(2,4-*cis* and *trans*)-Gigantecinone and 4-Deoxygigantecin, Bioactive Nonadjacent Bis-Tetrahydrofuran Annonaceous Acetogenins, from *Goniothalamus giganteus*

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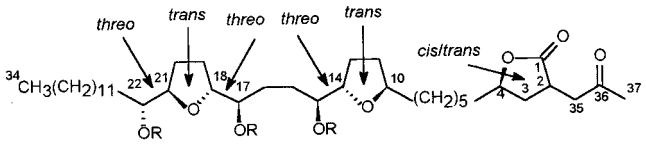
Two new acetogenins, (2,4-*cis* and *trans*)-gigantecinone (**1**), isolated as a mixture, and 4-deoxygigantecin (**2**), a known acetogenin whose absolute stereochemistry has not been determined previously, were isolated using activity-directed fractionation, from the bark of *Goniothalamus giganteus*. A key step in solving their absolute stereochemistries was the preparation of 1,4-diol formaldehyde acetal derivatives (**1b** and **2a**). Using the advanced Mosher ester method and circular dichroism, the absolute stereochemistries of **1** and **2** were revealed and were found to be the same as that of gigantecin (**3**), which supports a common biogenetic origin. Both **1** and **1b** showed potent and selective cytotoxicities against the PC-3 human prostate adenocarcinoma cell line. Against yellow fever mosquito larvae, **1** and **2** were more potent than rotenone in pesticidal activity. Longimicin C and a mixture of (2,4-*cis* and *trans*)-isoannonacin were also isolated for the first time from this species.

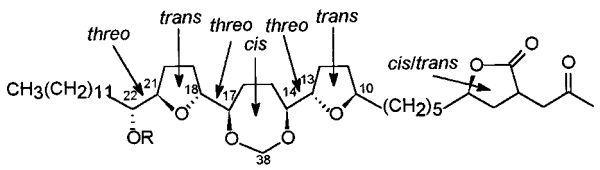
The Annonaceous acetogenins are a relatively new class of promising anticancer, antiinfective, and pesticidal natural compounds that are potent inhibitors of oxidative and substrate-level ATP production.[1,2] Structurally, most of these long-chain fatty-acid derivatives may be classified into three major groups: the mono-tetrahydrofuran (THF), adjacent bis-THF, and nonadjacent bis-THF subclasses. Although the last is generally less biologically active than the adjacent bis-THF subclass, some of them, for example, bullatalicin, show promising in vivo antitumor efficacy in athymic mice.[3] *Goniothalamus giganteus* Hook. f., & Thomas (Annonaceae) is a tree native to Thailand; in our further bioactivity-directed search of its bark for antitumor compounds,[1,4] guided by lethality to brine shrimp larvae (BST),[5] a mixture of two new cytotoxic acetogenins was isolated and identified as (2,4-*cis* and *trans*)-gigantecinone (**1**), the ketolactones of the parent compound gigantecin (**3**).[6] Other known acetogenins, 4-deoxygigantecin (**2**),[7] (2,4-*cis* and *trans*)-isoannonacin,[8] and longimicin C,[9] were also isolated; longimicin C is the first asimicin-like, adjacent bis-THF acetogenin to be isolated from this species.

Annonaceous acetogenins usually have numerous chiral centers, and chirality plays an important role in directing their bioactivities (e.g., differing at only one chiral center out of eight) significant differences are evident in the biological activities between the diastereoisomers asimicin (C-24 *R*)[10] and bullatacin (C-24 *S*).[1,2,11] Thus, the determination of the absolute stereochemistries and not just the relative stereochemistries of the Annonaceous acetogenins is crucial if we hope to understand better their structure−activity relationships.

