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Saponin and Sapogenol. XVII.[1] Structure of Sakuraso-saponin, a Pentaglycoside of Protoprimulagenin A from the Root of *Primula sieboldi* E. MORREN

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Sakuraso-saponin (3), a pentaglycoside of protoprimulagenin A (1), has been isolated from the root of *Primula sieboldi* E. MORREN (Primulaceae, Japanese name: sakurasō). Complete acid hydrolysis of sakuraso-saponin (3) furnished primulagenin A (2), glucose, galactose, rhamnose, and glucuronic acid, while the ultraviolet irradiation of sakuraso-saponin liberated the genuine aglycone protoprimulagenin A (1) in a good yield.

On the bases of the chemical and physicochemical investigations of the prosapogenols: PS-1 (4), PS-2 (5), PS-3 (6), and PS-4 (7), and a pentaglycoside PS-5 (8), in addition to those of sakuraso-saponin (3), the full structure of sakuraso-saponin has been established as 3-O-{4-O-[α-L-rhamnopyranosyl(1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl(1→4)]-2-O-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl}-protoprimulagenin A (3), which is a first structure determination of the primulaceous saponins.

It has been shown that the primulaceous plants contain a rich amount of saponins,[3] however, a study on the saponin of *Primula elatior* L. has been an only example of the structure elucidation although the full structure has not yet been established.[4] As a continuative study on the saponin constituents of the Japanese primulaceous plants, we have previously isolated a saponin named sakuraso-saponin from the root of *Primura sieboldi E.* MORREN (Japanese name: sakurasō) and have elucidated by virtue of the periodate degradation that protoprimulagenin A (1) is the genuine sapogenol of sakuraso-saponin rather than primulagenin A (2).[3] Afterwards, we have also reported that the ultraviolet irradiation is an effective method to cleave the glycoside linkage of sakuraso-saponin to furnish the genuine sapogenol protoprimulagenin A (1) and have simultaneously elucidated that an uronic acid in the oligosaccharide portion of sakuraso-saponin is directly attached to protoprimulagenin A *via* a uronide linkage.[5,6] The present paper deals with the full account on the structure elucidation of sakuraso-saponin (3).[7]

Since the saponin isolated in the previous paper[3] was noticed to be in a carboxylate form (infrared (IR) spectrum in Nujol: 1615 cm^{-1}), it was treated with a cationic ion-exchange resin and recrystallized from methanol to afford colorless needles of sakuraso-saponin (3), mp 267—270° (IR (Nujol): 3375 (br) cm^{-1} (hydroxyl) and 1725 cm^{-1} (carboxyl)).

Complete acid hydrolysis of sakuraso-saponin (3) furnished primulagenin A (2) as the aglycone and glucose, galactose, rhamnose, and glucuronic acid as the carbohydrate ingredients, whereas, on mild acid hydrolysis, 3 furnished four prosapogenols which were designated in the

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[1] Part XVI: I. Kitagawa, K.S. Im, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), 24, 1260 (1976).

[2] Location: *133-1, Yamada-kami, Suita, Osaka, 565, Japan.*

[3] I. Kitagawa, A. Matsuda, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), 20, 2226 (1972).

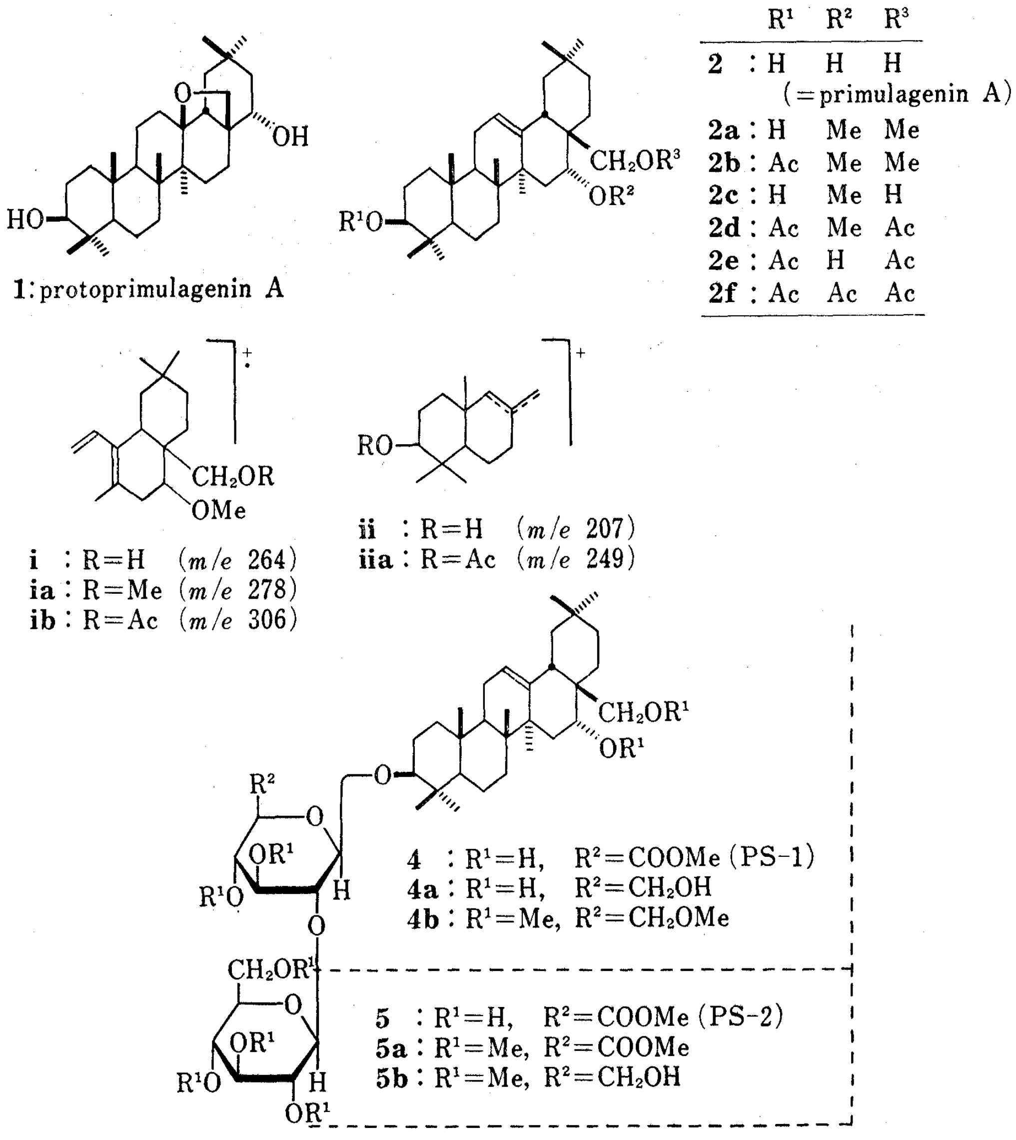
[4] R. Tschesche and G. Wulff, "Fortschritte der Chemie organischer Naturstoffe," Vol. 30, ed. by W. Herz, H. Grisebach, and G.W. Kirby, Springer-Verlag, Wien, New York, 1972, p. 461.

[5] *a*) I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Tetrahedron Letters*, 1973, 3997; *b*) I. Kitagawa, M. Yoshikawa, Y. Imakura, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), 22, 1339 (1974).

[6] It has recently been found that the photolysis of the glucuronide linkage in sakuraso-saponin is effected using a Vycor filter with an improved yield (see Experimental).

[7] Presented at the Annual Meeting of Pharmaceutical Society of Japan, Nishinomiya, April, 1975. Abstract Paper II-249.

order of increasing polarity as PS-1 (**4**), PS-2 (**5**), PS-3 (**6**), and PS-4 (**7**), and a pentaglycoside PS-5 (**8**), which possesses the same carbohydrate composition as the parent saponin (**3**) but the aglycone of which has been converted to primulagenin A (**2**) and the carboxylic function in the glucuronide moiety being methylated.



