Chem. Pharm. Bull.
20(8)1729—1734(1972)

UDC 547.92.02 : 581.192.582.572.2

Studies on the Constituents of Ophiopogonis Tuber. II.[1] On the Structure of Ophiopogonin B

Akihiro Tada and Junzo Shoji

School of Pharmaceutical Sciences, Showa University[2]

(Received January 12, 1972)

The chemical structure of ophiopogonin B, $C_{39}H_{62}O_{12}\cdot 2H_2O$, mp 269—271°, $[\alpha]_D^{15}$ $-105.5°$ (pyridine), a main glycoside isolated from the tuber of *Ophiopogon japonicus* Ker-Gawler var. *genuinus* Maxim. (Liliaceae) was established to be ruscogenin (1)-α-L-rhamnopyranosyl ($1_{rham}\rightarrow 2_{fuc}$)-$\beta$-D-fucopyranoside as represented by formula (Ia).

It should be noted that ophiopogonin B is one of the examples of the spirostane type glycosides whose sugar moiety links to the hydroxyl group other than C_3-hydroxyl group of the aglycone.

As we reported in the previous paper,[1] ophiopogonin B is one of the tuber glycosides of *Ophiopogon japonicus* Ker-Gawler var. *genuinus* Maxim. (Liliaceae), which was isolated in a pure form from the butanol soluble fraction of methanol extracts of the dried crude drug by repeated chromatography on silica gel. It is a ruscogenin diglycoside of which the sugar composition is one mole each of D-fucose and L-rhamnose. In the present paper, the structure elucidation of ophiopogonin B which leads to the assignment of the structure (Ia) has been described.

The infrared (IR) spectrum of ophiopogonin B (Ia), $C_{39}H_{62}O_{12}\cdot 2H_2O$, colorless needles, mp 269—271°, $[\alpha]_D^{15}-105.5°$ (in pyridine) indicates the presence of the isospiroketal ring system[3] and hence the sugar moiety must be combined with the hydroxyl group at C_1 or/and C_3 of rescogenin. Ophiopogonin B (Ia) affords a hexaacetate (Ib), $C_{51}H_{74}O_{18}$, colorless needles, mp 230°, $[\alpha]_D^{20}-63.6°$ (in chloroform) by usual acetylation.

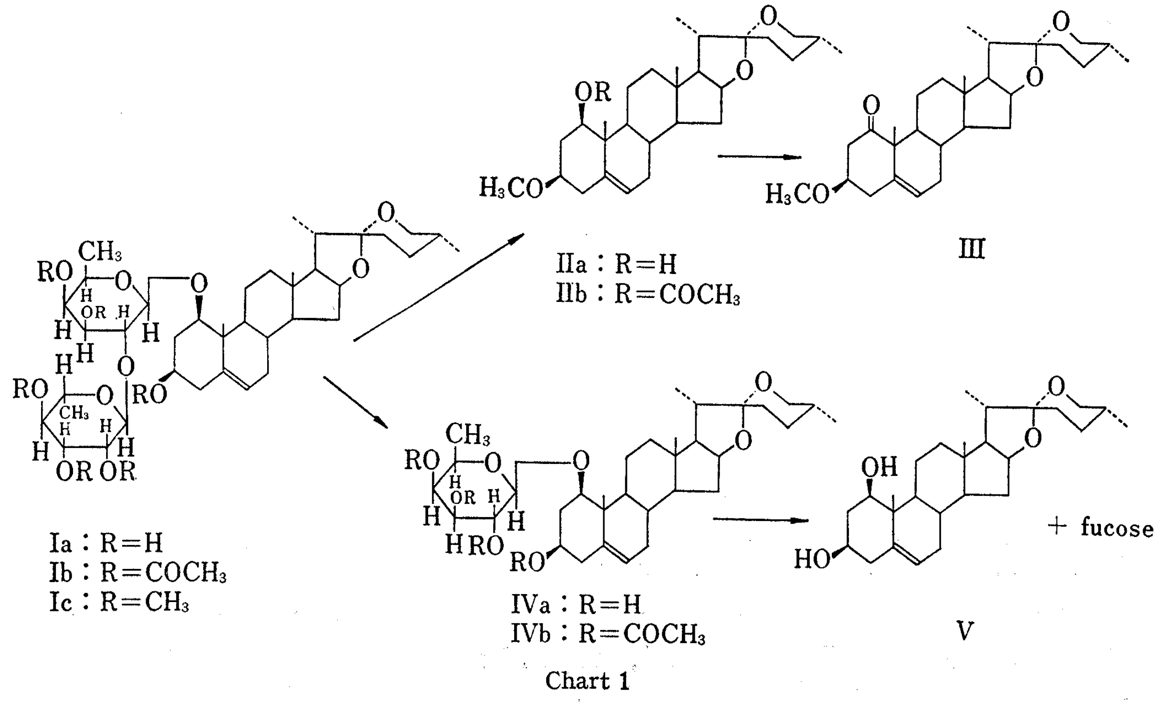

Chart 1

---

[1] Part I: H. Kato, S. Sakuma, A. Tada, S. Kawanishi, and J. Shoji, *Yakugaku Zasshi*, **88**, 710 (1968).

