GIGANENIN, A HIGHLY POTENT MONOTETRAHYDROFURAN ACETOGENIN AND 4-DEOXYGIGANTECIN FROM GONIOTHALAMUS GIGANTEUS

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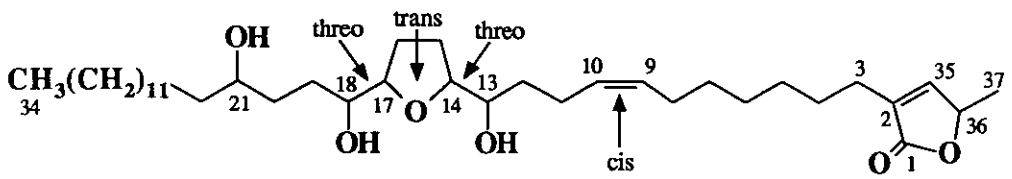
Abstract- Giganenin, a novel monotetrahydrofuran acetogenin with a double bond along the hydrocarbon chain, and 4-deoxygigantecin, a new nonadjacent bistetrahydrofuran acetogenin, have been isolated from the bark of Goniothalamus giganteus (Annonaceae) by the use of brine shrimp lethality for bioactivity-directed fractionation. The structures were elucidated based on spectral and chemical methods. Giganenin, which shows selective and highly potent cytotoxicities to human tumor cells in culture (ED_{50} values as low as 5.80×10^{-8} μg/ml) and toxicity to brine shrimp, is the most cytotoxic of the monotetrahydrofuran acetogenins reported thus far. 4-Deoxygigantecin is also cytotoxic to human tumor cells and toxic to brine shrimp.

In our bioactivity-directed search for antitumor natural products, two major classes of bioactive compounds have been found in the bark extracts of Goniothalamus giganteus Hook. f. & Thomas (Annonaceae) from Thailand. One class is the styryllactones, including altholactone, goniothalamin,[1] goniotriol,[2] goniofufurone, goniopypyrone, 8-acetylgoniotriol,[3] 9-deoxygoniopypyrone, 7-epigoniofufurone, goniodiol,[4] goniobutenolides

A and B, and goniofupyrone.[5] Most of these compounds show marginal cytotoxicities against human tumor cell lines, but several have unique heterocyclic structures. The other class is the Annonaceous acetogenins, including several monotetrahydrofuran acetogenins, goniothalamicin, annonacin,[6] gigantetracin, and gigantriocin,[7] as well as the potent nonadjacent bistetrahydrofuran acetogenin, gigantecin.[8] Most of these acetogenins are significantly cytotoxic to certain human tumor cell lines. Our continued investigation of this plant, using the brine shrimp assay, has now yielded giganenin (**1**), a novel monotetrahydrofuran acetogenin with a double bond in the hydrocarbon chain between the α,β-unsaturated γ-lactone and the tetrahydrofuran ring. This is the first reported Annonaceous acetogenin containing a double bond in the hydrocarbon chain although such compounds have been predicted as biogenetic precursors.[9] 4-Deoxygigantecin (**5**), a new nonadjacent bistetrahydrofuran analogue of gigantecin (**9**), was also isolated and characterized.



1 R = H **2** R = Ac **3** R = TMS **4** R = d-TMS

Giganenin (**1**) was isolated as an amorphous solid, mp 60-62°C, $[\alpha]^{25}_D$ = + 21.4° (c 0.23 in MeOH). The molecular weight was indicated by peaks at m/z 607 (MH^+) in the isobutane CIms and FABms, as well as 822 (M^+) and 849 (M^+) in the EIms of its tri-trimethylsilyl (TMS) derivative (**3**) and tri-perdeuterotrimethylsilyl (d-TMS) derivative (**4**). The HRFABms measurement gave m/z 607.4889 for the MH^+ (calcd 607.4937), corresponding to the molecular formula, $C_{37}H_{60}O_6$. The presence of an α,β-unsaturated γ-lactone was suggested by an ir carbonyl absorption band at 1751 cm^{-1}, an uv (MeOH) λ_{max} at 207 nm (log ε 3.77), four proton resonances at δ 6.97 (q, H-35), 4.97 (qq, H-36), 2.23 (tt, H-3), and 1.38 (d, H-37) in the ^1H nmr spectrum and five carbon resonances at δ 173.82 (C-1), 148.86 (C-35), 134.16 (C-2), 77.42 (C-36), and 19.23 (C-37) in the ^{13}C nmr spectrum (Table 1). These are characteristic spectral features for the α,β-unsaturated γ-lactone fragment without a 4-OH moiety in the Annonaceous acetogenins.[9]

