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REISHI, *GANODERMA LUCIDUM* AND *GANODERMA TSUGAE*: BIOACTIVE SUBSTANCES AND MEDICINAL EFFECTS

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Reishi, *Ganoderma* sp., has been known in Japan, China, and other countries as a food and raw material for the development of drugs. Recent studies have shown that the carcinostatic substance in Reishi is a polysaccharide, β-(1→3)-D-glucan. This polysaccharide seems to haye promise as a new type of carcinostatic agent which might be useful in immunotherapy. Unlike chemicals used in chemotherapy, it has few toxic side effects because its effect is based on immunological enhancement in the host. Reishi also seems to contain other substances which reduce blood pressure, blood cholesterol, and blood sugar levels; and inhibit platelet aggregation, etc. Some lanostane triterpenoids have been isolated from *Ganoderma lucidum* and *G. applanatum*, and drimane sesquiter-

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penoids from *Cryptoporus volvatus*. These are highly oxidized compounds which show interesting biological activities.

ORIGIN AND EFFECT OF THE REISHI

Mannentake (*Ganoderma lucidum* Fr. Krast) is a species of basidiomycetes which belongs to Polyporaceae (or Ganodermaceae) of Aphyllophorales. Its fruiting body is called "Reishi" in Japanese. In China it is called "Lingzhi" and is divided into four families: Ganoderma, Haddowia, Amauroderma, and Humphreya. These are further classified into 86 species (32, 33).

Reishi has other names, such as Mannentake, Saiwaitake, Sakikusa, Saegusa, Kamishiba, Gyokurai, Kisshotake, Sankei, Fushiso, and Zuishi. It is seldom found in the wild. In fact, in ancient times, a person who found this mushroom in the field or mountain and presented it to the Imperial Court was well rewarded For centuries, this mushroom has been regarded in the Orient as an effective medicinal. The virtues of its extracts have been handed down from generation to generation. It has been highly valued as a "cancer cure."

Recent studies (1, 2) have revealed that the carcinostatic substance in Reishi is a polysaccharide, β-(1→3)-D-glucan. This substance appears to act as a new type of a carcinostatic agent (immunotherapy) because its effect is based on enhancement of the host's immune system. Unlike general carcinostatic agents (chemical therapy), it appears to be nontoxic. In addition, Reishi contains other substances which may enhance various functions of homeostasis and physis, such as reduction of blood pressure and blood sugar level, elimination of cholesterol, antithrombotic reaction (platelet aggregation inhibiting activity), hepatitis healing, and robustness (3, 4).

Reishi, because of its perceived health benefits, has gained wide popularity as a health food, in both Japan and China. Even if the same strain of Mannentake is used, its fruiting body (Reishi) will exhibit delicate changes in shape, luster and color, flesh quality, bitterness, etc., depending on culture conditions, such as temperature, light, moisture, carbon dioxide concentration, and the production site. Such fruiting bodies are divided into Seishi (blue), Sekishi (red), Ohshi (yellow), Hakushi (white), Shishi (violet), and Kokushi (black) according to their luster and color; or into Rokkakushi (deer antler Reishi), Gyukakushi (ox horn Reishi), Unshi (cloud-like Reishi), and Nikushi (meat-like Reishi) based on their shape. The quality and contents of these varieties vary from one another, and it is rather difficult to evaluate and judge based only on analytical data of a "representative" sample.

ARTIFICIAL CULTIVATION OF REISHI

Artificial culture and cultivation of Mannentake were attempted initially by T. Henmi et al., in 1937. Its mass production was first achieved by Y. Naoi in

1971 by cultivating the spawn using pots containing sawdust. Since then use of bed logs or sawdust has become established practice. For some purposes a bagasse spawn bed method and bottle or bag cultivation may be used. Usually, for mass cultivation of high-quality Reishi, either outdoors or indoors, bed logs of white oak (*Quercus monogolica*), oak (*Quercu acutissma*), Japanese chestnut (*Castanea* sp.), Japanese apricot (*Prunus mume*), etc., are used.

The 1988 production of Reishi in Japan was estimated to be about 250 tons dry weight. Reishi cultivation has also prospered in China, Taiwan, Korea, and Thailand. In addition, attempts are being made to obtain useful cellular materials or to produce effective substances from cultured mycelia.

PHARMACOLOGICALLY ACTIVE COMPONENTS IN REISHI

General Chemical Components in Reishi

The major components and the free amino acids in Mannentake fruiting body (Reishi) have been determined (5). Both qualitative and quantitative differences are found in the composition of naturally grown and artificially cultivated samples of Reishi, depending on the lines, places of the production, cultivation conditions, etc.

