CHEMICAL STUDIES ON CRUDE DRUG PROCESSING RED GINSENG AND WHITE GINSENG

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INTRODUCTION

Ginseng is one of most well-known crude drugs which have been used in both processed and unprocessed forms such as red ginseng and white ginseng. However, most of the chemical studies on ginseng were concentrated on white ginseng.[1] In the other words, until recently, no study has been reported on the chemical comparison of white ginseng and red ginseng in relation to their biological activity difference.[2]

During the course of our chemical studies on bioactive principles of naturally occurring drug materials, we have been engaged in chemical characterizations of crude drug processings. Recently, we have comparatively analyzed the chemical constituents of red ginseng and white ginseng. This paper deals with the chemical elucidation of characteristic constituents of both ginsengs: *e.g.* the isolations of cytotoxic ginsenoside Rh_2 (7) from red ginseng[3] and water-soluble malonyl-ginsenosides Rb_1 (9), Rb_2 (10), Rc (11), and Rd (12) from white ginseng.[4]

CHARACTERISTIC CONSTITUENTS OF RED GINSENG

1. Isolation

The methanol extractives of red ginseng and white ginseng (6 years old, cultivated in Nagano Prefecture, Japan) were fractionated through the ordinary procedure as shown in Chart 1 to provide an ether-soluble portion, an ether-insoluble portion, and a water-soluble portion. Each fraction was then compared in detail by TLC and TLC scanning profile analyses.

Since the ether-soluble portion of red ginseng was found to contain several characteristic ingredients, the fraction was further separated by combination of various chromatography. Thus, repeated chromatography using silica gel and Bondapak C_{18} columns of the ether-soluble portion provided sterols, daucosterin, panaxynol, a new acetylenic compound named panaxytriol (8), a new glucoside named ginsenoside Rh_2 (7), and several mixtures of a pair of ginsenoside C-20 isomers. Respective isomers were further separated by chromatography of their acetates. Additional crops of slightly polar ginsenosides were obtained from the ether-insoluble portion together with polar ginsenosides Ro, Rb_1, Rb_2, Rc, Rd, Re, Rf, and Rg_1 which are known constituents of white ginseng.

Finally, we were able to isolate 20(S)-ginsenoside Rg_3 (1), ginsenoside Rg_3 (2), ginsenoside Rg_2 (3), 20(R)-ginsenoside Rg_2 (7), and panaxytriol (8) in the yields as shown in Chart 1. Among them, 1, 4, 6, 7, and 8 (underlined) were new compounds characteristically isolated from red ginseng.

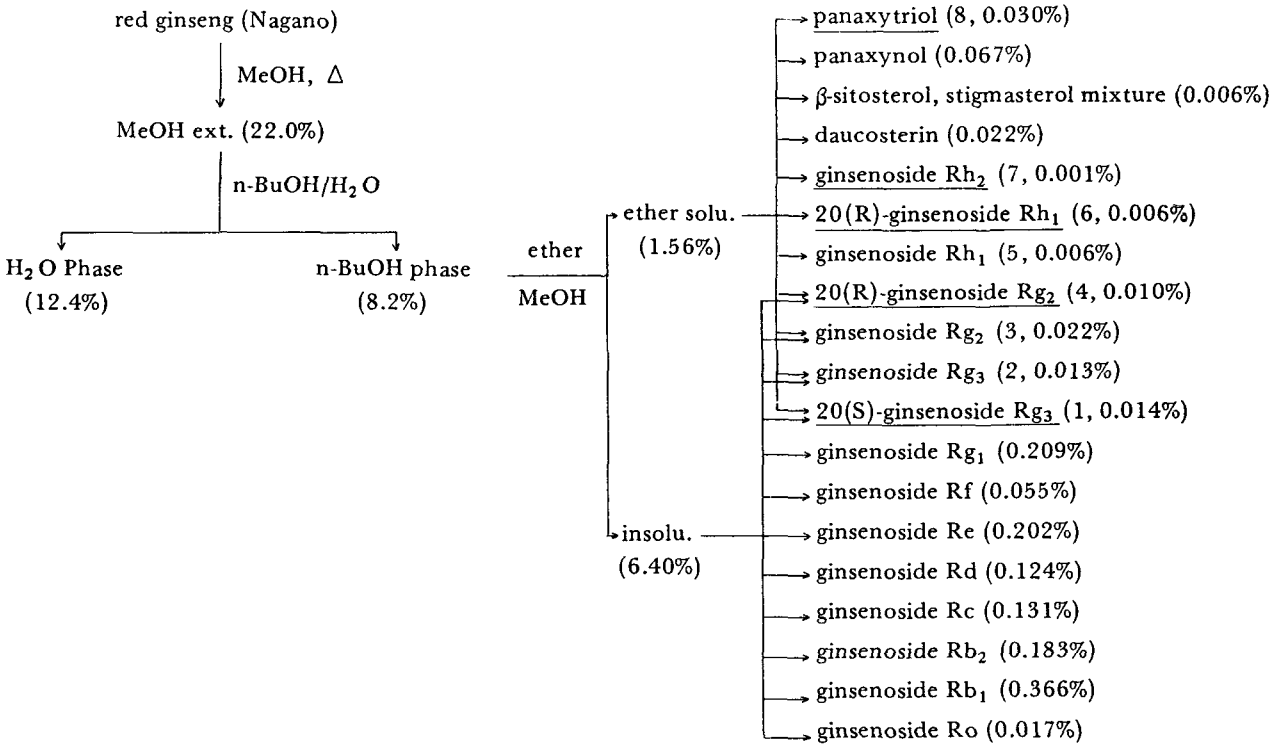


Chart 1: The Isolation Scheme for the Chemical Constituents of red ginseng
The Underlines denote the Characteristic Constituents.

