Three New Saponins from the Fresh Rhizomes of *Polygonatum kingianum*

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Further studies on the fresh rhizomes of *Polygonatum kingianum* led to the isolation of one new spirostanol saponin (25*R*)-kingianoside G (1), and two pairs mixture of 25*R* and 25*S* stereoisomeric spirostanol saponins (25*R*,*S*)-pratioside D_1 (2a, 2b) and (25*R*,*S*)-kingianoside A (3a, 3b), among them 2b and 3b were new spirostanol saponins, together with another two known compounds, disporopsin (4) and daucosterol (5). The structures of the new saponins were determined by detailed analysis of their 1D and 2D NMR spectra, and chemical evidences.

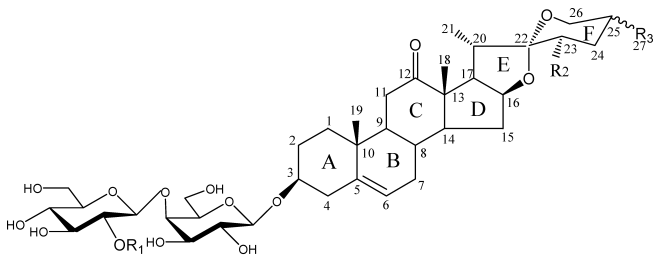
Key words *Polygonatum kingianum*; spirostanol saponin; identification; stereoisomeric

In the course of our phytochemical studies on the rhizomes of *Polygonatum kingianum*, we had previously reported five pairs of 25*R* and 25*S* stereoisomeric furostanol saponins from the rhizomes of *Polygonatum kingianum*.[1)] A further study led to the isolation of three new steroidal saponins (25*R*)-kingianoside G (**1**), (25*S*)-pratioside D_1 (**2b**) and (25*S*)-kingianoside A (**3b**), along with four known compounds pratioside D_1 (**2a**),[2)] kingianoside A (**3a**),[3)] disporopsin (**4**)[4)] and daucosterol (**5**).[5)] Compounds **2a** and **5** were reported for the first time from the rhizomes of *Polygonatum kingianum*. This paper describes the structural elucidation of the new saponins on the basis of extensive spectral analysis, including 2D NMR spectral data and chemical evidences.

Results and Discussion

The crude saponin fraction of *Polygonatum kingianum* was fractionated by a combination of macroporous resin, silica-gel and octa decil silica (ODS) silica-gel column chromatography and semi-preparative HPLC to afford compounds **1**—**5**. Compounds **2a**, **3a**, **4** and **5** were identified as pratioside D_1,[2)] kingianoside A,[3)] disporopsin[4)] and daucosterol,[5)] by comparison of their NMR spectral data with those in the literatures.

