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Saponins of Pericarps of Chinese *Sapindus delavayi* (Pyi-shiau-tzu), a Source of Natural Surfactants

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From pericarps of *Sapindus delavayi* (Chinese name: Pyi-shiau-tzu, Japanese name: Hishoushi) collected in Yunnan, China, several monodesmosides, **2**—**4** and **8**—**10** were isolated, all of which have already been isolated from pericarps of *S. mukurossi* (Japanese name: Enmeihi). Besides these known saponins, saponins **11** (a new saponin named hishoushi-saponin A), **13** and **14** (a new saponin named hishoushi-saponin Ee) were isolated from Pyi-shiau-tzu and their structures were elucidated to be as follows: **11**, oleanolic acid 3-*O*-α-L-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside; **13**, oleanolic acid 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (identical with the prosapogenin of the saponins from *Clematis chinensis*); **14**, an acetate of sapindoside B (**3**) in which the acetyl group is located at the 3-hydroxyl group of the terminal xylosyl unit. It was found that the content of acylated saponins such as **14** in Pyi-shiau-tzu is higher than that in Enmeihi. Two diacetylated saponins, **15** and **16**, were isolated from Pyi-shiau-tzu not in an impure state and their structures were deduced by nuclear magnetic resonance spectrometry. It is noteworthy that in contrast to Enmeihi, no bisdesmoside was detected in Pyi-shiau-tzu.

Keywords——*Sapindus delavayi*; pericarps; Sapindaceae; saponin; hederagenin; oleanolic acid; monodesmoside; Pyi-shiau-tzu; ^{13}C-NMR acetylation shift; acetylated saponin

An Asian folk medicine "延命皮 (Japanese name: Enmeihi)," pericarps of *Sapindus mukurossi* GAERTN. (Sapindaceae, Japanese name of this plant: Mukurozi) has traditionally been used as an expectorant. However, because of the potent surfactant properties of its extract, it is currently utilized as an excellent source of natural surfactants rather than as a medicine. From this folk medicine, a number of saponins of hederagenin (**1**) have been isolated.[1] Of these saponins, the purified major monodesmosides, saponin A (**2**), sapindoside B (**3**) and saponin C (**4**) are sparingly soluble in water, though the water solubilities of these monodesmosides are greatly increased by the co-occurring bisdesmosides, mukurozi-saponins X (**5**), Y_1 (**6**) and Y_2 (**7**).[1] Acyclic sesquiterpene oligoglycosides, which also enhanced the water solubilities of these monodesmosides, were isolated from this folk medicine as well.[2] Further, it has been found that solutions of these monodesmosides solubilized with the aid of the bisdesmosides or the sesquiterpene oligoglycosides cause remarkable promotion of the absorption of antibiotics from rat intestine or rectum.[3]

S. delavayi (FRANCH.) RADLK., is a tall tree distributed in Yunnan and Sichuan, China and grows at higher altitude than *S. mukurossi*. Pericarps of this plant (皮哨子, Chinese name: Pyi-shiau-tzu, Japanese name: Hishoushi) are also used as a source of natural surfactants, being exported from China to Japan as a substitute for Enmeihi. As a continuation of our studies on oligoglycosides as natural surfactants, the isolation and identification of the saponins of Pyi-shiau-tzu have been conducted.

A methanolic extract of Pyi-shiau-tzu was subjected to chromatography on highly

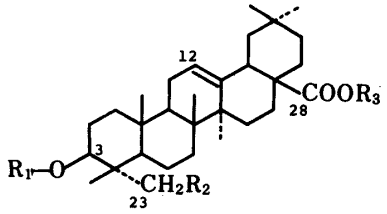
porous polymer and the resulting saponin fraction was separated by repeated chromatography to give nine saponins, I—IX, in yields of 2.3, 2.7, 0.4, 0.008, 0.03, 0.04, 0.7, 1.2 and 0.3%, respectively. Of these saponins, I, II, III, VI, VII and IX were identified as **2**, **3**, **4**, sapindoside A (**8**), mukurozi-saponins E_1 (**9**) and G (**10**), respectively, all of which have already been isolated from Enmeihi.[1]

