Two New Steroidal Saponins from Dried Fermented Residues of Leaf-Juices of *Agave sisalana* forma Dong No. 1

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In a previous paper, we reported the isolation and structure determination of three new steroidal saponins, dongnosides C (3), D (2) and E (1) from the dried fermented residues of leaf-juices of *Agave sisalana* forma Dong No. 1. In a continuing study on this plant, two additional new major steroidal saponins, named dongnosides B (4) and A (5), were obtained. Their structures were characterized respectively as tigogenin 3-*O*-α-L-rhamonpyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside and 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside on the basis of chemical and physicochemical evidence.

Keywords *Agave sisalana*; Agavaceae; steroidal saponin; spirostanol glycoside; tigogenin; dongnoside

Agave sisalana PERRINE is a plant of Agave Linne genus, Agavaceae family. It was introduced from abroad and has been cultivated in the south of China, named "Agave Dong No. 1." Its leaves are a very important source of hard fiber and raw material used to synthesize steroidal hormones. In a previous paper, we have reported the isolation and structure elucidation of three new steroidal saponins, dongnoside C (**3**), D (**2**) and E (**1**) from Agave Dong No. 1.[1] As a further study, we have isolated two more new steroidal saponins, named dongnosides B (**4**) and A (**5**), and have characterized their structures.

The dried fermented residues[2] (3.0 kg) of leaf-juices of Agave Dong No. 1 were refluxed with petroleum ether (petrol, 60—80 °C), and the petrol-insoluble residue was extracted with methanol (MeOH). The MeOH extract was evaporated under reduced pressure to give a residue (774 g) which was separated by column chromatography (CC) and preparative thin layer chromatography (TLC) over silica gel (Si gel), to provide compounds **1** (110 mg), **2** (1.25 g), **3** (186 mg), **4** (1.30 g) and **5** (3.60 g).

The saponins B (**4**) and A (**5**) were positive in the Liebermann–Burchard reaction, but negative to the Ehrlich reagent.[3] They showed the characteristic absorption band[4] of 25*R* spirostanol steroid [985, 920 < 900 (in intensity), 870 cm^{-1}] and hydroxyl group absorptions (3440—3460 and 1050—1080 cm^{-1}) in the infrared (IR) spectrum, suggesting that saponins **4** and **5** are the glycosides of the 25*R* spirostanol derivative.

Dongnoside B (**4**), a white powder (mp 275—277 °C), $[\alpha]_D$ −50.8° (pyridine), showed peaks due to a $[M-H]^-$ ion at *m/z* 1209 in the negative fast atom bombardment mass spectrum (negative FAB-MS), and the elementary analysis of **4** indicated the molecular formula $C_{57}H_{94}O_{27}$. Acid hydrolysis of **4** afforded rhamnose, galactose and glucose as the sugar components, and an aglycone, colorless needles (MeOH), mp 206—208 °C, $[\alpha]_D$ −51.7° (pyridine). The electron impact mass spectrum (EI-MS) showed a molecular ion peak at *m/z* 416 and a characteristic base peak at *m/z* 139 ($C_9H_{15}O$) derived from the spiroketal side chain of the steroidal sapogenol.[5] The molecular formula $C_{27}H_{44}O_3$ was determined by elementary analysis and EI-MS. Comparison of carbon-13 nuclear magnetic resonance (^{13}C-NMR) data for the aglycone (Table I) with those of tigogenin,[6] indicated aglycone was tigogenin, (5α,25*R*)-3β-hydroxy-spirostane. In the negative FAB-MS spectrum, in addition to a molecular ion peak, fragment ion peaks at *m/z* 1063 and 901 were found, which were ascribable to fragments due to the loss of terminal rhamnose and rhamnosyl-hexosyl residues, respectively. Saponin **4** was acetylated in the usual manner to yield a peracetate, $[\alpha]_D$ −27.0° (MeOH), showing fragment peaks at *m/z* 561 $[(Rha-Hex\text{-})Ac_6]^+$, 331 $[(terminal\ Hex\text{-})Ac_4]^+$ and 273 $[(terminal\ Rha\text{-})Ac_3]^+$ in the EI-MS. The above evidence suggested that **4** was a tigogenin pentaglycoside consisting of 4 mol of hexose and 1 mol of rhamnose in which 1 mol

TABLE I. ^{13}C-NMR Chemical Shifts for Aglycone Moieties of Saponins **4**, **5** and Prosapogenins **4b**, **4c**, **4d**, **5e** and Tigogenin (Pyridine-d_5)

