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Gaultherins A and B, two lignans from *Gaultheria yunnanensis*

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Abstract

Two lignans, trivially named gaultherins A (**1**) and B (**2**), were isolated from the roots of *Gaultheria yunnanensis* (Ericaceae). Their structures were deduced as 5-methoxy-(+)-isolariciresinol-9,9′-diacetate (**1**) and (+)-lyoniresinol-9,9′-diacetate (**2**), based upon physicochemical properties, spectral analyses and chemical degradation.

Keywords: *Gaultheria yunnanensis*; Ericaceae; Roots; Lignans; Gaultherin A; Gaultherin B

1. Introduction

Gaultheria yunnanensis (Franch.) Rehd. from the family Ericaceae is a herbal medicine widely used in the southwest district of China for the treatments of rheumatoid arthritis, swelling pain, trauma, chronic tracheitis, cold and vertigo. Its volatile oil called 'wintergreen oil' possesses antipyretic and analgesic effects (Jiangsu New Medical College, 1977; ICPP, 1992). However, studies of the composition of this drug have not been reported previously. Recently we have systematically investigated the chemical constituents of the title plant. In this paper, we describe the isolation and structural elucidation of two new natural lignans, gaultherins A (**1**) and B (**2**).

2. Results and discussion

The chloroform soluble fraction from the 95% EtOH extract of the roots of *G. yunnanensis* was chromatographed on silica gel, following by repeated polyamide column chromatography to afford two lignans, gaultherins A (**1**) and B (**2**).

Gaultherin A (**1**), a white powder, has a molecular formula of $C_{25}H_{30}O_9$ based on [M^+] at m/z 474 and elemental analyses as well as ^1H, ^{13}C NMR data. The UV spectrum of **1** showed absorption maxima at 225 and 283 nm. Its IR spectrum exhibited bands at 3410 cm^{-1} (hydroxyl group), 1717 cm^{-1} (carbonyl group), 1607 and 1508 cm^{-1} (aromatic nucleus). The ^1H, ^{13}C NMR and DEPT spectra of **1** possessed signals characteristic of an aryl-tetralin type lignan (Tables 1 and 2). Alkaline hydrolysis of **1** furnished **1a**, which was identified as 5-methoxy-(+)-isolariciresinol by comparison with published spectral and physical data (Vecchiett, Ferrari, Orsini, & Pelizzoni, 1974). The four signals at δ 170.9, 171.1, 20.7 and 20.8 appeared in the ^{13}C NMR spectrum and the proton signals at δ 2.02 (3H, s) and 2.06 (3H, s) confirmed that **1** had two acetyl groups. Inferred from the presence of proton signals at δ 7.04 (1H, s) and 7.21 (1H, s) (phenolic hydroxyl group), which disappeared when D_2O was added, the two acetyl groups should be located at C-9 and C-9′. Furthermore, the shifts observed in the ^{13}C NMR spectrum of **1** (as compared with **1a**) for the sig-

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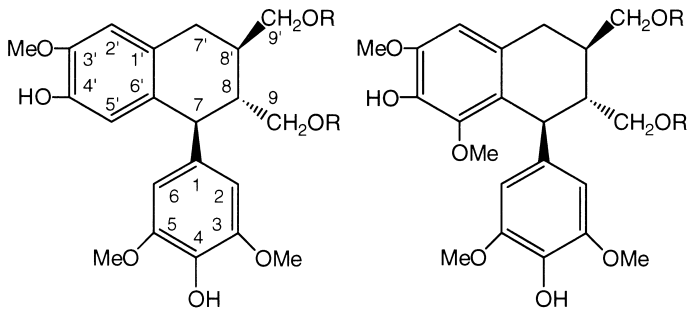

1. R=CH_3CO 2. R=CH_3CO

1a. R=H 2a. R=H

nals of C-9 (+1.9) and C-9′ (+2.2) revealed the presence of two acetyl groups at the C-9 and C-9′ positions. The absolute configuration of **1** and its derivative **1a** could be determined by examining their CD spectra (Sakakibara, Hiroji, Ina, & Yasue, 1974; Vecchiett et al., 1979; Ohashi, Watanabe, Okumura, & Kitagawa, 1994). CD spectra of **1** and **1a** indicated the absolute configurations of the aryl substituent at C-7 are *S*. From the above results, the structure of **1** was established as 5-methoxy-(+)-isolariciresinol-9,9′-diacetate.

