Asimin, Asiminacin, and Asiminecin: Novel Highly Cytotoxic Asimicin Isomers from *Asimina triloba*

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Activity-directed fractionation of the stem bark extracts of the North American paw paw tree, *Asimina triloba* (Annonaceae), has yielded three further acetogenins: asimin (**2**), asiminacin (**3**), and asiminecin (**4**). **2–4** are structural isomers of asimicin (**1**), which is a potent inhibitor of mitochondrial NADH:ubiquinone oxidoreductase, and thus exhibits potent antitumor and pesticidal effects. **2–4** have the same carbon skeleton and configurations as those of **1**, but they have the third hydroxyl group located at C-10, C-28, and C-29, respectively, rather than at C-4. The determinations of the hydroxyl group locations were largely based on mass spectral analyses of TMSi and TMSi-d_9 derivatives. **2–4** all showed highly potent cytotoxicities (ED_{50} values as low as $<10^{-12}$ μg/mL) with notable selectivities for the HT-29 human colon cancer cell line. The presence of a third hydroxyl at C-4, C-10, C-28, or C-29, as in **1–4**, greatly enhances the bioactivity of 4-deoxyasimicin (**5**).

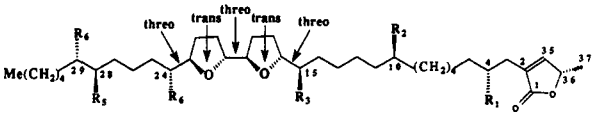
Introduction

Asimicin (**1**) (Figure 1) was the first acetogenin isolated from the seeds and stem bark of the North American paw paw tree, *Asimina triloba* 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 subsequently led us to additional novel bioactive acetogenins, including the very active adjacent bis-tetrahydrofuran (THF) compound, trilobacin.[2] The less active mono-THF acetogenins, (2,4-*cis* and *trans*)-annonacin-A-one, (2,4-*cis* and *trans*)-gigantetrocinone and (2,4-*cis* and *trans*)-isoannonacin have been more recently isolated.[3] The absolute configuration of asimicin (**1**) has now been defined,[4] and the mechanism of action of **1** and other Annonaceous acetogenins is via inhibition of NADH: ubiquinone oxidoreductase (complex I) in mitochondrial electron transport systems.[5–7]

Further activity-directed fractionation of the ethanolic extract of the stem bark, using the brine shrimp lethality test (BST) to monitor fractionation,[8,9] has now revealed three novel adjacent bis-THF acetogenins: asimin (**2**), asiminacin (**3**), and asiminecin (**4**) (Figure 1). An HPLC normal-phase silica gel column eluted with a methanol/tetrahydrofuran/hexane solvent system was indispensible in the isolation work. Compounds **2–4** are structurally identified as asimicin (**1**) isomers with the C-4 hydroxyl group moved to the C-10, C-28, and C-29 positions, respectively. These three compounds are nearly identical in their ^{1}H- and ^{13}C-NMR spectra. The determinations of the hydroxyl group positions were based on the mass spectral analysis of their tri-TMSi and tri-TMSi-d_9 derivatives. Cell culture data showed that these compounds have highly potent in vitro cytotoxicities against three human tumor cell lines in our in-house cytotoxicity tests.

Results and Discussion

Isolation. In searching for new bioactive acetogenins from the F005 fraction, which was partitioned from the EtOH extract of the stem bark,[2,3] the more polar column fractions from the most active pools (P_7–P_9) were investigated. The fraction sample was subjected to open column and chromatotron chromatography with gradient elutions using MeOH and CH_2Cl_2 and 0–5% of MeOH in a mixture of $CHCl_3$ and hexane (2:3). Fractions 14–19 on TLC plates exhibited an unique spot which failed to be further resolved on additional chromatotron plates. At first this material was considered as a single compound. The ^{1}H-NMR spectrum appeared to represent a single compound, but expansion of the proton signals of the terminal methyl groups showed an overlap of several compounds. A satisfactory separation was then achieved by using HPLC. Four separated HPLC peaks (A–D) were obtained by using 8-μm normal-phase silica gel HPLC columns with gradient elution of 8–12% of a mixture of MeOH and tetrahydrofuran (9:1) in hexane. Peak C was in pure form and gave compound **4**. Further resolution of peak A was satisfactorily achieved to give compounds **2** and **3** in pure form by decreasing the MeOH percentage to 5–10% and increasing the ramping time.



