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BIOLOGICALLY ACTIVE ACETOGENINS FROM STEM BARK OF *ASIMINA TRILOBA*

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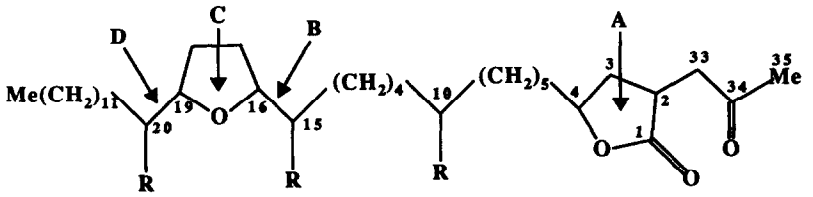
Key Word Index—*Asimina triloba*; Annonaceae; stem bark; acetogenins; *cis*- and *trans*-annonacin-A-one; *cis*- and *trans*-gigantetrocinone; *cis*- and *trans*-isoannonacin; squamolone.

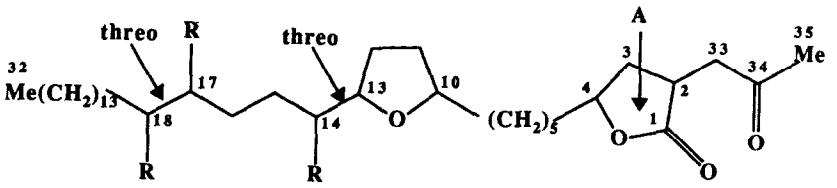
Abstract—In continuing our research with cytotoxic and pesticidal components from the stem bark of the North American paw paw, *Asimina triloba*, the novel cytotoxic monotetrahydrofuran Annonaceous acetogenins, *cis*- and *trans*-annonacin-A-one, *cis*- and *trans*-gigantetrocinone and *cis*-isoannonacin, in addition to the known compounds, *trans*-isoannonacin and squamolone, have been identified. Brine shrimp lethality testing was used to direct the fractionation. The structures were elucidated by spectral analysis and/or chemical synthesis. These acetogenins have potent cytotoxicities against the human tumour cell lines of A-549 (lung carcinoma), MCF-7 (breast carcinoma) and HT-29 (colon adenocarcinoma).

INTRODUCTION

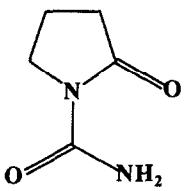
Asimina triloba (paw paw) is one of several species of the genus *Asimina* which are distributed in the eastern regions of the United States. Aside from its delicious fruits, its medicinal values were first recognized by the report of asiminine, purportedly an emetic alkaloid from its seeds, and another alkaloid, analobine, which is contained in the bark and was once used in medicine [1]. The discovery in our laboratory of the acetogenin, asimicin, and its significant cytotoxic and pesticidal properties [2] initiated our interest in the bioactive components of the bark of this plant. Subsequent investigation led to the isolation and structural elucidation of the new cytotoxic acetogenin, trilobacin, which is a diastereoisomer of asimicin, and five other cytotoxic compounds: bullatacin, bullatacinone, *N-p*-coumaroyltyramine, *N-trans*-feruloyltyramine and (+)-syringaresinol [3]. Further efforts, using brine shrimp lethality (BST) directed fractionation [4, 5] of the ethanol extract of the stem bark, have now resulted in the isolation of the novel monotetrahydrofuran acetogenins, *cis*- and *trans*-annonacin-A-one (**1**, **2**,) *cis*- and *trans*-gigantetrocinone (**3**, **4**), both as *cis*/*trans* mixtures at the ketolactone moiety, and *cis*-isoannonacin (**5**),

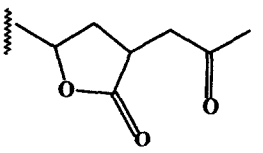


Compounds	A	B	C	D	R
1	*cis*	*threo*	*trans*	*erythro*	OH
1a	*cis*	*threo*	*trans*	*erythro*	OAc
2	*trans*	*threo*	*trans*	*erythro*	OH
2a	*trans*	*threo*	*trans*	*erythro*	OAc
5	*cis*	*threo*	*trans*	*threo*	OH
5a	*cis*	*threo*	*trans*	*threo*	OAc
6	*trans*	*threo*	*trans*	*threo*	OH
6a	*trans*	*threo*	*trans*	*threo*	OAc

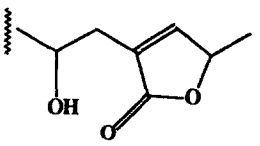


Compounds	A	R
3	*cis*	OH
3a	*cis*	OAc
4	*trans*	OH
4a	*trans*	OAc





Fragment A



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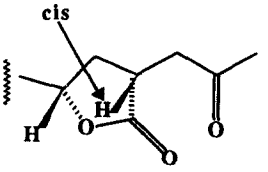

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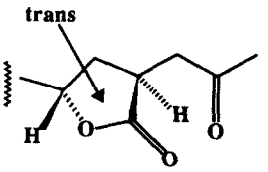

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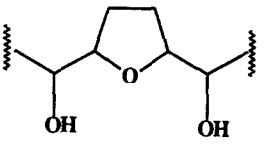

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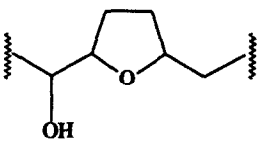


Fragment F

together with the known *trans*-isoannonacin (**6**) which is mixed with the *cis* stereoisomer **5**, and a known amide compound, squamolone (**7**).

