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Longifolicin, Longicoricin, and Gigantetroneninone, Three Novel Bioactive Mono-Tetrahydrofuran Annonaceous Acetogenins from *Asimina longifolia* (Annonaceae)

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Abstract—Longifolicin (**1**), longicoricin (**2**) and (2,4-*cis* and *trans*)-gigantetroneninone (**3**), three novel bioactive mono-tetrahydrofuran (THF) γ-lactone acetogenins, were isolated from the leaves and twigs of *Asimina longifolia* (Annonaceae) by directing the fractionation with the brine shrimp lethality test (BST). The structures were elucidated based on spectroscopic and chemical methods. Compounds **1–3** showed selective and potent cytotoxicities to certain human tumor cell lines. Published by Elsevier Science Ltd

Introduction

Asimina longifolia K. (Annonaceae), commonly known as long leaf paw paw, is a small tree native to the southeastern United States. The EtOH extract of the leaves and twigs showed potent toxicity in the brine shrimp lethality test (BST).[1,2] Previous bioactivity-directed investigation of the plant materials led to the isolation and structural elucidation of two novel cytotoxic acetogenins [longicin and (2,4-*cis* and *trans*)-goniothalamicinone], as well as nine known mono-tetrahydrofuran (THF) acetogenins [annonacin, isoannonacin, xylomaticin, gigantetrocin A and B, muricatetrocin A and B, gigantetrocin-A-one and goniothalamicin].[3] Further efforts, using the BST to direct the fractionation of the ethanol extract of the leaves and twigs, have now resulted in the isolation of three additional novel mono-THF acetogenins, longifolicin (**1**), longicoricin (**2**) and (2,4-*cis* and *trans*)-gigantetroneninone (**3**) (Fig. 1). Also, three known acetogenins, asimicin, corosolin and gigantetronenin, were isolated for the first time from this species;[4–9] asimicin is the only bis-THF acetogenin found, so far, in this species. The structures and absolute stereochemistries were determined by 1-D and 2-D NMR and MS before and after making certain chemical derivatives.

Results and Discussion

Compound **1** was isolated as a colorless wax. Its molecular weight was suggested by the mass peaks at *m/z* 580 $[M]^+$ in the EIMS and *m/z* 581 $[MH]^+$ in the CIMS. The HRCIMS gave *m/z* 581.4793 for the $[MH]^+$ ion (calcd 581.4781), corresponding to the molecular formula $C_{35}H_{64}O_6$.

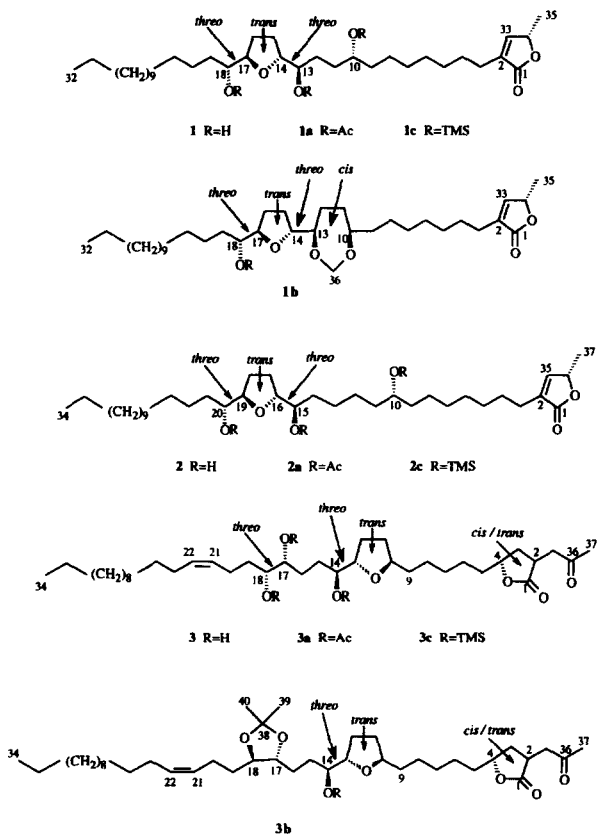


Figure 1. Structures of **1**, **1a**, **1b**, **1c**, **2**, **2a**, **2c**, **3**, **3a**, **3b**, and **3c**.

Key words: *Asimina longifolia*, Annonaceae, acetogenins, longifolicin, longicoricin, gigantetroneninone.

The spectral data of **1** showed an IR carbonyl absorption at 1750 cm^{-1}, a UV (MeOH) λ_{max} at 228 nm (log ε 3.70), four resonances at δ 6.99 (q, H-33), 5.00 (qq, H-34), 1.41 (d, H-35), and 2.26 (tt, H-3) in the ^1H NMR spectrum and five peaks at δ 174.00 (C-1), 148.88 (C-33), 134.29 (C-2), 77.42 (C-34), and 19.19 (C-35) in the ^{13}C NMR spectrum (Table 1). These are all characteristic spectral features for the methylated α,β-unsaturated γ-lactone fragment, without the presence of an OH group at the C-4 position, as commonly found among the annonaceous acetogenins.[4-6]

