Full Papers

Five Novel Mono-tetrahydrofuran Ring Acetogenins from the Seeds of *Annona muricata*

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Bioactivity-directed fractionation of the seeds of *Annona muricata* L. (Annonaceae) resulted in the isolation of five new compounds: *cis*-annonacin (**1**), *cis*-annonacin-10-one (**2**), *cis*-goniothalamicin (**3**), arianacin (**4**), and javoricin (**5**). Three of these (**1**−**3**) are among the first *cis* mono-tetrahydrofuran ring acetogenins to be reported. NMR analyses of published model synthetic compounds, prepared cyclized formal acetals, and prepared Mosher ester derivatives permitted the determinations of absolute stereochemistries. Bioassays of the pure compounds, in the brine shrimp test, for the inhibition of crown gall tumors, and in a panel of human solid tumor cell lines for cytotoxicity, evaluated relative potencies. Compound **1** was selectively cytotoxic to colon adenocarcinoma cells (HT-29) in which it was 10 000 times the potency of adriamycin.

This paper presents the isolation and structural elucidation of five new cytotoxic Annonaceous acetogenins from the seeds of *Annona muricata* L. (Annonaceae); these are components of a potent extract that offers a special potential for use as a new, biodegradable pesticide. *A. muricata*, also known as "sour sop" or "guanabana," is a popular table fruit cultivated throughout the tropical regions of the world.[1] This fruit also forms the basis of a well-developed juice industry in both Latin America and the countries of tropical Asia. By weight, the seeds of *A. muricata* make up from 3 to 7% of the fresh fruit,[2, 3] and thousands of tons of the fruit are processed each year. For example, in the Philippines alone, 500 000 trees of fruit-bearing age produce an estimated 8500 tons of fruit annually.[4] The large biomass of seeds that results from this commercial activity is presently being discarded because these seeds are toxic and, therefore, are unsuitable for animal feed or as a source for vegetable oil. Thus, the pursuit of the active components from the seeds was undertaken both because of their bioactivity and because the widespread availability of the cultivated plant material makes it a commercially viable resource, circumventing a major drawback to the commercialization of natural products from higher plants found only in the wild.

The toxicity of all crude extracts, major pools, and pure compounds used in the bioactivity directed isolation of the active principles from the extracted seed material was evaluated by the brine shrimp lethality test (BST)[5,6] as the directing assay. Initial screening in the BST of the defatted seeds of *A. muricata* showed the ethanolic extracts to be highly active. Our bioactivity-directed fractionation of this plant material previously led to the isolation of seven known mono-

tetrahydrofuran (THF) ring acetogenins, including the annonacin series,[7−9] the muricatetrocins and gigantetrocins,[10] and muricatacin,[11] a possible oxidation product derived from the annonacin series which provided insights into the absolute stereochemistry of these compounds. Cavé and co-workers previously reported the isolation, structure determination, and biological activities[12] of four new mono-THF ring acetogenins, murisolin,[13] corossolin, corossolone,[14] and solamin,[15] from the seeds. This paper presents five new diastereomeric mono-THF acetogenins, *cis*-annonacin (**1**) and *cis*-annonacin-10-one (**2**), which are structurally related to the known compounds of the annonacin series, and *cis*-goniothalamicin (**3**), arianacin (**4**), and javoricin (**5**), which are structural isomers of goniothalamicin (**6**).[16]
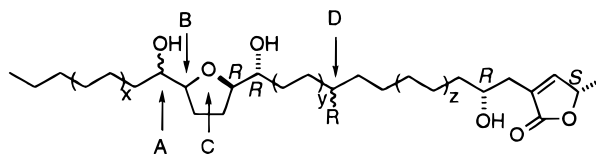

	A	B	C	D	x	y	z	R
1	S	S	cis	R	4	2	1	OH
2	S	S	cis	-	4	2	1	=O
3	S	S	cis	R	5	1	1	OH
4	R	R	trans	S	4	1	2	OH
5	R	R	trans	R	4	1	2	OH
6	R	R	trans	R	5	1	1	OH

Results and Discussion

The IR, UV, NMR, and MS of the known compound goniothalamicin (**6**),[16] as well as *cis*-annonacin (**1**), *cis*-annonacin-10-one (**2**), *cis*-goniothalamicin (**3**), arianacin (**4**), and javoricin (**5**), all showed the following features indicative of α,β-unsaturated γ-lactone acetogenins with
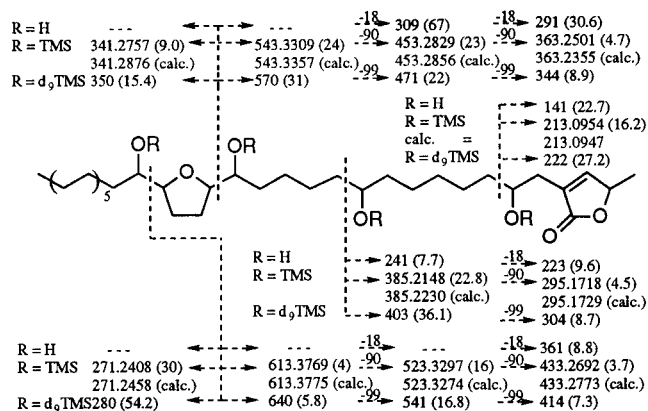
M+H$^+$ hr-fabms 597.4730 found, calculated 597.4730

Figure 1. All diagnostic EIMS fragment ions, in m/z, of **1**, with percent intensities reported in parentheses. Losses of m/z 90 indicate the loss of TMSiOH neutrals.
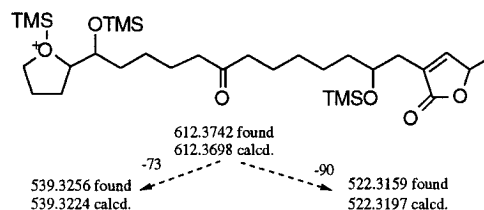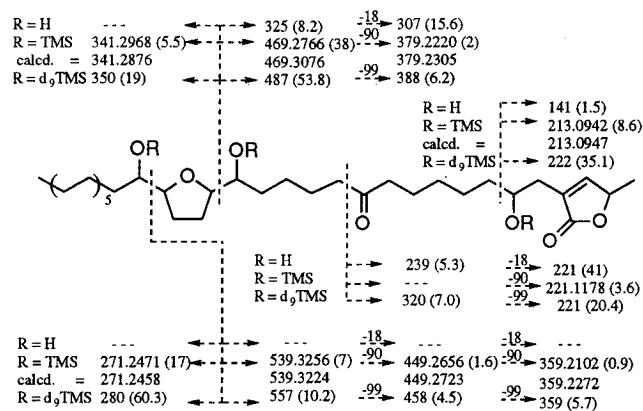

Figure 2. All diagnostic EIMS fragment ions, in m/z, of **2**, with percent intensities reported in parentheses. Losses of m/z 90 indicate the loss of TMSiOH neutrals.

a C-4 hydroxyl: FTIR absorption at ~3400 (OH stretch), 1750 cm^{-1} (lactone C=O), and a strong aliphatic C−H absorption below 3000 cm^{-1}; an absorption maximum (λ max) in the UV spectrum at ~215 ± 3 nm for each compound; characteristic ^{1}H and ^{13}C NMR resonances for H-3/C-3, H-4/C-4, H-33/C-33, H-34/C34, H-35/C-35, C-1 and C-2;[17,18] and strong absorption in the aliphatic region of the ^{1}H NMR, as well as alcohol and ether C−H methine resonances in the region δ 3.40−3.81. HRMS fragment peaks of the TMSi derivatives were within the acceptable mass range to confirm the presence of the α,β-unsaturated γ-lactones (m/z 213.0947) in all six compounds.[19]

The molecular weight of each compound was determined by HRFABMS; compounds **1** and **3−6** each gave an MH$^+$ within ±3 mmu of 597.4730 (calcd), corresponding to the formula $C_{35}H_{64}O_7$ + H$^+$, while **2** produced a lower molecular weight MH$^+$ at m/z 577.4453. The lower molecular weight produced by **2** is attributable to the loss of H$_2$O from the protonated parent compound and corresponded to $C_{35}H_{61}O_6$ ([M + H]$^+$ − H$_2$O) (577.4468 calcd). The carbon skeletons of

1 and **2** were determined by EIMS of their TMSi derivatives, and assignments of the peak fragments are presented in Figures 1 and 2, respectively.

