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ACYCLIC SESQUITERPENE OLIGOGLYCOSIDES FROM PERICARPS OF *SAPINDUS MUKUROSSI*

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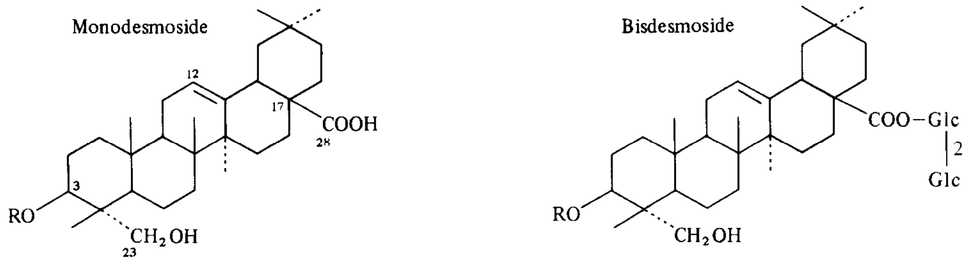
Key Word Index—*Sapindus mukurossi*; Sapindaceae; sesquiterpene oligoglycoside; mukuroziosides Ia, Ib, IIa, IIb; mukurozidiol; natural surfactants.

Abstract—New acyclic sesquiterpene oligoglycosides named mukuroziosides Ia, Ib, IIa and IIb were isolated from the pericarp of *Sapindus mukurossi* and their structures were elucidated. These oligoglycosides showed a potent solubilizing effect on the co-occurring saponins (monodesmosides), which are sparingly soluble in water in the pure state.

INTRODUCTION

The pericarp of *Sapindus mukurossi* Gaertn. (Sapindaceae; Japanese name: Enmeihi) is used in traditional Oriental medicine as an expectorant, as well as a source of natural surfactant. Several saponins have been isolated from this crude drug, as shown in Fig. 1 [1]. Of these saponins, monodesmosides such as **1**–**3** are sparingly soluble in water in the pure state, although these monodesmosides can be extracted from the crude drug with water and are soluble in water as a crude glycoside mixture. Consequently, we expected that the crude glycoside mixture from this crude drug would contain substances which solubilize these monodesmosides. Indeed, it was found that the water solubilities of these monodesmosides were greatly increased by the co-occurring bisdesmosides, mukurozi-saponins X (**4**), Y_1 (**5**) and Y_2 (**6**) [1] (Fig. 1). It was also found that solutions of these monodesmosides solubilized with the aid of the bisdesmosides produced a remarkable enhancement of the absorption of β-lactam type antibiotics such as sodium ampicillin (ABPC) from rat intestine and rectum [2]. Since the contents of these bisdesmosides in the crude drug are fairly low, we also looked for other solubilizing principles in the crude glycoside mixture. The present paper deals with the



	R	
1	$-\text{Ara (p)} \overset{2}{-} \text{Rha} \overset{3}{-} \text{Ara (p)}$	**6**
2	$-\text{Ara (p)} \overset{2}{-} \text{Rha} \overset{3}{-} \text{Xyl}$	**5**
3	$-\text{Ara (p)} \overset{2}{-} \text{Rha} \overset{3}{-} \text{Ara (f)}$	
	$-\text{Ara (p)} \overset{2}{-} \text{Rha}$	**4**

Ara (p): α - L - arabinopyranosyl
Ara (f): α - L - arabinofuranosyl
Rha: α - L - rhamnopyranosyl
Xyl : β - D - xylopyranosyl

Fig. 1. Saponins of *Sapindus mukurossi*.

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isolation and structure elucidation of four surface-active sesquiterpene oligoglycosides, named mukuroziosides Ia (**7**), Ib (**8**), IIa (**9**) and IIb (**10**).

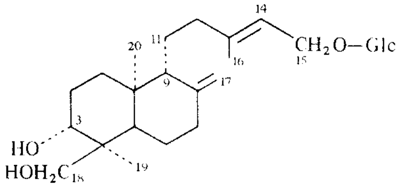
RESULTS AND DISCUSSION

The defatted crude drug was extracted with methanol and the methanol extract subjected to CC on highly porous polymer to yield a non-saponin glycoside fraction, a bisdesmoside fraction and a monodesmoside fraction. The non-saponin glycoside fraction was further chromatographed on silanized silica gel and then on silica gel, affording **7**–**10** in yields of 0.6, 0.4, 1.5 and 2.5‰, respectively.

