CHEMICAL AND BIOLOGICAL STUDIES ON THE BIOLOGICALLY ACTIVE NATURAL PRODUCTS IN KOREA

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In Korea, there are more than 3,500 plant species which had traditionary been used as folklore medicines in primary health care. Having rich folkloric experience on the efficacies and toxicological aspects of the plant medicines, many Korean scientists are investigating the pharmalogical effects and the phytochemical components of the plants. Bioactivities are monitored using various in *vitro* and *in vivo* assay techniques.

Among several successfull cases of these studies, two independent studies carried out in my laboratory will be described.

1. Chemical and biological studies on sedative principle of *Zizyphus* plant

Sanjoin, the seed of *Zizyphus vulgaris* var. *spinosus* (Rhamnaceae) is one of the most important herbal medicine used to treat insomnia without any fear for toxicity, drug depending and addiction in the oriental medicine (1-3). In the earlier studies on the pharmalogical aspects of this drug hypnotic (4), tranquilizing (5), sedative (6-9), analgesic (6), antiinflammatory (6), antiarrhythmic (10) and hypotensive (11) activities have been described. Some papers described isolation of saponins (9) and flavonoids (8,9) as active principles for the sedative activity. However the effective dosage of the isolated flavonoids or saponins seemed to be somewhat higher than that expected for the pure effective components. Major tranquilizing activity of the alkaloidal fraction of sanjoin has been reported, but active principle has not been isolated (5).

In these connection we monitored hypnotic or sedative activity of sanjoin by measuring the prolongation of sleeping time of mice induced by hexobarbital. Alkaloidal fraction showed sedative activity as shown in Table II. Based on this data we isolated sixteen alkaloids from sanjoin (12-19).

A. Structure of the alkaloids:

With combined flash column chromatography, preparative TLC and semipreparative HPLC methods, sixteen alkaloids were isolated from sanjoin (Table I). Sanjoinine-A, B, D, F, G1 and sanjoinenine are cyclopeptide alkaloids, while sanjoinine-G2 was identified as open chain analogue of cyclopeptide alkaloid. Nuciferine, nornuciferine, norisocorydine, N-methylasimilobine, caaverine and zizyphusine are aporphine alkaloids, coclaurine and juzirine are benzylisoquinolines and lysicamine is oxoaporphine. The structures of isolated alkaloids are summarized in Scheme 1 and 2.

Compound	Formula	MW	mp.	yield(%)[a)]
sanjoinine-A (frangufoline)	$C_{31}H_{42}N_4O_4$	534	249	$6.0x10^{-3}$
sanjoinine-B	$C_{30}H_{40}N_4O_4$	520	212-4	$5.5x10^{-6}$
sanjoinine-D	$C_{32}H_{46}N_4O_5$	566	256-8	$4.0x10^{-5}$
sanjoinine-F	$C_{31}H_{42}N_4O_5$	550	228-9	$1.3x10^{-4}$
sanjoinine-G1	$C_{31}H_{44}N_4O_5$	552	236-8	$5x10^{-5}$
sanjoinine-G2	$C_{30}H_{42}N_4O_5$	538	182	$1.6x10^{-4}$
sanjoinenine	$C_{29}H_{35}N_3O_4$	489	281-2	$2.2x10^{-4}$
sanjoinine-E(Nuciferine)	$C_{19}H_{21}NO_2$	295	166	$2.7x10^{-5}$
sanjoinine-Ia(Nornuciferine)	$C_{18}H_{19}NO_2$	281	155-7	$1.2x10^{-4}$
sanjoinine-Ib(Norisocorydine)	$C_{19}H_{21}NO_4$	327	184	$8.7x10^{-5}$
sanjoinine-K(+coclaurine)	$C_{17}H_{19}NO_3$	285	159-161	$1.4x10^{-3}$
N-methylasimilobine	$C_{18}H_{19}NO_2$	281	193-5	$5.0x10^{-6}$
Cadaverine	$C_{17}H_{17}NO_2$	267	204	$6.8x10^{-5}$
Ziziphusine	$C_{20}H_{24}NO_4$	426	214-6	$6.2x10^{-3}$
Juzirine	$C_{17}H_{15}NO_3$	281	203-5	$9.4x10^{-5}$b)
Lysicamine	$C_{18}H_{13}NO_3$	291	212	$1.2x10^{-4}$b)

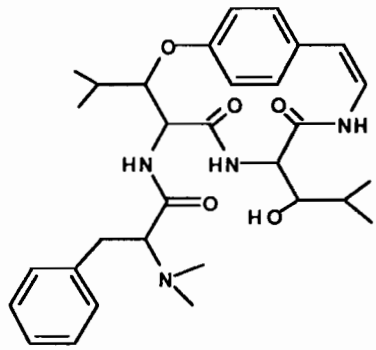
a) isolation yield

b) contents in sanjoin determined by HPLC

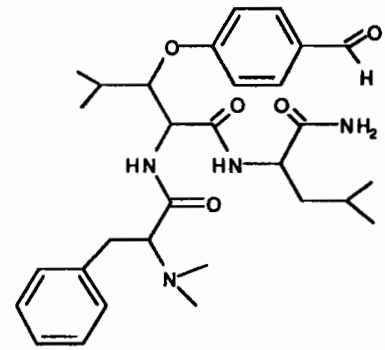
Table I : Alkaloids isolated from sanjoin.

