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Saponins of Pericarps of *Sapindus mukurossi* Gaertn. and Solubilization of Monodesmosides by Bisdesmosides

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From pericarps of *Sapindus mukurossi* (Japanese name: Enmei-hi), saponins of hederagenin (**1**), **2**—**7**, **9**, **11** and **12** were isolated along with sapindosides A (**10**) and B (**8**), both of which have already been isolated from this crude drug. Saponins **7** and **9** were identified as α-L-arabinopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside and α-L-arabinofuranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside of **1**, respectively, both of which were recently isolated from *Lecaniodiscus cupanoides* by Sandberg *et al.*

New saponins named mukurozi-saponins E_1 (**11**) and G (**12**) were proved to be the mono-acetate of **8** and the di-acetate of **7**, respectively. By means of mass and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, the locations of the acetyl groups of **11** and **12** were established to be at the 4-hydroxyl group of the xylosyl unit of **8** and at the 3,4-hydroxyl groups of the terminal arabinosyl unit of **7**, respectively. The other new saponins, named mukurozi-saponins X (**6**), Y_1 (**5**) and Y_2 (**4**), were revealed to be β-sophorosyl esters of **10**, **8** and **7**, respectively, by comparison of the mass and ^{13}C NMR spectra with those of the β-sophorosyl ester of **1** (**13**), which was synthesized from **1** as a reference compound for the study of the anomalous glycosylation shifts. Studies on the structures of the remaining saponins, **2** and **3**, are in progress. The water solubilities of the monodesmosides, **7**—**9**, which cause remarkable enhancement of the absorption of sodium ampicillin from rat intestine and rectum, were greatly increased by the bisdesmosides, **4**—**6**.

Keywords——Sapindus mukrossi; Sapindaceae; Enmei-hi; Saponin of hedragenin; mukurozi-saponin E_1, G, X, Y_1, Y_2; β-sophorosyl ester of hederagenin; ^{13}C NMR; solubilizing effect; monodesmoside; bisdesmoside

The Japanese folk medicine "Enmei-hi," pericarps of *Sapindus mukurossi* Gaertn. (Sapindaceae; "Japanese name: Mukurozi"), has been used as an expectorant. The isolation and structural elucidation of several sapoins of hedragenin (**1**) from this crude drug were reported by Chirva *et al.*[2] A pharmacological study on the crude saponin fraction of this drug was reported by Takagi *et al.*,[3] and very recently, Yata *et al.* of our institute disclosed the remarkable enhancement of absorption of an antibiotic, sodium ampicillin, from rat intestine or rectum by this crude saponin fraction. We have therefore carried out further identification of saponins of this drug.

The dried drug was defatted by extraction with benzene and then extracted with hot methanol. The resulting methanolic extract was subjected to repeated chromatography on columns of highly porous resin, silica gel and silanized silica gel, affording saponins **2**—**12** in decreasing order of polarities on silica gel thin layer chromatography (TLC), in yields of 0.06, 0.09, 0.06, 0.04, 0.06, 0.98, 0.94, 0.45, 0.08, 0.04 and 0.04%, respectively.

Taking account of the glycosylation shifts[4] of the carbon signals of **1**,[5] we concluded from the ^{13}C nuclear magnetic resonance (NMR) spectra that **7**—**12** are 3-*O*-glycosides of **1** (monodesmosides) (Table Ia) and that **10** must be formulated as the 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside of **1**, which has already been isolated from this drug (Sapindoside A),[2] and also from *Hedera helix* (α-hedrin),[6] *Kalopanax septemlobus*,[7] *Akebia quinata*,[8,9] *Hedera*

rhombea,[10] *Pulsatilla cernua*[11] and *Lecaniodiscus cupanoides*.[12] The identification of **10** was confirmed by comparison of the physical constants and the ^{13}C NMR spectrum with those of an authentic sample isolated from *A. quinata*.

On mineral acid hydrolysis, **8** yielded arabinose, rhamnose and xylose, and its ^{13}C NMR spectrum suggested that **8** can be formulated as 3-*O*-β-D-xylopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside of **1**, which has already been isolated from this crude drug(sapindoside B)[2] and also from *A. quinata*[9] and *L. cupanoides*.[12] The identification was established by comparison of the physical constants and the ^{13}C NMR spectrum with those of an authentic sample isolated from *A. quinata*.

