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Chinese Journal of Natural Medicines 2014, **12**(3): 0225−0228

Chinese Journal of Natural Medicines

Chemical investigation of the roots of *Polygala sibirica* L.

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Available online 20 Mar. 2014

[ABSTRACT]

AIM: To investigate the chemical constituents of the roots of *Polygala sibirica* L. (Polygalaceae)

METHOD: The isolation was performed by solvent extraction and various chromatographic techniques, including silica gel, Sephadex LH-20, ODS, semi-preparative HPLC, and preparative TLC. The chemical structures were elucidated based on extensive spectroscopic analysis, including HR-ESI-MS and 1D- and 2D-NMR spectroscopic data.

RESULTS: A total of sixteen compounds, including five xanthones (**5**, **7**–**10**), five saccharide esters (**1**, **3**, **4**, **12**, **13**), two flavonoids (**14**, **16**), two triterpenoids (**11**, **15**), one phenylpropanoid (**6**), and one benzophenone glycoside (**2**) were isolated. Their structures were determined as sibiricose A7 (**1**), sibiriphenone A (**2**), polygalatenoside A (**3**), polygalatenoside C (**4**), lancerin (**5**), 3, 4, 5-trimethoxycinnamic acid (**6**), 6-hydroxy-1, 2, 3, 7-tetramethoxyxanthone (**7**), 1, 3, 7-trihydroxy-2-methoxyxanthone (**8**), onjixanthone II (**9**), 1, 2, 3, 6, 7-pentamethoxyxanthone (**10**), presenegenin (**11**), 3'-*O*-3, 4, 5-trimethoxycinnamoyl-6-*O*-4-methoxy benzoyl sucrose (**12**), tenuifoliside C (**13**), 5, 3'-dihydroxy-7, 4'-dimethoxyflavonol-3-*O*-*β*-D-glucopyranoside (**14**), tenuifolin (**15**), and rhamnetin 3-*O*-*β*-D-glucopyranoside (**16**).

CONCLUSION: Compounds **1** and **2** are two new compounds from *P. sibirica*.

[KEY WORDS] *Polygala sibirica*; Polygalaceae; Sibiricose A7; Sibiriphenone A

[CLC Number] R284.1 **[Document code]** A **[Article ID]** 2095-6975(2014)03-0225-04

Introduction

The root of *Polygala sibirica* L. (Polygalaceae), as one of the authorized sources of Polygalae Radix (‘*Yuanzhi*’ in Chinese) in the Chinese Pharmacopoeia (2010 edition), is a well-known traditional Chinese medicine (TCM), commonly used as a tonic, sedative, and expectorant agent in China. As a continuation of a search for biologically active constituents from the genus *Polygala* [1–28], the roots of *P. sibirica* were phytochemically investigated, which led to the isolation of sixteen constituents. Herein the isolation and structural elucidation of all of the isolates, including two new compounds (**1** and **2**) and fourteen known compounds (**3**–**16**) are described.

Identification

Sibiricoside H (**1**) was isolated as a yellow powder, with a molecular formula of $C_{17}H_{22}O_9$, which was determined by HR-ESI-MS: *m/z* 393.116 4 $[M + Na]^+$ (Calcd. 393.115 6 for $C_{17}H_{22}O_9Na$). $[\alpha]_D^{20}$ +28.1 (*c* 0.27, MeOH). IR (KBr): 3 446 (OH), 1 630, 1 513 cm^{-1}. The ^{1}H- and ^{13}C NMR data were very similar to those of the known tenuifoliside D [29], except that a methoxy signal was absent at C-4', replaced by a hydroxyl group proton (Table 1). Furthermore, the HMBC correlation from H-6 (δ 4.20) to C-9' (δ 169) was observed, confirming the linkage of the aglycone and the sugar moiety (Fig. 1). Therefore, the structure of compound **1** was identified as 6-*O*-(*E*)-sinapoylpoligalitol, and named as sibiricose A7.

Sibiriphenone A (**2**) was isolated as a pale yellow pow-

[Received on] 14-Apr.-2013

[Research funding] This project was financially supported by the National Key Technology R & D Program “New Drug Innovation” of China (Nos. 2012ZX09301002-002-002, 2012ZX09304-005), special funds for scientific research on traditional Chinese medicine (No. 201307002), and National Science Fund for Excellent Young Scholars (No. 81222051).

