Journal of Traditional Medicines 17, 1−11, 2000

Human intestinal bacteria responsible for the metabolism of saikosaponins

Meselhy R. MESELHY

Institute of Natural Medicine, Toyama Medical and Pharmaceutical University

(Accepted November 30, 1999.)

Abstract

Metabolism of saikosaponins by human intestinal bacteria is discussed in view of the results obtained during the last years. Saikosaponins (SAPs) were converted to prosaikogenins and saikogenins in order by a human fecal suspension and by the bacterial strains, *Eubacterium* sp. A-44, *Bifidobacterium* sp. Saiko-1 and Saiko-2, isolated from human feces. From *E.* sp. A-44, two glycosidases responsible for the stepwise hydrolysis of SAPs were isolated and characterized as saikosaponin-hydrolyzing β-D-glucosidase and prosaikogenin-hydrolyzing β-D-fucosidase. After the oral administration of saikosaponin b_1 (Sb_1) to germ-free rats, no metabolites were detected in their plasma, cecal contents and cumulative feces. However, considerable amounts of prosaikogenin A and saikogenin A were detected when Sb_1 was orally given to conventional or the *E.* sp. A-44-infected gnotobiote rats. Fecal and cecal activities of hydrolyzing saikosaponins were found in the gnotobiote and conventional rats. These findings provided direct evidence that saikosaponins-hydrolyzing bacteria are necessary for the appearance of their biologically active metabolites in the systemic circulation.

Key words Saikosaponins, Human intestinal bacteria, *Bifidobacterium* sp. Saiko, *Eubacterium* sp. A-44, Gnotobiote rat.

I. Introduction

Since most of natural medicines are administered orally, they inevitably come in contact with intestinal bacteria in the digestive tract before their components act on the target organ or are absorbed into the body. One of the most significant and well-documented reactions carried out by intestinal bacteria is that of glycoside hydrolysis. Following the oral administration, glycosides which remain unabsorbed from the upper intestine are hydrolyzed to the respective aglycones. These aglycones may have pharmacological or toxicological significance. Metabolic activation of sennosides by intestinal bacteria is a quite important process of inducing their laxative effects.[1-3] After oral administration of several foodstuffs containing cyanogenetic glycosides, intestinal bacteria were implicated in the liberation of methylazoxymethanol, the hepatotoxic and carcinogenic metabolite of cycacin,[4] and in the liberation of cyanide from amygdalin and linamarin.[5]

Over the past 20 years, metabolism of major glycosides of natural medicines, sennosoides (from Rhei Rhizoma),[1-3,6] paeoniflorin (from Paeoniae Radix),[7] ginsenosides (from Ginseng Radix),[8] glycyrrhizin (from Glycyrrhizae Radix),[9] and *C*-glycosides (barbaloin, homoorientin, aloesin, safflor yellow B, mangiferin and bergenin)[10-14] by human intestinal bacteria has been investigated, and many of which produced biologically active metabolites.

Various bacterial glycosidases, such as β-glucosidase from *Bifidobacterium* sp. SEN for sennosides,[15] or *Eubacterium* sp. A-44 for geniposide,[16] and β-glucuronidase from *Eubacterium* sp. GLH for glycyrrhizin,[17] are assumed to take part in the metabolism of glycosides.

Saikosaponins (SAPs) are the major triterpene

*〒930-0194 富山市杉谷2630
富山医科薬科大学和漢薬研究所 細胞資源工学部門
2630 Sugitani, Toyama 930-0194, Japan

glycosides in Bupleuri Radix (the dried root of *Bupleurum* sp.) which is used widely in traditional Chinese medicines as antipyretic, analgesic and anti-inflammatory agents for the treatment of influenza, fever, and menestrual disorders.[18] The contents of SAPs in the radix were variable (1.24-4.86 %) and decreased with increase in the root diameters.[19] These components have been extensively investigated for their pharmacological activity, as anti-inflammatory,[20] anti-hypercholesteremic[21] and hepatoprotective agents.[22] So far detailed information concerning their metabolism and pharmacokinetics is available only in a few cases. Shimizu *et al.*[23] reported that saikosaponins a, b_1, b_2, c, and d were metabolized by rat gastric juice and mouse intestinal bacteria *in vitro*. After oral administration to rats, saikosaponin a and its metabolites were detected in the blood stream and excreted in feces.[24,25] However, there has been no report on the metabolism of saikosaponins by human intestinal bacteria and bacterial species capable of metabolizing saikosaponins have not been isolated so far.

In the course of our studies on the metabolism of crude drug components by human intestinal bacteria,[26-33] the present study was designed to: a) isolate bacterial strains from human feces capable of metabolizing saikosaponins, b) isolate and characterize saikosaponin-hydrolyzing glycosidase(s) responsible for the metabolism of saikosaponins from human intestinal bacteria, and c) investigate the metabolic fates of saikosaponins, through a pharmacokinetic study of saikosaponin b_1 (**1**), using germ-free, conventional, and gnotobiote rats.

II. Isolation of human intestinal bacteria capable of hydrolyzing saikosaponins[34]

Metabolism of saikosaponins by human fecal suspension (HFS)

When saikosaponins were, separately incubated with HFS, it was apparent that saikosaponins examined were effectively and completely metabolized to the corresponding prosaikogenins and saikogenins by a fecal suspension of humans. Saikosaponins a (**1**), b_1 (**3**), b_2 (**4**) and d (**2**) were converted to the corresponding prosaikogenins F (**6**), A (**8**), D (**9**) and G (**7**) and saikogenins F (**10**), A (**12**), D (**13**) and G (**11**) in order. Saikosaponins a (**1**), b_1 (**2**) and b_2 (**5**) were completely hydrolyzed after 24 hr, but complete hydrolysis of saikosaponin d (**2**) was achieved after 48 hr. In the case of saikosaponin c (**5**), only saikogenin E (**14**) was detected without formation of

