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TWO NEW SECOIRIDOID GLYCOSIDES FROM THE ROOTS OF *Picrorhiza scrophulariiflora*

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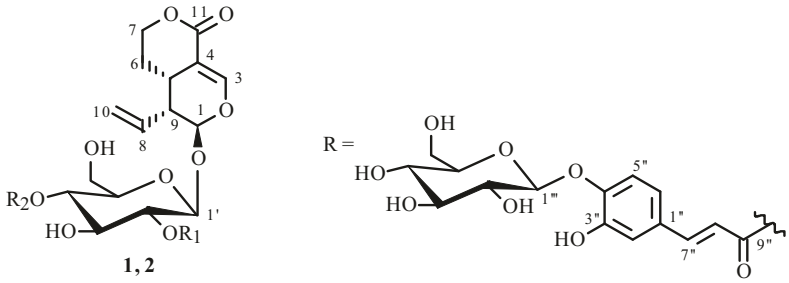
*Two new secoiridoid glycosides, named picrosecoside A (**1**) and picrosecoside B (**2**), have been isolated from the underground part of* Picrorhiza scrophulariiflora*. Their structures were elucidated on the basis of spectroscopic evidence.*

Keywords: *Picrorhiza scrophulariiflora*, secoiridoid glycosides, picrosecosides A and B.

Picrorhiza scrophulariiflora 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 are used in traditional chinese medicine for the treatment of "damp-heat" dysentery, jaundice, and "steaming of bone" [1]. This paper describes the structural elucidation and identification of two new secoiridoid glycosides, named picrosecoside A (**1**) and picrosecoside B (**2**).

Compound **1**, a pale yellow amorphous powder, has the molecular formula $C_{31}H_{38}O_{17}$ on the basis of HR-ESI-MS (*m/z* 705.1944 $[M + Na]^+$). IR absorptions (KBr, ν_{max}, cm^{-1}): 3436, 1693, 1628, 1611, and 1509 suggested the existence of a hydroxyl, an α,β-unsaturated ester, and aromatic groups in compound **1**. The ^1H NMR and ^{13}C NMR spectra of compound **1** were quite similar to those of picrosecosides I and II [2].

The ^1H NMR spectrum of compound **1** (Table 1) showed the presence of an acetal proton [δ 5.37 (1H, d, J = 1.2 Hz)] (the J value for H-1 indicated that it was β-positioned [3]), an oxygenated methylene proton [3.98 (1H, m), 4.25 (1H, d, J = 9.2 Hz)], an olefinic proton [7.38 (1H, d, J = 2.4 Hz)], a methylene protons [1.42 (1H, ddd, J = 3.6, 12.0, 16.4 Hz), 1.70 (1H, d, J = 12.0 Hz)], two methine protons [2.69 (1H, m); 2.65 (1H, dd, J = 8.0, 12.0 Hz)], and one terminal vinyl group [5.22 (1H, dd, J = 2.0, 9.0 Hz), 5.31 (1H, dd, J = 2.0, 16.8 Hz); 5.41 (1H, m)]. The above results indicated the presence of a sweroside moiety [3], a 1,3,4-trisubstituted aromatic ring [7.09 (1H, br.s); 7.05 (2H, br.d, J = 8.0)], and a pair of *trans* olefinic protons at δ 7.48 and 6.24 with a coupling constant of 16.0 Hz; these signals indicated the presence of a *trans*-caffeoyl moiety [4, 5]. The two signals at [δ 4.82 (1H, d, J = 8.0 Hz)] and [4.64 (1H, d, J = 8.4 Hz)] were assigned to the anomeric proton of glucose, indicating that compound **1** have two glucose moieties. Acid hydrolysis of **1** liberated D-glucose, which was identified by TLC comparison with the authentic sugars. The relatively large J value for the two anomeric protons indicated that the glucoside linkage was β (Table 1).



