SOME CHEMICAL STUDIES ON GINSENG

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The root and rhizome of several species of *Panax* are used as drugs in Chinese medicine. Korean Ginseng, the root of Panax ginseng C. A. Meyer, is most well-known and widely used among the peoples of East and South East Asian countries since ancient times.

In the old medical literatures in China, the sedative and stimulative effects of Ginseng were suggested.

American Ginseng, the root of *Panax quinquefolium* L. is also used among Chinese peoples for the similar purpose.

San-chi Ginseng which is cultivated in southern part of China is used as a remedy of bruise and haemorrhage. The botanical identification of the original plant of San-chi is suspending, but recently the name, *Panax Sanchi* Hoo or *Panax pseudoginseng* var. *notoginseng*(Burk.) Hoo et Tseng[1], has been proposed.

The rhizome of a Japanese wild growing plant, *Panax pseudo-ginseng* (Will.) subsp. *japonicum* Hara [2](= *P. japonicum* C. A. Meyer) is used sometimes in Japan as a drug named "Chikusetsu-ninjin" shows a little difference from Korean Ginseng. Similar species of *Panax pseudo-ginseng* are growing in the Circum Pacific Region of Asia from East Himalayas to Japan.

The cultivation of *Panax ginseng* was first introduced to Japan in 1728, and the production of Ginseng has later been developed in Nagano, Shimane and Fukushima pref.

In spite of the very high popularity of Ginseng since olden times, the effective principle of this drug was obscure until recently either chemically or pharmacologically.

Garrique[3] first studied in 1858 American Ginseng to isolate a crude saponin which was named panaquilon.

Some Japanese chemists, Asahina (1906)[4], Kondo (1915)[5] and Kotake (1930)[6] and their co-workers reported the isolation of saponins or prosapogenin of Ginseng, but they were not able to elucidate the chemical structures in detail.

Recently general interest to Ginseng has been raised among peoples not only in Asia but also in Russia, and other East and West European countries. Several papers on chemical studies of Ginseng saponins and sapogenins were published by Hörhammer[7], Wagner-Jauregg,[8] Kochetkov-Elyakov[9], Lin,[10] and ourselves[11] appeared almost simultaneously in 1961–1962.

Some other works on a long chain acetylenic compound and peptides were also published by Japanese[12] and German workers[13].

We demonstrated by thin layer chromatography (TLC) the occurrence of several saponins in Ginseng, which were named ginsenoside Rx (x = 0, a, b_1, b_2, c, d, e, f, g_1, g_2·····) by the sequence of