RESULTS AND DISCUSSION

The most active fraction F005 was obtained from the ethanol extract (F001) of the dried stem bark by partition between methylene chloride and water, and subsequently between hexane and 90% aqueous methanol under the direction of BST [4, 5]. The methanol extract (F005) was submitted to successive column, chromatotron and HPLC chromatographies leading to the isolation of the monotetrahydrofuran acetogenins: *cis*- and *trans*-annonacin-A-one (**1**, **2**), *cis*- and *trans*-gigantetrocinone (**3**, **4**), *cis*- and *trans*-isoannonacin (**5**, **6**) and a diamide compound, squamolone (**7**). As previously observed with such ketolactones [6], the *cis*- and *trans*- diastereoisomeric mixtures of **1** and **2**, **3** and **4**, and **5** and **6** could not be resolved by normal chromatographic techniques.

The mixture of *cis*- and *trans*-annonacin-A-one (**1**, **2**) was isolated as an amorphous powder. CI-mass spectrometry gave a $[M+H]^+$ at *m/z* 597 indicating a M_r of 596. The molecular formula was established to be $C_{35}H_{64}O_7$ by the high resolution FAB-mass spectrum (glycerol, $[M+H]^+$, 597.4718; calcd for $C_{35}H_{65}O_7$, 597.4730). The IR spectrum showed strong absorption at 1754 cm^{-1} for a γ-lactone and 1718 cm^{-1} for a ketone. Compounds **1** and **2** were transparent under UV light at 205 nm suggesting that the lactone ring is not α,β-unsaturated. Compared to the NMR data of the mixture of *cis*- and *trans*-bullatacinone [6], the ^1H and ^{13}C NMR spectra of **1** and **2** clearly indicated the presence of a ketolactone moiety (fragment A), which is isomeric with the butenolide structure (fragment B) of bullatacin [6]. In the ^1H NMR spectrum (Table 1), the resonances at δ4.53 and 4.37, with combined integrations of one proton, were assigned to H-4 and suggested the presence of *cis*- and *trans*-diastereoisomers at the ketolactone ring moiety, as is typical with these ketolactones [6]. In the ^{13}C NMR (Table 2), signal pairs at δ178.7 and 178.2, 44.2 and 43.8, 79.3 and 78.8, and 205.5 and 205.5 were assigned to C-1, C-2, C-4, and C-34, respectively; they confirmed the presence of *cis*- and *trans*-isomers. The absolute configuration of C-4 was suggested as *R* by comparison of ^1H and ^{13}C NMR spectral data with those of *cis*- and *trans*-bullatacinone of known chirality [7]. Thus, the *cis*- and *trans*-subunits can be described by the fragments C and D. The further assignments of H-2, H-3a, H-3b, H-33a and H-33b were

Table 1. ^{1}H NMR spectral data of compounds **1**, **1a**, **2**, **2a**, **5** and **5a** (500 MHz, δ, $CDCl_3$)

H	1	1a	2	2a	5	5a
2	3.08 *m*	3.08 *m*	3.00 *m*	3.00 *m*	3.08 *m*	3.08 *m*
3a	2.58 *ddd* (12.3, 9.4, 5.6)	2.58 *dddd* (12.3, 9.4, 5.6)	2.20 *ddd* (12.9, 9.6, 3.4)	2.20 *m* (12.9, 9.6, 3.4)	2.58 *ddd* (12.3, 9.4, 5.6)	2.58 *ddd* (12.3, 9.4, 5.6)
3b	1.45 *m*	1.45 *m*	1.95 *m*	1.95 *m*	1.45 *m*	1.45 *m*
4	4.37 *dddd* (10.7, 7.4, 5.4, 5.4)	4.37 *dddd* (10.7, 7.4, 5.4, 5.4)	4.53 *dddd* (8.3, 8.2, 5.7, 3.2)	4.53 *dddd* (8.3, 8.2, 5.7, 3.2)	4.37 *dddd* (10.7, 7.4, 5.4, 5.4)	4.37 *dddd* (10.7, 7.4, 5.4, 5.4)
5–8	1.25–1.60 *m*	1.25–1.60 *m*	1.25–1.60 *m*	1.25–1.60 *m*	1.25–1.62 *m*	1.25–1.62 *m*
9,11,14	1.39 *m*	1.40–1.60 *m*	1.39 *m*	1.40–1.60 *mm*	1.39 *m*	1.40–1.60 *m*
10	3.57 *m*	4.81 *m*	3.57 *m*	4.81 *m*	3.57 *m*	4.82 *m*
12–13	1.25–1.60 *m*	1.25–1.60 *m*	1.25–1.60 *m*	1.25–1.60 *m*	1.25–1.62 *m*	1.25–1.62 *m*
15	3.38 *m*	4.81 *m*	3.38 *m*	4.81 *m*	3.38 *ddd* (6.6, 5.7, 5.5)	4.82 *m*
16	3.79 *m*	3.94 *m*	3.79 *m*	3.94 *m*	3.78 *ddd* (6.6, 6.7, 6.3)	3.94 *ddd* (6.8, 6.7, 6.3)
17	1.98, 1.61 *m*	1.93, 1.70 *m*	1.98, 1.61 *m*	1.93, 1.70 *m*	1.96, 1.66 *m*	1.93, 1.70 *m*
18	1.90, 1.55 *m*	1.93, 1.70 *m*	1.90, 1.55 *m*	1.93, 1.70 *m*	1.96, 1.66 *m*	1.93, 1.70 *m*
19	3.79 *m*	3.94 *m*	3.79 *m*	3.94 *m*	3.78 *ddd* (6.8, 6.7, 6.3)	3.94 *ddd* (6.8, 6.7, 6.3)
20	3.85 *m*	4.89 *m*	3.85 *m*	4.89 *m*	3.38 *ddd* (6.6, 5.7, 5.5)	4.82 *m*
21–31	1.25–1.60 *m*	1.25–1.60 *m*	1.25–1.60 *m*	1.25–1.60 *m*	1.25–1.62 *m*	1.25–1.62 *m*
32	0.86 *t* (6.9)	0.86 *t* (6.9)	0.86 *t* (6.9)	0.86 *t* (6.9)	0.86 *t* (6.9)	0.86 *t* (6.9)
33a	3.02 *dd* (18.5, 3.5)	3.02 *dd* (18.5, 3.5)	2.98 *dd* (18.5, 3.4)	2.98 *dd* (18.5, 3.4)	3.02 *dd* (18.5, 3.5)	3.02 *dd* (18.5, 3.5)
33b	2.64 *dd* (15.3, 8.6)	2.64 *dd* (15.3, 8.6)	2.56 *dd* (19.5, 10.6)	2.56 *dd* (19.5, 10.6)	2.64 *dd* (15.3, 8.6)	2.64 *dd* (15.3, 8.6)
35	2.17 *s*	2.17 *s*	2.17 *s*	2.17 *s*	2.17 *s*	2.17 *s*
OAc-10	—	2.03 *s*	—	2.03 *s*	—	2.02 *s*
OAc-15	—	2.02 *s*	—	2.02 *s*	—	2.05 *s*
OAc-20	—	2.06 *s*	—	2.06 *s*	—	2.05 *s*