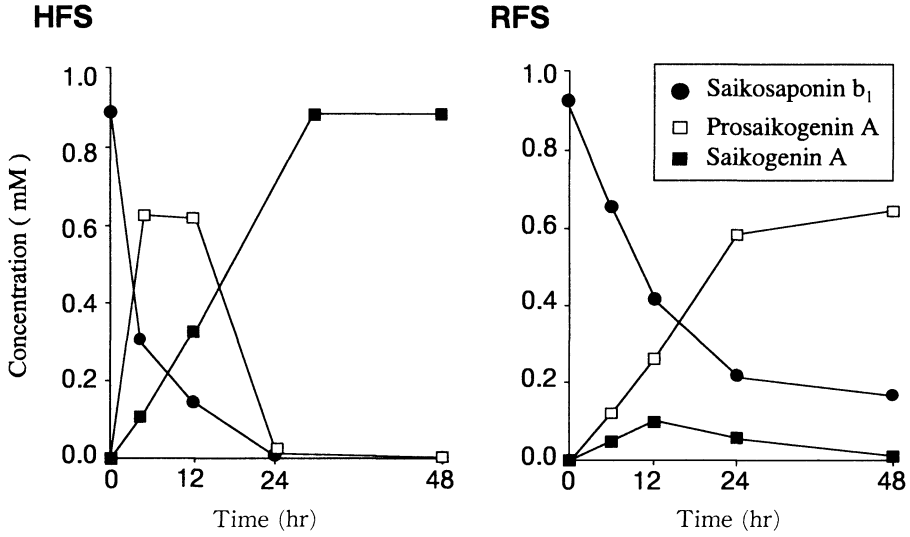

Fig. 1 Time course of metabolism of saikosaponin b_1 (**3**) by human and rat fecal suspensions. Saikosaponins **1**, **2** and **4** gave similar profiles. Saikosaponins and their metabolites were determined by TLC-densitometry [silica gel plates 60 F_{254}; solvent system, $CHCl_3$-MeOH-H_2O (3 : 1 : 0.1) ; detection, 262 nm for **3** and **4**, and their metabolites, λ_{max} 600 nm "sample" relative to 700 nm "reference" for **1**, **2** and **5**, and their metabolites].
HFS, human fecal suspension; RFS, rat fecal suspension.

intermediate prosaikogenins. Shimizu *et al.*[23] reported similar results for the metabolism of saikosaponins a and d by animal intestinal flora. However, the transformation of these compounds by a mixture of bacteria from human feces was much faster than by that from rat feces under the same conditions (Fig.1).

Isolation of human intestinal bacteria capable of hydrolyzing saikosaponins

Of 31 defined bacterial strains isolated from human feces and screened for their ability to metabolize saikosaponins, only *Eubacterium* sp. A-44 could metabolize saikosaponins a (**1**), b_1 (**3**), b_2 (**4**) and d (**2**) to the corresponding prosaikogenins, and the produced prosaikogenins F (**6**) and A (**8**) were further transformed to the corresponding saikogenins (Chart 1). However, saikosaponin c (**16**) was not metabolized by this strain.

Although most of these strains had β-D-glucosidase activity which hydrolyzes various glucosides, such as *p*-nitrophenyl β-D-glucoside, paeoniflorin,[35] sweroside[36] and geniposide,[37] they did not hydrolyze saikosaponins except for one strain, *Eubacterium* sp. A-44 which showed significant ability to transform most saikosaponins to their corresponding saikogenins.

For the purpose of isolating bacterial strain(s) capable of hydrolyzing SAPs, a 250-fold diluted HFS, which showed appreciable ability to hydrolyze saikosaponins, was seeded onto BL agar plates. Colonies were picked up and screened for their ability to hydrolyze saikosaponin a (**1**), used as representative for saikosaponins. As a result, two colonies (strains X and Z) showed ability to transform saikosaponin a (**1**) to **6**. These bacteria also hydrolyzed saikosaponins b_1 (**3**), b_2 (**4**) and d (**2**) to the corresponding prosaikogenins (Chart 1).

Although Y and Z had the ability to hydrolyze **1**-**4** in this study, attempts to isolate any bacterial species capable of hydrolyzing saikosaponin c (**5**) in a direct or stepwise manner were unsuccessful. However, the possibility of isolating them still remains.

Characterization of the isolated strains

The biochemical characteristics of the isolates (X and Z) are as shown in Table I. Both were gram-positive bud-forming rods and strict anaerobes, forming brown colonies when cultured on BL agar plates. Acetic acid and lactic acid were produced when D-glucose was fermented by X and Z. In addition, both strains had fructose 6-phosphate phosphoketolase activity. These findings lead us to confirm that both strains belong to the genus *Bifidobacterium*. Accordingly, we named them *Bifidobacterium* sp. Saiko-1 and

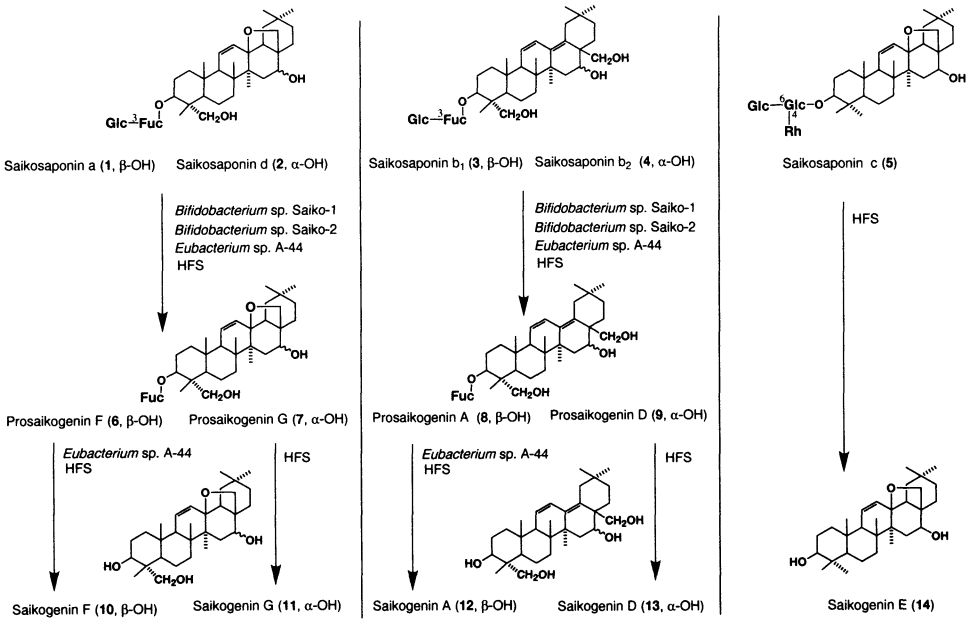

Chart 1 Metabolic pathway of saikosaponins by human intestinal bacteria and human fecal suspension (HFS)

Table I Characteristics of the isolates X and Z

Characteristic	Strain X	Strain Z	A-44
Gram stain	Gram-positive	Gram-positive	Gram-positive
Colony on BL agar	Brown	Brown	Milky-white
Aerobic growth	——	——	——
Major fatty acids	Lactic acid	Lactic acid	Lactic acid
	Acetic acid	Acetic acid	Acetic acid
Fructose-6-phosphate phosphoketolase	+	+	——
β-Glucosidase	+	+	+
Species	*Bifidobacterium*	*Bifidobacterium*	*Eubacterium*

+, positive; ——, negative.

