Comparative Studies on the Constituents of Ophiopogonis Tuber and Its Congeners. VIII. Studies on the Glycosides of the Subterranean Part of *Ophiopogon japonicus* KER-GAWLER cv. Nanus. (2)[1)]

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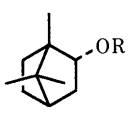
Two monoterpene glycosides, tentatively named OJV-I (1) and OJV-II (2), and eight steroidal glycosides, tentatively named OJV-III (3), OJV-IV (4), OJV-V (5), OJV-VI (6), OJV-VII (7), OJV-VIII (8), OJV-IX (9) and OJV-X (10), were isolated from the butanol-soluble fraction of the fresh subterranean part of *Ophiopogon japonicus* KER-GAWLER cv. Nanus. Among these compounds, 1, 2, 3, 4, 5, 6 and 7 were identified as *l*-borneol *O*-β-D-glucopyranoside, *l*-borneol *O*-β-D-apiofuranosyl (1→6)-β-D-glucopyranoside, ophiopogonin B, glycoside C, ophiopogonin D, Ls-10, and ruscogenin 1-*O*-sulfate, respectively. The structures of compounds 8, 9, and 10 were established to be (23*S*,24*S*,25*S*)-23,24-dihydroxyruscogenin 1-*O*-[α-L-rhamnopyranosyl(1→2)] [β-D-xylopyranosyl(1→3)]-α-L-arabinopyranoside 24-*O*-β-D-fucopyranoside, (23*S*,24*S*,25*S*)-23,24-dihydroxyruscogenin 1-*O*-[α-L-2,3,4-tri-*O*-acetylrhamnopyranosyl(1→2)] [β-D-xylopyranosyl(1→3)]-α-L-arabinopyranoside 24-*O*-β-D-fucopyranoside, and (23*S*,24*S*,25*S*)-23,24-dihydroxyruscogenin 1-*O*-[α-L-4-*O*-acetylrhamnopyranosyl(1→2)] [β-D-xylopyranosyl(1→3)]-α-L-arabinopyranoside 24-*O*-β-D-fucopyranoside, respectively.

Keywords *Ophiopogon japonicus* cv. Nanus; monoterpene glycoside; steroidal glycoside; Ophiopogonis Tuber; Liliaceae; (23*S*,24*S*,25*S*)-23,24-dihydroxyruscogenin

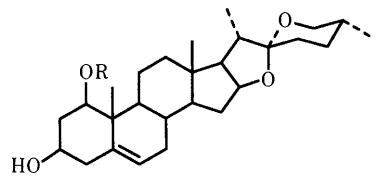
In the preceding paper,[2a)] we reported the isolation and structure elucidation of nine homoisoflavonoids from the subterranean part of *Ophiopogon japonicus* KER-GAWLER cv. Nanus. This plant is an evergreen perennial and is cultivated in our country as a garden plant. The present paper is concerned mainly with the isolation and structure elucidation of two monoterpene glycosides and eight steroidal glycosides of the subterranean part of *O. japonicus* KER-GAWLER cv. Nanus (Liliaceae; Japanese name; chaboryuunohige). These glycosides are tentatively named OJV-I (**1**), OJV-II (**2**), OJV-III (**3**), OJV-IV (**4**), OJV-V (**5**), OJV-VI (**6**), OJV-VII (**7**), OJV-VIII (**8**), OJV-IX (**9**), and OJV-X (**10**). The glycosides described here were isolated from the methanolic extract of the fresh subterranean part of the plant harvested at Kyoto Herbal Garden, Central Research Division, Takeda Chemical Industries, Ltd. in October 1989. The methanol extract was treated by the method described in the experimental section.

OJV-I (**1**) and OJV-II (**2**) were colorless needles and the structures were deduced to be *l*-borneol *O*-β-D-glucopyranoside (**1**) and *l*-borneol *O*-β-D-apiofuranosyl(1→6)-*O*-β-D-glucopyranoside (**2**), which have been isolated from the commercially available Ophiopogonis tuber imported from China by Kaneda *et al.*[3)] Identifications of **1** and **2** were achieved by comparison of their general properties and ^{13}C-NMR spectra with an authentic specimen.

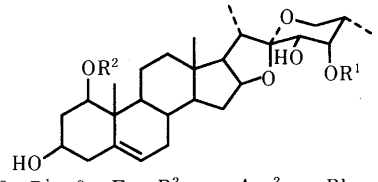
OJV-III (**3**) and OJV-V (**5**) are colorless needles and positive in the Liebermann–Burchard reaction. Both compounds showed strong absorption bands of hydroxyl groups and the characteristic absorption bands of the (25*R*)-spiroketal moiety in IR spectrum. ^{13}C-NMR spectrum of the former showed two anomeric carbon signals at δ 100.4, 101.6 and that of the latter showed three anomeric carbon signals at δ 100.4, 101.7, 106.5. On acidic hydrolysis, **3** afforded ruscogenin, D-fucose and L-rhamnose, while **5** gave ruscogenin, D-fucose, L-rhamnose and D-xylose. Consequently, the structure of **3** was established to be rusco-



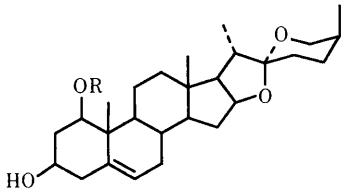
1 : R=β-D-Glc
2 : R=β-D-Glc^6-β-D-Api



3 : R=β-D-Fuc^2-α-L-Rha(=ophiopogonin B)
5 : R=β-D-Fuc^2-α-L-Rha(=ophiopogonin D)
β-D-Xyl^3
7 : R=SO_3M



8 : R^1=β-D-Fuc, R^2=α-L-Ara^2-α-L-Rha
β-D-Xyl^3
8a : R^1=H, R^2=α-L-Ara^2-α-L-Rha
8b : R^1=H, R^2=α-L-Ara^2-α-L-Rha
β-D-Xyl^3
8c : R^1=R^2=H
9 : R^1=β-D-Fuc, R^2=α-L-Ara^2-α-L-2,3,4-tri-*O*-Ac-Rha
β-D-Xyl^3
10 : R^1=β-D-Fuc, R^2=α-L-Ara^2-α-L-4-*O*-Ac-Rha
β-D-Xyl^3



4 : R=β-D-Fuc^2-α-L-Rha(=glycoside C)
6 : R=β-D-Fuc^2-α-L-Rha(=Ls-10)
β-D-Xyl^3

genin 1-*O*-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside (=Ophiopogonin B[2b]) isolated from Ophiopogonis tuber, while that of **5** was concluded to be ruscogenin 1-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-fucopyranoside (=Ophiopogonin D[2c]) isolated from the same crude drug by direct comparisons with the respective authentic samples.