Table 2. ^{13}C NMR spectral data of compounds **1–5** (125 MHz, δ, $CDCl_3$)

C	1	2	3	4	5
1	178.2	178.7	178.2	178.7	178.2
2	43.8	44.2	43.8	44.3	43.8
3	25.2–37.4	25.2–37.4	25.2–36.7	25.2–36.7	25.3–37.5
4	78.8	79.3	78.9	79.3	78.9
5–9	25.2–37.4	25.2–37.4	25.2–36.7	25.2–36.7	25.3–37.5
10	71.5[a]	71.5[a]	79.2	79.2	71.8
11–12	25.2–37.4	25.2–37.4	25.2–36.7	25.2–36.7	25.3–37.5
13	25.2–37.4	25.2–37.4	81.8	81.8	25.3–37.5
14	25.2–37.4	25.2–37.4	74.6[a]	74.6[a]	25.3–37.5
15	74.3	74.3	25.2–36.7	25.2–36.7	74.0[a]
16	82.2[b]	82.2[b]	25.2–36.7	25.2–36.7	82.6[b]
17	25.2–37.4	25.2–37.4	74.4[a]	74.4[a]	25.3–37.5
18	25.2–37.4	25.2–37.4	74.4[a]	74.2[a]	25.3–37.5
19	82.2[b]	82.2[b]	25.2–36.7	25.2–36.7	82.6[b]
20	71.7[a]	71.7[a]	25.2–36.7	25.2–36.7	74.0[a]
21–31	25.2–37.4	25.2–37.4	25.2–36.7	25.2–36.7	25.3–37.5
32	14.2	14.2	14.2	14.2	14.2
33	25.2–37.4	25.2–37.4	25.2–36.7	25.2–36.7	25.3–37.5
34	205.5	205.5	205.5	205.5	205.5
35	22.7	22.7	22.7	22.7	22.7

[a,b]Assignments are interchangeable.

based on the analysis of the ^{1}H–^{1}H NMR (COSY) spectrum of **1** and **2**. Since the ketolactone subunit (fragment A) has previously been chemically converted from the butenolide subunit (fragment B) [6], the *cis* and *trans* isomeric structures can be considered as either *in vivo* biosynthetic products, or as artifacts, chemically converted from fragment B during the isolation procedures.

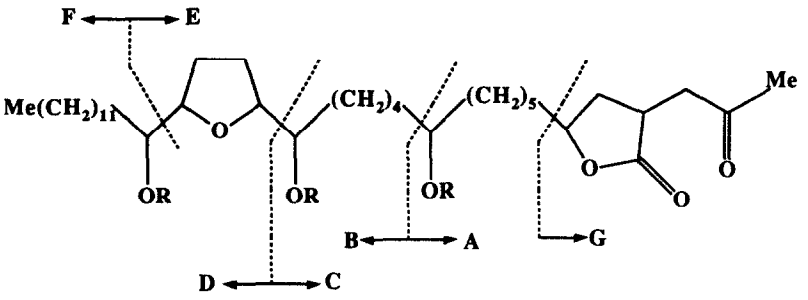
The remaining part of the structure of **1** and **2** exhibited identical ^{1}H and ^{13}C NMR signals for a long aliphatic chain, a single monotetrahydrofuran (THF) ring and three hydroxyl groups. In the CI-mass spectrum, a series of peaks at *m/z* 579, 561 and 543, arising from the successive losses of three molecules of water, were observed, confirming the presence of the three hydroxyl groups. The hydroxyl groups were also identified by the broad IR absorption band at 3364 cm^{-1} and by the three acetate methyl signals at δ2.05, 2.03 and 2.02 in the ^{1}H NMR spectrum of the triacetate derivatives, **1a** and **2a** (Table 1). The hydroxyls were further confirmed by the $[M]^+$ fragment at *m/z* 812 and three successive losses of TMSiOH (*m/z* 90) in the EI-mass spectrum of the triTMSi derivatives of **1** and **2** (Fig. 1).

In the ^{1}H–^{1}H NMR (COSY) spectrum of **1** and **2**, the multiplet signals at δ3.77 and 3.84 integrated for one proton each and exhibited coupling cross-peaks with the multiplet resonance of two protons at δ3.77 and with methylene signals at δ1.40 in the long aliphatic chain; after acetylation, these protons shifted downfield to δ4.56 (1H), 3.95 (2H) and 4.89 (1H) indicating the presence of a THF ring with two flanking hydroxyl groups (fragment E). The relative configuration in this THF ring moiety was established as *threo, trans, erythro* by comparison of the ^{1}H and ^{13}C NMR spectra of **1**, **1a**, **2** and **2a** with the reported spectral data of annonacin-A which has known relative configurations [8]. The configuration of the chiral centre at C-10 remains undetermined.

