Saponins from the Processed Rhizomes of *Polygonatum kingianum*

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Two new spirostanol saponins, named kingianoside H (1) and kingianoside I (2), were isolated from the processed rhizomes of *Polygonatum kingianum*, along with a known triterpenoid saponin ginsenoside-Rc (3), four known spirostanol saponins Tg (4), (5), polygonatoside C_1 (6) and ophiopogonin C′ (7). The structures of the new compounds were elucidated by detailed spectroscopic analyses, including 1D and 2D NMR techniques and chemical methods. Compounds 3 and 5 were first reported from the genus *Polygonatum*. Compounds 4, 6 and 7 are reported for the first time from the processed *Polygonatum kingianum*.

Key words *Polygonatum kingianum*; processing; steroidal saponin; triterpenoid saponin; identification

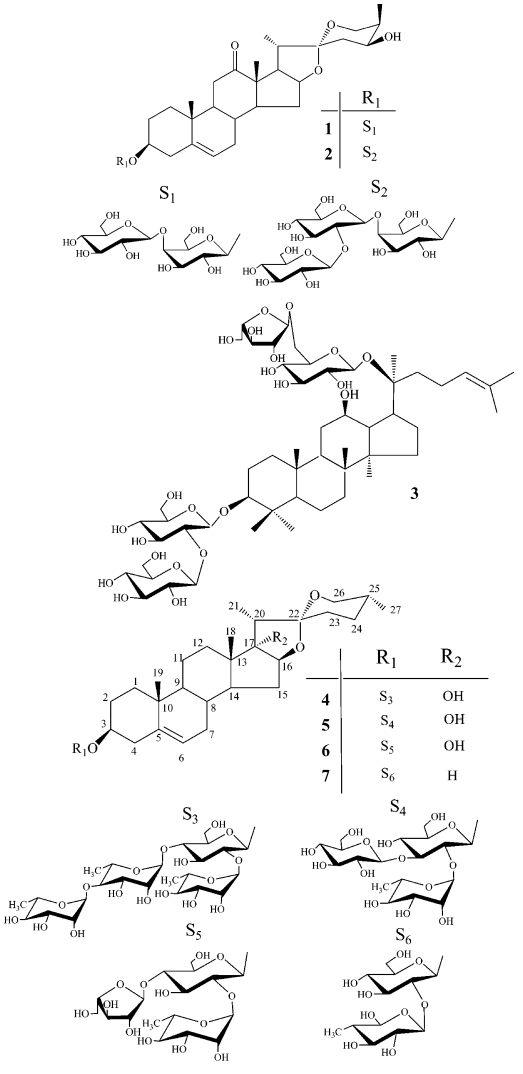
The rhizomes of *Polygonatum kingianum* Coll. *et* Hemsl. (Liliaceae), one of the original plants known as Huang-jing in traditional Chinese medicine, were used as a tonic remedy to treat lung troubles and ringworm.[1)] Clinically, the processed rhizomes products (processed with yellow rice wine) were routinely used, for it is widely believed that this process would enhance the effect of tonic remedy.

Previous phytochemical investigations on the fresh rhizomes of several *Polygonatum* species have resulted in the isolation of steroidal saponins and triterpenoid saponins,[2—8)] but no systematic study on the chemical constituents of the processed products has been reported so far. We had previously reported some steroidal saponins from the fresh rhizomes of *P. kingianum*.[9,10)] As a part of our ongoing research, we focused on investigating the variation chemical constituents of the fresh and the processed rhizomes. Our preliminary phytochemical studies on processed *P. kingianum* had indicated that the amounts of certain chemical constituents are higher in processed *P. kingianum* than those in fresh *P. kingianum*. Meanwhile, some other chemical constituents are lower and a few are new. Furthermore, the ultra performance liquid chromatograph (UPLC)-MS profile of the processed products revealed that a few constituents that were not reported previously. Therefore, our detailed chemical investigation on the processed *P. kingianum* led to the isolation of two new spirostanol saponins, along with a known triterpenoid saponin and four known spirostanol saponins. Compounds **3** and **5** were reported for the first time from the genus *Polygonatum*. These compounds are reported from the processed *P. kingianum* for the first time. In this paper, we describe the isolation and structure elucidation of the two new saponins on the basis of extensive spectral analyses, including 1D (dimensional) and 2D NMR spectral data and chemical evidences.

Results and Discussion

The crude extract of the processed *P. kingianum* was fractionated by using a combination of macroporous resin, silica-gel and octadecyl silica (ODS) silica-gel column chromatography and semi-preparative HPLC to afford compounds **1**—**7**.