[2] Location: *Hatanodai, Shinagawa-ku, Tokyo.*

[3] E.S. Rothman, M.E. Wall, and C.R. Eddy, *J. Amer. Chem. Soc.*, **74**, 4013 (1952).

Ophiopogonin B permethylate (Ic), $C_{45}H_{74}O_{12}$, mp 210—213°, $[\alpha]_D^{20}$ $-75.5°$ (in chloroform), prepared by the Hakomori's method[4] gives, on methanolysis, an aglycone $C_{28}H_{44}O_4$ (IIa), mp 225—228°, $[\alpha]_D^{25}$ $-108.9°$ (in chloroform) and a mixture of methylated sugars. Compound IIa indicates the presence of isospiroketal ring system by IR spectrum and two angular methyl groups (δ=0.78, 3H, s, δ=1.02, 3H, s), two secondary methyl groups (δ=0.78, 3H, d, δ=0.98, 3H, d), one O-methyl group (δ=3.32, 3H, s), and one olefinic proton (δ=5.52, 1H, m) by nuclear magnetic resonance (NMR) spectrum.

Compound IIa yields a monoketone (III), $C_{28}H_{42}O_4$, mp 185—187°, $[\alpha]_D^{20}$ $-62.7°$ (in chloroform), IR ν_{max}^{KBr} 1712 cm^{-1} and acetylation of IIa with acetic anhydride-pyridine affords monoacetate (IIb), $C_{30}H_{46}O_5$, mp 177—180°, $[\alpha]_D^{25}$ $-86.0°$ (in chloroform). The above results indicate that IIa is ruscogenin monomethyl ether and that the two monosaccharides of ophiopogonin B form a disaccharide which is combined with the hydroxyl group at C_1 or C_3 of ruscogenin. The sugar portion of the methanolysate is fractionated by chromatography on silica gel eluted with ethyl acetate and identified with methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside and methyl 3,4-di-O-methyl-D-fucopyranoside by comparing with an authentic sample by thin-layer chromatography (TLC) and gas-liquid chromatography (GLC).

Therefore the disaccharide portion of ophiopogonin B should have a L-rhamnopyranosyl (1→2)-D-fucopyranoside structure. To prove the site of the sugar linkage to ruscogenin with two hydroxyl groups, the syntheses of two isomeric ruscogenin monomethyl ethers and their derivatives have been undertaken and the synthetic compounds are compared with the aglycone (IIa) and its derivatives.

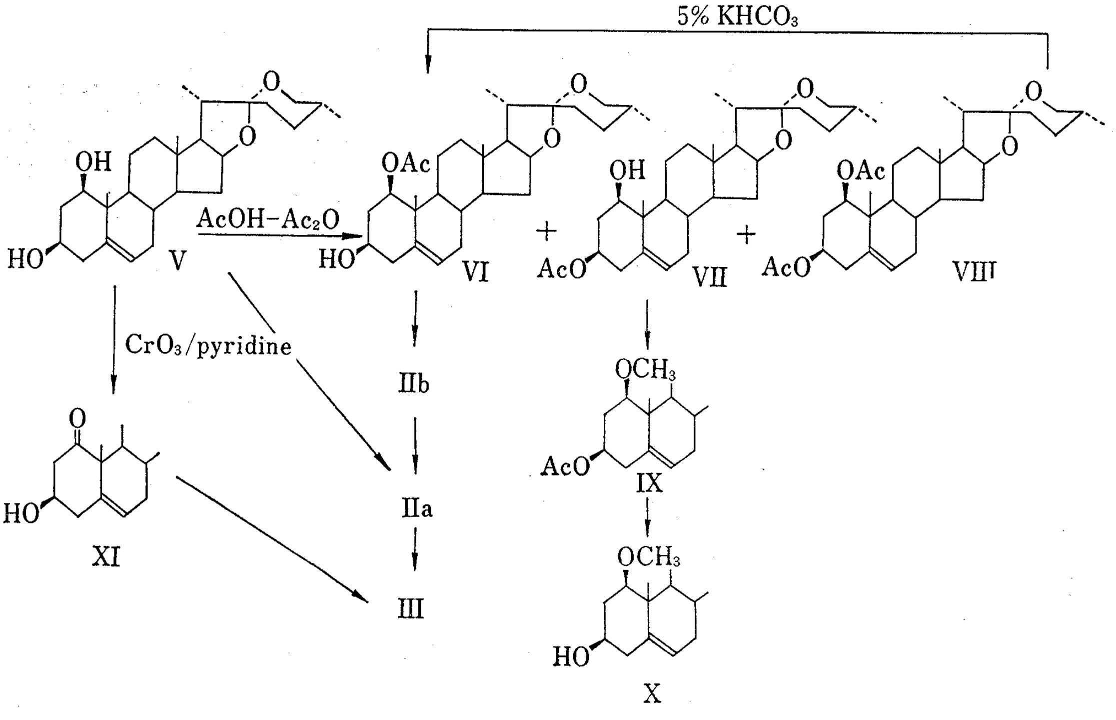

Chart 2

When ruscogenin (25R-spirost-5-ene-1β,3β-diol) (V) was heated by the Kemp's method[5] with acetic acid and acetic anhydride in the sealed tube at 60° for 18 hr, ruscogenin diacetate (VIII) and two isomeric monoacetates ($C_{29}H_{44}O_5$) were formed and the individual isomers, mp 203—205°, $[\alpha]_D^{27}$ $-93.3°$ (in chloroform), (VI) and mp 228—230°, $[\alpha]_D^{27}$ $-105.9°$ (in chloroform), (VII)[5] were separated by chromatography on silica gel. The compound VI was

