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STEROID SAPONINS FROM *POLYGONATUM KINGIANUM*

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Key Word Index—*Polygonatum kingianum*; Liliaceae; rhizome; steroid saponins; gentrogenin; kingianosides A, B, C and D.

Abstract—Four new steroid saponins, kingianosides A–D, were isolated from the rhizome of *Polygonatum kingianum*, together with two known steroid saponins. On the basis of chemical and spectral evidence, the structures of kingianosides A–D were established as gentrogenin 3-*O*-*β*-D-glucopyranosyl(1→4)-*β*-D-galactopyranoside, gentrogenin 3-*O*-*β*-D-glucopyranosyl(1→4)-*β*-D-fucopyranoside, 26-*O*-*β*-D-glucopyranosyl-22-hydroxy-25(*R*)-furost-5-en-12-on-3*β*,22-diol 3-*O*-*β*-D-glucopyranosyl(1→4)-*β*-D-galactopyranoside and 26-*O*-*β*-D-glucopyranosyl-22-hydroxy-25(*R*)-furost-5-en-12-on-3*β*,22-diol 3-*O*-*β*-D-glucopyranosyl(1→4)-*β*-D-fucopyranoside, respectively.

INTRODUCTION

The rhizome of *Polygonatum kingianum* Coll. et Hemsl. (Liliaceae), one of the original plants known as 'Huang-jing' in traditional Chinese medicine, is used as a tonic and a remedy to lung troubles and ringworm [1]. So far various steroid saponins have been isolated from several *Polygonatum* species [2–10], although no chemical studies have been performed on *P. kingianum*. As part of our studies on steroid saponins existing in Liliflorae plants, we have undertaken the chemical investigation of this plant, which has led to the isolation and structure elucidation of four new steroid saponins, kingianosides A–D (**1**–**4**), together with funkioside C (**5**) [11] and the 25(*R*)-epimer of Po-8 (**6**) from *P. officinalis* [8]. The results are presented in this paper.

RESULTS AND DISCUSSION

The crude saponin fraction of *P. kingianum* was chromatographed by a combination of silica gel and high porous polymer MCI gel CHP 20P, to afford saponins **1**–**6**.

Saponin **1**, which has a molecular formula of $C_{39}H_{60}O_{14}$ determined from the high resolution negative FAB mass spectrum, was hydrolysed with 5% sulphuric acid–ethanol (1:1) to yield an aglycone and a prosapogenin. This aglycone was identified as gentrogenin (**7**) by comparison of ^{13}C NMR [12], mp [13] and $[\alpha]_D$ [13] data with those of an authentic sample. Prosapogenin was readily assigned as gentrogenin 3-*O*-*β*-D-galactopyranoside (**8**) based on the analyses of FAB mass, ^1H and ^{13}C NMR spectra. It was observed that the glycosylation at the hydroxy group of C-3 of the aglycone resulted in a downfield shift (6.3 ppm) of the *α*-carbon (C-3) and upfield shifts of the *β*-carbons (1.3 ppm and 2.8 ppm for C-2 and C-4, respectively).

Complete acid hydrolysis of saponin **1** afforded **7** as its genuine sapogenin and galactose and glucose in a ratio of 1:1 as sugar components. In the ^1H NMR spectrum of **1**, two anomeric proton signals were observed at δ4.87 (1H, *d*, $J=7.3$ Hz) and 5.28 (1H, *d*, $J=8.1$ Hz), diagnostic of the *β*-configuration of two sugars. Comparison of the ^{13}C NMR spectrum with that of **8** indicated that the terminal glucose was attached to C-4 of the inner galactose. This was further supported by the results of partially methylated alditol acetate analysis [14, 15], which revealed the presence of 4-linked hexose (1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylhexitol) and terminal hexose (1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol). Therefore, the structure of **1** was elucidated to be gentrogenin 3-*O*-*β*-D-glucopyranosyl (1→4)-*β*-D-galactopyranoside, and was named kingianoside A.

