[Chem. Pharm. Bull. 25(11)3049—3055(1977)] UDC 547.918.02.05 : 581.192

Studies on the Constituents of Ophiopogonis Tuber. IV.[1] On the Structures of Ophiopogonin A, B′, C, C′, and D′

Yoshiaki Watanabe, Shuichi Sanada, Akihiro Tada, and Junzo Shoji

School of Pharmaceutical Sciences, Showa University[2]

(Received April 4, 1977)

Ophiopogonin A, B′, C, C′ and D′, the minor oligosides of Ophiopogonis tuber (tuber of *Ophiopogon japonicus* Ker-Gawler var. *genuinus* Maxim.), have been isolated. The structures of these oligosides have been established as follows: ophiopogonin A(III): ruscogenin 1-*O*-[(3-*O*-acetyl)-α-L-rhamnopyranosyl(1→2)]-β-D-fucopyranoside, ophiopogonin B′(IV): diosgenin 3-*O*-[(4-*O*-acetyl)-α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-glucopyranoside, ophiopogonin C (V): mono-*O*-acetylophiopogonin D, ophiopogonin C′ (VI): diosgenin 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside (=prosapogenin A of dioscin), ophiopogonin D′(VII): diosgenin 3-*O*-[α-L-rhamnopyranosyl-(1→2)][β-D-xylopyranosyl(1→3)]-β-D-glucopyranoside.

Keywords——ophiopogonis tuber; *Ophiopogon japonicus*; Liliaceae; spirostanol glycoside; ophiopogonin; diosgenin; ruscogenin; column chromatography; NMR; Klyne's rule

In our previous papers, it had been reported that the structures of two major glycosides of ophiopogonis tuber (tuber of *Ophiopogon japonicus* Ker-Gawler var. *genuinus* Maxim.; Liliaceae), namely ophiopogonin B[3] (I) and D[1] (II), were established to be ruscogenin 1-*O*-α-L-rhamnopyranosyl (1→2)-β-D-fucopyranoside and ruscogenin 1-*O*-[α-L-rhamnopyranosyl (1→2)]-[β-D-xylopyranosyl (1→3)]-β-D-fucopyranoside, respectively. It is remarkable that the sugar moiety of both spirostanol glycosides is attached to the hydroxyl group at C-1, not at C-3 in the ordinary steroidal saponin. The present paper deals with the structure determination of ophiopogonin A, B′, C, C′, and D′ leading to the assignment of the structures III, IV, V, VI, and VII.

Five new steroidal glycosides were obtained from the methanolic extract of the tuber as shown in Chart 1.

The general properties of ophiopogonin A, B′, C, C′, and D′ are given in Table I. Infrared (IR) and nuclear magnetic resonance (NMR) spectra of ophiopogonin A, B′, and C show the presence of one *O*-acetyl group in each glycoside.

On acid hydrolysis, both ophiopogonin A and C gave ruscogenin as an aglycone, while ophiopogonin B′, C′, and D′ afforded diosgenin. The monosaccharide components of each glycoside are listed in Table II and the optical rotations of each phenylosazone derivative of monosaccharides were measured.

Acetylation of ophiopogonin A, B′, C, and D′ with acetic anhydride and pyridine furnished ophiopogonin A pentaacetate, ophiopogonin B′ heptaacetate, ophiopogonin C heptaacetate and ophiopogonin D′ octaacetate, respectively. The identities of ophiopogonin A pentaacetate with ophiopogonin B hexaacetate,[3] ophiopogonin B′ heptaacetate with ophiopogonin D′ octaacetate and ophiopogonin C heptaacetate with ophiopogonin D octaacetate were established by direct comparisons. On the other hand, deacetylation of ophiopogonin A, B′, and C with 0.5% potassium hydroxide in ethanol gave ophiopogonin B,[3] D′, and D[1] respectively. Based on

1) Part III: A. Tada, M. Kobayashi, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **21**, 308 (1973).
2) Location: *Hatanodai, Shinagawa-ku, Tokyo.*
3) A. Tada and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **20**, 1729 (1972).