OJV-IV (**4**) and OJV-VI (**6**) are colorless needles and positive in the Liebermann–Burchard reaction. Both compounds showed strong absorption bands of hydroxyl groups in the IR spectrum and the characteristic absorption bands and signals of the (25*S*)-spiroketal moiety in the IR and ^{13}C-NMR spectra. ^{13}C-NMR spectrum of the former showed two anomeric carbon signals at δ 100.4, 101.6 and that of the latter showed three anomeric carbon signals at δ 100.4, 100.7, 106.5. On acidic hydrolysis, **4** and **6** afforded the mixture of ruscogenin and (25*S*)-ruscogenin as the common aglycone. Furthermore, the former afforded D-fucose and L-rhamnose, while the latter afforded D-fucose, L-rhamnose and D-xylose. As we pointed out in the previous paper,[2d] a part of (25*S*)-ruscogenin was assumed to be converted into (25*R*)-ruscogenin on acidic hydrolysis. Consequently, the structure of **4** was concluded to be (25*S*)-ruscogenin 1-*O*-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside (=glycoside C[2e]) isolated from *Liriope platyphylla* WANG *et* TANG, while that of **6** was established to be (25*S*)-ruscogenin 1-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-fucopyranoside (=Ls-10[2f]) isolated from *L. spicata* var. *prolifera* by the detailed examination of ^{13}C-NMR spectra of the both compounds. Finally, **4** and **6** were identified as glycoside C and Ls-10 by direct comparison with respective authentic samples.

OJV-VII (**7**) are colorless needles and positive in the Liebermann–Burchard reaction. The IR spectrum of **7** shows absorption bands of a hydroxyl group, characteristic absorption bands of (25*R*)-spiroketal moiety and a S–O stretching absorption band at 1210 cm^{-1}. To confirm the sulfate group, **7** was heated with pyridine to afford ruscogenin and sulfate ion. The ^{13}C-NMR spectrum of **7** showed that the C-1 carbon signals appeared at 7.3 ppm lower on the field than that of ruscogenin, but no shift of C-3 carbon signal was observed. Consequently, the structure of **7** was deduced to be ruscogenin 1-sulfate, which has been reported as an enzymatic hydrolysate of glycoside G,[2e] but this is believed to be the first report of ruscogenin having a sulfate ion on the C-1 hydroxyl group isolated from the natural resources.

OJV-VIII (**8**) is obtained as colorless needles and is positive in the Liebermann–Burchard reaction. The IR spectrum of **8** showed hydroxyl absorption bands, while the ^{13}C-NMR spectrum showed four anomeric carbon signals at δ 100.4, 101.7, 105.9, 106.2 and the characteristic spiroketal carbon signals at δ 111.8 (C-22). Accordingly, **8** was suggested to be a spirostanol tetraglycoside. On hydrolysis with 2 N hydrochloric acid in 50% dioxane, **8** gave D-fucose, L-rhamnose, D-xylose and L-arabinose, but the thin layer chromatogram (TLC) of the aglycone fraction showed several spots caused by the further conversion of the genuine aglycone. A mild acidic hydrolysis with 0.1 N hydrochloric acid in 50% dioxane was carried out and **8** afforded two kinds of partial hydrolysis products, Pro-VIIIa (**8a**), Pro-VIIIb (**8b**) and the genuine aglycone (**8c**).

The structure of **8c** was elucidated as follows. The molecular weight was determined by electron impact ionization mass spectrometry (EIMS) which showed a M^+ ion at *m/z* 462. By the comparative studies of the ^{13}C-NMR spectrum of **8c** with those of ruscogenin and (25*S*)-ruscogenin, the A—E ring carbon signal of **8c** showed the same pattern as those of ruscogenin and (25*S*)-ruscogenin, while two methine carbon signals, δ 68.8 (C-23) and 73.2 (C-24), were observed in place of two methylene carbon signals of ruscogenin (δ 32.1 (C-23) and 29.5 (C-24)) and of (25*S*)-ruscogenin (δ 26.5 (C-23) and 26.3 (C-24)).[2e] Accordingly, the structure of **8c** assumed that two hydroxyl groups were substituted in the F-ring of ruscogenin or (25*S*)-ruscogenin. Spindecoupling experiments by ^{1}H-NMR spectrometry of **8c** were carried out. Based on the above experiments, the locations of two hydroxyl groups were proved to be on C-23 and C-24, and the configurations of the C-24 hydroxyl group and C-25 methine proton were axial ($J_{H24,H25}$=2.5 Hz, $J_{H25,H26(eq.)}$=5.0 Hz, $J_{H25,H26(ax.)}$=11.5 Hz), respectively. The coupling constant of the protons of C-23 and C-24 appeared to be 3.5 Hz, and the steric relationship between both hydroxyl groups was chemically investigated. The formation of an acetonide between the C-23 and C-24 hydroxyl groups with acetone and *p*-toluenesulfonic acid revealed that the stereochemistry of C-23 and C-24 hydroxyl groups was concluded to be in the *cis* form. The structure of **8c** was established to be (23*S*,24*S*,25*S*)-23,24-dihydroxyruscogenin, which is believed to be a new compound obtained from natural resources.

Pro-VIIIa (**8a**), a white powder, was deduced to be a spirostanol diglycoside by the examination of the ^{13}C-NMR spectrum which shows two anomeric carbon signals. On hydrolysis with 2 N hydrochloric acid in 50% dioxane, **8a** afforded L-rhamnose and L-arabinose. Finally, the structure of **8a** was deduced by examination of ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum of **8a** octaacetate, which was obtained by acetylation with 4-dimethylaminopyridine, pyridine and acetic anhydride. It was proved that the C-1 hydroxyl group of the aglycone (δ 3.70, 1H, m) and that of C-2 of the arabinose (δ 4.28, 1H, dd, 7.5, 10.0 Hz) have not been acetylated, and the structure of **8a** was established to be (23*S*,24*S*,25*S*)-23,24-dihydroxyruscogenin 1-*O*-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside.

Pro-VIIIb (**8b**), a white powder, was deduced to be a spirostanol triglycoside by examination of its ^{13}C-NMR spectrum, which shows three anomeric carbon signals. On acidic hydrolysis under the same codition described above, **8b** afforded L-rhamnose, D-xylose and L-arabinose. The structure of **8b** was deduced by the examination of ^{1}H–^{1}H COSY spectrum of a decaacetate of **8b**, which was obtained by the same acetylation method as described above.