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Chart 1

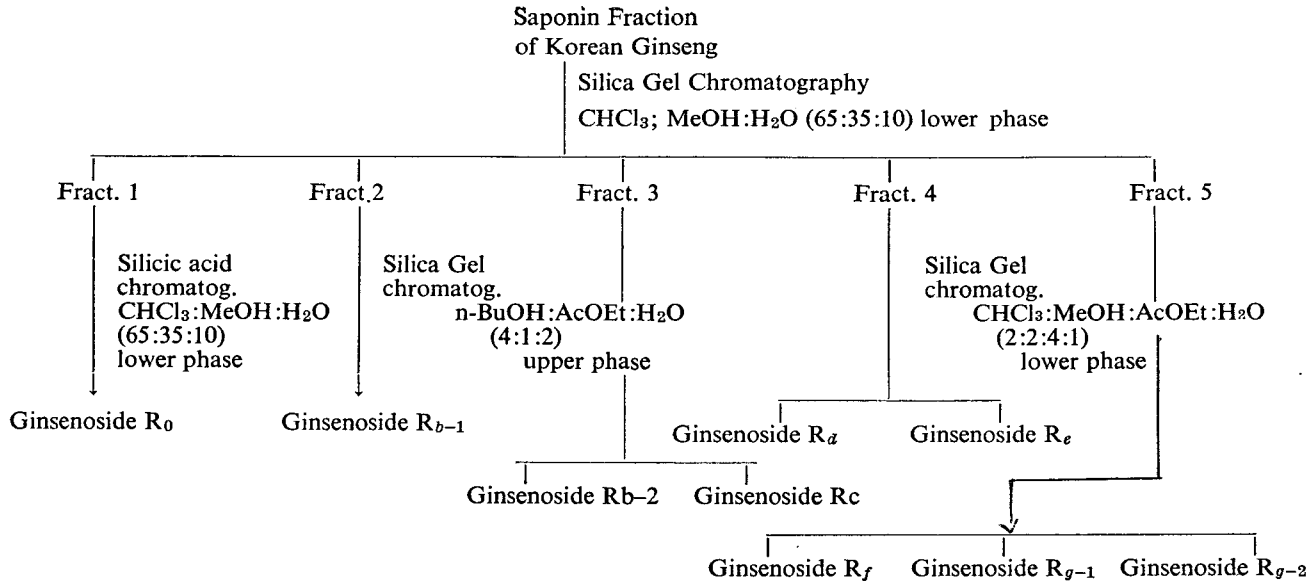
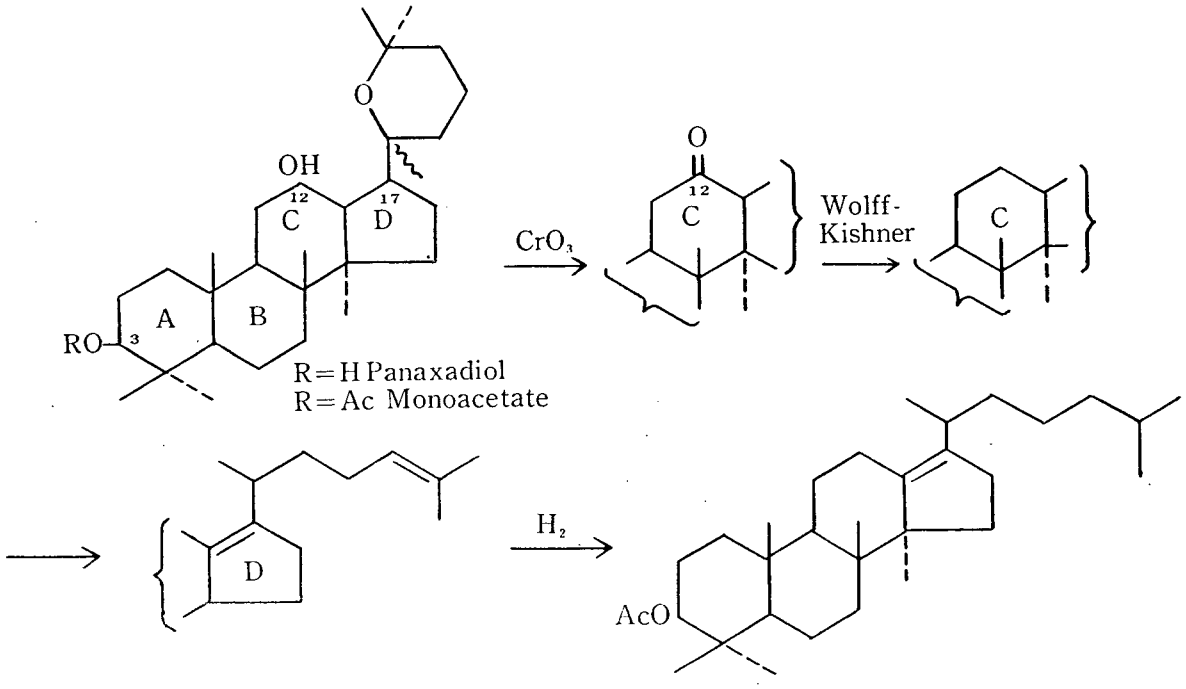


Chart 2



spots on TLC from the bottom to the top.

The comparative TLC experiments showed that Korean Ginseng, American Ginseng and San-chi Ginseng gave similar but slightly different patterns of TLC while Japanese Chikusetsu-ninjin and the rhizomes of other *P. pseudoginseng* spp. revealed a remarkably different pattern.

On acid hydrolysis of Ginseng saponin mixture showed the formation of two main sapogenins named panaxadiol and panaxatriol along with oleanolic acid as a minor sapogenin.[14]

The components of Ginseng saponins, Ginsenoside Rx, were separated by column chromatography as shown below[15].