B. Sedative activity

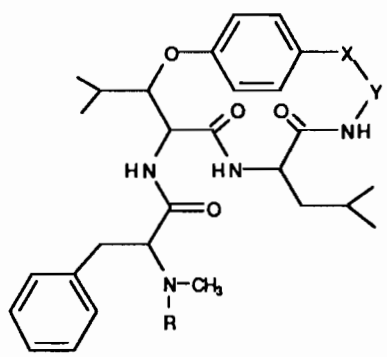
Oral administration of methanol extract of sanjoin prolonged sleeping time induced by hexobarbital by more than 67% compared to the control group (Table II). Isolated alkaloids were subjected to the sedative activity test by monitoring prolongation of sleeping time induced by hexobarbital in mice. Sanjoinine- A, which was identified as frangufoline (1), and nuciferine showed strong sedative activity while zizyphusine and coclaurine did not (Table III). Nuciferine has been already reported as having major tranquilizing activity (20). The sedative activity of frangufoline at a dose of 3 mg/kg is potent enough to establish that cyclopeptide alkaloids are the active principle of sanjoin and this is the first finding of sedative activity from cyclopeptide alkaloids.

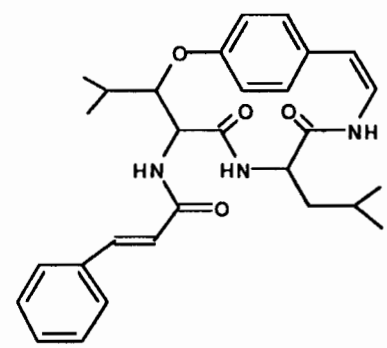


Sanjoinine-F



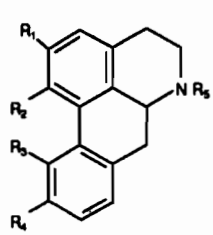
Sanjoinine-G2

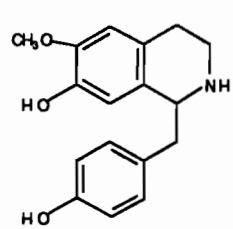




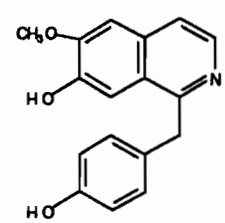
Sanjoinenine

Scheme 1 : Cyclopeptide alkaloids from sanjoin.

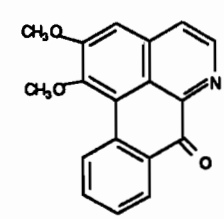




(+)-Coclaurine



Juzirine



Lysicamine

Nuciferine	R_1R_2=OCH_3 R_3R_4=H R_5=CH_3
N-methylasimilobine	R_1=OH R_2=OCH_3 R_3R_4=H R_5=CH_3
Nomuciferine	R_1R_2=OCH_3 $R_3R_4R_5$=H
Norisocorydine	$R_1R_2R_4$=OCH_3 R_3=OH R_5=H
Caaverine	R_1=OCH_3 R_2=OH $R_3R_4R_5$=H
Zizyphusine	R_1R_2=OH R_3R_4=OCH_3 R_5=$(CH_3)_2$

Scheme 2 : Isoquinoline alkaloids from sanjoin.

Fraction (dose)	Control	Sample
MeOH ext. (1.0 g/kg)	27.7 ± 7.6	46.5 ± 17.3
Benzene Alkaloid Fr. (50 mg/kg)	23.6 ± 11.8	29.2 ± 11.7
Ether Fr. (0.5 g/kg)	27.7 ± 7.6	30.3 ± 11.0
Butanol Fr. (0.5 g/kg)	28.8 ± 16.3	41.2 ± 15.4
Water Fr. (O.5 g/kg)	28.8 ± 16.3	27.5 ± 14.3

Table II : Sedative activity of fractions of sanjoin.

Samples were orally administered 60 min before hexobarbital -Na (50 mg/kg) i.p. injection, sleeping time in min., n = 6-7, mean ± S.E.

