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Saponin and Sapogenol. XII.[1] Mi-Saponin A and Mi-Saponin B, Two Major Bisdesmosides from the Seed Kernels of *Madhuca longifolia* (L.) Macbride

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On the basis of chemical and physicochemical evidence, two major saponins named Mi-saponin A and Mi-saponin B, which were isolated previously from the seed kernels of *Madhuca longifolia* (L.) Macbride (syn. *Bassia longifolia* L.), have been elucidated to be expressed respectively as 3-O-β-D-glucopyranosyl-28-O-[α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl]-protobassic acid (**9**) and 3-O-β-D-glucopyranosyl-28-O-{3-O-β-D-apio-D-furanosyl-4-O-[α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl]-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl}-protobassic acid (**10**).

In the recent years,[3] we reported the isolation of three saponins named Mi-saponin A, B (two majors), and C (minor) from the seed kernels of *Madhuca longifolia* (L.) Macbride (syn. *Bassia longifolia* L.) and elucidated on the basis of the chemical evidence and the soil bacterial hydrolysis that protobassic acid (**1**) and Mi-glycoside I (**2**) are, respectively, the common genuine sapogenol and prosapogenol of these saponins. In this paper, we wish to present the chemical and physicochemical evidence which is consistent with the structures **9** and **10** for Mi-saponin A and Mi-saponin B, respectively.

Mi-Saponin A (9)

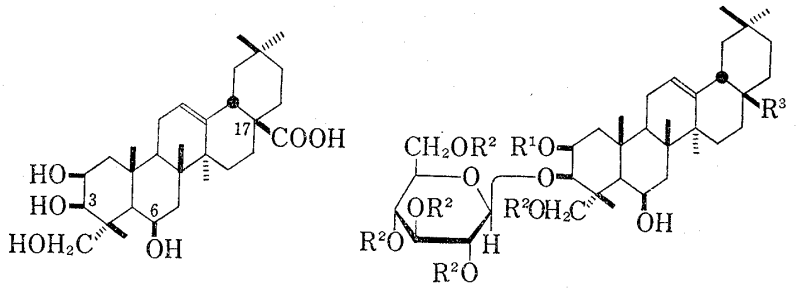
The infrared (IR) spectrum of Mi-saponin A (**9**) shows the ester carbonyl absorption band (1737 cm^{-1}) along with the strong hydroxyl band (3385 cm^{-1}). As reported previously,[3b] the soil bacterial hydrolysis[4] of Mi-saponin A (**9**) afforded protobassic acid (**1**) and Mi-glycoside I (**2**) as the major hydrolysates, while the acid hydrolysis of **9** liberated rhamnose, arabinose,[5] xylose, and glucose as the carbohydrate ingredients. On alkaline treatment, **9** yielded **2** as the sole prosapogenol (identified as the methyl ester pentaacetate **2a**). It has been presumed therefore that three monosaccharide moieties except glucose, which connects to 3β-OH of **1** (as in **2**), constitute the oligosaccharide chain attached to 17β-COOH of **1** through the ester-glycoside linkage. In the other words, Mi-saponin A (**9**) has been considered to be the bisdesmoside.[6]

Methylation of **9** with CH_3I/dimethyl sulfoxide(DMSO)/NaH[7] gave the pentadeca-O-methyl derivative (**9a**), which exhibits the hydroxyl (weak) and ester carbonyl absorption bands in its IR spectrum (3550 (w) and 1745 cm^{-1} in CCl_4). The retained free hydroxyl in **9a** is assumed to be the one at C-6 in the aglycone part. Methanolysis of **9a** yielded methyl 2,3,4-tri-O-methyl-rhamno-

1) Part XI: I. Kitagawa, H. Suzuki, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **23**, 2087 (1975).
2) Location: *133–1, Yamada-kami, Suita, Osaka, 565, Japan.*
3) *a*) I. Kitagawa, A. Inada, I. Yosioka, R. Somanathan, and M.U.S. Sultanbawa, *Chem. Pharm. Bull.* (Tokyo), **20**, 630 (1972); *b*) I. Yosioka, A. Inada, and I. Kitagawa, *Tetrahedron*, **30**, 707 (1974).
4) *a*) I. Yosioka, M. Fujio, M. Osamura, and I. Kitagawa, *Tetrahedron Letters*, **1966**, 6303; *b*) I. Yosioka, K. Imai, Y. Morii, and I. Kitagawa, *Tetrahedron*, **30**, 2283 (1974), and the preceding papers of the series cited therein.
5) Defined as L-arabinose by measuring the specific rotation.
6) R. Tschesche and G. Wulff, *Fortschr. Chem. Org. Naturstoffe*, **30**, 461 (1973).
7) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

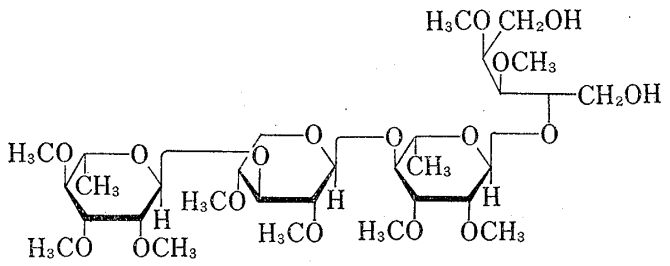
pyranoside, methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, methyl 2,4-di-O-methyl-xylopyranoside, methyl 2,3-di-O-methyl-rhamnopyranoside, and methyl 3,4-di-O-methyl-arabinopyranoside, which were isolated and identified respectively with the authentic specimens by thin-layer and gas-liquid chromatography (TLC, GLC).

Reduction of **9a** with $LiAlH_4$ in tetrahydrofuran furnished two products: **3** from the aglycone part and the nona-O-methyl derivative (**4**) from the oligosaccharide chain. The product **3** carries the free hydroxyl but no carbonyl function as shown by its IR spectrum. In the proton magnetic resonance (PMR) spectrum of **3**, are observed the signals due to six methoxyls and the anomeric proton (δ 4.20, d, J=7 Hz), and is also observed a one-proton multiplet at δ 4.45 ($W_{h/2}$=8 Hz) which is assignable to 6α-H in the triterpenoid part.[3b] Methanolysis of **3** gave methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, thus the structure **3** being rationalized.



