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Determination and Conversion of Saikosaponins from Bupleuri Radix

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Abstract—Various determination methods of saikosaponins in Bupleuri Radix are discussed, and the content of each saikosaponin which is original and/or artifact is rearranged. The changes of saikosaponin a and d into saikosaponin b_1 and b_2, respectively, under gastric pH, were studied.

Keywords—Saikosaponins · Bupleuri Radix · convertion · gastric pH · determination method.

Bupleuri Radix is recorded as "昆胡" in 'Sinnong-Boncho-Kyong'(神農本草經) and since that time, has been utilized in many prescrip tions in many oriental medicinal books. In 'Bang-Yak-Hap-Pyon'(方藥合編)[1], there are the below contents. That is, "柴胡 味苦 瀉肝火寒熱往來瘧疾可". This means that this crude drug is concerned with liver and/or febrile diseases. "外感生用 內傷酒炒 咳汗蜜水炒 肝膽火 猪膽炒" implies that the processing method is different according to the usage. In addition, there is "惡皂角 畏藜蘆 忌銅鐵" in the book. This means that the combination of Gleditsiae Spina and Veratri Rhizoma et Radix with Bupleuri Radix must be considered carefully, and that this crude drug must be treated without copper and iron. There are many crude drugs as the above, we all are sensible of our responsibility for investigating them scientifically or with modern meanings, developing oriental medicines for new therapeutics, and further, contributing to human health.

Bupleuri Radix which contains much fat/oil and is less lignified is regarded as an excellent product. The components of Bupleuri Radix are as fol lowed; fat/oil (about 2% including linoleic, linolenic, oleic and stearic acid), steroids (α-spinasterol, stigmasterol, Δ^7- and Δ^4-stigmasterol), sugar (adonitol), coumarin ((-)anomalin), and saikosaponins and their aglycones. Among them, up to now, saikosaponins are regarded as major active components and standard materials in the quality control of preparations.

As the above mentioned, from ancient times, the usage of Bupleuri Radix was different according to the processing, and at present, this is also admitted as an important fact that saikosaponins are converted chemically in the course of decoction. There are many reports concerned with the conversion of saikosaponins and about the determination using this character.

Chemical Change of Saikosaponins

Saikosaponins are composed of saikosaponin a and its two acetates whose genin is saikogenin F, saikosaponin c and e whose genin is saikogenin E, and saikosaponin d and its two acetates whose genin is saikogenin G. These saikosaponins are converted into dienes and/or methoxyl compounds in the course of extraction or acid

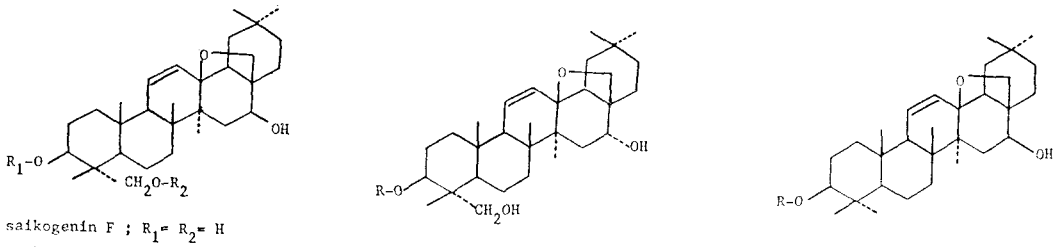

Fig. 1. Saikosaponins and their aglycones from Bupleuri Radix.

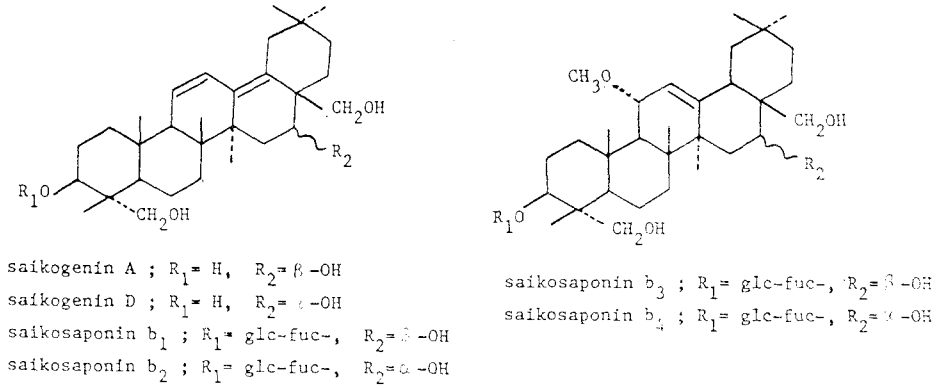

Fig. 2. Artificial saikosaponins and saikogenins.

