New Steroidal Constituents from the Bulbs of *Lilium candidum*

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Eight spirostanol saponins, including four new compounds, and two known furostanol saponins were isolated from the fresh bulbs of *Lilium candidum*. The structures of new compounds were determined to be (25*R*,26*R*)-26-methoxyspirost-5-ene-3β,17α-diol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside}, (25*R*,26*R*)-26-methoxyspirost-5-ene-3β,17α-diol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[6-*O*-acetyl-β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside}, (25*R*,26*R*)-26-methoxyspirost-5-ene-3β,17α-diol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside} and (25*S*)-spirost-5-ene-3β,27-diol 3-*O*-{*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside}, respectively, on the basis of spectroscopic analysis, including two-dimensional NMR techniques, and the result of hydrolysis. The inhibitory activity of the isolated saponins on Na^+/K^+ ATPase was evaluated.

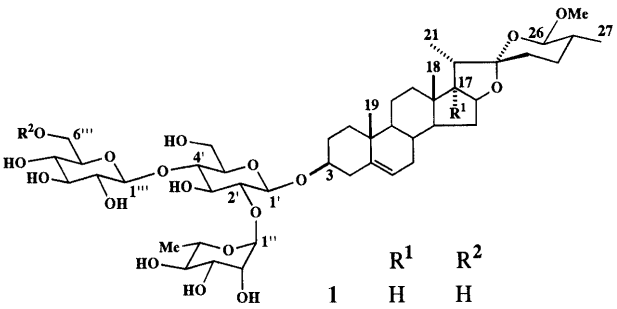
Key words *Lilium candidum*; Liliaceae; bulb; steroidal saponin; spirostanol saponin; Na^+/K^+ ATPase inhibition

Lilium candidum grows throughout Europe and goes by the name of white Madonna lily. As part of our systematic study on the steroidal constituents with biological activity from plants of the genus *Lilium*,[1] we have now undertaken an investigation of the fresh bulbs of *L. candidum* and isolated eight spirostanol saponins, four of which appeared to be new compounds, and two known furostanol saponins. This paper deals with the identification and structural assignment of the isolated saponins on the basis of spectroscopic analysis, including two-dimensional NMR techniques, and the result of hydrolysis, and their inhibitory activity on Na^+/K^+ ATPase.

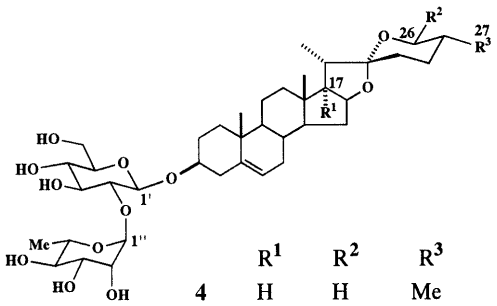
The methanolic extract of the fresh bulbs of *L. candidum* (2.0 kg) was partitioned between 1-butanol and H_2O. The 1-butanol-soluble portion was subjected to successive chromatographies on a silica gel column followed by a reversed-phase [octadecylsilanized (ODS) silica gel] column to afford **1** (14.1 mg), **2** (86.3 mg), **3** (16.7 mg), **4** (5.0 mg), **5** (3.3 mg), **6** (8.2 mg), **7** (21.8 mg), **8** (14.4 mg), **9** (54.7 mg) and **10** (20.3 mg).

Compounds **1**, **4**—**6**, **9** and **10** were known steroidal saponins and identified as (25*R*,26*R*)-26-methoxyspirost-5-en-3β-ol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside},[2] (25*R*)-spirost-5-en-3β-ol (diosgenin) 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside},[3] (25*S*)-spirost-5-ene-3β,27-diol (isonarthogenin) 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside},[4] (25*R*,26*R*)-26-methoxyspirost-5-en-3β-ol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside},[5] (25*R*)-26-*O*-β-D-glucopyranosyl-22-*O*-methylfurost-5-ene-3β,22ξ,26-triol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside}[6] and (25*R*)-26-*O*-β-D-glucopyranosyl-22-*O*-methylfurost-5-ene-3β,22ξ,26-triol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside},[7] respectively.