Medicinal Effects of Reishi Extract with Hot Water

The hot water extract (ca. 10% dry matter) obtained from Reishi seems to have some medicinal properties. Little could be done to isolate the pure substances having these medicinal properties until recently, when the culture and cultivation of Mannentake became possible. The results of this research are described below. Antitumor substances whose properties have been studied in depth are described in detail in the article Antitumor-Active Substances from Mushrooms.

Bitter Terpenoids

The fruiting body of Mannentake (Reishi) is extremely bitter, a characteristic not found in any other mushroom. The bitterness varies in degree depending on the place of production, cultivation conditions, its strain, etc. This bitterness is not found in cultured mycelia or substances produced in the culture medium, and Kokushi (black Reishi) does not contain the bitter substance. Though the relationship between bitterness and pharmacological effects is not fully understood, the bitterness serves as a marker for pharmacological evaluation and classification of *Ganoderma* sp. (3–12).

The bitter components and related compounds, highly oxidized lanostane triterpenoids, have been isolated from the extracts of Reishi by various chromatographic procedures (6, 7), and their bitterness was evaluated. Some other triterpenoids isolated from Mannentake have been studied for their antiallergy action (11), antiandrogen action (12), antihypertensive action (12), etc.

Steroids

Ergosterol (provitamin D_2) has been reported in concentrations of 0.3–0.4% in Reishi (13). However, further analysis has confirmed that the main component of the steroid fraction is 24-methylcholesta-7,22-dien-3-β-ol. Ergosterol and 24-methylcholest-7-en-3-β-ol are subcomponents. Recently, ganodesterone has also been isolated.

Nucleotides

Like other mushrooms, Reishi contains adenosine, 5′-GMP, 5′-XMP, RNA, etc., components which contribute to umami (14). It has recently been found that nucleosides which contain adenosine and guanosine, found in the water/alcohol extract of Reishi, possess a platelet aggregation inhibition action (antithrombotic activity) (26).

Hypoglycemic Proteoglycans

High hypoglycemic activity both in normal mice and in alloxan-induced hyperglycemic mice has been induced by two polysaccharide–protein complexes, Ganoderan B and C, by their i.p. administration to male mice of Std:ddY strain. These complexes were obtained by adding ethanol to a hot water extract of Reishi and separating the precipitated polymer substances by column chromatography (15).

Ganoderan B is a glucan–protein (55.4:44.4 w/w), with $[\alpha]_D$ $-25.8°$ and molecular weight 400,000.

Ganoderan C is a galactoglucan–protein (72.5:25.5 w/w), with its $[\alpha]_D$ $-20.1°$ and molecular weight 400,000. Polysaccharides of both complexes were chiefly composed of β-(1→6)- and β-(1→3)-glucan chains.

We have isolated, from Reishi fractions of water-soluble polysaccharides, a 3% ammonium-oxalate-soluble heteropolysaccharide, and a 5% NaOH-soluble peptidoglycan. These were further separated into several fractions by various chromatographic methods. Strong antitumor activity (1) and hypoglycemic activity were found (15) in certain fractions of the heteropolysaccharides.

No correlation was found between the antitumor activity of these active peptidoglycans and their antihyperglycemic activity or between the ratio composition of polysaccharides and proteins in the complex. Further research is needed to determine the source of these two activities.

Blood Pressure Stabilizing Components

Reishi has been assumed to have both hypotensive and hypertensive properties (homeostasis) (12, 16–18). A peptidoglycan (molecular weight, 100,000) having a mild hypotensive effect on Wister rats and SHR rats (congenitally hypertensive) has been isolated from a hot water extract of Reishi. According to one report, the blood pressure of about half the patients with hypertension was reduced when a Reishi extract was administered. It has been reported that a hypertension-related angiotensin-I-converting enzyme was inhibited by ganoderic acids (B, D, F, H, R, S, and Y), ganoderal A, and ganoderol A and B.

Germanium (Ge) Component

Crude drugs obtained from ginseng, a polyporaceae, treasured as an Oriental medicinal have high Ge content (19). The ability to concentrate Ge was confirmed in Mannentake (20). Recently, a polysaccharide β-(1→3)-D-glucan and its protein complex with high antitumor activity have been isolated from Mannentake. Correlation between this antitumor activity (interferon-inducing activity) and Ge contents is also of interest because Ge is said to neutralize pain during the final stages of cancer.

We have analyzed the Ge content and mineral composition in natural and cultivated mushroom samples of Polyporales and cultivated Reishi collected at various sites in Japan, by means of an inductively coupled plasma analysis (ICP method). The Ge concentrations in the samples analyzed were low (10 ~ 100 ppb; average, 50 ppb). None of the samples contained high Ge concentrations as previously reported (10 ~ 4000 ppm). However, it has been shown that the Ge content in Reishi was significantly higher when cultivated using a bed log which had absorbed organic Ge (Ge-132).

