ASITRIBIN AND ASIMINENINS A AND B, NOVEL BIOACTIVE ANNONACEOUS ACETOGENINS FROM THE SEEDS OF *ASIMINA TRILOBA*

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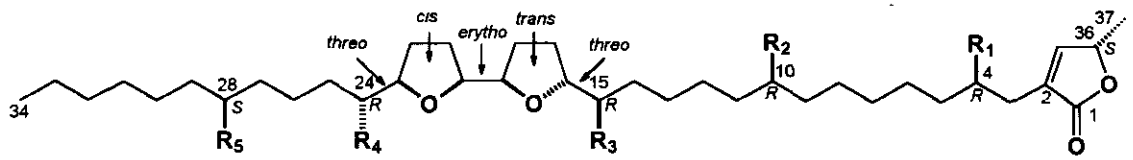
Abstract- A novel bioactive adjacent bis-tetrahydrofuran (THF) acetogenin, asitribin (**1**), and two novel bioactive mono-THF acetogenins with double bonds, asiminenins A (**2**) and B (**3**), were isolated from the seeds of *Asimina triloba* (Annonaceae) by directing the fractionation with brine shrimp lethality. In addition, asimicin, bullatacin, trilobacin (**4**) and trilobin (**5**), which are known, and parviflorin, which is known but is new in this species, were obtained **1** has an *erythro* configuration between the two THF rings, as do trilobacin (**4**) and trilobin (**5**), and is a structural isomer of **4** and **5** **1** showed potent cytotoxicities among six human solid tumor cell lines with notable selectivity for the lung cell line (A-549) **2** and **3** also exhibited potent and selective bioactivities across the six cell lines. **3** with the *trans* configuration of the mono-THF ring was more bioactive and selective than **2** with the *cis* configuration.

Asimicin was the first acetogenin isolated from the seeds and stem bark of the North American paw paw tree, *Asimina triloba* (L.) Dunal (Annonaceae).[1] Its highly potent antitumor and pesticidal activities suggested promising future medicinal and agricultural applications for this group of compounds. Further studies of the stem bark by Zhao *et al.* led to seven novel potently bioactive bis-THF acetogenins including trilobacin (**4**) and trilobin (**5**),[2-5] as well as five new, less potent, mono-THF ketolactones.[6] The mechanism of action of the Annonaceous acetogenins is *via* inhibition of NADH· ubiquinone oxidoreductase (complex I) in mitochondrial electron transport systems and the inhibition of NADH oxidase in the plasma membranes of tumor cells.[7-9] From the seeds, we have recently identified five new acetogenins, murisolin A, 16,19-*cis*-murisolin, asimilobin, and *cis*- and *trans*-murisolinones, in addition to murisolin and *cis*- and *trans*-bullata-

cinones.[10,11]

To continue our search for new anticancer and pesticidal constituents in this plant, the EtOH extract of the seeds was further investigated, using brine shrimp lethality (BST) to direct the fractionation.[12,13] This work has resulted in the isolation of a novel bioactive adjacent bis-THF acetogenin, named asitribin (**1**), and two new bioactive mono-THF acetogenins with double bonds, asiminenins A (**2**) and B (**3**); asimicin, bullatacin, trilobacin (**4**) and trilobin (**5**), which are known from the stem bark, were also found in the seeds, and parviflorin, which is known but is new in this species, was also isolated



Compounds	R_1	R_2	R_3	R_4	R_5
1	H	H	OH	OH	OH
1a	H	H	OAc	OAc	OAc
1b	H	H	OTMSi	OTMSi	OTMSi
1r	H	H	(*R*)-OMTPA	(*R*)-OMTPA	(*R*)-OMTPA
1s	H	H	(*S*)-OMTPA	(*S*)-OMTPA	(*S*)-OMTPA
4	OH	H	OH	OH	H
5	H	OH	OH	OH	H

Figure 1 Structures of asitribin (**1**), its derivatives (**1a**, **1b**, **1r**, and **1s**), trilobacin (**4**), and trilobin (**5**).

Compound **1** (Figure 1), mp 71-72°, was obtained as a colorless powder. The molecular formula was deduced as $C_{37}H_{66}O_7$ by the HRFABms which gave the $[MH]^+$ ion at *m/z* 623 4887 (Calcd 623.4886) The ir spectrum showed characteristic absorptions of α,β-unsaturated γ-lactone (1757 cm^{-1}, C=O) and OH (3448 cm^{-1}) functional groups The presence of the methylated α,β-unsaturated γ-lactone moiety was confirmed by the ^1H nmr resonance peaks at δ 6 99 (q, H-35), 5.00 (qq, H-36), and 1.41 (d, H-37), and ^{13}C nmr resonances at δ 173 91 (C-1), 134.34 (C-2), 148.83 (C-35), 77 39 (C-36) and 19.22 (C-37) (Table 1). Spectral comparisons of **1** with trilobacin (**4**), the 4- OH isomer, indicated that the H-3 signals appeared as a multiplet peak at δ 2.26 (tt, 2H) in the ^1H nmr spectrum of **1** instead of at δ 2.49 (dddd, Ha-3) and 2 37(dddd, Hb-3) in the ^1H nmr spectrum of **4**, and the upfield shift of the proton signals for H-35 and H-36 suggested that the 4-OH group was absent in the structure of asitribin (**1**); this is similiar to observations with the series of monohydroxyated 4-deoxyasimicin and 4-deoxybullatacin isomers.[3,4] Adjacent bis-THF rings with two flanking OH groups were suggested in **1** by the signals for protons at δ 3.84 (2H, H-16 and 23), 3.97 (H-19) and 4 08 (H-20) in the ^1H nmr spectrum and by the signals for four methine carbons at δ 83.01 (C-16), 81.69 (C-19), 80 97 (C-20) and 82.48 (C-23) (Table 1).[2-5] The signals for the oxygenated carbons of THF rings that lack adjacent OH groups usually appear at *ca.* δ 79; examples of such acetogenins are gigantecin, bullatalicin, gigantetrocin A, *cis*- and *trans*-bulladecinones and asimilobin [11,14-17]

