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Structures of Toxic Steroidal Saponins from *Narthecium asiaticum* MAXIM.

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The full structures of the two steroidal saponins from *Narthecium asiaticum* MAXIM. we previously identified as toxic substances by monitoring the toxicity in guinea pigs were phytochemically reinvestigation on the aerial parts of the plant. The desired toxic saponins (**6, 7**) were isolated together with two known lignan glucosides (**1, 2**), a known flavonoid glucoside (**3**), a new furanone glucoside (**4**), a known steroidal saponin (**5**) and a new steroidal saponin (**8**). The structures of the new furanone glucoside, toxic saponins and new saponin were determined on the basis of spectroscopic data and acid- or enzymatic-catalyzed hydrolysis to be (*S*)-5-β-D-glucopyranosyloxy-4-methoxyfuran-2(5*H*)-one (**4**), (25*R*,*S*)-5β-spirostan-3β-ol 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-[α-L-arabinopyranosyl-(1→3)]-β-D-galactopyranoside} (**6**), (25*R*,*S*)-5β-spirostan-3β-ol 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-galactopyranoside} (**7**) and (24*S*,25*R*)-5β-spirostane-3β,24-diol 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-*O*-[α-L-arabinopyranosyl-(1→3)]-β-D-galactopyranoside} (**8**), respectively.

Key words *Narthecium asiaticum*; Liliaceae; furanone glucoside; toxic steroidal saponin; spirostanol saponin

Narthecium asiaticum MAXIM. (Japanese name: kinkouka) is widely distributed in wet moorlands in the mountainous areas of the Tohoku district of Japan. An outbreak of *N. asiaticum* poisoning was reported in range cattle in the autumn of 1975.[1] We previously identified two steroidal saponins as the toxic substances by monitoring the toxicity in guinea pigs. The saponins were found to cause toxicity, diarrhea, proteinuria, hematuria and death in guinea pigs and were believed to have caused the outbreak of poisoning in the cattle. The structures were suggested to be (25*R*,*S*)-5β-spirostan-3β-ol glycosides by preliminary spectral inspection and hydrolysis,[2] however, the full structure remained to be determined. To clarify the full structures of the toxic steroidal saponins from *N. asiaticum*, phytochemical reinvestigation was made on the aerial parts of the plant, and the desired toxic saponins (**6, 7**) in adequate amounts for structural elucidation were isolated; two known lignan glucosides (**1, 2**), a known flavonoid glucoside (**3**), a new furanone glucoside (**4**), a known steroidal saponin (**5**) and a new steroidal saponin (**8**) were also isolated. The structures of the new furanone glycoside, toxic saponins and new saponin were determined on the basis of spectroscopic data and the products formed on acid or enzymatic hydrolysis. This paper describes details of the processes used.

The 1-butanol-soluble phase of the methanolic extract of *N. asiaticum* (13 kg, fresh weight) was chromatographed on silica-gel to give five fractions (I—V). Further chromatographic separations of fractions III and IV gave compounds **1** (46 mg), **2** (549 mg), **3** (33 mg), **4** (1.72 g), **5** (62 mg), and **6** (874 mg), **7** (26 mg) and **8** (32 mg), respectively.

Compounds **1**, **2**, **3** and **5** were identified as (+)-syringaresinol *O*-β-D-glucopyranoside,[3] (+)-pinoresinol *O*-β-D-glucopyranoside,[4] chrysoeriol 7-*O*-β-D-glucopyranoside,[5] and (25*R*,*S*)-5β-spirostan-3β-ol 3-*O*-{*O*-β-D-glucopyranosyl-(1→2)-β-D-galactopyranoside},[6]respectively. The physical and spectral characteristics of the known compounds were consistent with those previously

ly reported.

Compound **4** was obtained as an amorphous solid, [α]$_D$ +24.6° (methanol). The molecular formula was assigned as $C_{11}H_{16}O_9$ by the negative-ion FAB-MS (*m*/*z* 291
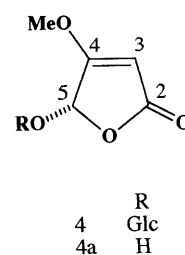

