Research on Ginseng in Our Laboratory

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This talk is to give a brief review on my works on Korean ginseng carried out in our laboratory for more than ten years.

Korean ginseng has been understood as the anti-ageing medicinal plant and also as one of the tonic remedies, since it has been known as the elixir of life in the orient ever since thousands of years ago.

Recent studies on ginseng were concerned mainly with the chemistry and pharmacology of ginsenosides[1-3]. Those studies on the ginsenosides were triggered by the publication of Brekhman's theory that glycosides in ginseng exhibit adaptogenic activity[4]. When we summarize those pharmacological data on ginsenosides, we can easily arrive at a conclusion that Brekhman's adaptogenic activity theory on ginsenosides has been rationalized again by the evidences from the data obtained by other pharmacologists. Those whole pharmacological data may be summarized as the tonic nature.

The anti-ageing nature of ginseng were substantiated for the first time by our recent studies which were published under the article of anti-oxidant activity of ginseng[5-10].

Our first approach to the ginseng studies was started with the *in vitro* screening test on the anti-inflammatory activity of some plant extracts. The inhibitory activity of plant extracts to the heat denaturation of serum albumin was adopted as the anti-inflammatory parameter for the screening[11,12].

Among many plant extracts, Araliaceae and Umbelliferae including ginseng showed strong positive reaction to the *in vitro* test. By monitoring the inhibitory activity we could finally isolate the effective components in a crystalline state. Finally the anti-inflammatory activities of the crystalline substances were confirmed by routine animal experiment, the rat paw carrageenin edema test. At the end of structural analysis we found that the effective components were identical with ginsenosides Rg_1 and Re whose structures have already been established by forerunner, S. Shibata *et al.*

At that time we found by literature survey that the phytochemical studies on ginsenoside Rx were almost finished and many scientists also reported various pharmacological activities of ginsenosides[14,15].

Based on this background we started to synthesize the radio-labelled ginsenosides which enabled us to start our metabolic studies on ginsenosides, one of the most important research fields of ginseng in a sense of R & D of new drug.

Synthesis of radio-labelled ginsenosides

We established the procedures for the synthesis of ^{14}C- and ^{3}H-labelled ginsenosides[16,17](Fig. 1). Carbon fourteen was labelled to the side chain of ginsenosides Rg_1 by combination of the preparation of trisnoraldehyde by a sequence of oxidative removal of isopropyl fragment from side chain and reversion to ^{14}C-labelled ginsenoside Rg_1 by Wittig synthesis using ^{14}C-isopropyl-Wittig reagent.

The chemical yield of this procedure was fairly good but the radio-chemical yield was very poor due to poor incorporation of ^{14}C-isopropyl moiety to the Witting-reagent arising from the instability of ^{14}C-isopropyl-iodide. Our next approach to synthesize tritium labelled ginsenosides was very successful with very high radio-chemical yield(Fig.2).

The ginsenosides-12-ketone derivatives were

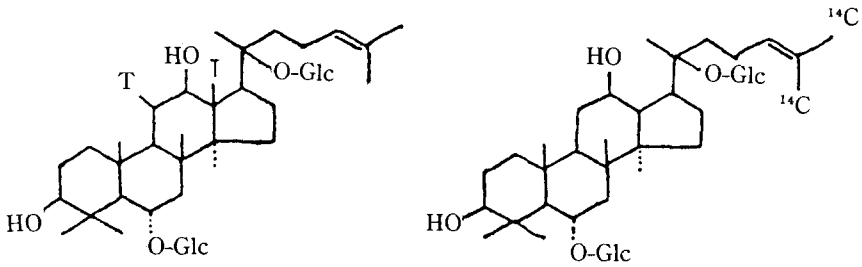
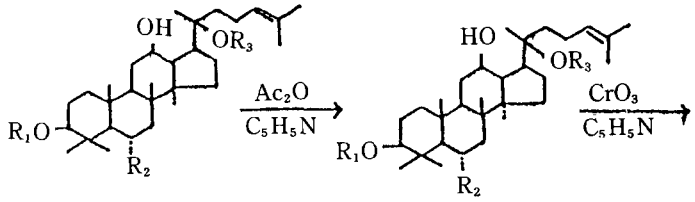
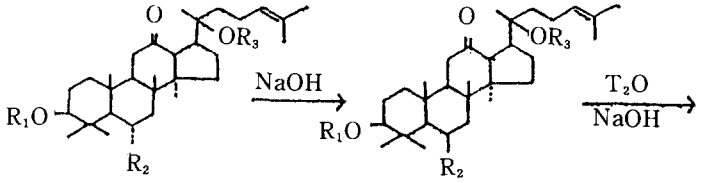


