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Steroidal saponins from the bulbs of *Lilium candidum*

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Abstract

Five new spirostanol saponins and a new furostanol saponin were isolated from the fresh bulbs of *Lilium candidum*. Their structures were elucidated on the basis of spectroscopic analysis, including two-dimensional NMR spectroscopic techniques and the result of acid hydrolysis. The isolated saponins contained a branched triglycoside moiety assigned as *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranose with the formation of an *O*-glycosidic linkage to C-3 of the aglycone as the common structural feature. The inhibitory activity of the saponins on Na^+/K^+ ATPase was evaluated.

Keywords: *Lilium candidum*; Liliaceae; Bulbs; Steroidal saponins; Spirostanol saponins; Furostanol saponin; Na^+/K^+ ATPase inhibition

1. Introduction

Lilium candidum is the well known 'white Madonna lily' with pure white bowl-shaped flowers that grows throughout Europe. Its original home is thought to be in the Balkans and over the centuries it has been cultivated and spread by man to other locations. The bulbs were long regarded as a medicinal material effective for the treatment of burns and swellings, being applied externally. So during the Middle Ages, *L. candidum* was preserved in monastery gardens. A survey of the literature showed that previous phytochemical studies carried out on *L. candidum* demonstrated the occurrence of dimeric pyrroline derivatives (Haladová, Eisenreichová, Bučková, Tomko, & Uhrín, 1988; Eisenreichová et al., 1992), 3-methylsuccinoylflavone (Bučková, Eisenreichová, Haladová, Uhrín, & Tomko, 1988) and pyrrolidinylflavone (Mašterová, Uhrín, & Tomko, 1987) as the characteristic components. Our systematic studies on the chemical constituents of the lily bulbs available from the markets in Japan have revealed that several species contained abundant steroidal saponins, some of which were unique in structure and that the distribution and structures of the saponins varied from species to species (Mimaki, Sashida, Nakamura, Nikaido, & Ohmoto, 1993; Nakamura, Mimaki, Nishino, & Sashida, 1994; Mimaki et al., 1994; Satou, Mimaki, Kuroda, Sashida, & Hatakeyama, 1996). As part of our chemical investigation on the *Lilium* plants, we have now examined the bulbs of *L. candidum* paying attention to the steroidal constituents, which resulted in the discovery of five new spirostanol saponins (**1**–**5**) and a new furostanol saponin (**6**). This paper deals with the structural elucidation of the new saponins by analysis of spectroscopic data and the result of acid hydrolysis. The inhibitory activity exhibited by the isolated saponins on Na^+/K^+ ATPase is also reported.

2. Results and discussion

Compound **1** was obtained as an amorphous solid, $[\alpha]_D$ −89.6° (methanol) and assigned the molecular

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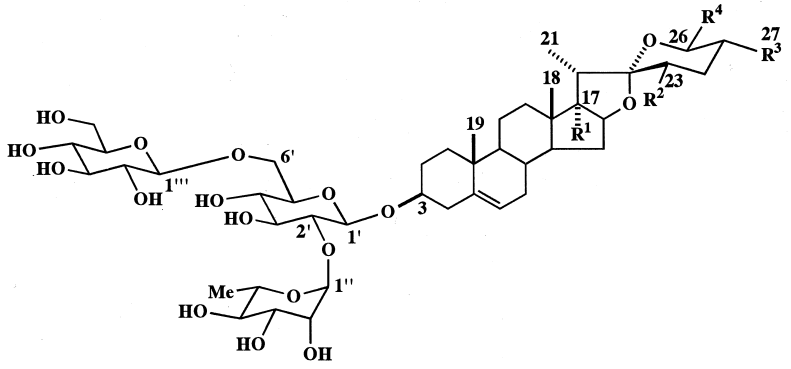
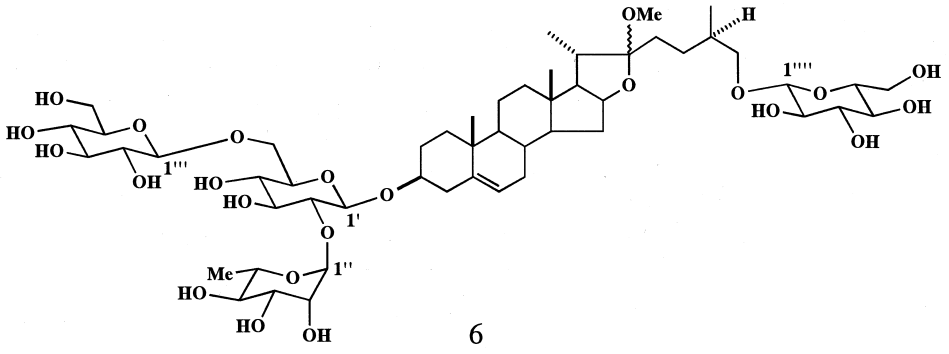


