Comparative Studies on the Constituents of Ophiopogonis Tuber and Its Congeners. I. Studies of the Constituents of the Subterranean Part of *Liriope platyphylla* Wang et Tang. (1)[1)]

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Eight steroidal glycosides, tentatively named glycosides A(1), B(2), C(3), D(4), E(5), F(6), G(7) and H(8), were isolated from the methanol extract of the subterranean part of *Liriope platyphylla* Wang et Tang (Liliaceae). The structures of these glycosides were established as ruscogenin 3-O-α-L-rhamnopyranoside (1), 25(S)-ruscogenin 1-O-β-D-fucopyranosido-3-O-α-L-rhamnopyranoside· (2), 25(S)-ruscogenin 1-O-α-L-rhamnopyranosyl-(1→2)-β-D-fucopyranoside (3), ruscogenin 3-O-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranoside (4), a mixture of 3-O-[α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-glucopyranosides of diosgenin and yamogenin (=a mixture of ophiopogonin D′ and its 25(S)-isomer, 5), a mixture of 3-O-β-chacotriosides of diosgenin and yamogenin (=a mixture of dioscin and its 25(S)-isomer, 6), ruscogenin 1-sulfate 3-O-α-L-rhamnopyranoside (7), and 26-O-β-D-glucopyranosyl-22-O-methylfurost-5-ene-3β,26-diol 3-O-β-chacotrioside (=methyl proto-dioscin, 8).

In a series of papers on the constituents of Ophiopogonis Tuber (tuber of *Ophiopogon japonicus* Ker–Gawler: Liliaceae), we have reported the isolation and structure elucidation of several steroidal glycosides[2)] and homoisoflavonoids.[3)] One of the congener crude drugs of Ophiopogonis Tuber is the tuber of *Liriope platyphylla* Wang et Tang (Japanese name: Yaburan), but the constituents of this drug have not been investigated. The present paper deals mainly with the isolation and structure elucidation of eight steroidal glycosides, tentatively named glycosides A, B, C, D, E, F, G and H in order of increasing polarity, of the subterranean part of the title plant, leading to the assignment of the structures 1, 2, 3, 4, 5, 6, 7 and 8, respectively.

Eight steroidal glycosides were obtained from the methanolic extract of the fresh subterranean part of *L. platyphylla* harvested at Tokyo Metropolitan Medicinal Plants Garden in February 1981, as shown in Chart 1.

Glycoside A(1), $C_{33}H_{52}O_8 \cdot H_2O$, is positive in the Liebermann–Burchard reaction, and it shows a strong absorption band of hydroxyl groups and characteristic absorption bands of the 25(R)-spiroketal moiety in the infrared (IR) spectrum.[4)] On acetylation with acetic anhydride and pyridine, 1 gave a tetraacetate (9), $C_{41}H_{60}O_{12}$, and hydrolysis of 1 with 2 N hydrogen chloride in 50% dioxane gave L-rhamnose and an aglycone, $C_{27}H_{42}O_4$, colorless needles, mp 205—207°C, which was identified as ruscogenin (10) by direct comparisons with an authentic sample.[2a)] Accordingly, 1 was assumed to be a ruscogenin monorhamnoside, and the location of the sugar moiety, on either the C-1 or C-3 hydroxyl group, was established as follows. Methylation of 1 by Hakomori's method[5)] afforded a tetra-O-methyl derivative (11), $C_{37}H_{60}O_8$, which was methanolyzed to give per-O-methyl-L-rhamnopyranoside and an aglycone, colorless needles, mp 196—197°C. The aglycone was identified as ruscogenin 1-O-methyl ether by comparing it with authentic samples of ruscogenin 1-O-methyl and 3-O-methyl ethers.[2b)] Based on the [1]H- and ^{13}C-nuclear magnetic resonance (NMR) spectra of 1 and 11, the configura-tion of L-rhamnose was assigned to be α,[6)] and the structure of

subterranean parts of *Liriope platyphylla* WANG et TANG (6 kg)

| 1. extd. with hot MeOH (81×4)
| 2. concentrated *in vacuo*

MeOH ext. (1.2 kg)

| partitioned between Et₂O and H₂O

Et₂O layer (42 g)   H₂O layer

| extd. with *n*-BuOH
| satrd. with H₂O

BuOH layer   H₂O layer
| evaporated *in vacuo*
residue (80 g)

| chromatographed on Avicel

solv. CHCl₃   solv. CHCl₃-MeOH-H₂O   solv. MeOH
                         (70:16:10 v/v,
                         lower phase)

36 g   25 g   14 g

chromatographed   chromatographed
on silica gel   on silica gel
solv. CHCl₃-MeOH-H₂O   solv. CHCl₃-MeOH-
(7:2:0.1 v/v)   H₂O (7:3:0.4 v/v)

Fr. 1   Fr. 2

chromatographed   chromatographed
on silica gel   on silica gel
solv. CHCl₃-MeOH-H₂O   solv. CHCl₃-MeOH-
(7:1:0.1 v/v)   H₂O (7:2:0.1 v/v)

chromatographed
on silica gel
solv. CHCl₃-MeOH-
AcOEt-H₂O
(2:2:4:1 v/v,
lower phase)

1   2   3   4   5   6   7   8
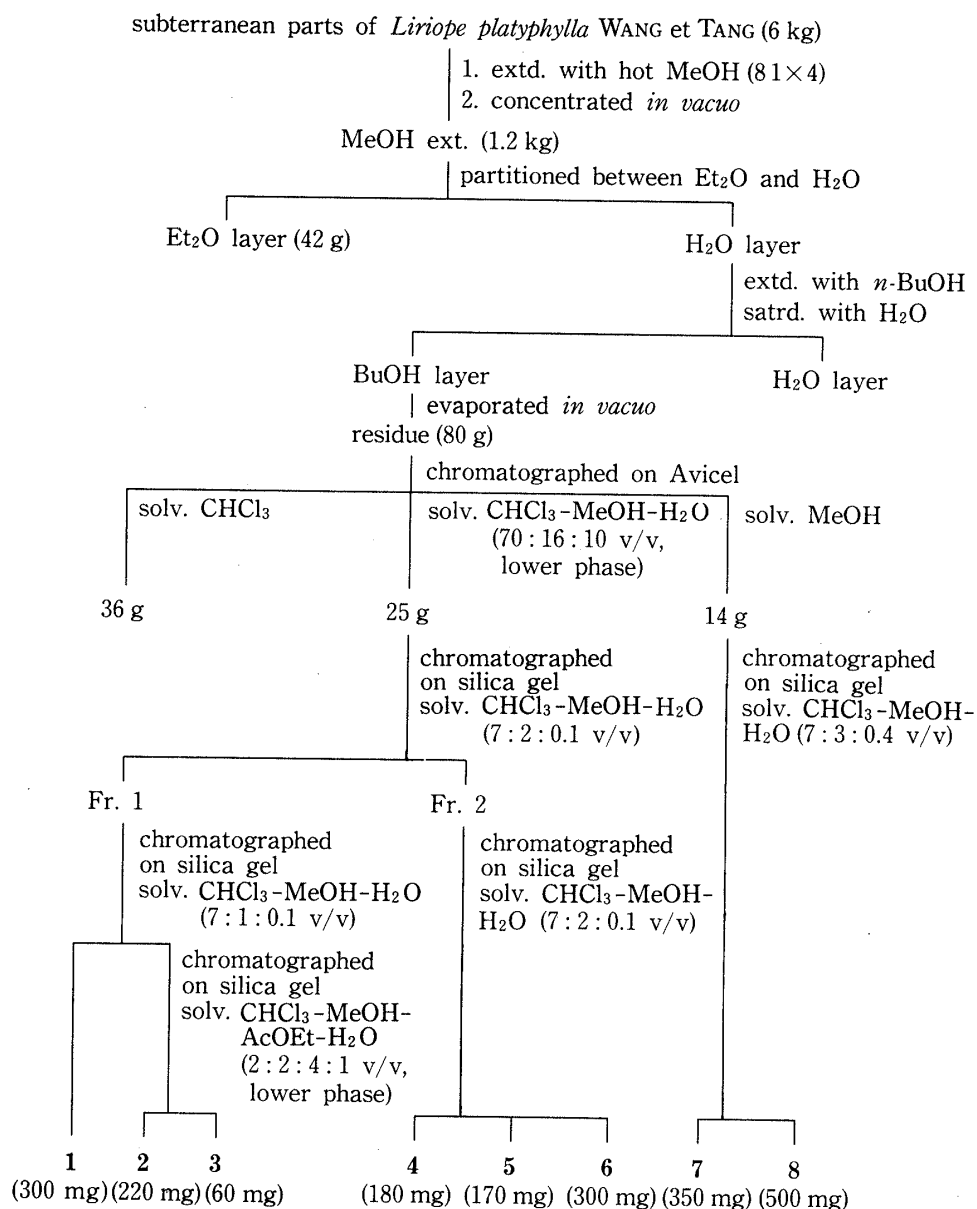(300 mg)(220 mg)(60 mg)   (180 mg) (170 mg) (300 mg)(350 mg) (500 mg)

Chart 1

glycoside A was established to be ruscogenin 3-*O*-α-L-rhamnopyranoside (1) as shown in Chart 2. ^{13}C-NMR chemical shifts of 25(R)-, 25(S)-ruscogenin and their glycosides are shown in Table I.