Compounds	R_1	R_2	R_3	R_4	R_5	R_6
Asimicin (1)	OH	H	OH	OH	H	H
Asimin (2)	H	OH	OH	OH	H	H
Asimin triacetate (2a)	H	OAc	OAc	OAc	H	H
Asiminacin (3)	H	H	OH	OH	OH	H
Aciminacin triacetate (3a)	H	H	OAc	OAc	OAc	H
Asiminecin (4)	H	H	OH	OH	H	OH
Asiminecin triacetate (4a)	H	H	OAc	OAc	H	OAc
4-Deoxyasimicin (5)	H	H	OH	OH	H	H

Figure 1. Chemical structures of **1–5** and the triacetate derivatives **2a–4a**. Note added in proof: the absolute configurations of **2–4** were determined by Mosher's method and were identical to those of **1**.[4]

Chemistry. Compound **2** was obtained as a colorless wax. A CIMS (isobutane) peak at *m/z* 623 indicated the molecular weight of 622. HR FABMS (glycerol) found the molecular ion peak at *m/z* 623.4907 which closely matched the exact mass 623.4887 calculated for the

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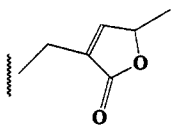
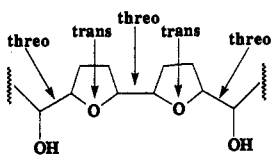
Table 1. NMR Data for Asimin (2) and Its Triacetate Derivative 2a

H/C no.	^{1}H-NMR (500 MHz, $CDCl_3$, δ in ppm, J in hertz) 2	2a	^{13}C-NMR (125.75 MHz, $CDCl_3$, δ in ppm)
1	-	-	173.80
2	-	-	134.20
3	2.26 tt (7.8, 1.6)	2.26 tt (7.8, 1.6)	31.94–22.73
9, 11	1.44 m	1.46–1.64 m	37.43, 37.45
10	3.59 m	4.86 m	71.84
14, 25	1.65 m	1.46–1.64 m	33.39, 33.46
15, 24	3.40 m	4.86 m	73.97, 74.08
16, 23	3.85 m	3.99 m	83.05, 83.17
17a, 18a, 21a, 22a	1.98 m	1.95 m	31.94–22.73
17b, 18b, 21b, 22b	1.65 m	1.78 m	31.94–22.73
19, 20	3.85 m	3.91 m	81.76, 81.81
4–8, 12–13, 26–33	1.24–1.60 m	1.20–1.38 m	31.94, 29.66, 29.64, 29.57, 29.37, 29.30, 29.14, 29.03, 29.02, 28.39, 27.41, 25.73, 25.70, 25.64, 25.20, 22.73
34	0.878 t (7.0)	0.879 t (7.0)	14.18
35	6.99 q (1.5)	6.99 q (1.5)	148.82
36	5.00 qq (6.8, 1.7)	5.00 qq (6.8, 1.7)	77.41
37	1.41 d (6.5)	1.41 d (7.0)	19.27
15-OAc		2.08 s	
24-OAc		2.08 s	
10-OAc		2.04 s	

molecular formula $C_{37}H_{66}O_7$. An IR absorption band at 1752 cm^{-1} and UV λ_{max} at 215 nm and the ^{1}H-NMR spectrum peaks (Table 1) at δ 6.99 (q, H-35), 5.00 (qq, H-36), as well as ^{13}C-NMR resonances (Table 1) at δ 173.80 (C-1) 134.20 (C-2), 148.82 (C-35), 77.41 (C-36), 19.27 (C-37), and 25.20 (C-3), suggested the presence of an α,β-unsaturated γ-lactone ring as previously reported in trilobacin and asimicin (1).[2] Spectral comparisons of compound 2 and asimicin (1) clearly indicated that the H-35 and H-36 proton signals and the C-35 and C-36 carbon resonances of 2 were shifted slightly upfield and the C-1 and C-2 signals were shifted downfield; the H-3a and H-3b peaks [at δ 2.34 (dddd) and 2.45 (dddd)], characteristic in the ^{1}H-NMR spectrum of 1,[1] were missing in 2, and the appearance of a multiplet peak at δ 2.26 (tt, 2H) suggested the absence of a hydroxyl group at the C-4 position. Therefore, the presence of the typical subunit, fragment A, was substantiated.[10,11]

