Gonionenin: A New Cytotoxic Annonaceous Acetogenin from *Goniothalamus giganteus* and the Conversion of Mono-THF Acetogenins to Bis-THF Acetogenins

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A new Annonaceous acetogenin, gonionenin (1), has been isolated from the bark of *Goniothalamus giganteus* (Annonaceae). C-21/22 double bonds in 1 and in gigantetronenin (10) were oxidized and then cyclized to give pairs of adjacent bis-THF (4 and 7) and nonadjacent bis-THF (11 and 14) acetogenins, respectively. This is the first reported preparation of bis-THF Annonaceous acetogenins through double-bond epoxidation and cyclization from mono-THF acetogenins. The resulting bis-THF compounds (4, 7, 11, 14) showed enhanced bioactivities, versus 1 and 10, with 11 and 14 being over a billion times more cytotoxic than adriamycin against certain solid tumor cell lines.

Introduction

Goniothalamus giganteus Hook. f. et Thomas (Annonaceae) is a tropical tree widely distributed in southeast Asia. It has been called "black medicine" and has great repute as a drug among the Malays.[1] Extracts of the bark, obtained from Thailand, showed toxicities in the brine shrimp test (BST) and showed murine toxicities in the 3PS (P388) leukemia bioassay.[2] From the ethanol extract of the bark, 11 highly cytotoxic Annonaceous acetogenins have been isolated and reported by our group,[3] and among them, four have a double bond in the aliphatic chain. This is a relatively rare feature in the Annonaceous acetogenins, and of over 90 acetogenins, only one more, bullatenin from *Annona bullata*, has been found to have a double bond in the chain.[4]

In this paper, we report a new mono-THF acetogenin, gonionenin (1), from the bark of *G. giganteus*, which also has a double bond in the aliphatic chain. The C-21/22 double bonds in gonionenin (1) and another mono-THF acetogenin, gigantetronenin (10), previously isolated from this plant by our group, were oxidized with *m*-chloroperbenzoic acid to give epoxides which then were cyclized using perchloric acid with OH groups appropriately located to form another THF ring and give a pair of adjacent bis-THF (4 and 7) and a pair of nonadjacent bis-THF (11 and 14) acetogenins, respectively. The reactions were facile and the yields of the products were high. This conversion of mono-THF acetogenins to adjacent or nonadjacent bis-THF acetogenins not only conclusively determined the position of the double bonds in the molecules but also significantly increased the cytotoxic potencies against certain human solid tumor cell lines (Table 3). These reactions mimic proposed biogenetic pathways leading to the THF rings of Annonaceous acetogenins.[4] This is the first reported preparation of bis-THF Annonaceous acetogenins through epoxidation and cyclization from mono-THF acetogenins possessing a chain double bond.

Results and Discussion

Gonionenin (1) was isolated as a white wax, mp 87-88 °C, $[\alpha]_D$ +19.5 (*c* 0.22, MeOH). The molecular formula of 1 was established to be $C_{37}H_{66}O_7$ by HRFABMS (glycerol) which gave *m/z* 623.4881 for the MH^+ (calcd 623.4887). Spectral characteristics of 1 and its acetate and TMS derivatives, including ^1H NMR (Table 1), ^{13}C NMR (Table 2), and MS (Scheme 1) data, suggested that 1 is a mono-THF Annonaceous acetogenin possessing a double bond on the aliphatic chain.

The IR spectrum of 1 contained a prominent absorption peak for hydroxyls at 3453 cm^{-1}; this peak and sequential losses of four molecules of H_2O from the MH^+ in the CIMS indicated that 1 has four hydroxyl groups. These were confirmed by the preparation of an acetyl derivative (2). 2 gave ^1H NMR peaks at δ 2.08 (6H, 2OAc), 2.04 (3H, OAc), and 2.03 (3H, OAc), and two multiple proton resonances at δ 5.10 (1H) and 4.87-4.83 (3H) corresponding to the downfield shifts of four protons on secondary hydroxyl-bearing carbons as compared to 1.

The IR carbonyl absorption band in 1 at 1754 cm^{-1}, the UV absorption λ_{max} (MeOH) at 209 nm (log ϵ, 4.05), the proton resonances at δ 7.19, 5.06, 3.84, 2.53 2.40, and 1.42, and the carbon signals at δ 174.6, 151.8, 131.0, 78.0, 69.8, and 19.1 provided characteristic spectral features for an α,β-unsaturated γ-lactone fragment with a 4-OH.[4,5]

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Table 1. ^{1}H NMR (500 MHz, $CDCl_3$) Data[a,b] of 1, 2, 4, 5, 7, 8, 11, 12, 14, and 15 [δ, ppm (J = Hz)]

