GIGANTETROCIN AND GIGANTRIOCIN: TWO NOVEL BIOACTIVE ANNONACEOUS ACETOGENINS FROM GONIOTHALAMUS GIGANTEUS

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Abstract - The structural elucidations of gigantetrocin and gigantriocin, two novel bioactive monotetrahydrofuran acetogenins with vicinyl hydroxyls are presented. These compounds are significantly and selectively cytotoxic to human tumor cells in culture and toxic to brine shrimp, and they inhibit the formation of crown gall tumors on potato discs, showing good potential for in vivo antitumor activities.

Our previous bioactivity-directed studies of Goniothalamus giganteus Hook. f. and Thomas (Annonaceae) have yielded the bioactive compounds altholactone (syn: goniothalenol, a furano-2-pyrone), goniothalamin (a styrylpyrone), and pinocembrin (a flavanone);[1] goniotriol (a 5,6-dihydroxy-styryl-2-pyrone);[2] goniothalamicin, annonacin, and gigantecin (three acetogenins);[3,4] and goniofufurone (a furano-2-furanone); goniopypyrone (a pyranopyrone), and 8-acetylgoniotriol.[5] In our continuing investigation of this plant as a source of bioactive compounds, two novel monotetrahydrofuran acetogenins, gigantetrocin (**1**) and gigantriocin (**5**), have been isolated from the partitioned ethanolic extract of the stem bark. The brine shrimp lethality test (BST) was used to direct the fractionations. These compounds are members of the diversely bioactive group of compounds referred to as the Annonaceous acetogenins.[6]

Gigantetrocin (**1**) was isolated as an amorphous solid (mp 80-81°C, $[\alpha]^{25}_D$ = +10.3°). The high resolution chemical ionization (isobutane) mass spectra (HRCIms) gave m/z 597.4750 (calcd 597.4730) for the MH^+, corresponding to the molecular formula, $C_{35}H_{64}O_7$. The presence of four hydroxyl moieties was suggested by four successive losses of water (m/z 18) from the molecular ion in the CIms. In addition, the ir spectrum contained a broad absorption band at 3448 cm^{-1}, consistent with the presence of hydroxyl groups. The existence of four hydroxyl groups was confirmed by the preparation of a tetraacetate derivative (**2**) (acetic anhydride/ pyridine) and a tetratrimethylsilyl derivative (**3**) [TMSi, bis(trimethylsilyl)acetamide in pyridine)] as evidenced by MH^+ ions corresponding to $[MH+ 4\ Ac]^+$ and $[MH+ 4\ TMSi]^+$ in the CIms.

A prominent ir carbonyl absorption at 1737 cm^{-1} and a uv λ max at 209 nm (ε = 15,136) suggested

the presence of the α,β - unsaturated γ-lactone. Expected resonances in the ^{1}H nmr (Table 1) and ^{13}C nmr (Table 2) confirmed the presence of an α,β - unsaturated γ-lactone as well as the presence of a hydroxyl group at C(4) .[6,7] In addition to the resonances due to the oxygenated carbons of the lactone and the four hydroxylated carbons at δ 74.42, 74.39, 74.22, and 69.78, the ^{13}C nmr showed two resonances at δ 81.75 and 79.25 also due to oxygen bearing carbons. These resonances and their corresponding ^{1}H nmr resonances at δ 3.87 and 3.79 were directly analogous to similar signals of one of the nonadjacent rings in gigantecin,[4] indicating that **1** possessed a single tetrahydrofuran ring with a single hydroxyl group adjacent to the ring.

Table 1. ^{1}H Nmr chemical shifts (ppm)[a] for **1**, **2**, **4**, **5**, and **6**.