Table II Comparative biochemical characteristics of the isolates X and Z

Compound	*B.* sp. Saiko-1 (Strain X)	*B. breve* ss *breve**	*B.* sp. Saiko-2 (Strain Z)	*B. adolescentis**
D-Arabinose	—	—	++	++
D-Xylosew	W	—	++	++
L-Rhamnose	—	—	W	—
D-Ribose	++	++	++	++
D-Glucose	++	++	++	++
D-Mannose	—	++	W	+—
D-Fructose	++	++	++	++
Maltose	++	++	++	++
Cellobiose	++	++	++	++
Lactose	++	++	++	++
Trehalose	—	—	++	+—
Melibiose	++	++	++	++
Raffinose	++	++	++	++
Melezitose	—	—	++	++
Starch	++	+—	++	++
Inulin	++	+—	—	+—
D-Mannitol	++	++	++	+—
D-Sorbitol	++	++	++	+—
Inositol	—	—	—	—
Esculin	++	++	++	++
Salicin	++	++	++	++
Amygdalin	—	++	—	++

−, negative reaction (pH 6.0 or above); w, weak reaction (pH 5.5−6.0); ++, positive reaction (pH 5.5 or below); +—, 11−89 % positive but may exhibit a negative reaction.
*Mitsuoka, T. (see reference 26)

Saiko-2, respectively. In the former strain, the ability of sugar fermentation was quite similar to that of *Bifidobacterium breve* ss *breve*[38,39] except for the cases of D-xylose, D-mannose and amygdalin (Table II). On the other hand, a pattern of sugar fermentation by *B.* sp. Saiko-2 was close to that shown by *B. adolescentis*[38,39] except for L-rhamnose and amygdalin. Both strains showed appreciable β-D-glucosidase activity when assayed with *p*-nitrophenyl β-D-glucoside as a substrate.

These findings suggested that a population of bacterial species, in human intestine, capable of hydrolyzing saikosaponins may be low and the three strains, *Eubacterium* sp. A-44, *Bifidobacterium* sp. Saiko-1 and Saiko-2 were responsible for the hydrolysis of most saikosaponins. These reactions were considered to be catalyzed by unique enzyme(s) which have not been isolated so far from human intestinal bacteria.

III. Intestinal bacterial enzymes responsible for the metabolism of saikosaponins[40]

β-D-Glucosidase and β-D-fucosidase activities

Commercially available emulsin from almond and rat liver homogenate, which was reported to contain both β-D-glucosidase and β-D-fucosidase activities,[41-43] could not hydrolyze saikosaponins or prosaikogenins after incubation for 24 h (Table III). The three bacterial strains were shown to have potent β-D-glucosidase activity, and only *E.* sp. A-44 showed appreciable β-D-fucosidase activity. However, the β-D-fucosidase activity of *E.* sp. A-44 was one third of the β-D-glucosidase activity. In the case of *B.* sp. Saiko-1, a weak β-D-fucosidase activity was detected for *p*-nitrophenyl β-D-fucoside in spite of no hydrolysis of prosaikogenins, and no activity was detected for *B.* sp. Saiko-2.

Since more than 80 % of saikosaponin-hydrolyzing activities was found in the supernatant of *E.* sp. A-44 obtained after centrifugation of the sonicated cells, it was evident that these enzymes are located in the intracellularly-soluble fraction of the organism. The bacterial supernatant showed hydrolyzing activities toward saikosaponins a, b_1, b_2, d and prosaikogenins A and F, but not prosaikogenins D and G, the same as that seen for the native cells. Moreover, appreciable β-D-glucosidase and β-D-fucosidase activities toward *p*-nitrophenyl β-D-glucoside and *p*-nitrophenyl β-D-fucoside, respectively, were also seen.

Through Butyl-Toyopearl 650M column chromatography (Fig. 2), this supernatent yielded two *p*-nitrophenyl β-D-glucoside-hydrolyzing activities and two *p*-nitrophenyl β-D-fucoside-hydrolyzing activities which were detected in four different fractions. These results indicated the presence of two kinds of β-D-glucosidase and two kinds of β-D-fucosidase enzymes. One of the β-D-glucosidases was capable of hydrolyzing saikosaponin a but not prosaikogenin F, and one of the β-D-fucosidases had prosaikogenin F-hydrolyzing activity but no saikosaponin a-hydrolyzing activity. Further purification of these enzymes was carried out through a column of Sephacryl S-300. By two steps of purification, the β-D-glucosidase and the β-D-fucosidase were purified 50-fold and 40-fold, respectively, with specific activities of 4.9 and 1.1 μmol/min/mg, respectively.

Properties of SHGase and PHFase

Using a series of standard proteins, the apparent molecular weight of saikosaponin-hydrolyzing β-D-glucosidase (SHGase) was estimated to be about 100 kDa using a column of Sephacryl S-300, and that of prosaikogenin-hydrolyzing β-D-fucosidase (PHFase) was shown to be about 130 kDa. The optimum pH of

Table III Enzymatic activity of saikosaponin-hydrolyzing bacterial strains

	Enzymatic activity (nmol/min/ml)			
	Saikosaponin a (**1**)	Prosaikogenin F (**6**)	pNPG	pNPF
Bifidobacterium sp. Saiko-1	4.04+0.086	N.D.	++	w
Bifidobacterium sp. Saiko-2	4.08+0.047	N.D.	++	N.D.
Eubacterium sp. A-44	5.26+0.211	1.34+0.097	++	++
Emulsin (from almond)	N.D.	N.D.	++	++
Rat liver homogenate	N.D.	N.D.	++	++

Enzymatic activity were determined using the disrupted cell of each bacterium, or sonicated liver homogenate suspended in 50 mM K-phosphate buffer (pH 7.25). Quantity of protein was determined by Lowry's method. Mean±S.D. are given.
pNPG, *p*-nitrophenyl β-D-glucoside; pNPF, *p*-nitrophenyl β-D-fucoside; W, weak activity; ++, strong activity; N.D., not detected.

