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Studies on the Constituents of Panacis Japonici Rhizoma. V.[1] The Structures of Chikusetsusaponin I, Ia, Ib, IVa and Glycoside P_1

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Chikusetsusaponin I, Ia, Ib, IVa, and glycoside P_1, the minor saponins and fatty-acylglycoside of Panacis japonici rhizoma (rhizome of *Panax japonicum* C. A. Meyer) have been isolated. The structure of these saponins have been established as **1**, **2**, **3**, **4**, and **5**, respectively. Chikusetsusaponin I is identical with ginsenoside-Rg_2, isolated from *P. ginseng* C. A. Meyer, while chikusetsusaponin IVa is identical with saponin C, isolated from *P. pseudoginseng* subsp. *himalaicus* var. *angustifolius*.

In our previous papers,[1,3a-c] it has been reported that three kinds of major saponins of "Chikusetsu Ninjin" (rhizome of *Panax japonicum* C.A. Meyer; Araliaceae), namely chikusetsusaponin III, IV and V, were isolated and established to be 20*S*-protopanaxadiol-3-[β-D-glucopyranosyl (1→2)] [β-D-xylopyranosyl (1→6)]-β-D-glucopyranoside, β-D-glucopyranosyl oleanate-(3)-α-L-arabinofuranosyl (1→4)-β-D-glucuronopyranoside and β-D-glucopyranosyl oleanate-(3)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranoside, respectively. In the present paper, the isolation and structure elucidation of chikusetsusaponin I, Ia, Ib, IVa and glycoside P_1 which leads to the assignment of the structure **1**, **2**, **3**, **4** and **5** is described.

The rhizoma of *Panax japonicum* C.A. Meyer were extracted with hot methanol and the extract was treated as shown in Chart 1. The thin-layer chromatograms (TLC) of each fraction on silica gel H are shown in Fig. 1. The general properties of chikusetsusaponin I, Ia, Ib, IVa and glycoside P_1 are given in Table I.

On acid hydrolysis both chikusetsusaponin Ib and IVa gave oleanolic acid (**6**) as an aglycone, chikusetsusaponin I gave panaxatriol (**7**),[4] and chikusetsusaponin Ia gave panaxadiol (**8**),[5] while glycoside P_1 gave β-sitosterol, campesterol, stigmasterol and palmitic acid. The monosaccharide components of each glycoside are listed in Table II. The aglycones and monosaccharides were identified by TLC and gas-liquid chromatography (GLC), respectively.

The genuine aglycones of chikusetsusaponin I and Ia were established to be 20*S*-protopanaxatriol (**9**)[4] and 20*S*-protopanaxadiol (**10**)[6] by Smith's oxidative degradation.[7]

It has been pointed out that the 20-O-glycosyldammarane type glycoside is readily hydrolyzed with 50% aqueous acetic acid being accompanied by the equilibrated epimerization of the 20-*tert*-hydroxyl group.[6,8] On the partial hydrolysis with 50% acetic acid, no hydrolysis of the glycosyl linkage was observed in case of chikusetsusaponin I and Ia. Furthermore, the infrared (IR) spectra of O-methyl derivatives of both saponin (**11**, **12**) prepared by the Hakomori's method show the hydroxyl absorption at 3388 cm^{-1} corresponding to the strongly

1) Part IV: N. Kondo, Y. Marumoto, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **19**, 1103 (1971).
2) Location: *1-5-8 Hatanodai, Shinagawaku, Tokyo.*
3) *a*) N. Kondo and J. Shoji, *Yakugaku Zasshi*, **88**, 325 (1968); *b*) N. Kondo, J. Shoji, N. Nagumo, and N. Komatsu, *Yakugaku Zasshi*, **89**, 846 (1969); *c*) N. Kondo, K. Aoki, H. Ogawa, R. Kasai, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **18**, 1558 (1970).
4) Y. Nagai, O. Tanaka, and S. Shibata, *Tetrahedron*, **27**, 881 (1971).
5) M. Nagai, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **19**, 2349 (1971).
6) M. Nagai, T. Ando, N. Tanaka, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **20**, 1212 (1972).
7) F. Smith, G.W. Hay, and B.A. Lewis, "Method in Carbohydrate Chemistry," Vol. 5, Academic Press, New York and London, 1965, p. 361.
8) S. Shibata, T. Ando, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **14**, 1157 (1966).

