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Chemical and Biochemical Aspects of Dammarane Triterpene Glycosides of Korean Ginseng*

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The Korean ginseng (*Panax ginseng* C.A. MEYER), which has been known more than 2000 years, occupies the most important place among the tonic remedies for the Far Eastern Asian peoples. According to the folkloric experiences, its long life of tonic nature is due to the innocuousness, even with medication of unusual high dosage. Thus, a number of scientific reports appeared in order to establish the scientific supports for the efficacy of the Korean ginseng as a drug. Until recent decades, ginseng researches have found no definite guideline of study, because no reproducibilities and dose dependent response were obtained in every pharmacological screening test.

A few years ago, Brekhman *et al.*[1] reviewed the divergent articles on the ginsengstudies and proposed a hypothesis on the pharmacology of *Panax ginseng*. It describes that dammarane glycoside, one main component of *Panax ginseng* may exhibit an adaptogenic activity. Adaptogenic activity was defined essentially as the nonspecific normalizing activity irrespective of the direction of foregoing pathological shift. Although the validity of the hypothesis is still not completely recognized on the basis of modern experimental pharmacology, it is so much suggestive that it triggered intensive research on the chemical, pharmacological, and biochemical problems of the components. Many scientists have involved in these fields of research since then. In Japan remarkable achievements were made by Shibata on the phytochemical problems of dammarane glycosides, by Takagi *et al.* on the pharmacological problems, by Oura *et al.* on the physiological activities, in the U.S.S.R. by Elyakov on phytochemistry, and in Bulgaria by Petkov on pharmacology of the glycosides.

On the other hand, the authors have also participated in the studies of the chemical and biochemical problems of ginseng saponins in order to elucidate the mechanism of ginseng pharmacology which is said to be a panacea.

Comprehensive reviews on the chemistry[2], pharmacology[1,3], and biological activities[4,5] of various components of ginseng have already appeared elsewhere. Present review concerns with the further summary of the chemical, pharmacological, and biological studies on the ginseng saponins and a possible mechanism of action of ginseng components.

Chemistry of Dammarane Triterpene Glycosides—Elyakov *et al.*[6] found six glycoside

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spots on the one dimensional thin layer chromatogram of ginseng saponin fraction and designated them as panaxoside A, B, C, D, E, and F in the order of decreasing R_f values. Each panaxosides were further isolated by silicagel column chromatography and their partial structures were reported[7]. The panaxosides were divided into two groups: Panaxoside A, B and C belong to protopanaxatriol group and panaxoside D, E and F to protopanaxadiol group. The former group is less polar glycosides and has less number of sugar moiety than the latter's. The subsequent work with the same glycoside fraction by Shibata *et al.*[8] showed the presence of thirteen glycoside spots on the two dimensional thin layer chromatogram, and designated them as ginsenoside Rx (x; o, a, b_1, b_2, c, d, e, f, g_1, g_2, g_3, h_1, and h_2) in the increasing order of R_f-values. He isolated them by using preparative thin layer chromatography, silica-gel column chromatography, and droplet counter current distribution process. Their complete structures elucidated are shown in Table I[9,10].

Table I—Configurations of dammarane glycosides of *Panax ginseng*.

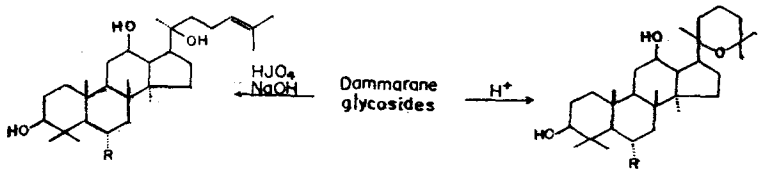
Ginsenoside Rx	Aglycone	C_3-Sugars	C_6-Sugars	C_{20}-Sugars
R_0	Oleanolic acid	β-Glucose-β-1→2-Glucose	β-Glu*	
Rb_1	Protopanaxadiol	β-Glucose-β-1→2-Glucose		β-Glu-β-1→6-Glu
Rb_2	Protopanaxadiol	β-Glucose-β-1→2-Glucose		β-Arap-β-1→6-Glu
Rc	Protopanaxadiol	β-Glucose-β-1→2-Glucose		β-Araf-β-1→6-Glu
Rd	Protopanaxadiol	β-Glucose-β-1→2-Glucose		β-Glucose
Re	Protopanaxatriol		β-Rham-β-1→2-Glu	β-Glu
Rf	Protopanaxatriol		β-Glu-β-1→2-Glu	
Rg_1	Protopanaxatriol		β-Glu	β-Glu
Rg_2	Protopanaxatriol		β-Rham-β-1→2-Glu	

* This is linked to the carboxyl radical at C_{28}.

