J Korean Med Sci 2001; 16(Suppl): S28-37
ISSN 1011-8934

Chemistry and Cancer Preventing Activities of Ginseng Saponins and Some Related Triterpenoid Compounds

More than 25 dammarane-type tetracyclic triterpenoid saponins have been isolated from ginseng, the root and rhizome of *Panax ginseng* C.A. Meyer (Araliaceae). The genuine sapogenins of those saponins, 20(S)-protopanaxa-diol and -triol, were identified as 20(S) 12β-hydroxy-and 20(S) 6α,12β-dihydroxy-dammarenediol-II, respectively. There are two types of preparations from ginseng: white ginseng prepared by drying after peelling off and red ginseng prepared by steaming and drying. Some partly deglycosylated saponins such as ginsenoside Rh-1, Rh-2, and Rg-3 are obtained from red ginseng as artifacts produced during steaming. Several workers studied the metabolic transformation by human intestinal bacteria after oral administration of ginsenoside Rb-1 and Rb-2 and found that the stepwise deglycosylation yielded compound K and finally 20(S)-protopanaxadiol. Ginsenoside Rg-1 was converted into 20(S)-protopanaxatriol via ginsenoside Rh-1. Yun et al. in Korea conducted the epidemiological case-control studies of ginseng and suggested its cancer preventing activities. Kitagawa et al. demonstrated in vitro that ginsenosides, especially 20(R)-ginsenoside Rg-3, specifically inhibited cancer cell invasion and metastasis. Azuma et al. found that ginsenoside Rb-2 inhibited tumor angiogenesis, and Kikuchi et al. reported that ginsenoside Rh-2 inhibited the human ovarian cancer growth in nude mice. Recently, ginsenoside Rg-3 was produced as an anti-angiogenic anti-cancer drug in China. The aforementioned reports suggest that less glycosylated protopanaxadiol derivatives are effective in cancer prevention. Apart from Ginseng tetracyclic triterpenoid saponins, some oleanane-type pentacyclic triterpenoid compounds showed the anti-carcinogenic activity in the two-stage anti-cancer-promotion experiments in vitro and in vivo.

Key Words : *Ginseng; Ginsenoside; Epidemiologic Studies; Case-Control Studies; Neoplasm Metastasis; Angiogenesis Factors*

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INTRODUCTION

Ginseng, the root and rhizome of *Panax ginseng* C.A. Meyer (Araliaceae), has been used as a drug by the people in the Eastern Asia regions for some 2000 yr. Panax is derived from panacea, which means cure-all and longevity. Ginseng has long been known multifunctional as both tonic and sedative agent as described in the oldest Chinese Materia Medica, the Shen Nung Ben Cao Jing, written in the 1st century by an unknown author. American Ginseng (*P. quinquefolium* L.), growing in the North-Eastern parts of the United States and Canada, and San-chi Ginseng (*P. notoginseng* Burk.), growing in Yun-nan Province in China and Northern parts of Vietnam, have also been used for the similar purpose. Japanese Chikusetsu Ginseng (*P. japonicus* C.A. Meyer) has been used in Japanese Kampo medicine as an alternative to ginseng. Vietnamese ginseng (*P. vietnamensis* Ha et Grushv). which had been used as a secret drug among the local minorities was found in the 1970s in mountainous regions of the middle parts of Vietnam. Some other species of *P. pseudoginseng* have been used as a folk medicine among the local people in the Himalayan regions and the middle parts of China.

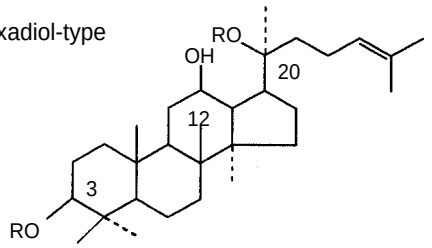
The pioneering works on the pharmacology of ginseng were carried out by Petkov (1959, 1961, 1968) and Brekhman (1957, 1969), and its chemistry by Asahina (1906), Kondo (1915, 1920), and Kotake (1930, 1932). Following these works, my coworkers and I began to investigate of the chemistry of saponins and sapogenins of white ginseng (*Panax ginseng* C.A. Meyer cultivated in Maruko, Nagano Prefecture, Japan) in the early 1960s (1, 2).

CHEMICAL STRUCTURES OF GINSENG SAPONINS AND SAPOGENINS

We proposed for the first time the chemical structures of panaxa-diol and -triol as the acid hydrolysis products of the

Table 1. Some representative Ginseng saponins including saponins of some Ginseng congeners

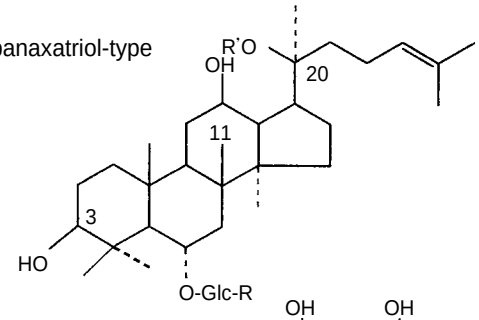
20(S) Protopanaxadiol-type



		R	R′
Ginsenoside	R_{b-1}	-Glc^2-Glc	-Glc^6-Glc
	R_{b-2}	-Glc^2-Glc	-Glc^6-Ara(p)
	R_c	-Glc^2-Glc	-Glc^6-Ara(f)
	R_d	-Glc^2-Glc	-Glc
	R_{g-3}	-Glc^2-Glc	-H
	R_{h-2}	-Glc	-H

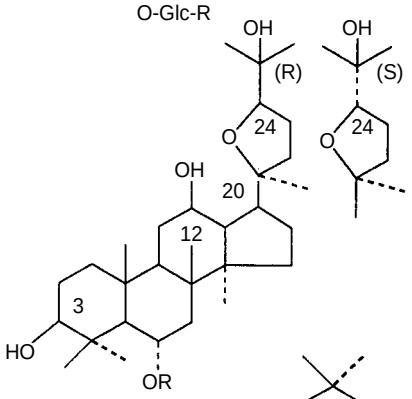
malonyl Glc^2-Glc^6-OOC-CH_2-COOH, (p): pyranosyl, (f): furanosyl

