

Full Paper

## Three New Caffeoyl Glycosides from the Roots of *Picrorhiza Scrophulariiflora*

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**Abstract:** From the underground parts of *Picrorhiza scrophulariiflora*, three new caffeoyl glycosides, scrocaffeside A-C (**1-3**), together with two caffeic acid derivatives, 4-*O*- $\beta$ -D-glucopyranosyl caffeic acid (**4**) and 4-methoxycaffeic acid (**5**) and a phenylethanoid glycoside, scroside D (**6**), were isolated. Their structures were elucidated on the basis of chemical and spectroscopic evidence and comparisons with literature data of related compounds.

**Keywords:** *Picrorhiza scrophulariiflora*, caffeoyl glycosides, scrocaffeside A-C.

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### Introduction

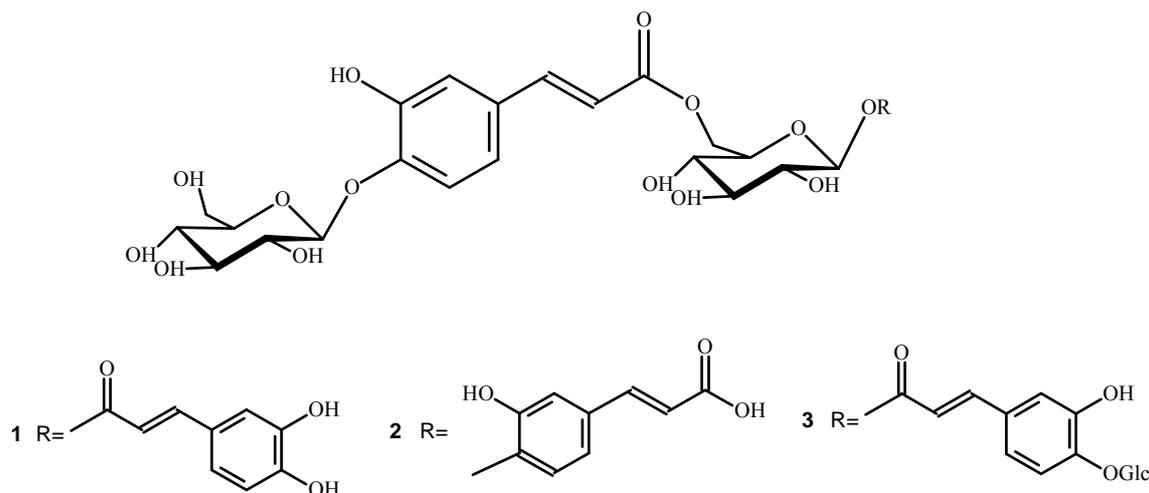
The plant *Picrorhiza scrophulariiflora* (*Scrophulariaceae*) grows in the high altitude regions (over 4400 m) in the southeast of Tibet and the northwest of Yunnan in China. The roots of this plant are used in traditional Chinese medicine for the treatment of damp-heat dysentery, jaundice and steaming bone disorder [1]. Previous phytochemical investigations of this plant led to the isolation of terpenoids [2, 3, 4], iridoid glycosides [5, 6, 7], phenolic glycosides and phenylethanoid glycosides [8-14]. Here we report the isolation and characterization of three new caffeoyl glycosides **1-3**, as well as three

known compounds.

## Results and Discussion

The three known compounds were identified as 4-*O*- $\beta$ -D-glucopyranosyl caffeic acid (**4**) [15], 4-methoxycaffeic acid (**5**) [16] and scroside D (**6**) [17] by comparing their physical and spectroscopic data with literature values.