Glycoside B (2), $C_{39}H_{62}O_{12} \cdot 2H_2O$, is positive in the Liebermann–Burchard reaction, and it shows a strong absorption band of hydroxyl groups and characteristic absorption bands of the 25(S)-spiroketal moiety in the IR spectrum at 985, 920, 900, 855 cm^{-1} (intensity; 920>900, 25(S)-spiroketal).[4] On hydrolysis with 2 N hydrogen chloride, 2 gave L-rhamnose, D-fucose, and an aglycone, $C_{27}H_{42}O_4$, colorless needles, mp 194—196°C, 212—214°C (double mp), which was acetylated with acetic anhydride and pyridine to afford a diacetate, $C_{31}H_{46}O_6$. The chemical and physical properties of the aglycone and its acetate appeared to be identical with those of 25(S)-ruscogenin (12) and its acetate reported by González *et al.*[7] Finally, the aglycone and its acetate were identified by direct comparisons with authentic samples prepared by catalytic hydrogenation of neoruscogenin.[8] The locations of rhamnose and fucose linkages were proved as follows. Methylation of 2 by Hakomori's method afforded a
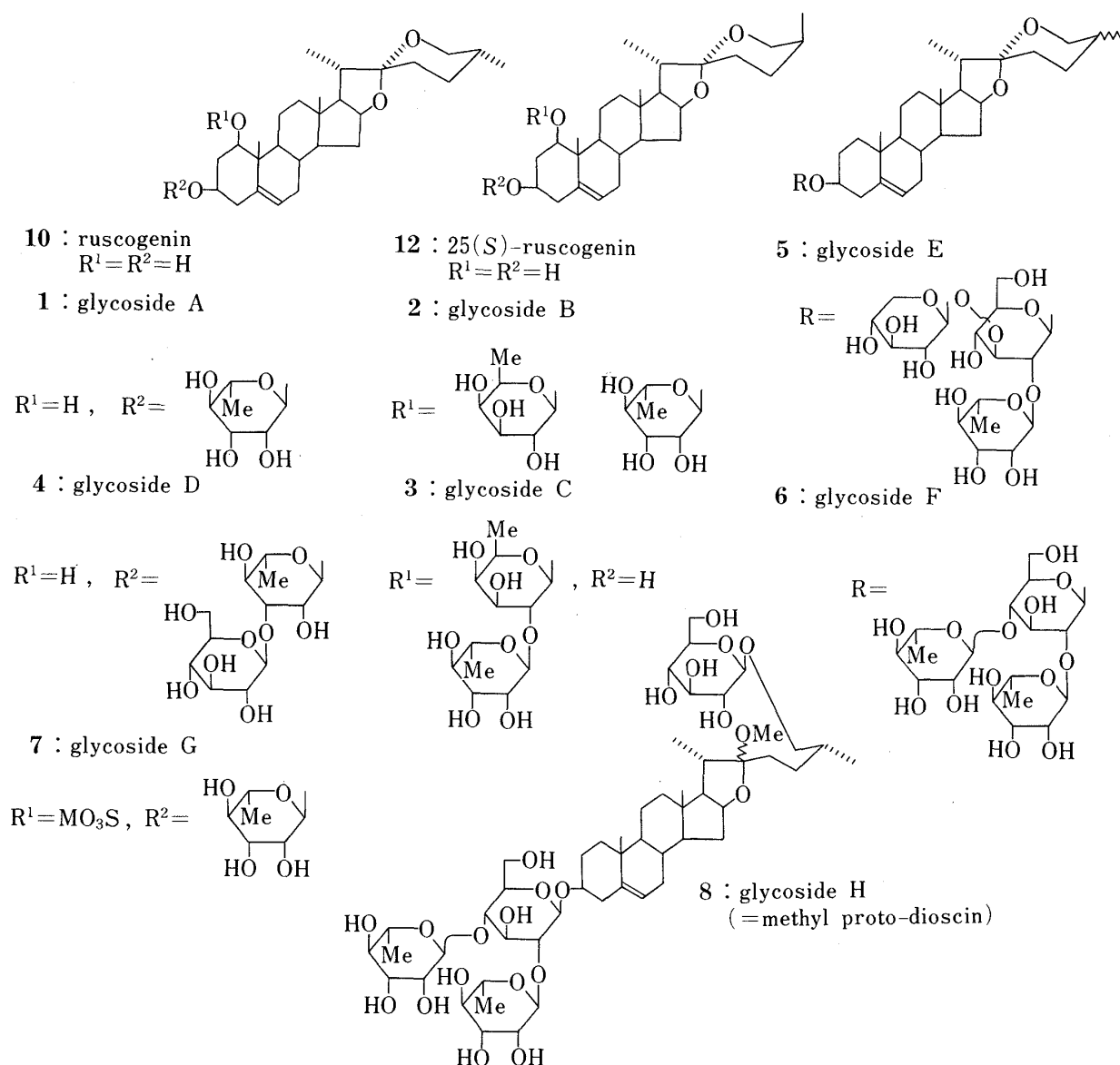
Chart 2

hexa-O-methyl derivative (**13**), $C_{45}H_{74}O_{12}$, which was methanolyzed to give per-O-methyl-L-rhamnopyranoside, per-O-methyl-D-fucopyranoside and **12**. The above result suggests that rhamnose and fucose of **2** are linked with the C_1- and C_3-hydroxyl groups, respectively, or *vice versa*. To prove the location of each sugar moiety, **2** was partially hydrolyzed to afford a prosapogenin (**14**), $C_{33}H_{52}O_8 \cdot H_2O$, which gave **12** and L-rhamnose on acid hydrolysis. The location of the rhamnose linkage with **12** was determined to be the C_3-hydroxyl group by comparative analysis of the ^{13}C-NMR spectra of ruscogenin and its 25-isomeric glycosides. Based on the glycosidation shifts[9] of the carbon signals of the A and B rings of **10** and **12**, the prosapogenin of **2** (25(*S*)-ruscogenin monorhamnoside) was deduced to be 25(*S*)-ruscogenin 3-O-L-rhamnopyranoside (Table I). The configurations of the sugar moieties were assigned as α-rhamnose and β-fucose by the same method as described for **1**. Consequently, the structure of **2** was established to be 25(*S*)-ruscogenin 1-O-β-D-fucopyranosido-3-O-α-L-rhamnopyranoside (**2**) as shown in Chart 2.

