Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 3, pp 549–555, 1997

PII: S0968-0896(96)00268-4
0968-0896/97 $17.00 + 0.00

4-Deoxyannomontacin and (2,4-*cis* and *trans*)-Annomontacinone, New Bioactive Mono-tetrahydrofuran Annonaceous Acetogenins from *Goniothalamus giganteus*

Feras Alali, Lu Zeng, Yan Zhang, Qing Ye, D. Craig Hopp, Jon T. Schwedler and Jerry L. McLaughlin*

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907, U.S.A.

Abstract—4-Deoxyannomontacin (**1**) and a mixture of (2,4-*cis* and *trans*)-annomontacinone (**2**), new bioactive mono-tetrahydrofuran (THF) γ-lactone and keto-lactone acetogenins, respectively, as well as five known mono-THF acetogenins [xylomaticin, longifolicin, longicoricin, (2,4-*cis* and *trans*)-gigantetrocinone, and (2,4-*cis* and *trans*)-gigantetroneninone], were isolated from the bark of *Goniothalamus giganteus* (Annonaceae) by activity-directed fractionation using the brine shrimp lethality test (BST). The structures were elucidated based on spectroscopic and chemical methods. The absolute stereochemistries of **1** and **2** were determined by the advanced Mosher ester method and by circular dichroism (CD). Determination of the absolute stereochemistry at C-10 as *R* for **1** is the first example of the direct determination of the absolute stereochemistry of a carbinol position isolated from other functional groups in the annonaceous acetogenins. **1** and **2** showed selective and potent cytotoxicities to certain human tumor cell lines and were comparable to the activity of rotenone against yellow fever mosquito larvae.

Introduction

The annonaceous acetogenins are a relatively new class of compounds. The first, uvaricin, was isolated in 1982.[1] They are C_{35}–C_{37} fatty acid derivatives with long chain hydrocarbon portions connecting a variable number of tetrahydrofuran (THF) or tetrahydropyran (THP) rings and terminated with a 2,4-disubstituted-γ-lactone (sometimes rearranged to a 2,4-disubstituted ketolactone) moiety. As very potent mitochondrial inhibitors, the annonaceous acetogenins are a class of promising anticancer, antiinfective, and pesticidal natural compounds. They are found only in the Annonaceae, and, so far, over 230 of them have been found from the genera *Annona*, *Asimina*, *Goniothalamus*, *Rollinia*, *Uvaria*, and *Xylopia*.[2–6]

Goniothalamus giganteus Hook. f. & Thomas (Annonaceae) is a tropical tree distributed in southeast Asia. It has a great reputation as a drug among the Malays.[7] The bark of this plant, obtained from Thailand, showed toxicities in the brine shrimp lethality test (BST) and murine toxicities in the 3PS (P388) leukemia bioassay.[8] Seventeen bioactive annonaceous acetogenins have been previously isolated from the bark.[9–19] In our further bioactivity-directed search for antitumor compounds, two new bioactive acetogenins, 4-deoxyannomontacin (**1**) and a mixture of (2,4-*cis* and *trans*)-annomontacinone (**2**), have been isolated. Also, five known mono-THF acetogenins [xylomaticin, longifolicin, longicoricin, (2,4-*cis* and *trans*)-gigantetrocinone, and (2,4-*cis* and *trans*)-gigantetroneninone], were isolated for the first time from this species.[20–22] The structures and absolute stereochemistries were determined by 1-D and 2-D NMR, MS, and CD 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 peak at *m/z* 609 $[MH]^+$ in the CIMS. The HRCIMS gave *m/z* 609.5069 for the $[MH]^+$ ion (calcd 609.5094) corresponding to the molecular formula $C_{37}H_{68}O_6$.

Compound **1** showed an IR carbonyl absorption at 1742 cm^{-1}, a UV (MeOH) λ_{max} at 228 nm (log 2.86), four resonances at δ 6.99 (q, H-35), 5.00 (qq, H-36), 1.41 (d, H-37), and 2.26 (tt, H-3) in the ^1H NMR spectrum and five peaks at δ 174.00 (C-1), 148.88 (C-35), 134.25 (C-2), 77.41 (C-36), and 19.17 (C-37) 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 some of the annonaceous acetogenins.[2–6]

The presence of three OH moieties in **1** was suggested by a prominent OH absorption at 3443 cm^{-1} in the IR

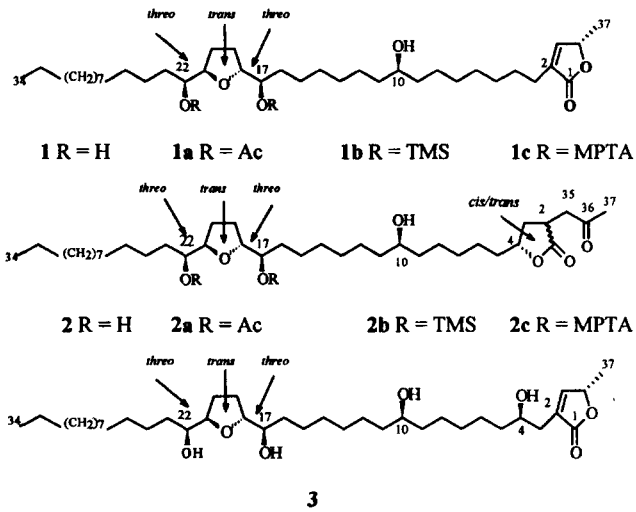

Figure 1. Structures of **1**, **1a**, **1b**, **1c**, **2**, **2a**, **2b**, **2c**, and **3**.