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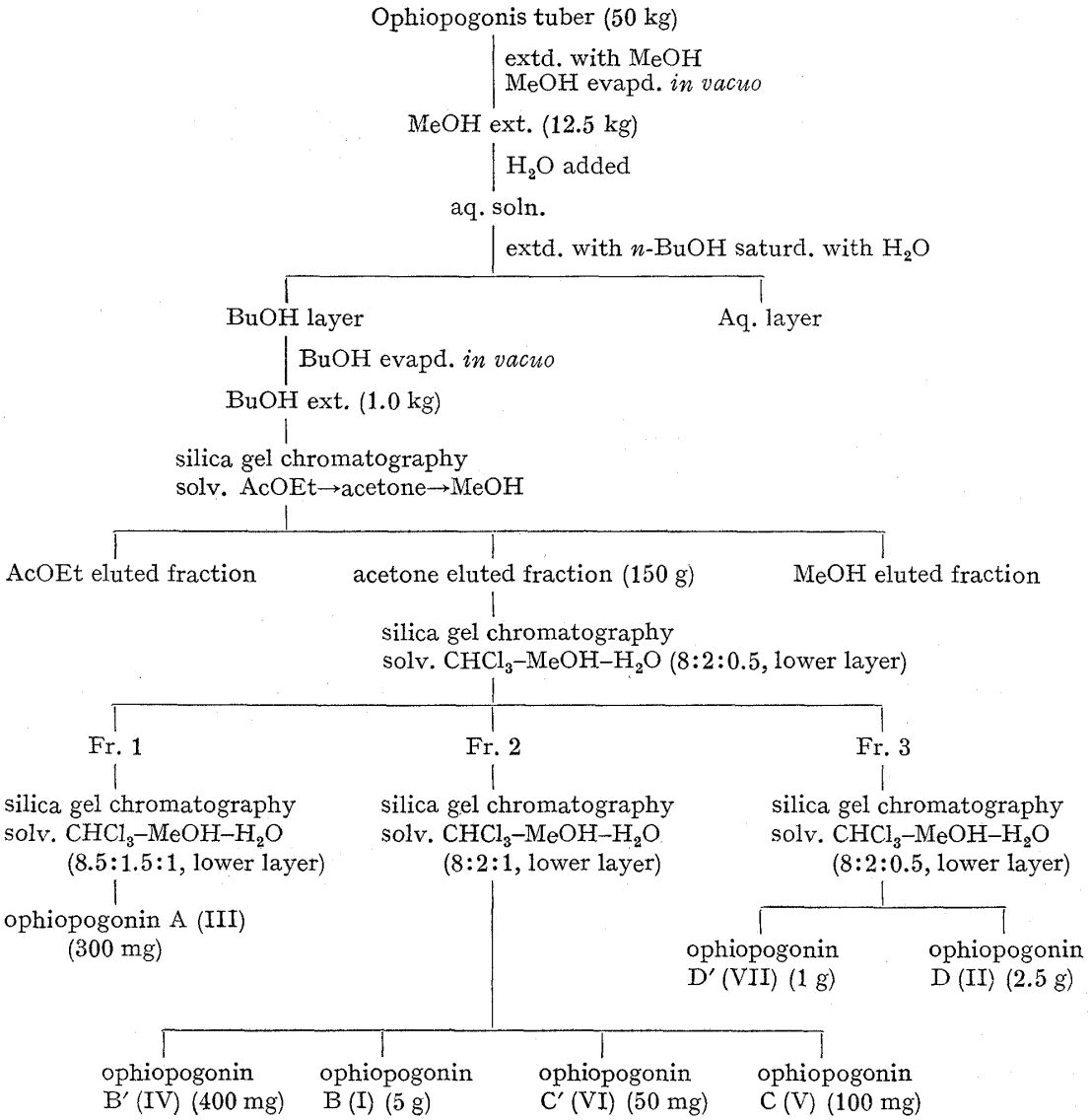

Chart 1

the accumulated evidences, ophiopogonin A, B′, and C were found to be identical with the monoacetates of ophiopogonin B, D′, and D, respectively.

Partial hydrolysis of ophiopogonin D′ with 0.4 N sulfuric acid gave three prosapogenins, namely prosapogenin D'_1(=ophiopogonin C′)(VI), D'_2(VIII), and D'_3(IX). On hydrolysis with 2 N hydrogen chloride in 50% dioxane, prosapogenin D'_1 gave diosgenin, glucose and rhamnose, while prosapogenin D'_3 afforded diosgenin and glucose. The physical and chemical properties of prosapogenin D'_1 and D'_3 suggested that both prosapogenins must be identical with prosapogenin A of dioscin(=diosgenin 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside[4,5]) and trillin(=diosgenin 3-*O*-β-D-glucopyranoside[4]), respectively. The identities of prosapogenin D'_1 with prosapogenin A and prosapogenin D'_3 with trillin were proved by direct comparison with authentic samples which were kindly given us from Prof. Kawasaki.

Furthermore, prosapogenin D'_2 gave diosgenin, glucose and xylose on hydrolysis with 2 N hydrogen chloride. After methylation of prosapogenin D'_2 by Hakomori's method,[6] hexa-*O*-

4) T. Tsukamoto, T. Kawasaki, and T. Yamauchi, *Pharm. Bull.* (Japan), **4**, 35 (1956); T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **10**, 703 (1962).
5) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1070, (1968).
6) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

