Agric. Biol. Chem., **50** (7), 1679～1688, 1986

Fractionation and Characterization of Antitumor Polysaccharides from *Maitake, Grifola frondosa*†

Takashi MIZUNO, Keiko OHSAWA, Naomi HAGIWARA and Reiko KUBOYAMA

Department of Agricultural Chemistry, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422, Japan

Received May 30, 1985

Three groups of polysaccharides from the edible mushroom "Maitake," the cultured fruiting body of *Grifola frondosa*, were extracted with hot water, 3% NH_4-oxalate (100°C), and 5% sodium hydroxide solution (30°C) in this order. The three fractions, FI, FII and FIII, were divided into several sub-fractions using various chromatographic techniques.

The fractions with host-mediated antitumor activity were as follows:

Water-soluble β-(1→3)-D-glucan: FI_0-a-β_1, $[\alpha]_D$ +9° (water), $\overline{MW}$ 1,000,000, N 0%, degree of branching=3, average chain-length=5, ID_{50} (the dose level, mg/kg in mice, inhibiting tumor growth in 50% of animal controls)=5.8.

Water-soluble acidic β-D-glucan: FA-1a-β_1, $[\alpha]_D$ +5° (water), $\overline{MW}$ 500,000, component sugars; Glc 82.4% and GlcUA 8.8%, ID_{50}=12.9.

Water-insoluble acidic xyloglucan: FII-3, $[\alpha]_D$ +56° (NaOH), $\overline{MW}$ 50,000, component sugars; Glc : Xyl=100 : 82, GlcUA 16.5%, ID_{50}=23.8.

Acidic hetero-glycan: FIII-1a, $[\alpha]_D$ +6° (NaOH), $\overline{MW}$ 100,000～250,000, protein content 3.8%, component sugars; Glc : Xyl : Man : Fuc=100 : 58 : 34 : 14, GlcUA 20.4%, ID_{50}=16.1.

Acidic glycoproteins: FIII-2a, FIII-2b and FIII-2c: $[\alpha]_D$ +58°, +43°, −11° (NaOH); $\overline{MW}$ 1,000,000, 70,000～100,000, 20,000～50,000; ID_{50}=38.5, 13.9, 9.3 respectively; component sugars; major=Glc, minor=Fuc, Xyl, Man, and Gal.

None of the polysaccharides intraperitoneally active against mouse-implanted Sarcoma 180 had any activity orally.

Grifola frondosa (*maitake* in Japanese) is a *Basidiomycete* fungus belonging to the order *Aphyllopherales*, and family *Polyporaceae*. This edible mushroom is a delicacy equal to *Tricholoma matsutake* (*matsutake*) for aroma and taste. In Japan, natural *maitake* used to be collected in mountain areas, but recently it has been cultured in bags or in bottles on wood shavings.

The antitumor polysaccharides[5] produced by *Basidiomycetes* have been receiving attention, and reports have appeared concerning β-glucans[6～8] of *G. frondosa*, and the water and alkaline-soluble β-glucans isolated from *G. umbellata*.[9～11]

We previously tried to fractionate the polysaccharides of the *Polyporus* fungus, which is known in traditional Japanese-Chinese medicine as a cure for cancer, and have studied the chemical structure of its carcinostatically active polysaccharide.[1～5]

In this paper, we investigated the most efficient methods for fractionating the water-soluble and insoluble polysaccharides from the cultured edible mushroom *maitake* and their antitumor activity.

MATERIALS AND METHODS

Fruiting body of *Grifola frondosa* (*maitake*). In April, 1982, about 40 kg of fresh *maitake* (100～200 g/bottle, Photo. 1) cultured in polyethylene jars (about 500 ml in capacity) using *Pterocarpus indicus* and *Fagus crenata* sawdust as the basal medium was obtained from Fuji Seito

† Studies on the Host-mediated Antitumor Polysaccharides. Part IX. For Part VIII, see ref. 2.

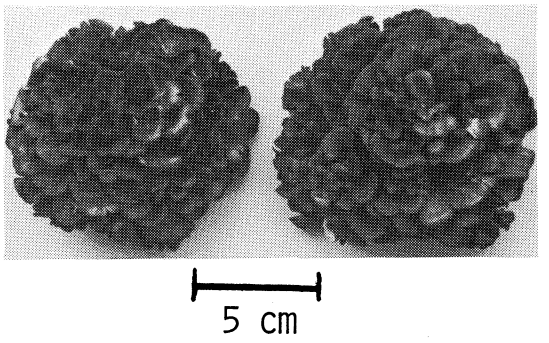

PHOTO. 1. The Fine Tasting Edible Mushroom *Maitake*, the Fruiting Bodies of Cultured *Grifola frondosa*.

Co., Shimizu City, Shizuoka Prefecture. The *maitake* were immediately crushed in a mixer with 5～6 volumes of ethanol, and the sediment after filtration was extracted 8 times, 3 hours each time, in 85% ethanol at 80°C, to remove the smaller substances such as free sugars. Residue I as shown in Fig. 1 was obtained as 6.4% of the fresh fruiting bodies.

Extraction and fractionation of polysaccharides. Details of the extraction and the fractionation of water-soluble polysaccharides are shown in Figs. 1 and 2, while that of water-insoluble polysaccharides are shown in Fig. 1.