A new saponin IV named hishoushi-saponin A (**11**) afforded oleanolic acid (**12**), arabinose and rhamnose on acid hydrolysis. In the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **11**, signals due to the aglycone moiety appeared at almost the same positions as those of 3-*O*-glycosylated oleanolic acid, such as the saponin from *Anemone rivularis* BUCH.-HAM.,[4] and the sugar carbon signals were found to be almost superimposable on those of **2** except for slight deshielding of the anomeric carbon of the inner arabinosyl unit (Table I). These observations led to the formulation of **11** as oleanolic acid 3-*O*-α-L-arabinopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

Saponin V (**13**) afforded **12**, arabinose, rhamnose and xylose on acid hydrolysis. The carbon signals due to the aglycone moiety of **13** were almost superimposable on those of **11** and the sugar carbon signals of **13** appeared at almost the same positions as those of **3** except for a slight downfield shift of the anomeric carbon signal due to the inner arabinosyl unit (Table I). Based on these results, structure of **13** was established as oleanolic acid 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside, which is identical with the prosapogenin of the saponins from *Clematis chinensis*.[5]

The thin layer chromatogram (TLC) of the methanolic extract (Fig. 1) of Pyi-shiau-tzu indicated the presence of a number of compounds showing higher *Rf* values than the monodesmosides (**2**—**4**, *etc.*), and the contents of these compounds relative to those of the monodesmosides are higher than in Enmeihi. Treatment of the methanolic extract with weak alkali resulted in the disappearance of these TLC spots and an increase of the content of the monodesmosides, **2**—**4**, so that most of these less polar substances seem to be acylated monodesmosides such as **9** and **10**. However, isolation of these acylated saponins in a completely homogeneous state was difficult, probably due to ready acyl migration or deacylation during the process of separation. Saponin VIII, a new acylated saponin named hishoushi-saponin Ee (**14**), was obtained from this fraction. A proton signal at δ 1.96 (3H, s) as well as carbon signals at δ 21.1 (CH_3) and 170.6 (C=O) indicated the presence of an acetoxyl group in **14**. Acid hydrolysis of **14** gave arabinose, rhamnose and xylose, and mild alkaline hydrolysis of **14** yielded **3**. The mass spectrum (MS) of its trimethylsilyl (TMSi) ether exhibited fragment ions at *m/z* 609 [(rhamnosyl-xylosyl)$(TMSi)_4$Ac] and 319 [xylosyl-$(TMSi)_2$Ac], indicating that the acetyl group is located on the terminal xylosyl unit. On going from **3** to **14**, the carbon signal due to C-3 of the terminal xylosyl unit of **3** was displaced downfield by 0.9 ppm and those due to C-2 and -4 were shifted upfield by 2.0 and 1.8 ppm, respectively, while other carbon signals remained almost unshifted (Table I). It follows that the acetyl group of **14** is located at the 3-hydroxyl group of the terminal xylosyl unit. The hemolytic activity of this acetylated saponin (**14**) was found to be stronger than that of its parent saponin (**3**) (Fig. 2).

From the above less polar fraction, two additional acylated saponins, **15** (yield: 0.08%) and **16** (0.008%), were isolated. Although these saponins could not be obtained in a completely pure state for elemental analysis, their structures were tentatively proposed mainly on the basis of NMR spectrometry. The presence of two acetoxyl groups in each compound was shown by proton nuclear magnetic resonance (^{1}H-NMR) signals [**15**, δ 1.93 and 1.96 (3H, s each); **16**, δ 1.92 and 1.97 (3H, s each)] as well as ^{13}C- NMR signals (Table I). On mild alkaline hydrolysis, **15** and **16** yielded **3** and **4**, respectively. On going from **3** to **15**, C-2, -3 and -5 of the terminal xylosyl unit of **3** were shielded by 2.2, 2.8 and 4.2 ppm, respectively, while other carbon signals remained at almost the same positions (Table I). This indicated that **15** is a



