Three New Adjacent Bis-tetrahydrofuran Acetogenins with Four Hydroxyl Groups from *Asimina triloba*

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Three new adjacent bis-tetrahydrofuran ring Annonaceous acetogenins with four hydroxy groups, bullatetrocin (**1**), 10-hydroxyasimicin (**2**), and 10-hydroxytrilobacin (**3**), were isolated by activity-directed fractionation from the stem bark of *Asimina triloba*. Their structures were established on the basis of chemical and spectral evidence. The absolute stereochemistry at the C-10 hydroxy position was determined by converting **2** and **3** to their ketolactone isomers, 2,4-*cis*/*trans* 10-hydroxyasimicinones and 2,4-*cis*/*trans* 10-hydroxytrilobacinones, respectively. The bioactivities of the new compounds against brine shrimp larvae and six human solid-tumor cell lines are reported, and structure−activity relationships between trihydroxylated and tetrahydroxylated acetogenins are discussed. In addition to **1**−**3**, gigantetrocin A, 2,4-*cis*/*trans*-gigantetrocin A-ones, annonacin, and annonacin A were also isolated for the first time from this species.

Bioactivity-directed isolation using the brine shrimp lethality test (BST)[1,2] has led to the discovery of approximately 40 bioactive acetogenins from the seeds and stem bark of the paw paw, a native North American tree, *Asimina triloba* (L.) Dunal (Annonaceae).[3−11] As part of our continuing efforts to find new antitumor agents, we have isolated three new acetogenins (**1**−**3**, Chart 1) from this plant. The structures were identified as adjacent bis-tetrahydrofuran (bis-THF) ring acetogenins by NMR and MS spectroscopic techniques and chemical derivatization. The absolute configurations of **1**−**3** were solved through their respective per-Mosher ester analyses.[12] In addition to **1**−**3**, gigantetrocin A, *cis*/*trans*-gigantetrocin A-ones, annonacin, and annonacin A were also isolated for the first time from this species.[13]

Results and Discussion

Compound **1** was isolated as a colorless wax, and its molecular formula was determined to be $C_{37}H_{66}O_8$ by HRFABMS of the $[MH]^+$ at *m*/*z* 639.4827 (calcd 639.4836). The structural determination of **1** was based on the analyses of NMR and MS fragmentation data. The presence of an adjacent bis-THF ring with one flanking hydroxyl group on both sides was indicated by the ^{1}H- and ^{13}C-NMR signals at δ 3.40 (H-15), 74.2 (C-15); 3.85 (H-16), 83.3 (C-16); 3.85 (H-19), 82.5 (C-19); 3.93 (H-20), 82.2 (C-20); 3.93 (H-23), 82.8 (C-23); and 3.86 (H-24), 71.5 (C-24) (Tables 1 and 2). The presence of a 4-deoxy-α,β-unsaturated methyl-γ-lactone moiety was indicated by signals at δ 2.27 (H-3), 25.2 (C-3); 6.99 (H-35), 148.8 (C-35); 5.00 (H-36), 77.4 (C-36); and 1.41 (H-37), 19.2 (C-37). The *threo*/*trans*/*threo*/*trans*/*erythro* relative stereochemistry in the adjacent bis-THF moiety from C-15 to C-24 was established on the basis of the close similarity of the ^{1}H- and ^{13}C-NMR data of **1** and published values of bullatacin,[12] indicating that **1** is a bullatacin-type acetogenin.

Table 1. ^{1}H-NMR (*J* in Hz) Data of Bullatetrocin (**1**), Its Tetraacetate (**1a**), and Its Acetonide Derivative (**1b**)

position	**1**	**1a**	**1b**
3	2.27 tt (7.5, 1.5)	2.26 tt (7.5, 1.5)	2.26 brt (7.5)
4	1.53 m	1.20−1.50	1.53 m
5−13	1.22−1.50 m	1.20−1.50	1.22−1.70 m
14	1.41 m	1.64 m	1.41 m
15	3.40 m	4.85 m	3.41 m
16	3.85 m	3.98 m	3.85 m
17	1.63 m, 1.98 m	1.63 m, 1.96 m	1.63 m, 1.99 m
18	1.63 m, 1.98 m	1.63 m, 1.96 m	1.63 m, 1.99 m
19	3.85 m	3.91 m	3.85 m
20	3.93 m	3.91 m	3.92 m
21	1.59 m, 1.98 m	1.60 m, 1.96 m	1.59 m, 1.98 m
22	1.59 m, 1.89 m	1.59 m, 1.94 m	1.59 m, 1.89 m
23	3.93 m	3.98 m	3.92 m
24	3.86 m	4.89 m	3.85 m
25	1.41 m	1.64 m	1.41 m
26−29	1.22−1.50 m	1.20−1.50	1.22−1.70
30	1.42	1.65	1.41
31	3.65 m	4.87[a]	3.89 m[a]
32	3.65 m	4.80[a]	4.05 m[a]
33	1.42 m	1.65	1.42 m
34	0.928 t (7.0)	0.902 t (7.0)	0.918 t (7.0)
35	6.99 ddd (1.5,1.5,1.5)	6.99 ddd (1.5,1.5,1.5)	6.99 ddd (1.5,1.5,1.5)
36	5.00 qq (7.0, 1.8)	5.00 qq (7.0, 1.8)	5.00 qq (7.0, 1.8)
37	1.41 d (7.0)	1.41 d (7.0)	1.41 d (7.0)
15-OAc		2.08 s	
24-OAc		2.05 s	
31-OAc		2.04 s	
32-OAc		2.04 s	
acetonyl methyl			1.36 s 1.43 s

[a] Interchangeable assignments within same column.

