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The Absolute Configuration of Adjacent Bis-THF Acetogenins and Asiminocin, A Novel Highly Potent Asimicin Isomer from *Asimina triloba*

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Abstract—A novel acetogenin, asiminocin (**1**), was isolated by activity-directed fractionation from the stem bark of the paw paw tree, *Asimina triloba*. By spectral and chemical methods, **1** was identified as (30*S*)-hydroxy-4-deoxyasimicin. The absolute configuration of **1**, along with those of previously reported acetogenins asimin, asiminacin, bullatin, (30*S*)-bullanin, and (30*R*) bullanin, was determined by Mosher ester methodology. Compound **1** was highly inhibitory to three human solid tumor cell lines with over a billion times the potency of adriamycin.

Introduction

As an annonaceous plant, the North American native paw paw tree, *A. triloba* (L.) Dunal, has received much attention since the highly potent acetogenin, asimicin, was isolated from the EtOH extract of its seeds and stem bark.[1] Our continued investigation of this plant has led to the discovery of 29 bioactive components including, 15 adjacent bis-THF acetogenins which can be divided into three types:[2] the asimicin type [asimin (**2**), asiminacin (**3**), asiminecin (**4**), asimilobin and parviflorin];[3–5] the bullatacin type [bullatacin, bullatin (**5**), squamocin, motrilin and bullanin (**6a** and **6b**)];[3,6] and the trilobacin type (trilobacin, trilobin and asitribin).[3,7,8] In addition, 10 mono-THF acetogenins[5,8–10] and four bioactive nonacetogenin compounds[3,9] have been isolated. Among the adjacent bis-THF acetogenins, the trilobacin type compounds, which, so far have been reported only from this species, possess the unique relative configuration of *threo/trans/erythro/cis/threo* between the two carbinol centers in the bis-THF moiety and thus they represent a new stereochemical type of adjacent bis-THF subunit.[7] The brine shrimp lethality test (BST)[11,12] and human solid tumor cell (A-549, MCF-7, HT-29)[13–15] cytotoxicity data of these 12 adjacent bis-THF acetogenins have shown some interesting structure–activity relationships. The three types of bis-THF acetogenins (asimicin, bullatacin and trilobacin) exhibit the same level of potent bioactivities and distantly hydroxylated compounds (i.e. with the C-4 hydroxyl group relocated at the C-10, C-28, C-29 or C-30 positions, respectively) have shown enhancement of potency within the series of each type.[4,6,7]

Key words: *Asimina triloba*, Annonaceae, acetogenins, asiminocin, absolute configuration.

In this paper, we report recent work on the isolation of a novel asimicin type acetogenin, asiminocin (**1**), which was identified as (30*S*)-hydroxyl-4-deoxyasimicin. Compound **1** showed higher activity against the three human solid tumor cell lines tested than the other members in the asimicin series, providing additional evidence for the structure–activity relationships. The absolute configuration of **1**, along with those of the previously reported acetogenins **2–5**, **6a** and **6b** was determined by Mosher ester methodology.[16–19] The analyses of ^{1}H NMR data of per-(*S*)-MTPA and per-(*R*)-MTPA Mosher esters of bullanin revealed, interestingly, that the C-30 position has both *S* and *R* configurations, suggesting the existance of two diastereomers, i.e. (30*S*)-bullanin (**6a**) and (30*R*)-bullanin (**6b**). The *S* configuration at C-36 for all of these acetogenins (**1–5**, **6a** and **6b**) was predicted by the comparisons of their CD spectra with that of squamocin, which is of known absolute configuration.[20] Additionally, unexpected positive $\Delta\delta_H$ values were noticed at H-16 in the bis-THF ring moiety of the MTPA derivatives of the bullatacin type acetogenins (**5**, **6a** and **6b**). Careful analyses of their ^{1}H NMR data together with those of the mono-MTPA derivatives of bullatacinone and uvaricin[19] suggests that the steric compression between the MTPA group and the THF rings causes this irregular arrangement of $\Delta\delta_H$ values.

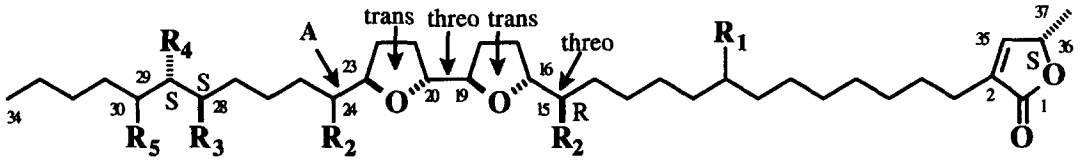
Results and Discussion

Asiminocin (**1**; Fig. 1) was isolated as a colorless wax. The M_r of **1** was determined as 622 by the mass peaks at *m/z* 623 (MH^+) and 645 (MNa^+) in the low-resolution FABMS spectrum. The HR-FABMS gave *m/z* 623.4865 for the MH^+ ion (calcd 623.4887) corresponding to the molecular formula, $C_{37}H_{66}O_7$.

