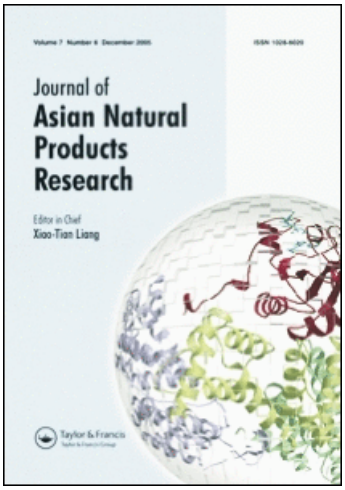

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

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To cite this Article Xu, De-Ping , Hu, Chang-Ying and Zhang, Yang(2009) 'Two new steroidal saponins from the rhizome of *Polygonatum sibiricum*', Journal of Asian Natural Products Research, 11: 1, 1 — 6
To link to this Article: DOI: 10.1080/10286020802513681
URL: http://dx.doi.org/10.1080/10286020802513681

Journal of Asian Natural Products Research
Vol. 11, No. 1, January 2009, 1–6

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(*Received 19 October 2007; final version received 23 September 2008*)

Two new furostanol saponins, polygonoides A (**1**) and B (**2**), along with three known compounds, were obtained from the ethanolic extract of the rhizome of *Polygonatum sibiricum* Redoute. On the basis of acid hydrolysis and comprehensive spectroscopic analyses, the structures of polygonoides A and B were elucidated as (25*R*)-26-*O*-β-D-glucopyranosyl-furost-5,22(23)-dien-3β,26-diol-3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (**1**) and 22α-(propionyloxy)-furost-5-en-3β,20α-diol-3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (**2**).

Keywords: *Polygonatum sibiricum*; furostanol saponins; polygonoide A; polygonoide B

1. Introduction

Polygonatum sibiricum grows wild and is cultivated as a traditional medicinal herb and foodstuffs in China. As a common Chinese medicine, *P. sibiricum* is considered to have the functions of replenishing vital essence, removing dryness, promoting secretion of fluid, and quenching thirst. It has been used traditionally for hundreds of years to treat many diseases, especially tuberculosis and diabetes [1,2]. In southern China, it is cooked with meats and porridge as nutrition foods. The public, including many Chinese doctors, believe that *P. sibiricum* using as a food ingredient is safe. In the acute toxicity test, the dose of *P. sibiricum* in mice is 64.5 g/kg (crude drug), equivalent to 150 times the human dose, which does not induce any toxicity sign or death in mice [3,4]. Some studies showed that *P. sibiricum* reduced serum cholesterol, triglyceride, and blood glucose levels in hyperlipemia patients and Wistar fatty rats [3]. In the present research, water-soluble extract of *P. sibiricum* can significantly lower hyperglycemia caused by starch loading in both normal and diabetic mice, improved the glucose tolerance in diabetic mice. It is therefore likely that the active components for treating diabetes, at least in part, are aqueous soluble [4].

Some steroid and triterpenoid saponins have been isolated from *P. sibiricum* [5]. But the constituents responsible for the inhibition of glucose absorption have not been identified. In our study, on the bioactive constituents of this plant, we have characterized five steroid saponins. In this paper, the isolation and structural elucidation of two new furostanol saponins, along with three known compounds, are presented.

2. Results and discussion

The *n*-butanolic fraction from EtOH extract of the fresh root of *P. sibiricum* was chromatographed on macroporous adsorption resin AB-8, reversed-phase silica gel, and MCI gel CHP 20P to afford five steroid saponins (**1**–**5**).

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ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020802513681
http://www.informaworld.com

