Pergamon

PII: S0031-9422(97)00545-1

Phytochemistry, Vol. 47, No. 1, pp. 79–85, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

STEROIDAL SAPONINS FROM THE LEAVES OF *CORDYLINE STRICTA*

YOSHIHIRO MIMAKI,* MINPEI KURODA, YOKO TAKAASHI and YUTAKA SASHIDA*

School of Pharmacy, Tokyo University of Pharmacy and Life Science, Horinouchi 1432-1, Hachioji, Tokyo 192-03, Japan

(*Received* 12 *May* 1997)

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

Abstract—Three new spirostanol saponins and two new furostanol saponins were isolated from the fresh leaves of *Cordyline stricta*. Their structures were elucidated on the basis of spectroscopic analysis, including various 2D-NMR techniques, hydrolysis, and by comparison of spectral data of known compounds. Two of the isolated saponins contained a new branched triglycoside moiety assigned as *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-xylopyranose with the formation of an *O*-glycosidic linkage to C-1 of the aglycone. © 1997 Elsevier Science Ltd

INTRODUCTION

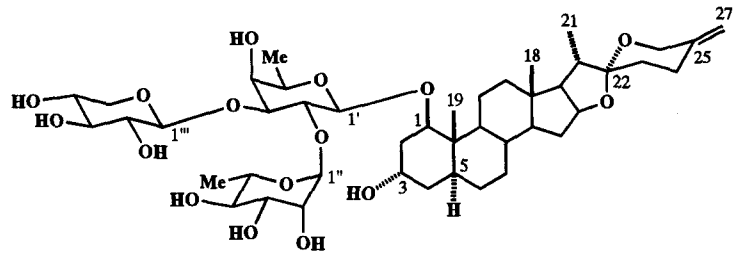
Previously, we have reported the isolation and structural assignment of four new spirostanol glucosides, three new furostanol glucosides and a new pregnane glucoside from the fresh leaves of *Cordyline stricta*, which is the first example of the isolation of the steroidal glucosides from a plant of the genus *Cordyline* [1]. Further phytochemical analysis of the plant material has resulted in the isolation of three new spirostanol saponins and two new furostanol saponins. This paper deals with the structural determination of the new saponins based on spectroscopic analysis, including various 2D-NMR techniques, hydrolysis, and by comparison of spectral data of known compounds.

RESULTS AND DISCUSSION

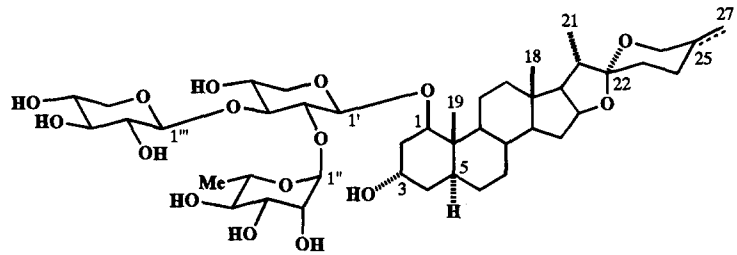
The 1-butanol-soluble part of the methanolic extract of *C. stricta* leaves was separated by silica gel column chromatography into five fractions. Compounds **1**–**5** were isolated from the most polar fraction (fr. V) after a series of chromatographic separations.

Compound **1** ($C_{44}H_{70}O_{16}$) was obtained as an amorphous solid, $[\alpha]_D$ −54.5° (methanol). Its ^1H NMR spectrum in pyridine-d_5 showed signals for three steroid methyls at δ 1.24 (*s*), 1.03 (*d*, *J* = 6.9 Hz) and 0.86 (*s*), an exomethylene group at δ 4.80 and 4.76 (each *br s*), and three anomeric protons at δ 6.40 (*br s*), 4.99 (*d*, *J* = 7.5 Hz) and 4.74 (*d*, *J* = 8.0 Hz). Acid hydrolysis of **1** with 1 M hydrochloric acid in dioxane–water (1:1) gave a steroidal sapogenin (**1a**) ($C_{27}H_{42}O_4$), together with D-xylose, D-fucose and L-rhamnose in a ratio of 1:1:1. Analysis of the ^1H NMR and ^{13}C NMR data led to identification of **1a** as 5α-spirost-25(27)-ene-1β,3α-diol, that is, 1β-hydroxycrabbogenin, which has been isolated from a saponified extract of *C. stricta* leaves [2]. The locations of the glycosidic linkages of **1** were elucidated by analysis of the 2D-NMR spectra, which were measured in a mixed solvent of pyridine-d_5 and methanol-d_4 (11:1) to remove exchangeable protons and minimize signal overlap. Complete assignment of the ^1H NMR signals of the three monosaccharides and the aglycone were achieved by inspection of the ^1H–^1H COSY spectrum, combined with the HOHAHA data, starting from the easily distinguished anomeric protons as shown in Tables 1 and 2. The HMQC spectrum correlated all the proton signals with those of the corresponding one-bond coupled carbons. The ^1H NMR assignment and comparison of the ^{13}C NMR shifts with literature values [3, 4], taking into account the known effect of *O*-glycosylation and the result of acid hydrolysis suggested that the saccharide sequence was *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-fucopyranose. The confirmative evidence for the triglycoside structure and its linkage position to the aglycone was obtained by the observation of ^1H–^{13}C long-range correlation from each anomeric proton across the glycosidic bond to the carbon of either the substituted monosaccharide or the agly-