With the contiguous carbon skeletons of **1** and **2** determined, the elucidation of the relative and absolute stereochemistries remained to be ascertained. The relative configuration was determined by a straightforward comparison of the NMR shifts of the naturally occurring compounds with those of six synthetic model mono-THF diol analogues.[20] Comparison of the ^{1}H NMR signals for the ring methylenes at δ 1.94 and 1.76 and the slight downfield shift of the ether methylene protons to δ 3.82 (Table 1) indicated that the rings in both **1** and **2** bore the *threo-cis-threo* configuration. The hydroxyl moieties of the *threo-cis-threo* ring should both

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Resonances (δ) for **1**, **1a** and **2**

position	*cis*-annonacin (**1**) δ ^{1}H (J in Hz)	δ ^{13}C	**1a** δ ^{1}H (J in Hz)	*cis*-annonacin-10-one (**2**) δ ^{1}H (J in Hz)	δ ^{13}C
1		174.6			174.6
2		131.0			131.0
3a	2.38, ^{1}H, ddt (15.1, 8, 1.4 Hz)	33.4	2.38, 1H, ddt (15.1, 8, 1.4 Hz)	2.38, 1H, ddt (15.1, 8, 1.4 Hz)	33.4
3b	2.51, 1H, dddd (15.1, 4.0, 1.4 Hz)		2.51, 1H, dddd (15.1, 4.0, 1.4 Hz)	2.51, 1H, dddd (15.1, 4.0, 1.4 Hz)	
4	3.82, 1H, m	69.8	3.86, 1H, m	3.82, 1H, m	69.8
5−8	1.1−1.6, m	22−38	1.1−1.6, m	1.1−1.6, m	22−38
9	1.1−1.6, 2H, m	22−38	1.1−1.6, 2H, m	2.42, 2H, t (J = 7.2)	42.5^a
10	3.59, 1H, m	71.6	3.53, 1H, m		211.3
11	1.1−1.6, 2H, m	22−38	1.1−1.6, 2H, m	2.41, 2H, t (J = 7.2)	42.6^a
12−14	1.1−1.6, 6H, m	22−38	1.1−1.6, 6H, m	1.1−1.6, 6H, m	22−38
15	3.42, 1H, m	74.3	3.40, 1H, m	3.42, 1H, m	74.3
16	3.82, 1H, m	82.7	3.79, 1H, m	3.82, 1H, m	82.7
17a,b	1.94, 1H, m	28.2	2.00, 1H, m	1.94, 1H, m	28.2
	1.76, 1H, m		1.69, 1H, m	1.76, 1H, m	
18a,b	1.94, 1H, m	28.2	2.00, 1H, m	1.94, 1H, m	28.2
	1.76, 1H, m		1.69, 1H, m	1.76, 1H, m	
19	3.82, 1H, m	82.7	3.79, 1H, m	3.82, 1H,m	82.7
20	3.42, 1H, m	74.3	3.40, H, 1H, m	3.42, 1H, m	74.3
21−31	1.1−1.6, 22H, m	22−38	1.1−1.6, 22H, m	1.1−1.6, 22H, m	22−38
32	0.88, 3H, t (J = 6.7)	14.1	0.88, 3H, t (J = 6.7)	0.88, 3H, t (J = 6.7)	14.1
33	7.18, 1H, q (J = 1.4)	151.8	7.18, 1H, q (J = 1.4)	7.18, 1H, q (J = 1.4)	151.8
34	5.01, 1H, qq, 6.4, 1.4	78.0	5.01, 1H, qq, 6.4, 1.4	5.01, 1H, qq, 6.4, 1.4	78.0
35	1.38, 3H, d, (J = 6.8)	19.2	1.38, 3H, d, (J = 6.8)	1.38, 3H, d, (J = 6.8)	19.2
36			4.71, 2H, dd (J =16.9, 6.9)		

Table 2. ¹H-NMR Shifts (δ) for Individual Mono-MTPA Esters of *cis*-Annonacin (**1**) Referenced to TMS

MTPA deriv **1**, 4-OH MTPA	MTPA config	proton chemical shifts ($\Delta\delta = \delta_S - \delta_R$)							carbinol config at C-4
		H(15) δ	H(10) δ	H(4) δ	H(3$_{a,b}$) δ	H(33) δ	H(34) δ	H(35) δ	
	S	3.43	3.59	5.33	2.59, 258	6.73	4.87	1.29	
	R	3.43	3.57	5.3	2.68, 2.60	6.97	4.91	1.32	R
	Δδ	(0)	+(0.02)		−(0.09, 0.02)	−(0.24)	−(0.04)	−(0.03)	

1, 10-OH MTPA	MTPA config	proton chemical shifts ($\Delta\delta = \delta_S - \delta_R$)							config at C-10
		H(18$_{a,b}$) δ	H(17$_{a,b}$) δ	H(16) δ	H(15) δ	H(10) δ	H(4) δ	H(3$_{a,b}$) δ	
	S	1.94, 1.74	1.94, 1.74	3.79	3.36	5.08	3.84	2.53, 2.40	
	R	1.95, 1.75	1.95, 1.75	3.81	3.40	5.07	3.82	2.52, 2.39	R
	Δδ	−(0.01, 0.01)	−(0.01, 0.01)	−(0.02	−(0.04)		+(0.02)	+(0.01, 0.01)	

1, 15-OH MTPA	MTPA config	proton chemical shifts ($\Delta\delta = \delta_S - \delta_R$)							config at C-15
		H(20) δ	H(19) δ	(H18$_{a,b}$)^a δ	H(17$_{a,b}$)^a δ	H(16) δ	H(14)^a δ	H(10) δ	
	S	3.30	3.75	1.82, 1.65	1.65, 1.92	4.00	1.65	3.59	
	R	3.35	3.85	1.92, 1.71	1.92, 2.01	4.01	1.60	3.50	R
	Δδ	−(0.05)	−(0.10)	−(0.10, 0.06)	−(0.27, 0.09)	−(0.01)	+(0.05)	+(0.09)	

1, 20-OH MTPA	MTPA config	proton chemical shifts ($\Delta\delta = \delta_S - \delta_R$)							config at C-20
		H(20) δ	H(19) δ	H(18$_{a,b}$) δ	H(17$_{a,b}$) δ	H(16) δ	H(15) δ	H(10) δ	
	S	5.04	4.01	1.65, 1.92	1.82, 1.65	3.85	3.36	3.58	
	R	5.05	3.99	1.92, 2.01	1.92, 1.71	3.75	3.31	3.58	S
	Δδ		+(0.02)	+(0.27, 0.09)	+(0.10, 0.06)	+(0.10)	+(0.05)	0	

