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Comparative Studies on the Constituents of Ophiopogonis Tuber and Its Congeners. II.[1] Studies on the Constituents of the Subterranean Part of *Ophiopogon planiscapus* NAKAI. (1)

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Seven steroidal glycosides, tentatively named glycosides A, B(**1**), C(**2**), D(**3**), E(**4**), F(**5**) and G(**6**), were isolated from the methanol extract of the subterranean part of *Ophiopogon planiscapus* NAKAI (Liliaceae). The structures of these glycosides were established as so-called β-sitosterol-β-D-glucopyranoside, diosgenin 3-*O*-α-L-rhamnopyranoxyl(1→2)-β-D-glucopyranoside (=prosapogenin A of dioscin) (**1**), diosgenin 3-*O*-[α-L-rhamnopyranosyl(1→2)]-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside(=deltonin) (**2**), 26-*O*-β-D-glucopyranosyl-22-hydroxyfurost-5-ene-3β,26-diol 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside (**3**), ruscogenin 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-α-L-arabinopyranoside (**4**), 26-*O*-β-D-glucopyranosyl-22-hydroxyfurost-5-ene-3β,26-diol 3-*O*-[α-L-rhamnopyranosyl(1→2)]-[β-D-glucopyranosyl(1→4)]-β-D-glucopyranoside (=22-hydroxyl form of deltoside) (**5**) and 26-*O*-β-D-glucopyranosyl-22-hydroxyfurost-5-ene-1β,3β,26-triol 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-α-L-arabinopyranosides (**6**), respectively. The relationship of steroidal glycosides of *Ophiopogon japonicus* KER-GAWLER, *O. planiscapus* NAKAI and *Liriope platyphylla* WANG *et* TANG, which are considered to be the plants of origin of the crude drug, Ophiopogonis Tuber, is also discussed. This is believed to be the first report of steroidal glycosides having a sulfate group on the sugar moiety.

Keywords——Ophiopogonis Tuber; *Ophiopogon planiscapus*; Liliaceae; spirostanol glycoside; furostanol glycoside; sulfated steroidal glycoside; diosgenin; ruscogenin

In the preceding paper[1] isolation and structure elucidation of eight steroidal glycosides from the subterranean part of *Liriope platyphylla* WANG *et* TANG, one of the congener crude drugs of Ophiopogonis Tuber, were reported. As we pointed out in the preceding paper, the steroidal glycosides of *Ophiopogon japonicus* KER-GAWLER consist of ruscogenin and diosgenin glycosides, while those of *Liriope platyphylla* are glycosides of ruscogenin, diosgenin and their 25(*S*)-isomers. However, it must be emphasized that only one glycoside, namely ophiopogonin D′, is common to both plants. Further, the present report provides only the second example of isolation of a sulfated steroidal glycoside from Liliaceous plants.[2]

The present paper is mainly concerned with the isolation and structure elucidation of seven steroidal glycosides of the subterranean part of *Ophiopogon planiscapus* NAKAI (Liliaceae; Japanese name: Ohbajanohige), the tuber of which may be used as a congener crude drug of Ophiopogonis Tuber. These glycosides are tentatively named glycosides A, B, C, D, E, F and G in order of increasing polarity. As the constituents of the title plant, diosgenin and ruscogenin were detected in the hydrolysate of the total glycosides of the subterranean part,[3] and quercetin 3-*O*-glucopyranoside-7-*O*-rutinoside[4] was found in preliminary experiments.

The seven steroidal glycosides obtained here were isolated from the methanolic extract of the fresh subterranean part of *O. planiscapus* NAKAI harvested at Tokyo Metropolitan

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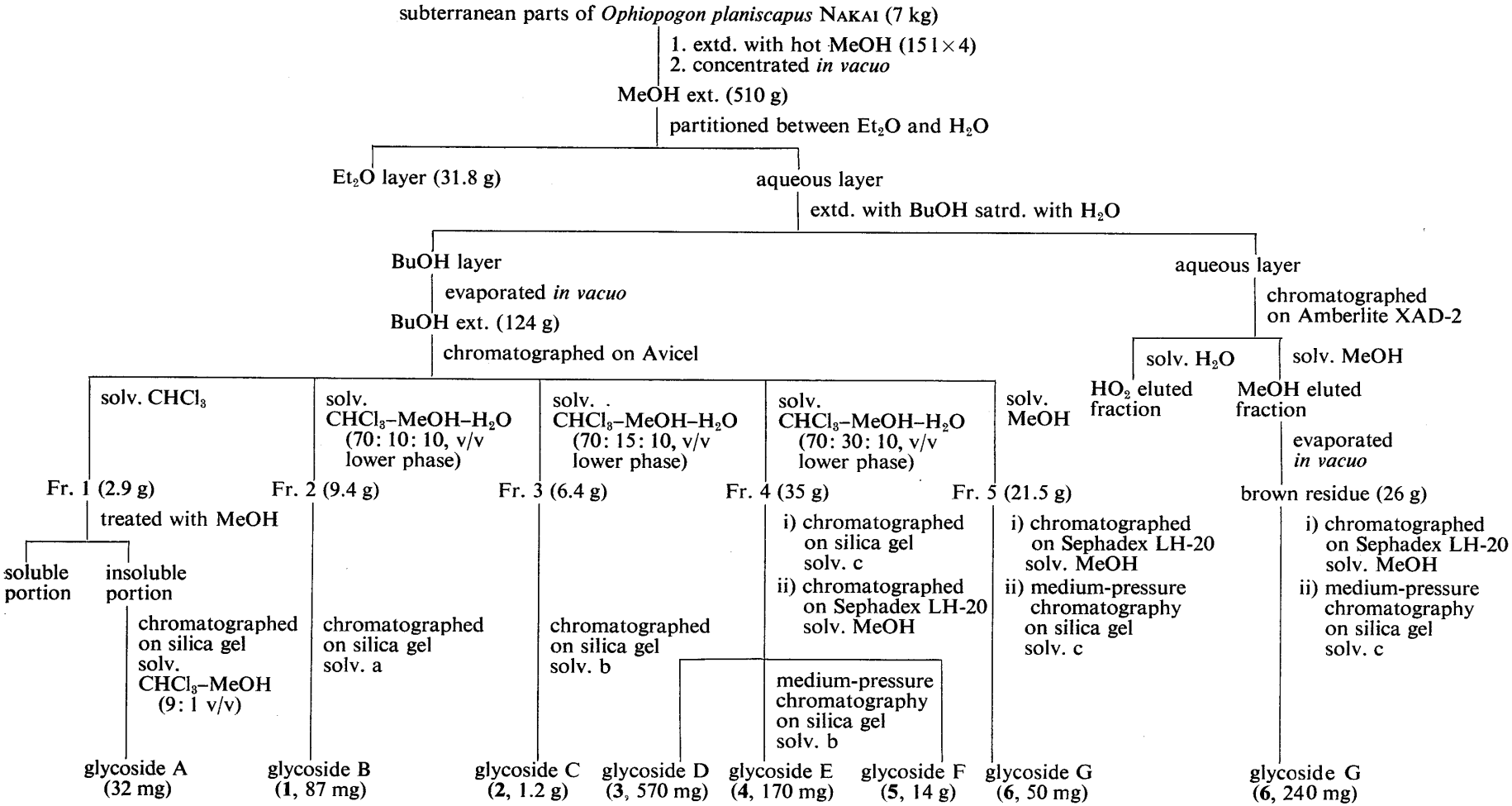

Chart 1

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Medicinal Plants Garden in October 1981, as shown in Chart 1.