spectrum and was confirmed by three successive losses of H_2O (*m/z* 18) from the $[MH]^+$ in the CIMS and the preparations of the tri-acetate (**1a**) and the tri-trimethylsilyl (TMSi) derivatives (**1b**). Compound **1a** gave three singlet proton peaks at δ 2.04 (10-OAc), 2.07 (17-OAc), and 2.07 (22-OAc), and a multiplet at δ 4.85 (H-10, H-17, and H-22) corresponding to the downfield shifts of three protons on acetylated secondary OH-bearing carbons. Furthermore, the ^{13}C NMR of **1** showed three resonances due to oxygen-bearing carbons at δ 71.88 (C-10), 74.01 (C-17), and 74.01 (C-22), 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 3.40 (H-17), 3.80 (H-18), 3.80 (H-21), and 3.40 (H-22), and the carbon peaks at δ 82.66 (C-18) and 82.61 (C-21); these were directly analogous to similar peaks of other mono-THF acetogenins with two flanking hydroxyl groups, such as annomontacin (**3**) and corossoline.[23,24]

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). The assignments of the peaks in the ^1H NMR spectrum of **1** were based on 1-D ^1H and 2-D COSY.

The stereochemistries at C-17/C-18 and C-21/C-22 in **1** were concluded to be *threo*, and the stereochemistry of the THF ring was determined as *trans* by comparison with model compounds of known relative configuration, synthesized by both Harmarge et al.[25] and Fujimoto et al.,[26] as well as by comparisons with the reported data for annomontacin (**3**)[23] and corossoline.[24] The absolute configurations of C-10, C-17, and C-22 were determined using advanced Mosher ester methodology.[27,28] The (*S*)- and (*R*)-methoxy(fluoromethyl) phenylacetic acid (MPTA) esters (Mosher esters) of **1** were prepared. COSY ^1H NMR analysis of these derivatives allowed the assignment of the absolute configuration at C-17 and C-22 as both *R* (Table 1); it then followed that those at C-18, C-21 were both *R* considering their relative stereochemistry. Determination of the absolute stereochemistry at C-10 as *R* was accomplished using the Mosher ester method; this was achieved by assigning H-4 in both (*R*)- and (*S*)-Mosher esters using 2-D COSY (Table 1).

The absolute stereochemistry at C-36 of **1** was determined by CD data. It is reported that a negative Cotton

Table 1. ^{13}C NMR and ^1H NMR (δ, *J* in Hz) of **1** and its *S*- and *R*-Mosher esters

	^{13}C NMR **1** (125 MHz)	**1** (*J* in Hz)	^1H (500 MHz) *S*-MPTA	*R*-MPTA	$\Delta\delta_H$ $\delta_S-\delta_R$
1	174.00	–	–	–	
2	134.25	–	–	–	
3	25.13	2.26 t (7.0)	2.26	2.25	+0.01
4	27.37	1.53 m	1.55	1.53	+0.02
5–8	25.13–31.88	1.25–1.70 m	1.25–1.70	1.25–1.70	
9	37.45	1.42 m	1.40	1.41	
10	71.88	3.58 m	5.07	5.03	C-10*R*
11	37.45	1.42 m	1.40	1.41	
12–15	25.13–31.88	1.25–1.70 m	1.25–1.70	1.25–1.70	
16	33.43	1.41 m	1.56	1.54	+0.02
17, 22	74.01	3.40 q (5.5)	4.97	5.03	C-17*R*, C-22*R*
18, 21	82.66, 82.61[a]	3.80 q (7.5)	3.92	4.01	–0.09
19a/b	28.73	1.69 m, 1.98 m	1.37, 1.65	1.57, 1.91	–0.20, –0.26
20a/b	28.73	1.69 m, 1.98 m	1.37, 1.65	1.57, 1.91	–0.20, –0.26
23	33.43	1.41 m	1.56	1.54	+0.02
24–33	22.65–31.88	1.25–1.70 m	1.25–1.70	1.25–1.70	
34	14.07	0.88 t (7.5)	0.88	0.88	
35	148.88	6.99 q (1.5)	6.99	6.99	
36	77.41	5.00 qq (7,2)	5.00	5.00	
37	19.17	1.41 d (6.5)	1.41	1.41	

[a]Signals may be interchangeable.

