of *Solanum* spp.; special examples for seeds are given in the text

***Solanum* species**	**Organ**	**Saponin**	**Aglycone**	**Sequence** *Abbreviations* – ara = α-L-arabinose; gal = β-D-galactose; glu = β-D-glucose; qui = β-D-quinovose; rha = α-L-rhamnose; xyl = β-D-xylose	**References**
S. aculeatissimum JACQ.	Roots	Aculeatiside A	Nuatigenin	rha-(1→2$_{glu}$)-rha-(1→4$_{glu}$)-glu-(1→3β) (β-chacotriose)	(1)
S. anguivi LAM.	Fruits	Anguivioside III	(25*R*),22α*O*-spirost-5-en-3β,23α,26β-triol	rha-(1→2$_{glu}$)-xyl-(1→3$_{glu}$)-glu-(1→3β)	(2)
S. chrysotrichum C.H.WRIGHT	Leaves	Saponin SC-2	Chlorogenin	xyl-(1→3$_{qui}$)-qui-(1→**6**α)	(3)
S. hispidum PERS.	Leaves	No trivial name	Neochlorogenin	xyl-(1→3$_{qui}$)-qui-(1→**6**α)	(4)
S. hispidum PERS.	Leaves	Hispinin C	Hispigenin (22β*O*)	rha-(1→3$_{rha}$)-rha-(1→**6**α)	(5)
S. indicum L.	Roots	Indioside E	Diosgenin	rha-(1→2$_{gal}$)-xyl-(1→3$_{gal}$)-gal-(1→3β)	(6)
S. laxum STEUD.	Aerial parts	Luciamin	(**25*S***),22α*O*-spirost-5-en-3β,15α-diol	rha-(1→2$_{gal}$)-[glu-(1→2$_{glu}$)-glu-(1→4$_{gal}$)]-gal-(1→3β)	(7)
S. nigrum L.	Aerial parts	Solanigroside D	(25*R*),22α*O*-3β,23α-dihydroxy-5α-spirostan-26-one	ara-(1→2$_{glu}$)-[xyl-(1→3$_{glu}$)]-glu-(1→4$_{gal}$)-[rha-(1→2$_{gal}$)]-gal-(1→3β)	(8)
S. sisymbrifolium LAM.	Roots	No trivial name	Isonuatigenin	rha-(1→2$_{gal}$)-glu-(1→3$_{gal}$)-gal-(1→3β) (ß-solatriose)	(9)
S. torvum SW.	Fruits	Torvoside K	Neosolaspigenin	rha-(1→3$_{qui}$)-qui-(1→3β)	(10)
S. torvum SW.	Aerial parts / fruits	Torvoside A	26-Degluco-torvoside A	rha-(1→3$_{qui}$)-qui-(1→**6**α)	(11); (12)

References: (1) Saijo et al. 1983; (2) Honbu et al. 2002; (3) Zamilpa et al. 2002; (4) Gonzalez et al. 2004; (5) Chakravarty et al. 1979; (6)Yahara et al. 1996b; (7) Soulé et al. 2000; (8) Zhou et al. 2006; (9) Ferro et al. 2005; (10) Iida et al. 2005; (11) Yahara et al. 1996a; (12) Arthan et al. 2002, 2006

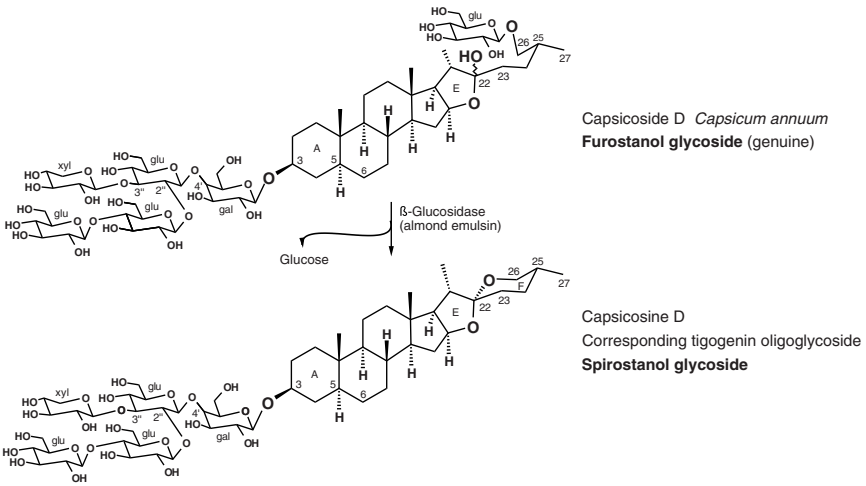


Fig. 7.12 Capsicoside D, a constituent of *Capsicum* seed, as an example for a genuine furostanol glycoside as a non-haemolytic *proto* congener which may be selectively hydrolyzed by a specific β-glycosidase present in another compartment of the seed. In case of herbivore attack this compartmentalization does not exist any longer thus resulting in a corresponding hydrolyzation (glucosyl residue at *O*-26 is liberated). This permits a spontaneous closure of the F-ring leading to the formation of the strongly haemolytic spirostanol glycoside capsicosi**n**e D (proved experimentally by use of almond emulsin; Yahara et al. 1994). The single rings within the respective penta- and hexacyclic systems are marked conventionally with capital letters *from left to right*

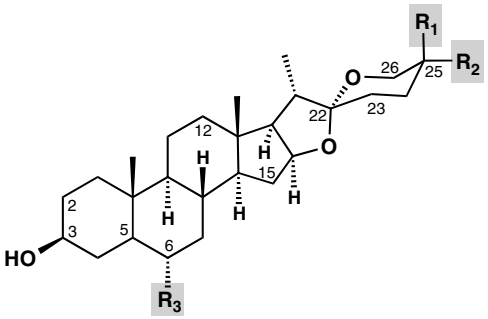
Occurrence in Non-solanaceous Taxa. Steroidal saponins are widespread in the plant kingdom, especially within the monocots (e.g., Agavaceae, Asparagaceae, Dioscoreaceae, Liliaceae). However, they also occur – though much rarer – within the dicots (e.g., Fabaceae, Plantaginaceae, Zygophyllaceae), where triterpenoid saponins are more frequent [occurrence documented in about 60 families (Teuscher and Lindequist 1994)].

7.7.1 Discovery and Structure Elucidation

7.7.1.1 Steroidal Sapogenins (Fig. 7.13)

Many sapogenins occurring in the Solanaceae had been discovered already in other plant families, e.g., diosgenin, yamogenin (genus *Dioscorea* L., Dioscoreaceae) (Marker et al. 1940, 1943), chlorogenin (genus *Chlorogalum* KUNTH, Hyacinthaceae) (Liang and Noller 1935), hecogenin (genus *Hechtia* KLOTZSCH, Bromeliaceae), the first steroidal sapogenin with a carbonyl group (Marker et al. 1943), digalogenin, digitogenin, gitogenin, tigogenin (genus *Digitalis* L., Plantaginaceae).

The first steroidal saponin, digitonin, was isolated by Schmiedeberg (1875) from "digitalin", an industrially produced extract obtained from the leaves of the purple foxglove, *Digitalis purpurea* L. Its sapogenin digitogenin was separated by Kiliani



Substituents				5α-Spirostane derivatives	Spirost-5-ene derivatives
R_1	R_2	R_3	Other substitutions		
H	CH_3	H	–	Tigogenin [(**25*R***)-5α-spirostan-3β-ol]	Diosgenin [(**25*R***)-spirost-5-en-3β-ol]
H	CH_3	OH	–	Chlorogenin	
H	CH_3	H	2α-OH, 15β-OH		**(25*R*)-Spirost-5-en-2α,3β,15β-triol**
H	CH_3	H	2α-OH, 17α-OH		**(25*R*)-Spirost-5-en-2α,3β,17α-triol**
H	CH_3	H	15α-OH, 23α-OH		**Scopologenin**
H	CH_3	H	15β-OH, 23α-OH		**Anosmagenin**
H	CH_3	H	15-Oxo, 14**β**-H; 23α-OH		**15-Dehydro-14β-anosmagenin**
H	CH_3	H	23α-OH, 26β-OH		**(25*R*)-Spirost-5-en-3β,23α,26β-triol**
H	CH_3	H	24β-OH		**Parquigenin**
H	CH_3	H	12-Oxo	Hecogenin	
H	CH_3	H	3-Deoxy-3-oxo, 12β-OH	**Hispidogenin**	
H	CH_3	OH	23ß-OH	**Solaspigenin**	
H	CH_3	H	2α-OH	Gitogenin	
H	CH_3	H	15β-OH	Digalogenin	
H	CH_3	H	2α-OH, 15β-OH	Digitogenin	
OH	CH_3	H	–	**[Isocaelagenin]**	**[(25*S*)-Isonuatigenin]**
CH_3	H	H	–	Neotigogenin [(**25*S***)-5α-spirostan-3β-ol]	Yamogenin [(**25*S***)-spirost-5-en-3β-ol]
CH_3	H	H	15α-OH		(**25*S***)-Spirost-5-en-3β, 15α-diol
CH_3	H	H	15α-OH, 23α-OH		(**25*S***)-Spirost-5-en-3β, 15α,23α-triol
CH_3	H	OH	–	**Neochlorogenin**	
CH_3	H	OH	23β-OH	**Paniculogenin**	
CH_3	OH	H	**–**		**[(25*R*)-Isonuatigenin]**
CH_3	H	OH	3-Deoxy-3-oxo	**Solagenin**	
CH_{33}	H	H	3-Deoxy-3-oxo, 12α-OH	**Torvogenin**	
CH_3	H	H	3-Deoxy-3-oxo, 12-oxo	**Sisalagenone**	

Fig. 7.13 Steroidal sapogenins of the 22α*O*-spirostanol type occurring in the genera *Solanum* and/or *Cestrum*, respectively; (for examples of species see Table 7.1). Compounds with names highlighted in **bold** were discovered in certain solanaceous species; if not, they had been discovered already before in other plant families. Compounds in parentheses do not represent the genuine aglycone but a rearranged stable derivative of an unstable genuine one (for details see text). For references see Table 7.1

(1890, 1911). The constitution of this aglycone was elucidated finally by Marker and Rohrmann (1939), the structure of its saponin digitonin by Tschesche and Wulf (1963). Discovery of further *Digitalis* sapogenins and progress in structure elucidation of them were contributed mainly by the studies of Windaus in the 1920s (discovery of gitogenin: Windaus and Brunken 1925), Jacobs in the 1930s (discovery of tigogenin: Jacobs and Fleck 1930; Jacobs and Simpson 1935), Marker in the 1930s/1940s (structure elucidation of gitogenin: Marker and Rohrmann 1939), and Tschesche in the 1950s/1960s (digalogenin from the seeds of *D. purpurea*; Tschesche and Wulff 1961).

Nevertheless, there are also sapogenins, which were discovered – predominantly in the 1960s – in solanaceous species with the consequence that most of them were named correspondingly (Fig. 7.13). There are some exceptions which got deviant names (e.g., andesgenin, neochlorogenin, nuatigenin); if comprehensible these deviations are explained below.

Steroidal Sapogenins Found in Solanaceous Species. The majority of structurally elucidated solanaceous saponins are characterized by aglycones of the 22α*O*-spirostanol type. They may be 5α-cholestanes as well as cholest-5-ene derivatives. 25*R* derivatives are dominating beside several 25*S* epimeric congeners (Fig. 7.13). However, some 22β*O*-spirostanol type congeners were also discovered (Fig. 7.14). Another important group of saponins is characterized by aglycones of the furostanol type; again 5α*H*- as well as 5,6-dehydro derivatives may occur. Finally, a few unusual though structurally related types are included: potential precursors of the common types just mentioned, e.g., cholestanes (Figs. 7.16–7.18), as well as probable metabolites, e.g., pregnanes (Fig. 7.15).

Like their alkaloidal congeners (Sect. 7.8), these sapogenins were (i) sometimes isolated directly as such from the plants co-occurring with their saponins/glycosides, (ii) obtained by hydrolysis of the latter or (iii) may be known as such but the knowledge on their corresponding glycosides may be incomplete. Finally, occurrence of aglycones without saponins might be possible.

Known 22α*O*-Spirostanol Type Sapogenins also Detected in Solanaceous Species. Early detections of sapogenins in *Solanum* spp. already known from other plant families were reported on tigogenin (*S. dulcamara* L.; Marker et al. 1943), diosgenin (*S. xanthocarpum* SCHRAD. & WENDL.; Sato and Latham 1953), and yamogenin (*S. tuberosum* L.; Schreiber 1957). Digitogenin and gitogenin could be isolated from the berries of *Cestrum laevigatum* SCHLTDL. (Canham and Warren 1950a). Further examples are given in Fig. 7.13 and Table 7.1. For furostanols see below ["Steroidal saponins (glycosides)"].

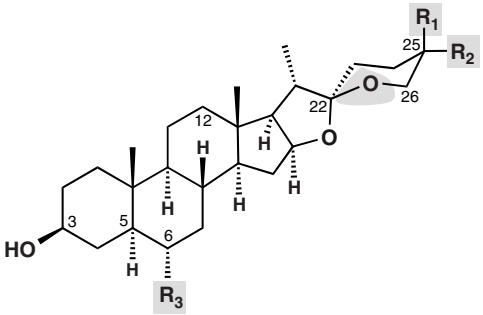
Novel 22α*O*-Spirostanol Type Sapogenins Discovered in Solanaceous Species. A considerable number of such aglycones were discovered in the Solanaceae. They are highlighted by bold types in Fig. 7.13. Thus, two closely related sapogenins were found in the leaves of *S. paniculatum* L. (Ripperger et al. 1967b). One of them turned out to be the 25*S*-epimer of the already known chlorogenin. It was named neochlorogenin because such epimers were named traditionally

according to their 25*R* forms together with the prefix "neo". The other one, paniculogenin, could be structurally elucidated as the 23α-hydroxy derivative of neochlorogenin. The Indian medicinal plant *S. hispidum* PERS. is another interesting source for novel sapogenins: Solaspigenin, the 23*R* isomer (23β-hydroxy) of paniculogenin, and the corresponding 22β*O* congener (neosolaspigenin; see below) could be isolated from the leaves (Chakravarty et al. 1980). Furthermore, 3-deoxy-3-oxo derivatives could be isolated from the berries/seeds (see below). Parquigenin is an example for the contribution of the genus *Cestrum* (*C. parqui* L'HÉRIT.; Baqai et al. 2001), scopologenin for the genus *Scopolia* (*S. japonica* MAXIM.; Okamura et al. 1992) to the spectrum of such aglycones from the Solanaceae.

Dehydro Derivatives (Spirostanones/Spirostenones). A number of natural 3-dehydro (3-deoxy-3-oxo) derivatives of spirostanols, i.e., **spirostan-3-ones,** were discovered in solanaceous species though their 3β-hydroxy congeners had been already known from other plant families. For example, hispidogenin, a positional isomer (3 = O,12β-OH) of hecogenin, could be isolated from the berries of *S. hispidum* (Maiti and Mookherjea 1965) and solagenin, the 3-dehydro congener of neochlorogenin, from the seeds (Chakravarty et al. 1979). In contrast, the roots are characterized by the content of steroidal alkaloids (Sect. 7.8). Two further spirostane-3-ones were discovered in the fruits of *S. torvum* Sw., which are commonly available in certain Asian markets and used as a vegetable, e.g., in Thai cuisine (Iida et al. 2005): Torvogenin, i.e., (25*S*)-12α-hydroxy-5α-spirostan-3-one, and the corresponding 3,12-dione sisalagenone (Morales Méndez et al. 1970), a 3-dehydro derivative of sisalagenin, known from *Agave sisalana* PERRINE ex ENGELM., Agavaceae (Callow and James 1955). (22*R*,25*R*)- as well as (22*R*,25*S*)-6α-hydroxy-5α-spirostan-3-one were isolated from the green berries of *S. scorpioideum* RUSBY (Usubillaga and Meccia 1987). Chlorogenone, the 3,6-dione of chlorogenin, as well as its 25*S*-epimer, neochlorogenone, were identified as natural metabolites for the first time in the unripe fruits of *S. meridense* BITT. ex PITTIER (De Valeri and Usubillaga 1989) and *S. torvum* Sw. (Cuervo et al. 1991), respectively. (25*R*)-3β-Acetoxy-5α-spirostan-6-one, also isolated from the green berries of *S. meridense*, may be considered as a closely related metabolite; the acylation of the 3β-hydroxyl – unique with regard to steroidal sapogenins – is preventing any glycosidation. Diosgenone, a **spirost-4-en-3-one**, and its presumable precursor tumaquenone, a furostanol-26-monoglucoside, both constituents of the aerial parts of *S. nudum* HUMB. & BONPL. ex DUN., showed a double bond as part of ring A, i.e., in conjugation to the carbonyl group (Saez et al. 1998). Such a partial structure is common for certain steroidal hormones, e.g., cortisol/cortisone, progesterone, testosterone. A curious aglycone forming a δ-lactone, (22*R*,25*R*)-3β,15α,23α-trihydroxy-**5α-spirostan-26-one,** has been discovered as part of a bioside, soladulcoside A, from the aerial parts of *S. dulcamara* L. (Yamashita et al. 1991); later it could be also detected in three novel saponins, solanigrosides C – E, from the aerial parts of *S. nigrum* L. (Zhou et al. 2006). Its 22*S*-configurated 5,6-dehydro derivative could be identified as aglycone of the β-chacotrioside foliumin A, isolated from the aerial parts of *S. amygdalifolium* STEUD. (Vázquez et al. 1999).

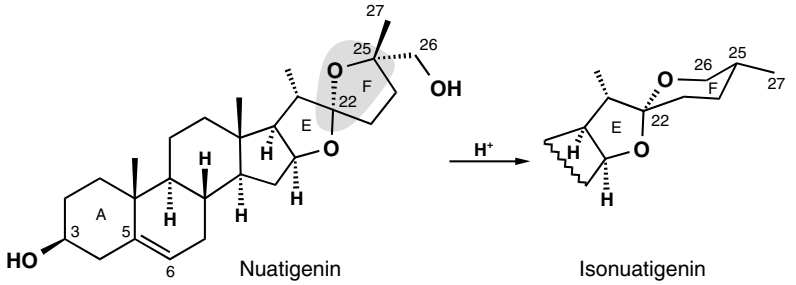
C/D *cis* Configuration. Usual spirostanol ring junctions and configurations (B/C *trans*, C/D *trans*, D/E *cis*) implicate C-13β*CH*$_3$, C-14α*H* in steroidal sapogenins. However, 15-dehydro-**14β**-anosmagenin, a constituent of *S. vespertilio* Ait., endemic to the Canary islands, was found to be a unique exception since it turned out to be **C/D *cis*** configurated [13β*CH*$_3$, 14**β***H*; Fig. 7.15]**.** Anosmagenin (15β,23α-dihydroxy-diosgenin) itself showed C/D *trans* configuration which is prevalent for naturally occurring steroids (Gonzalez et al. 1974). It may be assumed that the rare presence of a carbonyl group (C-15) in the neighbourhood of 14α*H* in the presumptive genuine precursor (15-dehydro-anosmagenin) enables spontaneous keto-enol tautomerism with the consequence of equilibrium in favour of 14β*H*.

Novel 22β*O*-Spirostanol Type Sapogenins Discovered in Solanaceous Species (Fig. 7.14). 22β*O* configurated spirostanols are very rare. Hispigenin, the first sapogenin of this type encountered in nature, was discovered in the leaves of *S. hispidum* Pers. in co-occurrence with its already known 22α*O* isomer paniculogenin (Chakravarty et al. 1978). During the structure elucidation procedure of torvoside L, a saponin from the fruits of *S. torvum* Sw., the configuration of hispigenin at C-23 has been revised recently from an originally supposed axial hydroxy group to equatorial, i.e., from 23*S* to 23*R* (Iida et al. 2005). Structural research work with torvoside K in the same study led also to a revision in case of neosolaspigenin, again already discovered about 25 years before as a constituent of the leaves of *S. torvum* (Chakravarty et al. 1979, 1980; Mahmood et al. 1983). This sapogenin, originally supposed to be a 22α*O* configurated spirostanol, finally turned out to be a 22β*O* congener. Furthermore, the aglycone of another saponin, torvoside J, in the



Substituents				5α-Cholestane derivatives
R_1	R_2	R_3	Other substitutions	
H	CH_3	OH	23β-OH	**(22*R*,23*S*,25*S*)-5α-Spirostan-3β,6α,23-triol**
H	CH_3	OH	23α-OH	**Hispigenin** (22*R*,23*R*,25*S*)
CH_3	H	OH	23β-OH	**Neosolaspigenin** (22*R*,23*S*,25*R*)

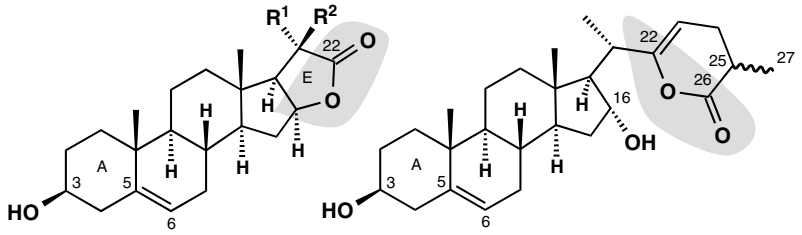
Fig. 7.14 Steroidal sapogenins of the 22β*O*-spirostanol type occurring in *Solanum hispidum* and *S. torvum*, respectively (for details and references see Table 7.1). Neosolaspigenin was erroneously characterized as 22α*O*-spirostanol type in the original report; for solaspigenin see Fig. 7.13



Nuatigenin
Genuine aglycone

Isonuatigenin
Spirostanol

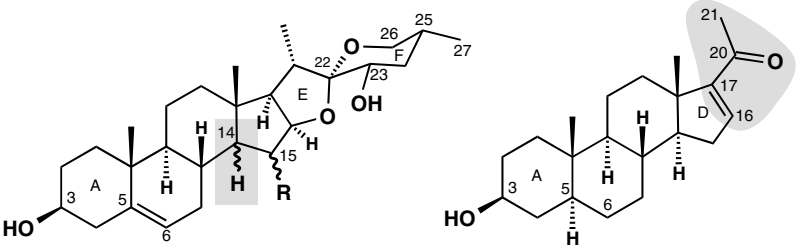
Solanum sisymbriifolium



$R_1 = CH_3$ $R_2 = H$ Vespertilin
$R_1 = OH$ $R_2 = CH_3$ 20*S*-Hydroxy-vespertilin

Bajamarin

Solanum vespertilio



R = ß-OH 14 αH Anosmagenin
R = =O 14ß*H* 15-Dehydro-14ß-anosmagenin

Solanum vespertilio

3ß-Hydroxy-5α-pregn-16-en-20-one
(aglycone of a 3-O-ß-lycotetraoside)

Solanum lycopersicum var. *cerasiforme*

Fig. 7.15 Unusual steroidal sapogenins; *highlighted in grey*: (i) 22,25-epoxide bridge (nuatigenin), (ii) lactone type metabolites (vespertilins, bajamarin), (iii) C/D *trans* configuration (15-dehydro-14β-anosmagenin), (iv) acetyl residue at C-17 including conjugated 16,17-double bond characterizing this *allo*pregnenolone aglycone

study of Iida et al., represents a third 22β*O* spirostanol characterized as the 25*S* epimer of neosolaspigenin.

Unusual Steroidal Sapogenins/Saponins. A 22*S*,25*S*-epoxy-furost-5-ene type sapogenin, [(25*S*)-]nuatigenin (Fig. 7.15), obtained from the roots of *S. sisymbrifolium* LAM., a species occurring in Central Paraguay with the local common name "Nuati-Pyta" (red prickle), which contributed to the trivial name, was discovered and structurally elucidated as part of "saponin P" by Tschesche and Richert (1964). In contrast to most other furostanol glycosides characterized as 3β,26-bisdesmosides *without ring F*, i.e., C-23–C-27 form an open chain, nuatigenin-based glycosides represent 3β,26-bisdesmosides *with such a ring F* formed by C-22–C-25 and therefore spiro-attached to ring E ("pseudospirostanol"). However, free [(25*S*)]-nuatigenin – like all common furostanols – is easily rearranged by mineral acids, e.g., during acidic hydrolysis of the genuine saponins, to a same spirostanol, [(25*S*)-]isonuatigenin. For corresponding 25-epimers see below (genus *Vestia* WILLD.). A comparable situation was found for the corresponding 5α,6-dihydro congeners isocaelagenin/caelagenin from *S. jamaicense* MILL. (Döpke et al. 1976).

From the green berries of a tree occurring in the Venezuelan Andes, *S. hypomalacophyllum* BITT., a 22,26-*epoxy*cholestane type sapogenin, (20*S*,22*R*,25*R*)-3β-hydroxy-5α-cholestan-22,26-epoxy-4,23-dione was isolated. It was named andesgenin according to the occurrence of the plant (González et al. 1975). Trivial names for metabolites using the species epithet had already been given to two steroidal *alkaloids* isolated from leaves and green berries of this plant before (solaphyllidine, solamaladine). Andesgenin may be considered as an *O*-homologous congener of 22,26-*epimino*cholestanes, a specific type of steroidal alkaloids frequently occurring in *Solanum* spp., e.g., just this solaphyllidine in that species (see Sect. 7.8.1.3).

The budding tuber of *S. tuberosum* L., potato, accumulated barogenin, a 16-oxo analogue of dormantinone (see also Sect. 7.8.3), i.e., a derivative of cholesterol with three additional oxygen functions (C-16, C-22, C-26) still lacking the spirostanol partial structure characterizing all sapogenins summarized in Fig. 7.13 (Kaneko et al. 1977a). Similar derivatives, 3β,16α,26-trihydroxy-5α-cholestan-22-one [saponins: abutilosides C (3β-monodesmoside), D, F (3β,26-bisdesmosides)] and its 5,6-dehydro derivative [saponins: abutilosides E, G (3β,26-bisdesmosides)], were discovered as constituents of the fresh roots of *S. abutiloides* (GRISEB.) BITT. & LILLO, a well-studied species (Tian et al. 1996; Yoshimitsu et al. 2000). Further structurally related metabolites, abutilosides L – N, characterized by a 22*S*,25*S*-epoxy-furost-5-ene type aglycone like nuatigenin, could be isolated from the fresh fruits (Yoshimitsu et al. 2003). The remaining congeners, abutilosides A, B, H–K, and O, belong to steroidal glycoalkaloids (Sect. 7.8.1.7 and 7.8.1.8). Two cholest-5-en-16, 22-dione type glycosides, anguiviosides XV and XVI, were discovered in the fruits of *S. anguivi* LAM. (Fig. 7.16). In addition, the latter metabolite showed an unusual linkage (C-23 → *O* → C-26) forming a non-anellated tetrahydrofuran ring; this was also determined for a furostanol bisdesmoside congener, anguivioside XI.

Three unique **lactone** type sapogenins (Fig. 7.15), bajamarin, the δ-lactone of 3β,16α,22-trihydroxy-cholesta-5,22-dien-26-oic acid, as well as vespertilin and

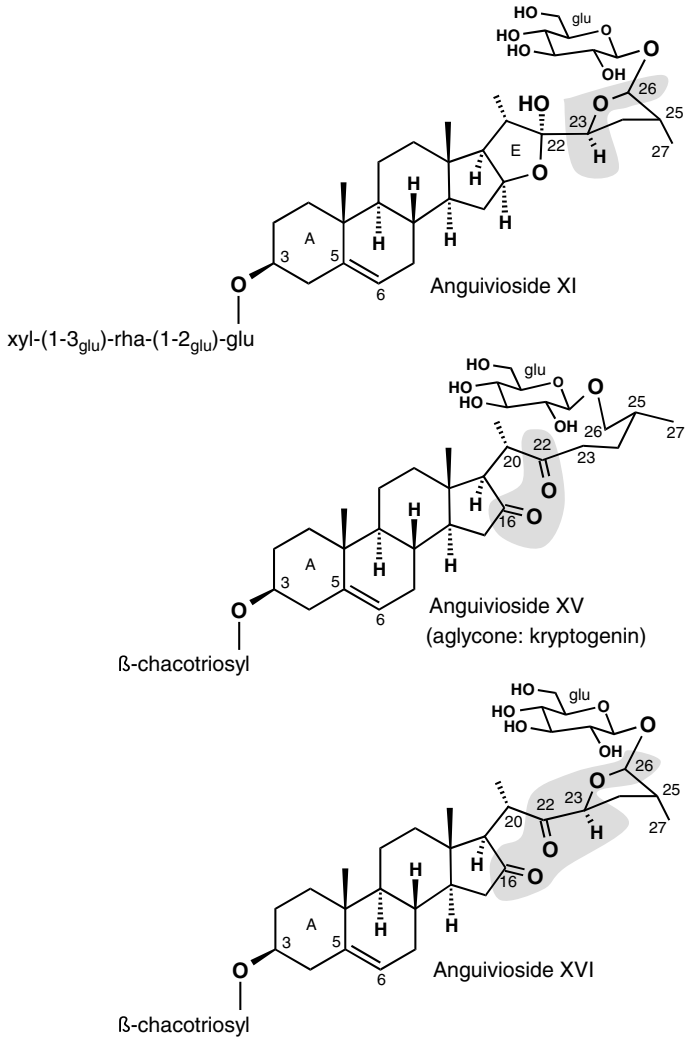


Fig. 7.16 Steroidal saponins with unusual aglycones from the fruits of *Solanum anguivi*: anguivioside XI (unusual linkage between C-23 and C-26) and two cholest-5-en-16,22-dione type glycosides, anguiviosides XV and XVI, i.e., both lack ring E, normally a characteristic of steroidal sapogenins (unusual partial structures *highlighted in grey*). For structure of chacotriose see Fig. 7.22

20*S*-hydroxyvespertilin, two γ-lactones of 3,16-dihydroxy- and 3,16,20-trihydroxypregnen-5-en-20-carboxylic acid, could be characterized as constituents of the fruits of *S. vespertilio* AIT. (Gonzalez et al. 1971, 1972, 1973).

Pregnane Type Sapogenins/Saponins. 5α-Pregn-16-en-3β-ol-20-one was isolated from *Solanum pimpinellifolium* JUSSL. sub nom. *Lycopersicon pimpinellifolium* (JUSSL.) MILL. by Schreiber and Aurich (1966) and also by Bennett et al. (1967). The authors speculated that this aglycone could be a metabolite of the steroidal glycoalkaloid tomatine. Heftmann and Schwimmer (1972) added that

this specific pregnenolone could also be a degradation product of neotigogenin in the same species. Possibly due to more careful isolation procedures a corresponding "tomato pregnane" glycoside, i.e., 5α-pregn-16-en-3β-ol-20-one (*allo*pregnenolone) β-lycotetraoside (Fig. 7.15) has been isolated recently from the overripe fruits of *S. lycopersicum* L. var. *cerasiforme* sub nom. *Lycopersicon esculentum* MILL. var. *cerasiforme* (DUN.) ALEF., Cherry tomato (Fujiwara et al. 2005). This constituent might be a metabolite of common (*N*-free or *N*-containing) steroidal glycosides. Depending on the developmental stage of the fruit a metabolic sequence tomatine (a glycoalkaloid of *immature* fruits) → esculeoside A [a glycoalkaloid of *ripe* fruits] → 5α-pregn-16-en-3β-ol-20-one glycoside (*overripe* fruits) proposed by the authors seems to be plausible. This is supported by the interesting fact that the carbohydrate (β-lycotetraosyl) moiety remained unchanged in all these compounds. For details on tomatine and esculeoside A see Figs. 7.22 and 7.24. A homologous situation could be observed with 5,6-dehydro derivatives though with another carbohydrate chain: Pregna-5,16-dien-3β-ol-20-one β-chacotrioside has been identified as a constituent of the underground parts of *S. sodomaeum* L. in co-occurrence with the saponin dioscin, i.e., diosgenin β-chacotrioside, and the glycoalkaloid solamargine, i.e., solasodine β-chacotrioside (Fig. 7.22) (Ono et al. 2006a), suggesting that the latter two metabolites might be degraded each to the former. A 20,22-seco type glycoside, the 16-aminoacyloxypregnenolone esculeoside D, may be a metabolite of corresponding glycoalkaloids. Nigrumoside A (Fig. 7.18, route B), another – though *N*-free – 16-acyloxypregnenolone, discovered as constituent of the aerial parts of *S. nigrum* L. (Zhu et al. 2001d), might be a metabolite of an oligofurostanoside type congener, uttroside B (Sharma et al. 1983). Both compounds represent β-lycotetraosides.

7.7.1.2 Oligosaccharide Moieties of the Glycosides

Generally there are no structural differences with regard to this topic between steroidal saponins and their alkaloidal congeners, e.g., β-solatriose, β-chacotriose, β-lycotetraose. They are also frequent oligosaccharides here. Due to (i) the fact that they were discovered as carbohydrate moieties of glycoalkaloids and (ii) the higher diversity and significance of these metabolites this topic is discussed in detail in Sect. 7.8.1. β-D-Galactose, β-D-glucose, β-D-quinovose, α-L-rhamnose, and β-D-xylose are more or less frequent monosaccharide units. However, it should be pointed out that solanigrosides D and E, two branched pentasaccharides isolated from *S. nigrum*, have been determined to contain an arabinose unit each which is extremely rare as a component of solanaceous steroidal saponins.

7.7.1.3 Steroidal Saponins (Glycosides) (Table 7.2)

The vast majority of structurally elucidated solanaceous saponins are **spirostanol monodesmosides**, characterized by one carbohydrate chain comprising usually 2–5 monosaccharide units which is attached glycosidically to the hydroxyl at C-3β of the aglycone, thus forming an acetal linkage. However, in case of several 6α-hydroxylated

aglycones this carbohydrate chain, though regularly confined here to only two monosaccharide units, may be linked to the latter position instead of C-3β, e.g., *S. chrysotrichum* C.H. Wright, *S. hispidum* Pers. The fruits of *S. torvum* Sw. have even shown the presence of such 6α-glycosides with a free 3β-hydroxy substituent (26-degluco-torvoside A) and also with its 3-dehydro (3-deoxy-3-oxo) congener (26-degluco-torvoside H) (Arthan et al. 2006). On the other hand, there are **furostanol bisdesmosides**, usually characterized by an attachment of one glucose unit to the hydroxyl at C-26 *in addition* to the oligoside chain at C-3β or C-6α. Co-occurrence of both structural types, spirostanol monodesmoside and furostanol bisdesmoside, was often observed, e.g., tomatosides A and B from the seeds of *Solanum lycopersicum* L. (Shchelochkova et al. 1980), spirostanol glycosides I–IV and furostanol glycosides V–VIII from the stems of *S. dulcamara* L. (Murakami et al. 1981), capsicoside D and capsicosine D from the seeds of *Capsicum annum* L. (Fig. 7.12); Yahara et al. 1994), indiosides A–E from the fruits and roots of *S. indicum* L. (Yahara et al. 1996b), dioscin and protodioscin from the underground parts of *S. sodomaeum* L. (Ono et al. 2006a). This is not surprising, because furostanol bisdesmosides are supposed to be biologically inactive storage forms of steroidal saponins liberating the corresponding toxic spirostanol monodesmosides for protection in case of being wounded due to herbivore attack or pathogen infections, e.g., protodioscin → dioscin (Heftmann 1983 and references therein; Kalinowska et al. 2005 and references therein); torvoside A → 26-degluco-torvoside A (Arthan et al. 2006); for details see Sect. 7.7.3.2. Of course, chemical hydrolysis during isolation and/or structural elucidation procedures of steroidal saponins, furostanols as well as spirostanols, in any case leads to the corresponding spirostanol aglycones. Free furostanol aglycones are subjected to spontaneous cyclization forming their spiroketal congeners. Apparently, this was the reason why spirostanol glycosides were detected much more frequently.

Unusual Bisdesmosides. A rare *spirostanol* 3β,24-bisdesmoside, based on (24*S*)-2α,24-dihydroxy-diosgenin, was discovered in the leaves of *Cestrum nocturnum* L. (Mimaki et al. 2002). Beside a branched pentasaccharide [gal, glu, xyl (1:3:1)] it was determined to contain a glucose unit as the second carbohydrate moiety – like common furostanol 3β,26-bisdesmosides – though at C-24. Recently, an unexpected structural variation of a *furostanol* bisdesmoside has also been detected in case of solanigroside F, a constituent of the aerial parts of *S. nigrum* L.: in addition to the normal carbohydrate chain at C-3β, a glucose unit was attached glycosidically at C-23α of the aglycone (Zhou et al. 2006).

Steroidal Saponin Analogues Containing a Uronic Acid Instead of a Sugar. Metabolites containing glucuronic acid were discovered as constituents of *S. lyratum* Thunb.: A 22α-methoxyfurost-5-en-3β,26-diol-based bisdesmoside (glucosyl at C-26), characterized by one β-D-glucuronic acid unit linked (1→3β) to the aglycone and one rhamnose unit linked to this acid monomer ($1\rightarrow2_{\text{glucuronic acid}}$) as well as diosgenin 3-*O*-β-D-glucuronic acid were isolated from the aerial parts along with "normal" glycosides (tigogenin 3-*O*-D-glucopyranoside and a furostanol glycoside; Yahara et al. 1986). Bioassay-directed fractionation of the cytotoxic fraction of the whole plant led to further diosgenin derivatives containing glucuronic acid or glucuronic acid methyl ester as one unit (Sun et al. 2006).

7.7.1.4 Co-occurrence of Steroidal Sapogenins/Saponins and Alkamines/Glycoalkaloids

Though studies were often performed with regard to only one of these two groups of metabolites – depending on the field of interest of the corresponding authors – it may be assumed that the other one is also present in (almost) all *Solanum* species which are found to be either sapogenin/saponin-positive or alkamine/glycoalkaloid-positive. In this connection it must also be taken into account that isolation and analysis procedures for the two groups are different. The presence of the other group is not easily announced in a certain procedure. However, there are many proves for the assumption mentioned above. Thus, three chemovarieties of the Eurasian *S. dulcamara* L. showed co-occurrence of pairs of corresponding sapogenins and alkaloids each (see also Sect. 7.8.): (i) tigogenin/soladulcidine, both characterized by 5α*H* and 25*R* configuration; (ii) diosgenin/solasodine (5,6 double bond, 25*R*); (iii) yamogenin/tomatidenol (5,6 double bond, 25*S*) (Sander 1963a and references therein; Willuhn 1966 and references therein; Lee et al. 1994). Another example for such an occurrence is given by *S. lyratum* Thunb. which is closely related to *S. dulcamara*: again a tigogenin-based saponin, degalactotigonin, as well as soladulcidine were detected as constituents of the leaves (Ye et al. 2001). As a third example *S. sodomaeum* L. shall be added. Again diosgenin-based saponins like dioscin are accompanied by the solasodine-based glycoalkaloid solamargine (Ono et al. 2006a). Even a 2α-hydroxy substituted pair, gitogenin/2α-hydroxysoladulcidine, could be discovered as constituents of the roots of *Lycianthes biflora* (Lour.) Bitt. (Ripperger and Porzel 1992).

The yamogenin content of fruits of *S. dulcamara* increased during ripening (in red ripe berries up to 0.75% dry weight). Its content in leaves of flowering and fruiting plants was higher than the content of tomatidenol. However, the opposite was observed in old stems (Willuhn and Koethe 1981).

7.7.2 Occurrence in the Solanaceae

In contrast to the alkamines/glycoalkaloids (Sect. 7.8.) their *N*-free homologues could be detected already in basal branches of the family tree, i.e., certain members of the Schwenckioideae and Petunioideae.

Schwenckioideae. Tigogenin and gitogenin could be identified as aglycones of glycosides found as constituents of the roots of *Schwenckia americana* L (Kapundu and Delaude 1988).

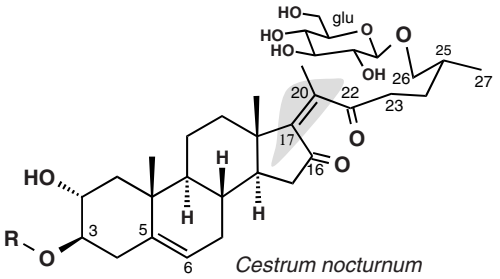
Petunioideae. The seeds of *Petunia* × *hybrida* (Hook.) Vilm. were found to contain 11 saponins altogether. The spirostane type petuniosides A, C, and E were characterized by tigogenin as the aglycone, their congeners B, D, and F by gitogenin. (25*R*)-5α-Furostan-3β,22α,26-triol (corresponding to tigogenin) was identified as genuine aglycone of petuniosides I, L, and N, (25*R*)-5α-furostan-2α,3β,22α,26-tetraol (corresponding to gitogenin) as the one of the congeners K

and M. Glucose and galactose in diverging ratios represent the oligosaccharide moiety at C-3β of the petuniosides, forming, e.g., monogalactosides (1→3β), diglycosides [(glu-(1→4)-gal-(1→3β)], triglycosides [glu-(1→2)-glu-(1→4)-gal-(1→3β)] (Shvets et al. 1995c, d).

Unfortunately, the Russian authors apparently did not know that Japanese authors had claimed the same trivial names (petuniosides A–D) for ergostane glycosides isolated from the fresh aerial parts of the same species just one year before (Shingu et al. 1994; see Sect. 7.11). This led to unpleasant confusions if SciFinderScholar™ 2006 (Chemical Abstracts Service) is used. Abstracts of the Russian authors are mixed with structures elucidated by the Japanese authors.

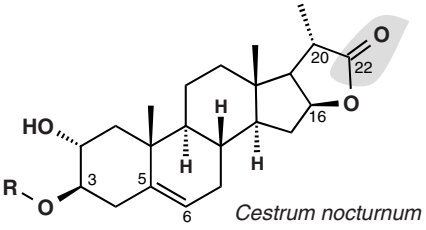
Cestroideae s.s. *Cestreae clade.* Several species of the genus *Cestrum* are cultivated as ornamental garden plants. There are a remarkable number of reports on the occurrence of steroidal glycosides/saponins just with regard to such species. Nevertheless, the vast majority of *Cestrum* spp., the third largest solanaceous genus (175 spp.), still remains to be investigated. A number of aglycones known already from other families were detected in this genus, though some of the corresponding glycosides turned out to be novel natural compounds. From the fresh green berries of *Cestrum parqui* L'HÉRIT., "willow leaf jessamine" [common names according to Hunziker (2001): "duraznillo negro" (Argentina), "palqui" (Chile)], and of *C. laevigatum* SCHLTDL., "inkberry plant", respectively, digitogenin and tigogenin were determined as part of steroidal saponins (Canham and Warren 1950a, b). Beside these two aglycones, digalogenin was found as a constituent of the leaves and fruits of *C. parqui* (Bianchi et al. 1963). Gitogenin and tigogenin were determined as constituents of *C. elegans* (BRONGN.) SCHLTDL. (Kereselidze et al. 1970); the saponin digitonin, known from *Digitalis purpurea* L., Plantaginaceae, could be also characterized in this species sub nom. *C. purpureum* STANDL. (Karawya et al. 1972). The tigogenin-based pentasaccharide diurnoside, detected in the fresh leaves of *C. diurnum* L., whose name is based on the fact that its flowers are fragrant during the day, is characterized by the branched carbohydrate sequence glu-(1→3_{glu})-glu-(1→2_{glu})-{xyl-(1→3_{glu})}-glu-(1→4_{gal})-gal-(1→3β) (Ahmad et al. 1993). Furthermore, tigonin, another tigogenin-based saponin known again from *D. purpurea*, could be identified (Karawya et al. 1972). Dioscin, a diosgenin-based ß-chacotrioside well-known from *Dioscorea* spp., Dioscoreaceae, turned out to be also a constituent of the leaves of *Cestrum pallidissimum* BACKER (Mi et al. 2002).

(25*R*)-Isonuatigenin, discovered in a *Vestia* sp. (see below), was detected also in *Cestrum parqui* L'HÉRIT. (Torres et al. 1988). More than one decade later, the new aglycone parquigenin was discovered as moiety of two saponins isolated from the aerial parts. It turned out to be (24*S*,25*S*)-spirost-5-ene-3β,24-diol. The corresponding saponins were determined to be a branched tetrasaccharide [rha:glu (2:2)], parquisoside A, and a branched pentasaccharide [rha:glu (3:2): {[rha-(1→4_{rha})-rha-(1→2_{glu})]-glu-(1→4_{rha})-rha-(1→4_{glu})}-glu-(1→3β)], parquisoside B, respectively (Baqai et al. 2001). Recently, 5α-spirostan-3α,12β,15α-triol and a pseudosapogenin, 26-*O*-(3′-isopentanoyl)-β-D-glucopyranosyl-5α-furost-20(22)-en-3β,26-diol (Fig. 7.17), have been discovered. The latter metabolite included two unique structural features,



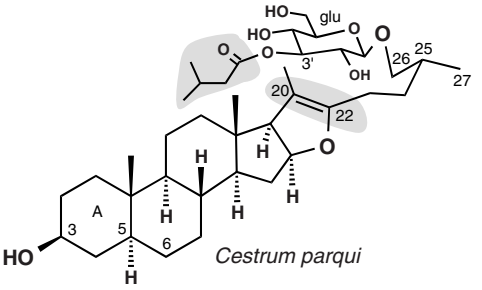
"3ß,26-Bisdesmoside **6**-2002"

R = glu-(1-3$_{glu}$)-glu-(1-2$_{glu}$)-[xyl-(1-3$_{glu}$)]-glu-(1-4$_{gal}$)-gal-(1-3ß)



"22-Homopregnane **8**-2002"

R = glu-(1-3$_{glu}$)-glu-(1-2$_{glu}$)-[xyl-(1-3$_{glu}$)]-glu-(1-4$_{gal}$)-gal-(1-3ß)



26-*O*-(3-Isopentanoyl)-ß-D-glucopyranosyl-
5α-furost-20(22)-en-3ß,26-diol

Fig. 7.17 Unusual steroidal glycosides, unfortunately lacking trivial names, from two *Cestrum* species; structural peculiarities are *highlighted in grey*

(i) an acylated glucosyl residue (at C-3′*O*) and (ii) a C-20/C-22 double bond (D'Abrosca et al. 2005).

Phytochemical studies of the leaves of *C. nocturnum* L., whose name is based on the fact that its flowers are fragrant only at night ("lady of the night", "dama de noche"), led to a rich source for *novel* steroidal sapogenins/saponins and related glycosides. In the nineties a saponin remarkably showing an unusual 2α-hydroxy

substituent was identified, a (25*R*)-spirost-5-en-2α,3β-diol pentaoside named nocturnoside A [glu-(1→3$_{glu}$)-glu-(1→2$_{glu}$)-{xyl-(1→4$_{glu}$)}-glu-(1→3$_{gal}$)-gal-(1→3β)] (Ahmad et al. 1991) beside a novel diosgenin tetraoside, nocturnoside B [{rha-(1→4$_{rha}$)-rha-(1→2$_{rha}$)}-rha-(1→4$_{glu}$)-glu-(1→3β)] (Ahmad et al. 1995). Recently, more comprehensive studies have been performed by Mimaki et al. (2001, 2002). Unfortunately, none of the numerous metabolites discovered by these authors have got a trivial name. Eight spirostanol saponins ("saponin **3**-2001" – "saponin **9**-2001"; "3β,24*S*-bisdesmoside **1**-2002") were characterized. With one exception ("saponin **8**-2001") all metabolites showed an unusual 2α-hydroxy substituent at the aglycone. This was also true for "furostanol 3β,26-bisdesmoside **2-**2002", "pseudofurostanol 3β,26-bisdesmoside **3-**2002" (20,22-dehydro derivative), two pregnane saponins ["3β,26-bisdesmoside **4**-2002" (16ß-acyl-2α,3β-hydroxypregn-5-en-20-one, an ester with 5 carbon atoms apparently derived from the original side chain of cholesterol); "monodesmoside **5**-2002" (2α,3β-dihydroxypregna-5,16-dien-20-one)], two cholestane glycosides ["3β,26-bisdesmoside **6**-2002" (Fig. 7.17); "3β,26-bisdesmoside **7**-2002"] and a unique "pregnane-carboxylic acid γ-lactone glycoside **8**-2002" [2α,16β-dihydroxypregnen-5-en-20-carboxylic acid γ-lactone, i.e., a $\mathbf{C_{22}}$ metabolite (22-*homo*pregnane) (Fig. 7.17) (Mimaki et al. 2002). Another very unusual substitution of the spirostanol skeleton at C-17 could be elucidated in the case of "saponin **4**-2001" and "saponin **7**-2001", respectively. Both share the same aglycone, (25*R*)-spirost-5-en-2α,3β,17α-triol. An isomeric novel steroidal sapogenin, again very unusual with regard to the substitution at C-15, was discovered in case of "saponin **6**-2001": (25*R*)-spirost-5-en-2α,3β,15β-triol. The carbohydrate moieties glycosidically linked to the 3β-position of the corresponding aglycone are represented by the tetrasaccharide glu-(1→2$_{glu}$)-[xyl-(1→3$_{glu}$)]-glu-(1→4$_{gal}$)-gal-(1→3β) for "saponin **3**-2001" and "saponin **4**-2001", respectively. The same sequence increased by a further glucose unit, 1→3-linked to the terminal glucose, thus forming a corresponding pentasaccharide, was determined for "saponin **5**-2001" – "saponin **7**-2001". A more diverging tetrasaccharide, rha-(1→2$_{glu}$)-[rha-(1→4$_{rha}$)-rha-(1→4$_{glu}$)]-glu-(1→3β), was found in case of "saponin **8**-2001" and "saponin **9**-2001", respectively. Five spirostanol glycosides, again without trivial names, four diglycosides [rha-(1→2$_{gal}$)-gal-(1→3β)] as well as one triglycoside [glu-(1→4$_{gal}$)-rha-(1→2$_{gal}$)-gal-(1→3β)], which were distinguished with regard to their aglycones from all other solanaceous saponins by two unique properties, could be identified as constituents of the leaves of *C. sendtenerianum*. (i) All of them were characterized by a hydroxyl group at C-1β forming a curious 1β,2α,3β-trihydroxy substitution (two 5α-spirostanes as well as two spirost-5-enes). Furthermore, (ii) three out of four showed a 25(27)-exomethylene group (Haraguchi et al. 2000).

The monotypic genus *Vestia*, endemic to Chile and closely related to *Cestrum*, is represented by *V. foetida* (Ruiz & Pav.) Hoffm., "palqui negro", "huevil" (Hunziker 2001). Hydrolysis of the saponin mixture isolated from the unripe berries of this shrub (sub nom. *V. lycioides* Willd.) yielded diosgenin (Faini et al. 1980) and (25*R*)-isonuatigenin as well as a mixture of (25*R*)-nuatigenin and (25*S*)-isonuatigenin (Faini et al. 1984; Torres et al. 1988).

Nicotianoideae. ***Nicotianeae clade.*** Four neotigogenin-based monodesmosides, nicotianosides A [glu-(1→3β)], B [rha-(1→2)-glu-(1→3β)], C {[rha-(1→2)]-[rha(1→4)]-glu-(1→3β), and D {[rha-(1→2)]-[rha(1→3)]-[rha(1→4)]-glu-(1→3β)}, were isolated from the seeds of *Nicotiana tabacum* L. In addition, three (25*S*)-5α-furostan-3β,22α,26-triol bisdesmosides corresponding to their spirostane congeners B–D, nicotianosides E (C-26: glucoside; carbohydrate chain at C-3β identical to B), F (to C), and G (to D), could be characterized. (Shvets et al. 1994, 1995b, e, 1996b). Interestingly, nicotianoside C could also be detected in the seeds of *Allium tuberosum* RTL., Alliaceae (Sang et al. 2000).

Solanoideae. ***Hyoscyameae clade.*** Eight glycosides, named atroposides A–H, were characterized as constituents of the seeds of *Atropa belladonna*. Diosgenin (B, D, E, H) and tigogenin (A, C, E, G), respectively, were found to be the aglycones; galactose, glucose, and rhamnose in various ratios formed the oligosaccharide moieties (Shvets et al. 1995a, 1996a).

Certain atroposides turned out to be identical to already known compounds: atroposides A, C, and E to certain petuniosides (see *Petunia* × *hybrida*); furthermore, C also to capsicoside B_2, D to capsicoside B_3 (see *Capsicum annuum*), F to funkioside D (see *Scopolia japonica*). Atroposide E was again detected as one of the constituents of *Solanum lyratum* (stems; Murakami et al. 1981) and *S. dulcamara* (Lee et al. 1994).

Together with the diosgenin-based funkioside D, already known from *Funkia ovata* SPRENG., Liliaceae (Mashchenko et al. 1977), two novel glycosides, scopolosides I and II, were identified in the fresh underground parts of *Scopolia japonica* MAXIM. Their carbohydrate chains, [I: glu-(1→4)-gal-(1→3β)] and [II: glu-(1→2)-glu-(1→4)-gal-(1→3β)] were identical with those of certain petuniosides, the one of congener II also with funkioside D. However, their aglycone turned out to be a novel diosgenin derivative, scopologenin, characterized by two additional hydroxy groups (C-15α, C-23α; Okamura et al. 1992).

Solaneae clade. Steroidal sapogenins/saponins are common constituents within the genus *Solanum* as already mentioned above. There are lots of reports on this topic reviewed, e.g., by Heftmann (1983 and references therein) and Hegnauer (1973, 1990 and references therein). Many examples have been discussed already in detail (Sect. 7.7.1). In addition, a selection of further examples is given in the following paragraphs.

Like in the case of other genera, the seeds of economically useful *Solanum* spp. were studied extensively due to the fact that their constituents may be of special interest with regard to growth-stimulating activities and/or prevention of virus/fungi attacks. Tomatoside B, a neotigogenin-based trisaccharide [glu-(1→2_{glu})-glu-(1→4_{gal})-gal-(1→3β)], and the corresponding furostanol type congener, the 3β,26-bisdesmosidic tomatoside A (glucosyl at C-26), were isolated from the seeds of *Solanum lycopersicum* sub nom. *Lycopersicum esculentum* (Shchelokova et al. 1980). These two metabolites were also found as constituents of *S. lyratum* THUNB. (Murakami et al. 1981). A number of spirostanol type monodesmosides, melongosides A–H (Kintya and Shvets 1984), K (Shvets and Kintya 1984), and L/M

(Kintya and Shvets 1985a), were identified in the seeds of *S. melongena* L. The corresponding aglycones were represented by tigogenin (e.g., A, L) and diosgenin (e.g., M), respectively. Moreover, three bisdesmoside congeners, melongosides N–P, belong to the constituents of the seeds. Their aglycones were identified as (25*R*)-5α-furostan-3β,22α,26-triol (N, P) and (25*R*)-furost-5-en-3β,22α,26-triol (O), respectively (Kintya and Shvets 1985b). The melongosides include di-, tri-, tetra-, and pentasaccharides (linked to C-3β of the aglycones) which consist of diverging ratios of glucose, galactose, and rhamnose as monomeric units; as usual, bisdesmosides show an additional glucosyl residue at C-26. Four yamogenin-based monodesmosides, tuberosides A–D as well as three (25*S*)-5α-furost-5-en-3β,22α,26-triol-based bisdesmosides, tuberosides E–G, corresponding to their spirostane congeners B–D were isolated from the seeds of *Solanum tuberosum* L., e.g., two disaccharides C and F containing an identical carbohydrate moiety [rha-(1→2)-gal-(1→3β)], the bisdesmoside F characterized by a glucosyl residue at C-26 (Kintya and Prasol 1991; Mashchenko et al. 1995).

A very unpleasant, confusing situation is given with regard to the term "tuberoside" combined with different capital letters, because novel steroidal saponins or other cholesterol-derived glycosides isolated from other organisms were named later in exactly the same manner by a number of authors, e.g., in case of *Allium tuberosum* ROTTL. ex SPRENG. (Alliaceae), *Polianthes tuberosa* L. (Agavaceae), *Ullucus tuberosus* CALDAS (Chenopodiaceae) or even Chinese truffles (*Tuber indicum* COOKE & MASSEE).

Besides their presence in seeds steroidal saponins may occur in all other plant organs; examples are given in Table 7.2.

A furostanol saponin yielding – after acidic hydrolysis – a stabilized aglycone, which is unable to rearrange to a corresponding spirostanol due to a methylation of the hydroxyl group at C-22, could also be detected in *Solanum* spp.: Methyl*proto*dioscin A, already known from different species of other families, was detected as a constituent of the flowering aerial parts of *S. rostratum* DUN., buffalo bur nightshade. As indicated by its name this saponin represents a derivative of the 3β,26-bisdesmosidic, i.e., C-26-glucosylated, counterpart of dioscin (diosgenin β-chacotrioside) known from *Dioscorea* spp. (Dioscoreaceae) (Bah et al. 2004).

Capsiceae clade. A 3β,26-bisdesmosidic furostanol glycoside named "capsicoside" was the first saponin discovered in a species of the genus *Capsicum*, *C. annuum* L. (seeds). Its enzymatic partial cleavage led to the corresponding spirostanol glycoside, unfortunately named "capsico**sine**" which – beside the close similarity to capsicoside – suggests wrongly an alkaloid (Tschesche and Gutwinski 1975). Hydrolysis of several capsicosides isolated a decade later from the roots afforded gitogenin (A_1, B_1, C_1), tigogenin (A_2, B_2, C_2), and diosgenin (A_3, B_3, C_3) respectively (Gutsu et al. 1986, 1987a, b). Two further glycosides, named capsicosines D_1 and E_1, again yielded gitogenin (Gutsu and Kintya 1989). Furthermore, five novel congeners were discovered by another group (Yahara et al. 1994) – unfortunately again named capsicosides combined with the same capitals though without numbers. They were isolated from

the seeds (capsicosides A – C) and roots (capsicoside D, *proto*-degalactotigonin) of two varieties of *C. annuum* and could be characterized as bisdesmosidic furostanol glycosides. Degalactotigonin, yielded as the respective spirostanol glycoside together with glucose by enzymatic hydrolysis of its *proto* precursor, had been found originally in *Solanum nigrum* L. (Saijo et al. 1982). On acidic hydrolysis the genuine glycosides of *C. annuum* liberated – beside sugars – gitogenin (capsicosides A–C) and tigogenin (capsicoside D, *proto*-degalactotigonin). In addition, three minor components, capsicosides E–G, could be isolated from the seeds of *C. annuum* var. *acuminatum* FINGERH. (Iorizzi et al. 2002).

The genuine glycosides isolated by Yahara et al. and Iorizzi et al. are represented by a branched tetra- (B, *proto*-degalactotigonin) or pentasaccharide (A, D, E–G; sequence see below) beside a straight-chain trisaccharide (C). All sapogenins were linked to their carbohydrate chain through a galactose unit. The remaining monomers of these eight saponins were formed by glucose save one xylose unit each in case of D and *proto*-degalactotigonin. The genuine furostanol glycoside capsicoside A was established to be 26-*O*-β-D-glucopyranosyl (25*R*)-5α-furostan-2α,3β,22ζ-triol3-*O*-ß-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1→2)-[β-D-glucopyranosyl(1→3]-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside. It turned out to be identical to "capsicoside" TSCHESCHE with the consequence of a structure revision in favour of Yahara's version. Sharing the carbohydrate chain with congener A the minor metabolites form a 5α-furost-25(27)-en-2α,3β,22ζ, 26-tetraol-based bisdesmoside (capsicoside E), a (25*R*)-5α-furost-20(22)-en-2α, 3β,26-triol (F), and a (25*R*)-5α-furostan-3β,22ζ,26-triol congener (G); the latter compound corresponds to 2-deoxycapsicoside A. In contrast, the carbohydrate chains of the capsicosides/capsicosines isolated by Gutsu et al. lacked this dominance of glucose units, e.g., xyl-(1→3_{glu})-glu-(1→4_{gal})-gal-(1→3β) or [gal-(1→2_{glu}]-xyl-(1→3_{glu})-glu-(1→4_{gal})-gal-(1→3β).

Recently, another *Capsicum* saponin, CAY-1, a gitogenin-based branched pentaoside {[glu-(1→3_{glu})-glu-(1→3_{glu})]-glu-(1→2_{glu})-glu-(1→4_{gal})-gal-(1→3β)} has been isolated from commercially available dry fruits of C. *frutescens* L., Cayenne pepper/chilli (De Lucca et al. 2002; Renault et al. 2003).

Neogitogenin (aerial parts) and gitogenin (roots) were isolated from *Lycianthes biflora* (LOUR.) BITT. (Ripperger 1990; Ripperger and Porzel 1992). Three novel furostanol bisdesmosides, lycianthosides A–C, were discovered as constituents of the leaves of a traditional Mesoamerican edible plant, *Lycianthes synanthera* (SENDTN.) BITT. The common oligoside linked to C-3β of the aglycones was formed by β-chacotriose, well-known from many *Solanum* glycosides, e.g., steroidal saponins from *S. aculeatissimum* JACQ., steroidal alkaloids like solamargine or chaconine (Sect. 7.8). Structure elucidation of the aglycone of lycianthoside B, furost-5-en-3β,17α,22α,25,26-pentol, revealed a high degree of oxygenated substitutions. Its congener C was found to be the 17-deoxy derivative of B, i.e., the corresponding tetraol. Finally, lycianthoside A corresponded to 22α-methoxy-furost-5-en-3β,17α,26-triol. It could be proved that the latter metabolite was a genuine one, in spite of the fact that the isolation procedure included the use of methanol (Piccinelli et al. 2005).

7.7.3 Biosynthesis

7.7.3.1 Sapogenins (Aglycones)

Most of the early work on the biosynthesis of sapogenins was done on species from the Dioscoreaceae. It was argued that "…. there is no reason to assume that a different process operates in Solanaceae." (Heftmann 1983 and references therein). In 1965 it could be demonstrated that cholesterol is a precursor of diosgenin. (25*R*)-26-Hydroxycholesterol was also converted to this sapogenin but not to its 25*S*-epimer yamogenin. Dependent on the introduction of the hydroxyl group at either C-26 (25*R*) or C-27 (25*S*) of cholesterol the result turned out to be diosgenin or yamogenin. The pathway to tigogenin was proposed to proceed via 5α-cholestan-3-one and 5α-cholestan-3β-ol. A metabolite of the latter, (25*S*)-5α-cholestan-3β,27-diol turned out to be the key intermediate in the biosynthesis of neotigogenin in *S. lycopersicum*. The following intermediates are characterized by further, stepwise oxidation at C-16 and C-22, respectively, followed by cyclization to the spiroketal system with its rings E and F (Heftmann and Weaver 1974 and references therein; Heftmann 1983 and references therein).

7.7.3.2 Mono- and Bisdesmosides

"It is generally assumed that steroidal saponins are accumulated and stored in vacuoles of plant cells mainly in the form of bisdesmosidic, furostane-type glycosides and they are converted to their spirostane-type counterparts only after damage of plant tissue" (Kalinowska et al. 2005). This is the reason why bisdesmosides are often named with the prefix "proto" in conjugation with the term for their corresponding monodesmoside, e.g., protodioscin. Recently, Arthan et al. (2006) have purified a furostanol glycoside 26-*O*-β-glucosidase to homogeneity – from the leaves of *Solanum torvum* – which was highly specific for cleavage of the glucose unit attached to the C-26 hydroxyl of torvoside A and its 3-dehydro congener torvoside H (Arthan et al. 2002) yielding the corresponding 26-degluco-torvosides (for a comparable reaction see Fig. 7.12).

This is an example for another kind of names for bisdesmosides vs corresponding monodesmosides. The confusion is enlarged by a third alternative, the integration of both structural types within the same principal name for a group, e.g., indiosides A–E (Yahara et al. 1996b).

Though the role of furostanol bisdesmosides as "protosaponins" is generally accepted, it is still discussed controversially, whether furostanol bisdesmosides are biogenetic precursors of spirostanol monodesmosides or vice versa. Thus, *one hypothesis* claims a biogenetic sequence cholesterol → stepwise oxidation at C-26 (or C-27), C-16, and C-22 → *subsequent cyclization forming rings E and F*, i.e., spirostanol aglycone → spirostanol monodesmoside → opening of ring F → subsequent glucosylation of the resulting C-26 hydroxyl group → furostanol

bisdesmoside → storage. Glycosylation of complete spirostanol aglycones, assumed in this hypothesis, might be supported experimentally by the results obtained with a number of more or less specific UDP-glucose-dependent glucosyltransferases acting on the C-3 hydroxyl group of spirostanols in certain species of other plant families (Kalinowska et al. 2005). However, it has to be taken into account that this step would represent only the initial one, because the carbohydrate chain usually comprises 2–5 sugar units. Two similar though separate enzymes of these glucosyltransferases were found to be present in almost all organs of *S. melongena*, eggplant/aubergine. One enzyme catalyzed the transfer of activated glucose to diosgenin, the other one to its *N*-containing congener solasodine (Paczkowski et al. 1998). Recently, a first glucosyltransferase involved in steroidal saponin biosynthesis, obtained from *S. aculeatissimum*, could be cloned. This enzyme, SaGT4A, did not show a strict substrate specificity; it catalyzed the 3-*O*-glucosylation of diosgenin, nuatigenin, and tigogenin as well as of steroidal alkaloids like solanidine, solasodine, and tomatidine. Anyhow, it turned out to be involved in plant defence system, since it was accumulated intensively as a wounding response (Kohara et al. 2005). Furthermore, it was found that in intact plant cells an opening of ring F of a spirostanol aglycone with subsequent glucosylation of the hydroxyl group at C-26 is possible. However, if one takes into account the idea of the most parsimonious possibility this would be in favour of the *alternative hypothesis*, i.e., the biogenetic sequence cholesterol → stepwise oxidation at C-26 (or C-27), C-16, and C-22 → *subsequent cyclization to ring E only* → furostanol 26*O*-glucoside → furostanol-3β,26-bisdesmoside → storage. Only after damage of plant tissue subsequent de-glucosylation (C-26*O*) would lead to a spontaneous cyclization forming ring F, i.e., the spirostanol monodesmoside according to Fig. 7.12.

Finally, a *third hypothesis* was proposed which required a 3β,26-bisdesmoside before any cyclization to rings E and F could have happened, i.e., a bisdesmoside including an aglycone which is characterized by an open-chained cholest-5-en-3β,16,22-triol or a corresponding 5αH congener. In fact, such a metabolite, anguivioside A (Fig. 7.18), could be discovered in the fruits of *S. anguivi* Lam. (Zhu et al. 2001d) in co-occurrence of spirostanol type congeners, e.g., anguivioside III (Table 7.2; Honbu et al. 2004); that may be interpreted as a support of the third hypothetical pathway. This is also true for abutilosides D and F, both 3β,**16**α,26-trihydroxy-5α-cholestan-22-one 3β,26-bisdesmosides and their 5,6-dehydro congeners, abutilosides E and G, discovered as constituents of *S. abutiloides* (Griseb.) Bitt. & Lillo (Yoshimitsu et al. 2000) in co-occurrence of (i) abutiloside C, the still open-chained 3β-monodesmoside corresponding to congener D (Tian et al. 1996) and (ii) protodioscin as well as solamargine (Tian et al. 1997). Thus, certain abutilosides were discussed as important key intermediates in the biogenesis of steroidal saponins (D–G) and steroidal alkaloids (H – K) (Yoshimitsu et al. 2003).

These findings show that glycosidation at both ends of the aglycone can happen already at an early stage of the pathway, i.e., before any additional ring closure (E, F) takes place. However, whether only intermediates with 16β-hydroxy substituents (e.g., anguivioside A) are predestined for cyclization (E) or also their analogues with

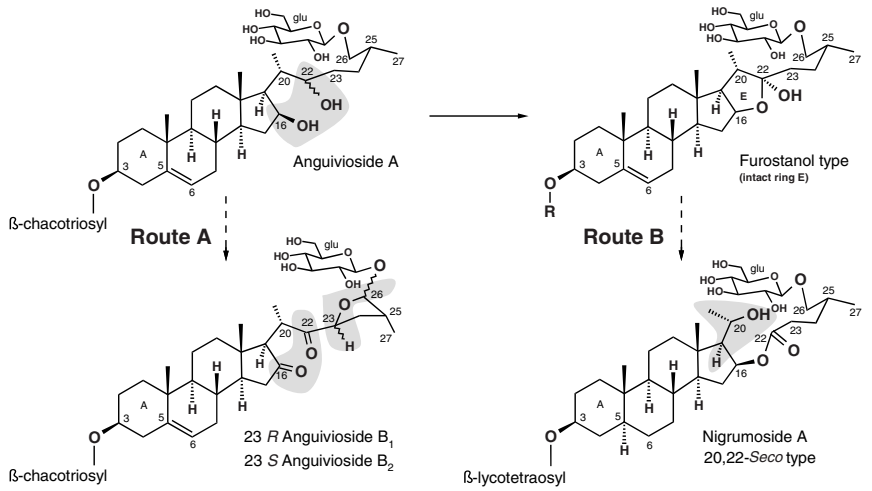


Fig. 7.18 Alternative hypotheses for the biosynthesis of steroidal saponins based on the isolation of unusual (i.e., lacking ring E) metabolites: (i) anguiviosides A (cholest-5-en-16,22-diol type glycoside), B_1, and B_2 (cholest-5-en-16,22-dione type glycosides = "16,22-dicarbonyl type") from the fruits of *Solanum anguivi* (route A), (ii) nigrumoside A ("20,22-seco type") from the aerial parts of *S. nigrum* (route B); unusual partial structures *highlighted in grey* (Zhu et al. 2001d). See also Fig. 7.16

16α-hydroxy groups (e.g., abutilosides D–G), remains to be established. Configurative rearrangement at C-16 (α-OH → β-OH) – presumably precondition for cyclization with a C-22 carbonyl – might occur via the corresponding 16-dehydro derivative (carbonyl function), since the occurrence of 16,22-diones is proven, e.g., anguiviosides B_1 and B_2 (Fig. 7.18). Steroidal aglycones with 16α-substitution are known at least within the steroidal alkamines, e.g., etioline, 22-isoteinemine; both are assumed to be intermediates in the biosynthesis of tomatidenol (16β*O*-configurated !) as well as solanidine (16β*N*-configurated !) (see Fig. 7.23). It seems to be remarkable in this connection that the *N*-containing abutilosides A, B, I, J, and K are also characterized by 16α-configurated hydroxyl groups, whereas their congener O shows 16β-OH configuration (Fig. 7.21). An alternative possibility for this cyclization could happen vice versa, i.e., by reaction of a C-16 carbonyl and a C-22 hydroxyl group.

7.7.4 Significance

7.7.4.1 Toxicology

Since saponins in general are absorbed by the intestines of mammalians to only a small extent, they are normally not very poisonous if taken perorally. Therefore, many crude saponin drugs or corresponding extracts – especially those which contain triterpenoid saponins – are used therapeutically without obvious problems. In

contrast, saponins show high toxicity when applied parenterally. This is especially due to the ***haemolytic property*** of saponins which may be very strong in case of steroidal monodesmosides. In contrast, their bisdesmosidic analogues show only weak or even no activity. Apparently, this second glycosidation at the opposite part of the molecule leads to an almost complete loss of the typical properties of saponins. Moreover, active steroidal saponins cause rapid haemolysis, whereas triterpenoid saponins give a slower effect (Hänsel and Sticher 2007). In any case, at least a disaccharidic carbohydrate chain is necessary for the typical properties of (neutral) saponins (Teuscher et al. 2004). Haemolysis is caused by a decrease of the surface tension between the aqueous phase and the lipid phase of the membrane of the erythrocytes. The lipids are emulsified and lifted out of the membrane. This allows the entry of Na^+ ions and water molecules into the cell and on the other hand the leakage of K^+ ions with the final consequence of membrane bursting and the leakage of haemoglobin into the plasma (Hänsel and Sticher 2007).

The membranolytic activity of saponins can cause a damage of intestinal mucosal cells by altering cell membrane permeability and interfere with active transport. One consequence might be that the uptake of antigens is increased (Piccinelli et al. 2005 and references therein).

Saponin-containing plants are fish poisons and therefore used in certain developing countries for fishing. Fishes die due to hydraemia caused by a pathologic increase of the permeability of gill epithelia (Hänsel and Sticher 2007). *S. panduraeforme* E. MEY is used in Mozambique as a fishing poison, fruits and leaf juice of *S. rugosum* DUN. for the same purpose in Brazil (Neuwinger 1996).

7.7.4.2 Pharmacology

Antiviral Activity against Human Pathogens. Spirostanol glycosides rather than congeners with furostane, solasodane, and ergostane frameworks turned out to be remarkably active against herpes simplex virus type 1 (HSV-1). The composition of the oligosaccharide side chain was crucial (Ikeda et al. 2000).

Antifungal Activity against Human Pathogens. CAY-1 was shown to be a potent fungicide for the germinating conidia of human pathogenic *Aspergillus* spp., e.g., *A. flavus* LINK, *A. fumigatus* FRESEN., *A. niger* TIEGH, with IC_{90} values between 3 and 20 µM and furthermore for potential human pathogenic yeasts (immunocompromised humans), e.g., *Candida albicans* (C.H. ROBIN) BERKHOUT (IC_{90}: 6.2 µM). This saponin may become a candidate for an effective therapy against invasive fungal infections since these concentrations are below the threshold for mammalian cell cytotoxicity (De Lucca et al. 2002). Its mechanism of action – to cause pores in fungal membranes with the consequence of lysis of the fungal cells – is different from those of clinically established drugs like amphotericin B and itraconazole. An in vitro study on potential synergism of CAY-1 with these drugs revealed an additive-synergistic interaction against non-germinated and germinating conidia of *A. fumigatus* and *A. niger*. Furthermore, an excellent synergy between CAY-1 and

amphotericin B could be observed (De Lucca et al. 2006). However, it has not yet been determined whether CAY-1 prevents diseases in *Capsicum* plants themselves (Duke et al. 2003). An interesting example for the development of evidence-based remedies from traditional herbal medicine has been reported in case of *Solanum chrysotrichum,* a medicinal plant used in many rural communities by the indigenous Mayan people in the highlands of Chiapas/Mexico for the treatment of resistant skin mycosis. A number of spirostane saponins (SC-1 to SC-6) could be characterized (Zamilpa et al. 2002). A comparison of two series of monodesmosides with a rare C-6α linkage of their carbohydrate moiety instead of C-3β allowed interesting structure-activity relationships: Certain 25*R*-spirostanol-based saponins, constituents of *S. chrysotrichum*, and their 25*S* configurated epimers, constituents of *S. hispidum*, revealed the relevance of the specific structure of the carbohydrate moiety for activity rather than the configuration at C-25. Thus, in vitro metabolites with the motif xyl-(1→3)-qui were ten-fold more active against, e.g., *Trichophyton mentagrophytes* than their congeners involving rha-(1→3)-qui (Gonzalez et al. 2004). Saponin SC-2 (Table 7.2) turned out to be the most active congener. A double blind and randomized clinical trial performed to evaluate the potency of a shampoo containing a standardized extract from the leaves of *S. chrysotrichum* has achieved high clinical and mycological effectiveness combined with absence of severe adverse effects in the treatment of Pityriasis capitis, dandruff, associated with the yeasts *Malassezia furfur* (C.H. Robin) Baill. and/or *M. globosa* (Herrera-Arellano et al. 2004). This trial has been controlled with a shampoo containing the established synthetic antifungal agent ketoconazole (2%). No significant difference between both patient groups could be observed. Similar positive results were obtained in the treatment of Tinea pedis, athlete's foot.

Cytotoxic/Antitumor Activity. A comprehensive study on cytotoxic activities of 20 steroidal saponins from *Solanum* spp. against various cell lines with the aim to elucidate structure/activity relationships revealed that glycosides with a spirostanol aglycone and chacotriose as the carbohydrate moiety showed the best activity. Furthermore, the presence of a terminal rhamnose turned out to be crucial (Nakamura et al. 1996). Three constituents of the leaves of *Cestrum nocturnum*, "saponin **5**-2001", "saponin **7**-2001", and "furostanol 3β,26-bisdesmoside **2**-2002", exhibited strong in vitro cytotoxic activity against human oral squamous cell carcinoma (HSC-2) cells equivalent to a clinically important drug, doxorubicin. A number of further saponins from the same species were inactive (Mimaki et al. 2002).The chacotrioside dioscin tested in 10 cancer cell lines implanted at the intraperitoneal and subcutaneous compartments of athymic mice (in vivo hollow fiber model) turned out to be a remarkably active compound, indicating its potential to function as cancer chemotherapeutic agent (Mi et al. 2002). Recently, it has been demonstrated that dioscin as well as its congeners diosgenin 3-*O*-β-solatrioside and protodioscin exhibited stronger antiproliferative activity in vitro against human promyelocytic leukaemia (HL-60) cells than cisplatin, an important synthetic drug therapeutically used against different cancer diseases (Ono et al. 2006a).

In contrast, only a moderate cytotoxic activity in vitro against a number of tumor cell lines was found for its aglycone, diosgenin, as well as for the corresponding rhamnosyl-(1→2)-glucuronic acid derivative (Sun et al. 2006). Degalactotigonin, a tigogenin-based, i.e., spirostanol saponin isolated from *S. nigrum* and checked using different human solid tumor cell lines, e.g., isolated from colon, prostrate, breast (Hu et al. 1999) and liver carcinoma, glioma (Zhou et al. 2006), respectively, showed considerable in vitro cytotoxicity in a range of 0.25–4.5 μM, whereas solanigrosides C–H, congeners with single minor substitutions (15α-OH, 17α-OH, 23α-OH, and =O at C-26, respectively) turned out to be inactive (Zhou et al. 2006). No cytotoxicity was observed for the 6α,26-bisdesmoside torvoside H and the corresponding spirostanol yielded by enzymatic hydrolysis of the genuine glycoside (Arthan et al. 2002). All these examples demonstrate that a pronounced cytotoxic activity depends on certain structural characters of specific saponins with regard to both parts of the molecules, aglycone and carbohydrate moieties.

Steroidal **sapogenins**, like diosgenin, increase biliary cholesterol secretion by direct effects on the intrahepatocytic regularity mechanism (Neuwinger 1996 and references therein).

7.7.4.3 Biological Pest Control and Ecology

Antiviral Activity against Plant Pathogens. "Tomatonin", an undetermined tomato saponin, as well as capsicoside/capsicosine (sensu Tschesche and Gutwinski 1975) showed antiviral activities in vitro at concentrations of 0.005–0.008% on a model of tobacco mosaic virus (TMV), "tomatonin", tomatoside, and capsicoside even in vivo at a concentration of 0.05% (Balashova et al. 1984). Structural requirements for this activity were a spirostanol type aglycone linked to an oligosaccharide involving 4–6 monomers (Choban et al. 1987). Petunioside treatment contributed to the enhancement of viral resistance of tobacco and cucumber plants (Shvets et al. 1995c). Prevention of virus attacks on tobacco by nicotianosides was also observed (Shvets et al. 1996b).

Antifungal Activity against Plant Pathogens. In an early study tigogenin-based as well as yamogenin-based saponins extracted from *Solanum dulcamara* showed remarkable inhibitory effects toward a few predominantly plant pathogenic fungi, e.g., *Piricularia oryzae* Cav., *Alternaria solani* (Ell. & Mart.) Jones & Grout (Wolters 1968). Cucumber seeds treated with Moldstim, a commercial product based on the total extract of furostane type glycosides from *Capsicum annuum* seeds, sown in presence of a number of soil-borne pathogenic fungi, resulted in healthy plantlets with biomass accumulation and development rhythm of vegetative organs superior to untreated check. This was also true for Ecostim, another commercial product based on glycosides of *Solanum lycopersicum* seeds (Chintea et al. 1998). In another study a number of steroidal glycosides turned out to be exogenous inducers of wheat resistance to fungal metabolites of the pathogenic fungus *Fusarium oxysporum* Schltdl.: Moldstim; Ecostim; nicotianosides E and F; melongoside O; atroposide N as well as the total glycosides from *Atropa belladonna*

seeds (Lupashku et al. 2004). A study on structure-activity relationships with regard to antifungal properties revealed that the motif "genin-gal-glu-glu-rha" mediated the fungicidal activity of spirostanols whereas a shorter carbohydrate moiety led to a loss of activity; furostanol glycosides were also inactive (Dimoglo et al. 1985).

Growth Regulation/Influence on Plant Resistance. Growth stimulating activities were established for the nicotianosides (Shvets et al. 1996b). Tomato seed treatment with melongoside P and nicotianoside E turned out to enhance plant resistance against diseases like macrosporiosis, bacterial black spot, and wilt. These compounds also increased yield capacity and improved fruit quality (Shvets et al. 1996c). The furostanol type tuberosides (potato seeds) increased tomato hybrid seed output, seed-producing capacity, and germination energy of vegetable and crop seeds (Mashchenko et al. 1995). Capsicosine/capsicoside were shown to exert stimulating effects on sowing qualities of spring wheat, *Triticum aestivum* L. (Poaceae), i.e., on biomass growth, development of generative organs, and total raise grain weight primarily due to an increase in grain number (Volynets et al. 2002). Delayed effect of seed pre-sowing treatment with capsicosin(e) on germination and seedling initial growth of a certain variety could be reported, whereas capsicoside had no effect (Goncharik et al. 2004). The effectiveness of combining seed treatments with application of Moldstim (see above), to vegetating plants during budding and flowering resulted in the increase and improvement of fruit quality of greenhouse tomatoes (Matevosyan et al. 2001).

Deterrent Activity. Three saponins from *Solanum laxum*, luciamin (Table 7.2) as well as its 23*S*-hydroxy congeners laxumins A (LC_{50}: 4.3 µM) and B (LC_{50}: 6.1 µM), were shown to be toxic to and/or to exhibit deterrent activity against the worst cereal aphid pest, *Schizaphis graminum* RONDANI (greenbug), Homoptera: Aphididae (Ferreira et al. 1996; Soulé et al. 1999, 2000). The difference to potato glycoalkaloids (solanine/chaconine: 137–138 µM) with regard to this specific biological activity is remarkable (see also Sect. 7.8.4).

7.8 Steroidal Alkaloids/Glycoalkaloids (C_{27} Isoprenoids)

Authorities. There will be no addition of the corresponding authorities to the species epithets in the text of Sect. 7.8, since they are added to the species names in the list of Tables 7.3 and 7.4.