The carbon skeleton and placement of the THF ring at C-16 to C-19, and the placement of the three hydroxyl groups at C-10, C-15 and C-20 along the hydrocarbon chain were determined based on the EI mass spectral analysis of **1** and **2** and their triTMSi derivatives (Fig. 1). Thus, the structures of **1** and **2** were defined as illustrated and named *cis*-annonacin-A-one (**1**) and *trans*-annonacin-A-one (**2**), honouring the parent acetogenin, annonacin-A [8].

Cis- and *trans*-gigantetrocinone (**3**, **4**) showed a $[M+H]^+$ ion at *m/z* 597 in the CI-mass spectrum indicating a M_r of 596. The molecular formula was established as $C_{35}H_{64}O_7$ by the high resolution FAB-mass spectrum (glycerol, $[M+H]^+$, 597.4715, calcd for $C_{35}H_{65}O_7$, 597.4730). The ketolactone moiety with the familiar *cis* and *trans* diastereoisomeric structure was then easily characterized by IR, UV, ^{1}H and ^{13}C NMR spectral comparisons with similar data of compounds **1** and **2**. In the ^{13}C NMR spectrum (Table 2) the oxygen-bearing carbon resonances at δ81.3 and 79.2 and the ^{1}H NMR peaks at δ3.86 and 3.78, integrating for one proton each in the ^{1}H NMR spectrum (Table 3), again suggested a single THF ring on the long aliphatic chain.

The existence of three hydroxyl groups in **3** and **4** was again obvious by a broad absorption band at 3418 cm^{-1} in the IR spectrum, the resonance at δ3.40 (3H) in the ^{1}H NMR spectrum (Table 3) and three peaks at δ74.6, 74.4, 74.2 in the ^{13}C NMR spectrum (Table 2). In addition, the ^{1}H NMR spectrum of the mixed triacetate derivatives, **3a** and **4a** (Table 3), showed three acetate methyl groups at δ2.05 (3H) and 2.06 (6H), further confirming the presence of three hydroxyl groups. A



M+/MTMSi+		A	B	C	D	E	F	G
R=H	596	241		327		397		141
	578 (a)			309 (a)		379 (a)		
	560 (a)			291 (a)		361 (a)		
	542 (a)							
R=TMS	812	313	499	471	341	541	271	141
	722 (b)	223 (b)	409 (b)	381 (b)	251 (b)		181 (b)	
	632 (b)		319 (b)	291 (b)				
	542 (b)							

(a): loss of H_2O (*m/z* 18); (b): loss of TMSi-OH (*m/z* 90)

Fig. 1. Diagnostic EI-MS fragment ions of *cis*- and *trans*-isoannonacin-A-one (**1**, **2**), and *cis*- and *trans*-isoannonacin (**5**, **6**) and their triTMSi derivatives.

Table 3. ^{1}H NMR spectral data of compounds **3**, **3a**, **3b**, **4**, **4a** and **4b** (500 MHz, δ, $CDCl_3$)

H	3	3a	4	4a	3b	4b
2	3.08 *m*	3.08 *m*	3.00 *m*	3.00 *m*	3.08 *m*	3.00 *m*
3a	2.58 *ddd*	2.58 *ddd*	2.20 *ddd*	2.20 *ddd*	2.58 *ddd*	2.58 *m*
	(12.3, 9.4, 5.6)	(12.3, 9.4, 5.6)	(12.9, 9.6, 3.4)	(12.9, 9.6, 3.4)	(12.3, 9.4, 5.6)	(12.3, 9.6, 3.4)
3b	1.45 *m*	1.45 *m*	1.95 *m*	1.95 *m*	1.45 *m*	1.95 *m*
4	4.37 *dddd*	4.37 *dddd*	4.53 *dddd*	4.53 *dddd*	4.37 *dddd*	4.53 *dddd*
	(10.7, 7.4, 5.4, 5.4)	(10.7, 7.4, 5.4, 5.4)	(8.3, 8.2, 5.7, 3.2)	(8.3, 8.2, 5.7, 3.2)	(10.7, 7.4, 5.4, 5.4)	(8.3, 8.2, 5.7, 3.2)
5–8	1.20–1.65 *m*	1.20–1.65 *m*	1.20–1.65 *m*	1.20–1.65 *m*	1.20–1.65 *m*	1.20–1.65 *m*
9	1.44 *m*	1.40 *m*	1.44 *m*	1.40 *mm*	1.44 *m*	1.44 *m*
10	3.86 *m*	3.83 *m*	3.86 *m*	3.83 *m*	3.86 *m*	3.86 *m*
11	2.03, 1.58 *m*	2.00, 1.51 *m*	2.03, 1.58 *m*	2.00, 1.51 *m*	2.03, 1.58 *m*	2.03, 1.58 *m*
12	1.98, 1.60 *m*	1.95, 1.55 *m*	1.98, 1.60 *m*	1.95, 1.55 *m*	1.98, 1.60 *m*	1.98, 1.60 *m*
13	3.78 *ddd*	3.93 *ddd*	3.78 *ddd*	3.93 *ddd*	3.78 *ddd*	3.78 *ddd*
	(7.2, 7.1, 7.0)	(7.2, 7.1, 7.0)	(7.2, 7.1, 7.0)	(7.2, 7.1, 7.0)	(7.2, 7.1, 7.0)	(7.2, 7.1, 7.0)
14	3.40 *m*	4.78 *m*	3.40 *m*	4.78 *m*	3.41 *m*	3.41 *m*
15	2.28, 1.60 *m*	1.55 *m*	2.28, 1.60 *m*	1.55 *m*	2.28, 1.60 *m*	2.28, 1.60 *m*
16	1.73, 1.50 *m*	1.55, *m*	1.73, 1.50 *m*	1.55, *m*	1.73, 1.50 *m*	1.73, 1.50 *m*
17	3.40 *m*	4.94 *m*	3.40 *m*	4.94 *m*	3.47 *m*[a]	3.47 *m*[a]
18	3.40 *m*	4.94 *m*	3.40 *m*	4.94 *m*	3.65 *m*[a]	3.65 *m*[a]
19	1.45 *m*	1.45 *m*	1.45 *m*	1.45 *m*	1.45 *m*	1.45 *m*
20–31	1.20–1.65 *m*	1.20–1.65 *m*	1.20–1.65 *m*	1.20–1.65 *m*	1.20–1.65 *m*	1.20–1.65 *m*
32	0.85 *t* (6.9)	0.85 *t* (6.9)	0.85 *t* (6.9)	0.85 *t* (6.9)	0.87 *t* (6.9)	0.87 *t* (6.9)
33a	3.02 *dd*	3.02 *dd*	2.98 *dd*	2.98 *dd*	3.02 *dd*	2.98 *dd*
	(18.5, 3.5)	(18.5, 3.5)	(18.2, 3.4)	(18.2, 3.4)	(18.5, 3.5)	(18.2, 3.5)
33b	2.64 *dd*	2.64 *dd*	2.56 *dd*	2.56 *dd*	2.64 *dd*	2.56 *m*
	(15.3, 8.6)	(15.3, 8.6)	(19.5, 10.6)	(19.5, 10.6)	(15.3, 8.6)	(19.5, 10.6)
35	2.17 *s*	2.17 *s*	2.17 *s*	2.17 *s*	2.17 *s*	2.17 *s*
		2.05 *s* (OAc-14)		2.05 *s* (OAc-14)	1.20–1.65 (Me)	1.20–1.65 (Me)
		2.06 *s* (OAc-17)		2.06 *s* (OAc-17)	1.20–1.65 (CH_2)	1.20–1.65 (CH_2)
		2.06 *s* (OAc-18)		2.06 *s* (OAc-18)	1.07 *t* (7.0, Me)	1.07 *t* (7.0, Me)