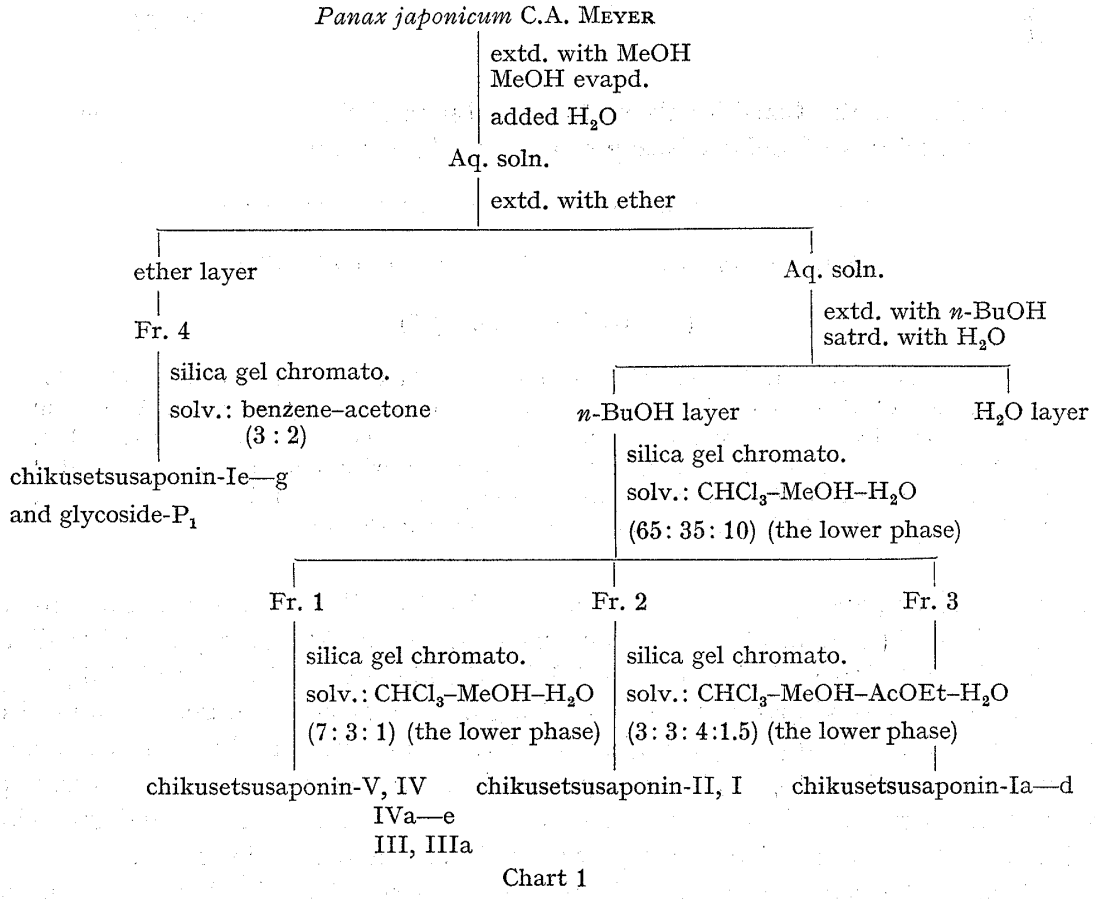


Chart 1

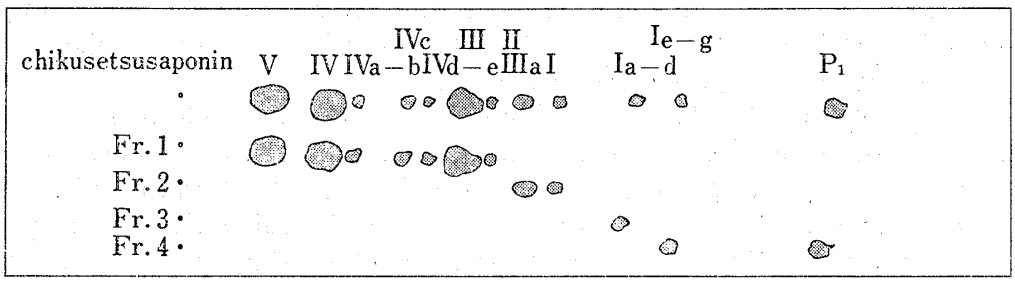


plate: silica gel H
solvent: $CHCl_3$–MeOH–H_2O (65: 35: 10 the lower phase)

Fig. 1. Thin-Layer Chromatograms of Chikusetsusaponins

hydrogen bonded C-20 hydroxyl group with C-12 O-methyl group.[9] These observations revealed that both saponin have no glycosyl linkage at the C-20 hydroxyl group of their dammarane type aglycones. The general properties of O-methylated chikusetsusaponin I and Ia are summarized in Table III.

The methanolyses of O-methylated chikusetsusaponin I and Ia with methanolic hydrogen chloride gave methyl 3,4,6-tri-O-methylglucoside and methyl 2,3,4-tri-O-methylrhamnoside from the former, while methyl 2,3,4-tri-O-methylglucoside and methyl 2,3,4-tri-O-methylxyloside from the latter.

All the properties of chikusetsusaponin I and its O-methyl ether (**11**) mentioned above strongly suggest that this saponin must be identical with ginsenoside-Rg_2,[9] the saponin of *Panax ginseng* C. A. Meyer. The identity of both compounds has been proved by the direct

9) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **22**, 2407 (1974) and the references cited therein.

Table I

Chikusetsusaponin	Properties	mp (°C)	$[\alpha]_D$ (c in MeOH; °C)	Formula	IR (KBr) cm^{-1}
I	colorless needles (EtOH)	189—191	+4.96° (1.01;30°)	$C_{42}H_{72}O_{13}$	3400 (OH) 1630 (C=C)
Ia	colorless needles ($CHCl_3$: MeOH:AcOEt: H_2O=3:3:4:1.5)	194	−3.51° (1.42;16°)	$C_{41}H_{70}O_{12}$	3400 (OH) 1630 (C=C)
Ib	colorless prism (CH_3COCH_3)	177	−17.98° (1.02;24°)	$C_{47}H_{74}O_{18}$	3400 (OH) 1730 (COOH, COOR) 1630 (C=C)
IVa	colorless needles (aq. BuOH: AcOEt =1:1)	221	+21.07° (0.85;17°)	$C_{42}H_{66}O_{14}$	3400 (OH) 1730 (COOR) 1630 (C=C)
Glycoside P_1	a white powder ($CHCl_3$: MeOH=1:1)	(162)	−45.37° (in $CHCl_3$, 1.41;20°)	$C_{50}H_{88}O_7$ $C_{51}H_{90}O_7$	3360 (OH) 1733 (COOR) (in CCl_4)

Table II

Chikusetsusaponin	Aglycone (genuine aglycone)	Sugar components
I	panaxatriol (20*S*-protopanaxatriol)	glucose rhamnose
Ia	panaxadiol (20*S*-protopanaxadiol)	glucose xylose
Ib	oleanolic acid	glucose glucuronic acid arabinose
IVa	oleanolic acid	glucose glucuronic acid
Glycoside P_1	β-sitosterol campesterol stigmasterol	glucose (palmitic acid)

comparison. On the other hand, the properties of chikusetsusaponin Ia and its O-methyl ether (**12**) suggested that this saponin must be identical with a prosapogenin of chikusetsusaponin III (desglucosylchikusetsusaponin III). To confirm the position of the glycosyl moiety in chikusetsusaponin Ia, O-methylated chikusetsusaponin Ia was catalytically reduced on PtO_2 to give a hepta-O-methyldihydrochikusetsusaponin Ia, which afforded 12-O-methyldihydroprotopanaxadiol[8] on hydrolysis with concentrated hydrogen chloride at room temperature. The identity of chikusetsusaponin Ia with desglucosylchikusetsusaponin III has been proved by direct comparison with the authentic sample which was obtained by enzymatic partial hydrolysis of chikusetsusaponin III.