Compound **2** was isolated as an amorphous solid, $[\alpha]_D$ −42.1° (MeOH–H_2O, 1 : 1). The molecular formula was deduced as $C_{46}H_{74}O_{19}$, which had one more oxygen atom than **1**, from an $[M-H]^-$ peak observed at *m/z* 929 in the negative-ion FAB-MS, the ^{13}C-NMR data showing a total of 46 carbon signals, and from the result of elemental analysis. The



	R^1	R^2
1	H	H
2	OH	H
3	OH	Ac



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

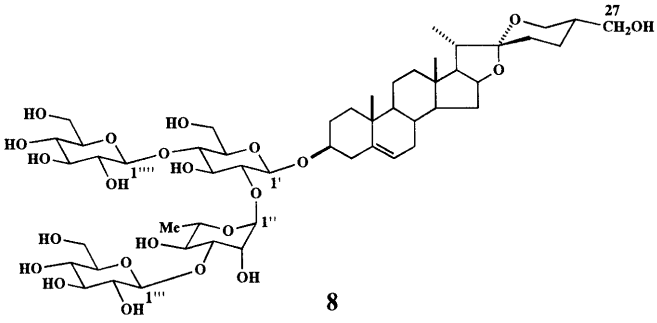


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Chart 1

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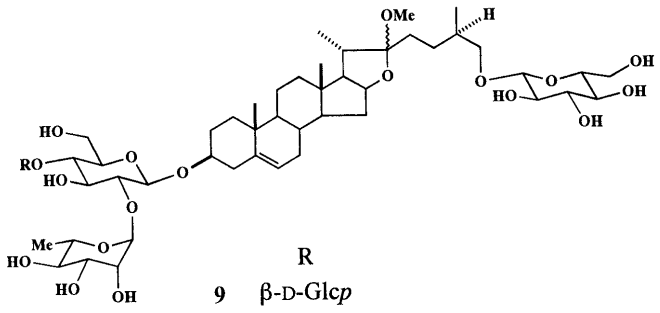

	R
9	β-D-Glc*p*
10	H

Chart 2

glycosidic nature of **2** was shown by strong IR absorptions at 3405 and 1030 cm^{-1}. The ^{1}H-NMR spectrum in pyridine-d_5 displayed signals for four steroid methyl groups at δ 1.24 (3H, d, J=7.1 Hz), 1.09 (3H, s), 0.96 (3H, d, J=6.4 Hz) and 0.95 (3H, s), a methoxyl group at δ 3.45 (3H, s), an olefinic proton at δ 5.29 (1H, br d, J=4.8 Hz), and three anomeric protons at δ 6.24 (1H, d, J=1.3 Hz), 5.12 (1H, d, J=7.9 Hz) and 4.94 (1H, d, J=6.7 Hz), which were essentially analogous to those of **1**. The identity of the branched triglycoside moiety linked to C-3 of the aglycone of **2** with that of **1** was shown by the precise agreement of their ^{13}C-NMR shifts. The presence of an additional hydroxyl group in **2** was suggested by the appearance of a quaternary carbon signal at δ 90.2,[8] accompanied by downfield shifts of the signals due to C-13 [δ 45.2 (+4.7 ppm)], C-16 [δ 90.3 (+8.9 ppm)] and C-20 [δ 44.8 (+2.8 ppm)] on comparison of the ^{13}C-NMR spectrum of **2** with that of **1**. This was confirmed by the isolated AB_3 spin system due to H-20 [δ 2.30 (1H, q, J=7.1 Hz)] and Me-21 [δ 1.24 (3H, d, J=7.1 Hz)] in the ^{1}H-NMR spectrum, and by two- or three-bond coupled ^{1}H–^{13}C correlations from the carbon signal at δ 90.2 to the H-16 [δ 4.57 (1H, t-like, J=7.0 Hz)], Me-18 [δ 0.95 (3H, s)], H-20 and Me-21 resonances in the ^{1}H-detected heteronuclear multiple-bond connectivities (HMBC) spectrum. A nuclear Overhauser effect (NOE) correlation between Me-18 and H-20 in the phase-sensitive NOE correlation spectroscopy (PHNOESY) spectrum gave evidence of the α-orientation of the C-17 hydroxyl group. In the ^{13}C-NMR spectrum of **2**, the signals attributable to C-12 (δ 32.1), C-14 (δ 53.1) and C-21 (δ 9.5) were displaced upfield by 7.7, 3.5 and 5.5 ppm, respectively, in comparison with those of **1**, and this was thought to be caused by the 1,3-diaxial-like interactions with the C-17α hydroxyl group. 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.[5] Thus, the structure of **2** was determined to be (25*R*,26*R*)-26-methoxyspirost-5-ene-3β,17α-diol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside}.