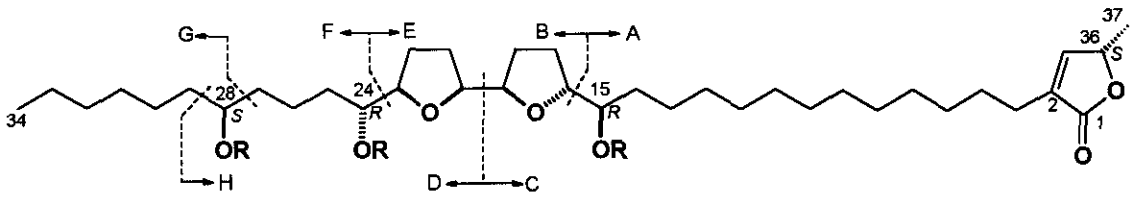
In the CIms of **1**, a series of peaks at *m/z* 605, 587, and 569, arising from the successive losses of three molecules of H_2O, were observed, confirming the presence of the three OH groups The OH groups were also identified by the formation of the triacetate (**1a**) which gave the expected molecular ion, M^+ at *m/z* 748 in the EIms, and exhibited two singlet proton peaks at δ 2 03 (3H) and 2.08 (6H) in the ^1H nmr (Table 1). The hydroxyls were further confirmed by the $[M]^+$ ion at *m/z* 743 in the triacetate derivative (**1a**) of **1**. The placements of the bis-THF ring system and the three OH groups of **1** along the aliphatic chain were determined based on the ms fragmentation pattern of the triTMSi derivative (**1b**) (Figure 2). The third OH of **1** was determined to exist at C-28 as in squamocin and asiminacin [3,18]

Table 1 ^1H Nmr (500 MHz) spectral data of **1** and **1a** and ^{13}C nmr (125 MHz) spectral data of **1** ($CDCl_3$, δ)

	δ_H (Hz)		δ_C		δ_H (Hz)		δ_C
No	**1**	**1a**	**1**	No	**1**	**1a**	**1**
1	-	-	173.91	23	3 84m	3 96m	82 48[a]
2	-	-	134 34	24	3.42m	4 85m	73 56
3	2 26tt(7 8,1 3)	2 26tt(7.8,1.3)	25 16	25	1 40-1 50m	1 42-1 58m	33 66
4	1 54m	1 54m	27 38	26	1 30m	1.29m	21.74
5-13	1 26br s	1.25br s	25.82-29.67	27	1.40-1.50m	1.42-1.58m	37 63[d]
14	1 40-1.50m	1 42-1 58m	34 37	28	3.59m	4 85m	71 77
15	3 36m	4 85m	74.58	29	1 40-1.50m	1.42-1 58m	37.31[d]
16	3 85m	3 96m	83.01[a]	30	1 30m	1.29m	25.65
17a	1 70m	1.60m	28.88[b]	31	1 26br s	1.25br s	29 73
17b	1.95m	2.04m	-	32	1.26br s	1.25br s	31 85
18a	ca 1.76-1 92[a]	ca 1 89-2.04[a]	28 20[b]	33	1 26br s	1.25br s	22.62
18b	ca 1 76-1 92[a]	ca.1 89-2 04[a]	-	34	0.88t(7.5)	0.88t(7.0)	14.09
19	3 97m[b]	3 81m[b]	81.69[c]	35	6 99q(1 5)	6 98q(1.5)	148 83
20	4 08m[b]	3.96m[b]	80 97[c]	36	5 00qq(6 8,1 4)	5.00qq(6 8,1.4)	77 39
21a	1 65m[a]	1.63m[a]	26.85[b]	37	1.41d(7.0)	1 41d(7 0)	19 22
21b	2 05m[a]	2.10m[a]	-	15-OAc		2.08s	
22a	1 70m	1.60m	28.25	24-OAc		2 08s	
22b	1 95m	2.04m	-	28-OAc		2 03s	

a,b,c,d Assignments may be interchangeable in each column.