(1) *DEAE-cellulose (Cl^-) column chromatography.* FI was dissolved in a small amount of water, passed through the column (2.6 × 90 cm) and separated into two fractions, namely the non-adsorbing neutral polysaccharide fraction (FI_0), and the adsorbed acidic polysaccharide fraction (FA). Then FA was fractionated into 4 sub-fractions by DEAE-cellulose (2.6 × 90 cm) using gradient elution of 0 to 2 M NaCl, FA-1, FA-2, FA-3, and FA-4 in Fig. 2.

(2) *Gel filtration.* The glycan was fractionated according to its molecular size on a Sephadex G-100 column (3 × 120 cm) or Sepharose CL 4B column (2.6 × 90 cm) as shown in Figs. 2 and 3.

(3) *Affinity chromatography.* Using a Con A-Sepharose CL 4B column (3 × 30 cm), α-glucans (adsorbed) and β-glucans (unadsorbed) were separated from the FI_0 fraction, and the acidic α-glucan (adsorbed) and the acidic β-glucan (unadsorbed) from FA-1 (Figs. 2 and 3).

Structural analysis of polysaccharides

(1) *Composition of constituent sugars and other constituents.* The polysaccharides were completely hydrolyzed by heating in 0.5～1 M H_2SO_4 at 100°C for 3～6 hours. The monosaccharides produced were reduced with $NaBH_4$, and then acetylated with acetic anhydride and pyridine. The constituent sugars were detected as alditol acetates by the standard GC method. The total sugars were measured by the phenol sulfuric acid method,[15] and the reducing sugars by the Somogyi–Nelson method.[16] Uronic acid was assayed by the carbazol-sulfuric acid method[17] or by the method of Misaki *et al.*[12] The content of nitrogen was measured by elemental analysis, and protein was assayed by the method of Lowry *et al.*[19]

(2) *Physicochemical properties.* The $[\alpha]_D$ was measured by a PM 101-type Union Giken automatic polarimeter, filter paper electrophoresis was done using a PA type Fuji Riken apparatus, the molecular weight ($\overline{MW}$) was measured using ultracentrifugation and gel filtration using a Sepharose CL 4B column with standard dextran (Pharmacia Co., Ltd.), and the reactivity with concanavalin A was measured using the agar gel double-diffusion method.[20]

(3) *NMR spectrum.* The PMR was measured using a Varian EM-390 type 90 MHz and a Jeol JNM GX-400 MHz instrument, and the ^{13}C-NMR was measured using a Varian XL-100 A-type 25.2 MHz-FT instrument. For both spectrum measurements, the chemical shift was observed in deuterium oxide using TPS as an internal standard.[1～4]

(4) *IR spectrum.* The IR spectrum was obtained using a Nihon Bunko IR-A 102-type instrument by the KBr tablet method native glycans, and by the CCl_4 method for methylates.

(5) *Methylation analysis.* Each polysaccharide completely methylated by the Hakomori method[21] was hydrolyzed with 90% formic acid and 0.25 M sulfuric acid. The partially methylated sugars were reduced with $NaBH_4$ or $AlLiH_4$, followed by acetylation with an acetic anhydride and pyridine mixture into alditol acetates. The partially methylated alditol acetates were identified and measured by the standard GC and GC-MS methods.[1～4]

(6) *Enzymic hydrolysis.* A sample of exo-(1→3)-β-D-glucanase, an extracellular enzyme produced by the *Basidiomycete* species QM 806, was purified by the Huotari method[23] into three sub-fractions E-I, -II, and -III, which were homogenous on polyacrylamide gel electrophoresis. The β-D-glucans were then digested with the purified enzyme. The digests were examined by the PPC method, by gel filtration using a Biogel P-2 column (1.6 × 86 cm), and by HPLC using a PNH_2-10/S 2504 column (0.4 × 25 cm) on Shimadzu LC-3A instrument with an RID-4 detector and C-RIA chromatopack.

Antitumor test. Antitumor activity was measured by intraperitoneal (*i.p.*) and oral (*p.o.*) administration to mice bearing Sarcoma 180.[1～4,18]

Experimental animals. Host animals were female, 7-week-old ICR/JCL mice (SPF) purchased from Japan Clera Co., which were used for our study after a week in an animal house at 23°±2°C room temperature and 55±5% relative humidity.