The ^{1}H–^{1}H COSY spectrum of a decaacetate of **8b** revealed that the C-1 hydroxyl group of the aglycone (C_1-H, δ 3.67 (1H, m)) and C-2 (C_2-H, δ 4.38 (1H, dd, *J*=6.5, 8.0 Hz)) and C-3 (C_3-H, δ 4.18 (1H, dd, *J*=4.0, 8.0 Hz)) hydroxyl groups of the arabinose have not been acetylated. Consequently, the structure of **8b** was established to be (23*S*,24*S*,25*S*)-23,24-dihydroxyruscogenin 1-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-α-L-arabinopyranoside.

The structure of **8** was deduced by the examination of the ^{1}H–^{1}H COSY spectrum of a dodecaacetate of **8**, which

was obtained by the same acetylation method as described above. The ^1H–^1H COSY spectrum of a dodecaacetate of **8** revealed that the C-1 (C_1-H, δ 3.67 (1H, m)) and C-24 (C_{24}-H, δ 4.33 (1H, br s)) hydroxyl groups of the aglycone and C-2 (C_2-H, δ 4.25 (1H, dd, J=7.5, 8.0 Hz)) and C-3 (C_3-H, δ 4.18 (1H, dd, J=3.5, 7.5 Hz)) hydroxyl groups of the arabinose have not been acetylated. Based on the J_{C1-H1} coupling constants, the configurations of L-rhamnose (170 Hz), L-arabinose (158 Hz), D-xylose (160 Hz) and D-fucose (158 Hz) were assigned to be α, α, β and β,[4] respectively. Consequently, the structure of **8** was established to be (23*S*,24*S*,25*S*)-dihydroxyruscogenin 1-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-α-L-arabinopyranoside 24-*O*-β-D-fucopyranoside as shown in the chart.

Several spirostanols having two hydroxyl groups on the F-ring, namely trillenogenin, epitrillenogenin, 21-deoxyltrillenogenin and their glycosides from *Trillium kamtschaticum* PALL[5] and dracogenin from *Dracaena draco*[6] (Liliaceae), have been reported, and the new aglycone, described above, is the fifth report on the characteristic spirostanol and its glycosides.

OJV-IX (**9**) was a white powder and was positive in the Liebermann–Burchard reaction. The IR spectrum of **9** showed the absorption bands of hydroxyl groups and *O*-acetyl groups, while that of the ^{13}C-NMR spectrum showed four anomeric carbon signals, spirostanol carbon signals and six signals assigned to three *O*-acetyl groups. Based on the above described results, the structure of **9** was assumed to be a tri-*O*-acetate of spirostanol glycoside. On deacetylation of **9** with 5% potassium carbonate in 50% methanol, **9** afforded **8**, which was identified by direct comparisons with **8**.

The protons, H-2, H-3 and H-4 of rhamnose moiety, were observed at a lower field region (δ 5.95 (H-2), 5.83 (H-3) and 5.50 (H-4)) in the ^1H–^1H COSY spectrum of **9**. Thus, the three *O*-acetyl groups were deduced to be combined with the hydroxyl groups of rhamnose. Finally, the structure of **9** was concluded to be (23*S*,24*S*,25*S*)-23,24-dihydroxyruscogenin 1-*O*-[α-L-2,3,4-tri-*O*-acetylrhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-α-L-arabinopyranoside 24-*O*-β-D-fucopyranoside.

OJV-X (**10**) was a white powder and was positive in the Liebermann–Burchard reaction. The IR spectrum of **10** showed the absorption bands of hydroxyl groups and *O*-acetyl groups, while that of the ^{13}C-NMR spectrum showed two carbon signals assigned to one *O*-acetyl group together with four anomeric carbon signals and spiroketal carbon signals. Accordingly, **10** was deduced to be a monoacetate of spirostanol tetraglycoside. On deacetylation under the same condition as in the case of **9**, **10** afforded **8**, which was identified by direct comparisons with **8**. Only the H-4 of rhamnose moiety was observed at a lower field (δ 5.65) in the ^1H–^1H COSY spectrum of **10**. This observation led us to conclude that the acetyl group was combined with the C-4 hydroxyl group of L-rhamnose. Consequently, the structure of **10** was established to be (23*S*,24*S*,25*S*)-23,24-dihydroxyruscogenin 1-*O*-[α-L-4-*O*-acetylrhamnopyranosyl(1→2)] [β-D-xylopyranosyl(1→3)]-α-L-arabinopyranoside 24-*O*-β-D-fucopyranoside.

In this paper we described the isolation and structural elucidation of three new spirostanol glycosides, whose new aglycone has a pair of *cis*-hydroxyl groups on the F-ring at C-23 and C-24. It is very interesting that one of the variations of *Ophiopogon japonicus* KER-GAWLER contains unique constituents, and we are urged to examine the biological activity of new compounds by comparing the known constituents of *O. japonicus* KER-GAWLER and its congeners.

Experimental

All melting points were determined on a Yanaco micro-melting point apparatus (hot-stage type) and are uncorrected. Optical rotations were measured with a JASCO DPI-140 polarimeter. IR spectra were recorded with a JASCO IR-810, and NMR spectra were recorded with a JEOL GX-400 spectrometer (400 MHz for ^1H-NMR and 100 MHz for ^{13}C-NMR). Chemical shifts are given a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. Gas-liquid chromatography (GLC) was run on a Shimadzu GC-6A unit equipped with a flame ionization detector. Experimental conditions for sugars: (a) column, 5% SE-52 on chromosorb W 3 mm × 3 m; column temp., 180 °C; injection temp., 200 °C; carrier gas N_2, 1.2 kg/cm^2; samples, trimethyl silyl (TMS) ether. (b) column, 5% SE-52 on chromosorb W 3 mm × 2 m; column temp., 170 °C, injection temp., 180 °C; carrier gas N_2, 1.1 kg/cm^2; samples, TMS ether.