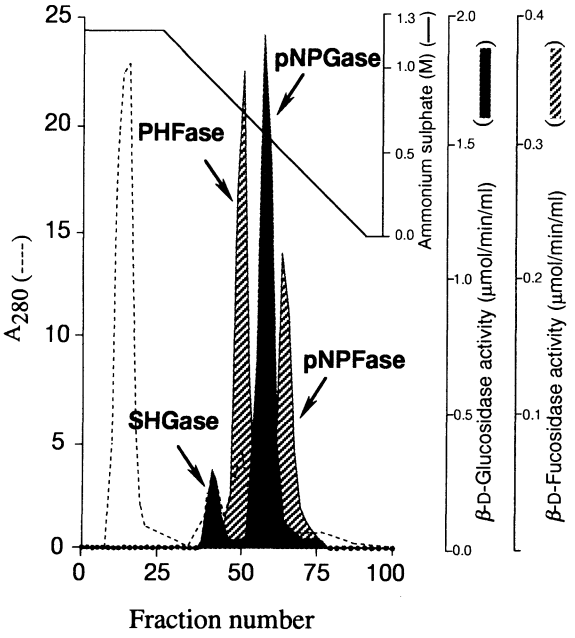

Fig. 2 Butyl-Toyopearl 650M column chromatography of a crude enzyme preparation from *Eubacterium* sp. A-44.
SHGase, saikosaponin-hydrolyzing β-D-glucosidase; PHFase, prosaikogenin-hydrolyzing β-D-fucosidase; pNPGase; *p*-nitrophenyl-hydrolyzing β-D-glucosidase; p-NPFase, *p*-nitrophenyl-hydrolyzing β-D-fucosidase.

saikosaponin-hydrolyzing activity of β-D-glucosidase was found to be pH 6.0 when *p*-nitrophenyl β-D-glucoside and saikosaponin a were used as substrates. However, that of PHFase was found to be pH 6.5 using *p*-nitrophenyl β-D-fucoside and prosaikogenin F as substrates. The substrate specificity of the two glycosidases, SHGase and PHFase, toward various glycosides relative to *p*-nitrophenyl β-D-glucoside-hydrolyzing activity (100 %) was determined. The hydrolyzing activities of the SHGase were 41.4 % for geniposide, 17.4 % for saikosaponin a, about 15 % for both saikosaponin b_1 and b_2, whereas a weaker activity was observed for saikosaponin d. However, fucosidase, galactosidase, and prosaikogenins-hydrolyzing activities were not detected. These data were in accordance with those reported for geniposide-hydrolyzing β-D-glucosidase isolated previously from the same bacterium.[16]

On the other hand, the hydrolyzing activities of PHFase for *p*-nitrophenyl β-D-glucoside, *p*-nitrophenyl β-D-galactoside and prosaikogenins A and F were 32.0 %, 22.2 %, 8.6 % and 8.2 % respectively, relative to that shown for *p*-nitrophenyl β-D-fucoside (100 %), suggesting a broad substrate specificity of this β-D-fucosidase. However, hydrolyzing activities for saikosaponins, prosaikogenins D and G were not observed. Many investigators reported that purified β-D-fucosidase has variable β-D-glucosidase and/or β-D-galactosidase activities.[44-46] For example, β-D-fucosidase isolated from the giant African snail seeds of *Dalbergia cochinchinesis* PIERRE showed β-D-glucosidase and β-D-galactosidase activities of 58 and 42 % relative to β-D-fucosidase activity, respectively. These enzymes showed acidic optima (around 5), relative to an optimum pH of 6.5 for PHFase. Likewise, the enzyme from bovine liver had the above activities of 77 and 64 %, and that from sheep liver, 66 and 40 %, respectively. In comparison with these reported β-D-fucosidases, PHFase seems to be a relatively specific β-D-fucosidase.

The activities of the two glycosidases were inhibited by various sugars and other related compounds. *p*-Nitrophenyl β-D-glucoside-hydrolyzing activity of SHGase was effectively inhibited by D-glucose and D-glucono δ-lactone, but not by L-glucose, D-fucose, L-fucose and D-galactose. Similarly, *p*-nitrophenyl β-D-fucoside-hydrolyzing activity of PHFase was inhibited by D-fucose, and weakly inhibited by D-glucose, D-glucono δ-lactone, D-galactose and D-galactono δ-lactone.

By incubation with both glycosidases, most saikosaponins were hydrolyzed to the corresponding saikogenins *via* prosaikogenins, by elemination of

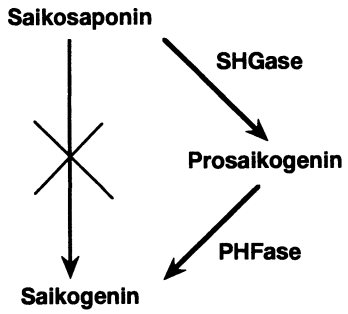

Chart 2 Stepwise metabolism of saikosaponins by intestinal bacterial enzymes
SHGase, saikosaponin-hydrolyzing β-D-glucosidase; PHFase, prosaikogenin-hydrolyzing β-D-fucosidase

their sugars in a stepwise manner (Chart 2).

IV. Metabolism and pharmacokinetics of saikosaponin b_1 *in vivo*[47]

For the purpose of investigating the metabolic fates of saikosaponins, a pharmacokinetic study of saikosaponin b_1 (Sb_1) was performed using conventional (CVL), germ-free (GF) and *E.* sp. A-44-infected gnotobiote (GN) rats.

It was apparent that considerable amounts (about 83 % of the administered dose) of the unabsorbed Sb_1 (**3**) remained in the cecal contents of the GF rats 4 hr after the oral administration (Table IV). However, no metabolite was detected in the cecal contents and cumulative feces. These findings suggested that orally administered saikosaponins are poorly absorbed from the gastrointestinal tract. This was further confirmed by the low intestinal permeability of Sb_1 assessed by the *in vitro* everted sac method, where the permeability decreased in the order of saikogenin A (Sg A, **12**), prosaikogenin A (pSg A, 8) and Sb_1 (**3**). On the other hand, Sb_1, pSg A (8) and Sg A (**12**) were recovered from cecal contents of the CVL rats 4 hr after the administration, and also detected in the cumulative feces within 10 hr (Table IV). Likewise, Sb_1 and its metabolites, 8 and **12** were recovered from the cecal contents of the *E.* sp. A-44-infected GN rats 4 hr after the administration (Table IV). However, only two metabolites 8 and **12** were recovered after 10 hr. Furthermore, **3**, 8 and **12** were also detected in cumulative feces of GN rats within 10 hr. (Table IV).

