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Saponin and Sapogenol. XXIV.[1] The Structure of Mi-Saponin C, a Bisdesmoside of Protobassic Acid from the Seed Kernels of *Madhuca longifolia* (L.) MACBRIDE

ISAO KITAGAWA, KIYOHARU SHIRAKAWA, and MASAYUKI YOSHIKAWA

Faculty of Pharmaceutical Sciences, Osaka University[2]

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The structure of a minor saponin named Mi-saponin C (**10**), which was previously isolated together with two major saponins Mi-saponin A (**4**) and Mi-saponin B (**5**) from the seed kernels of *Madhuca longifolia* (L.) MACBRIDE (Sapotaceae), has been investigated. On the basis of chemical and physicochemical evidence, the structure of Mi-saponin C has been established as 3-O-β-D-glucopyranosyl-28-O-{[3-O-α-L-rhamnopyranosyl-4-O-β-D-glucopyranosyl]-β-D-xylopyranosyl (1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl}-protobassic acid (**10**). Mi-saponin A (**4**), B (**5**), and C (**10**) are bisdesmosides of an olean-12-ene-type sapogenol protobassic acid (**1**).

Keywords——Mi-saponin A; Mi-saponin B; Mi-saponin C; protobassic acid; bassic acid; Mi-glycoside I; *Madhuca longifolia* (L.) MACBRIDE; bisdesmoside of oleanene-type triterpenoid; crude hesperidinase

In our continuing chemical studies on saponins (oligoglycosides of triterpenoids and steroids) which originate in natural resources such as various plant materials, sea cucumber, and starfish, we isolated three saponins named Mi-saponin A, Mi-saponin B, and Mi-saponin C from the seed kernels of *Madhuca longifolia* (L.) MACBRIDE (Sapotaceae). In the initial study, we elucidated that all three saponins contain protobassic acid (**1**) and Mi-glycoside I (**2**) as the common genuine sapogenol and prosapogenol, respectively.[3] Later on, we clarified the structures of two major bisdesmosides: Mi-saponin A (**4**) and Mi-saponin B (**5**).[4] This paper deals with the chemical and physicochemical evidence which is in good accord with the structure (**10**) for the minor bisdesmoside Mi-saponin C.[5]

The infrared (IR) spectrum of Mi-saponin C (**10**) shows the strong absorption band due to hydroxyl together with that of ester carbonyl as observed in the IR spectra of Mi-saponin A (**4**) and Mi-saponin B (**5**).[4] On acid hydrolysis with aqueous 10% sulfuric acid, Mi-saponin C (**10**) furnished bassic acid (**3**), which is an artifact sapogenol secondarily formed from the genuine sapogenol protobassic acid (**1**),[3] and the carbohydrate ingredient which comprises arabinose, glucose, rhamnose, and xylose as detected by paper partition chromatography (PPC). The carbohydrate ingredients were further confirmed by methanolysis of Mi-saponin C (**10**) with 9% hydrogen chloride in dry methanol and by successive gas liquid chromatographic (GLC) analysis of the trimethylsilyl (TMS) derivatives.

On the other hand, soil bacterial hydrolysis of Mi-saponin C (**10**) afforded protobassic acid (**1**) and Mi-glycoside I (**2**) as reported previously,[3b] while alkaline treatment of Mi-saponin C (**10**) resulted in formation of Mi-glycoside I (**2**, identified as the methyl ester (**2a**)) as the sole prosapogenol. It has become clear that Mi-saponin C (**10**) is a bisdesmoside of proto-

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[1] Part XXIII: I. Kitagawa, M. Yoshikawa, and A. Kadota, *Chem. Pharm. Bull.* (Tokyo), **26**, 484 (1978).

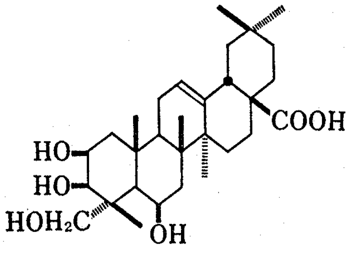
[2] Location: *133-1, Yamada-kami, Suita, Osaka 565, Japan.*

[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).

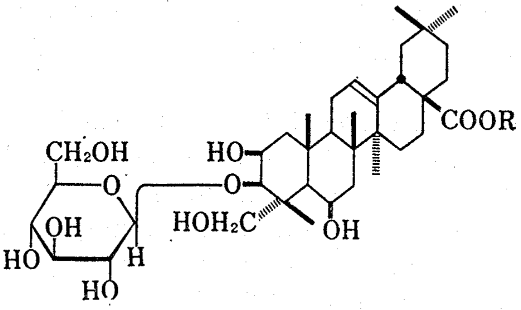
[4] I. Kitagawa, A. Inada, and I. Yosioka, *Chem. Pharm. Bull.* (Tokyo), **23**, 2268 (1975).

[5] I. Kitagawa, M. Yoshikawa, and K. Shirakawa, presented at the 97th Annual Meeting of Pharmaceutical Society of Japan, held at Tokyo, April 1977, Abstract Paper II-218.

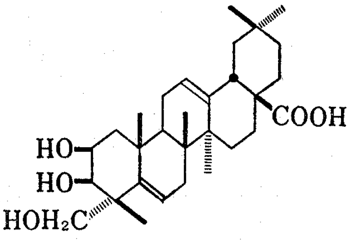
bassic acid which possesses a glucopyranosyl moiety connected at 3β-OH and an oligosaccharide moiety connected at 17β-COOH through an ester-glycoside linkage.



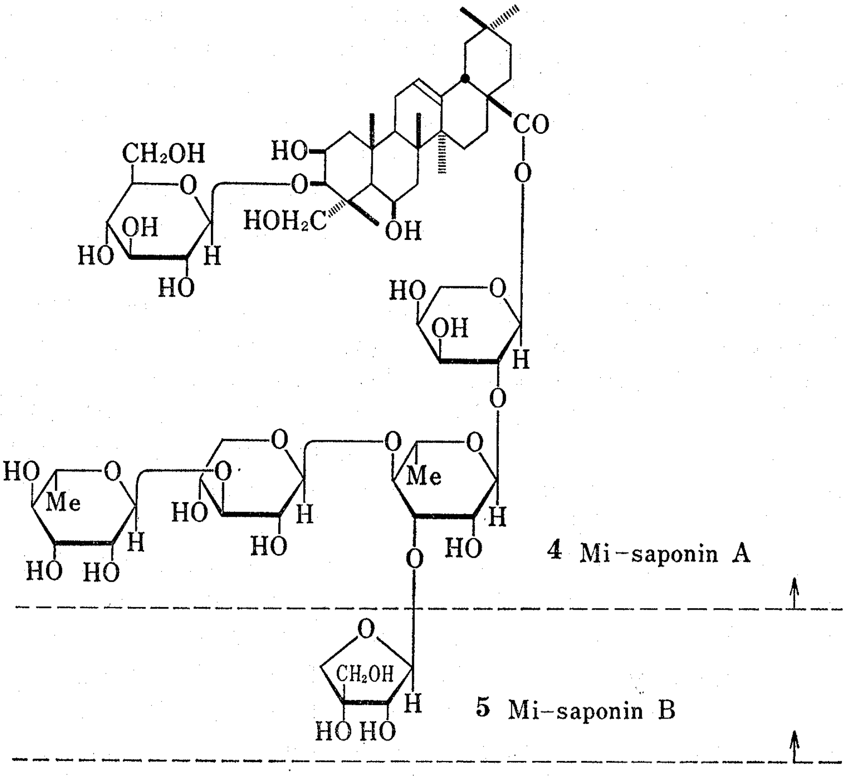
1 protobassic acid.