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Table I

Ophiopogonins	Properties	mp (°C)	$[\alpha]_D$ (c, solv., °C)	Formula	IR (KBr) cm^{-1}	NMR $\delta^{C_5D_5N}_{TMS}$ (ppm)
A (Ⅲ)	Colorless needles (EtOH–H_2O)	182—184	−89.7 (0.27, Py, 18)	$C_{41}H_{64}O_{13}\cdot 3H_2O$	3400 (broad OH) 1720 (ester) 982, 920, 900, 865 (intensity, 900>920 isospiroketal)	0.75 (3H, m, CH_3) 0.90 (3H, s, CH_3) 1.10 (3H, d, J=6Hz, CH_3) 1.43 (3H, s, CH_3) 1.52 (3H, d, J=6Hz, CH_3) 1.72 (3H, d, J=6Hz, CH_3) 2.00 (3H, s, $OCOCH_3$)
B′(Ⅳ)	Colorless needles (EtOH)	245—248 (dec.)	−86.65 (0.67, Py, 20)	$C_{46}H_{72}O_{12}\cdot 2H_2O$	3400 (broad OH) 1735 (ester) 980, 920, 900, 865 (intensity, 900>920 isospiroketal)	0.77 (3H, m, CH_3) 0.87 (3H, s, CH_3) 1.08 (3H, s, CH_3) 1.18 (3H, d, J=6Hz, CH_3) 1.78 (3H, d, J=6Hz, CH_3) 2.06 (3H, s, $OCOCH_3$)
C (Ⅴ)	Colorless needles (EtOH–H_2O)	238—240	−93.33 (0.15, Py, 28)	$C_{46}H_{72}O_{17}\cdot 4H_2O$	3420 (broad OH) 1715 (br., ester) 982, 920, 900, 862 (intensity, 900>920 isospiroketal)	0.75 (3H, m, CH_3) 0.90 (3H, s, CH_3) 1.08 (3H, d, J=6Hz, CH_3) 1.42 (3H, s, CH_3) 1.50 (3H, d, J=6Hz, CH_3) 1.72 (3H, d, J=6Hz, CH_3) 2.05 (3H, s, $OCOCH_3$)
C′(Ⅵ)	Colorless needles (MeOH)	238—240 (dec.)	−99.20 (0.21, Py, 21)	$C_{39}H_{62}O_{12}$	3400 (broad OH) 980, 920, 900, 865 (intensity, 900>920 isospiroketal)	0.77 (3H, m, CH_3) 0.87 (3H, s, CH_3) 1.08 (3H, s, CH_3) 1.18 (3H, d, J=6Hz, CH_3) 1.70 (3H, d, J=6Hz, CH_3)
D′(Ⅶ)	Colorless needles (EtOH–H_2O)	255—257 (dec.)	−41.34 (0.17, Py, 18)	$C_{44}H_{70}O_{16}\cdot 2H_2O$	3400 (broad OH) 980, 920, 900, 865 (intensity, 900>920 isospiroketal)	0.77 (3H, m, CH_3) 0.87 (3H, s, CH_3) 1.08 (sH, s, CH_3) 1.18 (3H, d, J=6Hz, CH_3) 1.78 (3H, d, J=6Hz, CH_3)

Py=pyridine.

Table II

Ophiopogonins	Aglycones	Sugar components
A (Ⅲ)	Ruscogenin	Fucose Rhamnose (Acetyl)
B′(Ⅳ)	Diosgenin	Glucose Xylose (Acetyl) Rhamnose
C (Ⅴ)	Ruscogenin	Fucose Xylose (Acetyl) Rhamnose
C′(Ⅵ)	Diosgenin	Glucose Rhamnose
D′(Ⅶ)	Diosgenin	Glucose Xylose Rhamnose

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methylprosapogenin D′$_2$ was methanolyzed by the same method described in the preceding papers. The methanolysate was examined by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC) to identify diosgenin, methyl 2,4,6-tri-*O*-methyl-D-glucopyranoside and per-*O*-methyl-D-xylopyranoside. The difference of molecular rotation[7] between prosapogenin D′$_2$ and prosapogenin D′$_3$ is $-97°$ ($[M]_D$ of methyl D-xylopyranoside: α form $=+249°$; β form $=-107°$) and the coupling constant (7 Hz) of the anomeric proton of hexa-*O*-methyl-prosapogenin D′$_2$ in NMR spectrum indicated that the configuration of D-xylose is β-form. Based on the experimental data, prosapogenin D′$_2$ and ophiopogonin D′ were established to be diosgenin 3-*O*-β-D-xylopyranosyl(1→3)-β-D-glucopyranoside and diosgenin 3-*O*-[α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-glucopyranoside, respectively.