Compounds **1** and **2** were found to be new saponins and their structures elucidated by 1D and 2D NMR in combination with MS studies. Compounds **3**—**7** were known saponins and identified by comparison of their NMR data with those reported in the literature. Compound **3** is a known triterpenoid saponin and its structure was identified as (20*S*)-



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protopanaxadiol-3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside-20-*O*-*α*-L-arabinofuranosyl-(1→6)-*β*-D-glucopyranoside (ginsenoside-Rc).[11] Compounds **4**—**7** are known spirostanol saponins and their structures were identified as (25*R*)-spirost-5-en-3*β*,17*α*-diol-3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→4)-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside (Tg),[12] (25*R*)-spirost-5-en-3*β*,17*α*-diol-3-*O*-*β*-D-glucopyranosyl-(1→3)-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside,[13] (25*R*)-spirost-5-en-3*β*,17*α*-diol-3-*O*-*α*-L-arabinofuranosyl-(1→4)-[*α*-L-rhamnopyranosyl-(1→2)]-*β*-D-glucopyranoside (polygonatoside C_1)[14] and (25*R*)-spirost-5-en-3*β*-ol-3-*O*-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranoside (ophiopogonin C′),[15] respectively.

Compound **1** was obtained as a white amorphous powder. It gave positive Liebermann–Burchard and negative Ehrlich reagent tests, which suggested that **1** was a spirostanol saponin. The molecular formula was determined as $C_{39}H_{60}O_{15}$ by the negative-ion HR-electrospray ionizatoin (ESI)-MS (*m*/*z* 767.3854 $[M-H]^-$). The positive ion FAB-MS also showed the characteristic fragment ion peaks at *m*/*z*: 769.4 $[M+H]^+$, 607.3 $[M+H-162]^+$, 445.2 $[M+H-162-162]^+$, suggesting the existence of two hexose units in the molecule. Compound **1** was hydrolyzed with acid to afford D-galactose and D-glucose. The ^{1}H-NMR spectrum of **1** revealed the presence of two singlet methyl signals at δ 0.89 (3H, s, 19-CH_3) and 1.07 (3H, s, 18-CH_3), and two doublet methyl signals at δ 1.29 (3H, d, J=6.6 Hz, 27-CH_3) and 1.38 (3H, d, J=6.6 Hz, 21-CH_3), which were characteristic of spirostanol saponin methyls. Furthermore, an olefinic proton at δ 5.27 (H, br s, H-6) was assigned, same for two anomeric protons at δ 4.86 (1H, d, J=7.2 Hz) and 5.28 (1H, d, J=8.4 Hz). The ^{13}C-NMR spectrum of **1** showed two anomeric carbon signals at δ 102.9 and 107.2, as well as two olefinic carbon signals at δ 140.8 and 121.4. Comparing the ^{13}C-NMR data of **1** with that of pratioside D_1,[4] significant differences of chemical shifts in F-ring (δ 109.3, 31.7, 29.2, 30.5, 67.0, 17.3) indicated that **1** had one hydroxyl group attached at F-ring of the spirostanol skeleton. The ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum was carefully inspected to assign the structure of the F-ring part, with the three-proton doublet signal at δ 1.29 (3H, d, J=6.6 Hz) attributable to 27-CH_3, being used as the starting point of analysis. As a result, the structural fragment of the F-ring, $-C_{(23)}H_2-C_{(24)}H(-O-)-C_{(25)}H$ ($-C_{(26)}H_2-O-)-CH_{3(27)}$, was revealed and the location of one hydroxyl group at C-24 was evident. The proton signals of H-23$_{ax}$ at δ 2.15 dd (J=12.0, 12.6 Hz) and signal of H-26$_{ax}$ at δ 2.15 br d (J=9.6 Hz) gave evidence for the 24*S* and 25*R* configurations. The ^{1}H- and ^{13}C-NMR data for the F-ring of **1** was identical with those of the compound **2**[16] in the literature, also provided evidence for the 24*S* and 25*R* configurations. In the heteronulcear multiple bond coherence (HMBC) spectrum of **1** (Fig. 1), the long range correlations between the carbon signal δ 66.4 (C-24) and δ 2.05 (H-23) and 3.58 (H-26) were confirmed the location of the one hydroxyl group at C-24. Furthermore, the