	R^1	R^2	R^3	R^4
1	H	H	Me	H
2	H	H	CH_2OH	H
3	H	OH	Me	H
4	H	H	Me	OMe
5	OH	H	Me	OMe



6

formula $C_{45}H_{72}O_{17}$ by the ^{13}C NMR data (Table 1), negative-ion FABMS showing an $[M–H]^-$ ion at m/z 883 and elemental analysis. The glycosidic nature of **1** was shown by strong IR absorptions at 3405 and 1040 cm^{-1}. The ^1H NMR spectrum of **1** (pyridine-d_5) showed two three-proton singlet signals at δ 1.05 and 0.83 and two three-proton doublets at δ 1.15 ($J=7.0$ Hz) and 0.70 ($J=5.6$ Hz), which were recognized as typical steroid methyls. Furthermore, signals for three anomeric protons at δ 6.37 (1H, br s), 5.10 (d, $J=7.8$ Hz) and 4.98 (d, $J=7.4$ Hz) and an olefinic proton at δ 5.31 (br d, $J=5.0$ Hz) could be readily assigned. Acid hydrolysis of **1** with 1 M hydrochloric acid in dioxane–H_2O (1:1) led to the production of D-glucose and L-rhamnose as the carbohydrate components and a steroidal sapogenin identified as (25*R*)-spirost-5-en-3β-ol, that is, diosgenin (Agrawal, Jain, Gupta, & Thakur, 1985). The above data were clearly indicative of **1** being a diosgenin triglycoside. The $^1H–^1H$ COSY experiments allowed the sequential assignment of the resonances for the triglycoside residue, starting from the easily distinguished anomeric protons. Multiplet patterns and measurements of coupling constants confirmed the presence of two β-D-glucopyranosyl ($^4C_1$) units and one α-L-rhamnopyranosyl ($^1C_4$) unit (Table 2). The HMQC spectrum correlated all the proton resonances with those of the corresponding one-bond coupling carbons. The one glucose and rhamnose residues were shown to be the terminal units by the absence of any glycosylation shift for their carbon resonances, which implied that the triglycoside had a branched sequence. In the HMBC spectrum, the anomeric proton signals at δ 6.37 (rhamnose), 5.10 (terminal glucose) and 4.98 (inner glucose) showed $^1H/^{13}C$ long-range correlations traversing the glycosidic linkage with the δ 77.5 (C-2 of inner glucose), 69.8

Table 1
^{13}C NMR spectral data for compounds **1**–**6**[a]