Lectins

The term "Lectin" (from the Latin "legere," to pick up, choose) is defined as a carbohydrate–protein of nonimmune origin which agglutinates cells or precipitates polysaccharides or glycoconjugates (21). Many species of animals, plants, and microorganisms contain lectins. However, relatively few studies have been conducted on lectins found in mushrooms (22–24).

Investigation of lectins from *Ganoderma lucidum* are now being carried out at our laboratory. We have isolated lectins from the fruiting body and the mycelium of this fungus (25). The two lectins have different molecular weights and amino acid compositions. This mycelium-lectin is the first isolated from other than the fruiting body of higher fungi.

Antithrombotic Compounds

Some active compounds have been isolated from mushrooms as platelet aggregation inhibitors (31). We have also isolated and identified adenosin, guanosin, and their derivatives as potent inhibitors from the 80% ethanol extract of Reishi (31).

ANTITUMOR SUBSTANCES IN REISHI

Cytotoxic Terpenoids

Mannentake (fruiting body and mycelium) contains various low molecular weight components. Monosaccharides, sugar alcohols, oligosaccharides, amino acids, organic acids, steroids, lipids, terpenoids (9), coumarin, tannin, etc., are among the low molecular weight components extracted with water/alcohol, water/acetone, water, etc.

It has been reported that triterpenoids isolated from cultured mycelia of Mannentake (ganoderic acid-R, -T, -U, -V, -W, -X, -Y, and -Z) showed a cytotoxicity-based carcinostatic effect on hepatoma cells *in vitro*.

Antitumor Polysaccharides

Among polymer components in Reishi, many polysaccharides were extracted with hot water, ammonium oxalate solution, alkali solution, dimethyl sulfoxide (DMSO) solution, etc., and separated by various chromatographic methods. These fractions were used for the screening of host-mediated antitumor activities (BRM substances and immunotherapeutic agents) using Sarcoma 180/mice, i.p. or p.o. method. Strong antitumor activities were found in various hetero-β-D-glucans having a β-(1→3)-D-glucan chain as the active site, such as β-D-glucan, glucurono-β-D-glucan, arabinoxylo-β-D-glucan, xylo-β-D-glucan, manno-β-D-glucan, and xylomanno-β-D-glucan, as well as in their protein complexes. These polysaccharides, especially in Reishi, will be examined for their possible use as new antitumor agents (immunity enhancers) because they have no major side effects.

In addition, polysaccharides having immunomodulated antitumor activities or anti-inflammatory functions are contained not only in water-soluble β-D-glucans but also in hemicellulose (the so-called dietary fiber), which is water insoluble. The polysaccharides present in hemicellulose can be extracted with alkali or DMSO in high yield.

In addition to these active polysaccharides, many other polysaccharides have been isolated or reported to exist, such as α-(1→6); α-(1→4)-D-glucan (glycogen-like polysaccharide), fucogalactan, mannofucogalactan, fucoxylomannan, and xylomannoarabino-galactan. None of these showed antitumor activity.

Because these nonactive polysaccharides exist together with the active β-D-glucans in Reishi, it may be that they are concerned with solubility, protection and digestion, and absorption of the active glucans.

Like the other mushrooms, cell wall polysaccharides of Reishi are composed not of cellulose but chiefly of chitin and β-D-glucans. The chitin (fungal chitin) is basically the same as the animal chitin in shrimp, crabs, insects, etc. Mushroom chitin and chitosan are water insoluble. When *N*-acetylchito-oligosaccharides (DP, 2-8) and chito-oligosaccharides (DP, 2-8) obtained by hydrolyzing chitins and chitosans with an acid or an enzyme were analyzed in the Sarcoma 180/mice, i.p. method, no antitumor activity was found (26).

Extracellular Polysaccharides of Mannentake, *G. lucidum*

Polysaccharides were produced extracellularly when Mannentake mycelia were cultured with shaking in a liquid medium (pH 6.8) containing monosaccharides (glucose, galactose, mannose, xylose, etc.) and disaccharides (sucrose, maltose and lactose) as the carbon source (5%), malt extracts (0.4%), yeast extracts (0.1%), and mineral salts (2).

Dried polysaccharides thus obtained were separated into water-insoluble (47%) and -soluble (53%) fractions. The water-insoluble polysaccharide fraction contained a β-(1$\rightarrow$3)-D-glucan having β-(1$\rightarrow$6) branches (branching ratio, 1:27). When this glucan was administered to mice (10 mg/kg $\times$ 10, i.p.) it showed high antitumor activity, with the suppression ratio percentage of tumor proliferation being 92% and the complete regression ratio being 4/6. The antitumor activity of this glucan was improved by conversion to the polyol polysaccharide (5 mg/kg $\times$ 10, i.p. administration showed the suppression ratio of 97% and the regression ratio of 5/7). The water-soluble fraction contained a heteroglucan composed of glucose, mannose, and galactose (1.0:0.5:0.13 in molar ratio) having no antitumor activity.