proton	1	2	4	5	7	8	11	12	14	15
3a	2.53 ddd	2.56 ddd	2.52 ddd	2.56 ddt	2.52 ddd	2.56 ddd	2.53 ddd	2.56 ddd	2.52 ddd	2.56 ddd
3b	2.40 ddt	2.52 ddt	2.40 ddt	2.52 ddt	2.40 ddt	2.53 ddt	2.40 ddt	2.52 ddt	2.40 ddt	2.53 ddt
4	3.84 m	5.10 m	3.84 m	5.09 m	3.84 m	5.10 m	3.84 m	5.10 m	3.84 m	5.10 m
10	3.63 m	4.83 m	3.62 m	4.84 m	3.61 m	4.82 m	3.88 m	3.85 m	3.88 m	3.85 m
11	1.44 m	1.56 m	1.70, 1.48	1.55 m	1.71, 1.48	1.55 m	2.02, 1.52	1.97, 1.44	2.02, 1.50	1.97, 1.44
12	1.48 m	1.56 m	1.56 m	1.55 m	1.58 m	1.55 m	1.98, 1.66	1.97, 1.56	1.98, 1.60	1.95, 1.54
13	3.45 m	4.87 m	3.44 m	4.84 m	3.44 m	4.85 m	3.80 m	3.96 m	3.80 m	3.95 m
14	3.82 m	3.97 m	3.85 m	3.98 m	3.91 m	4.08 m	3.41 m	4.84 m	3.42 m	4.83 m
15	2.00, 1.68	1.95, 1.55	1.98, 1.65	1.95, 1.55	1.98, 1.69	1.97, 1.55	1.72, 1.44	1.55 m	1.69, 1.48	1.57 m
16	2.00, 1.68	1.95, 1.55	1.98, 1.65	1.95, 1.78	1.98, 1.69	1.86, 1.65	1.72, 1.44	1.55 m	1.72, 1.58	1.57 m
17	3.82 m	3.97 m	3.85 m	3.90 m	3.90 m	3.92 m	3.41 m	4.83 m	3.47 m	4.86 m
18	3.43 m	4.83 m	3.85 m	3.90 m	3.90 m	3.84 m	3.80 m	3.96 m	3.83 m	3.95 m
19	1.48 m	1.56 m	1.98, 1.65	1.95, 1.78	1.94, 1.82	1.86, 1.72	1.98, 1.66	1.95, 1.56	1.94, 1.78	1.90, 1.56
20	2.19 m	2.00 m	1.98, 1.65	1.95, 1.55	1.94, 1.82	1.86, 1.72	1.98, 1.66	1.95, 1.56	1.94, 1.78	1.90, 1.56
21	5.36 ddd (11, 7, 7)	5.32 ddd (11, 7, 7)	3.85 m	3.98 m	3.89 m	3.94 m	3.80 m	3.96 m	3.83 m	3.95 m
22	5.39 ddd (11, 7, 7)	5.37 ddd (11, 7, 7)	3.39 m	4.84 m	3.37 m	4.88 m	3.41 m	4.83 m	3.40 m	4.86 m
23	2.04 m	2.00 m	1.40 m	1.55 m	1.46 m	1.55 m	1.44 m	1.39 m	1.45 m	1.39 m
34	0.88 t (7)	0.88 t (7)	0.88 t (7)	0.88 t (7)	0.88 t (7)	0.88 t (7)	0.88 t (7)	0.88 t (7)	0.88 t (7)	0.88 t (7)
35	7.19 q (1.5)	7.08 q (1.5)	7.19 q (1.5)	7.08 q (1.5)	7.19 q (1.5)	7.08 q (1.5)	7.19 q (1.5)	7.08 q (1.5)	7.19 q (1.5)	7.08 q (1.5)
36	5.06 qq (7, 1.5)	5.01 qq (7, 1.5)	5.06 qq (7, 1.5)	5.01 qq (7, 1.5)	5.06 qq (7, 1.5)	5.01 qq (7, 1.5)	5.06 qq (7, 1.5)	5.01 qq (7, 1.5)	5.06 qq (7, 1.5)	5.01 qq (7, 1.5)
37	1.44 d (7)	1.40 d (7)	1.43 d (7)	1.40 d (7)	1.43 d (7)	1.40 d (7)	1.43 d (7)	1.40 d (7)	1.43 d (7)	1.40 d (7)
4-OAc		2.03 s		2.03 s		2.03		2.02		2.02 s
10/14-OAc		2.04 s		2.04 s		2.04		2.07		2.07 s
13/17-OAc		2.08 s		2.08 s		2.08		2.07		2.08 s
22-OAc		2.08 s		2.08 s		2.09		2.09		2.09 s

[a] Assignments based on ^{1}H–^{1}H COSY, single-relayed COSY, and double-relayed COSY in $CDCl_3$, TMS as the standard. [b] Signals of other methylene protons appear between δ 1.70 and 1.21 m.

Table 2. ^{13}C NMR (125 MHz, $CDCl_3$) Data of 1, 4, 7, 11, and 14

C	1[a]	4[b]	7[c]	11[d]	14[e]
1	174.6	174.5	174.5	174.5	174.5
2	131.0	131.1	131.1	131.1	131.1
3	33.3	33.4	33.4	33.4	33.4
4	69.8	69.8	69.9	69.9	69.9
5	37.3	37.4	37.5	37.3	37.3
6	25.5	25.5	25.5	25.5	25.5
8	25.6	25.7	25.6	26.2	26.2
9	37.2	37.3	37.3	35.5	35.5
10	71.5	71.6	71.6	79.3	79.2
11				32.4	32.4
12	33.5	33.6	33.6	28.4	28.4
13	74.3	74.1	74.6	82.0	81.9
14	82.6	82.8	82.8	74.3	74.4
17	82.7	81.7	81.7	74.1	74.3
18	73.6	81.9	81.3	82.7	82.7
19	33.4				
20	23.3				
21	128.8	83.2	82.6	82.7	82.8
22	130.7	74.1	73.8	74.4	74.3
23	27.3	33.4	34.4	33.5	34.2
24		25.9	25.9	25.6	25.8
32	31.9	31.9	31.9	31.9	31.9
33	22.7	22.7	22.7	22.7	22.7
34	14.2	14.2	14.2	14.2	14.2
35	151.8	151.8	151.8	151.8	151.8
36	78.0	78.0	78.0	78.0	78.0
37	19.1	19.1	19.2	19.2	19.1