1: R_1 = R, R_2 = H
2: R_1 = H, R_2 = R

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TABLE 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data of Compounds **1** and **2** (DMSO-d_6, δ, ppm, J/Hz)

C atom	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
Secoiridoid				
1	95.5	5.37 (d, J = 1.2)	95.7	5.45 (d, J = 1.2)
3	150.7	7.38 (d, J = 2.4)	151.3	7.50 (d, J = 2.4)
4	104.9		104.9	
5	27.2	2.69 m	26.7	3.06 m
6	24.1	1.42 (ddd, J = 3.6, 12.0, 16.4), 1.70 (d, J = 12.0)	24.2	1.52 (ddd, J = 4.0, 12.0, 16.0), 1.78 (d, J = 12.0)
7	67.6	3.98 m; 4.25 (d, J = 9.2)	67.6	4.28 m; 4.37 (d, J = 8.8)
8	131.9	5.41 m	132.3	5.50 (ddd, J = 8.0, 10.0, 16.8)
9	41.0	2.65 (dd, J = 8.0, 12.0)	41.4	2.70 (dd, J = 8.0, 12.0)
10	120.5	5.22 (dd, J = 2.0, 9.0), 5.31 (dd, J = 2.0, 16.8)	120.3	5.27 (dd, J = 2.0, 9.0), 5.35 (dd, j = 2.0, 16.8)
11	163.6		164.5	
Caffeoyl				
1″	128.6		128.6	
2″	115.0	7.09 br.s	115.0	7.17 br.s
3″	146.6		146.8	
4″	147.4		147.5	
5″	115.8	7.05 (br.d, J = 8.0)	116.0	7.12 (br.d, J = 8.0)
6″	120.8	7.05 (br.d, J = 8.0)	120.8	7.12 (br.d, J = 8.0)
7″	144.5	7.48 (d, J = 16.0)	144.9	7.48 (d, J = 16.0)
8″	115.8	6.24 (d, J = 16.0)	115.9	6.24 (d, J = 16.0)
9″	165.6		165.7	
Glc-1				
1′	95.4	4.82 (d, J = 8.0)	98.0	4.64 (d, J = 8.0)
2′	73.2	4.65 (dd, J = 9.2, 8.0)	73.2	3.15 m
3′	73.5	3.51 m	73.6	3.52 m*
4′	70.2	3.18 (dd, J = 8.8, 9.2)	71.2	4.66 (dd, J = 9.6, 11.6)
5′	77.5	3.34 m*	74.9	3.52 m*
6′	60.8	3.50 m*; 3.71 m*	60.7	3.49 m*; 3.71 m*
Glc-2				
1‴	101.5	4.64 (d, J = 8.4)	101.5	4.79 (d, J = 7.2)
2‴	75.8	3.34–3.50 m*	75.8	3.30 m*
3‴	73.2	3.34–3.50 m*	73.2	3.31 m*
4‴	69.7	3.16 m*	69.8	3.17 m*
5‴	77.2	3.34–3.35 m*	77.2	3.35 m*
6‴	60.7	3.50 m*; 3.71 m*	60.7	3.49 m*; 3.71 m*

*Signal pattern unclear due to overlapping.

The ^{13}C NMR spectrum of **1** (Table 1) showed the presence of one carbonyl carbon (δ 165.6) of the caffeoyl moiety, eight olefinic carbons (147.4–115.0), and characteristic signals of two glucopyranosyl groups (101.5–60.7). The chemical shift of the anomeric carbon (δ 101.5) suggested that glucose 1 was glycosylated with phenol; in addition, the aglycone moiety containing ten carbon signals showed the presence of the sweroside moiety (95.5, 150.7, 104.9, 27.2, 24.1, 67.6, 131.9, 41.0, 120.5, 163.6).

The HMBC (Fig. 1) correlation between 3″-OH and C-2″, H-1‴ and C-4″ demonstrated that glucose 2 was connected to the C-4″ oxygen atom. The correlation between H-2′ and the carbonyl carbon and the downfield shift of the oxygenated methenyl proton at δ 4.65 (H-2′) suggested that the *trans*-caffeyl moiety was attached at C-2′ of the glucose 1. The relative configuration of **1** was determined by analysis of the NOESY spectra. The correlation between H-5 (δ 2.69) and H-9 (2.65) showed that the two protons were positioned on the same side. Thus, the structure and relative stereochemistry of **1** were determined to be those shown in Fig. 1.

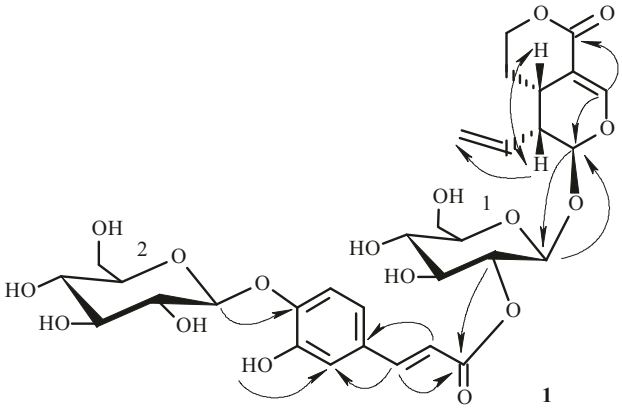

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Fig. 1. Key HMBC and NOESY correlations of **1**.

