IDENTIFICATION AND DETERMINATION OF GINSENG SAPONINS, PROSAPOGENINS AND SAPOGENINS FROM CRUDE DRUG PREPARATIONS FOR QUALITY CONTROL

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ABSTRACT

Ginseng saponins have been known as main active principles and analyzed as the index components in ginseng and its products for quality control. But it is generally difficult to analyze the saponins in crude drug preparations. Saponins, Prosapogenins and sapogenins of crude drug preparation were identified by TLC and determined quantitatively by HPLC. Prosapogenins - Rg_3, - Rg_2 and $\triangle^{20}$ - prosapogenin were extracted with ethyl acetate from 50% acetic acid hydrolyzates of saponin fractions and identified by TLC with lower phase of $CHCl_3$/MeOH/H_2O(65 : 35 : 10, v/v)on silica gel plate, and quantified by HPLC on Lichrosorb - NH_2 column with CH_3CN/H_2O(90 : 10, v/v). Sapogenins, panaxadiol and panaxatriol, were extracted with ethyl ether from 7% - sulfuric acid hydrolyzates of saponin fractions and identified by TLC with chloroform/acetone(1 : 1, v/v) on silica gel plate, and quantified by HPLC on μ - Bondapak C_{18} column with CH_3CN/MeOH/$CHCl_3$(83 : 10 : 7, v/v). These analyses of prosapogenins and sapogenins are more useful for quality control than those of saponins in crude drug preparations such as So - Shi - Ho - Tang(小柴胡湯), Sa - Kun - Ja - Tang (四君子湯), Yook - Kun - Ja - Tang(六君子湯) and In - Sam - Tang(人參湯)

INTRODUCTION

From the ancient times oriental people have traditionally used crude drug preparations, mostly as tang(decoction, 湯), to prevent or cure their diseases. But recently crude drug preparations have been changed to the types of extract granule and drink, etc. for the convenient dosage of the preparations. Therefore, quality controls for the effective components of crude drug preparations have been performed in manufacturing fatories and inspection authorities. Ginseng saponins, which have been known to occur in the plants of *Panax* genus, have many pharmacological efficacies and have attracted a great deal attention as the effective components.[1, 2]

Ginseng saponins are dammarane - type triterpenoid glycosides whose aglycones are bonded with glucose, rhamnose, arabinose or xylose[2, 3]. Saponins of ginseng are generally different from those of other plants not only in chemical structures but also in pharmacological efficacies[2]. From Korean red ginseng, the chemical structures of 25 ginsenosides[2, 4] have been determined so far and total saponins or major saponins such as ginsenosides - Rb_1, - Rb_2, - Rc, - Rd, - Re and - Rg_1 are analyzed for the quality control of ginseng and its products[5]. Therefore, studies on the determination of ginseng saponin by weighing[1, 6], colorimetry[7, 9], preparative TLC[9], TLC - scanner[10, 11], rod - TLC - FID[12, 13], GLC[14, 15], HPLC[16 - 18], radiochemistry[19] and immunochemical assay[21], etc. were reported. But only a few studies were carried out on the analysis of ginseng saponin compounds in crude drug preparations. Accordingly, these studies as a part of studies on the quality control for crude drug preparations, were performed to establish the conditions for the analysis of ginseng saponin, prosapogenin and sapogenin.

MATERIALS AND METHODS

Ginseng and crude drugs : Red ginseng was made of 6 - year - old ginseng in Korea Tobacco and Ginseng Corporation and crude drugs were purchased from wholesale medicinal herb-store under the confirmation of professional advice to use for the studies.

Reagents : HPLC grade(E. Merck Co.) of acetonitrile, n - butanol and distilled water for HPLC analysis, silica gel 60 precoated aluminum sheet (E. Merck, Art. 5554, layer thickness 0.2mm) for TLC and silica gel(E. Merck Co., 70 - 230mesh)for column chromatography were used.

Crude drug preparations : Crude drugs were mixed in a vessel according to the ratios described in chinese traditional prescription and 10 times volume of water(v/w) was added to extract at 75±2℃ for 8 hours, then 5 times volume of water added twice more to extract as the above descrived method and centrifuged on 10,000rpm(9,200×g). Supernatant was evaporated to 40% of water content under 70℃ to give water extract. Corn starch was added to the water extract at the same amount to each other, mixed and dried at 60℃ to make the granule of So - Shi - Ho - Tang(小柴胡湯), Sa - Kun - Ja - Tang(四君子湯), Yook - Kun - Ja - Tang(六君子湯) and In - Sam - Tang(人蔘湯).