The relative stereochemistry around the bis-THF rings was determined by comparing the ^1H and ^{13}C nmr signals of **1** and its triacetate (**1a**) with those of model compounds of known relative stereochemistry [19-21] The comparison suggested that the relative stereochemistries at C-15/16 and C-23/24 were *threo* from the signals for H-15 and H-24 at δ 3.36 and 3.42 in **1** and from the signals for the protons of the acetyl methyl of C-15 and C-24 at δ 2.08, and H-15 and H-24 at δ 2 03 in **1a**.[19,20] The relative stereochemistry between C-19 and C-20 was determined as *erythro* by the ^1H nmr signals for H-19 and H-20 at δ 3 97 and 4.08 in **1**, which are interchangeable, and at δ 3 81 and 3 96 in **1a**. So far, only two acetogenins, trilobacin (**4**) and trilobin (**5**), have been found that have an *erythro* relationship between the two THF



R	$MH^+/M(Ac)_3^+$ /$M(TMSi)_3^+$	A	B	C	D	E	F	G	H
H	623,605(a)	295,	327,309(a),	365,	257,239(a),	435*,	187,179,	155*,137*	537,519
(CIms)	587(a),569(a)	277(a)	291(a)	347(a)	221(a)	417*	151	197*	501
Ac	748,688(b)	337*,	411,351(b),	407,	341,281(b),	477*,	271,211,	137*	663,603,
(EIms)	628(b),568(b)	277(b)*	291(b)	347(b)	221(b)	417(b)	151	187,	543
TMS_1	838*.748(c)*	367,	471,381(c),	437,	401,311(c),	507,	331,241(c),	97(c)	753,663(c),
(EIms)	658(c)*,568(c)	277(c)	291(c)	347(c)	221(c)	417(c)	151(c)		573(c)

Figure 2 Diagnostic CIms and EIms fragmentation of **1** and its triacetate and tri-TMSi derivatives. (a) loss of H_2O (*m/z* 18), (b) loss of HAc (*m/z* 60); (c) loss of TMSi-OH (*m/z* 90) Ions indicated with asterisk (*) were not observed.

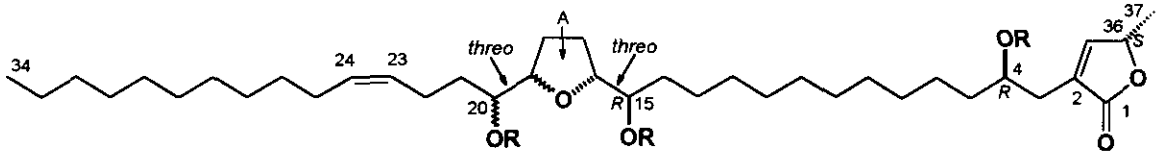
rings, the signals for the adjacent protons of the two rings were at *ca.* δ 4.0 and 3.9, respectively [2,5] On the other hand, the signals for the adjacent protons of the two THF rings with *threo* configurations between the two THF rings are observed at *ca.* δ 3.85 [14-16] ^1H and ^{13}C Nmr chemical shifts around the bis-THF rings were quite similiar to those of trilobin (**5**),[5] which has the relative stereochemistry of *threo/trans/erythro/cis/threo* between C-15 and C-24, except that the resonance of H-24 was shifted downfield (*ca.* 0 3 ppm) due to the 28-OH group in **1**, the third OH is at C-10 in **5** [5] Thus, the relative stereochemistries around the THF rings from C-15 to C-24 were concluded to be *threo/trans/erythro/cis/threo*. To determine the absolute stereochemistry of the carbinol centers at C-15, C-20 and C-28 in **1**, the tri -(*R*)- and -(*S*)-methoxytrifluoromethylphenylacetic acid (MTPA) esters (Mosher esters) (**1r** and **1s**) were prepared and analyzed by ^1H-^1H COSY.[22,23] The ^1H nmr chemical shift data of **1r** and **1s** showed that the absolute configurations at C-15 and C-20 are *R* and that at C-28 is *S* (Table 2). Consequently, the absolute configuration of **1** is C-15*R*, C-16*R*, C-19*R*, C-20*S*, C-23*R*, C-24*R*, and C-28*S*. The absolute con-

Table 2 ^1H Nmr chemical shifts (δ) for the determination of absolute configurations at C-15, C-24 and C-28 of the tri-(*S*)- and -(*R*)-MTPA esters of **1**.

MTPA	δ_H												
Config	14-Hab	16-H	17-Hab	18-Hab	19-H	20-H	21-Hab	22-Hab	23-H	25-Hab	27-Hab	29-Hab	34-H
R	1 44-	3 93	1 59	1 78	3 76	3 76	1 66	1 59	3 93	1 44-	1.44-	1 44-1.56	0.88
	1 56		1 94	1.96			2 02	1 94		*1.56*	*1 56*	*1.52-1 68*	
S	1 52-	3 93	1.46	1 72	3 63	3 69	1 46	1 51	3 96	1 52-	1 52-	_	0.86
	1.68		1 82	1 78			1 78	1.89		1.68	1 68		
	+	*0*[a]	-0.13	-0.06	-0 13	-0 07	-0.20	-0.08	-0 03	+	+		-0 02
			-0.12	-0 18			-0.24	-0 05					
Carbinol Config	C-15*R*									C-24*R*		C-28*S*	

figuration at C-36 is quite likely *S* as recently determined in **5** by cd [5] **1** is, thus, 28-hydroxy-4-deoxy-trilobacin and we named it asitribin.