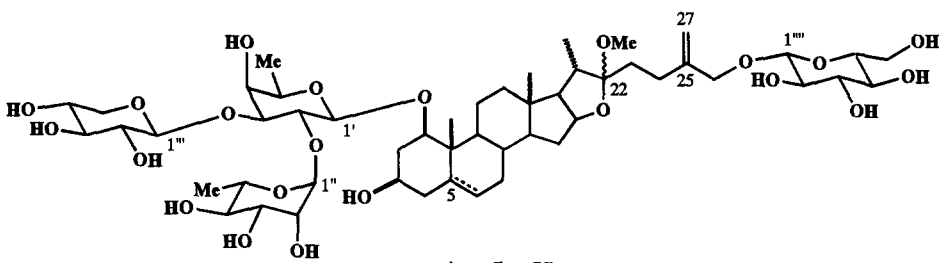
* Authors to whom correspondence should be addressed.



1



2 25*S*
3 $\Delta^{25(27)}$



4 5α-H
5 Δ^{5}

cone. In the HMBC spectrum, the anomeric proton signals at δ 6.27 (rhamnose), 4.92 (xylose) and 4.71 (fucose) exhibited correlations with the carbon signals at δ 73.4 (C-2 of fucose), 86.0 (C-3 of fucose) and 81.3 (C-1 of aglycone), respectively. Thus, the structure of **1** was characterized as 5α-spirost-25(27)-ene-1β,3α-diol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-fucopyranoside}.

The ^{1}H NMR spectrum of compound **2** ($C_{43}H_{70}O_{16}$) showed signals due to four steroid methyls at δ 1.23

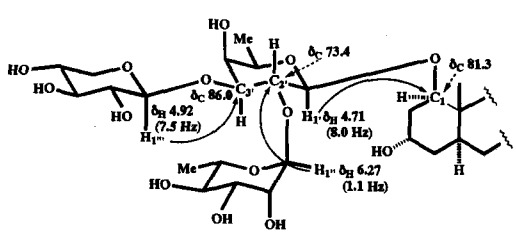


Fig. 1. HMBC correlations of the saccharide portion of **1**.

(*s*), 1.14 (*d*, *J* = 6.9 Hz), 1.07 (*d*, *J* = 7.0 Hz) and 0.92 (*s*), and three anomeric protons at δ 6.47 (*br s*), 4.94 (*d*, *J* = 7.6 Hz) and 4.72 (*d*, *J* = 7.7 Hz). Acid hydrolysis of **2** with 1 M hydrochloric acid yielded a steroidal sapogenin, identified as (25*S*)-5α-spirostane-1β,3α-diol (cordylagenin) by its physical properties and spectral data [1, 5], together with D-xylose and L-rhamnose in a ratio of 2:1. The structure of the saccharide moiety and its linkage position to the aglycone were determined by the same procedures as described for **1**. All the ^{1}H and ^{13}C NMR signals were assigned by the concerted use of the ^{1}H–^{1}H COSY, HOHAHA and HMQC spectra, suggesting that **2** contained a terminal xylopyranosyl unit, a terminal rhamnopyranosyl unit and a disubstituted xylopyranosyl unit. In the HMBC spectrum, the anomeric proton signals at δ 6.36 (rhamnose), 4.86 (terminal xylose) and 4.69 (substituted xylose) exhibited correlations with the carbon signals at δ 75.9 (C-2 of substituted xylose), 88.8 (C-3 of substituted xylose) and 80.9 (C-1 of agly-

Table 1. ^{1}H NMR and ^{13}C NMR chemical shift assignment of the aglycone moiety of compound **1** in pyridine-d_5–methanol-d_4 (11:1)