Extraction and Isolation The BuOH-soluble fraction (310.0 g) described in the preceding paper[2a] was subjected to column chromatography on Sephadex LH-20 with MeOH to separate it into five fractions (Fr.-A—Fr.-E). After Fr.-B was subjected to the column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (70 : 25 : 0.4, v/v) to separate it into seven fractions (Fr.-B-1—Fr.-B-7), Fr.-B-2 was further purified by the same column chromatography with $CHCl_3$–MeOH–H_2O (97 : 3 : 0.1, v/v) followed with $CHCl_3$–MeOH–AcOEt–H_2O (2 : 1.5 : 4 : 1, v/v) to afford OJV-IX (**9**, 250 mg). Fraction-B-5 was separated into four fractions (Fr.-B-5-1—Fr.-B-5-4) by column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (75 : 25 : 0.4, v/v). Fraction-B-5-2 was purified by silica gel column chromatography with $CHCl_3$–MeOH–AcOEt–H_2O (2 : 2.5 : 4 : 1, v/v) and by HPLC (column; Fuji-gel Chromatorex-ODS, 5 μm, i.d. 20 × 250 mm, solvent; 85% MeOH) to afford OJV-II (**2**, 300 mg), OJV-V (**5**, 150 mg), OJV-VI (**6**, 20 mg), OJV-VII (**7**, 90 mg) and OJV-X (**10**, 400 mg). Fraction-C was separated into six fractions (Fr.-C-1—Fr.-C-6) by silica gel column chromatography with $CHCl_3$–MeOH–AcOEt–H_2O (2 : 1.5 : 4 : 1, v/v). Fraction-C-5 was purified by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (85 : 15 : 2, v/v) and by HPLC under the same condition applied for Fr.-B-5-2 to afford OJV-I (**1**, 70 mg, OJV-III (**3**, 300 mg) and OJV-IV (**4**, 300 mg). Fraction-D was separated into five fractions (Fr.-D-1—Fr.-D-5) by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (75 : 25 : 0.4, v/v). Fraction-D-2 was purified by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (80 : 20 : 2, v/v) followed with $CHCl_3$–MeOH–AcOEt–H_2O (2 : 3 : 4 : 2, v/v) to afford OJV-VIII (**8**, 400 mg).

Properties OJV-I (**1**): Colorless needles (from EtOH), mp 122—126 °C (dec.), $[\alpha]_D^{23}$ −8.6° (c=0.31, MeOH) (lit.[3] mp 125—127 °C, $[\alpha]_D^{20}$ −7.3° (c=0.6, MeOH)). IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1080, 1025.

OJV-II (**2**): Colorless needles (from EtOH), mp 178—181 °C (dec.), $[\alpha]_D^{23}$ −53.4° (c=0.44, MeOH) (lit.[3] mp 183—185 °C, $[\alpha]_D^{20}$ −52.6° (c=0.6, MeOH)). IR ν_{max}^{KBr} cm^{-1}: 3400, 2980, 1075, 1020.

OJV-III (**3**) (Ophiopogonin B): Colorless needles (from MeOH), mp 270—272 °C (dec.), $[\alpha]_D^{23}$ −104.6° (c=0.46, pyidine) (lit.[2b] mp 269—272 °C, $[\alpha]_D^{15}$ −105.5° (c=0.31, pyridine)). IR ν_{max}^{KBr} cm^{-1}: 3500—3400, 990, 920, 900, 850 (intensity 920<900, (25*R*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: 84.3 (C1), 38.4 (C2), 68.6 (C3), 44.3 (C4), 139.7 (C5), 124.7 (C6), 33.7 (C7), 32.6 (C8), 51.2 (C9), 43.4 (C10), 24.5 (C11), 41.0 (C12), 40.8 (C13), 57.7 (C14), 33.3 (C15), 81.4 (C16), 63.5 (C17), 17.4 (C18), 15.4 (C19), 42.5 (C20), 15.6 (C21), 109.4 (C22), 32.4 (C23), 29.9 (C24), 31.1 (C25), 67.2 (C26), 17.8 (C27); fucose (→^{1}aglycone) 100.4 (C1), 75.0 (C2), 77.0 (C3), 73.5 (C4), 71.3 (C5), 17.7 (C6); rhamnose (→^{2}fucose) 101.6 (C1), 72.8 (C2), 72.9 (C3), 74.6 (C4), 69.3 (C5), 19.4 (C6).

OJV-IV (**4**) (Glycoside C): Colorless needles (from MeOH), mp 198—201 °C (dec.), $[\alpha]_D^{23}$ −88.8° (c=0.48, pyridine) (lit.[2e] mp 201—203 °C, $[\alpha]_D^{18}$ −89.6° (c=0.48, pyridine)). IR ν_{max}^{KBr} cm^{-1}: 3600—3400, 985, 920, 900, 855 (intensity 920>900, (25*S*)-spiroketal). ^{13}C-NMR (C_5D_5N) δ: 84.3 (C1), 38.4 (C2), 68.8 (C3), 44.3 (C4), 139.8 (C5), 124.5 (C6), 33.7 (C7), 32.6 (C8), 51.3 (C9), 43.4 (C10), 24.5 (C11), 41.0 (C12), 40.8 (C13), 57.7 (C14), 33.0 (C15), 81.5 (C16), 63.6 (C17), 17.4 (C18), 15.5 (C19), 43.0 (C20), 15.3 (C21), 109.9 (C22), 27.0 (C23), 26.8 (C24), 28.1 (C25), 65.2

(C26), 16.9 (C27); fucose (→1aglycone) 100.4 (C1), 75.0 (C2), 77.0 (C3), 73.5 (C4), 71.3 (C5), 17.7 (C6); rhamnose (→2fucose) 101.6 (C1), 72.8 (C2), 72.9 (C3), 74.6 (C4), 69.3 (C5), 19.4 (C6).

OJV-V (**5**) (Ophiopogonin D): Colorless needles (from MeOH), mp 262—265 °C (dec.), $[\alpha]_D^{23}$ −106.4° (*c*=0.53, pyridine) (lit.[2c] mp 263—265 °C, $[\alpha]_D^{14}$ −107.9° (*c*=0.66, pyridine)). IR ν_{max}^{KBr} cm^{-1}: 3600—3200, 980, 920, 900, 860 (intensity 920<900, (25*R*)-spiroketal). ^{13}C-NMR (C_5D_5N) *δ*: 84.3 (C1), 38.0 (C2), 68.4 (C3), 43.9 (C4), 139.7 (C5), 124.6 (C6), 33.2 (C7), 32.2 (C8), 50.7 (C9), 50.0 (C10), 24.1 (C11), 40.6 (C12), 40.3 (C13), 57.3 (C14), 32.5 (C15), 81.4 (C16), 63.2 (C17), 16.8 (C18), 14.7 (C19), 42.5 (C20), 14.9 (C21), 109.2 (C22), 32.4 (C23), 29.3 (C24), 30.5 (C25), 66.9 (C26), 17.3 (C27); fucose (→1aglycone) 100.4 (C1), 74.7 (C2), 85.7 (C3), 72.5 (C4), 71.0 (C5), 17.1 (C6); rhamnose (→2fucose) 101.7 (C1), 72.6 (C2), 72.7 (C3), 74.3 (C4), 69.3 (C5), 19.1 (C6); xylose (→3fucose) 106.5 (C1), 73.6 (C2), 78.2 (C3), 70.9 (C4), 66.9 (C5).

