New Constituents from Stems of *Goniothalamus amuyon*

Yu-Hsuan LAN,[a] Fang-Rong CHANG,[a] Yu-Liang YANG,[b] and Yang-Chang WU*,[a]

[a] *Graduate Institute of Natural Products, Kaohsiung Medical University; Kaohsiung, 807 Taiwan: and* [b] *Institute of Biological Chemistry, Academia Sinica; Taipei, 115 Taiwan.* Received January 24, 2006; accepted April 21, 2006

Two new compounds, goniothalesacetate (1) and goniothalesdiol A (2) together with goniodiol-7-monoacetate, goniodiol-8-monoacetate, leiocarpin C, liriodenine, griffithazanone A, 4-methyl-2,9,10-(2*H*)-1-azaanthracencetrione, velutinam and aristolactam BII were isolated and characterized from the stems of *Goniothalamus amuyon*. Structures of new compounds were determined by spectral analysis.

Key words *Goniothalamus amuyon*; styryllactone; goniothalesacetate; goniothalesdiol A

In the course of our investigation for bioactive compounds from Annonaceae natural resources,[1—4] we have studied the stems of *Goniothalamus amuyon* (BLANCO) MERR., which is not only indigenous to southern Taiwan near the coastal region but also the only species of *Goniothalamus* in Taiwan. The seeds were reported to be useful in the treatment of edema and rheumatism.[5] Literature surveys indicated that two major types of bioactive compounds, styryllactones and acetogenins, have been found from *Goniothalamus* species. In this study, we report herein the isolation and structure elucidation of two new compounds, goniothalesacetate (**1**) and goniothaesdiol A (**2**), together with eight known compounds, include three styryllactones, goniodiol-7-monoacetate,[4] goniodiol-8-monoacetate,[4] leiocarpin C,[6] and five alkaloids, liriodenine,[3] griffithazanone A,[7] 4-methyl-2,9,10-(2*H*)-1-azaanthracencetrione,[7] velutinam[8] and aristolactam BII.[8]

Compound **1** was isolated as yellow oil. The presence of hydroxyl and methoxyl groups were suggested in EI-MS by two fragment peaks at *m*/*z* 321 $[M-OH]^+$ and 307 $[M-OCH_3]^+$. Its molecular formula, $C_{17}H_{22}O_7$, was obtained by HR-FAB-MS (Found *m*/*z*: 339.1441, Calcd: 339.1444). The UV spectrum of **1** revealed the maximum absorption at 210 nm. The IR absorptions at 3433 and 1743 cm^{-1} were in accordance with hydroxyl and carbonyl groups.

The ^{1}H-NMR signals at δ 7.30—7.50 (5H) represented a mono-substituted phenyl moiety. Five oxygen-bearing methine carbons were suggested by the ^{1}H-NMR (δ 4.00, 4.05, 4.28, 4.72, 5.10) and ^{13}C-NMR (δ 78.1, 81.1, 82.8, 83.2, 87.9). One methylene group, two methoxy groups, and one acetyl group were also indicated at δ 2.55 (2H), δ 3.51 (3H), δ 3.68 (3H), and δ 1.97 (3H) in the ^{1}H-NMR spectrum, respectively. These groups were also confirmed in the ^{13}C-NMR spectrum.

Further spectral evidence was required to confirm the structure of **1**. The COSY spectrum showed coupling correlations through the sequence of H-2 to H-7 (Fig. 2). The HBMC spectrum (Fig. 2) showed cross peaks between the aromatic signals (H-2′, 6′) and C-2, and between H-2 and C-1′, which indicated the aromatic ring was connected to C-2. In addition, the HMBC cross peaks between δ_H 4.72 (H-2) and δ_C 83.2 (C-3), δ_H 5.10 (H-4) and δ_C 87.9 (C-2), δ_C 83.2 (C-3) and δ_C 82.8 (C-5) were also observed. The HMBC correlations from δ_H 4.00 (H-6) to δ_C 59.3 (OCH_3-6) and δ_H 3.51 (OCH_3) to δ_C 78.1 (C-6) showed that the methoxyl group was linked to C-6. In addition, a cross peak between δ_H 5.10 (H-4) and δ_C 171.7 (C-9) indicated the acetyl group was located on C-4. Accordingly, an ether linkage and a free hydroxyl should be assigned on positions 2, 3, or 5. The ^{1}H-NMR spectrum of the acetate derivative (**3**) confirmed that the hydroxyl group was located on C-3 according to the obvious down-field shift of H-3 (from δ 4.05 to 5.04) after acetylation of **1**. Therefore, a 2,5-ether linkage can be confirmed. The plane structure of **1** was determined as methyl 3-(3-acetoxy-4-hydroxy-5-phenyloxolan-2-yl)-3-methoxypropanoate.