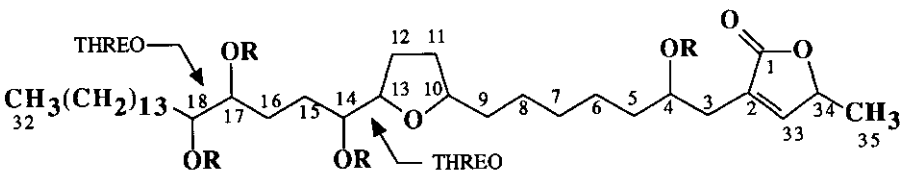
Proton	**1**	**2**	**4**	**5**	**6**
3a	2.38 (dddd) 3a, 3b (15.0) 3a, 4 (8.0) 3a, 33 (1.5) 3a, 34 (1.5)	2.48 (dddd) 3a, 3b (15.0) 3a, 4 (8.0) 3a, 33 (1.5) 3a, 34 (1.5)	2.38 (dddd) 3a, 3b (15.0) 3a, 4 (8.0) 3a, 33 (1.5) 3a, 34 (1.5)	2.26 (br t)	2.26 (br t)
3b	2.51 (dddd) 3b, 3a (15.0) 3b, 4 (4.0) 3b, 33 (1.5) 3b, 34 (1.0)	2.53 (dddd) 3b, 3a (15.0) 3b, 4 (4.0) 3b, 33 (1.5) 3b, 34 (1.0)	2.51 (dddd) 3b, 3a (15.0) 3b, 4 (4.0) 3b, 33 (1.5) 3b, 34 (1.0)	2.26 (br t)	2.26 (br t)
4	3.83 (m)	5.07 (m)	3.83 (m)	1.26-1.70 (m)	1.26-1.70 (m)
5-9	1.26-1.70 (m)	1.26-1.70 (m)	1.26-1.70 (m)	1.26-1.70 (m)	1.26-1.70 (m)
10	3.87 (m)	3.82 (m)	3.86 (m)	3.88 (m)	3.83 (m)
11	1.50-2.10 (m)	1.50-2.10 (m)	1.50-2.10 (m)	1.50-2.10 (m)	1.50-2.10 (m)
12	1.50-2.10 (m)	1.50-2.10 (m)	1.50-2.10 (m)	1.50-2.10 (m)	1.50-2.10 (m)
13	3.79 (m)	3.92 (m)	3.76 (m)	3.81 (q) 13, 12 (7.0) 13, 14 (7.0)	3.93 (m)
14	3.38-3.42 (m)	4.78 (m)	3.38 (m)	3.43 (m)	4.79 (m)
15-16	1.20-1.70 (m)	1.20-1.70 (m)	1.20-1.70 (m)	1.20-1.70 (m)	1.20-1.70 (m)
17	3.38-3.42 (m)	4.94 (m)	3.55-3.61 (m)	3.43 (m)	4.95 (m)
18	3.38-3.42 (m)	4.94 (m)	3.55-3.61 (m)	3.43 (m)	4.95 (m)
19-31	1.20-1.70 (m)	1.20-1.70 (m)	1.20-1.70 (m)	1.20-1.70 (m)	1.20-1.70 (m)
32	0.88 (t) 32, 31 (6.8)	0.88 (t) 32, 31 (6.8)	0.86 (t) 32, 31 (6.8)	0.88 (t) 32, 31 (6.8)	0.86 (t)
33	7.17 (q)	7.06 (q)	7.16 (q)	6.99 (q) 33, 34 (1.5) 33,3(1.5, 1.5)	6.96 (q)
34	5.04 (qq)	4.99 (qq)	5.04 (qq)	5.00 (qq) 34, 35 (7.0) 34, 33 (1.5)	4.99 (q)
35	1.42 (d) 35, 34 (6.8)	1.38 (d) 35, 34 (6.8)	1.42 (d) 35, 34 (6.8)	1.38 (d) 35, 34 (7.0)	1.38 (d) 35, 34 (7.0)
4 Ac	---	2.00 (s)	---	---	---
14 Ac	---	2.07 (s)	---	---	2.07 (s)
17 Ac	---	2.05 (s)[b]	---	---	2.05 (s)[b]
18 Ac	---	2.06 (s)[b]	---	---	2.06 (s)[b]
Acetonyl methyls	---	---	1.37 (s)	---	---

a) In $CDCl_3$ at 500 MHz b) Signals may be interchangeable

Table 2. ^{13}C Nmr chemical shifts (ppm)[a] for **1** and **5**.

