Furostanol Saponins from the Fresh Rhizomes of *Polygonatum kingianum*

Jie ZHANG,[a,b] Bai-Ping MA,*[,a] Li-Ping KANG,[a] He-Shui YU,[a] Yun YANG,[b] Xian-Zhong YAN,[c] and Fang-Ting DONG[c]

[a] *Beijing Institute of Radiation Medicine; Beijing 100850, People's Republic of China:* [b] *Henan Collage of Traditional Chinese Medicine; Zhengzhou 450008, People's Republic of China: and* [c] *The National Center of Biomedical Analysis; Beijing 100850, People's Republic of China.* Received November 11, 2005; accepted April 4, 2006

Ten furostanol saponins were isolated as five pairs of 25*R* and 25*S* epimers from the fresh rhizomes of *Polygonatum kingianum*. Seven of them were identified as new compounds, (25*S*)-kingianoside D (2), (25*S*)-kingianoside C (4), (25*R*,22)-hydroxylwattinoside C (5), kingianoside E (7), (25*S*)-kingianoside E (8), kingianoside F (9) and (25*S*)-kingianoside F (10), together with three known saponins, kingianoside C (1), kingianoside D (3), and 22-hydroxylwattinoside C (6). The structures of the new saponins were determinded by detailed analysis of their 1D and 2D NMR spectra, and by comparison of the spectral data with those reported.

Key words *Polygonatum kingianum*; Liliaceae; steroidal saponin; furostanol saponin

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 lung troubles and ringworm.[1)] It was planted mainly in the provinces of Yunnan, Guizhou and Guangxi. The chemical constituents of rhizomes of several *Polygonatum* species have been studied, and the isolation and identification of steroidal saponins have also been reported by several groups.[2—6)] Further phytochemical analysis of the rhizomes of *Polygonatum kingianum* with attention to the water-soluble steroidal glycoside constituents led to the isolation of five pairs of stereoisomeric steroidal furostanol saponins, seven new saponins named (25*S*)-kingianoside D (**2**), (25*S*)-kingianoside C (**4**), (25*R*,22)-hydroxylwattinoside C (**5**), kingianoside E (**7**), (25*S*)-kingianoside E (**8**), kingianoside F (**9**), (25*S*)-kingianoside F (**10**), and together with three known saponins, kingianoside D (**1**), C (**3**), and 22-hydroxylwattinoside C (**6**). This paper describes the structural determination of the seven new saponins by detailed analysis of their 1D and 2D NMR spectra, and by comparison of spectral data of some known compounds.

Results and Discussion

The crude saponin fraction of *Polygonatum kingianum* was fractionated by a combination of macroporous resin and silica gel RP-C18 column chromatography and preparative HPLC to afford compounds **1**—**10**. Compounds **1**, **3**, and **6** were identified as kingianoside D, kingianoside C,[4)] and 22-hydroxylwattinoside C,[7)] respectively, based on their NMR spectral data and by comparison of their physical properties with those reported in literature.

Compound **2** was obtained as a white amorphous powder, which gave positive Liebermann–Burchard and Ehrlish reagent tests. It suggested **2** to be a furostanol saponin. Its molecular formula was assigned as $C_{45}H_{72}O_{19}$ on the basis of the ^{13}C-NMR data (Tables 1, 2), high-resolution FAB-MS (*m/z* 939.4609 $[M(C_{45}H_{72}O_{19})+Na]^+$) and FAB-MS (*m/z* 899 $[M+H-H_2O]^+$). Furthermore, the prominent fragments at *m/z* 737 $[M+H-H_2O-162]^+$, 591 $[M+H-H_2O-162-146]^+$, and 429 $[M+H-H_2O-162-146-162]^+$ attribute to the sequential loss of a methylpentose and two hexose residues. **2** was hydrolyzed with acid to afford D-fucose and D-glucose. The ^{1}H-NMR spectrum of **2** showed two singlet methyl signals at δ 0.94 and 1.14, and two doublet methyl signals at δ 1.02 (J=6.6 Hz) and 1.53 (J=7.2 Hz), which were recognized as typical steroid methyls. Moreover, signals for three anomeric protons at δ 4.78 (1H, d, J=7.8 Hz), 4.81 (1H, d, J=7.8 Hz), and 5.21 (1H, d, J=7.8 Hz), and an olefinic proton at δ 5.31 (H, br s, H-6), could be readily assigned. The ^{13}C-NMR spectrum of **2** showed three anomeric carbons at δ 102.7, 105.0 and 107.0. The ^{1}H–^{1}H COSY (^{1}H–^{1}H shift correlation spectroscopy), HSQC (heteronuclear single quantum correlation) and HMBC (heteronuclear multiple bond correlation) spectra enabled the glucose residue at C-26 and the disaccharide moiety at C-3 to be assigned. In regard to the glycosidic moiety, in the HMBC spectrum, the anomeric proton signals of fucose (δ 4.78, H-1), glucose (δ 5.21, H-1′) and glucose (δ 4.81, 26-O-Glc H-1‴) showed correlations with C-3 of the aglycone (δ 77.6), C-4 of fucose (δ 83.3) and C-26 of the aglycone (δ 75.2), respectively. A comparison of the data for **2** with those of **1** indicated that both compounds possessed similar aglycone and sugar chains.

