Comparative Studies on the Constituents of Ophiopogonis Tuber and Its Congeners. VI.[1a] Studies on the Constituents of the Subterranean Part of *Liriope spicata* var. *prolifera* and *L. muscari*. (1)

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Seven steroidal glycosides, tentatively named glycosides Ls-1, Ls-2 (1), Ls-3 (2), Ls-4 (3), Ls-5 (4), Ls-6 (5) and Ls-7 (6) were isolated from the subterranean part of *Liriope spicata* (THUMB.) LOUR. var. *prolifera* Y. J. MA (Liliaceae) and three steroidal glycosides, tentatively named glycosides Lm-1, Lm-2 (7), Lm-3 (8) were isoalted from *L. muscari* (DECN.) BAILEY (Liliaceae). Glycosides Ls-1 and Lm-1 were identified as so-called β-sitosterol β-D-glucopyranoside. The structures of eight glycosides (1—8) were established as (25*S*)-ruscogenin 1-*O*-β-D-fucopyranosido-3-*O*-α-L-rhamnopyranoside (1) (=glycoside B from *L. platyphylla* WANG. *et* TANG.), (25*S*)-ruscogenin 1-*O*-β-D-xylopyranosido-3-*O*-α-L-rhamnopyranoside (2), (25*S*)-ruscogenin 1-*O*-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranoside (3), (25*S*)-ruscogenin 1-*O*-[(2-*O*-acetyl)-α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-fucopyranoside (4), (25*S*)-ruscogenin 1-*O*-[(3-*O*-acetyl)-α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-fucopyranoside (5), yamogenin 3-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-glucopyranoside (6), ruscogenin 1-*O*-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside (7) and ruscogenin 1-*O*-[β-D-glucopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-fucopyranoside (8), respectively.

Keywords *Liriope spicata* var. *prolifera*; *Liriope muscari*; Liriaceae; Ophiopogonis Tuber; steroidal glycoside, ruscogenin; yamogenin

In the preceding papers[1] we reported the isolation and structural elucidation of the constituents of Ophiopogonis Tuber and its congeners, namely *O. planiscapus* NAKAI, *O. ohwii* OKUYAMA, *O. jaburan* (KUNTH) LODD., *O. chekiangensis* K. KIMURA *et* H. MIGO, and *Liriope platyphylla* WANG *et* TANG. The present paper deals mainly with the isolation and structural elucidation of steroidal glycosides of the subterranean part of *Liriope spicata* (THUMB.) LOUR. var. *prolifera* Y. J. MA (Chinese name: Hubei maidong) harvested in Hubei province, China, and the tuber of *L. muscari* (DECN.) BAILEY (Chinese name: Duanting shan maidang) harvested in Fujian province, China.

The dried subterranean part of *L. spicata* var. *prolifera* and the dried tuber of *L. muscari* were individually extracted with hot methanol and both methanol extacts were treated by the method described in the experimental section. Seven steroidal glycosides, tentatively named Ls-1, Ls-2 (**1**), Ls-3 (**2**), Ls-4 (**3**), Ls-5 (**4**), Ls-6 (**5**) and Ls-7 (**6**) were isolated from the former, and three steroidal glycosides, tentatively named glycosides Lm-1, Lm-2 (**7**), Lm-3 (**8**) were isolated from the latter.

Glycosides Ls-1 and Lm-1 gave glucose and a mixture of β-sitosterol, campesterol and stigmasterol on acid hydrolysis. Therefore, glycosides Ls-1 and Lm-1 were concluded to be a mixture of glucopyranosides of β-sitosterol, campesterol and stigmasterol.

Glycoside Ls-2 (**1**) is positive in the Liebermann–Burchard reaction. It shows a strong absorption band of hydroxyl groups and characteristic absorption bands of a (25*S*)-spiroketal moiety in the infrared (IR) spectrum.[2] On hydrolysis with 2N hydrochloric acid in 50% dioxane, **1** gave D-fucose, L-rhamnose and an aglycone. The absolute configuration of these sugars were determined using the method of Oshima *et al.*[3] The aglycone of **1** was acetylated with acetic anhydride and pyridine to afford ruscogenin diacetate and (25*S*)-ruscogenin diacetate. The epimerisation of (25*S*)-spirostanol to (25*R*)-spirostanol under acidic conditions is well known,[4] therefore, from its chemical and physical properties, the structure of **1** was suggested to be identical with glycoside B from *L. platyphylla* WANG. *et* TANG.[1b] Finally, glycoside Ls-2 was identified as glycoside B (=(25*S*)-ruscogenin 1-*O*-β-D-fucopyranosido-3-*O*-α-L-rhamnopyranoside) by comparisons of thin-layer chromatographic (TLC) behavior, melting point, IR and ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectra with an authentic sample.

Glycosides Ls-3 (**2**) $C_{38}H_{60}O_{12}\cdot 3/2H_2O$ and Ls-4 (**3**) $C_{38}H_{60}O_{12}\cdot 2H_2O$ are positive in the Liebermann–Burchard reaction, and they showed strong absorption bands of hydroxyl groups and characteristic absorption bands of a (25*S*)-spiroketal moiety in the IR spectrum. On hydrolysis with 2N hydrochloric acid in 50% dioxane, **2** and **3** gave D-xylose, L-rhamnose and the same aglycone, which was acetylated with acetic anhydride and pyridine to afford ruscogenin diacetate and (25*S*)-ruscogenin diacetate. The location of xylose and rhamnose linkages of **3** were proven as follows. Methylation of **3** by Hakomori's method[5] afforded a hexa-*O*-methyl derivative, which was methanolyzed to give methyl 3,4-di-*O*-methylxylopyranoside, per-*O*-methylrhamnopyranoside and 3-*O*-methylated aglycone. Based on the above result and $J_{C_1-H_1}$ coupling constants of anomeric carbon signals, the structure of **3** was deduced to be (25*S*)-ruscogenin 1-*O*-α-L-rhamnopyranosyl(1→2)-β-D-xylopyranoside. On partial hydrolyses, **2** and **3** afforded the same prosapogenin, which was deduced to be (25*S*)-ruscogenin 1-*O*-β-D-xylopyranoside. In the ^{13}C-NMR spectra of **2**, the glycosilation shifts on C_1 and C_3 carbon signals of aglycone[1b] were observed, so that the sugar moieties were linked both to the C_1 and C_3 hydroxyl groups of (25*S*)-ruscogenin. Based on the $J_{C_1-H_1}$ coupling constants of anomeric carbon signals, the structure of **2** was established to be (25*S*)-ruscogenin 1-*O*-β-D-xylopyranosido-3-*O*-α-L-rhamnopyranoside.

