The Alkaloids

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PREFACE

Once again, this volume of ''*The Alkaloids: Chemistry and Biology*'' is comprised of four quite different chapters, from three different continents, on mechanisms of cytotoxic action, the calystegines, strychnine synthesis, and substituted quinoline alkaloids. This diversity reflects the need to see alkaloids as a class of natural product having tremendous biological potential and of continued broad scientific interest.

Numerous alkaloids have over the past 35 years shown cytotoxic activity. For the first time, Wink, in Chapter 1, discusses the diverse mechanisms through which various alkaloid classes, and individual compounds within those classes, effect their activity. It is also made apparent that alkaloids offer opportunities to overcome drug resistance, which is the nemesis of many therapeutic regimens and requires more detailed studies.

While the tropane alkaloids are best known for pharmaceutical agents such as cocaine and atropine, the development of the polyhydroxy tropane alkaloids, the calystegines, has brought new challenges. In Chapter 2, the advances in the isolation, structure determination, synthesis, biosynthetic pathways, and biology of this relatively new group of alkaloids are discussed by Biastoff and Dräger.

Small molecules with a high number of stereocenters offer challenges to synthetic organic chemists which are almost irresistable, and thus become model systems for the evolution of new methodologies. In Chapter 3, one of these archetypical alkaloids, strychnine, is reviewed solely from the perspective of recent synthetic efforts by Shibasaki and Ohshima.

Finally, in Chapter 4, a new group of alkaloids, those with a quinoline nucleus and various alkyl, aryl, and alkylaryl side chains, are discussed by the Brazilian group of da Silva, Soares, Fernandes, and Vieria from the perspectives of biosynthesis, biogenesis, and distribution in the plant, marine, and fungal environments.

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CHAPTER 1

Molecular Modes of Action of Cytotoxic Alkaloids: From DNA Intercalation, Spindle Poisoning, Topoisomerase Inhibition to Apoptosis and Multiple Drug Resistance

Michael Wink*

Contents

I. INTRODUCTION

Plants produce a high diversity of secondary metabolites (SM), and among them, alkaloids are a most prominent class. Over 21,000 alkaloids have been identified,

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which constitute the largest group among the nitrogen-containing SM (including 700 non-protein amino acids, 100 amines, 60 cyanogenic glycosides, 100 glucosinolates, and 150 alkylamides). However, the class of SM without nitrogen is even larger (more than 25,000 terpenoids, 7000 phenolics and polyphenols, 1000 polyacetylenes, fatty acids, waxes, and 200 carbohydrates) (1–4). Alkaloids are widely distributed in the plant kingdom, especially among angiosperms (more than 20% of all species produce alkaloids), and are less common in gymnosperms, lycopods, horsetails, mosses, and algae (3,5,6). Alkaloids also occur in bacteria, fungi, many marine animals (sponges, slugs), arthropods, amphibians, birds, and mammals (3,7–9).

Alkaloids are apparently important for the fitness of the organism that produces them. One of the main functions is that of chemical defence against herbivores or predators. Some alkaloids have antibacterial, antifungal, and antiviral activities in addition. In many cases, a single alkaloid can exhibit more than one biological function. During evolution, the constitution of alkaloids has been modulated so that they usually contain more than one active functional group allowing them to interact with several molecular targets. Therefore, a pleiotropic effect is a common theme in alkaloids and other SM (2,3,7,8,10–13). The multiple functions that alkaloids can exhibit include a few physiological tasks: sometimes, toxic alkaloids also concomitantly serve as nitrogen-storage and nitrogen-transport molecules. The ecological functions will not be reviewed in this chapter as they were discussed in previous reviews (2,7,8,10,11).

II. MOLECULAR TARGETS OF SECONDARY METABOLITES

In order to deter, repel, or inhibit the diverse range of potential enemies, ranging from arthropods, and vertebrates to bacteria, fungi, and viruses, alkaloids must be able to interfere with important cellular and molecular targets. A short overview of these potential targets is given in Figures 1 and 2. The modulation of a molecular target will negatively influence its communication with other components of the cellular network, especially proteins (cross-talk of proteins), or elements, or signal transducers. As a consequence, the metabolism and function of cells, tissues, organs, and eventually the whole organism will be affected. Although we know the structures of many SM, our knowledge concerning their molecular mode(s) of action is largely fragmentary and incomplete. Such knowledge is, however, important in order to understand the functions of SM for the producing organism, and for the rational utilization of SM in medicine or plant protection. Whereas many SM interact with multiple targets and thus have unspecific broad activities, others are highly specific, and interact exclusively with a particular target. SM with broad activities interact mainly with proteins, biomembranes, and DNA/RNA (Table I).

Among broadly active alkaloids, a distinction can be made between those that are able to form covalent bonds with proteins and nucleic acids, and those which modulate the conformation of proteins and nucleic acids by non-covalent bonding. Covalent bonds can be formed with reactive functional groups of SM, such as

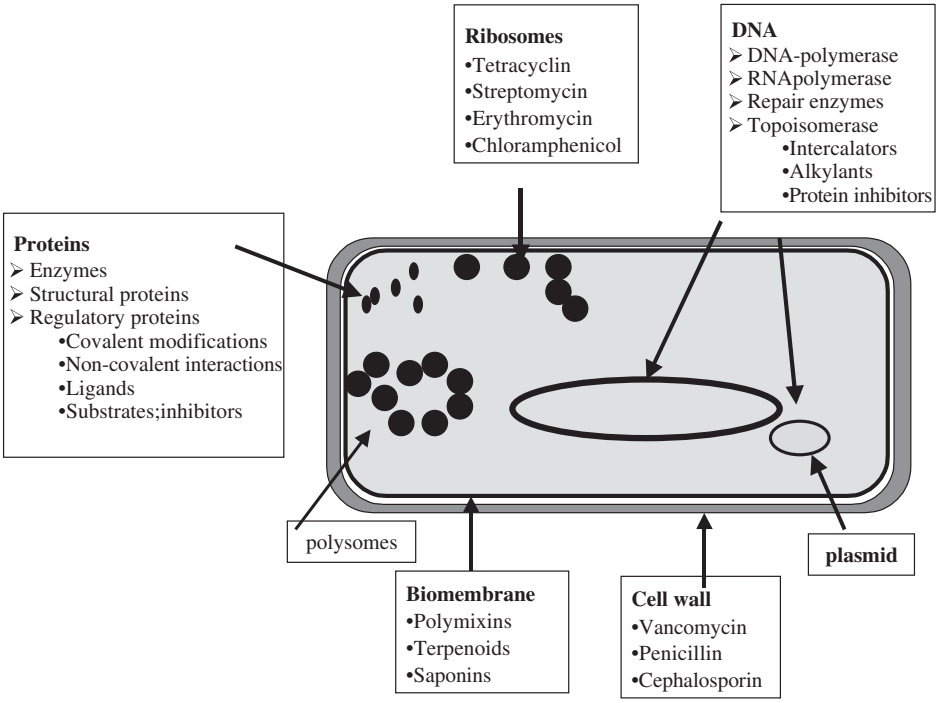

Figure 1 Molecular targets in bacteria that can be affected by natural products.

aldehydes, epoxides, SH groups, phenolic radicals, activated double bonds, and exocyclic or terminal methylene groups (Table I) (4,13,14).

Non-covalent bonds, especially hydrogen bonds, ionic bonds, hydrophobic interactions, and van der Waals forces are weak individually, but can be powerful if they work co-operatively together. For example, a tannin typically has several (more than 10) phenolic hydroxyl groups that can form hydrogen bonds with proteins and nucleic acids. Furthermore, these OH groups may dissociate under physiological conditions to form phenolate ions that can form ionic bonds with positively charged amino acid residues, such as those from lysine, arginine, and histidine. Phenolic hydroxyl groups are a common theme in many SM, mostly in phenylpropanoids, flavonoids, tannins, and polyketides, and they also occur in some alkaloids (especially in the isoquinoline and quinoline alkaloids). These OH groups are crucial for the biological activity of phenolics.

Nitrogen-containing compounds, such as alkaloids, amines, and peptides usually have positively charged *N*-atoms (under physiological conditions) in their molecules that can form ionic bonds with negatively charged amino acid residues of glutamic and aspartic acid in proteins. Both the covalent and non-covalent interactions will modulate the three-dimensional protein structure, i.e., the conformation that is so important for their bioactivities. A conformational change is usually associated with a loss or reduction in the activity of a protein,

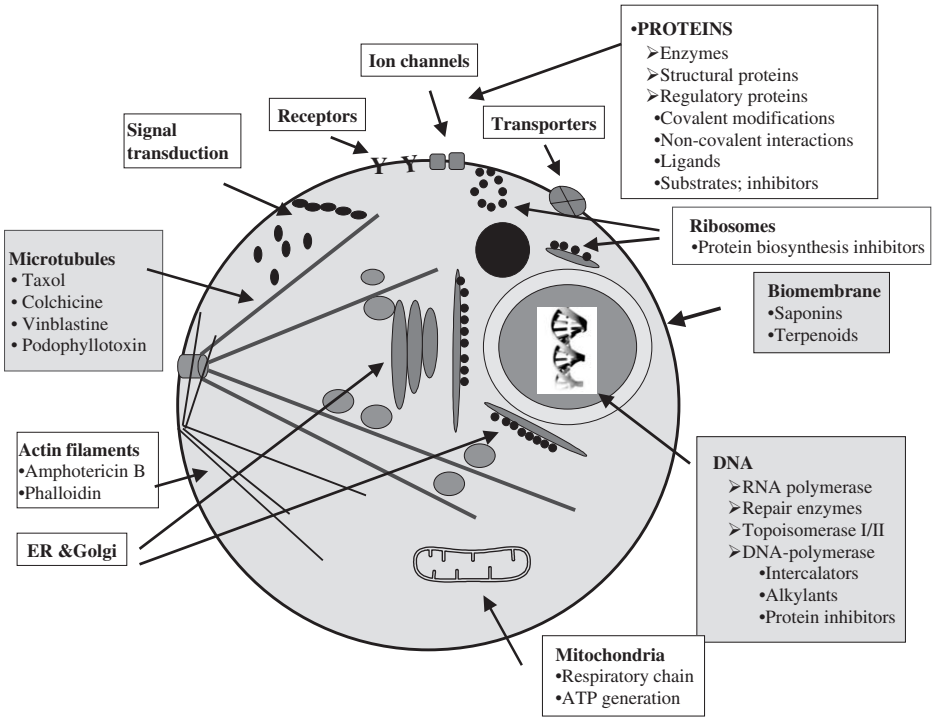

Figure 2 Molecular targets in animal and human cells that can be affected by natural products.

inhibiting enzyme, or receptor activity, or interference with the very important protein–protein interactions (4,13,14).

Lipophilic compounds, such as the various terpenoids, tend to associate with other hydrophobic molecules in a cell; these can be biomembranes or the hydrophobic core of many proteins and of the DNA double helix (4,13,14). In proteins, such hydrophobic and van der Waals interactions can also lead to conformational changes, and thus protein inactivation. A major target for terpenoids, especially saponins, is the biomembrane. Saponins can also change the fluidity of biomembranes, thus reducing their function as a permeation barrier. Saponins can even make cells prone to leak, which immediately leads to cell death. This can easily be seen in erythrocytes; when they are attacked by saponins these cells burst and release hemoglobin (hemolysis) (4,13,14).

These pleiotropic, multitarget bioactivities are not specific, but are nevertheless effective, and this is what is critical in an ecological context. Compounds with pleiotropic properties have the advantage that they can attack any enemy that is encountered by a plant, be it a herbivore, a bacterium, fungus, or virus. These classes of compounds are seldom unique constituents; quite often plants produce a mixture of SM, often both phenolics and terpenoids, and thus exhibit both covalent and non-covalent interactions. These activities are probably both additive, and synergistic (13,14).

Table I Interactions of secondary metabolites with proteins and biomembranes

Mode of action	Class of secondary metabolite
Covalent modifications	
Reaction of aldehyde groups with amino groups	Iridoids, terpenoids with aldehyde groups
Reaction of isothiocyanate groups with amino and SH groups	Mustard oils, (isothiocyanates)
Reaction of α,β-unsaturated carbonyl groups with SH groups	Sesquiterpene lactones, phenylpropanoids, monoterpenes
Reaction of allylsulfides with SH groups	Allicin
Reaction of epoxides with proteins and DNA	Valepotriates, metabolically activated metabolites
Reaction with metal ions	Quinones, naphthoquinones
Non-covalent bonds	
Ionic bonds	Phenolics, tannins, bases, acids
Hydrogen bonds	Phenolics, tannins, anthraquinones
Van der Waals and hydrophobic interactions	Lipophilic compounds, such as terpenoids
Disturbance of membrane fluidity	
Hydrophobic/amphiphilic interactions	Saponins and other terpenoids

Pharmacologists clearly prefer SM that interact with a single target in a specific way (monotarget substances) because dose response and structure activity relationships can be much more easily determined than for non-specific, multitarget compounds. Many alkaloids fall in the class of specific modulators, and have been modified during evolution in such a way that they mimic endogenous ligands, hormones, or substrates. We have termed this selection process ''evolutionary molecular modeling'' (7,8,11,12). Many alkaloids are neurotoxins that were selected for defence against animals. These compounds have the advantage for the plants producing them that they are usually not toxic for the producing organism (as plants have no nerves). On the other hand, plants need special capacities to produce and store the non-specific multitarget SM (7). Table II lists the potential neuronal targets that can be affected by alkaloids. Extensive reviews on this topic have been published (8,10,11). Since neuronal signal transduction is a very critical target in animals, and its modulation usually leads to toxic effects, many alkaloids are indeed strong (even deadly) poisons, or have mind-altering and hallucinogenic properties.

III. CYTOTOXICITY OF ALKALOIDS

In this review, the emphasis is placed on the cytotoxic properties of alkaloids and their underlying modes of action. For other properties of alkaloids, see the

Table II Molecular targets of alkaloids in neuronal signal transduction (10–12,16)

Target	Selected alkaloids
Neuroreceptor	
Muscarinic acetylcholine receptor	Hyoscyamine and other tropane alkaloids; arecoline; berbamine and other isoquinoline alkaloids; sparteine and other quinolizidine alkaloids; cryptolepine; pilocarpine
Nicotinic acetylcholine receptors	Nicotine; boldine and other aporphine alkaloids; C-toxiferine; coniine and other piperidine alkaloids; cytisine and other quinolizidine alkaloids; epibatidine; tubocurarine
Adrenergic receptors	Berbamine, berberine, and other isoquinoline alkaloids; cinchonidine and other quinoline alkaloids; corynanthine, yohimbine, and other indole alkaloids; emetine; ephedrine; ergometrine and related ergot alkaloids
Dopamine receptor	Ergocornine and related ergot alkaloids; bulbocapnine and related aporphine alkaloids; anisocycline, stylopine, and related protoberberine alkaloids; salsolinol and related isoquinolines
GABA receptor	Bicuculline, cryptopine, hydrastine, and related isoquinoline alkaloids; securinine; harmaline and related beta-carboline alkaloids
Glycine receptor	Corymine, strychnine, and related indole alkaloids
Glutamate receptor	Histrionicotoxin and related piperidines; ibogaine and related indole alkaloids; nuciferine and related aporphine alkaloids
Serotonin receptor	Akuammine and related indole alkaloids; annonaine, boldine, and related aporphine alkaloids; berberine and related protoberberine alkalodis; ergotamine, LSD, and related ergot alkaloids; psilocybine, bufotenine, *N,N*-dimethyltryptamine, and related indoles; harmaline and related beta-carboline alkaloids; kokusagine and related furoquinoline alkaloids; mescaline; ibogaine and other monoterpene indole alkaloids
Adenosine receptor	Caffeine, theobromine, and other purine alkaloids
Opiate receptor	Morphine and related morphinan alkaloids; akuammine, mitragynine, ibogaine, and related indole alkaloids
Acetylcholine esterase	Galanthamine; physostigmine and related indole alkaloids; berberine and related protoberberine alkaloids; vasicinol and related quinazolines;

Table II. (*Continued*)

Target	Selected alkaloids
	huperzine; harmaline, and related beta-carboline alkaloids; demissine and related steroidal alkaloids
Monoamine oxidase	Harmaline and related beta carbolines; carnegine, salsolidine, *O*-methylcorypalline and related isoquinoline alkaloids; *N*,*N*-dimethyltryptamine and related indole alkaloids
Neurotransmitter uptake (transporter)	Ephedrine and related phenylalkyl amines; reserpine, ibogaine, and related indole alkaloids; cocaine; annonaine and related aporphine alkaloids; arecaidine; norharman and related beta-carboline alkaloids; salsolinol and related isoquinoline alkaloids
Na^+, K^+ channels	Aconitine and related diterpene alkaloids; veratridine, zygadenine, and related steroidal alkaloids; ajmaline, vincamine, ervatamine, mitragynine, and other indole alkaloids; dicentrine and other aporphine alkaloids; gonyautoxin; paspalitrem and related indoles; phalloidine, quinidine, and related quinoline alkaloids; sparteine and related quinolizidine alkaloids; saxitoxin; strychnine; tetrodotoxin
Ca^{++} channels	Ryanidine; tetrandrine, berbamine, antioquine, and related bis-isoquinoline alkaloids; boldine; caffeine and related purine alkaloids; cocaine; corlumidine and other indole alkaloids
Adenylate cyclase	Ergometrine and related ergot alkaloids; nuciferine and related aporphine alkaloids
cAMP phosphodiesterase	Caffeine and related purine alkaloids; papaverine; chelerythrine, sanguinarine, and related benzophenanthridine alkaloids; colchicine; infractine and related indole alkaloids
Protein kinase A (PKA)	Ellipticine and related indole alkaloids
Protein kinase C (PKC)	Cepharanthine and related bis-isoquinoline alkaloids; michellamine B and related isoquinoline alkaloids; chelerythrine and related benzophenanthridine alkaloids
Phospholipase (PLA_2)	Aristolochic acid and related aporphine alkaloids; berbamine and related bis-isoquinoline alkaloids

previous reviews (3,7,8,10,11). The body of information regarding the cytotoxic activities of SM and alkaloids is fast-growing since many compounds have been, and are presently, being screened in many academic and industrial laboratories for potential anticancer and antiviral activities. Data published during the last 12 years (i.e., after the 1993 review; 10) are so numerous (more than 1,000 entries in data bases) that a tabulation would be beyond the scope of this review. Rather, this review will highlight the molecular modes of action that underlie the cytotoxicity of alkaloids. Cytotoxic compounds are potentially interesting directly on their own or as lead compounds for the development of new anticancer drugs, for drugs against parasites, such as trypanosomes and *Plasmodium* sp. (12,15), and against viral infections.

IV. MOLECULAR MODES OF ACTION OF CYTOTOXIC ALKALOIDS

Cytotoxicity occurs as a result of the molecular interactions of an alkaloid with one or several important targets present in a cell (Figures 1 and 2). The main targets include DNA, RNA, and the associated enzymes and processes (i.e., replication, repair, transcription, DNA polymerase, RNA polymerase, reverse transcriptase, repair enzymes, topoisomerase, telomerase), protein biosynthesis, protein conformation, biomembranes, and membrane proteins (for reviews, see (10,11,16)).

Cell biologists distinguish between necrotic and apoptotic cell death. If a cell is lysed by saponins or other detergents, or when it is mechanically wounded or exposed to physical stress (heat, freezing, hypoxia) then it dies quickly by necrosis. Although macrophages have to rush in to clear away the debris, often an inflammation results. Apoptosis, or programmed cell death, was discovered about 35 years ago in 1972 by Kerr, Wyllie, and Currie (17), and is a central mechanism in the development of most organisms. The pathway functions naturally to generate shapes and to control the number of cells in various tissues.

Apoptosis is characterized by nuclear chromatin condensation, cytoplasmic shrinking, a dilated endoplasmic reticulum, membrane blebbing, and the formation of apoptotic bodies. Programmed cell death is clean, quick, and involves a predictable sequence of structural changes that cause a cell to shrink and to be rapidly digested by macrophages or neighboring cells.

Apoptosis can be induced by many substances, among them several natural products, such as alkaloids, that primarily interact with an important molecular target such as DNA or microtubules. Most of the anticancer drugs presently used in cancer therapy lead to apoptosis. The molecular targets associated with cell death and cytotoxicity are discussed in the next section.

A. Interactions with DNA, RNA, and Associated Enzymes

1 DNA Alkylation and Intercalation

DNA is a central target of all organisms, which can be affected by certain molecules (Figures 1 and 2). Some molecules can form covalent bonds with DNA bases, the so-called alkylating agents. Alkylating compounds often attach to the

*N*6 of guanine. Some anticancer chemotherapeutic agents act as alkylating agents, including cisplatin, nitrosourea derivatives, and nitrogen mustards. These compounds lead to cytotoxicity by alkylating guanine units in their *N*6-position, which eventually leads to strand breaks. Such modifications are removed by the repair enzyme, alkylguanine–DNA alkyl transferase (AGT). In this process, the alkyl residue is transferred to a cysteine in the active site of the enzyme (18). If these modifications are not repaired by the abundant and active repair enzymes of a cell, mutations arise. These mutations include nucleotide exchanges (transitions, transversions) and deletions. Deletions are especially harmful, as they result in frameshift mutations. The corresponding proteins (in coding genes) are then translated in the wrong frame and result in nonsense proteins. Therefore, frameshift mutations mostly lead to a loss of function. Several alkaloids with alkylating properties have been described, among them pyrrolizidine alkaloids, aristolochic acids, and cycasin are the most prominent cases (16) (Table III). These alkaloids are known to be mutagenic and teratogenic, and may induce cancer (10,11,16).

Intercalating compounds, that are planar and lipophilic, can insert between base pairs and thereby stabilize the double helix in a way that the replication and transcription process is disturbed. Intercalation usually leads to frameshift mutations. If these mutations occur in the heterochromatin, which does not code for genes, they are usually without much consequence. In protein coding genes, a nucleotide exchange in the third codon position (a so-called silent mutation) is also without much consequence because of the degenerated genetic code. However, a mutation which changes the amino acid sequence of a protein or which influences promoters and other regulatory sequences can have detrimental effects. These negative effects can include disregulation of metabolism, tumor growth, or even cell death.

Intercalating compounds also directly inhibit DNA replication and transcription. RNA is basically single stranded, but most RNA molecules have double-stranded stem structures because of complementary base pairing. These double-stranded regions can also be intercalated. Therefore, many intercalating SM are also inhibitors of DNA and RNA polymerases, of reverse transcriptase, and even of DNA topoisomerases and possibly telomerase.

Table III provides an overview of alkaloids that interfere with DNA, RNA, and associated proteins. Alkaloids with a planar and polycyclic structure are good candidates for DNA intercalation. Protonable ring nitrogens can stabilize the alkaloid–DNA complex by binding to the negatively charged DNA surface (19,20). Such properties are abundant in isoquinoline, quinoline, and indole alkaloids that are synthesized from the aromatic amino acids phenylalanine, tyrosine, and tryptophan (11,12,16).

2 DNA Topoisomerase I and II

DNA topoisomerase I and II play important roles in DNA replication and transcription. Their inhibition usually leads to cell-cycle arrest and cell death by apoptosis. During replication, DNA needs to be uncoiled. In order to avoid torsions and rotations, DNA is cleaved by DNA topoisomerase I, which forms

Table III Interaction of alkaloids with DNA, RNA, and associated proteins (for earlier papers, see refs. 10,11)

Alkaloid	Source	Effect	References
Alkaloids derived from tryptophan			
Camptothecin	*Camptotheca acuminata* (Cornaceae)	DNA intercalator; topoisomerase I inhibitor; stabilization of topo I/DNA/CPT complex; cell-cycle arrest in G_2/M phase; apoptosis induction	(80,81,83)
Cinchonine	*Cinchona* sp. (Rubiaceae)	DNA intercalation; inhibition of DNA polymerase, reverse transcriptase	(12,16)
Cinchonidine	*Cinchona* sp. (Rubiaceae)	DNA intercalation; inhibition of DNA polymerase, reverse transcriptase	(12,16)
Cryptolepine	*Cryptolepis sanguinolenta* (Asclepiadaceae)	Intercalation of GC rich DNA, inhibition of topoisomerase II (stabilizes topo II-DNA complexes); stimulates DNA cleavage, cell-cycle arrest; apoptostic; forms G-quadruplexes with telomeric DNA	(82,84–87)
Dercitin	*Dercitus* (sponge)	DNA intercalator; inhibitor of DNA polymerase	(88)
Dictamine	*Dictamnus* sp. (Rutaceae)	DNA intercalation; mutagenic	(89)
Ellipticine	*Ochrosia elliptica* (Apocynaceae)	DNA intercalator; inhibition of topoisomerase II and telomerase; apoptotic; mutagenic	(16,19,90,91)
Evolitrine	Rutaceae	DNA intercalation; mutagenic	(89)
γ-Fagarine	Rutaceae	DNA intercalation; mutagenic	(89)
Harmine	*Peganum harmala* (Zygophyllaceae)	DNA intercalation; inhibition of topoisomerase I; antitumor activity; DNA strand break induction; inhibition of DNA polymerase and reverse transcriptase	(12,16,92–95)
Harmaline	*Peganum harmala* (Zygophyllaceae)	DNA intercalation; inhibition of DNA polymerase and reverse transcriptase	(12,16)
10-Hydroxy-camptothecin	*Camptotheca acuminata* (Cornaceae)	Topoisomerase I inhibitor; cell-cycle arrest in G_2/M phase; apoptosis induction	(78,79)

Kokusaginine	Rutaceae	DNA intercalation; mutagenic	(89)
Luotonine A	*Peganum nigellastrum* (Zygophyllaceae)	Inhibitor of topoisomerase I; cytotoxic	(96)
Maculine	Rutaceae	DNA intercalation; mutagenic	(89)
Matadine	*Strychnos gossweileri* (Loganiaceae)	DNA intercalation; topoisomerase II inhibition	(25)
Neocryptolepine	*Cryptolepis sanguinolenta* (Asclepiadaceae)	Intercalation of GC-rich DNA; topoisomerase II inhibition; cytotoxic, antibacterial, antiparasitic	(86,97,98)
Norharman	*Peganum harmala* (Zygophyllaceae)	DNA intercalation; inhibition of DNA polymerase and reverse transcriptase	(12,16)
Quinine	*Cinchona* sp. (Rubiaceae)	DNA intercalation; inhibition of DNA polymerase and reverse transcriptase	(12,16)
Quinidine	*Cinchona* sp. (Rubiaceae)	DNA intercalation; inhibition of DNA polymerase, reverse transcriptase	(12,16)
Serpentine	*Rauwolfia serpentina* (Apocynaceae)	DNA intercalation; topoisomerase II inhibition	(25)
Skimmianine	Rutaceae	DNA intercalation; mutagenic	(89)
Tangutorine	*Nitraria tangutorum* (Zygophyllaceae)	Induction of cyclin kinase inhibitor p21; inhibition of topoisomerase II expression	(99)
Usambarensine	*Strychnos usambarensis* (Loganiaceae)	DNA intercalator; apoptotic; cytotoxic, antiparasitic	(25)
Alkaloids derived from phenylalanine/tyrosine			
Actinodaphnidine	*Cassytha filiformes* (Lauraceae)	DNA intercalation; inhibition of topoisomerases; cytotoxic, antitrypanosomal activity	(100)
Aristolactam glucoside	*Aristolochia* sp. (Aristolochiaceae)	DNA intercalator	(101–103)
Aristolochic acid	*Aristolochia* sp. (Aristolochiaceae)	Activation by NADPH:CYP reductase leading to formation of DNA adducts; mutagenic and carcinogenic	(16,104)
Berbamine	*Berberis* sp. (Berberidaceae)	DNA intercalation; inhibition of DNA polymerase, reverse transcriptase	(12,16)

Table III (*Continued*)

Alkaloid	Source	Effect	References
Berberine	*Berberis* sp. (Berberidaceae)	DNA intercalation; binding to single stranded poly (rA); inhibitor of topoisomerase I and II; inhibition of DNA polymerase, reverse transcriptase	(12,16,20,105–107)
Berberrubine	*Berberis* sp. (Berberidaceae)	DNA intercalator; topoisomerase II inhibitor (stabilizing topo II cleavable complexes); inhibition of catalytic activity	(107–111)
Boldine	*Peumus boldo* (Monimiaceae)	DNA intercalation; inhibition of DNA polymerase, reverse transcriptase	(12,16,100,112)
Bulbocapnine	*Corydalis* sp. (Fumariaceae)	DNA intercalation	(100,112)
Burasaine	*Burasaia* sp. (Menispermaceae)	Probably DNA intercalation; cytotoxic	(49)
Cassythine	*Cassytha filiformes* (Lauraceae)	DNA intercalation; inhibition of topoisomerases; cytotoxic; antitrypanosomal activity	(100)
Chelerythrine	*Chelidonium majus* (Papaveraceae)	When activated by CYP, DNA adducts can be generated	(104)
Coptisine	Ranunculaceae	DNA intercalator	(111,113)
Coralyne	Papaveraceae	DNA intercalator (GC rich sequences), topoisomerase I inhibitor	(114–117)
Dicentrine	*Dicentra* sp. (Fumariaceae)	Minor groove DNA intercalator; inhibition of topisomerase II; mutagenic	(16,100,112,118)
Dicentrinone	*Ocotea leucoxylon* (Lauraceae)	Inhibitor of topoisomerase I	(119)
Ecteinascidin 743	*Ecteinascidia turbinate* (tunicate)	DNA alkylation; covalent bonding with 2′-amino group of guanine; cell-cycle arrest	(120,121)
Emetine	*Psychotria ipecacuanha* (Rubiaceae)	DNA intercalation; inhibition of DNA polymerase, reverse transcriptase, and protein biosynthesis; apoptotic	(12,16)

Epiberberine	*Coptis chinensis* (Ranunculaceae)	Inhibitor of topoisomerase	(110)
Fagaronine	*Fagara zanthoxyloides* (Rutaceae)	Major groove DNA intercalator; inhibition of topoisomerase I and II; antitumor	(122–125)
Glaucine	Papaveraceae	DNA intercalation	(100)
Groenlandicine	*Coptis chinensis* (Ranunculaceae)	Inhibition of topoisomerase I	(110)
Haemanthamine	*Lycoris radiata* (Amaryllidaceae)	Complex formation with RNA	(126)
Isocorydine	Papaveraceae	DNA intercalation	(100,112)
Jatrorrhizine	Ranunculaceae	DNA intercalator	(111,113)
Liriodenine	*Cananga odorata* (Annonaceae)	Inhibition of topoisomerase II (catalytic inhibitor); mutagenic	(16,127)
Lycobetaine	Amaryllidaceae	Inhibition of topoisomerase II; cytotoxic	(128)
Lycorine	*Narcissus* sp. (Amaryllidaceae)	Complex formation with RNA	(126)
Nitidine	*Toddalia asiatica* (Rutaceae)	DNA intercalator; inhibition of topoisomerase I and II; antitumor compound	(124,129)
Palmatine	Ranunculaceae	DNA intercalator	(111,113)
Roemerine	*Roemeria* sp. (Papaveraceae)	Mutagenic	(16)
Salsolinol	*Salsola* sp. (Chenopodiaceae)	In combination with Cu(II) causes DNA strand breaks; ROS formation; cytotoxic	(130)
Sanguinarine	*Sanguinaria canadensis* (Papaveraceae)	DNA intercalator; intercalation of double stranded GC-rich RNA; when activated by CYP, DNA adducts can be generated; inhibition of DNA polymerase, reverse transcriptase; clastogenic; mutagenic	(12,16,104,106,113,131–135)
Tetrandrine	*Stephania tetrandra* (Menispermaceae)	DNA alkylating; mutagenic; carcinogenic; apoptotic	(16)

Table III (*Continued*)

Alkaloid	Source	Effect	References
Alkaloids derived from ornithine/arginine			
Clivorine	*Ligularia hodgsonii* (Asteraceae)	Metabolic activation by CYP3A1 and CYP3A2;	(136,137)
Monocrotaline	*Crotalaria* sp. (Fabaceae)	CYP3A substrate; CYP oxidation gives dehydromonocrotaline which forms DNA–DNA interstrand and DNA–protein cross-links	(138–140)
Pyrrolizidine alkaloids	Asteraceae, Boraginaceae	Metabolic activation by CYP3A; generation of reactive dehydropyrrolizidines that can alkylate DNA; DNA–DNA interstrand and DNA–protein cross-link formation; mutagenic, carcinogenic	(16,138,141,142)
Miscellaneous alkaloids			
Acronycine	*Acronychia baueri* (Rutaceae)	DNA alkylation; intercalator; carcinogen, apoptotic	(144–148)
Actinomycin D	*Streptomyces* sp.	DNA intercalating; inhibitor of DNA polymerase, apoptotic; antineoplastic, antimicrobial	(22)
Ascididemin	*Cystodytes dellechiajei* (ascidian)	DNA intercalation; inhibition of topoisomerase II; SH-dependent oxidative DNA cleavage	(85,149–152)
Batzelline D	*Zyzzya fuliginosa* (sponge)	Topoisomerase II inhibitor	(153)
Cycasin	*Cycas* and related Cycadaceae	Activated methylazoxymethanol is a DNA methylating agent, i.e. mutagenic and carcinogenic	(16)
Cystodytin A, J	*Cystodytes* sp. (ascidian)	DNA intercalator; topoisomerase II inhibitor; cytotoxic	(154)
Eilatin	*Cystodytes* sp. (ascidian)	DNA intercalator; topoisomerase II inhibitor; cytotoxic	(77)

Kuanoniamine	*Cystodytes* sp. (ascidian)	DNA intercalator; topoisomerase II inhibitor; cytotoxic	(154)
Lamellarin	*Lamellaria* (mollusk)	DNA intercalator; inhibitor of topoisomerase I	(73,155–157)
Lobeline	*Lobelia* sp. (Campanulaceae)	DNA intercalation; inhibition of DNA polymerase, reverse transcriptase	(12,16)
Mahanine, murrayanol	*Murraya koenigii* (Rutaceae)	Inhibition of topoisomerase I and II; antimcrobial and molluscicidal activities	(158)
Makaluvamine A, F	*Zyzzya fuliginosa* (sponge)	DNA intercalator; topoisomerase II inhibitor; cytotoxic; antitumor activity	(73,159)
Popolophuanone F	marine sponges	Inhibitor of topoisomerase II	(160)
Prodigiosin	*Serratia marcescens* (Bacteria)	DNA intercalator; inhibition of topoisomerase I and II; induction of apoptosis	(161)
Shermilamine	*Cystodytes* sp. (ascidian)	DNA intercalator; topoisomerase II inhibitor; cytotoxic	(154)
Tsitsikammamine	*Tsitsikamma pedunculata* (sponge)	DNA intercalator; topoisomerase I inhibitor	(162)

single-strand nicks. This enzyme is an important target in tumor cells, which is also affected by some alkaloids, such as camptothecin and derivatives that are important chemotherapeutic agents in tumor treatment (21). DNA topoisomerase II cleaves and religates both DNA strands.

Drugs that attack topoisomerases either inhibit the enzymes (catalytic inhibitor) or they stabilise the fragile and normally transient ''cleavable ternary complexes'' by preventing religation. Such complexes are converted to lethal lesions when the cell tries to use the damaged DNA templates (22–24). The DNA intercalating alkaloids cryptolepine, matadine, and serpentine tightly bind DNA and stabilize the DNA–topoisomerase-II complex. In this way, they stimulate the cleavage of DNA by topoisomerase II (25). Intercalators with a ''deep intercalation mode'' mainly affect topoisomerase I, whereas drugs with an ''outside binding mode'' are topoisomerase II inhibitors (23). A number of alkaloids affect both topoisomerase I and II, such as certain benzophenanthridine, pyridoindole, indenoquinolone, and acridine alkaloids (23). Table III lists the alkaloids that have been reported to interfere with DNA topoisomerases. As mentioned above, many of these alkaloids have alkylating and intercalating properties.

3 Telomeres and Telomerase

Chromosomes are bordered by telomeres. The telomeres are made of tandem repeats of short DNA sequences such as TTAGGG. They are added to the chromosomes by telomerase, which is a reverse transcriptase and uses a RNA template as a primer (Figure 3). Telomeres comprise between 5000 and 10,000 nucleotides and appear to protect the chromosomes from the attack of exonucleases, from recombination and end-to-end fusion. The 3′-end of telomeres consists of 100 to 200 unpaired bases (26). Since telomeres are guanidine-rich, they have the possibility to form stacked guanine quadruplexes by Hoogsten-hydrogen-bonding (27). Telomerase is only active in embryonic, stem, and germline cells. In somatic cells it is turned off, with the consequence that telomere lengths get shorter and shorter following cell division and during life. It is speculated that aging and death result when telomeres become too short, so that essential genes are destroyed by exonucleases. Although primary cell cultures cannot be maintained in cultivation for more than 70 cell divisions, cancer cells are immortal and can be cultivated for dozens of years. The obvious difference is due to the fact that telomerase is still active in many cancer cells, but silent in primary cultures. About 80–85% of tumors express telomerase (28). Thus telomerase is associated with cell immortality and cancer, which might be connected with the ability of telomerase to prevent apoptosis by stabilizing telomeres.

This observation has triggered the search for compounds that can inhibit telomerase. Special attention has been given to G-quadruplexes, since telomeres that form stabilized quadruplexes cannot be elongated by telomerase (29). Several intercalating compounds stabilize G-quadruplexes and thus inhibit telomerase; among these compounds are acridine alkaloids and anthraquinones (30–32). Recently, some natural alkaloids were found, that can inhibit telomerase. These include ellipticine, a known intercalating substance and experimental anticancer agent. Ellipticine and 9-hydroxyellipticine interact with topoisomerase II

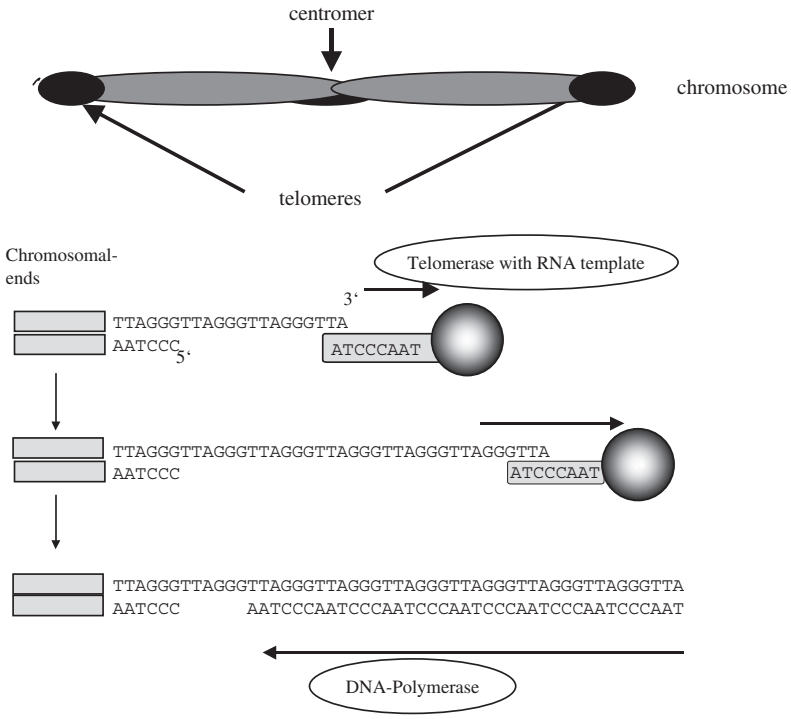

Figure 3 Schematic outline of telomeres and telomerase action.

and telomerase (19,33). Cryptolepine, neocryptolepine, and the bis-dimethylaminoethyl derivative of quindoline (a quinoline alkaloid) from *Cryptolepis sanguinolenta* (Asclepiadaceae) recognize triplex and quadruplex DNA structures. They are weak telomerase inhibitors with modest cytotoxicity (34,35). Also, the well-established DNA intercalating protoberberine alkaloids, such as berberine, act as moderate telomerase inhibitors (36). From marine sources, dictyodendrins A–E have been isolated from the sponge *Dictyodendrilla verongiformis*, and are strong telomerase inhibitors (37).

Telomerase is a relatively new drug target. It might be of some interest to evaluate intercalating and G-quadruped stabilizing compounds for their potential telomerase inhibiting activity.

B. Interactions with the Cytoskeleton

During cell division, the duplicated chromatids that are localized in the equatorial plane have to be separated and pulled apart into the daughter cells. This process is achieved by a complex interaction of microtubules. Microtubules are elements of the cellular cytoskeleton and are polymers of tubulin. Tubulin dimers form protofilaments when GTP is present and several protofilaments organize themselves into microtubules. When GDP dominates, the microtubules

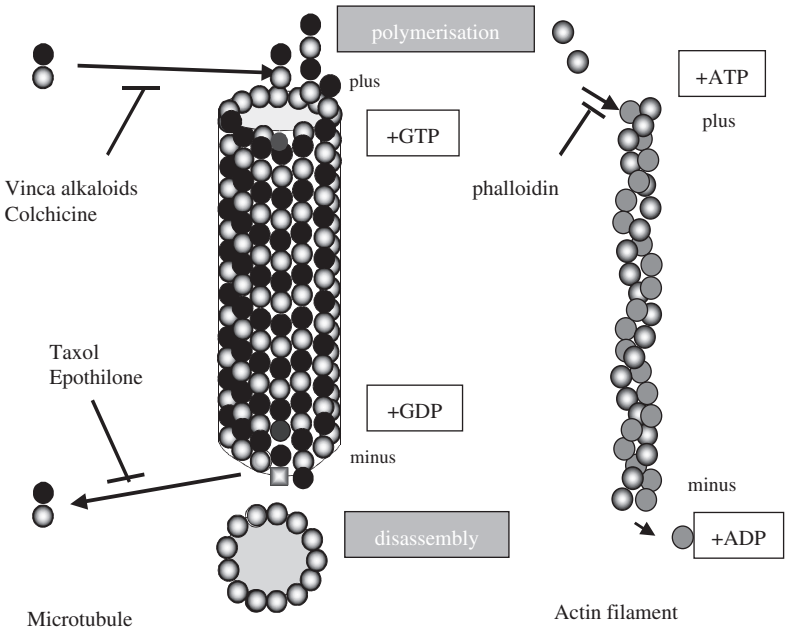

Figure 4 Illustration of the assembly and disassembly of microtubules and actin filaments.

disassemble (Figure 4). Even minor alterations to microtubule dynamics can engage the spindle checkpoint and that leads to cell-cycle arrest at mitosis and eventually to apoptosis (38,39).

Since cancer cells show a faster cell division rate than differentiated normal cells, the inhibition of microtubule activity in mitotic cells is a major target for anticancer drugs (Table IV). Anticancer agents that modulate or inhibit microtubules have a broad spectrum of activity against hematological and solid tumors. Most of the anticancer drugs that come from nature interfere with microtubules. Well-known examples are the bis-monoterpene indole alkaloids vinblastine and vincristine that are produced by *Catharanthus roseus* and paclitaxel (taxol) from *Taxus brevifolia* and *T. baccata*. Apparently, specific binding sites for these substances exist, that can interfere with microtubule dynamics (growth or shortening of microtubules) (40).

Vincristine binds to tubulin heterodimers and inhibits tubulin polymerization. The result is a depolymerization of microtubules and/or the formation of abnormal tubulin polymers. Other alkaloids with similar activities are colchicine and its derivative colcemide that occur in *Colchicum* species. Colchicine is too toxic and cannot be used in cancer therapy, whereas colcemide (demecolcine) has been used in the treatment of skin cancer.

Colchicine has an application in agriculture to induce polyploidization and in medicine to treat gout. In the latter case, apoptosis inhibits the movement of macrophages that are essential in the development of gout by blocking microtubule formation (3).

Table IV Interference of alkaloids with the assembly and disassembly of microtubules and actin filaments

Alkaloid	Source	Effect	References
Chelidonine	*Chelidonium majus* (Papaveraceae)	DNA intercalation; spindle poison; activation of caspase-3; DNA fragmentation; depolarization of mitochondrial membranes, apoptotic	(51,163,164)
Colchicine	*Colchicum* sp. (Colchicaceae)	Inhibition of S–S crosslinks between tubulin units	(165)
Cryptophycins	Marine sources	Inhibition of tubulin polymerization	(166–168)
Cytochalasin B	*Phoma* sp. (fungi)	Inhibition of actin filament assembly	(16)
Diazonamide A	*Diazona angulata* (ascidian)	Inhibition of tubulin polymerization	(169)
Dolostatin 10	*Dolabella auriculata*	Inhibition of tubulin polymerization	(170)
Epothilone A and B	Myxobactera	Inhibitor of microtubule depolymerization; activation of polymerization	(174)
Evodiamine	*Euodia hortensis* (Rutaceae)	Inhibition of tubulin polymerization; induction of cell-cycle arrest in G_2/M phase	(171)
Latrunculin A, B	*Latrunculia magnifica* (sponge)	Inhibition of actin filament assembly	(16)
Maytansine	*Maytenus* sp. (Celastraceae)	Inhibition of S–S crosslinks between tubulin units; antimitotic, anticancer drug	(165)
Misakinolide A	marine sources	Action on actin cytoskeleton	(172)
Nomofungin	endophytic fungus	Disruption of microfilaments	(173)
Noscapine	*Papaver somniferum* (Papaveraceae)	Tubulin binding; inhibition of microtubule assembly; induction of polyploidy	(119,174)
Paclitaxel	*Taxus* sp. (Taxaceae)	Inhibitor of microtubule depolymerization; activation of polymerization	(174–178)
Phalloidin	*Amanita phalloides* (fungi)	Stabilization of actin filaments, inhibition of actin depolymerization	(16)
Sanguinarine	Papaveraceae	Interaction with cytoskeleton	(179)
Vincristine, vinblastine	*Catharanthus roseus* (Apocynaceae)	Inhibition of S–S crosslinks between tubulin units; forming spiral filaments of tubulin	(165,180,181)
Vitilevuamide	*Didemnum cuculiferum* (ascidian)	Inhibition of tubulin polymerization; cytotoxic	(182)
Welwistatin	cyanobacteria	Inhibition of tubulin polymerization	(165,183)

Paclitaxel and other taxanes accelerate tubulin polymerization by stabilizing already assembled microtubules and thus preventing the depolymerization of microtubules. The fungal metabolites epothilone A and B share some of the binding characteristics of taxanes (40,41).

Microtubule-targeting drugs arrest cell-cycle progression, which eventually leads to apoptotic cell death (see below). Anticancer drugs that target microtubules have serious side effects, notably neurological and hematological toxicities. These inhibitors are often lipophilic and can induce drug resistance due to an activation of P-glycoprotein (P-gp) and other membrane pumps (see below). Table IV provides an overview of alkaloids known to interfere with microtubules.

Actin filaments are polymers of G-actin that polymerizes when ATP is present (Figure 4). Actin filaments are important for the establishment of the cell's architecture and the integration into apoptosis tissues. They should be a good target for SM. Surprisingly, only a small number of SM have been reported that inhibit actin filament formation, among them the alkaloids from the toadstool, *Amanita phalloides* (Table IV) such as the phalloidins, and the fungal metabolite amphotericin B.

C. Induction of Apoptosis

Many cells that are no longer of use are eliminated by apoptosis (see Table IV above). Apoptosis can also be induced by a number of toxins and appears to be the major mechanism of cytotoxicity caused by SM. Many polyphenols, terpenoids, saponins, and also alkaloids apparently can induce apoptosis.

Apoptosis can be induced by two pathways: the extrinsic pathway starts with an activation of death receptors on the cell surface, which leads to the activation of caspases (Figure 5). Death receptors are a subgroup of the tumor necrosis factor (TNF) receptor family that have an intracellular death domain. Death receptors include CD95, TRAIL-R1, and TRAIL-R2 (TNF-related apoptosis inducing ligand). The stimulated death receptors activate an adaptor protein FADD (Fas-associated death domain protein), which, in turn, activates the inactive form of caspase-8 (cysteine-aspartyl-specific proteases). Caspase-8 activates procaspase-3 and also the protein Bid (a member of the Bcl-2 family) that can stimulate and amplify the intrinsic pathway.

The intrinsic pathway is triggered by the permeabilization of mitochondrial membranes releasing cytochrome c and reducing ATP levels (42). Cytochrome c and other apoptotic factors form a complex with Apaf-1 (apoptotic protease activating factor) and inactive caspase-9. This complex has been termed apoptosome and leads to the activation of caspase-9 (43,44). Activated caspases-8 and -9 can activate pro-caspase-3 to caspase-3. The activated caspases cleave cellular proteins (e.g., proteins of the cytoskeleton) via caspase-activated DNAse (CAD) and also chromatin. As a consequence, a sequence of morphological and biochemical degradation steps sets in. During apoptosis a DNAse, Endo G, a aspase-independent apoptotic protein, is activated. If the DNA of apoptotic cells is analyzed by gel electrophoresis, a typical ladder pattern of fragmented chromosomes can be observed.

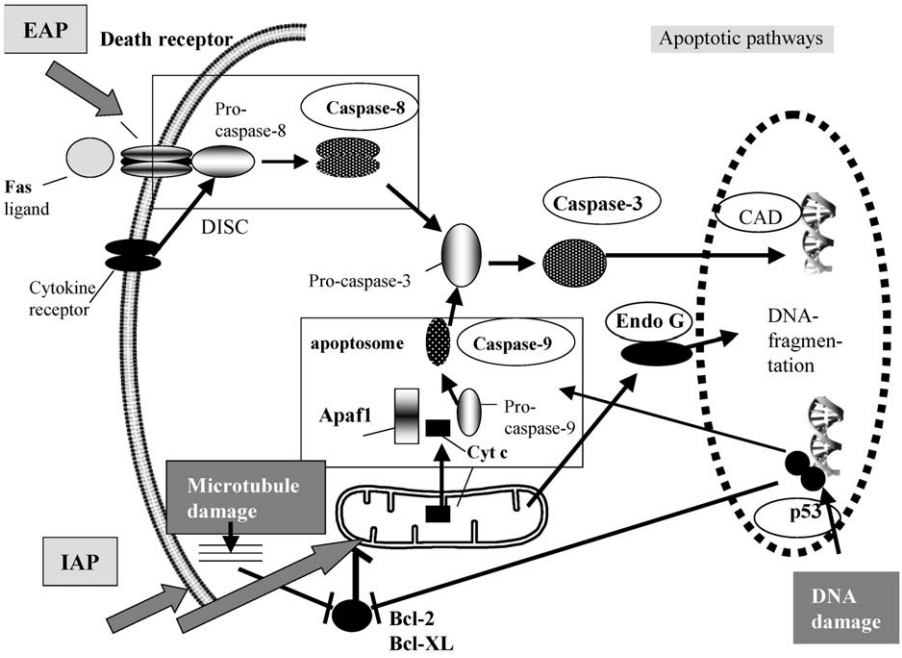

Figure 5 Schematic illustration of apoptosis with a focus on the extrinsic and intrinsic pathways.

Bcl-2 is an anti-apoptotic protein that keeps caspases in an inactive state and regulates the mitochondrial pathway. Overexpression of the *bcl-2* gene may confer resistance to chemotherapeutic drugs. A down-regulation of Bcl-2 and Bcl-XL enhances apoptosis. Agents that interfere with microtubules (such as spindle poisons) apparently inactivate Bcl-2 (46,47).

Some alkaloids have oxidizing properties and lead to the production of reactive oxygen species (ROS). The formation of ROS is associated with DNA damage, and cell cycle and mitochondrial disturbance.

DNA damage, which can also be caused by alkylating and intercalating compounds or gamma-irradiation and other signals, can stimulate the tumor suppressor gene p53 (45). As a consequence, Apaf-1 becomes activated, triggering apoptosis (Figure 5). P53 is a stress sensor that is modified by post-translational modifications. Activated p53 reacts as a transcription factor for pro-apoptotic proteins, including BAX, and death receptors (CD95, TRAIL). Loss of p53 function is associated with tumor aggressiveness and resistance to anti-cancer treatments.

On inhibition of the apoptotic pathway, as by the loss of p53 or Apaf-1 function, pre-cancerous cells survive and proliferate, and eventually form a tumor. A number of alkaloids have been described recently that exhibit anti-apoptotic effects. Huperzine A from *Huperzia serrata* (Lycopodiaceae) blocks apoptosis via the inhibition of ROS formation and caspase-3 activation (48). In the case of sampangine, the apoptotic effects could be blocked by the administration of a

ROS quencher, such as *N*-acetylcysteine, vitamin C, and vitamin E (49,50). Apoptosis is a very complex process in which many factors and interactions are still unknown. Therefore, the scheme drawn in this review (Figure 5) must necessarily be incomplete. A full treatment would be beyond the scope of this review.

Searching for new drugs with anticancer activities, those SM with apoptotic properties are of major interest. Table V lists these alkaloids that have been reported to have apoptotic properties. Not all alkaloids are apoptotic. In a more systematic approach, seventy alkaloids from several biogenetic groups were tested in this laboratory with leukemia cells (HL-60; Jurkat T-cells). Several protoberberine and benzophenanthridine alkaloids (berberine, chelerythrine, cheldonine, sanguinarine), homoharringtonine, noscapine, indole alkaloids (harmine, quinine, vincristine, vinblastine, ellipticine), emetine, piperine, and colchicines apparently induce apoptosis, whereas the tropane, quinolizidine, piperine, pyridine, purine, steroidal, and diterpene alkaloids were not active up to a concentration of 100 μM (51). The active alkaloids have in common that they intercalate DNA, and in consequence inhibit DNA and RNA polymerase, topoisomerases, and even ribosomal protein biosynthesis (12), or bind to tubulin/microtubules, thus acting as spindle poisons.

D. Interactions with ABC Transporters and Cytochrome p450

During evolution, herbivorous or predatory animals had to cope with the SM present in their food (3,10,52–54). In this review, only the adaptations of mammals will be considered in more detail, as they are relevant to pharmacology and to medicine.

In order to overcome the intestinal and blood brain barrier, plants have evolved lipophilic toxins that can enter the body by simple diffusion. As a counter measure, animals have evolved a powerful set of detoxification mechanisms. These involve reactions known in pharmacology as phase I and phase II reactions. In phase I, a lipophilic molecule is made more hydrophilic by introducing hydroxyl groups. This reaction is catalyzed by enzymes of an enzyme family, known as cytochrome p450 oxidases (CYP); other CYP (among them. CYP1A1, CYP1A2, CYP3A4, and CYP2D6 are the most important enzymes) cleave *N*-methyl, *O*-methyl, or methylene groups in order to obtain a more hydrophilic or more readily accessible substrate (55,56). In the human genome, about 57 active CYP genes are known (57). The polymorphism of CYPs that can metabolize drugs is substantial, and the regulation of the corresponding genes is only partly known. These enzymes, which occur in intestinal epithelia and in the liver, are inducible by SM that have entered the body. In Phase II, the hydroxylated compounds are conjugated with polar molecules, such as glutathione, sulfate, or glucuronic acid. These conjugates are eliminated via the kidneys and urine. That means, on exposure to lipophilic SM (among them several alkaloids, Table VI), enzymes are often induced (such as the cytochrome p450 system) and that activation that can inactivate the toxins. Several SM carry

Table V Induction of apoptosis by alkaloids

Alkaloid	Source	Effect	References
Alkaloids derived from tryptophan			
Camptothecin	*Camptotheca acuminata* (Cornaceae)	Topoisomerase I inhibitor; apoptosis induction in S-phase	(184)
Cinchonidine	*Cinchona pubescens* (Rubiaceae)	Weak DNA intercalation; activation of caspase-3; DNA fragmentation; depolarization of mitochondrial membranes; apoptotic	(51)
Cryptolepine	*Cryptolepis sanguinolenta* (Asclepiadaceae)	Cell-cycle arrest in G_2/M phase; PARP cleavage; cytochrome c release; upregulation of p53; DNA intercalation; activation of caspase-3, -9; PARP cleavage; DNA fragmentation; apoptotic; clastogenic potential	(39,51,85,171,184,186–190)
Ellipticine	*Ochrosia elliptica* (Apocynaceae)	Inhibition of cell cycling (G_2/M arrest); activation of Fas/Fas ligand pathway; induction of mitochondrial apoptotic pathway with caspase-9 activation; activation of caspase-3; DNA fragmentation; mitochondrial membrane depolarization; inhibitor of protein kinases which phosphorylate p53; bax activation	(19,51,90,91,191–194)
Ergotamine	*Claviceps purpurea*	Weak DNA intercalation; DNA fragmentation; slightly apoptotic	(51)
Evocarpine	*Evodia rutaecarpa* (Rutaceae)	Apoptotic	(195)
Evodiamine	*Evodia rutaecarpa* (Rutaceae)	Induction of cell-cycle arrest and apoptosis; tubulin inhibitor	(196–198)

Table V (*Continued*)

Alkaloid	Source	Effect	References
Harmine	*Peganum harmala* (Zygophyllaceae)	Inhibitor of cyclin dependent kinases; inhibition of Bcl-2 expression; upregulation of death receptor Fas, some DNA intercalation; activation of caspase-3; DNA fragmentation; depolarization of mitochondrial membranes, apoptotic	(51,111)
9-Hydroxyellipticine	*Ochrosia elliptica* (Apocynaceae)	Apoptotic; inhibition of p53 phosphorylation; inhibition of cdk2 kinase	(193)
10-Hydroxycamptothecin	*Camptotheca acuminata* (Cornaceae)	Topoisomerase I inhibitor; cell-cycle arrest in G_2/M phase; apoptosis induction	(78)
Isostrychnopentamine	*Strychnos usambarensis* (Loganiaceae)	Caspase-3, -9 activation, cell-cycle arrest in G_2–M phase, p21 induction	(199)
Neocryptolepine	*Cryptolepis sanguinolenta* (Asclepiadaceae)	Cell-cycle arrest in G_2/M phase; PARP cleavage; cytochrome c release; p53 upregulation	(85)
Quinine	*Cinchona pubescens* (Rubiaceae)	Weak DNA intercalation; cell-cycle arrest; activation of caspase-3; DNA fragmentation; depolarization of mitochondrial membranes, apoptotic	(51,200)
Sungucine	*Strychnos icaja* (Loganiaceae)	Caspase-3, -9 activation, cell-cycle arrest in G_1; PARP cleavage	(199,201)
Usambarensine	*Strychnos usambarensis* (Loganiaceae)	Caspase activation; apoptotic	(25)
Vinblastine, Vincristine	*Catharanthus roseus* (Apocynaceae)	DNA intercalation; spindle poison; phosphorylation of Bcl-2 and Bcl-XL by JNK; induction of p53; activation of caspase-3; DNA fragmentation; apoptotic; mitochondrial pathway; ROS production	(51,186,187–190,202–205)

Alkaloids derived from phenylalanine/tyrosine			
Antofine and related alk.	*Cynanchum* sp. (Asclepiadaceae)	Cell-cycle arrest in G_2/M phase, cytotoxicity	(206,207)
Berbamine	Ranunculaceae	Apoptotic	(208,209)
Berberine	*Berberis vulgaris* (Berberidaceae)	DNA intercalator; enzyme inhibitor; apoptotic, cell-cycle arrest in G_0–G_1-phase; caspase-3 activation; depolarization of mitochondrial membranes; downregulation of telomerase; antineoplastic, antimalarial	(51,107,133,134,210–212)
Cepharanthine	*Stephania cepharantha* (Menispermaceae)	Caspase-3 activation; induction of apoptosis; apoptosis protection; radical scavenger	(213–217)
Chelerythrine	*Chelidonium majus* (Papaveraceae)	DNA intercalation; release of cytochrome c; Bcl-XL inhibitor; activation of caspase-3; DNA fragmentation; depolarization	(51,163,164)
Chelidonine	*Chelidonium majus* (Papaveraceae)	DNA intercalation; spindle poison; activation of caspase-3; DNA fragmentation; depolarization of mitochondrial membranes, apoptotic	(51,163,164)
Colchicine	*Colchicum autumnale* (Colchicaceae)	Spindle poison; activation of caspase-3; DNA fragmentation; apoptotic; activation of JNK/SAPK; ROS production; inhibition of mitochondrial electron chain	(51,187,205,218)
Emetine	*Psychotria ipecacuanha* (Rubicaeae)	Protein, DNA and RNA synthesis inhibition; DNA intercalation; induction of apoptosis; caspase-3 activation; DNA fragmentation; depolarization of mitochondrial membranes	(51,219–222,328)
Harringtonine	*Cephalotaxus hainanensis* (Cephalotaxaceae)	Apoptosis induction	(223)

Table V (*Continued*)

Alkaloid	Source	Effect	References
Homoharringtonine	*Cephalotaxus harringtonia* (Cephalotaxaceae)	Apoptotic antileukemia drug; DNA fragmentation; caspase-3 activation; cytochrome c release; cleavage of poly(ADP-ribose) polymerase (PARP); bax upregulation; Bcl-2 downregulation	(51,224–226)
Isoharringtonine	*Cephalotaxus hainanensis* (Cephalotaxaceae)	Apoptosis induction , Bcl-2 downregulation	(227)
Liriodenine	*Liriodendron* sp. (Magnoliaceae)	Cell-cycle arrest in G_2/M phase; reduction of cyclin-dependent kinases; caspase activation; apoptotic	(228)
Lycorine	*Narcissus* sp. (Amaryllidaceae)	Cell-cycle arrest in G_2/M phase; caspase-8, -9, -3 activation; Bcl-2 downregulation	(229)
6–Methoxydihydro-sanguinarine	*Hylomecon hylomeconoides* (Papaveraceae)	Activation of caspase-8, -9, -3; PARP cleavage; release of cyt c; downregulation of Bcl-2; upregulation of p53 and bax; ROS generation, apoptotic	(246,247)
Noscapine	*Papaver somniferum* (Papaveraceae)	Tubulin binding; inhibition of microtubule assembly; cell-cycle arrest; activation of c-Jun NH2-terminal kinases (JNK); DNA fragmentation of mitochondrial membranes, apoptotic; ROS production	(51,230–235)
Pancratistatin	*Pancratium littorale* (Amaryllidaceae)	Apoptotic via mitochondria; cell-cycle arrest	(236–237)
Papaverine	*Papaver* sp. (Papaveraceae)	Low activation of caspase-3; DNA fragmentation; apoptotic	(51)

Piperine	*Piper nigrum* (Piperaceae)	Activation of caspase-3; DNA fragmentation; depolarization of mitochondrial membranes, apoptotic	(51)
Protopine	*Chelidonium majus* (Papaveraceae)	Activation of caspase-3; DNA fragmentation; slightly apoptotic (1 μM)	(51)
Sampangine	*Cananga odorata* (Annonaceae)	Induction of ROS formation, which disturb cell cycle and mitochondria, leading to apoptosis; antiparasitic	(49,50)
Sanguinarine	*Sanguinaria canadensis* (Papaveraceae)	Cell-cycle arrest in G_0–G_1-phase; induction of apoptosis in mitochondrial pathway, downregulation of cycline and cycline-dependant kinase; PARP cleavage; caspase activation; glutathione depletion; ROS production; depolarization of mitochondrial membranes; activation of caspase-3	(51,133,238–245)
Sinoculine	*Stephania sutchuensis* (Menispermaceae)	Cytotoxic, apoptotic	(248)
Tetrandrine	*Stephania tetrandra* (Menispermaceae)	Activation of caspase-3 dependant mitochondrial apoptosis pathway; activation of Endo G; PARP cleavage	(209,249–251)
Thaliblastine	*Thalictrum* sp. (Ranunculaceae)	Apoptotic DNA fragmentation	(252)
Alkaloids derived from ornithine/arginine			
Monocrotaline	*Crotalaria* sp. (Fabaceae)	Mutagenic; apoptotic;	(143)
Retrorsine	*Senecio* sp. (Asteraceae)	Apoptotic in liver cells, bax upregulation; Bcl-xL downregulation; cytochrome c release	(143)

Table V (*Continued*)

Alkaloid	Source	Effect	References
Alkaloids derived from lysine			
Matrine	*Sophora* sp. (Fabaceae)	Inhibition of DNA synthesis; cell arrest in G_1 phase; induction of apoptosis	(253)
Alkaloids with a terpenoid backbone			
Cyclopamine	*Veratrum album* (Liliaceae)	Blocks intracellular Shh signalling; apoptotic	(254)
Paclitaxel	*Taxus* sp. (Taxaceae)	Cell-cycle arrest in G_2/M phase; apoptotic; Bcl-XL phosphorylation; caspase-3 activation; PARP cleavage; caspase-8 activation (CD95/CD95-L independent)	(255–257)
Solamargine	*Solanum* sp. (Solanaceae)	Cytotoxicity; apoptotic;	(258)
Miscellaneous alkaloids			
Acronycine	*Acronychia baueri* (Rutaceae)	DNA alkylation; intercalator; carcinogen, apoptotic	(144–146)
Actinomycin D	*Streptomyces* sp.	DNA intercalating; inhibitor of DNA dependent RNA polymerase; DNA fragmentation; apoptotic; antineoplastic	(260–262)
Asciddemin	*Cystodytes dellechiajei* (ascidian)	DNA intercalation, inhibition of topoisomerase II; PARP cleavage; caspase-3 activation; apoptotic	(263)
Caffeine	*Coffea arabica* (Rubiaceae)	Slightly apoptotic; cell-cycle arrest; p53 dependence	(264–265)
Capsaicin	*Capsicum frutescens* (Solanaceae)	Apoptotic; Bcl dependent	(266)
Cycloheximide	*Streptomyces griseus*	Protein biosynthesis inhibitor; DNA fragmentation; caspase activation; apoptotic	(51,220,262)

Daunomycin	*Streptomyces peuceticus*	DNA intercalator; caspase-3 activation; DNA fragmentation; p53 activation	(51,267,268)
Lamellarin D	*Didemnum obscurum* (ascidian)	Promotion of apoptosis via mitochondrial pathway	(155)
Mahanine	*Micromelum minutum* (Rutaceae)	Induction of apoptosis by activating mitochondrial pathway and caspase-3; -8, -8 and -9; PARB cleavage; cytochrome c release; generation of ROS;	(269,270)
1-Methoxy-canthin-6-one	*Ailanthus altissima* (Simaroubaceae)	Induction of apoptosis	(259)
Polycarpine	*Polycarpa aurata* (ascidian)	Apoptotic; activation of p53	(271)
Preussin	marine sources	Apoptotic; caspase activation; cytochrome c release	(272)
Prodigiosin	*Serratia marcescens* (Bacteria)	DNA intercalating; inhibition of topoisomerase I and II; induction of apoptosis	(161)
Staurosporine	*Streptomyces* sp.	Kinase inhibitor, ROS formation; caspase-3 activation; mitochondrial dysfunction; Apoptosis	(273,274)
Theophylline	*Camellia sinensis* (Theaceae)	Slightly apoptotic, downregulation of Bcl-2;	(275,276)
Valinomycin	*Streptomyces fulvissimus*	Apoptotic, DNA fragmentation, depolarization of mitochondrial membranes	(51,277,278)