Fig.1. *Tritium and carbon-14 radio-labelled ginsenoside Rg_1.*



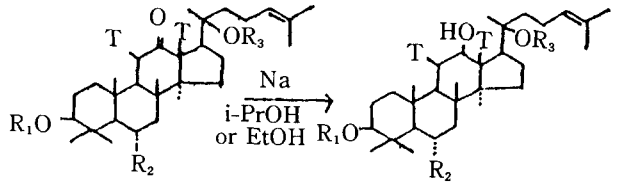
① Rb_1 : R_1=Glc-Glc, R_2=M, R_3=Glc-Glc
② Re : R_1=H, R_2=O-Glc-Rham, R_3=Glc
③ R_1 : R_1-H, R_2=O-Glc, R_3=Glc

④ R_1=Glc(Ac)$_3$-Glc(Ac)$_4$, R_2=H_1, R_3=Glc(Ac)$_3$-Glc(Ac)$_4$
⑤ R_1=H, R_2=O-Glc(Ac)$_3$-Rham(Ac)$_3$, R_3=Glc(Ac)$_4$
⑥ R_1=H, R_2=O-Glc(Ac)$_4$, R_3=Glc(Ac)$_4$



⑦ R_1=Glc(Ac)$_3$-Glc(Ac)$_4$, R_2=H, R_3=Glc(Ac)$_3$-Glc(Ac)$_4$
⑧ R_1=H, R_2=O-Glc(Ac)$_3$-Rham(Ac)$_3$, R_3=Glc(Ac)$_4$
⑨ R_1=H, R_2=O-Glc(Ac)$_4$, R_3=Glc(Ac)$_4$

⑩ R_1=Glc-Glc, R_2=H, R_3=Glc-Glc
⑪ R_1=H, R_2=O-Glc-Rham, R_3=Glc
⑫ R_1=H, R_2=O-Glc, R_3=Glc



⑬ R_1=Glc-Glc, R_2=H, R_3=Glc-Glc
⑭ R_1=H, R_2=O-Glc-Rham, R_3=Glc
⑮ R_1=H, R_2=O-Glc, R_3=Glc

Fig.2. *Synthesis of tritium labelled ginsenosides.*

prepared by combination of partial acetylation and chromium oxide oxidation. The C_{13}-H and C_{11}-H in the ketone derivative were easily replaced with tritium on heating in alkaline tritium oxide. The tritium labelled ketones could be reduced with Na in isopropanol to obtain the final products, ^{3}H-labelled ginsenosides. After chromatographic purification, we could obtain high yields of raido-chromatographically pure samples suitable for biological studies[19,20].

Metabolic fate of ginsenosides

When 200 mg of ginsenosides were administered by intravenous route to 2 kg rabbit, more

than 70% was excreted within 6 hours by renal route forming dipolar excretion curve. Therefore retention of ginsenosides in body did not exceed 50 mg/2 kg body weight in rabbit. For a long time, many pharmacological data on ginsenosides have been reported and many scientists have had a deep interests on whether the ginsenosides may be absorbed from gastro-intestinal tract and then finally reach to a pharmacological concentration in the target organs on which ginsenosides were reported to play some biological acitivities. In response to this interests, the experiments for the gastro-intestinal absorption, organ distribution and urinary excretion were carried out several times after oral administration of ^{3}H-ginsenosides of mice(Table 1). Consistent data could not be obtained for the absorption rate, probably due to individual physiological variation of mice. A wide-ranging change in the oral doses of ^{3}H-ginsenosides in each experiment would be another involved factor for the inconsistent GIT-absorption rate, but the retention of radio-activity in tissue was fairly constant in each experiment irrespective of changes in the oral doses. When the oral doses of ^{3}H-ginsenosides were increased to much higher level than the theoretically calculated pharmacological doses based on Chiness medical dosage, the greater part of the radio-active substances was remained in GIT(Fig.3). The radio-chromatogram of GIT-remaining substances showed highly complicated pattern probably due to hydrolytic interaction of gastric acidity[18-21].

