New Steroidal Saponins from the Bulbs of *Allium giganteum* Exhibiting Potent Inhibition of cAMP Phosphodiesterase Activity

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A new spirostanol saponin and two new furostanol saponins were isolated from the fresh bulbs of *Allium giganteum* together with several known saponins. The structures of the new compounds were determined on the basis of spectroscopic data, the products formed on hydrolysis and by comparing their properties with those of known compounds. The isolated saponins and their derivatives were evaluated for inhibitory activity by examining their action on cAMP phosphodiesterase as a primary screening test to identify new medicinal agents.

Keywords *Allium giganteum*; Liliaceae; steroidal saponin; spirostanol saponin; furostanol saponin; cAMP phosphodiesterase inhibition

Allium giganteum (Liliaceae) is native to central Asia and one of the most spectacular species of all the *Allium* plants.[1] Previously, we have reported the isolation of several polyhydroxylated spirostanol saponins.[2] Re-investigation of the bulbs led to the isolation of a new polyhydroxylated spirostanol saponin and two new furostanol saponins together with several previous reported saponins. This paper reports the structural assignments of the new saponins, based on spectroscopic data, products formed on hydrolysis, and by comparing them with known compounds. Furthermore, the inhibitory activity on cAMP phosphodiesterase of the isolated saponins and their derivatives is discussed.

The concentrated 1-butanol-soluble fraction of the methanolic extract of *A. giganteum* bulbs was repeatedly subjected to column chromatography on silica-gel, Diaion HP-20 and octadecylsilanized (ODS) silica-gel to yield compounds **1**—**7**.

Compounds **2**, **5** and **6** are known spirostanol saponins and their structures were assigned as 3-*O*-acetyl-(25*R*)-5α-spirostane-2α,3β,5α,6β-tetrol (alliogenin) 2-*O*-β-D-glucopyranoside,[2a] (25*R*)-5α-spirostane-2α,3β,6β-triol (agigenin) 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-[4-*O*-(*S*)-3-hydroxy-3-methylglutaryl-β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}[3] and agigenin 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} (aginoside),[4] respectively. Compound **7** was identified as the corresponding 22-methoxy-furostanol saponin of **6**, that is, 22-*O*-methyl-26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostane-2α,3β,6β,22ξ,26-pentol 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside}.[3]

Compound **1** was obtained as a white amorphous powder, $[\alpha]_D$ −50.0° (methanol). The molecular formula, $C_{35}H_{56}O_{13}$, was determined by negative-ion FAB-MS, which showed an $[M-H]^-$ ion at *m/z* 683, and elemental analysis. The IR spectrum of **1** showed the characteristic absorption bands of hydroxyl groups at 3400 cm^{-1} and a carbonyl group at 1715 cm^{-1}. The ^{1}H-NMR spectrum of **1** showed two three-proton singlet signals at δ 1.54 and 0.88, indicating the presence of two angular methyl groups, as well as two three-proton doublet signals at δ 1.15 (J=6.8 Hz) and 1.07 (J=6.5 Hz) assignable to secondary methyl groups. In addition, the presence of an acetyl group and a β-glucosyl group in **1** was readily demonstrated by the ^{1}H- and ^{13}C-NMR spectra [acetyl: δ 2.12 (3H, s), and δ 170.9 (C=O) and 21.6 (Me); β-glucosyl: δ 5.18 (1H, d, J=7.7 Hz, anomeric proton), and δ 103.1, 75.4, 78.3, 71.8, 78.5 and 63.0 (C-1′—C-6′)]. The above spectral features exhibited a close similarity to those of **2**. Alkaline treatment of **1** with 3% sodium methoxide in methanol gave the corresponding deacetyl derivative (**1a**), $C_{33}H_{54}O_{12}$. Following enzymatic hydrolysis of **1a** with β-glucosidase, it was recovered unchanged after 72 h. Hydrolysis with hesperidinase was able to cleave the β-glucosyl group of **1a** to give an aglycon (**1b**) and D-glucose. Compound **1b**, which has the molecular formula, $C_{27}H_{44}O_7$, deduced from high-resolution EI-MS, has one more oxygen atom than (25*R*)-5α-spirostane-2α,3β,5α,6β-tetrol (**2a**), the aglycon of **2**, indicating that **1b** contained five hydroxyl groups. The ^{13}C assignments of **1b** were closely related to those of **2a** except for the signals due to the F-ring carbons. Furthermore, in the ^{1}H-NMR spectrum of **1b**, the shift values and multiplicities of the signals due to the hydroxymethine protons,