1 : protobassic acid

2 : R^1=R^2=H, R^3=COOH (Mi-glycoside I)
2a : R^1=H, R^2=Ac, R^3=$COOCH_3$
3 : R^1=R^2=CH_3, R^3=CH_2OH



4

Chart 1

On the other hand, methanolysis of **4** furnished methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 2,4-di-O-methyl-xylopyranoside, methyl 2,3-di-O-methyl-rhamnopyranoside (TLC, GLC), and an unidentified product which has been assumed to be 3,4-di-O-methyl-arabitol on the basis of the comparison with methanolysis of **9a** described above. In conjunction with the conclusion resulted from the enzymatic hydrolysis of **9** (*vide infra*), the structure **4** has been reasoned for the nona-O-methyl-tetrasaccharide and the PMR analysis of **4** offers the further support for the formulation. Thus, the PMR spectrum ($CDCl_3$) of **4** shows the signals due to two rhamnose-methyls, nine methoxyls, and three anomeric proton signals at δ 4.60 (d, J=8 Hz), δ 4.98 (br. s), and δ 5.21 (br. s), among which the doublet at δ 4.60 suggests the presence of β-xylopyranoside linkage (taking Cl conformation) in **4**. The anomeric configurations of remaining two rhamnopyranosides have been assigned as α by application of the Klyne's empirical rule[8] to the partial hydrolysis products as shown later (Table I).

It has become evident so far that i) among five monosaccharides contained in Mi-saponin A (**9**), all the sugars but glucose are incorporated into the linear oligosaccharide moiety which

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

attaches to 17β-COOH of protobassic acid (**1**) through the ester-glycoside linkage, and ii) L-arabinose is the reducing end group in the oligosaccharide chain, while one of two rhamnose moieties is the non-reducing terminal.

In order to shed light on the mode of combination of the monosaccharide ingredients in Mi-saponin A (**9**), the enzymatic hydrolysis of **9** and its derivative (**7**) has been undertaken. Thus, enzymatic hydrolysis of **9** using crude hesperidinase afforded, along with protobassic acid (**1**), three prosapogenols designated as AH-1 (**5**), AH-2 (**7**), and AH-3 (**8**), whereas the subsequent enzymatic hydrolysis of **7** using crude takadiastase A yielded another prosapogenol designated as AHT-1 (**6**). All these prosapogenols retain the ester carbonyl function along with hydroxyl as shown by the IR spectra.

Methylation of AH-1 (**5**) with CH_3I/DMSO/NaH furnished the hexa-O-methyl derivative (**5a**) which, on methanolysis, liberated methyl 2,3,4-tri-O-methyl-arabinopyranoside (TLC, GLC). Since the PMR spectrum of **5b**, the hexaacetate of **5**, shows a one-proton doublet at δ 5.55 (J=5.5 Hz) which is ascribable to an anomeric proton in the ester-glycoside linkage,[4a,9] it has become obvious that the L-arabinose moiety[5] (Cl conformation) in **9** connects with α-orientation directly to 17β-COOH of protobassic acid (**1**).

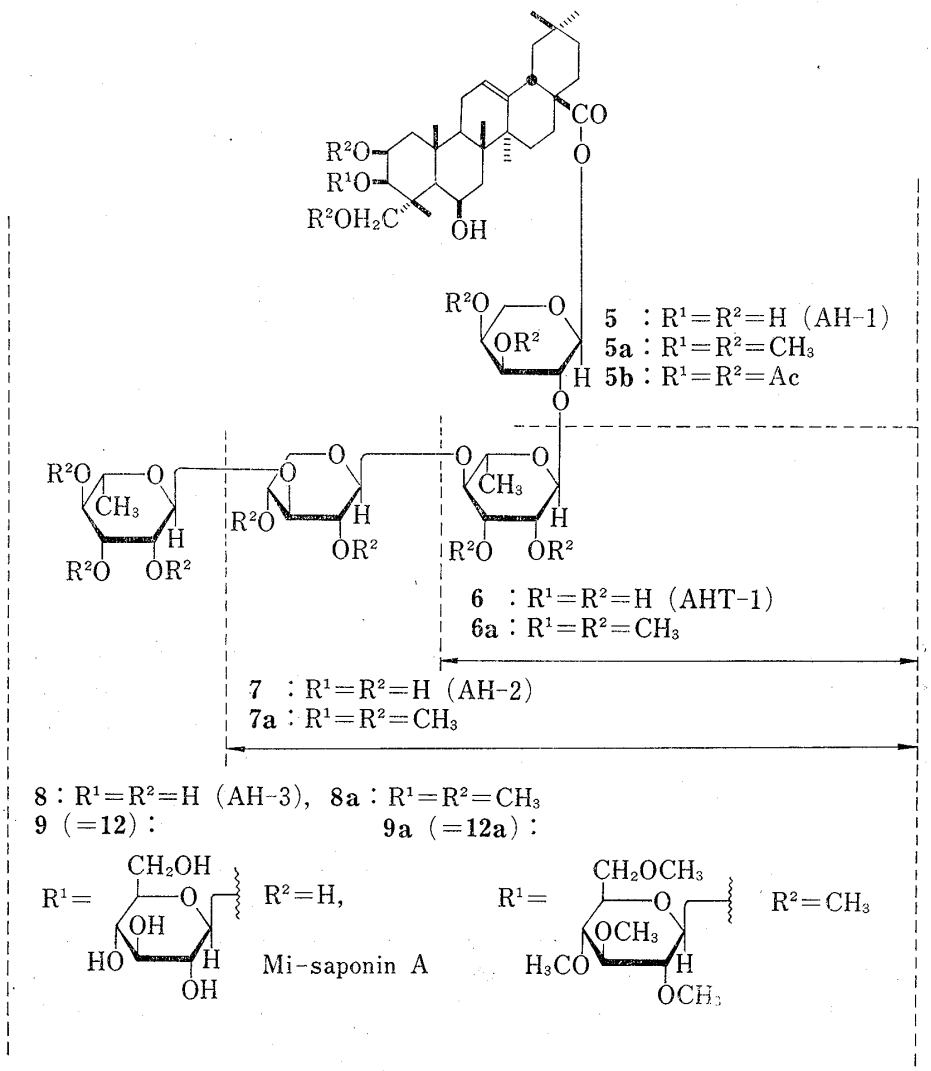


Chart 2

9) The anomeric proton in the PMR spectrum of **5** taken in d_5-pyridine is observed at δ 6.15 (d, J=5.5 Hz).

Methylation of the other prosapogenols (**6**, **7**, and **8**) as above gave the octa-O-methyl derivative (**6a**), the deca-O-methyl derivative (**7a**), and the dodeca-O-methyl derivative (**8a**), respectively. In the PMR spectrum of **7a**, are observed the signals due to ten methoxyls, three anomeric proton signals at δ 4.60 (d, J=8 Hz), δ 5.02 (br. s), and δ 5.78 (d-like), and a multiplet assignable to 6α-H in the triterpenoid portion at δ 4.40 ($W_{h/2}$=8 Hz).[3b]

Next, the three methylated derivatives (**6a**, **7a**, and **8a**) were subjected to methanolysis and the following methylated monosaccharides were identified (TLC, GLC): *i.e.*, methyl 2,3,4-tri-O-methyl-rhamnopyranoside and methyl 3,4-di-O-methyl-arabinopyranoside from **6a**, methyl 2,3,4-tri-O-methyl-xylopyranoside, methyl 2,3-di-O-methyl-rhamnopyranoside, and methyl 3,4-di-O-methyl-arabinopyranoside from **7a**, and methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 2,4-di-O-methyl-xylopyranoside, methyl 2,3-di-O-methyl-rhamnopyranoside, and methyl 3,4-di-O-methyl-arabinopyranoside from **8a**.

