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# STEROIDAL SAPONINS FROM THE STEMS OF *DRACAENA CONCINNA*

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**Key Word Index**—*Dracaena concinna*; Agavaceae; stems; steroidal saponins; furostanol saponins; spirostanol saponins.

**Abstract**—A total of thirteen steroidal saponins, including four new furostanol saponins, were isolated from the fresh stems of *Dracaena concinna*. The structures of new saponins were determined by detailed analysis of their $^1$H and $^{13}$C NMR spectra, hydrolysis, and by comparison of spectral data of known compounds. Two of the new furostanol saponins are unique in structure possessing a 4α-hydroxyl group on the furostanol skeleton.


## INTRODUCTION

The family Agavaceae with more than 480 species has a distribution in the tropic and subtropic dry climate regions throughout the world. The occurrence of steroidal saponins in several Agavaceae plants is well documented [1, 2]. We have already made phytochemical screening of the three Agavaceae plants, *Nolina recurvata* [3–5], *Sansevieria trifasciata* [6, 7] and *Cordyline stricta* [8, 9], and isolated a variety of new steroidal saponins and pregnane glycosides.

As part of our program of the chemical investigation of Agavaceae plants, we have now examined the fresh stems of *Dracaena concinna* which is native to Mauritius and cultivated as an excellent foliage plant. This study has resulted in the isolation of a total of thirteen steroidal saponins, including four new furostanol saponins. This paper describes the structural determination of the four new furostanol saponins by detailed analysis of their $^1$H and $^{13}$C NMR spectra, hydrolysis, and by comparison of spectral data of known compounds.

## RESULTS AND DISCUSSION

The 1-butanol-soluble phase of the methanolic extract of *D. concinna* stems was chromatographed on Diaion HP-20 and silica gel to give saponin fractions. Each fraction was subjected to silica gel and octadecylsilanized (ODS) silica gel column chromatography, and to preparative HPLC to furnish compounds **1**–**13**.

Compounds **1**–**9** were known steroidal saponins and identified as (25*R*)-spirost-5-en-3β-ol (diosgenin) 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside} (**1**) [10], diosgenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside} (**2**) [11], (25*R*)-spirost-5-ene-1β,3β-diol (ruscogenin) 1-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside} (**3**) [12], ruscogenin 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-sulfo-α-L-arabinopyranoside} (**4**) [13], (23*S*,24*S*,25*S*)-spirost-5-ene-1β,3β,23,24-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranoside} (**5**) [14], (23*S*,24*S*,25*S*)-spirost-5-ene-1β,3β,23,24-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranoside} 24-*O*-β-D-fucopyranoside (**6**) [14], 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25*R*)-furost-5-ene-3β,22ξ,26-triol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside} (**7**) [15], 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-(25*R*)-furost-5-ene-3β,22ξ,26-triol 3-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside} (**8**) [16] and 26-*O*-β-D-glucopyranosyl-22-*O*-methylfurosta-5,25(27)-diene-1β,3β,22ξ,26-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-fucopyranoside} (**9**) [9], respectively.

Compound **10** ($C_{46}H_{76}O_{18}$) was obtained as an amorphous solid, $[\alpha]_D$ −45.0° (methanol). Its $^1$H NMR spectrum in pyridine-$d_5$ showed signals for three steroid methyls at δ 1.25 (*s*), 1.11 (*d*, *J* = 6.8 Hz) and

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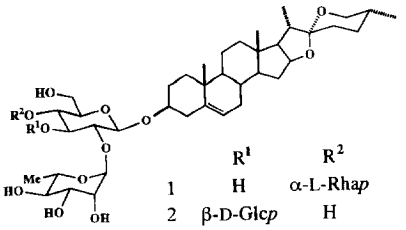
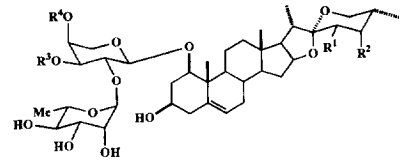
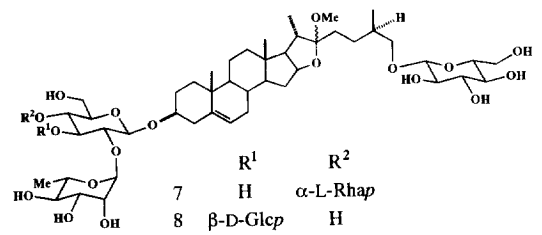


| | $R^1$ | $R^2$ |
|---|---|---|
| 1 | H | α-L-Rha*p* |
| 2 | β-D-Glc*p* | H |



| | $R^1$ | $R^2$ | $R^3$ | $R^4$ |
|---|---|---|---|---|
| 3 | H | H | H | H |
| 4 | H | H | H | $SO_3^-$ |
| 5 | OH | OH | β-D-Xyl*p* | H |
| 6 | OH | O-β-D-Fuc*p* | β-D-Xyl*p* | H |

