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Comparative Studies on the Constituents of Ophiopogonis Tuber and Its Congeners. III.[1] Studies on the Constituents of the Subterranean Part of *Ophiopogon ohwii* OKUYAMA and *O. jaburan* (KUNTH) LODD.

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Six steroidal glycosides, tentatively named glycosides O-1, O-2 (**1**), O-3 (**2**), O-4 (**3**), O-5 (**4**) and O-6 (**5**), were isolated from the subterranean part of *Ophiopogon ohwii* OKUYAMA (Liliaceae) and another six steroidal glycosides, tentatively named glycosides J-1, J-2 (**6**), J-3 (**7**), J-4 (**8**), J-5 (**9**) and J-6 (**10**), were isolated from the subterranean part of *O. jaburan* (KUNTH) LODD. Glycosides O-1 and J-1 were identified as so-called β-sitosterol β-D-glucopyranoside. The structures of **1**—**10** were established as ophiopogonin B (**1**), ophiopogonin D (**2**), ruscogenin 1-*O*-α-L-rhamnopyranosyl-(1→2)-4-*O*-sulfo-β-D-fucopyranoside (**3**), ruscogenin 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-α-L-arabinopyranoside (=glycoside E of *O. planiscapus* NAKAI) (**4**), 26-*O*-β-D-glucopyranosyl 22-hydroxy-25(*R*)-furost-5-en-1β,3β,26-triol 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-β-D-fucopyranoside (**5**), ophiopogonin D (=glycoside O-3 (**2**)) (**6**), a mixture of 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-β-D-fucopyranosido-3-*O*-β-D-glucopyranosides of ruscogenin and neoruscogenin (**7**), neoruscogenin 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-α-L-arabinopyranosido-3-*O*-β-D-glucopyranoside (**8**), a mixture of 26-*O*-β-D-glucopyranosyl 22-hydroxy-25(*R*)-furost-5-en-1β,3β,26-triol 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-β-D-fucopyranosido-3-*O*-β-D-glucopyranoside and 26-*O*-β-D-glucopyranosyl 22-hydroxyfurost-5,25(27)-dien-1β,3β,26-triol 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-β-D-fucopyranosido-3-*O*-β-D-glucopyranoside (**9**), and 26-*O*-β-D-glucopyranosyl 22-hydroxyfurost-5,25(27)-dien-1β,3β,26-triol 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-α-L-arabinopyranosido-3-*O*-β-D-glucopyranoside (**10**). It is interesting that the main saponins, ophiopogonins B and D, found in Ophiopogonis Tuber (obtained from *O. japonicus* KER-GAWLER) were also found in *O. ohwii* OKUYAMA and the latter saponin was also found in *O. jaburan* (KUNTH) LODD. Further, several steroidal glycosides carrying sulfate on the sugar moiety were found in Liliaceous plants belonging to the genus Ophiopogon.

Keywords——Ophiopogonis Tuber; *Ophiopogon ohwii*; *Ophiopogon jaburan*; Liliaceae; spirostanol glycoside; furostanol glycoside; sulfated steroidal glycoside

In a series of papers on the constituents of Ophiopogonis Tuber and its congeners, we have reported the isolation and structure elucidation of eight steroidal saponins from the subterranean part of *Liriope platyphylla* WANG *et* TANG[2] and six steroidal saponins from *Ophiopogon planiscapus* NAKAI.[1] The present paper deals mainly with the isolation and structure elucidation of steroidal glycosides of the subterranean parts of Liliaceous plants, *Ophiopogon ohwii* OKUYAMA (Japanese name: Nagabajanohige) harvested at Kyoto Herbal Garden, Central Research Division, Takeda Chemical Industries, Ltd. and *O. jaburan* (KUNTH) LODD. (Japanese name: Noshiran) harvested at Yachiyo Farm, Keisei Rose Nursery Inc.

Fresh subterranean parts of *O. ohwii* OKUYAMA and *O. jaburan* (KUNTH) LODD. were each extracted with hot methanol and both methanol extracts were treated by the method

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described in the experimental section. Six steroidal glycosides, tentatively named glycosides O-1, O-2 (**1**), O-3 (**2**), O-4 (**3**), O-5 (**4**) and O-6 (**5**) were isolated from the former, and six steroidal glycosides, tentatively named glycosides J-1, J-2 (**6**), J-3 (**7**), J-4 (**8**), J-5 (**9**) and J-6 (**10**), were isolated from the latter.

Glycosides O-1 and J-1 were positive in the Liebermann–Burchard reaction, and, on hydrolysis with 2 N hydrogen chloride, each compound gave glucose and a mixture of β-sitosterol, campesterol and stigmasterol, which were identified by gas liquid chromatography (GLC). Accordingly, glycosides O-1 and J-1 were concluded to be so-called β-sitosterol β-D-glucopyranoside.

Glycosides O-2 (**1**), O-3 (**2**) and J-2 (**6**) were positive in the Liebermann–Burchard reaction, and they showed strong absorption due to hydroxyl groups and characteristic absorption bands of a 25(*R*)-spiroketal moiety in the infrared (IR) spectra.[3] On hydrolysis with 2 N hydrochloric acid in 50% dioxane, **1** gave ruscogenin,[4] fucose and rhamnose, while **2** and **6** afforded ruscogenin, fucose, rhamnose and xylose. From the chemical and physical properties, **1** appeared to be identical with ophiopogonin B,[5] while **2** and **6** were suggested to be identical with ophiopogonin D,[6] both of which were reported by us as constituents of Ophiopogonis Tuber. Finally, glycoside O-2 was identified as ophiopogonin B (=ruscogenin 1-*O*-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside) and glycosides O-3 and J-2 were proved to be identical with ophiopogonin D (=ruscogenin 1-*O*-[α-L-rhamnopyranosyl-(1→2)][β-D-xylopyranosyl-(1→3)]-β-D-fucopyranoside) by comparisons of thin-layer chromatographic (TLC) behavior, melting point, IR and ^{13}C-nuclear magnetic resonance (^{13}C-NMR) spectra.