The alkaloids exist as a mixture in sanjoin, therefore some drug interactions such as additivity, synergistic or counteracting interaction could be postulated between alkaloids. To clarify these possibilities, frangufoline 1 was co-administered with nuciferine and coclaurine respectively. Table IV shows that the additivity of sedative activity exists between 1 and nuciferine, while Table V indicates that coclaurine does not enhance the activity of 1. As a result, the potent sedative activity of butanol fraction of sanjoin could not be explained by the high content of zizyphusine. Butanol fraction contains minor isoquinoline alkaloids such as caaverine, N-methylasimilobine and norisocorydine. It is highly probable that some part of the sedative activity of the butanol fraction may be contributed by these minor alkaloids.

	Frangufoline	Nuciferine	Zizyphusine	Coclaurine
Control	16.3 ± 9.8	27.8 ± 10.4	20.6 ± 2.1	20.6 ± 2.1
3 mg/kg	26.1 ± 13.1	33.3 ± 13.8		
10 mg/kg	30.6 ± 19	52.4 ± 17.5	20.0 ± 5.9	16.8 ± 4.3
33 mg/kg			22.2 ± 6.9	16.1 ± 8.0

Table III : Effect of alkaloids from sanjoin on hexobarbital induced sleeping time.

Samples were orally administered 1 hour before hexobarbital-Na (50 mg/kg) i.p. injection. Sleeping time in min., n=6-7, mean ±S.E.

Control	FR (1 mg/kg)	FR (1 mg/kg) + NU(2.5mg/kg)	FR (1 mg/kg)+ NU (5 mg/kg)	NU(5mg/kg)
32.1 ± 23.8	50.8 ± 11.0	46.1 ± 11.3	97.5 ± 31.0	83.3 ± 19.5

Table IV : Effect of co-administration of frangufoline and nuciferine on sleeping time*.

FR : frangufoline, NU : nuciferine

*Samples were administred i.p. 30 mins before hexobarbital-Na (50 mg/kg) i.p. injection. Sleeping time in min. n=7, mean ± S.E.

Control	FR (1 mg/kg)	FR (1 mg/kg)+ CO (10 mg/kg)	CO (10 mg/kg)
34.1 ±11.5	47.8 ±17.5	50.7±11.4	38.6 ±13.8

Table V : Effect of co-administration of frangufoline and coclaurine.
FR : frangufoline, CO : coclaurine, Conditions as in Table IV.

C. Heat Induced epimerization of Frangufoline.

Old Oriental Materia Medica (1-3) described that the roasting of sanjoin potentiates its hypnotic activity and traditionally roasted sanjoin has been used for the hypnotic purpose. To verify the traditional roasting of sanjoin bears any relevance on the activity of the alkaloids, the sanjoin alkaloids were subjected to heat treatment in sanjoin oil. Aporphine alkaloids such as nuciferine and nornuciferine produced a dehydrogenated product of lysicamine and this reaction was irreversible. However frangufoline 1 produced heat-induced artifact of sanjoinine-Ah1 2 which is interconvertable with 1 at high temperature.

Sedative activity of lysicamine, which is a heat-induced artifact of nornuciferine, is less potent than the original alkaloid of nornuciferine as shown in Table VI. Sanjoinine-Ah1 2, however showed increased activity than 1 itself (Table VII). This result reflects traditional heat process of sanjoin to the molecular level.

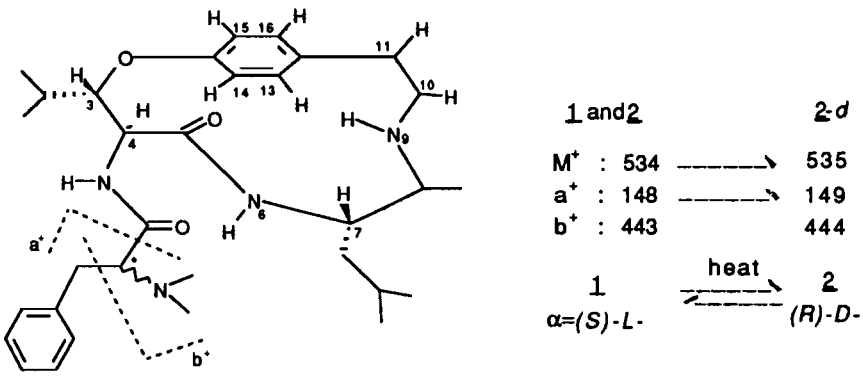
Control	LY (1 mg/kg)	LY (3 mg/kg)	NR (3 mg/kg)
29.5 ± 11.1	34.3 ±18.5	44.7 ± 15.4	46.4 ± 17.8

Table VI : Effect of Lysicamine (LY) and Nornuciferine (NR) on sleeping time.
Samples in 1% CMC were administered i.p. 30 mins before hexobarbital-Na (50 mg/kg) i.p. injection. Sleeping time in min., n=7, mean ±S.E.

