Solubilizing Properties of Ginseng Saponins

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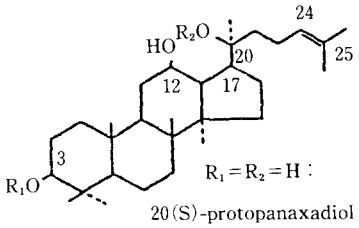
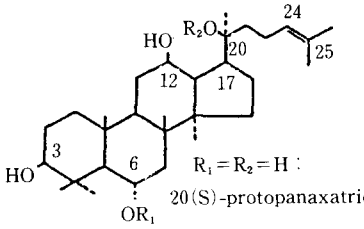
Acidic Saponins of Ginseng

A number of neutral dammarane saponins have been isolated from Ginseng roots(Fig.1) [1,2,3]. Besides these saponins, an acidic saponin of oleanolic acid named ginsenoside -Ro which has a glucuronide unit in the sugar moiety, was also isolated from Ginseng(Fig.2) [4]. This saponin is identical with chikusetsusaponin V which was first obtained from rhizomes of *Panax japonicus* by Shoji *et al*[5].

Recently, Kitagawa *et al*[6]. reported the isolation of acidic malonates of dammarane saponins of 20(S)-protopanaxadiol, malonyl-ginsenosides-Rb_1, -Rb_2, -Rc and -Rd in relatively high yields from Ginseng(Fig.1). These malonyl-ginsenosides are readily demalonylated to give the parent neutral ginsenosides during the steaming process,



20(S)-protopanaxadiol

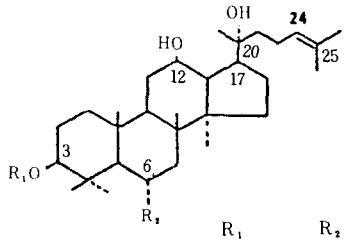


20(S)-protopanaxatriol

	R_1	R_2
ginsenoside-Ra_1 :	-glc(2-1) glc	-glc(6-1) arap(4-1) xyl
ginsenoside-Ra_2 :	-glc(2-1) glc	-glc(6-1) araf(2-1) xyl
ginsenoside-Ra_3 :	-glc(2-1) glc	-glc(6-1) glc(3-1) xyl
ginsenoside-Rb_1 :	-glc(2-1) glc	-glc(6-1) glc
ginsenoside-Rb_2 :	-glc(2-1) glc	-glc(6-1) arap
ginsenoside-Rb_3 :	-glc(2-1) glc	-glc(6-1) xyl
ginsenoside-Rc :	-glc(2-1) glc	-glc(6-1) araf
ginsenoside-Rd :	-glc(2-1) glc	-glc
* 20(S)- ginsenoside-Rg_3 :	-glc(2-1) gic	-H
*ginsenoside-Rh_2 :	-glc	-H
quinquenoside-R1 :	-glc(2-1) glc(6)jAc	-glc(6-1) glc
*ginsenoside-Rs_1 :	-gic(2-1) glc(6) Ac	-glc(6-1) arap
*ginsenoside-Rs_2 :	-glc(2-1) glc(6) Ac	-glc(6-1) araf
*malonyl-ginsenoside-Rb_1 :	-glc(2-1) glc(6) Ma	-glc(6-1) glc
*malonyl-ginsenoside-Rb_2 :	-glc(2-1) glc(6)Ma	-glc(6-1) arap
*malonyl-ginsenoside-Rc :	-glc(2-1) glc(6) Ma	-glc(6-1) araf
*malonyl-ginsenoside-Rd :	-gic(2-1) glc(6) Ma	-glc
notoginsenoside-R4 :	-glc(2-1) glc	-glc(6-1) glc(6-1) xyl

glc : β-D-glucopyranosyl
xyl : β-D-xylopyranosyl
arap : α-L-arabinopyranosyl
araf : α-L-arabinofuranosyl
rha : α-L-rhamnopyranosyl
Ac : acetyl
Ma : malonyl

