Two Phenylpropanoid Glycosides from *Neopicrorhiza scrophulariiflora*

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Two new phenylpropanoid glycosides, scrophulosides A (1) and B (2), were isolated from the rhizomes of *Neopicrorhiza scrophulariiflora* (Scrophulariaceae), along with two known compounds, androsin (3) and picroside I. Their structures were elucidated on the basis of both chemical and spectroscopic data.

Key words *Neopicrorhiza scrophulariiflora*; Scrophulariaceae; phenylpropanoid glycoside; scrophuloside A; scrophuloside B

Neopicrorhiza scrophulariiflora (PENNELL) HONG (basionym. *Picrorhiza scrophulariiflora* PENNELL) is distributed throughout the high altitude (>4400 m) regions in southeastern Tibet and the northwestern Yunnan Province of China.[1)] Its dried rhizomes have been used for the treatment of asthma, jaundice and arthritis in traditional medicines of China, Tibet, Nepal and India.[2,3)] Earlier investigations of this plant led to the isolation of triterpenoids, iridoid glycosides, phenolic glycosides and phenylethanoid glycosides.[3—6)] Recently, the Ministry of Health, Labour and Welfare of Japan began to study the reclassification of the raw materials exclusively used as pharmaceuticals,[7)] and a number of herbs were investigated for their toxicity and secondary metabolites. As part of this study, we have isolated two new phenylpropanoid glycosides, scrophulosides A (**1**) and B (**2**), along with two known compounds, androsin (**3**) and picroside I, from the rhizomes of this plant of herbal medicines market in Japan. This paper describes the isolation and structural elucidation of the new phenylpropanoid glycosides.

The MeOH extract of the dried rhizomes of *N. scrophulariiflora* was partitioned with dichloromethane, ethyl acetate, and 1-butanol successively. Upon repeated column chromatography and preparative HPLC, the ethyl acetate soluble portion yielded two new compounds, scrophulosides A (**1**) and B (**2**), along with two known compounds, androsin (**3**) and picroside I. Identification of the known compounds was accomplished by comparison of their spectral data with those in the literature.[1,8)]

Scrophuloside A (**1**) was obtained as a colorless amorphous powder. Its molecular formula was determined to be $C_{24}H_{26}O_{10}$ by the $[M+Na]^+$ ion peak at *m/z* 497.1391 (Calcd for $C_{24}H_{26}O_{10}Na$ 497.1424) in the HR-ESI-MS. UV (222, 270, 300 nm) and IR (3410, 1701, 1635, 1605, 1514 cm^{-1}) absorptions suggested the existence of hydroxyl, α,β-unsaturated ester and aromatic ketone groups in **1**. The ^{1}H-NMR spectrum of **1** indicated the presence of a 1,4-disubstituted aromatic ring [δ 7.44 (2H, d, J=8.6 Hz, H-2″, 6″), δ 6.81 (2H, d, J=8.6 Hz, H-3″, 5″)] and a 1,2,4-trisubstituted aromatic ring [δ 7.51 (1H, d, J=2.0 Hz, H-3), δ 7.41 (1H, dd, J=8.6, 2.0 Hz, H-5), δ 7.12 (1H, d, J=8.6 Hz, H-6)], an acetyl group (δ 2.29), a methoxy group (δ 3.87) and a pair of *trans* olefinic protons at δ 7.58 and 6.32 with a coupling constant of 16.1 Hz (Table 1). The ^{13}C-NMR spectrum of **1** showed the presence of two carbonyl carbons (δ 197.9, 167.4), fourteen olefinic carbons (δ 160.1—110.8), and characteristic signals of a hexose unit (δ 100.3, 76.5, 74.3, 73.4, 70.6, 63.3) (Table 1). The sugar component was identified as D-glucopyranoside by enzymatic hydrolysis of **1**. The relatively large J value (7.8 Hz) of the anomeric proton (δ 5.03) of the glucosyl moiety indicated that the glucoside linkage was β. The HMBC correlation between H-1′ and C-1 demonstrated that the glucosyl moiety was connected to the C-1 oxygen atom (Fig. 2). The ^{1}H- and ^{13}C-NMR spectra of **1** were quite similar to those of androsin (**3**),[1)] except for the signals originating from the phenylpropanoid ester group, which was determined to be a *para*-coumaroyl group by analysis of the ^{13}C-NMR (δ 167.4, 160.1, 145.4, 130.0×2, 125.8, 115.7×2, 113.8), H–H COSY, and HMBC spectra (Table 1). The downfield shift of the oxygenated methylene