The comparison of ^1H and ^{13}C NMR spectral data of **1** (Table 1) with those of previously reported asimicin type adjacent bis-THF acetogenins **2–4**[4] indicated that the structure of **1** was very similar to those of **2–4** including the adjacent bis-THF unit with a flanking hydroxyl group at each side, the α,β-unsaturated γ-lactone ring and the absence of the hydroxyl group at C-4. The major difference observed between the ^1H NMR spectra of **1** and **2–4** was the chemical shift of the terminal methyl, which was located further downfield at δ 0.907 for **1** (Table 1), suggesting that the third hydroxyl group of **1** could be closer to the terminal methyl.[2,6] The presence of three hydroxyl groups was concluded by the strong IR hydroxyl absorption band at 3445 cm^{-1} and by the proton resonances at δ 3.40 (2H) and 3.59 (1H), and the carbon resonances at δ 74.07, 73.99 and 71.92 (Table 1), as well as the three acetate methyl groups at δ 2.08 (6H) and 2.04 (3H) in the ^1H NMR spectrum of the triacetate derivative (**1a**; Table 1).

The placements of these hydroxyl groups and the adjacent bis-THF ring unit along the aliphatic chain were determined again by the EIMS spectral analysis of the tri-TMSi (**1b**) and the tri-TMSi-d_9 (**1c**) derivatives (Fig. 2). As in compounds **2–4**, the bis-THF ring with its two flanking hydroxyls was located in **1** from C-15 to C-24. That the third hydroxyl group was situated at C-30 was concluded by the fragment ion at *m/z* 159 in the EIMS spectrum of the tri-TMSi derivative (**1b**; Fig. 2) and further confirmed by the HREIMS which showed the exact mass at *m/z* 159.1202 very closely matching the anticipated fragment formula $C_8H_{19}SiO$ (calcd 159.1205). This result was also supported by the mass ion at *m/z* 168 in the EIMS spectrum and the exact mass at *m/z* 168.1768 (calcd 168.1770 for $C_8H_{10}D_9SiO$) of its tri-TMSi-d_9 derivative (**1c**; Fig. 2).

The relative stereochemistry in the adjacent bis-THF moiety was subsequently determined to be, as in compounds **2–4**, *threo/trans/threo/trans/threo* from C-15 to C-24, by comparison of ^1H NMR data of relevant protons of **1** and its triacetate (**1a**) with those of model compounds of known relative configurations.[21–24] The absolute configurations of the carbinol centers in the bis-THF unit were again determined as (15*R*), (16*R*), (19*R*), (20*R*), (23*R*) and (24*R*) by the analyses of the ^1H NMR spectra of the (*R*)-MTPA and (*S*)-MTPA ester derivatives, **1d** and **1e** (Table 2), using Mosher's methods.[16–19] The prediction of the absolute configuration of stereogenic centers located in the middle of the hydrocarbon chain (e.g. with C-28 and C-29 hydroxylated acetogenins) has been described by Fujimoto et al.[25] By focusing on the chemical shift of the terminal methyl group, the oxymethine chiral centers, i.e. at the C-26, C-28 and C-29 positions, of acetogenins have been determined by the advanced Mosher ester method. The configuration at C-30 was assigned as *S* based on the proton resonance comparison of the terminal methyl group in **1d** and **1e** (Table 2). The absolute configuration at C-36 in the butenolide ring has been directly determined as *S* only for uvaricin[26] and squamocin.[20,27,28] However, all acetogenins are predicted as having the (36*S*) [or (34*S*) in the 35 carbon acetogenins] configuration by comparisons of NMR data of the diagnostic protons and carbons with those of uvaricin and squamocin.[2,29,30] Recently, a novel use of Mosher ester data for determining the relative configuration between C-4 and C-36 of the 4-hydroxylated annonaceous acetogenins has been described by Hoye et al.[31,32] Unfortunately, this method is not applicable to the determination of the absolute configuration of C-36 in **1** due to the absence of the C-4 hydroxyl group. However, a negative Cotton effect at 236 nm in the CD spectrum of squamocin is attributable to the (36*S*) configuration in the γ-lactone moiety and provides a useful method for the determination of the C-36 configuration in other annonaceous acetogenins which lack a C-4



Asiminocin	**1**	R_1=H	R_2=OH	A= *threo*	R_3=H	R_4=H	R_5= ▬ OH
	1a	R_1=H	R_2=Ac	A= *threo*	R_3=H	R_4=H	R_5= ▬ Ac
	1b	R_1=H	R_2=TMSi	A= *threo*	R_3=H	R_4=H	R_5= ▬ TMSi
	1c	R_1=H	R_2= TMSi-d_9	A= *threo*	R_3=H	R_4=H	R_5= ▬ TMSi-d_9
	1d	R_1=H	R_2=(*R*)-MTPA	A= *threo*	R_3=H	R_4=H	R_5= ▬ (*R*)-MTPA
	1e	R_1=H	R_2=(*S*)-MTPA	A= *threo*	R_3=H	R_4=H	R_5= ▬ (*S*)-MTPA
Asimin	**2**	R_1= ▬ OH	R_2=OH	A= *threo*	R_3=H	R_4=H	R_5=H
Asiminacin	**3**	R_1=H	R_2=OH	A= *threo*	R_3=OH	R_4=H	R_5=H
Asiminecin	**4**	R_1=H	R_2=OH	A= *threo*	R_3=H	R_4=OH	R_5=H
Bullatin	**5**	R_1= ▬ OH	R_2=OH	A= *erythro*	R_3=H	R_4=H	R_5=H
30*S*-Bullanin	**6a**	R_1=H	R_2=OH	A= *erythro*	R_3=H	R_4=H	R_5= ▬ OH
30*R*-Bullanin	**6b**	R_1=H	R_2=OH	A= *erythro*	R_3=H	R_4=H	R_5= ▬ OH

Figure 1. Chemical structures of **1–5**, **6a** and **b**, and the asiminocin (**1**) derivatives **1a–1e**.