Ginsenoside Rb_1, b_2, c, and d belong to protopanaxadiol glycosides consisting of common prosapogenin, protopanaxadiol-3β-diglucoside[8]. And ginsenoside Re, f, g_1, and g_2 belong to protopanaxatriol glycosides[10]. The chemical structure of ginsenoside Ra, g_3, h_1, and h_2 were not elucidated as yet.

While the authors attempted to isolate the substances having a property of stabilizing serum protein on heat denaturation, two dammarane triperpene glycosides were obtained in fine crystalline form and they were designated as panax sponin A (mp 208~10) and C (mp 196~202°) in the decreasing order of R_f-values. The partial structure of these saponins were determined to be protopanaxatriol diglucoside for panax saponin A[11] and protopanaxatriol-rhamno-diglucoside for panax saponin C[12]. The ultimate identifications of these saponins were conducted by direct comparison with the authentic sample of ginsenoside Rg_1 and Re which were kindly supplied by Shibata. Some controversies on the structure of ginsenoside Re remain. As shown in Table I, Shibata proposed an oligoside structure[10], protopanaxatriol-20S-β-glucosyl-6β-rhamno-β-1→2-glucoside, for the structure of ginsenoside Re. On the other

hand, we reported previously that panax saponin C consumes 6 moles of periodate. This suggested either the monoside structure or 1→6 type oligoside structure. The gas-chromatogram of methylated sugars obtained by permethylation of panax saponin C followed by methanolysis showed only the peaks of methyl-α-2, 3, 4, 6-tetra-methoxy-glucopyranose and methyl-2, 3, 4-trimethoxy-rhamnopyranoside with 2 : 1 ratio. Based on these results, the authors concluded that panax saponin C possessed the monoside as its partial structure. Further confirmation on the complete structure of the saponin is under progress.



protopanaxadiol (Ⅰ) : R = H

protopanaxatriol (Ⅱ) : R = OH

panaxadiol (Ⅲ) : R = H

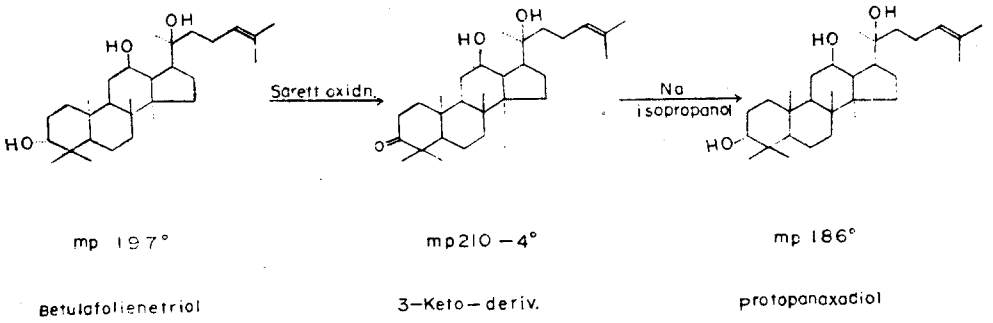
panaxatriol (Ⅳ) : R = OH

Scheme 1

Chemical studies on the dammarane triterpene sapogenins were conducted by Shibata[13~15] and Elyakov[16], independently. In the early days of ginseng chemistry, G.B. Elyakov and Shibata proposed a conflicting theory on the structures of panaxatrial and protopanaxatriol, however lately G.B. Elyakov withdrew his theory on the structures. The genuine sapogenins are protopanaxadiol and protophanaxatriol. These can be obtained by the Smith's degradation process[17], namely, periodate oxidation of sugar moiety followed by alkali treatment. The common acid catalyzed hydrolysis may produce artifact sapogenins, panaxadiol and panaxatriol by concurrent cyclization of side chain and by the epimerization of 20S to 20R configuration[18]. The chemical structures of these sapogenins were determined by usual spectral analysis and chemical analysis and finally by X-ray diffractometry.

Dammarane triterpenes are distributed in the plants of *Panax* genus[19], *Betulaceae*[20] and *Dipterocarpaceae* genus. The dammarane triterpene glycoside and their genuine aglycones are found only in the plants of Panax genus. The authors noticed once the structure of betulafolienetriol isolated from the unsaponificable fraction of fresh leaves of *Betula alba* by Fisher *et al*[20]., because it was found to be C_3-epimer of protopanaxadiol. The authors isolated the compound from the leaves of *Betula latifolia* Komarov[22] and transformed it to protopanaxadiol[23] by following simple chemical process.

