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Solubilizing Properties of Saponins from *Sapindus mukurossi* GAERTN.

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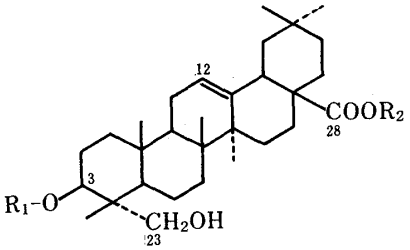
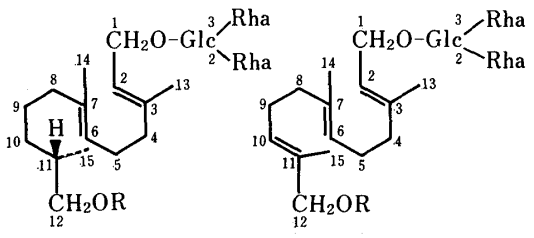
Mukurozi-saponins Y_1 and Y_2, bisdesmosides from pericarps of *Sapindus mukurossi*, greatly increased the water solubilities of the co-occurring monodesmosides. The mode of solubilizing properties of these saponins were investigated. These bisdesmosides also increased the solubilities of Yellow OB and progesterone in phosphate buffer.

Keywords——*Sapindus mukurossi*; Sapindaceae; Enmeihi; saponin; bisdesmoside; solubilizing effect; surface tension; hemolysis; surfactant; progesterone

Sapindus mukurossi GAERTN. (Sapindaceae), a tall tree (Japanese name: Mukurozi), grows in Japan and South-East Asia. Pericarps of this tree, which have been used as a crude drug (Japanese name: Enmeihi, Chinese name: Wu-huan-zi), are currently used in Japan as a source of natural surfactants rather than for medicinal purposes. A number of saponins of hederagenin (**1**) were isolated from this crude drug as shown in Chart 1.[1] Of these saponins, monodesmosides such as **2**—**4** are sparingly soluble in water in the pure state, although these monodesmosides are fairly soluble in water as a crude glycoside mixture. In studies to identify the solubilizing agents for these monodesmosides in the glycoside mixture of this crude drug, it was preliminarily reported that the water solubilities of these monodesmosides were greatly increased in the presence of a mixture of the co-occurring bisdesmosides, mukurozi-saponins Y_1 (**5**), Y_2 (**6**) and X(**7**) (Chart 1).[1] The co-occurring sesquiterpene oligoglycosides, mukuroziosides Ia, Ib, IIa and IIb (Chart 1),[2] also increase the water-solubilities of the monodesmosides. It is noteworthy that solutions of these monodesmosides solubilized with the aid of the bisdesmosides produce a remarkable enhancement of the absorption of



	R_1	R_2
1	-H	-H
2	-Ara(p)-(2—1)-Rha-(3—1)-Ara(p)	-H
3	-Ara(p)-(2—1)-Rha-(3—1)-Xyl	-H
4	-Ara(p)-(2—1)-Rha-(3—1)-Ara(f)	-H
5	-Ara(p)-(2—1)-Rha-(3—1)-Xyl	-Glc-(2—1)-Glc
6	-Ara(p)-(2—1)-Rha-(3—1)-Ara(p)	-Glc-(2—1)-Glc
7	-Ara(p)-(2—1)-Rha	-Glc-(2—1)-Glc



R

mukurozioside Ia: -Glc-(2—1)-Rha: mukurozioside Ib

IIa: -Glc-(2—1)-Rha: IIb

(3—1)-Rha

Ara(p): L-arabinopyranosyl
Ara(f): L-arabinofuranosyl
Glc: D-glucopyranosyl
Rha: L-rhamnopyranosyl
Xyl: D-xylopyranosyl

Chart 1

antibiotics from rat intestine and rectum.[3]

Other crude saponin mixtures such as saponins of *Camelia, Digitalis, Quillaia* and *Sapindus* have been used as surfactants, and investigation of the surface-active properties of saponins should also be of interest in relation to the biological activities of folk medicines. However, studies on surface-active properties have been limited to crude saponin mixtures, and no work has been done on purified saponins except for glycyrrhizin (**8**).[4] In connection with the possible biopharmaceutical utilization of saponins of Enmeihi, the present paper reports the solubilizing properties of the bisdesmosides, **5** and **6**.