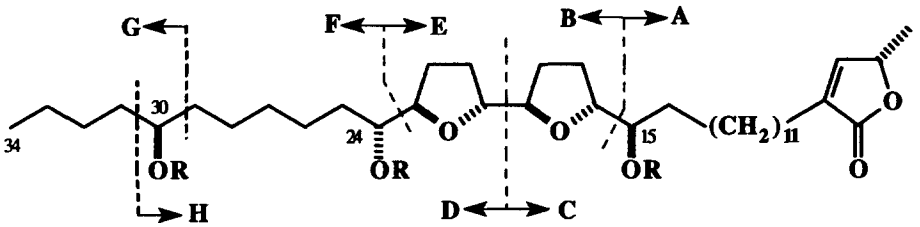
Table 1. NMR data of asiminocin (**1**) and its triacetate (**1a**)

H/C No.	^{1}H NMR (500 MHz, $CDCl_3$, δ in ppm, *J* in Hz)		^{13}C NMR (125.75 MHz, $CDCl_3$, δ in ppm)
	1	**1a**	**1**
1	-	-	173.83
2	-	-	134.24
3	2.26 *tt* (7.8, 1.6)	2.26 *tt* (7.8, 1.6)	25.20
4	1.22–1.72 *m*	1.46–1.64 *m*	27.41
5–13	1.22–1.72 *m*	1.46–1.64 *m*	29.20 – 29.75[a]
14, 25	1.43 *m*	1.46–1.64 *m*	33.45, 33.34
15, 24	3.40 *m*	4.85 *m*	74.07, 73.99
16, 23	3.86 *m*	3.98 *m*	83.15, 83.07
17a, 18a, 21a, 22a	1.98 *m*	1.95 *m*	29.02, 28.40
17b, 18b, 21b, 22b	1.66 *m*	1.78 *m*	29.02, 28.38
19, 20	3.86 *m*	3.90 *m*	81.80, 81.78
26–28	1.22–1.72 *m*	1.20–1.38 *m*	25.69, 25.60, 25.54
30	3.59 *m*	4.85	71.92
29, 31	1.44 *m*	1.46–1.64 *m*	37.18, 37.38
32	1.22–1.72 *m*	1.20–1.38 *m*	27.87
33	1.22–1.72 *m*	1.20–1.38 *m*	22.80
34	0.907 *t* (7.0)	0.880 *t* (7.0)	14.13
35	6.99 *q* (1.5)	6.99 *q* (1.5)	148.79
36	5.00 *qddd* (6.8, 1.7, 1.7, 1.7)	5.00 *qddd* (6.8, 1.7, 1.7, 1.7)	77.40
37	1.41 *d* (7.0)	1.41 *d* (7.0)	19.26
15-OAc		2.08 *s*	
24-OAc		2.08 *s*	
29-OAc		2.04 *s*	

[a]Signals in methylene envelope: δ 29.75, 29.71, 29.63, 29.62, 29.54, 29.34 and 29.20.

hydroxyl.[20] A negative Cotton effect at 237 nm (ε = −866) was observed in the CD spectrum of **1**; compared with squamocin [negative Cotton effect at 235 nm (ε = −973) as measured in our laboratory], **1** is, therefore, determined as having the (36*S*) configuration. From the above data, it was concluded that **1** is a new member of the asimicin type acetogenins and it was named asiminocin (Fig. 1).

The absolute configurations of **2–5**, **6a** and **6b** were determined by the analyses and comparisons of their ^{1}H NMR spectra of the per-(*S*)-MTPA and per-(*R*)-MTPA derivatives (Table 2). All these isolated compounds (**2–5**, **6a** and **6b**) showed negative Cotton effects at 237 nm (ε = −962) for **2**, 235 nm (ε = −847) for **3**, 237 nm (ε = −1171) for **4**, 237 nm (ε = −1174) for **5** and 238 nm (ε = −916) for **6a** and **6b** in their CD



R	M$^+$	A	B	C	D	E	F	G	H
H		295		365	(257)	(435)	187		
(**1**)				347 (a)	239 (a)	417 (a)	169 (a)		
(EIMS)				329 (a)	221 (a)	399 (a)	151 (a)		
TMSi	(838)	367	(471)	437	(401)	507	331	159	781
(**1b**)	748 (b)		381 (b)	347 (b)	311 (b)	417 (b)	241 (b)		691 (b)
(EIMS)	658 (b)		291 (b)			399 (a)	151 (b)		601 (b)
TMSi-d_9	(865)	376	(489)	446	419	516	349	168	808
(**1c**)	766 (c)	277 (c)	390 (c)	347 (c)	320 (c)	417 (c)	250 (c)		709 (c)
(EIMS)	667 (c)		291 (c)	329 (a)		399 (a)	151 (c)		
	568 (c)								

(a): loss of H_2O (*m/z* 18); (b): loss of TMSiOH (*m/z* 90); (c): loss of TMSiOH-d_9 (*m/z* 99).

Figure 2. Diagnostic mass fragmentation ions of asiminocin (**1**) and its tri-TMSi and tri-TMSi-d_9 derivatives (**1b** and **c**).