Experimental tumor. Solid Sarcoma 180, kindly supplied

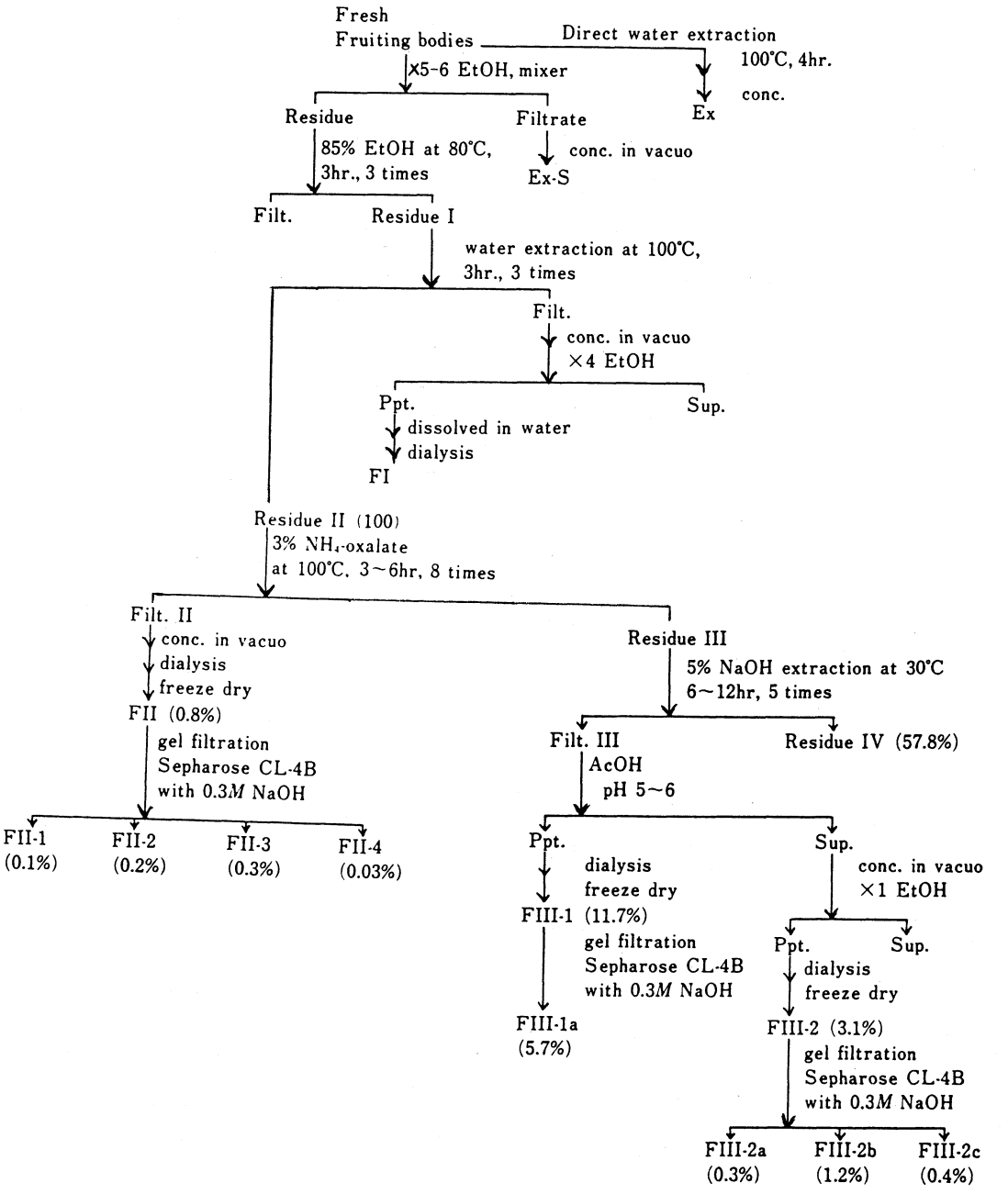

FIG. 1. Fractional Preparation of Some Polysaccharides from the Fruiting Bodies of *Grifola frondosa*.

by the National Cancer Center Research Institute (Dr. G. Chihara), was subcultured as the ascites type in ICR/JCL mice, and the tumor cells were harvested from the abdominal cavities of the mice 6~7 days after implantation. Living cells were counted ($2\sim5\times10^6$ cells), and then reduced to the desired cell counts by dilution with cold physiological saline solution before being used in the experiment.

Test for antitumor activity evaluation. Each group of experimental animals consisted of 5 to 6 mice, into which 2×10^6 Sarcoma 180 cells were inoculated at a subcutaneous site in the breast. Three days after inoculation, 1~100 mg/kg/day of polysaccharides dissolved or sus-