The structures of chikusetsusaponin Ib and IVa were elucidated as follows. Chikusetsusaponin IVa, which is composed of oleanolic acid, glucose and glucuronic acid, is suggested to have an ester linkage by IR spectrum, so that the per-O-methylation was carried out by the Kuhn's method. Per-O-methylchikusetsusaponin IVa (**13**) shown in Table III was treated with lithium aluminium hydride to afford erythrodiol 2,3,4-tri-O-methyl-β-D-glucoside (**14**),[10] 2,3,4,6-tetra-O-methyl-D-glucose and 2,3,4,6-tetra-O-methyl-D-sorbitol, which were identified with the authentic samples. The result suggests that one glucose residue is attached to the carboxyl group of either oleanolic acid or glucuronic acid in ester form. The properties of chikusetsusaponin IVa and per-O-methylchikusetsusaponin IVa agree with saponin C,[10] a saponin of *Panax pseudoginseng* subsp. *himalaicus* var. *angustifolius*, proposed by Kondo,

10) N. Kondo and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **23**, 3282 (1975).

et al. The identity of both chikusetsusaponin IVa and saponin C has been proved by the direct comparison.

Chikusetsusaponin Ib composed of oleanolic acid, glucuronic acid, glucose, and arabinose was methylated with diazomethane in methanol to afford a monomethyl ester (**15**), $C_{48}H_{76}O_{18}$,

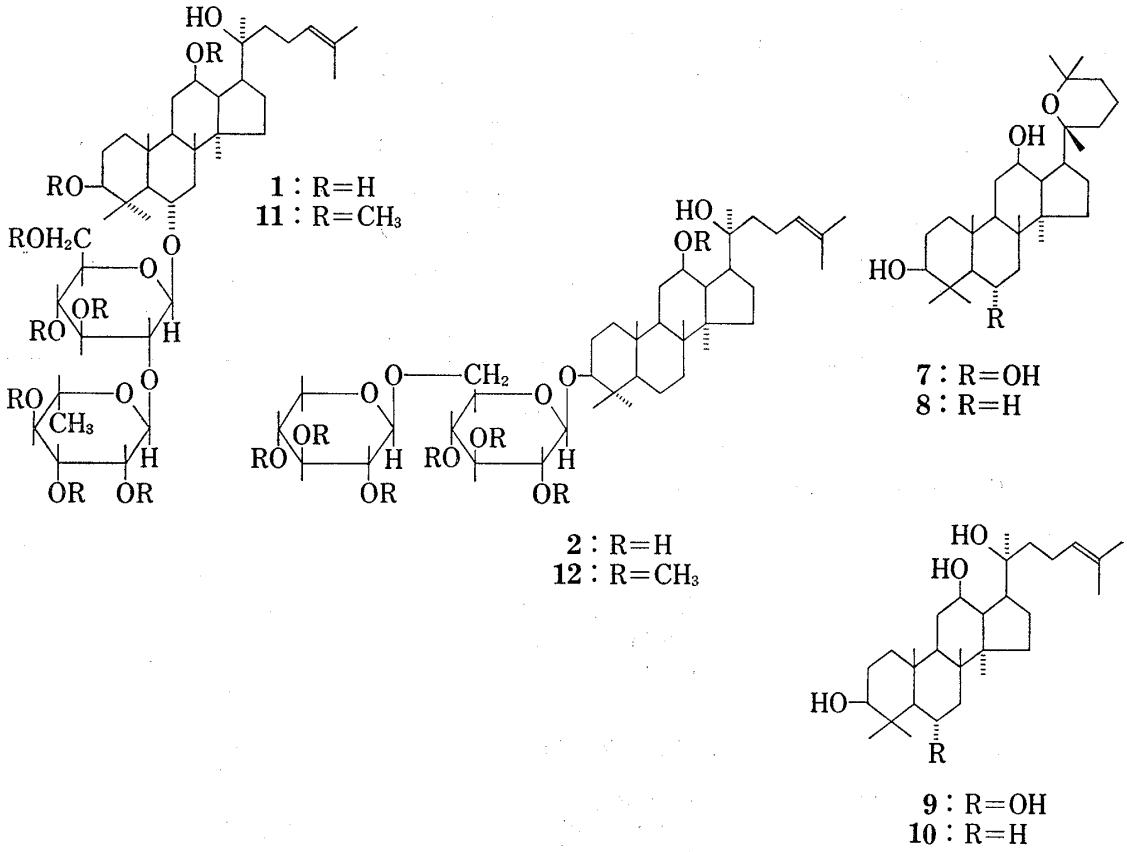


Chart 2

TABLE III

O-Methylated chikusetsusaponin	Properties	mp (°C)	$[\alpha]_D$ (c in $CHCl_3$; °C)	Formula	IR (state) (cm^{-1})	NMR (in $CDCl_3$) anomeric $H_{(\delta)}$
I	colorless needles (acetone)	126	±0.00° (1.01;15°)	$C_{50}H_{88}O_{13}$	3388 (CCl_4) OH band	4.60 (1H, d, J=7 Hz) 5.35 (1H, s, broad)
Ia	a white powder (Aq. methanol)	(74)	−17.24° (1.33;16°)	$C_{48}H_{84}O_{12}$	3388 (CCl_4) OH band	4.28 (1H, d, J=7 Hz) 4.35 (1H, d, J=7 Hz)
Ib	colorless needles (methanol)	83	−9.91° (0.71;24°)	$C_{57}H_{94}O_{18}$	1745 (nujol) ester band	4.15 (1H, d, J=8 Hz) 5.06 (1H, s) 5.30—5.45 (1H overlapped on $H\rangle C=C\langle$)
IVa	colorless needles (EtOH: CH_3COCH_3=1:1)	151	+17.60° (0.84;17°)	$C_{50}H_{82}O_{14}$	1750 (Nujol) ester band	4.33 (1H, d, J=7 Hz) 5.35—5.45 (1H overlapped on $H\rangle C=C\langle$)