Experimental

Materials——**2** was extracted from commercial Enmeihi as described in the previous paper.[1] Because of the low yields from this crude drug, **5** and **6** were prepared from the corresponding monodesmosides, **3** and **2**, respectively, as described below.

Yellow OB (**9**) purchased from Tokyo Kasei Kogyo Co., Ltd. was used after recrystallization from EtOH. Progesterone (**10**, special grade) was obtained from Sigma Chem. Co. Purified glycyrrhizin (**8**) was supplied by Tokiwa Phytochemical Co., Ltd.

Preparation of 5 and 6——**3** (1.5 g) was acetylated with Ac_2O (5 ml) and anhydrous C_5H_5N (10 ml) at room temperature overnight. The excess reagents were evaporated off *in vacuo* and the residue was purified by column chromatography on silica gel (solvent: C_6H_6–CH_3COCH_3 (5 : 1)), affording the peracetate of **3** (yield 89%). α-Acetobromosophorose was prepared from sophorose by the procedure reported previously.[1,5] The peracetate of **3** (1.8 g) and Ag_2CO_3 on Celite (1.8 g) were added to a solution of α-acetobromosophorose (1.4 g) in anhydrous CH_2ClCH_2Cl (40 ml).[6] The mixture was heated under reflux for 3 h and then passed through a column of Celite (eluted with $CHCl_3$). The eluate, after being concentrated to dryness, was deacetylated with 0.5 N BaO solution in anhydrous MeOH (50 ml) under stirring at room temperature overnight. The mixture was deionized with Amberlite MB-3 and concentrated to dryness. The residue was purified by liquid chromatography on a reversed-phase column (TSK-gel ODS-120A; solvent, 70% MeOH) and then on silica gel (Kieselgel 60H (Merck); solvent, AcOEt–MeOH–H_2O (8 : 2 : 1 and then 7 : 2 : 1)), affording **5** in a yield of 28% from **3**.

6 was prepared from **2** in the same way as above in a yield of 42%. The purity and identification of both the synthesized bisdesmosides were confirmed by carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrometry in C_5D_5N.[1]

Determination of Solubilizing Effects of 5 and 6 on 2——A solution of **2** in MeOH containing **5** or **6** was concentrated to complete dryness. The residue was taken up in 2 ml of H_2O and after sonication for several minutes, the mixture was shaken at 37 °C for 24 h. If necessary to remove undissolved saponin, the mixture was filtered through a 0.5 μm filter (Millipore Corporation). The content of **2** in the resulting solution was determined by dual-wavelength thin layer chromatography (TLC)-densitometry on a Shimadzu CS-910 (or CS-930) TLC scanner, as described in the previous paper.[1]

Measurement of Surface Tension——Surface tension curves of **5** and **6** were obtained with a Wilhelmy-type tensiometer (Shimadzu surface tensiometer, type ST-1).

Solubilization of 9 by 5, 6 and 8——A solution of a mixture of **9** (1 mg) with various amounts of **5**, **6** or **8** in MeOH was concentrated to complete dryness. The residue was taken up in 2.5 ml of 1/80 M phosphate buffer (pH 6.5, ionic strength 0.02, 2.5 ml). After sonication for several minutes, the mixture was shaken at 37 °C for 72 h and then filtered through Toyo GC-50 glass fiber filter paper. The filtrate was diluted with an equal volume of MeOH and subjected to colorimetry (Hitachi spectrometer type 124). A 50% aqueous methanolic phosphate buffer solution of **9** showed an absorption maximum at 460 nm. The calibration curve of **9** for optical density (OD) at 460 nm in 1/80 M phosphate buffer (pH 6.5)–MeOH (1 : 1) was found to be linear up to a concentration of 100 μM, passing through the origin.

Solubilization of 10 by 5 and 6——The solubilizing effect was determined in the same way as for **9**. After filtration, the resulting filtrate was diluted with an equal volume of EtOH and subjected to quantitative analysis by absorption measurement at 241 nm. Since **5** and **6** also show absorption near 241 nm (end-absorption due to the double bond), the concentration of **10** in the diluted filtrate was determined by subtracting OD_{241} of **5** or **6** in 50% ethanolic buffer solution (at the same concentration as that of the test solution) from OD_{241} of the resulting solution of **10**. The calibration curve of **10** determined by measuring OD_{241} in 50% aqueous ethanolic phosphate buffer showed good linearity up to a concentration of 60 μM, and passed through the origin.