Outline. Glycoalkaloids represent the glycosidic form of certain steroidal alkamines (basic aglycones). Some authors integrate glycoalkaloids into steroidal saponins ["basic saponins" in comparison to "neutral saponins" = common saponins (Sect. 7.7)] In fact, they share *some* basal physicochemical and biological properties with their *N*-free congeners. However, this integration would separate glycoalkaloids from steroidal alkamines which would be disadvantageous in some respects, e.g., since most of the latter are only known as such, i.e., unknown as

Table 7.3 Steroidal alkaloids (alkamines) alphabetically listed within the different structural types: For 41 out of altogether 115 compounds from *Solanum* spp. (113 compounds), *Lycianthes biflora*, and *Saracha punctata* (one compound each) at least one corresponding glycoalkaloid was found and structurally elucidated, i.e., for 36% of the alkamines also corresponding glycosides are known

Alkamines (terms used in references)	**Structural remarks** [Fig…..(deriv.): Cross-reference for basal structure]	**Discovery in (glycoalkaloid[a])**	**Ref.**
Spirosolanes			
3-Hydroxy-spirosolanes	One basic centre		
Esculeogenin A	(22α*N*,25*S*)-5α-spirosolan-3β,23α,27-triol [= (23*S*)-23,27-dihydroxy-soladulcidine] Fig. 7.24 (deriv.)	*Solanum lycopersicum* L. sub nom. *Lycopersicum esculentum* MILL. var. *cerasiforme* (DUN.) ALEF. (esculeoside A[b])	(1)
(23*S*,24*R*)-23,24-Dihydroxy-soladulcidine	Fig. 7.24 (deriv.)	*S. lycopersicum* L. sub nom. *L. esculentum* MILL. (lycoperoside G[c])	(2)
Isoesculeogenin A	(22β*N*,25*S*)-5α-spirosolan-3β,23α,27-triol [= (23*R*)-23,27-dihydroxy-tomatidine] Fig. 7.24 (deriv.)	*S. lycopersicum* L. sub nom. *L. esculentum* MILL. (lycoperoside F[b])	(2)
(23*S*)-23-Acetoxysoladulcidine	Fig. 7.19 (deriv.)	*S. lycopersicum* L. sub nom. *L. esculentum* MILL. (lycoperoside B)	(3)
25-Acetoxysolasodine	Fig. 7.19 (deriv.)	*S. robustum* WENDL. (25-acetoxyrobustin)	(3)
(23*S*,25*S*)-23-Acetoxy-5α,22α*N*-spirosolan-3β-ol	Fig. 7.19 (deriv.)	*S. lycopersicum* L. sub nom. *L. esculentum* MILL. (lycoperoside C)	(3)
(23*R*)-23-Acetoxytomatidine	Fig. 7.19 (deriv.)	*S. lycopersicum* L. sub nom. *L. esculentum* MILL. (lycoperoside A)	(3)
"23-*O*-Acetyl-12β-hydroxysolasodine"	20-Epimer of 23ζ-acetoxy-12β-hydroxy-solasodine[d]; Fig. 7.9 (deriv.)	*S. nigrum* L.	(3)
O-Acetylsolasodine	Fig. 7.19 (deriv.)	*S. umbelliferum* ESCHS.	(3)
22,25-Diepisolasodine	Fig. 7.19 (deriv.)	*S. sycophanta* DUN. (22, 25-diepisycophantine)	(4)
12β,27-Dihydroxysolasodine	Fig. 7.19 (deriv.)	*S. nigrum* L. (ß-chacotrioside)	(3)
2α-Hydroxysoladulcidine	Fig. 7.19 (deriv.)	*Lycianthes biflora* (LOUR.) BITT.	(3)
15α-Hydroxysoladulcidine	Fig. 7.19 (deriv.)	*S. dulcamara* L.	(5)
15β-Hydroxysoladulcidine	Fig. 7.19 (deriv.)	*S. dulcamara* L.	(5)
(23*R*)-23-Hydroxysoladulcidine	Fig. 7.19 (deriv.)	*S. panduraeforme* DRÈGE	(3)

(continued)

Table 7.3 Steroidal alkaloids (alkamines) alphabetically listed within the different structural types: For 41 out of altogether 115 compounds from *Solanum* spp. (113 compounds), *Lycianthes biflora*, and *Saracha punctata* (one compound each) at least one corresponding glycoalkaloid was found and structurally elucidated, i.e., for 36% of the alkamines also corresponding glycosides are known (continued)

Alkamines (terms used in references)	**Structural remarks** [Fig…..(deriv.): Cross-reference for basal structure]	**Discovery in (glycoalkaloid[a])**	**Ref.**
15α-Hydroxysolasodine	Fig. 7.19 (deriv.)	*S. dulcamara* L.	(5)
15β-Hydroxysolasodine	Fig. 7.19 (deriv.)	*S. dulcamara* L.	(5)
21-Hydroxysolasodine	Fig. 7.19 (deriv.)	*S. sycophanta* Dun. (21-hydroxysycophantine)	(4)
N-Hydroxysolasodine	Fig. 7.19 (deriv.)	*S. robustum* Wendl. (*N*-hydroxy-robustine)	(3)
(25*R*)-3β,12β-Dihydroxy-22α*N*-spirosol-5-en-27-oic acid	Oxidation product (C-27: $CH_2OH \rightarrow COOH$) of 12β,27-dihydroxysolasodine	*S. nigrum* L.	(3)
15α-Hydroxytomatid-5-en-3β-ol	Fig. 7.19 (deriv.)	*S. dulcamara* L.	(5)
15α-Hydroxytomatidine	Fig. 7.19 (deriv.)	*S. dulcamara* L.	(5)
N-Methylsolasodine	Fig. 7.19 (deriv.)	*S. nigrum* L.	(3)
N-Nitrosotomatidine	Fig. 7.19 (deriv.)	*S. lycopersicum* L. sub nom. *L. esculentum* Mill.	(3)
15-Oxosoladulcidine	Fig. 7.19 (deriv.)	*S. dulcamara* L.	(5)
Soladulcidine	(25*R*)-5α,22α*N*-Spirosolan-3β-ol Fig. 7.19	*S. dulcamara*[e] L. (soladulcine A and B)	(3); (6)
Solanaviol	12β-Hydroxysolasodine Fig. 7.19 (deriv.)	*S. aviculare* Forst. (β-solatrioside)	(3); (7)
Solaparnaine	27-Hydroxysolasodine Fig. 7.19 (deriv.)	*S. asperum* Vahl	(3)
Solasodine	(25*R*)-22α*N*-Spirosol-5-en-3β-ol, Fig. 7.19	*S. sodomaeum*[f] L. (solasonine)	(6); (8)
Solaverol A	(23*S*)-23-Hydroxysolasodine Fig. 7.19 (deriv.)	*S. verbascifolium* L. (solaverin I / II)	(3)
Solaverol B	(23*S*)-23,27-Dihydroxysolasodine Fig. 7.19 (deriv.)	*S. verbascifolium* L. (solaverin III)	(3)
"(3β,5α,22α,25*R*)-Spirosolan"	(5α,22α,25*R*)-Spirosolan-3β-ol	*S. lyratum* Thunb.	(9)
Tomatidenol	Tomatid-5-en-3β-ol	*S. tuberosum* L., *S. dulcamara*[g] L. (α- / β-solamarine; soladulcamarine)	(5); (6)
Tomatidine	5α-Tomatidan-3β-ol Fig. 7.19	*S. lycopersicum* L. sub nom. *L. esculentum* Mill. (tomatine)	(6); (8)

(continued)

Table 7.3 Steroidal alkaloids (alkamines) alphabetically listed within the different structural types: For 41 out of altogether 115 compounds from *Solanum* spp. (113 compounds), *Lycianthes biflora*, and *Saracha punctata* (one compound each) at least one corresponding glycoalkaloid was found and structurally elucidated, i.e., for 36% of the alkamines also corresponding glycosides are known (continued)

Alkamines (terms used in references)	**Structural remarks** [Fig…..(deriv.): Cross-reference for basal structure]	**Discovery in (glycoalkaloid[a])**	**Ref.**
3-Oxo-spirosolanes (spirosolan-3-ones)	One basic centre		
Solasodenone	Solasod-4-en-3-one [(25*R*)-22α*N*-spirosol-4-en-3-one]; Fig. 7.19 (deriv.)	*S. hainanense* Hance	(7)
(25*R*)-5β,22α*N*-Spirosolan-3-one	5β-Isomer of solasodan-3-one, Fig. 7.19 (deriv.)	*S. aviculare* Forst.	(3)
(25*S*)-5α,22β*N*-Spirosolan-3-one	5α-Tomatidan-3-one, Fig. 7.19 (deriv.)	*S. lycopersicum* L. × *S. hirsutum* Dun. [syn.: *L. esculentum* Mill. × *L. hirsutum* (Dun.) Macbride]	(3)
3-Amino-spirosolanes	Two basic centres		
Soladunalinidine	3-Deoxy-3β-amino-tomatidine, Fig. 7.19 (deriv.)	*S. dunalianum* Gaudich.	(7)
(22*S*,25*S*)-5α-Spirosolan-3α-amine	22β*N* Fig. 7.20 (deriv.)	*S. arboreum* Humb. & Bonpl. ex Dun.	(10)
(22*S*,25*S*)-Spirosol-5-en-3β-amine	22β*N* Fig. 7.20 (deriv.)	*S. arboreum* Humb. & Bonpl. ex Dun.	(10)
(25*R*)-5α,22α*N*-Spirosolan-3β-amine	3-Deoxy-3β-amino-soladulcidine Fig. 7.20	*S. triste* Jacq.	(3)
(25*R*)-22α*N*-Spirosol-5-en-3β-amine	3-Deoxy-3β-amino-solasodine, Fig. 7.20	*S. triste* Jacq.	(3)
(25*S*)-22β*N*-Spirosol-5-en-3α-amine	Fig. 7.20 (deriv.)	*S. triste* Jacq.	(3)
(25*R*)-22α*N*-Spirosol-5-en-3α-amine	Fig. 7. 20 (deriv.)	*S. triste* Jacq.	(3)
Solanidanes	Indolizidine moiety		
3-Hydroxy-solanidanes	One basic centre		
O(23)-Acetylleptinidine	See Leptinidine	*S. chacoense* Bitt. (leptines I / II)	(5)
Demissidine	Fig. 7.19	*S. demissum* Lindl. (demissine)	(6); (8)
5α,6-Dihydroleptinidine	23β-Hydroxydemissidine Fig. 7.19 (deriv.)	*S. lyratum* Thunb. (β-lycotrioside/-tetraoside)	(3)
Leptinidine	23β-Hydroxysolanidine Fig. 7.19 (deriv.)	*S. chacoense* Bitt. (leptinines I / II)	(5)
"23,24-(2-methyl-tetrahydrofuran)-solanidine"	C-23/C-24: Part of a 2-methyltetrahydrofurane moiety (anellation)	*S. cornifolium* Humb. & Bonpl. ex Dun.	(11)

(continued)

Table 7.3 Steroidal alkaloids (alkamines) alphabetically listed within the different structural types: For 41 out of altogether 115 compounds from *Solanum* spp. (113 compounds), *Lycianthes biflora*, and *Saracha punctata* (one compound each) at least one corresponding glycoalkaloid was found and structurally elucidated, i.e., for 36% of the alkamines also corresponding glycosides are known (continued)

Alkamines (terms used in references)	**Structural remarks** [Fig…..(deriv.): Cross-reference for basal structure]	**Discovery in (glycoalkaloid[a])**	**Ref.**
5β-Solanidan-3α-ol	Fig. 7.19 (deriv.)	*S. tuberosum* L.	(5)
(22*R*,25*R*)-Solanid-5-en-3β-ol	22,25-Diepisolanidine	*S. vernei* Bitt. & Wittm.	(3)
Solanidine	Fig. 7.19	*S. tuberosum*[h] L. (solanine)	(6); (8)
3-Amino-solanidanes	Two basic centres; amino group at C-3 acylated in certain metabolites[i]		
Isosolanogantamine	3α Isomer of solanogantamine	*S. giganteum* Jacq.	(3); (7)
Solanogantamine (syn.: solanopubamine)	3β-Amino analogue of 5α,6-dihydroleptinidine, Fig. 7.19 (deriv.)	*S. giganteum* Jacq., *S. pubescens* Willd.	(3); (7)
Solanogantine	22β*H*,25α*H*-Isomer of solanogantamine	*S. giganteum* Jacq.	(7)
Solanopubamide A	3(*N*)-Formyl derivative of solanogantamine	*S. pubescens* Willd.	(3)
Solanopubamide B	3(*N*)-Acetyl derivative of solanogantamine	*S. pubescens* Willd.	(3)
22,26-Epiminocholestanes (solacongestidine type)			
3-Hydroxy-22,26-epiminocholestanes	One basic centre		
16-*O*-Acetyletioline	Fig. 7.19 (deriv.)	*S. havanense* Jacq. (havanine)	(3)
20,25-Bisisoetioline	Fig. 7.19 (deriv.)	*S. canense* Rydb., *S. fraxinifolium* Dun.	(3)
Capsimine	(22*R*,25 *R*)-22,26-Epiminocholest-5-en-3β,16α-diol	*S. capsicastrum* Link (β-D-glucoside)	(3)
Deacetoxysolaphyllidine	See solaphyllidine	*S. hypomalacophyllum* Bitt. ex Pittier	(3); (7)
Deacetylsolaphyllidine	See solaphyllidine	*S. ecuadorense* Bitt.	(7)
Etioline	Isomer of 25-isoetioline Fig. 7.19 (deriv.)	*S. havanense*[j] Jacq. (etiolinine)	(3); (7)
25-Isoetioline	Fig. 7.19	*S. canense* Rydb.	(3)
20-Isosolafloridine	Fig. 7.19 (deriv.)	*S. abutiloides* (Griseb.) Bitt. & Lillo	(3)
25-Isosolafloridine	Fig. 7.19 (deriv.)	*S. callium* C.T.White ex R.J.Henderson	(7)
22-Isoteinemine (syn.: schlechtendamine)	25-Isomer of capsimine, i.e. (22*R*,25*S*)	*S. capsicastrum*[j] Link (capsicastrine), *S. schlechtendalianum* Walp.	(3); (12)

(continued)

Table 7.3 Steroidal alkaloids (alkamines) alphabetically listed within the different structural types: For 41 out of altogether 115 compounds from *Solanum* spp. (113 compounds), *Lycianthes biflora*, and *Saracha punctata* (one compound each) at least one corresponding glycoalkaloid was found and structurally elucidated, i.e., for 36% of the alkamines also corresponding glycosides are known (continued)

Alkamines (terms used in references)	**Structural remarks** [Fig…..(deriv.): Cross-reference for basal structure]	**Discovery in (glycoalkaloid[a])**	**Ref.**
23-Oxosolacongestidine	Fig. 7.19 (deriv.)	*S. congestiflorum* Dun.	(7)
Solacongestidine	Fig. 7.19	*S. congestiflorum* Dun. (solacongestine)	(5); (7)
Solafloridine	Fig. 7.19	*S. congestiflorum* Dun. (solaflorine)	(5); (7)
Solaphyllidine	(22*R*,23*S*,25*R*)-16α-Acetoxy-3β,23-dihydroxy-22,26-epimino-5α-cholestan-4-one	*S. hypomalacophyllum* Bitt. ex Pittier	(7)
Solaverbascine	(22*S*,25*R*)-22,26-Epiminocholest-5-en-3β,16β-diol	*S. verbascifolium* L.	(7)
Teinemine	22,25-Bisisomer of capsimine: 22*S*,25*S*	*S. capsicastrum*[j] Link (isocapsicastrine)	(3)
3-Oxo-22,26-epimino-cholestanes (22,26-epiminocholestan-3-ones)			
Solanudine	(22*R*,23*S*,25*R*)-4,23-Dihydroxy-22,26-epiminocholest-4-en-3-one	*S. nudum* Humb. & Bonpl. ex Dun.	(3)
Solaquidine (ketal deriv. of a 3-oxo congener)	(22*S*,25*R*)-3α,3β-Dimethoxy-22,26-epimino-5α-cholestane	*S. pseudoquina* St. Hil.	(3); (7)
3-Amino-22,26-epimino-cholestanes	Two basic centres		
Episolacapine	Fig. 7.20	*S. pseudocapsicum* L.	(3)
Isosolacapine	Fig. 7.20	*S. capsicastrum* Link, *S. pseudocapsicum* L.	(3)
Isosolaseaforthine	Fig. 7.20	*S. seaforthianum* Andr.	(7)
Sarachine	3β-Amino-22,26-epiminocholest-5-ene (C-22 configuration undetermined)	*Saracha punctata* Ruiz & Pav.	(13)
Solacallinidine	3-Amino analogue of 25-isosolafloridine, Fig. 7.19 (deriv.)	*S. callium* C.T.White ex R.J. Henderson	(7)
Solacapine	Fig. 7.20	*S. pseudocapsicum* L.	(3)
Solaseaforthine	Fig. 7.20	*S. seaforthianum* Andr.	(7)
23,26-Epiminocholest-23(N)-en-22-ones	3-Hydroxy deriv. only (no 3-amino congeners)		
"24-Oxosolacongestidine"	(20ξ,25*R*)-23,26-epimino-3β-hydroxy-5α-cholest-23(*N*)-en-22-one[d]	*S. congestiflorum* Dun.	(7)
5α,6-Dihydrotomatillidine	Fig. 7.21	*S. tomatillo* Phil. f.	(5); (7)

(continued)

Table 7.3 Steroidal alkaloids (alkamines) alphabetically listed within the different structural types: For 41 out of altogether 115 compounds from *Solanum* spp. (113 compounds), *Lycianthes biflora*, and *Saracha punctata* (one compound each) at least one corresponding glycoalkaloid was found and structurally elucidated, i.e., for 36% of the alkamines also corresponding glycosides are known (continued)

Alkamines (terms used in references)	**Structural remarks** [Fig…..(deriv.): Cross-reference for basal structure]	**Discovery in (glycoalkaloid[a])**	**Ref.**
Solamaladine	(20*S*,25*R*)-23,26-epimino-3β-hydroxy-5α-cholest-23(*N*)-en-4,22-dione	*S. hypomalacophyllum* Bitt. ex Pittier	(3); (7)
Solaspiralidine	(20*R*,25ξ)-23,26-epimino-3β,16α-dihydroxy-cholest-5,23(*N*)-dien-22-one[d]	*S. spirale* Roxb.	(3)
Tomatillidine	Fig. 7.21	*S. tomatillo* Phil. f.	(5); (7)
α-Epiminocyclo-hemiketals/-ketals (solanocapsine type)			
3-Hydroxy-α-epiminocyclo-hemiketals/-ketals	One basic centre		
Aculeamine (*ketal*)	3-Deamino-3β-hydroxy-23(*O*)-methyl-solanocapsine	*S. aculeatum* St.Lag.	(3)
3-Deamino-3β-hydroxy-solanocapsine (*hemiketal*)	Fig. 7.20 (deriv.)	*S. aculeatum* St.Lag.	(3)
22,26-Epimino-16β,23-epoxy-23α-ethoxy-5α,25α*H*-cholest-22(*N*)-en-3β,20α-diol (*ketal*)	Fig. 7.20 (deriv.)	*S. lycopersicum* L. × *S. hirsutum* Dun. [syn.: *L. esculentum* Mill. × *L. hirsutum* (Dun.) Macbride]	(3)
22,26-Epimino-16β,23-epoxy-23α-methoxy-5α,25α*H*-cholest-22(*N*)-en-3β,20α,27-triol (*ketal*)	Fig. 7.20 (deriv.)	*S. lycopersicum* L. sub nom. *L. esculentum* Mill. var. *cerasiforme* (esculeoside C[k])	(14)
Esculeogenin B (*hemiketal*)	(22β*H*,25α*H*)-22,26-Epimino-16β,23-epoxy-5α-cholestan-3β,23α,27-triol	*S. lycopersicum* L. sub nom. *L. esculentum* Mill. (esculeoside B[k])	(1)
22-Isopimpifolidine (*hemiketal*)	(22β*H*)-Isomer of pimpifolidine, Fig. 7.23	*S. pimpinellifolium* Jussl. sub nom. *L. pimpinellifolium* (Jussl.) Mill. (lycoperoside H[l])	(2); (3)
Pimpifolidine (*hemiketal*)	22,26-Epimino-16β,23-epoxy-5α,22α*H*, 25α*H*-cholestan-3β,23α-diol, Fig, 7.23 (deriv.)	*S. pimpinellifolium* Jussl. sub nom. *L. pimpinellifolium* (Jussl.) Mill.	(3)
Solanocardinol (*hemiketal*)	Identical to pimpifolidine ?	*S. neocardenasii* Hawk. & Hjert. (β-lycotetraoside)	(3)

(continued)

Table 7.3 Steroidal alkaloids (alkamines) alphabetically listed within the different structural types: For 41 out of altogether 115 compounds from *Solanum* spp. (113 compounds), *Lycianthes biflora*, and *Saracha punctata* (one compound each) at least one corresponding glycoalkaloid was found and structurally elucidated, i.e., for 36% of the alkamines also corresponding glycosides are known (continued)

Alkamines (terms used in references)	**Structural remarks** [Fig…..(deriv.): Cross-reference for basal structure]	**Discovery in (glycoalkaloid[a])**	**Ref.**
3-Amino-α-epiminocyclo-hemiketals/-ketals	Two basic centres		
7β-Hydroxy-*O*-methyl-solanocapsine (*ketal*)	Fig. 7.20 (deriv.)	*S. pseudocapsicum* L.	(3)
O-Methylsolanocapsine (*ketal*)	Fig. 7.20 (deriv.)	*S. pseudocapsicum* L.	(3)
Solacasine (*ketal*)	22,26(*N*)-Dehydro-23(*O*)-methyl-solanocapsine Fig. 7.20 (deriv.)	*S. pseudocapsicum* L.	(7)
Solanocapsine (*hemiketal*)	3β-Amino-22,26-epimino-16β,23-epoxy-5α,22α*H*,25ß*H*-cholestan-23β-ol Fig. 7.20	*S. pseudocapsicum* L.	(5)
Solanoforthine (*hemiketal*)	Fig. 7.20	*S. seaforthianum* Andr.	(7)
3-Aminospirostanes (jurubidine-type)	***One*** basic centre (at **C-3**); 22α***O*** (not 22α*N* !)		
Antillaridine	5,6-Dehydrojurubidine Fig. 7.21	*S. antillarum* O.E. Schulz	(3)
Antillidine	3α-Isomer of antillaridine Fig. 7.21 (deriv.)	*S. antillarum* O.E. Schulz	(3)
Isojuripidine	6α-Hydroxyisojurubidine Fig. 7.21 (deriv.)	*S. paniculatum* L.	(7)
Isojurubidine	(25*R*)-Isomer of jurubidine Fig. 7.21 (deriv.)	*S. paniculatum* L.	(7)
Isopaniculidine	9α-Hydroxyisojurubidine Fig. 7.21 (deriv.)	*S. paniculatum* L.	(7)
Jurubidine	(25*S*)-5α,22α*O*-Spirostan-3β-amine, Fig. 7.21	*S. paniculatum* L. (jurubine)	(5)
Juripidine	Fig. 7.21	*S. hispidum* Pers.	(3)
Neopaniculidine	9α-Hydroxy-(25*R*)-isojurubidine Fig. 7.21 (deriv.)	*S. paniculatum* L.	(5)
Paniculidine	9α-Hydroxyjurubidine Fig. 7.21 (deriv.)	*S. paniculatum* L. (paniculine)	(5)
26(N)-Acylamino-cholestan-22-ones			
Aglycone of abutilosides A / I	Fig. 7.21	*S. abutiloides* (Griseb.) Bitt. & Lillo (abutilosides A / I)	(3); (15); (16)

(continued)

Table 7.3 Steroidal alkaloids (alkamines) alphabetically listed within the different structural types: For 41 out of altogether 115 compounds from *Solanum* spp. (113 compounds), *Lycianthes biflora*, and *Saracha punctata* (one compound each) at least one corresponding glycoalkaloid was found and structurally elucidated, i.e., for 36% of the alkamines also corresponding glycosides are known (continued)

Alkamines (terms used in references)	**Structural remarks** [Fig…..(deriv.): Cross-reference for basal structure]	**Discovery in (glycoalkaloid[a])**	**Ref.**
Aglycone of abutiloside B	Fig. 7.21	*S. abutiloides* (Griseb.) Bitt. & Lillo (abutiloside B)	(3)
Aglycone of abutiloside H	Fig. 7.21	*S. abutiloides* (Griseb.) Bitt. & Lillo (abutiloside H)	(16)
Aglycone of abutilosides J / K	Fig. 7.21	*S. abutiloides* (Griseb.) Bitt. & Lillo (abutilosides J / K)	(16)
16-Aminoacyloxy-pregnenolones			
Aglycone of abutiloside O	Fig. 7.21	*S. abutiloides* (Griseb.) Bitt. & Lillo, *S. sodomaeum* L. (abutiloside O)	(17)
Aglycone of esculeoside D[b]	Fig. 7.21	*S. lycopersicum* sub nom. *L. esculentum* var. *cerasiforme* (Dun.) Alef. (esculeoside D)	(14)

[a] If known and structurally elucidated

[b] The genuine glycoside is characterized by an acetoxy group at C-23 and an unusual 27-*O*-β-D-glycopyranosyl residue in addition to the 3(*O*)-β-D-lycotetraosyl moiety

[c] The genuine glycoside lycoperoside G is characterized by an acetoxy group at C-23 and an unusual 24-*O*-β-D-glycopyranosyl residue in addition to the 3(*O*)-β-D-lycotetraosyl moiety

[d] Unknown configuration is designated ξ

[e] Also detected in *Lycianthes biflora* (Lour.) Bitt. (3)

[f] Also detected in *Cestrum purpureum* Stand., *Nicotiana plumbaginifolia* Viv. (7), and *Lycianthes biflora* (3) (Solanaceae) as well as in the genera *Fritillaria* L. and *Lilium* L. (3) (Liliaceae)

[g] Also detected in the genus *Fritillaria* L. (7) (Liliaceae)

[h] Also detected in *Capsicum annuum* L. (Solanaceae) and in the genera *Fritillaria* L., *Notholirion* Wall. ex Voigt, *Korolkowia* Regel, *Rhinopetalum* Fisch. ex Alex., and *Veratrum* L. (3), integrated into the traditional family Liliaceae s.l. (*Veratrum* is nowadays divided off and assigned to the Melanthiaceae)

[i] Acylation leads to non-basic *N*

[j] Also detected in the genus *Veratrum* L. (nowadays: Melanthiaceae; traditionally: Liliaceae s.l.)

[k] The genuine glycoside is characterized by an unusual 27-*O*-β-D-glycopyranosyl residue in addition to the 3(*O*)-β-D-lycotetraosyl moiety

[l] In contrast to the aglycone this glycoalkaloid was identified from *Solanum lycopersicum* (2)

References

(1) Fujiwara et al. 2004 / Yoshizaki et al. 2005; (2) Yahara et al. 2004 / Yoshizaki et al. 2005; (3) Ripperger 1998 and references therein; (4) Usubillaga et al. 1997; (5) Schreiber 1968 and references therein; (6) Prelog and Jeger 1960 and references therein; (7) Ripperger and Schreiber 1981 and references therein; (8) Prelog and Jeger 1953 and references therein; (9) Lee et al. 1997; (10) Maxwell et al. 1996; (11) Coy-Barrera et al. 2005; (12) Ferrer et al. 1998; (13) Moretti et al. 1998; (14) Ono et al. 2006b; (15) Tian et al. 1997; (16) Yoshimitsu et al. 2002; (17) Ono et al. 2006a and references therein

aglycones of naturally occurring glycosides. Steroidal alkaloids are common metabolites of certain solanaceous genera, especially *Solanum*; however, they do not occur in the Convolvulaceae.

Occurrence in Non-solanaceous Taxa. Steroidal alkaloids with an intact C_{27} alkamine skeleton (sometimes called "*Solanum* alkaloids") as metabolites of the plant kingdom are confined to the Solanaceae and to the Liliaceae s.l. (including Melanthiaceae), respectively. In spite of the fact that they are not related (nested within the eudicots and the monocots, respectively) these two families share the occurrence of a few alkamines, e.g., solanidine, solasodine (see different footnotes of Table 7.3).

Predominance of *Solanum* and Abundance of Knowledge. The genus *Solanum* is the vastly predominating taxon for steroidal alkaloids within the Solanaceae though some erratic examples for certain species in other genera are also known. *Solanum* comprises approximately 1350 species which represents an average value of different estimations discussed in detail later (see Sect. 7.8.2). This number corresponds to half of the species in the family. Thus, *Solanum* is one of the largest genera of flowering plants (Bohs 2006). Phytochemical literature on this topic is extremely voluminous due to:

1. The huge number (422) of *Solanum* spp. already proven to contain steroidal alkaloids (for details on the count see Sect. 7.8.2)
2. The vastly diverging structural composition of this class of metabolites
3. The great significance of very important, extensively cultivated glycoalkaloid-positive food plants, especially:

   - *Solanum tuberosum* (potato), worldwide in the fourth position in crop production after wheat, maize, and rice (Hawkes 1999)
   - *S. lycopersicum* (syn.: *Lycopersicum esculentum*; tomato), world production of the fruits: 26% of the potato tuber production (Hawkes 1999)
   - *S. melongena* (brinjal eggplant/aubergine), world production of the fruits: 7% of the potato tuber production (Daunay et al. 2001)

The structural diversity mentioned above is represented by 118 glycoalkaloids, i.e., glycosidic steroidal alkaloids and 115 non-glycosidic steroidal alkaloids, i.e., alkamines without carbohydrate chain. 39 Alkamines have turned out to serve as aglycones of glycoalkaloids, i.e., for the remaining 76 alkamines no glycosides have been discovered until now (Table 7.3). Thus, we have knowledge about altogether 194 structurally elucidated steroidal alkaloids.

Strategy. Therefore, it must be beyond the scope of this book to present this topic exhaustively. Fortunately, there exists a series of excellent updating reviews (Prelog and Jeger 1953, 1960; Schreiber 1968; Ripperger and Schreiber 1981; Ripperger 1998). All numbers of species and different groups of metabolites given in this chapter are based on the information from these reviews, updated from original reports for the period since then (Table 7.4).

Table 7.4 Update and additions to the catalogue of species in the reviews of Schreiber (1968), Ripperger and Schreiber (1981), and Ripperger (1998) on the occurrence of steroidal alkaloids within the genus *Solanum*

Species	Organ	Glycoalkaloids	Corresponding alkamines (cross-reference to Table 7.3)	References
S. abutiloides (Griseb.) Bitt. & Lillo	Fresh roots	Abutilosides A, B, H – K, O	26(*N*)-Acylaminocholestan-20-ones (A, B, H – K); 16-aminoacyloxypregnenolones (O); soladulcidine[a]	Tian et al. 1996, 1997; Yoshimitsu et al. 2002; Ono et al. 2006a
S. aculeastrum Dun.	Berries; root bark	Berries: solamargine, β-solamarine; root bark: solaculine A, solasodine tetraoside[b]	Solasodine; tomatidenol (β-solamarine, solaculine A)	Wanyonyi et al. 2002, 2003 (the solasodine tetraoside)
S. agrarium SendtN.	Green berries	Unknown	Solasodine	Agra and Bhattacharyya 1999
S. americanum Mill.	Leaves	Solasonine	Solasodine	Vidal Aldana and Noguiera Lima 1999
S. amygdalifolium Steud.	Aerial parts	Solasonine	Solasodine	Vázquez et al. 1999
S. arboreum Humb. & Bonpl. ex Dun.	Aerial parts	Impossible	Soladunalinidine, two further 3-aminospirosolanes	Maxwell et al. 1996
S. arundo Mattei	Root bark	Arudonine	Solasodine	Fukuhara et al. 2004
S. asterophorum Mart.	Green berries; leaves	Unknown	Solasodine (berries); isojuripidine (leaves)	Agra and Bhattacharyya 1999 (berries); De Cassia Meneses Oliveira et al. 2006
S. baturitense Huber	Green berries	Unknown	Solasodine	Agra and Bhattacharyya 1999
S. brusquense Smith & Downs	Fruits	Solasonine, solamargine	Solasodine	Leonart and Moreira 1984
S. capsicoides All.	Green berries	Unknown	Solasodine	Agra and Bhattacharyya 1999
S. cornifolium Humb. & Bonpl. ex Dun.	Aerial parts	Unknown	"23,24-(2-Methyltetrahydrofuran)-solanidine"	Coy-Barrera et al. 2005
S. crinitum Lam.	Fruits; young branches	Solasonine	Solasodine	Esteves-Souza et al. 2002; Sarmento da Silva et al. 2005

(continued)

Table 7.4 Update and additions to the catalogue of species in the reviews of Schreiber (1968), Ripperger and Schreiber (1981), and Ripperger (1998) on the occurrence of steroidal alkaloids within the genus *Solanum* (continued)

Species	Organ	Glycoalkaloids	Corresponding alkamines (cross-reference to Table 7.3)	References
S. grandifolium MORTON		Unknown	Solasodine	Kerber et al. 1993
S. indicum L.	Fruits	Solamargine, solasonine	Solasodine	Yahara et al. 1996b
S. iopetalum (BITT.) HAWK.	Tuber, leaves	Solanine, chaconine	Solanidine	Sarquis et al. 2000
S. jabrense AGRA & M.NEE	Fruits	Unknown	Solasodine	Esteves-Souza et al. 2002; Sarmento da Silva et al. 2005
S. lycopersicum L. sub nom. *Lycopersicum esculentum* MILL.	Ripe fruits	Dehydrotomatine; esculeosides A[c], B[d]; lycoperosides F – H	Tomatidenol (dehydrotomatine); 3-hydroxyspirosolanes (esculeoside A, lycoperosides F, G); 3-hydroxy-α-epiminocyclohemiketals (esculeoside B; lycoperoside H: 22-isopimpifolidine)	Friedman et al. 1997 (dehydrotomatine); Fujiwara et al. 2004 (esculeosides); Yahara et al. 2004 (lycoperosides)
S. lycopersicum L. sub nom. *L. esculentum* MILL. var. *cerasiforme* (DUN.) ALEF.	Ripe fruits	Esculeosides A, C, D	See 3-hydroxyspirosolanes (A), 3-hydroxy-α-epiminocyclohemiketals (esculeoside C); 16-aminoacyloxypregnenolones (D)	Fujiwara et al. 2003 (A); Ono et al. 2006b (C, D)
S. lyratum THUN.	Leaves	Solalyratines A, B	Soladulcidine	Lee et al. 1997; Ye et al. 2001
S. orbignianum SENDT.	Aerial parts	Leptinines I, II	Leptinidine	Coelho et al. 1998
S. pseudocapsicastrum, winter cherry	Root bark	Solateinemine	Teinemine	Gan and Lin 1997
S. rhytidoandrum SENDT.	Fruits	Unknown	Solasodine	Silva et al. 2005
S. schlechtendalianum WALP	Roots	Unknown	22-Isoteinemine ("schlechtendamine"), solacongestidine, 5,6-dihydrotomatillidine	Ferrer et al. 1998

Table 7.4 Update and additions to the catalogue of species in the reviews of Schreiber (1968), Ripperger and Schreiber (1981), and Ripperger (1998) on the occurrence of steroidal alkaloids within the genus *Solanum* (continued)

Species	Organ	Glycoalkaloids	Corresponding alkamines (cross-reference to Table 7.3)	References
S. sodomaeum L.	Underground parts	Abutiloside O (corrected structure)	Aglycone see Fig. 7.21	Ono et al. 2006a
S. stagnale MORIC.	Green berries	Unknown	Solasodine	Agra and Bhattacharyya 1999
S. stipulaceum ROEM.	Green berries	Unknown	Solasodine	Agra and Bhattacharyya 1999
S. stramoniifolium JACQ.	Green berries	Unknown	Solasodine	Agra and Bhattacharyya 1999
S. sycophanta DUN.	Fruits	Solamargine, solasonine, sycophantine; 21-hydroxysycophantine; 22,25-diepisycophantine	Solasodine, 21-hydroxysolasodine; 22,25-diepisolasodine	Usubillaga et al. 1997
S. suaveolens KUNTH. & BOUCHÉ	Aerial parts	Solasuaveoline, dihydrosolasuaveoline, isosolasuaveoline	Solasodine	Ripperger and Porzel 1997
S. vespertilio AIT.	Aerial parts	Unknown	Solasodine, Tomatidenol	Gonzalez et al. 1973

[a] No corresponding glycoalkaloid determined

[b] [rha-(1→2)]-[glu-(1→4)-rha-(1→4)]-glu-(1→3ß)-solasodine

[c] Cultivar 'Momotaro' (pink colour type)

[d] Cultivar 'Italian San Marzano' (red colour type)

Nevertheless, there will be some deviations from these numbers of species if one simply adds the catalogues of those reviews and the species of Table 7.4. This is due to the following reasons:

1. Double numbering of species (synonyms of scientific names for the same species) has been avoided to the best of the author's knowledge; in this connection 25 South American cultivated potatoes recognized as independent species by Bukasov and/or Juzepczuk in the 1930s have been neglected. These species names are not integrated either by Hawkes (1990) or by Nee (1999) in the list of *Solanum* species of the New World. Hunziker (2001) criticized the Russian botanists as "typical representatives of the "splitting" philosophy in plant taxonomy". For further details see Sect. 7.8.1.
2. Results with regard to hybrids are not taken into account (with the exception of the cultivated potatoes).
3. This is also true for invalid species names (in the reviews indicated by quotation marks).
4. Double numbering of identical compounds with different trivial names has been avoided.
5. Secondary or tertiary glycosides co-occurring with their genuine (primary) one in the same species are not taken into consideration (for details see below).

The reviews mentioned above are also used in order to limit the number of references, e.g., in Table 7.3 or in the text, indicated by the additional term "references therein" behind the review-author's name.

7.8.1 Discovery and Structure Elucidation

The scientific history of this class of metabolites started almost 190 years ago with the isolation of solanine from the berries of the type species of the genus *Solanum*, the Eurasian herb *S. nigrum* L., black nightshade (Desfosses 1820, 1821) as well as from *S. tuberosum* L., potato (Baup 1826). Four decades later, its glycosidic character including galactose, glucose, and rhamnose as well as the basic character of its aglycone solanidine was elucidated (Zwenger and Kind 1859, 1861). Based on the first successful structure elucidations of steroids at all in the early 1930s – cholic acid by Wieland and Dane as well as cholesterol by Windaus – it was shown that solanidine also belongs to this class of metabolites (Soltys and Wallenfels 1936). However, the complete structure of solanine could only be elucidated two decades later, after "solanine" had turned out to be a mixture of two solanidine-based glycoalkaloids, (α-)solanine and (α-)chaconine (Kuhn and Löw 1954, 1957; Kuhn et al. 1955a, b).

Structural Composition of Glycoalkaloids (Common Aspects). Like their *N*-free congeners [steroidal saponins (Sect. 7.7)], glycoalkaloids represent glycosides. In contrast to the former class of metabolites their aglycones are characterized by the

formal integration of a nitrogen atom into a C_{27}-(cholestane)-skeleton and/or by substitution of the hydroxyl of a 3-hydroxycholestane derivative by an amino group. Thus, these metabolites become basic.

Alkamines. Steroidal alkamines were (i) sometimes isolated directly as such from the plants co-occurring with their corresponding glycoalkaloids. However, usually they were (ii) obtained by acid hydrolysis of the latter, occasionally by enzymatic hydrolysis. Others (iii) may be well-known as such but the knowledge on their corresponding glycosides may be incomplete or even zero. Finally, certain metabolites (iv) apparently exist only as alkamines, especially – but not exclusively – 3-amino-3-deoxy congeners. Thus, the term "steroidal alkamine" is not synonymous with "steroidal aglycone".

All types of steroidal alkamines, potential aglycones or not, share the original C_{27} of the cholestane skeleton, however diverging in the manner of rearrangement (conjugation and/or cleavage) of the side-chain (C-20–C-27). Due to recent discoveries and insights a subdivision and increase of the traditional types is proposed [digits in brackets represent the corresponding number of structurally elucidated and characterized metabolites occurring in the Solanaceae (order according to Table 7.3)]:

- Spirosolanes (44)
  - 3-Hydroxy-spirosolanes (34)
  - 3-Oxo-spirosolanes (spirosolan-3-ones) (3)
  - 3-Amino-spirosolanes (7)
- Solanidanes (13)
  - 3-Hydroxy-solanidanes (8)
  - 3-Amino-solanidanes (5)
- 22,26-Epiminocholestanes (solacongestidine type) (25)
  - 3-Hydroxy-22,26-epiminocholestanes (16)
  - 3-Oxo-22,26-epiminocholestanes (2)
  - 3-Amino-22,26-epiminocholestanes (7)
- 23,26-Epiminocholest-23(*N*)-en-22-ones (5)
- α-Epiminocyclo[hemi]ketals (solanocapsine type) (13)
  - 3-Hydroxy-α-epiminocyclo[hemi]ketals (8)
  - 3-Amino-α-epiminocyclo[hemi]ketals (5)
- 3-Aminospirostanes (jurubidine type) (9)
- 26(*N*)-Acylamino-cholestan-22-ones (4)
- 16-Aminoacyloxy-pregnenolones (2)

Most metabolites of all types and subtypes are characterized by the unchanged tetracyclic 5α-androstane or androst-5-ene moiety (C_{19}) of the 5α-cholestane or cholest-5-ene skeleton (C_{27}) though substitutions at these C_{19} moieties may occur, e.g., hydroxyl groups at different positions. Furthermore, it should be pointed out that 5α-saturated as well as 5-unsaturated congeners of almost all structural types/subtypes have been detected. In contrast to the conserved (cholesterol-based) stereochemistry of the androstane/androstene moiety and normally also of C-20, stereoisomerism is frequent with regard to C-22 and C-25, respectively. Though altogether 115 alkamines could be identified, only a few show a considerable distribution within the genus *Solanum*; they

belong to the spirosolane- and solanidine-type, respectively. Thus, many alkamines may represent apomorphic or autapomorphic characters.

7.8.1.1 Spirosolanes

This structural type is the most common one by far with regard to the number of diverging structures as well as to the principal occurrence in the Solanaceae. It can be subdivided into two stereoisomeric subgroups, 25*R*- and 25*S*-spirosolanes. The methyl group at C-25 is in equatorial position in both series; (25*R*)-spirosolanes are 22α*N*- and (25*S*)-spirosolanes are 22β*N*-configurated. Spirosolanes are the *N*(22)-analogues of spirostanes (see Sect. 7.7.); both types share a C-22 spiro structure (C-22 represents the common member of the rings E/F).

3-Hydroxy-spirosolanes (Fig. 7.19). Solasodine is the dominating congener of the 25*R*-series (22α*N*-configurated) and even the most frequent individual aglycone of all types of solanaceous glycoalkaloids. It was detected unequivocally in 207 out of 367 steroidal alkaloid-positive *Solanum* spp. (56%).

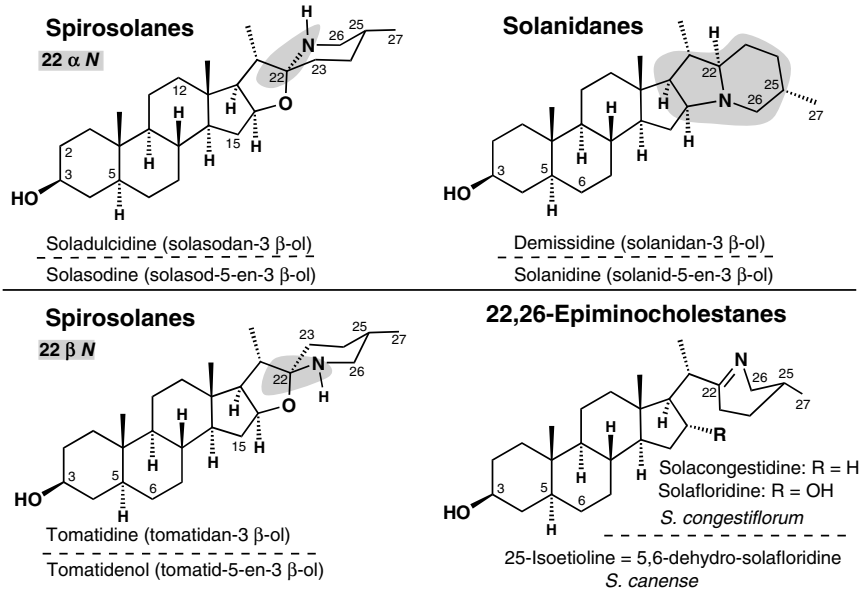


Fig. 7.19 Characteristic examples for major structural *types* of steroidal alkamines hydroxylated at C-3; except solacongestidine / solafloridine all these compounds represent more or less frequent aglycones of steroidal glycoalkaloids (order of frequency in species of the genus *Solanum*: Solasodine > solanidine > tomatidine > tomatidenol > demissidine > soladulcidine). Solafloridine was also detected in *S. abutiloides*, *S. umbellatum*, *S. verbascifolium. Highlighted in grey*: stereoisomerism at C-22 of spirosolanes and anellated (fused) indolizidine moiety of solanidanes, respectively. *Dotted lines* separate the structures given together with their trivial and/or chemical names (*above*) from structurally related 5,6-dehydro congeners (*below*)

The predominant role of solasodine becomes evident when a huge study on its determination in living *Solanum* spp. and herbarium specimens by an almost specific radioimmunoassay is considered which allowed the detection of minute concentrations of solasodine-based glycosides in crude extracts (Weiler et al. 1980). The aim of that study was to select candidates for the industrial production of that alkamine (see Sect. 7.8.4.4). Plant material of 83 species was determined to contain it in concentrations of 0.11–4.4%. An additional 143 *Solanum* species were determined to contain 0–0.1%. Unfortunately, due to the aim of this study mentioned above, no differentiation was documented between species with low concentrations of solasodine (<0.1%) and zero. Of course, subtraction of those species which were analyzed also in other studies – therefore already integrated in the counting mentioned above – would be necessary. Furthermore, it has be taken into account that, due to the cross reaction of the solasodine antiserum with tomatine (solasonine 100%, solamargine 110%, tomatine 104%) one has to be careful with the interpretation of the study. Nevertheless, since tomatine occurs comparably rarer than solamargine/solasonine in general (25 v. 120 spp.; for details see below) most of those species might be solasodine-based-positive rather than tomatidine-based-positive.

In contrast to solasodine, its 5α,6-dihydro derivative soladulcidine plays a minor role. It was detected in only 10 *Solanum* spp. The two most frequent members of the 25*S*-series (22β*N*-configurated) are tomatidenol (36 spp.) and its 5α,6-dihydro derivative tomatidine (36 spp.), respectively. The latter alkamine was discovered as aglycone of the glycoalkaloid tomatine, isolated from *S. lycopersicum* sub nom. *Lycopersicum esculentum* (Fontaine et al. 1948, empirical formula: Fontaine al. 1951), solasodine as aglycone of solasonine from the African shrub *S. sodomaeum* (Sodom's apple, Devil's apple) (Oddo 1929 and references therein), and soladulcidine as aglycone of soladulcine from *S. dulcamara* (European bittersweet, woody or climbing nightshade) (Tuzson and Kiss 1957; Schreiber 1958a). The complete structure elucidation of these three alkamines was achieved in the past fifties and sixties by degradation to already known steroids (e.g., to pregnane derivatives) and by conversion to or partial syntheses from steroid sapogenins (Sato et al. 1957; Prelog and Jeger 1960 and references therein; Schreiber 1968 and references therein). Tomatidenol was discovered as a minor congener in the sprouts of *S. tuberosum* (Schreiber 1957) and as the main alkamine from the epigeal vegetative parts of a chemovariety of *S. dulcamara* (Schreiber 1968 and references therein). Besides these four 3-hydroxyspirosolanes their 15α-hydroxy congeners, i.e., 3,15-dioxygenated spirosolanes, were found as aglycones of glycoalkaloids in the roots of this chemovariety.

3-Oxo-spirosolanes (Fig. 7.19 (deriv.)). These alkamines are oxidation products at C-3 of a solasodine derivative, the 5β-isomer of soladulcidine, and tomatidine. Thus, they are unable to form glycoalkaloids.

3-Amino-spirosolanes (Fig. 7.20). These metabolites are characterized by an additional basic centre (second amino group at C-3). Again members from both series, 25*R* (22α*N*) and 25*S* (22β*N*), respectively, were discovered. Interestingly, six out of seven congeners are constituents of two arboreal species of circum-Carribean distribution (*S. arboreum*, *S. triste;* Nee 1999).

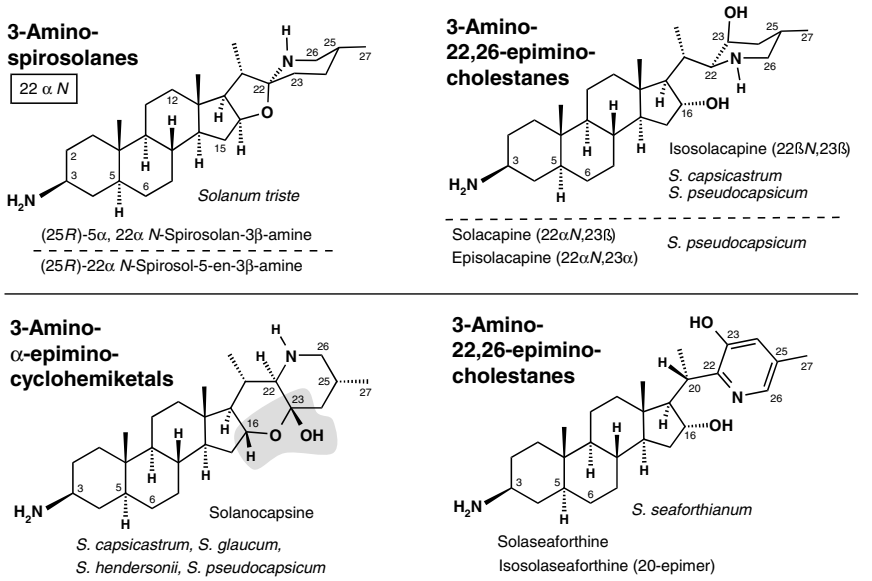


Fig. 7.20 Three types of steroidal alkamines with two basic centres and their occurrence in the genus *Solanum. Highlighted in grey*: cyclohemiketal moiety (full ketal: -OCH_3 or -OCH_2CH_3 instead of -OH; examples in Table 7.3). *Dotted lines* separate the respective structures given together with their trivial and/or chemical names (*above*) from the structurally related 5,6-dehydro congener in case of 3-aminospirosolanes and from corresponding isomers in case of 3-amino-22,26-epiminocholestanes (*below*). The 5,6-dehydro derivative of solanocapsine, solanoforthine, is another constituent of *S. seaforthianum* demonstrating the co-occurrence of different structural types

7.8.1.2 Solanidanes

This structural type is characterized by the integration of the side-chain (C-20–C-27) of the original cholestane skeleton (including the amino-*N* by conjugation at C-16) into a terminally anellated indolizidine moiety. Thus, a unique hexacyclic system is formed.

3-Hydroxy-solanidanes (Fig. 7.19). The longest known *Solanum* alkamines at all are solanidine and its 5α,6-dihydro derivative demissidine, both belonging to this structural type. The empirical formula of solanidine, which could be detected not only as the aglycone of solanine but also as free alkamine in the sprouts of *S. tuberosum*, was determined by Schöpf and Herrmann (1933), its constitution by Prelog and Szpilfogel (1942) as well as by Craig and Jacobs (1943). Demissidine, occurring in glycosidic form (demissine) in *S. demissum*, a Mexican species, was structurally elucidated by Kuhn and Löw (1947). Solanidine-based glycosides or this alkamine itself are frequently occurring metabolites (detected in 63 *Solanum* spp.), whereas demissidine-based glycosides are rather rare (28 *Solanum* spp.). It is remarkable that solanidine- and

demissidine-based glycoalkaloids seem to co-occur only in a minority of these species (10 out of 28: *S. boliviense* DUN., *S. canasense* HAWK., *S. commersonii* DUN., *S. demissum* LINDL., *S. leptophyes* BITT., *S. megistacrolobum* BITT., *S. sanctae-rosae* HAWK., *S. sepicula* DUN., *S. toralapanum* CARD. & HAWK., *S. tuberosum* L.).

Small amounts of the 22*R*,25*R* epimer of solanidine [(22*R*,25*R*)-solanid-5-en-3β-ol = 22,25-diepisolanidine] were obtained from the tubers of certain clones of *S. vernei* BITT. & WITTM. (Van Gelder and Scheffer 1991).

Leptinidine and (*O*)23-acetylleptinidine were discovered as aglycones of the glycosidic leptines and leptinines, respectively, in the leaves of a certain accession of *S. chacoense,* another South American species (Kuhn and Löw 1961a, b). These compounds got their plant-species-unrelated names, because the glycoalkaloids turned out to be resistance factors against the Colorado potato beetle, *Leptinotarsa decemlineata* SAY, Coleoptera: Chrysomelidae (Kuhn and Löw 1957). Another congener, 5α,6-dihydroleptinidine was isolated from the Chinese species *S. lyratum* (Ripperger 1998 and references therein). Leptinidine and dihydroleptinidine represent 23β-hydroxy derivatives of solanidine and demissidine, respectively. A demissidine derivative of unique structure with a C-23/C-24-*anellated* (fused) 2-methyltetrahydrofurane moiety, neither named with a trivial term nor with a chemically correct one, has been found in *S. cornifolium*, a species endemic to north-western South America (Coy-Barrera et al. 2005).

3-Amino-solanidanes (Fig. 7.19 (deriv.)). This subtype is rather rare; to date such metabolites have only been detected in two Old World *Solanum* spp. Interestingly, in contrast to their 3-hydroxy congener 5α,6-dihydroleptinidine, both 3-amino isomers, solanogantamine (3β-amino form) as well as isosolanogantamine (3α-amino form), are natural compounds occurring even in the same species, *S. giganteum*. Solanopubamides, unusual *N*(3)-acylated congeners of solanogantamine, were discovered in *S. pubescens*.

7.8.1.3 22,26-Epiminocholestanes (Solacongestidine Type)

Metabolites of this type show a structural character in which the side-chain (C-20–C-27) of the cholestane skeleton is rearranged to an *N*-containing six-membered ring without being conjugated to C-16 – in contrast to the preceding types of steroidal alkaloids. Such rings may be saturated or characterized by a double bond between C-22 and *N*. Saturated congeners again may occur in 22α*N*- or 22β*N*-configurated form.

3-Hydroxy-22,26-epiminocholestanes (Fig. 7.19). The first members of this subtype, solacongestidine and solafloridine (16α-hydroxysolacongestidine), were discovered as constituents of *S. congestiflorum* (Schreiber 1968; Ripperger and Schreiber 1981 and references therein). Etioline, an alkamine isolated first from *Veratrum grandiflorum* (MAX.) LOESEN, Melanthiaceae, could be detected also in two *Solanum* spp. of very different distribution: *S. havanense* (Caribbean islands) and

S. spirale (China to Australia). 20-Isosolafloridine and 20,25-bis-isoetioline share an unusual (20*R*)-configuration (Ripperger 1998 and references therein). All these metabolites show a double bond between C-22 and *N*, whereas solaphyllidine, the major alkaloid of *S. hypomalacophyllum*, and solaverbascine are saturated congeners (22α*N*). C-4 of the former compound represents an unusual carbonyl character. 22-Isoteinemine, also discovered in the genus *Veratrum* and later detected in *S. capsicastrum*, false Jerusalem cherry / winter cherry, is an example for 22β*N*-configurated 3-hydroxy-22,26- epiminocholestanes.

3-Oxo-22,26-epiminocholestanes. The structure of solaquidine, a metabolite isolated from the green berries of *S. pseudoquina* (South America), is characterized by a C-3-involving (dimethyl) ketal group which is unique for natural steroids.

3-Amino-22,26-epiminocholestanes (Fig. 7.20). Seven congeners of this rare subtype, characterized by the presence of an additional basic residue, were discovered in total, three of them as constituents of *S. pseudocapsicum,* Jerusalem cherry, an ornamental of neotropical origin cultivated worldwide: Solacapine and its 23α-epimer episolacapine belong to the 22α*N*-series whereas isosolacapine represents the 22β*N*-epimer (Ripperger 1998 and references therein). Furthermore, (20*S*)-solaseaforthine and its (20*R*)-epimer isosolaseaforthine were isolated from *S. seaforthianum,* again an ornamental of neotropical origin widely cultivated in tropical and subtropical areas (Nee 1999). Both show an unusual (aromatic) 2-linked 3-hydroxy-5-methylpyridine moiety at C-20. Solacallinidine should be mentioned as an aglycone of the glycoalkaloids from the Australian species *S. callium*. It represents the 3-amino analogue of 25-isosolafloridine, a 3-hydroxy-22,26-epiminocholestane, which is also a constituent of this species (Ripperger and Schreiber 1981 and references therein). Finally, a 5,6-dehydro derivative was discovered as a constituent of *Saracha punctata* Ruiz & Pav. (Moretti et al. 1998).

7.8.1.4 23,26-Epiminocholest-23(*N*)-en-22-ones (Fig. 7.21)

Diverging from the preceding ones, the metabolites of this small group of alkaloids are characterized by a double bond between C-23 and *N* with the consequence of an only five-membered ring and an exocyclic carbonyl function (C-22). To date only a few 3-hydroxy derivatives are known. The first members, tomatillidine and its 5α,6-dihydro congener, were discovered as aglycones of minor glycoalkaloids isolated from the leaves of *S. tomatillo* of Chilean origin. Originally interpreted as 22,26-epiminocholestanes (Schreiber 1968 and references therein), this was corrected later (Ripperger and Schreiber1981 and references therein). This rare structural type was also found in a paleotropical species represented by solaspiralidine, a metabolite from the roots of *S. spirale*, a species found in China and Celebes (Sulawesi)/Indonesia.

7.8.1.5 α-Epiminocyclohemiketals/-ketals (Solanocapsine Type)

3-Amino-α-epiminocyclohemiketals/-ketals (Fig. 7.20). Solanocapsine, the first metabolite of this type, was discovered almost 80 years ago in the leaves of *S. pseudocapsicum* (Breyer-Brandwijk 1929; Barger and Fraenkel-Conrat 1936). It turned out to be the first 3-amino type steroidal alkaloid of all. The latter authors could determine already the important fact that the molecule contained two nitrogen atoms. The correct empirical formula was established by Schlittler and Uehlinger (1952). Its structure containing an unusual epiminocyclohemiketal moiety was elucidated by Schreiber and Ripperger (1960) with the exception of the stereochemistry at C-25. Later Schreiber and Ripperger (1962) succeeded in isolating this alkaloid additionally from the leaves of *S. capsicastrum* and S. *hendersonii*. Alkylation of the hydroxyl at C-23 of such natural hemiketals leads to (full) ketals, e.g., solanocapsine → *O*-methylsolanocapsine, both occurring in S. *pseudocapsicum*.

3-Hydroxy-α-epiminocyclohemiketals/-ketals. 3-Hydroxy analogues of the preceding subgroup were only discovered in the 1980s (*S. aculeatum,* endemic to Caribbean islands) and nineties (*S. pimpinellifolium* sub nom. *Lycopersicum pimpinellifolium* of South American origin), respectively.

7.8.1.6 3-Aminospirostanes (Jurubidine Type) (Fig. 7.21)

These 3-amino analogues of spirostane type sapogenins (see Sect. 7.7) are lacking the basic centre *N*(22) in favour of an oxygen. "Paniculidine", the first member of this group, was discovered in the roots of *S. paniculatum* (Meyer and Bernoulli 1961), a species with a distribution including Argentina/Brazil/Paraguay. However, it turned out to be a mixture of 9α-hydroxyjurubidine (since then named paniculidine) and 9α-hydroxy-(25*R*)-isojurubidine (neopaniculidine) (Ripperger et al. 1967a). Jurubidine itself was also found as one of several congeners in the same species. It got its trivial name according to indigenous terms for *S. paniculatum*, the ethnomedicinally used Brazilian drug "jurubeba"/"juripeba."

7.8.1.7 26(*N*)-Acylamino-cholestan-22-ones (Fig. 7.21)

A series of steroids, named abutilosides, was discovered in *S. abutiloides* (Ohmura et al. 1995; Tian et al. 1996, 1997; Yoshimitsu et al. 2002), a species from Argentina/Bolivia. The congeners A, B, and H–K form this group of steroidal alkamines (for abutiloside O see next group; for C–G see Sect. 7.7.1.1). With the exception of abutiloside H, a cholest-5-ene, all metabolites discussed here represent 5α-cholestane derivatives. These metabolites are also lacking the basic centre *N*(22) and in contrast to the other groups of steroidal alkamines (save 3-aminospirostanes) the nitrogen atom is not integrated into a heterocyclic ring. Instead, an

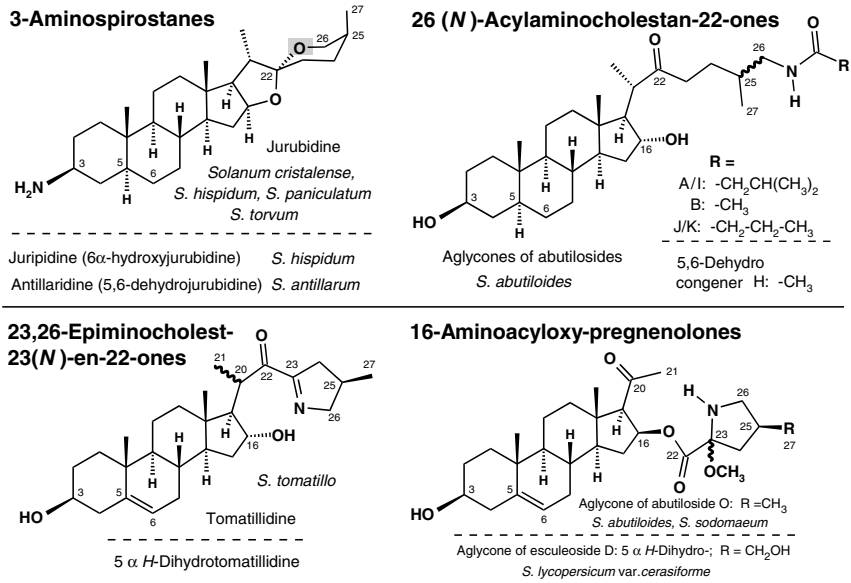


Fig. 7.21 Unusual structural types of steroidal alkamines. *Wavy lines* indicate unknown configuration. *Highlighted in grey*: oxygen instead of nitrogen in other structural types. *Dotted lines* separate structures given together with their trivial names (above) from structurally related congeners (below)

almost unchanged side-chain (C-20–C-27) of the cholestane/cholestene skeleton has been conserved (see also Sect. 7.8.3). This is apparently due to the *N*-acyl group (A/I: isovaleroyl; B/H: acetyl; J/K: *n*-butyroyl) which prevents cyclization: de-*N*-acylation of the aglycone of abutiloside A yielded solafloridine (see 3-hydroxy-22,26-epiminocholestanes) by 22,*N*-cyclization (Tian et al. 1997). Like 23,26-epiminocholest-23(*N*)-en-22-ones the abutilosides of the present type show a C-22 carbonyl group. Though stereochemistry at C-25 is still unproved the co-occurrence of other (25*R*)-congeners (soladulcidine, solafloridine) in the roots of *S. abutiloides* suggests the same for abutilosides. To date metabolites of this type, which is still closely related to cholesterol, seem to be confined to *S. abutiloides*.

7.8.1.8 16-Aminoacyloxy-pregnenolones (Fig. 7.21)

This recently discovered unique group is the only one lacking an intact C_{27} (cholestane) skeleton. As a consequence of an apparent cleavage between C-20 and C-22 a C_{21} skeleton (pregnane) is formed. However, the missing *N*-containing C_6 unit seems to be rearranged and integrated again via an ester bond at C-16. To date only two metabolites are known: (i) The aglycone of abutiloside O which was discovered in the fruits of *S. abutiloides* and the underground parts of *S. sodomaeum*, respectively (Yoshimitsu et al. 2003; Ono et al. 2006a), and (ii) the aglycone of

esculeoside D from the ripe fruit of cherry tomato, *Solanum lycopersicum* sub nom. *L. esculentum* var. *cerasiforme* (Ono et al. 2006b).

7.8.1.9 Oligosaccharide Moieties of Glycoalkaloids

The following sugars were identified as components of the oligosaccharidic moiety of glycoalkaloids: D-glucose, D-galactose, L-rhamnose, D-xylose, L-arabinose. In contrast to the other sugars, arabinose is very rare, e.g., in soladulcamarine, a tetrasaccharidic glycoalkaloid (aglycone: tomatidenol) from *Solanum dulcamara,* and in an unnamed solanidine trioside from *S. wrightii*. Genuine glycoalkaloids usually show tri- or tetrasaccharidic moieties in which the linkages are characterized by β-glycosidically attached D- and α-glycosidically attached L-monosaccharides, respectively. All combinations of the above mentioned monosaccharides were observed; frequent linkages are 1→2, 1→3, 1→4. Glucose, rhamnose, and xylose, respectively, may be present twice in an oligosaccharide moiety, e.g., glucose in tomatine (e.g., *S. lycopersicum*), rhamnose in solamargine (e.g., *S. marginatum*) (Fig. 7.22), xylose in polyanine, a tomatidine-based trisaccharidic (polyatriose) glycoalkaloid, discovered in *S. polyadenium* (Schreiber 1968 and references therein).

The most frequent oligosaccharide moieties, named *Solanum*-based by trivial terms, are branched, e.g., the trioses are linked with their inner monosaccharide unit (1→3) to the corresponding aglycones (Fig. 7.22):

- β-Solatriose [*O*-α-L-rhamnopyranosyl-(1→2_{gal})-*O*-β-D-glucopyranosyl-(1→3_{gal})-β-D-galactopyranose], e.g., in solanine (e.g., *S. tuberosum*), solasonine (e.g., *S. sodomaeum*)
- β-Chacotriose [*O*-α-L-rhamnopyranosyl-(1→2_{glu})-*O*-α-L-rhamnopyranosyl-(1→4_{glu})-β-D-glucopyranose], e.g., in chaconine (e.g., *S. chacoense*), solamargine (e.g., *S. marginatum*)
- β-Lycotetraose [*O*-β-D-glucopyranosyl-(1→2_{glu})-*O*-β-D-xylopyranosyl-(1→3_{glu})-*O*-β-D-glucopyranosyl-(1→4_{gal})-β-D-galactopyranose] (Kuhn et al. 1957) in tomatine/dehydrotomatine (e.g., *S. lycopersicum*), demissine (e.g., *S. demissum*); in the case of this tetraose the oligosaccharide is linked with its terminal galactose (1→3) to the corresponding aglycones, whereas the neighboured unit (inner glucose) represents the branching point (Fig. 7.22).

Another trivial-named trisaccharide, β-polyatriose [*O*-β-D-xylopyranosyl-(1→2_{glu})-*O*-β-D-xylopyranosyl-(1→3_{glu})-*O*-β-D-glucopyranose], mentioned already above, is much rarer.

7.8.1.10 Glycoalkaloids (Glycosides; Specific Aspects) (listed according to Table 7.5)

The accurate counting amounts to 118 structurally elucidated glycoalkaloids in the genus *Solanum*, based on 39 aglycones. Secondary or tertiary glycosides, e.g., β- and

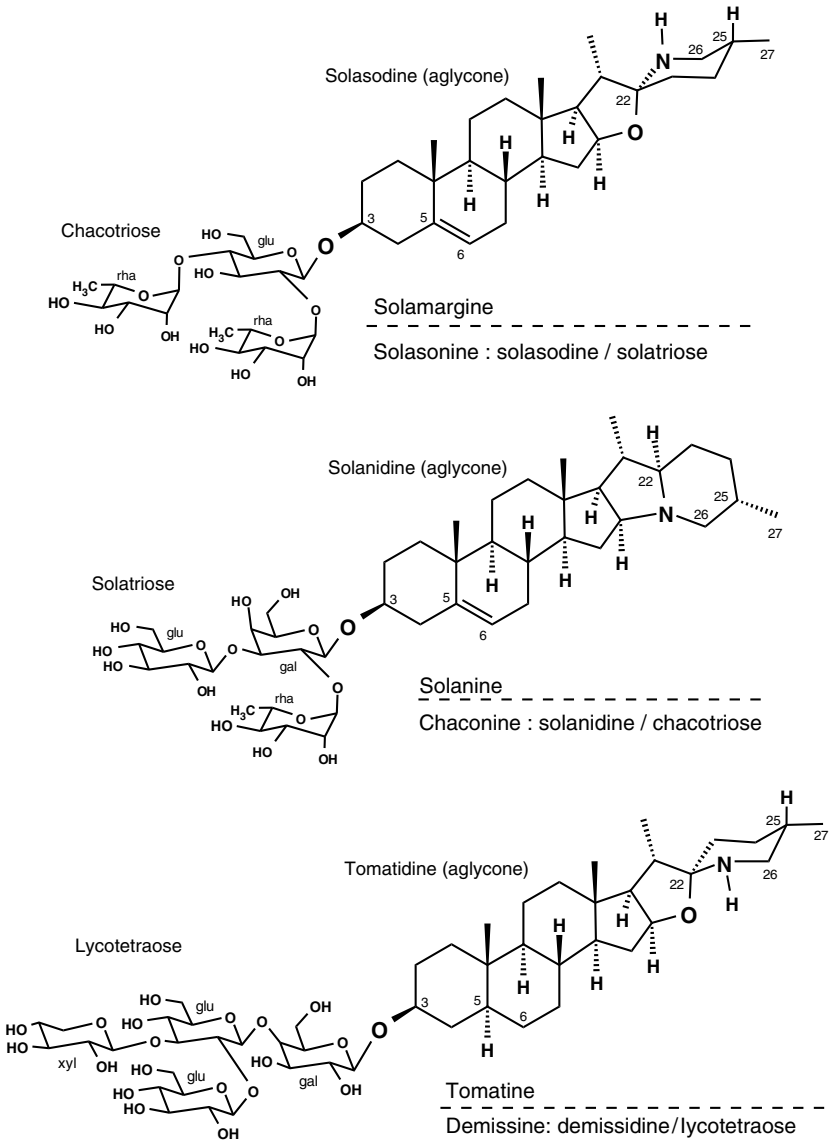


Fig. 7.22 The six most frequent glycoalkaloids of the genus *Solanum*; further solanaceous detections: solasonine in *Cestrum parqui*, solanine in *Capsicum annuum,* tomatine in *Lycianthes rantonnetii. Dotted lines* separate the structures given together with their trivial names (*above*) from related congeners (*below*)

γ-solanine or solasurine (solamargine minus one rhamnose unit) remained uncounted due to reasons discussed below. The great majority of these genuine glycoalkaloids (97 out of 118) were detected only in one species each. Eleven glycoalkaloids could be found in two species. The remaining 10 congeners with an

Table 7.5 Most frequent glycoalkaloids in the genus *Solanum* in the order of frequency; listed: all alkaloids with an occurrence in >2 species; solacauline added as a rare example for a straight chain (not branched) oligosaccharide moiety

Glycoalkaloid				Carbohydrate moiety (oligosaccharide)		
Trivial name[a]	**Detected in … species**	**Structure: Fig.**	**Aglycone**[b]	**Sequence** gal = β-D-galactose; glu = β-D-glucose; rha = α-L-rhamnose; xyl = β-D-xylose	**Branched ?**	**Trivial name**[c]
Solamargine	111	7.22	Solasodine	rha-(1→2$_{glu}$)-rha-(1→4$_{glu}$)-glu-(1→3β)	yes	β-Chacotriose
Solasonine[d]	107	7.22	Solasodine	rha-(1→2$_{gal}$)-glu-(1→3$_{gal}$)-gal-(1→3β)	yes	β-Solatriose
Solanine	42	7.22	Solanidine	rha-(1→2$_{gal}$)-glu-(1→3$_{gal}$)-gal-(1→3β)	yes	β-Solatriose
Chaconine	31	7.22	Solanidine	rha-(1→2$_{glu}$)-rha-(1→4$_{glu}$)-glu-(1→3β)	yes	β-Chacotriose
Tomatine	25	7.22	Tomatidine	glu-(1→2$_{glu}$)-xyl-(1→3$_{glu}$)-glu-(1→4$_{gal}$)-gal-(1→3β)	yes	β-Lycotetraose
Demissine	19	7.22	Demissidine	glu-(1→2$_{glu}$)-xyl-(1→3$_{glu}$)-glu-(1→4$_{gal}$)-gal-(1→3β)	yes	β-Lycotetraose
α-Solamarine	12		Tomatidenol	rha-(1→2$_{gal}$)-glu-(1→3$_{gal}$)-gal-(1→3β)	yes	β-Solatriose
Commersonine	8		Demissidine	glu-(1→2$_{glu}$)-[glu-(1→3$_{glu}$)]-glu-(1→4$_{gal}$)-gal-(1→3β)	yes	β-Commertetraose
Dehydrocommersonine	4		Solanidine	glu-(1→2$_{glu}$)-[glu-(1→3$_{glu}$)]-glu-(1→4$_{gal}$)-gal-(1→3β)	yes	β-Commertetraose
Isoanguivine	4		Solasodine	rha-(1→2$_{gal}$)-xyl-(1→3$_{gal}$)-gal-(1→3β)	yes	
Solacauline	1		Solanidine	glu-(1→4$_{xyl}$)-xyl-(1→4$_{xyl}$)-xyl-(1→3β)	no	

[a] In the early literature there was a differentiation between α-, β-, γ-, δ-glycoalkaloids, e.g,. α-solanine as the term for the glycoalkaloid with the highest number of monosaccharide units, i.e., the trioside β-solatriose, ß-solanine for the corresponding bioside (trioside *minus* rha), γ-solanine for the corresponding monoside (bioside *minus* glu); in modern literature, e.g., α-solanine is named solanine (for the reasons see text); however, α-solamarine is termed correctly, because β-solamarine is another *genuine* tomatidenol trioside with a different carbohydrate chain

[b] Structures see Fig. 7.19

[c] Structures see Fig. 7.22

[d] Including "solasodamine" and "β-solanigrine" which turned out to be identical to solasonine (Briggs et al. 1961; Schreiber 1968)

occurrence in >2 species are based on only five aglycones glycosidically conjugated with two tetraoses and four trioses, respectively.

Traditional terms like β-solanine, γ-solanine, analogously β-, γ-chaconine and others had been used for (secondary or tertiary) glycosides diminished by one or two monosaccharide units compared with the corresponding primary (genuine) α-glycoside. Nowadays α-glycoalkaloids are usually named as such, i.e., without Greek letters (e.g., solanine), and the designation of lower congeners (β- or γ-glycosides) is avoided. It is questionable whether the latter are really genuine constituents of plants; their isolation might have been caused by enzymatic hydrolysis during drying (disintegration of compartmentalization) if not by chemical hydrolysis due to the isolation procedure. Dependent on the stage of plant development, they may be also biogenetic precursors of the primary glycoalkaloid or normal metabolites yielded by specific glycosidases as could be demonstrated (Schreiber 1968 and references therein).

Solanine and **solasonine** belong to the most widely distributed glycoalkaloids (Table 7.5). A first differentiation between these two metabolites had been achieved by Oddo and Colombano (1905), who could find out that the "solanine" from the berries of *S. sodomaeum* was not identical with that of *S. tuberosum* (Colombano 1908 and references therein; Oddo 1929 and references therein). Since the 1960s it has been known that this major glycoalkaloid of the former species, meanwhile named solasonine, shares the sugar moiety (solatriose) with solanine but differs with regard to the aglycone (solasodine vs solanidine; structural types: spirosolanes vs solanidanes). Their corresponding chacotriose congeners, **solamargine** and **chaconine**, respectively, also belong to the most widely distributed glycoalkaloids. Solamargine was discovered in the green berries of *S. marginatum* L.f. (Briggs et al. 1952), chaconine as a constituent of the wild potato *S. chacoense*, named according to its geographic occurrence (Gran Chaco, Argentina/Paraguay). The fresh leaves were found to contain 3.0–3.5 g/kg of solanine/chaconine (50:50), a 10- to 12-fold higher concentration than detected in the leaves of *S. tuberosum* (Kuhn et al. 1955b).

It is remarkable that the solasodine-based glycoalkaloids solamargine/solasonine were shown to co-occur in 98 *Solanum* spp. Solamargine (without solasonine) was detected in a further 13 spp., solasonine alone in 9 spp. Thus, in altogether 120 species at least one of these two glycoalkaloids was identified. A comparable situation – though on a lower level – turned out to exist with the solanidine-based glycoalkaloids solanine/chaconine (co-occurrence: 31 *Solanum* spp.; solanine alone: 11 spp.; chaconine alone: none). Thus, many species are able to synthesize glycoalkaloids with solatriose moieties and in addition congeners with chacotriose. However, generally these solanidine-based compounds do not co-occur with their solasodine-based analogues (exceptions see Sect. 7.8.2).

"Solanine" was discovered in *Solanum lycopersicum* (syn.: *Lycopersicon esculentum*) by Fôdéré and Hecht (before 1884). Sattler (1912) reported that "solanine" is present in all parts of the tomato plant during all stages of development. Decades later this tomato "solanine" was named **tomatine**. Fontaine et al. (1948) succeeded in isolating crystalline tomatine from *S. pimpinellifolium* sub nom. *L. pimpinellifolium*, a wild tomato plant. This enabled to elucidate its structure in part, tomatidine as the aglycone and a tetrasaccharide side chain composed of xylose, galactose, and two glucose units,

later identified as lycotetraose (Kuhn et al. 1957). Decades later the corresponding tomatidenol-based glycoalkaloid dehydrotomatine could be elucidated as the main impurity of commercial tomatine (purity: 88%) (Friedman et al. 1997; Ono et al. 1997). **Demissine**, another lycotetraoside, was discovered by Kuhn and Löw (1947) in *S. demissum* as already mentioned. **α-Solamarine**, detected in 12 species, as well as its very rare congener β-solamarine represent tomatidenol triosides with different monosaccharide units and conjugations [rha-(1→2$_{gal}$)-glu-(1→3$_{gal}$)-gal-(1→3β) = β-solatriose) vs. rha-(1→2$_{glu}$)-rha-(1→4$_{glu}$)-glu-(1→3β)]. Thus, they are different *genuine* glycoalkaloids, both discovered in a certain chemovariety of *S. dulcamara*. **Commersonine**, a second demissidine-based glycoalkaloid, and its solanidine-based congener **dehydrocommersonine** share commertetraose as a unique carbohydrate chain. These two rather rare alkaloids are named according to the first finding of the former as a constituent of *S. commersonii* Dun. (Osman et al. 1976). A very high diversity in the concentration of commersonine has been determined in 39 accessions of this species from 21 geographically distinct areas of Uruguay ranging from 23.0 to 26,013 mg/kg fresh weight (Pianzzola et al. 2005). Dehydrocommersonine was isolated from root tissue initiated from a callus culture of a commersonine-positive chemovariety of *S. chacoense* (Zacharius and Osman 1977). For further glycoalkaloids see Sect. 7.8.2.

7.8.2 Occurrence in the Solanaceae (Table 7.3)

7.8.2.1 Genus *Solanum*

According to Hunziker (2001), the large genus *Solanum* comprises ca. 1,033 known species. However, he did not include *Cyphomandra* and *Lycopersicon* spp., altogether almost 50 species, which have been transferred meanwhile to *Solanum*. Estimations including them range between >1200 and approximately 1500 spp. (Nee et al. 1999; Judd et al. 1999; Knapp et al. 2004; Bohs 2006), "making its taxonomy a gargantuan and virtually impossible task" (Nee et al. 1999). "*Solanum* ….. desperately needs a world-wide infrageneric classification, and most of the sections require additional studies of various kinds. In addition, we must reconcile traditional nomenclature with cladistics, a contentious topic at this time ….." (Nee 2001). The Planetary Biodiversity Inventory (PBI) *Solanum* project (http://www.nhm.ac.uk/solanaceaesource/) with its aim to provide a worldwide species-level taxonomic treatment available electronically on the internet will also elucidate the real number within reasonable time frames (for details see also Knapp et al. 2004). In spite of the difficulties described above, in the following a chemotaxonomic overview on selected large and important taxa of the genus is given. Particular attention should be paid to recent DNA-based (molecular) cladistic analyses here which in these cases are – at least in part – compatible with traditional classifications. Cladistic analyses based on morphological characters are also integrated. The presence or absence of certain steroidal alkaloids in different taxonomic units may be of additional interest for the classification.

Steroidal alkaloids *with a completely elucidated structure*, alkamines and glycoalkaloids taken together, were detected in 367 *Solanum* spp. Based on an average estimation of 1350 species in the genus according to the numbers given by the different authors cited above, this means ca. 27%. However, there are at least 55 additional species which were found to be also characterized by the occurrence of steroidal alkaloids, though their specific structures are still unidentified. There are no unequivocal reports that any *Solanum* spp. was found to be steroidal alkaloid-negative.

In a study already mentioned above using a radioimmunoassay for the determination of solasodine-based glycoalkaloids including herbarium specimens of 226 species, the solasodine content of 143 spp. was reported to be 0–0.1% (dry weight) (Weiler et al. 1980). This indicates that there were species with a zero content of solasodine-based glycoalkaloids. Unfortunately, it was not specified between zero and >0–0.1% for reasons already mentioned. However, zero for solasodine does not mean zero for other steroidal alkaloids which were not recorded by this assay, save tomatidine-based glycoalkaloids (tomatine showed cross reaction). 58 out of those possibly solasodine-negative 143 species turned out to be steroid-alkaloid-positive in other, traditional-analytical studies. Several of such species were even shown to contain solasodine-based alkaloids. Other data for the remaining 85 spp. are lacking. Another point is that roots contain frequently more complex mixtures of steroidal alkaloids than epigeal parts of the plants (Hegnauer 1973), whereas Weiler et al. used the latter (herbarium specimens). In a comprehensive GC-MS-based study on the content of certain steroidal alkaloids (demissidine, solanidine, tomatidine, tomatidenol) in the tubers of 75 spp. belonging to subsect. *Potatoe* at least one of these metabolites could be detected in all of these species save four. Again this does not mean that these exceptions are really alkaloid-negative since only tubers were checked (Petersen et al. 1993).

Thus, it may be assumed, that steroidal alkaloids, i.e., alkamines and/or glycoalkaloids, represent a common and consistent trait in the genus *Solanum*. This was already assumed by Heftmann (1983) ("It is quite possible that they occur in all representatives of this genus") and Hegnauer (1990) ("*Solanum* alkaloids are hardly absent in any species of the genus".

Solasodine-based Glycoalkaloids. As already mentioned above, altogether 120 *Solanum* spp. were shown to produce at least 1 of the 2 major solasodine-based glycoalkaloids solamargine/solasonine. The corresponding alkamine solasodine (mostly obtained after hydrolyzation of undetermined glycoalkaloids) was characterized in *additional* 87 spp. enhancing the number of solasodine-positive species to 207. This corresponds to 56% of all *Solanum* spp. ever investigated phytochemically with sufficient results, i.e., with completely elucidated structures of at least one alkaloid (367 spp.). The percentage may be even much higher if one takes into account the radioimmunoassay of Weiler et al. (see above).

Some further solasodine-based, rare glycoalkaloids are known. One example is given by anguivine/isoanguivine, both discovered – besides solamargine – in *S. anguivi* LAM. (subgen. *Leptostemonum*). They represent *triosides* conjugated both to their common aglycone (1→3) by an inner hexose unit (glucose and galactose,

respectively) which in turn is $1\rightarrow2_{rha}$-linked to a rhamnose and furthermore $1\rightarrow3_{xyl}$-linked to a xylose unit (Ripperger 1998 and references therein). Another example is presented by solaradinine and its congener solashabanine, both discovered in the roots of *S. laciniatum* AIT., kangaroo apple, an Australian species (subgen. *Archaeosolanum*), characterized by the content of solamargine/solasonine in the epigeal parts. Whereas the trivial name of solaradinine is based on its occurrence in the corresponding plant organ, the latter was named apparently in honour of one of the authors (Bite and Shabana 1972; Ripperger 1998 and references therein). Both glycoalkaloids represent derivatives of the *trioside* solasonine (Fig. 7.22), in which a second glucose is 1→6-linked to the already present glucose unit to form the *tetraoside* solashabanine, whereas two further glucose units, 1→6-linked to each other, are $1\rightarrow2_{glu}$-linked to solasonine to form solaradinine. Thus, the latter is an example for *pentaosides* which are really rare in the group of glycoalkaloids. Arudonine, isolated from the root bark of *S. arundo* MATTEI, turned out to be another *tetraoside*, this time derived from the trioside solamargine characterized by an additional sugar unit (xylose) attached to the C-3 hydroxy group of β-chacotriose (Fukuhara et al. 2004). Finally, robustine, a glycoalkaloid from *S. robustum* WENDL. (subgen. *Leptostemonum*), should be mentioned as another example with an again branched *tetraoside* moiety, characterized by glucose as the monosaccharide unit, which is connected directly to C-3 of solasodine (instead of galactose in the examples mentioned above). Remarkably, (i) the remaining three monosaccharide units are linked each to this central glucose moiety and (ii) besides two rhamnose units ($1\rightarrow2_{glu}$- and $1\rightarrow4_{glu}$-linked, respectively) an extremely rare arabinose unit is $1\rightarrow3_{glu}$-linked (Ripperger and Porzel 1994). Unfortunately, the term "robustine" for this glycoalkaloid is another example for a hasty designation of a natural metabolite, since this name had been already chosen for a furoquinoline alkaloid from *Haplophyllum robustum* BUNGE (Rutaceae) three decades before (Fakhrutdinova et al. 1965), meanwhile detected in several further rutaceous species. To avoid confusions it would be preferable to name the solanaceous alkaloid "solarobustine".

A preliminary cladistic analysis of Australasian species of the *Solanum* subgenera *Archaeosolanum* MARZELL (8 spp. included) and *Leptostemonum* (DUN.) BITT. (106 spp. included), based on morphological characters resulted in the assumption that both subgenera are monophyletic (Lepschi and Symon 1999). Phytochemical studies on 7 spp. of *Archaesolanum* could be found in the literature: All of these species were solasonine/solamargine-positive whereas no other alkaloids were reported (*S. aviculare* FORST. f., S. *capsiciforme* (DOMIN) BAYLIS, *S. laciniatum* AIT., *S. linearifolium* GERASIMENKO., S. *simile* F.V. MUELL., *S. symonii* HJ. EICHL., *S. vescum* F.V. MUELL.). *S. aviculare* and *S. laciniatum*, both shrubs endemic to Australia and both commonly called "kangaroo apples", are closely related species. Due to their high content of solasodine-based alkaloids especially in the unripe fruits the cultivation of both played an important role in promising agroindustrial projects in the recent past (see Sect. 7.8.4.4).

Subgenus *Leptostemonum* comprises almost one third (ca. 350–450 spp.) of the genus with a *worldwide* distribution (Levin et al. 2006). The majority of species belonging to this largest subgenus are armed with epidermal prickles which led to

the term "spiny solanums". A total of 29 species were phytochemically checked out of those 106 *Australasian* spp. already mentioned; 8 of them were found to be solamargine/solasonine-positive, 19 species solasodine-positive. This implicates that there were only two species (as far as checked) lacking solasodine-based alkaloids: *S. dimorphospinum* C.T. WHITE and *S. dunalianum* GAUDICH. Both species are characterized by tomatidine-based alkaloids, the latter in addition by soladunalinidine, the 3-amino analogous congener of tomatidine (Table 7.3).

A similar picture appeared as a result of a corresponding phytochemical evaluation based on the list of the same subgenus involving 178 species from 10 sections occurring in the New World [sect. 15. – 24. of the taxonomic synopsis published by Nee (1999)]. Phytochemical data of 43 spp. (out of 178) are available in the literature. Solamargine/solasonine were detected in 15 spp., solasodine in additional 25 spp. Thus, altogether 40 out of 43 turned out to be solasodine-positive. Only three species could be characterized by different alkaloids: *S. hispidum* PERS., *S. paniculatum* L. (both by aminospirostanes), and *S. polytrichum* MORIC. (by solanine/chaconine). Thus, 24% of the species of all New World species belonging to this subgenus and – by chance – a similar percentage of every of its section (save one) were checked with regard to the occurrence of steroidal alkaloids. It is conspicuous that in such New World species of the large subgenus *Leptostemonum* – like in the Australasian relatives – solasodine-based alkaloids apparently are dominating by far, too. Thus, altogether 67 out of 72 phytochemically checked species from both provenances were found to be solasodine-positive.

The only one of the above-mentioned 10 New World sections, for which no data are available, is sect. 19. *Herposolanum* BITT. (eight spp.). Interestingly, Hunziker (2001) could not accept the transfer of this section to subgen. *Leptostemonum* by Nee and listed it under "Sections of uncertain subgeneric position".

A four-gene study of evolutionary relationships in section *Acanthophora* characterized by ferocious needle-like prickles at all vegetative parts led to the conclusion that its traditional circumscription (based on Nee 1979) is not a monophyletic one. However, the majority of its taxa comprise a monophyletic lineage (sister to sect. *Lasiocarpa*) (Levin et al. 2005). Anyhow, a check-up with regard to the occurrence of glycoalkaloids shows that all species of the strict consensus tree as far as phytochemically studied, i.e., 17 out of 23 species, are characterized by solasodine-based alkaloids. This includes (i) 7 out of 11 species belonging to the new sect. *Acanthophora* s.s., (ii) 3 out of 4 species belonging to sect. *Lasiocarpa*, and (iii) 7 out of 8 members from the remaining part of the subgenus.

A few data on total glycoalkaloid concentration in ripe fruits are mentioned in the study of Levin et al. in connection with a potential correlation between seed-dispersal and these metabolites: *S. mammosum* L. showed "almost 50 times more" than *S. capsicoides* ALL., *S. myriacanthum* DUN. a "much higher content" than *S. viarum* DUN., and *S. acerifolium* HUMB. & BONPL. a "low content". It might be assumed that these alkaloids are also solasodine-based in case of *S. myriacanthum* as proven for the remaining four species; however, this is speculative.

Recently, an enlarged, comprehensive phylogenetic study on numerous, *world-wide* distributed species of subgenus *Leptostemonum* using DNA sequence data

from two nuclear regions (ITS and the granule-bound starch synthase gene) and one chloroplast spacer region has been published (Levin et al. 2006). In addition to Australasian and American species this study involved endemic species from continental Africa, Madagascar, and Canary Islands, predominantly united in a large Old World clade. The subgenus turned out to be monophyletic – given the limited though large sampling – when two species groups (*S. wendlandii* group, *S. nemorense* group) are excluded (subgenus *Leptostemonum* s.s.). 10 clades (including the species-rich Old World clade already mentioned) have been defined which in part correspond to traditionally circumscribed groups or sections of species. The study comprised 102 species within these clades; reports on 46 steroid-alkaloid-positive species are to be found in the literature. A total of 45 species contained solasodine-based metabolites: Solasomargine/solasonine were detected in 26 spp., the corresponding aglycone solasodine in further 19 spp. Reports on the content of solamargine/solasonine and/or solasodine are existent for species of all of the 10 clades. This completeness is also true from the phytogeographic point of view (occurrence of steroidal alkaloids in endemic species from Madagascar is unknown). The only phytochemically checked species, for which solasodine or its glycoalkaloids were not reported until now, is represented by *S. paniculatum* (/Torva clade) which was characterized to be jurubine-positive as already mentioned above.

In the study of Levin et al. (2006) the /Torva clade included all 5 species sampled from the traditional *S. torvum* group, but there are only phytochemical reports on two of them: *S. paniculatum* and *S. torvum*, respectively. Interestingly, the rare 3-aminospirostan-type glycoalkaloid jurubine was discovered in both species, though the former one is of neotropical and the latter of paleotropical origin. However, in contrast to *S. paniculatum* co-occurrence with solasonine was detected in case of *S. torvum* (Schreiber 1968 and references therein).

As a result of the phytochemical evaluation – based on all of the four classifications discussed above – it may be concluded chemotaxonomically that presence of solasodine-based alkaloids and absence of demissidine-/solanidine-based congeners are consistent traits of the subgenus *Leptostemonum* s.s. (sensu Levin et al.) as well as s.l.

A number of species cultivated because of their *edible fruits* belong to the subgenus *Leptostemonum*; these species are solasodine-positive without exception though (almost ?) free of such alkaloids in the ripe fruit:

- *S. aethiopicum* L., common name: scarlet eggplant; cultivated in many parts of tropical Africa and also in Brazil; beside fruits leaves, when glabrous, are eaten as spinach; alkaloids: solasodine; [wild ancestor: *S. anguivi* Lam. agg.; alkaloids: solasodine-based glycoalkaloids (solamargine, angiuvine, isoanguivine)] (Daunay et al. 2001)
- *S. betaceum* Cav. [syn.: *Cyphomandra betacea* (Cav.) Sendtn.], tree tomato/tamarillo; origin: Bolivia/Argentina; cultivated worldwide in subtropical regions; alkaloids: solasodine, tomatidenol
- *S. macrocarpum* L., gboma eggplant/nakati omuzungu; cultivated throughout tropical Africa and also in some SE Asian countries; beside fruits leaves,

when glabrous, are eaten as spinach; alkaloids: solamargine/solasonine; [wild ancestor: *S. dasyphyllum* THONN., alkaloids: see text] (Daunay et al. 2001)
- *S. melongena* L., aubergine/brinjal eggplant; origin: Presumably India/Birma, cultivated mainly in China > India > Turkey > Egypt > Japan > Italy; alkaloids: solamargine/solasonine; [putative wild ancestor: *S. incanum* L. agg.; alkaloids: solamargine, solasonine] (Daunay et al. 2001)
- *S. quitoense* LAM., naranjilla/lulo; cultivated in Colombia/Ecuador; alkaloids: solamargine/solasonine
- *S. sessiliflorum* DUN., cocona; originated and cultivated in Peru/Brazil/Venezuela/Colombia; alkaloids: solasodine

The last two closely related species belong to the /Lasiocarpa clade in the study of Levin et al. (2006) corresponding to sect. 24. *Lasiocarpa* (DUN.) D'ARCY (Nee 1999). The eggplants *S. aethiopicum* (sect. *Oliganthes*), *S. macrocarpum*, and *S. melongena* (sect. 16. *Melongena* both) are closely related Old World species.

Furthermore, four species of that subgenus are/were cultivated because of their *high content of solasodine-based alkaloids* in the fruits (promising agroindustrial projects in the recent past; see Sect. 7.8.4.4):

- *S. khasianum* C.B. CLARKE; origin: India, cultivated also there; alkaloids: solamargine (= "khasianine")/solasodine (= "solakhasianine") (Mühlenbeck et al. 2002 and references therein)
- *S. mammosum* L.; origin: Venezuela/Bolivia; cultivated (i) as an ornamental (common name: Nipple fruit, due to its typically pear-shaped, yellow fruit with nipple-like protrusions ("*mammosum*" !) and (ii) because of its alkaloid content, e.g., in Indonesia (common name: Terong susu) (Indrayanto et al. 1998 and references therein). This species is one out of six solasodine-positive species of the /Acanthophora clade (Levin et al. 2006)
- *S. marginatum* L. f.; supposedly native to Ethiopia (Nee 1999), cultivated in, e.g., Ecuador.
- *S. viarum* DUN. sub nom. "*S. khasianum* C.B.CLARKE var. *chatterjeeanum* SENGUPTA" which is probably an error, i.e., this taxon, cultivated in India, is not a variety of *S. khasianum* (Mühlenbeck et al. 2002); origin: South America

Occurrence of Solasodine-based Glycoalkaloids as well as Solanidine-based Congeners in the Same Species or Sample. Co-occurrence of solasonine/solamargine on one hand and solanine on the other *in the same sample* is reported for only a few cases. Unripe fruits of *S. arundo* MATTEI (Saleh 1973), ripe fruits of *S. dasyphyllum* SCHUM. & THONN. (further content: tomatidenol; Adesina 1985), fruits of *S. dubium* L. (El Kheir and Salih 1979), different organs of *S. erianthum* G.DON (Moreira et al. 1980). However, since the identification of the alkaloids seems to be not absolutely unequivocal due to limited analytical possibilities in those reports (e.g., only identification by TLC comparison), reinvestigation and potential reconfirmation ought to be necessary taking into account the significance of this specifically unusual co-occurrence. If these results will be reconfirmed, infraspecific or morphogenetic variability may be the reason. The whole plant of

S. dasyphyllum except for roots had already been reported in a former study to contain seven tomatidenol-based glycoalkaloids, e.g., soladulcamarine, solamarines, with no mentioning of solasodine- and/or solanidine based glycosides (Coune and Denoel 1975; Coune 1977). Thus, it might be that the latter compounds, which were reported in the study of Adesina, were assumed to be present due to similar analytical behaviour, e.g., TLC comparison. In case of *S. arundo* the presence of solasodine-based glycoalkaloids (but not of solanine) has been confirmed in a recent paper though in another plant organ (root bark; Fukuhara et al. 2004). Tubers of a certain accession of *S. vernei* BITT. & WITTM. turned out to contain unequivocally solasodine-based glycoalkaloids beside solanidine-based major congeners (Van Gelder and Scheffer 1991).

Controversial Reports on Occurrence of Solasodine-based Glycoalkaloids or Solanidine-based Congeners in Certain Species. There are controversial reports on *S. berthaultii* BITT.: Schreiber (1963) detected solanine/chaconine in the leaves of this species. Gregory et al. (1981) found *solasodine* as the major aglycone in hydrolysates obtained also from the leaves (glycosides probably solamargine/solasonine), but no solanine/chaconine. Petersen et al. (1993) were again not able to find solasodine in contrast to solanidine; however, they used the tubers for analysis. Another case is documented with regard to *S. wrightii* BENTH.; this arboreal species, a native of Bolivia, was introduced into different other countries. Therefore, there are three reports on this species from different provenances: In a first study from Portugal the identification of solanidine in the leaves together with rhamnose, arabinose, and glucose was reported (Alves et al. 1961). However, the analysis of an Egyptian sample of the fruits resulted in the detection of solasodine-based alkaloids (Fayez and Saleh 1967). This was confirmed by isolation of solamargine/solasonine from an Indonesian sample of unripe fruits (Indrayanto et al. 1985). It may be speculated that *S. berthaultii* as well as *S. wrightii* are characterized by different chemovarieties (infraspecific variability) like in the case of *S. dulcamara* (see below). Thus, the diverging results might be explained by the specific accessions used. Alternatively, these species may be examples for morphogenetic variability (see also *S. dulcamara*).