Gaultherin B (**2**), a white powder, had a molecular formula of $C_{26}H_{32}O_{10}$ deduced from its EIMS at *m/z* 504, elemental analyses and ^1H and ^{13}C NMR data. The IR spectrum of **2** had bands at 3425 cm^{-1} (hydroxyl group), 1726 cm^{-1} (carbonyl group) and 1607, 1496 cm^{-1} (aromatic ring). Its ^1H and ^{13}C NMR spectra were very similar to those of **1** except for C-5′ at δ 146.9 and the additional carbon signal at δ 59.4 and proton signal at δ 3.34 (3H, s), which were assigned to OCH_3-5′. Therefore, compound **2** was a methoxy derivative of **1**. Alkaline hydrolysis of **2** afforded **2a**, determined to be (+)-lyoniresinol from its spectral features and physicochemical properties (Sakakibara et al., 1974; Vecchiett et al., 1979). The absolute stereochemistry of **2**, which is the same as those of (+)-lyo-

Table 1
^{13}C NMR spectral data for compounds **1**, **1a**, **2** and **2a** (100 MHz in acetone-d_6)[a]

Carbon	**1**	**1a**	DEPT	**2**	**2a**	DEPT
1	135.9	137.4	C	138.7	137.8	C
2	107.8	107.8	CH	107.0	106.5	CH
3	148.8	148.6	C	148.4	147.7	C
4	135.6	135.3	C	135.2	134.8	C
5	148.8	148.6	C	148.4	147.7	C
6	107.8	107.8	CH	107.0	106.5	CH
7	48.5	48.6	CH	42.7	40.4	CH
8	44.3	44.4	CH	45.4	46.8	CH
9	64.0	62.1	CH_2	65.8	62.7	CH_2
1′	127.4	128.4	C	128.5	128.8	C
2′	112.1	112.0	CH	107.1	107.0	CH
3′	146.8	146.5	C	148.0	147.0	C
4′	145.6	145.3	C	137.9	137.3	C
5′	116.5	116.8	CH	146.9	146.6	C
6′	133.1	133.9	C	125.3	125.1	C
7′	33.3	33.7	CH_2	33.2	32.3	CH_2
8′	36.7	40.4	CH	37.1	39.3	CH
9′	67.1	64.9	CH_2	67.5	64.9	CH_2
OMe-3	56.7	56.7	CH_3	56.8	56.4	CH_3
OMe-5	56.7	56.7	CH_3	56.8	56.4	CH_3
OMe-3′	56.2	56.2	CH_3	56.4	55.9	CH_3
OMe-5′				59.4	59.2	CH_3
CH_3CO	20.7, 20.8		CH_3, CH_3	20.7, 20.8		CH_3, CH_3
CO	170.9, 171.1		C, C	171.0, 171.2		C, C

[a] The assignment was based upon COSY and HETCOR experiments.

Table 2
The ^{1}H NMR data for compounds **1**, **1a**, **2** and **2a** (400 MHz in acetone-d_6)[a]