Saponin **2** showed a quasi-molecular ion peak at *m/z* 735.4000 [M $(C_{39}H_{60}O_{13})$-H]$^-$ in the high resolution negative FAB mass spectrum. Upon acid hydrolysis, it yielded **7** as aglycone and fucose and glucose in a ratio of 1:1 as sugar components. As L-fucose was also reported, although rare, occurring in natural glycosides [16], we had to determine the absolute configuration of the fucose. According to Oshima's method, which converts sugars to the corresponding TMS ether of *α*-L-methylbenzylaminoalditols [17], this fucose was determined to be the common D-form. Sugar sequence analysis (partially methylated alditol acetate by GC-mass spectrometry) [14, 15] revealed the presence of 4-linked fucose (1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-6-deoxyhexitol) and terminal glucose (1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol). The ^1H NMR spectrum of **2** exhibited two anomeric

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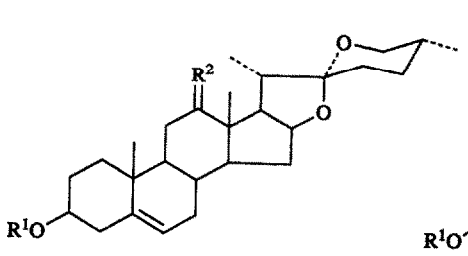
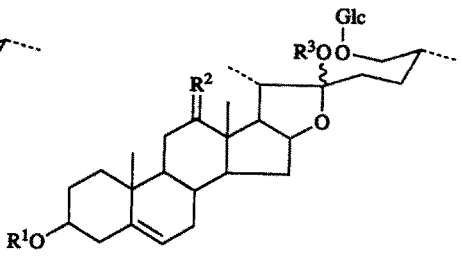


	R^1	R^2
1	-Gal (4←1) Glc	O
2	-Fuc (4←1) Glc	O
5	-Gal (4←1) Glc	H_2
7	-H	O
8	-Gal	O



	R^1	R^2	R^3
3	-Gal (4←1) Glc	O	H
3a	-Gal (4←1) Glc	O	Me
4	-Fuc (4←1) Glc	O	H
4a	-Fuc (4←1) Glc	O	Me
6	-Gal (4←1) Glc	H_2	H
6a	-Gal (4←1) Glc	H_2	Me

Table 1. ^{13}C NMR chemical shifts of aglycone moieties in pyridine-d_5 (ppm)

C	7*	1	8	2	3	3a	4	4a	5	6	6a
1	36.8	37.0	37.0	37.0	37.0	37.0	37.1	37.1	37.5	37.5	37.5
2	31.3	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.3	30.3	30.3
3	71.4	77.9	77.7	77.7	77.8	77.8	77.7	77.7	78.1	78.1	78.1
4	42.0	39.1	39.2	39.1	39.1	39.1	39.1	39.1	39.3	39.3	39.3
5	140.6	140.8	140.8	140.9	140.8	140.9	140.9	141.0	141.0	141.0	141.0
6	121.2	121.4	121.4	121.4	121.5	121.4	121.5	121.4	121.7	121.7	121.7
7	31.6[a]	31.8[a]	31.8[a]	31.8[a]	31.8	31.8[a]	31.8	31.8[a]	32.3	32.3	32.3
8	30.8	30.9	30.9	30.9	30.9	30.9	30.9	30.9	31.7	31.7	31.6
9	52.2	52.3	52.3	52.3	52.4	52.3	52.4	52.3	50.3	50.4	50.3
10	37.2	37.6	37.6	37.6	37.6	37.6	37.6	37.6	37.1	37.1	37.1
11	37.4	37.6	37.6	37.5	37.6	37.5	37.6	37.5	21.1	21.1	21.1
12	213.4	212.7	212.6	212.6	212.8	212.6	212.8	212.6	39.9	39.9	39.8
13	54.8	55.0	55.0	55.0	55.3	55.4	55.4	55.3	40.5	40.8	40.8
14	56.0	56.0	56.0	56.0	55.9	56.0	56.0	55.9	56.7	56.6	56.6
15	31.5[a]	31.7[a]	31.6[a]	31.6[a]	31.8	31.5[a]	31.8	31.5[a]	32.2	32.5	32.2
16	79.2	79.7	79.7	79.7	79.7	80.0	79.7	79.9	81.1	81.3	81.3
17	53.3	54.1	54.1	54.1	54.8	55.3	54.8	55.3	62.9	63.9	64.2
18	15.9	15.9	15.9	15.9	16.0	15.9	16.0	15.9	16.4	16.4[a]	16.3
19	19.0	18.8	18.8	18.8	18.8	18.8	18.8	18.8	19.4	19.4	19.4
20	42.3	42.7	42.7	42.7	41.3	41.1	41.3	41.1	42.0	40.7	40.5
21	13.2	13.9	13.9	13.9	15.2	14.9	15.2	15.0	15.0	16.5[a]	16.3
22	109.3	109.4	109.4	109.4	110.8	112.8	110.8	112.8	109.3	110.7	112.7
23	31.4[a]	31.8[a]	31.8[a]	31.8[a]	37.1	30.7	37.1	30.7	31.8	37.2	30.8
24	28.8	29.2	29.2	29.2	28.4	28.2	28.4	28.2	29.3	28.4	28.2
25	30.2	30.6	30.6	30.6	34.3	34.3	34.3	34.2	30.6	34.3	34.2
26	66.9	67.0	67.0	67.0	75.2	75.2	75.2	75.2	66.9	75.2	75.2
27	17.1	17.3	17.3	17.3	17.4	17.1	17.4	17.1	17.3	17.5	17.2
OMe						47.4			47.3		