In ^{1}H–^{1}H COSY and HSQC spectra (Table 3) of **2**, the protone signals of C-26 were observed at δ 4.08 (1H, 26-Ha) and δ 3.49 (1H, 26-Hb) in **2**, instead of the proton singals at δ 3.95 (1H, 26-Ha) and δ 3.61 (1H, 26-Hb) in **1**, and the difference (Δab) of the proton signals at C-26 of **2** was 0.59 and that of **1** was 0.34. Then the configuration of the methyl group at C-25 of **2** is *S* and that of **1** is *R*.[8,9)] Thus, these data led us to assign the structure of **2** as (25*S*)-[(3-*O*-β-D-glucopyranosyl-(1→4)-β-D-fucopyranosyl)oxy]-26-[(β-D-glucopyranosyl)oxy]-22ξ-hydroxyfurost-5-en-12-one, which is a new furostanol saponin, and named (25*S*)-kingianoside D.

Compound **4** was obtained as a white amorphous powder, which gave positive Liebermann–Burchard and Ehrlish reagent tests. It suggested **4** to be a furostanol saponin. **4** was hydrolyzed with acid to afford D-glucose and D-galactose. Its molecular formula was assigned as $C_{45}H_{72}O_{20}$ on the basis of the ^{13}C-NMR data (Tables 1, 2), high-resolution FAB-MS (*m/z* 955.4542 $[M(C_{45}H_{74}O_{20})+Na]^+$) and FAB-MS (*m/z* 915 $[M+H-H_2O]^+$), which was the same as that of **3**. Meanwhile, the ^{1}H- and ^{13}C-NMR (Tables 1—3) spectra of **4** were shown superimposable with those of **3**, except for the proton signals of C-26 at δ 4.08 (1H, 26-Ha) and δ 3.49 (1H, 26-Hb) in **4**, instead of the proton singals at δ 3.94 (1H, 26-Ha)