Glycoside O-4 (**3**), $C_{39}H_{61}O_{12}\cdot SO_3M$, is positive in the Liebermann–Burchard reaction and potassium rhodizonate test.[7] Based on the 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,[8] **3** was suggested to be a sulfate of a spirostanol glycoside. On heating with pyridine–dioxane,[9] **3** gave a desulfated glycoside, which afforded ruscogenin, fucose and rhamnose on acidic hydrolysis. Based on the TLC behavior, and IR and ^{13}C-NMR spectra, the desulfated glycoside was suggested to be identical with ophiopogonin B (=glycoside O-2) and this was confirmed by direct comparisons with an authentic sample. The location of the sulfate group was determined by comparative analyses of ^{13}C-NMR spectra. The corresponding carbon signals of the ring A of ruscogenin and rhamnose of ophiopogonin B and **3** were nearly superimposable, but a low-field shift of the C_4-carbon signal of fucose and slight high-field shifts of both the C_3- and C_5-carbon signals of fucose were observed. Furthermore, enzymatic hydrolysis of **3** with crude pectinase afforded rhamnose and a prosapogenin (**11**), which showed an S–O stretching absorption band at 1215 cm^{-1} in the IR spectrum and gave a positive reaction in the potassium rhodizonate test. As in the case of **3** described above, **11** was desulfated by heating with pyridine–dioxane to afford a desulfated prosapogenin (**12**), which no longer shows an S–O stretching absorption band in the IR spectrum. By comparative analyses of the ^{13}C-NMR spectra[1] of **11** and **12**, the location of the sulfate group was deduced to be the C_4-hydroxyl group of fucose, because a low-field shift of the C_4-carbon signal (+6.0 ppm) and high-field shifts of both the C_3-carbon signal (−0.7 ppm) and C_5-carbon signal (−0.6 ppm) were observed. Consequently, the structure of glycoside O-4 was established to be ruscogenin 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-β-D-fucopyranoside.

Glycoside O-5 (**4**), $C_{38}H_{59}O_{12}\cdot SO_3M$, is positive in the Liebermann–Burchard reaction and in the potassium rhodizonate test. The IR spectrum of **4** shows strong absorption of hydroxyl groups, characteristic absorption bands of a 25(*R*)-spiroketal moiety and an S–O stretching absorption band. On acidic hydrolysis, **4** gave ruscogenin, arabinose and rhamnose, while on refluxing with pyridine–dioxane, **4** afforded its desulfated derivative (**13**).

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Furthermore, enzymatic hydrolysis of **4** with crude pectinase afforded rhamnose and a prosapogenin (**14**), which showed an S–O stretching absorption band in the IR spectrum. The general properties of **4**, **13** and **14** appeared to be identical with those of glycoside E,[1,10] desulfated glycoside E[1,11] and a prosapogenin of glycoside E, respectively, reported by us as constituents of *Ophiopogon planiscapus* NAKAI. Finally, glycoside O-5, desulfated glycoside O-5 and a prosapogenin of glycoside O-5 were identified as ruscogenin 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-α-L-arabinopyranoside, ruscogenin 1-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside and ruscogenin 1-*O*-(4-*O*-sulfo)-α-L-arabinopyranoside, respectively, by comparisons of TLC behavior and ^{13}C-NMR spectra.

Glycoside O-6 (**5**), $C_{45}H_{73}O_{18}\cdot SO_3M$, is positive in the Liebermann–Burchard reaction, the Ehrlich reaction[12] and the potassium rhodizonate test. The IR spectrum of **5** shows an S–O stretching absorption band, but it does not show any characteristic spiroketal absorption band. Based on the easy chemical conversion of **5** into an *O*-methyl derivative by refluxing with methanol,[1] **5** was suggested to be a sulfated furostanol glycoside. On enzymatic hydrolysis with almond emulsin, **5** afforded glucose and a prosapogenin, which was identified as glycoside O-4 (**3**) by comparisons of TLC behavior and IR and ^{13}C-NMR spectra. Consequently, the structure of glycoside O-6 was established to be 26-*O*-β-D-glucopyranosyl 22-hydroxy-25(*R*)-furost-5-en-1β,3β,26-triol 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-β-D-fucopyranoside.