Position	^{1}H	J (Hz)	^{13}C
1	4.35 *dd*	11.5, 4.3	81.3
2 ax	2.20 *ddd*	12.5, 12.5, 2.7	35.4
eq	2.45 *br dd*	12.5, 4.3	
3	4.25 *br d*	2.7	66.1
4 ax	1.61 *br dd*	13.5, 13.5	37.3
eq	1.52 *br d*	13.5	
5	1.98		39.6
6 ax	1.25		28.9
eq	1.33		
7 ax	0.92 *dddd*	13.1, 13.1, 13.1, 3.5	32.7
eq	1.59		
8	1.60		36.8
9	1.19		55.5
10	—		42.7
11 ax	1.36		23.9
eq	3.20 *br dd*	13.8, 3.2	
12 ax	1.23		40.8
eq	1.57		
13	—		40.6
14	1.14		57.3
15 α	2.00		32.5
β	1.40		
16	4.50 *q*-like	6.8	81.6
17	1.79 *dd*	8.6, 6.8	63.3
18	0.83		17.0
19	1.20		7.8
20	1.93		42.1
21	1.01 *d*	7.0	14.9
22	—		109.6
23 a	1.78		33.3
b	1.74		
24 ax	2.68 *ddd*	13.0, 13.0, 5.4	29.1
eq	2.23 *br d*	13.0	
25	—		144.7
26 a	4.43 *br d*	12.1	65.1
b	3.99 *br d*	12.1	
27 a	4.80 *br s*		108.7
b	4.77 *br s*		

All the signals were assigned by the ^{1}H–^{1}H COSY, HOHAHA and HMQC spectra.

cone), respectively. The structure of **2** was thus formulated as (25*S*)-5α-spirostane-1β,3α-diol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-xylopyranoside}.

Compound **3** ($C_{43}H_{68}O_{16}$) was suggested to be a spirostanol saponin closely related to **2** from its spectral data. It only differed from **2** in the presence of an exomethylene group [δ_H 4.80 and 4.77 (each *br s*); δ_C 144.5 (C) and 108.6 (CH_2)] instead of the C-27 secondary methyl group, and the ^{1}H NMR shifts due to the exomethylene protons and the ^{13}C NMR signals due to the spiroacetal moiety (E and F ring parts) of **3** were in good agreement with those of **1**. The structure of **3** was assigned as 5α-spirost-25(27)-ene-1β,3α-diol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-xylopyranoside}.

Compound **4** ($C_{51}H_{84}O_{22}$) was shown to be a 22-methoxyfurostanol saponin by Ehrlich's test [6, 7] and the ^{1}H and ^{13}C NMR spectra [δ_H 3.25 (3H, *s*); δ_C 112.4 (C) and 47.3 (Me)] [8]. Enzymatic hydrolysis of **4** with β-glucosidase gave the corresponding spirostanol saponin (**4a**) and glucose, and acid hydrolysis with 1 M hydrochloric acid gave an aglycone (**4b**), together with D-glucose, D-xylose, D-fucose and L-rhamnose in a ratio of 1:1:1:1. The ^{1}H NMR spectrum of **4b** showed signals for three steroid methyls at δ 1.14 (*s*), 1.07 (*d*, J = 6.9 Hz) and 0.91 (*s*), and an exomethylene group at δ 4.82 and 4.79 (each *br s*), along with providing evidence for the presence of two equatorial oriented hydroxyl groups [δ 4.00 (*br m*, $W_{1/2}$ = 21.2 Hz) and 3.73 (*dd*, J = 11.3, 4.0 Hz)]. The above ^{1}H NMR data and inspection of the ^{13}C NMR spectrum allowed identification of **4b** as 5α-spirost-25(27)-ene-1β,3β-diol, that is, australigenin [9]. The triglycoside structure of **4a** was revealed to be same as that of **1** by comparison of their ^{1}H and ^{13}C NMR spectra, and the linkage to the aglycone C-1 hydroxyl group was shown by comparison of the ^{13}C NMR spectrum of **4a** with that of **4b**; the signal due to the aglycone C-1 was shifted to a lower field by 5.5 ppm, whereas the signal due to C-2 was moved to a higher field by 6.2 ppm by *O*-glycosylation. The structure of **4** was characterized as 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-5α-furost-25(27)-ene-1β,3β,22ξ,26-tetrol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-fucopyranoside}.

