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TWO LINEAR ACETOGENINS FROM *GONIOTHALAMUS GARDNERI*

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Key Word Index—*Goniothalamus gardneri*; Annonaceae; roots; acetogenin; gardnerilins A and B.

Abstract—Two new acetogenins, gardnerilins A and B, have been isolated from the roots of *Goniothalamus gardneri*. Both are C_{35} acetogenins containing non-tetrahydrofuran rings. Their structures have been established on the basis of spectral evidence. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Since epoxyrollins A and B, the first two acetogenins without a tetrahydrofuran (THF) ring, were reported in 1990 [1], 16 non-THF acetogenins have been isolated [2, 6]. These compounds are considered to be precursors in the formation of the THF acetogenins. Our investigations on the ethanolic extract of the roots of *Goniothalamus gardneri* resulted in the isolation of two novel non-THF acetogenins, gardnerilins A (**1**) and B (**2**), characterized by the presence of six and four hydroxyls in the aliphatic chain, respectively.

RESULTS AND DISCUSSION

Gardnerilin A (**1**) was obtained as a white amorphous powder. The FAB mass spectrum gave an $[M+H]^+$ at m/z 615 and the molecular formula was established to be $C_{35}H_{66}O_8$ on the basis of elemental analysis, mass and NMR data. A prominent IR carbonyl absorption at 173.2 cm^{-1} suggested the presence of an α,β-unsaturated γ-lactone group. The NMR spectra of **1** (CD_3OD) showed resonances at δ 7.39 (*d*, H-33), 5.13 (*dq*, H-34), 3.83 (*m*, H-4), 2.46 (*ddd*, H-3a), 2.38 (*dd*, H-3b), 1.43 (*d*, H-35), and six ^{13}C NMR (CD_3OD) resonances at δ 176.5 (C-1), 154.4 (C-33), 131.5 (C-2), 79.8 (C-34), 70.4 (C-4) and 19.1 (C-35), which confirmed the existence of a γ-methyl α,β-unsaturated γ-lactone with a C-4-OH moiety, in common with most Annonaceous acetogenins [3–6]. The presence of six OH group in **1** was evidenced by signals at δ 3.83 (1H), 3.57 (1H), 3.44 (4H) in the ^1H NMR spectrum and resonances due to oxygen-bearing carbons at δ 70.4, 72.4, 75.2, 75.3, 75.4 and 75.7 in the ^{13}C NMR spectrum. However, the lack of a THF ring along the aliphatic chain was indicated by the absence of any corresponding THF ether proton and carbon signals in the NMR spectra.

The location of the hydroxyl groups was established by EI mass fragmentation analyses of **1** and its TMSi derivative **1a** (Fig. 1); this compound contained two 1,2-diols in the aliphatic chain. The formation of an acetonide derivative **1b** further supported this conclusion. The ^1H NMR ($CDCl_3$) signals for the dioxolane ring protons at δ 3.59 (*m*, 2H) and 3.61 (*m*, 2H), and the signals for the acetonyl methyl protons, showing a singlet peak at δ 1.37, suggested *threo*-configurations for the two diols [7]. The absolute stereochemistry of C-4 was determined using Mosher ester methodology [8]. Analysis of the chemical shift differences of **1bs** and **1br** around the γ-lactone ring moiety exhibited negative results for H-3, H-33, H-34 and H-35, suggesting an *R*-configuration for C-4 (Table 1). The magnitude of the $\Delta\delta_H(\delta_S\text{-}\delta_R)$ values for H-33 and H-34 were 0.23 ppm and 0.04 ppm, respectively, showing that the C-34 is of the usual *S*-configuration [9]. The 34*S*-configuration of compound **1** was also confirmed by the existence of a negative Cotton effect at 240 nm in the CD spectrum [10–12].