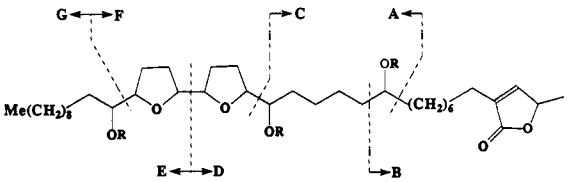


Fragment A



Fragment B

The integrations for four protons in the multiplet signal at 3.85 ppm (H-16, 19, 20, and 23) and for two protons in the broad peak at 3.40 ppm (H-15 and 24), in the ^{1}H-NMR spectrum (Table 1) of 2, indicated that 2 is an adjacent bis-THF acetogenin.[10,11] The ^{13}C-NMR spectrum (Table 1), likewise, showed three pairs of closely located resonance peaks, at δ 83.05 and 83.17, 81.76 and 81.81, and 73.97 and 74.08, which confirmed the presence of a moiety having adjacent bis-THF rings with two flanking hydroxyl groups. These proton and carbon signals were very similar to those observed with asimicin (1).[1,10] In order to determine the relative configuration between the adjacent chiral centers in this fragment, a triacetate derivative (2a) was made. The ^{1}H-NMR data of 2a (Table 1) showed three separated oxygen-bearing methine signals; two of these peaks were integrated for two protons each at the resonances of δ 3.91 and 3.99 arising from H-19, 20 and H-16, 23, respectively;



R	M$^+$/MH$^+$	A	B	C	D	E	F	G
H (CIMS)	623 605 (a) 587 (a) 569 (a)		225	311 (a) 293 (a) 275 (a)		241* 223* (a)		171
TMS (EIMS)	838* 748 (b) 658* (b) 568* (b)	643 553 (b) 463* (b) 273* (b)	297 207* (b)	455 365 (b) 275* (b)		313* 223* (b)	595 505 (b) 415 (b)	243 153* (b)
D_9-TMS (EIMS)	865* 766 (c) 667 (c) 568 (c)	670 571 (c) 472 (c) 373* (c)	306 207* (c)	473 374 (c) 275 (c)	543 444 (c) 345 (c)	322* 223* (c)	613 514 (c) 415 (c)	252 153* (c)

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

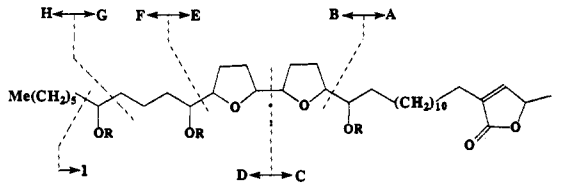
Figure 2. Diagnostic mass fragmentation ions of asimin (2) and its tri-TMSi and tri-TMSi-d_9 derivatives.

the third signal, which integrated for three oxygen-bearing methine protons, was located at δ 4.86 and had shifted downfield due to the acetylation of three hydroxyl groups; two of the hydroxyls were those flanking both sides of the bis-THF ring system. This chemical shift pattern closely matched the relative configuration of a *threo-trans-threo-trans-threo* system which is identical to that of asimicin (1) and is represented by fragment B.[12] A slight separation of the three pairs of oxygen-related carbons hinted the influence of the third hydroxyl group which, thus, was probably located near the bis-THF ring system.

The presence of the three hydroxyl groups in 2 was easily confirmed by the IR absorption at 3438 cm^{-1} and the proton signals at δ 3.40 (2H) and 3.59 (1H). In the CIMS spectrum (Figure 2), the observation of a series of fragments at m/z 605, 587, and 569, due to successive losses of three molecules of water, further confirmed the presence of three hydroxyl groups. Additional evidence was the three acetate methyl signals at δ 2.04 (3H) and 2.08 (6H) in the ^{1}H-NMR spectrum of 2a and the fragment peaks at m/z 689, 629, and 569 arising from the successive losses of three acetic acids (m/z 60) from the molecular ion at m/z 749 in the CIMS spectrum of 2a. Two hydroxyl groups were those flanking the THF ring, and the third hydroxyl, which exhibited its methine proton signal at δ 3.59 and its carbon resonance at δ 71.84, was predicted to be nearby in the aliphatic chain.