---

[4] S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
[5] J.A. Kemp, Brit. Patent 963482 (1959) [*C.A.*, **61**, 8382*b* (1964)].

also obtained from ruscogenin diacetate on mild hydrolysis with 5% potassium bicarbonate at room temperature. On methylation with methyl iodide and silver oxide in dried benzene, VI was converted to ruscogenin 3-methyl ether 1-acetate, $C_{30}H_{46}O_5$ (IIb), mp 173—175°, $[\alpha]^{25}_D$ −86.0° (in chloroform), whereas compound VII provides ruscogenin 1-methyl ether 3-acetate, $C_{30}H_{46}O_5$ (IX), mp 199—200° (from methanol), $[\alpha]^{21}_D$ −31.9° (in chloroform). Compounds IIb and IX were deacetylated with lithium aluminium hydride in absolute ether to afford ruscogenin 3-methyl ether (IIa), $C_{28}H_{44}O_4$, mp 223—225°, $[\alpha]^{20}_D$ −114.8° and ruscogenin 1-methyl ether (X), $C_{28}H_{44}O_4$, mp 195—197°, $[\alpha]^{25}_D$ −112° (in chloroform), respectively. Furthermore, product IIa was oxidized with Jones reagent$^{6)}$ in acetone with ice-cooling to form 3β-methoxy-25R-spirost-5-en-1-one, $C_{28}H_{42}O_4$ (III).

According to the Kemp's method$^{5)}$ ruscogenin was treated with chromium trioxide in pyridine and the resulted 25R-spirost-5-en-1-on-3β-ol (XI),$^{5)}$ $C_{27}H_{40}O_4$, mp 221°, $[\alpha]^{21}_D$ −35.0° (in ethanol), was methylated with methyl iodide and silver oxide in absolute benzene to afford 3β-methoxy-25R-spirost-5-en-1-one (III), $C_{28}H_{42}O_4$, mp 185—187°.

The aglycone of ophiopogonin B permethylate and its derivatives are identified with IIa, IIb, and III, respectively, by direct comparisons (mixed mp, IR and co-chromatography).

The conjunction of the sugar moiety with the 1β-hydroxyl group of ruscogenin to form ophiopogonin B has since been verified. Partial hydrolysis of Ia with 0.1 N sulfuric acid-50% ethanol gives proophiopogonin B (IVa), $C_{33}H_{52}O_8$, mp 238—240°, $[\alpha]^{25}_D$ −87.7° (in pyridine), which yields ruscogenin (V) and fucose on hydrolysis while on acetylation with acetic anhydride and pyridine affords tetraacetate (IVb), $C_{41}H_{60}O_{12}$, $[\alpha]^{21}_D$ −72.2° (in chloroform).

The configurations of two sugars are assigned by the comparison of chemical shifts and the coupling constants of each anomeric protons and by the application of Klyne's rule$^{7)}$ to compare the molecular optical rotation of Ia, IVa, and V. The results are summarized in Table I.

TABLE I

Rhamnose→fucose	NMR anomericH δ 5.25(broad singlet)		
	$[M]_D$·ophiopogoninB−$[M]_D$·proophiopogoninB=−237.8°		α
Fucose→ruscogenin	NMR anomericH δ 4.18 (J=8cps)		
	$[M]_D$·proophiopogoninB−$[M]_D$·ruscogenin=−6.5°		β
	methyl-α-L-rhamnopyranoside	$[M]_D$ −111°	
	methyl-β-L-rhamnopyranoside	$[M]_D$ +170°	
	methyl-α-D-fucopyranoside	$[M]_D$ +338°	
	methyl-β-D-fucopyranoside	$[M]_D$ −25°	

In conclusion the complete structure of ophiopogonin B is defined as ruscogenin (1)-α-L-rhamnopyranosyl(1_{rham}→2_{fuc})-β-D-fucopyranoside, Ia.

It should be noted that ophiopogonin B is one of the examples of the spirostane type glycoside, namely yononin,$^{8)}$ tokoronin,$^{9)}$ compound X$^{10)}$ (*Dioscorea Tokoro* MAKINO), convallasaponin B, glucoconvallasaponin B,$^{11)}$ convallasaponin D$^{12)}$ (*Convallaria keisukei* MIQ.), neotokorogenin-1-α-arabinoside$^{13)}$ (*Dioscorea tenuipes* FRANCH. *et* SAVAT.), paniculonin A, paniculonin B$^{14)}$ (*Solanum paniculatum* L.), whose sugar moiety links to the hydroxyl group other than C_3-hydroxyl group of the aglycone.

The study on the other glycosides of ophiopogonis tuber will be published in the near future.