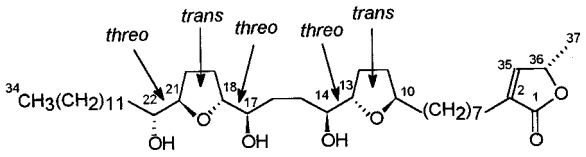
The mixture of (2,4-*cis* and *trans*)-gigantecinone (**1**) was isolated as a whitish wax in approximately a 1:1 ratio. Its molecular weight was suggested by the molecular ion peak at *m*/*z* 639 $[MH]^+$ in the CIMS. The HRCIMS gave *m*/*z* 639.4822 for the $[MH]^+$ ion (calcd 639.4836), corresponding to the molecular formula $C_{37}H_{66}O_8$. Spectral characteristics of **1** and its derivatives, including ^{1}H-NMR (Table 1), ^{13}C-NMR (Table 2), and EIMS (Figure 1) data, suggested that it belongs to



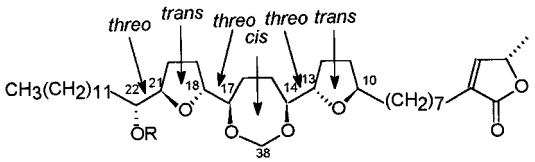
1 R= H **1a** R= Ac



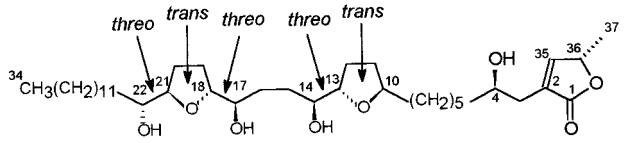
1b R= H **1c** R= (*S*)-MPTA **1d** R= (*R*)-MPTA



2 R= H



2a R= H **2b** R= (*S*)-MPTA **2c** R= (*R*)-MPTA



3 R= H

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Table 1. ^{1}H-NMR Spectral Data of **1** and **1a**−**d** [δ ppm (J = Hz)]

proton	**1**	**1a**	**1b**	**1c**	**1d**	$^{\Delta\delta}$**1c**−**1d**
2 *cis*	3.02 m	3.02 m	3.02 m	3.02 m	3.02 m	
trans	3.03 m	3.03 m	3.03 m	3.03 m	3.03 m	
3a *cis*	1.48 m	1.48 m	1.48 m	1.47 m	1.47 m	
trans	2.24 m	2.23 m	2.22 m	2.23 m	2.22 m	
3b *cis*	2.61 m	2.61 m	2.61 m	2.61 m	2.61 m	
trans	1.99 m	1.99 m	1.99 m	1.99 m	1.99 m	
4 *cis*	4.39 dddd	4.39 m	4.39 m	4.38 m	4.38 m	
trans	4.54 dddd	4.54 m	4.54 m	4.54 m	4.54 m	
5a *cis*	1.76 m	1.74 m	1.76 m	1.76 m	1.76 m	
trans	1.71 m	1.70 m	1.71 m	1.71 m	1.71 m	
5b *cis*	1.60 m	1.62 m	1.60 m	1.60 m	1.60 m	
trans	1.58 m	1.58 m	1.58 m	1.58 m	1.58 m	
6−9	1.20−1.50	1.20−1.50	1.20−1.50	1.20−1.50	1.20−1.50	
10	3.88 m	3.86 m	3.95 m	3.94 m	3.94 m	
11a	1.35−1.99		2.01 m	2.01 m	2.01 m	
11b	1.35−1.99		1.62 m	1.63 m	1.62 m	
12a	1.35−1.99		1.96 m	1.97 m	1.96 m	
12b	1.35−1.99		1.66 m	1.66 m	1.62 m	
13	3.81 m	3.96 m	4.00 m	4.00 m	4.01 m	−0.01
14	3.42 m	4.83 m	3.66 m	3.58 m	3.62 m	−0.04
15a, 16a	1.35−1.75	1.35−1.64	1.87 m	1.82 m	1.92 m	−0.10
15b, 16b	1.35−1.75	1.35−1.64	1.79 m	1.72 m	1.82 m	−0.10
17	3.42 m	4.83 m	3.63 m	3.58 m	3.62 m	−0.04
18	3.81 m	3.96 m	4.01 m	3.93 m	4.01 m	−0.08
19a	1.35−1.99	1.50−1.99	1.96 m	1.76 m	1.93 m	−0.17
19b	1.35−1.99	1.50−1.99	1.66 m	1.62 m	1.78 m	−0.16
20a	1.35−1.99	1.50−1.99	1.97 m	1.92 m	2.03 m	−0.11
20b	1.35−1.99	1.50−1.99	1.64 m	1.52 m	1.59 m	−0.07
21	3.81 m	3.96 m	3.84 m	4.08 m	4.08 m	∼0
22	3.42 m	4.83 m	3.39 m	5.06 m	5.05 m	*R*[a]
23	1.35−1.68		1.40 m	1.63 m	1.49 m	+0.14
24−33	1.35−1.68		1.20−1.40	1.20−1.40	1.20−1.40	
34	0.88 t (7.0)	0.88 t (7.0)	0.88 t (7.0)	0.88 t (7.0)	0.88 t (7.0)	
35a *cis*	2.61 dd 18.3, 9.2	2.61 dd		2.61 dd		
trans	2.67 dd 18.5, 9.5	2.67 dd		2.67 dd		
35b *cis*	3.10 dd 18.5, 3.5	3.10 dd		3.10 dd		
trans	3.05 dd 18.5, 3.4	3.05 dd		3.05 dd		
37	2.20 s	2.20 s		2.20 s		
38a			5.26 d (7.5)	5.20 d (7.5)	5.22 d (7.5)	−0.02
38b			4.62 d (7.5)	4.56 d (7.0)	4.59 d (7.5)	−0.03
OAc-14		2.09 s				
OAc-17		2.07 s				
OAc-18		2.07 s				

