Pergamon

Tetrahedron Letters, Vol. 37, No. 31, pp. 5449-5452, 1996

0040-4039/96 $15.00 + 0.00

PII: S0040-4039(96)01191-4

GIGANTRANSENINS A, B, AND C, NOVEL MONO-THF ACETOGENINS BEARING *TRANS* DOUBLE BONDS, FROM *GONIOTHALAMUS GIGANTEUS* (ANNONACEAE)

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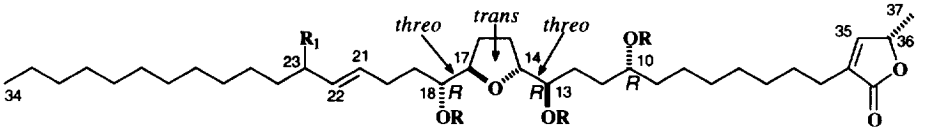
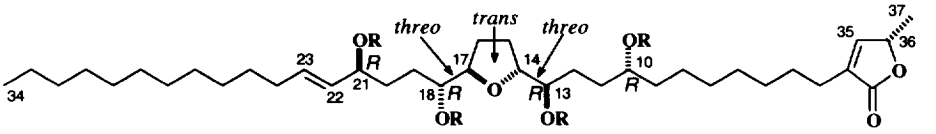
Abstract: Three novel bioactive acetogenins, gigantransenins A (**1**), B (**2**), and C (**3**), were isolated from the bark of *Goniothalamus giganteus* (Annonaceae). **1-3** are C-37 mono-tetrahydrofuran (THF) acetogenins each having a *trans* double bond; their absolute structures were elucidated by spectral and spectroscopic analyses and derivatizations. No Annonaceous acetogenins having *trans* double bonds have been reported previously. **1-3** showed selective inhibitory effects on the human breast tumor cell line (MCF-7) that are comparable with the potency of adriamycin. Copyright © 1996 Elsevier Science Ltd

The Annonaceous acetogenins are a relatively new class of compounds. Their diverse bioactivities have attracted more and more interest worldwide. Over 230 acetogenins, belonging to 23 different types, usually having mono-tetrahydrofuran (THF), adjacent or nonadjacent bis-THF, nonadjacent THF and tetrahydropyran (THP), or tri-THF rings, have been found in 26 species of the Annonaceae.[1] So far, 22 acetogenins bearing *cis* double bonds have been reported; one of these, coriadienin,[2] isolated recently from *Annona coriacea* Mart. has two *cis* double bonds and is considered to be important as a biogenetic precursor in the biosynthesis of certain acetogenins. In our search, directed by the brine shrimp lethality test (BST),[3] for bioactive components from the bark of *Goniothalamus giganteus* Hook. f. Thomas,[4] three novel mono-THF acetogenins, gigantransenins A (**1**), B (**2**), and C (**3**), were isolated. The structures of **1- 3** were determined by MS, ^{1}H NMR, and ^{13}C NMR. The absolute stereochemistries of their chiral centers were determined by Mosher ester and CD spectroscopic



	1	**1a**	**1s**	**1r**	**2**	**2a**	**2s**	**2r**
R	H	TMSi	(*S*)-MTPA	(*R*)-MTPA	H	TMSi	(*S*)-MTPA	(*R*)-MTPA
R_1	-OH	-OTMSi	-O(*S*)MTPA	-O(*R*)MTPA	···OH	···OTMSi	···O(*S*)MTPA	···O(*R*)MTPA



	3	**3a**	**3s**	**3r**
R	H	TMSi	(*S*)-MTPA	(*R*)-MTPA

methods, respectively.[5,6] **1-3** are the first examples of acetogenins having *trans* double bonds; such compounds have been proposed as biogenetic precursors of certain acetogenins bearing *erythro* configurations on the hydrocarbon side of their THF ring systems. **1-3** showed significant inhibitory effects among six human solid tumor cell lines with selectivity for the breast cell line (MCF-7) at potencies comparable to adriamycin.

Table 1. Characteristic ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) Data of **1**, **2**, **1s**, **1r**, **2s**, and **2r**.