	R_1	R_2
ginsenoside-Re :	-glc(2-1) rha	-glc
ginsenoside-Rf :	-glc(2-1) glc	-H
ginsenoside-Rg_1 :	-gic	-glc
ginsenoside-Rg_2 :	-glc(2-1) rha	-H
ginsenoside-Rh_1 :	-glc	-H
20-gluco-ginsenoside-Rf :	-glc(2-1) glc	-glc
notoginsenoside-R1 :	-glc(2-1) xyl	-glc



	R_1	R_2
*20(R)-ginsenoside-Rg_2 :	-H-O-glc(2-1)	rha
*ginsenoside-Rg_3 :	-glc(2-1) glc	-H
*20(R)-ginsenoside-Rh_1 :	-H-O-glc	

Fig.1. *Dammarane Saponins of Ginseng.*
**only from Red Ginseng*
***only from White Ginseng*

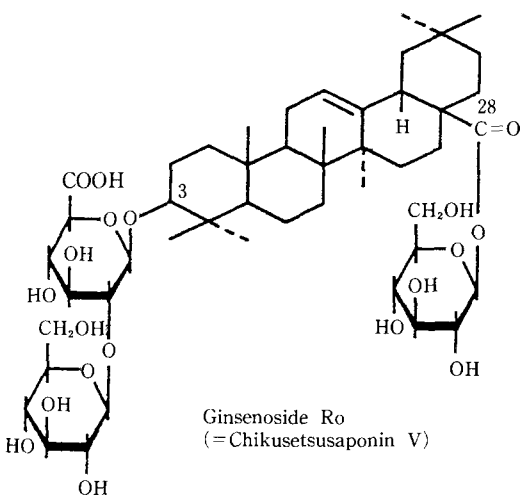


Fig. 2 *Oleanolic acid glucronide saponin of Ginseng.*

being not isolated from Red Ginseng.

A number of analytical and pharmacological studies on the neutral dammarane saponins of Ginseng, have been reported. However, analytical investigation of the acidic Ginseng saponins has not appeared in the literature. In the present symposium, HPLC analysis of these acidic saponins and solubilizing property of ginsenoside-Ro are reported.

Analysis of Acidic Saponins of Ginseng by High Performance Liquid Chromatography(HPLC)

HPLC analysis of neutral Ginseng saponins on an ODS column in the reverse phase mode as well as on a NH_2-column and a silica gel column in the normal phase mode, have been studied extensively. Recently, we have found that borate ion-exchange mode HPLC[7] and the normal phase mode HPLC on a column of newly developed hard spherical hydroxyaptite [8], are also useful for the separation of neutral Ginseng saponins.

Analysis of the acidic saponins of Ginseng did not afford satisfactory result by HPLC on an ODS column under the condition for the neutral dammarane saponins. Recently, it was found that sufficient separation of both acidic and neutral ginsenosides was obtained by the reverse phase mode HPLC on an ODS column using aqueous CH_3CN containing KH_2PO_4 as a mobile phase.

It was also disclosed that the excellent separation of acidic and neutral ginsenosides by the normal phase mode HPLC on a NH_2

Table 1. *Contents(%) of ginsenosides in ginseng*

saponin	wild Ginseng			cultivated Ginseng		
	rhizomes	main roots	lateral roots	rhizomes	main roots	lateral roots
Rg1	0.45	0.52	0.38	0.38	0.45	0.25
Re	0.47	0.19	0.87	0.57	0.35	1.40
Rf	0.15	0.08	0.17	0.15	0.11	0.17
Rd	0.07	0.04	0.49	0.16	0.08	0.52
Rc	0.47	0.32	1.50	0.47	0.31	1.50
M-Rc	0.34	0.15	0.64	0.35	0.23	0.84
Rb2	0.45	0.33	1.70	0.57	0.37	1.80
M-Rb2	0.40	0.20	0.83	0.42	0.30	1.10
Rb1	1.40	1.20	2.90	0.88	0.55	2.00
M-Rb1	1.30	0.63	1.30	0.69	0.41	1.20
Ro	3.40	1.10	0.68	1.80	0.50	0.62
total	8.90	4.76	11.46	6.44	3.66	11.40

(M- : malonyl-ginsenoside)

-column, was furnished also by using a mobile phase containing H_3PO_4.