2 R=H (Mi-glycoside I)
2a R=Me



3 bassic acid



4 Mi-saponin A

5 Mi-saponin B

Chart 1

The enzymatic hydrolysis of Mi-saponin C (**10**) using crude hesperidinase[4] furnished protobassic acid (**1**) and four prosapogenols designated as CH-1 (**6**), CH-2 (**7**), CH-3 (**8**), and CH-4 (**9**). Among these prosapogenols, the structures of CH-1, CH-2, and CH-3 have been elucidated by respective direct comparison (mixed mp, IR, and thin-layer chromatography (TLC)) with previously clarified prosapogenols (AH-1 (**6**), AH-2 (**7**), and AH-3 (**8**)) of Mi-saponin A (**4**).[4] In order to make sure of these identifications, CH-1 (**6**) was acetylated and the resulting hexaacetate was identified with AH-1 hexaacetate (**6b**) by IR and TLC. In the case of CH-2 (**7**) and CH-3 (**8**), they were methylated with methyl iodide-dimethyl sulfoxide-sodium hydride[6] to give a deca-O-methyl derivative (**7a**) and a dodeca-O-methyl derivative (**8a**), respec-

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[6] S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

tively. Methanolysis followed by TLC and GLC analysis has shown that **7a** yielded methyl 2,3,4-tri-O-methyl-xylopyranoside, methyl 2,3-di-O-methyl-rhamnopyranoside, and methyl 3,4-di-O-methyl-arabinopyranoside while **8a** gave 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, thus the structure (**7**, **8**) of CH-2 and CH-3 being further substantiated.

The IR spectrum of fourth prosapogenol (CH-4) (**9**) exhibits the strong hydroxyl absorption band in addition to the ester carbonyl absorption band. Methanolysis of the pentadeca-O-methyl derivative (**9a**), which was prepared as for **7a** and **8a**, furnished methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, methyl 2,3-di-O-methyl-rhamnopyranoside, methyl 3,4-di-O-methyl-arabinopyranoside, and methyl 2-O-methyl-xylopyranoside (*vide infra* for the identification).

In the proton magnetic resonance (PMR) spectrum of **9a**, the signals due to six tertiary methyls (in the sapogenol portion) and fifteen methoxyls are observed in addition to four anomeric proton signals: a one-proton doublet ($J=7$ Hz) at δ 4.59 assignable to a xylopyranoside anomeric proton, one-proton each of two broad singlets ($W_{h/2}=5$ Hz and 6 Hz, respectively) at δ 4.85 and δ 5.06 which are assignable to two rhamnopyranoside anomeric protons, and a one-proton broad singlet ($W_{h/2}=4$ Hz)[7] at δ 5.60 due to the anomeric proton of ester-type arabinopyranoside linkage. However, the anomeric proton signal of the glucopyranoside moiety is not detected because of overlapping with other signals. Based on the above mentioned evidence, the structure of CH-4 (**9**) has become clear except the anomeric configuration of the glucopyranoside linkage. CH-4 (**9**) corresponds to an isomer of Mi-saponin A (**4**)[4] in regard to the location of the glucopyranoside moiety and is readily discriminated from **4** by TLC comparison.

Methylation of Mi-saponin C (**10**) as above afforded an octadeca-O-methyl derivative (**10a**). In the IR spectrum of **10a**, a weak absorption band due to 6β-OH (in the sapogenol) and an ester carbonyl absorption band are observed as in the case of **9a** and the methylated derivatives of Mi-saponin A (**4**) and Mi-saponin B (**5**).[4] In the PMR spectrum of **10a**, four anomeric proton signals of an arabinoside, two rhamnosides, and a xyloside are readily assignable, but, here again, the anomeric proton signals due to two glucopyranoside moieties are unassignable as in the case of **9a**.

On methanolysis, **10a** furnished same kinds of methylated methyl glycosides as obtained from **9a**. However, GLC analysis has shown that **10a** gave two times amount of methyl 2,3,4,6-tetra-O-methyl-glucopyranoside as compared with that from **9a**.

Lithium aluminum hydride reduction of **10a** furnished a glucoside derivative (**11**) and a methylated oligosaccharide (**12**). The structure of the glucoside (**11**) has been confirmed by identification with the corresponding glucoside which was previously obtained by similar reduction of the methylated derivatives of Mi-saponin A (**4**) and Mi-saponin B (**5**).[4]

The IR spectrum of **12** shows the hydroxyl absorption band while the PMR spectrum exhibits the signals due to two rhamnose-methyls (δ 1.14, 6H, d, $J=7$ Hz) and twelve methoxyls. The anomeric proton signal of the glucopyranoside linkage, which has not been detected in the PMR spectra of **9a** and **10a**, is clearly observed in this case at δ 4.16 as a

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7) In the PMR spectra of the methylated derivatives (**7a**, **8a**, **9a**, and **10a**), the signal due to the anomeric proton of the ester-type α-L-arabinopyranoside linkage is observed as a broad singlet ($W_{h/2}=4$—6 Hz). However, in the case of the acetate (**6b**), the anomeric proton signal is observed as a doublet ($J=5.5$ Hz).[4] These findings are well explained by assigning different conformation for the arabinopyranoside moiety in these derivatives[8]: *e.g.* $^1C_4$ conformation[9] for the methylated derivatives and $^4C_1$ conformation[9] for the acetate.

8) K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), 17, 1369 (1969).

9) Rules for conformation nomenclature for five- and six-membered rings in monosaccharides and their derivatives, *J. Chem. Soc. Chem. Commun.*, 1973, 505.