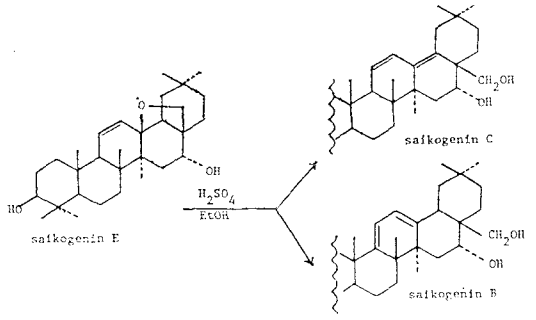

Fig. 3. Conversion of saikogenin E into saikogenin B and C[10].

treatment.

From saikosaponin a, saikosaponin b_1, saikogenin A (diene) and saikosaponin b_3 (methoxyl derivative) are made. Saikosaponin b_2, saikogenin D (diene) and saikosaponin b_4 (methoxyl derivative) are made from saikosaponin d. Saikogenin E is changed into two diene derivatives, saikogenin C and B, when treated with acid (Fig. 1~3).

In addition, saikosaponin a and d are converted into saikosaponin b_3 and b_4 respectively, when treated with 0.05% TsOH/MeOH. Saikosaponin b_3 and b_4 are converted into saikosaponin a and d with 0.05% TsOH/dioxane, and into saikosaponin b_1 and b_2 with 5% HCl/MeOH[2].

Determination Method of Saikosaponins

The first determination of saikosaponins was a colorimetry by using *p*-dimethylaminobenzaldehyde by A. Akahori *et al.*[2], afterwards, they introduced the preparative TLC method. S. Hiai *et al.*[6] also determined the contents of saiko-

saponins by means of colorimetry that saikosaponin a and d colored in greenish blue and saikosaponin c in red purple by vanillin-sulfuric acid.

Droplet counter current chromatography has been known as an excellent method for separation of natural products. O. Hideaki *et al.*[7] used this method with the solvent system, chloroform-benzene-ethylacetate-methanol (45-2-3-60-40), for separation and quantitative analysis of saikosaponins, and showed a good separation of saikosaponin a, c and d.

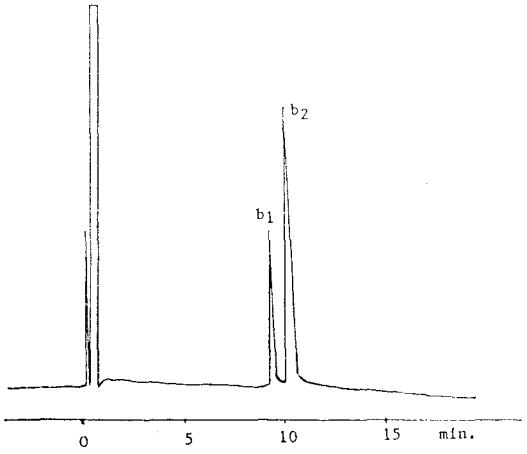

Fig. 4. High performance liquid chromatogram of saikosaponin b_1 and b_2.
column; Lichrosorb NH_2 (4mm i.d. 25cm)
eluent; $CHCl_3$: MeOH : H_2O: 2%NaOH(40: 10:1:0.1)
flow rate; 1.2ml/min.
detector; UV 254nm
temp; room temperature

H. Kimata *et al.*[8] applied HPLC with ODS column to analysis of saikosaponins after they changed saikosaponin a and d into saikosaponin b_1 and b_2 respectively, with treatment of acid. H. Kaizuka *et al.*[9] utilized silica column instead of ODS column but did not gain a good separation of saikosaponin a, c and d.