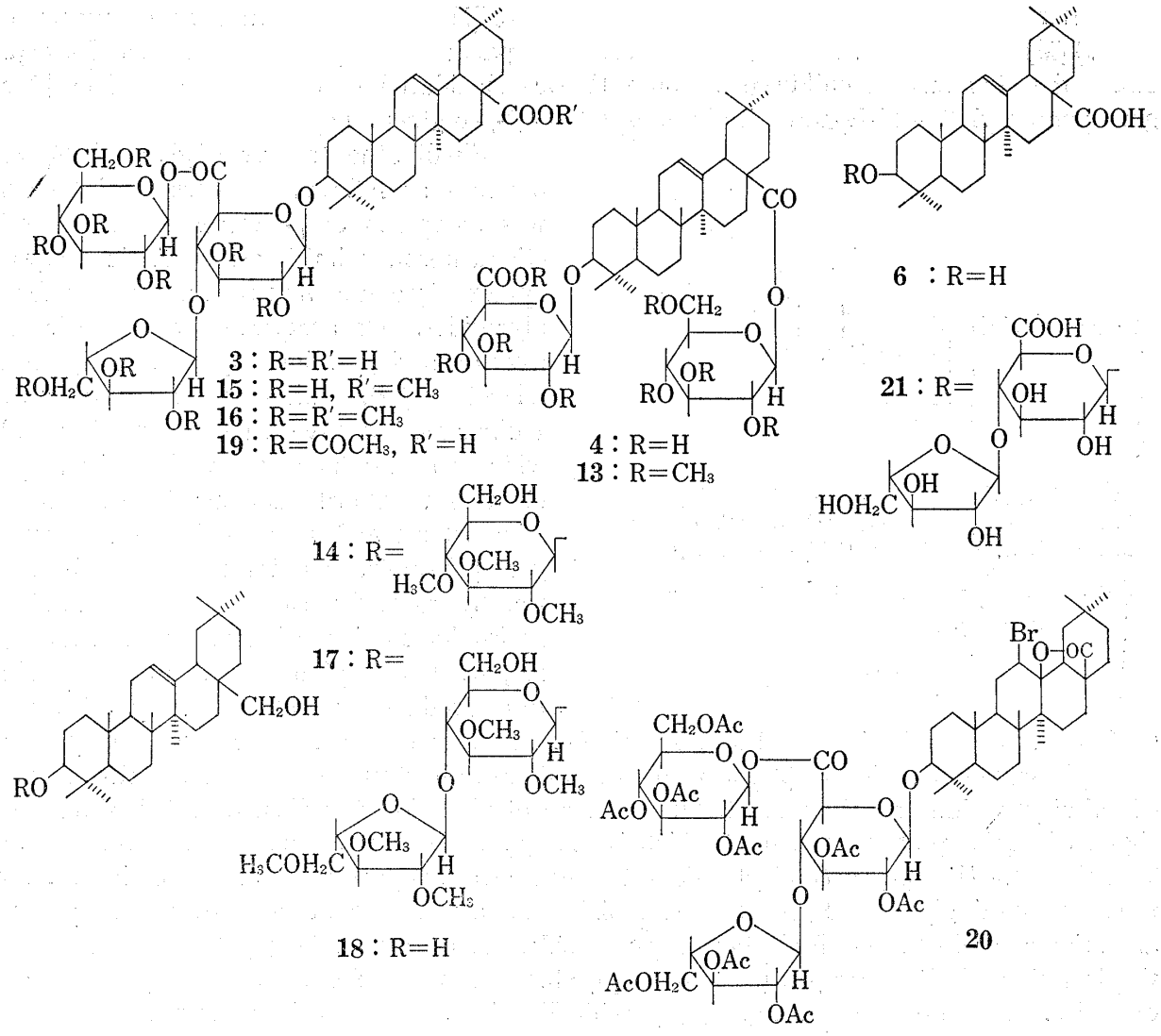
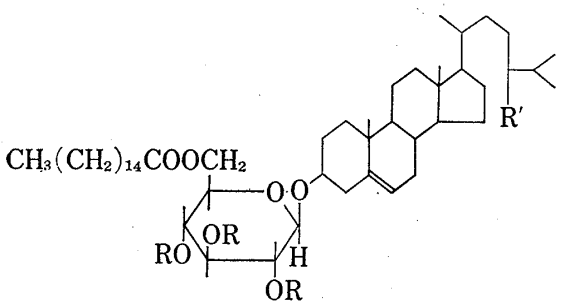


Chart 3

IR_{max}^{Nujol} cm^{-1}: 1740, 1730 (ester), which revealed the presence of methyl ester group at 3.80 ppm ($COOC\underline{H}_3$) in NMR spectrum. As is shown in Table III, chikusetsusaponin Ib gave a per-O-methyl ether (**16**), which afforded erythrodiol bioside (**17**),[3b,10] 2,3,4,6-tetra-O-methylglucose and 2,3,4,6-tetra-O-methylsorbitol by reductive cleavage with lithium aluminium hydride. Methanolysis of erythrodiol bioside with 2N hydrogen chloride gave erythrodiol (**18**), methyl 2,3-di-O-methylglucoside and methyl 2,3,5-tri-O-methylarabinofuranoside, which were identified with the authentic samples. Based on the foregoing experiments, erythrodiol bioside obtained from chikusetsusaponin Ib was established to be identical with that of chikusetsusaponin IV by the direct comparison. The results of degradative experiments about chikusetsusaponin Ib are all parallel with those of chikusetsusaponin IV, so that the former was assumed to be an isomer of the latter. To establish the structure of chikusetsusaponin Ib, a nonaacetate of chikusetsusaponin Ib (**19**), $C_{65}H_{92}O_{27}$, was treated with bromine to form a monobromolactone (**20**),[11a,b] $C_{65}H_{91}O_{27}Br$, which shows the γ-lactonic absorption at 1780 cm^{-1} in IR spectrum. The result obtained above suggests that glucose residue of chikusetsusaponin Ib attached to the carboxyl group of glucuronic acid in ester form in place of C-28 carboxyl group of oleanolic acid. The configuration of glucose was deduced to be β by the comparison of

11) *a*) S.W. Pelletier, N. Adityachaudhury, M. Tomaz, and J.J. Raynald, *J. Org. Chem.*, **30**, 4234 (1965); *b*) Y. Tsukitani, S. Kawanishi, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **21**, 791 (1973).

molecular rotations between chikusetsusaponin Ib($[M]_D=-166.6°$) and prosapogenin of Ib (**21**) ($[M]_D=-55.8°$) obtained from the former by partial hydrolysis with alkaline. Consequently, the structure of chikusetsusaponin Ib was elucidated to be oleanolic acid-(3)-[β-D-glucopyranosyl (1→6)]-[α-L-arabinofuranosyl (1→4)]-β-D-glucuronopyranoside.