	Tigogenin	**4b**	**4c**	**4**	**4d**	**5**	**5e**
C -1	37.5	37.1	37.1	37.2	37.1	37.2	37.2
C -2	32.5	30.5	30.6	29.9	30.5	30.6	29.9
C -3	70.6	77.1	77.5	77.4	77.5	77.4	77.4
C -4	39.3	34.7	34.8	34.8	34.7	34.8	34.8
C -5	45.2	44.8	44.8	44.6	44.6	44.6	44.6
C -6	29.1	28.8	28.9	28.9	28.9	28.9	28.9
C -7	32.5	32.3	32.4	32.4	32.4	32.2	32.4
C -8	35.4	35.2	35.3	35.2	35.3	35.2	35.2
C -9	54.6	54.3	54.5	54.4	54.3	54.4	54.4
C-10	35.9	35.7	35.8	35.8	35.8	35.8	35.8
C-11	21.1	21.2	21.3	21.3	21.2	21.3	21.3
C-12	40.3	40.0	40.2	40.1	40.1	40.1	40.1
C-13	40.8	40.7	40.8	40.7	40.7	40.8	40.8
C-14	57.6	56.4	56.5	56.4	56.4	56.4	56.4
C-15	32.1	32.1	32.1	32.1	32.1	32.1	32.2
C-16	81.1	81.1	81.1	81.1	81.1	81.1	81.1
C-17	63.1	63.0	63.0	63.0	62.9	63.0	63.0
C-18	16.7	16.6	16.6	16.6	16.6	16.6	16.6
C-19	12.5	12.2	12.3	12.3	12.3	12.3	12.3
C-20	42.0	41.9	42.0	42.0	41.9	42.0	42.0
C-21	15.0	14.9	15.0	15.0	15.0	15.0	15.0
C-22	109.2	109.1	109.2	109.2	109.2	109.2	109.2
C-23	31.9	31.7	31.8	31.8	31.8	31.8	31.8
C-24	29.3	29.2	29.2	29.2	29.2	29.2	29.2
C-25	30.6	29.9	29.9	30.0	29.8	30.0	30.6
C-26	66.9	66.9	66.9	66.9	66.8	66.9	66.8
C-27	17.3	17.3	17.3	17.3	17.3	17.3	17.3

Table II. ^{13}C-NMR Chemical Shifts for Sugar Moieties of Saponins **4**, **5** and Prosapogenins **4b**, **4c**, **4d**, and **5e** (Pyridine-d_5)

	4b	**4c**	**4d**	**4**	**5e**	**5**
Gal						
1	102.4	102.3	102.3	102.4	102.4	102.4
2	73.4	73.2	73.1	73.1	73.1	73.1
3	75.2	75.0	75.2	75.2	75.0	75.0
4	80.0	78.9	81.3	81.4	80.8	80.8
5	76.0	76.7	76.0	76.6	75.4	76.5
6	61.0	60.4	60.6	60.6	60.6	60.6
Glc						
1	107.1	105.1	104.3	104.1	104.0	103.9
2	75.4	75.5	80.1	80.1	79.9	79.8
3	78.4	86.0	88.1	87.9	88.4	88.0
4	72.2	70.2	70.7	70.6	69.0	70.6
5	78.7	78.1	77.4	77.3	78.6	78.0
6	63.0	61.5	62.3	62.2	62.3	62.0
Glc′						
1			104.7	104.7	104.8	104.7
2			75.4	75.3	75.3	75.5
3			78.5	77.1	78.4	77.2
4			71.5	78.5	71.6	78.1
5			77.4	76.0	78.1	75.5
6			62.1	61.1	63.0	61.1
Glc″						
1		106.8	104.5	104.9	104.4	104.1
2		75.6	75.2	75.5	75.3	75.3
3		78.9	78.5	78.4	86.8	86.9
4		71.7	70.8	70.9	70.7	70.7
5		78.4	77.3	77.9	77.5	77.4
6		62.9	62.9	62.9	62.0	62.9
Xyl						
1					106.0	106.1
2					75.4	75.3
3					77.7	77.4
4					70.7	70.3
5					67.0	67.0
Rha						
1				102.6		102.6
2				72.4		72.5
3				72.6		72.7
4				73.8		73.9
5				70.4		69.1
6				18.5		18.5