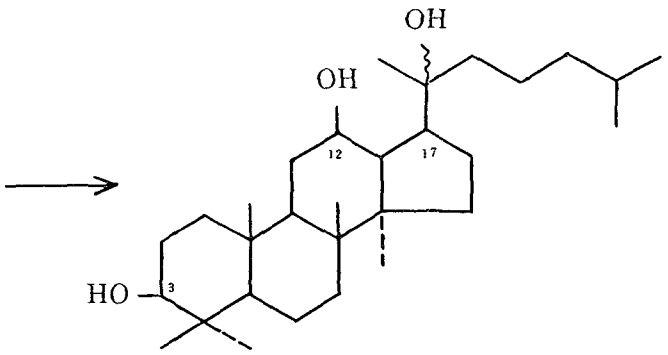
Panaxadiol,[11] $C_{30}H_{50}O_3$, m.p. 250°C, $[\alpha]_D+1°$, was proved to be a tetracyclic triterpene by the con-

version into isotricallenyl acetate.

The presence of a trimethyltetrahydropyrane ring structure in its molecule was revealed by a mass spectral fragment, m/e 127.

The trimethyltetrahydropyrane ring of panaxadiol was shown to be formed secondarily by a ring closure of an open side chain attached to C (17) in D-ring, since hydrogenated ginsenosides Rb_1,b_2,c mixture afforded dihydroprotopanaxadiol.[16]

Chart 3



Dihydroprotopanaxadiol

Dihydroprotopanaxadiol was correlated stereochemically to dammarandiol-I which is isolated from Dammar resin (The resin of *Shorea wiesueri, Hopea odorata,*[17] *Vateria acuminata* (Dipterocarpaceae)) by elimination of a hydroxyl at C (12).

Dammaranediols I and II are stereochemical epimers at C (20), but the chirality at C (20) has been remained unsettled.[18]

The chirality at C (20) of panaxadiol which was proved to be same with dammaranediol-I as above has been established as being R by the following reactions, starting from panaxadiol in obtaining (−) methyl cinenate which was prepared from R-(−) *linalool*[19].

On mild hydrolysis of ginsenoside Rb_1, b_2 and c by the Smith degradation using $NaIO_4$ followed by $NaBH_4$ and finally with 2N H_2SO_4 at room temperature, an epimer of 20R-protopanaxadiol was obtained.[20]

On the other hand 20R-protopanaxadiol was fromed by dehydrochlorination with base from a chlorine containing sapogenin prepared earlier by Kotake.[6]

The epimeric sapogenin having S-configuration at C (20) is rather labile forming readily an equilibrium mixture of S and R epimers.

Thus the chirality at C (20) of dammarandiol-I and dammarandiol-II has been established as being R and S, respectively.

Betulafolienetriol[21] which was isolated from the leaves of white birch in its ester form at C (3) α OH corresponds to dammaranediol-II. Thus 20S-protopanaxadiol is 3-epi-betulafolienetriol (= 12β-hydroxydammarenediol-II)

On acid hydrolysis, ginsenoside Rg_1, m.p. 196° $[\alpha]_D$ + 32° (pyr.) afforded panaxatriol which has