1: protoprimulagenin A

	R^1	R^2	R^3
2	H	H	H
	(=primulagenin A)		
2a	H	Me	Me
2b	Ac	Me	Me
2c	H	Me	H
2d	Ac	Me	Ac
2e	Ac	H	Ac
2f	Ac	Ac	Ac

i : R=H (*m/e* 264)
ia : R=Me (*m/e* 278)
ib : R=Ac (*m/e* 306)

ii : R=H (*m/e* 207)
iia : R=Ac (*m/e* 249)

4 : R^1=H, R^2=COOMe (PS-1)
4a : R^1=H, R^2=CH_2OH
4b : R^1=Me, R^2=CH_2OMe

5 : R^1=H, R^2=COOMe (PS-2)
5a : R^1=Me, R^2=COOMe
5b : R^1=Me, R^2=CH_2OH

Chart 1

The IR spectrum (Nujol) of PS-1 (**4**) shows the absorption bands at 3410 (br) cm^{-1} (hydroxyl) and 1740 (br) cm^{-1} (ester carbonyl) while the proton magnetic resonance (PMR) spectrum of **4** (taken in pentadeutero(d_5-)pyridine) shows a signal due to a methoxycarbonyl (δ 3.66, s) which has been induced during the methanolic acid treatment of 3[1,8] and presents also in the other prosapogenols.

Sodium borohydride ($NaBH_4$) reduction of PS-1 gave a product (**4a**, IR: no ester carbonyl), which furnished primulagenin A (**2**) and glucose on acid hydrolysis and was converted to a hexa-O-methyl derivative (**4b**) on methyl iodide (CH_3I)/dimethyl sulfoxide (DMSO)/sodium hydride (NaH) treatment.[9] The PMR spectrum of **4b** shows an anomeric proton signal at δ 4.12 (d, J=7.0 Hz), thus indicating that the glucose moiety (Cl form) in PS-1 is attached with

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[8] I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **22**, 3010 (1974).
[9] S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

a β linkage. A multiplet observed at δ 5.13 ($W_{h/2}=8.0$ Hz) assignable to an olefinic proton at C-12 suggests that the aglycone part in PS-1 is primulagenin A (**2**) which was formed secondarily during the acid hydrolysis by opening of the parent 13β,28-oxide moiety in protoprimulagenin A (**1**). On methanolysis, **4b** liberated methyl 2,3,4,6-tetra-O-methyl-glucopyranoside and a methylated aglycone which has been assigned as 16,28-di-O-methyl-primulagenin A (**2a**) on the following bases: i) The IR spectrum (carbon tetrachloride (CCl_4)) shows the presence of a hydroxyl (3625 cm^{-1}), ii) The PMR spectrum shows two three-proton singlets at δ 3.17 and 3.24 due to two methoxyls and a one-proton triplet-like signal at δ 5.07 due to an olefinic proton at C-12, iii) The mass spectrum gives a rich molecular ion peak at *m/e* 486 and two prominent ion peaks at *m/e* 278 (**ia**) and 207 (**ii**) both of which are considered to be derived *via* the reverse Diels-Alder type fragmentation of the ring C,[10,11] iv) The PMR spectrum of the monoacetate (**2b**, IR (CCl_4): no hydroxyl, 1736, 1245 cm^{-1}) shows the signals due to an acetoxyl (δ 1.96, 3H, s), two methoxyls (δ 3.17, & 3.26, 3H each, both s), 3α-H geminal to 3β-OAc (δ 4.42, 1H, t-like),[11] and an olefinic proton at C-12 (δ 5.15, 1H, t-like), and v) The mass spectrum of **2b** also gives a molecular ion peak at *m/e* 528 and two fragment ions at *m/e* 278 (**ia**) and 249 (**iia**) which are derived similarly as in the spectrum of **2a**. Consequently, the structure of PS-1 has been elucidated to be the methyl ester of primulagenin A 3-O-β-D-glucuronopyranoside (**4**).

PS-2 (**5**) is a glucoside of PS-1 (**4**). The IR (Nujol, 3372 (br), 1741 (br) cm^{-1}) and PMR (3H, s, at δ 3.70 in d_5-pyridine) spectra of **5** indicate the presence of similar functions as in **4**. A nona-O-methyl derivative (**5a**) obtained by methylation of **5** possesses an ester carbonyl but no free hydroxyl (IR) and the PMR spectrum of **5a** shows the signals due to two anomeric pro-

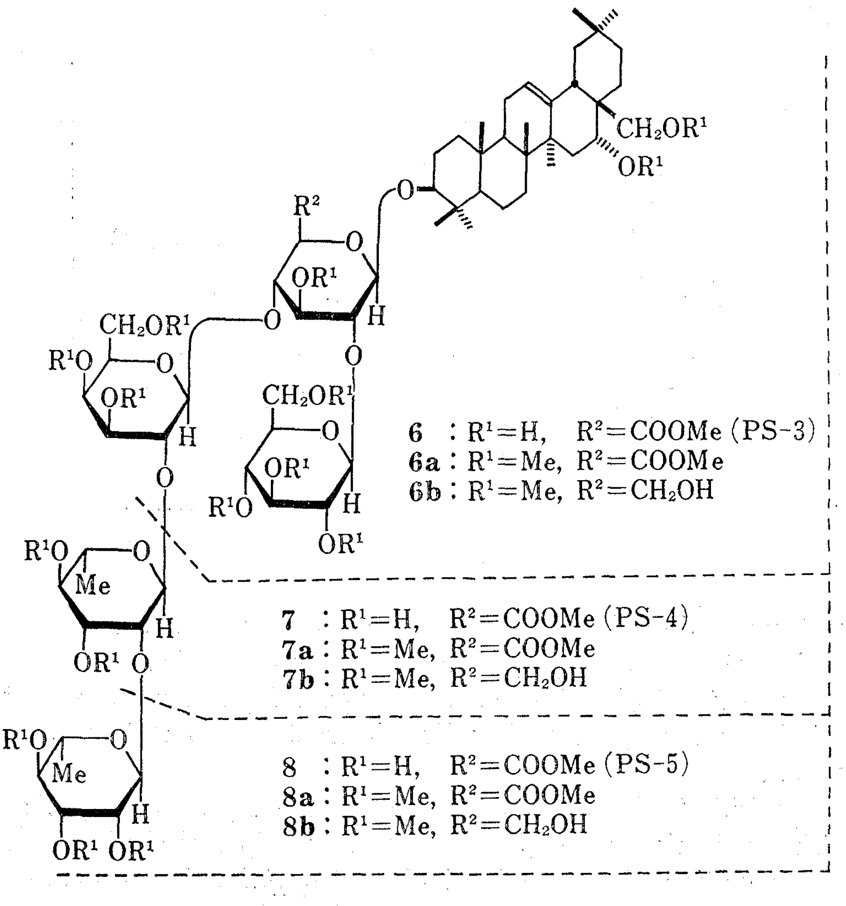

Chart 2

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[10] H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. 2, Holden-Day Inc., San Francisco, 1964, p. 121.

[11] I. Yosioka, T. Nishimura, A. Matsuda, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), 18, 1610 (1970).

tons at δ 4.28 and 4.52 (1H each, both d, J=7.0 Hz, suggesting the β linkage respectively in the Cl form) and an olefinic proton at C-12 (δ 5.15, 1H, m, $W_{h/2}$=8.0 Hz). Lithium aluminum hydride ($LiAlH_4$) reduction of **5a** gave a product (**5b**, IR (CCl_4): hydroxyl but no ester carbonyl), which, on methanolysis, furnished 16,28-di-O-methyl-primulagenin A (**2a**), methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, and methyl 3,4-di-O-methyl-glucopyranoside, thus the structure **5** being assigned to PS-2.

PS-3 (**6**), which corresponds to a galactoside of PS-2 (**5**), possesses hydroxyls (IR) and a methoxycarbonyl (IR, PMR). It was converted by methylation to a dodeca-O-methyl derivative (**6a**) which retains the methoxycarbonyl in **6** but possesses no free hydroxyl (IR) as in **5a**. The PMR spectrum of **6a** (taken in a 1: 3 mixture of hexadeutero (d_6-)benzene–CCl_4) shows three anomeric proton signals at δ 4.30 (1H, d, J=6.0 Hz), 4.71, and 4.83 (1H each, both d, J=7.0 Hz, β linkage) and an olefinic proton (C-12) signal at δ 5.16 (m, $W_{h/2}$=9.0 Hz). $LiAlH_4$ reduction of **6a** gave a product (**6b**, IR (CCl_4): hydroxyl but no ester carbonyl), which, on methanolysis, furnished 16,28-di-O-methyl-primulagenin A (**2a**), methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, methyl 2,3,4,6-tetra-O-methyl-galactopyranoside, and methyl 3-O-methyl-glucopyra oside. Therefore, PS-3 is expressed as **6**.