each of rhamnose and hexose was located at the terminal. The ^{1}H-NMR spectrum of **4** exhibited five anomeric proton signals, among which four doublet signals at δ 4.87 (J=7.3 Hz), 5.11 (J=7.7 Hz), 5.27 (J=7.7 Hz) and 5.56 (J=7.7 Hz) suggested all β-configurations of glucosyl and galactosyl moieties, while one singlet signal at δ 5.73 was attributable to the α-one of the rhamnosyl moiety. Partial hydrolysis of **4** with 0.5 N HCl in 50% EtOH provided four prosapogenins, **4a**, **4b**, **4c** and **4d**. The former three were the same as the hydrolyzed products of **1**,[1] identified with 3-*O*-monoglycoside, 3-*O*-diglycoside and 3-*O*-triglycoside of tigogenin, respectively. Compound **4d** showed the presence of four anomeric carbons at δ 102.3, 104.3, 104.5 and 104.7 in the ^{13}C-NMR spectrum (Table II), and the peracetate of **4d** gave a fragment peak at m/z 331. On acid hydrolysis, **4d** liberated glucose and galactose. The above evidence indicated that **4d** is a 3-*O*-tetraglycoside with a terminal hexose. In comparing of the ^{13}C-NMR spectrum (Table II) of **4d** with **4c**, **4d** showed signals due to one additional mole of glucose compared to **4c**, as well as a downfield shift (+4.6 ppm) at C-2 of the inner glucose, indicating that **4d** should possess 1 mol more of glucose

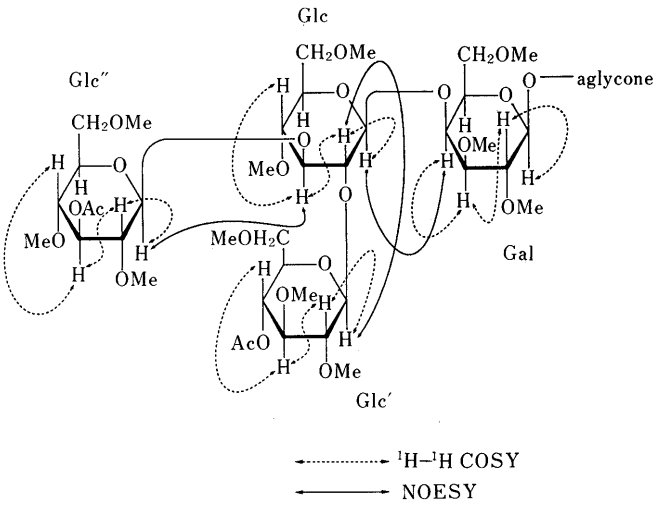
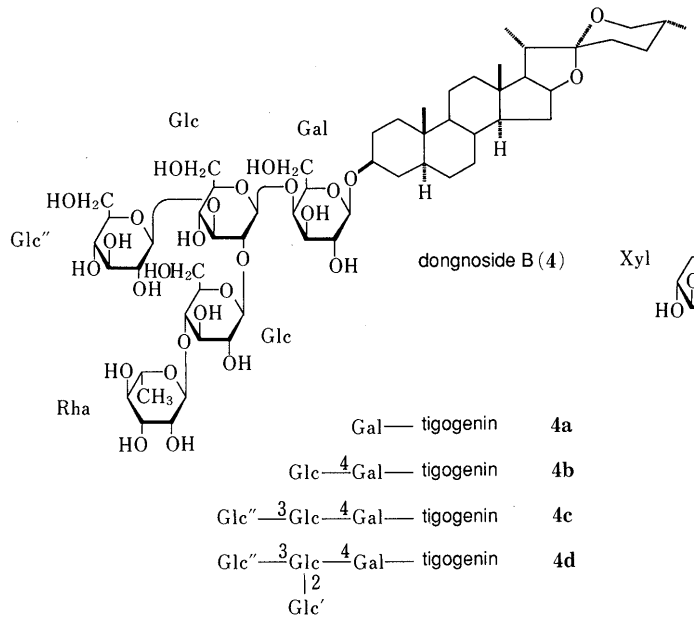


Fig. 1. ^{1}H–^{1}H COSY and NOESY of 5-M-1

than **4c** and that the terminal glucose branched at C-2 OH of the inner glucose. Thus, **4d** was characterized as tigogenin 3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside. The ^{13}C-NMR spectrum (Table II) of **4** showed increased signals due to the terminal rhamnose compared to those of **4d**, and displayed that the signal due to C-4 of one glucose shifted by +7.0 ppm, suggesting that a rhamnose was attached to the C-4 OH of one glucose in **4**. The question of whether the terminal rhamnosyl residue combines to C-2 or C-3 glucose remains unsolved in this stage; however, this could be settled after determining for the structure of the following compound, dongnoside A (**5**).