Glycosides Ls-5 (**4**) $C_{46}H_{72}O_{17}\cdot 3H_2O$ and Ls-6 (**5**), $C_{46}H_{72}O_{17}\cdot 4H_2O$ are positive in the Liebermann–Burchard reaction. Both compounds showed strong

absorption bands of hydroxyl groups, an esteric group, and the characteristic (25*S*)-spiroketal moiety in the IR spectrum. On hydrolysis with 2 N hydrochloric acid in 50% dioxane, **4** and **5** gave D-fucose, L-rhamnose, D-xylose and the same aglycone, which was acetylated with acetic anhydride and pyridine to afford ruscogenin diacetate and (25*S*)-ruscogenin diacetate. Each ^{13}C-NMR spectrum of **4** and **5** shows an acetyl group signal at δ 21.7 and 170.7 ppm. On treatment with alkaline, **4** and **5** afforded the common deacetylated derivative, which shows the same *Rf* value with the deacetylated product of glycoside B obtained from *Ophiopogon chekiangensis*.[1a] Based on the comparative analysis of the ^{13}C-NMR spectra, the structure of the sugar moiety of **4** is deduced to be identical with that of glycoside B *i.e.* [(2-*O*-acetyl)-α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-fucopyranose, while the structure of the oligosaccharide moiety of **5** was suggested to be [(3-*O*-acetyl)-α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-fucopyranose. The acyl migration between C-2 and C-3 hydroxyl groups of rhamnopyranoside is well known, therefore, the relationship between acetyl groups of **4** and **5** were proven by the next reaction. Each solution of **4** and **5** in pyridine was heated at 80 °C for 70 h[6] and the reaction mixture was examined by TLC. Each reaction mixture was confirmed to be the mixture of nearly equal amounts of **4** and **5**. Consequently, the structures of **4** and **5** were established to be (25*S*)-ruscogenin 1-*O*-[(2-*O*-acetyl)-α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-fucopyranoside and (25*S*)-ruscogenin 1-*O*-[(3-*O*-acetyl)-α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-fucopyranoside, respectively.

Glycoside Ls-7 (**6**), $C_{44}H_{70}O_{16}\cdot H_2O$ is positive in the Liebermann–Burchard reaction, and it shows a strong absorption band of hydroxyl groups and the characteristic (25*S*)-spiroketal moiety in the IR spectrum. On acidic hydrolysis **6** gave L-rhamnose, D-glucose, D-xylose and an aglycone, which was acetylated with acetic anhydride and pyridine to afford diosgenin acetate and yamogenin acetate.