Mineral acid hydrolysis of **7** afforded arabinose and rhamnose, while hydrolysis with crude Pectinase[13] yielded **10**. The mass spectrum (MS) of its acetate exhibited fragment ions at *m/z* 259 (arabinosyl $(Ac)_3$) and 489 ((arabinosyl-rhamnosyl) $(Ac)_5$). Methylation of **7** followed by methanolysis gave methyl 2,3,4-tri-*O*-methyl-arabinopyranoside, methyl 2,4-di-*O*-methyl-rhamnopyranoside and methyl 3,4-di-*O*-methyl-arabinopyranoside, leading to the formulation of **7** as 3-*O*-α-L-arabinopyranosyl(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside of **1**, which was recently isolated from *L. cupanoides*.[12] The identification was confirmed by comparison of the physical constants and the ^{13}C NMR spectrum with the reported data.

Saponin **9** yielded arabinose and rhamnose on mineral acid hydrolysis, while hydrolysis with crude Pectinase,[13] afforded **10**. The MS of its acetate exhibited fragment ions at *m/z* 259 (arabinosyl $(Ac)_3$) and 489 ((arabinosyl-rhamnosyl) $(Ac)_5$). Permethylation of **9** followed by methanolysis gave methyl 2,3,5-tri-*O*-methyl-arabinofuranoside, methyl 2,4-di-*O*-methyl-rhamnopyranoside and methyl 3,4-di-*O*-methyl-arabinopyranoside. On going from **10** to **9**, a set of carbon signals due to an α-arabinofuranosyl unit appeared in the spectrum of **9** and a signal at δ 72.4 assignable to C-3 of the α-rhamnosyl unit of **10** was displaced upfield, being observed at δ 79.2. These findings led to the identification of **9** as 3-*O*-α-L-arabinofuranosyl-(1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranoside of **1**, which was recently isolated from *L. cupanoides* by Sandberg *et al.*[12] though their assignment of C-3 of the rhamnosyl unit should be revised as shown in Table Ib. As already observed for Ginseng saponins (ginsenosides-Rb_1, Rb_2, Rb_3 and Rc),[14] it should be noted that the glycosylation shift for a glycosylated carbon (C-3 of the rhamnosyl unit of **9**) signal produced by α-arabinofuranosylation is generally less than those produced by α-arabinopyranosylation (in the case of **7**)[15] and β-xylopyranosylation (in the case of **8**).

The new monodesmosides, **11** and **12**, were named mukurozi-saponins E_1 and G, respectively. On mineral acid hydrolysis, **11** yielded arabinose, rhamnose and xylose. The presence of an acetoxyl group in **11** was demonstrated by a proton signal at δ 1.94 (3H, s) and a set of carbon signals at δ 20.7 (1C, q) and 170.4 (1C, s). On alkaline saponification, **11** gave **8** and the MS of its trimethylsilyl (TMSi) ether showed a fragment ion at *m/z* 319, 277 which was assignable to terminal xylosyl $(TMSi)_2(Ac)$ unit. On going from **8** to **11**, the carbon signal due to C-4 of the terminal xylosyl unit of **8** was deshielded by 1.9 ppm and signals due to C-3 and -5 were shielded by 3.5 and 3.8 ppm, respectively, while other carbon signals remained almost unshifted (Table Ib). It follows that **11** can be formulated as the mono-acetate of **8**, and the acetyl group is located at the 4-hydroxyl group of its terminal xylosyl unit.

Proton signals at δ 1.97 and 2.06 (3H each, s) as well as carbon signals at δ 20.9 (2C, q) and 170.6 (2C, s) indicated the presence of two acetoxyl groups in **12**. Alkaline saponification of **12** afforded **7** and the MS of the TMSi ether of **12** exhibited a fragment ion at *m/z* 289 (arabinosyl (TMSi) $(Ac)_2$). On going from **7** to **12**, carbon signals due to C-2 and -5 of the terminal arabinosyl unit of **7** were displaced upfield, while all other signals remained almost unshifted. These results indicated that **12** is a di-acetate of **7**, in which the acetoxyl groups are located at the 3- and 4-hydroxyl groups of the terminal arabinosyl unit. The appearance of the C-3 and -4 signals of the terminal arabinosyl unit of **12** at almost the same positions as those of the corresponding carbon signals of **7** can be explained in terms of compensation of the acylation

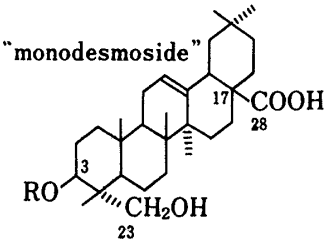
shift due to the acetylation of the vicinal hydroxyl groups.