All spectral features of compound **5** ($C_{51}H_{82}O_{22}$) showed a close similarity to those of **4**. On comparison of the ^{1}H NMR spectrum of **5** with that of **4**, an olefinic proton signal appeared at δ 5.60 (*br d*, J = 5.6 Hz), accompanied by a downfield shift of 19-Me by 0.18 ppm in the ^{1}H NMR spectrum of **5**. Furthermore, the aliphatic carbon signals at δ 43.2 (CH) and 28.9 (CH_2) due to C-5 and C-6, respectively, were displaced by a pair of olefinic carbon signals at δ 139.7 (C) and 124.7 (CH) in the ^{13}C NMR spectrum of **5**. These data implied that **5** was a 5,6-dehydro derivative of **4**. Acid hydrolysis of **5** with 1 M hydrochloric acid gave an aglycone, identified as spirost-5,25(27)-diene-1β,3β-diol (neoruscogenin) [4, 10], together with D-glucose, D-xylose, D-fucose and L-rhamnose. The above data were consistent with **5** being the corresponding Δ^5-furostanol saponin of **4**. The structure of **5** was shown to be 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}.

Compounds **1**–**5** are new naturally occurring steroidal saponins, among which **2** and **3** contain a new branched triglycoside moiety assigned as *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-xylopyranose.

EXPERIMENTAL

General. 1D-NMR (ppm, J Hz): Bruker AM-400, 400 MHz for ^{1}H NMR and 2D-NMR: Bruker AM-

Table 2. ^{1}H NMR and ^{13}C NMR chemical shift assignment of the saccharide moiety of compounds **1** and **2** in pyridine-d_5–methanol-d_4 (11:1)

	1				**2**		
Position	^{1}H	*J* (Hz)	^{13}C	Position	^{1}H	*J* (Hz)	^{13}C
1′	4.71 *d*	8.0	100.4	1′	4.69 *d*	7.7	100.5
2′	4.53 *dd*	9.5, 8.0	73.4	2′	4.03 *dd*	8.8, 7.7	75.9
3′	4.01 *dd*	9.5, 2.9	86.0	3′	3.88		88.8
4′	4.16 *br d*	2.9	72.8	4′	3.89		69.5
5′	3.60 *br q*	6.3	70.8	5′a	4.19		66.5
6′	1.43 *d*	6.3	17.1	b	3.36 *dd*	10.1, 10.1	
1″	6.27 *d*	1.1	101.6	1″	6.36 *br s*		101.5
2″	4.70 *dd*	3.5, 1.1	72.5	2″	4.65 *br d*	2.6	72.3
3″	4.48 *dd*	9.4, 3.5	72.4	3″	4.41 *dd*	9.5, 2.6	72.2
4″	4.19 *dd*	9.4, 9.4	74.2	4″	4.18 *dd*	9.5, 9.5	74.0
5″	4.75 *dq*	9.4, 6.2	69.4	5″	4.67 *dq*	9.5, 6.0	69.6
6″	1.70 *d*	6.2	19.1	6″	1.70 *d*	6.0	19.1
1‴	4.92 *d*	7.5	106.6	1‴	4.86 *d*	7.6	105.1
2‴	3.82 *dd*	8.6, 7.5	74.7	2‴	3.86 *dd*	8.7, 7.6	74.6
3‴	3.98 *dd*	8.6, 8.6	78.2	3‴	3.95 *dd*	8.7, 8.7	78.2
4‴	4.03 *ddd*	10.1, 8.6, 5.3	70.9	4‴	4.02		70.6
5‴a	4.23 *dd*	11.3, 5.3	67.1	5‴a	4.19		67.1
b	3.62 *dd*	11.3, 10.1		b	3.61 *dd*	10.9, 10.5	

500, 500 MHz for ^{1}H NMR. CC: silica gel (Fuji-Silysia Chemical), octadecylsilanized (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 μm) for prep. HPLC and a TSK-gel ODS-Prep column (Tosoh, 4.6 mm i.d. × 250 mm, ODS, 5 μm) for analytical HPLC.