Glycoside A, colorless needles, is positive in the Liebermann–Burchard reaction, and on hydrolysis with 2 N hydrochloric acid in 50% dioxane, it affords glucose and so-called β-sitosterol. The aglycone fraction was examined by gas-liquid chromatography (GLC), and β-sitosterol, campesterol and stigmasterol were identified by comparision with authentic samples. Accordingly, glycoside A was concluded to be a mixture of glucosides of β-sitosterol, campesterol and stigmasterol.

Glycoside B (**1**), $C_{39}H_{62}O_{12}$, is positive in the Liebermann–Burchard reaction, and it shows strong absorption of hydroxyl groups and characteristic absorption bands of a 25(*R*)-spiroketal moiety in the infrared (IR) spectrum.[5] On acetylation with acetic anhydride and pyridine, **1** gave a hexaacetate (**7**), $C_{51}H_{74}O_{18}$, and hydrolysis of **1** with 2 N hydrochloric acid in 50% dioxane gave D-glucose, L-rhamnose and an aglycone, $C_{27}H_{42}O_3$, colorless needles, mp 203—204 °C, which was identified as diosgenin (**8**) by direct comparisons with an authentic sample.[6] Accordingly, **1** was assumed to be either diosgenin rhamnosylglucoside or glucosylrhamnoside. The chemical properties of **1** and its acetate appeared to be identical with prosapogenin A of dioscin and its acetate[7] reported by us as constituents of Ophiopogonis Tuber (tuber of *Ophiopogon japonicus* KER-GAWLER). Finally, glycoside B and its acetate were identified as diosgenin 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside (**1**) and its hexaacetate by mixed fusion and by comparisons of thin-layer chromatographic (TLC) behavior, and IR and nuclear magnetic resonance (NMR) spectra.

Glycoside C (**2**), $C_{45}H_{72}O_{17} \cdot H_2O$, is positive in the Liebermann–Burchard reaction, and it shows strong absorption of hydroxyl groups and characteristic absorption bands of a 25(*R*)-spiroketal moiety in the IR spectrum. On acetylation with acetic anhydride and pyridine, **2** afforded a nonaacetate (**9**) and hydrolysis of **2** with 2 N hydrochloric acid gave D-glucose, L-rhamnose and diosgenin (**8**). The ^{13}C-NMR spectrum of **2** shows three anomeric carbon signals at δ 100. 3, 101.7 and 105.1 ppm, and one secondary methyl carbon signal of rhamnose at δ 18.6 ppm. Accordingly, **2** was assumed to be a diosgenin trioside, and the trisaccharide is constituted of two glucose moieties and one rhamnose moiety. To determine the structure of the oligosaccharide moiety, **2** was partially hydrolyzed with 0.3 N sulfuric acid to afford two prosapogenins, **2a** and **2b** (**10**). The former, **2a**, gave D-glucose, L-rhamnose and diosgenin (**8**) on acid hydrolysis. Based on the physical properties, **2a** and its acetate were suggested to be identical with glycoside B (**1**) and its hexaacetate (**7**), and this was confirmed by mixed fusion and by comparing TLC behavior and IR and ^{1}H-NMR spectra. The latter, **10**, $C_{39}H_{62}O_{13} \cdot 3/2H_2O$, gave D-glucose and diosgenin on acid hydrolysis and methylation of **10** by Hakomori's method[8] afforded a hepta-*O*-methyl derivative (**11**), which gave per-*O*-methyl-D-glucopyranoside, methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside and diosgenin (**8**) on methanolysis. Consequently, the structure of **10** was established to be diosgenin 3-*O*-D-glucopyranosyl(1→4)-D-glucopyranoside and the configurations of both glucose moieties were assigned as β on the basis of the coupling constants (each 7 Hz) of the anomeric protons. Finally, the structure of **2** was concluded to be diosgenin 3-*O*-[α-L-rhamnopyranosyl(1→2)]-[β-D-glucopyranosyl(1→4)]-β-D-glucopyranoside. Compound **2** and its acetate were proved to be identical with deltonin and its acetate reported by Paseshnichenko *et al.*,[9] by mixed fusion and by comparing TLC behavior, and IR and NMR spectra.

Glycoside D (**3**), $C_{45}H_{74}O_{18} \cdot H_2O$, a white powder from aqueous acetone, is positive in the Liebermann–Burchard reaction and in the Ehrlich reaction.[10] The IR spectrum of **3** does not show any characteristic spiroketal absorption bands and the ^{13}C-NMR spectrum shows characteristic furostanol carbon signals as reported by Hirai *et al.*[11]

On acid hydrolysis, **3** gave diosgenin (**8**), D-glucose and L-rhamnose, while enzymatic hydrolysis with almond emulsin afforded D-glucose and a prosapogenin, $C_{39}H_{62}O_{12}$, which showed characteristic absorption bands corresponding to a 25(*R*)-spiroketal in the IR

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spectrum. This prosapogenin was proved to be identical with glycoside B (**1**) by comparing TLC behavior, melting points, and IR and ^{13}C-NMR spectra. Consequently, the structure of **3** was established to be 26-*O*-*β*-D-glucopyranosyl 22-hydroxyfurost-5-ene-3*β*,26-diol 3-*O*-*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranoside, and the furostanol structure was proved by derivation of the 22-hydroxyl group of **3** to a 22-methoxyl group by refluxing with metanol for 5 h. Glycoside D has been obtained from *Dioscorea gracillima* MIQ. and designated as PPD by Kiyosawa *et al.*[10] or compound A by Kawasaki *et al.*[12]