ANTITUMOR-ACTIVE POLYSACCHARIDES OF THE FRUITING BODIES AND MYCELIA OF *GANODERMA TSUGAE* FUNGI

Antitumor-Active Polysaccharides from Chinese Mushroom "Songshan Lingzhi," the Fruiting Body of *Ganoderma Tsugae*

A systematic method of extraction, fractionation, and purification of polysaccharides with antitumor activity from "Songshan Lingzhi" (*Ganoderma tsugae*) was established; see Figures 1–3. Seven glycans with strong antitumor activities were obtained from 14 water-soluble and 15 water-insoluble fractions. They were FI_0-a, FA-1, FII-1, FIII-2, and FII-2-a, -b, and -c. FI_0-a and FA-1 were protein-containing glucogalactans associated with mannose and fucose. FII-1 was (1$\rightarrow$3)-β-D-glucan with lower protein content. The water-insoluble FIII-2-a,

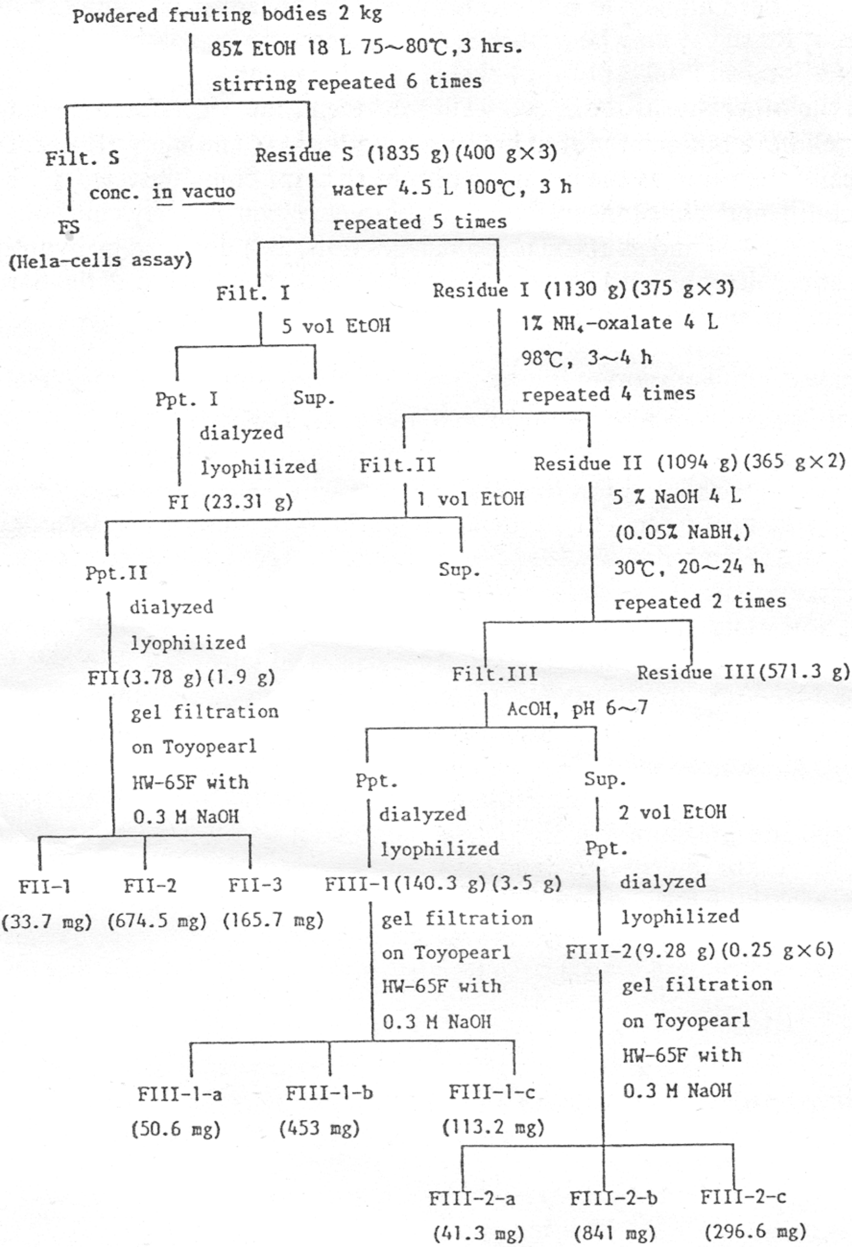

Figure 1. Fractionation of antitumor polysaccharides from the fruiting bodies of *Ganoderma tsugae*.