Demissidine/Solanidine- and Tomatidine/Tomatidenol-based Glycoalkaloids. The centre of occurrence of these solanidanes and 22β*N*-spirosolanes is given within sect. 10. *Petota* DUMORT. In his list of *Solanum* species in the New World Nee (1999) recognized (according to Hawkes 1990) 232 species in this section. They are arranged in two subsections (*Estolonifera*, *Potatoe*; Table 7.6). Subsect. *Estolonifera* is represented by nine South American non-tuber-bearing species (ser. 1. and 2.) and nine South American tomato species of the former genus *Lycopersicum* (ser. 3. *Neolycopersicon*).

The tomatoes are integrated here, since they have been shown to be nested within a monophyletic larger genus *Solanum* as results of DNA-based as well as morphological-character-based phylogenetic analyses (e.g., Bohs and Olmstead 1997; Olmstead et al. 1999) though this is still discussed controversially (e.g., Nee 1999; Hunziker 2001). For history of tomato classification including molecular systematics see Marshall et al. (2001), Peralta and Spooner (2001), Darwin et al. (2003).

Table 7.6 Distribution and frequency of steroidal alkamines (aglycones) in the series of sect. *Petota* DUMORT (genus *Solanum*)

Sect. 10. *Petota* DUMORT [classification according to Hawkes (1990; fide Nee) and Nee (1999)]	**No. of spp. investigated phytochemically vs. known spp.**	**Solanidane-type**		**Spirosolane-type**		**Variants of rDNA ETS**[c] according to Volkov et al. (2003) (no. of spp. included)
		Demissidine[a]	Solanidine[a]	Tomatidine[a]	Tomatidenol[b]	
Subsect. *Estolonifera* HAWK.						
Ser. 1. *Etuberosa* BUK. & KAMERAZ.	2/5		1	1		A (1)
Ser. 2. *Juglandifolia* (RYDB.) HAWK.	3/4		1	2		
Ser. 3. *Neolycopersicon* (CORRELL) CHILD[d]	7/9			7	3	
Subsect. *Potatoe* G.DON						
Superser. *Stellata* HAWK. (POLYPHYLETIC)						
Ser. 1. *Morelliformia* HAWK.	0/2					
Ser. 2. *Bulbocastana* (RYDB.) HAWK.	0/1					A (1)
Ser. 3. *Pinnatisecta* (RYDB.) HAWK.	5/10	3	2	3	1	A (3)
Ser. 4. *Polyadenia* CORRELL	2/2	1		2		A (1)
Ser. 5. *Lignicaulia* HAWK.	0/1					
Ser. 6. *Circaeifolia* HAWK.	1/3			1	1	B (1)
Ser. 7. *Commersoniana* BUK.	1/2	1	1			B (1)
Ser. 8. *Olmosania* OCHOA	0/1					
Ser. 9. *Yungasensa* CORRELL	4/6	1	3	1		C (1)
Superser. *Rotata* HAWK. (MONOPHYLETIC)						
Ser. 10. *Cuneolata* HAWK.	1/3		1			
Ser. 11. *Megistacroloba* CÁRD. & HAWK.	6/11	4	5	2	1	C (1)
Ser. 12. *Maglia* BITT.	1/1		1			C (1)
Ser. 13. *Tuberosa* (RYDB.) HAWK.: wild spp.	28/65[e]		27		1	C (11)
do. cultivated spp.	7/7	2	7	1	4	C (3)
Ser. 14. *Conicibaccata* BITT.	0/23					C (1)
Ser. 15. *Piurana* HAWK.	0/12					
Ser. 16. *Ingifolia* OCHOA	0/2					
Ser. 17. *Acaulia* JUZ.	1/2	1	1	1		C (1)
Ser. 18. *Longipedicellata* BUK.	4/6		4	1		
Ser. 19. *Demissa* BUK.	4/6	1	4	1	1	C (2)

[a] These steroidal alkamines were detected predominantly after acidic hydrolyzation of the corresponding glycoalkaloids demissine (demissidine-based), solanine/chaconine (solanidine-based), and tomatine (tomatidine-based), respectively

[b] Tomatidenol was detected as such with one exception. This may be due to methodological grounds, i.e., it does not mean that tomatidenol-based glycoalkaloids (solamarines) are absent. However, in sect. *Petota* they were only detected in *S. sogarandinum* OCHOA (series 11.; Ripperger 1998 and references therein)

[c] ETS = external transcribed spacer (for details see text)

[d] This series comprises 9 *Solanum* species assigned in the past to the former genus *Lycopersicum* (*Lycopersicon*) MILL.

[e] The 28. phytochemically checked species (*S. neocardenasii*) is characterized by the occurrence of the β-lycotetraoside of solanocardinol (Table 7.3) instead of solanine/chaconine, the glycoalkaloids of all remaining 27 spp. An additional 23 species described by Ochoa could not be evaluated by Hawkes; thus, the number of known species might be 65 + 23

In these reports the tomatoes are classified on the section level of *Solanum* [*Solanum* L. sect. *Lycopersicum* (MILL.) WETTST.]. Nevertheless, even recent phytochemical studies as well as reports on molecular systematics (Marshall et al. 2001) still use the genus name *Lycopersicum* for wild and cultivated tomatoes, at least due to the huge number of historical references.

Ser. 3. *Neolycopersicon*, according to Spooner et al. (1993) on a section level (sect. *Lycopersicum*), comprises – beside the cultivated *Solanum lycopersicum* (syn.: *Lycopersicum esculentum*) – eight related wild species. Most parsimonious reconstructions divided the monophyletic clade based on the nuclear ITS rDNA region into three subclades (Marshall et al. 2001):

- /"esculentum": *Solanum cheesmaniae* (RILEY) FOSB., *S. chmielewskii* (RICK et al.) SPOONER et al., *S. lycopersicum* (including also var. *cerasiforme*), *S. neorickii* SPOONER et al. (syn.: *Lycopersicum parviflorum* RICK et al.), *S. pimpinellifolium* JUSSL.
- /"peruvianum": *S. chilense* (DUN.) REICHE, *S. peruvianum* L.
- /"hirsutum": *S. hirsutum* (DUN.) MACBRIDE, *S. pennellii* CORRELL.; this subclade is the basal one and thus sister of the two others

These data were congruent with those from morphological studies and results obtained by other molecular methods. However, above all, the study – with more species involved than before – confirmed that the re-classification of *Lycopersicum* turns *Solanum* into a monophyletic group. The species of subclade /"esculentum" were found to be self compatible in contrast to those of both remaining subclades. Red fruits are a character of only three species, *S. cheesmaniae*, *S. lycopersicum*, *S. pimpinellifolium*, forming a monophyletic subclade of /"esculentum", whereas the remaining species are green fruited. From the phytochemical point of view all of the nine species are characterized by the dominating occurrence of the major glycoalkaloid tomatine. This is a convincing chemotaxonomic addition to the morphological and molecular data. Tomatidenol and/or its corresponding glycoalkaloid dehydrotomatine were detected in only four species (*S. cheesmaniae*, *S. lycopersicum*, *S. pimpinellifolium* (/"esculentum") and *S. pennellii* (/"hirsutum"). However, the late discovery of this congener (1994/95) as an impurity of "tomatine" (Friedman 2002 and references therein) might be the reason, why it had not been detected in more *Lycopersicum* spp., since most of them had been already analyzed decades ago. The absence of solanidine- as well as of solasodine-based alkaloids in all of these nine species is also a very remarkable trait.

Of course, the cultivated species *S. lycopersicum* represents the tomato species with the largest number of papers by far. The major alkaloid tomatine (tomatidine β-lycotetraoside) is accompanied by its minor congener dehydrotomatine (tomatidenol-based) in all parts of the plant. Three further minor components (0.00015–0.0005% fresh weight), the stereoisomeric 23-acetoxyspirosolan-3β-ol β-lycotetraosides, lycoperosides A–C (aglycones see Table 7.3) were isolated from leaves and fruits (Yahara et al. 1996c). Lycoperoside D, isolated together with its congeners A–C, turned out to be identical to γ-tomatine. A comprehensive review on tomato glycoalkaloids was published by Friedman (2002). However, in the

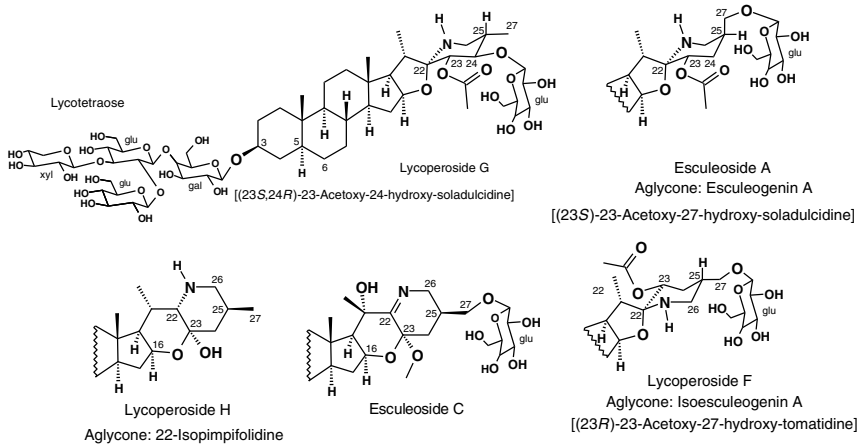


Fig. 7.23 Certain esculeosides (constituents of overripe fruits of *Solanum lycopersicum* var. *cerasiforme*, Cherry tomato) and certain lycoperosides (constituents of ripe fruits of *S. lycopersicum*), bisdesmosidic glycoalkaloids (save lycoperoside H) with structurally diverging types of aglycones: (i) Spirosolane type: lycoperoside G (22α*N*), esculeoside A (22α*N*); lycoperoside F = 22β*N* isomer of esculeoside A; (ii) α-Epiminocyclo(hemi)ketal type: lycoperoside H (*hemiketal*), esculeoside C (*ketal*). *In parentheses*: *Genuine* aglycones still containing an acetyl group at C-23*O*, lost by hydrolysis of the glycosides together with the carbohydrate chains

meantime further alkaloids, *again* β-*lycotetraosides*, have been discovered as constituents of *ripe* fruits of this species (tomato) and of its var. *cerasiforme* (Cherry tomato), respectively: Lycoperosides F–H (Yahara et al. 2004) as well as a novel group of major congeners, esculeosides A–C (Fujiwara et al. 2004). Both groups of glycoalkaloids turned out to be inhomogeneous with regard to the structural types of their aglycones: (i) tomatidine type 3,27-bisdesmosides (e.g., lycoperoside F), (ii) soladulcidine type 3,24- and 3,27-bisdesmosides (e.g., lycoperoside G, esculeoside A), (iii) 3-hydroxy-α-epiminocyclohemiketal/-ketal type monodesmosides [esculeosides B/C, lycoperoside H, (iv) 16-aminoacyloxypregnenolone type (esculeoside D). For structures see Fig. 7.23; further details: Table 7.3 (alkamines) and Table 7.4 (glycoalkaloids).

Recently, the trivial names esculeogenin A and B have been given to the corresponding aglycone of esculeoside A and B, respectively, (Table 7.3) with the advantage of avoidance of the rather complicated chemical term used before (Yoshizaki et al. 2005; Noguchi et al. 2006). It would be reasonable to proceed in a corresponding manner with regard to its congeners C–E. The aglycone of lycoperoside F turned out to be isoesculeogenin A, the 22*R*,23*R*,25*S*-isomer of esculeogenin A (22*S*,23*S*,25*S*), i.e., 22β*N* vs. 22α*N* (Yoshizaki et al. 2005).

For further alkaloids from *S. lycopersicum* (*N*-nitrosotomatidine), *S. pimpinellifolium* (pimpifolidines), and unique steroidal alkamines from the hybrid *S. lycopersicum* × *S. hirsutum* see Table 7.3. These metabolites may represent autapomorphic characters.

There are two reports on structurally elucidated steroid alkaloids in two out of five species of ser. 1. *Etuberosa* Buk. & Kameraz. (solanidine in *S. etuberosum*

LINDL.; tomatidine in *S. brevidens* PHIL.). In ser. 2. *Juglandifolia* (RYDB.) HAWK., comprising four species, two species were investigated phytochemically (solanidine in *S. ochranthum* DUN. sub nom. *S. caldasii* DUN., tomatine in *S. lycopersicoides* DUN.). A third species, *S. juglandifolium* DUN., checked by the above mentioned radioimmunoassay (Weiler et al. 1980) might be characterized by the content of tomatidine rather than by solasodine.

Subsect. *Potatoe* comprises the "true potatoes with their underground stems (stolons) carrying the tubers" (Hunziker 2001). It is subdivided into the superseries *Stellata* HAWK. (nine series) and *Rotata* HAWK. (eight series). They have a common origin as has been proven recently in a phylogenetic study on molecular evolution of rDNA external transcribed spacer of sect. *Petota* (Volkov et al. 2003). This study confirms the monophyletic origin of superser. *Rotata* whereas superser. *Stellata* turned out to be obviously polyphyletic. Since specific phytochemical data are available for almost all 30 spp. included in that study, a corresponding assignment shall be made in the following, extended by further species which were not involved in the phylogenetic study.

This phylogenetic study is based on the 5′ external transcribed spacer (ETS) region of ribosomal DNA. Three variants could be distinguished (last column in Table 7.6): (i) the ancestral organization (variant A) was detected for ser. *Etuberosa* (non-tuber-bearing species) and tuber-bearing wild potatoes of Central America (*Stellata* ser. 2.–4.); (ii) variant B characterized by duplication of a conservative element in a variable region in the ETS was found in *Stellata* ser. 6. and 7., respectively; (iii) variant C with further duplications could be discovered for ser. 11.–14., 17., and 19., i.e., the monophyletic superser. *Rotata* and in addition ser. 9. *Yungasensa* (out of the polyphyletic superser. *Stellata*).

Apparently due to the large interest in cultivated potatoes their wild relatives are *comparably* well-studied with regard to their ability of synthesizing glycoalkaloids (e.g., Schreiber 1963; Petersen et al. 1993 and references therein). A total of 65 out of 166 species listed by Nee in this subsection were investigated phytochemically; 31 were characterized by the occurrence of solanine/chaconine. In additional 25 species solanidine was detected as aglycone of undetermined glycoalkaloids (which – at least in part – might have been also solanine/chaconine). Thus, altogether 56 out of 65 species checked turned out to be solanidine-positive. Hence, subsect. *Potatoe* represents first and foremost a centre for the occurrence of this aglycone and its glycosides, respectively – a fact of chemotaxonomic significance. However, this may be differentiated still a little bit. As already mentioned, this subsection is arranged in two superseries, *Stellata* (ser. 1.–9.) and *Rotata* (ser. 10.–19.). The species are of Central American (ser. 1.–4., 18., 19.) and South American origin (ser. 5.–17.), respectively. Occurrence of solanidine-based alkaloids in superser. *Stellata* (polyphyletic) was shown in only 6 out of 13 glycoalkaloid-positive species. In contrast, 50 out of 52 glycoalkaloid-positive species of superser. *Rotata* (monophyletic) turned out to be solanidine-positive.

Studies on some taxa of superser. *Stellata* led to "weak" results from the phytochemical point of view. A species of ser. 2. *Bulbocastana, S. bulbocastanum* DUN., is remarkable since it showed a very low *foliar* glycoalkaloid level

(13 mg/100 g fresh weight). Thus, identification of any specific metabolite failed (Tingey et al. 1978; Gregory et al. 1981). Even GC-MS analysis of the *tubers* did not lead to the detection of any aglycone (Petersen et al. 1993). This was also true for *tubers* of *S. lignicaule* VARGAS, nested within the monotypic ser. 5. *Lignicaulia*, as well as of *S. capsicibaccatum* CÁRD. (ser. 6. *Circaeifolia*). Reports on the potential content in *other organs* of these species are not available.

There are phytochemically more fruitful reports for other taxa of superser. *Stellata*, especially for the larger series 3. (*Pinnatisecta*). Both, solanidane-type [demissidine (**D**)-, solanidine (**S**)-based alkaloids] and 22β*N*-spirosolanes [tomatidine (**Ti**)-, tomatidenol (**To**)-based alkaloids], could be detected in contrast to ser. *Neolycopersicon* (only **Ti/To**). However, co-occurrence of both types within one species is rare [{**D/S**}/**Ti**: *S. jamesii* TORR. (ser. 3.); **D/Ti**: *S. polyadenium* GREENM. (ser. 4.)]. Solanidanes only were detected in two species [**D/S** in *S. cardiophyllum* LINDL.; **D** in *S. ehrenbergii* (BITT.) RYDB. (ser. 3.)]. 22β*N*-spirosolanes only were found in four species [**Ti/To**: *S. nayaritense* (BITT.) RYDB. (ser. .3.), *S. circaeifolium* BITT. (ser. 6.); **Ti**: *S. pinnatisectum* DUN. (ser. 3.); *S. lesteri* HAWK & HJERT. (ser. 4.). Results for four spp. of the advanced *Stellata* ser. 9. *Yungasensa* (comprising six spp.) are available: No species showed co-occurrence of both structural types. Solanidanes only were detected in three spp. (**D/S**: *S. chacoense* BITT.; **S**: *S. huancabambense* OCHOA, *S. tarijense* HAWK.), whereas a 22β*N*-spirosolane was detected only in one species (**T**: *S. arnezii* CÁRD.).

The following larger series nested within superser. *Rotata* are well-studied: 11. *Megistacroloba*, 13. *Tuberosa*, 18. *Longipedicellata*, 19. *Demissa*. 72 species were assigned by Nee (1999) to ser.13. *Tuberosa*. Not less than 27 wild and 7 cultivated, altogether 34 out of 35 species investigated phytochemically were found to produce solanine/chaconine (20 spp.) or solanidine (14 spp.). However, it may be assumed that the latter species are also able to synthesize the corresponding glycoalkaloids. Depending on the scientific and technical possibilities sometimes only the aglycone could be elucidated, especially in reports of former times.

S. neocardenasii HAWK. & HJERT. represents that species for which an occurrence of solanidine was not yet reported. Instead, the α-epiminocyclohemiketal-type solanocardinol β-lycotetraoside was discovered (Table 7.3). Remarkably, further steroidal aglycones and glycoalkaloids seem to be rare in this series; if there occur any, then only in minor concentrations. Thus, tubers of a certain accession of *S. vernei* BITT. & WITTM. turned out to contain solasodine-based glycoalkaloids beside solanidine-based major congeners as already mentioned above (Van Gelder and Scheffer 1991). Small amounts of tomatidenol could also be detected; this was true again for dark sprouts of *S. tuberosum* (Schreiber 1963). Furthermore, this aglycone was detected by GC-MS analyses of tubers from the also cultivated species *S* × *ajanhuiri*, *S.* × *curtilobum*, and *S.* × *juzepczukii*, whereas tomatidine was found in a certain accession of *S. tuberosum* ssp. *andigena*.

Hawkes recognized seven cultivated potato species known in the Andes (Hunziker 2001):

- *S.* × *ajanhuiri* JUZ. & BUK., a diploid hybrid of the cultivated *S. stenotomum* and *S. megistacrolobum* BITT.; the latter, a frost-resistant wild species, is a member

of ser. 11. *Megistacroloba* and characterized by the occurrence of solanidine, tomatine, demissine, and commersonine

- *S.* × *chaucha* Juz. & Buk., a hybridogenic triploid
- *S.* × *curtilobum* Juz. & Buk., a natural pentaploid hybrid between *S.* × *juzepczukii* Buk. and *S. tuberosum* L. ssp. *andigena*
- *S.* × *juzepczukii* Buk., a natural triploid hybrid of *S. stenotomum* and *S. acaule* Bitt., a frost-resistant wild species of ser. 17. *Acaulia* and characterized by the occurrence of demissine/tomatine beside solanidine
- *S. phureja* Juz. & Buk. (diploid)
- *S. stenotomum* Juz. & Buk. (diploid), considered to be the species from which all other cultivated species arose
- *S. tuberosum* L. ssp. *andigenum* (Juz. & Buk) Hawk. [syn.: *S. andigenum* Juz. & Buk.], indigenous to the Andes from Venezuela to northern Chile/Argentina, supposed to be a tetraploid hybrid of *S. stenotomum* and another frost-resistant wild potato from Peru, *S. sparsipilum* (Bitt.) Juz. & Buk. (Raker and Spooner 2002 and references therein); the worldwide crop, ssp. *tuberosum*, indigenous to south-central Chile, is supposed to be evolved from ssp. *andigenum*. Another wild species from Peru, *S. leptophyes* Bitt., was proposed as an alternative parental candidate beside *S. stenotomum* (Hawkes 1999). These species as well as two further presumptive progenitors, *S. gourlayi* Hawk. and *S. spegazzinii* Bitt., respectively (Volkov et al. 2003) – all members of ser. 13. *Tuberosa* – are also producers of solanidine-based alkaloids.

Solanine and chaconine are the primary glycoalkaloids of all these cultivated species. Both metabolites together represent at least 95% of all the glycoalkaloids in commercial potatoes (Friedman and McDonald 1997). The formation of demissine in *S.* × *curtilobum* as well as in *S.* × *juzepczukii* in addition to solanine/chaconine is contributed apparently by their parentage from *S. acaule*. The occurrence of minor concentrations of non-solanidine-based alkaloids in certain cultivars of *S. tuberosum* might be caused also by crossing with wild species in order to transfer desired traits, e.g., resistance against herbivores or pathogens by alien steroidal alkaloids. Thus, up to 80% of *S. tuberosum* cultivars contain genetic material of the Mexican wild potato S. *demissum* (Volkov et al. 2003). Such breeding lines, cultivated partly since ancient times (potatoes were domesticated in the Bolivian-Peruvian Andes at least 7000 years ago) may contribute to the problem that "the origin of the tetraploid cultivated potato *S. tuberosum* still remains a subject of debate…….. due to the mosaic constitution of cultivated potato genomes" (Volkov et al. 2003). "In traditional Andean farming systems, there is ample room for mixing wild and weedy species with cultivated potatoes….. Farmers select and maintain varieties on factors such as yield, disease resistance, storage longevity, and taste…. Because of this selection within a loose agroecosystem, the species of the Andean potatoes likely cross and form a large plastic genepool." (Raker and Spooner 2002).

Somatic hybrids between a *S. tuberosum* line and an accession of the non-tuber-bearing wild potato species *S. brevidens* (see above) were checked with regard to the formation of parental-type and novel glycoalkaloid aglycones. The leaves of the

cultivated species contained solanidine (400 mg/kg dry weight), the leaves of the wild species tomatidine (>8000 mg/kg). In the leaves of the somatic hybrids demissidine could be detected which was absent in both parental species (Laurila et al. 1996). The authors proposed an interesting hypothesis for the occurrence of this new metabolite: The hydrogenase of *S. brevidens*, responsible for the formation of tomatidine from its (probable) precursor teinemine, is also able to hydrogenate the corresponding double bond in solanidine (origin: *S. tuberosum* genome) resulting in demissidine production.

Dominance of solanine/chaconine and absence or at least unusual rareness of other steroidal alkaloids are also consistent traits for ser. 18. *Longipedicellata* and ser. 19. *Demissa*, save *S. demissum* LINDL. This species is characterized by the dominant occurrence of demissidine (5α-dihydrosolanidine)- and tomatidine-based tetraoside type glycoalkaloids (demissine/commersonine; tomatine), respectively. However, solanidine could also be detected in a certain accession by GC-MS analysis (Petersen et al. 1993). Ser. 11. *Megistacroloba* is the only one of all *Rotata* series (cultivated species of ser. 13. *Tuberosa* excluded for reasons discussed above) which turned out to possess a more diverse alkaloid profile: Co-occurrence of solanidine (**S**) and demissidine (**D**)-based alkaloids (demissine/commersonine) was detected in four out of six species (*S. boliviense* DUN., *S. toralapanum* CÁRD. & HAWK, *S. megistacrolobum* BITT., *S. sanctae-rosae* HAWK.). The tomatidine (**T**)-based additional glycoalkaloid tomatine was found in the latter two of these species. In contrast, *S. raphanifolium* CÁRD. & HAWK. (**S**-based solanine/chaconine) and *S. sogarandinum* OCHOA (tomatidenol-based alkaloids α-/β-solamarine) are characterized by a poorer alkaloid profile. Conspicuously, solasodine-based glycoalkaloids like solamargine/solasonine were almost never detected in any species of the large subsect. *Potatoe*. Even GC-MS analysis of 50 species of this subsection turned out to be solasodine-negative with regard to tubers in all of these taxa (Petersen et al. 1993).

There are a few species which are reported to contain solanine beside solamargine/solasonine (see above). Beside those rare examples there are only two species, *S. agrimoniifolium* RYDB. and *S. columbianum* DUN., respectively, which are equivocal with regard to this point. These species belong to the large ser. 14. *Conicibaccata* BITT. (23 spp. listed by Nee plus another 17 spp. not evaluated by Hawkes/Nee) which is otherwise unexplored phytochemically. Due to the analytical method applied in this case it is not clear, whether glycoalkaloids of these two species are solasodine- or tomatidine-based (Weiler et al. 1980). However, the latter would be more probable in the context of the whole subsect. *Potatoe*. Unfortunately, there are also no phytochemical reports with regard to another large series [ser. 15. (12 spp.)] beside some small series (1., 2., 5., 8.).

Anyhow, the *presence* of solanidine-/demissidine- and/or tomatidine/tomatidenol-based, i.e., 22β*N*-spirosolane-type alkaloids as well as the *absence* of solasodine-based, i.e., 22α*N*-spirosolane-type congeners are apparently consistent traits of the sect. *Potatoe*. They are of unequivocal chemotaxonomic relevance. The unusually predominating role of solanidine-based alkaloids, even present in five out of six species of the phytochemical more complex ser. *Megistacroloba*, as well as the almost complete lack of solasodine-based congeners are interesting parallel traits to the occurrence of the variant C of rDNA ETS.

Finally, some phytochemical remarks with regard to subgenus *Solanum*, sect. 5. *Dulcamara* (MOENCH) should be added. First, there is a number of South American species widely cultivated as ornamentals in tropical and subtropical areas (Nee 1999; Hunziker 2001). Thus, at least from the safety point of view, knowledge about steroidal alkaloids in such species ought to be of interest like in other cultivated species mentioned already above:

- *S. amygdalifolium* STEUD. (syn.: *S. angustifolium* LAM.), common names: duraznillo enreda, jazmín de Córdoba; alkaloids: "solangustine", a not well-characterized glucoside of "solangustidine" which was regarded as "an isomer of solasodine" (Tutin and Clewer 1914; Schreiber 1968); more recently the presence of solasonine in the aerial parts was proved (Vázquez et al. 1999).
- *S. angustifidum* BITT., jazmín de Córdoba; alkaloids: Unknown
- *S. laxum* SPRENG. (syn.: *S. jasminoides* PAXT., *S. boerhaaviifolium* SENDT.), potato vine; alkaloids: solamargine, solasonine
- *S. seaforthianum* ANDR., St Vincent lilac; alkaloids: solanoforthine (3-amino-α-epiminocyclohemiketal), solaseaforthine, isosolaseaforthine (3-amino-22,26-epiminocholestanes) (Table 7.3, Fig. 7.20)

Intraspecific and Morphogenetic Variability. Another point of interest is given by the type species of the section just discussed, *S. dulcamara*. Due to its traditional use in European and other countries as a medicinal plant this species is one of the best-studied of the genus (Schreiber 1958a; Boll and Andersen 1962; Sander 1963a, b; Willuhn 1966; 1967; Ehmke and Eilert 1993 and references therein). It turned out to be characterized by the occurrence of three chemovarieties: (i) solasodine-based glycoalkaloids (solamargine, solasonine; rare occurrence of this chemovariety), (ii) soladulcidine-based congeners [trisaccharadic soladulcines (α-, β-, γ-), soladulcidine tetraoside; occurrence: drier European continental climates], (iii) tomatidenol-based congeners (soladulcamarine, solamarines; occurrence: humid Atlantic climate of Western Europe). Surprisingly, these characters were confined to leaves and stems.

Soladulcamarine, later also detected as a constituent of the leaves of *S. dasyphyllum* SCHUM. & THONN. (Coune 1977), is characterized – beside glucose and 2 rhamnose units – by the rare presence of arabinose (Baggesgaard-Rasmussen and Boll 1962).

Flowers and fruits of any of these chemovarieties of *S. dulcamara* showed exclusively solasodine-based glycoalkaloids, whereas roots contained the additional aglycone tomatidine and/or 15α-hydroxylated congeners of all four aglycones mentioned. Even 15β-hydroxy-soladulcidine and -solasodine were detected (Table 7.3). Two further soladulcidine-based glycoalkaloids, soladulcines A (β-chacotrioside) and B (β-lycotetraoside), were isolated and structurally elucidated from the aerial parts of this species in Japan (Lee et al. 1994).

Another economically important edible fruit is yielded by *S. muricatum* AIT., subgen. *Solanum* [syn.: subgenus *Potatoe* (G.DON) D'ARCY], sect. *Basarthrum* BITT., common name: Pepino/pepino dulce. The species is cultivated also out of the Americas (e.g., New Zealand). It is not known in the wild, but originates undoubtedly in Andean countries (Nee 1999). Steroidal alkaloids are produced unequivocally by

the plant though they have not yet been identified specifically. Furthermore, beside those species mentioned already to be cultivated for their edible organs, i.e., berries or glabrous leaves with low or almost no content of steroidal alkaloids, such parts of many other *Solanum* spp., cultivated, semicultivated or wild, are eaten on all continents. According to Daunay et al. (2001 and references therein) these species are mentioned mainly in papers dealing with ethnobotany.

7.8.2.2 Occurrence in Other Genera (Fig. 3.18; Table 7.7)

There are reports on only a few erratic occurrences of steroid alkaloids out of the genus *Solanum*. This is not surprising for two genera belonging to the /Capsiceae clade, ***Capsicum*** and ***Lycianthes***, which are closely related to *Solanum*. Solanine was identified in all organs of a certain variety of *C. annuum* (Gutsu et al. 1984). Two *Lycianthes* spp. showed different steroid alkaloids. The still more advanced genus ***Saracha*** (/Physaleae clade, /Iochrominae subclade), is represented by *S. punctata* Ruiz & Pav. From the leaves of this shrub, occurring in the humid highland forests between Venezuela and Bolivia, a novel 3β-amino-22,26-epiminocholestane-type alkamine, sarachine, could be isolated which is the first natural 5,6-dehydro derivative of this specific type. However, the C-22 configuration still

Table 7.7 Occurrence of steroidal alkaloids in species of other solanaceous genera (out of *Solanum*)

Species	Alkamines	Glycoalkaloids	References
Capsicum annuum L., red/bell pepper, chilli, paprika	Solanidine	Solanine	(1); (2)
Cestrum elegans (Brongn.) Schltdl. sub nom. *C. purpureum* Standl.	Solanidine, solasodine		(3)
Cestrum parqui L'Hérit., willow leaf jessamine, duraznillo negro	Solasodine	Solasonine	(4)
[*Cyphomandra betacea* (Cav.) Sendt. (valid name: ***Solanum betaceum*** Cav.); tree tomato, tamarillo]	[Solasodine, tomatidenol]		[(1)]
Lycianthes biflora (Lour.) Bitt.	2α-hydroxysoladulcidine, soladulcidine, solasodine		(5)
L. rantonnetii (Carr.) Bitt. sub nom. *S. rantonnei* Carr., blue potato bush	Tomatidine	Tomatine	(1)
Nicotiana plumbaginifolia Viv.	Solasodine	Solaplumbine	(6)
Saracha punctata Ruiz & Pav.	Sarachine (3β-amino-22,26-epiminocholestane-type)		(7)

References: (1) Schreiber 1968 and references therein; (2) Gutsu et al. 1984; (3) Karawya et al. 1972; (4) Silva et al. 1962; (5) Ripperger and Porzel 1992; (6) Singh et al. 1974; (7) Moretti et al. 1998

remains to be determined (Moretti et al. 1998). The remaining genera belong to much more basal clades which turns the occurrence into a more unexpected result. ***Cestrum*** (/Cestreae clade, Cestroideae s.s.): Silva et al. (1962) found solasonine in *C. parqui* L'HÉRIT. Solanidine and solasodine were reported to be constituents of *C. elegans* (BRONGN.) SCHLTDL. sub nom. *C. purpureum* STANDL. whereas *C. auranti-acum* LINDL. and *C. diurnum* L. turned out to be devoid of any steroidal alkaloid (Karawya et al. 1972). ***Nicotiana*** (/Nicotianeae clade, Nicotianoideae): Very surprisingly, a solasodine-based bioside (glucose, rhamnose), solaplumbine, was reported as a constituent of the aerial parts of *Nicotiana plumbaginifolia* VIVIANI (Singh et al. 1974); this species is well-known to contain nicotinoids in leaves and roots (see Table 3.4.).

Reports with negative results are rather rare though not without interest. One of these rare examples should be added: No glycoalkaloids could be detected in *Lycium ruthenium*, *Nicandra physalodes* (L.) GAERTN., *Physalis alkekengi* L., and *P. peruviana* L. (Tukalo 1964).

7.8.2.3 Co-occurrence of Steroidal Alkaloids/Glycoalkaloids and Withasteroids or Tropanes (Fig. 3.18)

Co-occurrence of steroidal alkaloids/glycoalkaloids and withasteroids has been demonstrated in two *Solanum* spp., *S. ciliatum, S. sisymbrifolium* (see also Sect. 7.10.3). Co-occurrence of steroidal alkaloids/glycoalkaloids and tropanes is confined to tropan-3-one, 3-tropanols/*nor*tropanols, and calystegines in the case of the genus *Solanum* and *Capsicum annuum* (Tables 3.1 and 3.8). There are no reports with regard to these unacylated tropanes/*nor*tropanes in the glycoalkaloid-positive species of the remaining genera. Apparently solanaceous species which synthesize steroidal alkaloids/glycoalkaloids have at least lost the ability to acylate 3-tropanols. This is true for both subclasses of tropane alkaloids of Fig. 3.18.

7.8.3 Biosynthesis

Glycoalkaloid biosynthesis begins – as has been reported comprehensively for *Solanum tuberosum* by Friedman and McDonald (1997) – during germination reaching a peak during the flowering period. Synthesized independently in all organs and parts of the plant, the highest levels of the alkaloids are detectable in flowers, unripe fruits, sprouts, and new leaves, while levels in older ones decrease. Tomato plants, *S. lycopersicum*, also show their highest glycoalkaloid concentrations in the flowers. No glycoalkaloids were found in their dormant seeds, traces in *S. laciniatum* seeds, but high concentrations in *S. aculeatissimum* seeds (Heftmann 1983 and references therein). In contrast to, e.g., tropane alkaloids, glycoalkaloids formed in roots and tubers are not transported upwards to epigeal parts. This is also true for transport between other organs (Friedman and McDonald 1997), e.g., from

shoots to roots studied with *S. dulcamara* (solasodine- as well as tomatidine-type strains; Willuhn 1967), from vegetative organs into fruits studied with *S. lycopersicum* (Friedman 2002 and references therein).

Alkamines. Steroidal alkamines and steroidal sapogenins may co-occur in the same plant. This was documented in many cases (for examples see Table 7.8). Thus, it is not surprising that there are several similarities concerning the biosynthesis of both groups. The exact pathway "cholesterol → steroidal alkamines" has not been fully proven (Friedman and McDonald 1997). Dormantinol, a sapogenin discovered as a constituent of dormant (sic!) budding stages of *Veratrum grandiflorum* (MAX.) LOESEN, Melanthiaceae (traditionally: Liliaceae s.l.), originally supposed to be an early precursor in solanidine biosynthesis of *Veratrum* (Kaneko et al. 1976, 1977b), was also assumed to be involved in the biogenesis of *Solanum* steroidal alkamines (Friedman and McDonald 1997; Friedman 2002). The next

Table 7.8 Co-occurrence of steroidal alkaloids and sapogenins (examples)

Species	Steroidal alkamine	Steroidal sapogenin	References
Cestrum parqui	Solasodine	Tigogenin Digalogenin Digitogenin	Schreiber 1968 and references therein
Solanum abutiloides (GRISEB.) BITT. & LILLO	Aglycones of abutilosides C – G (see Subsect. 7.7.1)	22*S*,25*S*-Epoxy-furost-5-enes (saponins: abutilosides L – O)	Yoshimitsu et al. 2003
S. dulcamara L.	Soladulcidine Solasodine Tomatidenol	Tigogenin Diosgenin Yamogenin	Willuhn (1966) and references therein
S. laciniatum AIT.	Soladulcidine Solasodine Tomatidenol	Tigogenin Diosgenin Yamogenin	Schreiber 1968 and references therein
S. lycopersicum L.	Tomatidine	Neotigogenin Tigogenin	Schreiber 1968 and references therein
S. mammosum L.	Solasodine	Diosgenin	Indrayanto et al. 1998 and references therein
S. melongena L.	Solasodine	Diosgenin	Paczkowski et al. 1998 and references therein
S. nigrum L.	Solasodine	Tigogenin	Schreiber 1968 and references therein
S. panduraeforme E.MEY	Tomatidenol	Yamogenin	Döpke et al. 1987
S. paniculatum L.	Jurubidine	Neochlorogenin Paniculogenin	Schreiber 1968 and references therein
S. pimpinellifolium JUSSL.	Tomatidine	Neotigogenin	Schreiber 1968 and references therein
S. spirale ROXB.	Tomatidenol	Yamogenin	Quyen et al. 1987
S. tuberosum L.	Solanidine Tomatidenol	Yamogenin	Schreiber 1968 and references therein

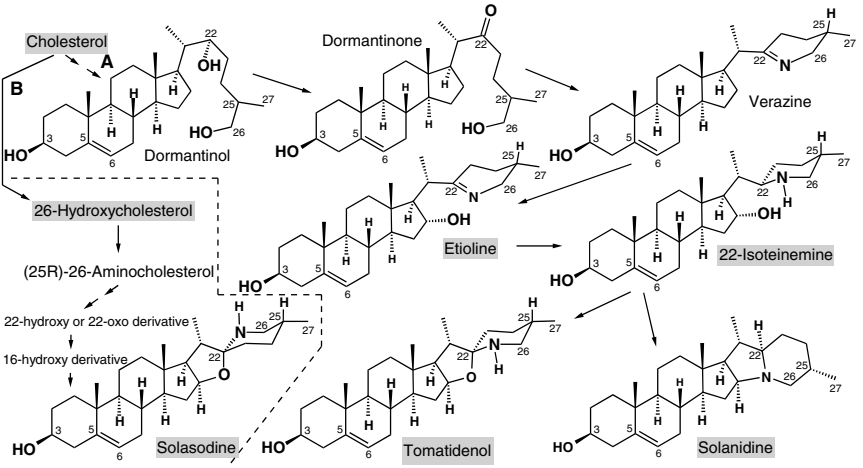


Fig. 7.24A,B Hypothetical biogenetic pathway of: **A** tomatidenol and solanidine proposed by Kaneko et al. (1976, 1977b, c) and **B** solasodine according to Tschesche and Brennecke (1980); the structure of the originally hypothesized "teinemine" as a precursor of tomatidenol/solanidine turned out to be 22-isoteinemine (Ripperger 1998). *Highlighted in grey*: metabolites detected in *Solanum* spp., thus supporting the hypothetical pathways

step in this sequence led to the 22-oxo derivative dormantinone (pathway A in Fig. 7.24). Verazine, a minor alkaloid discovered again in budding *V. grandiflorum*, should represent the first pathway-specific alkamine. This metabolite has not yet been detected in a solanaceous species in contrast to the following two alkaloids. (i) Etioline, discovered in the same *Veratrum* species, named due to the fact that it was discovered in the early stage of etiolation, and proposed as an intermediate in the solanidine biosynthesis (Kaneko et al. 1971), was also found in *Solanum* spp., e.g., *S. havanense* (Table 7.3). (ii) The structure given for the following metabolite of the pathway, termed "teinemine" by Kaneko et al. (1977c), later turned out to be identical to 22-isoteinemine [vice versa the alkaloid originally named "isoteinemine" is now teinemine (Ripperger 1998)]. Consequently, the intermediate in Fig. 7.24 has been termed 22-isoteinemine in contrast to former depictions (e.g., Heftmann 1983; Friedman and McDonald 1997; Friedman 2002). Both isomers were detected in, e.g., *S. capsicastrum* (Table 7.3). They were named according to the Ainu term "teine" for *Veratrum* (Ripperger and Schreiber 1981).

An alternative pathway was proposed for 22α*N*-spirosolanes, e.g., solasodine (pathway B in Fig. 7.24). Incorporation studies with labelled potential precursors administered to *S. laciniatum* indicated that the introduction of nitrogen occurs immediately after the hydroxylation at C-26 [forming (25*R*)-26-aminocholesterol] and before a further oxidation of the side chain. Afterwards, probably oxygenation of C-22 takes place as a prerequisite for the formation of ring F. Incorporation studies with the labelled 22,26-epiminocholestane solacongestidine (Fig. 7.19) and its

(22*S*)-22,*N*-dihydro congener showed that both compounds were converted – presumably via hydroxylation at C-16 followed by closure of ring E – to soladulcidine (Fig. 7.19) by *S. dulcamara*. In parallel assays with labelled (22*S*:25*R*)-22,26-epiminocholest-5-en-3ß-ol and its 16β-hydroxy derivative conversion to solasodine by *S. laciniatum* could be demonstrated. Both results are in favour of a primary formation of ring F followed by closure of ring E, homologous to the biosynthesis of tomatidenol/solanidine (pathway A in Fig. 7.24) (Tschesche and Spindler 1978; Tschesche and Brennecke 1980; Heftmann 1983; Petersen et al. 1993). Replacement of the terminal hydroxyl (C-26) by an amino group (Tschesche et al. 1976) is achieved by an exchange reaction which involves amino acids such as glycine, alanine or arginine as donor molecules (Heftmann 1983 and references therein). The configuration of the spirostanol alkaloids is apparently determined by the C-26 hydroxylating enzyme, since labelled (25*R*)-26-hydroxycholesterol applied to *S. lycopersicum* was incorporated only into soladulcidine but not into tomatidine (Tschesche et al. 1976).

However, there remains to be answered, at what stage of the pathway the corresponding 5,6-saturated congeners of solasodine, tomatidenol, and solanine, i.e., soladulcidine, tomatidine, and demissidine, are formed by enzymatic hydrogenation. Different postulations were published: (i) At an early stage, i.e., cholesterol → cholestanol (= 5,6-dihydrocholesterol; Petersen et al. 1993), (ii) as a final reaction, i.e., e.g., tomatidenol → tomatidine (Friedman 2002), or (iii) somewhere at an intermediate stage of the pathway. Even a vice versa reaction might be, i.e., dehydrogenation of saturated aglycones, e.g., tomatidine → tomatidenol. Variations are imaginable dependent on the species.

Results obtained by the group of Nohara from the fresh roots of *S. abutiloides* strongly support the hypothesis of Petersen et al., though on the level of the corresponding glycosides. From the fresh roots of this species, Ohmura et al. (1995) could isolate a glycosidic 26(*N*)-acylaminocholestan-22-one, the first 26-aminocholestanol glycoside, which was named later abutiloside A (aglycone: Fig. 7.21; Tian et al. 1997). In addition abutiloside B, its 26(*N*)-acetamino congener (aglycone: Fig. 7.21), and abutiloside C, the corresponding 26-hydroxy derivative (see also Sect. 7.7.1.1), could be characterized (Tian et al. 1996). De-*N*-acylation of the aglycone of abutiloside A yielded the 22,26-epiminocholestane type congener solafloridine (Fig. 7.19) by 22,*N*-cyclization. These abutilosides, 16α-hydroxy derivatives – like etioline, an intermediate in the biogenetic pathway of solanidine – though on a glycoside level, were regarded to be key intermediates in the biogenesis of 22α*N*-spirosolanes and other metabolites (cholesterol → → → abutiloside C → A and/or C → B). They co-occurred with the 5,6-saturated aglycones soladulcidine and solafloridine but also with the 5,6-dehydro type glycoalkaloid solamargine. Solasodine, the aglycone of the latter metabolite, might be formed – at least in *S. abutiloides* – by dehydrogenation during this pathway at an unknown stage as an alternative of the formation by pathway B discussed above (Fig. 7.24; Tschesche et al. 1976). The discovery of abutiloside H (Fig. 7.21, Yoshimitsu et al. 2002), the 5,6-dehydro derivative of abutiloside B, in the same species lends support to this hypothesis.

Abutiloside C represents an *N*-free congener, i.e., a steroidal saponin. In this connection the co-occurrence of protodioscin/diosgenin (Tian et al. 1997) is also of biogenetic relevance for this sister group of metabolites (see Sect. 7.7).

Steroidal Glycoalkaloids. The monosaccharide unit directly conjugated to the hydroxyl group at C-3β of the alkamine is predominantly provided by galactose (e.g., solatriose, lycotetraose) or glucose (e.g., chacotriose) (structures: Fig. 7.22). Consequently, UDP-galactose- and UDP-glucose-dependent specific glycosyltransferases could be characterized in *Solanum tuberosum*, *S. melongena*, and *S. lycopersicum* with preferred acceptor molecules such as solanidine, solasodine, and tomatidine, respectively (Kalinowska et al. 2005 and references therein). Thus, UDP-galactose:solanidine galactosyltransferase (SGT1) could be characterized as the enzyme for the biosynthesis of γ-solanine. UDP-glucose:solanidine glucosyltransferase (SGT2) catalyzes correspondingly the one of γ-chaconine (McCue et al. 2006). Such enzymes turned out to be almost completely inactive towards phytosterols as substrates. UDP-glucose:solasodine glucosyltransferase from *S. melongena* displayed only very limited activity in connection with solanidine or demissidine (Kalinowska et al. 2005 and references therein). Furthermore, UDP-glucose:diosgenin glucosyltransferase, a clearly distinguishable enzyme, proved to be present in this species suggesting that 3-*O*-glucosylation of steroidal sapogenins and alkaloids – at least in this species – is catalyzed by two separate enzymes (Paczkowski et al. 1998). Recently, potato β-solanine/β-chaconine rhamnosyltransferase (SGT3), the enzyme catalyzing the terminal step in formation of the potato glycoalkaloid triose side chains, could be identified (McCue et al. 2007). Thus, this last step in the specific triose formation for *both* branches of the two predominant potato glycoalkaloids, (α-)solanine and (α-)chaconine, seems to be catalyzed by the same enzyme (formation of a branched trisaccharide by linkage at C-2 of the corresponding hexose unit which in turn is conjugated to the aglycones). However, the enzymes for the second step (γ-glycoalkaloids → β-glycoalkaloids) – a rhamnosyltransferase and a glucosyltransferase, respectively – are still unknown.

Influence of Light Exposure. Of course, the environment may influence the composition of steroidal alkaloids qualitatively as well as quantitatively. One example is given by the influence of light. As mentioned already *S. vernei* is able to produce minor concentrations of solasodine-based alkaloids beside major solanidine-based congeners. Interestingly, the solasodine portion of the tubers was enlarged 13 times when plants of a certain clone of this species were grown under short-day instead of long-day photoperiods, whereas the solanidine portion was reduced to 60%. Thus, the solasodine-content increased up to almost 50% of the solanidine-content (long-day: 4%). Furthermore, tomatidenol could be also detected under short-day photoperiods though being undetectable under long-day conditions. Direct light exposure of *S. phureja* tubers and cultivated *S. tuberosum* tubers increased similarly the concentrations of solanine/chaconine in both species. In addition it induced the synthesis of the tomatidenol-based glycosides α- and β-solamarine in the tubers of the wild species. The latter metabolites were not detectable in tubers stored in

darkness (Griffiths et al. 2000). The total alkaloid concentration of non-sprouted potato tubers of a number of cultivars was steadily increasing with time during exposure (15 days) to sodium or fluorescent light with no indication of cessation. Synthesis of solanine was enhanced increasingly over that of chaconine (Percival 1999). Such studies are of importance with respect to consumer safety (for more details see also Friedman and McDonald 1997 and references therein).

Catabolic Metabolism of Glycoalkaloids. Degradation of glycoalkaloids, a common process during the maturation of *Solanum* berries and apparently in favour of fruit/seed dispersal, e.g., by birds, is well-studied in case of tomatoes. The change from green (unripe) to red (ripening) coloured berries, which is caused by the degradation of chlorophyll as well as by the synthesis of the tetraterpenes β-carotene/lycopene (Sect. 7.12) and accompanied by the corresponding increase in the fruit size, leads to an increasing metabolization of tomatine (Friedman 2002 and references therein). This major glycoalkaloid was assumed to be degraded to 5α-pregn-16-en-3β-ol-20-one [5α-pregn-16-enolone or *allo*pregnenolone; Fig. 7.15], discovered in fruits of *S. pimpinellifolium* JUSSL. sub nom. *Lycopersicum pimpinellifolium* (JUSSL.) MILL. as they ripen (Schreiber and Aurich 1966). However, no radioactive trace of this metabolite could be detected in the ripened fruit after the labelled corresponding aglycone, tomatidine, had been administered to a green fruit (Bennett et al. 1967). One reason for this contradictory result might be the fact that the tomatidine moiety *when integrated into the tomatine molecule* (Fig. 7.22) is degraded whereas the carbohydrate chain remains intact (Friedman 2002 and references therein). Thus, it may be that *free* tomatidine is not attackable by the metabolizing enzyme. In this connection it is interesting to take into account that green fruits apparently do not contain tomatidine but tomatine degradation products. Anyhow, labelled tomatine injected into ripe tomato fruits was shown to be converted to *allo*pregnenolone (Heftmann 1983 and references therein). 23-Hydroxylation was proposed as an initial step into the metabolizing process (Schreiber and Aurich 1966). Indeed, hydroxylation at C-23 of an aglycone may cause a chemical destabilization of the rings E and F (aza-oxa-spirane structure; Tschesche and Spindler 1978) with the consequence of an easy non-enzymatic conversion to a corresponding pregnane derivative as has been demonstrated recently (Noguchi et al. 2006). In the same report the corresponding β-lycotetraoside of *allo*pregnenolone has been isolated from over-ripe fruits of Cherry tomatoes (*S. lycopersicum* var. *cerasiforme*) that showed decreasing amounts of esculeoside A (Fig. 7.23; Table 7.3), a 23-*O*-substituted 22α*N*-spirosolan-type congener of tomatine (Fujiwara et al. 2005; Noguchi et al. 2006). Degradation of glycoalkaloids may also occur in the plant in order to avoid autotoxicity: Different organs of *S. tuberosum* including tubers were found to contain enzymes that cleave individual monosaccharides from (α-)solanine and (α-)chaconine (Friedman 2006 and references therein). Solasodenone (4-solasoden-3-one, Table 7.3) and 3β-hydroxy-5α-pregnan-16-one were isolated from *S. hainense* and proposed to be degradation products of solasodine (Heftmann 1983 and references therein).

7.8.4 Significance

7.8.4.1 Toxicology

Early reports on pharmacologic/toxicological studies of solanine and solanidine from potatoes were published already in the last third of the nineteenth century [fatal poisoning of pigs by solanine (Dragendorff 1868); experiments with the glycoalkaloid and its alkamine by Balmannya (1874) and Husemann (1875)]. Solanine and chaconine are considerably poisonous natural products. Their lethal doses were estimated to range from 1.75 mg/kg to 6 mg/kg body weight (apparently by parenteral application). This implicates that their acute toxicity is at least the same as for strychnine (Grunenfelder et al. 2006 and references therein). LD_{50} values in mice (i.p.) given in the literature for solanine differ in a range of 32–75 mg/kg. Values for a mixture of solasodine-based glycoalkaloids isolated from the fruits of *S. sodomeum* (33% solamargine, 33% solasonine, 33% mono- and diglycosides) were found to be 30 mg/kg in mice and 41 mg/kg in rats, respectively. The corresponding LD_{50} by gastric intubation in mice was 550 mg/kg (Cham et al. 1987). Due to different parameters, e.g., malabsorption (caused by partial enzymatic and/or acidic hydrolysis to less poisonous aglycones in the gastrointestinal tract or binding to sterols in the diet) and quick excretion, acute peroral toxicity of glycoalkaloids is much lower than parenteral toxicity [e.g., LD_{50} values of solanine/rats/peroral vs intraperitoneal (i.p.) application 590:71 mg/kg; / mice / >1000:34 mg/kg] (Friedman 2006 and references therein). The present toxicological knowledge with regard to humans including fatal poisonings is mainly due to the ingestion of glycoalkaloids from potatoes. A corresponding summary of such reports was published by Friedman and McDonald (1997). However, there also toxicological reports on other *Solanum* spp.; two examples will be given below.

Glycoalkaloids are characterized by at least two principal toxic mechanisms: They cause (i) disruption of cell membrane and (ii) inhibition of acetyl cholinesterase (AChE) as well as butyryl cholinesterase (BuChE). They share the former mechanism with steroidal saponins (Sect. 7.7.4), whereas the latter one is confined to the *N*-containing congeners. The unshared electron pair on the ring nitrogen of the aglycone is assumed to be required for formation of bioactive iminium ions. Thus, glycoalkaloids are characterized by an increased poisonous potency. However, severe gastrointestinal necrosis rather than inhibition of acetylcholinesterases turned out to be the mechanism by which subchronic toxicity studies with hamsters, an appropriate animal model with regard to the comparability with humans, led to deaths. Weak pulse, but high pulse rate, shallow, but rapid breathing, delirium, and coma are symptoms of an acute poisoning which are contributed to an inhibition of AChE in the central nervous system. Furthermore, nausea, vomiting, diarrhoea, abdominal pain, fever, and disorientation have been observed (Friedman 2006 and references therein).

Teratogenicity and embryotoxicity caused by glycoalkaloid-containing diet could be observed in animal studies. However, the mechanism of action is not yet

really understood. Alterations in ion channels are discussed, since glycoalkaloids were shown to interfere with transport of Ca^{++} across the cell membrane. On the other hand, the membrane disrupting effect – also given in case of (*N*-free) steroidal saponins – does not seem to be relevant, since the nitrogen on the steroid moiety is required for teratogenicity and is also involved in binding to membrane receptor sites (Friedman 2006 and references therein). Implication of a C-5/C-6 double bond was found to be a key structural factor in glycoalkaloid-induced mammalian teratogenesis rather than the stereochemistry at C-22 (Gaffield and Keeler 1993). Teratogenic potencies after peroral application were shown to be in the order chaconine (43) > solanine (32) = solanidine (32) > tomatidine (0) (relative potencies in Syrian hamsters given in parentheses, the *Veratrum* alkaloid jervine defined to be 100). The aglycone solanidine turned out to be as potent as the corresponding glycoalkaloid in this specific effect. This is remarkable, since solanidine is supposed to be the least toxic in all effects within the sequence α-solanine > β-solanine > γ-solanine > solanidine (Friedman and McDonald 1997 and references therein). Whether perorally consumed glycoalkaloids are teratogenic for humans remains to be elucidated (Friedman 2006).

Inhibition of Cholinesterases. Dietary ingestion of steroidal glycoalkaloids can initiate a cholinergic syndrome in humans (Krasowski et al. 1997). Cholinesterases represent enzymes inactivating the neurotransmitter acetylcholine at the synapses of the cholinergic system by hydrolysis to choline and acetate. The cholinesterase inhibiting effect of potato glycoalkaloids was discovered in the fifties of the past century (Pokrovskii 1956; Orgell and Vaidya 1958). Chaconine and solanine turned out to be not only strong, but also equal potent inhibitors of AChE (IC_{50} values: 14–17 μM) as well as BuChE (IC_{50}: 0.07–0.17 μM). A carbohydrate chain is crucial for this activity, since their common aglycone solanidine is inactive (Friedman 2006 and references therein). Since chaconine turned out to be stronger than solamargine though both glycoalkaloids share the same carbohydrate side chain (*chacotriose*) the structure of the aglycone apparently is decisive (Roddick et al. 1990). Later it was demonstrated that solamargine as well as β-solamarine, again a *chacotrioside* (tomatidenol-based), showed no AChE-inhibiting activity at a concentration of up to 100 μM in contrast to chaconine which was highly active. This does not mean that solamargine and β-solamarine are inactive, however, their activities are very much reduced (Roddick 1989). The *solatriosides* solanine and solasonine were characterized by properties which corresponded to their respective *chacotrioside* congener, i.e., highly and slightly inhibitory, respectively (Roddick et al. 2001). Thus, it may be concluded that the contribution of AChE-mediated effects to toxicity is of less significance in those solanaceous species which are characterized by the content of the solasodine-based or tomatidenol-based glycoalkaloids. In contrast, tomatine was shown to be an effective inhibitor of AChE though with lower activity than chaconine/solanine. The corresponding aglycones solanidine (see also above), tomatidine, and solasodine produced only slight to negligible inhibition (Roddick 1989).

With regard to defensive strategies of the plant glycoalkaloids substitute tropane alkaloids such as hyoscyamine/atropine and scopolamine which do not occur in

Solanum species. Like certain tropanes glycoalkaloids – especially solanidine- and tomatidine-based ones – are also able to interact with the cholinergic system of potential herbivores though in a different manner. These tropanes are competitive antagonist at all subtypes of muscarinic receptors (acetylcholine is their physiological agonist). Glycoalkaloids are inhibitors of acetylcholine degrading enzymes.

Glycoalkaloids are even more effective as defensive weapons since they may attack at diverging targets ("dirty drugs"). In contrast to the tropanes mentioned above they are also able to disturb other parts of the cholinergic systems, i.e., at the *nicotinic* receptors (Sect. 3.3.6) which mediate neurotransmission (i) at the neuromuscular junction, (ii) at the autonomic ganglia of both, sympathicus and parasympathicus, and (iii) at some sites in the central nervous system. In this connection it is of relevancy that co-administration of potato glycoalkaloids (30–100 nM) with another cholinesterase inhibitor, mivacurium, to rabbits led to additive inhibition. This curare-like drug belongs to the group of stabilizing muscular relaxants and is clinically used as an anaesthetic to secure muscular relaxation in certain larger surgical operations (e.g., thorax surgery). Furthermore, cholinesterase inhibition of the potato alkaloids slows mivacurium metabolism with the consequence of a prolongation of the recovery time from drug-induced muscular paralysis (McGehee et al. 2000; for further reports on adverse drug reactions in this connection see Krasowski et al. 1997 and references therein). Therefore, it might be useful to avoid any potato-containing food a few days before operations.

Inhibition of insect AChE from housefly, mosquito, and German cockroach by chaconine was measured to be high in contrast to Colorado potato beetle [IC_{50} values 9–35 vs 863 μM (Friedman 2006 and references therein)]. This finding elucidates at least in part, why the glycoalkaloids of *Solanum tuberosum* are unable to protect the plant against the beetle to a sufficient extent (see below) in contrast to tomatine (Friedman 2002 and references therein).

Membrane-disrupting Effects. The ability to disrupt membranes of cells and organelles, e.g., the outer membrane of mitochondria (Keukens et al. 1996), represents the other known toxic mechanism of glycoalkaloids. It is assumed that this is a consequence of their complex binding to free 3β-hydroxy sterol components of membranes, e.g., cholesterol, ergosterol, with the consequence of permeabilizing such membranes (Keukens et al. 1996; Roddick et al. 2001). Plant sterols such as β-sitosterol had a greater affinity for the glycoalkaloids than cholesterol and ergosterol, respectively. However, this difference did not significantly affect tomatine-induced membrane disruption, the glycoalkaloid which showed a higher potency than chaconine and solanine (Friedman 2002).

In detail, the mechanism leading to disruption seems to include the following steps: (i) Insertion of the alkamine moiety of glycoalkaloids into the membrane bilayer, (ii) complex formation with membrane sterols, (iii) rearrangement of the membrane structure resulting (iv) in membrane disruption and (v) leakage of the cell constituents. High-pH-enhancement of glycoalkaloid disruption of membranes apparently derives from the pH-dependent sterol-binding properties of the plant metabolites (Roddick 1989). The solanidine-based glycoalkaloids chaconine/

solanine are more toxic than the corresponding solasodine-based ones (solamargine/solasonine). This indicates that the specific structure of the aglycone is of decisive significance for the toxicity of an alkaloid rather than the carbohydrate moiety. Chaconine caused the severest cell disruptions of these four glycoalkaloids, whereas solanine turned out to be less toxic as far as this effect is concerned. The latter alkaloid showed only little lytic properties and less teratogenicity. Thus, in this case activity is influenced by the specific structure of the carbohydrate moiety (β-chacotrioside > β-solatrioside). This is also true for the two dominating solasodine-based alkaloids: bovine erythrocytes turned out to be lysed more by solamargine than by solasonine (100% haemolysis at 20 μM vs 80% at 100 μM). However, both alkaloids combined synergized in their effects, even with concentrations of the individual metabolites below the activity threshold. Interestingly, that finding is congruent with similar findings made for the two major solanidine-based glycoalkaloids. The results obtained with erythrocytes were confirmed in an artificial assay: Solamargine caused significant disruption of synthetic lipid membrane vesicles (phosphatidylcholine/cholesterol liposomes) at a concentration <50 μM, whereas solasonine was inactive at up to 150 μM. Thus, again the chacotrioside was effective rather than the solatrioside. In combination, again both alkaloids gave rise to a pronounced synergism. The frequent co-occurrence of glycoalkaloid pairs in *Solanum* spp., either the solanidine-based one (chaconine/solanine) or the solasodine-based one (solamargine/solasonine), is assumed to be of special interest with regard to metabolic cost aspects of chemical defence (Roddick et al. 1990). Alternative to the potential significance of synergistic effects concerning the conspicuous occurrence of glycoalkaloid pairs in such plants another possibility has been discussed recently (Friedman 2002). One metabolite of a pair might be more active against one set of pests and the other for a different set. Both aspects might be also relevant. Due to the fact that natural co-occurrence of solanidine-based glycoalkaloids and their solasodine-based derivatives is rare, it is perhaps rather of academic interest that (i) the combined solatriosides (solanine/solasonine) caused no lysis, (ii) the combined chacotriosides (chaconine/solamargine) showed additive effects, and (iii) the contrasting combinations chaconine/solasonine as well as solamargine/solanine, again caused synergistic effects. The latter combination, an extremely rarely occurring pair, was detected, e.g., in *S. dubíum* (Roddick et al. 1990). β-Solamarine (tomatidenol β-chacotrioside) was less active than the two other chacotriosides chaconine and solamargine. However, again synergistic effects could be observed, if one of the inactive solatriosides, solanine or solasodine, was combined with β-solamarine. Chaconine, solamargine, and β-solamarine seemed to show comparable haemolytic activity. Modification of chacotriose-containing glycoalkaloids led to further interesting structure/activity relationships (Roddick et al. 2001). Another comprehensive study with different membrane-containing systems revealed the following order of potency in two test systems [(i) haemolysis of erythrocytes, measured as haemoglobin release; (ii) induction of enzyme leakage in a human epithelial colon carcinoma cell line, measured as lactate dehydrogenase (LDH) release]: Tomatine > chaconine > solanine (Keukens et al. 1996). Furthermore, results concerning the outer membrane of rat liver mitochondria (measured adenylate kinase

released from the inter membrane space between outer and inner membrane) led to the conclusion that all of these glycoalkaloids – in the same order like mentioned above – were also more effective in permeabilizing these membranes than digitonin (see Sect. 7.7). The authors concluded that the outer membrane was not solubilized but only permeabilized. In conclusion, tomatine is apparently even more effective than chaconine, the most potent glycoalkaloid of the other studies already mentioned above which were carried out without this tomatidine-based glycoside.

Appendix: Ingestion of *S. dulcamara* (754 cases) were reported in the 1993 annual report of the American Association of Poison Control centers (Table 20 of the report), apparently without major or even death outcome (Table 22A of the report). Though solanine in contrast to many other steroidal glycoalkaloids has never been detected as a constituent of *S. dulcamara* (neither berries nor any other organ) this report used the term "solanine" in an inappropriate manner, in this case apparently instead of "steroidal alkaloids/glycoalkaloids" (1853 exposures including the above cited number of 754 for *S. dulcamara*, altogether without major or even death outcome; cited from Table 22A of the report; Litovitz et al. 1994). This inappropriate use of the term "solanine" is not a single case. In order to avoid confusions, medicinal sciences should realize and accept that "solanine" is *not* a term for a class of metabolites, but a single, specific compound. Instead, the class ought to be cited as "steroidal *Solanum* alkaloids".

Safety of *Solanum* Tubers and Fruits in Human Diet. As a result of the development of numerous breeding lines since ancient times, tubers of *S. tuberosum* show the lowest concentrations of glycoalkaloids of all plant organs. Sprouts were found to contain 2000–10,000 mg/kg fresh weight, flowers 2150–5000 mg/kg, leaves 230–1450 mg/kg, tuber peel 150–850 mg/kg, tuber flesh 12–110 mg/kg (Friedman and McDonald 1997). However, the presence of glycoalkaloids in all commercially available potatoes – though with diverging concentrations dependent on the specific cultivar and provenance – have led to safety guidelines proposing maximum levels to 200 mg/kg of fresh weight. Thus, "the total glycoalkaloid levels and individual glycoalkaloid compositions of current potato cultivars do not represent a toxicological or teratogenic hazard" (Gregory et al. 1981). Most commercial cultivars contain less than 120 mg/kg; such concentrations are in favour of smell/taste. Levels >140 mg/kg cause a bitter taste, >200 mg/kg unpleasant sensations in the mouth (Lachman et al. 2001 and references therein). Peeling before cooking removes nearly all of chaconine/solanine (ratio for peel: ~2), since these metabolites are located mainly in and close to the peel (Friedman 2006). However, mechanical damage during the harvest process or during wrong treatment in kitchens (e.g., a longer time interval between peeling/cutting and cooking) cause increasing levels of glycoalkaloids. Any damage induces biosynthesis of these metabolites in potato tubers whereas immediate cooking inactivates the corresponding enzymes necessary for synthesis. Furthermore, exposure to light causes greening of tuber parts and enhances alkaloid levels. But there is no link between both metabolic processes. Inhibitors of chlorophyll biosynthesis did not show any significant effect on light-enhanced alkaloid accumulation (Edwards et al. 1998).

Recently, the glycoalkaloid content of the flesh of even the greenest tubers of four specific cultivars was found to be well below the limit considered unsafe for human consumption (Grunenfelder et al. 2006).

Interestingly, the tubers of certain potato varieties grown organically turned out to contain statistically significantly higher glycoalkaloid levels than the corresponding conventional ones. Not exposed to pesticides and/or herbicides, organically grown potatoes seem to compensate by synthesizing higher levels of natural resistant factors (Friedman 2006 and references therein). The antisense DNA derived from SGT (see Sect. 7.8.3) can be used to limit expression of the SGT gene with the consequence of limiting the synthesis of chaconine/solanine in potato (Moehs et al. 1998).

Numerous studies/reviews on safety of potatoes have been published since decades (e.g., Friedman and McDonald 1997; Grunenfelder et al. 2006; Friedman 2006). Green tomato fruits, beside red ones also consumed in certain countries (as such, "pickled green", "fried-green tomatoes"), were shown to contain 50–80 mg tomatine/kg. If green fruits were stored, a fast metabolization of this glycoalkaloid during ripening and colour change occurred. Very high tomatine content (500–5000 mg/kg dry weight), present in fruits from certain cherry tomato plants, could be consumed without acute toxic effects by Peruvian indigenous people. This led to the assumption that this glycoalkaloid should be much safer for humans than chaconine/solanine (Friedman 2002 and references therein).

Beside tomatoes, the ripe fruits of a remarkable number of cultivated and wild *Solanum* spp., especially from the subgenus *Leptostemonum*, are edible since their low content of glycoalkaloids is normally negligible (for details see Sect. 7.8.2.1). However, there are also occasional reports on intoxications especially by wild species, e.g., consumption of pickled fruits of *Solanum triflorum* NUTT., a weedy species growing in Canada, caused serious gastro-intestinal sickness in some people, although these fruits were generally considered edible. This consumption cannot be recommended, since solamargine and solasonine were shown to accumulate predominantly in the fruits (up to 8 mg/g dry weight; Schulz et al. 1992).

Intoxications of Cattle. *S. glaucophyllum* DESF. [subgen. *Bassovia* (AUBL.) BITT.], duraznillo blanco, common in the wild of Bolivia/Paraguay/Brazil/Argentina, turned out to be responsible for economic losses caused by poisoning of cattle due to its content of solasodine-based glycoalkaloids (Nee 1999). Recently, Bizimenyera (2003) published a first report on acute poisoning of cattle (yearling heifers) after eating berries of *S. dasyphyllum* sub nom. *S. macrocarpon* L. ssp. *dasyphyllum*, a wild, non-edible African eggplant containing tomatidenol-based glycoalkaloids, with serious (e.g., severe dysentery, exudative dermatitis) and – in part fatal – consequences. Gastro-intestinal disturbances are assumed to be the result of membrane disruption. A possible collapse of the barrier function of intestinal cells might cause an increased absorption with the consequence of higher uptake into the blood stream (Keukens et al. 1996). High-glycoalkaloid diets in animal feeding studies induced a haemolytic anaemia, apparently due to a decrease in erythrocyte and haemoglobin levels (Friedman 2006 and references therein).

7.8.4.2 Pharmacology

Solanum species containing steroidal alkaloids have been and often are still used ethnomedicinally since ancient times. There are numerous reports in the literature, e.g., on *S. dulcamara* (Ehmke and Eilert 1993 and references therein), *S. incanum* L. and *S. torvum* Sw. (Neuwinger 1996 and references therein) as well as on a large number of further *Solanum* spp. e.g., with regard to Africa (Neuwinger 2000 and references therein), specifically for Uganda (Bukenya and Carasco 1999), Northeast of Brazil (Agra and Bhattacharyya 1999), and Florida (Austin 2004 and references therein). However, the following must be confined to modern scientifically proven results.

Antitumor Activity. Extracts obtained from *Solanum* spp. have been used to treat cancer for centuries (Cham et al. 1987) and are still used, e.g., in India as well as in Chinese medicine from *S. dulcamara* (Ehmke and Eilert 1993 and references therein). An early scientific report on a specific glycoalkaloid isolated from this species, β-solamarine, which proved a tumor-inhibiting activity (Sarcoma 180, mice) was published by Kupchan et al. (1965a). Many reports are available on solamargine and solasonine, e.g., Saijo et al. (1982), Ono et al. (2006a). The mechanism of such effects was assumed to be same as one of the two main mechanisms of glycoalkaloids responsible for their toxicity, i.e., disruption of sterol-containing cell membranes (e.g., Daunter and Cham 1990). However, further mechanisms of action seem to be involved (see below). Again solamargine as well as its aglycone solasodine were found to be part of the cytotoxic principle of *Solanum incanum* fruits against certain human cancer cell lines (Lin et al. 1990). Cham et al. (1987) demonstrated an in vivo activity of a mixture of solasodine-based glycoalkaloids (4×8 mg/kg) in mice against Sarcoma 180 (%T/C: 254; 11 out of 12 animals surviving after eight weeks). Such a mixture of purified alkaloids – applied in a cream formulation at 0.005% – turned out to be also effective in clinical trials against human non-malignant (keratoses) and malignant skin lesions (basal and squamous cell carcinomas, respectively) (Cham and Meares 1987; Cham 2000). Solamargine turned out to be the main antineoplastic agent in six cultured human solid tumor cell lines in vitro rather than its *N*-free *Solanum nigrum* congener degalactotigonin (Hu et al. 1999); see also sect. 7.7.4. For further reports see Roddick et al. (2001 and references therein) and Friedman et al. (2005 and references therein).

Solamargine was reported to induce cell death by apoptosis in human hepatoma cell systems (Kuo et al. 2000). Detailed mechanisms of action have been discovered, when solamargine was found to display cytoxicity in certain human lung cancer cells (3–7 μM). This glycoalkaloid turned out to sensitize lung adenocarcinoma cells through tumor necrosis factors (TNFs) and mitochondria-mediated pathways. It caused release of cytochrome c, down-regulation of anti-apoptotic proteins Bcl-2 and Bcl-x_L, increase of caspase-3 activity, and DNA fragmentation. Thus, it might be a potential anticancer agent for TNFs- and Bcl-2-related resistance of human lung cancer cells. Addition of solamargine to cisplatin-treated cells synergistically enhanced different caspase activities which might open a possibility to overcome cisplatin-resistance

(Liang et al. 2004; Liu et al. 2004). DNA-damaging activity was also observed for solasodine and *O*-acetylsolasodine, both isolated as genuine metabolites – together with solasodine 3-*O*-β-D-glucopyranoside – from whole plants of *S. umbelliferum* Eschs. The former two compounds exhibited significant activity toward DNA repair-deficient yeast mutants. It was hypothesized that this DNA-modifying activity of the two alkaloids may be due to the spiro-aminoacetal function which may be cleaved, producing an electrophilic iminium species capable of alkylating DNA. This hypothesis was supported by the fact that two synthetic derivatives, *N*-acetyl- and *N*,*O*-diacetylsolasodine, unable to produce an electrophilic iminium intermediate, turned out to be not active at a dose of 8000 µg/mL (Kim et al. 1996).

Tomatine (IC_{50} values 13 and 302 µg/L, respectively) turned out to be three times more toxic than dehydrotomatine (34 and 872 µg/L) in two different cell lines (Ono et al. 1997). Cytotoxic activity against various tumor cell lines was also reported for esculeosides A and B, both considered as metabolites of tomatine formed in ripe tomato fruits (Fujiwara et al. 2003, 2004).

Interestingly, its aglycone, tomatidine, may benefit cancer chemotherapy by inhibiting multidrug resistance in human cancer cells. It has been speculated that cell disruption of cancer cells might facilitate the effect of established chemotherapeutic drugs (Friedman 2002 and references therein).

Anticarcinogenic effects of pure chaconine and solanine as well as artificial and natural ratios of both glycoalkaloids – the latter obtained by extraction of tubers from five potato cultivars – were determined against human cervical, liver, lymphoma, and stomach cancer cells. Chaconine was more active than solanine. All mixtures reduced growth of all cell lines tested. Mixtures with a ratio in favour of solanine or 0.5:0.5 showed synergistic effects, those with a ratio in favour of chaconine (0.7:0.3) an additive one when used against liver cancer cells (Friedman et al. 2005). The authors suggested that synergistically active combinations might offer therapeutic or preventive advantages. The destruction of normal liver cells turned out to be lower than that of liver cancer cells. Anticarcinogenic potency was – beside the concentration of the alkaloids and their artificial or natural ratio – dependent on the nature of cancer cells in a range of two orders of magnitude (0.117–11.7 nmol/mL). In another study of the same group published one year before (Lee et al. 2004) several glycoalkaloids and their aglycones were checked against a colon and a liver cancer cell line, respectively. Furthermore, in case of chaconine, solanine, and tomatine all step-wise obtainable hydrolysis products were investigated, e.g., the genuine glycoalkaloid α-tomatine, the trisaccharide β-tomatine, the disaccharide γ-tomatine, the monosaccharide δ-tomatine and their common aglycone tomatidine. In addition, three further glycoalkaloids, dehydrocommersonine, solamargine, and solasonine as well as the aglycone demissidine were involved. All compounds including the aglycones turned out to be active; the glycoalkaloids were more potent than their hydrolysis products. The in vitro potencies of α-chaconine and α-tomatine in these cancer cell systems were higher than the ones of the clinically established drugs doxorubicin, an agent intercalating with DNA, and camptothecin, a topoisomerase I inhibitor. 22,26-Epiminocholestane-type alkaloids such as the aglycones capsimine and etioline as well as the isoteinemine-based glycoside capsicastrine were found to

inhibit significantly certain human hepatoma cells in vitro to a higher extent than solasonine (Gan et al. 1993).

Liver Protecting Effects. Khasianine [= β_2-solamargine, i.e., (α-)solamargine without attachment of rhamnose at C-2 of its glucosyl unit] as well as the 3-*hydroxy*-22,26-epiminocholestane-type alkaloids capsimine and capsimine-3-*O*-β-D-glucoside exhibited remarkable protection in mice against the pronounced hepatotoxicity of carbon tetrachloride (Gan et al. 1993). Similarly, an in vivo hepatoprotective effect at 20 mg/kg of the total alkaloid fraction of *Solanum pseudocapsicum* leaves – this species is characterized by 3-*amino*-22,26-epiminocholestane-type alkaloids – has been documented in rats (Vijayan et al. 2003).

Spasmolytic Effect. Isojuripidine, an alkamine of the 3-aminospirostan type, showed a spasmolytic effect in guinea-pig ileum due to a partially blockade of calcium influx through voltage-operated calcium channels (De Cassia Meneses Oliveira et al. 2006).

Immune System Stimulating Effects. Another potential beneficial effect by glycoalkaloids for mammalians might be given by enhancement of innate immunity defence mechanisms. Mice prophylactically treated with low doses of water-alcohol extracts prepared from sprouts of *Solanum tuberosum* were rendered resistant to an otherwise lethal challenge with *Salmonella typhimurium*, the typhoid fever causative organism. The prophylactic effect lasted for longer than one week following a single treatment administered either parenterally (i.p.) or per os. This was also true for very small doses of chaconine and solanine, whereas neither solanidine nor the *N*-free steroidal sapogenin diosgenin showed any protective activity. It could be confirmed that the glycoalkaloids do not possess direct antibiotic properties (Gubarev et al. 1998).

Antifungal Effects. In an early comprehensive study six steroidal alkaloids/glycoalkaloids, i.e., soladulcidine, a tetraoside of this aglycone, tomatidine, tomatine, solasodine, and tomatidenol, were checked for antifungal activity (15 species). Though these microorganisms represented predominantly plant pathogens, the true target was a pharmaceutical one. All compounds turned out to show marked activity against *Claviceps purpurea* TULASNE, *Sclerotinia fruticola* (WINT.) REHN, *Piricularia oryzae* CAV., *Rhizooctonia solani* KÜHN, and *Polyporus versicolor* FRIES. On the other hand no activity was exhibited in case of *Botrytis allii* MUNN, *Fusarium conglutinans* WOLL., *F. oxysporum* SCHLECHT., *Coniophora cerebella* (PERS.) DUBY, and *Fomes officinalis* NEUM. Solasodine and tomatidenol only were active toward *Aspergillus clavatus* DESM., *F. bulbigenum* COOKE & MASSEE, and *Alternaria solani* (ELL. & MART.) JONES & GROUT. Soladulcidine, tomatidine and its tetraosides were active toward *Trichotecium roseum* LK. ex FR. Usually the glycosides showed stronger activity, but in certain species the lipophilic aglycones were superior to the former compounds (Wolters 1964). Tomatine had already demonstrated activity against human pathogenic fungi before. In addition solasonine/solamargine and solanine, respectively, were checked toward the same fungi with comparably low effects (Wolters 1968). The activity of glycoalkaloids against a number of fungal plant pathogens was also studied. Mycelium development in

Rhizoctonia solani KÜHN ("Mycelia Sterilia") as well as in *Phoma medicaginis* MAL. & ROUM (Fungi imperfecti, Sphaeropsidales, Phomaceae), common name black stem of lucerne, was inhibited by solamargine; solasonine turned out to be inactive against the former fungus. Combinations of 50 µM of each alkaloid, led to synergistic effects with the consequence of significant inhibition of both fungi (Fewell et al. 1994). Spore germination in *Alternaria brassicicola* (SCHWEIN.) WILTSHIRE (Deuteromycotina, Hyphomycetales, Dematiaceae), common name black spot, and *P. medicaginis* as well as growth of these and two other species, *R. solani* and *Ascobolus crenulatus* P. KARST. (Pezizales, Ascobolaceae), in liquid culture were inhibited by both potato alkaloids with chaconine as the more potent compound. Again synergistic effects could be observed since concentrations of solanine below the inhibitory threshold led to total inhibition in combination with concentrations of chaconine which – taken as a single compound – were also below the inhibitory threshold (Fewell and Roddick 1997). Thus, as single compounds both chacotriosides, chaconine and solamargine, showed potent activity rather than their solatrioside congeners. On the other hand, solanidine was highly toxic for *Saccharomyces cerevisiae*, baker's yeast, in contrast to the corresponding glycoalkaloids (Kalinowska et al. 2005).

Solacongestidine, a 22,26-epiminocholestane-type alkaloid, turned out to be very active with remarkable minimal inhibitory concentrations (in vitro) against a number of human pathogenic yeasts and dermatophytes, e.g., *Candida albicans* (0.8 µg/mL), *Cryptococcus albidus* (0.78 µg/mL), *C. neoformans* (1.56 µg/mL), *Trichophyton rubrum* (0.4 µg/mL). Its congener solafloridine was less active against *Candida albicans* and almost inactive against *T. rubrum*; solasodine, tomatidine, tomatillidine [23,26-epiminocholest-23(*N*)-en-22-one type], and solanocapsine (3-amino-α-epiminocyclohemiketal type) exhibited even much lower activities. However, solacongestidine was inactive against different *Aspergillus* spp. in vitro. Mice infected with *C. albicans* showed prolonged survival time when treated with this alkaloid (Kusano et al. 1987).

Antiprotozoal Effects. Sarachine was found to inhibit the growth of *Leishmania brasiliensis* promastigotes (100% at 25 µM) causing leishmaniasis, and of *Trypanosoma cruzi* epimastigotes (50% at 25 µM) in vitro. Furthermore, it showed a strong in vitro antiplasmodial activity (IC_{50} value: 25 nM) (Moretti et al. 1998). Recently, efficacy and mechanisms of solasonine- and solamargine-induced cytolysis on two strains of *T. cruzi* have been studied. Both alkaloids effectively lysed epimastigotes as well as the bloodstream form trypomastigotes, though solamargine was more potent. The authors concluded from their results that the mechanism involved was largely independent of rhamnose receptor-specific interactions (though rhamnose is part of the carbohydrate moiety of both glycoalkaloids; Hall et al. 2006).

Molluscicidal Activity. Elimination of molluscs which transmit cercariae is an important aspect of health prevention especially in Third World countries. Solasonine/solamargine were found to be toxic to *Lymnaea cubensis* PFEIFFER (Pulmonata: Lymnaeidae) / (LC_{100}: 10 ppm) and *Biomphalaria glabrata* SAY (Gastropoda: Planorbidae) / (LC_{100}: 25 ppm), the intermediate host of *Schistosoma*

mansoni (Trematoda) causing schistosomiasis (bilharziasis) of the liver/intestine. The corresponding aglycone was inactive. This is also true for tomatidine though its glycoalkaloid tomatine was even more active than solasonine/solamargine (Alzerreca and Hart 1982). In contrast, solanine turned out to be inactive toward *B. glabrata* (Marston and Hostettmann 1985 and references therein). However, methanolic extracts obtained from *S. asperum* L.C. Rich, *S. paludosum* Moric., *S. sisymbrifolium* Lam., and *S. stipulaceum* Roem. & Schult., all of these known to produce solasodine-based glycoalkaloids, out of altogether 13 Brazilian *Solanum* species checked turned out to possess significant activity against this snail (LD_{50} 20–50 µg/ml. (Silva et al. 2005). An additional report has added some further suitable species, e.g., *S. jabrense* Agra & M. Nee, *S. parabainum* Agra. The authors concluded that lacking activity of alkaloidal extracts from a number of species "…. might be due to differences in number, type and the specific interglycosidic linkages of the sugars that are attached to the steroidal aglycones …." (Silva et al. 2006). However, detailed information on the specific alkaloidal composition of the extracts in both reports is still lacking. Solamargine and β-solamarine, isolated from the berries of the East African *S. aculeastrum* Dun., showed 100%-molluscicidal activity at 12.5 ppm against the African freshwater snail *B. pfeifferi* Krauss, like *B. glabrata* an important intermediate host of *Schistosoma mansoni*. The mixture of both alkaloids (1:1) killed 100% at 8 ppm. In contrast, solaculine A (a tomatidenol tetraoside) from the root bark of the same species was inactive. The authors also concluded that this activity is affected probably by the number and type of sugars as well as interglycosidic linkages rather than by the type of aglycone (Wanyonyi et al. 2002, 2003). An aqueous extract of *Solanum nigrum* leaves – this species is characterized by different structural types of alkaloids, e.g., solasodine- and tomatidenol-based glycoalkaloids – was active against three Egyptian snail species [*B. alexandrina* Ehrenberg, *Bulinus truncatus* Audouin (both Gastropoda: Planorbidae), *Lymnaea natalensis* Krauss (Pulmonata: Lymnaeidae)] which are intermediate hosts of parasites causing again schistosomiasis or facscioliasis (Ahmed and Ramzy 1997). Chaconine as well as solanine deterred feeding of another snail, *Cryptomphalus aspersus* O.F. Müller sub nom. *Helix aspersa* L. (Gastropoda: Helicidae). The former alkaloid was more active, but combined both metabolites interacted synergistically (Smith et al. 2001).

7.8.4.3 Resistance of *Solanum* Species Against Pests

The predominating scientific opinion is that glycoalkaloids play a minor role in protection against fungi (Friedman and McDonald 1997) in spite of their undeniable antifungal properties (see above). Even tomatine-rich green tomatoes did not inhibit the growth of different fungal pests. This has been explained with unsuitable pH values (4.0–4.5) given on the cell surfaces of the fruit pulp, since a suitable value is above ~6. The nonprotonated alkaloid seems to be more effective rather than the protonated form. Furthermore, it has been suggested that activities of tomatine are a function of the ergosterol content of the fungal cell membranes. On the other hand,

certain fungi, e.g., *Phytophthora infestans*, causing the late blight of potato and other solanaceous species, and *Pythium aphanidermatum*, which lacked ergosterol in their membranes, nevertheless were inhibited by tomatine. This observation led to the assumption that another mechanism of action than binding to sterols might be responsible for this effect. Just these two species are the only fungal pathogens for *Solanum lycopersicum* which are not able to degrade tomatine. Certain other organisms are characterized by different tomatine-inducible enzymes, tomatinases, which are able to cleave the genuine alkaloid in different manners. The tomatinase of certain pathogens, e.g., *Fusarium oxysporum* f. sp. *lycopersici*, catalyzed the cleavage into the aglycone, tomatidine, and the complete carbohydrate moiety, i.e., lycotetraose. Others were shown to produce enzymes, which detoxify (α-)tomatine either by removing only the terminal xylose resulting in the final formation of β_1-tomatine (*Botrytis cinerea*) or alternatively by removing glucose, thus producing β_2-tomatine (e.g., *Septoria lycopersici*). Consequently, tomato pathogens turned out to be less susceptible to tomatine than the nonpathogens (Friedman 2002; ref. therein). Many additional aspects, especially with regard to tomatoes and potatoes, were published in comprehensive reviews (Friedman and McDonald 1997; Friedman 2002, 2006).

Glycoalkaloids also seem to have little or even no effect toward bacterial pathogens. Finally, they are not assumed to impart much resistance to nematodes. However, solamargine killed in vitro 100% adults and microfiliariae of *Setaria cervi* (Nematoda: Filarioidea), bovine filarial worm, at 4 mg/mL as well as microfiliariae in vivo (100 mg/kg p.o. in 4 phases, rats) (Ghosh et al. 1994).

A different situation seems to be given in case of insect pests. Plants with low initial levels produced – attacked by insects – higher levels of glycoalkaloids as foliar metabolites. This is also true for potato plants attacked by the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) though their glycoalkaloids are ineffective against this pest (Friedman and McDonald 1997 and references therein). According to Tingey (1984) elevation of total glycoalkaloid content alone does not necessarily result in a corresponding increase in resistance. The importance of the presence of specific glycoalkaloids was pointed out. Thus, in contrast to chaconine/solanine in cultivated potato plants other foliar glycoalkaloids such as leptines discovered in a certain accession of a wild potato species, *S. chacoense*, provide resistance to *L. decemlineata* (Kuhn and Löw 1957; Sinden et al. 1980). This resistance is also given by the presence of demissine in *S. demissum* (Kuhn et al. 1955a). Leptine I – infiltrated into leaves of *S. tuberosum* – turned out to inhibit completely feeding by the potato beetle at the very low concentration of 1 μM, whereas the major potato alkaloids inhibited only ~50% at 6 mM. Demissine and tomatine were intermediate in activity. Deacetylation of leptines – resulting in the formation of leptinines – led to a loss of the strong repellency (Stürckow and Löw 1961). Commersonine and dehydrocommersonine were also found to be more active than chaconine/solanine. Interestingly, it was discovered that leptines seem to act independently of commersonine/dehydrocommersonine (Tingey 1984).

It has been suggested that the potato beetle – toxic and distasteful for potential predators – sequesters glycoalkaloids from the foliage of potatoes or other glycoalkaloid-containing solanaceous species. However, the repellency to predators is

probably caused by a toxic dipeptide rather than by excretion of host plant glycoalkaloids or their potential metabolites produced by the beetle. Solanine or chaconine could not be detected in larvae, adult beetles or in the hemolymph but in their excrements (Armer 2004).

Significant correlation of foliar total glycoalkaloid concentrations of 10 wild potato species and potato leafhopper [*Empoasca fabae* HARRIS (Homoptera: Cicadellidae)] resistance suggested that their content of glycoalkaloids may be a significant factor in the defence against this pest. *S. polyadenium* GREENM. (688 mg/100 g fresh weight) turned out to be resistant in contrast to S. *bulbocastanum* DUN. (13 mg/100 g) (Tingey et al. 1978). *S. polyadenium* is characterized by the content of demissine and tomatine, the remaining species by solanine/chaconine, save *S. bulbocastanum* (alkaloidal profile unknown). It was demonstrated that the worst cereal aphid pest, *Schizaphis graminum* RONDANI (Homoptera: Aphididae), greenbug, was sensitive to tomatine (LC_{50}: 7.3 µM) in contrast to its aglycone tomatidine and the glycoalkaloids solanine and chaconine. On the other hand, tomatine did not strongly affect the potato aphid, *Macrosiphum euphorbiae* THOMAS, the most important potato virus vector which is responsible for severe losses in potato tuber production. It was suggested that the insufficient sensitivity of *M. euphorbiae* was caused by co-evolutionary adaptation between host and herbivore (Soulé et al. 1999 and references therein). Larvae of *Earias insulana* BOISD. (Lepidoptera: Noctuidae), spiny bollworm, did not pupate, if they were fed on diets containing 0.1% solasonine or tomatine as well as 0.05% solamargine (Weissenberg et al. 1986). Larval growth of the *Tribolium castaneum* HERBST (Coleoptera: Tenebrionidae), red flour beetle, and *Manduca sexta* L. (Lepidoptera: Sphingidae), tobacco hornworm, was inhibited by diets containing 1 µmol/g tomatine in contrast to the corresponding aglycone. Solamargine and solasodine were active against the former insect only. Interestingly, the effect caused by solamargine and tomatine could be completely abolished, if the same concentration of cholesterol and/or sitosterol was added. However, this was not the case with solasonine, presumably due to its much reduced sterol binding capacity compared with the former glycoalkaloids (Weissenberg et al. 1998). Involvement of glycoalkaloids in resistance to other insects was discussed by Tingey (1984).