Proton	**1**	**1a**	**2**	**2a**
2	6.47 (1H, s)	6.46 (1H, s)	6.39 (1H, s)	6.29 (1H, s)
6	6.47 (1H, s)	6.46 (1H, s)	6.39 (1H, s)	6.29 (1H, s)
7	3.96 (1H, d, J=9.2 Hz)	3.94 (1H, d, J=10.1 Hz)	4.19 (1H, d, J=6.3 Hz)	4.23 (1H, d, J=5.8 Hz)
8	1.86 (1H, m)	1.83 (1H, m)	1.96 (1H, m)	1.86 (1H, m)
9	3.92 (2H, m)	3.71 (2H, m)	3.97 (2H, m)	3.26 (2H, m)
2′	6.69 (1H, s)	6.65 (1H, s)	6.60 (1H, s)	6.54 (1H, s)
5′	6.22 (1H, s)	6.22 (1H, s)		
7′	2.82 (1H, dd, J=15.5, 5.9 Hz), 2.06 (1H, dd, J=15.0, 10.3 Hz)	2.70 (1H, dd, J=15.3, 5.6 Hz), 2.18 (1H, dd, J=14.9, 10.1 Hz)	2.60 (1H, dd, J=14.9, 4.9 Hz), 2.20 (1H, dd, J=14.6, 11.5 Hz)	2.58 (1H, dd, J=14.8, 4.6 Hz), 2.38 (1H, dd, J=14.0, 11.8 Hz)
8′	2.21 (1H, m)	1.95 (1H, m)	1.36 (1H, m)	1.44 (1H, m)
9′	4.07 (1H, m), 4.19 (1H, m)	3.39 (1H, m), 3.82 (1H, m)	4.11 (1H, m), 4.20 (1H, m)	3.45 (1H, m), 3.85 (1H, m)
OMe-3	3.77 (3H, s)	3.77 (3H, s)	3.73 (3H, s)	3.64 (3H, s)
OMe-5	3.77 (3H, s)	3.77 (3H, s)	3.73 (3H, s)	3.64 (3H, s)
OMe-3′	3.81 (3H, s)	3.79 (3H, s)	3.99 (3H, s)	3.77 (3H, s)
OMe-5′			3.34 (3H, s)	3.35 (3H, s)
CH_3CO	2.02 (3H, s), 2.06 (3H, s)		1.97 (3H, s), 2.06 (3H, s)	
Ar-OH	7.04 (1H, s), 7.21 (1H, s)	7.09 (1H, s), 7.31 (1H, s)	7.13 (1H, s), 7.35 (1H, s)	7.16 (1H, s), 7.39 (1H, s)

[a] The assignment was based upon COSY and HETCOR experiments.

niresinol and **1**, **1a**, was established based on its CD spectrum. Thus, the structure of **2** was elucidated to be (+)-lyoniresinol-9,9′-diacetate.

3. Experimental

3.1. General procedures

M.p.'s were measured using XT_4 microscope apparatus and are uncor. IR spectra were recorded using Perkin-Elmer 983. CD and UV spectra were measured by JASCO-720W and UV-260, respectively. The ^{1}H and ^{13}C NMR spectra were run at 400 and 100 MHz, respectively, with TMS as int. standard. EIMS were obtained at 70 eV.

3.2. Plant material

The roots of *G. yunnanensis* were purchased from the market of Guiyang, the capital of Guizhou province of the People's Republic of China in 1995. A voucher specimen was deposited in the Division for Pharmacognostical Biotechnology, School of Pharmaceutical Sciences, Beijing Medical University, Beijing, China.

3.3. Extraction and isolation

Powdered roots (5 kg) were refluxed with 95% EtOH at 60°C for 3 h (three times). The combined solutions were evaporated under red. pres. to give an extract (500 g), which was suspended in water and then partitioned successively with petroleum ether, chloroform, EtOAc and BuOH. The chloroform fraction (30.5 g) was chromatographed over silica gel eluting with chloroform–EtOAc mixtures of increasing polarity to obtain part A (8.2 g), B (1.2 g), C (5.6 g), D (10.8 g) and E (2.6 g). Part B was subjected to repeated polyamide column chromatography eluting with acetone–H_2O (1:3) to furnish **1** (35 mg) and **2** (28 mg).

