Gigantetronenin and Gigantrionenin: Novel Cytotoxic Acetogenins from Goniothalamus giganteus

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GIGANTETRONENIN AND GIGANTRIONENIN: NOVEL CYTOTOXIC ACETOGENINS FROM *GONIOTHALAMUS GIGANTEUS*

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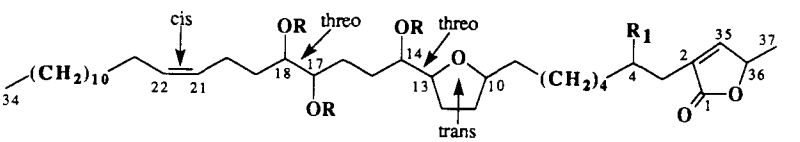
ABSTRACT.—Gigantetronenin [**1**] and gigantrionenin [**6**], two new monotetrahydrofuran Annonaceous acetogenins each possessing a double bond along the hydrocarbon chain, have been isolated from the bark of *Goniothalamus giganteus* by the use of brine shrimp lethality for bioactivity-directed fractionation. The structures were elucidated based on spectroscopic and chemical methods. Compounds **1** and **6** both show selective and potent cytotoxicities to human tumor cells in culture as well as toxicity to brine shrimp. A known cytotoxic acetogenin, annomontacin [**11**], was also isolated from this plant. The biogenetic pathway of the acetogenins from *G. giganteus* is discussed.

Several Annonaceous acetogenins, which represent a new class of extremely potent compounds, have been recently isolated from the Annonaceae (1). The compounds act by inhibiting complex I (NADH-Q reductase) of mitochondrial electron transport systems at or near subnanomolar concentrations (2–4), and this action likely explains their potent antitumor, pesticidal, and other biological effects (1). In our bioactivity-directed search for new antitumor natural products, two major classes of bioactive compounds have been found in the bark of *Goniothalamus giganteus* Hook f. & Thomas (Annonaceae) from Thailand. One class is the styryllactones, and most of these compounds are bioactive but show only modest cytotoxicities against certain human cancer cell lines (5–9). The other class is the Annonaceous acetogenins which are pesticidal and are much more cytotoxic to the cell lines (10–13). We report here from this plant the structures and cytotoxic activities of two new monotetrahydrofuran Annonaceous acetogenins, gigantetronenin [**1**] and gigantrionenin [**6**], and a known bioactive acetogenin, annomontacin [**11**] (14). The structures were determined by 1D and 2D nmr and ms, and, especially, after making certain chemical derivatives. The new acetogenins **1** and **6** each have a double bond along the hydrocarbon chain. The presence of double bonds is a new feature of the Annonaceous acetogenins (13) and provides evidence to support the proposed polyketide biogenetic pathway (1).

RESULTS AND DISCUSSION

The mol wt of gigantetronenin [**1**] was indicated by peaks at *m/z* 623 $[MH]^+$ in both the cims and fabms. The hrfabms gave *m/z* 623.4872 for the $[MH]^+$ (calcd 623.4886) corresponding to the molecular formula $C_{37}H_{66}O_7$. The existence of an α,β-unsaturated γ-lactone was suggested by an ir carbonyl absorption at 1734 cm^{-1}, a



1 R=H, R_1=OH
2 R=Ac, R_1=OAc
3 R=TMSi, R_1=TMSiO
4 R=*d*-TMSi, R_1=*d*-TMSiO
6 R=H, R_1=H
7 R=Ac, R_1=H
8 R=TMSi, R_1=H
9 R=*d*-TMSi, R_1=H

uv λ max at 208 nm (log ε 3.78), six resonances at δ 7.19 (H-37), 5.07 (H-36), 2.53 (H_a-3), 2.40 (H_b-3), 3.85 (H-4), and 1.44 (H-37) in the ^{1}H-nmr spectrum, and six peaks at δ 174.62 (C-1), 151.88 (C-35), 131.06 (C-2), 78.01 (C-36), 69.86 (C-4), and 19.08 (C-37) in the ^{13}C-nmr spectrum (Table 1). These are all characteristic spectral features for the methyl α,β-unsaturated γ-lactone fragment with a 4-OH moiety Annonaceous acetogenins (1).

