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STEROIDAL GLUCOSIDES FROM LEAVES OF *CORDYLINE STRICTA*

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Key Word Index—*Cordyline stricta*; Agavaceae; leaves; steroidal glucosides; steroidal saponins; spirostanol saponins; furostanol saponins; pregnane glucoside.

Abstract—A phytochemical study on the leaves of *Cordyline stricta* yielded four new spirostanol saponins, three new furostanol saponins and a new pregnane glucoside. The structures were determined by detailed analysis of their ^{1}H and ^{13}C NMR spectra, acid-catalysed hydrolysis and by comparison with spectral data of known compounds. Each of the isolated steroidal compounds contained a 3α-hydroxyl group bearing a glucose as the common structural feature. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus *Cordyline* with *ca* 20 species is distributed in south-east Asia, Australia and New Zealand. Although, several steroidal sapogenins have been isolated from the neutral fraction of saponified extracts of *C. manners-suttoniae* [1], *C. terminalis* var. *petiolaris* [1], *C. banskii* [1], *C. indivisa* [1], *C. pumilio* [1], *C. cannifolia* [2], *C. stricta* [1–3], *C. australis* [1, 4] and *C. rubra* [1, 5], no steroidal saponins appear to have been isolated from a *Cordyline* species. *Cordyline stricta* is an evergreen shrub native to Australia and an extract of the leaves have been used as a haemostatic in traditional Chinese medicine. A methanolic extract of *C. stricta* leaves has been chemically investigated and the structures of four new spirostanol saponins, three new furostanol saponins and a new pregnane glucoside isolated from the extract are reported in the present paper.

RESULTS AND DISCUSSIONS

The 1-butanol-soluble fraction of the methanolic extract of *C. stricta* leaves was fractionated by a combination of silica gel, octadecylsilanized (ODS) silica gel and Diaion HP-20 column chromatography, and preparative HPLC resulted in the isolation of four new spirostanol saponins (**1**–**4**), three new furostanol saponins (**5**–**7**) and a new pregnane glucoside (**8**).

Compound **1** ($C_{33}H_{54}O_9$) was obtained as an amorphous solid, $[\alpha]_D$ −55.2° (methanol). Its ^{1}H NMR spectrum showed two singlet signals at δ 1.05 and 0.88 (each 3H), indicating the presence of two angular methyl groups, as well as two doublet signals at δ 1.11 (3H, J = 6.9 Hz) and 1.07 (3H, J = 7.1 Hz) assignable to secondary methyl groups. The structure of **1** based upon a (25*S*)-spirostanol derivative was suggested by the above ^{1}H NMR data, a quaternary carbon signal at δ 109.7 in the ^{13}C NMR spectrum [6] and by the characteristic IR absorptions at ν_{max} 980, 915, 890 and 840 cm^{-1} with the absorption at 915 cm^{-1} being of greater intensity than that at 890 cm^{-1} [7–9]. In addition, the presence of a β-D-glucopyranosyl moiety in **1** was readily recognized by the appearance of an anomeric proton signal at δ 4.93 (1H, *d*, J = 7.8 Hz) in the ^{1}H NMR spectrum and also by the characteristic six signals at δ 102.6 (CH), 75.3 (CH), 78.7 (CH), 71.7 (CH), 78.3 (CH) and 62.9 (CH_2) in the ^{13}C NMR spectrum. Acid hydrolysis of **1** with M hydrochloric acid in dioxane–H_2O gave an aglycone (**1a**) and D-glucose. The ^{1}H NMR spectrum of **1a** displayed two exchangeable proton signals at δ 5.84 (1H, *br s*) and 5.39 (1H, *br d*, J = 5.1 Hz), which disappeared on addition of methanol-d_4, accompanied by a change in the signals at δ 4.39 (1H, *m*) and 4.36 (1H, *br s*) to δ 4.29 (1H, *dd*, J = 11.4, 4.7 Hz) and 4.30 (1H, *br s*), respectively, indicating the presence of two hydroxyl groups with axial and equatorial orientations. The above data and inspection of the IR and ^{13}C NMR spectra identified **1a** as (25*S*)-5α-spirostane-1β,3α-diol, that is, cordylagenin, which have been isolated as the major sapogenin from the leaves of *C. cannifolia* after treatment with acid [2]. The linkage position between the β-D-glucose and the aglycone was established by comparison of the ^{1}H NMR spectrum of **1** with that of the corresponding peracetyl derivative (**1b**). The signal for 1-H of the aglycone was shifted to lower field by 0.78 ppm by *O*-acetylation appearing at δ 5.05 (*dd*, J = 11.6, 3.4 Hz), whereas that for 3-H at δ 4.16 (*br s*) was little affected in the

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^{1}H NMR spectrum of **1b**, clearly accounting for the β-D-glucose moiety linkage to the C-3α(ax) hydroxyl position. The structure of **1** was thus characterized as (25*S*)-5α-spirostane-1β,3α-diol 3-*O*-β-D-glucopyranoside.

All spectral properties of **2** ($C_{33}H_{52}O_9$), **3** ($C_{33}H_{52}O_9$) and **4** ($C_{33}H_{54}O_{10}$) were closely similar to those of **1**; their structures were determined by the following spectral comparison and chemical method.

On comparison between the ^{1}H and ^{13}C NMR spectra of **2** with those of **1**, signals due to the C-27 secondary methyl group, which were observed at δ_H 1.07 (3H, *d*, J = 7.1 Hz) and δ_C 16.3 in **1**, were replaced by the signals assignable to an exomethylene group at δ_H 4.82 and 4.78 (each 1H, *br s*), and δ_C 144.5 (C) and 108.6 (CH_2) in **2**. Hydrogenation of **2** over platinum oxide under a hydrogen atmosphere gave **1**, indicating that **2** was the 25,27-dehydro derivative of **1**. The structure of **2** was therefore shown to be 5α-spirost-25(27)-ene-1β,3α-diol 3-*O*-β-D-glucopyranoside.