The presence of three OH moieties in **1** was suggested by a prominent OH absorption at 3400 cm^{-1} in the IR spectrum and confirmed by three successive losses of H_2O (*m/z* 18) from the $[MH]^+$ in the CIMS, and the preparation of a tri-acetate derivative (**1a**), and a tri-trimethylsilyl (TMSi) derivative (**1c**). Compound **1a** gave three singlet proton peaks at δ 2.04 (10-OAc), 2.08 (13-OAc) and 2.08 (18-OAc), and a multiplet at δ 4.84 (H-10, H-13, and H-18) corresponding to the downfield shifts of three protons on secondary OH-bearing carbons. Furthermore, the ^{13}C NMR of **1** showed three resonances due to oxygen-bearing carbons at δ 71.61 (C-10), 74.32 (C-13) and 74.03 (C-18), indicating the existence of three secondary OH moieties. The presence of a mono-THF ring, with two OH groups flanking the ring, was suggested by proton resonances at δ 3.45 (H-13), 3.83 (H-14), 3.82 (H-17), and 3.41 (H-18), and the carbon peaks at δ 82.71 (C-14) and 82.59 (C-17); these were directly analogous to similar peaks of other mono-THF acetogenins with two flanking OH groups, such as corisolin and goniothalamicin.[7,10]

The placements of the mono-THF ring system and the three OH groups of **1** along the aliphatic chain were determined based on the EIMS fragmentation pattern of **1** and its tri-TMSi derivative (Fig. 2). HRCIMS identified the peak at 297.1889 in **1c** as $C_{16}H_{29}O_3Si$ (calcd 297.1886); the important fragment confirmed the location of the third OH at C-10. The assignment of the ^1H NMR spectrum of **1** was based on the ^1H—^1H COSY and single and double relayed ^1H—^1H COSY.

The stereochemistries at C-13/C-14 and C-17/C-18 in **1** were concluded to be *threo*, and the stereochemistry of the THF ring was determined as *trans* by comparison with model compounds synthesized by Harmarge et al.[11] as well as by comparisons with corisolin[7] and goniothalamicin.[10] To determine the relative configuration at C-10/C-13, the formal (formaldehyde acetal) derivative (**1b**) was prepared using the method described by Gu et al.[12] The CIMS and EIMS of **1b** confirmed that the formal acetal had formed between the two OH groups at C-10 and C-13. Gu et al. demonstrated that the acetal moiety which is formed connects the diols but does not change the stereochemistries of their carbinol centers. Therefore, significant differences in the ^1H NMR signals between the acetal protons in the *cis* (at ca. δ 5.26 and 4.63 as two doublets) or *trans* (at ca. δ 4.96 as a singlet) configurations of the cyclic formal derivatives then permit the assignment of the relative stereochemistries of the diols in the parent compounds.[12] The acetal

Table 1. NMR data of **1**, **1a** and **1b**

H/C No.	^1H-NMR 500 MHz, δ in ppm, *J* in Hz			^{a}C-NMR 125 MHz, δ in ppm
	1	**1a**	**1b**	**1**
1	—	—	—	174.00
2	—	—	—	134.29
3	2.26 tt (7.8, 1.6)	2.26 tt (7.8, 1.6)	2.26 tt (7.8, 1.6)	22.65–37.50
4–9	1.22–1.72 m	1.22–1.72 m	1.22–1.72 m	22.65–37.50
10	3.63 m	4.84 m	3.66 m	71.61
11–12	1.22–1.72 m	1.22–1.72 m	1.60–1.84 m	22.65–37.50
13	3.45 m	4.84 m	3.66 m	74.32
14	3.83 m	3.96 m	3.99 dt (6.8, 6.7)	82.71
15a, 16a	2.00 m	1.94 m	1.98 m	22.65–37.50
15b, 16b	1.68 m	1.78 m	1.62–1.70 m	22.65–37.50
17	3.82 m	3.96 m	3.84 dt (6.8, 6.8)	82.59
18	3.41 m	4.84 m	3.39 m	74.03
19–31	1.22–1.72 m	1.22–1.72 m	1.22–1.72 m	22.65–37.50
32	0.88 t (7.0)	0.88 t (7.0)	0.88 t (7.0)	14.08
33	6.99 q (1.5)	6.99 q (1.5)	6.99 q (1.5)	148.88
34	5.00 qq (6.7, 1.7)	5.00 qq (6.7, 1.7)	5.00 qq (6.7, 1.7)	77.42
35	1.41 d (7.0)	1.41 d (7.0)	1.41 d (7.0)	19.19
36a	—	—	5.15 d (7.0)	—
36b	—	—	4.61 d (7.0)	—
10-OAc	—	2.04 s	—	—
13-OAc	—	2.08 s	—	—
18-OAC	—	2.08 s	—	—

Compd	R	MH*/ M(TMSi)$_3$*	A	B	C	D	E	F
1	H	581, 563[a] 545[a], 527[a]	225 207[a]	—	283, 265[a] 247[a]	297	353,335[a] 317[a]	—
1c	TMSi	796, 706[b] 616[b], 526[b]	297	499	427, 337[b] 247[b]	369	497, 407[b] 317[b]	299
2	H	609, 591[a] 573[a], 555[a]	225 207[a]	—	311, 293[a] 275[a]	297	381, 363[a] 345[a]	—
2c	TMSi	824, 734[b] 644[b], 554[b]	297	527	455, 365[b] 275[b]	369	525, 435[b] 345[b]	299

[a]Loss of H_2O (*m/z* 18); [b]loss of TMSiOH (*m/z* 90).