The existence of three hydroxyl moieties was obvious by an ir hydroxyl absorption at 3423 cm^{-1}, three successive losses of water (m/z 18) from MH^+ in both the isobutane CIms and FABms, m/z 589 ($MH^+ - H_2O$), 571 ($MH^+ - 2\ H_2O$), and 553 ($MH^+ - 3\ H_2O$), and the preparation of a triacetate derivative (**2**) (acetic anhydride in pyridine), a tri-TMS derivative (**3**) [bis(trimethylsilyl)acetamide in pyridine], and a tri-d-TMS derivative (**4**). **2**

gave three singlet peaks at δ 2.081 (3H, OAc), 2.079 (3H, OAc), and 2.043 (3H, OAc) and two multiple resonances at δ 4.86 (H-13,18) and 4.83 (H-21) corresponding to the downfield shift of three protons on secondary hydroxyl-bearing carbons in **1** after acetylation. The EIms of **3** and **4** showed three successive losses of TMSOH (- 90), 732 (M^+ - TMSOH), 642 (M^+ - 2 TMSOH), and 552 (M^+ - 3 TMSOH), or d-TMSOH (- 99), 750 (M^+ - d-TMSOH), 651 (M^+ - 2 d-TMSOH), and 552 (M^+ - 3 d-TMSOH), from the molecular ion, respectively. Furthermore, the ^{13}C nmr spectrum of **1** showed three resonances due to oxygen-bearing carbons at δ 74.32, 73.50, and 71.85 and absence of a signal characteristically resonating at ca. δ 69,[9] indicating the existence of three secondary hydroxyl moieties without a 4-OH. The presence of a monotetrahydrofuran ring with two hydroxyl groups adjacent to the ring was suggested by proton resonances at δ 3.80 (H-14, 17) and 3.41 (H-13, 18) and carbon resonances at δ 82.62 (C-14), 82.58 (C-17), 74.32 (C-13), and 73.50 (C-18), which were directly analogous to similar resonances of other monotetrahydrofuran acetogenins, such as annonacin,[6,10] goniothalamicin,[6] and squamone.[11] The ^1H nmr spectrum of **1** showed two interesting resonances at δ 5.37 (dt, J = 10.91, 7.03 Hz) and 5.32 (dt, J = 10.91, 6.86 Hz) (the J values were measured by selective decoupling), suggesting the presence of an isolated cis double bond; this group was further confirmed by two carbon resonances at δ 130.76 and 128.82.

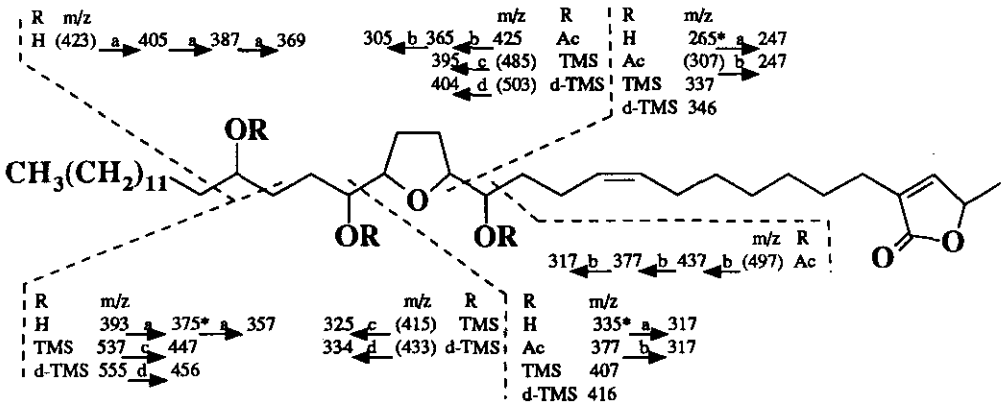


Figure 1. Diagnostic EIms fragment ions of giganenin (**1** R = H), triacetate derivative (**2** R = Ac), tri-trimethylsilyl derivative (**3** R = TMS), and tri-perdeutero-trimethylsilyl derivative (**4** R = d-TMS). The elemental compositions of fragments marked with an asterisk were confirmed through exact mass measurements. Peaks in parentheses were not seen. Letters above the arrows represent (a) loss of H_2O (m/z 18), (b) loss of AcOH (m/z 60), (c) loss of TMSOH (m/z 90), and (d) loss of d-TMSOH (m/z 99).

