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A NEW TYPE OF CYTOTOXIC ANNONACEOUS ACETOGENIN: GIGANIN FROM *GONIOTHALAMUS GIGANTEUS*

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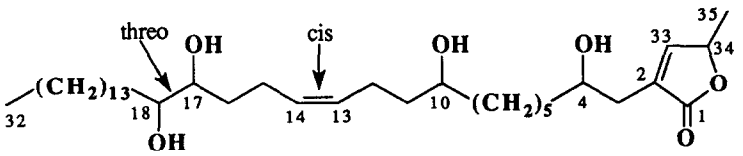
Abstract: A new cytotoxic Annonaceous acetogenin, giganin (**1**), was isolated from the bark of *Goniothalamus giganteus* (Annonaceae). This compound represents a new type of Annonaceous acetogenin lacking tetrahydrofuran or epoxide rings along the aliphatic chain, and its structure was elucidated by ^{1}H and ^{13}C NMR, MS, COSY, and single-relayed COSY.

The bark of *Goniothalamus giganteus* Hook. f. & Thomas (Annonaceae) from Thailand showed murine toxicity in the 3PS leukemia assay.[1] Ten highly cytotoxic Annonaceous acetogenins were previously isolated from this plant material in our laboratory.[2] In our further bioactivity-directed search for antitumor compounds, a new cytotoxic Annonaceous acetogenin, giganin (**1**), was isolated from the same plant material, and its structure has been determined by MS, ^{1}H and ^{13}C NMR, COSY, single-relayed COSY, and chemical derivatization. This compound is the first Annonaceous acetogenin without tetrahydrofuran or epoxide rings along the aliphatic chain, but it contains an isolated double bond in the appropriate place to serve as the biogenetic precursor of certain other acetogenins which possess epoxide or tetrahydrofuran rings.[3]



Giganin (**1**)

The molecular weight of giganin (**1**, $[\alpha]^{25}_{D}$ + 22.8, c 0.35 in $CHCl_3$) was determined by dominant peaks at m/z 581 (MH^+) in both the FABMS and CIMS. The HRFABMS gave m/z 581.4772 for the MH^+ (calcd 581.4781) corresponding to the molecular formula, $C_{35}H_{64}O_6$. The existence of four OH groups was indicated by an IR OH absorption at 3375 cm^{-1}, four successive losses of H_2O (m/z) from MH^+ in both the

FABMS and CIMS, and the preparation of a tetra-TMS derivative (**1a**). As with the other acetogenins, the presence of the methyl substituted α,β-unsaturated γ-lactone was suggested by the IR (ν_{max} 1732 cm^{-1}), UV (λ_{max} 209 nm, log ε 3.27), and the corresponding proton and carbon resonances in the ^1H and ^{13}C NMR spectra (Table 1) of **1**.[3] However, the lack of a tetrahydrofuran ring along the aliphatic chain was indicated by the absence of any corresponding tetrahydrofuran ether proton and carbon signals in the NMR spectra. Instead, the existence of an isolated cis double bond was evidenced by two proton multiple resonances around δ 5.38 (with J values of 11.6, 9.1, and 10.5 Hz, measured by double-resonance selective decoupled ^1H NMR) and two carbon peaks at δ 130.1 and 129.9.

Table 1. NMR Data [δ, (J/Hz)] of Giganin (**1**) and Its Butanonide

Atom	^1H NMR of **1** (500 MHz, $CDCl_3$)	^1H NMR of butanonide of **1** (500 MHz, $CDCl_3$)	^{13}C NMR of **1** (125 MHz, $CDCl_3$)
1	-	-	174.6
2	-	-	131.1
3	2.52 dddd (15.0,4.0,1.5) 2.40 dddd (15.0,8.0,1.5)	2.53 brd (15.0) 2.41 brdd (15.0,8.0)	33.6*
4	3.84 m	3.85 m	69.9
5	1.48 m	1.20 - 1.65 m	37.2*
6-8	1.20 - 1.40 m	1.20 - 1.65 m	29.8-29.7, 29.4, 29.3, 25.8, 25.5, 23.2
9	1.44 m	1.20 - 1.65 m	33.5*
10	3.58 m	3.58 m	70.5
11	1.52 m	1.20 - 1.65 m	36.8*
12	2.38 m, 2.08 m	2.36 m, 2.04 m	32.0
13,14	5.38 m	5.35 m, 5.39 m	130.1, 129.9
15	2.38 m, 2.08 m	2.36 m, 2.04 m	32.0
16	1.54 m	1.20 - 1.65 m	37.4*
17,18	3.40 m	5.12 m	74.4, 73.3
19	1.44 m	1.20 - 1.65 m	33.2*
20-31	1.20 - 1.40 m	1.20 - 1.65 m	29.8-29.7, 29.4, 29.3, 25.8, 25.5, 23.2, 22.8
32	0.88 t (7.0)	0.88 t (7.0)	14.2
33	7.19 q (1.5)	7.19 bs	151.8
34	5.06 qq (7.0,1.2)	5.06 q	78.0
35	1.44 d (7.0)	1.44 t (7.0)	19.2
Me-CH_3	-	1.32 s	-
Et-CH_2	-	1.64 q (7.0)	-
Et-CH_3	-	0.88 t (7.0)	-

*Signals may be interchangeable.

