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Antitumor Activity and Structural Characterization of Glucans Extracted from Cultured Fruit Bodies of *Grifola frondosa*

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Polysaccharides in the cultured fruit body of *Grifola frondosa* were extracted successively with hot water, aqueous zinc chloride, and cold and hot aqueous sodium hydroxide containing urea. The antitumor effect and structural features of the extracted compounds were examined. The above extraction gave water-soluble glucan fractions together with water-insoluble heteroglycan fractions. The heteroglycan fractions were composed of glucose, xylose and mannose. The zinc chloride extraction gave only a small quantity of polysaccharide. The structural characteristics of these polysaccharides were deduced from the results of gel filtration, methylation, enzymic degradation, and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy. The hot water extract contained a large amount of α-1,4-glycosidic linkages (about 50%) digestable by α-amylase, and also contained α-1,6-, β-1,6-, and β-1,3-linkages. The water-soluble glucan fractions extracted with cold and hot sodium hydroxide contained more 6-branched β-1,3-linkages. Methylation analysis suggests the presence of one 6-branched glucosyl unit in every three 3-substituted β-glucosyl units. All of these water-soluble and insoluble fractions showed potent antitumor activity against the solid form of Sarcoma 180 tumor in ICR mice. The activity of the hot sodium hydroxide extractable, water-soluble fraction was the strongest. The antitumor activity was positively correlated with the content of 6-branched β-1,3-glucan in these water-soluble fractions.

Keywords——polysaccharide; cultured fungi; *Grifola frondosa*; antitumor agent; β-1,3-glucan

Grifola frondosa is a fungus belonging to the Basidiomycetes, Aphyllophorales, Polyporaceae. The fruit body has been used as an edible mushroom. Recently, Oikawa *et al.* have established a cultivation method for production of the fruit body of the fungus,[1)] and we reported that a hot water extract of the fruit body showed potent antitumor activity against Sarcoma 180 solid form tumor in ICR mice.[2)]

The fungi belonging to Basidiomycetes contain various kind of antitumor glucans extractable with various solvents, such as water, sodium carbonate, and sodium hydroxide. Kato *et al.* have established a successive extraction method for glucan fractions from *Grifola umbellata* and *G. frondosa*, and reported the structure of a glucan extracted with aqueous zinc chloride from *G. frondosa* as 6-branched β-1,3-glucan.[3)] Kuroda *et al.* also reported the structure of a glucan extracted with hot water from *G. frondosa*.[4)] However, detailed examinations of the structure and antitumor activity of glucans extracted with various solvents from the fungus have not yet been performed.

This paper is concerned with the antitumor activity and the structural characterization of polysaccharide fractions extracted successively from the cultured fruit body of *G. frondosa* with various solvents.

Materials and Methods

Mice——Male ICR mice, 8 weeks old and weighing 27—30 g, were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka.

Tumor——Sarcoma 180 tumor cells in ascites form were kindly supplied by Dr. T. Sasaki, The National Cancer Center Research Institute, Japan, and were maintained by weekly passage in ICR mice.

Evaluation of Antitumor Activity——The antitumor activity was evaluated against the solid form of sarcoma 180 tumor cells. Tumor cells (5×10^6) were inoculated subcutaneously into the right groin of mice. Each fraction was administered for 10 consecutive days from 24 h after the tumor inoculation. Five weeks after tumor inoculation, the mice were sacrified. The inhibition was calculated as follows:

$$\left(1 - \frac{\text{average tumor weight of the treated group}}{\text{average tumor weight of the control group}}\right) \times 100\ (\%)$$

Isolation of Each Polysaccharide Fraction——Polysaccharide fractions were prepared from powdered (Chart 1) or cut (Chart 2) fruit bodies of *G. frondosa*. After being washed with water, the fruit bodies were lyophilized, and then pulverized to a powder or cut into a pieces of about 150 mg each. The powdered or cut fragments were refluxed several times with 80% methanol (or 80% ethanol) to remove fatty materials.

The defatted material (50 g) was extracted with water (600 ml) in an autoclave (1 kg/cm^2, 121 °C) for 1 h. The extract was dialyzed against water and concentrated to a small volume. The polysaccharide fractions were precipitated by adding 4 vol of ethanol (PF-1 and CF-1). The precipitates were dried by washing with acetone and then ether. The extraction was repeated 5 to 7 times.

The residue of water extraction (powder) was then extracted similarly in an autoclave with 10% aqueous zinc chloride. The extracts were dialyzed extensively against tap water and distilled water. A precipitate formed during the dialysis was collected by centrifugation (PF-2). The supernatant solution was concentrated and subjected to hot water extraction (PF-3).