[a] Signals of other methylene carbons appear approximately at δ 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, and 28.8. [b] Signals of other methylene carbons appear approximately at δ 29.8, 29.7, 29.6, 29.5, 29.4, 29.0, and 28.4. [c] Signals of other methylene carbons appear approximately at δ 30.0, 29.8, 29.7, 29.5, 29.5, 29.3, 28.5, and 28.3. [d] Signals of other methylene carbons appear approximately at δ 30.0, 29.7, 29.6, 29.4, 28.8, 28.7, and 28.4. [e] Signals of other methylene carbons appear approximately at δ 30.6, 29.8, 29.7, 29.6, 29.5, 29.4, 28.4, 28.2, and 28.1.

The presence of the mono-THF ring with a flanking OH group on each side was indicated by the proton signals at δ 3.82 (2H, H-14 and 17), 3.45 (H-13), 3.43 (H-18), 2.00 (2H, H-15a and 16a), and 1.68 (2H, H-15b and 16b) in 1 and at δ 4.87 (H-13), 4.83 (H-18), and 3.97 (2H, H-14 and 17) in 2 and the carbon resonances at δ 82.7, 82.6, 74.3, and 73.6 in 1. These NMR data also indicated that the relative stereochemistries of the carbon centers C-13/14 and C-17/18 were *threo* and the configuration across the THF ring (C-14/17) was *trans*, by comparisons with series of model compounds of known relative stereochemistry.[6] The carbon skeleton and the placement of the THF ring were determined on the basis of the EIMS fragmentation of the TMS derivative (3) of 1 (Scheme 1).

The slight downfield shifts, in the ^{1}H NMR spectrum of 1, of both H-13 (at δ 3.45, usually 3.41) and H-10 (at δ 3.63, usually 3.60) indicated that the fourth OH is located close to one of the THF flanking OH's; the same effects were also observed in goniothalamicin[3a] and the 12-hydroxybullatacinones.[7] In the ^{1}H–^{1}H double-relayed COSY spectrum, the correlation cross peaks between δ 3.45 and 3.63 were clearly observed, placing the fourth OH at C-10, which was also supported by the observation of EIMS fragments of 3 at *m/z* 385 and 313 (Scheme 1). The double-relayed COSY result also helped in suggesting the full assignments of proton chemical shifts around the THF ring.

The presence of an isolated double bond in 1 was determined by the proton signals at δ 5.39 and 5.36 and the carbon signals at δ 130.7 and 128.8. When the protons were selectively decoupling at δ 2.19 (H-20), which showed correlation cross peaks with the proton at δ 5.36 (H-21) in the COSY spectrum, the latter became a doublet (J = 11.0 Hz). This indicated that the double bond has a *cis*

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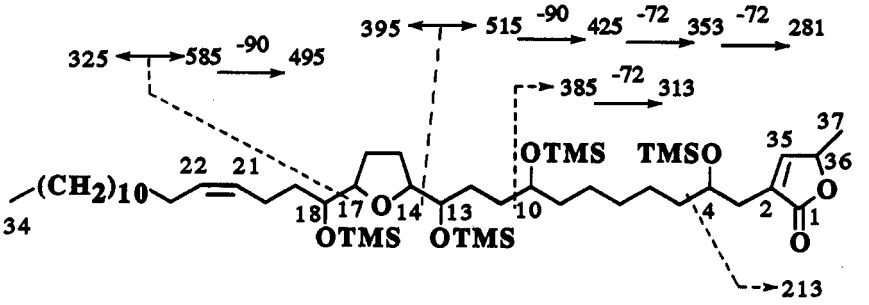
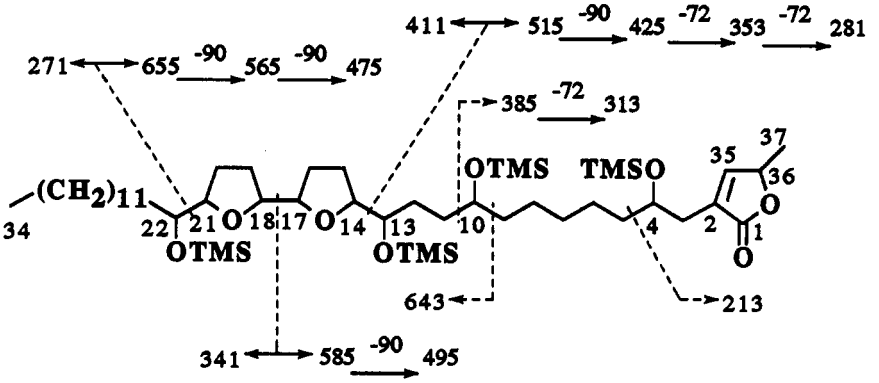
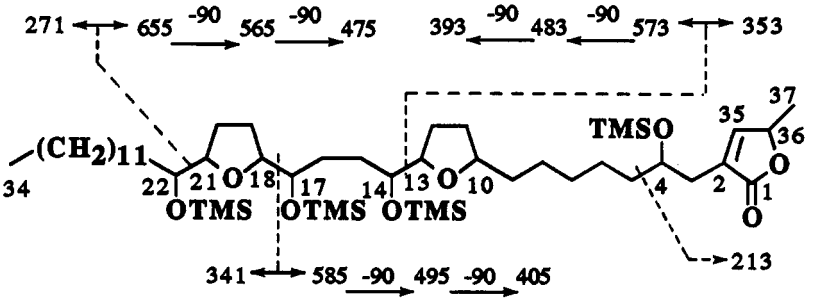
Scheme 1