Hemolysis——Hemolytic activities of solutions of **2** and solutions of **2** solubilized with a mixture of **5**, **6** and **7** (7 : 7 : 1)[1] in isotonic phosphate buffer (pH 7.4) against commercial sheep erythrocytes were determined in the same manner as reported previously.[7]

TABLE I. Solubilizing Effects of Mukurozi-saponin Y_1 (**5**) and Y_2 (**6**) on Monodesmoside **2** in Water at 37 °C

Concn. of bisdesmosides mg/ml (mM)		Amount of **2** added mg/ml	Concn. of **2** mg/ml (mM)
6	0.2 (0.17)	11	0.5[a] (0.57)
	0.6 (0.50)	17	2.5[a] (2.83)
	1.0 (0.83)	8	8 (9.07)
	1.0	15	15 (17.0)
	1.0	21	21 (23.8)
	1.0	23	17[a] (19.3)
	1.0	24	16[a] (18.1)
	1.0	30	15[a] (17.0)
	1.0	40	4[a] (4.54)
	1.0	59	2[a] (2.27)
	1.4 (1.16)	29	29 (32.9)
	1.8 (1.49)	35	35 (39.7)
5	0.2 (0.17)	12	0.3[a] (0.34)
	0.6 (0.50)	18	2.5[a] (2.83)
	1.0 (0.83)	24	11[a] (12.5)
	1.4 (1.16)	29	29 (32.9)
	1.8 (1.49)	35	35 (39.7)
5, **6** mix (1 : 1)	1.0	27	3.5[a] (3.97)

a) Saturated solution.

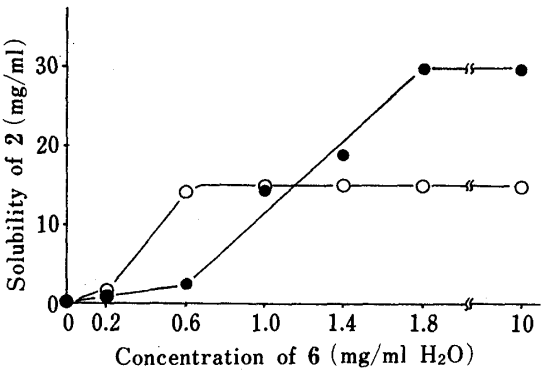


Fig. 1. Solubilizing Effect of Mukurozi-saponin Y_2 (**6**) on Monodesmoside **2**

—●—, 30 mg/ml of **2**; —○—, 15 mg/ml of **2**.

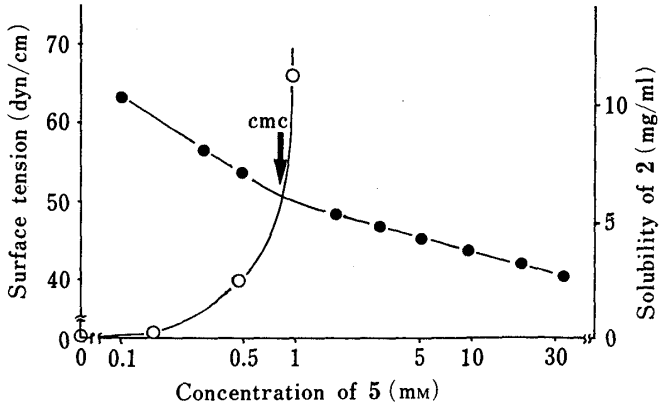


Fig. 2. Surface Tension of an Aqueous Solution of Mukurozi-saponin Y_1 (**5**) at 16 °C and Solubility Curve of Monodesmoside **2** in an Aqueous Solution of **5** at 37 °C

—○—, solubility curve; —●—, surface tension.