Gardnerilin B (**2**) was obtained as a white amorphous powder. The CI mass spectrum gave an $[M+H]^+$ at m/z 583, showing a molecular formula of $C_{35}H_{66}O_6$ assigned by elemental analysis. The presence of an α,β-unsaturated γ-lactone with a C-4-OH group was indicated by IR absorption at 3377 cm^{-1} (OH stretch), 1744 cm^{-1} (lactone C═O), six ^1H NMR ($CDCl_3$) signals at δ 7.20 (H-33), 5.06 (H-34), 3.84 (H-4), 2.48 (H-3a), 2.38 (H-3b) and 1.43 (H-35), and six ^{13}C NMR ($CDCl_3$) resonances at δ 174.7 (C-1), 152.0 (C-33), 131.0 (C-2), 78.1 (C-34), 69.8 (C-4) and 19.1 (C-35).

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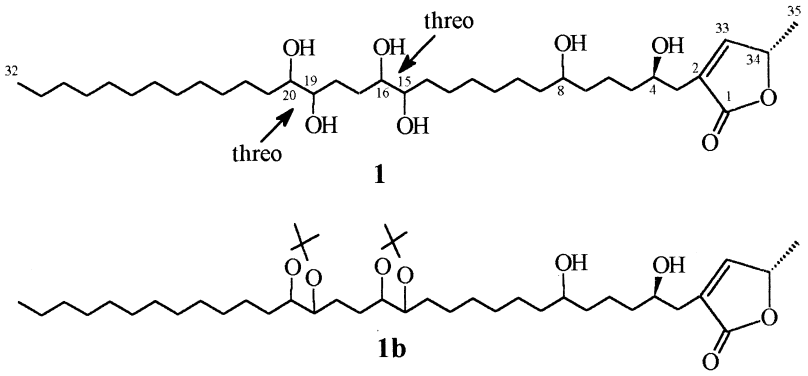


These are all characteristic spectral features for an α,β-unsaturated γ-lactone with a 4-OH group in Annonaceous acetogenins [3–6]. Four successive losses of H_2O from the $[M+H]^+$ in the CI mass spectrum of **2** (m/z 565, 547, 529 and 511) revealed the existence of four OH groups, and these were confirmed by signals at δ 3.84 (1H), 3.59 (1H), 3.40 (2H) in the ^{1}H NMR spectrum and the corresponding resonances due to oxygen bearing carbons at δ 69.8, 71.8, 74.4 and 74.6 in the ^{13}C NMR spectrum. The location

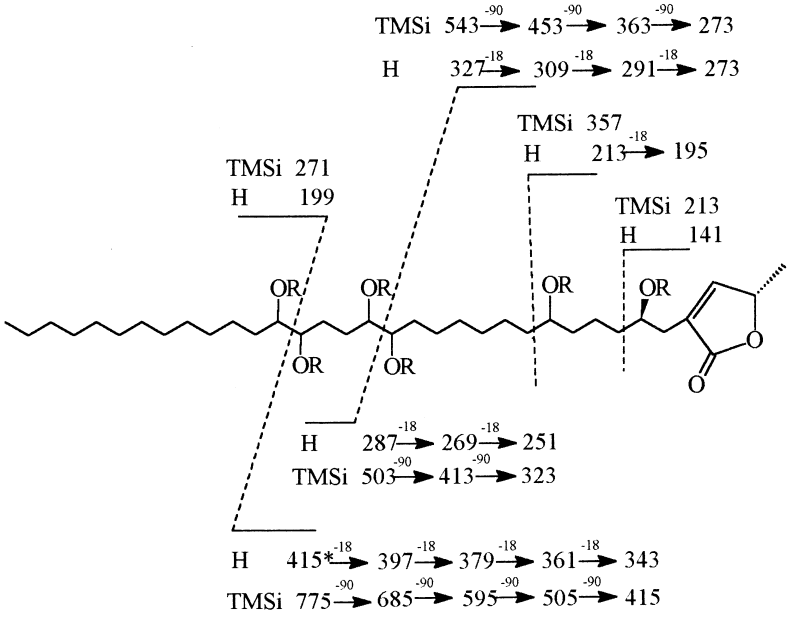
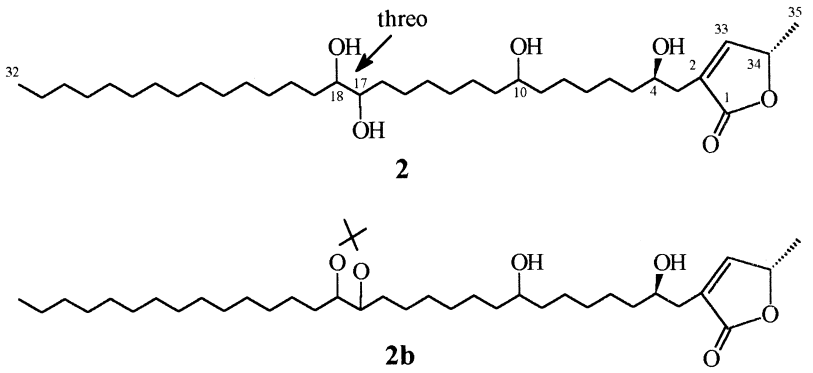