Compound **2**, mp 58-59°, was obtained as an amorphous powder. A molecular weight at *m/z* 607 in the CIms (isobutane) spectrum of **2** (Figure 4) indicated a molecular weight of 606. The HRCIms (isobutane) spectrum showed an exact mass peak at *m/z* 607.4926, which matched the molecular formula $C_{37}H_{66}O_6$ (Calcd 607.4938). Spectral characteristics of **2** and its acetate (**2a**) and TMSi derivatives (**2b**), including ^{1}H nmr (Table 2), ^{13}C nmr (Table 2), and ms (Figure 4) data, suggested that **2** is a mono-THF Annonaceous acetogenin possessing a double bond in the aliphatic chain **2** showed six resonances at δ 7 19 (H-35), 5.06 (H-36), 3.83 (H-4), 2.40 (Ha-3), 2 53 (Hb-3) and 1 44 (H-37) in the ^{1}H nmr, and six peaks at δ 174 63 (C-1), 151.79 (C-35), 131.16 (C-2), 77.96 (C-36), 69.95 (C-4) and 19 08 (C-37) in the ^{13}C nmr spectrum (Table 3). These are all characteristic spectral features for the methylated α,β-unsaturated γ-lactone fragment, bearing a 4-OH, as is prevalent in many of the Annonaceous acetogenins [14-16] The uv (228 nm) and ir (1756 cm^{-1}) spectral data also supported the presence of the γ-lactone moiety, which was confirmed by a positive reaction to Kedde's reagent.[1,24,25]

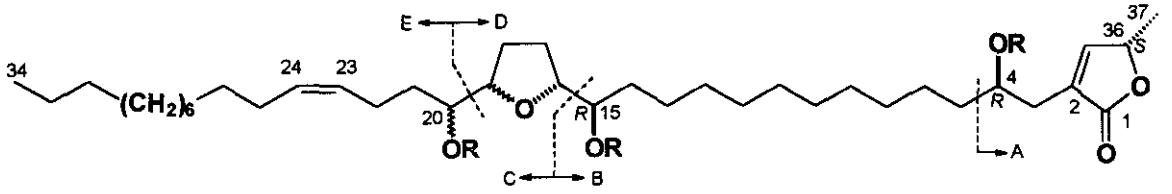


Compounds	A	R	Compounds	A	R
2	*cis*	H	**3**	*trans*	H
2a	*cis*	Ac	**3a**	*trans*	Ac
2b	*cis*	TMSi	**3b**	*trans*	TMSi
2r	*cis*	(*R*)-MTPA	**3r**	*trans*	(*R*)-MTPA
2s	*cis*	(*S*)-MTPA	**3s**	*trans*	(*S*)-MTPA

Figure 3. Structures of asuminenins A (**2**) and B (**3**) and their derivatives.

The ir spectrum of **2** contained an absorption peak for hydroxyls at 3411 cm^{-1}, this peak and sequential losses of three molecules of H_2O from the MH^+ in the CIms indicated that **2** has three OH groups. These were confirmed by the preparation of the triacetate derivative (**2a**) **2a** gave ^{1}H nmr peaks at δ 2 08 (6H, 2 OAc) and 2.03 (3H, OAc), and two multiplet proton resonances at δ 5 10 and 4 89 (2H) corresponding to the downfield shifts of three protons on secondary OH-bearing carbons as compared to **2.** The presence of the mono-THF ring with a flanking OH group on each side was indicated by the proton signals at δ 3 83 (2H, H-16 and 19), 3.43 (H-15), 3.43 (H-20), 1 94 (2H, H-17b and 18b) and 1 76 (2H, H-17a and 18a) in **2** and at δ 4.89 (2H, H-15 and 20) and 3.96 (2H, H-16 and 19) in **2a** and the carbon resonances at δ 82 68 (C-19), 82.61 (C-16), 74 31 (C-15), and 73 82 (C-20) in **2** These nmr data also indicated that the relative stereochemistries of the carbon centers at C-15/16 and C-19/20 were *threo* and the configuration across the

THF ring (C-16/19) was *cis*, by comparisons with series of model compounds of known relative stereochemistry.[19-21,26] The *cis* configuration of the mono-THF ring with two flanking OH groups has been recently reported in 16,19-*cis*-murisolin.[10] The carbon skeleton and the placement of the THF ring were determined on the basis of the EIms fragmentation of the triTMSi derivative (**2b**) of **2** (Figure 4)



R	Compound	$MH^+/M(Ac)_3^+$ /$M(TMSi)_3^+$	A	B	C	D	E
H (CIms)	**2**	607,589(a), 571,553(a)	141, 123(a)	311,293(a), 275(a)	295, 277(a)	381,363(a), 345(a)	225, 207(a)
H (CIms)	**3**	607,589(a), 571,553(a)	141, 123(a)	311,293(a), 275(a)	295, 277(a)	381,363(a), 345(a)	225, 207(a)
Ac (EIms)	**2a**	732,672(b), 612(b),552(b)	183, 123(b)	395,335(b), 275(b)	337, 277(b)	465,405(b), 345(b)	267*, 207(b)*
Ac (EIms)	**3a**	732,672(b), 612(b),552(b)	183*, 123(b)	395,335(b), 275(b)	337, 277(b)	465,405(b), 345(b)	267*, 207(b)*
TMSi (EIms)	**2b**	822,732(c), 642(c),552(c)	213, 123(c)	455,365(c), 275(c)	367, 277(c)	525,435(c), 345(c)	297, 207(c)
TMSi (EIms)	**3b**	822,732(c), 642(c),552(c)	213, 123(c)	455,365(c), 275(c)	367, 277(c)	525,435(c), 345(c)	297, 207(c)

Figure 4. Diagnostic CIms and EIms fragmentation ions of **2** and **3** and their triacetates (**2a**, **3a**) and tri-TMSi derivatives (**2b**, **3b**) (a). loss of H_2O (*m/z* 18); (b) loss of HAc (*m/z* 60), (c): loss of TMSi-OH (*m/z* 90). Ions indicated with asterisk (*) were not observed.