The ^{1}H and ^{13}C NMR spectra of **3** also showed signals for an exomethylene group at δ_H 5.28 (1H, *d*, J = 1.9 Hz) and 5.03 (1H, *d*, J = 1.4 Hz), and δ_C 153.2 (C) and 107.5 (CH_2), as in the case of **2**. A spin-decoupling experiment in the ^{1}H NMR of **3** revealed that the signal at δ 4.70, assignable to 16-H, was coupled to 17-H (δ 2.67) with a J value of 7.2 Hz, which in turn coupled to the exomethylene protons with J values of 1.9 and 1.4 Hz. The above data were consistent with the structural assignment of **3** as (25*S*)-5α-spirost-20-ene-1β,3α-diol 3-*O*-β-D-glucopyranoside.

In the ^{1}H NMR spectrum of **4**, the three-proton doublet signal due to 27-Me was missing but a deshielded three-proton singlet signal could be recognized at δ 1.56 (3H). Furthermore, 26-H_2 were observed as an *ABq*-like signal at δ 4.15 and 3.62 (each 1H, *d*, J = 10.3 Hz). On comparison between the ^{13}C NMR spectrum of **4** with that of **1**, the signal due to the C-25 carbon, which was observed at δ 27.5 (CH) in **1**, appeared at δ 66.4 as a quaternary carbon signal, accompanied by downfield shifts of the signals due to C-23 (+4.0 ppm), C-24 (+9.2 ppm), C-26 (+4.9 ppm) and C-27 (+8.7 ppm). Thus, the introduction of a hydroxyl group onto C-25 was evident. Takeda *et al.* reported that the IR spectral bands from 1100 to 800 cm^{-1} were useful in distinguishing between 25*R*- and 25*S*-hydroxyspirostanols [10]. The absorptions at ν_{max} 920 cm^{-1} (strong) and 840 cm^{-1} observed in the IR spectrum of **4** was consistent with the C-25*R* configuration. The above data allowed the identification of **4** as (25*R*)-5α-spirostane-1β,3α,25-triol 3-*O*-β-D-glucopyranoside.

Compound **5** ($C_{40}H_{68}O_{14}$) was shown to be a 22-methoxyfurostanol saponin using Ehrlich's test [11, 12] and from its ^{1}H and ^{13}C NMR spectra [δ_H 3.26 (3H, *s*); δ_C 112.7 (C) and 47.3 (Me)] [6]. The ^{1}H NMR spectrum showed two anomeric proton signals at δ 4.91 (1H, *d*, J = 7.7 Hz) and 4.85 (1H, *d*, J = 7.7 Hz), as well as four steroid methyls at δ 0.81 and 0.74 (each 3H, *s*), and 1.17 (3H, *d*, J = 6.9 Hz) and 1.06 (3H, *d*, J = 6.7 Hz). Enzymatic hydrolysis of **5** with β-glucosidase gave the corresponding spirostanol saponin (**5a**) and glucose, and acid hydrolysis with M hydrochloric acid gave an aglycone (**5b**) and glucose. The ^{1}H NMR spectrum of **5b** provided evidence for the presence of an axial hydroxyl group [δ 5.55 (1H, *br s*), which disappeared on addition of methanol-d_4; δ 4.29 (1H, *br s*)]. The ^{1}H NMR data and inspection of the IR and ^{13}C NMR spectra allowed the identification of **5b** as (25*S*)-5α-spirostan-3α-ol, that is, 3-*epi*-neotigogenin [1]. The structure of **5** was thus assigned as 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25*S*)-5α-furostane-3α,22ξ,26-triol 3-*O*-β-D-glucopyranoside.

Compound **6** ($C_{40}H_{68}O_{15}$) was also a 22-methoxyfurostanol saponin. The ^{1}H NMR and ^{13}C NMR spectra, and enzymatic hydrolysis, which gave **1** and glucose, confirmed the structure of **6** to be 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25*S*)-5α-furostane-1β, 3α,22ξ,26-tetrol 3-*O*-β-D-glucopyranoside.

Compound **7** ($C_{39}H_{64}O_{14}$) was suggested to be a furostanol saponin related to **6** from its spectral data. It differed from **6** in the presence of an olefinic group (δ_C 152.3 and 103.7). Furthermore, the 21-Me methyl doublet signal observed at δ 1.06 (3H, *d*, J = 7.0 Hz) in the ^{1}H NMR spectrum of **6** was absent from that of **7** but was replaced by a methyl singlet at δ 1.59 (3H, *s*). Acid hydrolysis of **7** with M hydrochloric acid yielded **1**, **1a** and glucose. The above data were consistent with **7** being the corresponding $\Delta^{20(22)}$-furostanol saponin of **6**. Its structure was thus formulated as 26-*O*-β-D-glucopyranosyl-5α-furost-20(22)-ene-1β, 3α,26-triol 3-*O*-β-D-glucopyranoside.