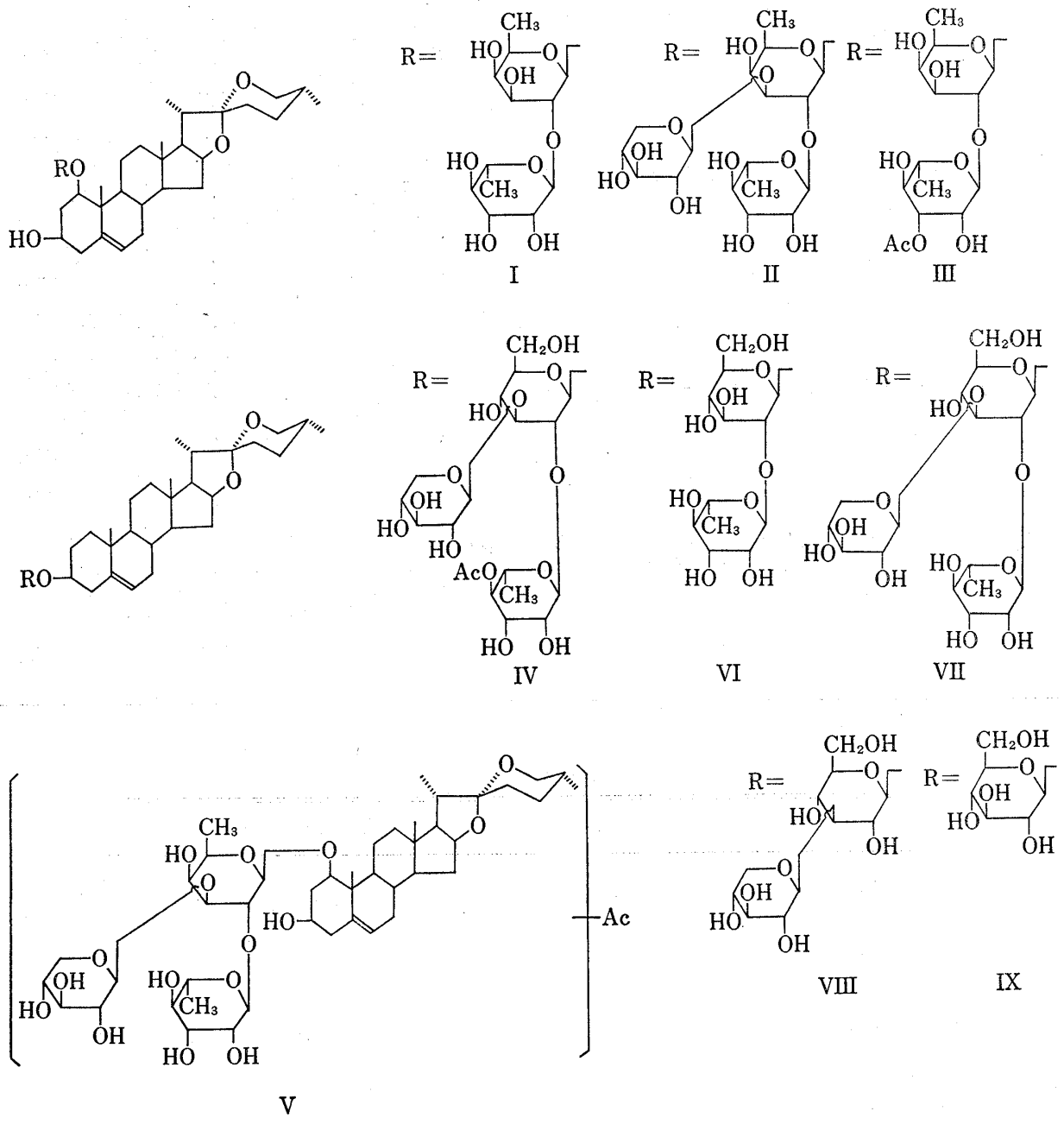

Chart 2

7) W. Klyne, *Biochem. J.*, **47**, xli (1950).

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Although ophiopogonin C has been deduced to be a monoacetate of ophiopogonin D, the location of acetyl group has not been defined due to the shortage of the material.

The chemical and physical properties of ophiopogonin C′ and prosapogenin D'_1(=prosapogenin A of dioscin) strongly resemble. Direct comparison of both compounds established the identity.

The structures of ophiopogonin A and B′ were established as follows.

To confirm the location of the acetyl group in each glycoside, ophiopogonin A and B′ were methylated by Kuhn's method[8] to afford penta-*O*-methylophiopogonin A and hepta-*O*-methylophiopogonin B′. On methanolysis, penta-*O*-methylophiopogonin A gave 3-*O*-methylruscogenin,[3] methyl 2,4-di-*O*-methyl-L-rhamnopyranoside and methyl 3,4-di-*O*-methyl-D-fucopyranoside, while hepta-*O*-methylophiopogonin B′ afforded diosgenin, per-*O*-methyl-D-xylopyranoside, methyl 2,3-di-*O*-methyl-L-rhamnopyranoside and methyl 4,6-di-*O*-methyl-D-glucopyranoside. Consequently, the locations of each acetyl group in ophiopogonin A and B′ are assigned to be C_3-hydroxyl group of rhamnose and C_4-hydroxyl group of rhamnose by comparison of methanolysates of per-*O*-methylophiopogonin B with penta-*O*-methylophiopogonin A and octa-*O*-methylophiopogonin D′ with hepta-*O*-methylophiopogonin B′. Finally, the structures of ophiopogonin A and B′ were established to be ruscogenin 1-*O*-[(3-*O*-acetyl)-α-L-rhamnopyranosyl(1→2)]-β-D-fucopyranoside and diosgenin 3-*O*-[(4-*O*-acetyl)-α-L-rhamnopyranosyl(1→2)] [β-D-xylopyranosyl(1→3)]-β-D-glucopyranoside, respectively.

As we described in the preceding papers, ophiopogonis tuber is one of the famous drugs in Chinese medicine, which is said to be effective as expectorant, antitussive and others, but the active principle has not been known. The unique chemical structure of ophiopogonin has urged us to study the biological activity of these glycosides and the result will be reported in the forthcoming paper.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a Yanagimoto OR-50 automatic polarimeter. IR spectra were obtained with a Hitachi Model EPI-2 and NMR spectra were taken at 90 MHz with a Hitachi Model R-22 High Resolution NMR spectrometer and chemical shifts are given in δ (ppm) scale with tetramethyl silane as the internal standard. GLC was run on a Shimadzu GC-6A with flame ionization detector. Paper partition chromatography (PPC) was conducted on Tôyô Roshi No. 51 using the upper layer of *n*-BuOH–AcOH–H_2O (4: 1: 5) as the solvent and aniline hydrogen phthalate as the spray reagent. TLC was performed on Kieselgel H (Merck) by using the following solvent system: a) hexane–acetone (2: 1), b) $CHCl_3$–MeOH–H_2O (65: 35: 10, lower layer), c) $CHCl_3$–MeOH–H_2O (7: 3: 1, lower layer). Detection was made by spraying 10% H_2SO_4 followed by heating.