Table VI Interaction of alkaloids with detoxification mechanisms in mammals (cytochrome p450 and ABC transporters)

Alkaloid	Source	Effect	References
Alkaloids derived from tryptophan			
Ajmalicine, serpentine	*Rauvolfia serpentina* (Apocynaceae)	Substrate of CYP2D6, but not of CYP3A4	(279)
Bromocryptine	*Claviceps purpurea* (fungi)	Reversal of P-gp mediated drug resistance	(280)
Brucine	*Strychnos* sp. (Loganiaceae)	Inducer of CYP2B3	(281)
Camptothecin	*Camptotheca acuminata* (Cornaceae)	Substrate for ABC2 transporter in *Botrytis cinerea*; for PMR5 from *Penicillium digitatum*, AtrBp from *Aspergillus nidulans*; induction of MDR overexpression	(282–286)
Cinchondine	*Cinchona pubescens* (Rubiaceae)	P-gp substrate	(287)
Cinchonine	*Cinchona pubescens* (Rubiaceae)	Reversal of P-gp mediated drug resistance	(288,289)
Conoduramine	*Peschiera laeta* (Apocynaceae)	P-gp binding	(290)
Coronaridine, Heyneanine	*Tabernanthe iboga* (Apocynaceae)	Reversal of P-gp mediated drug resistance in vincristine-resistant KB cells	(291)
Dihydroergocryptine	*Claviceps purpurea*	Metabolization by CYP3A4	(292)
Ergotamine	*Claviceps purpurea*	P-gp substrate	(68,287)
		Metabolism by CYP3A4	(293)
Harmine	*Banisteriopsis caapi* (Malpighiaceae)	Inhibition of CYP2D6; *O*-demethylation by CYP1A1 and CYP2D6	(294,295)
Ibogaine	*Tabernanthe iboga* (Apocynaceae)	*O*-methylation by CYP2D6	(296)
Indole-3-carbinol	Many Brassicaceae	Downregulation of upregulated P-gp; dietary adjuvant in MDR cancer treatment	(67)
		Activates CYP1A1	(297)
Kopsamine, pleiocarpine	*Kopsia dasyrachis* (Apocynaceae)	Reversal of P-gp mediated drug resistance	(291)
Kopsiflorine	*Kopsia dasyrachis* (Apocynaceae)	Reversal of P-gp mediated drug resistance	(298)
Quinidine	*Cinchona pubescens* (Rubiaceae)	P-gp substrate	(287)

Quinine	*Cinchona pubescens* (Rubiaceae)	Reversal of P-gp mediated drug resistance, P-gp substrate	(155,287,288)
		Substrate of CYP2D6	(299)
Rescinnamine	*Rauvolfia serpentina* (Apocynaceae)	P-gp substrate	(287)
Reserpine	*Rauvolfia serpentina* (Apocynaceae)	Substrate for bacterial ABC transporters; reversal of multidrug resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) strains (NorA MDR pump); P-gp substrate	(287,300,301)
Rutaecarpine	*Evodia rutaecarpa* (Rutaceae)	Reversal of P-gp mediated drug resistance	(302)
		Substrate of CYP1A and CYP2B; induction of CYP1A1, CYP1A2, CYP2B, CYP2E1	(303–306)
Strychnine	*Strychnos* sp. (Loganiaceae)	Inducer of CYP2B1, CYT2B2	(307)
Vincristine, Vinblastine	*Catharanthus roseus* (Apocynaceae)	Substrate of P-gp; P-gp substrate in BBB; induction of MDR overexpression	(308,309)
		Substrate of CYP3A4	(310–313)
Voacamine	*Peschiera fuchsiaefolia* (Apocynaceae)	Reversal of P-gp mediated drug resistance; enhancement of doxorubicin cytotoxicity	(314)
		P-gp substrate in BBB	(202,290,308,315)
Alkaloids derived from phenylalanine/tyrosine			
Berbamine	*Berberis* sp. (Berberidaceae)	P-gp substrate in BBB, and in Caco2 cells	(287,308)
Berberine	*Hydrastis canadensis* (Ranunculaceae)	Substrate of P-gp and bacterial ABC transporters; P-gp substrate in BBB	(308,317,318)
		inhibition of CYP1A1; substrate of CYP2C9, CYP2D6, CYP3A4	(319,320)
Boldine	*Peumus boldo* (Monimiaceae)	P-gp substrate	(287)
Californine	*Eschscholtzia californica* (Papaveraceae)	*N*-demethylation by CYP3A2; demethylenation by CYP2D1 and CYP2C11	(321)
Cephaeline	*Psychotria ipecacuanha* (Rubicaeae)	*O*-demethylation by CYP3A4, CYP2D6	(322)
Cepharanthine	*Stephania cepharantha* (Menispermaceae)	Inhibition of P-gp; enhancement of sensitivity of K562 cells towards doxorubicin and vincristine; apoptotic	(215,216,323,324)

Table VI (*Continued*)

Alkaloid	Source	Effect	References
Chelerythrine	*Zanthoxylum clava-herculis* (Rutaceae)	Reversal of drug resistance in methicillin-resistant *Staphylococcus aureus* (MRSA)	(325)
		Inhibition of CYP1A1; detoxification by CYP1A	(326)
Colcemide	*Colchicum autumnale* (Colchicaceae)	Spindle poison; P-gp substrate	(327)
Colchicine	*Colchicum autumnale* (Colchicaceae)	Spindle poison; apoptotic; P-gp substrate	(62,327)
Emetine	*Psychotria ipecacuanha* (Rubicaeae)	P-gp substrate; induction of MDR overexpression	(328)
		O-demethylation by CYP3A4, CYP2D6	(322)
Fangchinoline	*Stephania tetrandra* (Menispermaceae)	Reversal of P-gp mediated drug resistance; P-gp inhibition	(65,329)
Galanthamine	*Galanthus nivalis* (Amaryllidaceae)	Metabolization by CYP2D6 and CYP3A4	(330)
Glaucine	*Papaver* sp. (Papaveraceae)	P-gp substrate	(287)
Homoharringtonine	*Cephalotaxus harringtonia* (Cephalotaxaceae)	Induction of MDR overexpression, P-gp substrate	(331,332)
Hydrastine	*Hydrastis canadensis* (Ranunculaceae)	Substrate of CYP2C9, CYP2D6, CYP3A4	(320)
5–Methoxyhydnocarpine	*Hydnocarpus kurzii* (Flacoutiaceae)	Inhibitor of NorA MDR pump in *Staphylococcus aureus*	(316)
Palmatine	Ranunculaceae; Berberidaceae	Substrate of bacterial ABC transporters	(317)
Protopine	*Eschscholtzia californica* (Papaveraceae)	Demethylenation by CYP2D1 and CYP2C11	(321)
Roemerine	*Annona senegalensis* (Annonaceae)	P-gp substrate; reversal of P-gp mediated drug resistance	(333)
Sanguinarine	*Sanguinaria canadensis* (Papaveraceae)	Apoptotic; intercalator; reversal of P-gp mediated drug resistance	(245,334)
		Inhibition of CYP1A1, detoxification by CYP1A	(326)
Tetrandrine	*Stephania tetrandra* (Menispermaceae)	Reversal of P-gp mediated drug resistance; enhancement of sensitivity of KBv200 cells towards paclitaxel, docetaxel, vinblastine, doxorubicin; P-gp substrate in BBB	(65,185,202,308)

Thaliblastine	*Thalictrum* sp. (Ranunculaceae)	Apoptotic, DNA; reversal of MDR phenotype	(252,335)
Alkaloids derived from ornithine/arginine			
Clivorine	*Ligularia hodgsonii* (Asteraceae)	Metabolic activation by CYP3A1 and CYP3A2	(136,137)
Monocrotaline	*Crotalaria* sp. (Fabaceae)	CYP3A substrate; CYP oxidation to alkylating dehydromonocrotaline	(138–140)
Nicotine, cotinine	*Nicotiana tabacum* (Solanaceae)	Metabolization by CYP2A6; inhibition of CYP2E1; CYP1A1 inducer	(336–339)
Pervilleine B, C, F	*Erythroxylum pervillei* (Erythroxylaceae)	Reversal of P-gp mediated drug resistance in KB-V1 cells and in NCr n/nu mice	(340–343)
Retrorsine	*Senecio* sp. (Asteraceae)	Induction of CYP1A1, 1A2, 2EI, 2B1/2	(143)
Senecionine	*Senecio* sp. (Asteraceae)	CYP2B and CYP3A4 oxidation to alkylating dehydroderivatives	(344–346)
Alkaloids derived from lysine			
Lobeline	*Lobelia* sp. (Campanulaceae)	P-gp substrate	(287)
Tetrahydrorhombifoline	*Lupinus* sp. (Fabaceae)	P-gp substrate	(287)
Sparteine	*Cytisus scoparius* (Fabaceae)	Metabolization by CYP	(347)
Alkaloids with a terpenoid backbone			
Aconitine	*Aconitum napellus* (Ranunculaceae)	P-gp substrate	(287)
Cyclopamine	*Veratrum album* (Liliaceae)	Apoptotic; inhibitors of P-gp; reversal multidrug resistance in NCI AdrR cells to adriamycin and vinblastine	(348)
Lycaconitine	*Aconitum pseudo-laeve* (Ranunculaceae)	MDR inhibitor in nKBV20c cells	(349)
Paclitaxel	*Taxus* sp. (Taxaceae)	P-gp substrate; induction of MDR overexpression	(327,350)
		CYP3A substrate	(312,351)
Tomatidine	*Lycopersicon esculentum* (Solanaceae)	Inhibitors of P-gp; reversal of multidrug resistance in NCI AdrR cells to adriamycin and vinblastine	(348)
Miscellaneous alkaloids			
Ardeemins	Marine	Reversal of multidrug resistance	(352)
Caffeine	*Coffea arabica* (Rubiaceae)	Demethylation by CYP1A	(353)

Table VI (*Continued*)

Alkaloid	Source	Effect	References
Capsaicin	*Capsicum frutescens* (Solanaceae)	P-gp substrate	(287)
Cycloheximide	*Streptomyces griseus*	Substrate for ABC2 transporter in *Botrytis cinerea*	(283)
Dipiperamide A,B,C,D, E	*Piper nigrum* (Piperaceae)	Inhibition of CYP3A4	(354,355)
Lamellarin D	*Didemnum obscurum* (ascidian)	Promotion of apoptosis via mitochondrial pathway; reversal of multidrug resistance	(73,356)
Pilocarpine	*Pilocarpus jaborandi* (Rutaceae)	Substrate of CYP2A6; CYP2B	(137,357)
Piperine	*Piper nigrum* (Piperaceae)	Inhibition of CYP activity and CYP induction	(358)

methylenedioxyphenyl groups, such as in berberine and hydrastine, which are assumed to be inhibitors of cytochrome p450 enzymes.

The induction of CYP is important from a biological perspective and is also of clinical importance. If patients are treated with natural drugs, an activation of the CYP system could lead to an enhanced elimination of other drugs taken concomitantly and thus to drug resistance. An example is St. John's wort (*Hypericum perforatum*), a potent antidepressive drug that has been shown to activate the CYP. In conclusion, the knowledge about CYP genes and their polymorphisms is of great importance for effective drug therapy and for drug development. Especially interesting is the role of CYP in the metabolic activation of SM and of carcinogens.

Another line of counter defence that can be activated on exposure to lipophilic drugs (mostly lipophilic cations) are the ATP binding cassette (ABC) transporters, that are present in most cells, and which are especially active in intestinal, liver, kidney, and endothelial epithelia (58,59). These ATP-driven transporters can pump lipophilic compounds out of the cell, either back to the gut lumen or into the blood system, thus reducing the intracellular concentration of potentially toxic compounds. These effects were discovered during chemotherapy of cancer patients who developed resistance against a cytotoxic drug. It transpired that the tumor cells were able to pump out the lipophilic alkaloids (such as Vinca alkaloids, taxanes, and anthracycline derivatives) at almost the same speed as they were entering the tumor cells. Activated cells were resistant to vincristine and to several other lipophilic drugs (cross-resistance or multidrug resistance). Therefore, a major obstacle to the successful chemotherapy of tumors is multidrug resistance.

Two types of efflux pumps, which belong to the ABC transporter family, have been described (Figure 6): (1) P-gp (molecular weight 170 kDa) or MDR protein (multiple drug resistance protein) that is encoded by the MDR1 gene. P-gp is an efflux pump directed to the gut lumen, and (2) MRP 1 and 2 (multiple resistance-associated protein; 190 kDa) that are encoded by the *MRP1* and *MRP2* genes. MRP transports drugs conjugated to glutathione (GSH), and also unmodified cytostatics in the presence of GSH, usually into the blood system (59,60). Figure 5 illustrates the structure of P-gp; the substrates appear to bind to P-gp, at least in the early stages, in the cytoplasm (61).

Most modulators of ABC transporters act by binding to membrane transport proteins (especially P-gp and MRP) and inhibit their drug-effluxing activity, or by indirect mechanisms related to phosphorylation of the transport proteins, or the expression of the mdr1 and mrp1 genes.

ABC transporters are also important at the blood–brain barrier (BBB). The BBB only allows the entry of small lipophilic substances by passive diffusion. However, the uptake of lipophilic compounds in the brain is relatively low due to the high activity of P-gp, MRP, and organic anion transporting polypeptides (OATPs). These transporters catalyse a rapid efflux of lipophilic xenobiotics from the CNS (62). In order to allow therapeutic agents to reach the CNS, inhibitors of ABC transporters are of medicinal importance.

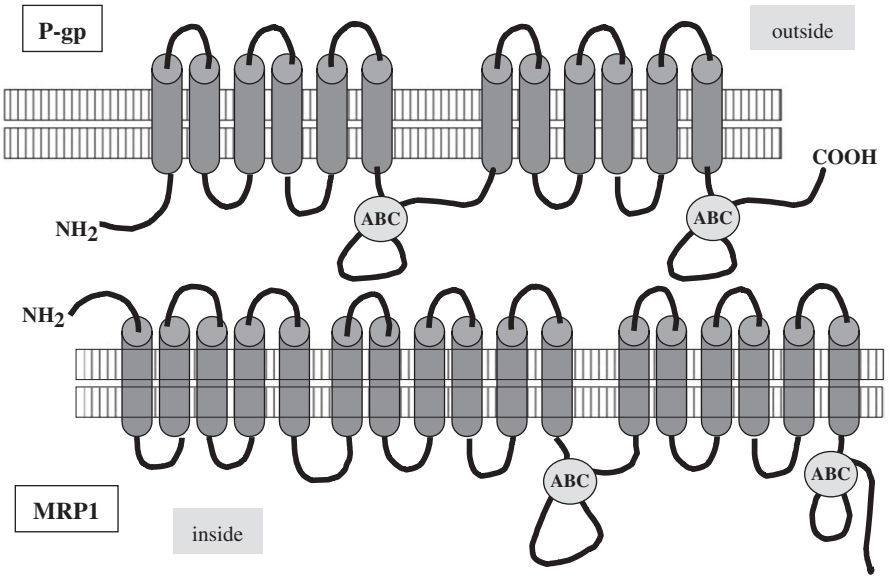

Figure 6 Molecular structure of ABC transporters, such as P-gp and MRP1.

Several of the pathogenic human parasites (*Plasmodium*, *Leishmania*, and *Trypanosoma*) often develop resistance to prophylactic and therapeutic compounds, such as quinolines, naphthoquinones, sesquiterpene lactones, and others. The underlying bases are membrane glycoproteins that are orthologous to the human P-gp, which can be induced and activated. The search for compounds that can reverse the drug resistance in parasites, also includes alkaloids (63).

A few compounds have been discovered that can reverse P-gp-dependent multidrug resistance *in vitro* and *in vivo*, so-called chemosensitizers (64). For example, synthetic derivatives of the bisbenzylisoquinoline alkaloids fangchinoline and tetrandrine apparently inhibit P-gp activity, but do not induce it. They enhanced the cytotoxicity of vinblastine much more than verapamil, a known P-gp substrate (65). Such activities might have a wider distribution among natural products; diallyl sulfide, which is released from garlic after enzymatic cleavage of alliin, is able to down-regulate P-gp in K562 cells that were resistant to Vinca alkaloids (66). The same applies to indole-3-carbinol, which is a common metabolite in the Brassicaceae. Such compounds might be used as a dietary adjuvant in the treatment of cancer for reversal of multiple drug resistance (67). A number of SM are both MDR1/P-gp chemosensitizers and CYP3A inhibitors. Such simultaneous inhibitions contribute to variation in drug–drug interactions and can be relevant for chemotherapy (68).

Table VI summarizes the evidence of alkaloids, which can serve as P-gp substrates and might be useful in strategies to reverse drug resistance in cancer cells and parasites. Since the induction of ABC transporters has evolved as a resistance mechanism against lipophilic defence chemicals, it is not surprising

that it can be induced by chemotherapeutic drugs, and by many SM, including alkaloids.

It is interesting to note that the intracellular transport of some alkaloids in plants, such as berberine, also appears to be catalysed by plant ABC transporters (69,70,359). It was shown that many alkaloids are transported by alkaloid/H^+ antiporters (71). At that time, ABC transporters were unknown. Since these antiporters were ATP-dependent, it might be worthwhile to revisit alkaloid transport mechanisms in plants (359).

V. CONCLUSIONS

Alkaloids comprise a class of SM with fascinating properties. Alkaloids are interesting from an evolutionary and ecological point of view, and they are relevant for medicine and potentially for plant protection (e.g., insecticidal pesticides).

As a follow-up of the human genome project, functional genomics will identify many of those human genes that are involved in disease and health disorders. Many of the genes will encode proteins that can be used as drug targets. So far, only a small number of human genes have been used as drug targets for the development of new therapeutics. In the future, much larger sets of new targets will become available. While searching for potential lead compounds, natural products will certainly become even more important than they are today.

Alkaloids have evolved in plants as powerful defence compounds against herbivores. Although many of them interfere with neuroreceptors and neuronal signal transduction, several others have cytotoxic properties, directed against animal and microbial cells. The present review has tried to summarize some of the molecular targets that are involved in this process. Alkaloids that interfere with microtubules, with DNA (intercalation, topoisomerase inhibition), with protein biosynthesis, and with membrane stability, apparently induce apoptosis, thus leading to cell death. It is very likely however, that many more targets exist that have not been detected, or are not yet correctly understood in this context.

Because of the evolutionary selection processes, we can safely conclude that it is very likely that alkaloids and other SM produced by plants, animals, and microbes could be relevant molecules when searching for bioactive compounds against new drug targets. Many cytotoxic compounds, such as pyrroles, pyrazines, imidazoles, and other *N*-heterocyclic alkaloids, have been discovered in marine organisms, such as sponges, ascidians, tunicates, and molluscs; several have been tested as novel antitumor agents (72–77). As sessile marine organisms cannot run away in the event of a predator or microbial attack they had to evolve potent chemical defence systems. In order to discover interesting active compounds from Nature, we only have to search by selecting the appropriate organisms and using intelligent screening assays, including high throughput procedures. In analogy to the mining for gold, this field has been termed bioprospecting- the search for biological gold.

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CHAPTER 2

Calystegines

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I. INTRODUCTION

It was a surprise when, a few years ago, a novel group of alkaloids was detected in the medicinal plant *Atropa belladonna* (deadly nightshade, Solanaceae). The plant is renowned, and has been widely utilised for its tropane alkaloid content for many years. The structures of the new alkaloids were elucidated as non-esterified, nortropane skeletons with three or four hydroxyl groups, and were named as calystegines A_3 (**1**), B_1 (**2**), and B_2 (**3**) (1). In the course of that investigation, the first observation of the alkaloids had been as spots on paper electrophoresis from extracts of the roots of *Calystegia sepium* (hedge bindweed) and *Convolvulus arvensis* (field bindweed), both in the family Convolvulaceae; the calystegines received their name from the plant, *C. sepium* (2).

The hydroxylated structures were reminiscent of other alkaloidal glycosidase inhibitors of plant and microbial origin. These have mostly hydroxylated pyrroline or piperidine rings, and in some cases bicyclic structures, like the indolizidine alkaloids castanospermine (**4**) and swainsonine (**5**) (3,4). Piperidines, like nojirimycin and 1-deoxynojirimycin (**6**), were initially detected in the course of the search for new antibiotic compounds in *Streptomyces* strains (5). Other glycosidase inhibitors were identified from plants, such as fagomine from buckwheat, *Fagopyrum esculentum* (6) or swainsonine from *Swainsona canescens* (7). Glycosidase inhibitory activity was determined for the calystegines soon after their detection (8), and this intensified interest in these alkaloids.

Up to now, the calystegines were found in plants, not in microorganisms, and predominantly in those plants that contain well-known and medicinally significant tropane alkaloids. These alkaloids have been in the focus of biochemical interest for a long time, and their biosynthesis has been the subject of many enzymatic and genetic investigations. As the calystegines are formed partially by the same biosynthetic steps, some of the biosynthetic enzymes and genes are already known.

Reviews on the calystegines were compiled previously, or were included in reviews on glycosidase inhibitors and polyhydroxylated alkaloids (4,9–13). In this review, emphasis will be placed on the following:

- Recent publications on the calystegines
- Biosynthetic enzymes and genes
- Specific properties different from other glycosidase inhibitors

II. STRUCTURES AND CHEMICAL PROPERTIES

After the examination of numerous species in the families Solanaceae and Convolvulaceae, and of *Morus* species in the Moraceae, 14 different alkaloids named as calystegines are distinguished, in addition to two, naturally occurring calystegine glycosides (Figure 1). Assignment of individual structures into the sections of calystegines A, B, and C depends on the number of three to five hydroxyl groups, respectively. Calystegines of section N possess a bridgehead

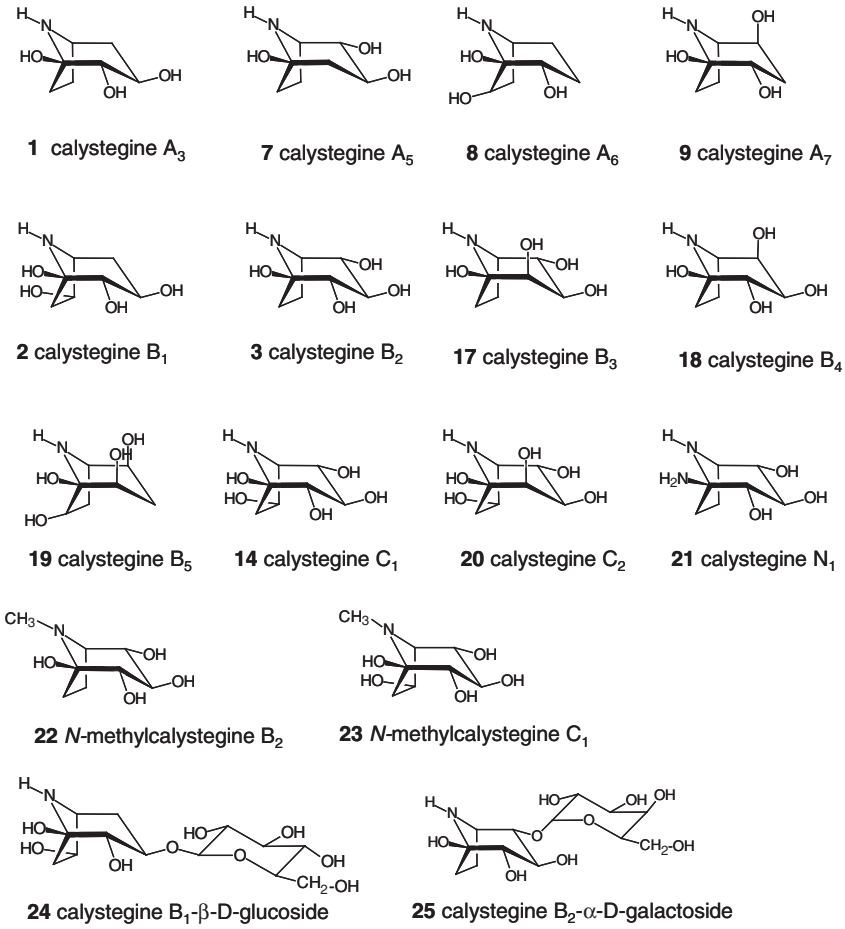


Figure 1 Structures of the calystegines.

amino group and comprise a single representative only. Numbering of individual calystegines within each section basically follows the date of the first isolation and identification. However, calystegines A_1, A_2, and A_4 were numbered as HPLC peaks and their structures are not further elucidated (1).

Subsequently, the calystegines of the A-group were numbered as A_5 (**7**), A_6 (**8**), and A_7 (**9**). Calystegine A_5 was identified in *C. sepium* (14), and may well be identical with one of the presumed calystegines A_1, A_2, or A_4. Besides the calystegines, a number of nortropanes are known that also carry several hydroxyl groups, but no bridgehead hydroxyl (Figure 2). They are not named or numbered as calystegines, partially because they had been assigned other names before the calystegines were detected, e.g., (−)-erycibelline (**10**) and baogongten C (**11**) (15,16). Further di- and trihydroxynortropane alkaloids were found in the course of alkaloid investigations of *Erythroxylum* species; they were mostly esterified and were named as nortropane alkaloid esters accordingly (17–20).

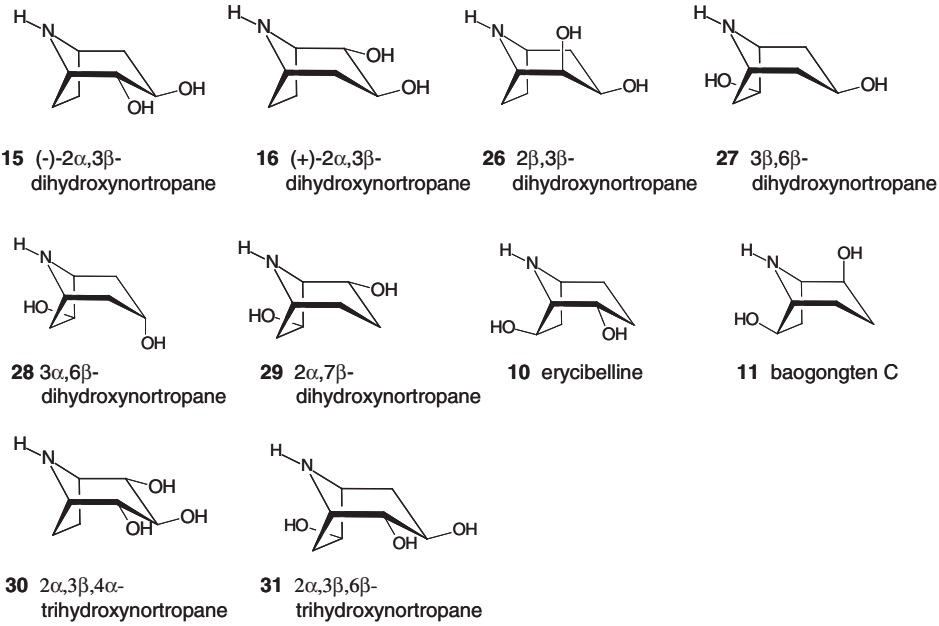


Figure 2 Structures of nortropane alkaloids with two or three hydroxyl groups.

Alkaloids in Figure 2 are characterised by carrying no bridgehead hydroxyl group, which renders them stronger bases than the 1-hydroxy aminoketal structures (Table I). They mostly have calculated pK_a values higher than 9, and approach those of the strong bases pseudotropine (**12**) and norpseudotropine (**13**) that are listed for comparison. For the aminoketal structures with a hydroxyl in position 1 there was concern whether the bicyclic system is stable, or whether it opens to give the monocyclic 4-aminocycloheptan-1-one structure. Studies with synthetic calystegines and analogous alkaloids indicated that the number and position of the hydroxyl groups are critical for the equilibrium between monocyclic and bicyclic configuration (21). All naturally occurring calystegines showed a clear preference for the bicyclic structure, but ring-opening and isomerisation cannot be excluded, and may contribute to the multitude of calystegine structures (see Section IIIC). Optical rotations are reported for many calystegines, but the absolute configuration was determined only for the naturally occurring calystegine B_2 (**3**) to be 1*R*, 2*S*, 3*R*, 4*S*, 5*R* (22–24). For other calystegines detected subsequently, optical rotation values established that they were chiral, and absolute configurations were concluded from their similarity to calystegine B_2 (**3**). From a biosynthetic perspective, this may be justified for calystegines within one plant. However, calystegines with contrasting chirality may also occur in taxonomically distant plant families. Although calystegine B_2 (**3**) from *Morus bombycis* leaves (25) and calystegine C_1 (**14**) from *M. alba* roots (26) revealed the same optical rotation as the corresponding calystegines from the Solanaceae species *Physalis alkekengi* (27), the isolation of (−)- and (+)-2α,3β-dihydroxynortropanes (**15** and **16**) from

Table I Chemical and Physical Properties of Calystegines and Related Compounds

	Alkaloid name	Structure	Optical rotation	CAS no.	Molecular weight	pK_a[a]	Solubility (water)[b]	OWPC[c]
1	Calystegine A_3	8-Azabicyclo[3.2.1]octane-1,2,3-triol (1*R*,2*S*,3*R*,5*R*)	–	131580-36-4	159.18	8.27±0.70	H_2O pH10 vs	−1.091±0.427
7	Calystegine A_5	8-Azabicyclo[3.2.1]octane-1,3,4-triol (1*R*,3*S*,4*S*,5*R*)	?	165905-26-0	159.18	8.27±0.70	H_2O pH10 vs	−1.162±0.415
8	Calystegine A_6	8-Azabicyclo[3.2.1]octane-1,2,7-triol (1*S*,2*S*,5*S*,7*S*)	?	177794-04-6	159.18	8.00±0.70	H_2O pH10 vs	−1.213±0.429
9	Calystegine A_7	8-Azabicyclo[3.2.1]octane-1,2,4-triol (1*R*,2*S*,4*S*,5*R*)	–	197565-90-5	159.18	8.10±0.70	H_2O pH10 vs	−1.284±0.416
2	Calystegine B_1	8-Azabicyclo[3.2.1]octane-1,2,3,6-tetrol (1*R*,2*S*,3*R*,5*S*,6*R*)	?	127414-86-2	175.18	7.22±0.70	H_2O pH10 vs	−1.715±0.462
3	Calystegine B_2	8-Azabicyclo[3.2.1]octane-1,2,3,4-tetrol (1*R*,2*S*,3*R*,4*S*,5*R*)	+	127414-85-1	175.18	7.34±0.70	H_2O pH10 vs	−1.072±0.488
17	Calystegine B_3	8-Azabicyclo[3.2.1]octane-1,2,3,4-tetrol (1*R*,2*R*,3*R*,4*S*,5*R*)	+	178231-95-3	175.18	7.34±0.70	H_2O pH10 vs	−1.072±0.488
18	Calystegine B_4	8-Azabicyclo[3.2.1]octane-1,2,3,4-tetrol (1*R*,2*S*,3*R*,4*R*,5*R*)	–	184046-85-3	175.18	7.30±0.70	H_2O pH10 vs	−1.072±0.488
19	Calystegine B_5	8-Azabicyclo[3.2.1]octane-1,2,4,7-tetrol (1*R*,2*R*,4*S*,5*R*,7*S*)	+	197565-91-6	175.18	7.06±0.70	H_2O pH10 vs	−1.580±0.469
14	Calystegine C_1	8-Azabicyclo[3.2.1]octane-1,2,3,4,6-pentol (1*R*,2*S*,3*R*,4*S*,5*R*,6*R*)	+	156705-04-3	191.18	6.30±0.70	H_2O pH10 vs	−1.585±0.525
20	Calystegine C_2	8-Azabicyclo[3.2.1]octane-1,2,3,4,6-pentol (1*R*,2*R*,3*R*,4*S*,5*R*,6*R*)	–	190957-44-9	191.18	6.30±0.70	H_2O pH10 vs	−1.585±0.525
21	Calystegine N_1	1-Amino-8-azabicyclo[3.2.1]octane-2,3,4-triol (1*R*,2*S*,3*S*,4*R*,5*S*)	?	177794-03-5	174.20	9.72±0.70	H_2O pH10 vs	−1.483±0.570
22	*N*-Methyl-calystegine B_2	8-Methyl-8-aza-bicyclo[3.2.1]octane-1,2,3,4-tetrol (1*R*,2*S*,3*R*,4*S*,5*R*)	?	184045-65-6	189.21	7.28±0.70	H_2O pH10 s (0.39 mol/L)	−1.108±0.465
23	*N*-Methyl-calystegine C_1	8-Methyl-8-aza-bicyclo[3.2.1]octane-1,2,3,4,6-pentol (1*R*,2*S*,3*R*,4*S*,5*R*,6*R*)	+	197449-07-3	205.21	6.24±0.70	H_2O pH10 s (0.34 mol/L)	−1.498±0.506

Table I (*Continued*)

	Alkaloid name	Structure	Optical rotation	CAS no.	Molecular weight	pK_a[a]	Solubility (water)[b]	OWPC[c]
24	3-*O*-β-D-Glucopyranosylcalystegine B_1	1,2,6-Trihydroxy-8-azabicyclo[3.2.1]oct-3-yl-β-D-glucopyranoside	?	176678-49-2	337.32	6.91 ± 0.70	H_2O pH10 vs	−4.035 ± 0.474
25	4-*O*-α-D-Galactopyranosylcalystegine B_2	3,4,5-Trihydroxy-8-azabicyclo[3.2.1]oct-2-yl-α-D-galactopyranoside	+	201463-91-4	337.32	6.96 ± 0.70	H_2O pH10 vs	−3.522 ± 0.495
15	(−)-2α,3β-Dihydroxynortropane	8-Azabicyclo[3.2.1]octane-2,3-diol (1*S*,2*R*,3*R*,5*R*)	−	455949-40-3	143.18	9.94 ± 0.60	H_2O pH10 vs	−0.811 ± 0.319
16	(+)-2α,3β-Dihydroxynortropane	8-Azabicyclo[3.2.1]octane-2,3-diol (1*R*,2*S*,3*S*,5*S*)	+	357426-28-9	143.18	9.94 ± 0.60	H_2O pH10 vs	−0.811 ± 0.319
26	2β,3β-Dihydroxynortropane	8-Azabicyclo[3.2.1]octane-2,3-diol (1*S*,2*S*,3*R*,5*R*)	−	455949-41-4	143.18	9.94 ± 0.60	H_2O pH10 vs	−0.811 ± 0.319
27	3β,6β-Dihydroxynortropane	8-Azabicyclo[3.2.1]octane-3,6-diol (1*R*,3*S*,5*S*,6*R*)	−	366803-73-8	143.18	9.64 ± 0.60	H_2O pH10 vs	−1.327 ± 0.319
28	3α,6β-Dihydroxynortropane	8-Azabicyclo[3.2.1]octane-3,6-diol (1*R*,3*R*,5*S*,6*R*)-	?	357426-29-0	143.18	9.64 ± 0.60	H_2O pH10 vs	−1.327 ± 0.319
29	2α,7β-Dihydroxynortropane[d]	8-Azabicyclo[3.2.1]octane-2,7-diol, (1*R*,2*S*,5*S*,7*S*)	+	357426-27-8	143.18	9.65 ± 0.60	H_2O pH10 vs	−1.378 ± 0.319
10	Erycibelline[d]	8-Azabicyclo[3.2.1]octane-2,7-diol (1*R*,2*S*,5*S*,7*S*)	−	107633-95-4	143.18	9.65 ± 0.60	H_2O pH10 vs	−1.378 ± 0.319
11	Baogongten C	8-Azabicyclo[3.2.1]octane-2,6-diol (1*R*,2*S*,5*R*,6*S*)	?	107259-50-7	143.18	9.65 ± 0.60	H_2O pH10 vs	−1.449 ± 0.290
30	2α,3β,4α-Trihydroxynortropane	8-Azabicyclo[3.2.1]octane-2,3,4-triol (1*R*,2*S*,3-*exo*,4*R*,5*S*)	?	455325-05-0	159.18	9.01 ± 0.70	H_2O pH10 s (0.93 mol/L)	−1.282 ± 0.405
31	2α,3β,6β-Trihydroxynortropane	8-Azabicyclo[3.2.1]octane-2,3,6-triol (1*S*,2*R*,3*R*,5*S*,6*R*)	−	455325-03-8	159.18	8.89 ± 0.70	H_2O pH10 vs (1.34 mol/L)	−1.765 ± 0.392
6	1-Deoxynojirimycin	2-(Hydroxymethyl)-3,4,5-piperidinetriol (2*R*,3*R*,4*R*,5*S*)	+	19130-96-2	163.17	7.99 ± 0.70	H_2O pH10 vs	−2.097 ± 0.415

12	Pseudotropine	8-Methyl-8-azabicyclo[3.2.1]octan-3β-ol		135-97-7	141.21	10.80 ± 0.40	H_2O pH10 vs (2.55 mol/L)	−0.102 ± 0.261
13	Norpseudotropine	8-Azabicyclo[3.2.1]octan3β-ol		501-33-7	127.18	10.86 ± 0.40	H_2O pH10 vs (3.88 mol/L)	−0.098 ± 0.265
49	Isofagomine	5-(Hydroxymethyl)-3,4-piperidinediol (3*R*,4*R*,5*R*)	+	169105-89-9	147.17	9.17 ± 0.70	H_2O pH 10 vs (3.57 mol/L)	−1.803 ± 0.383
5	Swainsonine	Octahydro-1,2,8-indolizinetriol (1*S*,2*R*,8*R*,8a*R*)	−	72741-87-8	173.21	7.41 ± 0.70	H_2O pH 10 s (0.38 mol/L)	−0.786 ± 0.417

[a] Dissociation constants, expressed as pK_a values.

[b] Solubility in water at 25°C: vs = very soluble > 1 mol/L; s = soluble 0.1–1 mol/L.

[c] Octanol–water partition coefficient, expressed as log *P*; pK_a and log P from calculation using advanced chemistry development (ACD) software Solaris V 8.19 (1994–2006 ACD) published in *Chemical Abstracts*.

[d] Inconsistencies between stereochemical nomenclature here and formulae for **10** and **29** in Fig. 2 following *Chemical Abstracts*. From the information available the disagreement cannot be resolved.

M. alba fruits (28) and *C. soldanella* (29), respectively, shows that the opposite chirality may occur in different plants. Caution with absolute configuration assignment is therefore advised after only NMR structure elucidation. All calystegines and other di- or trihydroxynortropane alkaloids are hydrophilic; their octanol–water partition coefficient is well below zero (Table I). In liquid–liquid extraction systems between water and organic phase they will remain in the aqueous phase, even if the pH is elevated above 7. This property prevented their detection in former times, as classical alkaloid analysis includes liquid extraction of free alkaloid bases by organic solvents from an alkaline water extract.

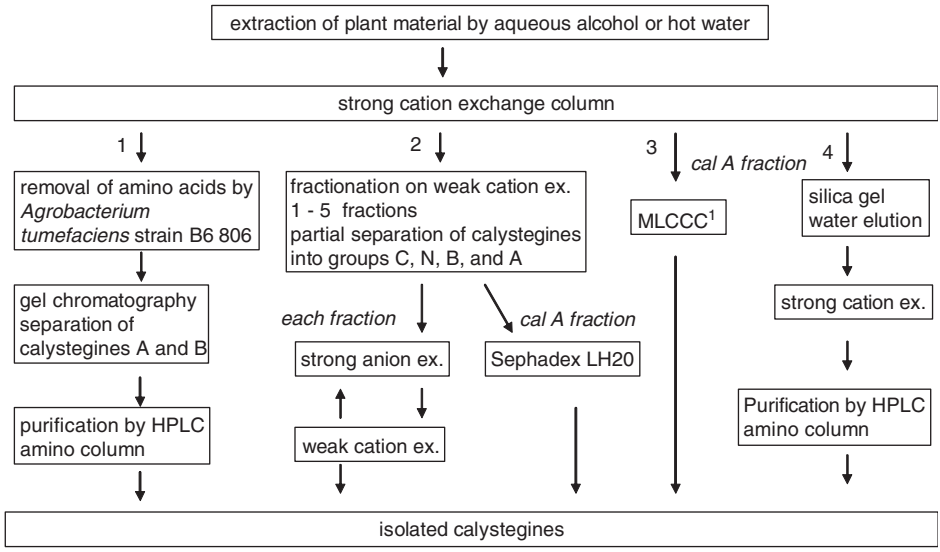
III. ISOLATION AND ANALYSIS OF CALYSTEGINES

A. First Description

Calystegines were initially detected in the course of a search for opines and other nutritional mediators in plant roots. Opines are hydrophilic molecules composed of an amino acid, typically glutamic acid, glutamine, ornithine, or arginine, and a sugar alcohol or a carboxylic acid moiety (30). They are found in crown gall tumours or adventitious roots that are formed by plant tissues after *Agrobacterium* infection. Opine identification from plant tissues served as proof for successful transformation by *Agrobacterium* before the polymerase chain reaction (PCR) was invented and applied. Opine analysis was done by paper electrophoresis and subsequent silver nitrate reduction for detection. Calystegines were detected as silver-positive spots and isolated by a series of chromatographic, electrophoretic, and biological enrichment steps. An aqueous extract of the roots of *C. sepium* was subjected to strong cation exchange chromatography for basic alkaloid enrichment. Amino acids were catabolised from the concentrate by the addition of *Agrobacterium tumefaciens* strain B6 806. The remaining calystegines of the groups A and B were then separated by gel filtration on GF02. Final isolation was achieved by HPLC on an amino column (Zorbax NH_2 6 μ) (Figure 3, method 1) (2). The procedure was never repeated in the course of other calystegine isolation attempts. Other strains of *A. tumefaciens* readily catabolised calystegines, thus the biological enrichment step appeared prone to failure (Mielke and Dräger, unpublished).

B. Isolation Procedures

Subsequent calystegine isolations followed enrichment schemes that were developed for the isolation of other polyhydroxy alkaloids, such as swainsonine (**5**) and 1-deoxynojirimycin (**6**) from *S. canescens* (Fabaceae) (7) and from *Streptomyces* strains (31), respectively. Due to the hydrophilicity and the basic nature of those alkaloids, they were purified from aqueous extracts by a sequence of ion exchange resin columns that were designed to bind the alkaloids as cations, or to adsorb contaminating anions, while the alkaloids were eluted with water (Figure 3, method 2). An overview of the procedures was expertly summarised by Fellows and Fleet (32). Calystegines of the A-group are the most difficult to



[1]MLCCC multilayer coil countercurrent chromatography.

Figure 3 Isolation schemes for the calystegines from plant material. See text for methods 1–4.

isolate by that procedure because they elute from cation exchange columns after the calystegines of the C- and B-groups, and mostly as a broadened zone rather than as sharp peaks. Therefore, two alternative methods for the purification of calystegine A_3 (**1**) were adopted. One procedure involves a Sephadex LH20 column, yielding calystegine A_3 by elution with *n*-butanol–acetic acid–water–methanol (10:10:90:15) (27). The other option for calystegine A_3 purification is multilayer coil countercurrent chromatography (MLCCC) (33).

Calystegine A_3 (**1**), different from calystegine B_2 (**3**), is sufficiently transferred from alkaline water (0.1 M ammonia) into an organic phase consisting of *n*-butanol by repeated liquid–liquid extraction, but a substantial yield of calystegine A_3 is achieved only after an MLCCC operating time of 20 h (Figure 3, method 3). A modified isolation scheme was developed for di- and trihydroxynortropanes from *M. alba* fruits (28). After the established concentration step by strong cation exchange chromatography, a silica gel column was eluted stepwise starting with pure chloroform, adding methanol, then switching to methanol–water mixtures, and ending with pure water elution. Calystegines were eluted with the aqueous fractions. They were separated on a cation exchange column (Dowex 50W-X4) adjusted to pH 5.7 with a formic acid–ammonia buffer and gradient elution by increasing ammonia (0–2.8%) in water. Purification of the individual calystegines was achieved by final preparative HPLC on an amino column eluted with 80% acetonitrile in water and refractive index detection (Figure 3, method 4).

Through these methods, 26 calystegines, calystegine-like nortropanes, and calystegine glycosides were isolated to date (Table II). The isolation methods for polyhydroxy alkaloids appear laborious and tedious. However, alternative

Table II First reports of structure elucidation of calystegines and related alkaloids

	Alkaloid name	CAS no.	First isolation reported from	Plant family and organ	References
1	Calystegine A_3	131580-36-4	*Calystegia sepium*	Convolvulaceae, root	(1)
7	Calystegine A_5	165905-26-0	*Physalis alkekengi*	Solanaceae, root	(27)
8	Calystegine A_6	177794-04-6	*Hyoscyamus niger*	Solanaceae, whole plant	(35)
9	Calystegine A_7	197565-90-5	*Lycium chinense*	Solanaceae, root	(36)
2	Calystegine B_1	127414-86-2	*C. sepium*	Convolvulaceae, root	(1)
3	Calystegine B_2	127414-85-1	*C. sepium*	Convolvulaceae, root	(1)
17	Calystegine B_3	178231-95-3	*P. alkekengi*	Solanaceae, root	(27)
18	Calystegine B_4	184046-85-3	*Scopolia japonica*	Solanaceae, root	(37)
19	Calystegine B_5	197565-91-6	*L. chinense*	Solanaceae, root	(36)
14	Calystegine C_1	156705-04-3	*Morus alba*	Moraceae, root	(26)
20	Calystegine C_2	190957-44-9	*Duboisia leichhardtii*	Solanaceae, leaf	(38)
21	Calystegine N_1	177794-03-5	*H. niger*	Moraceae, whole plant	(35)
22	*N*-Methylcalystegine B_2	184045-65-6	*L. chinense*	Solanaceae, root	(36)
23	*N*-Methylcalystegine C_1	197449-07-3	*L. chinense*	Solanaceae, root	(36)
24	3-*O*-β-D-Glucopyranosylcalystegine B_1	176678-49-2	*Nicandra physalodes*	Solanaceae, fruit	(39)
25	4-*O*-α-D-Galactopyranosylcalystegine B_2	201463-91-4	*M. alba*	Moraceae, leaf	(40)
15	(-)-2α,3β-Dihydroxynortropane	455949-40-3	*M. alba*	Moraceae, fruit	(28)
16	(+)-2α,3β-Dihydroxynortropane	357426-28-9	*C. soldanella*	Convolvulaceae, whole plant	(29)
26	2β,3β-Dihydroxynortropane	455949-41-4	*M. alba*	Moraceae, fruit	(28)
27	3β,6β-Dihydroxynortropane	366803-73-8	*M. alba*	Moraceae, fruit	(40)
28	3α,6β-Dihydroxynortropane	357426-29-0	*Duboisia leichhardtii*	Solanaceae, aerial parts	(29)
29	2α,7β-Dihydroxynortropane	357426-27-8	*C. soldanella*	Convolvulaceae, whole plant	(29)
10	Erycibelline	107633-95-4	*Erycibe elliptilimba*	Convolvulaceae, stem	(15)
11	Baogongten C	107259-50-7	*E. obtusifolia*	Convolvulaceae, organ not reported	(16)
30	2α,3β,4α-Trihydroxynortropane	455325-05-0	*M. alba*	Moraceae, fruit	(28)
31	2α,3β,6β-Trihydroxynortropane	455325-03-8	*M. alba*	Moraceae, fruit	(28)

purification schemes are not available for these hydrophilic compounds. Recently, the isolation of sugar-mimic alkaloids from mulberry latex for testing their insect toxicity was performed using the same procedures (34). Structure elucidation was achieved by proton and carbon NMR, and high-resolution mass spectrometry (HRMS). Detailed NMR and MS data were summarised, together with the respective methods in 1996, when nine calystegines were identified (12).

C. Analysis and Quantitation of Calystegines in Plant Material

Analytical methods for the calystegines also have to take into consideration that the alkaloids are hydrophilic, i.e., not amenable to HPLC separation on reversed phase columns. Further, they lack a chromophore for visible (VIS) or ultraviolet (UV) light, and detection by the widely applied diode array or fixed wavelength UV–VIS detectors fails, except for high calystegine concentrations. For calystegine isolation, separation by HPLC with amino columns was monitored by refractive index detection, but this method also is not sufficiently sensitive for the analysis of small quantities of calystegines. Attempts to analyse for calystegines from plant tissues therefore followed the methods that evolved for polyhydroxy alkaloids (41,42), since the analytical problems were common for the whole group of alkaloids. The preferred methods were thin layer chromatography (TLC), replacing paper electrophoresis used initially, and gas chromatography after derivatisation of the hydroxyl groups. For TLC detection, silver nitrate reduction followed by alkaline ethanol proved optimal for the calystegines (33), other than Ehrlich's reagent (4-dimethylamino-benzaldehyde in acidified ethanol) used for most other polyhydroxy alkaloids (43). Derivatisation prior to gas chromatography was done by silylation, either with strong silylating agents, such as *N*-methyl-*N*-(trimethylsilyl)fluoroacetamide (MSTFA) (8), or under milder conditions with hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) (33), the major difference being the silylation of the secondary nitrogen under the strong derivatisation conditions. Milder silylation conditions yielded complete conversion of calystegines and enabled quantitation of the alkaloids in plant tissues. A third derivatisation procedure applied to the calystegines consists of two steps: first methoximation by methoxyamine hydrochloride in pyridine followed by MSTFA as silylating agent. Methoxyamine hydrochloride transforms carbonyl groups into methyloximes, and MSTFA transfers trimethylsilyl groups onto every hydroxyl and primary or secondary amine group (44). Calystegines under these conditions reacted quantitatively and gave two signals proving that methoximation took place, which caused two separable *cis* and *trans* isomers from one carbonyl group (Richter, Kopka, Dräger, to be published). A carbonyl group in the calystegines is assumed to exist in the monocyclic heptameric ring form (Figure 4) that, in pyridine solution, exists in sufficient quantity in equilibrium with the bicyclic form to enable reaction with methoxyamine hydrochloride and concomitantly pull the equilibrium towards the monocyclic form until the reaction is complete.

Current methods for calystegine analysis have been reviewed either together with other polyhydroxy alkaloids (45), or together with tropane alkaloids (46).

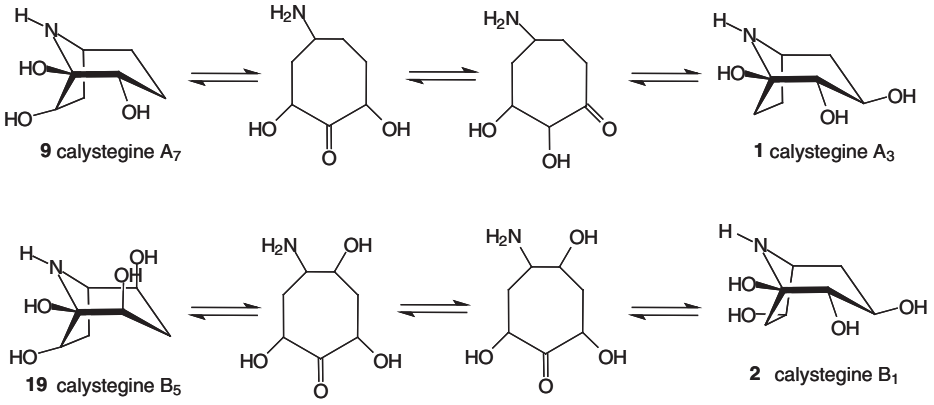


Figure 4 Possible contributions to the equilibrium between bicyclic aminoketal rings and monocyclic aminoheptanones and tautomerisations to calystegine structure variation.

Since then, no additional analytical strategies for the calystegines have been observed. Gas chromatography analysis is the standard method for calystegine quantitation, yet, due to the preceding enrichment and purification step by cation exchange cartridges and subsequent drying and silylation, it is tedious, time consuming, and prone to losses.

D. Desirable Future Developments

From a theoretical perspective, capillary electrophoresis (CE) appears an ideal option for the analysis of calystegines, as they are water-soluble, charged, and should migrate easily in the electric field, but again the lack of UV light absorption of the compounds prevented straightforward analysis by CE. Two methods were published that circumvented the problem. Calystegines were complexed with borate that is also used to complex sugars, and the complexes were separated by CE and detected by UV light (47). Borate complexes absorb UV light at 191 nm, but the specific extinction was low, and the limits of detection for individual calystegines correspondingly high. Alternatively, calystegines, after CE separation in a non-complexing buffer solution, were detected by electrochemical oxidation, which is a sensitive, but not very robust, way of detection (48). In addition, the combination of instruments, CE with electrochemical detection, is not commercially available. Thus, no CE separation method for the calystegines has made its way into routine analysis. However, with an appropriate detection system available, calystegine measurements by CE should be faster and easier.

MS coupled to HPLC appears an attractive alternative for detection after liquid chromatography, and was successfully applied for the detection of polyhydroxy alkaloids other than calystegines, as comprehensively reviewed by Molyneux and co-workers (45). Among the transfer and ionisation techniques from HPLC to the mass spectrometer, atmospheric pressure chemical ionisation

(APCI) was identified as superior to electrospray ionisation. While electrospray ionisation enables detection in the nanogram range, detection levels for APCI in the positive mode were at picogram levels. Compared with the negative ion mode, APCI in the positive mode generated ions more effectively from the polyhydroxy alkaloids, but the deprotonated molecular ions produced in the negative mode of APCI were more characteristic for the distinction between isomeric alkaloids. With the HPLC–MS combination and the detection problem solved, the separation of the polyhydroxy alkaloids on HPLC columns remains a challenge. Twelve polyhydroxy alkaloids from several structural classes were separated on a column designed for carbohydrate analysis with an acetonitrile–water gradient (49,50). Swainsonine (**5**), one of the less hydrophilic indolizidine alkaloids (logarithmic octanol–water partition coefficient log *P* 0.768), could be chromatographed on a reversed phase column with an isocratic mixture of 5% methanol in 20 mM aqueous ammonium acetate (51). These results are encouraging for attempts at calystegine analysis by HPLC–MS, bearing in mind that liquid chromatography may save a lot of time and effort during sample preparation.

IV. OCCURRENCE OF CALYSTEGINES IN THE PLANT KINGDOM

A. Chemotaxonomy

Alkaloidal glycosidase inhibitors are found in higher plants, as well as in fungi or bacteria. For example, swainsonine (**5**) occurs in the Fabaceae plants *S. canescens* (7), *Astragalus lentiginosus*, spotted locoweed (52), and *A. oxyphysus*, diablo locoweed (53), and as well in fungi of the classes Basidiomycetes and Deuteromycetes, i.e., *Rhizoctonia leguminicola* (54,55) and *Metarhizium anisopliae* (56,57). Fungal endophytes of *Astragalus* and *Oxytropis* species, probably of the genus *Embellisia*, also produced swainsonine in culture (58). For other alkaloidal glycosidase inhibitors, e.g., for 1-deoxynojirimycin (**6**), occurrence in several unrelated plants and bacteria was demonstrated. These were, e.g., *Bacillus amyloliquefaciens, B. polymyxa,* and *B. subtilis* (59,60), *B. subrutilus* (61), *Streptomyces lavandulae* ssp. *trehalostaticus* (31), and plants of the genus *Morus* (Moraceae) (62), *Omphalea queenslandiae* and *Endospermum medullosum* (Euphorbiaceae) (63), *Hyacinthus orientalis* (Hyacinthaceae) (64), and *Adenophora triphylla* var. *japonica* (Campanulaceae) (65). Other polyhydroxylated alkaloids, such as castanospermine (**4**), were found only in the Fabaceae (66). Comprehensive reviews on alkaloidal glycosidase inhibitors summarise their detection in plants, fungi, and microorganisms (4,13,67). To date, the calystegines were isolated only from higher plants, and within the angiosperms investigated so far, calystegines occur exclusively in dicotyledonous plants.

Calystegines were initially detected in the Convolvulaceae, and today this plant family is the most thoroughly investigated for the occurrence of calystegines. There were earlier reports of tropane alkaloids in the Convolvulaceae. Tropine esters and nortropine esters with vanillic acid and veratric acid had been isolated from *Convolvulus* species, *C. erinacius, C. krauseanus,* and *C. lineatus* and reported between 1965 and 1977 (68–70). Several Convolvulaceae root tissues,

C. sepium included, were reported in 1973 not to contain tropane alkaloids, but cuscohygrine, a putative by-product of tropane alkaloid formation, was detected in the roots (71). In a later study in 1995, tropinone (**32**), tropine (**33**) and pseudotropine (**12**) were isolated from the aerial parts of *C. arvensis* (72). From *C. siculus* and *C. sabatius* ssp. *mauritanicus* three tropan-3α-ol esters were isolated (73). Apart from early findings in *C. sepium* and *C. arvensis* (1,2,8), data on calystegine occurrence in Convolvulaceae result from the investigations of Schimming and Eich, together with many co-workers (14,74). Convolvulaceae plants are distributed worldwide and comprise *ca*. 1600 species classified in 57 genera. Recent molecular cladistic and phylogenetic analysis of Convolvulaceae confirmed the monophyly of the family (75). The subsequent Convolvulaceae reclassification including phylogenetic aspects revealed 12 separate tribes that are well supported by applying traditional taxonomic characters and by sequence comparisons of multiple molecular marker genes (76). From the 57 Convolvulaceae genera, 29 were examined for calystegines, comprising 129 species from all 12 tribes. Calystegines were found in 62 species from 22 genera, which are listed in Table III. The original studies (14,74) contain an impressive amount of additional data, e.g., species that were found not to contain calystegines, and detailed descriptions of the evolutionary aspects of the plants and the tissues that were examined.

Solanaceae plants are in a sister family to the Convolvulaceae (75), but the family is larger, comprising 2460 species in 102 genera. Seven Solanaceae subfamilies are distinguished containing 14 tribes (94–96), and the Solanoideae is by far the largest subfamily with 1925 species in 61 genera, comprising also the large genus *Solanum* with 1250–1700 species, depending on the taxonomy applied (97,98). Solanaceae plants have not been screened systematically for calystegines; rather, individual plants were investigated that were either known as sources for medicinal tropane alkaloids, such as *Atropa*, *Hyoscyamus*, *Datura,* and *Duboisia* species, or that were used in the traditional pharmacopoeia of China, such as *P. alkekengi* and *Lycium chinense* roots. Several other Solanaceae plants were examined, among them species whose fruits or tubers serve as vegetables. Tomatoes (*Lycopersicon esculentum* syn. *Solanum lycopersicon*), aubergines (*S. melongena*), cape gooseberries (*P. peruviana*), and potatoes (*S. tuberosum*) are staple foods for human nutrition in most regions of the world, and contain calystegines in the organs that are consumed (80), and in other parts of the plants. In total, the occurrence of calystegines is documented for 13 Solanaceae genera out of 102, covering 25 species out of 2460. Most of the Solanaceae species that were reported to possess calystegines (Table III) belong to the same subfamily, the Solanoideae; only *Brunfelsia* is grouped in the subfamily Petunioideae, and *Duboisia* belongs to the subfamily Anthocercidoideae. Among the prominent Solanaceae species, *Nicotiana tabacum* was shown to contain neither calystegines nor other tropane alkaloids.