The carbon skeleton and placement of the tetrahydrofuran ring and the three hydroxyl groups along the hydrocarbon chain were determined based on EIms spectral analysis of **1** - **4** (Figure 1). Fragments in the EIms at m/z 265 (loss of water giving m/z 247) and 335 (loss of water giving m/z 317) for **1**, m/z 437, 425, 377, and 247 for **2**, m/z 407, 395, 337, and 325 for **3**, and m/z 416, 404, 346, and 334 for **4** clearly positioned the tetrahydrofuran ring at C-14 along the hydrocarbon chain and supported the assignment of two hydroxyl groups at C-13 and C-18 adjacent to the tetrahydrofuran ring as indicated by the nmr data. The position of the remaining hydroxyl moiety was illustrated by EIms fragments at m/z 393, 375, 357, 405, 387, and 369 for **1**, m/z 537 and 447 for **3**, and m/z 555 and 465 for **4**. The three major fragments of **1** were confirmed by HREIms measurements which gave m/z 265.1800 for $C_{16}H_{25}O_3$ (calcd 256.1803), 335.2226 for $C_{20}H_{31}O_4$ (calcd 335.2222), and 375.2530 for $C_{23}H_{35}O_4$ (calcd 375.2535).

Table 1. Nmr data for giganenin (**1**) and its triacetate (**2**).

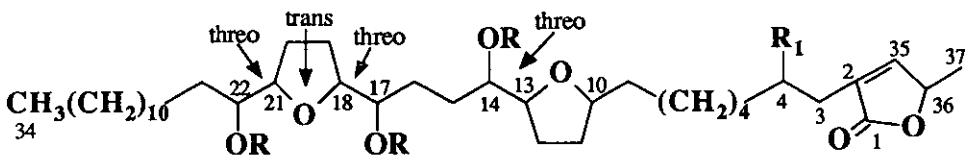
Atom	^{1}H Nmr [ppm,(J/Hz)] of **1** (500 MHz, $CDCl_3$)	^{1}H Nmr [ppm,(J/Hz)] of **2** (500 MHz, $CDCl_3$)	^{13}C Nmr (ppm) of **1** (125 MHz, $CDCl_3$)
1	------	------	173.82
2	------	------	134.16
3	2.23 tt (7.7, 1.5)	2.26 tt (7.8, 1.6)	25.17
4	1.52 m*	1.62 - 1.22 m	27.38
5-6	1.66 - 1.22	1.62 - 1.22 m	29.76 - 29.10
7	1.30 m*	1.62 - 1.22 m	31.94**
8	2.01 q (7.1)	2.04 m	27.26
9	5.37 dt (10.9, 7.0)	5.38 dt (10.8, 7.1)	130.76
10	5.32 dt (10.9, 6.9)	5.31 dt (10.8, 7.1)	128.82
11	2.15 m	2.10 m	25.71
12,19	1.43 m*	1.62 - 1.22 m	33.54** and 37.50**
13,18	3.41 m	4.86 m	74.32 and 73.50
14,17	3.80 m	3.98 m	82.62 and 82.58
15,16	1.97 m and 1.69 m	1.98 m and 1.60 m	28.77 and 28.75
20,22	1.41 m* and 1.50 m*	1.62 - 1.22 m	33.44** and 31.94**
21	3.60 m	4.83 m	71.85
23-31	1.66 - 1.22 m	1.62 - 1.22 m	29.76 - 29.10
32-33	1.66 - 1.22 m	1.62 - 1.22 m	23.33 and 22.71
34	0.85 t (6.8)	0.88 t (6.5)	14.16
35	6.97 q (1.5)	6.99 q (1.6)	148.86
36	4.97 qq (6.9, 1.5)	4.99 qq (6.8, 1.6)	77.42
37	1.38 d (6.9)	1.41 d (6.8)	19.23
Ac-13,18	------	2.081 s, 2.079 s	------
Ac-21	------	2.043 s	------

* Determined by trace function in the ^{1}H-^{1}H COSY nmr. ** Signals may be interchangeable.