[a] Absolute configuration of carbinol center.

Table 2. ^{13}C-NMR Spectral Data of **1**

carbon	**1** *cis*	**1** *trans*	carbon	**1** *cis*	**1** *trans*
1	178.3	178.8	18	82.7	
2	43.8	44.2	19−20	25.2−36.7	
3 a,b	25.2−36.7		21	82.7	
4	79.3	78.8	22	74.4[a]	
5−9	25.2−36.7		23−32	25.2−36.7	
10	79.2		33	22.6	
11−12	25.2−36.7		34	14.1	
13	82.0		35	25.2−36.7	
14	74.0[a]		36	205.62	205.57
15−16	25.2−36.7		37	36.7	
17	74.2[a]				

[a] Signals are interchangeable.

the *trans*/*threo*/*threo*/*trans*/*threo* group; a rare class of bioactive nonadjacent bis-THF acetogenins.[1]

The IR spectrum of **1** showed strong absorptions at 1749 cm^{-1} for a γ-lactone carbonyl and at 1708 cm^{-1} for a ketone carbonyl. Under UV light at 225 nm, **1** was transparent, suggesting that the lactone is not α,β-unsaturated. In comparison with (2,4-*cis* and *trans*)-isoannonacin,[8] (2,4-*cis* and *trans*)-bullatacinone,[11] and (2,4-*cis* and *trans*)-annomontacinone,[4] the ^{1}H- and ^{13}C-NMR spectra of **1** clearly indicated the presence of a ketolactone moiety. In the ^{1}H-NMR spectrum of **1** (Table 1), the resonances at δ 4.39 and 4.54, with combined integrations for one proton, were assigned to H-4 and suggested the presence of a mixture of the (2,4-*cis* and *trans*)-diastereoisomers, as is typical with these ketolactones. In the ^{13}C-NMR spectrum (Table 2), signal pairs at δ 178.3 and 178.8, 43.8 and 44.2, 79.3 and 78.8, and 205.6 and 205.6 were assigned to C-1, C-2, C-4, and C-36, respectively, and confirmed the presence of the mixture of (2,4-*cis* and *trans*)-isomers. The ^{1}H-NMR assignments of H-2, H-3a, H-3b, H-5a, H-5b, H-35a, and H-35b were based on the analysis of the

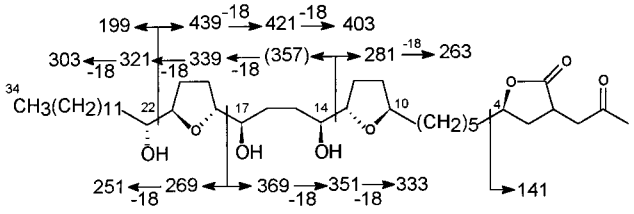

Figure 1. Diagnostic mass fragmentation ions of **1**. EIMS of **1**; losses of H_2O indicated by −18 *m*/*z*.