In all, four hydroxyl groups in **1** were present; in addition to those at C-15 and C-24, the others were observed at δ 3.65 (2H, H-31, H-32) and δ 71.6, 71.7 in the ^{1}H- and ^{13}C-NMR spectra, respectively. This was further confirmed by the observation of four acetyl methyl signals at δ 2.037, 2.044, 2.05, and 2.08 in the ^{1}H-NMR spectrum of the tetraacetate (**1a**). The formation of an acetonide derivative (**1b**) suggested that a 1,2-diol unit existed in **1**. This 1,2-diol was deduced to possess an *erythro* configuration from a direct comparison of its NMR chemical shifts (as well as the shifts of

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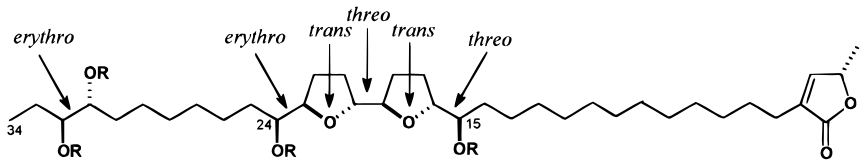
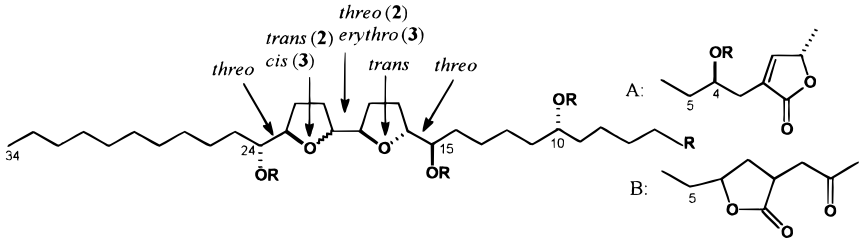
Chart 1



1 R=H **1a** R=Ac **1c** R=TMSi **1d** R=(*S*)-MTPA **1e** R=(*R*)-MTPA



2	A	R=H	**2a**	R=TMSi	**2b**	R=(*S*)-MTPA	**2c** R=(*R*)-MTPA
2d	B	R=H	**2e**	R=(*S*)-MTPA	**2f**	R=(*R*)-MTPA	
3	A	R=H	**3a**	R=TMSi	**3b**	R=(*S*)-MTPA	**3c** R=(*R*)-MTPA
3d	B	R=H	**3e**	R=(*S*)-MTPA	**3f**	R=(*R*)-MTPA	

Table 2. ^{13}C-NMR Data of Bullatetrocin (**1**), 10-Hydroxyasimicin (**2**), and 10-Hydroxytrilobacin (**3**)

position	**1**	**2**	**3**
1	173.8	174.6	174.7
2	134.2	131.2	131.2
3	25.2	33.4	33.4
4	27.4	69.9	69.9
5	29.3−29.8	37.4	37.4
6−8	29.3−29.8	25.5−29.7	25.5−29.7
9	29.3−29.8	37.2	37.3
10	29.3−29.8	71.7	71.8
11	29.3−29.8	37.3	37.3
12−13	29.3−29.8	25.5−29.7	25.5−29.7
14	33.3	33.4	33.5
15	74.2	74.1	74.5
16	83.3	83.1	83.2
17	28.4	28.3	25.5−29.7
18	28.9	28.9	25.7
19	82.5	81.8	81.6
20	82.2	81.8	80.9
21	29.2	29.0	25.8
22	25.0	28.3	27.0
23	82.8	83.2	82.6
24	71.5	74.0	73.9
25	32.5	33.4	34.2
26	22.0	25.6	25.6
27−29	29.3−29.8	25.5−29.7	25.5−29.7
30	37.2	25.5−29.7	25.5−29.7
31	71.5[a]	25.5−29.7	25.5−29.7
32	71.6[a]	31.9	31.9
33	39.7	22.7	22.7
34	14.1	14.1	14.1
35	148.8	151.8	151.8
36	77.4	78.0	78.0
37	19.2	19.1	19.1

[a] Interchangeable assignments.