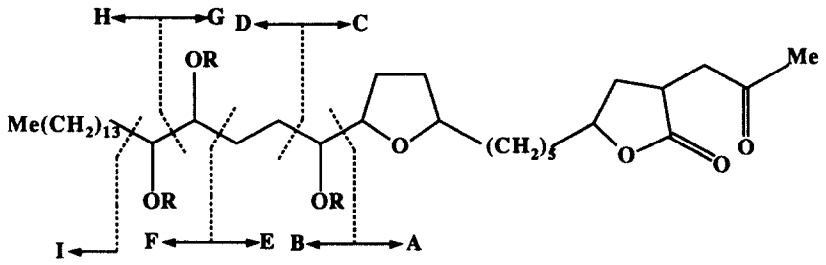
[a]Assignments may be interchangeable.

series of peaks at *m/z* 579, 561 and 543, corresponding to the successive losses of three H_2O (*m/z* 18) molecules in the CI-mass spectrum (Fig. 2), and the mass fragments at *m/z* 812 for the $[M]^+$, and *m/z* 722, 632, and 542 due to successive losses of three TMSiOH (*m/z* 90) in the triTMSi derivative EI-mass spectrum (Fig. 2), provided further evidence that three hydroxyl groups were present.

In the ^{1}H–^{1}H NMR (COSY) spectrum of **3** and **4**, the resonance at δ3.40 (3H), attributed to the hydroxyl-bearing methine protons, showed cross-peak coupling with the quartet resonance at δ3.78, which is one of the two methine protons of the oxygen-bearing carbons of the THF ring; another methine multiplet signal at δ3.86 exhibited correlation cross-peaks with aliphatic methylene resonances at δ1.98, 1.58 and 1.41, suggesting only one flanking hydroxyl group adjacent to the THF ring (fragment F). This assumption was strongly supported by the COSY spectrum of the mixed triacetates (**3a** and **4a**) in which the hydroxyl group-related methine protons became partially separated and shifted downfield from δ3.40 (3H) to δ4.94 (2H) and 4.78 (1H); the only correlation peak related to the hydroxyl-bearing methine protons and oxygen-bearing methine protons in the THF ring was found between the resonances at δ4.78 and 3.93, which is the THF ring methine proton that shifted from δ3.78.

The placement of the THF ring and the hydroxyl groups of **3** and **4** were determined based on the EI-mass spectral analysis of the triTMSi derivatives (Fig. 2). It was obvious that the THF ring is located from C-10 to C-13 and the three OH groups are situated on the C-14, C-17 and C-18 positions. In the double-relayed COSY spectrum of the mixed acetates (**3a** and **4a**), the double-relayed correlation cross-peak between the acetate-bearing methine protons of H-14 at δ4.78 and H-17 at δ4.94 was obvious, confirming that two methylene groups separate these two hydroxyl groups. The correlation cross-peak appearing between resonances at δ3.83 and 3.93 was consistent with the assignment of these signals to the THF ring oxygen-bearing methine protons at H-10 and H-13, respectively.

The relative configuration between C-13 and C-14 was determined as *threo* by comparing the ^{13}C NMR resonances of **3** and **4** at δ74.6 and 81.8 for C-14 and C-13, and the ^{1}H NMR signals at δ3.78 (H-13) and 3.40 (H-14) with those of model compounds of known relative stereochemistry [9]. The *threo* relationship was further supported by comparing the proton resonance at δ4.78 for H-14 of **3a**



M+/MTMSi+	A	B	C	D	E	F	G	H	I
R=H 596	281	315	311		339	257	369	227	197
578 (a)	263 (a)	297 (a)	293 (a)		321 (a)	239 (a)	351 (a)	209 (a)	
560 (a)		279 (a)					333 (a)		
542 (a)		261 (a)							
R=TMS 812	281	531	383	429	411	401	513	299	197
722 (b)	263 (a)	441 (b)	293 (b)	339 (b)	321 (b)	311 (b)	423 (b)	209 (b)	
632 (b)		351 (b)		249 (b)		221 (b)	333 (b)		
542 (b)		261 (b)							

