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Legume Saponin of *Gleditsia japonica* Miquel. II. Desmonoterpenyl Glycoside of Echinocystic Acid

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Three triterpenoid saponins were isolated from legumes of *Gleditsia japonica* cv. 'Saponifera' (Leguminosae). These saponins are rare examples of triterpenoid saponins containing monoterpenes. The desmonoterpenyl compound, $C_{74}H_{120}O_{38}$, $[\alpha]_D^{23}$ $-42.3°$ (MeOH), which was obtained from them by alkali hydrolysis with K_2CO_3, was characterized as a 3,28-O-bisglycoside on the basis of physical data and degradation products.

Keywords——bisdesmoside; echinocystic acid; triterpenoid saponin; *Gleditsia japonica*; Leguminosae

In the preceding paper[2] it was reported that there are two types of *Gleditsia japonica* Miq.; one of them, named *G. japonica* cv. 'Saponifera', contains a great deal of saponin and the other contains very little, if any. Moreover, we reported the isolation of three new triterpenoid saponins, *Gleditsia* saponins B, C and D (GS–B, GS–C and GS–D), from the legume of *G. japonica* cv. 'Saponifera.' The prosapogenin, which was obtained from them by alkali hydrolysis with 20% potassium hydroxide, was characterized as echinocystic acid 3-O-glycoside (GS–C″), I.

Afterwards, Shibata *et al.* indicated[3] that two monoterpene carboxylic acids are attached to the molecule of GS–C, and at least one of them is an α,β-unsaturated carboxylic acid. We examined[4] the structures of GS–B and GS–D, and it was ascertained that similar monoterpene carboxylic acids are attached to them. These monoterpenes are unstable and are detached from the saponins or decomposed on treatment with even a weak base, such as potassium carbonate or potassium bicarbonate.

This paper describes the structure elucidation of the desmonoterpenyl compound, GS–C′ (II), which was obtained from GS–C by alkali hydrolysis with 5% potassium carbonate in ethanol. GS–C′ (II), $C_{74}H_{120}O_{38}$, $[\alpha]_D^{23}$ $-42.3°$ (in methanol), obtained as a white powder from methanol–ether, was hydrolyzed with 2N sulfuric acid to afford echinocystic acid and four kinds of sugars (glucose, arabinose, xylose and rhamnose). II was methylated by Hakomori's method[5] to afford the permethylate (III). Its nuclear magnetic resonance (NMR) spectrum showed eight anomeric proton signals at δ 4.31 (d, $J=7$ Hz), 4.60 (d, $J=7$ Hz), 4.64 (d, $J=5$ Hz), 4.73 (d, $J=8$ Hz), 4.77 (d, $J=7$ Hz), 4.91 (broad s), 5.25 (broad s), 5.63 (d, $J=7$ Hz). On methanolysis of III with 2N hydrogen chloride in dried methanol, seven kinds of methylated monosaccharides were obtained and identified by comparison with authentic samples on thin-layer chromatography (TLC) and gas liquid chromatography (GLC) as shown in Table I. Reductive cleavage of the permethylate (III) with lithium aluminum hydride afforded

1) Location: *Nakauchi-cho 5, Misasagi, Yamashina-ku, Kyoto, 607, Japan.*
2) T. Konoshima, H. Fukushima, H. Inui, K. Sato, and T. Sawada, *Phytochemistry*, in press.
3) K. Koyama, T. Okuyama, K. Takahashi, and S. Shibata, Abstracts of papers, The 25th Annual Meeting of the Japanese Society of Pharmacognosy, Fukuoka, Oct. 1978, p. 11; Y. Okada, K. Koyama, T. Okuyama, K. Takahashi, and S. Shibata, Abstracts of Papers, The 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, Aug. 1979, p. 166.
4) T. Konoshima, K. Sato, M. Yonezawa, and T. Sawada, The 99th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, Aug. 1979, p. 165.
5) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