The accumulated evidence mentioned above has led us to formulate the structures of **4** (one of the $LiAlH_4$ reduction products of **9a**), AH-1 (**5**), AHT-1 (**6**), AH-2 (**7**), and AH-3 (**8**), and consequently to propose the structure of Mi-saponin A as **9** in which the anomeric configurations are undefined yet except those in the β-glucopyranoside and β-xylopyranoside linkages. The β orientations in the glucopyranoside and xylopyranoside have been revealed by the corresponding anomeric proton signal patterns (d, J=7—8 Hz) in the PMR spectra of **3**, **4**, **7a**, and **9a**. The rest of the anomeric configurations in **9** has been assigned by application of the Klyne's rule[8,10] to **8**, **7**, **6**, **5**, and **1** (Table I). In addition, the α-arabinopyranoside linkage in **9** has already been approved by the PMR analysis of **5b** as mentioned above.

Accordingly, the structure of Mi-saponin A has been expressed as 3-O-β-D-glucopyranosyl-28-O-[α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl]-protobassic acid (**9**).

Table I. Comparison of the Molecular Rotation Differences

	$[\alpha]_D$	$[M]_D$	$\Delta[M]_D$
Protobassic acid (**1**)	+22.7° (pyridine)[a]	+114°	
			−31°
AH-1 (**5**)	+13.0° (MeOH)	+83°	
			−114°
AHT-1 (**6**)	−4.0° (MeOH)	−31°	
			−40°
AH-2 (**7**)	−7.7° (MeOH)	−71°	
			−109°
AH-3 (**8**)	−17.0° (MeOH)	−180°	

a) insufficiently soluble in MeOH
$[M]_D$: methyl α-L-arabinopyranoside +29°; methyl β-L-arabinopyranoside +403°[10a]
methyl α-L-rhamnopyranoside −111°; methyl β-L-rhamnopyranoside +170°[10b]
methyl α-D-xylopyranoside +249°; methyl β-D-xylopyranoside −107°[10b]

Mi-Saponin B (10)

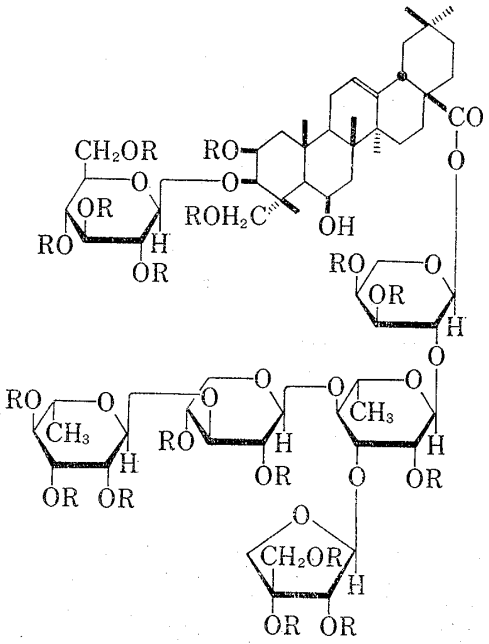
Mi-saponin B (**10**) possesses the ester carbonyl function (1754 cm^{-1}) and hydroxyls (3380 cm^{-1}) as shown by its IR spectrum and gave protobassic acid (**1**) and Mi-glycoside I (**2**) by the soil bacterial hydrolysis as in the case of Mi-saponin A (**9**).[3b] On complete acid hydrolysis, Mi-saponin B (**10**) liberated glucose, rhamnose, xylose, arabinose, and an undefined monosaccharide which was considered to be apiose,[11] while, on alkaline treatment, **10** gave Mi-glycoside I (**2**) as the sole prosapogenol (identified as **2a**). It follows therefore that all the

10) *a*) M. Kimura, M. Tohma, I. Yoshizawa, and H. Akiyama, *Chem. Pharm. Bull.* (Tokyo), **16**, 25 (1968); *b*) M. Kimura, M. Tohma, and I. Yoshizawa, *ibid.*, **16**, 1228 (1968).
11) Elucidated to be D-apiose on the basis of the following experiments (*e.g.*, methanolysis of **10a** and **11**).

monosaccharide ingredients of **10** except glucose, which links to 3β-OH of **1**, connect to 17β-COOH through the ester-glycoside bonding as in Mi-saponin A (**9**).

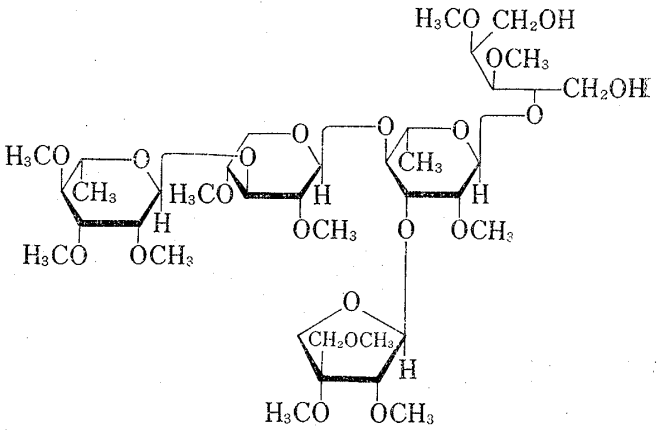
Methylation of **10** with CH_3I/DMSO/NaH furnished the heptadeca-O-methyl derivative (**10a**), which retained free 6β-OH in the triterpenoid portion (IR: 3450 cm^{-1} (w)) as in **9a**. Methylated monosaccharides generated by methanolysis of **10a** were isolated and identified by TLC and GLC with methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, methyl 2,4-di-O-methyl-xylopyranoside, methyl 3,4-di-O-methyl-arabinopyranoside, and methyl 2-O-methyl-rhamnopyranoside,[12] respectively. Methyl 2,3,4-tri-O-methyl-D-apio-D-furanoside should have been included in the total methanolysis products as is apparent from the elucidated structure of Mi-saponin B (**10**), however, the apiofuranoside could not be identified at this stage of experiments because of the overlapping on TLC and GLC. One of the distinct differences between the total methanolysis products of **9a** and those of **10a** is a fact that methyl 2-O-methyl-rhamnopyranoside was isolated from **10a** in place of 2,3-di-O-methyl-rhamnopyranoside from **9a**.