*Measured in $CDCl_3$.

[a]Signals may be interchanged within each column.

proton signals at δ4.81 (1H, *d*, J = 7.7 Hz) and 5.21 (1H, d, J = 7.7 Hz). The ^{13}C NMR spectrum of **2** indicated that it was a 3-*O*-glycoside of **7** [12], for the signal of C-3 was shifted downfield at δ77.7, whereas the signals of C-2 and C-4 were displaced upfield at δ30.0 and 39.1, respectively. Furthermore, a typical signal at δ83.3 arising from the glycosylation at the hydroxyl of C-4 of fucose was confirmed. Based on the above evidence, the structure of **2** was proved to be gentrogenin 3-*O*-β-D-glucopyranosyl(1→4)-β-D-fucopyranoside, and was named kingianoside B.

Saponins **3** and **4**, which were obtained as homogenous states as described in Experimental, gave a red colour with Ehrlich's reagent [18]. Their molecular formulae were $C_{45}H_{72}O_{20}$ and $C_{45}H_{72}O_{19}$, respectively, from their high resolution negative FAB mass spectra. Both

Table 2. ^{13}C NMR chemical shifts of sugar moieties in pyridine-d_5 (ppm)

C		1	8	2	3	3a	4	4a	5	6	6a
3-*O*-Gal	1	102.9	103.2		102.9	102.9			102.9	103.0	102.9
	2	73.4	72.7		73.5	73.5			73.4	73.5	73.4
	3	75.4[a]	75.5		75.4[a]	75.5[a]			75.4[a]	75.4[a]	75.4[a]
	4	80.0	70.4		80.0	80.0			79.9	80.0	80.0
	5	75.9[a]	77.0		75.9[a]	76.0[a]			75.9[a]	76.0[a]	75.9[a]
	6	61.0	62.6		61.0	61.0			61.0	61.0	61.0
Fuc	1			102.7			102.7	102.7			
	2			73.0			73.0	73.0			
	3			76.2[a]			76.2[a]	76.3[a]			
	4			83.3			83.3	83.3			
	5			70.6			70.6	70.6			
	6			17.6			17.7	17.7			
Glc	1	107.1		107.0	107.1	107.2	107.0	107.0	107.0	107.2	107.2
	2	75.2		75.6[a]	75.2[b]	75.2	75.6[a]	75.6[a]	75.2	75.2[b]	75.2[b]
	3	78.7		78.6	78.7[c]	78.8[b]	78.6	78.7	78.7	78.8[c]	78.8[c]
	4	72.3		71.7	72.3	72.4	71.7	71.7	72.3	72.4	72.3
	5	78.4		78.6	78.5	78.5	78.6	78.6	78.4	78.5	78.5
	6	63.2		62.9	63.2	63.2	62.9	63.0	63.1	63.2	63.2
26-*O*-Glc	1				104.9	105.0	104.9	105.0		105.0	105.0
	2				75.3[b]	75.2	75.2	75.2		75.3[b]	75.3[b]
	3				78.6[c]	78.7[b]	78.6	78.7		78.6[c]	78.7[c]
	4				71.8	71.8	71.8	71.8		71.8	71.8
	5				78.5	78.5	78.5	78.5		78.5	78.5
	6				62.9	63.0	62.9	63.0		62.9	62.9