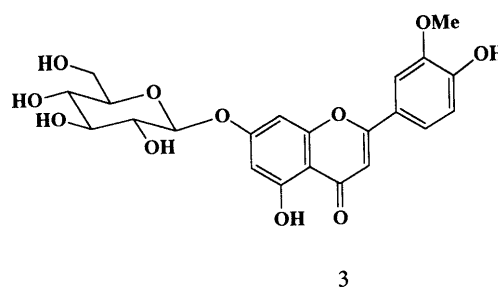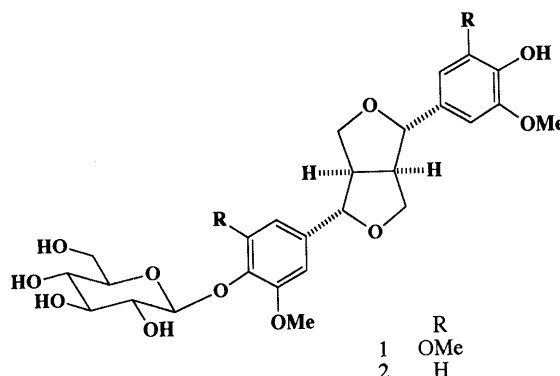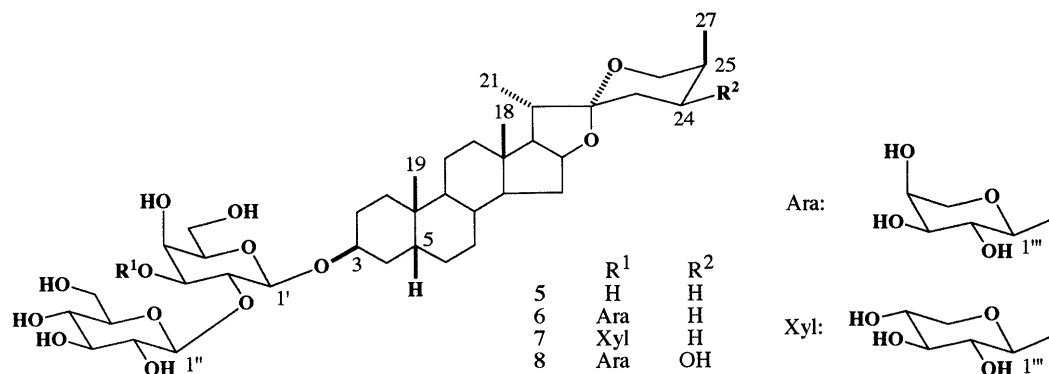|	R
1	OMe
2	H

3

	R
4	Glc
4a	H

Chart 1

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Chart 2

$[M-H]^-$), ^{13}C-NMR data (11 carbons) and elemental analysis. Absorptions at 3380, 1775 and 1635 cm^{-1} in the IR spectrum of **4** were indicative of the presence of several hydroxyl groups and a conjugated γ-lactone moiety; the latter was well supported by the UV absorption at λ_{max} 224 nm (log ε 4.11) due to π–π^* transition. The ^{1}H-NMR spectrum exhibited two one-proton singlets at δ 6.47 and 5.42, and a three-proton singlet at δ 3.71 assignable to a methoxyl group. The sequential proton signals were assigned to a β-glucose from their shift values and the multiplicities (J values) of the individual protons; six signals at δ 103.5 (CH), 74.9 (CH), 79.2 (CH), 71.0 (CH), 78.3 (CH) and 62.2 (CH$_2$) in the ^{13}C-NMR spectrum supported the ^{1}H-NMR data. Enzymatic hydrolysis of **4** with β-D-glucosidase produced an aglycone (**4a**), $C_5H_6O_4$, and D-glucose. The molecular formula of **4a** required 3 degrees of unsaturation. The conjugated carbonyl group accounted for 2 degrees, and consequently, **4a** must form a cyclic structure. In the ^{13}C-NMR spectrum of **4a**, the signal at δ 95.3 (CH) was shifted upfield by 2.9 ppm as compared with that of **4** (δ 98.2), indicating that each signal at δ 98.2 in **4** and at δ 95.3 in **4a** was due to an acetal carbon, and that the β-D-glucopyranose was linked to this position in **4**. The data presented above confirmed **4** to possess a 5-β-D-glucopyranosyloxyfuran-2(5H)-one structure. The olefinic proton at δ 6.47 and the acetal proton at δ 5.42 appeared as singlet signals; no coupling relation could be detected between them. The methoxyl group was thus concluded to be deposited on C-4 of the furanone. Compound **4** showed a negative Cotton effect at λ_{max} 224 nm ($\Delta\varepsilon$ −10.3) associated with the π–π^* transition in the CD spectrum. This sign was consistent with the S configuration at C-5 on application of the empirical rule for α,β-unsaturated γ-lactones found by Uchida and Kuriyama.[7] The aglycone (**4a**) showed no Cotton effect and no specific rotation. The 5-hydroxyfuran-2(5H)-one derivatives are known to be present in equilibrium with the corresponding open-chain acid.[8] Accordingly, the structure of **4** was shown to be (S)-5-β-D-glucopyranosyl-oxy-4-methoxyfuran-2(5H)-one.