The hot water extract (cut) and zinc chloride extract (powder) residues were then each extracted by stirring with 10% sodium hydroxide containing 5% urea for 24 h at 4 °C. The extracts were neutralized with acetic acid and dialyzed extensively against tap and distilled water. The precipitates produced during dialysis were treated as in the case of PF-2 (PF-4 and CF-4). The supernatants were concentrated and treated as described for the hot water extraction (PF-5 and CF-5). The extraction was repeated 3 times.

The residue (cut) was extracted at 65 °C for 1 h with buffer of the same composition and by the same procedures as used for cold alkali extraction. The precipitate was designated as CF-6 and the supernatant as CF-7.

Total Sugar Content——Total sugar contents were determined as anhydroglucose by the phenol–sulfuric acid method using D-glucose as a standard.[5]

Total Protein Content——Total protein contents were determined as bovine serum albumin by the Lowry–Folin method.[6]

Component Sugar Analysis——Each fraction (1 mg) was hydrolyzed wih 1 M trifluoroacetic acid (1 ml) at 100 °C for 5 h. The hydrolysate was evaporated to dryness, and the residue was derivatized to alditol acetates in the usual way, and analyzed with a Shimadzu GC-6A instrument fitted with glass column (0.3 × 200 cm) of 3% OV225 on Uniport KS under the following conditions: the column temperature was increased at 4 °C/min from 170 to 250 °C at a flow rate of 60 ml per min of N_2. The molar ratio of alditol acetates was determined from the peak heights.

Susceptibility to α-Amylase——Each fraction (9 mg) dissolved in 10 mM Tris-HCl buffer pH 6.9 (20 ml) was digested with α-amylase (Wako Pure Chemical Ltd.) (1 mg) at 37 °C for 6 h. Under these reaction conditions maximum reducing power was obtained. The reaction was terminated by heating at 100 °C for 3 min, then the reaction mixture was placed in a dialysis tube and dialyzed against water. The non-dialyzable fraction was concentrated. The sugar content of the non-dialyzable fraction was determined by the phenol–sulfuric acid method.

For preparing α-amylase-digested CF-1, the reaction was performed under similar conditions on a large scale. The non-dialyzable fraction was passed through a membrane filter (XM-50, Mw 50000, Amicon Co., Ltd.) and the non-passed fraction (α-amy CF-1) was tested for antitumor activity.

Methylation Analysis of Each Polysaccharide Fraction——Each polysaccharide fraction was methylated twice by the method of Hakomori. The fully *O*-methylated product was formylized with 90% HCOOH (2 ml) at 100 °C for 10 h in a sealed tube. After removal of HCOOH by evaporation, the residue was hydrolyzed with 1 M trifluoroacetic acid at 100 °C for 5 h, then the mixture was evaporated to dryness. The resulting partially *O*-methylated sugars were reduced with $NaBH_4$ at room temperature for 2 h to the corresponding alditols, and then acetylated as described above. Gas liquid chromatography (GLC) of the partially *O*-methylated alditol acetates was carried out by using a glass column (0.3 × 200 cm) packed with 3% OV-225 at increasing temperature (4 °C/min) from 150 to 230 °C. The molar ratio of each *O*-methylated sugar was calculated based on 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol as 1.0.

GLC-mass spectrum (MS) of the partially *O*-methylated alditol acetates was carried out on the same column under the same conditions. The electron impact mass spectra were recorded on Hitachi M-80 instruments.

Carbon-13 Nuclear Magnetic Resonance (NMR) Spectral Analysis——Carbon-13 NMR spectra were recorded at 27 °C for solutions in 0.2 M NaOD (internal standard, sodium 2,2,3,3-tetradeuterio-3-trimethylsilylpropionate (TSP)) and for solutions in DMSO-d_6 at 60 °C with a JEOL-FX 200 (for carbon-13 at 50.1 MHz) spectrometer. The spectra were obtained in the pulsed Fourier-transform mode with complete proton decoupling.

Results

Compositions of Fractions Extracted with Various Solvents

Polysaccharide fractions were prepared by two extraction procedures as shown in Charts 1 and 2. The 1st procedure, by which polysaccharide fractions were extracted from the sclerotina of *G. umbellata* and the fruit body of *G. frondosa*,[3] is shown in Chart 1. The starting material was "powdered" fruit body of cultured *G. frondosa*. As shown in Table I, hot water (PF-1) and cold alkali (PF-4,5) extractions yielded materials corresponding to about 10 and 13% of the dry weight, respectively. However, zinc chloride extraction (PF-2,3) yielded only 0.6%, suggesting that zinc chloride extraction is ineffective for preparing polysaccharide fractions from cultured *G. frondosa*. Hot water extraction gave a water-soluble glucan fraction (PF-1), and cold alkali extraction gave both a water-soluble glucan fraction (PF-5) and a water-insoluble heteroglycan fraction (PF-4). The water-soluble glucan fractions (PF-1,3,5) showed higher carbohydrate contents than the water-insoluble fractions (PF-2,4). When α-amylase digestion of these fractions was performed, only PF-1 was digestable.