Glucose and rhamnose were identified in the acid hydrolysate of **10**, while hydrolysis of **10** with a mixture of crude pectinase and crude hesperidinase [3] yielded an acid-unstable aglycone, $C_{15}H_{26}O_2$ (**11**) and a partially hydrolysed product (**12**). The ^{1}H NMR of **11** (100 MHz, $CDCl_3$) indicated the presence of one $-CH_2OH$ on an olefinic carbon carrying a proton [δ4.13 (2H, *d*, *J* = 7 Hz)], one $-CH_2OH$ on an olefinic carbon having no proton [δ3.98 (2H, *s*)], three protons, each on an olefinic carbon linked to a methylene group [δ5.12 (1H, *t*, *J* = 5 Hz) and 5.40 (2H, *t*, *J* = 5 Hz)] and three methyls, each on an olefinic carbon having no proton [δ1.68 (3H, *s*), 1.66 (3H, *s*) and 1.61 (3H, *s*)]. The carbon signals of all-*trans*-farnesol (**13**) were assigned by comparison with those of geraniol [4]. Comparison of the ^{13}C NMR spectrum of **11** (Table 1) with that of **13** as well as with the spectra of 9-hydroxylinalool and 1-hydroxylinalool reported by Tschesche *et al.* [5] led to the formulation of **11** as 12-hydroxy-all-*trans*-farnesol. The diacetate of **11** has already been isolated from *Tanacetum odessanum* (Compositae) [6].

On going from **11** to **12**, carbon signals due to C-1–C-3 were displaced by +6.7, −3.7 and +2.8 ppm, respectively, while other carbon signals remained almost unshifted. These glycosylation shifts were consistent with those observed for goshonoside F1 (**14**) [7], and thus **12** can be assigned as the 1-*O*-glycoside of **11**. The EI mass spectrum of acetylated **12** showed ions at *m/z* 791 and 273 attributable to [Glc-(Rha)$_2$Ac$_8$] and [(Rha)Ac$_3$], but no ions due to [(Glc)Ac$_4$], [(Glc-Rha)Ac$_6$] and [(Rha-Rha)Ac$_5$]. This indicated the presence of a branching-type sugar moiety, -Glc-(Rha)$_2$ in **12**. Permethylated **12** was subjected to sequence analysis [8–10] (acid hydrolysis and sodium borohydride reduction of the resulting sugar units, followed by acetylation and subsequent GC/MS analysis of the products), affording 1,2,3,5-tetra-*O*-acetyl-4,6-di-*O*-methylhexitol (**15**) formed from 2,3-linked glucose and 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methylhexitol (**16**) from terminal rhamnose. Carbon signals due to the sugar moiety of **12** were observed at the positions expected for 2,3-di-*O*-α-rhamnopyranosyl-β-glucopyranoside [11, 12] (Table 2). These results led to the formulation of **12** as 1-*O*-α-rhamnopyranosyl-(1 → 2)-[α-rhamnopyranosyl-(1 → 3)]-β-glucopyranoside of **11**.

On going from **12** to **10**, carbon signals due to C-10–C-12 and C-15 of the aglycone moiety were displaced by +4.2, −3.2, +7.1 and +1.0 ppm, respectively. These glycosylation shifts were consistent with those observed between 8-hydroxygeraniol (**17**) and its β-glucoside (**18**; prepared from **17**), indicating that both the 1- and 12-hydroxyl groups of **10** are glycosylated. The EI mass spectrum of acetylated **10** showed ions at *m/z* 791 and 273, also observed in the spectrum of acetylated **12** (*vide supra*), but no ions due to Glc-Glc, Glc-Rha, Rha-Rha and terminal Glc. The sequence analysis of permethylated **10** afforded **15** and **16**. The sugar carbon signals of **10**, which



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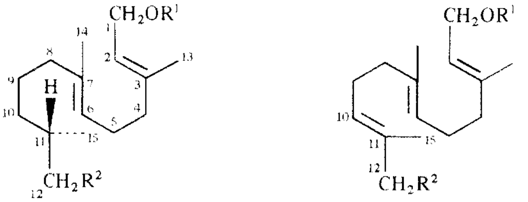