The stereochemistry of **1** was established by the NOESY spectrum. The cross peaks of H-2/H-5 were in agreement with *cis*-relationship for the tetrahydrofuranal moiety. The presence of a correlation between H-2 and H-4 and without correlation between H-3 and H-5 indicated that H-4 has the same orientation with H-2 and H-5. These correlations showed the relative configuration of H-2/H-3, H-3/H-4, and H-4/H-5 as *erythero*, *threo*, and *threo*. The H-5 and H-6 were also determined to be in the *threo* configuration as the result of $J_{5/6}$ (7.2 Hz) value.[9] In order to determine of the absolute stereochemistry of **1**, (*R*)- and (*S*)-methoxy fluoromethyl phenylacetic acid (MTPA) esters of **1** (**1r**, **1s**) were prepared. The ^{1}H-NMR data (see Table 2) of **1r** and **1s** indicated the absolute configuration of C-3 to be *R*. Thus, the chiral centers of **1** were evidenced as *2R*,3*R*,4*S*,5*S*,6*R*. From the foregoing spectral analyses, the structure **1** was established and named goniothalesacetate.

Compound **2** was isolated as white powder. Its molecular formula, $C_{14}H_{18}O_5$, was obtained by HR-FAB-MS (Found *m*/*z*: 267.1234, Calcd: 267.1232). The UV spectrum of **2** re-

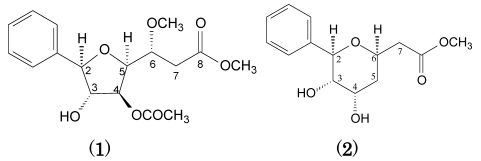

Fig. 1. Structure of Goniothalesacetate (**1**) and Goniothalesdiol A (**2**)

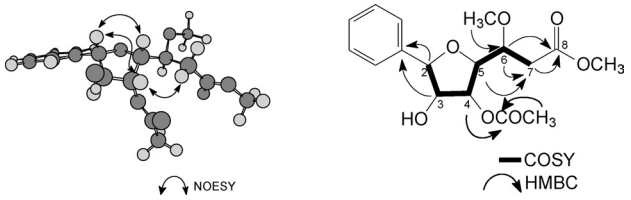

Fig. 2. Key NOESY Correlations and COSY and Key HMBC Correlations for Goniothalesacetate (**1**)

* To whom correspondence should be addressed. e-mail: yachwu@kmu.edu.tw

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data for **1** and **2** in CD_3OD and $(CD_3)_2CO$[a)]

	1		**2**	
	H	C	H	C[b)]
2	4.72 (1H, d, J=4.4 Hz)	87.9	4.87 (1H, d, J=8.0 Hz)	73.4
3	4.05 (1H, dd, J=4.4, 2.4 Hz)	83.2	3.68 (1H, dd, J=8.0, 3.6 Hz)	85.7
4	5.10 (1H, dd, J=4.2, 2.4 Hz)	81.1	4.47 (1H, ddd, J=5.6, 3.6, 2.4 Hz)	72.8
5	4.28 (1H, dd, J=7.2, 4.2 Hz)	82.8	1.77 (1H, ddd, J=13.6, 4.8, 2.4 Hz)	39.8
			2.35 (1H, ddd, J=13.6, 8.4, 5.6 Hz)	
6	4.00 (1H, td, J=7.2, 4.4 Hz)	78.1	4.38 (1H, dddd, J=8.4, 7.2, 6.4, 4.8 Hz)	74.4
7	2.60 (1H, dd, J=15.4, 4.4 Hz)	37.4	2.61 (1H, dd, J=15.2, 6.4 Hz)	40.3
	2.55 (1H, dd, J=15.4, 7.2 Hz)		2.75 (1H, dd, J=15.2, 7.2 Hz)	
8		173.2		172.3
6-OCH_3	3.47 (3H, s)	59.3		
8-OCH_3	3.67 (3H, s)	52.3		51.8
4-$OCOCH_3$	1.97 (3H, s)	171.7		
		20.7		
1′		141.7		147.7
2′, 6′	7.50 (2H, d, J=7.2 Hz)	126.8	7.40 (2H, m)	126.2
3′, 5′	7.37 (2H, t, J=7.2 Hz)	128.9	7.31 (2H, m)	128.4
4′	7.30 (1H, d, J=7.2 Hz)	128.2	7.28 (1H, m)	127.8