a compound (IV) and methylated oligosaccharide (V). Methylated monosaccharides obtained from IV by methanolysis (methyl 2,3,4-tri-O-Me-D-xylopyranoside, methyl 3,4-di-O-Me-L-arabinopyranoside and methyl 2,3,4-tri-O-Me-D-glucopyranoside) were identified by comparison with methylated monosaccharides obtained from the permethylate of the prosapogenin, GS-C'' (I). Compound (V), $C_{40}H_{74}O_{22}$, syrup, $[\alpha]_D^{28}$ $-65.5°$ (in chloroform), showed the presence of four anomeric protons (4.64 (d, $J=7$ Hz), 4.70 (d, $J=7$ Hz), 4.87 (broad s), 5.05 (broad s)) in the NMR spectrum. On methanolysis of V in the manner described for III, five methylated monosaccharides were obtained as shown in Table I. Furthermore, methyl 3,4-di-O-Me-D-glucopyranoside was obtained from III by methanolysis, and 3,4-di-O-Me-D-glucitol was obtained from V. Therefore, it was concluded that glucose of the oligosaccharide moiety (V) was attached at the C_{28}-carboxyl group of echinocystic acid, and has two side chains of sugars at the C_2 and C_6 positions.

Table I. O-Methylated Monosaccharides obtained by Methanolysis

	III	IV	I[a]	V	VI	VII	VIII	IX
/Me 2,3,4-tri-O-Me-D-glu. pyr.	+	+	+	−	−	−	−	−
Me 3,4-di-O-Me-D-glu. pyr.	+	−	−	−	−	−	−	−
Me 3,4-di-O-Me-L-ara. pyr.	+	+	+	−	−	−	−	−
Me 2,3,4-tri-O-Me-D-xyl. pyr.	+	+	+	+	−	−	−	−
Me 2,4-di-O-Me-D-xyl. pyr.	+	−	−	+	+	−	−	−
Me 2,3,4-tri-O-Me-L-rha. pyr.	+	−	−	+	+	+	+	+
Me 2,3-di-O-Me-L-rha. pyr.	+	−	−	+	+	+	−	−
3,4-Di-O-Me-D-glucitol[b]	−	−	−	+	+	+	+	−
1,2,3,4,5-Penta-O-Me-D-glucitol	−	−	−	−	−	−	−	+

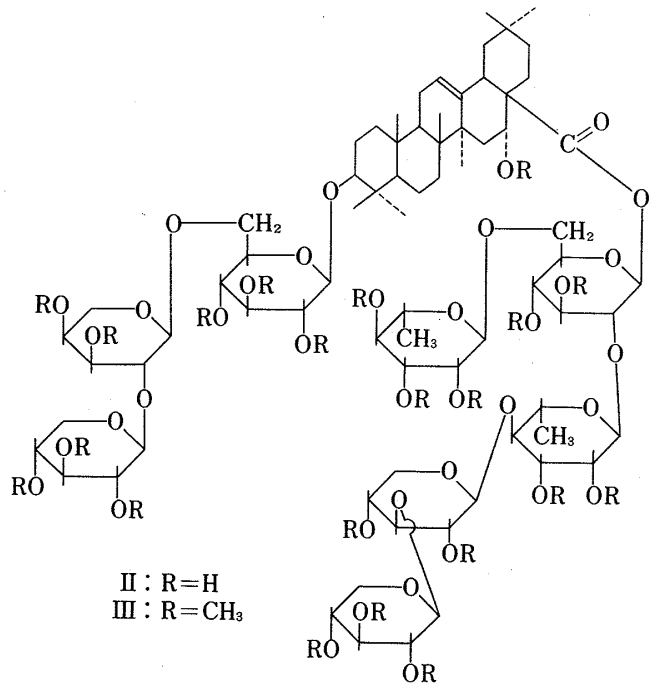
a) Permethylate of I.
b) Presumed to be this compound on the basis of TLC and GLC results.