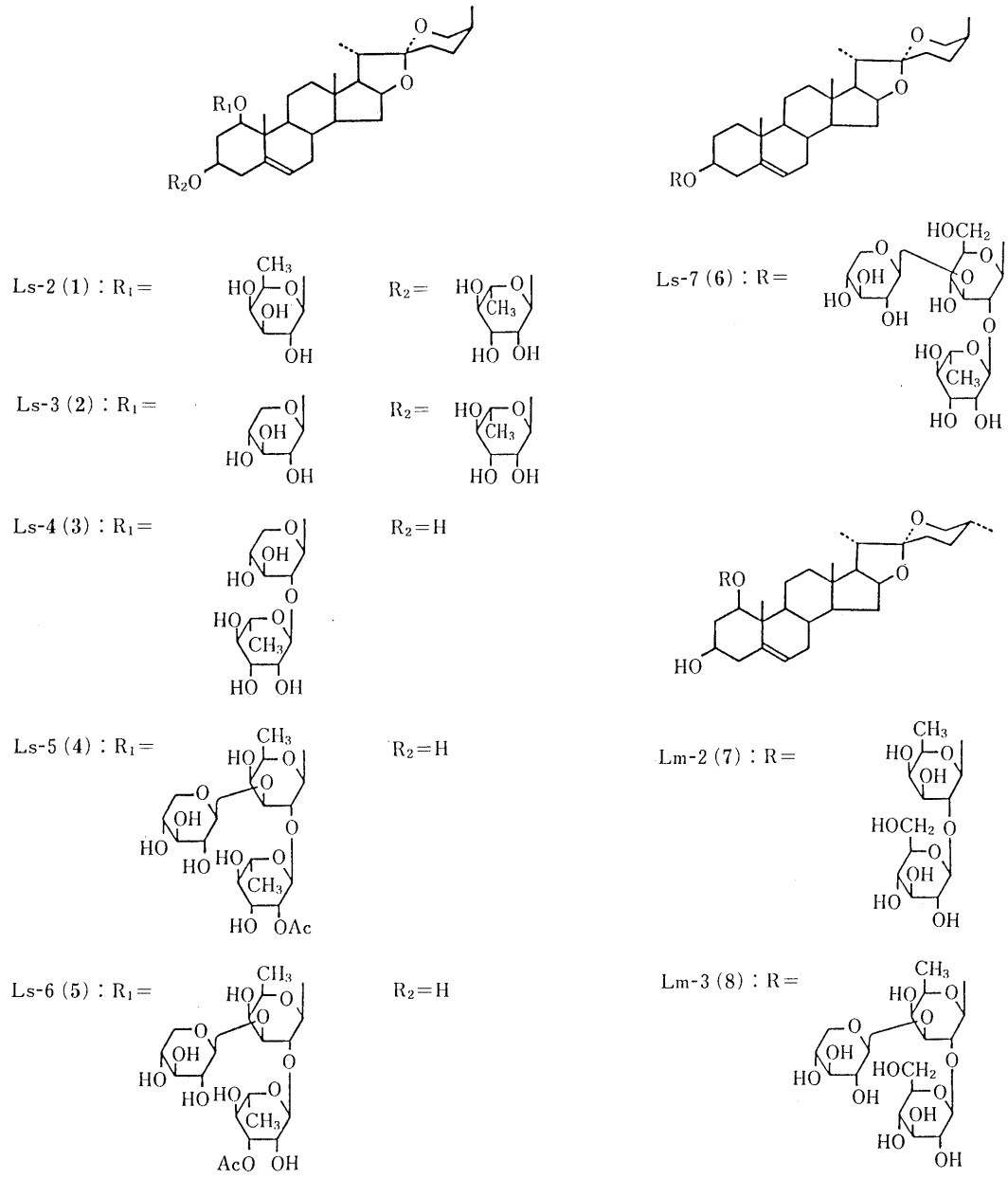

Chart 1

Based on the comparative analysis of the ^{13}C-NMR spectra, the aglycone was concluded to be yamogenin and the sugar moiety of **6** is the same as that of Glycoside E from *L. platyphylla* WANG *et* TANG.[1b] To confirm the sugar sequences, **6** was methylated by Hakomori's method and the octa-*O*-methyl derivative of **6** was methanolyzed to afford per-*O*-methylrhamnopyranoside, per-*O*-methylxylopyranoside and methyl 4,6-di-*O*-methylrhamnopyranoside. Based on the $J_{C_1-H_1}$ coupling constants of anomeric carbon signals, the structure of **6** was established to be yamogenin 3-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-glucopyranoside.

Glycoside Lm-2 (**7**), $C_{39}H_{62}O_{14}\cdot 2H_2O$ is positive in the Liebermann–Burchard reaction, and it shows a strong absorption band of hydroxyl groups and characteristic absorption bands of a (25*R*)-spiroketal moiety in the IR spectrum.[2] On acidic hydrolysis, **7** gave D-fucose, D-glucose and ruscogenin. Methylation of **7** by Hakomori's method afforded a hepta-*O*-methyl derivative, which was methanolyzed to give ruscogenin 3-*O*-methyl ether, per-*O*-methylglucopyranoside and methyl 3,4-di-*O*-methylfucopyranoside. Based on the $J_{C_1-H_1}$ coupling constants of anomeric carbon signals, the structure of **7** was established to be ruscogenin 1-*O*-β-D-glucopyranosyl(1→2)-β-D-fucopyranoside.

Glycoside Lm-3 (**8**), $C_{44}H_{70}O_{17}\cdot 3/2H_2O$ is positive in the Liebermann–Burchard reaction, and it shows a strong absorption band of hydroxyl groups and characteristic absorption bands of a (25*R*)-spiroketal moiety in the IR spectrum. On acidic hydrolysis **8** gave D-fucose, D-glucose, D-xylose and ruscogenin. Methylation of **8** by Hakomori's method afforded a nona-*O*-methyl derivative (**12**), which was methanolyzed to give ruscogenin 3-*O*-methyl ether, per-*O*-methylglucopyranoside, per-*O*-methylxylopyranoside and methyl 4-*O*-methylfucopyranoside. Partial hydrolysis of **8** afforded a prosapogenin, which was methylated by Hakomori's method. The resulting hexa-*O*-methyl derivative was methanolyzed to give ruscogenin 3-*O*-methyl ether, per-*O*-methylxylopyranoside and methyl 2,4-di-*O*-methylfucopyranoside. Consequently, the sugar moiety of **8** was concluded to be [β-D-glucopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-fucopyranose. Based on the ^{1}H- and ^{13}C-NMR spectra of **8** and **12**, the structure of **8** was established to be ruscogenin 1-*O*-[β-D-glucopyranosyl-(1→2)][β-D-xylopyranosyl(1→3)]-β-D-fucopyranoside.

It is very interesting that *Liriope platyphylla* contains both (25*R*)- and (25*S*)-spirostanol glycosides, while *L. spicata* var. *prolifera* contains only (25*S*)-spirostanol glycosides and *L. muscari* contains only (25*R*)-spirostanol glycosides. In this paper we have described the isolation and structural elucidation of four new steroidal glycosides of *L. spicata* var. *prolifera* and two new steroidal glycosides of *L. muscari*.

In China, *L. spicata* var. *prolifera* and *L. muscari* are sometimes used as the original plants of Ophiopogonis Tuber, and it would be of interest to compare the pharmacological activity of these crude drugs.