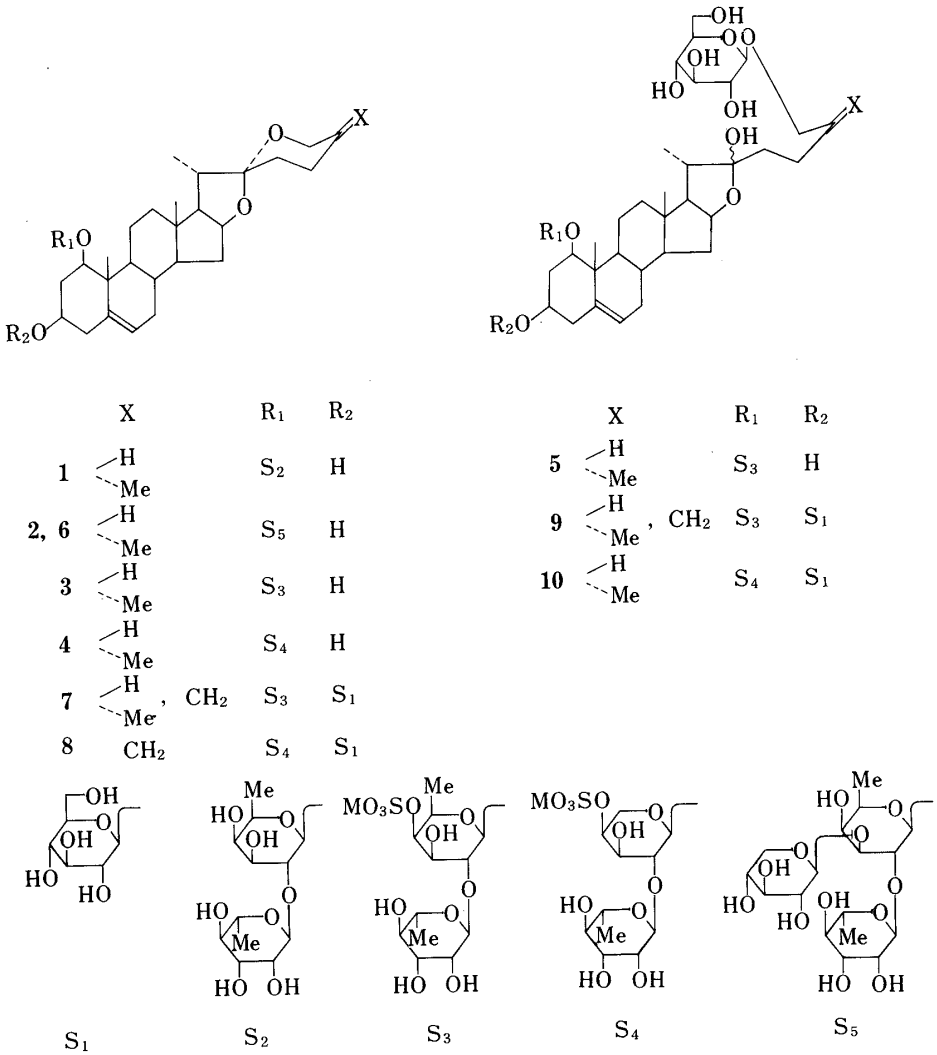

Chart 1

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Glycoside J-4, (**8**), $C_{44}H_{67}O_{17}\cdot SO_3M$, is positive in the Liebermann–Burchard reaction and potassium rhodizonate test, and it shows strong absorption of hydroxyl groups, characteristic absorption bands of a spiroketal moiety and an S–O stretching absorption band at 1230 cm^{-1} in the IR spectrum. On heating with pyridine–dioxane, **8** gave a desulfated glycoside (**15**), which no longer shows an S–O stretching absorption band in the IR spectrum. On acidic hydrolysis, **15** afforded neoruscogenin,[13] arabinose, rhamnose and glucose. The ^{13}C-NMR spectrum of **15** showed three anomeric carbon signals at δ 100.1, 101.4 and 102.6. By comparative analyses of the ^{13}C-NMR spectra of **15** and neoruscogenin, the sugar moieties of **15** are suggested to be conjugated to the C_1- and C_3-hydroxyl groups of neoruscogenin, because low-field shifts of the C_1-carbon signal (+4.8 ppm) and C_3-carbon signal (+5.7 ppm) and high-field shifts of the C_2-carbon signal (−9.1 ppm), C_4-carbon signal (−3.8 ppm) and C_{10}-carbon signal (−0.5 ppm) were observed. On enzymatic hydrolysis with almond emulsin for two weeks, **8** gave glucose and a prosapogenin (**16**), which gives a positive reaction in the potassium rhodizonate test and shows an S–O stretching absorption band in the IR spectrum. Compound **16** was treated with pyridine–dioxane as described above to afford a desulfated prosapogenin (**17**), which did not show an S–O stretching absorption band in the IR spectrum. On acidic hydrolysis, **17** gave neoruscogenin, arabinose and rhamnose, and the sugar composition of **17** was shown to be identical with that of desulfated glycoside of glycoside E obtained from *O. planiscapus* NAKAI.[1] In the ^{13}C-NMR spectra of **16**, **17** and glycoside E, the chemical shifts of carbon signals due to the ring A and sugar moieties of these glycosides agreed well with each other. Based on the results described above, the locations of the sulfate groups of **8** and **16** were deduced to be the C_4-hydroxyl group of arabinose, and the structure of **8** was deduced to be neoruscogenin 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-α-L-arabinopyranosido-3-*O*-β-D-glucopyranoside.

Glycoside J-6 (**10**), $C_{50}H_{79}O_{23}\cdot SO_3M$, is positive in the Liebermann–Burchard reaction, the Ehrlich reaction and the potassium rhodizonate test. The IR spectrum of **10** shows strong absorption of hydroxyl groups and an S–O stretching absorption band, but it does not show any absorption of a spiroketal moiety. Based on the easy chemical conversion of **10** into an *O*-methyl derivative by refluxing with methanol, **10** was suggested to be a sulfated furostanol glycoside. On enzymatic hydrolysis with almond emulsin, **10** gave glucose and glycoside J-4 (**8**) described above. Consequently, glycoside J-6 was concluded to be a proto-type glycoside of glycoside J-4 (**8**) and the structure of **10** was established to be 26-*O*-β-D-glucopyranosyl 22-hydroxyfurost-5,25(27)-dien-1β,3β,26-triol 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-α-L-arabinopyranosido-3-*O*-β-D-glucopyranoside.

Glycosides J-3 (**7**) and J-5 (**9**) each show one spot on TLC, and both glycosides are positive in the Liebermann–Burchard reaction and the potassium rhodizonate test. Furthermore, **9** is positive in the Ehrlich reaction and the IR spectrum shows a strong hydroxyl absorption band and an S–O stretching band, but no absorption band of a spiroketal moiety. Based on the color reactions and the easy chemical conversion of **9** into an *O*-methyl derivative by refluxing with methanol, **9** was suggested to be a sulfated furostanol glycoside. On enzymatic hydrolysis with almond emulsin, **9** afforded glucose and **7**, which showed hydroxyl, spiroketal and S–O absorption bands in the IR spectrum. The ^{13}C-NMR spectrum of **7** indicated that **7** might be a mixture of glycosides of 25(*R*)-spirostanol and $\Delta^{25(27)}$-spirostanol with the same sugar moieties. On refluxing with pyridine–dioxane, **7** afforded a desulfated glycoside (**18**), which gave fucose, rhamnose, glucose and a mixture of ruscogenin and neoruscogenin on acid hydrolysis. Both aglycones show one spot on TLC, and after acetylation each aglycone acetate was separated by column chromatography and identified. Consequently, **7** was concluded to be a mixture of ruscogenin and neoruscogenin glycosides. In order to elucidate the structure of the sugar moiety, **18** was methylated by Hakomori's method[14] to afford the nona-*O*-methyl derivative of **18**, which was methanolyzed

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to afford per-*O*-methylrhamnopyranose, per-*O*-methylglucopyranose, methyl 3,4-di-*O*-methylfucopyranoside and a mixture of ruscogenin and neoruscogenin. Furthermore, **7** was hydrolyzed with almond emulsin for two weeks to afford glucose and a prosapogenin (**19**), which was positive in the potassium rhodizonate test. The ^{13}C-NMR spectrum of **19** was almost superimposable on that of glycoside O-4 (**3**) except for the signals corresponding to the aglycone. In conclusion, the location of the sulfate group was deduced to be on the C_4-hydroxyl group, and **7** and **9** were established to be a mixture of 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-β-D-fucopyranosido-3-*O*-β-D-glucopyranosides of ruscogenin and neoruscogenin, and a mixture of 26-*O*-β-D-glucopyranosyl 22-hydroxy-25(*R*)-furost-5-en-1β,3β,26-triol 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-β-D-fucopyranosido-3-*O*-β-D-glucopyranoside and 26-*O*-β-D-glucopyranosyl 22-hydroxyfurost-5,25(27)-dien-1β,3β,26-triol 1-*O*-α-L-rhamnopyranosyl(1→2)-4-*O*-sulfo-β-D-fucopyranosido-3-*O*-β-D-glucopyranoside, respectively.

