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STEROIDAL SAPONINS FROM *NOLINA RECURVATA* STEMS AND THEIR INHIBITORY ACTIVITY ON CYCLIC AMP PHOSPHODIESTERASE

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Key Word Index—*Nolina recurvata*; Agavaceae; steroidal saponins; cyclic AMP phosphodiesterase inhibition.

Abstract—Seven steroidal saponins were isolated from the stems of *Nolina recurvata*, five of which appeared to be new compounds and were assigned as spirosta-5,25(27)-diene-1β,3β-diol (neoruscogenin) 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside}, (25*S*)-spirost-5-ene-1β,3β-diol [(25*S*)-ruscogenin] 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside}, neoruscogenin 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-fucopyranoside}, 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)]-α-L-arabinopyranoside} and 26-*O*-β-D-glucopyranosylfurosta-5,20(22),25(27)-triene-1β,3β,26-triol 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside}. The isolated saponins were evaluated for their inhibitory activity on cyclic AMP phosphodiesterase to identify new compounds with medicinal potential.

INTRODUCTION

The family Agavaceae with more than 580 species has a distribution in the tropic and subtropic dry climate regions throughout the world. The occurrence of steroidal saponins in several Agavaceae plants, especially those belonging to the representative genera, *Agave* and *Yucca*, is well documented [1].

Nolina recurvata is an Agavaceae plant indigenous to Mexico with a stem swelling in the shape of a bottle gourd in the proximal part. Previously, we have performed phytochemical examination of the swelling part and isolated a series of polyhydroxylated spirostanol saponins [2] and cholestane glycosides [3]. Further analysis of the plant material led to the isolation of seven steroidal saponins, five of which appeared to be new compounds. This paper refers to the identification and structural assignments of the isolated saponins based on spectroscopic analysis and some chemical transformations, and their inhibitory activity on cyclic AMP phosphodiesterase.

RESULTS AND DISCUSSION

Fractionation of the methanolic extract, obtained from the fresh stems of *N. recurvata*, by a combination of column chromatographic methods over silica-gel, octadecylsilanized (ODS) silica-gel and porous ion-exchange resin (Diaion HP-20), and finally by preparative HPLC, resulted in the isolation of compounds **1** (11.7 mg), **2** (26.2 mg), **3** (150 mg), **4** (31.2 mg), **5** (9.1 mg), **6** (317 mg) and **7** (27.5 mg).

Compounds **1** and **2** were identified by their IR, ^{1}H NMR, ^{13}C NMR and FAB mass spectral data as spirosta-5,25(27)-diene-1β,3β-diol (neoruscogenin) 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside} [4] and (25*S*)-spirost-5-ene-1β,3β-diol [(25*S*)-ruscogenin] 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-fucopyranoside} [5].

Compound **3** was obtained as an amorphous solid, $[\alpha]_D$ −63.8° (methanol). The molecular formula was assigned as $C_{43}H_{66}O_{16}$ by the negative-ion FAB mass spectrum showing an $[M]^-$ ion at *m/z* 838, ^{13}C NMR with 43 carbon signals and elemental analysis. The glycosidic nature of **3** was suggested by the strong absorption bands at 3410 and 1035 cm^{-1} in the IR spectrum. The identity of the aglycone structure of **3** with that of **1** was confirmed by the ^{1}H NMR spectrum of **3**, which showed three methyl proton signals at δ 1.05 (*d*, *J* = 6.8 Hz), 1.43 (*s*) and 0.85 (*s*), an olefinic proton signal at δ 5.59 (*br d*, *J* = 5.3 Hz) and exo-methylene proton signals at δ 4.80 and 4.77 (each *br s*), and by acid-catalysed hydrolysis of **3** with 1M hydrochloric acid in dioxane–H_2O (1:1), giving neoruscogenin (**3a**) [6] together with L-arabinose, L-rhamnose

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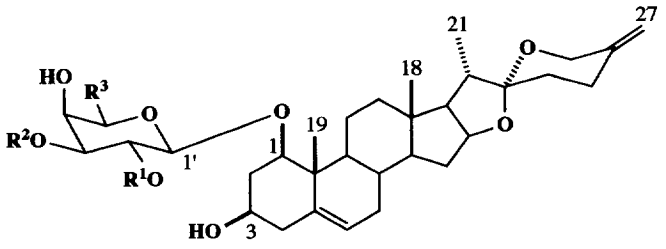
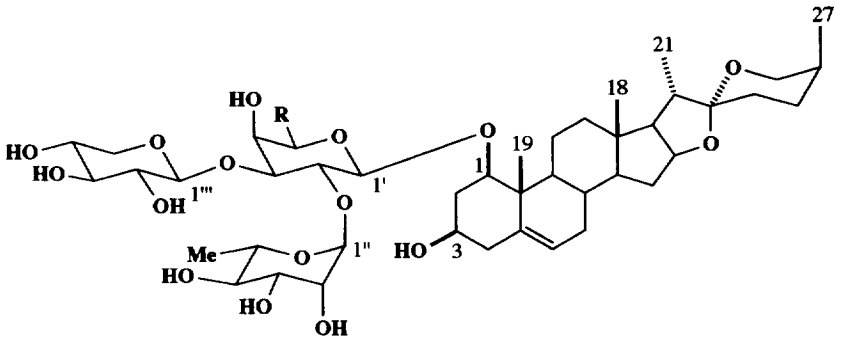


