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Studies on the Constituents of *Aspidistra elatior* BLUME. II.[1] On the Steroidal Glycosides of the Leaves. (1)

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Six steroidal glycosides were isolated from the fresh leaves of *Aspidistra elatior* BLUME (Liliaceae) and the structures of these glycosides, tentatively named glycosides A (**1**), B (**2**), C (**3**), D (**4**), E (**5**) and F (**6**), were established to be neopentologenin 5-*O*-*β*-D-glucopyranoside (**1**), 26-*O*-*β*-D-glucopyranosyl 22-methoxy-5*β*-furostane-1*β*,3*β*,4*β*,5*β*,26-pentaol 5-*O*-*β*-D-glucopyranoside (**2**), aspidistrin (**3**), 26-*O*-*β*-D-glucopyranosyl 22-methoxy-5*β*-furostane-1*β*,2*β*,3*β*,4*β*,5*β*,26-hexaol 5-*O*-*β*-D-glucopyranoside (**4**), methyl proto-aspidistrin (**5**) and magnesium 26-*O*-*β*-D-glucopyranosyl 22-methoxy-5*β*-furostane-1*β*,3*β*,4*β*,5*β*,26-pentahydroxy-2*β*-yl-sulfate monohydroxide (**6**), respectively. The last compound is the first sulfated steroidal glycoside to be isolated from a Liliaceous plant.

Keywords——steroidal glycoside; *Aspidistra elatior*; Liliaceae; leaf; spirostanol glycoside; furostanol glycoside; sulfated steroidal glycoside; diosgenin; neopentologenin; aspidistrin

In the previous paper[1] we reported the isolation of five steroidal compounds from the underground part of *Aspidistra elatior* BLUME (Liliaceae). The structures of four of them have been elucidated to be aspidistrin (=diosgenin 3-*O*-*β*-D-lycotetraoside, **3**), proto-aspidistrin, methyl proto-aspidistrin (**5**), and 1*β*,2*β*, 3*β*,4*β*,5*β*-pentahydroxyspirost-25(27)-ene (=$\Delta^{25(27)}$-pentologenin or $\Delta^{25(27)}$-neopentologenin). The present paper describes the isolation and structure elucidation of six steroidal glycosides, tentatively named glycosides A, B, C, D, E and F, of the leaves of the same plant, leading to the assignment of the structures **1**, **2**, **3**, **4**, **5** and **6**, respectively.

The six steroidal glycosides were obtained from the methanolic extract of the fresh leaves of *A. elatior* BLUME harvested at Yamashina, Kyoto, in August 1981, as shown in Chart 1.

Glycoside A (**1**), $C_{33}H_{54}O_{12}$, showed a strong absorption band of hydroxyl groups and characteristic absorption bands of the 25(*S*)-spiroketal moiety in the infrared (IR) spectrum.[2] On hydrolysis with 1 N sulfuric in 50% ethanol, **1** gave glucose and an aglycone (**1a**), $C_{27}H_{44}O_7$, which showed hydroxyl and 25(*S*)-spiroketal absorption bands in the IR spectrum.

Acetylation of **1a** with acetic anhydride and pyridine gave a tetraacetate (**1b**), $C_{35}H_{52}O_{11}$, which showed a hydroxyl absorption band in the IR spectrum and four acetoxyl signals in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum. Based on the chemical and physicochemical properties described in the experimental section, **1a** and **1b** were inferred to be neopentologenin and its acetate, and this was confirmed by direct comparisons with authentic samples obtained from *Rhodea japonica* (THUNB,) ROTH.,[3] which is taxonomically in an intimate relationship with the present plant.

The configuration of glucose in **1** was assigned as *β* from the coupling constant of the signal at *δ* 5.21 ppm (J=7 Hz) in the ^{1}H-NMR spectrum. Based on the ^{1}H-NMR chemical shift of the anomeric proton and carbon-13 nuclear magnetic resonance (^{13}C-NMR) chemical shift (*δ* 97.4 ppm) of the anomeric carbon of glucose, the sugar moiety of **1** was suggested to be