**Figure 1.** Compounds Scrocaffeside A (**1**), Scrocaffeside B (**2**) and Scrocaffeside C (**3**)



Scrocaffeside A (**1**) was obtained as a white amorphous powder, with  $[\alpha]_D -33.7^\circ$  ( $c$  0.18, MeOH) and its molecular formula was determined as  $C_{30}H_{34}O_{17}$  by the  $[M+H]^+$  quasi-ion peak at  $m/z$  667.1824 (calc. for  $C_{30}H_{35}O_{17}$ : 667.1810) in the HR-ESIMS. In the IR spectrum, **1** exhibited bands at 3293 (hydroxy group), 1708 (C=O in conjugated esters), 1632 (C=C in  $\alpha,\beta$ -unsaturated acid derivatives), 1599 and 1515  $cm^{-1}$  (aromatic ring), and its UV spectrum showed absorption maxima at 249 and 379 nm.

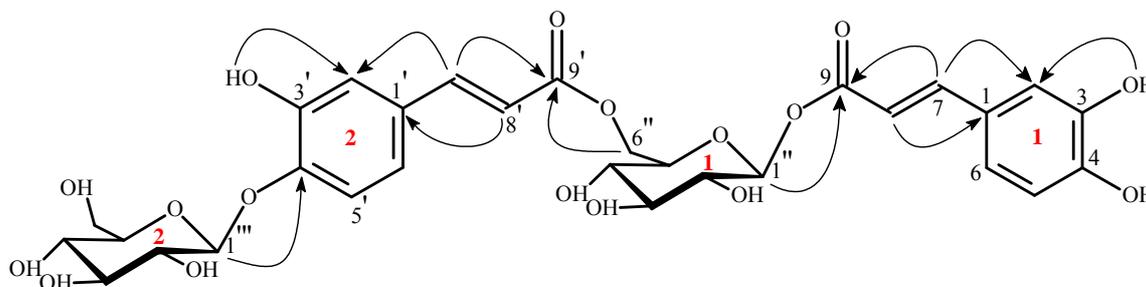
The  $^1H$ -NMR spectrum of **1** (Table 1) exhibited the characteristic signals of two (*trans*)-caffeoyl units and two  $\beta$ -glucose units [anomeric protons at  $\delta_H$  5.50 (1H, d,  $J$  = 8.0 Hz) and 4.78 (1H, d,  $J$  = 6.8 Hz)]. Twenty nine signals were exhibited in the  $^{13}C$ -NMR spectrum. The carbon resonances at  $\delta_C$  115.8 represent two methines, based on the HMQC experiment. Comparison of the  $^{13}C$ -NMR data with those of **4** also suggested the presence of two glucopyranosyl and two (*trans*)-caffeoyl moieties [15].

The presence of the glucopyranosyl and (*trans*)-caffeoyl moieties were further confirmed by the acid hydrolysis of **1**, which resulted in a release of glucose and caffeic acid, identified by TLC comparison with the authentic samples. The configuration of the glucopyranosyl was assigned to be  $\beta$ -D- according to the procedure of Oshima, Yamauchi and Kumanotani [18] and the coupling constant of the anomeric proton [19].

The detailed analysis of  $^1H$ -NMR, TCOSY,  $^{13}C$ -NMR, HSQC and HMBC spectra of **1** fixed the connections between the moieties. The deshielded shifts of H-1'' ( $\delta_H$  5.50) and H-6'' ( $\delta_H$  4.39, 4.14) indicated that the caffeoyl moieties were attached to C-1'' and C-6'', respectively. This was confirmed by HMBC correlations between H-1'' ( $\delta_H$  5.50) and C-9 ( $\delta_C$  165.3), and between H-6'' ( $\delta_H$  4.39 and

4.14) and C-9' ( $\delta_C$  166.5). A HMBC correlation between another anomeric proton H-1''' ( $\delta_H$  4.78) and C-4' ( $\delta_C$  147.5) demonstrated that the remaining glucopyranosyl was connected to C-4'. Therefore, the structure of scrocaffeside A (**1**) was concluded to be *E*-caffeoyl-6-*O*-[4-*O*-( $\beta$ -D-glucopyranosyl) *E*-caffeoyl]- $\beta$ -D-glucopyranoside, for which the trivial name scrocaffeside A is proposed.