	R^1	R^2	R^3
1	α-L-Rha*p*	H	H
3	α-L-Rha*p*	β-D-Xyl*p*	H
3d	H	β-D-Xyl*p*	H
5	α-L-Rha*p*	β-D-Xyl*p*	Me

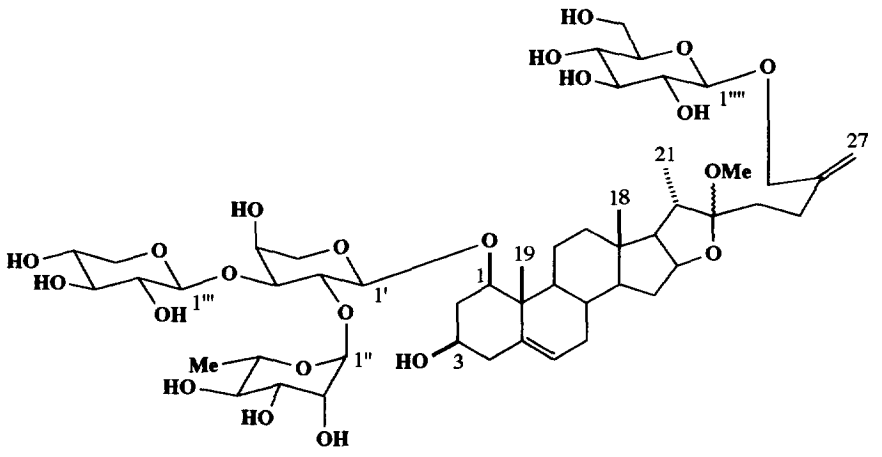


	R
2	Me
4	H

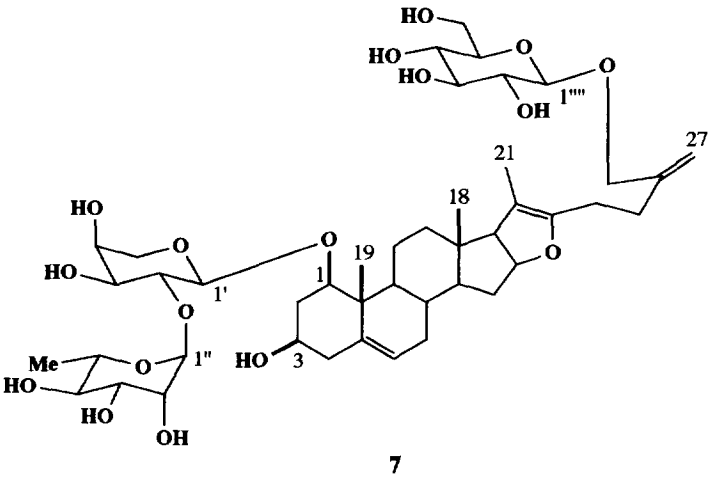
and D-xylose in a ratio of 1 : 1 : 1. On comparison of the whole ^{13}C NMR spectrum of **3** with that of **1**, a set of five additional signals, corresponding to a terminal β-D-xylopyranosyl unit appeared at δ 106.5 (CH), 74.6 (CH), 78.2 (CH), 71.0 (CH) and 67.0 (CH_2), and the signal of the C-3 of the inner arabinose was markedly displaced downfield by 8.6 ppm. Furthermore, in the ^1H NMR spectrum of the acetyl derivative (**3b**) of **3** prepared with acetic anhydride in pyridine, the H-2 and H-3 protons of the arabinose and the H-1 of the aglycone appeared at δ 4.10 (*dd*, *J* = 9.1, 7.6 Hz), 3.50 (*dd*, *J* = 9.1, 3.7 Hz) and 3.55 (*dd*, *J* = 12.1, 4.0 Hz), respectively, whereas other hydroxymethine protons of the saccharide part and the H-3 proton of the aglycone appeared at lower fields than δ 4.9 by *O*-acylation. The above data accounted for the saccharide structure as 2,3-branched L-arabinose embracing L-rhamnose at C-2 and D-xylose at C-3, or D-xylose at C-2 and L-rhamnose at C-3, and its linkage to the aglycone C-1 hydroxyl group. Mild acid hydrolysis of **3** with 0.2M hydrochloric acid gave two partial hydrolysates, **3c** and **3d**; the former was revealed to be identical to **1**. The ^{13}C NMR spectrum of **3d** showed the presence of a terminal β-D-xylopyranosyl unit (δ 107.0, 75.5, 78.2, 71.1 and 67.3) and a 3-substituted α-L-arabinopyranosyl unit (δ 102.3, 71.6, 83.5, 69.3 and 67.5) [7, 8]. Accordingly, the structure of **3** was determined to be neoruscogenin 1-*O*-{*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-α-L-arabinopyranoside}.