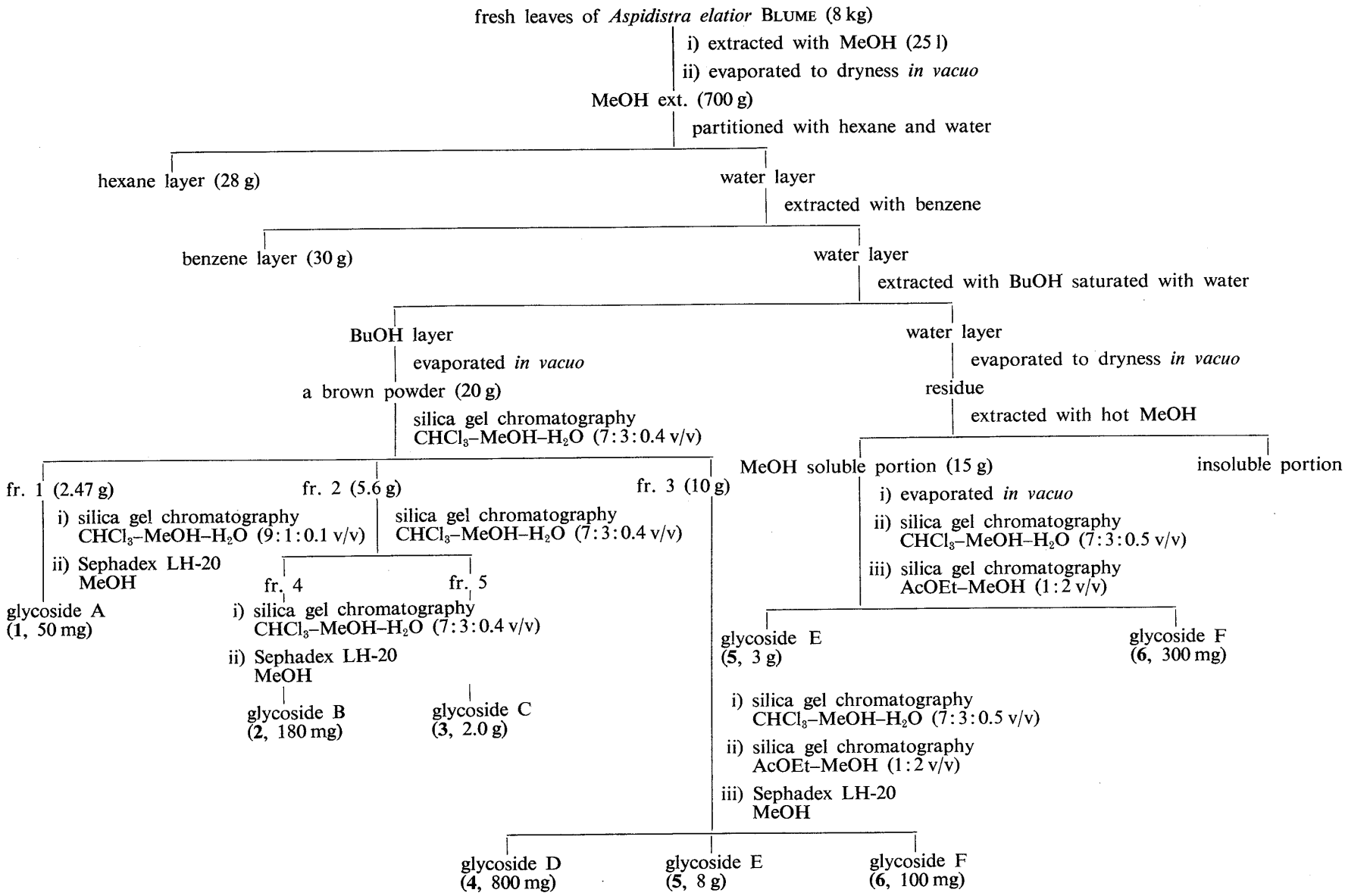
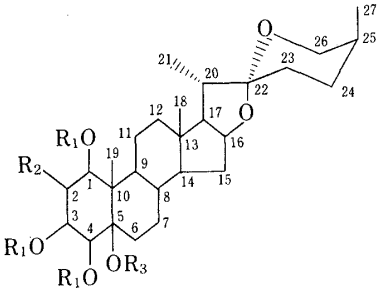
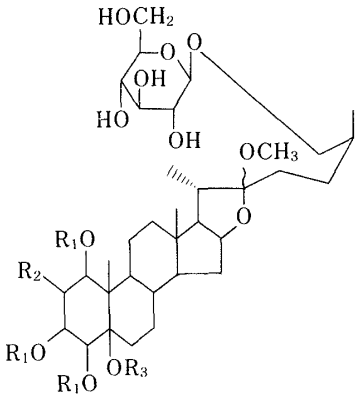


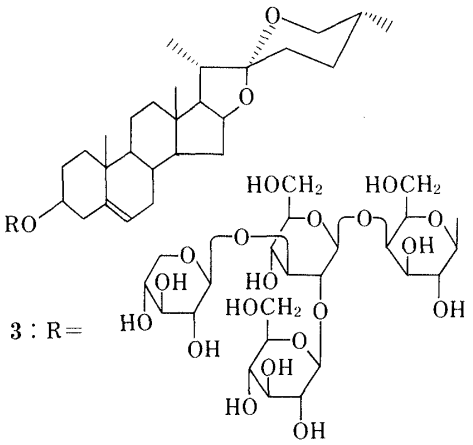
Chart 1

linked with a tertiary hydroxyl group of the aglycone.[4] Furthermore, in the ^{13}C-NMR spectra of **1** and **1a**, the signal of the C_5 carbon of the former was shifted about 10 ppm to lower field than that of the latter, and the C_4 and C_6 carbon signals of **1** were shifted by 0.7 and 5.1 ppm to higher field than those of **1a**, respectively. These shifts can be reasonably explained in terms of glycosidation shift and the location of glucose may be deduced to be at the C_5 hydroxyl group of neopentologenin. Finally, by comparing the ^{13}C-NMR spectra, glycoside A (**1**) was shown to be identical with neopentologenin 5-*O*-*β*-D-glucopyranoside, which had been isolated from *Rhodea japonica* (THUNB.) ROTH. and named compound R_6[5] by Konishi *et al.*





1 : R_1=H, R_2=OH, R_3=*β*-glucose
1a : R_1=R_3=H, R_2=OH
1b : R_1=Ac, R_2=OAc, R_3=H
2a : R_1=R_2=R_3=H
2b : R_1=R_2=H, R_3=*β*-glucose
2c : R_1=Ac, R_2=R_3=H
6a : R_1=R_3=H, R_2=OSO_3MgOH
6b : R_1=Ac, R_2=OSO_3MgOH, R_3=H
6c : R_1=Ac, R_2=OH, R_3=H
6d : R_1=Ac, R_2=OSO_2Me, R_3=H
2 : R_1=R_2=H, R_3=*β*-glucose
4 : R_1=H, R_2=OH, R_3=*β*-glucose
6 : R_1=R_3=H, R_2=OSO_3MgOH



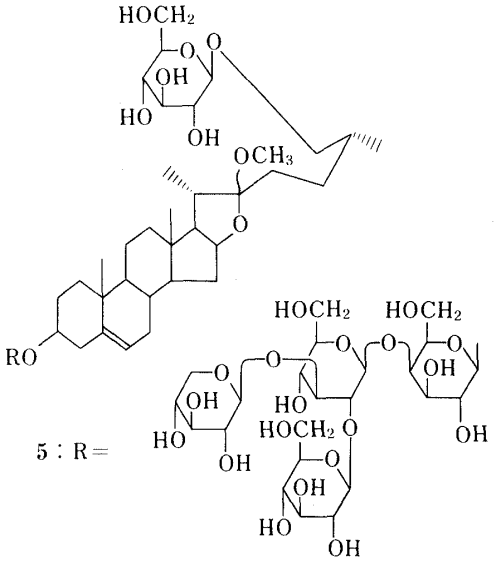


Chart 2

Glycoside D (**4**), $C_{40}H_{68}O_{18}$, is positive to the Ehrlich reagent[6] and its ^{13}C-NMR spectrum showed characteristic carbon signals of furostanol.[1] It showed strong hydroxyl absorption bands in the IR spectrum and the ^{1}H-NMR spectrum showed a methoxyl signal at *δ* 3.21 ppm. On enzymatic hydrolysis with *β*-glucosidase, **4** afforded glycoside A (**1**) and D-glucose. Based on the results described above, **4** was assumed to be a furostanol glycoside of glycoside A (**1**). The ^{13}C-NMR spectrum of **4** showed two signals corresponding to anomeric