The structure of PS-4 (**7**), which is a rhamnoside of PS-3 (**6**), has been elucidated similarly. In the PMR spectrum of PS-4 (in d_5-pyridine), a doublet due to a rhamnose methyl is observed at δ 1.31 (J=6.0 Hz) in addition to a singlet due to a methoxycarbonyl at δ 3.68. On methylation, PS-4 was converted to a tetradeca-O-methyl derivative (**7a**, IR (CCl_4): no hydroxyl), the PMR spectrum of which shows the signals due to a rhamnose methyl (δ 1.27, 3H, d, J=6.5 Hz) and three anomeric protons (at δ 4.46, 1H, d, J=6.0 Hz, and at δ 4.55 and 4.88, 1H each, both d, J=7.0 Hz) suggesting the β linkage (Cl form), and a two-proton multiplet (δ 5.17, $W_{h/2}$=9.0 Hz) which is an overlapped signal of an olefinic proton at C-12 and an anomeric proton on the rhamnoside linkage. A reduction product (**7b**), obtained by $LiAlH_4$ treatment of **7a**, furnished on methanolysis 16,28-di-O-methyl-primulagenin A (**2a**), methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, methyl 3-O-methyl glucopyranoside, methyl 3,4,6-tri-O-methyl-galactopyranoside, and methyl 2,3,4-tri-O-methyl-rhamnopyranoside. Consequently, the structure of PS-4 is assigned as **7**, in which the anomeric configuration at the rhamnoside linkage has followed the application of the Klyne's rule[12]: $[M]_D(\mathbf{7})$-$[M]_D(\mathbf{6})=-99.6°$; $[M]_D$ (methyl α-L-rhamnopyranoside)$=-109°$; $[M]_D$(methyl β-L-rhamnopyranoside)$=+169°$.[1,13]

PS-5 (**8**) contains two rhamnose units in its molecule as shown by the PMR spectrum taken in d_5-pyridine: two three-proton doublets at δ 1.37 and 1.57 (both d, J=6.0 Hz). The glucuronide carboxyl in **8** is also methylated as exhibited by the IR (Nujol, 1747 (br) cm^{-1}) and the PMR spectra (δ 3.71, 3H, s). The PMR spectrum of hexadeca-O-methyl derivative (**8a**), which was prepared from PS-5 by complete methylation as above, shows the signals due to two rhamnose methyls at δ 1.27 (6H, d, J=6.5 Hz), three anomeric protons at δ 4.44 (1H, d, J=6.0 Hz), δ 4.54 and 4.88 (1H each, both d, J=7.0 Hz), two anomeric protons on the rhamnoside linkages at δ 5.04 (2H, br.s) and an olefinic proton at C-12 (δ 5.20, 1H, m, $W_{h/2}$=9.0 Hz). On reduction with $LiAlH_4$, **8a** was converted to **8b**, which, on methanolysis, afforded 16,28-di-O-methyl-primulagenin A (**2a**), methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, methyl 3-O-methyl-glucopyranoside, methyl 3,4,6-tri-O-methyl-galactopyranoside, methyl 2,3,4-tri-O-methyl-rhamnopyranoside, and methyl 3,4-di-O-methyl-α-L-rhamnopyranoside (**9**).[14]

Since the authentic sample was unavailable, the structure (**9**) has been deduced on the following bases. Thus, the molecular composition $C_9H_{18}O_5$ has been elucidated by high resolution mass spectrometry. The IR spectrum (CCl_4) of **9** shows the hydroxyl absorption band at 3560

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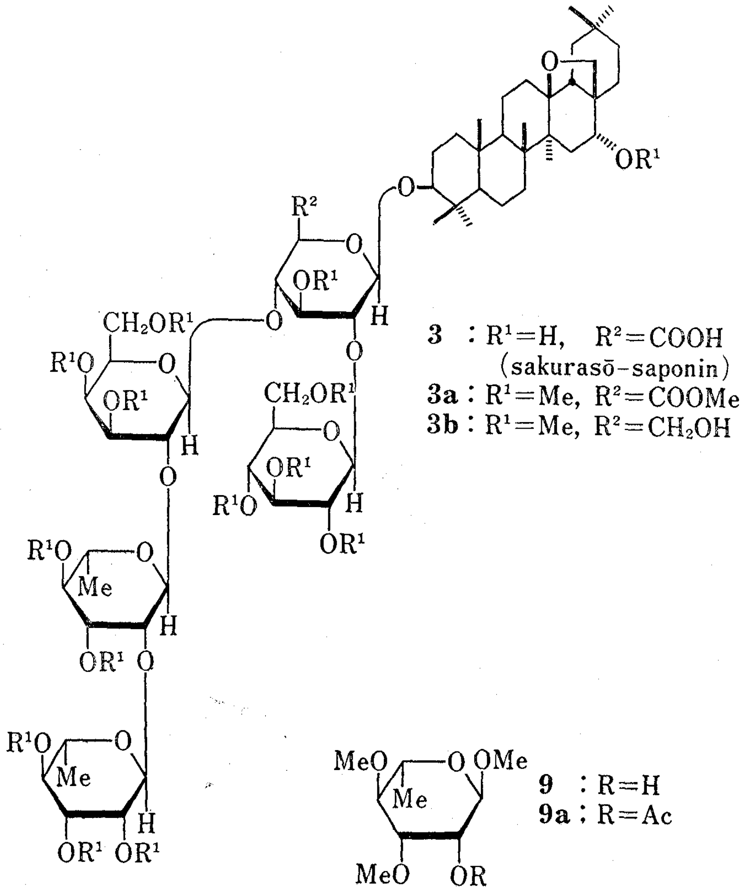
12) W. Klyne, *Biochem. J.*, **47**, xli (1950).

13) H. Okabe and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 514 (1972).

14) H. Okabe, N. Koshito, K. Tanabe, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **19**, 2394 (1971). The β anomer was also isolated as the minor (see Experimental). The pyranoside structure has been deduced from a fact that above described PS-4 (7) contains a terminal rhamnopyranoside moiety.

cm^{-1} whereas the PMR spectrum shows a three-proton doublet at δ 1.20 (J=6.0 Hz, secondary methyl), three three-proton singlets at δ 3.27, 3.42, and 3.43 (three methoxyls), a double doublet at δ 3.83 (J=2.0 & 4.0 Hz, 2-H), and a doublet at δ 4.49 (J=2.0 Hz, 1-H). The vicinal location of the latter two protons was confirmed by the decoupling experiments. Acetylation of **9** gave a monoacetate (**9a**) which carries no free hydroxyl (IR (CCl_4): 1740 cm^{-1}). The PMR spectrum of **9a** shows the presence of a newly formed acetoxyl (δ 2.07, 3H, s) and its geminal proton (δ 5.10, 1H, d.d, J=2.0 & 4.0 Hz, 2-H) in addition to the presence of three methoxyls, one secondary methyl, and an anomeric proton (δ 4.43, d, J=2.0 Hz, 1-H). Here again, the vicinal location of two protons at C-1 and C-2 was confirmed by the decoupling experiments as above. It is noted that the signal due to 2-H in **9a** is observed at the 1.27 ppm lower position than that of 2-H in **9**.

In addition to the above described evidence, the application of the Klyne's rule, $[M]_D$(**8**)-$[M]_D$(**7**)=−109.6°, has established the structure of PS-5 as **8** including the anomeric configuration at the terminal rhamnoside linkage.