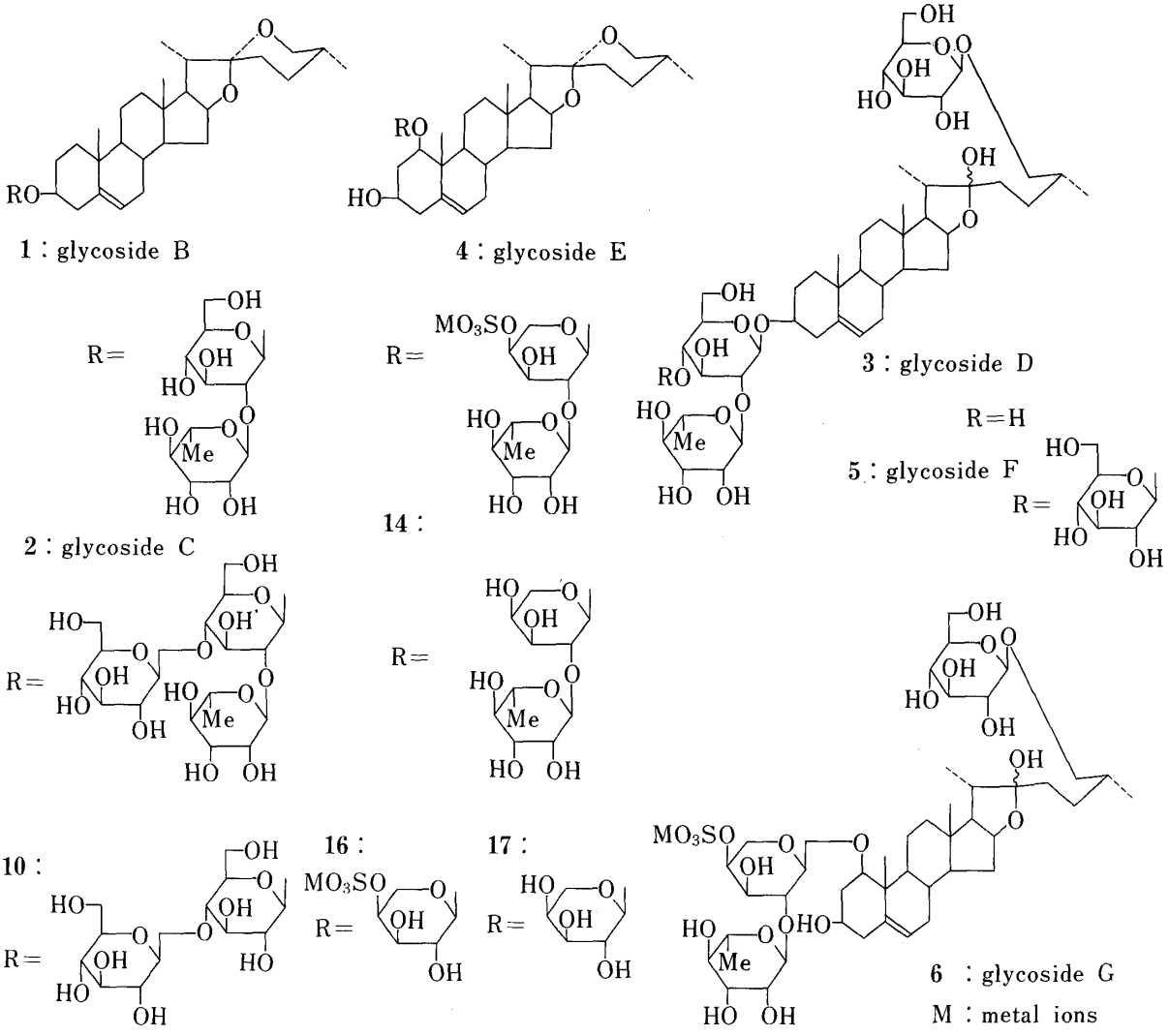

Chart 2

Glycoside E (**4**), colorless needles, $C_{38}H_{59}O_{11}\cdot SO_3M\cdot 3H_2O$, is positive in the Liebermann–Burchard reaction and it shows strong absorption of hydroxyl groups, characteristic absorption bands of a 25 (*R*)-spiroketal moiety and an S–O stretching absorption band at 1215 cm^{-1} in the IR spectrum.[13] On acidic hydrolysis, **4** gave ruscogenin (**13**),[14] L-arabinose and L-rhamnose, while on heating with pyridine–dioxane,[15] **4** gave desulfated glycoside E (**14**), $C_{38}H_{60}O_{11}\cdot 3/2H_2O$. A part of the reaction mixture was subjected to paper partition chromatography (PPC). The spot of sulfate ion was detected by spraying test solution of barium chloride and potassium rhodizonate.[16] The structure of **14** was established as follows. Methylation of **14** by Hakomori's method afforded a hexa-*O*-methyl derivative (**15**). Methanolysis of **15** gave per-*O*-methyl-L-rhamnopyranoside, methyl 3,4-di-*O*-methyl-L-

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arabinopyranoside and an aglycone, colorless needles, mp 222—224 °C, which was identified as ruscogenin 3-*O*-methyl ether by mixed fusion and by comparing its TLC behavior with that of an authentic sample.[17] Accordingly, the structure of **14** assumed to be ruscogenin 1-*O*-L-rhamnopyranosyl(1→2)-L-arabinopyranoside and the configurations of rhamnose and arabinose were assigned as α on the basis of ^{13}C-NMR analysis.[18]

The location of the sulfate group was revealed to be at the C_4-hydroxyl group of arabinose by chemical investigation and analysis of ^{13}C-NMR spectra. On enzymatic hydrolysis with crude pectinase, **4** gave L-rhamnose and a prosapogenin (**16**), which was positive in the potassium rhodizonate reaction. The IR spectrum of **16** showed an S–O stretching absorption band at 1215 cm^{-1} and, on heating in pyridine–dioxane, **16** afforded a desulfated derivative (**17**). By comparative analyses of the ^{13}C-NMR spectra of **4**, **14**, **16** and **17**, the location of the sulfate group was deduced to be the C_4-hydroxyl group of arabinose, because lower field shift of the C_4-carbon signal (+6.1—6.2 ppm) and higher field shifts of both the C_3-carbon signal (−1.0—1.2 ppm) and C_5-carbon signal (−1.6—1.8 ppm) were observed. Finally, the structure of glycoside E was established to be ruscogenin 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-α-L-arabinopyranoside.

Glycoside F (**5**), $C_{51}H_{84}O_{23}$, is positive in the Liebermann–Burchard reaction and in the Ehrlich reaction. The IR spectrum of **5** does not show any characteristic spiroketal absorption band and its ^{13}C-NMR spectrum shows characteristic furostanol carbon signals. On acidic hydrolysis, **5** gave diosgenin, D-glucose and L-rhamnose, while enzymatic hydrolysis with almond emulsin afforded D-glucose and a prosapogenin, $C_{45}H_{72}O_{17}\cdot H_2O$, which showed characteristic 25(*R*)-spiroketal absorption bands in the IR spectrum. This prosapogenin was proved to be identical with glycoside C (**2**) by direct comparison of TLC behavior, and IR and ^{13}C-NMR spectra with those of authentic samples. Furthermore, the furostanol skeleton of **5** was confirmed chemically by refluxing with methanol to introduce an *O*-methyl group at the C-22 hydroxyl group. Finally, the structure of glycoside F was established to be 26-*O*-β-D-glucopyranosyl 22-hydroxyfurost-5-ene-3β,26-diol 3-*O*-[α-L-rhamnopyranosyl(1→2)]-[β-D-glucopyranosyl(1→4)]-β-D-glucopyranoside. This compound was obtained by Sviridov *et al.*[19] as a mixture of C_{22}-hydroxyl and C_{22}-methoxyl compounds under the name of deltoside.