20(S) Protopanaxatriol-type



		R	R′
Ginsenoside	R_e	-Rha	-Glc
	R_f	-Glc	-H
	R_{g-1}	-H	-Glc
	R_{g-2}	-Rha	-H
	R_{h-1}	-H	-H
Notoginsenoside	R_1	-Xyl	-Glc

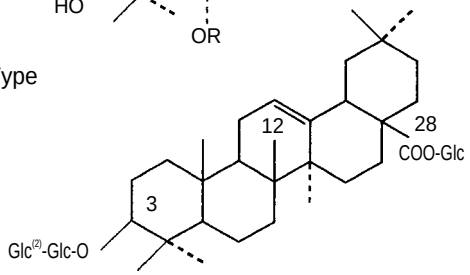
Ocotillol-type



			R
Pseudoginsenoside	F_{11}	24(R)	-Glc^2-Rha
Majonoside	R_1	24(S)	-Glc^2-Glc
	R_2	24(S)	-Glc^2-Xyl

Ocotillol-type saponins are found in American Ginseng and in higher content in Vietnamese Ginseng.

Oleanane-Type



Ginsenoside R_0
=Chikusetsusaponin V

ginseng saponin mixture (3-5) and those of protopanaxa-diol and -triol as the genuine sapogenins (6-12). At that time, several glycosides of these genuine sapogenins have been isolated and named ginsenosides Rx (x=0, a-1, a-2, b-1, b-2, b-3, c, d, e, f, 20-gluco-f, g-1, g-2, h-1...X corresponds to the sequence of Rf value of the spots on the TLC from the bottom to the top (8). The fundamental skeleton of the genuine sapogenins is dammarane-type tetracyclic triterpene, and that of only ginsenoside Ro is oleanane-type pentacyclic triterpene, which is a minor component in ginseng.

Today, more than 25 dammarane-type saponins have been identified as the characteristic principles of white and red Ginseng. White Ginseng prepared by drying after peelling off contains malonyl ester of ginsenosides Rb-1, Rb-2, Rc, and Rd. In red ginseng prepared by steaming and drying, the malonyl group, which is originally attached at the 6″-position of glucosyl moiety of the above mentioned ginsenosides, is released, and the glycosyl moiety at $C_{(20)}$-OH is partly lost to yield ginsenoside Rh-1, Rh-2, and Rg-3 as the artifacts. The acetyl group remains at the 6″-position of glucosyl moiety of some saponins in red Ginseng, such as quinquenoside-R1, ginsenoside Rs-1 and Rs-2, because steaming inactivates the deacetylating enzyme. Some representative dammarane-type saponins of ginseng, which are classified into 20(S)-protopanaxa-diol and -triol groups (13), are selected and formulated below (Table 1).

ISOLATION AND SEPARATION OF GINSENG SAPONINS

For the investigation of chemistry, ginseng was extracted

with methanol and the aqueous suspension of methanolic extracts was subjected to column chromatography over amberlite XAD-2, Diaion MCI Gel HP20 or Kogel BG4600. After removing saccharides and amino acids with water, the columns were eluted with methanol to obtain a saponin fraction. Those ginseng saponins, ginsenoside Rx, were separated on TLC and HPLC. A water-containing silica gel column and the water-containing solvent systems over Senshu-Pak Aquasil SS452N yielded a good separation of ginsenosides (14). The average yields of representative saponins from ginseng and its congeners are tabulated below [cited from Tanaka's data (13)] (Table 2).

Acid degradation of ginsenosides

We obtained for the first time panaxa-diol and -triol by acid (conc. HCl) hydrolysis of the total ginseng saponin mixture. Mass spectrometry revealed the trimethylpyrane ring system at m/e 127 and the tetracyclic triterpene fragment at m/e 341, and the latter ring system was proved by the chemical conversion into isotirucallenol. The pyrane-ring in panaxadiol and -triol is formed secondarily due to the ring closure of the open side-chain of the genuine sapogenins, protopanaxa-diol and -triol. The stereochemistry of OH at $C_{(20)}$ is initially S, and is converted into *R* by the acid treatment. Acid hydrolysis of diol-type Ginseng saponin with conc. HCl at room temperature yielded chlorinated sapogenin. The dehydrochlorination of this chloride with potassium *tert* butoxide gave protopanaxadiol. The fundamental genuine sapogenins of Ginseng were proved to be 20(S)12β-hydroxy- and 20(S) 6α,12β-dihydroxy-dammarenediol-II, respectively.

Table 2. Average yields (%) of saponins from Ginseng and its congeners

		White Ginseng	Red Ginseng	American Ginseng	San-Chi Ginseng
20(s) Protopanaxadiol-type					
Ginsenoside	R_{b-1}	0.5	0.4	1.8	1.8
	R_{b-2}	0.2	0.2	0.03	+
	R_c	0.3	0.1	0.3	+
	R_d	0.2	0.036	0.5	0.2
	R_{g-3}	0.0003	0.014 (20R) 0.015 (20S)	–	–
	R_{h-2}	–	0.001	–	–
20(s) Protopanaxatriol-type					
Ginsenoside	R_e	0.2	0.2	1.0	0.2
	R_f	0.05	0.07	–	–
	R_{g-1}	0.2	0.3	1.9	0.2
	R_{g-2}	0.014	0.01 (20R) 0.02 (20S)	0.008	0.03
	R_{h-1}	0.0015	0.007 (20R) 0.006 (20S)	–	0.01
Oleanane-Type					
Ginsenoside	R_0	0.02	0.045	0.07	–

PHARMACOLOGICAL ACTIVITIES OF GINSENG

Since ancient times ginseng has been used as a supplemental drug for the physical and mental void symptoms to recover homeostasis. It is neither a mere tonic nor a remedy for a certain disease. Brekhman expressed the action of ginseng as adaptogen, and Fulder called ginseng a harmony drug.