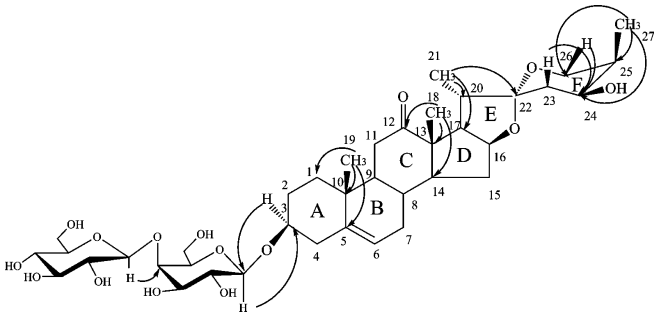

Fig. 1. The Key HMBC Correlations of Compound **1**

Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **1** and **2** (δ in Pyridine-d_5)[a]

Position	**1**		**2**	
	δ_C	δ_H J (Hz)	δ_C	δ_H J (Hz)
Aglycone				
1	37.0	1.47 m, 0.87 m	37.0	1.47 m, 0.86 m
2	30.0	2.06 m, 1.63 m	30.0	2.06 m, 1.65 m
3	77. 7	3.83 m	77.7	3.85 m
4	39.1	2.67 m, 2.35 m	39.1	2.67 m, 2.40 m
5	140.8	—	140.8	—
6	121.4	5.27 br s	121.4	5.27 br s
7	31.7	1.46 m, 1.84 m	31.7	1.46 m, 1.84 m
8	30.9	1.82 m	30.9	1.80 m
9	52.3	1.29 m	52.3	1.29 m
10	37.6	—	37.6	—
11	37.5	2.50 m, 2.28 dd (6.0, 14.4)	37.5	2.49 m, 2.27 br d (9.0)
12	212.5	—	212.5	—
13	54.9	—	54.9	—
14	56.0	1.41 m	56.0	1.41 m
15	31.5	2.06 m, 1.58 m	31.5	2.06 m, 1.58 m
16	80.1	4.45 m	80.1	4.45 m
17	53.6	2.78 dd (6.6, 8.4)	53.6	2.78 dd (7.2, 8.4)
18	15.8	1.07 s	15.8	1.07 s
19	18.8	0.89 s	18.8	0.89 s
20	43.2	1.97 m	43.2	1.97 m
21	13.7	1.38 d (6.6)	13.7	1.38 d (6.6)
22	111.5	—	111.5	—
23	36.0	2.05 m, 2.15 dd (12.6, 12.0)	36.0	2.05 m, 2.15 dd (12.6, 12.0)
24	66.4	4.62 m	66.4	4.62 m
25	35.9	2.03 m	35.9	2.03 m
26	64.6	4.03[b] o, 3.56 br d (9.6)	64.6	4.04 br d (10.2), 3.58 br d (10.2)
27	9.7	1.29 d (6.6)	9.7	1.28 d (7.8)
Glycone				
Gal				
1	102.9	4.86 d (7.2)	102.7	4.88 d (7.2)
2	73.5	4.38 dd (7.2, 9.0)	73.3	4.48 m
3	75.4	4.23 m	75.6	4.09 m
4	80.0	4.69 br s	81.0	4.57 m
5	76.0	4.04 m	76.8	4.06 m
6	61.0	4.23 m, 4.63 m	60.4	4.73 m, 4.19 m
Glc				
1	107.2	5.28 d (8.4)	105.2	5.14 d (7.8)
2	75.2	4.13 dd (8.4, 9.0)	86.2	4.15 m
3	78.7	4.22 m	78.5	4.27 m
4	72.3	4.06 m	71.9	3.96 m
5	78.5	4.01 m	78.2	3.97 m
6	63.2	4.20 m, 4.60 m	63.2	4.09 m, 4.62 m
Glc′				
1			107.0	5.23 d (7.8)
2			75.2	3.98 m
3			77.8	4.12 m
4			70.3	4.22 m
5			79.0	3.81 m
6			61.6	4.57 m, 4.37 m

a) The assignments were based on the ^{1}H-NMR, ^{13}C-NMR, ^{1}H–^{1}H COSY, HSQC and HMBC experiments. *b*) o: overlapping, indicates overlapping signals.

anomeric proton signals at δ 4.86 (H-1 of the galactose) and 5.28 (H-1 of the glucose) showed correlations with the carbon signals at δ 77.7 (C-3) and 80.0 (C-4 of the galactose), respectively. The full assignments of these sugar signals were confirmed by ^{1}H–^{1}H COSY, heteronuclear single quantum coherence (HSQC) and HMBC experiments. Therefore, the structure of **1** was determined to be (24*S*, 25*R*)-3β,24-dihydroxy-spirostan-5-en-12-one-3-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, and named kingianoside H.