Fig. 1. Diagnostic EI mass fragment ions (m/z) of compounds **1** and **1a**.

Table 1. Partial ^{1}H NMR chemical shifts (δ) of the (*S*)- and (*R*)-Per-MTPA Mosher esters of **1b** and **2b**

Proton	**1bs** δ_H	**1br** δ_H	$\Delta\delta_H(\delta_S-\delta_R)$	**2bs** δ_H	**2br** δ_H	$\Delta\delta_H(\delta_S-\delta_R)$
H-8 (10)	5.09	5.05	+0.04	5.07	5.05	+0.02
H-4	5.33	5.35	*R**	5.32	5.33	*R**
H-3a	2.58	2.65	−0.07	2.58	2.64	−0.06
H-3b	2.56	2.59	−0.03	2.56	2.58	−0.02
H-33	6.73	6.96	−0.23	6.73	6.96	−0.23
H-34	4.87	4.91	−0.04	4.87	4.91	−0.04
H-35	1.28	1.31	−0.03	1.28	1.31	−0.03

* Absolute configuration of carbinol centre.



of the hydroxyl groups was established by EI mass spectrometry of **2** and its TMSi derivative **2a** (Fig. 2). To determine the relative configuration at C-17/C-18, the acetonide derivative **2b** was prepared. The acetonyl methyls appeared at δ 1.381 and 1.383, and the dioxolane ring protons appeared at δ 3.64. The observations indicated that the 1,2-diol has the *threo*-configuration [7]. Absolute stereochemistries at C-4 and C-34 in **2** were assigned by analysing the per-Mosher ester derivatives (**2bs** and **2br**) of **2b** [8]. The values 0.23 ppm and 0.04 ppm of $\Delta\delta_H(\delta_S\text{-}\delta_R)$ for H-33 and H-34, respectively, indicated that the C-4 and C-34 chiral centres were of the usual 4*R*, 34*S*-configuration [9]. The CD spectrum of compound **2** also exhibited a negative Cotton effect at 240 nm; thus, the structure of **2** is supported as having the 34*S*-configuration [10–12].

Compound **1** gave cytotoxic IC_{50} values against KB, HCT-8 and Bel 7402 human tumour cell lines of >10, >10 and 3.6 μg ml^{-1}, respectively; while IC_{50} values of compound **2** against KB, HCT-8 and Bel 7402 were 5.5, 4.2 and 8.5 μg ml^{-1}, respectively. Increasing the amount of hydroxylation to six free hydroxyls in compound **1** decreases potency significantly. Thus, a certain median level of polarity may be important to the biological activity of acetogenins.

EXPERIMENTAL

Mps: uncorr. IR: KBr. ^{1}H and ^{13}C NMR: Bruker AM500 spectrometer.

Plant material

Roots of *G. gardneri* Hoof f. et. Thoms were collected from DiaoLo mount, Hainan Province, People's Republic of China, in July 1993. Identification was confirmed by Prof. Wan Zhi Song, Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences, P. R. China, where a voucher specimen has been deposited.