Extraction and Isolation of Ophiopogonins——Ophiopogonis tuber (dried tuber of *Ophiopogon japonicus* KER-GAWLER var. *genuinus* MAXIMOWICZ) was crushed and treated as shown in Chart 1. The BuOH extractives were submitted to column chromatography over silica gel with AcOEt, acetone and MeOH. The fraction eluted with acetone was rechromatographed on silica gel with $CHCl_3$–MeOH–H_2O (8: 2: 0.5, lower layer) to afford three fractions (Fr. 1—Fr. 3). Fr. 1 was separated by rechromatography on silica gel with $CHCl_3$–MeOH–H_2O (8.5: 1.5: 1, lower layer) to give ophiopogonin A. Fr. 2 and Fr. 3 were submitted to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (8: 2: 1, lower layer) for Fr. 2 and $CHCl_3$–MeOH–H_2O (8: 2: 0.5, lower layer) for Fr. 3 to afford ophiopogonin B′, B, C′ and C from the former and ophiopogonin D′ and D from the latter, respectively.

Properties of Ophiopogonins——The general properties of ophiopogonins are listed in Table I. Ophiopogonin A (III): *Anal.* Calcd. for $C_{41}H_{64}O_{13}\cdot 3H_2O$: C, 60.13; H, 8.61. Found: C, 60.62; H, 8.17. Ophiopogonin B′ (IV): *Anal.* Calcd. for $C_{46}H_{72}O_{17}\cdot 2H_2O$: C, 59.21; H, 8.21. Found: C, 59.58; H, 7.88. Ophiopogonin C (V): *Anal.* Calcd. for $C_{46}H_{72}O_{17}\cdot 4H_2O$: C, 57.54; H, 8.24. Found: C, 57.52; H, 7.97. Ophiopogonin D′ (VII): *Anal.* Calcd. for $C_{44}H_{70}O_{16}\cdot 2H_2O$: C, 59.31; H, 8.40. Found: C, 59.46; H, 8.24.

Hydrolysis of III, IV, V, VI and VII——III, IV, V, VI and VII were refluxed with 2 N HCl (4 N HCl–50% dioxane=1: 1 v/v) on a water bath for 4 hr, respectively. Each reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ extractive was washed with water, dried on anhyd. Na_2SO_4 and

8) R. Kuhn, *Angew. Chem.*, 67, 32 (1955).

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evaporated. IV, VI and VII afforded the same aglycone, colorless needles from acetone, mp 204—206°, IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 980, 920, 900, 865 (intensity 900>920, isospiroketal), NMR (in $CDCl_3$) δ: 0.78 (3H, d, J=6 Hz, CH_3), 0.80 (3H, s, CH_3), 0.98 (3H, d, J=6 Hz, CH_3), 1.04 (3H, s, CH_3), which was identified with diosgenin by TLC (solvent a, *Rf* 0.56), a mixed fusion and by comparing IR and NMR spectra. III and V gave ruscogenin as an aglycone[1,3] which was identified by TLC (solvent a, *Rf* 0.10), and by direct comparisons.

The aqueous layer was neutralized with Amberlite IR-4B and evaporated *in vacuo* to dryness. Each residue was examined by PPC, TLC and GLC. PPC: *Rf* 0.06 (glucose), 0.14 (xylose), 0.22 (rhamnose), 0.19 (fucose). TLC (solvent b): *Rf* 0.08 (glucose), 0.12 (xylose), 0.17 (rhamnose), 0.15 (fucose). GLC (column: 5% SE-52 on Chromosorb W 3 mm×2 m, column temp.: 170°, injection temp.: 210°, carrier gas: N_2 1.0 kg/cm^2, sample: trimethyl silane (TMS) derivative): t_R(min) 14.9, 23.3 (glucose), 5.2, 6.6 (xylose), 3.7, 5.0 (rhamnose), 4.0, 5.3 (fucose). The aqueous solution of each residue was heated with phenylhydrazine hydrochloride and AcONa for 30 min on a water bath and treated as the usual manner. The product was purified by column chromatography on silica gel with hexane–acetone. D-Glucose phenylosazone: yellow needles from acetone, mp 210°, $[\alpha]_D^{26}$ −6.94° (c=0.95, pyridine–EtOH). D-Xylose phenylosazone: a yellow crystalline powder, (mp 158—160°), $[\alpha]_D^{26}$ −43.72° (c=0.77, EtOH). L-Rhamnose phenylosazone: a yellow crystalline powder, (mp 195°), $[\alpha]_D^{26}$ +65.70° (c=0.78, pyridine).