Table 1. *Urinary excretion and gastrointestinal remaining of tritium labelled ginsenosides.*

	Dose radioactivity μCi	Urinary excretion %	GIT remained %
^{3}H-Rb$_1$	12.87	1.32	69.15
	26.41	1.54	45.6
	52.83	1.56	94.28
^{3}H-Re	12.12	6.91	93.16
	24.24	–	91.49
^{3}H-Rg$_1$	16.38	4.08	89.36
	32.77	2.67	88.04
^{3}H-Rc	9.48	2.00	87.08
Prosapogenin	19.01	2.60	88.90

It was very interesting that the radio-

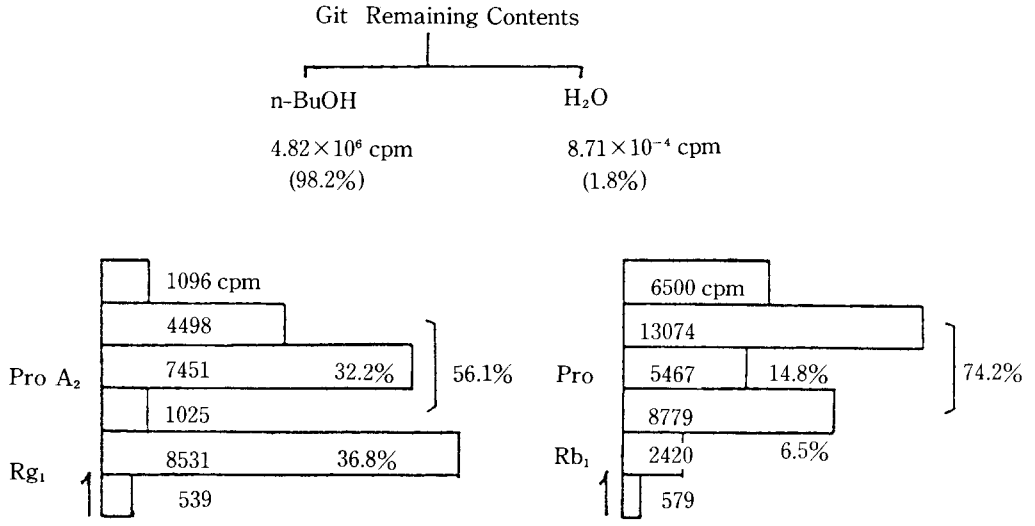


Fig.3. *Radiochromatograms of GIT remaining contents unabsorbed 26 hrs after oral administration of ^{3}H-ginsenoside Rg$_1$ and Rb$_1$.*

chromatogram of the urinary excretes from the same animal showed only intact ^{3}H-ginsenosides spot devoid of the degradation products found in GIT(Fig.4).

By cannulation experiment on bilary duct, we could find that both the intact ^{3}H-ginsenosides and the degradation products are absorbed from GIT, but that the excretion for them are different; the intact ^{3}H-ginsenosides through renal route and the degradation products through biliary route to intestine.

By biliary duct cannulation experiments, we could also find that the absorption of ^{3}H-ginsenosides were highly impeded by showing more than 95% of radio-activity in GIT even 26 hrs after oral administration of ^{3}H-ginsenosides.

The organ distribution of ^{3}H-ginsenosides was found to be pancellular(Table 2). The activity was found even in brain although its radio-activity was much weaker than that of other tissues. Based on the radio-activities of various tissues, the ginsenoside content retained in tissues was calculated to be in a range from some micromolar to nanomolar concentration which is much lower than the routine experimental concentration employed in the *in vitro* biological activity tests. The organ distribution of ginsenosides was also estimated quantitatively in the laboratory of Prof. U. Sankawa of the University of Tokyo by raido-immunoassay procedure which was developed in collaboration with us[22,23]. This result showed also a similar tendency to the data obtained by our radio-activity tracer studies(Fig.5).

The chemical natures of the degradation products in GIT were studied also in our laboratory. The C-20-glycosyl group is removed at the initial stage and then the chemical modification progresses to produce C-25 hydroxyl compound by hydration of the C-24 olefinic bond of the glycosides.

Anti-oxidant component of ginseng

Our new research topics on ginseng, the studies on the anti-oxidant components of ginseng, were created in our laboratory in 1977, based on the Brekhman's report that the adaptogenic activity of Eleutherococcus senticosus is believed to come from some lignane

