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LONGICIN AND GONIOTHALAMICINONE: NOVEL BIOACTIVE MONOTETRAHYDROFURAN ACETOGENINS FROM *ASIMINA LONGIFOLIA*

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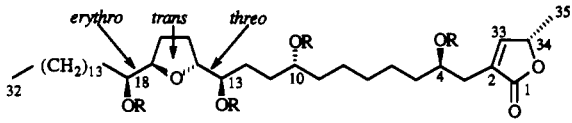
ABSTRACT.—Longicin [**1**] and (2,4-*cis* and *trans*)-goniothalamicinone [**2**], two new monotetrahydrofuran Annonaceous acetogenins, have been isolated from the leaves and twigs of *Asimina longifolia* (the long leaf paw paw) by the use of the brine shrimp lethality test for bioactivity-directed fractionation. The structures were elucidated based on spectroscopic and chemical methods. Compound **1** was converted to its ketolactone isomer, (2,4-*cis* and *trans*)-longicinone [**3**], to aid the stereochemical elucidation of **1**. Compounds **1–3** showed selective and potent cytotoxicities to certain human tumor cell lines, with the potency of **1** against pancreatic carcinoma (PaCa-2) over one million times that of adriamycin. Nine known cytotoxic acetogenins, annonacin, xylomaticin, isoannonacin, gigantetrocins A and B, muricatetrocins A and B, gigantetrocin-A-one and goniothalamicin, were also isolated for the first time from this species.

Asimina longifolia Kral (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). In our bioactivity-directed search for antitumor compounds, two new cytotoxic Annonaceous acetogenins, named longicin [**1**] and (2,4-*cis* and *trans*)-goniothalamicinone [**2**], were isolated from the plant material. Nine known monotetrahydrofuran (mono-THF) acetogenins [annonacin, isoannonacin, xylomaticin, gigantetrocins A and B, muricatetrocins A and B, gigantetrocin-A-one and goniothalamicin (3–5)] were also isolated for the first time from this species. The structures and absolute stereochemistries were determined by 1D and 2D nmr and ms before and after making certain chemical derivatives.

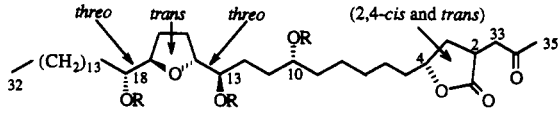
RESULTS AND DISCUSSION

The molecular weight of longicin [**1**] (Figure 1) was indicated by the peak at *m/z* 597 $[MH]^+$ in the cims. The high-resolution cims gave *m/z* 597.4719 for the $[MH]^+$ corresponding to $C_{35}H_{64}O_7$ (calcd 597.4730). The spectral data of **1** showed an ir carbonyl absorption at 1750^{-1}, a uv (MeOH) λ max at 228 nm (log ϵ 3.70), six resonances at δ 7.18 (H-33), 5.06 (H-34), 2.53 (H_a-3), 2.40 (H_b-3), 3.85 (H-4), and 1.44 (H-35) in the ^{1}H-nmr spectrum, and six peaks at δ 174.6 (C-1), 151.8 (C-33), 131.2 (C-2), 78.0 (C-34), 69.9 (C-4), and 19.1 (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 with a 4-OH moiety, as commonly found in the Annonaceous acetogenins (3–5).

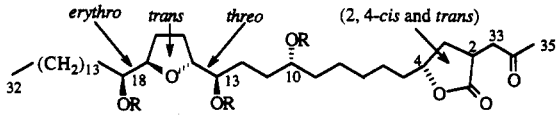
The existence of four OH moieties in **1** was obvious by an ir OH absorption at 3400 cm^{-1}, four successive losses of H_2O (*m/z* 18) from the $[MH]^+$ in the cims, and the



1 R=H **1a** R=Ac **1b** R=TMSi



2 R=H **2a** R=Ac **2b** R=TMSi



3 R=H **3a** R=Ac

FIGURE 1. Structures of longicin [**1**], (2,4-*cis* and *trans*)-goniothalamicinone [**2**], (2,4-*cis* and *trans*)-longicinone [**3**], and their acetate [**1a–3a**] and TMSi [**1b**, **2b**] derivatives.

