A New Secoiridoid Glycoside from Roots of *Picrorhiza scrophulariiflora*

ZHU Tong-fei[1], JIANG Ke[3], YAN Zhi-hui[1*], HU Juan-juan[1], ZOU Lian-chun[2], WANG Da-cheng[2], DENG Xu-ming[2]

1. Chongqing Medical and Pharmaceutical College, Chongqing 400030, China
2. College of Animal Science and Veterinary Medicine, Jilin University, Changchun 130062, China
3. No. 324 Hospital of PLA, Chongqing 400020, China

Abstract: **Objective** To study the chemical constituents in the roots of *Picrorhiza scrophulariiflora*. **Methods** The chemical constituents in the roots of *P. scrophulariiflora* were separated and purified with chromatographic methods. The structures were elucidated by spectroscopic methods and chemical analyses. **Results** A new secoiridoid glycoside, picrogentioside II (**1**) was successfully isolated from the roots of *P. scrophulariiflora*. **Conclusion** Compound **1** is a new secoiridoid glycoside.

Key words: picrogentioside II; *Picrorhiza scrophulariiflora*; Scrophulariaceae; secoiridoid glycoside
DOI: 10.3969/j.issn.1674-6348.2013.03.010

Introduction

Picrorhiza scrophulariiflora Pennell (Scrophulariaceae) grows in the high altitude regions (over 4400 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 treatments of damp-heat dysentery, jaundice, and steaming of bone (Pharmacopeia Committee of P. R. China, 2010). In the previous articles, we reported the isolation of six new secoiridoid glycosides and five new phenylethanoid glycosides from the roots of *P. scrophulariiflora* (Zhu *et al*, 2008; Zou *et al*, 2010). This paper describes the structural elucidation and identification of a new secoiridoid glycoside, named picrogentioside II (**1**).

Materials and methods

Plant materials

The roots of *Picrorhiza scrophulariiflora* Pennell were collected in October 2006 in Sichuan province, China, and identified by Prof. SUN Qi-shi (Shenyang Pharmaceutical University). The voucher specimen (20060304) has been deposited in the Herbarium of School of Traditional Chinese Materia Medica of Shenyang Pharmaceutical University, China.

General

The UV spectra were recorded on a Milton Roy Spectronic 1201 Spectrophotometer, and the FTIR spectra were measured on a Perkin-Elmer 157G Infrared Spectrophotometer. The optical rotations were obtained using a Perkin-Elmer 241-MC Polarimeter. The ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were obtained on a Bruker AV400 Spectrometer with DMSO-d_6 as the solvent and TMS as an internal standard. The HR-ESI-MS data were measured with a Bruker AOEXIII 7.0 TESLA FTMS. HPLC was conducted using reverse phase columns (Mighty Sil RP-18 and 8, Kantho Chemical Co., Ltd.) with the MeOH-H_2O solvent system. The column chromatography was carried out on silica gel (Qingdao Marine Chemical Company China, 200—300 meshes) and Sephadex LH-20 (Amersham Pharmacia Biotech AB). The silica GF254 for TLC was produced by Qingdao Marine Chemical Company, China and Merck Company.

Extraction and isolation

The dried ground roots (underground parts) of *P.*

First author: Zhu TF E-mail: 396435070@qq.com
Received: January 30, 2013; Revised: April 15, 2013; Accepted: May 25, 2013
Fund: National Key Project of Scientific and Technical Supporting Programs funded by Ministry of Science & Technology of China (2006BAD31B05)
Online time: July 17, 2013 Online website: http://www.cnki.net/kcms/detail/12.11410.R.20130717.1408.002.html

scrophulariiflora (3.0 kg) were successively extracted for 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 (bp 60—90 ℃), EtOAc, and *n*-BuOH. The *n*-BuOH layer was concentrated *in vacuo* to yield a viscous residue (500 g), which was then dissolved in water (2 L) and subjected to macroporous D-101 resin column chromatography 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 eluted with $CHCl_3$-MeOH of increasing polarity to give eight fractions (Frs. 1—8).