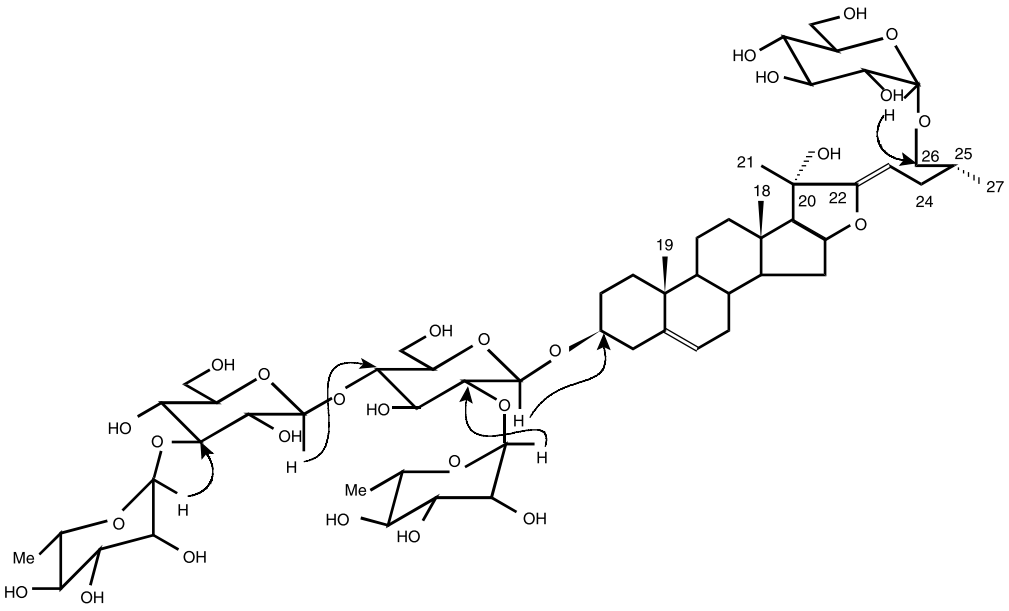

Figure 1. The structure and key HMBC correlations of compound **1**.

Compound **1** was isolated as a white amorphous powder and gave positive results for the Ehrlich reagent. The HR-ESI-MS showed an $[M - H]^-$ ion peak at *m/z* 1207.5732, corresponding to $C_{57}H_{92}O_{27}$. The ^{1}H NMR spectrum of **1** showed the presence of six methyl groups at δ 0.99 (3H, s), 1.14 (3H, s), 1.15 (3H, d, $J = 8.0$ Hz), 1.70 (3H, s), 1.80 (3H, d, $J = 7.4$ Hz), and 1.84 (3H, d, $J = 6.2$ Hz), five anomeric protons at δ 6.43 (1H, s), 5.91 (1H, s), 5.34 (1H, d, $J = 7.6$ Hz), 5.17 (1H, d, $J = 7.8$ Hz), and 4.94 (1H, d, $J = 7.6$ Hz), and two olefinic protons at δ 5.41 (1H, brs) and 5.30 (1H, brs). The ^{13}C NMR spectrum revealed 57 carbon signals, of which 30 were assigned to the sugar moieties and 27 to the aglycone moiety. These data indicated that compound **1** is a furostanol glycoside with five sugar moieties. A comparative study of the ^{13}C NMR spectrum of **1** with that of protodioscin [6] suggested the presence of an additional hydroxyl group and double bond in **1**. Detailed analysis of the HMQC and HMBC spectra revealed that the aglycone of **1** possesses the same partial structure in the A, B, C, and D rings as protodioscin (Figure 1). The difference between them in ring E, C-23, and C-24, the characteristic signal at δ 109.7 to the C-22 carbon of a spirostan skeleton [6] was not observed, downfield shift of C-20 (δ 77.0) (α effect), C-21 (δ 22.1) (β effect), implying a hydroxyl groups at C-20, downfield shift of C-22 (δ 169.3), C-23 (δ 91.6), C-24 (δ 29.8) (α effect), suggested that the double bond was at C-22 and C-23. These hypotheses were confirmed by the HMBC spectrum that showed correlations between the H-21 (δ 1.70) and the C-20 (δ 77.0), between H-23 (δ 5.30) and C-24 (δ 29.8). The resonances of the protons and carbons (C-24, C-26, C-27) around the C-25 center and the $^3J_{H.H}$ values (9.6, 3.5 Hz) between H-25 and H-26 provided the evidence for the *R* configuration of C-25 in **1** as described in several literature [6,7]. The geminal proton resonances of H_2-26 appeared at δ 3.61 and 3.98 ($\Delta ab = 0.37 < 0.48$ for 25R compound), and the result was in accordance with Agrawal's pattern [7]. The orientation of the C-21 methyl was determined to be