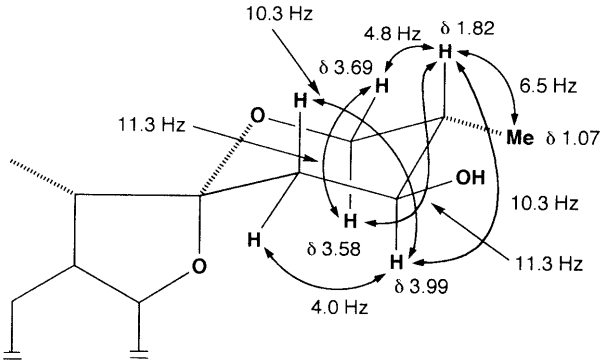


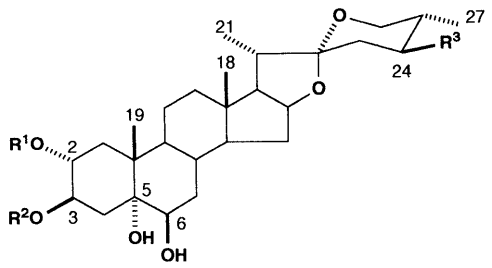
Fig. 1. ^{1}H-NMR Chemical Shifts and Spin-Coupling Constants of the F-Ring of **1b** in Pyridine-d_5

observed at δ 4.83 (1H, ddd, J=11.7, 9.1, 5.7 Hz), 4.44 (ddd, J=11.4, 9.1, 5.1 Hz) and 4.21 (1H, br s, $W_{1/2}$=8.2 Hz) were identical to those due to the H-3, H-2 and H-6 protons of **2a**. These data suggested the presence of 2α,3β,5α,6β-hydroxyl groups. An additional secondary hydroxyl group was suggested to be present on the F-ring of the spirostanol skeleton by the fragment ion peaks at m/z 155 and 131 in the EI-MS of **1b**.[5] Double resonance experiments in the ^{1}H-NMR spectrum of **1b**, starting from the signal at δ 1.07 (3H, d, J=6.5 Hz), attributable to the H-27 methyl protons, revealed the presence of a C-24 hydroxyl group. The large J values between the H-26 axial proton and H-25 proton (11.3 Hz), and between H-25 and H-24 (10.3 Hz) provided evidence for the 24S and 25S configurations (Fig. 1). Thus, the structure of **1b** was confirmed as (24S,25S)-5α-spirostane-2α,3β,5α,6β,24-pentol. On acetylation of **1** with acetic anhydride in pyridine, six additional acetyl groups were introduced into **1** (**1c**). In the ^{1}H-NMR spectrum of **1c**, the signals assignable to H-6 and H-24 were shifted downfield by 1.19 and 1.14 ppm, respectively, as compared with those of **1**, indicating that the C-2 and C-3 hydroxyl groups are substituted with glucose and acetic acid. In the ^{1}H-NMR spectrum of **1**, the signals at δ 2.80 (1H, dd, J=11.9, 11.0 Hz) and 2.34 (1H, dd, J=11.9, 6.4 Hz) were assigned to the H-4 axial and H-4 equatorial protons. The above assignments were based on the resonance of the H-4 axial proton at the unexpected lower field, which must be

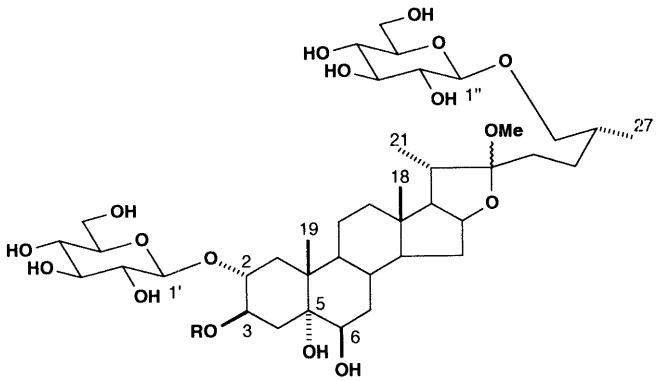
TABLE I. ^{13}C-NMR Spectral Data for Compounds **1**, **1a**, **1b** and **2**—**4**[a]