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 $C_{45}H_{70}O_{20}$ by the negative-ion HR-ESI-MS (*m*/*z* 929.4374 $[M-H]^-$) and FAB-MS (*m*/*z* 953.4 $[M+Na]^+$, 931.4 $[M+H]^+$). Furthermore, the prominent fragments at *m*/*z*: 769.2 $[M+H-162]^+$, 607.5 $[M+H-162-162]^+$ and 445.2 $[M+H-162-162-162]^+$ attribute to the sequential loss of three hexose residues, respectively. **1** was hydrolyzed with acid to afford D-galactose and D-glucose. The ^{1}H-NMR spectrum of **1** showed two singlet methyl signals at δ 0.85 (3H, s, 19-CH_3) and 1.27 (3H, s, 18-CH_3), and two doublet methyl signals at δ 0.71 (3H, d, J=6.0 Hz, 27-CH_3) and 1.40 (3H, d, J=7.2 Hz, 21-CH_3), which were recognized as typical spirostanol saponin methyls. Moreover, an olefinic proton at δ 5.25 (H, br s, H-6) could be readily assigned, and signals for three anomeric protons at δ 4.87 (1H, d, J=7.8 Hz), 5.14 (1H, d, J=7.8 Hz) and 5.23 (1H, d, J=7.8 Hz). The J values (>7 Hz) of three anomeric protons indicated the β-orientation at the anomeric center for the hexose.[6,7)] The ^{13}C-NMR spectrum of **1** showed three anomeric carbon signals at δ 102.8, 105.2 and 106.9. Comparing the ^{13}C-NMR data of **1** with that of polygonatoside C,[8)] significant differences of chemical shifts in F-ring (δ 112.3, 67.7, 39.1, 40.5, 63.4, 64.0) indicated that **1** had one hydroxyl group attached at F-ring of the spirostanol skeleton. Analysis of the ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY), heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) spectra identified all the proton and carbon signals arising from the F-ring part and gave unequivocal evidence that the location of the one hydroxyl group was at C-23 position. In the HMBC spectrum of **1** (Fig. 1), the correlations between the carbon signals δ 67.4 (C-23) and δ 1.78 (H-24) and 3.00 (H-20) were confirmed the location of the one hydroxyl group at C-23. Furthermore, in the HMBC spectrum, the anomeric proton signals at δ 4.87 (H-1 of the galactose), 5.12 (H-1 of the glucose) and 5.21 (H-1′ of the glucose) showed correlations with the carbon signals at δ 77.7 (C-3), 81.0 (C-4 of the galactose) and 86.1 (C-2 of the glucose), respectively. The ^{1}H- and ^{13}C-NMR data for the F-ring of **1** was identical with those of agamenoside A,[9)] indicating that **1** had the same 23*S* and 25*R* configurations as agamenoside A. Thus, the structure of **1** was determined to be (23*S*,25*R*)-spirostan-5-en-3β,23-dihydroxy-12-one-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopy-



	R_1	R_2	R_3
1	Glc	OH	eqMe
2a	Glc	H	eqMe
2b	Glc	H	axMe
3a	H	H	eqMe
3b	H	H	axMe

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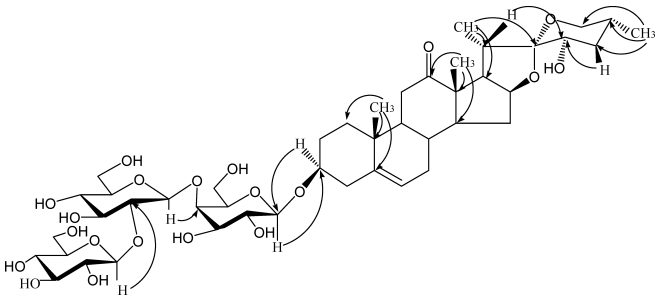

Fig. 1. The Key HMBC Correlations of Compound **1**

ranosyl-(1→4)-β-D-galactopyranoside, and named (25*R*)-kingianoside G.

Compound **2** was obtained as a white amorphous powder, and gave positive Liebermann–Burchard test. Its molecular formula was assigned as $C_{45}H_{70}O_{19}$ on the basis of HR-ESI-MS (negative) *m/z* 913.4414 $[M-H]^-$ (Calcd for $C_{45}H_{69}O_{19}$: 913.4438) and the FAB-MS (*m/z* 915.2 $[M+H]^+$). Furthermore, the prominent fragments at *m/z*: 753.2 $[M+H-162]^+$, 591.2 $[M+H-162-162]^+$ and 429.2 $[M+H-162-162-162]^+$ attribute to the sequential loss of three hexose residues, respectively. The ^{1}H-NMR spectrum of **2** showed one overlapping singlet methyl signal at δ 0.91 (6H, s, 19-CH_3), whereas another two singlet methyl signals at δ 1.09 (3H, s, 18-CH_3, 25*R*) and 1.08 (3H, s, 18-CH_3, 25*S*), and one overlapping doublet methyl signal at δ 1.34 (6H, d, J=6.6 Hz, 21-CH_3), another two doublet methyl signals at δ 0.68 (3H, d, J=5.4 Hz, 27-CH_3, 25*R*) and 1.06 (3H, d, J=7.2 Hz, 27-CH_3, 25*S*), which suggested **2** may be contained C-25*R* and C-25*S* isomers (**2a**, **2b**).[10,11] Furthermore, in the ^{1}H-NMR spectrum showed the molecular ratio of **2a** : **2b** was 2 : 1. The carbon signals at δ 26.4/31.8 (C-23), 26.2/29.2 (C-24), 27.5/30.5 (C-25), 64.5/67.0 (C-26) and 16.3/17.3 (C-27) in the ^{13}C-NMR spectrum also suggested **2** contained 25*R* and 25*S* isomers (**2a**, **2b**).[12—14] The ^{13}C-NMR spectrum of **2** showed three anomeric carbons signals at δ 102.7, 105.2 and 107.0. Comparing the ^{13}C-NMR spectrum data of **2a** with those of pratioside D_1,[2] indicated **2a** was pratioside D_1 and **2b** was (25*S*)-spirostan-5-en-12-one-3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, and named (25*S*)-pratioside D_1.