Using our pure ginsenoside preparations, Takagi and Saito showed that ginsenoside Rb-1 is central nervous system (CNS)-sedative and Rg-1 is CNS-stimulative. It is noted that oppositely acting principles are present in the same drug.

Table 3. Pharmacological effects of ginsenoside R_{b-1} and R_{g-1}

Ginsenoside R_{b-1}	Ginsenoside R_{g-1}
CNS-depressant action Anticonvulsive, analgesic, and antipyretic actions Antipsychotic action Inhibition of conditioned avoidance response (pole-climbing and shuttle box tests) Protection of stress ulcer (anti-stress action) Increase of gastrointestinal motility Weak anti-inflammatory action Potentiation of the NGF-mediated fiber production in chicken embryonic dosal root ganglia and sympathetic ganglia Anti-hemolytic action Acceleration of glycolysis, cholesterol synthesis (serum and liver) and nuclear RNA synthesis. Acceleration of serum protein synthesis	Weak CNS stimulant action A slight increase of motor activity. Potentiation of doubie blind performance (Y maze test) Behavioral effects Acceleration of acquisition of conditioned avoidance response and discrimination behavior (pole-climbing and Y maze tests), reversal learning (Y maze test), and one-trial passive avoidance learning (step down method) Antifatigue action Aggravation of stress ulcer

Table 4. Pharmacological actions of ginsenosides

	Ginsenoside
Anti-platelet aggregation	R_0, R_{g-1}, R_{g-2}
Fibrinolytic action	R_0, R_{b-1}, R_{b-3}, R_c, R_e, R_{g-1}, R_{g-2}
Stimulation of phagocytic action	R_0, R_{b-1}, R_{b-2}, R_c, R_{g-3}, R_{h-2}, R_e, R_{g-2}, R_{h-1}
Vasodilating action	R_{b-1}, R_d, R_{g-1}
Cholesterol and neutral lipid decreasing and HDL-cholesterol increasing effects	R_{b-1}, R_{b-2}, R_c
Stimulation of ACTH, corticosterone secretion	R_{b-1}, R_{b-2}, R_c, R_e
Stimulation of RNA polymerse, protein synthesis	R_{b-1}, R_c, R_{g-1}
Protection against ulcer induced by stress	Majonoside R_2
Recovering from disturbed sleep induced by stress	Majonoside R_2

This coincides with the old description of Ginseng actions in the Shen Nung Ben Cao Jing. Numerous reports have been published since then on the pharmacological and biological activities of ginsenosides Rx. Multifunctional activities of ginsenoside Rb-1 and Rg-1 and other members of ginseng saponins have been reported, which are summarized below (Table 3, 4).

METABOLISM OF GINSENG SAPONINS

In traditional Chinese medicine, Ginseng is usually taken orally. Therefore, the metabolism of ginseng saponins in the digestive organs should be investigated.

Micro-detection of ginsenosides by EIA (enzyme immuno assay) method combined with HPLC was developed by Kanaoka et al. (15). The time course difference of ginsenosides in the blood of healthy men after oral administration of red Ginseng extracts (250 mg each in 16 capsules=4 g) was examined by EIA-HPLC, and the result showed that ginsenoside Rb-1 was not detected in the blood. Therefore, the fate of ginsenosides in the digestive organs and their metabolic pathway were studied (16). First, ginsenoside Rb-1 was subjected to the anaerobic incubation with human intestinal bacteria, and the metabolites were then analyzed by TLC and HPLC. The result showed that ginsenoside Rb-1 underwent stepwise deglycosylation within 8 hr and ultimately was converted into 20(S)-protopanaxadiol via ginsenoside Rd, substance G-F_2 and compound K (16). In the same experimental process, ginsenoside Rg-1 was converted into protopanaxatriol via ginsenoside Rh-1. Ginsenoside Rb-1 was completely digested and converted into 20(S)-protopanaxadiol within 8 hr, whereas Rg-1 took 48 hrs to produce only 20% of 20(S)-protopanaxatriol. When red Ginseng powder was orally administered to a healthy man, compound K was detected in the serum after 8-12 hrs with some individual variation. It was also shown that ginsenoside Rb-1 was metabolized by the intestinal bacteria to compound K and then absorbed. Screening 31 species of human intestinal bacteria, all of which has the β-glucosidase activity, was carried out, and *Eubacterium* sp. A-44 (17) and *Provotella orise* (18) appeared to be responsible for metabolizing ginsenoside Rb-1.

Human intestinal microflora, which metabolize ginsenoside Rb-1 and Rb-2 to compound K (MI) and 20(S)-protopanaxadiol, were further extensively investigated by Bae et al. (19). *Eubacterium* sp. (Eu), *Streptococcus* sp. (Str), and *Bifidobacterium* sp. (Bif) metabolized ginsenoside Rb-1 to compound K via ginsenoside Rd, whereas *Fucobacterium* K-60 (Fuc) metabolized it to compound K via gypenoside XVII. Ginsenoside Rb-2 was metabolized to compound K via Rd or compound O by human intestinal microflora. Eu, Str, and Bif spp. metabolized Rb-2 to compound K via Rd rather than compound O. Fuc metabolized Rb-2 to compound K via compound O.

ANTICANCER ACTIVITIES OF GINSENG SAPONINS

Experimental and epidemiological studies of cancer prevention

It is probable that ginseng, as an agent for longevity, might be effective in preventing and suppressing cancer. However, the modern medical investigation had not yet reveal any reliable evidence for that.