*Plasma concentration of saikosaponin b_1 (**3**) and its metabolites after oral administration of **3** to CVL rats*

Figure 3A shows the plasma concentration-time courses of Sb_1 (**3**) and its metabolites (8 and **12**) after oral administration of **3** at a dose of 50 mg/kg to CVL rats. The maximal plasma concentration (C_{max}) of Sb_1 (**3**) was reached 30 min after the administration (129 pmol/ml), and Sb_1 (**3**) disappeared after 4 hr. In contrast, pSg A (8) and Sg A (**12**) could be detected after 4 and 6 hr, respectively. The plasma concentrations of both metabolites (8 and **12**) were reached the maximal values of 49.6 and 54.7 pmol/ml at 6 and 8 hr, respectively (Table V). Ten hours after the administration of Sb_1 (**3**), pSg A (8) disappeared from the rat plasma, but Sg A (**12**) (22.3 pmol/ml) was still detected. In the CVL rats, the sum of each AUC_{0-10hr} value of metabolites (8 and **12**) (22350 pmol/min/ml) was larger than that of Sb_1 (**3**) (12654 pmol/min/ml).

*Plasma concentration of saikosaponin b_1 (**3**) and its metabolites after oral administration of **3** to GF and GN rats*

Table IV Saikosaponin b_1 (**3**) and its metabolites (8 and **12**)* in cecal contents and feces** of CVL, GF and GN rats

Rat		Amount (μmol)		
		3	8	**12**
CVL rat :				
-cecal contents	4 hr	3.81±0.02	0.32±0.10	0.51±0.20
	10 hr	0.28±0.03	1.89±0.16	0.76±0.10
-feces		1.01±0.06	1.15±0.08	0.91±0.11
GF rat :				
-cecal contents	4 hr	4.93±0.34	N.D.	N.D.
	10 hr	2.73±0.08	N.D.	N.D.
-feces		1.08±0.26	N.D.	N.D.
GN rat :				
-cecal contents	4 hr	3.30±0.05	0.78±0.06	0.87±0.04
	10 hr	N.D.	0.82±0.05	1.85±0.25
-feces		0.85±0.08	0.84±0.03	0.90±0.15

*Determined by TLC-densitometry as above mentioned. **Determined in cumulative feces for 10 hr after the oral administration of **3** (50 mg/kg). Mean±S.E. (n=3) are given. CVL, conventional ; GF, germ-free ; GN, gnotobiote ; N.D., not detected.

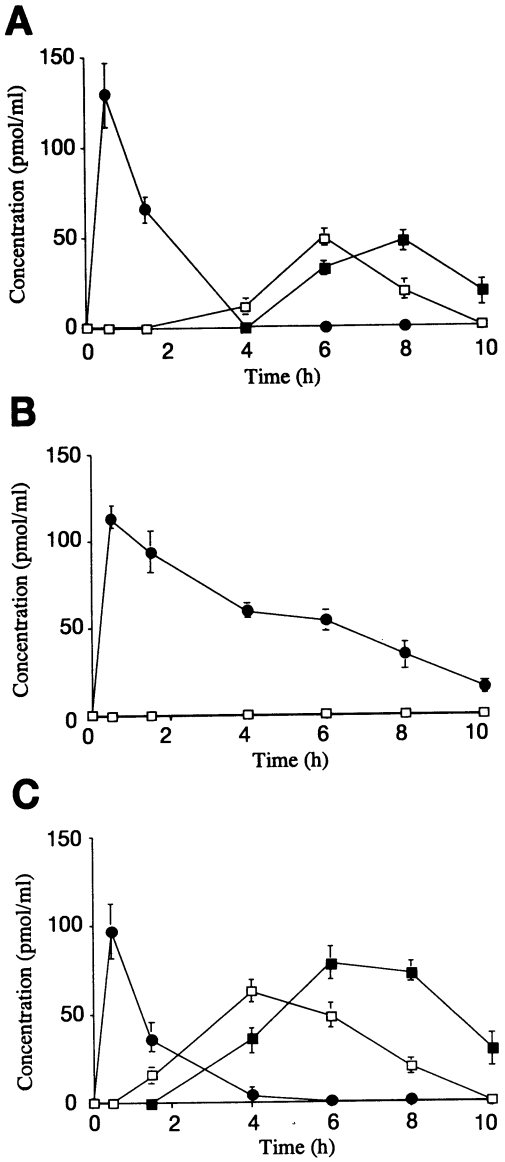

Fig. 3 Plasma-concentration-time cources of saikosaponin b_1 (●) and its metabolites, prosaikogenin (□) and saikogenin (■) in : conventional (A), germ-free (B), and gnotobiote rats (C) after the oral administration of saikosaponin b_1 at a dose of 50 mg/kg. Saikosaponin b_1 and its metabolites were determined by HPLC. Plasma (150 μl) were extracted with EtOH (15 ml) containing methyl benzoate (75 nmol, internal standard) and the EtOH-soluble fraction was evaporated to dryness. The residue was dissolved in 50 % aq. EtOH (30 μl) and filtered. Sample, 20 μl ; column, Develosil ODS-5 (4.6×150 mm) ; mobile phase, 38 % aq. MeCN containing 0.1 % trifuoroacetic acid; flow rate, 1.0 ml/min; detection, UV 250 nm.