β-configuration by the observed NOE correlation between β-oriented methyl proton at H-18 (δ 0.99) and the H-21 methyl proton at δ 1.70. The NOE correlation between H-21 and H-23 indicated that the Δ22(23) was cis-configuration, in accordance with the literature [8]. The H-24, H-25, H-26, H-27, and H-21 were assigned by $^{1}H-^{1}H$ COSY and HMQC spectra. As to the sugar moiety, acid hydrolysis of **1** afforded glucose and rhamnose as the sugar components. The ^{13}C NMR spectrum indicated the presence of five anomeric signals at δ 105.2, 105.0, 103.1, 102.2, and 100.5, which showed correlations with their corresponding anomeric protons at δ 5.17, 4.94, 5.91, 6.43, and 5.34, respectively, in the HMQC experiment. From the HMBC spectrum, a correlation between glc26-H-1 and the C-26 suggested that the glucose (anomeric carbon δ 105.0) was attached to the C-26 of the aglycone. The correlation between H-3 and glucose-1-C-1 confirmed that the glucose was attached to the C-3 (δ 78.5) of the aglycone. The correlations between rham-1-H-1 and glucose-1-C-2, between glucose-2-H-1′ and glucose-1-C-4 and between rham-2-H-1′ and glucose-2-C-3′, confirmed that the rhamnose-1 (anomeric carbon at δ 102.2) was attached to the C-2 of glucose-1, the glucose-2 (anomeric carbon at δ 105.2) was attached to the C-4 of glucose-1, and the rhamnose-2 (anomeric carbon at δ 103.1) was attached to the C-3′ of glucose-2. The glycosylation positions of sugar units were confirmed by TOCSY, HMQC, and HMBC experiments. The α-anomeric configurations for the two rhamnoses were determined by their C-5 data (δ 70.6, 69.7). The β-anomeric configuration of the three glucoses was determined from their large $^{3}J_{H1.H2}$ coupling constants (7.6, 7.8, and 7.6 Hz) [9,10]. Thus, the structure of **1** was elucidated as (25*R*)-26-*O*-β-D-glucopyranosyl-furost-5,22(23)-dien-3β,20,26-triol-3-*O*-α-L-rhamnopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside, named polygonoide A.

Compound **2** is a white amorphous powder, giving a positive coloration with Ehrlich reagent. The negative HR-ESI-MS of **2** showed a quasi-molecular ion peak at *m/z* 887.4316 $[M - H]^{-}$, corresponding to the molecular formula $C_{43}H_{69}O_{19}$. The ^{13}C NMR spectrum showed 43 carbon signals, of which 25 signals are assigned to the aglycone moiety, the other 18 signals due to the sugar residue. With regard to the aglycone moiety, the ^{13}C NMR spectral data for **2** suggested the presence of a carbonyl group (δ 179.2), a secondary methyl carbon (δ 30.2), and a methyl carbon (δ 18.6); moreover, the protons at δ 1.08 and the carbons at δ 179.2 showed the existence of propionyloxy group. Comparison of the signals from the aglycone moiety of **2** with those of **1** showed that the double bond between C-22 and C-23 in **1** was substituted by the hydroxyl group in **2**, which was confirmed by the presence of the proton and carbon signals at δ_H 1.08 and δ_C 179.2. Furthermore, the carbonyl carbon signal (δ 179.2) showed correlations with H-22 (δ 5.91) and H-25 (δ 1.08) in the HMBC spectrum, indicating that the propionyloxy group was attached to the C-22 in **2** (Figure 2).

The NOE correlation between H-22 and H-21 indicated the β-configuration of H-22 and α-configuration of the side chain attached to C-22. As for the sugar moiety, acid hydrolysis of **2** afforded glucose and rhamnose as the sugar components. The ^{13}C NMR spectrum indicated the presence of three anomeric signals (δ 103.0, 102.1, and 100.4), which showed correlations with their corresponding anomeric proton signals at δ 5.24, 6.45, and 5.16, respectively, in the HMQC experiment. From the HMBC spectrum, the correlation between H-3 (δ 4.12) and glucose-1-C-1 suggested that the glucose-1 was attached to the C-3 (δ 78.6) of the aglycone. The correlations between rham-H-1 and C-2 of glucose-1, between glucose-2-H-1′ and C-4 of glucose-1, revealed that the glucose-2 (anomeric carbon δ 103.0) was attached to the C-4 of glucose-1, rhamnose was attached to the C-2 of glucose-1. The glycosylation position of sugar units were confirmed by