Carbon	1	5
1	174.55	173.82
2	131.05	134.20
3	33.28	25.11
4	69.78	27.36
5	37.21	29.18-29.91
6	25.45	29.18-29.91
7-8	29.11-29.88	29.18-29.11
9	31.85[b]	31.90[b]
10	79.25	79.28
11	25.70[c]	25.67[c]
12	26.06[c]	26.09[c]
13	81.75	81.75
14	74.42[d]	74.42[e]
15	35.39[e]	35.53[e]
16	33.45[e]	33.45[e]
17	74.38[d]	74.39[d]
18	74.22[d]	74.22[d]
19	32.33[b]	32.33[b]
20-29	29.11-29.88	29.18-29.91
30	28.37	28.37
31	22.61	22.64
32	14.05	14.05
33	151.80	148.88
34	77.93	76.38
35	19.02	19.16

a) In $CDCl_3$ at 125 MHz
b-e)Values with the same superscript may be interchanged



1 **R = H**
2 **R = Ac**
3 **R = TMSi**

To establish the placement of the tetrahydrofuran ring, the placement of the hydroxyl group adjacent to the tetrahydrofuran ring, and the position of the remaining two hydroxyl groups along the hydrocarbon chain , mass spectral studies were undertaken . A comparison of the EIms of **1**, **2**, and **3** (Figure 1) established the carbon skeleton and the hydroxylation pattern for **1**. Fragments in the EIms of **3** at m/z 353 and corresponding signals in the EIms of **1** and **2** clearly positioned the tetrahydrofuran ring at C(10) along the hydrocarbon chain and allowed the assignment of the hydroxyl group at C(14) adjacent to the tetrahydrofuran ring. The position of the remaining two hydroxyl moieties vicinally at C(17) and C(18) was illustrated by fragments in the EIms of **3** at m/z 299 and 585. The later fragment showed three consecutive losses of TMSiOH to give the ions at m/z 495, 405, and 315. This fragmentation pattern was supported by the analogous fragmentations of **1** and **2**. The

successful preparation of an acetonide derivative (**4**) (p-toluenesulfonic acid in acetone) of **1** confirmed the existence of the vicinal hydroxyl groups. The CIms of **4** gave a MH+ at m/z 637 confirming the acetonide formation . The ^{1}H nmr of **4** also showed a downfield shift from δ 3.40 to 4.94 for two of the three protons on the hydroxyl bearing carbons which is consistent with their assignment as the vicinal hydroxyl groups.

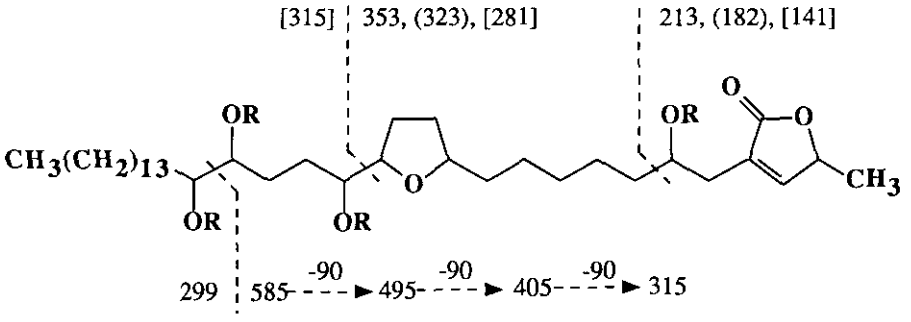
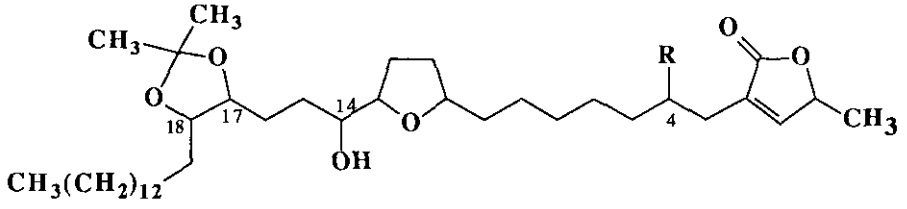


Figure 1. Diagnostic EIms fragment ions (m/z) of gigantetrocin (**1**), gigantetrocintetraacetate (**2**), and gigantetrocin-TMSi derivative **3.** Numbers in parentheses indicate fragment ions of **2**, and numbers in brackets indicate fragment ions of **1.** All other numbers are fragment ions of **3**; the ions of m/z -90 are indicative of losses of TMSiOH.