Table 2. ^{1}H NMR data of relevant protons of per-(*S*)-MTPA and per-(*R*)-MTPA esters of **1**–**5**, **6a** and **b**

Entry	MTPA configuration	Proton chemical shifts (δ_H)																
		C-10		C-28	C-29	C-30	Carbinol configuration	Adjacent bis-THF moiety										Carbinol configuration
		H-3	H-4	H-34	H-34	H-34		H-14	H-16	H-17	H-18	H-19	H-20	H-21	H-22	H-23	H-25	
1	*S* (**1d**)			/	/	0.82		1.57	3.94	1.84	1.73	3.77	3.77	1.73	1.84	3.94	1.39	
	R (**1e**)			/	/	0.88	C_{30}: *S*	1.48	3.99	1.94	1.84	3.93	3.93	1.84	1.94	3.99	1.37	C_{15}: *R*
	$\Delta\delta_H$ (δ_S -δ_R)			/	/	−0.06		+0.09	−0.05	−0.10	−0.11	−0.16	−0.16	−0.11	−0.10	−0.05	+0.02	C_{24}: *R*
2	*S*	2.256	1.54	/	/	/		1.57	3.94	1.84	1.73	3.77	3.77	1.73	1.84	3.94	1.39	
	R	2.250	1.51	/	/	/	C_{10}: *R*	1.48	3.99	1.94	1.84	3.93	3.93	1.84	1.94	3.99	1.37	C_{15}: *R*
	$\Delta\delta_H$ (δ_S -δ_R)	+0.006	+0.03	/	/	/		+0.09	−0.05	−0.10	−0.11	−0.16	−0.16	−0.11	−0.10	−0.05	+0.02	C_{24}: *R*
3	*S*	/	/	0.857	/	/		1.60	3.94	1.83 1.44	1.73 1.65	3.77	3.77	1.73 1.65	1.83 1.44	3.94	1.60	
	R	/	/	0.882	/	/	C_{28}: *S*	1.45	3.97	1.94 1.55	1.93 1.84	3.93	3.93	1.93 1.84	1.94 1.55	3.97	1.45	C_{15}: *R*
	$\Delta\delta_H$ (δ_S-δ_R)	/	/	−0.025	/	/		+0.15	−0.03	−0.11 −0.11	−0.20 −0.19	−0.16	−0.16	−0.20 −0.19	−0.11 −0.11	−0.03	+0.15	C_{24}: *R*
4	*S*	/	/	/	0.835	/		1.58	3.95	1.82 1.43	1.72 1.65	3.78	3.78	1.72 1.65	1.82 1.43	3.95	1.58	
	R	/	/	/	0.878	/	C_{29}: *S*	1.48	3.97	1.94 1.53	1.91 1.84	3.93	3.93	1.91 1.84	1.94 1.53	3.97	1.48	C_{15}: *R*
	$\Delta\delta_H$ (δ_S-δ_R)	/	/	/	−0.043	/		+0.10	−0.02	−0.12 −0.10	−0.19 −0.19	−0.15	−0.15	−0.19 −0.19	−0.12 −0.10	−0.02	+0.10	C_{24}: *R*
5	*S*	2.263	1.52	/	/	/		1.61	4.03	1.90 1.52	1.80 1.65	3.79	7.79	1.80 1.65	1.82 1.70	3.96	1.55	
	R	2.266	1.54	/	/	/	C_{10}: *S*	1.46	4.00	2.05 1.58	1.90 1.75	3.82	3.64	1.75 1.62	1.75 1.60	3.87	1.57	C_{15}: *R*
	$\Delta\delta_H$ (δ_S-δ_R)	−0.003	−0.02	/	/	/		+0.15	+0.03	−0.15 −0.06	−0.10 −0.10	−0.03	+0.15	+0.05 +0.03	+0.08 +0.10	+0.09	−0.02	C_{24}: *S*
6a	*S*	/	/	/	/	0.82 0.88	C_{30}:	1.60	4.03	1.90 1.52	1.80 1.65	3.79	3.79	1.80 1.65	1.82 1.70	3.98	1.55	
	R	/	/	/	/	0.88 0.82	**6a**: *S*	1.45	4.00	2.05 1.58	1.90 1.75	3.82	3.64	1.75 1.62	1.75 1.60	3.92	1.57	C_{15}: *R*
6b	$\Delta\delta_H$ (δ_S-δ_R)	/	/	/	/	−0.06 +0.06	**6b**: *R*	+0.15	+0.03	−0.15 −0.06	−0.10 −0.10	−0.03	+0.15	+0.05 +0.03	+0.08 +0.10	+0.06	−0.02	C_{24}: *S*

spectra. Compounds **2**–**5**, **6a** and **6b** are all, therefore, depicted as having the (36*S*) configuration.

As with **3** and **4**, the absolute configurations at the C-30 of **6a** and **6b** were predicted by the $\Delta\delta_H$ values of the terminal methyl group (C-34) in their Mosher esters. At first, the mixture of **6a** and **6b** was thought to be a pure compound, but the terminal methyl groups in the ^{1}H NMR spectra of both the (*S*)-MTPA and (*R*)-MTPA esters exhibited, interestingly, two separated triplets (Table 2). Careful analysis of the ^{13}C NMR spectrum of **6a** and **6b** revealed that the resonances from C-26 to C-31 were divided into pairs, one major and the other minor. Thus, this material was concluded to be a mixture of two diastereomers, the major one (**6a**) has the (30*S*) configuration and the minor one (**6b**) has the (30*R*) configuration; the same observation has previously been made with the (30*S*)- and (30*R*)-hydroxybullatacin isomers.[33]