All spectral properties of **4** ($C_{43}H_{68}O_{16}$) showed a close similarity to those of **3**. On comparison of the ^1H and ^{13}C NMR spectra of **4** with those of **3**, the exomethylene signals, which were observed at δ_H 4.80 and 4.77, and δ_C 144.5 (C) and 108.6 (CH_2) in **3**, were replaced by the signals assignable to a secondary methyl group at δ_H 1.09 (3H, *d*, *J* = 6.9 Hz) and δ_C 16.3 (Me) in **4**. Hydrogenation of **3** over platinum oxide under an H_2 atmosphere gave **4**. Thus, the structure of **4** was formulated as (25*S*)-ruscogenin 1-*O*-{*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-α-L-arabinopyranoside}.

Attempted assignments of the ^{13}C NMR signals of **5** ($C_{44}H_{68}O_{16}$) by referring to those of **2** and **3** established that **5** had the same sapogenol structure as **3**, and the same triglycoside constituent as **2**. The structure of **5** was readily assigned as neoruscogenin 1-*O*-{*O*-α-L-



6



7

rhamnopyranosyl-(1→2)-*O*-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-fucopyranoside}.

Compound **6** ($C_{50}H_{80}O_{22}$) was believed to be a 22-methoxyfurostanol saponin by Ehrlich's test [9, 10], and the ^1H and ^{13}C NMR spectra [δ_H 3.25 (3H, *s*); δ_C 112.4 (C) and 47.3 (Me)] [7]. Enzymatic hydrolysis of **6** with *β*-glucosidase gave D-glucose and **3**. The structure of **6** was shown to be 26-*O*-*β*-D-glucopyranosyl - 22 - *O* - methylfurosta - 5,25(27) - diene - 1*β*,3*β*,22*ξ*,26-tetrol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2) - *O* - [*β* - D - xylopyranosyl - (1→3)] - *α* - L - arabinopyranoside}.

Compound **7** ($C_{44}H_{68}O_{17}$) was also suggested to be a furostanol saponin by its spectral data. The presence of an additional olefinic group [δ 151.5 (C) and 104.2 (C)] and lack of the terminal *β*-D-xylose moiety attached to the C-3 hydroxyl group of the inner L-arabinose, as compared with **6**, were the differences recognized in the ^{13}C NMR spectral data of **7**. Furthermore, the 21-Me methyl doublet signal observed at δ 1.13 (*d*, $J = 6.7$ Hz) in the ^1H NMR spectrum of **6** was absent from that of **7** and was replaced by the methyl singlet at δ 1.58. The above data were consistent with **7** being the corresponding $\Delta^{20(22)}$-furostanol saponin with the terminal *β*-D-xylopyranose missing. The structure of **7** was characterized as 26-*O*-*β* - D - glucopyranosylfurosta - 5,20(22),25(27) - triene - 1*β*,3*β*,26-triol 1-*O*-{*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside}.

Compounds **3**–**7** are new steroidal saponins. The isolated saponins were evaluated for their inhibitory activity on cyclic AMP phosphodiesterase as a primary screening test to identify new medicinal agents [11, 12]. All the spirostanol saponins, **1**–**5**, exhibited considerable activity with the IC_{50} values ranging from 8.4–16.1 × 10^{-5} M, whereas the furostanol saponins, **6** and **7**, were far less potent, as shown in Table 2.

EXPERIMENTAL

General. NMR: Bruker AM-400 (δ, J Hz); CC: silica-gel (Fuji-Silysia Chemical), ODS silica-gel

Table 1. ^{13}C NMR spectral data for compounds **1**–**3**, **3a**, **3d**, **4**–**7** in pyridine-d_5