In 1980 at 3rd International Ginseng Symposium (Seoul), T. K. Yun and his coworkers reported the anticarcinogenic activity of orally administered red ginseng extracts to ICR mice (20). As the carcinogens, 9,10-dimethyl-1,2-benzanthracene (DMBA), urethane, N-2-fluorenylacetamide (FAA), and aflatoxin B_1 were respectively injected to the subscapular area of mice. After 26 weeks of DMBA injected group, no significant inhibitory effect of red ginseng extracts against adenoma was observed, but proliferation of lung adenoma was inhibited by 23% after 48 weeks. At the 28 weeks of urethane-injected group, red ginseng extracts decreased the incidence of lung adenoma by 22% and that of multiplicity by 31%. In the experiment by using FAA as a carcinogen, red ginseng extracts showed no significant inhibitory activity. At the 56 weeks of aflatoxin B_1 injected group, red ginseng extracts decreased the incidence of lung adenoma by 29% and that of hepatoma by 75%. These pre-clinical experiments demonstrated the anticarcinogenic activity of red ginseng extracts. Subsequently, T. K. Yun performed epidemiological studies among Korean people and demonstrated the non-organ-specific cancer-preventive activity of ginseng extracts. He investigated the cancer-preventive effect of ginseng in case-control studies, in which the number of subjects was extended from 905 to 1987 pairs. The results were reported in an international publication (21). In both the epidemiological studies, odds ratios of white ginseng powder intake (0.44, 0.30) and red ginseng extracts intake (0.45, 0.20) were remarkably reduced, and cancers were non-organ-specific. The intake of fresh ginseng slices, fresh Ginseng juice, and white ginseng tea did not decrease the risk for cancer. However, as the odds ratios show, the risk for cancer was rather unexpectedly low in the cases of intake of 1-3 times/year (0.62), 4-11 times/year (0.48), and 1 time/month or more (0.31). Overall, the risk for cancer decreased as the frequency and duration of Ginseng intake increased. The total lifetime intake of Ginseng (301-500) gave 0.33 odds ratios for male and 0.29 for female, which shows the dose-response relationship.

By using red ginseng extracts, the double blind placebo cohort study of the risk for liver cancer in chronic hepatitis virus C carriers will soon be started in Japan.

Induction of reverse transformation by ginsenoside Rh-2

Odashima et al. (22) reported in 1979 that ginsenoside fraction of Ginseng extracts induces reverse transformation of cancer cells. Subsequently, they found that ginsenoside Rh-2 obtained from red ginseng inhibited in vitro proliferation of lung cancer cells 3LL (mice), Morris liver cancer cells (rats), B-16 melanoma cells (mice), and HeLa cells (human). They also recognized that ginsenoside Rh-1 did not inhibit cancer cell proliferation but activated adenyl cyclase and promoted the melanin synthesis in melanoma cells, which might be related to reverse transformation activity (23). (See Table 1: Structural formula of ginsenoside Rh-1 and Rh-2)

Specific inhibition of cancer cell invasion by ginsenosides

Prevention of tumor cell invasion and metastasis is the most urgently required for the therapy of cancer.

Kitagawa et al. (24, 25) developed an in vitro model of tumor cell invasion and metastasis. Tumor cells were seeded on a monolayer of mesothelial or endothelial host cells. The number of tumor cells that penetrated through the monolayer indicated their capacity of invasion. The capacity of penetration of tumor cells in vitro was in parallel with that of in vivo implantation experiment. In the in vitro invasion system, 10% fetal calf serum (FCS) was essentially required for the culture medium. It was found that FCS could be replaced with 1-oleoyl-lysophosphatidic acid (LPA). By using this model, more than 10 kinds of ginsenosides Rx were tested for the inhibition of tumor cell invasion and metastasis, and ginsenoside Rg-3 was found to be the most effective in preventing invasion of clone MM1 obtained from AH130 rat ascites hepatoma cells, several kinds of cancer cells of human origin, and mouse melanoma B16 cells. Ginsenoside Rg-3 inhibited FCS-induced invasion of clone MM1 cells by 90% and LPA-induced invasion by 99%. LPA increased intracellular Ca^{++} in MM1-AH130 cells. 20(R)-ginsenoside Rg-3 (50 μM) suppressed the increase of Ca^{++} by 95.7%, and 20(S)-ginsenoside Rg-3 by 20%. The concentration of intracellular Ca^{++} in tumor cells was significantly correlated with their capacity of invasion.

Also, Kitagawa et al. (24, 25) investigated the effect of ginsenoside Rg-3 against peritoneal metastasis of intestinal adenocarcinoma induced by azoxymethane (AOM) together with bombesin, a peptide consisting of 14 amino acids and isolated from the skin of the frog, *Bombina bombina*. AOM was subcutaneously injected to male inbred Wistar rats in a dose of 7.4 mg/kg/week for the first 10 weeks. Then, bombesin was subcutaneously injected in a dose of 40 μg/kg/day from week 11 to week 45, and ginsenoside Rg-3 in a dose of 2.5 or 5 mg/kg/day from week 20 to week 45. The result showed that ginsenoside Rg-3 inhibited bombesin-promoted cancer metastasis by 78%, though it did not affect the growth or vascularity of intestinal cancer.

Azuma and Mochizuki (26) also reported the inhibitory activity of 20(S)- and 20(R)-ginsenoside Rg-3 and ginsenoside Rb-2 against pulmonary metastasis of B16-BL6 melanoma cells in vitro and in vivo (C57 BL/6 mice, 100 μg i.v.; 1,000 μg p.o.). It is noteworthy that oral administration of 20(S)-ginsenoside Rg-3 showed the effect weaker than 20(R)-ginsenoside Rg-3 and Rb-2 in the invasion experiment, whereas 20(S)-isomer of Rg-3 showed the strongest effect (65% inhibition) in pulmonary metastasis. 20(S) Rg-3 also inhibited the tumor growth in dose-dependent fashion, whereas 20(R) Rg-3 and Rb-2 did not.

Inhibition of tumor angiogenesis

When angiogenesis is induced, metastatic tumor cells grow rapidly. Therefore, inhibition of angiogenesis prevents tumor growth, proliferation, and secondary metastasis. Inhibition of angiogenesis and suppression of invasion, motion and proliferation of tumors, and growth of endothelial cells of blood vessel, are essentially required for cancer prevention.

