Notes

Two Diterpenoids from the Roots of *Gaultheria yunnanensis*

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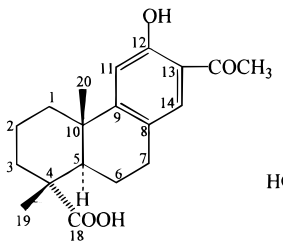
Two new diterpenoids, gaultheric acid (**1**) and gaultheronoterpene (**2**), were isolated from the roots of *Gaultheria yunnanensis*. Their structures were elucidated as 12-hydroxy-13-acetyl-8,11,13-podocarpatrien-18-oic acid (**1**) and 3*β*,12-dihydroxy-13-acetyl-4(18),8,11,13-podocarpatetraene (**2**) on the basis of spectral analysis.

Gaultheria yunnanensis (Franch.) Rehd. (Ericaceae) is a folk medicine widely used in the southwest and southern regions of the People's Republic of China for the treatment of rheumatoid arthritis, swellings and pain, trauma, chronic tracheitis, colds, and vertigo.[1] Previous studies have shown that the stems and leaves of *G. yunnanensis* contain a volatile oil, in which methyl salicylate is the major component (98.85% w/w).[2] We conducted a systematic phytochemical study on the roots of *G. yunnanensis* and have isolated and identified about 40 compounds including lignans, flavonoids, diterpenoids, triterpenoids, organic acids, coumarins, and sterols.[3] In the present paper, we describe the isolation and structural elucidation of two new diterpenoids, named gaultheric acid (**1**) and gaultheronoterpene (**2**).



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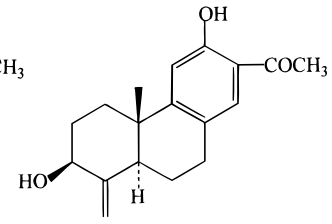

2

Gaultheric acid (**1**), mp 210−211 °C, was recrystallized from MeOH as colorless needles, and the molecular formula was assigned as $C_{19}H_{24}O_4$ on the basis of its HRMS at [M^+ + 1] *m*/*z* 317.1748 (calcd for $C_{19}H_{25}O_4$, 317.1554). The UV spectrum showed absorption maxima at 264 and 345 (aromatic ring) nm. Its IR spectrum exhibited bands at 1698 and 1642 cm^{-1} (a carboxylic acid group and a ketone group forming a hydrogen bond with the hydroxyl group at C-12) and at 1617, 1571, 1490 (aromatic ring) cm^{-1}. The presence of an aromatic ring was proved by signals at *δ* 6.80 (1H, s), 7.40 (1H, s), and 11.07 (Ar−OH, s) in its ^{1}H NMR spectrum and signals at *δ* 203.9, 183.1, 160.1, 157.9, 131.1, 126.2, 118.1, and 114.7 in the ^{13}C NMR spectrum.

Table 1. ^{1}H NMR Spectral Data of Compounds **1** and **2**[a]

proton(s)	**1**	**2**
H-1	1.39 m, 2.21 m	2.19 m, 2.27 m
H-2	1.62 m, 2.01 m	1.67 m,2.19 m
H-3	1.06 m, 2.24 m	4.09 m
H-5	1.52 dd (12.2,1.5)	2.17 m
H-6	2.18 m, 2.01 m	1.88 m, 2.88 m
H-7	2.76 m, 2.87 m	1.85 m, 2.90 m
H-11	6.80 s	6.91 s
H-14	7.40 s	7.45 s
H-18		4.80 s, 5.21 t
H-19	1.34 s	
H-20	1.12 s	1.01 s
$COCH_3$	2.58 s	2.60 s

[a] Values were recorded at 500 MHz (in $CDCl_3$ in the *δ* scale, with *J* values in Hz in parentheses).

