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Original article

Iridoid glycosides from the roots of *Scrophularia ningpoensis* Hemsl.

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ABSTRACT

Two new iridoid glycosides, named scrophularianoids A (**1**) and B (**2**), were isolated from the roots of *Scrophularia ningpoensis*. The chemical structures were established on the basis of extensive analyses of spectroscopic data. Compounds **1** and **2** were inactive in our preliminary *in vitro* myocardial protective bioassay.

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1. Introduction

The common figwort *Scrophularia ningpoensis* Hemsl. (Scrophulariaceae) is one of over 300 known species of the genus *Scrophularia*, which is well known for its variety of iridoids [1]. Its dried roots are used as anti-inflammatory agents for the treatment of fever, swelling, sore throat, and constipation [2,3]. A series of chemical constituents including iridoids, phenylethanoid glycosides, triterpene saponins, flavones, and volatile oils have been isolated from *S. ningpoensis* [4–6]. Our previous study on this plant led to the isolation of monoterpene pyridine alkaloids, iridoid glycosides, phenylpropanoid glycosides and phenolic acids, some of which showed inhibitory activity against KCl induced $[Ca^{2+}]_i$ increase in rat cardiomyocytes and cardio-protective effects against the apoptosis induced by H_2O_2 [7,8]. Further investigations on this plant have resulted in the isolation of two new iridoid glycosides, named scrophularianoids A (**1**) and B (**2**) (Fig. 1). In this paper, the isolation and structural elucidation of the two new compounds and their activity in an *in vitro* myocardial protective bioassay are reported.

2. Experimental

2.1. General

The dried roots of *S. ningpoensis* Hemsl. were collected from Pan'an, Zhejiang province, China, in September 2011, and authenticated by Zeng-Xi Guo, director pharmacist of TCM, at Zhejiang Institute for Food and Drug Control, Zhejiang Province, China. A voucher specimen was deposited at College of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, China.

2.2. Extraction and isolation

The dried roots of *S. ningpoensis* (25 kg) were chopped into small pieces and extracted with 70% EtOH under reflux twice for 2 h each time. The combined extracts were concentrated by evaporation to yield a residue of 5.6 kg, which was chromatographed on a D101 column eluted with EtOH/H_2O gradiently. The 50% EtOH eluted fraction (250.0 g) was then separated using silica gel column chromatography (CC) by gradient elution with CH_2Cl_2/MeOH (100:0 → 0:100) to afford 10 fractions. Fraction 8 (91.7 g) was fractionated on silica gel CC eluted with CH_2Cl_2/MeOH (95:5 → 0:100) to give 10 subfractions. Subfraction 5 (10.2 g) was applied to an ODS column using stepwise gradient mixtures of MeOH/H_2O (10:90 → 100:0) system. Subfraction 5.5 (1.7 g) was further passed over an HW-40 column with MeOH/H_2O (10:90 → 80:20) gradiently. Purification of the eluate of 10%

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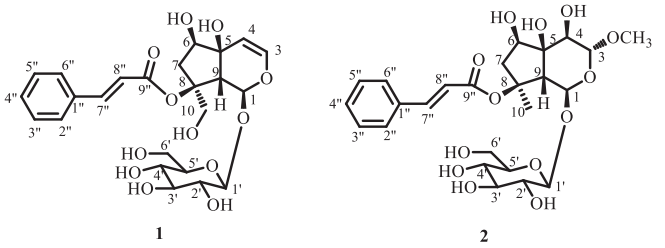

Fig. 1. Chemical structures of compounds **1** and **2**.

MeOH by semi-preparative HPLC with 44% MeOH gave **1** (7.0 mg) and **2** (5.4 mg).

2.3. Scrophularianoid A

Yellowish amorphous powder; $[\alpha]_D^{25}$ – 40.8 (*c* 0.5, MeOH). UV (MeOH) λ_{max} (log ε): 232 (3.16), 279 (4.26) nm; IR (KBr, cm^{-1}) ν_{max} 3366, 2936, 1711, 1637, 1577, 1496; HR-ESI-MS *m/z* 533.1637 $[M+Na]^+$, calcd. 533.1635 for $C_{24}H_{30}O_{12}Na$; ^1H and ^{13}C NMR data, see Table 1.