	R_1	R_2	R_3
1:	–H	–OH	–H
2:	–Ara(p)^{2}–Rha^{3}–Ara(p)	–OH	–H
3:	–Ara(p)^{2}–Rha^{3}–Xyl	–OH	–H
4:	–Ara(p)^{2}–Rha^{3}–Ara(f)	–OH	–H
5:	–Ara(p)^{2}–Rha	–OH	–Glc^{2}–Glc
6:	–Ara(p)^{2}–Rha^{3}–Xyl	–OH	–Glc^{2}–Glc
7:	–Ara(p)^{2}–Rha^{3}–Ara(p)	–OH	–Glc^{2}–Glc
8:	–Ara(p)^{2}–Rha	–OH	–H
9:	–Ara(p)^{2}–Rha^{3}–Xyl^{4}–Ac	–OH	–H
10:	–Ara(p)^{2}–Rha^{3}–Ara(p)^{3}–Ac, ^{4}–Ac	–OH	–H
11:	–Ara(p)^{2}–Rha^{3}–Ara(p)	–H	–H
12:	–H	–H	–H
13:	–Ara(p)^{2}–Rha^{3}–Xyl	–H	–H
14:	–Ara(p)^{2}–Rha^{3}–Xyl^{3}–Ac	–OH	–H
15:	–Ara(p)^{2}–Rha^{3}–Xyl^{3}–Ac, ^{4}–Ac	–OH	–H
16:	–Ara(p)^{2}–Rha^{3}–Ara(f)^{3}–Ac, ^{5}–Ac	–OH	–H

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

Chart 1

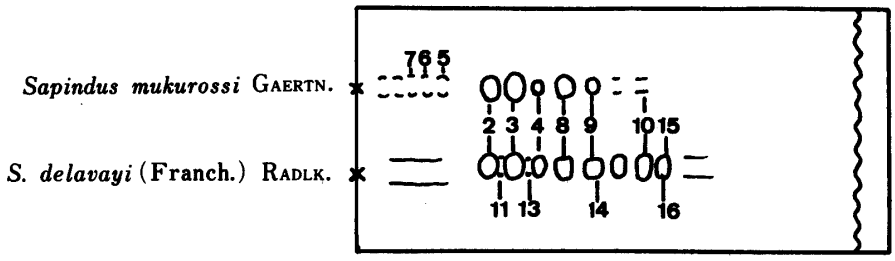


Fig. 1. TLC of MeOH Extracts of *Sapindus* spp.

Adsorbent, Silica gel $60F_{254}$ (Merck); solvent, EtOAc–EtOH–H_2O (8:2:1, homogeneous); detection, H_2SO_4.

diacetate of **3**, in which the acetyl groups are located at the 3- and 4-hydroxyl groups of the terminal xylosyl unit. On going from **4** to **16**, the carbon signals due to C-3 and -5 of the terminal arabinofuranosyl unit were displaced downfield by 1.9 ppm and those due to C-2 and -4 were shifted upfield by 1.3 and 6.0 ppm, respectively, while other carbon signals remained almost unshifted. Based on these findings, **16** was proposed to be a diacetate of **4**, in which the acetyl groups are located at the 3- and 5-hydroxy groups of the terminal arabinofuranosyl unit.

As already mentioned, the water solubilities of monodesmosides such as **2**—**5** from Enmeihi are greatly increased in the presence of the co-occurring bisdesmosides, **5**—**7**, as well as acyclic sesquiterpene oligoglycosides.[1,2] However, in contrast to Enmeihi, no bisdes-