OJV-VI (**6**) (LS-10): Colorless needles (from MeOH), mp 201—202 °C (dec.), $[\alpha]_D^{23}$ −93.4° (*c*=0.41, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200, 980, 920, 902, 865 (intensity 920>902, (25*S*)-spiroketal). ^{13}C-NMR (C_5D_5N) *δ*: 84.3 (C1), 38.1 (C2), 68.4 (C3), 44.0 (C4), 139.7 (C5), 124.6 (C6), 33.2 (C7), 32.2 (C8), 50.7 (C9), 43.5 (C10), 24.1 (C11), 40.6 (C12), 40.6 (C13), 57.3 (C14), 32.5 (C15), 81.2 (C16), 63.0 (C17), 16.8 (C18), 14.8 (C19), 42.8 (C20), 14.8 (C21), 109.8 (C22), 26.5 (C23), 26.2 (C24), 28.1 (C25), 65.2 (C26), 16.3 (C27); fucose (→1aglycone) 100.4 (C1), 74.7 (C2), 85.7 (C3), 72.5 (C4), 71.0 (C5), 17.1 (C6); rhamnose (→2fucose) 101.7 (C1), 72.6 (C2), 72.7 (C3), 74.3 (C4), 69.3 (C5), 19.1 (C6); xylose (→3fucose) 106.5 (C1), 73.6 (C2), 78.2 (C3), 70.9 (C4), 66.9 (C5).

OJV-VI (**7**): Colorless needles (from MeOH), mp 174—176 °C (dec.) (lit.[2e] mp 173—176 °C). IR ν_{max}^{KBr} cm^{-1}: 3600—3300, 1210, 982, 920, 900, 865 (intensity 920<902, (25*R*)-spiroketal). ^{13}C-NMR (C_5D_5N) *δ*: 85.5 (C1), 39.6 (C2), 67.9 (C3), 43.3 (C4), 139.6 (C5), 125.6 (C6), 33.1 (C7) 32.0 (C8), 50.0 (C9), 43.2 (C10), 23.7 (C11), 40.7 (C12), 40.3 (C13), 56.8 (C14), 32.4 (C15), 81.8 (C16), 63.3 (C17), 16.6 (C18), 14.7 (C19), 42.1 (C20), 15.0 (C21), 109.3 (C22), 32.0 (C23), 29.4 (C24), 30.7 (C25), 67.0 (C26), 17.3 (C27).

OJV-VIII (**8**): Colorless needles (from MeOH), mp 265—266 °C (dec.), $[\alpha]_D^{23}$ −35.2° (*c*=1.50, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500—3400. ^{1}H-NMR (C_5D_5N) *δ*: 1.00 (3H, s, 18-Me), 1.06 (3H, d, *J*=7.1 Hz, 27-Me), 1.11 (3H, d, *J*=7.1 Hz, 21-Me), 1.41 (3H, s, 19-Me), 1.49 (3H, d, *J*=6.5 Hz, rhamnose 6-Me), 1.67 (3H, d, *J*=6.5 Hz, fucose 6-Me). ^{13}C-NMR (C_5D_5N) *δ*: 83.7 (C1), 37.3 (C2), 68.3 (C3), 43.8 (C4), 139.8 (C5), 124.6 (C6), 33.1 (C7), 32.4 (C8), 50.5 (C9), 43.0 (C10), 24.0 (C11), 40.9 (C12), 40.6 (C13), 56.9 (C14), 32.0 (C15), 81.5 (C16), 61.6 (C17), 16.8 (C18), 15.1 (C19), 37.4 (C20), 12.9 (C21), 111.8 (C22), 69.8 (C23), 82.8 (C24), 35.4 (C25), 61.7 (C26), 14.8 (C27); arabinose (→1aglycone) 100.4 (C1, J_{C1-H1}=158 Hz), 74.3 (C2), 84.3 (C3), 69.5 (C4), 67.0 (C5); rhamnose (→2arabinose) 101.7 (C1, J_{C1-H1}=170 Hz), 72.4 (C2), 72.5 (C3), 74.2 (C4), 69.4 (C5), 19.0 (C6); xylose (→3arabinose) 106.2 (C1, J_{C1-H1}=158 Hz), 74.6 (C2), 78.0 (C3), 70.9 (C4), 66.7 (C5); fucose (→24aglycone) 105.9 (C1, J_{C1-H1}=158 Hz), 73.4 (C2), 75.2 (C3), 72.8 (C4), 71.5 (C5), 17.2 (C6). *Anal.* Calcd for $C_{49}H_{78}O_{20}\cdot 4H_2O$: C, 55.07; H, 7.56. Found: C, 55.56; H, 8.18.

OJV-IX (**9**): A white powder (from MeOH), mp 187—191 °C (dec.), $[\alpha]_D^{23}$ −30.2° (*c*=0.50, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500—3400, 1760. ^{1}H-NMR (C_5D_5N) *δ*: 0.97 (3H, s, 18-Me), 1.01 (3H, d, *J*=7.0 Hz, 27-Me), 1.07 (3H, d, *J*=7.0 Hz, 21-Me), 1.31 (3H, s, 19-Me), 1.38 (3H, d, *J*=6.5 Hz, rhamnose 6-Me), 1.43 (3H, d, *J*=6.5 Hz, fucose 6-Me), 1.92 (3H, s, OAc), 2.00 (3H, s, OAc), 2.14 (3H, s, OAc), 4.89 (1H, m, rhamnose 5-H), 5.50 (1H, dd, *J*=10.0, 10.5 Hz, rhamnose 4-H), 5.83 (1H, dd, *J*=3.5, 10.0 Hz, rhamnose 3-H), 5.95 (1H, m, rhamnose 2-H), 6.34 (1H, d, *J*=1.5 Hz, rhamnose 1-H). ^{13}C-NMR (C_5D_5N) *δ*: 83.9 (C1), 37.7 (C2), 68.1 (C3), 43.9 (C4), 139.5 (C5), 125.0 (C6), 33.1 (C7), 32.5 (C8), 50.4 (C9), 43.0 (C10), 24.0 (C11), 40.9 (C12), 40.5 (C13), 56.9 (C14), 32.1 (C15), 81.5 (C16), 61.69 (C17), 16.8 (C18), 15.0 (C19), 37.4 (C20), 13.1 (C21), 111.9 (C22), 69.8 (C23), 82.8 (C24), 35.5 (C25), 61.7 (C26), 14.8 (C27); arabinose (→1aglycone) 100.3 (C1), 71.0 (C2), 84.9 (C3), 69.8 (C4), 67.3 (C5); rhamnose (→2arabinose) 97.7 (C1), 70.4 (C2), 73.0 (C3), 72.2 (C4), 67.2 (C5), 18.2 (C6); xylose (→3arabinose) 106.4 (C1), 74.6 (C2), 78.3 (C3), 70.6 (C4), 66.5 (C5); fucose (→24aglycone) 105.9 (C1), 73.5 (C2), 75.2 (C3), 72.9 (C4), 71.6 (C5), 17.2 (C6), 170.4, 170.25, 170.30 (carbonyl carbons of acetyl groups), 20.6, 20.7 (C×2) (methyl carbons of acetyl groups). *Anal.* Calcd for $C_{55}H_{84}O_{23}\cdot 4H_2O$: C, 55.39; H, 7.39. Found: C, 55.72; H, 7.82.