* To whom correspondence should be addressed. e-mail: ma_bp@sohu.com

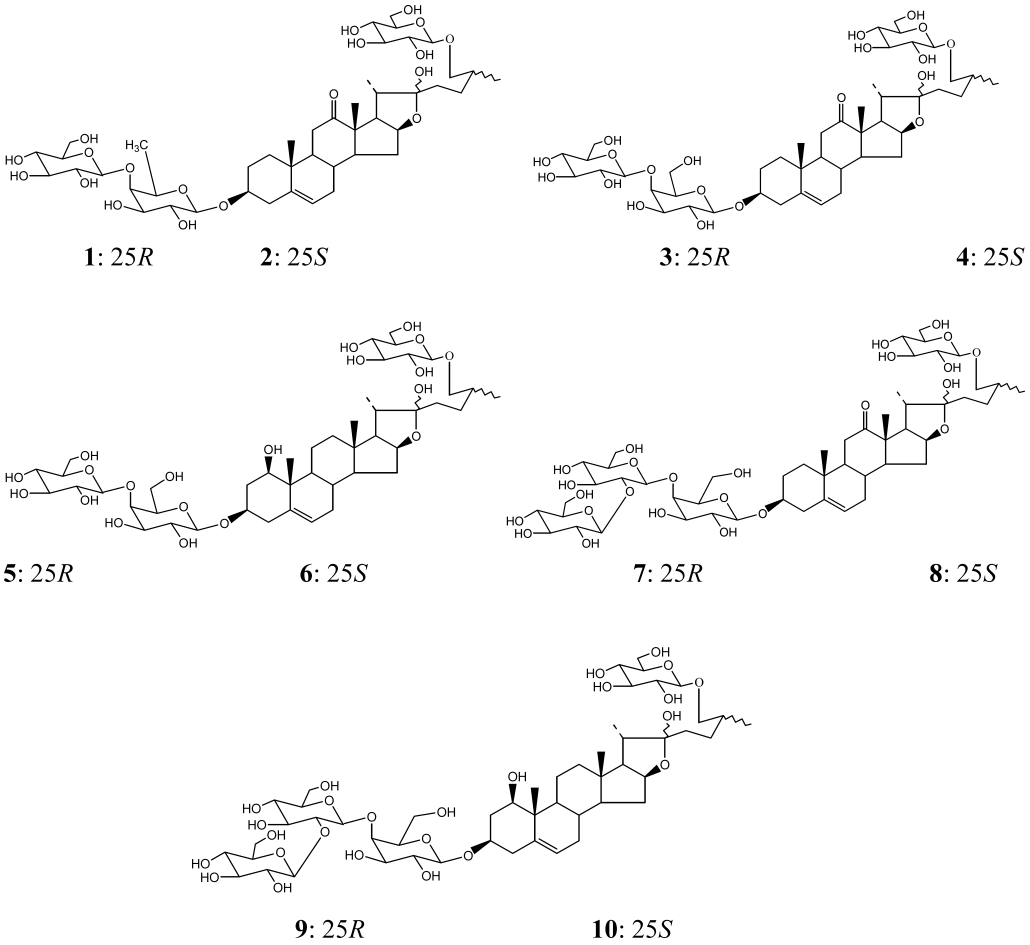

Fig. 1. Chemical Structures of Compounds **1**—**10**

and δ 3.61 (1H, 26-Hb) in **3**, which showed that the configuration of the methyl group at C-25 of **4** is *S* and that of **3** is *R*.[8,9] The 3,26-bisdesmoside structure of **4** was identified by an HMBC experiment, in which long-range correlations were observed between the H-1′ and the C-4 of galactose, between the H-1 of galactose and C-3, and between the H-1‴ and C-26. Finally, by comparison of the NMR data of **4** with those of **3**, the structure of **4** was determined to be (25*S*)-[(3-*O*-β-D-glucopyranosyl-(1→)-β-D-galactopyranosyl)oxy]-26-[(β-D-glucopyranosyl)oxy]-22ξ-hydroxyfurost-5-en-12-one, which is a new furostanol saponin, and named (25*S*)-kingianoside C.

Compound **5** was obtained as a white amorphous powder, which gave positive Liebermann–Burchard and Ehrlish reagent tests. It suggested **5** to be a furostanol saponin. **5** was hydrolyzed with acid to afford D-glucose and D-galactose. Its molecular formula was assigned as $C_{45}H_{74}O_{20}$ on the basis of the ^{13}C-NMR data (Table 1), high-resolution FAB-MS (*m/z* 957.4686 $[M(C_{45}H_{72}O_{20})+Na]^+$) and FAB-MS (*m/z* 917 $[M+H-H_2O]^+$), which was the same as those of **6**. Meanwhile, the ^{1}H- and ^{13}C-NMR (Tables 1—3) spectra of **5** were shown superimposable with those of **6**, except for the proton signals of C-26 at δ 3.61 (1H, 26-Ha) and δ 3.94 (1H, 26-Hb) in **5**, instead of the proton signals at δ 4.07 (1H, 26-Ha) and δ 3.47 (1H, 26-Hb) in **6**, which showed that the configuration of the methyl group at C-25 of **5** is *R* and that of **6** is *S*.[9] The 3,26-bisdesmoside structure of **5** was identified by an HMBC experiment, in which long-range correlations were observed between the H-1′ and the C-4 of galactose, between the H-1 of galactose and C-3, and between the H-1‴ and C-26. Finally, by comparison of the NMR data of **5** with those of **6**, the structure of **5** was determined to be (25*R*)-[(3-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-26-[(β-D-glucopyranosyl)oxy]-1β,3β,22ξ,26-tetrahydroxyfurost-5-ene (**5**), which is a new furostanol saponin, and named (25*R*,22)-hydroxylwattinoside C.

Compounds **7** and **8**, which were each isolated as a white amorphous power, were positive to Liebermann–Burchard and Ehrlish reagent tests. It suggested **7** and **8** to be furostanol saponins. **7** and **8** were found to have the same molecular formula $C_{51}H_{82}O_{25}$, which were determined from their HR-FAB-MS, FAB-MS and ^{13}C-NMR data (Tables 1, 2). The high-resolution FAB-MS of **7** and **8** gave an $[M+Na]^+$ ion at *m/z* 1117.5034 and 1117.5056, respectively. The positive-ion FAB-MS of **7** and **8** showed the feature peak at *m/z* 1077 $[M+H-H_2O]^+$. **7** and **8** were hydrolyzed with acid to afford D-glucose and D-galactose, respectively. In their ^{13}C-NMR spectra, the proton and carbon signals due to the sapogenol moiety of **7** and **8** were superimposable on those of **3** and **4** having a 3β,22,26-trihydroxyfurost-5-en-12-one 3,26-glycosidic structure. Comparing the ^{13}C-NMR data of the sugar moieties at C-3 of **7** and **8** with those of hecogenin 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside,[10] it was indicated that **7** and **8** had

