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Study on the Original Ginsenosides from the Fresh Root of *Panax ginseng* C.A. Meyer

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To study the pharmacologic effect of the original ginsenosides and the mechanism of their transformation and degradation in the course of ginseng processing, we isolated four chemical constituents from the fresh ginseng root cultivated in China, by means of different method from the document. On the basis of the physicochemical properties and spectroscopic analysis, three of them were elucidated as Malonyl-ginsenoside Rb_1, Rb_2, Rd. They were first isolated from the fresh ginseng root cultivated in China. Their structures were established to be 3-O-[-malonyl-β-D-glucopyranosyl(2→2)-β-D-glucopyranosy]-20-O-[β-glucopyranosyl(1→6)-β-D-glucopyranosyl]-20(s)-protopanaxadiol; 3-O-[6-o-malonyl-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]-20-O-[β-D-glucopyranosyl*F*-20(s)-protopanaxadiol.

MG-Rb_1: Colorless fine crystals, mp. 150~152℃, $IR\gamma_{max}^{KBr}cm^{-1}$: 3420, 2920, 1730, 1600, 1380, 1071, ^{1}HNMR (D_2O) δ (ppm): 0.78(3H), 0.84(3H), 0.89(6H), .0.13(3H), 1.32(3H), 1.61(3H), 1.67(3H), 3.61(2H). FAB-MS $[M+Na]^+$m/e: 1217, 1174, 1132, 1051, 997, 969, 951, 893, 875, 807, 798, 721, 451, 365.

MG-Rb_2: colorless fine crystals, mp. 148~150℃, $IR\gamma_{max}^{KBr}$ cm^{-1}; 3381, 2925, 1730, 1628, 1380, 1068. FAB-MS $[M+Na]^+$m/e: 1188, 1078, 969, 893, 875, 807, 789, 762, 621, 630, 641, 451, 365.

MG-Rd: Colorless fine crystals, mp. 158~160℃, IRV_{max}^{KBr} cm^{-1}: 3383, 2937,1730, 1600, 1383, 1074. FAB-MS$[M+N_a]^+$m/e: 1055, 969, 893, 875, 807, 789, 628, 673, 541, 349.

Analysis of *Panax ginseng* Polysaccharide by Alcian Blue Dye

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Polysaccharide contents in *Panax ginseng* roots were evaluated by a spectro-photometry, utilizing the complex formation of ginseng polysaccharide with alcian blue dye in 50 mM ammonium biphosphate, pH 4.2. The polysaccharide content in red ginseng was about three times more than that in fresh ginseng when both were extraced with water, and was increased about two times when red ginseng was extracted with an alkaline solution. The determination of polysaccharide in various parts of ginseng revealed that main roots contained the component more than fine roots. Fresh ginseng sections stained by the dye showed polysaccharide mainly found in cortex and cambium but not in epidermis.

Studies on the Chemmical Constitutensts of the Root and Leaves of *Panax quinquefolius* Linn (in China)

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From *Panax quinquefolium* Linn cultivated in Liaoning province (China) sixteen compounds (1～16) were isolated. The structures of these compounds were identified as palmitic acid (1), oleanolic acid (2), daucosterin (3), ginsenosides-Rh_1, -Rg_3, -Rg_2, -Rg_1, -Rf, -Re, -Rd, -Rb_2, -Rb_1, -Ro (4～13), sucrose (14), ginsengtrisaccharide (15) and a new saponin whose structure was elucidated to be 20 (s)-protopanaxadiol 3-O-β-O-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→6)-β-D-glucopynoside (16) on the basis of IR, Mass spectra (FD-MS, FAB-MS), ^{13}C-NMR and chemical evidences. The new saponin was named ginsenoside-RAO. The leaves of *Panax quinquefolius* Linn 3 compounds identified by means of macroreticular resin and TLC, as ginsenoside-Rb_1, Re and Rg_1.

Chemical Evaluation of *Panax species* Commercially Available in Taiwan

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Ginseng has been the most commonly used Chinese herbs since ancient time. In Taiwan, various grades of *Panax species* which belong to the following three major species are available:

1. *Panax ginseng* C.A. Meyer
   a. Red ginseng: Korean ginseng, Japanese ginseng
   b. White ginseng:
   c. Tong-Yang ginseng:
2. *P. quinquefolius* L.
3. *P. notoginseng* (Burk) Chen

The market price of the above mentioned ginseng species differs greatly through different sources. Korean ginseng is also divided into Heaven, Good, Tail and Cut grades from the morphology of the roots.

The yield of total water extract, polysaccharide, saponin and nonsaponin, nonpolysaccharide fractions of each ginseng species were evaluated by extraction or precipitation utilizing water, 1-butanol, and ethyl alcohol. Additionally, analysis of the content of each major ginsenosides (Rg_1, Re, Rd, Rc and Rb_1) by HPLC was also performed in order to provide chemical basis for identification of ginseng and their preparations.

The results showed that among the three ginseng species, *P.ginseng* had highest content of polysaccharide, followed by *P. quinquefolius* and *P. notoginseng.* On the other hand, *P. notoginseng* had the highest content of ginsenosides followed by *P. quinoefolius* and *P. ginseng.* However, ginseng from different sources, or various commercial grades of *P. ginseng* did not show significant difference in

their chemical compositions. The results from HLPC analyses also showed similar patterns.

Analysis of Neutral Ginsenosides of Wild and Cultivated *Panax ginseng* C.A. Meyer Roots Growing in Primorye/Region

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The composition and contents of neutral saponins of cultivated and wild *Panax ginseng* C.A. Meyer were analysed by high performance liquid chromatography (microcolumn liquid chromatograph "Milichrom", detection UV (202 nm). We have compared the saponin compositions as to the following major and minor saponins: Rg_1. Re, Rf, Rg_2, NG-R_2, Rb_1, Rc, Rb_2, Rd. There was no significant difference in the major saponins between wild and cultivated specimens.

Dammarane Type Saponins of Aerial Parts of *Panax ginseng* C.A. Meyer Cultivated in Promorye (Russia).

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The methanol extractive of leaves, flower-buds and fruits of cultivated primorye ginseng were studied. Plants were collected during few years in different regions. Qualitative and quantitative ginsenosides (NG-R_2, Rg_1, Re, Rf, Rb_1, Rb_2, Rc, Rd, F_2, Rg_2) were isolated from roots of ginseng. The ginsenoside Rg_2 was prepared by alkaline cleavege of ginsenoside Re. Epimerisation at C_{20} in these conditions was not observed.

Synthesis of the 3, 20-Di-O-β-D-Glucopyranosyl-Dammar-24-Ene-3β, 20 (s)-Diol-12-one (Chikusetsusaponin-Lt8)

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The 3, 20-di-O-β-D-glucopyranosyl-dammar-24-ene-3β, 20 (s)-diol-12-one (chikusetsusaponin-LT8 (1) is one of the major saponins of the leaves of *Panax japonicus* collected on the Japan Sea coast.