Table 2. NMR Data for Asiaminacin (3) and Its Triacetate Derivative 3a

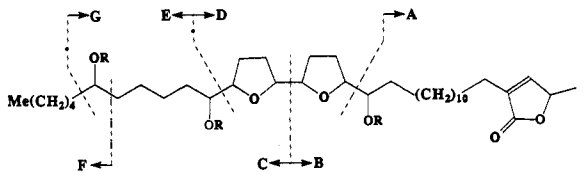
H/C no.	^{1}H-NMR (500 MHz, $CDCl_3$, δ in ppm, J in hertz) 3	3a	^{13}C-NMR (125.75 MHz, $CDCl_3$, δ in ppm)
1	–	–	173.83
2	–	–	134.25
3	2.26 tt (7.8, 1.6)	2.26 tt (7.8, 1.6)	31.88–22.67
14, 25	1.67 m	1.46–1.64 m	33.45, 33.22
15, 24	3.40 m	4.85 m	73.89, 74.07
16, 23	3.86 m	3.98 m	83.05, 83.19
17a, 18a, 21a, 22a	1.98 m	1.95 m	31.88–22.67
17b, 18b, 21b, 22b	1.67 m	1.79 m	31.88–22.67
19, 20	3.86 M	3.90 M	81.78, 81.85
27, 29	1.44 m	1.46–1.64 m	37.31, 37.56
28	3.60 m	4.85 m	71.73
4–13, 26, 30–33	1.22–1.72 m	1.20–1.38 m	31.88, 29.64, 29.55, 29.41, 29.34, 29.22, 29.04, 29.00, 28.40, 27.42, 25.71, 25.69, 25.21, 21.78, 22.67
34	0.882 t (7.0)	0.876 t (7.0)	14.15
35	6.99 q (1.5)	6.99 q (1.5)	148.78
36	5.00 qq (6.8, 1.7)	5.00 qq (6.8, 1.7)	77.40
37	1.41 d (6.5)	1.41 d (7.0)	19.27
15-OAc		2.08 s	
24-OAc		2.08 s	
28-OAc		2.03 s	



R	M^+/MH^+	A	B	C	D	E	F	G	H	I
H (CIMS)	623 605 (a) 587 (a) 569 (a)	295 277* (a)	327* 309 (a)	365 * 347 (a)	257 239 (a)	435*			115*	
TMS (EIMS)	838* 748 (b) 658* (b) 568* (b)	367 277* (b)	471 381* (b) 291 (b)	437 347 (b)		507 417 (b)	331 241 (b) 151* (b)		187	753 663 (b) 573* (b) 483* (b)
d_9-TMS (EIMS)	865* 766 (c) 667 (c) 568 (c)	376	489 390* (c) 291 (c)	446 347 (c)		516 417 (c)	349 250 (c) 151* (c)	669 570 (c) 471* (c)	196	780*

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

Figure 3. Diagnostic mass fragmentation ions of asiminacin (3) and its tri-TMSi and tri-TMSi-d_9 derivatives.



R	M^+/MH^+	A	B	C	D	E	F	G
H (CIMS)	623 605 (a) 587 (a) 569 (a)	295 277* (a)	365 347 (a)	257* 239 (a)	435* 417 (a)			551
TMS (EIMS)	838* 748 (b) 658* (b) 568* (b)	367	437 347 (b)	401* 311 (b)	507 417 (b)	331 241* (b) 151* (b)	173	767
D_9-TMS (EIMS)	865* 766 (c) 667 (c) 568 (c)	376	446 347 (c)	419*	516 417 (c)	349* 250 (c) 151 (c)	182	794 (695) (596) 497 (c)

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

Figure 4. Diagnostic mass fragmentation ions of asiminecin (4) and its tri-TMSi and tri-TMSi-d_9 derivatives.

MS spectral analysis has been a powerful probe for the determination of the carbon skeletons and placement of THF rings and functional groups along the aliphatic chains in the structural elucidation of these acetogenins.[10,11] Having determined the presence of fragments A and B and the three hydroxyl groups, the remaining portions of the structure of compound 2 consisted simply of two aliphatic chains. To establish the location of these fragments and hydroxyl groups along the aliphatic chains, the tri-TMSi and tri-TMSi-d_9 derivatives were prepared. Analysis of the EIMS spectra (Figure 2) of these derivatives determined that the adjacent bis-THF ring system was located at C-16 to C-23 and the three hydroxyl groups are at C-10, C-15, and C-24. Thus, the structure of compound 2 was established as that illustrated (Figure 1), and 2 was given the name of asimin.

Compound 3 was isolated as a wax in a similar amount as 2. The CIMS (isobutane) spectrum (Figure 3) of 3 showed a $[M + H]^+$ ion at m/z 623, indicating a molecular weight of 622, identical to the molecular weight of 2. The HR FABMS (glycerol) gave a peak for the molecular ion at m/z 623.4874, corresponding to the calculated exact mass of 623.4887 for the molecular formula $C_{37}H_{66}O_7$.