its acetate and acetonide derivatives) with literature data.[13] These data are quite characteristic and serve to distinguish between *threo* and *erythro* diols. Usually, the ^{1}H-NMR spectrum of an *erythro* diol shows an oxygenated methine signal at δ 3.60, while its *threo* isomer will appear at δ 3.40.[13] The chemical shift of δ 3.65 indicated an *erythro* diol for **1**, and this was supported by the close comparison of similar data of the methine, acetyl methyl, and acetonyl methyl protons of its acetate (**1a**) and acetonide (**1b**) compared to those of reported *erythro* 1,2-diols (Table 1). To confirm the configuration, differential NOE spectra were measured. After irradiation at δ 1.43, no NOE effect was observed for H-31 and H-32, while ca. 2% NOE enhancements were observed for H-31 and H-32 at δ 3.89 and 4.05, respectively, when the methyl group at δ 1.36 was irradiated. According to the results of Gu *et al.*,[14] a *cis*-configuration of the acetonide at H-31 and H-32 can only be obtained when an *erythro* 1,2-diol is present (Figure 1).

The placements of the hydroxyl groups and the adjacent bis-THF ring unit along the aliphatic chain were determined by the EIMS of **1** and its tetra-TMSi derivative (**1c**), where the diagnostic fragments at *m*/*z* 367, 437, 489, 507, and 419 were characteristic of the bis-THF ring system placed at C-16 to C-23 (Figure 2). A prominent fragment at *m*/*z* 131 for **1c** indicated that one of the 1,2-diol OH groups was located at C-32. To confirm the observed result from MS, a single-relayed proton−proton COSY spectrum was measured, and the cross peak between the terminal methyl (C-34) and the C-32 methine proton strongly supported the above conclusion.

The absolute stereochemistry of the carbinol centers was determined by advanced Mosher methodology using per-(*R*)- and per-(*S*)-methoxy (trifluoromethyl) phenylacetic acid (MTPA) to produce the *S* (**1d**) and *R* (**1e**) Mosher ester derivatives (Table 4). Analysis of the differences between **1d** and **1e**, the positive values of the THF ring protons (H-20 to H-23), the positive value of H-14, as well as the generally negative values for the other THF-ring moiety (H-16 to H-19), indicated *R* and *S* configurations for C-15 and C-24, respectively, which were identical to the reported data of bullatacin.[12] An irregular positive $\Delta\delta_{S-R}$ value for H-16 was observed in this case but was also found in some other bullatacin-type acetogenins; and this is explained as being due to the steric interactions between the bulky bis-THF rings and the 15-MTPA ester.[10,12] Similarly, the irregular positive $\Delta\delta_{S-R}$ value for H-25 (which showed a negative value for all the other bullatacin−type acetogenins

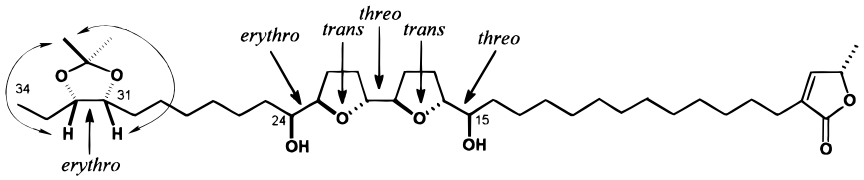


Figure 1. NOE correlations between one of the acetonide methyls and H-31/32 for **1b**.

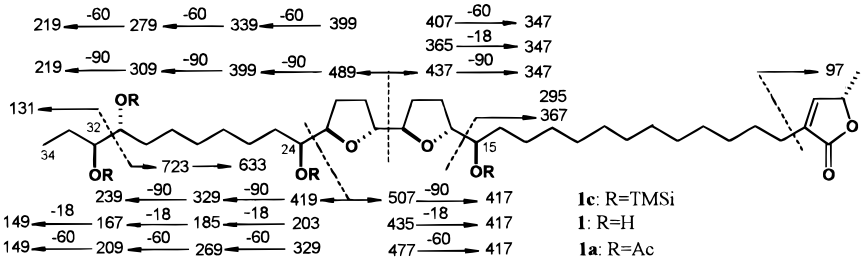


Figure 2. Diagnostic EIMS fragment ions (shown as *m/z* values) of **1** and its derivatives **1a** and **1c**.

Table 3. ^{1}H-NMR (*J* in Hz) Data of 10-Hydroxyasimicin (**2**), 2,4-(*cis/trans*)-10- Hydroxyasimicinones (**2d**), 10-Hydroxytrilobacin (**3**), and 2,4-(*cis/trans*)-10-Hydroxytrilobacinones (**3**)