C	1	2	3	3a	3d	4	5	6	7
1	83.5	84.4	83.8	78.1	83.8	83.8	84.4	83.7	83.5
2	37.4	38.1	37.5	44.0	37.7	37.5	38.1	37.4	37.4
3	68.3	68.3	68.2	68.1	68.1	68.2	68.3	68.3	68.2
4	43.9	43.9	43.9	43.6	43.8	43.9	43.9	43.9	43.9
5	139.7	139.6	139.6	140.4	139.6	139.5	139.7	139.6	139.6
6	124.7	124.8	124.7	124.3	124.7	124.7	124.7	124.7	124.7
7	32.0	32.1	32.0	32.3	32.0	32.0	32.1	32.0	32.1
8	33.2	33.1	33.1	33.0	33.1	33.1	33.1	33.1	32.9
9	50.4	50.6	50.3	51.4	50.3	50.4	50.6	50.4	50.4
10	42.9	42.9	42.9	43.6	42.9	42.9	42.9	42.9	43.1
11	24.1	24.0	24.1	24.2	23.8	24.1	24.0	24.0	24.2
12	40.3	40.5	40.2	40.6	40.3	40.3	40.4	40.2	40.2
13	40.3	40.2	40.2	40.3	40.2	40.2	40.3	40.4	42.9
14	56.9	57.2	56.8	57.0	56.8	56.8	57.2	56.8	55.1
15	32.4	32.4	32.4	32.4	32.4	32.4	32.4	32.4	34.7
16	81.5	81.2	81.5	81.4	81.5	81.2	81.5	81.5	84.5
17	63.0	63.0	63.0	63.2	63.1	62.8	63.1	64.2	64.6
18	16.7	16.8	16.7	16.6	16.7	16.7	16.8	16.7	14.5
19	15.1	15.0	15.0	15.0	15.0	15.0	15.0	15.1	15.1
20	41.9	42.5	41.8	41.9	41.9	42.4	41.9	40.5	104.2
21	15.0	14.8	14.9	13.9	14.8	14.8	14.9	16.1	11.8
22	109.5	109.7	109.4	109.5	109.4	109.7	109.5	112.4	151.5
23	33.3	26.4	33.2	33.3	33.3	26.4	33.2	31.6	31.1
24	29.0	26.2	29.0	29.0	29.0	26.2	29.0	28.1	24.7
25	144.6	27.6	144.5	144.5	144.5	27.5	144.6	146.9	146.3
26	65.0	65.0	65.0	65.0	65.0	65.0	65.0	72.0	71.7
27	108.6	16.3	108.6	108.6	108.6	16.3	108.6	111.1	111.6
OMe								47.3	
1′	100.3	100.5	100.5		102.3	100.5	100.5	100.4	100.3
2′	75.2	73.5	74.2		71.6	74.2	73.5	74.2	75.2
3′	75.9	85.6	84.5		83.5	84.5	85.6	84.5	75.8
4′	70.1	72.7	69.6		69.3	69.6	72.7	69.6	70.1
5′	67.3	70.8	67.1		67.5	67.1	70.9	67.1	67.3
6′		17.1					17.1		
1″	101.7	101.7	101.8			101.8	101.8	101.8	101.7
2″	72.6	72.5	72.5			72.5	72.6	72.5	72.5
3″	72.7	72.5	72.5			72.5	72.6	72.6	72.7
4″	74.3	74.3	74.1			74.1	74.3	74.1	74.2
5″	69.4	69.4	69.5			69.5	69.4	69.6	69.4
6″	19.0	19.1	19.1			19.1	19.2	19.1	19.0
1‴		106.6	106.5		107.0	106.5	106.7	106.4	
2‴		74.7	74.6		75.5	74.6	74.7	74.6	
3‴		78.3	78.2		78.2	78.3	78.4	78.3	
4‴		71.0	71.0		71.1	71.0	71.0	71.0	
5‴		67.1	67.0		67.3	67.0	67.1	66.9	
1⁗								103.9	103.8
2⁗								75.2	75.3
3⁗								78.6*	78.6*
4⁗								71.8	71.7
5⁗								78.5*	78.5*
6⁗								62.9	62.8

*Signals may be interchanged.

(Nacalai Tesque) and Diaion HP-20 (Mitsubishi-Kasei); TLC: precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) and RP-18 $F_{254}S$ (0.25 mm thick, Merck); HPLC: a Tosoh HPLC system (pump, CCPM; controller, CCP controller PX-8010; detector, UV-8000 or RI-8010) equipped with a Kaseisorb ODS-120-5 column (Tokyo-Kasei-Kogyo, 4.6 mm i.d. × 250 mm, ODS, 5 μm) for analytical HPLC, and a Kaseisorb

Table 2. Inhibitory activity of the isolated saponins on cyclic AMP phosphodiesterase

Compounds	IC_{50} ($\times 10^{-5}$ M)
1	8.4
2	10.3
3	9.2
4	8.7
5	16.1
6	45.6
7	127.0
Papaverine	3.0

ODS-120-5 column (Tokyo-Kasei-Kogyo, 10 mm i.d. × 250 mm, ODS, 5 μm) or a Chromatorex-ODS column (Fuji-Silysia Chemical, 10 mm i.d. × 250 mm, ODS, 5 μm) for preparative HPLC.

Chemicals. Beef heart phosphodiesterase and snake venom nucleotidase: Sigma. Cyclic [^{3}H] AMP: Radiochemical Center. All other chemicals used were of biochemical-reagent grade.

Plant material. Nolina recurvata was purchased from Exotic Plants Co Ltd (Japan) and the plant specimen is on file in our laboratory.