Compound 1



Compounds 4 and 7



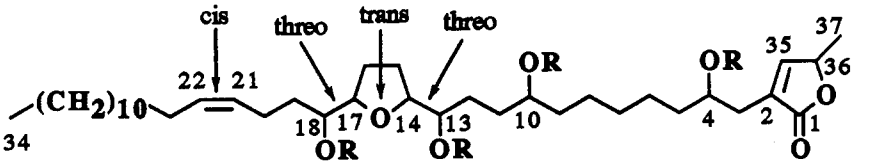
Compounds 11 and 14

configuration. The position of the double bond was determined at C-21/22 from the single-relayed COSY spectrum, which showed correlation cross peaks between H-18 (δ 3.43) and H-20 (δ 2.19), and the double-relayed COSY spectrum, which showed correlation cross peaks between H-18 (δ 3.43) and H-21 (δ 5.36). From the above spectral data, the structure of 1 was concluded (Chart 1) to be a new acetogenin, as illustrated, and the compound was named gonionenin.

Among Annonaceous acetogenins, most bis-THF compounds show much higher cytotoxic potencies than the mono-THF compounds.[4,8] The THF rings of these acetogenins are proposed to be biosynthesized from double bonds in the parent fatty acids through epoxide intermediates.[4] On the basis of this biogenetic proposal, 1 was oxidized by *m*-chloroperbenzoic acid (*m*-CPBA) to give the 21/22-epoxide (17) of 1. Then, 17 was cyclized using perchloric acid ($HClO_4$) to give a mixture of 4 and 7,[6,9] which were resolved by HPLC (Scheme 2). This reaction also served to confirm that the position of the double bond in 1 is at C-21/22.

(8) In rat liver mitochondrial bioassays, the bis-THF acetogenins also show higher activities than the mono-THF acetogenins (Landolt, J. L.; Ahammadsahib, K. I.; Hollingworth, R. M.; Barr, R.; Crane, F. L.; Buerck, N. L.; McCabe, G. P.; McLaughlin, J. L. *Chemico-Biol. Interact.* **1994**, submitted for publication). The Annonaceous acetogenins act as potent inhibitors of complex I in mitochondrial electron transport systems (Londershausen, M.; Leicht, W.; Lieb, F.; Moeschler, H.; Weiss, H. *Pestic. Sci.* **1991**, *33*, 427. Lewis, M. A.; Arnason, J. T.; B. Philogene, J. R.; Rupprecht, J. K.; McLaughlin, J. L. *Pestic. Biochem. Physiol.* **1993**, *45*, 15. Ahammadsahib, K. I.; Hollingworth, R. M.; Hui, Y.-H.; McGovren, J. P.; McLaughlin, J. L. *Life Sci.* **1993**, *53*, 1113).

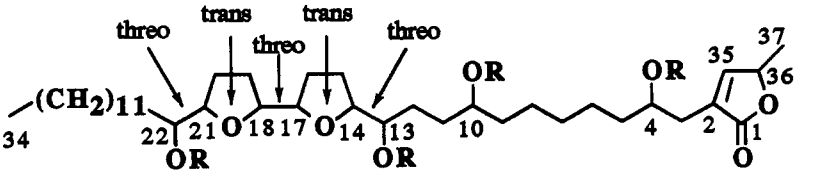
Chart 1. Structures of Compounds 1–9



1 Gonionenin, **R = H**

2 Gonionenin Tetraacetate, **R = Ac**

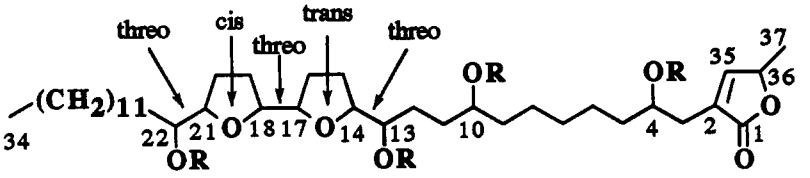
3 Tetra-TMS Derivative of Gonionenin, **R = TMS**



4 Cyclogonionenin T, **R = H**

5 Tetraacetate of Cyclogonionenin T, **R = Ac**

6 Tetra-TMS Derivative of Cyclogonionenin T, **R = TMS**



7 Cyclogonionenin C, **R = H**

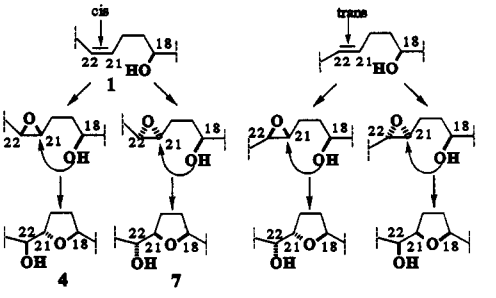
8 Tetraacetate of Cyclogonionenin C, **R = Ac**