Compounds **2** and **5** have their third hydroxyl group at the C-10 position, where determination of the absolute configuration is more challenging. Direct observation of the magnitude of chemical shift changes of H-9 and H-10 was impossible due to the overlapping of their proton signals in the COSY spectra of both MTPA esters. The chemical shifts of H-14 and H-15 were strongly affected by the Mosher residues at C-15 and C-24 and prevented their use as reference signals for the purpose of determination of the configuration at C-10. The only signals that could be used were those of the protons on the lactone ring side. The nearest assignable resonances are H-4 and H-3, which are six and seven bonds away from C-10, respectively. The $\Delta\delta_H$ values of the two methyl groups of the (*R*)-MTPA ester of the model compounds, 6-undecanol and 8-pentadecanol, were diagnostic (0.039 and 0.007 ppm) and useful for the prediction of the absolute stereochemistry of a carbinol center located five and seven bonds away from the observed protons.[34] Thus, the *R* configuration was proposed at the C-10 position for **2** and the *S* configuration was proposed for **5** by the $\delta_S-\delta_R$ values of H-4 and H-3 of their MTPA derivatives (Table 2). Similar results were recently observed in the determination of the absolute configuration at C-10 of trilobin [(10*R*)-hydroxy-4-deoxytrilobacin].[7] All the proton NMR data listed in Tables 1–3 were collected at 500 MHz by modern 2-D NMR techniques, which permit the identification of minor chemical shift differences within the digital resolution of 0.06 Hz per point in ^{1}H NMR spectra and less than 4 Hz per point in ^{1}H–^{1}H COSY spectra.

In our attempt to determine the absolute configurations of bullatin (**5**), (30*S*)-bullanin (**6a**) and (30*R*)-bullanin (**6b**), we noticed that the Δδ values of H-16 are irregularly positive. These unexpected data were also observed in the Mosher esters of bullatacin and the bullatacinones and no explanation was proposed.[19] This outcome, of course, could not be used for the determination of the absolute configuration of the bullatacin series of compounds. Re-analyses of the previously reported ^{1}H NMR chemical shift data of the 15-mono-(*S*)-MTPA and 15-mono-(*R*)-MTPA esters of the bullatacinones, uvaricin and hexepiuvaricin (Table 3) revealed that the Δδ values of the H-15 and H-16 were zero or very close to zero.[19] According to the Mosher's proposal of the configurational correlation model for the (*R*)-MTPA and the (*S*)-MTPA derivatives, the carbinyl proton and ester carbonyl and trifluoromethyl groups of the MTPA moiety lie in the same plane, i.e. the MTPA plane.[18] Thus, the Δδ value of the carbinyl proton, in these cases H-15, should be zero, since the anisotropic effects exerted by the carbonyl groups (the benzene group is far away) in both (*R*)-MTPA and (*S*)-MTPA esters are equal. H-16 receives a diamagnetic effect from the benzene ring, therefore, the distance between them in the (*R*)-MTPA and (*S*)-MTPA esters is the major factor which effects the Δδ value. Thus, the zero Δδ value of H-16 in these 15-mono-MTPA derivatives might be due to the steric compression between the bulky adjacent bis-THF rings and the MTPA moiety which compel the H-16 to be in the MTPA plane (Fig. 3). In the per-(*R*)-MTPA derivative of the bullatacin series, the benzene group of the MTPA residue at C-24 is positioned on the bis-THF ring side and added to the diamagnetic effect on H-16 makes it shift upfield, while the per-(*S*)-MTPA derivative has no such additional effect (Table 2). Hence, these steric interactions may cause an irregular positive value of the Δδ of H-16.

Table 3. ^{1}H NMR data of H-15 and H-16 of mono-MTPA esters of relevant compounds[a]

Compounds	MTPA	H-15 (δ_H)	H-16 (δ_H)
Uvaricin	(15*S*)	5.05	4.03
	(15*R*)	5.05	4.03
	δ_{S-R}	0	0
Hexepi-uvaricin	(15*S*)	5.05	4.03
	(15*R*)	5.05	4.03
	δ_{S-R}	0	0
Bullatacinone	(15*S*)	5.07	4.083
	(15*R*)	5.08	4.075
	δ_{S-R}	~ 0	~ 0
Bullatacinone	(24*S*)	3.37	3.89–3.78
	(24*R*)	3.37	3.64
	δ_{S-R}	0	(+)

[a]Chemical shift data see Ref. 19.

Asiminocin (**1**) exhibited very potent activities in the brine shrimp lethality test[11,12] and in human solid tumor cytotoxicity tests against the lung cancer (A-549), breast cancer (MCF-7) and colon cancer (HT-29) cell lines,[13–15] with ED_{50} values as low as $<10^{-12}$ μg mL^{-1} (Table 4). Compound **1** was nearly one billion times the cytotoxic potency of the standard reference, adriamycin. Compound **1** showed higher activity against the three tested human solid tumor cell lines than the other members in this asimicin series.[4] Annonaceous acetogenins powerfully inhibit ATP production at the cellular level through both the inhibition of the NADH-ubiquinone oxidoreductase (complex I) of mitochondrial electron transport systems[35,36] and the ubiquinone-linked NADH oxidase in the plasma membranes of tumor cells.[37] These combined actions may be linked to programmed cell

death (apoptosis)[38] and account for their high potencies in various bioassay systems.