The structure of the oligosaccharide moiety was deduced as follows. Attempted hydrolyses with cellulase, β-glucosidase and hesperidinase were unsuccessful. For that reason, we tried partial methanolysis[6] of V. On methanolysis with 0.5 N hydrogen chloride in dried methanol at room temperature, V afforded VI and VII. The NMR spectrum of VI showed the presence of three anomeric protons (4.66 (d, $J=7$ Hz), 4.87 (broad s), 5.06 (broad s)). A complete methanolysis of VI afforded methyl 2,3,4-tri-O-Me-L-rhamnopyranoside, methyl 2,3-di-O-Me-L-rhamnopyranoside, methyl 2,4-di-O-Me-D-xylopyranoside and a glucitol derivative. The NMR spectrum of VII showed the presence of two anomeric protons (4.87 (broad s), 5.09 (broad s)). On methanolysis, VII afforded methyl 2,3,4-tri-O-Me-L-rhamnopyranoside, methyl 2,3-di-O-Me-L-rhamnopyranoside and a glucitol derivative. Consequently, it was deduced that the oligosaccharide moiety (V) has the xylose[1]—[3]xylose[1]—[4]rhamnose sugar chain and rhamnose on the glucitol.

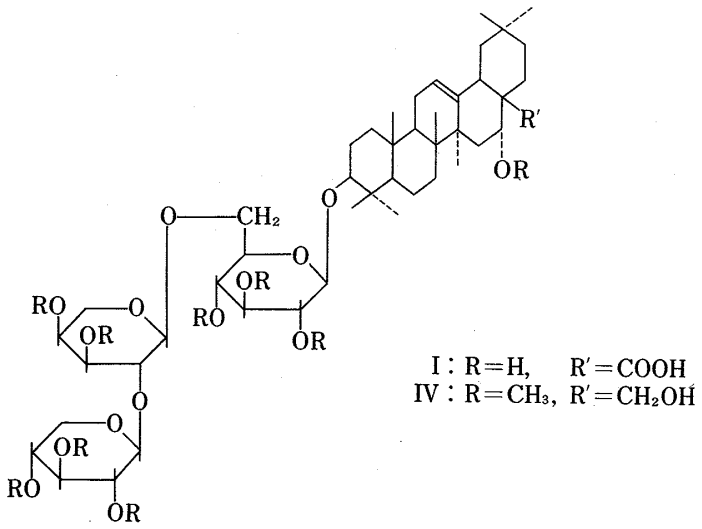
On methanolysis with 1 N hydrogen chloride in dried methanol at room temperature, VII afforded partially methanolyzed products, *i.e.*, methyl 2,3-di-O-Me-L-rhamnopyranoside and VIII. One anomeric proton was seen at δ 4.85 (broad s) in the NMR spectrum of VIII. Methyl 2,3,4-tri-O-Me-L-rhamnopyranoside and a glucitol derivative were obtained from VIII by complete methanolysis with 2 N hydrogen chloride. VIII was methylated by Hakomori's method to afford the permethylate (IX). IX was methanolyzed with 2 N hydrogen chloride to afford methyl 2,3,4-tri-O-Me-L-rhamnopyranoside and 1,2,3,4,5-penta-O-Me-D-glucitol, which were identified by comparison with authentic samples.

Therefore, in the oligosaccharide (V), rhamnose is attached at C_6 of the glucitol and xylosyl-(1→3)-xylosyl(1→4)-rhamnose is attached at C_2 of the glucitol.