Acetylation of III, IV, V and VII——III, IV, V and VII were dissolved in pyridine and Ac_2O, respectively. Each solution was allowed to stand overnight at room temperature. The reaction mixture was worked up as the usual manner and the product was purified by recrystallization. Ophiopogonin A pentaacetate: colorless needles from aqueous MeOH, mp 228—230°, $[\alpha]_D^{19}$ −65.2° (c=0.27, $CHCl_3$), *Anal.* Calcd. for $C_{51}H_{74}O_{18}$: C, 62.87; H, 7.75. Found: C, 62.92; H, 7.49. IR ν_{max}^{KBr} cm^{-1}: 1740 (ester), 982, 920, 900, 865 (intensity 900>920 isospiroketal). NMR (in $CDCl_3$) δ: 0.78 (3H, d, J=6 Hz, CH_3), 0.83 (3H, s, CH_3), 0.96 (3H, d, J=6 Hz, CH_3), 1.08 (3H, s, CH_3), 1.15 (3H, d, J=6 Hz, CH_3), 1.23 (3H, d, J=6 Hz, CH_3), 1.90—2.20 (3H×6 $OCOCH_3$). Ophiopogonin A pentaacetate was identified with ophiopogonin B hexaacetate[3] by direct comparisons. Ophiopogonin B′ heptaacetate: colorless needles from aqueous methanol, mp 167—170°, $[\alpha]_D^{19}$ −73.2° (c=0.27, $CHCl_3$). *Anal.* Calcd. for $C_{60}H_{86}O_{24}$: C, 60.49; H, 7.28. Found: C, 60.23; H, 7.34. IR ν_{max}^{KBr} cm^{-1}: 1745 (ester), 980, 920, 900, 865 (intensity 900>920, isospiroketal). NMR (in $CDCl_3$) δ: 0.80 (3H, s, CH_3), 0.97 (3H, d, J=6 Hz, CH_3), 1.02 (3H, s, CH_3), 1.20 (3H, d, J=6 Hz, CH_3), 1.26 (3H, d, J=6 Hz, CH_3), 1.98—2.18 (3H×8 $OCOCH_3$). Ophiopogonin C heptaacetate: colorless needles from aqueous MeOH, mp 184—186°. *Anal.* Calcd. for $C_{56}H_{86}O_{24}$: C, 60.48; H, 7.27. Found: C, 60.03; H, 7.46. IR ν_{max}^{KBr} cm^{-1}: 1750 (ester), 980, 920, 900, 865 (intensity 900>920, isospiroketal). NMR (in $CDCl_3$) δ: 0.78 (3H, d, J=6 Hz, CH_3), 0.83 (3H, s, CH_3), 0.96 (3H, d, J=6 Hz, CH_3), 1.08 (3H, s, CH_3), 1.15 (3H, d, J=6 Hz, CH_3), 1.23 (3H, d, J=6 Hz, CH_3), 2.00—2.25 (3H×7, $OCOCH_3$). Ophiopogonin C heptaacetate was identified with ophiopogonin D octaacetate derivated from ophiopogonin D[1] by direct comparisons. Ophiopogonin D′ octaacetate: colorless needles from aqueous MeOH, mp 167—170°, $[\alpha]_D^{19}$ −65.8° (c=0.36, $CHCl_3$). *Anal.* Calcd. for $C_{60}H_{86}O_{24}$: C, 60.49; H, 7.28. Found: C, 60.04; H, 7.46. IR ν_{max}^{KBr} cm^{-1}: 1745 (ester), 980, 920, 900, 865 (intensity 900>920, isospiroketal). NMR (in $CDCl_3$) δ: 0.80 (3H, s, CH_3), 0.97 (3H, d, J=6 Hz, CH_3), 1.02 (3H, s, CH_3), 1.19 (3H, d, J=6 Hz, CH_3), 1.23 (3H, d, J=5 Hz, CH_3), 1.98—2.18 (3H×8, $OCOCH_3$). Ophiopogonin D′ octaacetate was identified with ophiopogonin B′ heptaacetate by direct comparisons.