[a–c]Signals may be interchanged within each column.

showed three anomeric proton signals in the ^{1}H NMR spectra. In the ^{13}C NMR spectra of **3** and **4**, the signals due to either of their aglycone moieties were indicative of a 22-hydroxy-furost-5-en-12-on-3β, 22-diol one [19], while the signals due to their sugar moieties were identical with those of **1** and **2**, respectively, except for a set of additional signals corresponding to a β-glucopyranosyl unit. When allowed standing in methanol, **3** and **4** gave **3a** and **4a**, respectively, both of which showed a typical methoxy signal at δ3.27 in the ^{1}H NMR spectra and characteristic carbon signals of a 22-methoxy-furost-5-en-12-on-3β, 22-diol aglycone moiety in the ^{13}C NMR spectra [19]. Therefore, **3** and **4** were considered to be triglycosides possessing a 22-hydroxy-furost-5-en-12-on-3β, 22-diol aglycone moiety. It is known that furostanol saponins, which according to Marker [20] are precursors of spirostanol saponins, have two forms of 22-hydroxy and 22-methoxy in the course of extraction and isolation because of the interconversion in the presence of methanol and water. The former can be converted to the latter on refluxing with methanol and in contrast, the latter can be transformed back to the former on boiling with aqueous acetone or water [21]. In most cases, such a procedure has been used to obtain the homogenous saponin [19, 21]. It has also been suggested that the 22-methoxy furostanol saponins are secondary products formed from the corresponding 22-hydroxy form in extraction and isolation [21]. Thus compounds **3** and **4** are genuine saponins in this plant and **3a** and **4a** are artefacts.

On enzymatic hydrolysis, **3** and **4** afforded **1** and **2**, respectively, and glucose. While on acid hydrolysis, they gave the same aglycone **7** coupled with galactose and glucose in a ratio of 1 : 2 from **3** and fucose and glucose in a ratio of 1 : 2 from **4**. Furthermore, partially methylated alditol acetate analysis was employed to confirm the interglycosidic linkages. From these results, the structures of **3** and **4** were established, and they were named kingianosides C and D, respectively.

Saponin **5** was proved to be a diglycoside of diosgenin with the same sugar sequence as that of **1** on the basis of acid hydrolysis, FAB mass, ^{1}H and ^{13}C NMR spectra, identical to funkioside C isolated from *Funkia ovata* [11].

Saponin **6**, the genuine saponin existing in this plant, was obtained by the similar procedure to that used to obtain **3** and **4**. It was determined to be the corresponding furostanol glycoside of **5** by means of acid hydrolysis, chemical transformation, enzymatic hydrolysis, FAB mass, ^{1}H and ^{13}C NMR spectra. Compound **6** is the 25(*R*) epimer of Po-8 which was isolated as a mixture of 25(*R*, *S*) enantiomers from *Polygonatum officinalis* [8].

To the best of our knowledge, the examples of saponins bearing gentrogenin had not been reported until we isolated such glycosides from the title plant. This may provide some evidence for the chemotaxonomy of *Polygonatum* plants.

EXPERIMENTAL

Mps: uncorr. NMR spectra were measured in pyridine-d_5 unless otherwise noted and recorded at 270 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR (INEPT) using TMS as int. standard.

Plant materials. The rhizomes of *P. kingianum* Coll. et Hemsl. were collected in Dali, Yunnan province, China and identified by Prof. H. Li. A voucher specimen is deposited in the Herbarium of Kunming Institute of Botany, Academia Sinica.