Authors used NH_2 column contrary to the above and gained a good separation of saikosaponin b_1 and b_2, with shorter retention times in HPLC. Small amount of alkali solution in solvent system was an important factor for the excellent resolution (Fig. 4). We think this condition will be helpful to the quality control of Bupleuri Radix and its preparations.

Contents of Saikosaponins

Up to now, there are many reports about determination of saikosaponins from Bupleuri Radix and its preparations. Some of those results are rearranged as followed tables[2-5] (Table I～IV).

Table I shows saikosaponin d was extracted largely in the condition of 2% KOH in methanol. We can see that the original contents of saikosaponin a and d in methanol extract were double to triple of those in water extract, but the content of saikosaponin b_1 in water extract of chinese Bupleuri Radix was strangely much in Table II. Table III shows that the contents of saikosaponin a and d were the highest in '柴胡

Table I. Yields of saikosaponins by extraction with different conditions (TLC, A. Akahori et al.)

Condition	Solvent	Saikosaponin (mg/g) a	d
3g/120ml, 13hrs.	MeOH	3.81	1.87
Soxhlet	BuOH	1.93	0
7g/200ml, 1hr.	water (pH5.0)	0.74	0
water bath	water (pH 10.0)	0.77	0.15
	2% KOH-water	1.43	0.95
	2% KOH-MeOH	3.14	3.55

* Note : The used material is Chinese Bupleuri Radix

Table II. Saikosaponin content of commercial Bupleuri Radix by HPLC after TLC (S. Arichi et al.)

Source	Extract	a	b_1	b_3	d	b_2	b_4	(a)#	(d)#
Korean	MeOH ext.	3.80	—	0.09	2.84	0.25	0.25	3.89	3.34
	water ext.	1.11	0.06	0.03	0.13	0.91	0.07	1.20	1.11
Chinese	MeOH ext.	2.24	—	—	2.19	0.19	0.22	2.24	2.60
	water ext.	0.62	1.70	—	0.11	0.82	0.04	2.32	0.97
Japanese	MeOH ext.	1.83	—	—	1.57	0.17	0.11	1.83	1.85
	water ext.	0.58	0.01	—	0.08	0.59	—	0.59	0.67

* Note : The extraction condition was 7g/300ml, boiling for 30~60min. (S. Arichi et al.).
The unit of data is mg/g crude drug.
(a)# and (d)# are the sum of a, b_1 and b_3, and d, b_2 and b_4 repectively.

Table III. Content and composition of saikosaponins of Bupleuri Radix and its preparations (HPLC after TLC, S. Arichi et al.)

Sample	a	b_1	d	b_2 (mg/g)
Bupleuri Radix (Chinese)	0.46	0.24	0.09	0.84
So-Si-Ho-Tang (小柴胡湯)	0.40	0.46	0.10	0.10
Dae-Si-Ho-Tang (大柴胡湯)	0.25	0.57	0.12	0.90
Siho-Kyeji-Kongang-Tang (柴胡桂枝乾姜湯)	0.65	0.12	0.05	0.70
Siho-Kyeji-Tang (柴胡桂枝湯)	0.58	0.16	0.06	0.86
Siho-ga-Yonggol-Moryo-Tang (柴胡加龍骨牡蠣湯)	0.68	0.04	0.20	0.58

* Note : The extraction condition was the same as 'Sang-Han-Non' (傷寒論). The determination method was by HPLC after TLC of saikosaponin fractions.

Table IV. Contents of saikosaponins in the MeOH and water extracts according to age and a place of origin (HPLC, K. Shimizu et al.)