Compound **6** ($C_{44}H_{72}O_{17}$) was a main steroidal saponin contained in *N. asiaticum*. The ^{1}H-NMR spectrum exhibited four typical spirostan methyls; two appeared as singlets at δ 0.96 (19-Me) and 0.82 (18-Me) and the other two as doublets at δ 1.16 ($J=6.9$ Hz, 21-Me) and 1.08 ($J=6.9$ Hz, 27-Me). The above data and chromatographic

behavior of **6** were identical to those of the toxic steroidal saponin we isolated earlier.[2] The presence of three sugars in **6** was apparent from the three anomeric proton signals at δ 5.53 (d, $J=7.8$ Hz), 5.17 (d, $J=7.5$ Hz) and 4.91 (d, $J=7.8$ Hz). Three anomeric carbons were also observed at δ 106.4, 104.4 and 102.0 in the ^{13}C-NMR spectrum. Acid hydrolysis of **6** with 1 N hydrochloric acid in dioxane–H$_2$O (1:1) furnished a mixture of (25R)-5β-spirostan-3β-ol (smilagenin) and (25S)-5β-spirostan-3β-ol (sarsasapogenin),[9] and D-glucose, D-galactose and L-arabinose in a ratio of 1:1:1.[10] Assignments of the ^{13}C-NMR signals of the saccharide moiety of **6** were performed by comparison with those of authentic methyl glycosides, taking into account the downfield shifts due to O-glycosylation,[9,11] which indicated the existence of a terminal β-D-glucopyranosyl unit, a terminal α-L-arabinopyranosyl unit and a 2,3-branched β-D-galactopyranosyl unit (Table 1). The β-configurations of the anomeric centers of the D-glucose and D-galactose, and the α-configuration of that of L-arabinose were reinforced by the large $^3J_{1\text{-H},2\text{-H}}$ values (7 Hz<). Mild hydrolysis of **6** with 0.2 N hydrochloric acid at 95 °C for 30 min gave L-arabinose and a partial hydrolysate (**5**). The structure of the trisaccharide moiety of **6**, 2,3-branched D-galactose embracing D-glucose at C-2 and L-arabinose at C-3, was thus given. The complete structure of **6** was determined to be (25R,S)-5β-spirostan-3β-ol 3-O-{O-β-D-glucopyranosyl-(1→2)-O-[α-L-arabinopyranosyl-(1→3)]-β-D-galactopyranoside}.

The NMR data of **7** ($C_{44}H_{72}O_{17}$) showed that it possessed identical aglycone structure to **6** but slightly differed from it in terms of the saccharide structure. Attempted assignments of the 17 resonance lines of the saccharide carbons by referring to those of **6** allowed allocation of the 12 lines to a terminal β-D-glucopyranosyl unit and a 2,3-branched β-D-galactopyranosyl unit. The remaining 5 signals were presumed to be due to a terminal β-D-xylopyranosyl unit. Acid hydrolysis of **7** with 1 N hydrochloric acid gave a mixture of smilagenin and sarsasapogenin and D-glucose, D-galactose and D-xylose in a ratio of 1:1:1, and partial hydrolysis gave **5** and D-xylose. The structure of **7** was formulated as (25R,S)-5β-spirostan-3β-ol 3-O-{O-β-D-glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)]-β-D-galactopyranoside}.

The approximate ratio of the C-25R and C-25S isomers present in **5**—**7** was established as 3:4 from the integrals

Table 1. ^{13}C-NMR Spectral Data for Compounds 5—8[a]

C	5	6	7	8
1	30.9	30.7	30.7[b]	30.7
2	27.0[b]	27.0[b]	27.0[c]	27.0[b]
3	75.2	75.1	75.2	75.0
4	30.9	30.7	30.8[b]	30.7
5	36.9	36.5	36.6	36.5
6	26.8[b]	26.8[b]	26.7[c]	26.8[b]
7	26.8[b]	26.9[b]	26.9[c]	26.9[b]
8	35.5	35.6	35.6	35.6
9	40.2	40.3	40.3	40.3
10	35.2	35.3	35.3	35.3
11	21.2	21.2	21.1	21.1
12	40.3	40.3	40.3	40.3
13	40.9	40.9	40.9	40.9
14	56.5	56.5	56.4	56.4
15	32.2	32.2	32.2	32.1
16	81.3	81.4 (81.3)	81.3	81.7
17	63.0 (63.1)	63.0 (63.2)	63.0 (63.2)	62.6
18	16.6	16.6	16.5	16.5
19	24.0	24.0	23.9	23.9
20	42.5 (42.0)	42.5 (42.0)	42.5 (42.0)	42.6
21	14.9 (15.1)	14.9 (15.1)	14.9 (15.0)	14.8
22	109.7 (109.2)	109.7 (109.2)	109.7 (109.2)	111.4
23	26.2[c] (31.9)	26.2[c] (31.9)	26.2[d] (31.9)	36.1
24	26.4[c] (29.3)	26.5[c] (29.3)	26.4[d] (29.3)	66.5
25	27.5 (30.6)	27.6 (30.6)	27.5 (30.6)	35.9
26	65.1 (66.9)	65.1 (66.9)	65.1 (66.9)	64.6
27	16.3 (17.3)	16.3 (17.3)	16.3 (17.3)	9.7
1′	102.6	102.0	102.2	102.0
2′	81.9	77.6	77.8	77.6
3′	76.9	84.3	84.2	84.2
4′	69.8	69.8	69.8	69.8
5′	76.6	76.5	76.4	76.5
6′	62.2	62.2	62.1	62.2
1″	106.1	104.4	104.5	104.4
2″	75.5	76.4	76.4	76.4
3″	78.4	78.6	78.5[e]	78.6
4″	71.7	72.8	72.7	72.8
5″	78.0	77.8	77.8	77.8
6″	62.8	63.5	63.4	63.5
1‴		106.4	106.2	106.4
2‴		72.8	75.2	72.8
3‴		74.7	78.4[e]	74.7
4‴		69.8	71.0	69.8
5‴		67.5	67.2	67.5