(1) R_1=-Oglc R_2=O R_3=glc

(2) R_1=-OH R_2=O R_3=H

(3) R_1=···OH R_2=-OH R_3=H
(4) R_1=-OH R_2=-OH R_3=H
(5) R_1=-OAc R_2=-OH R_3=H
(6) R_1=-OAc R_2=O R_3=H
(7) R_1=-Oglc $(Ac)_4$ R_2=O R_3=glc $(Ac)_4$

The 12-ketone of 20 (s)-protopanaxadiol (2) which is readily obtainable from betulafolienetriol (3) via the 3-epibetulafolienetriol [=20 (s)-protopanaxadiol](4) and 3-acetoxy derivatives (5) and (6) is used as aglycon in synthesis of chikusetsusaponin-LT8. Condensation of (2) with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide in the presence of silver oxide or silver silicate in dichloroethane proceeds the acetylated glucoside (7) (63%). Deacetylation of (7) with methanolic O, 1N. sodium methoxide gives (1) in 95% yield.

The Analysis of Essential Oil Components of Kaesong Koryo Insam (*Panax ginseng* C.A. Meyer)

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The essential oil components obtained from roots of *Panax ginseng* C.A. Meyer were analysed by means of GC-MS. The essential oil dissolved and non-dissolved in water was analysed with the GC and the GC-MS using capillary column, with its components being identified by the tR and MS. Condition of GC:

Column (I) carbowax-20 m, 0.25 mm×50 m, column temperature 60～/200℃, 2℃/min. carrier gas He, 0.5 ml/min. S. ratio 100 : 1 Inj. temp. 230℃.

Column(II): SE-30, 0.25 mm×50 m, column temp. 80～220℃, 4℃/min, S. ratio 80: 1, Inj. temp. 250℃.

Condition of MS: EI 70 eV, Scan speed 2S/1～800, Scan range 20～400, Ion temp. 200℃. The essential oil includes more than 80 kinds of components i.e. α, β, γ, elemene, humulene, β-gurjnene, γ-cadinene, α-bisabolene, cyperene, etc.; its peculiar odour components were methylpirazine derivatives.

A Study on the Chemical Constituenets of Jilin Ginseng Volatile Oil

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Ginseng belongs to the Araliaceae plant (*Panax ginseng* C.A. Meyer). The ginseng in Jilin province is most famous in China as well as in foreign countries. Whether the ginseng is genuine or fake and good or bad in quality can usually be judged by its forms and smells at first. Since Garriques first isolated crude saponin from American ginseng many studies have been carried out on ginseng.

The investigation and application of Jilin genseng for the most part concentrated on ginseng saponin. The study on the volatile oil of ginseng did not begin until about twenty years ago. In this paper, the volatile chemical constituents of Jilin ginseng stems and leaves were collected and determined over and over again. The volatile oil can send forth delicate fragrance and we found the volatile oil can inhibit the cancer cells growth. The structure determination and quantitative analysis of the volatile oil were studied by a combination of HP-5 quartziferous capillary column gas chromatography and mass spectrometry in electron impact mode or with Fourier transform infrared spectrometry. Thirty eight important chemical constituents were identified, analytical results are listed in Table one. Among them, twent four kinds were definitely characterized, i.e., three monoterpenes and seventeen sesquiterpene hydrocarbons and four oxygen-containing sesquiterpenoids. They make up the characteristic constituents of the volatile oil in stems and leaves of Jilin ginseng. Prospective utilization of the volatile oil in view of their structures and their quantitative distribution was discussed. The stems and leaves of ginseng are the byproducts in a ginseng garden. The volatile oil extracted from it can be used as the additive for processing or making drink, cigarette, medicine and the reference for making up essence artificially. If the volatile oil is extracted firstly and then the ginsenoside from the stems and leave of ginseng not only the quality of ginsenoside can be raised but also the economic utilization. This result has an important value in economy and practice.

The Extraction and Analysis of the Ginseng Volatile Oil

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Using a gas chromatography-mass spectrum-computer (GC/MS/DS) device in the analysis of ginseng volatile oil is a preferable approach. However, what method to use for extracting the volatile oil is a preferable approach. However, what method to use for extracting the volatile oil and what conditions to choose for GC/MS conspicuously affects the separation and determination of this complex natural mixture. Here, with strict extraction controls, our restoration of the volatile oil has reached a rate of 0.96%. And, using an effective GC/MS/DS device under the most favorable conditions and with the reference data from the HP MS Chemstaton, we have determined 76 compounds. Moreover, the method used has proved stable in its repetition.

In order to achieve a high restoration of the volatile oil and a bumper determination of its ingredients, the following factors are of crucial significance:

1. The grinding method-preferably by hand instead of by hand electric grinder.
2. An adequate time for the saturation of ginseng and the circulation of ether
3. Enough times of the repetition of the extraction process.
4. An appropriate temperature for the reclamation of ether, which most directly affects the restoration of the volatile oil as well as the other low-boiling ingredients.
5. The setting of the most favorable GC/MS conditions, which will enhance the cylinder efficiency and MS sensitivity so as to ensure a disirable analysis outcome.

With all these precautions, especially the last two, we separated altogether 132 compounds, of which we determined 76, totalling 71.4%. We also find that as much as 26.79% of the volatile oil are sesquiter-

penes, and that the largest in ingredient is farnesene, which takes up 5.18% of the toal. Incidentally, those compounds that are not determined are mostly saturated hydrocarbons－a phenomenon that needs further probe.

Comparative Study on the Constituents of Essential Oil in Freeze-Dried Ginseng with Fresh, White and Red Ginseng

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Freeze-dried ginseng was a new product of ginseng processing. In order to make clear the qualitative characteristics, we carried out comparative study on the constitutions of essiential oil in the freeze-dried, the white, the red and the fresh ginseng produced in the same location. The experiment materials came from the ginseng farm and processing factory of Shongjiang River Forestry Bureau in Jilin Province. The determining means was JMSD300-JMA2000 Chromatography-Mass Spectrum-Computer simultaneous instrument.

Analytical results are shown as follows:

1. Fresh ginseng and its processing products contained sesqueterpenes as a mainpart in the constitution of essiential oil. Freeze-dried ginseng was similar with white ginseng in constituents while .ed ginseng changed much due to the high temperature in processing.