The ^{1}H NMR spectrum of **8** ($C_{27}H_{42}O_8$) showed two tertiary methyl proton signals at δ 1.07 and 0.99 (each 3H, *s*), an acetylmethyl proton signal at δ 2.21 (3H, *s*), an olefinic proton signal at δ 6.59 (1H, *dd*, J = 3.1, 1.6 Hz) and an anomeric proton signal at δ 4.95 (1H, *d*, J = 7.7 Hz). The presence of an α,β-unsaturated carbonyl group was indicated by the IR (ν_{max} 1650 cm^{-1}), UV [λ_{max} 241 nm (log ε 3.94)] and ^{13}C NMR [δ 196.2 (C=O), 155.8 (C) and 144.5 (CH)] spectra. Acid hydrolysis of **8** with M hydrochloric acid gave an aglycone ($C_{21}H_{32}O_3$) and glucose. The above data suggested that **8** was a glucoside of a pregnane with a 16-ene-20-one structure derived from a spirostanol by Marker's degradation [13]. On comparison between the ^{13}C NMR data of **8** with those of **1**, the ^{13}C signals due to the A and B parts, including C-19, were superimposable on those of **1**, indicating the presence of 1β,3α-hydroxyl group bearing a glucose at C-3. The 16-ene-20-one structure of **8** was well supported by agreement of the NMR data of the D-ring part, C-18 methyl and acetylmethyl groups with those of the pregnane glycosides previously isolated by us from *Sansevieria trifasciata* [14]. Accordingly, the structure of **8** was shown to be 1β,3α-dihydryoxy-5α-pregn-16-en-20-one 3-*O*-β-D-glucopyranoside.

Compounds **1**–**8** are new naturally occurring con-

stituents. This is believed to be the first report of the isolation of the steroidal glucosides from a plant of the genus *Cordyline*.

EXPERIMENTAL

General. NMR (ppm, *J* Hz): 400 MHz for ^{1}H NMR. CC: silica gel (Fuji-Silysia Chemical), ODS silica gel (Nacalai Tesque) and Diaion HP-20 (Mitsubishi-Kasei). TLC: precoated Kieselgel 60 F_{254} (Merck) and RP-18 $F_{254}S$ (Merck). HPLC: Capcell Pak C_{18} column (Shiseido, 10 mm i.d. × 250 mm, ODS, 5 μm) for prep. and a TSK-gel ODS-Prep column (Tosoh, 4.6 mm i.d. × 250 mm, ODS, 5 μm) for analytical; UV or RI detection.

Plant material. Purchased from Exotic Plants Co. Ltd, Japan; a specimen is on file in our laboratory.

Extraction and isolation. (Leaves fr. 8 kg) was extracted with hot MeOH (18 l × 2). The MeOH extract was concd under red. pres. and the viscous concentrate (680 g) partitioned between H_2O and *n*-BuOH. CC of the *n*-BuOH-soluble phase (320 g) on silica gel (600 g) and elution with a gradient mixt. of $CHCl_3$–MeOH system (9:1; 4:1; 2:1) and, finally, with MeOH, gave 5 frs (I–V). Fr. II (32 g) was subjected to CC on silica gel eluting with $CHCl_3$–MeOH (5:1) and ODS silica gel with MeOH–H_2O (17:3), and to prep. HPLC with MeOH–H_2O (9:1) to yield compounds **1** (668 mg), **2** (37.1 mg) and **3** (7.5 mg). Fr. III (28 g) was purified by CC on silica gel eluting with $CHCl_3$–MeOH (5:1) and ODS silica gel with MeOH–H_2O (7:3) and, finally, by prep. HPLC with MeCN–H_2O (2:3) to yield **4** (5.9 mg) and **5** (430 mg). Fr. IV (110 g) was passed through a Diaion HP-20 (375 g) column eluting with H_2O with increasing amounts of MeOH in H_2O and, finally, with MeOH. The 80% MeOH and MeOH eluate frs were combined and chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (20:10:1) to give frs IV(a)–IV(c). Fr. IV(a) was chromatographed on an ODS silica gel column eluting with MeOH–H_2O (7:3) to yield **8** (78.7 mg). Fr. IV(b) was purified by CC on ODS silica gel eluting with MeOH–H_2O (3:2) and prep. HPLC with MeCN–H_2O (3:7) to yield **6** (3.96 g) and **7** (335 mg).

Compound (**1**). Amorphous solid. $[\alpha]_D^{27}$ −55.2° (MeOH; *c* 0.11). Negative-ion FAB-MS *m/z* 593 [M−H]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2920 (CH), 1445, 1365, 1345, 1305, 1270, 1220, 1160, 1060, 1020, 980, 935, 915, 890, 840 (intensity 915 > 890, 25*S*-spiroacetal). ^{1}H NMR (pyridine-d_5): δ 4.93 (1H, *d*, *J* = 7.8 Hz, 1′-H), 4.52 (2H, overlapping, 16-H and 6′a-H), 4.38 (1H, *dd*, *J* = 11.6, 5.0 Hz, 6′b-H), 4.33 (1H, *br s*, 3-H), 4.27 (1H, *dd*, *J* = 12.0, 3.7 Hz, 1-H), 4.26 (1H, *dd*, *J* = 8.8, 8.8 Hz, 3′-H), 4.22 (1H, *dd*, *J* = 8.8, 8.8 Hz, 4′-H), 4.06 (1H, *dd*, *J* = 10.8, 2.8 Hz, 26a-H), 4.05 (1H, *dd*, *J* = 8.8, 7.8 Hz, 2′-H), 3.89 (1H, *ddd*, *J* = 8.8, 5.0, 2.3 Hz, 5′-H), 3.36 (1H, *br d*, *J* = 10.8 Hz, 26b-H), 1.11 (3H, *d*, *J* = 6.9 Hz, 27-Me), 1.07 (3H, *d*, *J* = 7.1 Hz, 21-Me), 1.05 (3H, *s*, 19-Me), 0.88 (3H, *s*, 18-Me). ^{13}C NMR: Table 1.