As determined with 2, the presence of a terminal α,β-unsaturated γ-lactone ring, without a 4-OH group (fragment A), and an adjacent bis-THF ring moiety (fragment B) was obvious from the ^{1}H- and ^{13}C-NMR spectra of 3 (Table 2) and by NMR spectral comparisons with compound 2. The presence of three OH groups was also recognized by the analysis of IR, ^{1}H-NMR, ^{13}C-NMR, and CIMS spectra of 3 and the CIMS spectrum of its triacetate derivative (3a). The relative configuration in the bis-THF ring moiety was also established as a *threo-trans-threo-trans-threo* system based on the ^{1}H-NMR spectral analysis of 3a.[12]

The placements of the adjacent bis-THF ring system (fragment B) and the three hydroxyl groups of 3 along the aliphatic chain were determined based on the fragmentation pattern of the tri-TMSi and tri-TMSi-d_9 derivatives of 3 in their EIMS spectra (Figure 4). It was again obvious that the bis-THF ring was located from C-16 to C-23, but the three hydroxyl groups were situated at the C-15, C-24, and C-28 positions. Hence, the only difference between 2 and 3 was that the third hydroxyl group was positioned at C-10 in compound 2, while compound 3 had its third hydroxyl group at C-28. Compound 3 was given the trivial name asiminacin.

Compound 4 was another waxy compound. A molecular ion peak at m/z 623 in the CIMS (isobutane) spectrum of 4 (Figure 4) once again indicated a molecular weight of 622. The HR CIMS (isobutane) spectrum showed an exact mass peak at m/z 623.4868, which matched the molecular formula $C_{37}H_{66}O_7$ (calculated 623.4887).

Table 3. NMR Data for Asiminecin (4) and Its Triacetate Derivative 4a

H/C no.	^{1}H-NMR (500 MHz, $CDCl_3$, δ in ppm, J in hertz) 4	4a	^{13}C-NMR (125.75 MHz, $CDCl_3$, δ in ppm)
1	-	-	173.83
2	-	-	134.25
3	2.26 tt (7.8, 1.6)	2.26 tt (7.8, 1.6)	31.94-22.69
14, 25	1.68 m	1.46-1.64 m	33.47, 33.33
15, 24	3.40 m	4.85 m	74.07, 73.87
16, 23	3.86 m	3.98 m	83.17, 83.05
17a, 18a, 21a, 22a	1.98 m	1.95 m	31.94-22.69
17b, 18b, 21b, 22b	1.68 m	1.78 m	31.94-22.69
19, 20	3.86 m	3.90 m	81.82, 81.78
28, 30	1.44 m	1.46-1.64 m	37.52, 37.33
29	3.60	4.85	71.81
4-13, 26-27, 31-33	1.22-1.72 m	1.20-1.38 m	31.94, 29.63, 29.54, 29.34, 29.21, 29.03, 29.02, 28.41, 23.39, 27.42, 25.70, 25.61, 25.58, 25.38, 22.69
34	0.891 t (7.0)	0.880 t (7.0)	14.11
35	6.99 q (1.5)	6.99 q (1.5)	148.79
36	5.00 qq (6.8, 1.7)	5.00 qq (6.8, 1.7)	77.41
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	

Table 4. Bioactivity Data of Compounds 1-5

compd	BST: LC_{50} (μg/mL) (95% confidence interval)	ED_{50} (μg/mL): human cancer cell lines A-549	MCF-7	HT-29
1	2.56×10^{-2} [$(1.54-4.25) \times 10^{-2}$]	84.3×10^{-4}	8.52×10^{-1}	$<10^{-12}$
2	4.6×10^{3} [$(2.9-7.4) \times 10^{-3}$]	7.99×10^{-9}	9.57×10^{-9}	$<10^{-12}$
3	5.7×10^{-3} [$(3.5-9.0) \times 10^{-3}$]	3.58×10^{-9}	$<10^{-12}$	$<10^{-12}$
4	4.9×10^{-3} [$(3.0-7.9) \times 10^{-3}$]	3.29×10^{-7}	2.74×10^{-9}	$<10^{-12}$
5[a]	5.4×10^{-1}	3.3×10^{-2}	2.5×10^{-3}	2.1
adriamycin	2.57×10^{-1} [$(1.89-5.3) \times 10^{-1}$]	1.04×10^{-4}	1.76×10^{-2}	1.53×10^{-4}