The present studies on the constituents of subterranean parts of *O. ohwii* OKUYAMA and *O. jaburan* (KUNTH) LODD. have been carried out as part of a series of comparative studies on the constituents of Ophiopogonis Tuber and its congeners, and twelve steroidal glycosides were isolated from the two plants. It was found that ophiopogonins B and D, the main constituents of Ophiopogonis Tuber, were also present in *O. ohwii* OKUYAMA and the latter saponin was also found in *O. jaburan* (KUNTH) LODD. Furthermore, some sulfated steroidal glycosides found in *Liriope platyphylla* WANG *et* TANG and *O. planiscapus* NAKAI were also found in *O. ohwii* OKUYAMA and *O. jaburan* (KUNTH) LODD. It is interesting that the sulfate group of the new steroidal glycosides is located on the sugar moieties, as in the case of steroidal glycosides found in *O. planiscapus* NAKAI. All the steroidal glycosides isolated from total subterranean parts of the two plants were also detected by TLC in the butanol extract of the tubers.

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., 210 °C; carrier gas N_2, 1.0 kg/cm^2; samples, trimethyl silyl (TMS) ether, (b) for *O*-methylated sugars: column, 5% neopenthyl glycol succinate (NPGS) on Shimalite 3 mm × 2 m; column temp., 170 °C; injection temp., 230 °C; carrier gas N_2, 1.0 kg/cm^2. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck) using the following solvents. (a) $CHCl_3$–MeOH–H_2O (7 : 1.5 : 0.1, v/v), (b) $CHCl_3$–MeOH–H_2O (7 : 2 : 0.2, v/v), (c) $CHCl_3$–MeOH–H_2O (7 : 3 : 0.4, v/v), (d) $CHCl_3$–MeOH–AcOEt–H_2O (2 : 2 : 4 : 1, v/v, lower phase). 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 spots were detected by spraying aniline hydrogen phthalate reagent. The solvent systems (solv. a, b, c and d) used for TLC were also applied for column chromatography.

Extraction and Isolation of Glycosides——i) The fresh subterranean parts of *Ophiopogon ohwii* OKUYAMA (4 kg) cultivated at Kyoto Herbal Garden, Central Research Division, Takeda Chemical Industries, Ltd. were crushed and extracted with hot MeOH (9 l × 4). The extract was combined and evaporated to dryness *in vacuo*. The residue (388 g) was dissolved in water and extracted with ether to afford the ether-soluble fraction (33.5 g). The aqueous layer was extracted with BuOH saturated with water, and the BuOH-soluble fraction was concentrated *in vacuo* to afford a brown powder (65.5 g), which was subjected to column chromatography on Avicel with $CHCl_3$, $CHCl_3$–MeOH–H_2O (70 : 20 : 10, v/v, lower phase) and then MeOH to obtain four fractions (Fr. 1—4). 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 (16 g) was combined with Fr. 4 (8.9 g) of the BuOH-soluble fraction described above. Fr. 1 (11.6 g) was subjected to column chromatography on silica gel with $CHCl_3$–MeOH (9 : 1, v/v) to afford glycoside O-1

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(β-sitosterol β-D-glucopyranoside, 131 mg). Fraction 2 was purified by silica gel column chromatography with solvent a to afford glycoside O-2 (**1**, 59 mg) and glycoside O-3 (**2**, 198 mg). Fraction 3 was separated by medium pressure column chromatography on Kieselgel H with solvent b to afford glycosides O-4 (**3**, 250 mg) and O-5 (**4**, 120 mg). Fraction 4 (24.9 g) was subjected to column chromatography on Sephadex LH-20 with MeOH followed by column chromatography on Kieselgel H with solvent c to afford glycoside O-6 (**5**, 432 mg).

ii) The fresh subterranean parts of *Ophiopogon jaburan* (KUNTH) LODD. (4 kg) cultivated at Yachiyo Farm, Keisei Rose Nursery Inc., were crushed and extracted with hot MeOH (9 l×4). The extract was combined and evaporated to dryness *in vacuo*. The residue (424 g) was dissolved in water and extracted with ether to afford the ether-soluble fraction (65.5 g). The aqueous layer was extracted with BuOH saturated with water, and the BuOH-soluble fraction was concentrated *in vacuo* to afford a brown powder (67.7 g), which was subjected to column chromatography on Avicel with $CHCl_3$, $CHCl_3$–MeOH–H_2O (70 : 15 : 10 and 70 : 30 : 10, v/v, lower phase) and then MeOH to obtain four fractions (Fr. 1—4). 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 (19 g) was combined with Fr. 4 (12 g) of the BuOH-soluble fraction described above. Fr.1 (6.7 g) was subjected to column chromatography on silica gel with $CHCl_3$–MeOH (9 : 1, v/v) to afford glycoside J-1 (β-sitosterol β-D-glucopyranoside, 141 mg). Fraction 2 (18.5 g) was purified by silica gel column chromatography with solvent a to afford glycoside J-2 (**6**, 174 mg). Fraction 3 (25 g) was subjected to column chromatography on Sephadex LH-20 with MeOH followed by medium pressure chromatography on Kieselgel H with solvent b to afford glycosides J-3 (**7**, 370 mg) and J-4 (**8**, 285 mg). Fraction 4 (31 g) was subjected to column chromatography on Sephadex LH-20 with MeOH followed by column chromatography on silica gel with BuOH saturated with H_2O to afford glycosides J-5 (**9**, 517 mg) and J-6 (**10**, 624 mg).