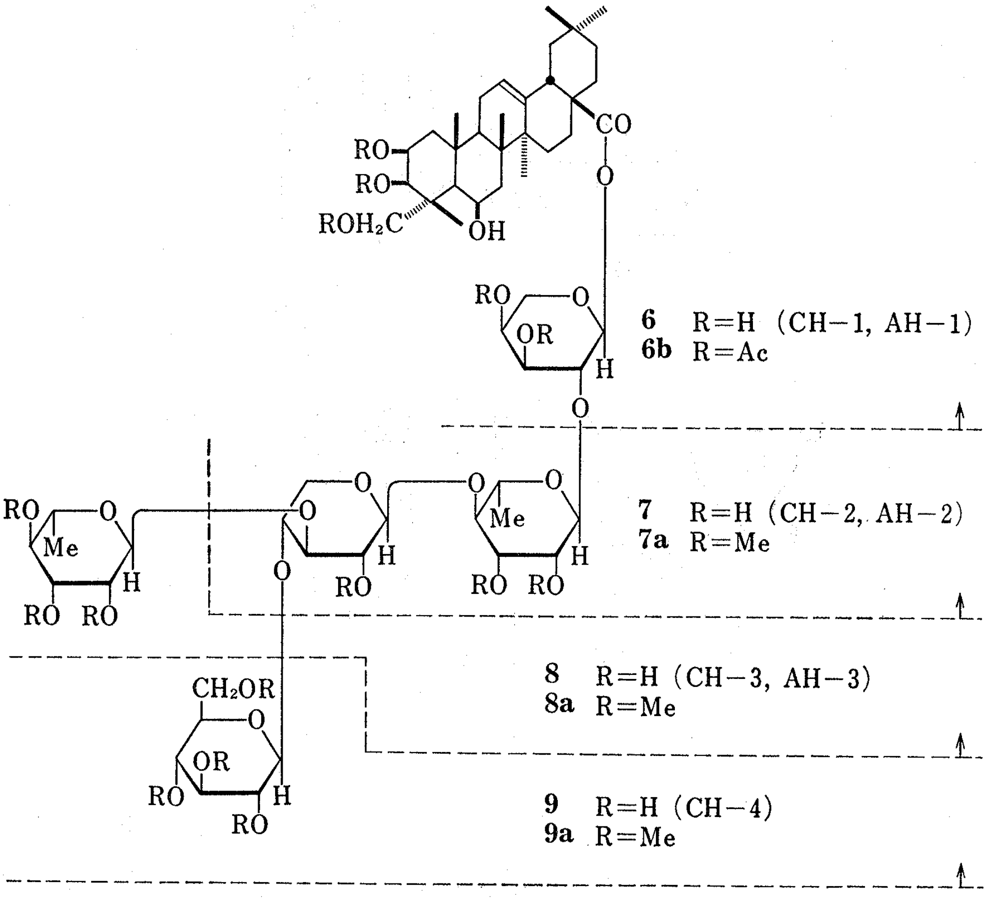

Chart 2

doublet ($J=7$ Hz) together with the signal due to the xylopyranoside anomeric proton at δ 4.60 (d, $J=8$ Hz). Consequently, the orientations of these glycoside linkages in CH-4 (**9**) and Mi-saponin C (**10**) have been assigned as β ($^4C_1$ conformation[9]). In addition, the signals due to two rhamnopyranoside anomeric protons are observed at δ 4.80 (br. s, $W_{h/2}=8$ Hz) and δ 5.06 (br. s, $W_{h/2}=10$ Hz), respectively. On methanolysis, **12** liberated methyl 2,3,4-tri-O-methyl-rhamnopyranoside, methyl 2,3,4,6-tetra-O-methyl-glucopyranoside, methyl 2,3-di-O-methyl-rhamnopyranoside, methyl 2-O-methyl-xylopyranoside (*vide infra*), and 3,4-di-O-methyl-arabitol.[4]

Based on the above described evidence, the structure of Mi-saponin C has been established as 3-O-β-D-glucopyranosyl-28-O-{[3-O-α-L-rhamnopyranosyl-4-O-β-D-glucopyranosyl]-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl}-protobassic acid (**10**).

As for methyl 2-O-methyl-α- and -β-xylopyranosides (**16**, **17**[10]) which were used for the above identification, the authentic samples were unavailable. Therefore, isolation of both xylopyranosides from the methanolysis mixture of **10a** was first attempted to clarify the structures. Although methyl 2-O-methyl-α-D-xylopyranoside (**16**) was obtained, isolation of the β-anomer (**17**)[10] was without success. **16**, thus isolated, analyses for $C_7H_{14}O_5$ by high resolution mass spectrometry. The IR spectrum of **16** shows the strong hydroxyl absorption band while the PMR spectrum shows the signals due to two methoxyls, 2-H (δ 3.10, d.d, $J=3$ and 10 Hz), and the anomeric proton (δ 4.76, d, $J=3$ Hz). The assignment of latter two signals has been confirmed by the decoupling experiments in hexadeutero(d_6)-acetone (Table I).

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[10] G.J. Robertson and T.H. Speedie, *J. Chem. Soc.*, **1934**, 824.

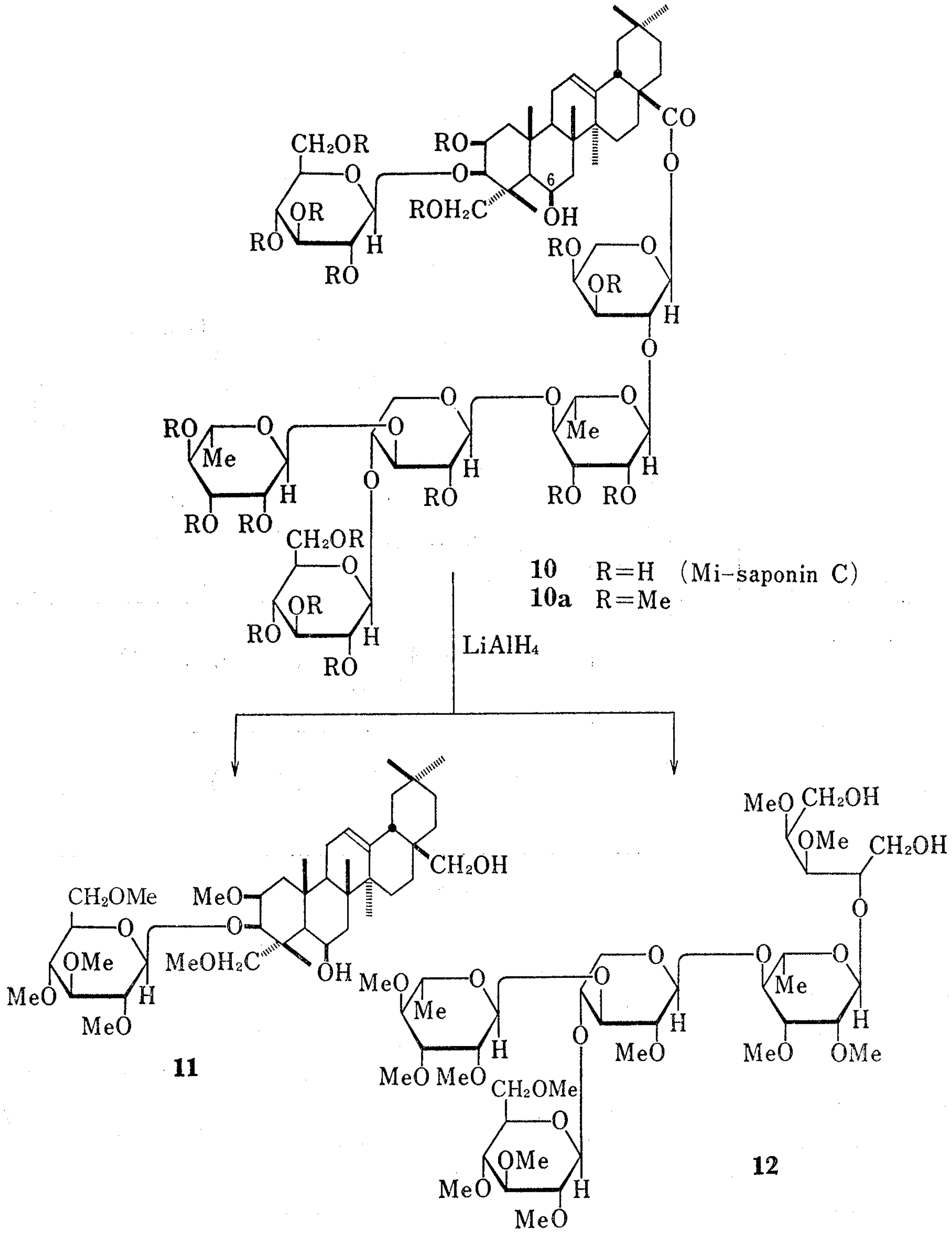

10 R=H (Mi-saponin C)
10a R=Me

Chart 3

Table I. Spin-decoupling Experiments of **16** (in d_6-acetone)[a]

Decoupled proton (δ)	Irradiated at δ	
	4.76	3.03
1-H (4.76, d, $J=3$ Hz)	—	singlet
2-H (3.03, d.d, $J=3$ and 10 Hz)	doublet ($J=10$ Hz)	—

[a] Abbreviations for Table I and II: d=doublet, d.d=doublet of doublet, d.d.d=doublet of doublet of doublet, m=multiplet.