3 : R^1=H, R^2=COOH
(sakurasō-saponin)
3a : R^1=Me, R^2=COOMe
3b : R^1=Me, R^2=CH_2OH

9 : R=H
9a : R=Ac

Chart 3

Finally, the structure of sakuraso-saponin (**3**) has been elucidated as based on the following evidence. Complete methylation of **3** gave a pentadeca-O-methyl derivative (**3a**, IR (CCl_4): no hydroxyl and ester carbonyl at 1757 cm^{-1}), the PMR spectrum of which shows the signals assignable to two rhamnose methyls (δ 1.40, 6H, d, J=6.5 Hz) and five anomeric protons (δ 4.52, d, J=6.5 Hz; δ 4.92, d, J=7.0 Hz; δ 5.25, d, J=7.0 Hz; δ 5.37, br. s; δ 5.42, br.s), but lacks the signal due to an olefinic proton found in the PMR spectra of the derivatives of PS-1 (**4**), PS-2 (**5**), PS-3 (**6**), PS-4 (**7**), and PS-5 (**8**). $LiAlH_4$ reduction of **3a** furnished **3b** (IR (CCl_4): 3584 (w) cm^{-1}, no ester carbonyl), the PMR spectrum of which shows five anomeric proton signals at δ 4.34, 4.53, and 4.90 (1H each, all d, J=7.0 Hz), and δ 5.04 (2H, br.s). On methanolysis, **3b** liberated

Table I. Chemical Shifts of 14α-Methyl Signal (δ Values at 90 MHz)

	in d_5-pyridine	in CCl_4	Δ
2	1.76	—[a]	—
2c	1.49	1.29	+0.20
2e[15]	1.73	1.36	+0.37
2f[15]	1.39	1.24	+0.15

a) not measured due to less solubility five methyl monosaccharides which were respectively identical with those obtained by methanolysis of **8b** and 16-O-methyl-primulagenin A (**2c**), the structure of the latter being confirmed on the following bases. Thus, the IR spectrum (CCl_4) of **2c** shows the hydroxyl absorption bands at 3631 and 3489 cm^{-1}, whereas the PMR spectrum ($CDCl_3$) shows the presence of a methoxyl (δ 3.23, 3H, s) and an olefinic proton at C-12 (δ 5.24, 1H, t-like). In the mass spectrum of **2c**, two prominent fragment ion peaks are observed at *m/e* 264 (**i**, base peak) and *m/e* 207 (**ii**) as expected[10,11] in addition to a rich molecular ion peak at *m/e* 472. The assignment of a methoxyl at C-16 in **2c** has followed the solvent induced PMR shift of the 14α-methyl signal. It appears at δ 1.29 in CCl_4 while at δ 1.49 in d_5-pyridine with a smaller shift value (0.20 ppm) than that in the case of 16α-hydroxy-olean-12-ene derivatives (Table I).[15] The PMR spectrum of the diacetate (**2d**, IR (CCl_4): no hydroxyl, 1743 cm^{-1}) shows the signals due to two acetoxyls (δ 2.02, 6H, s), a methoxyl (δ 3.27, 3H, s), a methylene at C-28 (δ 3.60 & 3.84, 1H each, ABq, $J=12.0$ Hz), 3α-H (δ 4.49, 1H, t-like), and an olefinic proton at C-12 (δ 5.23, 1H, t-like). The paramagnetically shifted δ values of the signals due to 28-H_2 and 3α-H are well explained by the acetylation of the geminal hydroxyls.[11] The mass spectrum of **2d** also supports the formulation by the ion peaks at *m/e* 556 (M^+), *m/e* 306 (**ib**), and 249 (**iia**).[10,11]

Sakuraso-saponin is, therefore, expressed as 3-O-{4-O-[α-L-rhamnopyranosyl (1→2)-α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→4)]-2-O-β-D-glucopyranosyl (1→2)-β-D-glucuronopyranosyl}-protoprimulagenin A (3), which is a first example of the structure determination of saponins which have been isolated from the primulaceous plants.[3,4]

Experimental[16]

Sakuraso-saponin (3)——A solution of saponin (0.50 g, isolated from the root of *Primula sieboldi*,[3] IR ν_{max}^{Nujol} cm^{-1}: 3510 (br, OH), 1615 (br, COO^-)) in MeOH (30 ml) was passed through a column of Dowex 50W×8 (H^+, 20 g) and evaporated under reduced pressure to give sakuraso-saponin (0.47 g). Three recrystallization from MeOH gave the analytical sample of **3**, mp 267—270°, $[\alpha]_D^{22}$ −39.0° (c=2.0, MeOH). *Anal.* Calcd. for $C_{60}H_{98}O_{27}\cdot 3H_2O$: C, 55.07; H, 8.22. Found: C, 55.05; H, 8.03. IR ν_{max}^{Nujol} cm^{-1}: 3375 (br, OH), 1725(COOH).

Photolysis of Sakuraso-saponin (3)——A solution of **3** (700 mg) in MeOH (700 ml) was irradiated internally for one hour with a high pressure mercury lamp (500 W, Eikōsha PIH-500) equipped with a Vycor filter. After neutralizing with 10% aq. K_2CO_3, the total solution was concentrated under reduced pressure to remove MeOH and diluted with water to give the precipitate which was collected by filtration and subjected to preparative TLC ($CHCl_3$–MeOH=25:1, detection by spraying water). Protoprimulagenin A (**1**, 189 mg, 73.7%) thus obtained was recrystallized from *n*-hexane–acetone and identified with the authentic sample[3] by mixed mp, IR (KBr), and TLC.

Complete Acid Hydrolysis of Sakuraso-saponin (3)——A solution of **3** (100 mg) in 20% aq. H_2SO_4–MeOH (18 ml–18 ml) was heated under reflux for 4 hr. The reaction mixture was then concentrated under reduced pressure to remove MeOH while adding water and the precipitated product was collected by filtration. The product was purified by preparative TLC ($CHCl_3$–MeOH=25:1, detection with water) to give primulagenin A (**2**, 19 mg, 51.9%) which was recrystallized from MeOH (colorless needles of mp 229—232°) and identified with the authentic sample[3] by mixed mp, IR (KBr), and TLC. The aqueous filtrate was made weakly acidic (pH *ca.* 6.0) with sat. aq. $Ba(OH)_2$ and the white precipitate ($BaSO_4$) was removed by a centrifuge (3000 cps, 30 min). The supernatant was concentrated under reduced pressure and the carbohydrate components thus remained were identified by paper partition chromatography (PPC) developing twice (20 hr each) with AcOEt–pyridine–AcOH–water (5:5:1:3) as rhamnose (*Rf*=0.84), glucose (0.72), galactose (0.65), and glucuronic acid

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15) I. Kitagawa, M. Yoshikawa, and I. Yosioka, *Tetrahedron Letters*, **1974**, 469.

16) Instruments used in the experimental section and the experimental conditions for chromatography were same as in our previous paper unless specified otherwise.[1] The conditions for gas liquid and thin-layer chromatography (GLC, TLC) were as follows: GLC: column (a)=15% polyneopentylglycol succinate on Chromosorb WAW (80—100 mesh), 3 mm×2 m; carrier gas: N_2 flow rate 30 ml/min; column (b)=3% silicone gum SE-30 on Chromosorb WAW (80—100 mesh), 3 mm×2 m; carrier gas: N_2 flow rate 15 ml/min; column (c)=2% silicone OV-17 on Chromosorb WAWDMCS (80—100 mesh), 3 mm×1 m, carrier gas: N_2 flow rate 20 ml/min. TLC: solvent (i)=benzene–acetone (3:1); solvent (ii)=benzene–acetone (2:1); solvent (iii)=benzene–acetone (1:2); solvent (iv)=benzene–MeOH (10:1); solvent (v)=$CHCl_3$–MeOH (5:1).

(0.42) (*cf.* galacturonic acid: *Rf*=0.34) (detection with aniline hydrogen phthalate). The carbohydrates were also identified by GLC as their trimethylsilyl (TMS) derivatives (silylation with trimethylsilyl chloride and hexamethyldisilazane in pyridine): column (b), column temp. 222°, carrier gas N_2 flow rate 30 ml/min, t_R(min): rhamnose (1′40″, 1′52″, 2′20″), glucose (2′57″, 3′48″, 4′01″, 5′11″), galactose (3′10″, 3′42″, 4′12″).