As already mentioned, malonyl-ginsenosides are rather unstable and afforded the corresponding neutral ginsenosides on heating in a solution. For example, heating solutions of malonyl ginsenosides-Rb_1, -Rb_2 and -Rc in 70% methanol at 80℃ for 7hr, resulted in about 60% demaloylation to give ginsenosides-Rb_1, -Rb_2 and -Rc, respectively. Accordingly, for the analysis of these malonyl-ginsenosides, the extraction of the material must be carried out at room temperature. The extract was carefully concentrated to dryness under reduced pressure. The residue was subjected to prechromatography on a column of highly porous polymer, MCI-gel CHP-20P, Mitsubishi Kasei Co., Ltd. and then analyzed by HPLC.

Saponin Contents of Wild Ginseng

The extensive studies on quantitative analysis of neutral ginsenosides in cultivated Ginseng have been reported. However, the analysis of saponins in the wild Ginseng has not been published. By means of HPLC in a variety of modes coupled with the two dimentional thin layer chromatography, identification of each saponin of the wild Ginseng collected in the North East province of China was substantiated. Then, quantitative analysis of neutral and acidic saponins was conducted by HPLC mentioned above.

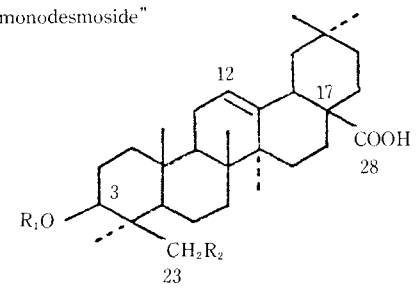
Comparison of the major saponin contents of the wild Ginseng with those of cultivated Ginseng are summarized in Table I. No significant difference in saponin composition was observed between wild and cultivated Ginseng. As already pointed out for cultivated Ginseng, contents of saponins in lateral roots and rhizomes of the wild Ginseng are also higher than those of main roots. It is noted that the content of malonyl-ginsenoside-Rb_1 was found to be higher than those of other dammarane saponins in every parts. It is also noteworthy that content of ginsenoside-Ro, the saponin of oleanolic acid, is unexpectedly high in the rhizomes, especially in the case of the wild Ginseng(3.4 %). This is important in the chemotaxonomical studies of Panax species by the saponin composition.

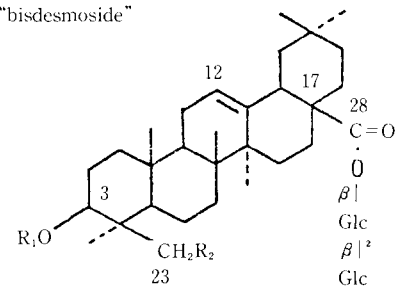
Malonyl-ginsenosides were detected also in American Ginseng and Sanchi-Ginseng by the HPLC.

Solubilizing Properties of Ginseng Saponins

The surface active properties of purified saponins have been investigated. A number of saponins of hederagenin were isolated from pericarps of *Sapindus mukurossi*(廷命皮)[9] and *S. delavayi*(皮哨子)[10]. These saponins can be classified into two groups; monodesmosides and corresponding neutral bisdesmosides; the formers have a sugar moiety at the 3-hydroxyl group and the 28-carboxyl group is unsubstituted, while the laters have two sugar moieties at both 3-hydroxyl and 28 -carboxyl groups(Fig.3). The monodesmosides, such as saponin A, are sparingly soluble in water in the pure state, though these monodesmosides are fairly soluble in water as a crude glycoside mixture. In the studies on the solubilizing agents for these monodesmosides in the glycoside mixture of this crude drug, it was found that the water solubilities of these monodesmosides were greatly increased in the presence of the co-occurring neutral bisdesmosides, mukurozisaponins Y1, Y2 and X[9, 11]. The co-occurring sesquiterpene oligolycosides named mukuroziosides Ia, Ib, IIa and IIb, also increased the water solubilities of the monodesmosides(Fig.4)[12]. It was also revealed that solutions of these monodesmosides solubilized with the aid of the co-occurring bisdesmosides, produced a remarkable enhancement of the absorption of antibiotics such as sodium ampicillin from rat intestine and rectum[13, 14]. This observation is noteworthy in view of biopharmaceutical significance of glycosides in the oriental traditional medicine.