Azuma (27) prepared a model of endothelial cell growth inhibition by using rat lung endothelial (RLE) cells adhered to a 96 hole-tissue culture plate coated with 1% gelatin. A series of ginsenoside Rx (x=b-2, d, e, g-1, and o) were tested by incubating them for 2 days. Cell proliferation was measured after TdR was added 4 hrs before the end of incubation. Ginsenoside Rb-2 inhibited RLE cell proliferation most potently. Therefore, ginsenoside Rb-2 was administered to C57BL/6 mice, which were intracutaneously transplanted with B16-BL6 cells, intravenously (100 μg), orally (2,000 μg), or injected to their tumor site on day 1 of tumor cell transplantation. On day 4, the number of newly formed blood vessels in tumor and the size of tumor were measured after intravenous injection of 1% Evans blue solution, showing in remarkable suppression of angiogenesis in every case of administration of ginsenoside Rb-2.

Inhibitory effects of ginsenoside Rh-2 on tumor growth in nude mice bearing human ovarian cancer cells

Kikuchi et al. (28) reported that oral administration (30 μM and 120 μM/day) of a red Ginseng saponin, ginsenoside Rh-2, to nude mice transplanted with human ovarian cancer cells (HRA) resulted in remarkable inhibition of HRA proliferation. The survival of tumor bearing mice was significantly increased (124 days in the control group, 198 days in the group treated with Rh-2 (120 μM/day)), which suggested that the host-mediated immuno-potentiation of ginsenoside Rh-2 activated NK-cells.

Nakata et al. (29) reported that oral administration of ginsenoside Rh-2 (0.4-1.6 mg/kg/day) showed not only the anti-tumor activity in HRA-transplanted nude mice but also a significant increase of their survival (60 days in the control group, 85 days in the group treated with Rh-2 (0.4

mg/kg/day). It is noteworthy that the anti-tumor activity of ginsenoside Rh-2 against HRA cells is superior when orally administered than intra-peritoneally injected. Oral administration of Rh-2 induced apoptosis in tumor cells and enhanced the NK-cell activity. It is suggested that the deglycosylated final product, protopanaxadiol, may be the real active anti-tumor compound at the site of tumor.

Chinese anti-cancer drug "Rg-3 Shenyi Jiaonang"

In May 2000, a Chinese newspaper reported that a new anti-cancer drug "Rg-3 Shenyi Jiaonang" appeared on the market. It was developed by Fu and Lu (30) in Dalian, China. The yield of ginsenoside Rg-3 from red ginseng was very low, so that the increase of its yield to 400-fold was necessary to produce this drug in a manufacturing scale. Clinical application of this drug was targeted on inhibition of tumor angiogenesis by suppressing its inducer in the endothelial cells of blood vessels, and then on prevention of adhering, invasion, and metastasis of tumor cells. Judging from the results of in vitro experiments, potentiation of the immunological activities of T-cells and NK-cells is also expected. No obvious side effects or cytotoxicities have yet been observed. It is instructed that two capsules of Rg-3 Shenyi (10 mg of 95% ginsenoside Rg-3/capsule) should orally be administered twice a day before each meal. The phase II clinical study of Rg-3 Shenyi capsules in lung cancer patients was reported by Lin H-S et al. of Guang-An Men Hospital at the 4th Annual Meeting of Chinese Society of Clinical Oncology, 2000 (http://www.37c.com.on/development/progress/200101/2001010156.html).

The results of the aforementioned investigations of ginseng and ginseng saponins made it clear that ginsenoside Rh-2 and Rg-3 possess the most promising anti-cancer activities. Both the compounds are produced from red ginseng, and their glucosyl group is less than that of other ginsenoside Rx members, which might be due to deglycosylation occurred by steaming during the processing of preparing red Ginseng. It is also proved that orally administered ginsenoside Rb-1 is metabolized by intestinal bacteria to a deglycosylated product, compound K (or M), which is found in the serum. Stepwise deglycosylation of ginsenoside Rx can be chemically performed in the laboratory. In 1966, the author and coworkers carried out mild acetic acid hydrolysis of the mixture of Rb-1, Rb-2, and Rc, obtaining a fairly good yield of 3-*o* (2β-D-glucopyranosyl-β-D-glucopyranosyl) 20(S)-protopanaxadiol, which is identical with ginsenoside Rg-3 isolated later from red Ginseng (31).

ANTI-TUMORIGENIC ACTIVITIES OF OTHER TRITERPENOID COMPOUNDS

Steroid is known as the source of various biologically active compounds such as sex and adrenocortical hormones, vitamin D series compounds, cardiac glycosides, cholesterol, bile acids, and toxic steroidal alkaloids. On the other hand, triterpenoid has ring structure similar to steroid and distributed abundantly in the plant kingdom has not so far been utilized in modern medicine. Only some triterpenoid principles are known to be in medicinal plants and traditional folklore medicine.

Glycyrrhizin (GL), a sweet tasting saponin of Licorice, the root of *Glycyrrhiza* spp., has been used as the anti-allergic, anti-inflammatory, and anti-hepatitis agent, especially in Japan. Glycyrrhetinic acid (GA), the sapogenin of GL, has also been used as the anti-inflammatory and anti-gastric ulcer agent. The anti-tumorigenic activity of GA (32) was investigated, since the skin-inflammatory action seemed to be involved in tumor promotion. There are two stages in the course of carcinogenesis: initiation and promotion. As the initiator for the experimental skin tumor system, 7,12-dimethylbenz[a]anthracene (DMBA) was applied to dorsal area of mice in a single dose that is under the threshold dosage for carcinogenesis. After some interval, 12-*o*-tetradecanoylphorbol 13-acetate (TPA), obtained from croton seed, was applied as the promoter to the same area by painting repeatedly in a very low dose (10^{-9}-10^{-8} M) for 18-20 weeks. Within a few weeks, formation of papilloma was ob-served in mice. Since the tumor promoting agents are mostly irritating compounds, naturally occurring anti-inflammatory agents were tested in vitro against TPA-promoted phospholipid metabolism. Incorporation of ^{32}Pi into phospholipids of HeLa cells was increased 4-fold by TPA, and GA (100 μM) inhibited the increase by 50% (32). Screening several other triterpenoid compounds was also carried out to investigate their anti-cancer activities. Oleanolic acid and hederagenin are the most abundantly distributed plant sapogenin in nature. By using these compounds as the starting materials, several chemically modified derivatives were prepared after a few steps of chemical reactions in prospects of increasing biological potency (Table 5) (33). In vivo test, the time course