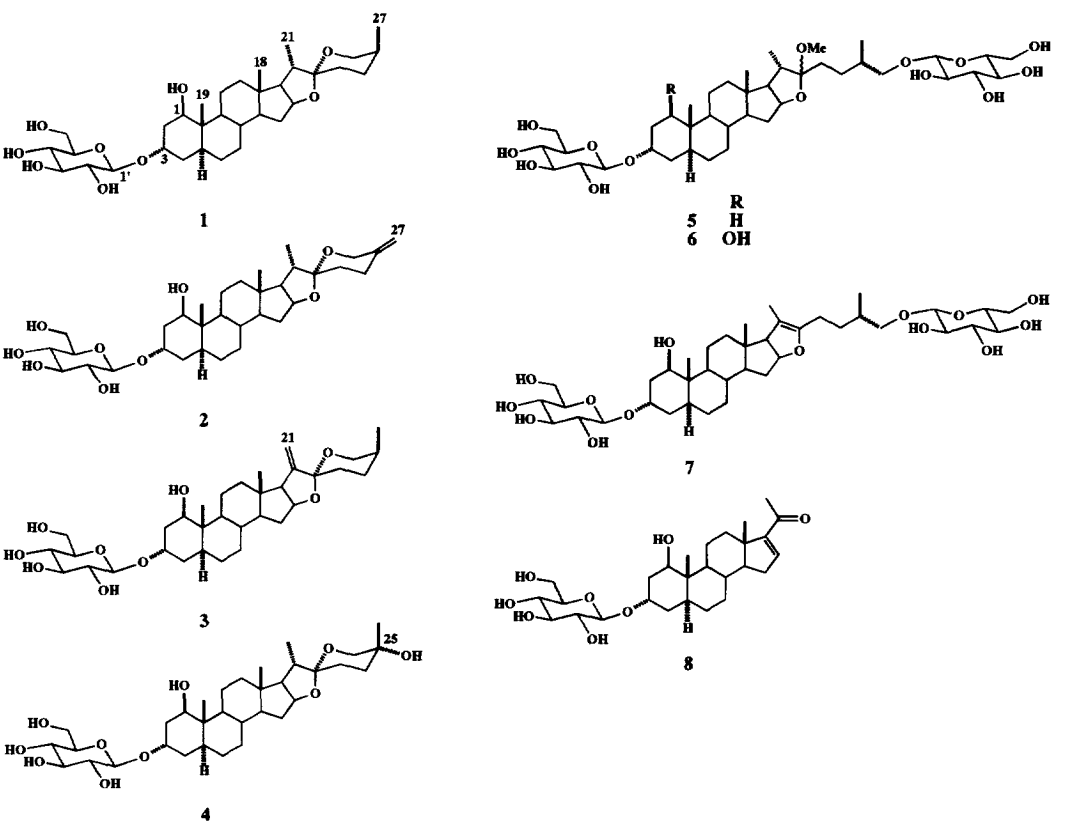

Table 1. ^{13}C NMR spectral data for compounds **1**, **1a**, **2**–**5**, **5a**, **6**–**8** and **8a** (pyridine-d_5)

C	1	1a	2	3	4	5	5a	5b	6	7	8	8a
1	73.7	74.1	73.7	73.7	73.7	32.7	32.7	32.8	73.7	73.7	73.5	73.8
2	37.1	41.4	37.1	37.1	37.1	25.6	25.6	29.9	37.1	37.1	37.1	41.3
3	73.9	66.2	73.9	73.8	73.8	73.1	73.1	65.5	73.8	73.8	74.0	66.2
4	35.1	37.1	35.1	35.1	35.1	35.0	35.0	36.9	35.1	35.1	35.1	37.1
5	39.1	38.9	39.1	39.1	39.1	39.5	39.5	39.4	39.1	39.2	39.3	39.1
6	28.7	28.9	28.7	28.6	28.7	28.8	28.7	29.0	28.7	28.7	28.6	28.9
7	32.5	32.8	32.5	32.4	32.4	32.4	32.4	32.6	32.4	32.7	32.1	32.4
8	36.0	36.2	36.0	36.4	36.0	35.2	35.2	35.4	36.0	35.8	34.5	34.7
9	55.1	55.8	55.1	55.0	55.1	54.0	54.0	54.7	55.1	55.1	55.6	56.3
10	42.6	43.1	42.6	42.6	42.6	36.1	36.1	36.5	42.6	42.6	42.7	43.2
11	24.8	24.9	24.8	24.5	24.8	20.8	20.8	21.0	24.8	25.0	24.9	25.0
12	40.9	41.0	40.9	39.8	40.9	40.1	40.2	40.3	40.8	40.6	36.0	36.0
13	40.4	40.4	40.5	42.6	40.5	41.1	40.8	40.8	40.8	43.4	46.2	46.2
14	56.8	56.9	56.8	57.0	56.8	56.5	56.6	56.6	56.7	55.1	56.8	56.8
15	32.3	32.4	32.3	33.0	32.3	32.0	32.0	32.1	32.3	34.7	32.5	32.5
16	81.2	81.2	81.4	81.2	81.3	81.4	81.2	81.2	81.3	84.4	144.5	144.5
17	63.2	63.1	63.3	60.6	63.2	64.4	62.9	62.9	64.6	64.9	155.8	155.8
18	16.8	16.8	16.7	15.2	16.8	16.6	16.6	16.7	16.7	14.6	16.3	16.3
19	6.5	6.5	6.5	6.5	6.5	11.6	11.6	11.6	6.5	6.5	6.5	6.5
20	42.5	42.5	41.9	153.2	41.5	40.5	42.5	42.5	40.5	103.7	196.2	196.3
21	14.8	14.9	15.0	107.5	14.9	16.3	14.8	14.9	16.3	11.8	27.1	27.1
22	109.7	109.7	109.4	107.5	109.3	112.7	109.7	109.7	112.7	152.3	—	—
23	26.2	26.2	33.2	26.3	30.2	30.9	26.2	26.2	31.0	31.4	—	—
24	26.4	26.4	29.0	26.6	35.6	28.2	26.4	26.4	28.2	23.6	—	—
25	27.5	27.5	144.5	27.8	66.4	34.5	27.5	27.5	34.5	33.7	—	—
26	65.1	65.0	65.0	66.1	70.0	75.0	65.1	65.1	75.0	75.2	—	—
27	16.3	16.3	108.6	16.5	25.0	17.6	16.3	16.3	17.6	17.2	—	—
OMe						47.3				47.3		
1′	102.6	—	102.7	102.6	102.6	102.6	102.6	—	102.6	102.6	102.7	—
2′	75.3	—	75.4	75.4	75.4	75.4	75.4	—	75.4	75.3	75.3	—
3′	78.7	—	78.7	78.7	78.7	78.7	78.7	—	78.7	78.7	78.7	—
4′	71.7	—	71.8	71.8	71.8	71.8	71.8	—	71.8	71.7	71.7	—
5′	78.3	—	78.4	78.4	78.4	78.4	78.4	—	78.4	78.4	78.3	—
6′	62.9	—	62.9	62.9	62.9	62.9	62.9	—	62.9	62.9	62.9	—
1″	—	—	—	—	—	105.1	—	—	105.1	105.2	—	—
2″	—	—	—	—	—	75.2	—	—	75.2	75.2	—	—
3″	—	—	—	—	—	78.6	—	—	78.6	78.6	—	—
4″	—	—	—	—	—	71.8	—	—	71.7	71.7	—	—
5″	—	—	—	—	—	78.5	—	—	78.5	78.5	—	—
6″	—	—	—	—	—	62.9	—	—	62.9	62.9	—	—