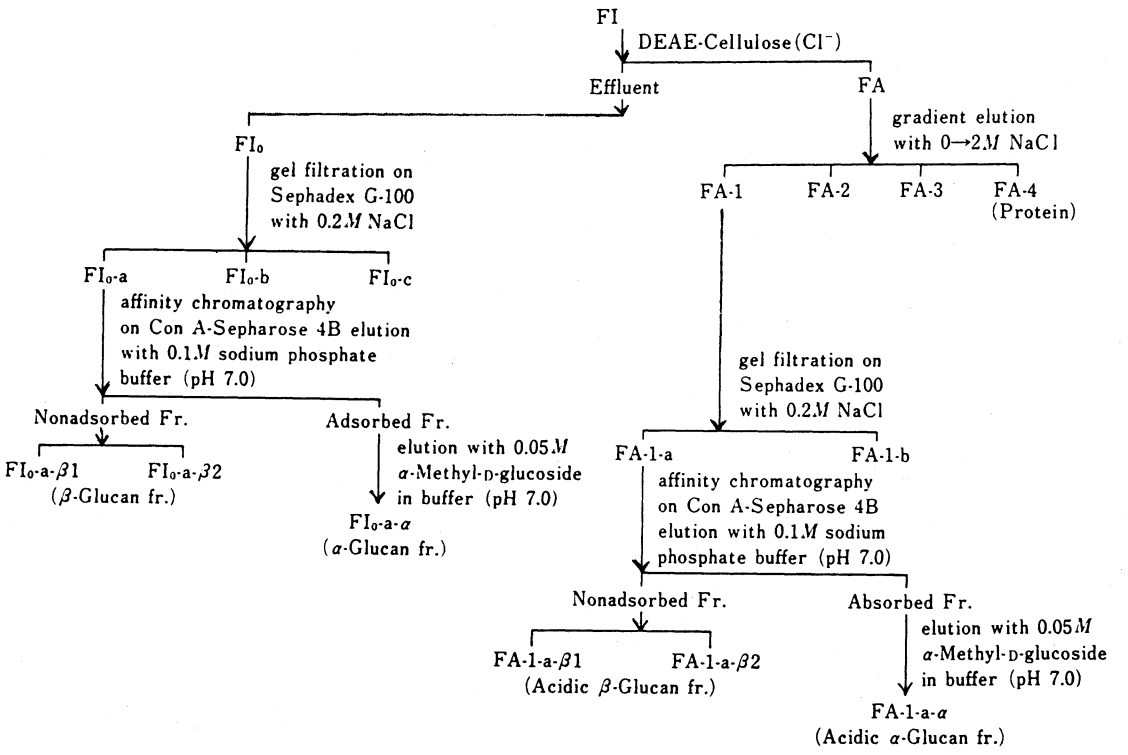

FIG. 2. Purification of Neutral Polysaccharide (FI_0) and Acidic Polysaccharide (FA-1) with Gel Filtration and Affinity Chromatography.

pended in sterilized physiological saline solution were administered once, intraperitoneally. Twenty-five days after polysaccharide administration, the size of the tumor (diameter in mm or weight in grams) was measured, and compared with that of the control group (to which only physiological saline solution had been administered) to calculate the suppression ratio percentage of tumor proliferation. Then, after 45 days, the complete regression ratio of the tumor (number of animals showing complete regression/5～6 animals) was examined 3 days after polysaccharide administration to discover any toxic effects of the polysaccharides. When the suppression ratio of tumor proliferation (%) was 0～25%, the antitumor activity was judged as ineffective (−), 26～50% as slightly effective (±), 51～75% as effective (+), 76～95% as considerably effective (++), and 96～100% as very effective (+++).

Calculation of the 50% inhibitory dose. On the basis of the above data on the doses of polysaccharides and tumor growth inhibitory ratio (%), the dose showing a 50% inhibitory effect was calculated as the ID_{50} (mg/kg body weight).

Antitumor test by oral administration (p.o.). In the experiment in which polysaccharides were administered *i.p.*, the samples showing evaluation better than "effective" were selected and administered orally in separate experiments. Sarcoma 180 tumor cells, 2×10^6 cells, were implanted subcutaneously in the breast of ICR/JCL mice, and a polysaccharide sample dissolved in physiological saline solution was administered orally once, 3 days after or 10 times, 3, 4, 6, 7, 8, 9, 10, 11, 13, and 14 days after implantation. After 24 days, the size of the tumor (diameter in mm) was measured to obtained the tumor proliferation inhibitory ratio percentage. After 45 days, the animals with complete regression were counted. Body weights were measured 3～15 days after tumor implantation to find if the polysaccharides were toxic.

RESULTS AND DISCUSSION

Fractionation and antitumor activity of polysaccharide

Each fraction of *maitake* polysaccharides during purification was examined for antitumor activity.

The hot-water extract (Ex) of *maitake* had an antitumor activity (Table I). After frac-