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus (hot-stage type) and are uncorrected. The optical rotations were measured with a JASCO DIP-140 polarimeter at 19 °C. The IR spectra were recorded with a Hitachi EPI-2 and the NMR spectra were recorded with a JEOL GX-400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. Gas-liquid chromatography (GLC) was run on a Shimadzu GC-6A unit equipped with a flame ionization detector. Experimental conditions (a) for sugars: column, 5% SE-52 on Chromosorb W 3 mm × 2 m; column temp., 180 °C; injection temp., 200 °C; carrier gas N_2, 1.0 kg/cm^2; samples, trimethylsilyl (TMS) ether, (b) for *O*-methylated sugars: column, 5% neopentylglycol succinate (NPGS) on Shimalite 3 mm × 2 m; column temp., 145 °C; injection temp., 165 °C; carrier gas N_2, 1.0 kg/cm^2; (c) for *O*-acetylated, *O*-methylated sugars: column, 5% NPGS on Shimalite 3 mm × 2 m; column temp., 180 °C; injection temp., 200 °C; carrier gas N_2, 1.5 kg/cm^2. TLC was performed on precoated Kiesel gel 60 F_{254} plates (Merck) using the following solvents. (a) hexane–acetone (2 : 1, v/v), (b) $CHCl_3$–MeOH–H_2O (80 : 20 : 2, v/v), (c) $CHCl_3$–MeOH–AcOEt–H_2O (2 : 2 : 4 : 1, v/v, lower phase). Detection was achieved by spraying 10% H_2SO_4 followed by heating.

Extraction and Isolation of Glycosides i) The dried tubers of *Liriope spicata* (THUMB.) LOUR. var. *prolifera* Y. J. MA (10 kg) harvested in Xiangyang country, Hubei province, China, were crushed and extracted with hot MeOH (12 l × 3). The extract was combined and evaporated to dryness *in vacuo*. The residue (1 kg) was dissolved in water and extracted with ether to afford the ether-soluble fraction (5 g). The aqueous layer was extracted with BuOH saturated with water, and the BuOH-soluble fraction was concentrated *in vacuo* to afford a brown residue (53 g), which was subjected to column chromatography on Sephadex LH-20 with MeOH, to afford three fractions (frs. I—III). Fraction I (21.4 g) was subjected to column chromatography on Avicel with $CHCl_3$–MeOH–H_2O (8 : 1 : 1, v/v, lower phase), $CHCl_3$–MeOH–H_2O (7 : 2 : 1, v/v, lower phase), and then MeOH, to give three fractions (frs. A—C). Fraction A (6 g) was rechromatographed on silica gel with $CHCl_3$–MeOH–H_2O (85 : 15 : 1, v/v) to obtain ten fractions (frs. 1—10). Fraction 2 (0.4 g) was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–AcOEt–H_2O (10 : 7 : 20 : 5, v/v, lower phase) to afford Ls-1 (60 mg). Fractions 6 and 7 were individually subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (80 : 20 : 2, v/v) to afford Ls-2 (**1**, 100 mg) from the former, and Ls-3 (**2**, 25 mg) from the latter.

ii) The dried thin roots of *L. spicata* var. *prolifera* (10 kg) were treated by the same way as described above to afford MeOH extract (1.3 kg), ether extract (60.5 g) and BuOH extract (168.9 g). The BuOH extract was subjected to column chromatography on Sephadex LH-20 with MeOH to afford five fractions (frs. i—v). Fraction ii (99.0 g) was subjected to column chromatography on Avicel with $CHCl_3$, $CHCl_3$–MeOH–H_2O (8 : 1 : 1, v/v, lower phase) and then MeOH, to afford three fractions (frs. a—c). Fraction b (35.0 g) was rechromatographed on silica gel with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2, v/v) to afford Ls-2 (**1**, 500 mg), Ls-3 (**2**, 50 mg), Ls-4 (**3**, 200 mg), Ls-5 (**4**, 30 mg), Ls-6 (**5**, 20 mg) and Ls-7 (**6**, 300 mg).

iii) The dried tubers of *L. muscari* (DECN.) BAILEY (10 kg) harvested in Xiangyou country, Fujian province, China, were treated by the same way as described above to afford MeOH extract (925 g), ether extract (4.2 g) and BuOH extract (39.5 g). The BuOH extract was subjected to column chromatography on Sephadex LH-20 with MeOH to afford three fractions (frs. I—III). Fraction II (24.1 g) was subjected to column chromatography on Avicel with $CHCl_3$, $CHCl_3$–MeOH–H_2O (8 : 1 : 1, v/v, lower phase) and finally MeOH was separated into three fractions (frs. A—C). Fraction A (12.9 g) was rechromatographed on silica gel with $CHCl_3$–MeOH–H_2O (9 : 1 : 0.1, v/v) to afford Lm-1 (100 mg). Fraction B (4.9 g) was rechromatographed on silica gel with $CHCl_3$–MeOH–H_2O (85 : 15 : 1, v/v) to afford five fractions (frs. 1—5). Fraction 2 was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (9 : 1 : 0.1, v/v) to afford Lm-2 (**7**, 200 mg). Fraction 4 was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (85 : 15 : 1, v/v) to afford Lm-3 (**8**, 1.00 g).

Properties of Glycosides Ls-1 and Lm-1 (So Called β-Sitosterol β-D-Glucopyranoside) Ls-1 and Lm-1, colorless needles from MeOH, mp 292—295 °C (dec.), were individually hydrolyzed with 2 N HCl and examined by GLC. aglycone; column, 5% OV-17 on Uniport K S, 3 mm × 2 m; column temp., 260 °C; injection temp., 280 °C; N_2 1.0 kg/cm^2. t_R (min) 18.4 (β-sitosterol), 16.6 (stigmasterol), 15.0 (campesterol). Each ratio of β-sitosterol, stigmasterol and campesterol was calculated to be about 7 : 2 : 1 for Ls-1 and 7 : 2 : 1 for Lm-1. sugar; condition a: t_R (min); 7.2, 10.8 (glucose).