TABLE 1. ^{1}H- (500 MHz) and ^{13}C- (125 MHz) Nmr Chemical Shifts (δ) for **1**, **1a**, **3**, and **3a** (coupling constants in Hz).

Position	^{1}H						^{13}C
	1	**1a**	**3** *(cis)*	**3** *(trans)*	**3a** *(cis)*	**3a** *(trans)*	**1**
1	—	—	—	—	—	—	174.6
2	—	—	3.04 m	3.00 m	3.04 m	3.00 m	131.2
3a	2.53 dddd (15, 3.3, 1.0, 1.0)	2.57 dddd (15, 3.3, 1.0, 1.0)	2.61 ddd (12.3, 9.4, 5.6)	2.23 ddd (12.9, 9.6, 3.4)	2.61 ddd (12.3, 9.4, 5.6)	2.23 ddd (12.9, 9.6, 3.4)	33.5
3b	2.40 dddd (15, 8.6, 1.0, 1.0)	2.51 dddd (15, 8.6, 1.0, 1.0)	1.40 m	1.96 m	1.40 m	1.96 m	
4	3.85 m	5.11 m	4.40 dddd (10.7, 7.4, 5.4, 5.4)	4.55 dddd (8.3, 8.2, 5.7, 3.2)	4.40 dddd (10.7, 7.4, 5.4, 5.4)	4.55 dddd (8.3, 8.2, 5.7, 3.2)	69.9
5–9	1.23–1.74 m	1.23–1.74 m	1.23–1.74 m		1.23–1.74 m		25.4–37.5
10	3.64 m	4.83 m	3.61 m		4.87 m		71.5
11–12	1.23–1.74 m	1.23–1.74 m	1.23–1.74 m		1.23–1.74 m		25.4–37.5
13	3.4 m	4.83 m	3.44 m		4.82 m		74.6
14	3.84 m	3.96 m	3.84 m		3.97 m		83.1
15–16	1.57–2.00 m	1.57–2.00 m	1.57–2.00 m		1.57–2.00 m		25.4–37.5
17	3.81 m	3.96 m	3.81 m		3.97 m		82.2
18	3.88 m	4.92 m	3.88 m		4.92 m		71.6
19–31	1.23–1.74 m	1.23–1.74 m	1.23–1.74 m		1.23–1.74 m		25.4–37.5
32	0.88 t (6.9)	0.88 t (6.9)	0.88 t (6.9)		0.88 t (6.9)		14.1
33a	7.18 q (1.5)	7.18 q (1.5)	2.61 dd (15.3, 8.6)	2.56 dd (19.5, 10.6)	2.61 dd (15.3, 8.6)	2.56 dd (19.5, 10.6)	151.8
33b			3.08 dd (18.5, 3.5)	3.00 dd (18.5, 3.4)	3.08 dd (18.5, 3.5)	3.00 dd (18.5, 3.4)	
34	5.06 qq (6.5, 1.5)	5.06 qq (6.5, 1.5)	—		—		78.0
35	1.44 d (6.5)	1.44 d (6.5)	2.20 s		2.20 s		19.1
OAc-4		2.02 s			—		
OAc-10 ...		2.03 s			2.03 s		
OAc-13 ...		2.07 s			2.07 s		
OAc-18 ...		2.05 s			2.05 s		

preparation of a tetra-acetate derivative [**1a**] and a tetra-trimethylsilyl (TMSi) derivative [**1b**]. Compound **1a** gave four singlet proton peaks at δ 2.02 (OAc), 2.03 (OAc), 2.05 (OAc), and 2.07 (OAc) and three multiplet resonances at δ 5.11 (H-4), 4.92 (H-18), and 4.83 (H-10, H-13), corresponding to the downfield shifts of four protons on secondary OH-bearing carbons. Furthermore, the ^{13}C-nmr spectrum of **1** showed four resonances due to oxygen-bearing carbons at δ 69.9 (C-4), 71.5 (C-10), 74.6 (C-13), and 71.6 (C-18), indicating the existence of four secondary OH moieties. The typical carbon resonance for the 4-OH is at δ 69 in the acetogenins (3–5). The presence of a mono-THF ring, with two OH groups flanking the ring, was suggested by ^{1}H-nmr resonances at δ 3.44 (H-13), 3.81, 3.84, and 3.88 (H-14, H-17, H-18), and the carbon peaks at δ 82.2 (C-17) and 83.1 (C-14); these were directly analogous to similar peaks of other mono-THF acetogenins with two flanking OH groups, such as annonacin A and annomuricin B (6,7).