The existence of four OH moieties in **1** was obvious by an ir OH absorption at 3436 cm^{-1}, four successive losses of H_2O (*m/z* 18) from the $[MH]^+$ in both the cims and fabms, and the preparation of a tetraacetate derivative **2**, a tetra-trimethylsilyl (TMSi) derivative **3**, and a tetraperdeutero-trimethylsilyl (*d*-TMSi) derivative **4**. Compound **2** gave four single proton peaks integrating for three protons each at δ 2.083 (OAc), 2.081 (OAc), 2.048 (OAc), and 2.025 (OAc) and three multiple resonances at δ 5.10 (H-4), 5.00 (H-17, -18), and 4.80 (H-14) corresponding to the downfield shifts of four protons on secondary OH-bearing carbons in **1** after acetylation. The cims of **2** showed four successive losses of HOAc (*m/z* 60) from the $[MH]^+$. The eims of **3** and **4** showed four successive losses of TMSiOH (*m/z* 90) or *d*-TMSiOH (*m/z* 99), respectively, from the $[M]^+$. Furthermore, the ^{13}C nmr of **1** showed four resonances due to oxygen-bear-

TABLE 1. Nmr Data for Gigantetronenin [**1**] and its Derivatives **2** and **5**.[a]

Position	^{1}H nmr [δ, (*J* in Hz)] of **1**	^{1}H nmr [d, (*J* in in Hz)] of **2**	^{1}H nmr [δ, (*J* in in Hz)] of **5**	^{13}C nmr (δ) of **1**
1	—	—	—	173.82
2	—	—	—	131.06
3	2.53 dt (15.1, 1.6)	2.56 dt (15.0, 1.6)	2.53 dt (15.0, 1.6)	33.34
	2.40 dd (15.1, 8.3)	2.51 dd (15.0, 8.0)	2.40 dd (15.0, 8.3)	—
4	3.85 m	5.10 m	3.85 m	69.86
5	1.75–1.40 m	1.75–1.40 m	1.48 m	37.24
6–8	1.40–1.22 m	1.40–1.22 m	1.40–1.22 m	26.11, 29.73–29.01
9	1.75–1.40 m	1.75–1.40 m	1.50 m	29.82
10	3.89 m	3.85 m	3.89 m	79.28
11–12	2.00 m, 1.60 m	2.00 m, 1.60 m	2.02 m, 1.57 m	27.26, 28.89
13	3.81 q (7.2)	3.95 q (6.6)	3.79 q (7.0)	81.73
14	3.45 m	4.80 m	3.41 br t (7.8)	74.88[b]
15	1.75–1.40 m	1.75–1.40 m	1.41 m	35.39
16	1.75–1.40 m	1.75–1.40 m	1.53 m	33.39
17	3.45 m	5.00 m	3.55 m	74.20[b]
18	3.45 m	5.00 m	3.63 m	74.06[b]
19	1.53 m	1.75–1.40 m	1.53 m	31.91
20	2.19 m	2.09 m	2.18 m	31.91
21	5.37 dt (10.9, 5.0)	5.28 dt (10.9, 7.0)	5.39 dt (10.8, 7.0)	128.95
22	5.40 dt (10.9, 4.0)	5.38 dt (10.9, 7.0)	5.33 dt (10.8, 7.0)	130.74
23	2.00 m	2.00 m	2.03 m	32.39
24–33	1.40–1.22 m	1.40–1.22 m	1.40–1.22 m	29.73–29.01, 22.68
34	0.88 t (7.0)	0.88 t (7.0)	0.88 t (7.0)	14.12
35	7.19 q (1.2)	7.08 q (1.2)	7.19 q (1.2)	151.88
36	5.07 qq (7.0, 1.2)	5.10 m	5.07 qq (7.0, 1.2)	78.01
37	1.44 d (7.0)	1.40 d (7.0)	1.44 d (7.0)	19.08
		2.081 s (3H, OAc)	1.32 s (3H, Me)	
		2.083 s (3H, OAc)	1.64 q (2H, 7.0, CH_2)	
		2.048 s (3H, OAc)	0.89 t (3H, 7.0, Me)	
		2.025 s (3H, OAc)		

[a]^{1}H-nmr (500 MHz) and ^{13}C-nmr (125 MHz) spectra were recorded in $CDCl_3$.
[b]Signals may be interchangeable.

ing carbons at δ 74.88, 74.20, 74.06, and 69.86, indicating the existence of four secondary OH moieties with a 4-OH (δ 69 is the typical carbon resonance for 4-OH in reported acetogenins) (1). The presence of a monotetrahydrofuran ring with one OH group adjacent to the ring was suggested by proton resonances at δ 3.89 (H-10), 3.81 (H-13), and 3.45 (H-14) and carbon peaks at δ 81.73 (C-13) and 79.28 (C-10); these were directly analogous to similar peaks of other monotetrahydrofuran acetogenins with one OH group adjacent to the ring, such as gigantetrocin and gigantriocin (12). The presence of an isolated cis double bond was suggested by two proton resonances at δ 5.40 (dt, J = 10.88, 4.00 Hz, H-22) and 5.37 (dt, J = 10.88, 5.00 Hz, H-21) (the J values were measured by double resonance selective decoupling experiments) and two carbon peaks at δ 130.74 and 128.95.