Table 1. ^{13}C-NMR Data for the Aglycone Moiety of Compounds **1**—**10** (Pyridine-d_5, 125 MHz)

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	37.0	37.0	37.0	37.0	77.8	77.8	37.0	37.0	77.8	77.8
2	30.0	30.0	30.0	30.0	41.1	41.1	30.0	30.0	41.1	41.1
3	77.6	77.6	77.8	77.7	75.3	75.3	77.9	77.9	75.3	75.3
4	39.1	39.1	39.1	39.1	39.7	39.8	39.1	39.1	39.8	39.8
5	140.9	140.9	140.8	140.7	139.2	139.2	140.8	140.8	139.3	139.3
6	121.4	121.4	121.5	121.5	125.1	125.1	121.5	121.4	125.1	125.1
7	31.8	31.8	31.8	31.8	32.3	32.3	31.8	31.8	32.3	32.3
8	30.9	30.9	30.9	30.9	32.9	32.9	30.9	30.9	32.9	32.9
9	52.4	52.4	52.3	52.3	51.3	51.3	52.3	52.3	51.3	51.3
10	37.6	37.6	37.6	37.5	43.6	43.7	37.6	37.6	43.6	43.6
11	37.6	37.6	37.6	37.5	24.2	24.2	37.6	37.6	24.2	24.2
12	212.8	212.8	212.8	212.8	40.5	40.5	212.8	212.8	40.6	40.5
13	55.4	55.3	55.4	55.3	40.6	40.6	55.3	55.3	40.7	40.6
14	55.9	55.9	55.9	55.9	56.8	56.8	55.9	55.9	56.8	56.8
15	31.8	31.8	31.8	31.8	32.7	32.7	31.8	31.8	32.7	32.7
16	79.7	79.7	79.7	79.7	81.1	81.1	79.7	79.7	81.1	81.1
17	54.8	54.8	54.8	54.8	64.1	64.1	54.8	54.8	64.1	64.1
18	16.0	16.0	16.1	16.0	16.7	16.7	16.1	16.0	16.7	16.7
19	18.8	18.8	18.8	18.8	13.7	13.7	18.8	18.8	13.7	13.7
20	41.3	41.3	41.3	41.3	40.7	40.8	41.3	41.3	40.7	40.8
21	15.2	15.2	15.2	15.2	16.5	16.5	15.2	15.2	16.5	16.5
22	110.8	110.8	110.8	110.8	110.7	110.7	110.8	110.8	110.7	110.7
23	37.1	37.1	37.1	37.1	37.3	37.2	37.1	37.1	37.3	37.2
24	28.4	28.3	28.4	28.3	28.4	28.4	28.4	28.3	28.3	28.2
25	34.3	34.4	34.3	34.4	34.3	34.5	34.3	34.4	34.3	34.5
26	75.2	75.2	75.2	75.2	75.2	75.4	75.2	75.2	75.4	75.4
27	17.4	17.5	17.4	17.5	17.5	17.5	17.4	17.5	17.5	17.5

Table 2. ^{13}C-NMR Spectral Data for the Sugar Moieties of Compounds **1**—**10** (Pyridine-d_5, 125 MHz)

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Gla-1			102.9	102.9	103.1	103.1	102.7	102.7	102.8	102.8
2			73.5	73.5	73.4	73.4	73.3	73.3	73.3	73.3
3			75.5	75.4	75.4	75.4	75.6	75.6	75.6	75.6
4			80.0	80.1	79.9	79.9	81.0	81.0	81.0	81.0
5			75.2	75.3	75.3	75.3	76.8	76.8	76.8	76.7
6			61.0	61.0	60.9	60.9	60.4	60.4	60.4	60.4
Fuc-1	102.7	102.7								
2	73.0	73.0								
3	76.3	76.3								
4	83.3	83.3								
5	70.6	70.6								
6	17.7	17.7								
Glc′-1	107.0	107.0	107.1	107.2	107.1	107.1	105.2	105.2	105.2	105.2
2	75.6	75.6	76.0	76.0	76.0	75.9	86.2	86.1	86.1	86.1
3	78.6	78.6	78.7	78.7	78.7	78.7	78.7	78.7	78.5	78.5
4	71.8	71.8	72.3	72.3	72.3	72.3	71.9	71.9	71.7	71.7
5	78.5	78.5	78.5	78.5	78.5	78.5	78.2	78.2	78.2	78.2
6	62.9	62.9	63.2	63.2	63.2	63.2	63.2	63.2	63.2	63.2
Glc″-1							107.0	107.0	107.0	106.9
2							75.3	75.3	75.1	75.1
3							77.7	77.7	77.7	77.7
4							70.4	70.4	70.3	70.3
5							79.0	79.0	78.9	78.9
6							61.7	61.7	61.6	61.6
Glc‴-1	105.0	104.9	105.0	105.0	105.2	105.0	105.0	105.2	105.2	105.2
2	75.3	75.2	75.3	75.3	75.0	75.0	75.0	75.0	75.0	75.0
3	78.7	78.7	78.8	78.8	78.5	78.5	78.7	78.7	78.6	78.6
4	71.8	71.7	71.8	71.7	71.7	71.7	71.8	71.8	71.8	71.8
5	78.5	78.5	78.7	78.7	78.6	78.6	78.5	78.5	78.5	78.5
6	62.9	62.8	62.9	62.8	62.9	62.9	62.9	62.9	62.8	62.8