9 Tetra-TMS Derivative of Cyclogonionenin C, **R = TMS**

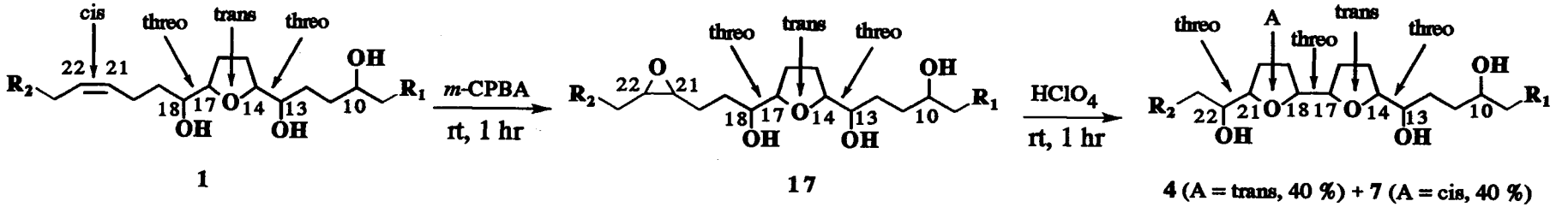
The structure of the carbon skeleton of **4** was confirmed by EIMS fragmentation of the TMS derivative (**6**, Scheme 1). All of the ^1H and ^{13}C NMR data of **4** were very similar to those of **1** except for those corresponding to the THF unit. The proton signals at δ 3.85 (4-H, H-14, 17, 18, and 21), 3.44 (H-13), and 3.39 (H-22) and carbon signals at δ 83.2 (C-21), 82.8 (C-14), 81.9 (C-18), 81.7 (C-17), and 74.1 (C-13 and 22) of **4** supported the existence of the adjacent bis-THF ring system.[4] The proton signals of this bis-THF unit at δ 4.84 (2H, H-13 and 18), 3.98 (2H, H-14 and 21), and 3.90 (2H, H-17 and 18) in the tetraacetate **5** of **4** showed a pseudosymmetrical unit like that of asimicin,[4] and both of the carbon chemical shifts of the flanking hydroxylated carbons in **4** appeared at δ 74.1 and indicated that the relative stereochemistries of this bis-THF unit are *threo/trans/threo/trans/threo* from C-13 to C-22,[6] the same as that of asimicin.[4]

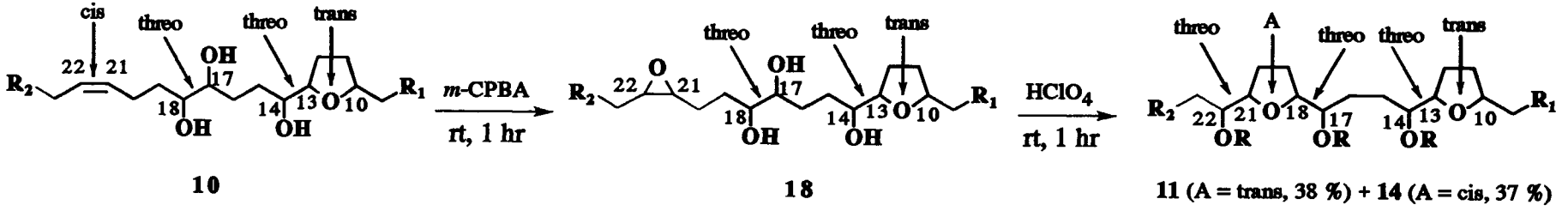
The carbon skeleton of **7** is the same as that of **4** (Scheme 1), and **7** differs from **4** only in the relative stereochemistry of the second THF ring (C-18/21) which should have the *cis* configuration.[9] In the ^1H NMR spectra of **4** and **7**, the

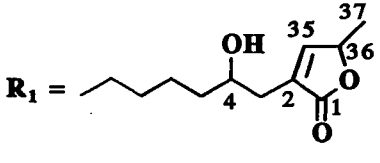
(9) The anticipated mechanism of this reaction, shown below, suggests that if the double bond at C-21/22 is *cis*, there is only a single pair of products and both have the *threo* configuration in carbon centers C-21/22; the *trans* double bond would similarly generate two products with the *erythro* configuration between C-21/22 (the absolute configurations may be the mirror images of the illustrations).



Scheme 2







$R_2 = CH_3(CH_2)_9CH_2$

signals corresponding to the protons on the oxygenated carbons of the THF rings were very similar, although those of the THF methylene protons (H-19 and 20) showed sharp differences (Table 1). H-19a and H-20a of **7** were located at δ 1.94, shifted upfield, and H-19b and H-20b of **7** were at δ 1.82, shifted downfield, compared with those of **4** and the methylene protons (H-15 and 16) of the other THF ring of **7** (at δ 1.98 and 1.65, respectively). This observation is very useful in the differentiation of *cis* from *trans* THF rings in the acetogenins.[10] The proton signals of the bis-THF unit of the tetraacetate **8** of **7** at δ 4.88 (H-22), 4.85 (H-13), 4.08 (H-14), 3.94 (H-21), 3.92 (H-17), and 3.84 (H-18) exactly matched with those of model bis-THF compounds with the *threo/trans/threo/cis/threo* configuration.[6b] Thus, **4** and **7** were determined to have the structures as illustrated in Chart 1. Both compounds are new to Annonaceous acetogenins, although they perhaps do not occur naturally, and **7** is the first acetogenin having the adjacent bis-THF ring system with a *threo/trans/threo/cis/threo* relative stereochemistry. **4** was named cyclogonionenin T and **7** was named cyclogonionenin C.