Mild Acid Hydrolysis of Sakuraso-saponin (3)——A solution of 3 (5.00 g) in anhydrous 10% methanolic HCl–MeOH (50 ml–500 ml) was heated under reflux for 35 min and neutralized with 20% aq. NaOH. The reaction mixture was then concentrated under reduced pressure to remove MeOH and extracted three times with *n*-BuOH saturated with water. The combined *n*-BuOH layer was washed with water and evaporated to dryness under reduced pressure to give a residue (4.00 g) which was mixed with silica gel (35 g) with the aid of MeOH, dried, put on a column of silica gel (600 g), and chromatographed. Successive elution with the $CHCl_3$–MeOH mixture (with increasing % of the latter solvent) gave primulagenin A (**2**, 411 mg),[3] PS-1 (**4**, 68 mg), PS-2 (**5**, 301 mg), PS-3 (**6**, 412 mg), PS-4 (**7**, 51 mg), and PS-5 (**8**, 247 mg). The other mixtures obtained during the chromatography were further subjected to repeated preparative TLC (developing with $CHCl_3$–MeOH=5: 1, $CHCl_3$–MeOH=2: 1, or $CHCl_3$–MeOH–water=65: 35: 10 (lower layer), and detection by spraying water) to give the additional amounts of **2** (12 mg), **4** (58 mg), **5** (43 mg), **6** (101 mg), **7** (64 mg), and **8** (46 mg). PS-1 (**4**), colorless needles of mp 227—229° (from $CHCl_3$–MeOH), $[\alpha]_D^{22}$ −27.9° (*c*=1.0, MeOH). *Anal.* Calcd. for $C_{37}H_{60}O_9$: C, 68.48; H, 9.32. Found: C, 68.18; H, 9.55. IR ν_{max}^{Nujol} cm^{-1}: 3410 (br, OH), 1740 (br, $COOCH_3$). PMR (d_5-pyridine, 90 MHz) δ: 0.87 (3H), 0.89 (6H), 0.98, 1.04, 1.19, 1.66 (3H each) (all s, *tert*-$CH_3\times7$), 3.66 (3H, s, $COOCH_3$). PS-2 (**5**), colorless needles of mp 234—235.5° (acetone–MeOH), $[\alpha]_D^{22}$ −28.4° (*c*=1.0, MeOH). *Anal.* Calcd. for $C_{43}H_{70}O_{14}\cdot2H_2O$: C, 60.97; H, 8.81. Found: C, 60.91; H, 8.74. IR ν_{max}^{Nujol} cm^{-1}: 3372 (br, OH), 1741 (br, $COOCH_3$). PMR (d_5-pyridine, 90 MHz) δ: 0.86, 0.93 (3H each), 1.04 (6H), 1.09, 1.20, 1.76 (3H each) (all s, *tert*-$CH_3\times7$), 3.70 (3H, s, $COOCH_3$). PS-3 (**6**), colorless needles of mp 286—287.5° (acetone), $[\alpha]_D^{22}$ −18.4° (*c*=1.0, MeOH). *Anal.* Calcd. for $C_{49}H_{80}O_{19}\cdot4H_2O$: C, 56.31; H, 8.49. Found: C, 56.49; H, 8.36. IR ν_{max}^{Nujol} cm^{-1}: 3352 (br, OH), 1743 (br, $COOCH_3$). PMR (d_5-pyridine, 90 MHz) δ: 0.82, 0.90 (3H each), 1.01 (6H), 1.07, 1.15, 1.72 (3H each) (all s, *tert*-$CH_3\times7$), 3.66 (3H, s, $COOCH_3$). PS-4 (**7**), fine crystals of mp 236—238° (EtOH–water), $[\alpha]_D^{22}$ −24.9° (*c*=1.0, MeOH). *Anal.* Calcd. for $C_{55}H_{90}O_{23}\cdot4H_2O$: C, 55.45; H, 8.29. Found: C, 55.25; H, 8.45. IR ν_{max}^{Nujol} cm^{-1}: 3375 (br, OH), 1745 (br, $COOCH_3$). PMR (d_5-pyridine, 90 MHz) δ: 0.81, 0.92, 0.96, 1.01 (3H each), 1.07 (6H), 1.72 (3H) (all s, *tert*-$CH_3\times7$), 1.31 (3H, d, *J*=6.0 Hz, *sec*-CH_3), 3.68 (3H, s, $COOCH_3$). PS-5 (**8**), colorless needles of mp 270—272° (EtOH), $[\alpha]_D^{22}$ −30.7° (*c*=1.0, MeOH). *Anal.* Calcd. for $C_{61}H_{100}O_{27}$: C, 57.90; H, 7.97. Found: C, 57.61; H, 8.26. IR ν_{max}^{Nujol} cm^{-1}: 3380 (br, OH), 1747 (br, $COOCH_3$). PMR (d_5-pyridine, 90 MHz) δ: 0.82, 0.92, 0.99, 1.03 (3H each), 1.11 (6H), 1.77 (3H) (all s, *tert*-$CH_3\times7$), 1.37, 1.57 (3H each, both d, *J*=6.0 Hz, *sec*-$CH_3\times2$), 3.71 (3H, s, $COOCH_3$).

$NaBH_4$ Reduction of PS-1 (4) giving 4a——To a solution of **4** (42 mg) in MeOH (6 ml) was added a suspension of $NaBH_4$ (50 mg) in MeOH (4 ml) and the total mixture was kept stirring at room temperature for one hour, treated with acetone (10 ml), Amberlite IRA-400 (OH^-, 5 g), and Dowex 50 W×8 (H^+, 5 g), successively. A product (41 mg) obtained by evaporation of the solvent under reduced pressure was purified by preparative TLC ($CHCl_3$–MeOH=5: 1, detection with water) to afford pure **4a** (34 mg), IR ν_{max}^{Nujol} cm^{-1}: 3380 (br, OH), no $COOCH_3$.

Acid Hydrolysis of 4a——A solution of **4a** (10 mg) in 20% aq. H_2SO_4–MeOH (2 ml–2 ml) was refluxed for 3 hr and concentrated under reduced pressure to remove MeOH while adding water. The precipitate thus obtained was collected by filtration and purified by preparative TLC ($CHCl_3$–MeOH=20: 1, water) to give primulagenin A (**2**, 4 mg) which was identified by direct comparison (mixed mp, IR (KBr), and TLC). The aqueous layer was neutralized with sat. aq. $Ba(OH)_2$, treated as above, and glucose (*Rf* 0.38) was identified by PPC (iso-PrOH: *n*-BuOH: water=7: 1: 2, detection with aniline hydrogen phthalate).

Methylation of 4a giving 4b——a) Dimsyl Carbanion: Prepared by adding NaH (1.0 g, washed with dry *n*-hexane several times beforehand) to DMSO (10 ml) and by keeping stirred under N_2 atmosphere at 80—85° for 45 min. *b*) To a solution of **4a** (40 mg) in DMSO (5 ml) was added the above prepared dimsyl carbanion (3 ml) and the total mixture was kept stirring under N_2 atmosphere at room temperature for one hour, treated with CH_3I (3 ml), and kept stirring in the dark overnight. The reaction mixture was poured into ice-water and extracted with ether (50 ml×3 times). The combined ether extract was washed with 10% aq. $Na_2S_2O_3$ and water and dried over $MgSO_4$. The product obtained by evaporation of the solvent was purified by preparative TLC (benzene–acetone=10: 1, water) to give the hexa-O-methyl derivative (**4b**, 18 mg). Crystallization of **4b** was failed and the analytical sample was made by precipitation from the methanolic solution with the addition of water. White powder (**4b**), $[\alpha]_D^{22}$ −7.9° (*c*=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{42}H_{72}O_8$: C, 71.55; H, 10.29. Found: C, 71.16; H, 10.45. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 2940, 1105. PMR (CCl_4, 90 MHz) δ: 0.80, 0.83, 0.85, 0.90, 0.92, 0.97, 1.26 (3H each, all s, *tert*-$CH_3\times7$), 3.17, 3.24, 3.32, 3.43, 3.51, 3.53 (3H each, all s, $OCH_3\times6$), 4.12 (1H, d, *J*=7.0 Hz, anom. H), 5.13 (1H, m, $W_{h/2}$=8.0 Hz, 12-H).

Methanolysis of 4b giving 16,28-Di-O-methyl-primulagenin A (2a)——A solution of **4b** (10 mg) in anhydrous 10% HCl–MeOH (2 ml) was heated under reflux for 2 hr, neutralized with Ag_2CO_3, and filtered to remove the precipitate (AgCl). Evaporation of the filtrate under reduced pressure gave a product (9 mg) which was subjected to preparative TLC (benzene–acetone=3: 1, detection with I_2 vapor) to give **2a** (5 mg) and methyl 2,3,4,-6-tetra-O-methyl-glucopyranoside [GLC: column (a), column temp. 180°, t_R(min): 4′54″ (minor), 6′49″ (major); column (b), column temp. 170°, t_R(min): 2′51″ (minor), 3′17″ (major). TLC: solvent (i), *Rf* 0.35 (major), 0.44 (minor); solvent (iv), *Rf* 0.45 (major), 0.53 (minor)]. Analytical sample of **2a** was prepared by recrystallization from acetone as colorless needles of mp 155—157°, $[\alpha]^{22}_{D}$ +9.8° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{32}H_{54}O_3$: C, 78.96; H, 11.18. Found: C, 78.77; H, 11.16. IR $\nu^{CCl_4}_{max}$ cm^{-1}: 3625 (OH), 2930, 1108. PMR (CCl_4, 90 MHz) δ: 0.74, 0.86, 0.89, 0.92, 0.94, 1.23, 1.28 (3H each, all s, *tert*-$CH_3 \times 7$), 2.71, 3.07 (1H each, ABq, J=9.0 Hz, 28-H_2), 3.17, 3.24 (3H each, both s, $OCH_3 \times 2$), 5.07 (1H, t-like, 12-H). Mass Spectrum m/e (%): 486 ((M^+, 26), 454 (M^+−MeOH, 23), 441 (M^+−CH_2OMe, 26), 409 (M^+−MeOH−CH_2OMe, 23), 278 (**ia**, 28), 246 (**ia**−MeOH, 33), 233 (**ia**−CH_2OMe, 98), 207 (**ii**, 16), 201 (**ia**−MeOH−CH_2OMe, 100), 189 (**ii**−H_2O, 35).