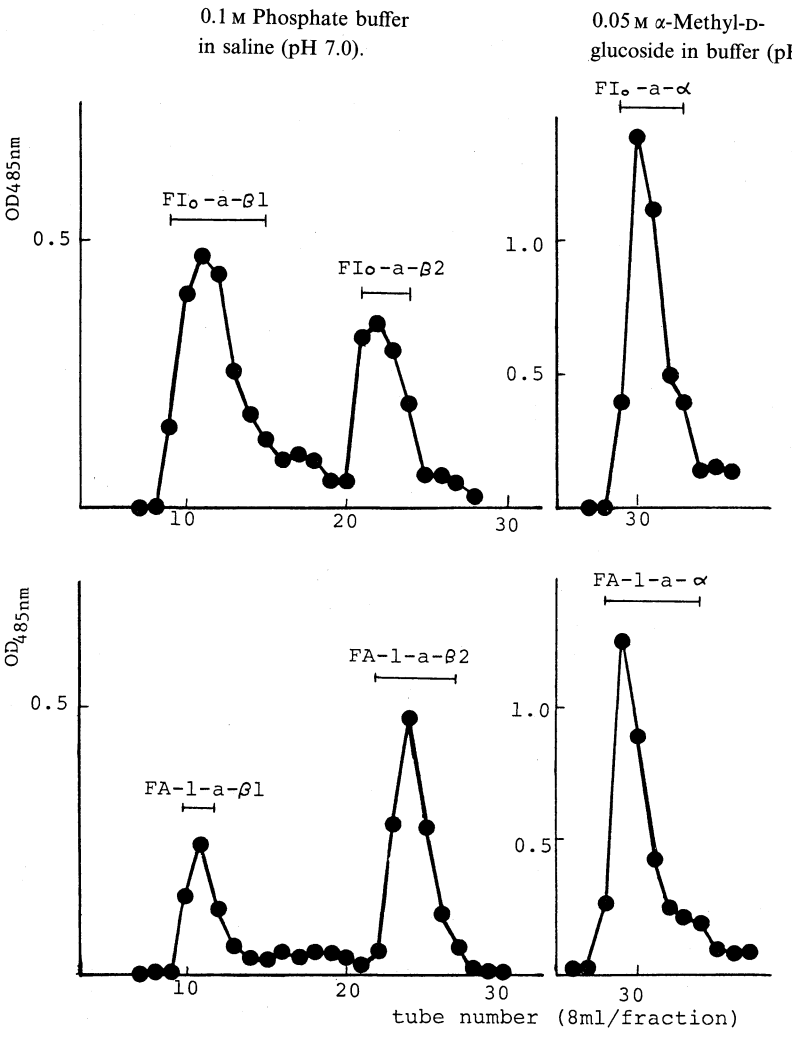

FIG. 3. Affinity Chromatography of FI_0-a and FA-1-a on a Con A-Sepharose 4B Column (3.0 × 25 cm).

tionation of water-soluble polysaccharides (FI) by several column chromatographic techniques, antitumor activity was concentrated into the FI_0 and FA-1 fractions. By further purification on a Con A-Sepharose column, two β-glucan fractions, FI_0-a-β_1 and FA-1a-β_1, had higher antitumor activities.

Of the water-insoluble polysaccharides, the ammonium oxalate extract, FII, was fractionated on a Sepharose CL 4B column into FII-1, -2, -3, and -4. Similarly the alkaline extract, FIII, was divided into FIII-1a, and FIII-2a, -2b, -2c. FII-3 and all fractions of FIII had higher activities (Table II). However, the ID_{50} value for the water-insoluble fraction was generally less active than that of the water-soluble fraction, just as in our study on *mannentake*, *Ganoderma lucidum*.[1)]

Structural Features of antitumor active water-soluble glucan

The constituents of FI_0 were Glc, Gal, Man, Xyl, and Fuc. The high molecular weight fraction FI_0-a consisted of only Glc without nitrogen. By affinity chromatography using a Con A-Sepharose 4B column, FI_0-a (30 mg) was fractionated into β-D-glucans, FI_0-a-β_1 (9 mg) and FI_0-a-β_2 (5 mg) obtained from the

TABLE I. ANTITUMOR ACTIVITY OF WATER-SOLUBLE POLYSACCHARIDE FROM *Grifola frondosa* AGAINST SARCOMA 180 IN MICE

Sample	Dose[a] (mg/kg/day) ×1	Average tumor diameter (mm) on day 25		Evaluation[c]	Complete regression on day 45	ID_{50}[b] (mg/kg)
		Treated/Control	Inhibition (%)			
Ex-S	10	20.0/19.6	−2	−	0/5	—
	100	15.3/19.6	22	−	0/5	
Ex	10	22.0/19.6	−12	−	0/5	67.6
	100	5.0/19.6	74	+	3/5	
FIo	8	20.9/19.6	−7	−	0/5	—
	80	22.1/19.6	−13	−	0/5	
FIo-a	2	22.8/19.6	−16	−	0/5	11.6
	20	2.7/19.6	86	++	4/5	
FIo-b	2	16.4/19.6	16	−	0/5	—
	20	17.3/19.6	12	−	0/5	
FIo-c	4	21.3/19.6	−9	−	0/5	—
	40	20.8/19.6	−6	−	0/5	
FA	8	19.5/19.6	1	−	0/5	50.6
	80	4.1/19.6	79	++	3/5	
FA-1	4	12.5/14.5	14	−	0/5	14.3
	40	0/14.5	100	+++	5/5	
FA-2	2	14.2/14.5	2	−	0/5	—
	20	13.9/14.5	4	−	0/5	
FA-3	8	14.8/14.5	−2	−	0/5	—
	80	12.8/14.5	12	−	0/5	

Sarcoma 180 cells (2×10^6) were inoculated subcutaneously into the breast region of female ICR/JCL mice.

[a] Injection was intraperitoneally on day 3.

[b] Dose level (mg/kg, mice) that inhibits tumor growth in 50% of the control.

[c] When the suppression ratio of tumor proliferation (%) was 0~25%, the antitumor activity was judgedas non-effective (−), 26~50% as a slightly effective (±), 51~75% as effective (+), 76~95% as considerably effective (++) and 96~100% as very effective (+++).

unadsorbed fraction, and α-D-glucan FI_0-a-α (14 mg) from the adsorbed fraction (Fig. 2).

β-D-Glucan FI_0-a-β_1 had an $[\alpha]_D$ of 9° (H_2O), an IR absorption peak of 890 cm^{-1}, a $\overline{MW}$ of about 1,000,000 (gel filtration), and 0% N; the results of methylation analysis were: 2,3,4,6-tetra-*O*-methyl Glc : 2,4,6-tri-*O*-methyl Glc : 2,4-di-*O*-methyl-Glc = 1.00 : 3.01 : 1.02 (molar ratio). The final products hydrolyzed by exo-(1→3)-β-D-glucanase were Glc : Gen (gentiobiose) = 2.2 : 1.0 (molar ratio). These results containing PMR and ^{13}C-NMR assignments indicated that FI_0-a-β_1 was a β-(1→3)-D-glucan branched with β-(1→6) linkage with 3 glucose residues, and an average chain length of 5 residues.