Extraction and isolation. Fresh stems of *N. recurvata* (2.5 kg) were extracted with hot MeOH. The MeOH extract was partitioned between *n*-BuOH and H_2O. CC of the *n*-BuOH-soluble fr. on silica-gel and elution with a gradient mixt of CH_2Cl_2–MeOH (9:1; 4:1; 2:1), and finally with MeOH, gave four frs (I–IV). Fr. I was further divided into three frs (Ia–Ic) by subjecting it to silica-gel CC eluting with $CHCl_3$–MeOH–H_2O (20:10:1). Fr. Ia was purified by an ODS silica-gel column eluting with MeOH–H_2O (9:1) and prep. HPLC with MeOH–H_2O (87:13) to give **1** (11.7 mg). Fr. II was chromatographed on silica-gel eluting with $CHCl_3$–MeOH–H_2O (20:10:1) and ODS silica-gel with MeOH–H_2O (7:3) to give **2**, **3**, **4**, **5** and **7**, each with a few impurities. Final purification was performed by HPLC eluting with MeOH–H_2O (4:1; 7:3) to furnish **2** (26.2 mg), **3** (150 mg), **4** (31.2 mg), **5** (9.1 mg) and **7** (27.5 mg) as pure compounds. Fr. III contained a considerable amount of saccharides, the removal of which was performed by CC on Diaion HP-20 with an increasing amount of MeOH in H_2O. The frs, eluted with 80% MeOH and 100% MeOH, were combined and chromatographed on silica-gel eluting with $CHCl_3$–MeOH–H_2O (20:10:1) and ODS silica-gel with MeOH–H_2O (7:3), and finally purified by prep. HPLC with MeOH–H_2O (4:1) to give **6** (317 mg).

Compound **1**. Amorphous solid, $[\alpha]_D^{27}$ −65.5° (MeOH: *c* 0.57). Negative-ion FABMS *m/z* 706 $[M]^-$, 559 $[M - \text{rhamnosyl}]^-$; IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2930 and 2850 (CH), 1450, 1375, 1340, 1255, 1230, 1195, 1135, 1040, 980, 960, 920, 875, 835, 815, 780, 700; ^{1}H NMR (pyridine-d_5): δ 6.36 (1H, *br s*, H-1″), 5.60 (1H, *br d*, *J* = 5.5 Hz, H-6), 4.87 (1H, *dq*, *J* = 9.4, 6.2 Hz, H-5″), 4.81 and 4.78 (each 1H, *br s*, H_2-27), 4.75 (1H, *br d*, *J* = 2.9 Hz, H-2″), 4.74 (1H, *d*, *J* = 7.4 Hz, H-1′), 4.66 (1H, *dd*, *J* = 9.4, 2.9 Hz, H-3″), 4.61 (1H, *dd*, *J* = 8.4, 7.4 Hz, H-2′), 4.52 (1H, *q*-like, *J* = 7.1 Hz, H-16), 4.46 and 4.02 (each 1H, *br d*, *J* = 12.1 Hz, H_2-26), 4.33 (1H, *dd*, *J* = 9.4, 9.4 Hz, H-4″), 4.28 (1H, *dd*, *J* = 12.1, 2.2 Hz, H-5′a), 4.16 (2H, overlapping, H-3′, -4′), 3.89 (1H, *m*, H-3), 3.85 (1H, *dd*, *J* = 11.9, 3.8 Hz, H-1), 3.67 (1H, *br d*, *J* = 12.1 Hz, H-5′b), 1.76 (3H, *d*, *J* = 6.2 Hz, Me-6″), 1.46 (3H, *s*, Me-19), 1.06 (3H, *d*, *J* = 6.9 Hz, Me-21), 0.87 (3H, *s*, Me-18).

Compound **2**. Amorphous solid, $[\alpha]_D^{27}$ −54.5° (MeOH: *c* 0.11). Negative-ion FABMS *m/z* 853 $[M - H]^-$, 721 $[M - \text{xylosyl}]^-$; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2945 (CH), 1445, 1375, 1220, 1155, 1125, 1060, 1040, 980, 915, 830, 810, 760, 695; ^{1}H NMR (pyridine-d_5): δ 6.35 (1H, *br s*, H-1″), 5.59 (1H, *br d*, *J* = 5.1 Hz, H-6), 4.98 (1H, *d*, *J* = 7.7 Hz, H-1‴), 4.67 (1H, *d*, *J* = 7.7 Hz, H-1′), 1.73 (3H, *d*, *J* = 6.1 Hz, Me-6″), 1.50 (3H, *d*, *J* = 6.2 Hz, Me-6′), 1.41 (3H, *s*, Me-19), 1.08 (3H, *d*, *J* = 6.9 Hz, Me-27), 1.06 (3H, *d*, *J* = 6.9 Hz, Me-21), 0.86 (3H, *s*, Me-18).