Although ginsenoside Rg_3 (2) was reported to be isolated from white ginseng by a Japanese group, 5 [a] its botanical origin seems to be obscure since the ginsenoside Rg_3 (2) was an only ginsenoside having unnatural 20(R) configuration isolated from white ginseng. In addition, we have been unsuccessful so far to isolate ginsenoside Rh_1 (5)[5 b] from white ginseng from Nagano Prefecture, Japan.

2. Chemical Structures

The chemical structures of above-mentioned characteristic constituents of red ginseng were determined as shown in Chart 2 on the bases of spectral properties (including ^{13}C NMR), chemical reactions (including methylation analyses), and enzymatic degradations.

Panaxytriol (8) is heptadec-1-ene-4,6-diyne-3,9,10-triol and ginsenoside Rh_2 (7) and 20(S)-ginsenoside Rg_3 (1) are glycosides of protopanaxadiol, while 20(R)-ginsenoside Rh_1 (6) and 20(R)-ginsenoside Rg_2 (4) are glycosides of protopanaxatriol.

From comparisons of the characteristic constituents of red ginseng with hitherto known constituents of white ginseng, we have presumed tat panaxytriol (8) might be derived from panaxydol (8a) *via* the epoxide ring cleavage during the processing. Furthermore, 20(S) - ginsenoside Rg_3 (1), 20(R)-ginsenoside Rg_2 (4), 20(R)-ginsenoside Rh_1 (6), and ginsenoside Rh_2 (7) have been presumed to be formed during the processing *via* the cleavage of the 20-O-glycosidic linkages of higher glycosides with or without isomerizations of the C-20 configurations.

3. TLC Comparisons of Ether-Soluble Portions of Various Red and White Ginsengs

Since the chemical constituents characteristic of red ginseng were identified, we next ex-

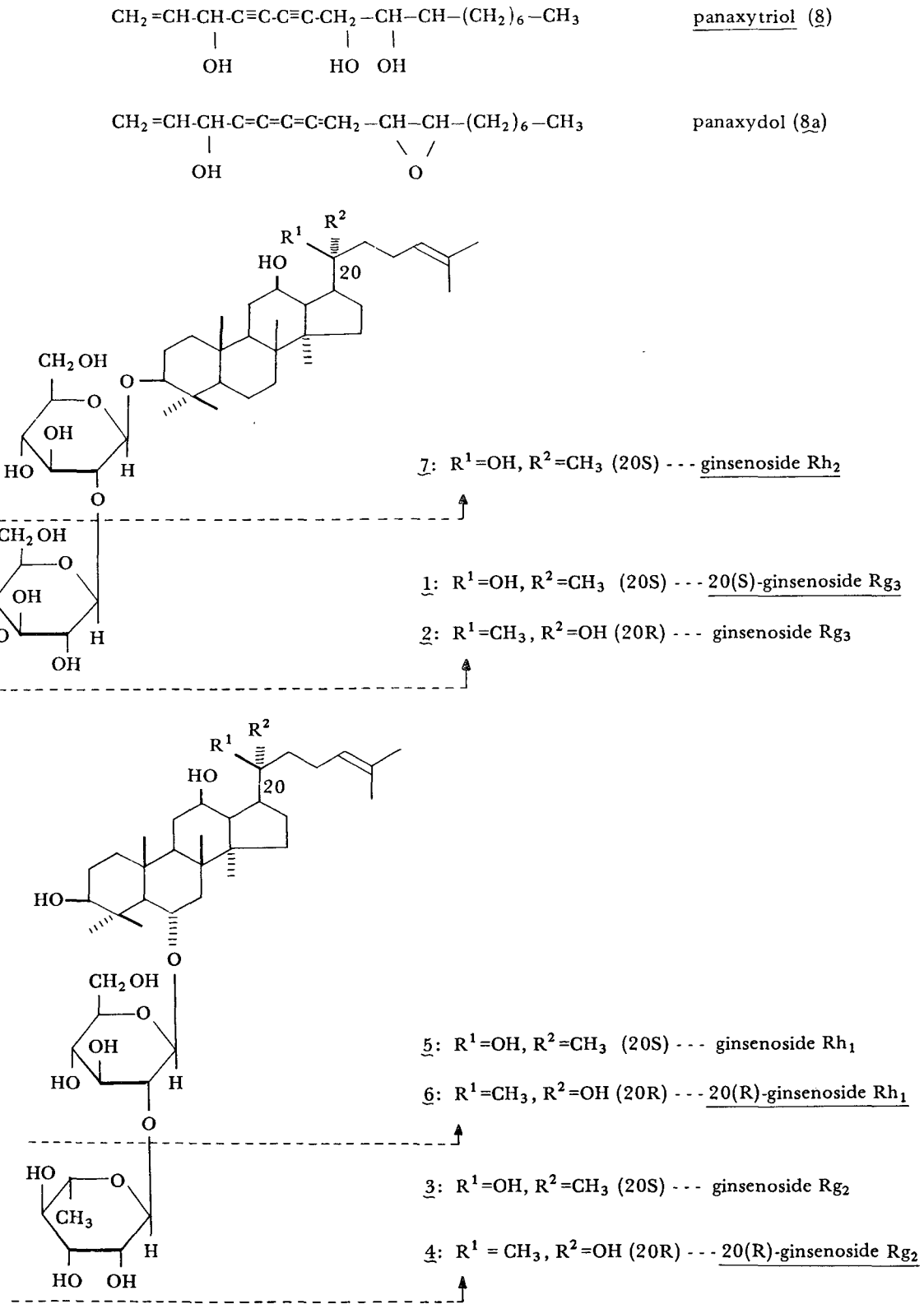


Chart 2: The Less-polar Constituents in Red Ginseng
(The underlines denote the characteristic constituents.)

amined the ether -soluble portions of various red and white ginsengs by TLC.