Acetylation of **16** gave **16b**, $C_{11}H_{18}O_7$ (high mass), which lacks the hydroxyl absorption band in its IR spectrum. In the PMR spectrum of **16b** (hexadeutero(d_6)-benzene), together with the signals due to two acetoxyls and two methoxyls, a two-proton multiplet (δ 3.56) due

Table II. Spin-decoupling Experiments of **16b** (in d_6-benzene)

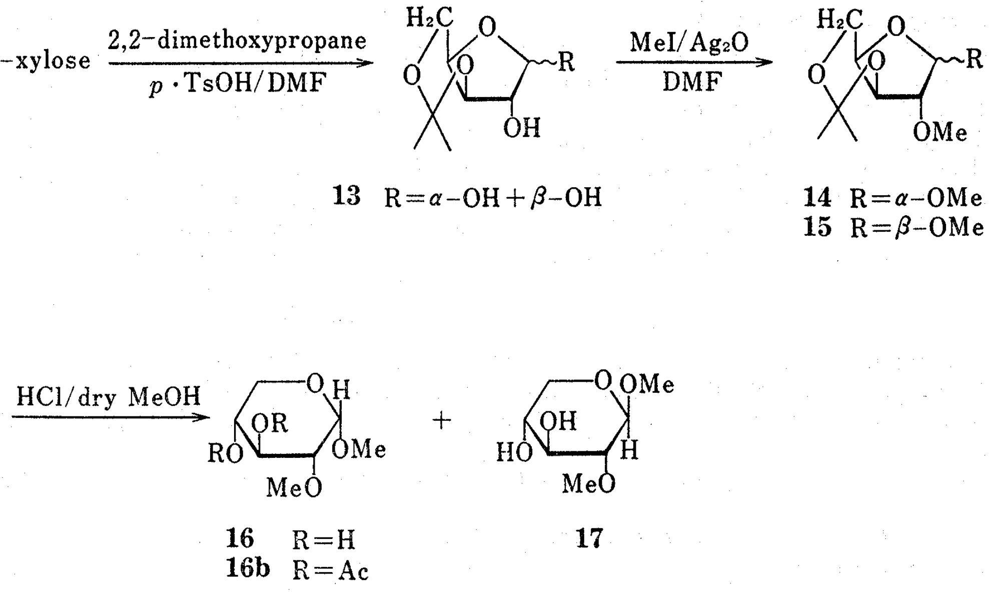
Decoupled proton (δ)	Irradiated at δ				
	4.66	3.20	5.58	5.02	3.56
1-H (4.66, d, $J=4$ Hz)	—	singlet			
2-H (3.20)[a]	deformed[b]	—	deformed[b]		
3-H (5.58, d.d, $J=10$ and 10 Hz)		doublet ($J=10$ Hz)	—	doublet-like[c]	
4-H (5.02, d.d.d, $J=6$, 9, and 10 Hz)			deformed[c]	—	doublet ($J=10$ Hz)
5-H_2 (3.56, m)				AB quartet ($J=10$ Hz)	—

a) The chemical shift (δ) of 2-H has been given on the basis of the decoupling experiments, since the signal of 2-H is partly overlapped by the signals of methoxyls.
b) The precise signal pattern is obscure due to partial overlapping with the signals of methoxyls.
c) Since the chemical shifts of 3-H and 4-H are close, the decoupling experiments were unsatisfactory.

to 5-H_2 and the anomeric proton signal (δ 4.66, d, $J=4$ Hz) are observed. In addition, the signals due to 4-H (δ 5.02, d.d.d, $J=6$, 9, and 10 Hz) and 3-H (δ 5.58, d.d, $J=10$ and 10 Hz), which have not been assigned in the PMR spectrum of **16**, are clearly observed and the assignments have been confirmed by the decoupling experiments (Table II).

On the basis of the above evidence, the structure of methyl 2-O-methyl-α-D-xylopyranoside (**16**) has been substantiated. However, since the anomeric counterpart (β-anomer, **17**)[10] has not been obtained in a pure form as mentioned above, in order to further confirm the structures, **16** and **17** have been synthesized through the following procedure.

Treatment of D-xylose with 2,2-dimethoxypropane and *p*-toluenesulfonic acid (*p*·TsOH) in dimethylformamide (DMF) furnished 3,5-O-isopropylidene-D-xylofuranose (**13**).[11] Subsequent methylation of **13** with methyl iodide and silver oxide[12] gave two products: **14** (α-anomer) and **15** (β-anomer). **14**, $C_{10}H_{18}O_5$ (high mass), lacks free hydroxyl as shown by its IR spectrum. The PMR spectrum of **14** shows the signals due to isopropylidene methyls, two methoxyls, and the anomeric proton (δ 5.08, d, $J=4$ Hz), thus the structure (**14**) being supported.



13 R=α-OH+β-OH

14 R=α-OMe
15 R=β-OMe

16 R=H
16b R=Ac

17

Chart 4

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11) M. Kiso and A. Hasegawa, *Carbohyd. Res.*, **52**, 95 (1976).
12) R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).

15, possesses the same molecular composition as **14**. The IR and mass spectra of **15** are quite resembled those of **14**. The PMR spectrum of **15** shows the signals due to isopropylidene methyls, two methoxyls, and the anomeric proton (δ 4.89, s), thus the structure (**15**) having a β-anomeric linkage being clarified.

Finally, methanolysis of **14** and **15** furnished respectively both of **16** and **17**, which have been identified with methyl 2-O-methyl-xylopyranoside (a mixture of α- and β-anomers) obtained above by methanolysis of **9a**, **10a**, and **12**.

Mi-saponin A (**4**),[4] Mi-saponin B (**5**),[4] and Mi-saponin C (**10**) are bisdesmosides of an olean-12-ene-type sapogenol protobassic acid (**1**). They contain a D-glucopyranosyl moiety connected at 3β-OH and an oligosaccharide moiety bound at 17β-COOH of the sapogenol, in a similar fashion as seen in the structures of saponins isolated from *Polygala senega* L. var. *latifolia* TORRY et GRAY (Polygalaceae, root)[13] and *Platycodon grandiflorum* A. DC (Campanulaceae, root).[14]

Recently, Yamahara, *et al.*[15] examined some biological activities of Mi-saponin which is the parent saponin mixture of Mi-saponin A (**4**), B (**5**), and C (**10**). They elucidated that Mi-saponin shows similar antiulcerogenic and anti-inflammatory activities as a saponin mixture isolated from Japanese horse chestnuts (the seeds of *Aesculus turbinata* BLUME, Hippocastanaceae),[16] which has been presumed to comprise the similar saponin ingredients as aescin.[17] They also demonstrated that the potency of anti-inflammatory activity of Mi-saponin is about one-fifth that of phenylbutazone, and hemolytic effect of Mi-saponin is much less than that of the saponin mixture of *A. turbinata*. Further investigation on some other biological activities of Mi-saponin is currently in progress by the same group.