On the other hand, $LiAlH_4$ reduction of **10a** gave **3** and the undeca-O-methyl-pentasaccharide (**11**). On methanolysis, **11** afforded methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 2,4-di-O-methyl-xylopyranoside, methyl 2-O-methyl-rhamnopyranoside, and methyl 2,3,4-tri-O-methyl-D-apio-D-furanoside[13] as identified by TLC and GLC. The latter methyl apiofuranoside derivative was further identified by referring the PMR data and $[\alpha]_D$ value to those of the reported data.[13] Here again, on the basis of the established structure of Mi-saponin B (**10**), 3,4-di-O-methyl-arabitol should have been liberated on the methanolysis as in the case of methanolysis of **4**, and although in fact an additional product was isolated, it was not identified due to the lack of authentic sample. The structure of the undeca-O-methyl-pentasaccharide has been depicted as **11** in connection with the partial hydrolysis of Mi-saponin B (**10**) as described below, and the structure **11** has also been supported by the PMR spectrum, in which, along with the signals due to two rhamnose-methyls and eleven methoxyls,



10 : R=H Mi-saponin B
10a : R=CH_3

Chart 3



11

Chart 4

12) The identification of methyl 2-O-methyl-rhamnopyranoside was further confirmed by preparing the 3,4-diacetate.
13) D.H. Ball, F.H. Bissett, I.L. Klundt, and L. Long, Jr., *Carbohyd. Res.*, **17**, 165 (1971).

are observed four anomeric proton signals at δ 4.52 (1H, d, J=8 Hz), δ 4.90 (1H, br.s), δ 5.11 (1H, br.s), and δ 5.19 (1H, br.s). The doublet at δ 4.52 is assignable to the anomeric proton of the β-xylopyranoside linkage (Cl conformation), and based on the comparison of the PMR data of **11** and **4**, the signals observed at δ 5.11, δ 4.90, and δ 5.19 are assigned to the anomeric protons of apiofuranoside, rhamnopyranoside, and rhamnopyranoside, and their anomeric configurations are elucidated to be β, α, and α, respectively as shown below.

It has become clear so far that among five monosaccharide ingredients of Mi-saponin B (**10**) connected to 17β-COOH, the reducing terminal is arabinose while the non-reducing terminals are one of two rhamnose moieties and apiose.

Finally, the structure of Mi-saponin B (**10**) has been established on the following basis. On mild acid hydrolysis, Mi-saponin B (**10**) was converted to a glycoside (**12**), mp 235—238°, which showed the identical physical properties (mp, TLC, and IR) with those of Mi-saponin A (**9**). To confirm the identity of the glycoside with **9**, the glycoside was methylated with CH_3I/DMSO/NaH to give the pentadeca-O-methyl derivative (**12a**). The product **12a** has been identified with the pentadeca-O-methyl derivative of Mi-saponin A (**9a**) by IR and PMR. Furthermore, the methylated monosaccharides liberated by methanolysis of **12a** were identified with those obtained similarly from **9a**. It follows therefore that apiose in Mi-saponin B (**10**), which is readily liberated on mild acid treatment, links to 3-OH of the non-terminal rhamnose moiety in Mi-saponin A (**9**).

In regard to the anomeric configurations in glucoside and xyloside linkages, they are defined as β on the similar basis of the PMR evidence as in the case of Mi-saponin A (**9**) (*vide supra*). Since Mi-saponin A (**9**) was obtained by the mild acid hydrolysis of Mi-saponin B (**10**), two rhamnose residues and arabinose in **10** are linked with α-orientation as in **9**. Moreover, the anomeric configuration of the β-apiofuranoside moiety in **10** has been disclosed on the basis of the $[M]_D$ comparison[8]: $[M]_D$ of **10a**—$[M]_D$ of **9a**=−175°; $[M]_D$ of methyl 2,3,4-tri-O-methyl-α-D-apio-D-furanoside=+239°; $[M]_D$ of methyl 2,3,4-tri-O-methyl-β-D-apio-D-furanoside=−163°.[13]

Accordingly, the structure of Mi-saponin B has been established as 3-O-β-D-glucopyranosyl-28-O-{3-O-β-D-apio-D-furanosyl-4-O-[α-L-rhamnopyranosyl(1→3)-β-D-xylopyranosyl]-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl}-protobassic acid (**10**). The natural occurrence of apiose in the oligosaccharide portion of saponin is quite rare and the present elucidation seems to be the second example.[14]

Experimental[15]

Isolation of Mi-Saponin A, B, and C——Silica gel (800 g) column chromatography of Mi-saponin (40 g)[3b] eluting with *n*-BuOH saturated with water furnished Mi-saponin A (**9**) (6.0 g), Mi-saponin B (**10**) (6.1 g), and Mi-saponin C (1.1 g) (in the order of elution) along with the mixtures of them (totally 20.0 g).

14) The first occurrence of apiose in saponin seems to be in the sugar moiety of platycodin D isolated from the root of *Platycodon grandiflorum* (Campanulaceae): A. Tada, T. Kaneiwa, J. Shoji, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), No. 11 (1975), "in press."

15) The following instruments were used for obtaining the physical data. Melting-points; Yanagimoto Micro-meltingpoint Apparatus and recorded uncorrected; Specific rotation: Rex Photoelectric Polarimeter (1=1 dm); IR spectra: Hitachi IR Spectrometer EPI-S2 or EPI-G3; Mass spectra: Hitachi RMU-6D Spectrometer; PMR spectra: Hitachi R-22 (90 MHz) NMR Spectrometer (in $CDCl_3$ unless specified otherwise and tetramethylsilane as the internal standard); GLC: Hitachi Type 063 Gas Chromatograph.

Silica gel (Camag D-5) was used for TLC and detection was made by 1% $Ce(SO_4)_2$/10% H_2SO_4. For preparative TLC, detection was made by spraying water or by keeping the developed TLC plate in the I_2 chamber. For column chromatography, silica gel (Merck, 0.05—0.2 mm) was used, and Toyo Filter Paper No. 50 was used for paper partition chromatography (PPC) and detection was made by spraying aniline hydrogen phthalate followed by heating.

Recrystallization from EtOAc saturated with water gave Mi-saponin A (**9**) of mp 235—238° (decomp., colorless fine crystals), $[\alpha]_D^{20}$ −33.1° (c=1.10, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3385 (br, OH), 1737 (–COO–). *Anal.* Calcd. for $C_{58}H_{94}O_{27}\cdot 2H_2O$: C, 55.31; H, 7.78. Found: C, 54.91; H, 7.59.