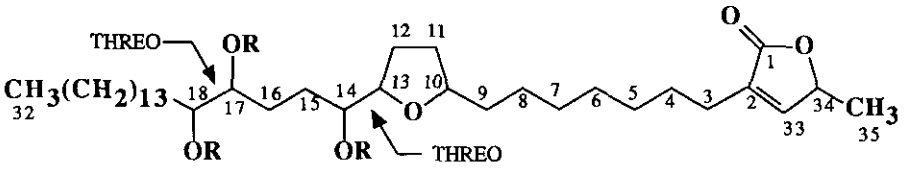
4 **R = OH**

9 **R = H**

The relative stereochemistry between C(13) and C(14) of **1** was determined by comparing the ^{13}C nmr signal for the hydroxylated carbon at C(14) with those of model compounds of known relative stereochemistry. This comparison indicated that the relative configuration between C(13) and C(14) was threo.[8] The threo assignment was further substantiated by resonances in the ^{1}H nmr of **1** at δ 3.87 (H-13) and 3.40 (H-14) indicative of a threo configuration.[8] A comparison of the ^{1}H nmr data of the acetate methyl of C(14) at δ 2.07 and H-14 at δ 4.78 of the tetraacetate of gigantetrocin (**2**) with a group of diacetyl dibutylated bis-tetrahydrofurans of known stereochemistry [9] also indicated a threo relationship. The relative stereochemistry between C(17) and C(18) of **1** was determined by comparing the ^{1}H-nmr signals for the methine protons of C(17) and C(18) with those of a group of threo and erythro diols.[10] The proton chemical shifts of δ 3.38-3.42 are indicative of a threo relationship between C(17) andC(18). The configuration of the remaining stereocenters at C(4), C(10), and C(34) are undefined.

Gigantriocin (**5**), a second new bioactive acetogenin, was also isolated as a white microcrystalline solid (mp 69-71°C, $[\alpha]^{25}_D$ = +18.0°, $CHCl_3$). The HRCIms (isobutane) gave an exact mass of 581.4776 (calcd 581.4740)

corresponding to the molecular formula $C_{35}H_{64}O_6$. This formula indicated that **5** was similar to **1** with one less hydroxyl group. The ^1H nmr (Table 1) and ^{13}C nmr (Table 2) of gigantriocin (**5**) and gigantetrocin (**I**) were essentially the same. However, **5** showed upfield shifts in both the ^1H nmr and ^{13}C nmr for the protons and carbons associated with the lactone ring, indicating the absence of the hydroxyl group at C(4). This was confirmed by the absence of the signal at δ 69.75 in the ^{13}C nmr of **5**; this signal is usually indicative of a hydroxyl group at C(4).[6,7] This was further substantiated by the presence of a two proton triplet at δ 2.26 in the ^1H nmr for the protons at C(3), instead of the complex geminal coupling pattern associated with C(3) when a hydroxyl group is present at C(4).



5 R = H
6 R = TMSi
7 R = TMSi-d_9
8 R = Ac

Mass spectral studies of **5** and its TMSi derivative (**6**) and perdeuterotrimethylsilyl derivative (**7**) [TMSi-d_9, bis(perdeuteriotrimethylsilyl)acetamide in pyridine] (Figure 2), showed that **5** possessed the same carbon skeleton and hydroxylation pattern as **1** with the exception of the C(4) hydroxyl group. Exact mass measurements for the fragments m/z 265.1810 (calcd 265.1805; $C_{16}H_{25}O_3$) and 299.2767 (calcd 299.2771; $C_{18}H_{39}OSi$) gave further credence to the positioning of the hydroxyl groups at C(18) and C(14). Furthermore, the ^1H nmr of **5** and the triacetate derivative (**8**) and the ^{13}C nmr of **5** indicated that the relative stereochemistries between C(13) and C(14), and C(17) and C(18) were threo, identical to **1**.[8-10] The synthesis of an acetonide derivative (**9**) of **5** was evidenced by a MH^+ of m/z 621 in the CIms; this derivative confirmed the presence of the vicinal hydroxyl groups. Therefore, the contigious atom structure of compound **5** would be identical to 4-desoxygigantetrocin; however, the stereochemistries at C(10) and C (34) remain undefined and could be different.