The presence of an isolated double bond in **2** was determined by the proton signals at δ 5.39 and 5.36 and the carbon signals at δ 130.77 and 128.96 When the protons were selectively decoupled at δ 2.20 (H-22) (which showed correlation cross peaks with the proton at δ 5 36 in the COSY spectrum), the proton at δ 5.36 became a doublet with *J*=11 Hz. This indicated that the double bond in **2** has a *cis* configuration [27] The position of the double bond was determined at C-23/24 from the single-relayed COSY spectrum, which showed correlation cross peaks between H-20 (δ 3.43) and H-22 (δ 2.20), and from the double-relayed COSY spectrum, which showed correlation cross peaks between H-20 (δ 3.43) and H-23 (δ 5.36) To determine the absolute stereochemistry of the carbinol centers at C-4, C-15 and C-20 in **2**, the tri-(*R*)- and -(*S*)-methoxytrifluoromethylphenylacetic acid (MTPA) esters (Mosher esters) (**2r** and **2s**) were prepared.[22,23] ^1H-^1H COSY analyses of these Mosher ester derivatives were then performed. The ^1H nmr chemical shift data of **2r** and **2s** showed that the absolute configurations at C-4 and C-15 are *R* and that at C-20 is *S* (Table 4). Hoye *et al.* synthesized (+)-*SS* (like) and (±)-*RS* (unlike) model butenolides and permitted the assignments of the relative configurations between C-4 and C-36 in acetogenins by using the magnitudes of the Δδ values for the ^1H and ^{19}F nuclei in their Mosher esters.[28,29] The $\Delta\delta_H$ values for H-35

Table 3 ^{1}H Nmr (500 MHz) spectral data of **2**, **2a**, **3** and **3a**, and ^{13}C Nmr (125 MHz) spectral data of **2** and **3** ($CDCl_3$, δ).

No	δ_H (Hz)				δ_C	
(H & C)	**2**	**2a**	**3**	**3a**	**2**	**3**
1	-	-	-	-	174 63	174.62
2	-	-	-	-	131.16	131 20
3a	2.40ddd(15.0,8 0,1 0)	2.51ddd(15.0,8.0,1.0)	2.40dd(15 0,8 5,1 0)	2.51ddd(15.0,8.0,1 0)	33 30	33.34
3b	2 53ddd(15.0,3 0,1 5)	2 57ddd(15 0,3.0,1.5)	2 53ddd(15 0,3.0,1 5)	2.57ddd(15.0,3 0,1 5)	-	-
4	3 83m	5.10m	3.84m	5 10m	69 95	70.01
5	1.48m	1.56m	1.48m	1.55m	37 37	37.41
6-13	1 26br s	1 25br s	1.26br s	1 26br s	25 52-29.70	25 54-29 73
14	1 48m	1 56m	1 48m	1.55m	34 07[a]	33 47
15	3 43m	4.89m	3 42m	4.87m	74 31	74 03
16	3.83m	3.96m	3 81m	3.98m	82 61	82 55
17a	1 76m	1 63m	1 68m	1.55m	28 07	28 71
17b	1 94m	1 90m	1 99m	1.95m	-	-
18a	1 76m	1 63m	1.68m	1.55m	28 07	28 71
18b	1.94m	1.90m	1.99m	1.95m	-	-
19	3 83m	3 96m	3 81m	3.98m	82 68	82 65
20	3 43m	4.89m	3 42m	4.87m	73 82	73 50
21	1.60m	1 56m	1 60m	1.58m	34.01[a]	33 47
22	2.20m	2 07m	2 20m	2.04m	23 41	23 31
23	5.36m	5 33m	5 36m	5.31m	128.96	128 92
24	5 39m	5.36m	5 39m	5 37m	130.77	130.83
25	2 04q(6 5)	1.99q(6.5)	2.04q(7.0)	1.99q(6.5)	27 22	27.23
26-32	1 26br s	1.25br s	1 26br s	1.25br s	28.73-31 89	29.32-31 91
33	1 30m	1.29m	1 30m	1.29m	22 66	22.68
34	0.88t(7 0)	0.88t(7.0)	0.88t(7 0)	0 88t(7.0)	14.09	14.11
35	7 19q(1 3)	7.08q(1.3)	7.19q(1 4)	7.08q(1 0)	151 79	151.78
36	5 06qq(7.0,1 5)	5.01qq(7.0,1.5)	5.06qq(6 5,1.0)	5.01qq(6.5,1 0)	77.96	77 97
37	1.44d(6 5)	1.40d(7.0)	1.44d(7 0)	1.40d(7.0)	19.08	19.11
4-OAc		2 03s		2.03s		
15-OAc		2.08s		2.07s		
20-OAc		2 08s		2.07s		

a) Assignments may be interchangeable

and H-36 in **2r** and **2s**, at 0 25 and 0.06, suggested that **2** has the 4*R*, 36*S* configuration, as is usual. The absolute configuration of **2** is C-4*R*, C-15*R*, C-16*R*, C-19*S*, C-20*S*, and C-36*S* Therefore, the structure of **2** was concluded to be a new acetogenin, as illustrated, and the compound was named asiminenin A.

