Interaction of Saponin of Bupleuri Radix with Ginseng Saponin: Solubilization of Saikosaponin-a with Chikusetsu-saponin V (= Ginsenoside-Ro)

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The possible presence of a solubilizing substance for saikosaponin-a, which is sparingly soluble in water, was investigated in Ginseng which is sometimes co-prescribed in decoctions of oriental traditional medicines. It was found that the water solubility of saikosaponin-a was not influenced in the presence of dammarane saponins but was remarkably increased in the presence of chikusetsusaponin V (= ginsenoside-Ro), a bisdesmoside of oleanolic acid. The solubilizing activity of chikusetsusaponin V was estimated by determining the surface tension of aqueous solutions. No influence of chikusetsusaponin V on the hemolytic activity of saikosaponin-a was observed.

Keywords——Bupleuri radix; saikosaponin-a; Ginseng saponin; chikusetsusaponin V; ginsenoside-Ro; solubilizing effect; surfactant; surface tension; hemolysis

Bupleuri radix (roots of *Bupleurum falcatum* L. and related species, Umbelliferae, Japanese name: Saiko) is a very important crude drug in the prescriptions of traditional oriental medicine (Kampo). From this crude drug, saikosaponin-a (1), -c (2), -d (3) and many other minor saponins have been isolated.[1] Anti-inflammatory action,[2] corticosterone secretion-inducing activities,[3] plasma cholesterol-lowering action[4] and inhibitory action against hepatic injury by D-galactosamine[5] were reported for 1 and 3, whereas such biological activities were not observed for 2. However, these active saponins (monodesmosides), 1 and 3 are sparingly soluble in water, so that their pharmacological activities have inevitably been investigated as suspensions or as solutions solubilized with the aid of a synthetic surfactant.

Recently, the monodesmosides 4, 5 and 6, which are hardly soluble in water, were isolated from pericarps of *Sapindus mukurossi* GAERTN. It was found that the solubility of these monodesmosides in water was remarkably increased by the coexisting bisdesmosides, mukurozi-saponins X (7), Y_1 (8), Y_2 (9).[6] However, substances which increase the water solubility of monodesmosides such as 1 and 3 could not be found in Bupleuri radix. In oriental traditional medicine, Bupleuri radix is not used alone but is prescribed with several other crude drugs in Kampo-decoctions. As a part of our studies on the interactions among compounds in oriental medicine, we have therefore searched for compounds which increase the water solubility of 1 in the crude drugs co-prescribed with Bupleuri radix.

Ginseng (roots of *Panax ginseng* C. A. MEYER) is sometimes used together with Bupleuri radix in Kampo-decoctions such as "Shosaiko-to." A number of saponins have been isolated from Ginseng.[7] A preliminary test demonstrated that the crude mixture of Ginseng saponins evidently increased the solubility of 1 in water. This solubilizing effect was not observed with the fraction of saponins of 20 (*S*)-protopanaxatriol (10) (fr. I) such as ginsenoside-Rg_1 (11), but was observed with the more polar saponin fraction (fr. II), which

consisted of dammarane and oleanane saponins (sapogenins: 20 (*S*)-protopanaxadiol (**12**) and oleanolic acid (**13**)). Further investigation of the purified saponins in fr. II disclosed that no significant solubilizing effect was apparent with saponins of **12**, the major bisdesmoside, ginsenoside-Rb$_1$ (**14**) and the more polar minor bisdesmosides, ginsenosides-Ra$_1$ (**15**) and -Ra$_2$ (**16**),[8] while one of the minor Ginseng saponins, chikusetsusaponin V (**17**)[9] (=ginsenoside-Ro,[10] the bisdesmoside of **13**) in fr. II remarkably increased the solubility of **1** in water. It is noteworthy that 1 ml of an aqueous solution containing 1.4 mg of **17** dissolved 4.08 mg of **1** at 37 °C, whereas the saturated concentration of **1** in water was 0.14 mg/ml (Table I and Fig. 1). It is noteworthy that fr. II increased the water solubility of **1** at a similar rate to that observed with **17** in spite of the low content of **17** in the fraction. This suggested the presence of other effective substances or of a cooperative solubilizing effect of saponins, though the details are unclear. It is also noteworthy that **17** was isolated from rhizomes of *Panax japonicus* C. A. MEYER (Japanese name: Chikusetsu-ninjin) in a higher yield (5.4%)[9] than that from Ginseng, and some oriental medical doctors prefer to prescribe Chikusetsu-ninjin rather than Ginseng for the Kampo-decoction "Shosaiko-to." No solubilizing effect on **1** was observed with the bisdesmosides **7**, **8** and **9**, which remarkably increased the solubility of the coexisting monodesmosides, **4**, **5** and **6**, of *Sapindus mukurossi* (*vide supra*).