No.	**1**		**2**		**1s**	**1r**		**2s**	**2r**	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_H	δ_H	$\Delta\delta_{1s-1r}$	δ_H	δ_H	$\Delta\delta_{2s-2r}$
1	173.5		173.5							
2	134.3		134.3							
3	25.2	2.26 tt (7)	25.2	2.26 tt (7)	2.26	2.25	-0.01	2.26	2.25	-0.01
4	27.4	1.52 m	27.4	1.52 m	1.54	1.52	-0.02	1.54	1.52	-0.02
10	71.7	3.64 m	71.6	3.64 m	4.95	4.92	*R*	4.95	4.92	*R*
11	33.5	1.44 m	33.4	1.44 m						
12	29.2	1.52 m	29.2	1.52 m	1.42	1.40	+0.02	1.42	1.40	+0.02
13	73.4	3.46 m	73.5	3.46 m	4.87	4.98	*R*	4.87	4.98	*R*
14	82.7	3.84 m	82.8	3.84 m	3.71	3.89	-0.18	3.71	3.89	-0.18
15	28.7	1.68 m	28.7	1.68 m	1.45	1.76	-0.31	1.46	1.78	-0.32
		2.02 m		2.02 m	1.23	1.43	-0.20	1.27	1.43	-0.15
16	28.5	1.68 m	28.7	1.68 m	1.66	1.87	-0.21	1.64	1.85	-0.21
		2.02 m		2.02 m	1.32	1.53	-0.21	1.38	1.53	-0.16
17	81.9	3.86 m	82.0	3.86 m	3.94	4.02	-0.08	3.92	3.97	-0.05
18	74.4	3.52 m	74.4	3.52 m	4.88	4.98	*R*	4.85	4.96	*R*
19	29.0	1.68 m	29.3	1.68 m	1.45	1.42	+0.03	1.45	1.42	+0.03
20	29.3	2.25 m	29.4	2.25 m						
21	127.5	5.72 td (7,15.5)	127.2	5.69 td (7,15.5)	5.58	5.59	-0.01	5.56	5.52	+0.04
22	136.1	5.58 dd (7, 15.5)	136.2	5.56 dd (7, 15.5)	5.44	5.47	-0.03	5.47	5.36	+0.11
23	72.8	4.08 q (7)	72.8	4.06 q (7)	5.34	5.32	*R*	5.30	5.32	*S*
24	37.4	1.48 m	37.2	1.48 m	1.55	1.48	+0.07	1.45	1.50	-0.05
					1.65	1.58	+0.07	1.57	1.60	-0.03
34	14.1	0.88 t (7)	14.1	0.88 t (7)						
35	148.8	6.99 q (1.5)	148.8	6.99 q (1.5)						
36	77.4	4.99 qq (2,7)	77.4	4.99 qq (2,7)						
37	19.2	1.41 d (7)	19.2	1.41 d (7)						

^{1}H and ^{13}C NMR spectra suggested that **1-3**[7] were typical mono-THF acetogenins bearing two flanking hydroxyls, having the *threo-trans-threo* configuration across the THF ring system and possessing an α,β-unsaturated γ-lactone without a 4-OH.[1] The successive losses of four molecules of H_2O (*m/z* 18) in their CIMS indicated that there are four hydroxyls in each molecule. These groups were also confirmed by the ^{13}C NMR spectra, in which four hydroxylated carbon signals were observed at δ 71.7, 72.8, 73.4, and 74.4 in **1**, at δ 71.6, 72.8, 73.5, and 74.4 in **2**, and at δ 71.6, 72.7, 74.1, and 74.3 in **3**.

The presence of a *trans* double bond and an adjacent hydroxyl in **1-3** was proven by decoupling and COSY experiments. Considering **1** as an example, when one hydroxymethine proton at δ 4.08 was irradiated, one double bond proton at δ 5.58 changed from a doublet of doublets to a doublet with a 15.5 Hz coupling constant;

Table 2. Characteristic ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) Data of **3**, **3s**, and **3r**.