As shown in Fig. 3B, the C_{max} of **3** (113 pmol /ml) was reached at 30 min after the oral administration of Sb_1 (**3**) at a dose of 50 mg/kg to GF rats. Sb_1 (**3**) (15.4 pmol /ml) was still detected in the plasma at 10 hr

Table V Pharmacokinetic parameters after oral administration of saikosaponin b_1 (**3**) at a dose of 50 mg/kg to CVL, GF and GN rats

Rat		Pharmacokinetic parameter		
		C_{max} (pmol/ml)	T_{max} (min)	$AUC_{0-10\,hr}$ (pmol/min/ml)
CVL rat:				
	3	129	30	12654
	8	49.6	360	9936
	12	54.7	480	12414
GF rat:				
	3	113	30	34308
	8	N.D.	N.D.	N.D.
	12	N.D.	N.D.	N.D.
GN rat:				
	3	100	30	6852
	8	59.4	240	17424
	12	65.6	360	22260

CVL, conventional ; GF, germ-free ; GN, gnotobiote ; N.D., not detected.

with AUC_{0-10hr} value of 34308 pmol/min/ml, while pSg A (**8**) and Sg A (**12**) were not detected (Table V). However, pSg A (**8**) and Sg A (**12**) were detected in the plasma of GN rats after the oral administration of Sb_1 (**3**), similar to the case of CVL rats (Fig. 2). Though, the AUC_{0-10hr} values of **8** and **12** (17424 and 22260 pmol/min/ml, respectively) were 2.0-2.6 fold larger than that of Sb_1 (**3**) (6852 pmol/min/ml) (Table V).

After oral administration of Sb_1 (**3**) to GF rats, less than 20 % of the administered dose was absorbed, but the AUC_{0-10hr} value was 2.5 times larger than that in the case of CVL and GN rats. This finding revealed that Sb_1 (**3**) was absorbed gradually during a long period of time (10 hr) in the GF rats, relative to the CVL rats (4 hr). The similar finding was reported for paeoniflorin administered orally to GF and CVL rats.[48] These findings confirmed that unabsorbed Sb_1 (**3**) reached cecum, and was hydrolyzed by intestinal bacteria to these metabolites (**8** and **12**), which were subsequently absorbed. Accordingly, we suggest that the intestinal bacteria also play a major role in the elimination of Sb_1 (**3**) as well as that of paeoniflorin.

Enzyme activities of fresh feces and cecal contents

Fresh feces and cecal contents of the GF rats showed no β-D-glucosidase, β-D-fucosidase, sai-

Table VI β-D-Glucosidase, β-D-fucosidase, saikosaponin b_1- and prosaikogenin A-hydrolyzing activities in cecal contents and feces of CVL, GF and GN rats*

Rat	Enzymatic activity (pmol/min/ml)			
	pNPGase	pNPFase	SHGase	PHFase
CVL rat:				
-cecal contents	8480±1278	1840±159	5.48±046	1.88±0.27
-feces	8060±6.5	3360±346	8.16±0.88	2.64±0.26
GF rat:				
-cecal contents	N.D.	N.D.	N.D.	N.D.
-feces	N.D.	N.D.	N.D.	N.D.
GN rat:				
-cecal contents	8810±889	2200±90	11.59±1.18	4.34±1.14
-feces	6670±774	2840±184	10.48±134	4.50±0.36

*Determined in cecal contents and fresh feces after sonication. Mean±S.E. (n=3) are given. CVL, conventional; GF, germ-free; GN, gnotobiote; pNPGase, *p*-nitrophenyl β-D-glucosidase; pNPFase, *p*-nitrophenyl β-D-fucosidase; SHGase, saikosaponin-hydrolyzing β-D-glucosidase; PHFase, prosaikogenin-hydrolyzing β-D-fucosidase; N.D., not detected.

kosaponin b_1-or prosaikogenin A-hydrolyzing activity (Table VI). In the case of the CVL and GN rats, each enzyme activity was found. However, both cecal saikosaponin b_1 (**3**)- and prosaikogenin A (**8**)-hydrolyzing activities of the GN rats (11.59 and 4.34 pmol/min/mg, respectively) were about 2-fold higher than those of CVL rats (5.48 and 1.88 pmol/min/mg, respectively).

V. Conclusion

The present study clarified the role of human intestinal bacteria in the metabolism of saikosaponins, the major components in Bupleuri Radix. It was clear that all saikosaponins examined were effectively and completely metabolized to the corresponding prosaikogenins and saikogenins by a fecal suspension of humans. Furthermore, the three bacterial strains, *Eubacterium* sp. A-44, *Bifidobacterium* sp. Saiko-1 and Saiko-2, isolated from human feces were found capable of metabolizing most saikosaponins tested. Although emulsin from almond and rat liver hemogenate showed no ability to metabolize these compounds, two glycosidases, SHGase and an *exo*-type PHFase, isolated from *E.* sp. A-44 were found responsible for the metabolism of saikosaponins in a stepwise manner. Both enzymatic activities were found in the feces and cecal contents of CVL and GN rats, but not in those of GF rats. These findings indicated that the present two enzymes from *E.* sp. A-44 may play an important role in the metabolism of saikosaponins in the human intestine.

Another piece of evidence was the finding that less than 20 % of Sb_1 (**3**) was absorbed from the gastrointestinal tract after its oral administration to GF rats, while considerable amounts was remained in the cecal contents, and its metabolites (**8** and **12**) were not detected. However, after oral administration of **3** to CVL and GN rats, pSgA (**8**) and Sg A (**12**) besides Sb_1 (**3**) were detected in the blood stream, cecal contents and feces. These findings confirmed that unabsorbed Sb_1 (**3**) reached cecum, and was hydrolyzed by intestinal bacteria to these metabolites (**8** and **12**), which were subsequently absorbed. This was supported by the observation that the t_{max} (time to C_{max}) of each metabolite was appreciably delayed (approx. at 4-8 hr), relative to that of Sb_1 (**3**) (30 min) when orally administered to the CVL or GN rats.

Of considerable significance, however, was the fact that various pharmacological effects,[21,49-51] such as anti-inflammatory and anti-hypercholesteremic actions have been reported for the metabolites from saikosaponins. Saikogenin A (**12**), the main metabolite of Sb_1 (**3**), was reported to show anti-inflammatory, analgesic, sedative, antitussive, hypothermic effects[13] and antitumor action. Accordingly, the findings obtained here were significant in establishing direct proof of the importance of intestinal bacteria

for the liberation of these metabolites and that rat cecum which contains the highest bacterial population was the site responsible for the appearance of these metabolites in the general circulation.

Nevertheless, I believe that metabolism of crude drug components in human intestine after oral administration should be investigated before a real understanding of the pharmacological activities of Kampo medicines can be achieved.

Acknowledgment

The author is grateful to Prof. Masao Hattori, Dr. Teruaki Akao and Dr. Hiroaki Kida, Toyama Medical and Pharmaceutical University for their sincere support and valuable assistance.

