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Studies on the Constituents of *Aspidistra elatior* Blume. I. On the Steroids of the Underground Part[1)]

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Five steroidal compounds were isolated from the dried underground part of *Aspidistra elatior* Blume (Liliaceae), and four of them were elucidated to be aspidistrin (diosgenin 3-*O*-β-lycotetraoside), proto-aspidistrin, methyl proto-aspidistrin, and 1β,2β,3β,4β,5β-pentahydroxyspirost-25(27)-ene ($\Delta^{25(27)}$-pentologenin or $\Delta^{25(27)}$-neopentologenin), on the basis of physical and chemical investigations. The remaining steroidal compound is suggested to be a new spirostanol compound.

Keywords——*Aspidistra elatior*; Liliaceae; steroid; diosgenin; $\Delta^{25(27)}$-pentologenin; $\Delta^{25(27)}$-neopentologenin; saponin; aspidistrin; proto-aspidistrin

Saponins and sapogenins in a Chinese drug "zhī zhū bào dàn" (Japanese name: Haran-kon), rhizome of *Aspidistra elatior* Blume (Liliaceae), have been investigated, and Takeda *et al.*[3)] reported the isolation of diosgenin, a sapogenin, a phytosterol and an unidentified amorphous saponin. Afterwards, Mori *et al.*[4)] reported the isolation of one steroidal saponin named aspidistrin and they established its structure to be diosgenin 3-*O*-β-lycotetraoside by comparing melting points (alone and on admixture), optical rotations, infrared (IR), nuclear magnetic resonance (NMR), and mass spectra, and *Rf* values on thin layer chromatography

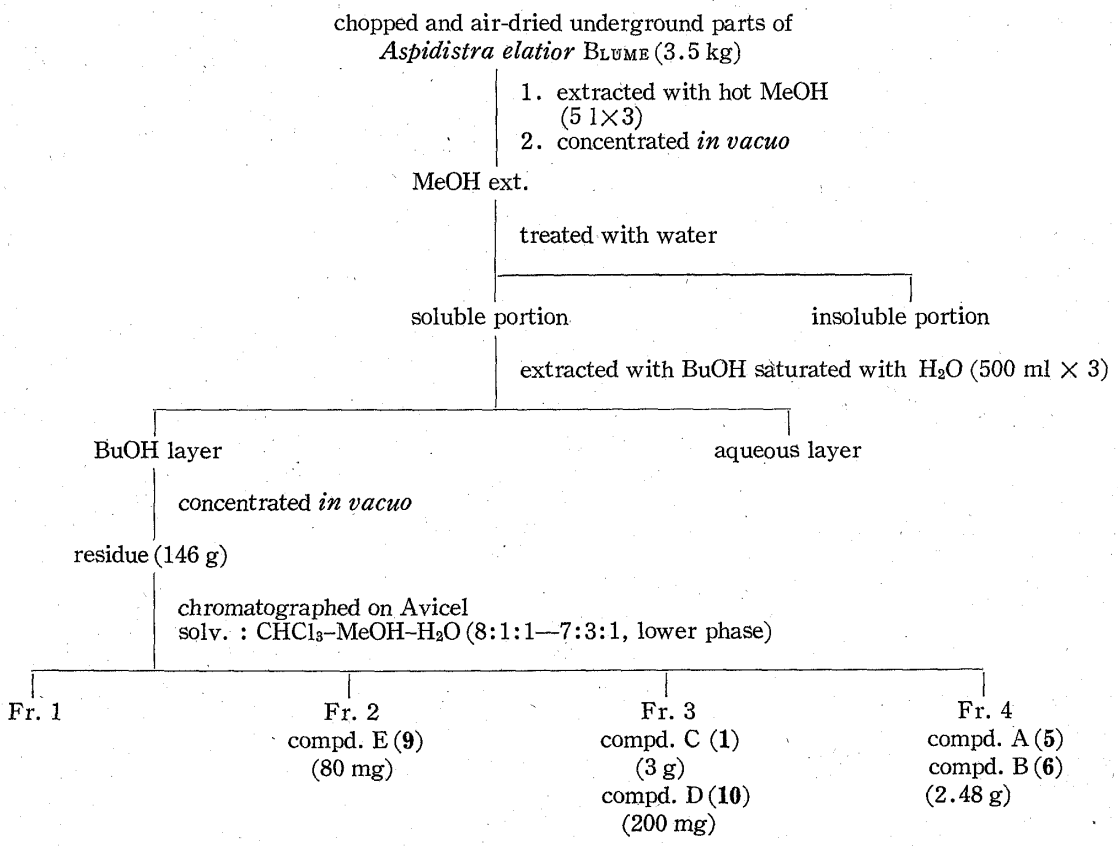

Chart 1

(TLC) of dihydroaspidistrin, tigogenin 3-*O*-β-lycotetraoside and their peracetates. In the same report, they suggested the existence of a furostanol bisglycoside, but the details have not yet been published. The present paper describes the isolation and structure elucidation of 22-hydroxyl and 22-methoxyl furostanol oligosides (compounds A and B) corresponding to aspidistrin and two new spirostanol derivatives (compounds D and E). A reinvestigation of the structure of aspidistrin (compound C) by chemical methods is also described. The results support the proposed structure.

The commercial cut rhizomes of *Aspidistra elatior* BLUME were extracted with hot methanol and the extract was fractionated by the method shown in Chart 1. TLC of the butanol extract revealed the presence of five steroidal compounds, which were tentatively named compounds A—E in order of decreasing polarity, and they were separated by Avicel column chromatography.

Compound C (**1**), $C_{50}H_{80}O_{22}$, is positive in the Liebermann–Burchard reaction, but negative to the Ehrlich reagent.[5] Compound C was predicted to be a tetraglycoside of 25(*R*)-spirostanol based on the characteristic 25(*R*)-spiroketal absorption band in the IR spectrum[6] and four anomeric proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra (Table I).