Table 5. Effects of olenane type triterpenes on the enhanced ^{32}Pi-incorporation into phospholipids of HeLa cells induced by TPA

Compound	Inhibition (%)
18β-Oleanolic acid	48.0
18β-Hederagenin	72.6
18β-Erythrodiol (18β-Olean-12-ene-3β, 28-diol)	81.1
18β-Olean-12-ene-3β,23,28-triol	100.0
18α-Olean-12-ene-3β,28-diol	100.0
18α-Olean-12-ene-3β,13,28-triol	100.0
18α-Olean-12-ene-3β,30-diol	81.3

HeLa cells were incubated with compound (25 μg/mL) and after 1 hr ^{32}Pi (20 μCi/culture) was added with or without TPA (50 nM). Incubation was continued for 4 hrs and then radioactivity incorporated into phospholipid fraction was measured.

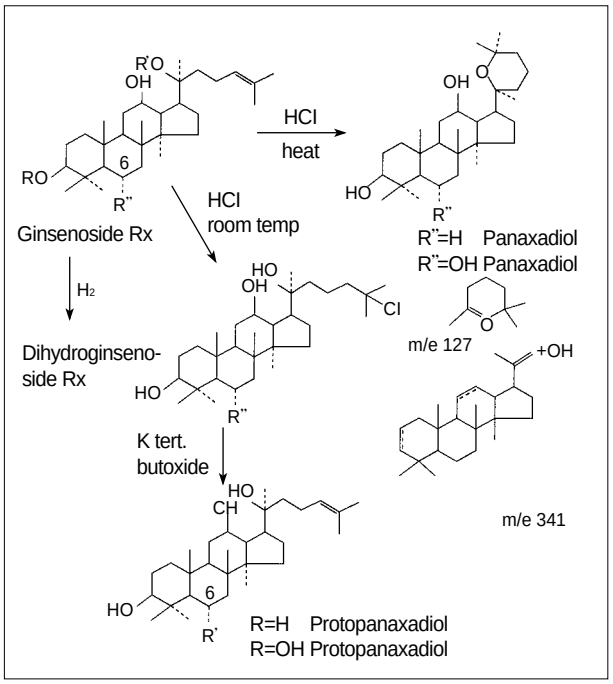


Fig. 1. Sapogenin and genuine sapogenin of Ginsenoside Rx.

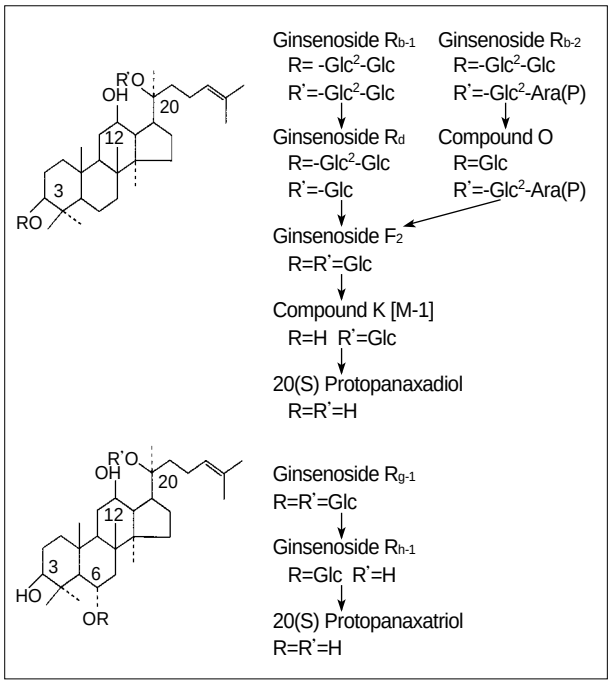


Fig. 2. Metabolic transformation of ginsenoside R_{b-1}, R_{b-2} and R_{g-1} by the intestinal bacteria.