Acid hydrolysis of **1**. A soln of **1** (30 mg) in M HCl (dioxane–H_2O, 1:1, 4 ml) was heated at 100° for 2 hr under an Ar atmosphere. After cooling, the reaction mixt. was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column and then chromatographed on silica gel eluting with a gradient mixt. of $CHCl_3$–MeOH (19:1; 1:1) to give an aglycone (**1a**) (14.6 mg) and a monosaccharide (7.7 mg). Compound **1a**. Amorphous solid. $[\alpha]_D^{29}$ −59.7° (MeOH; *c* 0.59). EI-MS *m/z* (rel. int.): 432 $[M]^+$ (4), 359 (9), 318 (9), 289 (20), 139 (100). **IR** ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2910 (CH), 1440, 1365, 1335, 1265, 1215, 1165, 1115, 1060, 1045, 1015, 980, 955, 930, 910, 885, 860, 840 (intensity 910 > 885, 25*S*-spiroacetal). ^1H NMR (pyridine-d_5): δ 5.84 (1H, *br s*, OH), 5.39 (1H, *br d*, J = 5.1 Hz, OH), 4.51 (1H, *q*-like, J = 7.2 Hz, 16-H), 4.39 (1H, *m*, 1-H), 4.36 (1H, *br* s, 3-H), 4.06 (1H, *dd*, J = 10.9, 2.6 Hz, 26a-H), 3.36 (1H, *br d*, J = 10.9 Hz, 26b-H), 1.13 (3H, *s*, 19-Me), 1.11 (3H, *d*, J = 7.0 Hz, 27-Me), 1.07 (3H, *d*, J = 7.1 Hz, 21-Me), 0.92 (3H, *s*, 18-Me). ^1H NMR (pyridine-d_5 + methanol-d_4): δ 4.50 (1H, *q*-like, J = 7.3 Hz, 16-H), 4.30 (1H, *br s*, 3-H), 4.29 (1H, *dd*, J = 11.4, 4.7 Hz, 1-H), 4.05 (1H, *dd*, J = 10.9, 2.6 Hz, 26a-H), 3.35 (1H, *br d*, J = 10.9 Hz, 26b-H), 1.10 (3H, *d*, J = 6.9 Hz, 27-Me), 1.08 (3H, *s*, 19-Me), 1.07 (3H, *d*, J = 7.0 Hz, 21-Me), 0.88 (3H, *s*, 18-Me). ^{13}C NMR: Table: 1. The monosaccharide was suggested to be glucose by direct TLC comparison with an authentic sample; R_f: 0.36 (*n*-BuOH–Me_2CO–H_2O, 4:5:1). The monosaccharide (2 mg) was diluted with H_2O (1 ml) and treated with (−)-α-methylbenzylamine (5 mg) and $Na[BH_3CN]$ (8 mg) in EtOH (1 ml) at 40° for 4 hr, followed by acetylation with Ac_2O (0.3 ml) in pyridine (0.3 ml). The reaction mixt. was passed through a Sep-

Pak C_{18} cartridge (Waters) with H_2O–MeCN (4:1; 1:1; 1:9, each 10 ml). The H_2O–MeOH (1:9) eluate fr. was further passed through a Toyopak IC-SP M cartridge (Tosoh) with EtOH (10 ml) to give a 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-dioxyalditol acetate derivative of the monosaccharide [15, 16], which was then analysed by HPLC under the following conditions: solvent, MeCN–H_2O (2:3); flow rate, 0.8 ml min^{-1}; detection, UV 230 nm. The derivative of D-glucose was detected. R_t (min): 24.16. As D-glucose was identified as the sugar component of **1**, it was assumed to be present in all of the isolated compounds.