The systematic search for calystegines in the Solanaceae family is not concluded, and many species and whole subfamilies, particularly those that are reported to contain tropane alkaloids, e.g., *Schizanthus* species in the Schizanthoideae (99,100) remain to be examined. In the whole angiosperm system, the

Table III Occurrence of calystegines in plant species

Plant family	Plant species	Plant part	Alkaloids[a]	References
Brassicaceae	*Aethionema grandiflora*	Leaf	**1**, **3**, **7**, **17**	(77)
	Brassica campestris	Leaf	**1**, **7**	(77)
	B. oleracea var. *gongylodes*	Leaf	**1**, **3**	(77)
	B. oleracea var. *silvestris*	Leaf	**1**, **3**, **7**, **17**	(77)
	B. oleracea var. *gemmifera*	Leaf	**1**	(77)
	B. nigra	Leaf	**1**, **3**, **7**	(77)
	Calepina irregulans	Leaf	**1**, **7**, **17**	(77)
	Camelina alyssum	Leaf	**1**, **3**	(77)
	C. sativa	Leaf	**1**, **3**, **7**, **17**	(77)
	Capsella bursa-pastoris	Leaf	**1**, **3**, **7**, **17**	(77)
	Cheiranthus cheiri	Leaf	**1**	(77)
	Cochlearia anglica	Leaf	**1**, **7**, **17**	(77)
	C. danica	Leaf	**1**, **7**, **17**	(77)
	C. glastifolia	Leaf	**1**, **7**, **17**	(77)
	C. groenlandica	Leaf	**7**, **17**	(77)
	C. megalosperma	Leaf	**7**, **17**	(77)
	C. officinalis	Leaf, root, flower	**3**, **7**, **17**	(77)
	C. polonica	Leaf	**7**, **17**	(77)
	C. pyrenaica	Leaf	**1**, **7**, **17**	(77)
	Crambe cordifolia	Leaf	**3**, **7**	(77)
	C. kotschyana	Leaf	**1**, **7**, **17**	(77)
	C. maritima	Leaf	**1**, **7**, **17**	(77)
	C. orientalis	Leaf	**1**, **7**, **17**	(77)
	Diplotaxis tenuifolia	Leaf	**1**, **7**, **17**	(77)
	Iberis amara	Leaf	**7**, **17**	(77)
	Hersperis matronalis	Leaf	**7**, **17**	(77)
	Lepidium sativum	Leaf	**1**, **7**	(77)
	Moricandia arvensis	Leaf	**1**, **7**, **17**	(77)
Convolvulaceae	*Argyreia androyensis*	Leaf	**2**, **3**, **14**, **18**	(14)
	A. capitata	Various parts	**2**, **3**	(14)
	A. hookeri	Leaf	**2**, **14**, **18**	(14)
	A. mollis	Various parts	**1**, **2**, **3**, **7**, **14**, **17**	(14)
	A. onilahiensis	Leaf	**2**, **3**, **14**, **18**	(14)
	A. vahibora	Leaf	**2**, **3**, **14**, **18**	(14)
	Aniseia martinicensis	Pericarp	**1**, **2**, **3**	(14)
	Bonamia semidigyna	Aerial parts	**3**	(14)
	B. spectabilis	Aerial parts	**3**, **18**	(14)
	Calystegia japonica	Root	**1**, **2**, **3**, **29**	(29)
	C. sepium	Leaf, flower, root, seedling	**1**, **3**	(78)
		Root culture	**1**, **2**, **3**, **29**	(1,8,79)

Table III. (*Continued*)

Plant family	Plant species	Plant part	Alkaloids[a]	References
	C. silvatica	Various parts	**1**, **2**, **3**, **7**, **18**	(14)
	C. soldanella	Whole plant	**1**, **2**, **3**, **16**, **29**, **30**	(29)
	Cardiochlamys madagascariensis	Leaf	**2**, **3**, **18**	(14)
	Convolvulus arvensis	Various parts	**1**, **7**, **2**, **3**, **17**, **18**	(14)
	C. caput-medusae	Aerial parts	**1**, **2**, **3**	(14)
	C. cneorum	Root	**1**, **2**, **3**, **7**	(14)
	C. elongates	Various parts	**1**, **2**, **18**	(14)
	C. glandulosus	Aerial parts	**2**, **3**	(14)
	C. graminetinus	Leaf	**3**	(14)
	C. humilis	Aerial parts	**1**, **2**, **3**	(14)
	C. sabatius ssp. *mauritanicus*	Aerial parts	**1**, **2**, **3**	(14)
	C. subauriculatus	Aerial parts	**2**, **3**	(14)
	C. tricolor ssp. *tricolor*	Flower	**2**, **3**, **7**	(14)
	Dichondra micrantha	Aerial parts	**1**, **3**, **17**, **18**	(14)
	D. sericea	Aerial parts	**3**	(14)
	Erycibe elliptilimba	Stem	**10**	(15)
	E. macrophylla	Leaf	**2**, **3**, **17**	(14)
	E. malaccensis	Leaf	**3**	(14)
	E. micrantha	Leaf	**1**, **2**, **3**, **14**	(14)
	E. obtusifolia		**11**	(15,16)
	E. parvifolia	Aerial parts	**3**	(14)
	E. rheedii	Leaf	**14**	(14)
	Evolvulus argyreus	Aerial parts	**3**	(14)
	Falkia repens	Various parts	**2**, **3**	(14)
	Hildebrandtia valo	Aerial parts	**1**, **3**, **17**	(14)
	Humbertia madagascariensis	Leaf	**1**, **18**	(14)
	Ipomoea alba	Various parts	**1**, **2**, **3**, **7**, **14**, **17**	(14)
	I. aquatica	Various parts	**1**, **2**, **3**, **18**	(14)
	I. batatas	Aerial parts	**2**, **3**, **29**	(29)
		Tuber	**1**, **2**, **3**, **14**	(80)
	I. cairica	Flower	**3**	(14)
	I. carnea	Aerial parts, flower, seed	**2**, **3**, **14**, **17**, **29**	(29,81,82)
		Seed	**19**	(81)
	I. chiriquiensis	Aerial parts	**2**, **3**	(14)
	I. eriocarpa	Various parts	**3**	(14)
	I. involucrata	Aerial parts	**2**, **17**	(14)
	I. lobata	Aerial parts	**2**, **3**, **14**	(14)
	I. obscura	Whole plant	**2**, **3**	(29)
	I. pes-caprae	Aerial parts	**3**	(29)
	I. polpha	Seed	**3**, **14**	(14)
	I. setifera	Various parts	**2**, **3**	(14)

Table III. (*Continued*)

Plant family	Plant species	Plant part	Alkaloids[a]	References
	I. squamosa	Various parts	**2**, **3**	(14)
	I. stolonifera	Aerial parts	**3**	(14)
	I. trichosperma	Various parts	**7**	(14)
	I. tricolor	Flower	**3**	(14)
	I. trifida	Root	**2**, **3**	(14)
	I. violacea	Various parts	**2**, **3**, **14**	(14)
	I. wrightii	Aerial parts	**3**	(14)
	I. sp. Q6 [aff. *calobra*]	Seed	**3**	(83)
	Iseia luxurians	Various parts	**2**, **3**, **14**	(14)
	Jacquemontia tamnifolia	Root	**1**, **2**, **3**, **7**, **14**	(14)
	Lepistemon urceolatum	Leaf	**14**	(14)
	Maripa panamensis	Aerial parts	**1**, **2**, **17**	(14)
	Merremia aurea	Various parts	**2**, **3**, **7**	(14)
	M. dissecta	Flower	**3**	(14)
	M. umbellata	Aerial parts	**2**, **17**	(14)
	Porana volubilis	Aerial parts	**2**, **3**, **14**, **17**	(14)
	Quamoclit angulata	Aerial parts	**2**, **3**, **29**	(29)
	Rapona tiliifolia	Fruit	**1**, **2**, **3**, **17**	(14)
	Stictocardia campanulata	Various parts	**1**, **3**	(14)
	S. mojangensis	Root	**1**, **2**, **3**	(14)
	Turbina abutiloides	Root	**1**, **2**, **3**	(14)
Erythroxylaceae	*Erythroxylum novogranatense*	Leaf, flower, fruit	**1**, **2**, **3**, **7**	(84)
	E. amazonicum		**1**, **2**, **3**	(84)
	E. andrei		**1**, **3**, **7**	(84)
	E. campestre		**1**, **3**, **7**	(84)
	E. citrifolium		**1**, **3**, **7**	(84)
	E. coca var. *coca*		**1**, **2**, **3**, **7**	(84)
	E. confusum		**1**, **3**	(84)
	E. cumanense		**1**, **3**	(84)
	E. cuneifolium		**1**, **3**, **7**	(84)
	E. deciduum		**1**, **2**, **3**, **7**	(84)
	E. densum		**3**	(84)
	E. *fimbriatum*		**1**	(84)
	E. foetidum		**1**, **3**	(84)
	E. glazioui		**1**, **3**, **7**	(84)
	E. gonocladum		**1**, **3**	(84)
	E. havanense		**1**, **3**	(84)
	E. hypoleucum		**3**	(84)
	E. aff. *impressum*		**1**, **3**	(84)
	E. lealcostae		**3**	(84)
	E. ligustrinum var. *ligustrinum*		**1**, **2**, **3**, **7**	(84)
	E. macrophyllum		**1**, **2**, **3**, **7**	(84)
	E. macrophyllum var. *savannarum*		**1**, **3**	(84)

Table III. (*Continued*)

Plant family	Plant species	Plant part	Alkaloids[a]	References
	E. magnoliifolium		**1**, **3**	(84)
	E. martii		**1**, **3**, **7**	(84)
	E. mattos-silvae		**3**	(84)
	E. mexicanum		**1**, **3**	(84)
	E. mikanii		**1**, **3**	(84)
	E. mucronatum		**1**, **3**	(84)
	E. myrsinites		**1**, **3**, **7**	(84)
	E. ochranthum		**1**, **3**, **7**	(84)
	E. orinocense		**1**, **2**, **3**	(84)
	E. ovalifolium		**1**	(84)
	E. passerinum		**1**, **3**, **7**	(84)
	E. pictum		**1**	(84)
	E. pulchrum		**1**, **3**, **7**	(84)
	E. rufum		**3**	(84)
	E. shatona		**3**, **7**	(84)
	E. suberosum		**1**, **3**, **7**	(84)
	E. subrotundum		**1**, **2**, **7**	(84)
Moraceae	*Morus alba*	Root bark	**2**, **3**, **14**, **27**	(26,40)
		Leaf, fruit	**3**, **25**	(40)
		Fruit	**15**, **26**, **27**, **31**	(28)
	M. bombycis	Leaf	**3**	(25)
Solanaceae	*Atropa belladonna*	Leaf, root, flower, fruit	**1**, **2**, **3**, **7**	(85)
		Root culture	**1**, **3**, **7**	(86)
	Brunfelsia nitida	Leaf	**1**, **2**, **3**, **14**	(87)
	Datura innoxia	Root culture	**1**	(88)
	D. stramonium	Root culture	**1**, **3**	(88)
	Duboisia leichhardtii	Leaf	**2**, **3**, **7**, **14**, **17**	(29,38)
		Leaf	**20**	(38)
		Aerial parts	**28**	(29)
	Hyoscyamus albus	Root culture	**1**	(89)
	H. aureus	Root culture	**1**	(89)
	H. muticus	Leaf	**1**, **3**	(90)
		Root culture	**1**	(89)
	H. niger	Leaf, root, flower	**1**, **2**, **3**, **7**, **17**, **18**	(35,85,89)
		Root culture	**1**	(89)
		Whole plant	**8**, **21**	(35)
	H. pusillus	Root culture	**1**	(89)
	Lycopersicon esculentum	Leaf, root, flower, stem, fruit	**1**, **3**, **7**	(80,88)
		Root culture	**1**, **3**, **7**	(88)
	Lycium chinense	Root	**1**, **2**, **3**, **7**, **8**, **9**, **14**, **17**, **18**, **19**, **20**, **21**, **22**, **23**	(36)
		Root	**1**, **3**	(87)

Table III. (*Continued*)

Plant family	Plant species	Plant part	Alkaloids[a]	References
	Mandragora autumnalis			
	M. officinarum	Leaf, root, flower, fruit	**1**, **2**, **3**	(85)
	Nicandra physalodes	Fruit	**2**, **24**	(39)
	Physalis alkekengi var. *francheti*	Root	**1**, **2**, **3**, **7**, **17**	(27)
		Fruit	**1**, **2**, **3**	(80)
	P. divaricata	Root culture	**1**, **2**, **3**, **7**	(88)
	Scopolia carniolica	Leaf, root, flower	**1**, **2**, **3**	(85)
	S. japonica	Root	**1**, **2**, **3**, **7**, **14**, **17**	(37)
	Solanum dulcamara	Leaf, whole plant	**1**, **2**, **3**, **7**, **21**	(29,91)
	S. melongena	Fruit	**1**, **3**	(80)
	S. sodomaeum	Leaf	**3**	(87)
	S. tuberosum	Leaf, root, flower, tuber	**1**, **3**, **17**, **18**	(85,92,93)
	Withania frutescens	Leaf	**1**, **2**, **3**, **14**	(87)
	W. somnifera	Leaf	**3**, **14**	(87)

[a] Alkaloids cited: Calystegines A_3 (**1**), A_5 (**7**), A_6 (**8**), A_7 (**9**), B_1 (**2**), B_2 (**3**), B_3 (**17**), B_4 (**18**), B_5 (**19**), C_1 (**14**), C_2 (**20**), and N_1 (**21**), *N*-Methylcalystegine B_2 (**22**) and C_1(**23**), Erycibelline (**10**), Baogongten C (**11**), (-)-2α,3β-Dihydroxynortropane (**15**), (+)-2α,3β-Dihydroxynortropane (**16**), 3-*O*-β-D-Glucopyranosylcalystegine B_1 (**24**), 4-*O*-α-D-Galactopyranosylcalystegine B_2 (**25**), 2β,3β-Dihydroxynortropane (**26**), (1*S*,3*S*,5*S*,6*R*)-8-Azabicyclo[3.2.1]octane-3,6-diol (**27**), (1*R*,3*R*,5*S*,6*R*)-8-Azabicyclo[3.2.1]octane-3,6-diol (**28**), (1*R*,2*S*,5*S*,7*S*)-8-Azabicyclo[3.2.1]octane-2,7-diol (**29**), 2α,3β,4α-Trihydroxynortropane (**30**), 2α,3β,6β-Trihydroxynortropane (**31**).

Solanaceae, together with the Convolvulaceae, form a cluster of tropane and calystegine alkaloid occurrence (Figure 5). They comprise most of the plant genera and species from which calystegines have been identified, but the search for calystegines within other angiosperms has not yet been performed representatively.

The Erythroxylaceae is a rather small plant family within the Malpighiales, comprising four genera and *ca*. 240 species. *Erythroxylum* is the largest genus, and more than 230 *Erythroxylum* species are distinguished. They are native to subtropical and tropical regions of South America, Africa, South East Asia, and Madagascar. The species of economic interest are the cultivated varieties of *E. coca* and *E. novogranatense* because of their cocaine content. Cocaine is a tropane alkaloid accumulated to appreciable amounts (up to 2% dry mass) only in these two *Erythroxylum* species (101), although traces of cocaine are reported from a few other *Erythroxylum* species (102–104). Alkaloids with a tropane alkaloid skeleton accumulate in *Erythroxylum coca* and *E. novogranatense*, and other species in the genus contain tropane alkaloid esters. They are mainly

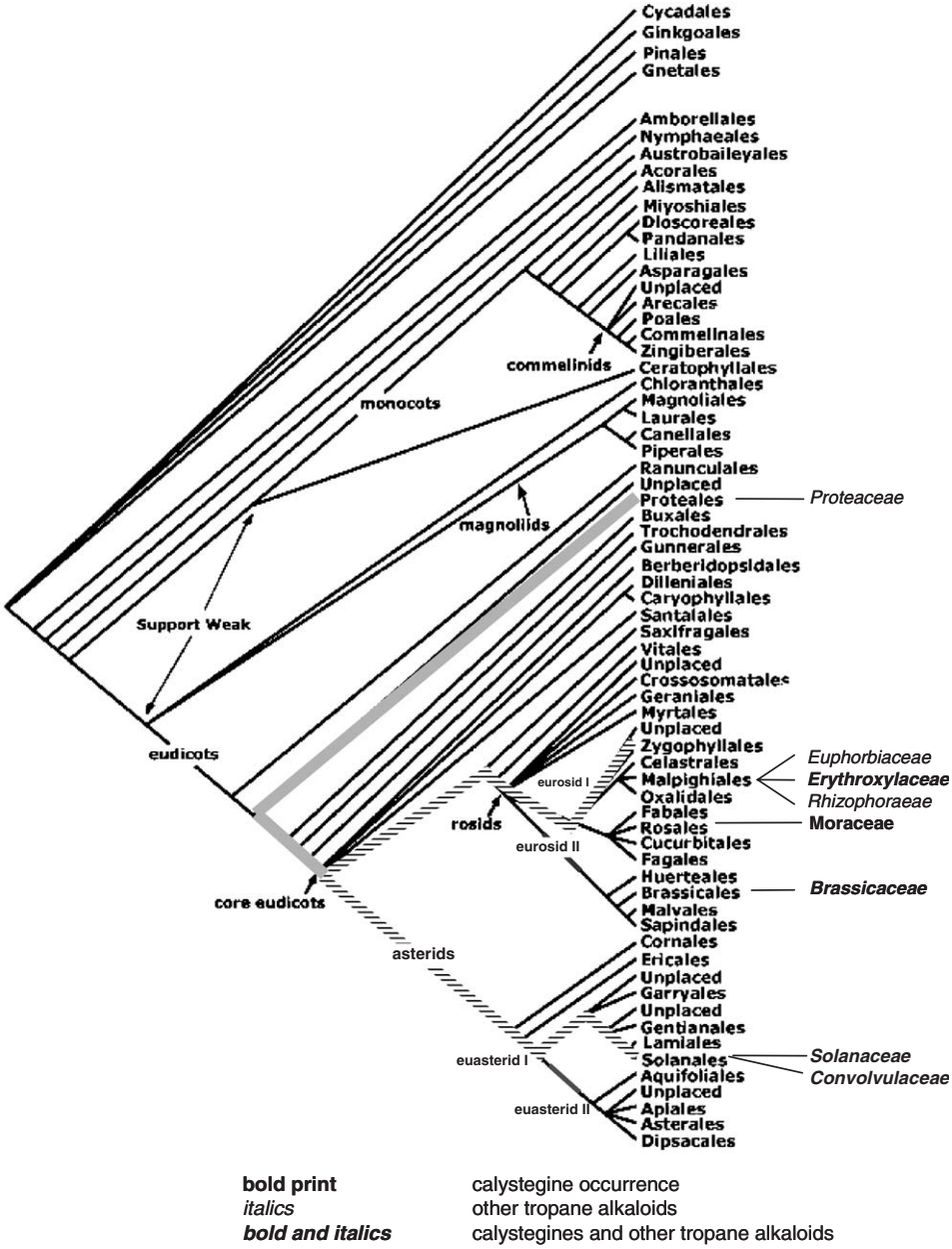


Figure 5 Occurrence of tropane alkaloids in the angiosperms. The tree diagram was taken from the angiosperm phylogeny website, Missouri Botanical Garden (http://www.mobot.org/MOBOT/Research/APweb/welcome.html) with kind permission of the author, Dr. Peter Stevens. The original tree on the website is linked on all terminal taxa to pages with detailed taxon characterisation. Tropane alkaloids were reported within a section of the eudicots, which is indicated by bold hatched lines. Bold grey lines enlarge the section for the inclusion of Proteaceae.

3α- and 3β-hydroxytropane and nortropane esters, summarised together with other tropane alkaloid occurrences by Lounasmaa and Tamminen in 1993 (105), and by Griffin and Lin in 2000 (106). Further tropane structures from the Erythroxylaceae, some of them with very interesting biological activity, such as reversal of multidrug resistance in carcinoma cells (107), were isolated later (17,18,108–112), and also a 1-hydroxy-3-tropylbenzoic acid ester was reported (113) with a structure reminiscent of calystegine hydroxylation at C1. It was therefore not surprising that most of the 46 *Erythroxylum* species that were analysed contained various mixtures and concentrations of calystegines (84).

The family Euphorbiaceae is closely related to the Erythroxylaceae according to the new cladistic structure of the angiosperms (Figure 5), and the Rhizophoraceae is another sister family to the Erythroxylaceae (114,115). Tropane alkaloids of diverging structures were obtained from these neighbouring families to the Erythroxylaceae (105,106). *Crossostylis* species (Rhizophoraceae) contained tropan-3α-yl cinnamate, tropan-3α-yl benzoate, tropan-3α-yl ferulate, and brugine (116). *Bruguiera sexangula* and *B. cylindrica* (Rhizophoraceae) are mangrove trees that gave the name to brugine and further thiophene carboxylic esters of tropane. Leaves of *B. cylindrica* and of *B. gymnorhiza* from greenhouse plants were found to contain calystegines (Brock and Dräger, to be published). Confirmation of calystegine occurrence and quantitation must be verified with plants from their natural habitats. Acetyl and benzoyl esters of hydroxytropanes were isolated from *Peripentadenia mearsi* (Euphorbiaceae) (117). Consequently, this family should receive more attention when seeking calystegines and other alkaloids with a tropane skeleton.

The Moraceae family lies within the order Rosales in the eurosid clade I, rather close to the cluster of Erythroxylaceae, Euphorbiaceae, and Rhizophoraceae (Figure 5). Calystegines were reported for *M. alba* and *M. bombycis* only (25,28,40). The family is much larger, however, with 38 genera and ∼110 species. This family should also be examined systematically for calystegine alkaloids.

The Brassicaceae is the largest family of the order Brassicales, with 3710 species in 338 genera, and is located in the eurosid clade II somewhere between the two major orders of tropane alkaloid occurrence, the Solanales and Malpighiales. Brassicaceae plants are well known for glucosinolates, not for alkaloids (118–120). Reports of cochlearine (**34**), a 3-hydroxybenzoic acid ester of tropine, in *Cochlearia officinalis* (121) and in *C. arctica* (now classified as *C. groenlandica*) (122) were hints to search for calystegine alkaloid occurrence in the family. They were detected in 18 species out of 43 examined, and were present in the basic genus *Aethionema*, illustrating the existence of tropane alkaloids early in the evolution of the Brassicaceae family (77).

The Proteaceae family awaits analysis for calystegines. Proteaceae plants were reported to contain several interesting tropane alkaloids; bellendine (123), darlingine, and 10-hydroxydarlingine (124) are pyranotropane bases, and knightoline has a 3α-acetoxy-2α-benzyltropane-6β-ol structure from *Knightia* species (125,126). *Agastachys odorata*, a Tasmanian Proteaceae species, was shown to contain 6β-acetoxy-3α-tigloyloxytropane and 3α-(4-hydroxybenzoyloxy)trop-6-ene, a naturally occurring tropene base. Proteales are not placed within the core

eudicots plant clade. They are assumed to have separated from the major group of eudicots more than 100 million years ago; estimates which rely on the fossil record of the family (127) and on recent molecular data (128). The increasing interest in the evolution of alkaloid biosynthetic pathways within the phylogeny of angiosperm plants should stimulate investigation of the metabolic steps of tropane alkaloid formation in the Proteaceae. Viewed together (Figure 5), calystegines were detected in a large fraction of the eudicot clade. If the Proteaceae are set separately, a smaller section of the occurrence of calystegines remains comprising the eurosids II and the asterids.

B. Calystegine Localisation in Plants

Calystegines were initially reported to be restricted to the roots (2), but later identified in all parts of plants. Composition and concentration of calystegines varies considerably among plants, and within one plant. For many species, a few selected tissues of interest were examined only, e.g., roots or fruits. From Table III some generalisations can be established:

- Calystegine B_2 (**3**) is the most abundant calystegine and occurs in almost all plants known to produce calystegines. Only some Convolvulaceae species contain calystegines without calystegine B_2. Calystegine B_2, due to the four equatorial hydroxyl groups, may be the most stable form within the calystegines of the B-group.
- Calystegines A_3 (**1**) and B_1 (**2**) also are widespread in calystegine-producing plants.
- Moraceae plants possess calystegines of the B-group and C_1 (**14**), and nortropane diols and triols, but do not contain calystegines of the A-group.
- Young meristematic tissues are often those with the highest concentrations of calystegines.

The distribution of calystegines among plant organs was specified for several species, but localisation of calystegines within plant cells has not been possible. Calystegines were described to act in the plant rhizosphere (see Section VIII), but excretion of calystegines has not been shown directly. Extracellular calystegines have never been found, in contrast to mulberry leaves, in which the latex is rich in alkaloidal glycosidase inhibitors, such as 1,4-dideoxy-1,4-imino-D-arabinitol, 1-deoxynojirimycin (**6**), and 1,4-dideoxy-1,4-imino-D-ribitol (34). Calystegines were not detected in the latex of Convolvulaceae plants (Sichhart and Dräger, to be published).

C. Quantities and Concentrations

The fruits, flowers, and young vegetative tissues are those with the highest concentrations of calystegines. Maximal concentrations were reported for young potato tuber sprouts, with more than 3 mg/g fresh mass (92).

A. belladonna and *Hyoscyamus* species are known for their content of the tropane alkaloids hyoscyamine (**35**) and scopolamine. Leaves of *A. belladonna*

typically contain 3 mg/g dry mass hyoscyamine (129), more than calystegines A_3 (**1**), B_1 (**2**), and B_2 (**3**) together (ca. 1.5 mg/g dry mass). Accordingly, in the roots of the plant, hyoscyamine concentration (2–3 mg/g dry mass) was higher than that of the total calystegine alkaloids (*ca.* 300 μg/g dry mass). But in cultured roots, the calystegines accumulated up to 4 mg/g dry mass, when 5% sucrose was applied, while hyoscyamine rose to 2.5 mg/g dry mass. Root cultures often contain higher calystegine concentrations than roots of the respective intact plants indicating that cultured roots contain a large proportion of young and proliferating root tissue. Tropane alkaloid biosynthetic enzymes were localised in the root pericycle and in the endodermis, tissues specific for young roots (130,131). In leaves of *Hyoscyamus muticus* plants (90) and in root cultures of *Hyoscyamus* species, similar concentrations of calystegines and hyoscyamine were observed (89,132). Obviously, a considerable fraction of the total tropane alkaloid content that is produced in these plants is devoted to calystegine formation, in particular in young tissues.

Other plants accumulate alkaloidal glycosidase inhibitors in low concentrations typically. Swainsonine (**5**) was measured in the leaves and flowers of *S. galegifolia* (Fabaceae) as 29 and 28 μg/g fresh mass, respectively (81). Transformed root cultures produced higher swainsonine levels (62 μg/g dry mass) than non-transformed root cultures (24 μg/g dry mass) or the roots of intact plants (9 μg/g dry mass) (133,134). *Astragalus* species (Fabaceae) may contain 3 mg swainsonine per gram dry mass in flowers, but usual concentrations range from 50 to 300 μg/g dry mass in leaves (135). Castanospermine (**4**), in *Castanospermum australe* (Fabaceae), was reported at 150–200 μg/g dry mass in seeds and at 12–16 μg/g dry mass in leaves (136). These data are comparable to calystegine concentrations in mature tissues. Young plant tissues containing extraordinarily high amounts of alkaloidal glycosidase inhibitors have not been reported for compounds other than calystegines. However, limitations to interpretation of the quantitative data for calystegines, and for most other glycosidase inhibitors in plants, must be considered. Many plant tissues were not examined quantitatively and, in some cases, as for *Erythroxylum* species, herbarium samples probably do not represent the full calystegine spectrum and quantity of the fresh tissues. Similar limitations may apply to greenhouse plants.

V. BIOSYNTHESIS

A. General Route of Calystegine Biosynthesis

The widespread occurrence of calystegine alkaloids in angiosperms provokes the question whether the biosynthetic steps and the intermediates are identical among all plants that contain calystegines. Investigations on calystegine biosynthesis in the Solanaceae profited substantially from the intensive research performed on tropane alkaloid biosynthesis in the Solanaceae, for which authoritative reviews are available (137–140). The first calystegine structures that were published from *C. sepium* and *A. belladonna* in 1990 (1), and the vast majority of calystegine structures, display an equatorial hydroxyl group at

carbon 3 of the nortropane skeleton, and suggest that they are products of pseudotropine after demethylation of the bridge nitrogen. Those calystegines that do not possess a 3-hydroxyl group, e.g., calystegines A_7 (**9**) and B_5 (**19**), may form non-enzymatically from other calystegines by ring-opening at the acid-labile aminoketal group, keto–enol tautomerism, and recyclisation to form the nortropane nucleus (Figure 4). A reduced form of the suggested monocyclic aminoheptanone intermediate was isolated from plant tissue (141), supporting the concept of an aminoketal rearrangement *in vivo*. The biosynthesis of calystegines was summarised in 1996 (12), and in 2004 (142), and since then the biogenetic pathway (Figure 6) has received further experimental support as discussed below. Dihydroxynortropanes without a hydroxyl group on carbon 3, and without an aminoketal functionality (**29**), pose a problem for the current scheme of calystegine biogenesis. Incorporation of labelled ^{15}N-tropinone into 2,7-dihydroxynortropane (**29**), however, revealed that the alkaloid was derived from tropinone in *C. sepium*, by whichever biosynthetic steps (78,79). At present, dehydration and double bond reduction of calystegines appears most probable.

The initial metabolic intermediates of the tropane skeleton formation are shared with those leading to nicotine (**36**); the *N*-methylpyrrolinium cation (**37**) is a precursor to both classes of alkaloids. *N*-Methylpyrrolinium cation formation begins with the decarboxylation of ornithine or arginine by ornithine decarboxylase (ODC, EC 4.1.1.17) and arginine decarboxylase (ADC, EC 4.1.1.19), respectively. The enzyme products enter the biosynthesis either directly as the ODC product putrescine (**38**), or via agmatine and *N*-carbamoylputrescine starting from arginine. In hairy root cultures of *Datura stramonium*, arginine was suggested to supply most of the putrescine for alkaloid biosynthesis (143). Putrescine (**38**) is an ubiquitous metabolite found in all living organisms. The first committed step for tropane alkaloid and nicotine biosynthesis is catalysed by *S*-adenosylmethionine (SAM)-dependent putrescine *N*-methyltransferase (Figure 6, PMT; see below).

Subsequently, *N*-methylputrescine (**39**) is assumed to be oxidatively deaminated by a diamine oxidase (DAO, EC 1.4.3.6). Enzyme activity oxidising *N*-methylputrescine was measured in tobacco tissues (144–147), and the protein was partially purified from *N. rustica* roots (148,149). A good affinity for *N*-methylputrescine (K_M 0.08–0.1 mM) was determined for the DAO from *N. tabacum* (149,150). The enzyme, however, did not behave specifically for the substrate, *N*-methylputrescine (**39**). DAO from *Brugmansia candida* hybrid root cultures oxidised *N*-ethylputrescine and *N*-(2-fluoroethyl)putrescine, as well as *N*-methylputrescine; *N*-*n*-propylputrescine and putrescine were oxidised with half-maximal velocity (151). The *N. tabacum* DAO apparently associates with *S*-adenosylhomocysteine (SAH) hydrolase in the plant tissues as part of a larger, multienzyme complex that may function as a nicotine metabolic channel (152). The oxidation product of *N*-methylputrescine is thought to undergo spontaneous cyclisation to form the reactive *N*-methylpyrrolinium cation (**37**), which enters nucleophilic condensation reactions. Nicotinic acid is the condensation partner on the way to nicotine (**36**). The enzyme catalysing this step is not known, nor are the enzyme(s) and condensation partner(s) on the way to tropinone (**32**) evident.

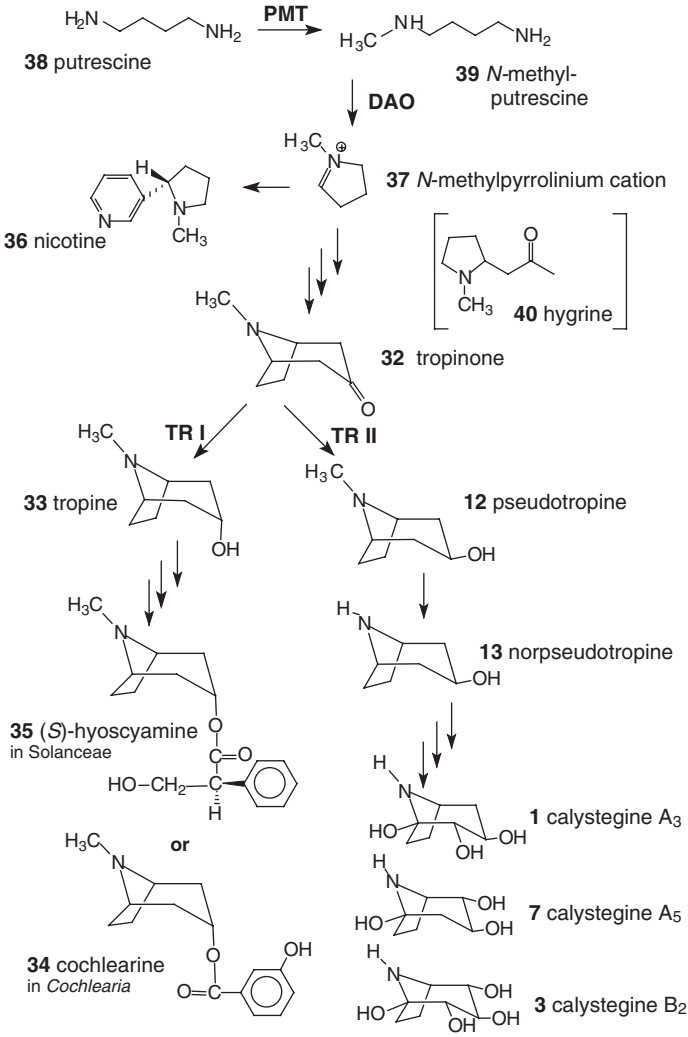


Figure 6 Tropane alkaloid biosynthetic pathway. Three arrows indicate that more than one enzymatic step leads to the next metabolite. PMT = putrescine *N*-methyltransferase; DAO = diamine oxidase; TRI = tropine forming tropinone reductase; TRII = pseudotropine forming tropinone reductase.

Hygrine (**40**) is found in many tropane alkaloid forming plants, e.g., *A. belladonna* (153), *H. albus* (154), other *Hyoscyamus* species (155), *D. ferox* (156), *D. innoxia* (157), and *E. coca* leaves (158–161). Hygrine or dehydrohygrine (162) were thought to be metabolites on the biosynthetic pathway to tropinone (**32**) (163), but this assumption was disapproved in 1972 based on feeding experiments with labelled hygrine (164), and was further discouraged by incorporation studies with hygrine and other labelled precursors (165). Studies of spontaneous

hygrine formation from cuscohygrine degradation support the view that hygrine is probably a by-product, rather than an intermediate, on the way to the calystegines and other tropane alkaloids (166).

The first intermediate with a tropane ring in the pathway is tropinone (**32**). It can be stereospecifically reduced by either of two related dehydrogenases, tropinone reductase I and II (TRI and TRII, see below). The alcohol with an axial hydroxyl group on carbon 3, tropine (**33**), is incorporated into (*S*)-hyoscyamine (**35**) and scopolamine, the 6,7-epoxy derivative of hyoscyamine. (*S*)-Hyoscyamine is formed by esterification of tropine and the phenylalanine-derived phenyllactic acid (167–169) to give littorine initially (170,171), which then rearranges to hyoscyamine (172–176). A cytochrome P450 gene, CYP80F1 identified from *H. niger* roots was expressed in yeast. The gene product catalysed the oxidation of (*R*)-littorine with rearrangement to hyoscyamine aldehyde, a putative precursor of hyoscyamine (177). Hyoscyamine is thereafter converted to the epoxide scopolamine by hyoscyamine-6β-hydroxylase (H6H, EC 1.14.11.11) (178–180). Tropine is also a part of cochlearine (**34**), the tropane alkaloid from the Brassicaceae (121). Pseudotropine (12), the 3-equatorial alcohol of the tropane skeleton, is the first specific precursor for the calystegines.

B. Putrescine *N*-Methyltransferase

Putrescine *N*-methyltransferase (PMT, EC 2.1.1.53) is the enzyme diverting the primary metabolite putrescine (**38**) into the biosynthesis of alkaloids, namely the nicotine alkaloids, the tropane alkaloids such as hyoscyamine (**35**), scopolamine, and cocaine, and the calystegines (Figure 6). The enzyme uses SAM as co-substrate and was first purified and measured from tobacco plant roots (144,181–183) and tobacco callus cultures (145). Root cultures of tropane alkaloid producing plants, *D. stramonium* (182,184,185), *H. albus* (186,187), and *H. niger* (188) contained PMT with similar properties as the enzyme from tobacco. The enzyme kinetics were described as ordered bi-bi with SAM binding first, followed by putrescine (185,188). Turnover values (K_{cat}) typically are between 0.1 and 0.4 s^{-1}, describing PMTs as rather slowly catalysing enzymes, like many other small molecule methyltransferases. The K_M values for putrescine were reported between 0.3 and 0.9 mM. Both values, K_M and V_{Max}, may suffer from inhibition by SAH, which is the product of SAM after methyl transfer. An enzyme-coupled colorimetric assay for methyltransferases was reported that utilises a recombinant adenosylhomocysteine nucleosidase (EC 3.2.2.9) for SAH hydrolysis into adenine and *S*-ribosylhomocysteine (189). Recombinant ribosylhomocysteinase (EC 3.2.1.148) then cleaves *S*-ribosylhomocysteine to form homocysteine, which is quantified after reaction with Ellman's reagent (5,5′-dithiobis-2-nitrobenzoic acid) leading to a yellow 2-nitro-5-thiobenzoic acid measured at 412 nm. A similar assay for methyltransferases may also be operated in a continuous manner (190). The method was adopted for PMT and showed considerably increased data for V_{Max} and decreased values of both K_M for putrescine and SAM for the identical enzyme preparation (191). In the course of the assay, the inhibitory product SAH was degraded to serve for enzymatic measurement and no longer

interfered with PMT activity *in vitro*. It may be speculated that kinetic data for many SAM-dependent methyltransferases that are feedback inhibited by SAH suffer from similar incorrectness, when their V_{Max} and K_M data were determined *in vitro* without SAH removal.

The first PMT cDNA sequence was obtained by subtractive hybridisation on cDNA banks exploiting different transcript levels between tobacco cultivars rich and poor (Burley 21) in nicotine (EMBL accession no. D28506) (192). Nicotine levels in several *N. tabacum* genotypes had been shown previously to correlate with PMT activity (193). Heterologous expression of cDNA isolated by subtractive hybridisation in an *E. coli* deletion mutant lacking the spermidine synthase gene resulted in the accumulation of *N*-methylputrescine, confirming the isolated cDNA coding for PMT. Further PMT cDNA sequences from *N. sylvestris* (EMBL accession no. AB004322, AB004323, AB004324) (194), and from other *Nicotiana* species, were found by the screening of genomic DNA libraries using 5′-terminal *pmt*-fragments as probes (195,196) that contain 33 base pairs repeated two to nine times (197). For *pmt* cloning from *H. niger* and *A. belladonna*, cDNA libraries from roots were screened with full length tobacco *pmt* probes (131,198).

Expression of the PMT gene is generally limited to root tissues (197). In *A. belladonna* roots, *pmt* was expressed specifically in the root pericycle (131), while *N. sylvestris* roots, endodermis, xylem, and outer cortex cells showed *pmt* expression (194). An interesting observation was made with *pmt* expression in *N. tabacum* leaf tissue. Stable transgenic lines of *N. tabacum* that carried a *pmt*-promoter-β-glucuronidase reporter gene fusion indicated *pmt* expression in mechanically wounded leaves localised around the wound site. Expression was transient with maximal levels immediately after wounding and diminishing after approximately 2–4 h. Wild-type plants of *N. tabacum* also showed *pmt* expression in leaves on wounding, as well as very low transcript levels in unwounded leaves (199). PMT in general is stress-responsive, and the gene is inducible by methyljasmonate in roots higher than in leaves (194,200–202). Consecutive nicotine accumulation is strongly enhanced by methyljasmonate in intact plants (203) and in cell cultures (204), and parallels *pmt* expression (202,205). Luciferase transient expression controlled by the promoter of the *N. sylvestris pmt* gene in tobacco bright-yellow-2 (BY-2) protoplasts proved that an 80 bp TATA proximal region was necessary and sufficient for the jasmonate response. A tetramer of the 24 bp sequence, including a T/G-box within the region, conferred jasmonate responsiveness to a cauliflower mosaic virus 35S minimal promoter (206).

Similar results were obtained for the promoter region of *N. tabacum pmt* and expression of β-glucuronidase gene fusion. A promoter fragment 111 bp upstream of the transcriptional start site was sufficient to confer methyljasmonate responsiveness, it contained a conserved G-box element (GCACGTTG) at positions −103 to −96 bp from the transcription start (207). Using automated transfection of *N. tabacum* BY-2 cells and automated evaluation of a luciferase reporter assay, two novel tobacco transcription factors were found, NtORC1 and NtJAP1, that positively regulate the putrescine *N*-methyltransferase (*pmt*) promoter (208). These two factors act synergistically to induce *pmt* transcriptional

activity. Regulation of nicotine (**36**) biosynthesis with an emphasis on PMT regulation was summarised (209). In contrast to the *Nicotiana pmt* promoters, no jasmonate responsive element was identified in the *A. belladonna pmt* promoter region (131). Correspondingly, hyoscyamine was not enhanced, and calystegines decreased on elicitation in root cultures of *A. belladonna* (86). Other tropane alkaloid plants, however, responded differently to methyljasmonate. In root cultures of *D. stramonium* (210), *Brugmansia suaveolens* (211), and *Scopolia carniolica* (212), accumulation of tropane alkaloids increased. Treatment of *H. muticus* root cultures with jasmonates enhanced the precursor putrescine (**38**) and the PMT product *N*-methylputrescine (**39**), but produced only a slight increase in tropane alkaloid tissue levels (213,214). In those studies, calystegines were not included in the measurements. In many other investigations, calystegine concentrations were observed to vary considerably among plants and within one plant. However, they appeared not inducible by stress, but were developmentally regulated. Accordingly, *pmt* expression was found responsive to auxin, and auxin responsiveness was observed in all plants. Genes for PMTs serving for nicotine (**36**), as well as for tropane alkaloid biosynthesis, were suppressed on auxin application in cell and root cultures (202,207,215,216).

Many of the data summarised so far indicate that PMT activity limits alkaloid production in the nicotine and tropane alkaloid pathway. It was therefore obvious to try *pmt* overexpression with the idea to enhance tropane alkaloid formation. Overexpression of *pmt* increased the nicotine content in *N. sylvestris*, whereas in *A. belladonna* plants, *pmt* overexpression triggered by the strong 35S-promoter caused higher *pmt* transcript levels, but hyoscyamine levels were comparable to those for the wild-type and vector control plants (217). Transgenic hairy root clones with a five-fold increase in *pmt* transcript levels similarly had alkaloid profiles, like the related wild-type roots. Methylputrescine accumulated in transgenic tissues and indicated that PMT activity was one of several limiting factors for tropane alkaloid biosynthesis. The *pmt* overexpressing root cultures, however, were no longer sensitive to auxin suppression of alkaloid biosynthesis (216). Accordingly, *pmt* overexpression on *Duboisia* hybrid hairy root cultures produced no significant increase in either tropane or pyridine-type alkaloids (218).

In contrast, hairy root cultures of *D. metel* and *H. muticus* overexpressing the *pmt* gene accumulated higher amounts of tropane alkaloids than control hairy roots (219). The results indicate that the same biosynthetic pathway in related plant species can be differently regulated, and overexpression of a given gene does not necessarily lead to a similar impact on alkaloid formation. When *pmt* and *h6h* cDNAs were overexpressed together in transgenic hairy root lines of *H. niger*, significantly higher levels of scopolamine were produced. The best of these lines contained 411 mg/L of scopolamine (220). The regulatory influence of PMT activity in *N. tabacum* plants was also illustrated, when *pmt* expression was suppressed by *pmt* antisense transformation (221). The antisense-*pmt* manipulation had no effect on the transcript levels of quinolinate acid phosphoribosyltransferase, regulating the synthesis of nicotinic acid, and which supplies the pyridine ring for both nicotine and anatabine synthesis. Regenerated plants contained

increased anatabine content that was interpreted as a result of an oversupply of nicotinic acid which, in the absence of a sufficient supply of *N*-methylpyrrolinium cation (**37**), is used to synthesise anatabine. Obviously, nicotine (**36**) is a defence alkaloid against insect herbivory because *N. sylvestris* plants with *pmt* antisense suppression had less nicotine, and were more readily consumed by larvae of *Manduca sexta* (222).

The amino acid sequences of PMT resemble those of plant spermidine synthases (SPDS, EC 2.5.1.16), and this ubiquitous enzyme of primary metabolism is considered to be the evolutionary ancestor of PMT (197). SPDS accept putrescine, the same substrate as PMT, but require a slightly different co-substrate, decarboxylated SAM (dcSAM). Cloning of plant spermidine synthase cDNA was fortuitously effected by the DNA sequence similarity when screening for *Nicotiana pmt* (197). Screening a cDNA library from potato sprouts for *pmt* likewise produced potato *spds* sequences (223). This enzyme is exclusively responsible for calystegine formation, as potato plants do not possess nicotine or tropane alkaloids, such as hyoscyamine (**35**) or scopolamine. SAM-dependent methyltransferases of various origins and activities (224–226), and small molecule methyltransferases from plants in particular (227), were aligned and revealed high degrees of sequence variability, yet conserved motifs were identified and postulated to participate in SAM binding. All of the motifs described for methyltransferases show little or no conservation in PMTs, which renders them insufficient for a sequence-based distinction between *pmt* and *spds*. Cloning of *pmt* demanded either cDNA bank screening or several rounds of nested PCR starting from small cDNA fragments, as performed for *Anisodus tanguticus pmt* (228). After cloning of eight additional PMT cDNA sequences from five Solanaceae plants and one Convolvulaceae plant that all contain calystegines, four short amino acid motifs (10–13 amino acids) that are indicative for PMTs only, and sufficiently different in SPDS sequences, were identified (88).

The three-dimensional structure of the PMT active site is not known. The protein structure of a *Thermotoga maritima* spermidine synthase obtained by protein crystallisation (229) suggested that those amino acids of the SPDS active site that were continuously different between PMTs and SPDS were responsible for the differences in co-substrate acceptance. Mutagenesis of those two amino acids in the *D. stramonium* PMT with the idea of changing PMT activity into spermidine synthase, however, resulted in a complete loss of catalytic activity (88). A refined protein model of PMT constructed as a consequence of the result discouraged the concept of comparable substrate and co-substrate binding in PMT and SPDS. Rather, the substrate putrescine binds differentially at the active sites of both enzymes.

C. Tropinone Reductases

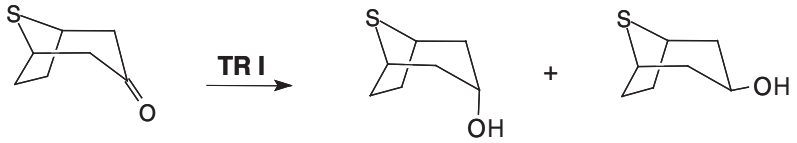
The molecular structures of most of the calystegines are characterised by an equatorial hydroxyl group at carbon 3, which is the typical feature of pseudotropine (**12**). TRII and the reduction product pseudotropine (Figure 6) had been isolated from *H. niger*, but could not be integrated into the tropane alkaloid

metabolism known previously (230,231). After the structure of calystegine was published, it was hypothesised that pseudotropine was the first specific metabolite on the pathway to the calystegines from tropinone (**32**) (89). Purification of two stereospecific TRs, TRI and TRII, from *D. stramonium* (232,233) and from *H. niger* (234) confirmed the assumption, as both plants contain the tropine esters hyoscyamine (**35**) and scopolamine, and the calystegines. Two separate, tropinone reducing, enzyme activities were confirmed in all Solanaceae plants that contained tropane alkaloids. No such activity was found in *N. tabacum* or in *Brassica campestris* (Brassicaceae) used as negative controls, while *C. sepium* showed TRII activity (234).

TRI and TRII are enzymes with similar protein properties, but with different catalytic and kinetic behaviour. Sequencing of cDNA coding for *D. stramonium* TRs revealed that the protein subunits consist of 273 (TRI) and 260 (TRII) amino acids and have a molecular mass of 29,615 and 28,310 Da, respectively (235). Amino acid sequence similarity (167 identical amino acid residues, 64%) and comparison of conserved amino acid motifs grouped both TRs into the large enzyme family of non-metal dependent short-chain dehydrogenases (236). Differences in tropinone (**32**) acceptance and fixation were suspected to be responsible for the selective formation of tropine (**33**) and pseudotropine (**12**), because reaction velocity, substrate affinity, and pH optima for TRI and TRII enzymes were systematically different (237). TRI catalysed the reduction of tropinone and the oxidation of tropine, whereas TRII was not, or only very slightly, active in catalysis of the oxidation reaction (233,234). In particular, pH dependency of the reaction velocities and K_M values differed characteristically between both enzymes. The catalytic constant K_{cat} of TRI from *D. stramonium* was elevenfold higher than that of TRII at pH 7.0. In buffers with acidic pH, TRI showed maximal reduction activity, but with a decreased affinity for tropinone, i.e., a very high K_M value. Together, this was taken as an indication that an acidic pH favored substrate turnover by TRI, but that uncharged tropinone was aligned better in the active centre of the enzyme (233).

Further evidence for differential substrate handling of both TRs was obtained from incubations with substrates analogous to the tropinone structure. Molecules with variations in charge and space requirements were accepted differentially by TRI and TRII. Quinuclidinone and 8-thiabicylo[3.2.1]octan-3-one (Figure 7, **41**), the sulfur analogue of tropinone, e.g., were efficiently reduced by all TRI enzymes, but were not accepted by TRIIs. Cloning and characterisation of a TRII from potato was considered as a proof for the specific role of TRII in calystegine formation (238). Although tropine and tropine ester are normally not present in potato, *tr*I-like sequences were also isolated from *S. tuberosum* tubers (239). The sequences showed 84–87% identity to other TRI proteins; expression in *E. coli* and product analysis confirmed tropine formation. Crystallisation and protein structure elucidation of TRI and TRII from *D. stramonium* by X-ray diffraction at 2.4 and 2.3 Å resolution was successful after heterologous enzyme protein expression in *E. coli* (240).

Modelling of the tropinone binding sites of TRI and TRII confirmed general protein folding of the two enzymes as almost identical, and demonstrated the



41 8-thiabicyclo[3.2.1] octan-3-one

42 8-thiabicyclo[3.2.1] octan-3α-ol 80%

43 8-thiabicyclo[3.2.1] octan-3β-ol 20%

Figure 7 Reduction of the sulfur analogue of tropinone by tropinone reductase I (TRI) to two isomeric products.

proposed two different modes of substrate fixation. In TRII, tropinone (**32**) is attached in the active centre by ionic interaction of the tropinone nitrogen to the side chain of glutamic acid (Glu156 in *D. stramonium* TRII). In the corresponding TRI, the nitrogen is repulsed by a histidine (His112) in the equivalent position, and tropinone is fixed in the opposite orientation by hydrophobic interactions. The reducing hydride from NADPH that occupies the same position in both TRs attacks tropinone to shift the alcohol group either to an equatorial or axial position (240–242). Thus, differential and selective fixation of the substrate tropinone by amino acids in the active centre explains how both enzymes succeed in stereospecific product formation. The higher reaction velocity of TRI was explained by the relatively loose substrate – and product – fixation caused by the charged nitrogen at the hydrophobic protein active site. Concurringly, 8-thiabicylo[3.2.1]octan-3-one (**41**) was reduced slowly by TRI (35% of maximal catalytic velocity), probably because it is non-charged, bound more strongly than tropinone, and may adopt several positions in the active site. Reduction of 8-thiabicylo[3.2.1]octan-3-one (**41**) yielded both stereochemical products, 8-thiabicylo[3.2.1]octan-3α-ol (**42**) with an axial hydroxyl group like tropine, and the corresponding β-configured alcohol (**43**) with the hydroxyl group position as in pseudotropine. Detailed elucidation of the catalytic mechanism of TRII was achieved by crystal saturation with the substrate tropinone and multiwavelength Laue X-ray diffraction (243). Transient structures were captured and demonstrated that a slight rotation of the product pseudotropine (**12**) on formation from the substrate was responsible for efficient catalysis. The concept of differential tropinone fixation in the active site of TRs was further supported by construction of chimeric TR enzymes and site-directed mutagenesis at those residues assigned as responsible for substrate fixation (244,245). Some of the enzymes with exchanged amino acids formed both tropine (**33**) and pseudotropine (**12**).

Tropinone (**32**) hardly accumulates in plant tissues, thus the two TRs were assumed to compete for the substrate (Figure 6). Metabolite flux into both arms of the tropane alkaloid pathway appears to be determined by the activity of the TRs. First evidence for this view was given by the inhibition of tropinone reduction to tropine in cultures of *D. stramonium* (246). More pseudotropine (**12**) was observed and decreased levels of tropine (**33**) and tropine products, e.g., acetyltropine and hyoscyamine (**35**) (247). In those studies, calystegine measurements were not included, but in *A. belladonna* root cultures, both hyoscyamine (**35**) and

calystegine A_3 (**1**) were observed after TRI inhibition by 8-thiabicylo[3.2.1]octan-3-one (**41**) (89). After 10 days, hyoscyamine had decreased considerably, while the concentration of calystegine A_3 was double that in control roots. Further analogues of tropinone with different alkylation on the nitrogen or with an enlarged ring (pseudopelletierine derivatives) were accepted as substrates by TRI and TRII to various degrees (151,248). Application of these analogues to root cultures again shifted the ratio of TRI to TRII-derived products. TR activities measured *in vitro* usually are higher than those of other tropane alkaloid pathway enzymes, and they are considered as not limiting for the total flux through the pathway (234). Yet, the ratio of products is influenced by the activity of both enzymes. The availability of TR coding sequences enabled overexpression of TRI and TRII in tropane alkaloid producing plant tissues for the detailed study of the regulatory impact of both enzymes. The first heterologous TRI expression in plants was achieved with a TRI cDNA cloned behind a strong 35S-promoter in the non-tropane alkaloid forming *N. tabacum*. Leaves of regenerated plants after tropinone (**32**) application produced tropine (**33**) and acetyltropine (249). *A. belladonna* was transformed with either *tr*I or *tr*II (250). The transformation effect on the alkaloid pattern in root cultures was stronger with *tr*I overexpression than with *tr*II. More tropine was found, and increased hyoscyamine accumulation resulted, from *tr*I overexpression.

D. Location of Biosynthesis and Possible Transport

TR overexpression results confirm that *in vivo*, the activities of TRI and TRII participate in tropane alkaloid biosynthesis regulation. However, they do not prove that both enzymes compete for their substrate tropinone (**32**) within the same tissues or cells. Rather, transcripts and proteins of both enzymes were localised in different tissues of *H. niger* root cultures. Reporter gene fusion to promoter regions of both TRs suggested expression in endodermis, pericycle, and in some cortex cells of mature roots, rather than in young roots (130). Immunostaining of TRI and TRII proved partial different localisation within the root diameter (251). The TRI protein signal was strong in the endodermis and in some cortex cells, while the TRII protein concentration was highest in the pericycle. Two other enzyme proteins of the tropane alkaloid pathway were localised in the pericycle of the roots, putrescine *N*-methyltransferase in *A. belladonna* (131) and hyoscyamine 6β-hydroxylase forming scopolamine in *H. niger* (252,253). TRI responsible for hyoscyamine (**35**) formation was absent from the pericycle, and requires metabolite transport between tissue layers in order to complete tropane alkaloid biosynthesis. As a consequence, transport, as well as enzyme activity, may be a limiting and regulating factor in alkaloid biosynthetic activity.

Early grafting experiments (254,255) and analysis of xylem sap (256) indicated that tropane and nicotine alkaloid biosynthesis in the Solanaceae takes place in the roots, and the alkaloids are transported into the aerial parts of the plant by the xylem. Tropane alkaloids can also be transported by the phloem sap (257). Isolated potato tubers, which are stem organs, also produce high amounts of calystegines during sprouting, proving that calystegine biosynthesis takes place

in non-rooted stem tissues that normally grow underground. Transcripts of potato *pmt* (223) and *tr*II (238) were isolated from sprouting tuber eyes. Immunostaining of both TRI and TRII in potato tissues showed differential localisation (239). TRI was detected in tuber tissues mostly at levels too low to be localised in individual cells. TRII was contained in cortex cells of root and stolon, and in phloem parenchyma. In tuber sprouts, TRII protein appeared in companion cells. Detailed transcript monitoring, protein analysis for potato PMT, TRI and TRII, in addition to calystegine measurement, indicated that PMT restricted the overall metabolic activity in the pathway (258). Tropinone (**32**) was present in low concentrations throughout the whole potato plant, and was assumed to be formed in the roots and distributed into the aerial parts of the plant and into the stolon and the developing tubers. TRs are active in all plant organs including the stolon, TRII activity exceeding that of TRI, except for tuber tissues. Strong TRII activity will impede tropinone to accumulate to high concentrations in tissues, indicating that tropinone supply at the location of TRII contributes to the overall metabolite flux into calystegine formation.

In the aerial parts of the plants, and in the stolon, tropinone is readily reduced to pseudotropine (**12**), which is then metabolised to the calystegines. In non-sprouting tubers, where no PMT, and only low TRII, activities were found, the major part of calystegine accumulation appears to be the result of tropinone reduction in the stolon and subsequent metabolism to calystegines. When tuber eyes sprout, a sudden decrease of calystegines in tuber peel is observed. Calystegines are transferred from the tuber peel to the eyes and thereafter to the sprouts. In addition, PMT and TRII localised in developing tuber sprouts will provide additional metabolites for calystegine formation in sprouts. Roots that contain high TRII activity and high calystegine levels may also export calystegines to aerial parts of the plants and to the tubers.

This model of calystegine accumulation involves many metabolite transport steps. Transport processes are an essential part of the regulatory instruments for biosynthesis of many alkaloids and other secondary compounds and are just being detected (259,260). In contrast to alkaloids, for which transport was attributed to ATP transporters (261,262) or pH-dependent efflux carriers (263), calystegines are hydrophilic sugar-mimic alkaloids. A plant sucrose transporter was identified that transports phenol glucosides (264), indicating that this type of transporter should receive attention when looking for calystegine transport.

E. Calystegines as Demethylated Tropane Alkaloids

The nortropane skeleton is a characteristic feature of most calystegines, implying that during the biosynthesis the *N*-methyl group of the initial tropane skeleton was lost. There are clues for the assumption that *N*-demethylation occurs as next metabolic step after formation of pseudotropine (**12**). Norpseudotropine (**13**) was isolated from *M. albus* fruits (28). In *C. sepium* root cultures, norpseudotropine was detectable on application of millimolar concentrations of external tropinone (**32**) (Sichhart, Meier, and Dräger, unpublished). In the Solanaceae, demethylation of the nitrogen in the tropane skeleton is not unusual. Several esterified

nortropane alkaloids were reported, e.g., norhyoscyamine (105). Nortropane alkaloids were also isolated from *Erythroxylum* species (17–20). Similar to the tropane alkaloid skeleton, nicotine (**36**) in several *Nicotiana* species is demethylated to yield nornicotine, e.g., in *N. otophora* (265), *N. tabacum* (266–268,268), *N. alata* (269), and *N. plumbaginifolia* (270). Oxidative methyl cleavage can be concluded from degradation of *N*-^{13}C-methyl-labelled nicotine, where the label was retrieved as carbon dioxide and in the amino acids serine and methionine. Folate is consequently assumed to assist in the transfer of the C1-group (271). Feeding (^{13}C,$^{2}H_3$-methyl)nicotine to *N. plumbaginifolia* suspension cell cultures indicated that the metabolite *N*′-formylnornicotine that was labelled at only ~6% of the level of nicotine was not a metabolite on the way to nornicotine (272). Results of several experiments feeding labelled nicotine were summarised (273). The long-standing hypothesis that a cytochrome P450 catalysed oxidation is the basis of nicotine demethylation was proven, when the CYP82E4 gene was isolated by a microarray-based selection strategy, which, after expression in yeast, revealed its function as a nicotine demethylase (274). For calystegine biosynthesis, demethylation of pseudotropine may be another rate-limiting step. TR activity in most tissues is higher than required for tropane alkaloid biosynthesis (234), and reduction products are esterified to acetic acid or tiglic acid (247), if they accumulate in excess on tropinone application. Norpseudotropine (**13**) is found in calystegine-forming tissues in traces only, if at all.

VI. CHEMICAL SYNTHESIS

The enantioselective preparation of compounds with several chiral carbon atoms poses high demands. For the calystegines that are of natural origin, calystegine A_3 (**1**), B_2 (**3**), B_3 (**17**), and B_4 (**18**) have been made available by synthetic procedures. For the preparation of calystegine A_3, two synthetic routes have been published. An approach for racemic calystegine A_3 started with aminocyclohexanone (21). Ring enlargement into 5-aminocyclohepten-2-one was achieved by a three-step procedure: silyl enol ether formation, Simmons–Smith cyclopropanation, and finally ring-opening with iron(III) chloride. Subsequent *trans*-dihydroxylation of the double bond and cyclisation to the nortropane skeleton yielded calystegine A_3. A different synthetic route starting from cycloheptatriene and proceeding via the 6-azido derivatives of *meso*-2-cycloheptene-1,4-diol provided both enantiomers of calystegine A_3, separately (275). The azido function was reduced to obtain the amine group, which was protected as a benzyl carbamate. Asymmetrisation was achieved by a *Pseudomonas cepacia* lipase, that selectively acylated one of the two hydroxyl groups of the aminocycloheptene ring. The remaining alcohol was substituted with phenyl selenide which, after oxidation to the selenoxide, underwent a sigmatropic rearrangement to give the corresponding 6-aminocycloheptene-3,4-diol. After stereospecific introduction of a keto group, the aminocycloheptanone-diols were converted to the bicyclic aminoketal structures of the respective enantiomers of calystegine A_3. The specific rotations of the calystegine A_3 enantiomers were +12.4° and −12.4° (*ca.* 0.63, water). The naturally occurring calystegine A_3 is assumed to be a metabolite

on the way to calystegine B_2 (**3**) and thus to have the same configuration as calystegine B_2, which is 1*R*,2*S*,3*R*,4*S*,5*R* with a (+)-rotation.

For the synthesis of calystegine B_2 (**3**), enantioselective synthetic routes were also described. They start from D-glucose for the selective preparation of the (−)- and (+)-calystegines B_2 (22–24,276–278). Similarly, further aldohexose epimers serve as starting material for further calystegines, D-galactose for calystegine B_3 (**17**) and D-mannose for calystegine B_4 (**18**) (279). The monosaccharide precursors introduced three hydroxyl groups in the particular desired configuration. Ring enlargement of the aldohexose was performed by various strategies and led to a protected polyhydroxyaminocycloheptanone, which after deprotection, underwent spontaneous cyclisation to the nortropane system. The ring enlargement was achieved after formation of a cyclohexanone intermediate from glucose by a Ferrier reaction (23,24). An alternative route to the aminocycloheptanone was the cycloaddition of chiral nitroso derivatives with cyclohepta-1,3-diene, which selectively provided a single stereoisomer (277). Intramolecular cycloaddition of an olefinic nitrile oxide obtained from D-glucose was used in the first approach to achieve the hydroxylated aminocycloheptanone (22). These methods suffered from low overall yields, again due to more than ten reaction steps in each case.

Ring-closing olefin metathesis with Grubb's catalyst after a zinc-mediated tandem reaction on a methyl 6-iodo-glycoside is an elegant method to lead to a protected hydroxylated aminocycloheptene (Figure 8).

Subsequent regioselective oxidation of the olefin then affords the corresponding aminocycloheptanone. The method is much shorter and more efficient than earlier procedures. The formation of the bicyclic nortropane structure occurs

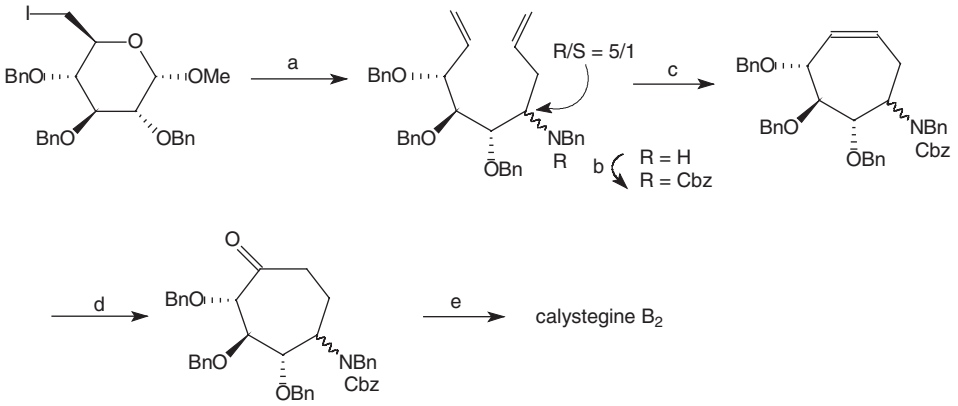


Figure 8 Synthesis of calystegine B_2 by ring-closing metathesis starting from a glucose molecule, in which all free hydroxyl groups were protected. Reagents and conditions from Skaanderup and Madsen (278). (a) Zn, $BnNH_2$, $CH_2 = CHCH_2Br$, THF, sonication, 40°C, (b) CbzCl, $KHCO_3$, EtOAc, H_2O, (c) 2 mol% $(PCy_3)(C_3H_4N_2mes_2)Cl_2Ru = CHPh$, CH_2Cl_2, room temperature, (d) BH_3 THF, THF, −40 to 0°C, then H_2O_2, NaOH, H_2O, 0°C, then Dess–Martin periodinane, CH_2Cl_2, room temperature, separation of ketone isomers, (e) H_2, $Pd(OH)_2/C$, THF, H_2O.

spontaneously on reductive deprotection. The synthesis of calystegine B_2 (**3**) has been achieved by this method (276,278), and calystegines B_3 (**17**) and B_4 (**18**) were synthetically prepared for the first time starting from the respective D-galactose and -mannose derivatives (279,280).

Synthetic strategies for compounds with structures analogous to the calystegines were also established. Polyhydroxylated 6-oxa-nor-tropanes were obtained from 5-deoxy-5-thioureido and 5-ureido-L-idofuranose precursors (281). Derivatives bearing different substituents at the nitrogen were prepared as well and tested for inhibition of several glycosidases. Strong and highly specific inhibition of bovine liver β-glucosidase was observed for 6-oxacalystegine B_2 analogues carrying aromatic pseudoaglyconic groups on the nitrogen. Further derivatives of a similar structure were prepared and evaluated systematically and proved that the analogues with a hydroxylation pattern of calystegines B_2 (**3**) and C_1 (**14**) were the most effective inhibitors of bovine liver β-glycosidase/β-galactosidase (282). Synthesis of calystegine analogues containing hydroxyl constituents on both the C6 and C7 bridge carbon positions was achieved starting with a cycloaddition of a nitroso compound generated *in situ* from the reaction of benzyl *N*-hydroxycarbamate and tetra-*n*-butyl-ammonium periodate with cycloheptadiene or cyclooctadiene, respectively. Surprisingly, the 6,7-dihydroxy product analogues exist mainly as the monocyclic keto-tautomer, in contrast to the bicyclic hemiaminal form reported for the majority of the calystegines isolated to date (283). Calystegine, tropane, and hydroxylated aminocycloheptane skeletons may be obtained from other cycloadduct reactions (284). *Endo*-selective intramolecular nitrone–alkene cycloadditions of hept-6-enoses controlled by a *trans*-acetonide to give bridged bicyclo[4.2.1]isoxazolidines were obtained and transformed into the respective mono- or bicyclic compounds.

VII. BIOCHEMICAL ACTIVITIES

A. Structure–Effect Relations between Calystegines and Glycosidases

Calystegines, like other monosaccharide-mimicking alkaloids, inhibit glycosidases due to their similarity to the pyranose moieties of the natural substrates. Biochemical activities and therapeutic applications for this class of natural compounds were expertly reviewed (4,9–11,13). The mode of glycosidase inhibition is competitive for most polyhydroxy alkaloids. Among the glycosidases, the inhibitory activity of polyhydroxylated alkaloids, such as the calystegines, is generally restricted to exoglycosidases cleaving at the non-reducing end of a saccharide chain, while endoglycosidases that cleave within a saccharide chain releasing oligo- or disaccharides mostly remain unaffected (13). For the calystegines in particular, the presence of an OH group at C2 of the nortropane skeleton is the minimal structure element required for inhibition (36). In general, a wide variety of carbohydrate-active enzymes other than glycosidases may bind polyhydroxy alkaloids (285). For example, liver glycogen phosphorylase was strongly inhibited by hydroxymethylpiperidine-3,4-diols (286). The pyrrolidine alkaloid 1,4-dideoxy-1,4-imino-D-ribitol purified from the mulberry tree (*M. alba*)

strongly inhibited the activities of eukaryotic DNA polymerases, but had almost no effect on the activities of prokaryotic DNA polymerases (287).

Calystegines B_1 (**2**) and C_1 (**14**) are strong inhibitors of β-galactosidase (36). Inhibitory activities of other calystegines towards β-galactosidase are somewhat weaker, and calystegines with axial OH groups in the piperidine ring, e.g., calystegines B_3 (**17**) and B_4 (**18**), did not inhibit β-galactosidase at all (36). Equatorial OH groups in the piperidine ring and a non-methylated *N*-bridge are important for optimal inhibition of both β-galactosidase and β-glucosidase (see below). Coffee bean α-galactosidase was strongly inhibited by calystegine B_2 (**3**) (K_i 0.86 μM) and *N*-methylcalystegine B_2 (**22**) (K_i 0.47 μM). *N*-methylation of calystegines enhanced inhibition strength towards α-galactosidase. Calystegines B_1 (**2**) and C_1 (**14**) did not show noteworthy inhibition towards this enzyme, but their C6-deoxy analogues, calystegines A_3 (**1**) and B_2 (**3**), inhibited α-galactosidase. Hence the OH group in the *exo* orientation at C6 of the nortropane skeleton interferes with binding to α-galactosidase. In the case of β-glucosidase, increasing the number of OH groups leads to stronger inhibition. The K_i value of calystegine C_1 towards almond β-glucosidase is only 0.45 μM. Enzymatic glycosylation of calystegines B_1 and B_2 led to a decrease of inhibition potency towards β-glucosidase, and α- or β-galactosidase. In contrast, 3-*O*-β-D-glucopyranosylcalystegine B_1 (**24**) was a non-competitive inhibitor (K_i 0.9 μM) of rice α-glucosidase (288). Calystegine B_4 (**18**) was a potent competitive inhibitor of pig kidney trehalase (K_i 1.2 μM), but almost inactive against fungal trehalase (37). Trehalose is the most widely distributed disaccharide in fungi, serving as an important storage compound (289). Thus, trehalase inhibitors are regarded as potential fungicides (10). It is noteworthy that calystegine N_1 (**21**), which is the C1 amino analogue of calystegine B_2, showed weak, non-competitive inhibition towards pig kidney trehalase (K_i 62 μM), while calystegine B_2 was a competitive inhibitor of this enzyme (K_i 5.3 μM) (35).

B. Mechanism of Inhibition

O-Glycosidases represent a structurally manifold group of enzymes. They are divided according to Bourne and Henrissat (290) into 85 families that differ in substrate specificity, and also in the hydrolytic mechanism. The oxygen orientation at the anomeric carbon of the sugar product released by the enzyme is either inverted or retained compared to its former orientation in the glycosidic bond. The anomeric carbon of a cyclic sugar is located in the semi-acetal (aldoses) or semi-ketal (ketoses) functionality. Anomeric chirality is created by ring closure of the sugar chain. Thus four transitions of former to new configuration are possible by glycolysis: axial → axial, axial → equatorial, equatorial → axial, equatorial → equatorial (291,292).