TABLE I. ^{13}C-NMR Chemical Shifts of Aspidistrin and Related Compounds[a]

Compounds		Diosgenin[b]	Glycoside C-a (**2**)	Glycoside C-b (**3**)	Glycoside C-c (**4**)	Aspidistrin (=Compound C (**1**))	Compound A (**5**)	Compound B (**6**)
Aglycone No.	1	38.0	37.7	37.6	37.5	37.6	37.4	37.6
	2	32.4	30.5	30.4	30.2	30.2	29.9	30.2
	3	71.4	78.4	78.4	78.4	78.5**	78.5	78.6
	4	34.6	39.6	39.4	39.3	39.4	39.1	39.4
	5	142.2	141.3	141.2	141.1	141.2	141.0	141.2
	6	121.0	121.7	121.6	121.5	121.6	121.4	121.6
	7	32.7	32.5	32.4	32.3	32.4	32.2	32.4
	8	32.0	31.9	31.9	31.7	32.0	31.6	31.8
	9	50.8	50.6	50.5	50.4	50.5	50.3	50.5
	10	37.3	37.3	37.2	37.1	37.2	36.9	37.2
	11	21.4	21.3	21.3	21.1	21.2	21.1	21.2
	12	40.2	40.1	40.1	39.9	40.0	39.8	39.9
	13	40.7	40.7	40.6	40.5	40.6	40.7	40.9
	14	57.0	56.9	56.8	56.7	56.8	56.5	56.8
	15	32.6	32.4	32.3	32.2	32.3	32.2	32.4
	16	81.3	81.2	81.2	81.1	81.2	80.7	81.2
	17	63.2	63.2	63.2	63.0	63.1	63.4	64.2
	18	16.5	16.5	16.4	16.3	16.4	16.3	16.3
	19	19.8	19.5	19.5	19.4	19.5	19.2	19.5
	20	42.2	42.2	42.1	42.0	42.1	40.4	40.6
	21	15.1	15.1	15.0	14.9	15.0	16.0	16.2
	22	109.4	109.4	109.3	109.2	109.3	110.7	112.8
	23	32.0	32.1	32.0	31.8	31.8	36.8	30.9
	24	29.5	29.5	29.4	29.2	29.4	28.1	28.3
	25	30.8	30.7	30.7	30.5	30.7	34.0	34.3
	26	67.1	67.1	67.0	66.9	67.0	75.1*	75.3**
	27	17.4	17.4	17.3	17.3	17.3	17.2	17.2
C_{22}-O$\underline{C}H_3$								47.4
C-3 Sugars								
Galactose	1		103.4	103.0	102.6	102.8	102.5	102.8
	2		72.9	73.5	73.1	73.1	72.8	73.1
	3		75.5	75.4	75.4	75.3*	75.1*	75.3**
	4		70.4	79.8	80.5	79.7	79.3	79.7
	5		76.9	75.9	76.4	76.0	75.7	76.0
	6		62.7	61.0	60.5	60.7	60.6	60.7

Compounds		Diosgenin[b]	Glycoside C-a (2)	Glycoside C-b (3)	Glycoside C-c (4)	Aspidistrin (=Compound C (1))	Compound A (5)	Compound B (6)
Glucose	1			107.0	104.8	104.9[c]	104.4[c]	104.9[c]
	2			75.2	85.5	81.2	80.7	81.2
(inner)	3			78.4	78.1	87.2	87.0	87.2
	4			72.4	71.7	70.7	70.5	70.7
	5			78.7	77.8	77.9	77.6	77.9
	6			63.1	61.7	62.7	62.3	62.7
Glucose	1				106.5	104.9[c]	104.4[c]	104.9[c]
	2				75.0	75.1*	74.8*	75.1**
	3				78.6	78.6**	78.1	78.6
	4				70.4	70.5	70.1	70.5
	5				77.4	77.5	77.1	77.5
	6				62.9	63.1	62.6	63.0*
Xylose	1					104.7	104.4[c]	104.9[c]
	2					75.6	75.2*	75.6
	3					78.6**	78.1	78.6
	4					71.3	71.1	71.3
	5					67.2	66.9	67.2
C-26 Sugar								
Glucose	1						104.4[c]	104.9[c]
	2						74.8*	75.1**
	3						78.1	78.6
	4						71.6	71.9
	5						77.8	78.2
	6						62.6	63.1*

a) Chemical shifts were measured in pyridine-d_5 at 50°C. Based on the T_1 values measured by an inversion recovery Fourier-transform (IRFT) method, each signal of the sugar moieties of **1**, **3** and **4** was assigned. Off-resonance spectra of each compound were also taken for the purpose of assignment. The signals marked * and ** may be reversed.

b) Each signal was assigned on the basis of the data measured in $CDCl_3$. Reference: H. Eggert and C. Djerassi, *Tetrahedron Lett.*, **1975**, 3635.

c) The signal intensities were determined by means of a gated decoupling technique, which is termed "NNE mode" in the JEOL FX 100 operation mannual (1980). Reference: S.J. Opella, N.J. Nelson and O. Jardetzky, *J. Chem. Phys.*, **64**, 2533 (1976).

On acidic hydrolysis with 2 N hydrogen chloride in 50% dioxane, **1** gave diosgenin, galactose, glucose, and xylose. Accordingly, **1** is considered to be a tetraglycoside of diosgenin and not the corresponding furostanol bisglycoside. Based on its physical and chemical properties, compound C was suggested to be aspidistrin, and this was confirmed by direct comparisons with an authentic sample kindly provided by Prof. Kawasaki, Kyushu University.