Experimental[18]

Isolation of Mi-Saponin C (10)——A total saponin mixture (80 g)[3b,4] was dissolved in a small amount of MeOH, mixed well with silica gel (100 g), and dried under reduced pressure. The mixture thus obtained was put on a column of silica gel (1.6 kg) and the column was eluted with *n*-BuOH saturated with water to give Mi-saponin A (**4**, 26.1 g), Mi-saponin B (**5**, 25.1 g), and Mi-saponin C (**10**, 5.4 g) successively. The mixtures of **4** and **5** (15.0 g) and **5** and **10** (2.1 g) were also obtained. Repeated crystallization from MeOH furnished Mi-saponin C (**10**) as colorless fine crystals (reported as white powder in our previous paper[3b,4]). Mi-saponin C (**10**), mp 254—256°, $[\alpha]_D^{19.5}$ $-37.2°$ (c=0.95, MeOH). *Anal.* Calcd. for $C_{64}H_{104}O_{32}\cdot 3H_2O$: C, 53.40; H, 7.70. Found: C, 53.47; H, 7.57. IR ν_{max}^{KBr} cm^{-1}: 3355 (br, OH), 1730 (–COO–).

Acid Hydrolysis of Mi-Saponin C (10)——A solution of **10** (50 mg) in MeOH (5 ml) was treated with aq. 10% H_2SO_4 (5 ml) and the total solution was heated under reflux for 8 hr. After removing MeOH under reduced pressure, the reaction mixture was diluted with water and extracted with AcOEt. The AcOEt layer was washed repeatedly with water, dried over $MgSO_4$, and evaporated under reduced pressure. Purification of the residue by preparative TLC (developing with $CHCl_3$–MeOH=4: 1) and by crystallization from $CHCl_3$–MeOH furnished colorless fine crystals (6 mg) which were identified with bassic acid (3) by IR (KBr) and TLC ($CHCl_3$–MeOH=4: 1; $CHCl_3$–MeOH–H_2O=10: 3: 1 (lower layer)). The aqueous layer was neutralized with Amberlite IR-45 (OH^- form), concentrated under reduced pressure, and subjected to PPC (iso-PrOH–*n*-BuOH–H_2O=7: 1: 2). Arabinose (*Rf*=0.44), rhamnose (0.59), xylose (0.49), and glucose (0.39) were identified.

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13) Y. Tsukitani and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), 21, 1564 (1973).

14) A. Tada, Y. Kaneiwa, J. Shoji, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), 23, 2965 (1975).

15) J. Yamahara, Y. Shintani, T. Konoshima, T. Sawada, and H. Fujimura, *Yakugaku Zasshi*, **95**, 1179 (1975).

16) I. Yosioka, A. Matsuda, K. Imai, T. Nishimura, and I. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1200 (1971).

17) G. Wulff and R. Tschesche, *Tetrahedron*, **25**, 415 (1969).

18) The instruments used in the experimental section and the experimental conditions for chromatography were same as in our previous paper[1] unless specified otherwise. The optical rotations were measured with JASCO DIP-181 Digital Polarimeter (l=1 dm). 9% HCl–dry MeOH used for methanolysis was prepared by adding AcCl (3 ml) dropwise into dry MeOH (20 ml). For TLC identification of **6** (CH-1, AH-1), **6b**, **7** (CH-2, AH-2), **8** (CH-3, AH-3), and methylated methyl glycosides, Pre-Coated TLC Plates (Silica Gel 60 F-254, 0.25 mm, Merck) were used.

Methanolysis followed by Trimethylsilylation of Mi-Saponin C (10)——A solution of **10** (5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 hr. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and filtered to remove the precipitate. The filtrate was evaporated to dryness under reduced pressure and the residue was dissolved in pyridine (0.1 ml) and treated with N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA, 0.2 ml). The mixture of TMS derivatives thus obtained was subjected to GLC analysis. The authentic TMS derivatives of L-arabinose, D-glucose, L-rhamnose, and D-xylose were prepared in the similar manner. GLC: 2% silicone SE-52 on chromosorb WAWDMCS (80—100 mesh), 2 m × 3 mm, column temp. 140°, carrier gas N_2, flow rate 40 ml/min. The following carbohydrates were identified (t_R): L-rhamnose (2′20″, 3′20″ (major)), L-arabinose (3′00″), D-xylose (4′35″ (major), 4′50″), and D-glucose (12′30″ (major), 13′35″).

Alkaline Treatment of Mi-Saponin C (10)——A solution of **10** (100 mg) in EtOH (20 ml) was treated with aq. 20% KOH (20 ml) and heated under reflux for 3 hr. The reaction mixture was diluted with water while removing EtOH under reduced pressure. The mixture was then neutralized with aq. 20% H_2SO_4 and extracted with *n*-BuOH. The *n*-BuOH layer was evaporated under reduced pressure. The residue thus obtained was dissolved in a small amount of MeOH, methylated with ethereal diazomethane, and left standing for 12 hr. Evaporation of the solvent followed by preparative TLC purification ($CHCl_3$–MeOH=2: 1) and crystallization from MeOH gave colorless needles (21 mg), mp 293—295°. The product was identified with Mi-glycoside I methyl ester (**2a**)[3] by IR (KBr), mixed mp, and TLC ($CHCl_3$–MeOH=2: 1; $CHCl_3$–MeOH–H_2O=7: 3: 1 (lower layer)).

Enzymatic Hydrolysis of Mi-Saponin C (10) with Crude Hesperidinase——A solution of **10** (1.24 g) in the Na_2HPO_4–citric acid buffer solution (pH 4.5, 160 ml) was treated with crude hesperidinase (Tanabe Pharm. Co., Lot No. N-30, 160 mg) and the total mixture was kept stirring at 31—33° for 9 days. The reaction mixture was extracted with *n*-BuOH and the *n*-BuOH extract was washed with water and evaporated to dryness under reduced pressure to give a product (600 mg). The product was then dissolved in a small amount of MeOH, mixed well with silica gel (5 g), dried, and put on a column of silica gel (90 g). Elution of the column with $CHCl_3$–MeOH–H_2O (7: 3: 1, lower layer) furnished **1** (16 mg), CH-1 (**6**, 9 mg), CH-2 (**7**, 83 mg), CH-3 (**8**, 32 mg), CH-4 (**9**, 132 mg), a mixture of **9** and **10** (135 mg), and **10** (85 mg, recovered). Crystallization from MeOH gave a pure sample of **1**, mp 310—312°, which was identified with the authentic sample[3] by IR (KBr), mixed mp, and TLC ($CHCl_3$–MeOH=4: 1). CH-1 (**6**), mp 195—198° (cryst. from AcOEt saturated with water) was identified with AH-1[3] by IR (KBr), mixed mp, and TLC ($CHCl_3$–MeOH–H_2O=10: 3: 1, lower layer, developing twice). CH-2 (**7**), mp 230—235° (cryst. from $CHCl_3$–MeOH), was identified with AH-2[3] by IR (KBr), mixed mp, and TLC ($CHCl_3$–MeOH–H_2O=7: 3: 1, lower layer, developing twice). CH-3 (**8**), mp 236—238° (cryst. from AcOEt saturated with water), was identified with AH-3[3] by IR (KBr), mixed mp, and TLC ($CHCl_3$–MeOH–H_2O=65: 35: 10, lower layer; *n*-BuOH–AcOH–H_2O=4: 1: 5, upper layer, developing twice for each solvent system). CH-4 (**9**), colorless fine crystals from MeOH, mp 223—225°, $[\alpha]_D^{27}$ −32.0° (c=0.23, MeOH). *Anal.* Calcd. for $C_{58}H_{94}O_{27} \cdot 3H_2O$: C, 54.48; H, 7.73. Found: C, 54.53; H, 7.89. IR ν_{max}^{KBr} cm^{-1}: 3420 (br, OH), 1740 (–COO–).