Glycoside G (**6**), $C_{44}H_{71}O_{17}\cdot SO_3M$, is positive in the Liebermann–Burchard reaction and in the Ehrlich reaction. The IR spectrum of **6** shows an S–O stretching absorption band at 1220 cm^{-1}, but does not show any characteristic spiroketal absorption bands. On acidic hydrolysis, **6** gave ruscogenin, L-arabinose, L-rhamnose and D-glucose, while enzymatic hydrolysis of **6** with almond emulsin afforded D-glucose and a prosapogenin, colorless needles, mp 218—220 °C (dec.), the IR spectrum of which showed characteristic absorption bands of 25(*R*)-spiroketal structure and an S–O stretching absorption at 1215 cm^{-1}. Based on the general properties, this prosapogenin was suggested to be identical with glycoside E (**4**) and this was confirmed by comparisons of TLC behavior, and IR and ^{13}C-NMR spectra. Finally, the configuration of the C_{26}-*O*-glucosyl moiety was deduced to be β by NMR analysis, and the structure of **6** was established to be 26-*O*-β-D-glucopyranosyl 22-hydroxyfurost-5-ene-1β,3β,26-triol 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-α-L-arabinopyranoside.

This is the first reported isolation of a protoruscogenin-type furostanol oligoglycoside, and further confirmation of the structure was carried out by Marker's degradation[20,21] of **6** to afford 1β,3β-dihydroxypregna-5,16-dien-20-one and a tetraacetate of methyl γ-methyl-δ-hydroxypentanoate β-D-glucopyranoside.

The seven steroidal glycosides of the whole subterranean part of *Ophiopogon planiscapus* NAKAI described above were also detected by TLC in the tuber of the same plant. It is interesting that even though the common aglycones of the steroidal glycosides of *O. planiscapus* NAKAI and *O. japonicus* KER-GAWLER were diosgenin and ruscogenin, the only

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common glycoside of both plants is prosapogenin A of dioscin.

Furthermore, sulfated steroidal oligoglycosides were found in both *Liriope platyphylla* and *O. planiscapus*, but the sulfate group of the former is located on the steroidal moiety, while that of the latter is conjugated with a hydroxyl group of the sugar moiety. As far as we know, this is the first reported example of the latter type of sulfated steroidal oligoglycosides.

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. The IR spectra were recorded with a Hitachi EPI-2 and the NMR spectra with a JEOL FX-100 spectrometer (100 MHz for ^{1}H-NMR and 25 MHz for ^{13}C-NMR). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. 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., 175 °C; injection temp., 220 °C; carrier gas N_2, 1.0 kg/cm^2; samples, trimethylsilyl (TMS) ether. (b) for *O*-methylated sugars: column, 5% neopentyl glycol succinate (NPGS) on Shimalite 3 mm × 2 m. TLC was performed on a precoated Kieselgel 60 F_{254} plate (Merck) using solvents (a) $CHCl_3$–MeOH–H_2O (7 : 1 : 0.1 v/v), (b) $CHCl_3$–MeOH–H_2O (7 : 1.5 : 0.1 v/v), (c) $CHCl_3$–MeOH–H_2O (7 : 3 : 0.4 v/v), and (d) hexane–acetone (2 : 1 v/v). Detection was achieved with 10% H_2SO_4 or Ehrlich reagent followed by heating. TLC for free monosaccharides was done on precoated Cellulose F plates (Merck) using a mixture of BuOH–AcOH–H_2O (4 : 1 : 5 v/v, upper layer) and detected by spraying aniline hydrogen phthalate reagent.

Extraction and Isolation of Glycosides——The fresh subterranean parts of *Ophiopogon planiscapus* NAKAI (7 kg), collected at Tokyo Metropolitan Medicinal Plants Garden, were crushed and extracted with hot MeOH (15 l × 4). The extract was combined and evaporated to dryness *in vacuo*. The residue (510 g) was dissolved in water and extracted with ether. The aqueous layer was extracted with BuOH saturated with water, and the BuOH-soluble fraction was concentrated *in vacuo* to afford a brown powder (124 g), which was subjected to column chromatography on Avicel with $CHCl_3$, $CHCl_3$–MeOH–H_2O (70 : 10 : 10—70 : 15 : 10—70 : 30 : 10 v/v, lower phase) and then with MeOH to obtain five fractions (Fr. 1—Fr. 5). Fr. 1 was separated into two fractions, the MeOH-soluble fraction and MeOH-insoluble fraction, and the latter was subjected to column chromatography on silica gel with $CHCl_3$–MeOH (9 : 1, v/v) to afford glycoside A (β-sitosterol β-D-glucopyranoside, 32 mg). Fr. 2 was purified by silica gel column chromatography with solvent a to afford glycoside B (**1**, 87 mg), and Fr. 3 was separated by column chromatography on silica gel with solvent b to afford glycoside C (**2**, 1.2 g). Fr. 4 was repeatedly subjected to column chromatography on silica gel with solvent c followed by column chromatography on Sephadex LH-20 with MeOH to afford three crude oligosides, namely glycosides D (**3**), E (**4**) and F (**5**). Crude glycosides D and F were separately refluxed with acetone–H_2O (4 : 1 v/v) for 10 h on a water bath to give pure glycoside D (**3**, 570 mg) and glycoside F (**5**, 14 g), respectively. Crude glycoside E (**4**) obtained above was purified by medium-pressure column chromatography on Kieselgel H (Type 60, Merck) using solvent b to afford pure glycoside E (170 mg). Fr. 5 was repeatedly purified by column chromatography on Sephadex LH-20 using MeOH and by medium-pressure column chromatography on Kieselgel H using solvent c to afford glycoside G (**6**, 50 mg).

Furthermore, the aqueous layer described above was evaporated *in vacuo* to remove remaining butanol and the residual aqueous solution was passed through a column of Amberlite XAD-2. The absorbate was eluted with MeOH and the eluate was concentrated to dryness *in vacuo*. The brown residue (26 g) was repeatedly subjected to column chromatography on Sephadex LH-20 with MeOH, and the glycoside G fraction was purified by medium-pressure column chromatography on Kieselgel H using solvent c to afford glycoside F (**6**, 240 mg).

Properties of Glycoside A (So-called β-Sitosterol β-D-Glucopyranoside)——Colorless needles from pyridine, mp 295—297 °C, IR ν_{max}^{KBr} cm^{-1}: 3550—3200 (OH), were refluxed with 2 N HCl in 50% dioxane for 2 h. The reaction mixture was extracted with $CHCl_3$ and the $CHCl_3$ layer was washed with water, dried over Na_2SO_4 and filtered. The filtrate was evaporated to dryness *in vacuo* and the residue was examined by GLC (column, 5% OV-17 on Uniport KS 3 mm × 2 m; column temp., 260 °C; injection temp., 280 °C; N_2 1.0 kg/cm^2). t_R (min) 11.8 (β-sitosterol), 10.3 (stigmasterol), 9.5 (campesterol). The ratio of β-sitosterol, stigmasterol and campesterol was calculated to be about 5 : 1 : 1. The aqueous layer described above was neutralized with Amberlite IR-45 and the neutral solution was evaporated to dryness *in vacuo*. The residue was examined by TLC (*Rf* 0.19 glucose).