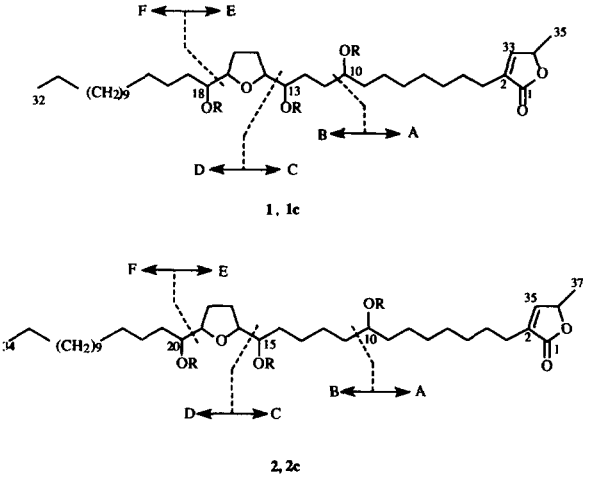


Figure 2. Diagnostic mass fragmentation ions of **1**, **1c**, **2**, and **2c**.

protons in **1b** were presented as a pair of doublets at δ 5.15 and 4.61 (J=7.5 Hz) in the ^{1}H NMR spectrum, indicating that the newly formed acetal ring possessed the *cis* relative configuration and thus, either an *S/S* or an *R/R* absolute configuration between C-10 and C-13 was revealed.

The absolute configuration of C-18 in **1b** was determined using advanced Mosher ester methodology.[13,14] The (*S*)- and (*R*)- methoxy(fluoromethyl) phenylacetic acid (MTPA) esters (Mosher esters) of **1b** were prepared. COSY ^{1}H NMR analysis of these derivatives allowed the assignment of the absolute configuration at C-18 as *R* (Table 2); it then followed that those at C-10, C-13, C-14, and C-17 were all *R* considering their relative stereochemistries. The absolute stereochemistry at C-34 was determined by CD data. It is reported that a negative Cotton effect at 236 nm in the CD spectrum of squamocin is attributed to the 36*S* configuration in the γ-lactone moiety.[15] CD of **1** showed a negative Cotton effect at 236.8 nm ($\Delta\varepsilon = -0.29$), compared with squamocin [negative Cotton effect at 235 nm ($\Delta\varepsilon = -0.33$)]; thus, the absolute stereochemistry at C-34 is proposed as *S*, and the structure of **1** was elucidated as illustrated (Figure 1) and named longifolicin.

Longicoricin (**2**) was also isolated as a colorless wax. The molecular formula, $C_{37}H_{68}O_6$, was established from the HRCIMS of the $[MH]^+$ (found *m/z* 609.5082, calcd 609.5094). The ^{1}H and ^{13}C NMR spectra (Table 3) exhibited signals characteristic of mono-THF acetogenins and, as with many other acetogenins and compound **1**, the existence of a methyl substituted α,β-unsaturated γ-lactone fragment without a 4-OH was indicated.[4-6] The presence of three OH moieties was again determined by three successive losses of H_2O (*m/z* 18) from the molecular ion in the CIMS, from preparations of triacetate (**2a**) and tri-TMSi (**2c**) derivatives, and by the characteristic IR, UV, and NMR data. The ^{1}H and ^{13}C NMR data of **2** showed the existance of a mono-THF ring with two adjacent hydroxyl groups, as with **1**.

The skeleton and placement of the THF ring and OH moieties along the aliphatic chain were determined based on the EIMS analysis of **2** and the TMSi derivative of **2** (Fig. 2). The EIMS fragmentation patterns clearly indicated that the OH groups were positioned at C-10, C-15, and C-20. HRCIMS again confirmed the positioning of the third OH at C-10. Compared with **1**, compound **2** has two more carbon units between the THF ring and the 10-OH group.

As with **1**, the relative stereochemistries around the THF ring were determined as *threo/trans/threo*, for C-15/C-16, C16/C-19, and C-19/C-20, by comparisons of corresponding NMR data of **2** and its acetate derivative (**2a**) with those of THF model compounds of known relative configuration.[11] The absolute stereochemistries of the carbinol stereocenters in **2** were also determined using advanced Mosher ester methodology (Table 4), and the absolute configurations were determined as C-10*R*, C-15*R*, C-16*R*, C-19*R*, and C-20*R*. CD of **2** showed a negative Cotton effect at 235.6 nm ($\Delta\varepsilon = -0.36$) and, as with **1**, this suggested that the absolute configuration at C-35 is *S*. Thus, the structure of **2** was determined as illustrated (Fig. 1), and it was named longicoricin.

(2,4-*cis* and *trans*)-Gigantetroneninone (**3**) was isolated as a mixture in the form of an amorphous waxy powder. The molecular weight of **3** was indicated by a peak at *m/z* 623 $[MH]^+$ in the CIMS. The HRCIMS gave *m/z* 623.4874 (calcd 623.4887) for the $[MH]^+$ corresponding to the molecular formula $C_{37}H_{66}O_7$. The IR spectrum showed a strong absorption at 1782 cm^{-1} for a γ-lactone carbonyl and 1726 cm^{-1} for a ketone carbonyl. Compound **3** was transparent under UV light at 220 nm suggesting that the lactone ring is not α,β-unsaturated. In comparison with (2,4-*cis* and *trans*)-isoannonacin[13,16] and (2,4-*cis* and *trans*)-goniothalamicinone,[3] the ^{1}H and ^{13}C NMR spectra of **3** clearly indicated the presence of a ketolactone moiety. In the ^{1}H NMR spectrum of **3** (Table 5) the resonances at δ 4.39 and 4.56, with combined integrations for one proton, were assigned to H-4 and suggested the presence of the mixture of (2,4-*cis* and *trans*)-diastereoisomers at the ketolactone ring moiety, as is typical with these ketolactones.[4-6] In the ^{13}C NMR (Table 6), signal pairs at δ 178.19 and 178.19, 43.74 and 44.20, 78.81 and 79.18, and 205.53 and 205.53 were assigned to C-1, C-2, C-4, and C-36, respectively; and they also confirmed the presence of the mixture of (2, 4-*cis* and *trans*)- isomers. The assignments of H-2,