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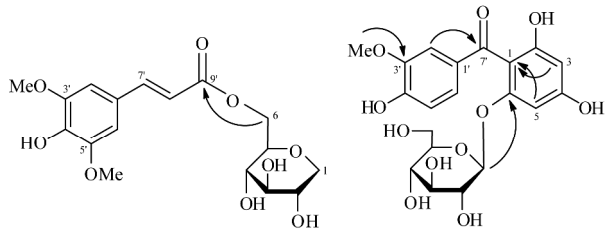
These authors have no any conflict of interest to declare.

der with a molecular formula of $C_{20}H_{22}O_{11}$, which was determined by the HR-ESI-MS data ($[M + Na]^+$, found: *m/z* 461.106 5, Calcd. 461.105 4). $[\alpha]_D^{20}$ –23.4 (*c* 2.75, MeOH). IR (KBr) ν_{max}: 3 418 (OH), 1 593, 1 514, 1 456 (aromatic ring) cm^{-1}. The D-glucose was detected in compound **2** by GC analysis after acid hydrolysis.

An ABX coupling system [δ 7.38 (1H, d, *J* = 1.5 Hz, H-2'), 6.74 (1H, d, *J* = 8.0 Hz, H-5'), and 7.25 (1H, dd, *J* = 1.5, 8.0 Hz, H-6')], two aromatic signals at δ 6.20 (1H, d, *J* = 2.0 Hz, H-3) and 6.03 (1H, d, *J* = 2.0 Hz, H-5), a methoxyl at



1 **2**

Fig. 1 structures and key HMBC correlations of compounds 1 and 2

Table 1 ^{1}H- and ^{13}C NMR data (500/125 MHz, CD_3OD) of compounds 1 and 2 (*J* in Hz)

No.	1		No.	2	
	δ_H	δ_C		δ_H	δ_C
1	3.13 (1H, t, *J* = 11.0) 3.84 (1H, dd, *J* = 5.5, 11.0)	71.0	1		110.1
			2		159.5
2	3.42 (1H, m)	71.3	3	6.03 (1H, d, *J* = 2.0)	98.1
3	3.36 (1H, m)	79.8	4		162.3
4	3.36 (1H, m)	71.8	5	6.20 (1H, d, *J* = 2.0)	95.8
5	3.42 (1H, m)	80.0	6		158.5
6	4.20 (1H, dd, *J* = 6.0, 12.0) 4.43 (1H, dd, *J* = 2.0, 12.0)	65.1	1'		132.4
		126.6	2'	7.38 (1H, d, *J* = 1.5)	113.1
1'		107.0	3'		148.5
2', 6'	6.85 (2H, s)	149.5	4'		152.9
3', 5'		139.6	5'	6.74 (1H, d, *J* = 8.0)	115.4
4'		147.2	6'	7.25 (1H, dd, *J* = 1.5, 8.0)	126.6
7'	7.56 (1H, d, *J* = 16.0)	115.7	3'-OMe	3.81 (3H, s)	56.4
8'	6.36 (1H, d, *J* = 16.0)	169.0	7'		197.3
9'		56.9	Glc-1	4.79 (1H, d, *J* = 7.5)	102.1
3', 5'-OMe	3.81 (6H, s)		2	3.02 (1H, t, *J* = 7.5)	74.6
			3	3.36 (1H, m)	78.1
			4	3.25 (1H, m)	71.0
			5	3.30 (1H, m)	77.7
			6	3.82 (1H, overlapped)	62.4
				3.63 (1H, dd, *J* = 5.5, 12.0)	

δ 3.81 (3H, s, 4-OMe), and a glucose anomeric signal at δ 4.79 (1H, d, *J* = 7.5 Hz) were observed in the ^{1}H NMR data of **2**. The value *J* = 7.5 Hz for the anomeric proton suggested the *β*-orientation of the glucose moiety (Table 2).

The ^{13}C NMR data of **2** (Table **1**) displayed a total of twenty carbon resonances, including a glucose moiety, one methoxyl, one 1, 3, 4-trisubstituted aromatic ring and one 1, 2, 4, 6-tetrasubstituted aromatic ring. These assignment, in combination with a carbonyl signal (δ 197.3) indicated **2** to be a benzophenone derivate.