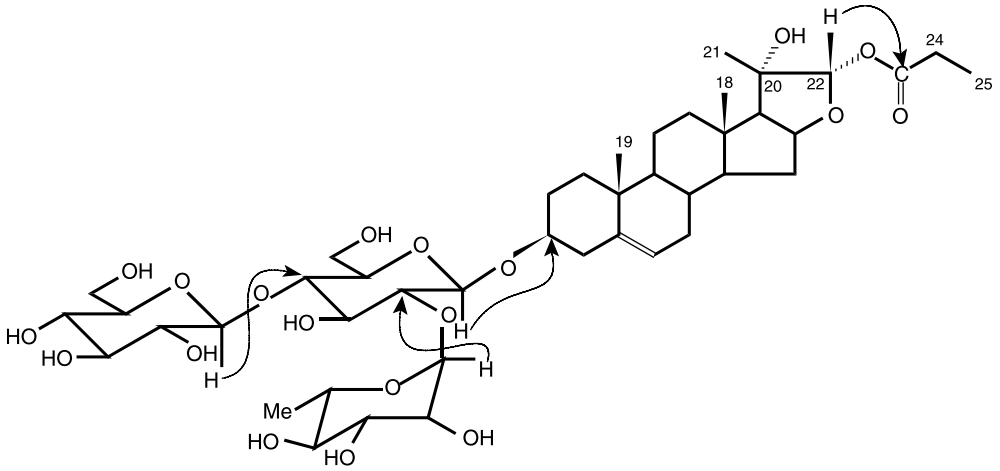

Figure 2. The structure and key HMBC correlations of compound **2**.

TOCSY, HMQC, and HMBC experiments. The α-anomeric configuration for the rhamnose was determined by its C-5 data (δ 69.7). The β-anomeric configuration of the two glucoses was determined from their large $^3J_{H1.H2}$ coupling constants (7.6, 6.7 Hz). According to the accumulated evidence above, the structure of **2** could be elucidated as 22α-(propionyloxy)-furost-5-en-3β,20α-diol-3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside, named polygonoide B.

The known compounds **3**, **4**, and **5** were identified as 3-*O*-β-D-glucopyranosyl(1→3)-β-D-glucopyranosyl(1 → 4)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-glucopyranoside-diosgenin, 3-*O*-β-D-α-L-rhamnopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-glucopyranoside-diosgenin, and 3-*O*-β-D-glucopyranosyl-(1 → 4)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-glucopyranoside-diosgenin by comparison of their spectral data with literature values [11].

3. Experimental

3.1 General experimental procedures

Melting points were measured with an X4 micro-melting point apparatus (uncorrected). The $[\alpha]_D$ values were obtained in MeOH at 20°C using a Perkin-Elmer 341 digital polarimeter. NMR spectra were recorded in pyridine-d_5 with a Bruker Avance 500 NMR spectrometer, with TMS as internal standard. HR-ESI-MS were recorded with a spectrometer JEOL JMS-DX302. Macroporous adsorption resin AB-8 (30–40 μm, Chemical Plant of Nankai University, Tianjing, China), Sephadex LH-20 (20–80 μm, Pharmacia Fine Chemical Co. Ltd, Uppsala, Sweden), MCI gel CHP20P (75–150 μm, Mitsubishi Kasei Co., Tokyo, Japan), and Cosmosil ODS (40–80 μm, Nacalai Tosoh Inc., Uetikon, Switzerland) were used for column chromatography. $HSGF_{254}$ (precoated plate, Qingdao Oceanic Chemical Co., Qingdao, China) was used for TLC analysis.

3.2 Plant material

The rhizome of *P. sibiricum* Redoute was collected from Xi'an, Shanxi Province of China in September 2004. The plant material was identified by Prof. Jingxian Yan (Institute of Botany North-West, Chinese Academy of Science).

3.3 Extraction and isolation

The fresh rhizome of *P. sibiricum* (15 kg) was extracted thrice (3 × 2 h) with EtOH–H_2O (4:1) at 80°C. The EtOH extracts were evaporated under reduced pressure to give a residue (1237 g) that was suspended in water

and then extracted with petroleum ether, ethyl acetate, and *n*-butanol successively. The *n*-butanolic fraction was subjected to macroporous adsorption resin AB-8 column chromatography, and eluted with an EtOH–H_2O (30:70–60:40) gradient system to afford five fractions. Fraction 3 eluted by 50% EtOH was subjected to column chromatography on Sephadex LH-20, eluted with H_2O–EtOH (9:1–8:2), and afforded fractions A (eluted by 10% EtOH) and B (eluted by 20% EtOH). Fraction A was subjected to column chromatography on reversed-phase ODS, using H_2O–EtOH (7:3–6:4) as the eluant to afford fractions I, II, and III. Fractions I, II, and III were further purified by repeated MCI gel CHP 20P column chromatography, respectively, using H_2O–EtOH 35% as the eluant, to give compounds **1** (67 mg), **2** (53 mg), and **3** (203 mg), respectively. Fraction B was subjected to reversed-phase ODS column chromatography with H_2O–EtOH solvent system (5:5–6:4) to get fractions IV and V, from which the final product compounds **4** (127 mg) and **5** (43 mg) were obtained by repeated MCI gel CHP 20P column chromatography.