Properties of Glycosides O-1 and J-1 (So-called β-Sitosterol β-D-Glucopyranoside)——Colorless needles from pyridine, mp 295—297 °C (dec.), 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; sample, TMS ether). 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 13 : 1 : 2 for *O. ohwii* and 2 : 1 : 1 for *O. jaburan*. Each 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 O-2, O-3, O-4, O-5, O-6, J-3, J-4, J-5 and J-6——Glycoside O-2 (**1**): colorless needles from EtOH, mp 270—272 °C (dec.) (lit.[5] mp 269—271 °C (dec.)), $[\alpha]_D^{21}$ −102.7° (*c*=0.42, pyridine) (lit.[5] $[\alpha]_D^{15}$ −105.5° (*c*=0.31)). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 985, 925, 905, 870 (intensity 925<905, 25(*R*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: fucose (1→aglycone) 100.4 (C_1), 74.9 (C_2), 76.8 (C_3), 73.3 (C_4), 71.1 (C_5), 17.2 (C_6); rhamnose (2→fucose) 101.6 (C_1), 72.6* (C_2), 72.7* (C_3), 74.4 (C_4), 69.2 (C_5), 19.0 (C_6). (* may be reversed). *Anal.* Calcd for $C_{39}H_{62}O_{12}\cdot H_2O$: C, 63.22; H, 8.71. Found: C, 63.02; H, 9.02. Glycosides O-3 (**2**) and J-2 (**6**): colorless needles from aqueous EtOH, mp 265—267 °C (dec.) (lit.[6] mp 263—265 °C (dec.)), $[\alpha]_D^{21}$ −105.8° (*c*=0.71, pyridine) (lit.[6] $[\alpha]_D^{14}$ −107.9° (*c*=0.66, pyridine)). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 983, 920, 900, 865 (intensity 920<900, 25(*R*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: fucose (1→aglycone) 100.5 (C_1), 81.2 (C_2), 85.6 (C_3), 73.3 (C_4), 70.9* (C_5), 17.1 (C_6); rhamnose (2→fucose) 101.4 (C_1), 72.3** (C_2), 72.5** (C_3), 74.2*** (C_4), 69.2 (C_5), 19.6 (C_6); xylose (3→fucose) 106.2 (C_1), 74.5*** (C_2), 77.9 (C_3), 70.7* (C_4), 66.8 (C_5). (*, **, *** may be reversed). *Anal.* Calcd for $C_{44}H_{70}O_{16}\cdot H_2O$: C, 60.53; H, 8.31. Found: C, 60.75; H, 8.73. Glycoside O-4 (**3**): colorless needles from MeOH, mp 228—231 °C (dec.), $[\alpha]_D^{21}$ −98.2° (*c*=0.33, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1215 (S–O), 985, 925, 905, 870 (intensity 925<905, 25(*R*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: 4-*O*-sulfo-fucose (1→aglycone) 100.3 (C_1), 76.0* (C_2), 75.7* (C_3), 79.1 (C_4), 70.6 (C_5), 17.3 (C_6); rhamnose (2→fucose) 101.5 (C_1), 72.3 (C_2), 72.3 (C_3), 74.3 (C_4), 69.3 (C_5), 18.9 (C_6). (* may be reversed). Glycoside O-5 (**4**): colorless needles from MeOH, mp 219—221 °C (dec.) (lit.[1] mp 220—221 °C (dec.)), $[\alpha]_D^{20}$ −80.7° (*c*=0.21, pyridine) (lit.[1] $[\alpha]_D^{23}$ −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 100.1 (C_1), 75.9* (C_2), 74.5 (C_3), 76.0* (C_4), 65.5 (C_5); rhamnose (2→arabinose) 101.2 (C_1), 72.1 (C_2), 72.1 (C_3), 74.0 (C_4), 69.3 (C_5), 18.7 (C_6). (* may be reversed). Glycoside O-6 (**5**): a white powder from aqueous acetone, (mp 183—185 °C (dec.)), $[\alpha]_D^{23}$ −50.8° (*c*=0.31, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1210 (S–O). ^{13}C-NMR (C_5D_5N) δ: 4-*O*-sulfo-fucose (1→aglycone) 100.1 (C_1), 76.0* (C_2), 75.9* (C_3), 79.0 (C_4), 70.5 (C_5), 17.3 (C_6); rhamnose (2→fucose) 101.3 (C_1), 71.9** (C_2), 72.2** (C_3), 74.2 (C_4), 69.2 (C_5), 18.8 (C_6); glucose (26→aglycone) 104.7 (C_1), 75.1 (C_2), 87.3 (C_3), 72.2** (C_4), 78.1 (C_5), 62.9 (C_6). (*, ** may be reversed). Glycoside J-3 (**7**): colorless needles from MeOH, mp 187—190 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1225 (S–O), 980, 920, 900, 880 (spiroketal). ^{13}C-NMR (C_5D_5N) δ: 4-*O*-sulfo-fucose (1→aglycone) 100.1 ($J_{C_1-H_1}$=158 Hz, β-C_1), 75.9* (C_2), 75.7* (C_3), 78.9 (C_4), 70.4 (C_5), 17.3 (C_6); rhamnose (2→fucose) 101.4 ($J_{C_1-H_1}$=170 Hz, α-C_1), 72.2 (C_2), 72.2 (C_3), 75.1** (C_4), 69.3 (C_5), 18.8 (C_6); glucose (3→aglycone) 102.4 ($J_{C_1-H_1}$=160 Hz, β-C_1), 75.2** (C_2), 78.2*** (C_3), 71.7 (C_4), 78.0*** (C_5), 62.7 (C_6). (*, **, *** may be reversed). Glycoside J-4 (**8**): a white powder from EtOH, (mp 234—