Table 3. Partial ^{1}H-NMR Data for Compounds **1**—**10** (Pyridine-d_5, 600 MHz)

Position	**1** (25*R*)	**2** (25*S*)	**3** (25*R*)	**4** (25*S*)	**5** (25*R*)	**6** (25*S*)	**7** (25*R*)	**8** (25*S*)	**9** (25*R*)	**10** (25*S*)
18	1.14 s	1.14 s	1.14 s	1.14 s	0.95 s	0.95 s	1.14 s	1.13 s	0.95 s	0.95 s
19	0.92 s	0.94 s	0.91 s	0.92 s	1.20 s	1.20 s	0.92 s	0.90 s	1.20 s	1.21 s
20	2.20 m	2.20 m	2.19 m	2.20 m	2.21 m	2.20 m	2.21 m	2.19 m	2.20 m	2.21 m
21	1.53 d 6.6	1.53 d 7.2	1.54 d 6.6	1.52 d 6.6	1.29 d 7.2	1.27 d 7.2	1.53 d 7.2	1.51 d 6.6	1.28 d 6.6	1.27 d 6.6
23	1.49 m	1.96 m	1.49 m	1.95 m	1.49 m	1.96 m	1.49 m	1.95 m	1.48 m	1.96 m
	2.02 m	2.05 m	2.02 m	2.03 m	2.02 m	2.05 m	2.02 m	2.05 m	2.02 m	2.05 m
24	1.66 m	1.67 m	1.66 m	1.67 m	1.67 m	1.65 m	1.66 m	1.67 m	1.67 m	1.67 m
	2.03 m	2.03 m	2.03 m	2.03 m	2.03 m	2.03 m	2.03 m	2.05 m	2.03 m	2.04 m
25	1.92 m	1.91 m	1.92 m	1.92 m	1.92 m	1.91 m	1.92 m	1.93 m	1.92 m	1.92 m
26	3.61 dd	3.49 dd	3.61 dd	3.49 dd	3.61 dd	3.47 dd	3.60 dd	3.48 dd	3.61 dd	3.49 dd
	6.0, 9.0	7.2, 9.0	6.0, 9.6	6.6, 9.0	6.0, 9.0	6.6, 9.0	6.0, 9.0	6.6, 9.0	6.0, 9.0	7.2, 9.0
	3.95 m	4.08 m	3.94 m	4.08 m	3.94 m	4.07 m	3.96 m	4.08 m	3.95 m	4.08 m
27	0.97 d 6.6	1.02 d 6.6	0.98 d 6.6	1.02 d 6.6	0.97 d 6.6	1.02 d 6.6	0.97 d 6.6	1.01 d 6.6	0.97 d 6.6	1.01 d 6.6
Gal-1			4.86 d 7.8	4.86 d 7.8	4.91 d 7.8	4.94 d 7.8	4.87 d 7.8	4.87 d 7.8	4.92 d 7.8	4.93 d 7.8
2			4.38 m	4.38 m	4.38 m	4.38 m	4.47 m	4.47 m	4.49 m	4.49 m
3			4.22 m	4.22 m	4.22 m	4.22 m	4.08 m	4.08 m	4.08 m	4.08 m
4			4.69 m	4.71 m	4.70 m	4.69 m	4.57 m	4.56 m	4.56 m	4.56 m
5			4.02 m	4.03 m	4.02 m	4.02 m	4.05 m	4.05 m	4.05 m	4.05 m
6			4.22 m	4.22 m	4.14 m	4.14 m	4.18 m	4.18 m	4.12 m	4.12 m
			4.64 m	4.65 m	4.63 m	4.63 m	4.74 m	4.73 m	4.71 m	4.71 m
Fuc-1	4.78 d 7.8	4.78 d 7.8								
2	4.34 m	4.33 m								
3	4.12 m	4.16 m								
4	4.14 m	4.16 m								
5	3.79 m	3.79 m								
6	1.60 d 6.0	1.61 d 6.6								
Glc′-1	5.20 d 7.8	5.21 d 7.8	5.28 d 7.8	5.28 d 7.8	5.27 d 7.8	5.27 d 7.8	5.13 d 7.8	5.13 d 7.8	5.13 d 7.8	5.12 d 7.8
2	4.06 m	4.08 m	4.13 m	4.12 m	4.11 m	4.10 m	4.12 m	4.14 m	4.11 m	4.10 m
3	4.22 m	4.22 m	4.22 m	4.22 m	4.22 m	4.22 m	4.25 m	4.26 m	4.26 m	4.25 m
4	4.22 m	4.22 m	4.05 m	4.04 m	4.05 m	4.22 m	3.95 m	3.94 m	3.94 m	3.94 m
5	3.95 m	3.93 m	4.02 m	4.02 m	4.01 m	4.01 m	3.96 m	3.96 m	3.96 m	3.96 m
6	4.36 m	4.38 m	4.19 m	4.21 m	4.16 m	4.16 m	4.08 m	4.10 m	4.10 m	4.10 m
	4.53 m	4.53 m	4.59 m	4.60 m	4.58 m	4.59 m	4.61 m	4.61 m	4.61 m	4.63 m
Glc″-1							5.21 d 7.8	5.22 d 7.8	5.20 d 7.8	5.19 d 7.8
2							4.06 m	4.05 m	4.02 m	4.02 m
3							4.12 m	4.12 m	4.12 m	4.12 m
4							4.21 m	4.21 m	4.20 m	4.20 m
5							3.82 m	3.80 m	3.76 m	3.76 m
6							4.36 m	4.36 m	4.33 m	4.32 m
							4.58 m	4.58 m	4.54 m	4.54 m
Glc‴-1	4.80 d 7.8	4.81 d 7.8	4.81 d 7.8	4.81 d 7.2	4.80 d 7.8	4.80 d 7.8	4.80 d 7.2	4.80 d 7.8	4.80 d 7.8	4.80 d 7.2
2	4.01 m	4.02 m	4.02 m	4.02 m	4.02 m	4.01 m	4.02 m	4.01 m	4.02 m	4.02 m
3	4.23 m	4.23 m	4.22 m	4.22 m	4.22 m	4.21 m	4.22 m	4.21 m	4.22 m	4.22 m
4	4.21 m	4.23 m	4.22 m	4.22 m	4.22 m	4.21 m	4.21 m	4.21 m	4.22 m	4.22 m
5	3.94 m	3.93 m	3.94 m	3.94 m	3.93 m	3.93 m	3.95 m	3.93 m	3.94 m	3.94 m
6	4.36 m	4.36 m	4.38 m	4.38 m	4.37 m	4.38 m	4.36 m	4.36 m	4.38 m	4.38 m
	4.52 m	4.53 m	4.54 d 11.4	4.55 m	4.54 m	4.53 m	4.55 m	4.56 m	4.53 m	4.54 m