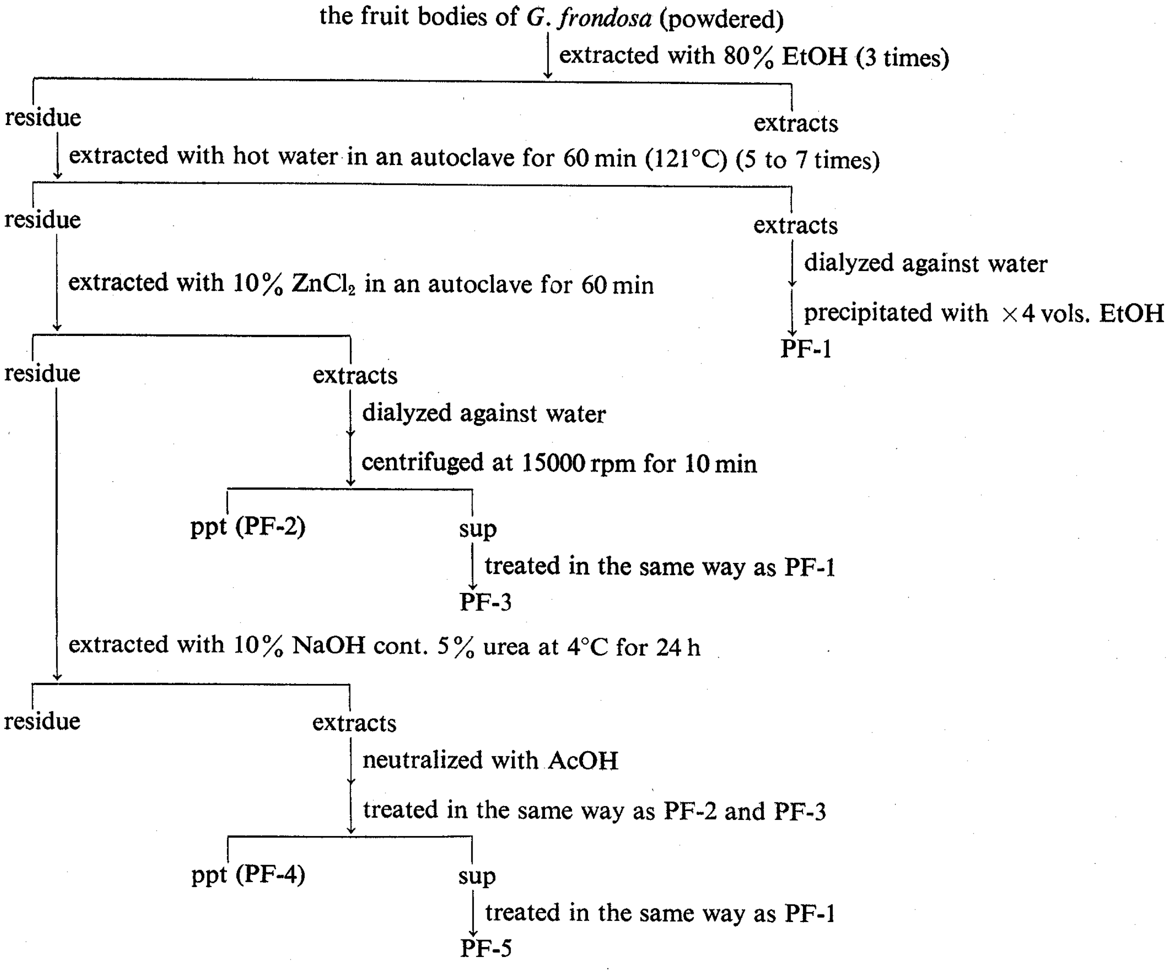

Chart 1. Isolation of the Polysaccharide Fractions from the Fruit Bodies of *G. frondosa* (Powdered)