Properties of Glycosides B, C, D, E, F and G——Glycoside B: colorless needles from MeOH, mp 239—242 °C (dec.), $[\alpha]_D^{23}$ −94.3° (c = 0.72, pyridine) (lit.[7] mp 238—240 °C (dec.), $[\alpha]_D^{21}$ −99.20° (pyridine)), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 980, 918, 900, 865 (intensity 918 < 900, 25 (*R*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: glucose ($\xrightarrow{3}$aglycone) 100.6 (C_1), 79.6 (C_2), 78.0 (C_3), 72.1 (C_4), 78.0 (C_5), 62.9 (C_6); rhamnose ($\xrightarrow{2}$glucose) 101.9 (C_1), 72.5 (C_2), 72.9 (C_3), 74.2 (C_4), 69.4 (C_5), 18.6 (C_6). *Anal.* Calcd for $C_{39}H_{62}O_{12}$: C, 64.79; H, 8.65. Found: C. 64.57; H, 8.86. Glycoside C: colorless needles from BuOH, mp 289—291 °C (dec.), $[\alpha]_D^{23}$ −90.3° (c = 0.92, pyridine) (lit.[9] mp 290—292 °C (dec.), $[\alpha]_D^{20}$ −98.7° (pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 980, 915, 900, 865 intensity 915 < 900, 25(*R*)-spiroketal).

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^{13}C-NMR (C_5D_5N) δ: glucose ($\xrightarrow{3}$aglycone) 100.3 (C_1), 78.5 (C_2), 76.2 (C_3), 82.2 (C_4), 77.6 (C_5), 62.3 (C_6); rhamnose ($\xrightarrow{2}$glucose) 101.7 (C_1), 72.4 (C_2), 72.9 (C_3), 74.3 (C_4), 69.4 (C_5), 18.6 (C_6); glucose ($\xrightarrow{4}$glucose) 105.1 (C_1), 75.0 (C_2), 78.4 (C_3), 71.5 (C_4), 77.7 (C_5), 62.4 (C_6). *Anal.* Calcd for $C_{45}H_{72}O_{17}\cdot H_2O$: C, 59.85; H, 8.26. Found: C, 59.35; H, 8.16. Glycoside D: a white powder from aqueous acetone, (mp 194—197 °C (dec.)), $[\alpha]_D^{23}$ −70.4° (c=0.62, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH). ^{13}C-NMR (C_5D_5N) δ: glucose ($\xrightarrow{26}$aglycone) 104.7 (β-C_1 $J_{C_1-H_1}$=155 Hz), 75.1 (C_2), 78.5 (C_3), 71.9 (C_4), 78.1 (C_5), 63.0 (C_6); glucose ($\xrightarrow{3}$aglycone) 100.5 (β-C_1 $J_{C_1-H_1}$=156 Hz), 79.5 (C_2), 78.1 (C_3), 72.1 (C_4), 77.9 (C_5), 62.9 (C_6); rhamnose ($\xrightarrow{2}$glucose) 101.8 (α-C_1 $J_{C_1-H_1}$=171 Hz), 72.4 (C_2), 72.8 (C_3), 74.2 (C_4), 69.3 (C_5), 18.5 (C_6). *Anal.* Calcd for $C_{45}H_{74}O_{18}$: C, 59.85; H, 8.26. Found: C, 59.56; H, 8.51. Glycoside E: colorless needles from MeOH, mp 220—221 °C (dec.), $[\alpha]_D^{23}$ −82.5° (c=0.77, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1215 (S–O), 980, 920, 900, 865 (intensity 920<900, 25(R)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: 4-*O*-sulfo-arabinose ($\xrightarrow{1}$aglycone) 100.2 (α-C_1, $J_{C_1-H_1}$=159 Hz), 75.9 (C_2), 74.5 (C_3), 76.0 (C_4), 65.5 (C_5); rhamnose ($\xrightarrow{2}$arabinose) 101.2 (α-C_1, $J_{C_1-H_1}$= 172 Hz), 72.1 (C_2), 72.1 (C_3), 74.0 (C_4), 69.3 (C_5), 18.6 (C_6). Glycoside F: a white powder from aqueous acetone, (mp 204—207 °C (dec.)), $[\alpha]_D^{23}$ −51.2° (c=0.72, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), ^{13}C-NMR (C_5D_5N) δ: glucose ($\xrightarrow{26}$aglycone) 104.9 (β-C_1, $J_{C_1-H_1}$=161 Hz), 75.0 (C_2), 78.5 (C_3), 71.9 (C_4), 78.3 (C_5), 63.0 (C_6); glucose ($\xrightarrow{3}$aglycone) 100.3 (β-C_1, $J_{C_1-H_1}$=156 Hz), 78.4 (C_2), 76.2 (C_3), 82.1 (C_4), 77.6 (C_5), 62.2 (C_6); rhamnose ($\xrightarrow{2}$glucose) 101.7 (α-C_1, $J_{C_1-H_1}$=172 Hz), 72.4 (C_2), 72.8 (C_3), 74.2 (C_4), 69.4 (C_5), 18.6 (C_6); glucose ($\xrightarrow{4}$glucose) 105.1 (β-C_1, $J_{C_1-H_1}$=161 Hz), 75.2 (C_2), 78.4 (C_3), 71.5 (C_4), 77.6 (C_5), 62.3 (C_6). *Anal.* Calcd for $C_{51}H_{84}O_{23}$: C, 57.50; H, 7.95. Found: C, 57.37; H, 8.14. Glycoside G: a white powder from aqueous acetone, (mp 212—215 °C (dec.)), $[\alpha]_D^{23}$ −55.2° (c=0.80, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1220 (S–O). ^{13}C-NMR (C_5D_5N) δ: glucose ($\xrightarrow{26}$aglycone) 104.5 (β-C_1, $J_{C_1-H_1}$=156 Hz), 75.0 (C_2) 78.1 (C_3), 71.7 (C_4), 77.9 (C_5), 62.7 (C_6); 4-*O*-sulfo-arabinose ($\xrightarrow{1}$aglycone) 100.1 (α-C_1, $J_{C_1-H_1}$=159 Hz), 75.9 (C_2), 74.5 (C_3), 76.0 (C_4), 65.5 (C_5); rhamnose ($\xrightarrow{2}$arabinose) 101.2 (α-C_1, $J_{C_1-H_1}$= 172 Hz), 72.1 (C_2), 72.1 (C_3), 73.9 (C_4), 69.4 (C_5), 18.7 (C_6).

Preparations of Sodium Salt of Glycosides E and G——An aqueous solution of glycoside E (50 mg) or G (50 mg) was passed through an Amberlite IR-120 column. The desalted solution was neutralized with 0.01 N NaOH and the neutral solution was concentrated to dryness *in vacuo*. The residue was purified by column chromatography on Sephadex LH-20 eluting with MeOH to afford the sodium salt of the glycoside. Sodium salt of glycoside E: colorless needles from MeOH, mp 218—220 °C (dec.), *Anal.* Calcd for $C_{38}H_{59}O_{11}\cdot SO_3Na\cdot 3H_2O$: C, 53.76; H, 7.72; S, 3.78. Found: C, 53.84; H, 7.33; S, 3.59. Sodium salt of glycoside G: a white powder from aqueous acetone, (mp 210—212 °C (dec.)), *Anal.* Calcd for $C_{44}H_{71}O_{17}\cdot SO_3Na\cdot 3H_2O$: C, 51.34; H, 7.54; S, 3.11. Found: C, 51.27; H, 6.99; S, 2.89.