Dongnoside A (**5**), white powder, (mp 265—270 °C), $[\alpha]_D$ −51.5° (pyridine), gave the molecular formula $C_{62}H_{102}O_{31}$ by elementary analysis. Acid hydrolysis of **5** with 2 N H_2SO_4 in 50% EtOH yielded tigogenin, glucose, galactose, xylose and rhamnose. The negative FAB-MS of **5** showed a molecular ion peak at m/z 1341 $[M-H]^-$ and fragment peaks due to $[M-H-Pen]^-$ at m/z 1209, $[M-H-Rha]^-$ at m/z 1195, $[M-H-Pen-Hex]^-$ at m/z 1047 and $[M-H-Rha-Hex]^-$ at m/z 1033. The EI-MS of **5**-peracetate gave fragment peaks at m/z 273 [(terminal Rha-)Ac_3]$^+$ and 259 [(terminal Pen-)Ac_3]$^+$, suggesting that **5** was composed of 4 mol of hexose and 1 mol each of rhamnose and xylose, and in addition, that the rhamnose and xylose were located at the terminal. In the ^{1}H-NMR spectrum, **5** exhibited five doublet signals at δ 4.84 (J=7.6 Hz), 5.06 (J=7.3 Hz), 5.08 (J=7.3 Hz), 5.16 (J=7.6 Hz) and 5.53 (J=7.3 Hz) assigned to the anomeric protons of glucose, galactose and xylose, and a singlet signal at δ 5.76 assignable to rhamnose, suggesting that rhamnose is α-linked and the other sugar moieties are β-linked. On partial hydrolysis, **5** afforded two main prosapogenins identical with **4** and **4d**. The ^{13}C-NMR spectrum (Table II) of **5** compared with that of **4** indicated an increase in signals due to 1 mol of xylose in **5** and its glycosylation shift (+8.4 ppm) at C-3 of the inner glucose. On the other hand, enzymatic hydrolysis of **5** with hesperidinase provided rhamnose and a prosapogenin, **5e**. A comparative study of the ^{13}C-NMR spectrum of **5** and **5e** suggested that signals due to a terminal rhamnose increased with a glycosylation



shift (+6.5 ppm) at C-4 of the inner glucose in **5**. Both the prosapogenin **5e** and **4** were pentaglycosides; however, the terminal sugar moeity and the linkage position were different. The **5e** had a terminal xylose attached to the C-3 OH of glucose, while **4** possessed a terminal rhamnose bound to the C-4 OH of another glucose. In order to solve the problem of which glycosyl side (glc′ or glc″) the terminal rhamnosyl or xylosyl moiety connected to in **5**, **5** was derived into permethyl ether (**5**-M) by Hakomori's method.[7] Compound **5**-M was partially acid hydrolyzed to give the products, which were separated and respectively acetylated to provide **5**-M-1 along with two minor products, **5**-M-2 and **5**-M-3. The positive FAB-MS showed **5**-M-1 to be a tetraglycosyl derivative and **5**-M-2 and **5**-M-3 to be pentaglycosyl derivatives. ^{1}H–^{1}H COSY and the differential NOE experiment disclosed that the structure of **5**-M-1 could be represented as shown in Fig. 1, in which two acetyl groups were introduced both to the C-4 OH (H-4, t, J=9.5, δ 4.81) of the one terminal glucosyl residue (Glc′ H-1, d, J=7.9, δ 5.13) and to the C-3 OH (H-3, t, J=9.0, δ 5.12) of another terminal glucosyl moiety (Glc″ H-1, d, J=7.7, δ 4.92), and that these two glucosyl residues were independently connected to the C-2 OH and C-3 OH of the inner glucosyl (Glc) moiety. Namely, terminal xylosyl and terminal rhamnosyl moieties were linked to the C-4 in Glc′ and C-3 in Glc″ in **5**. Therefore, the structure of dongnoside A (**5**) was represented as tigogenin 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, named dongnoside A.

Simultaneously, the structure of dongnoside B (**4**) was established to be tigogenin 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

From Agave Dong No. 1, five new spirostanol glycosides have been isolated. It is noteworthy that the crude drug used for the synthesis material of steroid hormones was firstly chemically disclosed in its pure state.