In general, the glycosidase catalytic mechanism involves protonation of the glycosidic oxygen by a donor group with subsequent formation of a planar oxocarbenium ion that is stabilised by another nucleophilic carboxylate residue in retaining hydrolases or by a solvent–nucleophile assisting carboxylate residue in inverting hydrolases (292,293). Recently, a β-glucosidase from *Thermotoga*

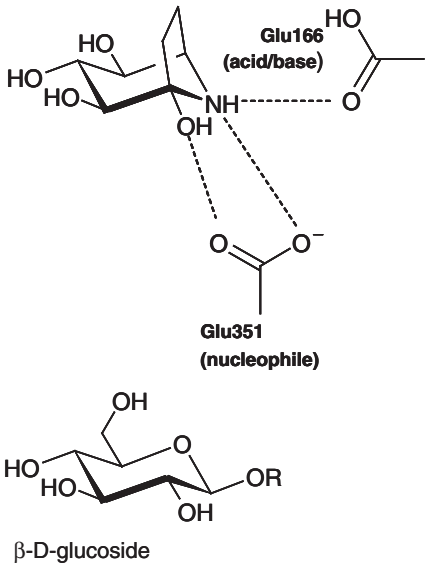


Figure 9 Calystegine B_2 in the active site of a *Thermotoga maritima* β-glucosidase. A β-D-glucoside is shown in an equivalent orientation to the inhibitor. Interactions between calystegine B_2 and the catalytic residues in the active site of β-glucosidase are indicated by hydrogen bonds in broken lines; protonation of calystegine B_2 is not shown.

maritima (TmGH1) was crystallised together with calystegine B_2 (PDB 2CBV) (294). The inhibitor occupies the active site in a relaxed chair conformation ($^4C_1$), wherein the calystegine B_2 nitrogen is at the position of the anomeric carbon atom of the substrate sugar moiety β-D-glucose (Figure 9). The *N*-protonated form of calystegine B_2 (**3**) was derived from K_i values, determined at different pH (294). Thus, the nitrogen is able to interact with both donator (acid/base) and nucleophile, forming ion pairs resembling the transition state to some extent. Nevertheless, the mode of interaction between calystegine B_2 and β-glucosidase must not be transferred to other glycosidases and should not be taken as the only valid mechanism. It should be kept in mind that even glycosidases with identical catalytic activity, e.g., trehalase (see above), might vary in structure both between species and between compartments of the same cell. It is difficult therefore to predict the inhibitory ability of a particular calystegine *in vivo* (13).

Predictions concerning glycosidases with different substrates appear much more difficult, almost impossible. Thus, further calystegine–glycosidase complexes may await elucidation in the future.

C. Toxicology of Calystegines

Calystegines occur in edible fruits and vegetables of the Solanaceae, Moraceae, and Convolvulaceae (for a summary, see Asano *et al*. (80)). Recently, they were also detected in edible cabbage species (77). Like other glycosidase inhibitors,

they must be considered as potential toxins inhibiting lysosomal glycosidases of human and animal cells. Alkaloids such as swainsonine (**5**) and castanospermine (**4**) are toxins because they inhibit lysosomal glycosidases involved in glycoprotein processing (295–297). Of course, inhibition of glycoprotein processing capacity offers a lot of opportunities for chemotherapy, e.g., as treatments against cancer, since oncogene products and cancer-specific proteins sometimes show unique glycosylation patterns (298). Treatment of several viral infections was intensively studied, as virus coat proteins need protein glycosylation in order to be functional (299,300). Many of these concepts were, and still are, under intensive investigation, and authoritative reviews are available (301–306). Indeed, calystegines B_1 (**2**) and C_1 (**14**) were also found to be potent inhibitors of liver lysosomal β-glucosidase from man and rat (80). Ikeda and co-workers (307) identified calystegines B_1 (**2**), B_2 (**3**), and C_1 (**14**) as potent inhibitors of isolated human glucocerebrosidase which is a lysosomal β-glucosidase (see following section). Moreover, calystegines occur in plants which cause intoxications in livestock after feeding, similar to lysosomal storage diseases, with vacuolated cells in different organs and neuronal damage (82,83,91) (Table IV).

Dorling and co-workers (308) report an ataxia syndrome in cattle, whose occurrence was implicated with calystegine-containing *Ipomoea* species that grew in the area of poisoning events. Recently, *Ipomoea carnea* toxicosis was examined with regard to calystegines (307,309). *I. carnea* also contains the indolizidine alkaloid swainsonine (**5**), which is a strong inhibitor of human lysosomal α-mannosidase (IC_{50} 0.04 μM (307)). Swainsonine is a model compound for the induction of lysosomal storage disease mannosidosis (310–312). Hueza and co-workers (309) fed rats with isolated calystegines B_1 (**2**), B_2 (**3**), B_3 (**17**), and C_1 (**14**), purified from the leaves of *I. carnea*. Calystegines did not produce any toxic effects, whereas swainsonine also purified from *I. carnea* caused typical cell

Table IV Calystegine-containing plants that were reported to cause poisoning in livestock

Plant	Animal species/trivial name of intoxication	Supposed toxic principles	References
Ipomoea carnea Convolvulaceae	Goats	Swainsonine, calystegines B_2 and C_1	(82)
I. muelleri and *I. lonchophylla* Convolvulaceae	Cattle	Several calystegines, swainsonine	(308)
Ipomoea sp. Q6 [aff. *calobra*] Convolvulaceae	Cattle and sheep	Swainsonine, calystegine B_2	(83)
Solanum dimidiatum Solanaceae	Cattle "Crazy Cow Syndrome"	Calystegine B_2	(91)
S. kwebense Solanaceae	Cattle "Maldronksiekte"	Calystegine B_2	(91)

vacuolation. The total aqueous extract from *I. carnea*, however, was more effective in causing toxic lesions in rat organs than the equivalent amount of isolated swainsonine. This may indicate that calystegines contribute to the toxicity of *I. carnea* leaf extract.

Surprisingly, Ikeda and co-workers (307) found an increased activity of β-glucosidase after incubation of normal human lymphoblasts with calystegines B_2 and C_1, applied via the culture media. They postulated that calystegines might act as chemical chaperones in the cells, protecting β-glucosidase from endoplasmic reticulum-associated degradation (see following section). Toxic effects caused by calystegines were not observed in these cells. In summary, toxicity of isolated calystegines has never been shown up to now. It was shown that inhibitory effects of calystegines on *isolated* enzymes differ dramatically from effects in intact cells. The question of whether calystegines can pass the plasma membrane, and, for practical reasons, whether they are resorbed from the intestines, remain to be answered. Other alkaloidal glycosidase inhibitors with toxicity potential, different from the calystegines, were shown to effectively cross biomembranes and act against intracellular glycosidases. The mobility of the calystegines across biomembranes would be a basic prerequisite for all further studies on the oral intoxication caused by calystegines.

D. Possible Medicinal Applications

The inhibition of intestinal digestive glycosidases decreases blood glucose peaks typically occurring after a meal rich in carbohydrates in diabetes patients. The α-glucosidase inhibitor acarbose, also named Bay g 5421, was found to reduce the post-prandial increase in blood glucose and the responding serum insulin levels in man (59,313,314). Acarbose (**44**, Figure 10) is a pseudotetrasaccharide and was the first glycosidase inhibitor introduced into therapy (Glucobay™/Precose™). Subsequently, two α-glucosidase inhibitors resembling monosaccharides, voglibose (**45**) (Basen™) and miglitol (**46**) (Diastabol™/Glyset™), enhanced the choices for the oral treatment of non-insulin dependent diabetes mellitus (10). Miglitol is the *N*-hydroxyethyl derivative of 1-deoxynojirimycin and, in contrast to acarbose, the compound is well absorbed after oral administration (315). It also reduces post-peak glucose after an oral glucose load, which is absorbed without enzymatic cleavage. The result was interpreted as a systemic hypoglycaemic effect of miglitol (316,317). Prevention or attenuation of diabetes by nutrition supplements and herbal medicine has been popularised more and more. Extracts of the roots and stem of *Salacia reticulata* (Celastraceae), containing the *S*-alkylthiophane derivative salacinol (**47**), are recommended in Ayurvedic medicine (318). In these compounds, the positively charged sulfur may mimic the oxocarbenium ion intermediate during glycoside hydrolysis (318). Castanospermine (**4**) was also found to be a competitive inhibitor of intestinal sucrase, and a non-competitive inhibitor of intestinal maltase (319,320).

The hypoglycaemic effect of Egyptian *M. alba* root bark extract (prepared with 70% ethanol) in streptozotocin-induced diabetic rats was reported by Singab and co-workers (321). An extract of *M. alba* root bark, prepared with 50%

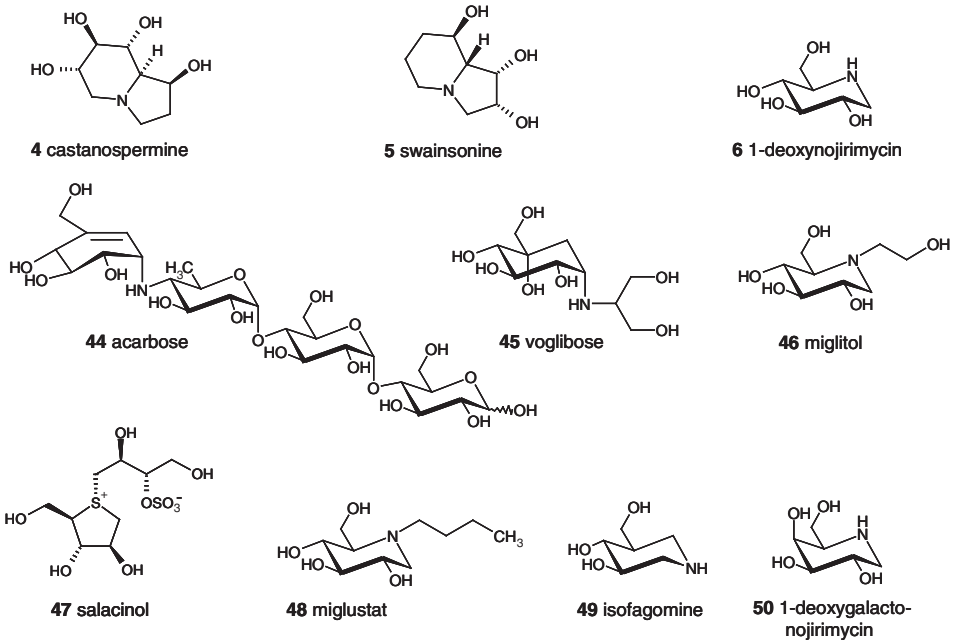


Figure 10 Structures of glycosidase inhibitors with toxicological or therapeutic relevance.

methanol, contained a range of polyhydroxylated, sugar-mimicking alkaloids (40). The major component was 1-deoxynojirimycin (**6**) (1.65 mg/g dry mass), an α-glucosidase inhibitor, which served as a template for the design of miglitol (10,322). Calystegines B_1 and B_2 were found in low concentrations compared to 1-deoxynojirimycin, 1.6 μg of calystegine B_1 per gram dry mass and 8.3 μg of calystegine B_2 per gram dry mass (40), suggesting that the observed hypoglycaemic effect was caused by 1-deoxynojirimycin. Hypoglycaemic effects of isolated polyhydroxy alkaloids were compared using pilocarpine-induced saliva secretion in streptozotocin-diabetic mice as a model (323). Calystegine B_2, in contrast to fagomine, showed no significant effects on these mice.

Mutations in the genes coding for the lysosomal glycosidases glucosylceramidase (glucocerebrosidase, EC 3.2.1.45) and α-galactosidase A (EC 3.2.1.22) cause the lysosomal storage diseases *Morbus Gaucher* type I and *M. Fabry*, respectively. *M. Gaucher* patients suffer from the accumulation of toxic glucosylceramide in lysosomes of macrophages (324,325). *M. Fabry* is a disorder of glycosphingolipid metabolism causing renal failure, strokes, and premature myocardial infarctions (326). The options for therapy of *M. Gaucher* type I were expanded by the introduction of Zavesca™ containing *N*-butyl-1-deoxynojirimycin, INN miglustat (**48**) (324,325). Platt and co-workers (327) established that *N*-butyl-1-deoxynojirimycin inhibited the glucosyltransferase (EC 4.2.1.80) involved in the biosynthesis of glucosylceramide and thus lowered the lysosomal deposition of residual compounds when membranes are degraded (''substrate

reduction therapy''). In addition to the inhibition of glycolipid biosynthesis, *N*-butyl-1-deoxynojirimycin inhibited the *N*-linked oligosaccharide processing enzyme α-glucosidase I. The galactose analogue *N*-butyl-1-deoxygalactonojirimycin was more selective, being a potent inhibitor of glycolipid biosynthesis, but showing no effect on the maturation of *N*-linked oligosaccharides, or on lysosomal glucerebrosidase (328). Miglustat is the first case of a glycosidase inhibitor applied as a glycosyltransferase inhibitor.

An interesting additional finding was the increased activity of the mutated glucocerebrosidase in transfected COS-7 cells with *N*-butyl-1-deoxynojirimycin at 10 μM in the culture medium, suggesting a chemical chaperone effect for the glycosidase protein (329,330). Chang and co-workers (331) accordingly reported increased activity of glucocerebrosidase in *M. Gaucher* fibroblasts after incubation with calystegines A_3 (**1**), B_1 (**2**), B_2 (**3**), and C_1 (**14**) among other inhibitors of this enzyme. The increase in activity was mediated by an increased level of mutant glucocerebrosidase protein in the cytoplasm and improved trafficking of the enzyme within the cell compartments. Thus, the chemical chaperone theory, postulated for calystegines by Ikeda and co-workers (307) based on the discovery of enhanced β-glucosidase activity in human lymphoblasts after incubation with calystegines, was confirmed.

Calystegines and other glycosidase inhibitors that exhibit specificity as folding helpers towards individual mutated human glycosidases may be termed ''pharmacological chaperones'' or shortly ''pharmacoperones'' (332). Pharmacoperones bind to the active site or another unique target structure on a certain protein and promote the correct folding (333). In the case of lysosomal enzymes, the enzyme output of the endoplasmic reticulum towards the Golgi apparatus is improved, eventually leading to better lysosomal enzyme equipment (334) (Figure 11). Calystegines similarly are able to stabilise mutant, but still active, glucocerebrosidase and prevent it from misfolding and subsequent degradation in the endoplasmic reticulum. Furthermore, calystegines showed only low toxicity towards cultured fibroblasts of *M. Gaucher* patients. Nevertheless, the most efficient inhibitor tested for increased activity of glucocerebrosidase in *M. Gaucher* fibroblasts (331) was isofagomine (**49**). Its effective concentration was half of that of calystegines B_1 and B_2.

1-Deoxygalactonojirimycin (**50**), a potent inhibitor of α-galactosidase, could be a supplement for the treatment of *M. Fabry*. The activity of α-galactosidase A in human *M. Fabry* lymphoblasts was enhanced when 1-deoxygalactonojirimycin was added to the culture medium (326). Oral administration of 1-deoxygalactonojirimycin in mice overexpressing human mutant α-galactosidase A enhanced enzyme activity in some organs (335). The strong α-galactosidase inhibitors calystegine B_2 (**3**) and *N*-methylcalystegine B_2 (**22**) may be candidates for the investigation of pharmacological chaperone activity towards mutant α-galactosidase A.

The identification of calystegines as pharmacological chaperones established their medicinal value and importance. Yet, any therapeutic success would require high bioavailability. After liberation from orally administered formulations, a substance must be resorbed from the intestines and released in the blood

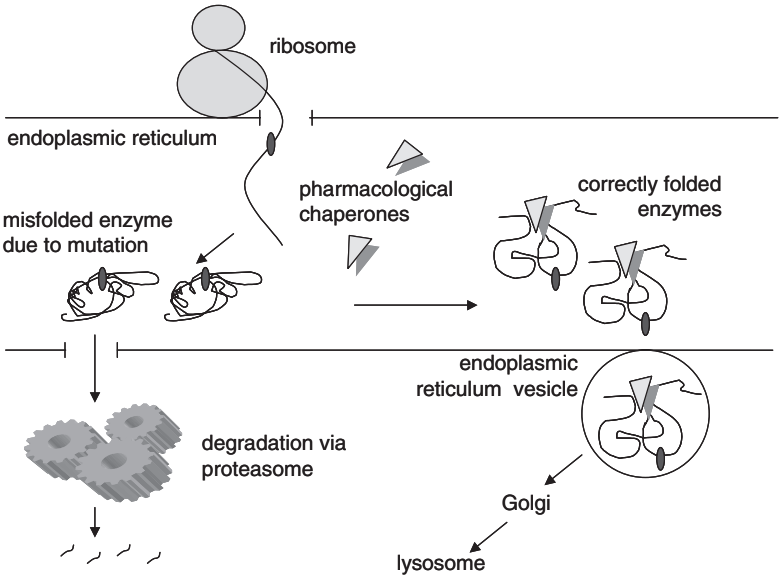


Figure 11 Pharmacological chaperones (grey triangles) bind to the active site of a protein, misfolded due to a mutation (black ellipse) and restore the functional protein conformation. Thus the protein is prevented from early degradation. The stable chaperone–protein complex is transferred by the endoplasmic reticulum vesicles to the Golgi and later to the lysosome, where the inhibitor is replaced by the natural substrate.

circulation, which demands either some kind of active or facilitated transport, or diffusion across several biomembranes. The question whether calystegines are resorbed from the intestines still needs to be answered. Moreover, incorporation of calystegines into endoplasmic reticulum, Golgi apparatus, and lysosomes of target cells is required for a possible function as pharmacoperones. In the case of hydrophilic alkaloids, two chemical properties appear important for transport across cell compartments: the octanol–water partition coefficient, expressed as log *P*, and the pK_a value.

The rapid incorporation of swainsonine (**5**) into cultured human fibroblasts does not appear to be carrier-mediated (336). Comparing the lipophilicity of swainsonine (**5**) to calystegines B_1 (**2**), B_2 (**3**), and isofagomine (**49**) (Table I) swainsonine possesses the highest log *P*, i.e., it is the most lipophilic alkaloid, which enables diffusion across biomembranes. On the other hand, isofagomine shows the highest pK_a value, i.e., it is the most basic alkaloid. Swainsonine is likely entrapped in the acidic environment of lysosomes by protonation (336). Intracellular pH values decrease within the trafficking pathway of lysosomal enzymes: endoplasmic reticulum (pH~7.1) → Golgi apparatus (pH 6.2–7.0) → lysosomes (pH ~4.5) (337). Thus isofagomine also should be entrapped effectively in the pH ambience of endoplasmic reticulum and the Golgi apparatus after entry into the respective compartment, although protonation on the other hand would conflict with membrane diffusion. Nevertheless, the strongly basic

isofagomine would be effectively prevented from diffusion out of the cell, thus presenting an explanation for the enhanced efficacy of isofagomine compared to calystegines B_1 and B_2 in the study of Chang and co-workers (331). In summary, polyhydroxylated alkaloids are relatively novel members of the large family of alkaloidal natural products with promising therapeutic properties (338), which, due to their hydrophilicity, will remain a challenge for rational drug design and pharmaceutical technology for the goal to create highly effective medicines.

VIII. ACTIVITY OF CALYSTEGINES IN PLANTS

Enzymatic processing of carbohydrates in plants is catalysed by a variety of carbohydrate-active enzymes, including glycosidases, glycosyl transferases, polysaccharide lyases, and carbohydrate esterases. Such enzymes may also be produced by microbial plant pathogens and insects and increase their virulence or herbivory, respectively. Plants have evolved proteinaceous inhibitors against glycosidases to modulate the activity of several of these enzymes (339). Low molecular mass compounds were suspected to possess a similar significance for plants, however, for calystegines there is no conclusive evidence for a positive contribution to plant survival or defence. Some experiments indicating a possible impact of calystegine on plant growth and defence are reported.

A. Plant Rhizosphere

The discovery of calystegines in the root exudates of *C. sepium* was accompanied by the disclosure of calystegine-metabolising bacteria, like *Rhizobium meliloti* 41 in the rhizosphere of this plant (2). Close association between calystegine-metabolising bacteria and calystegine-producing plants was confirmed (340). Growth of calystegine-metabolising *R. meliloti* 41 was stimulated in the presence of calystegines, while the growth of non-metabolising bacteria was not inhibited under the same conditions. It is noteworthy that *R. meliloti* 41 was able to metabolise naturally occurring (+)-calystegine B_2 (**3**), but not the synthetic enantiomer. Goldmann and co-workers (340) supposed that the calystegines function as nutrition mediators under natural conditions. *Sinorhizobium meliloti* Rm41 harbouring the plasmid that codes for the genes necessary to metabolise calystegines were shown to reach higher population levels in calystegine-positive plants than *S. meliloti* Rm41 without the plasmid (341). Using a TLC-based bioassay, Guntli and co-workers (342) found calystegine-metabolising bacteria also in the rhizosphere of the calystegine-negative plant *Zea mays* and they observed colonisation of sterile seedlings of *C. sepium* and *Z. mays* with calystegine-metabolising bacteria after planting in natural soil. In summary, the occurrence of calystegine-metabolising bacteria is not restricted to calystegine-producing plants, but the ability to metabolise calystegines appears to be valuable for bacteria in the rhizosphere of plants that produce calystegines. It may speculated that calystegines serve as an additional carbon source for specialised rhizobia, while the bacteria could provide benefits to the plant resulting in mobilisation of nutrients, biocontrol of pathogens, or detoxification of allelopathic soil compounds produced by

other plants. The manifold interactions between rhizosphere organisms and root exudates of plants were reviewed recently (343). The specific role of calystegines in the rhizosphere ecology remains to be discovered.

B. Allelopathic Activity

Plants excrete allelopathic substances in order to inhibit development of other plants in their vicinity. Goldmann and co-workers (340) observed germination of *Medicago sativa* seeds inhibited, and elongation of *M. sativa* hairy roots reduced, in the presence of (+)-calystegine B_2 (**3**), which is the natural enantiomer.

C. Insect Feeding Repellent Activity

Pupae and adults from the Lepidoptera, feeding on calystegine-containing plants, were reported to store calystegines and therefore to exhibit glycosidase inhibitory activity (344). Konno and co-workers (34) investigated the role of the latex of *M. alba* leaves in the defence against herbivorous caterpillars other than the silkworm *Bombyx mori*, which feeds on those leaves. The overall content of polyhydroxylated, sugar-mimicking alkaloids in the latex reaches 1.5–2.5%. Feeding experiments showed the latex to be toxic against caterpillars. The possible intake of calystegines by insects designates them as defence substances allowing only adapted insects to feed on those plants, like silkworms on *M. alba* leaves. Adapted insect species may further use calystegines to protect themselves, being then indigestible for predatory birds (344).

D. Anti-Nematode Activity

Polyhydroxylated alkaloids possess insecticide activity and also exhibit anti-nematode properties. Welter and co-workers (345) isolated the pyrrolidine derivative DMDP [(2*R*,5*R*)-bis(dihydroxymethyl)-(3*R*,4*R*)-dihydroxypyrrolidine] from the legume *Derris elliptica*. The substance affected several nematode species in different ways. In *Globodera* sp., mobility, cyst hatch, and root galling were inhibited (346). The activity of calystegines against nematodes would be worth investigating because they were claimed several times, although not directly shown, to be excreted into the plant rhizosphere (2,340).

E. Plant–Fungi Interactions

Höke and Dräger (347) found that calystegines do not inhibit the growth of endophytic fungi of *C. sepium*, nor the growth of the *C. sepium* pathogen *Stagonospora* sp. Infested plants did not show altered calystegine levels compared to control plants. In the course of the study, inhibition of *C. sepium* invertase by calystegine B_2 (**3**) was observed. This finding is a hint for the relationship between the calystegines and the carbohydrate metabolism in the plant.

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CHAPTER 3

Recent Studies on the Synthesis of Strychnine

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I. INTRODUCTION

The genus *Strychnos* is the numerically most important genus of the Loganiaceae family and comprises approximately 190 species of trees and lianas growing in the warm regions of Asia, America, and Africa (1–4). The toxicity of *Strychnos* was empirically well known in South-Eastern Asia and India from immemorial antiquity. Seeds of *Strychnos nux vomica*, which are the source of the notorious poison strychnine, were used as a traditional poison. Strychnine poisoning can be fatal to humans (a lethal dose of strychnine for an adult human is in the range of 30–100 mg), and can be introduced to the body by inhalation, swallowing, or absorption through the eyes or mouth. It produces some of the best-known, most dramatic, terrifying, and painful symptoms imaginable. The poisonous properties of *Strychnos* species were recognized in Europe as early as the sixteenth century and they were used as a pesticide, particularly for killing small vertebrates such as rodents.

In 1918, Pelletier and Caventou reported the isolation of strychnine, in pure form, as the principal toxin from *S. nux vomica* and *S. ignatii* (5,6). The extensive degradative and structural studies by Robinson et al. culminated in the elucidation of the correct structure of strychnine in 1946 (Figure 1) (7,8). The numbering system and ring labeling based on the biogenetic interrelationship of monoterpene indole alkaloids, as proposed by Le Men and Taylor, is used throughout this article (9). A year later, Woodward et al. independently suggested the same structure (10). In the early 1950s, two independent X-ray crystallographic analyses, by Robertson and Bevers (11,12), and Bijvoet (13), confirmed the relative configuration of strychnine. The absolute stereochemistry of strychnine was established by Peerdeman in 1956 with X-ray crystallographic analyses (14) and was confirmed by Schmid et al. in 1963 using a chemical method (15). Strychnine ranks as one of the most complex natural products of its size ($C_{21}H_{22}N_2O_5$, molecular weight (MW) = 334): only twenty-four skeletal atoms are assembled in seven rings, resulting in six contiguous stereogenic centers (five of them in the core cyclohexane E ring). Therefore, strychnine is recognized as the flagship alkaloid of the family of *Strychnos* alkaloids. Meanwhile, various other *Strychnos* alkaloids were also identified (2–4,16). The *Strychnos* alkaloids constitute an important group of architecturally complex and widely distributed monoterpenoid indole alkaloids. Selected representative *Strychnos* alkaloids are shown in Figure 2.

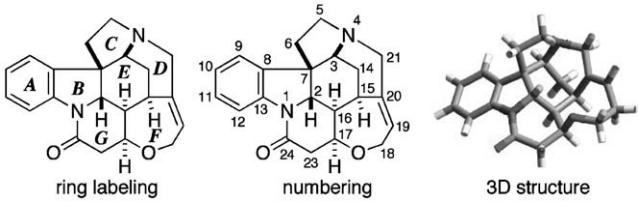


Figure 1 Structure of (−)-strychnine.

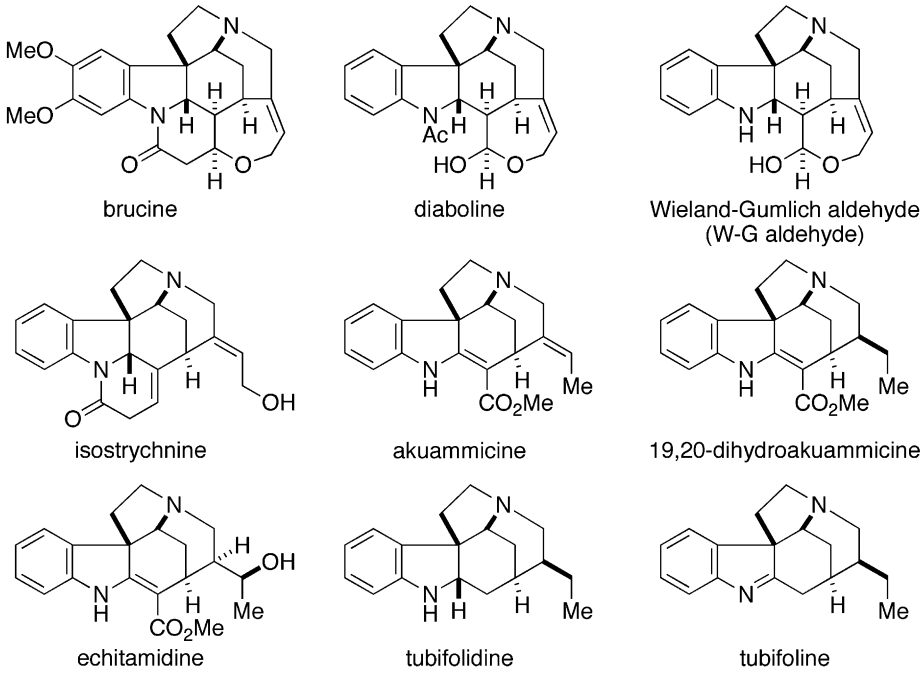


Figure 2 Selected representative *Strychnos* alkaloids.

Strychnine toxicity arises from the blocking of postsynaptic inhibition in the spinal cord and lower brain stem, where it acts as a prototypic competitive antagonist of the glycine receptor. This receptor, which is frequently referred to as the "strychnine-sensitive glycine receptor," displays nanomolar K_i values at recombinant and native receptors in binding and functional assays (17). In addition to its activity at these receptors, strychnine is a moderately potent, noncompetitive antagonist of muscle-type and neuronal heteromeric nicotinic acetylcholine receptors, where it has been shown to be a competitive antagonist of the neuronal homomeric α7 nicotinic receptor subtype (18). Finally, strychnine and brucine are also well-known allosteric modulators of muscarinic acetylcholine receptors. As a high-affinity and highly selective antagonist, as described above, strychnine has been useful as a tool for the structural characterization of those receptors, as well as in numerous biochemical studies of the nervous system.

The structural complexity of strychnine, coupled with its biological activity, has served as the impetus for numerous synthetic investigations. The first total synthesis, one of the most significant achievements in the history of organic synthesis, was reported by Woodward et al. in 1954 (19,20). Nearly 53 years after Woodward's pioneering work, 13 groups have achieved the synthesis of strychnine using novel procedures. The present paper provides a survey of the relevant literature to the end of 2006. The excellent review by Bonjoch and Solé (2) extensively covered the total synthesis of strychnine from 1954 to 1999: Woodward

(1954, 1963) (19,20), Magnus (1992, 1993) (21,22), Stork (1992) (23), Overman (1993) (24,25), Kuehne (1993 and 1998) (26,27), Rawal (1994) (28), Martin (1996/2001) (29,30), and Bonjoch/Bosch (1999) (31,32). Our aim, therefore, is to review the recent achievements by Vollhardt et al. (2000) (33,34), Mori et al. (2002) (35,36), Bodwell et al. (2002) (37), our own group (2002) (38,39), Fukuyama et al. (2004) (40), and Padwa et al. (2007) (41), with a brief overview of the earlier contributions. Total syntheses of other *Strychnos* alkaloids, such as akuammicine and tubifolidine, are generally not included in this review because most of methodologies used for those syntheses were later applied for the synthesis of strychnine.

II. AN OVERVIEW OF THE EARLIER SYNTHESES OF STRYCHNINE (1954–1999)

A. General Aspects

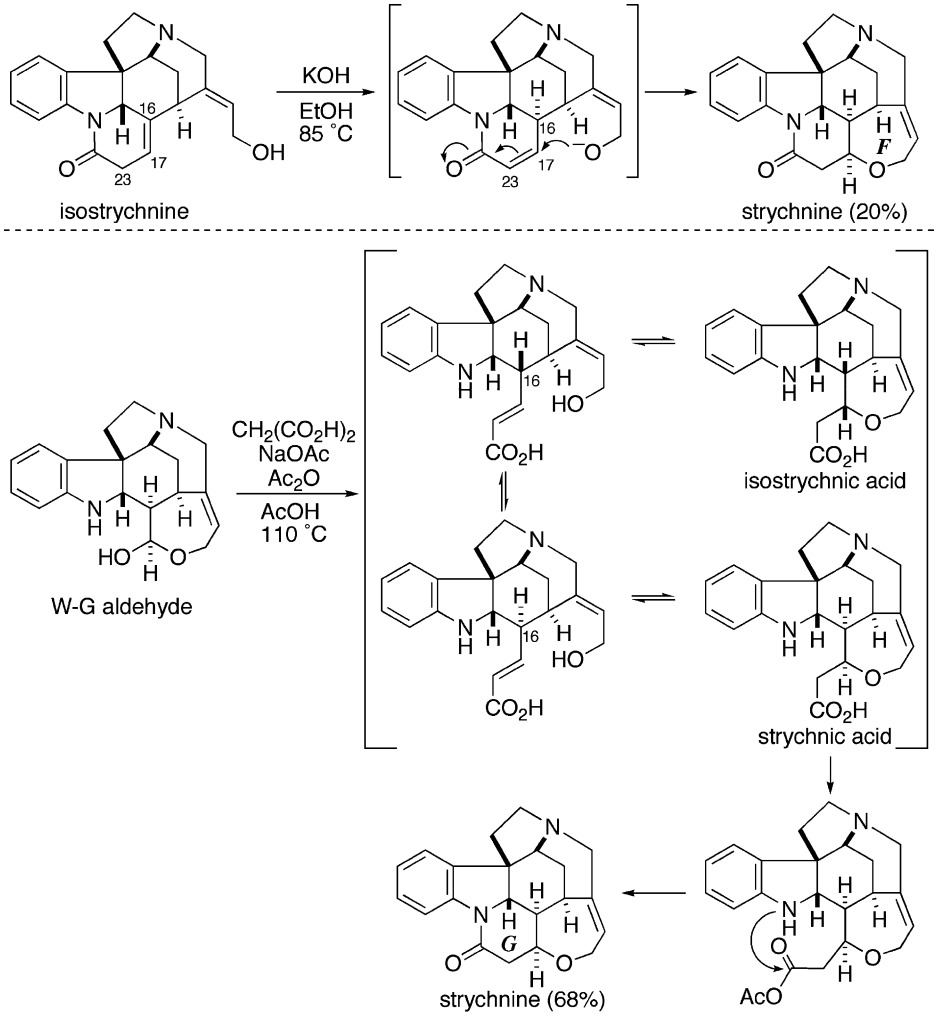
Following the first total synthesis of strychnine by Woodward et al. in 1954, eight total syntheses of strychnine were achieved in the 1990s. Three of them culminated in the enantioselective synthesis of the natural enantiomer, (−)-strychnine (24,25,27,31,32). This section presents an overview of the earlier syntheses of strychnine reported from 1954 to 1999 with a focus on the following three points: (i) the sequence of ring construction; (ii) the assembling of the CDE core ring, including the establishment of the C7 quaternary carbon center; and (iii) the way to access chiral intermediates. The interested reader will find some accounts of these early studies in the original papers, as well as in the previous reviews (2–4).

Through extensive degradation studies, started as early as the 1880s, it was revealed that isostrychnine (42) and Wieland–Gumlich (W–G) aldehyde (43–48), which are degradation products of strychnine, could be reconverted to strychnine in a single step by Prelog et al. in 1948 (49) and by Anet et al. (50), and by Robinson et al. in 1953 (51), respectively (Scheme 1). In the former case, treatment of isostrychnine with alcoholic potassium hydroxide induces isomerization of the C–C double bond from the C17–C16 position to the C23–C17 position to form an α,β-unsaturated lactam system with concomitant creation of the C16 stereocenter. Then, the following conjugate addition gives strychnine in 20% yield, with recovery of isostrychnine. In the latter case, treatment of W–G aldehyde with a mixture of malonic acid, sodium acetate, and acetic anhydride in acetic acid results in the direct formation of strychnine in 68% yield. On the basis of these precedents, all synthetic approaches so far reported have been directed to isostrychnine (six examples) or W–G aldehyde (nine examples).

B. Woodward's Relay Synthesis of (−)-Strychnine (1954)

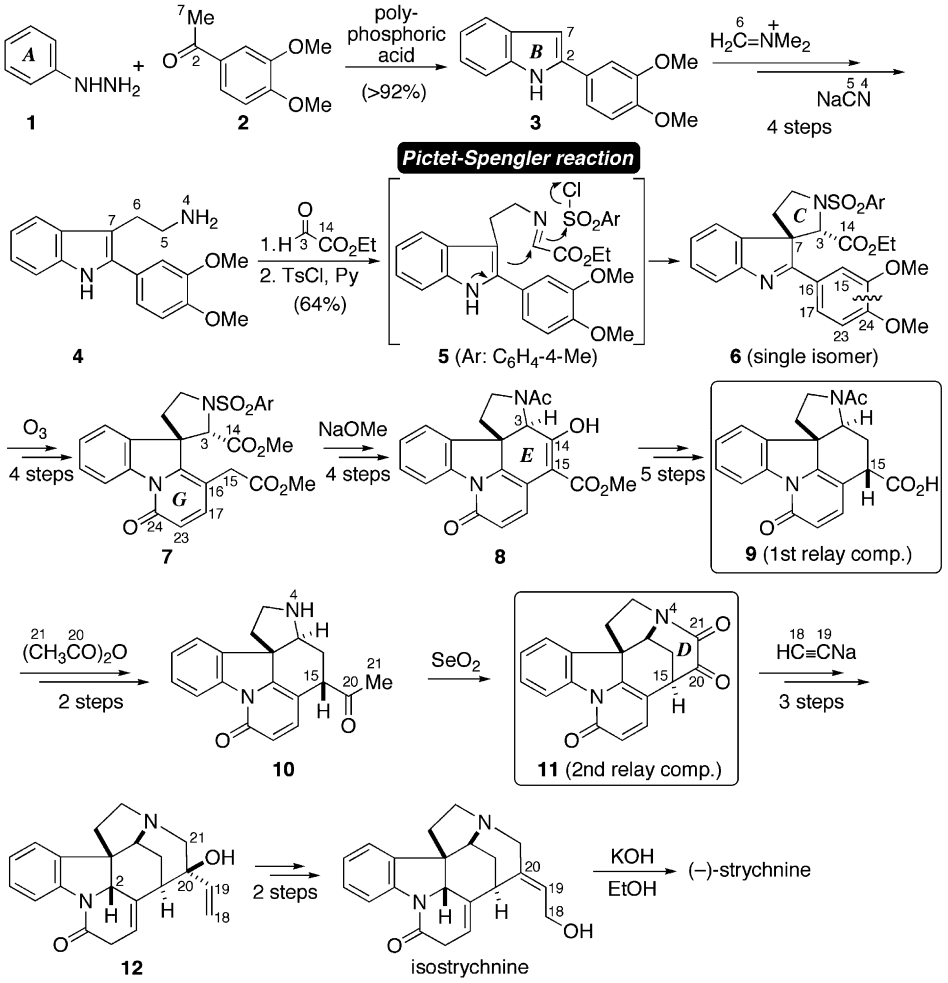
[**A** → A**B** → AB**C** → ABC**G** → ABC**E**G → ABC**D**EG → Isostrychnine → Strychnine] (19,20)

Woodward's strychnine synthesis began with a Fischer indole synthesis using phenylhydrazine (**1**) and acetoveratrone (**2**) [A → A**B**] (Scheme 2). In the later



Scheme 1 Reconversion of isostrychnine and Wieland–Gumlich aldehyde to strychnine.

stages of the synthesis, the veratryl group (–CH_2-C_6H_3-3,4–$(OMe)_2$) of **2** was used as parts of G, E, and D rings. After introduction of an aminoethyl moiety (N4–C6) at the C7 position, Pictet–Spengler reaction of the corresponding 2-veratryltryptamine (**4**) with ethyl glyoxylate was induced by 4-toluenesulfonyl chloride (TsCl) to give the spiroannulated compound **6** as the sole product [AB → AB**C**, C7 quaternary center]. The highly electron-rich veratryl derived from **2** was selectively cleaved by ozone at the bond between the two methoxy groups, and the resulting six-membered lactam formation afforded pyridone **7** [ABC → ABC**G**]. Prior to the E ring formation, the Ts group was changed to an acetyl group in three steps. Treatment with sodium methoxide induced epimerization at the C3



Scheme 2 Woodward's relay synthesis of (−)-strychnine.

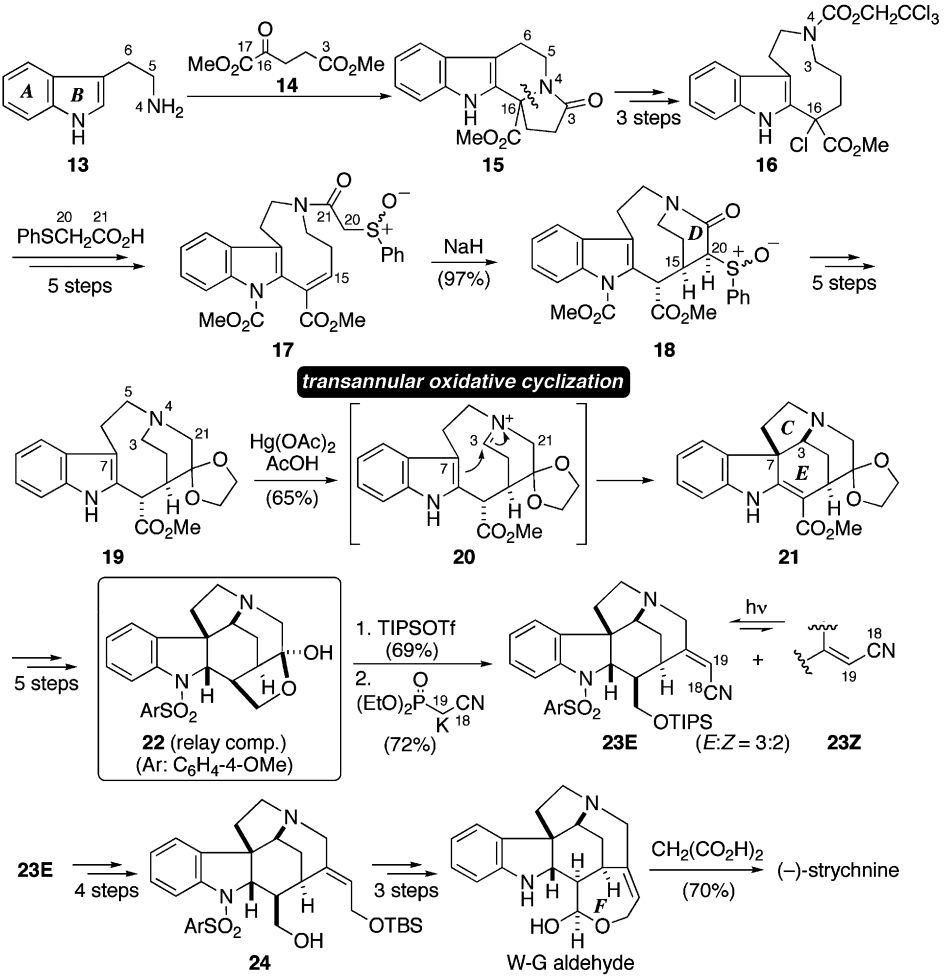
position, and subsequent Dieckmann cyclization gave enol ester **8** [ABCG→ ABCEG]. Deoxygenation at C14 via a sulfide intermediate, and hydrogenation of the C14–C15 double bond, gave the methyl ester of **9** (diastereomeric mixture at the C15 position). Then, epimerization at the C15 position under basic conditions provided the thermodynamically more stable carboxylic acid **9**, which was previously prepared by degradation of (−)-strychnine (52,53). The following transformations were performed using this optically pure, first relay compound **9**. Introduction of an acetyl group at the C15 position (54,55) and oxidation of the resulting methyl ketone **10** with SeO_2 directly gave the second relay compound **11** (52,53) [ABCEG→ABC**D**EG]. Diastereoselective addition of sodium acetylide to the ketone (C20), conversion to an allylic alcohol, reduction of the amide

carbonyl (C21), and stereoselective reduction of the pyridone ring by $LiAlH_4$ afforded **12**. Finally, rearrangement of the tertiary allylic alcohol to a primary allylic alcohol resulted in isostrychnine, which was then converted to (−)-strychnine by following the previously reported procedure (49).

C. Magnus' Relay Synthesis of (−)-Strychnine (1992)

[**AB** → AB**D** → AB**C**D**E** → ABCDE**F**: W–G aldehyde → Strychnine] (21,22)

Nearly 40 years after Woodward's pioneering work (19,20), Magnus et al. accomplished the second total synthesis of strychnine using a transannular oxidative cyclization (56,57), which simultaneously constructed the C and D rings, as the key reaction (Scheme 3). Magnus' strychnine synthesis began with



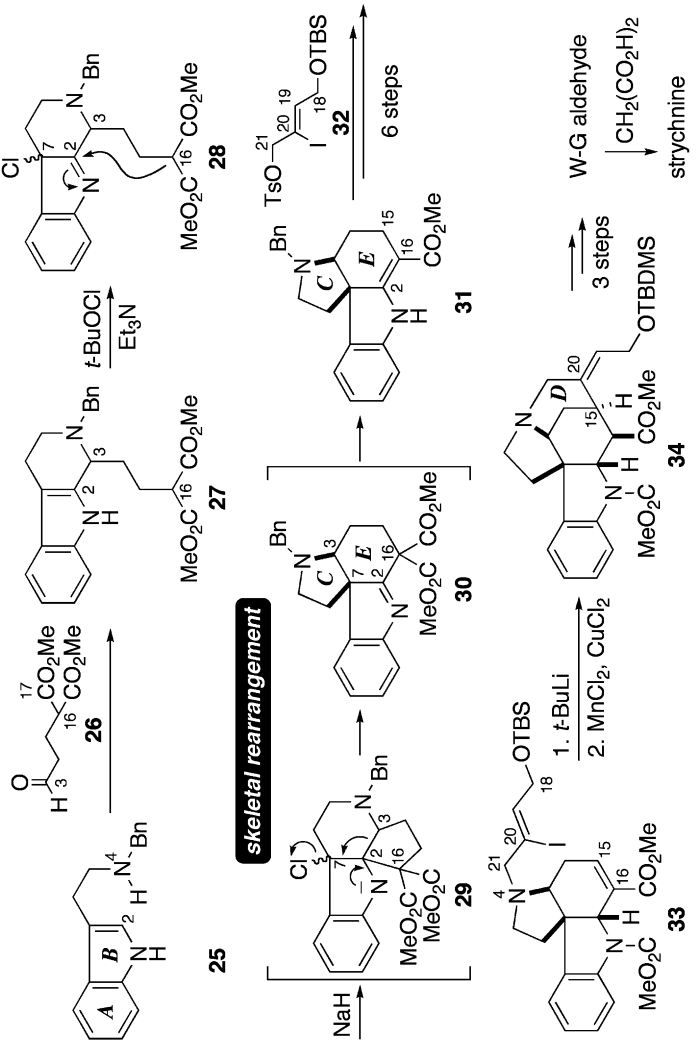
Scheme 3 Magnus' relay synthesis of (−)-strychnine.

the Pictet–Spengler reaction of tryptamine (**13**) with dimethyl 2-ketoglutarate (**14**) to yield lactam **15** directly (58–60). After conversion of this lactam to a tertiary amine, treatment with β,β,β-trichloroethyl chloroformate promoted N4–C16 bond cleavage to give a nine-membered cyclic compound **16** (61,62). Next, D ring formation was accomplished by the intramolecular conjugate addition of an α-sulfinyl amide to give the tetracyclic lactam **18** [AB → AB**D**]. For the construction of the CDE core ring system, Magnus employed the transannular oxidative cyclization, which was developed by Harley-Mason et al. for the synthesis of the pentacyclic *Strychnos* alkaloid tubifoline (56,57). Although dehydration of the tertiary amine functionality in **19** can give rise potentially to the three iminium ions (at C3, C5, and C21), treatment with mercuric acetate in acetic acid formed the iminium ion **20** selectively (C3:C21 = 17:1), yielding the desired pentacyclic compound **21** [ABD → AB**C**D**E**, C7 quaternary center]. The β-aminoacrylate **21** was then converted to the furanoside relay compound **22**, which is readily available from the degradation of W–G aldehyde. Optically pure **22** was transformed into **23** through a Horner–Wadsworth–Emmons reaction. While this process provided a mixture of geometrical isomers (*E*:*Z* = 3:2), the undesired *Z* isomer **23Z** could be converted to a mixture of **23E** and **23Z** by irradiation. The synthesis of W–G aldehyde was then achieved for the first time from the desired *E* isomer **23E** in seven steps [ABCDE → ABCDE**F**]. Finally, according to Robinson's procedure, W–G aldehyde was converted to strychnine (50).

D. Stork's Total Synthesis of Strychnine (1992)

[**AB** → AB**CE** → ABC**D**E → ABCDE**F**: W–G aldehyde → Strychnine] (23)

Stork's strychnine synthesis relies on the skeletal rearrangement of tetrahydro-β-carboline **27** to hexahydropyrrolo[2,3–*d*]carbazole **31** (63), which was previously developed by Massiot et al. (64) (Scheme 4). The key substrate **27** was prepared by the Pictet–Spengler reaction of *N*-benzyltryptamine (**25**) with aldehyde **26**. The tetrahydro-β-carboline **27** was first chlorinated at the C7 position by *t*-BuOCl (65–67) to give **28**, and then treatment of **28** with sodium hydride induced skeletal rearrangement (cleavage of the C2–C3 bond and formation of the C3–C7 bond), resulting in the direct formation of hexahydropyrrolo[2,3–*d*] carbazole **31** [AB → AB**CE**, C7 quaternary center] (64). Another key feature of this synthesis is the use of alkenyl iodide **32** as the source of the D and F rings, allowing *E* selective elaboration of the hydroxyethylidene substituent. Later, six groups employed this strategy in their strychnine syntheses (*vide infra*). Saturation of the C2–C16 double bond, generation of the C15–C16 double bond (68,69), and introduction of an *E* alkenyl iodide moiety (C18–C21) gave alkenyl iodide **33**. Treatment of **33** with *t*-BuLi promoted intramolecular conjugate addition, and the subsequent addition of $MnCl_2$ and $CuCl_2$ gave the pentacyclic compound **34** [ABCE → ABC**D**E]. Finally, the total synthesis of strychnine was accomplished in four steps via the W–G aldehyde (50).

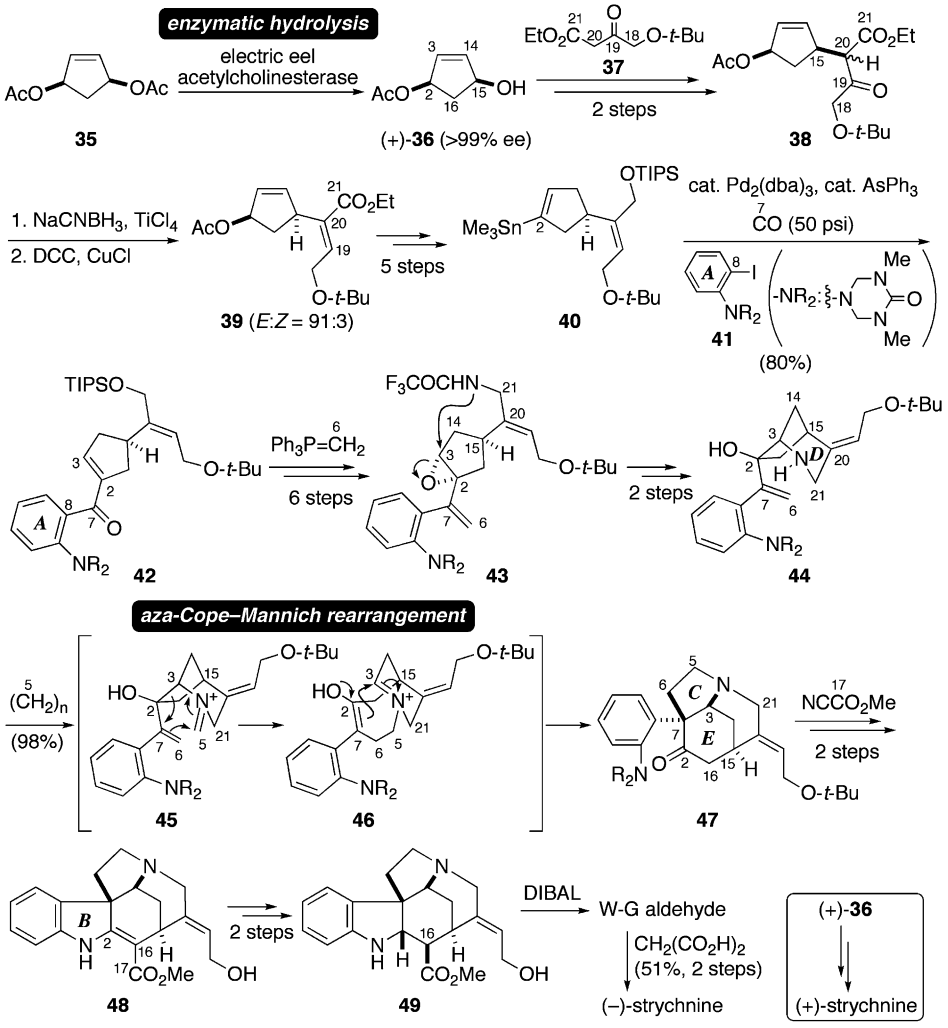


Scheme 4 Stork's total synthesis of strychnine.

E. Overman's Total Synthesis of (−)- and (+)-Strychnine (1993)

[**A***→A**D***→A**CDE***→A**B**CDE*→ABCDE**F**: W–G aldehyde*→(−)-Strychnine] (24,25)

In 1993, Overman achieved the first enantioselective total synthesis of (−)-strychnine using the optically pure monoacetate (+)-**36**, which was prepared by the enzymatic hydrolysis of **35** (70,71), as a starting material (Scheme 5). The key reaction in this synthesis is the cationic aza-Cope–Mannich rearrangement, which was previously developed by Overman et al. for the syntheses of various alkaloids, such as akuammicine (72–76), to assemble the CDE core ring system. The



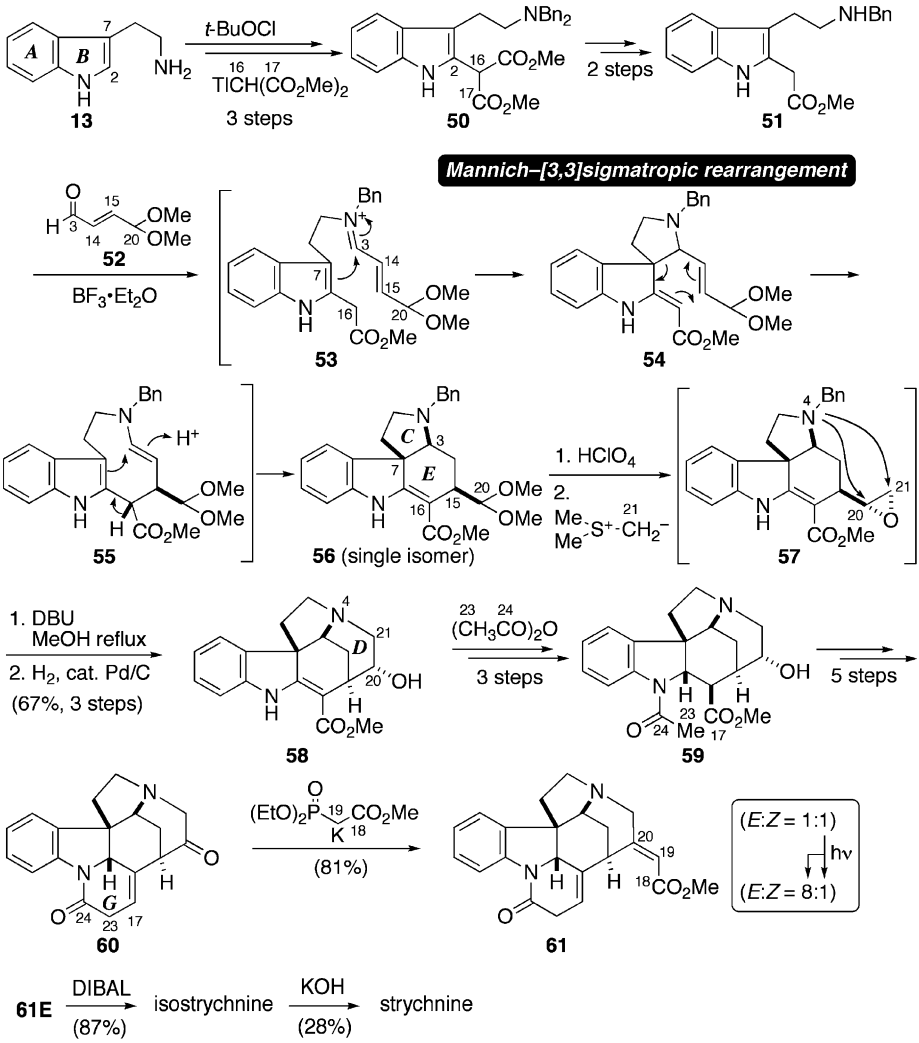
Scheme 5 Overman's total synthesis of (−)- and (+)-strychnine.

optically pure **36** was first converted to a β-keto ester **38** (77). *Anti*-selective reduction by the $NaCNBH_3$–$TiCl_4$ system (78) and *syn*-elimination by DCC–CuCl (79) gave the α,β-unsaturated ester **39** in a highly selective manner (*E*:*Z* = 91:3) (80). After conversion of the desired *E* isomer of **39** to the alkenyl stannane **40** in five steps, the A ring portion was introduced by a Pd-catalyzed carbonylative Stille coupling reaction (81,82). Stereoselective epoxidation of the C2–C3 double bond, Wittig methylenation of the ketone, and introduction of the N4 source at the C21 position afforded the trifluoroacetamide derivative **43**. Then, D ring formation was accomplished by heating with NaH to give the key intermediate **44** after removal of the trifluoroacetyl group [A* → A**D***] (83). The crucial domino reaction involving an aza-Cope rearrangement and a Mannich reaction was realized by heating **44** with excess paraformaldehyde to provide the azatricyclic ketone **47** in nearly quantitative yield [AD* → A**C**D**E***, C7 quaternary center] (72–76). Introduction of the C17 source at the C16 position (84) and acidic treatment gave (−)-18-hydroxyakuammicine (**48**) [ACDE* → A**B**CDE*]. Finally, the pentacyclic alkaloid **48** was transformed into (−)-strychnine via the W–G aldehyde (50), thereby achieving the first enantioselective total synthesis of (−)-strychnine. In addition, two years later, the total synthesis of unnatural (+)-strychnine was achieved from (+)-**36** using an analogous synthetic process (25).

F. Kuehne's Total Synthesis of Strychnine (1993)

[**AB** → AB**CE** → ABC**D**E → ABCDE**G** → Isostrychnine → Strychnine] (26)

A new domino condensation–electrocyclization reaction (85), which simultaneously constructs the C and D rings, is a crucial step in Kuehne's strychnine synthesis (Scheme 6). Starting from tryptamine (**13**), the C16–C17 unit was first introduced at the C2 position through chlorination by *t*-BuOCl (65–67), and nucleophilic addition of malonate to give the diester **50**. After conversion of **50** to **51** in two steps, Mannich-type condensation with aldehyde **52** was conducted with $BF_3 \cdot Et_2O$ to afford **54**. The spiro intermediate **54** simultaneously underwent a [3,3]sigmatropic rearrangement and an acid-catalyzed cyclization reaction to give the tetracyclic compound **56** as a single diastereomer [AB → AB**CE**, C7 quaternary center] (85). For the D ring formation, Kuehne employed an intramolecular epoxide opening reaction of **57**. Although the 5-exo epoxide opening reaction (formation of N4–C20 bond) was the kinetically favored process, refluxing in MeOH with DBU gave the thermodynamically more stable, six-membered (D ring) compound [ABCE → ABC**D**E]. After conversion of the key intermediate **58** to the *N*-acetyl compound **59**, heating with LiHMDS promoted G ring formation to give a β-keto lactam, which was then transformed into the β,γ-unsaturated lactam **60** [ABCDE → ABCDE**G**]. In a similar way as in Magnus' synthesis, the hydroxyethylidene side chain was introduced by a Horner–Wadsworth–Emmons reaction of ketone **60** to give a mixture of geometrical isomers (*E*:*Z* = 1:1). Finally, the *E* isomer **61E** was converted to strychnine via isostrychnine (49).

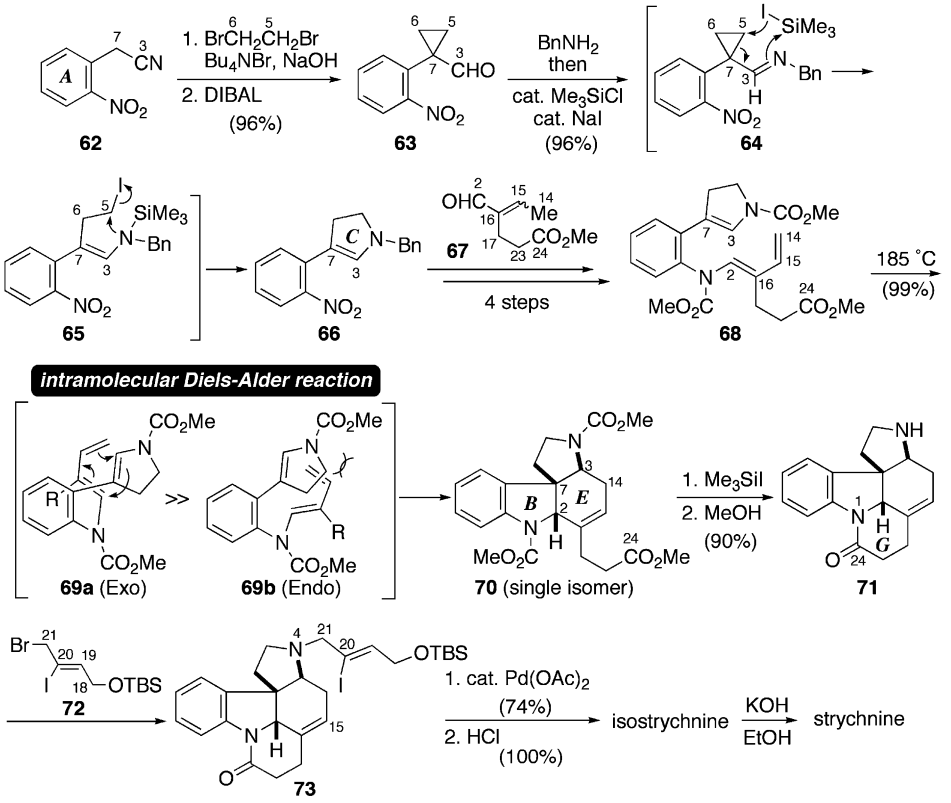


Scheme 6 Kuehne's total synthesis of strychnine.

G. Rawal's Total Synthesis of Strychnine (1994)

[**A** → A**C** → A**BCE** → ABCE**G** → ABC**D**EG → Isostrychnine → Strychnine] (28)

The key feature of Rawal's synthetic strategy is the use of an intramolecular Diels–Alder reaction for the construction of the ABCE ring system (Scheme 7), which was previously developed for the synthesis of 19,20-dehydrotubifoline (86). First, pyrroline **66** was synthesized from 2-nitrophenylacetonitrile (**62**) using the Stevens strategy (87,88), which involves cyclopropanation and cyclopropyl iminium ion rearrangement [A → A**C**]. Then, the diene moiety was assembled by



Scheme 7 Rawal's total synthesis of strychnine.

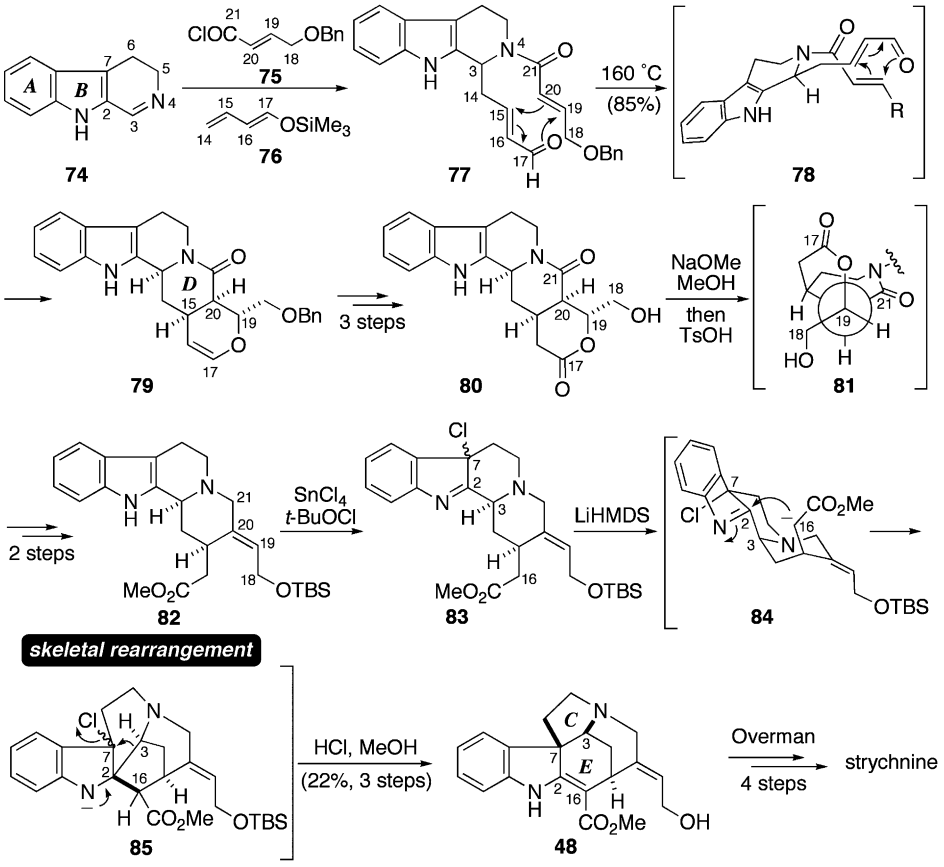
condensation with crotonaldehyde **67** to give diene **68** in four steps from **66**. Although both the diene and dienophile in **68** are electron rich, the desired Diels–Alder reaction (89,90) proceeded smoothly by heating (185–200°C), affording **70** as the sole product [AC→A**BC**E, C7 quaternary center]. After lactam (G ring) formation [ABCE→ABCE**G**] and *N*-allylation with allylic bromide **72**, D ring formation was accomplished by an intramolecular Heck reaction (91–95) of the resulting alkenyl iodide **73** [ABCEG→ABC**D**EG]. As compared with the anionic conjugate addition reported by Stork (~35%) (23), this process gave much higher yield (74%). Finally, removal of the *tert*-butyldimethylsilyl (TBS) group under acidic conditions afforded isostrychnine, which was then converted to strychnine using the conventional method (49).