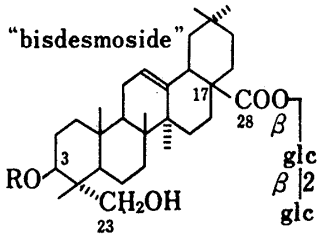
A comparison of the carbon signals of the new saponins, named mukurozi-saponins X(**6**), Y_1 (**5**), Y_2(**4**), Z_1(**3**) and Z_2(**2**), with those of **7**—**12** indicated that a signal due to a carboxyl group was deshielded by about 4 ppm in **2**—**6**, while other signals due to the aglycone moiety remained almost unshifted. This suggests that all of these saponins are 3,28-di-*O*-glycosides of **1** (ester type bisdesmosides).

On mineral acid hydrolysis, **6** gave arabinose, rhamnose and glucose, while alkaline saponification of **6** afforded **10**. The MS of the acetate of **6** showed fragment ions at *m/z* 273 (arabinosyl $(Ac)_3$), 489 ((arabinosyl-rhamnosyl) $(Ac)_5$), 331 (glucosyl $(Ac)_4$) and 619 ((glucosyl-glucosyl) $(Ac)_7$). These results revealed that **6** is a glucobiosyl ester of **10**.

As a part of our studies on the anomalous glycosylation shifts of 1,2-linked oligosaccharides, we prepared the β-sophorosyl ester of hederagenin (**13**) by glycosylation of diacetylhederagenin (**14**) with acetobromosophorose (**15**) in the presence of silver carbonate on Celite, followed by deacetylation with barium oxide in methanol. It was noted that the signals assignable to the glucosylated carbon (C-2 of the inner glucosyl unit) and the anomeric carbon of the terminal glucosyl unit of **13** appeared at δ 78.7 (or 78.0) and 104.5, respectively, being evidently more shielded than the corresponding signals of methyl β-sophoroside (see Table Ib). It is also significant that one of the C-6 signals of the sophorosyl moiety of **13** appeared at somewhat lower field (at δ 63.7) than usual (near δ 62). Comparison of the spectrum of **6** with those of **10** and **13** revealed that the carbon signals due to the sugar moiety of **6** unambiguously consisted of those of **10** and **13** (Table Ib), leading to the formulation of **6** as the β-sophorosyl ester of **10**.

Mineral acid hydrolysis of **5** gave arabinose, rhamnose, xylose and glucose, while **8** was obtained from **5** by alkaline saponification. The MS of the acetate of **5** exhibited *m/z* 331 (glucosyl $(Ac)_4$) and 619 ((glucosyl-glucosyl) $(Ac)_7$) along with the same fragment ions due to the sugar moiety as those of the acetate of **8** (*vide supra*). The carbon signals due to the sugar moiety of **5** appeared at almost the same positions as those of the combined signals of the





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7	: $\underset{\alpha}{-}$ara(p)$\underset{\alpha}{\overset{2}{-}}$rham$\underset{\alpha}{\overset{3}{-}}$ara(p)	: **4**
8	: $\underset{\alpha}{-}$ara(p)$\underset{\alpha}{\overset{2}{-}}$rham$\underset{\beta}{\overset{3}{-}}$xyl	: **5**
9	: $\underset{\alpha}{-}$ara(p)$\underset{\alpha}{\overset{2}{-}}$rham$\underset{\alpha}{\overset{3}{-}}$ara(f)	
10	: $\underset{\alpha}{-}$ara(p)$\underset{\alpha}{\overset{2}{-}}$rham	: **6**
11	: $\underset{\alpha}{-}$ara(p)$\underset{\alpha}{\overset{2}{-}}$rham$\underset{\beta}{\overset{3}{-}}xyl\overset{4}{-}$Ac	
12	: $\underset{\alpha}{-}$ara(p)$\underset{\alpha}{\overset{2}{-}}$rham$\underset{\alpha}{\overset{3}{-}}$ara(p)$\overset{3}{<}_{4}^{Ac}_{Ac}$	
1	: H	: **13**

ara(p): L-arabinopyranosyl rham: L-rhamnopyranosyl
ara(f) : L-arabinofuranosyl xyl : D-xylopyranosyl
glc : D-glucopyranosyl Ac : acetyl

Chart 1

TABLE Ia. ^{13}C NMR Chemical Shifts: Aglycone Moiety (in C_5D_5N)