2.4. Scrophularianoid B

Yellowish amorphous powder; $[\alpha]_D^{25}$ – 50.0 (*c* 0.5, MeOH). UV (MeOH) λ_{max} (log ε): 232 (3.41), 279 (4.26) nm; IR (KBr, cm^{-1}) ν_{max} 3370, 2926, 1691, 1635, 1578, 1514; HR-ESI-MS *m/z* 565.1904 $[M+Na]^+$, calcd. 565.1897 for $C_{25}H_{34}O_{13}Na$; ^1H and ^{13}C NMR data, see Table 1.

2.5. Sugar analysis

The absolute configuration of the sugar moiety was determined by the method reported by Tanaka et al. [9]. Compounds **1** and **2** (2 mg, each) were heated in 2 mol/L aqueous HCl for 2 h at 90 °C. The mixture was evaporated to dryness *in vacuo*, and then the residue was dissolved in H_2O and extracted with $CHCl_3$. The aqueous layer was collected. After drying *in vacuo*, the residue was dissolved in pyridine (1 mL) containing L-cysteine methyl ester (1 mg) (Sigma, USA) and the mixture was heated at 60 °C for 1 h. Then, *o*-tolyl isothiocyanate (5 μL) (Alfa Aesar, UK) was added to the mixture, which was heated at 60 °C for 1 h. The reaction mixture was directly analyzed by HPLC. Analytical HPLC was performed on a reversed-phase C18 column (250 mm × 4.6 mm i.d., 5 μm, Phenomenex, Gemini) at 35 °C with isocratic elution of 25% CH_3CN containing 0.1% formic acid for 40 min at a flow rate 0.8 mL/min. Peaks were detected by a UV detector at 250 nm. The derivatives of **1** and **2** both gave one peak at t_R 21.6 min. The derivatives of D-glucose and L-glucose (Sigma, USA) were subjected to the same method. The peaks were recorded at t_R 20.2 (L-glucose) and 21.6 (D-glucose) min, respectively.

Table 1
^1H and ^{13}C NMR data for compounds **1** and **2** (^1H: 400 MHz, ^{13}C: 100 MHz; in CD_3OD).

No.	**1**		**2**	
	δ_C	δ_H	δ_C	δ_H
1	92.7	5.95 d (0.8)	96.8	5.97 s
3	143.1	6.37 d (6.4)	98.2	4.99 d (8.0)
4	108.5	5.00 dd (6.4, 1.2)	71.2	3.19 d (8.0)
5	72.9		82.9	
6	78.0	3.80 t (4.0)	73.1	4.06 m
7	43.2	2.07 dd (14.0, 4.8)	47.2	2.41 m
		1.84 dd (14.0, 3.2)		2.25 m
8	79.3		88.1	
9	59.2	2.73 br s	57.0	2.79 br s
10	69.5	4.22 dd (11.6, 6.8)	23.1	1.62 s
OCH_3			57.5	3.54 s
1′	99.8	4.58 d (8.0)	100.3	4.68 d (7.6)
2′	74.7	3.22 m	74.8	3.28 m
3′	77.7	3.38 m	78.1	3.41 m
4′	71.8	3.30 m	72.2	3.33 m
5′	78.4	3.30 m	78.2	3.35 m
6′	62.9	3.86 br d (11.6)	63.4	3.93 dd (12.0, 2.0)
		3.66 m		3.70 m
1″	135.9		136.0	
2″	129.5	7.62–7.65 m	129.4	7.58–7.61 m
3″	130.2	7.40–7.42 m	130.2	7.39–7.41 m
4″	131.7	7.40–7.42 m	131.7	7.39–7.41 m
5″	130.2	7.40–7.42 m	130.2	7.39–7.41 m
6″	129.5	7.62–7.65 m	129.4	7.58–7.61 m
7″	146.9	7.75 d (16.0)	146.3	7.66 d (16.0)
8″	118.8	6.59 d (16.0)	120.2	6.49 d (16.0)
9″	168.6		168.7	