a) Spectra were measured in pyridine-d_5 and shifts for the C-25 isomers are shown in parentheses. b—e) Signals may be interchanged.
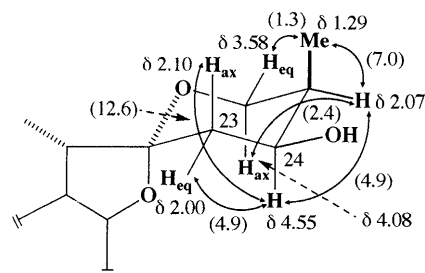

Fig. 1. ^{1}H-NMR Shifts of the F-Ring Part of 8 in Pyridine-d_5–Methanol-d_4 (10:1)
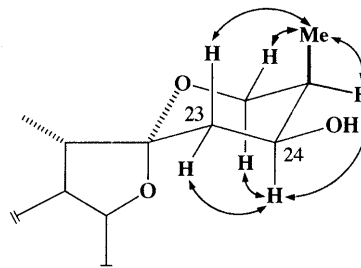
J values (Hz) are given in parentheses.

Fig. 2. NOEs of the F-Ring Part of 8 in Pyridine-d_5–Methanol-d_4 (10:1)

of the 27-Me proton signals.

Compound 8 ($C_{44}H_{72}O_{18}$) has one more oxygen atom than does 6. The ^{1}H-NMR spectrum of 8 resembled that of 6 with resonances for the 18-Me, 19-Me and 21-Me protons at δ 0.81 (s), 0.96 (s) and 1.19 (d, $J=6.9$ Hz), and the three anomeric protons at δ 5.54 (d, $J=7.8$ Hz), 5.18 (d, $J=7.5$ Hz) and 4.91 (d, $J=7.8$ Hz), while the 27-Me doublet signal observed at δ 1.08 (d, $J=6.9$ Hz) in 6 was shifted to lower field by 0.24 ppm to appear at δ 1.32 (d, $J=7.0$ Hz) in 8. The ^{13}C shifts of 8 also featured quite a similarity to those of 6 with exceptions of the signals due to the F-ring carbons; one of the methylene carbons at δ 26.2 and 26.5 assignable to C-23 and C-24, or C-24 and C-23, respectively, in 6 was replaced by the oxymethine signal at δ 66.5 and another methylene shifted to lower field to be observed at δ 36.1 in 8. Tracing out the ^{1}H–^{1}H coupling systems from the 27-Me signal through the

^{1}H–^{1}H correlation spectroscopy (COSY) spectrum combined with the homonuclear Hartmann-Hahn (HOHA-HA) spectrum, allowed the sequential assignments of the proton signals of the F-ring, giving rise to the partial structure, $-CH_2-CH(OH)-CH(CH_3)-CH_2-O-$. This data defined the location of the hydroxyl group at C-24. The configurations of C-24 and C-25 were determined by the ^{1}H-NMR parameters of the 24-H and 26-H_2 protons and NOE correlations. The 24-H appeared at δ 4.55 as a ddd signal coupling to 23$_{ax}$-H (δ 2.10) with a large J value of 12.6 Hz and to 23$_{eq}$-H (δ 2.00) and 25-H (δ 2.07) with small J values of 4.9 Hz. The 26-H_2 appeared at δ 4.08 and 3.58 and each coupled to 25-H with J values of 2.4 Hz and 1.3, respectively. Furthermore, in the phase-sensitive NOE correlation spectroscopy (PHNOESY) spectrum of 8, NOE correlations were detected from 24-H to 23$_{eq}$-H, 25-H and 26$_{ax}$-H, and from 27-Me to 23$_{ax}$-H, 25-H and 26$_{eq}$-H. These data were consistent with the 24S and 25R configurations and the chair-conformation of the F-ring as shown in Figs. 1 and 2. The definite assignments of the ^{1}H and ^{13}C signals due to the saccharide part of 8 were confirmed through detailed interpretation of the ^{1}H–^{1}H COSY, HOHAHA and heteronuclear multiple quantum coherence (HMQC) spectra (Table 2). The correlations between each anomeric proton and the three-bond coupled carbons across the glycosidic linkage detected in the heteronuclear multiple-bond correlation (HMBC) spectrum of 8 gave unambiguous evidence for the saccharide sequence and its linkage to C-3 of the aglycone as shown in Fig. 2.[12] The structure of 8 was entirely secured to be (24S,25R)-5β-spirostane-3β,24-diol 3-O-{O-β-D-glucopyranosyl-(1→2)-O-[α-L-arabinopyranosyl-(1→3)]-β-D-galactopyranoside}.