Animal tests found that the tumor inhibition was 100% by a single dose of 20 mg of this glucan/kg mice by the *i.p.* route as the complete tumor regression was 5/5 and $ID_{50} = 5.8$. This effect was similar to that of the β-D-glucans[1~4] obtained from other *Polyporous* fungi such as *mannentake*.

α-D-Glucan FI_0-a-α had an $[\alpha]_D$ of +156° (H_2O), with an IR absorption peak of 840 cm^{-1}, a $\overline{MW}$ of about 1,000,000 and consisted of only Glc. On the basis of the above data and the PMR and ^{13}C-NMR assignments with the results of methylation analysis it was shown that FI_0-a-α was an α-(1→4)-D-glucan with an

TABLE II. ANTITUMOR ACTIVITY OF WATER-INSOLUBLE POLYSACCHARIDES FROM *Grifola frondosa* AGAINST SARCOMA 180 IN MICE

Polysaccharide	Dose (mg/kg/day) × 1 *i.p.*	Average tumor diameter (mm) on day 25		Complete regression on day 25	Evaluation	ID_{50} (mg/kg)
		Treated/Control	Inhibition (%)			
FII	10	14.4/18.3	21	—	−	23.8
	100	0/18.3	100	5/5	+++	
FII-1	10	16.1/18.3	12	—	−	—
	100	16.6/18.3	9	—	−	
FII-2	10	16.4/18.3	10	—	−	—
	100	12.9/18.3	30	1/5	±	
FII-3	10	13.5/17.0	21	1/5	−	23.8
	100	0/17.0	100	5/5	+++	
FII-4	10	18.5/18.3	−1	—	−	—
	100	13.4/18.3	27	—	±	
FIII	10	12.8/18.3	30	2/5	±	16.7
	100	0/18.3	100	5/5	+++	
FIII-1	10	13.8/18.3	25	1/5	−	20.0
	100	2.2/18.3	88	4/5	++	
FIII-1a	10	12.7/18.3	31	1/5	±	16.1
	100	5.9/18.3	68	3/5	+	
FIII-2	10	8.9/17.0	48	2/5	±	10.4
	100	0/17.0	100	5/5	+++	
FIII-2a	10	14.8/17.0	13	1/5	−	38.5
	100	0/17.0	100	5/5	+++	
FIII-2b	10	10.9/17.0	36	1/5	±	13.9
	100	0/17.0	100	5/5	+++	
FIII-2c	10	7.8/17.0	54	3/5	+	9.3
	100	0/17.0	100	5/5	+++	

Sarcoma 180 cells (2×10^6) were inoculated subcutaneously into the breast region of female ICR/JCL mice. Administration of polysaccharide sample was intraperitonealy on day 3. ID_{50}: Dose level (mg/kg, mice) that inhibits tumor growth in 50% of the control.

α-(1→6) branch. No antitumor activity was detected.

FI_0-b was thought to be a kind of xylomannogalactoglycan consisting of Xyl, Man, Gal=8:10:11 with 0% N, and an $[\alpha]_D$ of +74° (H_2O). FI_0-c consisted of Xyl, Man, Gal, Glc=6:8:9:5, containing 0.9% protein (Lowry method) and with an $[\alpha]_D$ of +62° (H_2O). But neither FI_0-b nor FI_0-c showed any antitumor activity.

Fractionation and antitumor activity of water-soluble acidic heteroglycan

FA-1, -2 and -3 in Fig. 2 were acidic heteroglycans with mainly Glc as the component sugar, 0.4~0.8% protein, 6~18% uronic acid (confirmed to be GlcUA by the GC method[12]) and small amounts of Fuc, Xyl, Man, and Gal. FA-1, which had antitumor activity (Table I), was further fractionated into FA-1a and FA-1b (Fig. 2). The activity was found in FA-1a consisting of GlcUA and Glc, but not in FA-1b consisting of Fuc, Man, and Gal. By affinity chromatography using a Con A-Sepharose 4B

TABLE III. CHEMICAL ANALYSIS OF WATER-INSOLUBLE POLYSACCHARIDES FROM *Grifola frondosa*

Fraction	FII-1	FII-2	FII-3	FII-4	FIII-1a	FIII-2a	FIII-2b	FIII-2c
Yield (%) for Residue II	0.11	0.23	0.33	0.03	5.74	0.28	1.18	0.43
$\overline{MW}$ ($\times 10^{-4}$)[d]	200	20~40	5	1	10~25	100	7~10	2~5
N (%)	0	0	0	4.9	0.6	2.9	1.7	4.3
Total sugar[a] (%)	83	78	79	73	75	70	86	68
Uronic acid[b] (%)	9.1	13.5	16.5	11.8	20.4	13.5	10.0	9.8
Molar ratio of component sugar[c]								
Rha	7.3	—[e]	—	—	—	—	—	—
Fuc	12.8	—	3.3	9.6	13.7	8.2	—	3.0
Xyl	—	—	82.0	4.7	57.6	7.1	6.4	10.6
Man	8.9	2.0	2.3	9.8	33.7	5.3	4.7	6.1
Gal	14.2	3.8	5.4	14.5	—	7.2	—	4.7
Glc	100	100	100	100	100	100	100	100
$[\alpha]_D^{20}$ (NaOH), °	+123	+132	+56	+3	+76	+58	+43	−11