Experimental

Melting points were measured using a Boetius micromelting point apparatus and were uncorrected. Specific rotations were taken on a Perkin-Elmer-241 automatic digital polarimeter. The IR spectrum was recorded on a Shimadzu IR-410 (KBr). The ^{1}H- and ^{13}C-NMR spectra were taken on Bruker AM-400 and JEOL JNM-GX 400 (^{1}H: 400 Hz, ^{13}C: 100 Hz) NMR spectrometers, and chemical shifts are given as δ (ppm) with tetramethylsilane (TMS) as an internal standard. The symbols s, d, t, q, dd and br s denote singlet, doublet, triplet, quartet, double doublet and broad singlet, respectively. The coupling constant (J) values are given in Hertz (Hz). The EI-MS were measured on a Finnegan-4510 instrument. The FAB- and FD-MS were measured on JEOL MS-DX-300, ZAB-HS and 7070-HF instruments. The CC was carried out with Si gel (100—200 mesh, Quingdao, China) and Kieselgel 60 (230—400 mesh, Merck). The TLC was conducted on a precoated Si gel G and H (Qingdao, China) and Kieselgel 60 F_{254} plate (0.2 mm, Merck), and detection was achieved by spraying it with 10% H_2SO_4 following by heating.

Extraction and Separation The dried fermented solid (3 kg) of leaf-juice of *Agave sisalana* forma Dong No. 1 (Agavaceae) collected in Nanning was defatted with hot petrol ether. The defatted powder was extracted with hot MeOH until the extract was colorless. The extract (774 g) was subjected to CC on 100—200 mesh Si gel using $CHCl_3$–MeOH–H_2O (C–M–W. 7 : 3 : 0.5), and several fractions were obtained. The high polarity fractions were repeatedly subjected to low pressure CC on 10—40 μ Si gel H with C–M–W (7 : 3 : 1 lower phase) followed by preparative TLC with C–M–W (7 : 3 : 0.5) and crystallized with MeOH to afford compounds **1** (110 mg), **2** (1.25 g), **3** (186 mg), **4** (1.30 g) and **5** (3.60 g).

Dongnoside B (4) A white powder (mp 275—277 °C), $[\alpha]_D^{26}$ −50.8° (c=0.06, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3420—3450, 1060—1080 (–OH), 980, 920<900, 865 (25*R* spirostanol). ^{1}H-NMR (pyridine-d_5) δ: 0.64 (3H, s, CH_3-18), 0.70 (3H, d, J=5.1 Hz, CH_3-27), 0.82 (3H, s, CH_3-19), 1.15 (3H, d, J=7.0 Hz, CH_3-21), 1.69 (3H, d, J=6.2 Hz, Rha CH_3), 3.51—3.60 (2H, m, H_2-26), 4.87 (1H, d, J=7.3 Hz), 5.11 (1H, d, J=7.7 Hz), 5.27 (1H, d, J=7.7 Hz), 5.56 (1H, d, J=7.7 Hz), 5.73 (1H, br s). Negative FAB-MS m/z: 1209 $[M-H]^-$, 1063 $[M-H-Rha]^-$, 901 $[M-H-Hex-Rha]^-$, 739 $[M-H-Rha-2\times Hex]^-$, 577 $[M-H-Rha-3\times Hex]^-$. *Anal.* Calcd for $C_{57}H_{94}O_{27}\cdot 4H_2O$: C, 53.35; H, 7.96. Found: C, 52.91; H, 7.44. ^{13}C-NMR (pyridine-d_5): Tables I and II.

Acid Hydrolysis of 4 A solution of **4** (50 mg) in 2 N H_2SO_4–50% EtOH (5 ml) was refluxed on a water bath for 4 h. The reaction mixture was diluted with water (10 ml) and filtered. The precipitate was crystallized with MeOH to give an aglycone, colorless needles, mp 206—208 °C, $[\alpha]_D^{30}$ −51.7° (c=0.09, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3520, 1050, 1040 (–OH), 980, 960, 920<900, 865 (25*R* spirostanol). EI-MS m/z: 416 $[M]^+$, 401, 357, 344, 302, 287, 273, 139 (100), 115, 69. *Anal.* Calcd for $C_{27}H_{44}O_3\cdot H_2O$: C, 74.65; H, 10.60. Found: C, 74.97; H, 10.70. ^{13}C-NMR (pyridine-d_5): Table I. The aqueous layer was neutralized with solid $BaCO_3$, the filtrate was evaporated *in vacuo* and checked on TLC with a mixture of 9 ml of

C–M–W (7:3:1 lower phase) and 1 ml of AcOH.