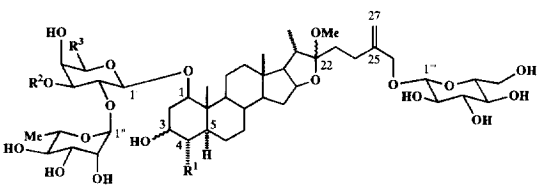


| | $R^1$ | $R^2$ |
|---|---|---|
| 7 | H | α-L-Rha*p* |
| 8 | β-D-Glc*p* | H |

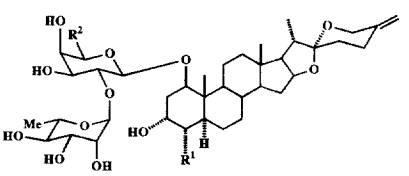
0.85 (*s*), an exomethylene group at $\delta$ 5.34 and 5.05 (each *br s*), and three anomeric protons at $\delta$ 6.41 (*br s*), 4.91 (*d*, $J = 7.8$ Hz) and 4.73 (*d*, $J = 7.7$ Hz). A positive colour reaction in Ehrlich's test [17, 18] and the characteristic $^1$H and $^{13}$C NMR signals at $\delta_H$ 3.25 (3H, *s*), and $\delta_C$ 112.4 (C) and 47.3 (Me) [19] were in favour of a 22-methoxyfurostanol saponin. On enzymatic hydrolysis of **10** with β-glucosidase, it liberated glucose to give the corresponding spirostanol saponin (**10a**). Acid hydrolysis with M hydrochloric acid in dioxane-$H_2O$ (1:1) gave a steroidal sapogenin ($C_{27}H_{42}O_4$) (**10b**), identified as 5α-spirost-25(27)-ene-1β,3α-diol (1β-hydroxycrabbogenin) [9, 20], together with D-glucose, D-fucose and L-rhamnose in a ratio of 1:1:1. The $^1$H NMR spectrum of **10a** exhibited two anomeric proton signals at $\delta$ 6.42 (*br s*) and 4.73 (*d*, $J = 7.8$ Hz). Attempted assignment of the $^{13}$C NMR signals arising from the saccharide moiety of **10a** by referring to those of the reported saponins [12, 14, 19] indicated the presence of a terminal α-L-rhamnopyranosyl unit and a 2-substituted β-D-fucopyranosyl unit in the molecule of **10a**, leading to the identification of the diglycoside as *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-fucopyranose. This diglycoside was shown to be linked to the aglycone C-1 hydroxyl group by comparison of the $^{13}$C NMR spectrum of **10a** with that of **10b**; the signal due to the aglycone C-1 was shifted to a lower field by 7.0 ppm, whereas the signal due to C-2 was moved to a higher field by 5.8 ppm by *O*-glycosylation. Thus, the structure of **10** was characterized as 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-5α-furost-25(27)-ene-1β,3α,22ξ,26-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-β-D-fucopyranoside}.

Compound **11** ($C_{45}H_{74}O_{18}$) was suggested to be a furostanol saponin closely related to **10** from its spectral properties. Enzymatic hydrolysis of **11** with β-glucosidase gave the corresponding spirostanol saponin (**11a**) and glucose, and acid hydrolysis with M hydrochloric acid gave **10b**, together with D-glucose, L-arabinose and L-rhamnose in a ratio of 1:1:1. Analysis of the $^1$H and $^{13}$C NMR spectra of **11a** confirmed that the diglycoside attached to C-1 of the aglycone corresponded to that of **3**. The structure of **11** was formulated as 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-5α-furost-25(27)-ene-1β,3α,22ξ,26-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-arabinopyranoside}.