Table 1. Preparations of crude drug extracts with ginseng and crude drugs

(unit : gram dosage/day)

Crude drug (生藥材)	So - Shi - Ho - Tang (小柴胡湯)	Sa - Kun - Ja - Tang (四君子湯)	Yook - Kun - Ja - Tang (六君子湯)	In - Sam - Tang (人蔘湯)
Ginseng Radix(紅蔘)	3.0	4.0	4.0	3.0
Bupleuri Radix(柴胡)	7.0	–	–	–
Pinellia Tuber(半夏)	5.0	–	4.0	–
Scutellariae Radix(黃芩)	3.0	–	–	–
Glycyrrhizae Radix(甘草)	2.0	1.5	1.5	3.0
Raw Ginger(生薑)	4.0	1.5	4.0	–
Zizyphi Fructus(大棗)	3.0	2.0	2.0	–
Atractylodes Rhizome(白朮)	–	4.0	4.0	3.0
Hoelen(茯苓)	–	4.0	4.0	–
Citrus Unshiu Peel(陳皮)	–	–	2.0	–
Ginger(乾薑)	–	–	–	3.0
Total	27.0	17.0	22.5	12.0

Isolation of the standards of prosapogenin and sapogenin : Prosapogenin - Rg_3 was obtained by the method of Kaku[23]. The mixture of ginsenosides - Rb_1, - Rb_2 and - Rc was hydrolyzed with 50% acetic acid and filtered. The filtrate was recrystalized with 60% dioxane and the supernatant was extracted with n - butanol, evaporated and subjected to column chromatography on silica gel using chloroform/methanol/water(75/25/10 to 70/30/10, lower phase) to give 20(R) - prosapogenin - Rg_3 and 20(S) - prosapogenin - Rg_3, respectively.

Ginsenoside - Re was hydrolyzed with 50% acetic acid and column chromatographed on silica gel using chloroform/methanol/ethyl acetate/water(2/2/4/1, lower phase) to give prosapogenin - Rg_2. Prosapogenin - Rg_2 was more purified by recrystalizing with etanol.

On the other hand, ginseng saponin mixture was hydrolyzed at 100℃ for 6 hours with 50% ethanolic 7% - sufuric acid to give sapogenin fraction[1,2], which was repeatedly subjected to column chromatography on silica gel using benzene/acetone(4 : 1) to yield panaxadiol and panaxatriol, respectively.

The chemical structures of the prosapogenins and sapogenins isolated were identified by the comparison of ^{13}C - NMR(75 MHz, d_5 - pyridine) data of authentic samples.

Isolation, identification and determination of ginseng saponin, prosapogenin and sapogenin in crude drug preparations

a) Fractionation of saponin, prosapogenin and sapogenin : Crude saponin[1] was obtained from n - butanol soluble fraction of 80% methanol extract of ginseng.

Prosapogenins[23] were obtained by refluxing crude saponin at 70℃ for 2 hours with 50% acetic acid and then extracting with ethyl acetate.

Sapogenins[1,2] were obtained by refluxing crude saponin at 100℃ for 6 hours with 50% ethanolic 7% sulfuric acid and then extracting with ethyl ether.

b) TLC of saponin, prosapogenin & sapogenin : Saponin and prosapogenin fractions were chromatographed on silica gel plate using Chloroform/Methanol/Water(65/35/10, lower phase) and sapogenin fraction was chromatographed on silicagel plate using chloroform : acetone(1 : 1, v/v).

The spots were visualized by spraying with 30% H_2So_4 and heating the plate at 110℃ for 15 min.

c) HPLC of saponin, prosapogenin and sapogenin : For the analysis of saponin, prosapogenin and sapogenin by HPLC were used waters Associates Model 510 and Differential Refractometer RI 410 detector. For the analysis of saponin and prosapogenin Lichrosorb NH_2 column(10um, 25cm×0.46mm, Merck Co.) was used with the solvent of acetonitrile/water/n - butanol(80/20/10, v/v)[5] and acetonitrile/water(90/10, v/v), respectively. For the analysis of sapogenin μ - Bondapak C_{18} column(3.9mm×30cm, Waters Co.) was used with the solvent of acetonitrile/water/methanol/chloroform(73/20/6/1, v/v).

RESULTS AND DISCUSSION

Identification and determination of ginseng saponin in crude drug preparations : Major ginseng saponins, "ginsenosides - Rb_1, - Rb_2, - Rc, - Rd, - Re and - Rg_1", etc were deteteted clearly in water extract of red ginseng, but not easily detected in crude drug preparations. From So - Shi - Ho - Tang which contains saikosaponins[24] in high amounts of Bupleuri Radix(Shi - Ho, 柴胡), especially, ginseng saponins were scarcely detected due to overlapping with saikosaponins. Moreover, ginseng saponins were decreased in crude drug preparations and then more hydrolyzed in these crude drug extracts manufactured by traditional extraction and evaporation method of high temperature to 100℃. As shown in Fig. 1, major saponins were decreased conspicuously and reduced to prosapogenins which have no glucosidic bonds at C - 20 position of sapogenin.