position	**2**	**2d**	**3**	**3d**
1				
2		3.04 m, 3.02 m		3.04 m, 3.02 m
3a	2.53 dddd (15.1,3.5,1.4,1.4)	2.60 m, 2.24 m	2.53 dddd (15.5,3.5,1.5,1.5)	2.60 m, 2.23 m
3b	2.41 dddd (15.1,8.3,1.4,1.4)	1.50 m, 1.98 m	2.40 dddd (15.5,8.0,1.5,1.5)	1.50 m, 1.98 m
4	3.86 m	4.39 m, 4.55 m	3.83 m	4.39 m, 4.56 m
5	1.49 m	1.75 m, 1.48 m,	1.49 m	1.75 m, 1.48 m,
		1.69 m, 1.57 m		1.69 m, 1.57 m
6−8	1.20−1.60 m	1.20−1.60 m	1.20−1.60 m	1.20−1.60 m
9	1.41 m	1.41 m	1.41 m	1.41 m
10	3.60 m	3.59 m	3.59 m	3.60 m
11	1.41 m	1.41 m	1.43 m	1.42 m
12−13	1.20−1.60 m	1.22−1.50 m	1.20−1.60 m	1.22−1.50 m
14	1.41 m	1.41 m	1.41 m	1.41 m
15	3.39 m	3.39 m	3.39 m	3.39 m
16	3.86 m	3.85 m	3.83 m	3.83 m
17	1.64 m, 1.98 m	1.64 m, 1.98 m	1.77 m, 1.96 m	1.77 m, 1.96 m
18	1.64 m, 1.98 m	1.64 m, 1.98 m	1.86 m, 1.94 m	1.86 m, 1.94 m
19	3.86 m	3.85 m	3.97 ddd (7.0,6.5,5.0)	3.97 ddd (7.0,6.5,5.0)
20	3.86 m	3.85 m	4.05 ddd (8.5,6.0,5.0)	4.05 ddd (8.5,6.0,5.0)
21	1.64 m, 1.98 m	1.64 m, 1.98 m	1.69 m, 2.04 m	1.69 m, 2.04 m
22	1.64 m, 1.98 m	1.64 m, 1.98 m	1.74 m, 1.96 m	1.74 m, 1.96 m
23	3.86 m	3.85 m	3.83 m	3.83 m
24	3.39 m	3.39 m	3.39 ddd (6.5,7.0,6.0)	3.39 m
25	1.41 m	1.41 m	1.41 m	1.41 m
26	1.22−1.60 m	1.22−1.60 m	1.22−1.60 m	1.22−1.60 m
27−31	1.22−1.60 m	1.22−1.60 m	1.22−1.60 m	1.22−1.60 m
32	1.22−1.60 m	1.22−1.60 m	1.22−1.60 m	1.22−1.60 m
33	1.22−1.60 m	1.22−1.60 m	1.22−1.60 m	1.22−1.60 m
34	0.88 t (7.0)	0.88 t (7.0)	0.88 t (7.0)	0.88 t (7.0)
35a	7.19 ddd (1.5,1.5,1.5)	3.11 dd (18.5, 3.5)	7.19 ddd (1.5,1.5,1.5)	3.11 dd (18.5, 3.5)
35b		2.61 dd (18.5, 8.5)		2.61 dd (18.5, 8.5)
36	5.06 qddd (7.0,1.5,1.5,1.5)		5.06 qddd (7.0,1.5,1.5,1.5)	
37	1.44 d (7.0)	2.20 s	1.44 d (7.0)	2.20 s

isolated so far) is likely caused by the diamagnetic effects of the benzene ring of the Mosher esters at C-31 and C-32. The difference in chemical shifts for the terminal methyl group with respect to the (*S*)- and (*R*)-Mosher ester derivatives indicated that C-32 possesses an *R* configuration. The C-31 position was, therefore, determined to be *S* due to the *erythro* configuration of the 1,2-diol at C-31 and C-32. Because the ent-isomers are never found in naturally occurring acetogenins, the absolute configuration of this new compound, bullatetrocin (**1**) is determined as 15*R*, 24*S*, 31*R*, and 32*S*; C-36 was assumed as *S*, as in all other acetogenins.[13]

Compound **2** was also isolated as a colorless wax, and the HRFABMS of **2** gave $[MH]^+$ at *m/z* 639.4855 (calcd 639.4836), corresponding to a molecular formula of $C_{37}H_{66}O_8$. The presence of an adjacent bis-THF ring unit with one flanking hydroxyl group on both sides in **2** was indicated by the ^{1}H- and ^{13}C-NMR resonances at δ 3.39 (H-15), 74.1 (C-15); 3.86 (H-16), 83.1 (C-16); 3.86 (H-19, 20), 81.8 (C-19, 20); 3.86 (H-23), 83.2 (C-23); and 3.39 (H-24), 74.0 (C-24) (Tables 2 and 3). These NMR data identified **2** as an asimicin-type acetogenin. The methylated α,β-unsaturated γ-lactone with a 4-OH group in **2** was indicated by ^{1}H- and ^{13}C-NMR signals at δ 7.19 (H-35), 151.8 (C-35); 5.06 (H-36), 78.0 (C-36); 3.86 (H-4), 69.9 (C-4); 2.53 (H-3a), 2.41 (H-3b), 33.4 (C-3); and 1.44 (H-37), 19.1 (C-37) (Tables 2 and 3).