No.	**3**		**3s**	**3r**		No.	**3**		**3s**	**3r**	
	δ_C	δ_H (*J* in Hz)	δ_H	δ_H	$\Delta\delta_{3s-3r}$		δ_C	δ_H (*J* in Hz)	δ_H	δ_H	$\Delta\delta_{3s-3r}$
1	173.5					17	82.5	3.83 m	3.94	3.99	-0.05
2	134.3					18	74.3	3.46 m	4.88	4.96	*R*
3	25.2	2.26 tt (7)	2.26	2.25	-0.01	19	29.3	1.46 m	1.44	1.41	+0.03
4	27.3	1.46 m	1.54	1.52	-0.02	20	33.7	1.68 m	1.63	1.64	-0.01
10	71.6	3.64 m	4.95	4.92	*R*	21	72.7	4.10 m	5.38	5.36	*R*
11	33.4	1.46 m				22	132.6	5.47 ddd (2,7,15.5)	5.47	5.36	+0.11
12	29.1	1.54 m	1.43	1.41	+0.02	23	127.5	5.65 ddd (2,7, 15.5)	5.70	5.61	+0.09
13	74.1	3.46 m	4.88	4.96	*R*	24	32.2	1.48 m	1.50	1.48	+0.02
14	82.5	3.83 m	3.74	3.89	-0.15				1.57	1.51	+0.06
15	28.7	1.68 m	1.49	1.78	-0.29	34	14.1	0.88 t (7)			
		2.02 m	1.27	1.40	-0.13	35	148.8	6.99 q (1.5)			
16	28.7	1.68 m	1.61	1.81	-0.20	36	77.4	5.00 qq (2,7)			
		2.02 m	1.40	1.55	-0.15	37	19.2	1.41 d (7)			

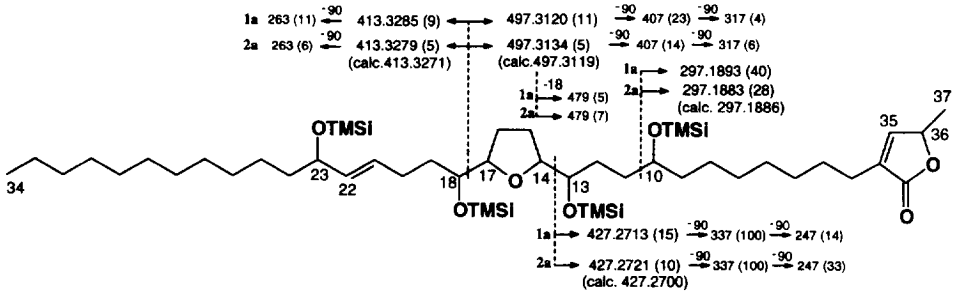


Figure 1. Mass fragmentations of **1a** and **2a**.

this demonstrated that one hydroxyl was located adjacent to a *trans* double bond.[1] Also, correlations between protons at δ 4.08 (H-23) and 5.58 (H-22) in **1**, δ 4.06 (H-23) and 5.56 (H-22) in **2**, and δ 4.10 (H-21) and 5.47 (H-22) in **3**, were observed in the COSY spectra. The MS fragments, *m/z* 297, 427, 497, of TMSi derivatives of **1-3** (Figures 1 and 2), placed one hydroxyl at C-10 and the THF rings at C-14. Double relayed COSY experiments also confirmed the correlations between δ 3.64 (H-10) and 3.46 (H-13) in each of the three compounds. Correlations, between protons at δ 3.52 (H-18) and 5.72 (H-21) in **1** and δ 3.52 (H-18) and 5.69 (H-21) in **2**, established five bond relationships for these two protons and permitted the placement of the double bond at C-21,22 and, thus, a hydroxyl at C-23. The MS fragment at *m/z* 283 in **3** established the placement of a hydroxyl at C-21 and, thus, the double bond at C-22,23.

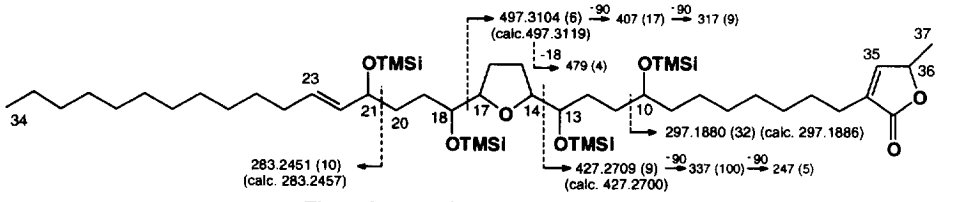


Figure 2. Mass fragmentations of **3a**.