Analytical sample of Mi-saponin B (**10**) was obtained by recrystallization from the mixture of *n*-BuOH saturated with water and MeOH as colorless needles of mp 250—253°, $[\alpha]_D^{20}$ −45.0° (c=1.22, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3380 (br, OH), 1754 (–COO–). *Anal.* Calcd. for $C_{63}H_{102}O_{31}\cdot 2H_2O$: C, 54.37; H, 7.67. Found: C, 54.59; H, 7.96. Mi-saponin C: white powder.[3b]

Acid Hydrolysis of Mi-Saponin A (9)——1) A solution of **9** (20 mg) in 5% H_2SO_4–EtOH (1: 1, 6 ml) was heated under reflux for 2 hr, diluted with water after cooling, concentrated under reduced pressure to remove EtOH, and extracted with EtOAc. The aqueous layer was taken, neutralized with resin Dowex 44 (OH^-), and evaporated under reduced pressure to give the residue which was subjected to PPC (iso-PrOH–*n*-BuOH–H_2O=7: 1: 2) and xylose, arabinose, rhamnose, and glucose were identified. 2) Mi-saponin A (**9**) (300 mg) was treated with 5% H_2SO_4–EtOH (1: 1, 20 ml) and the residue (120 mg) obtained by the similar treatment as above was subjected to preparative PPC (iso-PrOH–*n*-BuOH–H_2O=7: 1: 2, developing three times) and the fraction (20 mg) containing arabinose was taken and purified with small amount of charcoal to give pure arabinose (8.3 mg) of $[\alpha]_D^{14}$ +180.5° (c=0.83, H_2O, in the equilibrium state). L-Arabinose: $[\alpha]_D^{14}$ +185.6° (c=1.25, H_2O, in the equilibrium state).

Alkaline Treatment of Mi-Saponin A (9)——A solution of **9** (100 mg) in 20% aq. KOH–EtOH (1: 1, 40 ml) was heated under reflux for 3 hr. After cooling, the reaction mixture was diluted with water, neutralized with 5% H_2SO_4, concentrated under reduced pressure, and extracted with EtOAc. The EtOAc extractive (28 mg) was treated with CH_2N_2–ether and the methylated product (30 mg) was subsequently treated with Ac_2O (2 ml)–pyridine (3 ml) at room temperature for 36 hr. The product obtained by the usual work-up was crystallized from acetone to give colorless needles of Mi-glycoside I methyl ester pentaacetate (**2a**) (8 mg), identical with the authentic sample[3b] by mixed mp, TLC, and IR.

Mi-Saponin A Pentadeca-O-methyl Ether (9a)——A suspension of NaH (3 g, defatted with *n*-hexane beforehand) in dimethyl sulfoxide (DMSO) (50 ml) was heated at 80—100° for 30 min under N_2 atmosphere to furnish the solution of dimsyl carbanion. To a solution of **9** (450 mg) in DMSO (30 ml) was added the above prepared dimsyl carbanion (3 ml) and the total mixture was kept stirring under N_2 atmosphere for 1 hr, treated with CH_3I (3 ml) and stirred for further 3 hr in the dark. The reaction mixture was then poured into ice-water and extracted with EtOAc. The EtOAc solution was washed with 5% $Na_2S_2O_3$ and water, and treated as usual to give the product which was methylated again as above and the final product was purified by preparative TLC (benzene–acetone=3: 1) to give **9a** (187 mg, white powder), $[\alpha]_D^{18}$ −42.5° (c=1.41, $CHCl_3$). *Anal.* Calcd. for $C_{73}H_{124}O_{27}$: C, 61.13; H, 8.71. Found: C, 60.72; H, 8.92. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3550 (w, OH), 1745 (–COO–). PMR (δ)[16]: 4.62 (1H, d, J=8 Hz, anomeric H of xyloside), 5.02, 5.22 (1H, each, br. s, anomeric H's of rhamnosides), 5.78 (1H, d-like, $W_{h/2}$=4 Hz, anomeric H of arabinoside), 5.28—5.38 (1H, m, 12-H).

Methanolysis of 9a——A solution of **9a** (20 mg) in anhydrous 5% HCl–MeOH (3 ml) was heated under reflux for 2 hr. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and filtered to remove the precipitates. The residue obtained by evaporation of the filtrate was subjected to preparative TLC (benzene–acetone=3: 1) and the following methylated monosaccharides were isolated and identified by TLC and GLC with the authentic specimens.

Table II[17]

	TLC (Rf)[a]	GLC (t_R)[b]
Methyl 2,3,4-tri-O-methyl-rhamnopyranoside	0.74	2′14″
Methyl 2,3,4,6-tetra-O-methyl-glucopyranoside	0.70	4′59″
	0.53	7′24″
Methyl 2,4-di-O-methyl-xylopyranoside	0.36 (minor)	8′23″ (minor)
	0.26 (major)	12′15″ (major)
Methyl 2,3-di-O-methyl-rhamnopyranoside	0.36	8′23″
Methyl 3,4-di-O-methyl-arabinopyranoside	0.10	13′23″

a) TLC: benzene–acetone=3: 1
b) GLC: 15% ethyleneglycol succinate polyester on Uniport B (3 mm×1 m); column temp. 160°, N_2 flow rate 40 ml/min

16) The signal due to glucoside anomeric H is overlapped by the other signal.
17) For identification of the methylated monosaccharides, the conditions described here were applied for TLC and GLC hereafter unless specified otherwise.

$LiAlH_4$ Reduction of 9a and Methanolysis of 3 and 4——To a solution of **9a** (120 mg) in tetrahydrofuran (10 ml) was added $LiAlH_4$ (60 mg) and the total mixture was heated under reflux for 3 hr. After cooling, the mixture was treated with aqueous ether (to decompose excess $LiAlH_4$), extracted with ether and EtOAc successively.

The ether extract, after usual work-up, furnished the product (79 mg), which was purified by preparative TLC (benzene–acetone=3: 1) to give **3** (52 mg) as white powder (crystallization being failed), $[\alpha]_D^{19}$ +40.6° (c=0.65, $CHCl_3$). *Anal.* Calcd. for $C_{42}H_{72}O_{10}\cdot 1/2H_2O$: C, 67.56; H, 9.86. Found: C, 67.91; H, 10.07. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3640, 3500 (OH). PMR (δ): 0.92 (6H, s), 1.16, 1.28, 1.35, 1.62 (3H each, all s) (totally –C(CH$_3$)×6), 3.32, 3.40, 3.42, 3.56 (3H each, all s), 3.68 (6H, s) (OCH$_3$×6), 4.45 (1H, m, $W_{h/2}$=8 Hz, 6α-H), 5.20—5.35 (1H, m, 12-H), 4.20 (1H, d, J=7 Hz, 1′-H). A solution of 3 (8 mg) in anhydrous 6% HCl–MeOH (2 ml) was heated under reflux for 1 hr, and the methylated sugar thus obtained was identified with methyl 2,3,4,6-tetra-O-methyl-glucopyranoside by TLC and GLC.

The EtOAc extract was treated similarly and the product (34 mg) was purified by preparative TLC (benzene–MeOH=5: 1) to give an oily product **4** (28 mg), $[\alpha]_D^{19}$ −107.0° (c=0.40, $CHCl_3$). *Anal.* Calcd. for $C_{31}H_{58}O_{17}$: C, 52.95; H, 8.32. Found: C, 52.64; H, 8.47. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3450 (OH). PMR (δ): 1.25, 1.31 (3H, each, d, J=6 Hz, >CH–CH$_3$×2), 3.42, 3.47, 3.49, 3.51, 3.53 (3H each, all s), 3.57, 3.60 (6H each, all s) (OCH$_3$×9), 4.60 (1H, d, J=8 Hz, anomeric H of xyloside), 4.98, 5.21 (1H each, br. s, anomeric H's of rhamnosides). A solution of **4** (22 mg) in anhydrous 6% HCl–MeOH (2 ml) was heated under reflux for 1 hr. After cooling, the reaction mixture was neutralized with Ag_2CO_3, filtered to remove the precipitates, and evaporated under reduced pressure to furnish the products which were identified by TLC and GLC with methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 2,4-di-O-methyl-xylopyranoside, and methyl 2,3-di-O-methyl-rhamnopyranoside. A product, which was deduced to be 3,4-di-O-methyl-arabitol, was detected at the same time, however, the identification could not be made.