The carbon skeleton and placement of the mono-THF ring and four OH groups along the hydrocarbon chain of **1** were determined based on analysis of the eims spectral data (Figure 2). The mono-THF ring was determined to be at C-14, and the positions of the OH groups were assigned at C-4, C-10, C-13, and C-18 by analysis of the fragments in the eims spectrum of **1b**. The hreims of **1** gave an ion for $[C_{19}H_{37}O_4Si_2]^+$

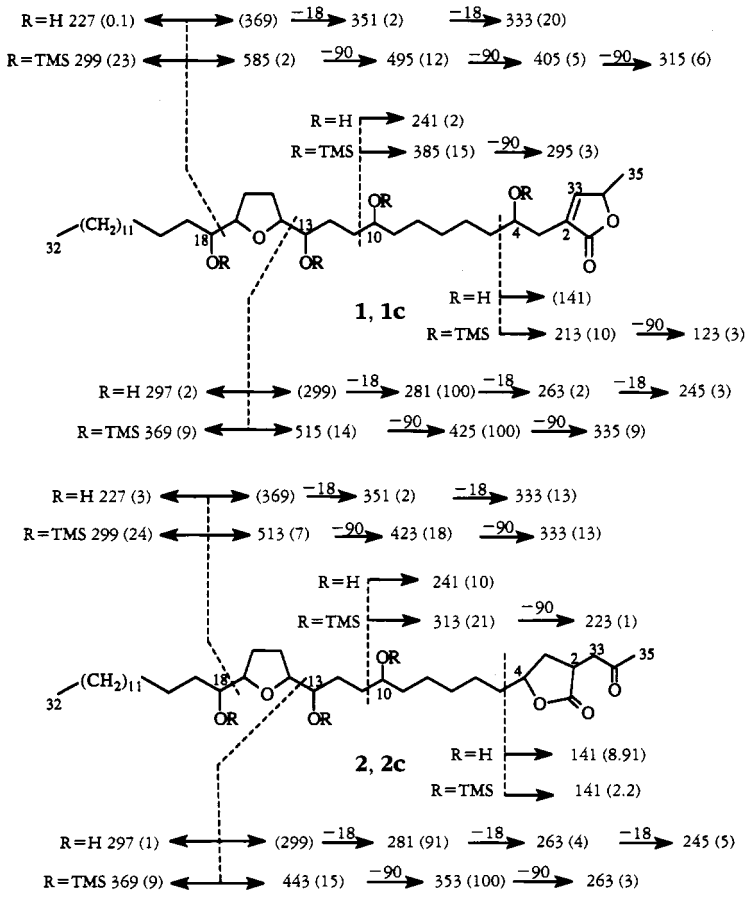


FIGURE 2. Diagnostic eims fragment ions of **1** and **2** (R=H) and **1b** and **2b** (R=TMSi) in *m/z*, with % intensities in parentheses. Losses of *m/z* 90 indicate the loss of TMSiOH neutrals and losses of *m/z* 18 indicate the loss of H_2O. Peaks in parentheses were not seen or were very weak.

at *m/z* 385.2222 (calcd 385.2230), and this fragment confirmed the placement of the OH group at C-10.

The stereochemistry at C-13/C-14 and C-17/C-18 was concluded to be threo and erythro, respectively, and the stereochemistry was determined as trans for the THF ring by comparison with model compounds synthesized by Cavé *et al.* (8), as well as by comparison with annonacin A (6), (2,4-*cis* and *trans*)-annonacin-A-one (9), and jetein (10).