Compound **12** ($C_{46}H_{76}O_{19}$) had one more oxygen atom than did **10**. Enzymatic hydrolysis of **12** with β-glucosidase gave the corresponding spirostanol saponin (**12a**) and glucose. The whole $^{13}$C NMR shifts of **12** featured quite a similarity to those of **10** with exceptions of the signals due to the A and B ring carbons. The methylene carbon signal at $\delta$ 37.4 assignable to C-4 in **10** was replaced by the oxymethine signal at $\delta$ 69.9 in **12**, accompanied by downfield or upfield shifts of the signals due to C-3, C-5 and C-6 by +5.3, +6.2 and −5.7 ppm, respectively; all other



| | $R^1$ | $R^2$ | $R^3$ | |
|---|---|---|---|---|
| 9 | H | β-D-Xyl*p* | Me | (3β) |
| 10 | H | H | Me | (3α) |
| 11 | H | H | H | (3α) |
| 12 | OH | H | Me | (3α) |
| 13 | OH | H | Me | (3β) |



| | $R^1$ | $R^2$ |
|---|---|---|
| 10a | H | Me |
| 11a | H | H |
| 12a | OH | Me |

signals, including those due to the saccharide moieties, were almost identical between **12** and **10**. Furthermore, tracing out the proton coupling systems from the 1-H signal through spin-decoupling experiment in the $^1$H NMR spectrum allowed the sequential assignment of the protons of the A-ring part, giving rise to the partial structure possessing oxygen atoms at C-1, C-3 and C-4. The above $^{13}$C and $^1$H NMR data were consistent with the presence of an additional hydroxyl group at C-4. The $^{13}$C NMR signal due to C-19 at $\delta$ 9.0 was in good agreement with that of **10**, giving evidence for the A/B *trans* (5$\alpha$-H) ring junction. The orientation of the oxygen atoms were located as 1$\beta$-equatorial, 3$\alpha$-axial and 4$\alpha$-equatorial, respectively, by the following $^1$H NMR parameters. The H-1 proton observed at $\delta$ 4.49 as a *dd* signal coupled to H-2$\beta$(ax) ($\delta$ 2.29) with a large $J$ value of 11.7 Hz and to H-2$\alpha$(eq) ($\delta$ 2.63) with $J = 4.1$ Hz. The H-4 proton resonated at $\delta$ 3.75 and coupled to H-3 ($\delta$ 4.30) with $J = 2.5$ Hz and H-5 ($\delta$ 2.00) with $J = 10.9$ Hz. Accordingly, the structure of **12** was assigned as 26-*O*-$\beta$-D-glucopyranosyl-22-*O*-methyl-5$\alpha$-furost-25(27)-ene-1$\beta$,3$\alpha$,4$\alpha$,22$\xi$,26-pentol 1-*O*-{*O*-$\alpha$-L-rhamnopyranosyl-(1 → 2)-*O*-$\beta$-D-fucopyranoside}.

Compound **13** ($C_{46}H_{76}O_{19}$) had the same molecular formula as **12**. Its $^{13}$C NMR spectrum indicated also the presence of a C-4 hydroxyl group and was suggestive of the stereoisomer of **12** with respect to the orientation(s) of the C-3 and/or C-4 hydroxyl group(s). Spin-decoupling experiments revealed that the H-4 proton at $\delta$ 3.73 coupled to H-3 ($\delta$ 3.87) and H-5 ($\delta$ 1.28) with large $J$ values of 12.9 and 9.8 Hz, respectively, and that, in turn, the H-3 proton coupled to H-2$\beta$(ax) ($\delta$ 2.34) with $J = 12.9$ Hz. The above data indicated the 3$\beta$-equatorial and 4$\alpha$-equatorial orientations of the hydroxyl groups. The H-1 and H-5 protons of **13** were shifted to upper fields by 0.44 and 0.72 ppm, respectively, as compared with those of **12**. This phenomenon was considered to be due to the absence of the 1,3-diaxial interactions between the C-3 hydroxyl group and the H-1$\alpha$(ax) and H-5$\alpha$(ax) protons in **13**, consistent with the $\beta$-equatorial orientation of the C-3 hydroxyl group. The structure of **13** was established as 26-*O*-$\beta$-D-glucopyranosyl-22-*O*-methyl-5$\alpha$-furost-25(27)-ene-1$\beta$,3$\beta$,4$\alpha$,22$\xi$,26-pentol 1-*O*-{*O*-$\alpha$-L-rhamnopyranosyl-(1→2)-*O*-$\beta$-D-fucopyranoside}.

Compounds **10**–**13** are new furostanol saponins, among which **12** and **13** are unique in structure possessing a 4$\alpha$-hydroxyl group on the furostanol skeleton.