2. 4 hydroazulenes were isolated from fresh ginseng essential oil for the first time. They were 1,4,9,9-tetramethyl-4,7-methano-octahydroazulene, 1,9,9-trimethyl-4-methylene-octahydroazulene, 1,1,4,7-tetramethyl-decahydro-cyclo-propa [e] azulene-4-ol and 2,2,4,8-tetramethyl-decahydro-4,8-methanoazulene-9-ol. And also 2 hydronaphthalenes were determined in fresh ginseng as the first time. They were 1,2,4,5,6,8a-octahydro-1,8a-dimethyl-7-(1-methyl ethenyl)-naphthalene and 1a,2,4,5,6,7a,7b-octahydro-1,1,7,7a-tetramethyl-1-hydro-cyclo-propa[a] naphthalene, which were perhaps produced from the rearangement of hydroazulenes in plant body. Azulenes, as antecedents of terpenes, would be ready to produce a series of sesqueterpenes.

3.Diene-arachidic acid with the effect of anti-tumors was first discovered in fresh ginseng with relative content of 12.37%.

4. Fresh ginseng didn't contain β- or γ-elemene. They were existing in the processing ginsengs as isomeric compounds from α-elemene.

5. Freeze-dried ginseng gave off the rich flavour mainly because it contained relative high content of sesqueterpenes, especial γ-elemene (11.8%), Freeze-dried ginseng was with 10 of flavour constituents (36.43%) while white ginseng only 8 (19.94%). That meant that the vacuum-freeze-dried method in processing decreased the lose of essiential oil. This point was very significant to its high quality.

6. The following three sesqueterpenes were found in Freeze-dried ginseng and red ginseng: 1-methyl-1-ethenyl-2-(1-methyl ethenyl)-4-methyl ethyl-cyclohexane, nerolidol and torreyol.

7. Trans-β-farnesene was contained relatively high in freeze-dried ginseng.

8. Since some constituenets in the essiential oil would happen isomerization or cyclozation in high temperature, we adopted cold extraction and purification. That was why we could determin out relative more constituents. So the research results reflect more completely the real state of essential oil consti-

tution in ginseng of each kind.

Determination of Volatile Oil in Ginseng Flower Bud and Study on the Indusive Complex of β-Cyclodextrin (β-CD)

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The volatile oil has been attracted from the Ginseng flower in our laberatery, recently 56 compounds have been determined by GC/MS in which the characteristic components of 19 sesquiterpenes is as high as 44.4% (see Table 1 and 2).

We also report here the study on β-CD inclusive complex of volatile oil in Ginseng flower. β-CD is a cyclicoligosaccharide, which is condensated by intermolecular 1,4-glycoside bonds of seven glucose molecules. Its cavity, whose internal diameter is 8 Å, is hydrophobic. Thus β-CD can include proper hydrophobic molecules or groups to form a inclusive complex. GFVO just can be included by β-CD, and this kind of inclusion complex is stable, slow-release and powdery, and it also can prevent volatility. So it can be applied as medicine.

The β-CD inclusion complex was analyzied by X-ray powder diffraction patterns, GC and thin-layer chromatography. The inclusion efficiency is above 90%. The inclusion has no selectivity and all the compounds which can be included are included. That provides a good method for the preparation of medicine.

The results show that it is an efficienct method for the application of Ginseng volatile oil.

A Study on Composition of Volatile Oil of Jilin Ginseng Flower Bud

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A total volatile oil of dried flower bud of Jinlin ginseng (FGVO) was obtained by ether extraction, and the yield was 0.2wt.%. 128 components were separated by Gas Chromatography (GC). Among them, 56 compounds and their area % (A%) were identified by Gas Chromatography-Mass Spectrometer (GC/MS) and Gas Chromatography-Fourier transform Infrared Spectrometer (GC/FTIR).

28 compounds including ester, aldehyde, ether, aromatic hydrocarbon, terpene, unsaturated aldehyde and ketone were identified when the column temperature programmed from 50℃ to 140℃ and they had 9.65% of FGVO's content. 19 sesquiterpenes were determined when the column temperature held at 140℃, their molecular weight are 204 and their molecular formulas are $C_{15}H_{24}$, they make up FAVO's

characteristic constituent. All sesquiterpenes had 44.40% of FAVO's content, and 20.87% for (Z) β-farnesene, the results of such high content had not been reported on other GVO's researches. The compounds of sesquiterpenoids, long chain alkene and alkyne, unsaturated acid and ester were separated above 140℃, and they had a content 32.90%.

A highly sensitive Ion Trap Detector(ITD) was adopted in GC/MS. 128 compositions were separated and 56 compounds were identified with ITD, GC/MS. FGVO's analyses were improved greatly by ITD technique. 20 of 56 compounds determined with GC/MS were confirmed further by GC/FTIR, which can distinguish isomers. Among the 56 compounds, 19 had not been reported on other GVO's researches, including seven sesquiterpenes (NO. 7,14,17,19,25～30,32,34,37,38,40,48,50,51,53). FGVO was separated into three groups of compounds by TLC (GF254, n-hexane: ethylacetate＝10.8 : 1). 19 sesquiterpenes were isolated in the first group (Rf＝0.91), five IR spectrograms among them are obtained first time with GC/FTIR. The others were two groups of polar compounds including ester aldehyde, ether and sesquiterpenoids, fatty acid. The result is very valuable for further isolation of single compound and study of their pharmacology.

It is found that FGVO could inhibit obviously the growth of SGC-803 body stomach cancer cell and mouse's liver cancer cell, but could not damage normal cell.

Studies on the Extraction, Isolation and Identification of Panaxydol from Ginseng

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An ester-soluble compenent of ginseng was extracted, isolated and identified with an improved method. Extracting the ginseng powder with ethyl acetate and washing the drying gel column with ethyl acetate-n-hexane(1 : 1) and ethyl acetate-n-hexane-chloroform respectively, and a fined pure substance was obtained. This substance was a light yellow colored oil. Thin layer determined it was a monomer, it was soluble in ethyl acetate, ester, chloroform and some other organic solvent, but not soluble in water. After being indentified by IR, ^{1}H-NMR (400 MHz, $CDCl_3$), ^{13}C-NMR (100 MHz, $CDCl_3$) and MS, it was determined to be panaxydol.

Studies on Water Soluble Polysaccharides Isolated From the Stem of *Panax ginseng* C.A. Meyer (II)
－Purification and Structural Approach to S-2A－

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Fraction S-2A was obtained from the crude polysaccharides isolated from the stem of *Panax ginseng* C.A. Meyer. Sepharose CL-4B column chromatography and electrophoresis showed that it is a homoge-

neous fraction. Its molecular weight is about 500,000. GC indicated S-2A is composed of Gal, GalA, Glc, Rha and Ara with molar ratios of 3.8 : 1 : 1.3 : 1.5 : 0.2.

Pectinase had no obvious effect on S-2A. Thus, there are no GalA-1αA GalA linkages in main chains. Periodate oxidation and Smith degradation showed excepting Ara the residues of other saccharides exist in the linkages in such a way that they were not oxidized. S-2A contains 4-O-substituted GalA and hexose which must be 2,3-O-unsubstituted.