H. Martin's Formal Synthesis of Strychnine (1996/2001)

[**AB**→AB**D**→AB**C**D**E**→ABCDE**F**: W–G aldehyde→Strychnine] (29,30)

In 1996, inspired by a transformation in the proposed biogenetic conversion of indole alkaloids (96–101), Martin developed a new biomimetic strategy to

Strychnos alkaloids, leading to the syntheses of akuammicine and a potential intermediate for strychnine synthesis (18-benzyloxyakuammicine) (29). Later, the formal synthesis of strychnine was realized by changing the 18-hydroxyl-protecting group from benzyl to TBS (Scheme 8) (30). Martin's synthesis features an intramolecular hetero Diels–Alder reaction (102,103), which was developed by his group for the syntheses of heteroyohimbinoid and corynantheoid alkaloids (104). Dihydro-β-carboline **74**, which was prepared from tryptamine (**13**) in two steps, was converted to the α,β-unsaturated aldehyde **77** by a vinylogous Mannich reaction with silyl dienol ether **76** (source of C14–C17 carbon atoms), in the presence of the acyl chloride **75** (source of C18–C21 carbon atoms). Heating of **77** induced the intramolecular hetero Diels–Alder reaction to give the pentacyclic adduct **79** via a transition state **78** [AB → AB**D**] (104). After the conversion of **79** to lactone **80**, basic treatment of **80** led to β-elimination to afford the *E*-configured, α,β-unsaturated amide, which was then transformed into the tetracyclic corynantheoid derivative **82**. The next key biomimetic skeletal rearrangement was



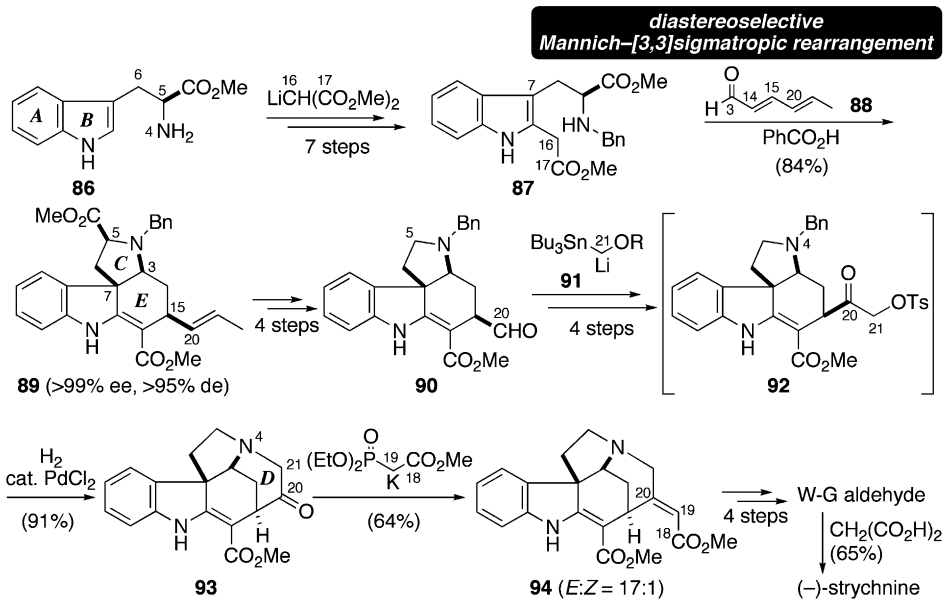
Scheme 8 Martin's formal synthesis of strychnine.

performed by following the Massiot strategy (64) (see also Stork's synthesis). Chlorination of **82** and basic treatment of the resulting chloroindolenine **83** gave simultaneous construction of the C and E rings (105) to give 18-hydroxyakuammicine (**48**) [ABD→AB**C**D**E**, C7 quaternary center] (24,25). Because Overman had previously converted **48** to strychnine in four steps (24,25), this process constitutes a formal synthesis of strychnine.

I. Kuehne's Total Synthesis of (−)-Strychnine (1998)

[**AB***→AB**CE***→ABC**D**E*→ABCDE**F**: W–G aldehyde*→(−)-Strychnine] (27)

After his racemic synthesis of strychnine (26), Kuehne also achieved an enantioselective synthesis of (−)-strychnine (Scheme 9). To avoid the low yield conversion of isostrychnine to strychnine, the second approach was directed to the W–G aldehyde. Starting from L-tryptophan methyl ester (**86**), the cyclization precursor **87** was prepared in seven steps in a similar way as in the previous racemic synthesis. The domino condensation–electrocyclization reaction of **87** with dienal **88** proceeded with quite high diastereoselectivity (>95% de) [AB*→AB**CE***, C7 quaternary center] (85). After conversion of the tetracyclic compound **89** to tosylate **92**, removal of the benzyl group resulted in the clean formation of the D ring [ABCE*→ABC**D**E*]. Unlike in the first synthesis, introduction of the hydroxyethylidene side chain by a Horner–Wadsworth–Emmons reaction of ketone **93** proceeded with high stereoselectivity (*E*:*Z* = 17:1). Finally, the *E* isomer **94E** was converted to (−)-strychnine via the W–G aldehyde (50).



Scheme 9 Kuehne's total synthesis of (−)-strychnine.

J. Bonjoch/Bosch's Total Synthesis of (−)-Strychnine (1999)

[**E**→**A**E→A**C**E*→AC**D**E*→A**B**CDE*→ABCDE**F**: W–G aldehyde*→(−)-Strychnine] (31,32)

In the 1990s, Bonjoch and Bosch developed a highly flexible strategy for the syntheses of various *Strychnos* alkaloids (106–109), such as (−)-tubifolidine, and these studies culminated in the enantioselective total synthesis of (−)-strychnine in 1999 (Scheme 10). Their strategy relied on a diastereoselective, double reductive amination of a prochiral product for the construction of the CE ring system of strychnine. First, the prochiral diketone **98** was prepared from 1,3-cyclohexadione **95** (source of the core E ring) in three steps including introduction of the A ring moiety and a Claisen rearrangement [E→**A**E, C7 quaternary center]. Ozonolysis of the terminal alkene in **98** gave the prochiral diketo aldehyde **99**, and treatment with (*S*)-1-phenylethylamine (96% ee) and $NaBH_3CN$ induced the double reductive amination, which involves (i) intermolecular reaction of a chiral amine with the aldehyde and (ii) diastereoselective intramolecular reaction of the resulting chiral diketo amine, to afford the octahydroindolone **100** (97:3 mixture of the *cis* diastereomer) [AE→A**C**E*] (110). For the closure of the D ring, a reductive Heck-type cyclization (91–95) was employed to give the key intermediate **102** [ACE*→AC**D**E*]. In a similar way as Overman's synthesis, the tricyclic ketone **102** was converted to (−)-strychnine in five steps (24,25).

III. RECENT SYNTHESES OF STRYCHNINE (2000–2006)

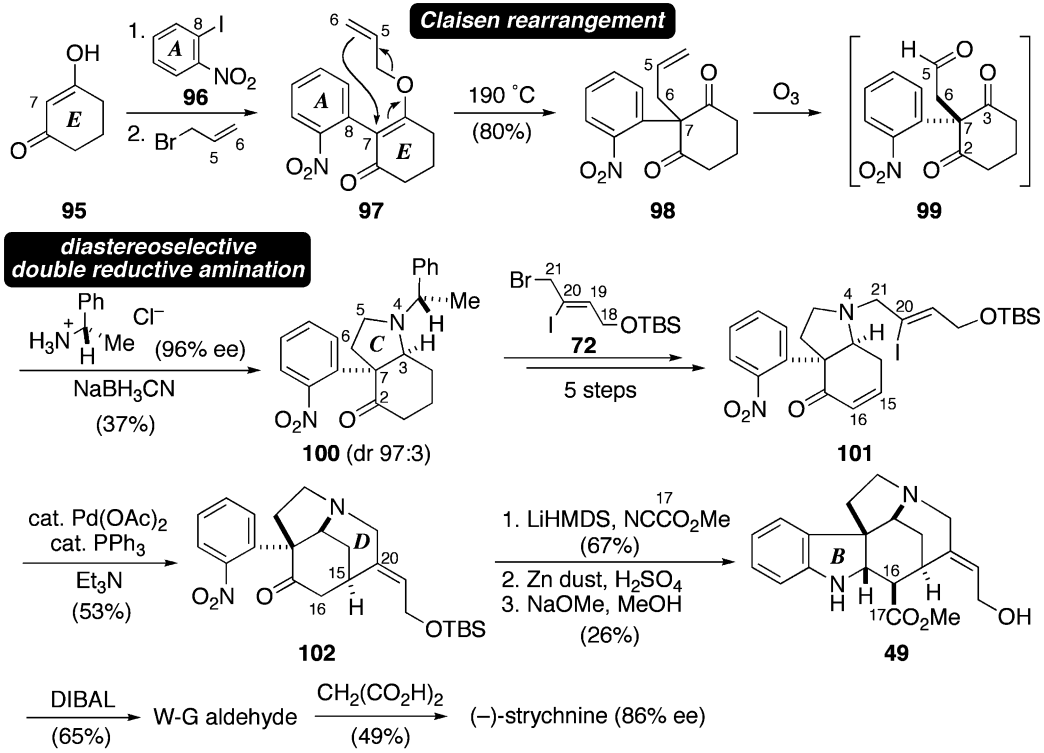
A. General Aspects

While nine syntheses, including those of both enantiomers, were achieved by the end of 1999, strychnine remains a popular target for demonstrating new reactions and novel synthetic strategies. In fact, recent total syntheses published between 2000 and 2006 also feature elegant applications of one or more key reactions that bring about a substantial increase in molecular complexity (*vide infra*).

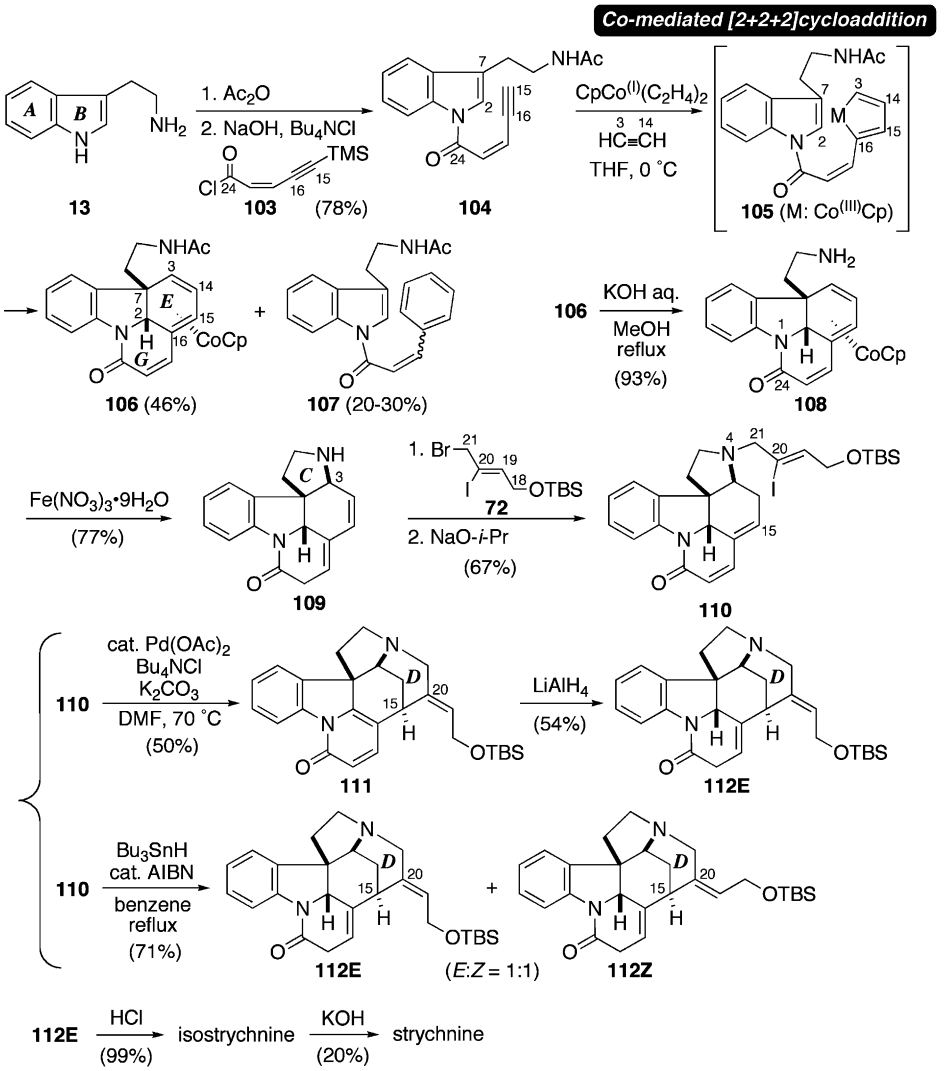
B. Vollhardt's Total Synthesis of Strychnine (2000)

[**AB**→AB**EG**→AB**C**EG→ABC**D**EG→Isostrychnine→Strychnine] (33,34)

Vollhardt's total synthesis of strychnine successfully demonstrated the power of the cobalt-mediated [2+2+2]cycloaddition (111) for the construction of complex polycyclic molecules (Scheme 11). This key reaction was originally developed by his group in the 1970s, and widely utilized for the syntheses of complex natural and unnatural products (112–116). Here, this reaction was used for the simultaneous closure of the E and G rings with formation of the C7 quaternary center (117–119). To accomplish total synthesis, five synthetic approaches were investigated, but only the successful one is shown here.



Scheme 10 Bonjoch/Bosch's total synthesis of (−)-strychnine.



Scheme 11 Vollhardt's total synthesis of strychnine.

Tryptamine (**13**) was first converted to enynoylindole **104** by the coupling with enynoyl chloride **103**, which was prepared from propiolic acid in five steps in 42% overall yield. The key [2+2+2]cycloaddition using **104** proceeded to give the tetracyclic Co complex **106** in 46% yield as a single diastereomer [AB→AB**EG**, C7 quaternary center] (117–119). This reaction may proceed as follows: (i) two ethylene ligands of low valent $CpCo^{(I)}(C_2H_4)_2$ are displaced by the terminal alkyne unit in **104** and acetylene; (ii) oxidative coupling gives $Co^{(III)}$ metallacyclopentadiene **105**; and (iii) either an alkyne-insertion or a Diels–Alder type cyclization (89,90) furnishes the stable 18e $Co^{(I)}$ complex **106**. This reaction also formed the undesired

by-product **107** by reaction of the terminal alkyne unit in **104** and two molecules of acetylene. In the next step of hydrolysis of the acetamide function in **106** under rather harsh conditions, the electron-donating and bulky CpCo moiety stabilizes the amide bond in the C ring, preventing hydrolysis of the N1–C24 bond. Oxidative demetalation of **108** with $Fe^{(III)}$ (117) led to a formal [1,8]-conjugate addition of the amine to the unsaturated lactam π-system, affording pentacyclic compound **109** in 77% yield [ABEG → AB**C**EG]. Alkylation of the N4 nitrogen with the allyl bromide **72** (28), followed by base-catalyzed isomerization of the diene unit into conjugation with the amide carbonyl, afforded vinyl iodide **110**.

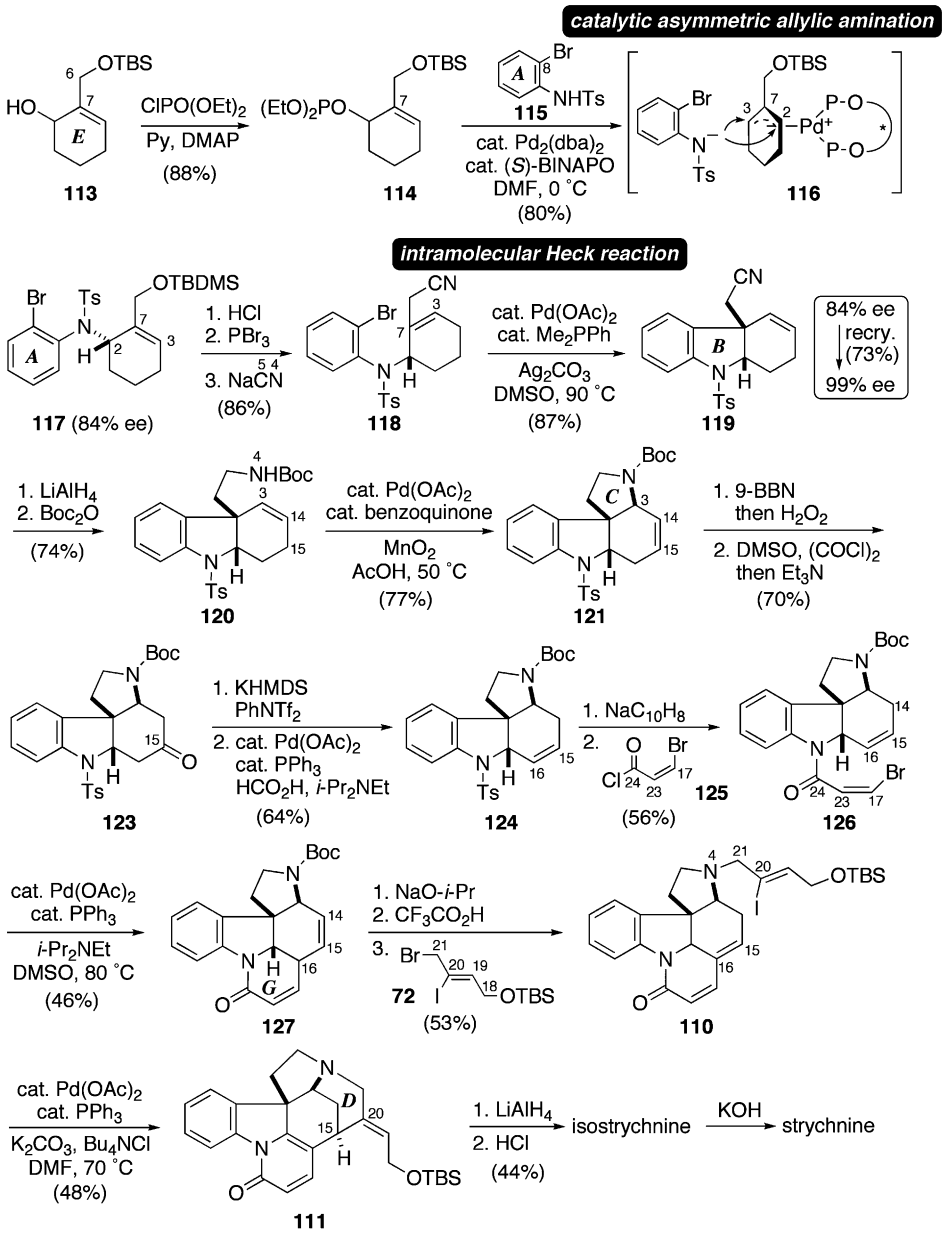
For the formation of the D ring, (i) metal–iodide exchange/conjugate addition (see Stork's approach), (ii) Pd-mediated Heck/anion capture sequences (see Rawal's and Bonjoch/Bosch's approaches), and (iii) radical-mediated closure were examined. After intensive examination, the first strategy proved to be fruitless. For example, Stork's conditions (23) gave poor yield (35%) and the reaction was difficult to reproduce. Next, a Heck-type reaction (91–95) was investigated. Intramolecular Heck reaction of **110** under Rawal's conditions (28) afforded the aromatic pyridone **111** in 50% yield through β-elimination of the C2 hydrogen [ABCEG → ABC**D**EG]. In an effort to suppress the β-elimination to obtain the more advanced compound **112E** directly, a reductive Heck reaction was examined. All of the attempts were, however, unsatisfactory. Alternatively, silylated isostrychnine **112E** was prepared in 54% yield by reduction of the Heck product **111** with $LiAlH_4$. Finally, the most successful result was obtained using the radical cyclization strategy. Reaction of **110** with Bu_3SnH and AIBN generated the hexacyclic product **112** in 71% yield. Although these conditions gave a 1:1 mixture of geometrical isomers, the two isomers were separated without difficulty by column chromatography. Thus, after the separation, the desired **112E** was converted to strychnine in two steps as in Rawal's synthesis (28). Thus, a short synthesis of strychnine (10 steps from tryptamine, 14 steps from propiolic acid) was realized (28).

C. Mori's Total Synthesis of (−)-Strychnine (2001)

[**E** → **A**E* → A**B**E* → AB**C**E* → ABCE**G*** → ABC**D**EG → Isostrychnine* → (−)-Strychnine] (35,36)

Pd-catalyzed reactions have been used quite often in the syntheses of natural products (120). Indeed, in Mori's total synthesis of (−)-strychnine, all cyclizations for the synthesis of (+)-isostrychnine were performed using Pd-catalyzed reactions, including the first enantioselective allylic substitution (121–124) (Scheme 12). The strategy used for this synthesis was previously developed by this group for the synthesis of (−)-tubifoline (124).

The substrate **114** for the key allylic substitution was prepared from allylic alcohol **113**, which acts as a source of the E ring. By treatment of **114** with $Pd^{(0)}$ in the presence of the nucleophile derived from **115**, the π-allylpalladium complex **116** would be formed. Because the Pd catalyst has a chiral ligand, the nucleophile should attack preferentially at either the C2 or C3 position, affording allyl amine **117** or *ent*-**117**. When (*S*)-BINAPO (125) was used as the chiral ligand, **117** was obtained in 84% ee [E → **A**E*]. After introduction of the C5–N4 unit by



Scheme 12 Mori's total synthesis of (−)-strychnine.

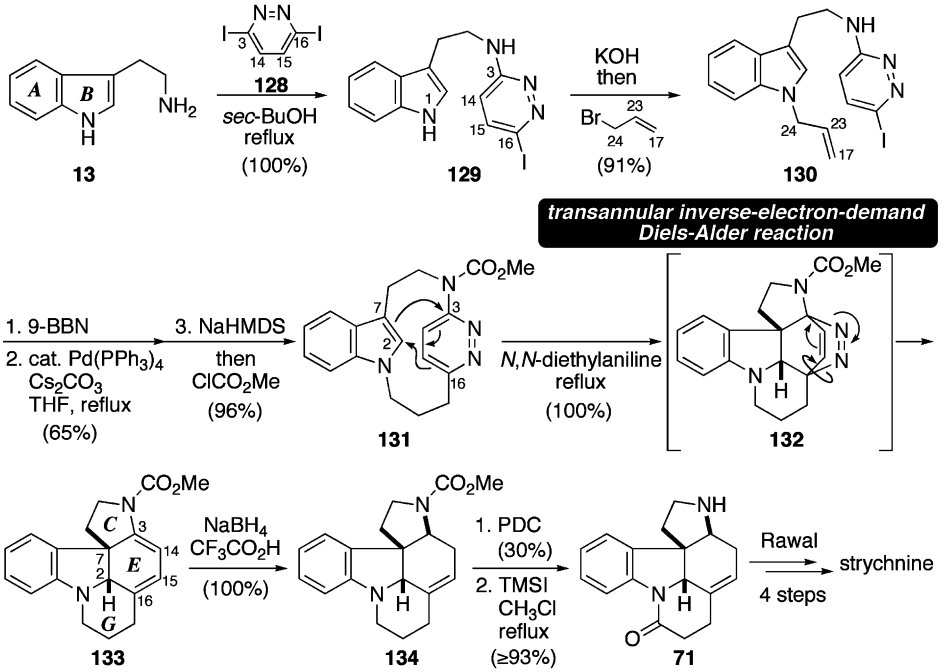
nucleophilic attack of cyanide to the corresponding allyl bromide, B ring formation was accomplished by intramolecular Heck reaction (91–95) of **118** to give indoline **119** in 87% yield [AE*→A**B**E*, C7 quaternary center]. At this stage, the enantiomeric excess was increased from 84 to 99% by recrystallization. Treatment

of the optically pure **119** with $LiAlH_4$ followed by protection of the resulting amine gave **120**. Then, Pd-catalyzed allylic oxidation (126) of **120** gave tetracyclic compound **121** in 77% yield [ABE* → AB**C**E*]. This cyclization may proceed through the nucleophilic attack of the N4 nitrogen on the $Pd^{(II)}$-coordinated C3–C14 double bond and β-elimination of the C15 hydrogen. Regioselective hydroboration of **121** with 9-BBN, followed by treatment with H_2O_2 and NaOH, gave an alcohol, which was oxidized to ketone **123**. The ketone functionality was converted to the C15–C16 double bond by regioselective formation of enol triflate (127,128) and Pd-catalyzed reduction (129). Then, the Ts group of the resulting compound **124** was removed by sodium naphthalenide and treatment with the acyl chloride **125** provided the monoalkylated compound **126**. The second intramolecular Heck reaction in this synthesis was conducted using **126** as a substrate to give pentacyclic compound **127** in 46% yield [ABCE* → ABCE**G***]. Finally, isomerization of the C14–C15 double bond to a C15–C16 double bond, removal of the Boc group, and N4 alkylation (28) provided the optically pure variant of Vollhardt's intermediate **110** (33,34). Thus, using the procedure of Vollhardt, the alkenyl iodide **110** was converted to (−)-strychnine in four steps (33,34).

D. Bodwell's Formal Synthesis of Strychnine (2002)

[**AB** → AB**CEG** → ABC**D**EG → Isostrychnine → Strychnine] (37)

In 2002, Bodwell reported the shortest synthesis of strychnine to date. As the premier method for the construction of functionalized and stereochemically complex, six-membered ring systems, the Diels–Alder reaction is the foundation of some of the most important achievements in chemical synthesis (89,90). For the synthesis of strychnine, Rawal already utilized an intramolecular Diels–Alder reaction, which led to E ring formation at the C7–C2 and C3–C14 bonds (Scheme 7). Vollhardt's E ring formation at the C2–C16 and C7–C3 bonds can also be categorized as Diels–Alder cyclization (Scheme 11). The feature of Bodwell's approach is the use of the small cyclophane (130–134), which did not have a high profile in the synthesis of natural products either as targets or as key intermediates, as a substrate of the key transannular inverse electron demand Diels–Alder reaction (IEDDA) (135) (Scheme 13). The indole–pyridazine pairing is particularly attractive because it should provide direct access to the carbazole skeleton, which is present in a wide array of indole alkaloids (136). However, attempts to perform intramolecular indole–pyridazine IEDDA reactions, in which a less activated pyridazine moiety was tethered to an indole system, were reported unsuccessful due to the lower reactivity of the pyridazine moiety (137). Thus, Bodwell assumed that the ''doubly tethered'' arrangement of diene and dienophile in cyclophane **131** would facilitate their reaction because the two aromatic systems of **131** can be held closely in a specific orientation with respect to one another (138,139). In fact, Bodwell previously succeeded in the transannular IEDDA of a doubly trimethylene-tethered cyclophane, affording a carbazole skeleton-containing pentacyclic compound (135).



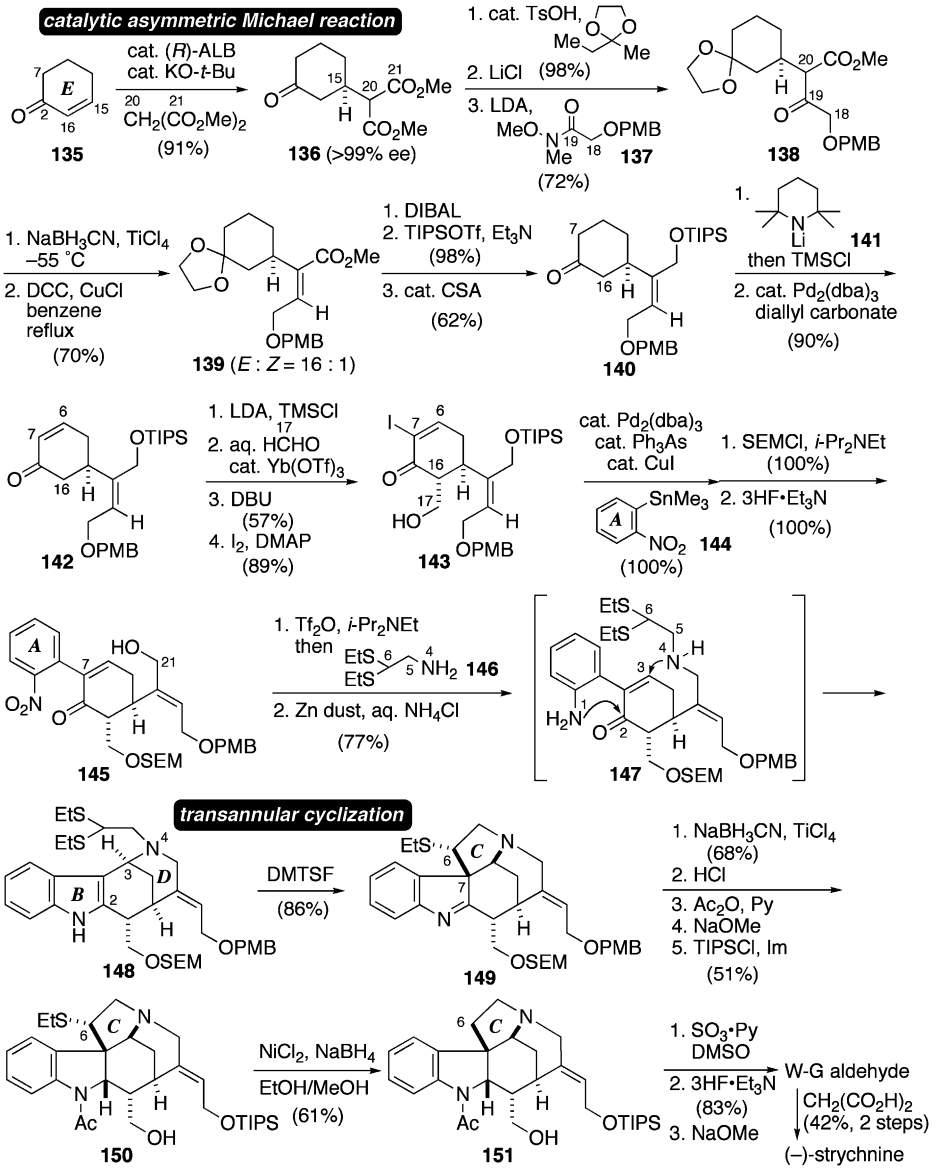
Scheme 13 Bodwell's formal synthesis of strychnine.

Bodwell's strychnine synthesis commenced with the reaction of tryptamine (**13**) with 3,6-diiodopyridazine (**128**) (140) to afford iodide **129**. After N1 allylation, the allyl compound **130** was subjected to a sequential hydroboration–intramolecular Suzuki–Miyaura coupling (141) to give the cyclophane. Protection of the secondary amine of the resulting cyclophane as a methyl carbamate resulted in the key substrate **131**. Heating the cyclophane **131** in *N*,*N*-dimethylaniline induced the transannular IEDDA to afford **132** and, following expulsion of N_2 from **132**, produced pentacyclic product **133** in quantitative yield [AB → AB**CEG**, C7 quaternary center] (135). Then, **133** was converted to Rawal's key intermediate **71** (28) by chemo- and stereoselective reduction of **133** with $NaBH_4/CF_3COOH$, oxidation of the tertiary amine to amide with PDC (142), and removal of the carbamate protecting group. Because strychnine was synthesized from **71** in four steps by Rawal (28), Bodwell's short formal synthesis (12 steps from tryptamine) was accomplished.

E. Shibasaki's Total Synthesis of (−)-Strychnine (2002)

[**E** → E* → **A**E* → A**BD**E* → AB**C**DE* → ABCDE**F**: W–G aldehyde* → (−)-Strychnine] (38,39)

In 2002, we achieved the total synthesis of (−)-strychnine using a catalytic asymmetric Michael reaction and a novel domino cyclization that simultaneously



Scheme 14 Shibasaki's total synthesis of (−)-strychnine.

constructed the B and D rings of strychnine (Scheme 14). Previously, we had developed the highly practical, catalytic asymmetric Michael reaction (143–151) based on the multi-functional catalyst concept (152–154). Only 0.1 mol % of (*R*)-ALB complex, which was prepared from $LiAlH_4$ and (*R*)-BINOL in a ratio of 1:2, completed the Michael reaction of dimethyl malonate with cyclohexenone (**135**)

to afford the enantiomerically pure Michael product **136** on a greater than kilogram scale (91% yield, >99% ee) (149). Using this Michael product as a starting material, we succeeded in the total synthesis of the *Strychnos* alkaloids (−)-tubifolidine and (−)-19,20-dihydroakuammicine (155,156). For the synthesis of strychnine, however, a different strategy was adopted as follows: (i) the C17 unit was introduced prior to B ring formation in consideration of F and G ring formation, and (ii) domino cyclization was employed to assemble the ABED ring system more efficiently. Although most of the other strategies for strychnine synthesis generated the C6–C7 bond in the early stage of synthesis, in order to utilize the Michael product **136** more efficiently in the synthesis, the C ring at the C6–C7 bond was assembled in the last stage.

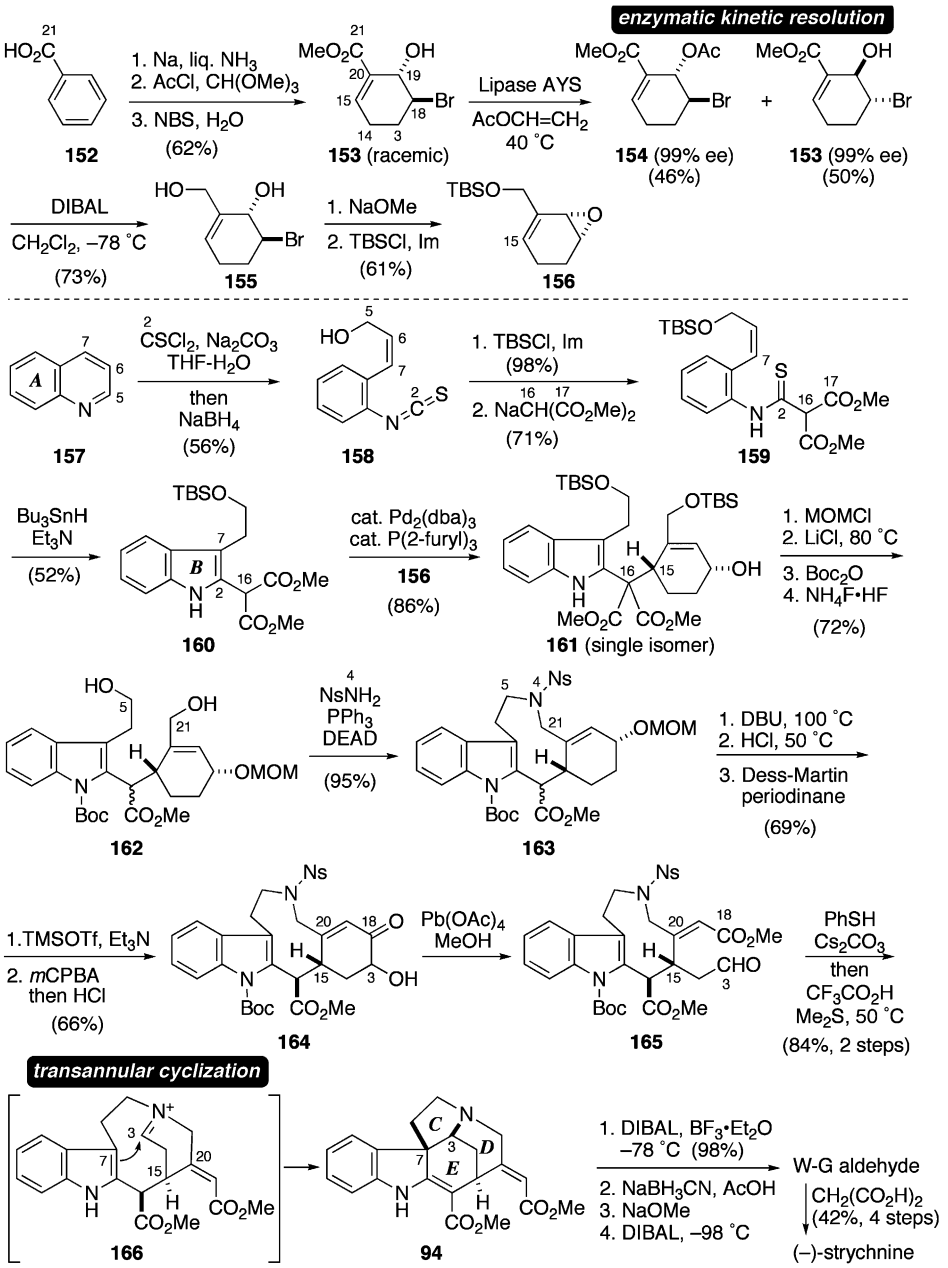
Starting from the enantiomerically pure product **136**, introduction of the additional two side chains to the core E ring was first conducted. The malonate moiety was converted to a β-keto ester through decarboxylation. By using modified Overman's conditions (24,25), the hydroxyethylidene substituent was constructed to give **139** in a highly *E*-selective manner (*E*:*Z* = 16:1). After conversion of **139** to the triisopropylsilyl (TIPS) ether **140**, regioselective silyl enol ether formation was facilitated by the action of bulky lithium amide **141** (C7:C16 = >6:1), and following a catalytic Saegusa–Ito reaction (157,158) provided enone **142**. Next, hydroxymethylation by the aldol reaction of an enol silyl ether in aqueous formamide (C16α:C16β = 3.5:1) (159,160), epimerization of the C16 stereocenter to the thermodynamically more stable α-isomer, and α-iodination of the enone functionality gave alkenyl iodide **143**. A Stille coupling reaction (81,82) of **143** with 4-nitrophenylstannane **144** proceeded smoothly [E*→**A**E*], and then protection of the primary alcohol as a 2-(trimethylsilyl)-ethoxymethyl (SEM) ether and removal of the TIPS group provided the key intermediate **145**. After introduction of the amine moiety (N4–C6) at the C21 position, treatment of the resulting compound with Zn dust provided the tetracyclic compound **148** in 77% yield from **145** [AE*→A**BD**E*]. This domino cyclization may include the following three reactions: (i) reduction of the nitro group to an amine by Zn dust; (ii) indole formation (N1–C2 bond formation); and (iii) 1,4-addition of the N4 amine (N4–C3 bond formation). The C ring formation by connecting the C6–C7 bond was conducted using the intramolecular electrophilic attack of a thionium ion, which was previously utilized for the synthesis of structurally simpler indole alkaloids by Bonjoch/Bosch's group (106,161,162) and our group (155,156), to give pentacyclic compound **149** in 86% yield [ABDE*→AB**C**DE*, C7 quaternary center]. Then, **149** was converted to **150** through reduction of the imine moiety using the $NaBH_3CN$–$TiCl_4$ system (78). Although desulfurization of **150** using conventional methods often induced undesired reactions, such as migration of the exocyclic olefin to an endocyclic olefin and over-reduction, the reaction using Ni borite (163) in an EtOH–MeOH mixed solvent system afforded the desired compound **151** with high chemoselectivity (>10:1). Finally, **151** was transformed into (−)-strychnine in four steps via W–G aldehyde (50).

F. Fukuyama's Total Synthesis of (−)-Strychnine (2004)

[**A** → A**B** → AB* → AB**CDE*** → ABCDE**F**: W–G aldehyde* → (−)-Strychnine] (40)

Fukuyama's total synthesis demonstrated the uniqueness of 2-nitrobenzenesulfonamide ($NsNH_2$) chemistry (164,165) in constructing a medium-sized cyclic amine. Indeed, this chemistry was successfully applied for the construction of the CDE core ring system (Scheme 15). Different from Magnus' oxidative strategy (Scheme 3), only the polycyclic iminium ion intermediate **166** was selectively obtained by the removal of the Ns group in **165** under very mild conditions. Another feature of this synthesis is the use of a cyclohexene unit as parts of the C, D, E, and F rings. In the last stage of the synthesis, cleavage of the cyclohexene ring at the C3–C18 bond in **164** allowed the formation of the α,β-unsaturated ester **165** with the *E*-configuration.

For the introduction of the desired cyclohexene unit, optically pure vinyl epoxide **156** was synthesized from benzoic acid (**152**). First, **152** was converted to the racemic bromohydrin **153** in three steps. Then, enzymatic kinetic resolution of the racemic **153** with lipase AYS provided the desired chiral bromohydrin acetate **154** (46% yield, 99% ee) along with the unreacted enantiomer (50% yield, 99% ee). After the reduction of **154** with DIBAL, treatment of the resulting product with base formed the epoxide. Subsequent protection of the primary alcohol with TBSCl gave the vinyl epoxide **156**. The indole unit was synthesized by a radical cyclization developed by Fukuyama (166,167). The requisite substrate **159** for the radical cyclization was prepared from quinoline (**157**) via isothiocyanate **158**. Treatment of **159** with Bu_3SnH and Et_3B at room temperature resulted in the formation of the 2,3-disubstituted indole **160** in 52% yield [A → AB]. Next, the cyclohexene unit **156** and indole unit **160** were coupled by a Pd-mediated reaction (168) to give **161** in 86% yield as a single isomer, in which the choice of catalyst and ligand was crucial. The coupling product **161** was then converted to diol **162** in four steps. Treatment of the diol **162** with $NsNH_2$ under Mitsunobu conditions (169) promoted a ring-closing, double *N*-alkylation to provide the desired nine-membered Ns-amide **163** in 95% yield (170,171). At this stage, the epimeric mixture at C16 was first equilibrated to the thermodynamically more stable β-ester. Then, removal of the methoxymethyl (MOM) group, followed by Dess–Martin oxidation (172) of the resulting alcohol, gave a ketone, which was converted to the α-hydroxyketone **164** through *m*-CPBA oxidation of the silyl enol ether moiety (173). Oxidative cleavage of the α-hydroxyketone **164** was performed by treatment with $Pb(OAc)_4$ to furnish aldehyde **165** bearing an α,β-unsaturated ester with the desired geometry. After removal of the Ns group from **165** with PhSH and Cs_2CO_3 (164,165), treatment with TFA and Me_2S induced a smooth transannular cyclization to give the Kuehne intermediate **94** (27) in 84% yield from **164** [AB* → AB**CDE***, C7 quaternary center]. Finally, by following Kuehne's procedure, **94** was converted to (−)-strychnine in five steps (27).

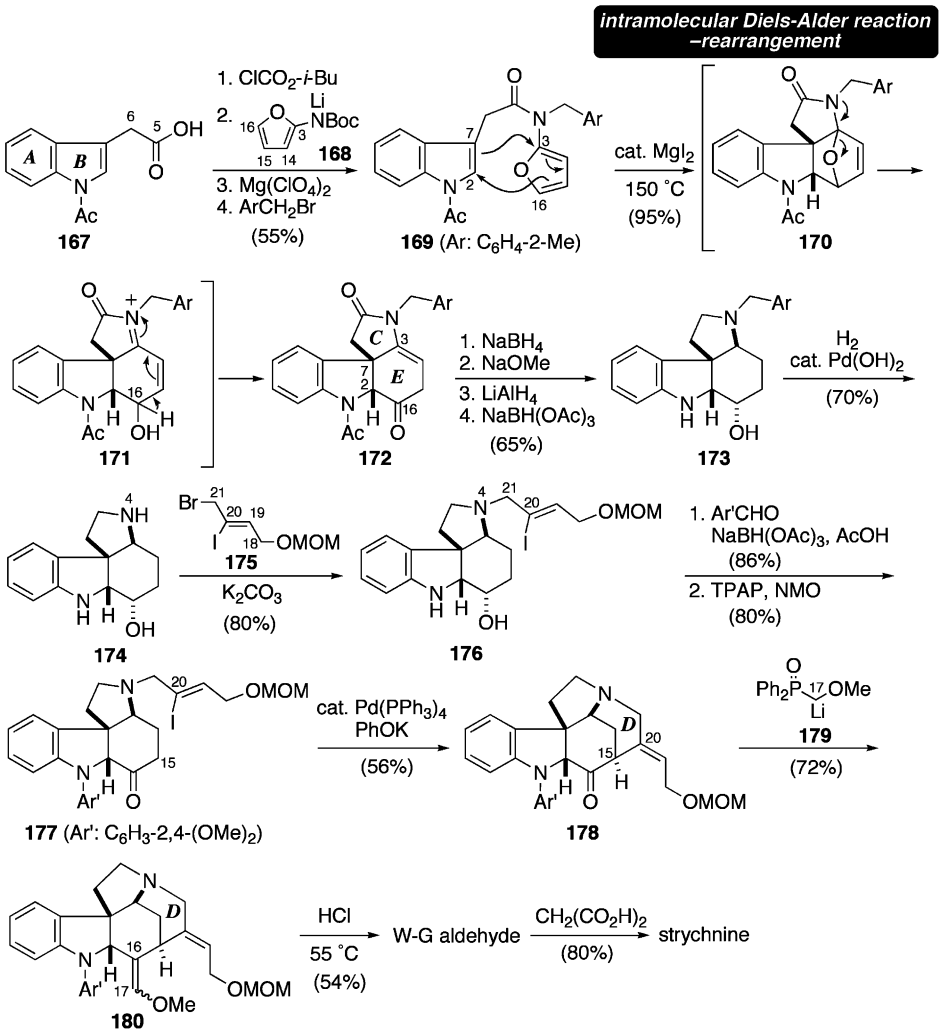


Scheme 15 Fukuyama's total synthesis of (−)-strychnine.

G. Padwa's Total Synthesis of Strychnine (2007)

[**AB** → AB**C**E → ABC**D**E → ABCDE**F**: W–G aldehyde → Strychnine] (41)

Very recently, Padwa reported a total synthesis of strychnine based on an intramolecular Diels–Alder reaction/rearrangement cascade, which was previously developed in his group to assemble the tetracyclic core ring system of indole alkaloids (Scheme 16) (174–178). Intramolecular cycloaddition reactions often benefit from higher reactivity and greater control of stereoselectivity relative to their intermolecular counterparts. Unlike Bodwell's IEDDA reaction (Scheme 13), the reaction of an electron-rich furan moiety with an *N*-acyl indole moiety required only a single tether. His synthesis also involved an



Scheme 16 Padwa's total synthesis of strychnine.

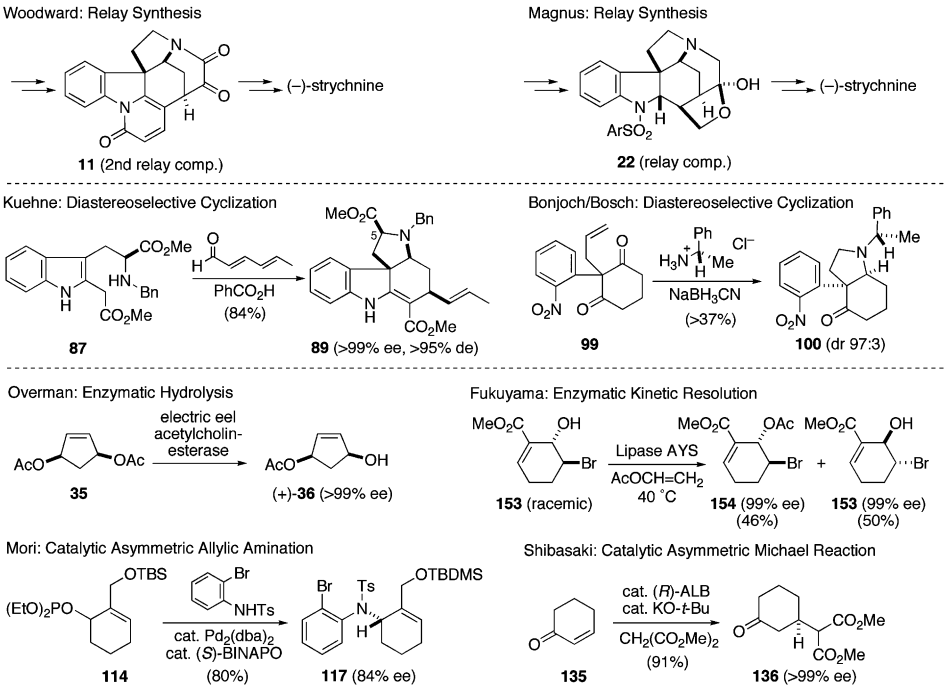
intramolecular, Pd-catalyzed, enolate-driven, cross-coupling reaction for the critical D ring formation.

The key mono-tethered substrate **169** (174) was prepared by acylation of **167** with **168**, followed by removal of the Boc group and subsequent *N*-alkylation with 1-bromomethyl-2-methyl benzene. The large 2-methylbenzyl group on the N4 amido nitrogen atom was expected to cause the reactive *s-trans* rotamer to be more highly populated and help promote the intramolecular Diels–Alder reaction (89,90). Indeed, heating the indolyl-substituted amidofuran **169** at 150°C in a microwave reactor for 30 min in the presence of catalytic amount of MgI_2 afforded the desired tetracyclic compound **172** in 95% yield [AB→AB**C**E, C7 quaternary center]. This domino sequence may proceed through: (i) nitrogen-assisted ring-opening of the initially formed cycloadduct **170** to produce the *N*-acyliminium ion **171**; (ii) subsequent deprotonation of the C16 hydrogen; and (iii) ketonization of the resulting enol. Next, **172** was converted to **174** through three types of reduction and removal of the acetyl and 2-methylbenzyl groups. Alkylation of the N4 amine nitrogen with alkenyl bromide **175**, protection of the N1 nitrogen atom as a dimethoxybenzylamine, and oxidation of the secondary alcohol using tetrapropylammonium perruthenate (TPAP) (179) afforded ketone **177**. Different from the previous strychnine syntheses that utilized a similar alkenyl iodide substrate (Schemes 4, 7, 10, 11, 12 and 13), Padwa employed an intramolecular, Pd-catalyzed, enolate-driven, cross-coupling reaction for the critical D ring formation (180–184). The coupling reaction of **177** with $Pd(PPh_3)_4$ and PhOK proceeded smoothly to furnish tetracyclic compound **178** in 56% yield [ABCE→ABC**D**E]. Although conversion of the keto group in **178** to the corresponding enol ether using phosphorane $MeOCH=PPh_3$ was unsuccessful, use of the sterically less demanding, and more nucleophilic, phosphine oxide reagent **179** provided enol ether **180**. Finally, acidic treatment of **180** provided W–G aldehyde, which was then converted to strychnine using the established procedure with the highest chemical yield (80%) (50).

IV. CONCLUDING REMARKS

The chemistry described herein should be highly useful for the preparation of indole alkaloids and for the synthesis of a variety of complex natural products. Although unsuccessful approaches are not described in this article, they also provide quite useful information. Before closing this article, we would like to examine the 15 total syntheses with emphases on: (i) the way to access optically active compounds (Scheme 17) and (ii) the polycyclization strategy (Scheme 18).

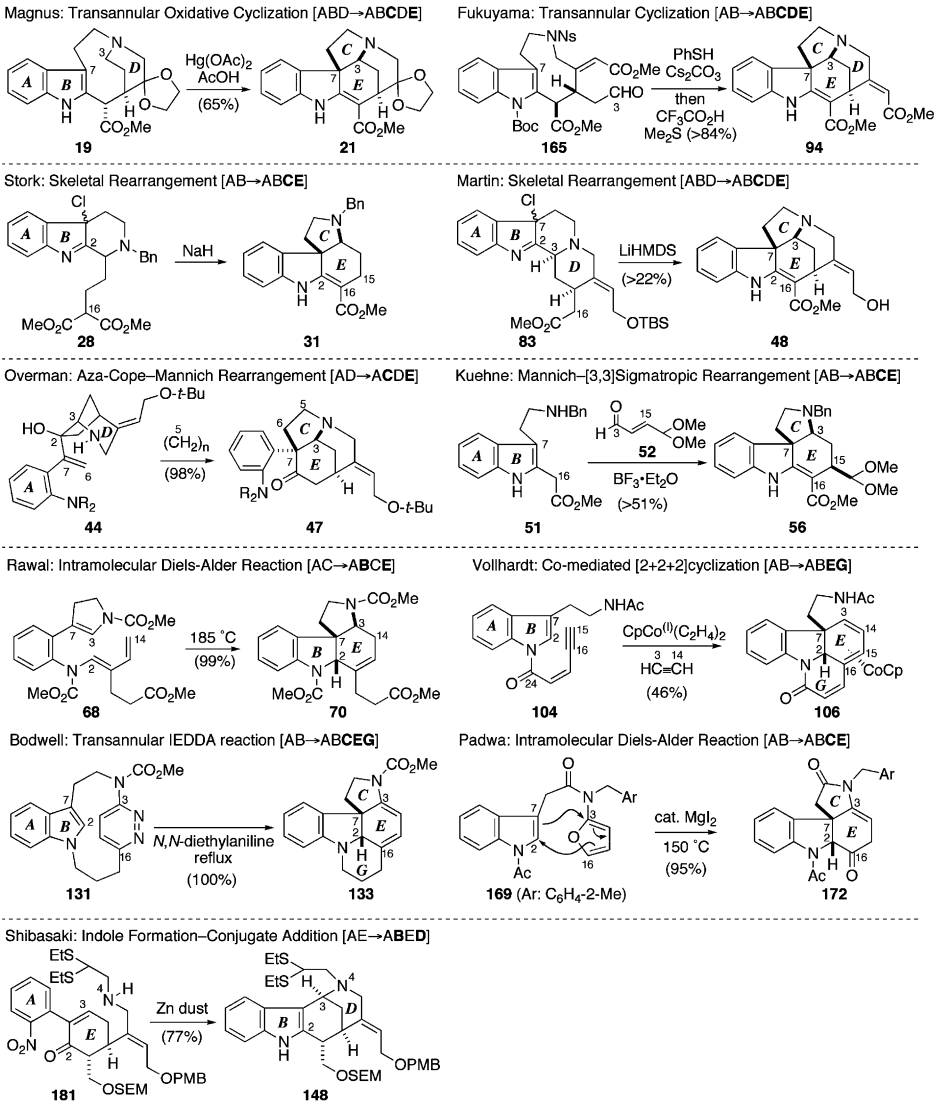
Molecular chirality is a principal element in nature that plays a key role in science and technology (185–187). Biological systems, in most cases, recognize enantiomers as different substances, and therefore two enantiomers will elicit different biological responses. The importance and practicality of asymmetric synthesis as a tool to obtain enantiomerically pure or enriched compounds is fully acknowledged in synthetic organic chemistry, medicinal chemistry, agricultural chemistry, natural products chemistry, the pharmaceutical and agricultural industries, etc. Among the fifteen syntheses of strychnine described above,



Scheme 17 Summary of enantioselective syntheses of (−)-strychnine.

two relay syntheses and six enantioselective syntheses culminated in the synthesis of the natural enantiomer (−)-strychnine (Scheme 17). Today, there are a variety of methods, but until the early 1970s, the classical resolution of racemates was the primary method used to obtain optically active compounds. Other methods involve transformation or derivatization of readily available natural chiral compounds. In the first synthesis by Woodward and the second synthesis by Magnus, relay compounds **11** and **22**, which were obtained from (−)-strychnine, were used for the final transformation into (−)-strychnine, respectively. Kuehne succeeded in the total synthesis of (−)-strychnine from optically pure L-tryptophan methyl ester, which is the so-called chiral pool method. Although the C5 stereocenter originating from the starting chiral source was eventually removed from an intermediate, the highly diastereoselective intramolecular chirality transfer reaction (**87** to **89**) permits the asymmetric synthesis. Bonjoch/Bosch's enantioselective synthesis also utilized an intramolecular chirality transfer strategy, in which two new stereocenters were constructed by the diastereoselective reductive, double amination reaction (**99** to **100**).

As compared with stoichiometric asymmetric syntheses, the use of catalytic asymmetric reactions for the syntheses of chiral compounds is a more desirable method in terms of atom economy. In principle, the chemical approach, which uses a small amount of a chiral catalyst, can produce optically active chiral materials in large quantities. In the early days, however, practical access to enantiomerically



Scheme 18 The polycyclization strategy in the syntheses of strychnine.

pure compounds from prochiral precursors in a catalytic manner was considered possible only by using biochemical or biological methods. In fact, the first enantioselective synthesis by Overman and recent enantioselective synthesis by Fukuyama employed enzymatic desymmetrization and enzymatic kinetic resolution, respectively. Such biological methods are powerful and environmentally benign processes. Recent tremendous progress in asymmetric catalysis has made it possible to accomplish enantioselective synthesis of (−)-strychnine using chiral artificial catalysts. Mori utilized the asymmetric allylic amination catalyzed by

the Pd–(*S*)-BINAPO complex. In our enantioselective synthesis, the asymmetric Michael reaction catalyzed by the (*R*)-ALB complex was used as the first step of the synthesis. In this manner, the recent growth of the area of enantioselective transformations with chemical catalysts and enzymes has greatly enhanced the overall potential of organic synthesis. Nowadays, asymmetric synthesis of single enantiomers is becoming a common practice in laboratories. It is hoped that efforts will be made to develop highly practical asymmetric catalysis in terms of substrate generality, catalyst efficiency, enantioselectivity, chemical yield, and reliability, even when applied to a large-scale process.

In terms of compatibility with our environment, the preservation of our resources, and the economical advantage, the efficiency of organic synthesis is becoming increasingly important. A general way to improve synthetic efficiency is the development of new methods that promote two or more bond formations in one process, involving a so-called domino reaction (188). Such methodology allows the formation of complex compounds starting from simple substrates in very few steps. Although many types of one-pot, multi-bond formations were utilized for strychnine synthesis, only polycyclization processes are summarized in Scheme 18. In most cases, the C and E rings were simultaneously constructed by a one-pot operation with the generation of the C7 spirocenter. Two of them culminated in simultaneous, three-ring formation (Bodwell and Fukuyama syntheses). The polycyclization reactions utilized for strychnine synthesis are classified according to the reaction type. Magnus and Fukuyama employed the transannular cyclization of similar iminium ion intermediates, leading to two-ring (C, E) and three-ring (C, D, E) formation, respectively. In Stork's and Martin's syntheses, Harley-Mason's skeletal rearrangement strategy was used to close the C and E rings. Domino cyclizations developed by Overman and Kuehne involve both [3,3]sigmatropic rearrangement and the Mannich reaction. Because intramolecular Diels–Alder type cycloaddition is one of the most powerful methods for the construction of functionalized six-membered ring systems, this type of reaction was also utilized for strychnine synthesis by Rawal, Bodwell, and Padwa, including Vollhardt's [2+2+2]cyclization. Our domino cyclization was rather different from others, in which the reduction of a nitro group acts as a trigger for B and D ring formation. In any event, one-pot, polycyclization processes made it possible to omit a number of steps in the synthesis. It is obvious that such one-pot, multi-bond formations are highly elegant and reduce the amount of waste produced in a synthesis, and conserve our resources. The total synthesis of complex natural products is a very powerful tool for supplying highly potent compounds and for developing highly practical and very useful asymmetric catalysis. We believe that the chemistry discussed herein will facilitate the development of the field of total synthesis of a wide variety of natural products.

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syntheses of *Strychnos* alkaloids described in this article, which were developed by our group. We also thank all of our co-workers, whose names are acknowledged on the publications from our laboratory cited here.

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CHAPTER 4

Alkyl, Aryl, Alkylarylquinoline, and Related Alkaloids

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Contents

I. INTRODUCTION

The distribution, chemistry, biogenesis, and taxonomic significance of quinoline alkaloids have been reviewed since 1953. Annual coverage has been provided by Openshaw (1953–1967), Snieckus (1972–1975), Grundon (1976–1990), Michael (1991–2005), in *The Alkaloids: Chemistry and Pharmacology* (R. H. F. Manske and

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H. L. Holmes, eds.), in *The Alkaloids*, published by The Chemical Society, London) and subsequently in *Natural Product Reports* (1–38). In this review, some of the latest developments in the group of natural products collectively known as the alkylquinolin/one alkaloids are presented. Emphasis is placed on their biogenesis, their biological activities, and their natural distribution.

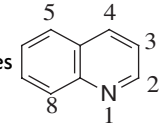
In the context of this review, the term alkylquinolin/one covers alkaloids of natural origin in which an amino acid, alkyl, or carboxyl group is attached through a C–C bond to an aromatic or a heterocyclic ring system. The review does not include 3-alkylquinolin-2(1*H*)-ones, which represent stages between the simple quinoline and the furo- or pyrano quinolin/one derivatives. Aryl- and alkylarylquinolin/ones are treated together here because they share common features in their biosynthesis. Tables I–XI list the different types of alkaloids, grouped as indicated in Figure 1 by their respective biogenetic origin, together with their sources (1–202). Pre-1953 research on alkylquinolin/ones was previously presented in other earlier reviews (1–40), and these are relevant for a discussion of the chemosystematics of these alkaloids. In order to bring up-to-date the distribution of these alkaloids, a literature search was carried out in *Chemical Abstracts* (to December, 2006). The biosynthesis of the alkaloids and their systematic value are discussed. In recent years, a number of alkylquinolin/ones have been found to possess interesting biological and pharmacological activities, and these are also discussed in the following sections.

II. BIOSYNTHESIS AND BIOGENESIS

Many 2-alkylquinoline/4(1*H*)-one alkaloids bear the trivial names pseudans because of their occurrence in bacteria of the genus *Pseudomonas*. For example, *P. aeruginosa* is a ubiquitous Gram-negative bacterial pathogen that causes infections in human hosts, in animals, and even in plants. This pathogen has been identified as one of the leading causes of nosocomial infections, and is responsible for fatal chronic lung infections in patients with cystic fibrosis (CF). *P. aeruginosa* coordinates its population behavior, such as biofilm formation and virulence factor production, by means of small extracellular signal molecules, so-called autoinducers, that are released into the environment under appropriate conditions. Since intercellular communication leads to cooperative and coordinated bacterial behavior in a cell density-dependent manner, it is referred to as quorum sensing.

A common feature of intercellular communication is the transcriptional activation of quorum-sensing-controlled genes when the bacterial signal molecules reach a certain threshold. *P. aeruginosa* produces two major cell-to-cell signals that are members of the *N*-acyl-homoserine lactones and 4-hydroxy-2-alkylquinolines. The latter include, in addition to *N*-oxides, 3,4-dihydroxy-2-heptylquinoline, and 4-hydroxy-2-heptylquinoline. The latter two molecules have been shown to be involved in intracellular communication.

Analysis of the underlying metabolic events of intercellular bacterial communication, and the elucidation of the biosynthesis of the signal molecules, might contribute to an understanding of, and provide an opportunity to

Table I Occurrence of quinolines and alkylquinolines in Rutaceae species 

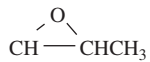
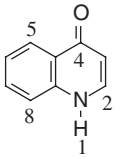
Alkaloid	Code	Substituents				Properties	Occurrence (plant part), (references)
		1	**2**	**3**	**4**		
Quinoline	A.1					^{13}C NMR (41)	*Angostura trifoliata*[a] (Willd.) T.S. Elias (bark) (1) *Citrus aurantium* L. (39) *Galipea officinalis*[a] J. Hancock (39)
3-Hydroxyquinoline (3-quinolol)	A.1			OH		C_9H_7NO ^{1}H NMR, MS (42)	*Ruta montana* Mill. (aerial parts) (42)
Quinaldine	B.1		Me				*Angostura trifoliata*[a] (Willd.) T.S. Elias (bark) (1) *Galipea officinalis*[a] J. Hancock (40)
2-*n*-Propylquinoline	B.1		*n*-C_3H_7			$C_{12}H_{13}N$ Oil, IR, ^{1}H, ^{13}C NMR, EIMS (43)	*Galipea bracteata*[a] (stems) (43) *Galipea longiflora*[a] K. Krause (stem, root bark, leaves) (44,45)
Chimanine B	B.1		(*E*) CH=CHCH$_3$			UV, EIMS, ^{1}H, ^{13}C NMR (44)	*Galipea longiflora*[a] K. Krause (stem, root bark, leaves) (44,45)
Chimanine D	B.1					UV, EIMS, ^{1}H, ^{13}C NMR (44)	*Galipea longiflora*[a] K. Krause (stem, root bark, leaves) (44,45)
2-*n*-Pentyl-quinoline (2-*n*-amylquinoline)	B.1		C_5H_{11}			$C_{14}H_{17}N$, b.p: 130-145°C/10 mm Oil, IR, ^{1}H, ^{13}C NMR, EIMS (43) GC-MS (46)	*Angostura trifoliata*[a] (Willd.) T.S. Elias (bark) (1) *Galipea bracteata*[a] (Nees & Mart.) Shult. (stems) (43) *Galipea longiflora*[a] K. Krause (stem, root bark, leaves) (44,45) *Galipea officinalis*[a] J. Hancock (trunk bark) (46)
2-(Pent-1-enyl)-quinoline	B.1		CH=CHC$_3$H$_7$			$C_{14}H_{15}N$ Oil, IR, ^{1}H, ^{13}C NMR, EIMS (43)	*Galipea bracteata*[a] (Nees & Mart.) Shult. (stems) (43)

Table I (*Continued*)

Alkaloid	Code	Substituents				Properties	Occurrence (plant part), (references)
		1	2	3	4		
4-Methoxyquinaldine	B		Me		OMe	NMR (47)	*Galipea officinalis*[a] J. Hancock (bark) (47) *Ruta montana* Mill. (aerial parts) (42)
Chimanine A	B		C_3H_7		OMe	^1H, ^{13}C NMR (44)	*Galipea longiflora*[a] K. Krause (stem, root bark, leaves) (44,45)
Chimanine C	B		(*E*) CH=CHCH$_3$		OMe	UV, EIMS, ^1H, ^{13}C NMR (44)	*Galipea longiflora*[a] K. Krause (stem, root bark, leaves) (44)
4-Methoxy-2-*n*-pentylquinoline (quinoleine)	B		C_5H_{11}		OMe	$C_{15}H_{19}NO$, b.p: 190–200°C/14 mm ^1H, ^{13}C NMR, MS (1,24,44)	*Angostura trifoliata*[a] (Willd.) T.S. Elias (bark) (1) *Galipea longiflora*[a] K. Krause (stem bark, stem, root bark, leaves) (24,44,45) *Zanthoxylum avicennae* (Lam.) DC. (49) *Galipea officinalis*[a] J. Hancock (bark) (50)
4-Methoxy-2-*n*-heptylquinoline	B		*n*-C_7H_{15}		OMe		*Zanthoxylum avicennae* (Lam.) DC. (49)
4-methoxy-2-(pent-1-enyl)quinoline	B		CH=CHC$_3$H$_7$		OMe	$C_{15}H_{17}NO$, Amorph., UV, ^1H NMR, MS (24)	*Galipea longiflora*[a] K. Krause (stem bark) (24)
4-Methoxy-2-(1-ethylpropyl)quinoline	B		CH$_3$CH$_2$CHCH$_2$CH$_3$		OMe	UV, IR, ^1H, ^{13}C NMR (51)	*Esenbeckia leiocarpa* Engl. (51)
4-Methoxy-2-(8-oxononyl)quinoline	B		(CH$_2$)$_7$COCH$_3$		OMe	$C_{19}H_{25}NO_2$, UV, IR, ^1H, ^{13}C NMR, HMBC, HR-MS (64)	*Ruta montana* Mill. (64)
1-Methyl-2-propyl-1,2,3,4-tetrahydroquinoline	B.1.1	Me	H, *n*-C_3H_7	H,H	H,H	GC–MS (46)	*Galipea officinalis*[a] J. Hancock (trunk bark) (46)
2-*n*-Pentyl-1,2,3,4-tetrahydroquinoline	B.1.1	H	H, *n*-C_5H_{11}	H,H	H,H	GC–MS (46)	*Galipea officinalis*[a] J. Hancock (trunk bark) (46)
(−)-Angustureine	B.1.1	Me	H, *n*-C_5H_{11}	H,H	H,H	$C_{15}H_{23}N$, Oil, $[\alpha]_D$ –7.12°, UV, EI-MS, ^1H, ^{13}C NMR, GC-MS (46,53)	*Galipea officinalis*[a] J. Hancock (bark, trunk bark) (46,53)

[a] *Angostura trifoliata* (syn. *Galipea officinalis* J. Hancock, see item III); *A. longiflora* (syn. *G. longiflora* K. Krause); *A. bracteata* (syn. *G. bracteata*) (268).