Chart 4

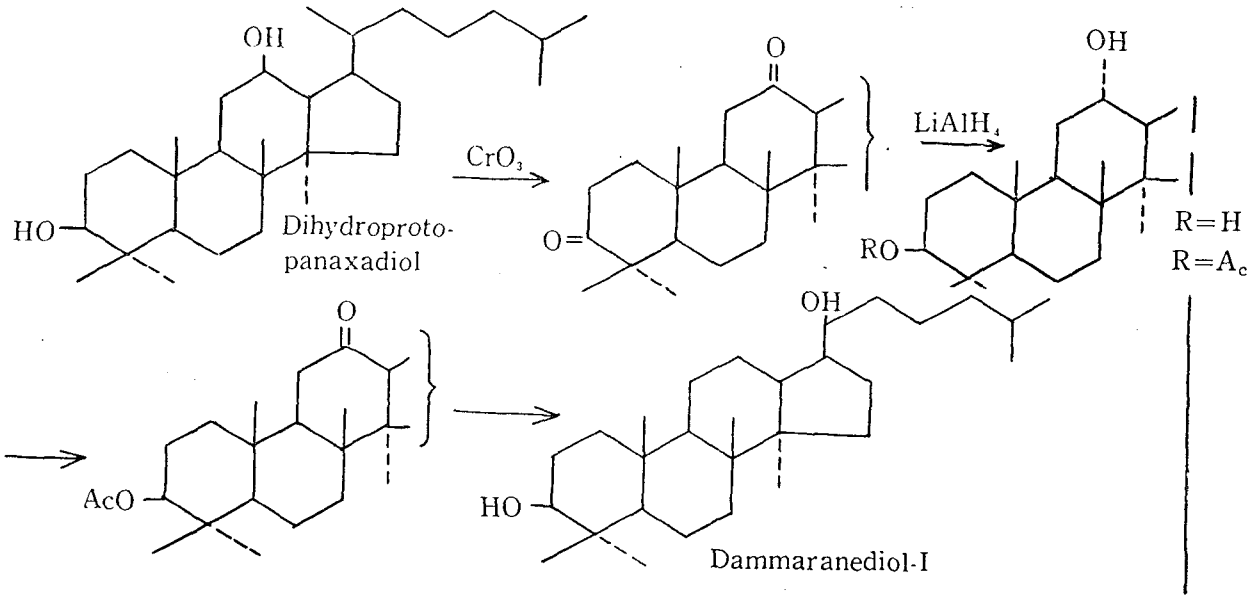
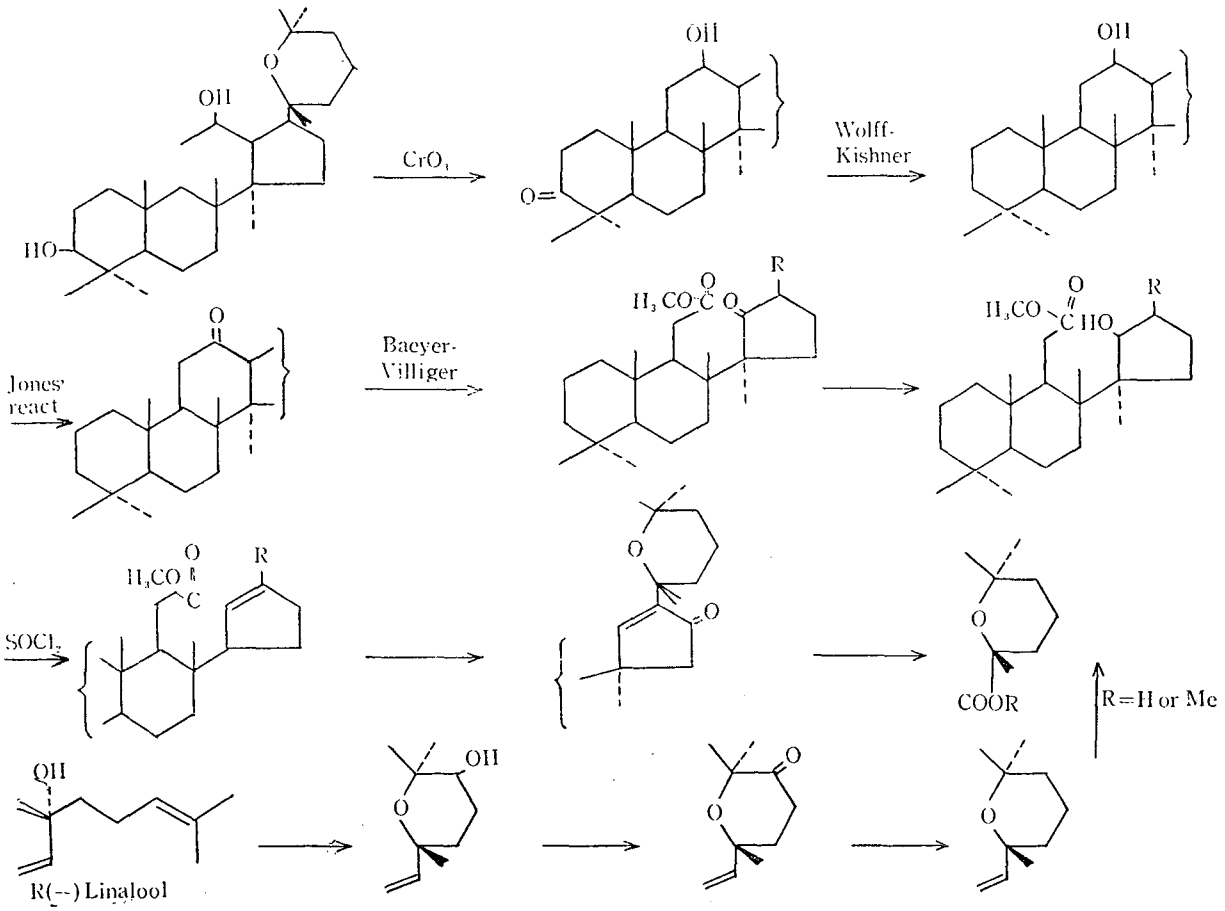


