**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...
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).

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

| Saponin Type       | R    | R’  |
|--------------------|------|-----|
| 20(S) Protopanaxadiol-type |
| Ginsenoside        | Rb-1 | Glc2-Glc |
|                    | Rb-2 | Glc2-Glc |
|                    | Rb-3 | Glc2-Glc |
|                    | Rb-4 | Glc2-Glc |
| Notoginsenoside    | R1   | Xyl  |
|                    | R2   | Glc  |

Malonyl Glc2-Glc-OOC-CH2-COOH, (p): pyranosyl, (f): furanosyl

| Saponin Type       | R    | R’  |
|--------------------|------|-----|
| 20(S) Protopanaxatriol-type |
| Ginsenoside        | Rb   | Rha |
|                    | R1   | Glc |
|                    | Rb-1 | H   |
|                    | Rb-2 | Rha |
| Notoginsenoside    | R1   | Xyl |
|                    | R2   | Glc |

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

Ginsenoside R1 = Chikusetsusaponin V

### 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 Sen-shu-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 was proven 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, protopanaxadiol 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)12-hydroxy- and 20(S)6,12-dihydroxy-dammarenediol-II, respectively.

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

| Saponin Type          | White Ginseng | Red Ginseng | American Ginseng | San-Chi Ginseng |
|-----------------------|---------------|-------------|------------------|-----------------|
| 20(s) Protopanaxadiol-type |               |             |                  |                 |
| Ginsenoside Rb-1      | 0.5           | 0.4         | 1.8              | 1.8             |
| Rb-2                  | 0.2           | 0.2         | 0.03             | +               |
| Rb-3                  | 0.3           | 0.1         | 0.3              | +               |
| Rc                    | 0.2           | 0.036       | 0.5              | 0.2             |
| Rb-3                  | 0.0003        | 0.014       | (20R)            | –               |
|                       |               | 0.015       | (20S)            | –               |
| Rb-1                  | –             | 0.001       | –                | –               |
| 20(s) Protopanaxatriol-type |             |             |                  |                 |
| Ginsenoside Rb-1      | 0.2           | 0.2         | 1.0              | 0.2             |
| Rb-2                  | 0.05          | 0.07        | –                | –               |
| Rb-1                  | 0.2           | 0.3         | 1.9              | 0.2             |
| Rb-2                  | 0.014         | 0.01 (20R)  | 0.008            | 0.03            |
|                       |               | 0.02 (20S)  |                  |                 |
| Rb-1                  | 0.0015        | 0.007       | (20R)            | 0.01            |
|                       |               | 0.006       | (20S)            |                 |
| Oleanane-Type         |               |             |                  |                 |
| Ginsenoside Rb-1      | 0.02          | 0.045       | 0.07             | –               |

### Table 3. Pharmacological actions of ginsenosides

| Ginsenoside | Anti-platelet aggregation | Fibrinolytic action | Stimulation of phagocytic action | Vasodilating action | Cholesterol and neutral lipid decreasing and HDL cholesterol increasing effects | Stimulation of ACTH, corticosterone secretion | Stimulation of RNA polymerase, protein synthesis | Protection against ulcer induced by stress | Recovering from disturbed sleep induced by stress |
|-------------|---------------------------|---------------------|----------------------------------|--------------------|--------------------------------------------------------------------------------|-----------------------------------------------|------------------------------------------------|-----------------------------------------------|------------------------------------------------|
| Rb-1        | Rb-1                      | Rb-1, Rb-2          | Rb-1, Rb-2, Rb-3, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb-3, Rb-2, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb, Rb-1, Rb-2 |
| Rb-2        | Rb-2                      | Rb-2, Rb-3, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb-3, Rb-2, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb, Rb-1, Rb-2 | Rb-1, Rb-2, Rb, Rb-1, Rb-2 |

### Table 4. Pharmacological actions of ginsenosides

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.
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 Kanaka 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, and compound K (16). In the same experimental process, ginsenoside Rg-1 was converted into protopanaxaxtriol 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 *Provetella 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 *Fuscobacterium* 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 B1 were respectively injected to the subcapsular 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 B1 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 tested for the inhibition of tumor cell invasion and metastasis of clone MM1-AH130 cells. 20(R)-ginsenoside Rh-1 showed the strongest effect (95.7%), and 20(S)-ginsenoside Rg-3 by 20%. The concentration of intracellular Ca$^{++}$ 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
yield of 3-
coworkers carried out mild acetic acid hydrolysis of the
cally performed in the laboratory. In 1966, the author and
product, compound K (or M), which is found in the serum.
is metabolized by intestinal bacteria to a deglycosylated
It is also proved that orally administered ginsenoside Rb-1
members, which might be due to deglycosylation occurred
their glucosyl group is less than that of other ginsenoside Rx
Both the compounds are produced from red ginseng, and
ginsenoside Rg-3 possess the most promising anti-cancer activities.
and ginseng saponins made it clear that ginsenoside Rh-2
sary 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,
metastasis of tumor cells. Judging from the
results of in vitro experiments, potentiation of the immuno-
logical activities of T-cells and NK-cells is also expected. No
obvious side effects or cytotoxocities 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.37.c.com.on/development/progress/200101/20010101
56.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 chemi-
ically 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
(2β-D-glucopyranosyl-3β-D-glucopyranosyl)
20(S)-protopanaxadiol, which is identical with ginsenoside
Rg-3 isolated later from red Ginseng (31).

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 neces-

Anti-Tumorigenic Activities of Other
Triterpenoid Compounds
Steroid is known as the source of various biologically active
compounds such as sex and adrenocortical hormones, vita-

Table 5. Effects of olenane type triterpenes on the enhanced
32P-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          |
| 18β-Olean-12-ene-3β,28-diol     | 100.0          |
| 18γ-Olean-12-ene-3β,13,28-triol | 100.0          |
| 18γ-Olean-12-ene-3β,30-diol     | 81.3           |

HeLa cells were incubated with compound (25 μg/mL) and after 1 hr
32P (20 μCl/culture) was added with or without TPA (50 nM). Incuba-
tion was continued for 4 hrs and then radioactivity incorporated into
phospholipid fraction was measured.
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-
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-

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+18β-Olean-12-ene-3β,23,28-triol tri-O-hemiphthalate sodium

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
are 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 trinemiphosphatate 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, tetra-cyclic 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 for pharmacological 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. Marumoto and T. Sato in Research Laboratory of Minophagen Pharmaceutical Co., Ltd.

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