Compound **2** was obtained as a pale yellow amorphous powder. Its molecular formula was determined as $C_{31}H_{38}O_{17}$ from the $[M + Na]^+$ quasi-ion peak at *m/z* 705.1994 in the HR-ESI-MS spectra. The ^1H NMR and ^{13}C NMR spectra of compound **2** were quite similar to those of **1** (Table 1), except for the signals originating from the linkage between the carbonyl carbon and the glucose 1. The HMBC correlation between H-4′ and the carbonyl carbon, and the downfield shift of the oxygenated methenyl proton at δ 4.66 (H-4′), suggested that the *trans*-caffeyl moiety was attached at the C-4′ oxygen atom. Accordingly, picrosecoside II was determined to have the structure **2**.

EXPERIMENTAL

General Experimetal Procedures. UV spectra were recorded on a Milton Roy Spectronic 1201 spectrophotometer, and FTIR spectra were measured on a PerkinElmer 157G infrared spectrophotometer. Optical rotations were obtained using a PerkinElmer 241-MC polarimeter. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained on a Bruker AV 400 spectrometer with DMSO-d_6 as the solvent and TMS as an 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. Column 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 produced by Qingdao Marine Chemical Company, China and Merck.

Plant Material. Roots of *Picrorhiza scrophulariiflora* were collected in October 2006 in Sichuan Province, China and identified by Prof. Qi-shi Sun (Shenyang Pharmaceutical University). A voucher specimen has been deposited in the Herbarium of the School of Traditional Chinese Medicine of Shenyang Pharmaceutical University, China.

Extraction and Isolation. The dried and ground roots (underground parts) of *Picrorhiza scrophulariiflora* (3.0 kg) were successively extracted three times with 90% EtOH under reflux. After removal of the solvent *in vacuo*, the residue (1.6 kg) was suspended in H_2O and then extracted successively with petroleum ether (br 60–90°C), EtOAc, and *n*-BuOH. The *n*-BuOH layer was concentrated *in vacuo* to give a viscous residue (500 g), which was then dissolved in water (2 L) and subjected to macroporous D-101 resin column chromatography and eluted successively with water and ethanol (water, 30%, 50%, and 100% ethanol). The 50% EtOH eluted fraction was evaporated *in vacuo* to yield a residue (140 g) that was subjected to silica gel column chromatography and eluted with mixtures of $CHCl_3$–MeOH of increasing polarity to give eight fractions (Frs.1–8).

Fraction 3 (28.5 g) was chromatographed over silica gel using a EtOAc–MeOH gradient system as the eluent to yield five fractions 3A–3E. Fraction 3B (276.6 mg) was purified by Sephadex LH-20 chromatography and further separated by reverse-phase HPLC using MeOH–H_2O (57:43/48:52) as the mobile phase to yield compounds **1** (18.4 mg) and **2** (15.6 mg).

Compound 1. Yellow amorphous powder. $[\alpha]_D^{25}$ –140.3° (*c* 0.45, MeOH). IR (KBr, ν_{max}, cm^{-1}): 3527, 3436, 3300, 2893, 1693, 1628, 1611, 1509, 1403. For ^1H and ^{13}C NMR spectra, see Table 1. HR-ESI-MS *m/z* 705.1944 ($[M + Na]^+$) (calcd for $C_{31}H_{36}O_{17}Na$ 705.1939).

Compound 2. Yellow amorphous powder. $[\alpha]_D^{25}$–185.6° (*c* 0.39; MeOH). IR (KBr, ν_{max}, cm^{-1}): 3530, 3436, 3298, 2889, 1701, 1628, 1609, 1509, 1413. For ^1H and ^{13}C NMR spectra, see Table 1. HR-ESI-MS *m/z* 705.1994 ($[M + Na]^+$) (calcd for $C_{31}H_{36}O_{17}Na$ 705.1981).

Acid Hydrolysis of 1 and 2. A solution of each compound (**1** and **2**) (6 mg) in 2 N TFA (3 mL) was refluxed at 100°C for 3 h. The reaction mixture was extracted with EtOAc. The EtOAc extract of compounds **1** and **2** was proved to contain caffeic acid by direct TLC comparison with authentic samples. D-glucose was found to be the only sugar present in the water part following the procedure of Oshima, Yamauchi, and Kumanotani [6].

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