Gigantetronenin (**10**) is a major acetogenin isolated from *G. giganteus* and also contains a mono-THF and an isolated chain double bond. Recently, **10** was also found in *Xylopia aromatica*.[11] In a suggested biogenetic pathway of the acetogenins from *G. giganteus*, Fang *et al.* suggested that **10** was possibly the precursor of gigantecin.[3b,4] To mimic this proposed biogenesis, **10** was treated with *m*-CPBA and then perchloric acid and produced a mixture of **11** and **14** (Scheme 2), which were separated by HPLC.

The disappearance of double-bond NMR signals of **10**, the proton signals at δ 3.83 (2H, H-18 and 21), 3.47 (H-17), and 3.40 (H-22), and carbon signals at δ 82.8 (C-21), 82.7 (C-18), and 74.3 (C-17 and 22) in **11** indicated the formation of a new THF ring, since both the ^1H and ^{13}C NMR data of the remainder of **11** were almost the same as those of **10**. The carbon skeleton was confirmed by the EIMS analysis of the TMS derivative **13** of **11** (Scheme 1). The proton signals of H-19a and -20a of this new THF were at δ 1.98 and those of H-19b and -20b were at δ 1.66; this suggested a *trans* arrangement for the THF (C-18/21)[6,9] and both of the carbon signals of the flanking hydroxyl-bearing carbons were at δ 74.3 and indicated the *threo* configuration for both carbon centers C-17/18 and C-21/22.[9] Thus, the structure of **11** (Chart 2) was concluded to be as illustrated. The skeleton and relative stereochemistries of **11** were exactly the same as those of gigantecin,[3,4] and co-TLC of **11** and gigantecin in several different solvent systems always showed them to be inseparable. Gigantecin, with low content, was isolated from *G. giganteus* previously by our group, was the first nonadjacent bis-THF acetogenin to be reported, and showed potent bioactivities in several bioassays.[3b] This conversion not only supports the hypothesis that gigantetronenin (**10**) may be the precursor of gigantecin (**11**) but also provides a new, more abundant, source of **11**.

The carbon skeleton of **14**, identical to that of **11**, was determined by the EIMS analysis of the TMS derivative **16** of **14**. **14** differs from **11** only in the stereochemistry of the newly formed THF ring (C-18/21). The proton signals of the THF methylene protons of **14** at δ 1.94 and 1.78 (Table 1), instead of at δ 1.98 and 1.65 corresponding to the *trans* THF arrangement,[9,10] indicated the *cis* configuration for this THF ring. This result also matched well with the suggested mechanism of the cyclization; since **11** has the *trans* configuration for this THF, **14** should have the *cis* configuration.[9] The structure of **14** (Chart 2), thus, was determined to be as illustrated; it represents a new type of nonadjacent bis-THF Annonaceous acetogenin and was named C-18/21-*cis*-gigantecin.

The bioassay data summarized in Table 3 indicates that all of the newly prepared bis-THF compounds are more bioactive than their parent compounds; the potent bioactivities of **11** and **14** are especially noteworthy and approach the levels previously observed with adjacent bis-THF acetogenins such as asimicin, trilobacin, and bullatacin.[4]

Experimental Section

Methods. Experimental procedures employed instruments and methods essentially as previously described.[3e,4a,b,7]

Bioassays. The extracts, fractions, and isolates were routinely evaluated for lethality to brine shrimp larvae (BST).[2a,b] Cytotoxicities against human solid tumor cells were measured at the Purdue Cell Culture Laboratory, Purdue Cancer Center, for the A-549 lung carcinoma,[12] MCF-7 breast carcinoma,[13] and HT-29 colon adenocarcinoma.[14]

Plant Material. 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, MD, where voucher specimens are maintained.

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 a 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 a MeOH layer (ca. 100 g of dry residue) and a hexane layer. The MeOH residue, which represented the most active fraction in the BST test (LC_{50} 15.1 μg/mL), was repeatedly chromatographed over silica gel columns directed by BST activity, using gradients of C_6H_6/EtOAc/MeOH, hexane/EtOAc, and $CHCl_3$/MeOH and purified by HPLC over silica gel, using hexane–MeOH–THF (90:9:1), to give a white wax of **1** (31 mg).

Oxidization and Cyclization of Gonionenin (1). To gonionenin (**1**, 20 mg, in 10 mL of CH_2Cl_2) was added *m*-chloroperbenzoic acid (*m*-CPBA, 13 mg), and the mixture was stirred for 1 h at room temperature. The mixture was washed using 1% $NaHCO_3$ (5 mL) and H_2O (2 × 5 mL), and the CH_2Cl_2 layer was dried *in vacuo* to give the 21/22-epoxide **17** of **1**; to **17** (in 10 mL of CH_2Cl_2) was added 30% perchloric acid ($HClO_4$, 5 μL). The mixture was stirred for another hour at room temperature to give a mixture of cyclogonionenins T and C (**4** and **7**). The mixture was washed using 1% $NaHCO_3$ (5 mL) and H_2O (2 × 5 mL), and the CH_2Cl_2 layer was dried *in vacuo* and resolved by HPLC to give 8 mg of **4** (yield 40%) and 8 mg of **7** (yield 40%).