[a] Data from Y.-H. Hui et al.[13]

The IR, UV, ^{1}H-NMR, and ^{13}C-NMR spectra of 4 (Table 3) were very similar to those of 2 and 3. Comparisons of these spectra gave the indication that 4 could also possess the identical structural skeleton as that of asimin (2) and asiminacin (3); this included the terminal α,β-unsaturated lactone ring (fragment A), the adjacent bis-THF ring system (fragment B), and three hydroxyl groups. The relative configuration in the bis-THF ring system was defined as having the *threo-trans-threo-trans-threo* pattern by the analysis of the usual diagnostic proton chemical shifts in the ^{1}H-NMR spectrum of the triacetate 4a.[12]

The EIMS diagnostic fragment peaks of the tri-TMSi and tri-TMSi-d_9 derivatives of 4 (Figure 4) again determined the location of the adjacent bis-THF ring system at C-16–C-23 and the two flanking hydroxyl groups at C-15 and C-24 which were identical to the structures of 2 and 3. The placement of the third hydroxyl group at C-29 was suggested by fragment ions at m/z 173 and 767 in the EIMS spectrum of the tri-TMSi derivative of 4 and was confirmed by the corresponding diagnostic ions at m/z 182 and 794 in the EIMS spectrum of the tri-TMSi-d_9 derivative of 4. Isolate 4 was given the trivial name asiminecin.

There are now five reported asimicin isomers: asimicin (1),[1] asimin (2), asiminacin (3), asiminecin (4), and 4-deoxyasimicin (5)[13] (Figure 1). Their structures are characterized by a terminal α,β-unsaturated γ-lactone ring and an adjacent bis-THF ring system at C-16–C-23 with two flanking hydroxyl groups at C-15 and C-24. They all have the identical pseudosymmetrical *threo-trans-threo-trans-threo* pattern of relative configuration in the bis-THF ring systems. The only difference among these structures is the location of the third hydroxyl group from C-4 [asimicin (1)] to C-10 [asimin (2)], C-28 [asiminacin (3)], and C-29 [asiminecin (4)] or the absence of the third hydroxyl group [4-deoxyasimicin (5)]. A careful comparison of ^{1}H-NMR spectra of 2–4 showed that all proton chemical shifts are identical except those of the terminal methyl groups (Tables 1–3). A clear implication is that the δ value of the terminal methyl group is affected by the location of the third hydroxyl group. Since the ^{1}H-NMR spectra of compounds 1 and 5 were measured by different researchers with different spectrometers and different internal references, a side-by-side comparison of terminal methyl chemical shifts of 1–5 was made by the remeasurement of ^{1}H-NMR spectra of 1–5 on a Varian VXR-500S (at 500 MHz) spectrometer in $CDCl_3$ with resonance signals referenced to TMS. The δ values of these terminal methyl groups have been assigned as 0.878 for 4-deoxyasimicin (5), asimicin (1), and asimin (2) and 0.882 for asiminacin (3) and 0.891 for asiminecin (4). The downfield shift effect on the terminal methyls was observed only when the hydroxylation is located along the C_{25-34} aliphatic chain, and the presence or absence of an hydroxyl group on the C_{3-14} aliphatic chain has no such effect. Interestingly, identical downfield shifts of terminal methyls have been recently observed in a series of C-4, 10, 28, 29, and 30 hydroxylated bullatacins,[14] as well as C-30, 31, and 32 hydroxylated bullatacinones.[15]

Bioactivity and SAR's. Bioactivity data of compounds 1-5, against three human tumor cell lines, are presented in Table 4. All of our in house, 7-day-run, human solid tumor cytotoxicity values were determined in the same run to eliminate possible variations; values for adriamycin, as a positive control in the same run, are also presented for comparison. Among these asimicin isomers, the three novel acetogenins 2–4 are very toxic in the brine shrimp

test (BST), and this test continues to be a convenient bench-top procedure to discover new cytotoxic compounds.[8,16,17] In the human tumor cell panel at the Purdue Cancer Center, 2–4 showed extremely potent cytotoxicities against A-549 (lung cancer),[18] MCF-7 (breast cancer),[19] and H-29 (colon cancer)[20] cell lines. Compounds 2–4 exhibited high selectivity against H-29 cancer cells with ED_{50} values as low as $<10^{-12}$ μg/mL. The overall activities of these three compounds (2–4) are as equally active as or more active than the previously reported compounds trilobacin, asimicin, and bullatacin[2] and much more active than 4-deoxyasimicin (5), which lacks a third hydroxyl.[13] These compounds are worthy of *in vivo* antitumor evaluation provided that larger quantities can be isolated.