^{a} Determined by 2D COSY ¹H NMR.
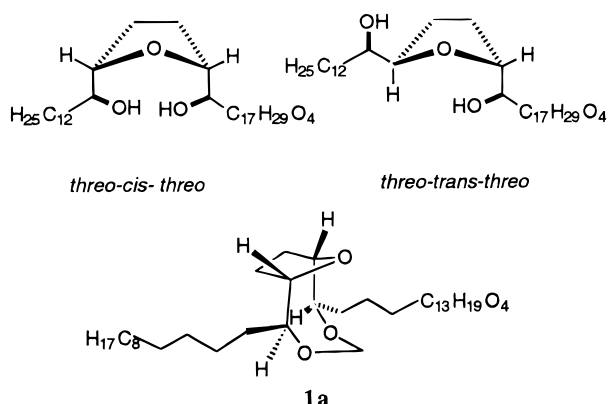
Figure 3. Spacial relationships between hydroxyl groups having *threo-cis-threo* and *threo-trans-threo* relative stereochemistries, with the structure of **1a** confirming the *threo-cis-threo* configuration of **1**.

reside on the same side of the plane defined by the THF ring (Figure 3), and the 16,19-*cis* assignment would be confirmed if these two hydroxyl groups could be bridged to form an acetal. The method of Gu *et al.* [21] for the

formation of the formal acetals of acetogenins from DMSO and TMSiCl was used. Both **1** and annonacin were subjected to this reaction; however, only **1** produced the expected adduct (**1a**), which was isolated in a 30% yield and confirmed the presence of a *cis* attachment of the ring (Figure 3) (Table 1).

The elucidation of the absolute configurations of the carbinol groups for **1** was determined by monoesterification with MTPA-Cl and NMR analysis using the method of Dale and Mosher as modified by Ohtani.[22–24] The compounds with mono-esters at C-4 and C-10 were assigned by their ¹H NMR spectra. Analysis of the esters indicated that both centers bore the *R* configuration (Table 2). Identification of the mono-MTPA ester at C-15 was straightforward due to the upfield (0.05 ppm) shift of the C-10 carbinol proton to δ 3.50 of the *R* ester. However, the *S* esters of C-15 and C-20 were virtually indistinguishable by ¹H NMR, so EIMS of the TMSi derivatives of these esters was used to distinguish between them before being analyzed by ¹H COSY NMR. The resonances and assignments of **1** are given in Table 2. The configuration of **2** was then assigned by com-

Table 3. ¹H NMR Chemical Shift (δ) Data for the Comparison of the Absolute Configuration About the Carbinol Centers of the *S* and *R* per-MTPA Ester Derivatives of Compounds **1** and **2**

MTPA deriv	MTPA config	proton chemical Shifts ($\Delta\delta = \delta_S - \delta_R$)						carbinol config at C-4
		H(5) δ	H(4) δ	H(3) δ	H(33) δ	H(34) δ	H(35) δ	
1	S	1.62	5.28	2.57, 2.52	6.69	4.83	1.26	
	R	1.60	5.34	2.65, 2.56	6.93	4.88	1.28	R
	Δδ	+(0.02)	a	−(0.08, 0.04)	−(0.24)	−(0.05)	−(0.02)	
2	S	1.62	5.28	2.57, 2.52	6.69	4.83	1.26	
	R	1.60	5.34	2.65, 2.56	6.93	4.88	1.28	R
	Δδ	+(0.02)	a	−(0.08, 0.04)	−(.24)	−(0.05)	−(0.02)	

MTPA deriv	MTPA config	H(21) δ	H(20) δ	H(19) δ	H(18$_{a,b}$) δ	H(17$_{a,b}$) δ	H(16) δ	H(15) δ	H(14) δ
1	S	1.30	5.06	3.86	(1.42, 0.82)	(1.81, 1.34)	4.06	4.87	1.59
	R	1.33	5.05	3.86	(1.46, 0.86)	(1.82, 1.37)	4.11	4.92	1.62
	Δδ	−(0.03)	a	−(0.01)	−(0.04, 0.04)	−(0.01, 0.03)	−(0.05)	−(0.05)	−(0.03)
2	S	1.30	5.07	3.86	(1.42, 0.84)	(1.82, 1.35)	4.09	4.91	1.69
	R	1.32	5/06	3.87	(1.45, 0.85)	(1.82, 1.36)	4.10	4.92	1.64
	Δδ	−(0.02)	a	−(0.01)	−(0.03, 0.01)	0	+(0.01)	a	+(0.05)

^{a} This is the position of the ester, therefore, no Δδ shift is given.

Table 4. [1]H- (500 MHz) and ^{13}C- (125 MHz) NMR Resonances (δ) for **3**−**5**