The spectral features of **3** ($C_{48}H_{76}O_{20}$) were quite similar to those of **2**. The existence of an acetyl group in the molecule of **3** was indicated by the IR (1730 cm^{-1}), ^{1}H-NMR [δ 2.06 (3H, s)] and ^{13}C-NMR [δ 170.9 (C=O), 20.7 (Me)] spectra. When **3** was treated with 4% potassium hydroxide in ethanol, it was hydrolyzed to yield **2**. Therefore, **3** was found to be a monoacetate of **2**. The ^{1}H- and ^{13}C-NMR resonances for the aglycone moiety of **3** completely agreed with those of **2**, suggesting that the ester linkage with acetic acid was formed at a hydroxyl group of the triglycoside moiety. The ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) experiment allowed the sequential assignment of the ^{1}H-NMR signals for the triglycoside residue, the easily distinguished anomeric protons at δ 6.23 (d, J=1.9 H), 5.07 (d, J=7.9 Hz) and 4.96 (d, J=7.8 Hz) being used as the starting point of analysis. Subsequent inspection of the ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectrum led to the correlation of all the proton resonances with those of the corresponding one-bond coupled carbons. On comparison of the ^{13}C-NMR shift thus assigned with those of **2**, the acylation shifts could be identified at C-6 and its adjacent carbon of C-5 of the terminal glucose moiety attached to C-4 of the inner glucose, the signal due to C-6 being shifted to lower field by 2.7 ppm, whereas the signal due to C-5 to upper field by 3.4 ppm. Furthermore, a ^{1}H–^{13}C long-range correlation was detected between the signals of the H-6b proton [δ 4.61 (dd, J=11.9, 8.3 Hz)] of the terminal glucose and the carbonyl carbon of the acetyl group. Accordingly, the acetyl group was shown to be linked to the terminal glucose C-6 hydroxy position, and the structure of **3** was assigned as (25*R*,26*R*)-26-methoxyspirost-5-ene-3β,17α-diol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[6-*O*-acetyl-β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside}.

The ^{1}H- and ^{13}C-NMR spectra of **7** ($C_{40}H_{64}O_{14}$) were completely identical to those of **3** with resonances for the aglycone moiety. However, the six signals due to the terminal glucopyranosyl moiety identified in the ^{1}H- and ^{13}C-NMR spectra of **3** were absent from those of **7**, accompanied by lack of a glycosylation shift observed at C-4 of the inner glucose moiety. The ^{13}C-NMR assignment of the saccharide moiety was superimposable on those of **6**. The structure of **7** was shown to be (25*R*,26*R*)-26-methoxyspirost-5-ene-3β,17α-diol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside}.