Acetylation of 2a giving 2b—— A solution of **2a** (25 mg) in Ac_2O (2 ml) and pyridine (2 ml) was left standing at room temperature for 3 hr, poured into ice-water, and neutralized with sat. aq. $NaHCO_3$. The reaction mixture was extracted with ether (30 ml × 3 times) and the ether extract was treated as usual to give a product which was once dissolved in MeOH and treated with water. The precipitate thus obtained was collected and crystallized from acetone to give **2b** (20 mg) as colorless plates of mp 179—180°, $[\alpha]^{22}_{D}$ +11.8° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{34}H_{56}O_4$: C, 77.22; H, 10.67. Found: C, 77.30; H, 10.65. IR $\nu^{CCl_4}_{max}$ cm^{-1}: no OH, 1736, 1245 (OAc). PMR (CCl_4, 90 MHz) δ: 0.84 (3H), 0.87 (6H), 0.90, 0.96, 1.24, 1.27 (3H each) (all s, *tert*-$CH_3 \times 7$), 1.96 (3H, s, OAc), 2.77, 3.07 (1H each, ABq, J=9.0 Hz, 28-H_2), 3.17, 3.26 (3H each, both s, $OCH_3 \times 2$), 4.42 (1H, t-like, 3α-H), 5.15 (1H, t-like, 12-H). Mass Spectrum m/e (%): 528 (M^+, 17), 496 (M^+−MeOH, 35), 483 (M^+−CH_2OMe, 24), 451 (M^+−MeOH−CH_2OMe, 26), 391 (M^+−MeOH−CH_2OMe−AcOH, 13), 278 (**ia**, 15), 249 (**iia**, 6), 246 (**ia**−MeOH, 27), 233 (**ia**−CH_2OMe, 84), 201 (**ia**−MeOH−CH_2OMe, 100), 189 (**iia**−AcOH, 34).

Methylation of PS-2 (5) giving 5a——To a solution of **5** (255 mg) in DMSO (10 ml) was added dimsyl carbanion (10 ml) and the total mixture was kept stirring under N_2 atmosphere at room temperature for one hour, treated with CH_3I (8 ml), and kept stirring in the dark overnight. The reaction mixture was then poured into ice-water and extracted with ether (120 ml × 4 times). The ether extractive obtained after treating as for **4b** was purified by preparative TLC (benzene–acetone=8: 1, water) to give the nona-O-methyl derivative (**5a**, 116 mg), amorphous, $[\alpha]^{22}_{D}$ −12.8° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{51}H_{86}O_{14}$: C, 66.35; H, 9.40. Found: C, 66.59; H, 9.43. IR $\nu^{CCl_4}_{max}$ cm^{-1}: no OH, 1752 ($COOCH_3$), 1101. PMR (CCl_4, 90 MHz) δ: 0.79, 0.84, 0.87, 0.90, 0.92, 0.99, 1.26 (3H each, all s, *tert*-$CH_3 \times 7$), 3.17, 3.24, 3.31 (3H each), 3.43 (6H), 3.50, 3.53, 3.56, 3.72 (3H each) (all s, $OCH_3 \times 9$), 4.28, 4.52 (1H each, both d, J=7.0 Hz, anom. H of glucuronide and glucoside), 5.15 (1H, m, $W_{h/2}$= 8.0 Hz, 12-H); PMR ($CDCl_3$) δ: 4.38, 4.61 (1H each, both d, J=7.0 Hz, anom. H of glucuronide and glucoside).

$LiAlH_4$ Reduction of 5a giving 5b——To a solution of **5a** (50 mg) in dry ether (10 ml) was added a suspension of $LiAlH_4$ (150 mg) in dry ether (5 ml) and the total mixture was kept stirring at room temperature for 1.5 hr, treated successively with ether, aq. ether, and 20% aq. H_2SO_4 (adjusted to pH *ca.* 5.0) and extracted with ether (50 ml × 4 times). The ether extract was washed with sat. aq. $NaHCO_3$ and water and dried over $MgSO_4$. The residue obtained after evaporation of the solvent was purified by preparative TLC (benzene–acetone=4: 1, water) to give **5b** (40 mg), IR $\nu^{CCl_4}_{max}$ cm^{-1}: 3540, 3380 (w, OH), no $COOCH_3$.

Methanolysis of 5b——A solution of **5b** (40 mg) in anhydrous 10% HCl-MeOH (2 ml) was heated under reflux for 1.5 hr and treated as for **4b**. Purification of the product by preparative TLC (benzene–acetone=2: 1, I_2) furnished **2a** (19 mg) [identified by IR (CCl_4) and TLC (benzene–acetone=15: 1; n-hexane–ether=2: 1)], methyl 2,3,4,6-tetra-O-methyl-glucopyranoside [identified as above and also by GLC with column (a), column temp. 200°, t_R(min): 2′54″ (minor), 3′35″ (major)], and methyl 3,4-di-O-methyl-glucopyranoside [GLC: column (a), column temp. 170°, t_R(min): 26′29″ (major), 30′26″ (minor); column temp. 200°, t_R(min): 12′55″ (major), 14′40″ (minor). TLC: solvent (iii), *Rf* 0.40].

Methylation of PS-3 (6) giving 6a——A stirred solution of **6** (98 mg) in DMSO (5 ml) was treated with dimsyl carbanion (5 ml) for one hour and with CH_3I (7 ml) as above. The product obtained after the similar treatment as for **5** was purified by preparative TLC (benzene–acetone=6: 1, water) to give **6a** (69 mg), amorphous, $[\alpha]^{22}_{D}$ −16.4° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{60}H_{102}O_{19}$: C, 63.92; H, 9.12. Found: C, 64.32; H, 9.21. IR $\nu^{CCl_4}_{max}$ cm^{-1}: no OH, 1755 ($COOCH_3$), 1102. PMR (C_6D_6: CCl_4=1: 3, 90 MHz) δ: 0.91 (9H), 0.93 (6H), 1.01 (3H), 1.31 (3H) (all s, *tert*-$CH_3 \times 7$), 3.17, 3.23, 3.30, 3.32, 3.44 (3H each), 3.48 (9H), 3.51, 3.52, 3.53, 3.64 (3H each) (all s, $OCH_3 \times 12$), 4.30 (1H, d, J=6.0 Hz, anom. H of glucuronide), 4.71, 4.83 (1H each, both d, J=7.0 Hz, anom. H of glucoside and galactoside), 5.16 (1H, m, $W_{h/2}$=9.0 Hz, 12-H); PMR ($CDCl_3$) δ: 4.49 (1H, d, J=6.0 Hz, anom. H of glucuronide), 4.76, 4.85 (1H each, both d, J=7.0 Hz, anom. H of glucoside and galactoside); PMR (CCl_4) δ: 4.32 (1H, d, J=6.0 Hz, anom. H of glucuronide), 4.69, 4.83 (1H each, both d, J=7.0 Hz, anom. H of glucoside and galactoside).

$LiAlH_4$ Reduction of 6a giving 6b——To a solution of **6a** (50 mg) in dry ether (10 ml) was added a suspension of $LiAlH_4$ (150 mg) in dry ether (2 ml) and the total mixture was kept stirring for 1.5 hr, treated as for **5a**, and subjected to preparative TLC (benzene–acetone=5: 1, water) to give **6b**, amorphous, IR $\nu^{CCl_4}_{max}$ cm^{-1}: 3600, 3350 (w, OH), no $COOCH_3$.