C	1	2	3	4	5	6
1	37.5	37.5	37.6	37.5	37.5	37.5
2	30.4	30.3	30.3	30.3	30.3	30.3
3	78.5	78.4	78.6	78.4	78.4	78.4
4	39.1	39.1	39.1	39.1	39.1	39.1
5	141.0	140.9	141.0	141.0	140.9	140.9
6	121.6	121.6	121.7	121.6	121.6	121.6
7	32.2	32.2	32.2	32.2	32.4	32.2
8	31.8	31.6	31.7	31.7	32.3	31.6
9	50.2	50.2	50.3	50.3	50.1	50.2
10	37.1	37.1	37.1	37.1	37.1	37.0
11	21.1	21.0	21.1	21.1	20.9	21.0
12	39.8	39.8	40.2	39.8	32.1	39.7
13	40.5	40.4	41.1	40.5	45.2	40.7
14	56.6	56.5	56.7	56.6	53.0	56.5
15	32.3	32.2	32.4	32.3	31.7	32.1
16	81.2	81.1	81.7	81.4	90.3	81.3
17	62.9	62.9	62.5	62.9	90.2	64.1
18	16.3	16.3	16.5	16.3	17.1	16.2
19	19.4	19.4	19.4	19.4	19.4	19.4
20	42.0	42.0	35.8	42.0	44.8	40.4
21	15.0	15.0	14.6	15.0	9.5	16.2
22	109.3	109.7	111.6	111.8	112.4	112.6
23	31.7	31.5	67.3	31.4	31.7	30.7
24	29.3	24.0	38.6	28.4	27.9	28.1
25	30.6	39.1	31.7	35.5	35.3	34.2
26	66.9	64.0	66.0	103.1	103.2	75.1
27	17.3	64.4	16.8	16.7	16.7	17.1
OMe				55.6	55.8	47.2
1′	100.7	100.7	100.6	100.7	100.6	100.6
2′	77.5	77.6	77.7	77.6	77.5	77.5
3′	79.5	79.4	79.3	79.5	79.5	79.4
4′	71.6	71.6	71.4	71.6	71.6	71.6
5′	76.9	76.8	76.8	76.8	76.8	76.8
6′	69.8	69.8	69.7	69.8	69.9	69.7
1″	102.0	102.0	102.0	102.0	102.0	102.0
2″	72.6	72.5	72.3	72.5	72.5	72.4
3″	72.8	72.8	72.6	72.8	72.8	72.8
4″	74.2	74.1	73.9	74.1	74.1	74.1
5″	69.5	69.4	69.5	69.4	69.4	69.4
6″	18.7	18.6	18.5	18.6	18.6	18.6
1‴	105.5	105.3	105.2	105.4	105.4	105.3
2‴	75.2	75.1	75.0	75.1	75.1	75.1
3‴	78.4	78.3	78.1	78.4	78.4	78.4
4‴	71.7	71.6	71.5	71.7	71.6	71.6
5‴	78.5	78.4	78.3	78.4	78.5	78.5
6‴	62.8	62.7	62.6	62.8	62.7	62.7
1⁗						104.9
2⁗						75.1
3⁗						78.3
4⁗						71.7
5⁗						78.4
6⁗						62.8

[a] Spectra were measured in pyridine-d_5, except for **3** in pyridine-d_5–methanol-d_4 (11:1).

(C-6 of inner glucose) and 78.5 (C-3 of aglycone) resonances, respectively. Thus, the structure of **1** was elucidated as (25*R*)-spirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside.

In the ^{13}C NMR spectra of **2**–**6**, the carbon shift assignment of the triglycoside moiety attached to C-3 of the aglycone was closely related to that of **1**, the shift value differences from **1** being less than ±0.3 ppm. This clearly indicated that the structure

Table 2
^{1}H NMR assignment of the triglycoside moiety of compound **1**[a]

position	^{1}H (ppm)	J (Hz)
1′	4.98 d	7.4
2′	4.20 dd	9.1, 7.4
3′	4.23 dd	9.1, 9.1
4′	4.13 dd	9.1, 9.1
5′	4.00 ddd	9.1, 5.4, 1.5
6′a	4.77 dd	10.8, 1.5
6′b	4.34 dd	10.8, 5.4
1″	6.37 br s	
2″	4.78 br d	3.4
3″	4.62 dd	9.3, 3.4
4″	4.35 dd	9.3, 9.3
5″	4.98 dq	9.3, 6.2
6″	1.79 d	6.2
1‴	5.10 d	7.8
2‴	4.04 dd	9.0, 7.8
3‴	4.21 dd	9.0, 9.0
4‴	4.24 dd	9.0, 9.0
5‴	3.93 ddd	9.0, 5.3, 2.2
6‴a	4.52 dd	10.8, 2.2
6‴b	4.37 dd	10.8, 5.3

[a] Spectrum was measured in pyridine-d_5.

of the triglycoside of **2**–**6** was the same as that of **1**.

The molecular formula of **2**, $C_{45}H_{72}O_{18}$, deduced from the ^{13}C NMR spectrum, negative-ion FABMS and elemental analysis, had one more oxygen atom than that of **1**. The ^{1}H NMR spectrum of **2** resembled that of **1** with resonances for the Me-18, Me-19 and Me-21 methyl groups at δ 0.84 (s), 1.05 (s) and 1.17 (d, J = 6.9 Hz), respectively. However, the Me-27 methyl doublet signal observed in the ^{1}H NMR spectrum of **1** was absent from that of **2** and was replaced by the oxymethylene signals at δ 3.74 (dd, J = 10.7, 5.3 Hz) and 3.66 (dd, J = 10.7, 7.4 Hz). The above data indicated that C-27 which was present as a methyl group in **1** was modified to a hydroxymethyl group in **2**. On treatment of **2** with 1 M hydrochloric acid, it liberated D-glucose and L-rhamnose, together with a steroidal sapogenin, which was assigned as (25*S*)-spirost-5-ene-3β,27-diol, that is, isonarthogenin (Minato, & Shimaoka, 1963; Blunden, & Patel, 1986). The structure of **2** was characterized as (25*S*)-27-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside.