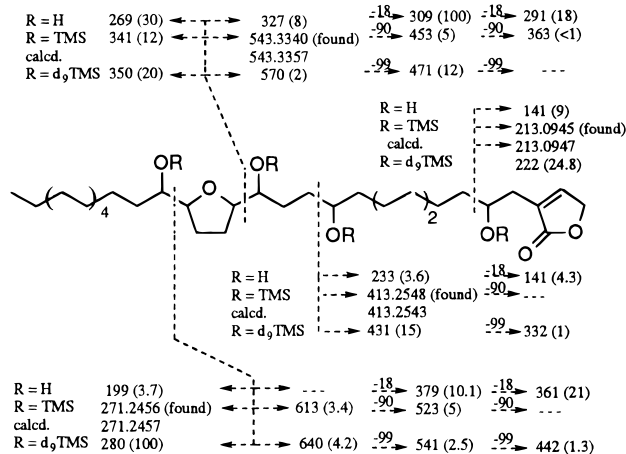| position	*cis*-goniothalamicin (**3**) [1]H	[13]C	arianacin (**4**) [1]H	[13]C	javoricin (**5**) and goniothalamicin (**6**) [1]H	[13]C
1		174.6		174.5		174.5
2		131.0		131.0		131.1
3a	2.38, 1H, ddt (15.1, 8, 1.6 Hz)	33.4	2.38, 1H, ddt (15.1, 8, 1.6 Hz)	33.4	2.38, 1H, ddt (15.1, 8, 1.6 Hz)	33.4
3b	2.51, 1H, ddd (15.1, 4.0, 1.6 Hz)		2.51, 1H, ddd (15.1, 4.0, 1.6 Hz)		2.51, 1H, ddd (15.1, 4.0, 1.6 Hz)	
4	3.82, 1H m	69.8	3.82, 1H m	69.9	3.82, 1H m	69.9
3 5−9 or **4, 5** 5−11	1.2−1.8, 10H, m	22−35	1.2−1.6, 10H, m	22−35	12.-1.6, 10H, m	22−35
3 10 or **4, 5** 12	3.65 1H, m	71.5	3.60	71.8	3.63	71.6
3 11, 12 or **4, 5** 13, 14	1.55−1.74, 4H, m	29.8−32.0	1.40−1.60, 4H, m	33.0−36.0	1.55−1.74, 4H, m	29.8−32.0
3 13 or **4, 5** 15	3.47, 1H, m	74.3	3.46, 1H, ddd (7.6, 7.6, 2.0 Hz)	74.4	3.46, 1H, ddd (8.0, 7.0, 3.0 Hz)	74.3
3 14 or **4, 5** 16	3.83, 2H, m	82.6	3.80, 1H, m	82.6	3.77−3.85, 1H, m	82.5
3 15,16a or **4, 5** 17,18a	1.76, 2H, m	26.1	1.67, 2H, m	28.2	1.67, 2H, m	28.2
3 15,16e or **4, 5** 17,18e	1.94, 2H, m	26.2	1.99, 2H, m	28.1	1.99, 2H, m	28.1
3 17 or **4, 5** 19	3.83, 1H, m	82.7	3.80, 1H, m	82.7	3.77−3.85, 1H m	82.7
3 18 or **4, 5** 20	3.41, 1H, dt (5.0, 6.7 Hz)	74.4	3.41, 1H, dt, 5.3, 6.4	74.1	3.41, 1H, dt, 5.3, 6.4	74.1
3 19−31 or **4, 5** 21−31	1.2−1.5, 26H, m	22.7−38	1.2−1.5, 26H, m	22.7−38	1.2−1.5, 26H, m	22.7−38
32	0.88, 3H, t (6.9 Hz)	14.2	0.88, 3H, t (6.9 Hz)	14.2	0.88, 3H, t (6.9 Hz)	14.2
33	7.17, 1H, q (1.6 Hz)	151.8	7.17, 1H, q (1.6 Hz)	151.8	7.17, 1H, q (1.6 Hz)	151.8
34	5.04, 1H, dq (6.8, 1.6 Hz)	78.0	5.04, 1H, dq (6.8, 1.6 Hz)	78.0	5.04, 1H, dq (6.8, 1.6 Hz)	78.0
35	1.42, 3H, d (6.8 Hz)	19.1	1.42, 3H, d (6.8 Hz)	19.1	1.42, 3H, d (6.8Hz)	19.1
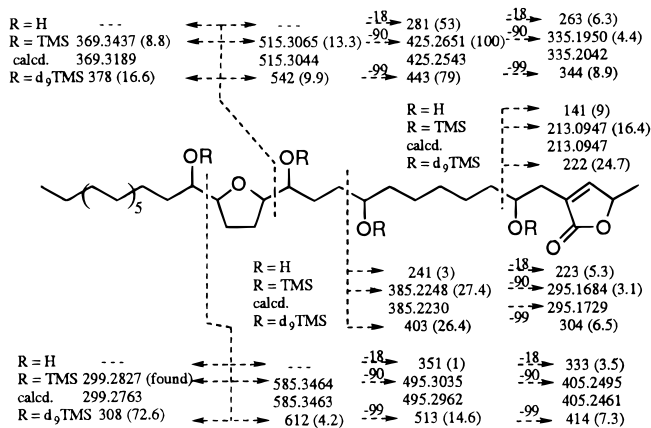
M+H^{+} hr-fabms 597.4731 found, calculated 597.4730

Figure 4. All diagnostic EIMS fragment ions, in *m/z*, of **3**, with percent intensities reported in parentheses. Losses of *m/z* 90 indicate the loss of TMSiOH neutrals.

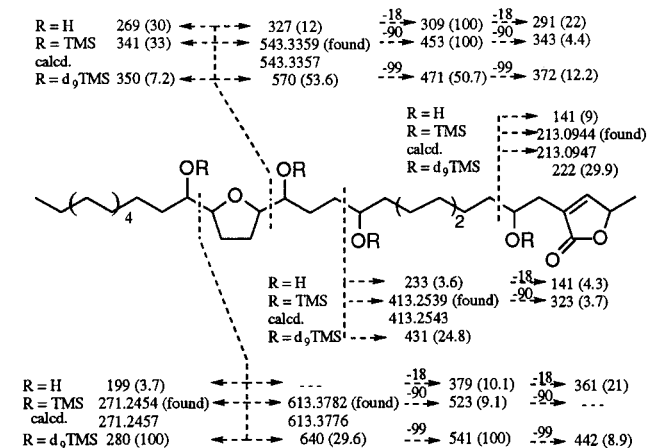M+H^{+} hr-fabms 597.4700 found, calculated 597.4730

Figure 5. All diagnostic EIMS fragment ions, in *m/z*, of **4**, with percent intensities reported in parentheses. Losses of *m/z* 90 indicate the loss of TMSiOH neutrals.

parison of its *per*-esters with those of **1** (Table 3). The absolute configurations of **1** and **2** are, thus, proposed as 15*R*, 16*R*, and 20*S* with **1** being 10*R*.

The four compounds, **3**-**6,** produced very similar ^{13}C and ^{1}H NMR spectra (Table 4). Compound **3**, isolated from impure **6**, was immediately recognizable as having the *threo-cis-threo* THF ring configuration by the diagnostic ^{1}H NMR resonances at δ 1.94 and 1.76, for the ether ring methylenes, and the ether methine resonances at δ 3.83. As they produced identical ^{13}C and ^{1}H NMR spectra (Table 4), the only indication that compounds **5** and **6** were discrete compounds was their different TLC mobilities (R_f = 0.37 in CH₂Cl₂:EtOAc: MeOH, 6:3:1, for **6** vs. 0.46 for **5**). Compound **4** shared the same R_f with **5** on TLC; however, its NMR spectra differed slightly from those of both **5** and **6**. EIMS of the TMSi derivatives from the four compounds produced spectra confirming that the pairs **3**, **6** and **4**, **5** were structural isomers (Figures 4−6). The major differences between the NMR spectra of **4** and **5** were exhibited in the slight upfield shift in the resonance found for the aliphatic chain alcohol methine at δ 3.63 in both **5** and

6 compared to δ 3.60 in **4**; this shift in the resonance was accompanied by a change in the fine splitting of the THF flanking alcohol methine, at approximately δ 3.46, from J = 8.0, 7.0, 3.0 Hz in both **5** and **6** to J = 7.6, 7.6, 2.0 Hz in **4**. Also observed were slight downfield shifts in the ^{13}C resonances for one ether methine and two alcohol methines from δ 82.5, 74.3, and 71.6 observed in **5** and **6**, to δ 82.6, 74.4, and 71.8 in **4**. These very minor shifts in the ^{1}H- and ^{13}C-NMR spectra had to arise from stereochemical differences; therefore, formal acetal derivatives of all four compounds were prepared (**3a**−**6a**), and the relative stereochemistries of the parent alcohols were assigned by symmetry considerations (Figure 7).[10] The spectra of both **5a** and **6a** proved to be identical, indicating that these two compounds share the same configuration (Table 5). Furthermore, the large difference in the shifts of the two formal protons in **3a**, **5a**, and **6a** of δ 4.61 and 5.15 (J = 7.5 Hz) showed that they resided in very different chemical environments, indicating a *cis* relationship between the alcohol centers at C-10 and C-13 of **3** and