COSY spectrum of **1**. The existence of three OH moieties was indicated by IR hydroxyl absorptions at 3389 and 3507 cm^{-1}, three successive losses of H_2O from the $[MH]^+$ in the CIMS, and the preparation of a triacetate derivative (**1a**). Compound **1a** gave three singlet proton peaks at δ 2.07 (OAc-17and OAc-22) and 2.09 (OAc-14) and a multiplet at δ 4.83 (H-14, H-17, and H-22), corresponding to the downfield shifts of three protons on acetylated secondary OH-bearing carbons (Table 1). Furthermore, the ^{13}C-NMR spectrum of **1** showed three resonances due to oxygen-bearing carbons at δ 74.0, 74.2, and 74.4, confirming the existence of three secondary hydroxyls. The presence of two non-adjacent THF rings was indicated by proton resonances at δ 3.88 (H-10) and 3.81 (H-13, H-18, and H-21) in **1**, and 3.86 (H-10) and 3.96 (H-13, H-18, and H-21) in **1a**, and carbon resonances at δ 79.2 (C-10), 82.0 (C-13), and 82.7 (C-17, C-21). The ^{1}H-NMR spectrum of **1** showed a multiplet that integrated for three protons at δ 3.42, corresponding to three oxygenated methines. The upfield shift of C-10 to δ 79.2 and H-10 to 3.88, indicated that there was no hydroxyl group adjacent to one side of one of the THF rings. These data are characteristic of protons on secondary hydroxyl-bearing carbons adjacent to a THF ring as found in most Annonaceous acetogenins.[1,2] Two of these hydroxyl groups were assigned as being adjacent to one THF ring, and one was established as being adjacent to the other THF ring.

The carbon skeleton and placement of the two THF rings and the three OH groups along the hydrocarbon chain were determined based on EIMS analysis (Figure 1) and by comparison with the EIMS spectral data of gigantecin (**3**)[6] (the parent compound) and 4-deoxygigantecin (**2**).[7] The relative stereochemistries at C-13/C-14, C-17/C-18, and C-21/C-22 were determined as *threo*, and the stereochemistries of the THF rings were determined as *trans*, by comparing ^{1}H- and ^{13}C-NMR data to those of the Cavé and Fujimoto models of known mono-THF relative stereochemistries.[12,13] To determine the relative configuration at C-14/C-17, the formal (formaldehyde acetal) derivative (**1b)** was prepared.[14] In this procedure, the acetal moiety that is formed connects the diols but does not change the stereochemistries of their carbinol centers.[14] Therefore, significant differences in the ^{1}H-NMR signals between the acetal protons in the *cis* (at ca. δ 5.26 and 4.63 as two doublets) or *trans* (at ca. δ 4.96 as a singlet) configuration of the cyclic formal derivatives then permit the assignment of the relative stereochemistries of the diols in the parent compounds. The acetal protons in **1b** were presented as a pair of doublets at δ 5.26 and 4.62 (J = 7.5 Hz) in the ^{1}H-NMR spectrum (Table 1), indicating that the newly formed acetal ring possessed the *cis* relative configuration; and, thus, either *S*/*R* or *R*/*S* relative stereochemistries between C-14 and C-17 were revealed.

The absolute configuration of C-22 in **1b** was determined using advanced Mosher ester methodology.[15,16] The (*S*)- and (*R*)-methoxy(fluoromethyl) phenylacetic acid (MPTA) esters (**1c**,**1d**) of **1b** were prepared (Table 1). COSY ^{1}H-NMR analysis of these derivatives allowed the assignment of the absolute configuration at C-22 as *R* (Table 1); this then allowed the assignment of the following chiral centers: 21*R*, 18*R*, 17*R*, 14*S*, 13*S*, and 10*R*. This is in full agreement with the structure of the parent compound, gigantecin (**3**),[6] which has been refined using X-ray crystallography.[17] Although Annonaceous acetogenins do not crystallize easily, preparation of their formaldehyde acetal derivatives is feasible and quick. All C-4 hydroxylated Annonaceous acetogenins, which are the parent compounds of 2,4-*cis* and *trans* ketolactone acetogenins, are C-4 *R*, and the configuration at C-4 does not change during translactonization.[18] Compound **1** was then identified as (2,4-*cis* and *trans*)-gigantecinone, the ketolactone mixture of the parent compound gigantecin (**3**), which is undoubtedly the precursor of **1** either naturally or as an extraction artifact.[18]