Due to 3α-axial and 12β-equatorial hydroxyl configuration of betulafolienetriol, C^3-partial keto-derivative could be produced. by controlling oxydation with limited amount of Sarret reagent. Reduction of the 3-keto-compound with metalic sodium in iso-propanol produces protopanaxadiol with good yield, Although the above process has no significance for industrial purpose at present, may warrant further study in order to exploit the natural resources



Scheme 2

for the chemical synthesis of ginseng component.

Pharmacological Significance of Dammarane Aglycone Composition—Noteworthy point in the concepts of adaptogenic activity is its normalizing property being always beneficial to host irrespective of foregoing pathological shift. The hypothesis of adaptogenic activity of ginseng glycosides was formulated by Brekhman[1], based on the experimental results conducted mainly by using the mixture of various glycosides. Therefore, the controversy on whether the dualistic pharmacological activities were originated from single dammarane glycoside molecule or shared of different molecule, should be answered. Brekhman suggests the former mechanism. On the other hand Takagi's[3] reports indicate apparently the latter by showing that glycosides of panaxadiol series display central nervous system (CNS) depressant activity and that glycosides of panaxatriol series give rise CNS stimulant activity. In general the structural analogues display their biological activities in a way of either antagonistic or analogic. It may be considered from the Takagi's results that the structural congeners of ginseng glycosides, namely, glycosides of protopanaxadiol series and protopanaxatriol series are antagonistic each other as far as CNS actions concerned. On the other hand, the authors reported that both series samples of dammarane glycosides had stimulant activity in protein synthesis. For the convenience of the argument, the pharmacological or biological activities of dammarane glycosides can be divided into two main categories; one is pan-cellular activity and the other is organ or tissue specific activity. Based on the various pharmacological and biochemical data concerning the dammarane glycosides of ginseng, the following hypothesis is speculated; pharmacological activities of both series of glycosides, protopanaxadiol series and protopanaxatriol series may be either antagonistic on their tissue specific activities and analogical on their pan-cellular activities. Thus the mixture of these two series of glycosides in an appropriate ratio, as appeared in crude extract of Korean ginseng, may be beneficial to the host by increasing the synthesis of some functional proteins due to the additive augmentation of pan-cellular activities, and with the disappearance of any significant behavioral symptoms due to the antagonism of tissue specific activities. Probably such two different activities may be one reason why so many conflicting data came out from classical behevioral tests with Korean ginseng. With such possibility,

Table II—Apparent content* of dammarane triterpene aglycones of Korean ginseng.

Medicinal part and age	Sapogenin content		Ratio (PD/PT)
	Panaxadiol	Panaxatriol	
Main root:**			
6-years	0.124	0.130	0.955
4-years	0.155	0.155	1.012
Side root:**			
6-years	0.556	0.380	1.465
4-years	0.474	0.316	1.500
Terrestrial part:***			
Folium:			
3-years	0.0188	0.187	0.100
4-years	0.017	0.167	0.102
6-years	0.016	0.157	0.102
Stem:			
3-years	0.0022	0.0222	0.100
4-years	0.0048	0.0476	0.101
6-years	0.0055	0.055	0.100

* The sapogenin content in this Table do not denote the actual sapogenin content of Korean ginseng, since the degradation of sapogenins during acid hydrolysis of glycosides was not considered in the calculation of the sapogenin contents.

** Sapogenin contents were assayed by combination of preparative thin layer chromatographic purification and vanillin-H_2SO_4 colour reaction.

*** Sapogenin contents were assayed by TLC densitometry.

the authors[25] examined the ratio of panaxatriol contents in the main root, fibrous side root, and terrestrial herbs of Korean ginseng.

As shown in Table II, the ratio of aglycone compositions shows significantly different values in the various part of ginseng root. In the main root portion of Korean ginseng, the ratio of panaxatriol content approaches nearly unit value, however, in the terrestrial part panaxatriol content is greater than the content of panaxadiol and in the fibrous side root panaxadiol content is predominant. Total glycoside content of the terrestrial part is not less than that of main root and that of fibrous side root is more than that of main root. In spite of higher glycoside content, the terrestrial part and the fibrous side root of Korean ginseng have not been utilized as the substitute of the main root in practice of the oriental medicine. If we make an assumption that the terrestrial part and the fibrous side root of ginseng has not been utilized in the oriental medicine owing to its side action originating from the unbalanced aglycone ratio, the real aspect of ginseng pharmacology which is beneficial to host might be understood through the pan-cellular activity, but not through the tissue specific activity. Considering the current trends of pharmacologist's view on the ideal drug which is now changing from the potency-first to the safety-first, the reason why the main root of Korean gin-

seng has been widely used as an miraculous drug may be understood through its properly balanced aglycone ratio. Assuming the aglycone ratio of ginseng as the decisive criterion for the indication of quality of various ginseng products, the ratio of panaxadiol to panaxatriol

Table III—The ratio of panaxadiol and panaxatriol contents in the dammarane glycosides of various ginseng species.