The carbon skeleton and placement of the tetrahydrofuran ring and four OH groups along the hydrocarbon chain were determined based on the eims spectral analysis of **1**–**4** (Figure 1). The existence of the vicinal OH groups was confirmed by the preparation of a butanonide derivative **5** of **1**. The fabms of **5** gave an $[MH]^+$ at m/z 677, confirming the butanonide formation. The ^1H nmr of **5** also showed two downfield shifts from δ 3.45 to 3.63 and 3.55 for two of the four protons on OH-bearing carbons, which was consistent with their asignment as the vicinal OH groups. The position of the double bond was determined by the eims of **1**–**4**, the COSY spectrum of **1**, and the double-relayed COSY of **5** to be across C-21 and C-22. The ms fragmentation data (Figure 1) indicated that the double bond was located beyond C-18. Correlation cross peaks were seen from H-21 (δ 5.37) to H-20 (δ 2.19), and the latter showed cross peaks to a multiplet containing several protons (H-15, -16, and -19) at δ 1.53 (assigned by a COSY trace function), which, in turn, showed cross peaks to another multiplet at δ 3.45 (H-14, -17, and -18) in the COSY spectrum of **1**. The COSY spectrum of **5** further supported this assignment because the H-16 and H-19 signals shifted slightly downfield after butanonylation of **1** and showed clearer correlation cross peaks from H-21 to H-18. Finally, the double-relayed COSY of **5** showed double-relayed correlation cross peaks from H-21 to H-18 and H-17 to H-14 and convincingly confirmed the assignments of the placements for the double bond and vicinal OH moieties.

The relative stereochemistry between C-13 and C-14 of **1** was determined as threo

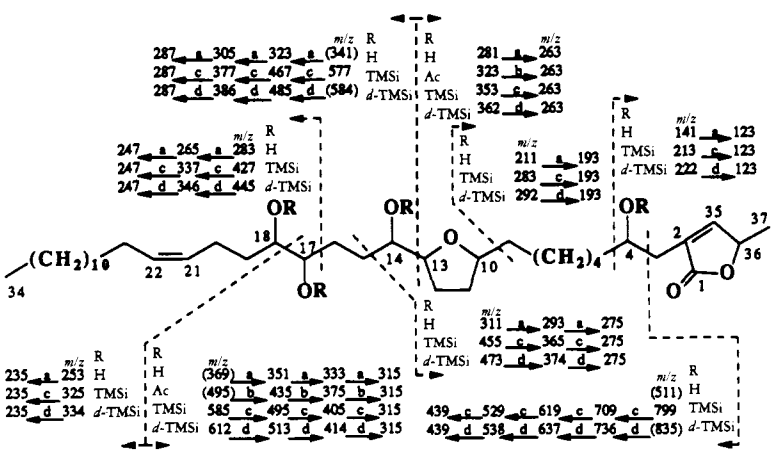


FIGURE 1. Diagnostic eims fragment ions of **1** (R = H), **2** (R = Ac), **3** (R = TMSi), and **4** (r = *d*-TMSi). Peaks in parentheses were not seen or were very weak. Letters above the arrows represent: (a) loss of H_2O (m/z 18), (b) loss of HOAc (m/z 60), (c) loss of TMSiOH (m/z 90), and (d) loss of *d*-TMSiOH (m/z 99).

by comparing the ^{13}C-nmr signal of **1** for C-14 (δ 74.88) and the ^{1}H-nmr resonances of **1** for H-13 (δ 3.81) and H-14 (δ 3.45) with those of model compounds of known relative stereochemistry (Born's technique) (15). The threo assignment was further substantiated by comparing the proton resonance of H-14 at δ 4.80 of **2** with a group of diacetyl dibutylated bistetrahydrofurans of known stereochemistry (Hoye's technique) (16,17). The relative configuration between C-17 and C-18 of **1** was suggested as threo by comparing the ^{1}H-nmr signals for H-17 and H-18 at δ 3.45 with those of a group of threo and erythro diols (18,19). The NOESY spectrum of **5** supported the threo assignment of the C-17/C-18, because the H-17 (δ 3.63) showed NOESY cross peaks with the methyl protons (s, δ 1.32) of the butanyl group and the H-18 (δ 3.55) showed NOESY cross peaks with the methylene protons (q, δ 1.64) of the butanyl group (Figure 2). This trans configuration of H-17/H-18 in **5** can from a vicinal diol with a threo configuration. Tlc examination of the butanonylation products as well as the ^{1}H nmr, COSY, and NOESY spectra of **5** indicated that only one diastereoisomer was the major product although the butanonylation reaction could give two diastereoisomers; this could be explained by an asymmetric inducing effect. The NOESY of **5** also suggested a trans configuration between C-10 and C-13, since it did not show cross peaks between the H-10 and H-13. The configuration of the chiral centers at C-4 and C-36 remains undefined. The ^{1}H- and ^{13}C-nmr data of **1** were assigned based on COSY and HETCOR spectra. Thus, the structure of **1** was determined as illustrated and named gigantetronenin.