Extraction and isolation

Dried and pulverized roots (10 kg) were extracted exhaustively with 95% EtOH and the solvent removed to yield extract F001 (1.4 kg) which was partitioned between H_2O and $CHCl_3$ (1:1), giving the H_2O-soluble for F002 (250 g) and the $CHCl_3$-soluble fr. F003 (615 g). F003 was then partitioned between 90% aq. MeOH and petrol (1:1) to yield a petrol-soluble fr. F004 (53 g) and an aq. MeOH-soluble fr. F005 (420 g). F005 (200 g) was applied to a column of silica gel

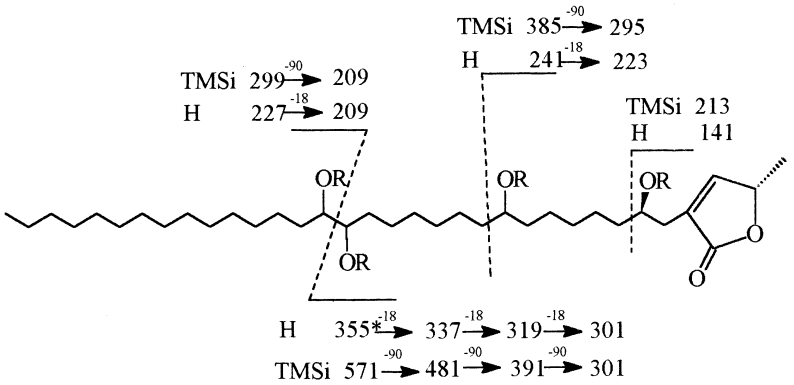


Fig. 2. Diagnostic EI mass fragment ions (m/z) of compounds **2** and **2a**.

(120–180 mesh) and eluted with $CHCl_3$ containing gradually increasing amounts of MeOH. Impure components were obtained according to their similar appearance on TLC analysis, and these were again subjected to repeated chromatography (300–400 mesh silica gel, gradients of $CHCl_3$–MeOH) to yield gardnerilin A (200 mg) and B (120 mg).

Bioassay

Cytotoxicity against human solid tumour cells was measured in 5-day MTT tests at the Department of Pharmacology, Institute of Materia Medsica, for KB nasopharyngeal carcinoma, HCT-8 colon adenocarainoma and Bel 7402 hepatoma cell lines.

Gardnerilin A (**1**). White amorphous powder, mp 94–95°. $[\alpha]_D^{11}$ +21.90° (*c* = 0.07, MeOH). Anal. calcd for $C_{35}H_{66}O_8$: C 68.40, H 10.75 (found: C 68.61, H 10.56). IR (KBr) ν_{max}: 3363, 2918, 2849, 1732, 1468, 1325, 1071, 852, 721 cm^{-1}. FAB-MS *m/z*: 615 $[M+H]^+$. EI-MS *m/z*: Fig. 1. CD (MeOH): $\Delta\varepsilon$ (nm) = −0.35 (240). ^1H NMR (500 MHz, CD_3OD): δ 0.94 (*t*, *J* = 6.8 Hz, H-32), 1.43 (*d*, *J* = 6.8 Hz, H-35), 1.29–1.80 (*m*, CH_2) 2.38 (*dd*, *J* = 8.1, 14.8 Hz, H-3b), 2.46 (*ddd*, *J* = 1.5, 2.8, 14.8 Hz, H-3a), 3.44 (*m*, H-15, 16, 19, 20), 3.57 (*m*, H-8), 3.83 (*m*, H-4), 5.13 (*dq*, *J* = 1.5, 6.8 Hz, H-34), 7.39 (*d*, *J* = 1.5 Hz, H-33). ^{13}C NMR (125 MHz, CD_3OD): δ 14.4 (C-32), 19.1 (C-35), 23.7–38.4 (CH_2), 70.4 (C-4), 72.4 (C-8), 75.2, 75.3, 75.4, 75.7 (C-15, 16, 19, 20), 79.8 (C-34), 131.5 (C-2), 154.4 (C-33), 176.5 (C-1). *TMSi derivative* (**1a**). Dry micro-amount samples of **1** were treated with N,O-*bis*(trimethylsilyl) acetamide (BSA) and pyridine (10:1) and heated at 70° for 30 min, EI-MS: Fig. 1.