As described above, Mori *et al.* proposed the structure of aspidistrin as diosgenin 3-*O*-β-lycotetraoside, based mainly on the qualitative and quantitative determination of the components of aspidistrin and mass spectrometric analysis of a peracetate of aspidistrin. After hydrogenation of aspidistrin over palladium charcoal in 60% alcohol, Mori *et al.* identified dihydroaspidistrin and its acetate as desgalactotigonin[7] and its acetate by direct comparisons but without detailed chemical experiments on the structure of the oligosaccharide moiety.

To prove the structure of **1**, we carried out further chemical investigations as follows. Methylation of **1** by Hakomori's method[8] afforded a per-*O*-methyl ether, which was methanolyzed to afford methyl 2,3,4-tri-*O*-methylxylopyranoside, methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, methyl 2,3,6-tri-*O*-methylgalactopyranoside and methyl 4,6-di-*O*-methylglucopyranoside. On partial hydrolysis with 0.2 N hydrogen chloride in 50% dioxane, **1** gave three prosapogenins, glycoside C-a (**2**), $C_{33}H_{52}O_8$, glycoside C-b (**3**), $C_{39}H_{62}O_{13}$, and glycoside C-c (**4**), $C_{45}H_{72}O_{18}$. Glycoside C-a afforded diosgenin and galactose, while glycosides C-b and C-c gave diosgenin, galactose and glucose on acidic hydrolysis. Finally, the structures of **2**, **3** and **4** were determined to be diosgenin 3-*O*-β-D-galactopyranoside, diosgenin 3-*O*-β-D-

glucopyranosyl(1→4)-β-D-galactopyranoside and diosgenin 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl(1→4)-β-D-galactopyranoside based on the analyses of the ^{13}C-NMR spectra (TableI). Thus, the structure of aspidistrin proposed by Mori *et al.* is supported by our additional experiments.

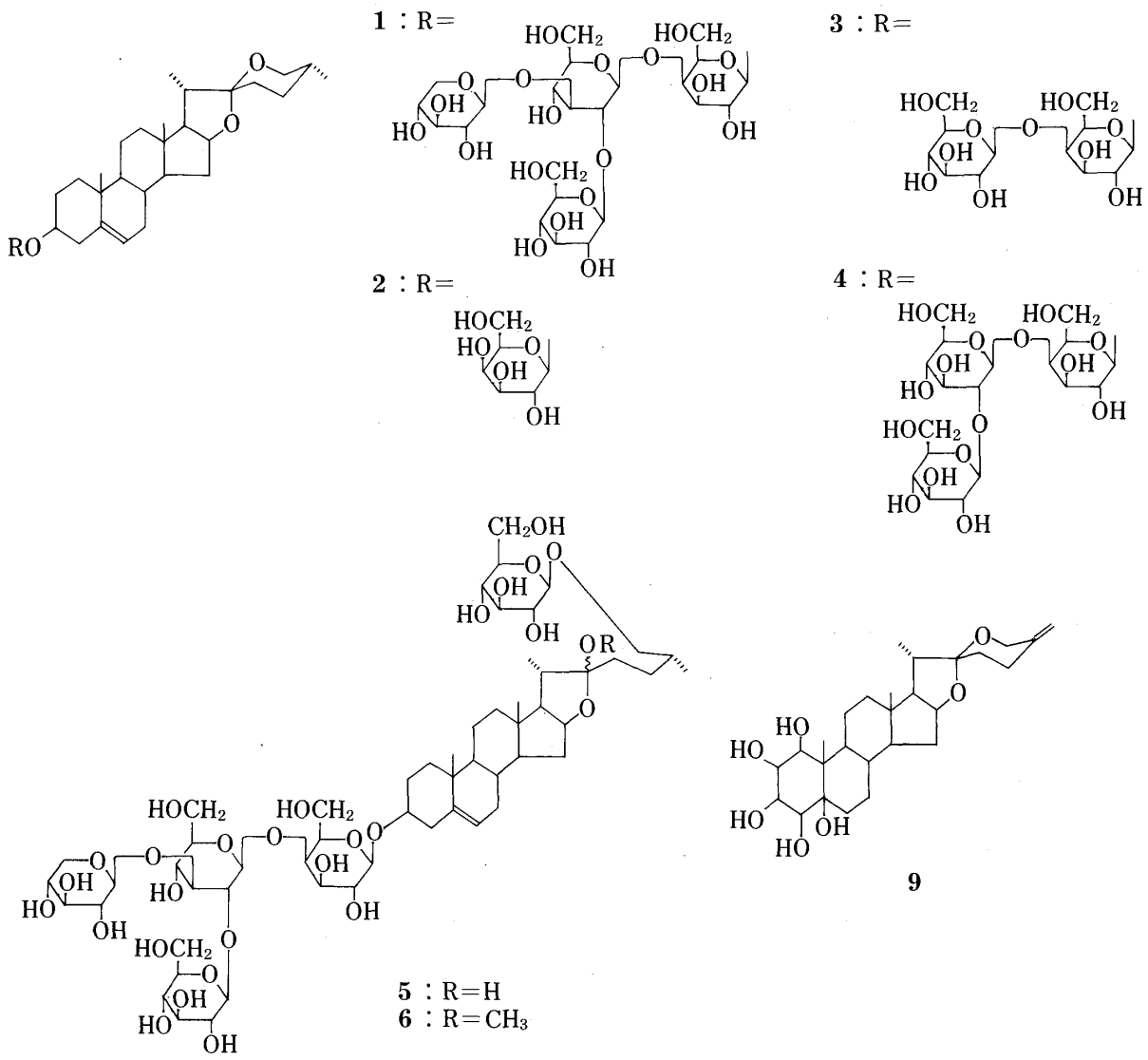

Chart 2

Compounds A (**5**, $C_{56}H_{92}O_{28}$) and B (**6**, $C_{57}H_{94}O_{28}$) were both assumed to be furostanol saponins on the basis of the positive coloration with the Ehrlich reagent. On boiling in aqueous acetone, **6** was converted to **5**, while **5** was converted to **6** on boiling in methanol.[9] The ^{13}C-NMR spectra of **5** and **6** show five anomeric carbon signals (Table I) and the ^{1}H-NMR spectrum of the latter is similar to that of the former except for a methoxyl signal at δ 3.30 ppm (3H, s, OCH_3). Therefore, **5** and **6** are considered to be 22-hydroxyl and 22-methoxyl furostanol pentaglycosides, respectively.