As shown in Fig. 1, TLC patterns of the ether-soluble portions of red ginsengs from Korea and Nagano Prefecture (Japan) were very similar. They differed significantly from TLC patterns of ether-soluble portions of white ginsengs from Korea, Nagano Prefectrue (Japan), and China.

TLC spots due to panaxytriol (8), ginsenoside Rh_1 (5), 20(R)-ginsenoside Rh_1 (6), and ginsenoside Rh_2 (7) were found as characteristic TLC spots of red ginsengs. However, TLC spots due to 20(S) - ginsenoside Rg_3 (1), ginsenoside Rg_3 (2), ginsenoside Rg_2 (3), and 20(R)-ginsenoside Rg_2 (4) were overlapped, so that these TLC conditions were unsatisfactory for evaluation of red and white ginsengs in regard to these four ginsenosides.

The isolation of these four ginsenosides (1, 2, 3, 4) were effected by chromatography of their acetates and it was found that red ginseng contained 20(S)-ginsenoside Rg_3 (1) ginsenoside Rg_3 (2), ginsenoside Rg_2 (3), and 20(R)-ginsenoside Rg_2 (4) whereas white ginseng contained only 3 among these four ginsenosides.

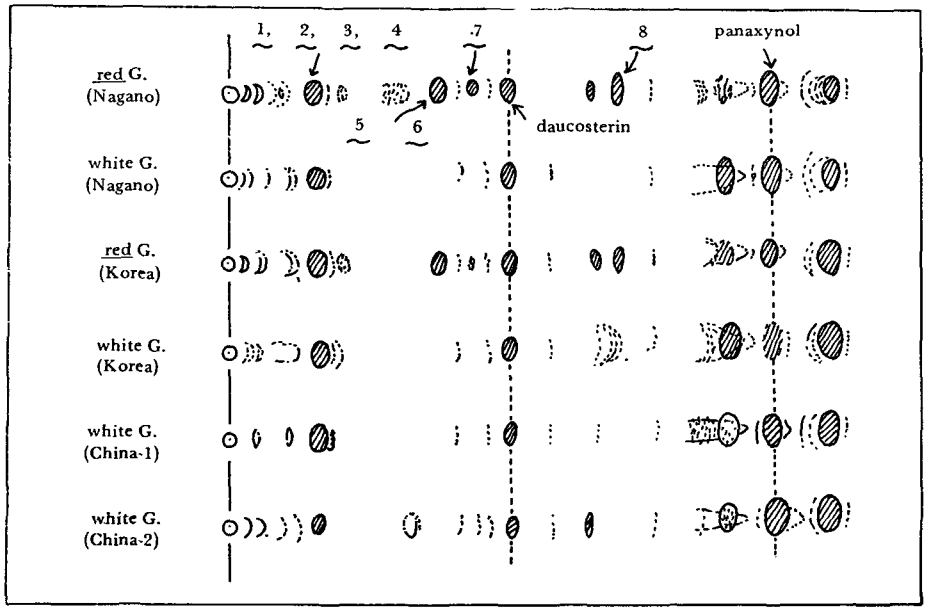
4. TLC Scanning Profiles of Ether-Soluble Portions of Various Red and White Ginsengs

The chemical ingredients of ether-soluble portions of various red and white ginsengs were examined by means of a TLC scanning method. As shown in Fig. 2, the shadowed portions in these profiles of red ginsengs indicated their characteristic constituents which were not found in the profiles of various white ginsengs.

Here again, it was confirmed by these TLC scanning profile analyses that red ginsengs differed significantly from white ginsengs in their ingredients of less polar ginsenosides (1, 2, 4-7) and an acetylenci compound (8).

5. Cytotoxic Activities of Ginsenoside Rh_2 (7)

During the course of the elucidation of respective biological activities of red and white ginsengs, it has been interestingly found by Prof. Odashima's group, Kanazawa Medical University, Japan that ginsenoside Rh_2 (7) exhibits cytotoxic activities while other ginsenosides isolated from red and white ginsengs do not show cytotoxicity.[6]



Pre-coated silica gel 60 F_{254} (0.25mm, Merck)
$CHCl_3$: MeOH:H_2O=7:3:1 (lower phase), 1% $Ce(SO_4)_2$ /10% H_2SO_4