Plant material. C. stricta 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 8.0 kg) was extracted with hot MeOH (18 l × 2). The MeOH extract was concd under red. pres., and the viscous concentrate (680 g) was 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 (9:1; 4:1; 2:1) and, finally, with MeOH gave 5 frs (I–V). Fr. V (90 g) was passed through a Diaion HP-20 (300 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 it was chromatographed on ODS silica gel eluting with MeOH–H_2O (3:2) to give frs V(a) and V(b). Fr. V(a) was subjected to CC on silica gel eluting with $CHCl_3$–MeOH–H_2O (30:10:1) to give **4** with a few impurities, final purification of which was established by prep. HPLC using MeCN–H_2O (7:13) as the solvent to yield **4** (127 mg) as a pure compound. Fr. V(b) was further fractionated by subjecting it to silica gel CC eluting with $CHCl_3$–MeOH–H_2O (30:10:1) into frs V(b-1)–V(b-3). Fr. V(b-1) was subjected to silica gel CC eluting with $CHCl_3$–MeOH–H_2O (30:10:1) and prep. HPLC with MeOH–H_2O (9:1) to give a mixt. of **2** and **3**, which was sepd by prep. TLC developing with $CHCl_3$–Et_2O–MeOH–H_2O (70:40:35:1) to yield **2** (54.8 mg) and **3** (10.4 mg) as pure compounds. Fr. V(b-2) was subjected to a silica gel column eluting with $CHCl_3$–MeOH–H_2O (30:10:1) and prep. HPLC with MeOH–H_2O (9:1) to yield **1** (47.4 mg). Fr. V(b-3) was purified by prep. HPLC eluting with MeCN–H_2O (7:13) to yield **5** (48.3 mg).

Compound **1**. Amorphous solid. $[\alpha]_D^{27}$ −54.5° (MeOH; *c* 0.29). Negative-ion FAB-MS *m/z* 853 $[M-H]^-$, 720 $[M\text{-xylosyl}]^-$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2905 and 2835 (CH), 1440, 1365, 1295, 1220, 1150, 1120, 1055, 1030, 965, 935, 910, 865, 825, 800. ^{1}H NMR (pyridine-d_5): δ 6.40 (1H, *br s*, 1″-H), 4.99 (1H, *d*, *J* = 7.5 Hz, 1‴-H), 4.80 and 4.76 (each 1H, *br s*, 27-H_2), 4.74 (1H, *d*, *J* = 8.0 Hz, 1′-H), 1.75 (3H, *d*, *J* = 6.1 Hz, 6″-Me), 1.45 (3H, *d*, *J* = 6.3 Hz, 6′-Me), 1.24 (3H, *s*, 19-Me), 1.03 (3H, *d*, *J* = 6.9 Hz, 21-Me), 0.86 (3H, *s*, 18-Me).

Acid hydrolysis of **1**. A soln of **1** (25 mg) in M HCl (dioxane–H_2O, 1:1, 5 ml) was heated at 100° for 1 hr under an Ar atmosphere. After cooling, the reaction mixt. was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column and chromatographed on silica gel eluting with a gradient mixt. of $CHCl_3$–MeOH (19:1; 1:1) to give an aglycone (**1a**) (3.4 mg) and a mixt. of monosaccharides (11.5 mg).

Table 3. ^{13}C NMR spectral data for compounds **1**, **1a**, **2**–**4**, **4a**, **4b** and **5** in pyridine-d_5