The position of the double bond was determined from the ^{1}H-^{1}H COSY and double relayed COSY spectra of **1** to be betweem carbons 9 and 10. Correlation cross peaks were seen from H-13 (δ 3.41) to H-12 (δ 1.43) which, in turn, showed cross peaks to H-11 (δ 2.15); then, H-11 showed cross peaks to one double bond proton, H-10 (δ 5.32). Because the H-12 signal was overlapped with some other proton signals, a double relayed COSY

spectrum (tau_1 = tau_2 = 0.08 sec) of **1** was measured to confirm this assignment. As the result, strong cross peaks between H-10 and H-13 were seen in the double relayed COSY spectrum. Several single relayed COSY spectra of **1** were also measured to eliminate other possible assignments. Meanwhile, the placement of the 21-OH was also confirmed by seeing double relayed correlation peaks between H-21 and H-18.

The relative stereochemistries between C-13/C-14 and C-17/C-18 were determined by comparing the ^{13}C nmr signals of **1** for the hydroxylated carbons at C-13 (δ 74.32) and C-18 (δ 73.50) as well as the ^{1}H nmr signals of **1** for H-13, 18 (δ 3.41) and H-14, 17 (δ 3.80) with those of model compounds of known relative stereochemistry, suggesting that the relative configurations between C-13/C-14 and C-17/C-18 were both threo.[12] The threo assignments were further substantiated by comparing proton resonances of **2** at δ 3.98 (H-14,17) and 4.86 (H-13, 18) with those of a group of diacetyl dibutylated bistetrahydrofurans of known stereochemistry.[13] The ^{1}H nmr signals of **2** at δ 3.98 for H-14 and H-17 suggested the trans configuration of these two protons.[14] The configurations of the chiral centers at C-21 and C-36 remain undefined. The ^{1}H nmr and ^{13}C nmr data of **1** were assigned based on the ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY. Thus, the structure of compound **1** was determined as illustrated and named giganenin.



5 R = H, R_1 = H **6** R = Ac, R_1 = H **7** R = TMS, R_1 = H **8** R = d-TMS, R_1 = H **9** R = H, R_1 = OH

4-Deoxygigantecin (**5**) was obtained as a whitish wax, mp 97 - 99 °C. $[\alpha]^{25}_D$ = 15.5° (c 0.2 in MeOH). The molecular weight was indicated by peaks at m/z 623 (MH^+) in the FABms and 749 (MH^+) in the isobutane CIms of its triacetate derivative (**6**). The HRFABms measurement gave m/z 623.4874 for MH^+ (calcd 623.4887), corresponding to the molecular formula, $C_{37}H_{66}O_7$. An ir carbonyl absorption band at 1751 cm^{-1}, an uv (MeOH) λ_{max} at 207 nm (log ε 3.93), four proton resonances at δ 6.98 (q, H-35), 4.99 (qq, H-36), 2.26 (tt, H-3, and 1.40 (d, H-37), and five carbon resonances at δ 173.18 (C-1), 148.84 (C-35), 134.19 (C-2), 77.44 (C-36), and 19.26 (C-37) (table 2) provided characteristic spectral features for the α,β-unsaturated γ-lactone fragment without a 4-OH.[9]

The existence of three hydroxyl moieties was indicated by a broad ir hydroxyl absorption band at 3442 cm^{-1} and the preparation of a triacetate derivative (**6**), a tri-TMS derivative (**7**), and a tri-d-TMS derivative (**8**). **6** gave three proton peaks at δ 2.090 (3H, OAc), 2.074 (3H,OAc), and 2.073 (3H, OAc), and a multiple proton resonance at δ 4.83 (H-14, 17, 22) corresponding to the downfield shift of three protons on secondary hydroxyl-bearing carbons in **5** after acetylation. The CIms of **6** showed three successive losses of AcOH (m/z 60) from MH^+, giving m/z 689 (MH^+ - AcOH), 629 (MH^+- 2 AcOH), and 569 (MH^+ - 3 AcOH). The EIms of **7** and **8** showed three successive losses of TMSOH (-90), 748 (M^+ - TMSOH), 658 (M^+ - 2 TMSOH), and 568 (M^+ - 3 TMSOH) or d-TMSOH (-99), 766 (M+ - d-TMSOH), 667 (M+ - 2 d-TMSOH), and 568 (M+ - 3 d-TMSOH), from the M^+, respectively. The ^{13}C nmr spectrum of **5** showed three resonances due to oxygen-bearing carbons at δ 74.44, 74.25, 74.08 and absence of a signal at ca. δ 69, indicating the existence of three secondary hydroxyl moieties without a 4-OH.[9] The presence of two tetrahydrofuran rings was indicated by proton resonances at δ 3.87 (1H) and 3.80 (3H), and carbon resonances at δ 82.68, 82.67, 82.00, and 79.29. The high similarity of the nmr data of **5** and gigantecin (**9**)[8] for the bistetrahydrofuran ring fragments hinted the existence of the same nonadjacent bis-tetrahydrofuran ring fragment with two hydroxyl groups adjacent to one ring and one hydroxyl group adjacent to the other ring.