Glycoside C (**3**), $C_{39}H_{62}O_{12} \cdot 1/2H_2O$, is positive in the Liebermann–Burchard reaction and it shows a strong absorption band of hydroxyl groups and 25(*S*)-spiroketal absorption

TABLE I. ^{13}C NMR Chemical Shifts of Ruscogenin and 25 (S)-Ruscogenin Glycosides[a]

Compounds	Ruscogenin glycosides					25 (S)-Ruscogenin glycosides			
	10	1	4	7	15	12	2	3	14
Aglycone									
No. 1	78.2	78.0	77.9	83.9	84.3	78.2	83.7	84.1	78.0
2	44.0	41.1	41.0	37.2	38.0	44.0	35.9	38.0	41.1
3	68.3	73.9	73.9	73.5	68.4	68.2	73.7	68.4	73.8
4	43.7	39.8	39.6	39.5	43.9	43.6	39.6	44.0	39.7
5	140.5	139.4	139.3	138.1	139.8	140.3	138.5	139.9	139.3
6	124.3	125.1	123.1	125.9	124.8	124.4	125.6	124.8	125.2
7	33.2	33.1	33.1	33.2	33.3	33.1	33.2	33.4	33.1
8	32.5	32.5	32.4	32.1	32.2	32.4	32.2	32.3	32.7
9	51.6	51.5	51.4	50.0	50.9	51.4	50.8	50.9	51.5
10	43.7	43.9	43.8	43.4	43.0	43.6	43.2	43.1	43.9
11	24.4	24.3	24.3	23.7	24.1	24.3	24.0	24.1	24.4
12	40.8	40.7	40.7	40.6	40.6	40.7	40.5	40.6	40.7
13	40.4	40.4	40.4	40.3	40.4	40.3	40.4	40.4	40.5
14	57.2	57.1	57.0	56.8	57.4	57.0	57.2	57.4	57.1
15	32.6	32.6	32.5	32.5	32.6	32.5	32.6	32.6	32.6
16	81.2	81.2	81.2	81.2	81.3	81.2	81.3	81.4	81.4
17	63.4	63.4	63.4	63.3	63.3	63.1	63.2	63.3	63.2
18	16.7	16.7	16.7	16.7	16.9	16.7	16.9	16.9	16.8
19	14.0	13.8	13.8	14.7[c]	15.0[c]	14.0	14.7	14.9[c]	13.9
20	42.2	42.2	42.2	42.1	42.2	42.6	42.7	42.7	42.8
21	15.1	15.1	15.0	15.0[c]	15.1[c]	15.0	14.8	15.2[c]	15.0
22	109.3	109.4	109.4	109.3	109.4	109.8	109.8	109.8	109.8
23	32.1	32.1	32.0	32.1	32.0	26.5	26.6	26.6	26.7
24	29.5	29.5	29.4	29.4	29.4	26.3	26.4	26.4	26.4
25	30.7	30.8	30.7	30.7	30.7	27.6	27.7	27.8	27.7
26	67.1	67.1	67.0	67.0	67.0	65.2	65.2	65.2	65.4
27	17.4	17.4	17.4	17.3	17.3	16.4	16.4	16.4	16.5
Fucose									
1					100.0		102.3	100.4	
2					76.7		72.3	76.8	
3					74.9		75.4	74.9	
4					73.3		72.6	73.4	
5					71.1		71.2	71.2	
6					17.1		17.3	17.1	
Rhamnose									
1		100.1	100.0	99.6	101.6		99.9	101.6	100.0
2		72.9[b]	71.7	72.7[b]	72.5[d]		72.9[b]	72.6[d]	72.8[b]
3		72.9[b]	83.7	72.7[b]	72.7[d]		72.9[b]	72.7[d]	72.8[b]
4		74.3	73.0	74.1	74.4		74.2	74.5	74.2
5		69.9	69.7	69.9	69.2		70.0	69.3	70.0
6		18.6	18.4	18.4	19.0		18.6	19.0	18.6
Glucose									
1			106.4						
2			75.9						
3			78.4[c]						
4			72.1						
5			78.3[c]						
6			62.7						

a) Chemical shifts were measured in pyridine-d_5 at 50°C.
b) The signal intensities were determined by means of the gated decoupling technique which is termeb "NNE mode" in the JEOL FX 100 operation manual (1980).
c,d) Assignments within any column may be reversed.

bands in the IR spectrum. On acidic hydrolysis, 3 gave L-rhamnose, D-fucose and 12. The ^{13}C-NMR chemical shifts of the carbons of the A and B rings of the aglycone suggested that the sugar moiety is linked only to the C_1-hydroxyl group of 12, so that the two monosaccharides, namely rhamnose and fucose, are combined to form a biose. Based on a comparative analysis

of the ^{13}C-NMR spectra, the sequence of two monosaccharides was deduced to be identical with that of ophiopogonin B (15),[2b] $i.e.$, α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside (Table I). This inference was supported by the chemical method. Methylation of 3 by Hakomori's method afforded a hexa-O-methyl derivative (16), $C_{45}H_{74}O_{12}$, which was methanolyzed to give per-O-methyl-L-rhamnopyranoside and methyl 3,4-di-O-methyl-D-fucopyranoside as sugar components. Accordingly, the structure of 3 was established to be 25(S)-ruscogenin 1-O-α-L-rhamnopyranosyl(1→2)-β-D-fucopyranoside (3) as shown in Chart 2.

Glycoside D (4), $C_{39}H_{62}O_{13} \cdot H_2O$, is positive in the Liebermann–Burchard reaction and it shows a strong absorption band of hydroxyl groups and 25(R)-spiroketal absorption bands in the IR spectrum. On acidic hydrolysis, 4 gave L-rhamnose, D-glucose and 10. Methylation of 4 by Hakomori's method afforded a hepta-O-methyl derivative (17), $C_{46}H_{76}O_{13}$, which was methanolyzed to give ruscogenin 1-O-methyl ether, per-O-methylglucopyranoside and methyl 2,4-di-O-methylrhamnopyranoside. The configurations of the sugar moieties were assigned as α-rhamnose and β-glucose based on analyses of the ^{13}C- and ^{1}H-NMR spectra. Consequently, the structure of 4 was established to be ruscogenin 3-O-β-D-glucopyranosyl(1→3)-α-L-rhamnopyranoside (4) as shown in Chart 2.

Glycoside E (5), $C_{44}H_{70}O_{16} \cdot H_2O$, colorless needles, is positive in the Liebermann–Burchard reaction and it shows an absorption band of hydroxyl groups and spiroketal absorption bands in the IR spectrum. On acidic hydrolysis 5 gave L-rhamnose, D-glucose, D-xylose, diosgenin (18)[2d] and a small amount of yamogenin (=25(S)-isomer of diosgenin, 19).[10] Separation of 18 and 19 from the aglycone mixture was carried out by acetylation followed by column chromatography over silica gel using CH_2Cl_2.[11] The ^{13}C-NMR spectrum of 5 suggests that 5 is a mixture of glycosides of 18 and 19,[12] but the sugar moieties of both glycosides are the same as that of ophiopogonin D' (20).[2d] To confirm the sugar sequences of both glycosides, 5 was methylated by Hakomori's method and the octa-O-methyl derivative of 5 (21) was methanolyzed to afford per-O-methyl-L-rhamnopyranoside, per-O-methyl-D-xylopyranoside and methyl 4,6-di-O-methyl-D-glucopyranoside. Consequently, 5 was concluded to be a mixture of 20 and yamogenin 3-O-[α-L-rhamnopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-glucopyranoside as shown in Chart 2 and the ratio of both glycosides was established to be about 4:1 by comparing the intensities of corresponding carbon signals of both glycosides by using the gated decoupling technique.[13]

Glycoside F (6), $C_{45}H_{72}O_{16} \cdot H_2O$, colorless needles, shows one spot on thin layer chromatography (TLC) and is positive in the Liebermann–Burchard reaction. The IR spectrum of 6 shows strong absorption bands of hydroxyl and spiroketal groups, while the ^{13}C-NMR spectrum revealed that 6 might be a mixture of two glycosides, which are the same oligosides of C_{25}(R)- and (S)-spiroketal isomers. On acidic hydrolysis, 6 afforded D-glucose, L-rhamnose, 18 and 19. Both aglycones were isolated and identified. As in the case of 5, the ^{13}C-NMR spectrum suggested that 6 is a mixture of glycosides of 18 and 19, but the sugar moieties of both glycosides are the same as that of dioscin (22).[14] To elucidate the sugar sequences of both glycosides, 6 was methylated by Hakomori's method to afford the per-O-methyl derivative of 6 (23), which was methanolyzed to afford per-O-methyl-L-rhamnopyranoside and methyl 3,6-di-O-methyl-D-glucopyranoside. Consequently, 6 was concluded to be a mixture of 22 and yamogenin 3-O-[α-L-rhamnopyranosyl(1→2)][α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside, as shown in Chart 2, and the ratio of the glycosides was estimated to be about 2:1 by ^{13}C-NMR spectrometry as described above.