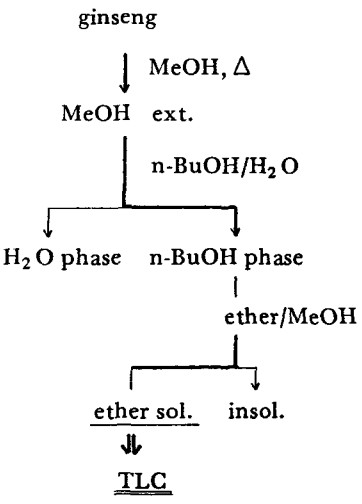


Fig. 1. TLC of Ether Soluble Portions of Various Ginsengs

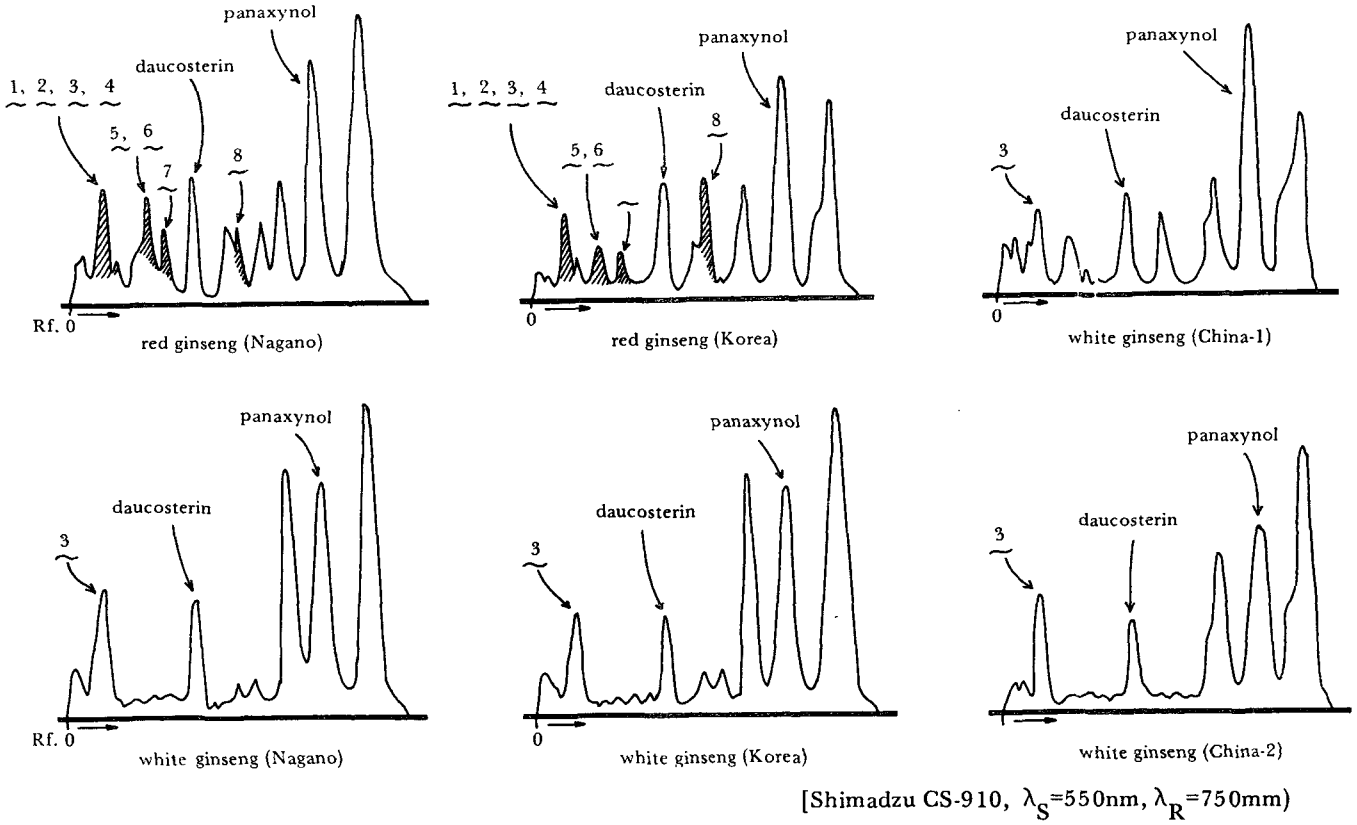


Fig. 2. TLC Scanning Profiles of Ether Soluble Portions of Various Ginsengs

The percent growths of 4 kinds of tumor cells, with or without addition of various ginsenosides in the culture media, were examined. As shown in Table 1, only ginsenoside Rh_2 (7) exhibited growth inhibitory effects on Lewis lung cancer cells (97.3% growth inhibition), Morris hepatoma cells (88.0% inhibition), B-16 Melanoma cells (60% inhibition), and HeLa cells (42.2% inhibition) at the concentration of 10μg/ml.

Table 1. Percent growth of tumor cells effected by addition by ginsenosides

Ginsenoside (10μg/ml)	Cell number (%)			
	3LL	MH_1C_1	B-16	HeLa
None	100.0	100.0	100.0	100.0
Rb_1	162.4	117.0	–	–
Rb_2	102.1	121.0	–	–
Rc	87.0	–	–	–
Rd	103.2	–	–	–
Rg_3	116.6	102.0	127.0	100.0
Rh_2	2.7	12.0	40.0	57.8
Re	95.1	–	–	–
Rg_1	107.9	97.0	–	–

3LL: Lewis lung cancer cells (from mouse), MH_1C_1: Morris hepatoma cells (from mouse), B-16;B-16 Melanoma cells (from mouse), HeLa:HeLa cells (from human)

Next, effects of ginsenosides Rh_1 (5) and Rh_2 (7) on growth of B-16 Melanoma cells were examined. Cells (10^5) were plated in Falcon dishes (35mm). 24hr after plating, ginsenoside Rh_1 (•) or Rh_2 (○) at various concentrations were added individually to separate dishes. At the termination of experiments on 4th day, the viable cells were detached with a 0.01% trypsin solution in phosphate-buffered saline and counted with an electronic particle counter. Each point on the curve represented an average of 5 samples. Similar results were obtained in these additional independent experiments.