Acetylation of Glycosides B and C——A solution of glycoside B (30 mg) or C (50 mg) in Ac_2O–pyridine (2 : 1 v/v, 1 ml per 10 mg of glycoside) was kept overnight at room temperature and the reaction mixture was treated in the usual way. Glycoside B hexaacetate (**7**): colorless needles from MeOH, mp 213—215 °C, $[\alpha]_D^{23}$ −70.2° (c=0.41, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1750 (ester), 985, 925, 905, 868 (intensity 925<905, 25 (R)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 0.79 (3H, s, CH_3; 3H, d, J=6 Hz, CH_3), 0.98 (3H, d, J=6 Hz, CH_3), 1.02 (3H, s, CH_3), 1.19 (3H, d, J=6 Hz, CH_3), 1.99, 2.01, 2.02, 2.13 (each 3H, s, $OCOCH_3\times 4$), 2.06 (6H, s, $OCOCH_3\times 2$). *Anal.* Calcd for $C_{51}H_{74}O_{18}$: C, 62.81; H, 7.65. Found: C, 62.42; H, 7.85. Glycoside C nonaacetate (**9**): colorless needles from EtOH, mp 145—147 °C, $[\alpha]_D^{24}$ −63.8° (c=0.74, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: 1750 (ester), 985, 915, 900, 865 (intensity 915<900, 25 (R)-spiroketal), ^{1}H-NMR ($CDCl_3$) δ: 0.79 (3H, s, CH_3; 3H, d, J=6 Hz, CH_3), 0.97 (3H, d, J=6 Hz, CH_3), 1.01 (3H, s, CH_3), 1.23 (3H, d, J= 6 Hz, CH_3), 1.99, 2.08 (each 6H, s, $OCOCH_3\times 2$), 2.01 (9H, s, $OCOCH_3\times 3$), 2.11, 2.12 (each 3H, s, $OCOCH_3$). *Anal.* Calcd for $C_{63}H_{90}O_{26}$: C, 59.90; H, 7.18. Found: C, 60.14; H, 7.48.

Hydrolysis of Glycosides B, C, D, E, F and G with 2 N HCl——A solution of glycoside B (30 mg), C (30 mg), D (30 mg), E (100 mg), F (30 mg) or G (30 mg) in 2 N HCl–50% dioxane (3 ml per 10 mg of glycoside) 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. Aglycone: the residue in the cases of glycosides B, C, D and F was purified by column chromatography on Sephadex LH-20 using $CHCl_3$ to afford colorless needles from acetone, mp 203—204 °C, IR ν_{max}^{Nujol} cm^{-1}: 3300 (OH), 980, 920, 900, 865 (intensity 920<900, 25(R)-spiroketal), TLC (solvent d) Rf 0.52. *Anal.* Calcd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.00; H, 10.26. The aglycones of glycosides B, C, D and F were identified as diosgenin by mixed fusion and by comparing TLC behavior, and IR spectra with those of an authentic sample. The residues of glycosides E and G were purified by column chromatography on silica gel using solvent d to afford colorless needles from MeOH, mp 205—207 °C, IR ν_{max}^{Nujol} cm^{-1}: 3300 (OH), 982, 920, 900, 865 (intensity 920<900, 25(R)-spiroketal), TLC (solvent d) Rf 0.10. *Anal.* Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 74.80; H, 10.04. The aglycones of glycosides E and G were identified as ruscogenin by mixed fusion and by comparing the TLC behavior and IR spectra with those of an authentic sample.

Sugars: The aqueous layer was neutralized with Amberlite IR-45 and concentrated to dryness *in vacuo*. The monosaccharides were examined by TLC and GLC. Glycosides B, C, D and F: TLC Rf 0.19 (glucose), 0.38 (rhamnose). GLC t_R (min) 3.5, 4.8 (rhamnose), 11.2, 17.4 (glucose). Glycosides E and G: TLC Rf 0.19 (glucose), 0.30 (arabinose), 0.38 (rhamnose). GLC t_R (min) 3.2, 3.7 (arabinose), 3.5, 4.8 (rhamnose), 11.2, 17.4 (glucose). Furthermore, each monosaccharide derived from glycoside E was isolated by column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (7 : 2 : 0.1 v/v) to afford L-arabinose and L-rhamnose. Arabinose: TLC Rf 0.30. $[\alpha]_D^{23}$

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+121.2° (c=0.43, H_2O), (lit.[22] L-arabinose $[\alpha]_D^{23}$ +104.5° (H_2O)). Rhamnose: TLC Rf 0.38, $[\alpha]_D^{23}$ +10.8° (c=0.37, H_2O), (lit.[23] L-rhamnose $[\alpha]_D^{23}$ +8.9° (H_2O)).

Partial Hydrolysis of Glycoside C——Glycoside C (**2**, 150 mg) was dissolved in 0.3 N H_2SO_4 in 50% EtOH (30 ml) and the solution was heated at 70 °C for 3 h. The reaction mixture was diluted with water (15 ml) and concentrated to 30 ml. After cooling, the precipitate was collected by filtration and subjected to column chromatography on silica gel using $CHCl_3$–MeOH–AcOEt–H_2O (2:2:4:1 v/v, lower layer) to afford prosapogenins **2a** and **2b** (**10**). Prosapogenin **2a**: colorless needles from MeOH, mp 237—240 °C (dec.), $[\alpha]_D^{25}$ −87.3° (c=0.41, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3200—3600 (OH), 980, 918, 900, 865 (intensity 918<900, 25(R)-spiroketal). Prosapogenin **2a** was hydrolyzed with 2 N HCl in 50% dioxane to afford diosgenin, glucose and rhamnose. On acetylation with Ac_2O–pyridine in the usual way, prosapogenin **2a** gave a hexaacetate, which was identified as glycoside B hexaacetate by mixed fusion and by comparing the TLC behavior, and IR and ^{1}H-NMR spectra with those of an authentic sample. Prosapogenin **2b** (**10**): colorless needles from n-BuOH, mp 269—272 °C (dec.), $[\alpha]_D^{23}$ −85.2° (c=0.47, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 982, 920, 902, 868 (intensity 920<902, 25(R)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: glucose ($\xrightarrow{3}$aglycone) 102.5 (C_1), 74.9 (C_2), 76.5 (C_3), 81.5 (C_4), 76.9 (C_5), 62.5 (C_6); glucose ($\xrightarrow{4}$glucose) 104.9 (C_1), 75.0 (C_2), 78.6 (C_3), 71.8 (C_4), 78.5 (C_5), 62.7 (C_6). *Anal.* Calcd for $C_{39}H_{62}O_{13}\cdot 3/2H_2O$: C, 61.15; H, 8.55. Found: C, 60.82; H, 8.46. On hydrolysis with 2 N HCl in 50% dioxane, prosapogenin **2b** afforded diosgenin and glucose. Hydrolysis products of prosapogenins **2a** and **2b** were identified by TLC and GLC. Diosgenin: TLC (solvent d) Rf 0.52. Glucose: TLC Rf 0.19. GLC t_R (min) 11.2, 17.4. Rhamnose: TLC Rf 0.38. GLC t_R (min) 3.5, 4.8.