N. ossifragum, a species found in Europe, caused a disease in lambs called "alveld",[2,13] and the two spirostanol saponins, narthecin and xylosin were identified

Table 2. ^{1}H- and ^{13}C-NMR Assignments of the Saccharide Moiety of **8**[a]

Position	^{1}H	J (Hz)	^{13}C
1′	4.86 d	7.8	101.7
2′	4.71 dd	9.8, 7.8	77.5
3′	4.25 dd	9.8, 3.4	84.4
4′	4.70 br d	3.4	69.8
5′	4.00 br t	6.1	76.5
6′	4.32 d	6.1	62.2
1″	5.47 d	7.9	104.3
2″	3.91 dd	9.0, 7.9	76.3
3″	4.09 dd	9.0, 9.0	78.5
4″	4.01 dd	9.0, 9.0	72.6
5″	3.64 dd	9.0, 6.3, 3.0	77.8
6″	4.38 dd	11.3, 3.0	63.4
	4.23 dd	11.3, 6.3	
1‴	5.12 d	7.5	106.4
2‴	4.39 dd	8.8, 7.5	72.7
3‴	4.03 dd	8.8, 3.1	74.6
4‴	4.20 br s		69.7
5‴	4.17 dd	11.8, 1.8	67.4
	3.70 br d	11.8	

a) Assignments were confirmed by the concerted use of the ^{1}H–^{1}H COSY, HOHAHA and HMQC spectra recorded in pyridine-d_5–methanol-d_4 (10 : 1).
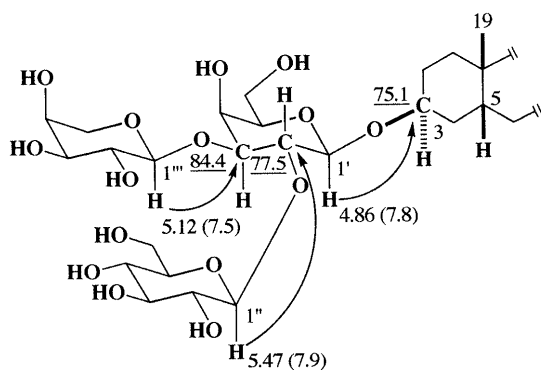
Fig. 3. HMBC Correlations of the Saccharide Moiety of **8** in Pyridine-d_5–Methanol-d_4 (10 : 1)

The HMBC spectrum was optimized for $^nJ_{C,H} = 8$ Hz. J values (Hz) in the ^{1}H-NMR spectrum are given in parentheses. Underlined figures indicate ^{13}C-NMR chemical shifts.

as toxins contained in the plant. Narthecin and xylosin were presumed to be sarsasapogenin trisaccharides composed of glucose, galactose and arabinose, and glucose, galactose and xylose, respectively. The sequences of the saccharides have been left undetermined. Unfortunately, we could not directly compare **6** and **7** with narthecin and xylosin, however, physical data reported in the literature[13] suggested that narthecin and xylosin do correspond to **6** and **7**, the identity of the toxins of *N. asiaticun* with *N. ossifragum*.

Experimental

Optical rotations were measured using a Jasco DIP-360 automatic digital polarimeter. IR spectra were recorded on a Hitachi 260-30 spectrophotometer and MS on a Hitachi M-80 or a VG AutoSpec E instrument. Elemental analysis was carried out using Perkin-Elmer 240B elemental analyzer. 1D NMR spectra were recorded on a Bruker AM-400 spectrometer (400 MHz for ^{1}H-NMR) and 2D NMR on a Bruker AM-500 instrument (500 MHz for ^{1}H-NMR) using the usual Bruker pulse program. Chemical shifts are given as δ-values with reference to tetramethylsilane (TMS), the internal standard. Silica-gel (Fuji-Silysia Chemical), Diaion HP-20 (Mitsubishi-Kasei), Sephadex

LH-20 (Pharmacia) and octadecylsilanized (ODS) silica-gel (Nacalai Tesque) were used for column chromatographies. TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm thick, Merck) and RP-18 F_{254} S (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. HPLC was performed using a Tosoh HPLC system (Tosoh: pump, CCPM; controller, CCP controller PX-8010; detector, UV-8000).