Acetylation of **1**. Compound **1** (41 mg) was acetylated with Ac_2O (0.5 ml) in pyridine (0.5 ml). After addition of H_2O, the reaction mixt. was extracted with Et_2O and the extract was chromatographed on silica gel eluting with hexane–Me_2CO (5:2) to give the peracetate (**1b**) (47.4 mg). Compound **1b**. Amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 2950 and 2850 (CH), 1755 (C=O), 1445, 1430, 1375, 1235, 1165, 1125, 1100, 1060, 1035, 995, 985, 960, 940, 910, 905, 850. ^1H NMR (pyridine-d_5): δ 5.76 (1H, *dd*, *J* = 9.6, 9.6 Hz, 3′-H), 5.51 (1H, *dd*, *J* = 9.6, 8.1 Hz, 2′-H), 5.49 (1H, *dd*, *J* = 9.6, 9.6 Hz, 4′-H), 5.06 (1H, *d*, *J* = 8.1 Hz, 1′-H), 5.05 (1H, *dd*, *J* = 11.6, 3.4 Hz, 1-H), 4.61 (1H, *dd*, *J* = 12.3, 5.1 Hz, 6′a-H), 4.49 (1H, *q*-like, *J* = 7.4 Hz, 16-H), 4.40 (1H, *dd*, *J* = 12.3, 2.4 Hz, 6′b-H), 4.16 (1H, *br s*, 3-H), 4.11 (1H, *ddd*, *J* = 9.6, 5.1, 2.4 Hz, 5′-H), 4.05 (1H, *dd*, *J* = 10.9, 2.6 Hz, 26a-H), 3.36 (1H, *br d*, *J* = 10.9 Hz, 26b-H), 2.42, 2.07, 2.06, 2.03 and 2.02 (each 3H, *s*, Ac), 1.14 (3H, *d*, *J* = 6.9 Hz, 27-Me), 1.07 (3H, *d*, *J* = 7.1 Hz, 21-Me), 0.96 (3H, *s*, 19-Me), 0.82 (3H, *s*, 18-Me).

Compound (**2**). Amorphous solid. $[\alpha]_D^{27}$ −45.5° (MeOH; *c* 0.44). Negative-ion FAB-MS *m/z* 591 [M-H]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 2910 (CH), 1440, 1365, 1220, 1165, 1150, 1065, 1035, 985, 950, 935, 915, 890, 865. ^1H NMR (pyridine-d_5): δ 4.97 (1H, *d*, *J* = 7.7 Hz, 1′-H), 4.82 and 4.78 (each 1H, *br s*, 27-H_2), 4.55 (2H, overlapping, 16-H and 6′a-H), 4.47 and 4.04 (each 1H, *br s*, *J* = 12.2 Hz, 26-H_2), 4.42 (1H, *dd*, *J* = 11.8, 4.9 Hz, 6′b-H), 4.35 (1H, *br s*, 3-H), 4.30 (1H, *dd*, *J* = 8.8, 8.8 Hz, 3′-H), 4.29 (1H, overlapping, 1-H), 4.25 (1H, *dd*, *J* = 8.8, 8.8 Hz, 4′-H), 4.08 (1H, *dd*, *J* = 8.8, 7.7 Hz, 2′-H), 3.91 (1H, *ddd*, *J* = 8.8, 4.9, 2.5 Hz, 5′-H), 1.07 (3H, *s*, 19-Me), 1.06 (3H, *d*, *J* = 7.1 Hz, 21-Me), 0.89 (3H, *s*, 18-Me). ^{13}C NMR: Table 1.

Catalytic hydrogenation of **2**. A mixt. of **2** (10 mg) and PtO_2 (5 mg) was stirred under an H_2 atmosphere at ambient temp. for 12 hr. The reaction mixt., after removal of PtO_2 by filtration, was subjected to prep. HPLC eluting with MeOH–H_2O (9:1) to furnish **1** (4.8 mg).

Compound **3**. Amorphous solid. $[\alpha]_D^{27}$ −32.2° (MeOH; *c* 0.21). Negative-ion FAB-MS *m/z* 591 [M-H]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2920 (CH), 1445, 1365, 1345, 1270, 1205, 1155, 1120, 1070, 1035, 1015, 980, 960, 935, 920, 890, 840. ^1H NMR (pyridine-d_5): δ 5.28 (1H, *d*, *J* = 1.9 Hz, 21a-H), 5.03 (1H, *d*, *J* = 1.4 Hz, 21b-H), 4.97 (1H, *d*, *J* = 7.7 Hz, 1′-H), 4.70 (1H, *ddd*, *J* = 7.2, 7.2, 4.6 Hz, 16-H), 4.55 (1H, *dd*, *J* = 11.3, 2.5 Hz, 6′a-H), 4.42 (1H, *dd*, *J* = 11.3, 5.1 Hz, 6′b-H), 4.34 (1H, *br s*, 3-H), 4.30 (1H, *dd*, *J* = 8.8, 8.8 Hz, 3′-H), 4.29 (1H, overlapping, 1-H), 4.25 (1H, *dd*, *J* = 8.8, 8.8 Hz, 4′-H), 4.11 (1H, *dd*, *J* = 11.0, 2.9 Hz, 26a-H), 4.08 (1H, *dd*, *J* = 8.8, 7.7 Hz, 2′-H), 3.91 (1H, *ddd*, *J* = 8.8, 5.1, 2.5 Hz, 5′-H), 3.39 (1H, *br d*, *J* = 11.0 Hz, 26b-H), 2.67 (1H, *ddd*, *J* = 7.2, 1.9, 1.4 Hz, 17-H), 1.10 (3H, *d*, *J* = 7.0 Hz, 27-Me), 1.05 (3H, *s*, 19-Me), 0.83 (3H, *s*, 18-Me). ^{13}C NMR: Table 1.