Extraction and isolation. The fresh rhizomes (10.5 kg) were extracted with hot MeOH. After removal of the solvent by evapn, the combined extracts were dissolved in H_2O, defatted with Et_2O, and then extracted with *n*-BuOH (satd with H_2O). The combined *n*-BuOH layers were concd to dryness to give a yellow residue (10.5 g). This residue was subjected to CC on MCI gel CHP 20P eluting with 10% MeOH and then with MeOH. The MeOH eluate was concd to dryness to afford a crude saponin fr. (6.8 g), which was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (50:10:1 to 20:10:1) and finally with MeOH to give frs I–VII in increasing order of polarity. Fr. II was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (70:10:1) to give β-sitosterol glucoside (25 mg). Fr. III was chromatographed on MCI gel CHP 20P with 85% MeOH to afford saponin **2** (18.5 mg). Fr. IV was subjected to CC on MCI gel CHP 20P with 85% MeOH to yield **1** (62 mg) and **5** (130 mg). Fr. V was purified on a MCI gel CHP 20P column with 70% MeOH to afford a mixture showing two spots on TLC. This mixture in 30% Me_2CO (2 ml) was heated at 85° for 20 hr and then concd to dryness to give saponin **4** (140 mg). Fr. VI was chromatographed on MCI gel CHP 20P eluting with 63%–70% MeOH to afford two pairs of mixtures, both of which showed two spots with close R_f values on TLC. After treating as that for **4**, the former yielded **3** (242 mg), while the latter gave **6** (310 mg).

Kingianoside A (**1**). Needles from MeOH, mp 250–252° (dec.), $[\alpha]_D^{20}$ −42.4° (MeOH; *c* 0.51); HRFABMS (neg.) *m/z*: 751.3915 $[M(C_{39}H_{60}O_{14})-H]^-$, calcd 751.3905; FABMS (neg.) *m/z*: 751 $[M-H]^-$, 589 $[M-Glc-H]^-$; ^1H NMR: δ0.70 (3H, *d*, *J* = 5.5 Hz, Me-27), 0.93 (3H, *s*, Me-19), 1.11 (3H, *s*, Me-18), 1.35 (3H, *d*, *J* = 6.6 Hz, Me-21), 4.87 (1H, *d*, *J* = 7.3 Hz, Gal H-1), 5.28 (1H, *d*, *J* = 8.1 Hz, Glc H-1), 5.29 (1H, *br s*, H-6).

Kingianoside B (**2**). Needles from MeOH, mp 271–274° (dec.), $[\alpha]_D^{20}$ −46.0° (MeOH; *c* 0.87); HRFABMS (neg.) *m/z*: 735.4000 $[M(C_{39}H_{60}O_{13})-H]^-$, calcd 735.3956; FABMS (neg.) *m/z*: 735 $[M-H]^-$; ^1H NMR: δ0.70 (3H, *d*, *J* = 5.5 Hz, Me-27), 0.94 (3H, *s*, Me-19), 1.11 (3H, *s*, Me-18), 1.36 (3H, *d*, *J* = 7.0 Hz, Me-21), 1.63 (3H, *d*, *J* = 6.6 Hz, Fuc Me), 4.81 (1H, *d*, *J* = 7.7 Hz, Fuc H-1), 5.23 (1H, *d*, *J* = 7.7 Hz, Glc H-1), 5.33 (1H, *br s*, H-6).

Kingianoside C (**3**). Powder, $[\alpha]_D^{20}$ −27.4° (pyridine; *c* 0.37); HRFABMS (neg.) *m/z*: 931.4515 $[M(C_{45}H_{72}O_{20})-H]^-$, calcd 931.4539; FABMS (neg.) *m/z*: 931 $[M-H]^-$; 769 $[M-Glc-H]^-$, 607 $[M-Glc-hexose-H]^-$; ^1H NMR: δ0.93 (3H, *s*, Me-19), 0.99 (3H, *d*, *J* = 6.6 Hz, Me-27), 1.16 (3H, *s*, Me-18), 1.55 (3H, *d*, *J* = 6.6 Hz, Me-21), 4.82 (1H, *d*, *J* = 7.7 Hz, 26-*O*-Glc H-1), 4.88 (1H, *d*, *J* = 7.7 Hz, Gal H-1), 5.28 (1H, *d*, *J* = 7.3 Hz, Glc H-1), 5.31 (1H, *br s*, H-6).