References

1) Sasaki, M., Yamauchi, K. and Kuwano, S. : Metabolic activation of sennoside A in mice. *Planta Med.* **37**, 370-378, 1979.
2) Lemli, J. and Lemmens, L. : Metabolism of sennoside and rhein in the rat. *Pharmacology* **20**, 50-57, 1980.
3) Dressen, M., Eyssen, H., Lemli, J. : The metabolism of sennoside A and B by the intestinal microflora : In vitro and in vivo studies on the rat and the mouse. *J. Pharm. Pharmacol.* **33**, 679-681, 1981.
4) Yang, M.G. and Mickelsen, O. : In "Toxic Constituents of Plant Food-stuffs" (I.E. Liener, Ed.), pp. 159-167. Academic Press, New York.
5) Smith, R.L. : In "A Symposium on Mechanisms of Toxicity" (W.N. Aldridge, Ed.), pp. 229-247. Macmiillian, London, 1971.
6) Hattori, M., Namba, T., Akao, T. and Kobashi, K. : Metabolism of sennosides by human intestinal bacteria. *Pharmacology* **36** (Suppl. 1), 172-179, 1988.
7) Hattori, M., Shu, Y.-Z., Shimizu, M., Hayashi, T., Morita, N., Kobashi, K., Xu, G.-J. and Namba, T. : Metabolism of paeoniflorin and related compounds by human intestinal bacteria. *Chem. Pharm. Bull.* **33**, 3838-3846, 1985.
8) Kanaoka, M., Akao, T. and Kobashi, K. : Metabolism of ginseng saponins, ginsenosides, by human intestinal bacteria. *J. Trad. Med.* **11**, 241-245, 1994.
9) Hattori, M., Sakamoto, T., Yamagishi, T., Sakamoto, K., Konishi, K., Kobashi, K. and Namba, T. : Metabolism of glycyrrhizin by human intestinal flora. II. Isolation and characterization of human intestinal bacteria capable of metabolizing glycyrrhizin and related compounds. *Chem. Pharm. Bull.* **33**, 210-217, 1985.
10) Akao, T., Che, Q., Kobashi, K., Hattori, M. and Namba, T. : A purgative action of barbaloin is induced by *Eubacterium* sp. Strain BAR, a human intestinal anaerobe, capable of transforming barbaloin to aloe-emodin anthrone. *Biol. Pharm. Bull.* **19**, 136-138 (1996).
11) Hattori, M., Shu, Y.-Z., El-Sedawy, A. I., Namba, T., Kobashi, K. and Tomimori, T. : Metabolism of homoorientin by intestinal bacteria. *J. Natural Prod.* **51**, 974-878, 1988.
12) Che, Q., Akao, T., Hattori, M., Kobashi, K. and Namba, T. : Metabolism of aloesin and related compounds by human intestinal bacteria: A bacterial cleavage of the *C*-glucosyl bond and the subsequent reduction of the acetonyl side chain. *Chem. Pharm. Bull.* **39**, 704-708, 1991.
13) Meselhy, M. R., Kadota, S., Hattori, M. and Namba, T. : Metabolism of safflor yellow B by human intestinal bacteria. *J. Natural Prod.* **56**, 39-45, 1993.
14) Hattori, M., Shu, Y.-Z., Tomimori, T., Kobashi, K. and Namba, T. : A bacterial cleavage of *C*-glycosyl bond of mangiferin and bergenin. *Phytochemistry* **28**, 1289-1290, 1989.
15) Yang, L., Akao, T., Kobashi, K. and Hattori, M. : Purification and characterization of a novel sennoside-hydrolyzing beta-glucosidase from *Bifidobacterium* sp. Strain SEN, a human intestinal anaerobe. *Biol. Pharm. Bull.* **19**, 705-709, 1996.
16) Yang, L., Akao, T. and Kobashi, K. : Purification and characterization of a geniposide-hydrolyzing β-glucosidase from *Eubacterium* sp. A-44, a strict anaerobe from human feces. *Biol. Pharm. Bull.* **18**, 1175-118, 1995.
17) Akao, T., Akao T. and Kobashi K. : Glycyrrhizin β-D-glucuronidase of *Eubacterium* sp. from human intestinal flora. *Chem. Pharm. Bull.* **35**, 705-710, 1987.
18) Tang, W. and Eisenbrand, G. : "Chinese drugs of plant origin" Springer-Verlag, Berlin, pp. 223-232, 1992.
19) Zhang, J. : Comparison on saikosaponin content in the root of *Bupleurm chinense* of different sizes. *Bull. Chin. Mat. Med.* **7**, 7-8, 1985.
20) Fuse, S., Shiotani, Y., Shimada, T., Terasawa, K. and Sagara, K. : Studies on the anti-inflammatory effects of the medical plant 'Saiko' (*Bupleurum falcatum* L.). *J. Trad. Med.* **11**, 206-213, 1994.
21) Yamamoto, M., Hayashi, Y. and Kumagai, A. : Further studies of anti-inflammatory and lipid metabolism-improving actions of saikosaponins and their aglycons, and excretion of saikosaponins. *J. Nissei Hospital* **22**, 9-12, 1994.
22) Abe, H., Sakaguchi, M., Yamada, M., Arichi, S. and Odashima, S. : Pharmacological actions of saikosaponins isolated from *Bupleurum falcatum*. *Planta Med.* **40**, 366-372, 1980.
23) Shimizu, K., Amagaya, S. and Ogihara, Y. : New derivatives of saikosaponins. *Chem. Pharm. Bull.* **33**, 3349-3355, 1985.
24) Fujiwara, K. and Ogihara, Y. : Pharmacological effects of oral saikosaponin a may differ depending on conditions of the gastrointestinal tract. *Life Science* **39**, 297-301, 1986.
25) Shimizu, K., Amagaya, S. and Ogihara, Y. : Structural transformation of saikosaponins by gastric juice and intestinal flora. *J. Pharmacobio-Dyn.* **8**, 718-725, 1985.
26) El-Mekkawy, S., Meselhy, M. R., Kawata, Y., Kadota, S., Hattori, M. and Namba, T. : Metabolism of strychnine *N*-oxide and brucine *N*-oxide by human intestinal bacteria. *Planta Med.* **59**, 347-350, 1993.
27) Meselhy, M. R., Kadota, S., Tsubono, K., Kusai, A., Hattori, M. and Namba, T. : Shikometabolins A, B, C and D, novel dimeric naphthoquinone metabolites obtained from shikonin by human intestinal bacteria. *Tetrahedron Lett.* **35**, 583-586, 1994.
28) Meselhy, M. R., Kadota, S., Tsubono, K., Hattori, M. and Namba, T. : Biotransformation of shikonin by human intestinal bacteria. *Tetrahedron* **50**, 3081-3098, 1994.
29) Meselhy, M. R., Nakamura, N. and Hattori, M. : Biotransformation of (-)-epicatechin 3-*O*-gallate by human intestinal bacteria. *Chem. Pharm. Bull.* **45**, 888-893, 1997.
30) Meselhy, M. R., Heikal, O. A., Akao, T., Khojimatov, O., Hattori, M., Ono, H. and Sadakane, C. : Disposition of paeoniflorin and

paeonimetabolin I in rats after oral administration of toki-shakuyaku-san (TS) and shakuyaku-kanzo-to (SK). *Natural Med.* **52**, 265-168, 1998.