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

the same sequence of sugar linkage at C-3 as the reference compound, and HMBC experiments on **7** and **8** showed long-range correlations between the following protons and carbons: H-1″ and C-2′, H-1′ and C-4 of galactose; H-1 of galactose and C-3; H-1‴ and C-26. The protons signals assignable to the 26-methylene group (26-Ha, δ 3.96; 26-Hb, δ 3.60) in the ^{1}H-NMR spectrum of **7** were very similar to those of **3**, while the protons signals assignable to the 26-methylene group (26-Ha, δ 4.08, 26-Hb, δ 3.48) in the ^{1}H-NMR spectrum of **8** were very similar to those of **4**. On the basis of the above evidence, the structures of **7** and **8** were formulated as (25*R*)-[(3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-26-[(β-D-glucopyranosyl)oxy]-22ξ-hydroxyfurost-5-en-12-one (**7**), named kingianoside E, and its 25*S*-isomer (**8**), named (25*S*)-kingianoside E.

Compounds **9** and **10**, which were each isolated as a white amorphous power, were positive to Liebermann–Burchard and Ehrlish reagent tests. It suggested **9** and **10** to be furostanol saponins. **9** and **10** were found to have the same molecular formula $C_{51}H_{84}O_{25}$, which were determined from their HR-FAB-MS, FAB-MS and ^{13}C-NMR data (Tables 1, 2). The high-resolution FAB-MS of **9** and **10** gave an $[M+Na]^+$ ion at *m/z* 1119.5201 and 1119.5219, respectively. The positive-ion FAB-MS of **9** and **10** showed the feature peak at *m/z* 1079 $[M+H-H_2O]^+$. **9** and **10** were hydrolyzed with acid to afford D-glucose and D-galactose, respectively. In the ^{1}H- and ^{13}C-NMR spectra, the proton and carbon signals due to the sapogenol moieties of **9** and **10** were superimposable with those of **5** and **6** having a furost-5-en-1β,3β,22,26-tetrol 3,26-glycosidic structure. Comparing the ^{13}C-NMR data of the sugar moieties at C-3 of **9** and **10** with those of **7**