**Figure 2.** Important HMBC correlations (H $\rightarrow$ C) for Scrocaffeside A (**1**)



Scrocaffeside B (**2**), a white amorphous powder, showed  $[\alpha]_D-23.5^\circ$  ( $c$  0.20, MeOH). Its molecular formula,  $C_{30}H_{34}O_{17}$ , was established by the  $[M+H]^+$  quasi-ion peak at  $m/z$  667.1830 (calc. for  $C_{30}H_{35}O_{17}$  667.1809) in the HR-ESIMS. The presence of  $\beta$ -D-glucopyranosyl and (*trans*)-caffeoyl moieties were confirmed using the same methods as for the structure elucidation of **1**. Close structural similarity of **2** and **1** followed from the general congruence of  $^1H$ - and  $^{13}C$ -NMR data (Table 1). Notable differences were signals from a (*trans*)-caffeoyl moiety in **2**. Comparison of the NMR spectra of **2** and **1** also showed a conspicuous deshielding of H-1'' ( $\Delta\delta_H$  0.64) and C-1'' ( $\Delta\delta_C$  7.1) of the central glucose core (Glc 1), indicating the different glycosidic linkage between the glucopyranosyl moiety and caffeoyl moiety 1. In the HMBC spectrum correlations between H-1'' (Glc 1-1) and C-4 demonstrated that the glucose 1 attached at C-4 of caffeoyl moiety 1. Thus, the structure of compound **2** was established as 4-*O*-[6-*O*-[4-*O*-( $\beta$ -D-glucopyranosyl) *E*-caffeoyl]- $\beta$ -D-glucopyranosyl] *E*-caffeic acid, and it was named scrocaffeside B.

Scrocaffeside C (**3**) was obtained as a white amorphous powder with  $[\alpha]_D-50.5^\circ$  ( $c$  0.17, MeOH). The molecular formula of  $C_{36}H_{44}O_{22}$  was assigned for **3** on the basis of HR-ESIMS at  $m/z$  829.2329  $[M+H]^+$  (calc. for  $C_{36}H_{45}O_{22}$  829.2338). The UV,  $^1H$ -NMR and the  $^{13}C$ -NMR spectroscopic data of **3** together with the chemical test results were very similar to those of **1**, suggesting a close relationship. Structural assessment of **3** was accomplished using a combination of NMR techniques, along with comparisons to the assignments of analogues **1** and **2**.

The  $^{13}C$ -NMR spectra experiments gave a total of 29 resonance lines, of which 12 signals ( $\delta_C$  102–60) could be assigned to three glucosyl moieties by the aid of DEPT and HMQC experiments. Analysis of  $^1H$ -NMR, TCOSY,  $^{13}C$ -NMR, HSQC and HMBC spectra of **3** indicated again the glycosidic linkages between the glucopyranosyl and caffeoyl moieties. The connections between glucose 1, glucose 2 and the two caffeoyl moieties of **3** shared the same pattern with those of **1**. The glucose 3 should be linked to C-4, as indicated by the HMBC crosspeak between this carbon ( $\delta_C$  147.7) and the anomeric proton ( $\delta_H$  4.80).

On the basis those data the structure of **3** was determined to be 1, 6-*di-O*-[4-*O*-( $\beta$ -D-glucopyranosyl) *E*-caffeoyl]- $\beta$ -D-glucopyranoside, and the compound was designated as scrocaffeside C.

**Table 1.**  $^{13}\text{C}$ - (100 MHz) and  $^1\text{H}$ - (400 MHz) NMR Data of **1**, **2** and **3** ( $\text{DMSO}-d_6$ )<sup>a</sup>