Enzymatic hydrolyses of **5** and **6** with almond emulsin (Sigma Chem. Co.) afforded glucose and aspidistrin. Consequently, **5** and **6** were inferred to be proto-aspidistrin (= 26-*O*-β-D-glucopyranosyl 22-hydroxyfurost-5-en-3β,26-diol 3-*O*-β-lycotetraoside) and methyl proto-aspidistrin (= 26-*O*-β-D-glucopyranosyl 22-methoxyfurost-5-en-3β,26-diol 3-*O*-β-lycotetraoside), and this view was confirmed by Marker's degradation[10] of the peracetate of **6** to provide 3β-hydroxypregna-5,16-dien-20-one (**7**) and methyl γ-methyl-δ-hydroxypentanoate

β-D-glucopyranoside tetraacetate (**8**). Therefore, **5** and **6** were identified as proto-aspidistrin and methyl proto-aspidistrin, respectively.

Compound E (**9**), $C_{27}H_{42}O_7$, colorless needles, is positive in the Liebermann–Burchard reaction and the IR spectrum shows strong hydroxyl and characteristic spiroketal absorption bands. On heating with pyridine and acetic anhydride, **9** gave a tetraacetate (**9a**), but under general acetylation conditions this reaction was not successful. The ^{1}H-NMR spectrum of **9a** reveals the presence of four methine groups carrying *O*-acetyl groups, and **9a** was deduced to be a 1,2,3,4-tetraacetoxy-5-hydroxyspirostane derivative by the spin–spin decoupling technique using europium tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octadionate)(= Eu(fod)$_3$) as a shift reagent. On irradiation at the double-doublet methine signal (δ 5.83 ppm, $J_1=J_2=3.5$ Hz, $>C\underline{H}_b$–OAc), the doublet methine signal at δ 5.61 ppm ($J=3.5$ Hz, $>C\underline{H}_a$–OAc) and the double-doublet methine signal at δ 5.30 ppm ($J_1=J_2=3.5$ Hz, $>C\underline{H}_c$–OAc) were decoupled to a singlet and a doublet, respectively. Furthermore, irradiation at the double-doublet methine signal (δ 5.30 ppm, $>C\underline{H}_c$–OAc) decoupled both the double-doublet methine signal at δ 5.83 ppm to a doublet and the doublet methine signal at δ 5.73 ppm ($J=3.5$ Hz, $>C\underline{H}_d$–OAc) to a singlet. Consequently, it is indicated that the four methine protons, namely H_a, H_b, H_c and H_d are adjacent to each other in the A-ring and that the fifth hydroxyl group may be located at C-5 of the A-ring.

The five hydroxyl groups of the A-ring were determined to have all α- or all β-configurations based on the facts that **9** afforded an orthoformate (**9b**) by reaction with trimethyl orthoformate in 4% hydrogen chloride–methanol, and that in the ^{1}H-NMR spectrum of **9a**, the coupling constants (J) of all signals of methine protons bearing an acetoxyl group are 3.5 Hz. On the other hand, the ^{1}H-NMR spectrum of **9a** shows a broad singlet at δ 4.74 ppm corresponding to two exomethylene protons and a pair of doublets (1H each, $J=13$ Hz) at δ 3.83 and 4.28 ppm corresponding to C-26 methylene protons,[11] while the ^{13}C-NMR spectrum shows a singlet at δ 143.5 ppm, a triplet at 108.5 ppm ($>C=CH_2$) and a quartet at δ 14.4 ppm corresponding to the C-21 methyl carbon. Based on the observations described above, the exomethylene group was deduced to be at C-25(27) and the structure of **9** was suggested to be 1α,2α,3α,4α,5α-pentahydroxyspirost-25(27)-ene or its 1β,2β,3β,4β,5β-isomer. The latter was isolated as a tetraacetate from "Senshokushichikon" by Takahira *et al.*[11] To distinguish these possibilities, **9a** was catalytically hydrogenated and the resulting 25(*R*)- and 25(*S*)-dihydro derivatives were examined by gas liquid chromatography (GLC). Two peaks were obtained at t_R (min) 27.0 and 28.0. The latter peak was identified as neopentologenin tetraacetate[12] by comparison with an authentic sample kindly given to us by Prof. Kawasaki, while the former peak may be pentologenin tetraacetate. Accordingly **9** was concluded to be $\Delta^{25(27)}$-pentologenin (=$\Delta^{25(27)}$-neopentologenin) which has five β-hydroxyl groups in the A-ring.

Compound D (**10**), colorless needles, mp 285—295°C (dec.), is positive in the Liebermann–Burchard reaction and the IR spectrum shows strong hydroxyl and characteristic spiroketal absorption bands. On heating in acetic acid, **10** afforded compound E (**9**), while on treatment with trimethyl orthoformate and 4% hydrogen chloride–methanol under the same conditions as described for **9**, **10** gave the same orthoformate (**9b**) as was derived from **9**. Therefore, compound D is suggested to be related to **9**. The details will be described in the next paper.

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 JASCO IRA-1 and Hitachi EPI-2 machines, and NMR spectra with a Hitachi R-22 (90 MHz) spectrometer and a JEOL FX 100 spectrometer (100 MHz for ^{1}H-NMR and 25 MHz for ^{13}C-NMR). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard. Mass spectra were recorded on a JEOL JMS-D 300 spectrometer. Unless otherwise noted, TLC was performed on precoated Kiesel gel F_{254} plates (Merck) with a mixture of $CHCl_3$–MeOH–H_2O (7: 3: 0.4 v/v) and detection

was achieved by spraying 10% H_2SO_4 followed by heating. Gas liquid chromatography (GLC) was run on a Shimadzu GC-6A unit equipped with a flame ionization detector.