Carbon	1	1a	1b	2	3	4
1	38.8	39.7	42.3	38.8	38.9	38.8
2	77.7	85.1	73.7[b]	77.7	77.7	77.6
3	75.0[b]	71.5	73.8[b]	75.0[b]	76.4	75.0[b]
4	37.8	40.4[b]	41.2	37.8	37.9	37.8
5	74.9	74.9	75.7	74.9	75.0	74.9
6	75.1[b]	75.2[c]	75.6	75.1[b]	75.0	75.1[b]
7	35.7	35.8	35.9	35.8	35.7	35.7
8	30.1	30.2	30.3	30.1	30.0	30.0
9	45.6	45.8	46.0	45.7	45.6	45.6
10	40.4	40.7	41.2	40.4	40.5	40.4
11	21.5	21.6	21.7	21.6	21.5	21.4
12	40.3	40.2[b]	40.5	40.4	40.5	40.2
13	41.0	41.0	41.0	41.0	41.4	41.3
14	56.2	56.4	56.4	56.2	56.1	56.1
15	32.3	32.3	32.3	32.3	32.3	32.3
16	81.6	81.6	81.6	81.2 (81.3)	81.4	81.4
17	62.8 (62.6)	62.8 (62.6)	62.8 (62.7)	63.2 (63.0)	64.4	64.4
18	16.6	16.7	16.7	16.7	16.3	16.3
19	17.9	18.1	18.5	17.9	18.0	17.9
20	42.3 (42.6)	42.3 (42.6)	42.3 (42.6)	42.0 (42.5)	40.5	40.4
21	15.0 (14.8)	15.0 (14.8)	15.0 (14.8)	15.0 (14.9)	16.6	16.6
22	111.8 (111.4)	111.8 (111.4)	111.8 (111.4)	109.2 (109.7)	112.6	112.6
23	41.8 (36.1)	41.8 (36.1)	41.9 (36.1)	31.9 (26.2)	30.8 (30.9)	30.8 (30.9)
24	70.6 (66.5)	70.6 (66.5)	70.6 (66.5)	29.3 (26.4)	28.2	28.2
25	40.0 (35.9)	39.9 (35.9)	40.0 (35.9)	30.6 (27.6)	34.2 (34.5)	34.2 (34.5)
26	65.3 (64.6)	65.3 (64.6)	65.3 (64.6)	66.9 (65.1)	75.3	75.2
27	13.6 (9.7)	13.6 (9.7)	13.6 (9.7)	17.3 (16.3)	17.2 (17.6)	17.2 (17.6)
Ac	170.9			170.9		170.9
	21.6			21.6		21.6
OMe					47.3	47.3
1′	103.1	104.7		103.1	103.4	103.1
2′	75.4	75.3[c]		75.4	75.3	75.3
3′	78.3[c]	78.5		78.3[c]	78.3[b]	78.3[c]
4′	71.8	71.9		71.9	71.8	71.8
5′	78.5[c]	78.5		78.5[c]	78.5[b]	78.5[c]
6′	63.0	62.8		63.1	62.9	63.0
1″					105.0 (105.1)	105.0 (105.1)
2″					75.2	75.2
3″					78.6[c]	78.6[d]
4″					71.8	71.8
5″					78.5[c]	78.5[d]
6″					62.9	62.9
1‴					132.0	
2‴					130.3	
3‴					128.7	
4‴					132.9	
5‴					128.7	
6‴					130.3	
7‴					166.8	

a) Spectra were measured in pyridine-d_5. Shift values for the C-25 isomers are shown in parentheses. *b*—*d*) Assignments may be reversed in each vertical column.



	R1	R2	R3
1	Glc	Ac	OH
1a	Glc	H	OH
1b	H	H	OH
2	Glc	Ac	H
2a	H	H	H
2b	Glc	H	H
3a	Glc	Bz	H



	R
3	Bz
4	Ac

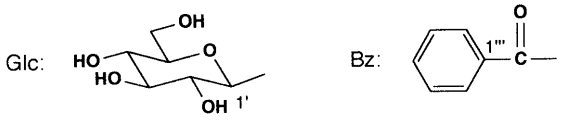


Chart 1

caused by interaction with the 6β-axial hydroxyl group, and movement of the signal upfield by more than 0.5 ppm following acetylation of the 6β-axial hydroxyl group. The signals at δ 6.14 (1H, ddd, J=11.0, 9.8, 6.4 Hz) and 4.70 (1H, dd, J=10.5, 9.8, 5.9 Hz) were assigned to the H-3 and H-2 oxymethine protons, respectively, by examination of the $^3J_{1H-1H}$ couplings from the H-4 protons through double resonance experiments. The signal at δ 6.14 was shifted upfield by 1.33 ppm on deacetylation of **1**. Thus, the glucose moiety was concluded to be located at the C-2 hydroxyl group and the acetyl group at the C-3 hydroxyl group. Accordingly, the structure of **1** was determined to be 3-*O*-acetyl-(24*S*,25*S*)-5α-spirostane-2α,3β,5α,6β,24-pentol 2-*O*-β-D-glucopyranoside.

Compound **3** was believed to be a 22-methoxyfurostanol saponin following Ehrlich's test[6] and examining the ^{1}H- and ^{13}C-NMR spectra [^{1}H-NMR: δ 3.25 (3H, s); ^{13}C-NMR: δ 112.6 (C) and 47.3 (Me)].[7] Enzymatic hydrolysis of **3** with β-glucosidase gave D-glucose and 3-*O*-benzoyl-(25*R*)-5α-spirostane-2α,3β,5α,6β-tetrol 2-*O*-β-D-glucopyranoside (**3a**).[2a] The structure of **3** was shown to be 3-*O*-benzoyl-22-*O*-methyl-26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostane-2α,3β,5α,6β,22ξ,26-hexol 2-*O*-β-D-glucopyranoside.

Compound **4** is also a 22-methoxyfurostanol saponin. The ^{1}H- and ^{13}C-NMR spectra along with the enzymatic hydrolysis of **4**, which gave D-glucose and **2**, showed the structure of **4** to be 3-*O*-acetyl-22-*O*-methyl-26-*O*-β-D-glucopyranosyl-(25*R*)-5α-furostane-2α,3β,5α,6β,22ξ,26-hexol 2-*O*-β-D-glucopyranoside.