Compound **2** was obtained as a white amorphous powder. It gave positive Liebermann–Burchard and negative Ehrlich reagent tests, which suggested that **2** was a spirostanol saponin. The molecular formula was determined as $C_{45}H_{70}O_{20}$ by the negative-ion HR-ESI-MS (*m/z* 929.4382 $[M-H]^-$). The positive ion FAB-MS also showed the characteristic fragment ion peaks at *m/z*: 931.4 $[M+H]^+$, 769.4 $[M+H-162]^+$, 607.3 $[M+H-162-162]^+$, 589.3 $[M+H-162-162-18]^+$, 445.3 $[M+H-162-162-162]^+$, 427.3 $[M+H-162-162-162-18]^+$, suggesting the existence of three hexose units in the molecule. Compound **2** was hydrolyzed with acid to afford D-galactose and D-glucose. The ^{1}H-NMR spectrum of **2** revealed the presence of two singlet methyl signals at δ 0.89 (3H, s, 19-CH_3) and 1.07 (3H, s, 18-CH_3), and two doublet methyl signals at δ 1.28 (3H, d, J=7.8 Hz, 27-CH_3) and 1.39 (3H, d, J=7.2 Hz, 21-CH_3), which were recognized as typical spirostanol saponin methyls. Furthermore, an olefinic proton at δ 5.26 (H, br s, H-6) was readily assigned, as well as signals for three anomeric protons at δ 4.88 (1H, d, J=7.2 Hz), 5.14 (1H, d, J=7.8 Hz) and 5.25 (1H, d, J=9.6 Hz). The ^{13}C-NMR spectrum of **2** showed three anomeric carbon signals at δ 102.7, 105.2 and 107.0, in addition, two olefinic carbon signals at δ 140.8 and 121.4. Analysis of the ^{1}H- and ^{13}C-NMR spectral data of **2** in comparison with those of **1** implied that the aglycone of **2** was the same as **1**. In the HMBC spectrum of **2**, a cross peak between the ^{1}H-NMR signal at δ 4.88 (H-1 of the galactose) and the carbon signal at δ 77.7 (C-3, aglycone) indicated glycosylation of the aglycone at C-3. Furthermore, the anomeric proton signals at δ 5.14 (H-1of the glucose) and 5.25 (H-1′ of the glucose) showed correlations with the carbon signals at δ 81.0 (C-4 of the galactose) and 86.2 (C-2 of the glucose), respectively. The full assignments of these sugar signals were confirmed by HSQC and ^{1}H–^{1}H COSY experiments. Thus, the structure of **2** was determined to be (24*S*,25*R*)-3β,24-dihydroxy-spirostan-5-en-12-one-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, and named Kingianoside I.

Experimental

General Methods The HR-ESI-MS was recorded on 9.4 T Q-FT-MS Apex Qe (Bruker Co.). FAB-MS: Micromass Zabspec. Optical rotations were measured with Perkin-Elmer 343 polarimeter. The NMR spectra were recorded with Varian UNITY*INOVA* 600 (599.8 MHz for ^{1}H-NMR and 150.8 MHz for ^{13}C-NMR), and the chemical shifts were given on δ (ppm) scale with tetramethylsilane as an internal standard. The HPLC analysis was performed using Agilent 1100 system (pump, quaternary pump. Detector, RID and DAD, U.S.A.), Apollo C_{18} (Alltech, 8.0 mm i.d.×250 mm, ODS, 10 μm, U.S.A.) and YMC-Pack ODS-A C_{18} (YMC, 4.6 mm i.d.×250 mm, ODS, 5 μm, Japan). The Gas chromatographic analysis was performed with an Agilent 6890 Series, gas chromatograph equipped with an H_2 flameionization detector. The column was an HP-5 capillary column (30 m×0.25 mm×0.25 μm) (Agilent, U.S.A.). Macroporous resin SP825 (Mitsubishi Chemical, Japan), silica-gel (Qingdao Haiyang Chemical Co., Ltd., China) and ODS silica-gel (120 Å, 50 μm, YMC) were used for chromatography.

Plant Material The material was collected from Fenggang county of Guizhou province, People's Republic of China in December 2006, and was identified as rhizomes of *Polygonatum kingianum* COLL. *et.* HEMSL. by Prof. Li-juan Zhang of the Tianjing University of Traditional Chinese Medicine. The processed products were processed according to the procedures from the Chinese pharmacopoeia: the dried fresh rhizomes of *P. kingianum* were mixed thoroughly with yellow rice wine with 5 : 1 ratio and kept in a container with cap tightly closed till the wine was all absorbed. The wine soaked rhizomes well and steamed thoroughly according to the specification of the procedures, afterwards samples were cooled to room temperature and cut to thin slices. Finally dried for 48 h at 50 °C and cooled to room temperature to become the processed samples. A voucher specimen (No. 061201) was deposited in the herbarium of Beijing Institute of Radiation Medicine, Beijing.