The surface-active properties of **17**, a natural anionic surfactant were investigated as follows. As shown in Fig. 2, the critical micelle concentration (cmc, about 0.1 mM, 0.1 mg/ml) of **17** was obtained by determining the surface tension of aqueous solutions; it was similar to that of sodium lauryl sulfate, a typical anionic surfactant. The evident increase of the solubility of **1** in the presence of **17** began to be observed near the cmc. It has been reported that the surface tension of solutions of surfactants is generally decreased by the presence of inorganic ions. The surface tension of a solution of **17** in saline was found to be lower than that in water, though no significant difference in cmc was noted between the two cases.

Figure 3 shows the influence of pH on the surface tension of solutions of **17** at the same ionic strength, indicating that the surface tension was decreased with increase of the hydrogen ion concentration. Figure 4 shows the surface tension curves in Tris–HCl buffer (pH 7.9) and
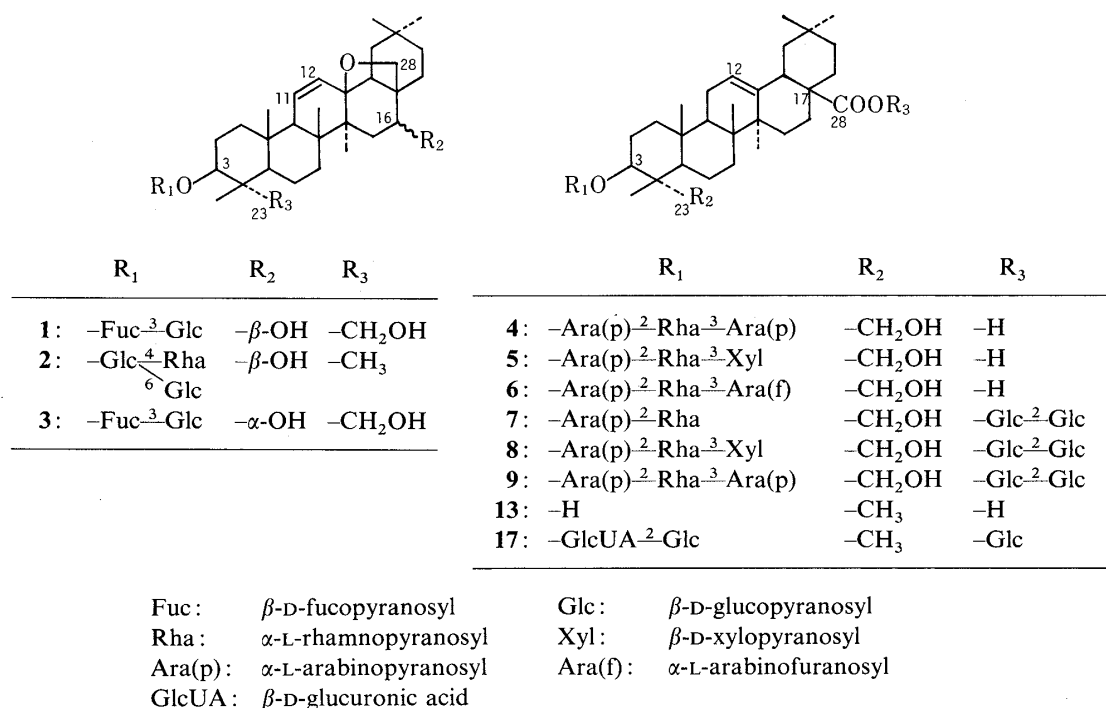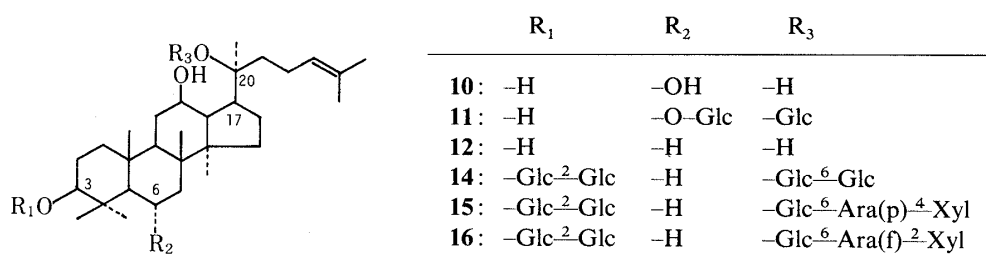