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236 °C (dec.)), $[\alpha]_D^{24}$ −81.3° (c=0.23, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1230 (S–O), 920, 880 (spiroketal). ^{13}C-NMR (C_5D_5N) δ: aglycone (=neoruscogenin) 83.0 (C_1), 34.9 (C_2), 73.9 (C_3), 39.8 (C_4), 138.5 (C_5), 125.5 (C_6), 33.2 (C_7), 32.2 (C_8), 50.3 (C_9), 43.1 (C_{10}), 24.0 (C_{11}), 40.2 (C_{12}), 40.2 (C_{13}), 56.7 (C_{14}), 32.5 (C_{15}), 81.5 (C_{16}), 62.9 (C_{17}), 16.6 (C_{18}), 14.9 (C_{19}), 42.1 (C_{20}), 14.9 (C_{21}), 109.4 (C_{22}), 33.4 (C_{23}), 29.0 (C_{24}), 144.6 (C_{25}), 65.0 (C_{26}), 108.4 (C_{27}). 4-*O*-sulfo-arabinose ($\xrightarrow{1}$aglycone) 99.9 ($J_{C_1-H_1}$=153 Hz, α-C_1), 75.9* (C_2), 74.8** (C_3), 76.0* (C_4), 65.7 (C_5); rhamnose ($\xrightarrow{2}$arabinose) 101.2 ($J_{C_1-H_1}$=172 Hz, α-C_1), 72.2 (C_2), 72.2 (C_3), 74.6** (C_4), 69.5 (C_5), 18.8 (C_6); glucose ($\xrightarrow{3}$aglycone) 102.5 ($J_{C_1-H_1}$=159 Hz, β-C_1), 75.1** (C_2), 78.1*** (C_3), 71.8 (C_4), 78.2*** (C_5), 62.8 (C_6). (*, **, *** may be reversed). Glycoside J-5 (**9**): a white powder from aqueous acetone, (mp 165—168 °C(dec.)). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1220 (S–O). Glycoside J-6 (**10**): a white powder from aqueous acetone, (mp 201—203 °C (dec.)), $[\alpha]_D^{24}$ −18.6° (c=0.80, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1220 (S–O). ^{13}C-NMR (C_5D_5N) δ: 4-*O*-sulfo-arabinose ($\xrightarrow{1}$aglycone) 99.8 (C_1), 76.0 (C_2), 75.0 (C_3), 76.0 (C_4), 65.7 (C_5); rhamnose ($\xrightarrow{2}$arabinose) 101.3 (C_1), 72.1 (C_2), 72.1 (C_3), 74.6 (C_4), 69.5 (C_5), 18.8 (C_6); glucose ($\xrightarrow{3}$aglycone) 102.3 (C_1), 75.0 (C_2), 78.1* (C_3), 71.8 (C_4), 78.2* (C_5), 62.7 (C_6); glucose ($\xrightarrow{26}$aglycone) 103.6 (C_1), 75.0 (C_2), 78.3* (C_3), 71.8 (C_4), 78.1* (C_5), 62.9 (C_6). (* may be reversed).

Preparations of Sodium Salt of 3, 4, 5, 8 and 10——An aqueous solution of **3** (100 mg), **4** (50 mg), **5** (100 mg), **8** (50 mg) or **10** (100 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 **3**: colorless needles from MeOH, mp 228—231 °C (dec.), *Anal.* Calcd for $C_{39}H_{61}O_{12}SO_3Na \cdot 2H_2O$: C, 54.40; H, 7.61; S, 3.72. Found: C, 54.46; H, 8.37; S, 3.60. Sodium salt of **4**: colorless needles from MeOH, mp 218—220 °C (dec.), *Anal.* Calcd for $C_{38}H_{59}O_{12}SO_3Na \cdot 2H_2O$: C, 53.89; H, 7.50; S, 3.79. Found: C, 53.84; H, 7.33; S, 3.59. Sodium salt of **5**: a white powder from aqueous acetone, (mp 185—186 °C (dec.)), *Anal.* Calcd for $C_{45}H_{73}O_{18}SO_3Na \cdot 2H_2O$: C, 51.91; H, 7.46; S, 3.08. Found: C, 51.72; H, 7.90; S, 3.53. Sodium salt of **8**: a white powder from EtOH, (mp 233—235 °C (dec.)), *Anal.* Calcd for $C_{44}H_{67}O_{17}SO_3Na$: C, 54.42; H, 6.98; S, 3.30. Found: C, 54.18; H, 7.24; S, 3.43. Sodium salt of **10**: a white powder from aqueous acetone, (mp 204—206 °C (dec.)), *Anal.* Calcd for $C_{50}H_{79}O_{23}SO_3Na$: C, 52.16; H, 6.92; S, 2.79. Found: C, 52.10; H, 7.54; S, 2.49.

Hydrolyses of 1, 2, 4, 6, 15, 17 and 18 with 2 N HCl——A solution of **1** (20 mg), **2** (40 mg), **4** (10 mg), **6** (50 mg), **15** (30 mg), **17** (5 mg) or **18** (60 mg) in 2 N HCl–50% dioxane (3 ml per 10 mg of glycoside) was refluxed for 2 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 **1**, **2**, **4** and **6** was purified by column chromatography on silica gel using hexane–acetone (2 : 1, v/v) to afford colorless needles from MeOH, mp 205—207 °C. IR ν_{max}^{Nujol} cm^{-1}: 3300 (OH), 980, 920, 900, 865 (intensity 920<900, 25(*R*)-spiroketal). TLC (solvent: hexane–acetone=2 : 1, v/v) *Rf* 0.10. The aglycones of **1**, **2**, **4** and **6** were identified as ruscogenin by mixed fusion and by comparing TLC behavior, and IR and ^{1}H-NMR spectra with those of an authentic sample.

On the other hand, the residues of **15** and **17** were purified by column chromatography on silica gel using hexane–acetone (2 : 1, v/v) to afford colorless needles from MeOH, mp 194—196 °C. IR ν_{max}^{Nujol} cm^{-1}: 3600—3200 (OH), 920, 880 (spiroketal). ^{1}H-NMR (C_5D_5N) δ: 0.81 (3H, s, CH_3), 0.84 (3H, d, J=7 Hz, CH_3), 1.05 (3H, s, CH_3), 4.77 (2H, $\Delta^{25(27)}$-CH_2), 5.55 (1H, m, C_6–H). The aglycones of **15** and **17** were both identified as neoruscogenin by comparisons of TLC behavior and IR and ^{1}H-NMR spectra.

The aglycone of **18** was acetylated with Ac_2O and pyridine in the usual way to afford a mixture of ruscogenin acetate and neoruscogenin acetate. The mixture was separated by column chromatography on silica gel using CH_2Cl_2. Ruscogenin acetate and neoruscogenin acetate were shown to be identical with authentic samples by mixed fusion and by comparing TLC behavior and IR and ^{1}H-NMR spectra. Neoruscogenin acetate: colorless needles from MeOH, mp 141—143 °C. IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1740 (ester), 910, 880 (spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 0.80 (3H, s, CH_3), 0.96 (3H, d, J=6 Hz, CH_3), 1.16 (3H, s, CH_3), 2.01 (3H, s, OAc), 2.02 (3H, s, OAc), 4.74 (2H, s, $\Delta^{25(27)}$-CH_2), 5.64 (1H, m, C_6–H). Ruscogenin acetate: colorless needles from MeOH, mp 198—200 °C. IR ν_{max}^{Nujol} cm^{-1}: 1735 (ester), 980, 920, 900, 868 (intensity 920<900, 25(*R*)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 0.78 (3H, s, CH_3; 3H, d, J= 6 Hz, CH_3), 0.96 (3H, d, J=6 Hz, CH_3), 1.16 (3H, s, CH_3), 2.01 (3H, s, OAc), 2.02 (3H, s, OAc), 3.46 (2H, m, C_{26}–H_2), 5.62 (1H, m, C_6–H).