	R¹	R²	
7	Glc $\overset{2}{-}$ Rha 3 Rha	O – Glc $\overset{2}{-}$ Rha	**8**
9	Glc $\overset{2}{-}$ Rha 3 Rha	O – Glc $\overset{2}{-}$ Rha 3 Rha	**10**
19	Glc $\overset{2}{-}$ Rha 3 Rha	OH	**12**
20	H	OH	**11**
	H	H	**13**

Glc: β - glucopyranosyl
Rha: α - rhamnopyranosyl

Table 1. ^{13}C NMR chemical shifts of aglycone moieties of **7–13**, **19** and **20** [25.15 MHz and 25.00 MHz (**20** at 270 MHz), CD_3OD, TMS as int. standard]

C	**20**	**11**	**19**	**12**	**7**	**8**	**9**	**10**	**13**	**13***
1	59.4	59.4	66.2	66.1	66.3	66.3	66.4	66.3	59.3	59.4
2	124.9[a]	125.0[a]	121.3	121.3	121.2	121.2	121.3	121.4	125.0[a]	123.4[a]
3	139.4	139.2	141.9	142.0	142.0	142.0	142.1	141.8	138.9	139.8
4	40.7	40.7[b]	40.6[b]	40.5	40.6[b]	40.3[b]	40.7[b]	40.4[b]	40.5	39.6
5	27.4[c]	27.3	27.1	27.3	27.2	27.4[c]	27.3	27.4	27.3[b]	26.3[b]
6	125.1[a]	125.3[a]	125.0	125.2	124.9	125.2	125.0	125.3	125.3[a]	123.8
7	136.4	136.0	136.3	136.0	136.4	135.9	136.4	136.0	135.6	135.4
8	40.7	40.5[b]	41.0[b]	40.5	41.0[b]	40.5[b]	41.0[b]	40.5[b]	40.5	39.6
9	26.4[c]	27.3	26.4	27.1	26.3	27.2[c]	26.3	27.4	27.6[b]	26.7[b]
10	33.9	126.5	33.8	126.4	34.3	130.2	34.4	130.6	125.0[a]	124.3[a]
11	36.8	136.0	36.7	135.8	34.7	132.5	34.7	132.6	131.7	131.2
12	68.5	69.0	68.4	68.9	76.4	75.8	76.7	76.0	25.9	25.7
13	16.2[d]	16.1	16.5[d]	16.5[d]	16.5[d]	16.5[d]	16.6[d]	16.2[d]	16.2	16.0[c]
14	15.9[d]	16.1	16.0[d]	16.2[d]	16.1[d]	16.2[d]	16.1[d]	16.1[d]	16.2	16.2[c]
15	17.1	13.7	17.1	13.8	17.4	14.7	17.4	14.8	17.8	18.7

[a,b,c,d] Signals may be interchangeable in each column.

*In $CDCl_3$.

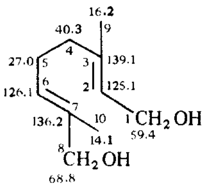
Table 2. ^{13}C NMR chemical shifts of sugar moieties of **7–10**, **12**, **18** and **19** (25.15 MHz and 25.00 MHz, CD_3OD, TMS as int. standard)