Isolation Fresh aerial parts of *N. asiaticum* (13 kg), collected at Yamagata prefecture, Japan, in July 1992, were cut into pieces and extracted with MeOH under reflux. The extract was concentrated almost to dryness under reduced pressure and the crude residue, after dilution with H_2O, was extracted with *n*-BuOH. The *n*-BuOH-soluble phase was fractionated on a silica-gel column, eluting with a gradient mixture of $CHCl_3$–MeOH (19 : 1; 9 : 1; 4 : 1), $CHCl_3$–MeOH–H_2O (20 : 10 : 1; 7 : 4 : 1) and finally with MeOH to give five fractions (I—V). Fraction III was further divided by a silica-gel column using $CHCl_3$–MeOH–H_2O (50 : 10 : 1) as the solvent system into four fractions (IIIa—IIId). Fraction IIIb was chromatographed on silica-gel, eluting with $CHCl_3$–MeOH–H_2O (60 : 10 : 1) and EtOAc–MeOH–H_2O (65 : 10 : 1), Sephadex LH-20 with MeOH, and on ODS silica-gel with MeOH–H_2O (2 : 3) to give compound **1** (46 mg). Fraction IIIc was chromatographed on silica-gel, eluting with $CHCl_3$–MeOH–H_2O (80 : 10 : 1) and ODS silica-gel with MeOH–H_2O (1 : 1; 2 : 3; 3 : 7) to give compounds **2** (549 mg) and **3** (33 mg). Chromatography of fraction IIId on silica-gel with $CHCl_3$–MeOH–H_2O (40 : 10 : 1) gave compound **4** (1.72 g) and a saponin fraction containing a considerable amount of saccharides. The removal of saccharides from it was performed by column chromatography on Diaion HP-20 with an increasing amount of MeOH in H_2O. The fraction eluted with MeOH was purified by ODS silica-gel column chromatography with MeOH–H_2O (4 : 1) and Sephadex LH-20 with MeOH to furnish compound **5** (62 mg). Fraction IV was subjected to column chromatographies on Diaion HP-20 with H_2O–MeOH, silica-gel with $CHCl_3$–MeOH–H_2O (30 : 10 : 1), and on ODS silica-gel with MeOH–H_2O (4 : 1) to yield compounds **6** (874 mg), **7** (26 mg) and **8** (32 mg).

The physical and spectral characteristics of **1**—**3** were consistent with those previously reported. Data refer to the literature shown in the text.

Compound 4 An amorphous solid, $[\alpha]_D^{25}$ +24.6° ($c = 0.25$, MeOH). *Anal.* Calcd for $C_{11}H_{16}O_9$: C, 45.21; H, 5.52. Found: C, 45.36; H, 5.35. Negative-ion FAB-MS m/z: 291 [M−H]$^-$, 129 [M−glucosyl]$^-$, UV λ_{max}^{MeOH} nm (log ε): 224 (4.11). CD ($c = 0.0001$, MeOH) $\Delta\varepsilon$: −10.3 (224) (negative maximum), +1.6 (247) (positive maximum). IR ν_{max}^{KBr} cm^{-1}: 3590, 3380 and 3120 (OH), 2920 (CH), 1775 (C=O), 1635 (C=C), 1450, 1375, 1305, 1280, 1250, 1155, 1100, 1075, 1060, 1035, 1005, 955, 925, 880, 865, 830, 775. ^{1}H-NMR (pyridine-d_5) δ: 6.47 (1H, s, 3-H), 5.42 (1H, s, 5-H), 5.29 (1H, d, $J = 7.8$ Hz, 1′-H), 4.48 (1H, dd, $J = 11.8$, 1.7 Hz, 6′a-H), 4.34 (1H, dd, $J = 11.8$, 4.7 Hz, 6′b-H), 4.27 (1H, dd, $J = 8.7$, 8.7 Hz, 3′-H), 4.22 (1H, dd, $J = 8.7$, 8.7 Hz, 4′-H), 4.09 (1H, dd, $J = 8.7$, 7.8 Hz, 2′-H), 3.91 (1H, ddd, $J = 8.7$, 4.7, 1.7 Hz, 5′-H), 3.71 (3H, s, OMe). ^{13}C-NMR (pyridine-d_5) δ: 178.3 (C-4), 170.6 (C-2), 103.5 (C-1′), 98.2 (C-5), 90.3 (C-3), 79.2 (C-3′), 78.3 (C-5′), 74.9 (C-2′), 71.0 (C-5), 62.2 (C-6′), 59.6 (OMe).