Table 2. ^{1}H NMR data of (*R*)- and (*S*)-MTPA derivatives of **1b**

	H-13	H-14	H-15	H-16	H-17	H-18	H-19
S	3.57	3.92	1.78, 1.67	1.93, 1.53	4.08	5.06	1.63
R	3.65	4.02	1.93, 1.80	2.03, 1.60	4.08	5.05	1.50
$\Delta\delta$ (*S*−*R*)	−0.08	−0.10	−0.15, −0.13	−0.10, −0.07	0	*R*[a]	+0.13

[a]Absolute configuration of carbinol center.

H-3a, H-3b, H-35a and H-35b were based on the analysis of the COSY spectrum of **3**.

The remaining part of the structure of **3** exhibited identical ^{1}H and ^{13}C NMR signals for a long aliphatic chain bearing a mono-THF ring and three OH groups. The existence of three OH moieties in **3** was observed by an IR hydroxyl absorption at 3450 cm^{-1}, three successive losses of H_2O (*m/z* 18) from the $[MH]^+$ in CIMS, and the preparation of triacetate (**3a**) and tri-TMS derivatives (**3c**). Furthermore, the ^{13}C NMR of **3** showed three resonances due to oxygen-bearing carbons at δ 74.40, 74.21, and 74.16, indicating the existence of three secondary hydroxyl moieties. The presence of a mono-THF ring with one hydroxyl group adjacent to the ring was suggested by proton resonances at δ 3.88 (H-10), 3.81 (H-13), and 3.44 (H-14) and carbon peaks at δ 79.20 (C-10), 81.73 (C-13), and 74.40 (C-14); these were directly analogous to similar peaks of other mono-THF acetogenins with one hydroxyl group adjacent to the ring, such as gigantrionenin and gigantetronenin.[8] The existence of the vicinal diol hydroxyl groups was confirmed by the preparation of the acetonide derivative (**3b**) of **3**. The ^{1}H NMR of **3b** showed two proton downfield shifts from δ 3.44 to 3.60 for two of the three methine protons on OH-bearing carbons which was consistent with their assignment as the vicinal diol OH groups. The presence of an isolated *cis* double bond was suggested by two proton resonances at δ 5.39 (dt,

Table 3. NMR data of **2** and **2a**

H/C No.	^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm, *J* in Hz)		^{a}C-NMR (125 MHz, $CDCl_3$, δ in ppm)
	2	**2a**	**2**
1	—	—	173.86
2	—	—	134.28
3	2.26 tt (7.8, 1.6)	2.26 tt (7.8, 1.6)	22.67–37.44
4–9	1.22–1.78 m	1.22–1.78 m	22.67–37.44
10	3.59 m	4.85 m	71.85
11–14	1.22–1.78 m	1.22–1.78 m	22.67–37.44
15	3.41 m	4.85 m	74.03
16	3.80 dt (7.0, 7.0)	3.97 m	82.65
17a, 18a	1.99 m	1.95 m	22.67–37.44
17b, 18b	1.68 m	1.68 m	22.67–37.44
19	3.80 dt (7.0, 7.0)	3.97 m	82.59
20	3.41 m	4.85 m	73.97
21–33	1.22–1.78 m	1.22–1.78 m	22.67–37.44
34	0.88 t (7.0)	0.88 t (7.0)	14.10
35	6.99 q (1.5)	6.99 q (1.5)	148.87
36	5.00 qq (6.7, 1.7)	5.00 qq (6.7, 1.7)	77.40
37	1.41 d (7.0)	1.41 d (7.0)	19.20
10-OAc	—	2.04 s	—
15-OAc	—	2.07 s	—
20/OAc	—	2.07 s	—

Table 4. ^{1}H NMR data of (*R*)- and (*S*)-Per-MTPA derivatives of **2**

	H-3	H-4	H-10	H-15	H-16	H-17	H-18	H-19	H-20	H-21
S	2.26	1.60	5.05	4.95	3.91	1.64, 1.39	1.64, 1.39	3.90	4.90	1.50
R	2.25	1.57	5.01	5.01	4.00	1.92, 1.58	1.92, 1.58	4.00	5.01	1.40
$\Delta\delta$ (*S*−*R*)	+0.01	+0.03	*R*[a]	*R*[a]	*R*[a]	−0.28, −0.19	−0.28, −0.19	*R*[a]	*R*[a]	+0.10

[a]Absolute configuration of carbinol center.

J=11.0, 4.0 Hz, H-22) and 5.37 (dt, J=11.0, 5.0 Hz, H-21) and two carbon peaks at δ 130.77 and 128.96.