Kingianoside D (**4**). Powder, $[\alpha]_D^{20}$ −13.4° (pyridine; *c* 0.62); HRFABMS (neg.) *m/z*: 915.4586 $[M(C_{45}H_{72}O_{19})-H]^-$, calcd 915.4589; FABMS (neg.) *m/z*: 915 $[M-H]^-$; 753 $[M-Glc-H]^-$, 607 $[M-Glc-Fuc-H]^-$; ^1H NMR: δ0.95 (3H, *s*, Me-19), 1.00 (3H, *d*, *J* = 6.2 Hz, Me-27), 1.17 (3H, *s*, Me-18), 1.56 (3H, *d*, *J* = 6.6 Hz, Me-21), 1.63 (3H, *d*, *J* = 6.2 Hz, Fuc Me), 4.81 (1H, *d*, *J* = 7.7 Hz, Fuc H-1), 4.83 (1H, *d*, *J* = 7.7 Hz, 26-*O*-Glc H-1), 5.23 (1H, *d*, *J* = 7.3 Hz, Glc H-1), 5.33 (1H, *br s*, H-6).

Funkioside C (**5**). Powder, $[\alpha]_D^{20}$ −60.2° (MeOH; *c* 0.59); FABMS (neg.) *m/z*: 737 $[M(C_{39}H_{62}O_{13})-H]^-$, 575 $[M-Glc-H]^-$; ^1H NMR: δ0.70 (3H, *d*, *J* = 5.5 Hz, Me-27), 0.84 (3H, *s*, Me-18), 0.89 (3H, *s*, Me-19), 1.15 (3H, *d*, *J* = 6.9 Hz, Me-21), 4.92 (1H, *J* = 7.7 Hz, Gal H-1), 5.31 (1H, *br s*, H-6), 5.33 (1H, *d*, *J* = 7.7 Hz, Glc H-1).

25(R) *Epimer of Po*-8 (**6**). Needles from Aq. Me_2CO, mp 198–200°, $[\alpha]_D^{20}$ −59.4° (pyridine; *c* 0.61); FABMS (neg.) *m/z*: 917 $[M(C_{45}H_{74}O_{19})-H]^-$; 755 $[M-Glc-H]^-$; ^1H NMR: δ0.91 (6H, *s*, Me-18 and 19), 0.99 (3H, *d*, *J* = 6.6 Hz, Me-27), 1.34 (3H, *J* = 7.0 Hz, Me-21), 4.82 (1H, *d*, *J* = 7.7 Hz, 26-*O*-Glc H-1), 4.90 (1H, *d*, *J* = 7.7 Hz, Gal H-1), 5.30 (1H, *d*, *J* = 7.7 Hz, Glc H-1), 5.31 (1H, *br s*, H-6).

Formation of **3a**, **4a** *and* **6a**. A soln of **3** (65 mg), **4** (80 mg) or **6** (80 mg) in MeOH (2 ml) was individually allowed to stand at room temp. overnight and then concd to dryness to yield **3a**, **4a** and **6a**.

Compound **3a**. Powder, ^1H NMR: δ0.93 (3H, *s*, Me-19), 1.00 (3H, *d*, *J* = 6.6 Hz, Me-27), 1.01 (3H, *s*, Me-18), 1.42 (3H, *d*, *J* = 7.0 Hz, Me-21), 3.27 (3H, *s*, OMe), 4.85 (1H, *d*, *J* = 7.3 Hz, 26-*O*-Glc H-1), 4.88 (1H, *d*, *J* = 7.3 Hz, Gal H-1), 5.30 (1H, *d*, *J* = 7.7 Hz, Glc H-1), 5.31 (1H, *br s*, H-6).

Compound **4a**. Powder, ^1H NMR: δ0.94 (3H, *s*, Me-19), 1.01 (3H, *d*, *J* = 6.6 Hz, Me-27), 1.09 (3H, *s*, Me-18), 1.43 (3H, *d*, *J* = 7.0 Hz, Me-21), 3.27 (3H, *s*, OMe), 4.81 (1H, *d*, *J* = 7.7 Hz, Fuc H-1), 4.86 (1H, *d*, *J* = 7.7 Hz, 26-*O*-Glc H-1), 5.30 (1H, *d*, *J* = 7.7 Hz, Glc H-1), 5.31 (1H, *br s*, H-6).

Compound **6a**. Powder, ^1H NMR: δ0.82 (3H, *s*, Me-18), 0.90 (3H, *s*, Me-19), 1.01 (3H, *d*, *J* = 5.6 Hz, Me-27), 1.20 (3H, *d*, *J* = 6.6 Hz, Me-21), 3.27 (3H, *s*, OMe), 4.86 (1H, *d*, *J* = 7.7 Hz, 26-*O*-Glc H-1), 4.90 (1H, *d*, *J* = 7.7 Hz, Gal H-1), 5.30 (1H, *d*, *J* = 7.7 Hz, Glc H-1), 5.31 (1H, *br s*, H-6).