Compound **3** was obtained as a white amorphous powder, and gave positive Liebermann–Burchard and negative Ehrlich reagent tests, which suggested that **3** was a spirostanol saponin. Its molecular formula was assigned as $C_{39}H_{60}O_{14}$ on the basis of the HR-ESI-MS (positive): *m/z* 775.3881 $[M+Na]^+$ (Calcd for $C_{39}H_{60}Na_1O_{14}$: 775.3875) and FAB-MS (*m/z* 775.3 $[M+Na]^+$). Furthermore, the prominent fragments at *m/z*: 753.2 $[M+H]^+$, 591.2 $[M+H-162]^+$ and 429.2 $[M+H-162-162]^+$ attribute to the sequential loss of two hexose residues, respectively. Analysis of the ^{1}H- and ^{13}C-NMR spectral data of **3** in comparison with those of **2** implied that the aglycone of **3** was the same as **2**. Compound **3** was a mixture of C-25*R* and C-25*S* isomers (**3a**, **3b**). In the ^{1}H-NMR spectrum of **3** showed the molecular ratio of **3a** : **3b** was 3 : 2. Comparing the ^{1}H- and ^{13}C-NMR spectral data of **3a** with those of kingianoside A,[3] indicating that **3a** was kingianoside A. Thus, the structure of **3b** was determined to be (25*S*)-spirostan-5-en-12-one-3-*O*-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside, and named (25*S*)-kingianoside A.

Experimental

General Methods HPLC 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). Gas chromatographic analysis was performed with an Aglient 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.). 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. Macroporous resin SP825 (Mitsubishi Chemical, Japan), AB-8 (Nan Kai 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 2004, and was identified as rhizomes of *Polygonatum kingianum* Coll. *et* Hemsl. by Prof. Jian-mei Huang of the Beijing University of Traditional Chinese Medicine. A voucher specimen (No. 040122) was deposited in the herbarium of Beijing Institute of Radiation Medicine, Beijing.

Extraction and Isolation The fresh rhizomes of *Polygonatum kingianum* (30.0 kg) were extracted for three times with 50% aqueous EtOH. The combined extract was concentrated under reduced pressure to give 2460 g of residue. Column chromatography of the extract was fractionated by macroporous resin AB-8 and eluted with a gradient mixture of Me_2CO–H_2O (1 : 9, 1 : 1 and 8 : 2), to give three fractions (Fr. A—C). Fraction B (16.8 g) was further purified on a macroporous resin SP825 column and eluted with a gradient mixture of Me_2CO–H_2O (2 : 8, 3 : 7, 2 : 3 and 8 : 2), to give four fractions, B_1 (0.8 g), B_2 (9.5 g), B_3 (5.0 g) and B_4 (1.5 g). A part of fraction B_3 (4.0 g) was subjected to column chromatography on ODS silica-gel with Me_2CO–H_2O (25 : 75, 40 : 60 and 60 : 40), to yield compound **4** (fractions B_3-18—22) (59.4 mg), and fractions B_3-30—34 was separated by semi-preparative HPLC with Me_2CO–H_2O (25 : 75), to yield compound **1** (18.2 mg). A part of fraction B_4 (1.4 g) was chromatographed on silica-gel with a $CHCl_3$–MeOH–H_2O solvent system (9 : 1 : 0.01→2 : 1 : 0.01). Finally, fractions B_4-11 and 12 was further separated by semi-preparative HPLC with Me_2CO–H_2O (85 : 15), to yield compounds **2** (7.6 mg) and **3** (16.4 mg), and fractions B_4-30—35 was subjected to recrystallization under room temperature, to yield compound **5** (95.4 mg).