[a] Calculated as glucose by the phenol–sulfuric acid method.
[b] Calculated as glucuronic acid by a modified carbazole method.
[c] Measured as alditol acetates by GLC and GC-MS.
[d] Measured by a gel filtration method.
[e] Nil.

column, acidic β-glucans (unadsorbed) FA-1a (21 mg) was purified into FA-1a-β_1 (8 mg) and FA-1a-β_2 (10 mg), and acidic α-glucan (adsorbed) FA-1a-α (2 mg).

FA-1a-β_1 had an $[\alpha]_D$ of +5° (H_2O), a $\overline{MW}$ of about 500,000, an IR peak of 890 cm^{-1}, and consisted of 82.4% Glc, 8.8% GlcUA, and 1.2% protein. In the antitumor test, the tumor growth inhibition was 100% by 40 mg/kg mice administered once by the *i.p.* route, the complete tumor regression was 5/5, and ID_{50} = 12.9.

FA-1a-α had an $[\alpha]_D$ of +108° (H_2O), a $\overline{MW}$ of 500,000, an IR peak of 840 cm^{-1}, and consisted of 84.8% Glc, 8.4% GlcUA, and 0.7% protein; we considered it to be an acidic α-D-glucan. However, no antitumor activity was found.

Fractionation and antitumor activity of water-insoluble heteroglycan

(1) *Fractionation and antitumor activity of FII.* Only FII-3 had antitumor activity (Table II). Table III shows the physicochemical properties and the composition of constituent sugars of FII-3 in comparison with those for the inactive FII-1, -2, and -4. FII-3 was thought to be a acidic xyloglucan, with an $[\alpha]_D$ of +56° (NaOH), containing Glc : Xyl = 100 : 82, 16.5% GlcUA, and with an average molecular weight of about 50,000. From its absorbance at 890 cm^{-1} in the IR spectrum, the main chain may have β-linkages. From the results (Table IV) of methylation analysis it was estimated to have a (1→6) and (1→2) branch on the β-(1→3)-glucan chain. The binding modes of the Xyl and GlcUA residues in this heteroglycan are now under investigation.

(2) *Fractionation and antitumor activity of FIII.* The heteroglycan fractions, FIII-1a and FIII-2a, -2b, and -2c had a tumor growth-inhibitory effect of 68~100%. FIII-1a was obtained at a higher yield and was a heteroglycan consisted of 28% Glc, 20% GlcUA, and Xyl, Man, and Fuc as component sugars. FIII-2a, -2b, and -2c might be a complex of heteroglycans consisted of mainly Glc and GlcUA, and small amounts of Fuc, Xyl, Man, and Gal, and with a small amount of protein.

TABLE IV. METHYLATION ANALYSIS OF THE GLUCAN CORES OF FII-2, FII-3, FIII-1a, FIII-2b, AND FIII-2c FROM *Grifola frondosa*

Peak	Alditol acetate of	T^a	Primary mass fragments (m/z)	Molar ratio[b]					Glucose residue
				FII-2	FII-3	FIII-1a	FIII-2b	FIII-2c	
A	2,3,4,6-M_4-Glc	1.00	205, 161, 117, 45	1.00	1.00	1.00	1.00	1.00	G→
B	2,4,6-M_3-Glc	1.96	233, 161, 117, 45	0.40	0.55	4.57	0.18	1.24	$\rightarrow_3$G→
C	2,3,6-M_3-Glc or 2,3,4-M_3-Glc	2.42		7.16	—	—	1.17	1.33	$\rightarrow_4$G→ or $\rightarrow_6$G→
D	2,6-M_2-Glc	3.84		—	—	—	0.29	—	$\rightarrow_4$G→ $\rightarrow_3$
D′	4,6-M_2-Glc	4.00		0.40	0.79	1.60	—	0.10	$\rightarrow_3$G $\rightarrow_2$
E	2,4-M_2-Glc	5.02	233, 189, 117	—	0.63	0.35	0.28	1.04	$\rightarrow_6$G→ $\rightarrow_3$
F	2,3-M_2-Glc	6.04		1.23	—	—	—	—	$\rightarrow_6$G→ $\rightarrow_4$

[a] Retention times of the corresponding alditol acetates on a 3% ECNSS-M column at 180°C, relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

[b] Molar ratio were corrected by using molar response factors for partially methylated alditol acetates according to carbon response.[24]

Antitumor activity of polysaccharide administered orally

The protein-bound β-D-glucan fraction obtained from *kawaratake* (mycelia of *Coriolus versicolor*)[13] and *enokitake* (fruiting bodies of *Flammulina veltipes*)[14] has antitumor activity when administered *p.o.* as well as *i.p.*

The polysaccharide fraction obtained from *maitake* with activity when administered *i.p.* did not have any antitumor activity when administered *p.o.* just as in the case of the active polysaccharides obtained from *mannentake* (fruiting bodies of *Ganoderma lucidum*).[1,2]

Acknowledgments. The *maitake* was a gift from the Fuji Seito Co. The carcinostatic test was done with the cooperation of Sankyo Co. Biological Research Laboratories, and elemental analysis and NMR measurements with the cooperation of the Analytical and Metabolic Research Laboratories of the same company. The authors extend their sincere thanks to the people involved.