Fr. 3 (28.5 g) was chromatographed over silica gel using an EtOAc-MeOH gradient system as the eluent to yield five fractions: Frs. 3A—3E. Fr. 3B (276.6 mg) was purified by Sephadex LH-20 chromatography and further separated by reversed-phase HPLC using MeOH-H_2O (57:43→48:52) as the mobile phase to yield compound **1** (13.7 mg).

Results and discussion

Compound **1** was obtained as yellow amorphous powder, $[\alpha]_D^{20}$ –236.5° (*c* 0.51, MeOH) and its molecular formula was determined as $C_{31}H_{36}O_{17}$ by the $[M + Na]^+$ quasi-ion peak at *m/z* 703.1861 (calcd. for $C_{31}H_{36}O_{17}Na$ 703.1849) in the HR-ESI-MS. UV (214 and 278 nm). And IR ν_{max}^{KBr} (cm^{-1}): 3437, 1703, 1629, 1607, 1513 absorptions suggested the existence of hydroxyl, α, β-unsaturated ester, and aromatic groups in compound **1**.

The ^{1}H-NMR spectrum of compound **1** (Table 1) showed the presence of an acetal proton [δ_H 5.60 (1H, d, J = 3.2 Hz), H-1], an oxygenated methylene proton [δ_H 5.00 (2H, m), H_2-7], two olefinic protons [δ_H 7.40 (1H, brs), H-3; 5.65 (1H, m), H-6], one methine proton [δ_H 3.32, (1H, m), H-9], and one terminal vinyl group [δ_H 5.20 (1H, dd, J = 2.0, 10.4 Hz, H_1-10, 5.22 (1H, dd, J = 2.0, 16.8 Hz), H_2-10 and 5.73 (1H, ddd, J = 6.8, 10.4, 16.8 Hz), H-8], due to one gentiopicroside moiety (Tian, Zhao, and Luan, 2007). Furthermore, the spectrum showed the presence of a *trans*-caffeoyl moiety (Zhu *et al*, 2008), which was evident from three aromatic H-atoms forming an ABX system [δ (A) 7.15, δ (B) 7.11, δ (X) 7.11], and two H-atoms of an *trans*-configured C=C bond at δ (H) 6.40, 7.51 (2d, J = 16.0 Hz each).

In addition, two anomeric H-atoms which were observed at δ_H 4.66 (1H, d, J = 8.0 Hz) and δ_H 4.78 (1H, d, J = 6.8 Hz) and combined with the ^{13}C-NMR signals at δ_C 101.6—60.7 (Table 1), are typical characters for

Table 1 ^{13}C-NMR (100 MHz) and ^{1}H-NMR (400 MHz) data of compound 1 (DMSO-d_6)

Positions	δ_C	δ_H	Positions	δ_C	δ_H
secoiridoid			7″	144.1	7.51, d, (16.0)
1	96.5	5.60, d, (3.2)	8″	116.6	6.40, d, (16.0)
3	148.6	7.40, brs	9″	165.9	
4	103.4		glucose-1		
5	124.9		1′	98.5	4.66, d, (8.0)
6	116.3	5.65, m	2′	70.9	3.18, m
7	69.1	5.00, m	3′	77.6	4.90, t, (9.2)
8	134.0	5.73, ddd, (6.8, 10.4, 16.8)	4′	67.8	3.34, m
9	44.4	3.32, m	5′	77.0	3.34, m
10	118.0	5.20, dd, (2.0, 10.4);	6′	60.7	3.47, m;
11	162.7		glucose-2		
caffeoyl			1‴	101.6	4.78, d, (6.8)
1″	128.7		2‴	75.8	3.34, m
2″	114.9	7.15, brs	3‴	73.2	3.31, m
3″	146.8		4‴	69.8	3.18, m
4″	147.3		5‴	77.2	3.34, m
5″	116.1	7.11, brs	6‴	60.7	3.47, m;
6″	120.7	7.11, brs			

two β-glucopyranosyl moieties. The sugar component was identified as *D*-glucopyranoside by co-HPLC analysis of the standard sugar (Oshima, Yamauchi, and Kumanotani, 1982). The relatively large *J* value of the two anomeric protons indicated that the glucoside linkage had a β-configuration.