	7	8	9	10	11	12	6	5	4	13
C- 1	38.9	38.9	38.8	38.9	38.9	38.9	39.1	39.0	38.8	38.7
C- 2	26.1	26.1	26.1	26.1	26.1	26.2	26.2	26.2	25.9	27.5
C- 3	81.2	80.9	81.3	81.0	81.2	81.3	81.0	81.0	80.9	73.2
C- 4	43.5	43.5	43.4	43.5	43.5	43.6	43.5	43.6	43.4	42.7
C- 5	47.7	47.5	47.8	47.7	47.6	47.7	47.6	47.5	47.4	48.1
C- 6	18.2	18.4	18.3	18.3	18.2	18.3	18.5	18.4	18.2	18.4
C- 7	33.2	33.2	33.2	32.8	33.2	33.2	33.1	33.1	32.9	33.1
C- 8	39.7	39.6	39.7	39.7	39.6	39.8	40.0	40.0	39.8	39.9
C- 9	48.1	48.1	48.1	48.1	48.1	48.1	48.2	48.2	48.0	48.4
C-10	36.8	36.8	36.8	36.8	36.8	36.9	36.8	36.8	36.7	37.1
C-11	23.7	23.7	23.7	23.7	23.7	23.8	23.8	23.8	23.6	23.7
C-12	122.5	122.3	122.6	122.5	122.5	122.5	122.5	122.5	122.5	122.5
C-13	144.6	144.4	144.7	144.7	144.6	144.8	144.4	144.4	144.2	144.3
C-14	42.0	42.0	42.0	42.0	42.0	42.2	42.0	42.0	41.9	42.0
C-15	28.2	28.2	28.3	28.2	28.2	28.3	29.3	29.1	28.9	29.0
C-16	23.7	23.7	23.7	23.7	23.7	23.8	22.9	23.0	22.9	23.0
C-17	46.5	46.5	46.6	46.6	46.6	46.7	47.0[a)]	47.0[a)]	46.8[a)]	46.9[a)]
C-18	42.0	41.8	42.0	42.0	42.0	42.2	41.8	41.7	41.9	42.0
C-19	46.5	46.5	46.6	46.6	46.6	46.7	46.1[a)]	46.1[a)]	45.9[a)]	46.2[a)]
C-20	30.9	30.8	30.9	31.0	30.9	31.0	30.7	30.7	30.5	30.7
C-21	34.2	34.1	34.2	34.2	34.2	34.2	33.9	34.0	33.9	34.0
C-22	33.2	33.2	33.2	33.2	33.2	33.2	32.4	32.3	32.0	32.3
C-23	64.1	63.9	64.1	64.0	63.9	64.2	64.0	63.9	63.6	67.6
C-24	14.0	14.1	13.9	13.9	14.0	14.1	14.1	14.2	13.8	13.0
C-25	16.0	16.1	16.0	16.0	16.0	16.1	16.1	16.1	15.9	15.9
C-26	17.4	17.4	17.4	17.4	17.4	17.6	17.5	17.5	17.3	17.5
C-27	26.1	26.1	26.1	26.1	26.1	26.2	26.2	26.2	25.9	26.1
C-28	180.1	179.8	180.2	180.2	180.1	180.5	176.5	176.2	176.2	176.4
C-29	33.2	33.2	33.2	33.2	33.2	33.2	33.1	33.1	32.9	33.1
C-30	23.7	23.7	23.7	23.7	23.7	23.8	23.8	23.8	23.6	23.7

a) Assignments in any column may be reversed.

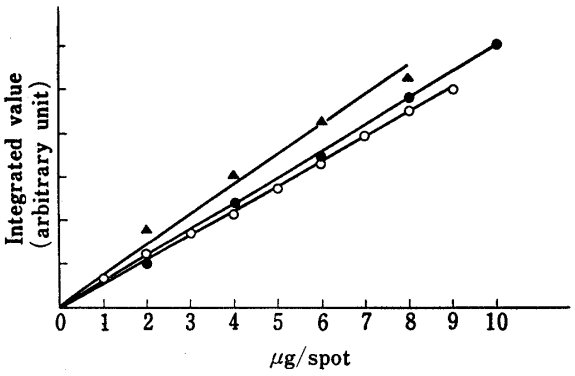

Fig. 1. Calibration Curves

Apparatus : Dual-wavelength TLC-scanner (Shimadzu CS-910).
Wavelength : λ_S 520 nm.
λ_R 760 nm.
Mode : reflection.
Linearizer : off.
—○—: 7 —▲—: 8 —●—; 9.