Methanolysis of 6b——A solution of **6b** (35 mg) in anhydrous 10% HCl–MeOH (2 ml) was heated under reflux and treated as for **4b**. Purification of the product by preparative TLC (benzene–acetone=2: 1, I_2) gave **2a** (14 mg, identified by IR (CCl_4) and TLC) and three methyl monosaccharides (15 mg) which were identified with methyl 2,3,4,6-tetra-O-methyl-glucopyranoside [GLC: column (a) and (b) and TLC as above], methyl 2,3,4,6-tetra-O-methyl-galactopyranoside [GLC: column (a), column temp. 170°, t_R(min): 7′34″; column (b), column temp. 170°, t_R(min): 3′05″ (minor), 3′30″ (major). TLC: solvent (i), *Rf* 0.24 (major), 0.35 (minor)], and methyl 3-O-methyl-glucopyranoside [GLC: column (b), column temp. 170°, t_R(min): 4′09″; column (c), column temp. 175°, t_R(min): 3′38″. TLC: solvent (iii), *Rf* 0.18 (major), 0.26 (minor); solvent (v), *Rf* 0.21].

Methylation of PS-4 (7) giving 7a——A solution of **7** (50 mg) in DMSO (5 ml) was treated with dimsyl carbanion (3 ml) for one hour and with CH_3I (3 ml) as above. The product obtained after the similar treatment as for **5** was purified by preparative TLC (benzene–acetone=5: 1, water) to give **7a** (23 mg) which was purified by repeated precipitation from the methanolic solution with water. **7a**, white powder, $[\alpha]_D^{22}$ $-18.5°$ (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{68}H_{116}O_{23}$: C, 62.75; H, 8.98. Found: C, 63.00; H, 9.00. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1760 ($COOCH_3$), 1097. PMR ($CDCl_3$, 90 MHz) δ: 0.84, 0.87 (3H each), 0.91 (12H), 1.29 (3H) (all s, *tert*-$CH_3\times 7$), 1.27 (3H, d, J=7.0 Hz, *sec*-CH_3), 3.22, 3.24 (3H each), 3.37 (6H), 3.47 (15H), 3.50 (9H), 3.57, 3.73 (3H each) (all s, $OCH_3\times 14$), 4.46 (1H, d, J=6.0 Hz, anom. H of glucuronide), 4.55, 4.88 (1H each, both d, J=7.0 Hz, anom. H. of glucoside and galactoside), 5.17 (2H, m, $W_{h/2}$=9.0 Hz, 12-H and anom. H of rhamnoside).

$LiAlH_4$ Reduction of 7a giving 7b——A solution of **7a** (10 mg) in dry ether (2 ml) was treated with $LiAlH_4$ (50 mg)–dry ether (2 ml) as for **5a**. Purification of the product by preparative TLC (benzene–acetone=4: 1, water) gave **7b** (8 mg), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3603 (w, OH), no $COOCH_3$.

Methanolysis of 7b——A solution of **7b** (18 mg) in anhydrous 10% HCl–MeOH (2 ml) was heated under reflux for 1.5 hr, treated as for **4b**, and the product was subjected to preparative TLC (benzene–acetone=2: 1, I_2) to give **2a** (6 mg, identified by IR (CCl_4) and TLC), methyl 2,3,4,6-tetra-O-methyl-glucopyranoside (GLC and TLC as above), methyl 3-O-methyl-glucopyranoside (GLC and TLC as above), methyl 3,4,6-tri-O-methyl-galactopyranoside [GLC: column (a), column temp. 180°, t_R(min): 15′03″ (major), 22′18″ (minor); column temp. 200°, t_R(min): 7′56″ (major), 11′18″ (minor). TLC: solvent (v), *Rf* 0.52 (minor), 0.57 (major)], and methyl 2,3,4-tri-O-methyl-rhamnopyranoside [GLC: column (a), column temp. 180°, t_R (min): 2′39″ (major), 3′30″ (minor); column temp. 200°, t_R(min): 1′43″ (major), 2′12″ (minor); column (b), column temp. 170°, t_R(min): 1′20″ (major), 1′52″ (minor). TLC: solvent (i), *Rf* 0.44 (minor), 0.57 (major)].

Methylation of PS-5 (8) giving 8a——A solution of **8** (130 mg), in DMSO (10 ml) was treated with dimsyl carbanion (7 ml) and CH_3I (5 ml) as above. The product was purified by preparative TLC (benzene–acetone=3: 1, water) followed by active charcoal treatment to give **8a** (81 mg), amorphous, $[\alpha]_D^{22}$ $-20.1°$ (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{76}H_{130}O_{27}$: C, 61.85; H, 8.88. Found: C, 62.02; H, 8.97. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1759 ($COOCH_3$), 1094. PMR ($CDCl_3$, 90 MHz) δ: 0.85 (6H), 0.87 (3H), 0.93 (6H), 1.01, 1.31 (3H each) (all s, *tert*-$CH_3\times 7$), 1.27 (6H, d, J=7.0 Hz, *sec*-$CH_3\times 2$), 3.20, 3.23, 3.33, (3H each), 3.42 (9H), 3.44 (12H), 3.45 (9H), 3.46, 3.52, 3.67 (3H each) (all s, $OCH_3\times 16$), 4.44 (1H, d, J=6.0 Hz anom. H of glucuronide), 4.54, 4.88 (1H each, both d, J=7.0 Hz, anom. H of glucoside and galactoside), 5.04 (2H, br. s, anom. H of two rhamnosides), 5.20 (1H, m, $W_{h/2}$=9.0 Hz, 12-H).

$LiAlH_4$ Reduction of 8a giving 8b——A solution of **8a** (40 mg) in dry ether (5 ml) was reduced with $LiAlH_4$ (100 mg)- dry ether (2 ml) as for **5a**. Purification of the product by preparative TLC (benzene–acetone=2: 1, water) gave **8b** (33 mg), amorphous, IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3588 (w, OH), no $COOCH_3$.

Methanolysis of 8b——A solution of **8b** (125 mg) in anhydrous 10% HCl–MeOH (12 ml) was heated under reflux for 2.5 hr and treated as above. The reaction product was dissolved in a small amount of MeOH and poured into water to give the precipitate which was collected by filtration and crystallized from acetone. **2a** (31 mg) thus obtained was identified by mixed mp, IR (CCl_4), and TLC. The aqueous filtrate was concentrated under reduced pressure to give a residue (82 mg) which was subjected to preparative TLC (benzene–acetone=2: 1, I_2) to furnish methyl 2,3,4,6-tetra-O-methyl-glucopyranoside (13 mg), methyl 3-O-methyl-glucopyranoside (15 mg), methyl 3,4,6-tri-O-methyl-galactopyranoside (16 mg), methyl 2,3,4-tri-O-methyl-rhamnopyranoside (14 mg) [identified by GLC and TLC], methyl 3,4-di-O-methyl-α-L-rhamnopyranoside (**9**, 14 mg), and methyl 3,4-di-O-methyl-β-L-rhamnopyranoside (0.9 mg). **9**, colorless oil, $[\alpha]_D^{23}$ $-74.0°$ (c=2.2, $CHCl_3$) (in lit.[14]) $[\alpha]_D^{25}$ $-99°$ (c=2.05, MeOH)). High resolution mass spectrum: Calcd. for $C_9H_{18}O_5$ (M^+): 206.115. Found: 206.115. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3560 (OH). PMR (CCl_4, 90 MHz) δ: 1.20 (3H, d, J=6.0 Hz, *sec*-CH_3), 2.40 (1H, br. s, 2-OH, exchangeable with D_2O), 3.27, 3.42, 3.43 (3H each, all s, $OCH_3\times 3$), 3.83 (1H, d.d, J=2.0 and 4.0 Hz; varied to d, J=4.0 Hz upon irradiation at δ 4.49, 2-H), 4.49 (1H, d, J=2.0 Hz, varied to s upon irradiation at δ 3.83, anom. H). Mass Spectrum *m/e* (%): 206 (M^+, 0.4), 175 (9), 162 (8), 119 (17), 101 (11), 88 (100), 87 (13), 75 (76), 74 (86), 72 (42).[17] GLC of **9** (with minor β-anomer): column (a), column temp. 185°, t_R (min): 4′30″ (major), 7′20″ (minor), column temp. 190°, t_R (min): 3′51″ (major), 5′52″ (minor). TLC of **9** (with minor β-anomer): solvent (ii), *Rf* 0.44 (minor), 0.58 (major). The β anomer of **9**: $[\alpha]_D^{23}$ $+57.7°$ (c=0.13, MeOH); 2-O-acetate, $[\alpha]_D^{23}$ $+109.0°$ (c=0.19, MeOH) (in lit.[18]): $[\alpha]_D^{20}$ $+38°$ (c=1.02, H_2O)).