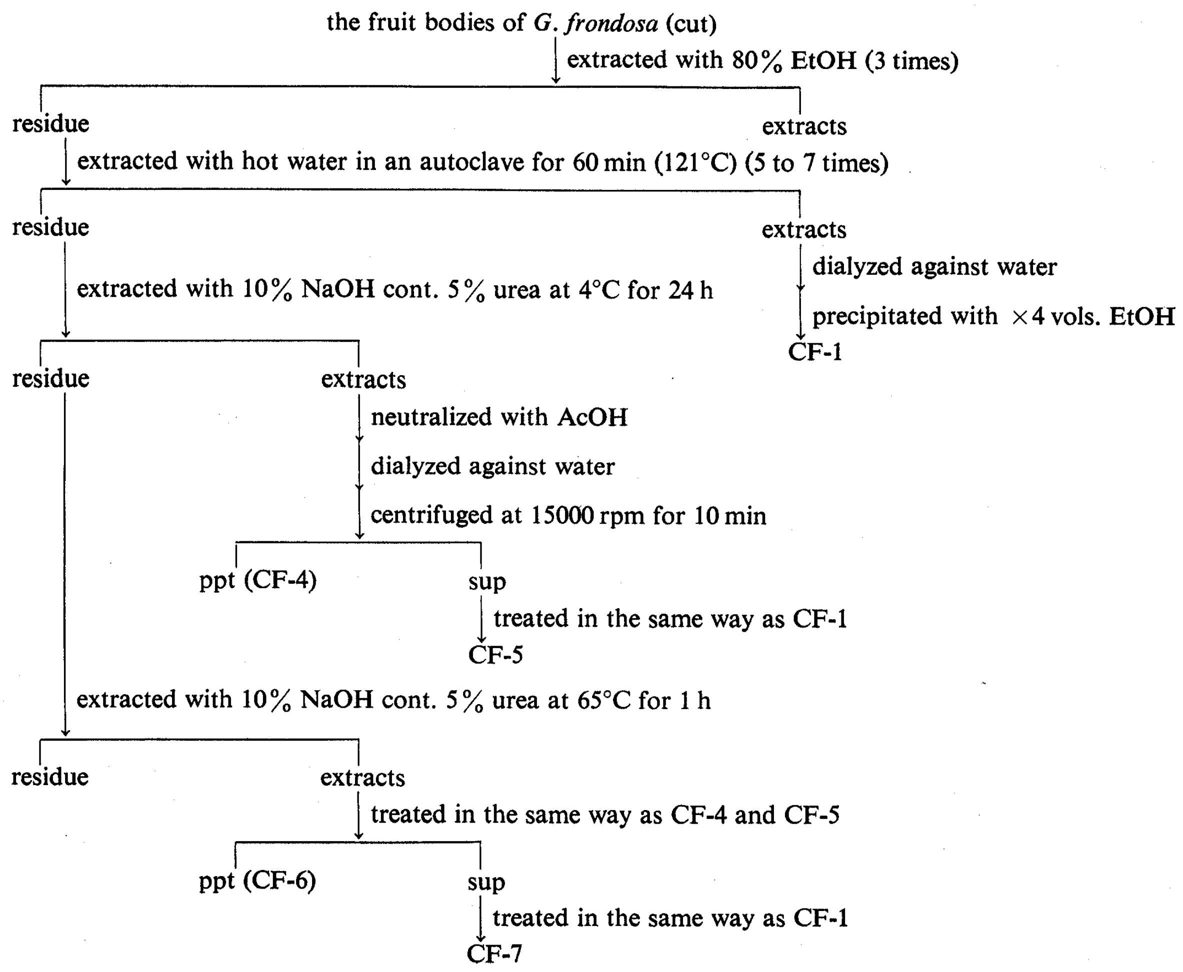

Chart 2. Isolation of the Polysaccharide Fractions from the Fruit Bodies of *G. frondosa* (cut)

TABLE I. Some Properties of Polysaccharide Fractions Extracted from the Fruit Bodies of *G. frondosa*

Fraction	Yield (g)[a]	Sugar (%)	Protein (%)	Component sugars[b]
PF-1	10.7	81	15	Glc
PF-2	0.1	23	18	Glc
PF-3	0.5	100	2	Glc
PF-4	8.4	52	50	Glc, Xyl, Man (6.4 : 1 : 1)
PF-5	4.7	71	15	Glc
CF-1	7.0	70	25	Glc
CF-4	5.6	100	10	n.d.[c]
CF-5	7.1	81	30	n.d.
CF-6	1.2	88	5	Glc, Xyl, Man (3.5 : 1 : 0.4)
CF-7	8.1	89	11	Glc

a) From 100 g of the fruit bodies.
b) Determined as alditol acetate derivatives by gas liquid chromatography.
c) Not determined.