Glycoside G (7), a white powder, $C_{33}H_{51}O_8 \cdot SO_3M$, is positive in the Liebermann–Burchard reaction and it shows a strong absorption band of hydroxyl groups, characteristic absorption bands of the 25(R)-spiroketal moiety and an S–O stretching absorption band at 1210 cm^{-1} in the IR spectrum.[15] Acetylation of 7 with acetic anhydride and pyridine afforded a tri-acetate, which has no hydroxyl absorption band, but shows an ester band (1740 cm^{-1}), an S–O stretching band (1220 cm^{-1}), and characteristic 25(R)-spiroketal absorption bands. To

confirm the presence of the sulfate group, **7** was heated with pyridine–dioxane[16] to afford **1**, which was acetylated to give a tetraacetate (**9**). A part of the reaction mixture was concentrated to dryness and the residue was subjected to paper partition chromatography (PPC). The spot of sulfate ion was detected by spraying a test solution of barium chloride and potassium rhodizonate.[15b,17] Consequently, **7** was proved to be a sulfate of **1** and the location of the sulfonyl group was established as follows. On enzymatic hydrolysis with crude pectinase (*Aspergillus niger*, SIGMA), **7** afforded L-rhamnose and a sulfated aglycone which was deduced to be ruscogenin 1-sulfate based on the results described above. Finally, the structure of **7** was established to be ruscogenin 1-sulfate 3-*O*-α-L-rhamnopyranoside (**7**) as shown in Chart 2. The metal ions bound to **7** were analyzed by atomic absorption spectrometry and Na^+, K^+, Ca^{2+} and others were detected, but the natural metal ion of glycoside G has not been investigated.

Glycoside H (**8**), $C_{52}H_{86}O_{22} \cdot H_2O$, is positive in the Liebermann–Burchard reaction and with the Ehrlich reagent.[18] The IR spectrum of **8** does not show any characteristic spiroketal absorption band, and its ^{13}C-NMR spectrum shows characteristic furostanol carbon signals as reported by Hirai *et al.*[19] On acidic hydrolysis, **8** gave **18**, D-glucose and L-rhamnose, while enzymatic hydrolysis with almond emulsin afforded D-glucose and a prosapogenin, $C_{45}H_{72}O_{16} \cdot H_2O$, colorless needles, mp 272—275°C (dec.), which showed characteristic absorption bands at 980, 920, 900, 865 cm^{-1} (intensity 920<900, 25(R)-spiroketal) in the IR spectrum. This prosapogenin was identified as **22** by comparisons of TLC behavior, melting point, and IR, ^1H- and ^{13}C-NMR spectra. On the other hand, the ^1H-NMR spectrum of **8** revealed the presence of an *O*-methyl group (δ 3.28 ppm, 3H, s); **8** was demethylated by refluxing it in acetone–water (7:3 v/v) to afford proto-dioscin (**24**). Finally, the structure of **8** was established to be 26-*O*-β-D-glucopyranosyl-22-*O*-methylfurost-5-ene-3β,26-diol 3-*O*-β-chacotrioside (=methyl proto-dioscin)[20] by direct comparison of its ^{13}C-NMR spectrum with that of an authentic sample.

The steroidal constituents of the whole subterranean part of *L. platyphylla* WANG et TANG described above were also found in the tuber of the same plant. It is interesting that the only common glycoside of *L. platyphylla* with those of Ophiopogonis Tuber is ophiopogonin D′. On the other hand, Ophiopogonis Tuber contains several glycosides of **10** and **18**, but *L. platyphylla* contains the glycosides of **10**, **18** and their C_{25}-isomers.

As described at the beginning of this paper, *L. platyphylla* is one of the congeners of Ophiopogonis Tuber, and it would be of interest to investigate the pharmacological activity of the new glycosides. Furthermore, this is only the second report of the isolation of a sulfated steroidal glycoside from a Liliaceous plant.[21]

Experimental

All melting points were determined on a Yanagimoto micro-melting point apparatus (hot-stage type) and are uncorrected. The optical rotations were measured with a Yanagimoto OR-50 polarimeter. The IR spectra were recorded with a Hitachi EPI-2 spectrometer and NMR spectra with a JEOL FX-100 spectrometer (100 MHz for ^1H-NMR and 25 MHz for ^{13}C-NMR). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. Atomic absorption spectra were recorded on a Hitachi 170-50A atomic absorption spectrophotometer. Gas liquid chromatography (GLC) was run on a Shimadzu GC-6A unit equipped with a flame ionization detector. Experimental conditions: (a) sugars: column, 5% SE-52 on Chromosorb W 3 mm × 2 m; column temp., 170°C; injection temp., 210°C; carrier gas N_2, 1.0 kg/ cm^2; samples, trimethyl silyl (TMS) ether, (b) *O*-methylated sugars: column, 5% NPGS on Shimalite 3 mm × 2 m; column temp., 145°C; injection temp., 200°C; carrier gas N_2, 1.0 kg/cm^2. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck) using the following solvents: (a) $CHCl_3$–MeOH–H_2O (7:1:0.1 v/v), (b) $CHCl_3$–MeOH–H_2O (7:2:0.1 v/v), (c) $CHCl_3$–MeOH–H_2O (7:3:0.4 v/v), (d) hexane–acetone (2:1 v/v) and (e) hexane–acetone (3:1 v/v). Detection was achieved by spraying 10% H_2SO_4 or Ehrlich reagent followed by heating. TLC for free monosaccharides was run on precoated Cellulose F plates (Merck) using a mixture of BuOH–AcOH–H_2O (4:1:5 v/v, upper layer) and spots were detected by spraying aniline hydro-

gen phthalate reagent.

Extraction and Isolation of Glycosides——The fresh subterranean parts of *Liriope platyphylla* WANG et TANG (6 kg) collected at Tokyo Metropolitan Medicinal Plants Garden were crushed and extracted with hot MeOH (8 l × 4). The extract was combined and evaporated to dryness *in vacuo*. The residue (1.2 kg) was dissolved in water and extracted with ether. The aqueous layer was extracted with BuOH saturated with water (2 l × 3) and the BuOH-soluble fraction was concentrated *in vacuo* to afford a brown powder (80 g), which was subjected to column chromatography on Avicel eluted with $CHCl_3$, $CHCl_3$–MeOH–H_2O (70 : 16 : 10 v/v, lower phase) and finally MeOH. The fraction eluted with $CHCl_3$–MeOH–H_2O (25 g) was rechromatographed on silica gel with solvent b to provide two fractions, Fr. 1 and Fr. 2. Fr. 1 was subjected to column chromatography on silica gel with solvent a to afford **1** (300 mg) and a mixture of **2** and **3**, this mixture was separated by column chromatography on silica gel with $CHCl_3$–MeOH–AcOEt–H_2O (2 : 2 : 4 : 1 v/v, lower phase) to afford **2** (220 mg) and **3** (60 mg). Fr. 2 was separated by column chromatography on silica gel with solvent b into **4** (180 mg), **5** (170 mg) and **6** (300 mg).

On the other hand, the methanol-eluted fraction described above was subjected to column chromatography on silica gel with solvent c followed by column chromatography on Sephadex LH-20 with MeOH to afford **7** (350 mg) and **8** (500 mg).

Properties of 1, 2, 3, 4, 5, 6, 7 and 8——**1**: Colorless needles from aqueous MeOH, mp 226—228°C (dec.), $[\alpha]_D^{19}$ −107.0° ($c=1.00$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 982, 920, 902, 865 (intensity 920<902, 25(R)-spiroketal). ^{1}H-NMR (C_5D_5N) δ: 0.72 (3H, br d, –CH–CH$_3$), 0.91 (3H, s, CH$_3$), 1.11 (3H, d, $J=6$ Hz, –CH–CH$_3$), 1.27 (3H, s, CH$_3$), 1.55 (3H, d, $J=6$ Hz, –CH–CH$_3$). ^{13}C-NMR (C_5D_5N) δ: Table. I 100.1 ($J_{C_1-H_1}=166$ Hz, α-anomeric carbon of rhamnose). *Anal.* Calcd for $C_{33}H_{52}O_8 \cdot H_2O$: C, 66.64; H 9.15. Found: C, 66.48; H, 8.98.