---

6) C. Djerassi, R.R. Engle, and A. Boers, *J. Org. Chem.*, **21**, 1547 (1956).
7) W. Klyne, *Biochem. J.*, **47**, xli (1950).
8) T. Kawasaki and K. Miyahara, *Tetrahedron*, **21**, 3633 (1965).
9) K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1369 (1969).
10) K. Miyahara, F. Isozaki, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1735 (1969).
11) M. Kimura, M. Tohma, I. Yoshizawa, and H. Akiyama, *Chem. Pharm. Bull.* (Tokyo), **16**, 25 (1968).
12) M. Kimura, M. Tohma, and I. Yoshizawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 1228 (1968).
13) I. Yasuda and A. Akahori, *Annual meeting of The Japanese Society of Pharmacognosy*, abstr. p. 43 (1971).
14) H. Ripperger and K. Schreiber, *Chem. Ber.*, **101**, 2450 (1968).

Experimental

All melting points were taken on Yanagimoto Micro Melting Point apparatus and are uncorrected. IR absorption spectra were obtained with Hitachi Model EPI-2. NMR spectra were measured with Japan Electron Co. JNM 4H-100 spectrometer and Hitachi model R-20 High Resolution NMR spectrometer with tetramethylsilane as an internal standard. The chemical shifts are reported in δ and the solvent used are indicated. Gas chromatograph used was Hitachi Model K-53 with hydrogen flame ionization detector and the conditions applied are as follows. Column, 10% SE-30 on chromosorb W, 3 mm × 1 m; column temperature, 170°; carrier gas, N_2, 20 ml/min. ORD curves were measured in solution using JASCO Optical Rotatory Dispersion Recorder Model ORD/UV-5. The *Rf* values of aglycone, its derivatives and methylated sugars were determined by TLC on silica gel H(Merck) using a mixture of benzene–acetone (4:1) as a solvent and 10% H_2SO_4 (spraying followed by heating) as a staining agent.

Ophiopogonin B(Ia)——This was obtained from the crude glycoside fraction of the tuber of *Ophiopogon japonicus* KER-GAWLER var. *genuinus* MAXIM. by repeated chromatography on silica gel with 5% MeOH–AcOEt saturated with water and recrystallized from EtOH as colorless needles, $C_{39}H_{62}O_{12}\cdot 2H_2O$, mp 269—271°, $[\alpha]_D^{15}$ $-105.5°$ ($c=0.31$ pyridine). IR ν_{max}^{Nujol} cm^{-1}: 3500—3300 (OH), 985, 920, 900, 864 (intensity 900>920,[15] isospiroketal).

Ophiopogonin B Hexaacetate(Ib)——Ophiopogonin B(I) was acetylated with Ac_2O and pyridine as reported in the previous paper to give its hexaacetate, $C_{51}H_{74}O_{18}$, colorless needles from aq. MeOH, mp 230°, $[\alpha]_D^{20}$ $-63.6°$ ($c=0.63$ $CHCl_3$), TLC: *Rf* 0.53.

Permethylation of Ophiopogonin B(Formation of Ic)——According to the Hakomori's method, NaH (500 mg) was stirred in dimethylsulfoxide (7 ml) at 70° for 1 hr in N_2 gas flow. To this reagent ophiopogonin B(730 mg) in dimethyl sulfoxide (15 ml) was added and the mixture was kept at room temperature for 10 min with stirring in N_2 gas flow. Methyl iodide (5 ml) was added and the reaction mixture was allowed to stand at room temperature for 3 hr with stirring. After dilution with water, the mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and concentrated to dryness. The syrupy residue (750 mg) was chromatographed on silica gel eluted with AcOEt-hexane (1:1) to give hexa-O-methylophiopogonin B, mp 210—213°, colorless needles from MeOH, $[\alpha]_D^{20}$ $-75.5°$ ($c=0.66$ $CHCl_3$). *Anal.* Calcd. for $C_{45}H_{74}O_{12}$: C, 66.78; H, 9.24. Found: C, 66.55; H, 9.29. IR $\nu_{max}^{CCl_4}$ cm^{-1}: OH (nil.), 982, 918, 898, 865, (intensity 898>918, isospiroketal). NMR (in $CDCl_3$) δ: 0.76 (3H, s, $\text{-}\overset{|}{\underset{|}{\mathrm{C}}}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$, 3H, d, $\text{-}\overset{|}{\mathrm{C}}\mathrm{H}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$), 0.94 (3H, d, $J=7$ cps $\text{-}\overset{|}{\mathrm{C}}\mathrm{H}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$), 1.01 (3H, s, $\text{-}\overset{|}{\underset{|}{\mathrm{C}}}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$), 1.23 (2 × 3H, d, $J=7.0$ cps $\text{-}\overset{|}{\mathrm{C}}\mathrm{H}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$), 3.29 (3H, s, $\mathrm{OC}\underline{\mathrm{H}}_3$), 3.40—3.50 (5 × 3H, s, $\mathrm{OC}\underline{\mathrm{H}}_3$), 4.18 (1H, d, $J=8$ cps anomeric H), 5.25 (1H, broad singlet, anomeric H), 5.50 (1H, m, $\rangle\mathrm{C{=}C}\langle^{\underline{\mathrm{H}}}$). TLC: *Rf* 0.30.