Oxidization and Cyclization of Gigantetronenin (10). Gigantetronenin (**10**) was available as previously isolated from *G. giganteus*.[3e] The oxidization and cyclization procedures were the same as described above with gonionenin (**1**): 90 mg of **10** gave 35 mg of gigantecin (**11**, yield 38%) and 35 mg of C-18/21-*cis*-gigantecin (**14**, yield 37%).

Gonionenin (1): white wax, mp 87–88 °C, $[\alpha]_D$ +19.5 (*c* 0.22, MeOH); UV λ_{max} (MeOH) 209 nm (log ε, 4.05); IR ν_{max} (film) 3454, 2910, 2852, 2361, 1732, 1468, 1317, 1108 cm^{-1}; HRFABMS

(10) Hoye *et al.* also obtained similar results in observing the ^1H NMR spectra of model mono-THF diol compounds (personal communication, T. R. Hoye).

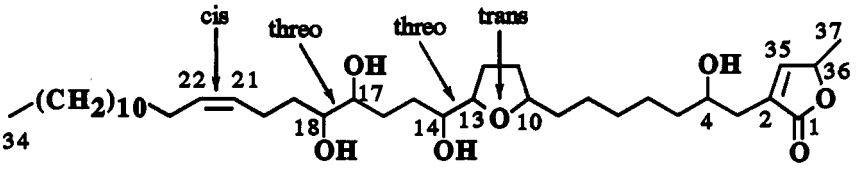
(11) Colman-Saizarbitoria, T.; Zambrano, J.; Ferrigni, N. R.; Gu, Z.-M.; Ng, J. H.; Smith, D. L.; McLaughlin, J. L. *J. Nat. Prod.* **1994** (in press).

(12) Giard, D. J.; Aronson, S. A.; Todaro, G. J.; Arnstein, P.; Kersey, J. H.; Dosik, H.; Parks, W. P. *J. Natl. Cancer Inst.* **1973**, *51*, 1417.

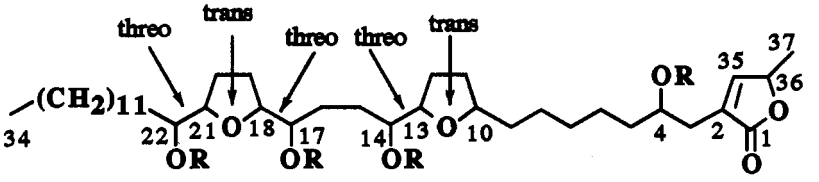
(13) Soule, H. D.; Vazquez, J.; Long, A.; Albert, S.; Brennan, M. *J. Natl. Cancer Inst.* **1973**, *51*, 1409.

(14) Fogh, J.; Trempe, G. New Human Tumor Cell Lines *in Human Tumor Cells, in vitro*; Fogh, J., Ed.; Plenum Press: New York, 1975; p 115.

Chart 2. Structures of Compounds 10–16



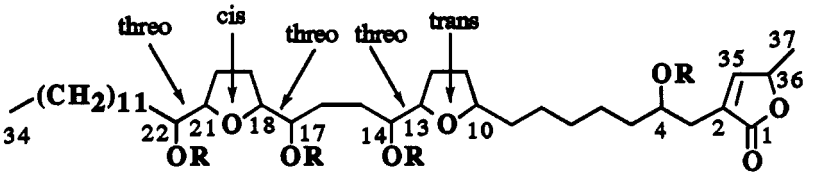
10 Gigantetronenin



11 Gigantecin, **R = H**

12 Gigantecin Tetraacetate , **R = Ac**

13 Tetra-TMS Derivative of Gigantecin, **R = TMS**



14 C-18/21-*cis*-gigantecin, **R = H**

15 C-18/21-*cis*-gigantecin Tetraacetate , **R = Ac**

16 Tetra-TMS Derivative of C-18/21-*cis*-gigantecin, **R = TMS**

Table 3. Bioactivities of Compounds 1, 4, 7, 10, 11 and 14[a]

compd	BST[b] LC_{50} (μg/mL)	A-549[c] ED_{50} (μg/mL)	MCF-7[d] ED_{50} (μg/mL)	HT-29[e] ED_{50} (μg/mL)
1	21.7	1.34×10^{-3}	4.54×10^{-3}	1.12×10^{-4}
4	1.95×10^{-1}	1.41×10^{-3}	1.19×10^{-3}	1.54×10^{-7}
7	2.71	1.07×10^{-3}	1.79×10^{-3}	4.08×10^{-5}
10	10.4	2.22×10^{-8}	1.49×10^{-8}	1.0×10^{-12}
11	3.44×10^{-2}	$<10^{-12}$	1.0×10^{-4}	$<10^{-12}$
14	2.49×10^{-1}	$<10^{-12}$	$<10^{-12}$	$<10^{-12}$
adriamycin[f]	8×10^{-2}	9.20×10^{-3}	2.31×10^{-1}	3.92×10^{-2}

[a] All the cytotoxicities against human tumor cell lines were tested in the same run. [b] Brine shrimp lethality test.[12] [c] Human lung carcinoma.[13] [d] Human breast carcinoma.[14] [e] Human colon adenocarcinoma.[15] [f] Positive control standard.