The HMBC correlations from H-3 (δ 6.03) to C-1 (δ 110.1), C-2 (δ 159.5), and C-4 (δ 162.3), from H-5 (δ 6.20) to C-1 (δ 110.1), C-3 (δ 98.1), C-4 (δ 162.3), and C-6 (δ 158.5), from the methoxy signal (δ 3.81) to the C-3' of aglycone (δ 148.5), from H-2' (δ 7.38) to C=O (δ 197.3), from H-6' (δ 7.25) to C=O (δ 197.3), and from the anomeric proton signal (δ 4.79) to the C-6 of aglycone (δ 158.5) determined that the methoxy group was linked at the C-3' position of the aglycone, while the C-2, C-4, and C-6 positions were substituted by hydroxyl groups, and the glucose was linked at the C-6 position (Fig. 1). Therefore, the structure of compound **2** was elucidated as 2, 4, 6, 4'-tetrahydroxy-3'-methoxybenzophenone-6-*O*-*β*-D-glucopyranoside, and named as sibiriphenone A.

Additionally, the fourteen known compounds were identified as polygalatenoside A (**3**) [30], polygalatenoside C (**4**) [30], lancerin (**5**) [31], 3, 4, 5-trimethoxycinnamic acid (**6**) [32], 6-hydroxy-1, 2, 3, 7-tetramethoxyxanthone (**7**) [33], 1, 3, 7-

trihydroxy-2-methoxyxanthone (**8**) [34], onjixanthone II (**9**) [33], 1, 2, 3, 6, 7-pentamethoxyxanthone (**10**) [33], presenegenin (**11**) [35], 3'-*O*-3, 4, 5-trimethoxycinnamoyl-6-*O*-4-methoxybenzoyl sucrose (**12**) [29], tenuifoliside C (**13**) [36], 5, 3'-dihydroxy-7, 4'- dimethoxyflavonol-3-*O*-*β*-D-glucopyranoside (**14**) [37], tenuifolin (**15**) [35], and rhamnetin 3-*O*-*β*-D-glucopyranoside (**16**) [38] by comparison of their NMR data with the literature data.

Experimental

General procedures

Optical rotations were determined with a Perkin-Elmer 243B digital polarimeter. IR spectra were achieved using a Nicolet-Avatar 360 infrared spectrometer using the KBr disc technique. NMR spectra were recorded on a Varian INOVA-500 and a JEOL JNM-A300 spectrometers with TMS as internal standard. ESI-MS spectra were obtained on an Applied-Biosystems QSTAR mass spectrometer and HR-ESI-MS spectra were obtained on Apex-II-FT-ICRMS spectrometer (Bruker Daltonics). The semi-preparative HPLC system consisted of a Waters-600E multi-solvent delivery pump and a Waters 2487 dual λ absorbance detector. The reversed-phase chromatography was carried out on a Waters preparative Nova-Pak HR C_{18} column (300 mm × 10 mm, 6 μm). The flow rate was 2.0 mL·min^{-1} and the UV detection was set at the dual wavelengths of 228 and 310 nm. Silica gel (100–200 and 200–300 mesh) employed in column chromatography (CC) were products of Qingdao Haiyang Chem. Co. Ltd.. HPD101 porous resin was a product of Tianjin Chem. Ind. Co. Ltd.. Sephadex LH-20 was supplied by Pharmacia. Octadecyl silica gel (ODS, 25–40 μm) and $HSGF_{254}$ TLC precoated silica gel plates were products of Merck (Darmstadt, Germany).

Plant material

The roots of *P. sibirica* were collected from Wutai County, Shanxi province, China in July 2006, and identified by Professor TU Peng-Fei. A voucher specimen (No. A20060715) is deposited in the herbarium of the Modern Research Center for Traditional Chinese Medicine, Peking University Health Science Center, Beijing China.