The 2nd procedure, which was used for preparing large quantities, is shown in Chart 2. For this purpose, "cut" fruit body was used. This procedure is a modification of that shown in Chart 1. Zinc chloride extraction was omitted, and hot alkali extraction of the residue of the cold alkali extracts was performed. As shown in Table I, in comparison with that of the 1st procedure, the yield of hot water extract (CF-1) was reduced to 7%, and that of cold alkali extracts (CF-4,5), especially the yield of the water-soluble, cold alkali-extracted glucan fraction (CF-5), was increased. Hot alkali extraction yielded a large amount of water-soluble glucan fraction (CF-7) and a small quantity of water-insoluble heteroglycan fraction (CF-6). All heteroglycan fractions (PF-4, CF-4, CF-6) were composed of Glc, Man, and Xyl.

Molecular Weight of Each Polysaccharide Fraction

Molecular weight of several major fractions were examined on a Sepharose CL-2B column equilibrated with 0.2 M NaOH. As shown in Fig. 1, water-soluble glucan fractions (PF-1, PF-5, CF-7) gave near symmetrical elution profiles and showed very widely distributed molecular weights. On the other hand, most of each water-insoluble heteroglycan fraction appeared to have an Mw of more than 20000000.

Antitumor Effect of Each Polysaccharide Fraction

The antitumor effect of each fraction was assayed by comparing the growth of Sarcoma 180 tumor cells in mice. Each fraction was administered at three doses (40, 400, or 4000 μg/mouse/d). The results are shown in Table II. It was found that all fractions showed growth

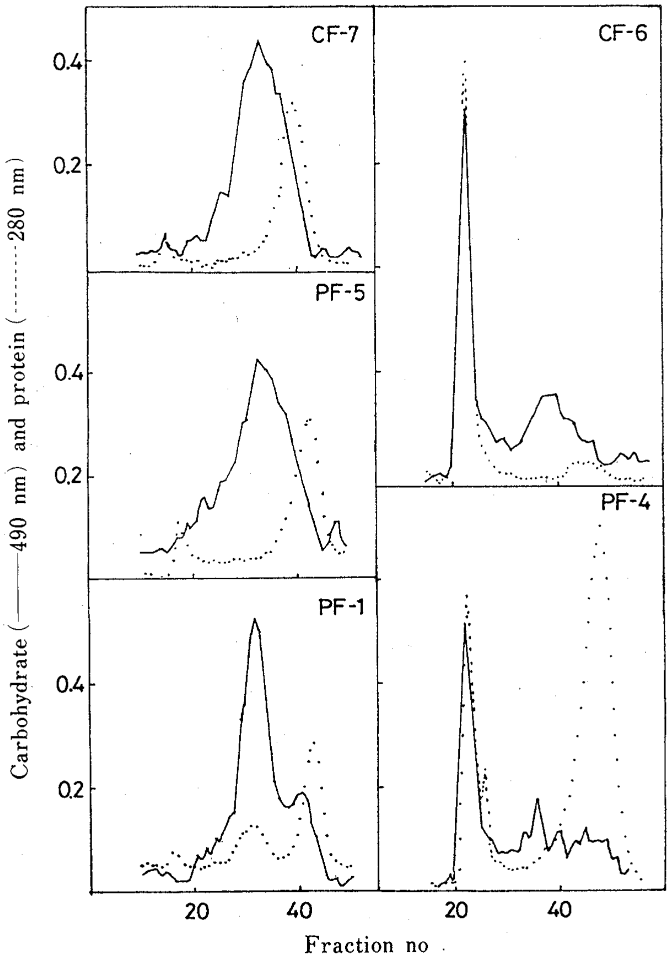

Fig. 1. Elution Profiles of Polysaccharide Fractions from a Column of Sepharose CL-2B

The column (1 × 60 cm) was equilibrated with 0.2 M NaOH, and 10 mg of material was applied. Fractions of 1.5 ml were collected and carbohydrate and protein contents were assayed by the phenol–H_2SO_4 method (----) and by ultraviolet absorption measurement (------), respectively. The void and bed volumes were at fr. 15 and fr. 48, respectively.

inhibition of more than 90% ($p<0.01$). Fractions CF-1,4, and 6 showed antitumor activity, giving more than 90% inhibition ratio at 4000 μg/mouse × 10 d. Fraction CF-5 gave more than 90% inhibition at 400 μg/mouse × 10 d. Furthermore, fraction CF-7 gave more than 90% inhibition at 40 μg/mouse × 10 d. α-Amylase-treated CF-1 also showed antitumor activity. These results suggest that each fraction contains antitumor polysaccharide, and the content of active polysaccharide was highest in the hot alkali-extracted, water-soluble fraction (CF-7).