Methanolysis of Ophiopogoninpermethylate(Ic)——The above permethylate (450 mg) was refluxed with 5% HCl in MeOH (45 ml) for 4 hr. The solution was neutralized with Ag_2CO_3 and evaporated to dryness. The residue (325 mg) was chromatographed on silica gel with AcOEt.

Aglycone——The crude aglycone was recrystallized from MeOH to provide pure aglycone(IIa), colorless needles, mp 225—228°, $[\alpha]_D^{25}$ 108.9° ($c=0.60$ $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97. Found: C, 75.41; H, 9.50. IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 980, 920, 900, 865 (intensity 900>920, isospiroketal). NMR (in $CDCl_3$) δ: 0.78 (3H, s, $-\overset{|}{\underset{|}{\mathrm{C}}}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$, 3H, d, $\text{-}\overset{|}{\mathrm{C}}\mathrm{H}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$), 0.98 (3H, d, $J=6$ cps, $\text{-}\overset{|}{\mathrm{C}}\mathrm{H}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$), 1.02 (3H, s, $\text{-}\overset{|}{\underset{|}{\mathrm{C}}}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$), 3.32 (3H, s, $\mathrm{OC}\underline{\mathrm{H}}_3$), 5.52 (1H, m, $\rangle\mathrm{C{=}C}\langle^{\underline{\mathrm{H}}}$). TLC: *Rf* 0.35.

Chromium Trioxide Oxidation of Aglycone IIa to Monoketone(III)——To a solution of aglycone IIa (48 mg) in acetone (20 ml) was added the Jones reagent (0.3 ml; CrO_3 2 g, H_2SO_4 3 g, H_2O 15 ml) dropwise with stirring at 0° and then kept at room temperature for 30 min. To the reaction mixture was added 0.5 ml of MeOH and diluted with 50 ml of water and then extracted with ether. The ether layer was washed with 5% $NaHCO_3$ and water, and dried over anhyd. Na_2SO_4. Evaporation of the solvent *in vacuo* gave white powder which was recrystallized from Me_2CO to give colorless plates (35 mg)(III), mp 185—187°, $[\alpha]_D^{20}$ $-62.7°$ ($c=0.51$, $CHCl_3$). *Anal.* Calcd. for $C_{23}H_{42}O_4$: C, 75.97; H, 9.56. Found: C, 76.17; H, 9.79. IR ν_{max}^{KBr} cm^{-1}: 1712 (six membered ketone), 983, 921, 900, 865 (intensity 900>921, isospiroketal). NMR (in $CDCl_3$) δ: 0.78 (3H, s, $\text{-}\overset{|}{\underset{|}{\mathrm{C}}}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$, 3H, d, $\text{-}\overset{|}{\mathrm{C}}\mathrm{H}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$), 0.98 (3H, d, $J=7$ cps $\text{-}\overset{|}{\mathrm{C}}\mathrm{H}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$), 1.28 (3H, s, $\text{-}\overset{|}{\underset{|}{\mathrm{C}}}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$), 2.65 (2H, d, $J=7$ cps $\text{-}\overset{|}{\mathrm{C}}\mathrm{H}\text{-}\overset{|}{\mathrm{C}}\underline{\mathrm{H}}_2\text{-CO-}$), 3.31 (3H, s, $\mathrm{OC}\underline{\mathrm{H}}_3$), 5.50 (1H, m, $\rangle\mathrm{C{=}C}\langle^{\underline{\mathrm{H}}}$). TLC: *Rf* 0.70. ORD ($c=0.2$, EtOH) $[\alpha]_D^{20}$ (mμ): $-720°$ (322) (peak), 0° (293), $+410°$ (277) (trough).

Acetylation of Aglycone IIa to Monoacetate (IIb)——Compound IIa (52 mg) was left to stand with pyridine (1 ml) and Ac_2O (1 ml) at room temperature for 48 hr. The reaction product (52 mg) was recrystallized from MeOH to provide monoacetate IIb as colorless needles, mp 177—180°, $[\alpha]_D^{25}$ $-86.0°$ ($c=0.65$ $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{46}O_5$: C, 74.03; H, 9.53. Found: C, 74.28; H, 9.44. IR ν_{max}^{KBr} cm^{-1}: 1740 (ester), 982, 918, 900, 864 (intensity 900>918, isospiroketal). NMR (in $CDCl_3$) δ: 0.78 (3H, s, $\text{-}\overset{|}{\underset{|}{\mathrm{C}}}\text{-}\mathrm{C}\underline{\mathrm{H}}_3$, 3H

---

[15] "900>920" means the absorbance of the 900 cm^{-1} band is stronger than that of 920 cm^{-1}.

d, $\text{-}\dot{C}H\text{-}C\underline{H}_3$), 0.95 (3H, d, $J=6$ cps $\text{-}\dot{C}H\text{-}C\underline{H}_3$), 1.13 (3H, s, $\text{-}\overset{|}{\underset{|}{C}}\text{-}C\underline{H}_3$), 2.01 (3H, s, $\text{-O-COC}\underline{H}_3$), 3.30 (3H, s, $OC\underline{H}_3$), 5.54 (1H, m, $\rangle C{=}C\langle^{\underline{H}}$). TLC: *Rf* 0.70.