TABLE I. ^{13}C-NMR Chemical Shifts of Glycosides A, B, D, F and Related Steroids[a]

Compounds		1a[b]	1b[b]	1	2[7]	4	6	6a	6b	6c	6d[c]
Aglycone No.	1	78.1	74.4	78.6[d]	73.4[d]	78.3[d]	75.0	75.2	76.5	77.6	74.7
	2	67.4	66.3	68.0[e]	34.0	68.1[e]	73.2[d]	73.2[d]	69.3	64.1	70.1[d]
	3	75.5	70.5	76.0	71.5	76.0	73.9[d]	73.6[d]	71.9	73.1	71.3[d]
	4	68.2	68.1	67.5[e]	68.0	67.6[e]	67.2	67.6	68.2	68.7	67.3
	5	77.9	75.9	87.3	87.8	87.3	78.2	78.9	75.9	75.8	75.3
	6	30.3	27.5	24.9	24.9	24.9	30.3	30.0	27.8	27.9	27.3
	7	28.4	29.3	28.4	28.3	28.1	27.9	27.9	29.2	29.2	28.5
	8	34.9	34.6	34.6	34.8	34.6	34.2	34.7	34.6	34.7	34.5
	9	45.4	45.4	46.3	46.8	46.2	45.2	45.5	45.7	45.9	45.6
	10	45.0	45.9	46.6	47.1	46.5	45.2	45.2	46.1	45.9	46.0
	11	21.6	21.8	21.6	21.6	21.7	21.2	21.0	21.6	22.1	21.7
	12	40.0	39.5	39.9	40.1	39.9	40.0	40.0	39.4	39.5	39.5
	13	40.6	40.4	40.5	40.8	40.8	40.3	40.4	40.3	40.5	40.3
	14	56.2	55.5	56.0	56.0	55.8	55.4	55.5	55.3	55.8	55.5
	15	32.2	32.0	32.1	32.4	32.2	32.2	31.9	31.9	32.0	31.7
	16	81.1	80.9	81.1	81.0	81.3	81.2	81.2	81.0	81.0	80.6
	17	62.8	62.5	62.8	64.2	64.2	63.9	62.7	62.3	62.6	61.9
	18	16.5	16.2	16.3	16.3	16.3	16.3	16.3	16.3	16.3	16.0
	19	13.7	12.5	13.6	13.6	13.6	13.5	13.6	12.5	12.7	12.2
	20	42.5	42.4	42.5	40.4	40.6	40.7	42.6	42.4	42.5	42.2
	21	14.8	14.7	14.8	16.5	16.5	16.1	14.8	14.7	14.8	14.3
	22	109.7	109.7	109.7	112.3	112.6	112.5	109.7	109.7	109.7	109.8
	23	26.4[d]	26.3[d]	26.3[f]	31.0	30.7	30.8	26.5[e]	26.3[d]	26.4[d]	26.0[e]
	24	26.2[d]	26.2[d]	26.2[f]	28.3	28.2	28.8	26.3[e]	26.2[d]	26.2[d]	25.8[e]
	25	27.5	27.9	27.5	34.4	34.3	34.2	27.6	27.5	27.5	27.1
	26	65.1	65.1	65.2	75.0	74.9	75.0	65.3	65.1	65.0	65.2
	27	16.3	16.3	16.5	17.4	17.3	17.3	16.3	16.3	16.5	16.3
–COCH$_3$			169.8						170.7	170.6	169.2
			170.1						170.7	170.6	169.2
			170.3						170.9	170.7	170.4
			170.5								
–COCH$_3$			20.5						20.7	20.7	20.7
			20.6						21.0	21.1	20.8
			20.7						21.0	21.1	20.8
			20.7								
–OCH$_3$					47.3	47.4	47.3			–SO$_2$CH$_3$	38.9
C-5 Sugar											
Glucose	1			97.4	97.1	97.2					
	2			75.6	75.5[d]	75.5					
	3			77.7	77.7	77.8					
	4			71.6	71.6	71.6					
	5			78.4[d]	78.2	78.2					
	6			62.6	62.5	62.6					
C-26 Sugar											
Glucose	1				104.8	104.8	104.8				
	2				75.0	74.9	75.0				
	3				78.2[e]	78.2[d]	78.1[e]				
	4				71.5	71.6	71.5				
	5				78.3[e]	78.3[d]	78.2[e]				
	6				62.7	62.7	62.7				

a) Chemical shifts were measured in pyridine-d_5 at room temperature.
b) Reference: K. Kudo, K. Miyahara and T. Kawasaki, the 101st Annual Meeting of the Pharmaceutical Society of Japan, April 1981, p. 493.
c) Chemical shifts were measured in $CDCl_3$ at room temperature.
d, e, f) Assignments may be reversed.

carbons of glucoses at δ 97.2 and 104.8 ppm, and the signals of the steroidal moieties of **1** and **4** were superimposable except for the C_{20}–C_{27} carbon signals. Consequently, the structure of glycoside D was concluded to be 26-*O*-β-D-glucopyranosyl 22-methoxy-5β-furostane-1β,2β,3β,4β,5β,26-hexaol 5-*O*-β-D-glucopyranoside (**4**).

Glycoside B (**2**), $C_{40}H_{68}O_{17}$, was suggested to be a furostanol glycoside based on the color reaction with the Ehrlich reagent. It showed a strong hydroxyl absorption band in the IR spectrum, and the ^{1}H-NMR spectrum showed a methoxyl signal at δ 3.27 ppm. On acidic hydrolysis, **2** gave glucose and an aglycone (**2a**), $C_{27}H_{44}O_6$, while on enzymatic hydrolysis with β-glucosidase, **2** gave a prosapogenin (**2b**), $C_{33}H_{54}O_{11}$, and glucose. The IR spectrum of **2b** showed strong hydroxyl and characteristic 25(*S*)-spiroketal absorption bands, and, on acidic hydrolysis, **2b** gave glucose and an aglycone (**2a**).