5: R=H, R'=CH_3 or C_2H_5 or C_2H_5 (Δ^{22})
22: R=$COCH_3$, R'=CH_3 or C_2H_5 or C_2H_5 (Δ^{22})
23: R=CH_3, R'=CH_3 or C_2H_5 or C_2H_5 (Δ^{22})
24: R=  –CO–, R'=CH_3 or C_2H_5 or C_2H_5 (Δ^{22})

Chart 4

Finally, the structure of glycoside P_1 was deduced as follows. Glycoside P_1, which was composed of β-sitosterol, campesterol, stigmasterol, palmitic acid, and glucose, was apparently assumed to be a mixture of three glycosides. On acetylation with acetic anhydride and pyridine, glycoside P_1 gave a triacetate (**22**), while on methylation by the Kuhn's method gave a trimethyl ether (**23**), which was methanolized with 2N hydrogen chloride to afford β-sitosterol, campesterol, stigmasterol and a partially O-methylated glucopyranosyl palmitate. The palmitate was hydrolyzed with 1N alcoholic potassium hydroxide to give palmitic acid and methyl 2,3,4-tri-O-methylglucoside. Each degradation product was identified by TLC and GLC with the authentic samples, respectively. Besides the degradation experiments, tribenzoyl ester of glycoside P_1 (**24**) was prepared and examined by NMR to confirm the position of palmityl residue on glucose. NMR spectrum of glycoside P_1 tribenzoate (**24**) shows the signal of H_2C-6 protons at δ 4.32 which is 0.28 ppm higher field than that of benzoate.[12] Therefore, the linkage of palmityl residue to glucose was assigned to be at C-6 of glucose, and the structure of glycoside P_1 has been established to be a mixture of (β-sitosterol, campesterol and stigmasterol)-β-D-(6-palmityl)-glucopyranoside. Recently, R. Aneja, *et al.*[12] has reported the isolation of sitosteryl-β-D-(6-O-fattyacyl)-glucopyranoside from the crude phosphatides of Maize. The direct comparison of glycoside P_1 with those glycosides has not been made, but both compounds are assumed to be identical.

The further studies on the minor saponins of *P. japonicum* C. A. Meyer are now in progress.

Experimental

All melting points were taken on a Yanagimoto micro melting point apparatus and uncorrected. IR spectra were measured with a Hitachi Model 215. NMR spectra were measured with a Hitachi Model R-20 High Resolution NMR spectrometer and a Hitachi Model R-22 High Resolution NMR spectrometer with tetramethylsilane as an internal standard. The chemical shifts are reported in δ and the solvent used are indicated. Gas chromatography used was a Hitachi Model K-53 with a hydrogen flame ionization detector.

The *Rf* value were determined by TLC on Silica gel H using solvent A: $CHCl_3$–MeOH–H_2O (65: 35: 10 the lower phase); solvent B: $CHCl_3$–MeOH–AcOEt–H_2O (3: 3: 4: 1.5 the lower phase); solvent C: $CHCl_3$–MeOH–H_2O (7: 3: 1 the lower phase); solvent D: hexane–acetone (3: 2); solvent E: hexane–acetone (3: 1); solvent F: hexane–acetone (5: 1); solvent G: hexane–acetone (2: 1); solvent H: benzene–acetone (4: 1); solvent I: benzene–acetone (18: 1); solvent J: benzene–acetone (20: 1), and spots were detected by spraying 10% H_2SO_4 followed by heating.

Extraction——The dried rhizoma were crushed and treated as shown in Chart 1. Each fraction was examined by TLC as shown in Fig. 1.

Isolation of Saponins and Glycoside P_1——The crude saponin fraction was submitted to column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (65: 35: 10 the lower phase) to afford three fractions (Fr.1—Fr.3). Fr.1 was purified by chromatography on silicic acid with $CHCl_3$–MeOH–H_2O (7: 3: 1 the lower phase) to give pure chikusetsusaponin-IVa. Fr. 2 and Fr. 3 were submitted to column chromatography on silica

12) R. Aneja and P.C. Harries, *Chem. and Physics of Lipids*, **12**, 351 (1974).

gel with $CHCl_3$–MeOH–AcOEt–H_2O (3: 3: 4: 1.5 the lower phase) to afford chikusetsusaponin I, from the former and chikusetsusaponin Ia and Ib from the latter, respectively. Fr. 4 (ether layer) was purified on silica gel with benzene–acetone (3: 2) to afford glycoside P_1.

Properties of Saponins and Glycoside P_1 (Table I)——Chikusetsusaponin I (**1**): *Anal.* Calcd. for $C_{42}H_{72}O_{13}\cdot 2H_2O$: C, 61.43; H, 9.35. Found: C, 61.67; H, 9.16. Chikusetsusaponin Ia (**2**): *Anal.* Calcd. for $C_{41}H_{70}O_{12}\cdot H_2O$: C, 63.70; H, 9.37. Found: C, 63.89; H, 9.07. Chikusetsusaponin Ib(**3**): *Anal.* Calcd. for $C_{47}H_{74}O_{18}$: C, 60.63; H, 8.44. Found: C, 60.66; H, 8.36. Chikusetsusaponin IVa (**4**): *Anal.* Calcd. for $C_{42}H_{66}O_{14}\cdot 2H_2O$: C, 60.81; H, 8.50. Found: C, 61.08; H, 8.29. Glycoside P_1 (**5**): *Anal.* Calcd. for $C_{51}H_{90}O_7$: C, 75.13; H, 11.13. (*cf.* $C_{50}H_{88}O_7$: C, 74.95; H, 11.07. $C_{51}H_{88}O_7$: C, 75.34; H, 10.90) Found: C, 75.56; H, 10.70.

Hydrolysis of Chikusetsusaponin I, Ia, Ib, IVa, and Glycoside P_1——(**1**), (**2**), (**3**), (**4**) were refluxed with 2N HCl (4N HCl: 50% dioxane=1: 1 v/v) on a water bath for 4 hr, respectively. Reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, dried on anhyd. Na_2SO_4 and evaporated. (**5**) was refluxed with 5% KOH in EtOH under N_2 gas flow for 4.5 hr. The reactant was diluted with water. The precipitate formed on cooling was collected by filtration and hydrolyzed with 2N HCl. The filtrate was extracted with ether to give a fatty acid. The aqueous layer was neutralized with Amberlite IR-4B (or Ag_2CO_3) and evaporated *in vacuo* to dryness. The $CHCl_3$ soluble fraction (genins), water soluble fraction (sugars) and fatty acid were identified by TLC, GLC and Mass spectrum by comparing with authentic sample. Genins: *Rf* (solvent E) 0.23 (oleanolic acid); *Rf* (solvent H) 0.29 (panaxadiol), 0.15 (panaxatriol), 0.67 (β-sitosterol, campesterol, stigmasterol). GLC (column: 3% SE-30 on chromosorb w 3 mm $\times$2 m; column temp.: 255°; carrier gas: N_2 30 ml/min; samples: TMS derivatives) t_R (min) 7.7 (β-sitosterol, about 68%), 7.1 (stigmasterol, about 15%), 6.5 (campesterol, about 17%). Sugars: *Rf* (solvent A) 0.13 (glucose), 0.03 (glucuronic acid), 0.20 (glucuronolactone), 0.18 (arabinose), 0.36 (rhamnose), 0.20 (xylose). GLC (column: 5% SE-52 on chromosorb w 6 mm $\times$2 m; column temp.: a) 155°, b) 150°; injection temp.: 265°; carrier gas: N_2 a) 1 kg/cm², b) 0.9 kg/cm²; samples: TMS derivatives) t_R (min) 12.8, 19.4 (glucose), 8.5 (glucuronolactone), 3.8, 4.2, 4.8 (arabinose), 4.2, 5.6 (rhamnose) (condition a), 7.5, 9.3 (xylose) (condition b). Fatty acid: *Rf* (solvent H) 0.52 (palmitic acid). GLC (column: 30% SE-30 on chromosorb 3 mm $\times$2 m; column temp.: 170°; injection temp.: 220°; carrier gas: N_2 38 ml/min; sample: methyl ester) t_R (min) 4.6 (methyl palmitate). Mass Spectrum: M^+ 256.