4-Deoxygigantecin (**2)** is a known compound from *Goniothalamus giganteus* whose absolute configuration has not been previously confirmed.[7] The planar structure of **2** was identified by comparing ^{1}H-NMR, ^{13}C-NMR, CIMS, and EIMS fragments to the literature values of **2**.[7] The formaldehyde acetal derivative (**2a**) was prepared to assign the relative stereochemistries across C-14/C-17. The acetal protons in **2a** were exhibited as a pair of doublets at δ 5.26 and 4.62 (J = 7.5 Hz) in the ^{1}H-NMR spectrum (Table 3) indicating a *cis*-C-14/C-17 arrangement. The per-Mosher ester derivatives (**2b**, **2c**) were then prepared for **2a** in order to determine the absolute stereochemistries across the THF rings. Analyzing the COSY spectra of **2b** and **2c** revealed an *R* configuration at C-22 (Table 3). This allowed the assignments of the following chiral centers as 21*R*, 18*R*, 17*R*, 14*S*, 13*S*, and 10*R*. The absolute stereochemistry at C-36 of **2** was determined by CD data. It is reported that a negative Cotton effect at 236 nm in the CD spectrum of squamocin is attributed to the 36*S* configuration in the γ-lactone moiety.[19] The CD spectrum of **2** showed a negative Cotton effect at 239 nm (ϵ −876.7) compared with squamocin [negative Cotton effect at 236 nm (ϵ −973)]; thus, the absolute stereochemistry at C-36 is proposed as *S*, as is common in all other reported Annonaceous acetogenins.[1,2] Thus, both compounds **1** and **2** have the same absolute stereochemistry as that of the parent compound, gigantecin (**3**); and this observation supports a similar biogenetic origin of these compounds.[1] The same inferences were made for 4-deoxyannomontacin, (2,4-*cis* and *trans*)-annomontacinone, and annomontacin.[4]

Two other known Annonaceous acetogenins, (2,4-*cis* and *trans*)-isoannonacin[8] and longimicin C,[9] were isolated for the first time from this genus. Longimicin C is the first bis-THF acetogenin with two flanking hydroxyls to be isolated from this plant.

The biological activities of **1**, **1b**, **2**, **2a**, and **3** are summarized in Table 3. These compounds were all active in the BST;[5] they also showed significant cytotoxicities in the 7-day MTT human solid tumor cytotoxicity tests at the Cell Culture Laboratory, Purdue Cancer Center. Although compound **3** is generally more cytotoxic, **1** and **2** were more selective across the six human tumor cell lines. Selectivities with **1** and **1b** were exhibited for the human prostate adenocarcinoma (PC-3). The activities of **1** and **1b** against PC-3 were 5−15 times the potency of adriamycin (Table 4). The formaldehyde acetal derivatives, **1b** and **2a**, showed activities approximately equivalent to those of the parent compounds. Compounds **1** and **2** showed potent and promising insecticidal activities slightly better than that of rotenone in the yellow fever mosquito larvae