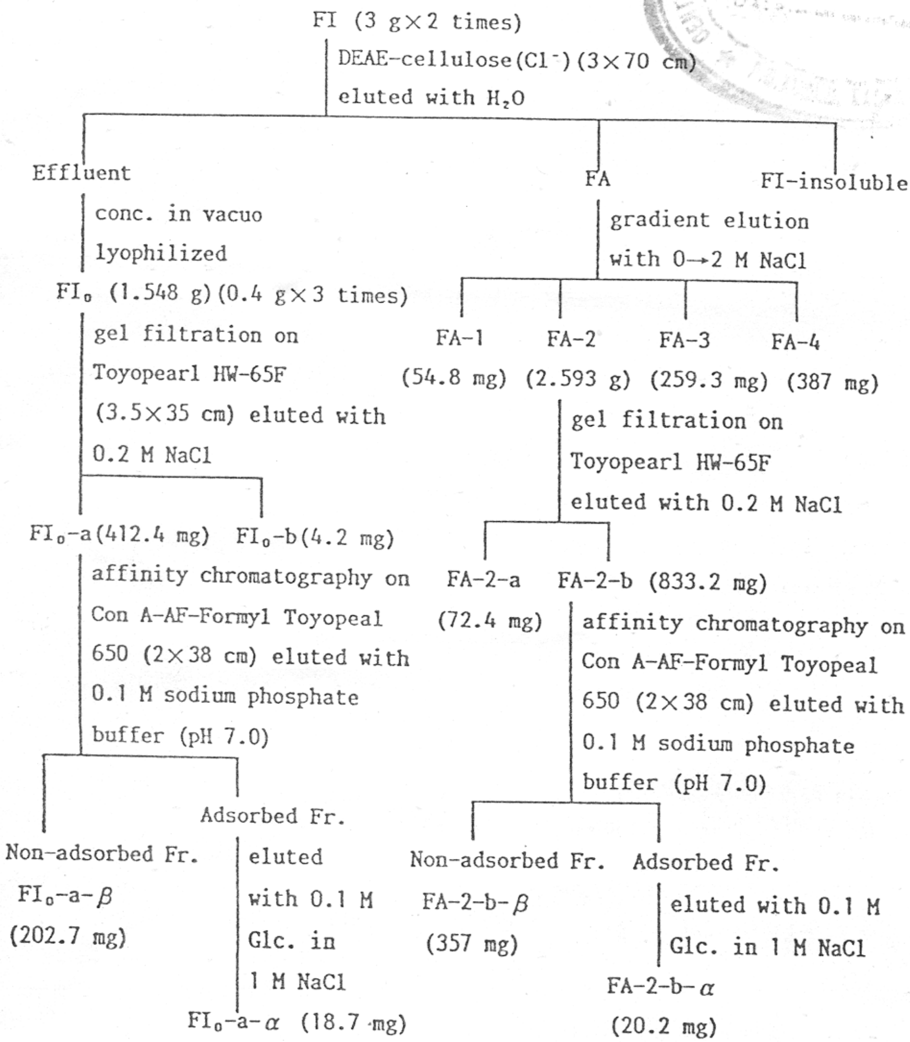

Figure 2. Fractionation of water-soluble polysaccharides (FI) from the fruiting bodies of *Ganoderma tsugae.*

-b, and -c extracted with alkali showed the strongest activity; they were a protein-containing (1→3)-β-D-glucan. Chemical properties and structure of each antitumor active polysaccharide was compared with that of three fungi of the *Ganoderma* family, namely, “Kofukitake” (*G. applanatum*), “Mannentake” (*G. lucidum*), and “Songshan Lingzhi” (*G. tsugae*).

The fruiting body of “Mannentake” (*Ganoderma lucidum*), a medicinal fungus belonging to the Polyporaceae family, is known as “Reishi.” Reishi assumes