Enzymatic Hydrolysis of 4 Compound **4** (200 mg) was dissolved in an AcOH/AcONa buffer (pH 5) (8 ml) with β-D-glucosidase (Tokyo-Kasei-Kogyo) (50 mg), and the mixture incubated at room temperature overnight. The crude products were chromatographed on silica-gel, using a discontinuous gradient from hexane–Me_2CO (1 : 1) to $CHCl_3$–MeOH (1 : 1) to yield an aglycone (**4a**) (80 mg) and D-glucose. D-Glucose was identified by direct TLC comparison with an authentic sample; Rf 0.40 (*n*-BuOH–Me_2CO–H_2O, 4 : 5 : 1). Compound **4a**: an amorphous solid, $[\alpha]_D^{25}$ ±0° ($c = 0.25$, MeOH). EI-MS m/z: 130 [M]$^+$, 84, 69. UV λ_{max}^{MeOH} nm (log ε): 223 (4.02). CD ($c = 0.0001$, MeOH) $\Delta\varepsilon$: no Cotton effect. IR ν_{max}^{KBr} cm^{-1}: 3250 and 3150 (OH), 2975 (CH), 1750 (C=O), 1655 (C=C), 1470, 1450, 1370, 1350, 1305, 1270, 1195, 1145, 1005, 975, 960, 935, 915, 840, 775. ^{1}H-NMR (pyridine-d_5) δ: 6.36 (1H, s, 3-H), 5.39 (1H, s, 5-H), 3.74 (3H, s, OMe). ^{13}C-NMR (pyridine-d_5) δ: 179.6 (C-4), 171.0 (C-2), 95.3 (C-5), 90.2 (C-3), 59.5 (OMe).

Compound 5 An amorphous solid, $[\alpha]_D^{25}$ −50.9° ($c = 0.25$, pyridine). Negative-ion FAB-MS m/z: 740 [M]$^-$, 577 [M−glucosyl]$^-$. ^{1}H-NMR (pyridine-d_5) δ: 5.27 (1H, d, $J = 7.7$ Hz, 1″-H), 4.91 (1H, d, $J = 7.6$ Hz, 1′-H), 1.16 (d, $J = 6.8$ Hz, 21-Me), 1.08 (d, $J = 7.1$ Hz, 27-Me), 0.98 (3H, s, 19-Me), 0.82 (s, 18-Me). Signals for C-25R isomer: 1.15 (d, $J = 6.9$ Hz, 21-Me), 0.83 (s, 18-Me), 0.71 (d, $J = 5.2$ Hz, 27-Me).

Compound 6 An amorphous solid, $[\alpha]_D^{25}$ −18.7° ($c = 0.25$, pyridine). Negative-ion FAB-MS m/z: 872 [M]$^-$, 740 [M−arabinosyl]$^-$, 710

[M−glucosyl]⁻, 577 [M−arabinosyl−glucosyl]⁻. IR ν_{max}^{KBr} cm⁻¹: 3390 (OH), 2940 (CH), 1445, 1375, 1170, 1130, 1055, 1000, 985, 965, 945, 915, 895, 845, 775. ¹H-NMR (pyridine-d_5) δ: 5.53 (1H, d, J=7.8 Hz, 1″-H), 5.17 (1H, d, J=7.5 Hz, 1‴-H), 4.91 (1H, d, J=7.8 Hz, 1′-H), 1.16 (d, J=6.9 Hz, 21-Me), 1.08 (d, J=6.9 Hz, 27-Me), 0.96 (3H, s, 19-Me), 0.82 (s, 18-Me). Signals for C-25R isomer: 1.15 (d, J=6.7 Hz, 21-Me), 0.83 (s, 18-Me), 0.71 (d, J=5.1 Hz, 27-Me).

Compound 7 An amorphous solid, $[\alpha]_D^{25}$ −31.1° (c=0.25, pyridine). Negative-ion FAB-MS m/z: 872 [M]⁻, 740 [M−arabinosyl]⁻; IR ν_{max}^{KBr} cm⁻¹: 3400 (OH), 2930 (CH), 1440, 1370, 1255, 1145, 1040, 975, 910, 890, 840, 795. ¹H-NMR (pyridine-d_5) δ: 5.59 (1H, d, J=7.8 Hz, 1″-H), 5.24 (1H, d, J=7.6 Hz, 1‴-H), 4.92 (1H, d, J=7.7 Hz, 1′-H), 1.16 (d, J=6.8 Hz, 21-Me), 1.08 (d, J=6.9 Hz, 27-Me), 0.97 (3H, s, 19-Me), 0.82 (s, 18-Me). Signals for C-25R isomer: 1.15 (d, J=6.7 Hz, 21-Me), 0.83 (s, 18-Me), 0.71 (d, J=5.1 Hz, 27-Me).

Compound 8 An amorphous solid, $[\alpha]_D^{25}$ −30.6° (c=0.25, pyridine). *Anal.* Calcd for $C_{44}H_{72}O_{18}\cdot 2H_2O$: C, 57.13; H, 8.28. Found: C, 56.83; H, 8.02. Negative-ion FAB-MS m/z: 887 [M−H]⁻. IR ν_{max}^{KBr} cm⁻¹: 3405 (OH), 2935 (CH), 1450, 1375, 1340, 1255, 1170, 1060, 995, 965, 895, 860, 775. ¹H-NMR (pyridine-d_5) δ: 5.54 (1H, d, J=7.8 Hz, 1″-H), 5.18 (1H, d, J=7.5 Hz, 1‴-H), 4.91 (1H, d, J=7.8 Hz, 1′-H), 1.32 (3H, d, J=7.0 Hz, 27-Me), 1.19 (3H, d, J=6.9 Hz, 21-Me), 0.96 (3H, s, 19-Me), 0.81 (3H, s, 18-Me).