Chemical Composition of Hot Water-extracted Glucan Fractions (CF-1 and PF-1)

The most abundant linkage in CF-1 was found to be α-1,4-, as deduced from the results of α-amylase digestion. This was also supported by the results of methylation analysis (Table III). CF-1 gave a large amount of 2,3,4- + 2,3,6-Me_3–Glc derivatives and the molar ratio of these derivatives was reduced after the α-amylase digestion. Furthermore, the ^{13}C-NMR spectrum of CF-1, when compared with the spectrum of starch, appeared to contain signals attributable to α-1,4-glucan (Fig. 2).

The most abundant linkage present in α-amylase-digested CF-1 was suggested to be β-1,6-linkage, because methylation analysis gave a large amount of 2,3,4- + 2,3,6-Me_3–Glc, and the major signals observed in the ^{13}C-NMR spectrum were very similar to those of islandican, which is a β-1,6-linear glucan obtained from *Penicillium islandicum* (Fig. 2). Furthermore, CF-1 contains β-1,3-linkage [^{13}C-NMR spectrum (89 ppm) and methylation analysis (2,4,6-Me_3–Glc)], and α-1,6-linkage [^{13}C-NMR spectrum (anomeric carbon at 101 ppm, signals asterisked)]. Thus, it is suggested that the major linkages present in CF-1 are

TABLE II. Antitumor Effect of Polysaccharide Fractions from *G. frondosa*[a)]

Sample	Dose (μg × 10)	No. of mice	Tumor weight (g, mean ± SD)	Inhibition (%)[b)]	C.R. (%)[b)]	Significance p[c)]
Control	—	9	5.87 ± 4.10	—	0	—
CF-1	40	9	4.09 ± 2.31	30.3	0	n.s.[f)]
	400	8	1.87 ± 2.22	68.1	0	0.05
	4000	10	0.07 ± 0.14	98.8	80.0	0.001
CF-4	40	9	5.00 ± 2.91	14.8	0	n.s.[f)]
	400	10	0.73 ± 0.94	87.6	30.0	0.01
	4000	9	0.29 ± 0.36	95.1	11.1	0.001
CF-5	40	9	2.14 ± 2.77	63.5	22.2	0.05
	400	10	0.30 ± 0.53	94.9	40.0	0.001
	4000	10	1.56 ± 1.58	73.4	0	0.01
CF-6	40	9	5.68 ± 3.26	3.2	0	n.s.[f)]
	400	10	1.50 ± 1.54	74.4	0	0.01
	4000	10	0.37 ± 0.56	93.7	30.0	0.001
CF-7	40	9	0.46 ± 0.06	92.2	44.4	0.01
	400	10	0.28 ± 0.21	95.2	10.0	0.001
	4000	10	1.05 ± 1.12	82.1	20.0	0.01
A-CF-1[d)]	40	10	3.31 ± 1.94	43.6	0	n.s.[f)]
	400	9	1.63 ± 2.30	72.2	22.2	0.02
	4000	10	0.31 ± 0.45	94.7	0	0.001
Amylase[e)]		5	10.02 ± 3.26	−70.7	0	n.s.[f)]

a) Sarcoma 180 tumor cells (5×10^6) were inoculated subcutaneously. Each sample was administered as saline solution by intraperitoneal injection.
b) Inhibition and C.R. (complete regression) were determined at 35 d after tumor inoculation.
c) The significance was evaluated according to Student's *t*-test. $p<0.05$ was taken as the criterion of a significant difference.
d) α-Amylase-treated CF-1. Experimental details are given in "Materials and Methods."
e) Calculated amount of α-amylase equivalent to α-amylase-treated CF-1 (4000 μg).
f) Not significant.

TABLE III. Gas Liquid Chromatography of Alditol Acetates Derived from the Methylated Polysaccharides

Alditol acetate of	CF-1	A-CF-1[a)]	PF-5	CF-5	CF-7
2,3,4,6-Me_4–Glc	1.0	1.0	1.0	1.0	1.0
2,4,6-Me_3–Glc	0.11	0.26	2.0	1.7	1.6
2,3,4- and/or 2,3,6-Me_3–Glc	4.7	1.2	0.53	2.8	1.9
2,6-, 3,6- and/or 4,6-Me_2–Glc	0.26	0.52	0	0.1	0.17
2,4-, and/or 3,4-Me_2–Glc	0.60	0.30	1.1	1.0	0.98
2,3-Me_2–Glc	0.17	0.35	0.0	0	0.11

a) α-Amylase-treated CF-1.