Samples	Panaxadiol*	Panaxatriol*	Ratio (panaxadiol/ panaxatriol)
Korean ginseng	0.65%	0.77%	0.844
Japanese ginseng	0.179	0.35	0.511
Chikusetzu ginseng	0.739	0.313	2.36
American ginseng	0.896	1.254	0.714
Canadian ginseng	0.896	0.739	1.212

* The aglycone contents were assayed by the combination of preparative thin layer chromatographic process and vanillin-H_2SO_4 color reaction. Calibration of the technique was checked by standard solution of authentic panaxadiol and panaxatriol samples.

Table IV—Time interval effect of PS-A on edema suppression.

Group No.	Doses mg/kg	Time interval[a](min)	Animals weight(gm)		Edema at the time after carrageenin administration				
					1	2	3	4	5(hrs.)
Control	CMC solution		133 s.e.±12	e	2.58	4.67	6.15	5.33	5.15
				r	—	—	—	—	—
				S.E.	0.555	1.03	0.600	0.795	0.633
				e	1.7	5.1	5.43	4.45	4.0
I	100	0	137	r	34.3	—	11	16.5	22.5
			s.e.±8.8	S.E.	0.26	1.09	1.17	1.05	0.9
				e	2.11	3.47	4.23	4.45	4.28
II	100	60	124	r	18.1	25.7	31.3	16.5	16.8
			s.e.±10.8	S.E.	0.83	0.545	0.90	1.06	0.835
				e	1.45	3.35	3.05	3.95	3.67
III	100	120	129	r	43.7	28.3	50.5*	26.0	28.7
			s.e.±10.2	S.E.	0.51	0.76	0.76	0.92	0.575
				e	2.41	3.50	3.43	2.66	3.93
IV	100	240	136	r	6.6	25.1	44.5*	50.1*	23.7
			s.e.±9.85	S.E.	0.64	0.286	0.71	0.59	0.62
				e	1.37	2.55	2.58	3.23	2.53
V	200	30	127	r	47.0	45.3	58.0*	39.5*	50.7*
			s.e.±8.75	S.E.	0.328	0.59	0.525	0.38	0.515

Abbreviation: CMC; carboxymethyl cellulose; edema volume×10, r; percent of edema reduction. S.E.; standard error of edema volume, s.e.; standard error of body weight of animals.

* Edema suppression is highly significant statistically. ($P<0.01$)

a) The length of time intervals between the medication of PS-A and carrageenin edema induction.

content in the various ginseng products cultivated by various countries was examined[26]. As shown in Table III, the quality of other ginseng are not comparable with Korean ginseng in respect of the ratio of panaxadiol to panaxatriol contents of dammarane glycosides.

Some Probelems of Biological Activities of Dammarane Glycosides—We reported previously the isolation of anti-inflammatory glycosides from the extract of *Panax ginseng*[11], and the data are shown in Table IV.

As shown in Table IV, the anti-inflammatory activity of panax saponin A showed the delayed and long lasting action. The long lasting activity was also checked in another experiment in which carrageenin edema induction was conducted one week after the medication of panax saponin A 100mg/kg, *p.o.* As one of possible mechanisms it may be considered that the anti-inflammatory activity of the saponin might result from the extended effect of some other primary action, such as the de novo synthesis of certain functional proteins. In this connection, the authors examined the stimulating effect of panax saponin A on the incorporation rate of ^{14}C-leucine into serum and liver protein of rat.

Table V—Effect of panax saponin A on the incorporation of ^{14}C-leucine into liver and serum protein.

Exp. No.	Materials	Dose (mg)	No. of mouse	Radio-activity (cpm/mg protein)	
				Serum (%)	Liver (%)
1	Control (saline)	—	3	642(100)	—
	PSA (triol)	1	3	828(129)	—
	Prostisol (diol)	1	3	942(147)	—
2	Control	—	3	629(100)	581(100)
	PSA	1	3	1313(208)	924(159)
	PSA	2	3	1515(241)	1043(179)
3	Control (saline)	—	3	1058(100)	830(100)
	PSA	1	3	1317(124)	1028(133)
4	Control (saline)	—	3	—	529(100)
	PSA	2	3	—	702(133)

Medication: Intraperitoneal injection.
^{14}C-leucine: 1μCi/mouse (wt.: *ca.* 20gm) 2hrs. before decapitation.
Saponins: 0.5~1.0% saline soln. 6hrs before decapitation.
Three mouse livers were pooled for each experimental or control group.