Compound **3** ($C_{45}H_{72}O_{18}$) had the same molecular formula as **2**. Acid hydrolysis of **3** with 1 M hydrochloric acid gave D-glucose and L-rhamnose, together with several unidentified artifactual sapogenols; no genuine aglycone could be obtained. Tracing out the proton spin-coupling systems from the signal assignable to the Me-27 methyl group at δ 0.73 (d, J = 6.4 Hz) through analysis of the ^{1}H–^{1}H COSY spectrum combined with the HOHAHA data led to assemble the structure of the F-ring part (C-23–C-27) as -$C_{(23)}$H(O−)–$C_{(24)}H_2$–$C_{(25)}$H(Me$_{(27)}$)–$C_{(26)}H_2$(O−)-, which was connected to C-22 by the observation of a $^3J_{C,H}$ correlation from H-26eq [δ 3.51 (dd, J = 10.9, 3.3 Hz)] to C-22 (δ 111.6). In the ^{13}C NMR spectrum of **3**, the signal due to C-23 at δ 67.4 in pyridine-d_5 was slightly shifted upfield by ca. 0.12 ppm on addition of a small amount of methanol-d_4, which was produced by the deuterium effect and gave confirmative evidence for the presence of the C-23 hydroxyl group. NOE correlations between the protons of H-26ax [δ 3.43 (dd, J = 10.9, 10.9 Hz)] and H-16 [δ 4.61 (ddd, J = 8.4, 7.2, 7.2 Hz)], H-26ax and Me-27, and H-25 (δ 1.80) and H-26eq in the phase-sensitive NOESY spectrum and spin-coupling constants between H-23 and H_2-24 ($^3J_{H\text{-}23,H\text{-}24ax}$ = 11.4 Hz, $^3J_{H\text{-}23,H\text{-}24eq}$ = 4.7 Hz) and between H-25 and H_2-26 ($^3J_{H\text{-}25,H\text{-}26ax}$ = 10.9 Hz, $^3J_{H\text{-}25,H\text{-}26eq}$ = 3.3 Hz) were consistent with the 22α, 23*S* and 25*R* configurations. The structure of **3** was assigned as (23*S*,25*R*)-23-hydroxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside.

The ^{1}H NMR spectrum of **4** ($C_{46}H_{74}O_{18}$) exhibited a characteristic three-proton singlet signal attributable to a methoxyl group at δ 3.52, as well as the signals for four steroid methyls at δ 1.14 (d, J = 7.0 Hz), 1.06 (s), 0.96 (d, J = 6.1 Hz) and 0.82 (s) and three anomeric protons at δ 6.34 (br s), 5.09 (d, J = 7.8 Hz) and 4.98 (d, J = 6.9 Hz). In the ^{13}C NMR spectrum of **4**, the signals due to C-22 (δ 111.8) and C-25 (δ 35.5) were shifted downfield, whereas the signals due to C-24 (δ 28.4) and C-27 (δ 16.7) were shifted slightly upfield in comparison with those of **1**. In addition, the resonance of the C-26 oxymethylene carbon, which was observed at δ 66.9 in **1**, was replaced by a signal at δ 103.1 (CH) in **4**. The above data and comparison of the whole ^{1}H and ^{13}C NMR signals of the aglycone moiety of **4** with those of the spirostanol saponins previously isolated by us from the several *Lilium* plants (Mimaki, & Sashida, 1991; Mimaki, Ishibashi, Ori, & Sashida, 1992; Mimaki et al., 1994; Nakamura et al., 1994) allowed the identification of the aglycone structure of **4** as (25*R*,26*R*)-26-methoxyspirost-5-en-3β-ol. The J value between the protons of H-25 and H-26 (J = 8.2 Hz) was consistent with the 25*R* and 26*R* configurations. The structure of **4** was formulated as (25*R*,26*R*)-26-methoxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside.