Extraction and Isolation of Steroids——The commercial cut underground parts of *Aspidistra elatior* BLUME (3.5 kg) were extracted with hot MeOH (5 l×3) and the extract was concentrated *in vacuo*. The residue (625 g) was suspended in water (2 l) and filtered. The filtrate was extracted with BuOH saturated with water (500 ml×3) and the BuOH-soluble fraction was concentrated *in vacuo* to afford a brown residue (146 g), which was subjected to column chromatography over Avicel with the lower phase of $CHCl_3$–MeOH–H_2O (8: 1: 1—7: 3: 1 v/v), yielding four fractions (Fr. 1—4). Fr. 2 was purified by rechromatography on silica gel with 5% MeOH–$CHCl_3$, followed by column chromatography on Sephadex LH-20 with $CHCl_3$–MeOH (7: 3 v/v). Compound E (**9**, 80 mg) was obtained as colorless needles from $CHCl_3$–MeOH–H_2O (7: 3: 0.4 v/v). Fr. 3 was treated with MeOH and the MeOH-soluble fraction was subjected to column chromatography on Sephadex LH-20 with MeOH, followed by column chromatography on silica gel with $CHCl_3$–MeOH–AcOEt–H_2O (10: 9: 20: 5 v/v, lower phase) to afford compound D (**10**, 200 mg) as colorless needles from $CHCl_3$–MeOH–H_2O (7: 3: 0.4 v/v). The MeOH-insoluble fraction was subjected to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (7: 3: 0.4 v/v) to afford compound C (**1**, 3.00 g) as colorless needles from BuOH saturated with water.

Fr. 4 was subjected to column chromatography on Sephadex LH-20 with MeOH, followed by repeated column chromatography on silica gel with BuOH saturated with water and with $CHCl_3$–MeOH–H_2O (7: 3: 0.4 v/v) to afford a mixture of compounds A and B (**5** and **6**, 2.48 g). The mixture (100 mg) was refluxed with 30% aqueous acetone (100 ml) for 48 h, then the reaction mixture was concentrated to 5 ml under reduced pressure. Next, 100 ml of acetone was added to the concentrated solution and the resulting white precipitate was collected by filtration. This white powder showed *Rf* 0.07 on TLC and gave a red-violet color with Ehrlich reagent.

On the other hand, a mixture of compounds A and B (500 mg) was refluxed in 1% methanolic acetone (100 ml) for 48 h. The reaction mixture was concentrated to 5 ml under reduced pressure, 100 ml of AcOEt was added and the resulting white precipitate was collected by filtration. This white powder showed *Rf* 0.12 on TLC and gave a red-violet color with Ehrlich reagent.

Properties of Compounds A (5), B (6), C (1), D (10) and E (9)——Compound A (**5**): A white powder from aqueous acetone, (mp 210—214°C (dec.)), $[\alpha]_D^{15.4}$ −64.0° (c=1.00, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3300 (OH). ^{1}H-NMR (pyridine-d_5) δ: 0.91 (6H, s, CH_3×2), ^{13}C-NMR (pyridine-d_5) δ: (Table I). *Anal.* Calcd for $C_{56}H_{92}O_{28}$: C, 55.43; H, 7.64. Found: C, 55.13; H, 7.65. Compound B (**6**): A white powder from MeOH–AcOEt, (mp 202—207°C (dec.)), $[\alpha]_D^{51.5}$ −63.4° (c=1.01, pyridine), IR ν_{max}^{KBr} cm^{-1}: 3600—3300 (OH), ^{1}H-NMR (pyridine-d_5) δ: 0.85 (3H, s, CH_3), 0.92 (3H, s, CH_3), 3.30 (3H, s, OCH_3). ^{13}C-NMR (pyridine-d_5) δ: (Table I). *Anal.* Calcd for $C_{57}H_{94}O_{28}$: C, 55.77; H, 7.73. Found: C, 55.43; H, 7.64. Compound C (**1**): Colorless needles from BuOH saturated with water, mp 263—268°C (dec.) (lit.[4] mp 265—267°C (dec.)), $[\alpha]_D^{15.8}$ −69.2° (c=1.07, pyridine) (lit.[4] $[\alpha]_D$ −68° (c=1.08, pyridine)), IR ν_{max}^{KBr} cm^{-1}: 3480—3350 (OH), 980, 922, 900, 868 (intensity 922<900, 25(*R*)-spiroketal). The IR spectrum was superimposable on that of an authentic sample of aspidistrin kindly given to us by Prof. Kawasaki, Kyushu University. ^{1}H-NMR (pyridine-d_5) δ: 0.72 (3H, broad d, J=6 Hz, CH_3), 0.86 (3H, s, CH_3), 0.88 (3H, s, CH_3), 1.15 (3H, d, J=6 Hz, CH_3), 4.80, 4.88, 5.16, 5.23 (1H each, d, J=7 Hz, anomeric H), 5.56 (1H, m, olefinic H), ^{13}C-NMR (pyridine-d_5) δ: (Table I), *Anal.* Calcd for $C_{50}H_{80}O_{22}\cdot H_2O$: C, 57.12; H, 7.88. Found: C, 57.22; H, 7.82. Compound D (**10**): Colorless needles from a mixture of $CHCl_3$–MeOH–H_2O (7: 3: 0.4 v/v), mp 285—295°C (dec.), IR ν_{max}^{Nujol} cm^{-1}: 3500—3380 (OH), 985, 920, 900, 855 (spiroketal), ^{1}H-NMR ($CDCl_3$–CD_3OD–D_2O) δ: 0.78 (3H, s, CH_3), 1.04 (3H, d, J=7 Hz, CH_3), 1.56 (3H, s, CH_3), 4.85 (2H, br s, >C=CH_2). Compound E (**9**): Colorless needles from $CHCl_3$–MeOH–H_2O (7: 3: 0.4 v/v), mp 298—300°C (dec.), IR ν_{max}^{Nujol} cm^{-1}: 3460—3380 (OH), 978, 920, 905, 865 (spiroketal), ^{1}H-NMR ($CDCl_3$–CD_3OD–D_2O) δ: 0.89 (3H, s, CH_3), 0.97 (3H, d, J=7 Hz, CH_3), 1.32 (3H, s, CH_3), 4.85 (2H, br s, >C=CH_2).