Table 1. ^{1}H- (500 MHz) and ^{13}C-NMR (125 MHz) Spectral Data for Compounds **1** and **2** in CD_3OD

Position	**1**		**2**	
	δ_C	δ_H *a)*	δ_C	δ_H *a)*
1	150.8		161.5	
2	149.3		116.0	7.12 (d, 9.2)
3	110.8	7.51 (d, 2.0)	130.3	7.86 (d, 9.2)
4	131.5		131.3	
5	123.1	7.41 (dd, 8.6, 2.0)	130.3	7.86 (d, 9.2)
6	114.9	7.12 (d, 8.6)	116.0	7.12 (d, 9.2)
7	197.9		197.9	
8	24.9	2.29 (s)	24.9	2.33 (s)
9	55.3	3.87 (s)		
1′	100.3	5.03 (d, 7.8)	100.0	5.03 (d, 7.2)
2′	73.4	3.57 (dd, 9.2, 7.8)	73.4	3.51 (m)
3′	76.5	3.52 (dd, 9.2, 8.9)	76.6	3.49 (m)
4′	70.6	3.41 (dd, 10.0, 8.9)	70.6	3.41 (m)
5′	74.3	3.78 (m)	74.3	3.79 (m)
6′	63.3	4.51 (dd, 11.8, 2.0)	63.2	4.53 (dd, 12.1, 2.3)
		4.37 (dd, 11.8, 7.8)		4.39 (dd, 12.1, 7.5)
1″	125.8		126.3	
2″	130.0	7.44 (d, 8.6)	110.3	7.19 (d, 2.0)
3″	115.7	6.81 (d, 8.6)	148.2	
4″	160.1		149.6	
5″	115.7	6.81 (d, 8.6)	115.3	6.82 (d, 8.3)
6″	130.0	7.44 (d, 8.6)	122.9	7.07 (dd, 8.3, 2.0)
7″			55.1	3.89 (s)
α	113.8	6.32 (d, 16.1)	114.0	6.39 (d, 16.0)
β	145.4	7.58 (d, 16.1)	145.8	7.61 (d, 16.0)
CO	167.4		167.5	

a) Multiplicity and J values in Hz are given in parentheses.

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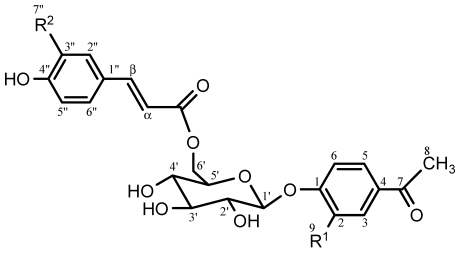

1 $R^1 = OCH_3$, $R^2 = H$

2 $R^1 = H$, $R^2 = OCH_3$

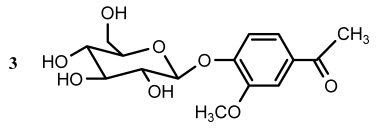

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Fig. 1. The Structures of Compounds **1**—**3**

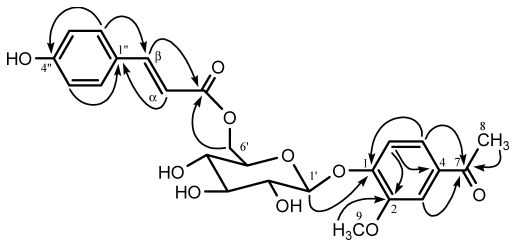

Fig. 2. Selected HMBC Correlations for **1**

protons at δ 4.51 and 4.37 (H_2-6′) suggested that the *para*-coumaroyl group was attached at C-6′. Moreover the *para*-coumaroyl group was determined to be connected to the C-6′ of the glucose moiety based on the correlation between the H_2-6′ and the carbonyl carbon (δ 167.4) seen in the HMBC spectrum (Fig. 2). Accordingly, scrophuloside A (**1**) was determined to have the structure shown in Fig. 1.