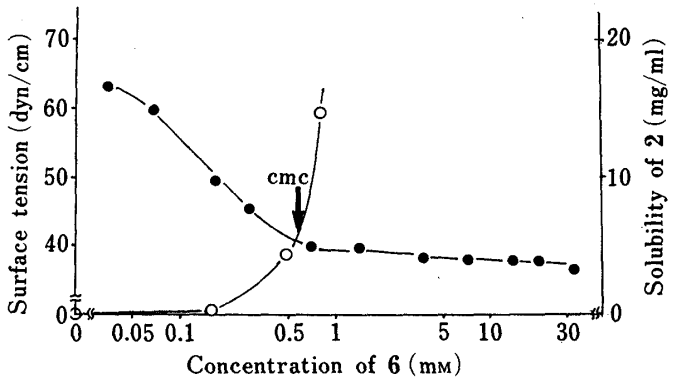


Fig. 3. Surface Tension of an Aqueous Solution of Mukurozi-saponin Y_2 (**6**) at 14.5 °C and Solubility Curve of Monodesmoside **2** in an Aqueous Solution of **6** at 37 °C

—○—, solubility curve; —●—, surface tension.

Results and Discussion

As already reported in the previous paper,[1] the saturated concentrations of purified monodesmosides in water at 37 °C are as follows: **2**, 0.017 mg/ml (0.019 mM); **3**, 0.039 mg/ml (0.044 mM); **4**, 0.0076 mg/ml (0.0086 mM). It was also reported that 1 ml of an aqueous solution containing 1 mg (0.1%) of a mixture of the bisdesmosides (**5**, **6** and **7**, 7:7:1) dissolved 5.7 mg of **2** (6.4 mM).[1] In the present study, the solubilizing effect of purified **5** and **6** on **2** as a representative of these monodesmosides was investigated.

No significant influence of the presence of the other monodesmosides on the water solubility of **2** was observed. The solubility of **2** in an aqueous solution of **5** or **6** depended strongly upon the amount of **2** used for the solubilizing test. As shown in Table I, when 21 mg of **2** was used for the test, a 0.1% (0.83 mM) aqueous solution of the corresponding bisdesmoside (**6**) completely dissolved the sample to give a 23.8 mM solution of **2**. In this case, the molar ratio of **6** to **2** is 1:29. Such a potent solubilizing effect has not been reported with usual synthetic surfactants. However, when more **2** was added, for instance 23, 30 or 40 mg, the concentration of **2** in a 0.1% solution of **6** decreased to 17, 15 and 4 mg/ml, respectively (Table I). This is presumably due to adsorption of the micelles of **6** with **2** on undissolved **2**.

As mentioned above, a 0.83 mM aqueous solution of **6** gave a 23.8 mM solution (21 mg/ml, 2.1%) of **2**. Figure 1 shows the ralationship between the concentration of **6** and the solubility of **2** when a definite amount of **2**, 15 or 30 mg, was added. To dissolve 15 mg of **2** completely in 1 ml of water (1.5%, 17.0 mM), at least 0.6 mg of **6** (0.06%, 0.49 mM) was required, while to obtain a 34.0 mM aqueous solution of **2** (30 mg/ml), the concentration of **6** should be more than 1.49 mM (0.18%). This suggests that the molar ratio of **2** to **6** in the soluble micelles decreases with increasing concentration of **2** and **6**.

As shown in Chart 1, **6** is the bisdesmoside having the same 3-*O*-sugar moiety as **2**, while **5**, an isomer of **6** differs from **6** only in the terminal monosaccharide unit of the 3-*O*-sugar moiety. The solubilizing effects of **5** and **6** on **2** at various concentrations of these bisdesmosides (Table I) indicated that the effect of **5** is similar to that of **6**. The solubilizing effect of the mixture of **5** and **6** (1:1) was evidently less than that of each bisdesmoside, and this finding requires further study.

The surface tension of an aqueous solution of **5** and **6** plotted against the concentration as well as the solubilizing effect on **2** is illustrated in Figs. 2 and 3 as a function of the concentrations of **6** and **5**, respectively. It was found that both **5** and **6** began to exhibit the solubilizing effect near the critical micelle concentration (cmc), like usual synthetic surfactants.