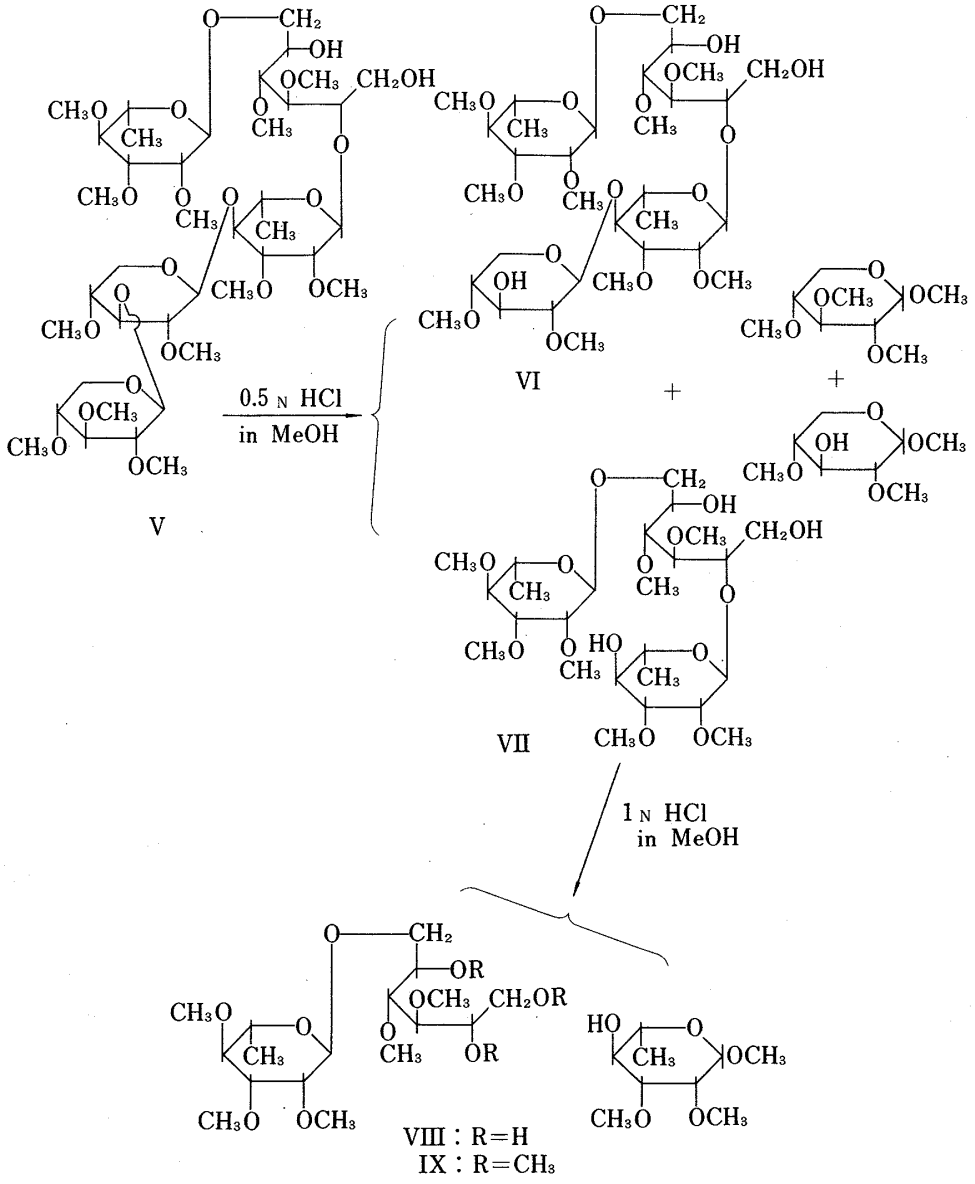
6) A. Tada, Y. Kaneiwa, and J. Shoji, *Chem. Pharm. Bull.*, 23, 2965 (1975).



II : R=H
III : R=CH$_3$



I : R=H, R′=COOH
IV : R=CH$_3$, R′=CH$_2$OH



V

VI + VII

VIII : R=H
IX : R=CH$_3$

Chart 1

The configuration of each sugar was deduced as follows. In the NMR spectrum of III, the anomeric proton at the lowest field, δ 5.63 (d, $J=7$ Hz), was assigned to the glucose attached at the C_{28}-carboxyl group of echinocystic acid. The configuration of xylose and rhamnose were deduced from the coupling constants of anomeric protons in the NMR spectra of III, V and VI, and from the molecular rotation differences[7] among the compounds. The two xyloses are of β-configuration and the two rhamnoses are of α-configuration, as shown in Table II.