The ^{1}H- and ^{13}C-NMR spectra showed that the isolated saponins contain small amounts of the corresponding C-25 isomers.

Table II. Inhibition of cAMP Phosphodiesterase Activity by Isolated Steroidal Saponins and Their Derivatives

Compounds	IC_{50} ($\times 10^{-5}$ M)
1	4.1
1a	6.9
1b	26.4
2	7.4
2a	—
2b	6.7
3	0.2
3a	9.7
4	0.5
5	2.4
6	7.5
7	4.4
Papaverine	3.0

The isolated saponins and their derivatives were evaluated for their inhibitory activity on cAMP phosphodiesterase as a primary screening test to identify new medicinal agents.[8] The IC_{50} values are listed in Table II. The new spirostanol saponin, **1**, exhibited inhibitory activity almost equal to that of a well-known inhibitor, papaverine. The spirostanol saponins, **1a**, **2**, **2b**, **3a** and **6** also showed considerable activity. The 3-hydroxy-3-methylglutaryl group at the xylose C-4 hydroxy position must lead to an enhancement of activity as shown when the IC_{50} values of **5** and **6** were compared. The aglycon, **1b**, was even less potent than **1** and **1a**, while **2a** exhibited no activity at all. Our previous investigations had revealed that several spirostanol saponins exhibited potent inhibitory activity while the corresponding furostanol saponins, bearing a glucosyl group at C-26, exhibited only weak activity or none at all.[9] In this present study, we have found that the furostanol saponins, **3**, **4** and **7**, were much more potent than the corresponding spirostanol saponins, **3a**, **2** and **6**, respectively. The structural peculiarity associated with the active furostanols is that they have several hydroxyl groups on the A and B rings. The detailed structure–activity relationships will be reported in the near future.

Experimental

Optical rotations were measured with a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a Hitachi 260-30 instrument and MS on a VG AutoSpec E instrument. NMR spectra were

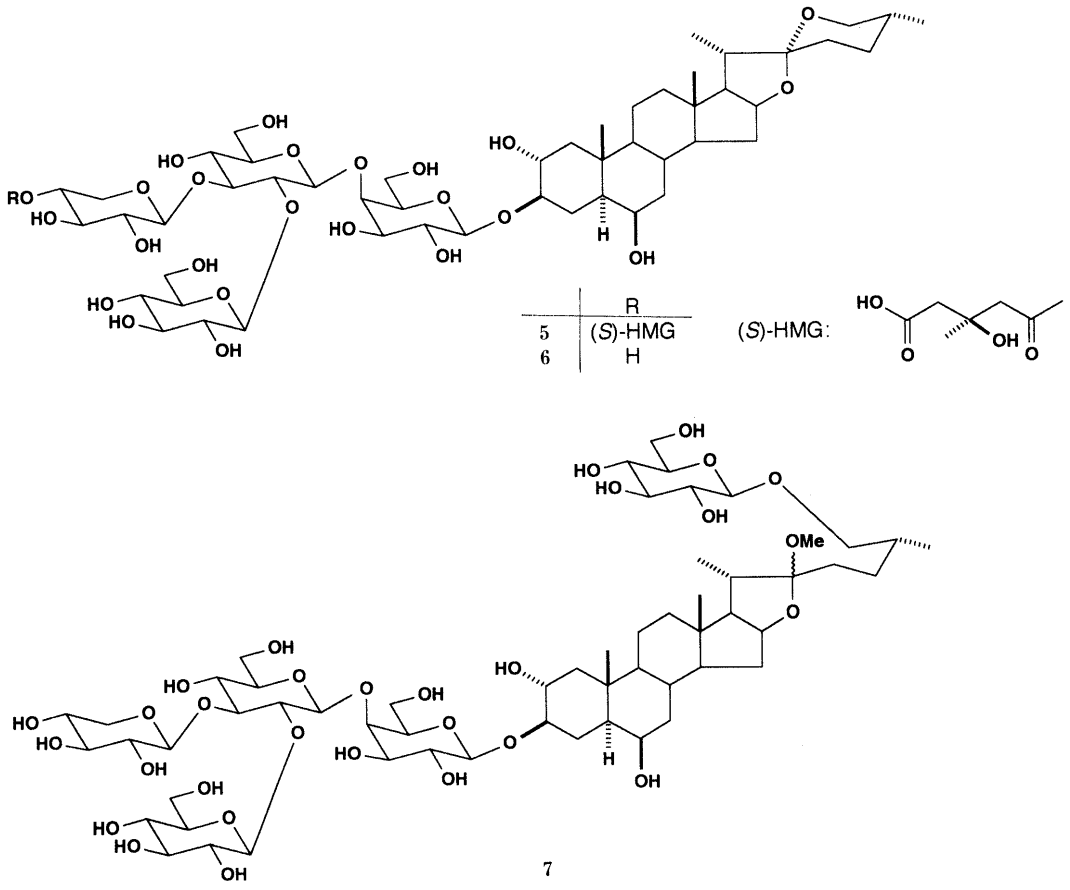


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Chart 2

recorded with a Bruker AM-400 spectrometer. Chemical shifts are given as δ-values with reference to the internal standard, tetramethylsilane (TMS). Silica-gel (Fuji-Silysia Chemical), Diaion HP-20 (Mitsubishi-kasei) and ODS silica-gel (Nacalai Tesque) were used for column chromatography. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. The liquid scintillation counter used was an Aloka LSC-903 instrument. Beef heart phosphodiesterase was purchased from Boehringer. Snake venom nucleotidase and cAMP were obtained from Sigma, and [^{3}H]cAMP from Radiochemical Center.