Scrophuloside B (**2**) was obtained as a colorless amorphous powder. Its molecular formula was determined to be $C_{24}H_{26}O_{10}$ by the $[M+Na]^+$ ion peak at *m/z* 497.1391 (Calcd for $C_{24}H_{26}O_{10}Na$ 497.1424) in the HR-ESI-MS, which was equivalent to that of **1**. UV (217, 275 nm) and IR (3410, 1709, 1636, 1600, 1515 cm^{-1}) absorptions also suggested the existence of hydroxyl, α,β-unsaturated ester and aromatic ketone groups in **2**. The ^{1}H-NMR spectrum of **2** indicated the presence of a 1,4-disubstituted aromatic ring [δ 7.86 (2H, d, J=9.2 Hz, H-3, 5), δ 7.12 (2H, d, J=9.2 Hz, H-2, 6)], a 1,2,4-trisubstituted aromatic ring [δ 7.19 (1H, d, J=2.0 Hz, H-2″), δ 7.07 (1H, dd, J=8.3, 2.0 Hz, H-6″), δ 6.82 (1H, d, J=8.3 Hz, H-5″)], an acetyl group (δ 2.33), a methoxy group (δ 3.89) and a pair of *trans* olefinic protons at δ 7.61 and 6.39 with a coupling constant of 16.0 Hz (Table 1). The ^{1}H- and ^{13}C-NMR spectra of **2** were quite similar to those of **1**, with all the key resonances in **2** having corresponding signals in **1**. This suggested that **2** also contained hexose, acetophenyl and phenylpropanoid ester groups. The ^{13}C-NMR spectrum of **2** and the HMBC correlation between H-1′ and C-1 (Fig. 3) demonstrated that the hexose unit (δ 100.0, 76.6, 74.3, 73.4, 70.6, 63.2) was connected to the C-1 oxygen atom (Table 1). The sugar component in **2** was demonstrated to be D-glucose, as in **1**. Analysis of the ^{13}C-NMR (δ 167.5, 149.6, 148.2, 145.8, 126.3, 122.9, 115.3, 114.0, 110.3, 55.1), H–H COSY, and HMBC spectra revealed that the phenylpropanoid ester at C-6′ of glucose was an *E*-feruloyl group (Fig. 3). On the basis of these data, scrophuloside B (**2**) was determined to have the structure shown in Fig. 1.

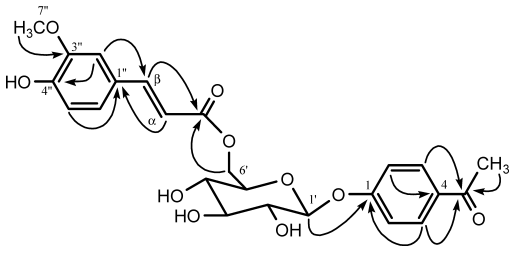

Fig. 3. Selected HMBC Correlations for **2**

Though several phenylpropanoid glycosides with a phenylethyl group at C-1′ of the glucose moiety have been reported,[4,6] this is the first time that one with an acetophenoyl group at C-1′ of glucose has been identified.

Scrophulosides A (**1**) and B (**2**) showed moderate cytotoxic activity on P-388 murine leukemia cells with IC_{50} values of 0.58 and 4.5 μg/ml, respectively.

Experimental

General Procedures Optical rotations were measured on a JASCO DIP-370 (Tokyo, Japan) digital polarimeter, UV spectra on a Shimadzu UV-2550 (Kyoto, Japan) spectrophotometer and IR spectra on a JASCO FTIR-5300 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded in CD_3OD on a JEOL ECA-500 (Tokyo, Japan) spectrometer, and chemical shifts were expressed in parts per million (ppm) relative to TMS as the internal standard. Mass spectra were obtained on a JEOL JMS-T100LC spectrometer. Preparative HPLC was carried out on a Shimadzu LC-8A with a Shimadzu SPD-6AV detector and a Wakosil 25C 18 column (20 mm i.d.× 250 mm, ODS, 10 μm, Wako).