OJV-X (**10**): A white poder (from MeOH), mp 229—233 °C (dec.), $[\alpha]_D^{23}$ −36.0° (*c*=0.50, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3500—3400, 1740. ^{1}H-NMR (C_5D_5N) *δ*: 0.97 (3H, s, 18-Me), 1.03 (3H, d, *J*=7.0 Hz, 27-Me), 1.07 (3H, d, *J*=7.0 Hz, 21-H), 1.36 (3H, s, 19-Me), 1.38 (3H, d, *J*=6.5 Hz, rhamnose 6-Me), 1.46 (3H, d, *J*=6.5 Hz, fucose 6-Me), 2.02 (3H, s, OAc), 4.56 (1H, dd, *J*=4.5, 9.5 Hz, rhamnose 3-H), 4.64 (1H, dd, *J*=1.5, 4.5 Hz, rhamnose 2-H), 4.80 (1H, m, rhamnose 5-H), 5.65 (1H, dd, *J*=9.5, 9.5 Hz, rhamnose 4-H), 6.29 (1H, d, *J*=1.5 Hz, rhamnose 1-H) ^{13}C-NMR (C_5D_5N) *δ*: 83.7 (C1), 37.3 (C2), 68.1 (C3), 43.9 (C4), 139.5 (C5), 124.8 (C6), 33.1 (C7), 32.4 (C8), 50.4 (C9), 42.9 (C10), 23.9 (C11), 40.9 (C12), 40.5 (C13), 56.8 (C14), 32.0 (C15), 81.4 (C16), 61.61 (C17), 16.7 (C18), 15.0 (C19), 37.3 (C20), 13.0 (C21), 111.8 (C22), 69.9 (C23), 82.7 (C24), 35.4 (C25), 61.56 (C26), 14.8 (C27); arabinose (→1aglycone) 100.5 (C1), 73.1 (C2), 84.9 (C3), 69.6 (C4), 67.0 (C5); rhamnose (→2arabinose) 100.8 (C1), 72.2 (C2), 69.7 (C3), 76.4 (C4), 66.9 (C5), 18.4 (C6); xylose (→3arabinose) 106.3 (C1), 74.4 (C2), 78.2 (C3), 70.8 (C4), 66.6 (C5); fucose (→24aglycone) 105.8 (C1), 73.4 (C2), 75.1 (C3), 72.8 (C4), 71.4 (C5), 17.1 (C6), 170.8 (carbonyl carbon of acetyl group), 21.0 (methyl carbon of acetyl group). *Anal.* Calcd for $C_{51}H_{80}O_{21}\cdot 4H_2O$: C, 55.62; H, 8.05. Found: C, 55.10; H, 7.83.

Hydrolysis of 3, 4, 5, 6, 8a, 8b and 8 Each solution of **3**, **4**, **5**, **6**, **8a**, **8b** and **8** (each *ca.* 1 mg) in 2 N HCl–50% dioxane 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 with Na_2SO_4. The $CHCl_3$ solution was filtered and the filtrate was evaporated to dryness. Each residue of **3**, **4**, **5**, **6**, **8a**, **8b** and **8** was acetylated with Ac_2O–pyridine. After the reaction mixture was treated in the usual way, each product was compared with ruscogenin diacetate and (25*S*)-ruscogenin diacetate by TLC (solvent CH_2Cl_2). Each acetylated aglycone of **3** and **5** showed the same *Rf* value as ruscogenin diacetate (*Rf* 0.31). Both acetylated aglycones **4** and **6** showed the same *Rf* value as (25*S*)-ruscogenin diacetate (*Rf* 0.34). Sugars: Each aqueous layer was neutralized with $NaHCO_3$ and concentrated to dryness *in vacuo*, and the monosaccharides were examined by GLC. **3** and **4**: t_R (min) 2.4, 3.2 (rhamnose), 2.8, 3.3 (fucose). **5** and **6**: t_R (min) 2.4, 3.2 (rhamnose), 2.8, 3.3 (fucose), 3.2, 4.1 (xylose). **8a**: t_R (min) 2.4, 2.8 (arabinose), 2.4, 3.2 (rhamnose). **8b**: t_R (min) 2.4, 2.8 (arabinose), 2.4, 3.2 (rhamnose), 3.2, 4.1 (xylose). **8**: t_R (min) 2.4, 2.8 (arabinose), 2.4, 3.2 (rhamnose), 2.8, 3.3 (fucose), 3.2, 4.1 (xylose).

Determination of Absolute Configuration of Sugars by High Performance Liquid Chromatography (HPLC) Each solution of **3** (1 mg), **4** (1 mg), **5** (1 mg), **6** (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-PACK C_{18} cartridge to give a sugar fraction. Each component sugar in the solution was derived to 1-((*S*)-*N*-acetyl-α-methylbenzylamino)-1-deoxyalditol acetate using the method of Asada *et al.*[7] and analyzed by normal-phase HPLC. Conditions of normal-phase HPLC: column, supermicro bead silica gel B-5, 5 μm (i.d. 10×250 mm); solvent, hexane–EtOH (9:1); flow rate, 4 ml/min; detection, ultraviolet (UV) (230 nm). t_R (min). **3** and **4**: L-rhamnose 22.8, D-fucose 24.9. **5** and **6**: L-rhamnose 22.8, D-fucose 24.9, D-xylose 37.7. **8**: L-arabinose 33.0, L-rhamnose 22.8, D-fucose 24.9, D-xylose 37.7.