Moiety	position	<b>1</b>		<b>2</b>		<b>3</b>	
		C	H (J, Hz)	C	H (J, Hz)	C	H (J, Hz)
Caffeoyl-1	1	125.3		128.9		128.4	
	2	114.9	7.06, br.s	115.1	7.12 *	115.1	7.18, br.s
	3	145.6		146.9		146.8	
	4	148.7		146.9		147.7	
	5	115.8	6.77, d, (8.4)	115.9	7.08, d, (8.8)	116.1	7.12, m
	6	121.7	7.02, br.d (8.4)	120.3	6.96, d, (8.4)	121.2	7.12, m
	7	146.6	7.57, d, (16.0)	143.5	7.38, d, (16.0)	146.0	7.62, d, (16.0)
	8	113.2	6.26, d, (16.0)	117.5	6.24, d, (16.0)	115.3	6.45, d, (16.0)
	9	165.2		167.7		165.1	
Caffeoyl-2	1'	128.6		128.5		128.6	
	2'	115.1	7.18, br.s	114.9	7.19, s	115.1	7.18, br.s
	3'	146.8		146.8		146.8	
	4'	147.5		147.5		147.5	
	5'	116.1	7.12, m	116.0	7.12 *	116.1	7.12, m
	6'	120.9	7.12, m	120.8	7.12 *	120.9	7.12, m
	7'	144.8	7.52, d, (16.0)	144.7	7.53, d, (16.0)	144.8	7.52, d, (16.0)
	8'	115.8	6.45, d, (16.0)	115.8	6.45, d, (16.0)	115.8	6.44, d, (16.0)
	9'	166.3		166.2		166.3	
Glc-1	1''	94.1	5.50, d, (8.0)	101.2	4.86, d, (6.8)	94.2	5.52, d, (8.0)
	2''	72.4	3.20-3.38, m *	75.5	3.30 *	72.4	3.17-3.39, m *
	3''	76.1	3.20-3.38, m *	73.9	3.71 *	76.1	3.17-3.39, m *
	4''	69.6	3.20-3.38, m *	69.7	3.20, m	69.6	3.17-3.39, m *
	5''	74.6	3.58, m	73.2	3.30 *	74.6	3.51, m
	6''	63.4	4.41, br.d, (11.2), 4.16, dd, (6.0, 12.0)	63.3	4.23, dd, (6.8, 11.6) 4.46, d, (11.2)	63.4	4.41, br.d, (11.2) 4.16, d d, (6.0, 12.0)
Glc-2	1'''	101.6	4.78, d, (6.8)	101.5	4.80, d, (6.8)	101.4	4.78, d, (6.0)
	2'''	75.8	3.20-3.38, m *	75.7	3.32 *	75.8	3.17-3.39, m *
	3'''	73.2	3.20-3.38, m *	73.3	3.32 *	73.2	3.17-3.39, m *
	4'''	69.8	3.20-3.38, m *	69.9	3.30 *	69.8	3.17-3.39, m *
	5'''	77.2	3.20-3.38, m *	77.1	3.32 *	77.2	3.17-3.39, m *
	6'''	60.7	3.71, br.d, (8.4) 3.47, m	60.7	3.48, dd, (5.6, 11.6) 3.71, d, (10.4)	60.7	3.70, br.d, (11.2) 3.47, m
Glc-3	1''''					101.5	4.80, d, (6.0)
	2''''					75.8	3.17-3.39, m *
	3''''					73.2	3.17-3.39, m *
	4''''					69.8	3.17-3.39, m *
	5''''					77.2	3.17-3.39, m *
	6''''					60.7	3.73, br.d, (11.2) 3.47, m

<sup>a</sup>Chemical shifts ( $\delta$ ) given in ppm; \* signal pattern unclear due to overlapping signals.

## Experimental

### General

UV spectra were recorded on a Milton Roy Spectronic 1201 spectrophotometer, and FTIR spectra were measured on a Perkin-Elmer 157G infrared spectrophotometer. Optical rotations were obtained using a Perkin-Elmer 241-MC polarimeter.  $^1\text{H-NMR}$  (400 MHz) and  $^{13}\text{C-NMR}$  (100 MHz) spectra were obtained on a Bruker AV400 spectrometer with  $\text{DMSO-}d_6$  as solvent and TMS as an internal standard. HR-ESIMS data were measured with a Bruker AOEXIII 7.0 TESLA FTMS. HPLC were carried out on the reverse phase columns (mighty sil RP-18 and 8, Kantho Chemical Co. Ltd) with the MeOH-H<sub>2</sub>O solvent system. Column chromatography was carried out on silica gel (Qingdao Marine Chemical Company China, 200-300 mesh), Sephadex LH-20 (Amersham Pharmacia Biotech AB). Silica GF254 for TLC was produced by Qingdao Marine Chemical Company China and Merck Company. All chemicals used were of biochemical reagent grade.