The EIMS of the TMSi derivative (**2a**) of **2** displayed intense ion peaks at *m/z* 543 and *m/z* 243 (Figure 3) and suggested the placement of bis-THF rings at C-16

Table 4. ^{1}H-NMR Data of the (*S*)- and (*R*)-Mosher Esters of **1d 1e**, **2b**, **2c**, **3b**, and **3c**

proton	H-14	H-16	H-17	H-18	H-19	H-20	H-21	H-22	H-23	H-25	H-34
S-MTPA (**1d**)	1.61	4.03	1.94,1.53	1.80,1.65	3.79	3.79	1.80,1.65	1.83,1.67	3.96	1.54	0.82
R-MTPA (**1e**)	1.46	4.00	2.01,1.58	1.89,1.75	3.82	3.63	1.75,1.62	1.76,1.59	3.86	1.53	0.90
$\Delta\delta_{S-R}$	+0.15	+0.03	−0.07,−0.05	−0.09,−0.10	−0.03	+0.16	+0.05,+0.03	+0.07,+0.08	+0.10	+0.01	−0.08
configuration		15*R*							24*S*		32*R*

proton	H-14	H-16, 23	H-17, 22	H-18, 21	H-19,20	H-25	H-5	H-3	H-35	H-36	H-37
S-MTPA (**2b**)	1.57	3.94	1.83	1.73	3.78	1.58	1.65	2.56, 2.60	6.72	4.86	1.28
R-MTPA (**2c**)	1.47	3.99	1.93	1.83	3.93	1.47	1.62	2.58, 2.67	6.96	4.91	1.30
$\Delta\delta_{S-R}$	+0.10	−0.05	−0.10	−0.10	−0.15	+0.11	+0.03	−0.02,−0.07	−0.24	−0.05	−0.02
configuration		15*R*			24*R*				4*R*		

proton	H-14	H-16	H-17	H-18	H-19	H-20	H-21	H-22	H-23	H-25	H-5	H-3	H-35	H-36	H-37
S-MTPA	1.61	3.97	1.78	1.80	3.64	3.71	1.76	1.90	3.93	1.61	1.65	2.56	6.72	4.85	1.28
(**3b**)			1.47	1.66			1.48	1.50				2.60			
R-MTPA	1.46	3.98	1.91	1.97	3.80	3.75	1.99	1.95	3.94	1.46	1.62	2.60	6.96	4.91	1.30
(**3c**)			1.58	1.80			1.67	1.58				2.67			
$\Delta\delta_{S-R}$	+0.15	−0.01	−0.13	−0.17	−0.16	−0.04	−0.22	−0.05	−0.01	+0.15	+0.03	−0.02	−0.24	−0.06	−0.02
			−0.11	−0.14			−0.18	−0.08				−0.07			
configuration		15*R*							24*R*				4*R*		

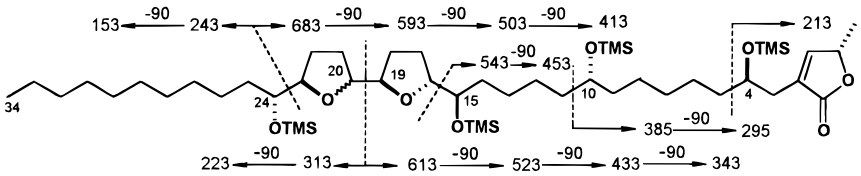


Figure 3. Diagnostic EIMS fragment ions (shown as *m*/*z* values) of **2a** (19/20 threo) and **3a** (19/20 erythro).

to C-23. The fragment at *m*/*z* 213 is a characteristic ion of an α,*β*-unsaturated *γ*-lactone with a 4-OH group present.[15] The position of the fourth OH group was determined by the fragment at *m*/*z* 385, which indicated that this hydroxyl was at C-10.[16]

The relative configuration of **2** was deduced as *threo*/*trans*/*threo*/*trans*/*threo* from C-15 to C-24 based on the similar chemical shifts in the ^{1}H- and ^{13}C-NMR spectra of **2** with those of the model compound, asimicin.[10] Again, the absolute stereochemistry of **2** was established using advanced Mosher ester methodology (Table 4). Analysis of differences between the (*S*)- and (*R*)-Mosher derivatives **2b** and **2c** around the *γ*-lactone ring moiety exhibited negative results for H-3, H-4, H-35, H-36, and H-37 and positive results for H-5, thus suggesting, an (*R*) configuration for C-4 (Table 4). The $\Delta\delta_H(S-R)$ values of the per-MTPA ester data around the bis-THF ring system were very similar to those of the related protons of asimicin, indicating that the absolute stereochemistry of the carbinol centers at C-15 and C-24 were (*R*) and (*R*), respectively. Because the chemical shifts of H-9 and H-11 were virtually indistinguishable by ^{1}H NMR, the absolute stereochemistry of the carbinol center at C-10 was not solvable by spectral analysis of the per-Mosher ester derivatives. Therefore, the mixture of 2,4-*cis*/*trans*-10-hydroxyasimicinones (**2d**) was prepared by translactonization of **2** with weak base. The resulting per-Mosher esters **2e** and **2f** of **2d** indicated that the absolute configuration at C-10 was *R* because positive values (+0.02 and +0.01) of $\Delta\delta_H(S-R)$ for H-4 were observed. Compound **2** was then identified as 10-hydroxyasimicin with the expected 4*R*, 10*R*, 15*R*, and 24*R* configurations. The configuration at C-36 was determined as *S* by the method of Hoye *et al.*[17] This compound was previously isolated by Fujimoto's research group from the seeds of *Annona reticulata*, but a compound name, spectral data, the stereochemistry at C-10, and biological evaluations were not reported.[18]