As shown in Table V, panax saponin A and Prostisol enhanced the incorporation rate of C^{14}-leucine into serum protein. Such effect of panax saponin A was confirmed by repeated experiments. The same effect by prostisol was fully investigated by Oura[27] previously, therefore, the compound was adopted as the reference for the biological activity. In Fig.1, the time course effect of Panax saponin A on the incoration rate of ^{14}C-leucine into liver protein is illustrated[24].

As shown in Fig. 1, both the time course graphs of graded doses showed maximum stimulation in the 4 hours group and the enhancement prolonged for a long time. The delayed and

long lasting tendency of the stimulation of protein synthesis shown in Fig. 1 is similar with that of anti-inflammatory activity in its long lasting and delayed actions. Based on the results of the time course experiment, it may be inferred that the stimulating activity of panax saponin A on protein synthesis may be closely correlated with the anti-inflammatory activity of the saponin. The stimulating activity of ginseng saponin on protein synthesis was also described by Oura. He separated an alcholic substance from the ginseng extract, which showed the stimulating activity on the protein synthesis, and designated the substance as prostisol[27].

Later studies on the prostisol demonstrated that it was the mixture of saponins of protopanaxadiol series. Prostisol exhibited the stimulating activities on the synthesis of bio-macromolecules such as messenger[28]RNA and DNA-dependent-RNA polymerase[29]. The earliest primary action of "Prostisol" was shown to be the stimulating activity on the DNA-dependent RNA-polymerase, namely, stimulation of protein synthesis at transcriptional level[30]. Based on these observations, Oura suggested the mechanism of hormone-like activity for the mode of action of ginseng saponins. However, when we compare the time course data of the authors [24] with that of Oura[27], it can be noted that the onset of maximum stimulation on protein synthesis is significantly different in their length of latent time. Panax saponin A has smaller molecular weight (mol. wt. 800) than that of prostisol (mol. wt.; approx. 1,100) and it shows the maximum stimulation at 4 hours after administration of the saponin, however, it was reported that the maximum stimulation of protein synthesis was attained at 8~12 hrs after the administration of prostisol. The apparent dependency of the latent time on the molecular wight of the saponin may suggest tacitly some information either on the molecular aspects of active forms of the saponin at the proximity of its receptor site and/or uptake and transport of these compounds by cells. Thus the mechanism of ginseng pharmacology is now being understood at the level of molecular interaction of ginseng components with the cellular components. In short, the biochemical studies on ginseng saponins published hitherto has been concerned only with the problems of the biological responses which are manifested by the administration of ginseng saponin. Reports concerned with the tracing the saponin in the biological system are not available at present. Therefore, the direct evidence for the presence of ginseng saponin at sub-cellular active site at the time of its maximum activity is desireable at present.

The Intestinal Absorption and the Route of Excretion—Although the intestinal absorption of the saponins is primary requisite for the understanding of the various pharmacological responses of the saponin, few is available with including our recent data[30]. Recently, the authors examined the intestinal absorption and the renal excretion of ginseng saponin. As shown in Fig. 2, panax saponin A appears rapidly in the blood, liver, and in the urine as unchanged form after the intravenous administration of the saponin. In another experiment, the unchanged panax saponin A was detected in the urine and blood of the rabbit 4 hrs after the oral administration of the saponin. Therefore, easy absorption and easy excretion of panax saponin are observed.

The unchanged saponin was also detected in the intestinal lumen of the animals to which the saponin was administred by intravenous injection. In order to find out the excretion route of

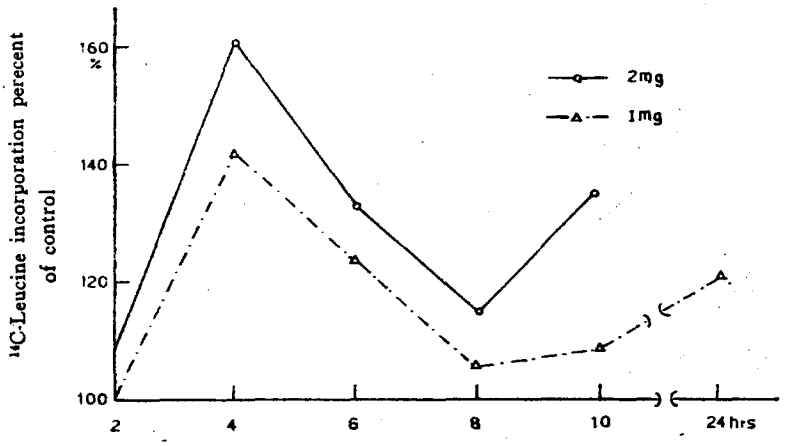


Fig. 1—Time course effect of PSA on the incorporation of ^{14}C-leucine into liver protein.
PSA (1 or 2mg/mouse) was administered intraperitoneally to mice at each designated time prior to killing and ^{14}C-leucine (1μCi/mouse) was injected intraperitoneally 2 hrs prior to killing the animals by decapitation. The specific radioactivity (cpm/mg protein) of liver protein was assayed as described in methods.[24] The control value was 529 cpm/mg protein. Three mouse livers were pooled for each experimental or control group.
—△— :2mg, —○— :1mg