Chart 5



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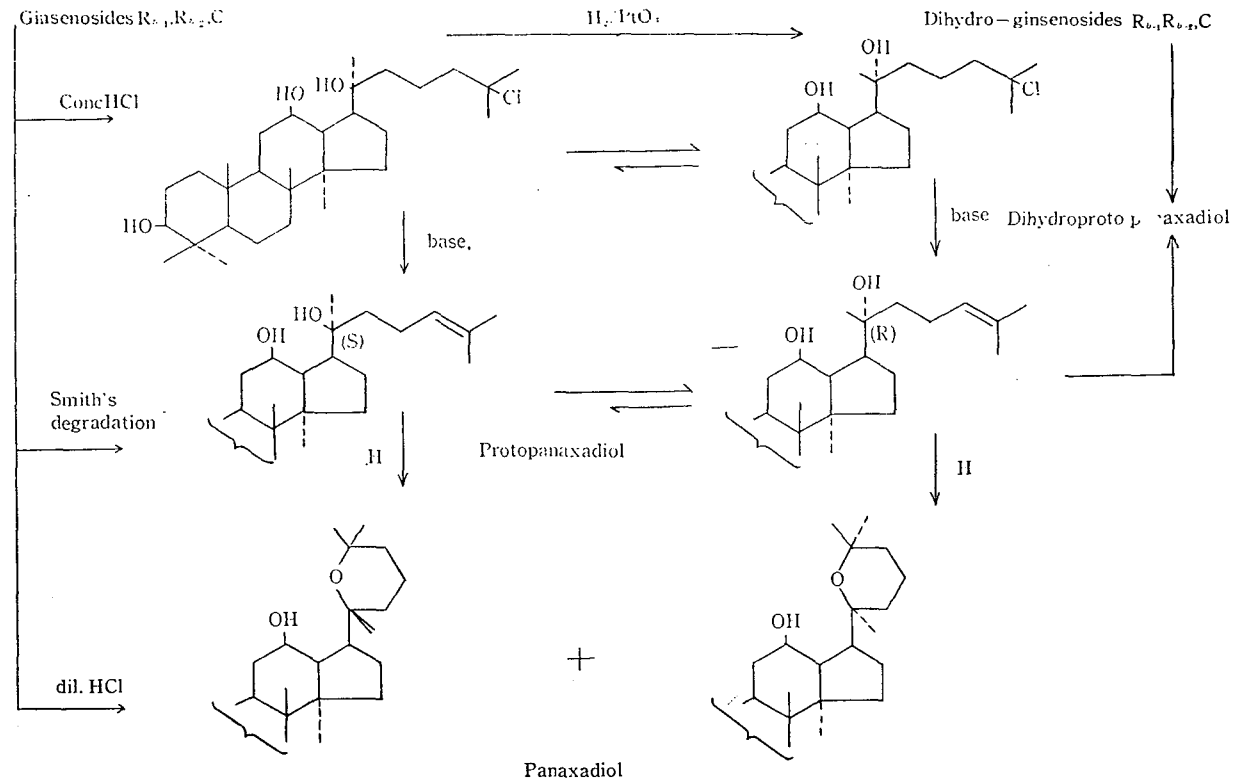
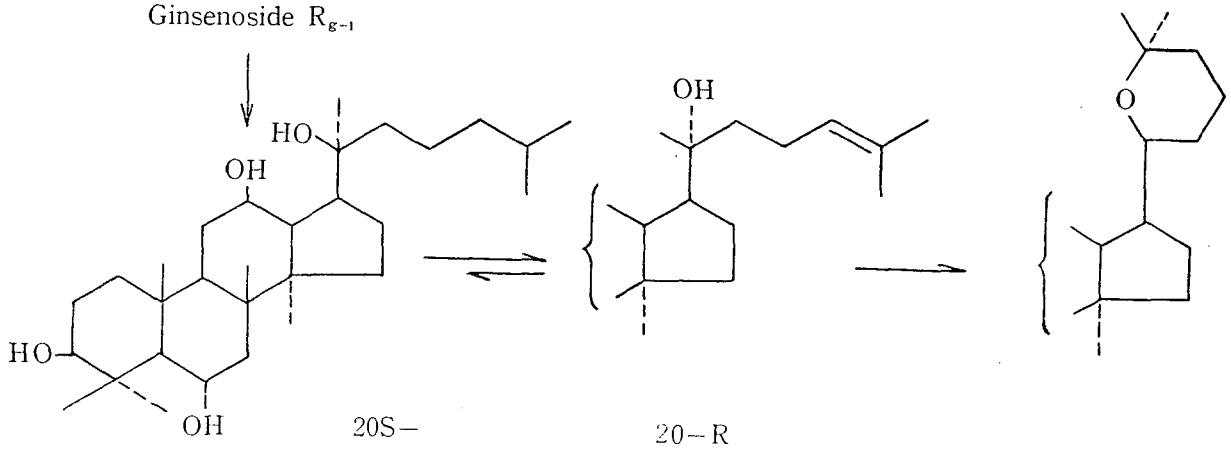