Enzymatic Hydrolyses of Glycosides D, E, F and G——i) An aqueous solution of glycoside D (70 mg in 15 ml), F (200 mg in 40 ml) or G (100 mg in 30 ml) was incubated with almond emulsin at 37 °C for 24 h. The precipitate was collected by filtration, dried and subjected to column chromatography on silica gel, while the aqueous filtrate was evaporated to dryness *in vacuo* and the residue was examined by TLC. The hydrolysate of each glycoside was characterized as follows. Glycoside D: A prosapogenin was obtained by elution with solvent a as colorless needles from MeOH, mp 239—242 °C (dec.), $[\alpha]_D^{24}$ −92.2° (c=0.31, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 980, 918, 900, 865 (intensity 918<900, 25(R)-spiroketal). *Anal.* Calcd for $C_{39}H_{62}O_{12}$: C, 64.79; H, 8.65. Found: C,64.51; H, 8.88. The product was identified as glycoside B by comparing TLC behavior (solvent c, Rf 0.40), mp, and IR and ^{13}C-NMR spectra. Glucose was detected in the aqueous filtrate by TLC (Rf 0.19). Glycoside F: A prosapogenin was obtained by elution with solvent b as colorless needles from BuOH, mp 289—291 °C (dec.), $[\alpha]_D^{23}$ −92.5° (c=0.42, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 980, 915, 900, 865 (intensity 915<900, 25(R)-spiroketal). *Anal.* Calcd for $C_{45}H_{72}O_{17}\cdot H_2O$: C, 59.85; H, 8.26. Found: C, 59.56; H, 8.29. The product was identified as glycoside C by comparing TLC behavior (solvent c, Rf 0.28), mp, and IR and ^{13}C-NMR spectra. Glucose was detected in the aqueous filtrate by TLC (Rf 0.19). Glycoside G: A prosapogenin was obtained by elution with solvent c as colorless needles from MeOH, mp 218—220 °C (dec.), $[\alpha]_D^{24}$ −80.4° (c=0.51, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1215 (S–O), 980, 920, 900, 865 (intensity 920<900, 25(R)-spiroketal). The product was identified as glycoside E by comparing TLC behavior (solvent c, Rf 0.14), mp, and IR and ^{13}C-NMR spectra. Glucose was found in the aqueous filtrate by TLC (Rf 0.19).

ii) Glycoside E (50 mg) in H_2O (20 ml) was incubated with crude pectinase (20 mg) prepared from *Aspergillus niger* (SIGMA) at 37 °C for 5 d. The reaction mixture was extracted with BuOH and the BuOH extract was concentrated *in vacuo*. The residue was purified by medium-pressure column chromatography on Kieselgel H using solvent b to afford a prosapogenin (**16**), colorless needles from MeOH, mp 208—210 °C (dec.), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1215 (S–O), 980, 920, 900, 865 (intensity 920<900, 25(R)-spiroketal), ^{13}C-NMR (C_5D_5N) δ: arabinose 101.7 (C_1), 72.7 (C_2), 73.6 (C_3), 75.8 (C_4), 65.7 (C_5). The aqueous layer was evaporated to dryness *in vacuo* and the residue was examined by TLC to identify rhamnose (Rf 0.39).

Methylation of 22-Hydroxyl Group of Glycosides D, F and G with Methanol——A methanolic solution (10 ml) of glycoside D (100 mg), F (100 mg) or G (30 mg) was refluxed for 5 h. The reaction mixture was evaporated to dryness *in vacuo* to afford the 22-O-methyl derivative. 22-O-Methylglycoside D: a white powder from acetone–MeOH, (mp 174—177 °C (dec.)), $[\alpha]_D^{23}$ −74.1° (c=0.85, pyridine). ^{1}H-NMR (C_5D_5N) δ: 3.28 (3H, s, OCH_3). *Anal.* Calcd for $C_{46}H_{76}O_{18}\cdot H_2O$: C, 59.08; H, 8.40. Found: C, 58.92; H, 7.99. 22-O-Methylglycoside F: a white powder from acetone–MeOH, (mp 194—197 °C (dec.)), $[\alpha]_D^{24}$ −59.3° (c=0.83, pyridine), ^{1}H-NMR (C_5D_5N) δ: 3.27 (3H, s, OCH_3). *Anal.* Calcd for $C_{52}H_{86}O_{23}$: C, 57.87; H, 8.03. Found: C, 57.88; H, 8.17. 22-O-Methylglycoside G: a white powder from acetone–MeOH, (mp 208—211 °C (dec.)), $[\alpha]_D^{23}$ −64.8° (c=0.47, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1220 (S–O), ^{1}H-NMR (C_5D_5N) δ: 3.24 (3H, s, OCH_3).

Solvolysis of Glycoside E and Its Prosapogenin (16)——A solution of glycoside E (100 mg) in pyridine–dioxane (4:1 v/v, 20 ml) was heated on a water bath at 80 °C for 5 h. The reaction mixture was evaporated to dryness *in vacuo* and the residue was chromatographed on Sephadex LH-20 with MeOH to afford desulfated glycoside E (**14**). A solution of prosapogenin (**16**) in pyridine–dioxane (4:1 v/v, 5 ml) was treated in the same manner as described above to afford a desulfated derivative (**17**). **14**: colorless needles from aq. MeOH, mp 266—267 °C (dec.), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 982, 920, 900, 865 (intensity 920<900, 25(R)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: arabinose ($\xrightarrow{1}$aglycone) 100.2 (C_1), 75.6* (C_2), 75.5* (C_3), 69.9 (C_4), 67.1 (C_5); rhamnose ($\xrightarrow{2}$arabinose) 101.6 (C_1), 72.5** (C_2), 72.7** (C_3), 74.3 (C_4), 69.4 (C_5), 18.9 (C_6) (assignments marked *, ** may be reversed). *Anal.* Calcd for

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$C_{38}H_{60}O_{11} \cdot 3/2H_2O$: C, 63.40; H, 8.82. Found: C, 63.37; H, 8.82. **17**: a white powder from MeOH, (mp 168—170 °C (dec.)), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 982, 920, 900, 865 (intensity 920<900, 25(*R*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: arabinose 102.2 (C_1), 72.8 (C_2), 74.8 (C_3), 69.6 (C_4), 67.5 (C_5).

On the other hand, a part of each reaction mixture described above was examined by paper partition chromatography [PPC; Tōyō Roshi No. 50 paper; BuOH–MeOH–H_2O (1 : 3 : 1 v/v); detection by spraying a solution of $BaCl_2$ (100 mg) in 70% MeOH (50 ml), then drying the filter paper and spraying a potassium rhodizonate (10 mg) solution in 50% MeOH (50 ml)]; sulfate ion in the hydrolysate was detected as yellow spot at *Rf* 0.31.