Control	FR(1mg/kg	FR(3mg/kg)	FR(10mg/kg)	SH(1mg/kg	SH(3mg/kg)	SH(10mg/kg)
Exp.1 18.3±8 Exp. 2 11.5±4.1	26.1±7.1	30.2±7.2 25.8±18.5	45.1±11.9	32.8±12.4	33.2±12.2 39.3±16.1	46.0±23.9

Table VII : Effect of Frangufoline (FR) and Sanjoinine-Ah1 (SH) on sleeping time.
Samples were administered i.p. 30 mins before hexobarbital-Na (50 mg/kg) i.p. injection. Sleeping time in mins, n=6-7, mean ±S.E.

Mass spectra of 1 and 2 were superimposable and ^{1}H and ^{13}C-NMR spectral patterns were also similar except minor differences in chemical shifts, but their physical properties are quite different. Frangufoline 1 showed mp. 247-9°C, $[\alpha]_D22$ = -183° (c=0.13 in MeOH), while sanjoinine-Ah1 2 showed mp. 218-220°C, $[\alpha]_D22$ = -203. Structure relationship of these two compounds was studied by deuterium exchange method. Deuterium labelled 2-*d* was prepared by heating 1 in CD_3OD/D_2O solvent. In a mass spectrum of 2-*d*, the molecular ion peak (M^+), a^+ and b^+ fragments in Scheme 3 were shifted by one mass unit upward, suggesting α-proton of the *N,N*-dimethyl-phenylalanine (DMPhe) moiety was exchanged with deuterium by a mechanism including carbanion intermediate.



Scheme 3 : Structure of Frangufoline 1 and Sanjoinine-Ah$_1$ 2.

Absolute configurations of 1 and 2 were studied by chromatographic and spectroscopic methods. GC analyses of diastereoisomeric derivatives of individual amino acid units in the hydrolysates of 1 and 2 revealed that the configurations of DMPhe and leucine moieties in 1 are (S)-L-forms, while those in 2 are (R)-D-form for DMPhe and (S)-L-form for leucine moiety. The configuration of β-oxyleucine moiety was determined as L-erythro (i.e. 3S, 4S) by NMR and molecular model study. NOE data suggested that sanjoinine-Ah1 2 has slightly distorted cyclic structure due to the inversion of chiral carbon of DMPhe moiety.

D. Ion Binding Activity of Frangufoline:

Interest on the ionophore active compounds has rapidly been expanded due to its potential biological activity through the modifiction of energy-linked transport of metal ions in microorganisms (21). Frangufoline 1 is a fourteen membered cyclopeptide alkaloid which is consisted of three amino acids and p-oxystyrylamine units. UV spectrum of 1 shows end-absorption with shoulder at 224 nm.

This shoulder disappeared by the addition of Ca^{2+} or Mg^{2+} ion as shown in Fig. 1, which suggests that frangufoline 1 has ion bonding activity to Ca^{2+} and Mg^{2+} ions, while Na^+, K^+, Ag^+ and Ba^{2+} ions did not affect the spectrum.

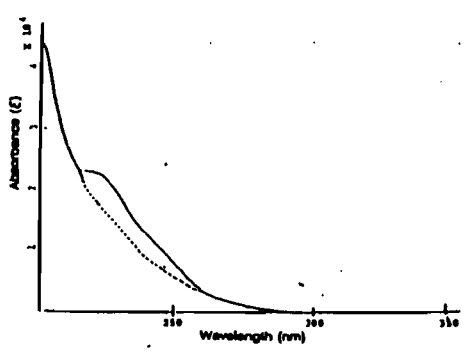


Fig.1: UV spectrum of frangufoline 1 in CH_3CN

——: 1 alone; -----: 1 and 1,8x10^{-3}M of Ca^{2+}(or Mg^{2+}) addition of Na^+, K^+, Ag^+, or Ba^{2+} ions leads no change in UV spectrum