Chart 7



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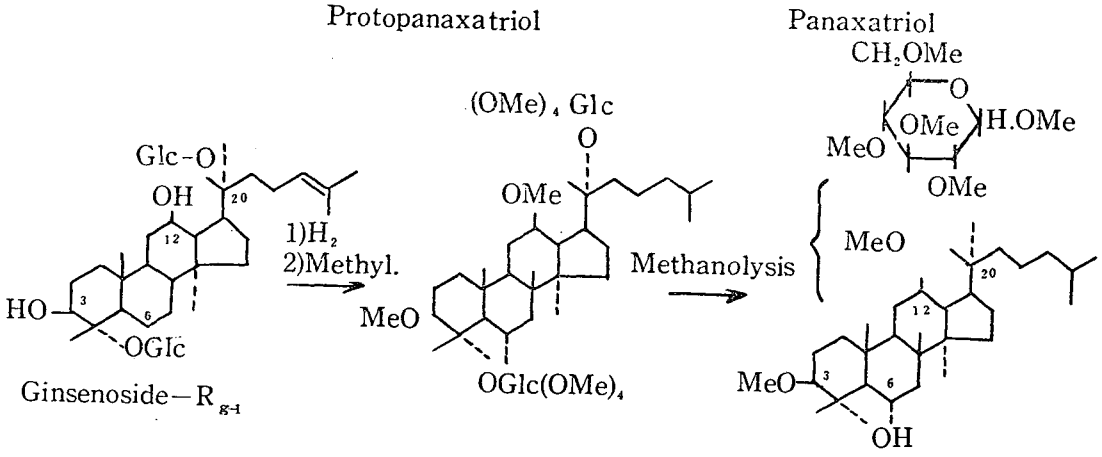
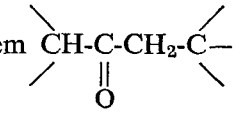


been proved to be 6α-hydroxypanaxadiol by the NMR and ORD experiments.

On the analogy panaxadiol and protopanaxadiol, the genuine sapogenin of ginsenoside Rg_1 should be 20S-protopanaxatriol.

On oxidation of panaxatriol, a diketonic compound was yielded, which showed the presence of 6-membered ring C = O by the IR absorption at 1720 cm^{-1}. One of the C = O groups is stereochemically hindered as it gave only monosemicarbazone. The Wolff-Kishner reduction of the diketonic compound afforded a monoketonic compound (IR: C = O 1714 cm^{-1}) to reveal that a less-hindered C = O was lost by the reduction.