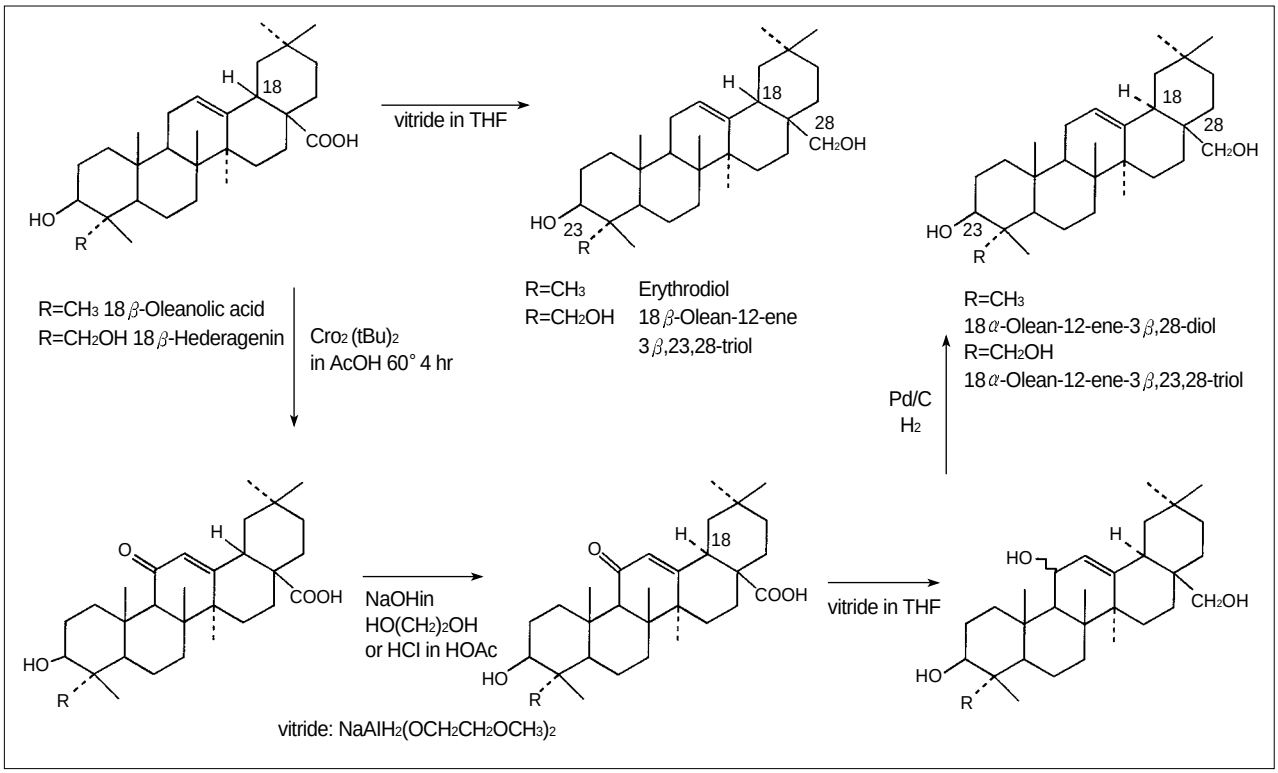


Fig. 3. Chemical conversion starting from oleanolic acid and hederagenin.

of skin tumor formation was observed in the group treated with DMBA/TPA with or without 18β- or 18α-olean-12-ene-3β,23,28-triol (Fig. 4).

Oral administration of triterpenoid compounds also demon-

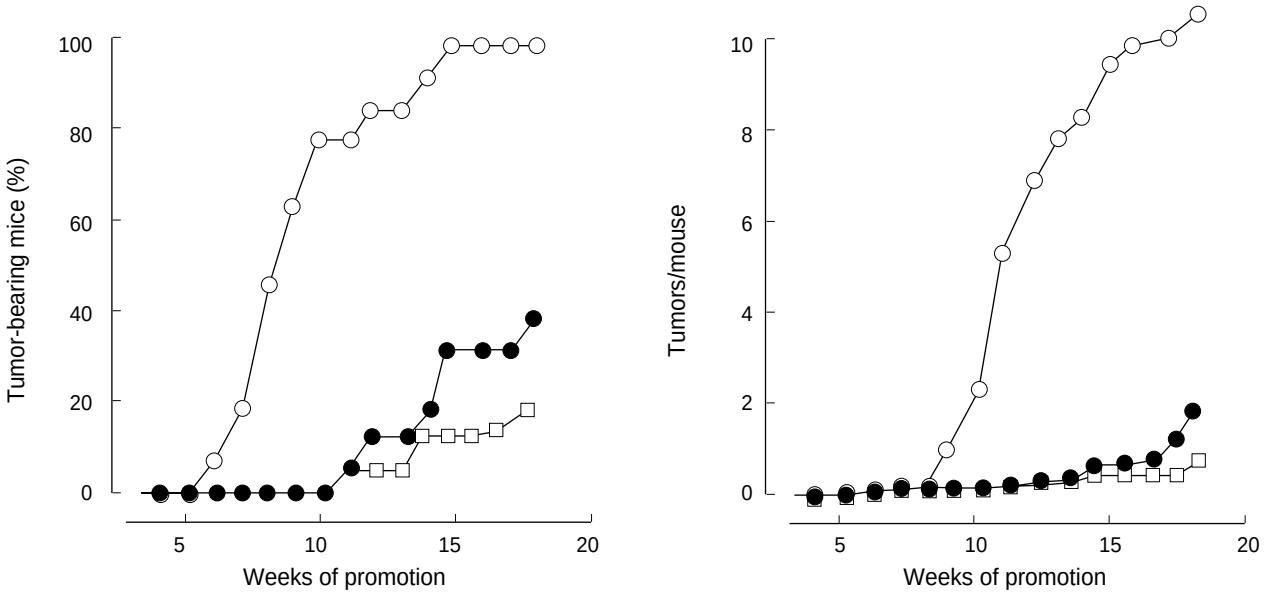


Fig. 4. Effect of triterpenoid compounds on the promotion of skin tumor formation by TPA in DMBA-initiated mice.
From 1 week after initiation by a single application of 100 μg of DMBA, 0.5 μg (0.81 nM) of TPA was applied twice a week.
The samples (81 nM) (molar ratio to TPA 100:1) was applied topically 40 min before each TPA application.
-○- group treated with DMBA+TPA
-●- group treated with DMBA+TPA+18β-Olean-12-ene-3β,23,28-triol
-□- group treated with DMBA+TPA+18α-Olean-12-ene-3β,23,28-triol