S-2A was hydrolyzed partially with H_2SO_4. All of the Ara was hydrolyzed finally. The untouched parts were mainly composed of Gal. This part and S-2A were methylated respectively and conducted G.C. -M.S. analysis. According to the experimental results, the main chains of S-2A are composed of (1-3) -linked Gal, some residues of which have side chains in position 6. The rest of the residues exist in the side chains. Most of Ara link in the external portions of S-2A.

The accetylated S-2A was subjected to oxidation with chromium trioxide. Most of the Rha were not oxidized, most of the Gal were oxidized, and the parts of the Glc were oxidized, which explained that the Rha residues have α configuration, whereas the Gal residues have β configuration, and Glc residues have both α and β configuration. ^{1}H-NMR spectrum of S-2A hydrolyzed partially contained the signals at 5.30, 5.19, 5.02, 4.82 and 4.55 ppm which were assigned to α-GlaA, α-Glc, α-Rha, β-Glc and β-Gal. ^{13}C-NMR spectrum had a signal at 105.9 ppm which was assigned to β-(1-3)-linked Gal.

Studies on the Polysaccharides Isolated from the Stems of *Panax quinquefolium*

－Purification and Structural Determination of PL-1－

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The crude water soluble polysaccharides isolated from the stems of *Panax quinquefolium* linn is a mixture o acidic heteroglycans which is composed of Ara, Rha, GalA, Gal and Glc. The fraction PL-1 was obtained from this crude polysaccharides by acidic alcohol fractionation. Sepharose CL-4B chromatography and deproteining with enzyme and Seveg method. PL-1 contains Ara, Xyl, Rha, GalA, Gal and Glc in the ratios of 2.13 : 1 : 0 : 0.75 : 2.5 : 2.88 : 3/25. It is a acidic heteroglycans the molecular weight of which is about 100,000. In addition, it contains 1.34% proteins.

After periodate oxidation, there were Ara, Glc, Gla and a little GalA in the products, but no Xyl and Rha. Therefore, Ara, Gal, Glc and GalA have the linkages that can not be oxidized by periodate oxidation, and Xyl and Rha have no the linkages of 3-o-substituted or 2,4-di-o-substituted. The products of Smith degradation had glycerol, glycerol acid, erythritol and erythrituronic acid, which showed Pl-1 has 2,3-o-unsubstituted pentoses and 4-o-substituted hexoses and hexuronic acid.

Pl-1 were hydrolyzed with 1 N H_2SO_4 and alcohol was added into the products. G.C. showed the preipitate contains 96.5% of total Gal, more than 98% total Ara and Rha. The IR spectrum of precipitate appeared absorption at 1260 and 1730 cm^{-1} which was assigned to estered GalA, and at 763 cm^{-1} which was assigned to Ara. Pectinase had no effect on PL-1.

According to above results. Ara, GlaA exist in the main chains. Parts of GalA are in the form of esterification. There are no GalA 1α4 · GalA links. These were consistent with methylation analysis and periodate oxidation.

Studies on the Polysacchardes Isolated from the Leaves of *Panax quinquefolium* Linn

－Isolation, Purification and Structural Analsis of PN－

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The water soluble polysaccharides were obtained from the leaves of *Panax quinquefolium* linn(Zuojia Research Unit of Special Products). It contains 73% saccharides and 14.8% proteins. A neutral polysaccharide was produced by freeze-drying, alcohol and acetone fractionation, and enzyme and Seveg method which was used to deproteinize.

PN appeared only one peak on a Sephadex G-100 column (1.5×90 cm). Electrophoresis also gave a single band. These explained that PN is homogeneous and its molecular weight is about 7400. It contains 97.8% saccharides and 2.2% proteins. Its optical rotation ($[\alpha]_D^{20}$, H_2O, c=0.1) is 675. IR spectrum has a absorption at 890 cm^{-1} which assigned to β-glycosidic linkages. G.C. determined that PN is composed of Glc, Gal, Xyl and Ara which have the ratios 8.25 : 1.5 : 0.75.

The results of periodate oxidation and Smith degradation showed Glc and Ara were oxidized completely and 75% of Gal and 83.3% of Xyl were oxidied, and the main chains of PN were oxidized. PN was hydrolyzed partially, then dialyzed. The results indicated most of Glc exist in the internal portions and Ara mainly exist in the external portion. ^{13}C-NMR spectrum had peak which was assigned (1-4) glycosidic links.

The main chains of PN are composed of (1-4) linked Glc. 25% residues of main chains have side chains in 0～6, that is, one of the four has a branch. Other residues link in the side chains.

Studies on the Free Nonprotein Amino Acids and N-γ Glutamyl Oligopeptides of *Panax species*

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Our studies were focused on nonprotein amino acids and peptides in the water-extract of *Panax species.*[1] From the free amino acids of *Panax ginseng* (Jilin, China), an inhibited neutransmitter, γ-aminobutyric acid (GABA), a neuroexcitotoxic and hemostatic nonprotein amino acid, β-oxa-lo-L-α, β-diaminopropionic acid (β-N-ODAP) were isolated in pure form by TLC, anion exchange chromatography and HPLC. They were identified by MS, ^1HNMR, $^{13}CNMR$, elementary analysis etc.[2,3] β-N-ODAP (dencichine) was also found in Korean red ginseng by Zhang *et al.* independently.[4] Ornithine and ethanolamine were also identified in the water-extract of ginseng.

Interesting results were found on the distribution of β-N-ODAP in different *Panax species. Panax ginseng* and *Panax notoginseng* contain relatively high quanitity of β-N-ODAP, *Panax quinquefolius* L. and red ginseng contain relatively a low quantity of β-N-PDAP. The β-N-ODAP is concentrated in rhizome of *Panax ginseng.* The main root of *Panax ginseng* contain relatively low quantity of β-N-ODAP. All *Panax species* contain almost no α-N-ODAP. So α-N-ODAP is very likely an artifact formed during separation. GABA was found to have a high quantity in the main root of Jilin white ginseng.[5] We

were particularly interested in obtaining oligopeptides with molecular weights below 2,000. After many trials, ginseng oligopeptides could be separated roughly into three parts: acidic (p-1-m), neutral (p-2-m) and basic (p-3-m).

A group of N-γ-glutamyl oligopeptides was found in the water-extract of ginseng. Oxidized glutathion. (GSSG) was isolated and identified from acidic part as the main constituent of small peptide mixture. An isomer of oxidized glutathione was isolated from the acidic peptides of 5th graded of ginseng, its structure was suggested to be $(\gamma\text{-Glu-Gly-CysS})_2$ which was identified by amino acid analysis, N- and C-terminal analysis etc. From the neutral part, a major constituent was identified as an oxidized glutathione amide, we are not sure that whether it was natural or an artifact during the isolation.