(a): loss of H_2O (*m/z* 18) (b): loss of TMSi-OH (*m/z* 90)

Fig. 2. Diagnostic EI-MS fragment ions of *cis*- and *trans*-gigantetrocinone (**3**, **4**) and their triTMSi derivatives.

and **4a** with a group of diacetyl dibutylated bis-THF's of known stereochemistry [10, 11]. The vicinal diol moiety was confirmed by the preparation of the butanonide derivatives (**3b** and **4b**) of **3** and **4**. The relative stereochemistry between these vicinal hydroxyl groups at C-17 and C-18 was suggested as *threo* by comparing the ^{1}H NMR resonances at δ3.40 for H-17 and H-18 and ^{13}C NMR signals at δ74.4 (C-17) and 74.2 (C-18) with those of a group of *threo* and *erythro* diols [12, 13] and also by ^{1}H and ^{13}C NMR spectral comparisons with similar compounds reported by our group [14]. Therefore, based on the spectral analysis, the structures of **3** and **4** were suggested as illustrated and named *cis*-gigantetrocinione (**3**) and *trans*-gigantetrocinone (**4**) in honour of the parent acetogenin, gigantetrocin [14].

The ^{1}H and ^{13}C spectra (Tables 1 and 2) of the mixture of stereoisomeric compounds **5** and **6** were essentially identical to the known compound, 'isoannonacin',which was originally isolated from *Annona densicoma* [15], except for the reported signals for the ketolactone moiety. As with the isomeric pairs of compounds **1** and **2**, and **3** and **4**, the *cis* and *trans* diastereoisomeric nature of **5** and **6** at the ketolactone moiety was obvious when the mixture was characterized by IR, UV, ^{1}H and ^{13}C NMR spectral comparisons of all of these compounds. The placement of the THF ring at C-16 to C-19, and the locations of the three hydroxyl groups at C-10, C-15 and C-20 were established by the EI-mass spectral analysis of triTMSi derivatives of **5** and **6** (Fig. 4). The stereochemical configuration at C-10 is still unknown. Thus, **5** and **6** were identified as *cis*- and *trans*-isoannonacin, respectively. Comparing with the reported spectral data, **6** is the same as the previously reported 'isoannonacin' [15] with the *trans* configuration; **5**, the *cis*-isoannonacin, is, thus, reported as a new member of the mono-THF acetogenin series.

Compound **7** was obtained as prisms from methylene chloride–hexane. Its structure was determined to be *N*-aminocarbonyl-2-oxopyrrolidine by IR, UV, CI-mass, EI-mass, ^{1}H and ^{13}C NMR spectral analysis. At first, this simple compound was thought to be an extraction artifact, but a search of the literature showed that **7** was previously isolated from *Annona squamosa* and named squamolone [16], but characterized as hexahydro-3H-1, 3-diazepine. The structure was corrected to that of **7** by Marquez *et al.* [17] and Brandner [18] after synthesis of both structures. The bioactivity of **7** and its occurrence in *Asimina* species have not been previously reported, although it has been found in another Annonaceous species, *Hexalobus crispiflorus* [19]. Synthesis of **7**, from urea and γ-aminobutyric acid, as previously reported [18, 20], confirmed the identification of our isolated material.

Biological activities of **1**–**7** are summarized in Table 4. These natural compounds are very active in the BST [4, 5]; the isolated acetogenins showed significant cytotoxicities against A-549 (human lung carcinoma) [21], MCF-7 (human breast carcinoma) [22] and HT-29 (human colon carcinoma) [23] in our seven-day human solid tumour cell *in vitro* tests. These cytotoxic activities are quite comparable to those of the positive control compound, adriamycin. The acetogenins act as powerful inhibitors of mitochondrial electron transport systems [24–26]; thus, when tumour cells require more energy than normal cells, *in vivo* antitumour activity can be elicited, this has been demonstrated in athymic mice bearing xenografts of A2780 human ovarian tumours [24].

EXPERIMENTAL

TLC sepns were made on silica gel 60 F-254 (0.25 mm) and visualized by spraying with 5% phosphomolybdic acid in EtOH and heating. Chromatotron plates (1 or

Table 4. Bioactivity data of compounds 1–7

Compounds	BST	Human cancer cell lines		
	LC_{50} ($\mu g\,ml^{-1}$) (95% confidence interval)	A-549 ED_{50} ($\mu g\,ml^{-1}$)	MCF-7 ED_{50} ($\mu g\,ml^{-1}$)	HT-29 ED_{50} ($\mu g\,ml^{-1}$)
1 & 2	0.29 (0.18–0.45)	3.39×10^{-2}	1.44	2.82×10^{-2}
3 & 4	0.13 (0.06–0.24)	9.74×10^{-3}	2.74×10^{-2}	5.49×10^{-4}
5 & 6	0.33 (0.21–0.52)	4.42×10^{-5}	$<10^{-3}$	1.70×10^{-1}
7	2.65 (0.63–5.58)	>10	>10	>10
Adriamycin	NT	1.48×10^{-3}	1.47×10^{-1}	6.69×10^{-3}

NT: Not tested.

2 mm) were prepd with silica gel 60 PF 254 containing gypsum and dried at 70° overnight. HPLC was carried out on a silica gel column (250 × 21 mm) equipped with a UV detector set at 220 nm.

Bioassays. The BST (*Artemia salina* Leach) was performed as described and modified in refs [4, 5]. Seven-day *in vitro* cytotoxicity tests (Table 4) against human tumour 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.

Plant material. Bark of *A. 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 Department herbarium.