Deacetylation of III, IV and V with 0.5% KOH——Each solution of III, IV and V in 0.5% KOH–EtOH was refluxed for 3 hr under N_2 gas flow. The reaction mixture was cooled, diluted with water and evaporated *in vacuo*. The aqueous solution was extracted with BuOH saturated with H_2O and the extractive was evaporated *in vacuo*. The residue was purified by recrystallization. Desacetylophiopogonin A: colorless needles from EtOH, mp 269—270°, $[\alpha]_D^{19}$ −98.7° (c=0.22, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3400 (broad, OH), 982, 920, 900, 865 (intensity 900>920, isospiroketal), NMR (in C_5D_5N) δ: 0.70 (3H, m, CH_3), 0.90 (3H, s, CH_3), 1.10 (3H, d, J=6 Hz, CH_3), 1.43 (3H, s, CH_3), 1.50 (3H, d, J=6 Hz, CH_3), 1.72 (3H, d, J=6 Hz, CH_3). Desacetylophiopogonin A was identified with ophiopogonin B by direct comparisons. Desacetylophiopogonin B′: colorless needles from aqueous MeOH, mp 255—257° (dec.), $[\alpha]_D^{21}$ −30.23° (c=0.73, pyridine). *Anal.* Calcd. for $C_{44}H_{70}O_{16}\cdot 2H_2O$: C, 59.31; H, 8.40. Found: C, 59.71; H, 8.29. IR ν_{max}^{KBr} cm^{-1}: 3400 (broad, OH), 980, 920, 900, 865 (intensity 900>920, isospiroketal), NMR (in C_5D_5N) δ: 0.77 (3H, m, CH_3), 0.87 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.18 (3H, d, J=6 Hz, CH_3), 1.78 (3H, d, J=6 Hz, CH_3). Desacetylophiopogonin B′ was identified with ophiopogonin D′ by direct comparisons. Desacetylophiopogonin C: colorless needles from aqueous EtOH, mp 250—252°,[9] $[\alpha]_D^{27}$ −100° (c=0.55, pyridine), IR ν_{max}^{Nujol} cm^{-1}: 3320 (broad, OH), 982, 920, 900, 862, (intensity 900>920, isospiroketal), NMR (in C_5D_5N) δ: 0.70 (3H, m, CH_3), 0.90 (3H, s, CH_3), 1.08 (3H, d, J=6 Hz, CH_3), 1.42 (3H, s, CH_3), 1.50 (3H, d, J=6 Hz, CH_3), 1.72 (3H, d, J=6 Hz, CH_3). Desacetylophiopogonin C was identified with ophiopogonin D by direct comparisons.

Partial Hydrolysis of VII——The solution of VII (200 mg) in 0.4 N H_2SO_4–50% EtOH (10 ml) was heated for 5 hr at 70° on a water bath. The reaction mixture was diluted with water (5 ml) and evaporated *in vacuo*

9) The melting point, 263—265°, reported in the previous paper[1] is erroneous and it was revised in this paper.

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to 10 ml. The aqueous solution was extracted with BuOH saturated with water and the extractive was evaporated *in vacuo*. The residue was fractionated by column chromatography over silica gel using $CHCl_3$–MeOH–H_2O (8: 2: 0.5 lower layer) to afford prosapogenin D'_1 (VI), D'_2 (VIII) and D'_3 (IX). Prosapogenin D'_1 (VI): colorless needles from MeOH, mp 238—243° (dec.), $[\alpha]_D^{21}$ −103.0° (c=0.21, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3400 (broad, OH), 980, 920, 900, 865 (intensity 900>920, isospiroketal). VI gave diosgenin, glucose and rhamnose on hydrolysis with 2 N HCl in 50% dioxane by the same procedure as described above. VI was identified with ophiopogonin C′ and a prosapogenin of dioscin given us from Prof. Kawasaki by comparing TLC (solvent c, *Rf* 0.53) and IR spectra. Prosapogenin D'_2 (VIII): colorless needles from MeOH, mp 250—252° (dec.), $[\alpha]_D^{18.5}$ −90.32° (c=0.29, pyridine), $[M]_D$=−600.24°. *Anal.* Calcd. for $C_{38}H_{60}O_{12}\cdot 3H_2O$: C, 57.27; H, 7.59. Found: C, 57.44; H, 7.62, IR ν_{max}^{KBr} cm^{-1}: 3450 (broad, OH), 980, 920, 900, 865 (intensity 900>920, isospiroketal). VIII gave diosgenin, glucose and xylose on hydrolysis by the same procedure described above. Prosapogenin D'_3 (IX): colorless needles from MeOH, mp 250—252° (dec.), $[\alpha]_D^{20}$ −87.2° (c=0.25, dioxane), $[M]_D$=−502.93°, IR ν_{max}^{KBr} cm^{-1}: 3450 (broad, OH), 980, 920, 900, 865 (intensity 900>920, isospiroketal). IX gave diosgenin and glucose on hydrolysis by the same procedure described above. Prosapogenin D_3' was identified with trillin given us from Prof. Kawasaki by comparing TLC (solvent c, *Rf* 0.69) and IR spectra.

Methylation of VII and VIII by Hakomori's Method——VII and VIII were methylated by the method described in the previous papers. Octa-*O*-methylophiopogonin D′: colorless needles from MeOH, mp 115—117°, $[\alpha]_D^{19}$ −33.3° (c=0.15, $CHCl_3$). *Anal.* Calcd. for $C_{52}H_{86}O_{16}$: C, 64.58; H, 8.96. Found: C, 64.21; H, 9.08. IR ν_{max}^{Nujol} cm^{-1}: 984, 918, 900, 865 (intensity 900>918, isospiroketal), NMR (in $CDCl_3$) δ: 0.80 (3H, s, CH_3), 0.96 (3H, d, J=7 Hz, CH_3), 1.03 (3H, s, CH_3), 1.22 (3H, d, J=6 Hz, CH_3), 1.32 (3H, d, J=6 Hz, CH_3), 3.33—3.68 (3H×8, OCH_3). Hexa-*O*-methylprosapogenin D'_2: colorless needles from MeOH, mp 143—145°, IR ν_{max}^{Nujol} cm^{-1}: 980, 920, 900, 865 (intensity 900>920, isospiroketal), NMR (in $CDCl_3$) δ: 0.80 (3H, s, CH_3), 0.95 (3H, d, J=6 Hz, CH_3), 1.03 (3H, s, CH_3), 1.22 (3H, d, J=6 Hz, CH_3), 3.40—3.70 (3H×6, OCH_3), 4.30 (1H, d, J=6 Hz, glucose anomeric H), 4.40 (1H, d, J=7 Hz, xylose anomeric H).