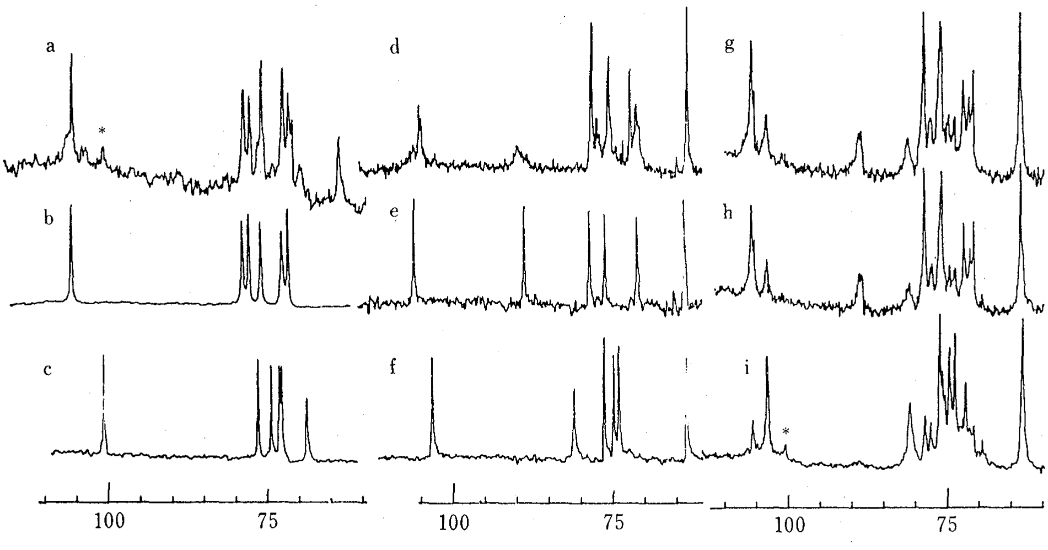

Fig. 2. ^{13}C-NMR Spectra of Polysaccharide Fractions in 0.2 M NaOD

a, α-amylase-treated CF-1; b, islandican (linear β-1,6-glucan); c, dextran T-2000; d, PF-5; e, curdlan; f, starch; g, CF-7; h, CF-5; i, CF-1. Values are given in ppm (TSP as 0.0 ppm).

α-1,4- and β-1,6. The results of methylation analysis and the ^{13}C-NMR spectra of CF-1 and PF-1 were quite similar to each other.

Composition of Alkali-extracted, Water-soluble Glucan Fractions (PF-5 and CF-5)

Methylation data and the ^{13}C-NMR spectra indicated that PR-5 and CF-5 are quite different from each other. This is presumably due to compositional differences of polysaccharides extracted from the powdered or cut materials, since the yield of PF-1 was greated than that of CF-1 (Table III).

The ^{13}C-NMR spectrum of PF-5 in 0.2 M NaOD solution was quite similar to that of lentinan[7)] and, therefore, suggested that PF-5 contains a large amount of C-6 branched β-1,3-glucan. Further, the ^{13}C-NMR spectrum of PF-5 in DMSO-d_6 characteristically showed a triplet signal due to substituted carbon 3 of a β-glucosyl unit and was quite similar to that of scleroglucan, which is also a C-6 branched β-1,3-glucan.[8)]

Methylation of PF-5 gave a small quantity of 2,3,4-+2,3,6-Me_3–Glc. However, this fraction was not digestable by α-amylase, as described above. The ^{13}C-NMR spectrum of PF-

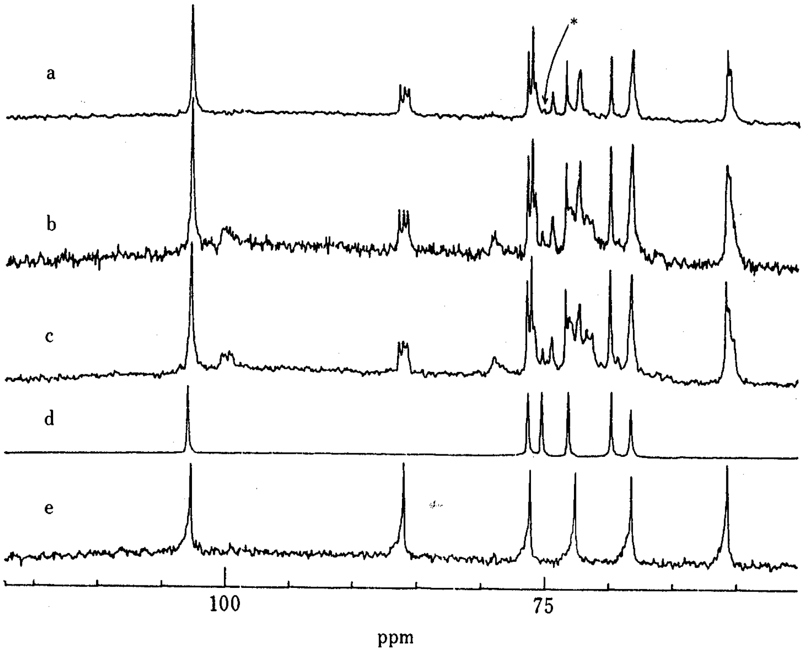

Fig. 3. ^{13}C-NMR Spectra of Polysaccharide Fractions in DMSO-d_6

a, PF-5; b, CF-5; c, CF-7; d, islandican; e, curdlan. Values are given in ppm (DMSO as 39.5 ppm).