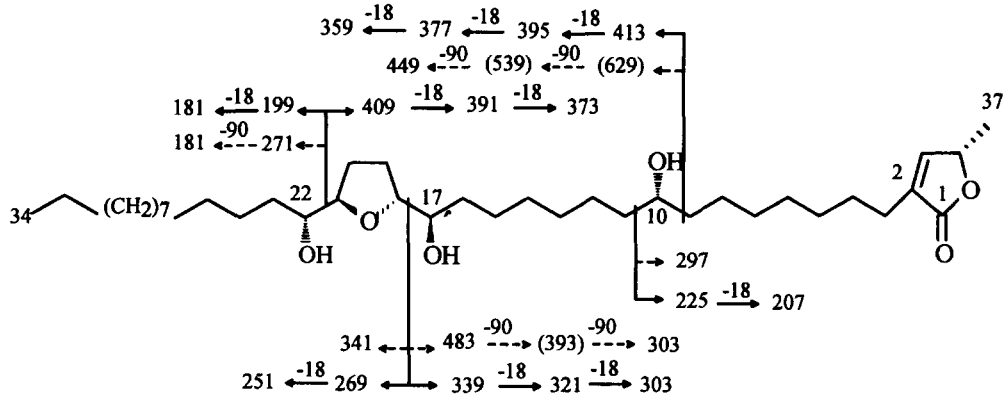

Figure 2. Diagnostic mass fragmentation ions of **1** and **1b**. EIMS of **1** (solid line); losses of H_2O indicated by 18 *m/z*. EIMS of **1b** (dashed line); losses of TMSiOH indicated by 90 *m/z*.

effect at 236 nm in the CD spectrum of squamocin is attributed to the 36*S* configuration in the γ-lactone moiety.[29] The CD of **1** showed a negative Cotton effect at 240 nm (= 0.49) compared with squamocin [negative Cotton effect at 236 nm (= 0.33)]; thus, the absolute stereochemistry at C-36 is proposed as *S* as is common in all other reported Annonaceous acetogenins. Thus, the structure of **1** was elucidated as illustrated (Fig. 1), and it was named 4-deoxyannomontacin after the parent compound annomontacin (**3**).[3,23]

The mixture of (2,4-*cis* and *trans*)-annomontacinone (**2**) was isolated in the form of an amorphous waxy powder. The molecular weight of **2** was indicated by a peak at *m/z* 625 for the $[MH]^+$ in the CIMS. The HRCIMS gave *m/z* 625.5061 (calcd 625.5043) for the $[MH]^+$ corresponding to the molecular formula $C_{37}H_{68}O_7$. The IR spectrum showed a strong absorption at 1754 cm^{-1} for a γ-lactone carbonyl and 1717 cm^{-1} for a ketone carbonyl. Compound **2** was transparent under UV light at 225 nm suggesting that the lactone ring is not α,β-unsaturated. In comparison with (2,4-*cis* and *trans*)-isoannonacin[30] and (2,4-*cis* and *trans*)-gigantetrocinone,[22] the ^1H and ^{13}C NMR spectra of **2** clearly indicated the presence of a ketolactone moiety. In the ^1H NMR spectrum of **2** (Table 2), the resonances at δ 4.40 and 4.55, 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.[31,32] In the ^{13}C NMR (Table 2), signal pairs at δ 178.33 and 178.85, 43.77 and 44.23, 79.32 and 78.87, 205.67 and 205.61 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, H-3a, H-3b, H-5a/b, H-35a, and H-35b were based on the analysis of the COSY spectrum of **2**.

The remaining part of the structure of **2** exhibited identical ^1H and ^{13}C NMR signals for a long aliphatic chain bearing a mono-THF ring and three OH groups. The existence of the three OH moieties was indicated by an IR hydroxyl absorption at 3433 cm^{-1}, three successive losses of H_2O (*m/z* 18) from the $[MH]^+$ in the CIMS, and the preparation of triacetate (**2a**) and tri-TMS derivatives (**2b**). Compound **2a** gave three singlet proton peaks at δ 2.04 (10-OAc), 2.07 (17-OAc), and 2.07 (22-OAc), and a multiplet at δ 4.85 (H-10, H-17, and H-22) corresponding to the downfield shifts of three protons on acetylated secondary OH-bearing carbons. Furthermore, the ^{13}C NMR of **2** showed three resonances due to oxygen-bearing carbons at δ 71.86, 74.00, and 74.06, indicating the existence of three secondary hydroxyls. The presence of a mono-THF ring with two OH groups flanking the ring, was suggested by proton resonances at δ 3.40 (H-17), 3.80 (H-18), 3.80 (H-21), and 3.40 (H-22), and the carbon peaks at δ 82.64 (C-18) and 82.57 (C-21); these directly matched similar peaks of other mono-THF acetogenins with two flanking OH groups such as annomontacin (**3**) and corossoline.[23,24]

The carbon skeleton and placement of the ring and three OH groups along the hydrocarbon chain were determined based on the EIMS spectral analysis of **2** (Fig. 3) and by comparison to the EIMS spectral data of annomontacin (**3**) (the parent compound) and 4-deoxyannomontacin (**1**).