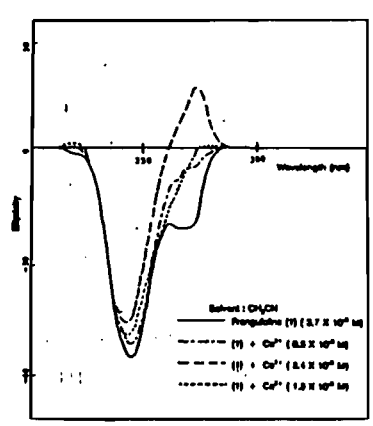


Fig. 2: CD spectrum of frangufoline

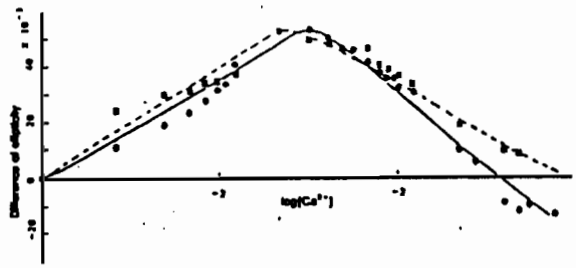
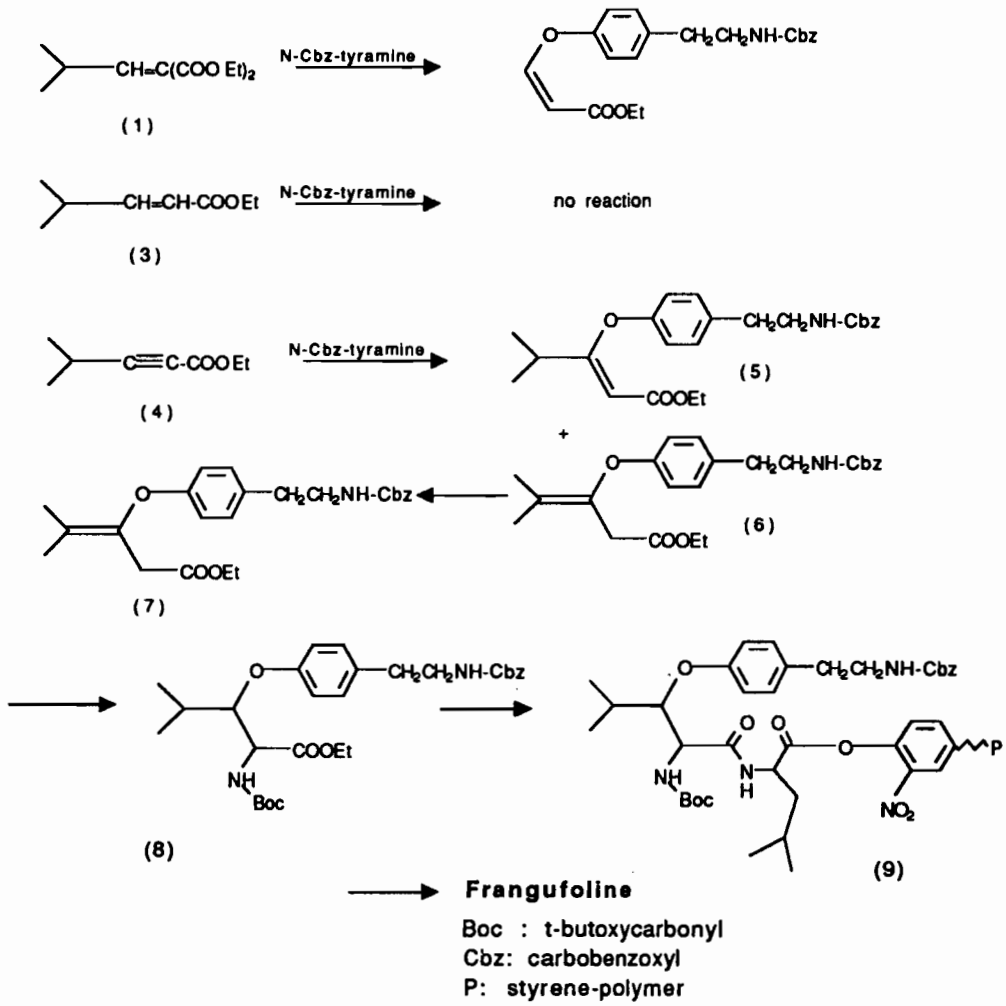


Fig.3: CD titration curve of frangufoline wityh $Ca(ClO_3)_2$

——: at 274nm; ----- at 252nm

The CD spectrum of 1 was markedly changed by the addition of calcium or magnesium ion, while it was not affected by sodium, potassium, silver or barium ion. This was consistent with the result of UV spectroscopic observation. The CD ellipticity ($\Delta\varepsilon$) 274 nm was varied from negative to positive and then negative again to the concentration of calcium ion. The plot of CD ellipticity ($\Delta\Delta\varepsilon$) at 252 nm and 274 nm to the log concentration of calcium ion shows two phases indicating at least two types of complex may be formed as the concentration of calcium increased.

E. Synthesis of Frangufoline.



Scheme 4 : Total synthetic procedure of Frangofuline.

Frangufoline 1 is constituted of L-Leucine, ß-oxyleucine, p-oxystrylamine and side chain, *N,N*-dimethylphenylalanine. The main points of general synthesis of cyclopeptide alkaloid such as frangufoline are :

a) the preparation of the ß-aryloxyamino acid,

b) the formation of an enamide, and

c) the generation of the macrocycle.

The strategy for the synthesis of 1 is shown in Scheme 4. In Scheme 4, attention should be drawn to etherification by Michael addition and cyclization using solid phase peptide synthesis. To begin with, we devised the synthetic route for compound **8** via Michael addition of N-cbz-tyramine to isobutylidene malonate **1** and subsequent Hoffmann rearrangement of one ethyl ester group.