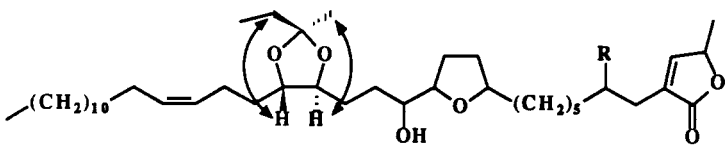


FIGURE 2. NOe's in **5** (R = OH) and **10** (R = H).

The mol wt of gigantrionenin [**6**] was indicated by peaks at *m/z* 607 $[MH]^+$ in both the cims and fabms, as well as 733 $[MH]^+$ in the cims of its triacetate derivative **7** and 822 $[MH]^+$ in the eims of its tri-TMSi derivative **8**. The hrfabms gave *m/z* 607.4919 for the $[MH]^+$ (calcd 607.4938) corresponding to the molecular formula $C_{37}H_{66}O_6$. This formula suggested that **6** was similar to **1** with one less OH group. The existence of three OH moieties was confirmed by an ir OH absorption at 3436 cm^{-1}, three successive losses of H_2O (*m/z* 18) from $[MH]^+$ in the fabms, and the preparation of a triacetate derivative **7**, a tri-TMSi derivative **8**, and a tri-*d*-TMSi derivative **9**. The ^{1}H- and ^{13}C-nmr data of **6** and **1** were essentially the same (Tables 1 and 2). However, **6** showed upfield shifts in both the ^{1}H- and ^{13}C-nmr signals (Table 2) for the protons and carbons associated with the lactone ring, indicating the absence of the OH group at C-4. This was confirmed by the absence of the carbon signal at δ 69.86 for **6**, and this signal is usually indicative of an OH group at C-4 (1). The absence of the 4-OH was further substantiated by the presence of a two-proton triplet at δ 2.26 for H-3, instead of the complex geminal coupling pattern associated with H-3 when an OH group is present at C-4. Again, the existence of an isolated cis double bond was indicated by two proton resonances at δ 5.39 (dt, 10.82, 3.90, H-22) and 5.36 (dt, 10.82, 4.51, H-21) and two carbon peaks at δ 130.78 and 128.95.

Ms analysis of **6–9** demonstrated that **6** possessed the same carbon skeleton and hydroxylation pattern as **1** with the exception of the 4-OH group (Figure 3). Hreims of the fragments *m/z* 265.1799 for $C_{16}H_{25}O_3$ (calcd 265.1804), 355.2212 for $C_{20}H_{31}O_4$

TABLE 2. Nmr Data for Gigantetronenin [**6**] and its Derivatives **7** and **10**.[a]