The spectral features of **5** ($C_{46}H_{74}O_{19}$) were essentially analogous to those of **4**. The presence of a hydroxyl group at C-17 was suggested by the appearance of a quaternary carbon signal at δ 90.2, accompanied by downfield shifts of the signals due to C-13 (+4.7

ppm), C-16 (+8.9 ppm) and C-20 (+2.8 ppm) on comparison of the ^{13}C NMR spectrum of **5** with that of **4** (Shimomura, Sashida, & Mimaki, 1989; Mimaki, Nakamura, Sashida, Nikaido, & Ohmoto, 1995). This was confirmed by the isolated AB_3 spin system due to H-20 [δ 2.29 (1H, q, J=7.2 Hz)] and Me-21 [δ 1.24 (3H, d, J=7.2 Hz)] in the ^1H NMR spectrum and by two- or three-bond coupled $^1H/^{13}C$ correlations from the carbon signal at δ 90.2 to H-16 [δ 4.57 (1H, dd, J=7.0, 6.4 Hz)], Me-18 [δ 0.94 (3H, s)], H-20 and Me-21 in the HMBC spectrum. An NOE correlation between Me-18 and H-20 indicated the α-orientation of the C-17 hydroxyl group. In the ^{13}C NMR spectrum of **5**, the upfield shifts of the carbon signals at C-12 (−7.7), C-14 (−3.6) and C-21 (−5.5) in comparison with that of **4** were considered to be caused by the C-17α hydroxyl group. The structure of **5** was shown to be (25*R*,26*R*)-17α-hydroxy-26-methoxyspirost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside.

Compound **6** ($C_{52}H_{86}O_{23}$) was shown to be a 22-methoxyfurostanol saponin by Ehrlich's test (Kiyosawa et al., 1968; Nohara, Miyahara, & Kawasaki, 1975), the ^1H NMR [δ 3.28 (3H, s)] and ^{13}C NMR [δ 112.6 (C) and 47.2 (Me)] spectra (Agrawal et al., 1985). The ^1H NMR spectrum showed four anomeric proton signals at δ 6.32 (br s), 5.08 (d, J=7.9 Hz), 4.96 (d, J=7.7 Hz) and 4.85 (d, J=7.7 Hz), as well as four steroid methyl proton signals at δ 1.20 (d, J=6.9 Hz), 1.05 (s), 1.01 (d, J=6.7 Hz) and 0.82 (s). Enzymatic hydrolysis of **6** with β-glucosidase gave **1** and D-glucose. Therefore, the structure of **6** was (25*R*)-26-(β-D-glucopyranosyloxy)-22-methoxyfurost-5-en-3β-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside.

Compounds **1**–**6** are new naturally occurring steroidal saponins and contain a branched triglycoside moiety assigned as *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranose with the formation of an *O*-glycosidic linkage to C-3 of the aglycone as the common structural feature.

The inhibitory activity of **1**–**6** on Na^+/K^+ ATPase was assayed and the result is listed in Table 3.

Table 3
Inhibitory activity of **1**–**6** on Na^+/K^+ ATPase

Compounds	IC_{50} ($\times 10^{-5}$ M)
1	2.2
2	–[a]
3	–
4	4.7
5	–
6	–
ouabain	0.1

[a] $> 1.0 \times 10^{-4}$ M.

Compounds **1** and **4** exhibited considerable activity with the IC_{50} values of 2.2×10^{-5} M and 4.7×10^{-5} M, respectively, while **2**, **3**, **5** and **6** were inactive, suggesting that introduction of hydroxyl group onto the aglycone significantly reduced the activity.

3. Experimental

3.1. General

NMR (ppm, *J* Hz): Bruker AM-400 (400 MHz for ^1H NMR), Bruker AM-500 (500 MHz for ^1H NMR), or Bruker DRX-500 (500 MHz for ^1H NMR). CC: silica gel (Fuji-Silysia Chemical) and octadecylsilanized (ODS) silica gel (Nacalai Tesque). TLC: precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) and RP-18 $F_{254}S$ (0.25 mm thick, Merck). HPLC: a Tosoh HPLC system (pump, CCPM; controller, CCP controller PX-8010; detector, UV-8000) equipped with a Kaseisorb LC ODS-120-5 column (Tokyo-Kasei-Kogyo, 4.6 mm i.d. × 250 mm, ODS, 5 μm). Ouabain sensitive dog kidney Na^+/K^+ ATPase: Sigma (USA). All other chemicals used were of biochemical reagent grade.

3.2. Plant material

The bulbs of *L. candidum* were provided from Hokkaido Experiment Station of Medicinal Plants (Japan). The bulbs were cultivated and a plant specimen is on file in our laboratory.