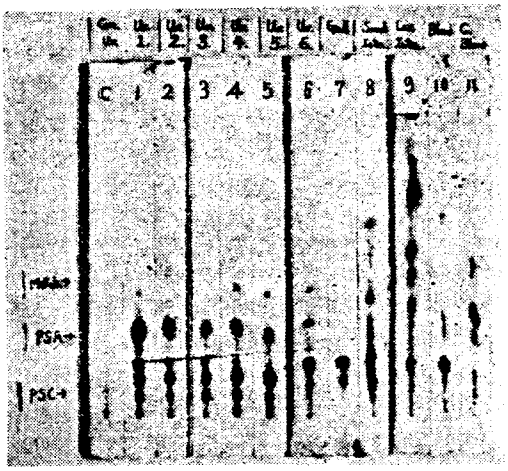


Fig. 2—Distribution of panax saponin A in various extra-cellular specimens. Panax saponin A was administered to the rabbit by intravenous injection, 200mg/2.0kg body wt. Panax saponin C(protopanaxatriol-rhamno-diglucoside) was added to every specimens as an internal references for the quantitative assay of panax saponin A. TLC, Solvent, $CHCl_3$: MeOH (3 : 1); colour reaction, H_2SO_4 spray and heat. C, control urine; 1, Urine 0~1 hr: 2, Urine 1~2 hr; 3, Urine 2~3hr; 4, Urine 3~4hr; 5, Urine 4~5hr; 6, Urine 5~6hr; 7, Gall bladder; 8, Small intestine; 9, Large intestine; 10, Blood; 11, Control blood.

panax saponin A in the intestinal lumen, rabbit was cannulated at bile duct and the intestinal content was analyzed to detect the presence of unchanged panax saponin A by thin layer chromatography. As shown in Fig. 3, the unchanged panax saponin A was detected only in the bile secretion with one unidentified spot which was considered probably as the metabolite of the saponin. Therefore, we concluded that panax saponin A in the intestinal lumen seems to appear through the excretion of bile duct, but not through the direct excretion of intestinal mucosa.

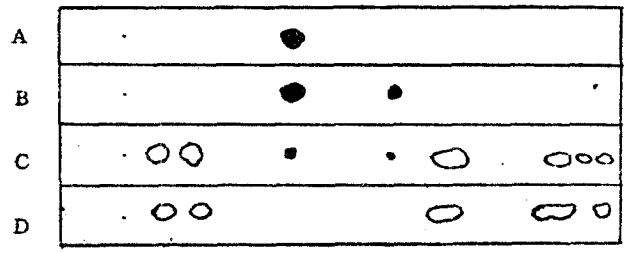


Fig. 3—Thin layer chromatogram of bile. Detection of panax saponin A in the intestinal lumen of bile duct cannulated rabbit. Panax saponin A was administered 200 mg/2kg by intravenous injection to the rabbit cannulated at bile duct. Intestinal content was collected 5hours after the administration of panaxsaponin A by intravenous injection. TLC samples of each specimen were prepared by ordinary butanol extraction. TLC; solvent; $CHCl_3$: MeOH (3 : 1), colour reaction; H_2SO_4 spray and heat. A, PSA; B, collected from bile juice; C, collected from small intestine; D, collected from large intestine.