Isolation Fresh bulbs of *A. giganteum* (8.4 kg), purchased from Daiichi-engei. Japan, were extracted with hot MeOH. After removing the solvent under reduced pressure, the concentrated material was suspended in H_2O and extracted with *n*-BuOH. The *n*-BuOH-soluble phase was chromatographed on silica-gel, first with CH_2Cl_2 alone, then with CH_2Cl_2–MeOH mixtures with an increasing proportion of MeOH (19:1, 9:1, 4:1, 2:1) and finally with MeOH alone to give five fractions (I—V). Fraction III was chromatographed on a Diaion HP-20 column with a mobile phase composed of H_2O gradually enriched with MeOH. The 80% MeOH and 100% MeOH fractions were combined and subjected to column chromatography on silica-gel with $CHCl_3$–MeOH (4:1) and EtOAc–MeOH (9:1, 4:1), and on ODS silica-gel with MeOH–H_2O (3:2, 9:1) to give compounds **1** (259 mg) and **2** (3.18 g). Fraction IV was passed through a Diaion HP-20 column. The 80% MeOH and 100% MeOH fractions were combined and chromatographed on silica-gel with $CHCl_3$–MeOH (4:1) and EtOAc–MeOH (4:1), and on ODS silica-gel with MeOH–H_2O (3:2) to give compound **3** (226 mg). Fraction V was passed through a Diaion HP-20 column. Chromatography of the 80% MeOH and 100% MeOH fractions on silica-gel with EtOAc–MeOH (4:1, 3:1, 2:1, 3:2), $CHCl_3$–MeOH (3:1), $CHCl_3$–MeOH–H_2O (20:10:1, 7:4:1) and $CHCl_3$–MeOH–H_2O–AcOH (20:10:1:1), and on ODS silica-gel with MeOH–H_2O (7:3) gave compounds **4** (714 mg), **5** (217 mg), **6** (119 mg) and **7** (314 mg).

Compound 1 A white amorphous powder, $[\alpha]_D^{25}$ −50.0° (c=0.10, MeOH). *Anal.* Calcd for $C_{35}H_{56}O_{13}\cdot H_2O$: C, 59.81; H, 8.32. Found: C, 60.04; H, 8.34. Negative-ion FAB-MS *m/z*: 683 $[M-H]^-$, 641 $[M-acetyl]^-$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2930 (CH), 1715 (C=O), 1450, 1375, 1260, 1155, 1030, 985, 950, 885, 800. ^{1}H-NMR (pyridine-d_5) δ: 6.14 (1H, ddd, J=11.0, 9.8, 6.4 Hz, H-3), 5.18 (1H, d, J=7.7 Hz, H-1′), 4.70 (1H, ddd, J=10.5, 9.8, 5.9 Hz, H-2), 4.59 (1H, q-like, J=7.3 Hz, H-16), 4.55 (1H, dd, J=11.7, 2.4 Hz, H-6′a), 4.42 (1H, dd, J=11.7, 4.9 Hz, H-6′b), 4.30 (1H, dd, J=8.8, 8.8 Hz, H-3′), 4.25 (1H, dd, J=8.8, 8.8 Hz, H-4′), 4.15 (1H, br s, $W_{1/2}$=8.1 Hz, H-6), 4.07—3.95 (2H, overlapping, H-24 and H-5′), 4.01 (1H, dd, J=8.8, 7.7 Hz, H-2′), 3.69 (1H, dd, J=11.1, 4.8 Hz, H-26a), 3.58 (1H, dd, J=11.1, 11.1 Hz, H-26b), 2.80 (1H, dd, J=11.9, 11.0 Hz, H-4$_{axial}$), 2.39 (1H, dd, J=10.5, 10.5 Hz, H-1$_{axial}$), 2.34 (1H, dd, J=11.9, 6.4 Hz, H-4$_{equatorial}$), 2.12 (3H, s, Ac), 1.54 (3H, s, H-19), 1.15 (3H, d, J=6.8 Hz, H-21), 1.07 (3H, d, J=6.5 Hz, H-27), 0.88 (3H, s, H-18). Signals for the C-25 isomer: δ 1.30 (d, J=6.9 Hz, H-27), 1.17 (d, J=6.7 Hz, H-21), 0.87 (s, H-18).