3. Results and discussion

Compound **1** was isolated as a yellowish amorphous powder, $[\alpha]_D^{25}$ – 40.8 (*c* 0.5, MeOH). The molecular formula $C_{24}H_{30}O_{12}$ was determined on the basis of HR-ESI-MS (*m/z* 533.1637 $[M+Na]^+$). Its UV spectrum exhibited the characteristic cinnamoyl chromophore absorption at 279 nm. Its IR spectrum displayed absorption bands at 1711 cm^{-1} for conjugated ester carbonyl group(s), 1637, 1577, and 1496 cm^{-1} for aromatic ring(s), and 3366 cm^{-1} for OH group(s). The ^1H NMR spectrum of **1** showed resonances for a cinnamoyl group at δ 7.62–7.65 (m, 2H, H-2″, 6″), 7.40–7.42 (m, 3H, H-3″, 4″, 5″), 7.75 (d, 1H, *J* = 16.0 Hz, H-7″), and 6.59 (d, 1H, *J* = 16.0 Hz, H-8″), and a sugar moiety including an anomeric proton signal at δ 4.58 (d, 1H, *J* = 8.0 Hz, H-1′) together with five proton signals at δ 3.22 (m, 1H, H-2′), 3.38 (m, 1H, H-3′), 3.30 (m, 2H, H-4′, 5′), 3.86 (br d, 1H, *J* = 11.6 Hz, H-6a′) and 3.66 (m, 1H, H-6b′). Remaining resonances indicated a characteristic iridoid skeleton at δ 5.95 (d, 1H, *J* = 0.8 Hz, H-1), 6.37 (d, 1H, *J* = 6.4 Hz, H-3), 5.00 (dd, 1H, *J* = 6.4, 1.2 Hz, H-4), 3.80 (t, 1H, *J* = 4.0 Hz, H-6), 2.07 (dd, 1H, *J* = 14.0, 4.8 Hz, H-7a), 1.84 (dd, 1H, *J* = 14.0, 3.2 Hz, H-7b), and 2.73 (br s, 1H, H-9), and two methylene protons at δ 4.22 (dd, 2H, *J* = 11.6, 6.8 Hz,). The ^{13}C NMR spectrum of **1** in combination with the DEPT and HSQC spectra indicated the presence of 24 carbons including a cinnamoyl group at δ 168.6, 146.9, 135.9, 131.7, 130.2 (×2), 129.5 (×2), and 118.8, a sugar moiety at δ 99.8, 78.4, 77.7, 74.7, 71.8, and 62.9, and an iridoid skeleton at δ 143.1, 108.5, 92.7, 79.3, 78.0, 72.9, 69.5, 59.2, and 43.2. After acid hydrolysis and derivatization of **1** using the reported method [9], an HPLC analysis of the derivatives revealed the presence of D-glucose. Additionally, the β-configuration was established based on the coupling constant (8.0 Hz) of the anomeric proton.

According to the ^1H and ^{13}C NMR data, an iridoid glycoside similar to harpagoside was proposed [2], except for the absence of a methyl group and the presence of an oxygenated methylene (δ 69.5) at C-8. HMBC correlation from δ 2.73 (H-9) to δ 69.5 further confirmed the above deduction. The partial structures of the iridoid, cinnamoyl, and glucose moiety were assigned by detailed analyses of ^1H–^1H COSY, HSQC and HMBC spectra. The glucose moiety was attached at C-1 of iridoid, based on the HMBC correlations observed at H-1/C-1′ and H-1′/C-1. Furthermore, the linkage of the cinnamoyl group to iridoid was established at C-8 by the HMBC long-range correlation at H-10/C-9″.