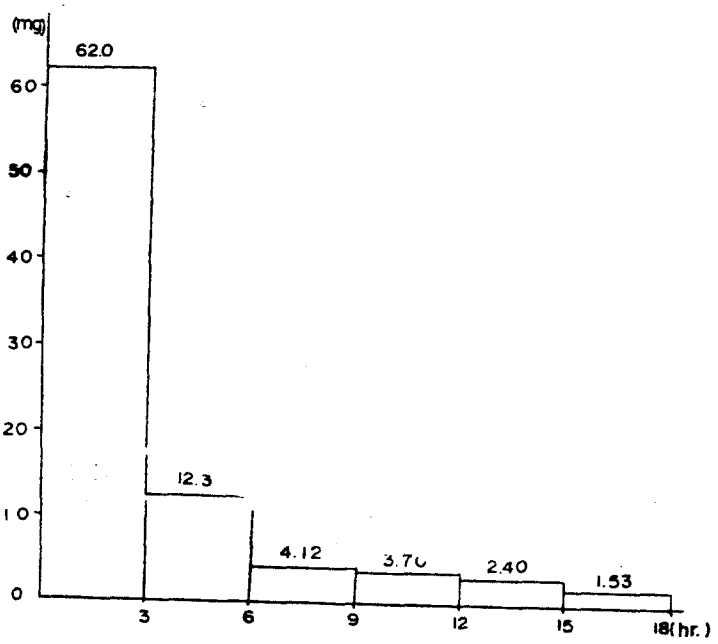


Fig. 4—Urinary excretion curve of unchanged panax saponin A. The saponin was administered 175 mg/1.75kg. to the rabbit by intravenous injection Unchanged panax saponin A was assayed by preparative thin layer chromatography and vanillin-H_2SO_4 colour reaction[23]. Panax saponin C was added quantitatively to each specimen as the internal references for the assay of panax saponin A.

The time course curve of urinary excretion of panax saponin A shows two phase, it constitutes rapidly decreasing curve from zero to six hour and slowly decreasing curve from six hour to the end of the experiment, as illustrated in Fig. 4.

Biphasic curve of urinary excretion of panax saponin A implies that this compound appear to exist both extra-cellularly and intra-cellularly *in vivo*. At present it is uncertain whether panax saponin A itself or its metabolites exhibits biological activities.

However, as shown in Fig. 2, an unidentified spot appeared on thin layer chromatogram from samples of urine and bile of the rabbit after the administration of panax saponin A. This unidentified spot is considered to be the metabolite of panx saponin A. In addition, this unidentified substance was excreted long time. Such long lasting excretion rate of this unidentified substance coincide with long lasting feature of pharmacolgical activities like the anti-inflammatory and stimulating activity of protein synthesis. so if we can put some co-relation between excretion rate and pharmacological activities of panax saponin A, the unidentified substance as metabolite of panax saponin A may play a important role. The excretion of unchanged panax saponin A was quantitatively estimated in the various extracellular specimens, and illustrated in Table VI.

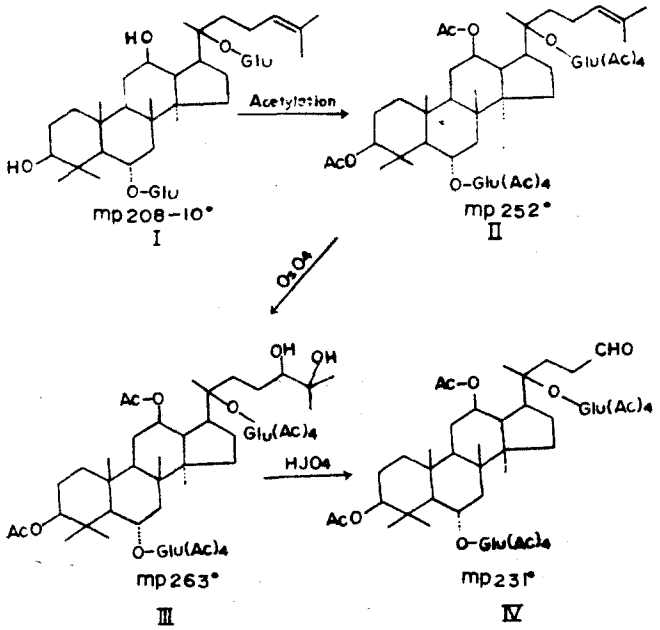
Table VI—Extra-cellular distribution of panax saponin A.

Route	Amount (mg)	Percent/total PSA
Urine	128.5	64.25
Small intestine	2.34	1.17
Large intestine	12.01	6.01
Blood	12.7 ?	6.35
Gall bladder	Detected	—
Total	155.55	77.78
Difference	44.45	22.23

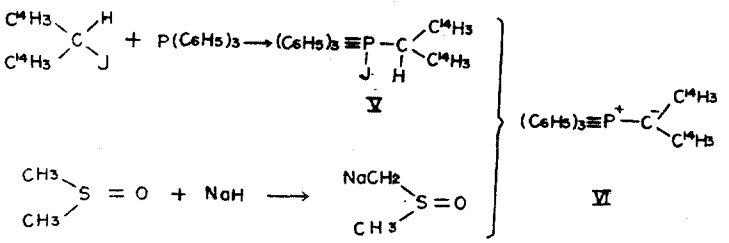
The content of unchanged panan saponix A in the various extra-cellular specimens were analyzed six hours after the intravenous administration of panax saponin A, 200mg/2kg of experimental rabbit.