Properties of Ls-2 (1), Ls-3 (2), Ls-4 (3), Ls-5 (4), Ls-6 (5), Ls-7 (6), Lm-2 (7) and Lm-3 (8) Ls-2 (**1**): Colorless needles from aqueous EtOH, mp 228—232 °C (dec.), $[\alpha]_D$ −104.7° (c = 0.60, pyridine), (lit. mp 225—227 °C, $[\alpha]_D$ −103.8°[1b]). Ls-3 (**2**): Colorless needles from aqueous EtOH, mp 265—267 °C (dec.), $[\alpha]_D$ −98.0° (c = 0.45, pyridine). IR

ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 982, 920, 902, 865 (intensity 920 > 902, (25*S*)spiroketal). ^{13}C-NMR (C_5D_5N) δ: aglycone ((25*S*)-ruscogenin) 83.2 (C_1), 35.8 (C_2), 73.9 (C_3), 39.9 (C_4), 138.3 (C_5), 125.6 (C_6), 33.7 (C_7), 32.5 (C_8), 51.0 (C_9), 43.5 (C_{10}), 24.6 (C_{11}), 41.0 (C_{12}), 40.8 (C_{13}), 57.3 (C_{14}), 32.9 (C_{15}), 81.5 (C_{16}), 63.5 (C_{17}), 17.3 (C_{18}), 15.1 (C_{19}), 43.1 (C_{20}), 15.4 (C_{21}), 109.9 (C_{22}), 27.1 (C_{23}), 26.8 (C_{24}), 28.2 (C_{25}), 65.5 (C_{26}), 16.9 (C_{27}); xylose (→^{1}aglycone) 102.4 ($J_{C_1-H_1}$ = 159 Hz; C_1), 75.6 (C_2), 79.0 (C_3), 71.6 (C_4), 67.8 (C_5); rhamnose (→^{3}aglycone) 100.1 ($J_{C_1-H_1}$ = 167 Hz; C_1), 73.14 (C_2), 73.10 (C_3), 74.5 (C_4), 70.3 (C_5), 19.1 (C_6). *Anal.* Calcd for $C_{38}H_{60}O_{12}\cdot 3/2H_2O$: C, 62.02; H, 8.63. Found: C, 62.26; H, 8.58. Ls-4 (**3**): Colorless needles from aqueous EtOH, mp 200—202 °C (dec.), $[\alpha]_D$ −84.2° (*c* = 0.71, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 980, 920, 902, 865 (intensity 920 > 902, (25*S*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: xylose (→^{1}aglycone) 101.7 ($J_{C_1-H_1}$ = 160 Hz; C_1), 80.3 (C_2), 76.9 (C_3), 71.8 (C_4), 67.6 (C_5); rhamnose (→^{2}xylose) 100.7 ($J_{C_1-H_1}$ = 168 Hz; C_1), 72.9 (C_2), 72.8 (C_3), 74.5 (C_4), 69.8 (C_5), 19.5 (C_6). *Anal.* Calcd for $C_{38}H_{60}O_{12}\cdot 2H_2O$: C, 61.27; H, 8.66. Found: C, 61.43; H, 8.57. Ls-5 (**4**): Colorless needles from aqueous MeOH, mp 209—211 °C (dec.), $[\alpha]_D$ −93.0° (*c* = 0.25, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1732 (ester), 980, 921, 902, 864 (intensity 921 > 902, (25*S*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: fucose (→^{1}aglycone) 100.4 (C_1), 73.0 (C_2), 85.3 (C_3), 72.7 (C_4), 71.4 (C_5), 17.6 (C_6); rhamnose (→^{2}fucose) 98.6 (C_1), 74.2 (C_2), 70.9 (C_3), 74.6 (C_4), 69.6 (C_5), 19.4 (C_6); xylose (→^{3}fucose) 106.0 (C_1), 75.1 (C_2), 78.3 (C_3), 71.0 (C_4), 67.4 (C_5). OAc (→^{2}rhamnose) 21.7, 170.7. *Anal.* Calcd for $C_{46}H_{72}O_{17}\cdot 3H_2O$: C, 58.03; H, 8.27. Found: C, 58.35; H, 8.03. Ls-6 (**5**): Colorless needles from aqueous EtOH, mp 181—184 °C (dec.), $[\alpha]_D$ −93.4° (*c* = 0.41, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1728 (ester), 980, 920, 902, 865 (intensity 920 > 902, (25*S*)-spiroketal), ^{13}C-NMR (C_5D_5N) δ: fucose (→^{1}aglycone) 100.5 (C_1), 74.1 (C_2), 85.8 (C_3), 73.0 (C_4), 71.4 (C_5), 17.6 (C_6); rhamnose (→^{2}fucose) 101.8 (C_1), 70.4 (C_2), 76.8 (C_3), 71.5 (C_4), 69.9 (C_5), 19.6 (C_6); xylose (→^{3}fucose) 106.5 (C_1), 75.1 (C_2), 78.6 (C_3), 71.1 (C_4), 67.4 (C_5). OAc (→^{3}rhamnose) 21.7, 170.7. *Anal.* Calcd for $C_{46}H_{72}O_{17}\cdot 4H_2O$: C, 57.01; H, 8.34. Found: C, 57.14; H, 7.87. Ls-7 (**6**): Colorless needles from aqueous MeOH, mp 279—282 °C (dec.), $[\alpha]_D$ −54.4° (*c* = 0.23, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 980, 920, 902, 860 (intensity 920 > 902, (25*S*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: aglycone: 38.1 (C_1), 30.7 (C_2), 78.8 (C_3), 39.7 (C_4), 141.0 (C_5), 121.8 (C_6), 32.9 (C_7), 32.3 (C_8), 50.9 (C_9), 37.7 (C_{10}), 21.7 (C_{11}), 40.4 (C_{12}), 41.0 (C_{13}), 57.2 (C_{14}), 32.7 (C_{15}), 81.5 (C_{16}), 63.2 (C_{17}), 16.9 (C_{18}), 20.0 (C_{19}), 43.0 (C_{20}), 15.4 (C_{21}), 109.9 (C_{22}), 28.1 (C_{23}), 27.1 (C_{24}), 26.8 (C_{25}), 65.5 (C_{26}), 16.9 (C_{27}); glucose (→^{3}aglycone) 100.5 ($J_{C_1-H_1}$ = 155 Hz, C_1), 77.6 (C_2), 82.1 (C_3), 71.1 (C_4), 78.8 (C_5), 62.4 (C_6); rhamnose (→^{2}glucose) 102.0 ($J_{C_1-H_1}$ = 167 Hz; C_1), 74.5 (C_2), 73.1 (C_3), 75.2 (C_4), 69.7 (C_5), 19.1 (C_6); xylose (→^{3}glucose) 105.9 ($J_{C_1-H_1}$ = 159 Hz; C_1), 76.5 (C_2), 78.0 (C_3), 72.7 (C_4), 67.7 (C_5). *Anal.* Calcd for $C_{44}H_{70}O_{16}\cdot H_2O$: C, 60.53; H, 8.31. Found: C, 60.37; H, 8.25. Lm-2 (**7**): Colorless needles from aqueous MeOH, mp 194—196 °C (dec.), $[\alpha]_D$ −93.6° (*c* = 0.59, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 982, 920, 902, 865 (intensity 920 < 902, (25*R*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: aglycone (ruscogenin) 83.5 (C_1), 37.9 (C_2), 68.7 (C_3), 44.0 (C_4), 139.9 (C_5), 124.4 (C_6), 33.6 (C_7), 32.6 (C_8), 51.0 (C_9), 43.5 (C_{10}), 24.3 (C_{11}), 41.0 (C_{12}), 40.8 (C_{13}), 57.5 (C_{14}), 33.0 (C_{15}), 81.5 (C_{16}), 63.6 (C_{17}), 17.3 (C_{18}), 15.5 (C_{19}), 42.6 (C_{20}), 15.7 (C_{21}), 109.4 (C_{22}), 32.4 (C_{23}), 29.9 (C_{24}), 31.2 (C_{25}), 67.3 (C_{26}), 17.7 (C_{27}); fucose (→^{1}aglycone) 100.1 ($J_{C_1-H_1}$ = 155 Hz; C_1), 82.6 (C_2), 75.3 (C_3), 72.4 (C_4), 71.3 (C_5), 17.7 (C_6); glucose (→^{2}fucose) 106.5 ($J_{C_1-H_1}$ = 156 Hz; C_1), 77.0 (C_2), 78.8 (C_3), 72.1 (C_4), 78.3 (C_5), 63.3 (C_6). *Anal.* Calcd for $C_{39}H_{62}O_{14}\cdot 2H_2O$: C, 59.22; H, 8.41. Found: C, 59.48; H, 8.51. Lm-3 (**8**): Colorless needles from aqueous EtOH, mp 292—295 °C (dec.), $[\alpha]_D$ −90.8° (*c* = 0.63, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 982, 920, 902, 865 (intensity 920 < 902, (25*R*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: fucose (→^{1}aglycone) 100.5 ($J_{C_1-H_1}$ = 155 Hz; C_1), 78.2 (C_2), 83.3 (C_3), 72.5 (C_4), 71.3 (C_5), 17.6 (C_6); glucose (→^{2}fucose) 106.3 ($J_{C_1-H_1}$ = 158 Hz, C_1), 76.6 (C_2), 78.8 (C_3), 72.5 (C_4), 78.4 (C_5), 63.8 (C_6); xylose (→^{3}fucose) 104.9 ($J_{C_1-H_1}$ = 157 Hz; C_1), 75.4 (C_2), 78.7 (C_3), 71.0 (C_4), 67.5 (C_5). *Anal.* Calcd for $C_{44}H_{70}O_{17}\cdot 3/2H_2O$: C, 58.84; H, 8.19. Found: C, 58.73; H, 8.48.