Allelopathic Effects. Arudonine was found to inhibit the growth of lettuce seedlings (*Lactuca sativa* L., Asteraceae) (Fukuhara et al. 2004).

7.8.4.4 Significance of Steroidal Alkaloids as Source for the Industrial Production of Steroidal Hormones

Diosgenin, used in huge amounts as the starting material for the industrial production of steroidal hormones (corticosteroids, sex hormones including oral contraceptives), became in short supply. However, in the early 1970s the bottleneck of its natural sources, especially certain wild *Dioscorea* spp. (Dioscoreaceae), and increasing prices induced efforts to discover other suitable plant steroids. Beside others solasodine, the *N*-homologue of diosgenin, turned out to be such a candidate as a new starting material in the partial synthesis of steroidal hormones, because it

also can be transformed chemically to key intermediates such as dehydropregnenolone acetate (Franz and Jatisatienr 1983 and references therein). This finding stimulated the search for potential *Solanum* sources which promised to be high-yielding producers of solasodine-based glycoalkaloids. An early study on 200 varieties, forms, and provenances of *S. nigrum* L. had been carried out with the aim to find suitable sources for such an industrial use (Schreiber 1958b). It could be demonstrated that *S. platanifolium* SIMS (Bhatnagar and Puri 1974) and *S. mammosum* L. (Telek et al. 1977; Indrayanto et al. 1998 and references therein) as well as *S. aviculare* FORST., *S. khasianum* C.B. CLARKE, *S. lacianatum* AIT., *S. marginatum* L.f., and *S. sodomeum* L. are characterized by high levels of solasodine-based glycoalkaloids in their fruits and/or leaves (Mann 1978; Franz and Jatisatienr 1983; Mühlenbeck et al. 2002 and references therein). Moreover, it was also the aim of a comprehensive study published by Weiler et al. (1980) to select candidates out of 226 *Solanum* spp. (~250 samples) for the industrial production of solasodine (for details see Sect. 7.8.1.1). The highest-yielding species of this study turned out to be *S. lacianatum* (up to 4.4%). A number of companies tried to use such promising *Solanum* spp. for commercial production (Trease and Evans 2002). However, phytosterols such as stigmasterol and sitosterol, obtainable from different easily available raw materials, e.g., the unsaponifiable fraction of soy bean oil, the pine pulp arising during production of cellulose (tall oil), meanwhile turned out to be a more suitable source of raw material from the cost-effective point of view.

7.9 Miscellaneous Rare Steroidal Metabolites

7.9.1 Homo-cholestane Glycosides (C_{27} + C_2/C_3)

Tagawa et al. (2003) could elucidate the unusual structure of three steroidal glycosides, aethiosides A–C, isolated from the fruits of *Solanum aethiopicum* L. (Solanaceae).These metabolites representing homologues of steroidal saponins were characterized by a *homo*-cholestane skeleton with an aromatized ring E. A plausible proposal for their biosynthesis was added starting from the conjugation of a polyhydroxycholesterol (on the 27*O*-glucoside level) and a C_2 unit (acetyl CoA or malonyl CoA) thus forming *homo*-cholestane glycosides (Fig. 7.25). The carbohydrate chain at C-3β of aethiosides A and C was determined to be β-chacotriosyl, well-known from steroidal glycoalkaloids. In the case of congener B the trioside contained xylose instead of rhamnose at C-4 of glucose.

7.9.2 Cardenolides

A surprising discovery was made when – due to a corresponding screening – an extract of the whole plant of *Nierembergia rigida* MIERS sub nom. *N. aristata* Auct. non MIERS displayed potent cytotoxic activity: Bioassay-guided fractionation yielded

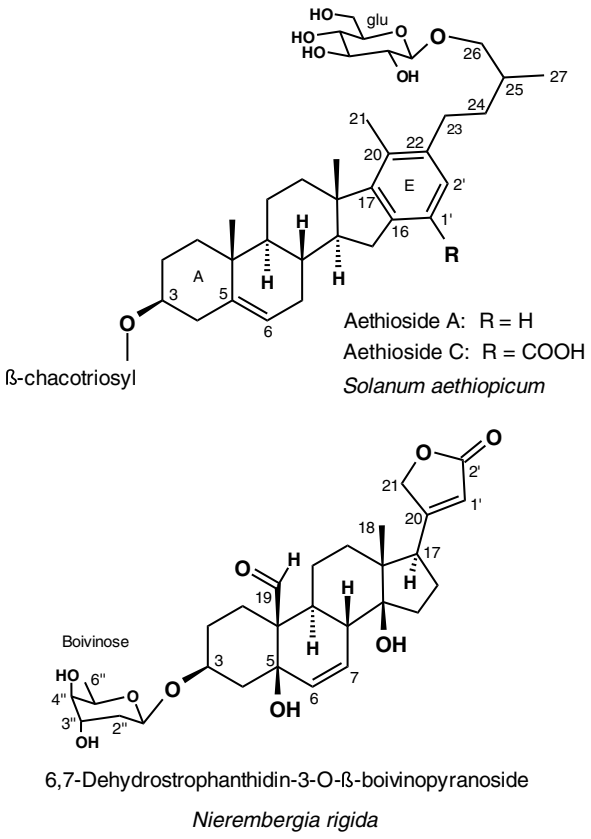


Fig. 7.25 Unique steroidal metabolites from two solanaceous species

three novel cardenolides, a group of cholesterol-derived metabolites which had been found never before in the Solanaceae (Gil et al. 1995). Cardiac glycosides occur in about 15 families, e.g., Apocynaceae, Plantaginaceae (eudicots), Convallariaceae (monocots); obviously, they do not occur in the Convolvulaceae. The cardenolides of *N. rigida* are characterized by unique 6,7-dehydro derivatives of the known aglycone strophanthidin and two 2,6-deoxy sugars, boivinose and oleandrose (Fig. 7.25).

7.9.3 Cholecalciferol/Vitamin D_3 and Congeners (C_{27} Isoprenoids)

Cholecalciferol, traditionally and erroneously named *vitamin* D_3, represents a *seco*steroidal hormone in humans. Biosynthesis of its active metabolite $1\alpha,25(OH)_2D_3$ proceeds via cholesterol → 7-dehydrocholesterol (liver) → cholecalciferol (irradiation

in the skin) → 25-hydroxy-cholecalciferol [25(OH)D_3; liver] → 1α,25-dihydroxy-cholecalciferol [1α,25$(OH)_2D_3$; kidney] (Fig. 7.26). Cholecalciferol regulates the calcium and phosphate balance in the body and is an essential factor in bone formation. Overfeeding leads to calcinosis, i.e., calcium deposition in human and animal

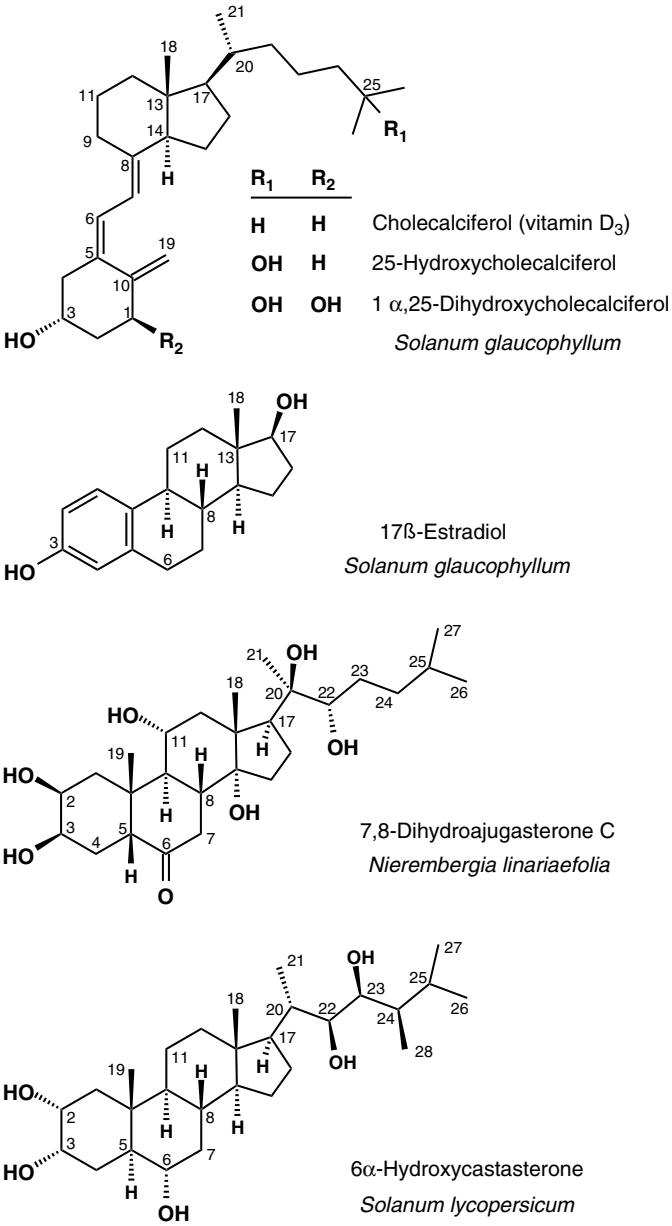


Fig. 7.26 Examples for the solanaceous occurrence of steroidal mammalian hormones (cholecalciferols, estrogens), insect hormones (ecdysteroids), and phytohormones (brassinosteroids)

tissues and dangerous consequences such as emaciation, extended lying, motional disorders, raised pulse, increased respiratory rate, impairment of fertility, and decreased vitality (Mello 2003 and references therein). Corresponding intoxications of grazing animals such as cattle, sheep, goats, pigs, horses, and others led to the discovery of calcinogenic plants. These are confined as far as known to four angiosperm families, namely Poaceae, Cucurbitaceae, Fabaceae, and above all Solanaceae (Boland et al. 2003 and references therein). Calcinogenic plants belong to the most noxious plants to animals in the world. They were found to contain active cholecalciferol as well as its metabolites known from humans and glycosides of both. It could be demonstrated that incubation of leaf extracts from a calcinogenic plant (*Solanum glaucophyllum* DESF. sub nom. *S. malacoxylon* SENDT.) with ruminal fluid of sheep leads to release of $1\alpha,25(OH)_2D_3$ from its glycosides (Boland et al. 1987). Thus, ingestion of glycosidic progenitors of cholecalciferol and its derivatives may lead to the physiologically active aglycones. However, free aglycones were found in concentrations higher than those of their glycosides in case of *Cestrum diurnum* (Prema and Raghuramulu 1996). Comprehensive reviews on "vitamin D compounds in plants" (Boland 1986; Boland et al. 2003) and "calcinosis – calcinogenic plants" (Mello 2003) have been published.

7.9.3.1 Occurrence in the Solanaceae

Nierembergia. Unclear results on the presence of a $1\alpha,25(OH)_2D_3$-like compound in *N. veitchii* BERKELEY ex HOOK. were obtained though administration of this species caused calcinosis in different animals (Boland et al. 2003 and references therein).

Cestrum. Leaves of *C. diurnum* were found to contain free aglycones (see above).

Nicotiana. Vitamin D_3 as well as $25(OH)D_3$ and $1\alpha,25(OH)_2D_3$were identified in the leaves of *N. glauca* GRAHAM (Skliar et al. 2000). Furthermore, 7-dehydrocholesterol known as a precursor in human cholecalciferol biosynthesis could also be detected. Furthermore, this precursor was detected in *Solanum glaucophyllum* sub nom. *S. malacoxylon* which led to the assumption that the biosynthesis of cholecalciferol and its congeners in plants might proceed on a similar pathway to that in humans (Aburjai et al. 1996).

Solanum. *S. glaucophyllum* (syn.: *S. malacoxylon*) is one of the most important poisonous plants of Argentina ("duraznillo blanco") and Brazil causing frequent calcinosis of grazing animals. Cholecalciferol, $25(OH)D_3$, and $1\alpha,25(OH)_2D_3$ were detected in the leaves. Furthermore, their corresponding fructoglucosides and other glycosides could be discovered. Variable contents in a range of 0.1–140 mg/kg dry weight were recorded (Weissenberg et al. 1993 and references therein). Cholecalciferol and $1\alpha,25(OH)_2D_3$ were also identified in leaf extracts of *S. lycopersicum* L. sub nom. *Lycopersicon esculentum* MILL., *S. melongena* L. (eggplant/aubergine), and S. *tuberosum* L. (potato) (Aburjai et al. 1998). Further calcinogenic *Solanum* spp. containing such metabolites are *S. eleagnifolium* CAV., *S. torvum*

SCHLTDL., *S. verbascifolium* L. (Boland et al. 2003 and references therein), and the Australian *S. esuriale* LINDL. (Mello 2003 and references therein).

7.9.4 Estrogens (C_{18} Isoprenoids)

The natural occurrence of animal steroid hormones, i.e., sex hormones and corticosteroids, in plants and their effects on plant growth, development, and flowering was reviewed by Geuns (1978). The detection of 17β-estradiol (Fig. 7.26) and estrone in leaves, flowers, and seeds of *Solanum glaucophyllum* DESF. (Milanesi et al. 2001) is an example for such an occurrence in the Solanaceae family. Bearing in mind that the physiological role of animal hormones in plants has been discussed controversially, first evidences on the existence of estrogen binding proteins in this species reported as a result of that study seem to be of significant relevance. This is emphasized by the observation that these proteins are structurally related to the mammalian ER α receptor subtype. Interestingly, *S. glaucophyllum* was shown previously to synthesize remarkable amounts of another (seco)steroid hormone, 1α,25-dihydroxy-vitamin D_3 and its congeners (see Sect. 7.9.3.1).

7.9.5 Ecdysteroids and Antagonists

7.9.5.1 Ecdysteroids/Phytoecdysones (C_{27} Isoprenoids)

More than 150 ecdysteroids, insect hormones or phytoecdysones stimulating moulting of caterpillars, pupa formation, and emergence from the pupa, have been structurally elucidated; all of them possess agonistic activity. Overall ca. 6% of plant species contain ecdysteroids though the distribution of such compounds in the plant kingdom is very uneven: There are families with many ecdysteroids-positive species and those containing very few (Savchenko et al. 2000 and references therein). Recently, it has been reported that even "…. over 250 ecdysteroid analogs have been identified so far in plants" (Ghosh and Laddha 2006).

Solanaceae. Phytoecdysones are at least as common in the Solanaceae as they are in plants in general (Savchenko et al. 2000 and references therein). Two hexahydroxy-6-ketosterols, the well-known ecdysterone (20-hydroxyecdysone, an insect hormone) and the novel 7,8-dihydroajugosterone C (Fig. 7.26) were isolated from the whole plant of *Nierembergia linariaefolia* GRAHAM sub nom. *N. hippomanica* auct. non MIERS (Pomilio et al. 1996). The structural difference of ecdysterone to the novel metabolite is given by the presence of a 7,8-double bond and a hydroxyl group at C-25 whereas 11α-OH is lacking. In order to check the accumulation of ecdysteroids or of ecdysteroids-antagonistic metabolites in the Solanaceae Savchenko et al. (2000) surveyed altogether 128 species from all subfamilies. On the basis of the co-occurrence of positive agonist bioassay and RIA responses they found *Browallia*

speciosa BENTH., *Nierembergia linariaefolia* GRAHAM sub nom. *N. hippomanica* auct. non MIERS var. *violacea*, "*N. solanacea"*, *Solanum scabrum* MILL. sub nom. *S. melanocerasum* ALL., and *S. nigrum* L. to be most significant. These species showed "a cocktail of ecdysteroids", of which 20-hyroxyecdysone (ecdysterone) and polypodine B (5β,20-dihydroxyecdysone) turned out to be major components.

Lower levels were detected by RIA also in several other species of the genera *Browallia, Nicandra, Nierembergia, Physalis, Solanum,* and *Withania*. If ecdysteroid-positive, solanaceous species in general apparently do not contain high levels. Flowers seem to contain the highest concentrations of all organs.

Convolvulaceae. Several phytoecdysones such as ecdysone, crustecdysone as well as the novel compounds muristerone A, kaladasterone, and calonysterone were isolated and structurally elucidated from Indian "kaladana" seeds (Canonica et al. 1972, 1973, 1975, 1977c). Kaladana is the common name for the seeds of apparently different *Ipomoea* species occurring in India named in dependence of local traditions. At least, there is much confusion in the literature with regard to the correct assigment to a certain species. Authentic specimens of seeds of *I. muricata* (syn.: *Calonyction muricatum*) and *I. hederacea*, both originally assumed to be the origin of those "kaladana" seeds extracted by the authors, turned out to be phytoecdysone-negative (Canonica et al. 1975). Thus, there is still considerable confusion about the origin. In their report of 1977 the authors classified the origin of their drug as *I. calonyction* (CHOISY) HALL. f. species nova; however, they still called the "….exact botanical classification …. a somewhat controversial matter." In a recent paper Ghosh and Laddha (2006) reported on a method "…. for the extraction of ecdysteroids from *Ipomoea hederacea* (kaladana) seeds." However, there is no real proof that these kaladana seeds are identical to those of Canonica et al. The "kaladana problem" is also true for the presence of ergoline alkaloids (see Sect. 4.2). Ten phytoecdysones were isolated from the aerial parts of *Porana discifera* C.K.SCHNEID. (Zhu et al. 2000).

7.9.5.2 Ecdysteroid Antagonists

The study of Savchenko et al. (2000) on solanaceous species described also ecdysteroid antagonist activity of solanaceous extracts. However, only weak activity was associated with a few of such extracts. Major withanolides were inactive, but they may be activated by metabolism after ingestion by invertebrate predators.

7.9.6 Brassinosteroids (C_{27} + C_1/C_2 Isoprenoids)

Brassinosteroids represent a class of phytohormones and are widespread in the plant kingdom. However, there are only a few reports on these metabolites in the two large Solanales families. Though their physiological effects on plants are not yet fully understood, it is obvious that they have specific effects on plant growth and development. Furthermore, they may protect plants from drought, extreme temperatures, heavy metals, salinity, and herbicidal injury. Bajguz and Tretyn

(2003) have published a comprehensive review on the chemical characteristic and distribution of these plant metabolites. There are C_{27}, C_{28}, and C_{29} brassinosteroids altogether derived from the 5α-cholestane skeleton. Vicinal hydroxyl groups at 2α,3α represent a general feature of biologically most active compounds. For details readers are referred to the review mentioned above.

Solanaceae. Three brassinosteroids, 6α-hydroxycastasterone (C_{28}) (Fig. 7.26) characterized by a 2α,3α-dihydroxy residue (Bishop et al. 1999), 6-deoxo-28-*nor*-cathasterone (C_{27}), and 6-deoxo-28-*nor*typhasterol (C_{27}) (Yokota et al. 2001) were discovered in a solanaceous species, *Solanum lycopersicum* L. sub nom. *Lycopersicon esculentum* MILL. Furthermore, the already known 6-deoxoteasterone as well as a number of closely related congeners of all four compounds could be detected in roots or shoots of this species.

Convolvulaceae. Castasterone (C_{28}) and 28-*nor*castasterone (C_{27}), both characterized by a 2α,3α-dihydroxy residue, were identified in the seeds of *Ipomoea purpurea* (L.) ROTH sub nom. *Pharbitis purpurea* (L.) VOIGT (Suzuki et al. 1985).

7.10 Withanolides/Withasteroids (C_{28} Isoprenoids)

Authorities. There will be no addition of the corresponding authorities to the species epithets in the text of Sect. 7.10, since they are added to the species names in the complete list of Table 7.9.

7.10.1 Discovery

The first withanolide, "withaferin", was discovered as a constituent of the leaves of *Withania somnifera* (L.) DUN. (Solanaceae), a perennial branched herbaceous species indigenous to the Old World, by Yarden and Lavie (1962). This metabolite represented a novel type of steroids characterized by an α,β-unsaturated lactone linked to C-17 of the sterane skeleton (Lavie et al. 1965a). However, "withaferin" turned out to be 2,3-dihydro-3-methoxywithaferin A co-occurring with withaferin A (Fig. 7.27) (Lavie et al. 1965b). Stereochemistry of the latter constituent could be determined by the same authors (Lavie et al. 1966). Independently, Kupchan et al. (1965b) discovered withaferin A also in the leaves of *Acnistus arborescens* (Solanaceae), a macrophyllic shrub or small evergreen tree of neotropical origin (Hunziker 2001). To date, about 400 withanolides or closely related congeners have been discovered in altogether 58 solanaceous species belonging to 22 genera (Table 7.9). Therefore, it must be beyond the scope of this book to discuss this topic extensively. It would need a monograph of its own. Fortunately, there exist a number of comprehensive reviews which comprise the topic from the chemical point of view (structures, structure elucidations, biosynthesis, synthetic achievements); furthermore, results on biological activities are included (Christen 1989; Glotter 1991; Ray and Gupta 1994; Anjaneyulu et al. 1998; Veleiro et al. 2005).

Table 7.9 Solanaceous species containing withanolides or closely related derivatives. Numbers in square brackets indicate the *first* mentioning of a species in a review: [1] Ray and Gupta 1994, [2] Anjaneyulu et al. 1998, [3] Veleiro et al. 2005; however, this does not mean that there may be no further mentioning and results in following reviews. Updating references for original reports on species not yet included in one of these three reviews are also given in square brackets; this may be also true occasionally for elder reports

Acnistus *arborescens* (L.) SCHLTDL. (syn.: *A. ramiflorus*) [1], [Veras et al. 2004a, b]
{*A. australis* see *Iochroma australe*}
{*A. breviflorus* see *Vassobia breviflora*}
{*A. lorentzii* see *Eriolarynx lorentzii*}
{*A. ramiflorus* see *A. arborescens*}

Brachistus *stramoniifolius* (KUNTH) MIERS [syn.: *Witheringia stramoniifolia* KUNTH] [Fang et al. 2003]

Browallia *viscosa* KUNTH [Rozkrutowa 1987]

Datura *ferox* L. [1]
D. metel L. [1]
D. metel var. *fastuosa* (BERNH.) DANERT sub nom. *D. fastuosa* L. [1][a]
D. quercifolia H.B.K. [1]
D. stramonium L. [1]; also sub nom. *"D. tatu**r**a"* [1], obviously *D. tatu**l**a*[a]
{*D. fastuosa* see *D. metel* var. *fastuosa*}
{*"D. tatu**r**a"* [1], obviously *D. tatu**l**a* s. *D. stramonium*}

Deprea *orinocensis* (KUNTH) RAF. [1]
D. procumbens[b] [1]
D. subtriflora (RUIZ & PAV.) D'ARCY [3]

Discopodium *penninervium* HOCHST. [Habtemariam 1997; Habtemariam and Gray 1998; Habtemariam et al. 1993, 2000]

Dunalia *brachyacantha* MIERS [3]
D. solanacea H.B.K. [3]
D. tubulosa (BENTH.) J.F.MACBR. [1]
{*D. australis* see *Iochroma australe*}

Eriolarynx *lorentzii* (DAMMER) HUNZ. sub nom. *Vassobia lorentzii* (DAMMER) HUNZ. (syn.: *Acnistus lorentzii*) [3]

Exodeconus *maritimus* (BENTH.) D'ARCY [3]

Hyoscyamus *niger* L. [Ma et al. 1999]

Iochroma *australe* GRISEB. sub nom. *Acnistus australis* (GRISEB.) GRISEB. [1]; furthermore sub nom. *Dunalia australis* (GRISEB.) SLEUMER [1][a]
{*I. coccinium* see *I. gesnerioides*}
{*I. fuchsioides* see *I. gesnerioides*}
I. gesnerioides (KUNTH) MIERS sub nom. *I. coccinium* SCHEIDWEILER [1]; furthermore sub nom. *I. fuchsioides* MIERS [1][a]

Jaborosa *araucana* PHIL. [3]
J. bergii HIERON. [1]
J. integrifolia LAM. [1]
J. laciniata (MIERS) HUNZ. sub nom. *Trechonaetes laciniata* MIERS [1]
J. leucotricha (SPEG.) HUNZ. [1]
J. magellanica (GRISEB.) DUSÉN [1]
J. odonelliana HUNZ. [1]
J. rotacea (LILLO) HUNZ. & BARBOZA [Nicotra et al. 2006]
J. runcinata LAM. [3]

(continued)

Table 7.9 Solanaceous species containing withanolides or closely related derivatives. Numbers in square brackets indicate the *first* mentioning of a species in a review: [1] Ray and Gupta 1994, [2] Anjaneyulu et al. 1998, [3] Veleiro et al. 2005; however, this does not mean that there may be no further mentioning and results in following reviews. Updating references for original reports on species not yet included in one of these three reviews are also given in square brackets; this may be also true occasionally for elder reports (continued)

J. sativa (MIERS) HUNZ. & BARBOZA [2]

Leucophysalis *viscosa* (SCHRAD.) HUNZ. sub nom. *Saracha viscosa* SCHRAD. [Ripperger and Kamperdick 1998]

Lycium *barbarum* L. sub nom. *L. halimifolium* MILL. [1]

L. chinense MILL. [1]

Nicandra *physalodes* (L.) GAERTN. [1] (not *N. physaloides*!)

Physalis *alkekengi* L. [1]

P. angulata L. [1], [Nagafuji et al. 2004; Abe et al. 2006]

P. chenopodifolia LAM.[c] [Maldonado et al. 2004]

P. cinerascens (DUN.) HITCHC. [Maldonado et al. 2005]

P. coztomatl MOC. & SESSÉ ex DUN.[c] [Pérez-Castorena et al. 2006]

P. curasavica[b] [1]

{*P. ixocarpa* see *P. philadelphica*}

P. lanceifolia [1] (*"P. lancifolia"*[b] in the original report is obviously wrong; two possibilities for authorities: NEES **or** RUGEL ex KUNZE)

P. minima var. *indica* (LAM.) C.B.CLARKE [1]

P. peruviana L.[c] [1]

P. philadelphica LAM.[c] (syn.: *P. ixocarpa* BROT. ex HORNEM.) [1][a]

P. pubescens L. [1]

P. solanaceus (SCHLTDL.) AXELIUS [Pérez-Castorena et al. 2004]

P. viscosa[d] [1]

Salpichroa *origanifolia* (LAM.) THELL [1]

{*Saracha viscosa* s. *Leucophysalis viscosa*}

Solanum *ciliatum* LAM. (*"S. cilistum"*[b], continuously spelled with an "s" in the original and following reports, but obviously wrong [Zhu et al. 2001a, b, c]

S. sisymbrifolium Lam. (*"S. sisymbiifolium"*[b], obviously wrong in the original report) [Niero et al. 2006]

{*Trechonaetes laciniata* s. *Jaborosa laciniata*}

Tubocapsicum *anomalum* (FRANCH. & SAV.) MAKINO [1], [Kiyota et al. 2007]

Vassobia *breviflora* (SENDTN.) HUNZ. sub nom. *Acnistus breviflorus* SENDTN. [1]

{*V.* lorentzii see *Eriolarynx lorentzii*}

Withania *aristata* (AITON) PAUQ. [1]

W. coagulans DUN. [1], [Atta-ur-Rahman et al. 1998a, b, 1999, 2003]

W. frutescens (L.) PAUQ. [1]; sub nom. *"W. arboritus"*[b] in [2]

W. obtusifolia V. TACKH. [1]

W. somnifera (L.) DUN. [1], [Matsuda et al. 2001; Zhao et al. 2002; Jayaprakasam and Nair 2003; Jayaprakasam et al. 2004; Choudhary et al. 2004; Kuroyanagi et al. 1999; Subbaraju et al. 2006; Lal et al. 2006]

Witheringia *coccoloboides* (DAMMER) HUNZ. [1]

W. solanacea L'HERIT. [Jacobo-Herrera et al. 2006]

[a] Doublet in [1]

[b] Authority unknown; species not listed by IPNI and w3TROPICOS

[c] Edible fruits

[d] Different authorities possible

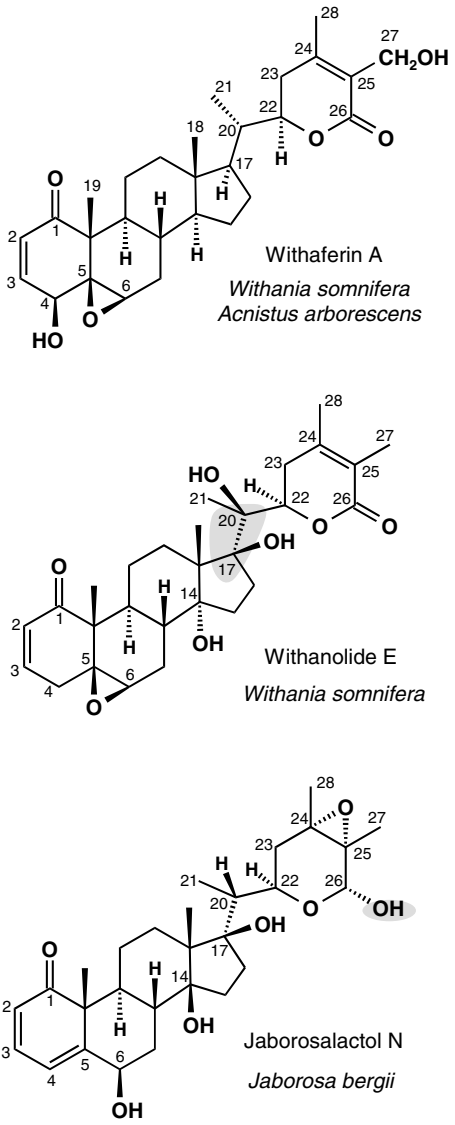


Fig. 7.27 Withaferin A as an example for withanolides with an unmodified β-oriented side chain at C-17, withanolide E as one for congeners with an unmodified α-oriented side chain (highlighted in grey), and jaborosalactol N as one for withanolide-type lactol derivatives (the corresponding hydroxyl group highlighted in grey; Monteagudo et al. 1988)

The reviews may also be useful as pathfinders for original literature in order to limit the number of references in this book. Some of these reviews include complete lists and/or structures of all withanolides already known at that time or corresponding updates (Glotter 1991; Ray and Gupta 1994; Anjaneyulu et al. 1998). Recent discoveries comprising exactly 100 novel compounds are summarized together with the corresponding original references in Tables 7.9 and 7.10.

The review of Veleiro et al. (2005) is confined to South American species; however, this is only of little disadvantage, since the majority of the withanolide-positive species are of South American origin (exceptions: species of the genera

Table 7.10 Recent discoveries of solanaceous withanolides not yet included in the reviews of Ray and Gupta (1994), Anjaneyulu et al. (1998), and Veleiro et al. (confined to South American species, 2005)

Species (Authorities see Table 7.9)	**Novel withanolides**	**References**
Acnistus arborescens	Withaphysalins **M – O** (type like withaphysalin **A** (Fig. 7.29)); 18*R*- and 18*S*-2,3-dihydrowithaphysalin F; Acnistins **I**, **K**, **L**	Veras et al. (2004a, b); Usubillaga et al. (2005)
Brachistus stramonifolius	Known physalins **B**, **F**, **H** (Fig. 7.29)	Fang et al. (2003)
Browallia viscosa	Known metabolites withaferin **A** (Fig. 7.27) and nicandrenone (Fig. 7.28)	Rozkrutowa (1987)
Discopodium penninervium	6α,7α-Epoxy-5α,17β-dihydroxy-1-oxo-witha-2,24-dienolide; three 5α,6α-epoxy-17α congeners; 17-epiacnistin **A**	Habtemariam and Gray (1998); Habtemariam et al. (1993, 2000)
Hyoscyamus niger	16α-Acetoxyhyoscyamilactol, [Known: hyoscyamilactol = Nic-**3**; daturalactone-4]	Ma et al. (1999)
Jaborosa rotacea	Jaborosalactones **26-37** (Fig.7.30)	Nicotra et al. (2006)
Leucophysalis viscosa sub nom. *Saracha viscosa*	Known physalins **D**, **F**, **H** (Fig. 7.29)	Ripperger and Kamperdick (1998)
Physalis angulata	Withaferin A-type physagulins **H – K; L – N**	Nagafuji et al. (2004); Abe et al. (2006)
P. chenopodifolia	Physachenolides **A – E** (18-oxygenated withanolide **E**-type metabolites)	Maldonado et al. (2004)
P. cinerascens	Cinerolide, 24,25-dihydrowithanolide **S**	Maldonado et al. (2005)
P. coztomatl	Physacoztolides **A – E** (18-oxygenated withaferin **A**-type metabolites)	Pérez-Castorena et al. (2006)
P. solanaceus	[Known physalins **A**, **B**, **D**, **F**]	Pérez-Castorena et al. (2004)
Solanum ciliatum sub nom. *S. "cilistum"*	Lactol-type cilistols **a**, **b**, **d**, **f**, **g**, **i**, **j**, **p** (Fig. 7.31), **pm**, **p1**, **q**, **t**, **u**, **v**, **w**, **y** (glucosides: **i**, **j**, **p**, **pm**, **p1**, **q**, **t**, **u,** **v**, **w**, **y** = 26-*O*-β-D-glucopyranosides)	Zhu et al. (2001a,b,c)
S. sisymbrifolium	Lactol-type cilistepoxide and cilistadiol	Niero et al. (2006)
Tubocapsicum anomalum	Acnistin **E**-type isotubocaposides **A – C** (**A** = branched triglucoside; **B** = gentiobioside; **C**: sophoroside)	Kiyota et al. (2007)
Withania coagulans	Withaferin **A**-type coagulins **H – R** (**K**, **L, N – Q** = 3β-*O*-β-D-glucopyranosides); one witha-2,5,24-trienolide and one witha-2,5,14,24-tetraenolide (withacoagulin)	Atta-ur-Rahman et al. (1998a, b, 2003)
W. somnifera	Withaferin **A**-type withanosides **I**, **III** (3β-*O*-β-D-glucopyranosides); **II**, **IV – VII** [gentiobiosides]	Matsuda et al. (2001)
do.	Five withanolides including withanosides **VIII – XI** (3β-*O*-β-D-glucopyranosides)	Zhao et al. (2002)

(continued)

Table 7.10 Recent discoveries of solanaceous withanolides not yet included in the reviews of Ray and Gupta (1994), Anjaneyulu et al. (1998), and Veleiro et al. (confined to South American species, 2005) (continued)

Species (Authorities see Table 7.9)	**Novel withanolides**	**References**
do.	Four withaferin **A**-type mono- or di-glucosides (linkages: 3β-*O* and/or 27-*O*) plus one extremely unusual aglycone [4-(1-hydroxy-2,2-dimethylcyclo-propanone)-2,3-dihydrowithaferin **A**]	Jayaprakasam and Nair (2003)
do.	One withaferin A-type 3ß-*O*-gentiobioside	Jayaprakasam et al. (2004)
do.	Two withaferin **A**-type withanolides	Choudhary et al. (2004)
do.	Three withaferin **A**-type withanolides including one 3β*O*-gentiobioside	Kuroyanagi et al. (1999)
do.	Four withaferin **A**-type metabolites including one 3β*O*-sulfate and one unusual 1,4-diene-3-one	Misra et al. (2005)
do.	One dimeric thiowithanolide (Ashwagandhanolide)	Subbaraju et al. (2006)
do.	(17α-)Isowithanone; 6α,7α-1α,3β,5α-trihydroxy-witha-24-enolide	Lal et al. (2006)
Witheringia solanacea	[Known physalins **B**, **D**, **F**]	Jacobo-Herrera et al. (2006)

Hyoscyamus, *Lycium, Tubocapsicum, Withania* as well as certain *Datura* and *Physalis* spp.).

Non-solanaceous Occurrence. Withanolides have been discovered also in certain *Tacca* spp. of the Taccaceae (taccalonolides; Huang et al. 2002) and *Ajuga* spp., e.g., *A. parviflora* BENTH., Lamiaceae (ajugins; Khan et al. 1999) as well as in some marine organism. Nevertheless, their occurrence in the Solanaceae is predominating by far.

7.10.2 Structure

"Withanolide" represents the term for the C_{28}-skeleton 22-hydroxyergostan-26-oic acid-22,26-olide; this δ-lactone residue containing structure is a theoretical one (Lavie et al. 1965a, b). Typical substitutions and other modifications for really existing metabolites are the following:

- Oxo group at C-1; instead less frequently a hydroxyl group
- Double bond C-2 → C-3; instead less frequently a hydroxyl group at C-3
- δ-Lactone (26 → 22*O*) often unsaturated (24,25)
- γ-Lactone moiety (26 → 23*O*) instead of δ-lactone, often also unsaturated

- Lactol moiety instead of lactone residue
- High degree of oxidation at many positions of the whole molecule (e.g., oxo groups, hydroxyl groups, epoxide substructures, hemiketals)
- High diversity with regard to such functionalizations with the consequence of oxidative cleavages and/or novel cyclizations

Unfortunately, the term "withanolide" ["withan…." from the genus *Withania*, "…. olide" = chemical term for a lactone] has been used in a diverging and therefore confusing manner. Beside (i) the structural term discussed above many authors have used the term "withanolides" (ii) *only* for compounds belonging to the original, simple, principal, and most abundant withaferin A-type (Fig. 7.27); its natural modifications resulting in compounds with more complex structural features have been classified as withaphysalins, physalins, nicandrenones, jaborols, ixocarpalactones, perulactones, acnistins, and miscellaneous congeners (Veras et al. 2004b and references therein). On the other hand, an increasing development has led to a broad acceptance of the term "withanolides" [iii] for *all* those ergostanes characterized by any lactone moiety linked to C-17 and even – though confusing – their derivatives, i.e., lactols. The reasonable proposal to call the whole class with all its diverging structures "withasteroids" (instead of "withanolides") used, e.g., in the review of Anjaneyulu et al. (1998) has not accomplished its final goal.

The following is representing only a selection of structural types due to reasons already mentioned above.

7.10.2.1 Metabolites with an Unmodified Withanolide Skeleton

Withanolides with an Unmodified β-Oriented Side Chain. This group of withanolides is a voluminous one comprising, e.g., metabolites from the genera *Withania, Datura, Dunalia, Lycium*. A classical example is represented by withaferin A (Fig. 7.27).

Withanolides with an Unmodified α-Oriented Side Chain. This group is less frequent though not rare; members were found, e.g., in the genera *Withania, Jaborosa*. The structures of withanolide E and the withanolide derivative jaborosalactol N are shown as examples in Fig. 7.27.

7.10.2.2 Metabolites with a Modified Withanolide Skeleton

Withanolides with an Aromatic Moiety (Fig. 7.28). (+)-Jaborol, a B-secowithanolide from *Jaborosa magellanica* (Fajardo et al. 1987) in which C-19 is retained, as well as jaborosalactone Q, a 19-*nor*withanolide from *J. leucotricha* (Veleiro et al. 1992), were found to contain a phenolic ring A each. Co-occurrence of jaborosalactone Q and congeners characterized by a hydroxymethyl substituent at C-10, i.e., a 19-hydroxyl group, is indicative of an oxidative degradation leading to a phenolic metabolite (Veleiro et al. 2005 and references therein). Withanolides with an unusual

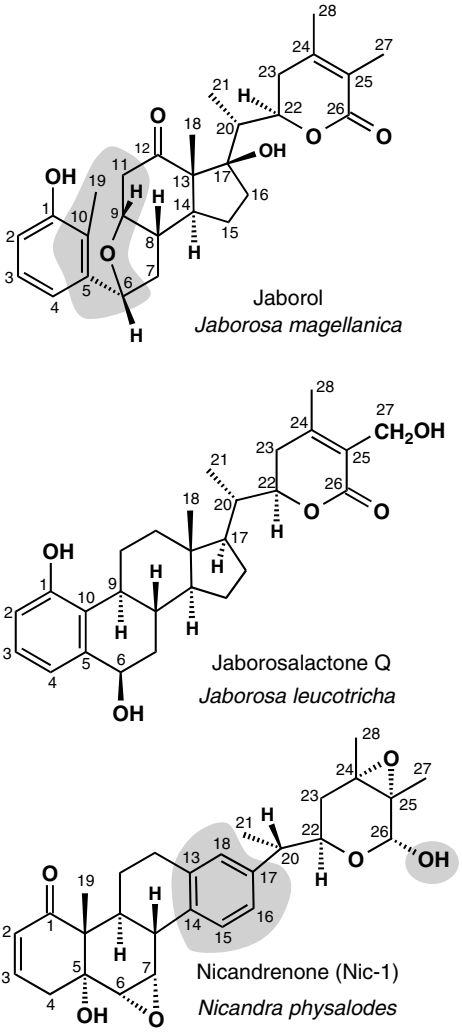


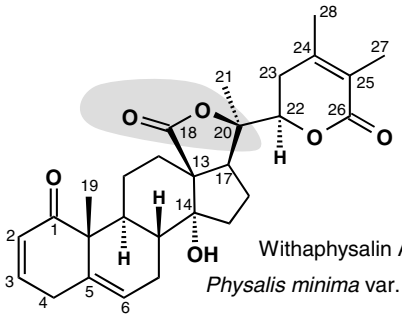
Fig. 7.28 A withanolide (jaborol), a 19-*nor*withanolide (jaborosalactone Q), and a lactol (nicandrenone; its corresponding hydroxyl group is *highlighted in grey*) characterized by an aromatic residue each (ring A and D, respectively). This is formed as a consequence of different oxidation steps: (i) the B-*seco*withanolide jaborol is probably generated by the cleavage of ring B (between C-9 and C-10) of a more classical withanolide precursor followed by the formation of a tetrahydrofuran moiety (C-6–C-9; *highlighted in grey)* and a phenolic ring A (Fajardo et al. 1987); (ii) in the case of jaborosalactone Q C-19 is lost thus forming a phenolic *nor*withanolide; (iii) after a cleavage of ring D of a more classical withanolide precursor its C-18 is probably rearranged thus forming an unusual aromatic ring D (*highlighted in grey*)

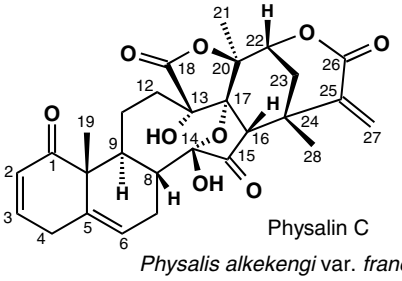
1,4-dien-3-one group such as 27-hydroxy-3-oxo-witha-1,4,24-trienolide ("compound **9**") found in the leaves of *Withania somnifera* (Misra et al. 2005) might be intermediates leading to aromatization in both cases. In contrast, nicandrenone, a metabolite of *Nicandra physalodes*, apple of Peru, is an example for a withanolide whose structure turned out to include an aromatic ring D (Begley et al. 1976). This constituent was discovered by Nalbandov et al. (1964) as a compound with strong insect repellent and mild insecticidal properties, although the authors were not able to elucidate its structure.

18-Functionalized Withanolides. Withacnistin (18-hydroxy-27-deoxy-withaferin A) was discovered along with withaferin A (Fig. 7.27) as a constituent of *Acnistus*

arborescens (Glotter 1991 and references therein). Withaphysalin A, a metabolite of *Physalis minima* var. *indica*, represents a withanolide with a slightly modified skeleton caused by further oxidation of C-18 to a carboxyl group (Fig. 7.29). Another functionalization finally even leads to a loss of C-18: A number of subtrifloralactones, discovered as constituents of *Deprea subtriflora*, represent 18-*nor*withanolides (Veleiro et al. 2005 and references therein).

13,14-Secowithanolides. Withaphysalin C, a derivative of withaphysalin A, as well as the physalins, e.g., physalins C and H (Fig. 7.29) belong to the most studied withanolides: 13,14-Seco congeners isolated from various *Physalis* spp. Due to an initial oxidative cleavage of the withanolide skeleton between C-13 and C-14 the main difference of withaphysalin C to its congener A is represented by a 13,14-epoxide moiety. This moiety is also a characteristic feature of the physalins





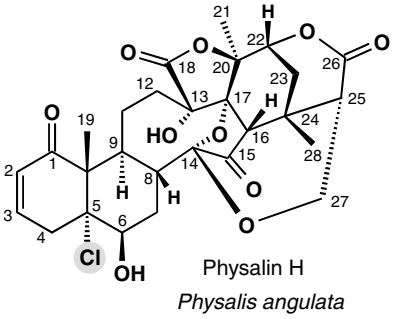


Fig. 7.29 Examples for withanolides from the genus *Physalis* characterized by an additional lactone moiety including C-13, C-17, C-20, and C-18 (lactonization caused by oxidation of the methyl group at C-13; *highlighted in grey* in case of withaphysalin A); physalins show an increased presence of polyfunctional positions leading to the most advanced group of withanolides in terms of the biogenetic oxidation level (molecules with 9–10 oxygen atoms; Makino et al. 1995). The revised structure of physalin H turned out to contain an unusual chlorine atom (*highlighted in grey*)

though they are much more modified with the consequence of rather complicated polycyclic structures with a really unusual content of oxygen atoms. All these metabolites still contain the additional γ-lactone residue caused by the functionalization of C-18.

21-Functionalized Withanolides. Acnistins E and A (4β-deoxyacnistin E) (Fig. 7.30) were assumed to be generated due to such a modification (Glotter 1991; Luis et al. 1994). After a hydroxylation of 21-methyl to the corresponding primary alcoholic hydroxyl group (21-hydroxywithanolides were discovered in *Datura metel*) this CH_2OH group might be transformed into a good leaving group, e.g., a phosphate ester. An external nucleophile (H_2O) might attack C-25 and lead to the

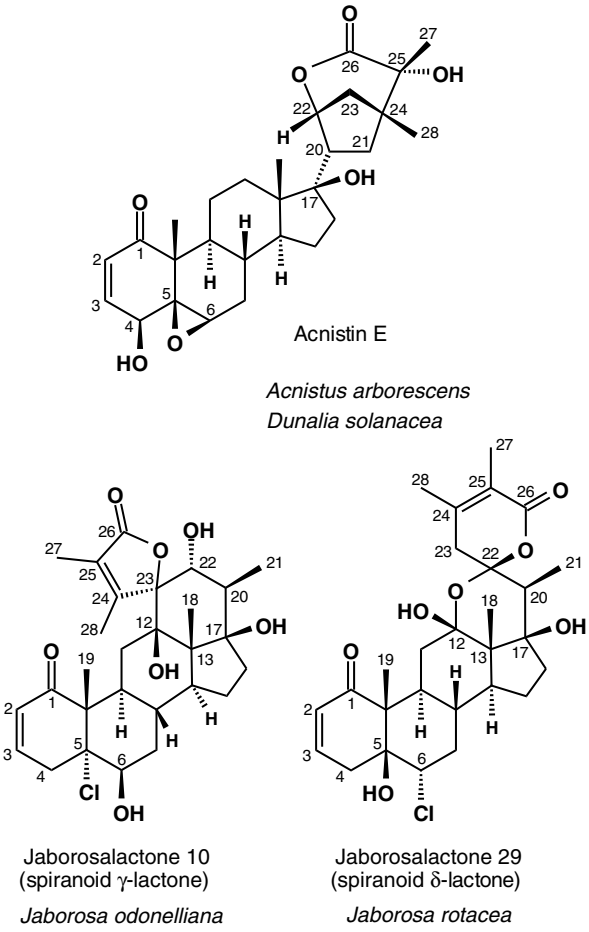


Fig. 7.30 Acnistin E, a withanolide with a modified skeleton due to a functionalization of 21-methyl (for explanation see text), and two highly functionalized jaborosalactones with characteristic spiranoid partial structures (C-23 and C-22, respectively)

formation of a new C-24 – C-21 bond thus displacing the 21-leaving group. The consequence would be the bicylic moiety of the acnistins.

Modifications Due to an Integration of C-12 into an Additional Ring System. Highly functionalized metabolites with characteristic spiranoid substructure have been discovered as constituents of *Jaborosa* spp., e.g., jaborosalactone 10 in *J. odonelliana* (Cirigliano et al. 2002 and references therein) and jaborosalactone 29 in *J. rotacea* (Nicotra et al. 2006) (structures: Fig. 7.30).

7.10.2.3 Withanolides with Unusual Hetero Atoms

Chlorowithanolides. Some withanolides, e.g., physalin H (Fig. 7.29) as well as jaborosalactones 10 and 29 (Fig. 7.30), turned out to contain chlorine substituents at C-5α or C-6β. Secondary metabolites with this substituent are generally very rare in the plant kingdom. Nevertheless, occurrence of 5,6-chlorohydrins together with the corresponding 5,6-epoxides is common among the withanolides (Nicotra et al. 2006 and references therein).

Thiowithanolides. Another hetero atom is present in the unusual sulfide linkage of withaperuvin H, a root constituent of *Physalis peruviana*, cape gooseberry/ground cherry (Oshima et al. 1989). The structure of this metabolite – closely related to withanolide E (Fig. 7.27) – is characterized by an unusual, anellated seven-membered *O*- and *S*- containing heterocyclic moiety including C-4 – C-6 of the withanolide skeleton (*O* linked to C-4β; *S* to C-6α). The authors assumed a biogenetic origin from 4β-hydroxywithanolide E by condensation with α-mercaptoacetaldehyde (O=CH–CH_2–SH) derived from L-cysteine. Such an *S*-linkage to C-6α has been identified also in case of ashwagandhanolide, a *homodimeric* thiowithanolide from the roots of *W. somnifera* (Subbaraju et al. 2006). The *S*-bridge is conjugating two identical withaferin A-type monomers. 2,3-Dihydrowithanone-3β-*O*-sulfate (sulfate of 5α,17α-dihydro-6α,7α-epoxy-3β-hydroxy-1-oxo-witha-24-enolide) has been discovered recently as another constituent of *W. somnifera* (Misra et al. 2005).

7.10.2.4 Withanolide Glycosides

The first withanolide glycosides, dunawithanins A and B (Fig. 7.31), were characterized by Adam et al. (1981) as constituents of the leaves of *Iochroma australe* sub nom. *Dunalia australis* (see also Adam et al. 1984). The aglycone of the dunawithanins turned out to be (20*R*,22*R*)-3β,20-dihydroxy-1α-acetoxy-witha-5,24-dienolide (physalolactone B). The 3β*O*-linked carbohydrate chains were determined as a triglucoside in case of dunawithanin A (two glucose units, 1→2 and 1→3 linked, respectively, to the glucose unit linked directly to the aglycone, thus forming a branched carbohydrate chain) and a diglucoside in case of dunawithanin B. These results were already published two years before Kirson et al. (1983) claimed that they found the first glycoside in the withanolide series, physalolactone

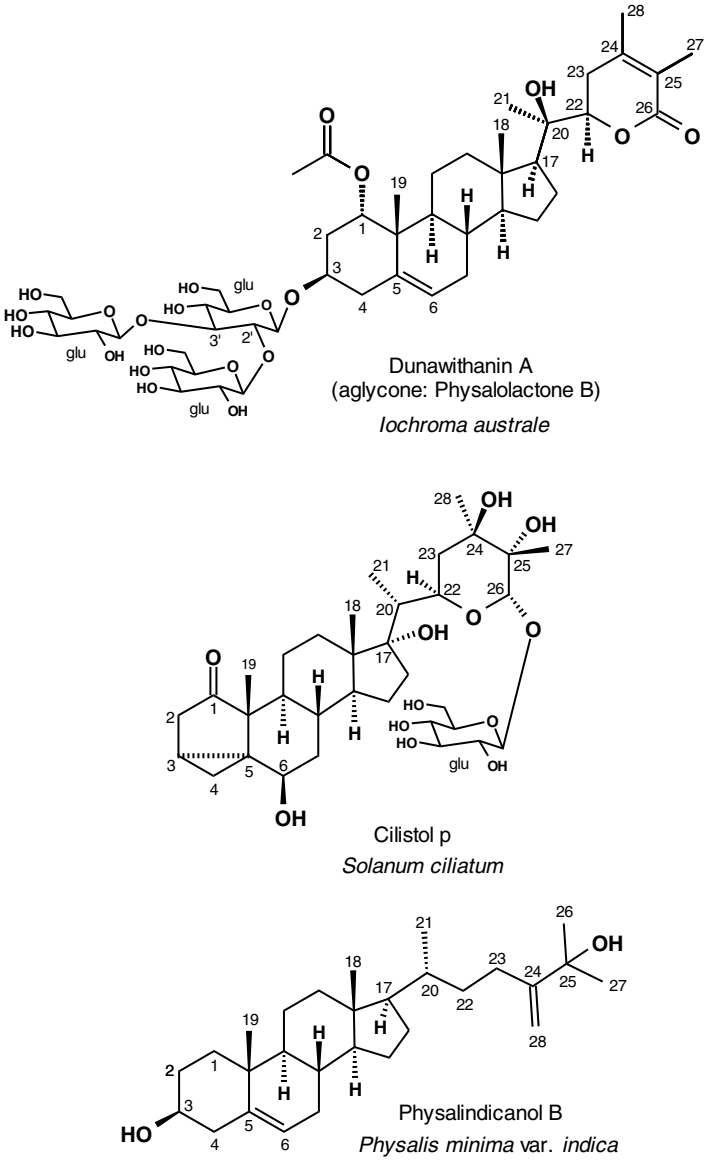


Fig. 7.31 Withasteroid glycosides with an unusual ring A (details in the text) and ergosta-5,24(28)-dien-3β,25-diol (physalindicanol B), a presumable precursor of the withanolide pathway

B 3-*O*-β-D-glucopyranoside, from the leaves of *Physalis peruviana*. Anjaneyulu et al. (1998) counted 20 isolated glycosides nested within their review. Only two further glycosides (constituents of *Dunalia brachyacantha*) could be integrated into the review of Veleiro et al. (2005 and references therein). However, recent structural elucidations of exactly 100 novel withanolides summarized in Table 7.10

include 37 glycosides, among them (beside 3β*O*-linked) 27*O*-linked lactone-type metabolites (Jayaprakasam and Nair 2003) as well as 26*O*-linked lactol-type derivatives [cilistols (Fig. 7.31)] (Zhu et al. 2001a, b, c). Isotubocaposides, di- and triglucosides isolated from *Tubocapsicum anomalum*, turned out to contain an aglycone with a C-17β-oriented bicyclic lactone skeleton like acnistin E (Fig. 7.30) whereas the remaining left part of the molecule including the carbohydrate chain is closely related to dunawithanin A (Fig. 7.31) (Kiyota et al. 2007).

Nevertheless, glycosides of withanolides are rather rare compared, e.g., with steroidal saponins and steroidal alkaloids. Remarkably, glucose represents the only carbohydrate unit of all known withasteroid glycosides again in contrast to the other steroidal metabolites just mentioned. In this connection it has to be pointed out that withanolides (sensu latiore) are representing the class of steroidal metabolites with the highest degree of structural diversity (confined to aglycones) as far as the plant kingdom is concerned.

7.10.2.5 Glycosidic Withanolide Congeners Without Lactone Cyclization

Bioassay-directed fractionation of extracts from the leaves of *Physalis peruviana* led to the discovery of two ester glycosides of 24-*E*-22ζ-acetoxy-1α,3β-dihydroxyergosta-5,24-dien-26-oic acid (Fig. 7.32). Beside glucose linked glycosidically to C-3β the carboxyl group turned out to be esterified with a triglucoside. The second compound is lacking the 6″-acetoxy residue in favour of a hydroxyl group. Both metabolites were strongly active against *Helicoverpa zea* Boddie, corn earworm (Lepidoptera: Noctuidae), an economic pest of numerous crops including tobacco and tomato (Waiss et al. 1993).

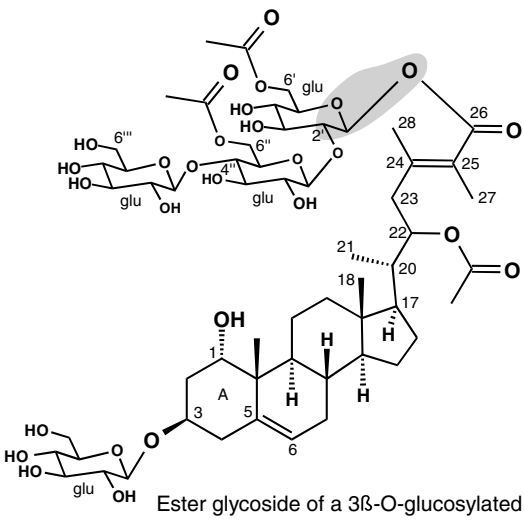


Fig. 7.32 Withanolide congener without lactone cyclization prevented by an acylation of the C-22 hydroxyl group and an ester glycosidation of the C-26 carboxyl group (*highlighted in grey*)

7.10.3 Occurrence in the Solanaceae (Table 7.9)

Withanolides have been reported predominantly as constituents of leaves/stems and roots; reports on other plant organs are rather rare, e.g., in fruits of *Physalis philadelphica* sub nom. *P. ixocarpa* ("tomatillos"; Veleiro et al. 2005 and references therein) and *Withania somniferum* (Abouh-Douh 2002; Lal et al. 2006). In this connection it is interesting to realize that withanolides are not very poisonous for mammals in contrast to other steroidal metabolites of the plant kingdom.

The occurrence of withanolides is confined to the large subfamily Solanoideae (Fig. 3.18) with a single exception (*Browallia viscosa*; see below). These metabolites have been discovered already in three out of four clades which altogether form the monophyletic basal clade of this subfamily:

- *Hyoscyamus niger* (Hyoscyameae clade)
- Nine *Jaborosa* spp. (Jaboroseae clade)
- *Lycium barbarum*, *L. chinense* (Lycieae clade)

However, the centre of the occurrence is represented by the most advanced solanaceous clades, i.e., four subclades of the monophyletic Physaleae clade comprising altogether 14 genera with withanolide-positive reports. This **occurrence in detail**, i.e., in genera and species, has been outlined in Tables 7.9 and 7.10. Recently, withasteroids have even been discovered in two *Solanum* species. However, they represent exclusively lactol-type derivatives (hydroxyl group at C-26α), i.e., to date no withanolides sensu stricto are known from this genus. Altogether 16 metabolites, named cilistols with different small letters (Table 7.10), were isolated from the leaves of *Solanum ciliatum* sub nom. *S. "cilistum"*. Eleven of these constituents turned out to be 26-*O*-glucosides (Zhu et al. 2001a, b, c). Cilistol **w** and **y** represent 3β-*O*-sulfates. Cilistol **p**, the 26-*O*-β-D-glucoside of (22*R*,24*R*,26*R*,26*S*)-22,26-epoxy-1-oxo-**3α,5α-cyclo**ergostane-6β,17α,24,25,26-pentaol (Fig. 7.31), as well as three congeners (cilistols **u**, **pm**, **p1**) are characterized by an unusual cyclopropane ring linking C-3 and C-5. A comprehensive scheme with a hypothetical biogenetic pathway of all cilistols proposed the 3β-*O*-sulfates as intermediates of this 3,5-cyclo-type (Zhu et al. 2001c).

Since neither the data base (IPNI) of the Royal Botanical Gardens, Kew/UK, nor the one of Missouri Botanical Garden (w3TROPICOS) are containing "*Solanum cilistum*", the author of the present book is supposing that this name was an error of the authors (instead of *S. ciliatum*). Furthermore, this led to the misplaced term "cilistols" with consequences for the naming of other constituents by further authors (see below). In this connection, it is to be regretted that in the appreciated review of Anjaneyulu et al. (1998) numerous species names are also corrupted.

Niero et al. (2006) have reported the identification of two novel cilistol derivatives, cilistepoxide and cilistadiol, from the leaves and stems of *S. sisymbrifolium*.

There is only one report on the occurrence of withanolides out of the Solanoideae, namely in *Browallia viscosa* ("0.02% nicandrenone and traces of withaferin A"; Rozkrutowa 1987). Therefore, it would be useful if this exceptional result – from the chemotaxonomic point of view – could be confirmed.

As demonstrated in Fig. 3.18, the majority of the Solanoideae clades between the basal and the most advanced ones is characterized by **the absence of withanolides/withasteroids and the presence of diverging secondary metabolites** such as tropane alkaloids (*Mandragoreae, Solandreae*) or steroidal alkaloids/glycoalkaloids (*Solaneae, Capsiceae*).

7.10.3.1 Co-occurrence of Withanolides/Withasteroids and Steroidal Alkaloids/Glycoalkaloids or Tropanes (Fig. 3.18)

Co-occurrence of lactol-type withasteroids and steroidal alkaloids/glycoalkaloids has been demonstrated in two *Solanum* spp. (*S. ciliatum, S. sisymbrifolium*). However, there is no report on co-occurrence of these alkaloids and withanolides. In a few taxa even withanolides co-occur surprisingly with different types of tropane alkaloids, i.e., hyoscyamine-type tropanes (four *Datura* spp., *Hyoscyamus niger*) or non-hyoscyamine-type tropanes and related alkaloids (*Nicandra physalodes, Physalis alkekengi*, *P. peruviana*, *Withania somnifera*); for details see Tables 3.1 and 3.8.

7.10.4 Biosynthesis

24-Methylene-cholesterol was proposed as a C_{28}-sterol precursor of the withanolides based on corresponding feeding experiments with *Withania somnifera* (Lockley et al. 1976). Isolated withaferin A and withanolide D showed radioactive incorporation of the administered supposed precursor whereas labelled 24-(*R,S*)-methyl-cholesterol failed to be incorporated. The discovery of two further C_{28}-sterols, physalindicanols A and B (Fig. 7.31), as constituents of the whole plant of *Physalis minima* var. *indica*, a species known for the occurrence of withanolides, led to the assumption that these sterols may also be putative intermediates (Sinha et al. 1987). Physalindicanol A turned out to be a 24-hydroxy derivative of ergosta-5,25-dien-3β,24ζ-diol, its congener B a 25-hydroxy derivative of 24-methylene-cholesterol [ergosta-5,24(28)-dien-3β,25-diol]. There are many additional plausible postulations on different sections of the complex withanolide pathway sensu latiore stimulated by steady discoveries of novel structures. However, reports on experimental results are rather rare. Readers are referred to corresponding extensive explanations in all reviews cited above. In favour of an appropriate comprehensibility with regard to rather difficult biogenetic/structural relationships the numbering system of the ergostane skeleton has been retained in Figs. 7.27–7.32.

7.10.5 Significance

Use of *Withania somnifera* as a valuable and very popular drug in Ayurvedic medicine (Ray and Gupta 1994) and *Physalis alkekengi* in Chinese medicine are two outstanding examples for an extensive significance of withanolide-containing

plants in traditional/folk medicine. Recently, a review on the medicinal evaluation of *Withania somnifera*, common Indian name "Ashwagandha", winter cherry, has been published by Kumar and Kushwaha (2006). It includes the literature with regard to all parts of the species and their potential to cure many painful and deadly diseases. Of course, there are also other withanolide-containing species ethnomedicinally used in many countries, especially from the Americas (e.g., Maldonado et al. 2004, 2005; Jacobo-Herrera et al. 2006).

Scientific history of the withanolides has been influenced extraordinarily by a broad interest in pharmacological properties based on the traditional use of corresponding plants. Already some early discoveries of withanolides were caused by bioassay-guided fractionation, e.g., withaferin A, since alcoholic extracts of *Acnistus arborescens* leaves showed significant tumor inhibitory activity (Kupchan et al. 1965b). Consequently, biological/pharmacological activities are topics in the reviews mentioned above. They focused on the following activities according to the corresponding knowledge at that time of their publication: immunomodulating properties, cytotoxic/tumor inhibiting activities, anti-inflammatory effects, hepatoprotective properties, antifungal and antibacterial activities, antifeedant and insecticidal properties (all reviews). In addition, Veleiro et al. (2005 and references therein) have summarized certain recently discovered ones such as antifertility effects or trypanocidal and leishmanicidal activities as well as phytotoxicity (15,21-cyclowithanolides from *Jaborosa bergii*). In particular, the induction of the phase II drug metabolizing enzyme quinone reductase (QR) by withanolides, used to determine their potential cancer chemopreventive property, turned out to be a promising field.

7.10.5.1 Novel Reports

There is still a continuous and remarkable interest in pharmacological activities of withanolides in general including new developments due to the discovery of novel effects.

Cytotoxic/Tumor Inhibiting Activities. Additional reports on such activities have been published on constituents of *Acnistus arborescens* (Veras et al. 2004a, b), *Brachistus stramoniifolius* (Fang et al. 2003), and *Discopodium penninervium* (Habtermariam 1997). Certain withanosides from *W. somnifera* showed significant neurite outgrowth activity at a concentration of 1 μM on a human neuroblastoma cell line (Zhao et al. 2002) which could be of interest indirectly. The dimeric thiowithanolide ashwagandhanolide displayed remarkable growth inhibition against a number of human cancer cell lines (Subbaraju et al. 2006).

Cancer Chemopreventive Properties. A summary of results on the topic "natural inhibitors of carcinogenesis" including a considerable number of withanolides/norwithanolides from *Physalis philadelphica* (syn.: *P. ixocarpa*), Mexican husk tomato / Mexican tomatillo (used as a vegetable and condiment in Mexican/Central American cuisine), and *Deprea subtriflora* has been published recently by Kinghorn et al. (2004 and references therein). This review especially comprises discussions

about structure-activity relationships obtained from the results on the QR induction activity already mentioned above (based on 12 withanolides and 12 18-*nor*withanolides). A specific review only with regard to these metabolites has been published by this group of authors in the same year (Su et al. 2004). Ixocarpalactone A, present in the edible part of the tomatillo (*P. philadelphica*), has shown potent antiproliferative activity in human colon cancer cells; furthermore, induction of apoptosis could be observed (Choi et al. 2006). This metabolite is characterized by the presence of a saturated 26,23 γ-lactone ring in a β-oriented side chain. Thus, tomatillos may have cancer chemopreventive properties.

Trypanocidal Activity. Withaferin A-type withanolides (physagulins) from *Physalis angulata* showed weak to considerable trypanocidal activity (physagulin D: 5 μM) against trypomastigotes, an infectious form of *Trypanosoma cruzi* and the etiologic agent for Chagas' disease (Abe et al. 2006).

Leishmanicidal Activity. Physalin H (Fig. 7.29), again a constituent of *P. angulata*, was found to possess potent leishmanicidal activity (Choudhary et al. 2006).

Further Biological Properties. Physalins B and F, isolated from *P. angulata*, have shown inhibitory activities on NF-κB activation at 16 and 8 μM, respectively (Jacobo-Herrera et al. 2006). This is of interest in connection with anti-inflammatory effects of withanolides. These physalins are structurally closely related to their congener H (Fig. 7.29): B as well as F are lacking substituents at C-5 and C-6 in favour of a 5,6-double bond with an additional 7β-OH in case of F. Furthermore, physalin B produced a marked inhibition with regard to tumor necrosis factor TNFα as well as to interleukins IL-6 and IL-12 in macrophages stimulated with lipopolysaccharide (LPS) and interferon IFNγ. It turned out to prevent the septic shock induced by LPS (Soares et al. 2003).

A number of withaferin A-type withanolides and withanosides have been shown to exhibit remarkable cyclooxygenase-2 (COX-2) inhibition. This activity turned out to be a selective one, since COX-1 was not concerned. Thus, these compounds might be candidates for novel anti-inflammatory drugs. Furthermore, COX-2 inhibitors could be of interest for the chemoprevention of various types of cancer (Jayaprakasam and Nair 2003). Withanolides from *W. somnifera* having a 4β-hydroxy-5β,6β-epoxy-2-en-1-one moiety turned out to induce cell differentiation (Kuroyanagi et al. 1999). Coagulin H (see *W. coagulans* in Table 7.10) was identified as a potential immunosuppressive candidate; it exhibited a powerful inhibitory effect on lymphocyte proliferation and Th-1 cytokine production. Furthermore, it was also a potent human IL-2 inhibitor. A molecular docking study predicted that this withanolide binds to the receptor binding site of IL-2 more effectively than the clinically established immune modulating drug prednisolone (Mesaik et al. 2006).

Five withanolides isolated from *Withania somnifera* [and *Ajuga bracteosa* BENTH. (Lamiaceae)] were shown to be novel natural cholinesterase (AChE) inhibitors with spasmolytic and calcium antagonistic properties. Thus, they represent leads or even possible drug candidates for treatment of Alzheimer's disease or related dementia (Choudhary et al. 2004, 2005). Three withanolides were found to be linear mixed-type inhibitors of AChE, the remaining two were non-competitive inhibitors, whereas all

five congeners turned out to be non-competitive inhibitors of butylcholinesterase. The scientific basis for anti-dementia drugs from *Withania somnifera* – though based on another mechanism of action – has also been discussed by Tohda et al. (2005). Based on their own results (obtained in vivo with rats), the authors are convinced that withanolide A, structurally closely related to withanolide E (Fig. 7.27; difference: no 5β,6β-epoxy group, instead 6α,7α-epoxy plus 4α-OH) and certain withanosides, e.g., 3β-*O*-β-D-glucopyranosides of different withanolide aglycones with an unmodified side chain, are important candidates for the therapeutic treatment of neurovegetative diseases, since these compounds have been shown to reconstruct neuronal networks. This has been confirmed recently in another study. Memory deficits of mice after Aβ(25-35)-injection were significantly improved and loss of axons, dendrites, and synapses prevented by the orally administered 3β*O*-gentiobioside withanoside IV (Kuboyama et al. 2006). Aβ(25-35) represents the neurotoxic β-sheet fragment of amyloid β. Sominone (1α,3β,27-trihydroxy-witha-5,24-dienolide), the aglycone and metabolite of withanoside IV, turned out to be the active principle. It could be shown that sominone reconstructed synapses in damaged neurons also in vitro. The authors concluded that the orally administered glycoside may ameliorate neuronal dysfunction in Alzheimer's disease.

Another 3β*O*-gentiobioside, withanoside VI (aglycone: 1α,3β,20β-trihydroxy-witha-5,24-dienolide), as well as withaferin A attenuated the tachyphylaxis to clonidine on electrically stimulated guinea-pig ileum in vitro (Matsuda et al. 2001).

7.11 Petuniasteroids (C_{28} Isoprenoids)

7.11.1 Discovery and Structures

Petuniasteroids, new types of ergostanoids with unusual functionalities, were discovered as constituents of the leaves and stems of *Petunia* × *hybrida* (HOOK.) VILM. by Elliger et al. (1988a, b). A first review on this topic was published already a few years later (Elliger and Waiss 1991). Meanwhile these ergostanoids may be divided into four subgroups with trivial names which indicate certain structural characteristics.

7.11.1.1 Petuniasterones

The members of this large subgroup are characterized by a keto group. Most of them are 3-oxo derivatives, e.g., petuniasterone A (Fig. 7.33; Elliger et al. 1988a), with a few exceptions [1-oxo, e.g., petuniasterone O; Elliger et al. 1989b]. Regularly they contain an allylic ketone functionality [1,4-dien-3-one residue except petuniasterone B (4-en-3-one)]. However, petuniasterone O is lacking any A-ring double bond. Several petuniasterones, usually discovered in *P.* × *hybrida*, show a unique bicyclic orthoester system on the side chain: orthoacetate, e.g., petuniasterones

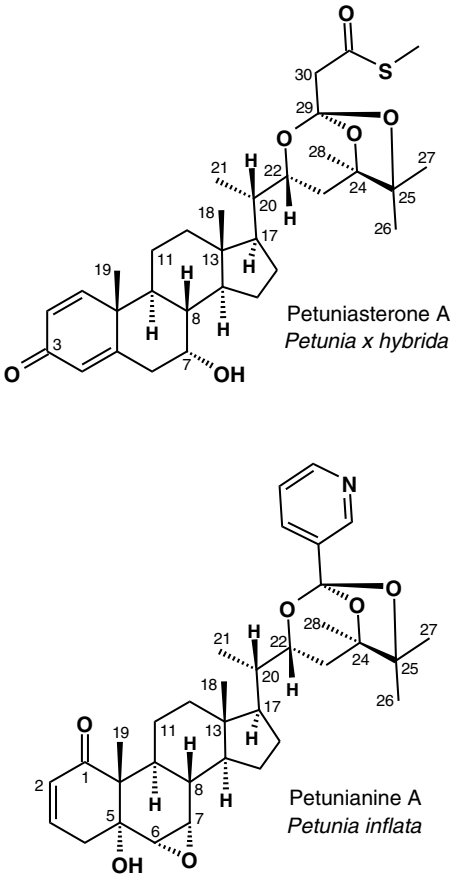


Fig. 7.33 Petuniasteroids, an ergostanoid type confined to the genus *Petunia* (Solanaceae)

D and O, or the unusual (methylthiocarbonyl)orthoacetate, e.g., petuniasterones A, N, Q, and R (Elliger et al. 1989a, 1992). *Petunia axillaris* (Lam.) Britton, Stern & Poggenb. and *P. parodii* Steere [syn.: *P. axillaris* ssp. *parodii* (Steere) Cabrera], respectively, were also found to synthesize orthoester-type petuniasterones (Elliger and Waiss 1989). In contrast, petuniasterones B and C, again discovered in *P.* × *hybrida*, show open C_{29} side chains with 24,25-epoxy moieties and a free hydroxyl group at C-22. Esters of such petuniasterones, e.g., 22*O*-[(methylthio)carbonyl]-acetates or 22*O*-nicotinates which are assumed to be biogenetic precursors of corresponding orthoesters were also discovered (Elliger et al. 1993). Three further petuniasterones were discovered by Moser et al. (1999).

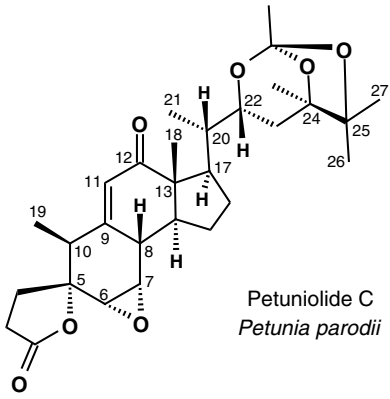
Compound N, detected in *P.* × *hybrida*, *P. integrifolia*, and *P. parodii* showed an unusual secoergostan structure: It turned out to be a D-ring-rearranged analogue of petuniasterone A with the result of an *O*-heterocyclic D-ring and an attachment of the side chain at C-16 instead of C-17. The latter carbon is now an exocyclic part of this chain (Elliger et al. 1989a). A number of minor congeners from *P.* × *hybrida* are characterized by normal, i.e., C-17-attached side chains with three hydroxyl groups (at C-22, C-24, C-25) (Elliger et al. 1988b).

7.11.1.2 Petuniolides

Like certain members of the preceding subgroup petuniolides are also characterized by orthoester residues. However, as their name is indicating their A-rings represent lactones as the consequence of an oxidative rearrangement of the original carbocyclic A-ring with loss of one carbon. This moiety is an unusual spirolactone. Furthermore, petuniolides have an 6α,7α-epoxy group, e.g., petuniolide C (Fig. 7.34). These compounds were discovered as foliage constituents of *P. parodii* [petuniolides A–G] and *P. integrifolia* (Hook.) Schinz & Thell. (petuniolide A). Orthoester moieties of congeners A and C are derived from acetic acid, those of B and D from propionic acid (Elliger et al. 1990).

7.11.1.3 Petunianines

Their trivial name is indicating that these compounds show an alkaloidal character due to the presence of a pyridine ring-bearing orthoester residue (orthonicotinate). Petunianines were detected in *P. inflata* Fries (Elliger et al. 1993). Petunianine A



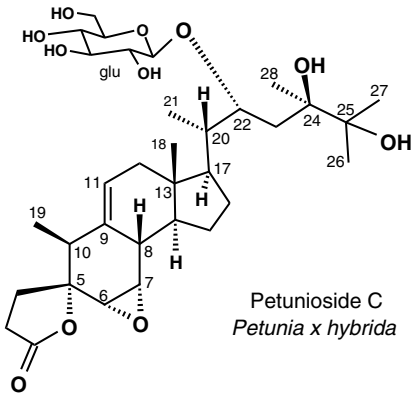


Fig. 7.34 A-rings rearranged spirolactone-type petuniasteroids confined to the genus *Petunia* (Solanaceae); biogenetic numbering according to cholesterol/ergosterol in spite of the loss of one carbon of the original A-ring

(Fig. 7.33) is almost analogous to petuniasterone O (Elliger et al. 1989b) and possesses A- and B-ring functionalization identical to that of certain withanolides (Elliger et al. 1993 and references therein). Petunianine B is analogous to petuniolide E, an orthotriacetate, i.e., it represents a spirolactone-type A-ring variation. Beside the orthonicotinates a series of mono- and di-esters of nicotinic acid (e.g., 22-nicotinate; 7,22-dinicotinate) derived from petuniasterones B and C could be structurally elucidated. Such congeners are assumed to be biogenetic precursors of the petunianines.

7.11.1.4 Petuniosides

From the fresh aerial parts of *P.* × *hybrida* a series of ergostanoid glycosides could be isolated which were termed petuniosides. Petunioside C (Fig. 7.34) represents the 22*O*-glucoside of a petuniolide with an open side chain [C-17-attached, with three hydroxyl groups (at C-22, C-24, C-25)]. Beside further open-chained congeners with similar structures petunioside A turned out to be a 3*O*-gentiobioside of an 1α,3β,5α,30-tetrahydroxy ergostanoid including a methylthiocarbonyl orthoester moiety (Shingu et al. 1994).

7.11.2 Ecological Significance

Larvae of *Helicoverpa zea* Boddie (syn.: *Heliothis zea*), corn earworm (Lepidoptera: Noctuidae), were found to show immediate distress after ingestion of only a few bites of *Petunia parodii* and to stop feeding exhibiting convulsive movements, diuresis, and eventually death (Elliger et al. 1990). Biologically active petuniasterones possess an ortho ester functionality attached at positions C-22, C-24, and C-25 (Elliger et al. 1992) not depending on the specific structure of the orthoester (Elliger et al. 1989a). Even the unusual D-ring rearranged petuniasterone N had about the same activity as its congener A (Elliger et al. 1989a). However, congeners B and C both lacking an orthoester residue did not appear to be active (Elliger et al. 1988b). Petuniolides B and D, also characterized by orthoesters, turned out to be 10 to 20 times more active than the most toxic petuniasterones (Elliger et al. 1990). Petuniolide C was shown to reduce the growth of the larvae of the lepidopteran insect to 50% at a dietary level of 3–4 mg/kg whereas the ED_{50} value of petuniasterone D was determined to be 130 mg/kg (Elliger et al. 1993). Petuniasterones had also different molluscicidal activities, again depending on the structural character of the side chains (Moser et al. 1999).

7.12 Tetraterpenoids/Carotenoids (C_{40} Isoprenoids)

The carotenoids are a large class of natural pigments. In plants they occur in the chromoplasts/chloroplasts. Their main role in photosynthesis is to protect chlorophyll against photooxidation. In addition, they act as accessory pigments because

they absorb light and funnel the energy to the photochemical reaction center just like chlorophyll b. They play a role as protectors against UV and short wavelength visible light. Thus, they are present in all green tissues. Furthermore, they are constituents of flowers and fruits where they are responsible for most of the yellow to red colours. Unsaturated C_{40} hydrocarbons are called carotenes; C_{40} congeners with oxygen functionalities are termed xanthophylls. Both subgroups taken together form the carotenoids sensu stricto. This term taken sensu latiore includes derivatives with higher or lower numbers of carbon atoms, e.g., degradation products generated during senescence, drying, and curing (tobacco). There are acyclic long-chained carotenoids and congeners with a 5- or 6-membered ring at one or both ends of the chains (Bramley 1997). A conjugated system of unusually frequent double bonds leads to their characteristic yellow to red colours. Though different geometric isomers (*cis/trans* = *E/Z*) are possible, *all-trans* forms are dominating. The most abundant natural carotene, the ubiquitous major carotenoid in chloroplasts of plants is represented by β-carotene (β,β-carotene). It is often accompanied by smaller amounts of β,ε-carotene (Britton et al. 2004). Neoxanthin (5′,6′-epoxy-6,7-didehydro-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,3′-triol), characterized by unusual double bonds between C-6 and C-7 as well as between C-7 and C-8, is occurring as a major carotinoid in all green leaves together with β-carotene, lutein [(3*R*,3′*R*,6′*R*)-β,ε-carotene-3,3′-diol], and violaxanthin (5,6:5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,3′-diol).

The biosynthesis of the carotenoids is well-studied including all enzymes involved (Al-Babili et al. 2000; Römer and Fraser 2005; Breitenbach and Sandmann 2005). The first part of the pathway including only acyclic carotenes with an increasing level of desaturation proceeds via intermediates in *cis* configuration: 15-*cis*-phytoene → 15-*cis*-phytofluene → 15,9′-*cis*-phytofluene → 9,15,9′-*tricis*-ζ-carotene → 9,9′-*dicis*-ζ-carotene → 7,9,9′-*tricis*-neurosporene → prolycopene (7,9,7′,9′-*tetracis*-lycopene) → *all-trans*-lycopene (Breitenbach and Sandmann 2005). There are different branches in the following pathway resulting in cyclizations of one or both terminal parts of the lycopene molecule catalyzed by corresponding cyclases. The *systematic* names of the resulting 5- or 6-membered rings are marked by Greek letters with the consequence of two letters for the *systematic* names of a carotenoid, e.g., ε,ψ-carotene. In contrast, *trivial* names of carotenes contain only one Greek letter, in the case of this example δ-carotene (Figs. 7.35 and 7.36).

7.12.1 Solanaceae

7.12.1.1 Discovery and Structural Elucidation

Wilstätter and Escher (1911) reported that “carotene” discovered in carrots, *Daucus carota* L. (Apiaceae), and lycopene (systematic name ψ,ψ-carotene; Fig. 7.35) discovered in tomatoes, *Solanum lycopersicum* L. (syn.: *Lycopersicon esculentum*

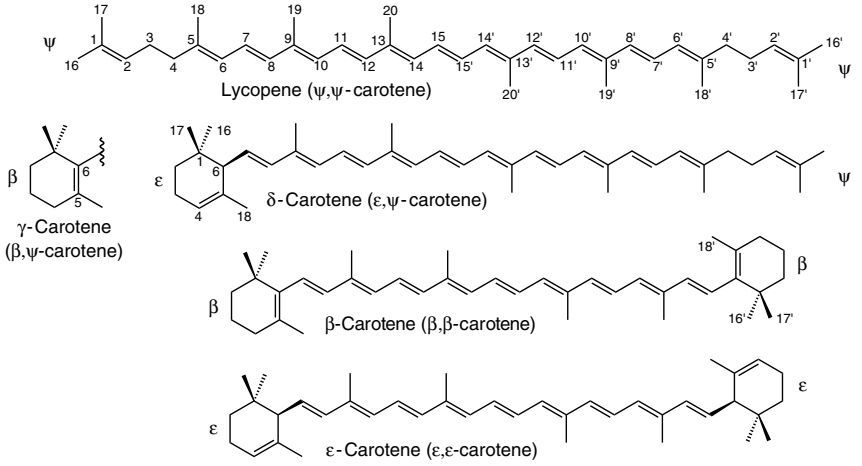


Fig. 7.35 Structures, numbering, trivial names, and systematic names (*in brackets*) of major carotenes; Greek letters at the terminal residues are identical with those of the systematic names. The systematic name for α-carotene (structure not shown) is (6′*R*)-β,ε-carotene

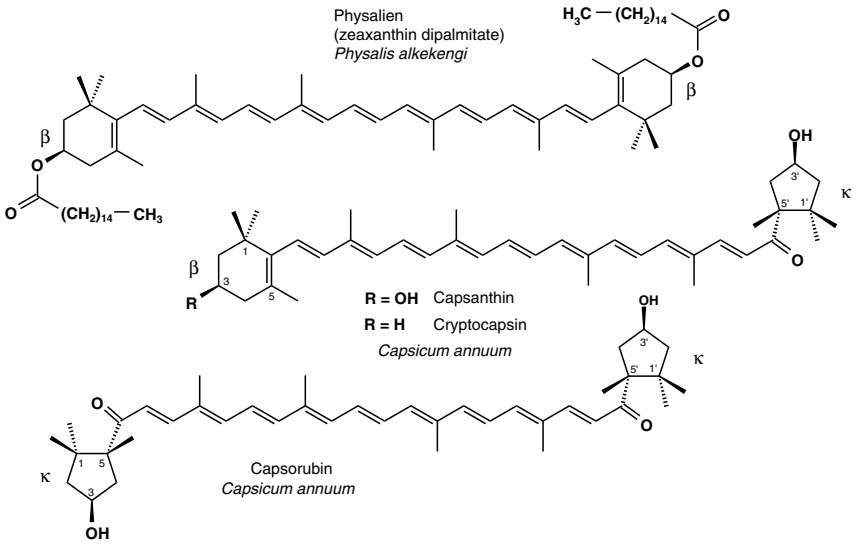


Fig. 7.36 Examples for carotenoids discovered in species of the Solanaceae; the Greek letter κ is indicating the rare cyclopentanol terminal group

MILL.), have the same molecular formula, but are not identical compounds. Beside lycopene two xanthophyll-type congeners were discovered in fresh tomatoes, lycoxanthin (ψ,ψ-carotene-16-ol) and lycophyll (ψ,ψ-carotene-16,16′-diol) (Zechmeister and Cholnoky 1936). Zechmeister and Cholnoky (1927) started their

extensive studies on paprika/red bell pepper (*Capsicum annuum* L.) coloring matters with a first report on the characterization of capsanthin. Structure elucidation needed many attempts and several decades before it turned out to be (3*R*,3′*S*,5′*R*)-3,3′-dihydroxy-β,κ-caroten-6′-one (Fig. 7.36) (Rüttimann et al. 1983 and references therein). The unusual cyclopentanol moiety (κ) was one of major reasons for the difficulties to determine its constitution and configuration. Two structurally related pigments of these fruits, capsorubin [(3*S*,5*R*,3′*S*,5′*R*)-3,3′-dihydroxy-κ,κ-caroten-6,6′-dione] and cryptocapsin [(3′*S*,5′*R*)-3′-hydroxy-β,κ-caroten-6′-one; Fig. 7.36], were discovered in 1934 and 1956, respectively, again by the group of Cholnoky (Rüttimann et al. 1983 and references therein). Remarkably, these three congeners seem to be confined to the genus *Capsicum*. In retrospect fruits of *S. lycopersicum* and *C. annuum* turned out to be a rich source for the discovery and/or isolation of carotenoids. Both represent the most frequent mentionings of solanaceous species and moreover one of the most frequent ones of the plant kingdom in the Carotenoids Handbook of Britton et al. (2004), the corresponding encyclopaedia. A third solanaceous species, *Physalis alkekengi* L., Chinese lantern, was another object for carotenoid research in the 1920s due to its red large calyces. At the same time when zeaxanthin [(3*R*,3′*R*)-β,β-carotene-3,3-diol] was discovered in yellow maize, *Zea mays* L. (Poaceae), this xanthophyll was also detected in the calyces and berries of *P. alkekengi* together with its dipalmitate which was named physalien (Fig. 7. 36) (Kuhn and Wiegand 1929). Its structural elucidation could be realized soon (Kuhn et al. 1930; Zechmeister and Cholnoky 1930). At the same time it was detected also as a constituent of the berries of another solanaceous species, *Lycium barbarum* L. sub nom. *L. halimifolium* Mill., boxthorn (Zechmeister and Cholnoky 1930). Recently, physalien has been determined to be a major carotenoid (31–56% of the total carotenoids) of different *Lycium* spp., e.g., *L. chinense* Mill. (Peng et al. 2005). β-Cryptoxanthin [(3*R*)-β,β-caroten-3-ol] was also discovered in *P. alkekengi* (Kuhn and Grundmann 1933). Later it was also detected in fruits of *Solanum betaceum* Cav. [syn.: *Cyphomandra betacea* (Cav.) Sendt.] (Britton et al. 2004 and references therein).

Violaxanthin → *all-trans*-neoxanthin → 9′-*cis*-neoxanthin → xanthoxin (C_{15}) are intermediates in the carotenoid pathway leading to the phytohormone abscisic acid (C_{15}). Mutants of *Nicotiana plumbaginifolia* Viviani and *Solanum lycopersicum* L. sub nom. *Lycopersicon esculentum* Mill. were involved in the elucidation of this biosynthesis (Dewick 1999; Oritani and Kiyota 2003 and references therein).

7.12.1.2 Occurrence

Mentionings in the Carotenoids Handbook (Britton et al. 2004) which indicate discovery in the corresponding species or suitable source for isolation

Order according to the handbook; systematic names in brackets, for structural comparison see Figs. 7.35 and 7.36; degradation products (apocarotenoids) are characterized by the prefix “apo” (see also Fig. 7.37).

Solanum lycopersicum* L. *(syn.: Lycopersicon esculentum* MILL.*)

γ-Carotene (β,ψ-carotene; Fig. 7.35)
β-Zeacarotene (7′,8′-dihydro-β,ψ-carotene)
Lycopene (ψ,ψ-carotene; Fig. 7. 35)
Phytofluene (7,8,11,12,7′,8′-hexahydro-ψ,ψ-carotene)
Phytoene (7,8,11,12,7′,8′,11′,12′-octahydro-ψ,ψ-carotene)
Lycoxanthin (ψ,ψ-caroten-16-ol), also in ripe berries of *S. dulcamara* L.
Lycopene 1,2-epoxide [(2*S*)-1,2-epoxy-1,2-dihydro-ψ,ψ-carotene]
ζ-Carotene epoxide [1,2-epoxy-1,2,7,8,7′,8′-hexahydro-ψ,ψ-carotene]
Phytoene epoxide [1,2-epoxy-1,2,7,8,11,12,7′,8′,11′,12′-decahydro-ψ,ψ-carotene]
Apo-6′-lycopenal (6′-apo-ψ-caroten-6′-al; *apocarotinoid*)
Apo-8′-lycopenal (8′-apo-ψ-caroten-8′-al; *apocarotinoid*)

Mutants of *S. lycopersicum*

ε-Carotene [(6*R*,6′*R*)-ε,ε-carotene; Fig. 7.35]
δ-Carotene [(6*R*)-ε,ψ-carotene; Fig. 7.35]
Prolycopene [(7*Z*,9*Z*,7′*Z*,9′*Z*) ψ,ψ-carotene]
γ-Carotene-1′,2′-epoxide (1′,2′-epoxy-1′,2′-dihydro-β,ψ-carotene)
δ-Carotene-1′,2′-epoxide (1′,2′-epoxy-1′,2′-dihydro-ε,ψ-carotene)

Lycopene accounts for >85% of the total tomato carotenoids in red-ripe fruits thus causing its characteristic colour (Lenucci et al. 2006 and references therein). This open-chain hydrocarbon with 11 conjugated and two isolated double bonds has the highest degree of unsaturation of all carotenoids. Its concentration varies from 30 to 200 mg/kg fresh wt (Topal et al. 2006). Phytoene, β-carotene, and lutein are further quantitatively remarkable constituents (Burns et al. 2003).