Rieser *et al.* have reported the determination of the absolute configuration of stereogenic carbinol centers in several Annonaceous acetogenins using Mosher ester methodology (11–13). The per-(*S*)- and (*R*)-methoxy(fluoromethyl)phenylacetic acid (MTPA) esters (Mosher esters) of **1** were prepared. COSY ^{1}H-nmr analysis of these derivatives allowed the absolute stereochemical assignment of C-4 as *R*. This result is identical to all acetogenins examined so far that possess an OH at C-4 (4,5,13). Recently, Hoye *et al.* determined the relative configuration between C-4 and C-34 by analysis of MTPA esters of model butenolides vs. the MTPA derivatives of 4-hydroxylated acetogenins (14). A comparison of the nmr data of the model compounds to those of **1** revealed that the relative stereochemistry between C-4 and C-34 in **1** was "unlike" (*RS* or *SR*) (Table 2). Thus, the absolute stereochemistry for C-34 is *S*, as is usual for all the known acetogenins.

TABLE 2. ^{1}H-Nmr Chemical Shifts for the Determination of the Absolute Configuration at C-4 and C-34 of the Tetra (*S*)- and (*R*)-MTPA Esters of **1**.

MTPA Derivative	MTPA Configuration	Proton Chemical Shifts δ (ppm) ($\Delta\delta_H=\delta_S-\delta_R$)							Carbinol Configuration
		H-5	H-4	H-3		H-33	H-34	H-35	
R,S-Synthetic ...	*S*	1.41	—	2.63	2.56	6.77	4.87	1.29	4*R*
Model	*R*	1.36	—	2.69	2.60	7.00	4.94	1.33	34*S*
Butenolide (13) .	Δδ	+0.05	—	−0.06	−0.04	−0.23	−0.07	−0.04	
	S	1.60	5.30	2.58	2.56	6.72	4.86	1.30	4*R*
1	*R*	1.57	5.35	2.65	2.60	6.94	4.90	1.32	34*S*
	Δδ	+0.03	−0.05	−0.07	−0.04	−0.22	−0.04	−0.02	

However, the Mosher ester procedure cannot be directly applied to acetogenins that have hydroxyls in close proximity to each other, such as in longicin [**1**] with a 1,4-diol at C-10/C-13. These hydroxyls are only two carbons apart, and, thus, the phenyl rings of the per-Mosher esters interfere with each other, making it difficult to assign accurately the complicated ^{1}H-nmr chemical shifts.

To determine the absolute stereochemistry for C-10, longicin [**1**] was converted to (2,4-*cis* and *trans*)-longicinone [**3**] by treatment with mild base. The (*S*)- and (*R*)-MTPA esters of **3** were then prepared. Analysis of the ^{1}H-nmr data of the per-Mosher ester derivatives of **3** determined that the chemical shift difference of the H-4 [$\Delta\delta_{H\text{-}4}$ (δ_S-δ_R)] of the *S*- (at δ 4.52, 4.36) and *R*- (at δ 4.51, 4.35) Mosher esters presented a positive value and suggested the *R* absolute configuration for C-10 (Table 3). Thus, the absolute configuration of the carbinol centers that are between the THF rings and the terminal lactones of acetogenins, such as **1**, can be defined by simply converting the butenolide acetogenin to its ketolactone isomer and then observing the chemical shift changes of H-4 in the ^{1}H-nmr spectra of the *S*- and *R*-Mosher esters.

The Mosher ester methodology also cannot be directly applied to the mono-THF ring with two flanking hydroxyls having the relative stereochemistry of threo, trans,

TABLE 3. ^{1}H-Nmr Chemical Shifts for the Determination of the Absolute Configuration at C-10 of the Tetra (*S*)- and (*R*)-MTPA Esters of **2** and **3**.