Extraction and Isolation The decoction pieces of processed *P. kingianum* (5.5 kg) were extracted for three times with 45% Me_2CO. The combined extract was concentrated under reduced pressure to give 72.0 g of residue. The extract was fractionated by macroporous resin SP825 and eluted with a gradient mixture of Me_2CO–H_2O (10 : 90, 40 : 60, 80 : 20), to give three fractions (Fr. A—C). Fraction A was further purified on a macroporous resin SP825 column and eluted with gradient mixtures of Me_2CO–H_2O (25 : 75, 35 : 65, 60 : 40), to give three fractions, A_1 (1.8 g), A_2 (0.9 g) and A_3 (4.0 g). A part of fraction A_3 (3.6 g) was chromatographed on silica-gel with a $CHCl_3$–MeOH–H_2O solvent system (15 : 1 : 0.01→2 : 1 : 0.01), and fractions A_3-96—106 was separated by semi-preparative HPLC with MeOH–H_2O (62 : 38), to yield compound **1** (8.9 mg), fractions A_3-107—156 was separated by semi-preparative HPLC with MeOH–H_2O (60 : 40), to yield compound **2** (9.6 mg), fractions A_3-205—220 were further separated by semi-preparative HPLC with MeCN–H_2O (22 : 78) to yield compounds **3** (7.3 mg). Fraction C (2.4 g) was subjected to column chromatography on ODS silica-gel with a MeCN–H_2O solvent system (45 : 55, 48 : 52, 52 : 48), to yield compound **4** (fractions C-71—73) (36.3 mg). Finally, fractions C-108—142 were further separated by semi-preparative HPLC with MeCN–H_2O (49 : 51) to yield compounds **5** (8.4 mg), **6** (9.7 mg) and **7** (18.7 mg).

Compound **1**: White amorphous power, $[\alpha]_D^{20}$ −34.3° (*c*=0.046, pyridine); ^{1}H- and ^{13}C-NMR: see Table 1. HR-ESI-MS (negative) *m/z*: 767.3854 $[M-H]^-$ (Calcd for $C_{39}H_{59}O_{15}$: 767.3867). FAB-MS *m/z*: 769.4 $[M+H]^+$, 607.5 $[M+H-162]^+$, 445.2 $[M+H-162-162]^+$

Compound **2**: White amorphous power, $[\alpha]_D^{20}$ −43.2° (*c*=0.038, pyridine); ^{1}H- and ^{13}C-NMR: see Table 1. HR-ESI-MS (negative) *m/z*: 929.4382 $[M-H]^-$ (Calcd for $C_{45}H_{69}O_{20}$: 929.4396). FAB-MS *m/z*: 931.4 $[M+H]^+$, 769.4 $[M+H-162]^+$, 607.3 $[M+H-162-162]^+$, 589.3 $[M+H-162-162-18]^+$, 445.3 $[M+H-162-162-162]^+$, 427.3 $[M+H-162-162-162-18]^+$.

Acid Hydrolysis of Compound 1 Compound **1** (about 2.0 mg) was treated in 1 M HCl (dioxane–H_2O, 1 : 1, 2 ml) at 100 °C for 1.5 h. The reaction mixture was neutralized with silver carbonate and evaporated to dryness under N_2 gas overnight. The residue was extracted with $CHCl_3$ and H_2O. Then, in monosaccharide mixture, glucose and galactose were detected by TLC analysis on a cellulose plate using *n*-BuOH–EtOAc–C_5H_5N–H_2O (6 : 1 : 5 : 4) as development and aniline-*o*-phthalic acid as detection, comparing with the authentic samples: glucose (*Rf* 0.46) and galactose (*Rf* 0.39). The determination of the configuration of sugar moieties followed the procedure was described in our previous paper.[10] The retention times of the derivatives for the standards were: t_R: 20.31 min (D-glucose derivative), t_R: 20.82 min (L-glucose derivative), t_R: 22.08 min (D-galactose derivative) and t_R: 22.65 min (L-galactose derivative). The retention times of the derivatives of D-glucose (D-glucose derivative) and D-galactose (D-galactose derivative) for compound **1** were 20.29 min and 22.03 min, respectively.

Compound **2** (about 2.0 mg) was subjected to acid hydrolysis as described for **1**. The derivatives of D-glucose (D-glucose derivative) and D-galactose (D-galactose derivative) were observed at 20.29 min and 22.03 min, respectively.

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