### Plant Material

Roots of *Picrorhiza scrophulariiflora* were collected in October 2006 in Sichuan Province of China and identified by Prof. Qi-shi Sun (Shenyang Pharmaceutical University). The voucher specimen has been deposited in the Herbarium of School of Traditional Chinese Medicines 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<sub>2</sub>O and then extracted successively with petroleum ether (b.p. 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 a macro-porous resin D-101 column chromatography eluting 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), which was subjected to silica gel column chromatography eluting with mixtures of CHCl<sub>3</sub>-MeOH of increasing polarity to give 8 fractions (frs.1-8).

Fraction 2 (43.0 g) was further separated by silica gel column chromatography, using an EtOAc-MeOH gradient as eluent, to afford six fractions (frs. 2A-2F). Fractions 2B (5.3 g) and 2C (4.6 g) were further purified separately by Sephadex LH-20 to yield compounds **4** (42.3 mg) and **5** (11.3 mg). Fraction 2D was purified by Sephadex LH-20 and further separated by reversed-phase HPLC using MeOH-H<sub>2</sub>O (57:43 and 44:56) as a mobile phase to afford compounds **1** (38.7mg) and **2** (15.9 mg). Fraction 3 (28.5 g) was chromatographed over silica gel using a EtOAc-MeOH gradient system as eluent to afford five fractions (frs. 3A-2E). Fraction 3B (6.7 g) was purified by Sephadex LH-20 chromatography and further separated by reversed-phase HPLC using MeOH-H<sub>2</sub>O (43:57) as a mobile phase to afford compounds **3** (17.6 mg) and **6** (18.4 mg).

*Scrocaffeside A (1)*: White amorphous powder;  $[\alpha]_D-33.7^\circ$ ; UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ): 379 (2.75), 249 (5.51); IR (KBr)  $\nu_{\max}$   $\text{cm}^{-1}$ : 3293, 2922, 2359, 1708, 1632, 1599, 1515; for  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data see Table 1; HR-ESIMS m/z: 667.1824 (calc. for  $\text{C}_{30}\text{H}_{35}\text{O}_{17}$ : 667.1810).

*Scrocaffeside B (2)*: White amorphous powder;  $[\alpha]_D-23.5^\circ$ ; UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ): 332 (2.35), 245 (5.50); IR (KBr)  $\nu_{\max}$   $\text{cm}^{-1}$ : 3324-2260, 2905, 2257, 1700, 1635, 1510; for  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data see Table 1; HR-ESIMS m/z: 667.1830 (calc. for  $\text{C}_{30}\text{H}_{35}\text{O}_{17}$ : 667.1809).

*Scrocaffeside C (3)*: White amorphous powder;  $[\alpha]_D-50.5^\circ$ ; UV (MeOH)  $\lambda_{\max}$  nm (log  $\epsilon$ ): 361 (2.51), 249 (5.50); IR (KBr)  $\nu_{\max}$   $\text{cm}^{-1}$ : 3309, 2918, 2255, 1711, 1634, 1510; for  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data see Table 1; HR-ESIMS m/z: 829.2329 (calc. for  $\text{C}_{36}\text{H}_{45}\text{O}_{22}$ : 829.2338).

### Acid hydrolysis of **1**, **2** and **3**

A solution of the compound (8 mg) in 2 N TFA (3 mL) was refluxed at  $100^\circ\text{C}$  for 3 h. The reaction mixture was extracted with EtOAc. The EtOAc extract was proven to contain caffeic acid by direct TLC comparison with authentic samples. D-Glucose was found as the only sugar present in the water part following the procedure of Oshima, Yamauchi and Kumanotani [18].

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*Sample Availability:* Available from authors.