Very recently, from Chinese traditional medicine, tubers of *Bolbostemma paniculatum*, Cucurbitaceae(土貝母), three neutral bisdes-

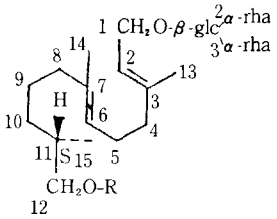


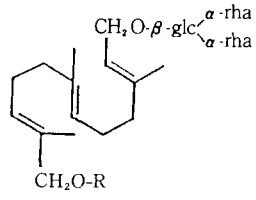


saponin A	: $\frac{}{\alpha}$Ara(p) $\frac{2}{\alpha}$Rha $\frac{3}{\alpha}$Ara(p)	−OH : mukurozi-saponin Y_2**
saponin B	: $\frac{}{\alpha}$Ara(p) $\frac{2}{\alpha}$Rha $\frac{3}{\beta}$Xyl	−OH : mukurozi-saponin Y_1**
saponin C	: $\frac{}{\alpha}$Ara(p) $\frac{2}{\alpha}$Rha $\frac{3}{\alpha}$Ara(f)	−OH
hishoushi-saponin A*	: $\frac{}{\alpha}$Ara(p) $\frac{2}{\alpha}$Rha $\frac{3}{\alpha}$Ara(p)	−H
hishoushi-saponin B*	: $\frac{}{\alpha}$Ara(p) $\frac{2}{\alpha}$Rha $\frac{3}{\beta}$Xyl	−H
saponin D_2	: $\frac{}{\alpha}$Ara(p) $\frac{2}{\alpha}$Rha	−OH : mukurozi-saponin X**
mukurozi-saponin E_1	: $\frac{}{\alpha}$Ara(p) $\frac{2}{\alpha}$Rha $\frac{3}{\beta}$Xyl $\frac{4}{}$Ac	−OH
hishoushi-saponin Ee*	: $\frac{}{\alpha}$Ara(p) $\frac{2}{\alpha}$Rha $\frac{3}{\beta}$Xyl $\frac{3}{}$Ac	−OH
mukurozi-saponin G	: $\frac{}{\alpha}$Ara(p) $\frac{2}{\alpha}$Rha $\frac{3}{\alpha}$Ara(p) $\frac{3}{4}$Ac, Ac	−OH

Fig.3. *Saponins from pericarps of Sapindus mukurossi Gaertn.(Japan)*[延命皮] *and S. delavayi(Franch.) Radlk.(Yunnan)*[皮哨子].
*only from *S. delavayi* (Franch.) Radlk. **only from *S. mukurossi* Gaertn.

mosides named tubeimosides I, II and III were isolated (Fig.5)[15, 16]. These bisdesmosides have a novel cyclic structure having 3-hydroxy -3-methyl-glutarate bridge, being named cyclic bisdesmosides. The potent solubilizing effect on the monodesmosides and on Yellow OB, a water insoluble synthetic dye, were observed with these cyclic bisdesmosides[15].





mukurozioside Ia -β-glc $\frac{2}{}$α-rha mukurozioside Ib -β-glc $\frac{2}{}$α-rha

mukurozioside IIa -β-glc $\frac{2\alpha\text{-rha}}{3\alpha\text{-rha}}$ mukurozioside IIb -β-glc $\frac{2\alpha\text{-rha}}{3\alpha\text{-rha}}$

Fig.4. *Sesquiterpene Glycosides of Sapindus mukurosi*(延命皮).