Alkaline Methanolysis of 1 Compound **1** (80 mg) was treated with 3% NaOMe in MeOH at room temperature for 1 h. The reaction mixture was passed through an Amberlite IR-120B (Organo) column and subjected to silica-gel column chromatography with $CHCl_3$–MeOH–H_2O (20:10:1) to give the deacetyl derivative (**1a**) (68.2 mg) of **1** as a white amorphous powder. Compound **1a**: $[\alpha]_D^{24}$ −88.0° (c=0.10, MeOH). Negative-ion FAB-MS *m/z*: 642 $[M]^-$. IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2925 (CH), 1445, 1375, 1205, 1160, 1035, 985, 950, 885. ^{1}H-NMR (pyridine-d_5) δ: 5.16 (1H, d, J=7.8 Hz, H-1′), 4.81 (1H, ddd, J=11.3, 8.8, 6.1 Hz, H-3), 4.60 (1H, dd, J=11.4, 2.1 Hz, H-6′a), 4.57 (1H, q-like, J=7.4 Hz, H-16), 4.38 (1H, ddd, J=11.5, 8.8, 5.3 Hz, H-2), 4.33 (1H, dd, J=11.4, 6.0 Hz, H-6′b), 4.30 (1H, dd, J=9.3, 9.3 Hz, H-3′), 4.23 (1H, dd, J=9.3, 9.3 Hz, H-4′), 4.16 (1H, br s, $W_{1/2}$=6.4 Hz, H-6), 4.11 (1H, dd, J=9.3, 7.8 Hz, H-2′), 4.09 (1H, ddd, J=9.3, 6.0, 2.1 Hz, H-5′), 3.99 (1H, ddd, J=10.7, 10.7, 4.8 Hz, H-24), 3.68 (1H, dd, J=11.3, 4.8 Hz, H-26a), 3.57 (1H, dd, J=11.3, 11.3 Hz, H-26b), 2.96 (1H, dd, J=11.8, 11.3 Hz, H-4$_{axial}$), 2.34 (1H, dd, J=11.8, 6.1 Hz, H-4$_{equatorial}$), 2.36 (1H, dd, J=11.5, 11.5 Hz, H-1$_{axial}$), 1.57 (3H, s, H-19), 1.15 (3H, d, J=6.9 Hz, H-21), 1.07 (3H, d, J=6.5 Hz, H-27), 0.88 (3H, s, H-18). Signals for the C-25 isomer: δ 1.30 (d, J=7.0 Hz, H-27), 1.17 (d, J=6.8 Hz, H-21), 0.87 (s, H-18).

Enzymatic Hydrolysis of 1a Compound **1a** (30 mg) was dissolved in AcOH/AcOK buffer (pH 4.3), hesperidinase (Sigma Chemical) (60 mg) added and the mixture incubated at room temperature for 72 h. The crude products were passed through a Diaion HP-20 column, firstly with H_2O–MeOH (4 : 1) as mobile phase and then with 100% MeOH to give the sugar and sapogenin fractions. The sugar fraction was chromatographed on silica-gel with $CHCl_3$–MeOH–H_2O (20 : 10 : 1) to give D-glucose (2.5 mg). The sapogenin fraction was purified by silica-gel column chromatography with $CHCl_3$–MeOH (4 : 1) to give the aglycon (**1b**) (9.7 mg) as a white amorphous powder. D-Glucose: $[\alpha]_D^{25}$ +51.5° (c=0.25, H_2O). TLC, Rf 0.39 (n-BuOH–Me_2CO–H_2O, 4 : 5 : 1). Compound **1b**: $[\alpha]_D^{30}$ −82.0° (c= 0.10, MeOH). EI-MS m/z (%): 480.3114 (5) [M^+, Calcd for $C_{27}H_{44}O_7$: 480.3087], 421 (6), 395 (10), 392 (14), 350 (12), 332 (27), 321 (15), 314 (16), 299 (7), 285 (8), 155 (100), 131 (19). IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2930 (CH), 1450, 1375, 1275, 1205, 1170, 1030, 990, 950, 895. ^{1}H-NMR (pyridine-d_5) δ: 4.83 (1H, ddd, J=11.7, 9.1, 5.7 Hz, H-3), 4.58 (1H, q-like, J=7.3 Hz, H-16), 4.44 (1H, ddd, J=11.4, 9.1, 5.1 Hz, H-2), 4.21 (1H, br s, $W_{1/2}$=8.2 Hz, H-6), 3.99 (1H, ddd, J=10.3, 10.3, 4.0 Hz, H-24), 3.69 (1H, dd, J=11.3, 4.8 Hz, H-26a), 3.58 (1H, dd, J=11.3, 11.3 Hz, H-26b), 3.05 (1H, dd, J=12.2, 11.7 Hz, H-4_{axial}), 2.46 (1H, dd, J=11.7, 11.4 Hz, H-1_{axial}), 2.39 (1H, dd, J=12.2, 5.7 Hz, H-$4_{equatorial}$), 2.16 (1H, dd, J=11.7, 5.1 Hz, H-$1_{equatorial}$), 1.82 (1H, m, H-25), 1.68 (3H, s, H-19), 1.16 (3H, d, J=6.9 Hz, H-21), 1.07 (3H, d, J=6.5 Hz, H-27), 0.91 (3H, s, H-18). Signals for the C-25 isomer: δ 1.30 (d, J=6.9 Hz, H-27), 1.17 (d, J=6.8 Hz, H-21), 0.89 (s, H-18).