2: Colorless needles from aqueous EtOH, mp 225—227°C (dec.), $[\alpha]_D^{19}$ −103.8° ($c=0.80$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 985, 920, 900, 855 (intensity 920>900, 25(S)-spiroketal). ^{1}H-NMR (C_5D_5N) δ: 0.86 (3H, s, CH$_3$), 1.08 (3H, d, $J=6$ Hz, –CH–CH$_3$), 1.14 (3H, d, $J=6$ Hz, –CH–CH$_3$), 1.17 (3H, s, CH$_3$), 1.55 (3H, d, $J=6$ Hz, –CH–CH$_3$), 1.70 (3H, d, $J=6$ Hz, –CH–CH$_3$). ^{13}C-NMR (C_5D_5N) δ: Table I. 102.3 ($J_{C_1-H_1}=155$ Hz, β-anomeric carbon of fucose), 99.9 ($J_{C_1-H_1}=166$ Hz, α-anomeric carbon of rhamnose). *Anal.* Calcd for $C_{39}H_{62}O_{12} \cdot 2H_2O$: C, 61.72; H, 8.77. Found: C, 61.99; H, 8.57.

3: Colorless needles from aqueous MeOH, mp 201—203°C (dec.), $[\alpha]_D^{18}$ −89.6° ($c=0.48$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 985, 920, 900, 855 (intensity 920>900, 25(S)-spiroketal). ^{1}H-NMR (C_5D_5N) δ: 0.89 (3H, s, CH$_3$), 1.08 (3H, d, $J=6$ Hz, –CH–CH$_3$), 1.14 (3H, d, $J=6$ Hz, –CH–CH$_3$), 1.44 (3H, s, CH$_3$), 1.52 (3H, d, $J=6$ Hz, –CH–CH$_3$), 1.75 (3H, d, $J=6$ Hz, –CH–CH$_3$). ^{13}C-NMR (C_5D_5N) δ: Table I. 100.4 ($J_{C_1-H_1}=156.3$ Hz, β-anomeric carbon of fucose), 101.6 ($J_{C_1-H_1}=169.7$ Hz, α-anomeric carbon of rhamnose). *Anal.* Calcd for $C_{39}H_{62}O_{12} \cdot 1/2H_2O$: C, 64.00; H, 8.54. Found: C, 63.81; H, 8.78.

4: Colorless needles from aqueous EtOH, mp 293—295°C (dec.), $[\alpha]_D^{19}$ −92.0° ($c=0.87$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 982, 920, 902, 865 (intensity 920<902, 25(R)-spiroketal). ^{1}H-NMR (C_5D_5N) δ: 0.70 (3H, br d, –CH–CH$_3$), 0.91 (3H, s, CH$_3$), 1.10 (3H, d, $J=6$ Hz, –CH–CH$_3$), 1.26 (3H, s, CH$_3$), 1.54 (3H, d, $J=6$ Hz, –CH–CH$_3$). ^{13}C-NMR (C_5D_5N) δ: Table I. 100.0 ($J_{C_1-H_1}=166.6$ Hz, α-anomeric carbon of rhamnose), 106.4 ($J_{C_1-H_1}=156.9$ Hz, β-anomeric carbon of glucose). *Anal.* Calcd for $C_{39}H_{62}O_{13} \cdot H_2O$: C, 61.88; H, 8.52. Found: C, 61.86; H, 8.63.

5: Colorless needles from aqueous EtOH, mp 254—256°C (dec.). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 982, 920, 902, 865 (spiroketal). ^{13}C-NMR (C_5D_5N) δ: glucose 100.2 (C_1), 77.2 (C_2), 82.9 (C_3), 70.7 (C_4), 78.5 (C_5), 62.1 (C_6); rhamnose 101.8 (C_1), 72.3 (C_2), 72.7 (C_3), 74.1 (C_4), 69.4 (C_5), 18.5 (C_6); xylose 105.5 (C_1), 74.8 (C_2), 77.2 (C_3), 70.8 (C_4), 67.2 (C_5). *Anal.* Calcd for $C_{44}H_{70}O_{16} \cdot H_2O$: C, 60.53; H, 8.31. Found: C, 60.17; H, 8.41.

6: Colorless needles from aqueous EtOH, mp 292—295°C (dec.), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 982, 920, 902, 865 (spiroketal). ^{13}C-NMR (C_5D_5N) δ: glucose 100.4 (C_1), 79.5 (C_2), 76.7 (C_3), 78.5 (C_4), 77.9 (C_5), 61.6 (C_6); rhamnose ($\overset{2}{\rightarrow}$glucose) 101.9 (C_1), 72.2 (C_2), 72.7 (C_3), 73.9 (C_4), 69.4 (C_5), 18.4 (C_6); rhamnose ($\overset{4}{\rightarrow}$glucose) 103.0 (C_1), 72.4 (C_2), 72.9 (C_3), 74.2 (C_4), 70.6 (C_5), 18.6 (C_6). *Anal.* Calcd for $C_{45}H_{72}O_{16} \cdot H_2O$: C, 60.93; H, 8.41. Found: C, 60.86; H, 8.51.

7: A white powder from EtOH, mp 300°C (dec.), $[\alpha]_D^{19}$ −60.4° ($c=1.01$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1210 (S–O), 982, 920, 902, 865 (intensity 920<902, 25(R)-spiroketal). ^{1}H-NMR (C_5D_5N) δ: 0.69 (3H, br d, –CH–CH$_3$), 0.84 (3H, s, CH$_3$), 1.18 (3H, d, $J=6$ Hz, –CH–CH$_3$), 1.24 (3H, s, CH$_3$), 1.55 (3H, d, $J=6$ Hz, –CH–CH$_3$). The metallic ions bound to the sulfate group were determined by atomic absorption spectroscopy (wavelengths: Na 589.0 nm; K 766.5 nm; Ca 422.8 nm) and the ratio of Na, K and Ca was about 6 : 3 : 1.

8: Colorless needles from MeOH, mp 184—187°C (dec.), $[\alpha]_D^{18}$ −90.3° ($c=0.76$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH). ^{1}H-NMR (C_5D_5N) δ: 0.83 (3H, s, CH$_3$), 1.01 (3H, d, $J=6$ Hz, –CH–CH$_3$), 1.05 (3H, s, CH$_3$), 1.60 (3H, d, $J=6$ Hz, –CH–CH$_3$), 1.78 (3H, d, $J=6$ Hz, –CH–CH$_3$), 3.28 (3H, s, OCH$_3$). ^{13}C-

NMR (C_5D_5N) δ: glucose $(\overset{26}{\rightarrow}$aglycone) 104.8 (C_1), 75.2 (C_2), 78.6 (C_3), 72.0 (C_4), 78.1 (C_5), 63.1 (C_6); glucose $(\overset{3}{\rightarrow}$aglycone) 100.4 (C_1), 79.5 (C_2), 76.8 (C_3), 78.2 (C_4), 77.9 (C_5), 61.6 (C_6); rhamnose $(\overset{2}{\rightarrow}$glucose) 101.9 (C_1), 72.4 (C_2), 72.7 (C_3), 73.8 (C_4), 69.4 (C_5), 18.4 (C_6); rhamnose $(\overset{4}{\rightarrow}$glucose) 103.0 (C_1), 72.4 (C_2), 72.8 (C_3), 74.2 (C_4), 70.5 (C_5), 18.6 (C_6). *Anal.* Calcd for $C_{52}H_{86}O_{22} \cdot H_2O$: C, 57.76; H, 8.20. Found: C, 57.93; H, 8.32.

Acetylation of 1——Glycoside A (**1**, 20 mg) was acetylated with Ac_2O–pyridine and the reaction mixture was treated in the usual way to afford an acetate, a white powder from MeOH, (mp 119—121°C), IR ν_{max}^{Nujol} cm^{-1}: OH (nil.), 1745 (ester), 980, 920, 900, 865 (intensity 920<900, 25(R)-spiroketal). ^{1}H-NMR (CDCl$_3$) δ: 0.78 (3H, s, CH$_3$; 3H, d, $J=6$ Hz, -CH-CH$_3$), 0.96 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.15 (3H, s, CH$_3$), 1.20 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.98, 2.15 (each 3H, s, -OCOCH$_3$), 2.04 (6H, s, -OCOCH$_3$×2). *Anal.* Calcd for $C_{41}H_{60}O_{12}$: C, 66.11; H, 8.12. Found: C, 66.17; H, 8.24.