Bupleuri radix(柴胡) is a very important drug in the oriental traditional medicine. From this drug, several monodesmosidic saponins named saikosaponins, were isolated and of these saponins, a variety of pharmacological activities were observed for saikosaponins-a and -d(Fig.6). However, these active monodesmosides are sparingly soluble in water, so that their pharmacological activities have inevitably been tested as suspensions or as solutions solubilized with the aid of a synthetic surfactant. In contrast to the case of Pericarps of *Sapindus mukurossi*, no substance which increase the water solubility of saikosaponins, was isolated from *Bupleuri radix*.

In oriental traditional medicine, *Bupleuri radix* is generally not used alone but is decocted with several other crude drugs as the herbal formulas. Ginseng is sometimes co-prescribed with *Bupleuri radix* in herbal formulas

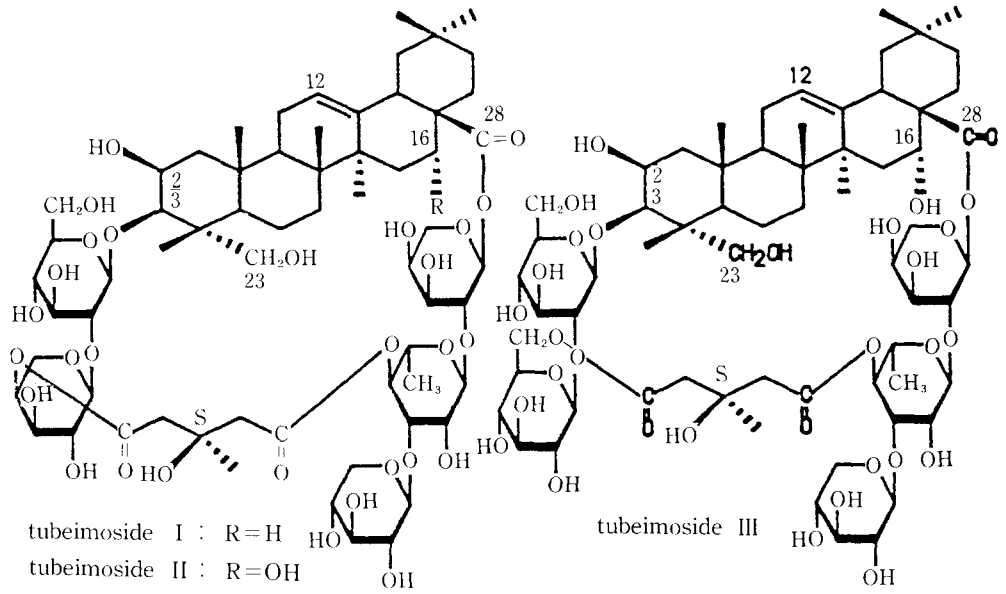
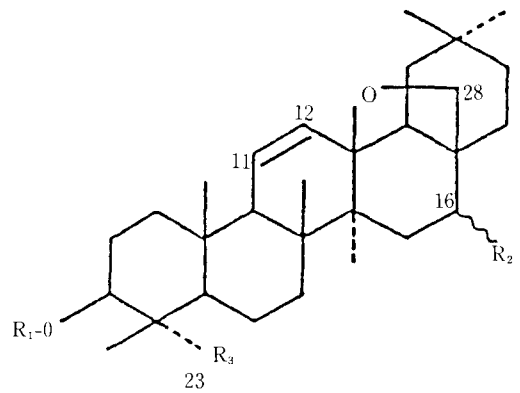


Fig.5. *Cyclic Bisdesmosides of Bolbostemma paniculatum* (土貝母).

such as "Minor Bupleurum Combination "(小柴胡湯). In Ginseng extract, we searched the substance which increases the water solubility of saikosaponin-a.

A suspension of water extract of Ginseng was extracted with 1-butanol saturated with water and the butanolic layer was dialyzed against water. It is noteworthy that the saponins of 20(S)-protopanaxatriol such as ginsenoside-Rg_1 are mostly dialyzed, while saponins of 20(S)-protopanaxadiol are hardly dialyzed, remaining in the non-dialyzed portion. This would be due to the formation of large size micelle. The solubilizing effect was observed not with the dialyzed portion but with the non-dialyzed portion. This non-dialyzed portion was further separated by chromatography to give a non-effective fraction, Fr.I and an effective fraction, Fr.II.