Acid Hydrolysis of 1——A solution of **1** (50 mg) in 4 ml of 2 N HCl–50% dioxane was heated on a boiling water bath for 2 h. The reaction mixture was diluted with water and extracted with $CHCl_3$ (20 ml×3). The $CHCl_3$ layer was washed with water, dried over Na_2SO_4 and evaporated to dryness. The residue (25 mg) was chromatographed on silica gel using hexane–AcOEt (6: 1 v/v) to afford a sapogenin (7 mg), which was recrystallized from acetone to give colorless needles, mp 207—212°C, IR ν_{max}^{Nujol} cm^{-1}: 3450 (OH), 980, 920, 888, 853 (intensity 920<888, 25(*R*)-spiroketal). ^{1}H-NMR ($CDCl_3$) δ: 0.77 (3H, d, J=6 Hz, CH_3), 0.78 (3H, s, CH_3), 0.93 (3H, d, J=6 Hz, CH_3), 0.99 (3H, s, CH_3), 4.33 (1H, m, >C<u>H</u>–OH), 5.32 (1H, m, olefinic H), MS m/z: Calcd for $C_{27}H_{42}O_3$: 414.313 (M^+). Found: 414.312. This compound was found to be identical with diosgenin by mixed melting point determination.

The aqueous layer was neutralized with $NaHCO_3$ and evaporated to dryness *in vacuo*, then the residue was examined by GLC (column, 5% SE 52 on Chromosorb W 3 mm×2 m; column temp. 160°C; injection temp. 180°C; carrier gas N_2, 1.2 kg/cm^2; samples, trimethyl silyl (TMS) ethers: t_R(min) 5.1, 6.3 (xylose), 9.2, 10.9 (galactose), 11.7, 15.6 (glucose)).

Partial Hydrolysis of 1 with 0.2 N HCl——Compound C (**1**, 1 g) was heated on a boiling water bath with 0.2 N HCl in 50% dioxane (100 ml) for 1 h. After cooling, the reaction mixture was neutralized with $NaHCO_3$ and evaporated to dryness *in vacuo*. The residue was chromatographed on silica gel, and elution with $CHCl_3$–

MeOH–H_2O (7: 3: 0.4 v/v) afforded diosgenin (140 mg), glycoside C-a (**2**, 120 mg), glycoside C-b (**3**, 170 mg) and glycoside C-c (**4**, 320 mg). Glycoside C-a: A white powder from MeOH, (mp 245—249°C (dec.)), *Anal.* Calcd for $C_{33}H_{52}O_8$: C, 68.71; H, 9.10. Found: C, 68.74; H, 9.24. Glycoside C-b: A crystalline powder from MeOH, (mp 253—257°C (dec.)), *Anal.* Calcd for $C_{39}H_{62}O_{13}\cdot H_2O$: C, 62.15; H, 8.53. Found: C, 61.87; H, 8.27. Glycoside C-c: Colorless needles from $CHCl_3$–MeOH, (mp 275—281°C (dec.)), *Anal.* Calcd for $C_{45}H_{72}O_{18}\cdot 3/2H_2O$: C, 58.23; H, 8.16. Found: C, 58.30; H, 7.97.

Acid Hydrolysis of Glycosides C-a, C-b and C-c——A solution of a glycoside (20 mg) in 4 ml of 1 N HCl–50% dioxane was heated on a boiling water bath for 2 h. After cooling, the reaction mixture was neutralized with $NaHCO_3$ and evaporated to dryness under reduced pressure. The residue was examined by GLC under the same conditions as described above. t_R(min): Glycoside C-a 9.2, 10.9 (galactose); Glycoside C-b 9.2, 10.9 (galactose); 11.7, 15.6 (glucose); Glycoside C-c 9.2, 10.9 (galactose), 11.7, 15.6 (glucose).

Methylation of 1 by Hakomori's Method——According to Hakomori's method, NaH (250 mg) was washed with anhydrous benzene followed by petroleum ether, and warmed with dimethylsulfoxide (DMSO, 10 ml) at 65°C in an oil bath for 1 h with stirring under N_2 flow, then a solution of **1** (100 mg) in a small amount of DMSO was added and the mixture was stirred for 1 h under 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 with MeOH, and then on silica gel with $CHCl_3$–MeOH (99: 1 v/v) to afford a per-*O*-methylate (**1a**, 74 mg), obtained as a resinous powder, (mp 90—93°C), IR ν_{max}^{Nujol} cm^{-1}: OH (nil.), ^{1}H-NMR ($CDCl_3$) δ: 0.71 (3H, s, CH_3), 0.72 (3H, d, J=6 Hz, CH_3), 0.94 (3H, d, J=7 Hz, CH_3), 1.03 (3H, s, CH_3), 3.32—3.63 (OCH_3), 4.07 (2H, d, J=7 Hz, anomeric H×2), 4.90 (1H, d, J=7 Hz, anomeric H), 4.99 (1H, d, J=7 Hz, anomeric H), 5.33 (1H, m, olefinic H).