Compound **3**. Amorphous solid, $[\alpha]_D^{27}$ −63.8° (MeOH; *c* 0.26). Anal. calcd for $C_{43}H_{66}O_{16} \cdot H_2O$: C, 60.27; H, 8.00%. Found: C, 60.08; H, 7.83%. Negative-ion FABMS *m/z* 838 $[M]^-$, 706 $[M - \text{xylosyl}]^-$, 692 $[M - \text{rhamnosyl}]^-$, 559 $[M - \text{xylosyl} - \text{rhamnosyl}]^-$; IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 2940 (CH), 1440, 1370, 1225, 1135, 1085, 1035, 975, 960, 915, 870, 830, 810, 775, 695; ^{1}H NMR (pyridine-d_5): δ 6.33 (1H, *br s*, H-1″), 5.59 (1H, *br d*, *J* = 5.3 Hz, H-6), 4.98 (1H, *d*, *J* = 7.5 Hz, H-1‴), 4.80 and 4.77 (each 1H, *br s*, H_2-27), 4.73 (1H, *d*, *J* = 7.3 Hz, H-1′), 1.74 (3H, *d*, *J* = 6.1 Hz, Me-6″), 1.43 (3H, *s*, Me-19), 1.05 (3H, *d*, *J* = 6.8 Hz, Me-21), 0.85 (3H, *s*, Me-18).

Acid hydrolysis of **3**. A soln of **3** (36.4 mg) in 1M HCl (dioxane–H_2O, 1:1, 6 ml) was heated at 100°C 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 $CHCl_3$–MeOH (19:1) followed by MeOH to give a steroidal sapogenin (**3a**) (6.9 mg) and saccharide mixt. (12.6 mg). Compound **3a**: amorphous solid, $[\alpha]_D^{24}$ −67.3° (MeOH; *c* 0.32). EIMS *m/z* 428 $[M]^+$, 410 $[M - H_2O]^+$; IR ν_{max}^{KBr} cm^{-1}: 3435 (OH), 2945, 2905 and 2845 (CH), 1445, 1370, 1225, 1035, 1015, 1000, 980, 955, 915, 890, 870, 830; ^{1}H NMR (pyridine-d_5): δ 6.23 (1H, *br s*, OH), 6.05 (1H, *d*, *J* = 4.1 Hz, OH), 5.63 (1H, *br d*, *J* = 5.7 Hz, H-6), 4.82 (1H, *br d*, *J* = 1.3 Hz, H-27a), 4.79 (1H, *br s*, H-27b), 4.55 (1H, *q*-like, *J* = 6.9 Hz, H-16), 4.47 and 4.03 (each 1H, *br d*, *J* = 11.8 Hz, H_2-26), 3.97 (1H, *m*, H-3), 3.83 (1H, *m*, H-1), 1.36 (3H, *s*, Me-19), 1.07 (3H, *d*, *J* = 7.0 Hz, Me-21), 0.93 (3H, *s*, Me-18). A 2 mg of the saccharide mixt. 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 3 hr followed by addition of AcOH (0.2 ml) and evaporation to dryness, the reaction mixt. was

acetylated with Ac_2O (0.3 ml) in pyridine (0.3 ml) at room temp. for 12 hr. The crude mixt. was passed through a Sep-Pak C_{18} cartridge (Waters) eluting with stepwise gradients of H_2O–MeCN (4:1; 1:1; 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 (20 ml) to give 1-[(*S*)-*N*-acetyl-α-methylbenzylamino]-1-deoxyalditol acetate derivatives of the monosaccharides, which were analysed by HPLC under the following conditions: column: Kaseisorb ODS-120-5 (Tokyo-Kasei-Kogyo, 4.6 mm i.d. × 250 mm, ODS, 5 μm); solvent: MeCN–H_2O (2:3); flow rate: 0.8 ml min^{-1}; detection: UV 230 nm. The derivatives of L-arabinose, D-xylose and L-rhamnose were detected; R_t 16.86 min (L-arabinose derivative), 17.89 min (D-xylose derivative), 26.30 min (L-rhamnose derivative).