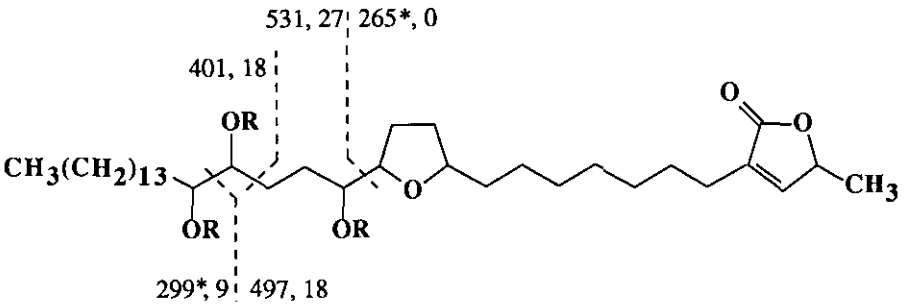


Figure 2. Diagnostic EIms fragment ions (m/z) of gigantriocin-TMSi derivative **6** and gigantriocin-TMSi-d_9 derivative (**7**). Numbers following the ion mass refer to mass shifts in the TMSi-d_9 derivative (**7**). Exact mass measurements (within 3 mmu) confirmed the elemental compositions of the proposed fragments marked with an asterisk.

Table 3 summarizes the biological activities of **1**, **5**, and their derivatives. Gigantetrocin and gigantriocin were both active in the brine shrimp lethality test (BST) [11] and inhibited the formation of crown gall tumors on potato discs.[12] The former is predictive of cytotoxicity, and the latter is predictive of 3PS (P388) in vivo murine antileukemic activity.[13] **1** and **5** were also significantly and selectively cytotoxic to human tumor cells in culture. Gigantriocin showed selectivity toward human lung carcinoma (A-549) versus human breast carcinoma (MCF-7) and human colon adenocarcinoma (HT-29). Gigantetrocin also exhibited selective cytotoxicity in A-549 and MCF-7 versus HT-29. In all bioassays, the acetate derivatives (**2** and **8**) showed a decrease in activity relative to their parent compounds, thus indicating an importance of free hydroxyl groups for optimal activity. Gigantetrocin was also more active than gigantriocin in every bioassay, indicating that the presence of a hydroxyl at C(4) results in an increase in activity. The cytotoxicities of the acetonide derivatives (**4** and **9**) generally showed decreases relative to their parent compounds; however, **9** showed an increase over **5** in activity toward the breast carcinoma (MCF-7). These findings are of interest from the standpoint of the structure activity relationships of these compounds.

Table 3. Bioactivities of compounds **1** and **5** and their derivatives.[a]

Compound	Assay				
	BST LC_{50}, μg/ml	PD % inhibition	A-549 ED_{50}, μg/ml	MCF-7 ED_{50}, μg/ml	HT-29 ED_{50}, μg/ml
1	0.60	66	3.48×10^{-3}	6.49×10^{-3}	1.24
2	>10	—	1.81×10^{-1}	7.26×10^{-1}	4.69
4	—	—	9.46×10^{-2}	2.56×10^{-2}	1.0×10^{-1}
5	5.6	53	2.69×10^{-2}	1.47	3.18
8	>10	—	8.06×10^{-1}	1.55	3.79
9	—	—	1.21	2.37×10^{-1}	>10
Adriamycin	—	—	6.84×10^{-3}	1.77×10^{-2}	4.16×10^{-3}

[a] Abbrevations: BST = Brine shrimp leathality test , PD = Inhibition of crown gall tumors on potato discs, A-549 = human lung carcinoma, MCF-7 = human breast carcinoma, HT-29 = human colon adenocarcinoma.
— = Tests were not conducted.

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