MTPA derivative	MTPA Configuration	Proton Chemical Shifts δ (ppm)				Carbinol Configuration at C-10
		H-4		H-5	H-10	
	S	4.53	4.37	1.68	5.02	
2	*R*	4.52	4.36	1.66	4.92	*R*
	Δδ	+0.01	+0.01	+0.02	+0.10	
	S	4.52	4.36	1.71	5.00	
3	*R*	4.51	4.35	1.68	5.01	*R*
	Δδ	+0.01	+0.01	+0.03	−0.01	

erythro. The two phenyl rings on the Mosher esters flanking the THF ring take the same orientation in the per-Mosher ester of **1**, making H-14, H-15, H-16, and H-17 receive both shielding and deshielding effects at the same time; thus, it was difficult to predict the absolute stereochemistry of the carbinol centers at C-13 and C-18. In the analysis of the ^{1}H-nmr and COSY data of the per-MTPA ester of **1**, the chemical shift difference of H-19 [$\Delta\delta_{H\text{-}19}$ (δ_S-δ_R)] of the *S*- and *R*-Mosher esters presented a negative value. By studying a molecular model of the per-Mosher ester derivative of **1**, it was anticipated that the chemical shift of H-19 would be significantly influenced by the C-18-OMTPA group, but much less by the C-13-OMTPA group. With this reasoning, we assigned the absolute configuration at C-18 as *S* and, therefore, at C-13 as *R*.

^{1}H-Nmr comparison studies were made with (*R*)-MTPA esters of stereochemically defined model compounds to confirm the above assignments. Two model compounds: di-(*R*)-MTPA esters of *erythro, threo, trans*-(2*R*,5*R*)-di(1-hydroxypentyl)-THF [**4**] and its antipode [**5**] were synthesized by the Fujimoto group (15). The chemical shifts of H-a and H-d of **4** and **5** (Figure 3) were compared with those of H-13 and H-18 in the per-(*R*)-Mosher ester of **1**. The chemical shifts of H-13 and H-18 were in agreement with those of H-a and H-d of **5**, rather than those of **4**, supporting our conclusion that **1** has the C-18*S*, C-13*R* absolute configuration. Thus, the absolute stereochemistry of **1** was established as C-4*R*, C-10*R*, C-13*R*, C-18*S*, and C-34*S*.

(2,4-*cis*- and *trans*)-Goniothalamicinone [**2**] were isolated in a mixture as an amorphous waxy powder. The mol wt of **2** was indicated by peaks at *m/z* 597 $[MH]^+$ in both the cims and the fabms. The hrcims gave *m/z* 597.4719 for the $[MH]^+$ (calcd 597.4730) corresponding to $C_{35}H_{64}O_7$. The ir spectrum showed a strong absorption at 1782 cm^{-1} for a γ-lactone carbonyl and at 1726 cm^{-1} for a ketone carbonyl. Compound **2** 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 (11,12,16) and 2,4-*cis*- and *trans*)-longicinone [**3**], as described above, the ^{1}H- and ^{13}C-nmr spectra of **2** clearly indicated the presence of a ketolactone moiety. In the ^{1}H-nmr spectrum of **2** (Table 4), the resonances at δ 4.40 and 4.58, with combined integrations for one proton, were assigned to H-4 and suggested the presence of a mixture of (2,4-*cis*- and *trans*)-diastereoisomers at the ketolactone ring moiety, as is typical with these ketolactones (3–

FIGURE 3. Structures of di-(*R*)-MTPA esters of erythro, threo, trans-(2*R*,5*R*)-di-(1-hydroxypentyl)-tetrahydrofuran [**4**] and its antipode [**5**] (15).

TABLE 4. ^{1}H- (500 MHz) and ^{13}C- (125 MHz) Nmr Chemical Shifts (δ) for **2** and **2a**.