Solvolysis of 7 The solution of **7** in pyridine–dioxane (4:1, v/v, 20 ml) was heated at 70 °C for 7 h. The reaction mixture was evaporated to remove the solvent. The residue was subjected to column chromatography on silica gel to give **7a**. **7a** was identified as ruscogenin by direct comparisons of mp, and by TLC behavior. A part of the reaction mixture described above was examined by paper partition chromatography (Paper: Toyo-roshi No. 5; solvent, BuOH–MeOH–H_2O (1:3:1, v/v); detection, the paper was sprayed with a solution of $BaCl_2$ (100 mg) in 70% MeOH (50 ml) then dried and sprayed with a potassium rhodizonate[8] ((10 mg) solution in 50% MeOH (50 ml)). Sulfate ion in the hydrolysate was detected as a yellow spot at *Rf* 0.32.

Partial Hydrolysis of 8 **8** (200 mg) was dissolved in 0.1 N HCl–50% dioxane (2.0 ml) and the solution was heated at 60 °C for 3 h. The reaction mixture was diluted with water and neutralized with $NaHCO_3$. The neutral solution was subjected to column chromatography on ODS, and then eluted with H_2O and MeOH, respectively. Furthermore, the MeOH eluent was chromatographed on silica gel using the solvents, in order, hexane–acetone (8:2, v/v), $CHCl_3$–H_2O–MeOH (8:2:0.2, v/v) and $CHCl_3$–MeOH–AcOEt–H_2O (2:2:4:1, v/v, lower phase), to afford three compounds (**8c**; 40 mg, **8a**; 25 mg, **8b**; 50 mg).

Properties of 8c, 8a and 8b **8c**: A white powder (mp 205—210 °C (dec.)). IR ν_{max}^{KBr} cm^{-1}: 3500—3400 (OH), 980, 920, 900, 860. EIMS *m/z*: 462 (M^+). ^{1}H-NMR (C_5D_5N) *δ*: 0.96 (3H, d, *J*=7.5 Hz, 27-Me), 1.02 (3H, s, 18-Me), 1.12 (3H, d, *J*=7.5 Hz, 21-Me), 1.28 (3H, s, 19-Me). 1.83 (1H, m, 25-H), 3.42 (1H, dd, *J*=10.5, 11.5 Hz, 26(ax.)-H), 3.71 (1H, d, *J*=3.5 Hz, 23-H), 3.80 (1H, dd, *J*=5.0, 11.5 Hz, 26(eq.)-H), 4.00 (1H, dd, *J*=2.5, 3.5 Hz,

24-H). ^{13}C-NMR (C_5D_5N) δ: 78.2 (C1), 43.9 (C2), 68.2 (C3), 43.7 (C4), 140.5 (C5), 124.3 (C6), 32.9 (C7), 32.3 (C8), 51.5 (C9), 43.6 (C10), 24.3 (C11), 40.9 (C12), 40.8 (C13), 57.1 (C14), 32.4 (C15), 83.0 (C16), 61.7 (C17), 16.7 (C18), 13.9 (C19), 36.9 (C20), 12.9 (C21), 112.7 (C22), 68.8 (C23), 73.1 (C24), 36.1 (C25), 60.8 (C26), 14.5 (C27).

8a: A white powder (mp 128—134 °C (dec.)). IR ν_{max}^{KBr} cm^{-1}: 3500—3400 (OH), 980, 915, 890, 840. ^{1}H-NMR (C_5D_5N) δ: 0.96 (3H, d, J=7.0 Hz, 27-Me), 0.99 (3H, s, 18-Me), 1.09 (3H, d, J=7.0 Hz, 21-Me), 1.37 (3H, s, 19-Me), 1.48 (3H, d, J=6.0 Hz, rhamnose 6-Me). ^{13}C-NMR (C_5D_5N) δ: 83.7 (C1), 37.4 (C2), 68.3 (C3), 43.8 (C4), 139.6 (C5), 124.7 (C6), 33.1 (C7), 32.3 (C8), 50.5 (C9), 43.0 (C10), 24.1 (C11), 40.74 (C12), 40.69 (C13), 57.0 (C14), 32.0 (C15), 83.1 (C16), 61.4 (C17), 16.8 (C18), 15.0 (C19), 36.9 (C20), 13.1 (C21), 112.7 (C22), 68.8 (C23), 73.1 (C24), 36.1 (C25), 60.7 (C26), 14.5 (C27); arabinose ($\rightarrow^1$aglycone) 100.4 (C1), 74.3 (C2), 84.3 (C3), 69.5 (C4), 67.0 (C5); rhamnose ($\rightarrow^2$arabinose) 101.7 (C1), 72.5 (C2), 72.5 (C3), 74.3 (C4), 69.4 (C5), 19.0 (C6); xylose ($\rightarrow^3$arabinose) 106.2 (C1), 74.6 (C2), 78.0 (C3), 70.9 (C4), 66.7 (C5).

8b: A white powder (mp 143—145 °C (dec.)). IR ν_{max}^{KBr} cm^{-1}: 3500—3400 (OH), 980, 920, 900, 880. ^{1}H-NMR (C_5D_5N) δ: 0.97 (3H, d, J=7.0 Hz, 27-Me), 1.00 (3H, s, 18-Me), 1.10 (3H, d, J=7.0 Hz, 21-Me), 1.37 (3H, s, 19-Me), 1.49 (3H, d, J=6.0 Hz, rhamnose 6-Me). ^{13}C-NMR (C_5D_5N) δ: 83.4 (C1), 37.4 (C2), 68.3 (C3), 43.9 (C4), 139.7 (C5), 124.7 (C6), 33.1 (C7), 32.3 (C8), 50.6 (C9), 43.0 (C10), 24.1 (C11), 40.8 (C12), 40.7 (C13), 57.0 (C14), 32.1 (C15), 83.1 (C16), 61. 5 (C17), 16.9 (C18), 15.1 (C19), 36.9 (C20), 13.0 (C21), 112.8 (C22), 68.8 (C23), 73.2 (C24), 36.2 (C25), 60.7 (C26), 14.6 (C27); arabinose ($\rightarrow^1$aglycone) 100.2 (C1), 75.6 (C2), 75.5 (C3), 70.0 (C4), 67.1 (C5); rhamnose ($\rightarrow^2$arabinose) 101.6 (C1), 72.5 (C2), 72.7 (C3), 74.3 (C4), 69.5 (C5), 18.9 (C6).