Compound **4**. Amorphous solid. $[\alpha]_D^{27}$ −25.3° (MeOH; *c* 0.30). Negative-ion FAB-MS *m/z* 610 [M]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2910 (CH), 1440, 1370, 1240, 1190, 1175, 1060, 1020, 985, 965, 920, 840. ^1H NMR (pyridine-d_5): δ 4.97 (1H, *d*, *J* = 7.7 Hz, 1′-H), 4.55 (2H, overlapping, 16-H and 6′a-H), 4.41 (1H, *dd*, *J* = 11.5, 4.8 Hz, 6′b-H), 4.35 (1H, *br s*, 3-H), 4.30 (1H, *dd*, *J* = 8.8, 8.8 Hz, 3′-H), 4.29 (1H, overlapping, 1-H), 4.25 (1H, *dd*, *J* = 8.8, 8.8 Hz, 4′-H), 4.15 and 3.62 (each 1H, *d*, *J* = 10.3 Hz, 26-H_2), 4.08 (1H, *dd*, *J* = 8.8, 7.7 Hz, 2′-H), 3.91 (1H, *ddd*, *J* = 8.8, 4.8, 2.4 Hz, 5′-H), 1.56 (3H, *s*, 27-Me), 1.14 (3H, *d*, *J* = 6.9 Hz, 21-Me), 1.07 (3H, *s*, 19-Me), 0.90 (3H, *s*, 18-Me). ^{13}C NMR: Table 1.

Compounds **5**. Amorphous solid. $[\alpha]_D^{27}$ −43.6° (MeOH; *c* 0.11). Negative-ion FAB-MS *m/z* 772 [M]$^-$. IR ν_{max}^{KBr}: 3420 (OH), 2930 (CH), 1445, 1375, 1250, 1190, 1160, 1070, 1030, 1015, 895, 840. ^1H NMR (pyridine-d_5): δ 4.91 (1H, *d*, *J* = 7.7 Hz, 1′-H), 4.85 (1H, *d*, *J* = 7.7 Hz, 1″-H), 3.26 (3H, *s*, OMe), 1.17 (3H, *d*, *J* = 6.9 Hz, 27-Me), 1.06 (3H, *d*, *J* = 6.7 Hz, 21-Me), 0.81 (3H, *s*, 18-Me), 0.74 (3H, *s*, 19-Me). ^{13}C NMR: Table 1.

Enzymatic hydrolysis of **5**. Compound (**5**) (64.8 mg) was treated with β-glucosidase (30 mg) in HOAc–NaOAc buffer (pH 5, 5 ml) at room temp. for 24 hr. The reaction mixt. was chromatographed on ODS silica gel eluting with MeOH–H_2O (17:3) to give the corresponding spirostanol saponin (**5a**) (16.9 mg) and glucose (8.4 mg). Compound (**5a**). Amorphous solid. $[\alpha]_D^{27}$ −36.0° (MeOH; *c* 0.10). Negative-ion FAB-MS *m/z* 577 [M-H]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2930 (CH), 1445, 1375, 1335, 1270, 1220, 1210, 1170, 1125, 1095, 1060, 1050, 1030, 985, 960, 955, 920, 895, 845. ^1H NMR (pyridine-d_5): δ 4.91 (overlapping with H_2O signal, 1′-H), 4.56 (1H, *dd*, *J* = 11.5, 2.4 Hz, 6′a-H), 4.52 (1H, *q*-like, *J* = 7.4 Hz, 16-H), 4.42 (1H, *dd*, *J* = 11.5, 5.3 Hz, 6′b-H), 4.29 (2H, overlapping, 3′-H and 4′-H), 4.26 (1H, *br s*, 3-H), 4.07 (1H, overlapping, 2′-H), 4.06 (each 1 H, *dd*, *J* = 10.9, 2.3 Hz, 26a-H), 3.95 (1H, *m*, 5′-H), 3.37 (1H, *br d*, *J* = 10.9 Hz, 26b-H), 1.15 (3H, *d*, *J* = 6.9 Hz, 27-Me), 1.08 (3H, *d*, *J* = 7.0 Hz, 21-Me), 0.82 (3H, *s*, 18-Me), 0.74 (3H, *s*, 19-Me). ^{13}C NMR: Table 1.

Acid hydrolysis of **5**. Compound **5** (45 mg) was subjected to acid hydrolysis as described for **1** and the reaction mixt. was chromatographed on silica gel eluting with $CHCl_3$–MeOH (19:1; 1:1) to give an aglycone (**5b**) (13.3 mg) and glucose (16.7 mg). Compound

5b. Amorphous solid. $[\alpha]_D^{29}$ −49.0° (MeOH, *c* 0.45). CI-MS *m/z* (rel. int.): 415 $[M-H]^+$ (31), 399 (24), 302 (22), 273 (40), 139 (100), 115 (27). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2920 (CH), 1445, 1375, 1335, 1300, 1270, 1245, 1220, 1165, 1125, 1095, 1060, 1050, 1030, 1010, 1000, 985, 960, 950, 915, 895, 845. ^1H NMR (pyridine-d_5): δ 5.55 (1H, *br s*, OH), 4.52 (1H, *q*-like, *J* = 7.3 Hz, 16-H), 4.29 (1H, *br s*, 3-H), 4.07 (1H, *dd*, *J* = 10.8, 2.7 Hz, 26a-H), 3.38 (1H, *br d*, *J* = 10.8 Hz, 26b-H), 1.16 (3H, *d*, *J* = 6.9 Hz, 27-H), 1.09 (3H, *d*, *J* = 7.0 Hz, 21-Me), 0.88 (3H, *s*, 19-Me), 0.83 (3H, *s*, 18-Me). ^{13}C NMR: Table 1.