Acetylation of **3**. Compound **3** (29.9 mg) was acetylated with Ac_2O (0.5 ml) in pyridine (0.5 ml) and the crude acetate was chromatographed on silica gel eluting with hexane–Me_2CO (3:1) to give the corresponding octaacetate (**3b**) (28.8 mg). Compound **3b**: amorphous solid. IR ν_{max}^{KBr} cm^{-1}: 2950 (CH), 1745 (C=O), 1430, 1365, 1240, 1220, 1135, 1075, 1040, 975, 955, 915, 870, 830, 695; ^1H NMR (benzene-d_6): δ 5.73 (1H, *dd*, *J* = 10.2, 3.6 Hz, H-3″), 5.65 (1H, *dd*, *J* = 3.6, 1.6 Hz, H-2″), 5.60 (1H, *d*, *J* = 1.6 Hz, H-1″), 5.55 (1H, *dd*, *J* = 8.6, 8.6 Hz, H-3‴), 5.52 (1H, *br d*, *J* = 5.2 Hz, H-6), 5.51 (1H, *dd*, *J* = 10.2, 10.2 Hz, H-4″), 5.19 (1H, *dd*, *J* = 8.6, 6.7 Hz, H-2‴), 5.12 (1H, *br dd*, 3.7, 1.9 Hz, H-4′), 5.02 (1H, *ddd*, *J* = 8.6, 8.6, 5.2 Hz, H-4‴), 4.91 (1H, *m*, H-3), 4.79 (1H, *d*, *J* = 6.7 Hz, H-1‴), 4.73 and 4.72 (each 1H, *br s*, H_2-27), 4.65 (1H, *dq*, *J* = 10.2, 6.2 Hz, H-5″), 4.54 (1H, *q*-like, *J* = 7.5 Hz, H-16), 4.51 and 3.98 (each 1H, *br d*, *J* = 11.9 Hz, H_2-26), 4.10 (1H, *dd*, *J* = 9.1, 7.6 Hz, H-2′), 4.04 (1H, *dd*, *J* = 11.6, 5.2 Hz, H-5‴a), 3.97 (1H, *d*, *J* = 7.6 Hz, H-1′), 3.88 (1H, *dd*, *J* = 13.0, 1.9 Hz, H-5′a), 3.55 (1H, *dd*, *J* = 12.1, 4.0 Hz, H-1), 3.50 (1H, *dd*, *J* = 9.1, 3.7 Hz, H-3′), 3.36 (1H, *d*, *J* = 11.6, 8.6 Hz, H-5‴b), 2.88 (1H, *br d*, *J* = 13.0 Hz, H-5′b), 1.97, 1.95, 1.92, 1.79 × 2, 1.74, 1.70 and 1.59 (each 3H, *s*, Ac × 8), 1.28 (3H, *d*, *J* = 6.2 Hz, Me-6″), 1.21 (3H, *s*, Me-19), 1.10 (3H, *d*, *J* = 6.9 Hz, Me-21), 0.94 (3H, *s*, Me-18).

Partial hydrolysis of **3**. A soln of **3** (60 mg) in 0.2M HCl (dioxane–H_2O, 1:1, 4 ml) was heated at 100°C for 30 min under an Ar atmosphere. After cooling, the reaction mixt. was neutralized by passing it through an Amberlite IRA-93ZU column and chromatographed on silica-gel eluting with $CHCl_3$–MeOH (4:1) to give two partial hydrolysates, **3c** (= **1**) (5.4 mg) and **3d** (6.3 mg). Compound **3d**: amorphous solid, $[\alpha]_D^{25}$ −43.1° (MeOH; *c* 0.26). Negative-ion FABMS *m/z* 692 $[M]^-$, 559 $[M - xylosyl]^-$; IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 2925 and 2850 (CH), 1445, 1370, 1225, 1175, 1155, 1060, 1040, 975, 955, 915, 870, 780; ^1H NMR (pyridine-d_5): δ 5.60 (1H, *br d*, *J* = 5.6 Hz, H-6), 5.30 (1H, *d*, *J* = 7.5 Hz, H-1‴), 4.81 and 4.78 (each 1H, *br s*, H_2-27), 4.79 (1H, *d*, *J* = 7.6 Hz, H-1′), 1.24 (3H, *s*, Me-19), 1.05 (3H, *d*, *J* = 6.9 Hz, Me-21), 0.84 (3H, *s*, Me-18).

Compound **4**. Amorphous solid, $[\alpha]_D^{27}$ −63.5° (MeOH; *c* 0.57). Anal. calcd for $C_{43}H_{68}O_{16}\cdot 3/2H_2O$: C, 59.50; H, 8.24%. Found: C, 59.65; H, 7.93%. Negative-ion FABMS *m/z* 840 $[M]^-$, 708 $[M - xylosyl]^-$, 694 $[M - rhamnosyl]^-$; IR ν_{max}^{KBr} cm^{-1}: 3425 (OH), 2950 (CH), 1450, 1375, 1225, 1135, 1090, 1040, 980, 915, 890, 870, 835, 810, 780, 700; ^1H NMR (pyridine-d_5): δ 6.33 (1H, *br s*, H-1″), 5.59 (1H, *br d*, *J* = 5.1 Hz, H-6), 4.98 (overlapping with H_2O signal, H-1‴), 4.73 (1H, *d*, *J* = 7.3 Hz, H-1′), 1.74 (3H, *d*, *J* = 5.8 Hz, Me-6″), 1.43 (3H, *s*, Me-19), 1.09 (3H, *d*, *J* = 6.9 Hz, Me-27), 1.06 (3H, *d*, *J* = 6.9 Hz, Me-21), 0.85 (3H, *s*, Me-18).

Catalytic hydrogenation of **3**. A mixt. of **3** (10 mg) and Pt_2O (2 mg) in MeOH (2 ml) was stirred under an H_2 atmosphere at ambient temp. for 12 hr. The reaction mixt., after removal of Pt_2O by filtration, was subjected to prep. HPLC eluting with MeOH–H_2O (9:1) to furnish **4** (9.2 mg).