In general, the Michael addtion of phenolic compound to double bond requires higher temperature (140°C) and longer reaction time (>100 hrs) (22). Under these reaction conditions, the reaction of N-cbz-tyramine and compound **1** yielded compound **2**, where isopropyl and an ethyl group were eliminated. It is likely that compound **2** resulted from a chemical process similar to -elimination reaction. Therefore, we attempted to react N-cbz-tyramine with trans-4-methyl-pent-2-enoate **3** in order to prevent such an elimination reaction. No reaction, however, proceeded under reaction conditions tried.

With the conception that Michael addition of phenolate ion to triple bonds has been known to give higher yields compared to double bonds (23), we tried to synthesize compound **5** and **6** by Michael addition reaction of ethyl-4-methyl-2-pentynoate **4** with N-cbz-tyramine. Compound **4** was successfully synthesized through a modified Wittig reaction (24) of isobutyraldehyde with a ylide formed from CBr_4, Ph_3P, Zn and subsequent electrophilic reaction with ethylchloroformate (yield 75%). Michael reaction of compound **4** with N-cbz-tyramine was carried out under mild conditions, and compound **6** was obtained as a major product and compound **5** as a minor product. The combined yield of compound **5** and **6** was 35%. The reduction of compound **6** was performed under mild conditions using Mg (turning)/MeOH reagents (25).

In summary, we achieved to synthesize an arylalkyl ether compound, and plan to synthesize frangufoline via formation of compound **8** and **9**.

2. Non-saponin constituents of Ginseng

In past twenty years, various pharmacological and phytochemical studies on ginseng have been conducted in my laboratory. A few representative studies have been concerned with :

1) synthesis and metabolic fates of radiolabeled ginsenosides (26,27),

2) radio-imunoassay for ginsenosides (28) and,

3) chemical studies on the gastro-intestinal metabolites of ginsenosides (29).

For detailed information, the reader is referred to the afore mentioned articles.

A. Anti-oxidant Component of Ginseng:

Our new research interests on Ginseng, the studies on the anti-oxidant components of ginseng, were prompted by the adaptogenic activity theory proposed by Brekhman (30). There were some intriguing features in the Brekhman's theory in that the reported adadaptogenic substances of ginseng and *Eleutherococcus senticosus* are panaxosides (dammarane triterpene glycosides) and lignan glycosides (phenolic glycosides), respectively. The active components of ginseng and *Eleutherococcus senticosus* are of two different chemical natures, although both plants belong to the same taxonomic family. It seemed possible for us that the anti-oxidant components of ginseng might be phenolic compounds. In order to understand the pharmalogical significance of biologically active anti-oxidant compounds, let us summarize the gerontologist's view on the cellular ageing (31).

Living cells produce very reactive and harmful free radical oxygen species such as

singlet oxygen molecules, superoxide anions and hydroxy radicals as a consequence of the consumption of oxygen for respiration. Although the greater part of them are quenched by some self-protective systems, a part of them leaks from the protective 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.

Once again the greater part of lipid peroxides are reduced to less reactive hydroxyacids, but a part of them leaks from the system and decomposes to produce highly reactive malondialdehyde. Malondialdehyde molecules thus produced bind nonspecifically to nearby biomacromolecules such as enzymes to produce lipofuscine pigments which will be accumulated in living cells in parallel with cellular ageing. The membrane damage caused by those free radical chain reaction products and the protein binding of malondialdehyde are believed to be the causes of various geriatric diseases. Therefore, the gerontologists assume biologically active anti-oxidants to be the anti-ageing agents.

Our first approach to the studies on the anti-ageing was started from screening on free radical quenching activities of 120 crude drugs and some cereal extracts, using diphenyl picryl hydrazyl (DPPH) as the free radical reagent. Almost more than 40% of plant extracts showed strong quenching activities. This widespread distribution of free radical quenching substances in the plant kingdom is very suggestive of the possible role of Chinese medicines which sometimes show dramatic therapeutic responses to some geriatric diseases.

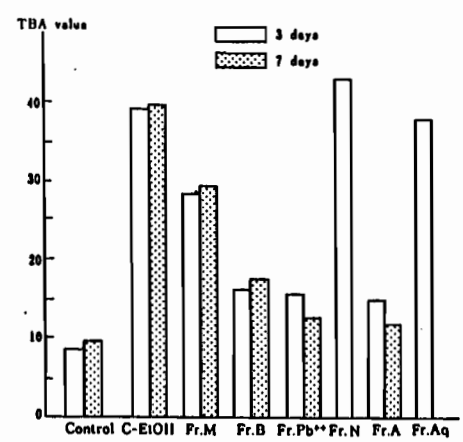
It was very difficult to select any specific plants from our *in vitro* screening data for the phytochemical studies to isolate the free radical quenching components, since flavonoids and tannins which are widely distributed in the plant kingdom seemed to exhibit free radical quenching activity in these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

In the subsequent approach, we selected 30 herbal drugs for an *in vitro* anti-oxidant activity screening test, referring to Chinese Material Medica Book in which their tonic or anti-ageing activities were strongly suggested.