Sugars: The aqueous layer was neutralized with Amberlite IR-45 and concentrated to dryness *in vacuo*. The monosaccharides were examined by TLC and GLC. **1**: TLC *Rf* 0.28 (fucose), 0.38 (rhamnose). GLC t_R (min) 5.1, 6.1 (fucose), 4.2, 5.7 (rhamnose). **2** and **6**: TLC *Rf* 0.28 (fucose), 0.38 (rhamnose), 0.31 (xylose). GLC t_R (min) 5.1, 6.1 (fucose), 4.2, 5.7 (rhamnose), 6.1, 7.7 (xylose). **4** and **17**: TLC *Rf* 0.30 (arabinose), 0.38 (rhamnose). GLC t_R (min) 4.1, 4.6 (arabinose), 4.2, 5.7 (rhamnose). **15**: TLC *Rf* 0.30 (arabinose), 0.38 (rhamnose), 0.19 (glucose). GLC t_R (min) 4.1, 4.6 (arabinose), 4.2, 5.7 (rhamnose), 14.2, 22.5 (glucose). **18**: TLC *Rf* 0.28 (fucose), 0.38 (rhamnose), 0.19 (glucose). GLC t_R (min) 5.1, 6.1 (fucose), 4.2, 5.7 (rhamnose), 14.2, 22.5 (glucose).

Enzymatic Hydrolyses of 3, 4, 5, 7, 8, 9 and 10——i) An aqueous solution of **3** (70 mg) in H_2O (15 ml) or **4** (30 mg) in H_2O (10 ml) was incubated with crude pectinase 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

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by column chromatography on silica gel using solvent b to afford the prosapogenin, **11** or **14**, respectively. **11**: a white powder from MeOH, (mp 224—225 °C (dec.)). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1215 (S–O), 980, 920, 905, 865 (intensity 920 < 905, 25(*R*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: 4-*O*-sulfo-fucose ($\xrightarrow{1}$aglycone) 102.2 (C_1), 72.5 (C_2), 74.8 (C_3), 78.7 (C_4), 70.8 (C_5), 17.6 (C_6). **14**: 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: 4-*O*-sulfo-arabinose ($\xrightarrow{1}$aglycone) 102.1 (C_1), 72.6 (C_2), 73.8 (C_3), 76.2 (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* 0.38) in each case.

ii) A solution of 100 mg of **5**, **9**, or **10** in 20 ml of water was incubated with almond emulsin at 37 °C for 24 h. The reaction mixture was extracted with BuOH and the BuOH extract was concentrated *in vacuo*. The residue was chromatographed on silica gel using solvent b for **5** and solvent c for **9** and **10** to afford a prosapogenin. The prosapogenins of **5**, **9** and **10** were identified as **3**, **7** and **8**, respectively, by comparing TLC behavior and IR and ^{13}C-NMR spectra.

iii) **8** or **7** (50 mg) was dissolved in 10 ml of water and incubated with almond emulsin at 37 °C for 2 weeks. The reaction mixture was extracted with BuOH and the BuOH extract was concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel using solvent b to afford the prosapogenin **16** or **19**, respectively. **16**: a white powder from EtOH, (mp 112—115 °C (dec.)). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1220 (S–O), 920, 880 (spiroketal). ^{13}C-NMR (C_5D_5N) δ: 4-*O*-sulfo-arabinose ($\xrightarrow{1}$aglycone) 100.2 (C_1), 75.9* (C_2), 74.8 (C_3), 76.0* (C_4), 65.3 (C_5); rhamnose ($\xrightarrow{2}$arabinose) 101.5 (C_1), 72.5 (C_2), 72.5 (C_3), 74.1 (C_4), 69.5 (C_5), 19.1 (C_6). **19**: a white powder from MeOH, (mp 145—147 °C (dec.)). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1220 (S–O), 985, 925, 900, 870 (spiroketal). ^{13}C-NMR (C_5D_5N) δ: 4-*O*-sulfo-fuco ($\xrightarrow{1}$aglycone) 100.3 (C_1), 76.0* (C_2), 75.6* (C_3), 78.7 (C_4), 70.4 (C_5), 17.3 (C_6); rhamnose ($\xrightarrow{2}$fucose) 101.3 (C_1), 72.3 (C_2), 72.3 (C_3), 74.0 (C_4), 69.1 (C_5), 18.8 (C_6). (* may be reversed).

Solvolyses of 3, 4, 7, 8, 11 and 16——A solution of **3** (50 mg), **4** (100 mg), **7** (100 mg), **8** (100 mg), **11** (20 mg) or **15** (30 mg) in pyridine–dioxane (4 : 1, v/v, 1 ml per 5 mg of glycoside) was heated on a water bath at 80 °C for 7 h. The reaction mixture was evaporated to dryness *in vacuo* and the residue was chromatographed on Sephadex LH-20 with MeOH to afford a desulfated glycoside. Desulfate of **3**: colorless needles from EtOH, mp 270—272 °C (dec.). The product was identified as **1** (=ophiopogonin B) by comparing TLC behavior, and IR and ^{13}C-NMR spectra. Desulfate of **4** (**13**): colorless needles from aqueous 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). (*,** may be reversed). *Anal.* Calcd for $C_{38}H_{60}O_{12} \cdot 1/2H_2O$: C, 63.57; H, 8.57. Found: C, 63.37; H, 8.82. Desulfate of **7** (**18**): colorless needles from MeOH, mp 218—220 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 982, 920, 900, 880 (spiroketal), ^{13}C-NMR (C_5D_5N) δ: fucose ($\xrightarrow{1}$aglycone) 100.1 (C_1), 75.2 (C_2), 76.7 (C_3), 73.2** (C_4), 71.0 (C_5), 17.2 (C_6); rhamnose ($\xrightarrow{2}$fucose) 101.4 (C_1), 72.5** (C_2), 72.7** (C_3), 74.5* (C_4), 69.2 (C_5), 19.0 (C_6); glucose ($\xrightarrow{3}$aglycone) 102.8 (C_1), 75.3* (C_2), 78.5 (C_3), 71.8** (C_4), 78.3 (C_5), 62.9 (C_6). (*, ** may be reversed). Desulfate of **8** (**15**): colorless needles from aqueous EtOH, mp 200—202 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 920, 880 (spiroketal). ^{13}C-NMR (C_5D_5N) δ: arabinose ($\xrightarrow{1}$aglycone) 100.1 (C_1), 75.7 (C_2), 75.7 (C_3), 70.0 (C_4), 67.2 (C_5); rhamnose ($\xrightarrow{2}$arabinose) 101.4 (C_1), 72.4 (C_2), 72.4 (C_3), 75.1 (C_4), 69.3 (C_5), 18.9 (C_6); glucose ($\xrightarrow{3}$aglycone) 102.6 (C_1), 75.1 (C_2), 78.4 (C_3), 71.7 (C_4), 78.4 (C_5), 63.1 (C_6). *Anal.* Calcd for $C_{44}H_{68}O_{17} \cdot H_2O$: C, 58.39; H, 8.02. Found: C, 58.45; H, 8.43. Desulfate of **11** (**12**): colorless needles from EtOH, mp 237—239 °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: fucose ($\xrightarrow{1}$aglycone) 102.4 (C_1), 72.4* (C_2), 75.5 (C_3), 72.7* (C_4), 71.4 (C_5), 17.4 (C_6). Desulfate of **16** (**17**): colorless needles from MeOH, mp 252—254 °C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 920, 880 (spiroketal). ^{13}C-NMR (C_5D_5N) δ: arabinose ($\xrightarrow{1}$aglycone) 100.2 (C_1), 75.6* (C_2), 75.5* (C_3), 70.0** (C_4), 67.2 (C_5); rhamnose ($\xrightarrow{2}$arabinose) 101.6 (C_1), 72.5*** (C_2), 72.7*** (C_3), 74.3 (C_4), 69.5** (C_5), 18.9 (C_6). (*, **, *** may be reversed).