The relative stereochemistries at C-17/C-18 and C-21/C-22 of **2** were determined to be *threo*, and the stereochemistry of the THF ring was determined as *trans* by comparing ^1H and ^{13}C NMR data to those of the Harmange et al.[25] and Fujimoto et al.[26] models of known mono-THF relative stereochemistries. The absolute stereochemistries of the carbinol centers of compound **2** were elucidated as C-10*S*, C-17*R*, C-18*R*, C-21*R*, and C-22*R* by using the advanced Mosher ester methodology (Table 2). While C-10 in compound **2** has a different absolute stereochemical assignment than that of compound **1**, due to the change in the priorities around C-10, it still has the same spatial orientation as that of compounds **1** and **3**. It is worth noting that compounds **1** and **2** have the same absolute stereochemistry as the parent compound, annomontacin (**3**), and this observation supports a common biogenetic origin of these compounds.

Table 2. ^{13}C NMR and ^{1}H NMR (δ, J in Hz) of **2** and its *S*- and *R*-Mosher esters

	^{13}C (125 MHz) 2		^{1}H (500 MHz)				$\Delta\delta_H$
	cis	*trans*	*cis*	*trans*	*S*-MPTA	*R*-MPTA	$\delta_S-\delta_R$
1	178.33	178.85	–	–	–	–	
2	43.77	44.23	3.04 m	3.00 m	3.04, 3.00	3.04, 3.00	
3a	35.29,[a] 35.52[a]		2.60 ddd (12.3, 9.4, 5.6)	2.23 ddd (12.9, 9.6, 3.4)	2.59	2.59	
3b			1.48 m	1.99 m	1.48	1.46	+0.02
4	79.32	78.87	4.39 ddt (10.7, 7.4, 5.4)	4.55 ddt (5.7, 3.2, 8.2)	4.38 *cis* 4.53 *trans*	4.36 *cis* 4.51 *trans*	+0.02 +0.02
5a/b	34.42, 36.67[a]		1.61–1.76 m	1.57–1.71 m	1.60, 1.71	1.59, 1.64	+0.01, +0.01
6–8	25.19–31.89		1.25–1.70 m		1.25–1.70	1.25–1.70	
9	37.45[a]		1.42 m		1.51–1.65	1.49–1.70	
10	71.86		3.58 m		5.06	5.03	C-10*S*
11	37.24[a]		1.42 m		1.51–1.65	1.49–1.70	
12–15	25.19–31.89		1.25–1.70 m		1.25–1.70	1.25–1.70	
16	33.45		1.42 m		1.57	1.54	+0.03
17, 22	74.00[a], 74.06[a]		3.40 q (5)		3.92	4.00	17*R*, 22*R*
18, 21	82.64[a], 82.57[a]		3.80 q (7.5)		3.92	4.02	–0.10
19a/b	28.72		1.69 m, 1.98 m		1.39, 1.66	1.59, 1.93	–0.20, –0.27
20a/b	28.72		1.69 m, 1.98 m		1.39, 1.66	1.59, 1.93	–0.20, –0.27
23	33.45		1.42 m		1.57	1.54	+0.03
24–33	25.19–31.89		1.25–1.70 m		1.25–1.70	1.25–1.70	
34	14.08		0.88 t (7)		0.88	0.88	
35a	36.67		3.11 dd (18.5, 3.0)	3.05 dd (10.2, 3.4)	3.11, 3.05	3.11, 3.05	
35b	36.67		2.64 dd (15.3, 8.6)	2.56 dd (19.5, 10.6)	2.64, 2.56	2.64, 2.56	
36	205.67	205.61	–	–	–	–	
37	22.66		2.20 s		2.20	2.20	

[a]Signals may be interchangeable.

Duret et al.[32] have experimentally demonstrated that the ketolactone annonaceous acetogenins are easily derived from the 4-OH-α,β-unsaturated γ-lactone acetogenins through translactonization. It is yet to be proven if this reaction is enzymatic, taking place in the plant cell, or an artifact of isolation and purification. Two possible mechanisms were proposed for this reaction via a cyclic orthoester using mild base; the first involves the hydrogen atom in the γ-position of the lactone and the second involves the hydrogen of the C-4 hydroxyl. In both mechanisms, the absolute configuration at C-4 is conserved after the reaction, and, since all C-4 hydroxyl acetogenins found, so far, have the *R* stereochemistry at C-4 including annomontacin (**3**) the parent compound of **2**, the *R* configuration has been assigned for C-4 in **2**. Consequently, the structure of **2** is proposed as illustrated, and it was named (2,4-*cis* and *trans*)-annomontacinone, honoring the parent acetogenin, annomontacin (**3**).[23]

The biological activities of **1–3** are summarized in Table 3. These compounds were all active in the BST; they also showed significant cytotoxicities against the human tumor cell lines in our seven-day MTT human solid-