The structure of **2** was studied by comparing its ^{13}C-NMR spectrum with those of neopentologenin (**1a**), and glycosides A (**1**) and D (**4**), and by referring to the ^{13}C-NMR spectral data on 5β-type steroidal sapogenins reported by Tori *et al.*[7] The ^{13}C-NMR spectrum of **4** showed signals of four secondary carbons bearing a hydroxyl group in the steroidal A-ring at δ 67.6, 68.1, 76.0, 78.3 ppm and one tertiary carbon (singlet) assigned to C_5 at δ 87.3 ppm, while **2** shows three secondary carbon signals at δ 68.0, 71.5 and 73.4 ppm, one methylene carbon signal at δ 34.0 ppm and one tertiary carbon signal corresponding to C_5 at 87.8 ppm. The chemical shift of the C_{19} methyl carbon of **2** (δ 13.6 ppm) may be reasonably explained by the presence of 1β-hydroxyl and 5β-hydroxyl or 5β-*O*-glucosyl groups as in the case of **1a** (13.7 ppm), **1** (13.6 ppm) and **4** (13.6 ppm). Accordingly, it is suggested that three hydroxyl groups of **2** are located on C_1, C_3 and C_4 in β-configuration, and the β-glucosyl group is linked with the C_5–hydroxyl group. The assignment of C_1–C_5 and C_{10} signals of **2** shown in Table I is consistent with this formulation in comparison with the assignments of compounds **1** and **4**, taking into consideration the effect of the 2β-hydroxyl group. Furthermore, the reason why the carbon signals of C_1 and C_3 of **2** are shifted by *ca.* 2 ppm to lower field than those of kitigenin[7] may be the effect of the glucose linked to the C_5 β–hydroxyl group. Consequently, **2a** was assumed to be identical with convallagenin B (C-25 epimer of kitigenin), reported by Kimura *et al.*[8] After acetylation of the sample with acetic anhydride–pyridine, the acetate (**2c**) was shown to be identical with convallagenin B triacetate by direct comparisons with an authentic sample derived from $\Delta^{25(27)}$-convallagenin B. Based on the above results, the structure of glycoside B was deduced to be 26-*O*-β-D-glucopyranosyl 22-methoxy-5β-furostane-1β,3β,4β,5β,26-pentaol 5-*O*-β-D-glucopyranoside (**2**).

Glycoside C, $C_{50}H_{80}O_{22}$, and glycoside E, $C_{57}H_{94}O_{27}$, were identified as aspidistrin (**3**) and methyl proto-aspidistrin (**5**) by the same method as described in the previous paper.[1]

Glycoside F (**6**), $C_{34}H_{58}MgO_{17}S$, is positive to the Ehrlich reagent and it showed strong hydroxyl absorption bands in the IR spectrum. Accordingly, **6** was suggested to be a furostanol glycoside. On enzymatic hydrolysis with β-glucosidase, **6** afforded D-glucose and a sapogenin, $C_{27}H_{44}MgO_{11}S$ (**6a**), which showed strong hydroxyl and characteristic 25(*S*)-spiroketal absorption bands in the IR spectrum. On acidic hydrolysis with 1 N hydrogen chloride in 50% ethanol, **6** gave D-glucose and neopentologenin (**1a**), while **6a** afforded **1a** alone. Furthermore, **1a** was formed from **6a** by heating on a boiling water bath with acetic acid for 30 min. The structure of **6a** was elucidated as follows. The IR spectra of **6** as well as **6a** showed strong absorption bands at 1200 and 1250 cm^{-1} due to S–O stretching vibration of sulfate. The presence of the sulfate group in **6a** was confirmed chemically. After treatments of **6** and **6a** with 1 N hydrogen chloride or acetic acid as described above, barium hydroxide was added to the mother liquor to form a white precipitate, which did not dissolve on being heated with hydrogen chloride or sodium hydroxide. Furthermore, the hydrolysates of **6** and **6a** were both positive to the potassium rhodizonate reagent.[9] Based on the above results, the sulfate group was suggested to be linked with the aglycone moiety. The location of the sulfate group

in **6** was decided as follows. The ^{1}H-NMR spectra of a triacetate (**6b**), $C_{33}H_{50}MgO_{14}S$, obtained by acetylation of **6a**, a desulfated compound (**6c**), $C_{33}H_{50}O_{10}$, obtained by heating **6b** with acetic acid, and neopentologenin tetraacetate (**1b**) obtained by acetylation of **6c** were comparatively analyzed. One proton signal of **6b** at δ 4.9 ppm (br) was shifted to δ 4.1 ppm (t, J=4 Hz) by solvolysis (**6c**), while this proton was shifted to δ 5.16 ppm (t, J=4 Hz) by acetylation (**1b**). Accordingly, the proton described above was considered to be a methine proton on the carbon carrying the sulfate group, and the coupling pattern of this proton signal indicated that the location of the sulfate group may be the C_2 or C_3 hydroxyl group. Taking into account the general concept that the axial proton signal appears at 0.1—0.7 ppm higher field than the equatorial proton signal, the sulfate group was deduced to be linked with the C_2–hydroxyl group. To confirm this, each methine proton signal of the steroidal A-ring of **6c** was assigned by the ^{1}H-NMR decoupling technique.

Furthermore, to elucidate the location of the sulfate group, the ^{13}C-NMR spectra were analyzed. As regards the substitution effect of a sulfate group in the ^{13}C-NMR spectrum, Vigon *et al.*[10] reported on the mesyl derivative of sugars and Kitagawa *et al.*[11] reported on holothurin B. Recently, Watanabe *et al.*[12] has reported that the α-carbon signal of sulfate of the steroidal A-ring shifts by *ca.* 5—6 ppm to lower field, while the β-carbon signal shifts to higher field. In the ^{13}C-NMR spectrum of **6b**, the C_2 carbon signal appeared at lower field by *ca.* 5.2 ppm than that of **6c**, while the C_1 and C_3 carbon signals appeared at higher field by 1.1 and 1.2 ppm than those of **6c**. The ^{13}C-NMR chemical shift of **6d** derived from **6c** by treatment with methanesulfonyl chloride was compatible with that of **6b** and the structure of **6b** was deduced to be 1,3,4-tri-*O*-acetylneopentologenin 2-sulfate.