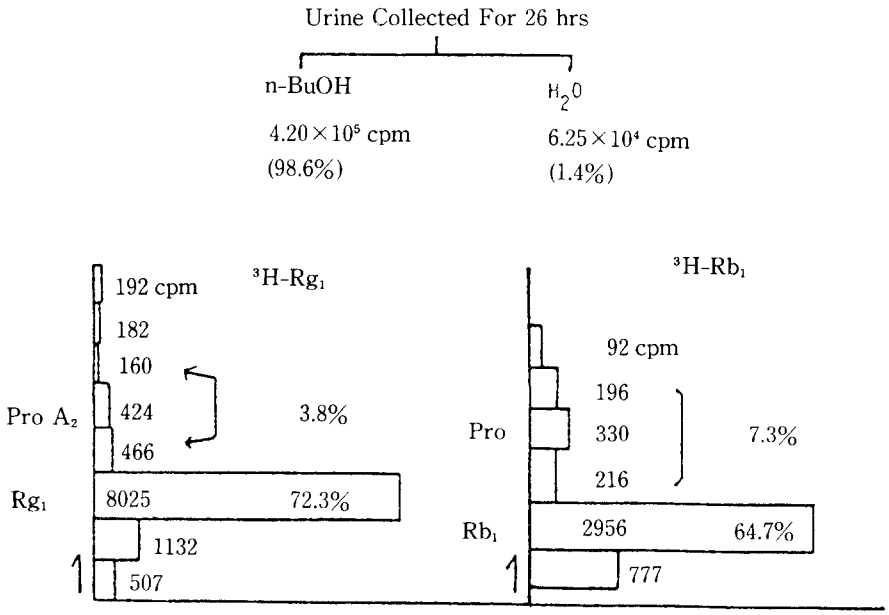


Fig.4. *Radiochromatograms of urinary excretion products of ^{3}H-ginsenoside Rg$_1$ and Rb$_1$ after oral administration.*

Table 2. *Organ distribution of ^{3}H-ginsenoside Rg_1, Re and Rb_1 after oral administration: Organs were harvested 30, 40 min after oral administration of ^{3}H-Rg_1 80.47 μCi/8.96 mg, ^{3}H-Re 75.06 μCi/6.3 mg, ^{3}H-Rb_1 76.93 μCi/6.34 mg per 25g b.wt mouse.*

	^{3}H-Rg_1			^{3}H-Re			^{3}H-Rb_1		
	cpm/100mg tissue	cpm/ organ	% of the dose	cpm/100mg tissue	cpm/ organ	% of the dose	cpm/100mg tissue	cpm/ organ	% of the dose
Blood	739	–	–	529	–	1.59×10^{-3}	1466	–	–
Brain	105	336	7.86×10^{-4}	189	637	2.79×10^{-3}	276	930	2.27×10^{-3}
Lung	8976	13284	3.1×10^{-2}	784	1113	9.73×10^{-4}	3569	5496	1.34×10^{-3}
Heart	1604	1300	3.04×10^{-3}	479	388	1.91×10^{-2}	964	743	1.81×10^{-3}
Kidney	1602	4870	1.13×10^{-2}	2598	7635	4.41×10^{-2}	2046	5892	1.44×10^{-2}
Liver	3998	49575	11.59×10^{-2}	1399	17594	4.36×10^{-4}	1562	20243	4.95×10^{-2}
Spleen	398	413	9.66×10^{-4}	87	174		106	212	5.18×10^{-4}
Muscle	280	–	–	166	–	–	1403	–	–

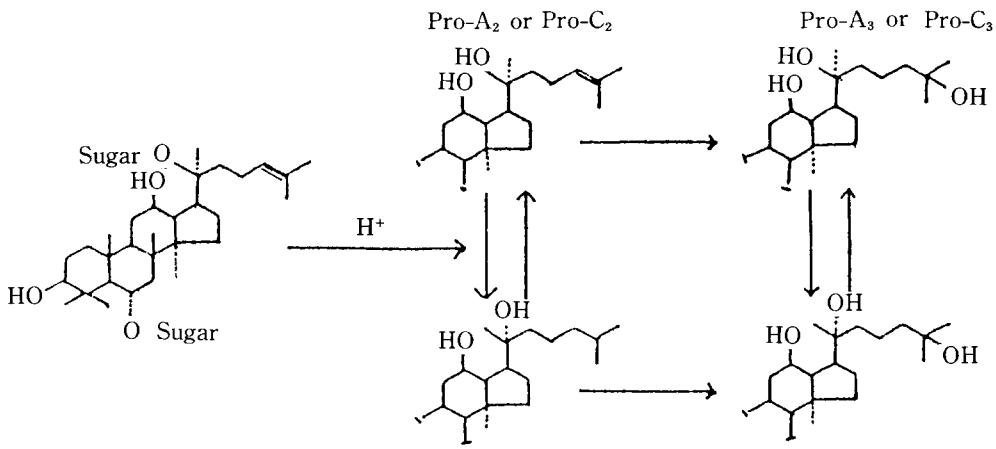


Fig.5. *The degraded products of ginsenoside in a physiological gastric conditions.*

glycosides which belong to phenolic substances. It was very interesting that the same adaptogenic activities were reported from the effective components of the different chemical nature; one from dammarane triterpene glycosides and the other from phenolic substances, although both plants belong to same taxonomic family. In order to understand the pharmacological significance of biologically active anti-oxidant substances, let us have a brief summary on the gerontologist's view on the cellular ageing.