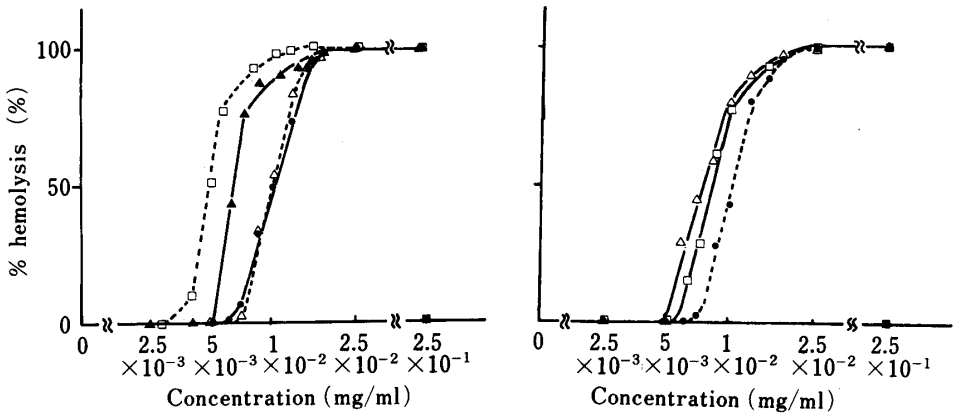


Fig. 2. Hemolysis by Monodesmosides

—●—, 2+6 (1 : 1, w/w); ---●---, 3+6 (1 : 1, w/w); ---△---, 4+6 (1 : 1, w/w); ---□---, 8+6 (5 : 8, w/w); —□—, 9+6 (1 : 1, w/w); —▲—, 10+6 (1 : 1, w/w); —△—, 14+6 (1 : 1, w/w); ■, 6.
Sheep erythrocytes. Isotonic phosphate buffer (pH 7.4, 37 °C). Incubation for 30 min.

TABLE I. ^{13}C-NMR Chemical Shifts of Sugar Moieties (in C_5D_5N)

	2	3	4	14	15	16	11	13
Ara(p)-1	104.4	104.3	104.4	104.5	104.7	104.6	105.5	105.0
(Inner) 2	75.2	75.4[a]	75.4	75.0	75.3	75.3	75.3	75.3
3	74.6	74.8	74.6	74.6	75.3	75.3	74.3	74.2
4	69.3	69.4	69.4	69.5	69.7	69.7	69.3[b]	69.1[b]
5	65.8	65.9	65.8	66.2	66.4	66.3	65.3	65.3
Rha-1	101.2	101.1	101.2	101.1	101.2	101.2	101.4	101.2
2	71.7	71.7	71.6	71.8	72.0	72.0	71.9	71.6
3	82.5	82.6	79.2	82.3	81.8	79.7	82.5	82.4
4	72.9	72.7	72.3	72.7	73.0	72.5	73.0	72.7
5	69.3	69.4	69.4	69.5	69.7	69.7	69.6[b]	69.5[b]
6	18.2	18.3	18.3	18.3	18.2	18.4	18.4	18.3
Ara(p)-1	107.0						107.0	
(Terminal) 2	72.9						73.0	
3	74.4						74.3	
4	69.3						69.3[b]	
5	66.9						66.7	
Xyl-1		107.1		106.9	106.6			106.9
2		75.2[a]		73.2	73.0			75.3
3		78.1		79.0	75.3			78.0
4		70.8		69.0	70.2			70.8
5		67.1		67.0	62.9			67.1
Ara(f)-1			110.8			111.2		
2			82.2			80.9[c]		
3			78.7			80.6[c]		
4			87.2			81.2[c]		
5			62.7			64.6		
$CH_3\underline{C}O$				170.6	170.3	170.7		
					170.3	170.7		
$\underline{C}H_3CO$				21.1	20.8	20.7		
					20.6	20.7		

a—*c*) Assignments in any column may be interchanged. Ara(p), α-L-arabinopyranosyl; Ara(f), α-L-arabinofuranosyl; Rha, α-L-rhamnopyranosyl; Xyl, β-D-xylopyranosyl.

mosides were detected in Pyi-shiau-tzu in the present study. Isolation of acyclic sesquiterpene oligoglycosides from Pyi-shiau-tzu is in progress.

Experimental

The NMR spectra were taken on JEOL JNM PFT-100 (^{1}H-NMR at 100 MHz and ^{13}C-NMR at 25.15 MHz) and JEOL FX-100 (^{1}H-NMR at 99.55 MHz and ^{13}C-NMR at 25.00 MHz) spectrometers in C_5D_5N, and chemical shifts are given on the δ(ppm) scale with tetramethylsilane as an internal standard.

MS were recorded on a JEOL 01-SG-2 mass spectrometer at 75 eV. Trimethylsilylation for MS: see previous paper.[1] Optical rotations were measured with a Union PM-101 automatic digital polarimeter in MeOH.