Table 3. ^{1}H NMR Spectral Data of **2** and **2a**−**c** [δ ppm (J = Hz)]

proton	**2**	**2a**	**2b**	**2c**	$^{\Delta\delta}$**2b**−**2c**
3	2.26 tt (7.8, 1.6)		2.26 tt		
4−9	1.22−1.72		1.22−1.72		
10	3.87 m	3.95 m	3.94 m	3.94 m	0.00
11, 12	1.70 m, 1.99 m	1.60−2.00	1.35−1.99		
13	3.80 m	4.00 m	4.00 m	4.02 m	−0.02
14	3.44 m	3.66 m	3.59 m	3.61 m	−0.02
15, 16	1.37−1.74	1.80 m, 1.90 m	1.72 m, 1.84 m	1.83 m, 1.93 m	−0.11, −0.09
17	3.40 m	3.63 m	3.59 m	3.61 m	−0.02
18	3.80 m	4.00 m	3.94 m	4.00 m	−0.06
19a	1.70 m, 2.00 m	1.96 m	1.77 m	1.93 m	−0.16
19b	1.70 m, 2.00 m	1.66 m	1.62 m	1.79 m	−0.17
20a	1.70 m, 2.00 m	1.97 m	1.92 m	2.04 m	−0.12
20b	1.70 m, 2.00 m	1.64 m	1.52 m	1.61 m	−0.09
21	3.80 m	3.84 m	4.08 m	4.08 m	0.00
22	3.40 m	3.39 m	5.05 m	5.05 m	*R*[a]
23	1.22−1.72	1.40 m	1.63 m	1.48 m	+0.15
24−33	1.22−1.72		1.22−1.72		
34	0.88 t (7.0)		0.88 t (7.0)		
35	6.99 q (1.5)		6.98 q (1.5)		
36	4.99 qq (7.0, 1.5)		4.99 qq (7.0, 1.5)		
37	1.40 d (7.0)		1.40 d (7.0)		
38a		5.26 d (7.5)	5.20 d (7.0)	5.22 d (7.0)	−0.02
38b		4.62 d (7.5)	4.56 d (7.0)	4.56 d (7.0)	0.00

[a] Absolute configuration of carbinol center.

Table 4. Bioactivities of Compounds **1**, **1b**, **2**, **2a**, and **3**

LC_{50} or ED_{50} (μg/mL)	**1**	**1b**	**2**	**2a**	**3**[9]	Adriamycin[i]	rotenone[j]
BST[a]	3.27	NT[k]	0.04	NT	222	NT	NT
YFM[b]	0.27	NT	0.68	NT	NT	NT	0.75
A-549[c]	2.14×10^{-1}	2.52×10^{-1}	1.31×10^{-1}	3.82×10^{-2}	2.19×10^{-7}	3.93×10^{-3}	NT
MCF-7[d]	>1	>1	1.0	4.95×10^{-1}	4.11×10^{-9}	3.69×10^{-1}	NT
HT-29[e]	>1	>1	1.43×10^{-1}	$1.83 \times ^{-1}$	2.68×10^{-4}	7.25×10^{-2}	NT
A-498[f]	2.12×10^{-1}	2.71×10^{-1}	3.28×10^{-1}	1.62×10^{-1}	NA[l]	1.13×10^{-3}	NT
PC-3[g]	1.08×10^{-3}	3.06×10^{-3}	1.50×10^{-1}	1.32×10^{-1}	NA	1.46×10^{-2}	NT
PACA-2[h]	>1	>1	3.93×10^{-1}	2.17×10^{-1}	NA	1.42×10^{-3}	NT

[a] LC_{50}: Brine shrimp lethality test.[5] [b] LC_{50}: Yellow fever mosquito larvae test.[20] [c] ED_{50}: Human lung carcinoma. [d] ED_{50}: Human breast carcinoma. [e] ED_{50}: Human colon adenocarcinoma. [f] ED_{50}: Human kidney carcinoma. [g] ED_{50}: Human prostate adenocarcinoma. [h] ED_{50}: Human pancreatic carcinoma, as previously described.[4] [i,j] Positive control standards. [k] NT: not tested. [l] NA: not available.

microtiter (YFM) assay[20] (Table 4). The ketolactone acetogenins may have the advantage of having a wider therapeutic index than their corresponding OH-4 parent acetogenin,[11] for example, (2,4-*cis* and *trans*)-bullatacinone is more effective, but less potent, than bullatacin in vivo against L-1210 murine leukemia.[3] Annonaceous acetogenins are potent inhibitors of oxidative ATP production via blocking at complex I in the mitochondria,[3,21] and, in addition, they act as potent inhibitors of the ubiquinone-linked plasma membrane NADH oxidase of cancerous cells; this second action decreases cytosolic ATP production.[22] The acetogenins also inhibit cells that are resistant to multiple druge due to ATP-dependent efflux mechanism.[23,24]