3.4. Gaultherin A (*1*)

A white powder from acetone–water (2:1), m.p. 159–160°C, $[\alpha]_D^{23}$ +30° (MeOH; *c* 0.08). Anal. calcd. for $C_{25}H_{30}O_9$, C: 63.28%, H: 6.37%; found C: 63.02%, H: 6.45%. UV λ_{max}^{MeOH} nm (log ε): 225 (2.68), 283 (0.91). IR ν_{max}^{KBr} cm^{-1}: 3410, 1717, 1607, 1508, 1461, 1427, 1268, 1210. EIMS *m/z* 474 [M^+, 96%], 414, 383, 354, 342, 339, 323, 314, 271, 200, 187, 167, 43 (100%). CD (MeOH; *c* 0.001): $[\theta]_{249}$+28007, $[\theta]_{255}$+26744, $[\theta]_{273}$+7324, $[\theta]_{288}$−9097. ^{13}C and ^{1}H NMR spectral data are given in Tables 1 and 2.

3.5. Gaultherin B (*2*)

A white powder from acetone–water (2:1), m.p. 120–121°C, $[\alpha]_D^{23}$ +40° (MeOH; *c* 0.05). Anal. calcd. for $C_{26}H_{32}O_{10}$, C: 61.90%, H: 6.39%; found, C: 61.65%, H: 6.52%. UV λ_{max}^{MeOH} nm (log ε): 218 (2.34), 274 (0.45). IR ν_{max}^{KBr} cm−^{1}: 3425, 1726, 1607, 1496, 1457, 1422. EIMS *m/z* 504 [M^+, 100%], 444, 413, 384, 372, 369, 353, 344, 301, 230, 217, 167, 43. CD (MeOH;

c 0.001): $[\theta]_{248}+19069$, $[\theta]_{254}+20584$, $[\theta]_{274}+3162$, $[\theta]_{288}-6081$. For ^{13}C and ^{1}H NMR spectral data see Tables 1 and 2.

3.6. Alkaline hydrolysis of gaultherin A (*1*) and B (*2*)

Gaultherin A (**1**, 12 mg) in 1 ml 0.8 N NaOH was heated at 80°C for 2 h. After cooling, the reaction mixture was neutralized with 1 N HCl and then extracted with EtOAc (3 times). The organic layers were evaporated to dryness under vacuum and the residue was applied to polyamide column purification eluting with acetone–H_2O (1:4) to afford **1a** (6 mg). By the same method, **2** (12 mg) afforded **2a** (7 mg).

3.7. 1a

Colorless needles recrystallized from acetone, m.p. 129–130°C, $[\alpha]_D^{23}$ +34° (MeOH; *c* 0.1). Anal. calcd. for $C_{21}H_{26}O_7$, C: 64.60%, H: 6.71%; found, C: 64.50%, H: 6.81%. UV λ_{max}^{MeOH} nm (log ε): 219 (2.26), 283(0.74). IR ν_{max}^{KBr} cm^{-1}: 3350, 1605, 1510, 1457, 1427, 1278, 1219. EIMS *m/z* 390 [M^+, 100%], 359, 341, 271, 217, 175, 167. CD (MeOH; *c* 0.001): $[\theta]_{250}+21123$, $[\theta]_{255}+19513$, $[\theta]_{274}+2612$, $[\theta]_{287}-1797$. ^{13}C and ^{1}H NMR spectral data are listed in Tables 1 and 2.

3.8. 2a

Colorless needle recrystallized from acetone, m.p. 117–118°C, $[\alpha]_D^{23}$ +68° (MeOH; *c* 0.1). Anal. calcd. for $C_{22}H_{28}O_8$, C: 62.85%, H: 6.71%; found, C: 62.96%, 6.31%. UV λ_{max}^{MeOH} nm (log ε): 219 (2.32), 278(0.40). IR ν_{max}^{KBr} cm^{-1}: 3380, 1598, 1490, 1463 1295, 1195. EIMS *m/z* 420 [M^+, 100%], 389, 371, 301, 247, 205, 167. CD (MeOH; *c* 0.001): $[\theta]_{248}+21108$, $[\theta]_{254}+22678$, $[\theta]_{273}+4593$, $[\theta]_{287}-1325$. ^{13}C and ^{1}H NMR spectral data are listed in Tables 1 and 2.

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