For Si gel column chromatography, Kieselgel 60 (Merck) and Kieselgel 60H (Merck) were used.

Extraction and Separation of Saponins——Pericarps of *Sapindus delavayi* (500 g) collected in Yunnan, China were extracted with hot MeOH. After removal of the solvent by evaporation, the MeOH ext. (297 g) was chromatographed on highly porous polymer, DIAION HP-20 (Mitsubishi Chem. Ind. Tokyo, Japan) (10, 50 and 75% aq. MeOH, MeOH successively and finally $CHCl_3$), affording the monodesmoside fraction (96.5 g) from the eluate with MeOH.

This monodesmoside fraction was separated by repeated column chromatography on Si gel (solvents: $CHCl_3$–MeOH–H_2O (90—30 : 10 : 1) EtOAc–MeOH–H_2O (16 : 2 : 1—15 : 2 : 1) or (65 : 10 : 1) or (200 : 10 : 1), all homogeneous), by reverse-phase column chromatography on Lichroprep RP-8 (Merck) (solvents: 70% or 80% aq. MeOH) and by high-performance liquid chromatography (HPLC) on TSK-Gel ODS-120A (solvents: 60% or 87% aq. MeOH), or LiChrosorb Si60 (solvents: EtOAc–MeOH–H_2O (65 : 10 : 1)), affording **2**, **3**, **4**, **8**, **9**, **10**, **11**, **13**, **14**, **15** and **16** in yields of 2.3, 2.7, 0.4, 0.04, 0.7, 0.3, 0.008, 0.03, 1.2, 0.08, and 0.008%, respectively.

Hishoushi-Saponin A (**11**): A white powder, $[\alpha]_D^{21}$ +3.1° (c=1.47, MeOH). *Anal.* Calcd for $C_{46}H_{74}O_{15}\cdot 2H_2O$: C, 61.17; H, 8.71. Found: C, 61.03; H, 8.60.

13: A white powder, $[\alpha]_D^{21}$ −6.7° (c=0.98, MeOH). *Anal.* Calcd for $C_{46}H_{74}O_{15}\cdot 3H_2O$: C, 60.00; H, 8.75. Found: C, 60.17; H, 8.68.

Hishoushi-Saponin Ee (**14**): A white powder, $[\alpha]_D^{26}$ −2.9° (c=1.01, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{17}\cdot H_2O$: C, 61.13; H, 8.34. Found: C, 61.33; H, 8.34.

Hydrolysis of Monodesmosides——A saponin (several mg) was heated with 3.5% HCl–dioxane (1 : 1, v/v) (1 ml) in a sealed tube in a boiling water bath for 3 h. The reaction mixture was diluted with H_2O and washed with $CHCl_3$. The aqueous layer was neutralized with Amberlite MB3 and concentrated, and the residue was subjected to TLC on silica gel with $CHCl_3$–MeOH–H_2O (6 : 4 : 1 homogeneous) using 2,3,5-triphenyltetrazolium chloride as a coloring reagent. For gas-liquid chromatography (GLC), the above residue was trimethylsilylated by the same procedure as used for MS. GLC was carried out on a Shimadzu GC-6A gas chromatograph (glass column, 2.6 mm × 2 m, 1.5% SE-30 on Chromosorb W; detector, FID; injection temp., 180 °C; column temp., 150 °C; carrier gas, N_2 at 40 ml/min). Methyl monosaccharides were identified by comparison of the retention times with those of authentic samples.

Deacetylation of 14—16——A solution of **14—16** in 2% KOH/MeOH was refluxed for 30 min. The reaction mixture was neutralized with Amberlite MB3 and concentrated to dryness, affording the corresponding parent saponin, **3** or **4**.

Hemolysis——Buffer and Preparation of Erythrocyte Solution: See previous paper.[6]

Solution of Saponins: Equivalent amounts of monodesmoside (**2—4**, **9**, **10** or **14**) and **6** were dissolved in MeOH. In the case of **8**, 5 mg of **8** and 8 mg of **6** were dissolved in MeOH. Then the solution was concentrated to dryness and the residue was dissolved in phosphate buffer.

Measurement of Hemolysis: See previous paper.[6]

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