ABSOLUTE STEREOCHEMISTRIES OF GIGANIN AND LONGANIN, BIOACTIVE NON-TETRAHYDROFURAN RING ANNONACEOUS ACETOGENINS FROM *ASIMINA LONGIFOLIA*

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Abstract-Two cytotoxic Annonaceous acetogenins, giganin (**1**) and longanin (**2**), were isolated from the leaves and twigs of *Asimina longifolia* (Annonaceae). These compounds represent the type of Annonaceous acetogenin lacking either tetrahydrofuran or epoxide rings along the aliphatic chain. Compound (**2**) is novel and giganin is previously known. The planar structures and absolute configurations of both were elucidated by ^{1}H and ^{13}C nmr and ms before and after certain chemical derivatizations. Compound (**2**) gave cytotoxic ED_{50} values comparable to those of adriamycin in a panel of human solid tumor cell lines.

The Annonaceous acetogenins are a class of promising anticancer, antiinfective, and pesticidal natural products. Over 220 acetogenins, usually belonging to mono-, bis-, and tri-tetrahydrofuran (THF) groups have been reported.[1] In our bioactivity-directed search for antitumor compounds, giganin (**1**) and longanin (**2**), two acetogenins without any tetrahydrofuran rings, have been isolated from the leaves and twigs collected from *Asimina longifolia* K. (Annonaceae) growing in Georgia. Longanin is novel to the literature. Giganin (**1**), the first non-THF ring acetogenin, was isolated from *Goniothalamus giganteus* Hook f. and Thomas by Fang *et al.* in 1993;[2] its absolute stereochemistry was previously undetermined. Herein, we describe the determination of the planar structures and absolute configurations of giganin (**1**) and longanin (**2**) by the use of spectroscopic methods and with the aid of chemical derivatizations.

Compound (**1**) was isolated as a colorless wax. Its planar structure was determined by ^{1}H, ^{13}C nmr

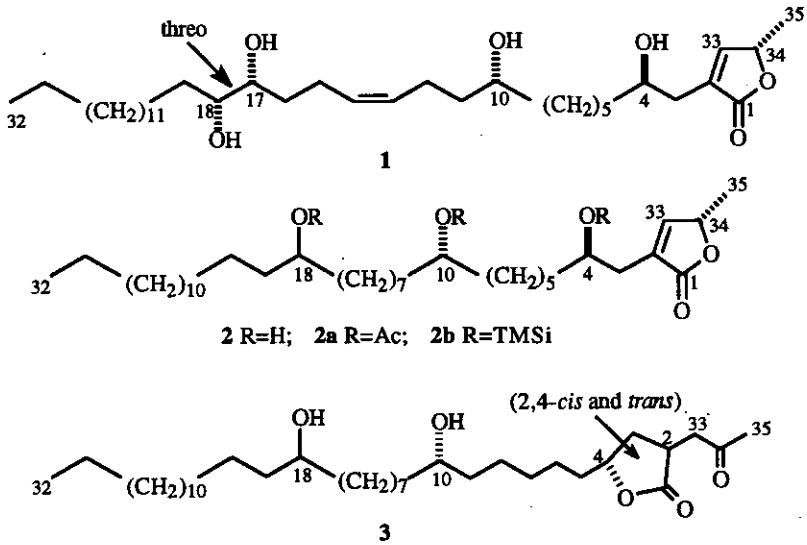
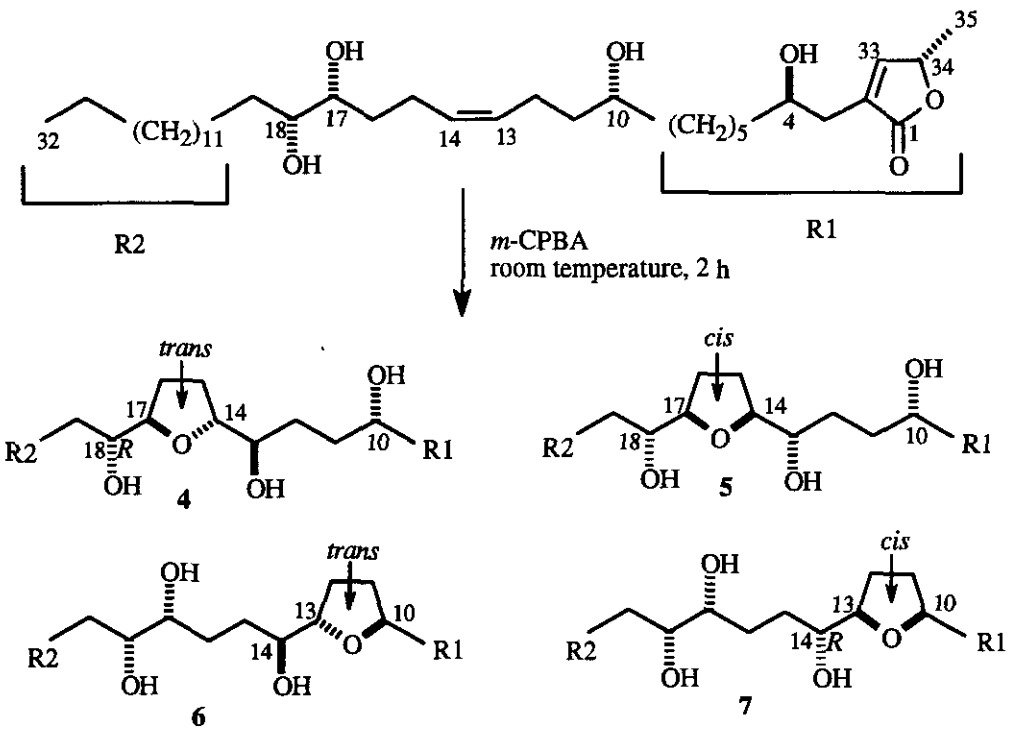