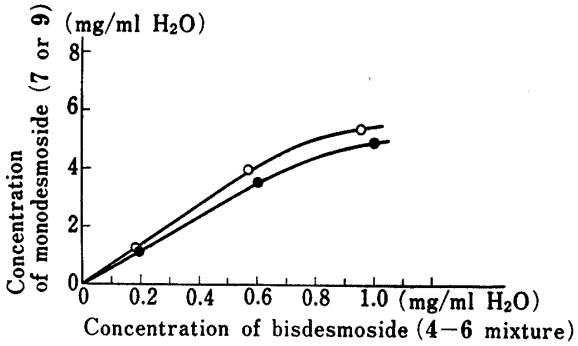

Fig. 2. Solubilizing Effect of Bisdesmoside (4—6 mixture) on Monodesmosides (7,9) [37°C, 24 h]

Saturated concs. in absence of bisdesmosides.
7: 0.017 mg/mlH_2O.
8: 0.039 mg/mlH_2O.
9: 0.0076 mg/mlH_2O.
—○—: 7 —●—: 9.

sugar moieties of **8** and **13**, showing that **5** is the β-sophorosyl ester of **8**.

On mineral acid hydrolysis, **4** gave arabinose, rhamnose and glucose, while alkaline saponification of **4** afforded **7**. The MS of the acetate of **4** showed the fragment ions due to the sugar moiety at m/z 331 and 619 along with those due to the fragmentation of the sugar moiety of **7**

TABLE Ib. ^{13}C NMR Chemical Shifts: Sugar Moiety (in C_5D_5N)

	7	8	9	10	11	12	6	5	4	13
ara-1	104.4	104.3	104.4	104.2	104.4	104.5	104.5	104.5	104.3	
(p) 2	75.2	75.4[a]	75.4	75.7	75.4[a]	75.3	75.9	75.5	75.2	
(Inner) 3	74.6	74.8	74.6	74.4	74.6	74.9	75.1	75.0	74.7	
4	69.3	69.4	69.4	69.2[a]	69.4	69.6[a]	69.6	69.6	69.2	
5	65.8	65.9	65.8	65.3	65.8	66.0	66.0	66.2	65.7	
rahm-1	101.2	101.1	101.2	101.4	101.2	101.2	101.5	101.3	101.2	
2	71.7	71.7	71.6	72.2[b]	71.7	71.8	72.5	71.9	71.7	
3	82.5	82.6	79.2	72.4[b]	82.3	82.1	72.5	82.9	82.8	
4	72.9	72.7	72.3	74.0	72.7	72.9	74.1	72.5	72.7	
5	69.3	69.4	69.4	69.6[a]	69.4	69.3[a]	69.9	69.6	69.2	
6	18.2	18.3	18.3	18.3	18.2	18.3	18.5	18.4	18.2	
ara-1	107.0					106.7			107.1	
(p) 2	72.9					70.1			72.7	
(Terminal) 3	74.4					73.9			74.3	
4	69.3					69.6[a]			69.2	
5	66.9					64.2			66.8	
xyl-1		107.1			106.7			107.4		
2		75.2[a]			75.1[a]			75.5		
3		78.1			74.6			78.3		
4		70.8			72.7			71.1		
5		67.1			63.3			67.3		
ara-1			110.8							
(f) 2			82.2							
3			78.7							
4			87.2							
5			62.7							
glc-1							93.6	93.6	93.4	93.6
(Inner) 2							79.0[a]	78.9[a]	78.6[a]	78.7[a]
3							78.2[a]	78.3[a]	77.9[a]	78.0[a]
4							70.7	70.7	70.5	70.6
5							78.2[a]	78.3[a]	78.6[a]	78.0[a]
6							64.0[b]	63.9[b]	63.6[b]	63.7[b]
glc-1							104.5	104.5	104.3	104.5
(Terminal) 2							75.5	75.5	75.5	75.8
3							78.2[a]	78.3[a]	77.9[a]	78.0[a]
4							72.5	72.8	72.7	72.7
5							79.0[a]	78.9[a]	78.6[a]	78.7[a]
6							62.0[b]	61.9[b]	61.7[b]	61.8[b]
CH_3CO-					170.4	170.6				
					20.7	20.9				

ara (p), α-L-arabinopyranosyl; rham, α-L-rhamnopyranosyl; xyl, β-D-xylopyranosyl; ara (f), α-L-arabinofuranosyl; glc, β-D-glucopyranosyl.
a, *b*) Assignments in any column may be exchangeable.

(*vide supra*). Comparison of the carbon signals of the sugar moiety of **4** with those of **7** and **13** in the same way as in the case of **5** and **6** led to the assignment of **4** as the β-sophorosyl ester of **7**.

Isolation of **5** and **7**—**10** from the same crude drug was recently also reported by Kitagawa *et al.*[16] Although the structure elucidation of **2** and **3** has not been completed as yet, sapindosides C, D and E isolated from this drug by Chirva *et al.*[2] have not been detected in the present study.