Compound **3**, mp 54-55°, was also obtained in an amorphous state. CIms gave a $[MH]^+$ at *m/z* 607 indicating a molecular weight of 606. The molecular formula was established to be $C_{37}H_{66}O_6$ by the HRCIms (MH^+ m/z 607 4938, calcd 607.4938) The ir, uv, and ms of **3** and its triacetate (**3a**) and triTMSi (**3b**) derivatives (Figure 4) were quite similiar to those of **2**, suggesting that **3** was a diastereomer of **2** The double bond in **3** was proven to be of the *cis* configuration using decoupling experiments as were performed with **2**, and the location also placed at C-23/24 since the double bond proton signal at δ 5.36

Table 4. ^{1}H Nmr chemical shifts for the determination of the absolute configurations at C-4, C-15 and C-20 of the tri-(*S*)- and -(*R*)-MTPA esters of **2** and **3**

MTPA	δH												
Config.	5-Hab	3-Hab	35-H	36-H	37-H	14-Ha	16-H	17-Ha	18-Ha	19-H	21-Hab	22-H	23-H
2r	1 56	2 60	6 97	4 91	1.31	1 30	3.89	1.46	1 32	4 11	1 40	1 96	5.38
	1.64	2 68				1.38		1.52	1.83		1 75		
2s	1.63	2 58	6 72	4 86	1.28	1.35	3.88	1.34	1 37	4 12	1 40	1 90	5 37
	1.69	2 58				1.43		1.44	1 84		1 56		
	+0.07	-0.02	-0.25	-0.05	-0.03	+0.05	-0.01	-0.12	+0 05	+0 01	0	-0 06	-0.01
	+0 05	-0.10				+0.05		-0.08	+0 01		-0 19		
3r	1.56	2.59	6.97	4 91	1 31	1.50	4 01	1.55	1.55	4 01	1.50	1.89	5.22
	1 65	2.68				1.61		1.93	1.93		1.61		
3s	1 63	2 58	6 72	4 86	1.28	1.60	3 93	1.38	1.38	3.93	1.60	2.04	5 28
	1.69	2 58				1.65		1 64	1.64		1.65		
	+0 07	-0 01	-0 25	-0 05	-0.03	+0 10	-0.08	-0 17	-0 17	-0.08	+0.10	+0.15	+0.06
	+0.04	-0.10				+0.04		-0.29	-0.29		+0.04		
Carbinol Config.	**2** ; C-4*R*					C-15*R*					C-20*S*		
	3 ; C-4*R*					C-15*R*					C-20*R*		

showed cross correlation to H-20 at δ 3 42. The relative stereochemistries of C-15/16 and C-19/20 were determined as described above and were the same as those of **2**. The methylene protons of H-17a and 18a in **3** were at δ 1 68 (shifted upfield), and those of H-17b and H-18b were at δ 1.99 (shifted downfield), compared with **2**, which has the *cis* configuration across the THF ring, indicating that **3** has the *trans* configuration of the mono-THF moiety [26] Thus, the relative stereochemistries around the THF ring from C-15 to C-20 were concluded to be *threo/trans/threo*. The absolute stereochemistries of the carbinol centers at C-4, C-15 and C-20 were determined to be *R* in **3r** and **3s** by using the advanced Mosher ester method (Table 4) [22,23] The absolute configuration of **3** is C-4*R*, C-15*R*, C-16*R*, C-19*R*, C-20*R*, and C-36*S*. Therefore, the structure of **3** is the same as that of asiminenin A (**2**) except that the mono-THF ring has the *trans* configuration, and C-19 and C-20 are *R* instead of *S* **3** was named asiminenin B.

Bioactivity data obtained with **1-3** are summarized in Table 5 **1** was very toxic to the brine shrimp larvae and showed potent and selective cytotoxicities to the human solid tumor cell lines, A-549 (lung carcinoma), MCF-7 (breast carcinoma), HT-29 (colon adenocarcinoma) and MIA PaCa-2 (pancreatic carcinoma). The

Table 5 Brine shrimp lethality and cytotoxicities in human solid tumor cell lines for **1-3**.

Compounds	BST[a] LC_{50} (μg/ml)	Cell Lines, ED_{50} values (μg/ml)					
		A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC-3[f]	MIA PaCa-2[g]
1	2.35×10^{-2}	2.25×10^{-10}	1.24×10^{-4}	$7\ 04\times10^{-5}$	1.69	1.30	1.25×10^{-4}
2	4.73×10^{-1}	$2\ 85\times10^{-4}$	2.39×10^{-3}	$8\ 12\times10^{-2}$	$6\ 26\times10^{-2}$	1.66	$8\ 58\times10^{-4}$
3	$5\ 82\times10^{-1}$	3.22×10^{-6}	3.61×10^{-6}	$6\ 94\times10^{-5}$	$5\ 72\times10^{-3}$	$3\ 66\times10^{-5}$	6.34×10^{-7}
Adriamycin[h]	NT	1.01×10^{-3}	1.03×10^{-2}	2.62×10^{-2}	3.66×10^{-3}	1.96×10^{-2}	1.32×10^{-3}