Compound **3** was isolated as a colorless oil, and the molecular formula was determined by HRFABMS of the $[MH]^+$ at *m*/*z* 639.4827, which was consistent with $C_{37}H_{66}O_8$ (calcd 639.4836). The presence of the methylated α,*β*-unsaturated *γ*-lactone with a 4-OH group was deduced from the NMR data (Tables 2 and 3). Comparison of the ^{1}H- and ^{13}C- NMR resonances with those of trilobacin and trilobin for the THF rings and flanking carbinol signals suggested that **3** had an adjacent bis-THF ring system possessing the trilobacin-type subunit, and the chemical shifts also indicated a relative configuration of *threo*/*trans*/*erythro*/*cis*/*threo* from C-15 to C-24 for **3** (Tables 2, 3).[7,8]

The positions of the adjacent bis-THF rings with their flanking hydroxyls and the fourth OH group in **3** were established by the EIMS of its tetra-TMSi derivative (**3a**). As shown in Figure 3, the fragments at *m*/*z* 543, 613, 313, and 243 located the THF rings at C-16 to C-23. An intense peak at *m*/*z* 385 was concluded to be due to fission between C-10 and C-11, and the fourth hydroxyl group was, thus, determined at C-10 as in **2**.

To elucidate the absolute stereochemistry, the pair of per-Mosher esters of **3** was prepared (**3b**, **3c**), and C-4, C-15, and C-16 were determined as *R*, *R*, and *R*, respectively (Table 4). As with **2**, the absolute stereochemistry of **3** at the 10-OH position was determined by converting **3** to its ketolactone isomeric mixture, 2,4-*cis*/*trans*-10-hydroxytrilobacinones (**3d**), and the two per-(*S*) (**3e**) and (*R*) (**3f**) Mosher ester derivatives were prepared to examine the $\Delta\delta_H(S-R)$ data. As with **2e** and **2f**, the difference values for H-4 were +0.02 and +0.02, respectively, indicating an *R* configuration at C-10 in **3**.

In addition to **1**−**3**, several known acetogenins were also isolated for the first time from this species. The structures of these known compounds were determined as gigantetrocin A; 2,4-*cis*/*trans*-gigantetrocin A-ones; annonacin; and annonacin A, based on the analyses of

Table 5. Bioactivity of Compounds **1**−**3**, Bullatacin , Bullanin, Asimicin, Asimin, Trilobacin, and Trilobin

compound	BST[a]	A-549[b]	MCF-7[c]	HT-29[d]	A-498[e]	PC-3[f]	PaCa-2[g]
1	3.1×10^{-1}	3.52×10^{-1}	4.97×10^{-1}	3.32×10^{-5}	>1	>1	>1
2	4.3×10^{-1}	6.73×10^{-1}	3.27×10^{-1}	7.58×10^{-3}	>1	5.27×10^{-1}	>1
3	2.6×10^{-1}	1.00×10^{-8}	1.88×10^{-8}	1.39	1.00×10^{-2}	3.78×10^{-1}	1.96×10^{-1}
bullatacin[21]	8.0×10^{-4}	1.25×10^{-13}	$<10^{-12}$	10^{-12}	NT	NT	NT
bullanin[11]	6.0×10^{-3}	3.11×10^{-14}	3.22×10^{-14}	4.77×10^{-14}	NT	NT	NT
asimicin[9]	2.7×10^{-2}	8.43×10^{-4}	8.52×10^{-1}	$<10^{-12}$	NT	NT	NT
asimin[9]	4.6×10^{-3}	7.99×10^{-9}	9.57×10^{-9}	$<10^{-12}$	NT	NT	NT
trilobacin[9]	8.7×10^{-3}	8.02×10^{-4}	3.39×10^{-1}	$<10^{-12}$	NT	NT	NT
trilobin[8]	9.7×10^{-3}	5.71×10^{-12}	2.95×10^{-12}	2.20×10^{-8}	NT	NT	NT
Adriamycin[i]	8.0×10^{-2}	1.78×10^{-3}	1.70×10^{-1}	4.28×10^{-3}	1.78×10^{-3}	1.89×10^{-2}	2.43×10^{-3}