The outlines of this study were reported at the 7th and 8th Symposia on Carbohydrates held in Osaka on August 28, 1984 and in Kyoto on July 23, 1985.

REFERENCES

1) T. Mizuno, N. Kato, A. Totsuka, K. Takenaka, K. Shinkai and M. Shimizu, *Nippon Nôgeikagaku Kaishi*, **58**, 871 (1984).
2) T. Mizuno, E. Suzuki, K. Maki and H. Tamaki, *Nippon Nôgeikagaku Kaishi*, **59**, 1143 (1985).
3) T. Mizuno *et al.*, Proceedings of the 7th and 8th Carbohydrate Symposia in Japan, A-21, p. 50 (1984) [Osaka] and A-23, p. 49 (1985) [Kyoto].
4) T. Mizuno *et al.*, *Bull. Fac. Agric. Shizuoka Univ.*, **30**, 41 (1980); *ibid.*, **31**, 49 (1981); *ibid.*, **32**, 29, 41 (1982); *Agric. Biol. Chem.*, **45**, 323 (1981); *Carbohydr. Res.*, **115**, 273 (1983); Proceedings of the 4th and 5th Carbohydrate Symposia in Japan, p. 48 (1981) [Tokyo] and p. 32 (1982) [Nagoya].
5) T. Mizuno, *Kagaku to Seibutsu*, **21**, 473 (1983); *ibid.*, **23**, 797 (1985); *Chemical Times*, **106**, 1902 (1982); *ibid.*, **107**, 1925 (1983).
6) K. Kato, T. Inagaki, H. Shibagaki, R. Yamauchi, K. Okuda, T. Sano and Y. Ueno, *Carbohydr. Res.*, **123**, 259 (1983).
7) K. Kato, T. Inagaki, T. Teranishi, R. Yamauchi, K. Okuda, T. Sano and Y. Ueno, *Carbohydr. Res.*, **124**, 247 (1983).
8) T. Miyazaki *et al.*, *Shinkin to Shinkinsyo*, **23**, 261 (1982); *ibid.*, **24**, 95 (1983); *Gendai Toyo Igaku*, **4**, 61 (1983); *Chem. Pharm. Bull.*, **32**, 1142 (1984); Proceedings of the 7th Carbohydrate Symposium in Japan, A-18, p. 44 (1984) [Osaka].
9) K. Kato, K. Mutoh, T. Egashira, M. Hiura and Y. Ueno, *Agric. Biol. Chem.*, **42**, 1073 (1978).
10) Y. Ueno, M. Abe, R. Yamauchi and K. Kato, *Carbohydr. Res.*, **87**, 257 (1980).
11) Y. Ueno, Y. Okamoto, R. Yamauchi and K. Kato, *Carbohydr. Res.*, **101**, 160 (1982).

12) Y. Sone, M. Kakuta and A. Misaki, *Agric. Biol. Chem.*, **42**, 417 (1978).
13) S. Tsukagoshi, *Gan to Kagakuryoho*, **1**, 251 (1974).
14) T. Ohkuma, K. Otagiri, T. Ikekawa and S. Tanaka, *J. Pharm. Dyn.*, **5**, 439 (1982).
15) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Robers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
16) N. Nelson and M. Somogyi, *J. Biol. Chem.*, **153**, 375 (1944); *ibid.*, **195**, 19 (1952).
17) T. Bitter and H. M. Mair, *Anal. Biochem.*, **4**, 330 (1962); J. T. Galambos, *Anal. Biochem.*, **19**, 119 (1967).
18) G. Chihara *et al., Nippon Rinshyo*, **27**, 1739 (1969); *Nature* (London), **222**, 687 (1969); *Cancer Res.*, **30**, 2776 (1970).
19) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
20) T. Mizuno *et al., Bull. Fac. Agric. Shizuoka Univ.*, **31**, 65 (1981); *Carbohydr. Res.*, **92**, 103 (1981); *J. Biochem.*, **89**, 1029 (1981).
21) S. I. Hakomori, *J. Biochem.*, **55**, 205 (1964); T. Narui, K. Takahashi, M. Kobayashi and S. Shibata, *Carbohydr. Res.*, **103**, 293 (1982).
22) J. X. Khym, "Methods in Carbohydrate Chemistry," Vol. 6, ed. by R. L. Whistler and J. N. BeMiller, Academic Press, 1972, p. 87.
23) F. I. Huotari, T. E. Nelson, F. Smith and S. Kirlwood, *J. Biol. Chem.*, **243**, 952 (1968).
24) D. D. Sweet, R. H. Shapiro and P. Albersheim, *Carbohydr. Res.*, **40**, 217 (1975).