***Capsicum annuum* L. [var. *longum*] (paprika/red bell pepper/red sweet pepper)**

Anhydrolutein I [(3*R*,6′*R*)-3′,4′-didehydro-β,γ-caroten-3-ol]
5,6-Diepilatoxanthin [5′,6′-epoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,5,6,3′-tetrol]
Cucurbitaxanthin A [3′,6′-epoxy-5′,6′-dihydro-β,β-carotene-3,5′-diol]
Cucurbitaxanthin B [5,6:3′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-3,5′-diol]
Cucurbitachrome 1 [5,8:3′,6′-diepoxy-5,8,5′,6′-tetrahydro-β,β-carotene-3,5′-diol]
Mutatoxanthin [5,8-epoxy-5,8-dihydro-β,β-carotene-3,3′-diol]
Cycloviolaxanthin [3,6:3′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene-5,5′-diol]
Cryptocapsin [(3′*S*,5′*R*)-3′-hydroxy-β,κ-caroten-6′-one; Fig. 7.36]
Capsanthin [(3*R*,3′*S*,5′*R*)-3,3′-dihydroxy-β,κ-caroten-6′-one; Fig. 7.36]
5,6-Diepicapsokarpoxanthin [3,5,6,3′-tetrahydroxy-5,6-dihydro-β,κ-caroten-6-one]
Capsanthin 5,6-epoxide [5,6-epoxy-3,3′-dihydroxy-5,6-dihydro-β,κ-caroten-6′-one]

Capsanthin 3,6-epoxide [3,6-epoxy-5,3′-dihydroxy-5,6-dihydro-β,κ-caroten-6′-one]
Capsanthone [(3*R*,5′*R*)-3-hydroxy-β,κ-caroten-3′,6′-dione]
Capsorubin [(3*S*,5*R*,3′*S*,5′*R*)-3,3′-dihydroxy-κ,κ-caroten-6,6′-dione; Fig. 7.36]
Apo-12′-capsorubinal [(3*S*,5*R*)-3-hydroxy-12′-apo-κ-caroten-12′-al; *apocarotinoid*]
Retro-C_{18}-dione (C_{18}; *apocarotinoid*)

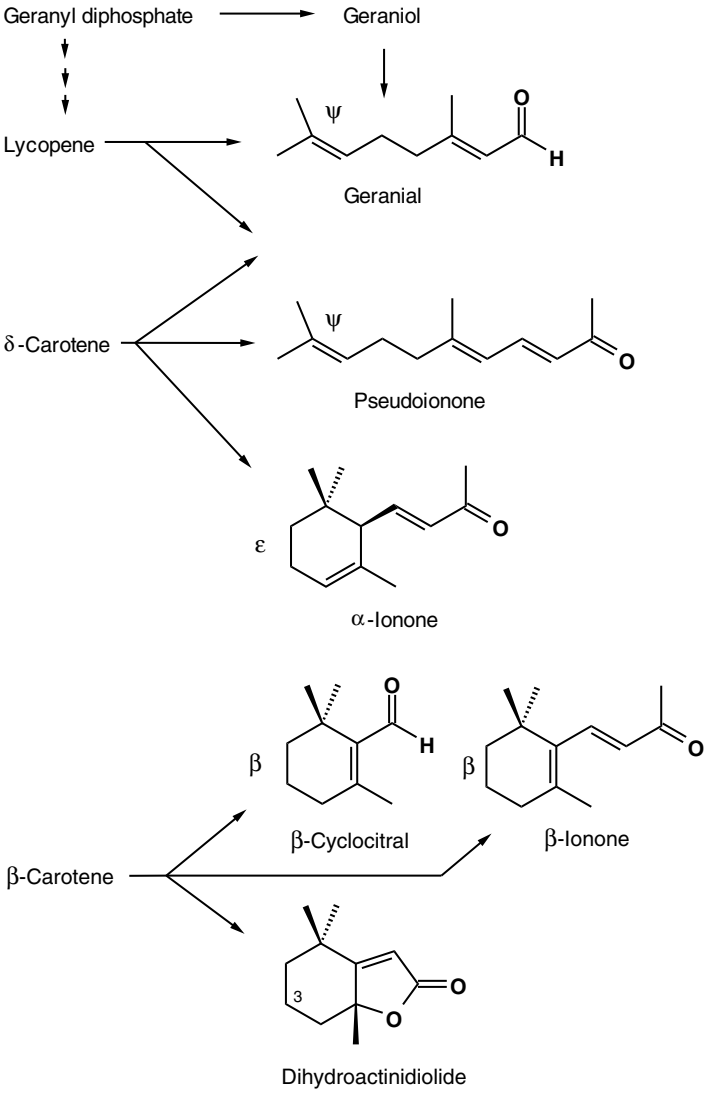


Fig. 7.37 In vivo degradation of carotenes in ripe fruits of *Solanum lycopersicum* leading to volatile monoterpenoids and C_{11} or C_{13} *nor*terpenoids; the alternative pathway lycopene → geranial represents a biochemical convergence to the classical route geranyl diphosphate → geraniol → geranial known from other plants containing essential oils

The ketocarotenoids cryptocapsin (synthesized via β-carotene → cryptoxanthin), capsanthin (via cryptoxanthin → zeaxanthin → antheraxanthin), and capsorubin (via antheraxanthin → violaxanthin) are formed during ripening in the red and orange fruits only (Fig. 7.36). Results were obtained with large-fruited varieties of *C. annuum* (bell peppers, red and yellow varieties) and also with small-fruited ornamental peppers of this species (white, yellow, orange, red) (Davies et al. 1970 and references therein). A comprehensive table on the occurrence of individual carotenoids in the fruits of colour varieties was integrated in that report. Less oxygenated carotenoids such as β-carotene, its 5,6-epoxide and cryptoxanthin were absent in orange fruits. Lutein turned out to be the most abundant carotenoid in green bell peppers with β-carotene, violaxanthin, and neoxanthin also as major pigments and different carotenes as minor ones (Curl 1964). Green, yellow, and red pepper varieties have been analyzed in detail again recently with updating results with regard to 13 specific carotenoids (Burns et al. 2003). Cycloviolaxanthin was found as a constituent of a black-fruited variety (Britton et al. 2004 and references therein).

***Solanum tuberosum* L.** Carotenoids, predominantly xanthophylls such as lutein, zeaxanthin, violaxanthin, only traces of carotenes, are present in the flesh of all potato cultivars. However, there are large quantitative differences: White-fleshed varieties were determined to contain 50–100 µg/100 g fresh weight, whereas yellow- to orange-fleshed ones may contain up to 2000 µg/100 g fresh weight. The colour of red and purple potatoes is caused by anthocyanins (Brown 2005 and references therein).

***Nicotiana tabacum* L.** There are no references concerning *Nicotiana* in the Carotenoids Handbook. Like in all green leaves characteristic major carotenoids such as β-carotene, lutein, violaxanthin, and neoxanthin as well as their biogenetic precursors are also present in *Nicotiana* leaves. Altogether 18 carotenoids have been identified in green tobacco plants (Weeks 1999 and references therein). "During the curing process, which involves air, heat, fire or sun-drying and leads to the creation of the typical tobacco aroma, the tobacco constituents are subjected to various enzymatic, microbial, photochemical and oxidative reactions" (Enzell et al. 1977). Carotenoids as well as diterpenoids (see Sect. 7.4.1) are precursors of such degradation products. "A large number of the tobacco flavour constituents can be viewed as carotenoid degradation products formed by oxidative cleavage of the polyene chain" (Enzell et al. 1977). They "….. are degraded drastically from 2000 ppm in a vigorously growing green plant to less than 100 ppm during senescence, harvesting, curing and storage" (Weeks 1999). Attacks at 6,7-, 7,8- and 9,10-double bonds in the polyene chain lead to C_9, C_{10}, C_{11} and C_{13} norisoprenoids (e.g., ionones, megastigma-dienones/-trienones, damascones, damascenones). Different reviews on *Nicotiana* contain comprehensive information with regard to the carotenoids as well as to numerous norisoprenoids generated from acyclic and cyclic carotenoids, e.g., Enzell et al. 1977; Leffingwell 1999). Obviously, many results have been obtained with cured tobacco, e.g., Bolt et al. (1983), rather than with fresh or carefully dried leaves. Thus, it is often unclear, whether a certain compound is a natural metabolite or an artefact; there may be a fluent transition.

Apocarotenoids/*Nor*carotenoids (→ Monoterpenoids/*Nor*isoprenoids) in Further Solanaceous Species. It has been demonstrated that different carotenoid pigmentation patterns in the fruits of certain varieties of *Solanum lycopersicum* L. sub nom. *Lycopersicum esculentum* MILL. (near-isogenic lines differing in fruit carotenogenesis genes) have profound effects on the *nor*isoprenoid and monoterpenoid aroma volatile compositions of the tomatoes (Lewinsohn et al. 2005). Noncyclic monoterpenoids, e.g., geranial, neral, and noncyclic *nor*isoprenoids, e.g., (*E*,*E*)-pseudoionone (C_{13}) (Fig. 7.37), geranyl acetone (C_{13}), farnesyl acetone (C_{18}) were formed by oxidative degradation of noncyclic tetraterpenoids such as lycopene which was the major pigment in wild-type red tomatoes. Tomatoes with β-carotene as the dominating carotene yielded monoterpenoids like β-cyclocitral and *nor*isoprenoids such as dihydroactinidiolide (C_{11}) and β-ionone (C_{13}) (Fig. 7.37). An orange-fleshed mutant accumulating high levels of the monocyclic δ-carotene revealed prominently α-ionone (Fig. 7.37) whereas a yellow-fleshed one being almost devoid of carotenoids in the fruits (due to a non-functional phytoene synthase) did not show *nor*isoprenoids or monoterpenoids. Such degradation products of carotenoids (apocarotenoids) contribute also to the aroma of species from other families, e.g., *Citrullus lanatus* (THUMB.) MATSUM. & NAKAI, watermelon (Cucurbitaceae) (Lewinsohn et al. 2005).

Studies on aroma generation in the lulo plant, *S. quitoense*, revealed that a number of non-volatile C_{13} *nor*isoprenoid glycosides accumulated in the leaves, e.g., (6*R*,9*R*)-13-hydroxy-3-oxo-α-ionol 9-*O*-β-D-glucopyranoside or (3*S*,5*R*,8*R*)-3,5-dihydroxy-6,7-megastigmadien-9-one 5-*O*-β-D-glucopyranoside. They represent precursors of volatile aglycones contributing to the flavour of the fruit due to hydrolyzation by glucosidases after the corresponding transport (Osorio et al. 2003). Many novel C_{13} *nor*isoprenoids (megastigmenes and megastigmadienes) have been identified as constituents of the *fresh* leaves of *Cestrum parqui* L'HÉRIT. (D'Abrosca et al. 2004, 2005). Megastigmenes and megastigmadienes are ionone derivatives lacking a terminal formyl group of the side-chain; in contrast to the ionones they possess at least one other oxygen functionality (frequently 3-oxo or 3-hydroxyl groups). Thus, they may represent metabolites of xanthophylls.

7.12.2 Convolvulaceae

7.12.2.1 Occurrence

***Cuscuta*.** Since the end of the nineteenth century it was known that the normal yellow-orange coloration of the holoparasitic genus *Cuscuta* is due to a high content of carotenoids (Temme 1883). About five decades later a considerable level of γ-carotene, some α- and β-carotene as well as traces of lycopene and rubixanthin [(3*R*)-β,ψ-caroten-3-ol] could be detected in *C. subinclusa* DURAND & HILG. and *C. salina* ENGELM. (Mackinney 1935). *C. australis* was found to contain β- and γ-carotene, α-carotene 5,6-epoxide, lutein, and taraxanthin (= lutein 5,6-epoxide) (Baccarini et al. 1965).

Ipomoea. The only convolvulaceous species cited in the Carotenoids Handbook is represented by *I. batatas* LAM., sweet potato, with two carotenoids, namely β-carotene diepoxide (5,6:5′,6′-diepoxy-5,6,5′,6′-tetrahydro-β,β-carotene) in a white-fleshed cultivar and luteochrome (5,6:5′,8′-diepoxy-5,6,5′,8′-tetrahydro-β, β-carotene; two isomers: 8*R* and 8*S*) as the major carotinoid in certain Brazilian cultivars. Furthermore, these sweet potatoes contained carotene and its 5,6-monoepoxide as well as ζ-carotene beside two minor constituents (Almeida and Penteado 1988). A specific variety used by Purcell and Walter (1968) showed β-carotene as the major component with phytoene, phytofluene, as well as α-, γ-, and ζ-carotenes as minor constituents. Recently, four carotenoids with an unusual 5,6-dihydro-5,6-dihydroxy-β-end group have been discovered as congeners of seven known metabolites in a yellow-fleshed sweetpotato cultivar popular in Japan: the ipomoeaxanthins A (5,6-dihydro-β,β-carotene-5,6,3′-triol), B (5,6,5′,6′-tetrahydro-β,β-carotene-5,6,5′,6′-tetrol), C1 (5′,8′-epoxy-5,6,5′,8′-tetrahydro-β,β-carotene-5,6-diol), C2 (8′-isomer of C1) (Maoka et al. 2007). Thus, congeners C1 and C2 show an unusual dihydrofurane moiety anellated at one terminal carbocycle due to the 5′,8′-epoxy group. Obviously, these novel carotenoids show a biogenetic relationship to β-cryptoxanthin-5′,6′-epoxide (congener A) and β-carotene-5,6,5′,6′-diepoxide, respectively. The latter compound was shown to be a minor carotenoid in white-fleshed sweet potato (see above). The major carotenoids in the leaves of *I. aquatica* FORSK., water spinach/water convolvulus, a popular Southeast and East Asian vegetable, were found to be β-carotene, lutein, lutein 5,6-epoxide (= taraxanthin), violaxanthin, and neoxanthin (Chen et al. 1991).

The apocarotenoid loliolide (3β-hydroxydihydroactinodiolide; for structural comparison see Fig. 7.37), a metabolite of violaxanthin (Ghosal et al. 1976), was isolated from the epigeal vegetative parts of *I. hederifolia* L. (Jenett-Siems 1996). Another apocarotenoid, β-damascenone, was identified as a foliage constituent of *I. pes-caprae* L. (Pongprayoon et al. 1992).

7.12.3 Significance

Ecological Role. From the ecological point of view carotenoids are one of the dominating classes of pigments in flowers (pollinator-plant interaction / zoidiogamy) and fruits (frugivores/endozoochory).

Provitamin A Compounds. As precursors of vitamin A certain carotenes play an essential role in human and animal diets, e.g., especially for visual pigments. Thus, mammalian β-carotene-15,15′-dioxygenase converts carotenes with at least one β-ionone moiety to *all-trans*-retinal (vitamin A aldehyde) which is necessary for the perception of light in, e.g., mammals. In contrast to the other provitamin A compounds, i.e., α- and γ-carotenes, β-carotene reveals two molecules of retinal. The visual pigment, the chromoprotein rhodopsin, is containing 11-*cis*-retinal obtained by isomerization of retinal (retinene isomerase). Retinal may be also transformed into *all-trans*-retinol (vitamin A) by retinol dehydrogenase (vice versa) and retinoic acid by retinal dehydrogenase. As an example for a suitable source for provitamin

A compounds the tuberous roots of *Ipomoea batatas* Lam., may be mentioned: 100 g of cooked sweet potatoes provides about 11.5 mg β-carotene or about four times the U.S. recommended daily allowance (Wang et al. 2005 and references therein). In contrast, potatoes are almost lacking carotenes (see above).

Other Health Benefits. Consumption of carotenoid-rich foods is associated with a reduced risk of developing several chronic diseases due to their antioxidant properties (e.g., Goñi et al. 2006 and references therein). This is especially true for lycopene due to the highest antioxidant activity among all dietary antioxidants. Thus, it is assumed to be protective in an especially distinctive manner against, e.g., cardiovascular diseases and certain types of cancer based on its ability to trap reactive hydroxyl and nitroxyl radicals, putative pathogenesis determinants of many degenerative diseases (damage of DNA, cells, and tissues) (Friedman 2002 and references therein). The most important source for this carotene is represented by tomato. In this connection it is of advantage that lycopene is fairly stable to heat processing with regard to usual cooking of tomatoes and to the production of sauces, ketchup, and tinned food (e.g., Lenucci et al. 2006 and references therein). However, some loss of color and biological activities due to isomerization (*cis* form) and oxidation during thermal processing is unavoidable (Topal et al. 2006 and references therein). On the other hand, bioavailability of lycopene in the human organism is increased in such products compared with the consumption of fresh tomatoes (Lenucci et al. 2006 and references therein). Bioaccessibility of lycopene, β-carotene and lutein in small and large intestine and in the gut in total has been studied recently (Goñi et al. 2006).

Lutein has been assumed to play a role in protection from age-related macular degeneration (AMD) and cataract (e.g., O'Donovan and Beatty 2006). Therefore, supplements of lutein and zeaxanthin have been recommended. However, an evaluation using the Food and Drug Administration's (FDA) evidence-based review system for health claims concluded "that no credible evidence exists for a health claim [on conventional foods and dietary supplements] about the intake of lutein or zeaxanthin (or both) and the risk of AMD or cataracts" (Trumbo and Ellwood 2006). Thus, this topic is still discussed controversially.

The fruit of *Lycium chinense* was found to afford significant protection against carbon tetrachloride-induced toxicity in cultures of rat hepatocytes. Zeaxanthin and physalein could be identified as active components; their activities turned out to be comparable to that of the clinically established drug silybin (Kim et al. 1997). An antispasmodic activity of the apocarotenoid β-damascenone was observed as a result of bioassay-guided fractionation of the leaves of *I. pes-caprae*. It turned out be as potent as the established drug papaverine (Pongprayoon et al. 1992).

References

Abdel-Gwad MM, El-Amin SM, El-Sayed MM, Refahy LA, Sabry WA (1997) Molluscidal saponins from *Cestrum parqui*. Al-Azar J Pharmaceut Sci 20:80–84

Abe F, Nagafuji S, Okawa M, Kinjo J (2006) Trypanocidal constituents in plants 6. Minor withanolides from the aerial parts of *Physalis angulata*. Chem Pharm Bull 54:1226–1228

Abou-Douh AM (2002) New withanolides and other constituents from the fruits of *Withania somnifera*. Arch Pharm Pharm Med Chem 6:267–276
Aburjai T, Bernasconi S, Manzocchi L, Pelizzoni F (1996) Isolation of 7-dehydrocholesterol from cell cultures of *Solanum malacoxylon*. Phytochemistry 43:773–776
Aburjai T, Al-Khalil S, Abuirjeie M (1998) Vitamin D_3 and its metabolites in tomato, potato, egg plant and zucchini leaves. Phytochemistry 49:2497–2499
Adam G, Chiên NQ, Khôi NH (1981) Dunawithanin A and B, first plant withanolide glycosides from *Dunalia australis*. Int Conf Chem Biotechnol Biol Act Nat Prod [Proc] 1st / 3. Bulg Acad Sci, Sofia, Bulgaria, pp 191–195
Adam G, Chiên NQ, Khôi NH (1984) Dunawithanins A and B, the first withanolide glycosides from *Dunalia australis*. Phytochemistry 23:2293–2297
Adesina SK (1985) Constituents of *Solanum dasyphyllum* fruit. J Nat Prod 48:147
Agra M de F, Bhattacharyya J (1999) Ethnomedicinal and phytochemical investigation of the *Solanum* species in the Northeast of Brazil. In: Nee M, Symon D, Lester RN, Jessop JP (eds) Solanaceae IV – Advances in taxonomy and utilization, Royal Botanic Gardens, Kew, UK, pp 341–343
Ahmad VU, Baqai FT, Fatima I, Ahmad R (1991) A spirostanol glycoside from *Cestrum diurnum*. Phytochemistry 34:511–515
Ahmad VU, Baqai FT, Ahmad R (1993) A tigogenin pentasaccharide from *Cestrum nocturnum*. Phytochemistry 30:3057–3061
Ahmad VU, Baqai FT, Ahmad R (1995) A diosgenin tetrasaccharide from *Cestrum nocturnum*. Z Naturforsch 50b:1104–1110
Ahmed AH, Ramzy MR (1997) Laboratory assessment of the molluscidal and cercaricidal activities of the Egyptian weed, *Solanum nigrum* L. Ann Trop Med Parasit 91:931938
Al-Babili S, Hugueney P, Schledz M, Welsch R, Frohnmeyer H, Laule O, Beyer P (2000) Identification of a novel gene coding for neoxanthin synthase from *Solanum tuberosum*. FEBS Lett 485:168–172
Almeida LB, Penteado MVC (1988) Carotenoids and pro-vitamin A value of white fleshed Brazilian sweet potatoes (*Ipomoea batatas* Lam.) J Food Compos Anal 1:341–352
Alves AC, Prista LN, Ferreira MA (1961) Isolation of a glycoside from *Solanum wrightii*. Garcia de Orta 9:713–720
Alzerreca A, Hart G (1982) Molluscicidal steroid glycoalkaloids possessing stereoisomeric spirosolane structures. Toxicol Lett 12:151–155
Anjaneyulu ASR, Rao DS, Lequesno PW (1998) Withanolides, biologically active natural steroidal lactones: A review. In: Atta-ur-Rahman (ed) Studies in natural products chemistry, vol 20 (part F). Elsevier, Amsterdam, NL, pp 135–261
Antonious GF, Kochhar TS (2003) Zingiberene and curcumene in wild tomato. J Envir Sci Health B38:489–500
Armer CA (2004) Colorado potato beetle toxins revisited: Evidence the beetle does not sequester host plant glycoalkaloids. J Chem Ecol 30:883–888
Arthan D, Svasti J, Kittakoop P, Pittayakhachonwut D, Tanticharoen M, Thebtaranonth Y (2002) Antiviral isoflavonoid sulfate and steroidal glycosides from the fruits of *Solanum torvum*. Phytochemistry 59:459–463
Arthan D, Kittakoop P, Esen A, Svasti J (2006) Furostanol glycoside 26-*O*-β-glucosidase from the leaves of *Solanum torvum*. Phytochemistry 67:27–33
Atta-ur-Rahman, Yousaf M, Gul W, Qureshi S, Choudhary MI, Voelter W, Hoff A, Jens F, Naz A (1998a) Five new withanolides from *Withania coagulans*. Heterocycles 48:1801–1811
Atta-ur-Rahman, Choudhary MI, Yousaf M, Gul W, Qureshi S (1998b) New withanolides from *Withania coagulans*. Chem Pharm Bull 46:1853–1856
Atta-ur-Rahman, Shabbir M, Yousaf M, Qureshi S, E-Shahwar D, Naz A, Choudhary MI (1999) Three withanolides from *Withania coagulans*. Phytochemistry 52:1361–1364
Atta-ur-Rahman, Dur-e-Shahwar D, Naz A, Choudhary MI (2003) Withanolides from *Withania coagulans*. Phytochemistry 63:387–390
Austin DF (2004) Florida Ethnobotany. CRC Press, Boca Raton, FL, USA

Baccarini A, Bertossi F, Bagni N (1965) Carotenoid pigments in the stem of *Cuscuta australis*. Phytochemistry 4:349–351

Baggesgaard-Rasmussen H, Boll PM (1962) Soladulcamarine, the alkaloidal glycoside of *Solanum dulcamara*. Acta Chem Scand 12:802–806

Bah M, Gutiérrez DM, Escobedo C, Mendoza S, Rojas JI, Rojas A (2004) Methylprotodioscin from the Mexican medical plant *Solanum rostratum* (Solanaceae). Biochem Syst Ecol 32:197–202

Bajguz A, Tretyn A (2003) The chemical characteristic and distribution of brassinosteroids in plants. Phytochemistry 62:1027–1046

Balashova IT, Verderevskaya TD, Kintya PK (1984) Antiviral activity of steroid glycosides on a model of tobacco mosaic virus (TMV). Sel'skokhozyaistvennaya Biol 83–86

Balmannya (1874) Versuche über die Wirkung des Solanins und Solanidins. Göttingen; fide Husemann et al. (1884)

Baqai FT, Ali A, Ahmad VU (2001) Two new spirostanol glycosides from *Cestrum parqui*. Helv Chim Acta 84:3350–3356

Barger LG, Fraenkel-Conrat HL (1936) Alkaloids from *Solanum pseudocapsicum*. J Chem Soc 1537–1542

Bartram S, Jux A, Gleixner G, Boland W (2006) Dynamic pathway allocation in early terpenoid biosynthesis of stress-induced lima beans leaves. Phytochemistry 67:1661–1672

Baup M (1826) Extrait d'une lettre sur plusieurs nouvelles substances. Ann Chim Phys 31:108–109

Begley MJ, Crombie L, Ham PJ, Whiting DA (1976) A new class of natural steroids, with ring D aromatic, from *Nicandra physaloides* (Solanaceae). X-Ray analysis of nic-10, and the structures of nic-1 ('nicandrenone'), -12, and -17. J Chem Soc Perkin I:304–307

Bennett RD, Lieber ER, Heftmann E (1967) Biosynthesis of neotigogenin and Δ^{16}-5α-pregnen-3β-ol-20-one from cholesterol in *Lycopersicon pimpinellifolium*. Phytochemistry 6:837–840

Bergenstraahle A, Borgaa P, Jonsson LMV (1996) Sterol composition and synthesis in potato tuber disks in relation to glycoalkaloid synthesis. Phytochemistry 41:155–161

Bhatnagar JK, Puri RK (1974) *Solanum platanifolium*, a new source of solasodine. Lloydia (later: J Nat Prod) 37:318–319

Bheemasankara Rao C, Suseela K, Subba Rao PV, Gopala Krishna P, Subba Raju GV (1984) Chemical examination of some Indian medicinal plants. Indian J Chem 23B:787–788

Bianchi E, Girardi F, Diaz F, Sandoval R, Gonzales M (1963) Components of the leaves and fruit of *Cestrum parqui*: Tigogenin, digalogenin, digitogenin, and ursolic acid. I. Ann Chim (Rome) 53:1761–1778

Birch AJ, Massy-Westropp RA, Wright SE, Kubota T, Matsuura T, Sutherland MD (1954) Ipomeamarone and ngaione. Chem Ind (London) 902

Bishop GJ, Nomura T, Yokota T, Harrison K, Noguchi T, Fujioka S, Takatsuto S, Jones JDG, Kamiya Y (1999) The tomato DWARF enzyme catalyzes C-6-oxidation in brassinosteroid biosynthesis. Proc Natl Acad Sci USA 96:1761–1766

Bite P, Shabana MM (1972) *Solanum* glycosides. VIII. Solashabanine and solaradinine. Acta Chim Acad Sci Hung 73:361–362

Bizimenyera ES (2003) Acute poisoning of Friesian heifers by *Solanum macrocarpon* L. ssp. *dasyphyllum*. Vet Hum Toxicol 45:222–223

Bloch CB, De Wit PJGM, Kuc J (1984) Elicitation of phytoalexins by arachidonic and eicosapentaenoic acids: a host survey. Physiol Plant Pathol 25:199–208

Bohlmann F, Zdero C (1978) New sesquiterpenes and acetylenes from *Athanasia* and *Pentzia* species. Phytochemistry 17:1595–1599

Bohlmann J, Stauber EJ, Krock B, Oldham NJ, Gershenzon J, Baldwin IT (2002) Gene expression of 5-*epi*-aristolochene synthase and formation of capsidiol in roots of *Nicotiana attenuata* and *N. sylvestris*. Phytochemistry 60:109–116

Böhm A, Jenett-Siems K, Kaloga M, Witte L, Eich E (1999) Bonaseminols, a novel type of benzofurans from *Bonamia semidigyna* (Convolvulaceae). Book of abstracts: Joint Meeting of American Society of Pharmacognosy, Association Française pour l'Enseignement et la

Recherche en Pharmacognosie, Gesellschaft für Arzneipflanzenforschung, Phytochemical Society of Europe, July 26–30, 1999. Leiden University, Division of Pharmacognosy, P 222
Bohs L (2006) The genus *Solanum*: views from the trees and the roots. Presentation, VI International Solanaceae Conference, Solanaceae Genomics Network, and 90th Annual Meeting of the Potato Association of America, Madison, Wisconsin, USA
Bohs L, Olmstead RG (1997) Phylogenetic relationships in *Solanum* (Solanaceae) based on ndhF sequences. Syst Bot 22:5–17
Boland RL (1986) Plants as source if vitamin D_3 metabolites. Nutr Rev 44:1–8
Boland RL, Skliar MI, Norman AW (1987) Isolation of vitamin D_3 metabolites from *Solanum malacoxylon* leaf extracts incubated with ruminal fluid. Planta Med 53:161–164
Boland RL, Skliar MI, Curino A, Milanesi L (2003) Vitamin D compounds in plants. Plant Sci 164:357–369
Boll P, Andersen B (1962) Alkaloidal glycosides from *Solanum dulcamara* III. Differentiation of geographical strains by means of thin-layer chromatography. Planta Med. 10:421–432
Bolt AJN, Purkis SW, Sadd JS (1983) A damascenone derivative from *Nicotiana tabacum*. Phytochemistry 22:613–614
Boyd MR, Wilson BJ (1972) Isolation and characterization of 4-ipomeanol, a lung-toxic furanosesquiterpenoid produced by sweet potatoes (*Ipomoea batatas*). J Agric Food Chem 20:428–430
Bramley PM (1997) Isoprenoid metabolism. In: Dey PM, Harborne JB (eds) Plant biochemistry. Academic Press, San Diego, USA, pp 417–437
Breitenbach J, Sandmann G (2005) ζ-Carotene *cis* isomers as products and substrates in the plant poly-*cis* carotenoids biosynthetic pathway to lycopene. Planta 220:785–793
Breyer-Brandwijk MG (1929) Bull Sci Pharmacol 36:541; fide Prelog & Jeger (1953)
Briggs LH, Brooker EG, Harvey WE, Odell AL (1952) *Solanum* alkaloids. VIII. Solamargine, a new alkaloid from *Solanum marginatum*. J Soc Chem 3587–3591
Briggs LH, Cambie RC, Hoare JL (1961) *Solanum* alkaloids. XV. Constituents of some *Solanum* species and a reassessment of solasodamine and solauricine. J Chem Soc 4645–4649
Britton G, Liaaen-Jensen S, Pfander H, Mercadante AZ, Egeland ES (2004) Carotenoids – Handbook. Birkhäuser Verlag, Basel, CH
Brown CR (2005) Antioxidants in potato. Am J Potato Res 82:163–172
Brown GD (1994) The sesquiterpenes of *Fabiana imbricata*. Phytochemistry 35:425–433
Bukenya ZR, Carasco JF (1999) Ethnobotanical aspects of *Solanum* L. (Solanaceae) in Uganda. In: Nee M, Symon D, Lester RN, Jessop JP (eds) Solanaceae IV – Advances in taxonomy and utilization, Royal Botanic Gardens, Kew, UK, pp 345–360
Burden RS, Rowell PM, Bailey JA, Loeffler RST, Kemp MS, Brown CA (1985) Debneyol, a fungicidal sesquiterpene from TNV infected *Nicotiana debneyi*. Phytochemistry 24:2191–2194
Burden RS, Loeffler RST, Rowell PM, Bailey JA, Kemp MS (1986) Cyclodebneyol, a fungitoxic sesquiterpene from TNV infected *Nicotiana debneyi*. Phytochemistry 25:1607–1608
Burka LT, Kuhnert L, Wilson BJ, Harris TM (1974) 4-Hydroxymyoporone, a key intermediate in the biosynthesis of pulmonary toxins produced by *Fusarium solani* infected sweet potatoes. Tetrahedron Lett 4017–4020
Burka LT, Kuhnert L, Wilson BJ, Harris TM (1977) Biogenesis of lung-toxic furans produced during microbial infection of sweet potatoes (*Ipomoea batatas*). J Am Chem Soc 99:2302–2305
Burka LT, Felice LJ, Jackson SW (1981) 6-Oxodendrolasin, 6-hydroxydendrolasin, 9-oxofarnesol and 9-hydroxyfarnesol, stress metabolites of the sweet potato. Phytochemistry 20:647–652
Burns J, Fraser PD, Bramley PM (2003) Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables. Phytochemistry 62:939–947
Buttery RG, Ling LC (1993) Volatile components of tomato fruit and plant parts: Relationship and biogenesis. ACS Sympos Ser No 525, American Chemical Society, Washington, DC, pp 23–34
Callow RK, James VHT (1955) Epimerisation at $C_{(25)}$ of steroid sapogenins: Sarsasapogenin, neotigogenin, and sisalagenin. J Chem Soc. 1671–1674
Canham PAS, Warren FL (1950a) Saponins.- I. Isolation of gitogenin and digitonin from *Cestrum laevigatum*. J South African Chem Inst 3:9–12

Canham PAS, Warren FL (1950b) Saponins.- II. Isolation of gitogenin and digitogenin from *Cestrum parqui*. J South African Chem Inst 3:63–65

Canonica L, Danieli B, Weisz-Vincze I, Ferrari G (1972) Structure of muristerone A, a new phytoecdysone. J Chem Soc, Chem Comm:1060–1061

Canonica L, Danieli B, Ferrari G, Krepinsky J, Rainoldi G (1973) Structure of calonysterone, an unsusually modified phytoecdysone. J Chem Soc, Chem Comm:737–738

Canonica L, Danieli B, Ferrari G, Krepinsky J, Weisz-Vincze I (1975) A novel method of isolation of phytoecdysones from kaladana seeds. Phytochemistry 14:525–527

Canonica L, Orsini F, Pelizzoni F, Ferrari G, Vecchietti V (1976) Aureoside, a new glycoside from *Operculina aurea* (Convolvulaceae). Gazz Chim Ital 106:889–894

Canonica L, Pelizzoni F, Ferrari G, Vecchietti V (1977a) Glycosides from *Operculina aurea* (Convolvulaceae). Isoaureoside and aniseoside. Gazz Chim Ital 107:223–227

Canonica L, Orsini F, Pelizzoni F, Zajotti A, Ferrari G, Vecchietti V (1977b) Glycosides from *Operculina aurea* (Convolvulaceae). III. New derivatives of *ent*-3α,16α,17- and *ent*-3β,16β, 17-kauranetriols. Gazz Chim Ital 107:501–502

Canonica L, Danieli B, Ferrari G, Krepinsky J, Haimova M (1977c) New phytoecdysones from kaladana. Structure of muristerone A and kaladasterone. Gazz Chim Ital 107:123–130

Cardeal ZL, Gomes da Silva MDR, Marriott PJ (2006) Comprehensive two-dimensional gas chromatography/mass spectrometric analysis of pepper volatiles. Rapid Commun Mass Spectrom 20:2823–2836

Carter CD, Gianfagna TJ, Sacalis JN (1989) Sesquiterpenes in glandular trichomes of a wild tomato species and toxicity to the Colorado potato beetle. J Agric Food Chem 37:1425–1428

Chakravarty AK, Dhar TK, Pakrashi SC (1978) Hispigenin, a novel 22β*O*-spirostane from *Solanum hispidum*. Tetrahedron Lett 19:3875–3878

Chakravarty AK, Saha CR, Pakrashi SC (1979) New spirostane saponins and sapogenins from *Solanum hispidum* seeds. Phytochemistry 18:902–903

Chakravarty AK, Dhar TK, Pakrashi SC (1980) Solaspigenin and neosolaspigenin, two new spirostane sapogenins from *Solanum hispidum*. Phytochemistry 19:1249–1251

Chakravarty AK, Mukhopadhyay S, Saha S, Pakrashi SC (1996) A neolignan and sterols in fruits of *Solanum sisymbrifolium*. Phytochemistry 41:935–939

Cham BE (2000) Anticancer medicinal compositions comprising solasodine glycosides. PCT Int Appl: 51 pp

Cham BE, Meares HM (1987) Glycoalkaloids from *Solanum sodomaeum* are effective in the treatment of skin cancers in man. Cancer Lett 36:111–118

Cham BE, Gilliver M, Wilson L (1987) Antitumour effects of glycoalkaloids isolated from *Solanum sodomaeum*. Planta Med 53:34–36

Chamberlain WJ, Schlotzhauer WS, Chortyk OT (1988). Chemical composition of non-smoking tobacco products. J Agric Food Chem 36:48–50

Chamy MC, Garbarino JA, Piovano M, López-Pérez JL, Nicoletti M, Gandolfo R, San Feliciano A (1997) 9-*epi*-Labdane diterpenoids from *Nolana rostrata* var. *rostrata*. Phytochemistry 45:797–800

Chamy MC, Piovano M, Garbarino JA (2002) Diterpenoids from *Nolana elegans*. Bol Soc Chil Quim 47:367–370

Chen BH, Yang SH, Han LH (1991) Characterization of major carotenoids in water convolvulus (*Ipomoea aquatica*) by open-column, thin-layer and high-performance liquid chromatography. J Chromatogr 543:147–155

Chen LJ, DeRose EF, Burka LT (2006) Metabolism of furans in vitro: Ipomeanine and 4-ipomeanol. Chem Res Toxicol 19:1320–1329

Chintea P, Buliga A, Mihaila M, Oprea M (1998) Effectiveness of some extracts of natural products in controlling pathogenic soil-borne fungi. Practice Oriented Results on Use and Production of Neem-Ingredients and Pheromones VIII, Proceedings of the Workshop, 8th, Hohensolms, Germany, Feb 16–18, pp 107–115

Choban IN, Dimoglo AS, Bersuker IB, Balashova IT, Kintya PK (1987) Structure-activity correlations for antiviral properties of steroidal glycosides. FECS Int Conf Chem Biotechnol Biol Act Nat Prod (Proc), 3rd. VCH, Weinheim, Germany, pp 431–435

Choi JK, Murillo G, Su BN, Pezzuto JM, Kinghorn AD, Mehta RG (2006) Ixocarpalactone A isolated from the Mexican tomatillo shows potent antiproliferative and apoptotic activity in colon cancer cells. FEBS J 273:5714–5723

Choudhary MI, Yousuf S, Nawaz SA, Ahmed S, Atta-ur-Rahman (2004) New cholinesterase inhibiting withanolides from *Withania somnifera*. Chem Pharm Bull 52:1358–1361

Choudhary MI, Nawaz SA, Haq ZuH, Lodhi MA, Ghayur MN, Jalil S, Riaz N, Yousuf S, Malik A, Gilani AH, Atta-ur-Rahman (2005) Withanolides, a new class of natural cholinesterase inhibitors with calcium antagonistic properties. Biochem Biophys Res Commun 334:276–287

Choudhary MI, Yousuf S, Samreen, Shah SAA, Ahmed S, Atta-ur-Rahman (2006) Biotransformation of physalin H and leishmanicidal activity of its transformed products. Chem Pharm Bull 54:927–930

Christen P (1989) Withanolide – Naturstoffe mit vielversprechendem Wirkungsspektrum. Pharmazie in unserer Zeit 18:129–139

Cirigliano AM, Veleiro AS, Oberti JC, Burton G (2002) Spiranoid withanolides from *Jaborosa odenelliana*. J Nat Prod 65:1049–1951

Coelho RM, De Souza MC, Sarragiotto MH (1998) Steroidal alkaloid glycosides from *Solanum orbignianum*. Phytochemistry 49:893–897

Colombano A (1908) On the solanine of the potato plant. Gazz Chim Ital 38:19–37

Coune C (1977) Etude phytochimique des Solanaceae d'Afrique Centrale. II. Les alcaloïdes de *Solanum dasyphyllum*. Planta Med 31:259–261

Coune C, Denoel A (1975) Phytochemical study of the Central African Solanaceae. I. Alkaloids of *Solanum dasyphyllum*. Planta Med 28:168–171

Coy-Barrera CA, Cuca-Suarez LE, Clara IOP (2005) A new steroidal alkaloid, two sterols and a pentacyclic triterpenoid isolated from *Solanum cornifolium*, section *Geminata*. Actualidades Biologicas (Medellin, Colombia) 27:131–134

Craig LC, Jacobs WA (1943) Veratrine alkaloids. XX. Further correlations in the veratrine group. The relationship between the veratrine bases and solanidine. J Biol Chem 149:451–464

Cuervo AC, Blunden G, Patel AV (1991) Chlorogenone and neochlorogenone from the unripe fruits of *Solanum torvum* SWARTZ. Phytochemistry 30: 1339–1341

Curl AL (1964) The carotenoids of green bell peppers. J Agric Food Chem 12:522–524

Czapek F (1925) Biochemie der Pflanzen, vol 3. Verlag von Gustav Fischer, Jena, Germany

D'Abrosca B, DellaGreca M, Fiorentino A, Monaco P, Oriano P, Temussi F (2004) Structure elucidation and phytotoxicity of C_{13} *nor*-isoprenoids from *Cestrum parqui*. Phytochemistry 65:497–505

D'Abrosca B, DellaGreca M, Fiorentino A, Monaco P, Natale A, Oriano P, Zarrelli A (2005) Structural characterization of phytotoxic of terpenoids from *Cestrum parqui*. Phytochemistry 66:2681–2688

Darwin SC, Knapp S, Peralta IE (2003) Taxonomy of tomatoes in the Galápagos Islands: Native and introduced species of *Solanum* section *Lycopersicon* (Solanaceae). Syst Biodivers 1:29–53

Das S, Ganguly SN, Mukherjee KK (1999) Fatty acids and phytochemical components of *Ipomoea* spp. seeds. Nat Prod Sci 5:121–123

Daunay MC, Lester RN, Gebhardt C, Hennart JW, Jahn M, Frary A, Doganlar S (2001) Genetic resources of eggplant (*Solanum melongena* L.) and allied species: a new challenge for molecular geneticists and eggplant breeders. In: Van den Berg RG, Barendse GWM, van der Weerden GM, Mariani C (eds) Solanaceae V – advances in taxonomy and utilization. Nijmegen University Press, Nijmegen, The Netherlands, pp 251–274

Daunter B, Cham BE (1990) Solasodine glycosides. In vitro preferential cytotoxicity for human cancer cells. Cancer Lett 55:209–220

Davies BH, Matthews S, Kirk JTO (1970) The nature and biosynthesis of the carotenoids of different colour varieties of *Capsicum annuum*. Phytochemistry 9:797–805

De Cassia Meneses Oliveira R, Lima JT, Ribeiro LAA, Silva JLV, Monteiro FS, Assis TS, Agra M de F, Silva TMS, Almeida FRC, Silva BA (2006) Spasmolytic action of the methanol extract and isojuripidine from *Solanum asterophorum* MART. (Solanaceae) leaves in guinea-pig ileum. Z Naturforsch 61c:799–805

De-Eknamkul W, Potduang B (2003) Biosynthesis of β-sitosterol and stigmasterol in *Croton sublyratus* proceeds via a mixed origin of isoprene units. Phytochemistry 62:389–398

De Lucca AJ, Bland JM, Vigo CB, Cushion M, Selitrennikoff CP, Peter J, Walsh TJ (2002) CAY-1, a fungicidal saponin from *Capsicum* sp. fruit. Med Mycol 40:131–137

De Lucca AJ, Bland JM, Boue S, Vigo CB, Cleveland TE (2006) Synergism of CAY-1 with amphotericin B and itraconazole. Microbiology 52:285–287

De Marino S, Borbone N, Gala F, Zollo F, Fico G, Pagiotti R, Iorizzi M (2006) New constituents of sweet *Capsicum annuum* L. fruits and evaluation of their biological activity. J Agric Food Chem 54:7508–7516

De Valeri B, Usubillaga A (1989) Sapogenins from *Solanum meridense*. Phytochemistry 28:2509–2511

Desfosses M (1820) Extrait d'une lettre. J Pharm 6:374–376

Desfosses M (1821) Extrait d'une lettre. J Pharm 7:414–417

Desjardins AE, Mccormick SP, Corsini DL (1995) Diversity of sesquiterpenes in 46 potato cultivars and breeding selections. J Agric Food Chem 43:2267–2272

Dewick PM (1999) The biosynthesis of $C_5 - C_{25}$ terpenoid compounds. Nat Prod Rep 16:97–130

Dimitriades E, Massy-Westropp RA (1984) The configuration of the sesquiterpenoid 4-hydroxymyoporone (athanagrandione). Phytochemistry 23:1325–1326

Dimoglo AS, Choban IN, Bersuker IB, Kintya PK, Balashova NN (1985) Structure-activity correlations for the antioxidant and antifungal properties of steroid glycosides. Bioorg Khim 11:408–413

Dominguez XA, Marroquin J, Coronado MM (1975) Ursolic acid and mannitol from *Leptoglossis texana*. Rev Latinoameric Quim 6:104

Döpke W, Sewerin E, Hess U, Nogueiras C (1976) Struktur und Stereochemie eines neuen Steroidsapogenins vom Spirostanoltyp aus *Solanum jamaicense*. Z Chem 16:104–105

Döpke W, Matos N, Duday S (1987) Über den Steroidalkaloid- und Sapogenin-Gehalt von *Solanum panduraeforme* E.Mey. Pharmazie 42:621–622

Dragendorff G (1868) Die gerichtlich-chemische Ermittelung von Giften in Nahrungsmitteln, Luftgemischen, Speiseresten, Körpertheilen etc. Verlag der Kaiserlichen Hofbuchhandlung H. Schmitzdorff, St. Petersburg, Russia, pp 314–317

Duke SO, Baerson SR, Dayan FE, Rimando AM, Scheffler BE, Tellez MR, Wedge DE, Schrader KK, Akey DH, Arthur FH, de Lucca AJ, Gibson DM, Harrison HF Jr, Peterson JK, Gealy DR, Tworkoski T, Wilson CL, Morris JB (2003) United States Department of Agriculture – Agricultural Research Service research on natural products for pest management. Pest Manag Sci 59:708–717

Duperon R, Thiersault M, Duperon P (1984) High level of glycosylated sterols in species of *Solanum* and sterol changes during the development of the tomato. Phytochemistry 23:743–746

Edwards EJ, Saint RE, Cobb AH (1998) Is there a link between greening and light-enhanced glycoalkaloid accumulation in potato (*Solanum tuberosum* L.) tubers ? J Sci Food Agric 76:327–333

Ehmke A, Eilert U (1993) *Solanum dulcamara* L. (Bittersweet): Accumulation of steroidal alkaloids in the plant and in different in vitro systems. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 21, medicinal and aromatic plants IV, Springer Verlag, Berlin, Germany, pp 339–352

El Imam YMA, Evans WC (1984) Tropane alkaloids of species of *Anthocercis, Cyphanthera* and *Crenidium*. Planta Med 50:86–87

El Imam YMA, Evans WC, Haegi L, Ramsey KPA (1991) Secondary metabolites of intergeneric hybrids of the Anthocercideae, family Solanaceae. Int J Pharmacog 29:263–267

El Kheir YM, Salih MH (1979) Investigation of the alkaloidal content of *Solanum dubium* L. growing in Sudan. Fitoterapia 50:255–258

Elliger CA, Waiss AC Jr (1989) Insect growth inhibitors from *Petunia* and other solanaceous plants. ACS Sympos Ser No 387, American Chemical Society, Washington, DC, pp 188–205

Elliger CA, Waiss AC Jr (1991) Insect resistance factors in *Petunia*. In: Hedin PA (ed) Naturally Occurring Pest Bioregulators. ACS Sympos Ser No 449, American Chemical Society, Washington, DC, pp 210–223

Elliger CA, Benson M, Haddon WF, Lundin RE, Waiss AC Jr, Wong RY (1988a) Petuniasterones, novel ergostane-type steroids of *Petunia hybrida* VILM. (Solanaceae) having insect-inhibitory activity. X-ray molecular structure of the 22,24,25-[(methoxycarbonyl)orthoacetate] of 7α,22,24,25-tetrahydroxyergosta-1,4-dien-3-one and of 1α-acetoxy-24,25-epoxy-7α-hydroxy-22-(methylthiocarbonyl)acetoxyergost-4-en-3-one. J Chem Soc, Perkin Transact I:711–717

Elliger CA, Benson M, Lundin RE, Waiss AC Jr (1988b) Minor petuniasterones from *Petunia hybrida*. Phytochemistry 27:3597–3603

Elliger CA, Haddon WF, Waiss AC Jr, Benson M (1989a) Petuniasterone N, an unusual ergostanoid from *Petunia* species. J Nat Prod 52:576–580

Elliger CA, Wong RY, Benson M, Waiss AC Jr (1989b) X-ray crystal structure of petuniasterone O, a novel ergostanoid from *Petunia parodii*. J Nat Prod 52:1345–1349

Elliger CA, Waiss AC Jr, Benson M, Wong RY (1990) Ergostanoids from *Petunia parodii*. Phytochemistry 29:2853–2863

Elliger CA, Waiss AC Jr, Benson M (1992) Petuniasterone R, a new ergostanoids from *Petunia parodii*. J Nat Prod 55:129–133

Elliger CA, Waiss AC Jr, Benson M, Wong RY (1993) Ergostanoids from *Petunia inflata*. Phytochemistry 33:471–477

Enzell CR, Wahlberg I, Aasen AJ (1977) Isoprenoids and alkaloids of tobacco. In: Zechmeister L, Herz W, Grisebach H, Kirby GW (eds) Progress in the chemistry of organic natural products, vol 34. Springer Verlag, Wien/A, pp 1–79

Esteves-Souza A, Sarmento da Silva TM, Alves CCF, de Carvalho MG, Braz-Filho R, Echevarria A (2002) Cytotoxic activities against Ehrlich carcinoma and human K562 leukemia of alkaloids and flavonoid from two *Solanum* species. J Brazil Chem Soc 13:838–842

Faini F, Torres R, Delle Monache F, Martini-Bettolo GB, Castillo M (1980) 1-Acetyl-3-carboxy-β-carboline, a new acid and other constituents of *Vestia lycioides*. Planta Med 38:128–132

Faini F, Torres R, Castillo M (1984) (25*R*)-Isonuatigenin, an unusual steroidal sapogenin from *Vestia lycioides*. Phytochemistry 23:1301–1303

Fajardo V, Freyer AJ, Minard RD, Shama M (1987) (+)-Jaborol, an unusual phenolic withanolide from *Jaborosa magellanica*. Tetrahedron 43:3875–3880

Fakhrutdinova IM, Sidyakin GP, Yunusov SY (1965) Alkaloids from *Haplophyllum robustum*. Structure of robustine, Khim Prirodn Soedin, Akad Nauk Uz SSR 107–109

Fang L, Chai HB, Castillo JJ, Soejarto DD, Farnsworth NR, Cordell GA, Pezzuto JM, Kinghorn AD (2003) Cytotoxic constituents of *Brachistus stramoniifolius*. Phytother Res 17:520–523

Farag MA, Paré PW (2002) C_6-Green leaf volatiles trigger local and systemic VOC emissions in tomato. Phytochemistry 61:545–554

Fayez MBE, Saleh AA (1967) Steroidal alkaloids of *Solanum wrightii* BENTH. Phytochemistry 6:433–436

Ferreira F, Soulé S, Vazquez A, Moyna P, Kenne L (1996) Steroid saponins from *Solanum laxum*. Phytochemistry 42:1409–1416

Ferrer A, Ferrer G, Perez C, Coll F, Borrego J, Jomarron I, Anaya H, Fuentes V (1998) Schlechtendamine, a new steroid alkaloid from *Solanum schlechtendalianum* WALP. Revista Cubana de Quimica 10:3–9

Ferro EA, Alvarenga NL, Ibarrola DA, Hellion-Ibarrola MC, Ravelo AG (2005) A new steroidal saponin from *Solanum sisymbrifolium* roots. Fitoterapia 76:577–579

Fewell AM, Roddick JG (1997) Potato glycoalkaloid impairment of fungal development. Mycol Res 101:597–603

Fewell AM, Roddick JG, Weissenberg M (1994) Interactions between the glycoalkaloids solasonine and solamargine in relation to inhibition of fungal growth. Phytochemistry 37:1007–1011

Fôdéré, Hecht (before 1884) Ann Chem Pharm 3:130; fide Husemann et al. (1884)

Fontaine TD, Irving GW Jr, Ma RM, Poole JB, Doolittle SP (1948) Isolation and partial characterization of crystalline tomatine, an antibiotic agent from the tomato plant. Arch Biochem 18:467–475

Fontaine TD, Ard JS, Ma RM (1951) Tomatidine, a steroid secondary amine. J Am Chem Soc 73:878–879
Franz C, Jatisatienr A (1983) Pflanzliche Steroid-Rohstoffe: Wird Solasodin das Diosgenin des nächsten Jahrzehnts? Dtsch Apoth Ztg 123:1069–1072
Friedman M (2002) Tomato glycoalkaloids: Role in the plant and in the diet. J Agric Food Chem 50:5751–5780
Friedman M (2006) Potato glycoalkaloids: Roles in the plant and in the diet. J Agric Food Chem 54:8655–8681
Friedman M, McDonald GM (1997) Potato glycoalkaloids: chemistry, analysis, safety, and plant physiology. Critic Rev Plant Sci 16:55–132
Friedman M, Kozukue N, Harden LA (1997) Structure of the tomato glycoalkaloid tomatidenol-3-β-lycotetraose (dehydrotomatine) J Agric Food Chem 45:1541–1547
Friedman M, Lee KR, Kim HJ, Lee IS, Kozukue N (2005) Anticarcinogenic effects of glycoalkaloids from potatoes against human cervical, liver, lymphoma, and stomach cancer cells. J Agric Food Chem 53:6162–6169
Fuchs A, Slobbe W, Mol PC, Posthumus MA (1983) GC/MS analysis of fungitoxic terpenoids from tobacco. Phytochemistry 22:1197–1199
Fujita M, Yoshizawa T (1989) Induction of phytoalexins by various mycotoxins and metabolism of mycotoxins in sweet potato tissues. Shokuhin Eiseigaku Zasshi 30:501–505
Fujiwara Y, Yahara S, Ikeda T, Ono M, Nohara T (2003) Cytotoxic major saponin from tomato fruits. Chem Pharm Bull 51:234–235
Fujiwara Y, Takaki A, Uehara Y, Ikeda T, Okawa M, Yamauchi K, Ono M, Yoshimitsu H, Nohara T (2004) Tomato steroidal alkaloid glycosides, esculeosides A and B, from ripe fruits. Tetrahedron 60:4915–4920
Fujiwara Y, Yoshizaki M, Matsushita S, Yahara S, Yae E, Ikeda T, Ono M, Nohara T (2005) A new tomato pregnane glycoside from the overripe fruits. Chem Pharm Bull 53:584–585
Fukuhara K, Shimizu K, Kubo I (2004) Arudonine, an allelopathic steroidal glycoalkaloid from the root bark of *Solanum arundo* Mattei. Phytochemistry 65:1283–1286
Gaffield W, Keeler RF (1993) Implication of C-5,C-6-unsaturation as a key structural factor in steroidal alkaloid-induced mammalian teratogenesis. Experientia 49:922–924
Gambaro V, Piovano M, Garbarino JA (1986) 9-Acetoxynerolidol from *Phrodus bridgesii*. Phytochemistry 25:739–740
Gan KH, Lin CN (1997) A steroidal glycoside from *Solanum pseudocapsicastrum*. Chin Pharmac J (Taipei) 49:315–320
Gan KH, Lin CN, Won SJ (1993) Cytotoxic principles and their derivatives of Formosan *Solanum* plants. J Nat Prod 56:15–21
Garbarino JA, Chamy MC, Gambaro V (1986) Labdane diterpenoids from *Nolana rostrata*. Phytochemistry 25:2833–2836
Garbarino JA, Chamy MC, Piovano M, Gambaro V (1988) Labdane diterpenoids from *Nolana filifolia*. Phytochemistry 27:1795–1796
Garbarino JA, Chamy MC, Montagna MP, Gambaro V (1993) Sesquiterpenoids from *Nolana coelestis*. Phytochemistry 32:987–989
Garcia Jiménez F, Pérezamador MC (1967) Corymbosin, a glucoside from *Turbina corymbosa*. Tetrahedron 23:2557–2561
Garcia Jiménez F, Collera O, Larios G, Taboada J, Pérezamador MC (1979) Revision of the structure of turbicorytin and corymbositin. Rev Latinoamer Quim 10:181–184
Garcia Jiménez F, Pérezamador C, Collera ZO (1993) *ent*-16α,17,19-Kauranetriol-17-*O*,19-*O*-di-*O*-β-D-glucopyranoside, a new glucoside from *Turbina corymbosa*. Tetrahedron 23:2557–2561
Gardner HW, Desjardins AE, McCormick SP, Weisleder D (1994) Detoxification of the potato phytoalexin rishitin by *Gibberella pulicaris*. Phytochemistry 37:1001–1005
Geuns JMC (1978) Steroid hormones and plant growth and development. Phytochemistry 17:1–14
Ghosal S, Singh AK, Chaudhuri RK (1976) Chemical constituents of Gentianaceae XX: Natural occurrence of loliolide in *Canscora decussata*. J Pharmaceut Sci 65:1549–1551

Ghosh D, Laddha KS (2006) Extraction and monitoring of phytoecdysteroids through HPLC. J Chrom Sci 44:22–26

Ghosh M, Sinhababu SP, Sukul NC, Sahu NP, Mahato SB (1994) Antifilarial effect of solamargine isolated from *Solanum khasianum*. Int J Pharmacog 32:184–190

Gibson RW, Pickett JA (1983) Wild potato repels aphids by release of aphid alarm pheromone. Nature (London) 302:608–609

Gil RR, Lin LZ, Chai HB, Pezzuto JM, Cordell GA (1995) Cardenolides from *Nierembergia aristata*. J Nat Prod 58:848–856

Giles JA, Schumacher JN (1961) Turkish tobacco. I. Isolation and characterization of α- and β-levantenolides. Tetrahedron 14:246–251

Glotter E (1991) Withanolides and related ergostane-type steroids. Nat Prod Rep 8:415–440

Goncharik NN, Volynets AP, Kintya PK (2004) The after-effect of steroid glycosides on seed quality and seedling growth of wheat (*Triticum aestivum* L.). Vestsi Natsy Akad Belarus, Ser Biyala Navuk 23–26

Goñi I, Serrano J, Saura-Calixto F (2006) Bioaccessibility of β-carotene, lutein, and lycopene from fruits and vegetables. J Agric Food Chem 54:5382–5387

Gonzalez AG, Garcia Francisco C, Freire Barreira R, Suarez Lopez E (1971) New sources of steroidal sapogenins. IX. *Solanum vespertilio*. Farmacia Nueva 37:905–908, 911–914

Gonzalez AG, Freire Barreira R, Garcia Francisco C, Salazar Rocio JA, Suarez Lopez E (1972) New natural source of steroidal sapogenins. XVII. Anal Quim 68:1063–1064

Gonzalez AG, Freire R, Francisco CG, Salazar JA, Suarez E (1973) 20*S*-Hydroxyvespertilin, a new steroid lactone from *Solanum vespertilio*. Tetrahedron 29:1731–1734

Gonzalez AG, Freire Barreira R, Garcia Francisco C, Salazar Rocio JA, Suarez Lopez E (1974) Determination of the structures of anosmagenin and 15-dehydro-14β-anosmagenin, two new spirostanic sapogenins of *Solanum vespertilio*. Anal Quim 70:250–253

Gonzalez AG, Francisco CG, Freire R, Hernández R, Salazar JA, Suarez E, Morales A, Usubillaga A (1975) Andesgenin, a new steroid sapogenin from *Solanum hypomalacophyllum*. Phytochemistry 14:2483–2485

González M, Zamilpa A, Marquina S, Navarro V, Alvarez L (2004) Antimycotic spirostanol saponins from *Solanum hispidum* leaves and their structure-activity relationships. J Nat Prod 67:938–941

Grace MH, Saleh MM (1996) Hepatoprotective effect of daturaolone isolated from *Solanum arundo*. Pharmazie 51:593–595

Gregory P, Sinden SL, Osman SF, Tingey WM, Chessin DA (1981) Glycoalkaloids of wild, tuber-bearing *Solanum* species. J Agric Food Chem 29:1212–1215

Griffiths DW, Bain H, Deighton N, Robertson GW, Finlay M, Dale B (2000) Photo-induced synthesis of tomatidenol-based glycoalkaloids in *Solanum phureja* tubers. Phytochemistry 53:739–745

Gross D (1977) Phytoalexine und verwandte Pflanzenstoffe. In: Zechmeister L, Herz W, Grisebach H, Kirby GW (eds) Progress in the chemistry of organic natural products, vol 34. Springer Verlag, Wien/A, pp 187–247

Grunenfelder LA, Knowles LO, Hiller LK, Knowles NR (2006) Glycoalkaloid development during greening of fresh market potatoes (*Solanum tuberosum* L.). J Agric Food Chem 54:5847–5854

Gubarev MI, Enioutina EY, Taylor JL, Visic DM, Daynes RA (1998) Plant-derived glycoalkaloids protect mice against lethal infection with *Salmonella typhimurium*. Phytother Res 12:79–88

Guishan T, Pingsheng X, Zhiyong D, Guocheng T (1991) Studies on the chemical compounds of *Ipomoea batatas* Lam. Nat Prod Res Develop 7:44–46

Gutsu EV, Kintya PK (1989) Steroidal glycosides from the roots of *Capsicum annuum*. IV. Structure of capsicosides C2 and C3. Khim Prir Soed 582–584

Gutsu EV, Kintya PK, Lazur'evskii GV, Balashova NN (1984) Steroidal alkaloids and glycosides of *Capsicum annuum* L. Rastitel'nye Resursy 20:127–130

Gutsu EV, Kintya PK, Lazur'evskii GV (1986) Steroid glycosides of *Capsicum annuum* root. I. The structure of capsicosides A1, B1, and C1. Khim Prir Soed 708–712

Gutsu EV, Kintya PK, Lazur'evskii GV (1987a) Steroid glycosides of *Capsicum annuum* root. II. Structure of capsicosides A2 and B2. Khim Prir Soed 242–246

Gutsu EV, Shvets SA, Kintya PK, Lazur'evskii GV (1987b) Steroidal glycosides of *Capsicum annuum* L. roots. The structure of capsicosines D1, E1. FECS Int Conf Chem Biotechnol Biol Act Nat Prod (Proc), 3rd. VCH, Weinheim, Germany, pp 436–440

Habtemariam S (1997) Cytotoxicity and immunosuppressive activity of withanolides from *Discopodium penninervium*. Planta Med 63:15–17

Habtemariam S, Gray AI (1998) Withanolides from the roots of *Discopodium penninervium*. Planta Med 64:275–276

Habtemariam S, Gray AI, Waterman PG (1993) 16-Oxygenated withanolides from the leaves of *Discopodium penninervium*. Phytochemistry 34:807–811

Habtemariam S, Skelton BW, Waterman PG, White AH (2000) 17-Epiacnistin-A, a further withanolide from leaves of *Discopodium penninervium*. J Nat Prod 63:512–513

Hall CA, Hobby T, Cipollini M (2006) Efficacy and mechanisms of α-solasonine- and α-solamargine-induced cytolysis on two strains of *Trypanosoma cruzi*. J Chem Ecol 32:2405–2416

Hänsel R, Sticher O (2007) Pharmakognosie – Phytopharmazie, 8th edn. Springer, Berlin Heidelberg New York

Haraguchi M, Mimaki Y, Motidome M, Morita H, Takeya K, Itokawa H, Yokosuka A, Sashida Y (2000) Steroidal saponins from the leaves of *Cestrum sendtenerianum*. Phytochemistry 55:715–720

Hashimoto K, Kawagishi H, Nakayama T, Shimizu M (1997) Effect of capsianoside, a diterpene glycoside, on tight-junctional permeability. Biochim Biophys Acta Biomembranes 1323:281–290

Hawkes JG (1990) The potato: evolution, biodiversity and genetic resources. Smithsonian Institution Press, Washington, DC

Hawkes JG (1999) The economic importance of the family Solanaceae. In: Nee M, Symon D, Lester RN, Jessop JP (eds) Solanaceae IV – advances in taxonomy and utilization. Royal Botanic Gardens, Kew, pp 1–8

Heemann V, Brümmer U, Paulsen C, Seehofer F (1983) Composition of the leaf surface gum of some *Nicotiana* species and *Nicotiana tabacum* cultivars. Phytochemistry 22:133–135

Heftmann E (1983) Biogenesis of steroids in Solanaceae. Phytochemistry 22:1843–1860

Heftmann E, Schwimmer S (1972) Degradation of tomatine to 3β-hydroxy-5α-pregn-16-en-20-one by ripe tomatoes. Phytochemistry 11:2783–2787

Heftmann E, Weaver ML (1974) 26-Hydroxycholesterol and cholest-4-en-3-one, the first metabolites of cholesterol in potato plants. Phytochemistry 13:1801–1803

Hegnauer R (1973) Chemotaxonomie der Pflanzen, vol 6. Birkhäuser Verlag Basel, Switzerland, pp 420–430; 446–449

Hegnauer R (1990) Chemotaxonomie der Pflanzen, vol 9. Birkhäuser Verlag Basel, Switzerland, pp 585–588

Henrici A (1996) Neuartige Sekundärstoffe unterschiedlichster Struktur aus tropischen Convolvulaceen. Dissertation Fachbereich Pharmazie, Freie Universität Berlin, Germany

Herrera-Arellano A, Jiménez-Ferrer E, Vega-Pimentel AM, Martínez-Rivera MdlA, Hernández-Hernández M, Zamilpa A, Tortoriello J (2004) Clinical and mycological evaluation of therapeutic effectiveness of *Solanum chrysotrichum* standardized extract on patients with Pityriasis capitis (dandruff). A double blind and randomized clinical trial controlled with ketoconazole. Planta Med 70:483–488

Honbu T, Ikeda T, Zhu XH, Yoshihara O, Okawa M, Nafady AM, Nohara T (2002) New steroidal glycosides from the fruits of *Solanum anguivi*. J Nat Prod 65:1918–1920

Hu K, Kobayashi H, Dong AJ, Jing YK, Iwasaki SG, Yao XS (1999) Antineoplastic agents. Part 3. Steroidal glycosides from *Solanum nigrum*. Planta Med 65:35–38

Huang Y, Liu JK, Mühlbauer A, Henkel T (2002) Three novel taccalonolides from the tropical plant *Tacca subflaellata*. Helv Chim Acta 85:2553–2558

Hunziker AT (2001) Genera Solanacearum – the genera of Solanaceae illustrated, arranged according to a new system. A.R.G.Gantner Verlag, Ruggell, Lichtenstein

Husemann T (1875) Arch Exp Path Pharm 4:369; fide Husemann et al. (1884)
Husemann A, Hilger A, Husemann T (1884) Die Pflanzenstoffe in chemischer, physiologischer, pharmakologischer und toxikologischer Hinsicht, vol 2. Julius Springer, Berlin, pp. 1148–1149
Hussain S, Ahmed E, Malik A, Jabbar A, Arshad M (2005) Phytochemical studies on *Cressa cretica*. J Chem Soc Pak 27:296–298
Iida Y, Yanai Y, Ono M, Ikeda T, Nohara T (2005) Three unusual 22-β-*O*-23-hydroxy-(5α)-spirostanol glycosides from the fruits of *Solanum torvum*. Chem Pharm Bull 53:1122–1125
Ikeda T, Ando J, Miyazono A, Zhu XH, Tsumagari H, Nohara T, Yokomizo K, Uyeda M (2000) Anti-herpes virus activity of *Solanum* steroidal glycosides. Biol Pharm Bull 23:363–364
Indrayanto G, Cholies N, Wahyudi (1985) Influence of fruit size of *Solanum wrightii* on its solasodine content. Planta Med 51:470
Indrayanto G, Sondakh R, Syahrani A, Utami W (1998) *Solanum mammosum* L. (Terong Susu): In vitro culture and the production of steroidal alkaloids and other secondary metabolites. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry vol 41, medicinal and aromatic plants IV. Springer, Berlin, Germany, pp 395–414
Inoue H, Kato N, Uritani I (1977) 4-Hydroxydehydromyoporone from infected *Ipomoea batatas* root tissue. Phytochemistry 16:1063–1065
Iorizzi M, Lanzotti V, De Marino S, Zollo F, Blanco-Molina M, Macho A, Muñoz E (2001) New glycosides from *Capsicum annuum* L. var. *acuminatum*. Isolation, structure determination, and biological activity. J Agric Food Chem 49:2022–2029
Iorizzi M, Lanzotti V, Ranalli G, de Marino S, Zollo F (2002) Antimicrobial furostanol saponins from the seeds of *Capsicum annuum* L. var. *acuminatum*. J Agric Food Chem 50:4310–4316
Irvine WJ, Woollen BH, Jones DH (1972) Bombiprenone from *Nicotiana tabacum*. Phytochemistry 11:467–469
Ishi M (1933) The carotenoids and some lipoids of *Ipomoea reptans* (L.) Poir. Experiment Station Record (U.S. Department of Agriculture) 71:559
Itoh T, Tamura T, Matsumoto T (1977) Triterpene alcohols in the seeds of Solanaceae. Phytochemistry 16:1723–1726
Izimitani Y, Yahara S, Nohara T (1990) Novel acyclic diterpene glycosides, capsianosides A – F and I – V from *Capsicum* plants. Chem Pharm Bull 38:1299–1307
Jackson DM, Severson RF, Johnson AW, Herzog GA (1986) Effect of cuticular duvane diterpenes from green tobacco leaves on tobacco budworm (Lepidoptera: Noctuidae) oviposition. J Chem Ecol 12:1349–1359
Jackson DM, Severson RF, Sisson VA, Stephenson MG (1991) Ovipositional response of tobacco budworm moths (Lepidoptera: Noctuidae) to cuticular labdanes and sucrose esters from the green leaves of *Nicotiana glutinosa* L. (Solanaceae). J Chem Ecol 17:2489–2506
Jacobo-Herrera NJ, Bremner P, Márquez N, Gupta MP, Gibbons S, Muñoz E, Heinrich M (2006) Physalins from *Witheringia solanacea* as modulators of the NF-κB cascade. J Nat Prod 69:328–331
Jacobs WA, Fleck EE (1930) Tigogenin, a *Digitalis* sapogenin. J Biol Chem 88:545–550
Jacobs WA, Simpson JCE (1935) The *Digitalis* sapogenins. J Biol Chem 110:429–438
Jayaprakasam B, Nair MG (2003) Cyclooxygenase-2 enzyme inhibitory withanolides from *Withania somnifera* leaves. Tetrahedron 59:841–849
Jayaprakasam B, Strasburg GA, Nair MG (2004) Potent lipid inhibitors from *Withania somnifera* fruits. Tetrahedron 60:3109–3121
Jenett-Siems K (1996) Phytochemische Untersuchungen an Windengewächsen der Gattungen *Calystegia*, *Convolvulus*, *Ipomoea* und *Merremia* unter besonderer Berücksichtigung des Alkaloidvorkommens. Dissertation Fachbereich Pharmazie, Freie Universität Berlin, Germany
Jenett-Siems K, Siems K, Witte L, Eich E (2001) Merrekentrones A – D, ipomeamarone-like furanosesquiterpenes from *Merremia kentrocaulos*. J Nat Prod 64:1471–1473
Jiménez-Escrig A, Santos-Hidalgo AB, Saura-Calixto F (2006) Common sources and estimated intake of plant sterols in the Spanish diet. J Agric Food Chem 54:3462–3471
Joshi BS, Rane DF (1967) Structure and synthesis of corymbosin, a flavone from *Webera corymbosa*. Tetrahedron Lett. 4579–4581

Judd WS, Campbell CS, Kellogg EA, Stevens PF (1999) Plant systematics – a phylogenetic approach. Sinauer Associates, Sunderland, MA, USA
Kalinowska M, Zimowski J, P&acedil;czkowski C, Wojciechowski ZA (2005) The formation of sugar chains in triterpenoid saponins and glycoalkaloids. Phytochem Rev 4:237–257
Kamiwatari T, Setoguchi S, Takamine K, Ogata S (2005) Content of monoterpene alcohols in stressed sweet potatoes and the flavor property of imu-shochu. Nippon Jozo Kyokaishi 100:520–526
Kaneko K, Watanabe M, Kawakoshi Y, Mitsuhashi H (1971) Etioline as important precursor in solanidine biosynthesis in *Veratrum grandiflorum*. Tetrahedron Lett 4251–4254
Kaneko K, Tanaka MW, Mitsuhashi H (1976) Origin of nitrogen in the biosynthesis of solanidine by *Veratrum grandiflorum*. Phytochemistry 15:1391–1393
Kaneko K, Terada S, Yoshida N, Mitsuhashi H (1977a) Structure of barogenin from *Solanum tuberosum*. Phytochemistry 16:791–793
Kaneko K, Tanaka MW, Mitsuhashi H (1977b) Dormantinol, a possible precursor in solanidine biosynthesis from budding *Veratrum grandiflorum*. Phytochemistry 16:1247–1251
Kaneko K, Tanaka MW, Takahashi E, Mitsuhashi H (1977c) Teinemine and isoteinemine, two new alkaloids from *Veratrum grandiflorum*. Phytochemistry 16:1620–1622
Kapundu M, Delaude C (1988) Sapogenins of *Schwenckia americana* L. Bull Soc Roy Sci Liège 57:561–565
Karawya MS, Rizk AFM, Hammouda FM, Diab AM, Ahmed ZF (1972) Phytochemical investigation of certain *Cestrum* species growing in Egypt. Act Chim Acad Sci Hungar 72:317–322
Kashiwaga T, Mikagi E, Mekuria DB, Boru AD, Tebayashi SI, Kim CS (2005) Ovipositional deterrent on mature stage of sweet pepper, *Capsicum annuum*, against *Liriomyza trifolii* (Burgess). Z Naturforsch 60c:739–742
Kato N, Imaseki H, Nakashima N, Uritani I (1971) Structure of a new sesquiterpenoid, ipomeamaronol, in diseased sweet potato root tissue. Tetrahedron Lett 843–846
Kawaguchi Y, Ochi T, Takaishi Y, Kawazoe K, Lee KH (2004) New sesquiterpenes from *Capsicum annuum*. J Nat Prod 67:1893–1896
Kawashima Y (1996) Flavors and fragrance materials kept in traditional folklores. Foods Food Ingred J Jpn 169:29–36
Kennedy BS, Nielsen MT, Severson RF, Sisson VA, Stephenson MK, Jackson DM (1992) Leaf surface chemicals from *Nicotiana* affecting germination of *Peronospora tabacina* Adam sporangia. J Chem Ecol 18:1467–1479
Kerber VA, Moreira EA, Gomes EC, Weiss FA, Vieira RF (1993) Qualitative and quantitative evaluation of steroidal alkaloids in three *Solanum* species – (*S. grandifolium*, *S. lacerdae*, and *S. lycocarpum*) with reference to solasodine. Rev Brasil Farm 74:67–69
Kereselidze EV, Pkheidze TA, Kemertelidze EP (1970) Steroid sapogenins from *Cestrum elegans* and *Cestrum parqui*. Khim Prir Soedin 6:379
Kessler A, Baldwin I (2001) Defensive function of herbivore-induced plant volatile emission in nature. Science 291:2141–2144
Keukens EAJ, de Vrije T, Jansen LAM, de Boer H, Janssen M, de Kroon AIPM, Jongen WMF, de Kruijff B (1996) Glycoalkaloids selectively permeabilize cholesterol containing biomembranes. Biochim Biophys Act 1279:243–250
Khan PM, Malik A, Ahmad S, Nawaz HR (1999) Withanolides from *Ajuga parviflora*. J Nat Prod 62:1290–1292
Kiliani H (1890) Über die Zusammensetzung des Digitonins. Ber 23:1555–1560
Kiliani H (1911) Digitonin, Digitogensäure und ihre Oxydationsprodukte. Ber 43:3574–3579
Kim SY, Kim HP, Huh H, Kim YC (1997) Antihepatotoxic zeaxanthins from the fruits of *Lycium chinense*. Arch Pharmacol Res 20:529–532
Kim YC, Che QM, Gunatilaka AAL, Kingston DGI (1996) Bioactive steroidal alkaloids from *Solanum umbelliferum*. J Nat Prod 59:283–285
Kinghorn AD, Su BN, Jang DS, Chang LC, Lee D, Gu JQ, Carcache-Blanco EJ, Pawlus AD, Lee SK, Park EJ, Cuendet M, Gills JJ, Bhat K, Park HS, Mata-Greenwood E, Song LL, Jang M, Pezzuto (2004) Natural inhibitors of carcinogenesis. Planta Med 70:691–705

Kintya PK, Prasol TI (1991) Steroidal glycosides from seeds of *Solanum tuberosum*. Tuberosides C and D. Khim Prir Soed 586–587

Kintya PK, Shvets SA (1984) Steroid glycosides of *Solanum melongena* seeds. Structure of melongosides A, B, E, F, G, and H. Khim Prir Soed 610–614

Kintya PK, Shvets SA (1985a) Melongoside L and melongoside M, steroidal saponins from *Solanum melongena* seeds. Phytochemistry 24:197–198

Kintya PK, Shvets SA (1985b) Melongoside N, O and P: steroidal saponins from seeds of *Solanum melongena*. Phytochemistry 24:1567–1569

Kirson I, Glotter E, Ray AB, Ali A, Gottlieb HE, Sahai M (1983) Physalolactone B 3-*O*-β-D-glucopyranoside, the first glycoside in the withanolide series. J Chem Res, Synopses:120–121

Kiyota N, Shingu K, Yamaguchi K, Yoshitake Y, Harano K, Yoshimitsu H, Ikeda T, Nohara T (2007) New C_{28} steroidal glycosides from *Tubocapsicum anomalum*. Chem Pharm Bull 55:34–36

Knapp S, Bohs L, Nee M, Spooner DM (2004) Solanaceae – a model for linking genomics with biodiversity. Comp Funct Genom 5:285–291

Kohara A, Nakajima C, Hashimoto K, Ikenaga T, Tanaka H, Shoyama Y, Yoshida S, Muranaka T (2005) A novel glucosyltransferase involved in steroid saponin biosynthesis in *Solanum aculeatissimum*. Plant Mol Biol 57:225–239

Kojima M, Uritani I (1981) Abnormal secondary metabolites in plants. In: Natori S, Ikekawa N, Suzuki M (eds) Advances in natural products chemistry. Kodansha Ltd, Tokyo, Wiley, New York, pp 178–194

Krasowski MD, McGehee DS, Moss J (1997) Natural inhibitors of cholinesterase: Implications for adverse drug reactions. Can J Anaesth 44:525–534

Kubota T (1958) Volatile constituents of black-rotted sweet potato and related substances. Tetrahedron 4:68–86

Kubota T, Matsuura T (1956) Synthesis of (±)-ipomeamarone [(±)-ngaione]. Chem Ind (London) 521–522

Kuboyama T, Tohda C, Komatsu K (2006) Withanoside IV and its active metabolite, sominone attenuate Aβ(25-35)-induced neurodegeneration. Eur J Neurosci 23:1417–1426

Kuc J (1982) Phytoalexins from the Solanaceae. In: Bailey JA, Mansfield JW (eds) Phytoalexins. Wiley, New York, pp 81–105

Kuhn R, Grundmann C (1933) Kryptoxanthin, ein Xantophyll der Formel $C_{40}H_{56}O$. Ber 66B:1746–1750

Kuhn R, Löw I (1947) Demissin, ein Alkaloidglykosid aus den Blättern von *Solanum demissum*. Ber 80:406–410

Kuhn R, Löw I (1954) Zur Konstitution des Solanins. Angew Chem 66:639–640

Kuhn R, Löw I (1957) Neue Alkaloidglykoside in den Blättern von *Solanum chacoense*. Angew. Chem 69:236

Kuhn R, Löw I (1961a) Zur Konstitution der Leptine. Ber 94:1088–1095

Kuhn R, Löw I (1961b) Zur Konstitution des Leptinidins. Ber 94:1096–1103

Kuhn R, Wiegand W (1929) Der Farbstoff der Judenkirsche (*Physalis Alkekengi* und *Physalis Franchetti)*. Helv Chim Acta 12:499–506

Kuhn R, Winterstein A, Kaufmann W (1930) Konjugierte Doppelbindungen. XII. *Physalis*-Farbstoff. Ber 63B:1489–1497

Kuhn R, Löw I, Trischmann H (1955a) Die Konstitution des Solanins. Ber 88:1492–1507

Kuhn R, Löw I, Trischmann H (1955b) Die Konstitution des α-Chaconins. Ber 88:1690–1693

Kuhn R, Löw I, Trischmann H (1957) Die Konstitution der Lycotetraose. Ber 90:203–218

Kumar P, Kushwaha RA (2006) Medicinal evaluation of *Withania somnifera* (L.) Dunal (Ashwagandha). Asian J Chem 18:1401–1404

Kuo KW, Hsu SH, Li YP, Lin WL, Liu LF, Chang LC, Lin CC, Lin CN, Sheu HM (2000) Anticancer activity evaluation of the *Solanum* glycoalkaloid solamargine. Triggering apoptosis in human hepatoma cells. Biochem Pharmacol 60:1865–1873

Kupchan SM, Barboutis SJ, Know JR, Lau Cam CA (1965a) Beta-solamarine: Tumor inhibitor isolated from *Solanum dulcamara*. Science 150:1827–1828

Kupchan SM, Doskotch RW, Bollinger P, McPhail AT, Sim GA, Renauld JAS (1965b) The isolation and structural elucidation of a novel steroidal tumor inhibitor from *Acnistus arborescens*. J Am Chem Soc 87:5805–5806

Kuroyanagi M, Shibata K, Umehara K (1999) Cell differentiation inducing steroids from *Withania somnifera*. Chem Pharm Bull 47:1646–1649

Kusano G, Takahashi A, Sugiyama K, Nozoe S (1987) Antifungal properties of *Solanum* alkaloids. Chem Pharm Bull 35:4862–4867

Lachman J, Hamouz K, Orsák M, Pivec V (2001) Potato glycoalkaloids and their significance in plant protection and human nutrition – Review. Rostlinná Vyroba 47:181–191

Lal P, Misra L, Sangwan R, Tuli R (2006) New withanolides from fresh berries of *Withania somnifera*. Z Naturforsch 61b:1143–1147

Laurila J, Laakso I, Valkonen JPT, Hiltunen R, Pehu E (1996) Formation of parental-type and novel glycoalkaloids in somatic hybrids between *Solanum brevidens and S. tuberosum*. Plant Sci 118:145–155

Lavie D, Glotter E, Shvo Y (1965a) Constituents of *Withania somnifera*. III. The side chain of withaferin A. J Org Chem 30:1774–1778

Lavie D, Glotter E, Shvo Y (1965b) Constituents of *Withania somnifera*. IV. The structure of withaferin A. J Chem Soc 7517–7531

Lavie D, Greenfield S, Glotter E (1966) Constituents of *Withania somnifera*. VI. The stereochemistry of withaferin A. J Chem Soc C 1753–1756

Lee JH, Kiyota N, Ikeda T, Nohara T (2006) Acyclic diterpene glycosides, capsianosides VIII, IX, X, XIII, XV and XVI from the fruits of paprika *Capsicum annuum* L. var. *grossum* Bailey and jalapeño *Capsicum annuum* L. var. *annuum*. Chem Pharm Bull 54:1365–1369

Lee KR, Kozukue N, Han JS, Park JH, Chang EY, Baek EJ, Chang JS, Friedman M (2004) Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells. J Agric Food Chem 52:2832–2839

Lee YY, Hashimoto F, Yahara S, Nohara T, Yoshida N (1994) Steroidal glycosides from *Solanum dulcamara*. Chem Pharm Bull 42:707–709

Lee YY, Hsu FL, Nohara T (1997) Two new soladulcidine glycosides from *Solanum lyratum*. Chem Pharm Bull 45:1381–1382

Leffingwell JC (1999) Basic chemical constituents of tobacco leaf and differences among tobacco types. In: Davis DL, Nielsen MT (eds) Tobacco – production, chemistry and technology. Blackwell Science, Oxford, UK, pp 265–284

Lenucci MS, Cadinu D, Taurino M, Piro G, Dalessandro G (2006) Antioxidant composition in cherry and high-pigment tomato cultivars. J Agric Food Chem 54:2606–2613

Leonart R, Moreira EA (1984) Solasodine in *Solanum brusquense* Smith & Downs. Tribuna Farmaceut 51–52:10–25

Lepschi BJ, Symon DE (1999) A preliminary cladistic analysis of Australasian *Solanum* and *Lycianthes*. In: Nee M, Symon D, Lester RN, Jessop JP (eds) Solanaceae IV – advances in taxonomy and utilization. Royal Botanic Gardens, Kew, pp 161–170

Levin RA, Watson K, Bohs L (2005) A four-gene study of evolutionary relationships in *Solanum* section *Acanthophora*. Am J Bot 92:603–612

Levin RA, Myers NR, Bohs L (2006) Phylogenetic relationships among the "spiny solanums" (*Solanum* subgenus *Leptostemonum*, Solanaceae). Am J Bot 93:157–169

Lewinsohn E, Sitrit Y, Bar E, Azulay Y, Meir A, Zamir D, Tadmor Y (2005) Carotenoid pigmentation affects the volatile composition of tomato and watermelon fruits, as revealed by comparative genetic analyses. J Agric Food Chem 53:3142–3148

Li C, Zheng Y, Sun Y, Wu Z, Liu M (1988) Studies on the odoriferous volatile constituents of the flower of *Cestrum nocturnum* L. Youji Huaxue 8:357–361

Liang CH, Liu LF, Shiu LY, Huang YS, Chang LC, Kuo KW (2004) Action of solamargine on TNFs and cisplatin-resistant human lung cancer cells. Biochem Biophys Res Commun 322:751–758

Liang P, Noller CR (1935) Saponins and sapogenins. III. The sapogenins obtained from *Chlorogalum pomeridianum* (Liliaceae). J Am Chem Soc 57:525–527

Lin CN, Lu CM, Cheng MK, Gan KH (1990) The cytotoxic principles of *Solanum incanum*. J Nat Prod 53:513–516
Litowitz TL, Clark LR, Soloway RA (1994) 1993 Annual Report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. J Ermerg Med 12:546–584
Liu LF, Liang CH, Shiu LY, Lin WL, Lin CC, Kuo KW (2004) Action of solamargine on human lung cancer cells – enhancement of the susceptibility of cancer cells to TNFs. FEBS Lett 577:67–74
Lockley WJS, Rees HH, Goodwin TW (1976) Biosynthesis of steroidal withanolides in *Withania somnifera*. Phytochemistry 15:937–939
Luis JG, Echeverri F, González AG (1994) Acnistins C and D, withanolides from *Dunalia solanacea*. Phytochemistry 36:1297–1301
Lupashku GA, Sashko YF, Mashchenko NE, Kintya PK, Shvets SA (2004) Immunomodulating activity of steroid glycosides. Dok Ross Akad Sel'skok Nauk (4) 28–31
Ma CY, Williams ID, Che CT (1999) Withanolides from *Hyoscyamus niger* seeds. J Nat Prod 62:1445–1447
Mackinney G (1935) J Biol Chem 112:421; fide Baccarini et al. (1965)
Mahmood U, Thakur RS, Blunden G (1983) Neochlorogenin, neosolaspigenin, and solaspigenin from *Solanum torvum* leaves. J Nat Prod 46:427–428
Maiti PC, Mookherjea S (1965) Hispidogenin. Chem Ind 39:1653
Makino B, Kawai M, Ogura T, Nakanishi M, Yamamura H, Butsugan Y (1995) Structural revision of physalin H isolated from *Physalis angulata*. J Nat Prod 58:1668–1674
Maldonado E, Torres FR, Martínez M, Pérez-Castorena AL (2004) 18-Acetoxywithanolides from *Physalis chenopodifolia*. Planta Med 70:59–64
Maldonado E, AlvaradoVE, Torres FR, Martínez M, Pérez-Castorena AL (2005) Androstane and withanolides from *Physalis cinerascens*. Planta Med 71:548–553
Mander LN (2003) Twenty years of gibberellin research. Nat Prod Rep 20:49–69
Maniara G, Laine R, Kuc J (1984) Oligosaccharides from *Phytophthora infestans* enhance the elicitation of sesquiterpenoid stress metabolites by arachidonic acid in potato. Physiol Plant Pathol 24:177–186
Mann JD (1978) Production of solasodine for the pharmaceutical industry. In: Brady NC (ed) Advances in agronomy, vol 30. Academic Press, New York, pp 207–243
Maoka T, Akimoto N, Ishiguro K, Yoshinaga M, Yoshimoto M (2007) Carotenoids with a 5,6-dihydro-5,6-dihydroxy-β-end group, from yellow sweet potato "Benimasari", *Ipomoea batatas* Lam. Phytochemistry 68:1740–1745
Marker RE, Rohrmann E (1939) Sterols. LXXIII. Reactions of digitogenin and gitogenin. J Am Chem Soc 61:2724–2726
Marker RE, Tsukamoto T, Turner DL (1940) Sterols. C. Diosgenin. J Am Chem Soc 62:2542–2543
Marker RE, Wagner RB, Ulshofer PR, Wittbecker EL, Goldsmith DP, Ruof CH (1943) Sterols CLVII. Sapogenins LXIX. Isolation and structures of thirteen new steroidal sapogenins. New sources for known sapogenins. J Am Chem Soc 65:1199–1209
Marshall JA, Knapp S, Davey MR, Power JB, Cocking EC, Bennett MD, Cox AV (2001) Molecular systematics of *Solanum* section *Lycopersicum* (*Lycopersicon*) using the nuclear ITS rDNA region. Theor Appl Genet 103:1216–1222
Marston A, Hostettmann K (1985) Plant molluscicides. Phytochemistry 24:639–652
Mashchenko NE, Lazur'evskii GV, Kintya PK (1977) Steroidal glycosides. XVIII. Structure of funkiosides C and D from *Funkia ovata*. Khim Prir Soed:123–124
Mashchenko NE, Prasol TI, Kintya PK (1995) Steroidal glycosides from potato seeds and their biological activity. Book of abstracts, 210th ACS National Meeting, Chicago, IL, August 20–24, (Pt 1), AGFD-160
Matevosyan GL, Kudashov AA, Ezaov AK, Sotnik VG (2001) Effect of plant growth regulators on the growth, development, yield, and quality of tomatoes under greenhouse conditions. Agrokhimiya (11) 49–58
Matsuda H, Murakami T, Kishi A, Yoshikawa M (2001) Structures of withanosides I, II, III, IV, V, VI, and VII, new withanolide glycosides, from the roots of Indian *Withania somnifera* Dunal and inhibitory activity for tachyphylaxis to clonidine in isolated guinea-pig ileum. Bioorg Med Chem 9:1499–1507