As shown in Fig. 3, ginsenoside Rh_2 (7) showed growth inhibitory effects on B-16 Melanoma cells, while Rh_1 (5) did not show such an effect.

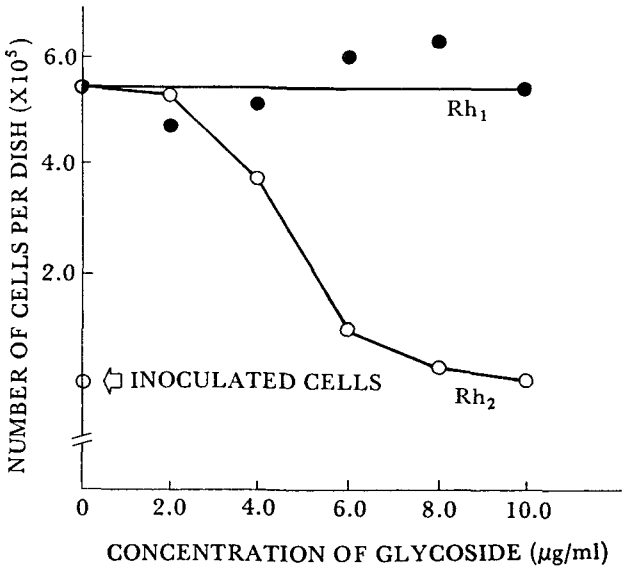


Fig. 3. Effects of ginsenosides Rh_1 (5) & Rh_2 (7) on growth of b-16 Melanoma Cells

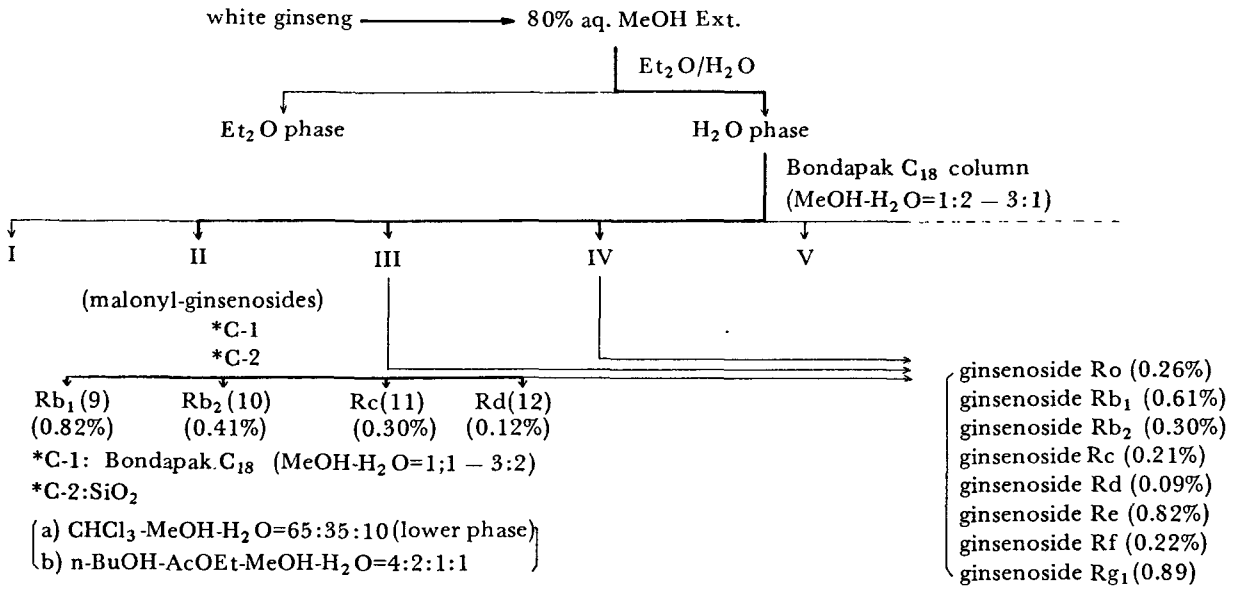


Chart 3: Isolation of Malonyl-Ginsenosides

CHARACTERISTIC CONSTITUENTS OF WHITE GINSENG

1. Isolation of Four New Malonyl-Ginsenosides

In preliminary examinations in detail of the water-soluble portions of red and white ginsengs, we have noticed the presence of several characteristic constituents in white ginseng. Since these constituents were readily transferred to the water-phase together with the carbohydrate ingredients in the ordinary n-butanol-water fractionation procedure, we have devised a new isolation procedure as shown in Chart 3.

Thus, white ginseng was first extracted with 80% aq. MeOH at room temperature. The extract was then partitioned into an ether-water mixture and the water-soluble portion was subjected to