Position	^{1}H				^{13}C	
	2 *(cis)*	**2** *(trans)*	**2a** *(cis)*	**2a** *(trans)*	**2** *(cis)*	**2** *(trans)*
1	—	—	—	—	178.7	178.2
2	3.04 m	3.00 m	3.04 m	3.00 m	44.2	43.8
3a	2.61 ddd (12.3, 9.4, 5.6)	2.23 ddd (12.9, 9.6 3.4)	2.61 ddd (12.3, 9.4, 5.6)	2.23 ddd (12.9, 9.6, 3.4)	25.4–37.5	
3b	1.40 m	1.96 m	1.40 m	1.96 m		
4	4.40 dddd (10.7 7.4 5.4, 5.4)	4.58 dddd (8.3, 8.2, 5.7, 3.2)	4.40 dddd (10.7, 7.4, 5.4, 5.4)	4.55 dddd (8.3, 8.2, 5.7, 3.2)	79.3	78.8
5–9	1.23–1.74 m		1.23–1.74 m		25.4–37.5	
10	3.65 m		4.84 m		71.6	
11–12	1.23–1.74 m		1.23–1.74 m		25.4–37.5	
13	3.45 m		4.84 m		74.4	
14	3.83 m		3.97 m		82.6	
15–16	1.57–2.00 m		1.57–2.00 m		25.4–37.5	
17	3.83 m		3.97 m		82.6	
18	3.41 m		4.84 m		74.0	
19–31	1.23–1.74 m		1.23–1.74 m		25.4–37.5	
32	0.88 t (6.9)		0.88 t (6.9)		14.2	
33a	2.61 dd (15.3, 8.6)	2.56 dd (19.5, 10.6)	2.61 dd (15.3, 8.6)	2.56 dd (19.5, 10.6)	151.8	
33b	3.08 dd (18.5, 3.5)	3.00 dd (18.5, 3.4)	3.08 dd (18.5, 3.5)	3.00 dd (18.5, 3.4)		
34	—		—		205.5	
35	2.20 s		2.20 s		22.7	
OAc-10			2.04 s			
OAc-13			2.08 s			
OAc-18			2.07 s			

5). In the ^{13}C-nmr spectrum (Table 4), signal pairs at δ 178.7 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, too, confirmed the presence of the mixture of (2,4-*cis*- and *trans*)-isomers. The absolute configuration of C-4 was suggested as *R* by comparison of ^{1}H- and ^{13}C-nmr spectral data with those of (2,4-*cis*- and *trans*)-bullatacinone of known chirality (10). The assignments of H-2, H-3a, H-3b, H-33a, and H-33b were based on analysis of the COSY spectrum of **2** (Table 4).

The remaining part of the structure of **2** exhibited identical ^{1}H- and ^{13}C-nmr signals for a long aliphatic chain bearing a mono-THF ring and three OH groups. In the cims spectrum, a series of peaks at *m/z* 579, 561, and 543, arising from the successive losses of three molecules of H_2O, were observed, confirming the presence of the three OH groups. The OH groups were also identified by the broad ir absorption band at 3450 cm^{-1} and by the three acetate methyl signals at δ 2.04, 2.07, and 2.08 in the ^{1}H-nmr spectrum of the triacetate derivative [**2a**].

The mono-THF ring, with the usual OH groups on each side, was indicated in **2** by ^{1}H-nmr chemical shifts at δ 3.45 (H-13), 3.83 (H-14, H-17), and 3.41 (H-18) and ^{13}C-nmr signals at δ 74.4 (C-13), 82.6 (C-14, C-17), and 74.0 (C-18). The placement of the mono-THF ring was determined to be at C-14 by diagnostic fragments at *m/z* 281 and 333 in the eims (Figure 2). The relative configuration in this THF ring moiety was established as threo, trans, threo by comparing the ^{1}H-nmr data of the acetate derivative [**2a**] with those of model compounds of known relative stereochemistry; the proton signals for H-13 and H-18 at δ 3.45 and 3.41 in **2** were shifted downfield in **2a** to δ 4.84 (8).

The position of the third OH group in **2** was assigned at C-10 by analysis of the fragments in the eims spectra of **2** and its TMSi derivative [**2b**] (Figure 2). The hreims gave an ion at *m/z* 313.1829 for $[C_{16}H_{29}O_4Si]^+$ (calcd 313.1835), which confirmed the position of the third OH at C-10.

To determine the absolute stereochemistry of compound **2**, the (*S*) and (*R*)-MTPA esters were prepared. COSY ^{1}H-nmr analysis of these derivatives allowed the absolute stereochemical assignment of the carbinol centers as C-10*R*, C-13*R*, and C-18*R* (Table 5), which is the same as that of goniothalamicin (13,17). Thus, the structure of **2** was defined as illustrated and named as (2,4-*cis*- and *trans*)-goniothalamicinone, based on the parent acetogenin, goniothalamicin (18).

TABLE 5. ^{1}H-Nmr Chemical Shifts for the Determination of the Absolute Configuration at C-13, C-18 of the Tetra (*S*)- and (*R*)-MTPA Esters of **2**.