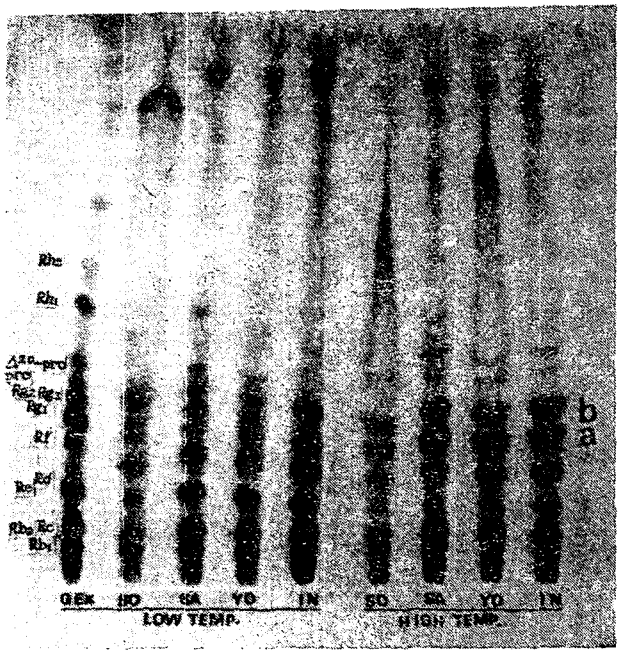

Fig. 1. Thin layer chromatograms of saponin fractions of crude drug extract granules.
a) Silica gel 60 plate developed in solvent system(chloroform/methanol/water=65 : 35 : 10, lower phase) and detected with 30% sulfuric acid spray and heating at 115℃ for 10 minutes.
b) Methods of extraction and concentration : LOW TEMP : extracted and concentrated at 75℃ water bath, HIGH TEMP : extracted and concentrated at 100℃ water bath.
c) The samples were as follows : GEX. water extract of red ginseng. SO : So - Shi - Ho - Tang, SA : Sa - Kun - Ja - Tang, YO : Yook - Kun - Ja - Tang, IN : In - Sam - Tang.
d) Spot a : mixture of prosaponin - Rg_3 and - Rg_2, Spot b : $\triangle^{20}$ - prosapogenin.

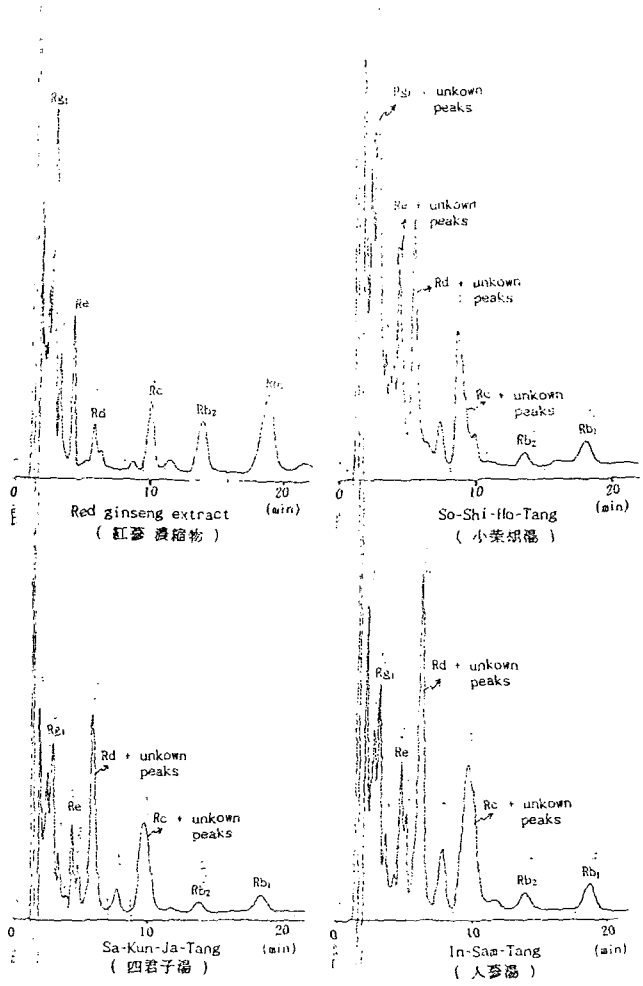

Fig. 2. HPLC chromatograms of saponin fractions from red ginseng extract and crude drug extract granules.
* HPLC conditions ; Column : Lichrosorb - NH_2(4.6 mm I.D×250mm, 10um), Mobile phase : acetonitrile/water/n - butanol(80 : 20 : 10, v/v), Flowrate : 1.0ml/min. Detector : RI

HPLC analysis showed good determination of 6 ginsenosides in ginseng extract. But in crude drug preparations the contents of ginsenosides - Rc, - Rd, - Re and - Rg_1 couldn't be determined because the peaks of saponins were overlapped with those of crude drug components.

As seen on the table 2, saponin contents were decreased remarkably in crude drug preparations compared with from ginseng. Among the preparations In - sam - tang," the simplest composition of crude drugs", showed the highest content of sapo-

Table 2. Contents of major ginseng saponins from the crude drug extract granules

(unit : mg/g)**

Content / Product	Ginsenoside					
	Rb_1	Rb_2	Rc	Rd	Re	Rg_1
Red ginseng extract(紅蔘 濃縮物)	4.65	2.25	2.29	1.01	2.76	3.64
So - Shi - Ho - Tang(小柴胡湯)	0.61	0.27	N*	N*	N*	N*
Sa - Kun - Ja - Tang(四君子湯)	0.98	0.47	N*	N*	1.48	1.96
Yook - Kun - Ja - Tang(六君子湯)	0.72	0.31	N*	N*	0.93	N*
In - Sam - Tang(人蔘湯)	1.58	0.77	N*	N*	2.43	2.57

*N : Not separated from the other components of crude drugs
** Ginseng saponin contents corresponding to 1g of red ginseng in the prescriptions of crude drug preparation

nin, while So - Shi - Ho - Tang did the lowest content of saponin. Ginsenosides - Rc, - Rd, - Re and - Rg_1 couldn't be determined by overlapping with other crude drug components.