Matthews D, Jones H, Gans P, Coates S, Smith LMJ (2005) Toxic secondary metabolite production in genetically modified potatoes in response to stress. J Agric Food Chem 53:7766–7776
Maxwell A, Pingal R, Reynolds WF, McLean S (1996) 3-Aminospirosolane alkaloids from *Solanum arboreum*. Phytochemistry 43:913–915
McCue KF, Allen PV, Shepherd LVT, Blake A, Whitworth J, Maccree MM, Rockhold DR, Stewart D, Davies HV, Belknap WR (2006) The primary in vivo steroidal alkaloid glucosyltransferase from potato. Phytochemistry 67:1590–1597
McCue KF, Allen PV, Shepherd LVT, Blake A, Maccree MM, Rockhold DR, Novy RG, Stewart D, Davies HV, Belknap WR (2007). Potato glycosterol rhamnosyltransferase, the terminal step in triose side-chain biosynthesis. Phytochemistry 68:327–334
McDowall FH (1925) Constituents of *Myoporum laetum* Forst ("the ngaio"). Part I. J Chem Soc, Transact 127:2200–2207
McGehee DS, Krasowski MD, Fung DL, Wilson B, Gronert GA, Moss J (2000) Cholinesterase inhibition by potato glycoalkaloids slows mivacurium metabolism. Anaesthesiology 93:510–519
Mehmood A, Malik A, Anis I, Khan PM, Riaz M, Makhmoor T, Choudhary MI (2002) Highly oxygenated triterpenes from the roots of *Atropa acuminata*. Nat Prod Lett 16:371–376
Mello JRB (2003) Calcinosis – calcinogenic plants. Toxicon 41:1–12
Mesaik MA, Zaheer-ul-Haq, Murad S, Ismail Z, Abdullah NR, Gill HK, Atta-ur-Rahman, Yousaf M, Siddiqui RA, Ahmad A, Choudhary MI (2006) Biological and molecular docking studies on coagulin-H: Human IL-2 novel natural inhibitor. Mol Immun 43:1855–1863
Meyer K, Bernoulli F (1961) Basische Inhaltsstoffe von *Solanum paniculatum*. Pharmaceut Acta Helv 36:80–96
Mi Q, Lantvit D, Reyes-Lim E. Chai H, Zhao W, Lee IS, Peraza-Sánchez S, Ngassapa LBS, Riswan S, Hollingshead MG, Mayo JG, Farnsworth NR, Cordell GA, Kinghorn AD, Pezzuto JM (2002) Evaluation of the potential cancer chemotherapeutic efficacy of natural product isolates employing in vivo hollow fiber tests. J Nat Prod 65:842–850
Miguel MA, Barroso (1994) Accumulation of stress metabolites in cell suspension cultures of *Hyoscyamus albus*. Phytochemistry 35:371–375
Milanesi L, Monje P, Boland R (2001) Presence of estrogens and estrogen receptor-like proteins in *Solanum glaucophyllum*. Biochem Biophys Res Commun 289:1175–1179
Mimaki Y, Watanabe K, Ando Y, Sakuma C, Sashida Y, Furuya S, Sakagami H (2001) Flavonol glycosides and steroidal saponins from the leaves of *Cestrum nocturnum* and their cytoxicity. J Nat Prod 64:17–22
Mimaki Y, Watanabe K, Sakagami H, Sashida Y (2002) Steroidal glycosides from the leaves of *Cestrum nocturnum*. J Nat Prod 65:1863–1868
Misra L, Lal P, Sangwan RS Sangwan NS, Uniyal GC, Tuli R (2005) Unusually sulfated and oxygenated steroids of *Withania somnifera*. Phytochemistry 66:2702–2707
Moehs CP, Allen PV, Rockhold DR, Stapleton A, Friedman M, Belknap W (1998) The potato genes for solanidine UDP-glucose glucosyltransferase and the use of antisense genes to limit glycoalkaloid biosynthesis. PCT Int Appl, 54 pp
Monteagudo ES, Burton G, Gonzalez CM, Oberti JC, Gros EG (1988) 14β,17β-Dihydroxywithanolides from *Jaborosa bergii*. Phytochemistry 27:3925–3928
Morales Méndez A, Cázares R, Romo J (1970) Components of *Solanum torvum*. Rev Latinoam Quim 1:1–6
Moreira E, Cecy C, Nakashima T, Cavazzani JR, Miguel OG, Krambeck R (1980) Solasodine in *Solanum erianthum* D.Don. Tribuna Farmac 48:24–43
Moreno-Murillo B, Fajardo MVM, Suárez MM (2001) Cytotoxicity screening of some South American Solanaceae. Fitoterapia 72:680–685
Moretti C, Sauvain M, Lavaud C, Massiot G Bravo JA, Muñoz V (1998) A novel antiprotozoal aminosteroid from *Saracha punctuata*. J Nat Prod 61:1390–1393
Morikawa T, Xu F, Matsuda H, Yoshikawa M (2006) Structures of new flavonoids, erycibenins D, E, and F, and NO production inhibitors from *Erycibe expansa* originating in Thailand. Chem Pharm Bull 54:1530–1534
Moser D, Klaiber I, Vogler B, Kraus W (1999) Molluscicidal and antibacterial compounds from *Petunia hybrida*. Pesticide Sci 55:336–339

Mühlenbeck U, Kortenbusch A, Barz W (2002) In vitro culture and the production of secondary metabolites in *Solanum khasianum*. In: Nagata T (ed) Biotechnology in agriculture and forestry, vol 51, medicinal and aromatic plants XII, Springer, Berlin, Germany, pp 268–280

Müller KO, Börger H (1940) Arb Biol Reichsanst Landwiss Forstwiss (Berlin) 23:189; fide Stoessl et al. (1976), Kojima and Uritani (1981)

Murai A, Sato S, Osada A, Katsui N, Masamune T (1982a) Biosynthesis from solavetivone of the phytoalexin rishitin in potato. Implicit role of solavetivone as an activator. J Chem Soc, Chem Commun 32–33

Murai A, Abiko A, Ono M, Masamune T (1982b) Synthesis of aubergenone, a sesquiterpenoid phytoalexin from diseases eggplants. Bull Chem Soc Jpn 55:1191–1194

Murai A, Yoshizawa Y, Miyazaki H, Masamune T, Sato N (1987) Biosynthesis of phytotuberin. Chem Lett 1377–1378

Murakami K, Saijo R, Nohara T, Tomimatsu T (1981) Studies on the constituents of *Solanum* plants. I. On the constituents of the stem parts of *Solanum lyratum*. Yagugaku Zasshi 101:275–279

Murofushi N, Yokota T, Takahashi N (1970) Isolation and structures of gibberellins from immature seeds of *Calonyction aculeatum*. Agric Biol Chem 34:1436–1438

Murofushi N, Yokota T, Takahashi N (1971) Structures of gibberellins A_{33} and A_{35} from immature seeds of *Calonyction aculeatum*. Agric Biol Chem 35:441–443

Murofushi N, Yokota T, Takahashi N (1973) Structures of kauranoic acids in *Calonyction aculeatum*. Tetrahedron Lett 789–792

Nagafuji S, Okabe H, Akahane H, Abe F (2004) Trypanocidal constituents in plants 4. Withanolides from the aerial parts of *Physalis angulata*. Biol Pharm Bull 27:193–197

Nagaoka T, Goto K, Watanabe A, Sakata Y, Yoshihara T (2001) Sesquiterpenoids in root exudates of *Solanum aethiopicum*. Z Naturforsch 56c:707–713

Nagase H, Nagaoka T, Watanabe A, Sakata Y, Yoshihara T (2001) Sesquiterpenoids from the roots of *Solanum aethiopicum*. Z Naturforsch 56c:181–187

Nakamura T, Komori C, Lee Y, Hashimoto F, Yahara S, Nohara T, Ejima A (1996) Cytotoxic activities of *Solanum* steroidal glycosides. Biol Pharm Bull 19:564–566

Nalbandov O, Yamamoto RT, Fraenkel GS (1964) Nicandrenone, a new compound with insecticidal properties, isolated from *Nicandra physalodes*. J Agric Food Chem 12:55–59

Nee M (1999) Synopsis of *Solanum* in the New World. In: Nee M, Symon D, Lester RN, Jessop JP (eds) Solanaceae IV – advances in taxonomy and utilization, Royal Botanic Gardens, Kew, UK, pp 285–333

Nee M (2001) Solanaceae systematics for the 21st century. In: Van den Berg RG, Barendse GWM, van der Weerden GM, Mariani C (eds) Solanaceae V – advances in taxonomy and utilization. Nijmegen University Press, Nijmegen, The Netherlands, pp 3–22

Neuwinger HD (1996) African ethnobotany – poisons and drugs. Chapman & Hall, London

Neuwinger HD (2000) African traditional medicine. A dictionary of plant use and applications. Medpharm Scientific Publ, Stuttgart, Germany

Nicotra VE, Ramacciotti NS, Gil RR, Oberti JC, Feresin GE, Guerrero CA, Baggio RF, Garland MT, Burton G (2006) Phytotoxic withanolides from *Jaborosa rotacea*. J Nat Prod 69:783–789

Niero R, Da Silva IT, Tonial GC, Camacho BDS, Gacs-Baitz E, Delle Monache G, Delle Monache F (2006) Cilistepoxide and cilistadiol, two new withanolides from *Solanum sisymbrifolium*. Nat Prod Res, A 20:1164–1168

Noguchi E, Fujiwara Y, Matsushita S, Ikeda T, Ono M, Nohara T (2006) Metabolism of tomato steroidal glycosides in humans. Chem Pharm Bull 54:1312–1314

Noma M, Suzuki F, Gamou K, Kawashima N (1982) Two labdane diterpenoids from *Nicotiana raimondii*. Phytochemistry 21:395–397

O'Donovan O, Beatty S (2006) Evidence that macular pigment protects against AMD and the relationship between macular pigment and serum and dietary levels of lutein and zeaxanthin. In: Motohashi N (ed) Lutein: prevention and treatment for age-related diseases. Transworld Research Network, Trivandrum, India; pp 257–279

Oddo G (1929) Über das Solanin. Ber 62:267–271

Oddo G, Colombano A (1905); fide Oddo (1929)
Ohmura E, Nakamura T, Tian RH, Yahara S, Yoshimitsu H, Nohara T (1995) 26-Aminocholestanol derivative, a novel key intermediate of steroidal alkaloids, from *Solanum abutiloides*. Tetrahedron Lett 36:8443–8444
Okamura S, Shingu K, Yahara S, Kohoda H, Nohara T (1992) Constituents of solanaceous plants. XXV. Two new steroidal glycosides from *Scopolia japonica* Maxim. Chem Pharm Bull 40:2981–2983
Olmstead RG, Sweere JA, Spangler RF, Bohs L, Palmer JD (1999) Phylogeny and provisional classification of the Solanaceae based on chloroplast DNA. In: Nee M, Symon DE, Lester RN, Jessop JP (eds) Solanaceae IV. Royal Botanic Gardens, Kew, pp 111–137
Ono H, Kozuka D, Chiba Y, Horigane A, Isshiki K (1997) Structure and cytotoxicity of dehydrotomatine, a minor component of tomato glycoalkaloids. J Agric Food Chem 45:3743–3746
Ono M, Nishimura K, Suzuki K, Fukushima T, Igoshi K, Yoshimitsu H, Ikeda T, Nohara T (2006a) Steroidal glycosides, from the underground parts of *Solanum sodomaeum*. Chem Pharm Bull 54:230–233
Ono M, Takara Y, Egami M, Uranaka K, Yoshimitsu H, Matsushita S, Fujiwara Y, Ikeda T, Nohara T (2006b) Steroidal alkaloid glycosides, esculeosides C and D, from the ripe fruit of Cherry tomato. Chem Pharm Bull 54:237–239
Orgell WH, Vaidya KA (1958) Inhibition of human plasma cholinesterase in vitro by extracts of solanaceous plants. Science 128:1136–1137
Oritani T, Kiyota H (2003) Biosynthesis and metabolism of abscisic acid and related compounds. Nat Prod Rep 20:414–425
Oshima Y, Hikino H, Sahai M, Ray A (1989) Withaperuvin H, a withanolide of *Physalis peruviana* roots. J Chem Soc, Chem Commun:628–629
Osman SF, Herb SF, Fitzpatrick TH, Schmiediche P (1976) Commersonine, a new glycoalkaloid from two *Solanum* species. Phytochemistry 15:1065–1067
Osorio C, Duque C, Batista-Viera F (2003) Studies on aroma generation in lulo (*Solanum quitoense*): Enzymatic hydrolysis of glycosides from leaves. Food Chem 81:333–340
Paczkowski C, Kalinowska, Wojciechowski ZA (1998) The 3-*O*-glucosylation of steroidal sapogenins and alkaloids in eggplant (*Solanum melongena*); evidence for two separate glucosyltransferases. Phytochemistry 48:1151–1159
Paschold A, Halitschke R, Baldwin IT (2006) Using 'mute' plants to translate volatile signals. Plant J 45:275–291
Pearce CM, Skelton NJ, Naylor S, Kanaan R, Kelland J, Oelrichs PB, Sanders JKM, Williams DH (1992) Parquin and carboxyparquin, toxic kaurene glycosides from the shrub *Cestrum parqui*. J Chem Soc, Perkin Trans 1 (Org Biorg Chem):593–600
Pedras MSC, Ahiahonu PWK (2005) Metabolism and detoxification of phytoalexins and analogs by phytopathogenic fungi. Phytochemistry 66:391–411
Peng Y, Ma C, Li Y, Leung KSY, Jiang ZH, Zhao Z (2005) Quantification of zeaxanthin dipalmitate and total carotenoids in *Lycium* fruits (Fructus Lycii). Plant Foods Hum Nutr 60:161–164
Peralta IE, Spooner DM (2001) Granule-bound starch synthase (GBSSI) gene phylogeny of wild tomatoes (*Solanum* L. section *Lycopersicon* [Mill.] Wettst. subsection *Lycopersicon*). Am J Bot 88:1888–1902
Percival G (1999) Light-induced glycoalkaloid accumulation of potato tubers (*Solanum tuberosum* L.). J Sci Food Agric 79:1305–1310
Pérez-Castorena AL, García M, Martínez M, Maldonado E (2004) Physalins from *Physalis solanaceous*. Biochem Syst Ecol 32:1231–1234
Pérez-Castorena AL, Oropeza EF, Vázquez AR, Martínez M, Maldonado E (2006) Labdanes and withanolides from *Physalis coztomatl*. J Nat Prod 69:1029–1033
Petersen HW, Mølgaard P, Nyman U, Olsen CE (1993) Chemotaxonomy of the tuber-bearing *Solanum* species, subsection *Potatoe* (Solanaceae). Biochem Syst Ecol 21:629–644
Pianzzola MJ, Zarantonelli L, González G, Fraguas LF, Vázquez A (2005) Genetic, phytochemical and biochemical analyses as tools for biodiversity evaluation of wild accessions of *Solanum commersonii*. Biochem Syst Ecol 33:67–78

Piccinelli AL, Salazar de Ariza J, Miranda RV, Mora SQ, Aquino R, Rastrelli L (2005) Three new furostanol saponins from the leaves of *Lycianthes synanthera* ("chomte"), an edible Mesoamerican plant. J Agric Food Chem 53:289–294

Pichersky E, Noel JP, Dudareva N (2006) Biosynthesis of plant volatiles: Nature's diversity and ingenuity. Science 311:808–811

Pokrovskii AA (1956) The effect of the alkaloids of the sprouting potato on cholinesterase. Biokhimiia 21:683–688

Pomilio AB, González MD, Eceizabarrena CC (1996) 7,8-Dihydroajugasterone C, norhygrine and other constituents of *Nierembergia hippomanica*. Phytochemistry 41:1393–1398

Pongprayoon U, Baeckstroem P, Jacobsson U, Lindstroem M, Bohlin L (1992) Antispasmodic activity of β-damascenone and *E*-phytol isolated from *Ipomoea pes-caprae*. Planta Med 58:19–21

Prelog V, Jeger O (1953) The chemistry of *Solanum* and *Veratrum* Alkaloids. In: Manske RHF, Holmes HL (eds) The alkaloids – chemistry and physiology, vol 3. Academic Press, New York, pp 247–314

Prelog V, Jeger O (1960) Steroidal alkaloids: The *Solanum* group. In: Manske RHF (ed) The alkaloids – chemistry and physiology, vol 7. Academic Press, New York, pp 343–361

Prelog V, Szpilfogel S (1942) Über Steroide und Sexualhormone. LXXIX. Über das 2-Äthyl-5-methyl-pyridine, ein Dehydrierungsprodukt des Solanidins. Helv Chim Acta 25:1306–1313

Prema TP, Raghuramulu N (1996) Vitamin D_3 and its metabolites in the tomato plant. Phytochemistry 42:617–620

Purcell AE, Walter WM Jr (1968) Carotenoids of centennial variety sweet potato, *Ipomoea batatas*. J Agric Food Chem 16:769–770

Quyen IT, Khoi NH, Suong NN, Schreiber K, Ripperger H (1987) Steroid alkaloids and yamogenin from *Solanum spirale*. Planta Med 53:292–293

Raguso RA, Levin RA, Foose SE, Holmerg MW, McDade LA (2003) Fragrance chemistry, nocturnal rhythms and pollination "syndromes" in *Nicotiana*. Phytochemistry 63:265–284

Raguso RA, Schlumpberger BO, Kaczorowski RL, Holtsford TP (2006) Phylogenetic fragrance patterns in *Nicotiana* sections *Alatae* and *Suaveolentes*. Phytochemistry 67:1931–1942

Raker CM, Spooner DM (2002) Chilean tetraploid cultivated potato, *Solanum tuberosum*, is distinct from the Andean populations: Microsatellite data. Crop Sci 42:1451–1458

Raulais D, Billet D (1970) Sur un nouveau sesquiterpène, isolé du bois de *Humbertia madagascariensis* Lamarck. Bull Soc Chim France 2401–2404

Ray AB, Gupta M (1994) Withasteroids, a growing group of naturally occurring steroidal lactones. In: Zechmeister L, Herz W, Kirby GW, Moore RE, Steglich W, Tamm C (eds) Progress in the chemistry of organic natural products, vol 63. Springer, Wien/A, pp 1–106

Reid WW (1979) The diterpenes of *Nicotiana* species and *N. tabacum* cultivars. In: Hawkes JG, Lester RN, Skelding AD (eds) The Biology and Taxonomy of the Solanaceae. Linn Soc Symposium Series No 7, Academic Press, London, pp 273–278

Renault S, De Lucca AJ, Boue S, Bland JM, Vigo CB, Selitrennikoff CP (2003) CAY-1, a novel antifungal compound from Cayenne pepper. Med Mycol 41:75–82

Ripperger H (1990) Steroid alkaloids and sapogenins from some *Solanum* and a *Lycianthes* species. Pharmazie 45:381–382

Ripperger H (1998) *Solanum* steroid alkaloids – an update. In: Pelletier SW (ed) Alkaloids: chemical and biological perspectives, vol 12. Elsevier Science, Amsterdam, The Netherlands, pp 103–185

Ripperger H, Kamperdick C (1998) First isolation of physalins from the genus *Saracha* of Solanaceae. Pharmazie 53:144–145

Ripperger H, Porzel A (1992) 2α-Hydroxysoladulcidine from *Lycianthes biflora*. Phytochemistry 31:725–726

Ripperger H, Porzel A (1994) Steroidal alkaloid glycosides from *Solanum robustum*. Liebigs Ann Chem 517–520

Ripperger H, Porzel A (1997) Steroidal alkaloid glycosides from *Solanum suaveolens*. Phytochemistry 46:1279–1282

Ripperger H, Schreiber K (1981) *Solanum* steroid alkaloids. In: Manske RHF, Rodrigo RGA (eds) The alkaloids – chemistry and physiology, vol 19. Academic Press, New York, pp 81–191

Ripperger H, Budzikiewicz H, Schreiber K (1967a) Jurubin, ein stickstoff-haltiges Steroidsaponin neuartigen Strukturtyps aus *Solanum paniculatum* L.; über die Struktur von Paniculidin. Ber 100:1725–1740

Ripperger H, Schreiber K, Budzikiewicz H (1967b) Isolierung von Neochlorogenin und Paniculogenin aus *Solanum paniculatum* L. Ber 100:1741–1752

Roberts DL, Rowland RL (1962) Macrocyclic diterpenes α- and β-4,8,13-duvatriene-1,3-diols from tobacco. J Org Chem 27:3989–3995

Roddick JG (1989) The acetylcholinesterase-inhibitory activity of steroidal glycoalkaloids and their aglycones. Phytochemistry 28:2631–2634

Roddick JG, Rijnenberg AL, Weissenberg M (1990) Membrane disrupting properties of the steroidal glycoalkaloids solasonine and solamargine. Phytochemistry 29:1513–1518

Roddick JG, Weissenberg M, Leonard AL (2001) Membrane disruption and enzyme inhibition by naturally-occurring and modified chacotriose-containing *Solanum* steroidal glycoalkaloids. Phytochemistry 56:603–610

Römer S, Fraser PD (2005) Recent advances in carotenoid biosynthesis, regulation and manipulation. Planta 221:305–308

Rozkrutowa B (1987) Phytochemical investigation on *Browallia viscosa*. FECS Int Conf Chem Biotechnol Biol Act Nat Prod [Proc], 3rd, 1985. VCH, Weinheim, Germany, pp 178–181

Rozkrutowa B (1991) Constituents of *Browallia grandiflora*. Fitoterapia 62:459

Rüttimann A, Englert G, Mayer H, Moss GP, Weedon BCL (1983) Synthese von (3*R*,3′*S*,5′*R*)-Capsanthin, (3*S*,5*R*,3′*S*,5′*R*)-Capsorubin, (3′*S*,5′*R*)-Kryptocapsin und einigen verwandten Verbindungen. Ein neuer Zugang zu optisch aktiven Fünfring-Carotinoidbausteinen durch Hydroborierung. Helv Chim Acta 66:1939–1960

Saez J, Cardona W, Espinal D, Blair S, Mesa J, Bocar M, Jossang A (1998) Five new steroids from *Solanum nudum*. Tetrahedron 54:10771–10778

Sahu NP, Chakravarti RN (1971) Constituents of the leaves of *Argyreia speciosa*. Phytochemistry 10:1949

Saijo R, Murakami K, Nohara T, Tomimatsu T, Sato A, Matsuoka K (1982) On the constituents of the immature berries of *Solanum nigrum* L. Yakugaku Zasshi 102:300–305

Saijo R, Fuke C, Murakami K, Nohara T, Tomimatsu T (1983) Two steroidal glycosides, aculeatiside A and B from *Solanum aculeatissimum*. Phytochemistry 22:733–736

Saiyed Z, Kanga DD (1936) Fruits of *Solanum xanthocarpum*. Proc Indian Acad Sci 4A:255–260

Saleh M (1973) Steroidal constituents of *Solanum arundo*. Planta Med 23:377–378

Sander H (1963a) Chemische Differenzierung innerhalb der Art *Solanum dulcamara* L. Planta Med. 11:303–316

Sander H (1963b) Über *Solanum dulcamara* L. 7. Mitt.: Abbau von Spirosolanolglykosiden in reifenden Früchten. Planta Med. 11:23–36

Sang S, Xia Z, Mao S, Lao A, Chen Z (2000) Studies on chemical constituents in seed of *Allium tuberosum* ROTTL. Zhongguo Zhonggyao Zazhi 25:286–288

Sannai A, Fujimori T, Kato K (1982) Isolation of (−)-1,2-dehydro-α-cyperone and solavetivone from *Lycium chinense*. Phytochemistry 21:2986–2987

Sarmento da Silva TM, Braz-Filho R, de Carvalho MG, Agra M (2002) 1,2,3,4-Tetrahydro-2-methyl-β-carboline and solavetivone from *Solanum jabrense*. Biochem Syst Ecol 30:1083–1085

Sarmento da Silva TM, Agra M, Bhattacharyya J (2005) Studies on the alkaloids of *Solanum* of northeastern Brazil. Rev Brasil Farmacog 15:292–293

Sarquis JI, Coria NA, Aguilar I, Rivera A (2000) Glycoalkaloid content in *Solanum* species and hybrids from a breeding program for resistance to late blight (*Phytophthora infestans*). Am J Potato Res 77:295–302

Sato Y, Latham HG Jr (1953) The isolation of diosgenin from *Solanum xanthocarpum*. J Am Chem Soc 75:6067

Sato Y, Latham HG Jr, Briggs LH, Seelye RN (1957) Conversion of tomatidine and solasodine into neotigogenin and diosgenin and into a common constituent, 5α-22,25-epoxyfurostan-3β-ol. J Am Chem Soc 79:6089–6090

Sattler E (1912) Beiträge zur Lebensgeschichte der Tomatenpflanze. Tübingen; fide Czapek (1925)

Savchenko T, Whiting P, Germade A, Dinan L (2000) Ecdysteroid agonist and antagonist activities in species of the Solanaceae. Biochem Syst Ecol 28:403–419

Schlittler E, Uehlinger H (1952) Das Sterolalkaloid Solanocapsin. Helv Chim Acta 35:2043–2044

Schmeda-Hirschmann G, Papastergiou F (1994) Sesquiterpenes from *Fabiana imbricata*. Phytochemistry 36:1439–1442

Schmeda-Hirschmann G, Jordan M, Gerth A, Wilken D, Hormazabal E, Tapia AA (2004) Secondary metabolite content in *Fabiana imbricata* plants and in vitro cultures. Z Naturforsch 59c:48–54

Schmiedeberg O (1875) [Digitonin] Arch Exp Path 3:18; fide Czapek (1925)

Schneider JA, Nakanishi K (1983) A new class of sweet potato phytoalexins. J Chem Soc Chem Commun 353–355

Schneider JA, Yoshihara K, Nakanishi K (1983) The absolute configuration of (+)-ipomeamarone. J Chem Soc Chem Commun 352–353

Schneider JA, Lee J, Naya Y, Nakanishi K, Oba K, Uritani I (1984) The fate of the phytoalexin ipomeamarone: Furanoterpenes and butenolides from *Ceratocystis fimbriata*-infected sweet potatoes. Phytochemistry 23:759–764

Schöpf C, Herrmann R (1933) Zur Kenntnis des Solanidins. Ber 66:298–305

Schreiber K (1957) Isolierung von Δ^5-Tomatidenol-(3β) und Yamogenin aus *Solanum tuberosum*. Angew Chem 69:483

Schreiber K (1958a) Die Alkaloide von *Solanum dulcamara* L. Planta Med. 6:94–97

Schreiber K (1958b) Über das Vorkommen von Solasodinglykosiden in *Solanum nigrum* L. und ihre industrielle Verwertung. Planta Med 6:435–439

Schreiber K (1963) Über die Alkaloidglykoside knollentragender *Solanum*-Arten. Kulturpflanze 11:422–450

Schreiber K (1968) Steroid alkaloids: The *Solanum* group. In: Manske RHF (ed) The alkaloids – chemistry and physiology, vol 10. Academic Press, New York, pp 1–192

Schreiber K, Aurich O (1966) Isolation of secondary alkaloids and 3-hydroxy-5-pregn-16-en-20-one from *Lycopersicon pimpinellifolium*. Phytochemistry 5:707–712

Schreiber K, Ripperger H (1960) Struktur des Solanocapsins. Experientia 16:536

Schreiber K, Ripperger H (1962) Isolierung von Solanocapsin aus *Solanum pseudocapsicum*, *Solanum capsicastrum* und *Solanum hendersonii*. Z Naturforsch 17b:217–221

Schreiber K, Ripperger H (1968) Isolierung von Jurubin, Neochlorogenin und Paniculogenin aus *Solanum torvum*. Kulturpflanze 15:199–204

Schulz D, Eilert U, Willker W, Leibfritz D, Ehmke A (1992) Steroidal glycoalkaloids from *Solanum triflorum*. Abstract Book, 40th Annual Congress on Medicinal Plant Research, Trieste, Italy, p 133

Sembdner G, Schreiber K (1965) Über die Gibberelline von *Nicotiana tabacum* L. Phytochemistry 41:49–56

Severson RF, Jackson DM, Johnson AW, Sisson VA, Stephenson MG (1991) Ovipositional behaviour of tobacco budworm and tobacco hornworm. Effects of cuticular components from *Nicotiana* species. ACS Sympos Ser vol 449, American Chemical Society, Washington, DC, pp 264–277

Severson RF, Eckel RVW, Jackson DM, Sisson VA, Stephenson MG (1994) Aphicidal activity of cuticular components from *Nicotiana tabacum*. ACS Sympos Ser vol 551, American Chemical Society, Washington, DC, pp 172–190

Sharma SC, Chand R, Sati OP, Sharma AK (1983) Oligofurostanosides from *Solanum nigrum*. Phytochemistry 22:1241–1244

Shchelochkova AP, Vollerner YS, Koshoev KK (1980) Tomatoside A from *Lycopersicum esculentum* seeds. Khim Prir Soed 533–540

Shih M, Kuc J, Williams EB (1973) Suppression of steroid glycoalkaloid accumulation as related to rishitin accumulation in potato tubers. Phytopathology 63:821–826

Shingu K, Fujii H, Mizuki K, Ueda I, Yahara S, Nohara T (1994) Ergostane glycosides from *Petunia hybrida*. Phytochemistry 36:1307–1314

Shvets SA, Kintya PK, (1984) Steroid glycosides. Structure of melongoside K from the seeds of *Solanum melongena*. Khim Prir Soed:668–669

Shvets SA, Kintya PK, Gutsu ON (1994) Steroidal glycosides from seeds of *Nicotiana tabacum*. I. Structure of nicotianosides A, B, and E. Khim Prir Soed: 737–742

Shvets SA, Latsterdis NV, Kintya PK (1995a) A chemical study on the steroidal glycosides from *Atropa belladonna* L. seeds. Book of abstracts, 210th ACS National Meeting, Chicago, IL, August 20–24, (Pt 1), AGFD-132

Shvets SA, Kintya PK, Gutsu ON (1995b) Steroidal glycosides from *Nicotiana tabacum* L. seeds and their biological activity. Book of abstracts, 210th ACS National Meeting, Chicago, IL, August 20–24, (Pt 1), AGFD-161

Shvets SA, Naibi MA, Kintya PK (1995c) Steroidal glycosides from *Petunia hybrida*. seeds and their biological activity. Book of abstracts, 210th ACS National Meeting, Chicago, IL, August 20–24, (Pt 1), AGFD-163

Shvets SA, Kintya PK, Naibi MA (1995d) Steroidal glycosides from *Petunia hybrida* seeds. II. Structure of petuniosides I, L and N. Khim Prir Soed:247–252

Shvets SA, Kintya PK, Gutsu ON, Grishkovets VI (1995e) Steroidal glycosides of *Nicotiana tabacum* seeds. II. Structure of nicotianosides C and F. Khim Prir Soed:396–401

Shvets SA, Latsterdis NV, Kintya PK (1996a) A chemical study on the steroidal glycosides from *Atropa belladonna* L. seeds. Adv Exp Med Biol 404:475–483

Shvets SA, Gutsu ON, Kintya PK (1996b) Steroidal glycosides from *Nicotiana tabacum* L. seeds and their biological activity. Adv Exp Med Biol 405:247–257

Shvets SA, Kintya PK, Gutsu ON (1996c) The influence of steroid glycosides from *Solanum melongena* L. and *Nicotiana tabacum* L. seeds on the yield capacity and quality of tomato fruits. Special Publication – Royal Society of Chemistry, 179 (Agri-Food Quality):104–106

Silva M, Mancinelli P, Cheul M (1962) Chemical study of *Cestrum parqui*. J Pharm Sci 51:289

Silva TMS, Batista MM, Câmara CA, Agra MF (2005) Molluscicidal activity of some Brazilian *Solanum* spp. (Solanaceae) against *Biomphalaria glabrata*. Ann Trop Med Parasit 99:4119–4125

Silva TMS, Câmara CA, Agra MF, de Carvalho MG, Frana MT, Brandoline SVPB, Paschoal LS, Braz-Filho R (2006) Molluscicidal activity of *Solanum* spp. of the Northeast of Brazil on *Biomphalaria glabrata*. Fitoterapia 77:449–452

Sinden SL, Sanford LL, Osman SF (1980) Glycoalkaloids and resistance to the Colorado potato beetle in *Solanum chacoense* BITTER. Am Potato J 57:331–343

Singh S, Khanna NM, Dhar MM (1974) Solaplumbin, a new anticancer glycoside from *Nicotiana plumbaginifolia*. Phytochemistry 13:2020–2022

Sinha SC, Ali A, Bagchi A, Sahai M, Ray AB (1987) Physalindicanols, new biogenetic precursors of C_{28}-steroidal lactones from *Physalis minima* var. *indica*. Planta Med 53:55–57

Skliar M, Curino A, Milanesi L, Benassati S, Boland R (2000) *Nicotiana glauca*: Another plant species containing vitamin D_3 metabolites. Plant Sci 156:193–199

Smith DB, Roddick JG, Jones JL (2001) Synergism between the potato glycoalkaloids α-chaconine and α-solanine in inhibition of snail feeding. Phytochemistry 57:229–234

Soares MB, Bellintani MC, Ribeiro IM, Tomassini TC, Ribeiro dos Santos R (2003) Inhibition of macrophage activation and lipopolysaccharide-induced death by seco-steroids purified from *Physalis angulata* L. Europ J Pharmacol 459:107–112

Soltys A, Wallenfels K (1936) Solanin und Solanidin. Ber 69b:811–818

Soulé S, Güntner C, Vázquez A, Argandoña V, Ferreira F, Moyna P (1999) Effect of *Solanum* glycosides on the aphid *Schizaphis graminum*. J Chem Ecol 25:369–374

Soulé S, Güntner C, Vázquez A, Argandoña V, Moyna P, Ferreira F (2000) An aphid repellent glycoside from *Solanum laxum*. Phytochemistry 55:217–222

Spooner DM, Anderson GJ, Jansen RK (1993) Chloroplast DNA evidence for the interrelationships of tomatoes, potatoes, and pepinos (Solanaceae). Am J Bot 80:676–688

Sticher O (2007) Triterpene einschließlich Steroide. In: Hänsel R, Sticher O (eds) Pharmakognosie – Phytopharmazie 8th edn. Springer, Heidelberg, Germany, pp 916–1022

Stoessl A, Unwin CH, Ward EWB (1972) Capsidiol, an antifungal compound from *Capsicum frutescens*. Phytopathol Z 74:141–152

Stoessl A, Stothers JB, Ward EWB (1975) A 2,3-dihydroxygermacrene and other stress metabolites of *Datura stramonium*. J Chem Soc, Chem Comm:431–432

Stoessl A, Stothers JB, Ward, EWB (1976) Sesquiterpenoid stress compounds of the Solanaceae. Phytochemistry 15:855–872

Stürckow B, Löw I (1961) Die Wirkungen einiger Solanaceen-Alkaloidglykoside auf den Kartoffelkäfer. Entomol Expt Appl 4:133–142

Su BN. Gu JQ, Kang YH, Park EJ, Pezzuto JM, Kinghorn AD (2004) Induction of phase II enzyme, quinone reductase, by withanolides and norwithanolides from solanaceous species. Mini-Rev Org Chem 1:115–123

Subbaraju GV, Vanisree M, Rao CV, Sivaramakrishna C, Sridhar P, Jayaprakasam B, Nair MG (2006) Ashwagandhanolide, a bioactive dimeric thiowithanolide isolated from the roots of *Withania somnifera*. J Nat Prod 69:1790–1792

Sun LX, Fu WW, Li W, Bi KS, Wang MW (2006) Diosgenin glucuronides from *Solanum lyratum* and their cytoxicity against tumor cell lines. Z Naturforsch 61c:171–176

Suzuki H, Noma M, Kawashima N (1983) Two labdane diterpenoids from *Nicotiana setchellii*. Phytochemsitry 22:1294–1295

Suzuki Y, Yamaguchi I, Takahashi N (1985) Identification of castasterone and brassinone from immature seeds of *Pharbitis purpurea*. Agric Biol Chem 49:49–54

Syu WJ, Don MJ, Lee GH, Sun CM (2001) Cytotoxic and novel compounds from *Solanum indicum*. J Nat Prod 64:1232–1233

Szafranek B, Chrapkowska K, Pawińska M, Szafranek J (2005) Analysis of leaf surface sesquiterpenes in potato varieties. J Agric Food Chem 53:2817–2822

Szafranek B, Chrapkowska K, Waligóra D, Palavinskas R, Banach A, Szafranek J (2006) Leaf surface sesquiterpene alcohols of the potato (*Solanum tuberosum*) and their influence on Colorado beetle (*Leptinotarsa decemlineata* Say feeding. J Agric Food Chem 54:7729–7734

Tagawa C, Okawa M, Ikeda T, Yoshida T, Nohara T (2003) Homo-cholestane glycosides from *Solanum aethiopicum*. Tetrahedron Lett 44:4839–4841

Takahashi N, Yokota T, Murofushi N, Tamura S (1969) Structures of gibberellins A_{26} and A_{27} in immature seeds of *Pharbitis nil*. Tetrahedron Lett 2077–2080

Takahashi N, Murofushi N, Yokota T (1972) Gibberellins in immature seed of moonflower (*Calonyction aculeatum*). Plant Growth Subst., Proc Int Conf, 7th, Springer, New York, pp 175–180

Telek L, Delphin H, Cabanillas E (1977) *Solanum mammosum* as a source of solasodine in the lowland tropics. Econ Bot 31:120–128

Temme F (1883) fide Baccarini et al. (1965)

Teuscher E, Lindequist U (1994) Biogene Gifte – Biologie, Chemie, Pharmakologie, 2nd edn. Gustav Fischer Stuttgart, Germany

Teuscher E, Melzig MF, Lindequist U (2004) Biogene Arzneimittel, 6th edn. Wissenschaftliche Verlagsgesellschaft, Stuttgart, Germany

Tian RH, Ohmura E, Yoshimitsu H, Nohara T, Matsui M (1996) Cholestane glycosides from *Solanum abutiloides*. Chem Pharm Bull 44:1119–1121

Tian RH, Ohmura E, Matsui M, Nohara T (1997) Abutiloside A, a 26-acylamino-3β,16α-dihydroxy-5α-cholestan-22-one glycoside from *Solanum abutiloides*. Phytochemistry 44:723–726

Tietze LF, Wegner C, Wulff C (1999) First total synthesis and determination of the absolute configuration of the stress factor (+)-hydroxymyoporone. Chem Eur J 5:2885–2889

Tingey WM (1984) Glycoalkaloids as pest resistance factors. Am Potato J 61:157–167

Tingey WM, Mackenzie JD, Gregory P (1978) Total foliar glycoalkaloids and resistance of wild potato species to *Empoasca fabae* (Harris). Am Potato J 55:577–585

Tofern B, Jenett-Siems K, Siems K, Jakupovic J, Eich E (1999) Arcapitins A – C, first dammarane-type triterpenes from the Convolvulaceae. Z Naturforsch 54c:1005–1010

Tohda C, Komatsu K, Kuboyama T (2005) Scientific basis for the anti-dementia drugs of constituents from Ashwagandha *(Withania somnifera*). J Tradit Med 22 (Suppl 1) 176–182

Tomiyama K, Sakuma T, Ishizaka N, Sato N, Katsui N, Takasugi M, Masamune T (1968) Phytopathology 58:115; fide Stoessl et al. (1983)

Topal U, Sasaki M, Goto M, Hayakawa K (2006) Extraction of lycopene from tomato skin with supercritical carbon dioxide: Effect of operating conditions and solubility analysis. J Agric Food Chem 54:5604–5610

Torres R, Modak B, Faini F (1988) (25*R*)-Isonuatigenin, an unusual steroidal sapogenin as taxonomic marker in *Cestrum parqui* and *Vestia lycioides*. Bol Soc Chil Quim 33:239–241

Trease D, Evans WC (2002) Pharmacognosy, 15th edn. W.B.Saunders, Edinburgh, UK, p 293

Trumbo PR, Ellwood KC (2006) Lutein and zeaxanthin intakes and risk of age-related macular degeneration and cataracts: An evaluation using the Food and Drug Administration's evidence-based review system for health claims. Am J Clin Nutr 84:971–974

Tsay YH, Silverton JV, Beisler JA, Sato Y (1970) The structure of carpesterol. J Am Chem Soc 92:7005–7006

Tschesche R, Brennecke HR (1980) Side chain functionalization of cholesterol in the biosynthesis of solasodine in *Solanum laciniatum*. Phytochemistry 19:1449–1451

Tschesche R, Gutwinski H (1975) Steroidsaponine mit mehr als einer Zuckerkette. X. Capsicosid, ein bisdesmosidisches 22-Hydroxyfurostanolglycosid aus den Samen von *Capsicum annuum* L. Ber 108:265–272

Tschesche R, Richert H (1964) Über Saponine der Spirostanolreihe – XI. Nuatigenin, ein Chologenin-Analogon des Pflanzenreiches. Tetrahedron 20:387–398

Tschesche R, Spindler M (1978) Zur Biogenese des Aza-Oxa-Spiran-Systems der Steroidalkaloide vom Spirosolan-Typ in Solanaceen. Phytochemistry 17:251–255

Tschesche R, Wulff G (1961) Saponine der Spirostanolreihe. VII. Über Digalogenin, ein neues Sapogenin aus den Samen von *Digitalis purpurea*. Ber 94:2019–2026

Tschesche R, Wulff G (1963) Über Saponine der Spirostanolreihe – IX. Die Konstitution des Digitonins. Tetrahedron 19:621–634

Tschesche R, Goossens B, Töpfer A (1976) Zur Einführung des Stickstoffs und zum gemeinsamen Vorkommen von 25(*R*)- und 25(*S*)-Steroidalkaloiden in Solanaceen. Phytochemistry 15:1387–1389

Tukalo EA (1964) Investigation of different varieties of the Solanaceae family for the presence of compounds with steroid structure. Izuch Ispol'z Lekarstv Rastit Resursov SSR (Leningrad: Med.): 288–290

Tutin F, Clewer HWB (1914) Constituents of *Solanum angustifolium*; isolation of a new glucoalkaloid, solangustine. J Chem Soc Transact 105:559–576

Tuzson P, Kiss Z (1957) Alkaloids of *Solanum*. II. Soladulcidine. Acta Chim Acad Sci Hungar 12:31–34

Uegaki R, Fujimori T, Kubo S, Kato K (1983) Sesquiterpenoid stress compounds from *Nicotiana rustica* inoculated with TMV. Phytochemistry 22:1193–1195

Uegaki R, Fujimori T, Kubo S, Kato K (1985) Stress compounds from *Nicotiana rustica* inoculated with TMV. Phytochemistry 24:2445–2447

Uegaki R, Kubo S, Fujimori T (1988) Stress compounds in the leaves of *Nicotiana undulata* induced by TMV inoculation. Phytochemistry 27:365–368

Usubillaga AN, Meccia G (1987) Steroidal sapogenins from *Solanum scorpioideum*. J Nat Prod 50:636–641

Usubillaga A, Aziz I, Tettamanzi MC, Waibel R, Achenbach H (1997) Steroidal alkaloids from *Solanum sycophanta*. Phytochemistry 44:537–543

Usubillaga A, Khouri N, Baptista JC, Bahsas A (2005) New Acnistins from *Acnistus arborescens*. Rev Latinoam Quim 33:121–127

Van Gelder WMJ, Scheffer JJC (1991) Transmission of steroidal glycoalkaloids from *Solanum vernei* to the cultivated potato. Phytochemistry 30:165–168

Vázquez A, Ferreira F, Moyna P, Kenne L (1999) Structural elucidation of glycosides from *Solanum amygdalifolium*. Phytochem Anal 10:194–197

Veleiro AS, Trocca CE, Burton G, Oberti (1992) A phenolic withanolide from *Jaborosa leucotricha*. Phytochemistry 31:2550–2551

Veleiro AS, Oberti JC, Burton G (2005) Chemistry and bioactivity of withanolides from South American Solanaceae. In: Atta-ur-Rahman (ed) Studies in natural products chemistry, vol 32 (part L). Elsevier, Amsterdam, NL, pp 1019–1052

Veras ML, Bezerra MZB, Lemos TLG, Uchoa DEA, Braz-Filho R, Chai HB, Cordell GA, Pessoa ODL, (2004a) Cytotoxic withaphysalins from the leaves of *Acnistus arborescens*. J Nat Prod 67:710–713

Veras ML, Bezerra MZB, Braz-Filho R, Pessoa ODL, Montenegro EC, Pessoa CdO, de Moraes MO, Costa-Lotufo LV (2004b) Cytotoxic epimeric withaphysalins from leaves of *Acnistus arborescens*. Planta Med 70:551–555

Verdonk JC, de Vos CHR, Verhoeven HA, Haring MA, van Tunen AJ, Schuurink RC (2003) Regulation of floral scent production in petunia revealed by targeted metabolomics. Phytochemistry 62:997–1008

Vidal Aldana M, Noguiera Lima C (1999) Isolation and characterization of a glycoside from fluid extracts of *Solanum americanum* MILL. Afinidad 56:393–396

Vijayan P, Prashanth HC, Vijayaraj P, Dhanaraj SA, Badami S, Suresh B (2003) Hepatoprotective effect of the total alkaloid fraction of *Solanum pseudocapsicum* leaves. Pharmaceut Biol 41:443–448

Volkov RA, Komarova NY, Panchuk II, Hemleben V (2003) Molecular evolution of rDNA external transcribed spacer and phylogeny of sect. *Petota* (genus *Solanum*). Mol Phylogenet Evol 29:187–202

Volynets AP, Shukanov VP, Goncharik NN (2002) Influence of steroid glycosides on grain productivity and sowing qualities of spring wheat seeds (*Triticum aestivum* L.). Vestsi Natsy Akad Navuk Belarusi, Ser Biyalag Navuk (3):10–12

Wagner G (1999) Leaf surface chemistry. In: Davis DL, Nielsen MT (eds) Tobacco – production, chemistry and technology. Blackwell Science, Oxford, UK, pp 292–303

Wahlberg I, Eklund AM (1992) Cembranoids, pseudopteranoids, and cubitanoids of natural occurrence. In: Zechmeister L, Herz W, Grisebach H, Kirby GW (eds) Progress in the chemistry of organic natural products, vol 59. Springer Verlag, Wien/A, pp 141–294

Wahlberg I, Ringberger T (1999) Smokeless tobacco. In: Davis DL, Nielsen MT (eds) Tobacco – production, chemistry and technology. Blackwell Science, Oxford, UK, pp 452–460

Wahlberg I, Wallin I, Narbonne C, Nishida T, Enzell CR (1981) Note on the stereostructure of thunbergol (isocembrol) and 4-*epi*isocembrol. Acta Chem Scand B35:65–68

Waiss A Jr, Elliger CA, Haddon WF, Benson M (1993) Insect inhibitory steroidal saccharide esters from *Physalis peruviana*. J Nat Prod 56:1365–1372

Wallin I, Narbonne C, Wahlberg I, Nishida T, Enzell CR (1980) Two new acyclic diterpenoids from *Nicotiana sylvestris*. Acta Chem Scand B34:391–396

Wang LT, Wang AY, Hsieh CW, Chen CY, Sung HY (2005) Vacuolar invertases in sweet potato: Molecular cloning, characterization, and Analysis of gene expression. J Agric Food Chem 53:3672–3678

Wang Y, Kays SJ (2002) Sweetpotato volatile chemistry in relation to sweetpotato weevil (*Cylas formicarius*) behaviour. J Am Soc Horticult Sci 127:656–662

Wanyonyi AW, Chhabra SC, Mkoji G, Eilert U, Njue WM (2002) Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum*. Phytochemistry 59:79–84

Wanyonyi AW, Tarus PK, Chhabra SC (2003) A novel steroidal alkaloid from *Solanum aculeastrum*. Bull Chem Soc Ethiopia 17:61–66

Ward EWB, Stoessl A (1972) Detoxification of capsidiol, an antifungal compound from peppers. Phytopathology 62:1186–1187

Weeks WW (1999) Relationship between leaf chemistry and organoleptic properties of tobacco smoke. In: Davis DL, Nielsen MT (eds) Tobacco – production, chemistry and technology. Blackwell Science, Oxford, UK, pp 304–312

Weiler EW, Krüger H, Zenk MH (1980) Radioimmunoassay for the determination of the steroidal alkaloid solasodine and related compounds in living plants and herbarium specimens. Planta Med 39:112–124

Weiss D, van der Luit A, Knegt E, Vermeer E, Mol JNM, Kooter JM (1995) Identification of endogenous gibberellins in petunia flower: induction of anthocyanin biosynthetic gene expression and the antagonistic effect of abscisic acid. Plant Physiol 107:695–702

Weissenberg M, Klein M, Meisner J, Ascher KRS (1986) Larval growth inhibition of the spiny bollworm, *Earias insulana*, by some steroidal secondary plant compounds. Entomol Exp Appl 42:213–217

Weissenberg M, Levy A, Wasserman RH (1993*) Solanum glaucophyllum* DESF. (duraznillo blanco): In vitro culture and the production of steroidal secondary metabolites. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 21, medicinal and aromatic plants IV, Springer, Berlin, Germany, pp 352–370

Weissenberg M, Levy A, Svoboda JA, Ishaaya I (1998) The effect of some *Solanum* steroidal alkaloids and glycoalkaloids on larvae of the red flour beetle, *Tribolium castaneum*, and the tobacco hornworm, *Manduca sexta*. Phytochemistry 47:203–209

Weyerstahl P, Christiansen C, Marschall H (1992) Isolation and synthesis of isohumbertiol, the first naturally occurring sesquiterpene alcohol with a humbertiane skeleton. Liebigs Ann Chem 1325–1328

Whitehead IM, Threlfall DR, Ewing DF (1989) 5-epi-Aristolochene is a common precursor of the sesquiterpenoid phytoalexins capsidiol and debneyol. Phytochemistry 28:775–779

Wijayanti L, Kobayashi M, Fujioka S, Yoshizawa K, Sakurai A (1995) Identification and quantification of abscisic acid, indole-3-acetic acid and gibberellins in phloem exudates of *Pharbitis nil*. Biosci Biotech Biochem 59:1533–1535

Willstätter R, Escher HH (1911) Die Farbstoffe der Tomate. Z Physiol Chem 64:47–61

Willuhn G (1966) Untersuchungen zur chemischen Differenzierung bei *Solanum dulcamara* L. I. Genetische Fixierung der unterschiedlichen Steroidalkaloidführung. Planta Med 14:408–420

Willuhn G (1967) Untersuchungen zur chemischen Differenzierung bei *Solanum dulcamara* L. II. Der Steroidgehalt in Früchten verschiedener Entwicklungsstadien der Tomatidenol- und Soladulcidin-Sippe. Planta Med 15:58–73

Willuhn G, Koestens J (1974) *Solanum dulcamara*. Triterpenoids and sterols from the petroleum ether extract of the leaves. Planta Med 25:115–137

Willuhn G, Koestens J (1975) Quantitative distribution of sterols and sterol derivatives in organs of *Solanum dulcamara*. Phytochemistry 14:2055–2058

Willuhn G, Koethe U (1981) Spirostanol-Gehalt und -Variabilität in oberirdischen Organen von *Solanum dulcamara* L. Dtsch Apoth Ztg 121:235–239

Wilson BJ, Yang DT, Boyd MR (1970) Toxicity of mould-damaged sweet potatoes (*Ipomoea batatas*). Nature (London) 227:521–522

Wilson DD, Son KC, Severson RF, Kays SJ, (1990) Effect of a pentacyclic triterpene from sweet potato storage roots on oviposition by the sweetpotato weevil (Coleoptera: Curculionidae). Environ Entomol 19:1663–1665

Windaus A, Brunken J (1925) Über das Vorkommen von Gitogenin in *Digitalis*-Blättern. Z Physiol Chem 143:33–47

Wolters B (1964) Beziehungen zwischen Struktur und antibiotischer Wirkung bei einigen Steroidalkaloiden. Arch Pharm 297:748–754

Wolters B (1968) Saponine als pflanzliche Pilzabwehrstoffe. Planta 79:77–83

Yahara S, Morooka M, Ikeda M, Yamasaki M, Nohara T (1986) Two new steroidal glucuronides from *Solanum lyratum*. Planta Med 52:496–498

Yahara S, Izumitani Y, Nohara T (1988) A novel acyclic diterpene glycoside, capsianside A, from *Capsicum annuum* var. *fasciculatum*. Tetrahedron Lett 29:1943–1946

Yahara S, Kobayashi N, Izumitani Y, Nohara T (1991) New acyclic diterpene glycosides, capsianosides VI, G and H from the leaves and stems of *Capsicum annuum* L. Chem Pharm Bull 39:3258–3260

Yahara S, Ura T, Sakamoto C, Nohara T (1994) Steroidal glycosides from *Capsicum annuum*. Phytochemistry 37:831–835

Yahara S, Yamashita T, Nozawa N (nee Fujimura), Nohara T (1996a) Steroidal glycosides from *Solanum torvum*. Phytochemistry 43:1069–1074

Yahara S, Nakamura T, Someya Y, Matsumoto T, Yamashita T, Nohara T (1996b) Steroidal glycosides, indiosides A–E, from *Solanum indicum*. Phytochemistry 43:1319–1323

Yahara S, Uda N, Nohara T (1996c) Lycoperosides A – C, three stereoisomeric 23-acetoxyspirosolan-3β-ol-β-lycotetraosides from *Lycopersicon esculentum*. Phytochemistry 42:169–172

Yahara S, Uda N, Yoshio E, Yae E (2004) Steroidal alkaloid glycosides from tomato (*Lycopersicon esculentum*). J Nat Prod 67:500–502

Yamashita T, Matsumoto T, Yahara S, Yoshida N, Nohara T (1991) Structures of two new steroidal glycosides, soladulcosides A and B from *Solanum dulcamara*. Chem Pharm Bull 39:1626–1628

Yang DTC, Wilson BJ, Harris TM (1971) The structure of ipomeamaranol: A new toxic furanosesquiterpene from moldy sweet potatoes. Phytochemistry 10:1653–1654

Yarden A, Lavie D (1962) Constituents of *Withania somnifera*. I. Functional groups of withaferin. J Chem Soc 2925–2927

Ye WC, Wang H, Zhao SX, Che CT (2001) Steroidal glycoside and glycoalkaloid from *Solanum lyratum*. Biochem Syst Ecol 29:421–423

Yokose T, Katamoto K, Park S, Matsuura H, Yoshihara T (2004) Anti-fungal sesquiterpenoid from the root exudate of *Solanum abutiloides*. Biosci Biotechnol Agrochem 68:2640–2642

Yokota T, Takahashi N, Murofushi N, Tamura S (1969a) Structures of new gibberellin glucosides in immature seeds of *Pharbitis nil*. Tetrahedron Lett 2081–2084

Yokota T, Takahashi N, Murofushi N, Tamura S (1969b) Isolation of gibberellin A_{26} and A_{27} and their glucosides from immature seeds of *Pharbitis nil*. Planta 87:180–184

Yokota T, Murofushi N, Takahashi N (1970) Structure of new gibberellin glucoside in immature seeds of *Pharbitis nil*. Tetrahedron Lett 1489–1491

Yokota T, Murofushi N, Takahashi N, Tamura S (1971a) Gibberellins in immature seeds of *Pharbitis nil*. II. Isolation and structures of novel gibberellins, gibberellins A_{26} and A_{27}. Agric Biol Chem 35:573–582

Yokota T, Murofushi N, Takahashi N, Tamura S (1971b) Gibberellins in immature seeds of *Pharbitis nil*. III. Isolation and structures of gibberellin glucosides. Agric Biol Chem 35:583–595

Yokota T, Murofushi N, Takahashi N, Katsumi M (1971c) Gibberellins in immature seeds of *Pharbitis nil*. IV. Biological activities of gibberellins and their glucosides in *Pharbitis nil*. Phytochemistry 10:2943–2949

Yokota T, Sato T, Takeuchi Y, Nomura T, Uno K, Watanabe T, Takatsuto S (2001) Roots and shoots of tomato produce 6-deoxo-28-cathasterone, 6-deoxo-28-nortyphasterol and 6-deoxo-28-norcastasterone. Phytochemistry 58:233–238

Yoshimitsu H, Nishida M, Nohara T (2000) Cholestane glycosides from *Solanum abutiloides*. III. Chem Pharm Bull 48:556–558

Yoshimitsu H, Nishida M, Yoshida M, Nohara T (2002) Four new 26-aminocholestane-type glycosides from *Solanum abutiloides*. Chem Pharm Bull 50:284–286

Yoshimitsu H, Nishida M, Nohara T (2003) Steroidal glycosides from the fruits of *Solanum abutiloides*. Phytochemistry 64:1361–1366

Yoshizaki M, Matsushita S, Fujiwara Y, Ikeda T, Ono M, Nohara T (2005) Tomato new sapogenols, isoesculeogenin A and esculeogenin B. Chem Pharm Bull 53:839–840

Zacharius RM, Osman SF (1977) Glycoalkaloids in tissue culture of *Solanum* species. Dehydrocommersonine from cultured roots of *Solanum chacoense*. Plant Sci Lett 10:283–287

Zamilpa A, Tortoriello J, Navarro V, Delgado C, Alvarez L (2002) Five new steroidal saponins from *Solanum chrysotrichum* leaves and their antimycotic activity. J Nat Prod 65:1815–1819

Zechmeister L, Cholnoky LV (1927) Über Paprika-Farbstoffe. Liebigs Ann 454:54–71

Zechmeister L, Cholnoky LV (1930) Zum Stand sauerstoffhaltiger Carotenoide in Pflanzen. Vorläufige Mitteilung. Z Physiol Chem 189:159–161

Zechmeister L, Cholnoky LV (1936) Lycoxanthin und Lycophyll, zwei natürliche Derivate des Lycopins. Ber 69B:422–429

Zhao J, Nakamura N, Hattori M, Kuboyama T, Tohda C, Komatsu K (2002) Withanolide derivatives from the roots of *Withania somnifera* and their neurite outgrowth activities. Chem Pharm Bull 50:760–765

Zhou X, He X, Wang G, Gao H, Zhou G, Ye W, Yao X (2006) Steroidal saponins from *Solanum nigrum*. J Nat Prod 69:1158–1163

Zhu XH, Takagi M, Ikeda T, Midzuki K, Nohara T (2001a) Withanolide-type steroids from *Solanum cilistum*. Phytochemistry 56:741–745

Zhu XH, Ando J, Takagi M, Ikeda T, Nohara T (2001b) Six new withanolide-type steroids from the leaves of *Solanum cilistum*. Chem Pharm Bull 49:161–164

Zhu XH, Ando J, Takagi M, Ikeda T, Yoshimitsu A, Nohara T (2001c) Four novel withanolide-type steroids from the leaves of *Solanum cilistum*. Chem Pharm Bull 49:1440–1443

Zhu XH, Tsumagari H, Honbu T, Ikeda T, Ono M, Nohara T (2001d) Peculiar steroidal saponins with opened E-ring from *Solanum* genera plants. Tetrahedron Lett 42: 8043–8046

Zwenger C, Kind A (1859) [Solanidin] Liebigs Ann Chem 109:244; fide Czapek (1925)

Zwenger C, Kind A (1861) [Solanidin] Liebigs Ann Chem 118:129; fide Czapek (1925)

8
Secondary Metabolites Derived from Fatty Acids and Carbohydrates

8.1 Fatty Acids and Their Derivatives

8.1.1 Fatty Acids

8.1.1.1 Occurrence in the Solanaceae

Seeds of species from this family contain 15–40% fatty oil. Linoleic acid [18:2 (n–6)] was found to be usually the major fatty acid component in genera such as *Atropa, Datura, Hyoscyamus, Physalis,* and *Solanum* (Hegnauer 1973 and references therein). In a comprehensive study the fatty acid composition of the seeds of 62 *Nicotiana* spp. (content: 25–40% on a dry weight basis) and of the leaves of 56 *Nicotiana* spp. (2.1–4.4%) were presented (Koiwai et al. 1983). α-Linolenic acid [18:3 (n–3)], the most abundant plant fatty acid, also was the dominant fatty acid in the leaves of all *Nicotiana* species (50–63%) whereas it predominated at least in the seeds of most species. Another report has been published with detailed information on the fatty acid composition of the seed oil of *Capsicum annuum* L. (Bekker et al. 2001). The fatty acid composition of the seed oils was also reported from, e.g, *Bouchetia anomala* (Miers) Britton & Rusby (Maestri and Guzman 1991), *Grabowskia duplicata* Arnott (Maestri et al. 1992), and *Phrodus microphyllus* (Miers) Miers (Maestri et al. 1994). Complete triglyceride types of seed oils from certain cultivated plants of the Solanaceae such as *Physalis pubescens* L., *P. philadelphica* Lam. sub nom. *P. ixocarpa* Brot., *C. annuum*, *Solanum lycopersicum* L. sub nom. *Lycopersicon esculentum* Mill., *S. melongena* L., and *S. nigrum* L. have been reported (Deineka and Deineka 2004).

Detailed results on the fatty acid composition of phospholipids and glycolipids (monogalactosyl- and digalactosyl-diacylglycerides) obtained from the seeds of *C. annuum* have been published recently (Asilbekova 2003, 2004). Glycolipids themselves from this species, e.g., capsoside A, have been isolated by two other groups (Iorizzi et al. 2001; de Marino et al. 2006). Tetra-, penta-, hexa-, and heptaacyl glycerides were characterized as constituents of stigmas from *Nicotiana tabacum* (Matsuzaki et al. 1983). As main hydroxy fatty acids 18:1-ω-hydroxy as well as 18:2-ω-hydroxy fatty acids have been identified. The terminal hydroxy

groups were esterified with other fatty acids (18:1 > 18:2 > 16:0, 18:0, 18:3). Later, the hydroxy and normal fatty acid distribution in stigmas of 51 *Nicotiana* spp. was reported in another comprehensive study (Koiwai and Matsuzaki 1988). Recently, two series of unique pseudoglycolipids named lanceolitols A1–A7 and B1–B7 have been discovered as constituents of the leaves of *Solanum lanceolatum* CAV. (Herrera-Salgado et al. 2005). 2-*O*-Acyl-D-*myo*-inositol moieties represent the aglycones of 1→1′-linked β-D-xylopyranosides (A series) and β-D-glucopyranosides (B series), respectively. The same straight-chain as well as methyl-branched even-numbered fatty acids (C_{12}–C_{20}) occur in both series as acyl residues. The lanceolitols were shown to possess anti-inflammatory activity (inhibition of phospholipase A_2 and cyclooxygenase-2).

C_6 Green Leaf Volatiles. The complexity of scent compounds produced by plants may be remarkable. Thus, e.g., altogether 125 volatile compounds from floral and vegetative organs of nine *Nicotiana* spp. have been characterized including (i) monoterpenoids (see Sect. 7.2.1), (ii) sesquiterpenoids (see Sect. 7.3.1), (iii) nitrogenous compounds (e.g., indole, methyl anthranilate), (iv) benzenoids (see Sect. 6.3.4.2), and (v) derivatives of the fatty-acid catabolism (Raguso et al. 2003). Among the latter metabolites several were products of the lipoxygenase (LOX) cascade: As has been shown for *N. attenuata* TORREY ex S. WATSON (Paschold et al. 2006) wounding or herbivory induced dioxygenation of linolenic acid [18:3 (n–3)] and linoleic acid [18:2 (n–6)], respectively, catalyzed by 13-LOX resulting in the formation of 13-hydroperoxy-α-linolenic acid (13-HPOT; T = triunsaturated) and 13-hydroperoxylinoleic acid (13-HPOD; D = diunsaturated), respectively. Catalyzed by hydroperoxidelyase 13-HPOT / 13-HPOD are cleaved yielding *Z*-3-hexenal and *n*-hexanal, respectively (with 12-oxo-dodec-9-enoic acid and 12-oxododecanoic acid, respectively, as the second product). These C_6-aldehydes may be reduced by alcohol dehydrogenase to the respective alcohols. *Z*-3-Hexen-1-ol was found to be esterified by acetyl-CoA. Furthermore, *Z*-3-hexenal may be isomerized to the corresponding *E*-isomer and/or *E*-2-hexenal. Such herbivory-induced C_6 green leaf volatiles have been detected in the study of Raguso et al. as components of the scent of, e.g., *N. plumbaginifolia* VIV. Such compounds were also detected as volatile components of fruits and leaves of tomato (Maneerat et al. 2002). Moreover, lipid derived volatiles of higher carbon numbers, e.g., 4,5-epoxy-(*E*)-2-decenal, were determined in the scent of these fruits (Buttery and Ling 1993).

Lipid-derived Oxylipins: Jasmonic and Tuberonic Acids. 13-HPOT is also a precursor of the phytohormon jasmonic acid in the so-called octadecanoid pathway. For jasmonates and related compounds involved in plant-herbivore interactions the reader is referred to a recent review (Halitschke and Baldwin 2005). *Nicotiana* spp. and potato are plant species which have been of specific significance for the elucidation of such relationships. Furthermore, aerial interaction of *Nicotiana attenuata* ("receiver") and sagebrush, *Artemisia tridentata* NUTT. (Asteraceae) ("emitter") is the best-documented example of between-plant signaling aboveground VOCs such as *cis*- and *trans*-epimers of methyl jasmonate, *cis*-3-hexenal, and *trans*-2-hexenal in nature (Baldwin et al. 2006 and references therein). Tuberonic acid (12-hydroxyjasmonic acid) glucoside which was found to stimulate

potato tuberization in concentrations as low as 3×10^{-8} as well as its methyl ester were detected in the leaves of *Solanum tuberosum* L. (Šimko et al. 1996 and references therein). For further oxylipins such as trioxygenated unsaturated fatty acids see, e.g., de Marino et al. 2006 and references therein.

Appendix. The long-chain saturated fatty alcohol n-hentriacontanol (myricyl alcohol; $C_{31}H_{64}O$), isolated from a *Cuatresia* sp., possibly *C. fosteriana* HUNZ. according to Hunziker (2001), showed in vivo antimalarial activity in mice (1992).

8.1.1.2 Occurrence in the Convolvulaceae

Seeds of species from this family usually contain about 10% fatty oil. By the 1950s its fatty acid composition of several species, e.g., *Convolvulus arvensis* L., *Cuscuta reflexa* ROXB., *Argyreia nervosa* (BURM. f.) BOJ. and some *Ipomoea* spp. was published (Hegnauer 1964 and references therein). In most cases stearic [18:0], oleic [18:1 (n–9)], and linoleic [18:2 (n–6)] acids represented the major components. Additionally a number of ornamental species such as *Convolvulus tricolor* L., *Ipomoea nil* (L.) ROTH, *I. purpurea* (L.) ROTH, *I.* × *sloteri* (HOUSE) OOSTSTR., and *I. coccinea* L., as well as *Turbina corymbosa* (L.) RAF. sub nom. *Rivea corymbosa* (L.) H.HALL. was investigated (Genest and Sahasrabudhe 1966). A corresponding analysis of six *Ipomoea* spp. from the Mexican wild belonging to sect. *Arborescentes* was reported (Pérez-Amador et al. 1992). Seed oil of *Rivea ornata* (ROXB.) CHOISY was found to contain vernolic acid (12,13-epoxy-octadec-*cis*-9-enoic acid) (Hosamani and Sattigeri 2000). Long-chain fatty acids characterized by one additional carbonyl function, e.g., cressatetratriacontanoic acid (29-oxotetratriacontanoic acid), were discovered as constituents of the aerial parts of *Cressa cretica* L. (Ramachandran et al. 2004) beside acyclic long-chain terpenoid alcohols acylated by unsaturated fatty acids (Ramachandran and Ali 2003).

8.1.2 Fatty Acid Amides and Aliphatic Monoamines

8.1.2.1 Occurrence in the Solanaceae

In the course of the search for tumor inhibitors of plant origin two fatty acid amides with long chain acyl residues (C_{16} and C_{18}), solapalmitine and solapalmitenine, were isolated from the whole plant of *Solanum tripartitum* DUN. by bioassay-guided fractionation (Kupchan et al. 1969). During structure elucidation procedures a degradation product could be isolated which was named solamine; it could be discovered later as a free natural product in *S. betaceum* together with its *N*-hexanoyl derivative solacaproine (see Sect. 5.1.4; Fig. 5.1). Solapalmitine, solamine, and solacaproine also turned out to be constituents of a number of further *Solanum* spp. (Evans and Somanabandhu 1980). *N*-[(−)-Jasmonoyl]-tyramine was discovered as a constituent of *Petunia* × *hybrida* pollen (Miersch et al. 1998). Beside two known congeners a novel ceramide,

(2*S*,3*S*,4*R*,9*E*)-1,3,4-trihydroxy-2-[(2′*R*)-2′-hydroxytetracosanoylamino]-9-octadecene, was identified as a constituent of the leaves and stems of *Physalis philadelphica* (Su et al. 2002). A cerebroside, i.e., a ceramide glycoside, was isolated from the leaves of *Datura metel* (Sahai et al. 1999).

Withanamides. Recently, a novel group of fatty acid amides, withanamides A–I, have been discovered in the fruits of *Withania somnifera* (L.) Dun. (Jayaprakasam et al. 2004). These unique metabolites are glycosides. The common moiety of their aglycones is represented by serotonin (5-hydroxytryptamine). Its side-chain amino group is conjugated to the carboxyl group of diverging long chain ω-1- or ω-2-hydroxyl fatty acids with 16 or 18 carbon atoms thus forming amides. These fatty acids may be saturated, di-, tri- and tetraunsaturated. The phenolic hydroxyl group of the serotonin portion is glycosidically linked to form di- or triglucosides (1 → 6). The withanamides turned out to be very potent lipid peroxidation inhibitors probably due to their hydroxylated long-chain acyl group. Thus, they are promising leads for the development of antioxidants.

8.1.2.2 Occurrence in the Convolvulaceae

Fatty acid amides such as palmitoylamide (16:0), stearoylamide (18:0), oleamide [18:1 (n–9)], and erucamide [22:1 (n–9)] were detected by GC/MS analysis as constituents of the aerial parts of *Evolvulus glomeratus* Choisy. Erucamide was also found in the aerial parts of *I. plebeia* R.Br. and *Operculina riedeliana* (Oliv.) Ooststr., respectively (Eich and Witte, unpublished results). These findings are remarkable in so far as such amides could not be detected in about 150 other convolvulaceous species belonging to 23 genera.

Aliphatic *N*,*N*-dimethylamines such as *N*,*N*-dimethyl-dodecylamine, *N*,*N*-dimethyl-tetradecylamine, *N*,*N*-dimethyl-hexadecylamine and *N*,*N*-dimethyl-octadecylamine also were discovered by GC/MS analysis in the aerial parts of a limited number of convolvulaceous species (Eich and Witte, unpublished results). In the cases of dodecylamine and tetradecylamine often co-occurrence with the corresponding *N*-monomethyl congeners was given. Such amines were found in the aerial parts of

- Cresseae
  - *Evolvulus glomeratus* Choisy (neotropical origin)
- Aniseieae
  - *Odonellia hirtiflora* (Mart. & Gal.) K.Rob. (neotropical)
- Convolvuleae
  - *Calystegia silvatica* (Kit.) Griseb. (central Europe)
  - *Convolvulus altheoides* L. (Mediterranean), *C. demissus* Choisy (Chile), *C. glandulosus* (Webb.) Hall. (Canary islands), *C. kilimandschari* Engl. (East Africa), *C. subauriculatus* (Burch.) Linding. (endemic to La Gomera/Canary islands)
- "Merremieae"
  - *Merremia gemella* (Burm.) Hall. f. (paleotropical)
  - *Operculina riedeliana* (Oliv.) Ooststr (paleotropical)

- Ipomoeeae
  - *Ipomoea eremnobrocha* D.F.Austin (neotropical), *I. plebeia* R.Br. (paleotropical), *I. regnellii* Meisn., *I. tricolor* Cav. cv. 'heavenly blue' (neotropical)

Although these metabolites have been detected in 6 tribes and in species from many different parts of the world they only occur erratically in this family: They could be only found in 14 species belonging to 7 genera out of 150 checked species belonging to 23 genera.

8.2 Secondary Carbohydrates

8.2.1 Occurrence in the Solanaceae

8.2.1.1 Sucrose Esters

Structure. From the cuticular waxes of a tobacco budworm resistant tobacco Severson et al. separated a series of polar, high molecular weight compounds from other major components (duvatrienediols, C_{25}–C_{36} alkanes) which turned out to be sucrose esters (Severson et al. 1985). Besides a series of low molecular aliphatic acids (up to C_8) especially acetic, 2-methylbutyric, and 3-methylvaleric acids were determined to be responsible for *O*-acylation at four positions (2,3,4,6) of the glucose moiety. The major component was characterized to be 6-*O*-acetyl-2,3,4-tri-*O*-[3-methylvaleryl]-α-D-glucopyranosyl-β-D-fructofuranoside. Investigations of the type B glandular trichome exudate from accessions of wild potato species revealed that complex mixtures of sucrose esters constitute the major portion of the non-volatile fraction (King and Calhoun 1988 and references therein). A comprehensive study including 54 *Nicotiana* spp. revealed that almost half of the species were able to synthesize sucrose esters on their leaf surfaces (Matsuzaki et al. 1988). Table 8.1 shows the occurrence and characterization of a number of di- to penta-acylated examples from four solanaceous genera. They include metabolites with acylated positions belonging to both monosaccharide units as well as congeners whose acyl substituents are confined to the glucose moiety.

Biosynthesis. Biosynthetic investigations showed that the branched amino acid pathway is used to form C_4 and C_5 branched acyl groups which are subsequently elongated by acetate to form the medium chain acyl groups (Steffens and Walters 1991). In the case of 2,3,4,1′-tetra-*O*-acylated sucrose metabolites a route proceeding in the glandular trichomes through 2,3,4-tri-*O*-acylation of the glucose moiety prior to 1-*O*-acylation of the fructose portion was assumed based on results with *Solanum hirsutum* sub nom. *Lycopersicon hirsutum* (King et al. 1990).

Occurrence Outside the Solanaceae. All reports of the isolation of acylated sucrose metabolites from the plant kingdom have been assumed to be from species belonging to the Solanaceae (Ovenden et al. 2005). However, a first report of such metabolites outside this family was published on *Spergula arvensis* L., corn spurrey

Table 8.1 Examples for the occurrence of sucrose esters in the Solanaceae and the basis for the structural diversity of such metabolites

Species	Organ	**Positions of *O-acylation*** (numbers without ′ belong to the glucose, with ′ to the fructose residues)	**Acyl residues** (positional isomers, i.e., substitution of the same acyl residues at different positions of the sugar moieties, may enlarge the diversity of metabolites; certain residues may occur twice or even three times in one specific metabolite)	References
Nicotiana acuminata (Graham) Hook.	Leaf surface	2,3,4,6,3′	Acetyl, propionyl, 2-methylbutyryl, 2-methylbutenoyl, 3-methylvaleroyl, 4-methylvaleroyl	Matsuzaki et al. 1991
N. glutinosa L.	Leaf (cuticular components)	2,3,4 and 2,3,4,3′	Acetyl, propionyl, *n*-butyryl, isobutyryl, 2-methylbutyryl, 3-methylbutyryl, valeroyl, 3-methylvaleroyl, 4-methylvaleroyl, hexanoyl, 4-methylhexanoyl, 5-methylhexanoyl, heptanoyl, 6-methylheptanoyl, octanoyl	Arrendale et al. 1990
N. tabacum L.	Leaf (cuticular components)	2,3,4,6	Like *Nicotiana glutinosa* though minus 6-methylheptanoyl and octanoyl	Severson et al. 1985, 1994
Petunia × *hybrida* (Hook.) Vilm.	Flower, flower bud	Not determined	Like *Nicotiana glutinosa* plus decanoyl	Son et al. 1994
do.	Aerial parts	2,3,4,6	2-Methylbutyryl, 3-methylbutyryl, pentanoyl, hexanoyl, heptanoyl, octanoyl	Moser et al. 1999
P. nyctaginiflora Juss.	Epigeal parts	2,3,4 and 2,3,4,6	Acetyl, hexanoyl, heptanoyl, 5-methylhexanoyl, 6-methylheptanoyl	Singh et al. 2003; Begum et al. 2004, 2005
Physalis nicandroides Schltdl. var. *attenuata* Waterf.	Fruit	2,3,3′ (nicandroses D, E) and 2,3,1′,3′ (nicandroses A–C)	Acetyl, isobutyryl, 2-methylbutyryl, decanoyl	Maldonado et al. 2006
P. viscosa L.	Flower, stem	2,3 (physaloside A)	3-Methyl-2-butenoyl, dodecanoyl	Ovenden et al. 2005
Solanum berthaultii Hawkes	Leaf	2,3,6,3′	Isobutyryl, 2-methylbutyryl, octanoyl	King et al. 1987
S. hirsutum (Dunal) Macbride sub nom. *Lycopersicon hirsutum* Dunal	Leaf	2,3,4 and 2,3,4,1′	2-Methylbutyryl, 3-methylbutyryl, isoundecanoyl, dodecanoyl	King et al. 1990

(Caryophyllaceae) (Peterson et al. 1998). Recently, further examples have been discovered in species of the Asteraceae, Cannaceae. and Polygalaceae (Maldonado et al. 2006 and references therein).

Biochemical Ecology. Sucrose esters were shown to possess properties affecting tobacco aphids, *Myzus nicotianae* BLACKMAN, Homoptera: Aphididae, in several ways, e.g., (i) influencing the acceptance or rejection of plants for colonization by alate migrant aphids, (ii) survival and fecundity of alate and apterous aphids (Severson et al. 1994). The LC_{50} value for a fraction containing 6-*O*-acetyl-2,3,4-tri-*O*-acylsucrose (acyl: C_3–C_7) was determined to be 0.25 µg. The same fraction as well as a 2,3,4-tri-*O*-acyl-3′-*O*-acetyl fraction, both obtained from *N. glutinosa*, stimulated tobacco budworm moths, *Heliothis virescens* FABRICIUS (Lepidoptera: Noctuidae), to oviposit on a tobacco budworm-resistant accession, when sprayed onto a leaf avoid of them (Jackson et al. 1991). Thus, these metabolites are contact ovipositional stimulants (Severson et al. 1991). Furthermore, molluscidal and antibacterial activities have been observed (Moser et al. 1999; Ovenden et al. 2005). Finally, tobacco seed germination and growth inhibiting activities were documented; this was also true for other plants (Matsuzaki et al. 1988).

8.2.1.2 Glucose Esters

Investigations of the type B glandular trichome exudate from numerous non-tuberous *Solanum* spp., wild *Nicotiana* spp., and *Datura metel* revealed the presence of 2,3-di-*O*- and/or 1,2,3-tri-*O*-acylated glucoses (King and Calhoun 1988). The principal esters of the latter species were identified as the respective di- and trihexanoyl derivatives. In general the same acyl components may occur as in the sucrose esters. A mixture of glucose esters from *Solanum pennellii* CORRELL sub nom. *Lycopersicon pennellii* (CORREL) D'ARCY was found to act as insect feeding deterrent (Steffens and Walters 1991). For further examples of glucose esters see King et al. 1993.

8.2.2 Occurrence in the Convolvulaceae

8.2.2.1 Aminoacyl Sugars

Recently a series of seven unknown aminoacyl sucrose derivatives has been discovered in the polar extracts from the tubers of *Ipomoea batatas* (L.) LAM., sweet potato (Dini et al. 2006). They share an acylation at C-2 of the glucose moiety. Their respective aminoacyl residue is represented by seven L-amino acids (glycine, alanine, valine, threonine, tyrosine, tryptophan, and histidine). Since these metabolites possess the structural prerequisite for sweetness the authors claim that they may widen the availability of natural sweeteners.

8.3 Resin Glycosides (Glycoresins)

8.3.1 Discovery and Structural Elucidation

8.3.1.1 Discovery and General Remarks

Resin glycosides are characteristic constituents of complex resins. They represent unique metabolites in the plant kingdom confined to the Convolvulaceae. Furthermore, they may be considered as the most specific compounds of the convolvulaceous secondary metabolism, since they occur frequently in this family and show a broad distribution. The history of their discovery was influenced mainly by the fact that they show intensive pharmacological effects, especially as drastic purgative remedies. Thus, such convolvulaceous crude drugs were used medicinally since ancient times worldwide. *Calystegia sepium* (L.) R.Br. (hedge bindweed), *Convolvulus arvensis* L. (field bindweed, possession vine), both common herbs of the temperate parts of the world, and *C. scammonia* L. (scammony), a herb of the countries of the Eastern Mediterranean, yielded resins applied in Europe and the Near East. *Operculina turpethum* (L.) Silva Manso, a paleotropical perennial herb, served as a source in India. Resins from several *Ipomoea* spp. and two species belonging to the genera *Merremia* and *Operculina*, respectively, traditionally were used in the ethnobotany of neotropical countries (Mannich and Schumann 1938; Jaretzky and Risse 1940a). Most of such cathartic crude drugs are derived from the roots which are especially rich in resin glycosides ("glycoresins"), i.e., 10–18% according to Pereda-Miranda and Bah (2003). However, in principle all parts of the plant may contain such metabolites.

Botanical textbooks have a tendency to point out that the Convolvulaceae in general are characterized by the occurrence of resins, e.g., "usually with lactifers, often with milky sap" (Judd et al. 1999). This tendency is apparently based on old sources. Thus, in his large work in three volumes "Allgemeine medizinisch-pharmazeutische Flora" Kosteletzky (1834) had already devoted himself in detail to this plant family and topic: "…… almost all (species) contain a pungent, purgative milky sap in different amounts in all organs, however especially in the root; therefore they are almost all suitable as remedies."

However, to date reports on structural elucidations of resin glycosides are confined to 34 species belonging to six genera, altogether listed in Table 8.2 together with the respective authorities for the species and the phytochemical references: *Ipomoea* (23 spp.), *Merremia* (3), *Convolvulus* (3), *Operculina* (2) *Cuscuta* (2), *Calystegia* (1). The vast majority of these plants are of tropical origin.