	19	**12**	**7**	**8**	**9**	**10**	**18**
Glc-1	103.6[e]	103.6[e]	103.6[e]	103.5[e]	103.7[e]	103.6[e]	102.4
(R^1) 2	80.3	80.3	80.3	80.3	80.3	80.2	75.0
3	87.8	87.7	87.7	87.6	87.8	87.9	78.1[f]
4	70.3[f]	70.3[f]	70.3[f]	70.3[f]	70.4[f]	70.4[f]	71.6
5	77.5	77.5	77.5	77.5	77.5	77.5	77.9[f]
6	62.5	62.4	62.5[h]	62.5[h]	62.5[h]	62.5	62.7
Glc′-1			103.3[i]	100.9[i]	103.2[i]	102.7[i]	
(R^2) 2			79.2	79.1	79.3	79.4	
3			78.7	78.9	88.2	88.1	
4			71.6	72.1	70.1[f]	70.1[f]	
5			77.5	77.5	77.5	77.5	
6			62.6[h]	62.6[h]	62.6[h]	62.5	
1	103.0[e]	103.0[e]	103.0[e]	102.9[e]	103.7[i]	103.6[i]	
	101.1[e]	101.0[e]	101.9[i]	102.0[i]	103.0[e]	103.0[e]	
			101.1[e]	100.9[e]	102.4[i]	101.2[e]	
					101.2[e]	100.9[i]	
2	72.1 (2C)[g]	72.1 (2C)[g]	72.2 (3C)[g]	72.1 (3C)	72.3 (4C)	72.3 (4C)	
Rha 3	72.3 (2C)[g]	72.3 (2C)[g]	72.3 (3C)[g]	72.1 (3C)	72.3 (4C)	72.3 (4C)	
Rha′ 4	73.6	73.5	73.8	73.7	73.5 (4C)	73.6 (4C)	
Rha″	73.4	73.4	73.5	73.5			
Rha‴			73.4				
5	70.6[f]	70.6[f]	70.6[f]	70.6[f]	70.7 (2C)[f]	70.7 (2C)[f]	
	70.1[f]	70.1[f]	70.1[f]	70.1[f]	70.4 (2C)[f]	70.4 (2C)[f]	
			69.7	69.7			
6	17.9	18.0	18.2	18.0 (2C)	18.3	18.0 (2C)	
	17.8	17.8	18.0	17.8	18.0	17.8 (2C)	
			17.8		17.9 (2C)		

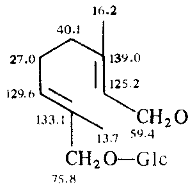
[e, f, g, h, i] Signals may be interchangeable in each column.

showed six anomeric carbon signals, were found to be identical with those of **12**, but duplicated (Table 2). Based on these results, **10** was formulated as the 1,12-*bis*-*O*-α-rhamnopyranosyl-(1 → 2)-[α-rhamnopyranosyl-(1 → 3)]-β-glucopyranoside of **11**.

The ^{13}C NMR spectrum of **8** showed five anomeric carbon signals (Table 2). Acid hydrolysis of **8** afforded glucose and rhamnose, and on enzymatic hydrolysis, **12** was obtained from **8**. The carbon resonances of the aglycone moiety of **8** appeared at almost the same positions as those of **10**, indicating that **8** is a 1,12-di-*O*-glycoside of **11**. The EI mass spectrum of acetylated **8** exhibited ions at *m*/*z* 561 [(Glc-Rha)Ac_6] together with the ions at *m*/*z* 791 and 273 observed for acetylated **12**.



17 (CD_3OD)

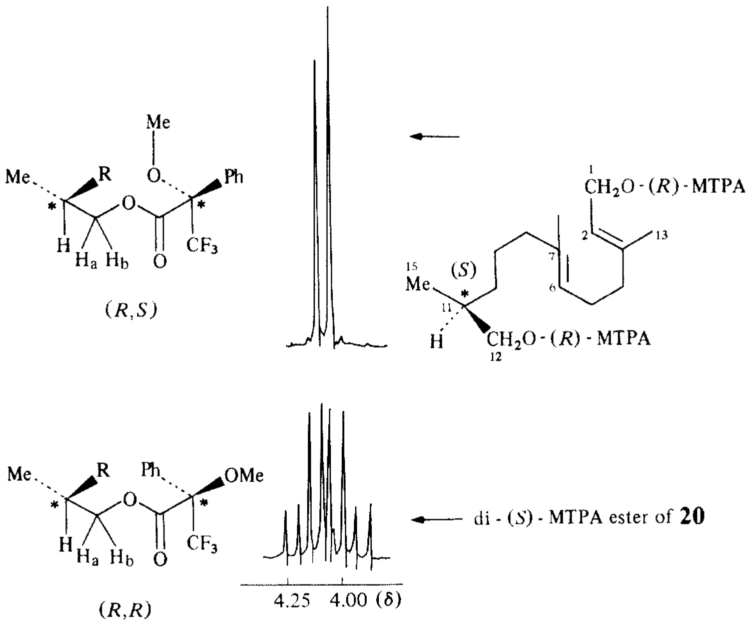


18 (CD_3OD)

Sequence analysis (*vide supra*) of permethylated **8** afforded 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylhexitol due to 2-linked glucose along with **15** and **16**. Further, it was found that the carbon signals associated with the sugar moiety of **8** consisted of signals due to 2-*O*-α-rhamnopyranosyl-β-glucopyranoside [13] together with those due to 2,3-di-*O*-α-rhamnopyranosyl-β-glucopyranoside. It follows that the structure of **8** can be assigned as the 12-*O*-α-rhamnopyranosyl-(1 → 2)-β-glucopyranoside of **12**.