Absolute stereochemistries at C-10,13,18, and 23 in **1** and **2**, and at C-10,13,18, and 21 in **3** were assigned by ^{1}H NMR analysis of per-Mosher ester derivatives (**1s**, **1r**, **2s**, **2r**, **3s**, and **3r**).[5] Using the positive and negative signs for $\Delta\delta_{s-r}$, the positions were concluded as C-10*R*, 13*R*, 14*R*, 17*R*, 18*R*, and 23*R* in **1**, C-10*R*, 13*R*, 14*R*, 17*R*, 18*R*, and 23*S* in **2**, and C-10*R*, 13*R*, 14*R*, 17*R*, 18*R*, and 21*R* in **3**. The stereochemistries at C-36 in **1-3** were determined by measuring CD spectra and comparing with model compounds.[6] Negative Cotton effects were observed at 236.0, 236.4, and 238.0 nm for **1-3**, respectively, and indicated that C-36 has the usual *S*-configuration in each compound.[6]

1 and **2** can be considered as biogenetic precursors for the biosynthesis of certain acetogenins bearing *erythro* configurations.[1] Their existence provides further evidence to substantiate the biogenetic pathways (Figure 3). **1-3** showed bioactivities in the BST LC_{50} 3.6, 5.8, and 4.2 μg/ml, and significant cytotoxicities towards six human solid tumor cell lines [A-549 (lung carcinoma) ED_{50} (μg/ml) 0.16, 0.21and 0.18,[8a] MCF-7 (breast carcinoma) 1.0×10^{-2}, 2.1×10^{-2} and 2.2×10^{-2},[8b] HT-29 (colon adenocarcinoma) 1.5, 1.4, and 1.3,[8c] A-498

(kidney carcinoma) 1.5, 1.6, and 1.5,[8a] PC-3 (prostate adenocarcinoma) 0.18, 0.71 and 1.5,[8d] PACA-2 (pancreatic carcinoma) 0.17, 1.5, and 1.1].[8e] Adriamycin as a positive control gave respective ED_{50} values (μg /ml) of $1.3x10^{-3}$, $1.3x10^{-2}$, $3.3x10^{-2}$, $7.3x10^{-2}$, $1.2x10^{-2}$, and $1.2x10^{-3}$. The selectivities (one to two orders of magnitude) of **1-3** on the breast cell line are unusual and are comparable to the potency of adriamycin. The acetogenins act as inhibitors of complex I in mitochondrial electron transport systems and of the plasma membrane NADH oxidase of tumor cells; they show potent *in vivo* antitumor effects, are active against multiple drug resistant cell lines, and are relatively nontoxic to noncancerous cells.[9]

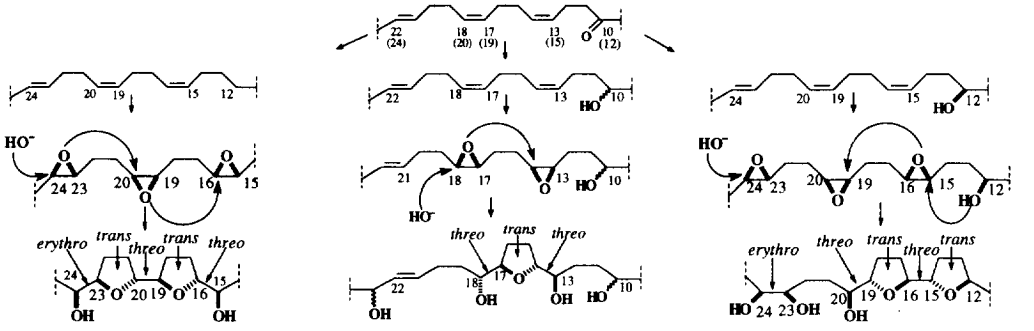


Figure 3. Hypothesis of the biogenetic pathways of the bullatacin and bulladecin types of acetogenins and of **1** and **2**.

Acknowledgment: This work was supported by R01 grant no. CA30909 from the National Cancer Institute, NIH.

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(*Received in USA* 30 *April* 1996; *revised* 7 *June* 1996; *accepted* 11 *June* 1996)