Experimental Section

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer 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. ^{1}H-NMR, ^{1}H−^{1}H COSY, and ^{13}C-NMR spectra were obtained on a Varian VXR-500S spectrometer. LRMS data were collected on a Finnigan 4000 spectrometer. HRCIMS were performed on a Kratos MS50 instrument. HPLC separations were performed with a Rainin Dynamax solvent delivery system (model SD-200) using a Dynamax software system and a Si 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 Si gel plates (0.25 mm), developed with $CHCl_3$−MeOH (20:1) and visualized with 5% phosphomolybdic acid in EtOH followed by heating.

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.

Bioassays. The bioactivities of extracts, fractions, and pure compounds were routinely assayed using a BST.[5] The yellow-fever mosquito larvae microtiter plate (YFM) assay[20] 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), MCF-7 (human breast carcinoma), HT-29 (human colon adenocarcinoma), A-498 (human kidney carcinoma), PC-3 (human prostate adenocarcinoma), and PACA-2 (human pancreatic carcinoma) as previously described.[4] Adriamycin was always used as a positive antitumor control in the same runs.

Extraction and Isolation. The stem bark was ground into a powder (10.7 kg) and percolated with 95% EtOH. The dry extract residue (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 Si 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 [(2,4-*cis* and *trans*)-isoannonacin and longimicin C] were isolated as colorless waxes and identified by 1D NMR and MS data as compared to literature values.[8,9]

(2,4-*cis* and *trans*)-Gigantecinone (1): a whitish wax (40 mg) ; $[\alpha]^{25}_D$ +23.3° (*c* 0.15, $CHCl_3$); IR (film on NaCl plate) 3507, 3389, 2908, 2837, 1749, 1708, 1461, 1190, 1167, 1049; ^{1}H-, and ^{13}C-NMR: see Tables 1 and 2, CIMS (isobutane) *m*/*z* $[MH]^+$ 639 (100), $[MH - H_2O]^+$ 621 (12), $[MH - 2H_2O]^+$ 603 (34), $[MH - 3H_2O]^+$ 585 (9); HRCIMS (isobutane) *m*/*z* 639.4822 for $C_{37}H_{66}O_8$ $[MH]^+$ (calcd 639.4836); EIMS see Figure 1.

Acetylation of 1. Compound **1** (1−2) mg was dissolved in 0.5−1.0 mL pyridine; 1 mL of anhydrous Ac_2O was added, and the mixture was set at room temperature for 4−8 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 derivative, **1a**, whose ^{1}H-NMR data are shown in Table 1.

Preparation of Mosher Esters. To the formaldehyde derivatives **1b** and **2a** (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 room temperature 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 Si gel (60−200 mesh) and eluted with 3 mL of 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. The similar use of (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride gave the (*R*)-Mosher esters. Both yields were typically higher than 90%. ^{1}H NMR chemical shifts of **1c**, **1d**, **2b**, and **2c** are given in Tables 1 and 2.

Preparation of Intramolecular Formal Acetal Derivatives (1b, 2a). A mixture of DMSO and TMSi (molar ratio 1.2:1) was mixed in 2 mL of benzene and placed in a refrigerator without stirring for 2 h to allow the formation of white crystals. The benzene was decanted, and the crystals were washed twice with CH_2Cl_2. These crystals were added stepwise to 0.5 mL of a $CHCl_3$ solution containing 10 mg of **1** and **2** at room temperature (a large excess of the crystals were added). The reaction was monitored by TLC at intervals of 3 h and was quenched with H_2O after 36−48 h. After workup by extraction with 5% aqueous $NaHCO_3$, the reaction mixture was purified by reversed-phase HPLC using CH_3CN−H_2O (75:25). The yield of **1b** and **2a** was 3 mg (30%) in each case, with some of the unreacted starting material recovered; for the ^{1}H-NMR data of **1b** and **2a**, see Tables 1 and 2.

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