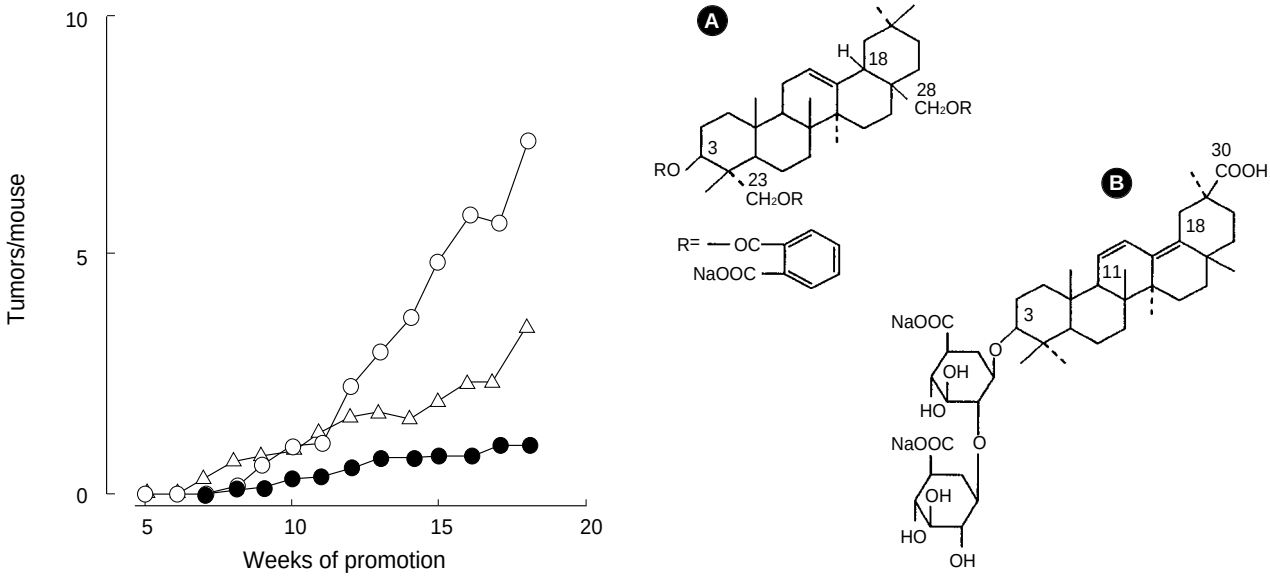


Fig. 5. Effect of per os administration of tri-terpenoid compounds on the promotion of skin tumor formation by TPA in DMBA-initiated mice.
-○- group treated with DMBA+TPA
-●- group treated with DMBA+TPA+18β-Olean-12-ene-3β,23,28-triol tri-O-hemiphthalate sodium
-△- group treated with DMBA+TPA+18β-Olean-11,13(18)-diene-3β,-ol-30-oic aicd 3-O-β-D-glucuropyranosyl(1→2)β-D-giucuronopyranoside sodium

strated the anti-tumor-promoting effects (34). 18β-Olean-12-ene-3β, 23-28-triol trihemiphthalate sodium (A) and olean-11,13(18)-diene-3β-ol-30-oic acid 3,O-β-D-glucuronopyranosyl (1→2) β-D-glucuronopyranoside sodium (B) were dissolved in water at the concentration of 0.5 g/1.2 L and given to mice ad libitum during the whole period of promotion stage. A single dose of DMBA (100 μg) was applied to dorsal area of 8-week-old female ICR mice to initi-

ate skin carcinogenesis. TPA (0.5 μg/painting) was applied to the same area twice a week for 18 weeks. The number of tumors in mice in the control group and in the treated group was counted every week (Fig. 5).

Another combination of initiator and promoter against other types of cancer was tested in the two-stage experiment. For lung cancer, the initiator was 4-nitro-quinoline N-oxide (4NQO) and the promoter was glycerol (5% in drinking water). The test compound was dissolved in drinking water (0.25 mg/mL). As mice drank 6-10 mL/day ad libitum, the total amount of the test compound was 1.5-2.5 mg/day. In this experiment, 18α-olean-12-ene-3β,23,28-triol trihemiphthalate sodium significantly inhibited formation of lung cancer in mice at week 18. Eighty five percent of mice in the control group bore lung tumors and 70% in the treated group. The mean number of tumors per mouse was 4.4 in the control group and 1.3 in the triterpenoid-treated group ($p<0.01$) (35).

Konoshima et al. (36) reported the anti-tumorigenic activity of ginsenoside Rg-1 isolated from the root of *Panax notoginseng* Burk. (San-chi Ginseng). By using Epstein-Barr virus activated by TPA, the in vitro inhibitory activity of Rg-1 was tested. In the experiments of DMBA-TPA-induced mice skin papilloma, 4NQO-glycerol-induced mice pulmonary tumors and N-nitrosodiethylamine (DEN)-phenobarbital-induced hepatic hyperplasia in mice, and topical application (for skin papilloma) or oral administration (for pulmonary and hepatic tumors) of Rg-1 showed the in vivo inhibitory effects.

Anti-tumor activities are the properties characteristic not only to Ginseng saponins but also to other pentacyclic triterpenes. It is noteworthy, however, that ginsenosides, tetracyclic dammarane-type triterpenoid saponins, could be applied safely due to their mostly non-toxic and non-hemolytic characteristics. Ginseng has been employed in Chinese medicine and folklore medicine in the Eastern Asia regions without any noticeable side effects for more than 2000 yr. Apart from triterpenoid saponins in ginseng, acetylenic alcohol obtained from Ginseng, especially panaxytriol, showed anti-cancer activity against melanoma B16 when intramuscularly administered (37).

ACKNOWLEDGEMENTS

I wish to thank my collaborators, Drs. O. Tanaka, J. Shoji, I. Kitagawa, and many other younger coworkers devoted to the chemical studies of ginseng, and its saponins and sapogenins at our laboratory in Department of Pharmacognosy and Phytochemistry, University of Tokyo, during the period of 1960-1976. Some of them are still continuing the study of ginseng at their own institutions after leaving my laboratory. For pharmacological studies of Ginseng, I am grateful to my colleagues, Dr. K. Takagi and Dr. H. Saito. For the recent anti-tumorigenic and cancer prevention studies of Ginseng saponins, their derivatives, and other triterpenoids, I am deeply indebted to Dr. H. Nishino and his coworkers in Kyoto Prefectural University of Medicine. I am also thankful to Mr. K. Hirabayashi, Drs. S. Iwata, H. Inoue, H. Matsumoto and T. Sato in Research Laboratory of Minophagen Pharmaceutical Co., Ltd.

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