3.3. Extraction and isolation

The plant material (fresh weight, 2.0 kg) was extracted with hot MeOH. The MeOH extract was concd under reduced pressure and the viscous concentrate was partitioned between H_2O and *n*-BuOH. The *n*-BuOH-soluble phase was fractionated by silica gel CC using a mobile phase composed of $CHCl_3$–MeOH (9:1; 6:1; 4:1; 3:1; 2:1; 1:1) and finally with MeOH alone to collect seven fractions (I–VII). Fr. VI was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (20:10:1) and ODS silica gel with MeOH–H_2O (4:1) to yield **1** (19.2 mg) and **4** (81.1 mg). Fr. VII was subjected to CC on silica gel eluting with $CHCl_3$–MeOH–H_2O (20:10:1) and ODS silica gel with MeOH–H_2O (8:3) and MeCN–H_2O (1:2) to yield **2** (61.9 mg), **3** (24.8 mg), **5** (33.2 mg) and **6** (41.4 mg).

3.4. Compound 1

Amorphous solid. $[\alpha]_D^{29}$ −89.6° (MeOH; *c* 0.27). (Found: C, 58.76; H, 8.68. Calc. for $C_{45}H_{72}O_{17}\cdot 2H_2O$: C, 58.68; H, 8.32%). Negative-ion FABMS *m/z* 883 $[M-H]^-$. IR ν_{max} (KBr) cm^{-1}: 3405 (OH), 2940 (CH),

1040. ^{1}H NMR (pyridine-d_5): δ 6.37 (1H, br s, H-1″), 5.31 (1H, br d, J=5.0 Hz, H-6), 5.10 (1H, d, J=7.8 Hz, H-1‴), 4.98 (1H, d, J=7.4 Hz, H-1′), 3.60 (1H, dd, J=10.7, 3.1 Hz, H-26eq), 3.51 (1H, dd, J=10.7, 10.7 Hz, H-26ax), 1.79 (3H, d, J=6.2 Hz, Me-6″), 1.15 (3H, d, J=7.0 Hz, Me-21), 1.05 (3H, s, Me-19), 0.83 (3H, s, Me-18), 0.70 (3H, d, J=5.6 Hz, Me-27).

3.5. Acid hydrolysis of *1*

A soln of **1** (3.2 mg) in 1 M HCl (dioxane–H_2O, 1:1, 2 ml) was heated at 100° for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column and chromatographed on silica gel eluting with $CHCl_3$–MeOH (9:1; 1:1) to give an aglycone (diosgenin, 0.9 mg) and a sugar fraction (1.1 mg). The sugar fraction was dissolved in H_2O (1 ml), to which (−)-α-methylbenzylamine (5 mg) and Na[BH_3CN] (8 mg) in EtOH (1 ml) were added. After being set aside at 40° for 4 h followed by addition of AcOH (0.2 ml) and evaporation to dryness, the reaction mixture was acetylated with Ac_2O (0.3 ml) in pyridine (0.3 ml) at room temperature for 12 h. The crude mixture was passed through a Sep-Pak C_{18} cartridge with H_2O–MeCN (4:1; 1:1, each 5 ml) mixtures as solvents. The H_2O–MeCN (1:1) eluate was further passed through a Toyopak IC–SP M cartridge (Tosoh) with EtOH (10 ml) to give a mixture of the 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides (Oshima, & Kumanotani, 1981; Oshima, Yamauchi, & Kumanotani, 1982), which was then analyzed by HPLC under the following conditions: solvent, MeCN–H_2O (2:3); flow rate, 0.8 ml min^{-1}; detection, UV 230 nm. The derivatives of D-glucose and L-rhamnose were detected. R_t (min): 30.94 (derivative of D-glucose); 34.92 (derivative of L-rhamnose).

3.6. Compound *2*

Amorphous solid. $[\alpha]_D^{27}$ −44.2° (MeOH–H_2O, 1:1; *c* 0.12). (Found: C, 57.81; H, 7.87. Calc. for $C_{45}H_{72}O_{18}\cdot 2H_2O$: C, 57.68; H, 8.17%). Negative-ion FABMS *m/z* 899 [M–H]$^-$. IR ν_{max} (KBr) cm^{-1}: 3405 (OH), 2940 (CH), 1035. ^{1}H NMR (pyridine-d_5): δ 6.32 (1H, br s, H-1″), 5.32 (1H, br d, J=5.0 Hz, H-6), 5.09 (1H, d, J=7.8 Hz, H-1‴), 4.96 (1H, d, J=6.9 Hz, H-1′), 3.74 (1H, dd, J=10.7, 5.3 Hz, H-27a), 3.66 (1H, dd, J=10.7, 7.4 Hz, H-27b), 1.78 (3H, d, J=6.2 Hz, Me-6″), 1.17 (3H, d, J=6.9 Hz, Me-21), 1.05 (3H, s, Me-19), 0.84 (3H, s, Me-18).