Acetylation of 8, 8a and 8b Each solution of **8** (10 mg), **8a** (10 mg) and **8b** (10 mg) in Ac_2O (0.5 ml)–pyridine (1.0 ml), dimethylaminopyridine (3 mg) stood still at room temperature overnight. The reaction mixtures were treated in the usual way to give **8**-Ac (7 mg), **8a**-Ac (5 mg) and **8b**-Ac (8 mg), respectively.

8-Ac: A white powder (mp 122—123 °C (dec.)). IR ν_{max}^{Nujol} cm^{-1}: 1760 (OAc). ^{1}H-NMR (C_5D_5N) δ: 0.95 (3H, d, J=7.0 Hz, 27-Me), 1.03 (3H, s, 18-Me), 1.11 (3H, d, J=7.0 Hz, 21-Me), 1.22 (3H, d, J=6.0 Hz, fucose 6-Me), 1.33 (3H, s, 19-Me), 1.40 (3H, d, J=6.0, rhamnose 6-Me), 3.67 (1H, m, C_1-H), 4.18 (1H, dd, J=3.5, 7.5 Hz, arabinose 3-H), 4.25 (1H, dd, J=7.5, 8.0 Hz, arabinose 2-H), 4.33 (1H, br s, C_{24}-H), 4.49 (1H, d, J=8.0 Hz, arabinose anomeric H), 5.03 (1H, d, J=8.0 Hz, fucose anomeric H), 5.09 (1H, d, J=6.0 Hz, xylose anomeric H), 5.70 (1H, d, J=5.0 Hz, rhamnose anomeric H).

8a-Ac: A white powder (mp 128—134 °C (dec.)). IR ν_{max}^{Nujol} cm^{-1}: 1760 (OAc). ^{1}H-NMR (C_5D_5N) δ: 0.96 (3H, d, J=7.0 Hz, 27-Me), 1.10 (3H, s, 18-Me), 1.14 (3H, d, J=7.0 Hz, 21-Me), 1.33 (3H, s, 19-Me), 1.49 (3H, d, J=6.0 Hz, rhamnose 6-Me), 3.70 (1H, m, C_1-H), 4.28 (1H, dd, J=7.5, 10.0 Hz, arabinose 2-H), 4.65 (1H, d, J=7.5 Hz, arabinose anomeric H), 5.58 (1H, d, J=5.0 Hz, rhamnose anomeric H).

8b-Ac: A white powder (mp 148—154 °C (dec.)). IR ν_{max}^{Nujol} cm^{-1}: 1755 (OAc). ^{1}H-NMR (C_5D_5N) δ: 0.93 (3H, d, J=7.0 Hz, 27-Me), 1.02 (3H, s, 18-Me), 1.11 (3H, d, J=7.0 Hz, 21-Me), 1.32 (3H, s, 19-Me), 1.40 (3H, d, J=6.0 Hz, rhamnose 6-Me), 3.67 (1H, m, C_1-H), 4.18 (1H, dd, J=4.0, 8.0 Hz, arabinose 3-H), 4.38 (1H, dd, J=6.5, 8.0 Hz, arabinose 2-H).

Deacetylation of 9 and 10 **9** (15 mg) and **10** (15 mg) were individually dissolved in 5% K_2CO_3–50% MeOH solution and stood at room temperature for 30 min. Each reaction mixture was diluted with water (20 ml) and chromatographed on ODS using solvent, water and MeOH, respectively. Each MeOH eluent was evaporated *in vacuo* to afford **8** (8 mg from **9**, 6 mg from **10**).

Acetonide of 8c A mixture of **8c** (10 mg), dry acetone (1.0 ml) and a catalytic amount of *p*-toluenesulfonic acid was allowed to stand at room temperature for 48 h. After being neutralized with sat. $NaHCO_3$ solution, the resulting mixture was extracted with ether. The organic layer was washed with water, dried with anhydrous Na_2SO_4, and evaporated. The residue was subjected to column chromatography on silica gel eluted with hexane–acetone (95 : 5, (v/v)) to afford the acetonide of **8c** (3.0 mg).

Acetonide of **8c**: Colorless needles (from MeOH, mp 243—245 °C (dec.)), ^{1}H-NMR ($CDCl_3$) δ: 0.89 (3H, s, 18-Me), 0.96 (3H, d, J=7.0 Hz, 27-Me), 1.02 (3H, d, J=7.0 Hz, 21-Me), 1.07 (3H, s, 19-Me), 1.36, 1.57 (each 3H, s, *gem*-dimethyl).

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References

1) A part of this work was presented at the 37th Annual Meeting of the Japanese Society of Pharmacognosy, Chiba, November 1990.
2) *a*) T. Asano, T. Murayama, Y. Hirai and J. Shoji, *Chem. Pharm. Bull.*, **41**, 391 (1993); *b*) A. Tada and J. Shoji, *ibid.*, **20**, 1729 (1972); *c*) A. Tada, M. Kobayashi and J. Shoji, *ibid.*, **21**, 308 (1973); *d*) B.-Y. Yu, Y. Hirai, J. Shoji and G.-J. Xu, *ibid.*, **38**, 1931 (1990); *e*) Y. Watanabe, S. Sanada, Y. Ida and J. Shoji, *ibid.*, **31**, 1980 (1983); *f*) B.-Y. Yu, Y. Hirai, J. Shoji and G.-J. Xu, Abstracts of Papers, 109th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1989, p. 183.
3) N. Kaneda, H. Nakanishi, T. Kurihashi and T. Katori, *Yakugaku-zasshi*, **103**, 1133 (1983).
4) K. Bock and C. Pederson, *J. Chem. Soc., Perkin Trans., 2*, **1974**, 293.
5) N. Fukuda, N. Imamura, E. Saito, T. Nohara and T. Kawasaki, *Chem. Pharm. Bull.*, **29**, 325 (1981).
6) *a*) A. G. Gonzarez, R. Freire, M. G. Garcia-Estrada, J. A. Salazar Rocio and E. Suarez Lopez, *Rev. Latinoamer. Qim.*, **3**, 8 (1972); *b*) A. G. Gonzarez, R. Freire Barreira, M. G. Garcia-Esterada, J. A. Salazar Rocio and E. Suarez Lopez, *An. Qim.*, **67**, 557 (1971).
7) Y. Asada, M. Ikeno, T. Ueoka and T. Furuya, *Chem. Pharm. Bull.*, **37**, 2747 (1989).
8) *a*) D. P. Burma, *Anal. Chim. Acta*, **9**, 513 (1953); *b*) J. J. Schneider and M. L. Lewbart, *J. Biol. Chem.*, **222**, 787 (1966).