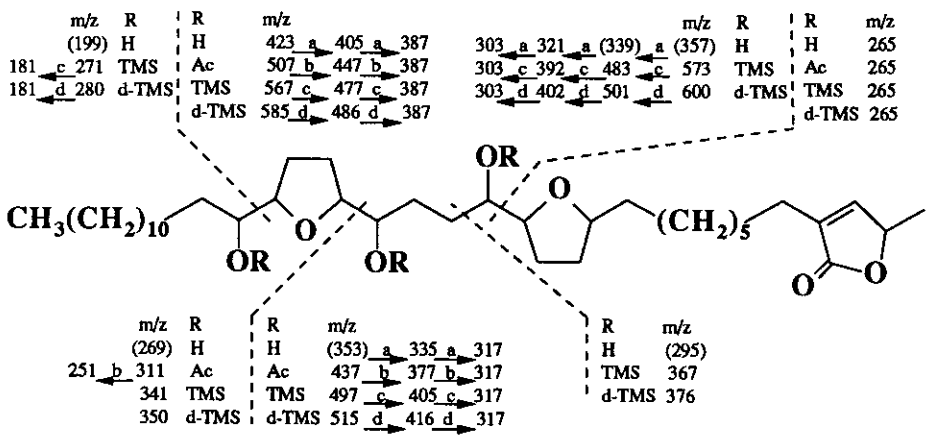


Figure 2. Diagnostic EIms fragment ions of 4-deoxygigantecin (**5** R = H), triacetate derivative (**6** R = Ac), tri-trimethylsilyl derivative (**7** R = TMS), and tri-perdeutero-trimethylsilyl derivative (**8** R = d-TMS). Peaks in parentheses were not seen. Letters above the arrows represent (a) loss of H_2O (m/z 18), (b) loss of AcOH (m/z 60), (c) loss of TMSOH (m/z 90), and (d) loss of d-TMSOH (m/z 99).

The carbon skeleton and placement of the tetrahydrofuran rings and the three hydroxyl moieties along the hydrocarbon chain of **5** were determined based on the EIms analysis of **5-8** (Figure 2). Fragments in the EIms at m/z 423, 335, and 265 for **5**, m/z 507, 437, 311, and 265 for **6**, m/z 573, 567 , 497, 367, 341, 271, and 265 for

7, and m/z 600, 585, 515, 376, 350, 280, and 265 for **8** clearly defined the positions of the tetrahydrofuran rings at C-10 and C-18 along the hydrocarbon chain and supported the assignment of two hydroxyl groups at C-17 and C-22 adjacent to one tetrahydrofuran ring and the third hydroxyl group at C-14 adjacent to the other tetrahydrofuran ring as indicated by the nmr data.

Table 2. Nmr data for 4-deoxygigantecin (**5**) and its triacetate (**6**).