Origin	Extract (age)	a	b_1	d	b_2 (mg/g)
Korean	MeOH (3 year)	3.07	0	2.21	0
	(2 year)	3.35	0	2.43	0
	water (3 year)	0.61	0	0	0.30
	(2 year)	0.90	0	0	0.54
Chinese	MeOH (3 year)	1.06	0	1.05	0
	(2 year)	1.09	0	1.04	0
	water (3 year)	0.34	0	0	0.38
	(2 year)	0.35	0	0	0.32
Japanese	MeOH (3 year)	2.46	0	1.96	0
	(2 year)	0.71	0	0.11	0
	water (3 year)	0.52	0	0	0.30
	(2 year)	0.45	0	0	0.33
Sosiho-Tang	(3 year)	0.29	0	0	0.26
(Korean)(柴胡湯)	(2 year)	0.27	0	0	0.20

* Note : The extraction condition was 8g/120ml 3% pyridine-MeOH, or 8g/120ml water by reflux for 1 hour. Sosiho-Tang contained 8g was extracted with 120ml water by reflux for 1 hour.

加龍骨牡蠣湯', but those of saikosaponin b_1 and b_2 were the highest in '大柴胡湯'. From the result, we can suggest that expected effects of each prescription may be changed even if in the level of saikosaponins. According to Table IV, the contents of saikosaponins in 3 year Bupleuri Radix from each country are nearly the same as 2 year Bupleuri Radix respectively. But the contents of saikosaponin a and d in the MeOH extract of 3 years old Bupleuri Radix japanese are strangely high. As shown in Table V, authors determined the contents of saikosaponin a and d through saikosaponin b_1 and b_2 respectively, on the basis that saikosaponin a and d are converted quantitatively into saikosaponin b_1 and b_2. The content of each saikosaponin in Korean Bupleuri Radix (*Bupleurum falcatum*) were nearly the same as results of other reports.

Table V. Contents of saikosaponin a and d in Korean Bupleuri Radix (*Bupleurum falcatum*) (% in crude drug)

Extract	Saikosaponin b_1 (=a) (%)	average	Saikosaponin b_2 (=d) (%)	average
MeOH ext.	0.62	0.62	0.35	0.36
	0.61		0.36	
	0.62		0.37	
Water ext.	0.30	0.30	0.21	0.22
	0.29		0.22	
	0.30		0.22	

Authors investigated the change of saikosaponin a and d into saikosaponin b_1 and b_2 under gastric pH by using our analysis condition with HPLC. Since saikosaponin a and d have the acid labile allyl oxide linkage, the conversion in the stomach may be a significant factor of study on biological activity and quality control of preparations including Bupleuri Radix. After traditional extraction, the content sequence of saikosaponin was $a>d>b_2>b_1$, but after treated with gastric pH for 2 hours, $a>b_2>b_1>d$. (Table VI). In other words, saikosaponin b_1 was 6.7% of the original content of saikosaponin a in extract, but 33% after treated with gastric pH. Saikosaponin b_2 was 32% of the original content of saikosaponin d in extract, but 63% when treated with gastric pH.

Discussion

Though method of extraction was a little different from each other, the content of saikosaponins differed largely according to a place of origin. This suggests that expected effects of preparations including Bupleuri Radix may be deviated even if in the same area of orient.

Period, water volume and temperature for extraction must be considered in the viewpoint of the content and chemical conversion of saikosaponins.

Saikosaponin a and d are different from saikosaponin b_1 and b_2 in biological activity.

Table VI. Change of saikosaponin a and d into saikosaponin b_1 and b_2 under gastric pH (Korean Bupleuri Radix) (% in crude drug)

	Saikosaponin							
	b_1 (%)	average	b_2 (%)	average	a (%)	average	d (%)	average
In extract	0.02	0.02	0.07	0.07	0.28	0.28	0.15	0.15
	0.02		0.06		0.28		0.16	
	0.02		0.07		0.28		0.15	
After treatment	0.10	0.10	0.14	0.14	0.20	0.20	0.08	0.08
	0.09		0.14		0.21		0.08	
	0.11		0.13		0.19		0.09	

For example, the activity of saikosaponin a and d on biomembrane and erythrocyte membrane are stronger than saikosaponin b_1 and b_2, but on Arthus' reaction, saikosaponin b_1 and b_2 are stronger. Accordingly, if we expect the effect of saikosaponin b_1, the utilization of '小柴胡湯' or '大柴胡湯' is more meaningful than Bupleuri Radix only.

In this connection, we think if we try to endow Bupleuri Radix preparations with scientific meaning, we have to analysis and explain preparations and their activities after conditions of manufacturing have become regular, that is, standardization.

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