	R$_1$	R$_2$	R$_3$
1:	–Fuc—³—Glc	–β-OH	–CH$_2$OH
2:	–Glc—⁴—Rha ⁶Glc	–β-OH	–CH$_3$
3:	–Fuc—³—Glc	–α-OH	–CH$_2$OH

	R$_1$	R$_2$	R$_3$
4:	–Ara(p)—²—Rha—³—Ara(p)	–CH$_2$OH	–H
5:	–Ara(p)—²—Rha—³—Xyl	–CH$_2$OH	–H
6:	–Ara(p)—²—Rha—³—Ara(f)	–CH$_2$OH	–H
7:	–Ara(p)—²—Rha	–CH$_2$OH	–Glc—²—Glc
8:	–Ara(p)—²—Rha—³—Xyl	–CH$_2$OH	–Glc—²—Glc
9:	–Ara(p)—²—Rha—³—Ara(p)	–CH$_2$OH	–Glc—²—Glc
13:	–H	–CH$_3$	–H
17:	–GlcUA—²—Glc	–CH$_3$	–Glc

Fuc:	β-D-fucopyranosyl	Glc:	β-D-glucopyranosyl
Rha:	α-L-rhamnopyranosyl	Xyl:	β-D-xylopyranosyl
Ara(p):	α-L-arabinopyranosyl	Ara(f):	α-L-arabinofuranosyl
GlcUA:	β-D-glucuronic acid		

Chart 1

Chart 2

	R$_1$	R$_2$	R$_3$
10:	–H	–OH	–H
11:	–H	–O–Glc	–Glc
12:	–H	–H	–H
14:	–Glc^2–Glc	–H	–Glc^6–Glc
15:	–Glc^2–Glc	–H	–Glc^6–Ara(p)^4–Xyl
16:	–Glc^2–Glc	–H	–Glc^6–Ara(f)^2–Xyl

TABLE I. Solubilizing Effect of Ginseng Saponins on Saikosaponin-a (1)

Concn. of bisdesmoside (mg/ml)		Concn. of **1** (mg/ml)
—		0.14
Fr. I	1.2	0.12
Fr. II	1.0	4.05
Fr. II	1.4	4.06
14	1.0	0.12
15	1.4	0.14
16	1.3	0.14
17	1.0	3.38
17	1.4	4.08

37 °C, 24 h, in H$_2$O.
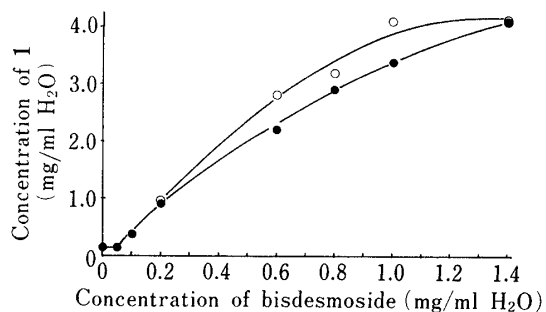
Fig. 1. Solubilizing Effects of Fr. II and Chikusetsusaponin V (**17**) on Saikosaponin-a (**1**) at 37 °C