Acetylation of CH-1 (6) giving 6b——A solution of **6** (10 mg) in pyridine (2 ml) was treated with Ac_2O (1 ml) and the total mixture was left standing at 30° for 24 hr. The reaction mixture was poured into ice-water and the precipitate was collected by filtration to give **6b** (8 mg) as white powder. **6b** was identified with the authentic sample[3] by IR (CCl_4) and TLC ($CHCl_3$–MeOH=50: 1; benzene–acetone=2: 1; benzene–MeOH=10: 1).

Methylation followed by Methanolysis of CH-2 (7)——To a solution of **7** (50 mg) in dimethyl sulfoxide (DMSO) (4 ml) was added dimsyl carbanion (4 ml)[19] and the total solution was kept stirring under N_2 atmosphere at room temperature for 1 hr. The mixture was treated with CH_3I (4 ml) and kept stirring similarly in the dark for further 3 hr. The reaction mixture was then poured into ice-water and extracted with AcOEt. The AcOEt extract was washed with aq. $Na_2S_2O_3$ and water successively, and dried over $MgSO_4$. Evaporation of the solvent under reduced pressure gave a syrupy product which was purified by preparative TLC (benzene–MeOH=10: 1) to give **7a** (35 mg). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500 (w, OH), 1745 (br, –COO–), 1100 (C–O–C). PMR (90 MHz, CCl_4, δ): 0.91, 0.96, 1.01, 1.09, 1.16, 1.22 (3H each, all s, *tert.* $CH_3 \times 6$), 3.26, 3.28, 3.31, 3.33 (3H each, all s), 3.41, 3.45 (6H each, both s), 3.51, 3.55 (3H each, both s) ($OCH_3 \times 10$), 4.51 (1H, d, J=6 Hz, anomeric H of xyloside), 4.90 (1H, br.s, $W_{h/2}$=5 Hz, anomeric H of rhamnoside), 5.32 (1H, br.s, $W_{h/2}$=8 Hz, 12-H), 5.61 (1H, br.s, $W_{h/2}$=4 Hz, anomeric H of arabinoside). A solution of **7a** (5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 hr. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and filtered to remove the precipitate. The filtrate was concentrated under reduced pressure and subjected to TLC and GLC analysis. Methyl 2,3,4-tri-O-methyl-xylopyranoside (I), methyl 2,3-di-O-methyl-rhamnopyranoside (II), and methyl 3,4-di-O-methyl-arabinopyranoside (III) were identified. TLC (benzene–MeOH=4: 1) (*Rf*): I (0.66), II (0.49), III (0.37, 0.34). GLC [15% ethyleneglycol succinate polyester on Uniport B (80—100 mesh), 2 m × 3 mm, column temp. 160°, carrier gas N_2, flow rate 30 ml/min] (t_R): I (3′03″, 3′51″ (major)), II (10′00″), III (17′30″).

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[19] I. Kitagawa, M. Yoshikawa, K.S. Im, and Y. Ikenishi, *Chem. Pharm. Bull.* (Tokyo), **25**, 657 (1977).

Methylation followed by Methanolysis of CH-3 (8)——A solution of **8** (25 mg) in DMSO (3 ml) was treated with dimsyl carbanion (2 ml) and CH_3I (1 ml) and the reaction mixture was worked up as for CH-2 (**7**). Purification of the product by preparative TLC (benzene–MeOH=10:1, developing twice) furnished **8a** (17 mg). IR $\nu_{\max}^{\mathrm{CCl_4}}$ $\mathrm{cm^{-1}}$: 3500 (w, OH), 1745 (br, –COO–), 1100 (C–O–C). PMR (CCl_4, δ): 0.93, 0.96, 1.02, 1.10, 1.20, 1.25 (3H each, all s, *tert.* $CH_3 \times 6$), 3.28, 3.31, 3.33, 3.36 (3H each, all s), 3.41, 3.44, 3.49 (6H each, all s), 3.51, 3.53 (3H each, both s) ($OCH_3 \times 12$), 4.54 (1H, d, $J=7$ Hz, anomeric H of xyloside), 4.85 (1H, br.s, $W_{h/2}=6$ Hz), 5.04 (1H, br.s, $W_{h/2}=6$ Hz) (anomeric H of rhamnoside × 2), 5.31 (1H, br.s, $W_{h/2}=8$ Hz, 12-H), 5.58 (1H, br.s, $W_{h/2}=6$ Hz, anomeric H of arabinoside). A solution of **8a** (5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 hr and worked up as for **7a**. TLC and GLC analysis of the product identified methyl 2,3,4-tri-O-methyl-rhamnopyranoside (IV), methyl 2,3-di-O-methyl-rhamnopyranoside (II), methyl 2,4-di-O-methyl-xylopyranoside (V), and methyl 3,4-di-O-methyl-arabinopyranoside (III). TLC (benzene–acetone=5:2) (*Rf*): IV (0.58), II (0.34), V (0.28), III (0.22). GLC (as for **7a**) (t_R): IV (3′00″), II (10′00″), V (11′15″, 15′35″ (major)), III (17′30″).

Methylation of CH-4 (9) giving 9a——A solution of **9** (62 mg) in DMSO (4 ml) was treated with dimsyl carbanion (4 ml) and CH_3I (4 ml) and the reaction mixture was worked up as for CH-2 (**7**). Preparative TLC purification (benzene–MeOH=10:1, developing twice) of the product gave **9a** (46 mg) which was crystallized from MeOH to furnish colorless fine crystals, mp 124—126°, $[\alpha]_D^{27}$ −35.5° (c=0.49, $CHCl_3$). *Anal.* Calcd. for $C_{73}H_{124}O_{27}$: C, 61.15; H, 8.72. Found: C, 60.94; H, 8.64. IR $\nu_{\max}^{\mathrm{CCl_4}}$ $\mathrm{cm^{-1}}$: 3500 (w, OH), 1748 (br, –COO–), 1100 (C–O–C). PMR (CCl_4, δ): 0.91, 0.95, 1.00, 1.09, 1.15, 1.23 (3H each, all s, *tert.* $CH_3 \times 6$), 3.25, 3.30, 3.33 (3H each, all s), 3.38 (9H, s), 3.40 (6H, s), 3.44 (9H, s), 3.50, 3.52 (6H each, both s) ($OCH_3 \times 15$), 4.59 (1H, d, $J=7$ Hz, anomeric H of xyloside), 4.85 (1H, br.s, $W_{h/2}=5$ Hz), 5.06 (1H, br.s, $W_{h/2}=6$ Hz) (anomeric H of rhamnoside × 2), 5.31 (1H, br.s, $W_{h/2}=8$ Hz, 12-H), 5.60 (1H, br.s, $W_{h/2}=4$ Hz, anomeric H of arabinoside).

Methanolysis of 9a——A solution of **9a** (5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 hr and worked up as for **7a**. TLC and GLC analysis of the product identified methyl 2,3,4-tri-O-methyl-rhamnopyranoside (IV), methyl 2,3,4,6-tetra-O-methyl-glucopyranoside (VI), methyl 2,3-di-O-methyl-rhamnopyranoside (II), methyl 3,4-di-O-methyl-arabinopyranoside (III), and methyl 2-O-methyl-xylopyranoside (**16**, **17**). TLC (benzene–MeOH=4:1) (*Rf*): IV (0.65), VI (0.60), II (0.49), III (0.37, 0.34), **17** (0.32), **16** (0.30). GLC [(i) as for **7a**] (t_R): IV (3′00″), VI (6′23″, 9′07″ (major)), II (10′00″), III (17′30″); GLC [(ii) 15% ethyleneglycol succinate polyester on Uniport B (80—100 mesh), 1 m × 3 mm, column temp. 185°, carrier gas N_2, flow rate 40 ml/min] (t_R): **17** (5′00″), **16** (7′05″).