Acid hydrolysis of **9** yielded glucose and rhamnose. Enzymatic hydrolysis of **9** with a mixture of crude hesperidinase and crude pectinase afforded a partially hydrolysed product (**19**) and an optically active aglycone named mukurozidiol, $C_{15}H_{28}O_2$ (**20**). The carbon signals due to C-1, C-8, C-13 and C-14 of **11** were observed at almost the same positions as those of **20**. However, on going from **11** to **20**, two olefinic carbon signals (C-10 and C-11) were replaced by signals due to one methylene carbon and one methine carbon and the C-15 signal was displaced upfield by 3.4 ppm, while signals assigned to C-8, C-9 and C-12 appeared at slightly shifted positions (Table 1). With regard to the ^1H NMR spectrum (100 MHz, $CDCl_3$), **20** exhibited two olefinic proton signals at δ5.41 (1H, *t*, $J = 5$ Hz) and 5.12 (1H, *t*, $J = 5$ Hz), and on going from **11** to **20**, the signals due to 12-CH_2O–(δ3.45, 2H, *d*, $J = 6$ Hz) and one of the three methyls (δ1.60 or 1.68, 3H, *s*) on olefinic carbon disappeared and instead, signals assignable to a $-CH_2O-$ (δ4.15, 2H, *d*, $J = 7$ Hz) and a methyl (δ0.92, 3H, *d*, $J = 6$ Hz) were observed, both of which must be located on a saturated methine carbon. It follows that **20** can be represented as 10,11-dihydro-**11**.

The chirality of C-11 of **20** was elucidated by ^1H NMR analysis of the optically active α-methoxy-α-trifluoromethylphenylacetate (MTPA ester) (Fig. 2). In 1977, Yasuhara and Yamaguchi reported the determination of the absolute configurations of primary carbinols with a chiral centre at the C-2 position by ^1H NMR spectroscopy of the MTPA esters with the use of a shift reagent [14]. Recently, it was found that in the case of MTPA esters of primary carbinols with a chiral carbon carrying H, Me and a linear alkyl group, such as **21**, **22** and **23** [14], the chirality can be determined from the coupling patterns of the primary carbinol protons without the aid of a shift reagent; the signal due to primary carbinol protons appears as a 2H doublet when the chiralities of MTPA and the methine carbon are different (combination of *R,S* or *S,R*). In contrast to this, in the case of the esters with the same chiralities (combination of *R,R* or *S,S*), the carbinyl proton signals appear as a pair of double doublets (1H each); in this case, the two protons of the primary carbinol are non-equivalent. It was observed that the spectrum of the (*R*)-MTPA ester of **20** prepared by Mosher and Dale's



21 R = ethyl
22 R = *n*-propyl
23 R = *n*-butyl

Fig. 2. ^1H NMR of $-\overset{|}{\underset{|}{^*C}}-CH_2O-MTPA$ (in $CDCl_3$).

procedure [15] corresponded to the former case and its (*S*)-MTPA ester exhibited primary carbinyl proton signals with the same coupling pattern (δ4.21 and 4.09, 1H each, *dd*, $J_1 = 10.6$, 5.5 Hz and $J_2 = 10.6$, 6.8 Hz; 270 MHz, $CDCl_3$) as that of the latter case. Consequently, the structure of **20** was established as 11(*S*)(*E*,*E*)-2,6-dodecadiene-3,7,11-trimethyl-1,12-diol.

Based on the glycosylation shifts of the allylic primary carbinol (*vide supra*) and saturated primary alcohols [16], a comparison of the aglycone carbon signals of **9** with those of **20** indicated that both the hydroxyl groups of **9** were glycosylated (Table 1). The EI mass spectrum of acetylated **9** showed the same ions associated with the sugar moiety as those of acetylated **10**, and the sugar carbon signals of **9** were found to be almost superimposable over those of **10**, leading to the formulation of **9** as the 1,12-*bis*-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucopyranoside of **20**.