The ^{1}H-NMR spectrum of compound **8** ($C_{51}H_{82}O_{23}$) showed signals for three steroid methyls at δ 1.16 (d, J=6.9 Hz), 1.07 (s) and 0.81 (s), and four anomeric protons at δ 6.21 (br s), 5.52 (d, J=7.7 Hz), 5.13 (d, J=7.8 Hz) and 4.92 (d, J=6.7 Hz). Four anomeric carbon signals and a quaternary carbon signal assignable to C-22 of the spirostanol skeleton could be observed at δ 106.9 (CH), 105.2 (CH), 101.7 (CH) and 100.0 (CH), and 109.7 (C), respectively, in the ^{13}C-NMR spectrum of **8**. Acid hydrolysis of **8** with 1 M hydrochloric acid in dioxane–H_2O (1 : 1) gave D-glucose and L-rhamnose as the carbohydrate compounds, together with a known steroidal sapogenin, (25*S*)-spirost-5-ene-3β,27-diol, that is, isonarthogenin.[9] The above data were indicative of **8** being isonarthogenin tetraglycoside. Mild acid hydrolysis of **8** with 0.2 M hydrochloric acid at 85 °C for 30 min gave a partial hydrolysate (**8a**), identified as isonarthogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside}, which we previously prepared from *Lilium brownii* var. *colchesteri*.[10] On comparison of the whole ^{13}C-NMR spectrum of **8** with that of **8a**, a set of six additional signals corresponding to a terminal β-D-glucopyranosyl unit could be identified in **8**. Furthermore, it was observed that the signal of the C-3 carbon of the rhamnose was markedly displaced downfield by 11.1 ppm to appear at δ 83.9, accompanied by slight upfield shifts of the signals due to its adjacent carbons of C-2 and C-4 by 0.7 and 1.1 ppm,

Table 1. ^{13}C-NMR Spectral date for Compounds **1**—**3**, **6**—**8** and **8a**[a)]

C	1	2	3	6	7	8	8a
1	37.5	37.5	37.5	37.5	37.5	37.4	37.5
2	30.1	30.1	30.1	30.2	30.1	30.1	30.2
3	78.1	78.1	78.1	77.9	77.8	78.2	78.2
4	38.9	38.9	38.9	39.0	38.9	39.1	39.0
5	140.8	140.8	140.8	140.9	140.8	140.9	140.9
6	121.8	121.8	121.8	121.7	121.7	122.0	121.8
7	32.2	32.5	32.5	32.2	32.4	32.2	32.3
8	31.7	32.3	32.3	31.7	32.3	31.6	31.7
9	50.4	50.2	50.2	50.3	50.2	50.2	50.4
10	37.1	37.1	37.1	37.1	37.1	37.1	37.2
11	21.1	20.9	20.9	21.1	20.9	21.1	21.1
12	39.8	32.1	32.2	39.8	32.1	39.8	39.9
13	40.5	45.2	45.2	40.5	45.2	40.4	40.5
14	56.6	53.1	53.1	56.7	53.1	56.6	56.7
15	32.3	31.7	31.7	32.3	31.6	32.2	32.3
16	81.4	90.3	90.4	81.4	90.2	81.1	81.2
17	62.9	90.2	90.3	62.9	90.2	62.8	63.0
18	16.3	17.1	17.1	16.3	17.1	16.3	16.3
19	19.4	19.4	19.4	19.4	19.4	19.4	19.4
20	42.0	44.8	44.8	42.0	44.8	42.0	42.1
21	15.0	9.5	9.5	15.0	9.5	15.0	15.0
22	111.8	112.5	112.5	111.8	112.4	109.7	109.7
23	31.4	31.7	31.7	31.4	31.6	31.5	31.6
24	28.3	27.9	28.0	28.3	27.9	24.0	24.1
25	35.5	35.3	35.3	35.5	35.3	39.1	39.2
26	103.1	103.3	103.3	103.1	103.2	64.0	64.1
27	16.6	16.7	16.7	16.7	16.7	64.4	64.5
OMe	55.6	55.9	55.9	55.6	55.8		
1′	100.0	99.9	99.9	100.3	100.3	100.0	100.0
2′	77.3	77.3	77.3	77.9	77.8	77.2	77.4
3′	77.7	77.7	77.7	79.6	79.6	77.5	77.8
4′	82.0	82.0	83.3	71.8	71.8	82.0	82.1
5′	76.2	76.2	76.0	78.2	78.2	76.2	76.2
6′	61.9	61.9	62.0	62.6	62.6	61.9	62.1
1″	101.8	101.8	102.0	102.1	102.0	101.7	101.8
2″	72.4	72.4	72.4	72.5	72.5	71.8	72.5
3″	72.8	72.8	72.8	72.8	72.8	83.9	72.8
4″	74.1	74.1	74.1	74.1	74.1	73.1	74.2
5″	69.4	69.5	69.5	69.5	69.4	69.2	69.5
6″	18.6	18.6	18.7	18.6	18.6	18.5	18.7
1‴	105.2	105.2	105.5			106.9	105.2
2‴	75.0	74.9	74.8			75.9	75.0
3‴	78.3	78.3	78.1			78.4	78.3
4‴	71.2	71.2	71.9			71.2	71.3
5‴	78.5	78.5	75.1			78.4	78.5
6‴	62.1	62.1	64.8			62.2	62.2
1⁗						105.2	
2⁗						74.9	
3⁗						78.4	
4⁗						71.2	
5⁗						78.4	
6⁗						62.0	
Ac			170.9				
			20.7				