The carbon skeleton and placement of the THF ring and three OH groups along the hydrocarbon chain were determined based on the EIMS spectral analysis of **3** (Fig. 3). The position of the double bond was determined, by EIMS and the COSY as well as the double-relayed COSY spectrum of **3**, to be across C-21 and C-22. The EIMS fragmentation data of **3** indicated that the double bond was located beyond C-18. The correlation cross peaks were seen from H-21 (δ 5.37) to H-20 (δ 2.19), and the latter showed cross peaks to a multiplet containing several protons (H-15, 16, and 19) at δ 1.53 (assigned by a COSY trace function), which, in turn, showed cross peaks to another multiplet at δ 3.44 (H-14, 17 and 18) in the COSY spectrum. To solve the overlapping between the signals for H-16 and H-19 with other protons, a relayed COSY experiment was applied. The double relayed correlation cross peaks from H-21 to H-18 and H-17 to H-14 confirmed the assignments of the placements for the double bond and vicinal diol OH moieties.

The relative stereochemistry between C-13 and C-14 of **3** was determined as *threo* by comparing the ^{13}C NMR signal for C-14 (δ 74.40) and the ^{1}H NMR resonances of **3** for H-13 (δ 3.81) and H-14 (δ 3.44) with those of model compounds of known relative stereochemistry (Born's technique).[17] The *threo* assignment was further substantiated by comparing the proton resonance of H-14 at δ 4.80 of **3a** with the group of diacetylated bistetrahydrofurans of known stereochemistry (Hoye's technique).[18,19] The relative configuration between C-17 and C-18 of the diol moiety in **3** was suggested as *threo* by comparing the ^{1}H NMR signals for H-17 and H-18 at δ 3.44 with those of the group of *threo* and *erythro* vicinal diols in compounds of known configuration.[20,21] The *threo* assignment of the vicinal diol at C-17/C-18 was, again, supported by a ^{1}H NMR singlet at δ 1.38 corresponding to two acetonyl methyl groups in the acetonide derivative (**3b**); only one ^{1}H NMR peak is shown for the two acetonyl methyl groups in the acetonide derivative of a *threo* vicinal diol compound, and two peaks are shown for those of an *erythro* vicinal diol isomer.[5,22]

The absolute stereochemistries of the carbinol centers of compound **3** were elucidated as C-10*R*, C-13*S*, C-14*S*, C-17*R* and C-18*R* by using the advanced Mosher ester methodology (Table 7). To determine the absolute stereochemistry of C-4 of compound **3**, gigan-

Table 5. ^{1}H NMR data of **3**, **3a** and **3b**

H	**3** (*cis*)	**3** (*trans*)	**3a** (*cis*)	**3a** (*trans*)	**3b** (*cis*)	**3b** (*trans*)
2	3.03 m	3.00 m	3.03 m	3.00 m	3.03 m	3.00 m
3a	2.60 ddd (12.3, 9.4, 5.6)	2.20 ddd (12.9, 9.6, 3.4)	2.60 ddd (12.3, 9.4, 5.6)	2.20 ddd (12.9, 9.6, 3.4)	2.60 ddd (12.3, 9.4, 5.6)	2.20 ddd (12.9, 9.6, 3.4)
3b	1.45 m	1.95 m	1.45 m	1.95 m	1.45 m	1.95 m
4	4.39 ddt (10.7, 7.4, 5.4)	4.56 ddt (5.7, 3.2, 8.2)	4.39 ddt (10.7, 7.4, 5.4)	4.56 ddt (5.7, 3.2, 8.2)	4.39 ddt (10.7, 7.4, 5.4)	4.56 ddt (5.7, 3.2, 8.2)
5–8	1.20–1.65 m		1.20–1.65 m		1.20–1.65 m	
9	1.44 m		1.44 m		1.44 m	
10	3.88 m		3.88 m		3.88 m	
11	2.03, 1.58 m		2.03, 1.58 m		2.03, 1.58 m	
12	1.98, 1.60 m		1.98, 1.60 m		1.98, 1.60 m	
13	3.81 ddd (7.5, 7.5, 7.5)		3.95 ddd (7.5, 7.5, 7.5)		3.79 ddd (7.5, 7.5, 7.5)	
14	3.44 m		4.80 m		3.44 m	
15–16	1.40–1.75 m		1.40–1.75 m		1.40–1.75 m	
17	3.44 m		5.00 m		3.60 m	
18	3.44 m		5.00 m		3.60 m	
19	1.53 m		1.53 m		1.53 m	
20	2.19 m		2.19 m		2.19 m	
21	5.37 dt (11.0, 5.0)		5.28 dt (11.0, 7.0)		5.32 dt (11.0, 5.0)	
22	5.39 dt (11.0, 4.0)		5.38 dt (11.0, 7.0)		5.39 dt (11.0, 4.0)	
23	2.03 m		2.03 m		2.03 m	
24–33	1.40–1.75 m		1.40–1.75 m		1.40–1.75 m	
34	0.88 t (8.0)		0.88 t (8.0)		0.88 t (8.0)	
35a	3.11 dd (18.5, 3.0)	3.05 dd (18.2, 3.4)	3.11 dd (18.5, 3.0)	3.05 dd (18.2, 3.4)	3.11 dd (18.5, 3.0)	3.05 dd (18.2, 3.4)
35b	2.64 dd (15.3, 8.6)	2.56 dd (19.5, 10.6)	2.64 dd (15.3, 8.6)	2.56 dd (19.5, 10.6)	2.64 dd (15.3, 8.6)	2.56 dd (19.5, 10.6)
37	2.20 s		2.20 s		2.20 s	
	—		2.08 s (14-OAc)		1.38 s (39-Me)	
	—		2.08 s (17-OAc)		1.38 s (40-Me)	
	—		2.08 s (18-OAc)			