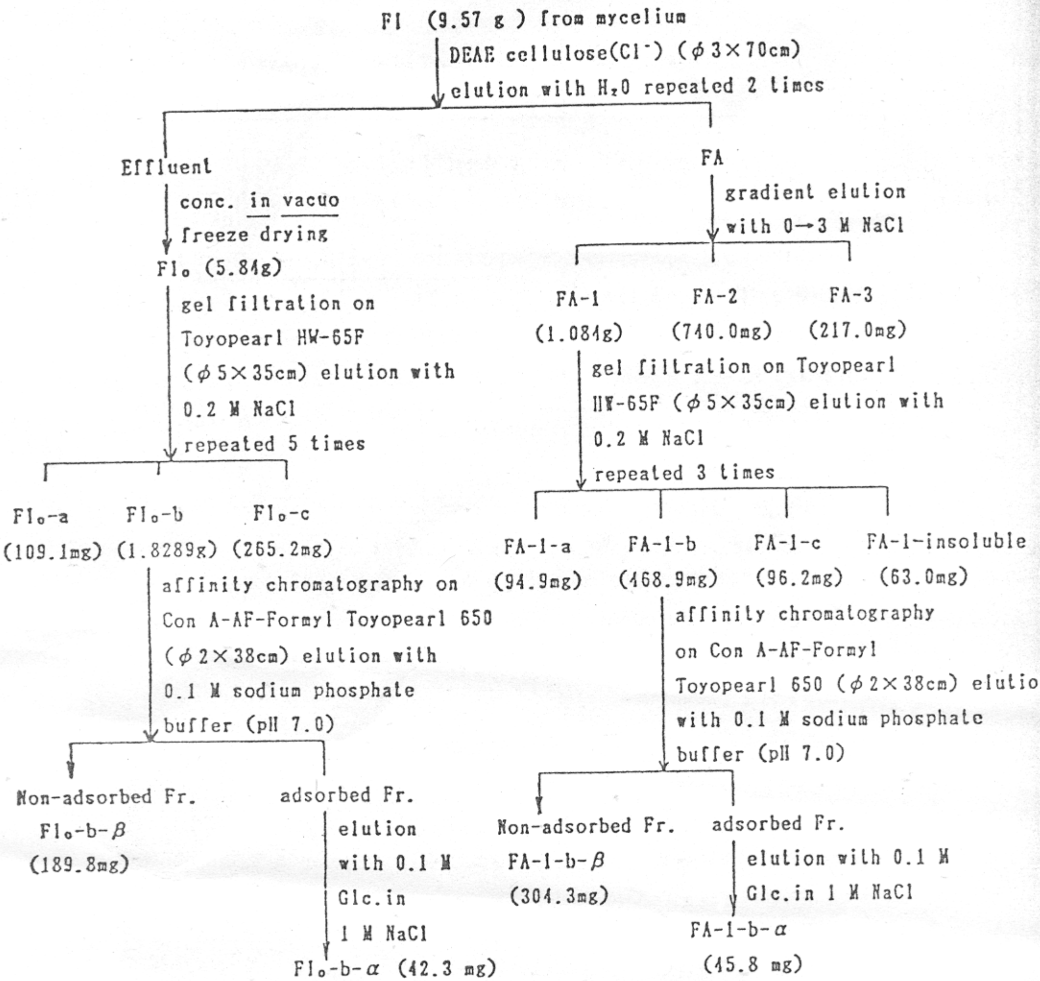

Figure 3. Fractionation of antitumor polysaccharides (FI) from mycelia of *Ganoderma tsugae*.

different colors and shapes depending on growing conditions. How these differences in taxonomy affect the chemical and pharmacological properties is not known. Recently, attempts have been made to classify them by differences in their chemical components and enzyme activities as well as by their morphological differences.

We have fractionated and purified various polysaccharides obtained from "Mannentake" (*Ganoderma lucidum*) (34–36) and "Kofukisarunokoshikake," "Juzetsu" ("tree tongue") (*G. applanatum*) (37), and have reported on their antitumor activities.

In this section, we report on the antitumor activities of the polysaccharides fractionated from the fruiting body of "Songshan Lingzhi" (*G. tsugae*) grown in China.

1. Extraction, fractionation, and yield of fruiting body polysaccharides: The yield of low molecular components (FS) from the dried fruiting body was 8.25% and that of water soluble polysaccharide FI was 1.94%, while that of FII extracted with 1% ammonium oxalate solution was as little as 0.34%. The yield of FIII extracted with 5% sodium hydroxide was 20.49%. Of FIII-1 precipitated in pH 6 with acetic acid, 9.22% was obtained from FIII, and 1.27% of FIII-2 precipitated with 2 volumes of ethanol was obtained from FIII.

2. Antitumor activity of polysaccharides: Water-soluble polysaccharides were fractionated into neutral and acidic polysaccharides by ion-exchange chromatography. The antitumor effect was markedly apparent with FI_0-a and FA-1 fractions. FI_0-a was further fractionated into FI_0-a-α and -β by affinity chromatography; however, these fractions did not show increase in activity. The higher molecular fraction FII-1, obtained from FII extracted with 1% ammonium oxalate solution by gel filtration, showed especially high activity. The highest-active fraction, FIII-2-a, was purified by gel filtration from FIII-2.

3. Physicochemical properties and structural features of antitumor polysaccharides: For FI_0-a, the tumor inhibition ratio was 95.5% and the life prolongation ratio was 209.9%. This polysaccharide fraction contains mainly galactose and glucose (1.72:1.00 molar ratio), with mannose and fucose as minor components. Protein content was 17.5%; it is a protein-containing glucogalactan. IR and NMR analyses showed a glucan chain of FI_0-a consisting mainly of $(1\rightarrow3)$-β-D-glucosidic linkages.