Acetonide derivative of gardnerilin A (**1b**). To **1** (30 mg) and *p*-toluene sulphonic acid (3 mg) in CH_2Cl_2 (2 ml) was added 2,2-dimethoxypropane (2 ml). The mixt. was stirred for 3 h at room temp. using TLC to monitor the conversion of **1** to **1b**. The product was then purified by prep. TLC (petrol–Me_2CO, 3:1) to give 22 mg of **1b**. ^1H NMR (500 MHZ. $CDCl_3$): δ 0.87 (*t*, *J* = 6.8 Hz, H-32), 1.20–1.90 (*m*, CH_2), 1.37 (*s*, $4\times CH_3$), 1.42 (*d*, *J* = 6.8 Hz, H-35), 2.40 (*dd*, *J* = 8.0, 15.0 Hz, H-3b), 2.52 (*ddd*, *J* = 1.5, 3.0, 15.0 Hz, H-3a), 3.59, 3.61 (*m*, H-8, 15, 16, 19, 20), 3.84 (*m*, H-4), 5.05 (*dq*, *J* = 1.5, 6.8 Hz, H-34), 7.17 (*d*, *J* = 1.5 Hz, H-33).

4,8-(S)-MTPA ester of **1b** (**1bs**). To **1b** (8 mg, in 2 ml CH_2Cl_2) was added 4-(dimethylamino) pyridine (2 mg), 25 mg (*S*)-(−)-α-(trifluoromethyl) phenylacetic acid (S-MTPA) and 1,3-dicyclohexylcarbodiimide (20 mg). The resulting mixt. was stirred at room temp. for 3 h. The reaction mixt. was then filtered and the filtrate concd and purified by prep. TLC to give product **1bs** (5 mg). ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (*t*, *J* = 6.8 Hz, H-32), 1.28 (*t*, *J* = 6.8 Hz, H-35), 1.378 (*s*, $4\times CH_3$), 1.25–1.65 (*m*, CH_2), 2.56 (*m*, H-3b), 2.58 (*m*, H-3a), 3.52, 3.53 (each *s*, $2\times OCH_3$), 3.59, 3.62 (*m*, H-15, 16, 19, 20), 4.87 (*dq*, *J* = 1.5, 6.8 Hz, H-34), 5.09 (*m*, H-8), 5.33 (*m*, H-4), 6.73 (*d*, *J* = 1.5 Hz, H-33), 7.39–7.53 (*m*, ArH).

4,8-(R)-MTPA ester of **1b** (**1br**). To **1b** (8 mg, in 2 ml CH_2Cl_2) was added 4-(dimethylamino) pyridine (2 mg), 25 mg (*R*)-(−)-α-(trifluoromethyl) phenylacetic acid (R-MTPA) and 1,3-dicyclohexylcarbodiimide (20 mg). The resulting mixt. was stirred at room temp. for 3 h. The reaction mixt. was filtered and the filtrate concd and purified by prep. TLC to give product **1br** (5 mg). ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (*t*, *J* = 6.8 Hz, H-32), 1.31 (*t*, *J* = 6.8 Hz, H-35), 1.373 (*s*, $2\times CH_3$), 1.377 (*s*, $2\times CH_3$), 1.17–1.81 (*m*, CH_2), 2.59 (*m*, H-3b), 2.65 (*m*, H-3a), 3.50, 3.54 (each *s*, $2\times OCH_3$), 3.59, 3.61 (*m*, H-15, 16, 19, 20), 4.91 (*dq*, *J* = 1.5, 6.8 Hz, H-34), 5.05 (*m*, H-8), 5.35 (*m*, H-4), 6.96 (*d*, *J* = 1.5 Hz, H-35), 7.39–7.52 (*m*, ArH).