C	1	1a	2	3	4	4a	4b	5
1	81.3	74.1	80.8	80.9	82.6	82.6	77.1	84.3
2	35.5	41.4	34.9	35.0	37.7	37.7	43.9	38.1
3	65.9	66.3	65.8	65.8	67.9	68.0	67.9	68.3
4	37.4	37.1	37.3	37.3	39.7	39.8	39.6	43.9
5	39.5	38.9	39.4	39.5	43.2	43.2	42.9	139.7
6	28.8	28.9	28.7	28.7	28.9	29.0	28.9	124.7
7	32.6	32.8	32.5	32.5	32.5	32.4	32.6	32.0
8	36.7	36.2	36.7	36.7	36.5	36.6	36.1	33.0
9	55.3	55.8	54.9	54.9	55.2	55.2	55.6	50.6
10	42.6	43.1	42.5	42.5	41.5	41.5	42.0	42.8
11	23.8	24.9	23.9	23.9	23.6	23.7	24.7	24.0
12	40.7	40.9	40.9	40.9	40.6	40.7	40.8	40.4
13	40.4	40.5	40.4	40.5	40.7	40.4	40.4	40.4
14	57.1	56.9	56.9	57.0	56.8	57.0	56.8	57.1
15	32.3	32.4	32.3	32.3	32.4	32.4	32.4	32.4
16	81.5	81.4	81.2	81.5	81.5	81.5	81.4	81.5
17	63.1	63.3	63.1	63.3	64.3	63.2	63.2	64.2
18	17.0	16.8	17.0	17.0	16.8	16.9	16.7	16.8
19	7.7	6.5	7.7	7.8	8.7	8.7	7.6	15.0
20	41.9	41.9	42.5	41.9	40.4	42.0	41.9	40.5
21	14.8	15.0	14.8	14.9	16.0	14.9	14.9	16.1
22	109.4	109.4	109.7	109.4	112.4	109.4	109.4	112.4
23	33.2	33.2	26.2	33.2	31.5	33.2	33.2	31.5
24	28.9	29.0	26.4	29.0	28.1	29.0	28.9	28.1
25	144.5	144.5	27.5	144.5	146.9	144.5	144.5	146.9
26	64.9	65.0	65.0	65.0	72.0	65.0	65.0	72.0
27	108.6	108.6	16.3	108.6	111.1	108.6	108.6	111.0
OMe					47.3			47.3
1′	100.4		100.5	100.5	99.7	99.7		100.5
2′	73.3		75.8	75.8	73.6	73.6		73.5
3′	85.8		88.9	88.9	85.8	85.7		85.6
4′	72.8		69.5	69.5	72.7	72.8		72.7
5′	70.8		66.6	66.6	70.8	70.9		70.8
6′	17.0				17.1	17.1		17.1
1″	101.6		101.5	101.5	101.6	101.7		101.8
2″	72.6		72.5	72.5	72.5	72.5		72.6
3″	72.6		72.5	72.5	72.5	72.6		72.6
4″	74.3		74.2	74.2	74.3	74.3		74.3
5″	69.3		69.6	69.6	69.4	69.4		69.4
6″	19.2		19.3	19.3	19.2	19.2		19.2
1‴	106.6		105.2	105.2	106.6	106.6		106.7
2‴	74.7		74.7	74.8	74.7	74.7		74.7
3‴	78.3		78.4	78.5	78.3	78.4		78.4
4‴	71.0		70.6	70.6	71.0	71.0		71.0
5‴	67.1		67.2	67.3	67.1	67.1		67.1
1⁗					103.8			103.9
2⁗					75.1			75.2
3⁗					78.6			78.6
4⁗					71.7			71.7
5⁗					78.5			78.5
6⁗					62.8			62.9

Compound **1a**. *Amorphous solid.* $[\alpha]_D^{29}$ −53.7° (MeOH; *c* 0.19). EI-MS *m/z* (rel. int.): 430 $[M]^+$ (1.5), 360 (12), 318 (24), 289 (28), 137 (100). IR ν_{max}^{kBr} cm^{-1}: 3435 (OH), 2920 and 2845 (CH), 1445, 1370, 1335, 1285, 1270, 1225, 1165, 1115, 1100, 1065, 1040, 1020, 990, 980, 955, 940, 920, 895, 875, 860. ^1H NMR (pyri-

dine-d_5): δ 4.82 and 4.78 (each 1H, *br s*, 27-H_2), 4.55 (1H, *q*-like, J = 6.8 Hz, 16-H), 4.48 and 4.04 (each 1H, *br d*, J = 12.2 Hz, 26-H_2), 4.38 (overlapping, 1-H, 3-H), 1.13 (3H, *s*, 19-Me), 1.06 (3H, *d*, J = 6.9 Hz, 21-Me), 0.93 (3H, *s*, 18-Me). The monosaccharide mixt. was suggested to be composed of xylose, fucose and rhamnose by direct TLC comparison with authentic samples. R_f (*n*-BuOH–Me_2CO–H_2O, 4:5:1): 0.66 (rhamnose); 0.51 (xylose); 0.48 (fucose). The mixt. (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; 1:9, 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 mixt. of 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides [11, 12], which were 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 derivatives of D-xylose, D-fucose and L-rhamnose were detected. R_t (min): 18.73 (D-xylose derivative); 20.80 (D-fucose derivative); 27.34 (L-rhamnose derivative).

Compound **2**. Amorphous solid. $[\alpha]_D^{28}$ −46.5° (MeOH; *c* 0.16). Positive-ion FAB-MS *m/z* 881 $[M+K]^+$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2930 (CH), 1445, 1370, 1215, 1165, 1125, 1060, 1035, 985, 915, 890, 860, 805. ^{1}H NMR (pyridine-d_5): δ 6.47 (1H, *br s*, 1″-H), 4.94 (1H, *d*, J = 7.6 Hz, 1‴-H), 4.72 (1H, *d*, J = 7.7 Hz, 1′-H), 1.78 (3H, *d*, J = 6.1 Hz, 6″-Me), 1.23 (3H, *s*, 19-Me), 1.14 (3H, *d*, J = 6.9 Hz, 27-Me), 1.07 (3H, *d*, J = 7.0 Hz, 21-Me), 0.92 (3H, *s*, 18-Me).