31) Abdel-Hafez, A. A., Meselhy, M. R., Nakamura, N., Hattori, M., Watanabe, H., Mohamed, T., Mahfouz, N. and El-Gendy, M. A. : Potent anticonvulsant paeonimetabolin-I derivatives obtained by incubation of paeoniflorin and thiol compounds with *Lactobacillus brevis*. *Chem. Pharm. Bull.* **46**, 1486-1487, 1998.
32) Abdel-Hafez, A. A., Meselhy, M. R., Nakamura, N., Hattori, M., Watanabe, H., Mohamed, T., Mahfouz, N. and El-Gendy, M. A. : Anticonvulsant activity of paeonimetabolin-I adducts obtained by incubation of paeoniflorin and thiol compounds with *Lactobacillus brevis*. *Biol. Pharm. Bull.* **22**, 491-497, 1999.
33) Kawata, Y., Ma, C., Meselhy, M. R., Nakamura, N., Wang, H., Hattori, M., Namba, T., Satoh, K. and Kuraishi, Y. : Conversion of aconitine to lipoaconitine by human intestinal bacteria and their antinociceptive effects in mice. *J. Trad. Med.* **16**, 15-23, 1999.
34) Kida, H., Nakamura, N., Meselhy, M. R., Akao, T. and Hattori, M. : Isolation and identification of human intestinal bacteria capable of hydrolyzing saikosaponins. *Journal of Trad. Med.* **14**, 34-40, 1997.
35) Shu, Y., Hattori., M., Akao, T. Kobashi, K. and Namba, T. : Metabolism of paeoniflorin and related compounds by human intestinal bacteria. III. Metabolic ability of intestinal bacterial strains and fecal flora from different individuals. *J. Med. Pharm. Soc. WAKAN-YAKU* **4**, 82-87, 1987.
36) El-Sedawy, A. I., Hattori, M., Kobashi, K. and Namba, T. : Metabolism of sweroside from *Swertia japonica* by human intestinal bacteria. *Shoyakugaku Zasshi* **44**, 122-126, 1990.
37) Kawata, Y. Hattori, M., Akao, T., Kobashi, K. and Namba, T. : Formation of nitrogen-containing metabolites from geniposide and gardenoside by human intestinal bacteria. *Planta Med.* **57**, 536-542, 1991.
38) Sneath, P. H., Mair, N. S., Sharpe, M. S. and Holt, J. G. : "Bergey's manual of systematic bacteriology". Williams & Wilkins, Baltimore, pp. 1418-1434, 1986.
39) Mitsuoka, T. : "A color atlas of anaerobic bacteria". Shoubunsha Co., Tokyo, pp. 146-155, 1980.
40) Kida, H., Akao, T., Meselhy, M. R. and Hattori, M. : Enzynes responsible for the metabolism of saikosaponins from *Eubacterium* sp. A-44, a human intestinal anaerobe. *Biol. Pharm. Bull.* **20**, 1274-1278, 1997.
41) Walker, D. E. and Axelrod, B. : Evidence for a single catalytic site on the "β-D-glucosidase-β-D-galactosidase" of almond emulsin. *Arch. Biochem. Biophys.* **187**, 102-107, 1978.
42) Heyworth, R. and Walker, P. G. : Almond-emulsin β-D-glucosidase and β-D-galactosidase. *Biochem. J.* **83**, 331-335, 1962.
43) Levvy, G. A. : The β-D-fucosidase activity of mammalian tissues and its relation to other glycosidases. *Nature* **187**, 1027, 1960.
44) Srisomsap, C., Svasti, J., Surarit, R., Champattanachai, V., Sawangareetrakul, P., Boonpuan, K., Subhasitanont, P. and Chokchaichamnankit, D. : Isolation and characterization of an enzyme with β-glucosidase and β-fucosidase activities from *Dalbergia cochinchinesis Pierre*. *J. Biochem.* **119**, 585-590, 1996.
45) Arai, K., Kanaseki, T. and Ohkuma, S. : Isolation of highly purified lysosomes from rat liver: identification of electron carrier components on lysosomal membranes. *J. Biochem.* **110**, 541-547, 1991.
46) Melgar, M. J., Cabezas, J. A. and Calvo, P. : Kinetic studies on β-D-fucosidase of *Littorina littoria* L. *Comp. Biochem. Physiol.* **80B**, 149-156, 1985.
47) Kida, H., Akao, T., Meselhy, M. R. and Hattori, M. : Metabolism and pharmacokinetics of orally administered saikosaponin b_1 in conventional, germ-free and *Eubacterium sp.* A-44-infected gnotobiote rats. *Biol. Pharm. Bull.* **21**, 588-593, 1998.
48) Takeda, S., Wakui, Y., Mizuya, Y., Ishihara, K., Amagaya, S., Maruno, M. and Hattori, M. : Gastrointestinal absorption of paeoniflorin in germ-free rats. *J. Trad. Med.*, **13**, 247-251, 1996.
49) Shibata, M., Yoshida, R., Motohashi, S. and Fukushima, M. : Pharmacological studies on *Buplerum falcatum* L. IV. Some pharmacological effects of crude saikoides, saikogenin A and syrupy residue. *Yakugaku Zasshi* **93**, 1660-1667, 1973.
50) Cheng, J. T. and Tsai, C.-L. : Anti-inflammatory effect of saikogenin A. *Biochem. Pharmacol.*, **35**, 2483-2487, 1986.
51) Yokoyama, H., Hiai, S. and Oura, H. : Chemical structures and corticosterone secretion-inducing activities of saikosaponins. *Chem Pharm. Bull.* **29**, 500-504, 1981.