Position	^{1}H nmr [δ, (*J* in Hz)] of **6**	^{1}H nmr [d, (*J* in in Hz)] of **7**	^{1}H nmr [δ, (*J* in in Hz)] of **10**	^{13}C nmr (δ) of **6**
1	—	—	—	174.02
2	—	—	—	134.22
3	2.26 br t (7.0)	2.26 br t (7.0)	2.26 tt (7.0, 1.4)	27.22
4	1.52 m	1.52 m	1.54 m	26.11
5–8	1.40–1.22 m	1.40–1.22 m	1.40–1.22 m	29.71–28.91
9	1.75–1.40 m	1.44 m	1.50 m	29.82
10	3.88 m	3.85 m	3.89 m	79.33
11–12	2.00 m, 1.55 m	1.95 m, 1.55 m	2.02 m, 1.60 m	27.33, 28.37
13	3.81 q (7.1)	3.95 q (7.0)	3.79 q (7.0)	81.76
14	3.44 m	4.80 m	3.41 br t (7.0)	74.43[b]
15	1.75–1.40 m	1.58 m	1.44 m	35.50
16	1.75–1.40 m	1.58 m	1.54 m	33.34
17	3.44 m	5.00 m	3.55 m	74.20[b]
18	3.45 m	5.00 m	3.63 m	74.04[b]
19	1.75–1.40 m	1.58 m	1.54 m	31.88
20	2.19 m	2.02 m	2.18 m	31.88
21	5.36 dt (10.8, 4.5)	5.28 dt (10.8, 7.0)	5.39 dt (10.8, 7.0)	128.99
22	5.39 dt (10.8, 4.0)	5.37 dt (10.8, 7.0)	5.34 dt (10.8, 7.0)	130.78
23	2.00 m	1.95 m	2.02 m	32.35
24–33	1.75–1.22 m	1.65–1.22 m	1.70–1.22 m	29.71–28.91, 22.64
34	0.88 t (7.0)	0.88 t (7.0)	0.88 t (7.0)	14.08
35	7.00 q (1.2)	6.99 q (1.2)	6.99 q (1.2)	149.03
36	5.00 qq (7.0, 1.6)	5.00 m	5.00 qq (7.0, 1.6)	77.48
37	1.41 d (7.0)	1.40 d (7.0)	1.41 d (7.0)	19.15
		2.060 s (6H, OAc)	1.32 s (3H, Me)	
		2.057 s (3H, OAc)	1.62 q (2H, 7.0, CH_2)	
			0.91 t (3H, 7.0, Me)	

[a]^{1}H-nmr (500 MHz) and ^{13}C-nmr (125 MHz) spectra were recorded in $CDCl_3$.
[b]Signals may be interchangeable.

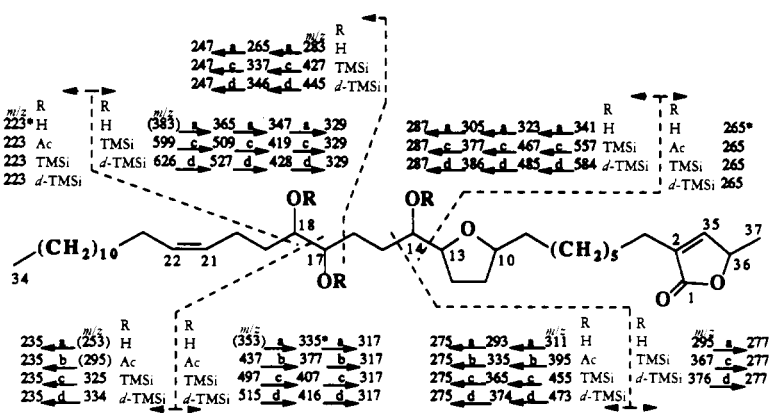


FIGURE 3. Diagnostic eims fragment ions of **6** (R = H), **7** (R = Ac), **8** (R = TMSi), and **9** (R = *d*-TMSi). The elemental compositions of fragments marked with an asterisk were confirmed through hreims. Peaks in parentheses were not seen or were very weak. Letters above the arrows represent: (a) loss of H_2O (*m/z* 18), (b) loss of HOAc (*m/z* 60), (c) loss of TMSiOH (*m/z* 90), and (d) loss of *d*-TMSiOH (*m/z* 99).

(calcd 335.2222), and 223.2420 for $C_{16}H_{31}$ (calcd 223.2426) gave further credence to the positioning of the OH groups at C-14, C-17, and C-18. The synthesis of a butanonide derivative **10** of **6** was evidenced by the ^{1}H-nmr and COSY spectra and an $[MH]^+$ of *m/z* 661 in the fabms of **10**, and confirmed the presence of vicinal OH groups. The position of the double bond was, again, determined from the COSY spectra of **6**, **7**, and **10** to be across C-21 and C-22. The stereochemistries between C-13 and C-14 as well as C-17 and C-18 were both determined to be threo, identical to **1**, by comparing the ^{1}H- and ^{13}C-nmr data (Table 2) with those of model compounds of known relative stereochemistry. As with **1**, the NOESY spectrum of **10** supported the threo assignment between C-17 and C-18 and suggested a trans configuration between C-10 and C-13 based on no observable nOe effect between H-10 and H-13 (Figure 2). The chiral center at C-36 remains undefined. Therefore, the structure of **6** was determined as 4-deoxy-**1** and given the trivial name gigantrionenin.

A third acetogenin was also isolated, and the structure was identified as the known compound annomontacin [**11**] (14) by ^{1}H- and ^{13}C-nmr, COSY, HETCOR, ms, and preparation of chemical derivatives, including the peracetate derivative **12**, per-TMSi derivative, and per-*d*-TMSi derivative.