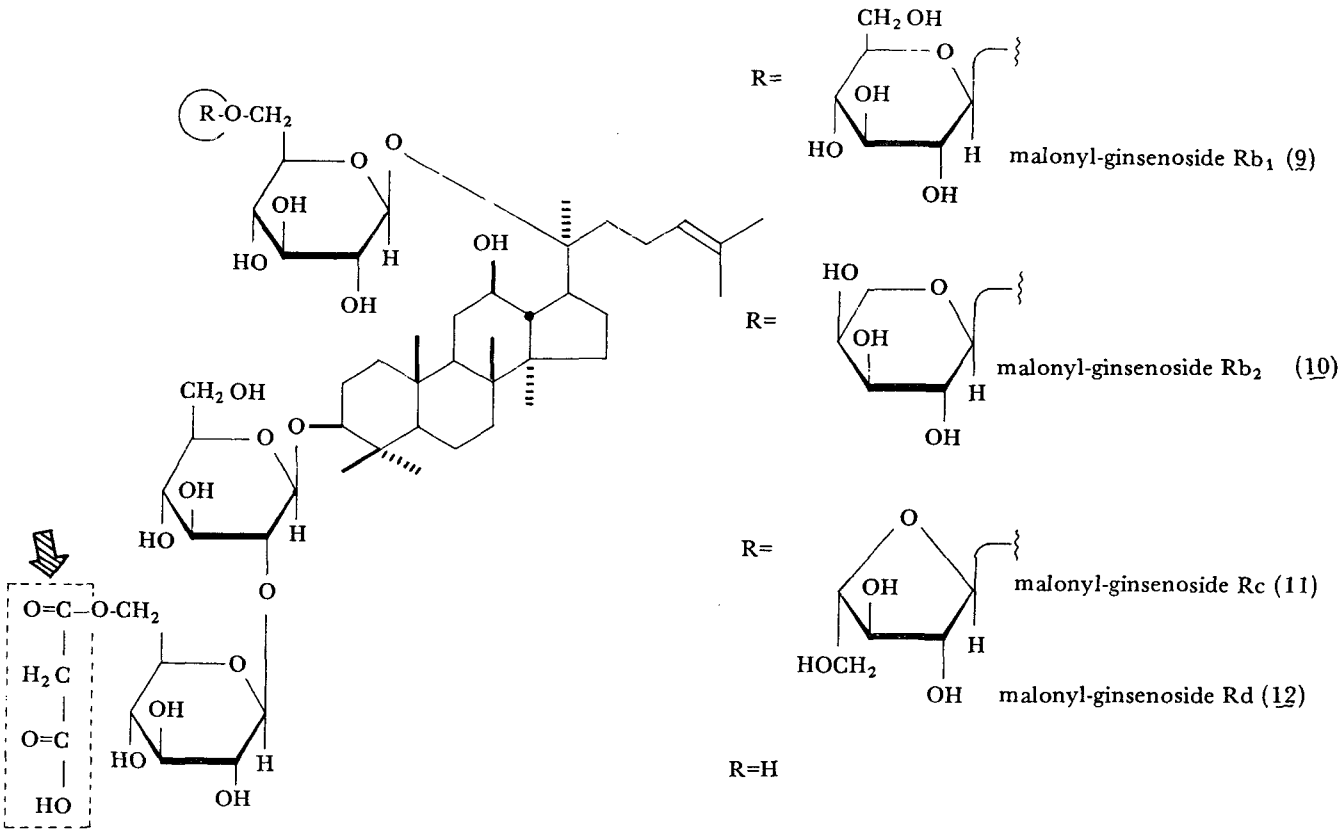


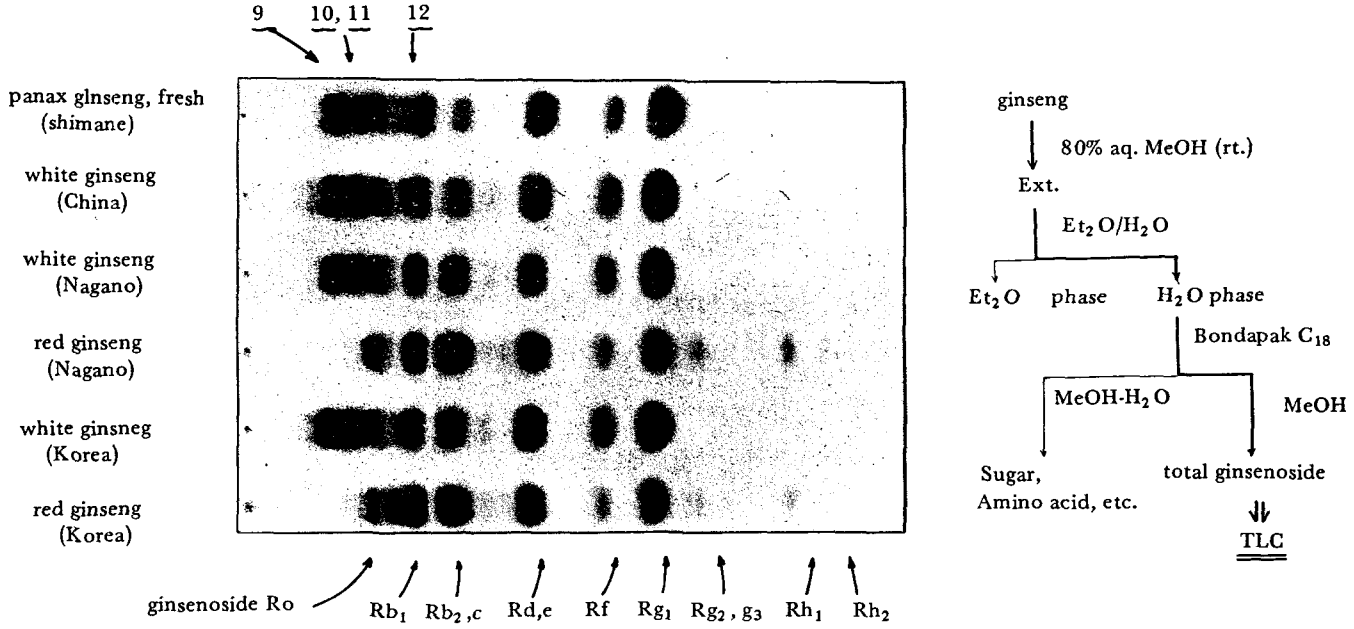
Chart 4: Malonyl-Ginsenosides from white ginseng