The detailed analysis on ^{1}H-NMR, TOCSY, ^{13}C-NMR, HMQC, and HMBC spectra of compound **1** fixed the connections between the various moieties. The HMBC (Fig. 1) correlation between H-1‴ (δ_H 4.78) and C-4″ (δ_C 147.3) demonstrated that glucose 2 was connected to the C-4″ oxygen atom. The downfield shift of the oxygenated methenyl proton at δ_H 4.90 (H-3′) suggested that the caffeoyl moiety was attached at C-3′ of the glucose 1. This was confirmed by HMBC correlation between H-3′ (δ_H 4.90) and the carbonyl carbon C-9″ (δ_C 165.9). The relative stereochemistry of compound **1** was determined from the NOESY spectrum. The correlation between H-1 (δ_H 5.60) and H-8 (δ_H 5.73) showed that the two protons were positioned on the same side. Thus, the structure of compound **1** was identified as in Fig. 2.

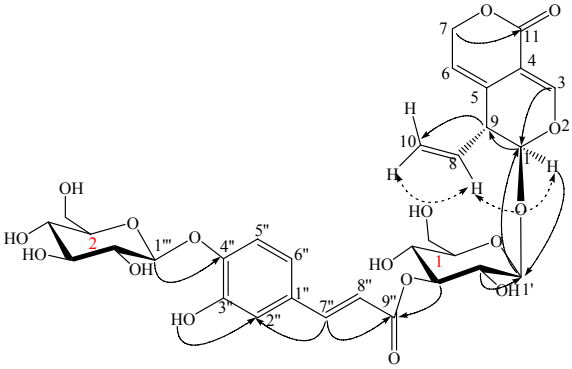

Fig. 1 HMBC (H→C) and NOESY (⤻) correlations for picrogentioside II

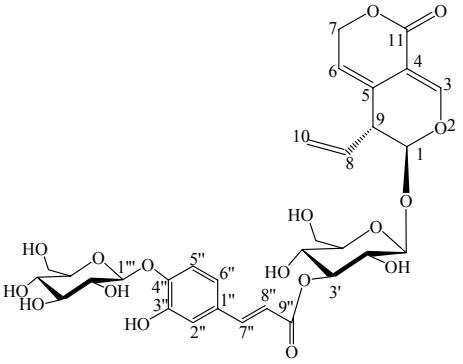

Fig. 2 Structure of picrogentioside II

References

Oshima R, Yamauchi Y, Kumanotani J, 1982. Resolution of the enantiomers of aldoses by liquid chromatography of diastereo-isomeric 1-(*N*-acetyl-α-methylbenzylamino)-1-deoxyalditol acetates. *Carbohydr Res* 107: 169-176.

Pharmacopeia Committee of P. R. China, 2010. *Pharmacopoeia of People's Republic of China*. China Medical Science and Technology Press: Beijing.

Tian J, Zhao YM, Luan XH, 2007. Chemical constituents of *Verbena officinalis* L. *Nat Prod Res Dev* 19: 247-249.

Zhu TF, Huang KY, Deng XM, Zhang Y, Xiang H, Gao HY, Wang DC, 2008. Three new caffeoyl glycosides from the roots of *Picrorhiza scrophulariilflora*. *Molecules* 13: 729-735.

Zou LC, Yan ZH, Zhu TF, Xiang H, Wang DC, Deng XM, 2010. Two new phenylethanoid glycosides and a new secoiridoid glycoside from the roots of *Picrorhiza scrophulariiflora*. *Chin Chem Lett* 21: 1103-1106.

Zou LC, Zhu TF, Gan SC, Wang DC, Zhang Y, Deng XM, 2008a. Two new secoiridoid glycosides from the roots of *Picrorhiza Scrophulariiflora*. *Chin Chem Lett* 19: 1224-1227.

Zou LC, Zhu TF, Xiang H, Yu L, Yan ZH, Gan SC, Wang DC, Zeng S, Deng XM, 2008b. New secoiridoid glycosides from the roots of *Picrorhiza scrophulariiflora*. *Molecules* 13: 2049-2057.