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SECOIRIDOID GLYCOSIDES FROM THE ROOTS OF *Picrorhiza scrophulariiflora*

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*A new secoiridoid glycoside, named picrogentioside D (**1**), has been isolated from the underground parts of* Picrorhiza scrophulariiflora*, together with three known compounds, picrogentioside C (**2**), scroneoside A (**3**), and scrophuloside B (**4**). Their structures were elucidated on the basis of spectroscopic evidence.*

Keywords: *Picrorhiza scrophulariiflora*, secoiridoid glycoside, picrogentioside D.

The plant *Picrorhiza scrophulariiflora* (Scrophulariaceae) grows in the high altitude regions (over 4,400 m) in the southeast of Tibet and the northwest of Yunnan in China. The roots of this plant, known to be rich in terpenoids, iridoid glycosides, phenolic glycosides, and phenylethanoid glycosides [1–7], are used in traditional Chinese medicine for the treatment of damp-heat dysentery, jaundice, and steaming of bone [8]. Here we report the isolation and characterization of a new secoiridoid glycoside, named picrogentioside D (**1**) (Fig. 1), as well as three known compounds, picrogentioside C (**2**) [5], scroneoside A (**3**) [6], and scrophuloside B (**4**) [7].

Picrogentioside D (**1**) is a yellow amorphous powder, $[\alpha]_D^{25}$ –130.2° (*c* 0.50, MeOH). The molecular formula $C_{26}H_{34}O_{14}$ was assigned for **1** on the basis of HR-ESI-MS at *m/z* 593.1845 $[M + Na]^+$ (calcd for $C_{26}H_{34}O_{14}Na$, 593.1841). Structural assessment of **1** was accomplished using a combination of NMR techniques, along with comparisons with the assignments of picrogentioside C [5].

The ^1H NMR spectrum of **1** (Table 1) showed the presence of three methoxyl groups [δ 3.55 (3H, s), 11-OCH_3; 3.81 (6H, s), 3″, 5″-OCH_3], an acetal proton [δ 5.52 (1H, d, J = 7.2 Hz), H-1], protons of an oxygenated methylene [δ 4.28 (1H, m), 4.19 (1H, m, H_2-7] and a methylene [δ 1.87 (1H, m), 1.96 (1H, m), H_2-6], two methine protons [δ 2.87 (1H, dd, J = 6.8, 12.0 Hz), H-5; 2.62 (1H, dd, J = 7.2, 12.8 Hz), H-9], an olefinic proton [δ 7.50, s, H-3], and one terminal vinyl group [δ 5.25 (1H, br.d, J = 10.8 Hz), 5.34 (1H, br.d, J = 17.2 Hz, H_2-10); 5.75 (1H, m, H-8)]. These signals indicated one secologanol moiety [9–11].

Furthermore, the spectrum showed the presence of a syringic acid moiety [12], which was evident from two aromatic H-atoms at δ 7.21 (2H, s, H-2″, 6″), and two methoxyl groups at δ 3.81 (6H, s, 3″, 5″-OCH_3). In addition, one anomeric H-atom was observed at δ 4.54 (1H, d, J = 8.0 Hz) which, combined with the ^{13}C NMR signals at δ 98.8–61.2 (Table 1), is typical for one β-glucopyranosyl moiety. The sugar component was identified as D-glucopyranoside by co-HPLC analysis of its 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivative with the same derivative of the standard sugar [13]. The relatively large J value of the two anomeric protons indicated that the glucoside linkage had a β-configuration.

Compared with the secologanol moiety [9–11] signals, the syringic acid moiety should be attached at C-7 of the secologanol moiety by the downfield shift of the C-7 protons at δ 4.19 and 4.28 (H_2-7) and of the C-7 signal at δ 63.1.

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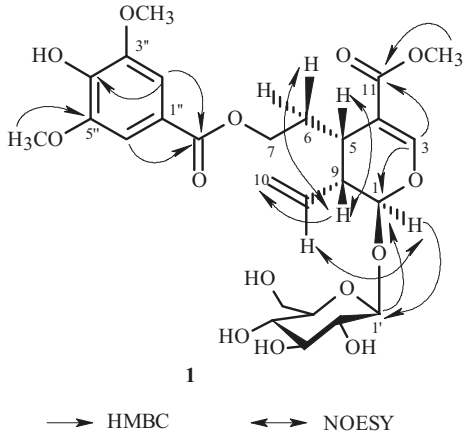
TABLE 1. ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR Data of **1** (DMSO-d_6, δ, ppm, J/Hz)