It was observed that although the monodesmosides **7**—**9** were sparingly soluble in water in the pure state, these saponins could be extracted from the crude drug with water in fairly good yields. The concentrations of purified **7**, **8** and **9** in the saturated aqueous solutions at 37°C were determined by TLC-densitometry to be only 0.019, 0.044 and 0.0086 μmol/ml (0.017, 0.039 and 0.0076 mg/ml), respectively. A search for the compounds in this drug which increase the solubilities of these monodesmosides in water showed that **7**—**9** showed remarkably higher solubilities ina solution of a mixture of the bisdesmosides, **4** — **6**. Fig. 2 shows the solubilizing

effect of the bisdesmoside mixture on the monodesmosides determined by TLC-densitometry. The saturated concentrations of **7** and **9** in a 0.1% solution of a mixture of **4** — **6** (ratio of **4**, **5** and **6**=7: 7: 1) were found to be 6.4 and 5.6 μmol/ml (5.7 and 5.0 mg/ml), respectively. The solubilizing effect on **8** could not be determined because of the formation of an emulsified solution with the mixture of the bisdesmosides. Studies on the solubilizing effects of the individual bisdesmosides as well as of other oligoglycosides which may have more potent solubilizing effects are in progress.

It was revealed the monodesmosides **7**—**9** showed remarkable promotion of the absorption of an antibiotic, sodium ampicillin, from rat intestine or rectum, while the bisdesmosides **4** — **6** did not. It is significant that a solution of each monodesmoside in a solution of a mixture of **4** — **6** also exhibited remarkable enhancement of the absorption.[17] The details of this biopharmaceutical study will be reported elsewhere.

Experimental

The NMR spectra were taken on a JEOL PFT-100 NMR spectrometer at 25.15 MHz for ^{13}C-NMR and 100 MHz for ^{1}H-NMR in pyridine-d_5 and chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard. Mass spectra were recorded on a JEOL 01-SG-2 mass spectrometer at 75 eV. Trimethylsilylation for MS: A sample of a saponin (1—2 mg) was heated with *N*-trimethylsilylimidazole (5 drops) in a sealed micro-tube at 80°C for 2—3 h. The reaction mixture was diluted with H_2O and then extracted with *n*-C_6H_{14}. The C_6H_{14} layer was washed with H_2O and concentrated to dryness by blowing N_2 gas over it at room temperature. The residue was subjected to MS. Acetylation for MS: A solution of a saponin (1—2 mg) in C_5H_5N (5—6 drops) and Ac_2O (2—3 drops) was allowed to stand at room temperature overnight. The reaction mixture was concentrated to dryness by blowing N_2 gas over it at room temperature and the residue was subjected to MS.

Extraction and Separation of Saponins——Pericarps of *Sapindus mukurossi* (100 g) were defatted by extraction with hot C_6H_6 and the residue was extracted with hot MeOH. After removal of the solvent by evaporation, the MeOH ext. (60 g) was chromatographed on highly porous polymer (MCI-HP20P, Mitsubishi Chemical Ind. Ltd.) (solvent: 10, 50, and 75% aqueous MeOH and finally MeOH), affording bisdesmoside fraction (7.5 g) from the eluate with 75% aqueous MeOH and monodesmoside fraction (10.5 g) from the eluate with MeOH.

Bisdesmoside fraction was separated by repeated column chromatography on silica gel (solvents: $CHCl_3$–MeOH–H_2O (20: 10: 1) and then EtOAc–EtOH–H_2O (10: 2: 1) or (8: 2: 1), all homogeneous), by reverse phase chromatography on RP-8 (Merck) (solvent: 65% or 58% MeOH) and finally by HLC on TSK-gel LS-410 (solvent: 65% MeOH), affording **2**—**6** in yields of 0.06, 0.09, 0.06, 0.04 and 0.06%, respectively. Monodesmoside fraction was chromatographed repeatedly on silica gel (solvents: EtOAc–EtOH–H_2O (15: 2: 1) or (30: 2: 1) and $CHCl_3$–MeOH–H_2O (45: 10: 1) or (65: 10: 1), all homogeneous, and C_6H_6–$(CH_3)_2CO$–H_2O (5: 8: 1, upper phase)), affording **7**—**12** in yields of 0.98, 0.94, 0.45, 0.08, 0.04 and 0.04% respectively.