Acid hydrolysis of **7** yielded glucose and rhamnose. On hydrolysis with crude naringinase [3], **7** afforded a partially hydrolysed product which was identical with **19** obtained from **9**. A comparison of the carbon signals of **19** with those of **20** and **12** indicated that **19** is the 1-*O*-glycoside of **20**, and the sugar moiety appears to be identical with that of **12**. The EI mass spectrum of acetylated **7** showed the same ions due to the sugar moiety as those of acetylated **8**. The aglycone carbon signals of **7** appeared at almost the same positions as those of **9** (Table 1) and its sugar carbon signals were almost superimposable on those of **8** (Table 2). These results led to the formulation of **7** as the 1-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 3)]-β-D-glucopyranoside-12-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside of **20**.

As already mentioned, the monodesmosides, **1**–**3** were remarkably solubilized in the presence of the bisdesmosides, **4**–**6**. For instance, 1 ml of an aqueous solution containing 1 mg of **6** dissolved 21 mg of the corresponding monodesmoside **1** at 37°, whereas the saturated concentration of **1** in water was only 0.017 mg/ml. It is surprising that 1 mM **6** can solubilize 29 mM **1**; such a potent solubilizing effect has not been reported with usual synthetic surfactants. The solubility of **1** was also found to be increased significantly by the co-occurring sesquiterpene oligoglycosides in the present study. The saturated concentration of **1** in 1 ml of an aqueous solution containing 1 mg of each mukurozioside at 37° was determined to be as follows: **7**, 5.1 mg/ml; **8**, 5.2 mg/ml; **9**, 2.3 mg/ml; **10**, 4.7 mg/ml. The surface tension of an aqueous solution of **10** is plotted against the concentration of **10** in Fig. 3. It is noteworthy that, unlike general surfactants, which have a distinct critical micelle concentration (cmc), **10** showed no apparent cmc.

EXPERIMENTAL

^1H NMR spectra were measured at 100 and 270 MHz, ^{13}C NMR at 25 and 68 MHz in CD_3OD or $CDCl_3$; chemical shifts are given on the δ(ppm) scale with TMS as int. standard. MS were recorded at 75 eV. Acetylation of sesquiterpene oligoglycosides for MS: was carried out according to ref. [1]. GC/MS conditions: He carrier gas at 50 ml/min; 1.5 m × 2.6 mm column packed with 5% ECNSS-M; isothermal 215°; injection temp. 240°; separator temp. 240°; ionization voltage 70 eV; accelerating voltage 1.5 kV. Optical rotations were measured at 16–24° in MeOH. For silica gel CC, Kieselgel 60 (Merck) and Kieselgel 60 H (Merck) were used. Surface tension was determined with a Wilhelmy-type tensiometer at 25°.

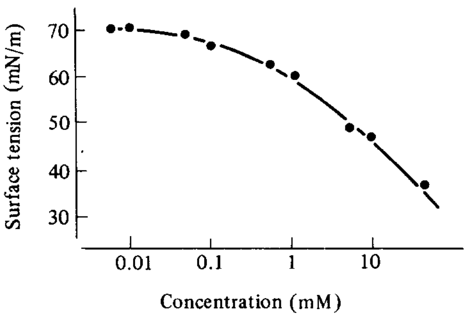


Fig. 3. Surface tension of **10** in water at 25°.

Plant material was purchased in a market in Japan and authenticated by Professor Itsuo Nishioka, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Kyushu University, to whom the authors express their thanks.

Extraction and separation of mukuroziosides. Pericarps of *S. mukurossi* (100 g) were defatted by extraction with hot C_6H_6 and the residue extracted with hot MeOH. After removal of solvent by evaporation, the MeOH extract (86 g) was chromatographed on highly porous polymer, DIAION HP-20 (Mitsubishi Chem. Ind. Tokyo, Japan) (10, 50 and 75% aq. MeOH successively and finally MeOH), affording the non-saponin glycoside fraction (12 g) from the eluate with 50% aq. MeOH.

This non-saponin glycoside fraction was separated by repeated CC on silica gel (EtOAc–MeOH–H_2O, 8:2:1 and 7:2:1, all homogeneous), by reverse-phase CC on LiChroprep RP-8 (Merck) (57% aq. MeOH) and finally by HPLC on TSK-Gel ODS-120A (60% or 55% aq. MeOH, 7.5 mm × 30 cm, detection, RI), affording **7**, **8**, **9** and **10** in yields of 0.6, 0.4, 1.5 and 2.5%, respectively.