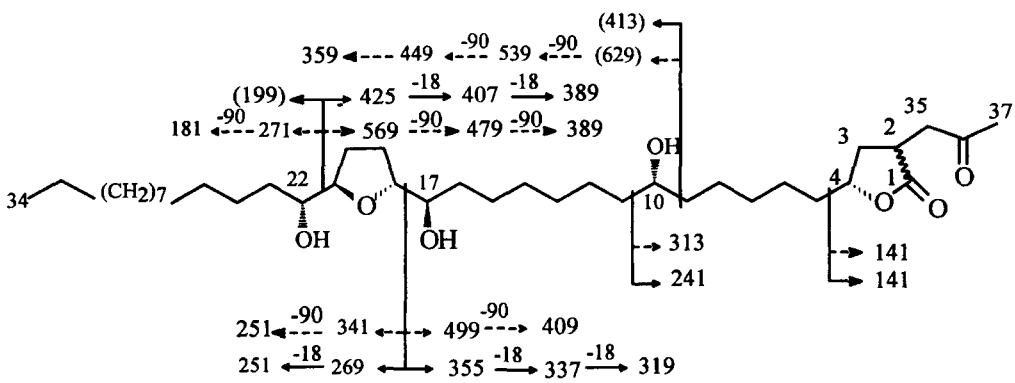

Figure 3. Diagnostic mass fragmentation ions of **2** and **2b**. EIMS of **2** (solid line); losses of H_2O indicated by 18 *m/z*. EIMS of **2b** (dashed line); losses of TMSiOH indicates by 90 *m/z*.

Table 3. Bioactivity of **1**, **2**, and **3**

Compounds		1	2	3[23b]	Adriamycin[i]	Rotenone[j]
BST[a] LC_{50} (μg/mL)		0.13	0.31	13	–	4.9×10^{-2}
YFM[b] LC_{50} (μg/mL)		1.30	1.00	–	–	1.2
Human	A-549[c]	6.45×10^{-7}	2.60	1.3×10^{-7}	4.40×10^{-3}	–
Tumor	MCF-7[d]	5.77×10^{-7}	3.21	2.3×10^{-8}	5.94×10^{-2}	–
Cell	HT-29[e]	1.41×10^{-1}	2.55×10^{-1}	3.4×10^{-2}	2.91×10^{-2}	–
Lines	A-498[f]	1.50×10^{-1}	1.44	–	7.20×10^{-3}	–
ED_{50}	PC-3[g]	1.73×10^{-1}	1.01	–	3.70×10^{-2}	–
(μg/mL)	PACA-2[h]	1.00×10^{-5}	6.78×10^{-1}	–	2.33×10^{-2}	–

[a]Brine shrimp lethality test.[8a,b]
[b]Yellow fever mosquito larva test.[33]
[c]Human lung carcinoma.[34a]
[d]Human breast carcinoma.[34b]
[e]Human colon adenocarcinoma.[34c]
[f]Human kidney carcinoma.[34a]
[g]Human prostate adenocarcinoma.[34d]
[h]Human pancreatic carcinoma.[34e]
[i,j]Positive control standard.

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. The relatively high cytotoxicity of **3**, in comparison to **1** and **2**, is probably due to the presence of the 4-OH group. Selectivity in **1** was exhibited for the human lung carcinoma (A-549),[34a] human breast carcinoma (MCF-7),[34b] and human pancreatic carcinoma (PACA-2).[34e] The activity of **1** against MCF-7 is over 100,000 times that of adriamycin, against A-549 over 10,000 times that of adriamycin, and against PACA-2 1000 times that of adriamycin. Compound **2** showed less potent activities than **1**; however, it exhibited comparable activity with that of adriamycin against the human colon adenocarcinoma (HT-29) cells.[34c] Compounds **1** and **2** showed potent activities in the yellow fever mosquito larvae microtiter (YFM) assay[33] comparable with that of rotenone. Generally, the ketolactone acetogenins are less active than the α,β-unsaturated γ-lactone acetogenins; however, they may have the advantage of being antitumor compounds with a wider therapeutic index than their corresponding 4-OH parent acetogenin.[32] All of the acetogenins tested, so far, decrease oxygen uptake in mitochondrial tests.[35] These results indicate that they act, at least in part, as potent inhibitors of ATP production via blocking at complex **I** in mitochondria.[36] In addition, they act as potent inhibitors of the ubiquinone-linked plasma membrane NADH oxidase of cancerous cells; this action decreases cytosolic ATP production.[37] The consequence of such ATP deprivation is apoptosis (programmed cell death).[38] The acetogenins also inhibit cells that are multiple drug resistant and offer an excellent potential for development as new antitumor agents.[39]

Experimental

Instumentation

Optical rotations were determined on a Perkin 241 polarimeter. IR spectra (film) were measured on a Perkin Elmer 1600 FTIR spectrometer. UV spectra were taken in MeOH on a Beckman DU-7 UV spectrophotometer. CD spectra were recorded on a JASCO Model J600 Circular Dichroism spectrometer. ^1H NMR, ^1H-^1H COSY, and ^{13}C NMR spectra were obtained on a Varian VXR-500S spectrometer. Low-resolution MS data were collected on a Finnigan 4000 spectrometer. Low resolution EIMS for TMS derivatives and high resolution CIMS were performed on a Kratos MS50. HPLC separations were performed with a Rainin Dynamax solvent delivery system (model SD-200) using a Dynamax software system and a silica-gel column (Dynamax 60-A 250 21 mm) equipped with a Dynamax absorbance detector (model UV-1) set at 225 nm. Analytical TLC was carried out on silica gel plates (0.25 mm), developed with $CHCl_3$–MeOH (20:1) and visualized with 5% phosphomolybdic acid in EtOH.