Figure 1. Structures of compounds **1**, **2**, **2a**, **2b**, and **3**.

and ms to be the same as that of giganin.[2] As in the previous work, the relative stereochemistry at C-17/C-18 of **1** was also resolved as *threo* by the preparation of the acetonide derivative. However, the absolute stereochemistry of giganin remained unknown. We approached this problem by using advanced Mosher ester methodology.[3] (*R*)- and (*S*)- MTPA derivatives of **1** were prepared and the absolute stereochemistry of C-4 was easily determined as *R*. Comparing the ^{1}H-nmr data of the MTPA derivatives of **1** with those of model butenolides synthesized by Hoye *et al.*,[4] the relative stereochemistry between C-4 and C-34 was revealed as "unlike" (*RS* or *SR*). Thus, the absolute stereochemistry for C-34 was determined as *S*, as is usual for all the known acetogenins.

The absolute stereochemistries of C-10, C-17, and C-18 of **1**, however, cannot be determined by the use of simple MTPA derivatives because of the lack of appropriate nearby reference protons. By studying the structure of giganin (**1**), it was noticed that the double bond is conveniently located two methylenes away from the 10-OH and the 17-OH. We assumed that if the double bond were oxidized to the epoxides, as in the proposed biogenetic pathways,[1] the 10-OH or the 17-OH group could attack the epoxide to form a pair of THF rings, one having a *cis*- and the other a *trans*- configuration. The hydroxyls which attack the epoxide would retain their absolute stereochemistries. Therefore, it would be possible to determine the stereochemistries at these positions by preparing the Mosher ester derivatives of these semi-synthesized compounds.

Gu *et al.*[5] and Zhang *et al.*[6] had similarily oxidized unsaturated mono- and bis-THF acetogenins into epoxides using *m*-chloroperbenzoic acid (*m*-CPBA) and then treated the epoxides with perchloric acid to make pairs of bis- and tri-THF acetogenins. In our study, we found that the reaction was able to be completed by treating with excess *m*-CPBA at room temperature for 2 hours. After the treatment of **1** with *m*-CPBA, compounds (**4-7**) were obtained and separated by hplc. Comparing the spectra of **4-7** to those of the model compounds and reference acetogenins,[1] **4-7** were revealed as goniothalamicin, *cis*-goniothalamicin, gigantetrocin-A, and *cis*-gigantetrocin-A, respectively. The Mosher esters of **4** and **7** were then prepared. C-18 was determined as *R* in **4**, and, thus, C-17 was deduced as *R* by relating the relative stereochemistry at C-17/C-18. C-14 was determined as *R* in **7**; therefore, C-10 was deduced as *R* by tracing the relative stereochemistry across the ring. In these ways, the absolute stereochemistries of giganin (**1**) were resolved as *R* for C-4, C-10, C-17, and C-18 and *S* for C-34.



Scheme 1. The Conversion of **1** to **4-7**.

Compound (**2**) was also isolated as another colorless wax. On the basis of spectral data, **2** was suggested to be a new acetogenin possessing the usual methyl substituted α, β-unsaturated γ-lactone with a 4-OH group.[1] The molecular formula of **2**, $C_{35}H_{66}O_5$ [determined by HRFABms which gave *m/z* 567.4983 for the MH^+(calcd

567.4989)], having only three indexes of hydrogen deficiency, indicated that there are no other unsaturations in the molecule except for the α, β-unsaturated γ-lactone ring. The lack of a tetrahydrofuran ring along the aliphatic chain was also indicated by the absence of any corresponding THF ether proton and carbon signals in the nmr spectra. Instead, the existance of three OH groups was indicated by the ir OH absorption at 3375 cm^{-1} and the preparation of tri-acetate (**2a**) and tri-TMSi derivatives (**2b**). Compound (**2a**) gave three multiplet resonances at δ 5.11 (H-4) and 4.86 (H-10, H-18), corresponding to the down field shifts of three protons on secondary OH-bearing carbons. Furthermore, the ^{13}C-nmr spectrum of **2** showed three resonances due to oxygen-bearing carbons at δ 69.9 (C-4), 71.7 (H-10), and 71.8 (C-18), indicating the existance of three secondary OH moieties.