The biological activities of **1**, **6**, and **11** and their derivatives are summarized in Table 3. Gigantetronenin [**1**], gigantrionenin [**6**], and annomontacin [**11**] were all active in the brine shrimp lethality test (BST) (20), which is predictive of cytotoxicity, and were also significantly and selectively cytotoxic to human solid tumor cells. All of the peracetate derivatives **2**, **7**, and **12** showed a decrease in activity relative to their parent compounds; this is the same as in previous reports (1).

TABLE 3. Bioactivities of **1**, **6**, and **11** and Their Acetate Derivatives **2**, **7**, and **12**.

Compound	BST[a] LC_{50} (μg/ml)	A-549[b] ED_{50} (μg/ml)	MCF-7[c] ED_{50} (μg/ml)	HT-29[d] ED_{50} (μg/ml)
1	9.4	4.71×10^{-3}	6.03×10^{-1}	5.37×10^{-2}
2	—	9.60×10^{-1}	5.97	1.60
6	13.9	3.94×10^{-3}	8.06	2.92×10^{-3}
7	—	3.59×10^{-1}	24.85	6.67×10^{-2}
11	12.5	7.72×10^{-3}	1.65×10^{-1}	2.58×10^{-3}
12	—	1.70×10^{-1}	90.39	14.87
Adriamycin[e]	8×10^{-2}	2.37×10^{-4}	1.08×10^{-2}	4.83×10^{-3}

[a]Brine shrimp lethality test.
[b]Human lung carcinoma.
[c]Human breast carcinoma.
[d]Human colon adenocarcinoma.
[e]Positive control standard.

Although no experimental work on the biosynthesis of the Annonaceous acetogenins has been reported, the biogenetic pathway of this class of compounds was proposed, and the precursors were predicted to be assembled by the linear combination of two- and three-carbon units (HOAc and propionic acid) via acetyl-CoA, malonyl-CoA, and propionyl-CoA, through mechanisms analogous to the well-known pathway for fatty acid biosynthesis (1). Based on this prediction, different arrangements of the tetrahydrofuran rings can be derived from epoxidation of triene, diene, and triene ketone intermediates followed by ring openings and closures. Stereochemistries of the tetrahydrofuran rings and adjacent OH groups depend on the regiochemistry of double bonds, the face of epoxidation, and the way to open and close the epoxide rings. The

acetogenins reported here, **1**, **6**, and **11**, as well as others reported previously from *G. giganteus*, including goniothalamicin [**13**] (10), gigantecin [**14**] (11), gigantetrocin [**15**] and gigantriocin [**16**] (12), giganenin [**17**] and 4-deoxygigantecin [**18**] (13), and giganin [**19**] (an unpublished non-THF acetogenin discovered by us recently) strongly support a polyketide pathway as hypothesized in Figure 4. Acetogenins such as **1**, **6**, and **19**, which contain a double bond in the appropriate place in the hydrocarbon chain, provide a logical sequence of intermediates to support this biogenetic proposal.