Hydrolysis of 1, 2, 3, 4, 5, 6 and 8 with 2 N HCl——A solution of **1** (50 mg), **2** (100 mg), **3** (10 mg), **4** (50 mg), **5** (70 mg), **6** (150 mg) or **8** (50 mg) in 2 N HCl–50% dioxane (3 ml per 10 mg of each glycoside) was refluxed for 3 h. The reaction mixture was diluted with water and extracted with CHCl$_3$. The CHCl$_3$ layer was washed with water and dried over Na$_2$SO$_4$. The CHCl$_3$ solution was filtered and the filtrate was evaporated to dryness.

Aglycones: The residues of **1** and **4** were crystallized from MeOH to afford colorless needles, mp 205—207°C, $[\alpha]_D^{18}$ $-112.4°$ $(c=0.31,$ pyridine). IR ν_{max}^{Nujol} cm^{-1}: 3300 (OH), 982, 920, 900, 865 (intensity 920<900, 25(R)-spiroketal). TLC (solvent e): Rf 0.19. *Anal.* Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.19; H, 10.15. Each aglycone was found to be identical with **10** by mixed fusion and by comparing the IR spectra. The residues of **2** and **3** were crystallized from MeOH to afford colorless needles, mp 194—196°C, 212—214°C (double mp), $[\alpha]_D^{18}$ $-108.1°$ $(c=0.37,$ pyridine). IR ν_{max}^{Nujol} cm^{-1}: 3300 (OH), 990, 920, 900, 850 (intensity 920>900, 25(S)-spiroketal). *Anal.* Calcd for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.80; H, 10.08. Each aglycone was found to be identical with **12** by mixed fusion and by comparisons of IR and ^{1}H-NMR spectra. Furthermore, each aglycone was acetylated with Ac_2O–pyridine, and the reaction mixture was treated in the usual way to afford colorless needles from MeOH, mp 182—185°C, $[\alpha]_D^{19}$ $-91.0°$ $(c=0.35,$ CHCl$_3$). IR ν_{max}^{Nujol} cm^{-1}: 1735 (ester), 985, 920, 905, 852 (intensity 920>905, 25(S)-spiroketal). ^{1}H-NMR (CDCl$_3$) δ: 0.78 (3H, s, CH$_3$), 0.98 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.06 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.15 (3H, s, CH$_3$), 2.01 (6H, -OCOCH$_3$), 3.29 (1H, d, $J=12$ Hz, C$_{26}$-H), 3.95 (1H, dd, $J=3$ Hz and 12 Hz, C$_{26}$-H), 5.62 (1H, m, $\diagup$C=C$\diagdown_H$) *Anal.* Calcd for $C_{31}H_{46}O_6$: C, 72.34; H, 9.01. Found: C, 72.19; H, 9.18. Each acetate was found to be identical with 25-(S)-ruscogenin diacetate by mixed fusion, TLC (solvent CH$_2$Cl$_2$, Rf 0.59 (25(S)-ruscogenin diacetate), 0.65 (ruscogenin diacetate)) and by comparing IR and ^{1}H-NMR spectra. The residues of **5** and **6** were acetylated with Ac_2O–pyridine, and each reaction mixture was treated in the usual way. Each acetylation product was subjected to column chromatography on silica gel with CH$_2$Cl$_2$ to separate diosgenin acetate and yamogenin acetate. Diosgenin acetate: Colorless needles from MeOH, mp 192—195°C. ^{1}H-NMR (CDCl$_3$) δ: 0.78 (3H, s, CH$_3$; 3H, d, $J=6$ Hz, -CH-CH$_3$), 0.98 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.02 (3H, s, CH$_3$), 2.00 (3H, s, -OCOCH$_3$), 3.30 (2H, m, 26-H$_2$). Yamogenin acetate: Colorless needles from MeOH, mp 173—176°C. ^{1}H-NMR (CDCl$_3$) δ: 0.78 (3H, s, CH$_3$), 0.98 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.02 (3H, s, CH$_3$), 1.06 (3H, d, $J=6$ Hz, -CH-CH$_3$), 2.00 (3H, s, -OCOCH$_3$), 3.24 (1H, d, $J=12$ Hz, C$_{26}$-H), 3.94 (1H, dd, $J=3$ Hz and 12 Hz, C$_{26}$-H). The acetates of diosgenin and yamogenin were each refluxed with 0.5% KOH in EtOH under an N$_2$ stream for 30 min. The reaction mixture was diluted with water, concentrated *in vacuo* to remove EtOH and then extracted with CHCl$_3$. The CHCl$_3$ solution was washed with water, dried over Na$_2$SO$_4$, and evaporated to dryness. **18**, colorless needles from acetone, mp 203—204°C, and **19**, colorless needles from MeOH, mp 201°C, were identified by TLC (2% AgNO$_3$–impregnated precoated Kieselgel 60 F$_{254}$ plates; solvent, CHCl$_3$–Et$_2$O=199:1; Rf 0.21 (yamogenin), 0.28 (diosgenin)) and by mixed fusion with an authentic sample. Furthermore, **18** and **19** in the hydrolysates of **5** and **6** were determined by GLC (column, 3% SE-30 on Chromosorb W, 3 mm×2 m; column temp., 260°C; injection temp., 330°C; carrier gas, N$_2$ 1.8 kg/cm^2; t_R(min), 14.8 (**18**), 15.7 (**19**)). The residue of **8** was recrystallized from acetone to afford **18**, mp 203—204°C, which was identified by TLC and by mixed fusion with an authentic sample.

Sugars: Each aqueous layer was neutralized with Amberlite IR 45 and concentrated to dryness *in vacuo*. The monosaccharides were examined by TLC and GLC (condition a). **1, 4** and **8**: TLC Rf 0.19 (glucose), 0.38 (rhamnose). GLC t_R(min) 5.2, 7.1 (rhamnose), 16.5, 24.8 (glucose). Furthermore, each neutralized aqueous layer was heated on a water bath with phenylhydrazine hydrochloride and AcONa for 30 min, and the reaction mixture was treated in the usual way. The product was purified by column chromatography on silica gel with solvent d to afford phenylosazones of D-glucose, L-rhamnose, D-fucose and D-xylose. D-Glucose phenylosazone: yellow needles from acetone, mp 209—210°C, $[\alpha]_D^{24}$ $-9.52°$ $(c=0.21,$ pyridine–EtOH), (lit.[22] mp 208°C, $[\alpha]_D$ $-1.5°$ (pyridine–EtOH)). L-Rhamnose phenylosazone: a yellow powder, (mp 168—170°C), $[\alpha]_D^{24}$ $+69.23°$ $(c=0.26,$ pyridine), (lit.[23] mp 184°C, $[\alpha]_D$ $+107°\rightarrow+60°$ (pyridine)). D-Fucose phenylosazone: a yellow powder from aq. EtOH, (mp 165—167°C), $[\alpha]_D^{24}$ $+67.41°$ $(c=0.52,$

pyridine), (lit.[24] mp 172°C, $[\alpha]_D + 70°$ (pyridine)). D-Xylose phenylosazone: yellow needles from aq. EtOH, mp 157°C, $[\alpha]_D^{24}$ $-43.77°$ ($c=0.60$, EtOH), (lit.[25] mp 153—155°C, $[\alpha]_D$ $-40.9°$ (EtOH)).

Determination of the Ratios of Diosgenin and Yamogenin Glycosides in 5 and 6——The ratios of diosgenin and yamogenin glycosides in **5** and **6** were estimated by ^{13}C-NMR using the gated decoupling technique.[13] Experimental conditions: temp. 50°C; pulse width 10 μs; pulse repetition 4 s. The ratios of signal intensities of the corresponding carbon signals of the glycosides were determined as follows. **5**: C-20 (3.8: 1), C-21 (3.8: 1), C-22 (4.3: 1), C-24 (3.9: 1), C-25 (4.2: 1), C-26 (4: 1). **6**: C-20 (2.2: 1), C-21 (1.9: 1), C-22 (2.2: 1), C-24 (2.3: 1), C-25 (2.1: 1), C-26 (1.8: 1). Based on the above results, the average ratios of diosgenin and yamogenin glycosides in **5** and **6** were calculated to be about 4: 1 for the former and 2: 1 for the latter.