Acetylation of 4 A solution of **4** (30 mg) in 3 ml Ac_2O–pyridine (2:1) was kept at room temperature for 2 d. The reaction mixture was poured into ice water and the precipitate was collected by filtration to afford the peracetate, **4**-A, a white powder, $[\alpha]_D^{22}$ −27.0° (c=0.06, MeOH). IR ν_{max}^{film} cm^{-1}: no OH, 1750 (C=O), 1225 (C–O), 980, 920<900, 840 (25*R* spirostanol). EI-MS *m/z*: 561 [(Rha−Hex-)Ac_6]$^+$, 331 [(terminal Hex-)Ac_4]$^+$, 273 [(terminal Rha-)Ac_3]$^+$.

Partial Acid Hydrolysis of 4 Compound **4** (1 g) was suspended in 0.5 N HCl–50% EtOH and was heated at 85 °C for 20 min. Partition of the reaction mixture was attained between $CHCl_3$ and water, followed by water-saturated 1-BuOH and water. The $CHCl_3$ layer and 1-BuOH layer were combined, evaporated *in vacuo*, and chromatographed on Si gel with C–M–W (7:3:1 lower phase) to afford **4a** (10.5 mg), **4b** (17.2 mg), **4c** (20.1 mg) and **4d** (154 mg). Compounds **4a**, **4b** and **4c** were identified with **1a**, **1b** and **1c**, respectively by ^{13}C-NMR. **4d**, ^{13}C-NMR (pyridine-d_5): Tables I and II. **4d**-A (peracetate), EI-MS *m/z*: 331 [(terminal Hex-)Ac_4]$^+$.

Dongnoside A (5) A white powder (mp 265—270 °C), $[\alpha]_D^{26}$ −51.5° (c=0.07, pyridine). IR ν_{mac}^{KBr} cm^{-1}: 3450—3470, 1060—1080 (–OH), 985, 920<900, 865, 815 (25*R* spirostanol). ^{1}H-NMR (pyridine-d_5) δ: 0.65 (3H, s, CH_3-18), 0.71 (3H, d, J=5.1 Hz, CH_3-27), 0.83 (3H, s, CH_3-19), 1.16 (3H, d, J=7.0 Hz, CH_3-21), 1.68 (3H, d, J=5.9 Hz, Rha CH_3), 3.51—3.62 (2H, m, H_2-26), 4.84 (1H, d, J=7.6 Hz), 5.06 (1H, d, J=7.3 Hz), 5.08 (1H, d, J=7.3 Hz), 5.16 (1H, d, J=7.6 Hz), 5.53 (1H, d, J=7.3 Hz), 5.76 (1H, br s). Negative FAB-MS *m/z*: 1341 [M−H]$^-$, 1209 [M−H−Pen]$^-$, 1195 [M−H−Rha]$^-$, 1047 [M−H−Hex−Pen]$^-$, 1033 [M−H−Hex−Rha]$^-$, 901 [M−H−Hex−Pen−Rha]$^-$, 739 [M−H-2×Hex−Pen−Rha]$^-$, 577 [M−H-3×Hex−Pen−Rha]$^-$, 413 [M−3H-4×Hex−Pen−Rha]$^-$. *Anal.* Calcd for $C_{62}H_{102}O_{31}\cdot 2H_2O$: C, 53.99; H, 7.69. Found: C, 53.96; H, 7.56. ^{13}C-NMR (pyridine-d_5): Tables I and II.

Acetylation of 5 A solution of compound **5** (30 mg) was acetylated in the same manner as **4** to afford the peracetate (**5**-A), a white powder, $[\alpha]_D^{22}$ −26.7° (c=0.09, MeOH). IR ν_{max}^{film} cm^{-1}: no OH, 1750 (C=O), 1225 (C–O), 980, 920<900, 840 (25*R* spirostanol). EI-MS *m/z*: 561 [(Rha−Hex-)Ac_6]$^+$, 547 [(Pen−Hex-)Ac_6]$^+$, 273 [(terminal Rha-)Ac_3]$^+$, 259 [(terminal Pen-)Ac_3]$^+$.