Methylation of III and IV by Kuhn's Method——According to the Kuhn's method,[8] the solution of III and IV in dimethylformamide was methylated with Ag_2O and methyl iodide at room temperature for 7 days with stirring. The reaction mixture was filtered and the filtrate was diluted with water and KCN (solid) was added to dissolve the silver salt. The reaction mixture was extracted with $CHCl_3$, and the $CHCl_3$ layer was washed with water, dried over Na_2SO_4 and evaporated. The residue was submitted to chromatography on silica gel using hexane–acetone (9: 1) as a solvent and each fraction was purified by reprecipitation from MeOH. Penta-*O*-methylophiopogonin A: a white powder from aqueous MeOH, (mp 70—72°), IR ν_{max}^{Nujol} cm^{-1}: 1745 (ester), 982, 920, 900 865 (intensity 900>920, isospiroketal), NMR (in $CDCl_3$) δ: 0.83 (3H, s, CH_3), 0.84 (3H, d, J=6 Hz, CH_3), 0.95 (3H, d, J=6 Hz, CH_3), 1.08 (3H, s, CH_3), 1.22—1.32 (3H×2, broad, CH_3), 2.08 (3H, s, $OCOCH_3$), 3.33—3.60 (3H×5, s, OCH_3), 4.18 (1H, d, J=8 Hz, fucose anomeric H), 5.25 (1H, d, J=1 Hz, rhamnose anomeric H). Hepta-*O*-methylophiopogonin B′: a white powder from MeOH, (mp 95—97°). *Anal.* Calcd. for $C_{53}H_{86}O_{17}$: C, 63.96; H, 8.71. Found: C, 63.76; H, 9.29. IR ν_{max}^{Nujol} cm^{-1}: 1735 (ester), 980, 920, 900, 865 (intensity 900>920, isospiroketal), NMR (in $CDCl_3$) δ: 0.80 (3H, s, CH_3), 0.96 (3H, d, J=6 Hz, CH_3), 1.03 (3H, s, CH_3), 1.23 (3H, d, J=6 Hz, CH_3), 1.32 (3H, d, J=6 Hz, CH_3), 2.08 (3H, s, $OCOCH_3$), 3.40—3.70 (3H×7, OCH_3), 4.30 (1H, d, J=6 Hz, glucose anomeric H), 4.40 (1H, d, J=6 Hz, xylose anomeric H), 5.38 (1H, d, J=1 Hz, rhamnose anomeric H).

Methanolyses of *O*-Methylophiopogonins with Methanolic 6% HCl——*O*-methylophiopogonins were methanolyzed with methanolic 6% HCl refluxing for 2 hr, respectively. Each reaction mixture was neutralized with Ag_2CO_3 and evaporated to dryness. The residue was examined by TLC (solvent a) and GLC (column: 5% NPGS glass column 3 mm×2 m, column temp.: 160°, injection temp.: 200°, carrier gas: N_2 1.0 kg/cm^2). Penta-*O*-methylophiopogonin A: *Rf* 0.25 (methyl 3,4-di-*O*-methylfucopyranoside), 0.29 (methyl 2,4-di-*O*-methylrhamnopyranoside); t_R(min) 5.1(methyl 2,4-di-*O*-methylrhamnopyranoside), 7.7(methyl 3,4-di-*O*-methylfucopyranoside). Hepta-*O*-methylophiopogonin B′: *Rf* 0.10 (methyl 4,6-di-*O*-methylglucopyranoside), 0.53 (methyl 2,3-di-*O*-methylrhamnopyranoside), 0.49, 0.53 (per-*O*-methylxylopyranoside); t_R 2.5, 3.1 (per-*O*-methylxylopyranoside), 2.8 (TMS derivative of methyl 4,6-di-*O*-methylglucopyranoside), 8.1 (methyl 2,3-di-*O*-methylrhamnopyranoside). Octa-*O*-methylophiopogonin D′: *Rf* 0.10 (methyl 4,6-di-*O*-methylglucopyranoside), 0.49, 0.53 (per-*O*-methylxylopyranoside), 0.47 (per-*O*-methylrhamnopyranoside); t_R 2.5, 3.1 (per-*O*-methylxylopyranoside), 2.8 (TMS derivative of methyl 4,6-di-*O*-methylglucopyranoside), 2.6, 3.0 (per-*O*-methylrhamnopyranoside). Hexa-*O*-methylprosapogenin D'_2: *Rf* 0.14 (methyl 2,4,6-tri-*O*-methylglucopyranoside), 0.49, 0.53 (per-*O*-methylxylopyranoside); t_R 2.5, 3.1 (per-*O*-methylxylopyranoside), 24.3 (methyl 2,4,6-tri-*O*-methylglucopyranoside).

Acknowledgement The authors express their gratitude to Professor T. Kawasaki of Kyushu University for his kind supply of the authentic samples. Thanks are also due to the members of Analytical Laboratory of this school for elemental analysis and for the measurement of IR and NMR spectra.

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