reversed phase silica gel (Bondapak C_{18}) column chromatography. Elution with methanol-water provided a mixture of total ginsenoside, from which four new water-soluble oligosides named malonyl-ginsenosides Rb_1, Rb_2, Rc, and Rd (9, 10, 11, 12) were effectively separated by repeated column chromatography using reversed phase and ordinary phase silica gel. By means of this new isolation procedure, four malonyl-ginsenosides (9, 10, 11, 12) were isolated in high yields together with known ginsenosides Ro, Rb_1, Rb_2, Rc Rd, Re, Rf, and Rg_1.

2. Chemical Structures of Four Malonyl-Ginsenosides

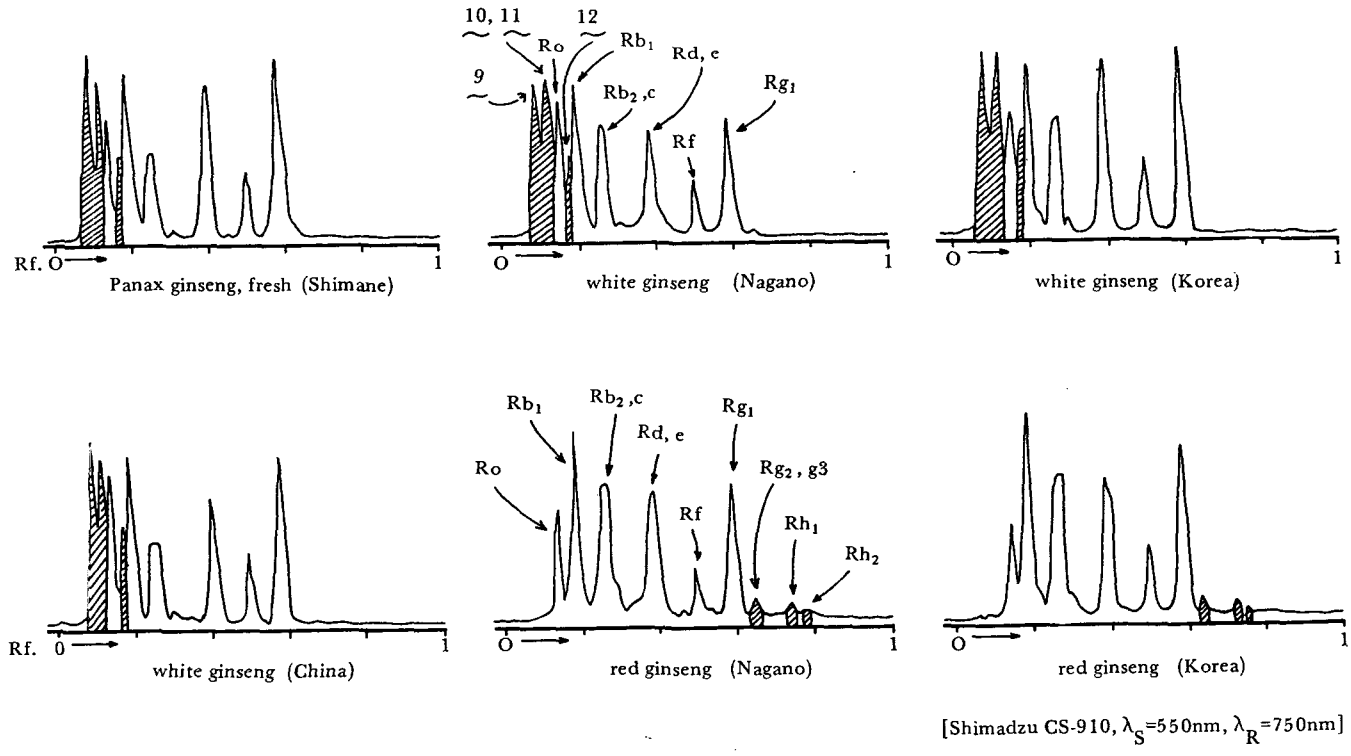
The structures of malonyl-ginsenosides Rb_1 (9), Rb_2 (10), Rc (11), and Rd (12) were determined on the basis of chemical and physicochemical evidence including ^{13}C NMR and secondary ion mass spectra (SIMS).

As shown in Chart 4, these four malonyl-ginsenosides (9, 10, 11, 12) contained a malonyl residue attached to the 6-hydroxyl function of the respective terminal glucosyl moieties. Interestingly, they are more readily soluble in water as compared with ordinary ginsenosides Rb_1, Rb_2, Rc, and Rd. Since these malonyl-ginsenosides possess a free carboxyl group in their structures, they behave as acidic saponins and their malonyl residues are partially hydrolyzed upon thermal treatment even under neutral conditions (*e.g.* hot methanol, hot water). Malonyl-ginsenosides are scarcely found in red ginseng from Nagano Prefecture in Japan, so that they are presumably decomposed during the processing to result in the formation of ordinary ginsenosides Rb_1, Rb_2, Rc, and Rd.