Table 5. ^{1}H NMR Resonances (δ) for the Formal Derivatives, **3a**, **4a**, **5a** and **6a**[a]

position (3a, 6a or 4a, 5a)	goniothalamicin formal (6a)	*cis*-goniothalamicin formal (3a)	arianacin formal (4a)	javoricin formal (5a)
10 or 12	3.66, m	3.63, 1H, tt (4.0, 3.8Hz)	3.62−3.68, 1H, m	3.66, m
11, 12 or 13, 14	1.6−1.83, 4H, m	1.75−2.0, 4H, m		1.6−1.83, 4H, m
13 or 15	3.74, 1H, m	3.98, 1H, ddd (4.2, 4.4, 6.5 Hz)	3.62−3.68, 1H, m	3.74, 1H, m
14 or 16	3.99, 1H, dt (8.5, 6.0 Hz)	3.67, 1H, dt (8.0, 4.2 Hz)	3.925, 1H, ddd (7.2, 6.4, 4.8 Hz)	3.99, 1H, dt (7.6, 6.0 Hz)
15a, 16a or 17a, 18a	1.80, 2H, m	1.80, 2H, m	1.60−1.80, 2H, m	1.80, 2H, m
15e, 16 or 17e,18e	1.99, 2H, m	1.96, 2H, m	1.99, 2H, m	1.99, 2H, m
17 or 19	3.84, 1H, dt (6.5, 7.0 Hz)	3.85, 1H, ddd (5.2, 5.2, 5.2 Hz)	3.82, 1H, dt (6.5, 7.0 Hz)	3.84, 1H, dt (6.5, 7.0 Hz)
18 or 20	3.385, 1H, dt (6.5, 5.4 Hz)	3.36, 1H, m	3.385, 1H, dt (6.5, 5.4 Hz)	3.385, 1H, dt (6.5, 5.4 Hz)
36a	4.61, 1H, d (7.4 Hz)	4.61, 1H, d (7.4 Hz)	4.81, 1H, d (5.1 Hz)	4.61, 1H, d (7.4 Hz)
36b	5.15, 1H, d (7.4 Hz)	5.15, 1H, d (7.4 Hz)	4.84, 1H, d (5.1 Hz)	5.15, 1H, d (7.4 Hz)

[a] Only those resonances differing from those of the parent compounds are presented.

M+H^{+} hr-fabms 597.4730 found, calculated 597.4730

Figure 6. All diagnostic EIMS fragment ions, in *m/z*, of **5**, with percent intensities reported in parentheses. Losses of *m/z* 90 indicate the loss of TMSiOH neutrals.

Figure 7. Formal derivative structures (**3a**−**6a**) of compounds **3**−**6** showing relative and absolute configurations.

6 (C-12 and C-15 of **5**). The formal protons of **4a** resonated much closer together, at δ 4.81 and 4.84, (*J* = 5.1 Hz), indicating similar magnetic environments and allowing assignment of *trans* relative configuration between the centers at C-12 and C-15 of **4**.

With the relative configuration of the five THF ring and carbon chain carbinol stereocenters determined, the absolute stereochemistries of **4**−**6** are defined by assignment of any one of the contiguous stereocenters. *Per*-MTPA esterification of compounds **3** and **6** was carried out. The resonances of the C-18 ester methine protons of the *R* and *S* derivatives were determined by COSY and single relay COSY NMR spectroscopy. Interpretation of the *R*-MTPA *per*-ester of **6** was straightforward, with a relay crosspeak to C-20 protons being quite visible at δ 1.14 in the aliphatic region of the spectrum, tracing back to the diagonal allowed assignment of δ 5.02 to the C-18 methine proton. ^{1}H COSY analysis of the same sample allowed assignment of δ 1.56 to the C-19 methylene protons. Analysis of the *S*-MTPA *per*-ester of **6** was somewhat more problematic. Correlations in the COSY spectra showed that the C-18 methine proton either resided at δ 4.93 and was coupled to methylene protons at δ 1.57, or it overlapped the C-34 lactone methine proton at δ 4.83 and showed a correlation with methylene protons at δ 1.45. However, C-10 protons clearly resonated at δ 4.98 and showed a crosspeak with C-9 and C-11 methylene protons centered on δ 1.55. In the relayed spectrum, the C-10 protons showed relay crosspeaks with C-12 protons
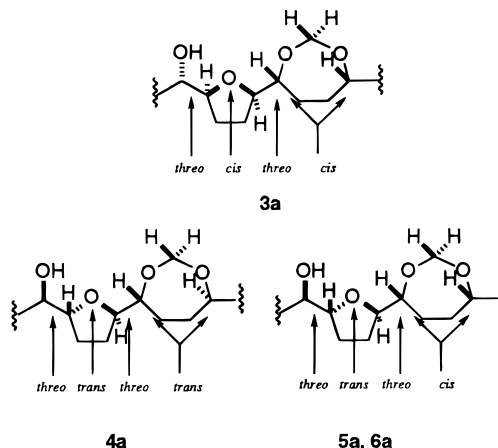
centered on δ 1.45; since a relay crosspeak also appeared with δ 4.86 to 1.55, the proton at C-13 was assigned the resonance at δ 4.86. On the basis of these observations, the C-18 proton was assigned the remaining resonance at δ 4.93. The COSY crosspeak at δ 1.57 could then be assigned to C-19, and the relay crosspeak at δ 1.38 was assigned to C-20. Analysis of this Mosher shift data, from these resonances, resulted in the assignment of the *R* configuration to the alcohol methine at C-18 of the parent compound. With the relative stereochemistries of the compounds **4a**−**6a,** respectively, defined as *threo-trans-threo-trans, threo-trans-threo-cis*, and *threo-trans-threo-cis*, the absolute configurations were assigned as 12*S*,15*R*,16*R*,19*R*,20*R* to **4**, 12*R*,15*R*,16*R*,19*R*,20*R* to **5**, and 10*R*,13*R*,14*R*,17*R*,-18*R* to **6** (Table 6). These results confirmed our previous report on the absolute stereochemistries of **6**.[21]

Stereochemical analysis of *cis*-goniothalamicin (**3**) was achieved in a similar manner. The *per*-(*S*)-MTPA ester methine protons produced discrete resonances with no overlap, and strong relay COSY crosspeaks to the highly shielded THF ring methylene of C-16 at δ 0.93 and to the aliphatic methylene resonance of C-20 at δ 1.13 allowed assignment of the C-18 ester methine to the resonance at δ 5.04. The shifts of the C-19 methylene at δ 1.27 and the C-17 ether methine at δ 3.87 were then assigned by their correlations to δ 5.04. Assignments of the (*R*)-MTPA ester methines were complicated by the high degree of overlap, at δ 4.88−4.91, of three proton resonances belonging to C-34, C-10 and an ester methine proton. The remaining ester methine proton resonated at δ 5.02. The downfield ester resonance at

Table 6. ^{1}H NMR Chemical Shift Data[a] (δ) for the Determination of the Absolute Configuration of Compounds **3** and **6** from the (*S*)- and (*R*)-MTPA Mosher Ester Derivatives

| MTPA deriv | MTPA config | proton chemical shifts ($\Delta\delta = \delta_S - \delta_R$) | | | | | | carbinol config at C-4 |
		H(5) δ	H(4) δ	H(3) δ	H(33) δ	H(34) δ	H(35) δ	
6	S	1.62	5.28	2.57, 2.52	6.69	4.83	1.26	
	R	1.60	5.34	2.65, 2.56	6.93	4.88	1.28	R
	$\Delta\delta$	+(0.02)		−(0.08, 0.04)	−(0.24)	−(0.05)	−(0.02)	
3	S	1.62	5.28	2.57, 2.52	6.69	4.83	1.26	
	R	1.60	5.34	2.65, 2.56	6.93	4.88	1.28	R
	$\Delta\delta$	+(.02)		−(0.08, 04)	−(0.24)	−(0.05)	−(0.02)	

| MTPA deriv | MTPA config | proton chemical shifts ($\Delta\delta = \delta_S - \delta_R$) | | | | | carbinol config at C-18 |
		H(20)	H(19)	H(18)	H(17)	H(16)	
6	S	1.38	1.57	4.93	3.92	1.63, 1.35	
	R	1.14	1.56	5.02	4.01	1.92, 1.60	R
	$\Delta\delta$	+(0.24)	+(0.01)		-(0.09)	−(0.29, 0.25)	
3	S	1.13	1.27	5.04	3.87	1.50, 0.93	
	R	1.60	1.30	5.02	3.84	1.40, 0.83	S
	$\Delta\delta$	−(0.47)	+(0.03)		+(0.03)	+(0.10, 0.10)	

[a] Chemical shifts >2.0 were derived from 1D-^{1}H NMR spectra, and shifts < δ 2.00 were determined from the center of the relevant COSY off-diagonal peaks because of the large degree of overlap among these resonances.