The biological activities (Table 4) of 4-deoxyasimicin (5)[13] were much less than that of asimicin (1)[1] in the A-549 and H-29 solid tumor cell lines and slightly more active in the MCF-7 cell line; this initially suggested that the hydroxyl group at the C-4 position was essential for potent activity. However, compounds 2–4, which have no 4-hydroxyl group in their structures, showed enhancement of activity vs that of asimicin (1) in the A-549 and MCF-7 cell lines. It appears that the substitution of the third hydroxyl group greatly enhances the cytotoxic activity and the third hydroxyl group substituted at the C-10, C-28, or C-29 positions is just as effective as or more effective than that at the C-4 position.

Experimental Section

Instrumentation. Melting point determinations were made 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 spectrometer. UV spectra were measured on a Beckman DU-7 UV spectrometer. ^1H- and ^{13}C-NMR spectra were recorded on a Varian VXR-500S (^1H at 500 MHz, ^{13}C at 125.75 MHz) spectrometer in $CDCl_3$ with resonance signals referenced to TMS. Low-resolution CIMS and EIMS data were collected on a Finnigan 4000 spectrometer. FAB MS, EIMS for TMSi and TMSi-d_9 derivatives, and exact mass measurements through peak matching were performed on a Kratos MS50 mass spectrometer. 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 a silica gel column (250 × 21 mm) equipped with a Rainin UV-1 detector set at 220 nm.

Chemicals. For preparation of tri-TMSi and tri-TMSi-d_9 derivatives, *N,O*-bis(trimethylsilyl)acetamide (BSA) and pyridine in silylation grade were purchased from Pierce Chemical Co. (USA); bis(trimethylsilyl-d_9)trifluoroacetamide (BSTF-A-d_{18}) was from Regis Chemical Co. (USA) under the brand name Deutero Regisil-d_{18}.

Derivatization. Tri-TMSi and tri-TMSi-d_9 derivatives were prepared by treatment of the isolated acetogenins with BSA for the tri-TMSi derivatives or BSTFA-d_{18} for the tri-TMSi-d_9 derivatives in the presence of pyridine. Approximately 10–50 μg 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 sample was treated with 2 μL of pyridine and 20 μL of BSA or BSTFA-d_{18} and 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/decade.

Bioassays. The brine shrimp (*Artemia salina* Leach) test (BST) was performed as modified.[8,9] Seven-day *in vitro* cytotoxicity tests against human tumor cell lines were carried out at the Purdue Cancer Center, using standard protocols for A-549 (human lung carcinoma),[18] MCF-7 (human breast carcinoma),[19] and HT-29 (human colon carcinoma)[20] with adriamycin as a positive control. The reported values (Table 4) were tabulated from the same run in order to facilitate comparison for the SAR's.

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

Extraction and Purification. The air-dried pulverized stem bark (15 kg) was extracted exhaustively with 95% EtOH and vacuum evaporated to yield extract F001 (1645 g) which was partitioned between H_2O and CH_2Cl_2 (1:1), giving a water-soluble fraction (F002, 5 g), CH_2Cl_2-soluble fraction (F003, 1560 g), and an insoluble interface (F004, 80 g). F003 was further partitioned between hexane and 90% MeOH aqueous solution and yielded the MeOH fraction (F005, 650 g) and the hexane fraction (F006, 905 g).

Directed by the BST bioassay, the most bioactive fraction, F005 (BST LC_{50} 7.151×10^{-1} μg/mL) (200 g) was further fractionated by open column chromatography on silica gel (8 kg, 60–200 mesh), eluting with hexane–EtOAc and EtOAc–MeOH gradients; 12 pools were made from the collected fractions according to their TLC patterns and evaluated by the BST bioassay. The most active pools (P_7–P_9) were combined (20 g) and subjected to further repeated separation by silica gel (1000 g, 230–400 mesh) column chromatography eluted with MeOH–$CHCl_3$ gradients. Further purifications of the most bioactive fractions F11–12 (1.2 g, BST LC_{50} 3.5×10^{-2} mg/mL) were carried out on Chromatotron plates (2 mm thick), eluted with hexane–CH_2Cl_2–MeOH (30:20:0–5). The white wax from the hexane–CH_2Cl_2–MeOH fractions was further resolved by HPLC eluted with 10% THF in MeOH–hexane gradients to yield the new acetogenins 2–4.