At first, based on the *Rf* value on thin layer chromatography (TLC), **6** was assumed to be a steroidal glycoside containing several monosaccharides. Besides the presence of the C_2 sulfate group, some other factor, for instance, the presence of metal, may be necessary to explain the strong polarity and the broad IR and NMR spectra of **6**. After treatment with ion exchange resin the *Rf* value of **6** on TLC (plate, silica gel; solvent, $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5 v/v)) was increased from 0.17 to 0.35. The retention of the sulfate group in the eluate was proved by the potassium rhodizonate test, and the eluate was neutralized with magnesium hydroxide or sodium hydroxide. In the former case glycoside F (**6**) was obtained and in the latter, the product was the sodium salt of **6**. On the other hand, the adsorbed metal ion was eluted with 1 N hydrogen chloride and the chloride salt gave a positive reaction with quinalizarin reagent.[13] Taking account of the elemental analysis and the result of quantitative analysis by atomic absorption spectroscopy, the strong polarity of **6** can be explained by the presence of the $-OSO_3Mg(OH)$ gorup. Based on the above results, the structure of glycoside F was suggested to be magnesium 26-*O*-β-D-glucopyranosyl 22-methoxy-5β-furostane-1β,3β,4β,5β,26-pentahydroxy-2β-yl-sulfate monohydroxide, as shown by formula **6**. The natural metal of this glycoside has not been identified.

Two steroidal constituents, namely aspidistrin (**3**) and methyl proto-aspidistrin (**5**), of the leaves of *Aspidistra elatior* BLUME described above are common to the subterranean parts, but the glycosides of neopentologenin, proto-neopentologenin and convallagenin B are unique steroidal constituents of the leaves. This is the first report of the isolation of a sulfated steroidal glycoside from Liliaceous plants.

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 an Union automatic polarimeter, model P-101 (cell length 1 cm). The IR spectra were recorded with a Shimadzu IR-27G spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were measured with a Varian CFT-20 NMR spectrometer at 20 MHz, and chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. Atomic absorption spectra were recorded on a Shimadzu AA-610S atomic

absorption spectrophotometer. Paper partition chromatography (PPC) of monosaccharides was performed on Tōyō Roshi No. 50 using the upper phase of BuOH–pyridine–H_2O (6:2:3 v/v)–pyridine (1 v) and detection was achieved by spraying aniline hydrogen phthalate solution followed by heating. TLC was performed on pre-coated Kieselgel 60 F_{254} plates (Merck) and detection was achieved by spraying the Ehrlich reagent or 10% H_2SO_4 followed by heating. Column chromatography was carried out on Kieselgel (0.02—0.2 mm, Merck) and Sephadex LH-20. Unless otherwise noted, solvents used for TLC and silica gel column chromatography were as follows: solv. a, $CHCl_3$–MeOH–H_2O (7:3:0.5 v/v); solv. b, $CHCl_3$–MeOH–H_2O (7:3:0.4 v/v); solv. c, AcOEt–MeOH (1:2 v/v); solv. d, benzene–acetone (3:1 v/v). MeOH was used as a solvent for Sephadex LH-20 column chromatography. Gas liquid chromatography (GLC) was run on a Shimadzu GC-6A unit equipped with a flame ionization detector.

Extraction and Isolation of Steroidal Glycosides——As shown in Chart 1, the fresh leaves of *Aspidistra elatior* BLUME (Liliaceae) (8 kg) harvested at Yamashina, Kyoto, in August 1981, were extracted with MeOH (25 l) and the extract was concentrated *in vacuo*. The residue (700 g) was dissolved in water and extracted with hexane followed with benzene. The aqueous layer was extracted with BuOH saturated with water, and the BuOH-soluble fraction was concentrated under reduced pressure to afford a brown powder (20 g), which was subjected to column chromatography over silica gel (300 g) with solv. b to provide three fractions (fr. 1—fr. 3). Fraction 1 (2.47 g) was separated by column chromatography on silica gel using $CHCl_3$–MeOH–H_2O (9:1:0.1 v/v) to afford glycoside A (**1**, 50 mg). Fraction 2 (5.6 g) was subjected to column chromatography on silica gel with solv. b to provide two fractions (fr. 4 and fr. 5). Fraction 4 and fr. 5 were individually purified by silica gel column chromatography with solv. b followed by Sephadex LH-20 column chromatography using MeOH to afford glycoside B (**2**, 180 mg) from fr. 4 and glycoside C (**3**, 2.0 g) from fr. 5. Fraction 3 (10 g) was subjected to column chromatography on silica gel with solv. a and rechromatography on a silica gel column with solv. c, followed by Sephadex LH-20 column chromatography to afford glycosides D (**4**, 800 mg), E (**5**, 8 g) and F (**6**, 100 mg).

On the other hand, the water-soluble fraction was evaporated to dryness under reduced pressure and the residue was extracted with MeOH. The MeOH-soluble fraction (15 g) was subjected to column chromatography on silica gel with solv. a followed by silica gel chromatography with solv. c to afford glycosides E (**5**, 3 g) and F (**6**, 300 mg).

Properties of Glycosides A, B, C, D, E and F——Glycoside A (**1**): Colorless needles from EtOH, mp 226—230 °C (dec.), $[\alpha]_D^{25}$ −73.4° ($c=0.98$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH), 980, 910, 890, 845 (intensity 910 > 890, 25(*S*)-spiroketal). ^{1}H-NMR (pyridine-d_5) δ: 0.82 (3H, s, CH_3), 1.07 (3H, d, $J=6$ Hz, CH_3), 1.13 (3H, d, $J=6$ Hz, CH_3), 1.68 (3H, s, CH_3), 5.21 (1H, d, $J=7$ Hz, anomer. H). *Anal.* Calcd for $C_{33}H_{54}O_{12}\cdot 3/2H_2O$: C, 59.17; H, 8.58. Found: C, 58.89; H, 8.57.

Glycoside B (**2**): A white powder from AcOEt–MeOH, (mp 187—191 °C (dec.)), $[\alpha]_D^{16}$ −43.2° ($c=1.11$, pyridine). IR ν_{max}^{Nujol} cm^{-1}: 3200—3500 (OH). ^{1}H-NMR (pyridine-d_5) δ: 0.84 (3H, s, CH_3), 1.05 (3H, d, $J=7$ Hz, CH_3), 1.15 (3H, d, $J=7$ Hz, CH_3), 1.66 (3H, s, CH_3), 3.27 (3H, s, OCH_3), 4.80 (1H, d, $J=7$ Hz, anomer. H), 5.23 (1H, d, $J=7$ Hz, anomer. H) *Anal.* Calcd for $C_{40}H_{68}O_{17}$: C, 58.55; H, 8.35. Found: C, 58.80; H, 8.40.