Partial Hydrolysis of Chikusetsusaponin I and Ia with 50% AcOH——Each saponin was heated with 50% AcOH for 4 hr at 70°. The aqueous solution was extracted with *n*-BuOH saturated with water and the solvent was evaporated *in vacuo*. In case of these, no significant change of the *Rf* value in TLC was observed revealing that no cleavage of the glycoside linkage occurred.

Oxidative Degradation of Chikusetsusaponin I and Ia with $NaIO_4$——Both saponins were treated by the method described in the previous paper.[3c] The products were purified by chromatography on silica gel eluted with *n*-hexane–acetone (2: 1) to afford 20*S*-protopanaxatriol and 20*S*-protopanaxadiol (mp 198°), which were identified by TLC and mixed fusion. TLC: *Rf* (solvent H) 0.09 (20*S*-protopanaxatriol, *cf.* 0.12, 20*R*-protopanaxatriol), *Rf* (solvent E) 0.16 (20*S*-protopanaxadiol, *cf.* 0.20, 20*R*-protopanaxadiol).

Methylation of Chikusetsusaponin I and Ia by Hakomori Method——Both saponins were methylated by the method described in the previous paper.[3c] The property of each O-methyl derivative was listed in Table III. O-Methylchikusetsusaponin I: mp 126°, *Anal.* Calcd. for $C_{50}H_{88}O_{13}$: C, 66.93; H, 9.88. Found: C, 66.62; H, 9.80. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3388 (OH), NMR (in $CDCl_3$) δ: 0.88—1.65 (3H$\times$9, C$\underline{H}_3$), 3.33—3.57 (3H$\times$8, OC$\underline{H}_3$), 4.60 (1H, d, J=7 Hz, anomer H), 5.15 (1H, b, $>C=C<^{\underline{H}}$), 5.35 (1H, bs, anomer H). TLC (solvent B): *Rf* 0.31. O-Methylchikusetsusaponin I was identified with O-methylginsenoside-Rg_2 by comparing TLC, IR and NMR spectra. O-Methylchikusetsusaponin Ia: (mp 74°), *Anal.* Calcd. for $C_{48}H_{84}O_{12}$: C, 67.57; H, 9.72. Found: C, 67.55; H, 9.39. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3388 (OH). NMR (in $CDCl_3$) δ: 0.85—1.12 (3H$\times$6, C$\underline{H}_3$), 1.65—1.75 (3H$\times$2, $>C=C<^{CH_3}_{\underline{CH}_3}$), 3.32—3.65 (3H$\times$7, OC$\underline{H}_3$), 4.28 (1H, d, J=7 Hz, anomer H), 4.35 (1H, d, J=7 Hz, anomer H), 5.14 (1H, b, $>C=C<^{\underline{H}}$).

Methanolysis of O-Methylchikusetsusaponin I and Ia with Methanolic 6% HCl——Both compounds were methanolyzed with methanolic 6% HCl refluxing for 2 hr, respectively. The reactant was neutralized with Ag_2CO_3 and evaporated to dryness. The residue was examined by TLC and GLC (column: 5% NPGS on chromosorb w 3 mm $\times$2 m; column temp.: 170°; carrier gas: N_2 1 kg/cm²). Methyl sugars of chikusetsusaponin I: *Rf* 0.19 (solvent G), t_R (min) 6.5 (methyl 3,4,6-tri-O-methylglucoside); *Rf* 0.53 (solvent G), t_R (min) 1.4 (methyl 2,3,4-tri-O-methylrhamnoside). Methylsugars of chikusetsusaponin Ia: *Rf* 0.20 (solvent E), t_R (min) 14.5 (methyl 2,3,4-tri-O-methylglucoside); *Rf* 0.22 (α), 0.48 (β), t_R (min) 2.6 (β), 3.1 (α) (methyl 2,3,4-tri-O-methylxyloside).

Catalytic Reduction of O-Methylchikusetsusaponin Ia——O-Methylchikusetsusaponin Ia was catalytically reduced on PtO_2 in EtOH and the solution was filtered. The filtrate was evaporated *in vacuo* and the residue was purified by reprecipitation from EtOH–H_2O. Dihydro-O-methylchikusetsusaponin Ia was examined by NMR. NMR (in $CDCl_3$) δ: 0.85—1.40 (3H$\times$8, s, C$\underline{H}_3$) (*cf.* O-methylchikusetsusaponin Ia).

Hydrolysis of Dihydro-O-methylchikusetsusaponin Ia——The compound was hydrolyzed with conc. HCl at room temperature for 7.5 hr. The reactant was diluted with water and extracted with ether. The extract was purified by preparative TLC (solvent H) and crystallized with aqueous acetone afford 12-O-methyldihydroprotopanaxadiol, colorless needles, mp 143—145°, which was identified with authentic sample by TLC, mixed fusion and IR spectra. TLC (solvent H) *Rf* 0.46. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3388 (hydrogen bonding OH), 3626 (free OH).