Living cells produce very reactive and harmful free radical oxygen species such as singlet oxygen, superoxide anion and hydroxy radicals in paralle with the consumpion of oxygen for their respiration[24]. Although a greater part of them is quenched by some self-protective system, a part of them leaks from the system and attacks the unsaturated fatty acids in various biomembranes, leading to the production of lipid peroxides and finally resulting in the decreased vital efficiency of the cell[25]. Once again a greater part of lipid peroxides is

reduced to less reactive hydroxyacids, but a part of them leaks from the system and decompose to produce highly reactive malondialdehyde.

This binds nonspecifically to nearby biomacromolecules such as enzyme to produce so called lipofuscine pigment which will be accumulated in living cells in paralle with cellular ageing[26]. The membrane damage caused by those free radical chain reaction products, and the protein binding of malondialdehyde are believed to be the cause of various geriatric diseases. Based on these backgrounds, the gerontologists assume biologically active anti-oxidants to be the anti-ageing agents[27,28].

Our first approach to the studies on the anti-ageing drug was started from the screening on free radical queenching activities of 120 crude drugs and some cereal extracts, using diphenyl picryl hydrazyl(DPPH) as the free radical reagent[29,30]. Almost more than 40% of plant extracts showed strong quenching activities[31].

This widespread distribution of free radical quenching substances in the plant kingdom is very suggestive of the possible role of Chinese medicine which shows sometimes a dramatic therapeutic response to geriatric disease.

It was very difficult to select any plant from our *in vitro* screening data for our phytochemical studies to isolate the free radical quenching components, since flavonoids and tannins which are distributed widely in the plant kingdom seem to exhibit free radical quenching activity in *in vitro* experiments.

In the subsequent approach, we selected 30 herbal drugs as the candidates for anti-oxidant activity screening test by animal experiments, reviewing Chinese Material Medica Book in which tonic or anti-ageing activity were suggested strongly.

Pharmacological doses of plant extracts were fed to mice and then acute toxic doses of 50% ethanol were administered orally to induce lipid peroxidation and the livers were removed for the assay of lipid peroxide content by Masugi's TBA-value procedure[32].

Some of the plant extracts including Panax ginseng showed strong inhibitory activities on the lipid peroxidation by ethanol intoxication[7]. Animals for blank test group showed TBA-values of around 10 units, and the ethanol group 38 units, whereas the animal group treated with ginseng extract only 12 units[5]. This dramatic biological data promoted us to isolate the effective components of ginseng, monitoring the anti-oxidant activity by the animal experiment(Fig.6).

The ether soluble acidic fraction and butanol soluble glycosidic fraction showed the strong activity whereas the ether soluble neutral fraction and highly polar water soluble fr. were devoid of the activity. The ether soluble acidic fraction was further purified to obtain the active substances in a pure crystalline state by silica gel column chromatography[5,8](Fig.7).

They were identified as simple substances, maltol, salicylic acid, vanillic acid and p-coumaric acid. Strong anti-oxidant activities

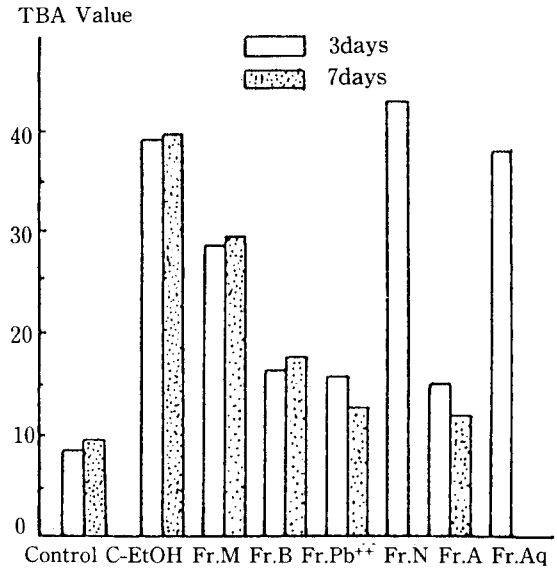
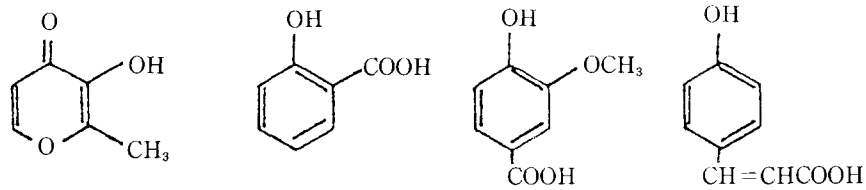


Fig.6. *Antioxidant activities of ginseng fractions.* C-EtOH:EtOH intoxication, Fr.M: methanol ex. Fr.B: supernatant of $Pb(Ac)_2$ treated saponin fr. Fr.Pb: precipitate of $Pb(Ac)_2$ treated saponin fr. Fr.N: neutral fr. of ether ex., Fr.A: acidic fr. of ether ex., Fr.Aq: finally water soluble fr.