Separation and identification of prosapogenins from ginseng saponin hydrolyzates : Ginseng saponins were hydrolyzed with 50% acetic acid at 70℃ for 60 minutes as seen on Fig. 3. As Shibata[2] and Kaku[23] reported, protopanaxadiol saponins—ginsenosides - Rb_1, - Rb_2, - Rc and Rd—gave prosapogenin - Rg_3 and $\triangle^{20}$ - prosapogenin and panaxatriol saponin—ginsenoside - Re—gave prosapogenin - Rg_2 with the acid hydrolysis of glucosidic bond at C - 20.

In other word, protopanaxadiol saponins—ginsenosides - Ra 1, - Rb_1, - Rb_2, - Rb_3, - Rc and Rd, etc.—have two glucoses at C - 3 but different sugars at C - 20. Therefore, if sugars at C - 20 are removed by hydrolysis, all the protopanaxadiol saponins will give the same prosapogenins with two glucoses only at C - 3. Accordingly, analysis of the prosapogenins is more useful for the quality control of a small quantity of ginseng saponins in crude drug preparation.

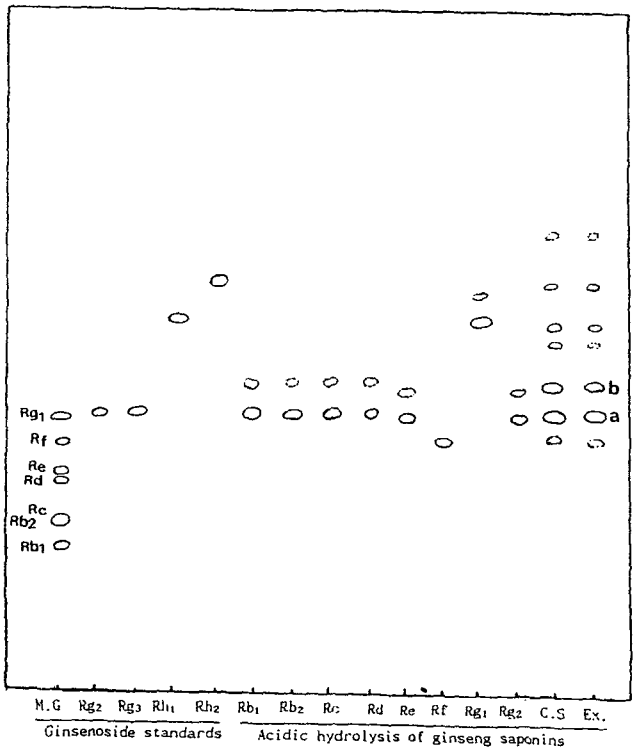

Fig. 3. Thin layer chromatograms of acidic hydrolyzates from ginseng saponins with 50% acetic acid.
a) Silica gel 60 plate developed in solvent system(chloroform/methanol/water=65 : 35 : 10, lower phase) and detected with 30% sulfuric acid spray and heating at 115℃ for 10 minutes.
b) The samples were as follows : M.G : major ginsenoside(mixture of ginsenoside - Rb_1, - Rb_2, - Rc, - Rd, - Re, - Rf, - Rg_2), C.S : ginseng crude saponin, Ex. : ginseng extract
c) Spot a : mixture of prosaponin - Rg_3 and - Rg_2, Spot b : $\triangle^{20}$ - prosapogenin.

And at the same time, prosapogenins from protopanaxatriol saponins can be also used in quality control of ginseng in crude drug preparations. When protopanaxatriol saponins are hydrolyzed, prosapogenin - Rg_2 is obtained from ginsenoside - Re, ginsenoside - Rf from 20 - gluco - ginsenoside - Rf and ginsenoside - Rh_1 from ginsnoside - Rg_1.

Prosapogenin mixtures were column chromatographed to give pure prosapogenins and analyzed by NMR. Glucosidic bonds at C - 20 position were hydrolyzed and two moles of glucoses at C - 3 position are found. As seen on Table 3 and Table 4, 20(R) - prosapogenin - Rg_3, 20(S) - prosapogenin - Rg_3 and $\triangle^{20}$ - prosapogenin are glycosides with two glucoses bonded to C - 3 position of protopanaxadiol, and prosapogenin - Rg_2 is a glycoside with a glucose and a rhamnose bonded to C - 6 of protopanaxatriol. Chemincal shifts at C - 23 have been reported to be 22 - 23 ppm, according to ^{13}C - NMR data[26] of protopanaxadiol and protopanaxatriol, which were genuine aglycones of ginseng saponins. However, the compound, $\triangle^{20}$ - prosapogenin, of Table 3 shows 25.6 ppm of chemical shift at C - 23, about 3 ppm lower than that of aglycones, suggesting that a double bond can occur around C - 23. ^{13}C - NMR data of the compound are almost identical to those of 20(S) ginsenoside - Rg_3 but two new signals of 140.1 ppm and 127.0 ppm at C - 20 and C - 22 tells the presence of a double hond.

Identification and determination of prosapogenins in crude drug preparations : Prosapogenins were detected on TLC from 50% acetic acid hydrolyzates of saponin fractions from ginseng extract, So - Shi - Ho - Tang, Sa - Kun - Ja - Tang, Yook - Kun - Ja - Tang and In - Sam - Tang. But the prosapogenins were not detected on TLC of contrast samples without ginseng(Fig. 4).