Enzymatic Hydrolysis of Chikusetsusaponin III with Pectinase——Chikusetsusaponin III (500 mg) was dissolved in McIlvaine buffer solution with addition 400 mg of pectinase (Sigma), which was adjusted to pH 4.2 and the mixture was kept at 38° for 48 hr. The reaction mixture was extracted with *n*-BuOH. The extract was evaporated *in vacuo* and the residue was crystallized from $CHCl_3$–MeOH–AcOEt–H_2O (3: 3: 4: 1.5, the lower phase) to afford prosapogenin, colorless needles, mp 194°, $[\alpha]_D^{20}$ −3.60° (c=1.41, MeOH). *Anal.* Calcd. for $C_{41}H_{70}O_{12}\cdot H_2O$: C, 63.70; H, 9.37. Found: C, 63.79; H, 9.09. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1630 (C=C), which was identified with chikusetsusaponin Ia by TLC (*Rf* 0.16, solvent C), mixed fusion and comparing IR spectra.

Methylation of Chikusetsusaponin IVa and Chikusetsusaponin Ib with CH_2N_2——Both saponins were treated as described in the previous paper[3b] to afford a monomethyl ester, a white powder from aqueous EtOH. The methylate of chikusetsusaponin IVa (mp 206—207°) was identified with saponin C monomethyl ester by TLC (*Rf* 0.53, solvent C) and by comparison of IR and NMR spectra.

Monomethyl ester of chikusetsusaponin Ib: (mp 172°), *Anal.* Calcd. for $C_{48}H_{76}O_{18}\cdot 4H_2O$: C, 56.90; H, 7.96. Found: C, 57.07; H, 7.70. IR ν_{max}^{Nujol} cm^{-1}: 1740, 1730 (COOR). NMR (in $CDCl_3$–CD_3OD) δ: 3.80 (3H×1, s, $COOC\underline{H}_3$).

Per-O-methylation of Chikusetsusaponin IVa and Chikusetsusaponin Ib——According to the previous paper,[1] both saponins were methylated by Kuhn method. The products were purified by chromatography on silica gel using *n*-hexane–acetone (5: 1). Per-O-methylchikusetsusaponin IVa was crystallized from EtOH–CH_3COCH_3 to give a colorless needles, mp 151°, $[\alpha]_D^{17}$ +17.60° (c=0.84, $CHCl_3$), *Anal.* Calcd. for $C_{50}H_{82}O_{14}$: C, 66.19; H, 9.01. Found: C, 66.41; H, 9.09. IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1750 (COOR). NMR (in $CDCl_3$) δ: 0.78—1.12 (3H×7, s, $C\underline{H}_3$), 3.34—3.78 (3H×8, s, $OC\underline{H}_3$), 4.33 (1H, d, J=7 Hz anomer H), 5.35—5.45 (2H, b, $>C=C<^{\underline{H}}$, anomer H). It was identified with per-O-methylsaponin C by comparing TLC (*Rf* 0.83, solvent H), IR and NMR spectra. Per-O-methylchikusetsusaponin Ib was crystallized from MeOH to give a colorless needles, mp 83°, $[\alpha]_D^{24}$ −9.91° (c=0.71, $CHCl_3$), *Anal.* Calcd. for $C_{57}H_{94}O_{18}$: C, 64.14; H, 8.88. Found: C, 64.71; H, 8.80. IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1745 (COOR). NMR (in $CDCl_3$) δ: 0.75—1.50 (3H×7, s, $C\underline{H}_3$), 3.30—3.75 (3H×10, s, $OC\underline{H}_3$), 4.15 (1H, d, J=8 Hz, anomer H), 5.06 (1H, s, anomer H), 5.30—5.45 (2H, b, $>C=C<^{\underline{H}}$, anomer H).

Reductive Cleavage of Per-O-methylate with $LiAlH_4$——Each permethylate was reduced by the method described in the previous paper.[1] The ether soluble fraction was purified by chromatography on silica gel using *n*-hexane–acetone (2: 1) followed by precipitation from dilute MeOH.

Per-O-methylchikusetsusaponin-IVa yielded erythrodiol 2,3,4-tri-O-methylglucoside, while per-O-methylchikusetsusaponin Ib gave erythrodiol bioside. Erythrodiol bioside: A white powder, (mp 113—114°), $[\alpha]_D^{25}$ +4.34° (c=1.61, $CHCl_3$). *Anal.* Calcd. for $C_{46}H_{78}O_{11}\cdot H_2O$: C, 66.96; H, 9.67. Found: C, 67.25; H, 9.38.

The $CHCl_3$ soluble fraction was examined by TLC to reveal the presence of two methylated monosaccharides, which were identified with authentic samples of 2,3,4,6-tetra-O-methylglucose and 2,3,4,6-tetra-O-methylsorbitol by TLC (solvent G) and GLC (column: 5% NPGS on chromosorb w 3 mm×2 m; column temp.: 160°; carrier gas: N_2 flow 1 kg/cm^2; samples: TMS derivatives). 2,3,4,6-Tetra-O-methylglucose: *Rf* 0.24, t_R (min) 3.2; 2,3,4,6-tetra-O-methylsorbitol: *Rf* 0.11, t_R (min) 4.8.

Methanolysis of Erythrodiol 2,3,4-Tri-O-methylglucoside and Erythrodiol Bioside——Each was refluxed with methanolic 2N HCl for 4 hr and the reactant was treated as usual. Erythrodiol 2,3,4-tri-O-methyl-D-glucoside yielded erythrodiol and methyl 2,3,4-tri-O-methylglucoside, while erythrodiol bioside gave erythrodiol, methyl 2,3,5-tri-O-methyl-L-arabinofuranoside and methyl 2,3-di-O-methyl-D-glucoside. Erythrodiol and methylsugars were identified with authentic samples by mixed fusion and by TLC (solvent G) and GLC (same condition as described above). Erythrodiol: colorless needles from acetone, mp 236°, *Rf* 0.60. Methyl 2,3,4-tri-O-methylglucoside: *Rf* 0.18; t_R (min) 10.0. Methyl 2,3,5-tri-O-methylarabinofuranoside: *Rf* 0.58; t_R (min) 1.8, 2.4. Methyl 2,3-di-O-methylglucoside: *Rf* 0.13, 0.08; t_R (min) 4.0, 5.2.

Acetylation of Chikusetsusaponin Ib——A solution of chikusetsusaponin Ib in pyridine and Ac_2O was allowed to stand for 48 hr at room temperature. The reactant was worked up as usual and the product was purified by chromatography on silica gel using benzene–acetone (5: 1) to afford a nonaacetate, a white powder from *n*-hexane, (mp 98°), $[\alpha]_D^{24}$ −12.5° (c=0.48 MeOH). *Anal.* Calcd. for $C_{65}H_{92}O_{27}\cdot H_2O$: C, 59.00; H, 7.16. Found: C, 59.20; H, 7.01, NMR (in $CDCl_3$) δ: 0.76—1.12 (3H×7, s, $C\underline{H}_3$), 2.02—2.12 (3H×9, s, $OCOC\underline{H}_3$).