(glycerol) obsd 623.4881 (MH^+), calcd for $C_{37}H_{66}O_7$ 623.4887. ^1H and ^{13}C NMR: see Tables 1 and 2, respectively.

21/22-Epoxide of gonionenin (17): white wax; FABMS 639 (MH^+); ^1H NMR (500 MHz, $CDCl_3$) δ 7.20 [q, 1H, J = 1.5 Hz, H-35], 5.06 [qq, 1H, J = 7.0, 1.5 Hz, H-36], 3.88–3.80 [m, 3H, H-4, H-14, and H-17], 3.65 [m, 1H, H-10], 3.50–3.44 (m, 2H, H-13 and H-18], 2.98–2.92 [m, 2H, H-21 and H-22], 2.52 [ddd, 1H, J = 15.0, 8.0, 1.5, 1.1 Hz, H-3a], 2.41 [ddt, 1H, J = 15.0, 8.0, 1.4 Hz, H-3b], 1.96–2.06 [m, 2H, H-11a and H-12a], 1.90–1.22 [m, 45H], 0.88 [t, 3H, J = 7.0 Hz, H-34].

Cyclogonionenin T (4): white wax, mp 61–62 °C, $[\alpha]_D$ +18.3 (c 0.46, MeOH); UV λ_{max} (MeOH) 210 nm (log ε, 4.01); IR ν_{max} (film) 3475, 2922, 2853, 2361, 1723, 1467, 1443, 1325, 1060 cm^{-1}; HRFABMS (glycerol) obsd 639.4808 (MH^+), calcd for $C_{37}H_{67}O_8$ 639.4836. ^1H and ^{13}C NMR: see Tables 1 and 2, respectively.

Cyclogonionenin C (7): white wax, mp 69–70 °C, $[\alpha]_D$ +3.0 (c 0.33, MeOH); UV λ_{max} (MeOH) 209 nm (log ε, 4.03); IR ν_{max} (film) 3406, 2925, 2854, 2359, 2341, 1754, 1462, 1071 cm^{-1}; HRFABMS (glycerol) obsd 639.4828 (MH^+), calcd for $C_{37}H_{67}O_8$ 639.4836. ^1H and ^{13}C NMR: see Tables 1 and 2, respectively.

21/22-Epoxide of gigantetronenin (18): white wax; FABMS 639 (MH^+); ^1H NMR (500 MHz, $CDCl_3$) δ 7.19 [q, 1H, J = 1.5 Hz, H-35], 5.06 [qq, 1H, J = 7.0, 1.5 Hz, H-36], 3.92–3.78 [m, 3H, H-4, H-10, and H-13], 3.52–3.40 (m, 3H, H-14, H-17, and H-18], 2.98–2.92 [m, 2H, H-21 and H-22], 2.52 [ddd, 1H, J = 15.0, 8.0, 1.5, 1.1 Hz, H-3a], 2.40 [ddt, 1H, J = 15.0, 8.0, 1.4 Hz, H-3b], 1.96–2.06 [m, 2H, H-11a and H-12a], 1.90–1.22 [m, 45H], 0.88 [t, 3H, J = 7.0 Hz, H-34].

Gigantecin (11): white wax, mp 109–110 °C, $[\alpha]_D$ +5.3 (c 1.8, MeOH); UV λ_{max} (MeOH) 212 nm (log ε, 3.60); IR ν_{max} (film) 3453, 2918, 2850, 2360, 2340, 1754, 1728, 1469, 1322, 1059 cm^{-1}; HRFABMS (glycerol) obsd 639.4841 (MH^+), calcd for $C_{37}H_{67}O_8$ 639.4836. ^1H and ^{13}C NMR: see Tables 1 and 2, respectively.

C-18/21-*cis*-Gigantecin (14): white wax, mp 89–90 °C, $[\alpha]_D$ +6.7 (c 1.5, MeOH); UV λ_{max} (MeOH) 212 nm (log ε, 3.74); IR ν_{max} (film) 3440, 2918, 2851, 1754, 1726, 1468, 1306 cm^{-1}; HRFABMS

(glycerol) obsd 639.4854 (MH^+), calcd for $C_{37}H_{67}O_8$ 639.4836. ^1H and ^{13}C NMR: see Tables 1 and 2, respectively.

Acetylations. Compounds **1**, **4**, **7**, **11**, and **14** (0.5 mg of each) were mixed with anhydrous pyridine/Ac_2O at rt overnight and, through the usual workup, gave ca. 0.5 mg of the tetraacetates **2**, **5**, **8**, **12**, and **15**, respectively. ^1H NMR data are shown in Table 1.

TMS Derivatizations. Compounds **1**, **4**, **7**, **11**, and **14** (ca. 0.3 mg of each) were treated with *N,O*-bis(trimethylsilyl)-acetamide (20 μL) and pyridine (2 μL) and heated at 70 °C for 30 min to yield the respective tetra-TMS derivatives **3**, **6**, **9**, **13**, and **16**. EIMS fragmentations are shown in Scheme 1.

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Supplementary Material Available: Copies of ^1H, ^{13}C, and 2D NMR spectra of **1**, **2**, **4**, **5**, **7**, **8**, **10**, **11**, **12**, **14**, **15**, **17**, and **18** (28 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.