Prosapogenin analysis was carried out with Lichrosorb - NH 2 column, RI detector and a mobile phase of acetonitrile/water(90 /10, v/v) as seen in Fig. 5. Correlations of the yields of prosapogenin - Rg_3, $\triangle^{20}$ - prosapogenin and prosapogenin - Rg_2 from total crude saponins were linear and their coefficients of correlation were close to r=1, suggesting that this is a good method.

But 20(S) - and 20(R) - epimers of prosapogenin - Rg_3 or - Rg_2 were determined together because these epimers were separated each other by HPLC. These prosapogenins in the extract granules of So - Shi - Ho - Tang, Sa - Kun - Ja - Tang, Yook - Kun - Ja - Tang and In - Sam - Tang were also determined by the same conditions of HPLC, and the results for determination of prosapogenins are shown in Table 5. This method also enabled the analysis of ginseng saponin compounds in the granules of So - Shi - Ho - Tang, Sa - Kun - Ja - Tang, Yook - Kun - Ja - Tang and In - Sam - Tang by determing the prosapogenins with HPLC. The transfer rates of prosapogenins from the preparations were good as 66.4 - 93.6% except the So - Shi - Ho - Tang granule. Choi[25] reported that ginsenoside - Rb_1

Table 3. Aglycone moieties of prosapogenins

Carbon No.	20(R) - Rg_3	20(S)G - Rg_3	$\triangle^{20}$ - pro.	20(S)G - Rg_2
C - 1	39.1	39.1	39.2	40.0
C - 2	26.7	26.7	27.4	27.7
C - 3	88.9	88.9	88.9	78.5
C - 4	40.0	39.9	39.7	41.1
C - 5	56.4	56.4	56.4	60.8
C - 6	18.4	18.4	18.4	74.1
C - 7	35.2	36.7	35.3	46.0
C - 8	36.9	39.1	36.9	39.6
C - 9	50.6	50.6	50.4	49.7
C - 10	39.7	39.7	39.7	39.4
C - 11	32.2	32.1	32.2	32.2
C - 12	70.7	71.6	71.5	71.0
C - 13	49.2	49.2	49.6	48.2
C - 14	51.8	51.8	51.0	51.7
C - 15	31.4	31.4	32.2	31.3
C - 16	26.7	26.8	26.7	27.0
C - 17	50.4	56.4	49.6	54.6
C - 18	16.6	16.6	16.0	18.7
C - 19	16.4	16.4	16.4	17.6
C - 20	71.6	73.0	140.1	73.0
C - 21	22.6	26.6	26.7	26.8
C - 22	43.3	35.2	127.0	35.7
C - 23	22.8	22.7	25.6	22.9
C - 24	126.1	126.0	123.8	126.0
C - 25	130.7	130.7	131.2	130.7
C - 26	25.8	25.8	25.6	25.8
C - 27	17.7	17.6	17.6	17.6
C - 28	28.1	28.1	28.0	32.0
C - 29	15.8	15.8	15.8	17.1
C - 30	17.3	17.3	17.0	16.9

Table 4. Sugar moieties of prosapogenins

Carbon No.		20(R) - Rg_3	20(S)G - Rg_3	$\triangle^{20}$ - pro.	20(S)G - Rg_2
3 - Glc	1	105.1	105.1	105.1	–
	2	83.5	83.4	83.3	–
	3	78.2	78.1	78.2	–
	4	71.6	71.6	71.6	–
	5	77.9	77.8	71.6	–
	6	62.8	62.8	62.8	–
Glc	1	105.1	106.0	105.9	–
	2	77.1	77.1	77.0	–
	3	78.3	78.3	78.3	–
	4	71.6	71.6	77.0	–
	5	77.9	77.8	78.0	–
	6	62.8	62.8	62.6	–
6 - Glc	1	–	–	–	101.7
	2	–	–	–	78.5
	3	–	–	–	79.4
	4	–	–	–	72.9
	5	–	–	–	78.3
	6	–	–	–	65.1
Rha	1	–	–	–	101.9
	2	–	–	–	72.7
	3	–	–	–	72.0
	4	–	–	–	74.3
	5	–	–	–	69.4
	6	–	–	–	18.7

Table 5. Contents of ginseng prosapogenins from crude drug extract granules hydrolyzed with 50% acetic acid (unit : mg/g)*

Sample	Content	Prosapogenin		
		- Rg_3	$\triangle^{20}$	Rg_2
Red ginseng ext(紅蔘 濃縮物)		10.35	5.12	2.34
So - Shi - Ho - Tang(小柴胡湯)		4.13	2.01	0.88
Sa - Kun - Ja - Tang(四君子湯)		6.91	3.96	1.45
Yook - Kun - Ja - Tang(六君子湯)		6.81	3.27	1.37
In - Sam - Tang(人蔘湯)		9.86	4.75	2.23

* Prosapogenin contents corresponding to 1g of red ginseng in the prescriptions of crude drug preparation.