Table II Occurrence of alkylquinolin-4(1*H*)-ones in Rutaceae species 

Alkaloid	Code	Substituents						Properties	Occurrence (plant part) (references)
		1	**2**	**3**	**6**	**7**	**8**		
1,2-Dimethylquinolin-4(1*H*)-one	Bo	Me	Me					$C_{11}H_{11}NO$, m.p.: 178–179°C, UV, IR (48)	*Platydesma campanulata* H. Mann (root, leaves, stem bark) (48) *Acronychia baueri* Schott. (39) *Ruta montana* Mill. (49)
1-Acetoxymethyl-2-methylquinolin-4(1*H*)-one	Bo	CH_2OAc	Me					$C_{13}H_{13}NO_3$, m.p.: 160–164°C, UV, IR ^1H, ^{13}C NMR, EIMS (50)	*Boronia lanceolada* (aerial parts) (50) *Boronia bowmanii* (51)
Leptomerine	Bo	Me	*n*-C_3H_7					$C_{13}H_{15}NO$, m.p.: 147–148°C, NMR	*Haplophyllum leptomerum* Lincz. & Vved. (aerial parts) (52)
1-Acetoxymethyl-2-*n*-propylquinolin-4(1*H*)-one	Bo	CH_2OAc	*n*-C_3H_7					$C_{15}H_{17}NO_3$, m.p: 112°C, IR, UV, NMR	*Boronia ternata* Endl. (leaves) (53)
Schinifoline[a]	Bo	Me	*n*-C_7H_{15}					$C_{17}H_{23}NO$, IR, UV, ^1H, ^{13}C NMR, COSY, ^1H–^{13}C COSY, NOESY (54)	*Zanthoxylum schinifolium* Sielbold & Zucc. (54,55)
1-Methyl-2-*n*-nonylquinolin-4(1*H*)-one	Bo	Me	*n*-C_9H_{19}					UV, IR, ^1H NMR, MS (11)	*Ruta graveolens* (aerial parts) (11,56)
								$C_{19}H_{27}NO$, mip.: 73–75°C, EIMS, ^1H NMR, RP-HPLC-MS (56)	*Haplophyllum acutifolium* (D.C.) G. Don fil. (57)
								m.p.: 71–73°C, HMBC, HSQC (63)	*Haplophyllum tuberculatum* (Forrsk.) A.L. Juss. (aerial parts) (58) *Boronia bowmanii* (51) *Euodia ruticarpa* (Juss.) Benth. (fruit) (59,60,61)
								m.p.: 69–70°C, $[\alpha]_D$ –52° ($CHCl_3$), UV, IR, ^1H, ^{13}C NMR, ESI-MS (61)	*Euodia sutchuenensis* (62) *Raulinoa echinata* R.S. Cowan (leaves) (63)

Table II (*Continued*)

Alkaloid	Code	Substituents						Properties	Occurrence (plant part) (references)
		1	2	3	6	7	8		
1-Acetoxymethyl-2-*n*-nonylquinolin-4(1*H*)-one	Bo	CH_2Oac	*n*-C_9H_{19}					$C_{21}H_{29}NO_3$ m.p.: 79°C, UV, IR, ^1H, ^{13}C NMR, EIMS	*Boronia bowmanii* (aerial parts) (51)
1-Methyl-2-(8-oxononyl)quinolin-4(1*H*)-one	Bo	Me	$(CH_2)_7COCH_3$					$C_{19}H_{25}NO_2$, UV, IR, ^1H, ^{13}C NMR, HMBC, HR-MS	*Ruta montana* Mill. (64)
1-Methyl-2-*n*-decylquinolin-4(1*H*)-one	Bo	Me	*n*-$C_{10}H_{21}$					EIMS, ^1H NMR, RP-HPLC-MS (56)	*Ruta graveolens* L. (aerial parts) (56) *Euodia ruticarpa* (Juss.) Benth. (60)
1-Methyl-2-(9-oxodecyl)quinolin-4(1*H*)-one	Bo	Me	$(CH_2)_8COCH_3$					$C_{20}H_{27}NO_2$. UV, IR, ^1H, ^{13}C NMR, HMBC, HR-MS	*Ruta montana* Mill. (64)
1-Methyl-2-*n*-undecylquinolin-4(1*H*)-one	Bo	Me	*n*-$C_{11}H_{23}$					$C_{21}H_{31}NO$, m.p.: 68.5–70°C. MS, IR (65); EIMS, ^1H NMR, RP-HPLC-MS (56) m.p.: 69–70°C IR, UV, MS, ^1H, ^{13}C NMR (66)	*Euodia ruticarpa* (Juss.) Benth. (leaves) (59,60,61,65,66) *Ruta graveolens* L. (aerial parts) (56)
1-Methyl-2-*n*-dodecylquinolin-4(1*H*)-one	Bo	Me	*n*-$C_{12}H_{25}$					m.p.: 75–76°C, UV, IR, EIMS, ^1H, ^{13}C NMR	*Euodia ruticarpa* (Juss.) Benth. (fruit) (59)
Dihydroevocarpine	Bo	Me	*n*-$C_{13}H_{27}$					$C_{23}H_{35}NO$, m.p.: 74–75°C, MS, UV, IR, ^1H, ^{13}C NMR (59,65,66)	*Euodia ruiecarpa* (Juss.) Benth. (fruits) (59,60,61,65,66) *Euodia officinalis* Dode (fruits) (67)
1-Methyl-2-*n*-tetradecylquinolin-4(1*H*)-one	Bo	Me	*n*-$C_{14}H_{29}$					$C_{20}H_{27}NO_2$, white powder, UV, IR, ^1H, ^{13}C NMR, HMBC, HR-FABMS	*Euodia ruticarpa* (Juss.) Benth. (fruits) (68)
1-Methyl-2-*n*-pentadecylquinolin-4(1*H*)-one	Bo	Me	*n*-$C_{15}H_{31}$					$C_{25}H_{39}NO$, m.p.: 80°C, MS, ^1H NMR, IR, UV (65) m.p.: 65–66°C, IR, UV, MS,^1H, ^{13}C NMR (66)	*Euodia ruticarpa* (Juss.) Benth. (leaves, fruits) (59,61,65,66) *Boronia álgida* F. Muell. (69)
1-Methyl-2-[(*Z*)-undec-5-enyl]quinolin-4(1*H*)-one	Bo	Me	$(CH_2)_4CH{=}CH(CH_2)_4CH_3$					UV, IR, EIMS, ^1H, ^{13}C NMR	*Euodia ruticarpa* (Juss.) Benth. (fruit) (59)

1-Methyl-2-[(*Z*)-undec-6-enyl]quinolin-4(1*H*)-one	Bo	Me	$(CH_2)_5CH{=}CH(CH_2)_3CH_3$	UV, IR, EIMS, ^1H, ^{13}C NMR (59)	*Euodia ruticarpa* (Juss.) Benth. (fruit) (59,60,66) *Euodia officinalis* Dode (fruits) (67)
1-Methyl-2-[(*Z*)-tridec-7-enyl]quinolin-4(1*H*)-one	Bo	Me	$(CH_2)_6CH{=}CH(CH_2)_4CH_3$	UV, IR, EIMS, ^1H, ^{13}C NMR	*Euodia ruticarpa* (Juss.) Benth. (fruit) (59)
1-Methyl-2-[(4*Z*)-tridec-4-enyl]quinolin-4(1*H*)-one	Bo	Me	$(CH_2)_3CH{=}CH(CH_2)_7CH_3$		*Euodia ruticarpa* (Juss.) Benth (60)
Evocarpine	Bo	Me	$(CH_2)_7CH{=}CH(CH_2)_3CH_3$	$C_{23}H_{33}NO$, m.p.: 34–38°C. UV, IR, ^1H NMR, MS (59,70)	*Euodia ruticarpa* (Juss.) Benth. (fruit) (59,60,61,66,70) *Euodia officinalis* Dode (fruits) (67)
1-Methyl-2-[(*Z*)-pentadec-10-enyl]quinolin-4(1*H*)-one	Bo	Me	$(CH_2)_9CH{=}CH(CH_2)_3CH_3$	$C_{25}H_{37}NO$, Oil, UV, IR, EIMS, ^1H, ^{13}C NMR (59)	*Euodia ruticarpa* (Juss.) Benth. (fruit) (59,60,66)
1-Methyl-2-[(*Z*)-pentadec-9-enyl]quinolin-4(1*H*)-one	Bo	Me	$(CH_2)_8CH{=}CH(CH_2)_4CH_3$	UV, IR, EIMS, ^1H, ^{13}C NMR	*Euodia ruticarpa* (Juss.) Benth. (fruit) (59)
1-Methyl-2-[(*Z*)-pentadec-6-enyl]quinolin-4(1*H*)-one	Bo	Me	$(CH_2)_5CH{=}CH(CH_2)_7CH_3$	$C_{25}H_{37}NO$, Oil, UV, IR, ^1H, ^{13}C NMR, MS (66)	*Euodia ruticarpa* (Juss.) Benth. (fruit) (60,66)
1-Methyl-2-[(4*Z*,7*Z*)-tridec-4,7-dienyl]quinolin-4(1*H*)-one	Bo	Me	$(CH_2)_3CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$	$C_{23}H_{31}NO$, Oil, UV, IR, MS, ^1H, ^{13}C NMR (66)	*Euodia ruitcarpa* (Juss.) Benth. (fruits) (60,61,66)
1-Methyl-2-[(6*Z*,9*Z*)-pentadec-6,9-dienyl]quinolin-4(1*H*)-one	Bo	Me	$(CH_2)_5CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$	$C_{25}H_{35}NO$, Oil, UV, IR, MS, ^1H, ^{13}C NMR (66)	*Euodia ruticarpa* (Juss.) Benth. (fruits) (60,61,66)
Leiokinine B [1-Methyl-2-(1-ethylpropyl)quinolin-4(1*H*)-one]	Bo	Me	$CH_3CH_2CHCH_2CH_3$	UV, IR, ^1H, ^{13}C NMR, APT (82) NMR, NOE, syntheses (79)	*Esenbeckia leiocarpa* Engl. (roots) (82)
2-*n*-Propylquinolin-4(1*H*)-one	Bo		*n*-C_3H_7		*Boronia ternata* Endl. (39)
2-*n*-Heptylquinolin-4(1*H*)-one	Bo		*n*-C_7H_{15}	UV, IR, EIMS, ^1H NMR, HPLC-MS	*Ruta graveolens* L. (aerial parts) (56)
2-*n*-Octylquinolin-4(1*H*)-one	Bo		*n*-C_8H_{17}	UV, IR, EIMS, ^1H, ^{13}C NMR, HPLC-MS	*Ruta graveolens* L. (aerial parts) (56)
2-(8-Oxononyl)quinolin-4(1*H*)-one	Bo		$(CH_2)_7COCH_3$	$C_{18}H_{23}NO_2$, UV, IR ^1H, ^{13}C NMR, HMBC, HR-MS	*Ruta montana* Mill. (64)
2-*n*-Nonylquinolin-4(1*H*)-one	Bo		*n*-C_9H_{19}	UV, IR, EIMS, ^1H, ^{13}C NMR, HPLC-MS (56)	*Boronia bowmanii* (51) *Ruta graveolens* L. aerial parts) (56)
				m.p.: 74–76°C, HMBC, HSQC. (63). m.p.: 138–139°C, $[\alpha]_D$ –156° (MeOH), UV, IR, ^1H, ^{13}C NMR, ESI-MS (61)	*Raulinoa echinata* R.S. Cowan (leaves) (63) *Euodia sutchuenensis* (62) *Euodia ruticarpa* (Juss.) Benth. (fruits) (61)

Table II (*Continued*)

Alkaloid	Code	Substituents						Properties	Occurrence (plant part) (references)
		1	**2**	**3**	**6**	**7**	**8**		
2-*n*-Decylquinolin-4(1*H*)-one	Bo		*n*-$C_{10}H_{21}$					UV, IR, EIMS, ^{1}H, ^{13}C NMR, HPLC-MS	*Ruta graveolens* L. (aerial parts) (56)
2-*n*-Undecylquinolin-4(1*H*)-one	Bo		*n*-$C_{11}H_{23}$					$C_{20}H_{29}NO$, UV, IR, EIMS, ^{1}H, ^{13}C NMR, HPLC-MS (56)	*Ruta graveolens* L. (aerial parts) (56)
								m.p.: 138–139°C, $[\alpha]_D$ –130° ($CHCl_3$), UV, IR, ^{1}H, ^{13}C NMR ESI-MS (61)	*Ptelea trifoliata* L. (71) *Euodia uticarpa* (Juss.) Benth. (fruits) (61)
2-*n*-Tridecylquinolin-4(1*H*)-one	Bo		*n*-$C_{13}H_{27}$					m.p.: 132–134°C, UV, IR, EIMS, ^{1}H, ^{13}C NMR	*Euodia ruticarpa* (Juss.) Benth. (fruit) (59)
Acutine	Bo		$(CH_2)_3CH{=}CHCH_2CH_3$					$C_{16}H_{19}NO$, m.p.: 122–123°C, UV, IR, NMR, MS (72,73,74)	*Haplophyllum acutifolium* (D.C.) G. Don fil. (aerial parts) (72,73,74)
2-(*n*-Nona-3,6-dienyl)quinolin-4(1*H*)-one	Bo		$(CH_2)_2CH{=}CHCH_2CH{=}CHCH_2CH_3$					$C_{18}H_{21}NO$, m.p.: 103°C, UV, IR, ^{1}H NMR, MS	*Vepris ampody* H. Perrier (leaves) (75)
Hapovine	Bo		$(CH_2)_5(CH{=}CH)_3CH_3$					$C_{24}H_{31}NO$	*Haplophyllum popovii* Korovin (76)
2-(*n*-9-Hydroxynonyl)quinolin-4(1*H*)-one	Bo		$(CH_2)_8CH_2OH$					$C_{16}H_{25}NO_2$, m.p.: < 50°C. UV, IR, ^{1}H NMR, MS	*Vepris ampody* H. Perrier (leaves) (75)
2-(*n*-10-Oxo-undecyl)quinolin-4(1*H*)-one	Bo		$(CH_2)_9COCH_3$					$C_{20}H_{27}NO_2$, m.p.: 126°C, UV, IR, ^{1}H NMR, MS	*Vepris ampody* H. Perrier (leaves) (75)
Malatyamine ethyl ester	Bo		$(CH_2)_5CO_2Et$ (or COOH)					$C_{21}H_{21}NO_3$, m.p.: 126°C, UV, IR, ^{1}H NMR, MS	*Haplophyllum carpadocicum* (whole plant) (77)
2-(12-Hydroxy-12-methyltridecyl)quinolin-4(1*H*)-one	Bo		$(CH_2)_{11}COH(CH_3)_2$					$C_{23}H_{35}NO_2$ UV, IR, ^{1}H, ^{13}C NMR, HSQC, ESI-MSMS, EA	*Dictyoloma vandellianum* A.H.L. Juss. (leaves) (78)
2-(14-Hydroxy-14,15-dimethylhexadecyl)quinolin-4(1*H*)-one	Bo		$(CH_2)_{13}COHCH_3CH(CH_3)_2$					$C_{27}H_{43}NO_2$, $[\alpha]_D$ +3.0° ($CHCl_3$), UV, IR, ^{1}H, ^{13}C NMR, HSQC, ESI-MSMS, EA	*Dictyoloma vandellianum* A.H.L. Juss. (leaves) (78)
2,3-Dimethylquinolin-4(1*H*)-one	Bo.2		Me	Me				$C_{11}H_{11}NO$, m.p.: 310-315°C, UV, IR, ^{1}H, ^{13}C NMR, EIMS (50)	*Boronia lanceolada* (aerial parts) (50) *Boronia bowmanii* (51)
1-Acetoxymethyl-2,3-dimethylquinolin-4(1*H*)-one	Bo.2	CH_2OAc	Me	Me				$C_{11}H_{11}NO$, m.p.: 175–178°C, UV, IR, ^{1}H, ^{13}C NMR, EIMS (50)	*Boronia lanceolada* (aerial parts) (50) *Boronia bowmanii* (51)

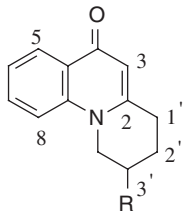
Leiokinine A	Bo.1	Me	n-C_3H_7	OMe				NMR, NOE, synthesis	*Esenbeckia leiocarpa* Engl. (79)
2-(10-Hydroxy-10-methyldodecyl)-3-methoxyquinolin-4(1*H*)-one	Bo.1		$(CH_2)_9COH(CH_3)CH_2CH_3$	OMe				$C_{23}H_{35}NO_3$, $[\alpha]_D$ +5.4° ($CHCl_3$), UV, IR, ^1H, ^{13}C NMR, HSQC, ESI-MSMS, EA	*Spathelia excelsa* (Krause) R.S. Cowan and Brizicky (leaves) (80)
2-(11-Hydroxy-11-methyldodecyl)-3-methoxyquinolin-4(1*H*)-one	Bo.1		$(CH_2)_{10}COH(CH_3)_2$	OMe				$C_{23}H_{35}NO_3$, UV, IR, ^1H, ^{13}C NMR, HSQC, ESI-MSMS, EA	*Spathelia excelsa* (Krause) R.S. Cowan and Brizicky (leaves) (80)
2-(12-Hydroxytridecyl)-3-methoxyquinolin-4(1*H*)-one	Bo.1		$(CH_2)_{11}COH(CH_3)$	OMe				$C_{23}H_{35}NO_3$, $[\alpha]_D$ +20.7° ($CHCl_3$), UV, IR, ^1H, ^{13}C NMR, HSQC, ESI-MSMS, EA	*Spathelia excelsa* (Krause) R.S. Cowan and Brizicky (leaves) (80)
2-(12-Hydroxy-12-methyltridecyl)-3-methoxyquinolin-4(1*H*)-one	Bo.1		$(CH_2)_{11}COH(CH_3)_2$	OMe				$C_{24}H_{37}NO_3$, UV, IR, ^1H, ^{13}C NMR, HSQC, ESI-MSMS, EA	*Dictyoloma vandellianum* A.H.L. Juss. (leaves) (78)
2-(12-Oxotridecyl)-3-methoxyquinolin-4(1*H*)-one	Bo.1		$(CH_2)_{11}CO(CH_3)$	OMe				$C_{23}H_{33}NO_3$, UV, IR, ^1H, ^{13}C NMR, HSQC, ESI-MSMS, EA	*Spathelia excelsa* (Krause) R.S. Cowan and Brizicky (leaves) (80)
2-(14-Hydroxy-14,15-dimethylhexadecyl)-3-methoxyquinolin-4(1*H*)-one	Bo.1		$(CH_2)_{13}COHCH_3CH(CH_3)_2$	OMe				$C_{28}H_{45}NO_3$, $[\alpha]_D$ +4.0° ($CHCl_3$), UV, IR, ^1H, ^{13}C NMR, HSQC, ESI-MSMS, EA	*Dictyoloma vandellianum* A.H.L. Juss. (leaves) (78)
6-Hydroxy-2-(3-hydroxy-3-methylbutyl)quinolin-4(1*H*)-one	Bo.3		$(CH_2)_2COH(CH_3)_2$		OH			$C_{14}H_{17}NO_3$, UV, IR, ^1H, ^{13}C NMR, HSQC, ESI-MSMS, EA	*Spathelia excelsa* (Krause) R.S. Cowan and Brizicky (leaves) (80)
7-Hydroxy-2-(3-hydroxy-3-methylbutyl)quinolin-4(1*H*)-one	Bo.3		$(CH_2)_2COH(CH_3)_2$			OH		$C_{14}H_{17}NO_3$, UV, IR, ^1H, ^{13}C NMR, HSQC, ESI-MSMS, EA	*Spathelia excelsa* (Krause) R.S. Cowan and Brizicky (leaves) (80)
1-Methyl-2-*n*-pentyl-8-methoxyquinolin-4(1*H*)-one	Bo.3	Me	n-C_5H_{11}				OMe	$C_{16}H_{21}NO_2$, Oil, UV, IR, ^1H, ^{13}C NMR, MS	*Esenbeckia almawillia* Kaastra (trunk bark) (81)
1-Methyl-2-*n*-hexyl-8-methoxyquinolin-4(1*H*)-one	Bo.3	Me	n-C_6H_{15}				OMe	$C_{17}H_{24}NO_2$, Oil, UV, IR, ^1H, ^{13}C NMR, MS	*Esenbeckia almawillia* Kaastra (trunk bark) (81)
1-Methyl-2-*n*-heptyl-8-methoxyquinolin-4(1*H*)-one	Bo.3	Me	n-C_7H_{15}				OMe	$C_{18}H_{27}NO_2$, Oil, UV, IR, ^1H, ^{13}C NMR, MS	*Esenbeckia almawillia* Kaastra (trunk bark) (81)
8-Methoxy-1-methyl-2-tridecylquinolin-4(1*H*)-one	Bo.3	Me	n-$C_{13}H_{27}$				OMe	$C_{24}H_{37}NO_2$, Oil, UV, IR, ^1H, ^{13}C NMR, MS	*Esenbeckia leiocarpa* Engl. (83)
1-Methyl-2,3-dicarbomethoxyquinolin-4(1*H*)-one	AC.2	Me	COOMe	COOMe					*Sarcomelicope dogniensis* (84)
(+)-Sarcomejine	AC.2	Me	CHOMeCOOMe	COOMe				$C_{16}H_{17}NO_6$, Amorph., UV, ^1H, ^{15}N, ^{13}C NMR, EIMS, HRMS	*Sarcomelicope megistophylla* T.G Hartley (bark) (85)

Table II (*Continued*)

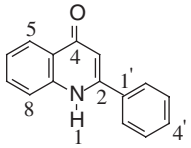
Alkaloid	Code	Substituents						Properties	Occurrence (plant part) (references)
		1	**2**	**3**	**6**	**7**	**8**		
Megistonine II	AC.4	Me	COOMe	OMe		OH	OMe	$C_{16}H_{17}NO_6$, Amorph., UV, ^{1}H, ^{13}C NMR, MS-DCI, HRMS, EI	*Sarcomelicope megistophylla* T.G. Hartley (bark) (86)
Megistonine I	AC.4	Me	COOMe	OMe		OH	Pr	$C_{14}H_{15}NO_6$, Amorph., UV, ^{1}H, ^{15}N, ^{13}C NMR, MS-DCI, HR-MS, EI	*Sarcomelicope megistophylla* T.G. Hartley (bark) (86)
(−)-Megistolactone (see Scheme 7)	AC.1								*Sarcomelicope megistophylla* T.G. Hartley (87) *Sarcomelicope follicularis* T.G. Hartley (88)
Cyclomegistine (see Scheme 7)	AC.3							$C_{18}H_{19}NO_7$, UV, MS-DCI, HRMS, HR-EIMS, ^{1}H, ^{13}C NMR, HMBC, ORTEP (89)	*Sarcomelicope megistophylla* T.G. Hartley (89) *Sarcomelicope follicularis* T.G. Hartley (88)

[a] Pr: prenyl group. The name schinifoline has been used for a different alkaloid (27).

Table III Occurrence of 2,4′-piperidine-3′-alkylquinolin-4(1*H*)-ones in Rutaceae species



Alkaloid	Code	Substituents		Properties	Occurrence (plant part) (references)
		R	**6**		
Dictyolomide A	Bo.4	$(CH_2)_2CH{=}CHCH_2CH_3$ (Z)		$[\alpha]_D$ +21° ($CHCl_3$), EIMS, ^1H, ^{13}C NMR, HMQC, HMBC (90)	*Dictyoloma peruvianum* Planch. (stem bark) (90) *Dictyoloma vandellianum* A.H.L. Juss. (leaves) (78)
Dictyolomide B	Bo.4	$CHOH(CH_2)_4CH_3$		$[\alpha]_D$ +32° (c 0.6, $CHCl_3$), EIMS, ^1H, ^{13}C NMR, HMQC, HMBC	*Dictyoloma peruvianum* Planch. (stem bark) (90)
(+)-6-Methoxydictyolomide A	Bo.4	$(CH_2)_2CH{=}CHCH_2CH_3$ (Z)	OMe	$C_{20}H_{25}NO_2$, $[\alpha]_D$ +22.0° ($CHCl_3$), UV, IR, ^1H, ^{13}C NMR, HSQC, HMBC, NOESY, ESI-MSMS, EA	*Dictyoloma vandellianum* A.H.L. Juss. (leaves) (78)

Table IV Occurrence of 2-arylquinolin-4(1*H*)-ones in Rutaceae species 

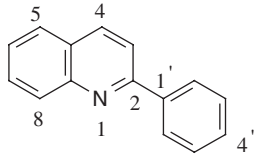
Alkaloids	Code	Substituents											Properties	Occurrence (plant part) (references)
		1	**3**	**5**	**6**	**7**	**8**	**2′**	**3′**	**4′**	**5′**	**6′**		
1	Do	Me											$C_{16}H_{13}ON$, m.p.: 144–145°C, Synthesis, IR, ^{1}H NMR, HR-MS, (91,93) m.p.: 118–120°C, ^{1}H, ^{13}C NMR, HMBC, HSQC (63)	*Balfourodendron riedelianum* Engl. (bark) (3,91) *Casimiroa edulis* Llave et Lex. (seeds, leaves) (92,94) *Haplophyllum foliosum* Vved. (fruits) (95) *Haplophyllum perforatum* (M. Bieb.) Kar. & Kir. (aerial parts) (96) *Haplophyllum leptomerum* Lincz. &Vved. (aerial parts) (52) *Raulinoa echinata* R.S. Cowan (leaves) (63) *Flindersia fournieri* Pancher & Sebert (stem bark) (97)
Reevesianine-A	Do	Me								OH			$C_{16}H_{13}NO_2$, m.p.: 322–326°C, UV, IR, MS, ^{1}H, ^{13}C NMR (98) Synthesis (93)	*Skimmia reevesiana* Fortune (root and stem bark) (98)
Folimidine	Do	Me							OH	OH			$C_{17}H_{15}NO_3$, IR, UV, NMR, MS	*Haplophyllum foliosum* Vved. (aerial parts) (99)
Norgraveoline	Do								OCH_2O				UV, NMR (100)	*Haplophyllum dubium* Korovin (aerial parts) (100) *Haplophyllum foliosum Vved.* (101)

Graveoline (Rutamine)	Do	Me				OCH_2O	$C_{17}H_{13}NO_3$, m.p.: 205°C (3) S	*Ruta graveolens* L. (herb, upper twigs, and flowers) (3,102–104)
							Synthesis, UV, IR, ^1H NMR, HR-MS (93,102,103)	*Ruta chalepensis* L. (aerial parts) (105)
								Ruta bracteosa DC. (aerial parts) (105)
								Ruta angustifolia Pers. (aerial parts) (106)
								Ruta chalepensis var. *latifolia* Salisb. (root and aerial parts) (107)
								Haplophyllum perforatum (M. Bieb.) Kar. & Kir. (aerial parts) (96)
								Haplophyllum dubium Korovin (aerial parts) (100)
								Haplophyllum foliosum Vved. (108)
3′-Hydroxy-graveoline	Do	Me				OH OCH_2O	$C_{17}H_{13}NO_4$, amorph., UV, IR, ^1H NMR, MS	*Ruta chalepensis* L. (aerial parts) (109)
2	Do	Me				OMe OCH_2O	$C_{18}H_{15}NO_4$, amorph., m.p.: 193–194°C, IR, ^1H, ^{13}C NMR, MS	*Esenbeckia grandiflora* Mart. (roots) (110)
3	Do	Me			OMe	OMe OCH_2O	$C_{19}H_{17}NO_5$, IR, ^1H, ^{13}C NMR, MS	*Esenbeckia grandiflora* Mart. (roots) (110)
Eduleine	Do	Me		OMe			$C_{17}H_{15}NO_2$, m.p.: 198–200°C, degradation and synthesis; IR, UV, NMR (92,111, 112,114)	*Lunasia amara* Blanco (leaves) (2,111); *L. quercifolia* (Warb.) Schum. & Lauterb. (bark) (3,112); *Casimiroa edulis* Llave et Lex. (bark, seeds, leaves) (92,94,113)
Lunamarine	Do	Me		OMe		OCH_2O	$C_{18}H_{15}NO_4$, m.p.: 245–247°C, IR, UV	*Lunasia amara* Blanco (leaves) (3,111)

Table IV (*Continued*)

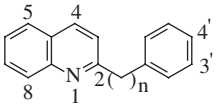
Alkaloids	Code	Substituents											Properties	Occurrence (plant part) (references)
		1	**3**	**5**	**6**	**7**	**8**	**2′**	**3′**	**4′**	**5′**	**6′**		
Eduline	Do	Me			OMe								$C_{17}H_{15}NO_2$, m.p.: 187–188°C, synthesis, IR, ^1H, ^{13}C NMR, HR-MS, UV (93,116,118)	*Casimiroa edulis* Llave et Lex. (seeds) (92,116); *Skimmia japonica* Thunb. (117); *Orixa japonica* Thunb. (leaves) (118); *Esenbeckia pentaphylla* (Macfad.) Griseb. (aerial parts) (119)
Reevesianine-B	Do	Me			OMe					OH			$C_{17}H_{15}NO_3$, m.p.: 304–306°C. UV, IR, MS, ^1H, ^{13}C NMR	*Skimmia reevesiana* Fortune; (root and stem bark) (98)
Lunasia I	Do	Me			OMe					OCH_2O			$C_{18}H_{15}NO_4$, m.p.: 230–233°C, synthesis	*Lunasia amara* Blanco; (bark) (120,121)
4	Do	Me		OH									$C_{16}H_{13}NO_2$, m.p.: 174–175°C, UV, IR, ^1H, ^{13}C NMR, EIMS (122,124)	*Lunasia quercifolia* Schum. & Lauterb. (122); *Skimmia japonica* Thunb. (123); *Casimiroa edulis* (seeds) (124)
Japonine	Do	Me	OMe		OMe								$C_{18}H_{17}NO_3$, m.p.: 143°C, UV, IR, ^1H NMR, EIMS (118,125)	*Orixa japonica* Thunb.; (leaves, stem) (118,125,126)
5	Do			OMe	OMe				OMe				$C_{18}H_{17}NO_4$, amorph., m.p.: 111-114°C, UV, IR, ^1H, ^{13}C NMR, EIMS	*Casimiroa edulis*; (leaves) (127)
6	Do			OMe	OMe				OMe	OMe			$C_{19}H_{19}NO_5$, amorph., m.p.: >100°C, UV, IR, ^1H, ^{13}C NMR EIMS	*Casimiroa edulis*; (leaves) (127)
7	Do			OMe	OMe			OMe	OMe			OMe	$C_{20}H_{21}NO_6$, amorph., m.p.: 143°C, UV, IR, ^1H, ^{13}C NMR EIMS	*Casimiroa edulis*; (leaves) (127)

Note: **1**: 1-Methyl-2-phenylquinolin-4(1*H*)-one; **2**: 1-Methyl-2-(3-methoxy-4,5-methylenedioxyphenyl)quinolin-4(1*H*)-one; **3**: 8-Methoxy-1-methyl-2-(3-methoxy-4,5-methylenedioxyphenyl)quinolin-4(1*H*)-one; **4**: 5-Hydroxy-1-methyl-2-phenylquinolin-4(1*H*)-one; **5**: 5,6-Dimethoxy-2-(3-methoxyphenyl)quinolin-4(1*H*)-one; **6**: 5,6-Dimethoxy-2-(3,4-dimethoxyphenyl)quinolin-4(1*H*)-one; **7**: 5,6-Dimethoxy-2-(2,3,6-trimethoxyphenyl)quinolin-4(1*H*)-one.

Table V Occurrence of 2-arylquinolines in Rutaceae species 

Alkaloids	Code	Substituents			Properties	Occurrence (plant part) (references)
		4	**3′**	**4′**		
2-Phenylquinoline	D.1				$C_{15}H_{11}N$, m.p.: 84°C, UV, ^1H NMR, MS (128); Synthesis, IR, ^1H NMR, HR-MS (93)	*Galipea longiflora*[a] K. Krause; (bark of stem) (45,128)
4-Methoxy-2-phenylquinoline	D	OMe			$C_{16}H_{13}NO$, m.p.: 66–67°C, Degradation and synthesis, UV, ^1H NMR, MS (2,128,129)	*Lunasia amara* Blanco; (leaves) (2,129); *Galipea longiflora*[a] K. Krause; (stem bark) (45,128)
Dubamine	D.1		OCH_2O		$C_{16}H_{12}NO_2$, m.p.: 96–97°C, Synthesis, IR, RMN ^1H, HR-MS (3,93,130–132)	*Haplophyllum dubium* Korovin; (leaves) (3, 130–132); *Haplophyllum latifolium* Kar. & Kir. (leaves, roots, and seeds) (133); *Dictamnus albus* L. (39)
Graveolinine	D	OMe	OCH_2O		$C_{17}H_{13}NO_3$, m.p.: 116–117°C, Synthesis; UV, IR, ^1H NMR, MS (111,134,135)	*Lunasia amara* Blanco (leaves) (3,111); *Ruta graveolens* L. (herb) (3,134); *Ruta chalepensis* L. (135); *Ruta chalepensis* var. *latifolia* Salisb. (root and aerial parts) (107,136)

[a] *Angostura longiflora* (syn., *G. longiflora* K. Krause); (268).

Table VI Occurrence of 2-alkylarylquinolines in rutaceae species 

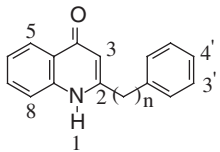
Alkaloids	Code	Substituents							Properties	Occurrence (plant part) (references)
		n	**1**	**2**	**3**	**4**	**3′**	**4′**		
2-(3,4-Dimethoxyphenylethyl)quinoline	E.1	2					OMe	OMe	$C_{19}H_{19}NO_2$, amorph. UV, ^{1}H NMR, MS, GC–MS (46,128)	*Galipea longiflora*[a] K. Krause; (bark of stem) (45,128); *Galipea officinalis*[a] J. Hancock; (trunk bark) (46)
Demethoxycusparine [2-(3,4-methylenedioxyphenylethyl) quinoline]	E.1	2					OCH_2O		$C_{18}H_{15}NO_2$, m.p.: 65°C, UV, IR, ^{1}H, ^{13}C NMR, EIMS, GC–MS (43,46,128)	*Galipea longiflora*[a] K. Krause; (stem bark) (45,128); *Galipea bracteata*[a] (Nees & Mart.) Shult. (stems) (43); *Galipea officinalis*[a] J. Hancock; (trunk bark) (46,137)
2-(3-Hydroxy-4-methoxyphenylethyl)-4-methoxyquinoline; or [(3-methoxy-4-hydroxyphenylethyl]- isomer	E	2				OMe	OH	OMe	GC–MS	*Galipea officinalis*[a] J. Hancock; (trunk bark) (46)
Cusparine	E	2				OMe	OCH_2O		$C_{19}H_{17}NO_3$, m.p.: 91–92°C, degradation and synthesis ^{1}H NMR, MS (1,128)	*Angostura trifoliata*[a] (Willd.) T. S. Elias (bark) (1); *Galipea longiflora*[a] K. Krause; (stem bark) (45,128); *Galipea officinalis*[a] J. Hancock; (trunk bark, bark) (50,137)

Galipoline	E	2				OH	OMe	OMe	$C_{19}H_{19}NO_3$, m.p.: 193°C, degradation and synthesis (1)	*Angostura trifoliata*[a] (Willd.) T.S. Elias (bark) (1); *Galipia officinalis*[a] J. Hancock (39)
Galipine	E	2				OMe	OMe	OMe	$C_{20}H_{21}NO_3$, Oil, degradation and synthesis, UV, ^1H, ^{13}C NMR, DCI/NH_3-MS, GC–MS (46,50,137)	*Angostura trifoliata*[a] (Willd.) T.S. Elias (bark) (1); *Galipea officinalis*[a] J. Hancock; (trunk bark, bark) (46,50,137)
2-(3,4-Methylenedioxystyryl)-4-methoxyquinoline	E	CH=CH				OMe	OCH_2O		$C_{19}H_{15}NO_3$, amorph., UV, RMN ^1H, MS	*Galipea longiflora*[a] K. Krause (bark of stem) (128)
2-(3,4-Dimethoxyphenylethyl)-1,2,3,4-tetrahydroquinoline	E.1.1	2	H	H	H,H	H,H	OMe	OMe	GC–MS	*Galipea officinalis*[a] J. Hancock (trunk bark) (46)
2-(3,4-Methylenedioxyphenylethyl)-1,2,3,4-tetrahydroquinoline	E.1.1	2	H	H	H,H	H,H	OCH_2O		GC–MS	*Galipea officinalis*[a] J. Hancock (trunk bark) (46)
(−)-Galipeine	E.1.1	2	Me	H	H,H	H,H	OH	OMe	$C_{19}H_{23}NO_2$, Oil, $[\alpha]_D$ −13.6°, UV, DCI/NH_3-MS, ^1H, ^{13}C NMR, GC–MS (46,53)	*Galipea officinalis*[a] J. Hancock (bark, trunk bark) (46,53)
3,4-Methylenedioxicuspareine or (−)-Galipinine (Allocuspareine)	E.1.1	2	Me	H	H,H	H,H	OCH_2O		$C_{19}H_{21}NO_2$, Oil, $[\alpha]_D$ −33.4° ($CHCl_3$), UV, ^1H, ^{13}C NMR, HREIMS, GC–MS (46,137); CD, HR-EI-MS, ^1H, ^{13}C NMR (50)	*Galipea officinalis*[a] J. Hancock (trunk bark, bark) (46,50,137)

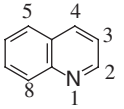
Table VI (*Continued*)

Alkaloids	Code	Substituents							Properties	Occurrence (plant part) (references)
		n	**1**	**2**	**3**	**4**	**3′**	**4′**		
Cuspareine	E.1.1	2	Me	H	H,H	H,H	OMe	OMe	$C_{20}H_{25}NO_2$, Oil, $[\alpha]_D$ –22.8° ($CHCl_3$), m.p.: 54–56°C, degradation and synthesis, UV, ^1H, ^{13}C NMR, DCI/NH_3-MS, GC–MS (46,137); COSY, NOE, ^1H, ^{13}C NMR (50)	*Angostura trifoliata* (Willd.) T.S. Elias (bark) (1); *Galipea officinalis*[a] J. Hancock (trunk bark, bark) (46,50,137)
2-[6-(3,4-Methylenedioxyphenyl) hexyl]-4-methoxyquinoline	E	6				OMe	OCH_2O		$C_{22}H_{24}NO_3$, m.p.: 163°C, UV,IR, ^1H, ^{13}C NMR, EIMS, HRFTMS	*Ruta chalepensis* L. (roots) (138)

[a] *Angostura trifoliata* (syn. *Galipea officinalis*, see item III); *A. longiflora* (syn. *G. longiflora* K. Krause); *A. bracteata* (syn. *G. bracteata*) (268).

Table VII Occurrence of 2-alkylarylquinolin-4(1*H*)-ones in rutaceae species 

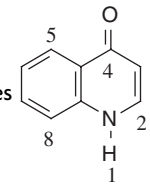
Alkaloids	Code	Substituents				Properties	Occurrence (plant part) (references) Plant species
		n	**5**	**3′**	**4′**		
2-[4-(3,4-Methylenedioxyphenyl)butyl]quinolin-4(1*H*)-one (Rutaverine)	Eo	4		OCH_2O		$C_{20}H_{20}NO_3$, m.p.: 224°C, IR, ^1H NMR, ESI-MS (139,140)	*Ruta graveolens* L. (leaves, upper twigs, and flowers) (104,139)
2-[6-(3,4-Methylenedioxyphenyl)hexyl]quinolin-4(1*H*)-one	Eo	6		OCH_2O		$C_{22}H_{24}NO_3$, m.p.: 163°C, UV, IR, ^1H and ^{13}C NMR, EIMS, HRFTMS	*Ruta chalepensis* L. (roots) (138)
1-Methyl-2-[6-(3,4-methylenedioxyphenyl)hexyl]-quinolin-4(1*H*)-one	Eo	6	Me	OCH_2O		$C_{23}H_2NO_3$, m.p.: 167–168°C, ^1H and ^{13}C NMR, HR-ESIMS	*Ruta graveolens* L. (leaves) (139)

Table VIII Occurrence of quinolines and alkylquinolines in non-rutaceous species 

Alkaloids	Code	Substituents								Properties	Occurrence (plant part) (references)
		1	2	3	4	5	6	7	8		
Quinoline	A.1									C_9H_7N, MS, ^1H, ^{13}C NMR	*Peganum harmala* L. (Zygophyllaceae) (141)
Quinaldine	B.1		Me							$C_{10}H_9N$	*Peganum harmala* L. (Zygophyllaceae) (141)
1,4-Dimethylquinolinium iodide	J.4	Me			Me						*Eichhornia crassipes* (Mart.) Solms (Pontederiaceae) (142)
Kynurenic acid	I		CO_2H		OH						*Ephedra pachyclada* ssp. *sinaica* (Riedl.) H. Freitag & M. Marie-Stolte (Ephedraceae) (stems) (143); *Ginkgo biloba* L. (Ginkgoaceae) (144)
6-Hydroxykynurenic acid	IO(6)		CO_2H		OH		OH			$C_{10}H_7NO_4$, m.p.: 287°C, PMR, IR, UV, MS.(145,147)	*Nicotiana tabacum* L. (Solanaceae) (leaves) (145); *Thapsia villosa* L. (Apiaceae) (fruits) (146); *Limonium perezii* F.T. Hubb. *L. gmelinii* (Willd.) Kuntze (Plumbaginaceae) (147); *Ephedra* spp. (Ephedraceae) (148)
6-Methoxykynurenic acid	IO(6)		CO_2H		OH		OMe			$C_{11}H_9NO_4$, m.p.: 298-300°C, UV, IR, ^1H NMR, HPLC, HR-EIMS, EIMS	*Ephedra pachyclada* ssp. *sinaica* (Reidl.) H. Freitag & M. Marie-Stolte (Ephedraceae) (stems) (143)
8-Hydroxyquinoline-4-carboxaldehyde	J.5				CHO				OH	$C_{10}H_7NO_2$, UV, ^1H NMR	*Broussonetia zeylanica* (Thw.) Corner (Moraceae) (timber) (150)

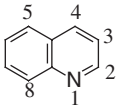
8-Hydroxyquinoline-4-carbaldehyde oxime	J.3.1				CH=NOH					OH	$C_8H_8N_2O_2$, m.p.: 220°C (151); ^1H, ^{13}C NMR, COSY, NOE, synthesis (152,153)	*Broussonetia zeylanica* (Thwait.) Corner (Moraceae) (151–153)
3-Phenylquinoline	A.1.2			Ph							$C_{15}H_{11}N$, m.p.: 43–44°C, synthesis, IR, ^1H, ^{13}C NMR, MS (159)	*Peganum nigellastrum* Bunge. (Zygophyllaceae) (aerial parts) (155)
3-(4-Hydroxy phenyl)quinoline	A.1.2			4-OH-Ph							$C_{15}H_{11}NO$, m.p.: 221–222°C, synthesis; IR, ^1H, ^{13}C NMR, MS (159)	*Peganum nigellastrum* Bunge. (Zygophyllaceae) (aerial parts) (155)
Quinoline-3-carboxamide	H[O]			$CONH_2$							$C_{10}H_8N_2O$, synthesis, ^1H NMR (156)	*Peganum nigellastrum* Bunge. (Zygophyllaceae) (154,156)
Tuberosine B	J.1.2	H	H,H	H,H	OH, COOH							*Allium tuberosum* Roxb. (Alliaceae) (157)
Broussonetine (see Scheme 9)	J.2.1										^1H, ^{13}C NMR	*Broussonetia zeylanica* (Moraceae) (210)
Luotonin F (see Scheme 4)	H										$C_{18}H_{11}N_3O_2$, m.p: 238–240°C, UV, IR, ESIMS,HR-EIMS, ^1H, ^{13}C NMR, HMQC, HMBC	*Peganum nigellastrum* Bunge. (Zygophyllaceae) (154)
3-(1*H*-Indol-3-yl)quinoline (see Scheme 4)	A.1.1										$C_{17}H_{12}N_2O$, m.p.: 173–175°C, ^1H NMR	*Peganum nigellastrum* Bunge. (Zygophyllaceae) (155)
2-Methyl-5,6,7,8-tetrahydroquinoline	[a]		Me			H,H	H,H	H,H	H,H			*Glycyrrhiza uralensis* Fisch. (Fabaceae) (158)
2,4-Dimethyl-5,6,7,8-tetrahydroquinoline	[a]		Me		Me	H,H	H,H	H,H	H,H			*Glycyrrhiza uralensis* Fisch. (Fabaceae) (158)

[a] See Scheme 19.

Table IX Occurrence of alkylquinolin-4(1*H*)-ones in non-rutaceous species 

Alkaloids	Code	Substituents							Properties	Occurrence (plant part) (references)
		1	2	3	5	6	7	8		
1-Acetoxymethyl-2-(10-acetoxyundecyl)quinolin-4(1*H*)-one	Bo	CH_2OAc	$(CH_2)_9CHOHCH_3$						$C_{25}H_{35}NO_5$, Oil, m.p.: 111-114°C, $[\alpha]_D$ -1° (MeOH), IR, ^1H, ^{13}C NMR, HREIMS	Aff. *Samadera* SAC-2825 (Simaroubaceae) (160)
Transtorine	Io		COOH						$C_{18}H_{17}NO_4$, Oil, UV, IR, ^1H, ^{13}C NMR, HREIMS	*Ephedra transitoria* (Ephedraceae) (aerial parts) (161)
Ephedralone	IoO(7)		COOH				OMe		$C_{11}H_9NO_4$, m.p.: 268°C, UV, IR, EI-MS, ^1H, ^{13}C NMR	*Ephedra alata* Decsne. (Ephedraceae) (162); *Castanea mollissima* Blume (Fagaceae) (273)
8-Methoxy-4(1*H*)-quinolone-2-carboxylic acid	IoO(8)		COOH					OMe		*Lappula squarosa* (Retz.) Dumort. (Boraginaceae) (202)
Melovinone	MPh.1		Me	OMe	$(CH_2)_5Ph$		OMe	OMe	$C_{24}H_{29}NO_4$, m.p.: 134–136°C, ^1H NMR, MS, UV, IR	*Melochia tomentosa* L. (Sterculiaceae) (168)
Melochinone (see Scheme 19)	MPh.2.1								$C_{22}H_{21}NO_2$, m.p.: 316–318°C, MS, IR, UV, ^1H, ^{13}C NMR, ORTEP	*Melochia tomentosa* L. (Sterculiaceae) (169)
Waltherione-A (see Scheme 19)	MPh.2.1.1								$C_{23}H_{23}NO_5$, m.p. : 206-207.5°C, $[\alpha]_D$ -25.5° ($CHCl_3$), HREIMS, IR, ^1H, ^{13}C NMR	*Waltheria douradinha* Saint-Hilaire (Sterculiaceae) (170)
17,18-Bis-nor-antidesmone (Hyeronine B)	M		Me	OMe	H, C_6H_{13}	H,H	H,H	= O	$C_{17}H_{26}NO_3$, LC-MS, CID-MS	*Antidesma* spp. (Euphorbiaceae) (163); *Hyeronima alchorneoides* Allemão (Euphorbiaceae) (163)

18-Nor-antidesmone	M	Me	OMe	H,C_7H_{15}	H,H	H,H	= O	$C_{18}H_{28}NO_3$, LC-MS, CID-MS	*Antidesma* spp. (Euphorbiaceae) (163); *Hyeronima alchorneoides* Allemão (Euphorbiaceae) (163)
(+)-O-Acetylhyeronimone	M	Me	OMe	H,C_8H_{17}	H,H	H,H	H,OAc	$C_{21}H_{33}NO_4$, m.p.: 75-77°C, $[\alpha]_D$ +178° ($CHCl_3$), UV, IR, ^1H, ^{13}C NMR, HREIMS	*Hyeronima alchorneoides* Allemão (Euphorbiaceae) (roots) (164)
(+)-Hyeronimone (8-Dihydroantidesmone)	M	Me	OMe	H,C_8H_{17}	H,H	H,H	H,OH	$C_{19}H_{31}NO_3$, m.p.: 85-86°C, $[\alpha]_D$ +115° ($CHCl_3$), IR, ^1H, ^{13}C NMR, HREIMS, UV, EIMS, ^1H, ^{13}C NMR, HMQC (163)	*Hyeronima alchorneoides* Allemão (Euphorbiaceae) (roots) (164); *Antidesma* spp. (Euphorbiaceae) (163)
8-Deoxoantidesmone	M	Me	OMe	H,C_8H_{17}	H,H	H,H	H,H	$C_{19}H_{32}NO_2$, LC-MS (TSQ), CID-MS, ESI-CID-TOF-MS	*Hyeronima alchorneoides* Allemão (Euphorbiaceae) (163); *Antidesma* spp. (Euphorbiaceae) (163)
(*S*)-(+)-Antidesmone (Hyeronine A)	M	Me	OMe	H,C_8H_{17}	H,H	H,H	= O	$C_{19}H_{29}NO_3$, Oil, UV, IR, HREIMS ^1H, ^{13}C NMR, ESI-CID-TOF-MS, NOE, HMBC, 2D INADEQUATE (166)	*Antidesma membranaceum* Müll. Arg. (165); *A. venosum* E.May. ex. Tul. (leaves) (166); *Antidesma* spp. (Euphorbiaceae) (166); *Hyeronima alchorneoides* Allemão (163); *H. oblonga* Cuatrec. (166); (Euphorbiaceae); *Thecacoris stenopetala* Müll. Arg. (Euphorbiaceae) (163)
(+)-(7*RS*)-7-(β-D-Glucopyranosyloxy)-antidesmone	M	Me	OMe	H, $(CH_2)_6CHOglCH_3$	H,H	H,H	= O	$C_{25}H_{40}NO_9$, UV, ESI-TOF-MS ^1H, ^{13}C NMR	*Antidesma membranaceum* (Euphorbiaceae) (leaves) (167)
(+)-(7RS)-8-Deoxo-7-(β-D-glucopyrano-syloxy)-antidesmone	M	Me	OMe	H, $(CH_2)_6CHOglCH_3$	H,H	H,H	H,H	$C_{25}H_{40}NO_9$, UV, HRESI TOF-MS, ^1H, ^{13}C NMR	*Antidesma membranaceum* Müll. Arg. (Euphorbiaceae) (bark) (167)
Echinoramine-I (see Scheme 2)	Ao.1								*Echinops ritro* L. (Asteraceae) (26)

Table X Occurrence of quinolines in fungi, bacteria, and marine organisms 

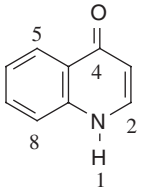
Alkaloids	Code	Substituents								Properties	Occurrence (references)
		1	2	3	4	5	6	7	8		
2-Pentyl-4-hydroxyquinoline	B		*n*-C_5H_{11}		OH					$C_{14}H_{17}NO$, m.p.: 141–142°C, UV, IR, ^1H NMR, MS (171)	*Alteromonas* sp. (marine bacterium) (171,172)
2-Heptyl-4-hydroxyquinoline	B		*n*-C_7H_{15}		OH					$C_{16}H_{21}NO$, m.p.: 146–147°C, (171); EIMS, ^{13}C NMR (188)	*Alteromonas* sp. (marine bacterium) (171,172); *Pseudomonas aeruginosa* (173,188)
2-Nonyl-4-hydroxyquinoline	B		*n*-C_9H_{19}		OH					GC–MS (173); $C_{24}H_{24}NO_2$, m.p.: 132°C, amorph., UV, IR, CIMS, EIMS, ^1H, ^{13}C NMR (181)	*Pseudomonas aeruginosa* (173); *Pseudomonas* (strain 1531-E7 – associated with sponge *Homophymia* sp.) (181)
2-Undecyl-4-hydroxyquinoline	B		*n*-$C_{11}H_{23}$		OH					GC–MS	*Pseudomonas aeruginosa* (173)
2-(Undec-1-enyl)-4-hydroxyquinoline	B		$CH{=}CH(CH_2)_8CH_3$		OH					GC–MS	*Pseudomonas aeruginosa* (173)
Kynurenic acid	I		CO_2H		OH					$C_{10}H_7NO_3$, m.p.: 196–198°C, IR, ^1H, ^{13}C NMR, APT, EI–MS	*Streptomyces griseofulvus* (174)
Helquinoline	B.1.1		H, Me	H_2	H, OH				CO_2H	$C_{12}H_{15}NO_3$, HR-EIMS, UV, IR, ^1H, ^{13}C NMR, HMQC, COSY, HMBC, NOESY	*Janibacter limosus* (175)
3-Hydroxyquinoline-2-carboxylic acid Calcium salt	IO(3).1		$CO_2^- Ca^{+2}$	OH						$C_{20}H_{20}N_2O_6Ca$, m.p.: 170°C. UV, IR, ^1H, ^{13}C NMR, APT, EIMS	*Streptomyces griseoflavus* subsp. (Go 3592) (174)
Xanthurenic acid	IO(8)		CO_2H		OH				OH		*Pseudomanas fluorescens* (ATCC 17400) (176) *Tridemnum* sp. (ascidian) (177)
Quinolobactin	IO(8)		CO_2H		OMe				OH	$C_{11}H_9NO_4$, ^1H, ^{13}C NMR	*Pseudomanas fluorescens* (ATCC 17400) (178)

Aurachin A (see Scheme 3)	A.1.F					$C_{25}H_{33}NO_3$, mp: 111–112°C, $[\alpha]_D$ –49.2° (MeOH), UV, IR	*Stigmatella aurantiaca* (Gram-negative bacterium) (179)
Aurachin B	B.1.F	O^-	Me	OH	Farn	$C_{25}H_{33}NO_2$, m.p.: 93–94°C, UV, IR	*Stigmatella aurantiaca* (Gram-negative bacterium) (179)
Aurachin F–I (see Scheme 3)	B.1.F.1A.F. A.F					(Patent)	*Stigmatella aurantiaca* (Gram-negative bacterium) (180)
2-Heptyl-4-hydroxyquinoline *N*-oxide	B	O^-	*n*-C_7H_{15}		OH	GC–MS	*Pseudomonas aeruginosa* (173)
2-Nonyl-4-hydroxyquinoline *N*-Oxide	B	O^-	*n*-C_9H_{19}		OH	$C_{24}H_{24}NO_2$, amorph., mp: 132°C, UV, IR, CIMS, EIMS, ^1H, ^{13}C NMR (181)	*Pseudomonas* (strain 1531-E7 – associated with sponge *Homophymia* sp.) (181) *Pseudomonas aeruginosa* (173)
2-(Hept-1-enyl)-4-hydroxyquinoline *N*-oxide	B	O^-	$CH{=}CH(CH_2)_4CH_3$		OH	GC–MS	*Pseudomonas aeruginosa* (173)
2-(Non-1-enyl)-4-hydroxyquinoline *N*-oxide	B	O^-	$CH{=}CH(CH_2)_6CH_3$		OH	GC–MS	*Pseudomonas aeruginosa* (173)
2-(Undec-1-enyl)-4-hydroxyquinoline *N*-oxide	B	O^-	$CH{=}CH(CH_2)_8CH_3$		OH	GC–MS	*Pseudomonas aeruginosa* (173)
Quinoline-4-carbaldehyde	J				COH		*Archangium gephyra* (bacterium strain Ar T205) (182)
Quinoline-4-carbaldoxime	J.3				CH=NOH		*Archangium gephyra* (bacterium strain Ar T205) (182); *Myxococcus virescens* (bacterium strain Mx v48) (182)
Quinoline-4-carboxylic acid	J.1				COOH		*Archangium gephyra* (bacterium strain Ar T205) (182)
4-Hydroxymethyl-quinoline	J.2				CH_2OH	$C_{11}H_9NO$, amorph., UV, MS, ^1H NMR (183)	*Polyporus sanguineus* (wood-rotting fungus) (183); *Polyporus versicolor* (183); *Archangium gephyra* (bacterium strain Ar T205) (182); *Myxococcus virescens* (bacterium strain Mx v48) (182)

Table X (*Continued*)

Alkaloids	Code	Substituents								Properties	Occurrence (references)
		1	2	3	4	5	6	7	8		
4,8-Dimethyl-6-hydroxyquinoline	J.4.1				Me		OH		Me	$C_{11}H_{11}NO$, amorph., UV, IR, EIMS, ^1H, ^{13}C NMR	*Lyngbya majuscula* (cyanobacterium) (184)
4,8-Dimethyl-6-*O*-(2,4-di-*O*-methyl-β-D-xylopyranosyl)quinoline	J.4.1				Me		Ogl		Me	$C_{18}H_{23}NO_5$, amorph., $[\alpha]_D$ –56.7° ($CHCl_3$), UV, IR, HR-EIMS, EIMS, ^1H, ^{13}C NMR (184)	*Lyngbya majuscula* (cyanobacterium) (184,185)
4-Chloromethylenyl-8-methyl-6-*O*-(2,4-di-*O*-methyl-β-D-xylopyranosyl)-1,2,3,4-tetrahydroquinoline	J.4.1.1				= CHCl		Ogl		Me	$C_{18}H_{24}NO_5Cl$, amorph., $[\alpha]_D$ -20.8° (MeOH), UV, IR, LRFABMS, HRFABMS, ^1H, ^{13}C NMR	*Lyngbya majuscula* (cyanobacterium) (185)
Quinocitrinine A (see Scheme 16)	G									^1H, ^{13}C NMR	*Penicillium citrinum* (VKM FW-800) (186)
Quinocitrinine B (see Scheme 16)	Go.1.1.1									^1H, ^{13}C NMR	*Penicillium citrinum* (VKM FW-800) (186)
7-Bromo-4-(2-ethoxyethyl)quinoline	J.1.1				$(CH_2)_2O$-CH_2CH_3			Br		$C_{13}H_{14}BrNO$, MS, IR, ^1H NMR, NOE	*Flustra foliacea* (L.) (149)
Trididemnic acid A	IO(8).1		CO_2H		OH	Bz			OH	$C_{17}H_{11}NO_6$, EIHRMS, ^1H NMR (synthesis of derivatives)	*Trididemnum* sp. (ascidian) (177)
Trididemnic acid B	IO(8).1		CO_2H		OH	Bz[a]			OH	$C_{17}H_{11}NO_7$, EIHRMS, ^1H NMR (synthesis of derivatives)	*Trididemnum* sp. (ascidian) (177)
Perspicamide A	IO(8)		CONHCH=CHPh-4-OH (*E*)		OH				OH	$C_{18}H_{14}N_2O_2$, UV, IR, LRESIMS, HRESIMS, ^1H, ^{13}C NMR, gHMBC, gCOSY	*Botrylloides perspicuum* (ascidian) (187)
Perspicamide B	IO(8)		CONHCH=CHPh-*p*-OH (*Z*)		OH				OH	$C_{18}H_{14}N_2O_2$, UV, IR, LRESIMS, HRESIMS, ^1H, ^{13}C NMR, gHMBC	*Botrylloides perspicuum* (ascidian) (187)

Notes: Farn: Farnesyl group; Bz: Benzoyl group.
[a] Bz: Benzoyl-4-OH.

Table XI Occurrence of alkylquinolin-4(1*H*)-ones in fungi, bacteria, and marine organisms 

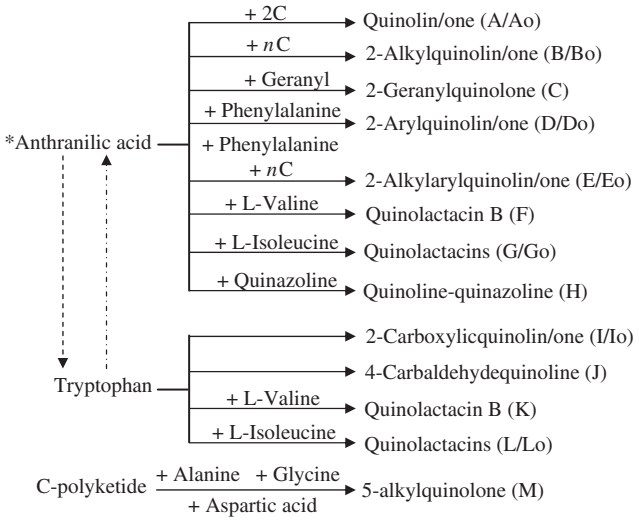
Alkaloids	Code	Substituents			Properties	Occurrence (references)
		1	2	3		
2-*n*-Nonylquinolin-4(1*H*)-one	Bo		*n*-C_9H_{19}		$C_{18}H_{25}NO$, m.p.: 134°C amorphous, UV, IR, CIMS, EIMS, ^1H, ^{13}C NMR	*Pseudomonas* (strain 1531-E7 – associated with sponge *Homophymia* sp.) (181)
2-*n*-Undecylquinolin-4(1*H*)-one	Bo		*n*-$C_{11}H_{23}$		$C_{20}H_{29}NO$, m.p.: 132°C amorphous, UV, IR, CIMS, EIMS, ^1H, ^{13}C NMR	*Pseudomonas* (strain 1531-E7 – associated with sponge *Homophymia* sp.) (181)
2-(Hept-1-enyl)quinolin-4(1*H*)-one	Bo		$CH{=}CH(CH_2)_4CH_3$		NMR, MS	*Pseudomonas aeruginosa* (189)
2- [(1*E*)-Undec-1-enyl]quinolin-4(1*H*)-one	Bo		$CH{=}CH(CH_2)_8CH_3$		$C_{20}H_{29}NO$, m.p.: 131°C, amorph., UV, IR, CIMS, EIMS, ^1H, ^{13}C NMR	*Pseudomonas* (strain 1531-E7 – associated with sponge *Homophymia* sp.) (181)
2-(Undec-3-enyl)quinolin-4(1*H*)-one	Bo		$(CH_2)_2CH{=}CH(CH_2)_6CH_3$		$C_{20}H_{27}NO$, m.p.: 114°C, UV	*Pseudomonas aeruginosa* (190)
3-Methyl-2-*n*-pentylquinolin-4(1*H*)-one	Bo.2		C_5H_{11}	Me	$C_{15}H_{19}NO$, m.p.: 222–223°C, UV, IR ^1H, ^{13}C NMR, HR-EIMS, EIMS	*Pseudomonas cepacia* (strain PC-II) (191)
3-Methyl-2-*n*-heptylquinolin-4(1*H*)-one	Bo.2		C_7H_{15}	Me	$C_{17}H_{23}NO$, m.p.: 227–228°C, UV, IR ^1H, HR-EIMS, EIMS, synthesis	*Pseudomonas cepacia* (strain PC-II) (191)
3-Methyl-2-*n*-nonylquinolin-4(1*H*)-one	Bo.2		C_9H_{19}	Me	$C_{19}H_{27}NO$, m.p.: 215–216°C, UV, IR ^1H, ^{13}C NMR, HR-EIMS, EIMS, synthesis	*Pseudomonas cepacia* (strain PC-II) (191)
3-Methyl-2-(hept-2-enyl)quinolin-4(1*H*)-one	Bo.2		$CH_2CH{=}CH(CH_2)_3CH_3$	Me	$C_{17}H_{21}NO$, m.p.: 225–227°C, UV, IR, ^1H, ^{13}C NMR, COSY, HMQC, HMBC, HREIMS, EIMS (191,192)	*Pseudomonas cepacia* (192) (strain PC-II) (191,193) *Pseudomonas* cells (194)
3-Methyl-2-(non-2-enyl)quinolin-4(1*H*)-one	Bo.2		$CH_2CH{=}CH(CH_2)_5CH_3$	Me	$C_{19}H_{25}NO$, m.p.: 197–198°C, UV, IR, ^1H, ^{13}C NMR, HREIMS, EIMS (191,192)	*Pseudomonas cepacia* (192) (strain PC-II) (191,193)
1-Hydroxy-2-(non-2-enyl)-3-methylquinolin-4(1*H*)-one	Bo.2	OH	$CH_2CH{=}CH(CH_2)_5CH_3$	Me	$C_{19}H_{25}NO_2$, Syrup, HRFAB-MS, FAB-MS, UV, IR, ^1H, ^{13}C NMR, HMBC	*Arthrobacter* sp. (strain YL-02729S) (195)

Table XI (*Continued*)

Alkaloids	Code	Substituents			Properties	Occurrence (references)
		1	**2**	**3**		
1-Methyl-2-(1-hydroxygeranyl)quinolin-4(1*H*)-one	Co	Me	$CHOHCH{=}CHCH_3(CH_2)_2CH{=}C(CH_3)_2$		$C_{20}H_{25}NO_2$, $[\alpha]_D$ +66.3° (MeOH), HREI-MS, UV, IR, ^1H NMR, ^{13}C NMR, LREI-MS	*Pseudonocardia* sp. (CL38489) (196)
1-Methyl-2-(3-hydroxy-3,7-dimethyl-octa-1,6-dienyl)quinolin-4(1*H*)-one	Co	Me	$CH{=}CHCOHCH_3(CH_2)_2CH{=}C(CH_3)_2$		$C_{20}H_{25}NO_2$, $[\alpha]_D$ –51.4° (MeOH), HREI-MS, UV, IR, ^1H NMR, LREI-MS	*Pseudonocardia* sp. (CL38489) (196)
2-Geranylquinolin-4(1*H*)-one	Co		Ger		$C_{19}H_{23}NO$, HREI-MS, UV, IR, ^1H NMR, LREI-MS	*Pseudonocardia* sp. (CL38489) (196)
1-Methyl-2-geranylquinolin-4(1*H*)-one	Co	Me	Ger		$C_{20}H_{25}NO$, HREI-MS, UV, IR, ^1H NMR, LREI-MS	*Pseudonocardia* sp. (CL38489), (196)
2-Geranyl-3-methylquinolin-4(1*H*)-one	Co.1		Ger	Me	$C_{20}H_{25}NO$, HREI-MS, UV, IR, ^1H, ^{13}C NMR, LREI-MS	*Pseudonocardia* sp. (CL38489) (196)
1-Methyl-2-geranyl-3-methylquinolin-4(1*H*)-one	Co.1	Me	Ger	Me	$C_{21}H_{27}NO$, HREI-MS, UV, IR, ^1H, ^{13}C NMR, LREI-MS	*Pseudonocardia* sp. (CL38489) (196)
1-Methyl-2-(6,7-epoxygeranyl)quinolin-4(1*H*)-one	Co.1	Me	Ger (6,7-epoxy)	Me	$C_{21}H_{27}NO$, $[\alpha]_D$ –17.5° (MeOH), HREI-MS, UV, IR, ^1H, ^{13}C NMR, LREI-MS	*Pseudonocardia* sp. (CL38489) (196)
1-Methylthiolmethyl-2-geranyl-3-methylquinolin-4(1*H*)-one	Co.1	CH_2SMe	Ger	Me	$C_{22}H_{29}NOS$, HREI-MS, UV, IR, ^1H, ^{13}C NMR, LREI-MS	*Pseudonocardia* sp. (CL38489) (196)
Aurachin C	Bo.F	OH	Me	Farn	$C_{25}H_{33}NO_2$, m.p.: 124–125°C, UV, IR	*Stigmatella aurantiaca* (Gram-negative bacterium), (179)
Aurachin D	Bo.F		Me	Farn	$C_{25}H_{33}NO$, m.p.: 165–168°C, UV, IR	*Stigmatella aurantiaca* (179)
Aurachin E (see Scheme 3)	Bo.F.1				(Patent)	*Stigmatella aurantiaca* (Gram-negative bacterium) (180)
(*S*)-(−)-Quinolactacin B (see Scheme 15)	F				$C_{15}H_{16}N_2O_2$, m.p.: 260–263°C, $[\alpha]_D$ –3.3° (DMSO), FAB-MS, HRFAB-MS, UV, IR, TLC, ^1H, ^{13}C NMR, HMBC, NOESY (197) Enantioselective synthesis (201)	*Penicillium* sp. (EPF-6) (197,198)
(9*S*, 1′*R*)-Quinolactacin A1 (see Scheme 16)	Go.1.1				$C_{16}H_{18}N_2O_2$, m.p.: 262–265°C, FAB-MS, HRFAB-MS, UV, IR, TLC, ^1H, ^{13}C NMR, PFG-HMBC, COSY (197); Enantioselective synthesis (201)	*Penicillium* sp. (EPF-6) (*197,198*); *Penicillium citrinum* (90648) (199)

(9*S*, 1′*S*)-Quinolactacin A2 (see Scheme 16)	Go	$C_{16}H_{18}N_2O_2$, m.p.: 262–265°C, $[\alpha]_D$ +17.9^0 (DMSO), FAB-MS, HRFAB-MS, UV, IR, TLC, ^1H, ^{13}C NMR, PFG-HMBC, COSY (197); Enantioselective synthesis (201)	*Penicillium* sp. (EPF-6) (197,198); *Penicillium citrinum* (90648) (199)
Quinolactacin C	Go.1	$C_{16}H_{18}N_2O_3$, m.p. :180–185°C, $[\alpha]_D$ +5.9°, (DMSO), HRFAB-MS, UV, IR, ^1H NMR, ^{13}C NMR (197)	*Penicillium* sp. (EPF-6) (197,198)
Quinolactacide (see Scheme 16)	Go.1.2	$C_{14}H_8N_2O_2$, m.p.: 373–374°C, EIMS, HR-EIMS, ^1H, ^{13}C NMR, HMQC, IR, DEPT, COSY, HMBC, UV	*Penicillium citrinum* Thom. (F 1539) (200)

Note: Farn: Farnesyl group.