Methanolysis of 1a——A solution of **1a** (10 mg) in methanolic 1 N HCl (5 ml) was refluxed for 5 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and the resulting precipitate was filtered off. The filtrate was concentrated to dryness and the residue was examined by GLC (column, 5% NPGS on Shimalite 3 mm×2 m; column temp. 155°C; injection temp. 180°C; carrier gas, N_2 1.2 kg/cm^2; samples, TMS derivatives): t_R(min) 1.8, 2.2 (methyl 2,3,4-tri-*O*-methylxylopyranoside), 4.0, 5.7 (methyl 2,3,4,6-tetra-*O*-methylglucopyranoside), 4.4, 5.2 (methyl 2,3,6-tri-*O*-methylgalactopyranoside), 6.6 (methyl 4,6-di-*O*-methylglucopyranoside).

Enzymatic Hydrolysis of Compounds A (5) and B (6)——A mixture of **5** and **6** (200 mg) in H_2O was incubated with almond emulsin (150 mg) at 37°C for 4 h. The precipitate was collected by filtration, dried and crystallized from BuOH saturated with water to afford compound C(**1**) as colorless needles, mp 263—268 °C (dec.), IR ν_{max}^{KBr} cm^{-1}: 3480—3350 (OH), 980, 922, 900, 868 (intensity 922<900, 25(*R*)-spiroketal), which were identical with aspidistrin. The aqueous filtrate was evaporated to dryness *in vacuo*. The residue was examined by TLC and only glucose was detected as a sugar component.

Acetylation of Compound B (6)——Compound B (**6**, 40 mg) was dissolved in pyridine (2 ml) and Ac_2O (1 ml) and the solution was allowed to stand overnight at room temperature. The solvents were removed by drying in a stream of nitrogen and the residue was purified by column chromatography on silica gel with $CHCl_3$–MeOH (19: 1 v/v) to afford compound B peracetate (43 mg) as a white powder from Et_2O–hexane, (mp 118—124°C), IR (Nujol): OH (nil.), *Anal.* Calcd for $C_{59}H_{126}O_{44}$: C, 56.26; H, 6.68. Found: C, 56.01; H, 6.82.

Marker's Degradation of Compound B Peracetate——A solution of compound B peracetate (500 mg) in acetic acid (20 ml) was refluxed for 1 h, then the reaction mixture was evaporated to dryness *in vacuo*. The residue was dissolved in 80% acetic acid (20 ml) and a solution of chromic trioxide (50 mg) in 80% acetic acid (2 ml) was added to the solution over 15 min under stirring and with cooling below 20°C. After the reaction mixture had been stirred for 3 h, excess reagent was decomposed with MeOH. The reaction mixture was diluted with water (20 ml), and extracted with ether. The ether solution was washed with water, dried over Na_2SO_4, and evaporated to dryness. The residue (470 mg) was refluxed with 5% K_2CO_3 in iso-PrOH (20 ml) for 1 h. The reaction mixture was diluted with water (20 ml) and extracted with BuOH saturated with water. The BuOH solution was washed with water and evaporated to dryness *in vacuo*. The residue was dissolved in 2 N HCl–50% dioxane (20 ml) and the solution was heated for 1 h on a water bath. The reaction mixture was diluted with water (20 ml) and extracted with $CHCl_3$. $CHCl_3$ solution was washed, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel using hexane–acetone (3: 1 v/v) to afford 3β-hydroxypregna-5,16-dien-20-one (**7**, 20 mg). **7**: Colorless plates from MeOH, mp 210—212°C, IR ν_{max}^{Nujol} cm^{-1}: 3440 (OH), 1655, 1580 (enone), ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, s, CH_3), 1.04 (3H, s, CH_3), 2.26 (3H, s, CH_3), 3.49 (1H, m, >C<u>H</u>–OH), 5.33 (1H, m, olefinic H), 6.71 (1H, m, olefinic H). High resolution MS (m/z): Calcd for $C_{21}H_{30}O_2$ (M^+) 314.224. Found: 314.229. Compound **7** was identified as 3β-hydroxypregna-5,16-dien-20-one by mixed melting point determination and by comparison of the TLC behavior (solvent: hexane–acetone (2: 1 v/v) *Rf* 0.51) and IR and ^{1}H-NMR spectra with those of an authentic sample.

The alkaline aqueous layer described above was acidified with acetic acid to pH 6 and extracted with BuOH saturated with water. The BuOH solution was evaporated to dryness *in vacuo* and the residue was

acetylated with Ac_2O (2 ml) and pyridine (2 ml) under heating for 1 h. The reaction mixture was evaporated to dryness *in vacuo*. The residue was dissolved in MeOH and methylated with CH_2N_2 in ether. Excess CH_2N_2 in the reaction mixture was decomposed with acetic acid and the solvent was evaporated off under reduced pressure. The residue was purified by column chromatography on silica gel using hexane–acetone (3: 1 v/v) to afford methyl γ-methyl-δ-hydroxypentanoate β-D-glucopyranoside tetraacetate (**8**, 20 mg). **8**: A colorless syrup, ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, d, J=6 Hz, CH–C<u>H</u>$_3$), 2.04 (3H, s, OAc), 2.05 (3H, s, OAc), 2.07 (3H, s, OAc), 2.11 (3H, s, OAc), 3.69 (3H, s, –C(=O)–O–CH_3), 4.52 (1H, d, J=7 Hz, anomeric H). Ms (*m/z*): 331, 243, 242, 200, 169, 157, 145, 140, 129.098 ($C_7H_{13}O_2^+$), 115, 109.