## EXPERIMENTAL

*General.* NMR (ppm, $J$ Hz): Bruker AM-400, 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 $F_{254}$ (0.25 mm thick or 0.5 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 or RI-8010) equipped with a CAPCELL PAK $C_{18}$ column (Shiseido, 10 mm i.d. × 250 mm, ODS, 5 $\mu$m) for prep. HPLC and a TSK-gel ODS-Prep column (Tosoh, 4.6 mm i.d. × 250 mm, ODS, 5 $\mu$m) for analytical HPLC.

*Plant material. D. concinna* was purchased from Exotic Plants Co Ltd, Japan, and the plant specimen is on file in our laboratory.

*Extraction and isolation.* The plant material (leaves, fresh weight 11.5 kg) was extracted with hot MeOH (20 l × 2). The MeOH extract was concd under red. pres. and the viscous concentrate (900 g) was partitioned between $H_2O$ and *n*-BuOH. The *n*-BuOH-soluble phase (420 g) was passed through a Diaion HP-20 column with increasing amounts of MeOH in $H_2O$. The 80% MeOH and MeOH eluate frs were combined and chromatographed on silica gel eluting with a stepwise gradient mixture of $CHCl_3$-MeOH (9:1; 4:1; 2:1) and, finally, with MeOH. Frs with the same TLC profile were combined. Five frs (I–V) were recovered. Frs III–V mainly contained steroidal saponins. Fr. III was subjected to silica gel CC eluting with $CHCl_3$-MeOH (5:1; 4:1; 3:1) and ODS silica gel CC with MeOH-$H_2O$ (17:3; 4:1) to give **5** (48.7 mg), and **1** and **3** with a few impurities. Final purification of **1** was established by prep. HPLC using MeOH-$H_2O$ (9:1), and that of **3** by prep. TLC developing with $CHCl_3$-MeOH-$H_2O$ (30:10:1) to yield **1** (328 mg) and **3** (48.4 mg), respectively, as pure compounds. Fr. V was further fractionated by subjecting it to ODS silica gel CC with MeOH-$H_2O$ (7:3) into four frs V(a)–V(d). Fr. V(b) was chromatographed on silica gel eluting with $CHCl_3$-MeOH-$H_2O$ (20:10:1) to yield **8** (43.0 mg). Fr. Vc was subjected to a silica gel column eluting with $CHCl_3$-MeOH-$H_2O$ (25:10:1) to yield **7** (300 mg). Fr. Vd was subjected to silica gel CC eluting with $CHCl_3$-MeOH (4:1) and ODS silica gel CC with MeOH-$H_2O$ (7:3; 3:2) and MeCN-$H_2O$ (1:3; 1:4) to collect **4** (123 mg), **2** and **9** with a few impurities, a mixture of **12** and **13**, and a mixture of **6**, **10** and **11**. Purification of **2** and **9** was achieved by means of prep. HPLC using MeOH-$H_2O$ (9:1) for **2** and MeCN-$H_2O$ (1:3) for **9** to furnish **2** (55.3 mg) and **9** (39.4 mg), respectively. Separation of **12** and **13** was carried out by prep. HPLC using MeCN-$H_2O$ (1:4) to yield **12** (47.2 mg) and **13** (22.0 mg). The mixture of **6**, **10** and **11** was subjected to prep. HPLC using MeCN-$H_2O$ (1:3) to result in the isolation of **6** (39.3 mg), **10** (170 mg) and **11** (87.6 mg) as pure compounds.

The physical and spectral characteristics of **1**–**9** were consistent with those previously reported. Data refer to the literature shown in the text.

*Compound* **10**. Amorphous solid. $[\alpha]_D^{27} -45.0°$ (MeOH; *c* 0.12). Negative-ion FAB-MS *m/z* 915 $[M-H]^-$. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 3420 (OH), 2920 (CH). $^1$H NMR (pyridine-$d_5$): $\delta$ 6.41 (1H, *br s*, 1″-H), 5.34 and 5.05 (each 1H, *br s*, 27-H$_2$), 4.91 (1H, *d*, $J = 7.8$ Hz, 1‴-H), 4.73 (1H, *d*, $J = 7.7$ Hz, 1′-H), 3.25 (3H, *s*,

OMe), 1.75 (3H, *d*, $J = 6.1$ Hz, 6″-Me), 1.45 (3H, *d*, $J = 6.3$ Hz, 6′-Me), 1.25 (3H, *s*, 19-Me), 1.11 (3H, *d*, $J = 6.8$ Hz, 21-Me), 0.85 (3H, *s*, 18-Me).