[a] brine shrimp lethality test. [b] human lung carcinoma. [c] human breast carcinoma. [d] human colon adenocarcinoma. [e] human kidney carcinoma. [f] human prostate adenocarcinoma. [g] human pancreatic carcinoma. [h] NT = not determined. [i] standard positive control. Bioactivity data other than **1**−**3** were taken from the literature.[8,9,11,21]

spectroscopic data and the EIMS of the TMSi derivatives of each compound.[13]

Among the large number of reported adjacent bis-THF ring acetogenins, bis-THF ring acetogenins with four OH groups are relatively rare. Those acetogenins with four OH groups generally show less potent activity against human solid tumor cell lines[15] and in the inhibition of yellow fever mosquito larvae.[19] This decrease in bioactive potency might be due to the extra OH, which would increase the polarity of the molecules and affect the distribution of the compounds in cells, leading to reduction of activity. Similar decreases of activity were also observed for those mono-THF ring acetogenins with five or six hydroxyls when compared with the four hydroxylated mono-THF ring molecules.[20]

The three new acetogenins, **1**−**3**, all showed significant activities in the brine shrimp lethality test (BST). Compounds **1** and **2** displayed selective cytotoxicities for the colon (HT-29) cells; and **3**, for the lung (A-549) and breast (MCF-7) cells in our panel of six human solid-tumor cell lines in a 7-day MTT test (Table 5). As mentioned previously, the biological activities of **1**−**3** are lower than those of the similar acetogenins bearing three hydroxyl groups. Compound **1** has the adjacent bis-THF ring in the same position and the same stereochemistry as that of bullatacin, but the latter exhibits much more potent activities (Table 5).[21] Bullanin is 30-hydroxy-4-deoxybullatacin and possesses an even closer structural resemblance to **1**, in which the third hydroxyl group is substituted at C-30 instead of a diol at C-31 and C-32 as in **1**. However, the ED_{50} values of bullanin were as low as $<10^{-12}$ μg/mL (Table 5).[11] Asimicin [with a 4(*R*)-OH] and asimin [a 4-deoxyasimicin with a 10-(*R*)-OH] are both very potent cytotoxic compounds for human tumor cell lines, while 10(*R*)-hydroxyasimicin (**2**) displayed much lower cytotoxic potencies compared with its three hydroxylated counterparts (Table 5).[9] Similar results were also found among trilobacin, trilobin (4-deoxy-10-hydroxytrilobacin), and 10(*R*)-hydroxytrilobacin (**3**) (Table 5).[8] Significant enhancement of cytotoxicity for the lung (A-549) and breast (MCF-7) carcinoma cell lines was observed when the C-4 hydroxyl (in trilobacin) was shifted to C-10 (in trilobin). Similarly, the presence of the C-10 hydroxyl group appears to make **3** more potent than trilobacin in these two cell lines, while **3** (with four hydroxyls instead of three) is less toxic when compared with trilobin. Thus, polarity seems to play an extremely important role in determining the cytotoxicity of the acetogenins. With the adjacent bis-THF ring acetogenins, the three hydroxylated molecules are much more potent than those with four hydroxyl groups. These data, therefore, provide useful information in the study of structure−activity relationships among these very potent bioactive compounds.

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. The IR data were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer. The LREIMS and HREIMS were obtained on Finnigan 4000 and on Kratos 50 spectrometers, respectively. The NMR spectra were recorded on a Varian VXR-500 (^{1}H at 500 MHz and ^{13}C at 125 MHz) or a Bruker ARX-300 (^{1}H at 300 MHz and ^{13}C at 75 MHz) spectrometer with $CDCl_3$ as solvent and TMS as internal reference. A Rainin HPLC system with Dynamax software and a Dynamax UV-1 variable wavelength detector was used for preparative separations.

Plant Material. The stem bark of *Asimina triloba* was collected from stands growing wild at the Purdue University Horticultural Research Farm, West Lafayette, Indiana. The species was identified by Dr. George R. Parker, Department of Forestry and Natural Resources, Purdue University. A voucher specimen is deposited in the Pharmacognosy Herbarium at Purdue University.

Bioassays. The brine shrimp (*Artemia salina* Leach) test (BST)[1,2] was routinely employed for evaluating the extracts, fractions, and isolated compounds from the title plant. Seven-day MTT *in vitro* cytotoxicity determinations against A-549 (human lung carcinoma),[22] MCF-7 (breast carcinoma),[23] HT-29 (colon adenocarcinoma),[24] A-498 (kidney carcinoma),[22] PC-3 (prostate adenocarcinoma),[25] and MIA PaCa-2 (pancreatic carcinoma)[26] human tumor cell lines were performed at the Cell Culture Laboratory, Purdue Cancer Center, using standard protocols, with Adriamycin as a positive control.