Mukurozioside Ia (**7**). White powder, $[\alpha]_D^{24}$ −79.1° (MeOH; *c* 1.0). (Found: C, 52.58; H, 7.79. $C_{45}H_{78}O_{24} \cdot H_2O$ requires: C, 52.47; H, 7.93%.) *Mukurozioside Ib* (**8**). White powder, $[\alpha]_D^{20}$ −70.4° (MeOH; *c* 0.97). (Found: C, 52.35; H, 7.63. $C_{45}H_{76}O_{24} \cdot H_2O$ requires: C, 52.11; H, 7.78%.) *Mukurozioside IIa* (**9**). White powder, $[\alpha]_D^{24}$ −70.7° (MeOH; *c* 1.0). (Found: C, 51.83; H, 7.79. $C_{51}H_{88}O_{28} \cdot 2H_2O$ requires: C, 51.66; H, 7.82%.) *Mukurozioside IIb* (**10**). White powder, $[\alpha]_D^{20}$ −66.7° (MeOH; *c* 1.0). (Found: C, 51.35; H, 7.52. $C_{51}H_{86}O_{28} \cdot 2\frac{1}{2}H_2O$ requires: C, 51.37; H, 7.69%.)

Acid hydrolysis of sesquiterpene oligoglycosides and identification of the resulting monosaccharides was carried out as described in previous paper [16]. GC analysis conditions: dual FID detector; carrier gas N_2 at 1.5 kg/cm^2; 2 m × 4 mm column packed with 2% SE30; isothermal 180°; injection and detector temp. 230°). Me monosaccharides were identified by comparison of the R_ts with those of authentic samples.

Enzymatic hydrolysis of **10**. A soln of **10** (500 mg), crude hesperidinase (2.5 g; Tanabe Pharm. Ind. Co. Ltd., Osaka, Japan) and crude pectinase (2.5 g; Tanabe Pharm. Ind. Co. Ltd., Osaka, Japan) in H_2O (50 ml, containing 5 ml toluene to prevent fungal contamination) was incubated at 37° for 5 days. The reaction mixture was extracted with Et_2O. The Et_2O layer was concd and the residue separated by CC on silica gel ($CHCl_3$–MeOH, 100:1–50:1) to give **11** (22 mg). The aq. layer was diluted with MeOH and centrifuged at 2500 rpm. The supernatant was concd to dryness and the residue chromatographed on DIAION HP-20 (H_2O and then MeOH). The MeOH eluate fraction was further separated by CC on silica gel (EtOAc–MeOH–H_2O, 6:2:1), affording **12** (90 mg) and unreacted **10** (81 mg). Compound **11**: colourless oil, $[\alpha]_D^{19}$ 0°(MeOH; *c* 0.67); HRMS at 70 eV: $[M]^+$. Found: 238.190. $C_{15}H_{26}O_2$ requires 238.193. Compound **12**: a white powder, $[\alpha]_D^{16}$ $-59.6°$ (MeOH; *c* 0.26). (Found: C, 55.18; H, 8.11. $C_{33}H_{56}O_{15} \cdot 1\frac{1}{2}H_2O$ requires: C, 55.06; H, 8.26%.)

Enzymatic hydrolysis of **8**. A soln of **8** (200 mg), crude hesperidinase (1.0 g) and crude pectinase (1.0 g) in H_2O (20 ml, containing 1 ml of toluene) was incubated at 37° for 6 days. The reaction mixture was chromatographed on DIAION HP-20 (H_2O and then MeOH). The MeOH eluate was separated by HPLC on μ-Bondapak C-18 (65% aq. MeOH, 8 mm × 10 cm; RI detection), affording **12** (3 mg).