3.3.1 *Polygonoide A (**1**)*

A white amorphous powder, mp 196–198°C, Ehrlich reagent positive, $[\alpha]_{20}^{D} = -27.0$ (MeOH, *c* 0.06). ^{1}H NMR (pyridine-d_5) δ: 0.99 (3H, s, H-18), 1.14 (3H, s, H-19), 1.15 (3H, d, $J = 8.0$ Hz, H-27), 1.70 (3H, s, H-21), 1.80 (3H, d, $J = 7.4$ Hz, Rham-2-H-6), 1.84 (3H, d, $J = 6.2$ Hz, Rham-1-H-6), 4.94 (1H, d,

Table 1. ^{13}C NMR spectral data of compounds **1** and **2** (in pyridine-d_5).

Aglycone	**1**	**2**	Sugar	**1**	**2**
1	37.7	37.6	Glc1-1	100.5	100.4
2	30.4	30.2	2	79.0	78.8
3	78.5	78.2	3	76.5	77.3
4	39.2	39.9	4	78.3	78.7
5	141.1	141.0	5	77.8	77.8
6	121.9	121.7	6	61.8	61.5
7	32.2	32.1	Glc2-1′	105.2	103.0
8	31.3	31.2	2′	74.3	75.0
9	50.4	50.2	3′	84.4	78.3
10	37.2	37.2	4′	70.0	69.2
11	20.8	20.5	5′	78.2	77.5
12	39.5	38.8	6′	62.1	63.0
13	40.6	40.5	Rha-1	102.2	102.1
14	57.2	56.0	2	72.0	72.6
15	33.7	32.4	3	72.6	72.9
16	79.0	82.8	4	75.4	74.3
17	68.0	64.4	5	69.7	69.7
18	13.7	13.8	6	18.7	18.8
19	19.6	19.5	Rha2-1′	103.1	
20	77.0	75.4	2′	71.9	
21	22.1	20.9	3′	72.5	
22	163.9	105.2	4′	75.1	
23	91.6	179.2	5′	70.6	
24	29.8	30.2	6′	18.9	
25	35.1	18.6	Glc26-1	105.0	
26	75.5		2	75.8	
27	17.6		3	78.7	
			4	72.1	
			5	78.6	
			6	63.1	

$J = 7.6$ Hz, Glc-26-H-1), 5.17 (1H, d, $J = 7.8$ Hz, Glc-2-H-1), 5.30 (1H, brs, H-23), 5.34 (1H, d, $J = 7.6$ Hz, Glc-1-H-1), 5.41 (1H, brs, H-6), 5.91 (1H, s, Rham-2-H-1), 6.43 (1H, s, Rham-1-H-1). The ^{13}C NMR spectral data are shown in Table 1. HR-ESI-MS: *m/z* 1207.5732 $[M - H]^-$ (calcd for $C_{57}H_{91}O_{27}$, 1207.5748).

3.3.2 *Polygonoide B (**2**)*

A white amorphous powder, mp 186–188°C, Ehrlich reagent positive, $[\alpha]_{20}^{D} = -32.2$ (MeOH, *c* 0.06). ^{1}H NMR (pyridine-d_5) δ: 0.90 (3H, s, H-18), 1.08 (3H, t, $J = 7.1$ Hz, H-25), 1.12 (3H, s, H-19), 1.28 (3H, s, H-21), 1.84 (1H, d, $J = 6.2$ Hz, Rham-H-6), 5.16 (1H, d, $J = 6.7$Hz, Glc-1-H-1), 5.24 (1H, d, $J = 7.6$ Hz, Glc-2-H-1), 5.39 (1H, d, $J = 3.6$ Hz, H-6), and 6.45 (1H, s, Rham-H-1). The ^{13}C NMR spectral data are shown in Table 1. HR-ESI-MS: *m/z* 887.4316 $[M - H]^-$ (calcd for $C_{43}H_{68}O_{19}$, 888.4355).

3.3.3 *Acid hydrolysis of **1***

A solution of **1** (10 mg) in 1N HCl (1 ml) was heated at 80°C for 2 h in a water bath. The sugar components were identified by TLC ($CHCl_3$–MeOH–Me_2CO–H_2O, 3:3:3:1), with R_f 0.46 as glucose and R_f 0.63 as rhamnose.

Acknowledgements

This work was supported by the Science and Technology Bureau of Zhuhai City, Guangdong Province, China.

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