Extraction and Isolation. A 3.0-g fraction of F005 obtained as described in previous papers[6−11] was repeatedly chromatographed over open Si gel columns using gradients of CH_2Cl_2−MeOH and then separated by repeated normal-phase HPLC (Dynamax-60 A 8 μm Si gel, 250 × 21.4 mm i.d. or 250 × 4.6 mm i.d.), eluted with hexane−90% MeOH/THF (8:2) to give a mixture containing **1**−**3**. The mixture was further resolved on a C_{18} column (250 × 21.4 mm i.d.) with a CH_3CN−H_2O (8:2) isocratic solvent system to yield, in sequence, **3** (4.4 mg), **1** (9.7 mg), and **2** (8.0 mg).

Bullatetrocin (1): colorless wax, $[\alpha]^{22}_D$ +16.3° (*c* 0.27, $CHCl_3$); IR (dry film) ν_{max} 3350, 2923, 2846, 1742,

1594, 1455, 1359, 1125, 1103 cm^{-1}; FABMS *m/z* 639 $[M + H]^+$; HRFABMS (GLY/S-GLY) *m/z* 639.4827 for $C_{37}H_{66}O_8$ $[M + H]^+$, calcd 639.4836; EIMS (Figure 1); ^1H and ^{13}C NMR (Tables 1, 2).

Formation of tetraacetate (1a). Compound **1** (1.5 mg) was acetylated with Ac_2O−pyridine (1:1) at room temperature overnight to give **1a**; ^1H-NMR data (Table 1).

Formation of Acetonide (1b). Compound **1** (4.0 mg) was dissolved in 2 mL of dry Me_2CO with 2 pellets of *p*-toluenesulfonic acid monohydrate, and the mixture was allowed to stand at room temperature for 5 h using TLC to monitor the conversion of **1** to **1b**. The product was then purified over Si gel in a small pipette eluted with hexane−Me_2CO (20:1) to give 1.5 mg of **1b**.

Formation of Tetra-TMSi Derivatives (1c, 2a, 3a). Approximately 10−50 μg of each pure compound were placed in a 100-μL conical reaction vial and dried in a vacuum desiccator over P_2O_5 for 24 h. Each sample was treated with 2 μL of pyridine and 20 μL of *N*,*O*-bis-(trimethylsilyl) acetamide and heated at 70 °C for 30 min.

Formation of per-(*R*)- and per-(*S*)-Mosher Ester Derivatives. Each compound (0.5−1 mg) was dissolved in 0.5 mL of dry CH_2Cl_2, and 0.2 mL of pyridine, and 0.2 mg of 4-dimethylaminopyridine, and 25 mg of (*R*)-(−)-methoxyl-α-(trifluoromethyl)-phenylacetyl chloride was sequentially introduced to this solution and the reaction mixture stored in a refrigerator overnight. The product was purified over a small pipette eluted with hexane, hexane−CH_2Cl_2, CH_2Cl_2, and CH_2Cl_2−EtOAc to give *S*-Mosher esters. *R*-Mosher esters were prepared in the same way using (*S*)-(+)-methoxyl-α-(trifluoromethyl)-phenylacetyl chloride reagent. ^1H NMR: **2e**, δ 4.53, 4.37 (H-4); **2f**, δ 4.51, 4.36 (H-4); **3e**, δ 4.53, 4.38 (H-4); **3f**, δ 4.51, 4.36 (H-4); ^1H-NMR data of **1d**, **1e**, **2b**, **2c**, **3b**, and **3c**, see Table 4.

10-Hydroxyasimicin (2): colorless wax, $[\alpha]^{22}_D$ +17.3° (*c* 0.22, $CHCl_3$); IR (dry film) ν_{max} 3376, 2922, 2856, 1740, 1600, 1467, 1318, 1116, 1074 cm^{-1}; FABMS *m/z* 639 $[M + H]^+$; HRFABMS *m/z* 639.4855 for $C_{37}H_{66}O_8$ $[M + H]^+$, calcd 639.4836; EIMS (Figure 2); ^1H- and ^{13}C-NMR data (Tables 2, 3).

Formation of 2,4-*cis/trans*-10-Hydroxyasimicinone (2d). Compound **2** (5 mg) was refluxed in 10 mL EtOH saturated with Na_2CO_3 for 4 h. The solution was then diluted with 90 mL of H_2O, and the EtOH was completely evaporated. The remaining H_2O solution was partitioned with $CHCl_3$ to give 4.0 mg of **2d**, which was prepared to form the per-Mosher esters (**2e**, **2f**).

10-Hydroxytrilobacin (3): colorless oil, $[\alpha]^{22}_D$ +8.3° (*c* 0.36, $CHCl_3$); IR (dry film) ν_{max} 3365, 2928, 2856, 1752, 1590, 1456, 1420, 1120, 1036 cm^{-1}; FABMS (GLY/S-GLY) *m/z* 639 $[M + H]^+$; HRFABMS (GLY/S-GLY) *m/z* 639.4827 for $C_{37}H_{66}O_8$ $[M + H]^+$, calcd 639.4836; EIMS (Figure 2); ^1H and ^{13}C NMR data (Tables 2 and 3); the procedures for the preparation of **3b**−**3f** were the same as those employed for **2b**−**2f**.

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