Compound **5**. Amorphous solid, $[\alpha]_D^{27}$ −45.0° (MeOH; *c* 0.44). Negative-ion FABMS *m/z* 852 $[M]^-$, 720 $[M - xylosyl]^-$, 706 $[M - rhamnosyl]^-$, 573 $[M - xylosyl - rhamnosyl]^-$; IR ν_{max}^{KBr} cm^{-1}: 3430 (OH), 2930 (CH), 1440, 1370, 1225, 1150, 1060, 1040, 980, 915, 875, 835, 700; ^1H NMR (pyridine-d_5): δ 6.37 (1H, *br s*, H-1″), 5.61 (1H, *br d*, *J* = 5.0 Hz, H-6), 5.00 (1H, *d*, *J* = 7.5 Hz, H-1‴), 4.81 and 4.77 (each 1H, *br s*, H_2-27), 4.69 (1H, *d*, *J* = 7.7 Hz, H-1′), 1.75 (3H, *d*, *J* = 6.1 Hz, Me-6″), 1.52 (3H, *d*, *J* = 6.3 Hz, Me-6′), 1.42 (3H, *s*, Me-19), 1.03 (3H, *d*, *J* = 6.9 Hz, Me-21), 0.87 (3H, *s*, Me-18).

Compound **6**. Amorphous solid, $[\alpha]_D^{26}$ −46.6° (MeOH; *c* 0.31). Anal. calcd for $C_{50}H_{80}O_{22}\cdot 3/2H_2O$: C, 56.65; H, 7.89%. Found: C, 59.52; H, 7.70%. Negative-ion FABMS *m/z* 1032 $[M]^-$, 901 $[M - xylosyl]^-$, 886 $[M - rhamnosyl]^-$, 754 $[M - xylosyl - rhamnosyl]^-$: IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2900 (CH), 1440, 1365, 1035, 970, 900, 825, 800, 770, 690; ^1H NMR (pyridine-d_5): δ 6.32 (1H, *br s*, H-1″), 5.60 (1H, *br d*, *J* = 5.4 Hz, H-6), 5.34 and 5.05 (each 1H, *br s*, H_2-27), 4.98 (1H, *d*, *J* = 7.4 Hz, H-1‴), 4.91 (overlapping with H_2O signal, H-1⁗), 4.74 (1H, *d*, *J* = 7.3 Hz, H-1′), 3.25 (3H, *s*, OMe), 1.73 (3H, *d*, *J* = 6.0 Hz, Me-6″), 1.42 (3H, *s*, Me-19), 1.13 (3H, *d*, *J* = 6.7 Hz, Me-21), 0.83 (3H, *s*, Me-18).

Enzymatic hydrolysis of **6**. Compound **6** (80 mg) was treated with β-glucosidase (Tokyo-Kasei-Kogyo, 60 mg) in AcOH/AcONa buffer (pH 5, 5 ml) at room temp. for 105 hr. The reaction mixt. was chromatographed on silica-gel eluting with $CHCl_3$–MeOH–H_2O (20:10:1) and to give **3** (52.8 mg) and D-glucose (12.5 mg).

Compound **7**. Amorphous solid, $[\alpha]_D^{26}$ −24.0° (MeOH; *c* 0.48). Anal. calcd for $C_{44}H_{68}O_{17}\cdot 2H_2O$: C, 58.39; H, 8.02%. Found: C, 58.35; H, 7.55%. Negative-ion FABMS *m/z* 868 $[M]^-$, 722 $[M - rhamnosyl]^-$, 705 $[M - glucosyl]^-$; IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 2925 (CH), 1450, 1350, 1260, 1220, 1130, 1065, 1045, 980, 940, 905, 835, 815, 780, 700; ^1H NMR (pyridine-d_5): δ 6.34 (1H, *br s*, H-1″), 5.60 (1H, *br d*, *J* = 5.5 Hz,

H-6), 5.36 and 5.06 (each 1H, *br s*, H_2-27), 4.92 (1H, *d*, $J = 7.9$ Hz, H-1‴), 4.74 (1H, *d*, $J = 6.9$ Hz, H-1′), 1.75 (3H, *d*, $J = 6.2$ Hz, Me-6″), 1.58 (3H, *s*, Me-21), 1.45 (3H, *s*, Me-19), 0.75 (3H, *s*, Me-18).

Assay of cyclic AMP phosphodiesterase activity. The phosphodiesterase activity was assayed by a modification of the method of Thompson and Brooker as described previously [13, 14]. The assay was a two-step isotopic procedure. Tritium-labelled cyclic AMP was hydrolysed to 5′-AMP by phosphodiesterase, and the 5′-AMP was then further hydrolysed to adenosine by snake venom nucleotidase. The hydrolysate was treated with an anion-exchange resin (Dowex AG1-X8; BIO-RAD) to adsorb all charged nucleotides, leaving [^{3}H]-adenosine as the only labelled compound to be counted.

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