Methylation of 1, 2, 3, 4, 5 and 6 by Hakomori's Method——According to Hakomori's method, NaH (100 mg) was defatted with anhydrous benzene followed by petroleum ether, then warmed with dimethyl-sulfoxide (DMSO, 10 ml) at 70°C in an oil bath for 1 h with stirring under an N_2 flow. A solution of **1** (100 mg) in dimethyl sulfoxide (DMSO) (5 ml) was then added and the mixture was stirred for 1 h under an N_2 flow. CH_3I (2 ml) was added to the solution and the reaction mixture was allowed to stand at room temperature for 1 h with stirring. After dilution with water, the reaction mixture was extracted with $CHCl_3$ and the organic layer was washed with water, dried and evaporated to dryness. The residue was chromatographed on Sephadex LH-20 and elution with $CHCl_3$ afforded a per-O-methylate of **1**. The per-O-methylates of **2, 3, 4, 5** and **6** were also obtained by the same procedure as described above. **11**: colorless needles from MeOH, mp 211—213°C, $[\alpha]_D^{20}$ $-124.4°$ ($c=0.45$, $CHCl_3$). *Anal.* Calcd for $C_{37}H_{60}O_8$: C, 70.22; H, 9.56. Found: C, 70.06; H, 9.98. IR ν_{max}^{Nujol} cm^{-1}: OH (nil.), 982, 918, 902, 865 (intensity 918<902, 25(R)-spiroketal). ^{1}H-NMR (CDCl$_3$) δ: 0.79 (3H, s, CH$_3$; 3H, d, $J=6$ Hz, -CH-CH$_3$), 0.94 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.03 (3H, s, CH$_3$), 1.28 (3H, d, $J=6$ Hz, -CH-CH$_3$), 3.29, 3.56 (each 3H, s, OCH$_3$), 3.50 (6H, OCH$_3\times2$), 4.90 (1H, d, $J=1$ Hz, rhamnose anomeric H). **13**: colorless needles from MeOH, mp 155—157°C, $[\alpha]_D^{17}$ $-106.9°$ ($c=0.73$, $CHCl_3$). *Anal.* Calcd for $C_{45}H_{74}O_{12}$: C, 66.97; H, 9.24. Found: C, 66.74; H, 9.47. IR ν_{max}^{Nujol} cm^{-1}: OH (nil.)., 990, 920, 900, 850 (intensity 920>900, 25(S)-spiroketal). ^{1}H-NMR (CDCl$_3$) δ: 0.78 (3H, s, CH$_3$), 0.99 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.06 (3H, s, CH$_3$), 1.08 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.25 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.28 (3H, d, $J=6$ Hz, -CH-CH$_3$), 3.50 (9H, OCH$_3\times3$), 3.51 (3H, s, OCH$_3$), 3.54 (6H, OCH$_3\times2$), 4.20 (1H, d, $J=6$ Hz, fucose anomeric H), 4.97 (1H, d, $J=1$ Hz, rhamnose anomeric H). **16**: colorless needles from MeOH, mp 197—200°C, $[\alpha]_D^{15}$ $-90.0°$ ($c=0.40$, $CHCl_3$). *Anal.* Calcd for $C_{45}H_{74}O_{12}$: C, 66.97; H, 9.24. Found: C, 66.52; H, 9.34. IR ν_{max}^{Nujol} cm^{-1}: OH (nil.), 985, 920, 900, 850 (intensity 920>900, 25(S)-spiroketal). ^{1}H-NMR (CDCl$_3$) δ: 0.78 (3H, s, CH$_3$), 0.99 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.03 (3H, s, CH$_3$), 1.07 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.24 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.27 (3H, d, $J=6$ Hz, -CH-CH$_3$), 3.34, 3.49, 3.51, 3.53 (each 3H, s, OCH$_3$), 3.46 (6H, OCH$_3\times2$), 4.20 (1H, d, $J=7$ Hz, fucose anomeric H), 5.24 (1H, d, $J=1$ Hz, rhamnose anomeric H). **17**: colorless needles from MeOH, mp 211—213°C, $[\alpha]_D^{20}$ $-62.3°$ ($c=0.53$, $CHCl_3$). *Anal.* Calcd for $C_{46}H_{76}O_{13}$: C, 66.00; H, 9.15. Found: C, 66.20; H, 9.31. IR ν_{max}^{Nujol} cm^{-1}: OH (nil.), 982, 920, 902, 865 (intensity 920<902, 25(R)-spiroketal). ^{1}H-NMR (CDCl$_3$) δ: 0.79 (3H, s, CH$_3$; 3H, d, $J=6$ Hz, -CH-CH$_3$), 0.96 (3H, d, $J=6$ Hz, -CH-CH$_3$), 1.03 (3H, s, CH$_3$), 1.29 (3H, d, $J=6$ Hz, -CH-CH$_3$), 3.28, 3.38 (3H each, s, OCH$_3$), 3.52 (9H, OCH$_3\times3$), 3.62 (6H, OCH$_3\times2$), 4.47 (1H, d, $J=6$ Hz, glucose anomeric H), 4.88 (1H, d, $J=1$ Hz, rhamnose anomeric H). **21**: colorless needles from MeOH, mp 112—114°C. *Anal.* Calcd for $C_{52}H_{86}O_{16}$: C, 64.58; H, 8.96. Found: C, 64.49; H, 9.19. IR ν_{max}^{Nujol} cm^{-1}: OH (nil.). ^{1}H-NMR (CDCl$_3$) δ: 3.38—3.61 (24H, OCH$_3\times8$), 4.28 (1H, d, $J=6$ Hz, xylose anomeric H), 4.38 (1H, d, $J=6$ Hz, glucose anomeric H), 5.25 (1H, d, $J=1$ Hz, rhamnose anomeric H). **23**: a white powder from MeOH, (mp 102—105°C). *Anal.* Calcd for $C_{53}H_{88}O_{16}$: C, 64.87; H, 9.04. Found: C, 64.52; H, 9.29. IR ν_{max}^{Nujol} cm^{-1}: OH (nil.). ^{1}H-NMR (CDCl$_3$) δ: 3.38—3.54 (24H, OCH$_3\times8$), 4.39 (1H, d, $J=6$ Hz, glucose anomeric H), 5.25 (1H, d, $J=1$ Hz, rhamnose anomeric H), 5.35 (1H, d, $J=1$ Hz, rhamnose anomeric H).

Methanolysis of 11, 13, 16, 17, 21 and 23 with Methanolic 5% HCl——A per-O-methyl ether, **11, 13, 16, 17, 21** or **23** was refluxed with methanolic 5% HCl (0.3 ml per 1 mg sample) for 2 h, then the reaction mixture was neutralized with Ag$_2$CO$_3$ and evaporated to dryness. The residue was chromatographed on silica gel and elution with solvent d afforded the aglycone. O-Methylated sugars in the methanolysates were examined by TLC and GLC. **11**: aglycone: ruscogenin 1-O-methyl ether, colorless needles from MeOH, mp 196—197°C, was identified by TLC (solvent: benzene–acetone=4: 1 v/v, Rf 0.25 (*cf.* ruscogenin 3-O-methyl ether Rf 0.37)) and by mixed fusion with an authentic sample. Sugar: TLC (solvent d) Rf; 0.48 (per-O-methylrhamnopyranoside). GLC (condition b) t_R(min); 2.8, 4.3 (per-O-methylrhamnopyranoside). **13**: aglycone: 25(S)-ruscogenin(identification TLC). Sugars: TLC (solvent d) Rf; 0.48 (per-O-methylrhamnopyranoside), 0.30, 0.40 (per-O-methylfucopyranoside). GLC (condition b) t_R(min); 2.8, 4.3 (per-O-methylrhamnopyranoside), 3.4, 4.9 (per-O-methylfucopyranoside). **16**: aglycone: 25(S)-ruscogenin 3-O-methyl ether (not identified). Sugars: TLC (solvent d) Rf; 0.48 (per-O-methylrhamnopyranoside), 0.28 (methyl 3,4-di-O-methylfucopyranoside). GLC (condition b) t_R(min); 2.8, 4.3 (per-O-methylrhamnopyranoside), 8.4 (methyl 3,4-di-O-methylfucopyranoside). **17**: aglycone: ruscogenin 1-O-methyl ether. Sugars:

TLC (solvent d) Rf; 0.32, 0.47 (per-O-methylglucopyranoside), 0.35 (methyl 2,4-di-O-methylrhamnopyranoside). GLC (condition b) t_R(min); 6.5, 9.7 (per-O-methylglucopyranoside), 7.3 (methyl 2,4-di-O-methylrhamnopyranoside). **21**: aglycones: diosgenin and yamogenin. Sugars: TLC (solvent d) Rf; 0.48 (per-O-methylrhamnopyranoside), 0.45, 0.50 (per-O-methylxylopyranoside), 0.12 (methyl 4,6-di-O-methylglucopyranoside). GLC (condition b) t_R(min); 2.8, 4.3 (per-O-methylrhamnopyranoside), 2.7, 3.4 (per-O-methylxylopyranoside), 11.1 (methyl 4,6-di-O-methylglucopyranoside). **23**: aglycones: diosgenin and yamogenin. Sugars: TLC (solvent d) Rf; 0.48 (per-O-methylrhamnopyranoside), 0.13 (methyl 3,6-di-O-methylglucopyranoside). GLC (condition b) t_R(min); 2.8, 4.3 (per-O-methylrhamnopyranoside), 8.4, 9.8 (methyl 3,6-di-O-methylglucopyranoside).