Enzymatic Hydrolysis of Mi-Saponin A (9) with Crude Hesperidinase——A solution of **9** (2.0 g) in the Na_2HPO_4–citric acid buffer solution (pH 4.0) (250 ml) was treated with crude hesperidinase (Tanabe Pharm. Co., Lot. No. N-30) (250 mg) and the total mixture was kept stirring at 31—33° for 70 hr and extracted with *n*-BuOH. The *n*-BuOH solution was then washed with water and evaporated under reduced pressure to give a product (1.1 g) which was subjected to silica gel (70 g) column chromatography eluting with *n*-BuOH saturated with water and protobassic acid (**1**) (87 mg), AH-1 (**5**) (24 mg), AH-2 (**7**) (621 mg), AH-3 (**8**) (22 mg) and their mixture (totally *ca.* 200 mg) were obtained.

AH-1 (**5**), mp 196.5—199° (colorless fine crystals from EtOAc saturated with water), $[\alpha]_D^{24}$ +13° (c=0.47, MeOH). *Anal.* Calcd. for $C_{35}H_{56}O_{10}\cdot 1/2H_2O$: C, 65.09; H, 8.89. Found: C, 64.97; H, 8.63. IR ν_{max}^{KBr} cm^{-1}: 3400 (br, OH), 1740 (–COO–). PMR (d_5-pyridine) δ: 0.89, 0.95, 1.20, 1.60, 1.92, 2.15 (3H each, all s) (–C(CH$_3$)×6), 6.15 (1H, d, J=5.5 Hz, 1′-H).

AH-2 (**7**), mp 236—238° (colorless needles from $CHCl_3$–MeOH), $[\alpha]_D^{29}$ −7.7° (c=1.05, MeOH). *Anal.* Calcd. for $C_{46}H_{74}O_{18}\cdot 3H_2O$: C, 57.01; H, 8.32. Found: C, 56.90; H, 8.76. IR ν_{max}^{KBr} cm^{-1}: 3375 (br, OH), 1750 (–COO–).

AH-3 (**8**), mp 236—238.5° (colorless fine crystals from EtOAc saturated with water), $[\alpha]_D^{26}$ −17.0° (c=0.57, MeOH). *Anal.* Calcd. for $C_{52}H_{84}O_{22}\cdot 3H_2O$: C, 56.09; H, 8.13. Found: C, 56.;34 H, 8.28. IR ν_{max}^{KBr} cm^{-1}: 3400, (br, OH), 1725 (–COO–).

Enzymatic Hydrolysis of AH-2 (7) with Crude Takadiastase A——A suspension of **7** (50 mg) in EtOH (3 ml) and the Na_2HPO_4–citric acid buffer solution (pH 4.0) (20 ml) was treated with crude takadiastase A (100 mg) and the total mixture was kept stirring at 31° for 5 days and extracted with *n*-BuOH. The *n*-BuOH solution was then washed with water, dried, and evaporated under reduced pressure to give a product (60 mg), which was purified by silica gel (5 g) column chromatography eluting with $CHCl_3$–MeOH–H_2O (65: 35: 10, lower layer). Recrystallization of the product from $CHCl_3$–MeOH furnished **6** (26 mg) as colorless needles of mp 235—238°, $[\alpha]_D^{29}$ −4.0° (c=0.45, MeOH). *Anal.* Calcd. for $C_{41}H_{66}O_{14}\cdot H_2O$: C, 61.47; H, 8.56. Found: C, 61.70; H, 8.99. IR ν_{max}^{KBr} cm^{-1}: 3450 (br, OH), 1751 (–COO–).

Methanolysis of AH-1 Hexa-O-methyl Ether (5a)——To a solution of **5** (10 mg) in DMSO (10 ml) was added dimsyl carbanion (1 ml) (prepared from 1 g of NaH and 10 ml of DMSO as above) and the total mixture was kept stirring under N_2 atmosphere for 1 hr, treated with CH_3I (0.5 ml), and kept stirring for further 3 hr in the dark. The mixture was poured into ice-water and extracted with EtOAc and the EtOAc extract, after ordinary work-up, furnished a product which was purified by preparative TLC (benzene–acetone=3: 1) to give **5a** (5 mg), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3400 (w, OH), 1722 (–COO–). A solution of **5a** (5 mg) in anhydrous 6% HCl–MeOH (2 ml) was heated under reflux for 2 hr, neutralized with Ag_2CO_3, and filtered to remove the precipitates. Evaporation of the filtrate yielded methyl 2,3,4-tri-O-methyl-arabinopyranoside being identified by TLC and GLC.

AH-1 Hexaacetate (5b)——Acetylation of **5** (40 mg) with Ac_2O (1.5 ml) and pyridine (3 ml) at 31° for 24 hr yielded a product which was purified by preparative TLC (benzene–acetone=3: 1) to give **5b** (white powder, 28 mg), $[\alpha]_D^{22}$ +33.0° (c=0.40, $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3550 (w, OH), 1748 (br, OAc, –COO–). PMR (δ): 0.90 (6H), 1.02, 1.07, 1.38, 1.57 (3H, each) (all s, –C(CH$_3$)×6), 1.99, 2.01, 2.05, 2.10 (3H each, all s), 2.04

(6H, s) (OAc×6), 3.55—4.10 (4H, m, 4α-C$\underline{H}_2$OAc, 5′-$\underline{H}_2$), 4.38 (1H, m, $W_{h/2}$=8 Hz, 6α-$\underline{H}$), 4.84 (1H, d, J=4 Hz, 3α-$\underline{H}$), 5.00—5.25 (3H, m, 2′,3′,4′-$\underline{H}_3$), 5.25—5.45 (2H, m, 12-$\underline{H}$, 2α-$\underline{H}$), 5.55 (1H, d, J=5.5 Hz, 1′-$\underline{H}$).

Methanolysis of AHT-1 Octa-O-methyl Ether (6a)——A solution of **6** (7 mg) in DMSO (10 ml) was treated with dimsyl carbanion (2 ml) (prepared from 1 g of NaH and 25 ml of DMSO) and the total mixture was kept stirring under N_2 atmosphere at room temperature for 1 hr, added with CH_3I (0.5 ml), and stirred for further 3 hr in the dark. After working-up as in the case of **5a**, was obtained **6a** (5 mg), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3430 (w, OH), 1733 (–COO–), which was dissolved in anhydrous 6% HCl–MeOH (3 ml) and heated under reflux for 2 hr. After cooling, the mixture was neutralized with Ag_2CO_3, filtered to remove the precipitates, and concentrated to give the products which were identified by TLC and GLC with methyl 2,3,4-tri-O-methyl-rhamnopyranoside and methyl 3,4-di-O-methyl-arabinopyranoside.