Partial acid hydrolysis of **1**. A soln of **1** (40 mg) in 5% H_2SO_4–EtOH (1:1, 6 ml) was heated 100° for 2.5 hr. After cooling, the reaction mixt. was subjected to CC on MCI gel CHP 20P eluting with H_2O and then with MeOH. The MeOH eluate was concd to dryness to afford a residue, which was chromtographed on silica gel with $CHCl_3$–MeOH (9:1) to give **7** (9.1 mg) and **8** (2.4 mg).

Gentrogenin (**7**). Needles from MeOH, mp 217–219° {ref. [13] mp 213–215°}, $[\alpha]_D^{20}$ −45.1° ($CHCl_3$; *c* 0.39) { ref. [13] $[\alpha]_D^{28}$ −56.0 ± 2° ($CHCl_3$, *c* 1.02)}; EIMS (70 eV) *m/z*: 428 $[M(C_{27}H_{40}O_4)]^+$, 410, 314, 296, 176, 139 (base peak), 126; ^1H NMR ($CDCl_3$): δ0.79 (3H, *d*, *J* = 6.2 Hz, Me-27), 1.07 (3H, *d*, *J* = 7.0 Hz, Me-21), 1.09 (3H, *s*, Me-19), 1.11 (3H, *s*, Me-18), 3.35 (1H, *dd*, *J* = 10.6, 11.0 Hz, H-26ax), 3.54 (2H, *m*, H-3 and H-26eq), 4.36 (1H, *m*, H-16), 5.39 (1H, *m*, H-6).

Prosapogenin (**8**). Powder from MeOH, FABMS (neg.) *m/z*: 589 $[M(C_{33}H_{50}O_9)-H]^-$; ^1H NMR: δ0.70 (3H, *d*, *J* = 6.2 Hz, Me-27), 0.94 (3H, *s*, Me-19), 1.11 (3H, *s*, Me-18), 1.37 (3H, *d*, *J* = 6.9 Hz, Me-21), 4.98 (1H, *d*, *J* = 7 7 Hz, Gal H-1), 5.29 (1H, *br s*, H-6).

Acid hydrolysis of **1**–**6**. A soln of each saponin (about 2 mg) in 2 N HCl–dioxane (1:1, 1 ml) was heated at 95° for 6 hr. The reaction mixt. was blowed to dryness with a N_2 stream. The residue was checked, respectively, for identification of aglycone and sugar moieties. Aglycone was identified with TLC by comparison with authentic samples, using $CHCl_3$ as development and 10% H_2SO_4 as detection. Saponins **1**–**4** gave gentrogenin (**7**) (R_f 0.09), while saponins **5** and **6** gave diosgenin (R_f 0.17). Sugars were checked with TLC and GLC. TLC was carried out on a HPTLC silica gel 50000 F_{254} plate using *n*-BuOH–*i*-PrOH–H_2O (10:5:4, homogenous) as development and anisaldehyde–H_2SO_4 as detection. R_f: Gal (0.24), Glc (0.29), Fuc (0.56). For GLC, the residue was further dried over P_2O_5 in a vacuum oven, trimethylsilylated with TMS-HT at room temp. for 10 min and then directly subjected to GLC analysis. GLC: glass column packed with 1% silicone OV-17 on Gaschrom Q (80–100 mesh), 3 mm × 2 m; detection: FID; column temp.: 140°; carrier gas: N_2 (40 ml min^{-1}). R_t (min): Glc (17.7, 29.4), Gal (14.5, 18.0), Fuc (5.5, 6.9).