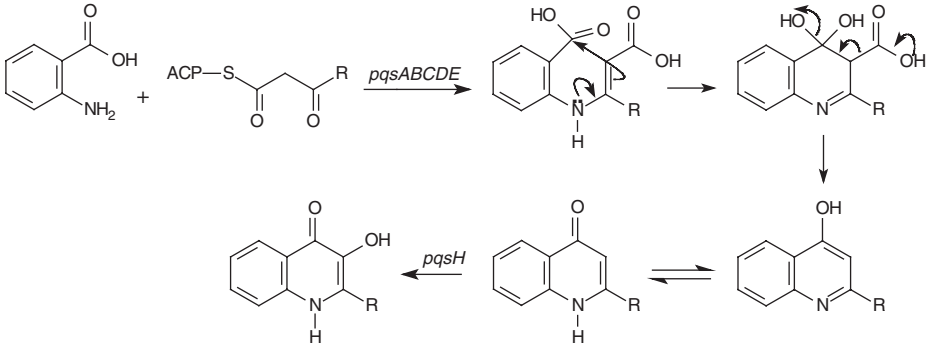
*Anthranilic acid may also be produced by metabolism of tryptophan

Figure 1 Biogenetic classification of the quinolin/one alkaloids.

interfere with, the control of virulence factor production. Moreover, a 2-heptyl-3-hydroxyquinolin-4(1*H*)-one biosynthetic gene cluster has been identified. This *pqsABCDE* operon codes for a putative coenzyme A ligase (*pqsA*), two β-keto-acyl-acyl carrier protein synthases (*pqsB*, *pqsC*), and a FabH1 homologous transacetylase (*pqsD*). On the other hand, *pqsE* seems to encode a response effectors protein which itself is not involved in the biosynthesis of 2-heptyl-3-hydroxyquinolin-4(1*H*)-one.

Although, it has clearly been demonstrated that the *pqsABCD* genes are essential for 2-heptyl-3-hydroxyquinolin-4(1*H*)-one biosynthesis, their enzymatic function remains to be elucidated (203–205). Previous studies strongly suggested that anthranilic acid and a β-keto-acid are precursors of 2-alkylquinolin-4(1*H*)-ones (206,207). Further studies, using feeding experiments with labeled isotopes, with detection by gas chromatography–mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy, confirmed that *P. aeruginosa* synthesizes 2-alkylquinolin-4(1*H*)-ones via a common biosynthetic pathway involving the ''head-to-head'' condensation of anthranilic acid and β-keto fatty acids (Scheme 1). Moreover, 2-heptyl-3-hydroxyquinolin-4(1*H*)-one biosynthesis seems to be dependent on an intact *pqsABCD* operon and on the availability of β-keto acids. Interestingly, at least some of these acids seem to be derived from a common pool of β-hydroxy-keto acids involved in rhamnolipid biosynthesis (205).

A search of completed and uncompleted microbial genomes revealed that several species belonging to the genera *Pseudomonas* and *Burkholderia*, as well as the plant pathogen *Ralstonia*, contain putative homologs of the *P. aeruginosa* pqs biosynthetic genes *pqsA*, *pqsC*, and *pqsD*, respectively. However, apart from *P. aeruginosa*, only strains of the primary human pathogen, *B. pseudomallei*, and



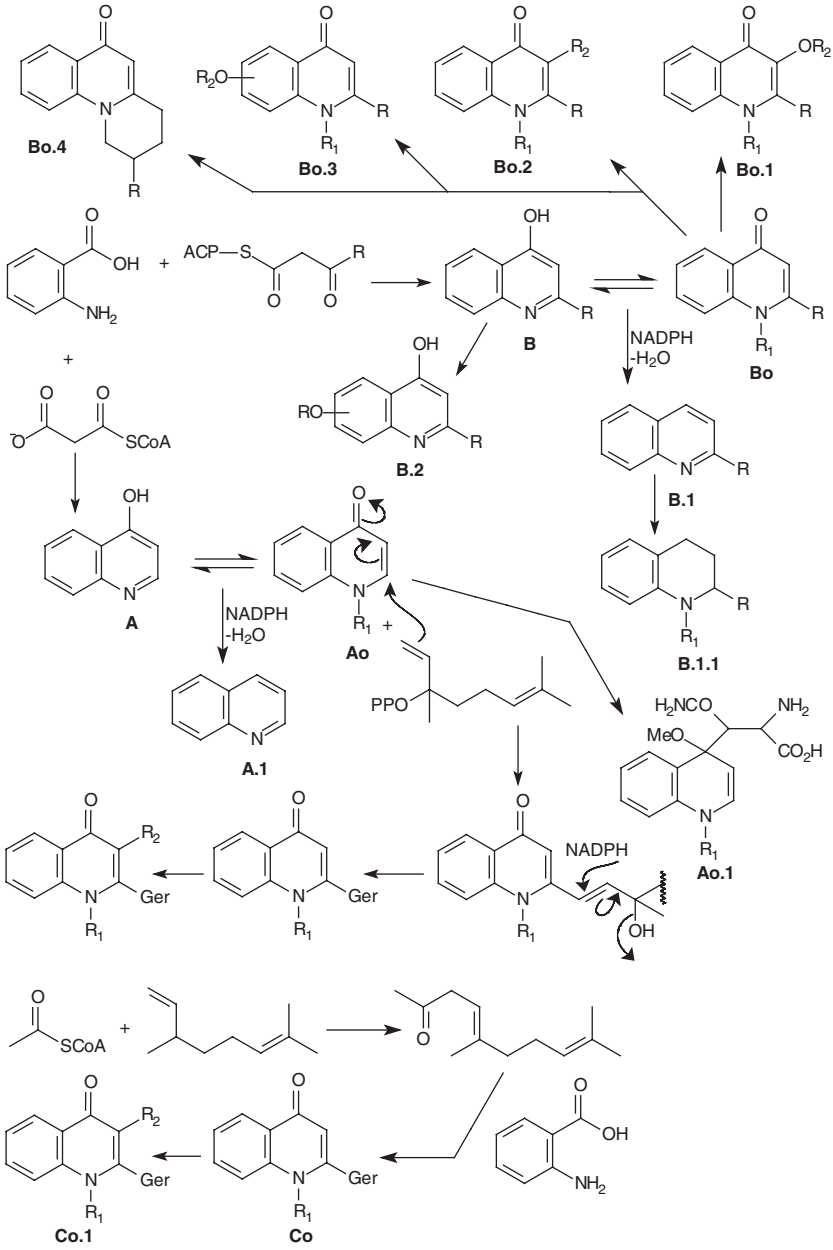
Scheme 1 Biosynthesis of a pseudan (205).

the serologically and genetically related *B. thailandensis*, were found to contain a complete, putative *pqsABCDE* operon located on chromosome 2. Interestingly, neither *B. pseudomallei* nor *B. thailandensis* possess a homolog of the *P. aeruginosa pqsH* gene. This gene facilitates the conversion of 2-heptylquinolin-4(1*H*)-one to 2-heptyl-3-hydroxyquinolin-4(1*H*)-one (Scheme 1) in *P. aeruginosa*, and its absence from *B. pseudomallei* would suggest that production of 3,4-hydroxy-2-heptylquinoline was unlikely in this organism (208).

No data are available on the biosynthesis of 2-alkylquinoline/4(1*H*)-one (B/Bo) alkaloids from anthranilic acid in plants. However, the structures of many of the alkaloids isolated, especially those obtained from plants in the family Rutaceae (Tables I and II), have provided substantial indirect evidence for the biogenetic route and structural interrelationships proposed in Scheme 2.

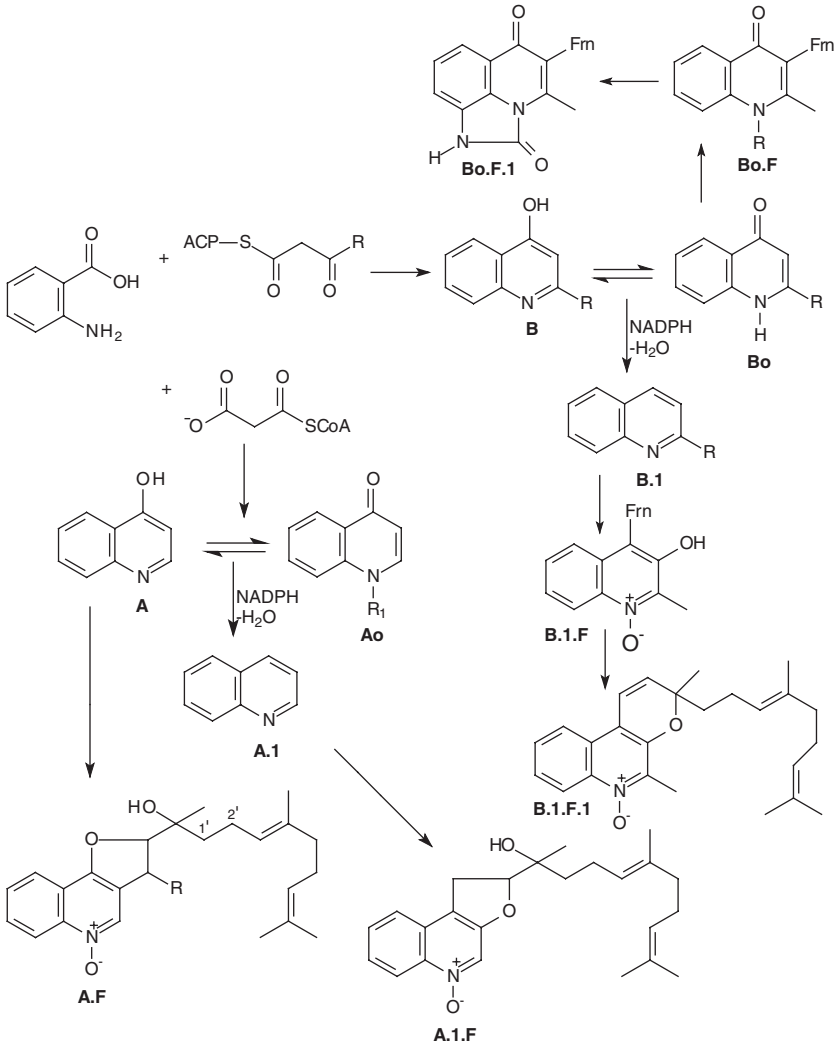
2-Geranylquinolin-4(1*H*)-ones were isolated from bacterium *Pseudonocardia* sp. CL38489 (Table XI) (196), and their biogenesis may involve the direct reaction between a quinoline or quinolin-4(1*H*)-one and geranyl diphosphate. However, chemical considerations do not justify this route, because quinoline and quinolin-4(1*H*)-one are insufficiently nucleophilic at C-2 for that reaction to occur readily. Another reasonable biogenesis for their formation may involve the direct reaction between a quinolin-4(1*H*)-one and linalyl diphosphate (LPP) by Michael addition at C-2 to give 1-methyl-2-(3-hydroxy-3,7-dimethyl-octa-1,6-dienyl)quinolin-4(1*H*)-one, which is naturally occurring (Co; Scheme 2). Therefore, these alkaloids are of uncertain biogenetic origin, and it is not known whether they are formed via a pathway involving the condensation of anthranilic acid and a β-keto intermediate with one acetate unit attached to the geranyl group. For convenience, the second proposal was codified (Co).

Aurachins A–I are sesquiterpenoid quinolin/one alkaloids from the myxobacterium *Stigmatella aurantiaca* (Tables X and XI). They were described in the patent literature as alkaloids produced when the organism was cultured in the presence of added anthranilic acid (180). Thus, from the biogentic pathways of quinolin/ones shown in Schemes 1 and 2, it can be concluded that alkylation by farnesyl diphosphate of positions C-3 or C-4, and subsequent oxidative cyclization, does occur in myxobacteria. The 3-position of quinolin-4(1*H*)-one is a



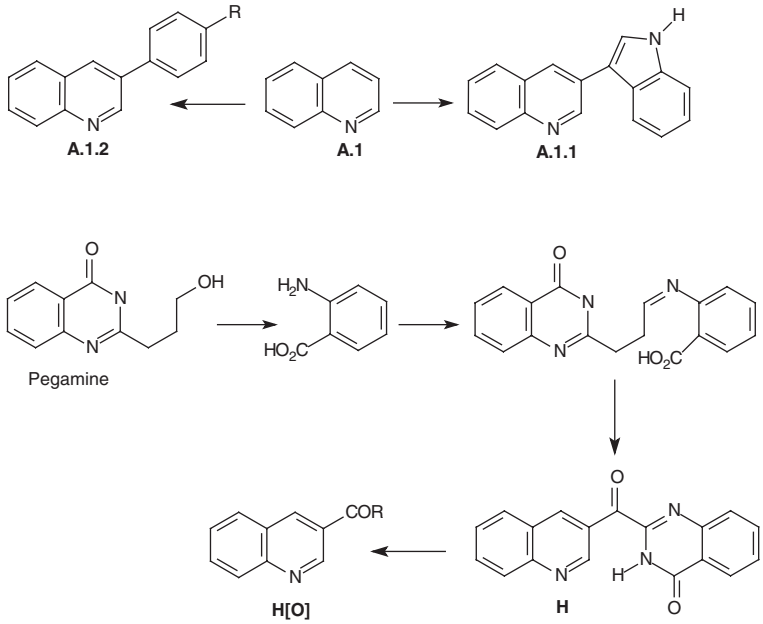
Scheme 2 Biogenesis of the quinolin/4(1*H*)-one alkaloids.

powerful nucleophilic center, and a farnesyl diphosphate should be readily introduced at this position (Bo.F, Scheme 3). Oxidation at C-3 of a quinoline ensures nucleophilicity at C-4 for alkylation, which can be followed by cyclization to a dihydrofuroquinoline (A.1.F, Scheme 3).



Scheme 3 Biogenetic pathway for the formation of the Aurachins (180), A (A.1.F), B (B.1.F), C (Bo.F; R = OH), D (Bo.F; R = H), E (Bo.F.1), F (B.1.F.1), G (A.F; R = H, 1′, 2′-dihydro), H (A.F; R = H), I (A.F; R = OMe).

3-Phenylquinolines (A.1.2), previously unknown as natural products, are surprising new metabolites of the Chinese medicinal plant *Peganum nigellastrum* (Zygophyllaceae; Table VIII) (155). The same plant source also yielded the unusual mixed alkaloids 3-(1*H*-indol-3-yl)quinoline (A.1.1) and quinoline–quinazoline (H). The biogenesis of the latter was plausibly suggested to be from pegamine, a quinazoline alkaloid found in *P. nigellastrum* (154). Oxidation of pegamine to the corresponding aldehyde, followed by imine formation with anthranilic acid, was suggested to produce an intermediate imine, cyclization,



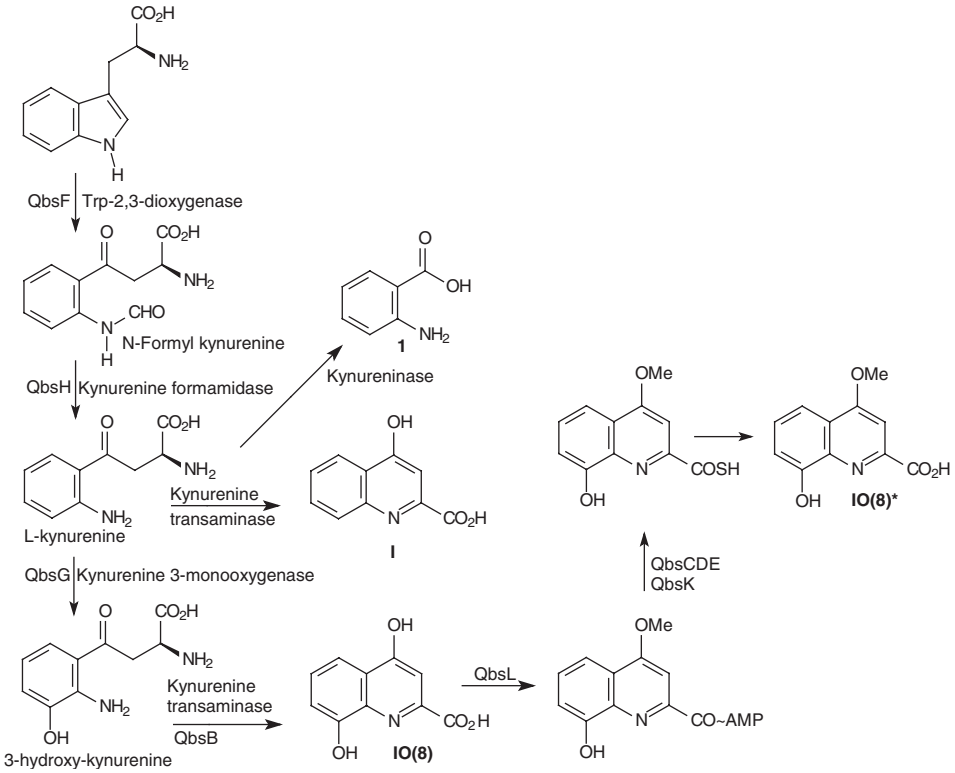
Scheme 4 Biogenesis of the 3-substituted quinolines.

and further elaboration of which leads to luotonin F (H) (Scheme 4). Thus, it seems likely that quinoline-3-carboxamide (H[O]) is the product of oxidative cleavage of luotonin F (H; Table VIII). Therefore, these alkaloids do not have the same biogenetic origin observed for other 3-substituted quinolines.

Phenylquinolines and 3-(1*H*-indol-3-yl)quinoline are probably derived from quinoline. However, a mechanism for their formation is not clear. They may be produced by the coupling of two phenolic systems, or a phenolic and an indole system, by means of free radical reactions, as occurs in the lignans found in the Zygophyllaceae (209) (Scheme 4).

The experiments discussed above on the biosynthesis of a pseudan have also shown that kynurenic acid is not the precursor of the 2-alkylquinolin-4(1*H*)-ones. Based on the putative function of the genes of the *qbs* operons (176), a pathway was proposed for the biosynthesis of kynurenic acid (I), xanthurenic acid [IO(8)], and quinolobactin [IO(8)*] in *Pseudomonas fluorescens* ATCC 17400 (Scheme 5). The first step, the oxidation of tryptophan to *N*-formylkynurenine, is likely to be catalyzed by the enzyme tryptophan 2,3-dioxygenase (TDO) (QbsF), which is a heme-dependent enzyme. The second step, the deformylation of *N*-formylkynurenine to L-kynurenine is catalyzed by kynurenine formamidase (KFA). The product of *qbsH*, a metal-dependent hydrolase found also in other bacterial genomes, is the likely candidate.

In support of this hypothesis, the activity of a homolog of QbsH from *Ralstonia metallidurans*, as a specific KFA has recently been demonstrated. A homolog of KFA is also found in the genome of *P. aeruginosa* (PA 2081) and in other

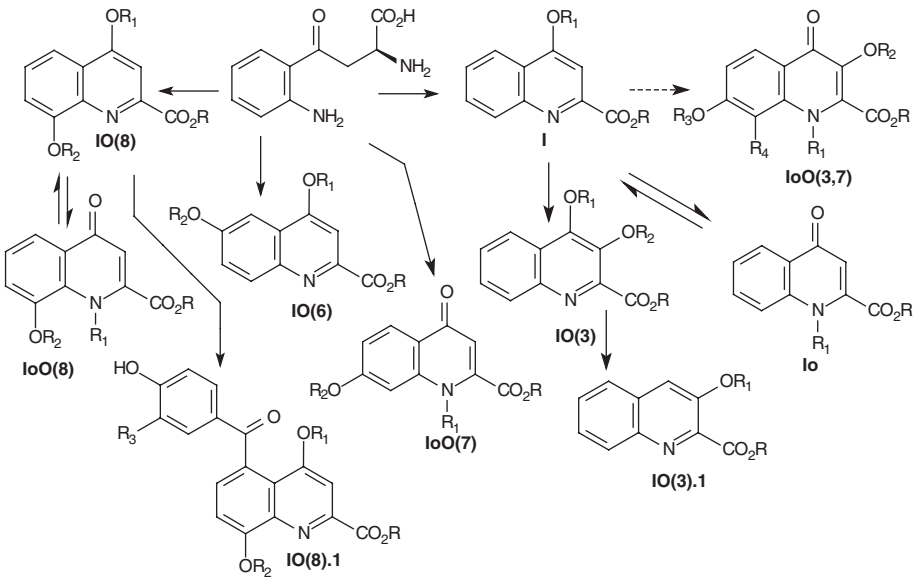


Scheme 5 Biogenetic pathway for the formation of anthranilic (**1**), kynurenic (I), and xanthurenic [IO(8)] acids, and quinolabactin [IO(8)*] (176).

fluorescent pseudomonads. In the following step, L-kynurenine could be transaminated into kynurenic acid by the kynurenine transaminases (KTE), or hydroxylated by a kynurenine 3-monooxygenase (KMO) homolog encoded by *qbsG*. In this context, it is interesting to note that in *P. aeruginosa*, and in other fluorescent pseudomonads, no KMO homolog can be found. However, a homolog of kynureninase (KYN, PA 2080 in *P. aeruginosa*) is known, indicating that these fluorescent pseudomonads have a biosynthetic pathway available for the degradation of tryptophan to anthranilate. After the action of KMO, 3-hydroxykynurenine could be transaminated into xanthurenic acid by the QbsB protein, which shows the highest similarity with KTE. A biogenetic pathway for the formation of quinolobactin from xanthurenic acid was also proposed. The bifunctional protein QbsL can activate xanthureic acid via its N-terminal AMP ligase domain, whereas the C-terminal domain of QbsL is responsible for the addition of the methyl group. Next, the QbsCDE proteins transfer sulfur from an unknown sulfur donor molecule (cysteine?). The participation and exact role of QbsK, a putative oxidoreductase, is still not clear at this juncture. Quinolobactin is proposed to result from the spontaneous hydrolysis of 8-hydroxy-4-methoxy-2-quinoline thiocarboxylic acid (176).

Kynurenic acid (I) is also found in higher plants (Table VIII), e.g. *Ephedra pachyclada* ssp. *sinaica* (Ephedraceae). 6-Hydroxy- and 6-methoxy derivatives [IO(6)] occur in *Limonium perezii*, *L. gmelinii* (Plumbaginaceae), *Ephedra* spp., and *E. pachyclada* ssp. *sinaica* (143,147,148). An undescribed species of ascidian (genus *Tridemnum*; Table X), harvested in the northeastern Pacific Ocean, has yielded xanthurenic acid [IO(8)] and two derivatives, the tridemnic acids A [IO(8).1] (R, R_1, R_2, R_3 = H) and B [IO(8).1] (R, R_1, R_2 = H, R_3 = OH), which represent a new family of ascidian alkaloids (177). These compounds likely are derived from kynurenic and xanthurenic acids, respectively, or from intermediate L-kynurenine by benzoylation at C-5 (Scheme 6). Recently, the xanthurenamides (Table X) were isolated from *Botrylloides perspicuum* (Australian ascidian), which can be formed by reacting xanthurenic acid and 1,2-dihydrotyramine [IO(8), R_1, R_2 = H, CO_2R = CONHCH = CH-*p*-OH-Ph] (187). Regarding the substitution pattern of quinolin/4(1*H*)-one-2-carboxylic acids other examples are cited in Tables VIII–X.

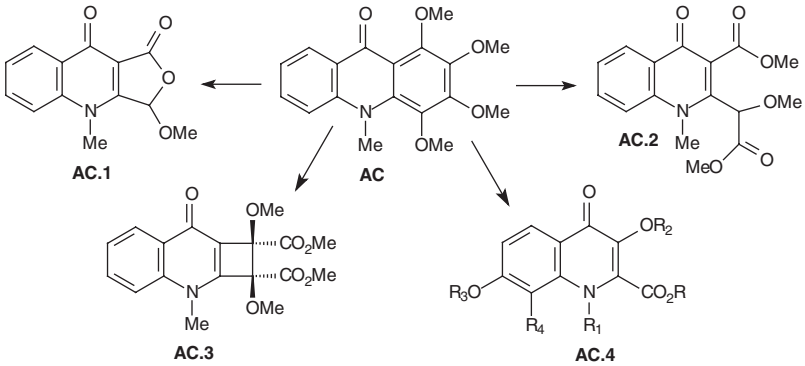
The bark of the New Caledonia tree *Sarcomelicope megistophylla* (Rutaceae) yielded an intriguing range of unprecedented quinoline alkaloids, all of which are conceivably formed from highly oxygenated acridones, such as melicopicine (AC), by oxidative cleavage of ring A (Scheme 7). The structural similarities are clearly apparent in (+)-sarcomejine (AC.2; Table II), (−)-megistolactone (AC.1), and cyclomegistine (AC.3) (87,89). The biosynthetic origins of megistonine I [IoO(3,7), or AC.4, R, R_1, R_2 = Me, R_3 = H, R_4 = 3,3-dimethylallyl] and II [IoO(3,7), or AC.4, R, R_1, R_2 = Me, R_3 = H, R_4 = OMe] are less obviously ascribable to acridone oxidation (86). Therefore, these alkaloids do not have the same biogenetic origin as observed for other alkylquinolin/ones.



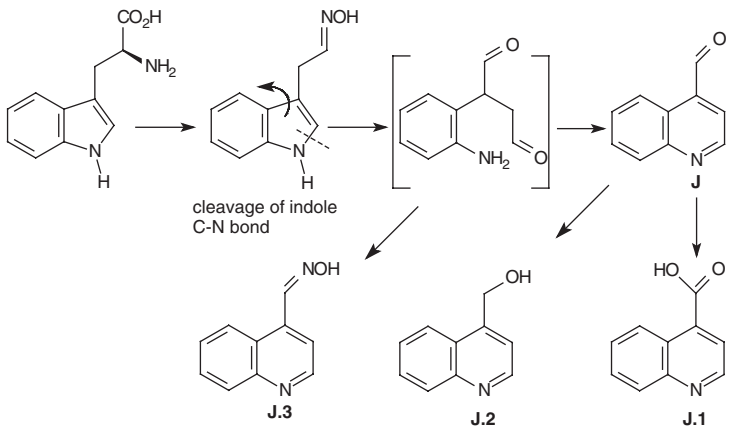
Scheme 6 Biogenesis of quinolin/4(1*H*)-one-2-carboxylic acid derivatives.

Feeding experiments with L-[1′-^{14}C]tryptophan in the myxobacterium *Archangium gephyra* Ar T205 indicated that quinoline-4-carbaldehyde (J; Table X), quinoline-4-carbaldoxime (J.3), quinoline-4-carboxylic acid (J.1), and 4-hydroxymethyl-quinoline (J.2) arise through a fundamental rearrangement of the indole nucleus of tryptophan (182) (Scheme 8).

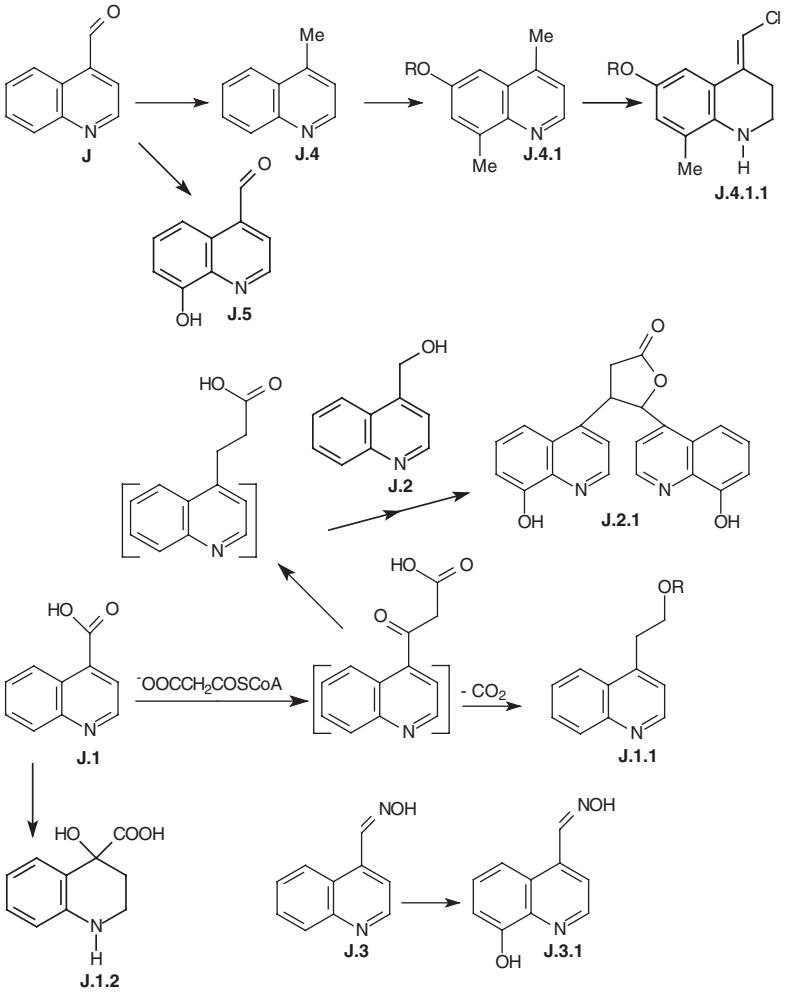
Quinoline-4-carbaldehyde derivatives also occur in higher plants (Table VIII). For example, 8-hydroxyquinoline-4-carbaldehyde oxime (J.3.1; Scheme 9) was isolated from *Broussonetia zeylanica* (Moraceae) (151–153). (+)-Tuberosine B (J.1.2), an unprecedented tetrahydroquinoline alkaloid, has been reported in the inaccessible Chinese literature as a new metabolite of *Allium tuberosum* (Alliaceae) (157). *Eichhornia crassipes* (Pontederiaceae) is an invasive plant and often jams rivers and lakes with uncounted thousands of tons of floating plant matter. It has yielded 1,4-dimethylquinolinium iodide (J.4) (142).



Scheme 7 Biogenesis of A-ring cleaved acridones.



Scheme 8 Biosynthesis of quinoline-4-carbaldehyde derivatives (182).



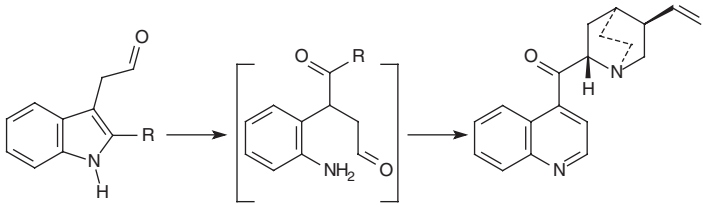
Scheme 9 Biogenesis of the 4-substituted quinolines.

The isolation of the first, naturally occurring, bromo-quinoline alkaloid, 7-bromo-4-(2-ethoxyethyl)quinoline (J.1.1) from the marine bryozoan *Flustra foliacea* was reported (149). The marine cyanobacterium *Lyngba majuscule* from Puerto Rican waters yielded a chlorine-containing tetrahydroquinoline alkaloid (*E*)-4-chloromethylenyl-8-methyl-6-*O*-(2,4-di-*O*-methyl-β-D-xylopyranosyl)-1,2,3,4-tetrahydroquinoline (J.4.1.1), together with 4,8-dimethyl-6-hydroxyquinoline (J.4.1) and 4,8-dimethyl-6-*O*-(2,4-di-*O*-methyl-β-D-xylopyranosyl)quinoline (J.4.1; Table X) (184,185). The authors also found a polyketide compound with the chloromethylene unit, leading to the hypothesis that the quinoline ring system is not derived from anthranilic acid, but perhaps from acetate and a suitable amino acid such as β-alanine. However, the isolation of 4-substituted quinolines cited above suggests that the biogenesis of these alkaloids involves a common

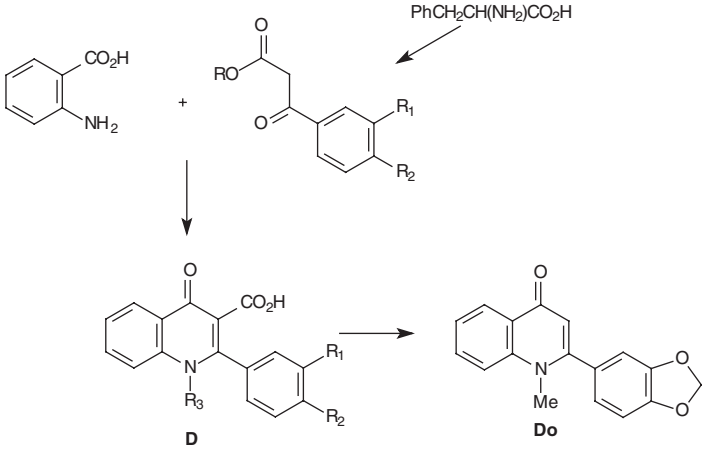
quinoline-4-carbaldehyde precursor (Scheme 9). Broussonetine (J.2.1), a dimer quinoline, has been isolated from *B. zeylanica*, together with 8-hydroxyquinoline-4-carbaldehyde oxime (J.3.1) (151,210), suggesting that in the former, the lactone portion is derived from quinoline-4-carboxylic acid and malonyl CoA (Scheme 9). However, these biogenetic proposals cannot yet be regarded as the main pathway, and remain to be tested experimentally.

The indole–quinoline rearrangement proposed in Scheme 8 for the quinoline-4-carbaldehyde derivatives is well-known for some monoterpenoid indole alkaloids (Scheme 10), e.g., the formation of cinchoninone from the Rubiaceae (211). The term pseudoquinoline can be used to distinguish this group of alkaloids, which are better regarded as monoterpenoid indole alkaloids, a large group found in the genera *Cinchona* and *Remijia*. Thus, they are not included in this review.

Blaschke-Cobet and co-workers (212) have shown that the non-anthranilate portion of the 2-arylquinolin-4(1*H*)-one of graveoline of *Ruta graveolens* is derived from the decarboxylation and deamination of phenylalanine. Feeding experiments with labeled isotopes in *R. graveolens* indicated that hydroxylation of the aromatic ring precedes cyclization to the quinolin-4(1*H*)-one system. A β-keto ester and a 3-carboxyquinolin-4(1*H*)-one are probable intermediates in the biosynthesis of graveoline (Do) (Scheme 11).

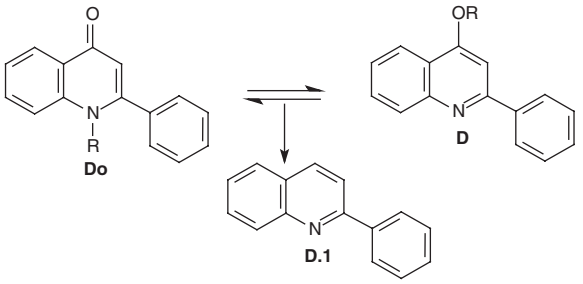


Scheme 10 Indole–quinoline rearrangement in the biogenesis of cinchoninone.

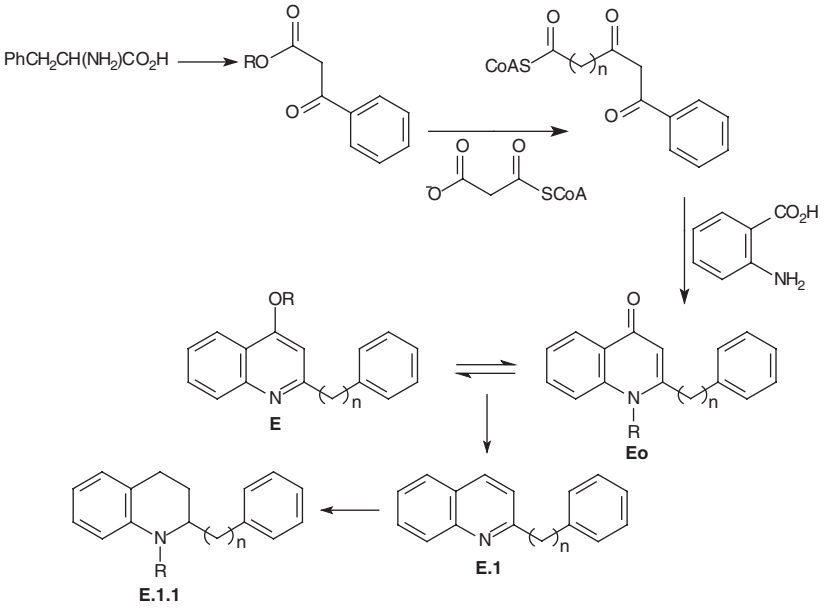


Scheme 11 Biosynthesis of graveoline (Do) in *Ruta graveolens* (212).

No data are available on the biosynthesis of alkaloids of the rutaverine type found in *R. graveolens* (Table VII), in which the quinolin-4(1*H*)-one and phenyl ring systems are separated by a multiple carbon chain; these are the 2-alkylarylquinolin-4(1*H*)-ones. A similar biogenetic pathway as that leading to graveoline (Do), but involving the condensation of a β-keto intermediate with more than one acetate unit to give a polyketide chain attached to the carboxylic acyl group of the amino acid can be proposed. This unit might then condense with anthranilate to form the 2-alkylarylquinolin-4(1*H*)-ones. Direct support for these biogenetic ideas, through the appropriate labeling studies, are minimal from plants in the Rutaceae. However, the structures of many aryl- and alkylaryquinolin/ones isolated from the rutaceous genera (Tables IV–VII), have provided substantial indirect evidence for the routes proposed in Schemes 12 and 13.



Scheme 12 Biogenesis of the arylquinolin/4(1*H*)-ones.

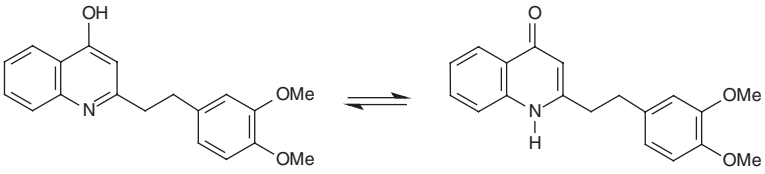


Scheme 13 Biogenesis of the alkylarylquinolin/4(1*H*)-ones.

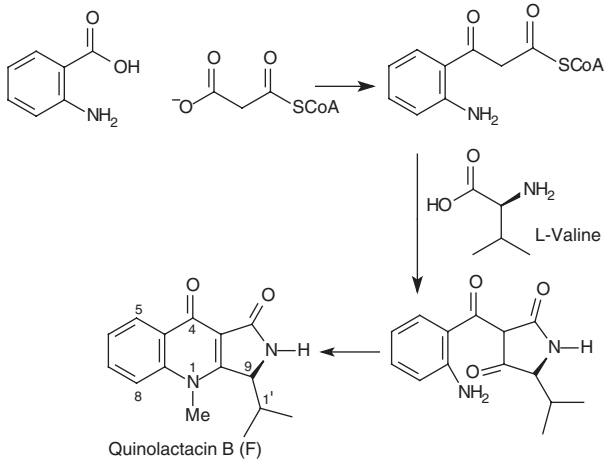
The coexistence of the NH-quinolin-4(1*H*)-one and 4-hydroxyquinoline isomers has been confirmed by spectrometric analysis. However, the exclusive NH-4-oxo nature of these alkaloids in solution phase (NMR) and solid state (IR and X-ray) has also been corroborated (213). For example, galipoline, isolated from *Angostura trifoliate* (Rutaceae) (Table VI), has the potential to exist in tautomeric equilibrium (Scheme 14), explaining a number of reports which have appeared showing the structure of galipoline (1,214), either as 2-(3, 4-dimethoxyphenylethyl)-4-hydroxyquinoline or as 2-(3,4-dimethoxyphenylethyl)-quinolin-4(1*H*)-one (40).

A number of reports have confirmed, by ^{13}C NMR, the carbonyl nature of the NH-2-alkylquinolin-4-one unit, indicating the predominance of the NH-oxo forms, which are widely distributed in rutaceous genera, and in *Pseudomonas* species (Table XI).

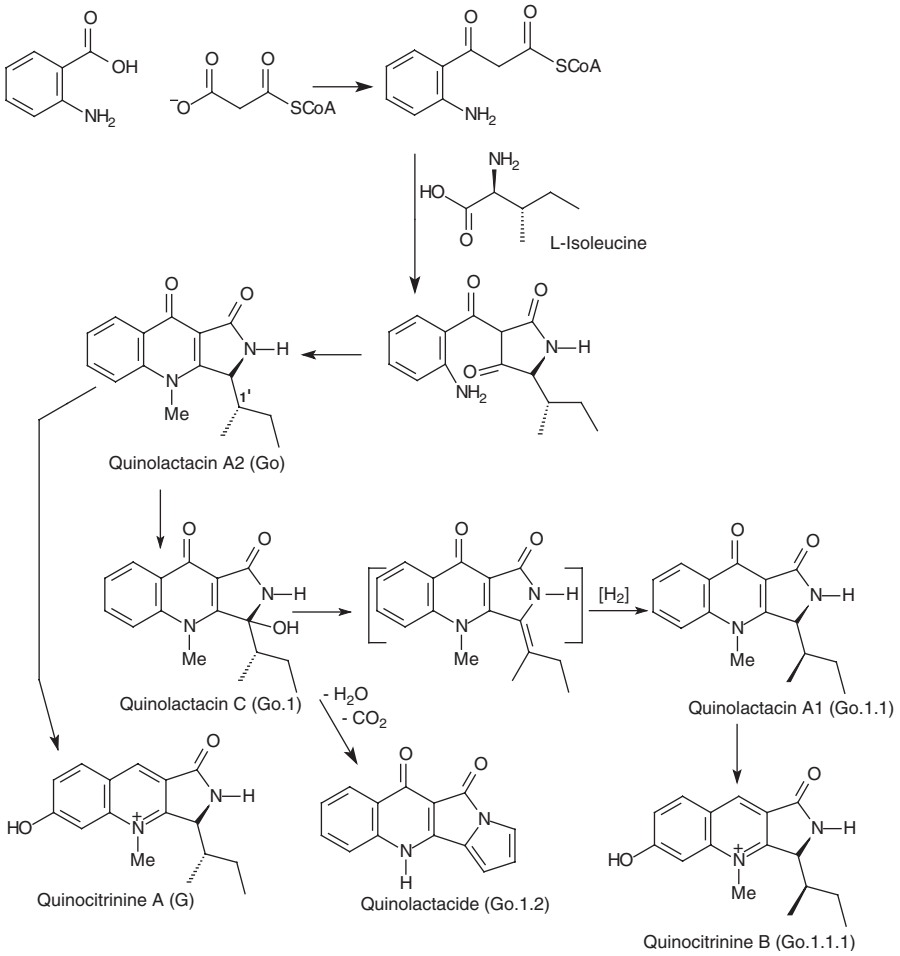
The novel quinolone antibiotics, quinolactacins A–C (Schemes 15 and 16; Tables X and XI), were first discovered from the cultured broth of *Penicillium* sp. EPF-6, which was isolated from the larvae of the mulberry pyralid (*Margaronia pyloalis* Welker) (197,198). More recently, the two quinolactacin A diastereomers were isolated from a solid-state fermentation of *P. citrinum* 90648 and named quinolactacin A1 and quinolactacin A2. The relative configuration of



Scheme 14 Tautomeric equilibrium of galipoline.



Scheme 15 Biogenesis of quinolactacin B from anthranilic acid.



Scheme 16 Biogenesis of quinolactacins A1, A2, and C, quinocitrinines A and B, and quinolactacide from anthranilic acid.

quinolactacin A2 is assumed to be the same as that of the originally assigned quinolactacin A from the cultured broth of *Penicillium* sp. EPF-6 on the basis of spectroscopic analysis. Quinolactacin A1 is the C-1′ diastereomer of A2 (199).

Further studies with *P. citrinum* VKM FW-800 obtained from the permafrost region of Northern Russia, led to the isolation of the alkaloids (+)-quinocitrinine A and (−)-quinocitrinine B, which are diastereomers at the C-1′ and C-9 stereocenters (186). These alkaloids were the first pyrrolo[3,4-*b*]quinoline-type representatives isolated from microbial sources. Their structures are unique in that a quinolone skeleton is conjugated with a γ-lactam ring. A biomimetic total synthesis of quinolactacin B was reported by Tatsuta's group (215). A new enantioselective synthesis of quinolactacins A2 and B, via the skeletal rearrangement of a β-carboline to the pyrrolo[3,4-*b*]quinolin-4(1*H*)-one system by an

oxidative process, confirmed the absolute configuration of quinolactacin B as assigned in the literature, namely, (*S*)-(−)-quinolactacin B (201).

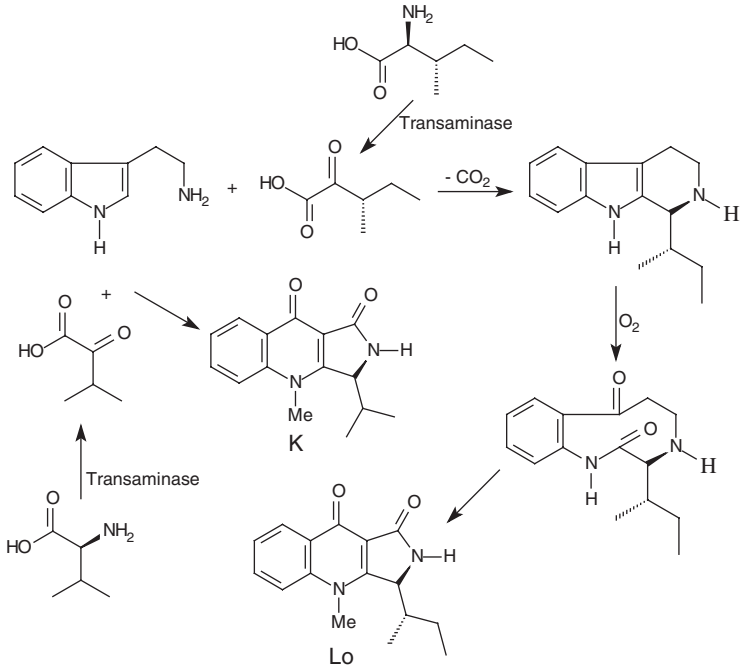
The absolute configurations of the quinolactacins A1 and A2, have not been clearly determined as yet. Quinolactacin A2 was obtained as a single enantiomer, and the stereochemistries at C-9 and C-1′ were each established as being of the *S* configuration (9*S*, 1′*S*). Through the same synthetic pathway described above, a pair of C-1′ diastereomers were obtained. Chiral HPLC was used for the separation of these two diastereomers. From the ^{1}H NMR of the diastereomeric mixture, the C-1′ diastereomer of quinolactacin A2 could be differentiated by comparison with the spectrum of A2. The spectrum was in agreement with the data of quinolactacin A1 reported by Kid coworkers in 2001 (199). This result confirmed that quinolactacin A1 is the C-1′ diastereomer of quinolactacin A2 (9*S*, 1′*R*) (201).

The first synthesis suggested that these alkaloids might be biogenetically derived from three components, the amino acids L-valine or L-isoleucine, anthranilic acid, and acetic acid (215) (Schemes 15 and 16).

Quinolactacin A1 does not appear to have the same biogenetic origin as considered for quinolactacin A2, since the other possible isoleucine stereoisomer is not generally found in living organisms. In addition, the β-carbon is insulated from the amino and carboxy groups which, by electron-withdrawing effects and resonance stabilization, promote epimerization at the α-position. Thus, the β-epimerization rates of the isoleucine are predicted to be extremely slow in comparison to their α-epimerization rates. Quinolactacin C was also found in the genus *Penicillium*, leading to the hypothesis that quinolactacin A1 is not derived from β-epimerization, but perhaps from the former by elimination of H_2O and subsequent hydrogenation producing the C-1′ diastereomer of quinolactacin A2 (Scheme 16). However, the requisite stereochemistry at the two stereogenic centers in quinolactacin C was not elucidated.

Recently a new quinolactacin with a pyrrole ring (quinolactacide Go.1.2) was isolated from *P. citrinum* Thom F 1539 (200). The additional ring could be formed from quinolactacin C by the oxidation of a methyl group at C-1′, decarboxylative elimination (–CO_2 and –H_2O), and subsequent oxidative cyclization (Scheme 16).

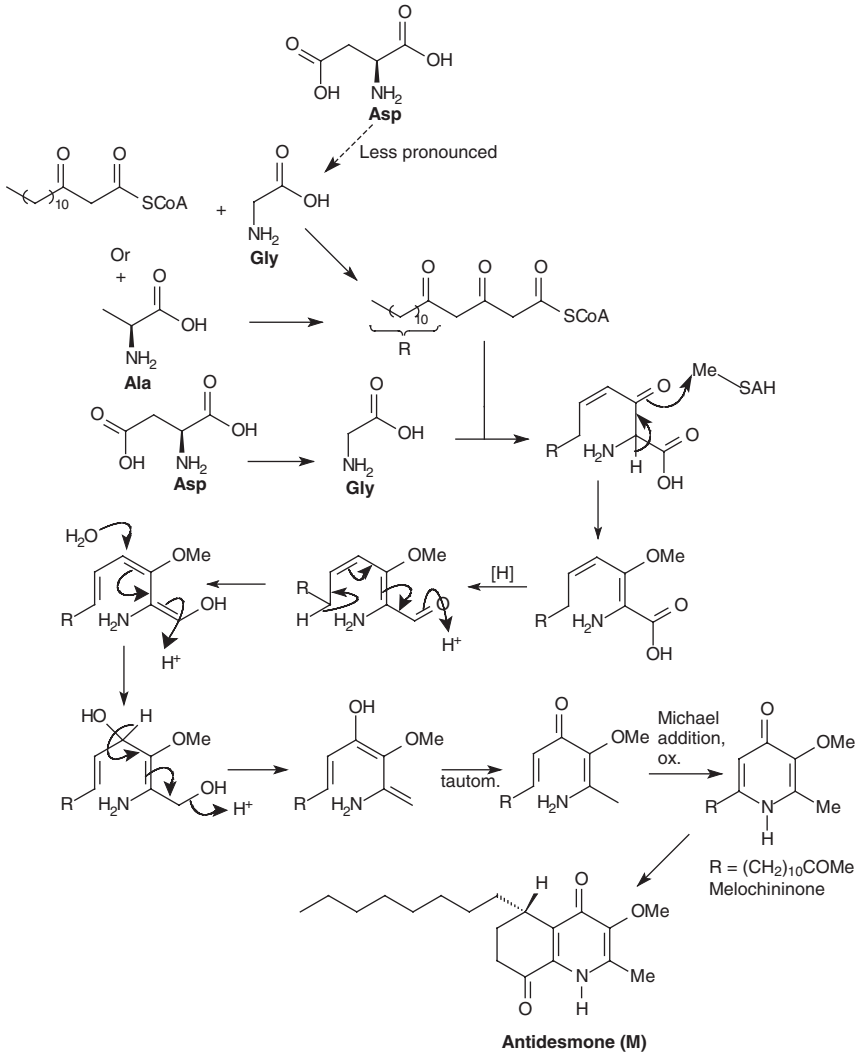
The second synthesis, which suggested that the pyrrolo[3,4-*b*]quinolin-4(1*H*)-one system is derived from tryptamine (201), is an alternative biogenetic pathway (Scheme 17). This route using the keto acid derived from the amino acid L-leucine (or L-valine) by transamination and tryptamine, under some stereochemical control, can also form the quinolactacins stereospecifically as the C-9 (*S*)-diastereomer (Scheme 17). Biological oxidation of the indole ring by TDO (see Scheme 5) could form the 3-hydroperoxy-β-carboline. Subsequent intramolecular addition of the 3-hydroperoxy group to an iminium ion at C-2, followed by heterolytic cleavage of the peroxide bond would give the dione intermediate, which can rearrange to the quinolin-4(1*H*)-one. For convenience, the first proposals were codified and used in Tables X and XI. Therefore, such a proposal cannot yet be regarded as the main pathway, and experimental evidence is needed.



Scheme 17 Biogenesis of quinolactacins A2 and B from tryptamine.

Antidesmone, known from several species of *Antidesma* and *Hyeronima* (Euphorbiaceae), is a quinolin-4(1*H*)-one alkaloid with an unusual substitution pattern (Table IX). It carries a linear aliphatic side chain that indicates at least a partial acetogenic origin for the carbon skeleton. Feeding experiments with ^{13}C- and ^{15}N-labeled precursors administered to cell cultures of *Antidesma membranaceum* yielded antidesmone, which was investigated by NMR spectroscopy (216). Accordingly, it was shown that antidesmone is constructed from a linear C_{16}-polyketide chain and a C_2 unit derived directly from glycine (Scheme 18). Acetate and, unexpectedly, alanine and glycine, were efficient sources of the polyketide chain, which indicated the conversion of both amino acids into acetyl-CoA. The transformation of glycine into acetyl-CoA apparently represents a new biosynthetic pathway. Very significantly, [U-$^{13}C_2$, ^{15}N]glycine appeared as an intact C_2N fragment in the pyridone ring, its CO_2H forming the 2-CH_3 substituent. Alanine, a more plausible source of the C_2N unit, was not incorporated at these positions at all. A final noteworthy feature of the feeding experiments was the specific incorporation of [U-$^{13}C_4$]aspartic acid into the 2-CH_3 substituent and, less prominently, into the acetate-derivative positions, which suggests the novel conversion of this amino acid into both acetyl-CoA and glycine. Glycine also served as the precursor of the methyl group, which probably occurs via *S*-adenosyl methionine (216).

Antidesmone is thus an uncharacteristic quinoline alkaloid, since its formation does not follow the biosynthetic route from either tryptophan or anthranilic



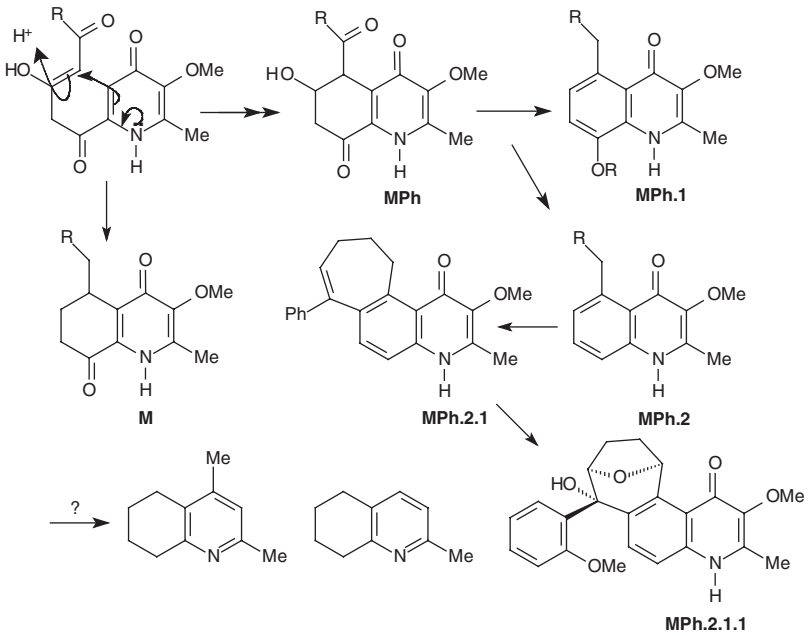
Scheme 18 Biosynthesis of antidesmone in cell cultures of *Antidesma membranaceum* (216).

acid (216). This study permitted a major structural revision of antidesmone alkaloids and the identical metabolite hyeronine A from *Hyeronima oblonga* from the same family, which had been assigned as an isoquinoline derivative (166). Thus, hyeronine A and B were included as quinolin-4(1*H*)-ones analogs (Table IX).

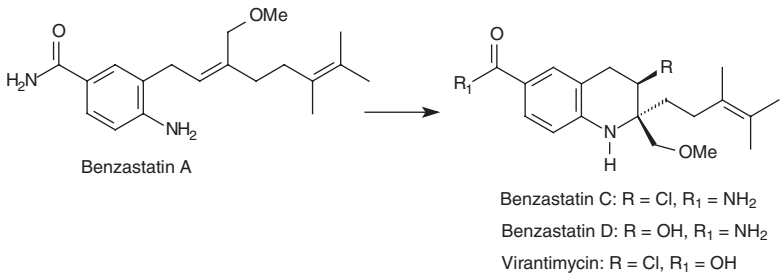
The carbocyclic ring is probably formed after the closure of the heterocycle, as suggested by the existence of the possible precursor analog melochininone (Scheme 18) from *Melochia pyramidata* (Sterculiaceae) (217,218). Cinnamoyl-CoA may be the starter unit of the polyketide chain, rather than acetyl-CoA, in

melochinone (MPh.2.1) from *M. tomentosa* (Table IX) (169), having a less saturated structure (Scheme 19) (216). Two unusual tetrahydroquinolines, 2-methyl-5,6,7,8-tetrahydroquinoline and 2,4-dimethyl-5,6,7,8-tetrahydroquinoline (Table VIII), were isolated from *Glycyrrhiza uralensis* (Fabaceae) (158), which are less obviously ascribable to alkaloids derived from a polyketide unit and glycine (Scheme 19).

The benzastatins, a group of tetrahydroquinoline antibiotics from the genus *Streptomyces*, appear to be biogenetically derived by the oxidative cyclization of another simpler metabolite, benzastatin A (Scheme 20) (219–224). Since these alkaloids do not have their origins in the fundamental tryptophan or anthranilic routes, they are not included in this review.



Scheme 19 Biogenesis of alkylquinolin-4(1*H*)-one alkaloids derived from a polyketide and glycine.



Scheme 20 Biogenesis of the benzastatins.

III. BIOLOGICAL ACTIVITY

Interest in the 2-substituted quinolin/ones alkaloids has accelerated rapidly with the finding that these alkaloids display marked leishmanicidal activity. Since then, a wide spectrum of other biological properties for these alkaloids has been discovered. In this review the latest developments disclosed for the biological activities of the 2-substituted quinolin/ones alkaloids are presented.

A. Antiprotozoal Activity

The need for novel and more selective agents to treat protozoal diseases remains. The classic examples of these diseases are Chagas disease, sleeping sickness, leishmaniasis, and malaria.

1 Leishmaniasis

Leishmaniasis is a group of tropical diseases caused by a number of species of protozoan parasites belonging to the genus *Leishmania*. In humans, *Leishmania* spp. cause a variety of clinical diseases, and these have been used by the World Health Organization as the basis to classify leishmaniasis into four clinical forms: visceral, mucocutaneous, cutaneous diffuse or disseminated, and cutaneous. Certain species of the parasite have been associated with the different clinical forms of the disease; *Leishmania donovani* complex, for example, causes visceral leishmaniasis, while the *Leishmania tropical* complex is known to induce cutaneous and cutaneous diffuse leishmaniasis in several countries of Latin America.

During its biological cycle, parasites of the *Leishmania* genus exist in two forms that develop in a different host, a flagellated extracellular form known as a promastigote and an intracellular one designated as an amastigote. The form that infects both humans and other vertebrate hosts is the promastigote. The vertebrate host is infected with the promastigote form of the parasite as a result of a sting by the vector insect (*Phlebotomus* and *Lutzomyia*). After this, the promastigotes are quickly phagocytized by the macrophages of the host and inside the promastigotes change to the amastigote form. The clinical manifestation of the disease is a consequence of the multiplication of the amastigotes inside the macrophages. Drugs currently used to cure leishmaniasis are derivatives of pentavalent antimony, which are potentially toxic and are generally administered via the parenteral route in a hospital setting for relatively long periods of time. In many endemic regions, the classic treatments are too expensive or are unavailable to the population suffering from cutaneous leishmaniasis (225).

Most of the studies directed towards the detection of plant secondary metabolites with leishmanicidal activity have been performed using the promastigote form of the parasite because it is easier to maintain under *in vitro* conditions. However, since the promastigote is not the infective form of the parasite in vertebrate hosts, evaluations done with them provide only an indicative value of the possible leishmanicidal activity of the metabolite tested. Such studies

must be complemented with an evaluation using intracellular amastigotes in macrophages. Interest in the 2-substituted quinolines has accelerated rapidly with the finding by Fournet and his colleagues in the early 1990s (44,45,227–229) that these alkaloids display marked leishmanicidal activity.

a. *Alkaloids from plant sources. Galipea* Aubl. is a neotropical genus of the Rutaceae, with around 14 species occurring from Costa Rica in Central America to Brazil and Bolivia (226). New combinations in this genus were made recently, and all of the *Galipea* species cited here belong to the genus *Angostura* (226). However, in order to facilitate the search of the original literature, all of the *Galipea* species are cited here as they appeared originally. *Galipea* species are known to contain several 2-alkyl- and 2-alkylarylquinolines (Table I). Poultices prepared from the stem bark of the tree *Galipea longiflora* have been used traditionally by the Chimane Indians of Bolivia for the treatment of cutaneous leishmaniasis.

The alkaloids chimanine B [2-(*E*)-prop-1-enylquinoline] and chimanine D [2-(1,2-*trans*-epoxypropyl)quinoline], isolated from *G. longiflora*, showed activities at an IC_{90} of 25 μg/mL against the promastigotes of *L. braziliensis*, while 2-*n*-propylquinoline and 4-methoxy-2-phenylquinoline showed activities at an IC_{90} of 50 μg/mL (44). When tested *in vivo* against cutaneous lesions caused by *L. amazonensis* and *L. venezuelensis*, 2-*n*-propylquinoline was more potent than the standard *N*-methylglucamine antimonite at a concentration of 100 mg/kg/day. The alkaloids 2-(3,4-methylenedioxyphenylethyl)quinoline, cusparine, 2-(3,4-dimethoxyphenylethyl) quinoline, chimanine B, 2-*n*-pentylquinoline, and 2-phenylquinoline, also isolated from *G. longiflora*, were as effective as the reference drug (45,227).

2-*n*-Propylquinoline, when administered orally (0.54 mmol/kg), suppresses by 99.9% the presence of *L. donovani* parasites in the liver of BALB/c mice after 10 days of treatment. Subcutaneous treatment with chimanine D for 10 days at 0.54 mmol/kg per day resulted in 86.6% parasite suppression in the liver (228). Further investigation on oral treatment and intralesional injection of chimanine B showed that it reduced lesion weight and parasite loads substantially, and demonstrated improved performance over the reference drug (229).

Additional studies have been undertaken on the *Galipea* alkaloids, chimanine B, 2-(pent-1-enyl)quinoline and 2-*n*-pentylquinoline (Table I) which showed modest activity against *L. amazonensis* and *L. infantum* amastigotes (231). Some of these findings have been reviewed, and 2-*n*-propylquinoline has been proposed as a new oral treatment for visceral leishmaniasis after it was found to have decreased intestinal P-glycoprotein activity in mice infected with *L. donovani* (230,232).