Solubility of **1**, 0.14 mg/ml H$_2$O; —○—, fr. II; —●—, **17**.
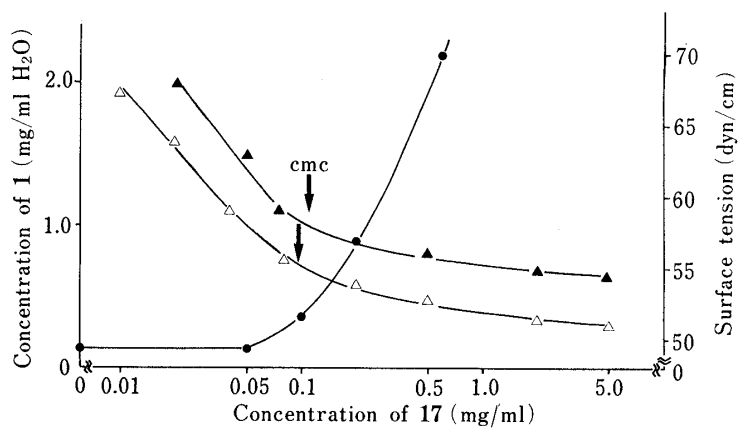
Fig. 2. Solubility Curve of Saikosaponin-a (**1**) in Solution of Chikusetsusaponin V (**17**) and Surface Tension of Solutions of **17** at 25 °C

—●—, solubility curve; —▲—, surface tension (in H$_2$O); —△—, surface tension (in saline).
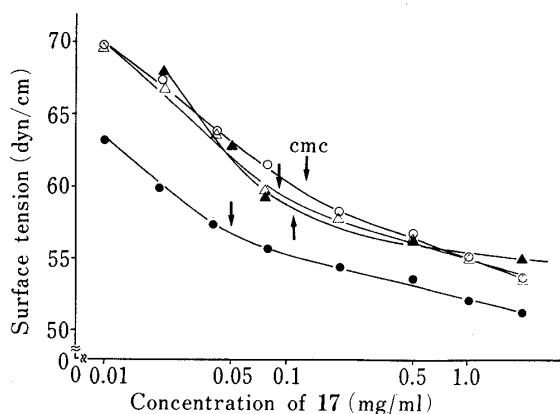
Fig. 3. Surface Tension of Solutions of Chikusetsusaponin V (17) at 25 °C

—▲—, H_2O; —●—, pH 4.2; —△—, pH 5.1; —○—, pH 5.5.
Ionic strength, 0.02; buffer, CH_3COOH–CH_3COOK–KCl.
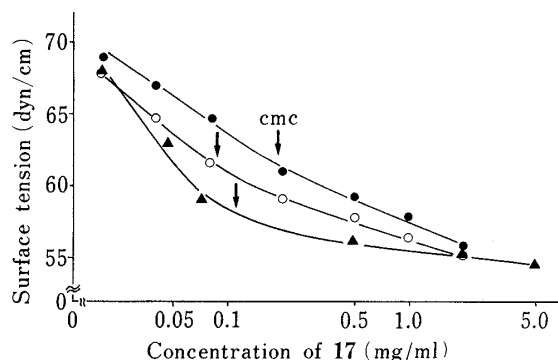
Fig. 4. Surface Tension of Solutions of Chikusetsusaponin V (17) at 25 °C

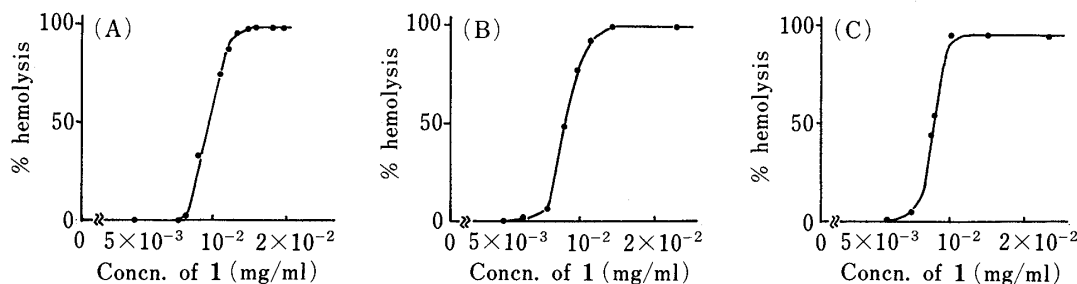—▲—, H_2O; —○—, pH 6.5 phosphate buffer (NaH_2PO_4–Na_2HPO_4); —●—, pH 7.9 Tris–HCl buffer.