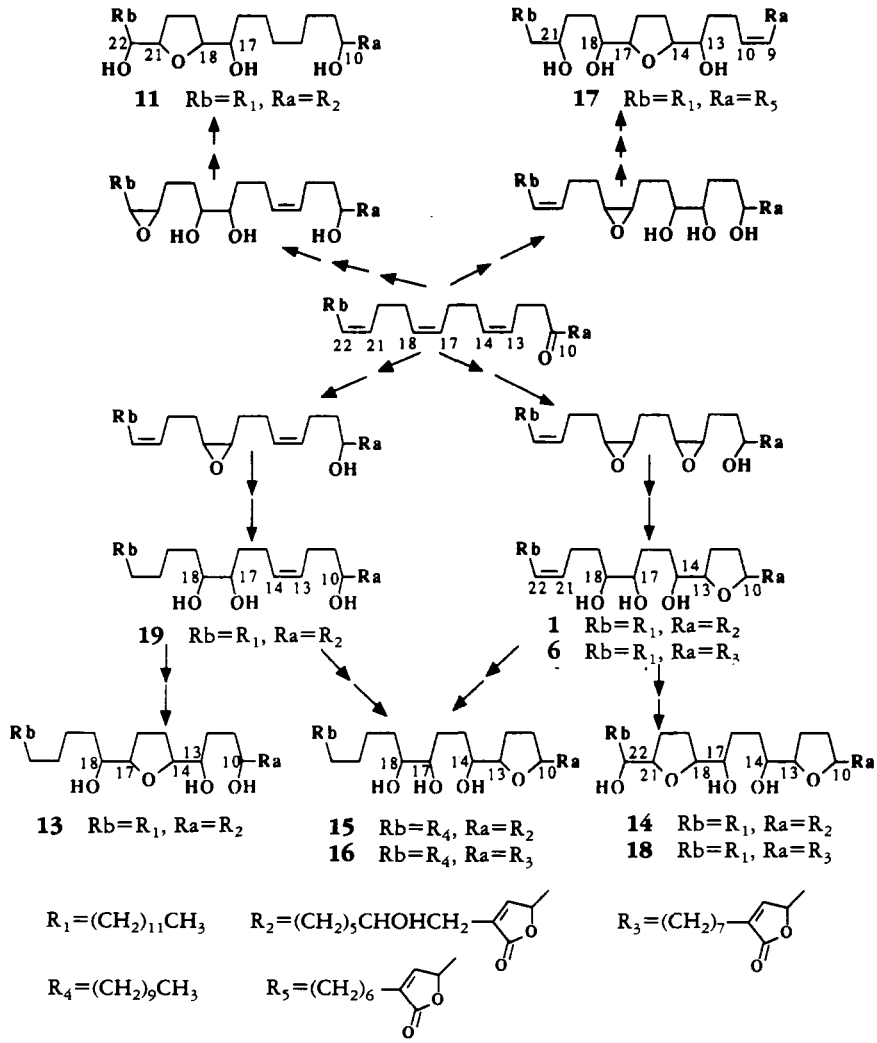


FIGURE 4. Hypothesis for the biogenetic pathways leading to the tetrahydrofuran rings of Annonaceous acetogenins from *Goniothalamus giganteus*, showing the possible positions of **1**, **6**, and **19** as intermediate compounds. Reaction steps involve simple epoxidation of double bonds, reduction of ketones to alcohols, hydration of epoxides or double bonds, and dehydration to form the rings.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's 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 EtOH on a Beckman DU-7 spectrophotometer. Ir spectra were obtained on salt

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 the Varian software systems.

PLANT MATERIAL AND BIOASSAYS.—The stem bark of *G. giganteus* (B-826538, PR-50604) was collected in Thailand in Sept. 1978 under the auspices of Dr. Robert E. Perdue, Medicinal Plant Laboratory, USDA, Beltsville, Maryland, where voucher specimens are maintained. Brine shrimp lethality (BST) was tested in our laboratory (20,21). The cytotoxicity tests against A-549 (human lung carcinoma), MCF-7 (human breast carcinoma), and HT-29 (human colon adenocarcinoma) cells were performed in the Purdue Cell Culture Laboratory, Purdue Cancer Center, using standard protocols with adriamycin as a positive standard control (22).

EXTRACTION AND ISOLATION.—The residue of the 95% EtOH crude extract of 4 kg of the stem bark was partitioned between H_2O and $CHCl_3$ to give an H_2O layer and a $CHCl_3$ layer. The residue of the $CHCl_3$ layer was partitioned between hexane and 10% H_2O in MeOH to give an MeOH layer, ca. 100 g dry residue, and a hexane layer. The MeOH residue, which was the most active fraction in brine shrimp lethality test (BST LC_{50} 15.1 μg/ml), was repeatedly chromatographed over Si gel columns and chromatotron separations directed by BST activity, using gradients of C_6H_6/EtOAc/MeOH, hexane/EtOAc, and $CHCl_3$/MeOH and purified by hplc [C-18 column, 15% H_2O in MeOH–*n*-PrOH (85:15)] to give three whitish waxes of **1** (100 mg), **6** (8 mg), and **11** (50 mg).

Gigantetronenin [**1**].—A whitish wax (100 mg): mp 57–59°; $[\alpha]^{25}D$ +10° (*c* = 0.2, $CHCl_3$); uv (MeOH) λ max 208 nm (log ε 3.78); ir (film on NaCl plate) 3436, 2918, 2849, 1734, 1466, 1321, 1086, 1066, 948, 854 cm^{-1}; fabms (glycerol) *m/z* (%) $[MH]^+$ 623 (24), $[MH-H_2O]^+$ 605 (1.9), $[MH-2H_2O]^+$ 587 (7.6), $[MH-3H_2O]^+$ 569 (5.7), $[MH-4H_2O]^+$ 551 (6.0); hrfabms *m/z* 623.4872 for $C_{37}H_{67}O_7$ $[MH]^+$ (calcd 623.4886); cims (isobutane) *m/z* (%) 623 (100), 605 (27), 587 (80), 569 (42), 551 (5.0), 351 (18), 333 (10), 311 (7), 281 (39), 263 (5), 253 (4), 141 (3); eims see Figure 1; ^{1}H and ^{13}C nmr see Table 1; COSY, double-relayed COSY (tau = 0.09 msec), and HETCOR in $CDCl_3$ (500 MHz).