The solubilizing effect of these bisdesmosides on compounds other than the monodesmosides was also investigated. Yellow OB (**9**), which is completely insoluble in water, has been used as a standard compound for examining the solubilizing effects of surfactants. As shown in Fig. 4, **9** was solubilized in phosphate buffer solutions of both the bisdesmosides more effectively than in the solution of **8**. It was also found that the solbulity of progesterone (**10**) in phosphate buffer was evidently increased by both the bisdesmosides (Fig. 5).

It has been reported[7] that the water-solubility of saikosaponin-a, an active principle of Bupleuri Radix, was strongly increased by chickusetsusaponin V (=ginsenoside Ro), an oleanolic acid saponin (bisdesmoside) from Panacis japonici Rhizoma, Ginseng Radix and other *Panax* spp. It was also found that chikusetsusaponin V has no influence on the hemolytic activity of saikosaponin-a. In the present study, the hemolytic activity of **2** in phosphate buffer (pH 7.4) against sheep erythrocytes was compared with that of **2** solubilized with a mixture of bisdesmosides, **5**, **6** and **7** (7:7:1); these solutions exhibited no hemolytic activity. As shown in Fig. 6, these bisdesmosides had no significant influence on the activity of **2**.

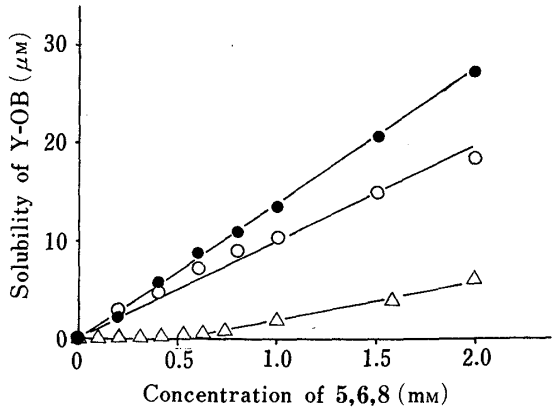


Fig. 4. Solubility of Yellow OB (**9**) in Phosphate Buffer Solutions of Mukurozi-saponins Y_1 (**5**), Y_2 (**6**) and Glycyrrhizin (**8**) at 37 °C
—○—, **5**; —●—, **6**; —△—, **8**.

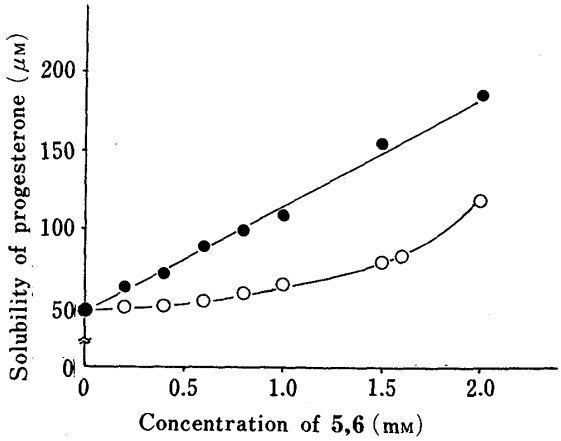


Fig. 5. Solubility of Progesterone (**10**) in Phosphate Buffer Solutions of Mukurozi-saponins Y_1 (**5**) and Y_2 (**6**) at 37 °C
—○—, **5**; —●—, **6**.

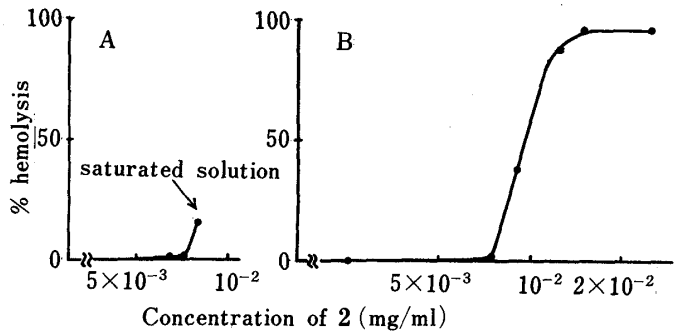


Fig. 6. Hemolysis by Monodesmoside **2**
Sheep erythrocytes were incubated in isotonic phosphate buffer (pH 7.4) at 37 °C for 30 min.
(A), **2**; (B), **2** solubilized with an equal amount of a mixture of **5**, **6** and **7** (7:7:1).

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