TWO NEW BENZOYL ESTERS OF GLUCOSE FROM *Lagotis yunnanensis*

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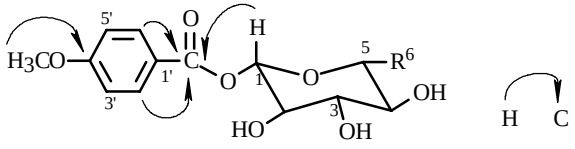
Two new benzoyl esters of glucose 1-O-*(*E*)-4′-methoxybenzoyl*-β-*D-glucopyranose (**1**) and 1*-O-*(*E*)-4′-methoxybenzoyl*-β-*D-gluconic acid (**2**) were isolated from* Lagotis yunnanensis*, together with six previously known iridoid glucosides. The structures of these compounds were elucidated on the basis of spectral analysis, including 2D NMR spectroscopy.*

Key words: *Lagotis yunnanensis*, benzoyl esters, 1-*O*-(*E*)-4′-methoxybenzoyl-*β*-D-glucopyranose, 1-*O*-(*E*)-4′-methoxybenzoyl-*β*-D-gluconic acid.

The genus *Lagotis* (Scrophulariaceae) is represented by 17 species in China, mostly growing in the southwestern part of the country on mountains 3000 meters above sea level or higher [1]. Several species, such as *L. yunnanensis*, *L. glauca*, *L. integra*, and *L. brachystachya*, are used in Tibetan folk medicine for the treatment of fever, hypertension, and acute and chronic hepatitis [2, 3]. As part of our studies of medicinal plants growing on the Yunnan-Tibet Plateau, *L. yunnanesis* W. W. Smith, an herbaceous plant collected in Deqin prefecture, southwestern part of China, was examined. In the previous papers, some chemical constituents from this plant were reported [4–7]. Continued studies on the same plant led to the isolation of two new benzoyl esters of glucose (**1** and **2**). The structures of these compounds were elucidated on the basis of spectral analysis, including 2D NMR spectroscopy.

Compound **1** was isolated as a white amorphous powder, ($C_{14}H_{18}O_8$). The IR spectrum showed characteristic absorptions for OH (3367 cm^{-1}, br), ester (1710 cm^{-1}), and the aromatic ring (1598, 1559, and 1508 cm^{-1}). The UV spectrum at 242 (3.70) and 265 (3.22) nm also confirmed the existence of these unsaturated functional groups.



1, 2

1: R = CH_2OH; **2:** R = COOH

Fig. 1. Structure and the key correlation in HMBC spectrum of **1** and **2**.

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TABLE 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data (acetone-d_6) of **1** and **2**

C atom	**1**		**2**	
	δ_C (DEPT)	δ_H	δ_C (DEPT)	δ_H
1	101.33 (CH)	5.09 (d, 1H, J = 7.8)	100.13 (CH)	5.08 (d, 1H, J = 7.5)
2	74.61 (CH)	3.26 (dd, 1H, J = 9.1, 7.8)	71.95 (CH)	3.26 (dd, 1H, J = 9.1, 7.5)
3	76.58 (CH)	3.41 (dd, 1H, J = 9.1, 8.0)	73.85 (CH)	3.41 (dd, 1H, J = 9.1, 8.7)
4	71.24 (CH)	3.25 (dd, 1H, J = 10.0, 8.0)	72.50 (CH)	3.25 (dd, 1H, J = 10.0, 8.7)
5	77.95 (CH)	3.31 (m, 1H)	71.97 (CH)	3.31 (d, 1H, J = 10.0)
6	62.57 (CH_2)	3.61 (dd, 1H, J = 12.0, 6.0) 3.90 (dd, 1H, J = 12.0, 2.0)	183.80 (C)	11.88 (s, 1H, COOH)
1′	123.20 (C)	-	123.27 (C)	-
2′,6′	132.02 (CH)	7.92 (dd, 2H, J = 8.6, 2.0)	131.43 (CH)	7.92 (dd, 2H, J = 8.6, 2.0)
3′,5′	116.94 (CH)	7.10 (dd, 2H, J = 8.6, 2.0)	116.36 (CH)	7.12 (dd, 2H, J = 8.6, 2.0)
4′	161.44 (C)	-	161.47 (C)	-
7′	166.15 (C)	-	166.17 (C)	-
MeO	52.10 (CH_3)	3.68 (s, 1H)	52.25 (CH_3)	3.72 (s, 1H)

From its NMR experiments (Table 1), the signals of a 4-disubstituted benzoyl (C-1′ to C-7′, H-2′, H-3′, H-5′, and H-6′) and a *β*-D-glucose (C-1 to C-6, H-1 to H-6) were observed. The ^{1}H NMR spectrum showed an AA′BB′ system for four aromatic protons at δ_H 7.92 (2H, dd, J = 8.6, J = 2.0 Hz, H-2′, 6′) and 7.10 (2H, dd, J = 8.6, J = 2.0 Hz, H-3′, 5′), and an aromatic methoxyl group at δ_H 3.68 (s, MeO). An anomeric proton at δ_H 5.09 (1H, d, J = 7.8 Hz, H-1) and a methylene group at δ_H 3.61 (1H, dd, J = 12.0, 6.0 Hz, H-6a) and 3.90 (1H, dd, J = 12.0, 2.0 Hz, H-6b) in the ^{1}H NMR spectrum, as well as ^{13}C NMR signals at δ_C 101.33 (C-1) and 62.57 (C-6), suggested the presence of a glucose moiety. Based on its coupling constant of anomeric proton (J = 7.9 Hz), a *β*-glucose was confirmed. In the case of an *α*-glucose, the coupling constant normally appears at approximately 3.6 Hz [8]. Detailed NMR data are presented in Table 1. In the HMBC spectrum (Fig. 1), the correlations between δ_H 3.68 (MeO) to δ_C 161.44 (C-4′) suggested that the benzoyl moiety was 4-methoxybenzoyl, while δ_H 5.09 (H-1) to δ_C 166.15 (C-7′) indicated that the 4-methoxybenzoyl group was attached to the C-1 position of the glucose.