C atom	δ_C	δ_H	C atom	δ_C	δ_H
1	95.7	5.52 (d, J = 7.2)	2″	106.8	7.21 (s)
3	152.0	7.50 (s)	3″	147.5	–
4	109.4	–	4″	140.6	–
5	30.4	2.87 (dd, J = 6.8, 12.0)	5″	147.5	–
6	29.1	1.96 (m); 1.87 (m)	6″	106.8	7.21 (s)
7	63.1	4.28 (m); 4.19 (m)	C=O	165.5	–
8	134.7	5.75 (m)	3″, 5″-OCH_3	56.0	3.81 (s)
9	43.3	2.62 (dd, J = 7.2, 12.8)	1′	98.8	4.54 (d, J = 8.0)
10	118.9	5.25 (br.d, J = 10.8)	2′	76.7	3.15 (m)
		5.34 (br.d, J = 17.2)	3′	73.0	2.95 (t, J = 8.0)
11	166.6	–	4′	70.0	3.02 (t, J = 9.2)
11-OCH_3	51.0	3.55 (s)	5′	77.3	3.25 (m)
1″	119.4	–	6′	61.2	3.43 (m); 3.69 (m)



1

HMBC NOESY

Fig. 1. Key HMBC and NOESY correlations of compound **1**.

The relative configuration of **1** was determined by analysis of the NMR coupling constants and with the aid of NOESY experiments (Fig. 1). A J (1, 9) value of 7.2 Hz suggested a *trans*-diaxial orientation for H-1 and H-9 [14–16], and the NOESY cross-peaks between H-5 and H-9, as well as between Hβ-6 and H-9, revealed that they were on the same side of the molecular plane (β). From the above data, the structure of **1** was established as shown in Fig. 1.

EXPERIMENTAL

General Experimetal Procedures. Optical rotations were obtained using a PerkinElmer 241-MC polarimeter. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a Bruker AV400 spectrometer with DMSO-d_6 as solvent and TMS as internal standard. HR-ESI-MS data were measured with a Bruker AOEXIII 7.0 Tesla FTMS. HPLC was conducted using reverse-phase columns (Mightysil RP-18 and 8, Kantho Chemical Co. Ltd.) with the MeOH–H_2O solvent system. Colum chromatography was carried out on silica gel (Qingdao Marine Chemical Company, China, 200–300 mesh) and Sephadex LH-20 (Amersham Pharmacia Biotech AB). Silica GF254 for TLC was obtained from Qingdao Marine Chemical Company, China and Merck.

Plant Material. Roots of *Picrorhiza scrophulariiflora* were collected in June 2014 in Sichuan Province, China and identified by Prof. Qing-Wen Sun (Guiyang College of Traditional Chinese Medicine). A voucher specimen has been deposited in the Chongqing Medical and Pharmaceutical College, China.

Extraction and Isolation. The dried and ground roots (underground parts) of *Picrorhiza scrophulariiflora* (2.5 kg) were successively extracted three times with MeOH under reflux. After removal of the solvent *in vacuo*, the residue (1.09 kg)

was suspended in H_2O and then extracted successively with *n*-hexane, EtOAc, and *n*-BuOH. The EtOAc layer was concentrated *in vacuo* to give a viscous residue (175 g), which was subjected to silica gel column chromatography eluting with mixtures of CH_2Cl_2–MeOH of increasing polarity to give eight fractions (Frs. 1–8). Fraction 7 (19.0 g) was further separated by silica gel column chromatography using CH_2Cl_3–MeOH gradient as eluent to afford five fractions (Frs. 6A–6E). Fraction 6B (2.3 g) was further purified by Sephadex LH-20 and further separated by preparative thin-layer chromatogram to yield compounds **1** (4.2 mg), **3** (3.3 mg), and **4** (4.7 mg). Fraction 6C was purified by Sephadex LH-20 and further separated by preparative thin-layer chromatogram to afford compound **2** (5.4 mg).

Compound 1. Yellow amorphous powder; $[\alpha]_D^{25}$–130.2° (*c* 0.50, MeOH). For ^1H and ^{13}C NMR spectra, see Table 1. HR-ESI-MS *m/z* 593.1845 $[M + Na]^+$ (calcd for $C_{26}H_{34}O_{14}Na$, 593.1841).

Acid Hydrolysis of 1. A solution of compound **1** (6 mg) in 2 N TFA (3 mL) was refluxed at 100°C for 3 h. The reaction mixture was extracted with EtOAc. D-Glucose was found to be the only sugar present in the water part following the procedure of Oshima, Yamauchi, and Kumanotani [13]. The H_2O layer was neutralized by passing through an ion exchange resin (Amberlite MB-3) column and concentrated under reduced pressure to dryness. The residue was dissolved in H_2O (1 mL), to which L-(–)-α-methylbenzylamine (5 mg) and $NaBH_3CN$ (8 mg) in EtOH (1 mL) were added. The mixture was stirred at 40°C for 4 h, then acidified by addition of glacial AcOH (0.2 mL) and evaporated to dryness. The resulting solid was acetylated with Ac_2O (0.3 mL) in pyridine (0.3 mL) for 24 h at room temperature. After evaporation, H_2O (1 mL) was added to the residue. The solution was passed through a Sep-Pak C18 cartridge and washed with H_2O and CH_3CN. The CH_3CN eluate was analyzed by HPLC under the following conditions: solvent, MeCN–H_2O (2:3); flow rate, 0.8 mL/min; detection, UV 230 nm. The derivative of D-glucopyranose was detected with t_R 17.3 min.

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