The rutaceous genus *Dictyoloma* Juss. contains only two species, *D. vandellianum* Adr. Juss. (syn. *D. incanescens* DC), which occurs in Brazil, and *D. peruvianum* Planch. from Peru (Engler, 1931; 233). Both species are also widespread in Bolivia. *D. peruvianum* is a small tree used in traditional medicine for the treatment of leishmaniasis. *D. vandellianum* has yielded 2-alkylquinolin-4(1*H*)-ones, and both species have afforded a group of piperidino[1,2-a]quinolin-4(1*H*)-one derivatives, including dictyolomide A and B (Table III). These alkaloids cause the total lysis of

the promastigotes of *L. amazonensis* at 100 μg/mL, and show minor activity on the promastigotes of *L. braziliensis* at the same concentration (90).

2 Chagas Disease

Chagas disease is a protozoosis caused by the flagellate protozoan *Trypanosoma cruzi*, which belongs to the Kinetoplastida order and the Trypanosomatidae family. Four main evolutionary forms can be identified during the *T. cruzi* life cycle. The trypomastigote is the infective flagellate form of the parasite found in the blood of the mammalian host (blood trypomastigote) and in the terminal part of the digestive and urinary tracts of vectors (metacyclic trypomastigote). Metacyclic trypomastigotes are more infective than blood trypomastigotes. The epimastigote is the replicative form of the parasite in the insect vector and in an acellular culture medium. The amastigote is the intracellular replicative form of the parasite in the vertebrate host. The spheromastigote is found in the stomach of the vector (234). Besides low efficacy, the drugs nifurtimox and benznidazole that are currently available have strong side effects. A number of 2-substituted quinolines were found to display antitrypanosomal activity.

a. *Alkaloids from plant sources.* The alkaloid chimanine B shows activity at an IC_{90} of 25 μg/mL against *T. cruzi* (Tulahuen), while 2-*n*-propylquinoline and chimanine D show activity at an IC_{90} of 50 μg/mL (44). Chimanine B was more potent than the standard drug benznidazole (IC_{90} of 50 μg/mL) (44). The effects of oral treatment with benznidazole and 2-*n*-propylquinoline were evaluated in BALB/c mice infected with *T. cruzi* chronically. The reference drug and 2-*n*-propylquinoline were administered 60 days post-infection for 30 days at 25 mg/mL. At 35 days post-treatment, the serological tests (ELISA) of the 2-*n*-propylquinoline-treated mice were significantly different from the controls ($p = 0.01$) and the benznidazole-treated mice ($p = 0.03$), while this was not the case at 85 days post-treatment (235). Further investigation has been undertaken on the *Galipea* alkaloids for evaluation as antiprotozoal agents. Chimanine B, 2-(pent-1-enyl)quinoline, and 2-*n*-pentylquinoline (Table I) showed modest activity against *T. brucei* trypomastigotes and *T. cruzi* amastigotes (231).

1-Methyl-2-nonylquinolin-4(1*H*)-one and 2-nonylquinolin-4(1*H*)-one, isolated from the Brazilian shrub *Raulinoa echinata* (Table II), showed moderate antitrypanosomal activity against the trypomastigote forms of *T. cruzi* with IC_{50} values of 134.9 and 100.9 μg/mL, respectively (63).

3 Malaria

Human malaria is caused mainly by four species of *Plasmodium*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. falciparum*. Of these four species, *P. falciparum* is responsible for the vast majority of the 300–500 million episodes of human malaria infestation worldwide, and accounts for 0.7–2.7 million deaths annually. *P. vinckei* was first isolated in 1952, although it was not recognized as an independent species until 1975. Four subspecies are recognized, *P. vinckei vinckei*, *P. vinckei petteri*, *P. vinckei lentum*, and *P. vinckei brucechwatti*. The parasite is readily grown in mice and laboratory-reared thicket rats, where it shows a preference for mature red blood cells. Infections are synchronous with a periodicity of 24 h. Malaria

parasites are transmitted by female *Anopheles* mosquitoes. *A. gambiae* is one of the best known species, because of its predominant role in the transmission of the most dangerous *P. falciparum*. *P. vinckei* may be transmitted by *Anopheles stephensi* mosquitoes over a wide temperature range. The parasites multiply within red blood cells, causing symptoms that include anemia, light headedness, shortness of breath, tachycardia, etc., as well as other general symptoms such as fever, chills, flu-like illness, and in severe cases, coma and death. Malaria infections are treated through the use of antimalarial drugs, such as chloroquine, mefloquine, or pyrimthamine. Drug resistance is increasingly common and a very serious clinical issue globally (236,237). These factors have increased the urgency of the search for novel antimalarial agents.

a. *Alkaloids from Plant Sources*. *G. longiflora* is also used as a traditional medicine in Bolivia for the treatment of recurrent fevers, such as malaria. The crude alkaloidal extract isolated from the bark of this species shows antiplasmodial activity in mice infected with the malaria-causing parasite *Plasmodium vinckei petteri*. The alkaloids identified in this extract, 2-propylquinoline, 2-pentylquinoline, chimanines B and D, 4-methoxy-2-phenylquinoline, and 2-(3,4-methylenedioxyphenylethyl)quinoline proved effective when tested separately against the parasite, while 2-pentylquinoline was especially active, showing approximately the same level of activity as the well-known antimalarial compound chloroquine (237).

Some *G. officinalis* tetrahydroquinolines, including cuspareine, galipeine, galipinine (Table VI), and angustureine (Table I), have been shown to possess antimalarial activity against both chloroquine-sensitive and chloroquine-resistant strains of the malaria parasite *P. falciparum*, with galipinine being the most active compound (IC_{50} 0.09–0.9 μg/mL for the resistant strain) (238).

b. *Alkaloids from Microbial Sources*. 2-*n*-Nonylquinolin-4(1*H*)-one, 2-*n*-undecylquinolin-4(1*H*)-one, and 2-[(1*E*)-undec-1-enyl]quinolin-4(1*H*)-one isolated from *Pseudomonas* sp., also showed activity against *P. falciparum* (ID_{50} 1–4.8 μg/mL) (181). These results are sufficiently encouraging to continue the development of 2-substituted quinolin/ones as antileishmanial, antitrypanosomal, and antimalarial drugs. The structural requirement for optimal antiprotozoal activity is principally the quinoline skeleton; for antimalarial activity, quinolin-4(1*H*)-ones and 1,2,3,4-tetrahydroquinolines also showed significant effect. In addition, this review shows that the plant species *Galipea bracteata*, *G. officinalis*, *Angostura trifoliata*, *Zanthoxyllum avicennae*, *Esenbeckia leiocarpa*, *Ruta montana*, *R. chalepensis*, *R. graveolens*, *Lunasia amara*, *Haplophyllum dubium*, *H. latifolium*, and the microbial species *P. aeruginosa*, *P. cepacia*, *P. fluorescens*, *Pseudonocardia* sp., *Arthrobacter* sp., *S. aurantiaca*, and *Streptomyces nitrosporeus* could be potential sources of lead compounds for the rational design of novel agents to treat protozoal diseases.

B. Molluscicidal Activity

Schistosomiasis or bilharzia is a disease affecting many people in developing countries. The most common way of acquiring schistosomiasis in developing

countries is by wading or swimming in lakes, ponds, and other bodies of water which are infested with the snails (usually of the *Biomphalaria*, *Bulinus*, or *Oncomelania* genus) that are the natural reservoir of the *Schistosoma* pathogen. *Schistosoma mansoni* is found in parts of South America and the Caribbean, Africa, and the Middle East; *S. haematobium* is in Africa and the Middle East; and *S. japonicum* is in the Far East. *S. mekongi* and *S. intercalatum* are focally in Southeast Asia and central West Africa, respectively. Freshwater snails of the genus *Biomphalaria* are intermediate snail hosts for the transmission of the medically important *S. mansoni*. Of the seven species of *Biomphalaria* transmitting schistosomiasis in the Western Hemisphere, *B. glabrata* is the most important and the best studied experimentally. It is found mostly in South America and the Greater and Lesser Antilles, where the snails occupy habitats that are often temporary due to frequent floods and droughts. They are dispersed to new habitats during times of flooding. A few countries have eradicated the disease, and many more are working towards it. Snail control by means of molluscicides is today considered as an auxiliary method within the integrated control of schistosomiasis (239).

a. *Alkaloids from Plant Sources*. Studies of the molluscicidal activity of 2-substituted quinolin/one alkaloids have been limited. Only three showed activity against *Biomphalaria glabrata*, 2-*n*-pentylquinoline, and 2-(pent-1-enyl) quinoline at 20 and 10 ppm, respectively, and the 2-alkylarylquinoline, 2-(3,4-methylenedioxy-phenylethyl)quinoline, at a concentration of 5 ppm (43). These alkaloids were isolated from *G. bracteata* (Tables I and VI). Clearly, this class of alkaloid deserves more attention as potential molluscicidal agents.

C. Antimicrobial Activity

Discovery of new antimicrobial substances is an important research objective, due to the continuing evolution of microbial resistance in medicine and agriculture. A number of plant 2-substituted quinolin/ones have shown potent antimicrobial activity. Their potential in medicine and in agriculture is discussed separately.

1 In Medicine

a. *Alkaloids from microbial sources*. 2-*n*-Heptyl-4-hydroxyquinoline and 2-*n*-pentyl-4-hydroxyquinoline (Table X), isolated from a marine pseudomonad *Alteromonas* sp., displayed antibiotic activity at 50 μg/disc against *Staphylococcus aureus* (<6 mm, >6 mm, respectively), *Escherichia coli* (no activity), *Vibrio harveyi* (<6 mm), and *Vibrio anguillarum* (<6 mm) (171).

Aurachins A–D (Tables X and XI), isolated from the Gram-negative bacteria *S. aurantiaca*, were active against numerous Gram-positive bacteria *Bacillus subtilis* (MIC 5, 2.5, 0.15, 0.15 μg/mL, respectively aurachins A–D), *S. aureus* (MIC 2.5, 1.25, 0.39, 0.39 μg/mL), *Arthrobacter aurescens* (MIC 0.19, 0.78, 0.19, 0.19 μg/mL), *Brevibacterium ammoniagenes* (MIC 0.39, 1.25, 0.05, 0.05 μg/mL),

Corynebacterium fascians (MIC 1.56, 1.56, 0.78, 0.78 μg/mL), *E. coli* (all MIC > 50 μg/mL). At higher concentrations they were also weakly active against the fungus, *Debaryomyces hansenii* and the yeast, *Saccharomyces cerevisiae* (179).

2-Heptyl-4-hydroxyquinoline *N*-oxide, obtained from *P. aeruginosa* (Table X), showed mild to strong activities against *S. aureus, B. sphaericus*, and *B. subtilis* (strong), *S. aureus, B. circulans, Listeria monocytogenes*, and *Kurthia zopfii* (moderate), *B. cereus, L. innocua, L. seeligeri*, and *L. welshimeri* (mild), and no activity against *B. licheniformis* (173). 1-Hydroxy-2-(non-2-enyl)-3-methylquinolin-4(1*H*)-one isolated from *Arthrobacter* sp. YL-02729S (Table XI) showed moderate activity against Gram-positive bacteria, including *B. subtilis* and multiple-drug resistant *S. aureus* and *S. epidermidis* (195).

The alkaloids 2-geranylquinolin-4(1*H*)-one, 1-methyl-2-geranylquinolin-4(1*H*)-one, 2-geranyl-3-methylquinolin-4(1*H*)-one, 1,3-dimethyl-2-geranylquinolin-4(1*H*)-one, 1-methylthiolmethyl-2-geranyl-3-methylquinolin-4(1*H*)-one, 1-methyl-2-(1-hydroxygeranyl)quinolin-4(1*H*)-one, 1-methyl-2-(3-hydroxy-3,7-dimethyl-octa-1,6-dienyl)quinolin-4(1*H*)-one, 1,3-dimethyl-2-(6,7-epoxygeranyl)quinolin-4(1*H*)-one, isolated from *Pseudonocardia* sp. CL38489 (Table XI), each proved to be active in inhibiting the growth of *Helicobacter pylori* (implicated in the formation of gastric and duodenal ulcers). The most potent compound was the epoxy derivative, which had a significant bactericidal effect (MIC 0.1 ng/mL), and an even more pronounced bacteriostatic effect (MIC 0.1 ng/mL). However, the most striking aspect of the activity of these compounds was their specificity. Their inactivity towards microorganisms other than *H. pylori* offers the prospects for therapeutic use as antiulcer agents because they are less likely to disturb the normal gastrointestinal microbial flora (196). 2-Nonyl-4-hydroxyquinoline *N*-oxide, isolated from *Pseudomonas* sp. (Table XI), was active against *S. aureus* (20 mm/20 μg) (181).

Quinolactacins A, B, and C, isolated from *Penicillium* sp. EPF-6 (Table XI), were inactive towards a wide range of bacteria, including *B. subtilis*, *S. aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, fungi *Candida albicans*, *S. cerevisiae*, *Aspergillus niger*, *A. fumigatus*, *Mucor hiemalis*, *Trichophyton interdigitale*, and the yeasts *Schizosaccharomyces pombe*, *Rhodotorula acuta*, and *Cryptococcus* sp. Very weak activity was apparent only against *A. niger* (198).

Similar alkaloids quinocitrines A and B, obtained from *P. citrinum* (Table X), showed moderate activity against *B. subtilis*, *S. aureus, E. coli, Enterococcus faecalis, Mycobacterium smegamatis*, *C. albicans,* and *Penicillium* sp., and no activity against *P. aeruginosa* and *Sporobolomyces almonicolor* (186). Helquinoline isolated from *Janibacter limosus* (Table X), showed moderate activity against *B. subtilis*, *Streptomyces viridochromogenes*, and *S. aureus*. But it was inactive against *E. coli, C. albicans*, and *Mucor miehei*, and the micro-algae *Chlorella vulgaris, C. sorokiniana*, and *Scenedesmus subspicatus* (175). Quinolactacin A2 isolated from *P. citrinum* (Table XI), was found to have moderate activity against *Candida neoformans* and *Aspergillus fumigatus*, and no activity against *C. albicans* and *A. niger* (240).

b. *Alkaloids from plant sources*. Transtorine, the keto tautomer of kynurenic acid (4(1*H*)-quinolone-2-carboxylic acid, Table IX) and obtained from *Ephedra transitoria* (Ephedraceae), inhibited the growth of the common bacteria *Enterobacter cloacae, P. aeruginosa*, and *S. aureus* (MIC 0.45, 0.5, and 0.38 mg/mL, respectively), but

was inactive towards several other bacteria (161). The similar alkaloid 8-methoxy-4(1*H*)-quinolone-2-carboxylic acid, isolated from *Lappula squarosa* (syn. *L. echinata*, Boraginaceae, Table IX) was found to have antibacterial activity against *P. aeruginosa* ATCC 27853, enteropathogenic *E. coli* (EPEC) 0111, *Klebsiella pneumoniae*, and *Staphylococcus epidermidis* (202).

The alkaloids isolated from *Galipea officinalis*, cusparine, galipine, and demethoxycusparine were found to be more active against *Mycobacterium tuberculosis* than the tetrahydroquinoline cuspareine (Table VI). However, the bulk of the anti-TB activity resided in the unidentified polar basic fraction from the bark extract (50,137).

Evocarpine, 1-methyl-2-[(*Z*)-tridec-7-enyl]quinolin-4(1*H*)-one, 1-methyl-2-undecylquinolin-4(1*H*)-one, dihydroevocarpine, 1-methyl-2-pentadecylquinolin-4(1*H*)-one, 1-methyl-2-[(4*Z*,7*Z*)-tridec-4,7-dienyl]quinolin-4(1*H*)-one, and 1-methyl-2-[(6*Z*,9*Z*)-pentadec-6,9-dienyl]quinolin-4(1*H*)-one from *Euodia ruticarpa* (Table II) showed strong antibacterial activity against *H. pylori*, both *in vitro* and *in vivo*, by inhibiting respiration. The results reinforced previous indications of this class of alkaloids as novel, potential therapeutic agents for the treatment of ulcers, as shown above with the alkaloids from microbial sources (68,241–243). The related alkaloids 1-methyl-2-*n*-nonylquinolin-4(1*H*)-one, 2-*n*-nonylquinolin-4(1*H*)-one, and 1,2-dimethyl-quinolin-4(1*H*)-one from *Boronia* (Table II) showed mild to moderate activities against *B. subtilis*, *S. aureus*, *Sarcina lutea*, exterotoxigenic *E. coli*, *Salmonella typhi*, and *Klebsiella* sp. (244).

Further investigation has been undertaken on the *Euodia ruticarpa* alkaloids (1-methyl-2-*n*-undecylquinolin-4(1*H*)-one, 1-methyl-2-[(*Z*)-undec-6-enyl]quinolin-4(1*H*)-one, 1-methyl-2-[(4*Z*,7*Z*)-tridec-4,7-dienyl]quinolin-4(1*H*)-one, 1-methyl-2-[(6*Z*,9*Z*)-pentadec-6,9-dienyl]quinolin-4(1*H*)-one, and 1-methyl-2-[(*Z*)-tridec-7-enyl]quinolin-4(1*H*)-one (Table II) for evaluation against *Mycobacterium fortuitum*, *M. smegmatis*, and *M. phlei*. The last alkaloid showed the most potent activity against all three mycobacteria with MIC values of 2 μg/mL (245).

The antibacterial activity of waltherione-A, isolated from *Waltheria douradinha* (Sterculiaceae) (Table IX) and its *O*-methylated derivative were evaluated by means of direct bioautography in a TLC bioassay. The former alkaloid was not active against all bacteria tested (*S. aureus*, *Streptococcus epidermidis*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Salmonella setubal*, and *E. coli*), while its *O*-methyl derivative was active against all of them. The detection limits were 25.0, 3.5, 6.5, 12.5, 12.5, and 6.5 μg, respectively (170).

2 In Agriculture

a. *Alkaloids from microbial sources*. 3-Methyl-2-(hept-2-enyl)quinolin-4(1*H*)-one and 3-methyl-2-(non-2-enyl)quinolin-4(1*H*)-one, obtained from *Pseudomonas cepacia* (Table XI), exhibited similar antibiotic activity against several fungal (*Pythium ultimum*, inhibitory activity at 10 μg/mL, 37.7 and 0%, respectively; *Fusarium oxysporum*, 34.4 and 41.1%; *Rhizoconia solani*, 50.9 and 58.1%; *Verticillium dahliae*, 82.8 and 74.9%; *Gaeumannomyces graminis*, 17.1 and 54.7%; *Pyricularia oryzae*, 70.8 and 49.9%; and *Cochliobolus miyabeanus*, 63.9 and 68.0%) and bacterial plant pathogens (*Agrobacterium tumefaciens*, MIC >50 and >20 μg/mL; *Corynebacterium*

michiganense, 10, 10 μg/mL; *Erwinia carotovora*, >50 and >20 μg/mL; *Pseudomonas solanacearum*, >50 and >20 μg/mL; and *Xanthomonas campestris* pv *oryzae*, 50 and >20 μg/mL) (192).

In a search for natural antagonists of the soil-borne pathogen *Phytophthora capsici*, which is responsible for "phytophthora blight" in red peppers (*Capsicum annuum*), the culture broth of *P. cepacia* strain PC-II was screened by bioactivity-guided fractionation. While 2-pentyl-3-methylquinolin-4(1*H*)-one, 2-heptyl-3-methylquinolin-4(1*H*)-one, 2-nonyl-3-methylquinolin-4(1*H*)-one, and 2-(non-2-enyl)-3-methylquinolin-4(1*H*)-one isolated from this extract proved effective when tested separately; 2-(hept-2-enyl)-3-methylquinolin-4(1*H*)-one (Table XI) was the most active against *P. capsici* and other fungal plant pathogens. Furthermore, when red pepper seeds were treated with this alkaloid before planting, their growth was significantly enhanced (191).

b. *Alkaloids from plant sources*. The antifungal activity of the *R. graveolens* alkaloids was evaluated against seven plant pathogenic fungi. 2-*n*-Nonylquinolin-4(1*H*)-one (Table II) inhibited the growth of *Colletotrichum acutatum* by 50% at 300 μM, which was comparable to benomyl (the standard fungicide) activity (33%) at the same concentration. This alkaloid also had similar activity against *C. fragariae*, but was less active than benomyl (76%). At 300 μM, it was active against *F. oxysporum*, inhibiting growth by 37%, but it was less fungitoxic than benomyl and captan (commercial fungicide) (77 and 100%, respectively). Against *Phomopsis obscurans* and *P. viticola* it gave 100% inhibition at 100 and 30 μM, respectively. However, it precipitated at 300 μM in both experiments, thereby reducing its effectiveness (139). 2-Nonylquinolin-4(1*H*)-one, isolated from Brazilian shrub *R. echinata* (Table II), was weakly fungicidal towards *Leucoagaricus gongylophorus*, a symbiotic fungus of leaf-cutting ants (63).

Two 2-alkylarylquinolin-4(1*H*)-one alkaloids (Table VII) were also evaluated. The activities against *C. acutatum* and *C. fragariae* of 1-methyl-2-[6-(3,4-methylenedioxyphenylhexyl]quinolin-4(1*H*)-one were similar to those of 2-alkylquinolin-4(1*H*)-one above, which inhibited the growth of both fungi *ca.* 57% at 300 μM. This alkaloid also showed activity against *C. gloeosporioides*, with 67.7% inhibition at 100 μM, and against *Botrytis cinerea* with high activity at 30–300 μM, similar to that of benomyl. At a concentration of 30 μM, 2-[4-3,4-methylenedioxyphenylbutyl]quinolin-4(1*H*)-one and 1-methyl-2-[6-3,4-methylenedioxyphenylhexyl]quinolin-4(1*H*)-one inhibited *P. obscurans* and *P. viticola* growth 100%, similar to the activity levels of the fungicide standards (139). The only 2-arylquinolin-4(1*H*)-one alkaloid evaluated was graveoline (rutamine, Table IV), which showed activity against *C. fragariae* and *F. oxysporum* at 300 μM, and *P. obscurans* at 100 μM, but was less active than the standard fungicides in all bioassay (139).

Further studies with these alkaloids using *in vivo* systems (fungi on plant tissue) are needed in order to further determine their potential as leads for commercial fungicides (139). Filamentous fungi of the genera *Botrytis*, *Colletotrichum*, *Fusarium*, and *Phomopsis* species are all considered to be major plant pathogens worldwide. Failure to control these fungi can result in serious economic losses to global agriculture. Anthracnose (caused by *Colletotrichum* spp.) and Phomopsis

stem and leaf blight (caused by *Phomopsis* spp.) are serious problems for fruit production in many areas of the world (139).

It is noteworthy that most of the quinolin/ones alkaloids which show high antimicrobial activity are of the 2-alkylquinolin-4(1*H*)-one type, suggesting that the species cited in Tables II and XI should be accorded high priority with respect to further investigation for antimicrobially active alkaloids.

D. Miscellaneous Biological Activities

1 Toxicity to Brine Shrimp

a. *Alkaloids from plant sources.* Evocarpine and dihydroevocarpine (Table II), well-known alkaloids from the medicinally important fruits of *Euodia ruticarpa*, showed toxicity in the brine shrimp test (LC_{50} 0.77 and 21.4 μg/mL, respectively) (246). Hyeronines A and B (Table IX) were also active in the brine shrimp lethality assay (LC_{50} 4 ppm for both) (247).

2 Inhibitory Effect on Germination

a. *Alkaloids from plant sources.* 2-*n*-Propylquinoline, 2-*n*-pentylquinoline, 2-(pent-1-enyl)quinoline (Table I), and 2-(3,4-methylenedioxyphenylethyl)quinoline (Table VI), isolated from *G. bracteata*, showed an inhibitory effect on shoot and root growth of lettuce (*Lactuca sativa*) at concentrations higher than 500 ppm, while at 100 ppm only the two latter alkaloids showed activity (43). Graveoline, from *R. graveolens* (Table IV), inhibited the germination of representative monocot and dicot seeds (bentgrass and lettuce, respectively), substantially impeded the growth of duckweed, an aquatic plant, and reduced cell division. These results suggest that alkyl-, aryl-, and alkylarylquinolines should be investigated as potential biodegradable, environmentally friendly herbicides (248).

3 Enzyme Inhibitory Activity

a. *Alkaloids from microbial sources*. Cytochromes *bo* and *bd*, two terminal respiratory oxidases found in *E. coli* and many other bacteria, catalyze the oxidation of ubiquinol by molecular oxygen. It was recently shown that the 2-alkylquinolin-4(1*H*)-one alkaloid aurachin C is an extremely potent inhibitor of the quinol oxidation sites of both enzymes. Binding appears to be far tighter than that of any previously described inhibitors known to affect the quinoline binding site. Aurachin D is also a highly effective inhibitor of cytochrome *bd* (249). Aurachin C and D were isolated from *S. aurantiaca* a Gram-negative bacteria (Table XI) (179). Twelve derivatives based on aurachin C and D were tested against cytochrome *bd* and *bo*, and they possessed inhibitory activity (249). 1-Hydroxy-2-(non-2-enyl)-3-methylquinolin-4(1*H*)-one, isolated from *Arthrobacter* sp. (Table XI), possessed potent inhibitory activity against lipoxygenase (195). Quinolactacins A1 and A2, isolated from *P. citrinum* (Table XI), were evaluated in an acetylcholinesterase assay and A2 showed 14-times higher inhibitory activity in a dose-dependent fashion with an IC_{50} of 280 μmol (199). Aurachins A–D (Tables X and XI) blocked NADH oxidation (179). They appear to be as potent inhibitors of the

eukaryotic respiration chain as 2-heptyl-4-hydroxyquinoline *N*-oxide from *P. aeruginosa* (250).

b. *Alkaloids from plant sources.* Evocarpine, 1-methyl-2-[(6*Z*,9*Z*)-pentadec-6,9-dienyl]quinolin-4(1*H*)-one, and 1-methyl-2-[4*Z*,7*Z*)-tridec-4,7-dienyl]quinolin-4(1*H*)-one isolated from *Euodia ruticarpa* fruits (Table II), were shown to be blockers of angiotensin II receptor binding (IC_{50} 43.4, 34.1, and 48.2 μM, respectively) (251). Evocarpine, 1-methyl-2-tetradecylquinolin-4(1*H*)-one, 1-methyl-2-[(4*Z*,7*Z*)-tridec-4,7-dienyl]quinolin-4(1*H*)-one and 1-methyl-2-[(6*Z*,9*Z*)-pentadec-6,9-dienyl]quinolin-4(1*H*)-one were tested as diacylglycerol acyltransferase (DGAT) inhibitors. The moderate activity of these alkaloids (IC_{50} 69.5, 23.8, 20.1, and 13.5 μM, respectively) indicates possible utility in the design of hypolipidemic and anti-obesity agents. These alkaloids were obtained from *E. ruticarpa* (Table II) (68). Another *Euodia* alkaloid, 1-methyl-2-undecylquinolin-4(1*H*)-one (Table II), has proved to be an irreversible and selective inhibitor of type B monoamine oxidase (K_i 0.01 μM), suggesting potential value in the treatment of neurological disorders such as Parkinson's and Alzheimer's diseases, and Huntington's chorea (252).

Leukotrienes, such as LTB_4, are highly potent mediators of inflammation and allergic reactions. They are formed in human neutrophils by 5-lipoxygenase (5-LOX) which catalyses the first step in the conversion of arachidonic acid. Experience has shown that secondary plant metabolites, especially those from medicinal herbs long used in traditional medicine against inflammatory disorders, are highly promising candidates for the discovery of new antiinflammatory leads. In order to obtain a fast method suitable for high throughput screening of herbal drugs and their constituents for leukotriene biosynthesis inhibitory activity, Adams and his colleagues (253) developed an *ex vivo* test system on a microtitre scale on the basis of a commercial LTB_4 ELISA immunoassay (EIA) kit using activated human leukocytes. The alkaloids isolated from *E. rutaecarpa* fruits, 1-methyl-2-*n*-nonylquinolin-4(1*H*)-one, 1-methyl-2-[(*Z*)-undec-6-enyl]quinolin-4(1*H*)-one, 1-methyl-2-[(4*Z*,7*Z*)-tridec-4,7-dienyl]quinolin-4(1*H*)-one, evocarpine, and 1-methyl-2-[(6*Z*,9*Z*)-pentadec-6,9-dienyl]quinolin-4(1*H*)-one (Table II), exhibited inhibitory activity on leukotriene biosynthesis in a bioassay using human polymorphonuclear granulocytes with IC_{50} values of 12.1, 10.0, 10.1, 14.6, and 12.3 μM, respectively (253).

4 Nuclear Factor of Activated T Cells – NFAT Inhibitor

a. *Alkaloids from plant sources.* The alkaloids 2-*n*-nonylquinolin-4(1*H*)-one, 2-*n*-undecylquinolin-4(1*H*)-one, 1-methyl-2-*n*-nonylquinolin-4(1*H*)-one, 1-methyl-2-undecylquinolin-4(1*H*)-one, dihydro-evocarpine, 1-methyl-2-pentadecylquinolin-4(1*H*)-one, evocarpine, 1-methyl-2-[(4*Z*,7*Z*)-tridec-4,7-dienyl]quinolin-4(1*H*)-one, and 1-methyl-2-[(6*Z*,9*Z*)-pentadec-6,9-dienyl]quinolin-4(1*H*)-one from fruits of *E. ruticarpa* (Table II), were found to affect the expression of NFAT (nuclear factor of activated T cells) and NF-κB proteins in Jurkat T cells without compromising cell viability. Excessive activation of these nuclear factors, which regulate the transcription of cytokine genes implicated in the immune response, can provoke undesirable reactions such as inflammation, autoimmunity, and transplant

rejection. The inhibitory activity against NFAT (IC_{50} 0.91–15.91 μM) improved with increasing length of the aliphatic substituent, although the *N*-methyl alkaloids 1-methyl-2-*n*-nonylquinolin-4(1*H*)-one and 1-methyl-2-undecylquinolin-4(1*H*)-one were less potent than their desmethyl analogs, 2-*n*-nonylquinolin-4(1*H*)-one and 2-*n*-undecylquinolin-4(1*H*)-one. These findings indicated interesting possibilities for developing new therapies based on quinolone leads for treating diseases of the immune system (61).

5 Inhibitory Effect on Tumor Necrosis Factor (TNF) Production

a. *Alkaloids from microbial sources*. Quinolactacins A, B, and C, isolated from *Penicillium* sp., were assayed for TNF production modulation. Only quinolactacin A inhibited LPS-induced TNF production by murine peritoneal macrophages in a dose-dependent manner with an IC_{50} of 12.2 μg/mL (198).

6 Anti-HIV Activity

a. *Alkaloids from plant sources*. 2-(Pent-1-enyl)quinoline and 2-*n*-pentylquinoline, obtained from *A. trifoliata*, *G. bracteata*, and *G. longiflora* (Table I) demonstrated *in vitro* activity against HIV-1 replication (IC_{50} 7.9 and 2.9 μM, respectively) (231). 2-*n*-Undecylquinolin-4(1*H*)-one, isolated from *R. graveolens* and *Pseudomonas* sp. (Tables II and XI), also showed activity against HIV-1 (IC_{50} 0.001 μg/mL) (181).

7 Cytotoxic Activity

a. *Alkaloids from plant sources*. In an investigation of the biological activity of *R. graveolens* metabolites, graveoline (Table IV) was found to be cytotoxic towards the HeLa cancer cell line (ED_{50} 3.35 μg/mL) (254). A related alkaloid from *G. longiflora*, 2-(3,4-methylenedioxyphenylethyl)quinoline (Table VI), inhibited the growth of cells infected with human T-lymphotropic virus type 1 (HLTV-1) at a concentration of 10 μM, but was less effective than a number of synthetic quinolines (255).

Some *G. officinalis* tetrahydroquinolines, including cuspareine, galipeine, galipinine (Table VI), and angustureine (Table I), were cytotoxic toward human fibroblast cells, and cuspareine was the most effective (IC_{50} 5.8–8.5 μg/mL) (238). A mixture of 1-methyl-2-[(*Z*)-pentadec-10-enyl]quinolin-4(1*H*)-one and 1-methyl-2-[(*Z*)-pentadec-9-enyl]quinolin-4(1*H*)-one, evocarpine, and dihydroevocarpine, isolated from *Euodia officinalis* (Table II), showed moderate cytotoxic activity against cultured human colon carcinoma (HT-29), human breast carcinoma (MCF-7) and human hepatoblastoma (HepG-2). These alkaloids were inactive against topoisomerases I and II (256).

b. *Alkaloids from microbial sources*. 2-Nonyl-4-hydroxyquinoline *N*-oxide, isolated from *Pseudomonas* sp. (Table X), showed cytotoxicity toward KB cells with an IC_{50} of less than 2 μg/mL (181). 1-Hydroxy-2-(non-2-enyl)-3-methylquinolin-4(1*H*)-one (Table XI), isolated from *Arthrobacter* sp., was cytotoxic to HeLa S3 cells *in vitro* (IC_{50} 0.59 μg/mL) (195). Quinocitrinines A and B, obtained from *P. citrinum* (Table X), showed moderate antiproliferative activity (IC_{50}, μg/mL) on L-929 cells (A: 33.2, B: 18.6), K-562 cells (A: 19.5, B: 7.8), and HeLa cells (A: >50, B: >59) (186).

8 Antimutagenic Activity

a. *Alkaloids from plant sources*. 5-Hydroxy-1-methyl-2-phenylquinolin-4(1*H*)-one, isolated from *Casimiroa edulis* (Table IV), were assayed for antimutagenic activity (*Salmonella typhimurium*, MMOC and EROD). However, it did not show promise as a chemopreventive agent (124).

9 Antiplatelet Activity

Platelets are a type of blood cell. They play a key role in normal blood clotting, the purpose of which is to stop bleeding. During the clotting process, platelets clump together to plug small holes in damaged blood vessels. The number of platelets in blood can be affected by many diseases. Platelets may be counted to monitor or diagnose diseases or identify the cause of excess bleeding. If the number is higher than normal (thrombocytosis), this may be associated with: Polycythemia vera, post-splenectomy syndrome, primary thrombocytosis, certain malignancies, early chronic myelogenous leukemia (CML), and anemia. Theoretically, antiplatelet agents can be developed that target each step in the platelet activation or inhibition mechanisms. For example, the antiplatelet activity of aspirin was discovered while studying cyclo-oxygenase inhibitors. Numerous new antiplatelet agents were developed based on their inhibitory effects on platelet activation. Nonetheless, the number of antiplatelet agents available for clinical use is still insufficient, and deleterious side effects are associated with most of the existing agents. Therefore, the search for an improved antiplatelet agent is still an important goal for the pharmaceutical industry.

a. *Alkaloids from plant sources*. During routine antiplatelet screening, Huang and colleagues (257) discovered that 2-phenylquinolin-4(1*H*)-one showed aspirin-like, antiplatelet activity. Since the physiochemical properties of this alkaloid are quite different from those of aspirin, and its antiplatelet activity had not been previously reported, they synthesized a series of potential isomers. The alkaloid 2-phenylquinolin-4(1*H*)-one inhibited arachidonic acid AA-induced platelet aggregation in a concentration-dependent manner. Its IC_{50} value was 9.63 μM, twice the potency of aspirin (IC_{50} 20.0 μM) (257). These data stimulated other synthetic efforts (258) and additional biological screening on rutaceous alkaloids. However, with respect to 1-methyl-2-(3,4-methylenedioxyphenyl)quinolin-4(1*H*)-one (graveoline) and 4-methoxy-2-(3,4-methylenedioxyphenyl)quinoline (graveolinine) isolated from *R. graveolens*, only the latter showed significant inhibition of platelet aggregation, induced by arachidonic acid and collagen (254).

10 Muscle Relaxant Activity

a. *Alkaloids from plant sources*. The ability of *Orixa japonica* extracts to act as relaxants of rat jejunum smooth muscle has been traced to two alkaloids, eduline and japonine (Table IV), the effects of which were comparable to that of the typical muscle relaxant papaverine (118).

11 Estrogenic Activity

a. *Alkaloids from plant sources*. Two, 2-substituted quinolin-4(1*H*)-ones from *Haplophyllum* species were evaluated for estrogenic activity in animals (estrogenic activity, effect on the menstrual cycle, and embryogenesis). The 2-alkylquinolin-4(1*H*)-one alkaloid acutine, at a dose of 10 mg/kg, increased the uterine mass by 21.5% without fluid, and the mass of the ovaries by 3.4%. However, 1-methyl-2-phenylquinolin-4(1*H*)-one exhibited only a weak estrogenic effect (259). The first alkaloid was isolated from *Haplophyllum acutifolium* (Table II), and the latter from *H. foliosum*, *H. perforatum*, and *H. leptomerum* (Table IV).

12 Antifeedant and Insecticide Activities

a. *Alkaloids from plant sources*. Leiokinine A and B (Table II), isolated from *E. leiocarpa*, showed moderate antifeedant activities against the pink bollworm *Pectinophora gossypiella* (79).

b. *Alkaloids from microbial sources*. Quinolactacide (Table XI), obtained from *P. citrinum*, demonstrated excellent insecticidal activity against green peach aphids (*Myzus persicae*) (200).

IV. ALKYL, ARYL, ALKYLARYLQUINOLINE, AND RELATED ALKALOIDS: THEIR DISTRIBUTION AND SYSTEMATIC SIGNIFICANCE

A. Distribution and Systematic Significance in Higher Plant Taxa

1 Rutaceae

The ''classical'' systematics of the Rutaceae elaborated by Engler (233,260) subdivided it into seven subfamilies of which the Rutoideae, Toddalioideae, and Aurantioideae, are large. All three sub-families were subdivided into a number of tribes and sub-tribes. The four remaining sub-families, Flindersioideae, Dictyolomatoideae, Spathelioideae, and Rhabdodendroideae, are all small and their relative positions in the family are contentious.

Saint-Hilaire (261–263) cited *Cusparia*, *Angostura*, and *Conchocarpus* among the synonyms of *Galipea* Aubl., and described several new species in *Galipea* and two in *Ticorea* Aubl. Those described in *Galipea* belong to *Conchocarpus*, and those described in *Ticorea* belong to *Galipea*. His erroneous circumscriptions of these genera were perpetuated by de Candolle (264), Jussieu (265), and Bentham and Hooker (266). Engler, in his treatments of the Rutaceae in Martius's *Flora Brasiliensis* (267) and in *Die Natürlichen Pflanzenfamilien* (233,268), defined these genera somewhat more clearly. He limited *Ticorea* to species with five fertile stamens by transferring to *Galipea* the two species (with two fertile stamens) described by Saint-Hilaire. He limited *Galipea* to species with connate carpels by excluding from it the apocarpus species, i.e., the species described by Saint-Hilaire (which, as noted above, belong in *Conchocarpus*) and the type of *Angostura*, which he called *Cusparia trifoliate* (Willd.) Engl. He recognized these

as one genus, *Cusparia*, still confounding *Angostura* and *Conchocarpus*. He also failed to realize that his *G. bracteata* (Nees & Mart.) Engl. was congeneric with his *C. trifoliate*.

These errors were rectified recently by Kallunki and Pirani (269) and the genus *Angostura* Roem. & Schult., as understood by Engler, was defined more narrowly (269). The species excluded from *Angostura* were recognized as species of *Conchocarpus* J. C. Mikan. Three new species of *Angostura* (*A. alipes* Kallunki, *A. quinquefolia* Kallunki, and *A. simplex* Kallunki) were described, and three new combinations in this genus were made [*A. bracteata* (Nees and Mart.) Kallunki, *A. granulosa* (Kallunki) Kallunki, and *A. longiflora* (K. Krause) Kallunki]. They recognized seven species of *Angostura*, the six latter and its type *A. trifoliata* (Willd.) T. S. Elias [syn., *Bonplandia trifoliata* Willd., *Cusparia febrifuga* Humb., *A. cuspare* Roem. & Schult., *Galipea cusparia* A. St.-Hil. ex DC., *B. angostura* Spreng., *G. officinalis* J. Hancock, *Sciuris officinalis* (J. Hancock) Oken, *C. trifoliata* (Willd.) Engl., *Portenschlagia trifoliata* Pohl ex Engl., *G. febrifuga* (Humb.) Baillon, *B. angostura* Rich. Ex B. D. Jacks, *C. officinalis* (J. Hancock) Engl., and *C. angostura* (Rich. ex B. D. Jacks) A. Lyons]. However, all of the *Galipea* species are cited here as they appear in the original literature. The major source of both 2-alkyl and 2-arylquinolines is the bark of the S. American tree *A. trifoliata* (Willd.) T. S. Elias (Tables I and VI).

Waterman (270), studying the systematic distribution of alkaloids, coumarins, limonoids, flavonoids, and chromones in the Rutaceae, proposed to regard those genera producing 1-benzyltetrahydroisoquinolines as the most primitive representatives within the family, which were termed the proto-Rutaceae. The evolutionary trend is for replacement of this alkaloid type by the apparently more ''modern'' anthranilate-derived alkaloids. Reduction or total lack of alkaloids in various groups of Rutaceae indicates further advances and evolutionary progress. These deductions were incorporated by Waterman (270) into a phylogenetic diagram (Figure 2), which is very useful for examination of the distribution of alkaloids and to assess their systematic significance.

The reported 2-alkyl-, 2-aryl- and 2-alkylaryl-quinolin/one alkaloids from the Rutaceae are derivatives of anthranilic acid. For a discussion of the distribution of these alkaloids Waterman's delimitation was accepted. These alkaloids occur widely in the Rutoideae genera (Table XII). The tribes Zanthoxyleae, Ruteae, Boronieae, and Cusparieae are chemically very similar, containing many of these alkaloids in common. However, the Cusparieae exhibit considerable variation in their alkaloid content. Found in this tribe are simple quinoline (A.1), 4-oxygenated 2-alkylquinolines (B), 2-alkylquinolines (B.1), 2-alkylquinolin-4(1*H*)-ones (Bo), (Figure 2a), 4-oxygenated 2-arylquinoline (D), 2-arylquinoline (D.1), 2-arylquinolin-4(1*H*)-ones (Do), 4-oxygenated 2-alkylarylquinolines (E), and 2-alkylarylquinolines (E.1) (Figure 2b). The occurrence of 2-alkylquinolines and 2-alkylarylquinolines containing the heterocycle reduced at C-2, C-3, and C-4 (B.1.1, E.1.1, respectively) are restricted to the Cusparieae. Moreover, the 2-alkylquinolin-4(1*H*)-ones oxygenated at C-3 (Bo.1) and in the anthranilate ring (Bo.3) apparently occur only in the Cusparieae genera.

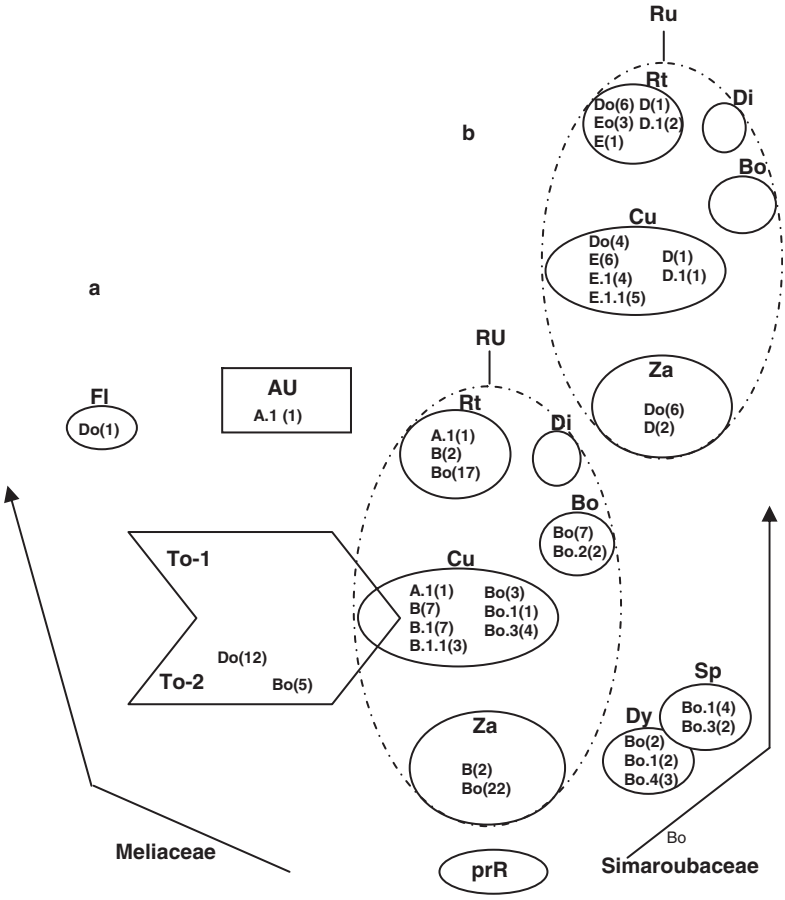


Figure 2 Schematic representation of Waterman's phylogenetic diagram of the Rutales; AU: Aurantioideae, RU: Rutoideae, Rt: Rutinae, Cu: Cusparieae, Di: Diosmeae, Bo: Boronieae, Za: Zanthoxyleae, To-1, To-2: Toddalioideae, Sp: Spathelioideae, Dy: Dictyolomatoideae, Fl: Findersioideae, prR: proto-Rutaceae (270). (a) Distribution of 2-alkylquinolin/ones, 2-aryl- and 2-alkylarylquinolin/ones. (b) In order to facilitate the analysis the shape representing Rutoideae (RU) was repeated to show the distribution of 2-aryl- and 2-alkylarylquinolin/ones in his tribes.

The Boronieae yielded simple 2-alkylquinolin-4(1*H*)-one alkaloids (Bo) and those possessing a methyl group at C-3 (Bo.2), which do not occur in the other Rutoideae tribes and are common in the bacteria (see Table XI). Genera in the Ruteae and Zanthoxyleae appear able to produce a considerable range of closely related 2-alkylquinolin-4(1*H*)-ones (Bo), with variation only in the aliphatic chains. The latter sub-tribe is relatively poor in arylated quinolines, and only 4-oxygenated 2-arylquinolines (D) and 2-arylquinolin-4(1*H*)-ones (Do) have been found. The former shares with Cusparieae the Do, E, D, and D.1 sub-types of

Table XII Number of alkaloids, classified into structural types (cf. Schemes 2, 12, 13), in the Rutaceae genera

Subfamily/tribe/genus	A.1	B	B.1	B.1.1	Bo	Bo.1	Bo.2	Bo.3	Bo.4	Do	D	D.1	Eo	E	E.1	E.1.1	AC.1	AC.2	AC.3	AC.4
Ru[a]-Zanthoxyleae																				
Zanthoxylum		2			1															
Euodia					20															
Orixa										2										
Lunasia										4	2									
Sarcomelicope																	1	2	1	2
Platydesma					1															
Ruteae																				
Boenninghausenia																				
Ruta	1	2			12					2	1		3	1						
Haplophyllum					5					4		1								
Dictamnus												1								
Boronieae																				
Boronia					7		2													
Cusparieae																				
Raulinoa					2					1										
Esenbeckia		1			1	1		4		3										
Galipea[a]		4	5								1	1		2	2					
Angostura[a]	1	2	2	3										4	2	5				
Dy[a]-*Dictyoloma*					2	2			3											
Fl[a]-*Flindersia*										1										
Sp[a]-*Spathelia*						4		2												
To[a]-Toddalieae																				
Balfourodendron										1										
Ptelea					1															
Casimiroa										7										
Vepris					3															
Acronychia					1															
Skimmia										4										
Au[a]-*Citrus*	1																			

[a] Ru: Rutoideae; Dy: Dictyolomatoideae; Fl: Flindersioideae; Sp: Spathelioideae; To: Toddalioideae; Au: Aurantioideae; New combinations in *Angostura* and *Galipea* were made recently, and all *Galipea* cited here belong in *Angostura* (268).

alkaloids, while the 2-alkylarylquinolin-4(1*H*)-ones (Eo) are at present unknown from any other source.

In contrast, the Toddalioideae seems to exhibit a markedly reduced ability to produce 2-substituted anthranilate alkaloids. The co-occurrence of Bo and Do in the genera of the Zanthoxyleae, Ruteae, Boronieae (except Do), Cusparieae, and Toddalieae (Figure 2; Table XII) suggests some affinity between the Rutoideae and Toddalioideae.

The Flindersioideae is a smaller sub-family of two genera, and only *Flindersia* has been reported to contain a 2-arylquinolin-4(1*H*)-one alkaloid (Do). The presence of only one alkaloid is of little chemosystematic value to clarify the classification of the subfamily. However, it does begin to reinforce its position close to the Toddalioideae in Waterman's diagram (Figure 2a).

The placements of the genera *Spathelia* L. and *Dictyoloma* Juss. have been doubtful since they were first described in Linnaeus (1762) and in de Jussieu (1825) (265), who assigned them to the Rutaceae. According to Bentham and Hooker (1862) (266), *Spathelia* and *Dictyoloma* belong to the Simaroubaceae. Engler (1874) (267) initially included these genera in the Simaroubaceae, but later moved them to the Rutaceae, placing *Spathelia* in the subfamily Spathelioideae and *Dictyoloma* in Dictyolomatoideae (Engler, 1931; 233). *Spathelia* and *Dictyoloma* are known to contain prenylated chromones and limonoids (78). However, prenylated chromones have only been reported from the genera *Spathelia*, *Dictyoloma*, and *Harrisonia*, as well as from the Cneoraceae and Ptaeroxylaceae (78). *Harrisonia* is a member of the Simaroubaceae, although it differs from other genera by its lack of quassinoids. Its limonoid constituents suggest a strong affinity with rutaceous genera such as *Spathelia* (78). Chromones have not been found in other Simaroubaceae or Rutaceae. Thus, according to Waterman (270), the co-occurrence of chromones in these taxa is phylogenetically significant by segregating them into a distinct group near the associated large family Rutaceae.

Analysis of DNA sequence data from members of the Cneoraceae, Meliaceae, Ptaeroxylaceae, Rutaceae, and Simaroubaceae showed that the Rutaceae are paraphyletic, with *Spathelia* and *Dictyoloma* (Rutaceae), *Harrisonia* (Simaroubaceae), *Cneorum* (Cneoraceae), and *Ptaeroxylon* (Ptaeroxylaceae) forming a clade sister to all other Rutaceae. Circumscription of Rutaceae to include all of these taxa was recommended (271,272). This analysis also indicated that the Simaroubaceae and the Meliaceae are the outgroups closest to the Rutaceae (271,272). The 2-alkylquinolin-4(1*H*)-one alkaloids that were recently isolated from *Dictyoloma vandellianum* showed strong similarities to the Zanthoxyleae, which contain several 2-alkylquinolin-4(1*H*)-ones. These data strongly supported Chase and Scott's taxonomic conclusions (271,272) and permitted Sartor *et al*. (78) to include the Dictyolomatoideae in Waterman's phylogenetic diagram (270) in a position between the proto-Rutaceae genera and the Spathelioideae, but close to the Zanthoxyleae (78). These taxa, as separated small figures (an expression of the number of species in the taxon), occupy a position between the advanced families Rutaceae and Simaroubaceae (Figure 2).

Further studies have been undertaken on *Spathelia excelsa* (80), and the finding of 2-alkylquinolin-4(1*H*)-ones showed similarities with the Zanthoxyleae, Ruteae, Boronieae, Cusparieae, Toddalieae, and Dictyolomatoideae providing firm support for moving the Spathelioideae close to, or within, the Dictyolomatoideae (Figure 2). Consideration of the biogenetic pathway for the 2-alkylated quinolones shows that the Dictyolomatoideae and Spathelioideae have in common some advanced alkaloids with the Cusparieae, e.g. Bo.1 and Bo.3. 2-Aryl- and 2-alkylarylquinolin/ones have otherwise been reported only from the latter, possible evidence of the derivation of these two subfamilies from an ancestor close to the more primitive Zanthoxyleae tribe.

Sarcomelicope species have yielded alkylquinoline alkaloids, which appear to be formed from highly oxygenated acridones by oxidative cleavage of ring A (Scheme 7). This genus is classified in the Zanthoxyleae. However, it does not appear to be able to produce the 2-alkylated and 2-arylated quinolines similar to those from other genera of the tribe. It contains diversified acridones, justifying the biogenetic pathway proposed in Scheme 7.

2 Simaroubaceae

The phytochemical analysis of two collections of a new species (SAC-2825), tentatively assigned as aff. *Samadera bidwillii* (Simaroubaceae), has yielded a limonoid, a quassinoid, three alkaloids (1-acetoxymethyl-2-(10ξ-acetoxyundecyl) quinolin-4(1*H*)-one and two acridones), and seven bicyclo-octane type lignans. The metabolites identified are collectively typical of the Rutales, but have a biosynthetic range never previously found together in a single species. Particularly noteworthy is the co-occurrence of limonoids and quassinoids in the same plant, which is currently unique to SAC-2825 (160). The presence of only one 2-alkylquinolin-4(1*H*)-one (Bo) in Simaroubaceae is of little chemosystematic value. However, it does reinforce its position near to the Dictyolomatoideae and the Spathelioideae in Waterman's diagram (Figure 2).

3 Families Distant from the Rutales

3-Phenylquinolines (A.1.2) are surprising metabolites of the Chinese medicinal plant *P. nigellastrum* (Zygophyllaceae), which likely are derived from quinoline, although a biogenetic pathway for their formation is not clear (Scheme 4). Quinoline (A.1) and 2-methylquinoline (quinaldine; B.1) have been isolated from *Peganum* sp. Interestingly, Engler (1896) (268) classified the genus *Peganum* in the Rutaceae. The same genus has also yielded unusual mixed alkaloids 3-(1*H*-indol-3-yl)quinoline (A.1.1) and quinoline–quinazoline (H), suggesting a clue to the link between the Rutales and the taxa placed in the Geraniales, such as the Zygophyllaceae. Dahlgren (273) included both orders in the superorder Rutanae.

Chemosystematic comments are of great value if the biosynthesis of the class of compounds in analysis is known, or if the compounds isolated provide evidence for a proposed route. 2-Alkylquinolin-4(1*H*)-ones with an unusual linear aliphatic side chain have been found in the Euphorbiaceae (M) and the Sterculiaceae (MPh.1, MPh.2). They are non-typical quinolones when compared

with those from the Rutaceae, since they did not follow a biosynthetic derivation from anthranilic acid. As discussed previously, they appear to be constructed from a linear C-polyketide chain and a C_2 unit derived directly from glycine (Scheme 19). This is not surprising, since these families do not even belong to the Rutanae, but, according to Dahlgren (273), to the superorder Malvanae, which are not closely related. In the Sterculiaceae the carbocyclic ring is aromatic, suggesting that cinnamoyl-CoA may be the starter unit of the polyketide chain. Thus, these phytochemical individualities strongly support Dahlgren's delimitation, which places the Euphorbiaceae in the order Euphorbiales, and the Sterculiaceae in the Malvales.

The sporadic occurrence of particular alkaloid types in unrelated taxa is a general phenomenon. Quinoline-2- and 4-carboxylic acid derivatives (I and J, respectively) are not formed via the common biosynthetic pathway involving the condensation of anthranilic acid and a β-keto intermediate. They are derived from tryptophan. The indole system is reactive toward electrophilic substitution at position 3, thus biological oxidation of the indole ring can form 3-hydroperoxy derivatives. Subsequent intramolecular addition of the 3-hydroperoxy group to an iminium ion at C-2, followed by heterolytic cleavage of the peroxide bond, gives the *N*-formyl kynurenine as an intermediate (Scheme 5). Alternatively, electrophilic addition of hydrogen at C-3, followed by Schiff base hydrolysis, gives an aniline derivative (Scheme 8). The former intermediate then affords quinoline-2-carboxylic acids (I), while the latter yields quinoline-4-carbaldehyde (J), which, by oxidation, leads to carboxylic acids (Schemes 5 and 8).

These alkaloids constitute two small groups, and only a few derivatives occur sporadically in microorganisms and higher plants. For example, 8-hydroxyquinoline-4-carbaldehyde oxime (J.3.1) and broussonetine (J.2.1), a dimeric quinoline, were isolated from *B. zeylanica* (Moraceae) (151), (+)-tuberosine B (J.1.2) from *A. tuberosum* (Alliaceae) (157), and 1,4-dimethylquinolinium iodide (J.4) (142) from *E. crassipes* (Pontederiaceae), families classified by Dahlgen in the distinct superorders, Malvanae, Lilianae, and Bromelianae, respectively. Kynurenic acid (I) is found in *E. pachyclada* ssp. *sinaica* (Ephedraceae), 6-hydroxy- and 6-methoxy derivatives [IO(6)] in *L. perezii*, *L. gmelinii* (Plumbaginaceae), *Ephedra* spp. and *E. pachyclada* ssp. *sinaica* (143,147,148), 8-methoxyquinolin-4(1*H*)-one-2-carboxylic acid [IoO(8)] in *L. squarosa* (Boraginaceae) (202) and transtorine (Io) in *Castanea mollissima* Blume (Fagaceae) (274). These families are very distant from each other. The Plumbaginaceae is included in the superorder Plumbaginanae, the Boraginaceae is placed in the Solannae, the Fagaceae is located in the Rosanae, and the Ephedraceae is a family of Gymnosperms.

B. Distribution and Systematic Significance in Microorganisms

1 Bacteria

The 2-alkylquinoline/4(1*H*)-one alkaloids isolated from some bacteria are typical of the Rutaceae. From the systematic standpoint, the co-occurrence of 2-*n*-nonylquinolin-4(1*H*)-one in *R. graveolens*, *R. echinata*, *Boronia bowmanii*, 2-*n*-undecylquinolin-4(1*H*)-one in the former, *Ptelea trifoliate* (Rutaceae), and

both in *Pseudomonas* (Proteobacteria) is astonishing. In addition, it is noteworthy that many of these alkaloids in bacteria were firmly established as derived from anthranilic acid, while the Rutaceae are, as yet, less biosynthetically investigated.

Analysis of the distribution of alkylquinolin/ones among the five genera of the Proteobacteria division showed that *Pseudomonas* spp. (Pseudomonadaceae) are the major sources of 2-alkylquinolin/4(1*H*)-ones [Bo (5), Bo.2 (5), B (8)]. There is a much less proliferation of this alkaloid type in *Alteromonas* (Alteromonadaceae) [B (2)]. Both produce these alkaloids with variation only in the aliphatic chains at C-2. These two families are included in the orders Pseudomonadales and Alteromonadales, respectively, and in the same class of Gammaproteobacteria (275). *Stigmatella*, which forms part of the Cystobacteraceae family (Myxococcales order and Deltaproteobacteria class), explore 2-alkylquinolin/4(1*H*)-ones chemistry along several different biosynthetic pathways, which leads to alkaloids containing a farnesyl moiety as a substituent [Bo.F (2), Bo.F.1 (1), B.1.F (1), B.1.F.1 (1), A.1.F (1), A.F (3), Scheme 3]. *Archangium* is also included in the family Cystobacteraceae. However, it has yielded 4-substituted quinolines derived (J, J.1, J.2, J.3) from tryptophan. In contrast, 4-substituted quinolines (J) could be taken as indicative of an affinity to the Myxococcales, where similar alkaloids occur in *Myxococcus* (Myxococcaceae) (J.2, J.3).

Of the four genera from the Actinobacteria division examined, notably *Pseudonocardia*, yield 2-alkylquinolin-4(1*H*)-ones with an uncommon substitution pattern containing a geranyl group at C-2 [Co (4), Co.1 (4)] instead of an aliphatic chain derived from β-keto fatty acids (Scheme 2). The four genera are included in the Actinomycetales order and the quinolin/ones are of sporadic occurrence in the three remaining genera. *Arthrobacter* (Micrococcaceae) contain 2-alkylquinolin-4(1*H*)-one with a methyl group at C-2 (Bo.2), while in *Janibacter* (Intransporangiaceae) alkylation of the quinoline skeleton appears at position C-8 as an oxidized methyl group (B.1.1). The presence of kynurenic acid (I) in *Streptomyces* (Streptomycetaceae) suggests this family as the more primitive, since this metabolite derives from L-kynurenine, the same precursor of anthranilic acid (Scheme 5).

Less is known of the occurrence of these alkaloids in the cyanobacteria. 4-Substituted quinolines [J.4.1 (2), J.4.1.1 (1)] have been recorded from only one genus *Lyngbya* (Oscillatoriaceae, Oscillatoriales order) (276) showing affinity to Proteobacteria.

It is premature to draw any definitive conclusions about the systematic significance of alkylquinolin/ones in bacteria on the basis of the presently insufficient phytochemical data. The above comments are only speculations.

2 Fungi

The quinolin/4(1*H*)-one alkaloids with a linear aliphatic side chain at C-2 appear to be absent in fungi. The wood-rotting fungi *Polyporus* grown in culture produce a 4-substituted quinoline (J.2). This genus is included in the Basidiomycota phylum, Basidiomycetes class and Polyporales order. The unrelated fungal genus *Penicillium* (Ascomycota phylum, Ascomycetes class and Eurotiales order) (277)

has yielded a novel class of quinolin/ones. They are based on the combination of amino acids L-valine and L-isoleucine, anthranilic acid, and acetic acid (215) (Schemes 15 and 16), or these amino acids and tryptamine (201) (Scheme 17). They constitute two small groups which can be considered as 2-substituted quinolin-4(1*H*)-ones: quinolactacins A1, A2, B, and C, quinolactacide; and 2-substitued quinolines: quinocitrinines A and B. Both alkaloid types are at present unknown from any other source. Thus, fungi from the Ascomycetes class deserve more attention in order to find new quinolactacins, since they showed interesting biological effects on tumor necrosis factor (TNF) (198).

C. Distribution and Systematic Significance in Marine Organisms

Marine organisms appear to be relatively poor in alkylquinolin/one alkaloids. In terms of numbers, thus far they are few. However, those alkaloids isolated constitute two new classes of alkylquinolines. One class is the 8-benzoylxanthurenic acid derivatives, termed tridemnic acids [IO(8).1], which occur in the ascidian *Tridemnum* species. The second class refer to *N*-(2-*p*-hydroxyphenyl)vinylxanthurenamide [IO(8), Perspicamides A and B], which have been found in the ascidian *Botrylloides* species. Thus, these biosynthetically related alkaloids suggest the close ties between *Tridemnum* and *Botrylloides*, genera of the Animalia kingdom, Chordata phylum, Tunicata subphylum, and Ascidiaceae class. *Botrylloides* belongs to the Stolidobranchia order and the Styelidae family, while *Tridemnum* is included in Aplousobranchia order and the Didemnidae family (278).

The bryozoan *Flustra* produces the 4-substituted quinoline (J.1.1), showing weak affinities with the two above ascidians. Its family, the Flustridae, is distant from those above, being classified in Animalia kingdom, Bryosoa phylum, Gymnolaemata class, Cheilostomata order (278).

V. SUMMARY AND CONCLUSIONS

The Rutaceae continues to be the primary source of new alkyl-, aryl-, and alkylarylquinolin/ones. In the past 17 years, the overall distribution of these alkaloid types within the family has changed little since the chemosystematics reviews by Waterman (270), Mester (40), and da Silva et al. (279). Alkylquinolones dominate the reported isolations with about 51% of the total, with arylquinolones (16%), alkylquinolines (15%), alkylarylquinolines (11%), arylquinolines (3%), alkylarylquinolones (2%), and quinolines (2%) as the significant structural groups contributing to the remainder of this class of alkaloids. The alkyl-, aryl-, and alkylarylquinolin/one alkaloids occur in 50 species belonging to 24 genera and 6 subfamilies. Despite the intensive chemical exploration of many species from other plants in the Rutales family, but not in the family Rutaceae, the first alkaloid alkylquinolone from a simaroubaceous plant (160) was not reported until 1997. Although many additional alkaloids have been reported, some of new structural types (Bo.4), substantial biosynthetic work on plant-derived alkylquinolin/ones has not yet been carried out. The biosynthesis

of some of these alkaloids in bacteria was firmly established as being derived from anthranilic acid.

Outside of the Rutales, alkyl-, aryl-, and alkylarylquinolin/ones have not been found, except for simple quinoline (A.1; only one) and 2-methylquinoline derivatives in the Zygophyllaceae, and only an atypical quinolone derivative (Ao.1) in the Asteraceae family. A few 3-phenylquinolines (2), 3-(1*H*-indol-3-yl)quinoline (1), and quinoline–quinazoline (1) alkaloids have been reported from only a single genus in the Zygophyllaceae. Tryptophan-derived quinolines in higher plants are confined to a few 2-carboxylicquinolin/ones (6) and 4-carbaldehydequinolines (5); the former found in the Ephedraceae (5), Boraginaceae (1), Fagaceae (1), Ginkgoaceae (1), Plumbaginaceae (1), Solanaceae (1), and Apiaceae (1), and the latter in the Moraceae (3), Alliaceae (1), and Pontederiacae (1). The number of quinolones derived from glycine and a polyketide is also limited. 5-Alkyl-2-methylquinolin-4(1*H*)-ones (8) occur in the Euphorbiaceae, and 5-alkyaryl-2-methylquinolin-4(1*H*)-ones ((3) in the Sterculiaceae.

Alkylquinolin/ones are well-known as typical alkaloids of three Proteobacteria and three Actinobacteria; the genus *Pseudomonas* yielded the majority (46%) of the total number of alkaloids reported (39). 2-Carboxylicquinolin/ones (4) and 4-carbaldehydequinolines (6) are minor constituents in both divisions of bacteria. More interesting are the quinolactacins (7), in which the second nitrogen is derived from L-valine or L-isoleucine, recently reported to occur only in the fungus *Penicillium*. Many of these diverse alkaloids have served directly as medicines or as lead compounds for the synthesis (258) of derivatives with an improved biological profile.

It is apparent from the summary view of the alkyl-, aryl-, and alkylarylquinolin/ones reported in the Rutaceae that they help to confirm the affinity between Rutoideae tribes and provide firm support for placing the Spathelioideae and the Dictyolomatoideae close to the more primitive Zanthoxyleae tribe. On the other hand, the bacteria and fungi are needed for more substantial chemical studies. When more data become available, it is likely that useful systematic correlations will emerge.

More detailed studies regarding the biosynthetic pathways of the alkyl-, aryl-, and alkylarylquinolin/ones in the Rutaceae and in bacteria are needed. Such studies would clarify the differences in the pathways based on their derivation from anthranilic acid in bacteria and in rutaceous plants.

Finally, this survey indicates that the Rutaceae, and various bacterial and fungal species offer considerable potential for the discovery of new or known alkaloids with significant and possibly valuable biological activities.

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