Atom	^{1}H Nmr [ppm,(J/Hz)] of **5** (500 MHz, $CDCl_3$)	^{1}H Nmr [ppm,(J/Hz)] of **6** (500 MHz, $CDCl_3$)	^{13}C Nmr (ppm) of **5** (125 MHz, $CDCl_3$)
1	------	------	173.18
2	------	------	134.19
3	2.26 tt (7.3, 1.6)	2.26 tt (7.3, 1.6)	25.20
4	1.35 - 1.21 m	1.38 - 1.21 m	27.42
5-7	1.35 - 1.21 m	1.38 - 1.21 m	29.75 - 29.40
8,24	1.35 - 1.21 m	1.38 - 1.21 m	26.21 and 25.65
9,23	1.74 - 1.37 m	1.63 - 1.44 m	32.43 and 31.96
10	3.87 m	3.86 m	79. 29
11,12	1.99 m and 1.70 m	1.99 m and 1.60 m	29.98* and 28.74*
13	3.80 m	3.97 m	82.00
14	3.44 m	4.83 m	74.08
15,16	1.74 - 1.37 m	1.63 - 1.44 m	35.61 and 33.47
17,22	3.40 m	4.83 m	74.44 and 74.25
18,21	3.80 m	3.97 m	82.68 and 82.67
19,20	1.99 m and 1.70 m	1.99 m and 1.60 m	28.79* and 28.44*
25-30	1.35 - 1.21 m	1.38 - 1.21 m	29.75 - 29.40
31,32	1.35 - 1.21 m	1.38 - 1.21 m	29.27 and 29.14
33	1.35 - 1.21 m	1.38 - 1.21 m	22.74
34	0.88 t (7.1)	0.88 t (7.1)	14.19
35	6.98 q (1.6)	6.99 q (1.6)	148.84
36	4.99 qq (7.0, 1.6)	5.00 qq (6.8, 1.6)	77.44
37	1.40 d (7.0)	1.41 d (7.0)	19.26
Ac-14	------	2.090 s*	------
Ac-17	------	2.074 s*	------
Ac-22	------	2.073 s*	------

* Signals may be interchangeable.

The relative configurations between C-13/C-14, C-17/C-18, and C-21/C-22 were all proposed to be threo by comparing the ^{13}C nmr signals of **5** for the hydroxylated carbons at C-14 (δ 74.08), C-17 (δ 74.44), and C-22 (δ 74.25) as well as the ^{1}H nmr signals of **5** for H-14, 17, 22 (δ 3.40-3.44), and H-13, 18, 21 (δ 3.80) with those of model compounds of known relative stereochemistry.[12] The threo assignments were further substantiated by comparing proton resonances of **6** at δ 3.97 (H-13, 18, 21) and 4.83 (H-14, 17, 22) with those of a group of diacetyl dibutylated bistetrahydrofurans of known relative stereochemistry.[13] The ^{1}H nmr signals of **6** at δ 3.97 for H-18 and H-21 suggested the trans configuration of these two protons.[14] The configurations of the chiral centers at C-10 and C-36 remain undefined. The ^{1}H and ^{13}C nmr data were assigned by comparison with those of gigantecin (**9**).[8] The same relative configurations of the chiral centers assigned so far and the similarities of the

ir and nmr data of **5** and **9** suggested that the two undefined chiral centers of **5** and those of **9** might be the same. thus, the structure of compound **5** was concluded to be as illustrated, and this new compound was named 4-deoxygigantecin.

Table 3. Bioactivities of compounds **1**, **2**, **5**, and **6**

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	89.02	6.97×10^{-7}	2.59×10^{-2}	5.80×10^{-8}
2	------	9.72×10^{-3}	10.48	1.78×10^{-4}
5	29.46	2.51×10^{-2}	5.38	3.58
6	------	$< 10^{-2}$	2.07	$< 10^{-2}$
Adriamycin[e]	8×10^{-2}	8.21×10^{-4}	3.27×10^{-1}	1.75×10^{-3}

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

Both giganenin (**1**) and 4-deoxygigantecin (**5**) were active in the brine shrimp lethality test (BST)[15] and also significantly cytotoxic to human tumor cells in culture (Table 3).[16] Especially, giganecin (**1**) exhibited highly potent and selective cytotoxicities in A-549 (human lung carcinoma) and HT-29 (human colon adenocarcinoma); it is the most potent antitumor monotetrahydrofuran acetogenin to have been reported at the present time. **1** showed from ten times to five thousand times the cytotoxic potency of adriamycin and would seem to be worthy of further evaluation for future development as an antitumor agent. The enhanced antitumor activity of **1**, relative to other monotetrahydrofuran acetogenins,[9] is likely due to the presence of the double bond and its location along the hydrocarbon chain, since that is its major structural difference from the other less active compounds. 4-Deoxygigantecin (**5**) showed selective cytotoxicity for A-549 (human lung carcinoma) cells, and its peracetate derivative (**6**) showed an unusual increase in activity across all three of these noted tumor cell lines.

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