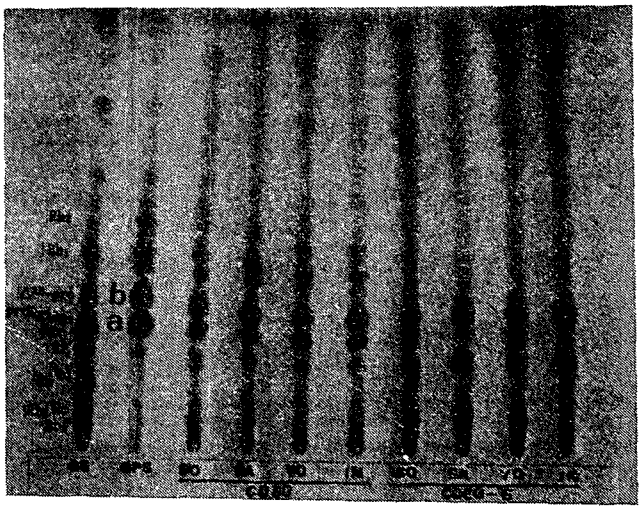

Fig. 4. Thin layer chromatograms of prosapogenins from various crude drug preparations.
a) Silica gel 60 plate developed in solvent system(chloroform : methanol : water=65 : 35 : 10, lower phase) and detected with 30% sulfuric acid spray and heating at 115℃ for 10 minutes.
b) The samples were as follows : GS : ginseng saponin, GPS : prosapogenins of red ginseng extract, So : So - Shi - Ho - Tang, SA : Sa - Kun - Ja - Tang, YO : Yook - Kun - Ja - Tang, IN - In - Sam - Tang.
c) Spot a : mixture of prosaponin - Rg_3 and - Rg_2, Spot b : $\triangle^{20}$ - prosapogenin.

in So - Shi - Ho - Tang was transferred by only 19.8% by saponin analysis and in the present experiment which prosapogenin - Rg_3 was determined instead of saponin, was also very low as 39.9%. Therefore, analysis of prosapogenin compounds in So - Shi - Ho - Tang which contain high amounts of saikosaponins of Bupleuri Radix need further improvement of the analysis.

Separation and identification of sapogenins of ginseng saponins : On the other hand, as Shibata[2] reported, ginseng saponins can be converted into two types of alycones, resulting from the cyclization of side chain at C - 20 position by hydrolyzing all the sugars honded to C - 3, C - 6 and C - 20 positions with 7% sulfuric acid in 50% ethanol. Namely, all the protopanaxadiol saponins give panaxadiol sapogenin, and all the protopanaxatriol saponins give panaxatriol sapogenin.

In order to examine the optimal hydrolysis time of the method, saponins were hydrolyzed with the times and then the yields of panaxadiol and panaxatriol were analyzed. The yields of the products increased till 5 hours of hydrolysis but found not to be any more later.

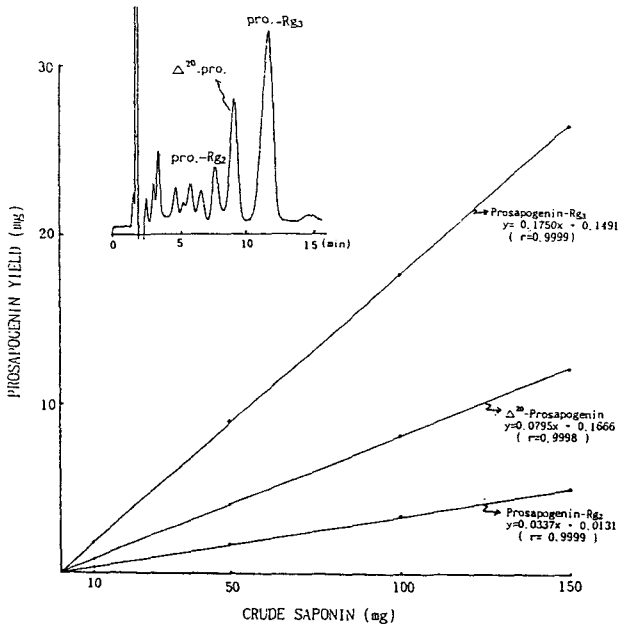

Fig. 5. Correlations of the prosapogenin yields from crude saponin by hydrolysis with 50% acetic acid
* HPLC conditions ; Column : Lichrosorb - NH_2(4.6 mm I.D×250mm, 10um), Mobile phase : acetonitrile/water/n - butanol(90 : 20 : 10, v/v), Flowrate : 1.0ml/min. Detector : RI

The end products of acid hydrolyzates were column chromatographed on silica gel to give sapogenins. Panaxadiol and panaxatriol with carbon numers of 30 were identified with NMR.

Table 6. Aglycone moieties of Panaxadiol and Panaxatriol

No.	Panaxadiol	Panaxatriol
C1	39.4	39.1
C2	28.6	27.0
C3	78.6	78.5
C4	39.4	38.9
C5	56.4	61.1
C6	19.8	68.6
C7	35.3	47.0
C8	40.1	40.1
C9	50.3	49.4
C10	37.5	39.2
C11	33.4	30.8
C12	70.3	69.8
C13	49.5	48.7
C14	51.5	51.0
C15	31.5	31.1
C16	27.5	26.4
C17	55.1	54.6
C18	16.6	18.2
C19	16.5	19.4
C20	73.4	73.1
C21	27.9	27.1
C22	36.1	35.7
C23	30.9	30.6
C24	36.8	36.4
C25	77.6	78.6
C26	25.5	25.1
C27	18.7	17.1
C28	30.9	32.9
C29	16.0	15.5
C30	17.4	17.0

Identification and determination of sapogenins in crude drug preparations : For the pretreament of sapogenin analysis in crude drug preparations, saponin fractions from the preparations were extracted and then hydrolyzed by 50% ethanolic 7% sulfuric acid. Because the direct hydrolysis of the preparations can cause to overlap the sapogenin spots with other components of crude drugs as seen in Fig. 8.