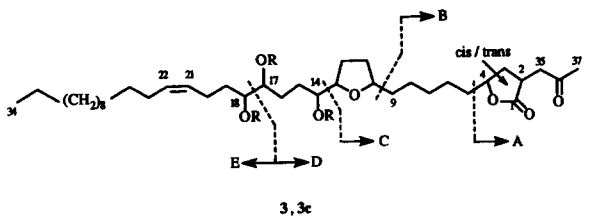
Table 6. ^{1}C NMR data of **3**

Carbon no.	**3** (*cis*)	**3** (*trans*)
1	178.19	178.19
2	43.74	44.20
3	25.14–36.67	25.14–36.67
4	78.81	79.18
5–9	25.14–36.67	25.14–36.67
10	79.20	79.20
11–12	25.14–36.67	25.14–36.67
13	81.73	81.73
14	74.40[a]	74.40[a]
15	25.14–36.67	25.14–36.67
17	74.16[a]	74.16[a]
18	74.21[a]	74.21[a]
19–20	25.14–36.67	25.14–36.67
21	128.96	128.96
22	130.77	130.77
23–33	25.14–36.67	25.14–36.67
34	14.05	14.05
35	25.14–36.67	25.14–36.67
36	205.53	205.53
37	22.62	22.62

[a]Signals may be interchangeable.

Compd	*R*	MH*/ M(TMSi)$_3$*	A	B	C	D	E
3	H	623, 605[a] 487[a], 569[a]	141	211 193[a]	281	369, 351[a] 333[a]	—
3c	TMSi	838, 748[b] 658[b], 568[b]	141	211	281	513, 423[b] 333[b]	325 235[b]

[a]Loss of H_2O (*m/z* 18); [b]loss of TMSiOH (*m/z* 90).



3, **3c**

Figure 3. Diagnostic mass fragmentation ions of **3** and **3c**.

tetronenin, a known compound which was also isolated in this plant, was converted to (2,4-*cis*- and *trans*)-gigantetroneninone by treatment with mild base. CD data for these two compounds were obtained, and negative Cotton effects at 223 nm were displayed by both compounds. Since the ^{1}H NMR spectrum of compound **3** agreed well with (2,4-*cis*- and *trans*)-gigantetroneninone, the two compounds are deduced to have the same absolute stereochemistry at C4. The proposed mechanism for translactonization shows that the absolute configurations of C4 of acetogenins is not changed during the reaction,[23] thus, it is concluded that the absolute stereochemistry of C4 of compound **3** is *R*, as was determined in gigantetronenin by using the advanced Mosher ester methodology. Thus, the structure of **3** was defined as illustrated and named as (2,4-*cis* and *trans*)-gigantetroneninone, honouring the parent acetogenin, gigantetronenin.[8]

Biological activities of **1–3** and **1b** are summarized in Table 8. These compounds were active in the BST; they also showed significant cytotoxicities against all six human tumor cell lines in our seven-day MTT human solid tumor cytotoxicity tests. Compound **3** was generally more cytotoxic, while **1** and **2** appeared to be more selective across the six human tumor cell lines. Selectivity in **1** was exhibited for the human lung carcinoma (A-549),[24] human breast carcinoma (MCF-7)[25] and, especially, human prostate adenocarcinoma (PC-3).[27] The activity of **1** against PC-3 is over one-million times that of adriamycin. The formal acetal (**1b**), however, showed less activities than **1** and it lost the selectivities as well. Compound **2**, which has two more carbon units than **1** between the THF ring and the 10-OH group, showed especially promising selectivity against the human prostate adenocarcinoma (PC-3).[27] Compounds **1** and **3** were shown to exert their effects through inhibition of oxygen uptake in the rat liver mitochondrial electron transport system at complex I with **1** giving $IC_{50}=14$ nM/mg protein (bullatacin used in the same run as a positive standard gave $IC_{50}=4$ nM/mg protein) and **3** giving $IC_{50}=30$ nM/mg protein (bullatacin used in the same run gave $IC_{50}=7$ nM/mg protein).[29,30] Recently, it was reported that the acetogenins can also inhibit the NADH oxidase that is prevalent in the plasma membranes of tumor, but not normal, cells; the resulting depletion of intracellular ATP levels induces in vivo antitumor

Table 7. H NMR data of (*R*)- and (*S*)-tri-MTPA derivatives of compound **3**

	H-10	H-11	H-12	H-13	H-14	H-15	H-16
S	3.84	1.46, 1.40	1.83, 1.35	3.87	4.84	1.52, 1.48	1.42, 1.57
R	3.72	1.44, 1.34	1.78, 1.33	3.86	4.88	1.57, 1.52	1.52, 1.60
$\Delta\delta$ ($S-R$)	+0.12	+0.02, +0.06	+0.02, +0.05	+0.01	*S*[a]	−0.05, −0.04	−0.10, −0.03
	H-16	H-17	H-18	H-19	H-20	H-21	H-22
S	1.42, 1.57	5.05	4.97	1.38, 1.46	1.93	5.40	5.17
R	1.52, 1.60	5.16	5.18	1.30, 1.43	1.89	5.38	5.16
$\Delta\delta$ ($S-R$)	−0.10, −0.03	*R*[a]	*R*[a]	+0.08, +0.03	+0.04	+0.02	+0.01

[a]Absolute configuration of carbinol center.