Bioassays

The bioactivities of extracts, fractions, and pure compounds were routinely assayed using a test for lethality to brine shrimp larvae (BST).[8a,b] The yellow fever mosquito larvae microtiter plate (YFM) assay[33] was used to determine the relative pesticidal activities of compounds **1** and **2**; rotenone was used as the positive pesticidal control standard. In vitro cytotoxicities, against human tumor cell lines, were carried out at the Purdue Cancer Center, Cell Culture Laboratory, using standard seven-day MTT assays for A-549 (human lung carcinoma),[34a] MCF-7 (human breast carcinoma),[34b] HT-29 (human colon adenocarcinoma),[34c] A-498 (human kidney carcinoma),[34a] PC-3 (human prostate adenocarcinoma),[34d] and PACA-2 (human pancreatic carcinoma).[34e] Adriamycin is always used as a positive antitumor control in the same runs.

Plant material

The stem bark of *Goniothalamus giganteus* (B-826538, PR-50604) was collected in Thailand in September 1978

under the auspices of Dr Robert E. Perdue, Medicinal Plant Laboratory, USDA, Beltsville, MD, where voucher specimens are maintained.

Extraction and isolation

The stem bark (10.7 kg) was ground into a powder and percolated with 95% ethanol. The dry extract (900 g) (F001) was partitioned between H_2O and CH_2Cl_2 to give a H_2O layer (F002) and a CH_2Cl_2 layer. The residue of the CH_2Cl_2 layer (430 g) (F003) was partitioned between 90% MeOH and hexane, giving a MeOH layer (400 g) (F005) and a hexane layer (30 g) (F006). The MeOH layer (F005) was the most active fraction in the BST (LC_{50} 1.02 μg/mL). Thus, a portion (190 g) of F005 was repeatedly chromatographed over open silica-gel columns directed by the BST test, using gradients of hexane–acetone, hexane–EtOAc and $CHCl_3$–MeOH, and purified by normal phase HPLC eluted with 10% THF in MeOH–hexane (4–6%) to give the colorless waxy compounds **1** and **2**. The known compounds [xylomaticin, longifolicin, longicoricin, (2,4-*cis* and *trans*)-gigantetrocinone and (2,4-*cis* and *trans*)-gigantetroneninone] were isolated as colorless waxes, and identified by 1-D NMR and MS data as compared to literature values.[2–5]

Preparation of Mosher esters

To an acetogenin (0.5–1 mg, in 0.5 ml of CH_2Cl_2), were sequentially added pyridine (0.1 mL), 4-(dimethylamino)pyridine (0.1 mg), and 15 mg of (*R*)-( )-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride. The mixture was stirred at rt from 4 h to overnight, checked with TLC to make sure that the reaction was complete, and passed through a disposable pipet (0.6 4 cm) containing silica gel (60–200 mesh) and eluted with 3 mL CH_2Cl_2. The CH_2Cl_2 residue, dried in vacuo, was redissolved in 1% $NaHCO_3$ (5 mL) and H_2O (2 5 mL); the CH_2Cl_2 layer was dried in vacuo to give the (*S*)-Mosher esters. Using (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride gave the (*R*)-Mosher esters. Both yields were typically higher than 90%. ^1H NMR chemical shifts of **1c** and **2c** are given in Tables 1 and 2.

Preparation of TMS derivatives

Compounds **1** and **2** (ca. 0.3 mg of each) were treated with *N,O*-bis(trimethylsilyl) acetamide (20 μL) and pyridine (2 μL) and heated at 70° C for 30 min to yield the respective tri-TMS derivatives, **1b** and **2b**. EIMS fragmentations are shown in Figures 2 and 3.

Preparation of acetylated derivatives

One to two milligrams of pure acetogenin, **1** and **2**, was dissolved in 0.5–1.0 mL 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 derivatives, **1a** and **2a**.

4-Deoxyannomontacin (1). A whitish wax (20 mg); $[\alpha]_D^{25}$ +10.9 (*c* 0.060, $CHCl_3$); UV(MeOH) λ_{max} 228 nm (log 2.86); IR (film on NaCl plate) 3443, 2921, 2850, 2360, 1742, 1455, 1374, 1318,1191, 1068, 1028 cm $^{-1}$; CIMS(isobutane) *m/z* (%) $[MH]^+$ 609 (78), $[MH\text{-}H_2O]^+$ 592 (35), $[MH\text{-}2H_2O]^+$ 574 (21), $[MH\text{-}3H_2O]^+$ 556 (5.9); HRCIMS (isobutane) *m/z* 609.5069 for $C_{37}H_{68}O_6$ $[MH]^+$ (calcd 609.5094); EIMS see Figure 2; ^1H and ^{13}C NMR: see Table 1.