Table II. Assignment of Configuration of Sugars

	NMR anomeric H (δ)	Difference of $[M]_D$
xylose[1]——[3]xylose	4.70 $J=7$ Hz β	(V)−(VI) −214[a] β
xylose[1]——[4]rhamnose	4.64 $J=7$ Hz β	(VI)−(VII) −126[b] β
	4.66 $J=7$ Hz β	
rhamnose[1]——[2]glucose	5.05 broad s.	(VII)−(VIII) −129[c] α
	5.06 broad s.	
rhamnose[1]——[6]glucose	4.87 broad s.	(IX)−P.M.G.[e] −130[d] α
	4.85 broad s.	

a) Me 2,3,4-tri-O-methyl-β-D-xylopyranoside[8] $[M]_D$ −150.
Me 2,3,4-tri-O-methyl-α-D-xylopyranoside $[M]_D$ +86.
b) Me 2,4-di-O-methyl-β-D-xylopyranoside[6] $[M]_D$ −158.
c) Me 2,3-di-O-methyl-α-L-rhamnopyranoside[9] $[M]_D$ −78.
d) Me 2,3,4-tri-O-methyl-α-L-rhamnopyranoside[9] $[M]_D$ −99.
Me 2,3,4-tri-O-methyl-β-L-rhamnopyranoside $[M]_D$ +230.
e) P.M.G.=1,2,3,4,5-penta-O-methyl-D-glucitol $[M]_D$ +15.

Thus the structure of the desmonoterpenyl compound, GS–C′ (II), obtained from GS–C is established as echinocystic acid-3-O-[β-D-xylopyranosyl(1→2)-α-L-arabinopyranosyl(1→6)-β-D-glucopyranoside]-28-O-[β-D-xylopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)][α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside.

Studies on the structures of the monoterpenes and positions of their attachment are in progress. GS–B and GS–D gave the same desmonoterpenyl compound, GS–C′ (II), as was obtained from GS–C by hydrolysis with a weak base, and so it is presumed that the differences among the structures of GS–B, C and D arise in the unidentified monoterpenes.

Experimental

Melting points are uncorrected. The NMR spectra were measured in $CDCl_3$ with TMS as an internal standard. GLC was carried out on 200×0.3 cm 15% PEGS on Chromosorb W; column temperature 195°; carrier gas N_2 (30 ml/min).

Properties of GS-B, GS-C and GS-D[10]——GS-B was a white powder, $[\alpha]_D^{30}$ −25.9° ($c=1.0$ MeOH), mp 187—189°. IR ν_{max}^{KBr} cm^{-1}: 3500—3600 (OH), 1725 (COOR). GS-C was a white powder, $[\alpha]_D^{19}$ −24.2° ($c=1.02$ MeOH), mp 192—193°. IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1725 (COOR). GS-D was a white powder, $[\alpha]_D^{30}$ −22.5° ($c=0.99$ MeOH), mp 185—188°. IR ν_{max}^{KBr} cm^{-1}: 3400—3600 (OH), 1725 (COOR).

Hydrolysis of GS-C with 5% K_2CO_3 in EtOH——A solution of GS-C (30 mg) in EtOH (10 ml) was treated with 5% K_2CO_3 (10 ml) and the mixture was refluxed for 1 hr. The reaction mixture was then cooled to room temperature and neutralized with Dowex 50W×8. The neutral solution was concentrated under reduced pressure, and the residue was extracted with AcOEt. The aqueous layer was extracted with *n*-BuOH saturated with water, and the organic layer was evaporated to dryness under reduced pressure. The residue was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (65:35:10) (the lower layer) to afford crude II (20 mg), which was precipitated from MeOH–Et_2O to give a white powder, mp 219—221°, $[\alpha]_D^{23}$ −42.3° ($c=0.98$ MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1720 (COOR). *Anal.* Calcd for $C_{74}H_{120}O_{38}\cdot CH_3OH$: C, 54.27; H, 7.68.