Methylation of Prosapogenin 2b (10) and Desulfated Glycoside E (14) by Hakomori's Method——According to Hakomori's method, NaH (50 mg) was defatted with anhydrous benzene followed with petroleum ether, then warmed with dimethylsulfoxide (DMSO, 5 ml) at 70 °C in an oil bath for 1 h with stirring under N_2 flow. A solution of **10** (30 mg) in DMSO (3 ml) was added and the mixture was stirred. After dilution with water, the reaction mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and evaporated to dryness. The residue was chromatographed on Sephadex LH-20 with $CHCl_3$ to afford a hepta-*O*-methyl derivative of **10** (**11**).

A solution of **14** (50 mg) in DMSO (3 ml) was treated in the same manner as described above to afford a hexa-*O*-methyl derivative of **14** (**15**). **11**: a white powder from methanol, (mp 100—103 °C), IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 985, 922, 905, 870 (intensity, 922<905, 25(*R*)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 3.37, 3.39, 3.62 (each 3H, s, OCH_3), 3.49, 3.54 (each 6H, s, $OCH_3 \times 2$), 4.31, 4.33 (each 1H, d, J=7 Hz, glucose anomeric H). **15**: colorless needles from MeOH, mp 200—202 °C, IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 982, 920, 900, 865 (intensity 920<900, 25(*R*)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 0.79 (3H, s, CH_3; 3H, d, J=6 Hz, CH_3), 0.95 (3H, d, J=6 Hz, CH_3), 1.05 (3H, s, CH_3), 1.25 (3H, d, J=6 Hz, CH_3), 1.28 (3H, d, J=6 Hz, CH_3), 3.34, 3.40, 3.49, 3.51 (each 3H, s, OCH_3), 3.45 (6H, s, $OCH_3 \times 2$), 5.12 (1H, d, J=1 Hz, rhamnose anomeric H), 5.54 (1H, d, J=6 Hz, arabinose anomeric H). *Anal.* Calcd for $C_{44}H_{72}O_{11}$: C, 68.00; H, 9.34. Found: C, 67.73; H, 9.84.

Methanolysis of Per-*O*-methyl Derivatives of 11 and 15——The per-*O*-methyl derivative, **11** (5 mg) or **15** (50 mg), was refluxed with methanolic 5% HCl (each 1.5 ml) for 2 h, and the reaction mixture was neutralized with Ag_2CO_3 then evaporated to dryness. The residue was examined by TLC and GLC. Aglycone: TLC (solvent d) *Rf* 0.52 diosgenin. **15**: TLC (solvent: benzene–acetone (4 : 1 v/v) *Rf* 0.37 ruscogenin 3-*O*-methyl ether. *cf.* ruscogenin 1-*O*-methyl ether *Rf* 0.25). *O*-Methylsugars: **11**, TLC (solvent d) *Rf* 0.39, 0.50 (per-*O*-methylglucopyranoside), 0.18 (methyl 2,3,6-tri-*O*-methylglucopyranoside). GLC (column temp., 160 °C; injection temp., 200 °C; carrier gas N_2 1.0 kg/cm^2). t_R (min) 2.6, 3.7 (per-*O*-methylglucopyranoside), 8.0, 10.7 (methyl 2,3,6-tri-*O*-methylglucopyranoside). **15**: TLC (solvent d) *Rf* 0.52 (per-*O*-methylrhamnopyranoside), 0.11 (methyl 3,4-di-*O*-methylarabinopyranoside). GLC (column temp., 150 °C; injection temp., 200 °C, carrier gas N_2 1.0 kg/cm^2) t_R (min) 1.7, 2.2 (per-*O*-methylrhamnopyranoside), 7.7 (methyl 3,4-di-*O*-methylarabinopyranoside).

Marker's Degradation of Glycoside G——A solution of glycoside G (120 mg) in Ac_2O–pyridine (1 : 1 v/v, 10 ml) was allowed to stand overnight at room temperature. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water and evaporated to dryness. The residue (115 mg) was dissolved in Ac_2O (10 ml) and the solution was refluxed for 1 h, then concentrated to dryness *in vacuo*. The residue was dissolved in acetic acid (2.4 ml) and AcONa (48 mg), and a solution of chromic trioxide (80 mg) in 40% AcOH (0.6 ml) was added over 15 min with stirring and cooling (below 15 °C).

After the reaction mixture had been stirred for 1 h, excess reagent was decomposed with MeOH. The reaction mixture was diluted with water (50 ml) and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water and evaporated to dryness. The residue was incubated at 30 °C for 3.5 h with water (1.5 ml) containing KOH (170 mg) and *tert*-BuOH (4 ml). The reaction mixture was evaporated *in vacuo* to remove *tert*-BuOH and the aqueous solution was extracted with *n*-BuOH. The BuOH solution was washed with water and evaporated to dryness *in vacuo*. The residue was dissolved in 2 N HCl in 50% dioxane (10 ml) and the solution was heated for 1 h on a water bath, then diluted with water (10 ml) and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water and evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel using solvent d to afford 1,3-dihydroxypregna-5,16-dien-20-one (8 mg), colorless needles from hexane, mp 233—234 °C, ^{1}H-NMR ($CDCl_3$) δ 0.93 (3H, s, CH_3), 1.07 (3H, s, CH_3), 2.26 (3H, s, $OCOCH_3$), 3.46 (2H, m, C_1–H and C_3–H), 5.56 (1H, m, C_6–H), 6.70 (1H, t, J=4 Hz, C_{16}–H). This product was identified by mixed fusion and by comparing the TLC behavior and ^{1}H-NMR spectra with those of an authentic sample derived from ruscogenin by Marker's degradation.

The alkaline aqueous layer described above was acidified with acetic acid to pH 6 and extracted with *n*-BuOH. The BuOH solution was evaporated to dryness *in vacuo* and the residue was acetylated with Ac_2O (1 ml) and pyridine (1 ml) under heating for 1 h. The reaction mixture was evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and methylated with CH_2N_2 in ether. Excess CH_2N_2 in the reaction mixture was decomposed with acetic acid and the solvent was evaporated off under reduced pressure. The residue was purified by column chromatography on silica gel using solvent d to afford methyl γ-methyl-δ-hydroxypentanoate β-D-glucopyranoside tetraacetate (7 mg) as a colorless syrup, ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, d, J=6 Hz, CH–CH_3), 2.01 (3H, s, $OCOCH_3$), 2.02 (3H, s, $OCOCH_3$), 2.05 (3H, s, $OCOCH_3$), 2.09 (3H, s, $OCOCH_3$), 3.66 (3H, s, $COOCH_3$), 4.52 (1H, d, J=7 Hz, anomeric H). MS *m/z*: 331, 243, 242, 200, 169, 157, 145, 140, 129.098 ($C_7H_{13}O_2^+$), 115, 109.

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(284 g) was extracted with hot MeOH. The extracts were combined and evaporated to dryness *in vacuo*. The residue was treated by the method described above. The butanol-soluble fraction was concentrated *in vacuo* to afford a brown powder (11.2 g), which was examined for the presence of glycosides B—G by TLC (solvent c). *Rf* 0.40 (glycoside B), 0.28 (glycoside C), 0.15 (glycoside D), 0.14 (glycoside E), 0.06 (glycoside F), 0.03 (glycoside G).

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