*Enzymatic hydrolysis of* **10**. Compound **10** (16 mg) was treated with β-glucosidase (20 mg) in HOAc-NaOAc buffer (pH 5, 5 ml) at room temp. for 12 hr. The reaction mixture was chromatographed on silica gel eluting with $CHCl_3$-MeOH-$H_2O$ (25:10:1) to give the corresponding spirostanol saponin (**10a**) (4.0 mg) and glucose (1.9 mg).

*Compound* **10a**. Amorphous solid. $[\alpha]_D^{29} -28.1°$ (MeOH; *c* 0.14). Negative-ion FAB-MS *m/z* 721 $[M-H]^-$. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3410 (OH), 2920 (CH). $^1H$ NMR (pyridine-$d_5$): δ 6.42 (1H, *br s*, 1″-H), 4.80 and 4.77 (each 1H, *br s*, 27-$H_2$), 4.73 (1H, *d*, $J = 7.8$ Hz, 1′-H), 1.77 (3H, *d*, $J = 6.1$ Hz, 6″-Me), 1.45 (3H, *d*, $J = 6.3$ Hz, 6′-Me), 1.26 (3H, *s*, 19-Me), 1.03 (3H, *d*, $J = 6.9$ Hz, 21-Me), 0.88 (3H, *s*, 18-Me). Glucose was identified by direct TLC comparison with an authentic sample. $R_f$ (*n*-BuOH-$Me_2CO$-$H_2O$, 4:5:1): 0.34.

*Acid hydrolysis of* **10**. A soln of **10** (20 mg) in M HCl (dioxane-$H_2O$, 1:1, 4 ml) was heated at 100° for 1 hr 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 a gradient mixture of $CHCl_3$-MeOH (19:1; 1:1) to give an aglycone (**10b**) (5.1 mg) and a mixture of monosaccharides (9.5 mg). The monosaccharide mixture was suggested to contain glucose, fucose and rhamnose by direct TLC comparison with authentic samples. $R_f$ (*n*-BuOH-$Me_2CO$-$H_2O$, 4:5:1): 0.62 (rhamnose); 0.52 (fucose); 0.36 (glucose). The mixture (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 mixture was passed through a Sep-Pak $C_{18}$ cartridge (Waters) with $H_2O$-MeCN (4:1; 1:9, each 10 ml). The $H_2O$-MeCN (1:9) eluate fr. was further passed through a Toyopak IC-SP M cartridge (Tosoh) with EtOH (10 ml) to give a mixture of 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides [21, 22], which were 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-fucose, D-glucose and L-rhamnose were detected. $R_t$ (min): 20.77 (D-fucose derivative); 24.22 (D-glucose derivative); 27.22 (L-rhamnose derivative).

*Compound* **11**. Amorphous solid. $[\alpha]_D^{27} -37.5°$ (MeOH; *c* 0.41). Negative-ion FAB-MS *m/z* 901 $[M-H]^-$. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3430 (OH), 2925 (CH). $^1H$ NMR (pyridine-$d_5$): δ 6.39 (1H, *br s*, 1″-H), 5.34 and 5.07 (each 1H, *br s*, 27-$H_2$), 4.91 (1H, *d*, $J = 7.8$ Hz, 1‴-H), 4.73 (1H, *d*, $J = 7.7$ Hz, 1′-H), 3.25 (3H, *s*, OMe), 1.76 (3H, *d*, $J = 6.1$ Hz, 6″-Me), 1.25 (3H, *s*, 19-Me), 1.12 (3H, *d*, $J = 6.8$ Hz, 21-Me), 0.83 (3H, *s*, 18-Me).