Glycoside C (**3**): Colorless needles from BuOH saturated with H_2O, mp 262—266 °C (dec.), $[\alpha]_D^{11}$ −84.2° ($c=0.76$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH), 980, 920, 895, 845 (intensity 920 < 895, 25(*R*)-spiroketal). This compound was identified as aspidistrin by TLC, IR and ^{1}H-NMR comparisons with an authentic sample.[1)]

Glycoside D (**4**): Colorless needles form MeOH, mp 195—198 °C (dec.), $[\alpha]_D^{32}$ −60.4° ($c=1.01$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH). ^{1}H-NMR (pyridine-d_5) δ: 0.81 (3H, s, CH_3), 1.04 (3H, d, $J=6$ Hz, CH_3), 1.14 (3H, d, $J=6$ Hz, CH_3), 1.70 (3H, s, CH_3), 3.21 (3H, s, OCH_3), 4.80 (1H, d, $J=7$ Hz, anomer. H), 5.23 (1H, d, $J=7$ Hz, anomer. H). *Anal.* Calcd for $C_{40}H_{68}O_{18}\cdot 2H_2O$: C, 55.03; H, 8.31. Found: C, 54.79; H, 8.37.

Glycoside E (**5**): A white powder from MeOH–AcOEt, (mp 202—207 °C (dec.)), $[\alpha]_D^{20}$ −68.4° ($c=1.33$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH). This compound was identified as proto-aspidistrin (22-OCH_3 type) by TLC, IR and ^{1}H-NMR comparisons with an authentic sample.[1)]

Glycoside F (**6**): A hygroscopic white powder from MeOH, (mp 230—236 °C (dec.)), $[\alpha]_D^{25}$ −34.8° ($c=1.15$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH), 1250, 1220 (sulfate). The magnesium ion bound to sulfate was determined by atomic absorption spectroscopy (wavelength: Mg 285.2 nm). *Anal.* Calcd for $C_{34}H_{58}MgO_{17}S\cdot 3H_2O$: C, 48.08; H, 7.60; Mg, 2.86. Found: C, 48.15; H, 7.60; Mg, 2.40 (atomic absorption spectroscopy).

Enzymatic Hydrolyses of 2, 4, and 6——Compounds **2** (50 mg), **4** (290 mg) and **6** (150 mg) were each incubated with almond emulsin at 37 °C for 24 h, and the product precipitated from the reaction mixture was collected by filtration and dried. The hydrolysis product of **2** was purified by recrystallization from MeOH to afford colorless needles (**2b**, 30 mg), mp 260—265 °C (dec.), $[\alpha]_D^{32}$ −44.1° ($c=0.93$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH), 985, 915, 895, 850 (intensity 915 > 895, 25(*S*)-spiroketal). *Anal.* Cacld for $C_{33}H_{54}O_{11}\cdot 3H_2O$: C, 58.21; H, 8.88. Found: C, 58.48; H, 8.76. Hydrolysis products of **4** and **6** were purified by column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (8:2:0.2 v/v). The former afforded colorless needles (**1**, 90 mg) from EtOH, mp 227—229 °C (dec.); this product was identified as glycoside A by mixed fusion, and TLC and IR comparisons. The latter gave hygroscopic colorless needles (**6a**, 80 mg) from MeOH, mp 274—276 °C (dec.), $[\alpha]_D^{23.5}$ −42.7° ($c=0.82$, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH), 1250, 1215 (sulfate), 980, 915, 880, 845 (intensity 915 > 880, 25(*S*)-spiroketal). *Anal.* Calcd for $C_{27}H_{44}MgO_{11}S\cdot 1/2H_2O$: C, 53.16; H, 7.44; Mg, 3.98. Found: C, 53.29; H, 7.81; Mg, 3.48.

The filtrate of the reaction mixture of **4** was concentrated *in vacuo* and the residue was chromatographed on

Sephadex LH-20 with MeOH to provide the starting material (**4**, 180 mg) and D-glucose (54.8 mg), $[\alpha]_D^{20}$ +52.8° (c=5.48, H_2O) (lit.[14] $[\alpha]_D$ +52.7°). The filtrate of the reaction mixture of **6** also afforded D-glucose (20.5 mg). Glucose in the reaction mixture was detected by TLC (solv. a, *Rf* 0.14) and PPC (Tōyō Roshi No. 50; solvent, upper phase of BuOH–pyridine–H_2O (6:2:3 v/v)+pyridine (1 v)), detection; aniline hydrogen phthalate. *Rf* 0.33).

Acid Hydrolyses of 1, 2, 2b, 6 and 6a——A solution of **1** (20 mg) in 1 N H_2SO_4–50% EtOH was refluxed for 6 h, and the reaction mixture was diluted with water. The precipitate was collected by filtration and purified by recrystallization from MeOH to afford colorless needles, **1a** (6.4 mg), mp 288—293 °C (dec.), $[\alpha]_D^{19}$ −62.5° (c=0.72, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH), 980, 910, 890, 843 (intensity 910>890, 25(*S*)-spiroketal). *Anal.* Calcd for $C_{27}H_{44}O_7$: C, 67.47; H, 9.23. Found: C, 67.16; H, 9.45. This compound was identified as neopentologenin by comparing the melting point and IR spectra.

The filtrate was neutralized with Amberlite IRA-410 and evaporated to dryness *in vacuo*. The residue was examined by TLC and PPC performed under the same conditions as described above. Glucose was detected.

Compounds **2** (10 mg) and **2b** (5 mg) were each refluxed with 2 N HCl–50% dioxane for 5 h. Each reaction mixture was diluted with water. The precipitate was collected by filtration and the residue was recrystallized from $CHCl_3$–MeOH to afford colorless needles, **2a** (5 mg from **2** and 2 mg from **2b**), mp 299—300.5 °C. IR ν_{max}^{KBr} cm^{-1}: 3200—3500 (OH), 986, 918, 900, 850 (intensity 918>900, 25(*S*)-spiroketal). Each filtrate was neutralized with Amberlite IRA-410 and evaporated to dryness under reduced pressure. The residue was examined by TLC and PPC, and only glucose was detected as a sugar component.