MS fragmentation analyses of **1** and **1a** demonstrated that the four OH groups were located at C-4, C-10, C-17, and C-18, as shown in Figure 1. The vicinal diol moiety (C-17 - C-18) was further confirmed by successfully making a butanonide derivative (butanone/p-toluenesulfonic acid) of **1** (^1H NMR data see Table 1). The position of the double bond was determined by the COSY and single-relayed COSY spectra of **1** to be across C-13 and C-14. The COSY spectrum of **1** showed coupling correlations of H_{10} - H_{11} - H_{12} - H_{13} and H_{14} - H_{15} - H_{16} - H_{17}. Because the signals of H-11 and H-16 were overlapped with some other proton signals, a single-relayed COSY experiment was applied to overcome this problem and to confirm the COSY assignment. As a result, single-relayed correlation cross peaks between H_{10} - H_{12} and H_{15} - H_{17} were clearly shown in the single-relayed COSY spectrum (Figure 2). The relative configuration of the vicinal diol (C-17/C-18) was suggested as threo by comparing the ^1H NMR signals for H-17 and H-18 at δ 3.40 with those

of a group of threo and erythro vicinal diols[4] and other vicinal diol-containing acetogenins,[3] such as gigantetrocin,[5] gigantriocin, gigantetronenin, and gigantrionenin.[2] The stereochemistries of the other three chiral centers at C-4, C-10, and C-34 in **1** remain undefined due to lack of sufficient sample to make derivatives.

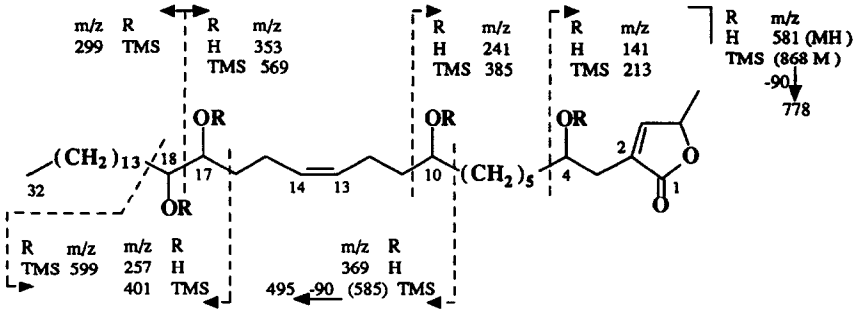


Figure 1. Diagnostic MS Fragments of Giganin (**1**, R=H)) and Its TMS-derivative (**1a**, R=TMS).

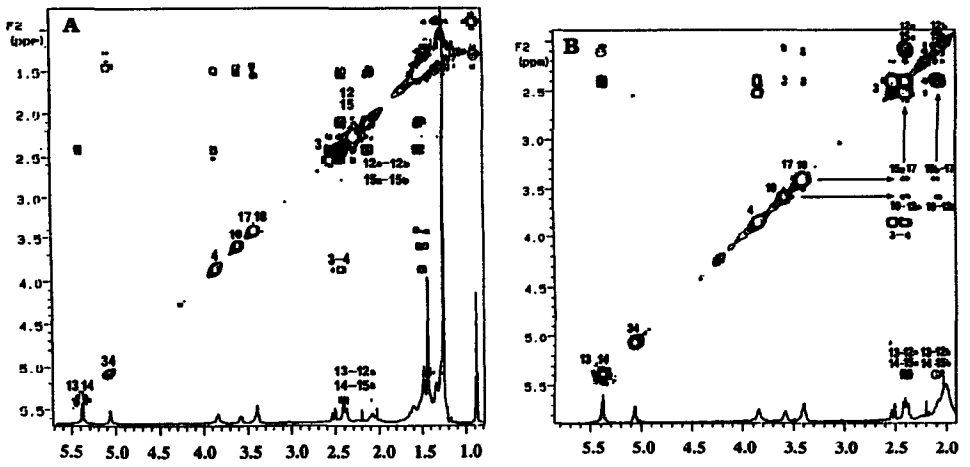


Figure 2. COSY (A) and Single-Relayed COSY (B) Spectra (500 MHz, $CDCl_3$) of Giganin (**1**)

Although giganin (**1**) was not significantly toxic to brine shrimp (BST LC_{50} 890 μg/ml),[6] it still exhibited good cytotoxicities to human lung carcinoma (A-549 ED_{50} 1.88 x 10^{-2} μg/ml), human breast carcinoma (MCF-7 ED_{50} 1.03 μg/ml), and human colon adenocarcinoma (HT-29 ED_{50} 6.88 x10^{-3} μg/ml).[7] This result suggests that tetrahydrofuran rings may not be required for the basic cytotoxic effects of acetogenins, but their presence can enhance the cytotoxic action, since the bis-tetrahydrofuran acetogenins

are usually more potent than the mono-tetrahydrofuran acetogenins.[3] These compounds are potent inhibitors of complex I in mitochondrial electron transport systems;[8] thus, their antitumor effects are elicited when tumor cells have greater energy demands than normal cells.

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