Bromination of Chikusetsusaponin Ib Nonaacetate——To a solution of Ib acetate and AcONa in 90% AcOH was added dropwise of bromine solution with stirring. The reactant was poured into water containing a little of sodium bisulfite and the resulted precipitate was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water and evaporated to give a solid which was purified by TLC chromatography on Silica gel

H using *n*-hexane–acetone (7:3) to afford a bromolactone, a white powder (mp 93—94°), IR $\nu_{max}^{CCl_4}$ cm^{-1}: OH (nil), 1780 (γ-lactone), 1730 (COOR). *Anal.* Calcd. for $C_{65}H_{91}O_{27}Br$: C, 56.49; H, 6.64. Found: C, 57.03; H, 6.88.

Partial Hydrolysis of Chikusetsusaponin Ib with 10% MeOH-KOH——Chikusetsusaponin Ib was refluxed with 10% KOH in MeOH under N_2 gas flow for 1 hr, and the reaction mixture was neutralized with dilute HCl and extracted with *n*-BuOH saturated with water. The extract was evaporated *in vacuo* and purified by reprecipitation from MeOH to give a prosapogenin, a white powder, (mp 195—197°), $[\alpha]_D^{29}$ −7.32° (c=0.87, MeOH). *Anal.* Calcd. for $C_{41}H_{64}O_{13}\cdot 4H_2O$: C, 58,47; H, 8.43. Found: C, 58.23; H, 8.19. IR ν_{max}^{Nujol} cm^{-1}: 1720 (COOH), 1688 (COOH).

Acetylation of Glycoside P_1——Glycoside P_1 was acetylated by the same method described above. The product was purified by chromatography on silica gel using benzene-acetone (18:1) and crystallized from ether to afford a triacetate, colorless needles, mp 123—125°, $[\alpha]_D^{20}$ +26.52° (c=0.57, $CHCl_3$), *Anal.* Calcd. for $C_{57}H_{96}O_{10}$: C, 72.72; H, 10.27. Found: C, 72.70; H, 9.86. IR $\nu_{max}^{CCl_4}$ cm^{-1}: OH (nil.), 1758 (COOR). NMR (in $CDCl_3$) δ: 0.68—1.00 (3H×7, b, C$\underline{H}_3$), 1.25 (s, −(C$\underline{H}_2$)$_n$−), 2.00—2.08 (3H×3, s, COOC$\underline{H}_3$), 3.70 (2H, bm, C_5'–H, C_3–H), 4.19 (2H, b, C_6'–C$\underline{H}_2$–), 4.55 (1H, d, J=7 Hz, C_1'–H), 4.80—5.20 (3H, d, C_2', C_3', C_4'–H), 5.34 (1H, b, >C=C<$^{\underline{H}}$).

Per-O-methylation of Glycoside P_1——Glycoside P_1 was methylated by Kuhn method and the mixture was purified by chromatography on silica gel using benzene–acetone (20:1) and crystallized from aqueous EtOH to afford a tri-O-methyl ether, colorless needles, mp 61°, $[\alpha]_D^{20}$ −5.36° (c=1.31, $CHCl_3$). *Anal.* Calcd. for $C_{54}H_{96}O_7$: C, 75.65; H, 11.28. Found: C, 75.86; H, 10.95. IR $\nu_{max}^{CCl_4}$ cm^{-1}: OH (nil.), 1735 (COOR), NMR (in $CDCl_3$) δ: 0.68—1.00 (3H×7, b, C$\underline{H}_3$), 1.25 (s, −(C$\underline{H}_2$)$_n$−), 3.52—3.65 (3H×3, s, OC$\underline{H}_3$), 4.20—4.45 (2H+1H, m, C_1'–H, C_6'–C$\underline{H}_2$–), 5.35 (1H, b, >C=C<$^{\underline{H}}$).

Methanolysis of O-Methylglycoside P_1 with Methanolic 2N HCl-Ether——O-Methylglycoside P_1 was methanolyzed with methanolic 2N HCl–ether (2:1 v/v) refluxing for 4 hr. The reaction mixture was neutralized with Ag_2CO_3 and evaporated to dryness. The residue was purified by chromatography on silica gel eluted with benzene–acetone (4:1) to afford sterols (β-sitosterol, stigmasterol,campesterol) and methyl glucopyranosylpalmitate. The sterols were identified with authentic samples by TLC and GLC.

Hydrolysis of Methyl Glucopyranosyl Palmitate with Methanolic 1N KOH-Ether——The palmitate was refluxed with methanolic 1N KOH in ether under N_2 flow for 1 hr. The reactant was diluted with water, then removed the organic solvent and neutralized with diluted HCl to pH 6.0, which was extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated *in vacuo* and the residue was purified by chromatography on silica gel using benzene–acetone (4:1) to afford palmitic acid and a methyl sugar. The latter was identified by TLC (solvent H) and GLC (column: 5% NPGS 3 mm×2 m on chromosorb w; column temp.: 165°; carrier gas: N_2 20 ml/min). Methyl 2,3,4-tri-O-methylglucoside: *Rf* 0.12, t_R (min) 18.1.

Tri-O-benzoyl Glycoside P_1——A solution of glycoside P_1 in anhydrous pyridine was cooled in an ice bath and treated dropwise with benzoyl chloride (excess). After 2 hr at room temperature, ice water was added and the mixture allowed to stand overnight. The precipitate was filtered, washed with water, and dried. Crystallization from acetone–methanol gave colorless prisms, mp 115°, $[\alpha]_D^{20}$ +9.01° (c=1.01, $CHCl_3$), IR ν_{max}^{Nujol} cm^{-1}: OH (nil), 1735 (COOR). NMR (in $CDCl_3$) δ: 0.70—1.00 (3H×7, C$\underline{H}_3$), 1.30 (s, −C$\underline{H}_2$–), 4.32 (2H, d, J=5 Hz, C_6'–C$\underline{H}_2$–) (*cf.* Tetra-O-benzoyl-β-sitosterol-D-glucoside: 4.60 (C_6'–C$\underline{H}_2$–)).

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