As shown in Table VI, unchanged panax saponin A distributed in the extra-cellular phase reached approximately 77% at six hours after the intravenous administration of the saponin. The difference between the total excretion of unchanged panax saponin A and total amount administered will probably correspond to the amount of metabolite and or the amount of slowly excreting cellular component of panax sapinon A. Therefore, researches concerned with the chemical nature of the metabolite and with the biological activity of the substance will have the greatest importance in study of ginseng for future.

Labelling of Radio-isotope into Panax Saponin A—Tracer compound labelled with radio-isotope is indispensable for the studies of metabolism of ginseng saponins. One of the authors[31] established a new radio-labelling synthetic procedure by which ^{14}C-isopropyl fragment can be



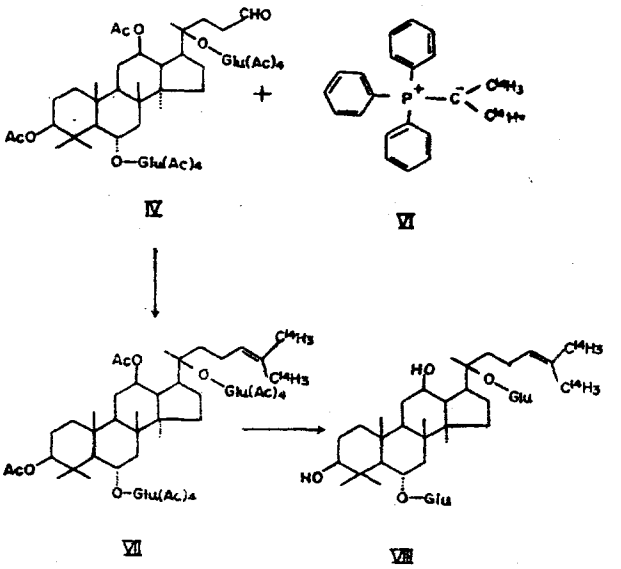
Scheme 3a—Preparation of trisnoraldehyde derivative of panax saponin A(ginsenoside Rg.)



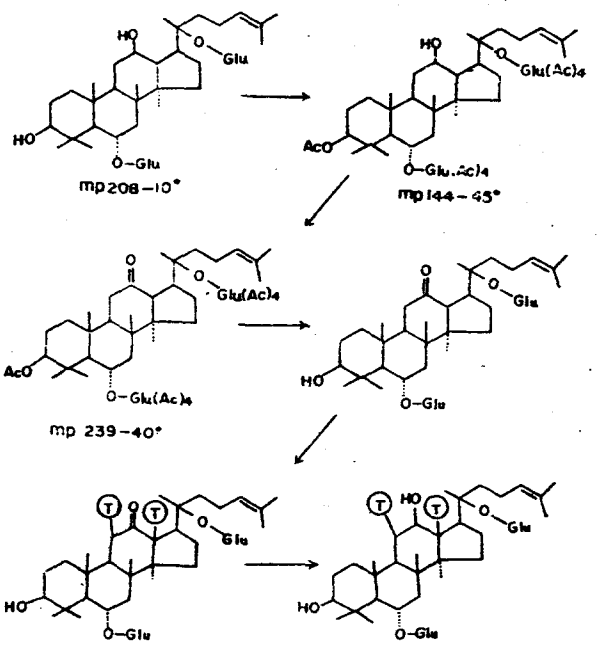
Scheme 3b— Preparation of ^{14}C-isopropyl-Witting reagent.

introduced to the side chain of ginseng saponin.

As shown in Scheme, the method constitutes three steps; the first step is the breakdown of side chain by osmium tetroxide oxidation followed by periodate oxidation, the second step is the synthesis of ^{14}C-carrying Wittig reagent, and the last step re-generation of the original saponin molecule labelled with ^{14}C-isopropyl fragment by condensation of the Wittig reagent with the trisnor-aldehyde derivative of the saponin. This experiment was conducted with panax saponin A with overall yield more than 41%. This method may be applicable to the every dammarane glycosides of panax ginseng. Radio-labelling on the side chain will have



Scheme 3c—Synthesis of panax saponin A(^{14}C).



Scheme 4—Tritium labelling of panax saponin A.

some danger of metabolic breakdown of labelled fragment during tracer studies *in vivo*. Therefore, labelling the saponin on polycyclic skeleton is more desirable. Recently the authors[32] established a new synthetic procedure labelling on polycyclic skeleton by tritum(^{3}H).

As shown in Scheme, the method constitutes the selective acetylation of hydroxyl function leaving one hydroxyl function in sapogenin unacetylated, oxidation of the unacetylated hydroxyl function by Sarett reagent, tritation of the resulting ketonic compound by keto-enol tautomerism and regeneration of labelled original saponin molecule by stereo-specific reduction. These two labelling procedures will offer a great help in the studies of metabolism of saponins of panax ginseng.

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