Methylation of Mi-Saponin C (10) giving 10a——A solution of **10** (500 mg) in DMSO (30 ml) was treated with dimsyl carbanion (8 ml) and CH_3I (5 ml) and the reaction mixture was worked up as for **7**. Purification of the product by preparative TLC (benzene–MeOH=4:1, developing twice) gave **10a** (144 mg, white powder), $[\alpha]_D^{27}$ −43.9° (c=0.12, $CHCl_3$). *Anal.* Calcd. for $C_{82}H_{140}O_{32}$: C, 60.12; H, 8.62. Found: C, 59.69; H, 8.85. IR $\nu_{\max}^{\mathrm{CCl_4}}$ $\mathrm{cm^{-1}}$: 3620 (w, OH), 1745 (br, –COO–), 1100 (C–O–C). PMR ($CDCl_3$, δ): 0.91, 0.96, 1.01, 1.09, 1.17, 1.25 (3H each, all s, *tert.* $CH_3 \times 6$), 3.29, 3.35, 3.37, 3.40. 3.43 (3H each, all s), 3.46 (6H, s), 3.50 (9H, s), 3.55, 3.57, 3.59, 3.62 (6H each, all s) ($OCH_3 \times 18$), 4.64 (1H, d, $J=7$ Hz, anomeric H of xyloside), 5.00 (1H, br.s, $W_{h/2}=6$ Hz), 5.25 (1H, br.s, $W_{h/2}=5$ Hz) (anomeric H of rhamnoside × 2), 5.37 (1H, br.s, $W_{h/2}=7$ Hz, 12-H), 5.79 (1H, br.s, $W_{h/2}=5$ Hz, anomeric H of arabinoside).

Methanolysis of 10a——A solution of **10a** (5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 2 hr. After cooling, working up of the reaction mixture as for **7a** gave a product, which was subjected to TLC and GLC analysis. Methyl 2,3,4-tri-O-methyl-rhamnopyranoside (IV), methyl 2,3,4,6-tetra-O-methyl-glucopyranoside (VI), methyl 2,3-di-O-methyl-rhamnopyranoside (II), methyl 3,4-di-O-methyl-arabinopyranoside (III), and methyl 2-O-methyl-xylopyranoside (**16**, **17**) were identified. TLC [(i) benzene–acetone=5:2] (*Rf*): IV (0.58), VI (0.50, 0.45), II (0.34), III (0.22), **17** (0.20), **16** (0.13); [(ii) $CHCl_3$–MeOH=10:1] (*Rf*): II (0.69), III (0.63), **17** (0.60), **16** (0.55). GLC [(i) 15% ethyleneglycol succinate polyester on Uniport B (80—100 mesh), 2 m × 3 mm, column temp. 180°, carrier gas N_2, flow rate 50 ml/min] (t_R): IV (5′12″ (major), 6′39″), VI (9′05″, 11′53″ (major)), II (13′31″), III (20′15″). GLC [(ii) as for **9a** (ii)] (t_R): **17** (5′00″), **16** (7′05″).

$LiAlH_4$ Reduction of 10a giving 11 and 12——To a solution of **10a** (80 mg) in tetrahydrofuran (5 ml) was added a suspension of $LiAlH_4$ (50 mg) in tetrahydrofuran (5 ml) and the total mixture was heated under reflux for 1 hr. After cooling, the reaction mixture was treated with aqueous ether, made weakly acidic with aq. 20% H_2SO_4, and extracted with AcOEt. The AcOEt extract was washed with water, dried over $MgSO_4$, and evaporated under reduced pressure. The residue thus obtained was purified by preparative TLC (benzene–MeOH=4:1) to furnish **11** (32 mg) and **12** (27 mg). **11** was identified with the authentic sample[4] by IR (CCl_4), TLC (benzene–MeOH=5:1; $CHCl_3$–MeOH=30:1; benzene–acetone=1:1), and PMR ($CDCl_3$). **12**, colorless oil, $[\alpha]_D^{27}$ −62.8° (c=0.57, $CHCl_3$). *Anal.* Calcd. for $C_{40}H_{74}O_{22}$: C, 52.97; H, 8.22. Found: C, 53.26; H, 8.33. IR $\nu_{\max}^{\mathrm{CCl_4}}$ $\mathrm{cm^{-1}}$: 3470 (OH), 1100 (C–O–C). PMR (CCl_4, δ): 1.14 (6H, d, $J=7$ Hz, rhamnose $CH_3 \times 2$), 3.35, 3.39 (3H each, both s), 3.41, 3.43, 3.45, 3.47 (6H each, all s), 3.55, 3.57 (3H each, both s) ($OCH_3 \times 12$), 4.16 (1H, d, $J=7$ Hz, anomeric H of glucoside), 4.60 (1H, d, $J=8$ Hz, anomeric H of xyloside), 4.80 (1H, br.s, $W_{h/2}=8$ Hz), 5.06 (1H, br.s, $W_{h/2}=10$ Hz) (anomeric H of rhamnoside × 2).

Methanolysis of 12——A solution of **12** (5 mg) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 hr. Working up of the reaction mixture as for **7a** gave a product which was subjected to TLC and GLC analysis. Methyl 2,3,4-tri-O-methyl-rhamnopyranoside (IV), methyl 2,3,4,6-tetra-O-methyl-glucopyranoside (VI), methyl 2,3-di-O-methyl-rhamnopyranoside (II), and methyl 2-O-methyl-xylopyranoside (**16, 17**) were identified by TLC and GLC. 3,4-Di-O-methyl-arabitol (VII)[4] was identified by TLC. TLC [(i) benzene–acetone=1: 1] (*Rf*): IV (0.80), VI (0.70), II (0.65), **17** (0.40), **16** (0.30), VII (0.10); [(ii) benzene–MeOH=4: 1] (*Rf*): IV (0.65), VI (0.60), II (0.49), **17** (0.32), **16** (0.30), VII (0.05); [(iii) benzene–acetone=1: 3] (*Rf*): VII (0.22); [(iv) $CHCl_3$–MeOH=5: 1] (*Rf*): VII (0.37), GLC [(i) 15% ethyleneglycol succinate polyester on Uniport B (80—100 mesh), 2 m×3 mm, column temp. 150°, carrier gas N_2, flow rate 50 ml/min] (t_R): IV (10′05″ (major), 15′07″), VI (21′13″, 30′35″ (major)), II (34′20″); GLC [(ii) as for **9a** (ii)] (t_R): **17** (5′00″), **16** (7′05″).

Isolation of Methyl 2-O-Methyl-α-D-xylopyranoside (16)——A solution of **10** (1.2 g) in DMSO (50 ml) was treated with dimsyl carbanion (20 ml) and CH_3I (15 ml) and the reaction mixture was worked up as for **7**. The oily product (1.3 g) was dried *in vacuo* and dissolved in 9% HCl–dry MeOH (20 ml). After heating under reflux for 2 hr, the reaction mixture was worked up as for **7a** to furnish an oily product (1.2 g). The product was then chromatographed on a silica gel (100 g) column developing with benzene–MeOH (10: 1). After evaporation of the solvent, a fraction (300 mg) containing **16** was partitioned into AcOEt–H_2O (1: 1) mixture. The aqueous layer was concentrated under reduced pressure and the residue was purified by preparative TLC (benzene–acetone=1: 1) to give **16** (80 mg), colorless oil, $[\alpha]_D^{22}$ +109.4° (c=1.4, MeOH). High resolution mass spectrum: Calcd. for $C_7H_{14}O_5$ (M^+): 178.084; Found: 178.083. IR ν_{max}^{film} cm^{-1}: 3400 (br, OH), 1050 (br, C–O–C). PMR (d_6-acetone, δ): 3.03 (1H, d.d, J=3 and 10 Hz, 2-H), 3.34, 3.40 (3H each, both s, $OCH_3\times 2$), 4.25 (2H, br.s, exchangeable with D_2O, $OH\times 2$), 4.76 (1H, d, J=3 Hz, 1-H). MS *m/e* (%): 178 (M^+, <1), 147 (12), 129 (14), 88 (53), 87 (100).