Pre-coated silica gel 60 F_{254} (0,25mm, Merck)
$CHCl_3$-MeOH-H_2O=65:35:10 (lower phase), twice, 1% $Ce(SO_4)_2$ /10% H_2SO_4

Fig. 4. TLC of Total Ginsenoside (From H_2O phase)



[Shimadzu CS-910, λ_S=550nm, λ_R=750nm]

Fig. 5. TLC Scanning Profiles of Total ginsenoside (From H_2O phase)

3. TLC Comparisons of Total Ginsenosides in Various Ginsengs[7])

The presence of water-soluble malonyl-ginsenosides was readily shown by TLC of total ginsenoside which was obtained by pre-treatment of water-soluble portion with reversed phase silica gel (Bondapak C_{18}). The total ginsenoside fractions obtained from various ginsengs (fresh, white, and red ginsengs from Japan, China, and Korea) were then examined by TLC and TLC scanning profile analyses.

As shown in Fig. 4, fresh and white ginsengs were shown to contain four malonyl-ginsenosides (9, 10, 11, 12). However, we could not find any spot due to a malonyl-ginsenoside in the total ginsenoside fractions of red ginsengs.

The presence of malonyl-ginsenosides in white ginseng was also shown by TLC scanning profile analyses of the total ginsenoside fractions. As shown in Fig. 5, various ginsenosides were found to contain more amount of malonyl-ginsenosides [Rb_1 (9), Rb_2 (10), Rc (11), and Rd (12), shown as shadowed peaks] than the amount of ordinary ginsenosides (Rb_1, Rb_2, Rc, and Rd) which have protopanaxadiol as their aglycones. In the other words, it has been shown that malonyl-ginsenosides are major glycosides of protopanaxadiol in white ginseng and they are decomposed to ordinary protopanaxadiol-ginsenosides during the processing.

CONCLUSION

In order to characterize chemically the processing of ginseng root, we comparatively analyzed the constituents of red and white ginsengs in detail. As a result, we found a new acetylenic compound named panaxytriol (8) and four new ginsenosides named 20(S)-ginsenoside Rg_3 (1), 20(R)-ginsneoside Rg_2 (4), 20(R)-ginsenoside Rh_1 (6), and ginsenoside Rh_2 (7) from red ginseng which are presumably formed during the processing. Among hitherto isolated ginsenosides, only ginsenoside Rh_2 (7) was found to exhibit cytotoxic activities against four kinds of cultured tumor cells.

On the other hand, we isolated four malonyl-ginsenosides Rb_1 (9), Rb_2 (10), Rc (11), and Rd (12) from the water-soluble portion of white ginseng. Since these malonyl-ginsenosides are scarcely found in red ginseng, their malonyl residues are presumably removed during the processing. We are currently investigating various properties of malonyl-ginsenosides.

Our comparative analyses of the chemical constituents of red and white ginsengs mentioned above were carried out for ginsengs harvested in the same area. In order to make these comparisons more precise, we recently analyzed red and white ginsengs which were prepared from a single *Panax ginseng* root.

Thus, a fresh root of *Panax ginseng* of 6 years old was harvested in September in Shimane Prefecture (Japan) and it was divided lengthwise into two. Each cut was respectively treated to prepare red and white ginsengs. Red and white ginsengs thus prepared were analyzed as mentioned above and we confirmed the similar chemical changes of the constituents occurring during the processings.[8])

Chong: You've shown that Rh_2 are cytotoxic. You've mentioned the Morris hepatoma, B-16 melanoma and Hella cells. What concentrations of those fractions were used when you found them to be cytotoxic? What do you think is clinical significance of what you've found?

Kitagawa; The ED_{50} concentration of Rh_2 on B-16 melanoma cell was 5μg/ml. But the yield of Rh_2 from Red Ginseng is quite low, 0.001%. Therefore, these findings can't be clinically associated with cytotoxicity of Red Ginseng itself.

Shibata: The compound Rh_2, which possesses more glucose, more sugar unit, shows no cytotoxicity. So why didn't you test 20(s)-protopanaxadiol?

Kitagawa: We had asked professor Odashima's group to test aglycon and they found that aglycon exhibits cytotoxic activity. But I don't know the recent results. This active mechanism will be presented by professor Odashima at the annual assembly of Cancer

Association of Japan in October.

Shibata: The reason of my question is that one of the recent findings by my collaborator in medical science reported that aglycon sapogenin shows inhibitory effect against cancer promoting actions, but saponin does not.

Tanaka: My comment is that when we take ginsenosides, 6% of that were decomposed by stomach juice.

생약에 관한 화학구조 - 홍삼 및 백삼 -

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생약의 화학적 특성에 대한 계속적인 연구가 이루어짐에 따라 우리는 홍삼 및 백삼의 화학성분을 상대적으로 규명하였다.

홍삼은 극성이 약한 분획에서 5개의 새로운 배당체 (20R-ginsenoside Rg_2, Rh_1;20R, 20S-ginsenoside Rg_3; ginsenoside Rh_2)와 새로운 아세칠렌 화합물 (Panaxytriol)을 함유하는 특징적인 성분들이 gins - enoside Rh1, Rg2와 함께 분리되었다.

ginsenoside Rh2는 배양된 종양세포에 대해 세포독소 효과를 보여주었다. 백삼은 수용성 분획에서 특징적인 성분이 있는 것으로 밝혀졌으며, 여기에서 malonly-ginsenosides Rb1, Rb2, Rc 및 Rd로 명명된 새로운 배당체 성분이 분리되었다.

Malona-ginsenosides는 백삼에서는 주요한 배당체이지만, 홍삼에서는 검출되지 않았다.

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