Table 7. Toxicity to *Artemia salina* (BST), Inhibition of Crown Gall Tumors on Potato Discs, and Cell Culture Activity of the Isolated Mono-THF Ring Annonaceous Acetogenins **1**−**6** and their Formal Derivatives **1a**, **3a**−**6a**

compound	BST LC50 (μg/mL)	PD[a] % crown gall tumor inhibition	A-549[b] IC$_{50}$ (μg/mL)	MCF-7[c] IC$_{50}$ (μg/mL)	HT-29[d] IC$_{50}$ (μg/mL)
F005	0.8		$<10^{-3}$	1.5×10^{-2}	$<10^{-3}$
cis-annonacin (**1**)	2.3	28	2.3×10^{-1}	1.18	1.0×10^{-8}
cis-annonacin formal (**1a**)		22	7.9×10^{-2}	2.6	2.1
cis-annonacin-10-one (**2**)	1.8	32	3.5×10^{-1}	2.9×10^{-1}	9.0×10^{-4}
cis-goniothalamicin (**3**)	5.2	47	1.3×10^{-1}	1.05	5.3×10^{-3}
cis-goniothalamicin formal (**3a**)		21	5.3×10^{-1}	5.2×10^{-1}	2.6
arianacin (**4**)	7.1	26	4.7×10^{-3}	4.0×10^{-1}	4.4
arianacin formal (**4a**)		28	2.0×10^{-2}	8.5	1.0
javoricin (**5**)	4.9	47	1.7×10^{-2}	2.3×10^{-1}	1.8
javoricin formal (**5a**)		31	2.0×10^{-2}	2.9	5.6
goniothalamicin (**6**)	0.6		8.0×10^{-3}	5.7×10^{-2}	1.1×10^{-3}
goniothalamicin formal (**6a**)			2.5×10^{-2}	1.03	1.13
adriamycin[e]			4.0×10^{-4}	2.7×10^{-2}	5.1×10^{-4}

[a] Potato disc assay. [b] Human lung carcinoma cell line. [c] Human breast carcinoma cell line. [d] Human colon adenocarcinoma cell line. [e] Typical value: run no. 182, Feb. 26, 1992.

δ 5.02 showed two weak relay correlations, one to a THF ring methylene proton at δ 0.83 and one to an aliphatic methylene proton at δ 1.60. This aliphatic chain methylene resonance (δ 1.60) and COSY correlations at δ 1.30 and 1.40 to δ 5.02 overlapped with the COSY crosspeaks to δ 4.91. All of the correlations to either δ 5.02 or 4.91 had absorption frequencies greater than the *S*-MTPA ester resonance for protons at C-19 and C-20 of δ 1.27 and 1.13, respectively. Both the C-19 and C-20 protons of the *R* ester resonated upfield of δ 1.27; therefore, $\Delta\delta_{S-R}$ was negative, allowing assignment of the *S* configuration to the center at C-18. The relative stereochemistry of *threo-cis-threo-cis* allowed assignment of the absolute stereochemistry as 10*R*,13*R*,14*R*,17*S*,18*S* to **3** (Table 6). All of the 4-OH annonaceous acetogenins, so far, are 4*R* and 36*S*.[24]

Table 7 summarizes the bioactivities of compounds **1**−**6**. All compounds showed significant activities in the BST and inhibited the growth of crown gall tumors on potato disks, which is suggestive of in vivo antitumor activity.[6] All of the compounds showed significant activity against the human solid tumor cells within one to three orders of magnitude of the potency of Adriamycin; however, **1** showed a selective inhibitory effect against the colon cell line that was four orders of magnitude greater than the potency of Adriamycin. 16,-19-*cis*-Murisolin, which has an OH group at C-10 of annonacin and is the only other mono-*cis*-THF aceto-

genin previously reported, is not selective for HT-29.[25] Comparison of the cytotoxicity values of these single ring compounds with other types of acetogenins shows that previously observed trends of structure−activity relationships continue to be seen. Specifically, the bis-THF ring compounds are more potent than nonadjacent bis-THF ring compounds, which themselves are more potent than the mono-THF ring compounds, which in turn are more potent than nonring compounds.[17,18] The acetogenins exert their biological effects, at least in part, through inhibition of mitochondrial electron transport (complex I)[26] and the inhibition of the plasma membrane NADH oxidase of tumor cells.[27]

Experimental Section

General Experimental Procedures. Rainin HPXL pumps and a Rainin Model UV-1 ultraviolet detector, controlled by Dynamax version 1.2 software, were used in all HPLC isolations. Mp determinations were made on a Scientfic Instruments Mel-Temp apparatus and are uncorrected. Optical rotation determinations were made on a Perkin-Elmer 241 polarimeter. FTIR spectra were obtained on a Perkin-Elmer 1420. ^{1}H-NMR, 2-D COSY, and NOESY spectra referenced to residual CHCl$_3$ in CDCl$_3$ unless otherwise stated and ^{13}C-NMR and HET-COR spectra referenced to CDCl$_3$ were obtained on a Varian VXR-500S. Low resolution EI and CI mass spectra were obtained on a Finnigan 4000. Low resolu-

tion EIMS of TMSi derivatives and all high resolution MS were obtained either by peak matching or by high resolution scanning with perfluorokerosene used as an internal standard, on a Kratos MS50.

In all CC the extracts were first adsorbed onto Celite before loading onto the open column. Si gel 60, 60–200 mesh (Selecto brand), purchased in bulk through Fischer Scientific, was used for open columns. Baker flash column Si gel, 40-μm diameter, was used for flash column chromatography. Chromatotron rotors were poured with Si gel 60 PF-254, no. 7749–3, containing gypsum, produced by Merck and purchased through EM Science. Si gel 60, precoated TLC plates, no. 5714, without a fluorescent indicator, produced by Merck and purchased through EM Science, were used for TLC evaluations. All solvents for chromatography, with the exception of THF, were of spectroscopic grade. The solvents used for extraction and partitions, as well as THF for chromatography, were reagent grade. TLC plates were visualized by spraying with 5% phosphomolybdic acid or 5% vanillin in H_2SO_4, followed by heating. Normal phase, analytical, and preparatory HPLC runs were obtained using Rainin columns packed with Dynamax-60A 8 μm Si gel. Reversed-phase HPLC was performed on Rainin 21.4-mm i.d. columns packed with Dynamax-60A 8 μm C18.