Table 8. Bioactivity data of compounds **1**, **1b**, **2** and **3**

Compounds		**1**	**1b**	**2**	**3**	Adriamycin[h]
BST[a] LC_{50} (μg/mL) (95% Confidence Interval)		3.52 (6.29/1.78)	15.64 (32.5/6.73)	1.56 (0.27/2.68)	0.11 (0.22/0.06)	—
Human	A-549[b]	1.13×10^{-6}	5.71×10^{-1}	1.04	1.0×10^{-6}	2.41×10^{-2}
Tumor	MCF-7[c]	1.23×10^{-5}	1.35	2.31	1.98	2.83×10^{-1}
Cell	HT-29[d]	1.23	2.70	1.36×10^{-3}	4.81×10^{-7}	1.92×10^{-2}
Line	A-498[e]	4.55×10^{-1}	4.76	1.71	1.12×10^{-7}	1.62×10^{-2}
ED_{50}	PC-3[f]	$<10^{-7}$	>10	3.04×10^{-6}	8.35×10^{-3}	2.80×10^{-1}
(μg/mL)	PACA-2[g]	4.22×10^{-3}	8.54×10^{-3}	1.36	1.79×10^{-7}	2.68×10^{-2}

[a]Brine shrimp lethality test;[1,2] [b]Human lung carcinoma;[24] [c]Human breast carcinoma;[25] [d]Human colon adenocarcinoma;[26] [e]Human kidney carcinoma;[25] [f]Human prostate adenocarcinoma;[27] [g]Human pancreatic carcinoma;[28] [h]Positive control standard.

effects and especially suggests potential synergistic effects with other chemotherapeutic agents in the treatment of the ATP dependent multi-drug resistance.[31]

Experimental

Plant material

The leaves and twigs of *A. longifolia* K. were collected in Georgia in September 1993 under the auspices of one of us (PRE), Curator of the Herbarium, University of Georgia, where voucher specimens are maintained.

Instrumentation

Melting points were determined on a Mettler FP5 hot-stage apparatus and are uncorrected. The optical rotations were determined on a Perkin-Elmer 241 polarimeter. UV spectra were taken in MeOH on a Beckman DU-7 spectrophotometer. IR spectra were obtained using NaCl plates on a Perkin Elmer 1600 FTIR spectrophotometer. CD spectra were performed on a JASCO Model J600 Circular Dichroism Spectropolarimeter. Low resolution MS were recorded on a Finnigan 4000 mass spectrometer. The exact masses were determined on a Kratos MS 50 mass spectrometer through peak matching. ^1H and ^{13}C NMR spectra were recorded on a Varian VXR-500S spectrometer, using the Varian software systems. HPLC was carried out with a Rainin HPLC instrument using the Dynamax software system and a Si gel column (250 × 21 mm) equipped with a Rainin UV-1 detector set at 220 nm.

Bioassays

The brine shrimp lethality test (BST) was conducted in our laboratory.[1,2] The cytotoxicity tests against A-549 (human lung carcinoma),[24] MCF-7 (human breast carcinoma),[25] HT-29 (human colon adenocarcinoma),[25] A-498 (human kidney carcinoma),[23] PC-3 (human prostate adenocarcinoma),[26] and PaCa-2 (human pancreatic carcinoma)[27] cells were performed in the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard protocols in seven day assays using MTT with adriamycin as a positive standard control.

Extraction, isolation and purification

The residue of the 95% EtOH crude extract of 4 kg of the leaves and twigs was partitioned between H_2O and CH_2Cl_2 to give a H_2O layer and a CH_2Cl_2 layer. The residue of the CH_2Cl_2 layer was partitioned between hexane and 10% H_2O in MeOH to give an aqueous MeOH layer and a hexane layer. The MeOH residue (141 g), which was the most active fraction in the BST (LC_{50} 17.26 μg/mL), was repeatedly chromatographed over Si gel columns and chromatotron separations, directed by BST activity, using gradients of hexane/$CHCl_3$/MeOH and hexane/acetone and, finally, purified by HPLC [Si gel column, 10% MeOH:THF (9:1) in hexane] to give the white waxes of **1**–**3**. Using the same methods, the three known compounds, as mentioned above, were also isolated. The known compounds were identified by these spectral data and direct comparisons.[4–6]

Preparation of TMSi derivatives

Tri-TMSi derivatives were prepared by treatment of pure acetogenins with *N,O*-bis-(trimethylsilyl) acetamide (BSA) 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 mL pyridine and 20 mL of BSA 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 sec/decade.

Preparation of per-(*S*) and per-(*R*)-mosher esters

0.5 mg of purified acetogenin, **1b**, **2**, or **3**, was dissolved in 0.5 mL of CH_2Cl_2, and sequentially, 0.2 mL pyridine, 0.2 mg 4-dimethylamino pyridine, and 25 mg of (*R*)-(−)α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (Aldrich) were added. The mixture was left at rt for 4 h and purified over a micro-column (0.6 × 6 cm) of Si gel eluted with 2 mL of CH_2Cl_2; the eluate was washed

with 1% $NaHCO_3$ (5 mL) and H_2O (5 mL × 2); the eluate was dried in vacuo to give the *S*-Mosher esters. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (Aldrich) gave the *R*-Mosher esters of **1b**, **2**, and **3**. Their pertinent ^1H NMR chemical shifts are given in Tables 2, 4, and 7.