Enzymic Hydrolysis of 5 A mixture of compound **5** (128.6 mg) and hesperidinase (50 mg) (from Penicillium species, Sigma Chemical Co.) in citrate buffer (pH 5.0, 4 ml) and dimethyl sulfoxide (DMSO, 1 ml) was incubated at 37 °C for 3 d. The reaction mixture was evaporated to dryness and dissolved in $CHCl_3$ and MeOH. The filtrate was chromatographed over Si gel with C–M–W (7:3:0.2) to afford **5e** (53.3 mg). ^{1}H-NMR (pyridine-d_5) δ: 0.64 (3H, s, CH_3-18), 0.70 (3H, d, J=5.1 Hz, CH_3-27), 0.83 (3H, s, CH_3-19), 1.14 (3H, d, J=7.0 Hz, CH_3-21), 3.49—3.61 (2H, m, H_2-26), 4.88 (1H, d, J=7.7 Hz), 5.10 (1H, d, J=7.3 Hz), 5.14 (1H, d, J=7.7 Hz), 5.21 (1H, d, J=7.7 Hz), 5.57 (1H, d, J=7.3 Hz). ^{13}C-NMR (pyridine-d_5): Tables I and II. **5e**-A (peracetate of **5e**), EI-MS *m/z*: 331 [(terminal Hex-)Ac_4]$^+$, 259 [(terminal Pen-)Ac_3]$^+$.

Permethylation of 5, Subsequent Partial Hydrolysis and Acetylation A solution of glycoside **5** (142 mg) in DMSO (3 ml) was added to the preheated (70 °C, 30 min) mixture of DMSO (3 ml) and NaH (200 mg) and was kept at 70 °C for another 20 min. Then CH_3I (4 ml) was added and kept at room temperature for 3 h. To the reaction mixture, water and $CHCl_3$ were added and shaken. The organic layer was concentrated to give a resinous liquid, to which ether and water were added. The organic layer was evaporated under reduced pressure to afford a residue, which was purified by Si gel CC with *n*-hexane–acetone (Hex–Ace, 4:1) as a solvent to provide a permethyl ether (**5**-M, 56 mg). IR no OH. ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, s, CH_3-18), 0.79 (3H, d, J=6.2 Hz, CH_3-27), 0.82 (3H, s, CH_3-19), 0.96 (3H, d, J=7.0 Hz, CH_3-21), 3.35, 3.37, 3.40, 3.42, 3.44, 3.46, 3.47, 3.50, 3.52 (×2), 3.55, 3.56 (×2), 3.57, 3.59 (×2), 3.61 (16×CH_3), 4.30 (1H, d, J=7.7 Hz, Gal H-1), 4.39 (1H, ddd, J=7.4, 7.4, 7.4 Hz, H-16), 4.73 (1H, d, J=7.3 Hz, Glc H-1), 4.79 (1H, d, J=7.3 Hz, Glc″ H-1), 4.81 (1H, d, J=7.7 Hz, Glc′ H-1), 4.99 (1H, d, J=7.7 Hz, Xyl H-1), 5.02 (1H, s, Rha H-1).

The permethyl ether (**5**-M, 49.4 mg) was partially hydrolyzed with 0.5 N HCl–MeOH (1.5 ml) for 60 min on the water bath. The usual work-up of the reaction mixture and separation by Si gel CC using Hex–Ace (4:1) as an eluting solvent afforded three main compounds, which were subsequently acetylated with Ac_2O and pyridine to yield the corresponding acetates, **5**-M-1 (14.5 mg), **5**-M-2 (6.5 mg) and **5**-M-3 (5.2 mg).

5-M-1: A white powder, positive FAB-MS *m/z*: 1326 [M+Na+H]$^+$, 1302 [M]$^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, s, CH_3-18), 0.79 (3H, d, J=6.6 Hz, CH_3-27), 0.81 (3H, s, CH_3-19), 0.96 (3H, d, J=7.0 Hz, CH_3-21), 2.09, 2.11 (each 3H, s, 2×OAc), 4.38 (1H, ddd, J=7.5, 7.5, 7.5 Hz, H-16), 4.31 (1H, d, J=7.3 Hz, Gal H-1), 3.56 (1H, m, Gal H-2), 3.08 (1H, dd, J=3.1, 10.1 Hz, Gal H-3), 4.08 (1H, d, J=3.1, Gal H-4), 4.74 (1H, d, J=7.3 Hz, Glc H-1), 3.75 (1H, t, J=7.7 Hz, Glc H-2), 4.05 (1H, t, J=8.4 Hz, Glc H-3), 3.21 (1H, t, J=9.3 Hz, Glc H-4), 5.13 (1H, d, J=7.9 Hz, Glc′ H-1), 3.02 (1H, t, J=8.4 Hz, Glc′ H-2), 3.31 (1H, t, J=9.3 Hz, Glc′ H-3), 4.81 (1H, t, J=9.5 Hz, Glc′ H-4), 4.92 (1H, d, J=7.7 Hz, Glc″ H-1), 3.01 (1H, t, J=8.1 Hz, Glc″ H-2), 5.12 (1H, t, J=9.0 Hz, Glc″ H-3), 3.15 (1H, t, J=9.1 Hz, Glc″ H-4).