	R_1	R_2	R_3
saikosaponin-a :	$-Fuc^{3}_{-}Glc$	$-\beta-OH$	$-CH_2OH$
saikosaponin-c :	$-Glc^{4}_{-}Rha$ (6→Glc)	$-\beta-OH$	$-CH_3$
saikosaponin-d :	$-Fuc^{3}_{-}Glc$	$-\alpha-OH$	$-CH_2OH$

Fig.6. *Saponins(monodesmosides) of Bupleuri Radix* (柴胡).

Concentration of saikosaponin-a in a solution was determined by the mild acid treatment and subsequent analysis of the resulting diene-saponin, saikosaponin-b_1, by dual wavelength TLC-densitometry at 256nm[17, 18]. The saturated concentration of saikosaponin-a in water at 37℃ is 0.10mg/m*l*, while in 0.1% solution of Fr.II, saikosaponin-a was dissolved at concentration of 4.9mg/m*l*. It was demonstrated by TLC that Fr.II contains saponins of 20(S)-protopanaxadiol, ginsenosides-Rb_1, -Rb_2, -Rc and -Rd together with the acidic saponin, ginsenoside-Ro, a

small amount of ginsenoside-Re and some minor saponins.

It was disclosed that among these saponins in Fr.II, no significant solubilizing effect was apparent with all of the dammarane saponins, while the glucuronide saponin, ginsenoside-Ro remarkably increased the solubility of saikosaponin-a in water. It was found that 1m*l* of 0.1 % solution of ginsenoside-Ro dissolved 6.2mg of saikosaponin-a at 37 ℃[19].

However, because of low content of ginsenoside-Ro in Fr.II, the solubilization of saikosaponin-a by Fr.II could not be explained only by the function of the effect of ginsenoside-Ro. The quantitative analysis of each saponin in Fr.II was conducted by HPLC mentioned above. The contents are as follows; ginsenosides-Rb_1: 24.2%, -Rb_2: 13.9%, -Rc: 14.4 %, -Rd: 6.5%, -Re: 2.9% and -Ro: 4.5%. This indicated that as for the concentration of ginsenoside-Ro, 0.1% solution of Fr.II corresponds to 0.0045% solution of ginsenoside-Ro. As already mentioned, 1m*l* of 0.1% solution of Fr.II dissolved 4.9mg of saikosaponin-a. However, no solubilizing effect was observed with 0.0045% solution of ginsenoside-Ro. This suggested the presence of other effective substances or the cooperative solubilizing effect of other saponins.

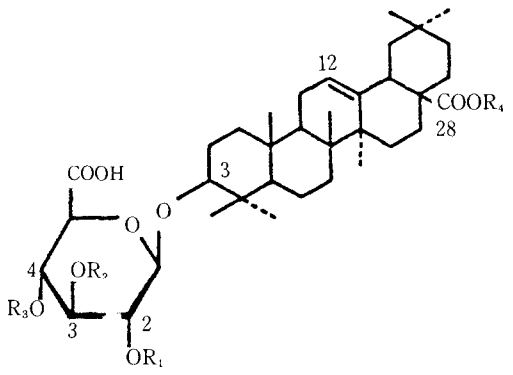
Based on the above analytical results of saponin contents in Fr.II, a mixture of ginsenosides-Rb_1, -Rb_2, -Rc, -Rd, -Re and -Ro in a ratio of 24.2:13.9:14.4:6.5:2.9:4.5 was prepared, being designated as G-mix, 0.664 mg of which is equivalent to 1mg of Fr.II with respect to the saponin composition. It was found that 0.0664% solution of G-mix exhibited almost the same solubilizing effect as that of Fr.II. It was also found that G-mix without ginsenoside-Ro which is a mixture of dammarane saponins, did not increase the water solubility of saikosaponin-a and this cooperative effect of the dammarane sapoin mixture can be substituted by each saponin of 20(S) -protopanaxadiol, ginsenosides-Rb_1, -Rb_2, -Rc or -Rd, not by saponin of 20(S)-protopanaxatriol, ginsenoside-Re of -Rg_1.