The sapogenin analysis was carried out with μ - Bondapak C_{18} column, RI detector and a mobile phase of acetonitrile/water/methanol/chloroform(73/20/6/1, v/v)as show in Fig. 9. Correlations of the yields of panaxadiol and panaxatriol were linear from total crude saponins and their correlative coefficients were close to r=1, suggesting that it is a good method.

Panaxadiol and panaxatriol of the hydrolyzates from the extract granules of So - Shi - Ho - Tang, Sa - Kun - Ja - Tang, Yook - Kun - Ja - Tang and In - Sam - Tang were determined by HPLC.

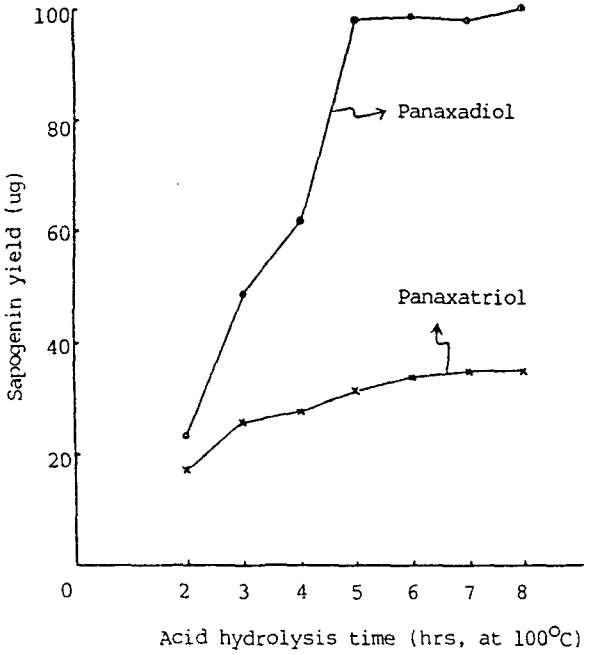

Fig. 6. Yields of sapogenins of on the times of acid - hydrolysis with 7% - sulfuric acid in 50% ethanol
* Five hundred ugs of ginseng crude saponins were hydrolyzed in each treatment

Saponins were transferred from the 4 kinds of preparations more by the hydrolysis after extraction of saponin fractions than before extraction of saponin as shown in Table 7. HPLC analysis also gave better determination of panaxadiol and panaxatriol in Sa - Kun - Ja - Tang. Yook - Kun - Ja - Tang and In - Sam - Tang than in So - Shi - Ho - Tang. It is probably due to the high content of saikosaponin in the preparation, to which high amount(7g) of Bupleuri Radix was added compared to 3g of ginseng based on the dose per day.

Accordingly, analysis of saponin compounds in So - Shi - Ho - Tang which contains high amounts of saikosaponins needs the improvement of pretreatment for the efficient fractionation of ginseng saponin. And the decrease of ginseng saponin components could be expected by the precipitations and absorptions

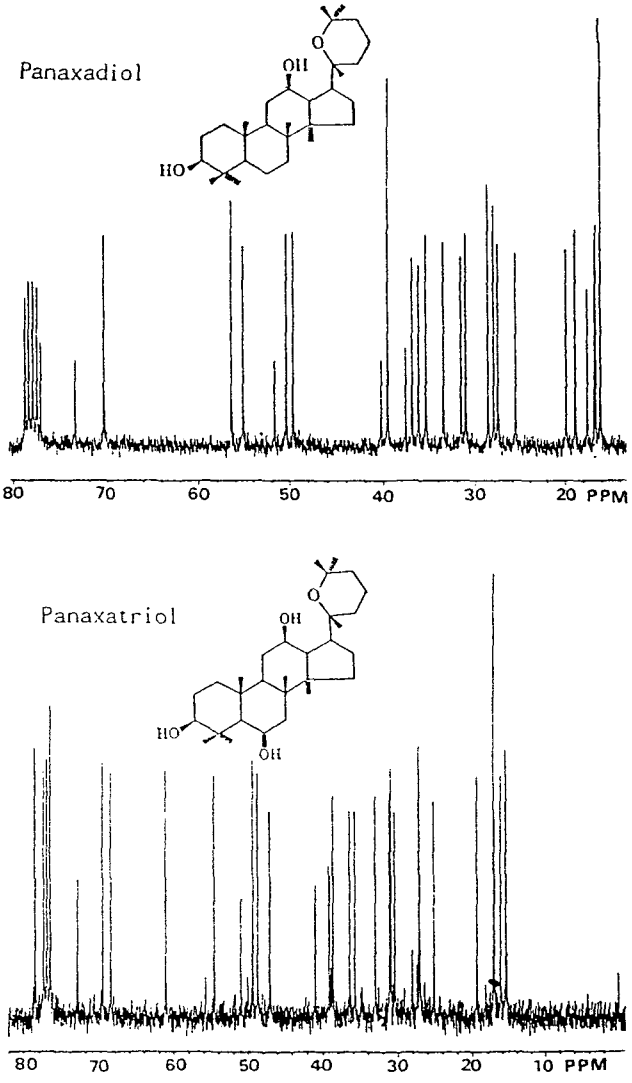

Fig. 7. C^{13} - NMR spectra of panaxadiol and panaxatriol

on the residue, etc in the manufacturing processes of extraction, filtration and centrifugation, etc.