Plant Material. The dried seeds (2.2 kg) of *Annona muricata* were obtained from the Dominican Republic through the Possum Trot Tropical Fruit Nursery, 14955 S. W. 214 St., Miami, FL 33187. A second collection of seeds (10.7 kg), from juice producers in various locations around the Caribbean region, was provided by Native Plants Inc. (now Agridyne Inc.), 417 Wakara Way, Salt Lake City, UT 84108.

Biological Evaluations. The extracts, fractions, and isolated compounds were routinely tested for toxicity in the BST[5]. Sea salt was prepared from 3.8% (w/v) Instant Ocean artificial sea salt (Instant Ocean Co., Cincinnati, OH) in distilled H_2O. Analysis of the data was performed by probit analysis on a Finney computer program to determine the lethal concentration to half of the test organisms (LC_{50}). Crude extracts resulting in LC_{50} values of less than 250 ppm or pure compounds with LC_{50} values of less than 40 ppm were considered significantly active. The isolated compounds were also evaluated by their ability to inhibit the growth of crown gall tumors on potato discs (PD)[28] inoculated with *Agrobacterium tumefaciens* carrying a tumor-inducing plasmid. Cytotoxicity to human solid tumor cells was evaluated at the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard protocols for A-549 lung carcinoma,[29] MCF-7 breast carcinoma,[30] and HT-29 colon adenocarcinoma[31] cell lines. Generally, cytotoxicity ED_{50} values less than 20 μg/mL for crude extracts and less than 4 μg/mL for pure compounds are considered active.

Extraction and Isolation. The seeds (2.2 kg) were oven-dried at 37 °C and ground through a 2 × 10 mm screen using a hammer mill. The seed meal was then defatted by Soxhlet extraction in hexane for 72 h. The hexane from the Soxhlet extraction was removed by rotary evaporation to yield an amber oil that was partitioned with 10% aq. MeOH. The resulting solid (18.7 g) from the aqueous MeOH layer was reserved. The defatted plant material was repeatedly percolated with 95% EtOH to yield 100 g of extract F001. Concentration of F001 caused the crystallization of a sugar mixture, which was removed by filtration. The remaining liquor was partitioned between CH_2Cl_2–H_2O (1:1). The H_2O soluble fraction (F002) was freeze-dried to yield a sticky yellow mass (30 g), while the CH_2Cl_2-soluble fraction was concentrated by rotary evaporation to yield F003 (63 g). F003 was then partitioned between 90% aqueous MeOH and hexane (1:1). The two phases were dried by rotary evaporation to yield the hexane soluble fraction F006 (35 g) and an aqueous MeOH-soluble fraction F005 (26 g), which was combined with the solid aqueous MeOH residue recovered (above) from defatting.

All fractions were subjected to the BST, with the most active fraction being F005 (BST, $LC_{50} = 1.6$ ppm). Flash column chromatography of F005 with 0–25% MeOH in CH_2Cl_2, eluted over Baker 40 μm Si gel, separated mixtures of active compounds from inert material as determined by the BST. Active fractions were pooled by activity and subjected to repeated chromatography, by chromatotron, in two solvent systems, CH_2Cl_2–EtOAc (3:2) and 1% MeOH, with polarity increasing in steps of 0.1% MeOH for every 50 mL of eluent passed over the rotor and $CHCl_3$–MeOH (99.5:0.5), with polarity increasing with 0.2% MeOH for every 25 mL of eluent until the composition reached 2% MeOH. Impure components were combined according to their similar appearance on TLC analysis, and these were again subjected to elution on the chromatotron in one of the two solvent systems described above. This repeated chromatography resulted in the isolation of impure annonacin, annonacin-10-one, and goniothalamicin. From these impure fractions, small quantities of the five new compounds were isolated by HPLC and assigned the trivial names *cis*-annonacin (24 mg, R_f 0.39 in CH_2Cl_2–EtOAc–MeOH, 6:3:1) **1**, *cis*-annonacin-10-one (20 mg, R_f 0.52 in CH_2Cl_2–EtOAc–MeOH, 6:3:1) **2**, *cis*-goniothalamicin (28 mg, R_f 0.37 in CH_2Cl_2–EtOAc–MeOH, 6:3:1) **3**, arianacin (16 mg, R_f 0.46 in CH_2Cl_2–EtOAc–MeOH, 6:3:1) **4**, and javoricin (18 mg, R_f 0.46 in CH_2Cl_2–EtOAc–MeOH, 6:3:1) **5**. A Rainin Dynamax-60A 8-μm silica support eluted with 7% MeOH–THF (9:1) in hexane was used and gave a near base line separation of the *cis* and *trans* diastereomers.

TMSi Derivatives of 1–6. The samples of less than 1 mg were dried *in vacuo* in the presence of P_2O_5 and KOH as drying agents. The dry sample was reacted with 20 μL of either the unlabeled *N,O*-bis(trimethylsilyl)acetamide (BSA) or d_9-BSA in pyridine (10:1). The reaction mixture was heated at 70 °C for 30 min to yield the trimethylsilyl (TMSi) ethers that were analyzed by EIMS.

MTPA Derivatives of 1–6. (*R*) or (*S*)-α-Methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA), 1 g, was dissolved in thionyl chloride and refluxed at 78 °C for 72 h. The reaction was determined to be complete by the shift in the IR spectrum of the carbonyl stretch, from 1732 cm^{-1} to 1791 cm^{-1}, with the concomitant loss of the low broad OH stretch in the region from 3000–3500 cm^{-1}. The unreacted thionyl chloride and HCl were removed by vacuum evaporation. The product MTPA chlorides were dissolved in 10 mL of dry CH_2Cl_2. About 0.1 mL of the reagent solution was added to a 4-mg sample of acetogenin dissolved in dry CH_2Cl_2 with a few

crystals of (dimethylamino)pyridine (DMAP) and one drop of dry pyridine to produce the ester. The reaction mixture was quenched and partitioned several times with saturated $NaHCO_3$ before purification of the resulting esters by normal-phase HPLC.

Formal Derivatives of 1 and 3−6. In a 20-mL scintillation vial, 10 mL of C_6H_6, 100 μL (790 μmol) of trimethylsilyl chloride, and 80 μL (1100 μmol) of DMSO were mixed well. The mixure was allowed to react for 2 h at room temperature, during which time a white, needle-like precipitate was formed. After the formation of the precipitate, the C_6H_6 was decanted, and the precipitate was quickly washed once with CH_2Cl_2. Approximately 12 mg (200 μmol) of acetogenin was dissolved into 5 mL of CH_2Cl_2 and added to the reaction vial containing the precipitate, causing the precipitate to dissolve. The vials were capped, and the reactions were allowed to continue for 48−72 h. The reaction was then quenched and partitioned several times with saturated $NaHCO_3$. The CH_2Cl_2 layer was concentrated and filtered, and the products were separated by normal-phase HPLC [7% MeOH−THF (9:1) in hexane]. Typical yields were on the order of 30%, with recovery of 30% of the starting material. This procedure was recently described for making the formal (formaldehyde acetal) derivatives of acetogenins.[21]