Pharmacological doses of plants extracts were fed to mice and then acute toxic doses of 50% ethanol were orally administered to induce lipid peroxidation. The animals were sacrified 12 hours later, and the livers were taken for the assay of lipid peroxide contents by Masugi's TBA procedure.

Some of the plant extracts including *Panax ginseng* showed strong inhibitory activities on the lipid peroxide formation after ethanol intoxication. 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. These dramatic biological data prompted us to isolate the effective components of ginseng by monitoring the anti-oxidant activity (32).

Ether soluble, acidic fr. and butanol soluble, glycosidic fr. showed strong activities, whereas ether soluble, neutral fr. and highly polar, water soluble fr. were devoid of the activity. The ether soluble, acidic fr. was further purified to obtain the active substances in pure crystalline states by silica gel column chromatography.



Antioxidant activities of ginseng fractions

C-EtOH :EtOH intoxication, Fr. m: methanol ex.; Fr. B : supernatant of Pb (Ac), treated saponin; Fr. Pb^{++}: precipitate of Pb (Ac), treated saponin fr.; Fr. N : neutral fr. of ether ex.; Fr. a : acidic fr. of ether ex.,: Fr. Aq : finally water-soluble fr.

They were identified as simple compounds, maltol, salicylic acid and vanillic acid (33). Interestingly, 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 suggests that maltol is the artefact produced by heat treatment of ginseng during the manufacturing process or red ginseng. Many other phenolic compounds such as ferulic acid and caffeic acid were detected from the ether soluble, acidic fr., but there are still more phenolic compounds unidentified yet.

Although further studies have to be done for the complete elucidation of chemical identities of the anti-oxidant components of ginseng, it is possible to assume the phenolic compounds to be the effective principles of the anti-oxidant activity of ginseng. Noteworthy is the fact that none of the purified ginsenosides showed anti-oxidant activity both *in vitro* and *in vivo* tests, whereas the semi-purified ginsenoside preparations showed strong activity. This must be due to the contamination of the impure ginsenoside preparations by the phenolic compounds.

In Chinese Materia Medica Book we can find the notice that ginseng is used in contraindication with iron.

	Control	Ethanol control	Sample, mg/30 g 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-coumaric 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 - Toxopherol acetate	10.1	38.5				11

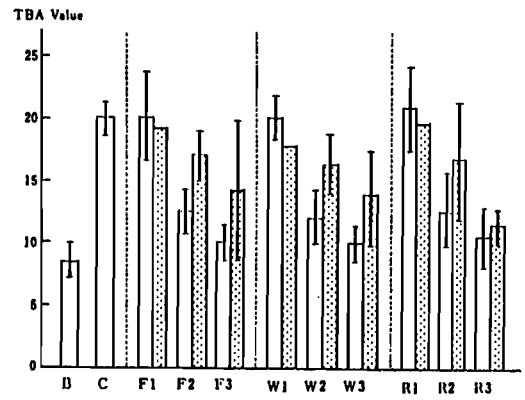
Anti-oxidant activities of ginseng components :

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 Am/g wet liver.

In fact, folkloric custom eliminates all iron-made tools in processing ginseng including decoction and in peeling fresh ginseng. This folkloric custom is very suggestive for our understanding the real character of the active principle of ginseng. In order to see whether the folkloric experiences were reproduced in our modern molecular pharmacological experiments, we added a very small amount of ferric ion in the extraction process of ginseng, instead of using iron vessel. It was found in our animal experiments that the anti-oxidant activity of the ginseng preparations pretreated with ferric ion was considerably reduced (34).

Effect of ferric iron treatment on the antioxidant activity of ginseng extracts

Blank column : non treated ginseng groups. Shaded colums ; 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 administred for 3 days before the induction of lipid peroxide by ethanol intoxication was conducted. Each group was consisted for 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/30 g body wt. mouse.



B. The anti-oxidant activity mechanisms of phenolic compounds (35).

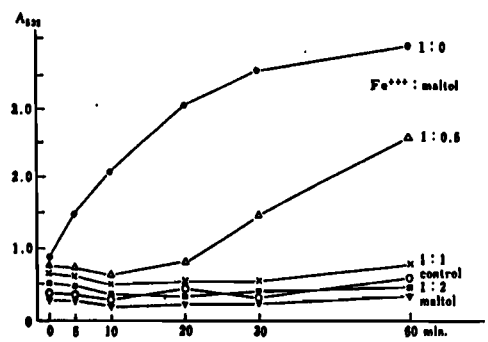
When we incubated liver homogenate, the lipid peroxide content gradually increased in proportion to incubation time due to the activation of NADPH-dependent, microsomal lipid peroxidation system. This increase was dramatically enhanced upon the addition of a small amount of ferric ion to the homogenated.