Maltol Salicylic acid vanillic acid *p*-Coumaric acid.

Fig.7. *Some phenolic compounds isolated from the ether-soluble acidic fraction of ginseng.*

of the former three crystalline substances were confirmed by the same animal experiments but p-coumaric acid was devoid of the activity. However, maltol was found only in the extract of red ginseng and the other components were found in the extracts of both red and white ginseng. This probably suggests that maltol is the artefact product produced by heat treatment of ginseng during the red ginseng manufacturing process. Still many other phenolic substances were detected on TLC, but these were not isolated yet[33].

Although the complete elucidation of chemical identities of the anti-oxidant components if ginseng is still awaiting further studies, we are assuming the phenolic substances to be the effective principles of anti-oxidant activity of ginseng.

Noteworthy is the fact that none of the purified ginsenosides show anti-oxidant activity both *in vitro* and *in vivo* animal tests whereas the semipurified ginsenoside samples shows strong activity. This must be due to the contamination of the impure ginsenoside samples by the phenolic substances(Table 3).

We are still trying to isolate some phenolic glycosides from butanol soluble fraction which is highly active in our anti-oxidant activity tests by animal experiments.

In the Chinese Materia Medica Book we can find the fact that ginseng is contraindicated with iron. Actually folkloric customs eliminate all iron-made tools in the processing of ginseng including decoction and peeling of fresh ginseng. This folkloric customs are very suggestive for our understanding of the real character

Table 3. *Antioxidant activities of ginseng components: Samples were administered orally to mice once daily for 3 days. Mice were starved for 8 hrs after last medication and lipid peroxidation was induced by ethanol intoxication and lipid peroxide content in the mouse liver was assayed by TBA method 12 hrs after ethanol intoxication. TBA values were expressed as A_{535}/g wet liver.*

	Control	Ethanol -control	Sample, mg / 30g b. wt			
			0.001	0.01	0.1	1
Maltol	10.4	31.6	30.7	19.5	12.6	11.8
Salicylic acid	9.7	25.3	21.6	18.2	14.7	13.3
Vanillic acid	10.1	23.4		19.8	15.2	14.4
P-coumalic acid	8.1	15.2	14.2		12.6	
Ginsenoside Rg_1	9	27.5				24
Ginsenoside Re	9	27.5				22.4
Ginsenoside Rb_2+Rc	9	27.5				28.3
Ginsenoside Rb_1	9	27.5				25
α-Tocopherol acetate	10.1	38.5				11

of the active principle of ginseng.

In order to see whether the folkloric experiences are reproduced in our modern moleuclar pharmacological experiments, we added a very small amount of ferric ion in the extraction process of ginseng instead of using iron vessel[6,9]. The ginseng extract containing ferric ion showed considerably decreased anti-oxidant activity in the animal experiment(Fig.8).

The anti-oxidant activity mechanism of phenolic substances

When we incubate liver homogenate, the lipid peroxide content is gradually increased in propotion to incubation time due to the activation of NADPH-dependent microsomal system[34] for lipid peroxidation. This increase was dramatically enhanced when a small amount of ferric ion was added to the homogenate. This dramatic increase was completely blocked by concomittant addition of two mole equivalent of maltol(Fig.9).

It was also found in our laboratory that maltol and ferric ion form a stable chelate with the binding ratio of two to one. Thus formation of a stable maltol-Fe^{+++}-chelate may be considered as the mechanism of anti-oxidant activity of maltol which deprives effectively the ferric ion from ADP-Fe^{+++} complex that plays as the initiation factor in the free radical chain reaction of microsomal system[34].

Anti-fatigue activity of ginseng

The anti-oxidant activity of ginseng may be somewhat concerned with other pharmacological activities which have been repeatedly reported as the ginseng efficacy by others. Such activities are as following; 1) protection from radiation injury, 2) protection of liver from drug, 3) prevention of hang over symptoms, 4) anti-fatigue activity, 5) anti-artherosclerosis and 6) anti-thrombosis etc... So we can have a question on whether those activities are arising from ginsenosides or phenolic sub-