Saponin **7**: White powder, $[\alpha]_D^{15}$ +12.1° (c=1.0, MeOH). *Anal.* Calcd for $C_{46}H_{74}O_{16}\cdot 4H_2O$: C, 57.84; H, 8.65. Found: C, 57.55, H, 8.66. Saponin **9**: White powder, $[\alpha]_D^{23}$ −10.2° (c=1.0, MeOH). *Anal.* Calcd for $C_{46}H_{74}O_{16}\cdot 9/2H_2O$: C, 57.30; H, 8.70. Found: C, 57.14; H, 8.46. Mukurozi-saponin E_1 (**11**): White powder, $[\alpha]_D^{23}$ −6.2° (c=1.0, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{17}\cdot 9/2H_2O$: C, 57.30; H, 8.52. Found: C, 57.23; H, 8.06. Mukurozi-saponin G (**12**): White powder, $[\alpha]_D^{23}$ +24.0° (c=0.96, MeOH). *Anal.* Calcd for $C_{50}H_{78}O_{18}\cdot 3H_2O$: C, 58.80; H, 8.29. Found: C, 58.45; H, 7.98. Mukurozi-saponin X (**6**): White powder, $[\alpha]_D^{17}$ −3.7° (c=1.1, MeOH). *Anal.* Calcd for $C_{53}H_{86}O_{22}\cdot 2H_2O$: C, 57.28; H, 8.16. Found: C, 57.39; H, 8.09. Mukurozi-saponin Y_1 (**5**): White powder, $[\alpha]_D^{21}$ −10.1° (c=0.98, MeOH). *Anal.* Calcd for $C_{58}H_{94}O_{26}\cdot 3H_2O$: C, 55.23; H, 7.99. Found: C, 54.87; H, 7.62. Mukurozi-saponin Y_2 (**4**): White powder, $[\alpha]_D^{21}$ −4.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{58}H_{94}O_{26}\cdot 4H_2O$: C, 54.45; H, 8.04. Found: C, 54.23; H, 7.87.

Hydrolysis of 4—12——A saponin (several mg) was heated with 4 N HCl/50% aqueous dioxane (1: 1 v/v) (1 ml) in a sealed tube on a boiling water bath for 4 h. The reaction mixture was diluted with H_2O and washed with $CHCl_3$. The aqueous layer was neutralized with Amberlite MB3, concentrated and subjected to TLC on silica gel with $CHCl_3$–MeOH–H_2O (65: 40: 10 homogeneous) using 2,3,5-triphenyltetrazolium chloride as a coloring reagent. For GLC, the above aqueous layer was concentrated to dryness and the residue was trimethylsilylated by the same procedure as used for MS. Gas liquid chromatography (GLC) was carried out on a Shimadzu GC-4A gas chromatograph (glass column, 4 mm × 2 m, 1.5% SE-30 on Chromosorb W; detector, FID; injection temperature, 200°C; column temperature, 180°C; carrier gas, N_2 at 1 kg/cm^2). Retention times (min): glucose, 8.3, 10.0, 12.2 and 14.9; xylose, 4.5 and 5.8; rhamnose 3.3 and 4.6; arabinose, 3.1.

Permethylation of 7 and 9 Followed by Methanolysis——According to Hakomori's method, a mixture of

NaH (100 mg) and DMSO (2.5 ml) was heated at 65°C for 2 h under N_2, and to this mixture was added a solution of the saponin **7** or **9** (50 mg) in DMSO (2 ml). The whole was stirred for 2 h at 65°C, then MeI (7 ml) was added to the solution. The mixture was further stirred for 30 min at 65°C, and then allowed to stand overnight at room temperature. After dilution with H_2O, the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O and concentrated to dryness *in vacuo*. The residue was purified by preparative TLC (solvent: C_6H_6–$(CH_3)_2CO$ (3: 1)), affording a permethylated saponin. Permethyl ether of **7**; infrared (IR) (CCl_4): no OH absorption; ^{1}H-NMR ($CDCl_3$): δ 4.34 (1H, d, J=5.4 Hz), 4.66 (1H, d, J=5.4 Hz), 5.12 (1H, d, J=1 Hz). Permethyl ether of **9**; IR (CCl_4): no OH absorption; ^{1}H-NMR ($CDCl_3$): δ 4.30 (1H, d, J=6 Hz), 5.09 (1H, d, J=2 Hz), 5.20 (1H, d, J=1 Hz).