Partial Hydrolysis of 2——Glycoside B (**2**, 70 mg) was dissolved in 0.2 N H_2SO_4 in 50% EtOH (20 ml) and the solution was heated at 60°C for 5 h. The reaction mixture was diluted with water (10 ml), concentrated to 20 ml, and cooled. The precipitate was collected by filtration and subjected to column chromatography on silica gel using solvent b to afford **14** (20 mg), a crystalline powder from aqueous MeOH, mp 173—175°C (dec.), $[\alpha]_D^{18}$ −115.2° ($c = 0.36$, pyridine). *Anal.* Calcd for $C_{33}H_{52}O_8 \cdot H_2O$: C, 66.64; H, 9.15. Found: C, 66.22; H, 9.02. IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 985, 920, 900, 855 (intensity 920>900, 25(S)-spiroketal). On hydrolysis with 2 N HCl in 50% dioxane, the prosapogenin of glycoside B afforded 25(S)-ruscogenin and rhamnose.

Preparation of Sodium Salt of 7——An aqueous solution of **7** (50 mg) was passed through an Amberlite IR 120 column and the desalted solution was neutralized with 0.01 N NaOH. The neutral solution was evaporated to dryness *in vacuo* and the residue was purified by column chromatography on LH-20 with MeOH to afford the sodium salt of **7** as colorless needles from EtOH, mp 190—193°C (dec.). *Anal.* Calcd for $C_{33}H_{51}$-$NaO_{11}S \cdot 2H_2O$: C, 55.44; H, 7.76; S, 4.49. Found: C, 55.14; H, 7.76; S, 4.59.

Acetylation of 7——Glycoside G (**7**, 30 mg) was acetylated with Ac_2O–pyridine and the reaction mixture was treated in the usual way to afford a triacetate, colorless needles from EtOH, mp 172—174°C (dec.), IR ν_{max}^{Nujol} cm^{-1}: OH (nil.), 1740 (ester), 1220 (S–O), 980, 920, 900, 865 (intensity 920<900, 25(R)-spiroketal).
^{1}H-NMR (CDCl$_3$) δ: 0.80 (3H, s, CH$_3$; 3H, d, $J = 6$ Hz, –CH–CH$_3$), 0.96 (3H, d, $J = 6$ Hz, –CH–CH$_3$), 1.08 (3H, s, CH$_3$), 1.22 (3H, d, $J = 6$ Hz, –CH–CH$_3$), 2.01, 2.07, 2.17 (each 3H, s, OCOCH$_3$).

Solvolysis of 7——A solution of **7** (50 mg) in pyridine–dioxane (4: 1 v/v, 20 ml) was heated on a water bath at 80°C for 5 h. The reaction mixture was evaporated to dryness *in vacuo* and the residue was chromatographed on silica gel with solvent b to afford colorless needles from aqueous MeOH, mp 226—228°C (dec.), $[\alpha]_D^{18}$ −105.3° ($c = 0.42$, pyridine). *Anal.* Calcd for $C_{33}H_{52}O_8 \cdot H_2O$: C, 66.64; H, 9.15. Found: C, 66.30; H, 9.27. The product was identified as glycoside A (**1**) by comparisons of mp, TLC behavior (solvent c, Rf 0.57) and IR spectra. Furthermore, an acetate of desulfated glycoside G, which was prepared by the usual method, was identified as glycoside A tetraacetate (**9**) by comparisons of TLC behavior (solvent e, Rf 0.45) and IR spectra.

On the other hand, a part of the reaction mixture described above was examined by paper partition chromatography (Toyo-roshi No. 50 paper; solvent, BuOH–MeOH–H_2O (1: 3: 1 v/v)). For 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 (10 mg) solution in 50% MeOH (50 ml); sulfate ion in the hydrolysate was detected as a yellow spot at Rf 0.32.

Enzymatic Hydrolysis of 7——A solution of **7** (100 mg) in McIlvaine buffer (pH 4.0, 50 ml) was incubated with crude pectinase prepared from *Aspergillus niger* (SIGMA, 50 mg) at 37°C for five d. The precipitate was collected by filtration, dried and subjected to column chromatography on silica gel with solvent b to afford ruscogenin 1-sulfate (15 mg) as a white powder from MeOH, (mp 173—175°C (dec.)), IR ν_{max}^{Nujol} cm^{-1}: 3320 (OH), 1210 (S–O), 982, 920, 900, 865 (intensity 920<900, 25(R)-spiroketal). Furthermore, the presence of rhamnose in the filtrate was detected by TLC (Rf 0.38) and GLC (condition a; t_R 5.2, 7.1).

Demethylation of 8 with Aqueous Acetone——A solution of **8** (50 mg) in a mixture of Me_2CO–H_2O (7: 3 v/v, 20 ml) was refluxed for 10 h. The reaction mixture was evaporated to dryness *in vacuo* to afford colorless needles from water, mp 190—194°C (dec.), $[\alpha]_D^{17}$ −80.2° ($c = 0.46$, pyridine) (lit.[20] mp 190—196°C (dec.), $[\alpha]_D$ −79.8°). *Anal.* Calcd for $C_{51}H_{84}O_{22} \cdot H_2O$: C, 57.39; H, 8.12. Found: C, 57.25; H, 8.25. ^{1}H-NMR: OCH$_3$ (nil). The product was identified as proto-dioscin by comparing the mp, TLC behavior (solvent c, Rf 0.12) and ^{13}C-NMR spectrum with those of an authentic sample.

Enzymatic Hydrolysis of 8——A solution of **8** (100 mg) in H_2O (20 ml) was incubated with almond emulsin (50 mg) at 37°C for 24 h. The precipitate was collected by filtration, dried and subjected to column chromatography on silica gel with solvent b to afford colorless needles from EtOH, mp 272—275°C (dec.), $[\alpha]_D^{18}$ −109.8° ($c = 0.84$, pyridine). *Anal.* Calcd for $C_{45}H_{72}O_{16} \cdot H_2O$: C, 60.93; H, 8.41. Found: C, 60.72; H, 8.40. IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 980, 920, 900, 865 (intensity 920<900, 25(R)-spiroketal). ^{1}H-NMR (C_5D_5N) δ: 0.72 (3H, br d, –CH–CH$_3$), 0.84 (3H, s, CH$_3$), 1.06 (3H, s, CH$_3$), 1.15 (3H, d, $J = 6$ Hz, –CH–CH$_3$), 1.64 (3H, d, $J = 6$ Hz, –CH–CH$_3$), 1.77 (3H, d, $J = 6$ Hz, –CH–CH$_3$). This compound was identified as dioscin by comparing the TLC behavior (solvent c, Rf 0.33), mp, and IR, ^{1}H-NMR and ^{13}C-NMR spectra with those of an authentic sample.

The aqueous filtrate was evaporated to dryness *in vacuo*. The presence of glucose in the residue was detected by TLC (Rf 0.19) and GLC (condition a, t_R(min) 16.5, 24.8).

Extraction and Identification of the Glycosides of the Tuber——The dried tuber of *Liriope platyphylla* WANG et TANG (50 g) was extracted with hot MeOH. The extracts were combined and evaporated to dryness *in vacuo*. The residue (12.5 g) was treated by the method described above. The butanol-soluble fraction was concentrated *in vacuo* to afford a brown powder (2.4 g), which was examined by TLC (solvent c). Rf: 0.57 (1), 0.49 (2), 0.47 (3), 0.42 (4), 0.36 (5), 0.33 (6), 0.19 (7), 0.12 (8).

References and Notes

1) This work was presented at the 102nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 1982.

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