Compound **1**: White amorphous power, $[\alpha]_D^{20}$ −44.6° (c=0.032, pyridine); ^{1}H- and ^{13}C-NMR: see Table 1. HR-ESI-MS (negative): *m/z* 929.4374 $[M-H]^-$ (Calcd for $C_{45}H_{69}O_{20}$: 929.4387). FAB-MS *m/z*: 931.4 $[M+H]^+$, 769.2 $[M+H-162]^+$, 607.5 $[M+H-162-162]^+$, 445.2 $[M+H-162-162-162]^+$.

Compound **2**: White amorphous power, $C_{45}H_{70}O_{19}$, HR-ESI-MS (negative): *m/z* 913.4414 $[M-H]^-$ (Calcd for $C_{45}H_{69}O_{19}$: 913.4438). FAB-MS: *m/z* 915.2 $[M+H]^+$, 753.2 $[M+H-162]^+$, 591.2 $[M+H-162-162]^+$, 429.2 $[M+H-162-162-162]^+$. ^{1}H-NMR (pyridine-d_5, 599.8 MHz), δ: 4.89 (H-Gal-1, overlap), 5.14 (2H, d, J=7.8 Hz, H-Glc-1) and 5.23 (2H, d, J=7.2 Hz, H-Glc′-1); **2a**: δ 0.91 (3H, s, 19-CH_3), 1.09 (3H, s, 18-CH_3), 0.68 (3H, d, J=5.4 Hz, 27-CH_3), 1.34 (3H, d, J=6.6 Hz, 21-CH_3); **2b**: δ 0.91 (3H, s, 19-CH_3), 1.08 (3H, s, 18-CH_3), 1.06 (3H, d, J=7.2 Hz, 27-CH_3), 1.34 (3H, d, J=6.6 Hz, 21-CH_3). ^{13}C-NMR: see Table 2.

Compound **3**: White amorphous power, $C_{39}H_{60}O_{14}$, HR-ESI-MS (positive): *m/z* 775.3881 $[M+Na]^+$ (Calcd for $C_{39}H_{60}Na_1O_{14}$: 775.3875). FAB-MS: *m/z* 775.3 $[M+Na]^+$, 753.2 $[M+H]^+$, 591.2 $[M+H-162]^+$, 429.2 $[M+H-162-162]^+$. ^{1}H-NMR (pyridine-d_5, 599.8 MHz), δ: 4.86 (2H, d, J=7.2 Hz, H-Gal-1) and 5.28 (2H, d, J=7.2 Hz, H-Glc-1); **3a**: δ 0.91 (3H, s, 19-CH_3), 1.09 (3H, s, 18-CH_3), 0.68 (3H, d, J=5.4 Hz, 27-CH_3), 1.30 (3H, d, J=6.6 Hz, 21-CH_3); **3b**: δ 0.91 (3H, s, 19-CH_3), 1.08 (3H, s, 18-CH_3), 1.06 (3H, d, J=7.2 Hz, 27-CH_3), 1.30 (3H, d, J=6.6 Hz, 21-CH_3). ^{13}C-NMR: see Table 2.

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 the solvent thoroughly driven out 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