Table 2. ^{13}C NMR Spectral Data of Compounds **1** and **2**[a]

carbon	**1** (DEPT)	**2** (DEPT)
C-1	39.0 (CH_2)	36.3 (CH_2)
C-2	19.8 (CH_2)	29.2 (CH_2)
C-3	37.2 (CH_2)	72.8 (CH)
C-4	43.9 (C)	151.8 (C)
C-5	52.3 (CH)	45.7 (CH)
C-6	20.9 (CH_2)	21.3 (CH_2)
C-7	31.1 (CH_2)	32.9 (CH_2)
C-8	126.2 (C)	125.7 (C)
C-9	157.9 (C)	156.3 (C)
C-10	39.4 (C)	39.9 (CH)
C-11	114.7 (CH)	114.9 (CH)
C-12	160.1 (C)	160.1 (C)
C-13	118.1 (C)	117.9 (C)
C-14	131.1 (CH)	131.3 (CH)
C-18	183.1 (C)	103.9 (CH_2)
C-19	28.7 (CH_3)	
C-20	22.9 (CH_3)	22.5 (CH_3)
$COCH_3$	203.9 (C)	203.9 (C)
$COCH_3$	26.6 (CH_3)	26.5 (CH_3)

[a] Values in ppm were recorded at 500 MHz (in $CDCl_3$). Assignments are from DEPT, ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, and HMBC experiments.

In the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of compound **1,** signals appeared for three methyl groups [δ_H 1.12 (3H, s), δ_C 22.9, δ_H 1.34 (3H, s), δ_C 28.7, and δ_H 2.58 (3H, s), δ_C 26.6], a ketone group (δ_C 203.9), a carboxylic acid group (δ_C 183.1), and a phenolic hydroxyl group [δ_H

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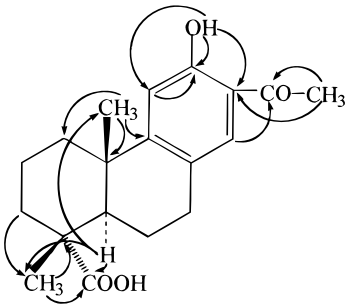

Figure 1. Prominent HMBC correlations for galtheric acid (**1**).

11.07 (1H, s)]. The above data, along with the appearance of 19 signals in the ^{13}C NMR spectrum, indicated that **1** is a diterpenoid with a tricyclic podocarpane-type skeleton.[4−7] The HMBC correlations of signals at δ 22.9 (C-20) with δ 1.39 (H-1) and δ 1.52 (H-5), and δ 28.7 (C-19) with δ 1.03 (H-3) and δ 1.52, δ 203.9 (C-17) with δ 2.58 ($COCH_3$), verified that the two methyl groups should be located at positions C-19 and C-20 and that the third one was from an acetyl group. In a similar manner, the carboxylic acid group, the ketone group, and the phenolic hydroxyl group were placed at positions C-18, C-13 and C-12, respectively, on the basis of the HMBC spectral data of **1** (Figure 1). The stereochemistry of **1** was determined by analyzing its phase-sensitive NOESY spectrum and by molecular modeling. In the NOESY spectrum of **1**, the NOE correlations of signals at δ 2.21 (H-1) with δ 1.12 (CH_3-20) and δ 6.80 (H-11), δ 2.01 (H-2) with δ 1.12, δ 1.52 with δ 2.24 (H-3), and δ 7.40 (H-14) with δ 2.58 ($COCH_3$) showed that compound **1** had H-5α and COOH-18α substituents (Figure 1). Therefore, **1** was assigned as 12-hydroxy-13-acetyl-8,11,-13-podocarpatrien-18-oic acid.