Determination of the absolute configuration of fucose [17]. A soln of saponin **2** (3 mg) in 2 N HCl–dioxane (1:1, 1 ml) was heated at 90° for 4.5 hr. After cooling, the reaction mixt. was diluted with H_2O and extracted with $CHCl_3$. The aq. layer was neutralized with Amberlite MB-3, concd to dryness to yield a

mixture of sugars. According to the Oshima's method, a soln of the sugar mixt. in H_2O (40 μl) and a soln of L-(−)-MBA (7 mg) and $NaBH_3CN$ (1.6 mg) in EtOH (40 μl) was kept at 40° for 3.5 hr. Several drops of Ac_2O were added, and the mixture evapd to dryness with a N_2 stream and further dried over P_2O_5 in a vacuum oven. To the residue was added TMS-HT (50 μl). After standing at room temp. for 20 min, the supernant was subjected to GLC analysis. Identification of peaks was made by comparing with those of the reaction product of an authentic sugar and L-(−)-MBA. GLC conditions, capillary column: PEG 20M 25 m × 0.22 mm; detection: FID; column temp.: 180°; carrier gas: He (2 kg cm^{-2}). R_t (min): D-Fuc (6.23), D-Glc (8.41)[authentic samples, D-Fuc (6.18), L-Fuc (6.54), D-Glc (8.41)].

Enzymatic hydrolysis of **3**, **4** *and* **6**. A soln of **3** (40 mg) and β-glucosidase (20 mg) in an acetic buffer (6 ml, pH = 4.3) was incubated at 37° for 2 hr. The soln was diluted with H_2O, and then filtered. The resulting residue was subjected to MCI gel CHP 20P. Elution with 80% MeOH recovered **3** (29 mg), and subsequent elution with 90% MeOH afforded **1** (7.3 mg). Identification was made by ^{13}C NMR spectrum. Glucose was detected in the concd filtrate by TLC as described above.

By the same procedure carried out for **3**, **6** (60 mg) yielded the corresponding spirostanol saponin **5** (12.6 mg) with a recovery of unreacted **6** (36 mg).

A soln of **4** (55 mg) and β-glucosidase (55 mg) in an acetic buffer (5 ml, pH = 4.3) was incubated at 37° for 10 hr. The reaction mixt. was directly subjected to MCI gel CHP 20P. Stepwise elution with H_2O, 70% and 85% MeOH gave glucose, **4** (20 mg) and **2** (30.5 mg).

Partially methylated alditol acetate analysis (GLC-MS) of **1**–**4** [14, 15]. To a soln of a saponin (3 mg) in DMSO (0.1 ml) was added a soln of *t*-BuOK (0.12 g) in DMSO (0.6 ml). The soln was sonicated at room temp. for 1 hr. To this soln was added Me I (0.8 ml) and the soln was further sonicated at room temp. for 1 hr. Excess Me I was removed by a N_2 stream. The reaction mixt. was diluted with H_2O, and then extracted with three 5-ml portions of $CHCl_3$. The $CHCl_3$ layer was dried over Na_2SO_4 and then concd to dryness. The residue was purified by a silica gel column [solvent used: $CHCl_3$ for **1** and **2**; $CHCl_3$–MeOH (100 : 1) for **3** and **4**]. The resulting permethylated product was hydrolysed with 0.5 N H_2SO_4 in 93% AcOH (0.5 ml) at 76° for 3 hr. The soln was neutralized with Amberlite IR-45 washing with H_2O (20 ml) and MeOH (15 ml). The combined washing was concd to dryness to afford a residue. To the residue was added 1% $NaBH_4$ (1 ml) and the soln was standing at room temp. for 1.5 hr. To this soln several drops of AcOH was added and the resulting H_3BO_3 was removed by co-distillation with MeOH. The residue was dried over P_2O_5 in a vacuum oven and then acetylated with Ac_2O (0.5 ml) at 100° for 4 hr. Excess of Ac_2O was removed by co-distillation with toluene. The methylated alditol acetate thus obtained was subjected to GC-MS glass column packed with 1.5% OV-210 on Chromosorb-W, 2 mm × 2 m; column temp.: 180°; carrier gas: He (40 ml min^{-1}); ionization voltage: 70 eV; accelerating voltage: 3 kV. *t*-Glc: R_t (4 min, 24 sec), *m/z*: 205, 161, 145, 129, 117, 101 (base peak), 87, 71, 45 and 43; 4-Gal: R_t (9 min, 9 sec), *m/z*: 233, 173, 161, 131, 129, 117 (base peak), 113, 101, 87, 71 and 45; 4-Fuc: R_t (5 min, 48 sec), *m/z*: 203, 143, 117 (base peak), 101, 87 and 43.

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