Preparation of acetylated derivatives

1–2 mg of pure acetogenin, **1–3**, was dissolved in 0.5–1.0 mL of pyridine; 1 mL of anhydrous Ac_2O was added, and the mixture was set at rt for 48 h. The mixture was then partitioned between H_2O and $CHCl_3$, and the organic layer was concentrated and subjected to Si gel microcolumn chromatography to afford the pure acetate derivatives.

Preparation of intramolecular formal acetal derivative (1b). A mixture of DMSO and TMSCl (molar ratio 1.2:1) was mixed in 2 mL of benzene and placed in a refrigerator without stirring for 2 h to allow the formation of white crystals. The benzene was decanted and the crystals were washed twice with CH_2Cl_2. These crystals were added stepwise to 0.5 mL of a $CHCl_3$ solution containing 16 mg of **1** at rt (a large excess of the crystals were added). The reaction was monitored by TLC at intervals of 2 h and was quenched with H_2O after 12 h. After work up by extractions with 5% aqueous $NaHCO_3$, the reaction mixture was purified by normal phase HPLC. The yield of **1b** was 5 mg (31%), with most of the unreacted starting material recovered. CIMS (isobutane) *m/z*: 593 $[MH]^+$ 563, 545. EIMS: 365, 347, 295, 297, 279. ^1H NMR: see Table 1.

Preparation of acetonide derivative (3b). 1.5 mg of **3** was added to 0.5 mL of HCl–acetone (0.7 mg HCl in 1 mL acetone) and left overnight at rt; the mixture was dried in vacuo to give the pure acetonide derivative (**3b**). ^1H NMR: see Table 5.

Preparation of (2,4-*cis*- and *trans*)-gigantetroneninone. 3 mg of gigantetronenin was dissolved in 15 mL of EtOH saturated with Na_2CO_3 and refluxed for 4 h, 80 mL of H_2O was added to the mixture and the EtOH was completely evaporated. The H_2O remaining was partitioned with CH_2Cl_2 to give 2.3 mg of (2,4-*cis*- and *trans*)-gigantetroneninone.

Longifolicin (1). A whitish wax (40 mg); mp 83 °C; $[\alpha]_D^{25}$ +13.0° (*c* 0.001, CH_2Cl_2); UV(MeOH) λ_{max} 228 nm (log ε 3.70); IR (film on NaCl plate) 3400, 2900, 2820, 1750, 1440, 1300, 1073 cm^{-1}; CIMS (isobutane) *m/z* (%) $[MH]^+$ 581 (100), $[MH-H_2O]^+$ 563 (42), $[MH-2H_2O]^+$ 545 (28), $[MH-3H_2O]^+$ 527 (2.0); HRCIMS (isobutane) *m/z* 581.4793 for $C_{35}H_{64}O_6$ $[MH]^+$ (calcd 581.4781), *m/z* 297.1889 for $C_{16}H_{29}O_3Si$ [fragment bearing 10-OH]$^+$ (calcd 297.1886); EIMS: see Figure 2; ^1H and ^{13}C NMR: see Table 1.

Longicoricin (2). A whitish wax (20 mg); mp 74–75 °C; $[\alpha]_D^{25}$ +12.0° (*c* 0.001, CH_2Cl_2); UV (MeOH) λ_{max} 222 nm (log ε 2.93); IR (film on NaCl plate) 3422, 2923, 2855, 1734, 1650, 1456, 1076 cm^{-1}; CIMS (isobutane) *m/z* (%) $[MH]^+$ 609, $[MH-H_2O]^+$ 591, $[MH-2H_2O]^+$ 573, $[MH-3H_2O]^+$ 555; HRCIMS (isobutane) *m/z* 609.5082 for $C_{37}H_{68}O_6$ $[MH]^+$ (calcd 609.5094), *m/z* 297.1889 for $C_{16}H_{29}O_3Si$ [fragment bearing 10-OH]$^+$ (calcd 297.1886); EIMS, see Figure 2; ^1H and ^{13}C NMR: see Table 3.

(2,4-*cis* and *trans*)-Gigantetroneninone (3). A whitish wax (50 mg); mp 98 °C; $[\alpha]_D^{25}$ +22.9° (*c* 0.01, CH_2Cl_2); UV (MeOH) λ_{max} 210 nm (log ε 4.00); IR 3450, 2900, 2853, 1782, 1726, 1457, 1386, 1218 1072 cm^{-1}; CIMS (isobutane) m/z (%) $[MH]^+$ 623, $[MH-H_2O]^+$ 605, $[MH-2H_2O]^+$ 587, $[MH-3H_2O]^+$ 572; HRCIMS (isobutane) *m/z* 623.4874 for $C_{37}H_{64}O_7$ $[MH]^+$ (calcd 623.4887), m/z 313.1829 for $C_{16}H_{29}O_4Si$ [fragment bearing 10-OH]$^+$ (calcd 313.1835); EIMS, see Figure 3; ^1H and ^{13}C NMR: see Tables 5 and 6.

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