Gaultheronoterpene (**2**) was also recrystallized from MeOH as colorless needles. It was assigned a molecular formula of $C_{18}H_{22}O_3$ on the basis of its HRMS $[M^+ + 1]$ at *m/z* 287.1621 (calcd for $C_{18}H_{23}O_3$ 287.1648). Its UV spectrum exhibited absorption maxima at 264 and 341 (aromatic ring) nm, and in the IR spectrum absorption bands appeared for an aromatic ring at ν_{max} 1613, 1556, and 1484 cm^{-1} and for a ketone at ν_{max} 1643 cm^{-1}. Analysis of its ^1H and ^{13}C NMR and DEPT spectra suggested that **2** was a derivative of **1** bearing an acetyl group at C-13 and a phenolic hydroxyl group at C-12, with the absence of a carboxylic acid group at C-18 and a methyl group at C-19. ^1H NMR signals at δ 4.80 (1H, s) and 5.21 (1H, t) and ^{13}C NMR signals at δ 151.8 (C) and δ 103.9 (CH_2), together with the appearance of signals at δ_H 4.09 (1H, m) and δ_C 72.8, indicated that **2** had a double bond and an additional hydroxyl group, respectively, in comparison to **1**. In the $^1H-^1H$ COSY spectrum, the correlations of δ 4.09 with δ 1.67 (H-2), δ 1.67 with δ 2.27 (H-1), δ 2.17 (H-5) with δ 1.88 (H-6), and δ 1.88 with δ 2.90 (H-7) indicated that the hydroxyl group was located at C-3 and the double bond was at the C-4 (18) position. The stereochemistry of **2** was also established by thorough analysis of its phase-sensitive NOESY spectrum and by molecular modeling. The key NOE correlations of signals at δ 2.27 with δ 6.91 (1H, s, H-11), δ 2.27 with δ 1.67, δ 1.67 with δ 4.09, δ 4.09 with δ 2.17, δ 1.88 with δ 2.90, and δ 2.90 with δ 7.45 (1H, s, H-14) indicated **2** to have a 3β-hydroxyl group and a 5α-hydrogen substituent. Thus, the structure of **2** was elucidated as 3β,-12-dihydroxy-13-acetyl-4(18),8,11,13-podocarpatetraene (**2**).

Experimental Section

General Experimental Procedures. Melting points were determined on a Mel-temp apparatus and are uncorrected. The UV spectra were recorded on a UV-240 spectrometer and IR spectra on a Perkin-Elmer 983 spectrometer. NMR spectra were run on a JEOL α-500 NMR spectrometer using TMS as internal standard. EIMS were recorded on AEI-MS-50 mass spectrometer, and HRMS was determined on Voyager Bio-spectrometry Workstation (ionization method: MALDI-TOF). CAChe Version 3.8 on Power Machintosh 7500/100 was used for molecular modeling.

Plant Material. The roots of *G. yunnanensis* were obtained in September 1995 from a market at Guiyang, Guizhou Province, People's Republic of China. A voucher specimen (acquisition no. 950814) has been deposited in the Division for Pharmacognostical Biotechnology, School of Pharmaceutical Sciences, Beijing Medical University, Beijing, People's Republic of China.

Extraction and Isolation. Powdered roots (15 kg) were refluxed with 95% EtOH at 60 °C. The ethanol extract was resuspended in water and successively extracted with petroleum ether, chloroform, ethyl acetate, and *n*-butanol. The solvent was removed from the petroleum ether fraction under vacuum, and then the gummy residue was chromatographed over silica gel (100−200 mesh) and eluted with mixtures of petroleum−EtOAc of increasing polarity. Compounds **1** (17 mg) and **2** (10 mg) were obtained by further chromatography over silica gel (200−300 mesh) using benzene−EtOAc (10:1) as eluting solvents and recrystallization from MeOH.

Gaultheric acid (1): colorless needles (MeOH); mp 210−211 °C; UV (EtOH) λ_{max} (log ϵ) 218 (4.34), 263 (2.90), 338 (sh) (0.80) nm; IR ν_{max} 3060, 2960, 1698, 1642, 1617, 1571, 1490, 1230, 767 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 316 $[M]^+$ (100), 301 (35), 256 (20), 255 (93), 213 (22), 199 (21), 175 (14), 43 (60); HRMS $[M^+ + 1]$ *m/z* 317.1748 (calcd for $C_{19}H_{25}O_4$ 317.1554).

Gaultheronoterpene (2): colorless needles (MeOH); mp 187−189 °C; UV (EtOH) λ_{max} (log ϵ) 219 (3.00), 262 (1.98), 339 (sh) (0.55) nm; IR ν_{max} 3459, 2930, 2850, 1643, 1484, 1306, 1194, 892, 791 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 286 $[M]^+$ (77), 271 (16), 253 (14), 227 (13), 216 (23), 211 (28), 201 (25), 199 (17), 189 (17), 149 (16), 43 (100); HRMS $[M^+ + 1]$ *m/z* 287.1621 (calcd for $C_{18}H_{23}O_3$ 287.1648).

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