7) W. Klyne, *Biochem. J.*, **47**, xli (1950).
8) O. Wintersteiner and A. Klingsberg, *J. Am. Chem. Soc.*, **71**, 939 (1949).
9) H. Okabe, N. Koshito, K. Tanaka, and T. Kawasaki, *Chem. Pharm. Bull.*, **19**, 2394 (1971).
10) The extraction and isolation were reported in the preceding paper.[1]

Found: C, 54.21; H, 8.00. The AcOEt layer was concentrated under reduced pressure, and several compounds were obtained. These compounds appeared to be monoterpenoids from the results of ^{13}C-NMR spectroscopy, but structure elucidations are in progress. Compound (II) was also obtained from GS-B and GS-D in the same manner.

Hydrolysis of II with 2 N H_2SO_4 in EtOH——A solution of II (100 mg) in EtOH (20 ml) was treated with 2 N H_2SO_4 (20 ml) and the mixture was refluxed for 3 hr. The solution was concentrated to 20 ml under reduced pressure, water was added, and the solution was extracted with Et_2O. The aqueous layer was neutralized with Amberlite IR 45, and concentrated to 1 ml. The residue was found to be a mixture of glucose, arabinose, xylose and rhamnose by PPC and TLC. PPC: solvent, iso-PrOH–*n*-BuOH–H_2O (7: 1: 2); detection with aniline hydrogen phthalate. TLC: solvent, AcOEt–iso-PrOH–H_2O (32: 12: 6); detection with aniline hydrogen phthalate. The organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. The residue was recrystallized from MeOH to give echinocystic acid (21 mg), mp 308—309°, which was identified by comparison with a sample obtained by the hydrolysis of GS-C (IR spectroscopy, TLC and mixed melting point determination).

Permethylation of II——According to Hakomori's method, NaH (500 mg) was stirred with dimethylsulfoxide (DMSO, 45 ml) at 80° for 30 min under N_2 gas. To this reagent (15 ml), a solution of II (100 mg) in DMSO (5 ml) was added, and the mixture was stirred for 1 hr at room temperature under N_2 gas. CH_3I (15 ml) was added and the whole was stirred for 3 hr at room temperature. The reaction mixture was poured into ice-water, and the mixture was extracted with Et_2O. The organic layer was washed with 5% $Na_2S_2O_3$ and water, dried over $MgSO_4$ and concentrated to afford a syrup (120 mg). This syrup was purified by preparative TLC (silica gel, benzene–acetone (5: 2)) to afford the permethylate (III), $[\alpha]_D^{11}$ −50.0° (c=0.86 $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: OH (nil), 1720 (COOR). NMR (30% pyridine-d_5 in $CDCl_3$) δ: 0.75—1.35 (21H, m, 7×C$\underline{H}_3$), 3.40—3.70 (63H, m, 21×OC$\underline{H}_3$), 4.31 (1H, d, J=7 Hz, anomeric H), 4.60 (1H, d, J=7 Hz, anomeric H), 4.64 (1H, d, J=5 Hz, anomeric H), 4.73 (1H, d, J=8 Hz, anomeric H), 4.77 (1H, d, J=7 Hz, anomeric H), 4.91 (1H, broad s, anomeric H), 5.25 (1H, broad s, anomeric H), 5.44 (1H, broad s, -C$\underline{H}$=C<), 5.63 (1H, d, J=7 Hz, anomeric H). *Anal.* Calcd for $C_{94}H_{160}O_{38}\cdot H_2O$: C, 58.91; H, 8.52. Found: C, 58.82; H, 8.78.

Methanolysis of III——A solution of III (30 mg) in methanolic 2 N HCl (5 ml) was refluxed for 2.5 hr. The reaction mixture was cooled to room temperature, neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness, and the residue was examined and identified (by comparison with authentic samples) by TLC and GLC. TLC: solvent, benzene–acetone (3: 1); detection with ceric sulfate. GLC (t_R); 1′39″, 2′28″ (methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside), 1′47″, 2′10″ (methyl 2,3,4-tri-O-methyl-D-xylopyranoside), 5′09″, 5′36″ (methyl 2,3-di-O-methyl-L-rhamnopyranoside), 5′36″, 7′20″ (methyl 2,4-di-O-methyl-D-xylopyranoside), 7′50″ (methyl 3,4-di-O-methyl-L-arabinopyranoside), 8′32″, 12′05″ (methyl 2,3,4-tri-O-methyl-D-glucopyranoside), 16′48″, 17′40″ (methyl 3,4-di-O-methyl-D-glucopyranoside).