Plant Material The rhizomes of *Neopicrorhiza scrophulariiflora* were purchased from Uchida Wakanyaku Co. Ltd. A voucher specimen was deposited in the National Institute of Health Sciences, Japan.

Extraction and Isolation The dried rhizomes of *N. scrophulariiflora* (300 g) were ground and extracted with hot MeOH (500 ml×5). The solvent was removed *in vacuo* to give a residue (73.8 g) which was suspended in H_2O (500 ml). The suspension was extracted successively with CH_2Cl_2 (500 ml×3), EtOAc (500 ml×3), and 1-BuOH (500 ml×3) and the solvent was removed *in vacuo* to afford CH_2Cl_2-soluble (6.3 g), EtOAc-soluble (8.9 g), and 1-BuOH-soluble (24.8 g) portions, respectively. The EtOAc-soluble portion was placed on a silica gel column and eluted sequentially with $CHCl_3$/MeOH mixtures (1 : 0, 50 : 1, 10 : 1, 5 : 1, 1 : 1, 0 : 1) to give ten fractions (frs. 1—10). Fraction 7 (857.6 mg) was further separated by reversed-phase HPLC using MeOH/H_2O (60 : 40, 1 : 0) to afford five fractions (frs. 7A—E). Repeated reversed-phase HPLC of fraction 7A (369.9 mg) using mixtures of either MeOH/H_2O or MeCN/H_2O afforded compounds **1** (17.6 mg) and **2** (6.6 mg). Fraction 8 (2.46 g) was subjected to reversed-phase HPLC using MeOH/H_2O (38 : 62) to afford picroside I (287.5 mg). Repeated reversed-phase HPLC of fraction 9 (1.9 g) using mixtures of either MeOH/H_2O or MeCN/H_2O afforded androsin (**3**) (29.9 mg).

Scrophuloside A (**1**): Colorless amorphous powder; $[\alpha]_D^{24}$=−29.5° (c= 0.88, MeOH); UV (MeOH) λ_{max} nm (log ε): 222 (4.39), 270sh (4.19), 300 (4.39); IR (KBr) ν_{max} cm^{-1}: 3410, 1701, 1635, 1605, 1514; ^{1}H- and ^{13}C-NMR spectra: see Table 1; HR-ESI-MS *m/z* 497.1391 $[M+Na]^+$ (Calcd for $C_{24}H_{26}O_{10}Na$ 497.1424).

Scrophuloside B (**2**): Colorless amorphous powder; $[\alpha]_D^{24}$=−36.0° (c= 0.50, MeOH); UV (MeOH) λ_{max} nm (log ε): 217 (4.31), 275 (4.18); IR (KBr) ν_{max} cm^{-1}: 3410, 1709, 1636, 1600, 1515; ^{1}H- and ^{13}C-NMR: see Table 1; HR-ESI-MS *m/z* 497.1399 $[M+Na]^+$ (Calcd for $C_{24}H_{26}O_{10}Na$ 497.1424).

Enzymatic Hydrolysis of 1 and 2 A solution of each compound (**1**, **2**) (2 mg) in H_2O (1.0 ml) and β-D-glucosidase (10 mg) from almonds was incubated at 37 °C for 25 h. The solution was subsequently washed with EtOAc and the aqueous layer was evaporated to yield a residue that showed a spot

indicative of glucose (*Rf* 0.18, $CHCl_3$–MeOH–H_2O 5 : 2 : 0.1) on silica gel TLC. The residue was converted to a thiazolidine derivative and analyzed by silica gel TLC (*Rf* 0.49, 0.37, $CHCl_3$–MeOH–H_2O 15 : 6 : 1).[9] Authentic thiazolidine derivatives obtained from D- and L-glucoses showed spots at *Rf* 0.49 and 0.37, and 0.45, respectively.

Cytotoxic Activity Assay The assay was performed in the same manner as described previously.[10]

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