Hydrolysis of 2—8 with 2 N HCl Each solution of **1** (3 mg), **2** (3 mg), **3** (3 mg), **4** (5 mg), **5** (5 mg), **6** (5 mg), **7** (25 mg) and **8** (25 mg) in 2 N HCl–50% dioxane was refluxed for 3 h. The reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water and dried over Na_2SO_4. The $CHCl_3$ solution was filtered and the filtrate was evaporated to dryness. Aglycones: Each residue of **1**—**8** was examined by TLC (solvent: hexane–acetone = 2 : 1, v/v). The hydrolysate from **1**—**5**, **7** and **8** showed the same *Rf* values as (25*S*)-ruscogenin and ruscogenin (*Rf* 0.31), while the residue from **6** showed the same *Rf* values as diosgenin and yamogenin (*Rf* 0.49). Furthermore, each aglycone was acetylated with Ac_2O–pyridine. After the reaction mixture was treated in the usual way, each product was compared with ruscogenin diacetate, (25*S*)-ruscogenin diacetate, yamogenin acetate and diosgenin acetate by TLC (solvent CH_2Cl_2). Each acetylated aglycone of **1**—**5** showed the same *Rf* value as (25*S*)-ruscogenin diacetate (*Rf* 0.31) and ruscogenin diacetate (*Rf* 0.34), while the acetylated aglycones of **7** and **8** showed the same *Rf* values as ruscogenin diacetate (*Rf* 0.34) and that of **6** showed the same *Rf* values as yamogenin acetate (*Rf* 0.38) and diosgenin acetate (*Rf* 0.41). Sugars: Each aqueous layer was neutralized with $NaHCO_3$ and concentrated to dryness *in vacuo*, and the monosaccharides were examined by GLC. **1**: t_R (min) 2.8, 3.3 (fucose), 2.4, 3.2 (rhamnose). **2** and **3**: GLC t_R (min) 2.4, 3.2 (rhamnose), 3.2, 4.1 (xylose). **4** and **5**: t_R (min) 2.8, 3.3 (fucose), 2.4, 3.2 (rhamnose), 3.2, 4.1 (xylose). **6**: t_R (min) 2.4, 3.2 (rhamnose), 3.2, 4.1 (xylose), 7.2, 10.8 (glucose). **7**: t_R (min) 2.8, 3.3 (fucose), 7.2, 10.8 (glucose). **8**: t_R (min) 2.8, 3.3 (fucose), 3.2, 4.1 (xylose), 7.2, 10.8 (glucose).