With FA-1 the favorable antitumor effect was evidenced by the inhibition ratio 89.9%, life prolongation ratio 248.0%, complete regression ratio 3/5, and mortality 2/5. FI_0-a is composed of polysaccharide and protein at 56.7:29.0 w/w. Its constituent sugars are Gal:Glc:Ara:Rha:Man:Fuc, present in molar ratios of 4.20:1.00:0.49:0.28:0.24:0.22. The average molecular weight is about 8000.

FII-1 is a $(1\rightarrow3)$-β-D-glucan with $(1\rightarrow)$-β-D-glucosyl-branched chains, molecular weight 110,000, $[\alpha]_D$ +8.8° (c = 0.5, 10% NaOH). Protein content was low, 5.5%.

The FII-2-a, -b, and -c fractions showed very high antitumor activities, especially the high molecular weight fraction FIII-2-a obtained by gel filtration from FIII-2. It exhibited the highest activity (inhibition ratio 100%, life prolongation ratio 267.5%, complete regression ratio 5/5, and mortality 0/5) among all polysaccharide fractions of "Songshan Lingzhi." FIII-2-a, -b, and -c are protein-containing glucans with glucan:protein ratios of 64.4–71.5:10.7–35.0 w/w, and average molecular weights in the range of 10–190 thousands. IR and NMR analyses indicate that these fractions are composed of $(1\rightarrow3)$-β-D-glucan chains.

The amino acid compositions of the protein moiety of polysaccharides with high antitumor activity have been listed. The constituent ratios of amino acids to total amino acids were highest for Ser and Thr in FI_0-a; Ser, Asp, Met, Gly, Pro, Thr, Cys, and Phe in FII-3; Ser, Met. Asp, Cys, and Phe in FIII-1-b; Arg, Gly, Ser, Asp, and Met in FIII-2-b; and Ser, Gly, Asp, Met. Cys, Pro, and Thr in FIII-2-c.

4. The comparison between antitumor polysaccharides obtained from Ganodermaceae mushrooms: Antitumor-active polysaccharides obtained from three species of the *Ganoderma* genus have been reported, namely, "Mannentake" or "Reishi" (*G. lucidum*) (32–35), "Kofukitake" (*G. applanatum*) (37), and "Songshan Lingzhi" (*G. tsugae*). Their antitumor activities have been described (38).

Several water-soluble and water-insoluble (soluble in salt or dilute alkali) polysaccharides with antitumor activity were obtained from three species of fungi (fruiting body and mycelium). They are (1→3)-β-D-glucan, homopolysaccharide or its protein complex; and hetero-β-D-glucan or its protein complex with heteroglycosidic chains of mannose, galactose, xylose, and fucose.

The active polysaccharide fractions of fruiting body or mycelium of three mushroom fungi were found to be markedly different in their component sugars, their protein moiety content, and their average molecular weight. The optimum dose of polysaccharide showing the highest activity seems to differ for the respective polysaccharides. This requires further study.

Antitumor-Active Protein-Containing Glycans from the Chinese Mushroom Songshan Lingzhi, *Ganoderma tsugae* Mycelium

The water-soluble polysaccharide FI, extracted from the mycelium of *Ganoderma tsugae*, was fractionated and purified by ion-exchange chromatography, gel filtration, and affinity chromatography. Sixteen polysaccharides were obtained and examined for their antitumor effects on Sarcoma 180/mice. Three active polysaccharides were obtained.

FI_0-b-α: This is a molecular weight 10,000, glucan–protein complex containing 25.8% protein. The inhibition ratio was 61.8% against the solid cancer Sarcoma 180/mice. The survival ratio was more than 194% as compared to the control group (100).

FA-1-a: This has a protein content of 33.0%; it is a glucan–protein complex having heteroglyco chains of galactose, mannose, fucose, xylose, and arabinose. The survival rate was comparatively good at 167%.

FA-1-b-α: Its molecular weight is 16,000; it is a complex of glycan:proteins = 42:58 w/w, consisting of glucose as a main component, associated with arabinose, mannose, xylose, and galactose. This showed 56% of the tumor inhibition ratio and more than 182% of the survival ratio as compared to the control.

A comparison of active glycan in the fruiting body and in the mycelium has been done. The previous section reports on glucogalactan protein complex, β-D-glucan, and β-D-glucan–protein complex isolation as the antitumor active polysaccharides obtained from the fruiting body of "Songshan Lingzhi" (*Ganoderma tsugae*); see section 1.5.1. Since then, the authors successfully mass cultured this fungus. From this cultured mycelium, we prepared the water-soluble polysaccharide and purified it by further fractionation. At the same time, the antitumor activity was tested and compared with the active polysaccharides obtained from fruiting bodies. The results of the comparative studies are now reported.