Acetate **2** *of gigantetronenin*.—Compound **1** (2 mg) was acetylated (Ac_2O/pyridine, 24 h, room temperature) and the mixture was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ extract, on concentration and Si gel microcolumn chromatography, afforded **2** (1.5 mg oil): cims (isobutane) *m/z* (%) $[MH-HOAc]^+$ 731 (100), $[MH-2HOAc]^+$ 671 (15), $[MH-3HOAc]^+$ 611 (13), $[MH-4HOAc]^+$ 551 (2); eims see Figure 1; ^{1}H nmr see Table 2.

Butanonide **5** *of gigantetronenin*.—Compound **1** (4 mg) was butanonylated (*p*-toluenesulfonic acid in butanone, stirring for 24 h at room temperature), and the mixture was directly purified on Si gel microcolumn chromatography to give **5** (2 mg oil): fabms (glycerol) *m/z* (%) $[MH]^+$ 677 (2.3), 605 (1.1), 587 (2.9); eims *m/z* (%) 587 (1.7), 569 (1.5), 551 (1.5), 475 (1.8), 457 (0.5), 395 (0.5), 377 (1.0), 323 (3.1), 305 (2.5), 287 (3.0), 281 (8.3), 263 (4.1), 141 (5.7); ^{1}H nmr see Table 1; COSY and NOESY (mixing time 0.4 sec) in $CDCl_3$ (500 MHz).

Gigantrionenin [**6**].—A whitish wax (8 mg): mp 55–57°; $[\alpha]^{25}D$ +17 (*c* = 0.2, $CHCl_3$); uv (MeOH) λ max 207 nm (log ε 4.00); ir (film on NaCl plate) 3436, 2922, 2851, 1755, 1457, 1318, 1072, 1026, 80 cm^{-1}; fabms (glycerol) *m/z* (%) $[MH]^+$ 607 (0.6); hrfabms *m/z* 607.4919 for $C_{37}H_{67}O_6$ (calcd 607.4938); cims (isobutane) *m/z* (%) 607 (1.7), $[MH-H_2O]^+$ 5.89 (0.1), $[MH-2H_2O]^+$ 5.71 (0.8), $[MH-3H_2O]^+$ 553 (0.2); eims see Figure 3; hreims *m/z* 335.2212 for $C_{20}H_{31}O_4$ (calcd 335.2222), 265.1799 for $C_{16}H_{25}O_3$ (calcd 265.1804), 223.2420 for $C_{16}H_{31}$ (calcd 223.2426); ^{1}H and ^{13}C nmr see Table 2; COSY in $CDCl_3$ (500 MHz).

Acetate **7** *of gigantrionenin*.—Compound **6** (1.5 mg) was acetylated by the same procedure as for **2** to give **7** (1 mg): cims (isobutane) *m/z* (%) $[MH]^+$ 733 (0.6), $[MH-HOAc]^+$ 673 (7.9), $[MH-2H_2O]^+$ 613 (1.7), $[MH-3HOAc]^+$ 553 (2.6), 265 (3.4), 247 (1.2), 223 (0.2); eims see Figure 3; ^{1}H nmr see Table 2; COSY in $CDCl_3$ (500 MHz).

Butanonide **10** *of gigantrionenin*.—Compound **6** (2 mg) was butanonylated by the same procedure as for **5** to give **10** (1 mg): fabms (glycerol) *m/z* (%) $[MH]^+$ 661 (3.3), 589 (2.7), 571 (6.6), 553 (3.3); eims *m/z* (%) 660 (2.0), 631 (1.5), 571 (7.1), 554 (6.2), 395 (1.5), 377 (1.0), 323 (10.8), 305 (9.2), 291 (12.9), 281 (8.1), 265 (30.0), 247 (15.9), 223 (18.9); ^{1}H nmr see Table 2; COSY and NOESY (mixing time 0.4 sec) in $CDCl_3$ (500 MHz).

Annomontacin [**11**].—Most of the physical, chemical, and spectroscopic data were essentially identical to those reported (14) except the mp 70–72° and the $[\alpha]^{25}D$ +18° (*c* = 0.45, $CHCl_3$), which were different from the previous results: mp 34–36° and $[\alpha]^{20}D$ +81° (*c* = 1.0, MeOH).

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