Determination of Absolute Configurations of Sugars by High Performance Liquid Chromatography (HPLC) Each solution of **1** (1 mg), **2** (1 mg), **3** (1 mg), **4** (1 mg), **5** (1 mg), **6** (1 mg), **7** (1 mg) and **8** (1 mg) in 2 N HCl–50% dioxane (2 ml) was heated in a sealed tube for 3 h at 100 °C. The reaction mixture was diluted with water and evaporated to remove dioxane. The solution was neutralized with Amberlite IRA-93ZU (OH^- form) and passed through a SEP-PAK C_{18} cartridge to give a sugar fraction. Each component sugar in the solution was derivated to 1-(*N*-acetyl-L-α-methylbenzylamino)-1-deoxyalditol acetate using the method of Asada *et al.*[7] and analyzed by normal- and reversed-phase HPLC. Conditions of normal-phase HPLC: column, supermicro bead silica gel B-5, 5 μm (10 × 250 mm); solvent, hexane–EtOH (9 : 1); flow rate, 4 ml/min; detection, ultraviolet (UV) (230 nm). t_R (min) **1**: D-fucose 24.9, L-rhamnose 22.8. (reference: L-fucose 21.6, D-rhamnose 20.5). Conditions of reversed-phase HPLC: column, Chromatorex-ODS DU0015MT (10 × 250 mm); solvent, 40% CH_3CN; flow rate, 3 ml/min; detection, UV (230 nm). t_R (min) D-fucose 31.1, L-rhamnose 41.3. (reference: L-fucose 34.0, D-rhamnose 41.3). 1-(*N*-acetyl-L-α-methylbenzylamino)-1-deoxyalditol acetates were identified by direct comparison with authentic speciments. **2** and **3**: normal-phase HPLC t_R (min) L-rhamnose 22.8, D-xylose 37.7. (reference: L-xylose 34.9); reversed-phase HPLC t_R (min) L-rhamnose 41.3, D-xylose 27.6. (reference: L-xylose 26.7). **4** and **5**: normal-phase HPLC t_R (min) D-fucose 24.9, L-rhamnose 22.8, D-xylose 37.7; reversed-phase HPLC t_R (min) D-fucose 31.1, L-rhamnose 41.3, D-xylose 27.6. **6**: normal-phase HPLC t_R (min) L-rhamnose 22.8, D-xylose 34.9, D-glucose 37.7. (reference: L-glucose 36.0); reversed-phase HPLC t_R (min) L-rhamnose 41.3, D-xylose 37.7, D-glucose 37.7. (reference: L-glucose 36.0). **7**: normal-phase HPLC t_R (min) D-fucose 24.9, D-glucose 37.7; reversed-phase HPLC t_R (min) D-fucose 31.1, D-glucose 36.6. **8**: normal-phase HPLC t_R (min) D-fucose 24.9, D-xylose 37.7, D-glucose 37.7; reversed-phase HPLC t_R (min) D-fucose 31.1, D-xylose 27.6, D-glucose 36.6.

Methylation of 3, 6, 7 and 8 by Hakomori's Method According to Hakomori's method, NaH (100 mg) was defatted by anhydrous benzene and petroleum ether, then warmed with dimethylsulfoxide (DMSO 10 ml) at 70 °C in an oil bath for 1 h with stirring under N_2 flow. A solution of **3** (50 mg) in dimethylsulfoxide (DMSO 5 ml) was added and the mixture was stirred for 1 h under N_2 flow. CH_3I (2 ml) was added to the solution and the reaction mixture was allowed to stand at room temperature for 1 h with stirring. After dilution with water, the reaction mixture was extracted with $CHCl_3$ and the $CHCl_3$ layer was washed with water, dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed on silica gel and eluted with hexane–acetone (5 : 1, v/v) afforded as per *O*-methylate of **3** (**9**). The per-*O*-methylates of **6**, **7** and **8** (**10**, **11** and **12**) were also obtained by the same procedure as described above. **9**: Colorless needles from MeOH, mp 158—160 °C. IR ν_{max}^{Nujol} cm^{-1}: OH (nil.), 982, 920, 902, 865 (intensity 920 > 902, (25*S*)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 3.34, 3.47, 3.48, 3.50, 3.52 3.59 (each 3H, s, OCH_3), 4.28 (1H, d, *J* = 8 Hz, xylose anomeric H), 5.31 (1H, d, *J* = 1 Hz, rhamnose anomeric H). **10**: Colorless syrup. IR ν_{max}^{Nujol} cm^{-1}: OH (nil.), 980, 920, 902, 860 (intensity 920 > 902, (25*S*)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 3.38—3.60 (24H, s, $OCH_3 \times 8$), 4.28 (1H, d, *J* = 8 Hz, xylose anomeric H), 4.39 (1H, d, *J* = 6 Hz, glucose anomeric H), 5.25 (1H, d, *J* = 1 Hz, rhamnose anomeric H). **11**: Colorless syrup. IR ν_{max}^{Nujol} cm^{-1}: OH (nil.), 982, 920, 902, 865 (intensity 920 < 902, (25*R*)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 3.34, 3.38, 3.47, 3.52, 3.54, 3.58, 3.62 (each 3H, s, OCH_3), 4.27 (1H, d, *J* = 8 Hz, fucose anomeric H), 4.60 (1H, d, *J* = 6 Hz, glucose anomeric H). **12**: Colorless needles from MeOH, mp 88—90 °C. IR ν_{max}^{Nujol} cm^{-1}: OH (nil.), 982, 920, 902, 865 (intensity 920 < 902, (25*R*)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 3.34, 3.38, 3.48, 3.52, 3.54, 3.55, 3.56, 3.58, 3.62 (each 3H, s, OCH_3), 4.21 (1H, d, *J* = 7 Hz, fucose