Acetylation of 9 giving 9a——A solution of **9** (25 mg) in Ac_2O (3 ml) and pyridine (1 ml) was left standing at room temperature for 10 hr, poured into ice-water, neutralized with sat. aq. $NaHCO_3$, and extracted with ether (50 ml × 4 times). Usual work-up of the ether extract gave a product (26 mg) which was purified by preparative TLC (benzene–acetone=4: 1, I_2) to give **9a** (23 mg), colorless glassy, $[\alpha]_D^{23}$ $-42.6°$ (c=1.98, MeOH). High resolution mass spectrum: Calcd. for $C_{11}H_{20}O_6$ (M^+): 248.126. Found: 248.126. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH,

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17) The fragmentation patterns agree well with those reported for the methyl hexopyranosides: J. Lönngren and S. Svennson, *Advan. Carbohyd. Chem. and Biochem.*, **29**, 41 (1974).

18) W.N. Haworth, E.L. Hirst, and E.J. Miller, *J. Chem. Soc.*, **1929**, 2469.

1757, 1238 (OAc). PMR (CCl_4, 90 MHz) δ: 1.23 (3H, d, J=6.0 Hz, *sec*-CH_3), 2.07 (3H, s, OAc), 3.29, 3.31, 3.46 (3H each, all s, $OCH_3 \times 3$), 4.43 (1H, d, J=2.0 Hz, varied to s upon irradiation at δ 5.10, anom. H), 5.10 (1H, d. d, J=4.0 and 2.0 Hz, varied to d, J=4.0 Hz upon irradiation at δ 4.43, 2-H). Mass Spectrum *m/e* (%): 248 (M^+, 0.3), 217 (M^+−MeO, 4, high mass: Calcd. for $C_{10}H_{17}O_5$ 217.108; Found 217.107), 204 (10), 129 (6), 116 (25), 101 (14), 88 (100), 75 (79), 74 (50), 72 (45).[17]

Methylation of Sakuraso-saponin (3) giving 3a——A solution of **3** (350 mg) in DMSO (8 ml) was treated with dimsyl carbanion (10 ml) for one hour and CH_3I (9 ml) as above. The product was mixed with silica gel (2 g), put on a column of silica gel (50 g), and chromatographed developing with benzene–acetone (8: 1). The eluate was evaporated and the residue was purified by repeated precipitation from the methanolic solution with water to give **3a** (243 mg), white powder, $[\alpha]_D^{22}$ −54.5° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{75}H_{128}O_{27}$: C, 61.62; H, 8.83. Found: C, 61.32; H, 8.75. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1757 ($COOCH_3$), 1094. PMR (C_6D_6, 90 MHz) δ: 0.91, 1.07 1.23 (6H each). 1.37 (3H) (all s, *tert*-$CH_3 \times 7$), 1.40 (6H, d, J=6.5 Hz, *sec*-$CH_3 \times 2$), 3.20, 3.23, 3.24, 3.37 (3H each), 3.39 (6H), 3.41 (3H), 3.46 (9H), 3.57, 3.60 (3H each), 3.64 (6H), 3.73 (3H) (all s, $OCH_3 \times 15$), 4.52 (1H, d, J=6.5 Hz, anom. H of glucuronide), 4.92, 5.25 (1H each, both d, J=7.0 Hz, anom. H of glucoside and galactoside), 5.37, 5.42 (1H each, both br. s, anom. H of two rhamnosides). PMR ($CDCl_3$, 90 MHz) δ: 4.46 (1H, d, J=6.5 Hz, anom. H of glucuronide), 4.55, 4.91 (1H each, both d, J=7.0 Hz, anom. H of glucoside and galactoside), 5.08 (2H, br. s, anom. H of two rhamnosides).

$LiAlH_4$ Reduction of 3a giving 3b——A solution of **3a** (130 mg) in dry ether (10 ml) was treated with $LiAlH_4$ (90 mg)- dry ether (2 ml) suspension as for **5a**. Preparative TLC (benzene–acetone=2: 1, water) of the product gave **3b** as white powder, $[\alpha]_D^{22}$ −35.3° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{74}H_{128}O_{26}$: C, 61.99; H, 9.00. Found: C, 61.62; H, 9.21. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3538 (w, OH), no $COOCH_3$. PMR (C_6D_6, 90 MHz) δ: 3.07, 3.21 (3H each), 3.26, 3.32, 3.38, 3.45 (6H each), 3.55 (3H), 3.61 (9H) (all s, $OCH_3 \times 14$), 4.87, 5.21 (1H each, both d, J=7.0 Hz, anom. H of glucoside and galactoside),[19] 5.28, 5.36 (1H each, both, br. s, anom. H of two rhamnosides). PMR ($CDCl_3$, 90 MHz) δ: 4.34, 4.53, 4.90 (1H each, all d, J=7.0 Hz, anom. H of two glucosides and galactosides), 5.04 (2H, br. s, anom. H of two rhamnosides).

Methanolysis of 3b——A solution of **3b** (55 mg) in anhydrous 9% HCl–MeOH (10 ml) was heated under reflux for one hour and treated as above. Preparative TLC (benzene–acetone=2: 1, I_2) of the product gave 16-O-methyl-primulagenin A (**2c**, 18 mg) and methyl monosaccharides (23 mg) which were identical with those obtained by methanolysis of **8b**: *i.e.* methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, methyl 3-O-methyl-glucopyranoside, methyl 3,4,6-tri-O-methyl-galactopyranoside, methyl 2,3,4-tri-O-methyl-rhamnopyranoside, and methyl 3,4-di-O-methyl-rhamnopyranoside (identified by GLC and TLC as above). **2c**, white powder, $[\alpha]_D^{22}$ +12.9° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{52}O_3$: C, 78.76; H, 11.09. Found: C, 78.73; H, 11.10. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3631, 3489 (OH), 2954, 2930, 1107, 1090. PMR (CCl_4, 90 MHz) δ: 0.74 (3H), 0.85 (6H), 0.91, 0.93, 0.95, 1.29 (3H each) (all s, *tert*-$CH_3 \times 7$), 3.28 (3H, s, OCH_3), 5.17 (1H, t-like, 12-H). PMR (d_5-pyridine) δ: 0.94 (6H), 0.96, 1.00, 1.02, 1.19, 1.49 (3H each) (all s, *tert*-$CH_3 \times 7$), 3.32 (3H, s, OCH_3), 5.30 (1H, t-like, 12-H). Mass Spectrum *m/e* (%): 472 (M^+, 43), 441 (M^+-CH_2OH, 10), 440 (M^+−MeOH, 8), 409 (M^+-CH_2OH−MeOH, 9), 264 (**i**, 99), 233 (**i**−CH_2OH, 100), 232 (**i**−MeOH, 26), 207 (**ii**, 32), 201 (**i**−CH_2OH−MeOH, 99), 189 (**ii**−H_2O, 31).

Acetylation of 2c giving 2d——A solution of **2c** (28 mg) in Ac_2O (2 ml) and pyridine (1 ml) was left standing at room temperature for 4 hr, poured into ice-water, and extracted with ether. Usual work-up of the ether extract gave a product which was purified by preparative TLC (benzene–acetone=15: 1, water) to furnish **2d** (19 mg). Recrystallization from acetone gave an analytical sample of mp 189—190° as colorless needles, $[\alpha]_D^{22}$ +17.1° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{35}H_{56}O_5$: C, 75.49; H, 10.14. Found: C, 75.35; H, 10.15. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 1743, 1245 (OAc). PMR (CCl_4, 90 MHz) δ: 0.87 (12H), 0.92, 0.96, 1.31 (3H each) (all s, *tert*-$CH_3 \times 7$), 2.02 (6H, s, OAc × 2), 3.27 (3H, s, OCH_3), 3.60, 3.84 (1H each, ABq, J=12.0 Hz, 28-H_2), 4.49 (1H, t-like, 3α-H), 5.23 (1H, t-like, 12-H). Mass Spectrum *m/e*(%): 556 (M^+, 7), 496 (M^+−AcOH, 15), 464 (M^+−AcOH−MeOH, 19), 306 (**ib**, 50), 274 (**ib**−MeOH, 19), 249 (**iia**, 19), 246 (**ib**−AcOH, 62), 233 (**ib**−CH_2OAc, 69), 214 (**ib**−AcOH−MeOH, 79), 201 (**ib**−CH_2OAc−MeOH, 100), 189 (**iia**−AcOH, 61).

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[19] The signal due to another glucoside anomeric proton was not observed since it was overlapped by the other carbinyl proton signals.