	Proton Chemical Shifts δ (ppm) ($\Delta\delta=\delta_S-\delta_R$)									
	H-12	H-13	H-14	H-15		H-16		H-17	H-18	H-19
S	1.58	4.94	3.94	1.67	1.47	1.50	1.27	3.77	4.88	1.48
R	1.50	4.98	4.00	1.91	1.57	1.86	1.48	3.95	4.92	1.42
Δδ (*S*-*R*)	+0.08	−0.04	−0.06	−0.24	−0.10	−0.36	−0.21	−0.18	−0.04	+0.06
Carbinol Configuration	C-13*R*									C-18*R*

Biological activities of **1** and **2** are summarized in Table 6. These natural compounds are very active in the BST, and they showed significant cytotoxicities against A-549 (human lung carcinoma) (19), MCF-7 (human breast carcinoma) (20), HT-29 (human colon adenocarcinoma) (21), A-498 (human kidney carcinoma) (19), PC-3 (human prostate adenocarcinoma) (22), and PaCa-2 (human pancreatic carcinoma) (23) cell lines in our seven-day MTT human solid tumor cytotoxicity tests. These cytotoxicity values are quite favorable compared to those of the positive control compound, adriamycin, with **1** generally being more active and exhibiting selective cytotoxicity of one million

TABLE 6. Bioactivities of Compounds **1–3**.

Compound		**1**	**2**	**3**	Adriamycin[h]
BST[a] LC_{50} (μg/ml)		0.11 (0.17/0.07)	0.14 (0.37/0.02)	0.46 (0.62/0.20)	—
Human Cancer Cell Line ED_{50} (μg/ml)	A-549[b]	1.77×10^{-6}	2.06×10^{-5}	1.23×10^{-4}	3.41×10^{-3}
	MCF-7[c]	>1	9.67×10^{-1}	1.86×10^{-1}	3.29×10^{-1}
	HT-29[d]	2.4×10^{-5}	4.05×10^{-4}	2.35×10^{-1}	1.16×10^{-2}
	A-498[e]	1.99×10^{-4}	2.91×10^{-1}	1.72×10^{-1}	1.97×10^{-3}
	PC-3[f]	4.26×10^{-3}	1.37×10^{-1}	2.01×10^{-1}	6.24×10^{-2}
	PaCa-2[g]	1.25×10^{-9}	1.33×10^{-3}	2.37×10^{-4}	1.95×10^{-3}

[a]Brine shrimp lethality test (1,2).
[b]Human lung carcinoma (19).
[c]Human breast carcinoma (20).
[d]Human colon adenocarcinoma (21).
[e]Human kidney carcinoma (19).
[f]Human prostate adenocarcinoma (22).
[g]Human pancreatic carcinoma (23).
[h]Postive control standard.

times that of adriamycin against the pancreatic cell line (PaCa-2). The acetogenins are powerful inhibitors of mitochondrial electron transport systems (5) and have recently been shown also to inhibit the NADH oxidase that is prevalent in the plasma membranes of tumor, but not normal, cells (24); this depletion of intracellular ATP levels induces in vivo antitumor effects and especially suggests potential synergistic effects with other chemotherapeutic agents in the treatment of multi-drug resistance (25).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps 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. Low-resolution ms were recorded on a Finnigan 4000 mass spectrometer. The exact masses were determined on a Kratos 50 mass spectrometer through peak matching. ^{1}H- and ^{13}C-nmr spectra were recorded on a Varian VXR-500S spectrometer, using 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.

PLANT MATERIAL.—The leaves and twigs of *A. longifolia* Kral were collected in Georgia in September 1993 under the auspices of one of us (P.R.E.), Curator of the Herbarium, University of Georgia, where voucher specimens are maintained.

EXTRACTION AND ISOLATION.—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 H_2O and CH_2Cl_2 layers. 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 assay (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/Me_2CO, and purified finally by hplc [Si gel column, 10% MeOH-THF (9:1) in hexane] to give **1** and **2** as white waxes. Using the same methods, the nine known compounds, as mentioned above, were also isolated. The known compounds were identified by spectral data and direct comparison (3–5).