Extraction of Polysaccharide from the Cultured Mycelium

Polysaccharides were isolated from fruiting bodies of *Ganoderma tsugae* collected at the foot of Mt. Changbai, Jilin, China. This fungus was supplied through the courtesy of the Jilin Province Biological Institute, China.

Liquid Medium: The culture medium contained glucose 30 g, peptone 3 g, potassium biphosphate 1.5 g, magnesium sulfate 0.7 g, and vitamin B_1 0.01 g dissolved in 1000 mL water (pH 7.0). The medium was sterilized by autoclaving at 121°C for 30 min.

Culture: The precultured strain was aeration-cultured in 0.5-ton tanks, then moved successively from 1-ton, to 5-ton, to 10-ton, to 20-ton tanks. The culture was grown at 26 ± 1°C, 7–10 days in each tank.

Extraction of Polysaccharides from Mycelium: After culture, 25 kg of fresh mycelia was obtained by filtration and centrifugation. Fifteen liters of water were added to the mycelia. The polysaccharides were extracted by heating to 100°C for 3 h, then filtered. To this filtrate, 4 volumes of 99% ethanol were added, and the precipitate was collected by centrifugation (3000 rpm, 10 min, at 5°C). The precipitate was washed with ethanol, then acetone, and finally with ethyl ether, and dried *in vacuo*. The crude polysaccharide FI was obtained. The yield was 450 g of a water-soluble grayish white powder.

Fractionation and Purification of Water-Soluble Polysaccharide

The FI fraction was dissolved in water and separated into a nonadsorbed part (FI_0) and an adsorbed part (FA) by ion-exchange chromatography on a column of DEAE–Cellulose (Cl^-) (30 × 70 cm).

FI_0 was fractionated into FI_0-a, -b, and -c, by gel filtration (0.2 M NaCl elution) on a column of Toyopearl HW-65F (3.5 × 35 cm). In addition, FI_0-b was further fractionated into FI_0-b-β (nonadsorbed) and FI_0-b-α (adsorbed) by affinity chromatography on Con A–AF–Formyl Toyopearl 650 column (2 × 38 cm).

FA was fractionated into Fa-1, -2, -3 by gradient elution with $0 \rightarrow 3$ M NaCl solution. FA-1 was further fractionated into FA-1-a, -b, -c, and FA-1-insoluble by gel filtration. FA-1-b was further fractionated into FA-1-b-β (nonadsorbed) and FA-1-b-α (absorbed) by affinity chromatography.

Fractionation and Antitumor Activity of Polysaccharides from Mycelia

Sixteen polysaccharides were obtained from mycelia as a result of fractionation and purification, and their antitumor activities were determined.

The antitumor activities of polysaccharides obtained from mycelia were low both in their tumor inhibition ratio and in their tumor complete regression ratio when compared with the polysaccharide fractions obtained from fruiting bodies (39). However, the survival ratio shown by FI_0, FI_0-b, FI_0-b-α, FA-1-a and -b, and FA-1-b-α were good.

The increase in the survival ratio of the hosts may be considered as an antitumor effect. This may indicate that although the polysaccharides could not inhibit the increase of the tumor cells completely, the host could survive although the tumor cells were still present.

Physicochemical Properties and Structural Features of Antitumor Polysaccharides

Physicochemical properties of the water-soluble polysaccharides obtained from mycelia and the amino acid composition of protein moiety were determined by IR, ^{1}H-NMR, and ^{13}C-NMR spectra. Among the water-soluble polysaccharides obtained from the mycelia, FI_0-a, FI_0-b-α, and FA-1-a and -b showed fairly good antitumor effects, and contained protein. They are protein-containing glucans.

Comparison of Active Polysaccharides in Mycelia and Fruiting Bodies

As previously reported (39), among water-soluble polysaccharides obtained from fruiting bodies, fractions having high activity were FI_0-a and FA-1. Both were heteropolysaccharides, of molecular weight 10,000 and consisting of galactose, glucose, mannose, and fucose. The highly active fractions obtained from mycelia are FI_0-a and FI_0-b-α, and both are protein-containing glucans, molecular weight 10,000. These polysaccharides, based on IR and NMR data, are α-glucans: IR peak of 840 cm^{-1}; ^{1}H-NMR spectra—an anomeric α-proton of α-1,4 (5.4 ppm) and α-1,6 (4.9 ppm) linkage; and ^{13}C-NMR spectra—C-1α (102.0), C-3 (82.5), C-5 (74.3), C-4 (72.8), and C-6 (63.5 ppm). Moreover, this is confirmed by the lower values (+63.5, +67.6) of the specific rotations.

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