Fractionation of Methylated Sugars——The methylated sugars were suggested to be methyl 3,4-di-O-methyl-D-fucopyranoside and methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside by TLC and GLC comparing with the authentic samples. TLC: *Rf* 0.42, 0.29 (methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside), 0.07, 0.00 (methyl 3,4-di-O-methyl-D-fucopyranoside). GLC: t_R 6.15, 7.00 (methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside), 8.20, 9.00 (methyl 3,4-di-O-methyl-D-fucopyranoside).

The sugar fraction of the methanolysate of ophiopgonin B permethylate was isolated by chromatography on silica gel eluted with AcOEt. Methyl 2,3,4-tri-O-methyl-L-α-rhamnopyranoside and methyl 3,4-di-O-methyl-α-D-fucopyranoside were obtained.

Methyl 2,3,4-tri-O-Methyl-L-α-rhamnopyranoside——Syrup, $[\alpha]_D^{18}$ $-18.1°$ ($c=1.1$ $CHCl_3$). TLC: *Rf* 0.42. GLC: t_R 6.15.

Methyl 3,4-di-O-Methyl-α-D-fucopyranoside——Colorless needles from petroleum ether, mp 101—103°, $[\alpha]_D^{18}$ $+133.7°$ ($c=0.25$ $CHCl_3$). It was identified with an authentic sample by mixed mp, TLC and GLC. (TLC: *Rf* 0.07, GLC: t_R 8.20).

Partial Hydrolysis of Ophiopogonin B——Ophiopogonin B (980 mg) was refluxed with 0.1N H_2SO_4 in 50% EtOH (50 ml) for 2 hr, EtOH was removed *in vacuo*, water was added and the precipitates were collected by filtration. The water insoluble product (600 mg) was placed on a silica gel column and eluted successively with a mixture of MeOH and AcOEt saturated with water (0—10%). Ruscogenin (180 mg), proophiopogonin B (25 mg) and ophiopogonin B (300 mg) were fractionated.

Proophiopogonin B(IVa)——Proophiopogonin B was crystallized from EtOH containing water as colorless needles, mp 238—240°, $[\alpha]_D^{25}$ $-91.0°$ ($c=0.47$ pyridine). *Anal.* Calcd. for $C_{33}H_{52}O_8$: C, 68.72; H, 9.09. Found: C, 68.43; H, 9.19. IR ν_{max}^{KBr} cm^{-1}: 3500—3300 (OH), 982, 920, 900, 865 (intensity 900>920, isospiroketal).

Acetylation of Proophiopogonin B to Tetraacetate(IVb)——Proophiopogonin B (20 mg) was left to stand with pyridine (1 ml) and Ac_2O (1 ml) at room temperature for 48 hr. The reaction product (18 mg) was recrystallized from EtOH containing water as colorless needles, mp 225—230°, $[\alpha]_D^{21}$ $-72.2°$ ($c=0.72$ $CHCl_3$). *Anal.* Calcd. for $C_{41}H_{60}O_{12}$: C, 66.11; H, 8.12. Found: C, 66.30; H, 8.22. IR ν_{max}^{KBr} cm^{-1}: 1750 (ester), 985, 918, 901, 865 (intensity 901>918, isospiroketal), TLC: *Rf* 0.66.

Hydrolysis of Proophiopogonin B——Proophiopogonin B (8 mg) was refluxed with 3N HCl (3 ml), dioxane (1 ml) and benzene (3 ml) for 4 hr. The reaction mixture was treated as usual and ruscogenin(V) was found in benzene layer (TLC: *Rf* 0.17) and fucose in aqueous layer (paper chromatogram: Toyo Roshi No. 50, solvent; BuOH–AcOH–H_2O (4:1:5, upper layer), spray reagent; aniline hydrogenphthalate, *Rf* 0.25).

Partial Acetylation of Ruscogenin(V)——According to the Kemp's method, ruscogenin (V, 600 mg) was acetylated with AcOH (14.4 ml) and Ac_2O (2.4 ml) in a sealed tube at 60° for 18 hr. The reaction mixture was diluted with ice-water and the precipitates (620 mg) were collected by filtration. Column chromatography of the crude product on silica gel using benzene–acetone (4:1) mixture provided ruscogenin diacetate(VIII, 130 mg, *Rf* 0.65), ruscogenin 3-acetate(VII, 250 mg, *Rf* 0.60), ruscogenin 1-acetate(VI, 50 mg, *Rf* 0.41) and ruscogenin (V, *Rf* 0.14).

Partial Hydrolysis of Ruscogenin Diacetate(VIII)——A solution of ruscogenin diacetate (22 mg) in MeOH (3 ml) and 5% $KHCO_3$ (2 ml) was left at room temperature overnight. The solvent was evaporated *in vacuo* and the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, dried and evaporated to a solid. Recrystallization of the residue from MeOH to afford ruscogenin 1-acetate (VI, 10 mg).