3.7. Acid hydrolysis of *2*

Compound **2** (5.1 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone (isonarthogenin, 1.2 mg) and a mixture of monosaccharides (1.9 mg). The monosaccharides were identified as D-glucose and L-rhamnose by HPLC analysis of their corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives.

3.8. Compound *3*

Amorphous solid. $[\alpha]_D^{26}$ −41.5° (pyridine; *c* 0.28). (Found: C, 55.82; H, 8.68. Calc. for $C_{45}H_{72}O_{18}\cdot 7/2H_2O$: C, 56.06; H, 8.26%). Negative-ion FABMS *m/z* 899 [M–H]$^-$. IR ν_{max} (KBr) cm^{-1}: 3405 (OH), 2930 (CH), 1040. ^{1}H NMR (pyridine-d_5): δ 6.32 (1H, br s, H-1″), 5.30 (1H, br d, J=5.0 Hz, H-6), 5.08 (1H, d, J=7.7 Hz, H-1‴), 4.95 (1H, d, J=7.1 Hz, H-1′), 3.56 (1H, dd, J=10.6, 3.1 Hz, H-26eq), 3.49 (1H, dd, J=10.6, 10.6 Hz, H-26ax), 1.77 (3H, d, J=6.2 Hz, Me-6″), 1.21 (3H, d, J=7.0 Hz, Me-21), 1.01 (3H, s, Me-19), 1.00 (3H, s, Me-18), 0.74 (3H, d, J=5.9 Hz, Me-27). ^{1}H NMR (pyridine-d_5–methanol-d_4, 11:1): δ 6.15 (1H, br s, H-1″), 5.29 (1H, br d, J=4.9 Hz, H-6), 4.98 (1H, d, J=7.8 Hz, H-1‴), 4.88 (1H, d, J=7.5 Hz, H-1′), 4.61 (1H, ddd, J=8.4, 7.2, 7.2 Hz, H-16), 3.79 (1H, dd, J=11.4, 4.7 Hz, H-23), 3.51 (1H, dd, J=10.9, 3.3 Hz, H-26eq), 3.43 (1H, dd, J=10.9, 10.9 Hz, H-26ax), 2.95 (1H, m, H-20), 2.05 (1H, H-24eq), 1.80 (1H, H-25), 1.73 (1H, H-24ax), 1.69 (3H, d, J=6.2 Hz, Me-6″), 1.16 (3H, d, J=7.0 Hz, Me-21), 0.98 (3H, s, Me-19), 0.97 (3H, s, Me-18), 0.73 (3H, d, J=6.4 Hz, Me-27). ^{13}C NMR (pyridine-d_5): δ 67.3795 (C-23). ^{13}C NMR (pyridine-d_5 + methanol-d_4): δ 67.2549 (C-23).

3.9. Acid hydrolysis of *3*

Compound **3** (4.8 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone fraction (1.5 mg) and a mixture of monosaccharides (2.2 mg). The monosaccharides were identified as D-glucose and L-rhamnose by HPLC analysis of their corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives.

3.10. Compound *4*

Amorphous solid. $[\alpha]_D^{27}$ −47.1° (MeOH–H_2O, 1:1; *c* 0.14). (Found: C, 58.53; H, 8.54. Calc. for $C_{46}H_{74}O_{18}\cdot 3/2H_2O$: C, 58.65; H, 8.24%). Negative-ion FABMS *m/z* 913 [M–H]$^-$. IR ν_{max} (KBr) cm^{-1}: 3405 (OH), 2945 (CH), 1035. ^{1}H NMR (pyridine-d_5): δ 6.34 (1H, br s, H-1″), 5.33 (1H, br d, J=4.9 Hz, H-6), 5.09 (1H, d, J=7.8 Hz, H-1‴), 4.98 (1H, d, J=6.9 Hz, H-1′), 4.49 (1H, dd, J=8.2 Hz, H-26), 3.52 (3H, s, OMe), 1.79 (3H, d, J=6.2 Hz, Me-6″), 1.14 (3H, d,

$J=7.0$ Hz, Me-21), 1.06 (3H, s, Me-19), 0.96 (3H, d, $J=6.1$ Hz, Me-27), 0.82 (3H, s, Me-18).