(2,4-*cis* and *trans*)-Annomontacinone (2). A whitish wax (4 mg) ; $[\alpha]_D^{25}$ 13.6 (*c* 0.022, $CHCl_3$); IR (film on NaCl plate) 3433, 2920, 2850, 1754, 1717, 1550, 1460, 1182, 1075, 727; CIMS (isobutane) *m/z* (%) $[MH]^+$ 625 (100), $[MH\text{-}H_2O]^+$ 607 (32), $[MH\text{-}2H_2O]^+$ 589 (27), $[MH\text{-}3H_2O]^+$ 571 (3); HRCIMS (isobutane) *m/z* 625.5061 for $C_{37}H_{68}O_7$ $[MH]^+$ (calcd 625.5043); EIMS see Figure 3; ^1H and ^{13}C NMR: see Table 2.

Acknowledgments

This investigation was supported by RO1 grant no. CA 30909 from the National Cancer Institute, National Institute of Health. Stipend support for Feras Alali was provided by a fellowship from the Jordan University of Science and Technology, Irbid, Jordan. Thanks are due to the Purdue Cell Culture Laboratory, Purdue Cancer Center, for the cytotoxicity testing. The authors thank Barbara Moreno for the help in conducting the YFM assay.

References

1. Jolad, S. D.; Hoffmann, J. J.; Schram, K. H.; Tempesta, M. S.; Kriek, G. R.; Bates, R. B.; Cole, J. R. *J. Org. Chem.* **1982**, *47*, 3151.

2. Rupprecht, J. K.; Hui, Y.-H.; McLaughlin, J. L. *J. Nat. Prod.* **1990**, *53*, 237.

3. Fang, X.-P.; Rieser, M. J.; Gu, Z.-M.; Zhao, G.-X.; McLaughlin, J. L. *Phytochem. Anal.* **1993**, *4*, 27.

4. Gu, Z.-M.; Zhao, G. X.; Oberlies, N. H.; Zeng, L.; McLaughlin, J. L. In *Recent Advances in Phytochemistry*; Arnason, J. T.; Mata. R.; Romeo, J. T., Eds.; Plenum: New York, 1995; Vol 29, pp 249–310.

5. Zeng, L.; Ye, Q.; Oberlies, N. H.; Shi, G.; Gu, Z.-M.; He, K.; Mclaughlin, J. L. *Nat. Prod. Rep.* **1996**, *13*, 275.

6. Cave, A. In *Phytochemistry of Plants Used in Traditional Medicine*; Hostettmann, K.; Marston, A.; Maillard, M.; Hamburger, M., Eds.; Clarendon: Oxford, 1995; pp 227–248.

7. Perry, L. M.; Metzger, J. *Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses*; MIT: Cambridge, MA, 1980; pp 18–21.

8. (a) Meyer, B. N.; Ferrigni, N. R.; Putnam, J. E.; Jacobson, L. B.; Nichols, D. E.; McLaughlin, J. L. *Planta Med.* **1982**, *45*,

31. (b) McLaughlin, J. L. In *Methods in Plant Biochemistry*; Hostettmann, K., Ed.; Academic: London, 1991; Vol 6, pp 1–33. (c) Geran, R. I.; Greenburg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 1.

9. Alkofahi, A.; Rupprecht, J. K.; Liu, Y.-M.; Chang, C.-J.; Smith, D. L.; McLaughlin, J. L. *Experientia* **1990**, *46*, 539.

10. Alkofahi, A.; Rupprecht, J. K.; Smith, D. L.; Chang, C.-J.; McLaughlin, J. L. *Experientia* **1988**, *44*, 83.

11. Fang, X.-P.; Anderson, J. E.; Smith, D. L.; McLaughlin, J. L.; Wood, K. V. *J. Nat. Prod.* **1992**, *55*, 1655.

12. Fang, X.-P.; Anderson, J. E.; Smith, D. L.; Wood, K. V.; McLaughlin, J. L. *Heterocycles* **1991**, *34*, 1075.

13. Fang, X.-P.; Song, R.; Gu, Z.-M.; Rieser, M. J.; Miesbauer, L. R.; Smith, D. L.; McLaughlin, J. L. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1153.

14. Fang, X.-P.; Rupprecht, J. K.; Alkofahi, A.; Hui, Y.-H.; Liu, Y.-M.; Smith, D. L.; Wood, K. V.; McLaughlin, J. L. *Heterocycles* **1991**, *32*, 11.

15. Gu, Z.-M.; Fang, X.-P.; Zeng, L.; McLaughlin, J. L. *Tetrahedron Lett.* **1994**, *35*, 5367.

16. Gu, Z.-M.; Fang, X.-P.; Zeng, L.; Song, R.; Ng, J. H.; Wood, K. V.; Smith, D. L.; McLaughlin, J. L. *J. Org. Chem.* **1994**, *59*, 3472.

17. Zhang, Y.; Zeng, L.; Woo, M.-H.; Gu, Z.-M.; Ye, Q.; Wu, F.-E.; McLaughlin, J. L. *Heterocycles* **1995**, *41*, 1743.