and **8**, it was indicated that **9** and **10** had the same sugar moieties at C-3 as **7** and **8**. The protons signals assignable to the 26-methylene group (26-Ha, δ 3.95; 26-Hb, δ 3.61) in the ^{1}H-NMR spectrum of **9** were very similar to those of **7**, while the protons signals assignable to the 26-methylene group (26-Ha, δ 4.08, 26-Hb, δ 3.49) in the ^{1}H-NMR spectrum of **10** were very similar to those of **8**. On the basis of the above evidence, the structures of **9** and **10** were formulated as (25*R*)-[(3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-26-[(β-D-glucopyranosyl)oxy]-1β,3β,22ξ,26-tetrahydroxyfurost-5-ene (**9**), named kingianoside F, and its 25*S*-isomer (**10**), named (25*S*)-kingianoside F.

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, ODS, 10 μm, U.S.A.) and YMC-Pack ODS-A C_{18} (YMC, 4.6 mm i.d.×250, ODS, 5 μm, Japan). HR-FAB-MS: VG ZAB-2f, 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 resins SP825 (Mitsubishi Chemical, Japan), AB-8 (Nan Kai Chemical Co., Ltd., China) and ODS-A silica-gel (120 Å, 50 μm, YMC) were used for chromatography.

Plant Material The material was collected from Fengang 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 (2460 g) was concentrated under reduced pressure. Column chromatography of the extract was performed on macroporous resin AB-8 and eluted with a gradient mixture of Me_2CO–H_2O (1 : 9, 1 : 1, 8 : 2), to give three fractions (Fr. A—C). Fraction B (16.8 g) was chromatographed on macroporous resin SP825 and eluted with a gradient mixture of Me_2CO–H_2O (2 : 8, 3 : 7, 2 : 3, 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_2 (8.5 g) was chromatographed on ODS silica-gel (50 μm) with Me_2CO–H_2O (15 : 85, 18 : 82, 20 : 80), to yield compounds **5** (19.4 mg) and **7** (64.1 mg), and other three fractions, C_1 (2.2 g), C_2 (3.0 g) and C_3 (1.2 g). Fraction C_3 was chromatographed by preparative HPLC with Me_2CO–H_2O (25 : 75), to yield compound **1** (30.0 mg), **2** (24.0 mg). Fraction C_2 was chromatographed by preparative HPLC with Me_2CO–H_2O (23 : 77), to yield compound **3** (137.6 mg), **4** (61.0 mg) and **6** (74.0 mg). Fraction C_1 was chromatographed by preparative HPLC with Me_2CO–H_2O (21 : 79), to yield compounds **8** (78.4 mg), **9** (20.0 mg) and **10** (28.0 mg).

Compound **2**: White amorphous powder, $[\alpha]_D^{20}$ −18.5° (c=0.065, pyridine); ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-FAB-MS (positive): *m/z* 939.4609 $[M(C_{45}H_{72}O_{19})+Na]^+$. Calcd 939.4566. FAB-MS *m/z*: 899 $[M+H-H_2O]^+$, 737 $[M+H-H_2O-162]^+$, 591 $[M+H-H_2O-162-146]^+$, 429 $[M+H-H_2O-162-146-162]^+$.

Compound **4**: White amorphous powder, $[\alpha]_D^{20}$ −42.3° (c=0.265, pyridine); ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-FAB-MS (positive): *m/z* 955.4542 $[M(C_{45}H_{74}O_{20})+Na]^+$. Calcd 955.4515. FAB-MS *m/z*: 915 $[M+H-H_2O]^+$, 753 $[M+H-H_2O-162]^+$, 591 $[M+H-H_2O-162-162]^+$, 429 $[M+H-H_2O-162-162-162]^+$.

Compound **5**: White amorphous powder, $[\alpha]_D^{20}$ −33° (c=0.400, pyridine); ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-FAB-MS (positive): *m/z* 957.4686 $[M(C_{45}H_{72}O_{20})+Na]^+$. Calcd 957.4671. FAB-MS *m/z*: 917 $[M+H-H_2O]^+$, 755 $[M+H-H_2O-162]^+$, 593 $[M+H-H_2O-162-162]^+$, 431 $[M+H-H_2O-162-162-162]^+$.