Cell chemistry studies indicated that ginseng peptides did not inhibit cell growth but affected the metabolism by modulating some enzyme system.

Comparison of Inogranic Element Contents Among Different Types of Ginsengs

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Ginseng is a famous precious medicine both at home and abroad. Along with changes in the surroundings and improvement of planting methods, the morphology of the root shapes gradually developed during the long process of growth and multiplication. Now there are many types of ginseng, such as DAMAYA(1), ERMAYA(2), CHANGBE(3), ZHUJIELU(4) and YUANLU(5). For the need of metal nutrients during seed growing, we have determined 24 kinds of inorganic elements in different types of JILIN ginseng by ICP-AEM method.

1. The additive amount of 21 kinds of inorganic elements in different types of JILIN ginseng, the summed amount of the essential macroelements Ca, P, Mg and trace elements Fe, Sr, Mn, Zn, Cr, Cu, Ni, V, Co and Mo are all in the following order: (3)〉(4)〉(1)〉(2)〉(5) This is in accord with the order of the content in percentage of glucosides in JI' AN ginseng determined by ZUOJIA Native-Product Research Institute. The summed contents of harmful trace elements Pb, Cd and Be are in the following order: (2)〉(3)〉(1)〉(4)〉(5) and are far below the toxication dosage.

2. The additive amount and the summed amount of 13 useful elements in different types of ginseng are all in the following order: rhizome〉fibrous root〉main root. Works done by JILIN Agriculture University have indicated that the total amount of ginsenosides in the rhizome is 2～3 times higher than that in the root. This value is identical with our result that contents of the inorganic elements in the rhizome are far higher than that in the main root. However, at present, the rhizomes, which account for 5～8% of the ginseng root, were discarded by some pharmaceutical factories during processing.

3. The root shape changes of ginseng were affected by the soil type more than hereditary factors. The effective components are the basis for evaluation of the quality of ginseng. The contents of inorganic elements in ginseng from JI'AN Ginseng Farm No. 1 are much higher than those from JI'AN Ginseng Farm No. 2. Ginseng's quality strongly dependes on fertility of the soil.

4. The Chinese scholars have founded that the order of decrease of Zn/Cu values in the serum of patients with deficiency syndromes is correspondent with the order of increase in Zn/Cu values

in tonic medicines, indicating that these tonics have the effect of improving the deficiency. Zn and Cu contents are relatively high in all types of ginseng, with an average Zn/Gu values of 3.1, which 1.83 times higher than the average value of 1.7 of Astragalus membranaceus and 27 other tonic medicines. It is not without reason to believe that ginseng are: (2)〉(4)〉(5)〉(3)〉(1). The order of Zu/Cu value is: fibrous root〉rhizome〉main root with the exception of (2).

5. It is worthy of pointing out that trace element Y was determined only in the fibrous root of (5) with a content as higher as 0.025 μg/g, but not for In is (5): Be was not determined in (5) and (3). La has not been determined in all types of ginsengs.

Study on Senility-Resity Component in Red Ginseng
－The Separation, Identification, Ration, Synthesis and Forming Mechanism of Maltol－

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3-Hydroxyl-2-Methyl-4-Pyrone was seperated with distillation method of water vapour and identified with the spectrum methods. Content of maltol was determined with colorimetric analysis and doublewavelength thin-layer chromatography scanning method.

The compound was synthesized with Brennan method of Prizer company and identified with the compound seperated from red ginseng. The compound was identified as a special compound through comparing tests with different varieties of processed ginseng from fresh ginseng of producing area. It is concluded that the compound appeared after first stoving (high temperature stoving) through the sign was first used discussing the influence of the parameter of processing technology on forming the compound. $A_2B_2D_2$ combination of processing technology parameter was selected that is beneficial to forming the compound in ginseng roots. The parameter is the basis of theory of processing correctly and improving the technology.

A Studies on the Chemical Components in Variety and Type of *Panax ginseng* in China

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Ginseng (*Panax ginseng* C.A. Mey.) has had some morphological changes, especially in its root form, from the wild, natural environment to the artificial, cultivated conditions and thus formed some different varieties and types. Ginseng growers and scientists have paid more attention to them and widely carried out investigation because they are valuable germplasm of breeding superior varieties. However, the studies on the content of fatty acids and on the Thin-Layer Chromatography (TLC) and content

of total saponin and group saponins have not been reported.

To provide a scientific basis for the breeding and bioengineering of ginseng, we conducted the systematic, comparative analysis and determination for the fatty acids and saponin in ten-year-old, mixed-grade white-dried ginseng roots of Da-Ma-Ya, Er-Ma-Ya, Yuan-Bang-Yuan-Lu, Chang-Bo, Zhu-Jie-Lu and yellow-fruit ginseng, the main varieties in Jinlin, China.

The reactions of total ginsenoside to lieberman-Burchard, Rosen-Heimer, Salkowski, Tschugaeff and Kahlenberg were positive. The comparative analysis on TLC of ginseng's total saponin showed that all the sample had the same results. The comparison of TLC of different group ginsenosides showed that the panaxadiol saponins were all purple, panaxitriol ones orange and oleanolic acid ones yellow. These are consistent with references.

The comparison of TLC of panaxasapogenol showed that the spots corresponding to panaxadiol, panaxitriol and oleanolic acid were purple, orange and yellow, respectively, indicating that these samples have the same panaxasapogenols.

The determination and comparative analysis on contents of ginseng's total saponin and group saponins the results were shown the contents of total saponin and three group saponins: panaxitriol ones: oleanolic acid ones are approximately 6 : 2 : 1 in the different varieties and types of ginseng.

The content of fatty acids of ginseng was compared and analyzed, the results are shown the content of non-saturated fatty acid in Zhu-Jie-Lu is the highest, 50.09% and that of saturated one in it the lowest, 30.26%. The contents of various fatty acids in the yellow-fruit ginseng are mediate.

A Comparative Study on Saponins and Amino Acids of Freeze-Dried Ginseng, Red Ginseng, Scalded Ginseng and Air-Dried Ginseng in *Panax quinquefolium*

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To enlarge the limits of medical treatment and health care for *Panax quinquefolium*, in 1992, we developed three new processed assortments *P. quinquefolium*, i.e. freeze-dried ginseng, red ginseng, and scalded ginseng. It was found by pharmacological test that these new assortments had not only the same pharmacological efficiency as the air-dried ginseng but also respectively particular pharmacological properties.