Acetylation of 1 Compound **1** (50 mg) was acetylated with Ac_2O in pyridine and the crude acetate was purified by silica-gel column chromatography with hexane–Me_2CO (2 : 1) to give the corresponding hexaacetate (**1c**) (42.7 mg) as a white amorphous powder. Compound **1c**: $[\alpha]_D^{29}$ −74.0° (c=0.10, $CHCl_3$). EI-MS m/z: 877 [M−MeCOO]$^+$ (0.4), 848 (0.6), 806 (0.6), 589 (0.5), 529 (0.6), 469 (4), 331 (15), 267 (4), 169 (49), 137 (100), 109 (52). IR ν_{max}^{KBr} cm^{-1}: 3490 (OH), 2950 (CH), 1740 (C=O), 1435, 1365, 1235, 1130, 1025, 985, 950, 900. ^{1}H-NMR (pyridine-d_5) δ: 5.96 (1H, q-like, J=9.0 Hz, H-3), 5.80 (1H, dd, J=9.6, 9.6 Hz, H-3′), 5.53 (1H, dd, J=9.6, 9.6 Hz, H-4′), 5.42 (1H, dd, J=9.6, 7.9 Hz, H-2′), 5.34 (1H, br s, $W_{1/2}$=8.1 Hz, H-6), 5.20 (1H, d, J=7.9 Hz, H-1′), 5.15 (1H, ddd, J=10.9, 10.9, 4.9 Hz, H-24), 4.61 (1H, dd, J=12.4, 4.6 Hz, H-6′a), 4.52 (1H, q-like, J=7.4 Hz, H-16), 4.46 (1H, dd, J=12.4, 2.1 Hz, H-6′b), 4.32 (1H, ddd, J=11.3, 9.0, 5.5 Hz, H-2), 4.21 (1H, ddd, J=9.6, 4.6, 2.1 Hz, H-5′), 3.61 (1H, dd, J=11.4, 4.9 Hz, H-26a), 3.50 (1H, dd, J=11.4, 11.4 Hz, H-26b), 2.18, 2.14, 2.12, 2.09, 2.06, 2.03, 2.01 (each 3H, s, Ac), 1.31 (3H, s, H-19), 1.15 (3H, d, J=6.9 Hz, H-21), 0.85 (3H, s, H-18), 0.77 (3H, d, J=6.5 Hz, H-27). Signals for the C-25 isomer: δ 1.08 (d, J=7.0 Hz, H-27), 0.87 (s, H-18).

Compound 3 A white amorphous powder, $[\alpha]_D^{22}$ −84.9° (c=0.11, MeOH). *Anal.* Calcd for $C_{47}H_{72}O_{18}$: C, 61.02; H, 7.85. Found: C, 60.64; H, 7.84. Negative-ion FAB-MS m/z: 924 [M]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2940 (CH), 1720 (C=O), 1465, 1390, 1330, 1295, 1155, 1040, 895, 715. ^{1}H-NMR (pyridine-d_5) δ: 8.45 (2H, dd, J=7.8, 1.8 Hz, H-2‴ and H-6‴), 7.46 (3H, H-3‴—H-5‴), 6.32 (1H, ddd, J=11.0, 9.2, 6.3 Hz, H-3), 5.24 (1H, d, J=7.7 Hz, H-1′), 4.91 (1H, ddd, J=12.1, 9.2, 5.8 Hz, H-2), 4.87 (1H, d, J=7.7 Hz, H-1″), 4.49 (1H, q-like, J=7.2 Hz, H-16), 3.25 (3H, s, OMe), 2.91 (1H, dd, J=11.8, 11.0 Hz, H-4_{axial}), 2.58 (1H, dd, J=11.8, 6.3 Hz, H-$4_{equatorial}$), 2.48 (1H, dd, J=12.1, 12.1 Hz, H-1_{axial}), 1.58 (3H, s, H-19), 1.18 (3H, d, J=7.1 Hz, H-21), 1.01 (3H, d, J=6.7 Hz, H-27), 0.87 (3H, s, H-18). Signals for the C-25 isomer: δ 4.86 (d, J=7.8 Hz, H-1″), 3.24 (s, OMe), 1.16 (d, J=7.1 Hz, H-21), 1.07 (d, J=6.7 Hz, H-27).