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

Aglycone	C	H, *J* (Hz)	Glycone	C	H, *J* (Hz)
1	37.0	1.47 m, 0.87 m	3-O-Gal		
2	30.0	2.06 m, 1.68 m	1	102.7	4.87 d (7.8)
3	77.7	3.85 m	2	73.3	4.48 m
4	39.1	2.66 m, 2.38 dd (12.0, 12.0)	3	75.6	4.09 m
5	140.8		4	81.0	4.57 m
6	121.4	5.25 br s	5	76.8	3.99 m
7	31.8	1.46 m, 1.83 m	6	60.5	4.72 m, 4.18 m
8	30.8	1.80 m	Glc		
9	52.3	1.31 m	1	105.2	5.12 d (7.8)
10	37.6		2	86.1	4.15 m
11	37.5	2.49 m, 2.26 d (14.4, 6.0)	3	78.5	4.27 m
12	212.9		4	71.9	3.96 m
13	55.6		5	78.2	3.98 m
14	56.0	1.45 m	6	63.2	4.09 m, 4.62 m
15	31.6	2.12 m, 1.70 m	Glc′		
16	80.3	4.60 m	1	107.0	5.21 d (7.8)
17	53.6	2.89 dd (7.2, 8.4)	2	75.2	4.06 m
18	16.1	1.27 s	3	77.9	4.11 m
19	18.8	0.85 s	4	70.4	4.22 m
20	36.4	3.00 m	5	79.0	3.81 m
21	13.7	1.40 d (7.2)	6	61.7	4.57 m, 4.35 m
22	111.9				
23	67.4	3.87 m			
24	38.7	2.10 m, 1.78 m			
25	31.7	1.80 m			
26	66.2	3.54 dd (3.6, 7.2), 3.48 m			
27	17.0	0.71 d (6.0)			

a) The assignments were based on the ^{1}H-NMR, ^{13}C-NMR, ^{1}H–^{1}H COSY, HSQC and HMBC experiments.

Table 2. ^{13}C-NMR Data of Compounds **2a**, **2b**, **3a** and **3b** (δ in Pyridine-d_5, 150 MHz)

Aglycone	**2a** (25*R*)	**2b** (25*S*)	**3a** (25*R*)	**3b** (25*S*)	Glycone	**2a** (25*R*)	**2b** (25*S*)	**3a** (25*R*)	**3b** (25*S*)
1	37.0		37.0		3-*O*-Gal-1	102.7		102.9	
2	30.0		30.0		2	73.3		73.5	
3	77.7		77.8	77.7	3	75.6		75.4	
4	39.1		39.1		4	81.0		80.0	
5	140.8		140.8		5	76.8		75.2	
6	121.4		121.4		6	60.4		61.0	
7	31.7		31.8		Glc′-1	105.2		107.2	
8	30.9		30.9		2	86.2		76.0	
9	52.3		52.3		3	78.5		78.8	
10	37.6		37.6		4	71.9		72.3	
11	37.5		37.5		5	78.2		78.5	
12	212.6		212.6		6	63.2		63.2	
13	55.0		55.0		Glc″-1	107.0			
14	56.0		56.0		2	75.2			
15	31.6		31.6		3	77.8			
16	79.7	79.8	79.7	79.8	4	70.4			
17	54.0	53.9	54.0	53.9	5	79.0			
18	15.9		15.9		6	61.6			
19	18.8		18.8						
20	42.6	43.1	42.7	43.1					
21	13.9	13.7	13.9	13.7					
22	109.3	109.8	109.3	109.8					
23	31.8	26.4	31.7	26.4					
24	29.2	26.2	29.2	26.2					
25	30.5	27.5	30.5	27.5					
26	67.0	64.5	67.0	64.5					
27	17.3	16.3	17.3	16.3					

galactose (*Rf* 0.39). Furthermore, the sugar residue in pyridine (1 ml) was added to L-cysteine methyl ester hydrochloride (3.0 mg) and kept at 60 °C for 1 h. Then HMDS-TMCS (hexamethyldisilazane-trimethylchlorosilane) (0.6 ml) was added to the reaction mixture and kept at again 60 °C for 0.5 h.[15)] The supernatant (1.0 ml) was analyzed by GC under the following conditions: Agilent Technologies 6890 gas chromatograph was the equipment carrying H_2 flame ionization detector and HP-5 capillary column (30 m×0.25 mm×0.25 μm). The conditions as followings: column temperature: 180 °C/250 °C; programmed increase, 15 °C/min; carrier gas: N_2 (1 ml/min); injection and detector temperature: 250 °C; injection volume: 4.0 μl,

split ratio: 1/50. The derivatives of D-glucose and D-galactose were detected, t_R: 20.31 min (D-glucose derivative) and 22.08 min (D-galactose derivative).

By the same procedures carried out for **2** and **3** (each about 2.0 mg). The derivatives of D-galactose and D-glucose were detected, t_R: 20.31 min (D-galactose derivative) and 22.08 min (D-glucose derivative).

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