*Enzymatic hydrolysis of* **11**. Compound **11** (45 mg) was treated with β-glucosidase (100 mg) in HOAc-NaOAc buffer (pH 5, 10 ml) at room temp. for 48 hr. The reaction mixture was subjected to silica gel CC eluting with $CHCl_3$-MeOH (3:1) and prep. TLC with $CHCl_3$-MeOH-$H_2O$ (30:10:1) to give the cor-

Table 1. $^{13}C$ NMR spectral data for compounds **10**, **10a**, **10b**, **11**, **11a**, **12**, **12a** and **13** in pyridine-$d_5$

| C | 10 | 10a | 10b | 11 | 11a | 12 | 12a | 13 |
|---|---|---|---|---|---|---|---|---|
| 1 | 81.0 | 81.1 | 74.1 | 80.4 | 80.5 | 80.2 | 80.3 | 81.8 |
| 2 | 35.5 | 35.6 | 41.4 | 35.0 | 35.1 | 33.5 | 33.6 | 34.7 |
| 3 | 65.9 | 65.9 | 66.3 | 65.8 | 65.8 | 71.2 | 71.2 | 75.0 |
| 4 | 37.4 | 37.4 | 37.1 | 37.3 | 37.3 | 69.9 | 69.9 | 74.6 |
| 5 | 39.4 | 39.4 | 38.9 | 39.4 | 39.4 | 45.6 | 45.6 | 49.8 |
| 6 | 28.8 | 28.8 | 28.9 | 28.8 | 28.8 | 23.1 | 23.1 | 22.9 |
| 7 | 32.6 | 32.7 | 32.8 | 32.6 | 32.6 | 32.4 | 32.4 | 32.4 |
| 8 | 36.6 | 36.7 | 36.2 | 36.4 | 36.5 | 36.4 | 36.5 | 36.2 |
| 9 | 55.3 | 55.3 | 55.8 | 55.0 | 55.0 | 55.5 | 55.5 | 55.5 |
| 10 | 42.6 | 42.6 | 43.1 | 42.6 | 42.6 | 42.6 | 42.6 | 42.6 |
| 11 | 23.7 | 23.8 | 24.9 | 23.7 | 23.8 | 23.3 | 23.3 | 23.3 |
| 12 | 40.6 | 40.8 | 40.9 | 40.7 | 40.8 | 40.6 | 40.7 | 40.6 |
| 13 | 40.7 | 40.5 | 40.5 | 40.6 | 40.3 | 40.8 | 40.4 | 40.7 |
| 14 | 57.0 | 57.1 | 56.9 | 56.8 | 56.9 | 57.0 | 57.1 | 56.8 |
| 15 | 32.3 | 32.4 | 32.4 | 32.3 | 32.3 | 32.3 | 32.3 | 32.4 |
| 16 | 81.5 | 81.5 | 81.4 | 81.4 | 81.4 | 81.5 | 81.5 | 81.5 |
| 17 | 64.3 | 63.2 | 63.3 | 64.3 | 63.2 | 64.3 | 63.1 | 64.3 |
| 18 | 16.9 | 17.0 | 16.8 | 16.7 | 16.8 | 16.9 | 16.9 | 16.8 |
| 19 | 7.7 | 7.7 | 6.5 | 7.7 | 7.7 | 9.0 | 9.0 | 10.1 |
| 20 | 40.4 | 41.9 | 41.9 | 40.4 | 41.9 | 40.4 | 41.9 | 40.4 |
| 21 | 16.0 | 14.8 | 15.0 | 16.1 | 14.9 | 16.0 | 14.8 | 16.0 |
| 22 | 112.4 | 109.4 | 109.4 | 112.4 | 109.4 | 112.4 | 109.4 | 112.4 |
| 23 | 31.5 | 33.2 | 33.2 | 31.5 | 33.1 | 31.5 | 33.1 | 31.5 |
| 24 | 28.1 | 29.0 | 29.0 | 28.0 | 28.9 | 28.0 | 28.9 | 28.0 |
| 25 | 146.8 | 144.5 | 144.5 | 146.8 | 144.5 | 146.8 | 144.5 | 146.8 |
| 26 | 72.0 | 65.0 | 65.0 | 72.0 | 64.9 | 72.0 | 64.9 | 72.0 |
| 27 | 111.0 | 108.6 | 108.6 | 111.0 | 108.6 | 111.0 | 108.6 | 111.0 |
| OMe | 47.3 | | | 47.3 | | 47.3 | | 47.3 |
| 1′ | 100.2 | 100.3 | | 100.4 | 100.5 | 100.2 | 100.3 | 99.7 |
| 2′ | 74.3 | 74.3 | | 74.9 | 74.9 | 74.3 | 74.3 | 73.9 |
| 3′ | 77.0 | 77.1 | | 76.3 | 76.3 | 77.0 | 77.0 | 76.9 |
| 4′ | 73.3 | 73.3 | | 70.3 | 70.3 | 73.3 | 73.3 | 73.3 |
| 5′ | 71.0 | 71.1 | | 67.5 | 67.5 | 71.1 | 71.1 | 71.2 |
| 6′ | 17.1 | 17.1 | | | | 17.1 | 17.1 | 17.1 |
| 1″ | 101.4 | 101.4 | | 101.5 | 101.5 | 101.4 | 101.4 | 101.4 |
| 2″ | 72.6 | 72.6 | | 72.6 | 72.5 | 72.6 | 72.6 | 72.5 |
| 3″ | 72.6 | 72.6 | | 72.6 | 72.6 | 72.6 | 72.6 | 72.5 |
| 4″ | 74.3 | 74.3 | | 74.2 | 74.2 | 74.3 | 74.3 | 74.2 |
| 5″ | 69.2 | 69.2 | | 69.3 | 69.3 | 69.2 | 69.2 | 69.2 |
| 6″ | 19.0 | 19.0 | | 19.0 | 19.0 | 19.0 | 19.0 | 19.0 |
| 1‴ | 103.8 | | | 103.8 | | 103.8 | | 103.8 |
| 2‴ | 75.1 | | | 75.1 | | 75.1 | | 75.1 |
| 3‴ | 78.6 | | | 78.6 | | 78.6 | | 78.6 |
| 4‴ | 71.7 | | | 71.7 | | 71.7 | | 71.7 |
| 5‴ | 78.5 | | | 78.5 | | 78.5 | | 78.5 |
| 6‴ | 62.8 | | | 62.8 | | 62.8 | | 62.8 |