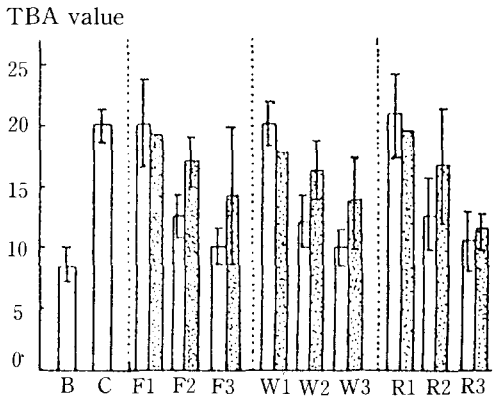


Fig.8. *The effect of ferric ion treatment on the antioxidant activity of ginseng extracts; Blank column: non treated ginseng groups. Shaded column; Ferric ion treated ginseng groups, B: blank, C: control(ethanol intoxication only). To F-1, F-2, F-3, W-1, W-2, W-3, R-1, R-2, and R-3 group animals, the ginseng samples were administered for 3 days before the induction of lipid peroxide by ethanol intoxication was conducted. Each group was consisted of 4-6 mice and the results are the mean value of four repeated experiments. The prefix F, W, and R denote fresh ginseng, white ginseng and red ginseng. The suffix-1, -2, and -3 denote the dosages of 0.2, 2.0, 20 mg ginseng/ 30g body wt. mouse.*

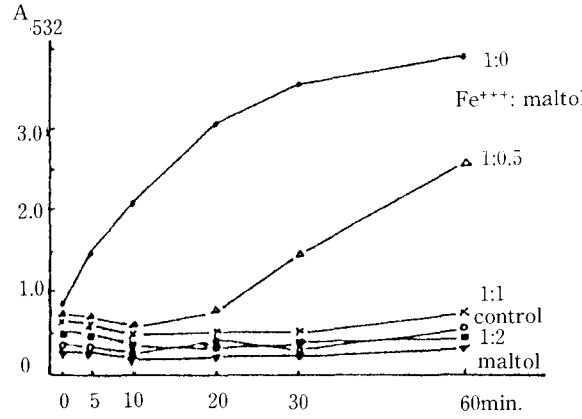


Fig.9. *Inhibitory effect of maltol on the Fe^{+++} -catalized oxidation of liver homogenate: 0.2 ml of 0.05M $FeCl_3 \cdot 6H_2O$ and given molar equivalent maltol in 0.175M KCl and 0.4 ml of 9% mouse liver homogenate were incubated at 37℃ for specified time. Lipid peroxide was measured by TBA method(A_{532}).*

Table 4. *Antifatigue activity of ginseng and its components: 20mice per group were administered with samples and two hours later subjected to swimming test in a 24℃ water pool. Mean swimming times were recorded.*

Group	Swimming time min.
Exp. 1.	Doses ; 0.22g/kg body wt. once daily for 5 days
Control	88.1±26.9
H_2O Ex.	107.6±41.3*
Et_2O fr.	131.9±43.0**
BuOH fr.	117.2±42.5**
H_2O fr.	96.0±28.7
Exp. 2. Doses ; 10mg/kg body wt.	
Control	108.4±55.7
Naltol	162.6±63.5**
Salicylic acid	167.5±69.4**
Vanillic acid	160 ±73.1**
Exp. 3.	Doses ; 10mg/kg body wt.
Control	122.4±44.6
Ginsenoside	
Rg_1	99.3±26
Re	125.5±41.6
Rb_1	128.4±46

$*p<0.05$, $**p<0.001$

stances. It is very difficult to give a decisive conclusion at present, but I will give a very suggestive data on this question.

Twenty years ago Brekhman I.I. reported the anti-fatigue activity of ginseng and panaxosides which was evaluated by the increase of swimming time of mice[4,35]. In our laboratory we reexamined the anti-fatigue activity of ginseng and ginsenosides by the same swimming test as Brekhman did[10,36]. The anti-fatigue activity distribution in various fraction of ginseng extract was very similar to that of anti-oxidant activity in the ginseng fractions. Brekhman's anti-fatigue activity was reproduced in our experiment on the ginseng extract and impure ginsenoside samples, but not on any purified ginsenosides which were isolated in our laboratory by repeated crystallization(Table 4).

On the other hand, maltol and phenolic aicds isolated as the anti-oxidant components of ginseng were strongly active in the prolongation of swimming time(Table 4).

Conclusion

The ginseng efficacies were found in old Chinese Materia Medica Book as followings; (神農本草經) 主補五藏, 安精神, 定魂魄, 止警悸, 除邪氣, 明目開心益智, 久服輕身延年.

Of these many kinds of efficacies, the last one could be explained in a modern scientific expression from our finding on anti-oxidant and anti-fatigue activities of phenolic substances in ginseng. We are assuming that some pharmacological activities reported under the name of ginsenosides may be due to the direct or indirect consequences of anti-oxidant activity of phenolic substances contained as impurities in ginsenoside samples. Therefore we don't consider ginseng as being a stimulant or depressant to some particular function but rather as being a cleaning substance for the unwanted sludges produced by abnormal oxidation in cell.