Early phytochemical reports had been published with regard to *Ipomoea purga* sub nom. *Ipomoea Schideana* Zuccar (Cadet de Gassicourt 1817; Kayser 1844) or sub nom. *Convolvulus Schiedeanus* Zuccar (Mayer 1852, 1854, 1855). The terms "jalap tuber/jalap resin" are derived from the original Mexican location (Jalapa) where the plant had been collected (exported from the port Vera Cruz which caused the term "Vera Cruz jalap" for the original drugs). Later substitutes of these crude

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family

Convolvulaceae-based trivial names of hydroxy fatty acids (HFA):

- Further hydroxy fatty acids lacking such trivial names are listed in the corresponding column of the table -

Braziliolic acid = possibly a trihydroxymyristic acid

Convolvulinolic acid = (11*S*)-hydroxytetradecanoic acid = 11-hydroxymyristic acid

Ipolearic acid = (3*S*,11*S*)-dihydroxyhexadecanoic acid = 3,11-dihydroxypalmitic acid

Ipurolic acid = (3*S*,11*S*)-dihydroxytetradecanoic acid = 3,11-dihydroxymyristic acid

Jalapinolic acid = (11*S*)-(+)-hydroxyhexadecanoic acid = 11-hydroxypalmitic acid

Operculinolic acid = 3,12-dihydroxyhexadecanoic acid = 3,12-dihydroxypalmitic acid

Turpetholic acid A = 3,12-dihydroxypentadecanoic acid

Turpetholic acid B = 4,12-dihydroxypentadecanoic acid

Turpetholic acid C = 4,12-dihydroxyhexadecanoic acid = 4,12-dihydroxypalmitic acid

***Sugars (monosaccharides)*:**

Hexose: glu = β-D-glucose; *methylpentoses*: rha = α-L-rhamnose, fuc = β-D-fucose, qui = β-D-quinovose; *pentose*: xyl = β-D-xylose

Short-chain aliphatic acids: aca = acetic acid; pra = propionic acid; nba = *n*-butyric acid; iba = isobutyric acid (2-methylpropionic acid); mba = (2*S*)-methyl-butyric acid; 3-mba = 3-methyl-butyric acid; hmba = 3-hydroxy-2-methyl-butyric acid (nilic acid; stereochemistry undetermined) / (−)-hmba = 2*R*,3*R* / (+)-hmba = 2*S*,3S; nva = *n*-valeric acid; iva = isovaleric acid; taa = trimethylacetic acid; cra = crotonic acid; tga = tiglic acid

Saturated straight-chain fatty acids: nha = *n*-hexanoic acid (caproic acid), noa = *n*-octanoic acid (caprylic acid), nda = *n*-decanoic acid (capric acid), 7-hda = 7-hydroxydecanoic acid, ndoa = *n*-dodecanoic acid (lauric acid); nhda = *n*-hexadecanoic acid (palmitic acid), noda = *n*-octadecanoic acid (stearic acid), neia = *n*-eicosanoic acid (arachidic acid)

Arylalkyl acids: cna = *trans*-cinnamic acid

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids** (*GA*)[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)**[b]	**Acids originally linked to sugars**[a]	**References**
Cuscuteae						
Cuscuta australis R.Br.	Seed	**EISP**: mixture of glycosidic ester-type oligomers (up to heptamers) with a core consisting of cuscutic acids $\mathbf{A_1}$ – $\mathbf{A_3}$	*GA*: cuscutic acids $\mathbf{A_1}$ – $\mathbf{A_3}$; *HFA* of $\mathbf{A_1}$: jalapinolic acid; *HFA* of $\mathbf{A_2}$, $\mathbf{A_3}$: convolvulinolic acid	$\mathbf{A_1}$, $\mathbf{A_2}$: glu, rha, fuc (1:1:1); $\mathbf{A_3}$: glu, rha (2:1)	**EISP**: aca, iba, mba, (−)-hmba, tga	Du et al. 1999

(continued)

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids** (*GA*)[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)[b]**	**Acids originally linked to sugars[a]**	**References**
C. chinensis LAM.	Seed (Chinese Pharmacopoeia: Cuscutae semen)	**EISP**: 2 acylated trisaccharides (cus-**1**, cus-**2**) together with a complex mixture of resin glycoside-like compounds; **ESP**: cuscutic resinoside A	**EISP**-*GA*: cuscutic acids **A – D;** *HFA* of **A – C** and cus-**1**: convolvulinolic acid; *HFA* of **D,** cus-**2**: jalapinolic acid; **ESP**-*GA*: unnamed; *HFA*: convolvulinolic acid	**EISP**: **A, D**: glu, rha (2:2), **B**: glu, rha, xyl (1:2:1), **C**: glu, rha, qui (1:2:1); cus-**1**, cus-**2**: glu, rha (1:2); **ESP**: 2x rha	**EISP** (in total): aca, pra, mba, (−)-hmba, tga; cus-**1**, cus-**2**: aca, (−)-hmba; **ESP:** (−)-hmba	Miyahara et al. 1996, Du et al. 1998; Umehara et al. 2004
Convolvuleae						
Calystegia soldanella (L.) ROEM. & SCHULT.	Root	**$CHCl_3$-SP**: soldanelline **A**, **B**	*GA*: soldanellic acids **A**, **B**; *HFA*: jalapinolic acid	**A**: glu, rha (4:1); **B**: glu, rha, qui (2:1:1)	**A**, **B**: mba, hmba[c], tga (1:1:1)	Gaspar 1999, 2001
Convolvulus al-sirensis HAMDAZIL	Not reported	**Ethanol-SP**	*HFA of main GA*: jalapinolic acid	glu, rha (3:1)	Not reported	Wagner et al. 1983
C. microphyllus SIEBER. ex SPRENG.	Aerial parts	**ESP**	*GA*: microphyllic acid; *HFA*: jalapinolic acid	glu, rha, fuc (1:3:1)	aca, pra, iba, nva, iva, tga	Wagner and Schwarting 1977

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids** (*GA*)[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)[b]**	**Acids originally linked to sugars[a]**	**References**
C. scammonia L.	Root (Scammoniae radix = scammony root)	Scammony resin (Scammoniae resina); **ESP**: Scammonins **I – VIII**; **EISP**: glycosides unnamed	**ESP**-*GA*: **I – VI**: scammonic acid **A**; **VII**: orizabic acid **A**; **VIII**: scammonic acid **B**; *HFA* of **I – VIII**: jalapinolic acid; **EISP**-*GA*: unnamed; *HFA*: ipurolic acid, convolvulinolic acid	**ESP / I – VI**: glu, rha, qui (1:1:2); **VII**: glu, rha, fuc, qui (1:1:1:1); **VIII**: glu, rha, qui (2:1:1) **EISP**: glu, rha, fuc	**ESP – I**: mba, tga (2x); **II, VII, VIII**: mba, tga; **III**: iba, tga; **IV**: tga (2x); **V**: iba; **VI**: tga **EISP**: aca, pra, iba, mba, nva, iva, tga	**EISP**: Shellard 1961c and references therein; **ESP**: Spirgatis 1858; Power and Rogerson 1912b; Bauer and Junge 1934; Noda et al. 1990, 1992a; Kogetsu et al. 1991
Merremia hungaiensis Lingelish. & Borza	Root	**ESP**: tuguajalapins **I – X** [groups of positional isomers: (**I – III**), (**IV – VI**), (**VII, VIII), (IX, X**)]; merremin = ester type dimer of **X**	*GA* of **I – X**: operculinic acid **A**; *HFA*: jalapinolic acid; *GA* of merremin: 2x operculinic acid A; *HFA*: 2x jalapinolic acid	**I – X**: glu, rha, fuc (1:3:1); merremin: glu, rha, fuc (2:6:2)	**I – III**: 3x nhda; **IV – VI**: nhda, noda (2:1); **VII, VIII**: nhda, neia (2:1); **IX, X**: 2x nhda; merremin: 4x nhda	Noda et al. 1994b, 1995
M. mammosa (Lour.) Hallier f.	Fresh tuber	**$CHCl_3$-SP of methanol extract**: merremosides **a – g**, **h_1**, **h_2**, mammosides **A**, **B**, **H_1**, **H_2**	*GA* of **a – g**: merremoside **i**; of **h_1**, **h_2**: merremoside **j**; *GA* of **A**, **B**: mammoside **I; H_1**, **H_2**: mammoside **J**; *common HFA*: jalapinolic acid	**a – g**: rha (4x), **h_1**, **h_2**: glu, rha (1:4); **A, B:** rha, fuc (3:1); **H_1**, **H_2**: glu, rha, fuc (1:3:1)	**b, d, e, g**: 2x iba; **a**: 2x mba; **c, f**: iba, mba; **B, H_2**: 2x iba; **H_1**: iba, mba; **A**: 2x mba	Kitagawa et al. 1988, 1989a, b, 1996a, b; 1997; Shibuya et al. 1989

(continued)

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids** (*GA*)[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)**[b]	**Acids originally linked to sugars**[a]	**References**
M. tuberosa (L.) RENDLE, also sub nom. ***Ipomoea tuberosa*** L. (woodrose)	Tuber (Jalapae brasiliensis tuber; see also *Operculina macrocarpa*)	Brazilian jalap (see also *Operculina macrocarpa*) **EISP**: woodrosins **I**, **II**	*GA*: woodrosinic acid **A**; *HFA*: jalapinolic acid	**I**, **II**: glu, rha (4:1)	**I**: 4x mba; **II**: iba, 3x mba	Shellard 1961b; Ono et al. 1993b
Operculina macrocarpa (L.) URBAN sub nom. *Ipomoea operculata* MART. & SPIX	Tuber (Jalapae brasiliensis tuber; see also *Merremia tuberosa*)	Brazilian jalap resin (Jalapae brasiliensis resina); **EISP**: "Brazilian convolvulin" (50%); **ESP**: "Brazilian jalapin" (5%): operculins **I – XVIII**	**EISP**: *Major GA*: "rhamnoconvolvulinic acid C *Graf*" (= "operculinic acid *Wagner*"), corresponding *HFA*: operculinolic acid (12-*O*-hexasaccharide); *minor HFA*: "braziliolic acid *Shellard*" (possibly a trihydroxymyristic acid) **ESP** / *GA* of operculins **I**, **II**, **V**, **VII**, **VIII**, **XIII – XV**: operculinic acid **A**; **III**, **IV**; **IX**, **X**, **XVI – XVIII:** operculinic acid **B; VI, XI, XII**: operculinic acid **C;** operculinic acids **D – F**: corresponding glycosides not yet identified (**G**: artefact of **A**); common *HFA* of operculinic acids **A – F:** jalapinolic acid	**EISP**: glu, rha (4:2) **ESP:** *operculinic acids* **A:** glu, rha, fuc (1:3:1); **B:** glu, rha (2:3); **C**: rha, fuc (3:1); **D**: glu, rha, xyl (1:3:1); **E**: glu, rha (1:3); **F**: rha, xyl (3:1)	**EISP**: aca, pra, mba, taa, nva, iva, tga, **ESP**: nda (**VII – X**, **XIV**, **XVII**), 2x nda (**II**, **IV**), ndoa (**VII – X**; **XIII**, **XV**, **XVI**, **XVIII**), 2x ndoa (**I**, **III, V, VI, XI, XII**)	Mannich and Schumann 1938[d]; Auterhoff and Demleitner 1955; **EISP**: Shellard 1961b; Graf et al. 1965; Graf and Bühle 1974a, b; Wagner and Kazmaier 1977 and references therein; **ESP**: Ono et al. 1989a, b, 1990b, c, 1991, 1992a

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids** (*GA*)[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)**[b]	**Acids originally linked to sugars**[a]	**References**
O. turpethum (L.) SILVA MANSO sub nom. *Ipomoea turpethum* (L.) R.BR.	Root	Turpethi resin (Turpethi resina), "Indian Jalap"; **ESP**; **EISP**; **Ethanol-SP**	**ESP**: *HFA*: jalapinolic acid. **Ethanol-SP**: *GA*: turpethinic acids **A – E**; *HFA* of **A**: turpetholic acid **A**; *HFA* of **B**: turpetholic acid **B**; *HFA* of **C**: turpetholic acid **C**; *HFA* of **D**: operculinolic acid; *HFA* of **E**: jalapinolic acid	**ESP** and **EISP**: glu, rha; **Ethanol-SP:** glu, rha (3:1)	**ESP** and **EISP**: nva, iva, tga; **Ethanol-SP**: pra, nba, iba, mba, hmba, nva, iva, tga, nda	**ESP** and **EISP**: Auterhoff and Demleitner 1955 and references therein; **Ethanol:** Wagner et al. 1978
Ipomoeeae						
Ipomoea arborescens HUMB. & BONPL. (section *Eriospermum*)	Root	**$CHCl_3$-SP**: arboresins **1 – 6**, murucins **1 – 9**	*GA of arboresins*: arboresinic acid; *GA of murucins*: murucinic acid; *common HFA*: jalapinolic acid	*Arboresins*: glu, rha (2:3); *murucins*: glu, rha, qui (1:3:1)	*Arboresins*: aca (**2**), pra (**3**), nba (**4**), mba (**5**), tga (**6**), hmba (**1 – 6**), ndoa (**1 – 6**); *murucins*: aca (**1, 6**), pra (**2**), nba (**3**), mba (**4**), hmba (**5, 6 – 8**), cra (**7**), tga (**8**), ndoa (**1 – 9**)	León et al. 2006

(continued)

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids** (*GA*)[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)**[b]	**Acids originally linked to sugars**[a]	**References**
I. bahiensis WILLD. ex ROEM. & SCHULT. (section *Eriospermum*)	Leaf	**Methanol-SP**: 4 glycosides **1a**, **1b**, **2a**, **2b**	*HFA* of **1a, 2a**: jalapinolic acid; *HFA of* **1b, 2b**: convolvulinolic acid	**1a**, **1b**, **2a**, **2b**: glu, rha, fuc (1:1:1)	**1a**, **1b**: hmba, tga; **2a**, **2b**: tga	Bieber et al. 1986
I. batatas (L.) LAM. cv. Simon (sweet potato) (section *Eriospermum*)	Tuber	**ESP**: simonins **I – V** [main component (48%): **IV**]	*GA* of **I**: operculinic acid **C**; *GA* of **II**: simonic acid **A**; *GA* of **III – V**: simonic acid **B**; common *HFA* of **I – V**: jalapinolic acid	**I**: rha, fuc (3:1); **II**: glu, rha (1:4); **III – V**: rha, fuc (4:1)	mba (**II**, **III**), noa (**V**), nda (**IV**), 2x nda (**I**), ndoa (**II – V**), cna (**I**)	Noda et al. 1992b; Pereda-Miranda and Bah 2003
I. carnea JACQ. **ssp.** ***fistulosa*** (CHOISY) D.F.AUSTIN sub nom. ***I. fistulosa*** MART. ex CHOISY (section *Eriospermum*)	Leaf	**Methanol-SP**: 4 glycosides **A – D**	*HFA* of **A**: jalapinolic acid; *HFA* of **B**: convolvulinolic acid; *HFA* of **C**: 7-hydroxydecanoic acid; *HFA* of **D**: ipurolic acid	**[A – D]**: glu, fuc, [qui + rha] (1.04:1:0.9)	**[A – D]**: aca, nba, iba, nva, iva, tga	Legler 1964

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids** (*GA*)[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)**[b]	**Acids originally linked to sugars**[a]	**References**
I. imperati (VAHL) GRISEB. sub nom. *I. stolonifera* (CYRILL) J.F.GMEL. (section *Erpipomoea*)	Whole plant	**ESP**: stoloniferins **I – XII**	*GA* of **I – III**, **VIII – X**: simonic acid **B**; *GA* of **IV –VII**: operculinic acid **A**; *GA* of **XI**, **XII**: operculinic acid **C**; *HFA* of **I – XII**: jalapinolic acid	**I – III**, **VIII – X**: rha, fuc (4:1); **IV – VII**: glu, rha, fuc (1:3:1); **XI**, **XII**: rha, fuc (3:1)	**III – V, VIII – XII**: mba; **I**: 2x mba; **II**: iba; **VI**: nha; **VII, VIII**: noa; **II – VII**, **IX, XI, XII**: nda; **X**: ndoa	Noda et al. 1994a, 1998
I. indica (BURM. f.) MERR. sub nom. ***I. learii*** PAXT. (section *Pharbitis*)	Whole plant	**Ethanol-SP**	*GA*: ipolearoside; *HFA*: ipolearic acid (11-*O*-tetrasaccharide)	glu, rha, fuc (1:2:1)	iba, mba, nha, noa, nda, ndoa	Sarin et al. 1973
I. lacunosa L. (section *Eriospermum*)	Root	**Petroleum ether-SP**	*Main HFA*: jalapinolic acid	glu, rha (2:5)	5 short-chain aliphatic acids	Wagner et al. 1983
I. leptophylla TORR. (section *Eriospermum*)	Leaf and stem	Leptophyllins **A**, **B**	*GA* of **A**, **B**: operculinic acid **A**; *HFA*: jalapinolic acid	**A**, **B**: glu, rha, fuc (1:3:1)	**A**, **B**: pra, ndoa; **A**: cna	Barnes et al. 2003
I. lonchophylla *J.* BLACK (section *Erpipomoea*)	Aerial parts	**Methanol-SP**: inseparable mixture of glycosides	*HFA of major GA*: ipurolic acid (11-*O*-hexasaccharides); *HFA of a minor GA*: ipolearic acid	*Major GA*: glu, rha, fuc, qui (2:1:1:2)	Differing numbers of not specified C_5 acids	MacLeod et al. 1997

(continued)

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids** (*GA*)[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)**[b]	**Acids originally linked to sugars**[a]	**References**
I.* × *multifida (Rafin.) Shinn. sub nom. ***Quamoclit* × *multifida*** Rafin. (section *Mina*)	Seed	**ESP**: multifidins **I**, **II**; quamoclins **I – IV**	*GA of multifidins* **I, II**: multifidinic acid **A**; *GA of quamoclins* **I – III**: quamoclinic acid A; *HFA of both groups*: convolvulinolic acid; **IV**: operculinic acid **A**; *HFA*: jalapinolic acid[e]	*Multifidins* **I**, **II**: glu, rha, qui (1:3:1); *quamoclins* **I –IV**: glu, rha, fuc (1:3:1)	*Multifidins*: mba (**I**, **II**), nda (**I**), ndoa (**II**); *quamoclins*: mba (**I – IV**), nda (**III**), ndoa (**I**, **II**, **IV**)	Ono et al. 1997
I. murucoides Roem & Schult. (section *Eriospermum*)	Root; flower	*Root* (CH_2Cl_2): murucins **1 – 5**; *flower* ($CHCl_3$): murucoidins **I – V**, stoloniferin **I**	*GA of murucins*: murucinic acid; *GA of murucoidins*: simonic acid **B** (**I – III**), operculinic acid **A** (**IV**, **V**); *GA of stoloniferin* **I**: simonic acid **B**; common *HFA*: jalapinolic acid	*Murucins*: glu, rha, qui (1:3:1); *murucoidins* **I – III,** *stoloniferin* **I**: rha, fuc (4:1); **IV**, **V**: glu, rha, fuc (1:3:1)	Murucins: aca (**1**), pra (**2**), nba (**3**), mba (**4**), hmba (**5**), ndoa (**1 – 5**); *murucoidins*: mba (**I**), iba / mba (**II**), 2x mba (**III – V**); *stoloniferin* **I**: 2x mba	León et al. 2005; Cherigo and Pereda-Miranda 2006
I. nil (L.) Roth sub nom. ***Pharbitis nil*** Choisy (section *Pharbitis*)	Seed	**Ethanol-SP**: "pharbitin"	*GA*: "pharbitic acid" = mixture of 2 major (pharbitic acids **C**, **D**) and two minor constituents (**A**, **B**); *HFA* of **C** and **D**: ipurolic acid (11-*O*-oligosaccharides); *HFA* of **B**: ipolearic acid (11-*O*-oligosaccharide)	Pharbitic acids **B**, **D**: glu, rha, qui (2:3:1); **C**: glu, rha, qui (2:2:1)	"Pharbitin": mba, (–)-hmba, nva, tga	Asahina and Terada 1919; Asahina and Shimidzu 1922; Kawasaki et al. 1971; Okabe et al. 1971; Okabe and Kawasaki 1972; Ono et al. 1990a

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids** (*GA*)[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)**[b]	**Acids originally linked to sugars**[a]	**References**
I. orizabensis (PELLET.) LED. ex STEUD. (section *Pharbitis*)	Root (Mexican scammony root = Scammoniae mexicanae radix)	Mexican scammonium resin (Scammoniae mexicanae resina), "Ipomoea resin" Shellard; **ESP**: orizabin (= "scammonin Auterhoff") (70%) / scammonins **I** and **II**, orizabins **I – XXI EISP**: "α-scammonin Auterhoff" (4%)	**ESP**-*GA*: orizabins **I – IV**: orizabic acid **A**; scammonins **I, II**, orizabins **V – XXI**: scammonic acid **A**; common *HFA*: jalapinolic acid; **EISP**-*HFA*: ipurolic acid and operculinolic acid	**ESP** and **EISP**: glu, fuc, rha (Shellard)**;** orizabins **I – IV**: glu, rha, fuc, qui (1:1:1:1); scammonins **I, II**, orizabins **V – XXI**: glu, rha, qui (1:1:2)	**ESP** and **EISP**: aca, pra, iba, mba, nva, iva, tga (Shellard); *orizabins*: iba (**II, IV, X – XIII**), mba (**I, V – IX, XIV, XVII**), 2x mba (**XVIII – XXI**), (+)-hmba (**I – IV, XI, XIII, XV, XVII, XIX, XXI**), (–)-hmba (**V – X, XII, XIV, XVI, XVIII, XX**); 2x (+)-hmba (**III**), tga (**I – IV, IX – XVII**)	Mayer 1854, 1855; Samuelson 1884; Power and Rogerson 1912a; Jaretzky and Risse 1940b and references therein; Auterhoff and Demleitner 1955 and references therein; Shellard 1961c; Noda et al. 1987; Hernández-Carlos et al. 1999; Pereda-Miranda and Hernández-Carlos 2002; Pereda-Miranda et al. 2006b

(continued)

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids** (*GA*)[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)**[b]	**Acids originally linked to sugars**[a]	**References**
I. orizabensis (PELLET.) LED. ex STEUD. sub nom. *I. tyrianthina* LINDL.	Root	**CH_2Cl_2-SP**: tyrianthins **1 – 7**; orizabins **I**, **XVIII**; scammonins **I**, **II**; stansins **3**, **5**	Common GA of all glycosides: scammonic acid **A**; *HFA*: jalapinolic acid	glu, rha, qui (1:1:2)	Tyrianthins **4**, **6**: nba; **1**, **6**: mba; **4**, **7**: hmba; **1 – 3**, **5**: 2x hmba; **2 – 4**: tga; *orizabins, scammonins, stansins*: see above	Mirón-López et al. 2007
I. pandurata (L.) G.F.W. MEYER (section *Eriospermum*)	Root	**Petroleum ether-SP**	*HFA of main GA*: jalapinolic acid	glu, rha (1:3)	5 short-chain aliphatic acids	Wagner et al. 1983
I. parasitica (KUNTH.) G.DON (section *Tricolores*)	Seed	4 glycosides	*HFA*: jalapinolic acid	fuc, qui, "a 6-deoxygulose"[f]	mba	Smith et al. 1964
I. pes-caprae L. (section *Erpipomoea*) (beach morning glory, railroad vine, bay hops) (section *Erpipomoea*)	Whole plant	**Hexane-SP**: pescapreins **I – IV**, stoloniferin **III**	*GA* of "pescaproside **E** *Srivastava*", "pescaproside **A** *Pereda-Miranda*" (a GA methyl ester), pescapreins **I – IV**, stoloniferin **III**: simonic acid **B**; *HFA*: jalapinolic acid	"Pescaproside **E** *Srivastava*", "pescaproside **A** *Pereda-Miranda*", **I – IV**, stoloniferin **III**: rha, fuc (4:1)	"Pescaproside **E** *Srivastava*": mba, ndoa; *pescapreins* **I – IV**: ndoa; **II**: mpa; **III**: mba; **IV**: nha; (**I**: no 2. acid); *stoloniferin* **III**: mba, nda	Christensen and Reese 1938; Srivastava et al. 1991; Pereda-Miranda et al. 2005

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids** (*GA*)[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)**[b]	**Acids originally linked to sugars**[a]	**References**
I. purga (WENDER.) HAYNE sub nom. *Exogonium purga* (WENDER.) BENTH. (section *Exogonium*)	Tuber (Tubera Jalapae)	Resina Jalapae = Mexican jalap resin (= Vera Cruz jalap resin); **ESP** = *jalapin*; **EISP** = *convolvulin;* **$CHCl_3$-methanol-SP** (afterwards alkaline hydrolysis → purgic acids)	**ESP**-*GA*: jalapinic acid; *HFA*: jalapinolic acid **EISP**-*GA*: complex oligosaccharidic acid with two components: (i) *"convolvulinic acid"* consisting of two *HFA*: ipurolic acid *plus* convolvulinolic acid; (ii) *"purginic acid"* consisting of two *HFA*: ipurolic acid *plus* "a hydroxylauric acid"[g]; purgic acid **A**, *HFA*: convolvulinolic acid; purgic acid **B**, *HFA*: jalapinolic acid	*Jalapinic acid*: glu, rha, fuc; *convolvulinic acid:* glu, rha, fuc, qui; *purginic acid*: rha; *purgic acids **A, B***: glu, rha, fuc, qui (2:1:1:2)	**ESP** and **EISP**: aca, pra, mba, nva, iva, tga[h]	Husemann et al. 1884 and references therein; Hoehnel 1896; Power and Rogerson 1910; Auterhoff and Demleitner 1955; Shellard 1961a; Graf et al. 1965; Singh and Stacey 1973; Pereda-Miranda et al. 2006b
I. purpurea (L.) ROTH, also sub nom. ***Pharbitis purpurea*** VOIGT (section *Pharbitis*)	Seed / aerial parts	**EISP** (seed): ipopurpuroside **ESP** (aerial part): marubajalapins **I – XI** [groups of positional isomers: (**I**, **II**), (**III – V**), (**VII – XI**)]	*HFA*: ipurolic acid, hydroxylauric acid (*Power*); **EISP** (seed) – *HFA*: ricinoleic acid / 12-hydroxystearic acid[i]; **ESP** (aerial part) – *GA* of **I – XI**: operculinic acid **E**; *HFA*: jalapinolic acid	**EISP**: glu, rha, qui; **ESP / I – XI**: glu, rha (1:3)	HCOOH, nba, mba (*Power*); **EISP**: "a steam volatile acid"; **ESP / I, II**: 2x noa; **III – V**: noa, nda; **VI**: 2x nda; **VII – XI**: 3x noa	Power and Rogerson 1909; Nikolin et al. 1978 (seed); Ono et al. 1992c, d (aerial parts)

(continued)

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids (*GA*)[a]** = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)[b]**	**Acids originally linked to sugars[a]**	**References**
I. quamoclit L. (section *Mina*)	Root	**ESP**	Main *GA*: quamoclitic acid; *HFA*: jalapinolic acid	rha, fuc (4:1)	aca, pra, nba, iba, nva, tga	Wagner et al. 1983
I. quamoclit L. sub nom. ***Quamoclit pennata*** (DESR.) BOJER (section *Mina*)	Seed	**ESP**: quamoclins **I – IV**	**I – III**: quamoclinic acid **A**; *HFA*: convolvulinolic acid; **IV**: operculinic acid **A**; *HFA*: jalapinolic acid	**I – IV**: glu, rha, fuc (1:3:1)	**I – IV**: mba; **III**: nda; **I**, **II**, **IV**: ndoa	Ono et al. 1992b
I. simulans HANB. (section *Exogonium*)	Tuber	Tampico jalap resin; **ESP**	**ESP**-*GA*: complex; *HFA*: jalapinolic acid	glu, rha, fuc	aca, pra, mba, iba, iva, tga	Spirgatis 1870; Shellard 1961c
I. squamosa CHOISY (section *Eriospermum*)	Leaf	Ipomoeassins **A – E**	5 *GA*; *HFA* of **A**, **B**: (11*S*)-hydroxy-4-oxo-tetradecanoic acid; *HFA* of **C**,**D**: (5*S*,11*S*)-dihydroxy-4-oxo-tetradecanoic acid; *HFA* of **E**: (5*S*)-acetoxy-(11*S*)-hydroxy-4-oxo-tetradecanoic acid	glu, fuc (1:1)	**A**, **C, D**: sugar-linked aca; **D**, **E**: *HFA*-linked aca (5-*O*-acetyl); **A – E:** tga, cna	Cao et al. 2005

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids (*GA*)**[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)**[b]	**Acids originally linked to sugars**[a]	**References**
I. stans Cav. (section *Pharbitis*)	Root	**Ethyl acetate-SP**: Less polar chromatographic fraction: 4 unnamed resin glycosides; more polar fraction: stansins **1 – 5**; **$CHCl_3$-methanol-SP** (afterwards alkaline hydrolysis → operculinic acid **B**)	Common *GA* of the 4 unnamed resin glycosides and the stansins **1 – 5**: scammonic acid **A**; operculinic acid **B**; *HFA of both GA*: jalapinolic acid	Scammonic acid **A**: glu, rha, qui (1:1:2); operculinic acid **B**: glu, rha (2:3)	Less polar glycosides: iba, mba, 3-mba, hmba; *stansins*: mpa (**1**, **4**), mba (**3**, **4**), 2x mba (**5**), 3-mba (**2**), 2x hmba (**1 – 3**)	Enriquez et al. 1992; Reynolds et al. 1995; León et al. 2004; Pereda-Miranda et al. 2006b
I. tricolor Cav. (section *Tricolores*)	Aerial parts	Monomers: tricolorins **A – G;** ester-type dimers: tricolorins **H – J**	*GA* of tricolorins **A – D**: tricoloric acid **A**; of **E**: tricoloric acid **B**; of **F**: tricoloric acid **C**; of **G:** unnamed; **H** = ester type dimer of **G** and tricoloric acid **C**; **I** and **J** = ester type dimers of **F** and tricoloric acid **C** linked at glu C-3 (**I**) / at C-6 (**J**); common *HFA*: jalapinolic acid	**A – D**: glu, rha, fuc (1:2:1); **E**: glu, rha, qui (1:2:1); **F**: glu, fuc, qui (1:1:1); **G**: glu, rha, fuc (1:1:1); **H**: [glu, fuc, qui (1:1:1)] *plus* [glu, rha, fuc (1:1:1)]; **I**, **J**: 2x [glu, fuc, qui (1:1:1)]	Tricolorins **A**, **D**, **E**: 2x mba; **B**: iba, mba; **C**: mba, (−)-hmba; **H**: mba; **F, G, I, J**: No short-chain aliphatic acids	Pereda-Miranda et al. 1993; Bah and Pereda-Miranda 1996, 1997; Castelli et al. 2002; Pereda-Miranda and Bah 2003; Pereda-Miranda et al. 2006a

(continued)

Table 8.2 Occurrence of structurally (at least almost completely) elucidated resin glycosides and glycosidic acids, respectively, in the Convolvulaceae family (continued)

Tribes (clades) *Species* ***(highlighted in bold: common ornamentals)***	**Plant organ (term of pharmacopoeias)**	**Solvent (ESP** = ether-soluble portion; **EISP** = ether-insoluble portion): **resins and resin glycosides**	**Glycosidic acids** (*GA*)[a] = hydroxy fatty acids (*HFA*)[b] linked to oligosaccharides	**Monosaccharides (sugars)**[b]	**Acids originally linked to sugars**[a]	**References**
I. turbinata LAG. sub nom. *I. muricata* (L.) JACQ. (section *Calonyction*)	Seed	**ESP**: muricatins **I – VIII**	*GA* of **I – V, VII**: muricatic acid **A**; **VI**: muricatic acid **B**; **VIII**: muricatic acid **C**; *HFA* of **I – VIII**: jalapinolic acid[j, k]	**I – V, VII**: rha, fuc, qui (1:1:2); **VI**: rha, qui (1:3, straight-chain); **VIII**: rha, qui (1:3, branched)	**I, III**: 2x mba; **II, IV**: iba, mba; **V, VI**: mba; **VII**: mba, (−)-hmba; **VIII**: no acid	Misra and Tewari 1952, 1953; Khanna and Gupta 1967; Noda et al. 1988a,b,c

[a] Yielded on alkaline hydrolysis of the corresponding glycoside

[b] Yielded on acid hydrolysis of the glycosidic acid

[c] Determined to be the unusual 2*S*,3*R* isomer based only on optical rotation

[d] In the original report of Mannich and Schumann (1938) the corresponding results on "convolvulin" had been assigned erroneously to Mexican jalap (*I. purga*) which was corrected by Shellard (1961b) and Graf et al. (1965); see also footnote[g]

[e] In addition multifidinic acid B was characterized on alkaline hydrolysis of the resin; however this glycosidic acid was not assigned to a certain resin glycoside; difference multifidinic acid B vs. A: Jalapinolic acid as *HFA* instead of convolvulinolic acid; i.e., the pentasaccharide is the same [glu, rha, qui (1:3:1)]

[f] Tentatively identified (see text)

[g] The reported determination of the structure of "convolvulinolic acid" from (supposed) *Mexican* (Vera Cruz) jalap as 3,12-dihydroxypalmitic acid by Votocek and Prelog (1929) and confirmed by Mannich and Schumann (1938) as well as by Auterhoff and Demleitner (1955) was shown by Shellard (1961b) to be an error: The former authors apparently had checked resin of *Brazilian* jalap instead resin of *Mexican* jalap due to confusion (basis: purchased resins instead of genuine tubers which would have been distinguishable without problems); see also footnote[d]

[h] Exogonic acid, discovered in "convolvulin" of *supposed* Mexican jalap by Mannich and Schumann (1938) and therefore named according to *Exogonium purga*, the invalid former synonym of *Ipomoea purga*, is *not* a constituent of this species but of *Operculina macrocarpa* (sub nom. *I. operculata*), ergo of Brazilian jalap (Shellard 1961b; Graf et al. 1965); see also footnotes [d] and [g]

[i] Identified only by TLC comparison

[j] "Muricatin B" identified by Khanna and Gupta (1967) is supposed to be a mixture of muricatic acids A and B or either of them (Noda et al. 1988a); "muricatin A" originally characterized by Misra and Tewari (1952, 1953) turned out to afford "muricatin B" together with *n*-caproic, palmitic, and stearic acids

[k] The original determination of the absolute configuration at C-11 of jalapinolic acid (*R*) was revised later to *S* (Shibuya et al. 1989; Ono et al. 1989a)

drugs, however harvested from other species, were also called "jalap" with the individual addition of their origin like Brazilian jalap, Tampico jalap, Indian jalap. The necessity of such substitutes was given due to an increasing lack of the original drug since there was a great need for the world market. Early reports were also published with regard to *I. orizabensis*, named after the Mexican town of Orizaba (Johnston 1840; Kayser 1844; Mayer 1854, 1855). Moreover, this is also true for *Convolvulus scammonia* (Spirgatis 1860). Mexican jalap, syn.: Vera Cruz jalap (*I. purga,* further syn.: *Exogonium purga*), Mexican scammony (*I. orizabensis*), and scammony (*C. scammonia*) together with Brazilian jalap [*Operculina macrocarpa* (sub nom. *I. operculata*) or alternatively, i.e., another source, *Merremia tuberosa* (sub nom. *I. tuberosa*)], represent the most important resin glycosides-containing crude drugs of the past two centuries. Therefore they were studied most intensively over decades. Nevertheless, due to the limited methodical possibilities and the highly complex chemistry of resin glycosides results obtained in the nineteenth century were not very elucidating, sometimes contradictory, and often erroneous (Husemann et al. 1884 compared with more recent results). "Many (of the earliest workers who investigated the nature of Vera Cruz jalap resin) considered the ether insoluble and ether soluble fractions to be definite chemical compounds, often ascribing definite molecular formulae to them" (Shellard 1961a). In this connection a report of Power and Rogerson (1910) on the chemical examination of jalap seems to be remarkable. These authors recommended that "…. all so-called active principles be dismissed from chemical literature as useless" due to the fact that "….. no one isolated substance can represent the purgative properties thereof."

Data given in Table 8.2 represent the correct present status of knowledge (see also its footnotes [d], [g], and [h], respectively). This is also true regarding the validity of the botanical terms of the species. As their respective authorities also are listed in that table such species will be cited in the following text without authorities.

Even during the first six decades of the past century several reports have to be regarded with caution. Shellard (1961a) elucidated commendably apparent errors with regard to the confusion of Mexican (Vera Cruz) jalap resin and Brazilian jalap resin in the results obtained by Votoček and Prelog (1929), Mannich and Schumann (1938), and Auterhoff and Demleitner (1955). The two genuine drugs (tubers of *Ipomoea purga* and *Operculina macrocarpa* / *Merremia tuberosa*, respectively) cannot be confused if checked by traditional methods of pharmacognosy (e.g., microscopy). However, these errors had been caused due to the fact that the (amorphous) resins used for the investigations had not been produced from such authentic genuine drugs in the corresponding laboratories. Instead commercially available resins had been purchased. On the other hand, Shellard (1961b) emphasized particularly that in his study "…. Brazilian jalap is obtained from the dried sliced tubercles of *Merremia tuberosa* and of *Operculina macrocarpa*." However, his report on three samples of this drug ["obtained commercially and their identity confirmed by comparison with (authentic) samples …… and by microscopic examination" before the preparation of the resins used for the phytochemical investigation] unfortunately did not show any differentiation concerning these two plant species.

Traditional nomenclature is another problem. Mayer (1854, 1855) termed the resin obtained from *I. purga* (sub nom. *C. Schideanus*) "convolvulin" and the one from *I. orizabensis* (sub nom. *C. orizabensis*) "jalapin". Possibly caused by wrong or misunderstood translation of the papers of Mayer (written in German) Shellard (1961a) claimed that "Mayer gave this fraction (ether insoluble portion of *I. purga*) the name convolvulin, reserving the name jalapin for the ether soluble portion." Unfortunately, this misunderstanding was transmitted to *Convolvulus scammonia* (Noda et al. 1990); afterwards the term "convolvulin" was used for ether insoluble fractions of all species in the past decades as well as "jalapin" for ether soluble fractions, at least by some authors, e.g., Ono et al. (1991, 1992b). Nevertheless, these terms have been avoided in Table 8.2 in favour of ESP (ether soluble portion) and EISP (ether insoluble portion) because (i) "convolvulin" / "jalapin" are not used any longer in the last ten years and (ii) different other solvents were used in more recent reports. Therefore the solubility/insolubility in the specific solvents is listed in the second column of the table (in bold). In this connection it is of historical interest that the first phytochemical report on a convolvulaceous resin (jalap) already pointed out that there is an ether soluble as well as an ether insoluble portion (Cadet de Gassicourt 1817).

Alternatively, Ono et al. (1993b) proposed to use the term "jalapin" for resin glycosides having intramolecular cyclic ester structures only and the term "convolvulin" for others, respectively. Again this alternative use of the terms has not been applied in Table 8.2, because to date there would be present almost exclusively "jalapins" sensu Ono. On the other hand just Japanese scientists still used the term "jalapins" regularly in the past nineties for ether-soluble glycosides, sometimes even in trivial names for single, specified compounds, e.g., marubajalapins (Ono et al. 1992c), tuguajalapins (Noda et al. 1994b).

8.3.1.2 Structural Composition

Traditionally, the components of resin glycosides were structurally elucidated by decomposition. Thus, *alkaline* hydrolysis of a resin glycoside causes its cleavage into (a) the corresponding glycosidic acid, a hydroxy fatty acid linked to an oligosaccharide, e.g., cuscutic acid B (top left in Fig. 8.1), pharbitic acid C (right in Fig. 8.2), and (b) short-chain aliphatic acids (vast majority of cases) and/or long-chain fatty acids (however, the latter may lack). *Acid* hydrolysis of a glycosidic acid provides (a) the corresponding hydroxy fatty acid and (b) those monosaccharides as individual compounds which have formed the units of the original oligosaccharide. Thus, resin glycosides are normally composed of (i) oligosaccharides, (ii) hydroxy fatty acids, and (iii) further organic acids. (i) and (ii) are linked to each other forming glycosidic acids which as a consequence of specific cyclization provide macrolactones. These may occasionally already represent final resin glycosides, e.g., muricatin VIII (Noda et al. 1988c), tricolorins F and G (Pereda-Miranda and Bah 2003). However, regularly monosaccharide units of such macrolactones are

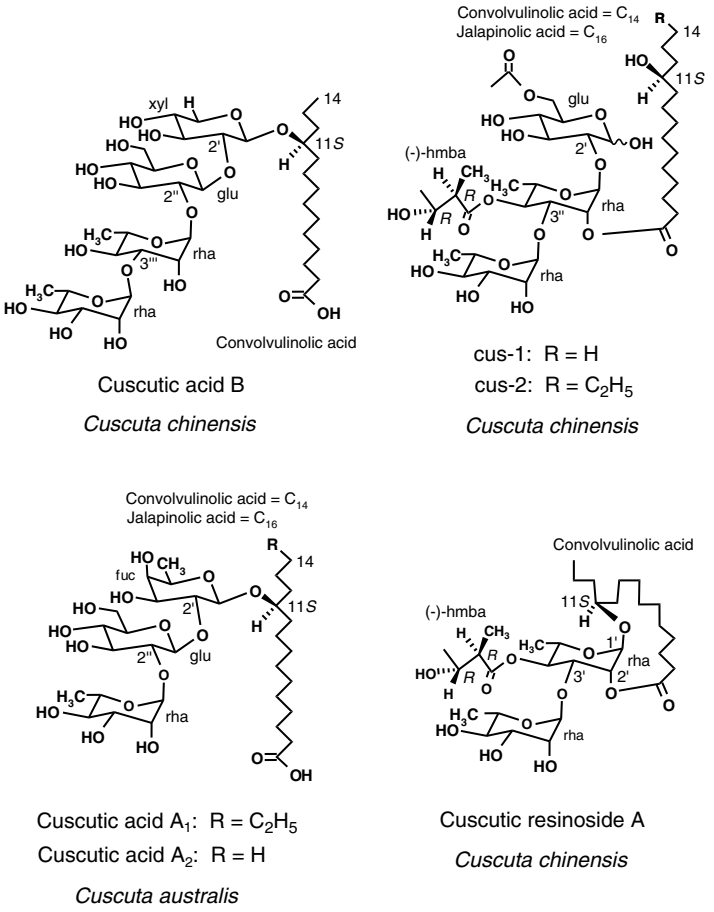


Fig. 8.1 Glycosidic acids (cuscutic acids), unique acylated trisaccharides without cyclization to a macrolactone structurally closely related to resin glycosides (cus-1, cus-2), and a real resin glycoside (cuscutic resinoside A) from the seeds of *Cuscuta* spp. (clearing up of the abbreviations concerning sugar units and acyl residues: see Table 8.2)

additionally acylated by specific acids. But it has to be taken into account that this must not be the correct sequence of the biosynthesis of resin glycosides which is not yet studied. The acylating step at certain positions of specific monosaccharide units may occur already at the stage of oligosaccharides or glycosidic acids. The discovery of cus-1 and cus-2, two acylated trisaccharides conjugated to hydroxy fatty acids surprisingly by reaction with the carboxyl group (structures top right in Fig. 8.1) in contrast to glycosidic acids (e.g., cuscutic acid B, top left) still offers another aspect. Cus-1 and cus-2, respectively, could be isolated from the seeds of *Cuscuta chinensis* without any preceding hydrolyzing procedure (Miyahara et al. 1996), thus representing genuine constituents.

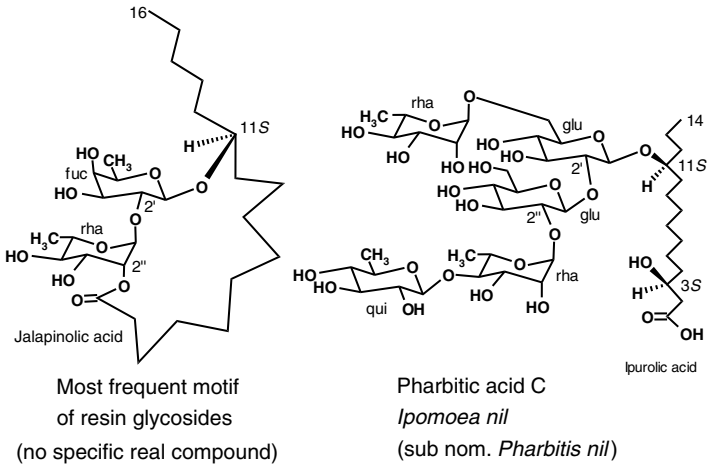


Fig. 8.2 Motif conserved in altogether 41 monomeric glycoresins belonging to 8 different groups of congeners which were discovered in 9 convolvulaceous species (for details see text; *left structure*); pharbitic acid C, provided by alkaline hydrolyzation of "pharbitin", a resin glycoside mixture from the seeds of *Ipomoea nil* (for the unusual linkage of its hydroxy fatty acid to an *inner* monosaccharide unit see text; *right structure*)

Monosaccharides as Residues of Oligosaccharides (obtained by acid hydrolysis of glycosidic acids). By 1844 Kayser was already able to detect "sugar" (he indirectly assigned it to glucose) after passing hydrochloric acid into the ethanolic solution of crude jalap resin. Spirgatis (1858) could find glucose in the acidic hydrolysate of scammony resin. Votoček reported on the identification of the methylpentose "rhodeose" (= fucose) as a structurally integrated component of the complex resin glycoside mixtures "convolvulin" from *Ipomoea purga* (Votoček 1901) and "jalapin" from *I. orizabensis* (Votoček and Vondraček 1903), respectively. Furthermore, he detected these two sugars and discovered a second methylpentose, rhamnose, as moieties of resin glycosides from *Operculina turpethum* sub nom. *Ipomoea turpethum* (Votoček and Kastner 1907). Finally, glucose, "rhodeose" (= fucose), and another methylpentose, "isorhodeose" (= quinovose), could be isolated after cleavage of "convolvulin" from *I. purga* (Votoček 1910). These four sugars later turned out to be the most important monosaccharidic building blocks of resin glycosides in general: D-Glucose and especially L-rhamnose are present in almost every oligosaccharidic moiety; either D-fucose or D-quinovose are also present in at least one resin glycoside of almost all species listed in Table 8.2, sometimes even present together in a specific compound or – more often – both in different compounds of the same species. It may even be assumed that all four monosaccharides are potential components of certain resin glycosides of every species synthesizing such compounds, since especially those species whose phytochemical results do not fit this assumption are not yet well-studied. In contrast to some other classes of glycosides, e.g., triterpenoid saponins, where D-xylose is a frequent monosaccharidic unit, this pentose is extremely rare in the present class.

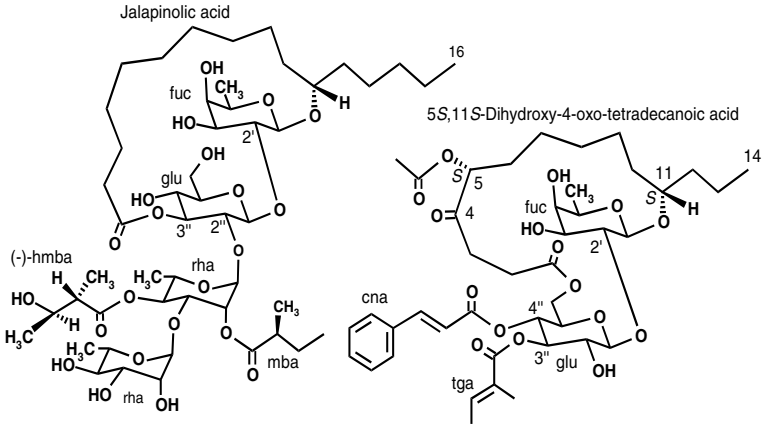
It was only detected in three glycosidic acids, cuscutic acid B from *Cuscuta chinensis* as well as operculinic acids D and F from *Operculina macrocarpa*. Interestingly, – by pure chance or not – to date no resin glycosides corresponding to these three glycosidic acids could be identified.

Oligosaccharides between two and seven monosaccharides could be identified as structural part of resin glycoside monomers, e.g., ipomoeassin E (disaccharide), tricolorin C (tetrasaccharide) (both: Fig. 8.3), murucin 5 (pentasaccharide; Fig. 8.4). Tetra- (48%) and pentasaccharides (41%) are dominating, tri- (6%), di-(3%), and hexasaccharides (2%) play a minor role whereas a heptasaccharide could only be found once.

Though all four frequent monosaccharides – or at least three (i.e., fucose and quinovose alternatively) – may occur together, but only *once* each, in one single specific resin glycoside, there are also many examples, where especially rhamnose but also glucose occur more than once (2 – 5×). Thus, rhamnose is present four times in certain resin glycosides each of, e.g., *Merremia mammosa*, *Ipomoea batatas*; the same situation is given for glucose in, e.g., *Calystegia soldanella*, *Merremia tuberosa*. Even quinovose is present three times in muricatins VI and VIII, isolated from the seeds of *Ipomoea turbinata* (Noda et al. 1988b, c).

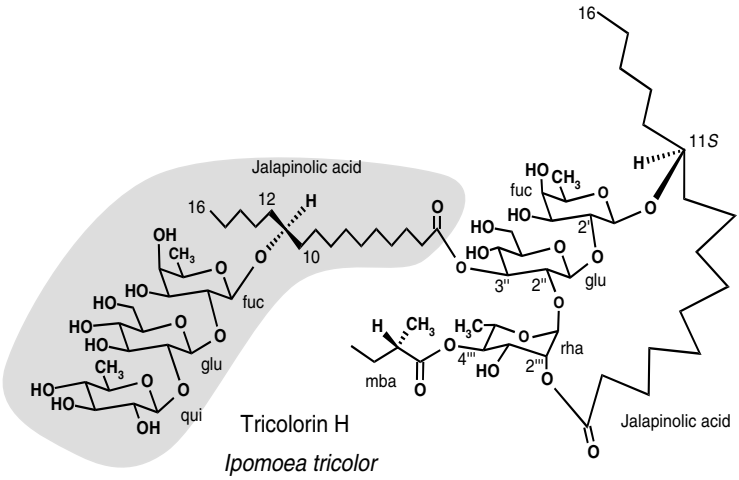
The linkages between the acetalic hydroxyl group of one monosaccharide unit and an alcoholic hydroxyl group of its immediate neighbour unit to form a disaccharide, trisaccharide etc. are principally possible at every hydroxyl of the latter, i.e., 1→2′, 1→3′, 1→4′, or 1→6′ (glucose) and 1→2′, 1→3′, or 1→4′ (methylpentoses, xylose), respectively. Indeed, all these possibilities are realized in resin glycosides or glycosidic acids with regard to rhamnose and quinovose. However, there are no structurally unequivocally elucidated examples known with glucose linked at C-4 in contrast to C-2, C-3, and C-6, respectively. Furthermore, in both remaining cases, fucose and xylose, there are reports only on examples linked at C-2 of these monosaccharides. The two epimeric methylpentoses quinovose and fucose are noticeably often though not generally linked directly to the hydroxyl group of the corresponding hydroxy fatty acid.

Beside the more frequent straight-chain oligosaccharides, e.g., in cuscutic acid B (Fig. 8.1), tricolorin C (Fig. 8.3), murucin 5 (Fig. 8.4), also branched ones occur. The first branched (tetrasaccharidic) macrolactone, muricatin VIII, isolated from the seeds of *Ipomoea turbinata* sub nom. *I. muricata*, was reported by Noda et al. (1988c) containing quinovose and rhamnose as terminal units each, both linked to the second inner quinovose unit (at C″-3 and C″-2, respectively). The corresponding glycosidic acid is called muricatic acid C. In a second example, operculinic acid A, the C-1‴→C-4″ linked inner rhamnose unit is further linked with two alcoholic hydroxyl groups to the terminal rhamnose unit (at C-4‴) and to the terminal glucose unit (at C-3‴), respectively (common glycosidic acid of tuguajalapin VII and operculin V; Fig. 8.5) (Ono et al. 1989a). Operculinic acid A is one out of two branched glycosidic acids which occur in more than one convolvulaceous species (the other: simonic acid B; see Table 8.3). Further examples for branched oligosaccharidic glycosidic acids are quamoclinic acid A (Ono et al. 1992b) and soldanellic acid B (Gaspar 2001).



Tricolorin C *Ipomoea tricolor* Ipomoeassin E *Ipomoea squamosa*

[Tricolorin A: (-)-hmba substituted by mba] [Ipomoeassin D: 4'-O-acetyl derivative of E]



Tricolorin H

Ipomoea tricolor

Fig. 8.3 Resin glycosides from the whole plant of *Ipomoea tricolor* and the leaves of *I. squamosa*, two neotropical vines: The structures of tricolorin C and of ipomoeassin E differ remarkably with regard to (i) the hydroxy fatty acid moieties, (ii) the position of their linkage to the oligosaccharide components, (iii) the number and – in part – the types of monosaccharides involved, and (iv) the other acyl residues. Tricolorin H represents a hetero*dimer* of two trisaccharides, tricoloric acid C (*highlighted in grey*) and an acylated (mba) derivative of the *monomeric* macrolactone tricolorin G (*right*), linked to each other by esterification of the jalapinolic acid moiety of tricoloric acid C at position C-3″ (glu) of the macrolactone part

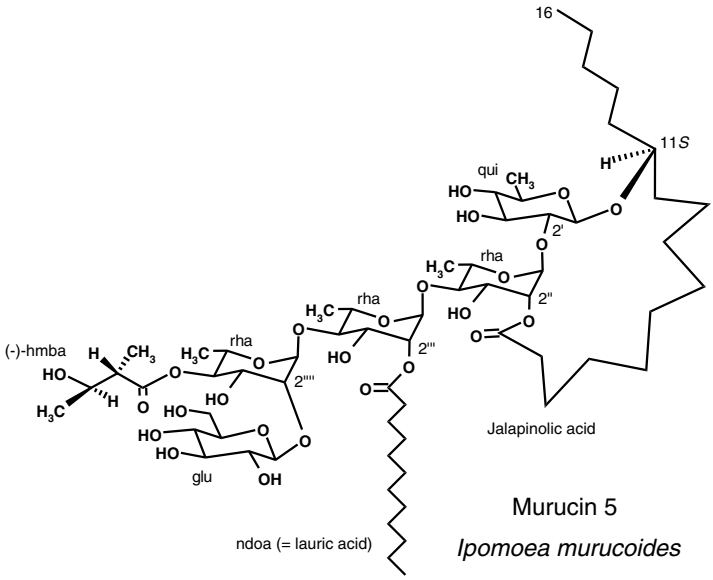
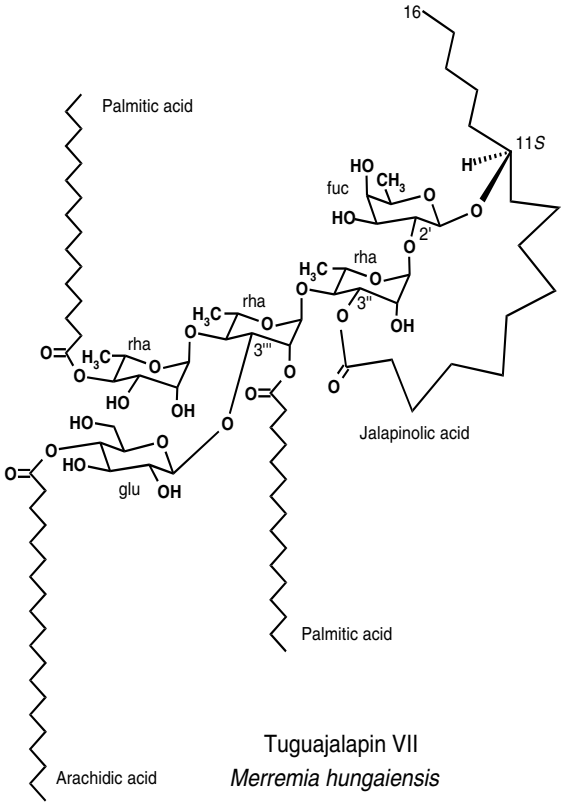


Fig. 8.4 Murucin 5, a resin glycoside from the roots of *Ipomoea murucoides*, an arboreal species growing in Mexico and Guatemala, as an example for a pentasaccharide of jalapinolic acid with a short-chain aliphatic acid residue and a saturated straight-chain fatty acid residue, respectively, linked to two different rhamnose moieties. The difference between this compound and its congeners (murucins 1–4) is given by diverging short-chain aliphatic acid residues at C-4 of the rhamnose moiety directly linked to glucose. The common corresponding (water-soluble) glycosidic acid, afforded by basic hydrolysis of the murucins, is named murucinic acid

A unique linkage is given in case of pharbitic acids B–D, because their hydroxy fatty acids are linked (C-1′→C-11) to an *inner* monosaccharide unit (glucose) (Fig. 8.2) (Ono et al. 1990a). Normally hydroxy fatty acids are linked to *final* monosaccharide units. However, it might be assumed that this unusual structure was caused by a *secondary* linkage of rhamnose to the *originally* final glucose moiety of a *primary* glycosidic acid (e.g., in case of the finally pentasaccharidic pharbitic acid C a postulated tetrasaccharidic precursor).

Hydroxy Fatty Acids (yielded by acid hydrolysis of glycosidic acids). A number of mono- and dihydroxy fatty acids named according to their unique convolvulaceous occurrence are linked with the oligosaccharide moieties of resin glycosides forming macrolactones. The overwhelming majority of such glycosides are characterized by the occurrence of jalapinolic acid which is involved in 31 (out of 35) species. It was discovered as a structural component of "jalapin", i.e., the resin of *Ipomoea orizabensis* sub nom. *Convolvulus orizabensis* (Mayer 1854, 1855); however, the molecular formula reported ($C_{32}H_{30}O_6$) later turned out to be incorrect, perhaps due to uncompleted hydrolyzation. The correct formula ($C_{16}H_{32}O_3$) was published by Samuelson (1884). However, today it is apparent that Spirgatis had been able to characterize



Tuguajalapin VII
Merremia hungaiensis

Operculin V: Both palmitoyl (C_{16}) residues substituted by lauroyl (C_{12})
Arachidoyl (C_{20}) residue substituted by H
Operculina macrocarpa

Fig. 8.5 Tuguajalapin VII, the largest known resin glycoside monomer (MM: 1771; $C_{98}H_{178}O_{26}$) and operculin V as examples for branched pentasaccharides conjugated to jalapinolic acid with saturated straight-long-chain fatty acid residues linked to different positions of different monosaccharide units. Both resin glycosides share a common glycosidic acid, operculinic acid A

this compound including the correct molecular formula on hydrolysis of scammony resin (*Convolvulus scammonia*) and of Tampico jalap resin (*Ipomoea simulans*) already clearly earlier (Spirgatis 1858, 1870, respectively). In the latter case he had named it "tampicolic acid." Many decades later jalapinolic acid was shown to be identical to 11-hydroxyhexadecanoic acid (11-hydroxypalmitic acid; Davies and Adams 1928; Bauer and Junge 1934). The absolute configuration of natural (+)-jalapinolic acid originally thought to be *R* (Noda et al. 1987) finally turned out to be *S* (Shibuya et al. 1989; Ono et al. 1989a). Convolvulinolic acid, named due to its occurrence as a structural component of "convolvulin", i.e., the resin of *I. purga*

Table 8.3 Glycosidic acids as components of resin glycosides occurring in more than one species (references see Table 8.2)

Glycosidic acid	**Oligosaccharide linked to jalapinolic acid (jal)** glu = β-D-glucose; rha = α-L-rhamnose; fuc = β-D-fucose; qui = β-D-quinovose	**Occurrence as component of resin glycosides (species) (discovery in bold)**
Murucinic acid	Straight-chain pentasaccharide: glu-(1→2)-rha-(1→4)-rha-(1→4)-rha-(1→2)-qui-(1→11)-jal; structure: see Fig. 8.4	*Ipomoea arborescens,* ***I. murucoides***
Operculinic acid A	Branched pentasaccharide: glu-(1→3)-[rha-(1→4)]-rha-(1→4)-rha-(1→2)-fuc-(1→11)-jal; structure: see Fig. 8.5	*Merremia hungaiensis,* ***Operculina macrocarpa,*** *I. imperati*, *I.* × *multifida*, *I. murucoides*
Operculinic acid B	Branched pentasaccharide: glu-(1→3)-[rha-(1→4)]-rha-(1→4)-rha-(1→2)-glu-(1→11)-jal	***O. macrocarpa***, *I. stans*
Operculinic acid C	Straight-chain tetrasaccharide: rha-(1→4)-rha-(1→4)-rha-(1→2)-fuc-(1→11)-jal	***O. macrocarpa***, *I. imperati*
Operculinic acid E	Straight-chain tetrasaccharide: rha-(1→4)-rha-(1→4)-rha-(1→2)-glu-(1→11)-jal	***O. macrocarpa***, *I. purpurea*
Orizabic acid A	Straight-chain tetrasaccharide: qui-(1→4)-rha-(1→2)-glu-(1→2)-fuc-(1→11)-jal	*Convolvulus scammonia*, ***I. orizabensis***
Scammonic acid A	Straight-chain tetrasaccharide: qui-(1→4)-rha-(1→2)-glu-(1→2)-qui-(1→11)-jal	***C. scammonia***, *I. orizabensis*, *I. stans*
Scammonic acid B	Straight-chain tetrasaccharide: glu-(1→4)-rha-(1→2)-glu-(1→2)-qui-(1→11)-jal	***C. scammonia***, *I. murucoides*
Simonic acid B	Branched pentasaccharide: rha-(1→3)-[rha-(1→4)]-rha-(1→4)-rha-(1→2)-fuc-(1→11)-jal	*I. batatas* (cv. Simon), *I. imperati, I. murucoides, I. pes-caprae*

(Mayer 1854, 1855), was again published with an incorrect molecular formula. Almost a century later convolvulinic acid was proved to be 11-hydroxytetradecanoic acid (11-hydroxymyristic acid; Kawasaki 1950). A further four decades later its configuration (11*S*) could be determined, again by Kawasaki's group (Ono et al. 1992b). This C_{14}-homologue of jalapinolic acid was detected in resin glycosides of only eight species. It is remarkable that convolvulinolic acid as a component of resin glycosides was found only in such species which – in addition – also contained jalapinolic acid-based glycosides. Some unusual monohydroxy fatty acids forming also macrolactones as moieties of resin glycosides were discovered in certain species: 7-hydroxydecanoic acid (*Ipomoea carnea* ssp. *fistulosa*; Legler 1964), "a hydroxylauric acid" (monohydroxydodecanoic acid; *I. purga*; Shellard 1961a), ricinoleic acid / 12-hydroxystearic acid (*I. purpurea*; Nikolin et al. 1978), (11*S*)-hydroxy-4-oxo-tetradecanoic acid (4-oxo-convolvulinolic acid; *I. squamosa*; Cao et al. 2005).

In contrast to the two dominating monohydroxy fatty acids, their dihydroxy congeners are of secondary importance. Ipurolic acid, its most prominent member, could be detected in only seven species. Named according to its discovery in *Ipomoea purpurea* (Power and Rogerson 1909) this component was structurally elucidated as 3,11-dihydroxymyristic acid (3,11-dihydroxytetradecanoic acid) by Asahina and Shimidzu (1922); the absolute configuration was determined many decades later to be 3*S*,11*S* (Ono et al. 1990a). Jalapinolic acid has *not* been found in only two (*I. lonchophylla*, *I. nil*) of these seven ipurolic acid-positive species (6 *Ipomoea* spp., 1 *Convolvulus* sp.) demonstrating again the dominant role of the former (i.e., monohydroxy) fatty acid. Ipolearic acid, the C_{16}-homologue of ipurolic acid, named according to its discovery in *I. learii* (valid syn.: *I. indica*; Sarin et al. 1973) is even more rare (additional occurrence: *I. lonchophylla*, *I. nil*). Interestingly, *I. indica* and *I. nil* are closely related species, both belonging to section *Pharbitis*. Another rare building block is represented by operculinolic acid, the 3,12-isomer of ipolearic acid. This acid was discovered by Votoček and Prelog (1929) but trivially named only 30 years later by Shellard (1961b) according to its occurrence in *Operculina macrocarpa*. It must be added that Votoček and Prelog believed erroneously to have isolated this compound from the resin of *Ipomoea purga*; however, Shellard was able to prove that it had been the resin from *O. macrocarpa*. Finally, there are also some unique components: Three turpetholic acids (A–C), 3,12- as well as 4,12-dihydroxy fatty acids from *O. turpethum* (Wagner et al. 1978), as well as (5*S*,11*S*)-dihydroxy-4-oxo-tetradecanoic acid and its (5*S*)-*O*-acetyl derivative from *I. squamosa* (Cao et al. 2005). Only one single trihydroxy fatty acid was found as part of a resin glycoside: braziliolic acid, possibly a trihydroxymyristic acid from *O. macrocarpa*, named according to the "Brazilian jalap resin" obtained from this species (Shellard 1961b).

Glycosidic Acids (yielded by alkaline hydrolysis of resin glycosides). A large number of glycosidic acids trivially named according to their convolvulaceous occurrence were discovered over the past 120 years. Even much earlier, Kayser (1844) had found out that the ether-insoluble portion of Mexican jalap resin (*Ipomoea purga*), possessed a glycosidic character and could be decomposed by acid hydrolysis in alcoholic-aqueous solution yielding glucose and "rhodeoretinol". Furthermore, Mayer (1854, 1855) reported the precipitation of glycosidic acids ("convolvulinic acid" and "jalapinic acid", respectively) by hydrolysis of "convolvulin", named after *Convolvulus Schiedeanus*, the synonym for *I. purga* used at that time, and of "jalapin", named after "jalap" though isolated from Mexican scammony ("false jalap"; *I. orizabensis*), i.e., not from authentic jalap (*I. purga*).

To date, 75 different glycosidic acids were identified and characterized structurally in altogether 36 species. This implicates that more than one glycosidic acid may (but must not) occur in the resin of the same species (up to five different ones in *Operculina turpethum*). Remarkably, most of the species apparently produce their own individual, specific glycosidic acids. However, altogether nine glycosidic acids, all of them involving jalapinolic acid as the core hydroxy fatty acid, were found in more than one species (Table 8.3), i.e., 88% of the glycosidic acids seem

to be confined to only one species. This high structural diversity is based on the integration of altogether six aspects: The building blocks, (i) different hydroxy fatty acids (to date at least known: 14) and (ii) different monosaccharides (5), enabling multiple possibilities of structural combination dependent on (iii) the number and (iv) the order of monosaccharides linked to each other forming hetero-oligosaccharides (di- to heptasaccharides), (v) the diverging positions of the linkages, e.g., 1→2, 1→3, including the possibility of straight-chain or branched oligosaccharides as well as (vi) the linkage of the concerning hydroxy fatty acid to them.

Resin Glycosides. The high structural diversity is still increased by the diverging possibilities of cyclization of glycosidic acids to the corresponding macrolactone. The first complete structures of resin glycosides, orizabins I–IV from the roots of *Ipomoea orizabensis* (Mexican scammony root) have been published by Noda et al. (1987). Since that report the complete structure of no less than a further 178 resin glycosides could be elucidated. In addition a large number of others are known at least in part, i.e., especially the corresponding glycosidic acids could be characterized. The last elucidating step from a glycosidic acid to the corresponding resin glycoside is often still missing due to the fact that many studies had been made in the 1960s and 1970s when methodological problems had stopped complete structure elucidations, e.g., pharbitic acids (*I. nil*), ipolearoside (*I. indica*). Though this has changed since the late 1980s, necessary reinvestigations of formerly studied species are still lacking.

Apparently the absolutely dominating positions, at which cyclization by the carboxyl group of glycosidic acids with its open oligosaccharide chain happens, are those which include the monosaccharide unit directly neighboured to the already hydroxy fatty acid-linked monosaccharide unit. Here there are two really existing, almost equivalent possibilities: The reaction with the C-2″-hydroxyl of the second monosaccharide unit forming an 18-membered lactone, e.g., murucin 5 (Fig. 8.4) or at C-3″ forming a 19-membered lactone, e.g., tuguajalapin VII / operculin V (Fig. 8.5). In a few cases this cyclization can occur between the first monosaccharide unit and the third one, thus forming a still larger macrolactone (23- and 24-membered, respectively). There are also reports on very rare alternatives. One example is represented by the so-called woodrosins, discovered in *Merremia tuberosa* and termed according to the plant's common name "woodrose" which had been given due to its large dried woody fruit with calyx resembling a rose. These branched pentasaccharidic resin glycosides show a hydroxy fatty acid cyclization with the first (glucose) and the fourth monosaccharide unit (glucose, at C-2⁗), thus forming an even 27-membered lactone. Another example is known from ipomoeassin E, discovered in *Ipomoea squamosa*, which shows such a cyclization with the first (fucose) and the second monosaccharide unit (glucose) by an unusual acylating linkage at C-6″ (20-membered lactone) (Fig. 8.3). An exotic example is represented by cuscutic resinoside A, provided by the seeds of *Cuscuta chinensis*: its glycosidic acid, convolvulinolic acid, is linked with both functional groups to the same monosacharide unit, rhamnose, at directly neighboured positions (C-2′ and C-3′, respectively). Thus, a 15-membered lactone is formed (Fig. 8.1)

(Umehara et al. 2004), the smallest possible macrocyclic lactone for such compounds. This metabolite has the lowest molecular weight (618 Da; $C_{31}H_{54}O_{12}$) of all known resin glycosides.

Although there is a high degree of structural diversity in the class of resin glycosides there are some motifs which are more or less frequent. Especially the occurrence of a disaccharidic core involving rhamnose-(1→2)-fucose linked to jalapinolic acid forming an 18-membered lactone by conjugation of its hydroxyl to fucose (C-1′→C-11) and acylation of the hydroxyl at C-2″ of rhamnose is worth mentioning (left structure in Fig. 8.2; Fürstner and Müller 1999). This motif represents an important part of the following congeners occurring identically in altogether nine species from the genera *Ipomoea* (7) and *Merremia* (2): leptophyllins A, B; mammosides H_1, H_2; murucoidins I–IV; operculins I, II, VI–VIII, XIII–XV; quamoclins I–IV; simonins III–V; stoloniferins V–X; tuguajalapins II–VIII, X (the quamoclins were found in two species).

So far there was only mention of such glycolipid *mono*mers. They may show a very varying molecular magnitude, as low as e.g., 618 Da (cuscutic resinoside A; see above) or 761 Da (ipomoeassin B, $C_{40}H_{57}O_{14}$; Fig. 8.3) up to 1771 Da (tuguajalapins VII/VIII, $C_{98}H_{178}O_{26}$; Fig. 8.5), the largest known congener. However, beside the monomeric tricolorins A–G recently also hetero*dimeric* congeners, tricolorins H–J, could be isolated from the whole plant of *Ipomoea tricolor* (Pereda-Miranda and Bah 2003). The right monomeric moiety of the heterodimeric tricolorin H (Fig. 8.3) represents a derivative of the monomeric resin glycoside G acylated by (2*S*)-methylbutyric acid at C-4‴ (rhamnose unit). This derivative, the intact macrolactone moiety of the heterodimer, is conjugated to its left part, tricoloric acid C (the glycosidic acid of tricolorin F), by an ester linkage between the carboxyl function of the latter and the alcoholic hydroxyl at C-3″ of the glucose unit of the former. Alternatively the methylbutyryl derivative of tricolorin G is substituted by tricolorin F without such a short-chain acyl residue [difference between F and G: quinovose (F) instead of rhamnose (G) as third monosaccharide unit]. The remaining part of the dimer is identical to H, thus forming the analogous tricolorin I. Finally, a positional isomer of I, named tricolorin J, could be discovered with an alternative ester linkage (hydroxyl of C-6″ of the glucose unit). Thus, alkaline hydrolysis of the tricolorins I and J would provide tricoloric acid C only whereas their congener H would yield (i) tricoloric acid C, (ii) another glycosidic acid (unnamed to date, therefore termed as "D" in Table 8.2) which is characterized by the trisaccharide sequence fucose-glucose-rhamnose, and (iii) (2*S*)-methylbutyric acid.

An analogous situation was discovered in *Merremia hungaiensis*. Beside the monomeric tuguajalapins I–X one *dimeric* congener of X, merremin ($C_{156}H_{280}O_{50}$; molecular weight: 2952 Da), could be characterized (Noda et al. 1995). One monomeric part, its macrolactone-involving, i.e., intact tuguajalapin X moiety, is conjugated to a second, however non-lactonic monomeric part. This part is represented by (i) operculinic acid A (the glycosidic acid of tuguajalapin X), which is (ii) acylated twice by palmitic acid (at C-2‴ of an inner rhamnose unit and C-4 of the terminal one). Both parts are conjugated by an ester linkage between the carboxyl function of the latter and the alcoholic hydroxyl at C-6 of the terminal glucose unit

of the former. (The only difference between tuguajalapin X and its congener VII, which is illustrated structurally in Fig. 8.5: X is lacking the arachidic acid residue at C-4 of the terminal glucose.)

Mannich and Schumann (1938) had hypothesized that "convolvulin" from *Ipomoea purga* sub nom. *Exogonium purga* (in reality these authors had checked *Operculina macrocarpa* instead of *I. purga* as already mentioned above) is characterized by "macromolecules" which are composed by monomeric resin glycosides which in turn are linked by "continued ester-like conjugation …… due to the reaction of one carboxyl group to the hydroxyl of the next one ….." etc. The authors estimated 20 conjugated monomers for such a macromolecule with a molecular weight of 31,018 Da. This hypothesis was supported by the results obtained by Legler (1964) with the resin from the leaves of *Ipomoea carnea* ssp. *fistulosa* sub nom. *I. fistulosa*. He reported that "….. the glycosidic acids are esterified with each other to form a mixture of closely related polyesters with an average molecular weight of about 25,000." Unfortunately, there are no recent papers on both *Ipomoea* species; thus, results obtained by modern methods of structure elucidation are still lacking. Almost all reports of the past thirty years on resin glycosides of other convolvulaceous species are confined to monomers (exceptions: the two reports on dimers mentioned already above). However, there was evidence that ether-insoluble resin glycosides obtained from the seeds of *Ipomoea nil* (sub nom. *Pharbitis nil*) had a molecular weight up to 20,000 Da and probably represent oligomers of acylated glycosidic acids with free carboxylic groups (Ono et al. 1993b). Furthermore, the last two (out of 27) papers of Miyahara's group on these metabolites, with regard to *Cuscuta chinensis* (Du et al. 1998) and *C. australis* (Du et al. 1999), respectively, are again in favour of the occurrence of at least oligomers. They were not able to isolate homogeneous compounds from the ether-insoluble resin glycoside-like fraction but they could characterize new glycosidic acids obtained by alkaline hydrolysis. Thus, in the latter case the authors drew the conclusion from their results that "…. the resin glycoside is considered to be a complex mixture of glycosidic ester-type oligomers (up to heptamers)."

Acids Originally Linked to Oligosaccharide Moieties of Resin Glycosides (yielded by alkaline hydrolysis). The vast majority of the structurally elucidated resin glycosides show acyl residues *O*-linked to certain positions of one or more monosaccharide units. There are three types of acids which may contribute to the structure: (i) short-chain aliphatic acids (C_2–C_5; for the most part volatile in steam), normally saturated, e.g. (2*S*)-methylbutyric acid, except 2-butenoic acid (only found once) and tiglic acid ; (ii) straight-chain saturated fatty acids (not volatile in steam), e.g. *n*-hexanoic acid (caproic acid), *n*-dodecanoic acid (lauric acid); (iii) arylalkyl acids, to date only represented by one example: *trans*-cinnamic acid. Occurring acids are completely listed in Table 8.2.

Certain short-chain aliphatic acids afforded by alkaline hydrolyzation of resin glycosides were discovered already at the turn to the twentieth century, e.g., 2-methyl-butyric acid (Hoehnel 1896), tiglic acid, 3-hydroxy-2-methyl-butyric acid (Kromer 1901). There was no information on such acids in the detailed monograph

"Die Pflanzenstoffe" (plant constituents) by Husemann et al. (1884) supporting the supposition that they were still undiscovered at that time as structural parts of resin glycosides whereas glycosidic acids and sugars were already known as such. The most frequent acyl residues of this type are provided by 2-methyl-butyric acid (detected in 24 out of 34 species) and its 3-hydroxy congeners. In contrast to early reports (up to the 1970s) in more recent reports these acids are often characterized also with regard to their absolute configuration. 2-Methylbutyric acid itself was only found in its *S*-form. 3-Hydroxy-2-methyl-butyric acid, named nilic acid according to its finding in the seeds of *Ipomoea nil* sub nom. *Pharbitis nil* (Asahina and Shimidzu 1922) represents the (−)-form (Kawasaki et al. 1971). Muricatin VII, isolated from the seeds of *Ipomoea turbinata* sub nom. *I. muricata* (Noda et al. 1988c), was the first resin glycoside whose structure was elucidated including the absolute configuration of its niloyl residue [(−)-2*R*,3*R*-form]. This form was also determined later in glycosides from *Cuscuta australis*, *C. chinensis*, *I. nil*, and *I. tricolor*. In some further species nilic acid had not yet been specified stereochemically (*Operculina turpethum*, *I. arborescens*, *I. bahiensis*, *I. murucoides*, *I. stans*). Surprisingly, the co-occurrence of two enantiomeric forms of nilic acid moieties, the already known (−)-2*R*,3*R*-form [= (−)-hmba, partial structure of cus-1/cus-2, top right in Fig. 8.1] as well as its (+)-2*S*,3*S*-antipode, could be proved in resin glycosides from the roots of *Ipomoea orizabensis*: Six pairs of diastereomeric compounds (orizabins IX/X, XI/XII, etc. to XX/XXI) resulting from esterification of the tetrasaccharide core by both forms of the *threo*-nilic acid enantiomers were characterized (Table 8.2; Pereda-Miranda and Hernández-Carlos 2002). Finally, a third isomer, 2*S*,3*R*-nilic acid, i.e., one of the *erythro* forms, was identified in the hydrolysate of the resin glycoside soldanelline B from the roots of *Calystegia soldanella* (Gaspar 2001).

The first long-chain fatty acids, provided by alkaline hydrolyzation of resin glycosides, *n*-decanoic (capric) and *n*-dodecanoic (lauric) acids, were reported to occur in the ether-soluble portion of the resin from the roots of *Ipomoea operculata* (Ono et al. 1989b). Examples for such organic acids as moieties of resin glycosides are structurally illustrated in Fig. 8.4 (murucin 5) and Fig. 8.5 (tuguajalapin VII, operculin V). They were found only in altogether 11 (out of 34) species (*Merremia hungaiensis*, *Operculina macrocarpa*, and 9 *Ipomoea* spp.). In principal, it may be about all even-numbered straight-chain saturated fatty acids between C_6 (*n*-hexanoic acid = caproic acid) and C_{20} (*n*-eicosanoic acid = arachidic acid) with one exception; C_{14} (*n*-tetradecanoic acid = myristic acid) has not yet been found as component of resin glycosides. Moreover, 7-hydroxydecanoic acid (7-hydroxycapric acid) was noticed (*I. carnea* ssp. *fistulosa*).

Simonin I from the tuber of *I. batatas*, ipomoeassins D/E from *I. squamosa* (Fig. 8.3), and leptophyllin A from the leaf/stem of *I. leptophylla* are the rare examples for a *trans*-cinnamoyl residue.

The degree of acylation is often very different and may depend in part on the number of monosaccharide units, e.g., 1 acyl residue/2 monosaccharide units (cuscutic resinoside A, Fig. 8.1), 2 acyl residues/2 monosaccharide units (ipomoeassin E, Fig. 8.3), 3/2 (ipomoeassin D, Fig. 8.3), 2/4 (scammonins, stoloniferin XI, tri-

colorin A/C, Fig. 8.3) 3/4 (simonin I), 1/5 (operculin XIII, pescaprein II); 2/5 (operculin I, quamoclin IV), 3/5 (tuguajalapins, Fig. 8.5). The most frequent occurrence is the one with 2 acyl residues/5 monosaccharide units. Occasionally it might be that even no acylation at all occurs, as already mentioned (muricatin VIII). There are several examples for two acyl residues at different positions of the *same* monosaccharide unit, e.g., ipomoeassins with a tigloyl residue at C-3″ and a *trans*-cinnamoyl residue at C-4″ of their glucose unit, tricolorins A–E with (2*S*)-methylbutyryl at C-2‴ and the same or another acyl at C-4‴ of the inner rhamnose unit (Fig. 8.3). Furthermore, there are several species which co-produce positional isomers by using different positions for acylation, e.g., operculins XI and XII where one *n*-dodecanoyl substituent is situated in the same rhamnose unit at C-2‴ and C-3‴, respectively, the remaining very complex structure being identical. Finally, it is worth mentioning that there is a unique example for an additional acyl substitution in the hydroxy fatty acid moiety (acetylation of the hydroxyl at C-5: ipomoeassin E).

In addition to the aspects (i)–(vi) already discussed above ("**Glycosidic Acids**") and many different possibilities for a cyclization to macrolactones the multiple variations contributed by acylation are able to increase once more considerably the structural diversity of monomeric resin glycosides. Thus, a huge number of congeners may occur in the whole family as well as a remarkable number in the same species, e.g. tuguajalapins, resin glycosides from the roots of *Merremia hungaiensis*, a species growing in China. Tuguajalapin VII is an example for a branched pentasaccharide of jalapinolic acid with three saturated straight-long-chain fatty acid residues linked to three different monosaccharide units (one inner and the terminal rhamnose as well as the terminal glucose moieties) (Fig. 8.5). The difference between this compound and its congeners (tuguajalapins I–VI, VIII–X) is given by the *presence or absence* of diverging fatty acid residues (C_{16}, C_{18}, C_{20}) at C-4 *or* C-6, respectively, of the glucose moiety (the palmitoyl residues at both rhamnose moieties are constant substituents). Thus, with the exception of two tuguajalapins which possess only these constant acyl substituents (IX, X) all compounds have an additional one. However, the macrolactone ring, formed by jalapinolic acid with one hydroxyl of the other inner rhamnose moiety, is expanded in tuguajalapins I, IV, and IX since the cyclization has happened at C-3″ (C-2″ = tuguajalapins II, III, V–VIII, X). The common corresponding glycosidic acid of all tuguajalapins, afforded by basic hydrolysis of these resin glycosides, is operculinic acid A.

In conclusion, it may be assumed that, beside numerous well-known monomeric resin glycosides (however, the vast majority of the convolvulaceous species is not yet investigated), numerous undiscovered oligomeric congeners might exist. Whether there are any convolvulaceous species which only produce monomers and alternatively others which only produce oligomers remains to be established, since even those species which may be considered as well-studied, might have conserved some secrets up to date. Thus, it has to be pointed out that many structure elucidations have been successful already, even in very difficult cases. But our knowledge is only representing the tip of the iceberg. There is still a large field of terra incognita waiting for endeavour.

8.3.2 Occurrence in the Convolvulaceae

As already mentioned, roots are especially resin glycoside-rich organs (up to 18%). However, these metabolites may occur in almost all parts of the plant. Fully matured seeds of *Ipomoea nil* (sub nom. *Pharbitis nil*) were found to contain ~3% "pharbitin". 94% of this amount could be determined in the cotyledons, mostly stored in many giant cells (among normal-sized mesophyll cells). Thus, these giant cells represent the main storage sites of the seed. Their formation reached the final size in the immature cotyledons 20 days after anthesis. Then the production of resin glycosides increased until seed maturation (Yokoyama and Wada 1987). In leaves of adult plants and open corollas these metabolites were present at a relatively low level.

Surprisingly, all species known to be glycoresin-positive belong to the **/Convolvuloideae** (subfamily) clade (Fig. 2.2) with the exception of the two *Cuscuta* spp. showing a special phylogenetic position. There is not a single report in the literature on any other species of the remaining parts of the family, neither of the large (subfamily) sister clade **/Dicranostyloideae** nor of the three basal clades (Erycibeae s.s., Cardiochlamyeae, Humbertieae). It might be speculated that this is due to the fact that these species are often not easily available in sufficient amounts (tiny herbs/rare occurrence/unapproachable endemic areas etc.) in contrast to many **/Convolvuloideae** ones. It might be also possible that such species had been checked, but the results were not published because they were found to be resin glycoside-negative. Furthermore, there are not even indications in detail on the occurrence of *resins* in floras, but only very general statements with the regard to the family, e.g., "…… often with milky juice" (van Ooststroom and Hoogland 1953), "…… the sap milky in some species" (Austin 1982), "…… cellules sécrétrices résineuses, latex blanc ou incolore souvent présent" (Deroin 2001).

The phytogeographic distribution of resin glycoside-containing species has been already discussed above in part. With regard to the genus *Ipomoea* it should be added that most of the species included in Table 8.2 are neotropical ones or show pantropical distribution with neotropical origin (*I. batatas*, *I. carnea* ssp. *fistulosa*, *I. multifida*, *I. nil*, *I. orizabensis*, *I. quamoclit*, *I. purpurea*, *I. quamoclit*, *I. tricolor*, *I. turbinata*). A further three pantropical species are of doubtful origin (*I. imperati*, *I. indica*, *I. pes-caprae*). Three (out of four) remaining species are of subtropical American origin (USA: *I. lacunosa*, *I. leptophylla*, *I. pandurata*). There is only one species of paleotropical origin (Australia: *I. lonchophylla*).

With the exception of *Calystegia* and *Operculina* most of the species belonging to the remaining genera (*Convolvulus*, *Cuscuta*, *Merremia* except *M. tuberosa*) are of Asian origin. This is also true for *O. turpethum* whereas *O. macrocarpa* (like *M. tuberosa*) is a neotropical species.

From an intrageneric point of view, glycoresin-positive members of seven (out of nine) traditionally accepted *Ipomoea* sections of American origin are listed in Table 8.2: *Eriospermum* (9 spp.), *Pharbitis* (5), *Erpipomoea*, *Exogonium*, *Mina*, *Tricolor* (2 each), *Calonyction* (1).

Among glycoresin-producing species are plants with very diverging habitus like vines with long-trailing stems, rooting at the nodes, e.g., *I. pes-caprae*, herbaceous, climbing, slender vines, e.g., *I. quamoclit*, large vines, suffrutescent at the base, e.g., *I. squamosa*, lianas with basally woody stems, e.g., *M. tuberosa*, and even two Mexican trees, *I. arborescens* and *I. murucoides* (Austin 1982; Austin and Huáman 1996).

It should be added, that there are reports on the occurrence of purgative resins in several further *Convolvulus* spp., e.g., *C. elongatus* WILLD., *C. hirsutus* M.B., *C. lanatus* VAHL., *C. siculus* L., *C. tricolor* L., and *Ipomoea* spp., e.g., *I. coccinea* L., *I. dumosa* (BENTH.) L.O.WILLIAMS, *I. hederacea* CHOISY, *I. hederifolia* L., *I. caudata* FEMALD sub nom. *I. hintonii* L.O.WILLIAMS, though they were not investigated phytochemically until now (Kosteletzky 1834; Jaretzky and Risse 1940b; Hilal et al. 1983; Hegnauer 1989).

Exogonic acid, a non-glycosidic dihydroxy fatty acid derivative (2-carboxymethyl-7-methyl-1,6-dioxa*spiro*-[4,4]-nonane; Fig. 8.6), represents a characteristic, apparently unique phytochemical indicator for *Brazilian* jalap, the resin of the roots of *Operculina macrocarpa* (invalid synonym: *Ipomoea operculata*) (Shellard 1961a). This constituent got its trivial name after *Exogonium purga*, the meanwhile invalid name of *Ipomoea purga*, because the discovering authors erroneously believed to have isolated it from *Mexican* jalap (Mannich and Schumann 1938). However, this compound is not involved as a structural component of the resin glycosides. Exogonic acid could be isolated as a free acid from this resin with a yield of 7% together with a smaller amount of 4-oxocaprylic acid (4-oxo-*n*-octanoic acid). Exogonic acid represented a mixture of four stereoisomers (Graf and Dahlke 1964). It turned out to consist predominantly of the *E*,*E* and *Z*,*Z*-diastereomers (2*S*,5*S*,7*R* and 2*S*,5*R*,7*R* configuration, respectively (Lawson et al. 1992). However, minor amounts of the *E*,*Z* and *Z*,*E* isomers (2*R*,5*S*,7*R* and 2*R*,5*R*,7*R*, respectively) could also be detected. Apparently, there exists an epimeric equilibrium in the resin due to the acidic nature of the latter, inducing epimerization.

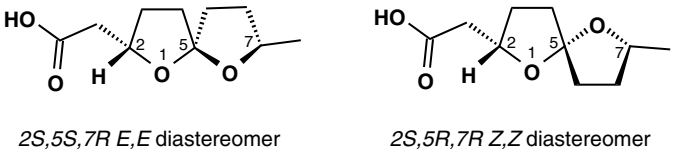


Fig. 8.6 Exogonic acid, a free component of Brazilian jalap resin (not linked structurally to its resin glycosides) exists as epimeric equilibrium of two major stereoisomers shown structurally together with two minor stereoisomers (2*R*,5*S*,7*R* / *E*,*Z* and 2*R*,5*R*,7*R* / *Z*,*E;* not shown)

8.3.3 Significance

8.3.3.1 Pharmacology and Toxicology

Saponin-like Effects. The powerful haemolytic effect, well-known from steroidal saponins (see Sect. 7.7.4) and their *N*-containing derivatives (see Sect. 7.8.4), is also a property of resin glycosides and – though diminished – still of glycosidic acids whereas hydroxy fatty acids like convolvulinolic and jalapinolic acids have a relatively weak haemolytic potency (Valette 1937b). A saponin-like effect of "convolvulin" (taken from *I. purga*) and "jalapin" (taken from *Operculina macrocarpa* sub nom. *I. orizabensis*) had already been reported by Heinrich (1918). (i) He found out that a 38,000-fold dilution of "jalapin" caused a total haemolysis of human blood (rabbit 1:14,000; wether 1:18,000). (ii) "Jalapin" also turned out to be a potent and specific fish poison (roach) being lethal in a dilution of 1:50,000, whereas the lethal dosage for "convolvulin" was double as much (1.25,000). These saponin-like effects had led to the assumption that certain convolvulaceous plants contain saponins as such. However, reports on alleged saponins, e.g., "ipomotocin" from the leaves of *Ipomoea carnea*, presumably ssp. *fistulosa*, (Tewari et al. 1964) or on even frequent occurrence in *Ipomoea* spp. and *Merremia* spp. (Nair et al. 1986), are lacking any unequivocal structural proof. The haemolytic effect observed might be caused by resin glycosides. Apparently, in contrast to the Solanaceae saponins are no convolvulaceous metabolites.

Ionophoric Properties. Mammosides B and H_1 as well as merremosides a – d and with increased activity congeners of the latter, i.e., f, g, h_1, and h_2 exhibited ionophoric activity against Na^+, K^+, and Ca^{++} ions in a human erythrocyte membrane method (Kitagawa et al. 1989a, c, 1996a). The best transporting activity was measured for Na^+ with merremoside g, for K^+ with the congeners f, h_1, h_2, and for Ca^{++} again with f. The authors concluded that the additional monosaccharide unit branching at C-3‴ shared by the congeners f, g, h_1, h_2, however lacking in the cases of a–d, might be the reason for the much better activity of the former ones. Furthermore, they argued that the cavities in the lactone rings are not large enough to trap metal ions. Instead, they proposed a molecular conformation that may be formed by the presence of the lactone rings providing an ion-trapping activity. In an artificial membrane model system merremosides a and h_1 were shown to possess the same properties (Kitagawa et al. 1989b). The difference between congeners a and h_1 in ion transport through the biological membrane may result from the more complex architecture of biological membranes over simple hydrophobic barriers. The ion-transport activities were completely lost by cleavage of the macrolactone moiety of the glycoresins.

Therapeutic Use. An early pharmacological study on convolvulaceous resins was published by Hagentorn (1857). Resin glycosides induce peristaltic movements in the small intestine resulting in numerous aqueous bowel movements within 1–2 h even after moderate dosages. However, the mechanism causing the purgative effect still remains to be elucidated (Pereda-Miranda and Bah 2003). Based on his

observation that water-insoluble "convolvulin" (from different species) could be readily dissolved in aqueous solutions of salts of bilic acids and that such solutions have a marked solvent power for lecithin, Valette (1937a) proposed that these compounds are able to dissolve lecithin from the epithelial cells of the intestine. This might be the reason for its irritation and as a consequence for the purgative effect which is confined to the intact resin glycoside molecules; glycosidic acids are inactive. Since even low overdosing may cause severe inflammations of the mucous membrane of both, intestine and colon, purgative convolvulaceous resins obviously have lost their former medicinal significance in the last third of the past century. This development was accelerated by the development of alternative remedies with less adverse effects. Williams (1970) pointed out that there was a great contrast between the United States Dispensatory, 24th edition (Osol and Farrar 1947) and the 26th edition (Osol et al. 1967), e.g., it was stated in the latter that preparations of jalap (*Ipomoea purga*) were no longer official and that preparations from Mexican scammony (*I. orizabensis*) were no longer included in a United States official compendium. Similar developments could be observed in other countries, e.g., the German pharmacopoeia of 1926 (DAB 6) (DAB 6 1926) contained four jalap monographs (*I. purga* sub nom. *Exogonium purga*: "Tubera Jalapae", "Resina Jalapae", "Pilulae Jalapae", "Sapo jalapinus") which were not included any longer in the next edition (DAB 7 1968). These four monographs had been already part of the first edition (Pharmacopoea Germanica 1872), together with a fifth ("Tinctura Resinae Jalapae") and two scammony monographs (*Convolvulus scammonia*: "Radix Scammoniae", "Resina Scammoniae").

Another again obsolete indication was the use of glycoresin-containing crude drugs as anthelmintics (Hernández-Carlos et al. 1999). However, there are almost no scientific reports on anthelmintic effects. The resin from the roots of *Convolvulus lanatus* was found to be inactive against the earthworm *Allolobophora caliginosa* Savigny, Oligochaeta: Lumbricidae, which is of course not a human pathogen (Hilal et al. 1983). It might be assumed that the anthelmintic effect observed in humans was an indirect one due to the drastic purgative properties.

Ethnomedicine. Since ancient times resin-containing convolvulaceous crude drugs, predominantly roots/tubers or resins prepared from the former had been used as drastic purgatives, especially in neotropical countries and in India (Wagner et al. 1978; Pereda-Miranda and Bah 2003). Still today, *Ipomoea purga*, *I. orizabensis*, and *I. stans* are "easily found in the numerous Mexican herbal markets or as an ingredient in over-the-counter products sold in health food stores in Mexico and the United States" (Pereda-Miranda et al. 2006b and references therein). Many species from the genera *Convolvulus*, *Cuscuta*, or *Ipomoea* were used again since ancient times also in the treatment of tumours/cancer in the traditional medicine of many peoples all over the world (Hartwell 1968).

The seeds of *Cuscuta australis* as well as of *C. chinensis* are still used as a tonic in Chinese traditional medicine (Miyahara et al. 1996). The roots of *Merremia hungaiensis* play a role in China against chronic hepatitis, children's tantrums and hernia (Noda et al. 1994b). The tuber of *M. mammosa* is said to be useful for

treating diabetes as well as affections of the throat and respiratory system in Indonesia (Kitagawa et al. 1988). The seeds of *Ipomoea turbinata* are used as a laxative and carminative in India (Noda et al. 1988a), the roots and leaves of *I. batatas* as a health food in Japan; they were claimed to be effective for different frequent, even severe complaints (Noda et al. 1992b). *I. quamoclit* is cultivated in India and used as an antiphlogistic drug (Wagner et al. 1983). Indigenous North Americans appreciated the enormous root of *I. leptophylla* (common name "man root" or "big root morning glory"; habitat open prairies) as remedies against nervousness and stomach ailments, early European settlers as a tonic (Barnes et al. 2003 and references therein). A review on the ethnobotany and ethnomedicine of glycoresin-containing species in Mexico since pre-Columbian times has been published recently (Pereda-Miranda and Bah 2003; see also Pereda-Miranda et al. 2006b).

Whether all ethnomedicinal applications would survive an evidence-based medicine is another question. Furthermore, if this would be the case, the question arises, whether such effects are based fully or in part on the unequivocal occurrence of resin glycosides. "Australian aborigines still apply the heated leaves …. (of the pantropical beach morning-glory *Ipomoea pes-caprae*) …. directly to wounds, skin infections, and inflamed sores, as well as to stings from poisonous fish, manta ray, and insects. Decoctions of the plant have a worldwide use in medicinal baths to treat fatigue, strain, arthritis, and rheumatism" (Pereda-Miranda et al. 2005). The leaves of *I. pes-caprae* have been used in the traditional medicine of many tropical countries for the treatment of inflammatory disorders. However, this species is an example for the possibility that other constituents [in this case phenylpropanoids (see Sect. 6.3.2.2)] may at least contribute to the activity of glycoresin-containing crude drugs (Pongprayoon et al. 1991).

8.3.3.2 Further Biological Effects

A scientific revival of and a new interest in resin glycosides caused by the discovery of novel biological effects, e.g., antibacterial, cytotoxic, phytotoxic activities, could be observed during the last two decades. This development has stimulated again phytochemical research on these compounds.

Antibacterial Effects. Valette and Liber (1938) reported that "convolvulin" and especially "jalapin" showed a bactericidal effect. In the 1960s it was noticed that resin glycosides show structural similarities to certain glycolipid bacterial metabolites with antibiotic properties (Smith et al. 1964; Khanna and Gupta 1967), e. g., glucoustilic acid from a species belonging to the Ustilaginales, a 15,16-dihydroxydecanoic acid whose two alcoholic hydroxyls are linked to two glucose molecules (Lemieux 1951). Moreover, ustilagic acid, a chemically heterogeneous mixture, apparently comparable to resin glycosides in the general makeup, provided – beside glucoustilic acid – steam-volatile and steam-non-volatile acids by alkaline hydrolysis. A list of references concerning glycolipids from bacteria, fungi, and yeasts was given by Fürstner and Müller (1999).