a) Chemical shift values are given in ppm, and J values in parenthese are given in Hz. Assignments were confirmed by ^{1}H–^{1}H COSY, HMQC, and HMBC experiments. *b*) ^{13}C-NMR 100 MHz, in $CDCl_3$.

Table 2. ^{1}H-NMR Spectral Data for **1r** and **1s** (in δ) in CD_3OD

	H-2	H-3	H-4	H-5	H-6	H-7
1s	4.940	5.345	5.284	4.242	4.016	2.529
1r	4.799	5.345	5.345	4.234	4.029	2.553
$\Delta\delta_{H(S-R)}$	+0.141	0.000	−0.061	+0.01	−0.01	−0.04

vealed the maximum absorption at 208 nm. The IR absorptions at 3409 and 1732 cm^{-1} indicated the presence of hydroxyl and carbonyl groups.

The existence of a mono-substituted phenyl moiety was indicated by the proton resonances at δ 7.29—7.45 (5H). Four oxygen-bearing methines were suggested by the ^{1}H-NMR (δ 3.68, 4.38, 4.47, 4.87) and ^{13}C-NMR (δ 72.8, 73.4, 74.4, 85.7). The NMR spectra showed the existence of two methylene groups at δ_H 1.88 and 2.34/δ_C 39.8 and δ_H 2.74 and 2.83/δ_C 40.3. A singlet at δ 3.71 (3H) and a carbon resonance at δ 51.8 also indicated the presence of a methoxyl group.

The coupling correlations through a sequence from H-2 to H-6 were found in the COSY spectrum. The HBMC cross peaks between aromatic signals (H-2′, H-6′) and C-2 as well as between H-2 and C-1′ indicated that the aromatic ring was connected to C-2. The HMBC correlations between δ_H 3.71 and δ_C 172.3 (C-8) as well as between δ 2.74/2.84 (H-7) and δ 172.3 (C-8) suggested that the acetic acid methyl ester tail was linked to C-6 as in **1**. However, the arrangements of the sequence from H-2 to H-6 are different in NMR spectra with those of **1**. On the basis of aforementioned spectral features, tetrahydropyranal central skeleton was proposed.

The stereochemistry of **2** was assigned by analysis of ^{1}H-NMR coupling constant and circular dichroism. The $J_{2/3}$ value (8.0 Hz) indicated an axial–axial position of H-2 and H-3.[10)] The observed $J_{3/4}$ value was 3.6 Hz, which indicated that H-3 and H-4 should be an axial–equitorial position, and the $J_{5/6}$ (8.4, 4.8 Hz) determined the conformation of H-6 as axial. Hence, the relative conformations H-2/H-3 and H-3/H-4 are assigned to be *erythero* and *erythero*. In addition, the $[\alpha]_D$ values and circular dichroism spectrum was measured; however, the data can not lead to the decision of the absolute configuration of **2**. The $[\alpha]_D$ values of glycopyranosylarenes had been reported in either positive and negative values. Therefore, the negative $[\alpha]_D$ value of **2** is an useless data in stereochemistry. Moreover, in comparison with the previous CD data of glycopyranosylarenes,[11)] the ignorable negative cotton effect ($\Delta\varepsilon$ −0.5) of **2** at the 250 nm region is also ambiguous to the structural elucidation. The Mosher's ester reaction also failed due to the insufficient amount of the sample. Thus, we predicted the absolute stereochemistry should follow the ratiocination in biosynthesis and assign as 2*R**,3*S**,4*S**, and 6*R**, respectively, which was named goniothalesdiol A. It is a new-type styryllactone derivative from the genus *Goniothalamus*.

According to the previous literatures,[12)] the possible partial biosynthesis pathway of styryllactones was published. Due to the unusual structural features of **1** and **2**, we supposed their possible biogenetic pathway (Fig. 3). By key hydroxylation procedures (steps **a**, **b**) passing through different oxidative cyclization, possible metabolites, including the new compounds **1** and **2**, deoxygoniopypyrone, and goniofupyrone, can be generated. In addition to our and previous supposition,[12)] the biogenetic originals of most styryllactone derivatives from *Goniothalamus* species can be completed. All proposed approaches were designed on the basis of refs. 12 and 13.