Compounds **6** (5 mg) and **6a** (2 mg) were each hydrolyzed with 1 N HCl–50% EtOH on a boiling water bath for 2 h. Each reaction mixture was cooled and the precipitate was collected by filtration. The products were recrystallized from MeOH to afford neopentologenin (**1a**) (1.5 mg from **6** and 0.4 mg from **6a**) as colorless needles; this product was identified by mixed fusion and TLC ($CHCl_3$–MeOH–H_2O (8:2:0.2 v/v)) comparison with an authentic sample. Each filtrate was neutralized with Amberlite IRA-410 and evaporated to dryness under reduced pressure. The residue was examined by PPC and TLC (solv. a). Glucose was found in the reaction mixture of **6**, but not in the reaction mixture of **6a**.

Furthermore, each filtrate was neutralized with aqueous NaOH and evaporated to dryness under reduced pressure. The residue was subjected to PPC (Tōyō Roshi, No. 50) developed with MeOH–H_2O (1:1 v/v) mixture. After drying in the air, the paper was sprayed with a solution of $BaCl_2$ (100 mg) in 70% MeOH (50 ml) and dried again in the air. The dried paper was colored by spraying a solution of potassium rhodizonate (10 mg) in 50% MeOH (50 ml). Each sample showed a single spot at *Rf* 0.64, which was identified as sulfate ion by comparison with a test solution of Na_2SO_4.

A part of the residue described above was dissolved in a small amount of water. Addition of a solution of $Ba(OH)_2$ gave a white precipitate, which was insoluble in hot 5% HCl or 10% NaOH.

Acetylation of 1a, 2a and 6a——Compound **1a** (105 mg) was acetylated with acetic anhydride and pyridine (1:1 v/v) by heating on a water bath for 5 h, while compounds **2a** (1 mg) and **6a** (105 mg) were acetylated with the same reagents at room temperature for 24 h.

The reaction mixture of **1a** was poured into ice-water and the precipitate was collected by filtration, dried and purified by column chromatography on silica gel using solv. d to afford a tetraacetate (**1b**, 100 mg), as cololress needles from MeOH, mp 161—163 °C, $[\alpha]_D^{32}$ −35.0° (c=1.00, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3580 (OH), 1750 (COOR), 980, 920, 890, 845 (intensity 920>890, 25(*S*)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, s, CH_3), 0.99 (3H, d, J=7 Hz, CH_3), 1.15 (3H, s, CH_3), 1.16 (3H, d, J=7 Hz, CH_3), 1.96, 2.10, 2.13, 2.14 (3H each, s, $COCH_3$), 3.27 (1H, d, J=11 Hz, C_{26}–H), 3.41 (1H, s, C_5–OH), 3.92 (1H, dd, J=2 Hz, 11 Hz, C_{26}–H), 4.34 (1H, br, C_{16}–H), 5.16 (1H, t, J=4 Hz, C_2–H), 5.47 (1H, d, J=4 Hz, C_4 or C_1–H), 5.52 (1H, d, J=4 Hz, C_1 or C_4–H), 5.62 (1H, t, J=4 Hz, C_3–H). *Anal.* Calcd for $C_{35}H_{52}O_{11}\cdot 1/2H_2O$: C, 63.90; H, 8.12. Found: C, 63.60; H, 8.29.

The reaction mixture of **2a** and **6a** were each poured into ice-water and the solution was extracted with BuOH saturated with water. The BuOH solution was evaporated to dryness under reduced pressure, and the product (crude **2c**) was identified as convallagenin B triacetate by TLC (2% $AgNO_3$-impregnated precoated high-performance thin-layer chromatography (HPTLC) plates; solvent CH_2Cl_2–acetone=9:1 v/v; *Rf* 0.49 convallagenin B triacetate; 0.59 25(*R*)-convallagenin B triacetate) and by GLC (column, OV-17 on Shimalite W 4 mm × 1.5 m; column temp., 285 °C; injection temp., 300 °C; carrier gas, N_2 1.0 kg/cm^2; t_R (min), 27.0 25(*R*)-convallagenin B triacetate, 28.0 convallagenin B triacetate).

The product derived from **6a** was subjected to column chromatography on silica gel using $CHCl_3$–MeOH–H_2O (9:1:0.1 v/v) to afford a tr acetate (**6b**, 130 mg) as a hygroscopic white powder from aqueous MeOH, (mp 210—212 °C (dec.)), $[\alpha]_D^{25}$ −32.6° (c=1.77, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3590, 3400—3500 (OH), 1735 (COOR), 1220—1270 (COOR, sulfate), 980, 920, 890, 846 (intensity 920>890, 25(*S*)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 0.75 (3H, s, CH_3), 0.96 (3H, d, J=7 Hz, CH_3), 1.14 (3H, s, CH_3), 1.21 (3H, d, J=7 Hz, CH_3), 2.08 (3H, s, $COCH_3$), 2.15 (6H, s, $COCH_3\times 2$), 3.26 (1H, d, J=11 Hz, C_{26}–H), 3.46 (1H, s, C_5–OH), 3.90 (1H, dd, J=2, 11 Hz, C_{26}–H), 4.35 (1H, br, C_{16}–H), 4.90 (1H, br, C_2–H), 5.60 (2H, br, C_4, C_1–H), 5.75 (1H, br, C_3–H). *Anal.* Calcd for $C_{33}H_{50}MgO_{14}S\cdot 2H_2O$: C, 51.93; H, 7.13; Mg, 3.18. Found: C, 51.59; H, 6.99; Mg, 2.78.

Solvolysis of 6, 6a and 6b with AcOH——Solutions of **6** (2 mg) and **6a** (2 mg) in acetic acid (5 ml) were each

refluxed for 30 min. The reaction mixtures were diluted with water and the precipitate was collected by filtration. The product derived from **6a** was identified as neopentologenin (**1a**) by TLC ($CHCl_3$–MeOH–H_2O (8 : 2 : 0.2 v/v)) and by mixed fusion. Both filtrates were examined by the method described above and sulfate ion was detected.