AH-2 Deca-O-methyl Ether (7a)——A solution of **7** (250 mg) in DMSO (12 ml) was treated with dimsyl carbanion (3 ml) (prepared from 1 g of NaH and 20 ml of DMSO), stirred under N_2 atmosphere for 30 min, added with CH_3I (2 ml), and stirred for further 3 hr in the dark. The reaction mixture was poured into ice-water, and extracted with EtOAc. The product obtained from the EtOAc extract was purified by preparative TLC to give **7a** (102 mg, white powder), $[\alpha]_D^{28}$ −17.8° (c=1.28, $CHCl_3$), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3450 (w, OH), 1735 (–COO–). PMR (δ): 0.92, 0.96, 1.10, 1.13, 1.55 (3H each, all s), 1.28 (br, 6H) (–$\overset{|}{C}$–C$\underline{H}_3$×6, >CH–C$\underline{H}_3$ in rhamnose), 3.31, 3.36, 3.39, 3.48, 3.58, 3.60 (3H each, all s), 3.45, 3.52 (6H each, s) (OC$\underline{H}_3$×10), 4.40 (1H, m, $W_{h/2}$=8 Hz, 6α-$\underline{H}$), 4.60 (1H, d, J=8 Hz, anomeric H of xyloside), 5.02 (1H, br. s, anomeric H of rhamnoside), 5.78 (1H, d-like, $W_{h/2}$=4 Hz, anomeric H of arabinoside), 5.27—5.46 (1H, m, 12-$\underline{H}$).

Methanolysis of 7a——A solution of **7a** (50 mg) in anhydrous 6% HCl–MeOH (3 ml) was heated under reflux for 1 hr. After cooling, the mixture was neutralized with Ag_2CO_3, filtered to remove the precipitates, and evaporated to dryness to give a product which was subjected to preparative TLC (benzene–acetone =3:1). Isolated methylated sugars were identified respectively with methyl 2,3,4-tri-O-methyl-xylopyranoside, methyl 2,3-di-O-methyl-rhamnopyranoside, and methyl 3,4-di-O-methyl-arabinopyranoside by TLC (benzene–acetone=3:1, benzene–MeOH=5:1) and GLC (a) 15% ethyleneglycol succinate polyester on Uniport B, 3 mm×1 m; column temp. 160°; N_2 flow rate 30 ml/min, (b) 15% neopentylglycol succinate on chromosorb WAW, 3 mm×2 m; column temp. 185°; N_2 flow rate 50 ml/min.).

Methanolysis of AH-3 Dodeca-O-methyl Ether (8a)——To a solution of **8** (10 mg) in DMSO (5 ml) was added dimsyl carbanion (1 ml) (prepared from 1 g of NaH and 10 ml of DMSO) and the mixture was stirred under N_2 atmosphere for 1 hr, treated with CH_3I (0.5 ml), and stirred for further 3 hr in the dark. The product obtained by the similar working-up as for **5a** gave **8a** (ca. 5 mg), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3400 (w, OH), 1730 (–COO–), which was dissolved in anhydrous 6% HCl–MeOH (3 ml) and heated under reflux for 1 hr. After cooling, the reaction mixture was neutralized with Ag_2CO_3, filtered to remove the precipitates, and evaporated to dryness to give the products, which were identified by TLC and GLC with methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 2,4-di-O-methyl-xylopyranoside, methyl 2,3-di-O-methyl-rhamnopyranoside, and methyl 3,4-di-O-methyl-arabinopyranoside.

Acid Hydrolysis of Mi-Saponin B (10)——A solution of **10** (20 mg) in 5% H_2SO_4–EtOH (1:1, 4 ml) was heated under reflux for 2 hr. After cooling, the mixture was diluted with water while removing EtOH under reduced pressure and extracted with EtOAc. The aqueous layer was neutralized with resin Dowex 44 (OH^-) and evaporated under reduced pressure to give the monosaccharide mixture which was subjected to PPC (iso-PrOH–*n*-BuOH–H_2O=7:1:2) and xylose, arabinose, rhamnose, and glucose were identified. The spot presumably due to apiose was also detected.

Alkaline Treatment of Mi-Saponin B (10)——A solution of **10** (100 mg) in 20% aq. KOH–EtOH (1:1, 60 ml) was heated under reflux for 3 hr. After cooling, the reaction mixture was diluted with water, neutralized with 5% H_2SO_4, concentrated under reduced pressure, and extracted with EtOAc. The EtOAc extractive (30 mg) was treated with CH_2N_2–ether and the methyl ester (32 mg) was acetylated with Ac_2O (2 ml)–pyridine (5 ml) at 31° for 40 hr, poured into water, and treated in the usual manner. Crystallization of the product from acetone furnished colorless needles (12 mg) of Mi-glycoside I methyl ester pentaacetate (**2a**) being identified by mixed mp, TLC, and IR.

Mi-Saponin B Heptadeca-O-methyl Ether (10a)——A solution of **10** (400 mg) in DMSO (15 ml) was treated with dimsyl carbanion (8 ml) (prepared from 3 g of NaH and 50 ml of DMSO) and the total mixture was stirred under N_2 atmosphere at room temperature for 2 hr, treated with CH_3I (3 ml), stirred for further 3 hr in the dark, poured into ice-water, and extracted with EtOAc. The EtOAc extract, after working-up as for **9**, gave a product which was methylated again as above and the final product (352 mg) was purified by preparative TLC (benzene–acetone=3:1) to give **10a** (white powder, 192 mg), $[\alpha]_D^{23}$ −49.4° (c=0.64, $CHCl_3$). *Anal.* Calcd. for $C_{80}H_{136}O_{31}$: C, 60.21; H, 8.60. Found: C, 59.61; H, 8.80. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3450 (w, OH), 1740 (–COO–). PMR (δ)[16]: 4.50 (1H, d, J=8 Hz, anomeric H of xyloside), 5.04, 5.19 (1H each, br. s, anomeric H's of rhamnosides), 5.12 (1H, br. s, anomeric H of apioside), 5.72 (1H, d-like, $W_{h/2}$=4 Hz, anomeric H of arabinoside), 5.24—5.46 (1H, m, 12-H).

Methanolysis of 10a——A solution of **10a** (34 mg) in anhydrous 9% HCl–MeOH (5 ml) was heated under reflux for 3 hr. After cooling, the mixture was neutralized with Ag_2CO_3, filtered to remove the pre-

cipitates, and concentrated to give the product which was subjected to preparative TLC (benzene–acetone =3: 1) and the following methylated monosaccharides were isolated and identified by TLC and GLC.