Acetylation of 16 giving 16b——A solution of **16** (80 mg) in pyridine (1 ml) was treated with Ac_2O (1 ml) and the total solution was left standing at 33—35° for 12 hr. The reaction mixture was poured into ice-water and extracted with ether. The ether extract was washed with water, dried over $MgSO_4$, and evaporated to dryness to give **16b** (70 mg), colorless oil, $[\alpha]_D^{16}$ +125.03° (c=7.0, MeOH). High resolution mass spectrum: Calcd. for $C_{11}H_{18}O_7$ (M^+): 262.105; Found: 262.105. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1760, 1242 (OAc), 1055 (C–O–C). PMR (d_6-benzene, δ): 1.76, 1.84 (3H each, both s, $OCOCH_3\times 2$), 3.12, 3.14 (3H each, both s, $OCH_3\times 2$), 3.20 (1H, 2-H, *cf.* Table II), 3.56 (2H, m, 5-H_2), 4.66 (1H, d, J=4 Hz, 1-H), 5.02 (1H, d.d.d, J=6, 9, and 10 Hz, 4-H), 5.58 (1H, d.d, J=10 and 10 Hz, 3-H); (d_6-acetone, δ): 1.96, 1.99 (3H each, both s, $OCOCH_3\times 2$), 3.38, 3.39 (3H each, both s, $OCH_3\times 2$), 4.90 (2H, m, 1-H, 4-H), 5.25 (1H, d.d, J=10 and 10 Hz, 3-H); ($CDCl_3$, δ): 2.01, 2.06 (3H each, both s, $OCOCH_3\times 2$), 3.41, 3.43 (3H each, both s, $OCH_3\times 2$), 4.84 (2H, m, 1-H, 4-H), 5.35 (1H, d.d, J=10 and 10 Hz, 3-H). MS *m/e* (%): 262 (M^+, <1), 231 (2), 142 (28), 87 (46), 43 (100).

3,5-O-Isopropylidene-D-xylofuranose (13)——To a suspension of D-xylose (2.0 g) in DMF (25 ml) was added *p*·TsOH·H_2O (30 mg) and 2,2-dimethoxypropane (5 ml). The total mixture was kept stirring at 40—45° for 3 hr. The resulting clear solution was neutralized with Amberlite IRA-400 (OH^- form) and filtered. The filtrate was concentrated below 60° under reduced pressure and the residue was chromatographed on a silica gel (200 g) column developing with benzene–MeOH (10: 1) to give **13** (270 mg), colorless oil. IR ν_{max}^{film} cm^{-1}: 3400 (OH), 845 (lit.[11]: IR ν_{max}^{film} cm^{-1}: 3370, 840). PMR ($CDCl_3$, δ): 1.38, 1.40, 1.43, 1.45 (totally 6H, all s, isopropylidene methyl×2), 3.80—4.40 (totally 5H, m), 5.22 (s), 5.62 (d, J=3 Hz) (totally 1H, anomeric H). [lit.[11]: PMR (60 MHz, $CDCl_3$, δ): 1.45 (isopropylidene methyls), 3.80—4.40 (ring protons), 5.20 (s), 5.67 (d, J=3.8 Hz) (β and α proton of C-1)].

Methylation of 13 giving 14 and 15——To a solution of **13** (250 mg) in DMF (2 ml) was added CH_3I (5 ml) and Ag_2O (800 mg) and the total mixture was kept stirring at 40° for 12 hr in the dark. The reaction mixture was filtered to remove the inorganic precipitate and the filtrate was diluted with AcOEt and washed repeatedly with water. The organic layer was dried over $MgSO_4$, and evaporated under reduced pressure. The product was then purified by column chromatography (silica gel 20 g) developing with benzene–acetone (10: 1) to furnish **14** (28 mg), **15** (21 mg), and a mixture of **14** and **15** (105 mg).[20] **14**, colorless oil, $[\alpha]_D^{26}$ +74.0° (c=0.6, MeOH). High resolution mass spectrum: Calcd. for $C_{10}H_{18}O_5$ (M^+): 218.115; Found: 218.115. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 2930, 1050, 850. PMR ($CDCl_3$, δ): 1.38, 1.41 (3H each, both s, isopropylidene methyl×2), 3.47 (6H, s, $OCH_3\times 2$), 5.08 (1H, d, J=4 Hz, anomeric H). MS *m/e* (%): 218 (M^+, 1), 203 (28), 187 (72), 101 (100). **15**, colorless oil, $[\alpha]_D^{26}$ −38.5° (c=0.4, MeOH). High resolution mass spectrum: Calcd. for $C_{10}H_{18}O_5$: (M^+) 218.115; Found: 218.115. IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH, 2930, 1080, 850. PMR ($CDCl_3$, δ): 1.39, 1.40 (3H each, both s, isopropylidene methyl×2), 3.42 (6H, s, $OCH_3\times 2$), 4.89 (1H, s, anomeric H). MS *m/e* (%): 218 (M^+, 1), 203 (17), 187 (62), 101 (58).

Methanolysis of 14 and 15 giving 16 and 17——i) A solution of **14** or **15** (5 mg each) in 9% HCl–dry MeOH (1 ml) was heated under reflux for 1 hr. Working up of the reaction mixture as for **7a** gave **16** and **17** which were identical with methyl 2-O-methyl-xylopyranoside obtained from the methanolysis mixture of **9a**, **10a**, and **12**.

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20) Due to the close *Rf* values, the chromatographic separation of **14** and **15** was difficult. Moreover, repeated TLC separation readily caused unfavorable partial degradation of the products.

ii) A mixture of **14** and **15** (*ca.* 1: 1, 40 mg) was dissolved in 9% HCl–dry MeOH (5 ml) and the solution was heated under reflux for 1 hr. Working up of the reaction mixture as for **7a** gave a product which was purified by preparative TLC (benzene–acetone=1: 1) to furnish **16** (12 mg) and **17** (11 mg). **16** was identified with methyl 2-O-methyl-α-D-xylopyranoside obtained above from **10a** by TLC (benzene–acetone=1: 1, *Rf*=0.30) and GLC [as for **9a** (ii), t_R=7′05″]. **17**, mp 110—111° (colorless needles from dry ether–dry EtOH) [lit.[10] mp 111—112° (dry ether–dry EtOH)], $[\alpha]_D^{28}$ −66.7° (c=0.15, $CHCl_3$) [lit.[10] −67.7° (c=1.37, $CHCl_3$)]. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH), 2930, 1058 (C–O–C). PMR (d_6-acetone, δ): 2.80 (1H, d.d, J=7 and 8 Hz, varied to d, J=8 Hz on irradiation at δ 4.16, 2-H), 3.44, 3.49 (3H each, both s, $OCH_3 \times 2$), 4.16 (1H, d, J=7 Hz, varied to s on irradiation at δ 2.80, 1-H).

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