Ruscogenin 1-Acetate(VI)——Ruscogenin 1-acetate was obtained as colorless needles from MeOH, mp 203—205°, $[\alpha]_D^{27}$ $-93.3°$ ($c=0.90$ $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.43; H, 9.25. IR ν_{max}^{Nujol} cm^{-1}: 3550 (OH), 1740 (acetyl), 980, 920, 898, 865 (intensity 898>920, isospiroketal). NMR (in $CDCl_3$) δ: 0.78 (3H, s, $\text{-}\overset{|}{\underset{|}{C}}\text{-}C\underline{H}_3$, 3H, d, $\text{-}\dot{C}H\text{-}C\underline{H}_3$), 0.95 (3H, d, $J=6$ cps $\text{-}\dot{C}H\text{-}C\underline{H}_3$), 1.13 (3H, s, $\text{-}\overset{|}{\underset{|}{C}}\text{-}C\underline{H}_3$), 2.01 (3H, s, $\text{-COC}\underline{H}_3$), 5.55 (1H, m, $\rangle C{=}C\langle^{\underline{H}}$).

Ruscogenin 3-Acetate(VII)——Ruscogenin 3-acetate was obtained as colorless needles from MeOH, mp 228—230°, $[\alpha]_D^{27}$ $-110.4°$ ($c=0.67$ $CHCl_3$). *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.96; H, 9.38. IR ν_{max}^{Nujol} cm^{-1}: 3540 (OH). 1740 (acetyl), 980, 920, 900, 868 (intensity 900>920, isospiroketal). NMR (in $CDCl_3$) δ: 0.78 (3H, s, $\text{-}\overset{|}{\underset{|}{C}}\text{-}C\underline{H}_3$, 3H, d, $\text{-}\dot{C}H\text{-}C\underline{H}_3$), 0.98 (3H, d, $J=6$ cps $\text{-}\dot{C}H\text{-}C\underline{H}_3$), 1.02 (3H, s, $\text{-}\overset{|}{\underset{|}{C}}\text{-}C\underline{H}_3$), 2.00 (3H, s, $\text{-OCOC}\underline{H}_3$), 5.52 (1H, m, $\rangle C{=}C\langle^{\underline{H}}$).

Methylation of Ruscogenin Monoacetates(VI and VII)——i) Ruscogenin 1-Acetate 3-Methyl Ether (IIb): Compound VI (70 mg) in anhydrous benzene (5 ml) was stirred with CH_3I (1 ml) and Ag_2O (1 g) at room temperature for 48 hr. Precipitates were filtered off, the benzene solution was evaporated to dryness and the residue (72 mg) recrystallized from MeOH afforded IIb as colorless needles, mp 173—175°, $[\alpha]_D^{20}$ $-86.0°$ ($c=0.69$ $CHCl_3$), *Rf* 0.70, *Anal.* Calcd. for $C_{30}H_{46}O_5$: C, 74.03; H, 9.53. Found: C, 74.10; H 9.47. Mixed mp with the natural product(IIb) showed no depression, the IR spectra were superimposable and co-chromatography gave single spot.

Ruscogenin 1-Methyl Ether 3-Acetate(IX)——Compound VII (160 mg) in dimethylsulfoxide (10 ml) was stirred with CH_3I (3 ml) and Ag_2O (2 g) at room temperature for 72 hr. Being diluted with water, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ solution was washed with 5% KCN solution and then water, dried and evaporated to a syrup (150 mg). The product was chromatographed on silica gel using benzene and benzene–acetone (9:1). The ruscogenin 1-methyl ether 3-acetate (IX) was isolated as colorless needles from MeOH, mp 199—200°, $[\alpha]_D^{20}$ $-31.9°$ ($c=0.65$ $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{46}O_5$: C, 74.03; H, 9.53. Found: C, 74.00; H, 9.31. IR ν_{max}^{Nujol} cm^{-1}: 1740 (acetyl), 984, 919, 900, 865 (intensity 900>919, isospiroketal). NMR (in $CDCl_3$) δ: 0.78 (3H, s, $-\overset{|}{\underset{|}{C}}-C\underline{H}_3$, 3H, d, $-\overset{|}{C}H-C\underline{H}_3$), 0.98 (3H, d, $J=6$ cps $-\overset{|}{C}H-C\underline{H}_3$), 1.02 (3H, s, $-\overset{|}{\underset{|}{C}}-C\underline{H}_3$), 2.01 (3H, s, $OCOC\underline{H}_3$), 3.25 (3H, s, $OC\underline{H}_3$), 5.52 (1H, m, $>C=C<\underline{H}$). TLC: *Rf*: 0.70.

Deacetylation of Ruscogenin Monomethyl Ether Monoacetates(IIb and IX)——i) Ruscogenin 3-Methyl Ether: Compound IIb (60 mg) in absolute ether (10 ml) was refluxed with $LiAlH_4$ for 25 min. The reaction mixture was diluted with water and extracted with ether. The ether solution was dried and evaporated to give a solid (42 mg). Recrystallization of the residue from MeOH gave ruscogenin 3-methylehter(IIa) as colorless needles, mp 223—225°. *Anal.* Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97. Found: C, 75.45; H, 10.00. It was identical with an authentic sample of IIa (mixed mp, IR and TLC). TLC: *Rf* 0.35.