responding spirostanol saponin (**11a**) (21.5 mg) and glucose (5.1 mg).

*Compound* **11a**. Amorphous solid. $[\alpha]_D^{29}$ −68.7° (MeOH; *c* 0.91). Positive-ion FAB-MS *m/z* 747 $[M+K]^+$. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420 (OH), 2920 (CH). $^1H$ NMR (pyridine-$d_5$): δ 6.39 (1H, *br s*, 1″-H), 4.81 and 4.77 (each 1H, *br s*, 27-$H_2$), 4.74 (1H, *d*, *J* = 7.7 Hz, 1′-H), 1.77 (3H, *d*, *J* = 6.1 Hz, 6″-Me), 1.26 (3H, *s*, 19-Me), 1.05 (3H, *d*, *J* = 6.9 Hz, 21-Me), 0.86 (3H, *s*, 18-Me).

*Acid hydrolysis of* **11**. Compound **11** (10 mg) was subjected to acid hydrolysis as described for **10** to give an aglycone (**10b**) (2.5 mg) and a mixture of monosaccharides (5.7 mg). The monosaccharides were identified as D-xylose, L-arabinose and L-rhamnose by direct TLC comparison with authentic samples and HPLC analysis of their corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives. $R_f$ (*n*-BuOH-$Me_2CO$-$H_2O$, 4:5:1): 0.64 (rhamnose); 0.48 (arabinose); 0.36 (glucose). $R_t$ (min): 17.63 (L-arabinose derivative); 24.27 (D-glucose derivative); 27.22 (L-rhamnose derivative).

*Compound* **12**. Amorphous solid. $[\alpha]_D^{29}$ −64.0° (MeOH; *c* 0.10). Negative-ion FAB-MS *m/z* 931 $[M-H]^-$. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420 (OH), 2915 (CH). $^1H$ NMR (pyridine-$d_5$): δ 6.41 (1H, *br s*, 1″-H), 5.34 and 5.05 (each 1H, *br s*, 27-$H_2$), 4.91 (1H, *d*, *J* = 7.8 Hz, 1‴-H), 4.78 (1H, *d*, *J* = 7.7 Hz, 1′-H), 4.49 (1H, *dd*, *J* = 11.7, 4.1 Hz, 1-H), 4.30 (1H, *br dd*, *J* = 3.4, 2.5 Hz, 3-H), 3.75 (1H, *dd*, *J* = 10.9, 2.5 Hz, 4-H), 3.25 (3H, *s*, OMe), 2.63 (1H, *ddd*, *J* = 13.8, 4.1, 3.4 Hz, 2eq-H), 2.29 (1H, *br dd*, *J* = 13.8, 11.7 Hz, 2ax-H), 2.00 (1H, *m*, 5-H), 1.74 (3H, *d*, *J* = 6.1 Hz, 6″-Me), 1.45 (3H, *d*, *J* = 6.3 Hz, 6′-Me), 1.33 (3H, *s*, 19-Me), 1.10 (3H, *d*, *J* = 6.9 Hz, 21-Me), 0.85 (3H, *s*, 18-Me).