Saponins and amino acids are important active matters in *P. quinquefolium*. The specimens from these assortments were determined by Model CS-930 TLC Scanner (Shimadzu, Japan), UV-240 Ultraviolet Spectro-photometer (Shimadzu, Japan) and 835-50 Hitachi Amino Acid Autoanalyser. Results showed that the total saponins content of the freeze-dried ginseng was 0.47% more than that of the air-dried ginseng, 0.47% more than that of the scalded ginseng, and 0.97% more than that of the red ginseng, indicating that the processing technology of the freeze-dried ginseng is advantageous to the preservation of saponins.

The determination results for single saponin are as follows (1) The TLC scanning spectra of these four specimens were similar, indicating that the sorts of the single saponin in these processed assortments are identical. (2) The content of Rb_1, R_0 and Rg_1 in these three new assortments was all less than that in the air-dried ginseng. (3) The content of Rb_2 in the freeze-dried ginseng and the scaled

ginseng was all markedly more than that in the air-dried ginseng. (4) The content of Rd was the most in the freeze-dried ginseng as compared with the other assortments. (5) The content of Re, Rg_1 and Rg_2 was the most in the red ginseng.

The determination results for amino acids showed that all of these four assortments had more than 16 sort of amino acids, which were the most in the freeze-dried ginseng. The content of the amino acids in the red ginseng and the scalded ginseng was all less than the air-dried ginseng. It indicats that the processing technology of the freeze-dried ginseng is advantageous to the preservation of amino acids.

Seperating and Identifing Fatty Acid in Active American Ginseng and White American Ginseng

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Methyl mixed fatty acid ester was obtained from Active American Ginseng and White American Ginseng through saponifying and esterifying free fatty acid and complex lipids and was seperated and identified with VG 707E-HF GS/MS Mass Spectrometer and was further quantitated with GC-7A Gas Chromatograph.

1. Eleven fatty acids were first seperated from Active American Ginseng and eight of them were identified as myristic acid, tetradecaroic acid, 12-Methy, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid and 6,9,12-octadecatricnoic acid. Ten of the eleven fatty acids, except 6,9,12-octadecatricnoic acid, were all seperated and seven of them were identified from White American Ginseng.

Table 1. Relative contents of partial fatty acids in active American Ginseng and white American Ginseng (%)

	Acive American Ginseng	White American Ginseng
Methyl myristate	0.01	0.15
Methyl tetradecaroate 12-Methy	0.02	0.07
Methyl palmitate	3.16	8.65
Methyl stearate	0.07	0.19
Methyl oleate	5.96	8.95
Methyl linoleate	87.30	76.08
Methyl linolenate	2.93	4.36
Methyl 6,9,12-Dctadecatricnonate	0.03	–
Methyl saturated fatty acid ester	3.62	9.06
Methyl unsaturated fatty acid ester	96.22	89.58
Weight of methyl mixed fatty acid ester/weight of dried American Ginseng	0.48	0.36

2. Quantitative analysis

The process method of Active American Ginseng is better than that of White American Ginseng because of remaining fatty acid, especially unsatured fatty acid.

Determination of Trace Germanium in Ginseng Products

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It has been testified by the experiments that the special curative effects of Ginseng and several other medical plants are due to their rich in germanium, which is a rare elements. Great attention has been laid on the measurment of germanium. The hydride gerneration technique was adopted in our Lab. to make germanium ion transferred to GeH in gas state which was absorbed and coloured by a mixed solution of silver nitrate, gelatin and organic solvent, and a new spectrophotometric method for the determination of traces of germanium was established. The high selectivity of this method can remove the interferences of most of the other ions. This method is rapid, simple, sensitive and reproducible. The apparent molar coefficient is $7.32\times10^4\,L\cdot mol^{-1}cm^{-1}$. We have got a satisfactory result on our analysis of the trace of germanium in the ginseng products.

The Anti-Cancer Action of Ginseng Saponin RH_2 and the Mixture of RH_2 and Organic Ge

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Ginseng Saponin Rh_2 is the active component of anti-cancer in Red Ginseng. It's content is 10^{-5}. Chinese research found Rh_2 in leaves of Ginseng. The author make saponin of Ginseng leaves to be inverted into Rh_2 by using chemical method (intered is 41.6%). The content of Rh_2 increase hundrends times so to use Rh_2 in clinic become possible. This thesis reports the pharmacological action of Rh_2 (inverted substance) and the mixture of it and organic Ge.

1. Rh_2 and the mixture inhibit the growth of cancer cell S_{180} sarcoma, ascites of EAC and hepar cancer H_{22} significantly in mice. The effective dosages are 15 mg/kg of Rh_2 and 15 mg. 7.5 mg/kg of the mixture of Rh_2 and organic Ge.

2. The research of mechanism of anticancer show that Rh_2 and the mixture inhibit the synthesis of DNA and RNA in cancer cells, improve immunization and modulate blood-sugar and blood-fat of cancer mice.

3. Using Feulgen's stain and Lei-MPV_2 microphotometer to determine the content of DNA in cancer cells. The results show that Rh_2 and mixture decrease the polyploid and increase the diploid of cancer

cells.

After administration 24 hr the content of DNA in cancer cells decrease. The synthesis of DNA is inhibited. The results also show that the polyploid cell invert to the diploid cell. It is possible to make cancer reverse to normal cells.

4. Rh_2 is a cancericidal. It kill Hela cell and inhibit multiplication of Clone of cell more significantly than 5-FU. The research show that Rh_2 is a anti-medicine with time-dependence. It is also a antimetabolite. Rh_2 stop fission of cancer in G and S storage to make the number of cell in GM stage decrease.

Effect on the Liver of *Panax Ginseng* (Kesong)

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Study on the effects of *Panax ginseng* on the liver was initiated in 1965, having been paid increasing concern especially because of the recent report on regression of cancerous hepatic gene into normal one.

In order to examine effects of *Panax ginseng* on hepatic glycogen and sinusoids in groups differently aged rats in weanling, prepuberty, maturity, and agedness groups were given pulverised ginseng in the dose of 0.8g per weight (kg) which was kneaded in to pill with wheat flour, administered before morning feed once a day, and then the profiles of glycogen and sinusoid of the liver were observed on the days after giving the pills for 1, 2, 3 and 7 days.

Weanling group: Decrease in the ratio of the pattern enriched in glycogen granules was initiated on the day after giving for 2 days, and the ratio of that pattern diminished from 70% to 30% in the 7 days-group. Increase in the ratio of the pattern of expanded sinusoid began to be recognized already in the 1 day-group, being most marked in the 3 days group with 75% ($P>0.001$).

Prepuberty group: Increase in the ratio of the pattern scanty of glycogen granules were never recognizable in the 1, 2, 3 days-group, and observed in the 7 days-group with 16.6% from 10%. Increased ratio of the pattern of expanded sinusoid were never noticeable in the 1, 2 days group, and began to be recognizable form the 3 days-group, being most pronounced in the 7 days-group with 58.3% from 30%.