In the biological assay, compound **1** did not show significant inhibition against several cancer cell lines, including Hep2G (human hepatocellular carcinoma), Hep3B (human hepatoma cells), MDA-MB-231 (human breast carcinoma)

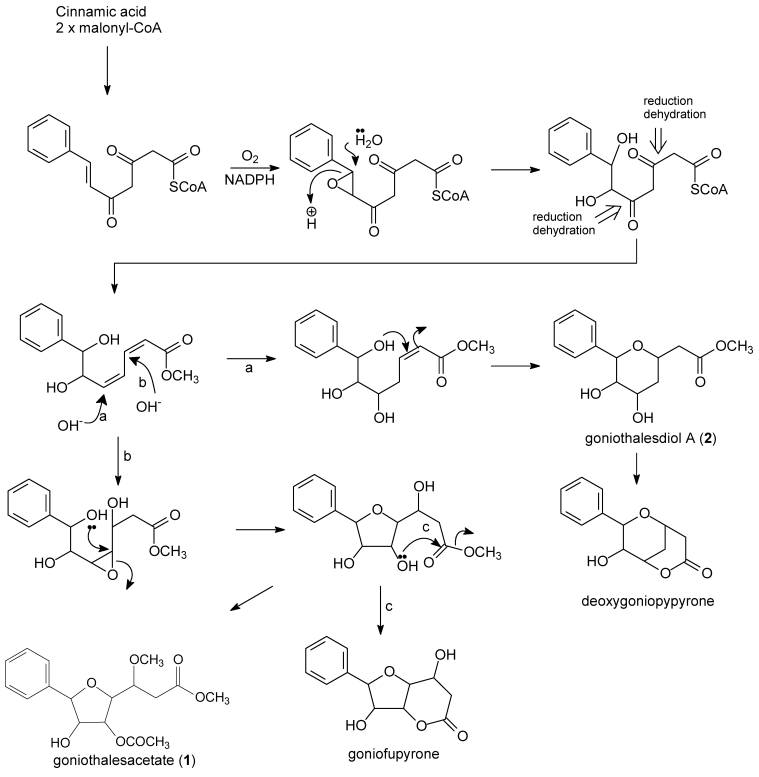

Fig. 3. Biosynthesis Pathway of **1** and **2**

and MCF-7 (human breast carcinoma) cells. The significant antiplatelet aggregation and cytotoxic activities of other known compounds had been reported in the previous studies.[4,8]

Experimental

Melting points were measured on a Yanagimoto micro-melting point apparatus and were uncorrected. The UV spectra were obtained on a Hitachi 200-20 spectrophotometer in CH_3OH solution. The IR spectra were recorded on a Mattson Genesis II spectrophotometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded with Varian NMR spectrometers. LR-EI-MS were collected on a JEOL JMS-SX/SX 102A mass spectrometer. HR-FAB-MS were collected on a Finnigan/Thermo Quest MAT 95XL mass spectrometer. TLC analysis was carried out on Si gel GF_{254} pre-coated plates with detection using 50% H_2SO_4 followed by heating on a hot plate.

Plant Material Fresh stems of *G. amuyon* (Blanco) Merr. were collected in Hengchun, Pingtung Hsien, Taiwan in September, 2001. The voucher specimen (Goniothalamus 1) is deposited in the Graduate Institute of Natural Products, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation The procedures employed for extraction and partition were described previously.[4] The $CHCl_3$ (Fr. C-1) residue was subjected to silica gel (2.5 kg, 11×53 cm) column chromatography, gradient eluting with *n*-hexane/$CHCl_3$/CH_3OH. The collected fractions were combined on the basis of their TLC characteristics to give 15 fractions after removal of solvents. Fraction 6, eluted with pure $CHCl_3$, was further separated by CC over silica gel with $CHCl_3$ to give velutinam (1.6 mg), liriodenine (6.2 mg), griffithazanone A (2.3 mg), 4-methyl-2,9,10,-(2*H*)-l-azaanthracencetrione (1.9 mg) and aristolactam BII (3.5 mg). Fraction 8 eluted with $CHCl_3$ and purified by preparative TLC to give goniodiol-7-monoacetate (1.1 mg) and goniodiol-8-monoacetate (0.9 mg). Fraction 9 was rechromatographed on silica gel eluting with $CHCl_3$ to afford **1** (45 mg).