*Enzymatic hydrolysis of* **12**. Compound **12** (30 mg) was treated with β-glucosidase (50 mg) in HOAc-NaOAc buffer (pH 5, 5 ml) at room temp. for 24 hr. The reaction mixture was subjected to silica gel CC eluting with $CHCl_3$-MeOH-$H_2O$ (25:10:1) to give the corresponding spirostanol saponin (**12a**) (17.8 mg) and glucose (4.9 mg).

*Compound* **12a**. Amorphous solid. $[\alpha]_D^{23}$ −84.4° (MeOH; *c* 0.87). Negative-ion FAB-MS *m/z* 737 $[M-H]^-$. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420 (OH), 2910 (CH). $^1H$ NMR (pyridine-$d_5$): δ 6.41 (1H, *br s*, 1″-H), 4.87 (1H, *dq*, *J* = 9.5, 6.1 Hz, 5″-H), 4.80 (1H, *br s*, 27-Ha), 4.78 (1H, *d*, *J* = 7.7 Hz, 1′-H), 4.77 (2H, overlapping, 27-Hb and 2″-H), 4.62 (1H, *dd*, *J* = 9.5, 3.3 Hz, 3″-H), 4.53 (1H, *dd*, *J* = 9.3, 7.7 Hz, 2′-H), 4.50 (1H, *q*-like, *J* = 7.4 Hz, 16-H), 4.48 (1H, *dd*, *J* = 11.7, 4.2 Hz, 1-H), 4.45 and 4.01 (each 1H, *br d*, *J* = 12.0 Hz, 26-$H_2$), 4.32 (1H, *dd*, *J* = 9.5, 9.5 Hz, 4″-H), 4.30 (1H, *br dd*, *J* = 3.5, 2.4 Hz, 3-H), 4.09 (1H, *dd*, *J* = 9.3, 3.2 Hz, 3′-H), 3.87 (1H, *br d*, *J* = 3.2 Hz, 4′-H), 3.75 (1H, *dd*, *J* = 10.9, 2.4 Hz, 4-H), 3.56 (1H, *br q*, *J* = 6.3 Hz, 5′-H), 2.63 (1H, *ddd*, *J* = 14.1, 4.2, 3.5 Hz, 2eq-H), 2.31 (1H, *br dd*, *J* = 14.1, 11.7 Hz, 2ax-H), 2.00 (1H, *m*, 5-H), 1.75 (3H, *d*, *J* = 6.1 Hz, 6″-Me), 1.45 (3H, *d*, *J* = 6.3 Hz, 6′-Me), 1.34 (3H, *s*, 19-Me), 1.02 (3H, *d*, *J* = 6.9 Hz, 21-Me), 0.88 (3H, *s*, 18-Me).

*Compound* **13**. Amorphous solid. $[\alpha]_D^{29}$ −64.8° (MeOH; *c* 0.11). Negative-ion FAB-MS *m/z* 931 $[M-H]^-$. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3425 (OH), 2915 (CH). $^1H$ NMR (pyridine-$d_5$): δ 6.40 (1H, *br s*, 1″-H), 5.34 and 5.05 (each 1H, *br s*, 27-$H_2$), 4.91 (1H, *d*, *J* = 7.7 Hz, 1‴-H), 4.78 (1H, *d*, *J* = 7.8 Hz, 1′-H), 4.05 (1H, *dd*, *J* = 11.9, 4.0 Hz, 1-H), 3.87 (1H, *ddd*, *J* = 12.9, 9.8, 5.0 Hz, 3-H), 3.73 (1H, *dd*, *J* = 12.9, 9.8 Hz, 4-H), 3.25 (3H, *s*, OMe), 2.79 (1H, *ddd*, *J* = 12.9, 5.0, 4.0 Hz, 2eq-H), 2.34 (1H, *ddd*, *J* = 12.9, 12.9, 11.9 Hz, 2ax-H), 1.73 (3H, *d*, *J* = 6.1 Hz, 6″-Me), 1.52 (3H, *d*, *J* = 6.3 Hz, 6′-Me), 1.33 (3H, *s*, 19-Me), 1.28 (1H, *m*, 5-H), 1.12 (3H, *d*, *J* = 6.8 Hz, 21-Me), 0.84 (3H, *s*, 18-Me).

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