Maturity group: Decreased ratio of the pattern abundant in glycogen granules could not be recognized in 1, 2 days-group, being observable from the 3 days-group to 7 days-group with 50% from 60%. Findings of enlarged sinusoid were never recognized in each group of days.

Agedness group: Increased ratio of the pattern scanty of glycogen was most noticeable in the 2 days-group with 62.5% from 8.3% ($P<0.02$). Findings of enlarged sinusoid were not observed like maturity group.

Additionally, such senile changes as lymphocytic infiltration and petty necroses in the hepatic lobules were found.

Thus, the effects of ginseng on the liver were somewhat varied depending upon age, duration of administration, while on the whole ginseng made sinusoid as microcirculation enlarged, glycogen decreased in the liver, indicating incubation of the liver into an active state.

The Reaction of Ginsenoside-Re with Different Age Integrate Human Red Blood Cell (RBS)

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Ginseng is a important anti-aging traditional Chinese medicine and contains ginsenoside -Ro, -Rb_1, -Rb_2, -Rc, -Rd, -Re, -Rf, -Rg_1, -Rg_2, etc which are main components of anti-aging function. In this paper, we research the antiaging mechanism of the ginsenoside Re using fluorescence-technique and find that the effect of Re on fluidity of different age RBC membranes is different.

The differnt age RBCs are separated by density gradient centrifugation. Briefy, fresh human RBCs are washed three times, then the RBCs are placed on the top of gradient solutions which is composed of five densities: 1.093, 0.090, 1.086, 1.080, 1.074. Density Fractionation is then accomplished by centrifugation at 13,000 rpm for 40 mins at 4℃. The top fraction and the bottom fraction are collected, washed three times with PBS buffer, and termed "young" and "old" RBC_S, respectively. The "young" and "old" RBC_S, are diluted into a hematocrit of 1% with PBS, and incubated with Re, which final concentration is 50 mg/L. After incubation one hour at 37℃ RBC_S are labeled with DPH. Fluidity of RBC membrances is measured by fluorescence-spectra and the results of experiment are, shown on the Table 1.

Table 1. Polarization value of old and young RBC_S

Concentration	0	50 mg/*l*
Young RBC	0.307	0.272
Odl RBC	0.328	0.3

The results indicate: (1) The fluidity of "old" RBC_S is smaller than "young" RBC_S; (2) Re can increase the fluidity of both "old" and "young" RBC membranes, fluidity increase in "young" RBC_S is 11.4% and in "old" RBC_S is 7.62%, (3) The extent of the fluidity of different age RBC membranes increment is different.

The possible explanation of the results is integrate RBC is always attacked easily by the free radicals of OH^-, O_2^-, thus the unsaturation degree of phospholipid is decreased and so is fluidity of membranes. Re may act as a trap of the free radicals, thus, the presence of Re inhibit the peroxidation of on unsaturated fatty acids on RBC membrances. For the "old" RBC membranes, the unsaturated fatty acids have been partly oxidized to saturation and Re only protect the other unsaturated fatty acids, so the protective effect of Re on the "old" RBC is smaller, but for the "young" RBC membranes, the unsaturated degree is higher. Re can protect the all unsaturated lipid acid, so the protective effect

of the Re on the "young" RBC ls larger and so is the increase of fluidity.

In summary, ginsenoside -Re has function of anti-aging and the effect is notable.

Single Channel Analysis on Calcium Channel Blockade Action of Panaxatriol Saponin Monomer Re

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Using the cell-attached configuration of the patch clamp technique, the single channel activities of T, L and B type calcium channels on cultured neonatal Wistar rat myocardiocytes was recorded respectively. Bath solution (mmol L^{-1}) aspartic potassium 140, EGTC 10, HEPES 10, pH 7.4 with KOH. Microelectrode filling solution (mmol.L ^{1}): $BaCl_2$110, HEPES 10. The cut-off frequencies of the low-pass filters built in the patch clamp amplifier and oscilloscope were set at 1 KHz and 10 KHz respectively. Larger than 10 GΩ seal resistance between microelectrode and cell membrance was used in experiment. When the membrane potential was stepped from −50 mV holding potential to flom V, the single channel activities of L type calcium channel could be recorded. Under the condition that the membrane potential was stepped from −70 mV holding potential to −10 mV, the single channel activites of T type calcium channel could be recorded. If the holding potential was kept at −70 mV without the application of step command, the single channel spontaneous activities of B type calcium channel could be recorded. Panaxatriol saponin monomer Re 50 μg·ml^{-1}, calcium channel blocker verapamil 37.5 μg·ml^{-1} or calcium channel activator BAY K 8644 5 μmol·L^{-1} was added into the bath solution respectively, the single channel activities of L, T and B type calcium channels were recorded before and after administration. The mean open time and close time were obtained by exponentially fitting the open time and close time histograms. The amplitude of Ba^{2+} current flowing through the calcium channel was obtained by fitting the current amplitude histograms with Gaussian curve. The open-state probability was obtained by dividing the sum of open time in each sample curve by the total sampling time of the curve. This experiment only recorded the inhibiting effect of Re on the L, T and B type calcium channels but also compared the Re with veramapil and Bay K 8644; therefore, this experiment proved the blockade effect of Re on the calcium channel. Its mechanism involved the decrease in open-state probability and the open time of calcium channels.

Influence of Ginsenoside-Rb_1 on the Phase Conversion Temperature of the Liposome DPPC

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Influence of a drug on the body, at first, is action of the drug on biocell's membrane. By means of interaction parameters of the drug with cell's membrane, researching characteristic of the drug is possible. Therefore, we investigated interaction of Ginsenoside-Rb_1 with model membrane liposome DPPC. The result is discovered that Rb_1 possesses function of improving cell's membrane.

The special construction of the lipid bilayer is a basic construction of life. Liposome DPPC (dipalmitoylphosphatidylcholine) has lipid bilayer construction. Consequently, it is applied widely as a model of natural membrane.

We investigated phase conversion temperature of the liposome DPPC by DSC (Fig. 1) and NMR (Fig. 2) technique. It is indicated from Fig. 1. When there is not Rb_1, there is a absorptive peak of the DPPC membrane on 41℃. It is main phase conversion. The temperature of ahead phase conversion is 36℃. When Rb_1 is added into, the main phaseconversion temperature of the liposome DPPC is decreased to 34℃, and ahead phase conversion is not obvious. It is indicated from Fig. 2 (a), (b) both peaks reach stable value on 41℃. That is main phase conversion temperature of it's. After Rb_1 is added, main phase conversion temperatur became to 34℃. The result of NMR is agreeable completely to DSC.

Conclusion: The ginsenoside-Rb_1 can reduce phase conversion temperature of the liposome DPPC, can increase it's softening and fludity. That is, it can improve conformation and function of the cell's membrane, possesses anti-decriptude and protecting illness function.