***cis*-Annonacin (1):** white, amorphous powder from hexane, 24 mg from 900 mg of impure annonacin; mp = 77 °C; $[\alpha]^{25}_D$ 10° (*c* 17, CHCl$_3$); UV (MeOH) λ_{max} 215 nm, ϵ 9700; IR ν_{max} 3395 cm^{-1} OH, 2920 and 2851 cm^{-1} CH, 1734 cm^{-1} lactone C=O, 1469 cm^{-1} CH; CIMS MH$^+$ *m/z* 597(14) with 4 × H$_2$O losses to 579 (100), 561 (68), 543 (32), and 525 (3); EIMS M$^+$ *m/z* 327 (0) with 3 × H$_2$O losses to yield 309 (100), 291 (30.6), and 273 (28); HRFABMS *m/z* 597.4738 found for C$_{35}$H$_{65}$O$_7$, 597.4730 calcd; ^{1}H NMR (500 MHz, CDCl$_3$) see Table 1; ^{13}C NMR (125 MHz, CDCl$_3$) see Table 1. *R* Mosher tetraester of **1**: 3.2 mg yellow oil; ^{1}H NMR (500 MHz, CDCl$_3$, referenced to TMS): characteristic resonances see Table 3; *S* Mosher tetraester of **1**: 2.2 mg yellow oil, ^{1}H NMR (500 MHz, CDCl$_3$, referenced to TMS) characteristic resonances see Table 3. Formal derivative of **1** (**1a**): white powder; ^{1}H NMR (500 MHz, CDCl$_3$) see Table 1. *R*- and *S*-Mosher monoesters of **1** at each individual OH position: colorless oils, 500 MHz ^{1}H NMR equivalent to those of the parent compound, with the exception of the resonances, in Table 2, and MTPA-OCH$_3$ resonances which ranged from δ 3.55 to 3.65.

***cis*-Annonacin-10-one (2):** white, amorphous powder from hexane (20 mg from 86 mg of impure annonacin-10-one); mp = 70 °C); $[\alpha]^{25}_D$ 6.2° (*c* 0.07, CHCl$_3$); UV (MeOH) λ_{max} 209 nm, ϵ 8,400; IR ν_{max} 3444 cm^{-1} OH, 2918 and 2850 cm^{-1} CH, 1750 cm^{-1}, 1705 lactone C=O, 1467 cm^{-1} CH; CIMS M$^+$ *m/z* 595(0) with 3 × H$_2$O losses to 577 (100), 559 (78), 541 (22), fragments at *m/z* 325 (8.2) with 1 H$_2$O loss to yield 307 (100); HRFABMS MH$^+$ *m/z* 577.4453 C$_{35}$H$_{63}$O$_7$-H$_2$O, 577.4468 calcd; ^{1}H NMR (500 MHz, CDCl$_3$) see Table 1; ^{13}C NMR (125 MHz, CDCl$_3$) see Table 1. *R* Mosher triester of **2**: 3.5 mg of yellow oil; ^{1}H NMR (500 MHz, CDCl$_3$, referenced to TMS) characteristic resonances see Table 3; *S* Mosher triester of **2**: 2.9 mg of oil; ^{1}H NMR (500 MHz, CDCl$_3$, referenced to TMS) characteristic resonances see Table 3.

***cis*-Goniothalamicin (3):** white, amorphous powder

from hexane (28 mg from 165 mg of impure goniothalamicin); mp = 80 °C; $[\alpha]^{25}_D$ = 7.2° (*c* 0.03, CHCl$_3$); UV (MeOH), λ_{max} 213 nm, ϵ 10 500; IR ν_{max} 3628 cm^{-1} OH, 2923 cm^{-1} CH, 1750 cm^{-1} lactone C=O, 1469 cm^{-1} CH; CIMS MH$^+$ *m/z* 597 (36) with 4 × H$_2$O losses to 579 (100), 561 (64), 543 (22), 525 (0); EIMS M$^+$ *m/z* 281 (53), 263 (6), 241 (3), 223 (5); HRFABMS MH$^+$ *m/z* 597.4738 for C$_{35}$H$_{65}$O$_7$, 597.4730 calcd ^{1}H NMR (500 MHz, CDCl$_3$) see Table 4; ^{13}C NMR (125 MHz, CDCl$_3$) see Table 4. formal derivative of **3** (**3a**): white powder, ^{1}H NMR (500 MHz, CDCl$_3$) see Table 5. *R* Mosher tetraester of **3**: colorless oil; ^{1}H NMR (500 MHz, CDCl$_3$, referenced to TMS) characteristic resonances see Table 6; *S* Mosher tetraester of **3**: colorless oil; ^{1}H NMR (500 MHz, CDCl$_3$, referenced to TMS) characteristic resonances see Table 6.

Arianacin (4): white, amorphous powder from hexane (18 mg from 2.1 g of extract); mp 64 °C; $[\alpha]^{25}_D$ = +12.5° (*c* 0.14, CHCl$_3$); UV (MeOH) λ_{max} 215 nm, ϵ 12 500; IR ν_{max} 3629 cm^{-1} OH, 2925 and 2847 cm^{-1} CH, 1734 cm^{-1} lactone C=O, 1469 cm^{-1} CH; CIMS MH$^+$ *m/z* 597 with four losses of H$_2$O 597 (56), 561 (100), 543 (60), 526 (23); EIMS M$^+$ *m/z* 327 (12), 309 (100), 291 (22) 199 (3.7), 269 (30); ^{1}H NMR (500 MHz, CDCl$_3$) see Table 4; ^{13}C NMR (125 MHz, CDCl$_3$) see Table 4. Formal derivative of **4** (**4a**): white wax; ^{1}H NMR (500 MHz, CDCl$_3$) see Table 5.

Javoricin (5): white, amorphous powder from hexane (16 mg from 2.1 g of extract); mp 70 °C; $[\alpha]^{25}_D$ = +13.6° (*c* 0.1, CHCl$_3$); UV (MeOH) λ_{max} 217 nm ϵ 11 800; IR ν_{max} 3450 cm^{-1} OH, 2924 and 2853 cm^{-1} CH, 1750 cm^{-1} lactone C=O, 1457 cm^{-1} CH; CIMS MH$^+$ *m/z* 597(4) with four losses of H$_2$O, 561 (100), 543 (38), 526 (12); EIMS M$^+$ *m/z* 327 (8), 309 (100), 291 (18); ^{1}H NMR (500 MHz, CDCl$_3$) see Table 4; ^{13}C NMR (125 MHz, CDCl$_3$) see Table 4. Formal derivative of **5** (**5a**): white wax; ^{1}H NMR (500 MHz, CDCl$_3$) see Table 5.

Goniothalamicin (6): white, amorphous powder from hexane (125 mg from 2.1 g of extract); mp 87 °C; $[a]^{25}_D$ = +15.5° (*c* 0.23, CHCl$_3$), IR ν_{max} 3450 cm^{-1} OH, 2916 and 2847 cm^{-1} CH, 1745 cm^{-1} lactone C=O, 1469 cm^{-1} CH; CIMS MH$^+$ *m/z* 597 (32) with four losses of H$_2$O 579 (100), 561 (44), 543 (9.2); EIMS M$^+$ *m/z* 281 (100), and 263 (7.5); ^{1}H NMR (500 MHz, CDCl$_3$) see Table 4; ^{13}C NMR (125 MHz, CDCl$_3$) see Table 4. *R*-Mosher tetraester of **6**: colorless oil; ^{1}H NMR (500 MHz, CDCl$_3$, referenced to TMS) characteristic resonances see Table 6; *S*-Mosher tetraester of **6**: colorless oil, ^{1}H NMR (500 MHz, CDCl$_3$, referenced to TMS) characteristic resonances see Table 6. Formal derivative of **6** (**6a**): white wax; ^{1}H NMR (500 MHz, CDCl$_3$) see Table 5.

References and Notes

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