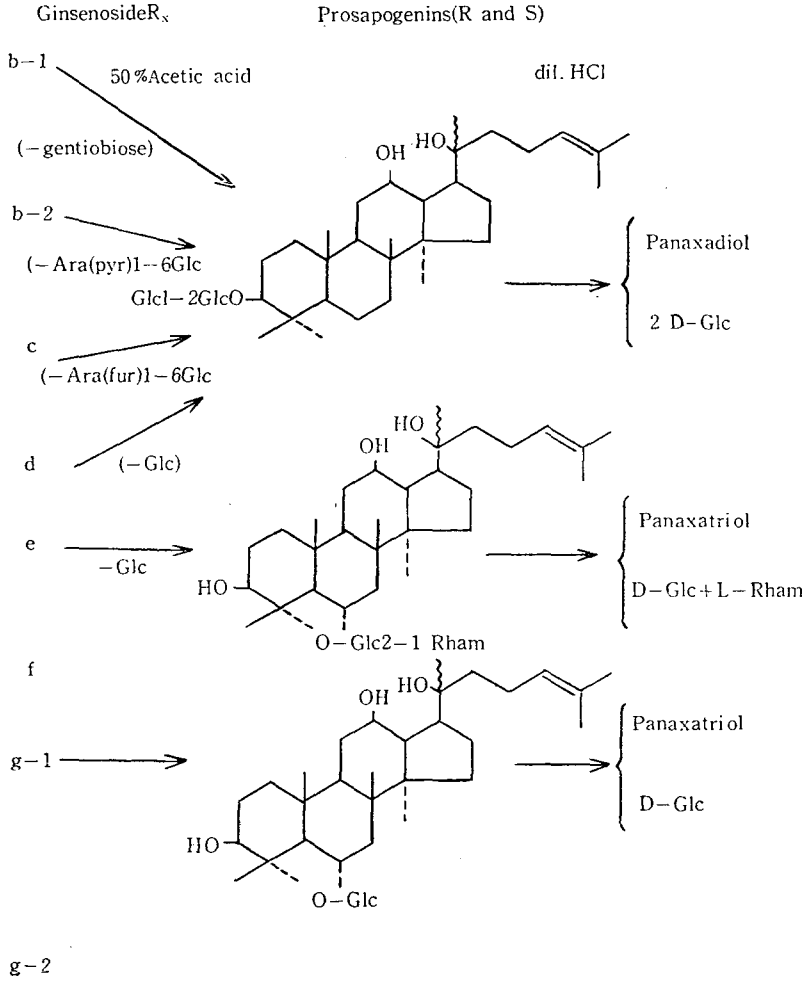
The NMR spectrum of this compound gave AB-type doublets at δ 2.60 and 1.80 (1H each) and a singlet at δ 2.19 (1H). On deuterization these signals disappeared to indicate that the active protons are located in a system  , which could only be represented by the system involving a C = O at the 6-position of B-ring of dammarane.

The initial hydroxyl at this position of panaxatriol resists against acetylation to reveal the α-(equatorial) configuration by the analogy with 6-α-hydroxyl of zeorin.

Ginsenoside R_{g-1} was fully methylated by the Hakomori method, and then methanolyzed to give methyl 2, 3, 4, 6-tetramethyl-D-glucose only as the sugar component.

Hydrogenated ginsenoside R_{g-1} was fully me-

Chart 9



thylated and hydrolyzed to form 3, 12-dimethyl ether of dihydroprotopanaxatriol. Thus one each molecule of D-glucosyl attached to 6 α and 20-hydroxyls of the saponin to show that ginsenoside R_{g-1} is 6,20-diglucoside of 20-S-protopanaxatriol.[22]

The identity of ginsenoside R_{g-1} with Elyakov's panaxoside A[23] was established by the direct comparison of authentic samples, though Elyakov proposed a different structure for it.

The O-glucosyl moiety at C (20) of ginsenoside R_{g-1} was readily hydrolyzed with 50% acetic acid to give a prosapogenin in which 6-0-glucosyl still remained.