Saikosaponin-d, another pharmacologically active saponin of Bupleuri radix, is more sparingly soluble in water than saikosaponin-a; the saturated concentration in water at 37℃ is 0.01mg/m*l*. It was observed that as in the case of saikosaponin-a, the water solubility of saikosaponin-d was significantly increased in the presence of Fr.II, ginsenoside-Ro or G-mix; 2.0mg/m*l* in 0.1% solutions of Fr.II, 3.1mg/m*l* in 0.0664% solution of G-mix and 5.0mg/m*l* in 0.05% solution of ginsenoside-Ro at 37℃. No solubilizing effect was observed with 0.0045% solution of ginsenoside-Ro and the same cooperation of dammarane saponins on the solubilizing effect of ginesnoside-Ro, was also observed as in the case of saikosaponin-a.

In continuing of this study, the relationship between structure and the solubilizing effect were investigated. It was noted that methylation or reduction to -CH_2OH of the glucuronide-carboxyl group of ginsenoside-Ro, resulted in the remarkable decrease of the solubilizing effect on saikosaponin-a.

From rhizomes of *Panax japonicus*(竹節人參), several analoguous glucuronide saponin of oleanolic acid, chikusetsusaponins IV and IVa *etc.*, have been also isolated together with ginsenoside Ro(=chikusetsusaponin V) by Shoji *et al* (Fig.7)[20]. From rhizomes of Chinese cucurbitaceous medicinal plants, *Hemsleya chinensis*(中華雪膽) and *Hemsleya macrosperma*(大種雪膽), we isolated glucuronide saponins of oleanolic acid named hemslosides, structures of which are closely related to chikusetsusaponins(Fig.7)[21, 22]. All of these chikusetsusaponins and hemslosides have the common partial structure, 3-0 -β-D-glucuronide of β-D-glucosyl oleanoate(=chikusetsusaponin IVa) and it was found that chikusetsusaponin-IV, hemsolsides-Ma2 and -Ma3 exhibited the similar solubilizing effect on saikosaponin-a to that of ginsenoside-Ro[22]. On the other hand, the neutral bisdesmosides such as tubeimosides

oleanolic acid saponins
a) *P. japonicus*(Araliaceae)
b) *H. macrosperma*(Cucurbitaceae)
c) *H. chinensis*(Cucurbitaceae)



from yield	a) (%)	b) (%)	c) (%)	R_1	R_2	R_3	R_4
chikusetsetsusaponin IVa	(trace)	(−)	(2.2)	: −H	−H	−H	−Glc
chikusetsusaponin IV	(0.4)	(−)	(−)	: −H	−H	−Ara(f)	−Glc
chikusetsusaponin V (=ginsenoside Ro)	(5.4)	(−)	(−)	: −Glc	−H	−H	−Glc
hemsloside Ma1	(−)	(0.72)	(3.3)	: −H	−Ara(p)	−H	−Glc
hemsloside Ma2	(−)	(0.20)	(−)	: −Xyl	−Ara(p)	−H	−Glc
hemsloside Ma3	(−)	(3.4)	(1.7)	: −Glc	−Ara(p)	−H	−Glc
hemsloside H_1	(−)	(−)	(0.41)	: −Glc	−Ara(p)	−H	−Glc$\overset{6}{-}$Glc

Fig.7. *Comparison of Saponins from Rhizomes of Panax japonicus, Hemsleya macrosperma* and *Hemsleya chinensis.*

(Fig.5) and mukurozi-saponins(Fig.3) mentioned above which exhibited potent solubilizing effect on acidic monodesmosides such saponin A(Fig.3), did not increase the water solubility of saikosaponin-a. These results suggested the important role of the glucuronide moiety of ginsenoside-Ro for the solubilizing effect on the neutral monodesmosides, saikosaponins.

The present study suggests that the saponin combination of Ginseng is highly attractive not only in the pharmacological activities but also in view of physico-pharmaceutics. The investigation of the solubilizing effect of Ginseng saponins on natural and synthetic medicines other than monodesmosidic saponins, is under progress.

Acknowledgement

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