The relative configuration of **1** was determined by comparison with reported literature values. The chemical shift of C-1 at δ 92.7 indicated a cis-fused iridoid by comparing with the chemical shift of C-1 (δ 100–105) in trans-fused iridoids [10]. The C-1 shift (δ 92.7) together with the difference between the C-3 and C-4 shifts

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($\Delta\delta$ 34.6) demonstrated a 6β-*O*-substituted iridoid compared to the C-1 shift ($\delta > 99$) and the difference between the C-3 and C-4 shifts ($\Delta\delta > 47$) for 6α-*O*-substituted compounds [11]. On the basis of the above evidence, **1** is believed to be a new iridoid glycoside, designated as scrophularianoid A.

Compound **2** was obtained as a yellowish amorphous powder, $[\alpha]_D^{25}$ − 50.0 (*c* 0.5, MeOH). HR-ESI-MS gave a quasimolecular ion peak at *m/z* 565.1904 $[M+Na]^+$, corresponding to the molecular formula $C_{25}H_{34}O_{13}$. Its UV absorption λ_{max} at 279 nm was indicative of a cinnamoyl chromophore. Its IR spectrum showed absorption bands of OH groups (3370 cm^{-1}), conjugated ester carbonyl group(s) (1691 cm^{-1}) and aromatic ring(s) (1635, 1578, 1514 cm^{-1}). The ^1H and ^{13}C NMR spectra of **2** were also similar to those of harpagoside [2]. Proton signals at δ 7.58–7.61 (m, 2H, H-2″, 6″), 7.39–7.41 (m, 3H, H-3″, 4″, 5″), 7.66 (d, 1H, *J* = 16.0 Hz, H-7″), and 6.49 (1H, d, *J* = 16.0 Hz, H-8″), together with carbon signals at δ 168.7, 146.3, 136.0, 131.7, 130.2 (×2), 129.4 (×2), and 120.2, suggested the presence of a cinnamoyl moiety. Proton signals at δ 4.68 (d, 1H, *J* = 7.6 Hz, H-1′), 3.28 (m, 1H, H-2′), 3.41 (d, 1H, *J* = 8.8 Hz, H-3′), 3.33 (m, 1H, H-4′), 3.35 (m, 1H, H-5′), 3.93 (dd, 1H, *J* = 12.0, 2.0 Hz, H-6a′) and 3.70 (m, 1H, H-6b′), together with carbon signals at δ 100.3, 78.2, 78.1, 74.8, 72.2, and 63.4, belonged to a sugar moiety. Acid hydrolysis followed by an HPLC analysis of the derivatives using an authentic sample as reference [9] confirmed the presence of D-glucose. Additionally, the configuration of the anomeric proton was deduced to be β based on the coupling constant (7.6 Hz) of the anomeric proton.

The remaining ^1H and ^{13}C NMR signals of **2** were similar to those of harpagoside, except for the absence of an olefinic bond at C-3 and C-4, and the presence of two oxygenated methines (δ 98.2, 71.2) and an additional methoxy group (δ 57.5). HMBC correlation from δ 3.54 (–OC$\underline{H}_3$) to δ 98.2 (C-3) indicated the attachment of the methoxy group at C-3.

The relative configuration of **2** was determined by comparison with **1** and further supported by NOESY analysis. The correlations between H-1 and H-10, H-10 and H-4, and H-4 and H-6 indicated that C_4-OH was in β-orientation. The α-configuration of C_3-OCH_3 was established based on the coupling constant ($J_{3,4}$ = 8.0 Hz). Thus, **2** is a new iridoid derivative and is named scrophularianoid B.

HR-ESI-MS, UV, IR, ^1H NMR, ^{13}C NMR, DEPT, ^1H–^1H COSY, HSQC, HMBC and NOESY spectra of compound **1**–**2** are supplied in Supporting information.

In our preliminary *in vitro* myocardial protective bioassay, compounds **1** and **2** were evaluated by the MTT assay. However, both of them showed little effect against H_2O_2-induced apoptosis in cardiomyocytes.

4. Conclusion

In conclusion, the 70% EtOH extract of the roots of *S. ningpoensis* gave two new iridoid glycosides, named scrophularianoids A (**1**) and B (**2**). The myocardial protective bioassay indicated that they both had little cardioprotective effect against the apoptosis induced by H_2O_2.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2014.05.007.

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