By the analogous treatment, other members of ginsenosides Rx were examined to prove their structures.

Ginsenoside R_o possesses exceptionally oleanolic acid as the sapogenin.

The oleanolic acid-type saponins were dominantly isolated from Japanese Chikusetsu-ninjin (*Panax pseudoginseng* subsp. *japonicum*)

On the analogy of ginsenoside-R_{g-1} almost all Ginseng saponins (ginsenoside Rx) have been established to be formulated as follows[15),24)]:

Ginsenosides Rx

b_1 20-S-Protopanaxadiol-3-[0-β-D-glucopyranosyl (1→2) β-D-glucopyranoside] -20-[0-β-D-glucopyranosyl (1→6) β-D-glucopyranoside]

b_2 20-S-Protopanaxadiol-3-[0-β-D-glucopyranosyl (1→2) β-D-glucopyranoside]-20-[0-α-L-arabinopyranosyl (1→6) β-D-glucopyranoside]

c 20-S-Portopanaxadiol-3-[0-β-D-glucopyranosyl (1→2) β-D-glucopyranoside]20-[0-α-L-arabinofuranosyl (1→6) β-D-gluco-

pyranoside]

d 20-S-Protopanaxadiol-3-[0-β-D-glucopyranosyl (1→2) β-D-glucopyranoside] 20-[0-β-D-glucopyranoside]

e 20-S-Protopanaxatriol-6-[0-p-α-L-rhamnopyranosyl (1→2) β-D-glucopyranoside]-20-[0-β-glucopyranoside]

f 20S-Protopanaxatriol-6-[0-β-D-glucopyranosyl (1→2) β-D-glucopyranoside]

g_1 20-S-Protopanaxatriol-6-[0-β-glucoside]-20-[0-β-D-glucoside]

g_2 20-S-Protopanaxatriol-6-[0-α-L-rhamnopyranosyl (1→2) β-D-glucopyranoside]

It would be noted that the principal saponins of Korean Ginseng are dammarane-type triterpenoid combining with sugars at 3, 6 or 20 positions.

These saponins are the first example of dammarane type triterpenes occurring in nature as the glucosidic form, and it is very characteristic among the natural triterpenoid and steriod saponins that the panaxatriol series saponins possess 6-0-glycosidic moiety with free 3-hydroxyl.

For the semimicro separation and also for qualitative or quantitative determination of plant glycosides, we have developed recently a new process which was initially adopted for biochemical preparations by Dr. Tanimura analystical chemistry department of our school and named Droplet counter Current Chromatography (DCCC).[25]

The DCCC apparatus consists of 500—1,000 thin glass tubings of a few mm in diameter which are connected one by one with thin "Junflon" tubings. The suitable solvent system for this process is selected in testing as used for thin layer chromatography. In the first place all the tubings are filled with the lower phase of some suitable immissible solvents, and the end of tubing system is connected with a fraction collector. Then a mixture of samples to be separated is dissolved in the solvent of the lower phase and changed to the first few tubings.

After setting up the apparatus completely, the upper phase of the immissible solvent system is pushed in slowly from one end of the apparatus using a small pressure pump to make small bubbles moving through the lower phase solvent. The whole process is carried out automatically for a certain time period to collect the fractions which show a well-separated chromatographical pattern by the colorimetrical determination on addition of phenol-H_2SO_4 as the colour reagent.

By this method, we have made a comparative study on Korean, American and San-chi Ginseng as well as Japanese Chikusetsu-ninjin to show their characteristic patterns of chromatograms which would be used for the identification of saponins and the determination of their contents.

Using our preparations of Ginseng saponins, ginsenosides Rx, we asked to Prof. K. Takagi and Dr. H. Saito of our school to develop pharmacological study, and to Dr. Yamamoto School of Medicine, Chiba University for biochemical investigation. The results of their studies are presented by themselves to this symposium.

Acknowledgements: This series of chemical studies on Ginseng saponins and sapogenins was initially started by Dr. M. Fujita and Dr. H. Itokawa who were in our department in 1960, and up to the present many younger coworkers have devoted their efforts to clarify the chemical structures of the active principles.

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