Fig. 5. Hemolysis by Saikosaponin-a (1)

Sheep erythrocytes, isotonic phosphate buffer (pH 7.4, 37 °C), 37 °C, 30 min incubation.
(A), 1; (B), 1+fr. II (1 : 1 w/w); (C), 1+chikusetsusaponin V (17) (1 : 1 w/w).

phosphate buffer (pH 6.5), which are generally used for physiological experiments. In these cases, especially in Tris–HCl buffer, 17 did not show a distinct cmc. This is presumably due to the decrease of stability of micelles resulting from the dissociation of the carboxyl group and/or interaction between the dissociated carboxyl group and a basic component of the buffer.

Strong hemolytic activity of 1 has been reported,[11] while 17 showed no hemolysis even at a high concentration of 5.1 mg/ml. As shown in Fig. 5, 17 as well as fr. II had no influence on the hemolytic activity of 1. Other pharmacological studies of 1 solubilized with 17 are in progress.

Experimental

Saponins——1 and 3 were isolated from Bupleurum falcatum L.,[12] while 17 was isolated from Panax japonicus C. A. Meyer[13] and 14, 15 and 16 were isolated from Panax ginseng C. A. Meyer[8] according to the reported procedures.

Separation of Fr. I and II——Roots of white ginseng (8 kg) were extracted with hot water (70 °C, 120 l, 1 h) twice. After removal of the water by evaporation, a suspension of the residue (1.9 kg) in water was extracted with 1-BuOH saturated with water. The BuOH layer was concentrated to dryness in vacuo, affording a crude saponin mixture (110 g). A solution of this mixture in H_2O was dialyzed through cellophane film against H_2O for two weeks. The dialyzed fraction was concentrated to dryness and the residue was chromatographed on highly porous polymer (DIAION, HP-20, Mitsubishi Chemical Ind., Co., Ltd.) (solvent: 10% aqueous MeOH and then MeOH). Concentration of the eluate with MeOH to gave fr. I (yield 0.24%). The non-dialyzed fraction was concentrated to dryness and the residue was chromatographed on DIAION HP-20 (solvent: 15 and 85% aqueous MeOH and finaly

MeOH). The eluate with 85% MeOH afforded fr. II (yield 0.37%).

Determination of Solubilizing Effects——A saturated aqueous solution of **1** was prepared by incubation of an excess of **1** in water at 37 °C for 24 h followed by filtration through a 0.5 μm filter (Millipore Corporation). A saturated solution of **1** in an aqueous solution of bisdesmoside or various fractions was prepared as follows. A solution of an excess of **1** in MeOH containing bisdesmoside was concentrated to complete dryness and the residue was incubated in water (5 ml) at 37 °C for 24 h. Each saturated solution was filtered as described above. The content of **1** in each saturated solution was determined by thin-layer chromatography (TLC)-densitometry according to the methods of described in a previous paper.[14]

Hemolysis——Buffer: 3.26 g of KH_2PO_4 and 18.97 g of $Na_2HPO_4 \cdot 2H_2O$ per 1000 ml of distilled water, pH 7.4 (37 °C, isotonic).

Preparation of Erythrocytes Solution: Commercial sheep erythrocytes were used within two weeks after collection. The blood was centrifuged at 2500 rpm for 15 min to discard plasma and buffy coat. The precipitated cells were then washed twice with saline and further washed once with phosphate buffer. The erythrocytes were then diluted with phosphate buffer to give a 10% suspension, which was used for experiments within 24 h.

Solution of Saponins: Equivalent amounts of **1** and **17** were dissolved in MeOH. Then this solution was concentrated to dryness and the residue was dissolved in phosphate buffer. In the same way, **1** was dissolved in a solution of fr. II.

Measurement of Hemolysis: Equivalent volumes of the 10% erythrocytes solution and a saponin solution were mixed and incubated for 30 min at 37 °C. The mixture was centrifuged at 2500 rpm for 10 min and the absorbance of the supernatant was measured at 410 nm after appropriate dilution. Complete hemolysis was obtained by diluting the solution with enough H_2O. Hemolysis of the sample solution was evaluated in terms of the percentage value based on complete hemolysis as 100% and 0% hemolysis as that caused by phosphate buffer instead of saponin solution.

Measurement of Surface Tension——Surface tension was determined with a Wilhelmy-type tensiometer (Shimadzu surface tensiometer type ST-1) at 25 °C.

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