Longicin [**1**].—Whitish wax (20 mg); mp 83°; $[\alpha]^{25}D$ +13.0° (c=1.0, CH_2Cl_2); uv (MeOH) λ max 228 (log ε 3.70) nm; ir (film on NaCl plate) ν max 3400, 2900, 2820, 1750, 1440, 1300, 1073 cm^{-1}; cims (isobutane) *m/z* $[MH]^+$ 597 (100), $[MH-H_2O]^+$ 579 (42), $[MH-2H_2O]^+$ 561 (100), $[MH-3H_2O]^+$ 543 (33), $[MH-4H_2O]^+$ 525 (3), 351 (3), 333 (1), 281 (3); hrcims (isobutane) *m/z* 597.4719 for $C_{35}H_{64}O_7$ $[MH]^+$ (calcd 597.4730), *m/z* 385.2222 for $C_{19}H_{37}O_4Si_2$ [fragment bearing OH-10]$^+$ (calcd 395.2230); eims, see Figure 2; ^{1}H- and ^{13}C-nmr data, see Table 1.

*(2,4-*cis *and* trans*)-Goniothalamicinone* [**2**].—Whitish wax (50 mg); mp 98°; $[\alpha]^{25}D$ +22.9° (c=1.0, CH_2Cl_2); uv (MeOH) λ max 210 (log ε 4.00) nm; ir ν max 3450, 2900, 2853, 1782, 1726, 1457, 1388, 1218, 1072 cm^{-1}; cims (isobutane) *m/z* $[MH]^+$ 597 (0.2), $[MH-2H_2O]^+$ 561 (20), $[MH-3H_2O]^+$ 543 (31), $[MH-4H_2O]^+$ 525 (0.4), 351 (1), 333 (5), 309 (32), 297 (0.6), 281 (100), 263 (1), 245 (1), 241 (5), 141 (2); hrcims (isobutane) *m/z* 597.4719 for $C_{35}H_{64}O_7$ $[MH]^+$ (calcd 597.4730), *m/z* 313.1829 for $C_{16}H_{29}O_4Si$ [fragment bearing OH-10]$^+$ (calcd 313.1835); eims, see Figure 2; ^{1}H- and ^{13}C-nmr data, see Table 1.

*(2,4-*cis *and* trans*)-Longicinone* [**3**].—Compound **1** (5 mg) was dissolved in EtOH saturated with Na_2CO_3 (15 ml) and refluxed for 4 h. The solution was diluted with H_2O (80 ml) and the EtOH was completely evaporated. The H_2O remaining was partitioned with CH_2Cl_2 to yield **3** (4.5 mg); ^{1}H-nmr data, see Table 1.

PREPARATION OF TMSi DERIVATIVES.—Tri-TMSi derivatives (**1b**, **2b**) were prepared by treatment of **1** and **2** with *N,O-bis*-(trimethylsilyl) acetamide (BSA) in the presence of pyridine. Approximately 10–50 μg of pure compound were placed in a 100-μl conical reaction vial and dried in a vacuum desiccator over P_2O_5 for 24 h. The sample was treated with 2 μl pyridine and 20 μl BSA and heated at 70° 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 MOSHER ESTER DERIVATIVES.—A 0.5-mg aliquot of purified acetogenin, **1** or **2**, was dissolved in 0.5 ml CH_2Cl_2, and sequentially, 0.2 ml pyridine, 0.2 mg 4-dimethylaminopyridine, and 25 mg of (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (Aldrich) were added. The mixture was left at room temperature 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 **1** and **2**. Their pertinent ^{1}H-nmr chemical shifts are given in Tables 3–5.

BIOLOGICAL TESTING.—The brine shrimp lethality test (BST) was conducted in our laboratory (1,2). The cytotoxicity tests against A-549 (human lung carcinoma) (18), MCF-7 (human breast carcinoma) (19), HT-29 (human colon adenocarcinoma) (20), A-498 (human kidney carcinoma) (18), PC-3 (human prostate adenocarcinoma) (21), and PaCa-2 (human pancreatic carcinoma) (22) cells were performed in the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard protocols in seven day assays and MTT with adriamycin as a positive standard control.

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