a) Spectra were measured in pyridine-d_5.

respectively, as compared with those of **8a**, indicating that the C-3 position of the rhamnose was the glycosylated position to which the additional D-glucose was linked. This was well supported by the agreement of the ^{13}C-NMR shifts of the rhamnose moiety of **8** with those of the steroidal saponins having a 3-*O*-glucosylated α-L-rhamnopyranosyl moiety.[11] The structure of **8** was formulated as isonarthogenin 3-*O*-{*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside}.

Table 2. ^{1}H-NMR Assignment of the Triglycoside Moiety of Compounds **2** and **3**[a)]

position	2		3	
	^{1}H (ppm)	*J* (Hz)	^{1}H (ppm)	*J* (Hz)
1′	4.94 d	6.7	4.96 d	7.8
2′	4.23		4.21 dd	8.8, 7.8
3′	4.22		4.27 dd	8.8, 8.8
4′	4.20 dd	8.9, 8.9	4.16 dd	8.8, 8.8
5′	3.84 ddd	8.9, 3.9, 2.6	3.88 ddd	8.8, 3.9, 2.4
6′ a	4.51 dd	12.1, 3.9	4.51 dd	12.1, 3.9
b	4.45 dd	12.1, 2.6	4.47 dd	12.1, 2.4
1″	6.24 d	1.3	6.23 d	1.9
2″	4.74 dd	3.5, 1.3	4.76 dd	3.5, 1.9
3″	4.58 dd	9.3, 3.5	4.59 dd	9.4, 3.5
4″	4.34 dd	9.3, 9.3	4.34 dd	9.4, 9.4
5″	4.95 dq	9.3, 6.2	4.93 dq	9.4, 6.2
6″	1.76 d	6.2	1.78 d	6.2
1‴	5.12 d	7.9	5.07 d	7.9
2‴	4.05 dd	9.2, 7.9	4.02 dd	9.3, 7.9
3‴	4.21 dd	9.2, 9.2	4.18 dd	9.3, 9.3
4‴	4.27 dd	9.2, 9.2	3.95 dd	9.3, 9.3
5‴	3.97 ddd	9.2, 5.1, 2.3	4.12 ddd	9.3, 8.3, 2.0
6‴ a	4.46 dd	11.5, 2.3	4.92 dd	11.9, 2.0
b	4.33 dd	11.5, 5.1	4.61 dd	11.9, 8.3
Ac			2.06 s	

a) Spectra were measured in pyridine-d_5.

Compounds **2**, **3**, **7** and **8** are new naturally occurring spirostanol saponins.

Compounds **1**, **4** and **6** were found to inhibit ouabain sensitive Na^+/K^+ ATPase with the IC_{50} values of 2.3×10^{-5}, 1.4×10^{-5} and 1.8×10^{-5} M, respectively, while the others were inactive (IC_{50} 1×10^{-4} M <). This result suggested that the introduction of a hydroxyl group onto the aglycone moiety significantly reduced the activity. It seems worth subjecting **1**, **4** and **6** to the screening of leukemia cell differentiation associated with Na^+/K^+ ATPase inhibition.[12]