Asimin (2): colorless wax (12 mg); $[\alpha]_D$ +26 ($CHCl_3$, 1 mg/mL); UV λ_{max}^{MeOH} (nm) 215; IR υ_{max}^{film} (cm^{-1}) 3438 (OH), 2925, 2855, 1752 (C=O), 1457, 1318, 1200, 1069, 954, 870; FAB HR-MS (glycerol) *m/z* 623.4907 ($[M + H]^+$, found) (623.4887 calcd for $C_{37}H_{66}O_7$); CIMS, EIMS (tri-TMSi derivative), and EIMS (tri-TMSi-d_9 derivative) *m/z* see Figure 2; ^1H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125.75 MHz, $CDCl_3$) see Table 1.

Asimin Triacetate (2a). Treatment of 2 (4 mg) with Ac_2O–pyridine (at room temperature, overnight) and subsequent workup gave 2a as a wax: IR υ_{max}^{film} (cm^{-1}) 2926, 2855, 1758, 1733, 1459, 1368, 1317, 1241, 1068, 1022, 951; CIMS (isobutane) *m/z* 749 $[M + H]^+$, 689 $[MH - CH_3COOH]^+$, 629 $[MH - 2CH_3COOH]^+$, 569 $[MH - 3CH_3COOH]^+$, 551, 517, 465, 421, 405, 345, 327, 219; ^1H-NMR (500 MHz, $CDCl_3$) see Table 1.

Asiminacin (3): colorless wax (12 mg); $[\alpha]_D$ +21.1 ($CHCl_3$, 3.8 mg/mL); UV λ_{max}^{MeOH} (nm) 215; IR υ_{max}^{film} (cm^{-1}) 3418 (OH), 2925, 2856, 1753 (C=O), 1457, 1319, 1200, 1069, 953, 872; FAB HR-MS (glycerol) *m/z* 623.4874 ($[M + H]^+$, found), (623.4887 calcd for $C_{37}H_{66}O_7$); CIMS, EIMS (tri-TMSi derivative), and EIMS (tri-TMSi-d_9 derivative) *m/z* see Figure 3; ^1H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125.75 MHz, $CDCl_3$) see Table 2.

Asiminacin Triacetate (3a). Treatment of 3 (4 mg) with Ac_2O–pyridine (at room temperature, overnight) and subsequent workup gave 3a as a wax: IR υ_{max}^{film} (cm^{-1}) 2929, 2855, 1753, 1732, 1456, 1369, 1317, 1241, 1072, 1020, 949; CIMS (isobutane) *m/z* 749 $[M + H]^+$, 689 $[MH - CH_3COOH]^+$, 629 $[MH - 2CH_3COOH]^+$, 569 $[MH - 3CH_3COOH]^+$, 551, 465, 423, 407, 351, 347, 329, 219; ^1H-NMR (500 MHz, $CDCl_3$) see Table 2.

Asiminecin (4): colorless wax (10 mg); $[\alpha]_D$ +22 ($CHCl_3$, 1 mg/mL); UV λ_{max}^{MeOH} (nm) 215; IR υ_{max}^{film} (cm^{-1}) 3419 (OH), 2925, 2856, 1751 (C=O), 1456, 1318, 1199, 1069, 953, 872; HR-CIMS (isobutane) *m/z* 623.4868 ($[M + H]^+$, found), (623.4887 calcd for $C_{37}H_{66}O_7$); CIMS, EIMS (tri-TMSi derivative), and EIMS (tri-TMSi-d_9 derivative) *m/z* see Figure 4; ^1H-NMR (500 MHz, $CDCl_3$), and ^{13}C-NMR (125.75 MHz, $CDCl_3$) see Table 3.

Asiminecin Triacetate (4a). Treatment of 4 (4 mg) with Ac_2O–pyridine (at room temperature, overnight) and subsequent workup gave 4a as a wax: IR υ_{max}^{film} (cm^{-1}) 2930, 2845, 1754, 1735, 1460, 1370, 1318, 1242, 1072, 1024, 953; CIMS (isobutane) *m/z* 749 $[M + H]^+$, 689 $[MH - CH_3COOH]^+$, 629 $[MH - 2CH_3COOH]^+$, 569 $[MH - 3CH_3COOH]^+$, 521, 425, 397, 391, 369, 331, 295, 271, 257, 217, 201, 169, 101; ^1H-NMR (500 MHz, $CDCl_3$) see Table 3.

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