Compound 4 A white amorphous powder, $[\alpha]_D^{28}$ −72.2° (c=0.11, MeOH). *Anal.* Calcd for $C_{42}H_{70}O_{18}$: C, 58.45; H, 8.18. Found: C, 58.00; H, 7.94. Negative-ion FAB-MS m/z: 862 [M]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2925 (CH), 1710 (C=O), 1445, 1370, 1255, 1155, 1065, 1025, 950, 890. ^{1}H-NMR (pyridine-d_5) δ: 6.14 (1H, ddd, J=11.4, 9.7, 6.2 Hz, H-3), 5.18 (1H, d, J=7.7 Hz, H-1′), 4.86 (1H, d, J=7.8 Hz, H-1″), 4.71 (1H, ddd, J=10.8, 9.7, 5.9 Hz, H-2), 4.47 (1H, q-like, J=7.4 Hz, H-16), 3.24 (3H, s, OMe), 2.81 (1H, dd, J=12.0, 11.4 Hz, H-4_{axial}), 2.40 (1H, dd, J=10.8, 10.8 Hz, H-1_{axial}), 2.35 (1H, dd, J=12.0, 6.2 Hz, H-$4_{equatorial}$), 1.53 (3H, s, H-19), 1.17 (3H, d, J=7.0 Hz, H-21), 1.01 (3H, d, J=6.6 Hz, H-27), 0.85 (3H, s, H-18). Signals for the C-25 isomer: δ 4.85 (d, J=7.8 Hz, H-1″), 3.23 (s, OMe), 1.15 (d, J=7.2 Hz, H-21), 1.06 (d, J=6.6 Hz, H-27).

Enzymatic Hydrolysis of 3 and 4 A solution of **3** (50 mg) and β-glucosidase (Tokyo-kasei-kogyo) (50 mg) in AcOH/AcONa buffer (pH 5) was stirred at room temperature for 24 h. The reaction mixture was chromatographed on silica-gel with a gradient mixture of $CHCl_3$–MeOH (9 : 1, 4 : 1) and with 100% MeOH to give the corresponding spirostanol saponin (**3a**) (15.9 mg) and D-glucose. Compound **4** (50 mg) was treated in the same way as **5** to give compound **2** (22.9 mg) and D-glucose.

Assay of cAMP Phosphodiesterase Activity The phosphodiesterase activity was assayed by a modification of the method of Thompson and Brooker as described previously.[8b,c] The assay consisted of a two-step isotopic procedure. Tritium-labelled cAMP was hydrolyzed to 5′-AMP by phosphodiesterase and the 5′-AMP was then further hydrolyzed to adenosine by snake venom nucleotidase. The hydrolysate was treated with an anion-exchange resin (Dowex AG1-X8; Bio-Rad) to adsorb all charged nucleotides leaving [^{3}H]adenosine as the only labelled compound to be counted.

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References

1) J. E. Bryan, "Bulbs," Vol. 1, Timber Press, Portland and Oregon, 1989, p. 69.
2) *a*) Y. Sashida, K. Kawashima, Y. Mimaki, *Chem. Pharm. Bull.*, **39**, 698 (1991); *b*) K. Kawashima, Y. Mimaki, Y. Sashida, *Phytochemistry*, **30**, 3063 (1991).
3) K. Kawashima, Y. Mimaki, Y. Sashida, *Phytochemistry*, **32**, 1267 (1993).
4) A. N. Kel'ginbaev, M. B. Gorovits, T. T. Gorovits, N. K. Abubakirov, *Khim. Prir. Soedin.*, **1976**, 480.
5) A. M. E. Abdel-Aziz, K. R. Brain, G. Blunden, T. Crabb, A. K. Bashir, *Planta Medica*, **56**, 218 (1990).
6) *a*) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa, T. Kawasaki, *Chem. Pharm. Bull.*, **16**, 1162 (1968); *b*) T. Nohara, K. Miyahara, T. Kawasaki, *ibid.*, **23**, 872 (1975).
7) P. K. Agrawal, D. C. Jain, R. K. Gupta, R. S. Thakur, *Phytochemistry*, **24**, 2479 (1985).
8) *a*) T. Nikaido, T. Ohmoto, T. Kinoshita, U. Sankawa, S. Nishibe, S. Hisada, *Chem. Pharm. Bull.*, **29**, 3586 (1981); *b*) T. Nikaido, T. Ohmoto, H. Noguchi, T. Kinoshita, H. Saitoh, U. Sankawa, *Planta Medica*, **43**, 18 (1981); *c*) T. Nikaido, T. Ohmoto, U. Sankawa, T. Tomimori, Y. Miyaichi, Y. Imoto, *Chem. Pharm. Bull.*, **36**, 654 (1988); *d*) H. Sakurai, T. Nikaido, T. Ohmoto, Y. Ikeya, H. Mitsuhashi, *ibid.*, **40**, 1191 (1992).
9) *a*) S. Kubo, Y. Mimaki, Y. Sashida, T. Nikaido, T. Ohmoto, *Phytochemistry*, **31**, 2445 (1992); *b*) K. Ori, Y. Mimaki, K. Mito, Y. Sashida, T. Nikaido, T. Ohmoto, A. Masuko, *ibid.*, **31**, 2767 (1992); *c*) Y. Mimaki, Y. Sashida, O. Nakamura, T. Nikaido, T. Ohmoto, *ibid.*, **33**, 675 (1993).