Gardnerilin B (**2**). White amorphous powder (120 mg), mp 65–66°. $[\alpha]_D^{11}$ +12.78° (*c* 0.11, MeOH). Anal. calcd for $C_{35}H_{66}O_6$: C, 72.16; H, 11.34 (found: C, 72.58; H, 11.17). IR (KBr) ν_{max}: 3377, 2918, 2851, 1744, 1468, 1072 cm^{-1}. CI-MS *m/z*: 583 $[M+H]^+$, 565 $[MH-H_2O]^+$, 547 $[MH-2H_2O]^+$, 529 $[MH-3H_2O]^+$, 511 $[MH-4H_2O]^+$. EI-MS *m/z*: Fig. 2. CD (MeOH): $\Delta\varepsilon$ (nm) = −0.35 (240). ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (*t*, *J* = 6.8 Hz, H-32), 1.42 (*d*, *J* = 6.8 Hz, H-35), 2.38 (*dd*, *J* = 8.0, 14.8 Hz, H-3b), 2.48 (*dd*, *J* = 4.1, 14.8 Hz, H-3a), 3.40 (*m*, H-17, 18), 3.59 (*m*, H-10), 3.84 (*m*, H-4), 5.05 (*q*, *J* = 6.6 Hz, H-34), 7.20 (*br s*, H-33). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1 (C-32), 19.1 (C-35), 22.7–37.1 (CH_2), 69.8 (C-4), 71.8 (C-10), 74.4, 74.6 (C-17, 18), 78.1 (C-34), 131.0 (C-2), 150.0 (C-33), 174.7 (C-1). *TMSi derivative* (**2a**). Compound **2** was treated as described for the prepn of TMSi gardnerilin A. EI-MS: Fig. 2.

Acetonide derivative of gardnerilin B (**2b**). ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (*t*, *J* = 6.7 Hz, H-32), 1.381, 1.383 (each *s*, $2\times CH_3$), 1.42 (*d*, *J* = 6.8 Hz, H-35), 2.40 (*dd*, *J* = 8.3, 15.1 Hz, H-3b), 2.53 (*dd*, *J* = 4.0, 14.8 Hz, H-3a), 3.69 (*m*, H-10, 17, 18), 3.82 (*m*, H-4), 5.06 (*q*, *J* = 6.7 Hz, H-34), 7.18 (*br s*, H-33).

4,10-(S)-MTPA ester of **2b** (**2bs**). ^1H NMR (500 MHz, $CDCl_3$): δ 0.87 (*t*, *J* = 6.8 Hz, H-32), 1.28 (*t*, *J* = 6.8 Hz, H-35), 1.375, 1.378 (each *s*, $2\times CH_3$), 2.56 (*m*, H-3b), 2.58 (*m*, H-3a), 3.54, 3.55 (each *s*, $2\times OCH_3$), 3.61 (*m*, H-17, 18), 4.87 (*q*, *J* = 6.8 Hz, H-34), 5.07 (*m*, H-10), 5.32 (*m*, H-4), 6.73 (*d*, *J* = 1.5 Hz, H-35), 7.39–7.54 (*m*, ArH).

4,10-(R)-MTPA ester of **2b** (**2br**). ^1H NMR (500 MHz, $CDCl_3$): δ 0.88 (*t*, *J* = 6.8 Hz, H-32), 1.31 (*t*, *J* = 6.8 Hz, H-35), 1.374, 1.378 (each *s*, $2\times CH_3$), 2.58 (*m*, H-3b), 2.64 (*m*, H-3a), 3.51, 3.53 (each *s*, $2\times OCH_3$), 3.60 (*m*, H-17, 18), 4.91 (*q*, *J* = 6.8 Hz, H-34), 5.05 (*m*, H-10), 5.33 (*m*, H-4), 6.96 (*d*, *J* = 1.5 Hz, H-35), 7.38–7.51 (*m*, ArH).

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