Experimental

Optical rotations were measured using a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded on a JASCO A-100 spectrophotometer and MS on a VG AutoSpec E instrument. Elemental analysis was carried out with an Elementar Vario EL elemental analyzer. NMR spectra were recorded on a Bruker AM-400 spectrometer (400 MHz for ^{1}H-NMR) or a Bruker DRX-500 (500 MHz for ^{1}H-NMR) using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as internal standard. silica gel (Fuji-Silysia Chemical), ODS silica gel (Nacalai Tesque) and Toyopearl HW-40 (Tosoh) 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 the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed using a Tosoh HPLC system comprising a CCPM pump, a CCP controller PX-8010, a UV-8000 detector and Rheodyne injection port with a 20 μl loop. A Capcell Pak C_{18} column (Shiseido, 4.6 mm i.d. ×250 mm, ODS, 5 μm) was used for HPLC analysis. Ouabain sensitive dog kidney Na^+/K^+ ATPase was obtained from Sigma (U.S.A.). All other chemicals used were of biochemical reagent grade.

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

Extraction and Isolation The plant material (fresh weight, 2.0 kg) was extracted with hot MeOH. The MeOH extract was concentrated 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 column chromatography 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). Fraction II was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (40 : 10 : 1) and ODS silica gel with MeOH–H_2O (4 : 1) and MeCN–H_2O (1 : 1) to give **3** (16.7 mg), **4** (5.0 mg) and **6** (8.2 mg). Fraction III was subjected to a silica gel column eluting with $CHCl_3$–MeOH–H_2O (40 : 10 : 1) and ODS silica gel with MeCN–H_2O (1 : 1; 5 : 7; 1 : 2) to give **7** (21.8 mg). Each of fractions IV and V was subjected to silica gel column chromatography eluting with $CHCl_3$–MeOH–H_2O (30 : 10 : 1) and ODS silica gel column chromatography with MeOH–H_2O (4 : 1) and MeCN–H_2O (5 : 7; 1 : 2) to result in the isolation of **5** (3.3 mg), and **1** (14.1 mg) and **2** (86.3 mg), respectively. Fraction VII was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (20 : 10 : 1), ODS silica gel with MeOH–H_2O (2 : 1) and MeCN–H_2O (1 : 2; 5 : 12), and on Toyopearl HW-40 with MeOH to provide **8** (14.4 mg), **9** (54.7 mg) and **10** (20.3 mg).

Compound **2**: Amorphous solid, $[\alpha]_D^{27}$ −42.1° (MeOH–H_2O, 1 : 1, c=0.14). Negative-ion FAB-MS *m/z*: 929 $[M-H]^-$. *Anal.* Calcd for $C_{46}H_{74}O_{19} \cdot 5/2H_2O$: C, 56.60; H, 8.16. Found: C, 56.83; H, 8.18. IR (KBr) ν_{max} cm^{-1}: 3405 (OH), 2940 (CH), 1030. ^{1}H-NMR (pyridine-d_5) δ: 6.24 (1H, d, J=1.3 Hz, H-1″), 5.29 (1H, br d, J=4.8 Hz, H-6), 5.12 (1H, d, J=7.9 Hz, H-1‴), 4.94 (1H, d, J=6.7 Hz, H-1′), 4.57 (1H, t-like, J=7.0 Hz, H-16), 4.55 (1H, d, J=8.2 Hz, H-26), 3.85 (1H, m, H-3), 3.45 (3H, s, OMe), 2.30 (1H, q, J=7.1 Hz, H-20), 1.76 (3H, d, J=6.2 Hz, Me-6″), 1.24 (3H, d, J=7.1 Hz, Me-21), 1.09 (3H, s, Me-19), 0.96 (3H, d, J=6.4 Hz, Me-27), 0.95 (3H, s, Me-18).