Enzymatic hydrolysis of **9**. A soln of **9** (480 mg), crude hesperidinase (1.0 g) and crude pectinase (1.0 g) in H_2O (50 ml, containing 5 ml of toluene) was incubated at 37° for 7 days. The reaction mixture was chromatographed on DIAION HP-20 (H_2O and then MeOH). The MeOH eluate was concd to dryness and the residue separated by reverse-phase CC on RP-8 (80% aq. MeOH), affording Frs I (**20**-rich fraction) and II (**19**-rich fraction). Fr. II was further hydrolysed with crude enzyme under the same conditions as above. This reaction mixture was chromatographed on DIAION HP-20 (H_2O and then MeOH). The MeOH eluate fraction was concd to dryness and the residue separated by CC on silica gel (EtOAc–MeOH–H_2O, 8:2:1), affording **19** (12 mg) and crude **20** fraction (Fr. III). Frs. I and III were combined and purified by HPLC on TSK-Gel ODS-120A (80% aq. MeOH, 7.5 mm × 30 cm; RI detection) and by CC on silica gel ($CHCl_3$–MeOH, 70:1, homogeneous), affording **20** (12 mg). Compound **20**: colourless oil, $[\alpha]_D^{15}$ $-4.5°$ (MeOH; *c* 0.60); HRMS at 70 eV: $[M+H]^+$. Found: 241.210. $C_{15}H_{29}O_2$ requires 241.217.

Enzymatic hydrolysis of **7**. A soln of **7** (160 mg) and crude naringinase (160 mg, Tanabe Pharm. Ind. Co. Ltd., Osaka, Japan) in H_2O (16 ml, containing a few drops of toluene) was incubated at 37° for 3 days. The reaction mixture was concd to dryness and the residue separated by CC on silica gel (EtOAc–MeOH–H_2O, 8:2:1) and then by reverse phase CC on RP-8 (75% aq. MeOH), affording **19** (90 ml). Compound **19**: white powder, $[\alpha]_D^{20}$ $-67.7°$ (MeOH; *c* 1.0). (Found: C, 52.50; H, 8.94. $C_{33}H_{58}O_{15} \cdot 3\frac{1}{2}H_2O$ requires C, 52.30; H, 8.64%.)

Sugar sesquence analysis by GC/MS. The permethylated oligoglycoside prepared by Hakomori's method [18], was treated with 90% HCO_2H (2 ml) at 100° for 1 hr. The reaction mixture was evapd and the residue heated with 0.13 M H_2SO_4 at 100° for 16 hr. The reaction mixture was neutralized with ion-exchange resin (Amberlite MB-3), and concd to *ca* 2 ml. This soln was treated with $NaBH_4$ (25 mg). After standing at room temp. for 2 hr, the reaction mixture was acidified by passage through a column of Dowex 50W-X1 (H^+ form) and concd to dryness. Boric acid in the residue was removed by co-distillation × 3 with MeOH. The resulting methylated alditol mixture was acetylated with Ac_2O–C_5H_5N (1:1, 1 ml) at 100° for 1 hr. The reagent was removed by co-distillation with toluene. The methylated alditol acetate mixture thus obtained was subjected to GC/MS [17].

Synthesis of **18**. A soln of **17** [19] (2.1 g, 0.01 mol), $Hg(CN)_2$ (10 g, 0.04 mol) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucosyl bromide (10 g, 0.024 mol) in dry toluene (200 ml) was refluxed for 3 hr. Insoluble $Hg(CN)_2$ was removed by filtration and the filtrate concd to dryness. The residue was deacetylated with 5% KOH in MeOH (200 ml) by standing for 30 min at room temp. and the resulting ppt removed by filtration. The filtrate was deionized with Amberlite MB-3 resin and concd to dryness. The residue was purified by CC on silica gel ($CHCl_3$–Me_2CO–MeOH, 4:2:1, homogeneous) and further subjected to CC on RP- 8 (50% aq. MeOH) to give **18** (1.35 g). Compound **18**: white powder, $[\alpha]_D^{20}$ $-30.3°$ (MeOH; *c* 2.18). (Found: C, 57.70; H, 8.58. $C_{16}H_{28}O_7$ requires C, 57.81; H, 8.49%.)

Determination of solubilizing effects. A satd aq. soln of **1** was prepared by incubation of an excess of **1** in water at 37° for **24** hr followed by filtration through a 0.5 μm filter (Millipore) to remove undissolved saponin. A satd soln of **1** in an aq. soln of mukuroziosides was prepared as follows. A soln containing an excess of **1** in MeOH containing a mukurozioside was concd to dryness and the residue incubated in H_2O at 37° for 24 hr. Each satd soln was filtered as described above. The content of **1** in each satd soln was determined by TLC-densitometry according to the method of ref. [1].

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