Compound **7**: White amorphous powder, $[\alpha]_D^{20}$ −28.9° (c=0.405, pyridine); ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-FAB-MS (positive): *m/z* 1117.5034 $[M(C_{51}H_{82}O_{25})+Na]^+$. Calcd 1117.5043. FAB-MS *m/z*: 1077 $[M+H-H_2O]^+$, 915 $[M+H-H_2O-162]^+$, 753 $[M+H-H_2O-162-162]^+$, 591 $[M+H-H_2O-162-162-162]^+$, 429 $[M+H-H_2O-162-162-162-162]^+$.

Compound **8**: White amorphous powder, $[\alpha]_D^{20}$ −30.5° (c=0.400, pyridine); ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-FAB-MS (positive): *m/z* 1117.5056 $[M(C_{51}H_{82}O_{25})+Na]^+$. Calcd 1117.5043. FAB-MS *m/z*: 1077 $[M+H-H_2O]^+$, 915 $[M+H-H_2O-162]^+$, 753 $[M+H-H_2O-162-162]^+$, 591 $[M+H-H_2O-162-162-162]^+$, 429 $[M+H-H_2O-162-162-162-162]^+$.

Compound **9**: White amorphous powder, $[\alpha]_D^{20}$ −36.7° (c=0.302, pyridine); ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-FAB-MS (positive): *m/z* 1119.5201 $[M(C_{51}H_{84}O_{25})+Na]^+$. Calcd 1119.5199. FAB-MS *m/z*: 1079 $[M+H-H_2O]^+$, 917 $[M+H-H_2O-162]^+$, 755 $[M+H-H_2O-162-162]^+$, 593 $[M+H-H_2O-162-162-162]^+$, 431 $[M+H-H_2O-162-162-162-162]^+$.

Compound **10**: White amorphous powder, $[\alpha]_D^{20}$ −43.0° (c=0.330, pyridine); ^{1}H- and ^{13}C-NMR: see Tables 1 and 2. HR-FAB-MS (positive): *m/z* 1119.5219 $[M(C_{51}H_{84}O_{25})+Na]^+$. Calcd 1119.5199. FAB-MS *m/z*: 1079 $[M+H-H_2O]^+$, 917 $[M+H-H_2O-162]^+$, 755 $[M+H-H_2O-162-162]^+$, 593 $[M+H-H_2O-162-162-162]^+$, 431 $[M+H-H_2O-162-162-162-162]^+$.

Acid Hydrolysis of Compounds 2, 4, 5, and 7—10 Compound **2** (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 1 M NaOH and filtered. The filtrate was extracted with $CHCl_3$ and H_2O. Then, the presence of D-glucose and D-fucose in the H_2O-souble fraction were identified by converting them into 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives,[11] which were analyzed by HPLC under the following conditions: Column: YMC-Pack ODS-A C_{18}, solvent: MeCN–H_2O (2 : 3), flow rate: 0.8 ml min^{-1}, detection: UV 230 mn. The derivatives of D-fucose and D-glucose were detected, t_R: 19.17 min (D-fucose derivative), 24.07 min (D-glucose derivative).

By the same procedures carried out for **4**, **5** and **7**—**10** (each about 2.0 mg). The derivatives of D-galactose and D-glucose were detected; t_R: 20.83 min (D-galactose derivative), 24.07 min (D-glucose derivative).

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References and Notes

1) Jiangsu New Medicinal College, "The Dictionary of Chinese Herbal Medicines," Shanghai People's Publishing Press, Shanghai, 1977, pp. 2041—2044.
2) Jin J. M., Zhang Y. J., Li H. Z., Yang C. R., *J. Nat. Prod.*, **67**, 1992—1995 (2004).
3) Li X. C., Yang C. R., Matsuura H., Kasai R., Yamasaki K., *Phytochemistry*, **33**, 465—470 (1993).
4) Li X. C., Yang C. R., Ichikawa M., Matsuura H., Kasai R., Yamasaki K., *Phytochemistry*, **31**, 3559—3563 (1992).
5) Yesilada E., Houghton P. J., *Phytochemistry*, **30**, 3405—3409 (1991).
6) Son K. H., Do J. C., Kang S. S., *J. Nat. Prod.*, **53**, 333—339 (1990).
7) Yang F., Shen P., Wang Y. F., Zhang R. Y., Yang C. R. *Acta Botanica Yunanica*, **23**, 373—380 (2001).
8) Agrawal P. K., *Magn. Reson. Chem.*, **42**, 990—993 (2004).
9) Agrawal P. K., *Steroids*, **70**, 715—724 (2005).
10) Xu Y. X., Chen H. S., Liang H. Q., Gu Z. B., Liu W. Y., Leung W. N., Li T. J., *J. Planta. Med.*, **66**, 545—550 (2000).
11) Mimaki Y., Kuroda M., Takaashi Y., Sashida Y., *Phytochemistry*, **47**, 1351—1356 (1998).