Compound **3**: Amorphous solid, $[\alpha]_D^{27}$ −36.7° (MeOH–H_2O, 1 : 1, c=0.15). Negative-ion FAB-MS *m/z*: 971 $[M-H]^-$, 929 $[M-Ac]^-$, 767 $[M-glucosyl-Ac]^-$. *Anal.* Calcd for $C_{48}H_{76}O_{20} \cdot 3/2H_2O$: C, 57.64; H, 7.96. Found: C, 57.69; H, 8.30. IR (KBr) ν_{max} cm^{-1}: 3445 (OH), 2935 (CH), 1730 (C=O), 1025. ^{1}H-NMR (pyridine-d_5) δ: 6.23 (1H, d, J=1.9 Hz, H-1″), 5.30 (1H, br d, J=4.9 Hz, H-6), 5.07 (1H, d, J=7.9 Hz, H-1‴), 4.96 (1H, d, J=7.8 Hz, H-1′), 4.57 (1H, t-like, J=6.8 Hz, H-16), 4.55 (1H, d, J=8.2 Hz, H-26), 3.86 (1H, m, H-3), 3.45 (3H, s, OMe), 2.30 (1H, q, J=7.1 Hz, H-20), 2.06 (3H, s, Ac), 1.78 (3H, d, J=6.2 Hz, Me-6″), 1.24 (3H, d, J=7.1 Hz, Me-21), 1.09 (3H, s, Me-19), 0.96 (3H, d, J=6.2 Hz, Me-27), 0.95 (3H, s, Me-18).

Alkaline Hydrolysis of 3 Compound **3** (3 mg) was treated with 4% KOH in EtOH (2 ml) at room temperature for 30 min. The reaction mixture was neutralized by passage through an Amberlite IR-120B column (Organo) and chromatographed on ODS silica gel using MeCN–H_2O (5:7) to yield **2** (2.6 mg).

Compound **7**: Amorphous solid, $[\alpha]_D^{26}$ −30.6° (pyridine, c=0.26). Negative-ion FAB-MS *m/z*: 767 $[M-H]^-$. *Anal.* Calcd for $C_{40}H_{64}O_{14} \cdot 2H_2O$: C, 59.69; H, 8.51. Found: C, 59.39; H, 8.19. IR (KBr) ν_{max} cm^{-1}: 3425 (OH), 2935 (CH), 1035. ^{1}H-NMR (pyridine-d_5) δ: 6.38 (1H, br s, H-1″), 5.32 (1H, br d, J=4.3 Hz, H-6), 5.04 (1H, d, J=7.5 Hz, H-1′), 4.55 (1H, d, J=8.2 Hz, H-26), 3.46 (3H, s, OMe), 2.30 (1H, q, J=7.1 Hz, H-20), 1.78 (3H, d, J=6.2 Hz, Me-6″), 1.25 (3H, d, J=7.1 Hz, Me-21), 1.10 (3H, s, Me-19), 0.97 (3H, d, J=6.3 Hz, Me-27), 0.96 (3H, s, Me-18).

Compound **8**: Amorphous solid, $[\alpha]_D^{26}$ −10.6° (pyridine, c=0.25). Negative-ion FAB-MS *m/z*: 1061 $[M-H]^-$, 899 $[M-glucosyl]^-$. *Anal.* Calcd for $C_{51}H_{82}O_{23} \cdot 5/2H_2O$: C, 55.27; H, 7.91. Found: C, 55.41; H, 8.34. IR (KBr) ν_{max} cm^{-1}: 3400 (OH), 2940 (CH), 1065, 1025. ^{1}H-NMR (pyridine-d_5) δ: 6.21 (1H, br s, H-1″), 5.52 (1H, d, J=7.7 Hz, H-1‴), 5.46 (1H, br d, J=4.7 Hz, H-6), 5.13 (1H, d, J=7.8 Hz, H-1⁗), 4.92 (1H, d, J=6.7 Hz, H-1′), 1.72 (3H, d, J=6.1 Hz, Me-6″), 1.16 (3H, d, J=6.9 Hz, Me-21), 1.07 (3H, s, Me-19), 0.81 (3H, s, Me-18).

Acid Hydrolysis of 8 A solution of **8** (5.5 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 2 ml) was heated at 100 °C for 2 h under an Ar atmosphere. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo) column, and chromatographed on silica gel using $CHCl_3$–MeOH (19 : 1; 9 : 1; 1 : 1) to give isonarthogenin as a genuine aglycone (1.9 mg) and a sugar fraction (2.1 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 °C 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 (Waters) with H_2O–MeCN (4 : 1; 1 : 1; 1 : 9, each 5 ml) mixtures as solvents. The H_2O–MeCN (1 : 9) 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,[13] which was then analyzed by HPLC under the following conditions: solvent, MeCN–H_2O (2 : 3); flow rate, 0.8 ml/min; detection, UV 230 nm. The derivatives of D-glucose and L-rhamnose were detected; t_R (min): 18.74 (derivative of D-glucose); 21.06 (derivative of L-rhamnose).