Extraction and purification. Each step of the extraction and fractionation was monitored by the BST. Air-dried pulverized stem bark (15 kg) was extracted exhaustively with 95% EtOH and vacuum evapd to yield extract F001 (1645 g) which was partitioned between H_2O and CH_2Cl_2 (1:1), giving a water soluble fr. (F002, 5 g), CH_2Cl_2 soluble. fr. (F003, 1560 g) and an insoluble interface (F004, 80 g). F003 was further partitioned between hexane and 90% MeOH aq. soln and yielded the MeOH fr. (F005, 650 g) and the hexane fr. (F006, 905 g).

The most bioactive fr. F005 (BST $LC_{50} = 7.151 \times 10^{-1}$ $\mu g\,ml^{-1}$) (200 g) was further fractionated by open CC on silica gel (60–200 mesh), eluting with hexane–EtOAc and EtOAc–MeOH gradients; 12 pools were made from the collected frs according to their TLC patterns and evaluated by the BST. The most active pools (P_7–P_9) were combined and subjected to further sepn on Chromatotron plates (2 mm thick), eluted with hexane–CH_2Cl_2–MeOH (6:4:0.1). Crude crystals of **7** formed in the hexane–CH_2Cl_2 frs. After recrystallization from $CHCl_3$–hexane (1:4), compound **7** was purified as crystalline prisms (4 mg). The powder from the hexane–CH_2Cl_2–MeOH frs was further resolved by HPLC eluted with hexane–10%THF in MeOH gradients to yield acetogenins **1–6** as the three *cis/trans* diastereoisomeric mixts.

Cis- *and* trans-*annonacin-A-one* (**1**, **2**). Powder (25 mg), mp 91–92°. $[\alpha]_D$ +20° ($CHCl_3$; *c* 0.2). UV λ_{max}^{MeOH} nm: 205. IR ν_{max}^{film} cm^{-1}: 3415 (OH), 2913, 2841, 1754 (C=O), 1718 (C=O), 1395, 1451, 1369, 1164, 1062, 856, 749. FAB HR-MS (glycerol) *m/z*: 597.4718 ($[M+H]^+$, found), (597.4730, calcd for $C_{35}H_{65}O_7$). CI-MS (isobutane) *m/z*: 597 $[M+H]^+$, 579 $[MH-H_2O]^+$, 561 $[MH-2H_2O]^+$, 543 $[MH-3H_2O]^+$, 309. FAB-MS (glycerol), *m/z*: 597 $[M+H]^+$, 579 $[MH-H_2O]^+$, 561 $[MH-2H_2O]^+$, 543 $[MH-3H_2O]^+$, 309. EI-MS *m/z*: see Fig. 1. EI-MS (triTMSi derivative) *m/z*: Fig. 1. ^1H NMR (500 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (125 MHz, $CDCl_3$): see Table 2.

Cis- *and* trans-*annonacin-A-one triacetates* (**1a**, **2a**). Treatment of **1** and **2** (4 mg) with Ac_2O–pyridine (room temp., overnight) and subsequent work-up gave **1a** and **2a** as a wax. IR ν_{max}^{film} cm^{-1}: 2928, 2857, 1771, 1736, 1461, 1371, 1242, 1164, 1023, 963. CI-MS (isobutane) *m/z* 723 $[M+H]^+$, 663 $[MH-MeCO_2H]^+$, 603 $[MH-2MeCO_2H]^+$, 543 $[MH-3MeCO_2H]^+$, 481, 421, 311. ^1H NMR (500 MHz, $CDCl_3$): see Table 1.

TriTMSi cis- *and* trans-*annonacin-A-one.* About 10–50 μg of **1** and **2** was placed in a 100 μl conical reaction vial and dried in a vacuum desiccator over P_2O_5 for 24 hr. The sample was treated with 2 μl pyridine (silylation grade) and 20 μl of *N,O*-bis-(trimethylsilyl)-acetamide (BSA) and heated at 70° for 30 min. EI-MS: see Fig. 1.

Cis- *and* trans-*gigantetrocinone* (**3**, **4**). Powder (18 mg), mp 91–92°. $[\alpha]_D$ +10° ($CHCl_3$; *c* 0.2). UV λ_{max}^{MeOH} nm: 205. IR ν_{max}^{film} cm^{-1}: 3418 (OH), 2918, 2848, 1758 (C=O), 1717 (C=O), 1592, 1462, 1437, 1357, 1167, 1063. FAB HR-MS (glycerol) *m/z*: 597.4715 ($[M+H]^+$, found), (597.4730, calcd for $C_{35}H_{65}O_7$). CI-MS (isobutane) *m/z*:

597 $[M+H]^+$, 579 $[MH-H_2O]^+$, 561 $[MH-2H_2O]^+$, 543 $[MH-3H_2O]^+$, 311. FAB-MS (glycerol) *m/z*: 597 $[M+H]^+$, 579 $[MH-H_2O]^+$, 561 $[MH-2H_2O]^+$, 543 $[MH-3H_2O]^+$, 311. EI-MS *m/z*: see Fig. 1. EI-MS (triTMSi derivative) *m/z*: see Fig. 2. ^{1}H NMR (500 MHz, $CDCl_3$): see Table 3. ^{13}C NMR (125 MHz, $CDCl_3$): see Table 2.

Cis- *and* trans-*gigantetrocinone triacetates* (**3a**, **4a**). Treatment of **3** and **4** (4 mg) with Ac_2O–pyridine (room temp., overnight) and subsequent work-up gave **3a** and **4a** as a wax. IR ν_{max}^{film} cm^{-1}: 2926, 2855, 1772, 1738, 1650, 1558, 1538, 1457, 1372, 1231, 1163, 1022, 950. CI-MS (isobutane) *m/z* 723 $[M+H]^+$, 663 $[MH-MeCO_2H]^+$, 603 $[MH-2MeCO_2H]^+$, 543 $[MH-3MeCO_2H]^+$, 561, 515, 281. ^{1}H NMR (500 MHz, $CDCl_3$): see Table 3.