References

1. H. Oura and S. Hiai, *Proc. Internat. Ginseng Symposium,* The Central Res. Inst. Office of Monopoly, ROK p.23(1974).
2. K. Takagi, *ibid,* p.119(1974).
3. S. Shibata, *ibid,* p.69(1974).
4. I.I. Brekhman and I.V. Dardymov, *Ann. Rev. Pharmacol.*, **9**,419(1969).
5. B.H. Han, M.H. Park, L.K. Woo, W.S. Woo and Y.N. Han, *Korean Biochem J.*, **12**(1), 33(1979).
6. B.H. Han and M.H. Park, *Korean J. Pharmacog.*, **9**,169(1978).
7. B.H. Han, S.Y. Yoo, M.H. Park and H.J. Lee, *ibid,* **10**, 108(1979).
8. B.H. Han, M.H. Park and Y.N. Han, *Arch. Pharm. Res.*, **4** (1), 53(1981).
9. B.H. Han, M.H. Park and Y.N. Han, *Program*

of the 32nd Ann. Convention of the Pharm. Soc. of Korea, P.110(1983).
10. B.H. Han, M.H. Park, Y.N. Han and S.C. Shin, *Yakhak Hoeji,* **28**(4), 231(1984).
11. B.H. Han, Y.N. Han and L.K. Woo, *J. Pharm. Soc. Korea,* **16**(3), 129(1972).
12. B.H. Han, K.S. Ryu, H.J. Chi and Y.N. Han, *Kor, J. Pharmcog.*, **3** (4), 205(1972).
13. Y. Nagai, O. Tanaka and S. Shibata, *Tetrahedron,* **27**, 881(1971).
14. S. Sanada, N. Kondo, J. Shoji, O. Tanaka and S. Shibata, *Chem. Pharm. Bull.,* **22**(2), 421(1974).
15. I. Kitagawa, T. Taniyama, T. Hayashi and M. Yoshikawa, *Chem. Pharm.Bull.*, **31**(9), 3353(1983).
16. L.K. Woo, *J. Pharm. Soc. Korea,* **17**, 123(1973).
17. B.H. Han and L.K. Woo, *Arch. Pharm. Res.*, **1,** 27(1978).
18. B.H. Han and I.M. Chang, *Korean J. Ginseng Sci.*, **2** (1), 17(1977).
19. B.H. Han, M.H. Park, L.K. Woo, S.K. Hong and D.H. Kim, *Program of the 31st Ann. Convention of the Pharm. Soc. of Korea,* p.128(1982).
20. B.H. Han, M.H. Park, S.K. Hong and D.H. Kim, *Program of the 32nd Ann. Convention of the Pharm. Soc. of Korea,* p.117(1983).
21. B.H. Han, E.B. Lee, U.C. Yoon and L.K. Woo, *Korean Biochem. J.,* **9,** 21(1976).
22. B.H. Han, M.H. Park, Y.N. Han, L.K. Woo, U. Sankawa, S. Yahara and O. Tanaka, *Arch. Pharm. Res.*, **4** (1), 25(1981).
23. B.H. Han, M.H. Park, Y.N. Han, L.K. Woo, U. Sankawa, S. Yahara and O. Tanaka, *Planta Medica,* **44**(3), 144(1982).
24. D.B. Menzel, *Ann. Rev. Pharmacol.,* **10**, 379(1970).
25. F. Masugi and T. Nakamura, *Nutrition and Food(Japan)*, **29**(7), 361(1976).
26. A. Wolf and A.M. Pappenheimer, *J. Neuropath. Exp. Neurol.,* **4,** 402(1945).
27. D. Harman, *J. Gerontol.,* **16**, 247(1961).
28. D. Harman, *ibid,* **23**, 476(1968).
29. M.S. Blois, *Nature,* **181,** 1199(1958).
30. A. Mellors and A.L. Tappel, *J. Biol. Chem.,* **24**(19), 4353(1966).
31. B.H. Han and M.H. Park, unpublished data.
32. F. Masugi and T. Nakamura, *Vitamin(Japan),* **51**(1), 21(1977).
33. K.J. Choi, *Thesis for Ph. D. Korea University, Seoul, Korea,* 1983.
34. B.A. Svingen, J.A. Buege, F.O.O'Neal and S.D. Aust, *J. Biol. Chem.,* **254** (13), 5892(1979).
35. I.I. Brekhman, *Symposium of Gerontology(Lugano),* p.5(1976).
36. B.H. Han, M.H. Park and Y.N. Han, *Program of the 31th Ann. Convention of the Pharm. Soc. of Korea,* p.128 and p.134(1982).