A solution of **6b** (40 mg) in acetic acid (12 ml) was heated on a boiling water bath for 30 min. After cooling, the reaction mixture was diluted with water and extracted with ether. The ether layer was washed with water, dried over Na_2SO_4 and evaporated on a water bath. The residue was subjected to column chromatography over silica gel with benzene–acetone (5 : 1 v/v) to afford a desulfated compound, **6c** (31.9 mg). **6c**: a white powder from aqueous MeOH (mp 171—174 °C), $[\alpha]_D^{25}$ −44.5° ($c=1.15$, MeOH). IR ν_{max}^{Nujol} cm^{-1}: 3550, 3300—3400 (OH), 1750 (COOR), 930, 920, 890, 845 (intensity 920 > 890, 25(*S*)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 0.77 (3H, s, CH_3), 0.98 (3H, d, $J=7$ Hz, CH_3), 1.14 (3H, s, CH_3), 1.15 (3H, d, $J=7$ Hz, CH_3), 2.09, 2.13, 2.15 (3H each, s, $COCH_3$), 3.26 (1H, d, $J=11$ Hz, C_{26}–H), 3.35 (1H, s, C_5–OH, disappears on addition of D_2O), 3.89 (1H, dd, $J=2$, 11 Hz, C_{26}–H), 4.10 (1H, t, $J=4$ Hz, C_2–H), 4.35 (1H, br, C_{16}–H), 5.38 (1H, d, $J=4$ Hz, C_4 or C_1–H), 5.47 (1H, d, $J=4$ Hz, C_1 or C_4–H), 5.57 (1H, t, $J=4$ Hz, C_3–H). On irradiation at δ 4.10 (1H, t) signal, a double signal at 5.47 ppm changed into singlet and a triplet signal at 5.57 ppm changed into a doublet ($J=4$ Hz), but the doublet signal at 5.38 ppm was not affected. *Anal.* Calcd for $C_{33}H_{50}O_{10}\cdot 1/2H_2O$: C, 64.36; H, 8.35. Found: C, 64.00; H, 8.33.

The aqueous layer was evaporated to dryness under reduced pressure. The residue was examined by PPC under the same conditions as described above, and showed a yellow spot at *Rf* 0.64 on the paper in the potassium rhodizonate test.

Acetylation of 6c——Compound **6c** (5 mg) was acetylated with acetic anhydride and pyridine (1 : 1 v/v) in a manner similar to that described above to afford a tetraacetate (**1b**, 3 mg), colorless needles from MeOH, mp 161—163 °C, $[\alpha]_D^{20}$ −52.6° ($c=0.38$, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 3590 (OH), 1755 (COOR), 985, 920, 890, 846 (intensity 920 > 890, 25(*S*)-spiroketal). The product was identified as **1b** by mixed melting point determination and TLC and IR spectral comparisons with an authentic sample.

Methanesulfonation of 6c with Methanesulfonyl Chloride——A solution of compound **6c** (80 mg) in pyridine (5 ml) was treated with methanesulfonyl chloride (5 drops) at 0 °C and the reaction mixture was kept overnight in a refrigerator. The reaction mixture was poured into ice-water and the precipitate was collected by filtration. The product was purified by column chromatography on silica gel with benzene–acetone (5 : 1 v/v) to afford the methanesulfonate of **6c** (**6d**, 50 mg) as a white powder from EtOH–H_2O, $[\alpha]_D^{10}$ −43.3° ($c=3.5$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350 (OH), 1750 (COOR), 1220 (CH_3SO_3-R), 980, 918, 890, 845 (intensity 918 > 890, 25(*S*)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 0.76 (3H, s, CH_3), 0.98 (3H, d, $J=7$ Hz, CH_3), 1.16 (3H, d, $J=7$ Hz, CH_3; 3H, s, CH_3), 2.10 2.15, 2.16 (each 3H, s, $COCH_3$), 3.02 (3H, s, CH_3SO_3-R), 3.25 (1H, d, $J=11$ Hz, C_{26}–H), 3.35 (1H, s, C_5–OH), 3.90 (1H, dd, $J=2$, 11 Hz, C_{26}–H), 4.35 (1H, br, C_{16}–H), 4.98 (1H, t, $J=4$ Hz, C_2–H), 5.49 (1H, d, $J=4$ Hz, C_4 or C_1–H), 5.70 (1H, d, $J=4$ Hz, C_1 or C_4–H), 5.77 (1H, t, $J=4$ Hz, C_3–H). *Anal.* Calcd for $C_{34}H_{52}O_{12}S$: C, 59.63; H, 7.65. Found: C, 59.54; H, 7.50.

Detection of Magnesium Ion in 6, 6a and 6b——An aqueous solution of **6** (50 mg) was passed through a column of Amberlite IR-120 (30 g) and eluted with water. The eluate was evaporated to dryness under reduced pressure and the residue was hydrolyzed. Sulfate ion was detected by means of the potassium rhodizonate test. The column of ion exchange resin was eluted with 1 N HCl and the eluate was evaporated under reduced pressure. The residue was examined by atomic absorption spectrophotometry (wavelength: Mg 285.2 nm) and by use of the quinalizarin reagent test (quinalizarin reagent solution A, 2 N NaOH solution; solution B, 10% HNO_3 solution; solution C, 0.02% quinalizarin solution in alcohol).

Compounds **6a** and **6b** were treated in the same manner as described above and both Mg^{2+} ion and sulfate ion were detected.

Preparation of Magnesium and Sodium Salts of 6——An aqueous solution (4 ml) of **6** (10 mg) was treated with ion exchange resin (IR-120B, 858 mg). The solution was filtered and the resin was washed with water repeatedly until the washings were neutral. Then 3 mg of $Mg(OH)_2$ was added to the filtrate with stirring and the excess reagent was filtered off (2.2 mg). The filtrate was evaporated to dryness under reduced pressure. An MeOH solution (0.5 ml) of the residue (9.5 mg) was treated with 5 ml of AcOEt to afford a white powder (9.0 mg), which was identified as **6** by comparing the mp (233—238 °C (dec.)), IR spectrum and elemental analysis with those of an authentic sample.

An aqueous solution (5 ml) of **6** (15 mg) was treated with ion exchange resin (IR-120B) in the same manner as described above and the filtrate was neutralized with 0.01 N NaOH. The neutral solution was evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and chromatographed on a column of Sephadex LH-20 to remove inorganic substances. The eluate was evaporated to dryness *in vacuo* and the residue was treated with a small amount of AcOEt to give the sodium salt of **6**, a white powder, (mp 230—233 °C (dec.)), $[\alpha]_D^{31}$ −28° ($c=0.5$, MeOH). *Anal.* Calcd for $C_{34}H_{57}NaO_{16}S\cdot 4H_2O$: C, 48.10; H, 7.72. Found: C, 48.25; H, 7.41.

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