TriTMSi cis- *and* trans-*gigantetrocinone*. About 10–50 μg of **3** and **4** was placed in a 100 μl conical reaction vial and dried in a vacuum desiccator over P_2O_5 for 24 hr. The sample was silylated as described for **1** and **2**. EI-MS: see Fig. 2.

Butanonide of cis- *and* trans-*gigantetrocinone* (**3b**, **4b**).Compounds **3** and **4** (2.5 mg) were butanonylated with 2 ml of butanone and 0.2 mg of *p*-toluenesulphonic acid, stirring for 24 hr at room temp. The oily product mixt. was purified by silica gel micro-CC to give the butanonide derivatives (1.5 mg). CI-MS (isobutane) *m/z*: 651 $[M+H]^+$, 623, 605, 579, 561, 543, 351, 281. ^{1}H NMR (500 MHz, $CDCl_3$): see Table 3.

Cis- *and* trans-*isoannonacin* (**5**, **6**). Powder (18 mg), mp 95–96°. $[\alpha]_D$ +20° ($CHCl_3$; *c* 0.2). UV λ_{max}^{MeOH} nm: 205. IR ν_{max}^{film} cm^{-1}: 3414 (OH), 2916, 2845, 1752 (C=O), 1717 (C=O), 1454, 1391, 1371, 1160, 1062, 864, 744. FAB HR-MS (glycerol) *m/z*: 597.4718 ($[M+H]^+$, found), (597.4730, calcd for $C_{35}H_{65}O_7$). CI-MS (isobutane) *m/z*: 597 $[M+H]^+$, 579 $[MH-H_2O]^+$, 561 $[MH-2H_2O]^+$, 543 $[MH-3H_2O]^+$, 309. FAB-MS (glycerol) *m/z*: 597 $[M+H]^+$, 579 $[MH-H_2O]^+$, 561 $[MH-2H_2O]^+$, 543 $[MH-3H_2O]^+$, 309. EI-MS *m/z*: see Fig. 1. ^{1}H NMR (500 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (125 MHz, $CDCl_3$): see Table 2.

Cis- *and* trans-*isoannonacin triacetates* (**5a**, **6a**). Treatment of **5** and **6** (4 mg) with Ac_2O–pyridine (room temp., overnight) and subsequent work-up gave **5a** and **6a** as a wax. IR ν_{max}^{film} cm^{-1}: 2927, 2856, 1772, 1736, 1654, 1560, 1509, 1460, 1372, 1242, 1163, 1023, 963. CI-MS (isobutane) *m/z* 723 $[M+H]^+$, 663 $[MH-MeCO_2H]^+$, 603 $[MH-2MeCO_2H]^+$, 543 $[MH-3MeCO_2H]^+$, 481, 421, 311. ^{1}H NMR (500 MHz, $CDCl_3$): see Table 1.

TriTMSi cis- *and* trans-*isoannonacin*. About 10–50 μg of **5** and **6** was placed in a 100 μl conical reaction vial and dried in a vacuum desiccator over P_2O_5 for 24 hr. The sample was silylated as described for **1** and **2**. EI-MS: see Fig. 1.

Squamolone (**7**). Prisms, mp 145–146° (lit., mp 145–146° [15]). IR ν_{max}^{film} cm^{-1}: 3377, 3213 (NH_2), 1714 (C=O), 1689 (C=O), 1589, 1358, 1267, 1181, 1092, 783. HR-CI-MS (isobutane) *m/z* (%): 129.0660 (100, $[M+H]^+$, found), (129.0664, calcd for $C_5H_8O_2N_2$). EI-MS *m/z* (%): 128 (40) $[M]^+$, 85 (95) $[C_4H_7ON]^+$, 84 (48) $[C_4H_6ON]^+$, 57 (100) $[C_3H_7N]^+$, 56 (98) $[C_3H_6N]^+$. ^{1}H NMR (500 MHz, $CDCl_3$): δ2.06 (2H, *tt*, *J* = 7.2, 8.1 Hz, H-4), 2.63 (2H, *t*, *J* = 8.1 Hz, H-3), 3.87 (2H, *t*, *J* = 7.2 Hz, H-5), 5.17 [1H, *br*, (NH_2)], 8.21 [1H, *br*, (NH_2)]. ^{1}H NMR (500 MHz, pyridine-d_5): δ1.65 (2H, *tt*, *J* = 7.2, 8.1 Hz, H-4), 2.44 (2H, *t*, *J* = 8.1 Hz, H-3), 3.79 (2H, *t*, *J* = 7.2 Hz, H-5), 8.41 [1H, *br*, (NH_2)], 8.58 [1H, *br*, (NH_2)]. ^{13}C NMR (125 MHz, $CDCl_3$): δ176.81 (C-2), 153.13 (C-6), 45.34 (C-5), 33.18 (C-3), 16.92 (C-4).

Synthesis of compound **7**. Two parts of urea (12.0 g) and one part of γ-aminobutyric acid (10.3 g) were dissolved in 5 parts (50 ml) of H_2O and acidified with conc. HCl to pH 3; the mixt. was heated at 110° for 16 hr at reflux while being maintained at pH 4 by gradual addition of conc. HCl. The H_2O soln was evapd and dried under vaccum, and the residue was subjected to silica gel (60–200 mesh) CC eluting with CH_2Cl_2–MeOH gradients. The dried intermediate was suspended in 15 ml of $POCl_3$ and reacted in Ar gas at room temp. for 48 hr. The product mixt. was sepd on a silica gel (230–400 mesh) column eluting with CH_2Cl_2–MeOH gradients. The crude crystals were recrystallized from CH_2Cl_2–hexane to give prisms of squamolone, mp 145°. The IR, ^{1}H NMR and ^{13}C NMR spectra of the synthetic product were identical with those of natural **7**.

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