Influence of Maltol on the Fluidity of Sheep Ghost Membrances and Liposomes Made of Lipids Extracted from Sheep Ghost

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Maltol is a water-soluble extracted from red Ginseng, which has been suggested to be an antioxidant and an anti-aging agent. In this paper we studied the effect maltol on the fluidity of sheep erythrocyte membrane and SUV liposomes composed of lipid mixture extracted from sheep ghost by using the techniques of fluorescence polarization and electron spin resonance (ESR).

1-[4-(Trimethylammonio) phenyl]-6-phenylhexa-1,3,5-treine(TMA-DPH) is a hydrophobic fluorescent probe, which is widely used in membrane fluidity studies of phospholipidic bilayers by fluorescence anisotropy. It was assumed to have a well-defined "wobbling in cone" motion, presumably remaining anchored to the phospholipid polar heads by it's positively charged group. With TMA-DPH (final concentration; 5×10^{-1} M) as a probe.

We found that maltol arised a decrease of the polarization value (P) by the probe inserted in both the sheep ghosts and the SUV liposomes. This fact indicated that maltol has effect on the polar heads of phospgholipids and can increase the fluidity of the hydrophilic region of membrane lipids. The results were shown in Table 1 and Table 2:

The ghost has been incubated with maltol of different concentration at 37℃ for two hours. Ghost concentration, 50 mg protein/L. The incubation condition is the same as Table 1. Lipid concentration 35.7 mg/L.

From Table 1 and Table 2, we can find that maltol decreased the P values of TMA-DPH inserted

Table 1. Flurescence polarization (P) of TMA-DPH labeling sheep ghosts

Maltol/lipid (mol/mol)	Control	0.5/1	1/1	1.5/1	2/1
P	0.386	0.383	0.368	0.367	0.363

Table 2. Fluorescence polarization (P) of TMA-DPH labeling SUV liposomes made of lipids extracted from sheep ghosts

Maltol/lipid (mol/mol)	Control	0.2/1	0.5/1	2/1
P	0.474	0.468	0.449	0.446

Table 3. Effects of maltol on the fluidity of 5NS-labeling sheep ghosts

Maltol/lipid (mol/mol)	Control	0.1/1	0.2/1	0.5/1	1/1
P	8.24	8.08	8.01	7.48	7.32

in both ghost membrane and liposomes. However, the extent of P values dropped by maltol is different in both systems. This difference may suggest that maltol interacts not only with membrane lipids but also with membrane proteins.

In addition, ESR spectra were recorded from sheep ghosts labeled with the spin probe 5-nitroxide stearic acid (5NS). And the rotation correlation time values (I) were used to evaluate the membrane lipid fluidity. The results that decreases with the addition of maltol also indicate that maltol arised an increase of fluidity of the polar head region of membrane lipids. The results were shown in Table 3. Maltol is incubated with ghost at 37℃ for two hours before measurement. Final concentration of 5NS, 10^{-6} M, Ghost concentration 4.0g protein/L.

Effect of Ginsenosides Rb_2, Rg_1, Rh_1 and Re on Proliferation of Cells *in Vitro*

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Ginseng saponins of roots (SRG) and ginsenosides Rb_1, Rg_1, Rd and Rh_1 were added to the culture medium a aging human fibroblasts and Hela cells for 3～6 days. All ginsenosides concentration of $1\times10^{2}\sim1\times10^{-3}$ μg/m*l* range were used. The presence of 1.0～0.01 μg/m*l* range Rb_1 and Rg_1 and Rg_1 increased the rate of clonal growth, whereas Re and Rh_1 had no significant effect on this parameter in aging human fibroblasts but all ginsenosides-treated Hela cells reduced the elonal growth rate compare to untreated controls. All ginsenosides increased the population proliferation rate of aging human fibroblasts but reduced the rate of parameter in Hela cells. Rb_1, Rg_1 and Rh_1 increased the rate of saturation density of aging human fibroblasts but Re and Rh_1 reduced the rate of parameter in Hela cells. Using the cytochemical methods, DNA content was determented with a cytospectrophotometer in aging humamn fibroblasts and Hela cells when all ginsenosides were added to the medium. The

results showed that Rb_1 and Rg_1 increased the DNA content compare to untreated controls and SRG in aging human fibroblasts but Re, Rh_1 and SRG reduced the DNA content compare to untreated controls. The results of cell cycle analysis showed that Rb_1 and Rg_1 increased the rate of cell number in G_2M phase and reduced the rate of cell number in G_1 phase in aging human fibroblasts but Re, Rh_1 and SRG reduced the rate of cell number in multiploid and increased the rate of cell number in dioploid of Hela cells. The results suggested that different ginsenosides effect on proliferation of identical state's cell was identical orientation but effect on proliferation of different state's cell was different orientation despite the different type sapogenins. Rb_1 and Rg_1 had significant effect on the proliferation of aging human fibroblasts and slightly effect on the Hela cells but Re and Rh_1 had significant effect on the proliferation of Hela cells and slightly effect on the aging human fibroblasts. These different ginsenosides had respective dose-reponse relations, each ginsenosides fixed dose's range was positive effect and the dose outside fixed range was negative effect on cell proliferation.

Decline of Atrial Natriuretic Peptide (ANP) Gene Expression during Aging Development and Effect of Ginsenoside on and Gene Expression in vivo in Rat

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1. The levels of atrial natriuretic peptide (ANP) gene expression in rat atria at 2～3, 14～18 and 24～26 months ages, and the effects of ginsenoside on r-ANP-gene expression by determing the concentration of ANP-mRNA were investigated. The male and female rats were abdominally (i.p.) injected with aqueous solution of ginsenosides prepared from ginseng stems as well as leaves (G-PSL) and ginseng roots (G-PR), 50 mg/kg body wt, once a day for 7 days. Atrial total RNA was extracted by cold phenol method. The ANP-mRNA contents were determined using the Northern blot and dot hybridization technique with α-32* P-labelled r-prepro-ANP-cDNA probe.

2. The ANP-mRNA contents of 14～18 months and 24～26 months rats were remarkably less than that of 2～3 months rats. The ANP-mRNA levels of male rat atria at 14～18 months and 24～26 months were about 15% and 40%, respectively. For female rat, the correspondences were 60% and 50%.

3. G-PSL and G-PR increased the ANP-mRNA content of male rats at 14～18 months 1 and 2 fold, respectively, G-PSL increased the ANP-mRNA content of male rats at 24～26 months 0.5 fold; whereas G-PSL and G-PR decreased the ANP-mRNA content in male rats of age 2～3 months. No apparent effects of ginsenoside on ANP gene expression was observed in female rats.

4. These results revealed that the ANP expression declined during aging development and ginsenosides possessed anti-aging effects in the heart endocrineous function aspect.