Reduction of III with $LiAlH_4$——A solution of III (70 mg) in THF (10 ml) was treated with 30 mg of $LiAlH_4$ and the mixture was refluxed for 3 hr. The excess $LiAlH_4$ was decomposed with wet ether under ice cooling. The reaction mixture was poured into water, and the mixture was extracted with ether and AcOEt, in that order. Each organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. From the ether extract, three O-methylated monosaccharides were obtained by methanolysis and found to be identical with samples obtained from the permethylate of the prosapogenin by TLC and GLC. The AcOEt extract was purified by preparative TLC with benzene–acetone (3 : 2) to afford methylated oligosaccharide (V) (25 mg), colorless oil, $[\alpha]_D^{28}$ −60.5° (c=1.00 $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH). NMR δ: 1.24—1.36 (3H×2, 2×C$\underline{H}_3$), 3.45—3.62 (36H, m, 12×OC$\underline{H}_3$), 4.64 (1H, d, J=7 Hz, anomeric H), 4.70 (1H, d, J=7 Hz, anomeric H), 4.87 (1H, broad s, anomeric H), 5.05 (1H, broad s, anomeric H). *Anal.* Calcd for $C_{40}H_{74}O_{22}$: C, 52.97; H, 8.22. Found: C, 53.10; H, 8.39.

Methanolysis of V——A solution of V in methanolic 2 N HCl was refluxed for 2 hr and the reaction mixture was worked up in the same way as for III. Methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside, methyl 2,3,4-tri-O-methyl-D-xylopyranoside, methyl 2,3-di-O-methyl-L-rhamnopyranoside and methyl 2,4-di-O-methyl-D-xylopyranoside were identified (by comparison with authentic samples) by TLC and GLC. A compound assumed to be 3,4-di-O-methyl-D-glucitol was also obtained, but its identity was not confirmed.

Partial Methanolysis of V——A solution of V (130 mg) in methanolic 0.5 N HCl (10 ml) was allowed to stand for 48 hr at room temperature. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated to dryness under reduced pressure. The residue was examined by TLC (solvent: benzene–acetone (10: 13)), and showed five main spots (*Rf* 0.25, 0.30, 0.50, 0.60 and 0.75). The mixture was fractionated by chromatography on silica gel. The product corresponding to *Rf* 0.75 was identified as methyl 2,3,4-tri-O-methyl-D-xylopyranoside, and the product corresponding to *Rf* 0.60 was identified as methyl 2,4-di-O-methyl-D-xylopyranoside by TLC and GLC, respectively. The product with *Rf* 0.50 was identical with compound (V) (20 mg). The products corresponding to *Rf* 0.25 and 0.30 were purified by preparative TLC (solvent: benzene–acetone (3: 2)) to afford VI (20 mg) and VII (50 mg). VI was a syrup, $[\alpha]_D^{34}$ −45.7° (c=1.00 $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH). NMR δ: 1.28 (3H, each d, J=6 Hz, 2×C$\underline{H}_3$), 3.47 (9H, s, 3×OC$\underline{H}_3$), 3.49 (9H, s, 3×OC$\underline{H}_3$), 3.54, 3.57, 3.59 (3H, each s, 3×OC$\underline{H}_3$), 4.66 (1H, d, J=6 Hz, anomeric H), 4.87 (1H, broad s, anomeric H), 5.06 (1H, broad s, anomeric H). *Anal.* Calcd for $C_{32}H_{60}O_{18}$: C, 52.45; H, 8.25. Found: C, 53.15; H, 8.66. VII was a syrup, $[\alpha]_D^{33}$ −36.4° (c=1.04 $CHCl_3$). IR $\nu_{max}^{CHCl_3}$