anomeric H), 4.80 (1H, d, $J=8$ Hz, glucose anomeric H), 4.82 (1H, d, $J=8$ Hz, xylose anomeric H).

Methanolysis of 9—12 with Methanolic 5% HCl Per-*O*-methyl ethers, **9—12**, were individually refluxed with methanolic 5% HCl (0.3 ml per 1 mg sample) for 2 h, then the reaction mixture was neutralized with Ag_2CO_3 and evaporated to dryness. The residue was dissolved in acetone and examined by GLC (condition b) and TLC (solvent: benzene–acetone = 4 : 1, v/v); *Rf* 0.60 (ruscogenin 1-*O*-methyl ether), 0.67 (ruscogenin 3-*O*-methyl ether) **9**: aglycone (TLC): 3-*O*-methyl (25*R*,25*S*)-ruscogenin. sugars (GLC): t_R (min); 3.1 (per-*O*-methylrhamnopyranoside); 9.4, 10.6 (methyl 3,4-di-*O*-methylxylopyranoside). **10**: sugars (GLC): t_R (min): 3.1 (per-*O*-methylrhamnopyranoside); 3.8 (per-*O*-methylxylopyranoside); The sugar fraction was acetylated with acetic anhydride and pyridine, and the reaction mixture was concentrated and examined by GLC (condition c) t_R (min): 16.2 (methyl 2,3-di-*O*-acetyl-4,6-di-*O*-methylglucopyranoside). **11**: aglycone (TLC): 3-*O*-methylruscogenin. sugars (GLC): t_R (min): 7.8, 11.7 (per-*O*-methylglucopyranoside); The sugar fraction was acetylated by the same method described above, and the acetyl derivative was examined by GLC (condition c) t_R (min): 3.7, 6.3 (methyl 2-*O*-acetyl-3,4-di-*O*-methylfucopyranoside). **12**: aglycone (TLC): 3-*O*-methylruscogenin. sugars (GLC): t_R (min): 3.2, 4.3 (per-*O*-methylxylopyranoside), 7.8, 11.7 (per-*O*-methylglucopyranoside); The sugar fraction was acetylated by the same method as described above, and the acetyl derivative was examined by GLC (condition c) t_R (min): 12.7, 20.5 (methyl 2,3-di-*O*-acetyl-4-*O*-methylfucopyranoside).

Partial Hydrolysis of 2, 3 and 8 **2** (1 mg), **3** (1 mg) and **8** (100 mg) were individually dissolved in 0.05 N HCl in MeOH and the solution was heated at 80 °C for 30 min. The reaction mixture was diluted with water, neutralized with $NaHCO_3$ and concentrated to dryness *in vacuo*. Each residue of **2**, **3** and **8** was dissolved in MeOH and filtered. Both filtrates derived from **2** and **3** were compared by TLC (solvent b), and they showed the presence of the same prosapogenin (*Rf* 0.33). The filtrate derived from **8** was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O = 85 : 15 : 1, v/v) to afford **13**. **13**: A white powder from aq. MeOH. (mp 211—213 °C (dec.)). ^{13}C-NMR (C_5D_5N) δ: fucose ($\rightarrow^1$aglycone) 102.3 (C_1), 75.7 (C_2), 85.1 (C_3), 72.3 (C_4), 71.41 (C_5), 17.7 (C_6); xylose ($\rightarrow^3$fucose) 107.1 (C_1), 75.7 (C_2), 78.3 (C_3), 71.37 (C_4), 67.5 (C_5). **13** was methylated by Hakomori's method to afford **14**. **14**: ^{1}H-NMR ($CDCl_3$) δ: 3.34, 3.47, 3.54, 3.55, 3.62, 3.61 (each 3H, s, OCH_3), 4.22 (1H, d, $J=7$ Hz, fucose anomeric H), 4.52 (1H, d, $J=8$ Hz, xylose anomeric H). **14** was methanolyzed with methanolic 5% HCl and examined by GLC (condition b); t_R (min) sugars: 3.2, 4.3 (per-*O*-methylxylopyranoside). After acetylation, the product was examined by GLC (condition c) t_R (min): 3.7, 4.7 (methyl 3-*O*-acetyl-2,4-di-*O*-methylfucopyranoside).

Alkaline Hydrolysis of 4 and 5 **4** (1 mg) and **5** (1 mg) were individually dissolved in 0.5% K_2CO_3–MeOH and heated in a water bath for 1 h. The reaction mixture was examined by TLC (solvent: $CHCl_3$–MeOH–AcOEt–H_2O = 10 : 10 : 20 : 5, v/v, lower phase). Deacetylates of **4** and **5** showed the same *Rf* value (0.28).

Interconversion of 4 and 5 Each solution of **4** and **5** in pyridine was heated at 80 °C for 70 h and examined by TLC (solvent b). Each solution of **4** and **5** was found to be transformed into a solution of nearly equal amounts of **4** and **5**. (*Rf* 0.44 (**4**), 0.40 (**5**)).

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