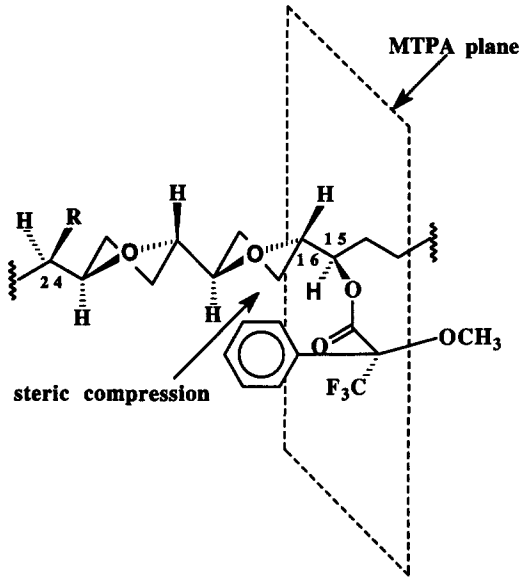
Experimental

Plant material

The stem bark of *A. triloba* (L.) Dunal (Annonaceae) was collected from stands growing wild at the Purdue Horticultural Research Farm, West Lafayette, IN, U.S.A. The identification was confirmed by Dr George R. Parker, Department of Forestry and Natural Resources, Purdue University, U.S.A. A voucher specimen of the bark is preserved in the pharmacognosy herbarium.

Instrumentation

Optical rotations were taken on a Perkin–Elmer 241 polarimeter. IR spectra were obtained on a Perkin–Elmer 1600 FTIR spectrometer. UV spectra were measured on a Beckman DU-7 UV spectrometer. CD spectra were performed on a JASCO Model J600 circular dichroism spectropolarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian VXR-500S (^{1}H at 500 MHz, ^{13}C at 125.75 MHz) spectrometer in $CDCl_3$ with proton signals referenced to TMS. Low-resolution CIMS and EIMS data were collected on a Finnigan 4000 spectrometer. FABMS, EIMS for TMSi and TMSi-d_9 derivatives and exact mass measurements through peak matching were performed on a Kratos MS50 mass spectrometer.



uvaricin: R=OAc
bullatacinone: R=OH

Figure 3. The MTPA plane at C-15 of 15-mono-MTPA derivatives of relevant compounds.

Chromatography

TLC separations were made on silica gel 60 F-254 (EM5717) glass plates (0.25 mm) and visualized by spraying with 5% phosphomolybdic acid in EtOH and heating. Chromatotron plates (1 or 2 mm) were prepared with silica gel 60 PF 254 containing gypsum and dried at 70 °C overnight. HPLC was carried out with a Rainin HPLC instrument using the Dynamax software system and silica gel columns (a 21.4 i.d. × 250 mm column and a 4.6 i.d. × 250 mm column) equipped with a Rainin UV-1 detector set at 215–230 nm.

Bioassays

The extracts, fractions and isolated compounds were routinely evaluated for lethality to brine shrimp larvae (BST) as described and modified.[11,12] 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),[13] MCF-7 (human breast carcinoma),[14] and HT-29 (human colon adenocarcinoma)[15] with adriamycin as a positive control.

Extraction, isolation and purification

The extraction procedures were the same as those described in our previous reports.[3,4,6,7,9] The most bioactive fraction, F005 (BST LC_{50} 7.151×10^{-1} μg mL^{-1}, 200 g) was applied to an open column on silica gel (8 Kg, 60–200 mesh), eluting with C_6H_{14}:EtOAc and EtOAc:MeOH gradients; five major fractions were made from the collected fractions according to their TLC patterns and evaluated by the BST bioassay. The most active fraction F3 (BST, LC_{50}: 0.7 μg mL^{-1}, 10 g) was subjected to further repeated separation by chromatotron chromatography with gradient elutions

Table 4. Bioactivity data of asiminocin (1)

Compounds	BST[a] LC_{50} (μg mL^{-1}) (95% confidence interval)	Human cancer cell lines (ED_{50} μg mL^{-1}) A-549[b]	MCF-7[c]	HT-29[d]
Asiminocin (1)	4.9×10^{-3} ($3.1 \times 10^{-3} - 7.8 \times 10^{-3}$)	3.1×10^{-12}	2.9×10^{-12}	$< 10^{-12}$
Adriamycin[e]	2.57×10^{-1} ($1.89 \times 10^{-1} - 5.3 \times 10^{-1}$)	1.84×10^{-2}	1.32×10^{-1}	3.60×10^{-2}

[a]BST: brine shrimp test.[11,12]
[b]A-549: human lung cancer.[13]
[c]MCF-7: human breast cancer.[14]
[d]HT-29: human colon cancer.[15]
[e]Standard reference antitumor compound.

using MeOH:CH_2Cl_2 and 0–5% of MeOH in a mixture of $CHCl_3$:C_6H_{14} (2:3). Fractions 10–19 (350 mg, BST, LC_{50} 3.5×10^{-2} μg mL^{-1}) on TLC plates exhibited an unique spot which failed to be further resolved on additional chromatotron plates. Further separation was achieved by HPLC using a normal phase silica gel HPLC column (21.4 i.d. × 250 mm) eluted with 10% THF in MeOH:C_6H_{14} gradients (5–15%), MeOH (0–1%) in the mixture of C_6H_{14}:EtOAc (2:1) and acetone in $CHCl_3$ (0–10%) to yield acetogenins asiminocin (**1**) along with compounds **2–5**, **6a**, **6b**, squamocin and motrilin.

Acetylation

The pure compounds (1–2 mg) were dissolved in 0.5–1.0 mL of pyridine; 1 mL of anhydrous Ac_2O was added, and the mixture was set at room temperature for 4–8 h. The mixture was then partitioned between H_2O and $CHCl_3$ and the organic layer was concentrated and subjected to silica gel microcolumn chromatography to afford the pure acetate derivatives.