Partial Acid Hydrolysis of 8 Compound **8** (3.8 mg) was treated with 0.2 M HCl (dioxane–H_2O, 1 : 1, 2 ml) at 85 °C for 30 min. After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU column and chromatographed on ODS silica gel using MeCN–H_2O (5 : 12) to give a partial hydrolysate (**8a**) (0.8 mg).

Assay of Na^+/K^+ ATPase Activity The Na^+/K^+ ATPase activity was assayed according to the reported method[14] with some modification. The reaction mixture composed of 50 mM Tris–HCl (pH 7.3, 37 °C), 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 dimethyl sulfoxide (DMSO), was incubated for 15 min at 37°C. 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 the following method. To the test solution was added 0.5% Na 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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References and Notes

1) Mimaki Y., Sashida Y., Nakamura O., Nikaido T., Ohmoto T., *Phytochemistry*, **33**, 675—682 (1993); Nakamura O., Mimaki Y., Nishino H., Sashida Y., *ibid.*, **36**, 463—467 (1994); Mimaki Y., Nakamura O., Sashida Y., Satomi Y., Nishino A., Nishino H., *ibid.*, **37**, 227—232 (1994); Satou T., Mimaki Y., Kuroda M., Sashida Y., Hatakeyama Y., *ibid.*, **41**, 1225—1230 (1996).
2) Mimaki Y., Ishibashi N., Ori K., Sashida Y., *Phytochemistry*, **31**, 1753—1758 (1992).
3) Watanabe Y., Sanada S., Ida Y., Shoji J., *Chem. Pharm. Bull.*, **31**, 3486—3495 (1983).
4) Mimaki Y., Sashida Y., *Phytochemistry*, **29**, 2267—2271 (1990).
5) Mimaki Y., Sashida Y., *Phytochemistry*, **30**, 937—940 (1991).
6) Idaka K., Hirai Y., Shoji J., *Chem. Pharm. Bull.*, **36**, 1783—1790 (1988).
7) Singh S. B., Thakur R. S., *J. Nat. Prod.*, **45**, 667—671 (1982).
8) Shimomura H., Sashida Y., Mimaki Y., *Phytochemistry*, **28**, 3163—3170 (1989); Mimaki Y., Nakamura O., Sashida Y., Nikaido T., Ohmoto T., *ibid.*, **38**, 1279—1286 (1995).
9) Minato H., Shimaoka A., *Chem. Pharm. Bull.*, **11**, 876—881 (1963); Blunden G., Patel A. V., *J. Nat. Prod.*, **49**, 687—689 (1986).
10) Mimaki Y., Sashida Y., *Chem. Pharm. Bull.*, **38**, 3055—3059 (1990).
11) Mimaki Y., Nakamura O., Sashida Y., Koike K., Nikaido T., Ohmoto T., Nishino A., Satomi Y., Nishino H., *Chem. Pharm. Bull.*, **43**, 971—976 (1995); Mimaki Y., Kuroda M., Nakamura O., Sashida Y., *J. Nat. Prod.*, **60**, 592—597 (1997); Mimaki Y., Kuroda M., Kameyama A., Yokosuka A., Sashida Y., *Chem. Pharm. Bull.*, **46**, 298—303 (1998).
12) Numazawa S., Shinoki M., Ito H., Yoshida T., Kuroiwa Y., *J. Cell. Physiol.*, **160**, 113—120 (1994).
13) Oshima R., Kumanotani J., *Chem. Lett.*, **1981**, 943—946; Oshima R., Yamauchi Y., Kumanotani J., *Carbohydr. Res.*, **107**, 169—176 (1982).
14) Esmann M., *Methods Enzymol.*, **156**, 105—115 (1988).