Acid hydrolysis of **2**. Compound **2** (10 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone (**2a**) (5.9 mg) and a mixt. of monosaccharides (4.0 mg). Physical and spectral data of **2a**: ref. [1]. The monosaccharides were identified as D-xylose 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); 0.55 (xylose). R_t (min): 18.63 (D-xylose derivative); 27.23 (L-rhamnose derivative).

Compound **3**. Amorphous solid. $[\alpha]_D^{27}$ −72.6° (MeOH; *c* 0.52). Positive-ion FAB-MS *m/z* 879 $[M+K]^+$. IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2915 (CH), 1445, 1365, 1225, 1155, 1060, 1035, 975, 935, 915, 865, 825, 805. ^{1}H NMR (pyridine-d_5): δ 6.48 (1H, *br s*, 1″-H), 4.80 and 4.77 (each 1H, *br s*, 27-H_2), 4.95 (1H, *d*, J = 7.6 Hz, 1‴-H), 4.73 (1H, *d*, J = 7.7 Hz, 1′-H), 1.79 (3H, *d*, J = 6.1 Hz, 6″-Me), 1.23 (3H, *s*, 19-Me), 1.10 (3H, *d*, J = 6.9 Hz, 21-Me), 0.93 (3H, *s*, 18-Me).

Compound **4**. Amorphous solid. $[\alpha]_D^{27}$ −20.0° (MeOH; *c* 0.11). Negative-ion FAB-MS *m/z* 1047 $[M-H]^-$. IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2925 (CH), 1445, 1370, 1160, 1065, 1040, 980, 910. ^{1}H NMR (pyridine-d_5): δ 6.37 (1H, *br s*, 1″-H), 5.34 and 5.05 (each 1H, *br s*, 27-H_2), 4.99 (1H, *d*, J = 7.4 Hz, 1‴-H), 4.91 (1H, *d*, J = 7.8 Hz, 1⁗-H), 4.60 (overlapping, 1′-H), 3.25 (3H, *s*, OMe), 1.74 (3H, *d*, J = 6.0 Hz, 6″-Me), 1.52 (3H, *d*, J = 6.3 Hz, 6′-Me), 1.23 (3H, *s*, 19-Me), 1.12 (3H, *d*, J = 6.7 Hz, 21-Me), 0.81 (3H, *s*, 18-Me).

Enzymatic hydrolysis of **4**. Compound **4** (50 mg) was treated with β-glucosidase (20 mg) in HOAc–NaOAc buffer (pH 5, 5 ml) at room temp. for 12 hr. The reaction mixt. was chromatographed on silica gel eluting with $CHCl_3$–MeOH–H_2O (20:10:1) to give the corresponding spirostanol saponin (**4a**) (13.7 mg) and D-glucose (6.5 mg). Compound **4a**. Amorphous solid. $[\alpha]_D^{27}$ −63.6° (MeOH; *c* 0.56). Negative-ion FAB-MS *m/z* 853 $[M-H]^-$, 722 [M-xylosyl]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2905 (CH), 1440, 1370, 1295, 1220, 1055, 1035, 970, 915, 865, 805. ^{1}H NMR (pyridine-d_5): δ 6.39 (1H, *br s*, 1″-H), 5.00 (1H, *d*, J = 7.5 Hz, 1‴-H), 4.79 and 4.76 (each 1H, *br s*, 27-H_2), 4.62 (overlapping, 1′-H), 1.75 (3H, *d*, J = 6.0 Hz, 6″-Me), 1.53 (3H, *d*, J = 6.2 Hz, 6′-Me), 1.24 (3H, *s*, 19-Me), 1.04 (3H, *d*, J = 6.9 Hz, 21-Me), 0.84 (3H, *s*, 18-Me).

Acid hydrolysis of **4**. Compound **4** (36 mg) was subjected to acid hydrolysis as described for **1** to give an aglycone **4b** (2.7 mg) and a mixt. of monosaccharides (15.8 mg). Compound **4b**. Amorphous solid. $[\alpha]_D^{29}$ −50.0° (MeOH; *c* 0.14). EI-MS *m/z* (rel. int.): 430 $[M]^+$ (1.4), 360 (11), 318 (19), 289 (23), 137 (100). IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2915 and 2845 (CH), 1445, 1370, 1225, 1175, 1040, 1020, 1000, 980, 955, 935, 920, 875. ^{1}H NMR (pyridine-d_5): δ 4.82 and 4.79 (each 1H, *br s*, 27-H_2), 4.57 (1H, *q*-like, J = 6.8 Hz, 16-H), 4.48 and 4.04 (each 1H, *br d*, J = 12.0 Hz, 26-H_2), 4.00 (1H, *br m*, $W_{1/2}$ = 21.2 Hz, 3-H), 3.73 (1H, *dd*, J = 11.3, 4.0 Hz, 1-H), 1.14 (3H, *s*, 19-Me), 1.07 (3H, *d*, J = 6.9 Hz, 21-Me), 0.91 (3H, *s*, 18-Me). The monosaccharides were identified as D-glucose, D-xylose, D-fucose 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): 0.61 (rhamnose); 0.54 (xylose); 0.51 (fucose); 0.38 (glucose). R_t (min): 18.66 (D-xylose derivative); 20.76 (D-fucose derivative); 24.20 (D-glucose derivative); 27.22 (L-rhamnose derivative).