Preparation of TMSi and TMSi-d_9 derivatives

Tri-TMSi and tri-TMSi-d_9 derivatives were prepared by treatment of the isolated acetogenins with *N,O*-bis-(trimethylsilyl)acetamide (BSA, in silylation grade were purchased from Pierce Chemical Company, U.S.A.) for the tri-TMSi derivatives, or bis-(trimethylsilyl)trifluoroacetamide-d_{18} (BSTFA-d_{18}, under the brand name Deutero Regisil-d_{18} from Regis Chemical Company, U.S.A.) for the tri-TMSi-d_9 derivatives in the presence of pyridine. Approximately 0.3 mg of pure compound was placed in a 100 μL conical reaction vial and dried in a vacuum desiccator over P_2O_5 for 24 h. The samples were treated with 2 μL pyridine and 20 μL of BSA or BSTFA-d_{18} and then heated at 70 °C for 30 min. The EIMS measurements of the derivatives were carried out at a resolution of 1500, scanning mass 900–100 at 30 s per decade.

Preparation and purification of per-(*S*) and per-(*R*)-Mosher esters

The isolated acetogenin (0.5–1 mg in 0.3 mL of CH_2Cl_2) was sequentially treated with pyridine (0.2 mL), 4-(dimethylamino)pyridine (0.5 mg) and 25 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride. The mixture was stirred at room temperature for 4 h, monitored by TLC and then passed through a silica gel open column (0.6 × 6 cm^2), in a disposable pipet and eluted with 3 mL of CH_2Cl_2 which was dried under a vacuum. The residue was dissolved in CH_2Cl_2 and washed with 1% $NaHCO_3$ (5 mL) and H_2O (2 × 5 mL); the organic layer was dried under vacuum to give the per-(*S*)-Mosher ester derivative. The per-(*R*)-Mosher ester was prepared by using (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride as the reagent. 1-D ^1H NMR spectra were recorded in $CDCl_3$ at 500 MHz with data size of 128 K and spectral width of 8 ppm (digital resolution: 0.06 Hz per point). 2-D ^1H–^1H COSY spectra were recorded in $CDCl_3$ at 500 MHz with both F1 and F2 data size of 2048 and spectral width of 8 ppm (digital resolution: <4 Hz per point).

Asiminocin (1). Colorless wax (5 mg), $[\alpha]_D$ +26° ($CHCl_3$, 1 mg mL^{-1}). UV, λ_{max}^{MeOH} nm: 215; CD: 237 nm (ε = −866); IR υ_{max}^{film} cm^{-1}: 3445 (OH), 2927, 28546 1753 (C=O), 1457, 1414, 1266, 1180, 1021, 926, 729; HR-FABMS (glycerol) *m/z* 623.4865 (MH^+, found), 623.4887, (calcd for $C_{37}H_{66}O_7$); EIMS, *m/z*: see Figure 2. ^1H NMR (500 MHz, $CDCl_3$), and ^{13}C NMR (125.75 MHz, $CDCl_3$): see Table 1.

Asiminocin triacetate (1a). Treatment of **1** (1 mg) with Ac_2O–pyridine (at room temperature, overnight) and subsequent work up gave **1a** as a wax. IR, ν_{max}^{film} cm^{-1}: 2932, 2848, 1755, 1737, 1461, 1372, 1320, 1238, 1069, 1026, 955; CIMS (isobutane) *m/z* 749 MH^+, 689 $[MH-CH_3COOH]^+$, 629 $[MH-2CH_3COOH]^+$, 569 $[MH-3CH_3COOH]^+$, 521, 425, 397, 391, 369, 331, 295, 271, 257, 217; ^1H NMR (500 MHz, $CDCl_3$): see Table 1.

Asiminocin-tri-TMSi (1b). Compound **1** (0.3 mg) was treated with BSA and subsequent work up afforded the tri-TMSi derivative (**1b**). EIMS: see Figure 2. HREIMS *m/z* 159.1202, $[C_8H_{19}SiO]^+$, 159.1205, (calcd for $C_8H_{19}SiO$).

Asiminocin-tri-TMSi-d_9 (1c). Compound **5** (0.3 mg) was treated with BSTFA-d_{18} and subsequent work up afforded the tri-TMSi-d_9 derivative (**5c**). EIMS: see Figure 2. HREIMS *m/z* 168.1768, $[C_8H_{10}D_9SiO]^+$, 168.1770, (calcd for $C_8H_{19}D_9SiO$).

Tri-(*S*)-MTPA esters of 1–5, 6a and 6b. Compounds **1–5**, **6a** and **6b** (0.5–1 mg) were treated with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride to give light yellow oily products. ^1H NMR (500 MHz, $CDCl_3$, TMS reference): see Table 2.

Tri-(*R*)-MTPA esters of 1–5, 6a and 6b. Compounds **1–5**, **6a** and **6b** (0.5–1 mg) were treated with (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride to give light yellow oily products. ^1H NMR (500 MHz, $CDCl_3$, TMS reference): see Table 2.

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References

1. Rupprecht, J. K.; Chang, C.-J.; Cassady, J. M.; McLaughlin, J. L.; Mikolajczak, J. L.; Weisleder, D. *Heterocycles* **1986**, *24*, 1197.

2. Gu, Z.-M.; Zhao, G.-X.; Oberlies, N. H.; Zeng, L.; McLaughlin, J. L. *Recent Advances in Phytochemistry*; Arnason, J. T.; Mata, R.; Romeo, J. T. Eds.; Plenum: New York, **1995**; Vol. 29, pp 249–310.