Table 1. ^{1}H- (500 MHz, $CDCl_3$, J in Hz) and ^{13}C-nmr (125 MHz, $CDCl_3$) Data of Longanin (**2**) and its Tri-acetate derivative (**2a**).

C/H No.	δ^{1}H (**2**)	δ^{1}H (**2a**)	δ^{13}C (**2**)
1	-	-	174.6
2	-	-	131.2
3	2.53 ddt (15, 3.3, 1.0) 2.40 ddt (15, 8.6, 1.0)	2.57 ddt (15, 3.3, 1.0) 2.51 ddt (15, 8.6, 1.0)	33.5
4	3.85 m	5.11 m	69.9
5	1.45 m	1.55 m	37.3
6-9	1.20-1.50 m	1.20-1.50 m	22.74-37.74
10	3.61 m	4.83 m	71.7
11-17	1.20-1.50 m	1.20-1.50 m	22.74-37.74
18	3.61 m	4.83 m	71.8
19-30	1.20-1.50 m	1.20-1.50 m	22.74-37.74
31	1.21 m	1.21 m	22.7
32	0.88 t (6.9)	0.88 t (6.9)	14.1
33	7.19 q (1.5)	7.19 q (1.5)	151.8
34	5.06 qq (6.5, 1.5)	5.06 qq (6.5, 1.5)	77.9
35	1.43 d (6.5)	1.43 d (6.5)	19.1
4-OAc	-	2.03 s	-
10-OAc	-	2.04 s	-
18-OAc	-	2.04 s	-

Ms fragmentation analyses of **2b** demonstrated that the three OH groups were located at C-4, C-10, and C-18. The hreims of **2b** gave ions for $[C_{18}H_{39}OSi]^+$ at *m/z* 299.2776 (calcd 299.2770) and for $[C_{16}H_{27}O_3Si]^+$ at *m/z* 295.1735 (calcd 295.2770), and these fragments confirmed the placement of the OH groups at C-10 and C-18, respectively.

The absolute stereochemistry at C-4 was determined as *R* by using advanced Mosher ester methodology,[3] and the absolute stereochemistry for C-34 was also determined as *S* by using Hoye's method.[4] To determine the absolute stereochemistry at C-10, which, as with **1**, could not be determined by the conventional Mosher ester

methodology because of the lack of the nearby reference protons, **2** was converted by translactonization to its ketolactone (**3**) by treatment with mild base. H-4 in **3**, which is six carbons away from H-10, could then serve as a convenient reference proton.[7] Analyses of the ^{1}H-nmr spectra of the per-Mosher ester derivatives of **3** then determined that the chemical shift difference of H-4 [δH-4 (δ_S-δ_R)] of the *S*- (at δ 4.52, 4.36) and *R*- (at δ 4.51, 4.35) Mosher esters presented a positive value and suggested the *S* absolute configuration for C-10. From the above data, the structure of **2** was determined as illustrated with the absolute stereochemistry at C-18 remaining undefined; **2** was named longanin, and **3** was named (2,4)-*cis* and *trans*-longaninone.

Compounds (**2**) and (**3**) were toxic to brine shrimp,[8] and they also showed significant cytotoxicities against human tumor cell lines (Table 2), with **3** being generally a little less active than **2**. The acetogenins act as inhibitors of complex I in mitochondrial electron transport systems[10] and as inhibitors of the plasma membrane NADH oxidase of tumor cells.[11] They show selectivity for tumorous vs. normal cells, and they are equally effective against drug resistant cells.[12]

Table 2. Bioactivity Data of Longanin (**2**) and (2,4)-*cis* and *trans*- Longaninone (**3**) and Adriamycin as the Positive Control Standard (ED_{50}, μg/ml).

Compounds	**2**	**3**	Adriamycin
BST[a]	12.3	37.6	NT
A-549[b]	4.91×10^{-2}	8.29×10^{-1}	3.51×10^{-2}
MCF-7[c]	3.39	2.48	3.87×10^{-1}
HT-29[d]	5.99×10^{-1}	3.09×10^{-1}	6.29×10^{-2}
A-498[e]	3.86×10^{-2}	7.63	3.23×10^{-2}
PC-3[f]	3.96×10^{-1}	4.39	1.41×10^{-1}
PaCa-2[g]	1.11×10^{-2}	1.58×10^{-1}	3.64×10^{-2}

a. Brine shrimp lethality test [8,9] b. Human lung carcinoma[9a] c.Human breast carcinoma[9b] d.Human colon adenocarcinoma[9c] e.Human kidney carcinoma[9a] f. Human prostate adenocarcinoma[9d] g.Human pancreatic carcinoma[9e]

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