On the other hand a part of each reaction mixture described above was subjected to the potassium rhodizonate test on filter paper and each test solution showed positive reaction, revealing the presence of sulfate ion.

Methylation of 18 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 an N_2 flow. CH_3I (3 ml) was added to a solution of **18** (30 mg) in DMSO (3 ml) and the mixture was stirred for 3 h. 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 nona-*O*-methyl derivative of **18**, a white powder from MeOH, (mp 107—108 °C). IR ν_{max}^{Nujol} cm^{-1}: OH (nil). ^{1}H-NMR ($CDCl_3$) δ: 3.40, 3.49, 3.52, 3.53, 3.58, 3.62 (each 3H, s, OCH_3), 3.46 (9H, s, $OCH_3 \times 3$), 4.19 (1H, d, J=7 Hz, fucose anomer. H), 4.47 (1H, d, J=7 Hz, glucose anomer. H), 5.28 (1H, d, J= 1 Hz, rhamnose anomer. H).

Methanolysis of Nona-*O*-methyl Derivative of 18——The nona-*O*-methyl derivative of **18** (5 mg) was refluxed with methanolic 5% HCl (2 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. *O*-Methylsugars: TLC (solvent, hexane–acetone (2 : 1, v/v) *Rf* 0.32, 0.47 (per-*O*-methylglucopyranose), 0.48 (per-*O*-methylrhamnopyranose), 0.28 (methyl 3,4-di-*O*-methyl-fucopyranoside). GLC t_R (min) 3.7, 5.2 (per-*O*-methylglucopyranose), 1.8 (per-*O*-methylrhamnopyranose), 5.7

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(methyl 3,4-di-*O*-methylfucopyranoside).

***O*-Methylation of the 22-Hydroxyl Group of 5, 9 and 10 with MeOH**——A methanolic solution (10 ml) of **5** (100 mg), **9** (100 mg) or **10** (100 mg) was refluxed for 5 h. The reaction mixture was evaporated to dryness *in vacuo* to afford the 22-*O*-methyl derivative. 22-*O*-Methyl derivative of **5**: a white powder from AcOEt–MeOH, (mp 205—208 °C (dec.)), $[\alpha]_D^{23}$ −24.6° (c=0.28, pyridine). ^{1}H-NMR (C_5D_5N) δ: 3.23 (3H, s, OCH_3). 22-*O*-Methyl derivative of **9**: a white powder from acetone–MeOH, (mp 185—187 °C (dec.)). ^{1}H-NMR (C_5D_5N) δ: 3.23 (3H, s, OCH_3). 22-*O*-Methyl derivative of **10**: a white powder from acetone–MeOH, (mp 202—205 °C (dec.)), $[\alpha]_D^{23}$ −24.6° (c=0.28, pyridine). ^{1}H-NMR (C_5D_5N) δ: 3.62 (3H, s, CH_3).

Extraction and Identification of the Glycosides of the Tuber——The fresh tubers of *Ophiopogon ohwii* (224 g) and *O. jaburan* (180 g) were extracted with hot MeOH. Each extract was 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 (8.9 g from the former and 7.5 g from the latter), which was examined for the presence of **1**—**10** (solvent, $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5, v/v)). *O. ohwii*: *Rf* 0.70 (**1**), 0.50 (**2**), 0.32 (**3**), 0.27 (**4**), 0.09 (**5**). *O. jaburan*: *Rf* 0.50 (**6**), 0.12 (**7**), 0.09 (**8**), 0.05 (**9**), 0.04 (**10**).

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10) The molecular formulae for glycosides E and G, $C_{38}H_{59}O_{11}SO_3M \cdot 3H_2O$ and $C_{44}H_{71}O_{17}SO_3M \cdot 3H_2O$, reported in the preceding paper[1] were erroneous, and they should be corrected to $C_{38}H_{59}O_{12}SO_3M \cdot 2H_2O$ and $C_{44}H_{71}O_{18}SO_3M \cdot 2H_2O$, respectively. Accordingly, the analytical data for these compounds should be amended as follows. Sodium salt of glycoside E: *Anal.* Calcd for $C_{38}H_{59}O_{12}SO_3M \cdot 2H_2O$: C, 53.89; H, 7.50; S, 3.79. Sodium salt of glycoside G: *Anal.* Calcd for $C_{44}H_{71}O_{18}SO_3M \cdot 2H_2O$: C, 51.45; H, 7.36; S, 3.12.
11) The molecular formula for desulfated glycoside E, $C_{38}H_{60}O_{11} \cdot 3/2H_2O$ reported in the preceding paper[1] was erroneous, and must be corrected to $C_{38}H_{60}O_{12} \cdot 1/2H_2O$. Accordingly, the analytical data for this compound should be amended as follows. Desulfated glycoside E: *Anal.* Calcd for $C_{38}H_{60}O_{12} \cdot 1/2H_2O$: C, 63.57; H, 8.57.
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