3. Zhao, G.-X.; Hui, Y.-H.; Rupprecht, J. K.; Wood, K. V.; McLaughlin, J. L. *J. Nat. Prod.* **1992**, *55*, 347.

4. Zhao, G.-X.; Miesbauer, L. R.; Smith, D. L.; McLaughlin J. L. *J. Med. Chem.* **1994**, *37*, 1971.

5. Woo, M. H.; Cho, K. Y.; Zhang, Y.; Zeng, L.; Gu, Z.-M.; McLaughlin, J. L. *J. Nat. Prod.* **1995**, *58*, 1533.

6. Zhao, G.-X.; Ng, J.; Kozlowski, J. F.; Smith, D. L.; McLaughlin, J. L. *Heterocycles* **1994**, *38*, 1897.

7. Zhao, G.-X.; Gu, Z.-M.; Zeng, L.; Chao, J.-F.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L. *Tetrahedron* **1995**, *51*, 7149.

8. Woo, M. H.; Zeng, L.; McLaughlin, J. L. *Heterocycles* **1995**, *41*, 1731.

9. Zhao, G.-X; Rieser, M. J.; Hui, Y.-H.; Miesbauer, L. R.; Smith, D. L.; McLaughlin, J. L. *Phytochemistry* **1993**, *33*, 1065.

10. Woo, M. H.; Zeng, L.; Ye, Q.; Gu, Z.-M.; Zhao, G.-X.; McLaughlin, J. L. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1135.

11. McLaughlin, J. L. In *Methods in Plant Biochemistry*, Hostettmann, K. Ed.; Academic: London, 1991; Vol. 6, pp 1–35.

12. Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobsen, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*, 31.

13. Giard, D. J.; Aaronson, S. A.; Todaro, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Natl. Cancer Inst.* **1973**, *51*, 1417.

14. Soule, H. D.; Vazquez, J.; Long, A.; Albert, S.; Brennan, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409.

15. Fogh, J. Ed., *Human Tumor Cells, In Vitro*, Plenum: New York, 1975; pp 115–159.

16. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.

17. Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143.

18. Ohtani, I.; Kusumi, T.; Kasman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092.

19. Rieser, M. J.; Hui, Y.-H.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, Z.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *26*, 10203.

20. Sahai, M.; Singh, S.; Singh, M.; Gupta, Y. K.; Akashi, S.; Yuji, R.; Hirayama, K.; Asaki, H.; Araya, H.; Hara, N.; Eguchi, T.; Kakinuma, K.; Fujimoto, Y. *Chem. Pharm. Bull.* **1994**, *42*, 1163.

21. Rupprecht, K. J.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod.* **1990**, *53*, 237.

22. Hoye, T. R.; Zhuang, Z.-P. *J. Org. Chem.* **1988**, *53*, 5578.

23. Hoye, T. R.; Hanson, P. R.; Kovelesky, A. C.; Ocain, T. D.; Zhung, Z. *J. Am. Chem. Soc.* **1991**, *113*, 9369.

24. Born, L.; Lieb, F.; Lorentzen, J. P.; Moescher, H.; Nonfon, M.; Sollner, R.; Wendisch, D. *Planta Med.* **1990**, *56*, 312.

25. Nishioka, S.; Araya, H.; Murasaki, C.; Sahai, M.; Fujimoto, Y. *Nat. Prod. Lett.* **1994**, *5*, 117.

26. Jolad, S. D.; Hoffmann, J. J.; Cole, J. R.; Barry, C. E.; Bates, R. B.; Linz, G. S.; Konig, W. A. *J. Nat. Prod.* **1985**, *48*, 644.

27. Born, L.; Lieb, F.; Lorentzen, J. P.; Moescher, H.; Nonfon, M.; Sollner, R.; Wendisch, D. *Planta Med.* **1990**, *56*, 312.

28. Araya, H.; Hara, N.; Fujimoto, Y.; Srivastava, A.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 388.

29. Rupprecht, K. J.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod.* **1990**, *53*, 237.

30. Fang, X.-P.; Rieser, M. J.; Gu, Z.-M.; Zhao, G.-X.; McLaughlin, J. L. *Phytochem. Anal.* **1993**, *4*, 27 and 49.

31. Hoye, T. R.; Hanson, P. R.; Hasenwinkel, L. E.; Ramirez, E. A.; Zhuang, Z. *Tetrahedron Lett.* **1994**, *35*, 8525.

32. Hoye, T. R.; Hanson, P. R.; Hasenwinkel, L. E.; Ramirez, E. A.; Zhuang, Z. *Tetrahedron Lett.* **1994**, *35*, 8529.

33. Gu, Z.-M; Zeng, L.; Schwedler, J. T.; Wood, K. V.; McLaughlin, J. L. *Phytochemistry* **1995**, *40*, 467.

34. Nishioka, S.; Araya, H.; Murasaki, C.; Sahai, M.; Fujimoto, Y. *Nat. Prod. Lett.* **1994**, *5*, 117.

35. Degli Esposti, M.; Ghelli, A.; Ratta, M.; Cortes, D. *Biochem. J.* **1994**, *301*, 161.

36. Hollingworth, R. M.; Ahammadsahib, K. I.; Gadelhak, G.; McLaughlin, J. L. *Biochem. Soc. Trans.* **1994**, *22*, 230.

37. Morre, D. J.; de Cabo, R.; Farley, C.; Oberlies, N. H.; McLaughlin, J. L. *Life Sci.* **1995**, *56*, 343.

38. Wolvetang, E. J.; Johnson, K. L.; Krauer, K.; Ralph, S. J.; Linnane, A. W. *FEBS Lett.* **1994**, *339*, 40.

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