The $CHCl_3$ layer (25 g) (Fr. C-2) was subjected to silica gel column chromatography (630 g, 7×27 cm) and eluted with gradient mixtures of $CHCl_3$/CH_3OH. The eluates were combined into 10 fractions on the basis of TLC monitoring. Fraction 9 was further purified by RP-HPLC (Hypersil ODS column, i.d. 21.2×250 mm, CH_3CN–water, 20 : 80, flow rate 3.5 ml/min; UV detector set at 210 nm) to give **2** (1.2 mg) and leiocarpin C (1.4 mg) (Hypersil ODS column, i.d. 21.2×250 mm, CH_3OH–CH_3CN–water, 10 : 10 : 80, flow rate 3.5 ml/min; UV detector set at 210 nm).

Goniothalesacetate (**1**): Yellow oil. $[\alpha]_D$ +1.03° (c=0.39, CH_3OH). ^{1}H-NMR (CD_3OD, 400 MHz) and ^{13}C-NMR (CD_3OD, 100 MHz) see Table 1. IR ν_{max} cm^{-1}: 3433, 2927, 1743, 1437, 1372, 1235, 1203, 1092, 1048. UV λ_{max} nm: 210 (log ε 3.87). FAB-MS m/z (rel. int. %): 339 ([M+H]$^+$, 75). EI-MS (70 eV) (rel. int. %): m/z=339 [M+H]$^+$ (4), 321 (2), 307 (3), 246 (18), 229 (23), 133 (27), 91 (70). HR-FAB-MS: Calcd for $C_{17}H_{22}O_7$ m/z [M+H]$^+$ 339.1444, Found 339.1441.

Goniothalesdiacetate (**3**): **1** (5.2 mg) was dissolved in acetic anhydride/pyridine and the reaction mixture left at room temperature for 12 h. The mixture on concentration and chromatograpgy afforded **3** (4.5 mg, 86%). ^{1}H-NMR (CD_3OD, 400 MHz) δ: 4.90 (1H, d, J=4.0 Hz, H-2), 5.04 (1H, dd, J=4.0, 2.0 Hz, H-3), 5.28 (1H, dd, J=4.0, 2.0 Hz, H-4), 4.30 (1H, dd, J=7.6, 4.0 Hz, H-5), 4.05 (1H, td, J=7.6, 4.0 Hz, H-6), 2.61 (1H, dd, J=16.0, 4.0 Hz, H-7b), 2.54 (1H, dd, J=16.0, 7.6 Hz, H-7b), 7.29—7.37 (5H, m, ph). ^{13}C-NMR (CD_3OD, 100 MHz) δ: 173.2, 171.4, 171.3, 140.5, 129.3, 129.0, 127.3, 86.2, 84.1, 83.9, 78.2, 78.1, 59.5, 52.3, 37.5, 20.7, 20.6. EI-MS (70 eV) (rel. int. %): m/z=349 [M−OCH_3]$^+$.

Goniothalesdiol A (**2**): White powder. ^{1}H-NMR ($(CD_3)_2CO$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) see Table 1. IR ν_{max} cm^{-1}: 3409, 2915, 2841, 2361, 1732, 1439, 1200, 1167, 1063. UV λ_{max} nm: 208. HR-FAB-MS: Calcd for $C_{14}H_{18}O_5$ m/z [M+H]$^+$ 267.1232, Found 267.1234. CD (c 3.75×10^{-3}, CH_3OH): $\Delta\varepsilon_{250}$ −0.50.

Preparation of (*R*)- and (*S*)-MTPA Esters of 1 Compound **1** (4.5 mg) was dried completely under vaccum. Deuterated pyridine (0.5 ml) and (*R*)-(−)-α-methoxy-α-(trifluoromethyl) phenylacetyl chloride (15 mg) were added under a N_2 gas stream. The mixture was stirred 2 h at room temperature and purified by HPLC with CH_3CN/H_2O to give (*R*)-MTPA ester **1r** (3.2 mg). By the same procedure, the (*S*)-MTPA ester **1s** (2.5 mg) was prepared. ^{1}H-NMR data of **1r** and **1s**, see Table 2.

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