Compound **6**. Amorphous solid. $[\alpha]_D^{27}$ −36.4° (MeOH; *c* 0.11). Negative-ion FAB-MS *m/z* 788 $[M]^-$: IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2910 (CH), 1445, 1370, 1250, 1155, 1065, 1015, 985, 935, 890, 835. ^1H NMR (pyridine-d_5): δ 4.96 (1H, *d*, *J* = 7.7 Hz, 1′-H), 4.84 (1H, *d*, *J* = 7.7 Hz, 1″-H), 3.26 (3H, *s*, OMe), 1.13 (3H, *d*, *J* = 6.9 Hz, 27-Me), 1.07 (3H, *s*, 19-Me), 1.06 (3H, *d*, *J* = 7.0 Hz, 21-Me), 0.87 (3H, *s*, 18-Me). ^{13}C NMR: Table 1.

Enzymatic hydrolysis of **6**. Compound **6** (45 mg) was treated with β-glucosidase (30 mg) in HOAc–NaOAc buffer (pH 5, 5 ml) at room temp. for 24 hr. The reaction mixt. was chromatographed on silica gel eluting with $CHCl_3$–MeOH (4:1) and ODS silica gel with MeOH–H_2O (9:1) to give **1** (22.1 mg) and glucose (8.7 mg).

Compound **7**. Amorphous solid. $[\alpha]_D^{27}$ −10.0° (MeOH; *c* 0.10). Negative-ion FAB-MS *m/z* 756 $[M]^-$. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2920 (CH), 1445, 1370, 1155, 1075, 1030, 940, 895. ^1H NMR (pyridine-d_5): δ 4.96 (1H, *d*, *J* = 7.7 Hz, 1′-H), 4.82 (1H, *d*, *J* = 7.7 Hz, 1″-H), 1.59 (3H, *s*, 21-Me), 1.06 (3H, *s*, 19-Me), 1.03 (3H, *d*, *J* = 6.6 Hz, 27-Me), 0.76 (3H, *s*, 18-Me). ^{13}C NMR: Table 1.

Acid hydrolysis of **7**. Compound **7** (50 mg) was subjected to acid hydrolysis as described for **1** and the reaction mixt. chromatographed on silica gel eluting with $CHCl_3$–MeOH (19:1; 1:1) to give **1** (3.1 mg), **1a** (3.2 mg) and glucose (12.5 mg).

Compound **8**. Amorphous solid. $[\alpha]_D^{27}$ +43.2° (MeOH; *c* 0.13). Negative-ion FAB-MS *m/z* 493 $[M-H]^-$. UV λ_{max}^{MeOH} nm (log ε): 241 (3.94). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2920 (CH), 1650 (C=O), 1580 (C=C), 1440, 1420, 1360, 1315, 1225, 1195, 1155, 1070, 1025, 990, 930, 890, 840, 825, 815. ^1H NMR (pyridine-d_5): δ 6.59 (1H, *dd*, *J* = 3.1, 1.7 Hz, 16-H), 4.95 (1H, *d*, *J* = 7.7 Hz, 1′-H), 4.54 (1H, *dd*, *J* = 11.7, 2.5 Hz, 6′a-H), 4.41 (1H, *dd*, *J* = 11.7, 4.9 Hz, 6′b-H), 4.34 (1H, *br s*, 3-H), 4.29 (1H, *dd*, *J* = 8.9, 8.9 Hz, 3′-H), 4.28 (1H, overlapping, 1-H), 4.24 (1H, *dd*, *J* = 8.9, 8.9 Hz, 4′-H), 4.06 (1H, *dd*, *J* = 8.9, 7.7 Hz, 2′-H), 3.91 (1H, *ddd*, *J* = 8.9, 4.9, 2.5 Hz, 5′-H), 2.21 (3H, *s*, 21-Me), 1.07 (3H, *s*, 19-Me), 0.99 (3H, *s*, 18-Me). ^{13}C NMR: Table 1.

Acid hydrolysis of **8**. Compound **8** (30 mg) was subjected to acid hydrolysis as described for **1** and the reaction mixt. chromatographed on silica gel eluting with $CHCl_3$–MeOH (39:1; 1:1) to give an aglycone (**8a**) (7.3 mg) and glucose (7.9 mg). Compound **8a**. Amorphous solid. $[\alpha]_D^{23}$ +43.5° (MeOH; *c* 0.29). EI MS *m/z* (rel. int.): 332 $[M]^+$ (27), 289 (21), 93 (21), 61 (100). UV λ_{max}^{MeOH} nm (log ε): 240 (3.86). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2905 and 2840 (CH), 1650 (C=O), 1580 (C=C), 1445, 1425, 1360, 1310, 1285, 1270, 1250, 1225, 1170, 1155, 1135, 1100, 1065, 1050, 1020, 1005, 995, 960, 930, 910, 890, 835, 815. ^1H NMR (pyridine-d_5): δ 6.60 (1H, *dd*, *J* = 3.1, 1.7 Hz, 16-H), 5.85 and 5.34 (each 1H, *br s*, OH × 2), 4.37 (2H, overlapping, 1-H and 3-H), 2.22 (3H, *s*, 21-Me), 1.14 (3H, *s*, 19-Me), 1.02 (3H, *s*, 18-Me). ^{13}C NMR: Table 1.

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