Resin prepared from the roots of *Convolvulus lanatus* showed a very weak antimicrobial activity against the human pathogens *Escherichia coli* (Gram-negative) and *Bacillus subtilis* (Gram-positive) (Hilal et al. 1983). Four resin glycosides from *I. bahiensis* (compounds 1a, 1b, 2a, 2b) exhibited moderate activity against several Gram-positive bacteria, e.g., *Bacillus subtilis*, *Staphylococcus aureus*, in contrast to Gram-negative human pathogens (Bieber et al. 1986). A preliminary screening of resin glycoside-containing fractions of *Ipomoea stans* showed moderate antibacterial activities towards *Staphylococcus aureus* and *Bacillus subtilis* (Reynolds et al. 1995).

A glycoresin-containing crude extract of the leaves/stems from *Ipomoea leptophylla* had shown moderate activity (92% inhibition at an initial test concentration of 150 µg/ml) against *Mycobacterium tuberculosis,* the pathogen causing tuberculosis. Bioassay-guided fractionation led to the isolation of leptophyllin A which appeared to be the major active component (Barnes et al. 2003).

The term "leptophyllin" chosen for the resin glycosides of *Ipomoea leptophylla* is an example for a carelessly created trivial name since there had been published metabolites before with the same name (even including the capitals A, B etc.) but different structures (piperidine alkaloids and coumarins, respectively) from two different species (*Cassia leptophylla* Voq., Fabaceae; *Apium leptophyllum* F.Muell. ex Benth., Apiaceae). Thus, to avoid confusion it would be better to integrate not only the epithet part of a species name (which may occur often in other genera, too) but also at least one or two syllables of the genus part, e.g., "ipoleptophyllin".

Moderate in vitro activities against *Mycobacterium tuberculosis* (MIC: 16–32 µg/ml) were also documented in a study with tricolorins A–E providing the rationale for the traditional use of *Ipomoea tricolor* in the treatment of tuberculosis (Rivero-Cruz et al. 2005).

A recent study including 22 resin glycosides (7 tricolorins, 2 scammonins, 13 orizabins) revealed further biological properties of certain congeners in the context of antibacterial activities (Pereda-Miranda et al. 2006a). The aim of this study has been to evaluate these compounds against a panel of *Staphylococcus aureus* strains possessing or lacking specific efflux pumps. Orizabins IX and XIX, which – in contrast to many other congeners – exhibited no antimicrobial activity themselves (MIC: > 256 µg/ml), turned out to display a strong synergistic effect in combination with the bactericidal synthetic fluorquinolone derivative norfloxacin (a gyrase inhibitor which is active especially against Gram-negative pathogens and used therapeutically against urinary tract infections). Thus, orizabin XIX reversed norfloxacin resistance fourfold for a certain strain of (the gram-positive human pathogen) *S. aureus* overexpressing a certain multidrug efflux pump. Its congener IX at 1 µg/ml completely inhibited bacterial growth in the presence of 2 µg/ml of norfloxacin. Orizabins IX and XV were nearly equipotent with respect to the inhibition of ethidium bromide efflux by *S. aureus* (ethidium bromide is an established substrate for efflux pumps). At certain concentrations (less than 10 µM) both orizabins turned out to be more efficacious than the well-established reference agent reserpine. Therefore, the authors claim that their results "…. open the possibility of using these compounds as leads for the development of more potent inhibitors of *S. aureus* multidrug efflux pumps." It must be added

that this study also involved interesting details on structure-activity relationships with respect to the antibacterial activity as such. Based on the results with those 22 congeners, aspects like the size of the lactone ring (not crucial for the potency), degree of acylation, and others are discussed.

Antifungal Effects. Tricolorins and orizabins have been proposed as promising lead structures for the development of antifungal agents due to their capability to inhibit fungal cell-wall synthesis. These compounds turned out to be potent in vitro inhibitors of 1,3-β-glucan synthase, an enzyme essential for the synthesis of the major fungal cell wall polymer and thus a specific target for an antifungal drug (Castelli et al. 2002). Orizabin XX (IC_{50} value: 62 µg/ml) and tricolorin A (IC_{50}: 85 µg/ml), respectively, were the most active compounds of their group of congeners. Resin glycosides from *I. bahiensis* exhibited only very low antifungal activity against human pathogens (Bieber et al. 1986). Resin glycosides in general are moderately inhibitory to phytopathogenic fungi (Duke et al. 2003).

Antitumor Effects. A total of 24 convolvulaceous species were listed as antitumor remedies used in the ethnomedicine of many peoples as already mentioned (Hartwell 1968). Surprisingly, in modern times the vast majority of them have been found to contain resin glycosides. Furthermore, again surprisingly all that species belong to those taxa which have turned out to produce resin glycosides (**/Convolvuloideae**, Cuscuteae) with the only exception of *Cressa cretica* L., cited by Dioscorides for "hot tumours of uterus". Thus, ethnomedicinal experiences seem to contribute clearly to a (negative) answer to the question "Are there resin glycosides as constituents in convolvulaceous taxa outside the **/Convolvuloideae**, Cuscuteae?"

Scientific research on this topic started in the 1950s, when preparations from *Ipomoea orizabensis*, checked in comparison with other cathartics from plants, were found to produce moderate histologically demonstrable damages in a sarcoma 37 model (Belkin et al. 1952). Some decades later, cytotoxicity was confirmed for certain extracts of *I. orizabensis*, *I. purga*, and *I. tricolor* in different human cancer cell lines (Pereda-Miranda 1995). Pure, single resin glycosides (orizabins, tricolorins) were shown to exhibit at least a weak cytotoxicity against different carcinoma cell lines (Hernández-Carlos et al. 1999; Pereda-Miranda and Bah 2003) with tricolorin A as the most interesting congener.

Aqueous-ethanolic extracts of the whole plant of *I. indica* sub nom. *I. learii* had shown significant activity against Walker carcinoma 256 in rats. This prompted Sarin et al. (1973) to isolate the active principle. It was characterized as a new glycosidic acid which was named ipolearoside. An unnamed resin glycoside "1a" isolated from *I. bahiensis* was found to exhibit considerable activity (7.5 mg/kg) against sarcoma 180 in mice (Bieber et al. 1986). Stansin 5 was shown to exhibit moderate to marginal cytotoxic activity against an ovarian carcinoma (ED_{50}: 1.5 µg/ml) and a cervical carcinoma cell line (4.0 µg/ml) in contrast to a colon carcinoma one (24.0 µg/ml) (León et al. 2004). Pescaproside A and pescapreins I–IV were found to be weakly cytotoxic to four human cancer

cell lines (ED_{50}: 5–20 µg/ml; Pereda-Miranda et al. 2005). Murucoidins I–III and V as well as stoloniferin I were inactive in nasopharyngeal (KB) and laryngeal carcinoma (Hep-2) cell systems whereas murucoidin IV exhibited marginal cytotoxicity (ED_{50}: 4 µg/ml) in the latter system (Chérigo and Pereda-Miranda 2006). However, murucins 2–5, 8, and 9 obtained from the same species (*Ipomoea murucoides*) and arboresins 3–5 (*I. arborescens*) were inactive in cytotoxicity tests against certain colon, cervical, and ovarian carcinoma cells. Their congeners, murucins 1 and 7 as well as arboresins 1 and 2 exhibited marginal activity (4–5 µg/ml) against the ovarian carcinoma cell line (León et al. 2005, 2006). The resin from the roots of *Convolvulus lanatus* was inactive against Ehrlich ascites carcinoma (Hilal et al. 1983).

Based on studies with the amphiphilic tricolorin A in its crystalline state it has been proposed that the cytotoxicity exhibited by active resin glycosides may be caused by their ability to perturb cell membranes by forming non-selective pores and/or their influence on the function of multidrug efflux pumps (Rencurosi et al. 2004). Also in *Sf9* cell membranes of the insect *Spodoptera frugiperda* J.E.Smith, Lepidoptera: Noctuidae, tricolorins induced current fluctuations suggesting the formation of non-selective pores (Villatoro-Vera et al. 2004). Based on such results it has been proposed "…. that the resin glycosides kill target cells, at least in part, by interacting with their plasma membrane to induce possible ion flux perturbation." Whether the formation of that pores and the ionophoretic effect mentioned above are causally related to each other has not yet been discussed.

Minor structural variations in a series of ipomoeassins and their semisynthetic acetyl derivatives caused significant differences with regard to cytotoxicity (A2780 human ovarian cell line) with IC_{50} values between 0.035 µM (ipomoeassin D) and 19.1 µM (*O*-3′, *O*-2″-diacetylipomoeassin D) (Cao et al. 2005). These results prompted the authors to argue that the hypothesis of Rencurosi et al. does not fully explain why closely similar compounds show such different cytotoxicities and that therefore the possibility is raised that other mechanisms of action are involved. Anyhow, for the present ipomoeassin D seems to be the most potent and therefore the most promising agent of all resin glycosides (IC_{50} values for comparison in the same system: 35 nM vs 0.8–2.4 nM for actinomycin D, an established anticancer drug, as the positive control). However, to date there are neither reports on preclinical animal experiments nor on clinical trials. On the other hand, cuscutic resinoside A, also a resin glycoside monomer, *stimulated* MCF-7 cell proliferation as well as T47D human breast cancer cells at a concentration of 10 µM (Umehara et al. 2004).

Antiserotonergic Activity. Merremosides b and d exhibited antiserotonergic activity with ED_{80} values (mice) of 10 µg/ml and 2 µg/ml, respectively (synthetic reference compound promethazine: 2 µg/ml) (Kitagawa et al. 1988).

Miscellaneous Effects. Recently, tyrianthin 6 was demonstrated (i) to result in antidepressant activity (mice, i.p.), (ii) to exhibit dose-dependent protective effects against pentylenetetrazole-induced seizures, and (iii) to produce relaxant effects on

spontaneous contractions in the isolated rat ileum. One of its congeners, scammonin 1, also showed the effects (ii) and (iii) whereas the effect of another one, scammonin 2, was confined to (iii). These metabolites have been isolated from the roots of *Ipomoea tyrianthina* LINDL. (Mirón-López et al. 2007). However, this is only an invalid synonym for *I. orizabensis* (Austin and Huáman 1996); see also Table 8.1.

8.3.3.3 Chemical Ecology

Allelopathic Effects. Anaya et al. (1990 and references therein) reported that *Ipomoea tricolor* is commonly grown in traditional agriculture of tropical zones in Mexico as a weed controller. Thus, after a certain period (2–3 months) as a cover crop in the sugar cane fields, when this species eliminates all other weeds, it is cut and incorporated into the soil as green manure. Based on this traditional experience the authors could demonstrate that a mixture of resin glycosides (tricolorins) from this Mexican morning glory obtained by bioassay-guided fractionation showed a very considerable allelopathic potential (Anaya et al. 1990, 1995). The major phytogrowth inhibiting agent turned out to be tricolorin A [63% of all tricolorins, i.e., A–J (Pereda-Miranda and Bah 2003)]. This compound strongly inhibited the radicle growth of an *Amaranthus* sp., Amaranthaceae, and an *Echinochloa* sp. (Poaceae) (IC_{50} values 12–37 μM); the corresponding inhibition of *Ipomoea purpurea* and *I. tricolor* itself was much less though significant. Seed germination of the *Amaranthus* sp. was also strongly inhibited, in case of the *Echinochloa* sp. to a lesser extent; both *Ipomoea* spp. were only slightly affected. In a greenhouse experiment the biomass of the *Echinochloa* sp. was diminished by the incorporation of plant material from *I. tricolor* into the soil. The authors concluded from their results a confirmation of the traditional experiences: "In the field, it is possible that the weed control effect of *I. tricolor* takes place in two stages, first, by the combination of the allelopathic potential of the living plant expressed through leaching by rain and competition (interference); and second, when the plant is cut and incorporated into the soil …." where the glycoresins are released from decaying matter. This is especially useful before the sowing of sugar cane, corn, or other crops.

Crude polar extracts of sweet potato (*I. batatas*) root periderm tissue also inhibited germination of different species; attempts to isolate the inhibiting principle failed (Peterson and Harrison 1991 and references therein). However, simonin IV, the major constituent from a certain variety of *I. batatas*, showed allelopathic potential including inhibition of radicle growth in a dose-dependent manner (Pereda-Miranda and Bah 2003 and references therein) though in a diminished extent compared to tricolorin A.

In this connection it is interesting to realize that tricolorin A was also shown to uncouple photophosphorylation in spinach chloroplasts in a potent manner (U_{50} = 0.33 μM) and to inhibit – in high concentrations (20 μM) – electron transport in photosystem II. Again the intact macrolactone moiety turned out to be crucial (Achnine et al. 1999). Glycoresins might be useful as leads for new herbicides (Vyvyan 2002).

Resistance to Insects. Laboratory evaluation of leaf extract of *Ipomoea carnea* ssp. *fistulosa* showed that such an extract (acetone as the solvent) has the potentiality to cause mortality and disrupt the development and growth of the malaria vector *Anopheles stephensii* LISTON (Diptera: Culicidae) (Saxena and Sumithra 1985). Experiments with the diamond moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) fed with resin glycosides from the periderm tissue of storage roots from sweet potato (*Ipomoea batatas*) showed highly significant negative correlations between levels and survival as well as levels and larval weight. However, it could be observed a significant positive correlation between glycoside level and developmental time of larvae. The authors concluded that "…. resin glycosides may contribute to the resistance in sweet potato breeding lines to soil insect pests" (Jackson and Peterson 2000). Later they confirmed this conclusion ("moderately inhibitory to insects"); however, other root periderm components (complex esters of still unknown structure and phenolic compounds like caffeic acid) turned out to be more active in bioassays (Duke et al. 2003). Resin glycosides and caffeic acid concentrations were significantly different among genotypes and between years (Mao et al. 2001). Interestingly, drought stress on sweet potato roots had no effect on resin glycosides content but significantly reduced the content of caffeic acid (Mao et al. 2004).

Protection Against Vertebrate Herbivores; Toxicoses in Livestock. The leaves of *Ipomoea carnea* ssp. *fistulosa* are very poisonous (Legler 1964) which is of some importance since this species, an evergreen, erect to scrambling shrub up to 3 m blooming throughout the year, is a pantropically occurring, cultivated ornamental. It is also used in hedges as a windbreak around houses and kraals in Mozambique (Molyneux et al. 1996) or as a protection for cultivated food plants from animals in Rajasthan/India (Saxena and Sumithra 1985). Furthermore, it often 'escapes' from cultivation (Meeuse and Welman 2000 and references therein). Intoxication – often of goats – is characterized by loss of appetite, soft faeces, and weight loss as well as by central nervous system signs as head shaking and hyperesthesia; even lethal events may occur (De Balogh et al. 1999). These effects have been attributed to alkaloidal components, the polyhydroxy alkaloids swainsonine and different calystegines (Haraguchi et al. 2003; see Section 3.5.). It is still unknown whether (and – if applicable – to what extent) resin glycosides contribute to the toxicity of this plant. This is of special interest since this species is also characterized by the high content of very diverging and complex glycoresins in the leaves (Legler 1964). Anyhow, this author had entitled his report "Die Bestandteile des *giftigen* Glykosidharzes aus *Ipomoea fistulosa*" ("The components of the *poisonous* glycoresins …."). A similar situation is given in the "outback" of Australia with regard to *I. lonchophylla* (common name: cow vine), a very common non-twining annual creeper occurring on clay soils. The plant also contains both classes of metabolites. Again calystegines and swainsonine have been detected (Dorling et al. 2004). On the other hand, resin glycosides have been found in this species which turned out to be toxic to mice (LD_{50}: 100 mg/kg; MacLeod et al. 1997). The former study has been induced by

an ataxia syndrome in cattle in north Western Australia, the latter study by the "dumb lamb syndrome" causing high mortality rates (~20%) among newly born lambs. In another study with *I. lonchophylla* results indicated that this plant may contain substances that affect the functional development of the foetal brain in ewes (Walker et al. 1992). The possibility that this plant has a greater impact in sheep populations with poor nutrition and in more extreme environmental conditions was discussed by the authors. Final conclusions on the extent of the contribution of resin glycosides as well as of polyhydroxy alkaloids in all such cases are impossible without pen feeding trials with the plants or preferably with pure compounds. Perhaps both classes of metabolites are responsible. Their principle toxicities are given with confidence.

8.3.4 Convolvulaceous Resin Glycosides versus Solanaceous Steroidal Glycoalkaloids (Sect. 7.8.1)

Based on the facts – coincidence or not – both large Solanales families show a strange parallelism with regard to the development of their most specific, most structurally diverse class of secondary metabolites: Both classes of compounds, resin glycosides (Convolvulaceae) and steroidal glycoalkaloids (Solanaceae), respectively:

- Are characterized by the occurrence of more or less uniform groups of respective aglycones (hydroxy fatty acids vs cholesterol-derivatives)
- Share the presence of straight-chain as well as branched oligosaccharides (sometimes mono- or disaccharides) forming *O*-glycosides with their corresponding aglycones
- Share glucose and rhamnose as frequent common monosaccharidic residues though there are specific differences with regard to the remaining frequent sugar units of the respective glycosides [fucose, quinovose (resin glycosides) vs galactose, xylose (glycoalkaloids)]
- Have been developed especially in *certain advanced* taxa, Cuscuteae / **Convolvuloideae** vs Solaneae / Capsiceae clades, thus representing apomorphic characters
- Are characteristic constituents of the largest genus in the respective family by far [*Ipomoea* (~650 spp.) vs *Solanum* (~1400 spp.)]
- Are lacking in almost all of the other clades
- Share biological activities like damaging of membranes causing haemolytic properties, fish toxicity, cytotoxicity, antifungal activity
- Share the fact that their aglycones are more or less biologically inactive which is not to be regarded as a matter of course for natural metabolites (examples for inactive glycosides: cyanogenic glycosides, glucosinolates
- Are at present of toxicological but not of therapeutic significance

References

Achnine L, Pereda-Miranda R, Iglesias-Prieto R, Moreno-Sanchez R, Lotina-Hennsen B (1999) Tricolorin A, a potent natural uncoupler and inhibitor of photosystem II acceptor side of spinach chloroplasts. Physiol Plant 106:246–252

Anaya AL, Calera MR, Mata R, Pereda-Miranda R (1990) Allelopathic potential of compounds isolated from *Ipomoea tricolor* Cav. (Convolvulaceae). J Chem Ecol 16:2145–2152

Anaya AL, Sabourin DJ, Hernandez-Bautista BE, Mendez I (1995) Allelopathic potential of *Ipomoea tricolor* (Convolvulaceae) in a greenhouse experiment. J Chem Ecol 21: 1085–1102

Arrendale RF, Severson RF, Sisson VA, Costello CE, Leary JA, Himmelsbach DS, van Halbeek H (1990) Characterization of the sucrose ester fraction from *Nicotiana glutinosa*. J Agric Food Chem 38:75–85

Asahina Y, Shimidzu T (1922) Chemische Untersuchung des Samens von *Pharbitis nil* Chois. II. Mitt. Chem Zentralbl 976

Asahina Y, Terada SX (1919) Constituents of the seeds of *Pharbitis nil* Chois. Yakugaku Zasshi 452:821

Asilbekova DT (2003) Lipids from *Capsicum annuum* seeds. Chem Nat Comp 39:528–530

Asilbekova DT (2004) Glycolipids from *Capsicum annuum*. Chem Nat Comp 40:115–117

Austin DF (1982) 165. Convolvulaceae. In: Harling G, Sparre B (eds) Flora of Ecuador, vol 15. Swedish Research Councils, Stockholm

Austin DF, Huáman Z (1996) A synopsis of *Ipomoea* (Convolvulaceae) in the Americas. Taxon 45:3–38

Auterhoff H, Demleitner H (1955) Vergleichende Untersuchungen an Convolvulaceen-Harzen. Arzneim Forsch 5:402–407

Bah M, Pereda-Miranda R (1996) Detailed FAB-mass spectrometry and high resolution NMR investigations of tricolorins A–E, individual oligosaccharides from the resins of *Ipomoea tricolor* (Convolvulaceae). Tetrahedron 52:13063–13080

Bah M, Pereda-Miranda R (1997) Isolation and structural characterization of new glycolipid ester type dimers from the resin of *Ipomoea tricolor* (Convolvulaceae). Tetrahedron 53:9007–9022

Baldwin IT, Halitschke R, Paschold A, von Dahl CC, Preston CA (2006) Volatile signalling in plant-plant interactions: "talking trees" in the genome era. Science 311:812–815

Barnes CC, Smalley MK, Manfredi KP, Kindscher K, Loring H, Sheeley DM (2003) Characterization of an anti-tuberculosis resin glycoside from the prairie medicinal plant *Ipomoea leptophylla*. J Nat Prod 66:1457–1462

Bauer KH, Junge R (1934) Zur Kenntnis des Skammoniumharzes. Arch Pharm 272:841–848

Begum AS, Rai UK, Singh S, Sahai M (2004) New pairs of acyl sucroses from *Petunia nyctaginiflora* Juss. J Indian Chem Soc 81:495–500

Begum AS, Singh AP, Sahai M, Singh S, Fujimoto Y (2005) Two novel acyl sucroses from *Petunia nyctaginiflora*. Indian J Chem 44B:648–650

Bekker NP, Ul'chenko NT, Glushenkova AI (2001) Physicochemical properties and composition of lipids from *Capsicum annuum*. Chem Nat Comp 37:131–133

Belkin M, Fitzgerald DB, Cogan GW (1952) Tumor-damaging capacity of plant materials. I. Plants used as cathartics. J Nation Canc Inst 13:139–155

Bieber LW, da Silva Filho AA, Corrêa Lima RMO, de Andrade Chiappeta A, do Nascimento SC, de Souza IA, de Méllo, Veith HJ (1986) Anticancer and antimicrobial glycosides from *Ipomoea bahiensis*. Phytochemistry 25:1077–1081

Buttery RG, Ling LC (1993) Volatile components of tomato fruit and plant parts: relationship and biogenesis. ACS Symposium Ser vol. 525, American Chemical Society, Washington, DC, pp 23–34

Cadet de Gassicourt L (1817) J pharmacie 3:495; fide Shellard EJ (1961a)

Cao S, Guza RC, Wisse JH, Miller JS, Evans R, Kingston DGI (2005) Ipomoeassins A–E, cytotoxic macrocyclic glycoresins from the leaves of *Ipomoea squamosa* from the Suriname rainforest. J Nat Prod 68:487–492

Castelli MV, Cortés JCG, Escalante AM, Bah M, Pereda-Miranda R, Ribas JC, Zacchino SA (2002) In vitro inhibition of 1,3-β-glucan synthase by glycolipids from convolvulaceous species. Planta Med 68:739–742

Chérigo L, Pereda-Miranda R (2006) Resin glycosides from the flowers of *Ipomoea murucoides*. J Nat Prod 69:595–599

Christensen BV, Reese JA (1938) A study of the leaves of *Ipomoea pes-caprae*. J Am Pharmaceut Ass 27:195–199

DAB 6 (Deutsches Arzneibuch, 6th edn) (1926) R v Decker's Verlag, G Schenck, Hamburg, Germany

DAB 7 (Deutsches Arzneibuch, 7th edn) (1968) Deutscher Apotheker-Verlag, Stuttgart / Govi-Verlag, Frankfurt, Germany

Davies LA, Adams R (1928) Structures of convolvulinolic and jalapinolic acids. Synthesis of 11-hydroxypentadecanoic and 11-hydroxyhexadecanoic acid. J Amer Chem Soc 50:1749–1755

De Balogh KIM, Dimande AP, van der Lugt JJ, Molyneux RJ, Naudé TW, Welman WG (1999) A lysosomal storage disease induced by *Ipomoea carnea* in goats in Mozambique. J Veter Diagn Inv 11:266–273

Deharo E, Sauvain M, Moretti C, Richard B, Ruiz E, Massiot G (1992) Antimalarial effect of n-hentriacontanol isolated from *Cuatresia* sp. (Solanaceae). Anal Parasit Hum Compar 67:126–127.

Deineka VI, Deineka LA (2004) Triglyceride types of seed oils. I. Certain cultivated plants of the Solabaceae family. Chem Nat Comp 40:184–185

De Marino S, Borbone N, Gala F, Zollo F, Fico G, Pagiotti R, Iorizzi M (2006) New constituents of sweet *Capsicum annuum* L. fruits and evaluation of their biological activity. J Agric Food Chem 54:7508–7516

Deroin T (2001) 171. Convolvulaceae. In: Morat P (ed) Flore de Madagascar et des Comores. Muséum National d'Histoire Naturelle, Paris

Dini I, Tenore GC, Trimarco E, Dini A (2006) Seven new aminoacyl sugars in *Ipomoea batatas*. J Agric Food Chem 54:6089–6093

Dorling PR, Colegate SM, Allen JG, Nickels R, Mitchell AA, Main DC, Madin B (2004) Calystegines isolated from *Ipomoea* spp. possibly associated with an ataxia syndrome in cattle in north Western Australia. In: Acamovic T, Stewart CS, Pennycott TW (eds) Poisonous plants and related toxins. CABI Publishing, Wallingford, UK, pp 140–145

Du XM, Kohinata K (née Tsuji), Kawasaki T, Guo YT, Miyahara K (1998) Resin glycosides. XXVI. Components of the ether-insoluble glycoside-like fraction from *Cuscuta chinensis*. Phytochemistry 48:843–850

Du XM, Sun NY, Nishi M, Kawasaki T, Guo YT, Miyahara K (1999) Components of the ether-insoluble resin glycoside fraction from the seed of *Cuscuta australis*. J Nat Prod 62:722–725

Duke SO, Baerson SR, Dayan FE, Rimando AM, Scheffler BE, Tellez MR, Wedge DE, Schrader KK, Akey DH, Arthur FH, de Lucca AJ, Gibson DM, Harrison HF Jr, Peterson JK, Gealy DR, Tworkoski T, Wilson CL, Morris JB (2003) United States Department of Agriculture – Agricultural Research Service research on natural products for pest management. Pest Manag Sci 59:708–717

Enriquez RG, León I, Perez F, Walls F, Carpenter KA, Puzzuoli FV, Reynolds WF (1992) Characterization, by two-dimensional NMR spectroscopy, of a complex tetrasaccharide glycoside isolated from *Ipomoea stans*. Can J Chem 70:1000–1008

Evans WC, Somanabandhu A (1980) Nitrogen-containing non-steroidal secondary metabolites of *Solanum*, *Cyphomandra*, *Lycianthes* and *Margaranthus*. Phytochemistry 19:2351–2356

Fürstner A, Müller T (1999) Efficient total synthesis of resin glycosides and analogues by ring-closing olefin metathesis. J Am Chem Soc 121:7814–7821

Gaspar EMM (1999) New pentasaccharide macrolactone from the European Convolvulaceae *Calystegia soldanella*. Tetrahedron Lett 40:6861–6864

Gaspar EMM (2001) Soldanelline B – the first acylated nonlinear tetrasaccharide macrolactone from the European Convolvulaceae *Calystegia soldanella*. Eur J Org Chem:369–373
Genest K, Sahasrabudhe MR (1966) Alkaloids and lipids of *Ipomoea*, *Rivea* and *Convolvulus* and their application to chemotaxonomy. Econ Bot 20:416–428
Graf E, Bühle H (1974a) Zur Struktur der Rhamnoconvolvulinsäure. I. Isolierung der Rhamnoconvolvulinsäure C und strukturbeweisende Synthese ihres Aglykons. Arch Pharm 307:628–635
Graf E, Bühle H (1974b) Zur Struktur der Rhamnoconvolvulinsäure. II. Untersuchung des Zuckeranteils von Rhamnoconvolvulinsäure C. Arch Pharm 307:636–643
Graf E, Dahlke E (1964) Über die Exogonsäure. Planta Med 12:293–295
Graf E, Dahlke E, Voigtländer HW (1965) Über die Convolvuline; neue Bausteine und Untersche idungsreaktionen. Arch Pharm 298:81–91
Hagentorn (1857) Pharmacologische Untersuchungen einiger Convolvulaceenharze, Dorpat (today Tartu/Estonia); fide Spirgatis (1860)
Halitschke R, Baldwin IT (2005) Jasmonates and related compounds in plant-insect interactions J Plant Growth Regul 23:238–245
Haraguchi M, Gorniak SL, Ikeda K, Minami Y, Kato A, Watson AA, Nash RJ, Molyneux RJ, Asano N (2003) Alkaloidal components in the poisonous plant, *Ipomoea carnea* (Convolvulaceae). J Agric Food Chem 51:4995–5000
Hartwell JL (1968) Plants used against cancer. A survey. Lloydia/J Nat Prod 31:158–163
Hegnauer R (1964) Chemotaxonomie der Pflanzen, vol 3. Birkhäuser Verlag, Basel, Switzerland, pp 557–558
Hegnauer R (1973) Chemotaxonomie der Pflanzen, vol 6. Birkhäuser Verlag, Basel, Switzerland, pp 439–440
Hegnauer R (1989) Chemotaxonomie der Pflanzen, vol 8. Birkhäuser Verlag, Basel, Switzerland, pp 321–322
Heinrich G (1918) Zur Kenntnis des biologischen Verhaltens von Convolvulin und Jalapin. Biochem Z 88:13–34
Hernández-Carlos B, Bye R, Pereda-Miranda R (1999) Orizabins V–VIII, tetrasaccharide glycolipids from the Mexican scammony root (*Ipomoea orizabensis*). J Nat Prod 62:1096–1100
Herrera-Salgado Y, Garduno-Ramirez ML, Vázquez L, Rios MY, Alvarez L (2005) *Myo*-inositol-derived glycolipids with anti-inflammatory activity from *Solanum lanceolatum*. J Nat Prod 68:1031–1036
Hilal SH, Haggag MY, Soliman FM, El-Kashoury ESA (1983) Phytochemical study and biological screening of *Convolvulus lanatus* Vahl. Egypt J Pharm Sci 24:139–148
Hoehnel M (1896) Ueber das Convolvulin, das Glycosid der Tubera Jalapae (*Ipomoea purga* Hayne). Arch Pharm 234:647–685
Hosamani KM, Sattigeri RM (2000) Industrial utilization of *Rivea ornata* seed oil. A moderate source of vernolic acid. Indust Crops Prod 12:93–96
Husemann A, Hilger A, Husemann T (1884) Die Pflanzenstoffe in chemischer, physiologischer, pharmakologischer und toxikologischer Hinsicht, vol 2. Verlag von Julius Springer, Berlin, Germany, pp 1138–1145
Iorizzi M, Lanzotti V, De Marino S, Zollo F, Blanco-Molina M, Macho A, Muñoz E (2001) New glycosides from *Capsicum annuum* L. var. *acuminatum*. Isolation, structure determination, and biological activity. J Agric Food Chem 49:2022–2029
Jackson DM, Peterson JK (2000) Sublethal effects of resin glycosides from the periderm of sweet potato storage roots on *Plutella xylostella* (Lepidoptera: Plutellidae). J Econ Entomol 93:388–393
Jackson DM, Severson RF, Sisson VA, Stephenson MG (1991) Ovipositional response of tobacco budworm moths (Lepidoptera: Noctuidae) to cuticular labdanes and sucrose esters from the green leaves of *Nicotiana glutinosa* L. (Solanaceae). J Chem Ecol 17:2489–2506
Jaretzky R, Risse E (1940a) Radix und Herba Convolvuli sepii ein einheimischer Ersatz für Tubera Jalapae. Arch Pharm 278:241–252

Jaretzky R, Risse E (1940b) Beiträge zur Chemie verschiedener Convolvulaceenharze. Arch Pharm 278:379–389

Jayaprakasam B, Strasburg GA, Nair MG (2004) Potent lipid peroxidation inhibitors from *Withania somnifera* fruits. Tetrahedron 60:3109–3121

Johnston JFW (1840) Philos Trans Roy Soc, London A 341; fide Noda et al. (1990)

Judd WS, Campbell CS, Kellogg EA, Stevens PF (1999) Plant systematics – a phylogenetic approach. Sinauer Associates, Inc, Sunderland, MA, USA, p 359

Kawasaki T (1950) Structure of convolvulinolic acid and its related compounds. Yakugaku Zasshi 70:485–490

Kawasaki T, Okabe H, Nakatsuka I (1971) Studies on resin glycosides. I. Reinvestigation of the components of pharbitin, a resin glycoside of the seeds of *Pharbitis nil* CHOISY. Chem Pharm Bull 19:1144–1149

Kayser GA (1844) Chemische Untersuchung des Jalappenharzes. Liebigs Ann Chem 51:81–105

Khanna SN, Gupta PC (1967) Structure of muricatin. Phytochemistry 6:735–739

King RR, Singh RP, Calhoun LA (1987) Isolation and characterization of 3,3′,4,6-tetra-*O*-acylated sucrose esters from the type B glandular trichomes of *Solanum berthaultii* HAWKES (PI 265857). Carbohydrate Res 166:113–121

King RR, Calhoun LA (1988) 2,3-Di-*O*- and 1,2,3-tri-*O*-acylated glucose esters from the glandular trichomes of *Datura metel*. Phytochemistry 27:3761–3763

King RR, Calhoun LA, Singh RP, Boucher A (1990) Sucrose esters associated with glandular trichomes of wild *Lycopersicon* species. Phytochemistry 29:2115–2118

King RR, Calhoun LA, Singh RP, Boucher A (1993) Characterization of 2,3,,4,3′-tetra-*O*-acylated sucrose esters associated with the glandular trichomes of *Lycopersicon typicum*. J Agric Food Chem 41:469–473

Kitagawa I, Shibuya H, Yokokawa Y, Baek NI, Ohashi K, Yoshikawa M, Nitta A, Wiriadinata H (1988) Structures of merremosides B and D, new antiserotonic resin-glycosides from the tuber of *Merremia mammosa*, an Indonesian folk medicine. Chem Pharm Bull 36:1618–1621

Kitagawa I, Baek NI, Ohashi K, Sakagami M, Yoshikawa M, Shibuya H (1989a) Mammosides B and H1, new ionophoric resin-glycosides from the tuber of *Merremia mammosa*, an Indonesian folk medicine. Chem Pharm Bull 37:1131–1133

Kitagawa I, Ohashi K, Koyama W, Kawanishi H, Yamamoto T, Nishino T, Shibuya H (1989b) A new method for measuring ionophoretic activity using a glass-cell apparatus equipped with artificial membranes. Chem Pharm Bull 37:1416–1418

Kitagawa I, Ohashi K, Kawanishi H, Shibuya H, Shinkai K, Akedo H (1989c) Ionophoretic activities of oligopeptide lactones and resin-glycosides in human erythrocytes. Chem Pharm Bull 37:1679–1681

Kitagawa I, Baek NI, Kawashima K, Yokokawa Y, Yoshikawa M, Ohashi K, Shibuya H (1996a) Indonesian medicinal plants. XV. Chemical structures of five new resin-glycosides, merremosides a, b, c, d, and e, from the tuber of *Merremia mammosa* (Convolvulaceae). Chem Pharm Bull 44:1680–1692

Kitagawa I, Baek NI, Kawashima K, Yokokawa Y, Yoshikawa M, Ohashi K, Shibuya H (1996b) Indonesian medicinal plants. XVI. Chemical structures of four new resin-glycosides, merremosides f, g, h_1, and h_2, from the tuber of *Merremia mammosa* (Convolvulaceae). Chem Pharm Bull 44:1693–1699

Kitagawa I, Ohashi K, Baek NI, Sakagami M, Yoshikawa M, Shibuya H (1997) Indonesian medicinal plants. XIX. Chemical structures of four additional resin-glycosides, mammosides A, B, H_1, and H_2, from the tuber of *Merremia mammosa* (Convolvulaceae). Chem Pharm Bull 45:786–794

Kogetsu H, Noda N, Kawasaki T, Miyahara K (1991) Scammonin III–VI, resin glycosides of *Convolvulus scammonia*. Phytochemistry 30:957–963

Koiwai A, Matsuzaki T (1988) Hydroxy and normal fatty acid distribution in stigmas of *Nicotiana* and other plants. Phytochemistry 27:2827–2830

Koiwai A, Suzuki F, Matsuzaki T, Kawashima N (1983) The fatty acid composition of seeds and leaves of *Nicotiana* species. Phytochemistry 22:1409–1412

Kosteletzky VF (1834) Allgemeine medizinisch-pharmazeutische Flora, vol 3. Verlag von Heinrich Hoff, Mannheim, Germany, pp 854–868

Kromer N (1901) Ueber die Bildung von α-Methyl-β-Oxybuttersäure $CH_3CH(OH)CH(CH_3)COOH$ bei der Einwirkung von Barythydrat auf Jalapin. Arch Pharm 239:373–384

Kupchan SM, Davies AP, Barbouts SJ, Schnoes HK, Burlingame AL (1969) Solapalmitine and solapalmitenine, two novel alkaloid tumor inhibitors from *Solanum tripartitum*. J Org Chem 34:3888–3893

Lawson EN, Jamie JF, Kitching W (1992) Absolute stereochemistry of exogonic acid. J Org Chem 57:353–358

Legler G (1964) Die Bestandteile des giftigen Glykosidharzes aus *Ipomoea fistulosa* Mart. et Chois. Phytochemistry 4:29–41

Lemieux RU (1951) Biochemistry of the Ustilaginales. III. The degradation products and proof of the chemical heterogeneity of ustilagic acid. Can J Chem 29:415–425

León I, Enriquez RG, Gnecco D, Villarreal ML, Cortés, DA, Reynolds WF, Yu M (2004) Isolation and characterization of five new tetrasaccharide glycosides from the roots of *Ipomoea stans* and their cytotoxic activity. J Nat Prod 67:1552–1556

León I, Enriquez RG, Nieto DA, Alonso D, Reynolds WF, Aranda E, Villa J (2005) Pentasaccharide glycosides from the roots of *Ipomoea murucoides*. J Nat Prod 68:1141–1146

León I, Mirón G, Alonso D (2006) Characterization of pentasaccharide glycosides from the roots of *Ipomoea arborescens*. J Nat Prod 69:896–902

MacLeod JK, Ward A, Oelrichs PB (1997) Structural investigation of resin glycosides from *Ipomoea lonchophylla*. J Nat Prod 60:467–471

Maestri DM, Guzman CA (1991) Characteristics of seed oils of *Nierembergia aristata* and *Bouchetia anomala* (Solanaceae). Anal Asoc Quim Argentina 79:251–257

Maestri DM, Zygadlo JA, Gunzman CA (1992) General composition of seed oils from Lycieae, Jaboroseae and Nicandreae species (Solanaceae). Anal Asoc Quim Argentina 80:439–443

Maestri DM, Lamarque AL, Zygadlo JA, Grosso NR, Bernardello LM, Galetto L, Guzman CA (1994) Seed oil and protein in Lycieae (Solanaceae). Anal Asoc Quim Argentina 82:237–241.

Maldonado E, Torres FR, Martínez M, Pérez-Castorena AL (2006) Sucrose esters from the fruits of *Physalis nicandroides* var. *attenuata*. J Nat Prod 69:1511–1513

Maneerat C, Hayata Y, Kozuka H, Sakamoto K, Osajima Y (2002) Application of the Porak Q column extraction method for tomato flavour volatile analysis. J Agric Food Chem 50:3401–3404

Mannich C, Schumann P (1938) Über Jalapenharz und dessen Hauptbestandteil, das Convolvulin. Arch Pharm 276:211–226

Mao L, Story RN, Hammond AM, Peterson JK, Labonte DR (2001) Effect of nitrogen on restistance of sweet potato to sweetpotato weevil (Coleoptera: Curculionidae) and on storage root chemistry. J Econ Entomol 94:1285–1291

Mao L, Jett L, Story RN, Hammond AM, Peterson JK, Labonte DR (2004) Influence of drought stress on sweetpotato resistance to sweetpotato weevil, *Cyclas formicarius* (Coleoptera: Curculionidae), and storage root chemistry. Florida Entomol 87:261–267

Matsuzaki T, Koiwai A, Kawashima N (1983) Isolation of tetra-, penta-, hexa- and heptaacyl glycerides from stigmas of *Nicotiana tabacum*. Agric Biol Chem 47:77–82

Matsuzaki T, Koseki K, Kawashima N (1988) Germination and growth inhibition of surface lipids from *Nicotiana* species and identification of sucrose esters. Agric Biol Chem 52:1889–1897

Matsuzaki T, Shinozaki Y, Suhara S, Tobita T, Shigematsu H, Koiwai A (1991) Leaf surface glycolipids from *Nicotiana acuminata* and *Nicotiana pauciflora*. Agric Biol Chem 55:1417–1419

Mayer W (1852) Ueber das Jalappaharz. Liebigs Ann Chem 83:121–153

Mayer W (1854) Vorläufige Notiz über zwei homologe Glucoside. Liebigs Ann Chem 92:125–129

Mayer W (1855) Ueber die sogenannten Jalappaharze. Liebigs Ann Chem 95:129–176

Meeuse ADJ, Welman WG (2000) Convolvulaceae. In: Germishuizen G (ed) Flora of Southern Africa, vol 28, part 1. National Botanical Institute, Pretoria, South Africa, pp 116–117

Miersch O, Knöfel HD, Schmidt J, Kramell R, Parthier B (1998) A jasmonic acid conjugate, *N*-[(−)-jasmonoyl]-tyramine, from *Petunia* pollen. Phytochemistry 47:327–329

Mirón-López G, Herrera-Ruiz M, Estrada-Soto S, Aguirre-Crespo F, Vázquez-Navarrete L, León-Rivera I (2007) Resin glycosides from the roots of *Ipomoea tyrianthina* and their biological acitivity. J Nat Prod 70:557–562

Misra AL, Tewari JD (1952) Chemical examination of seeds of *Ipomoea muricata*. III. J Indian Chem Soc 29:430–433

Misra AL, Tewari JD (1953) Chemical examination of *Ipomoea muricata* seeds. IV. J Indian Chem Soc 30:391–397

Miyahara K, DU XM, Watanabe M, Sugimura C, Yahara S, Nohara T (1996) Resin glycosides. XXIII. Two novel acylated trisaccharides related to resin glycoside from the seeds of *Cuscuta chinensis*. Chem Pharm Bull 44:481–485

Molyneux RJ, Nash RJ, Asano N (1996) The chemistry and biological activity of calystegines and related *nor*tropane alkaloids. In: Pelletier SW (ed) Alkaloids: chemical and biological perspectives, vol 11. Pergamon/Elsevier Science, London, pp 303–343

Moser D, Klaiber I, Vogler B, Kraus W (1999) Molluscicidal and antibacterial compounds from *Petunia hybrida*. Pesticide Sci 55:336–339

Nair GG, Daniel M, Sabnis SD (1986) Chemosystematics of *Ipomoea* Linn. and some related taxa. Curr Sci 55:961–965

Nikolin A, Nikolin B, Janković M (1978) Ipopurpuroside, a new glycoside from *Ipomoea purpurea*. Phytochemistry 17:451–452

Noda N, Ono M, Miyahara K, Kawasaki T, Okabe M (1987) Resin glycosides. I. Isolation and structure elucidation of orizabin I, II, III and IV. Genuine resin glycosides from the root of *Ipomoea orizabensis*. Tetrahedron 43:3889–3902

Noda N, Kobayashi H, Miyahara K, Kawasaki T (1988a) Resin glycosides. II Identification and characterization of the component organic and glycosidic acids of the crude resin glucoside from the seeds of *Ipomoea muricata*. Chem Pharm Bull 36:627–633

Noda N, Kobayashi H, Miyahara K, Kawasaki T (1988b) Resin glycosides. III. Isolation and structural study of the genuine resin glycosides, muricatins I–VI, from the seeds of *Ipomoea muricata*. Chem Pharm Bull 36:920–929

Noda N, Nishi M, Miyahara K, Kawasaki T (1988c) Resin glycosides. IV. Two new resin glycosides, muricatins VII and VIII, from the seeds of *Ipomoea muricata*. Chem Pharm Bull 36:1707–1713

Noda N, Kogetsu H, Kawasaki T, Miyahara K (1990) Resin glycosides. VI. Scammonins I and II, the resin glycosides of Radix Scammoniae from *Convolvulus scammonia*. Phytochemistry 29:3565–3569

Noda N, Kogetsu H, Kawasaki T, Miyahara K (1992a) Resin glycosides. XII. Scammonins VII and VIII, two resin glycosides from *Convolvulus scammonia*. Phytochemistry 31: 2761–2766

Noda N, Yoda S, Kawasaki T, Miyahara K (1992b) Resin glycosides. XV. Simonins I–V, ether-soluble resin glycosides (jalapins) from the roots of *Ipomoea batatas* (cv Simon). Chem Pharm Bull 40:3163–3168

Noda N, Takahashi N, Kawasaki T, Miyahara K, Yang CR (1994a) Stoloniferins I–VII, resin glycosides from *Ipomoea stolonifera*. Phytochemistry 36:365–371

Noda N, Tsuji K, Miyahara K, Yang CR (1994b) Resin glycosides. XXI. Tuguajalapins I–X, the resin glycosides having long-chain fatty acid groups from the root of *Merremia hungaiensis*. Chem Pharm Bull 42:2011–2016

Noda K, Tsuji K, Kawasaki T, Miyahara K, Hanazono H, Yang CR (1995) Resin glycosides. XXII. A novel resin glycoside, merremin (tuguajalapin X dimer), from *Merremia hungaiensis*. Chem Pharm Bull 43:1061–1063

Noda N, Takahashi N, Miyahara K, Yang CR (1998) Stoloniferins VIII–XII, resin glycosides from *Ipomoea stolonifera*. Phytochemistry 48:837–841

Okabe H, Kawasaki T (1972) Studies on resin glycosides. III. Complete structures of pharbitic acids C and D. Chem Pharm Bull 20:514–520

Okabe H, Koshito N, Tanaka K, Kawasaki T (1971) Studies on resin glycosides. II. Unhomogeneity of "pharbitic acid" and isolation and partial structures of pharbitic acids C and D, the major constituents of "pharbitic acid". Chem Pharm Bull 19:2394–2403

Ono M, Kubo K, Miyahara K, Kawasaki T (1989a) Operculin I and II, new ether-soluble resin glycosides ("jalapin") with fatty acid ester groups from Rhizoma Jalapae Brasiliensis (roots of *Ipomoea operculata*). Chem Pharm Bull. 37:241–244

Ono M, Kawasaki T, Miyahara K (1989b) Resin glycosides. V. Identification and characterization of the component organic and glycosidic acids of the ether-soluble crude resin glycosides ("jalapin") from Rhizoma Jalapae Brasiliensis (roots of *Ipomoea operculata*). Chem Pharm Bull 37: 3209–3213

Ono M, Noda N, Kawasaki T, Miyahara K (1990a) Resin glycosides. VII. Reinvestigation of the component organic and glycosidic acids of pharbitin, the crude ether-insoluble resin glycoside ("convolvulin") of Pharbitidis semen (seeds of *Pharbitis nil*). Chem Pharm Bull 38:1892–1897

Ono M, Fukunaga T, Kawasaki T, Miyahara K (1990b) Resin glycosides. VIII. Four new glycosidic acids, operculinic acids D, E, F, and G, of the ether-soluble crude resin glycosides ("jalapin") from Rhizoma Jalapae Brasiliensis (roots of *Ipomoea operculata*). Chem Pharm Bull 38:2650–2655

Ono M, Nishi M, Kawasaki T, Miyahara K (1990c) Resin glycosides. IX. Operculins I, II, V, VII and VIII, new ether-soluble resin glycosides of Rhizoma Jalapae Brasiliensis (the roots of *Ipomoea operculata*). Chem Pharm Bull 38:2986–2991

Ono M, Kawasaki T, Miyahara K (1991) Resin glycosides. XI. Operculins III, IV, IX, X, XVI, XVII and XVIII, new ether-soluble resin glycosides of Rhizoma Jalapae Brasiliensis (root of *Ipomoea operculata*). Chem Pharm Bull 39:2534–2539

Ono M, Fujimoto K, Kawata M, Fukunaga T, Kawasaki T, Miyahara K (1992a) Resin glycosides. XIII. Operculins VI, XI, XII, XIII, XIV and XV, the ether-soluble resin glycosides ("jalapin") from Rhizoma Jalapae Brasiliensis (roots of *Ipomoea operculata*). Chem Pharm Bull 40:1400–1403

Ono M, Kuwabata K, Kawasaki T, Miyahara K (1992b) Resin glycosides. XIV. Quamoclins I–IV, new ether-soluble resin glycosides (jalapin) from seeds of *Quamoclit pennata*. Chem Pharm Bull 40:2674–2680

Ono M, Ueguchi T, Murata H, Kawasaki T, Miyahara K (1992c) Resin glycosides. XVI. Marubajalapins I–VII, new ether-soluble resin glycosides from *Pharbitis purpurea*. Chem Pharm Bull 40:3169–3173

Ono M, Ueguchi T, Kawasaki T, Miyahara K (1992d) Resin glycosides. XVII. Marubajalapins VIII–XI, jalapins from the aerial part of *Pharbitis purpurea*. Yakugaku Zasshi 112:866–872

Ono M, Yamada F, Noda N, Kawasaki T, Miyahara K (1993a) Resin glycosides. XVIII. Determination by Mosher's method of the absolute configurations of mono- and dihydroxy fatty acids originated from resin glycosides. Chem Pharm Bull 41:1023–1026

Ono M, Nakagawa K, Kawasaki T, Miyahara K (1993b) Resin glycosides. XIX. Woodrosins I and II, ether-insoluble resin glycosides from the stems of *Ipomoea tuberosa*. Chem Pharm Bull 41:1925–1932

Ono M, Honda F (née Yamada), Karahashi A, Kawasaki T, Miyahara K (1997) Resin glycosides. XXV. Multifidins I and II, new jalapins, from the seed of *Quamoclit* × *multifida*. Chem Pharm Bull 45:1955–1960

Osol A, Farrar GF (eds) (1947) United States Dispensatory, 24th edn. JB Lippincott Company

Osol A, Robertson P, Altschule MD (eds) (1967) United States Dispensatory, 26th edn. JB Lippincott Company

Ovenden SPB, Yu J, Bernays J, Wan SS, Christophidis LJ, Sberna G, Tait RM, Wildman HG, Lebeller D, Lowther J, Walsh NG, Meurer-Grimes BM (2005) Physaloside A, an acylated sucrose ester from *Physalis viscosa*. J Nat Prod 68:282–284

Paschold A, Halitschke R, Baldwin IT (2006) Using 'mute' plants to translate volatile signals. Plant J 45:275–291

Pereda-Miranda R (1995) Bioactive natural products from traditionally used Mexican plants. In: Arnason JT, Mata R, Romeo JT (eds) Phytochemistry of medicinal plants. Plenum Press, New York, pp 83–112

Pereda-Miranda R, Bah M (2003) Biodynamic constituents in the Mexican morning glories: Purgative remedies transcending boundaries. Curr Top Med Chem 3:111–131

Pereda-Miranda R, Hernández-Carlos B (2002) HPLC isolation and structural elucidation of diastereomeric niloyl ester tetrasaccharides from Mexican scammony root. Tetrahedron 58:31453154

Pereda-Miranda R, Mata R, Anaya AL, Wickramaratne DBM, Pezzuto JM, Kinghorn AD (1993) Tricolorin A, major phytogrowth inhibitor from *Ipomoea tricolor*. J Nat Prod 56:571–582

Pereda-Miranda R, Escalante-Sánchez E, Escobedo-Martínez C (2005) Characterization of lipophilic pentasaccharides from beach morning glory (*Ipomoea pes-caprae*). J Nat Prod 68:226–230; Erratum (2006): J Nat Prod 69:862

Pereda-Miranda R, Kaatz GW, Gibbons S (2006a) Polyacylated oligosaccharides from medicinal Mexican morning glory species as antibacterials and inhibitors of multidrug resistance in *Staphylococcus aureus*. J Nat Prod 69:406–409

Pereda-Miranda R, Fragoso-Serrano M, Escalante-Sánchez E, Hernández-Carlos B, Linares E, Bye R (2006b) Profiling of the resin glycoside content of Mexican jalap roots with purgative activity. J Nat Prod 69:1460–1466

Pérez-Amador MC, García Argáez A, Amor Prats D, Murguía G, García Jiménez F, Márquez Alonso LC (1992) Estudio comparativo de asceites de semillas de seis especies de *Ipomoea* del grupo *Arborescentes* y de *I. carnea* Jacq. ΦYTON 53:71–75

Peterson JK, Harrison HF Jr (1991) Isolation of substance from sweet potato (*Ipomoea batatas*) periderm tissue that inhibits seed germination. J Chem. Ecol 17:943–951

Peterson JK, Snook ME, Harrison HF Jr, Mason PF (1998) Isolation and structural identification of sucrose esters from corn spurrey (*Spergula arvensis*): inhibition of seed germination. J Chem Ecol 24:1803–1816

Pharmacopoea Germanica (1872) R v Decker's Verlag, Berlin, Germany

Pongprayoon U, Baeckström P, Jacobsson U, Lindström M, Bohlin L (1991) Compounds inhibiting prostaglandin synthesis isolated from *Ipomoea pes-caprae*. Planta Med 57:515–518

Power FB, Rogerson H (1909) Chemical examination of *Ipomoea purpurea* Roth. Am J Pharmacy 80:251–286

Power FB, Rogerson H (1910) Chemical examination of jalap. J Am Chem Soc 32:80

Power FB, Rogerson H (1912a) Chemical examination of the root of *Ipomoea orizabensis*. J Chem Soc, Transact 101:1–26

Power FB, Rogerson H (1912b) Chemical examination of the root of scammony root and of scammony. J Chem Soc, Proc 101:398–412

Raguso RA, Levin RA, Foose SE, Holmerg MW, McDade LA (2003) Fragrance chemistry, nocturnal rhythms and pollination "syndromes" in *Nicotiana*. Phytochemistry 63:265–284

Ramachandran R, Ali M (2003) Isolation and characterization of acyclic terpenic constituents from *Cressa cretica* aerial parts. J Med Arom Plant Sci 25:81–90

Ramachandran R, Ali M, Mir SR (2004) Isolation and characterization of aliphatic constituents from *Cressa cretica* aerial parts. J Saudi Chem Soc 8:523–530

Rencurosi A, Mitchell EP, Cioci G, Pérez S, Pereda-Miranda R, Imberty A (2004) Crystal structure of tricolorin A: molecular rationale for the biological properties of resin glycosides found in some Mexican herbal remedies. Angew Chem Int Ed 43:5918–5922

Reynolds WF, Yu M, Enriquez RG, Gonzalez H, León I, Magos G, Villareal ML (1995) Isolation and characterization of cytotoxic and antibacterial tetrasaccharide glycosides from *Ipomoea stans*. J Nat Prod 58:1730–1734

Rivero-Cruz I, Acevedo L, Guerrero JA, Martinez S, Bye R, Pereda-Miranda R, Franzblau S, Timmermann BN, Mata R (2005) Antimycobacterial agents from selected Mexican medicinal plants. J Pharm Pharmacol 57:1117–1126

Sahai M, Manickam M, Gupta M, Srivastava A, Ray AB (1999) Characterisation of a cerebroside isolated from the leaves of *Datura metel*. J Indian Chem Soc 76:95–97

Samuelson C (1884) Chem Ztg 1543; fide Shellard (1961a)

Sarin JPS, Garg HS, Khanna NM, Dhar MM (1973) Ipolearoside: a new glycoside from *Ipomoea leari* with anti-cancer activity. Phytochemistry 12:2461–2468

Saxena SC, Sumithra L (1985) Laboratory evaluation of leaf extract of a new plant to suppress the population of malaria vector *Anopheles stephensii* Liston (Diptera: Culicidae). Curr Sci 54:201–202

Severson RF, Arrendale RF, Chortyk OT, Green CR, Thome FA, Stewart JL, Johnson AW (1985) Isolation and characterization of the sucrose esters of the cuticular waxes of green tobacco leaf. J Agric Food Chem 33:870–875

Severson RF, Jackson DM, Johnson AW, Sisson VA, Stephenson MG (1991) Ovipositional behaviour of tobacco budworm and tobacco hornworm. Effects of cuticular components from *Nicotiana* species. ACS Symposium Ser vol. 449, American Chemical Society, Washington, DC, pp 264–277

Severson RF, Eckel RVW, Jackson DM, Sisson VA, Stephenson MG (1994) Aphicidal activity of cuticular components from *Nicotiana tabacum*. ACS Symposium Ser vol. 551, American Chemical Society, Washington/DC, pp 172–179

Shellard EJ (1961a) The chemistry of some convolvulaceous resins. Part I. Vera Cruz Jalap. Planta Med 9:102–116

Shellard EJ (1961b) The chemistry of some convolvulaceous resins. Part 2. Brazilian Jalap. Planta Med 9:141–145

Shellard EJ (1961c) The chemistry of some convolvulaceous resins. Part III. Tampico, Ipomoea and Scammonia resins. Planta Med 9:146–152

Shibuya H, Kawashima K, Baek NI, Narita N, Yoshikawa M, Kitagawa I (1989) Synthesis of (11S)-(+)- and (11R)-(−)-jalapinolic acids. A revision of chemical structures of merremosides B and D. Chem Pharm Bull 37:260–262

Šimko I, Omer EA, Ewing EE, McMurry S, Koch JL, Davies PJ (1996) Tuberonic (12-OH-jasmonic) acid glucoside and its methyl ester in potato. Phytochemistry 43:727–730

Singh AP, Singh AK, Begum AS, Sahai M (2003) Two acyl sucroses from *Petunia nyctaginiflora*. Phytochemistry 63:485–489

Singh S, Stacey BE (1973) A new β-D-quinovoside from commercial *Ipomoea purga*. Phytochemistry 12:1701–1705

Smith CR Jr, Niece LH, Zobel HF, Wolff IA (1964) Glycosidic constituents of *Ipomoea parasitica* seed. Phytochemistry 3:289–299

Son KC, Severson RF, Pair SD, Kays SJ (1994) Comparison of the sucrose ester fatty acid components in flowers and flower buds of three *Petunia* × *hybrida* Hort. cultivars. Han'guk Wonye Hakhoechi 35:617–622

Spirgatis H (1858) N R P 7:9; fide Shellard (1961c)

Spirgatis H (1860) Ueber die Constitution des Scammoniumharzes. Liebigs Ann Chem 116:289–323

Spirgatis H (1870) N R P 19:452; fide Shellard (1961c)

Srivastava R, Sachdev K, Madhusudanan KP, Kulshreshtha DK (1991) Structure of pescaproside E, a fatty acid glycoside from *Ipomoea pescaprae*. Carbohydr Res 212:169–176

Steffens JC, Walters DS (1991) Biochemical aspects of glandular trichome-mediated insect resistance in the Solanaceae. ACS Symposium Ser vol. 449, American Chemical Society, Washington, DC, pp 136–149

Su BN, Misico R, Park EJ, Santarsiero BD, Mesecar AD, Fong HHS, Pezzuto JM, Kinghorn AD (2002) Isolation and characterization of bioactive principles of the leaves and stems of *Physalis philadelphica*. Tetrahedron 58:3453–3466

Tewari JP, Dutta KC, Mishra SS (1964) Phytochemical and pharmacological investigations of *Ipomoea carnea* (leaves). Labdev No 2:220–222

Umehara K, Nemoto K, Ohkubo T, Miyase T, Degawa M, Noguchi H (2004) Isolation of a new 15-membered macrocyclic glycolipid lactone, cuscutic resinoside A from the seeds of *Cuscuta chinensis*: A stimulator of breast cancer cell proliferation. Planta Med 70:299–304

Valette G (1937a) Hydrotropic action of Convolvulaceae resins on lecithin. Compt Rend Soc Biol 125:405–407
Valette G (1937b) Hemolytic power of Convolvulaceae resins and their products of hydrolysis. Compt Rend Soc Biol 125:407–409
Valette G, Liber A (1938) Bactericidal power of Convolvulaceae resin. Compt Rend Soc Biol 128:362–363
van Ooststroom SJ, Hoogland RD (1953) Convolvulaceae. In: van Steenis CGGJ (ed) Flora Malesiana, ser I, vol 4^4, Noordhoff-Kolff, Djakarta/Indonesia, pp 389–512
Villatoro-Vera RA, Bah M, Lorence A, Pereda-Miranda R (2004) Convolvulaceous resin glycosides induce non-selective pore formation in cell membranes. International Congress on Natural Products Research, Phoenix, AZ, USA, Book of Abstracts p 332, P369
Votoček E (1901) Rhodeose, eine Methylpentose des Convolvulins. Z. Zuckerind Böhmens 25:297–305
Votoček E (1910) Über die Glykosidsäuren des Convolvulins und die Zusammensetzung der rohen Isorhodeose. Ber 43:476–482
Votoček E, Kastner J (1907) Ein neues Rhamnosid aus *Ipomoea turpethum*. Z Zuckerind Böhmens 31:307–316
Votoček E, Prelog V (1929) Sur l'acide 3,12-dioxypalmitique, composant de l'acide rhamnoconvolvulique. Coll Trav Chim Tchécoslovaq 1:55–64
Votoček E, Vondraček R (1903) Über die Zucker des Jalapins und anderer vegetabilischer Glykoside. Z Zuckerind Böhmens 27:257–271, 333–340
Vyvyan JR (2002) Allelochemicals as leads for new herbicides and agrochemicals. Tetrahedron 58:1631–1646
Wagner H, Kazmaier P (1977) Struktur der Operculinsäure aus dem Harz von *Ipomoea operculata*. Phytochemistry 16:711–714
Wagner H, Schwarting G (1977) Struktur der Microphyllinsäure aus dem Harz von *Convolvulus microphyllus*. Phytochemistry 16:715–717
Wagner H, Wenzel G, Chari VM (1978) The turpethinic acids of *Ipomoea turpethum* L. Planta Med 33:144–151
Wagner H, Schwarting G, Varljen J, Bauer R, Hamdard ME, El-Faer MZ, Beal J (1983) Die chemische Zusammensetzung der Convolvulaceen-Harze. IV. Die Glykosidsäuren von *Ipomoea quamoclit*, *I. lacunosa*, *I. pandurata* und *Convolvulus al-sirensis*. Planta Med 49:154–157
Walker D, Bird A, Flora T, O'Sullivan B (1992) Some effects of feeding *Tribulus terrestris*, *Ipomoea lonchophylla*, and the seed of *Abelmoschus ficulneus* on fetal development and the outcome of pregnancy in sheep. Reprod Fert Develop 4:135–144
Williams LO (1970) Jalap or Veracruz jalap and its allies. Econ Bot 24:399–401
Yokoyama R, Wada K (1987) Pharbitin content in *Pharbitis nil*. Rep Fac Sci, Shizuoka Univ 21:77–88
Zhu W, Yang X, He H, Hao X (2000) Phytoecdysones from *Porana discifera*. Yunnan Zhiwu Yanjiu 22:351–357

Appendix
Color Plates of Solanales Species

The first half of the color plates (Plates 1–8) shows a selection of phytochemically prominent solanaceous species, the second half (Plates 9–16) a selection of convolvulaceous counterparts. The scientific name of the species in bold (for authorities see text and tables) may be followed (in brackets) by a frequently used though invalid synonym and/or a common name if existent. The next information refers to the habitus, origin/natural distribution, and – if applicable – cultivation. If more than one photograph is shown for a certain species there will be explanations for each of them.

Finally, section numbers of the phytochemical Chapters 3–8 are given, where the respective species are discussed. The individually combined occurrence of secondary metabolites from different structural classes characterizes every species. However, it has to be remembered that a small number of citations does not necessarily indicate a poorer secondary metabolism in a respective species compared with others; this may just be due to less studies being carried out.

Solanaceae

Plate 1a ***Anthocercis littorea*** (yellow tailflower): erect or rarely sprawling shrub (to 3 m); W- and SW-Australia; Sects. 3.1 / 3.4

Plate 1b, c ***Atropa belladonna*** (deadly nightshade): erect herbaceous perennial plant (to 1.5 m); Europe to central Asia (naturalized: N-USA; cultivated as a medicinal plant); **b** fruiting twig; **c** flowers, unripe (green) and ripe (black) berries; Sects. 3.1 / 3.3.2 / 3.4 / 3.5 / 6.5.2 / 7.5.1 / 7.7.2 / 7.7.4.3

Plate 1d ***Brugmansia versicolor*** (angel's trumpet): shrub or small tree (to 5 m); tropical parts of Ecuador west of the Andes (cultivated as an ornamental in tropical and subtropical regions); Sect. 3.4

Plate 2a ***Brunfelsia pauciflora*** (yesterday-today-tomorrow): shrub (to 2.4 m); Brazil (cultivated as an ornamental in tropical and subtropical regions); Sects. 3.1 / 6.6.2 / 6.7.3.2

Plate 2b *Capsicum annuum* var. *frutescens* (chili, Cayenne pepper): suffrutex (to 1.5 m); Neotropics, only known as a cultivated plant related to the wild species *C. chacoënse* and *C. annuum* var. *glabriusculum* (cultivated pantropically for the commercial production of pungent fruits); Sects. 3.3.2.2 / 3.4 / 3.5 / 6.3.4.1 / 6.4 / 6.6.3 / 6.7.1 / 7.2.1.1 / 7.3.1.1 / 7.3.1.2 / 7.4.1.2 / 7.5.1 / 7.6.1 / 7.7.1.3 / 7.7.2 / 7.7.4.2 / 7.7.4.3 / 7.8.2.2 / 7.12.1 / 8.1.1.1

Plate 2c *Cestrum diurnum* (day blooming Cestrum, day jessamine): shrub; tropical S-America (cultivated as an ornamental in tropical and subtropical regions); Sects. 3.3 / 6.7.1.1 / 7.7.2 / 7.8.2.2 / 7.9.3 / 7.9.3.1

Plate 2d *Cestrum elegans:* shrub; Mexico (cultivated as an ornamental in tropical and subtropical regions); Sects. 6.6.3.1 / 6.7.1.1 / 7.7.2 / 7.8.2.2 (Table 7.7)

Plate 2e *Cestrum parqui* (willow-leafed jessamine, green poisonberry): shrub (to 3 m); S-America (cultivated as an ornamental in subtropical regions, sometimes naturalized); Sects. 6.3.1.1 / 6.3.2.2 / 6.3.3.1 / 6.6.4.1 / 6.7.1.1 / 6.8.1.1 / 7.4.1.1 / 7.5.1 / 7.7 / 7.7.1.1 / 7.7.2 / 7.8.2.2 (Table 7.7) / 7.12.1.2

Plate 3a *Datura stramonium* (common thornapple, jimson weed): erect annual herb (to 1.2 m); temperate to tropical regions worldwide (cultivated as a medicinal plant); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.1.1 / 6.6.2 / 6.6.3.1 / 7.2.1.1 / 7.3.1.2 / 7.5.1 / 7.10.3 (Table 7.9)

Plate 3b–d *Duboisia myoporoides* × *D. leichhardtii*: cultivated as bushes in Queensland, Australia; {[*D. myoporoides* (poisonous corkwood, yellow basswood): tree (to 15 m); Queensland]; [*D. leichhardtii* (common names like *D. myoporoides*): small tree (to 3 m); Queensland]}: **b** inflorescence; **c** single bush; **d** plantation near Murgon/Queensland; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 5.1.4 / 7.5.1

Plate 4a *Hyoscyamus albus* (white henbane): erect annual or biennial herb (to 0.8 m); S-Europe to Near East; Sects. 3.1 / 3.4 / 3.5 / 6.6.1.1 / 6.6.3.1 / 6.7.1.1 / 7.3.1.2 / 8.1.1.1

Plate 4b *Hyoscyamus niger* (black henbane): erect annual or biennial herb (to 0.8 m); Europe, N-Africa, N-Asia, N-India; Sects. 3.1 / 3.4 / 3.5 / 6.6.3.1 / 6.7.1.1 / 6.8.1.2 / 7.10.3 / 8.1.1.1

Plate 4c *Iochroma gesnerioides* (syn.: *I. coccineum, I. fuchsioides*): shrub (to 4 m); Columbia, Ecuador; Sects. 6.7.1.1 / 7.10.3 (Table 7.9)

Plate 4d *Nicandra physalodes* (apple of Peru): erect annual herb (to 2 m); Peru to N-Argentina (cultivated as an ornamental in tropical and subtropical regions; often naturalized); Sects. 3.1 / 3.4 / 3.5 / 6.7.1.1 / 7.8.2.2 / 7.9.5.1 / 7.10.2.2 / 7.10.3.1

Plate 4e *Nicotiana glauca* (tree tobacco): shrub/small tree (to 6 m); S-America, introduced to many subtropical regions, semideserts, dry open countries; Sects. 3.3 / 7.4.1.4 / 7.9.3.1

Plate 5a *Nicotiana langsdorffii* (green flowering tobacco): erect annual herb; Brazil, Chile; Sects. 3.3 / 6.3.3.1 / 7.2.1.1 / 7.4.1.4

Plate 5b *Nicotiana sylvestris*: perennial; one parent of *N. tabacum*; Bolivia; Sects. 3.3 / 6.3.3.1 / 7.2.1.1 / 7.3.1.2 / 7.4.1.2 / 7.4.1.4

Plate 5c *Nicotiana tabacum* (tobacco): erect annual herb (to 3 m); only known as a cultivated plant, assumed to be a hybrid of the wild S-American species *N. sylvestris* and *N. tomentosiformis* (*N. tabacum*: cultivated almost worldwide for the production of tobaccos); Sects. 3.3 / 3.5 / 5.1.3 / 6.3.3.1 / 6.5.1 / 6.6.2 / 6.6.3.1 / 6.6.3.2 / 6.6.4 / 6.6.4.1 / 6.6.4.3 / 6.7.1.1 / 6.7.3.1 / 7 / 7.3.1.1 / 7.3.1.2 / 7.4.1.1 / 7.4.1.4 / 7.5.1 / 7.6.1 / 7.7.2 / 7.12.1.2 / 8.1.1.1

Plate 5d *Petunia* × *hybrida* (petunia): small perennial herb, obtained as a hybrid of the wild S-American species *P. axillaris* and *P. integrifolia* (*P.* × *hybrida*: cultivated almost worldwide as an ornamental); Sects. 3.5 / 6.3.3 / 6.3.3.1 / 6.5.1 / 6.6.2 / 6.6.3.1 / 6.6.4 / 6.6.4.3 / 6.7 / 6.7.1.1 / 6.7.3.1 / 7.3.1.1 / 7.4.1.1 / 7.7.2 / 7.11 / 8.1.2.1

Plate 6a *Salpiglossis sinuata* (velvet flower, painted tongue, palito amargo): perennial herb (to 1.2 m); Chile (cultivated as an ornamental in temperate regions of the world); Sects. 3.3 / 6.7.1.1

Plate 6b *Schizanthus pinnatus* (butterfly flower, mariposita): annual herb (to 0.5 m); Chile (cultivated as an ornamental); Sects. 3.1 / 3.4 / 7.6.1

Plate 6c *Solandra maxima* (goldcup vine, chalice vine): large woody climber (to 30 m) of the tropical rainforest; Neotropics (cultivated as an ornamental in tropical and subtropical regions); Sects. 3.1 / 3.4

Plate 6d *Solanum betaceum* (syn.: *Cyphomandra betacea*; tree tomato, tamarillo): tree; Neotropics (cultivated in subtropical regions of the world for edible fruits); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 5.1.4 / 7.8.2.1 / 7.12.1.1

Plate 7a *Solanum laciniatum* (kangaroo apple): shrub (to 4 m); S-Australia; Sects. 3.1 (Table 3.1) / 7.8.2.1 / 7.8.3 / 7.8.4.4 (Table 7.8)

Plate 7b *Solanum laxum* (syn.: *Solanum jasminoides*; potato vine, jasmine nightshade): semi-evergreen climber; Argentina, Brazil, Paraguay, Uruguay (cultivated as an ornamental in tropical and subtropical regions, sometimes naturalized); Sects. 7.7 (Tables 7.1, 7.2) / 7.7.4.3 / 7.8.2.1

Plate 7c *Solanum pseudocapsicum* (Jerusalem cherry): shrub (to 1.2 m); S-America (cultivated worldwide as an ornamental due to its red/orange-coloured fruits); Sects. 7.8.1.2 / 7.8.1.5 / 7.8.2 (Table 7.3) / 7.8.4.2

Plate 7d *Solanum sisymbrifolium* (viscid nightshade): erect annual herb (to 1.5 m); S-America; Sects. 3.1 (Table 3.1) / 6.8.1.1 / 7.7 (Tables 7.1, 7.2) / 7.10 (Tables 7.9, 7.10)

Plate 7e *Solanum tuberosum* (potato): herbaceous annual, only known as a cultivated plant; Bolivian/Peruvian Andes (ssp. *andigenum*), Chile (ssp. *tuberosum*), for details see Sect. 7.8.2.1; cultivated worldwide (agricultural production of tubers); Sects. 3.3 / 3.4 / 3.5 / 5.1.6 / 5.1.7 / 6.3.3.1 / 6.5.1 / 6.6.1.1 / 6.6.3.1 / 6.6.4.3 / 6.7.1.1 / 6.7.3.1 / 6.8.1.1 / 7.3.1.1 / 7.3.1.2 / 7.6.1 / 7.7.1.1 / 7.7.2 / 7.8 / 7.8.1 / 7.8.1.1 / 7.8.1.2 / 7.8.1.9 / 7.8.1.10 / 7.8.2.1 / 7.8.4.1 / 7.8.4.2 / 7.8.4.3 / 7.9.3.1 / 7.12.1.2 / 8.1.1.1

Plate 8a, b *Solanum wrightii* (potato tree); forest tree (to 20 m); Bolivia (cultivated pantropically for ornament): **a** tree; **b** inflorescence; Sects. 7.7 (Tables 7.1, 7.2) / 7.8.1.9 / 7.8.2.1

Plate 8c *Streptosolen jamesonii* (firebush): shrub (to 2 m); Ecuador, Peru (cultivated as an ornamental in tropical and subtropical regions); Sect. 3.3

Plate 8d *Withania somnifera*: perennial suffrutex; Africa, S-Europe to S-Asia, China (cultivated in India as a medicinal plant); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 6.6.3.1 / 7.6.1 / 7.10.1 / 7.10.2.2 / 7.10.2.3 / 7.10.3.1 / 7.10.4 / 7.10.5 / 7.10.5.1 / 8.1.2.1

Convolvulaceae

Plate 9a *Aniseia martinicensis*: herbaceous vine (moist habitats); Neotropics, naturalized in the Paleotropics; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 5.2.2

Plate 9b *Argyreia capitata*: large twiner (to 15 m); SE-Asia; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 5.2.3 / 5.2.4 / 6.7.1.2 / 6.7.2.2 / 7.5.2

Plate 9c *Argyreia mollis*: large twiner (to 10 m); SE-Asia; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.2.3.4 / 5.2.3 / 5.2.4 / 6.7.2.1

Plate 9d *Argyreia nervosa* (Hawaiian baby woodrose): large twiner (to 10 m); India, Sri Lanka (cultivated, sometimes naturalized in other tropical regions); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.2.3.4 / 5.2.3 / 5.2.4 / 6.1.2 / 6.7.2.1 / 8.1.1.2

Plate 9e–g *Bonamia spectabilis*: climbing shrub; Zaire to E- and SE-Africa, Madagascar: **e**,**f** blue-blooming form; **g** white-blooming form; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 6.1.2 / 6.8.2.3 / 6.8.2.5

Plate 10a *Convolvulus cneorum* (silverbush): suffrutex; Italian and Balkan regions (ornamental cultivars: subtropical countries); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 6.6.4.1

Plate 10b *Convolvulus dorycnium*: shrub with many, closely branched, greenish shoots (to 1 m); the photograph shows a fruiting plant; Greece, N-Africa; Sects. 3.1 / 3.3 / 3.4

Plate 10c *Convolvulus sabatius* ssp. *mauritanicus* (blue rock bindweed): perennial herb; Italy, N-Africa (ornamental cultivars: subtropical and temperate countries); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 6.6.4.1

Plate 10d *Cuscuta australis* (dodder): annual parasitic vine with (due to carotenoids) yellowish herbaceous stems twining around the host plants attached to them by haustoria; found from S-Europe/Africa to Australia; Sects. 3.1/ 3.3 / 3.4 / 3.5 / 7.12.2.1 / 8.3.1.2 / 8.3.3

Plate 10e *Erycibe rheedii*: large woody climber (to 20 m); Major Sunda Islands, Malaysia (peninsula); Sects. 3.1 / 3.3 / 3.4 / 3.5

Plate 11a *Evolvulus alsinoides*: annual or suffrutescent herbs (to 60 cm); Neotropics, widely naturalized in the Paleotropics; Sects. 3.1 / 3.3 / 3.4

Plate 11b *Ipomoea alba* (syn.: *Calonyction bona-nox*; moonflower/moonvine): herbaceous vine (to 5 m), woody basally; Neotropics, widely naturalized in the Paleotropics (cultivated worldwide as an ornamental); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 3.6 / 4.2.3.2 / 4.2.3.3 / 6.1.2 / 6.2.2 / 6.7.3.2 / 6.8.2.2 / 7.4.2.1

Plate 11c *Ipomoea aquatica* (water spinach): herbaceous annual vine, rooting at nodes, procumbent on wet ground or floating on water; Paleotropics (cultivated as a vegetable especially in Asia; sometimes naturalized in the Neotropics); Sects. 3.1 / 3.2.2 / 3.3 / 3.4 / 3.5 / 4.2.3.2 / 5.2.4 / 6.6.4.1 / 7.12.2.1

Plate 11d, e *Ipomoea asarifolia*: climbing and prostrate herbaceous perennial long-trailing vines, rooting at the nodes; Neotropics, tropical Africa, tropical Asia: **d** white-blooming form; **e** purple-blooming form; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.2.3.1 / 4.2.3.6 / 4.2.4 / 6.6.1.2 / 6.7.3.2

Plate 11f, 12a, b *Ipomoea batatas* (sweet potato): perennial herb, prostrate, rooting at nodes or twining (to 5 m); wild form in the Neotropics, cultivated throughout tropical and subtropical regions of the world (agricultural production of tubers), sometimes cultivated for horticultural purposes (ornamental: flowers and/or leaves): **11f** wild form; **12a** cultivar for tuber production; **b** ornamental cultivars; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.1.2 / 4.2.3.3 / 5.2.4 / 6.2.2 / 6.3.4.2 (Appendix) / 6.5.2 / 6.6.1.2 / 6.6.3.2 / 6.7.3.2 / 7.2.2 / 7.3.1.2 / 7.3.2 / 7.5.2 / 7.6.2 / 7.12.2.1 / 7.12.3 / 8.2.2.1 / 8.3.1.2 / 8.3.2 / 8.3.3.1 / 8.3.3.3

Plate 12c *Ipomoea cairica* (railway creeper): herbaceous vine; tropical Africa and Asia (cultivated and naturalized in many tropical and subtropical regions); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.1.2 / 4.2.3.2 / 6.7.3.2 / 6.8.2.2

Plate 12d *Ipomoea carnea* ssp. *fistulosa*: erect shrub (to 3 m), woody basally, herbaceous at tips; Neotropics, meanwhile pantropical through cultivation (cultivated as an ornamental and/or as a fence); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 3.5.3 (Appendix) / 4.2.3.2 / 6.6.3.2 / 6.7.1.2 / 6.7.3.2 / 8.3.1.2 / 8.3.2 / 8.3.3.1 / 8.3.3.3

Plate 12e *Ipomoea hederifolia* (scarlet morning-glory): annual herbaceous vine; Neotropics, introduced into the Paleotropics; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 3.7.1.1 / 4.2.3.2 / 6.6.4.1 / 6.7.3.2 / 7.3.2.1 / 7.12.2.1 / 8.3.2

Plate 13a *Ipomoea hildebrandtii*: subwoody shrublet (to 2.4 m); E-Africa; Sects. 3.1 / 3.3 / 3.4 / 4.2.3.1 / 4.2.3.6 / 4.2.4

Plate 13b *Ipomoea lobata* (syn.: *Mina lobata*; Spanish flag, firecracker vine): annual or perennial herbaceous vine with zygomorphic, red corollas later becoming pale yellow or whitish; Neotropics (cultivated worldwide as an ornamental, sometimes naturalized); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 3.7.1.1 / 4.2.3.2

Plate 13c, d *Ipomoea muelleri*: perennial herbaceous vine, stems trailing, twining at tips; NW- to central Australia; Sects. 3.1 / 3.3 / 3.4 / 4.2.3.1 / 4.2.3.6 / 4.2.5.3

Plate 13e, f *Ipomoea neei*: liana; Mexico to Panama; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 3.7.1.1

Plate 14a *Ipomoea nil* (syn.: *Pharbitis nil*; Japanese morning-glory): annual vine; Neotropics, meanwhile pantropical (cultivated as an ornamental worldwide, with unusual efforts and cultural background in Japan); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.1.2 / 4.2.3.2 / 5.2.5 / 6.3.3 / 6.7.3.2 / 6.7.3.3 / 6.8.2.1 / 6.8.2.3 / 7.4.2.1 / 8.1.1.2 / 8.3.1.2 / 8.3.2

Plate 14b, c *Ipomoea obscura*: herbaceous twiner (to 2 m); Paleotropics from E-Africa to N-Australia/Polynesia: **b** yellow to orange-blooming form; **c** cream to white-blooming form with purple centre; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.2.3.3 / 6.2.2 / 6.6.3.2 / 6.6.4.2

Plate 14d, e *Ipomoea pes-caprae* (beach morning glory, railroad vine, bay hops): herbaceous perennial long-trailing vine (to 30 m) with a thick taproot, rooting at nodes; pantropical (beaches): **d** ssp. *pes-caprae*; **e** ssp. *brasiliensis*; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.2.3.1 / 4.2.3.6 / 6.2.2 / 6.6.3.2 / 7.12.2.1 / 7.12.3 / 8.3.2 / 8.3.3.1

Plate 14f *Ipomoea setifera*: twining or decumbent vines; Neotropics, tropical Africa; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.2.3.1 / 4.2.3.6

Plate 15a *Ipomoea squamosa*: herbaceous vine, suffrutescent at base; Neotropics; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 8.3.1.2 / 8.3.2

Plate 15b *Ipomoea tricolor* cv. 'heavenly blue' (seeds: "badoh negro"): annual herbaceous vine (to 3 m); Mexico, meanwhile pantropical (ornamental cultivars: almost worldwide); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.2.1.2 / 4.2.2 / 4.2.3 / 4.2.3.1 / 4.2.3.6 / 4.2.4 / 4.2.5.2 / 6.5.3 / 6.7.3.2 / 8.1.2.2 / 8.3.1.2 / 8.3.2 / 8.3.3.2 / 8.3.3.3

Plate 15c *Jacquemontia pentantha*: herbaceous vine; Neotropics (widely cultivated as an ornamental); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 6.8.2.4

Plate 15d *Maripa panamensis*: woody liana (>30 m, stems to 30 cm in diameter); E-Panama, N-Colombia, N-Venezuela; Sects. 3.1 / 3.3 / 3.4 / 3.5

Plate 15e ***Merremia dissecta***: perennial herbaceous vine (to 4 m); Neotropics, meanwhile pantropical; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 6.2.2 / 6.6.1.2 / 6.6.3.2 / 6.6.4.1

Plate 15f ***Merremia guerichii***: perennial suffrutex; Namibia; Sects. 3.1 / 3.3 / 3.4

Plate 15g ***Merremia quinquefolia***: herbaceous twiner, woody and perennial basally; Neotropics; Sects. 3.1 / 3.2.2 / 3.3 / 3.4 / 3.7.1.3 / 4.1.2 / 4.2.3.6 / 6.1.2 / 6.6.4.1

Plate 15h ***Merremia tuberosa*** (woodrose): liana; Neotropics, cultivated as an ornamental (flowers and fruits, the latter = "woodrose"), sometimes naturalized in other tropical regions; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 6.6.1.2 / 6.6.4.1 / 8.3.1.1 / 8.3.1.2 / 8.3.2

Plate 16a ***Merremia vitifolia***: large twiner; tropical Asia; Sects. 3.1 / 3.3 / 3.4 / 6.2.2 / 6.3.4.2 / 6.6.4.1

Plate 16b ***Porana volubilis***: large woody twiner (to 20 m; diameter to 2 cm); SE-Asia (cultivated in gardens for ornamental purposes); Sect. 3.5

Plate 16c ***Stictocardia beraviensis***: woody liana; W-Africa to Madagascar; Sects. 3.1 / 3.3 / 3.4 / 4.2.3.5

Plate 16d ***Stictocardia mojangensis***: woody liana; W-Madagascar; Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.2.3.5 / 6.2.2 / 6.6.4.1

Plate 16e ***Turbina corymbosa*** (seeds: ololiuqui): liana, herbaceous at tips, woody at base; Mexico (cultivated for ornament in many tropical regions, sometimes naturalized); Sects. 3.1 / 3.3 / 3.4 / 3.5 / 4.2.1.2 / 4.2.3 / 4.2.3.5 / 4.2.3.6 / 4.2.4 / 4.2.5.2 / 7.4.2.1 / 8.1.1.2

Contribution of photographs to the color plates by Winfried Krautwurst (Mainz): 1c, 4b; Alex Espinosa (Panama City): 9a; Kristina Jenett-Siems (Berlin): 15f; Elisabeth Bäumel-Eich (Berlin): 1a, 2a, 2c–e, 3a–d, 4a, 4c–e, 5a–d, 6a–d, 7b–e, 8a–d, 10a–c, 11a–c, 11f, 12d, 13a, 13b, 13e, 13f, 14e, 14f, 15a–e, 15g, 15h, 16b, 16c, 16e; Eckart Eich (Berlin): 1b, 1d, 2b, 7a, 9b–g, 10d, 10e, 11d, 11e, 12a–c, 12e, 13c, 13d, 14a–d, 16a, 16d

Plate 1a *Anthocercis littorea*

Plate 1b *Atropa belladonna*

Plate 1c *Atropa belladonna*

Plate 1d *Brugmansia versicolor*

Plate 2a *Brunfelsia pauciflora*

Plate 2b *Capsicum annuum var. frutescens*

Plate 2c *Cestrum diurnum*

Plate 2e *Cestrum parqui*

Plate 2d *Cestrum elegans*

Plate 3a *Datura stramonium*

Plate 3b *Duboisia myoporoides* × *D. leichhardtii*

Plate 3c *Duboisia myoporoides* × *D. leichhardtii*

Plate 3d *Duboisia myoporoides* × *D. leichhardtii*

Plate 4a *Hyoscyamus albus*

Plate 4b *Hyoscyamus niger*

Plate 4c *Iochroma gesnerioides*

Plate 4d *Nicandra physalodes*

Plate 4e *Nicotiana glauca*

Plate 5a *Nicotiana langsdorffii*

Plate 5b *Nicotiana sylvestris*

Plate 5c *Nicotiana tabacum*

Plate 5d *Petunia* × *hybrida*

Plate 6a *Salpiglossis sinuata*

Plate 6b *Schizanthus pinnatus*

Plate 6c *Solandra maxima*

Plate 6d *Solanum betaceum*

Plate 7a *Solanum laciniatum*

Plate 7b *Solanum laxum*

Plate 7c *Solanum pseudocapsicum*

Plate 7d *Solanum sisymbrifolium*

Plate 7e *Solanum tuberosum*

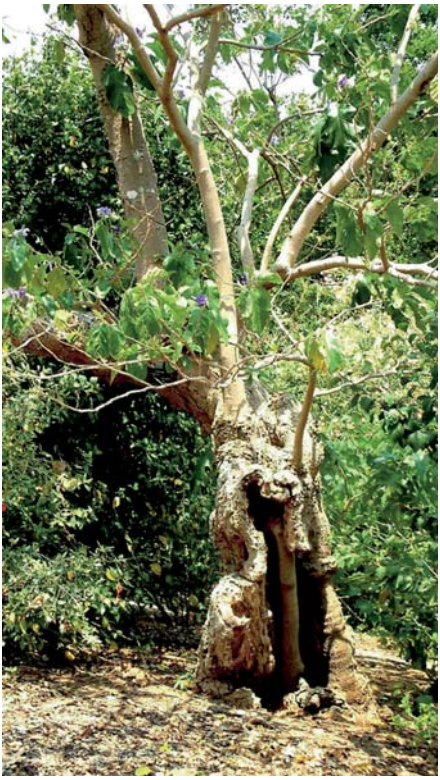


Plate 8a *Solanum wrightii*

Plate 8b *Solanum wrightii*

Plate 8d *Withania somnifera*

Plate 8c *Streptosolen jamesonii*

Plate 9a *Aniseia martinicensis*

Plate 9c *Argyreia mollis*

Plate 9e *Bonamia spectabilis*

Plate 9b *Argyreia capitata*

Plate 9d *Argyreia nervosa*

Plate 9f *Bonamia spectabilis*

Plate 9g *Bonamia spectabilis*

Plate 10a *Convolvulus cneorum*

Plate 10b *Convolvulus dorycnium*

Plate 10c *Convolvulus sabatius ssp. mauritanicus*

Plate 10d *Cuscuta australis*

Plate 10e *Erycibe rheedii*

Plate 11a *Evolvulus alsinoides*

Plate 11b *Ipomoea alba*

Plate 11c *Ipomoea aquatica*

Plate 11d *Ipomoea asarifolia*

Plate 11e *Ipomoea asarifolia*

Plate 11f *Ipomoea batatas*

Plate 12a *Ipomoea batatas*

Plate 12b *Ipomoea batatas*

Plate 12c *Ipomoea cairica*

Plate 12e *Ipomoea hederifolia*

Plate 12d *Ipomoea carnea ssp. fistulosa*

Plate 13a *Ipomoea hildebrandtii*

Plate 13b *Ipomoea lobata*

Plate 13c *Ipomoea muelleri*

Plate 13d *Ipomoea muelleri*

Plate 13e *Ipomoea neei*

Plate 13f *Ipomoea neei*

Plate 14a *Ipomoea nil*

Plate 14b *Ipomoea obscura*

Plate 14c *Ipomoea obscura*

Plate 14d *Ipomoea pes-caprae*

Plate 14e *Ipomoea pes-caprae*

Plate 14f *Ipomoea setifera*

Plate 15a *Ipomoea squamosa*

Plate 15b *Ipomoea tricolor*

Plate 15c *Jacquemontia pentantha*

Plate 15d *Maripa panamensis*

Plate 15e *Merremia dissecta*

Plate 15f *Merremia guerichii*

Plate 15g *Merremia quinquefolia*

Plate 15h *Merremia tuberosa*

Plate 16a *Merremia vitifolia*

Plate 16c *Stictocardia beraviensis*

Plate 16b *Porana volubilis*

Plate 16d *Stictocardia mojangensis*

Plate 16e *Turbina corymbosa*

Subject Index

B

C

I

N

Taxonomic Index