cm^{-1}: 3450 (OH). NMR δ: 1.30 (3H, d, $J=6$ Hz, $C\underline{H}_3$), 1.33 (3H, d, $J=6$ Hz, $C\underline{H}_3$), 3.45 (3H, s, $OC\underline{H}_3$), 3.47 (3H, s, $OC\underline{H}_3$), 3.48 (9H, s, $3\times OC\underline{H}_3$), 3.54 (3H, s, $OC\underline{H}_3$), 3.57 (3H, s, $OC\underline{H}_3$), 4.87 (1H, broad s, anomeric H), 5.09 (1H, broad s, anomeric H). *Anal.* Calcd for $C_{25}H_{48}O_{14}$: C, 52.43; H, 8.45. Found: C, 52.85; H, 8.71.

Methanolysis of VI——Compound (VI) was methanolized in the same way as III to give methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside, methyl 2,3-di-O-methyl-L-rhamnopyranoside and methyl 2,4-di-O-methyl-D-xylopyranoside. These methylated monosaccharides were identified (by comparison with authentic samples) by TLC and GLC. A compound assumed to be 3,4-di-O-methyl-glucitol was also seen on TLC, but its identity was not confirmed.

Methanolysis of VII——Compound (VII) was methanolized in the same was as III to give methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside and methyl 2,3-di-O-methyl-L-rhamnopyranoside (identified by TLC). A compound assumed to be 3,4-di-O-methyl-D-glucitol was also seen on TLC, but its identity was not confirmed.

Partial Methanolysis of VII——The solution of VII (60 mg) in methanolic 1 N HCl (9 ml) was allowed to stand for 40 hr at room temperature. The reaction mixture was treated as described above. The residue was examined by TLC (solvent: benzene–acetone (1: 4)) and showed main four spots (*Rf* 0.20, 0.25, 0.60, 0.75). The products corresponding to *Rf* 0.60 and 0.75 were identified as methyl 2,3-di-O-methyl-L-rhamnopyranoside. The product corresponding to *Rf* 0.25 was identical with compound (VII) (25 mg). The product corresponding to *Rf* 0.20 was purified by preparative TLC (solvent: benzene–acetone (1: 4)) to afford VIII (18 mg), colorless syrup, $[\alpha]_D^{27}$ $-20°$ ($c=0.75$ $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450 (OH). NMR δ: 1.28 (3H, d, $J=6$ Hz, $C\underline{H}_3$), 3.45 (3H, s, $OC\underline{H}_3$), 3.48 (9H, s, $3\times OC\underline{H}_3$), 3.53 (3H, s, $OC\underline{H}_3$), 4.85 (1H, broad s, anomeric H).

Methanolysis of VIII——Compound (VIII) was methanolized in the same way as in III to give methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside which was identified by TLC and GLC. A compound assumed to be 3,4-di-O-methyl-D-glucitol was also seen on TLC, but its identity was not confirmed.

Permethylation of VIII——VII (10 mg) was methylated by Hakomori's method, and the reaction mixture was treated as described above to afford IX (5 mg), colorless syrup, $[\alpha]_D^{22}$ $-26.3°$ ($c=1.07$ $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: OH (nil). NMR δ: 1.28 (3H, d, $J=6$ Hz, $C\underline{H}_3$), 3.37, 3.42, 3.45, 3.47 (3H, each s, $OC\underline{H}_3$), 3.54 (3H, s, $OC\underline{H}_3$), 4.85 (1H, broad s, anomeric H). MS (m/z): 440 (M^+), 235 ($C_{11}H_{23}O_5^+$), 189 ($C_9H_{17}O_4^+$).

Methanolysis of IX——IX was methanolized in the same way as III to give methyl 2,3,4-tri-O-methyl-L-rhamnopyranoside (t_R: 1′15″, 1′53″) and 1,2,3,4,5-penta-O-methyl-D-glucitol (t_R: 6′51″), $[\alpha]_D^{27}$ $+6°$ ($c=1.00$ $CHCl_3$), which were identified by comparison with authentic samples by TLC and GLC.

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