NT: Not tested [a]Brine shrimp test [2,13] [b]Lung carcinoma[30] [c]Breast carcinoma[31] [d]Colon adenocarcinoma[32] [e]Kidney carcinoma[30] [f]Prostate adenocarcinoma[33] [g]Pancreatic carcinoma[34] [h]Positive control standard.

potency of **1** against A-549 (lung) was ten-million times that of adriamycin. **2** and **3** exhibited less potent but selective cytotoxicities against the human tumor cell lines. The structural differences at C-19 and C-20 in **3** appear to account for one to five orders of magnitude more potency than **2**. This result is somewhat consistent with that previously observed for murisolin (*trans* configuration) and 16,19-*cis*-murisolin (*cis* configuration).[10]

EXPERIMENTAL

Mps were determined on a Mel-Temp apparatus and are uncorrected. Optical rotations were taken on a Perkin-Elmer 241 Polarimeter Ir spectra were obtained on a Perkin-Elmer 1600 FTIR spectrophotometer. Uv spectra were measured on a Beckman DU-7 UV spectrophotometer. ^{1}H, ^{13}C Nmr and COSY spectra were recorded on a Varian VXR-500S (^{1}H at 500 MHz, ^{13}C at 125 MHz) spectrometer in $CDCl_3$ with TMS as reference. Low resolution CIms and EIms data were collected on a Finnigan 4000 spectrometer EIms for TMSi derivatives and exact masses on ms measurements were obtained on a Kratos MS 50 spectrometer through peak matching. For tlc, silica gel 60 F-254 (EM 5717) glass plates (0 25 mm) were used and visualized by spraying with 5% phosphomolybdic acid in EtOH and heating. Hplc was carried out with a Rainin HPLC instrument using the Dynamax software system and a silica gel column (250 x 21 mm) equipped with a Rainin UV-1 detector set at 230 nm

Chemicals

For preparation of triTMSi derivatives, *N,O*-bis(trimethylsilyl)acetamide (BSA) and pyridine in silylation grade were purchased from Pierce Chemical Company (USA) Mosher reagents (*S*)-(+)- and (*R*)-(-)-α-(trifluoromethyl)phenylacetyl (MTPA) chloride, were obtained from the Aldrich Company

Derivatization

The triTMSi derivatives were prepared by treatment of the isolated acetogenins with BSA in the presence of pyridine. **2** or **3** was separately placed in a 100 μl conical reaction vial and dried in a vacuum desiccator over P_2O_5 for 24 hr, respectively The sample was treated with 2 μl pyridine (silylation grade) and 20 μl of *N,O*-bis(trimethylsilyl)acetamide (BSA) and heated at 70° for 30 min. EIms see Figures 3 and 4. The EIms measurements of the derivatives were carried out at a resolution of 1500, scanning mass 900-100 at 30 sec/decade Mosher esters were made by the advanced Mosher's methodology [22,23] To 1 mg of **1**, **2** or **3** in 0.5 ml of CH_2Cl_2 were sequentially added pyridine 0 2 ml, 4-(dimethylamino)pyridine 0.5 mg, and 12 mg of (*R*)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride, separately. The mixture was left at room temperature overnight and purified over a micro-column (0.6 x 6 cm) of silica gel (230-400 mesh) eluted with 3-4 ml of hexane-CH_2Cl_2 (1:2), the eluate was dried, CH_2Cl_2 (5 ml) was added and the CH_2Cl_2 was washed using 1% $NaHCO_3$ (5 ml x 3) and H_2O (5 ml x 2); the washed eluate was dried in vacuo to give *S*-Mosher esters of **1**, **2** and **3**, respectively. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride afforded the *R*-Mosher esters Their pertinent ^{1}H nmr chemical shifts are given in Tables 3 and 4

Bioassays

The extracts, fractions, and isolated compounds were routinely evaluated for lethality to brine shrimp larvae (BST).[12,13] Seven-day in vitro MTT cytotoxicity tests against human tumor cell lines were carried out at the Purdue Cancer Center, using standard protocols for A-549 (human lung carcinoma),[30] MCF-7 (human breast carcinoma),[31] HT-29 (human colon adenocarcinoma)[32], A-498 (human kidney carcinoma),[33] PC-3 (human prostate adenocarcinoma)[34] and MIA PaCa-2 (human pancreatic carcinoma)[34] with adriamycin as a positive control. The reported ED_{50} values in μg/ml (Table 5) were tabulated from the same run in order to facilitate comparisons for the SAR's.

Plant material

The seeds of *Asimina triloba* (L.) Dunal were collected from plantations of paw paw trees grown at the University of Maryland and were purchased from the Paw Paw Foundation. The identification was confirmed by R. Neal Peterson A voucher specimen of the seeds is preserved at the Department of Medicinal Chemistry and Pharmacognosy, Purdue University.