Table III[a)]

	TLC (*Rf*)	GLC (t_R)
Methyl 2,3,4-tri-O-methyl-rhamnopyranoside	0.74	2′13″
Methyl 2,3,4-tri-O-methyl-apiofuranoside	0.68	3′08″
Methyl 2,3,4,6-tetra-O-methyl-glucopyranoside	0.70	4′59″
	0.53	7′24″
Methyl 2,4-di-O-methyl-xylopyranoside	0.36 (minor)	8′52″ (minor)
	0.26 (major)	12′14″ (major)
Methyl 3,4-di-O-methyl-arabinopyranoside	0.10	13′21″
Methyl 2-O-methyl-rhamnopyranoside	0.09	21′15″

a) conditions of TLC and GLC were same as in Table II

The fraction containing methyl 2-O-methyl-rhamnopyranoside was acetylated with Ac_2O (0.2 ml)–pyridine (0.6 ml) at 30° for 15 hr. The 3,4-diacetate thus obtained was identified with the authentic sample by TLC (benzene–acetone=3: 1), and GLC (15% ethyleneglycol succinate polyester on Uniport B (3 mm ×1 m); column temp. 180°; N_2 flow rate 50 ml/min; t_R=14′58″).

$LiAlH_4$ Reduction of 10a——A solution of **10a** (125 mg) and $LiAlH_4$ (40 mg) in tetrahydrofuran (10 ml) was heated under reflux for 3 hr, treated with aqueous ether (to decompose excess $LiAlH_4$), and extracted with ether and EtOAc successively. The ether extract, after usual work-up, furnished a product (62 mg), which was purified by preparative TLC (benzene–acetone=3: 1) to give **3** (white powder, 48 mg) being identified by TLC, IR and PMR. The product, obtained from the EtOAc extract after ordinary work-up, was purified by silica gel (5 g) column chromatography eluting with ether–acetone (1: 1) to give an oily product (**11**, 38 mg), $[\alpha]_D^{24}$ −119.0° (*c*=0.65, $CHCl_3$). *Anal.* Calcd. for $C_{38}H_{70}O_{21}$: C, 52.89; H, 8.17. Found: C, 53.39; H, 7.83. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3450 (OH). Mass Spectrum *m/e* (%): 189 (63), 175 (13), 143 (100). PMR (δ): 1.23, 1.30 (3H each, d, *J*=6 Hz, >CH–C$\underline{H}_3$×2), 4.52 (1H, d, *J*=8 Hz, anomeric H of xyloside), 4.90, 5.19 (1H each, br. s, anomeric H's of rhamnosides), 5.11 (1H, br. s, anomeric H of apioside).

A solution of **11** (10 mg) in anhydrous 9% HCl–MeOH (1 ml) was heated for 2 hr under reflux and treated as for **4**. The methylated monosaccharides thus obtained were identified by TLC and GLC with methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 2,3,4-tri-O-methyl-D-apio-D-furanoside, methyl 2,4-di-O-methyl-xylopyranoside, and methyl 2-O-methyl-rhamnopyranoside.[12)] Another methylated substance, which was assumed to be 3,4-di-O-methyl-arabitol, was also obtained but not identified.

Isolation of Methyl 2,3,4-Tri-O-methyl-D-apio-D-furanoside——A solution of **11** (300 mg) in anhydrous 10% HCl–MeOH (15 ml) was heated under reflux for 2 hr. After cooling, the reaction mixture was neutralized with resin IR-45, and concentrated to give a product (250 mg), which was purified by preparative TLC (benzene–acetone=3: 1) to give methyl 2,3,4-tri-O-methyl-D-apio-D-furanoside (amorphous), $[\alpha]_D^{18}$ −59.1° (*c*=0.50, $CHCl_3$), IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, and identified with the authentic sample by TLC and GLC. PMR (δ)[18)]: 3.35, 3.38, 3.44, 3.48 (3H each, all s, OC$\underline{H}_3$×4), 3.56 (1H, d, *J*=2.5 Hz, 2-$\underline{H}$), 3.97, 4.00 (2H, ABq, *J*=11 Hz, 5-$\underline{H}_2$), 4.93 (1H, d, *J*=2.5 Hz, 1-$\underline{H}$). The PMR data and $[\alpha]_D$ value are coincided with those reported by Ball, *et al.*[13)]

Mild Acid Hydrolysis of Mi-Saponin B (10) giving 12 (=9)——A solution of **10** (3.5 g) in 0.2 N HCl–MeOH (1: 1, 120 ml) was left standing at 40° for 60 hr, poured into water, evaporated under reduced pressure to remove MeOH, neutralized with resin IR-45 and extracted with *n*-BuOH. Evaporation of the *n*-BuOH extract under reduced pressure furnished a product which was purified by silica gel (300 g) column chromatography eluting with *n*-BuOH saturated with water to give **12** (=Mi-saponin A (**9**), 450 mg), mp 235—238° (decomp., colorless fine crystals from EtOAc saturated with water), $[\alpha]_D^{26}$ −29.5° (*c*=0.44, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3380 (br, OH), 1735 (–COO–).

Methylation of 12 (=9)——A solution of **12** (300 mg) in DMSO (20 ml) was treated with dimsyl carbanions (3 ml) (prepared from 2 g of NaH and 20 ml of DMSO) and the mixture was kept stirring under N_2 atmosphere for 1 hr, added with CH_3I (3 ml), kept stirring for further 3 hr in the dark, poured into ice-water, and extracted with EtOAc. The product obtained from the EtOAc extract was purified by preparative TLC (benzene–acetone=3: 1) to give **12a** (=**9a**) (110 mg, white powder). *Anal.* Calcd. for $C_{73}H_{124}O_{27}$: C, 61.13; H, 8.71. Found: C, 61.13; H, 8.95. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3530 (w, OH), 1745 (–COO–). PMR (δ)[16)]: 4.60 (1H, d, *J*=8 Hz, anomeric H of xyloside), 5.01, 5.21 (1H each, br. s, anomeric H's of rhamnosides), 5.77 (1H, d-like, $W_{h/2}$= 4 Hz, anomeric H of arabinoside), 5.26—5.38 (1H, m, 12-H).

18) A part of the signals due to 4-H_2 was observed at δ 3.50 and 3.52, but the precise chemical shifts are obscure due to the overlapping by the OCH_3 signals.

Methanolysis of 12a (=9a)——A solution of **12a** (10 mg) in anhydrous 10% HCl–MeOH (3 ml) was heated under reflux for 1 hr. After cooling, the reaction mixture was neutralized with Ag_2CO_3, filtered to remove the precipitates, and concentrated under reduced pressure to give the methylated monosaccharides which were identified by TLC and GLC with methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, methyl 2,4-di-O-methyl-xylopyranoside, methyl 2,3-di-O-methyl-rhamnopyranoside, and methyl 3,4-di-O-methyl-arabinopyranoside.

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