Acetylation of Compound E (9)——Compound E (50 mg) was dissolved in pyridine (4 ml) and Ac_2O (2 ml) and the solution was heated on a boiling water bath for 2 h. The reaction mixture was poured into ice-water and the resulting precipitate was collected by filtration to afford a white powder (**9a**, 49 mg). Compound **9a** showed a single spot (*Rf* 0.29) on a TLC plate developed with hexane–acetone (3: 1 v/v), but attempts to recrystallize or reprecipitate **9a** from various organic solvents were not successful. Compound **9a**: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 1745 (ester), 1655 (C=C), 980, 922, 900, 880 (spiroketal), ^{1}H-NMR (12.7 mg in 0.3 ml of $CDCl_3$ and 3.7 mg of Eu(fod)$_3$) δ: 0.78 (3H, s, 18-CH_3), 0.96 (3H, d, J=7 Hz, 21-CH_3), 1.21 (3H, s, 19-CH_3), 2.00 (3H, s, OAc), 2.18 (3H, s, OAc), 2.21 (6H, s, OAc×2), 3.86 (1H, d, J=13 Hz, C_{26}–H), 4.31 (1H, d, J=13 Hz, C_{26}–H), 4.48 (1H, m, C_{16}–H), 4.76 (2H, br s, >C=CH_2), 5.30 (1H, dd, J_1=J_2=3.5 Hz, >CH–OAc), 5.61 (1H, d, J=3.5 Hz, >CH–OAc), 5.73 (1H, d, J=3.5 Hz, >CH–OAc), 5.83 (1H, dd, J_1=J_2=3.5 Hz, >CH–Ac). ^{13}C-NMR ($CDCl_3$) δ: 12.3 (q, C_{19}), 14.4 (q, C_{21}), 16.3 (q, C_{18}), 20.4, 20.5, 20.6, 20.7 (each q, –CO<u>C</u>H$_3$), 41.6 (d, C_{20}), 64.9 (t, C_{26}), 65.3, 67.3, 69.8, 74.2 (each d, C_1—C_4), 75.6 (s, C_5), 108.5 (t, C_{27}), 109.3 (s, C_{22}), 143.5 (s, C_{25}). High resolution MS (*m/z*): Calcd for $C_{35}H_{50}O_{17}$ (M^+) 646.335. Found: 646.332.

Hydrogenation of 9a——Compound **9a** (30 mg) in MeOH (50 ml) was shaken with 10% Pd/C (20 mg) under H_2 at room temperature for 24 h. The reaction mixture was filtered to remove Pd/C and the filtrate was concentrated to afford a dihydro derivative of **9a** (**13**, 28 mg) as a colorless syrup, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (OH), 1745 (ester), 980, 922, 900, 865 (spiroketal), ^{1}H-NMR ($CDCl_3$) δ: 0.78 (3H, s, CH_3), 0.96 (3H, d, J=7 Hz, CH_3), 1.14 (3H, d, J=7 Hz, CH_3), 1.15 (3H, s, CH_3), 1.94 (3H, s, OAc), 2.09 (3H, s, OAc), 2.11 (3H, s, OAc), 2.17 (3H, s, OAc), 3.27 (1H, d, J=10 Hz, C_{26}–H), 3.91 (1H, q, J=3, 10 Hz, C_{26}–H), 4.35 (1H, m, C_{16}–H), 5.17 (1H, dd, J_1=J_2=3.5 Hz, >CH–OAc), 5.48 (1H, d, J=3.5 Hz, >CH–OAc), 5.52 (1H, d, J=3.5 Hz, >CH–OAc), 5.63 (1H, dd, J_1=J_2=3.5 Hz, >CH–OAc).

Compound **13** was examined by GLC (column: 1.5% OV-17 on Shimalite W (AW-DMCS) 3 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 (pentologenin tetraacetate(?), not identified), 28.0 (neopentologenin tetraacetate).

Ortho Ester of Compounds E (9) and D (10)——Compound **9** (15 mg) was dissolved in methanolic 4% HCl (2 ml) and trimethyl orthoformate (2 ml), and the solution was allowed to stand at room temperature for 45 min. The reaction mixture was neutralized with Ag_2CO_3, then the precipitate was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The residue was purified by column chromatography on silica gel using $CHCl_3$–MeOH (97: 3 v/v) to afford an ortho ester (**14**, 12 mg) as colorless needles from MeOH. The same product, **14** (40 mg), was obtained from compound **10** (50 mg) by the same procedure. The identity of both products was proved by mixed melting point determination and by comparisons of IR and NMR spectra. Compound **14**: Colorless needles from methanol, mp 244—246°C, IR ν_{max}^{KBr} cm^{-1}: 3495—3280 (OH), 1650 (C=C), 980, 920, 900, 878 (spiroketal), ^{1}H-NMR ($CDCl_3$) δ: 0.78 (3H, s, CH_3), 0.95 (3H, d, J=7 Hz, CH_3), 1.42 (3H, s, CH_3), 3.77 (1H, br s, >C<u>H</u>–OH), 3.83 (1H, d, J=12 Hz, C_{26}–H), 4.14 (1H, br s, >C<u>H</u>–OH), 4.29 (1H, d, J=12 Hz, C_{26}–H), 4.45 (1H, m, C_{16}–H), 4.74 (2H, br s, >C=CH_2), 5.49 (1H, s, –C(–O–)(–O–)–H ortho ester), *Anal.* Calcd for $C_{28}H_{40}O_7 \cdot 3/2H_2O$: C, 65.21; H, 8.41. Found: C, 65.64; H, 8.22.

Treatment of Compound D (10) with Acetic Acid——Compound **10** (60 mg) was dissolved in AcOH (5 ml) and the solution was heated on a water bath for 30 min. After cooling, the reaction mixture was evaporated to dryness *in vacuo*, and the residue was acetylated with Ac_2O (2 ml) and pyridine (2 ml) by heating on a boiling water bath for 2 h. The reaction mixture was poured into ice water and the precipitate (30 mg) was collected by filtration. The product was identified as compound **9a** by comparing its TLC behavior (solvent: hexane–acetone (3: 1 v/v), *Rf* 0.29), and IR and ^{1}H-NMR spectra with those of an authentic sample.

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