However, concomittant addition of two mole equivalent of maltol completely abolished the enhancement effect by ferric ion. 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^{+3} chelate might be responsible for the anti-oxidant activity of maltol which effectively deprives the ferric ion from ADP-Fe^{+3} complex that plays a role as the initiation factor in the free radical chain reaction of microsomal enzyme system.

Inhibitory effect of maltol on the Fe^{+3}- catalized oxidation of liver homogenate :

0.2 ml of 0.05 M $FeCl_3$ $6H_3O$ and given molar equivalent maltol in 0.175 M KCl and 0.4 ml of 9% mouse liver homogenate were incubated at 37°C for specified time. Lipid peroxide was measured by TBA method (Am).



C. Anti-fatigue activity of ginseng (36).

The anti-oxidant activity of ginseng may be considered as an underlying mechanism for other pharmacological activities which have been repeatedly reported as the ginseng efficacies by others. Such activities are :

1) protection from radiation injury,
2) protection of liver from drug intoxication,
3) prevention of hangover symptoms,
4) anti-fatigue activity,
5) anti-artherosclerosis, and
6) anti-thrombosis, etc...

Here, we have a question whether those activities are arising from ginsenosides or from phenolic compounds. 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, I.I. Brekhman reported the anti-fatigue activity of ginseng and panaxosides which was evaluated by the increase of swimming time of mice. In our laboratory we reexamined the anti-fatigue activity of ginseng and ginsenosides by the same swimming test as Brekhman did. The anti-fatigue activity distribution in various fractions of ginseng extract was very similar to that of anti-oxidant activity in the ginseng fractions. In our experiment, Brekhman's anti-fatigue activity was successfully reproduced on the ginseng extract and on the impure ginsenoside preparations, but not on any purified ginsenosides. On the other hand, maltol and phenolic acids isolated as the anti-oxidant components of ginseng considerably prolonged the swimming time of mice.

Group	Swimming time min.	Group	Swimming time min.
Exp. 1.	Doses:0.22g/kg body wt. once daily for 3 days	Exp. 2.	Doses:10mg/kg body wt.
Control	88.1 ± 26.9	Control	108.4 ± 55.7
H_2O Ex.	107.6 ± 41.3·	Maltol	162.6 ± 63.5··
Et_2O fr.	131.9 ± 43.0··	Salicylic acid	165.5 ± 69.4··
BuOH fr.	117.2 ± 42.5··	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

Anti-fatigue activity of ginseng and its components :

Twenty mice per group were administered with samples and two hours later subjected to swimming test in a 24°C water pool. Mean swimming times were recorded.·$p<0.05$, ··$p<0.001$

D. Conclusion

The ginseng efficacies were found in old Chinese Materia Medica Book as followings : repairing the five viscera, harmonizing energies, strengthening the soul, allaying fear, removing toxic substances, brightening the eyes, opening the heart and improving thought (English translation by S. Fulder : The Root of Being, pp. 109, Hutchinson, London, 1980). Of these many efficacies, the last one could be explained in a modern scientific expression through our finding of anti-oxidant and anti-fatigue activities of phenolic compounds of ginseng reported heretofore under the name of ginsenosides might be due to direct or indirect consequences of anti-oxidant activity of phenolic compounds contained as impurities in ginsenoside preparations. Therefore, we considere the ginseng efficacy as being clearing effect for unwanted sludges such as lipid peroxides produced by abnormal oxidation in cells due to respiration during normal life rather than being a stimulant or a depressant to some particular function.

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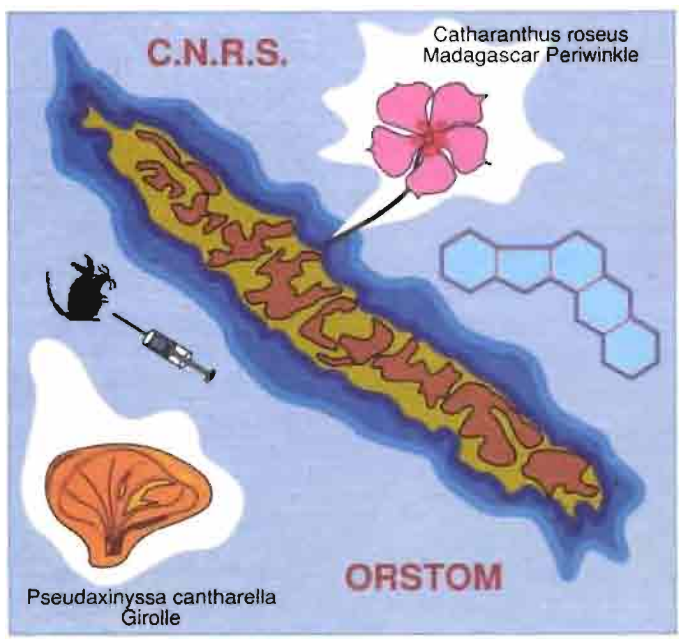

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