3.11. Compound **5**

Amorphous solid. $[\alpha]_D^{27}$ −42.1° (MeOH–H_2O, 1:1; *c* 0.14). (Found: C, 57.06; H, 8.23. Calc. for $C_{46}H_{74}O_{19}\cdot 2H_2O$: C, 57.13; H, 8.13%). Negative-ion FABMS *m/z* 929 $[M-H]^-$. IR ν_{max} (KBr) cm^{-1}: 3405 (OH), 2935 (CH), 1035. ^1H NMR (pyridine-d_5): δ 6.32 (1H, br s, H-1″), 5.29 (1H, br d, $J=5.0$ Hz, H-6), 5.07 (1H, d, $J=7.8$ Hz, H-1‴), 4.95 (1H, d, $J=7.4$ Hz, H-1′), 4.57 (1H, dd, $J=7.0$, 6.4 Hz, H-16), 4.55 (1H, d, $J=8.1$ Hz, H-26), 3.45 (3H, s, OMe), 2.29 (1H, q, $J=7.2$ Hz, H-20), 1.77 (3H, d, $J=6.2$ Hz, Me-6″), 1.24 (3H, d, $J=7.2$ Hz, Me-21), 1.09 (3H, s, Me-19), 0.96 (3H, d, $J=6.2$ Hz, Me-27), 0.94 (3H, s, Me-18).

3.12. Compound **6**

Amorphous solid. $[\alpha]_D^{29}$ −69.0° (MeOH; *c* 0.29). (Found: C, 56.48; H, 8.26. Calc. for $C_{52}H_{86}O_{23}\cdot 3/2H_2O$: C, 56.46; H, 8.11%). Negative-ion FABMS *m/z* 1077 $[M-H]^-$. IR ν_{max} (KBr) cm^{-1}: 3405 (OH), 2935 (CH), 1060, 1035. ^1H NMR (pyridine-d_5): δ 6.32 (1H, br s, H-1″), 5.33 (1H, br d, $J=5.0$ Hz, H-6), 5.08 (1H, d, $J=7.9$ Hz, H-1‴), 4.96 (1H, d, $J=7.7$ Hz, H-1′), 4.85 (1H, d, $J=7.7$ Hz, H-1⁗), 3.28 (3H, s, OMe), 1.78 (3H, d, $J=6.1$ Hz, Me-6″), 1.20 (3H, d, $J=6.9$ Hz, Me-21), 1.05 (3H, s, Me-19), 1.01 (3H, d, $J=6.7$ Hz, Me-27), 0.82 (3H, s, Me-18).

3.13. Enzymatic hydrolysis of **6**

Compound **6** (5.9 mg) was treated with β-glucosidase (5.2 mg) in HOAc/NaOAc buffer (pH 5, 2 ml) at room temperature for 12 h. The reaction mixture was chromatographed on ODS silica gel eluting with MeCN–H_2O (5:6) to yield **11** (4.1 mg) and D-glucose. D-Glucose: TLC, R_f 0.49 (*n*-BuOH–Me_2CO–H_2O, 4:5:1).

3.14. Assay of Na^+/K^+ ATPase activity

The Na^+/K^+ ATPase activity was assayed according to the reported method (Esmann, 1988) with some modification. The reaction mixture composed of 50 mM Tris–HCl (pH 7.3, 37°), 3 mM ATP, 4 mM Mg^{2+}, 130 mM Na^+, 20 mM K^+ and 0.02 units of Na^+/K^+ ATPase, with or without test compound dissolved in DMSO, was incubated for 15 min at 37°. The concentration of DMSO in the reaction mixture was held at 5%. The reaction was terminated by addition of 50% CCl_3COOH. The released inorganic phosphate was determined by a modification of the method of reference (Fiske, & Subbarow, 1925). To the test soln was added 0.5% sodium dodecyl sulfate, 0.1% 2,4-diaminophenol·2HCl in 1% Na_2SO_3 and 1% ammonium heptamolybdate in 1 M H_2SO_4. After 20 min, the absorbance at 660 nm was recorded.

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