5-M-2: A white powder, positive FAB-MS *m/z*: 1458 [M+Na+H]$^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, s, CH_3-18), 0.79 (3H, d, J=6.2 Hz, CH_3-27), 0.82 (3H, s, CH_3-19), 0.96 (3H, d, J=6.6 Hz, CH_3-21), 2.11 (3H, s, OAc), 4.39 (1H, ddd, J=7.7, 7.7, 7.7 Hz, H-16), 4.31 (1H, d, J=7.7 Hz, Gal H-1), 3.53 (1H, m, Gal H-2), 3.08 (1H, dd, J=3.7, 9.2 Hz, Gal H-3), 4.06 (1H, d, J=3.1, Gal H-4), 4.73 (1H, d, J=7.3 Hz, Glc H-1), 3.73 (1H, t, J=8.1 Hz, Glc H-2), 4.04 (1H, t, J=8.4 Hz, Glc H-3), 3.25 (1H, t, J=9.3 Hz, Glc H-4), 4.92 (1H, d, J=7.7 Hz, Glc′ H-1), 3.01 (1H, t, J=9.2 Hz, Glc′ H-2), 3.25 (1H, t, J=9.3 Hz, Glc′ H-3), 4.83 (1H, t, J=9.5 Hz, Glc′ H-4), 4.75 (1H, d, J=7.3 Hz, Glc″ H-1), 2.94 (1H, t, J=8.1 Hz, Glc″ H-2), 3.14 (1H, t, J=8.1 Hz, Glc″ H-3), 3.25 (1H, t, J=9.3 Hz, Glc″ H-4), 5.01 (1H, d, J=7.7 Hz, Xyl H-1), 3.08 (1H, t, J=9.3 Hz, Xyl H-2), 3.78 (1H, t, J=9.2 Hz, Xyl H-3), 3.05 (1H, m, Xyl H-4).

5-M-3: A white powder, positive FAB-MS *m/z*: 1472 [M+Na+H]$^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, s, CH_3-18), 0.79 (3H, d, J=6.2 Hz, CH_3-27), 0.81 (3H, s, CH_3-19), 0.96 (3H, d, J=7.0 Hz, CH_3-21), 1.29 (3H, d, J=6.2 Hz, Rha CH_3), 4.31 (1H, d, J=7.7 Hz, Gal H-1), 4.79 (1H, d, J=7.0 Hz, Glc H-1), 3.77 (1H, t, J=7.3 Hz, Glc H-2), 4.01 (1H, t, J=8.4 Hz, Glc H-3), 3.20 (1H, t, J=9.2 Hz, Glc H-4), 5.10 (1H, d, J=7.8 Hz, Glc′ H-1), 4.81 (1H, d, J=7.0 Hz, Glc″ H-1), 3.04 (1H, t, J=8.1 Hz, Glc″ H-2), 5.11 (1H, t, J=9.2 Hz, Glc″ H-3), 3.21 (1H, t, J=9.3 Hz, Glc″ H-4), 5.01 (1H, br s, Rha H-1).

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References and Notes

1) Y. Ding, Y. Y. Chen, D. Z. Wang and C. R. Yang, *Phytochemistry*, **28**, 2787 (1989).
2) It could be regarded that the coexisting furostanol glycosides were changed into the corresponding spirostanol glycosides by fermentation for about one week.
3) S. Kiyosawa, M. Hutoh, T. Komori, T. Nohara, I. Hosokawa and T. Kawasaki, *Chem. Pharm. Bull.*, **16**, 1162 (1968).
4) *a*) M. E. Wall, C. R. Eddy, M. L. McClennan and M. E. Klumpp, *Anal. Chem.*, **24**, 1337 (1952); *b*) C. R. Eddy, M. E. Wall and M. K. Scott, *ibid.*, **25**, 266 (1953); *c*) E. C. Rothman, M. E. Wall and C. R. Eddy, *J. Am. Chem. Soc.*, **74**, 4013 (1952).
5) H. Budzikiewicz, C. Djerassi and D. H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day Inc., San Francisco, 1964, p. 113.
6) P. K. Agrawal, D. C. Jain, R. K. Gupta and R. S. Thakur, *Phytochemistry*, **24**, 2479 (1985).
7) S. Hakomori, *J. Biol. Chem.*, **55**, 205 (1964).