Compound **5**. Amorphous solid. $[\alpha]_D^{27}$ −24.3° (MeOH; *c* 0.12). Negative-ion FAB-MS *m/z* 1045 $[M-H]^-$, 899 [M-rhamnosyl]$^-$. IR ν_{max}^{KBr} cm^{-1}: 3390 (OH), 2910 (CH), 1440, 1370, 1300, 1150, 1055, 1035, 975, 900, 825, 800. ^{1}H NMR (pyridine-d_5): δ 6.36 (1H, *br s*, 1″-H), 5.60 (1H, *br d*, J = 5.6 Hz, 6-H), 5.34 and 5.05 (each 1H, *br s*, 27-H_2), 4.99 (1H, *d*, J = 7.5 Hz, 1‴-H), 4.92 (overlapping with H_2O signal, 1⁗-H), 4.68 (1H, *d*, J = 7.8 Hz, 1′-H), 3.25 (3H, *s*, OMe), 1.73 (3H, *d*, J = 6.1 Hz, 6″-Me), 1.52 (3H, *d*, J = 6.3 Hz, 6′-Me), 1.41 (3H, *s* 19-Me), 1.11 (3H, *d*, J = 6.9 Hz, 21-Me), 0.85 (3H, *s*, 18-Me).

Acid hydrolysis of **5**. Compound **5** (20 mg) was subjected to acid hydrolysis as described for **1** to give

an aglycone **5a** (2.6 mg) and a mixt. of monosaccharides (7.5 mg). Physical and spectral data of **5a**: ref. [4]. D-Glucose, D-xylose, D-fucose and L-rhamnose in the mixt. of monosaccharides were identified by direct TLC comparison with authentic samples and HPLC analysis of their corresponding 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives.

Acknowledgements—We are grateful to Dr Y. Shida and Mrs C. Sakuma of the Central Analytical Center of Tokyo University of Pharmacy and Life Science for the measurements of the mass and 2D NMR spectra.

REFERENCES

1. Mimaki, Y., Takaashi, Y., Kuroda, M. and Sashida, Y., *Phytochemistry*, 1997, **45**, 1229.
2. Blunden, G., Jaffer, J. A., Jewers, K. and Griffin, W. J., *Tetrahedron*, 1981, **37**, 2911.
3. Asano, T., Murayama, T., Hirai, Y. and Shoji, J., *Chemical and Pharmaceutical Bulletin*, 1993, **41**, 566.
4. Mimaki, Y., Takaashi, Y., Kuroda, M., Sashida, Y. and Nikaido, T., *Phytochemistry*, 1996, **42**, 1609.
5. Jewers, K., Manchanda, A. H., Dougan, J., Nagler, M. J., Blunden, G. and Griffin, W. J., *Tetrahedron Letters*, 1974, 1475.
6. Kiyosawa, S., Hutoh, M., Komori, T., Nohara, T., Hosokawa, I. and Kawasaki, T., *Chemical and Pharmaceutical Bulletin*, 1968, **16**, 1162.
7. Nohara, T., Miyahara, K. and Kawasaki, T., *Chemical and Pharmaceutical Bulletin*, 1975, **23**, 872.
8. Agrawal, P. K., Jain, D. C., Gupta, R. K. and Thakur, R. S., *Phytochemistry*, 1985, **24**, 2479.
9. Blunden, G., Sitton, D., Beach, S. J. and Turner, C. H., *Journal of Natural Products*, 1984, **47**, 266.
10. González, A. G., Freire, R., García-Estrada, M. G., Salazar, J. A. and Suárez, E., *Tetrahedron*, 1972, **28**, 1289.
11. Oshima, R. and Kumanotani, J., *Chemistry Letters*, 1981, 943.
12. Oshima, R., Yamauchi, Y. and Kumanotani, J., *Carbohydrate Research*, 1982, **107**, 169.