Acid Hydrolysis of 6, 7 and 8 A solution of **6** (30 mg) in 1 N HCl (dioxane–H_2O, 1:1, 8 ml) was refluxed for 2 h under an Ar atmosphere. The reaction mixture was neutralized by passing it through an Amberlite IRA-93ZU (Organo) column, and then transferred to a silica-gel column, eluting with CHCl₃–MeOH (19:1; 1:1) to give an aglycone (**6a**) (10.3 mg), identified as (25S)-5β-spirostan-3β-ol (sarsasapogenin), containing a certain amount of the C-25 isomer (smilagenin) and a mixture of monosaccharides (10.2 mg). ¹H-NMR of **6a** (chloroform-d_1) δ: 4.40 (1H, m, 16-H), 4.11 (1H, br s, 3-H), 3.95 (dd, J=11.0, 2.7 Hz, 26a-H), 3.30 (br d, J=11.0 Hz, 26b-H), 1.08 (d, J=7.1 Hz, 21-Me), 0.99 (d, J=7.0 Hz, 27-Me), 0.98 (3H, s, 19-Me), 0.76 (3H, s, 18-Me). Signals for C-25R isomer: 3.47 (ddd, J=10.8, 4.3, 2.1 Hz, 26a-H), 3.37 (br d, J=10.8, 10.8 Hz, 26b-H), 0.96 (d, J=7.0 Hz, 21-Me), 0.79 (d, J=6.4 Hz, 27-Me). ¹³C-NMR of **6a** (chloroform-d_1) δ: 29.7, 27.8, 67.1, 33.6, 36.6, 26.6, 26.6, 35.3, 39.9, 35.3, 20.9, 40.3, 40.7, 56.5, 31.8, 81.0, 62.1 (62.3), 16.5, 23.9, 42.1 (41.6), 14.3 (14.5), 109.8 (109.3), 25.8ᵃ⁾ (31.4), 26.0ᵃ⁾ (28.8), 27.1 (30.3), 65.2 (66.9), 16.1 (17.1) (C-1—C-27; a) signals may be interchanged). The shift values in parentheses indicated the signals due to the C-25R isomer. The aglycone, **6a**, increased in intensity of the signals due to the C-25R isomer as compared with those of **6**. The (25S)-spirostanes are known to be converted into the (25R)-isomer through acid treatment.[14] Following this procedure, **7** (10 mg) and **8** (15 mg) were subjected to acid hydrolysis. Compound **7** gave **6a** (3.6 mg) and a monosaccharide mixture (2 mg) and **8** gave several unidentified decomposed compounds and a monosaccharide mixture (5.8 mg).

Determination of the Absolute Configurations of Sugars To each sugar mixture (2 mg) in H_2O, (−)-α-methylbenzylamine (8 mg) was added followed by Na[BH₃CN] (8 mg) in EtOH (1 ml). The mixture was left standing for 4 h at 40 °C, then acetylated with Ac₂O (0.3 ml) in pyridine (0.3 ml). The reaction mixture was passed through a Sep-Pak C_{18} cartridge (Waters), eluting with H_2O–MeCN (4:1; 1:1; each 10 ml), and then with MeCN (10 ml). The MeCN fraction was then passed through

a Toyopak IC-SP M cartridge (Tosoh), eluting with EtOH (10 ml), to give a mixture of the 1-[(S)-N-acetyl-α-methylbenzylamino]-1-deoxy-alditol acetate derivatives of the monosaccharides, which was then analyzed by HPLC under the following conditions: column, TSK-gel ODS-Prep (Tosoh, 4.6 mm i.d. × 250 mm, ODS, 5 μm); solvent, MeCN–H_2O (2:3); flow rate, 0.8 ml/min; detection, UV (230 nm). Derivatives of D-glucose, D-galactose and L-arabinose were detected in the sugar fractions of **6** and **8**, and D-glucose, D-galactose and D-xylose in that of **7**. t_R (min): D-glucose, 23.42; D-galactose, 19.92; L-arabinose, 17.29; D-xylose, 18.02.

Partial Hydrolysis of 6 and 7 A solution of **6** (300 mg) in 0.2 N HCl (dioxane–H_2O, 1:1, 8 ml) was heated for 30 min at 95 °C under an Ar atmosphere. The reaction mixture was neutralized by passing it through an Amberlite IRA-93ZU column, and chromatographed on silica-gel, eluting with CHCl₃–MeOH–H_2O (40:10:1) and then with CHCl₃–MeOH (1:1) to give **5** (24.2 mg) as a partial hydrolysate and L-arabinose. Compound **6**, 210 mg, was recovered unchanged. Following this procedure, **7** (5 mg) was subjected to partial hydrolysis to also give **5** and D-xylose.

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