Extraction and purification of acetogenins

The air-dried *Asimina triloba* seeds (6 Kg) were ground into a powder and then extracted with 95% EtOH (8 *l* x 4) at room temperature for 3 days and evaporated, under rotary vacuum, to yield 2 kg of the EtOH extract (F001, BST LC_{50} 2 48 μg/ml) which was partitioned between H_2O (3 *l*) and CH_2Cl_2 (3 *l* x 5), giving 298 g of the H_2O soluble fraction (F002, BST LC_{50} 1 66 x 10^2 μg/ml), 1.7 kg of the CH_2Cl_2 soluble fraction (F003, BST LC_{50} 8 19 x 10^{-1} μg/ml) and 2 g of an insoluble interface (F004). F003 was further partitioned between hexane (3 *l*) and 90% MeOH aq. soln (3 *l* x 6) and yielded 600 g of the MeOH fraction (F005, BST LC_{50} 1.43 x 10^{-1} μg/ml) and 1 1 kg of the hexane soluble fraction (F006, BST LC_{50} 1.05 x 10^2 μg/ml). Directed by the BST bioassay, the most bioactive fraction, F005 (440 g) was further fractioned by open column chromatography on silica gel (3 7 kg, 60-200 mesh), eluting with hexane-$CHCl_3$ and $CHCl_3$-MeOH gradients, 14 pools (from A to N) were made from the collected fractions, according to their tlc patterns, and evaluated by the BST bioassay. The most active pools, D (BST LC_{50} 6 85 x 10^{-2} μg/ml) and E (BST LC_{50} 1.49 x 10^{-1} μg/ml), were separately subjected to further repeated separation by Si gel (60-200 mesh) column chromatography eluted with hexane-acetone gradients Further purifications of the most bioactive fractions were carried out by hplc eluted with 10% THF in MeOH-hexane gradients (5-10%) to yield the three new acetogenins, **1-3** In addition, asimicin, bullatacin, trilobacin (4) and trilobin (5), which are known, and parviflorin that is known but is new in this species, were obtained.[14-16]

Asitribin (1)

Compound (**1**) was obtained as a colorless powder (6 mg), mp 71-72°. $[\alpha]_D$ + 15 0°(c = 0.1 mg/ml, CH_2Cl_2). Uv λmax CH_2Cl_2 nm· 234 (log ε = 3 55). Ir ν_{max} film (cm^{-1})· 3448 (OH), 2925, 2827, 1757 (C=O), 1589, 1073. HRFABms (glycerol) *m/z*: 623.4894 ([MH]$^+$, found), (required 623 4887, $C_{37}H_{66}O_7$) Cims, EIms (triTMSi derivative, **1c**) and EIms (triacetate derivative, **1b**) see Figure 3 ^{1}H Nmr (500 MHz, $CDCl_3$) see Table 1 ^{13}C Nmr (125 MHz, $CDCl_3$): see Table 1.

Asitribin triacetate (1a)

Treatment of compound (**1**) (1 mg) with Ac_2O (0 5 ml)-pyridine (0.5 ml) at room temperature, overnight and subsequent

workup gave **1a** as a colorless wax (0.8 mg). ^{1}H Nmr (500 MHz, $CDCl_3$): see Table 1.

Asiminenin A (2)

Compound (**2**) was obtained as a white powder (40 mg), mp 58-59°. $[\alpha]_D$ + 10.0°(c = 0.1 mg/ml, CH_2Cl_2) Uv λmax CH_2Cl_2 nm: 228 (log ε = 3 55). Ir ν_{max} film (cm^{-1}) 3411(OH), 2921, 2852, 1756 (C=O), 1588, 1078, 669 HRCIms (isobutane) *m/z*: 607 4926 ([MH]$^+$, found), (required 607.4938, $C_{37}H_{66}O_6$). CIms, EIms (triTMSi derivative, **2c**) and EIms (triacetate derivative, **2b**) see Figure 4. ^{1}H Nmr (500 MHz, $CDCl_3$): see Table 2 ^{13}C Nmr (125 MHz, $CDCl_3$) see Table 2.

Asiminenin A triacetate (2a)

Treatment of compound (**2**) (1 mg) with Ac_2O (0.5 ml)-pyridine (0.5 ml) at room temperature, overnight and subsequent workup gave **2a** as a colorless wax (0.9 mg). ^{1}H Nmr (500 MHz, $CDCl_3$) see Table 2.

Asiminenin B (3)

Compound (**3**) was also obtained as a colorless powder (15 mg), mp 54-55°. $[\alpha]_D$ + 17.0° (c = 0.1 mg/ml, CH_2Cl_2). Uv λmax CH_2Cl_2 nm: 230 (log ε = 2 93) Ir ν_{max} film (cm^{-1}): 3458(OH), 2918, 2850, 1731 (C=O), 1590, 1316, 1081, 669. HRFABms (glycerol) *m/z* 607 4938 ([MH]$^+$, found), (required 607.4938, $C_{37}H_{66}O_6$). CIms, EIms (triTMSi derivative **3b**) and EIms (triacetate derivative **3c**) see Figure 4 ^{1}H Nmr (500 MHz, $CDCl_3$): see Table 2. ^{13}C Nmr (125 MHz, $CDCl_3$) see Table 2.

Asiminenin B triacetate (3a)

Treatment of compound (**3**) (1 mg) with Ac_2O (0.5 ml)-pyridine (0.5 ml) at room temperature, overnight and subsequent workup gave **3a** as a colorless wax (0 8 mg) ^{1}H Nmr (500 MHz, $CDCl_3$): see Table 2.

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