A solution of the resulting permethylated saponin in 6.7% methanolic HCl (0.5 ml) was refluxed for 4 h. The reaction mixture was concentrated to dryness by blowing N_2 gas over it at room temperature. GLC: on a glass column of 20% butane-1,4-diol succinate on Chromosorb WAW, 4 mm×2 m; detector, FID; injection temperature, 200°C; column temperature, 185°C; carrier gas, N_2 1 kg/cm^2. Methyl 2,3,4-tri-*O*-methyl-arabinopyranoside, methyl 2,4-di-*O*-methyl-rhamnopyranoside, methyl 3,4-di-*O*-methyl-arabinopyranoside and methyl 2,3,5-tri-*O*-methyl-arabinofuranoside were identified by comparison of the retention times with those of authentic samples.

Deacetylation of 11 and 12——A solution of **11** or **12** in 2% KOH/MeOH was stirred for 30 min at room temperature. The reaction mixture was neutralized with Dowex 50W and concentrated to dryness, affording the corresponding parent saponin, **8** or **7**.

Hydrolysis of 7 and 9 with Crude Pectinase——A solution of **7** (80 mg) and crude pectinase (Tanabe Pharm. Ind. Co. Ltd., 160 mg) in McIlvaine buffer (pH 4, 50 ml, containing a few drops of toluene to prevent fungal contamination) was incubated at 37°C for 10 d, then the reaction mixture was heated for 5 min and concentrated to dryness *in vacuo*. The residue was purified by column chromatography on silica gel (solvent: $CHCl_3$–MeOH (14: 3)), giving the prosapogenin (**10**) as a white powder (28 mg).

Saponifications of Bisdesmosides (4—6)——A saponin (10 mg) was refluxed with 5% KOH/15% MeOH (2 ml) for 1 h. The reaction mixture was diluted with water and extracted with EtOAc–1-BuOH (2: 1). The organic layer was concentrated to dryness to give the corresponding monodesmosides, **7**, **8** or **10**.

Quantitative Analysis of 7—9——A MeOH solution of each saponin was spotted on a TLC plate (Silica gel $60F_{254}$ precoated, Merck) and developed with EtOAc–EtOH–H_2O (8: 2: 1, homogeneous). After being sprayed with 10% H_2SO_4, the plate was heated at 120°C for a few min, immediately covered with a glass plate of the same size to prevent color change of the spots and then subjected to TLC-densitometry; for conditions, see Fig. 1. Calibration plots of integrated values of spots against weight/spot were found to be linear for **7**—**9** up to a concentration of 8 μg/spot and the extrapolated plots passed through the origin, as indicated in Fig. 1. The ratio of **4**—**6** in a solution of a mixture of these saponins used for the assay of the solubilizing effect was determined in the same way (TLC developed with EtOAc: EtOH: H_2O, 5: 2: 1).

Determination of Solubilizing Effects——Saturated aqueous solutions of **7**—**9** were prepared by incubation of an excess of each saponin in H_2O (5 ml) at 37°C for 24 h followed by filtration through a 0.5 μm filter (Millipore Corporation) to remove undissolved saponin. Saturated solutions of the monodesmosides (**7** and **9**) in an aqueous solution of a mixture of the bisdesmosides (**4**—**6**) were prepared as follows. A solution of an excess of each monodesmoside in MeOH containing a mixture of **4**, **5** and **6** (ratio: 7: 7: 1) was concentrated to dryness and the residue was incubated in H_2O (5 ml) at 37°C for 24 h. Each saturated solution was filtered as described above. The concentration of the monodesmoside in each saturated solution was determined by TLC-densitometry under the conditions given in Fig. 1.

Synthesis of 13——Acetobromosophorose (400 mg) was added to a solution of 3,23-di-*O*-acetylhederagenin (200 mg) in 1,2-dichloroethane (20 ml) containing Ag_2CO_3-Celite (600 mg) from which moisture had first been removed by evaporation of about 5 ml of the solvent, and the mixture was refluxed for 2 h. After removal of the precipitate by filtration through Celite, the reaction mixture was concentrated to dryness. The residue was deacetylated by standing in anhydrous MeOH (10 ml) in the presence of BaO (380 mg) at room temperature for 1 h under stirring. The mixture was diluted with H_2O (70 ml) and extracted with 1-BuOH saturated with H_2O. The BuOH layer was concentrated to dryness and the residue was chromatographed on silica gel (solvent: $CHCl_3$–MeOH (35: 10)) to give **13** (230 mg) as colorless needles from $CHCl_3$–MeOH, mp 214—216°C, $[\alpha]_D^{13}$ +25.3° (c=1.6, MeOH). *Anal.* Calcd for $C_{42}H_{68}O_{14}\cdot 5H_2O$: C, 56.86; H, 8.86. Found: C, 57.16; H, 8.61.

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