18. Zeng, L.; Zhang, Y.; Ye, Q.; Shi, G.-E.; He, K.; McLaughlin, J. L. *Bioorg. Med. Chem.* **1996**, *4*, 1271.

19. Zeng, L.; Zhang, Y.; McLaughlin, J. L. *Tetrahedron Lett.* **1996**, *37*, 5449.

20. Colman-Saizarbitoria, T.; Zambrano, J.; Ferrigni, N. R.; Gu, Z.-M.; Ng, J. H.; Smith, D. L.; McLaughlin, J. L. *J. Nat. Prod.* **1994**, *57*, 486.

21. Ye, Q.; Alfonso, D.; Evert, D.; McLaughlin, J. L. *Bioorg. Med. Chem.* **1996**, *4*, 537.

22. Zhao, G. X.; Rieser, M. J.; Hui, Y. H.; Miesbauer, L. R.; Smith, D. L.; McLaughlin, J. L. *Phytochemistry* **1993**, *33*, 1065.

23. (a) Jossang, A.; Dubaele, B.; Cave, A.; Bartoli, M. H.; Beriel, H. *J. Nat. Prod.* **1991**, *54*, 967. (b) Rieser, M. J. *Annonaceous Acetogenins from the Seeds of Annona muricata.* PhD Thesis, Purdue University, West Lafayette, 1993; pp 60–184.

24. Cortes, D.; Myint, S. H.; Laurens, A.; Hocquemiller, R.; Leboeuf, M.; Cave A. *Can. J. Chem.* **1991**, *69*, 8.

25. Harmange, J. C.; Figadere, B.; Cave, A. *Tetrahedron Lett.* **1992**, *33*, 5749.

26. Fujimoto, Y.; Murasaki, C.; Shimada, H.; Nishioka, S.; Kakinuma, K.; Singh, S.; Singh, M.; Gupta, Y. K.; Sahai, M. *Chem. Pharm. Bull.* **1994**, *42*, 1175.

27. Rieser, M. J.; Fang, X. P.; Anderson, J. E.; Miesbauer, L. R.; Smith, D. L.; McLaughlin, J. L. *Helv. Chim. Acta* **1994**, *77*, 882.

28. Rieser, M. J.; Hui, Y.-H.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, A.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *144*, 10203.

29. Sahai, M.; Singh, S.; Singh, M.; Gupta, Y. K.; Akashi, S.; Yuji, R.; Hirayama, K.; Asaki, H.; Araya, H.; Hara, N.; Eguchi, T.; Kakinuma, K.; Fujimoto, Y. *Chem. Pharm. Bull.* **1994**, *42*, 1163.

30. Xu, L.-Z.; Chang, C.-J.; Yu, J.-G.; Cassady, J. M. *J. Org. Chem.* **1989**, *54*, 5418.

31. Hui, Y. H.; Rupprecht, J. K.; Anderson, J. E.; Liu, Y. M.; Smith, D. L.; Chang, C. J.; McLaughlin, J. L. *J. Nat. Prod.* **1989**, *52*, 463.

32. Duret, P.; Laurens, A.; Hocquemiller, R.; Cortes, D.; Cave, A. *Heterocycles* **1994**, *39*, 741.

33. Anonymous, World Health Organization, Report Ser. **1970**, *443*, 66.

34. (a) Giard, D. J.; Aaronson, S. A.; Todaro, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Natl. Cancer Inst.* **1973** *51*, 1417. (b) Soule, H. D.; Vazquez, J.; Long, A.; Albert, S.; Brennan, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409. (c) Fogh, J.; Trempe, G. In *Human Tumor Cells*; Fogh, J., Ed.; Plenum: New York, 1975; p 115. (d) Kaighn, M. E.; Narayan, K. S.; Ohnuki, Y.; Lechner, J. F.; Jones, L. W. *Invest. Urol.* **1979**, *17*, 16. (e) Yunis, A. A.; Arimura, G. K.; Russin, D. *Int. J. Cancer* **1977**, *19*, 128.

35. Landolt, J. L.; Ahammadsahib, K. I.; Hollingworth, R. M.; Barr, R.; Crane, F. L.; Buerck, N. L.; McCabe, G. P.; McLaughlin, J. L. *Chemico-Biol. Interact.* **1995**, *98*, 1.

36. Londershausen, M.; Leicht, W.; Lieb, F.; Moeschler, H.; Weiss, H. *Pestic. Sci.* **1991**, *33*, 427.

37. Morre, J. D.; Decabo, R.; Farley, C.; Oberlies, N. H.; McLaughlin, J. L. *Life Sci.* **1995**, *56*, 343.

38. Wolvetang, E. J.; Johnson, K. L.; Krauer, K.; Ralph, S. J.; Linnane, A. W. *FEBS Lett.* **1994**, *339*, 40.

39. Oberlies, N. H.; Croy, V. L.; Harrison, M. L..; McLaughlin, J. L. *Cancer Lett.*. (accepted).

(*Received in U.S.A.* 18 *September* 1996; *accepted* 29 *October* 1996)