Consequently, to identify and determine prosapogenins and sapogenins are more useful than to do saponins for quality control in these crude drug preparations.

Table 7. Contents of ginseng sapogenins from crude drug extract granules hydrolyzed with 50% etanolic 7% sulfuric acid

(unit : mg/g)*

Contents / Products	Direct hydrolysis of the granules		Hydrolysis of saponin fraction of the granules	
	Panaxadiol	Panaxatriol	Panaxadiol	Panaxatriol
Red ginseng ext(紅蔘 濃縮物)	7.85	2.09	9.37	2.67
So - Shi - Ho - Tang(小柴胡湯)	3.12	0.88	3.68	1.02
Sa - Kun - Ja - Tang(四君子湯)	5.15	1.30	6.18	1.67
Yook - Kun - Ja - Tang(六君子湯)	5.64	1.43	7.17	1.94
In - Sam - Tang(人蔘湯)	7.26	1.83	8.69	2.37

* Sapogenin contents corresponding to 1g of red ginseng in the prescriptions of crude drug preparations

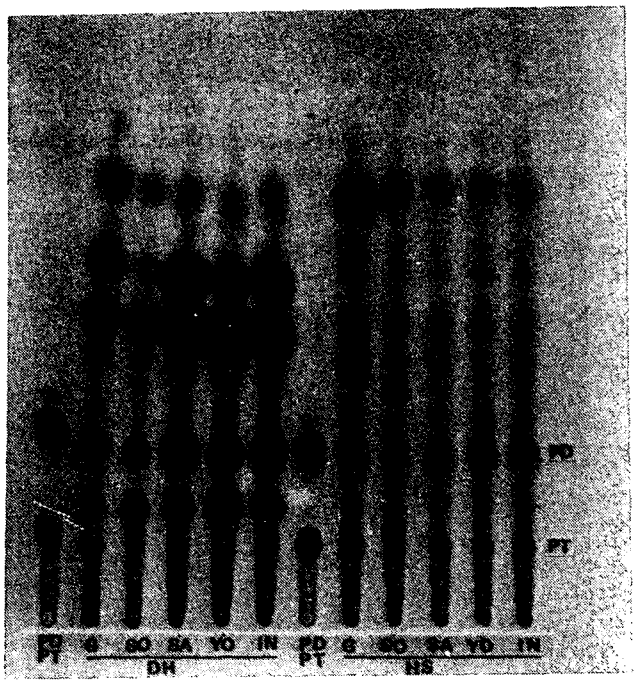

Fig. 8. Thin layer chromatograms of sapoganin fraction of crude drug extract granules.
a) Silica gel 60 plate developed in solvent system(chloroform : acetone = 1 : 1) and detected with 30% sulfuric acid spray and heating at 115℃ for 10 minutes.
b) Methods of hydrolysis : DH : direct hydrolysis of crud drug extract granule, HS : hydrolysis of saponin fraction from crude drug extract granule
c) The samples were as follows : PD : panaxadiol, PT : panaxatriol, So : So - Shi - Ho - Tang, SA : Sa - Kun - Ja - Tang, YO : Yook - Kun - Ja - Tang, IN : In - Sam - Tang

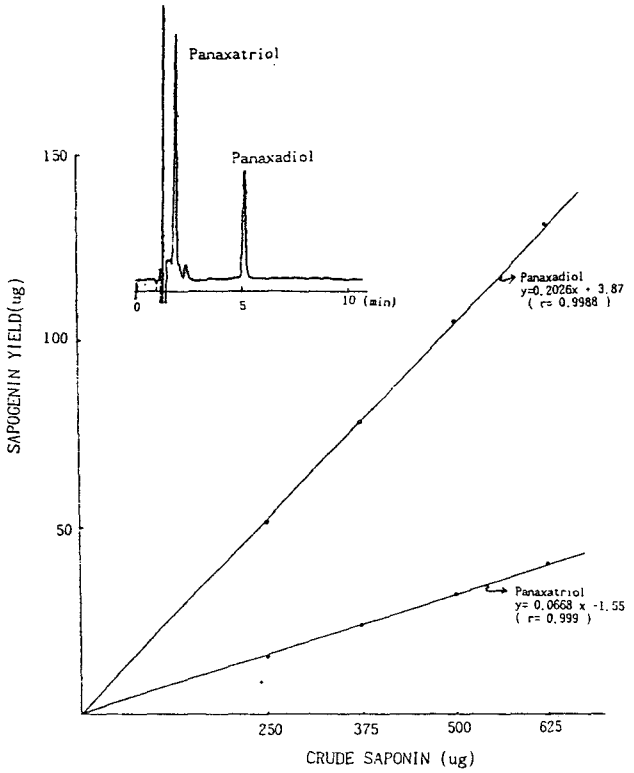

Fig. 9. Correlations of the sapogenin yields from ginseng crude saponin hydrolysis with 7% - sulfuric acid in 50% etanol.
* HPLC conditions : μ - Bondapack C_{18}(3.9mm I.D×300 mm),
Mobile phase : acetonitrile/water/methanol/chroroform(70 : 20 : 6 : 1, v/v), Flow rate : 1.5ml/min., Detector : RI

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