Therefore, the structure of compound **1** was elucidated as 1-*O*-(*E*)-4′-methoxybenzoyl-*β*-D-glucopyranose.

Compound **2** was also isolated as a white amorphous powder ($C_{14}H_{16}O_9$). The IR spectrum showed characteristic absorptions for COOH (3300–2510 cm^{-1}, br), ester (1708 cm^{-1}), and aromatic ring (1598, 1559 and 1510 cm^{-1}).

From the NMR spectrum (Table 1), other spectroscopic data of **2** were very similar to those of 1, except for a gluconic acid (C-1 to C-6, H-1 to H-6) in place of glucose moiety. The EIMS spectrum of **2** exhibited a molecular ion at *m/z* 328 and a characteristic peak at *m/z* 152 (M^+-176), which also correspond to the presence of a gluconic acid group. Therefore, the structure of compound **2** was elucidated as 1-*O*-(*E*)-4′-methoxybenzoyl-*β*-D-gluconic acid.

EXPERIMENTAL

General Methods. The $[\alpha]_D$ values was obtained on a JASCO-20C digital polarimeter. UV spectra were determined on a UV 210A spectrometer and IR spectra on a Bio-Red FTS-135 spectrometer. 1D and 2D NMR spectra were taken on a DRX-500 instrument with TMS as internal reference. EIMS were recorded on a VG Auto spec-3000 mass spectrometer.

Plant Material. The plant material was collected in Deqin Country, Yunnan Province, P. R. China, in September 2001, and was identified as *Lagotis yunnanensis* W. W. Smith. A voucher specimen was deposited in the School of Pharmacy, Yunnan University.

Extraction and Isolation. The dried whole plants (8.0 kg) were extracted four times with 95% EtOH (20 L × 4) at room temperature for 20 days, and the combined extracts were concentrated in vacuo. The residue was suspended in H_2O and then partitioned with $CHCl_3$ (1.5 L × 4) and *n*-BuOH (1.5 L × 6), successively. The *n*-BuOH extract (897.83 g) was subjected to chromatography over silica gel, eluting with $CHCl_3$–MeOH–H_2O (8:1:0.1, 6:1:0.1, 4:1:0.15, 2:1:0.2, 1:1:0.4 and 0:1:0), to afford seventeen fractions (A-Q). Fraction B (15.33 g) was purified by polyamide column chromatography (250 g) eluted with $CHCl_3$–MeOH (1:0→0:1) to give two fractions (1 and 2). Fraction 2 (2.34 g) was purified by silica gel column chromatography

(60 g) eluted with $CHCl_3$–MeOH (30:1→0:1) to give compound **1** (340 mg). Fraction H (45.89 g) was subjected to column chromatography over silica gel (800 g, 200–300 mesh), using $CHCl_3$–MeOH (60:1→ 0:1) as eluent to give four factions (1–4). Fraction 2 (15.36 g) was chromatographed over polyamide (450 g), eluting with $CHCl_3$–MeOH (60:1→0:1) to afford compound **2** (20 mg).

Compound 1. White amorphous powder. $[\alpha]_D^{26}$ –34.65 (*c* 0.218, MeOH). UV spectrum (MeOH, max, nm): 242, 265 (log ε 3.70, 3.22). IR spectrum (KBr, ν, cm^{-1}): 3367, 2982, 2948, 2834, 2527, 2053, 1710, 1598, 1559, 1508, 1454, 1419, 1115, 1033, 656. ^1H and ^{13}C NMR: Table 1. HREIMS: 314.2968 ($C_{14}H_{18}O_8$, calc. 314.2911). Mass spectrum (EI, 70 eV, *m*/*z*): 314 (19), 152 (100), 135 (95), 163 (10), 147 (9), 133 (10), 107 (9), 77 (18).

Compound 2. White amorphous powder. $[\alpha]_D^{26}$ –22.08 (*c* 0.114, MeOH). UV spectrum (MeOH, max, nm): 244 (3.80), 268 (3.45). IR spectrum (KBr, ν, cm^{-1}): 3300, 2950, 2854, 2586, 1708, 1598, 1559, 1510, 1432, 1419, 1121, 1029, 664. ^1H and ^{13}C NMR: Table 1. HREIMS: 328.2786 ($C_{14}H_{16}O_9$, calc. 328.2751). Mass spectrum (EI, 70 eV, *m*/*z*): 328 (15), 152 (100), 135 (90), 163 (18), 147 (12), 133 (16), 107 (9), 77 (16).

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