ii) Ruscogenin 1-Methyl Ether(X): Compound IX (40 mg) was deacetylated as above affording a crude methylether (30 mg), which was recrystallized from MeOH to give X as colorless needles mp 195—197°, $[\alpha]_D^{24}$ $-86.1°$ ($c=0.79$ $CHCl_3$). *Anal.* Calcd. for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97. Found: C, 75.50; H, 9.94. IR ν_{max}^{KBr} cm^{-1}: 3550 (OH), 980, 917, 898, 865 (intensity 898>917, isospiroketal). NMR (in $CDCl_3$) δ: 0.78 (3H, s, $-\overset{|}{\underset{|}{C}}-C\underline{H}_3$, 3H, d, $-\overset{|}{C}H-C\underline{H}_3$), 0.98 (3H, d, $J=6$ cps $-\overset{|}{C}H-C\underline{H}_3$), 1.04 (3H, s, $-\overset{|}{\underset{|}{C}}-C\underline{H}_3$), 3.28 (3H, s, $-OC\underline{H}_3$), 5.50 (1H, m, $>C=C<^{\underline{H}}$). TLC: *Rf* 0.24.

Direct Methylation of Ruscogenin(V)——Compound V (30 mg) in anhydrous benzene (5 ml) was stirred with CH_3I (1 ml) and Ag_2O (500 mg) at room temperature for 24 hr. Precipitates were filtered off, the benzene solution was evaporated to dryness and the residue (30 mg) was recrystallized from MeOH to afford IIa as colorless needles, mp 223—225°, which was identified with an authentic sample of IIa (mixed mp, IR, TLC). TLC: *Rf* 0.35.

Oxidation of Ruscogenin 3-Methyl Ether(IIa)——To a solution of IIa (40 mg) in acetone (10 ml) was added Jones reagent (0.2 ml) portionwise at 0° and stirred for 25 min. The reaction mixture was diluted with water and the solution was extracted with ether. The ether solution was washed with water, dried and evaporated to give a solid (31 mg) which was recrystallized from acetone to give colorless plates(III), mp 183—186°. *Anal.* Calcd. for $C_{28}H_{42}O_4$: C, 75.97; H, 9.56. Found: C, 75.77; H, 9.48. This compound was identified with an authentic sample of III by mixed mp, TLC (*Rf* 0.70) and IR comparison.

Chromium Trioxide Oxidation of Ruscogenin (25R-Spirost-5-en-1β,3β-diol, V)——According to the Kemp's method the solution of CrO_3 (350 mg) in pyridine (3.5 ml) was added to the solution of rescogenin (350 mg) in pyridine (3.5 ml) and stirred at room temperature for 20 hr. The reaction mixture was diluted with water and then extracted with ether. The ether layer was washed with water, and dried over anhyd. Na_2SO_4. Evaporation of the solvent *in vacuo* gave a white powder (150 mg, TLC: *Rf* 0.20, 0.38). The product was chromatographed on silica gel using benzene–acetone=4:1. The 25R-spirost-5-en-1-on-3β-ol(XI) was isolated and recrystallized from acetone to afford colorless plates (80 mg), mp 221°, $[\alpha]_D^{19}$ $-35.0°$ ($c=0.1$ EtOH). *Anal.* Calcd. for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41. Found: C, 75.66; H, 9.42. IR ν_{max}^{KBr} cm^{-1}: 3550 (OH), 1710 (C=O), 983, 921, 901, 866 (intensity 901>921, isospiroketal). NMR (in $CDCl_3$) δ: 0.80 (3H, s, $-\overset{|}{\underset{|}{C}}-C\underline{H}_3$, 3H, d, $-\overset{|}{C}H-C\underline{H}_3$), 0.98 (3H, d, $J=6$ cps $-\overset{|}{C}H-C\underline{H}_3$), 1.30 (3H, s, $-\overset{|}{\underset{|}{C}}-C\underline{H}_3$), 2.68 (2H, d, $J=8$ cps $-\overset{|}{C}O-C\underline{H}_2-\overset{|}{C}H-$), 5.60 (1H, $>C=C<^{\underline{H}}$). TLC: *Rf* 0.38.

Methylation of 25R-Spirost-5-en-1-on-3β-ol(XI)(Formation of 25R-Spirost-5-en-3-methoxy-1-one(III)) Compound XI (45 mg) in anhydrous benzene (2 ml) was stirred with CH_3I (1 ml) and Ag_2O (1 g) at room temperature for 48 hr. Precipitates were filtered off and the benzene solution was evaporated to dryness giving the residue (45 mg) which was recrystallized from acetone, colorless plates, mp 185—187°. *Anal.* Calcd. for $C_{28}H_{42}O_4$: C, 75.97; H, 9.56. Found: C, 75.79; H, 9.51. This compound was found to be identical with an authentic sample of III (mixed mp, IR, TLC). TLC: *Rf* 0.70.

Acknowledgement The authors express their gratitude to Prof. S. Shibata, Faculty of Pharmaceutical Sciences, University of Tokyo, for his encouragement throughout the course of this work.

We are grateful to Prof. T. Kawasaki, Faculty of Pharmaceutical Sciences, Kyushu University, for his kind supply of the authentic samples, and to Mr. M. Okano, Nippon Shinyaku Co., Ltd. for his kind supply of ruscogenin for synthesis.

Thanks are also due to Dr. M. Shimizu, Daiichi Seiyaku Co., Ltd. for NMR spectral measurement, to the members of Analytical Laboratory of the School of Pharmaceutical Sciences, Showa University for elemental analyses.