Extraction and isolation

The air-dried roots of *P. sibirica* (9.0 kg) were refluxed three times with 95% EtOH (70 L × 3) for 3 h each time. The extracts were combined and evaporated *in vacuo* to yield a residue (2.2 kg), most of which was suspended in water (6 L) and defatted with petroleum ether (8 L). The water layer was extracted successively with $CHCl_3$ (12 L) and *n*-BuOH (12 L) to afford the $CHCl_3$ extract (135 g) and *n*-BuOH extract (545 g), respectively. Then, the *n*-BuOH extract (500 g) was subjected to a HPD 101 resin CC, eluting with H_2O (16 L), 25% aqueous EtOH (25 L), 50% aqueous EtOH (40 L), and 70% aqueous EtOH (25 L) sequentially.

The 25% aqueous EtOH eluate (87 g) was subjected to silica gel (200–300 mesh) CC, eluting with $CHCl_3$–MeOH (10 : 1 to 1 : 1, *V*/*V*) to afford five fractions (Frs. I–V). Fr. I was subjected to an open ODS (25–40 μm) CC, eluting with a gradient of MeOH–H_2O to yield three subfractions (Frs. 1-1–1-3). Fr. 1-2 was further purified by semi-preparative HPLC (MeOH–H_2O, 28 : 72, *V*/*V*) to yield **1** (5 mg). Fr. II was applied on silica gel (200–300 mesh) CC, eluting with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2 to 65 : 35 : 10, lower layer) to obtain four subfractions (Frs. 2-1–2-4). Fr. 2-3 was subjected to ODS (25–40 μm) CC, eluting with a gradient of MeOH–H_2O to afford three fractions (Frs. 2-3-1–2-3-3), and Fr. 2-3-1 was subjected to Sephadex LH-20 CC (MeOH) to yield **2** (70 mg). Fr. III was fractionated by silica gel (100–200 mesh) CC, followed by ODS (25–40 μm) CC, Sephadex CC, and semi-preparative HPLC to yield **3** (61 mg). Fr. IV was subjected to silica gel (200–300 mesh) CC, followed by ODS (25–40 μm) CC, Sephadex LH-20 CC, and semi-preparative HPLC to obtain **4** (9 mg) and **5** (7 mg).

The 50% EtOH eluate (100 g) was loaded onto a silica gel (200–300 mesh) CC, eluting with a gradient of $CHCl_3$-MeOH (10 : 1 to 1 : 1) to afford six fractions (Frs. 50-1–50-6). Fr. 50-1 was subjected to Sephadex LH-20, followed by ODS (25–40 μm) CC and recrystallization to yield **6** (12 mg) and **7** (14 mg), and followed by semi-preparative HPLC to afford **8** (8 mg) and **9** (10 mg). Fr. 50-2 was introduced to ODS CC, followed by semi-preparative HPLC, to yield **10** (15 mg). Fr. 50-3 was subjected to Sephadex LH-20 (MeOH) CC to yield **11** (38 mg), and followed by ODS (25–40 μm) CC and semi-preparative HPLC to yield **12** (17 mg). Fr. 50-4 was subjected to Sephadex LH-20 (MeOH) CC, ODS (25–40 μm) CC, and then semi-preparative HPLC to yield **13** (14 mg) and **14** (94 mg). Fr. 50-5 was subjected to ODS (25–40 μm) CC to obtain **15** (72 mg). Fr. 50-6 was applied to ODS CC and semi-preparative HPLC to yield **16** (8 mg).

*Acid hydrolysis of compound **2***

Compound **2** (2 mg) was hydrolyzed with 2N aqueous CF_3COOH (5 mL) at 110 °C for 6 h in a sealed tube. After this period, the reaction mixture was diluted with H_2O (15 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined CH_2Cl_2 extracts were washed with H_2O and then evaporated in a vacuum. The residue was dissolved in pyridine (60 μL), and hexamethylsilane (60 μL) and trimethylsilane chloride (20 μL) were added, and stirred at 60°C for 30 min, and subjected to GC [HP-5 capillary column (28 m × 0.32 mm, ID.) D-glucose (12.45 min) was observed from compound **2**.

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Cite this article as: ZHOU Yu-Hong, ZHANG Shui-Ying, GUO Qiang, CHAI Xing-Yun, JIANG Yong, TU Peng-Fei. Chemical investigation of the roots of *Polygala sibirica* L. [J]. *Chinese Journal of Natural Medicines*, 2014, **12**(3): 225-228