5 in DMSO-d_6 at 60 °C shows signals probably attributable to a linear β-1,6-glucan (asterisked). These results suggest that the tri-*O* methyl derivative is 2,3,4-Me_3–Glc. The molar ratio of 2,4,6-Me_3–Glc to 2,4-Me_2–Glc in PF-5 was about 2 : 1 and, in the ^{13}C-NMR spectrum, carbon-3 of β-1,3-linked Glc gave a triplet signal. These results suggest that PF-5 is mainly composed of a glucan consisting of a β-1,3-linked main chain in which one of every three glucose residues is branched at the 6-carbon with a β-linkage.

The ^{13}C-NMR spectra of CF-5 in NaOD and DMSO-d_6 suggest that CF-5 contains C-6 branched β-1,3-glucan, because almost all of the signals observed in PF-5 were also predominant in CF-5. In addition, methylation and ^{13}C-NMR spectral analysis of CF-1, PF-5, and CF-5 suggest that CF-5 contains CF-1 as a contaminant. The molar ratio of 2,4,6-Me_3–Glc to 2,4-Me_2–Glc was higher than that of PF-5. However, the ^{13}C-NMR spectra suggest similarity of the 6-branched β-1,3-glucan in CF-5 and PF-5. Detailed chemical characterization is in progress.

Chemical Composition of Hot Alkali-extracted, Water-soluble Glucan Fraction (CF-7)

The ^{13}C-NMR spectrum of CF-7 was similar to those of CF-5 and PF-5. The ^{13}C-NMR spectrum and methylation analysis suggest that the most abundant component present in CF-7 is a partially carbon-6 branched β-1,3-glucan because of the presence of triplet signals due to carbon 3 of 3-substituted β-glucosyl units. It appears that not only CF-5 but also CF-7 contains water-extractable glucans, and that the contamination of water-extractable glucans were higher in CF-5 than in CF-7.

Discussion

As described in the results section, cutted fruit bodies of *G. frondosa* contain various kinds of water-soluble polysaccharides extractable with water and aqueous sodium hydroxide containing urea. The hot water extract contains a large amount of α-1,4-glucan, whereas the cold and hot alkali extracts contain a large amount of β-glucans. Among these glucan fractions, antitumor activity was strongest in the hot alkali extract, and weakest in the hot water extract.

The hot water extract and also the cold and hot alkali extracts showed potent antitumor activity against solid Sarcoma 180 tumor, and it is considered that the activity is due to 6-branched β-1,3-glucan. Structural analysis was performed by methylation and ^{13}C-NMR spectral analyses. The identification of triplet signals of a 3-linked β-glucasyl unit in ^{13}C-NMR spectroscopy using DMSO-d_6 as a solvent (Fig. 3, *ca.* 85 ppm) was the most important observation pointing to the 6-branched β-1,3-glucan structure. The integration of these signals from *G. frondosa* was similar to that of scleroglucan,[8] but different from that of PS-1426.[9] Both scleroglucan and PS-1426 are known to be 6-branched β-1,3-glucans, but these are fewer branching points in the former. Scleroglucan and PS-1426 were branched at every three and two 3-substituted β-glucosyl units, respectively. From the results of this and the methylation study, the glucan found in *G. frondosa* is considered to be branched at every three 3-substituted β-glucosyl units.

Antitumor polysaccharides have been identified in many kinds of Basidiomycetes and Ascomycetes (*e.g.*, lentinan, schizophyllan, PS-1426, TAK, and PSK). Lentinan, schizophyllan, PS-1426, and TAK are composed of only carbohydrates. On the other hand, PSK require a protein moiety for activity. The antitumor activity of *G. fronodosa* was suggested to be due to be presence of 6-branched β-1,3-glucan for the following reasons. (1) Antitumor activity was positively correlated with the proportion of 6-branched β-1,3-glucan in these fractions. (2) α-Amylase digestion of CF-1 did not affect the antitumor activity of CF-1 (Table II), so α-1,4-glucan is not involved in the antitumor activity of *G. frondosa.* (3) The antitumor activity of periodate-oxidized/borohydride-reduced CF-1 was similar to that of CF-1 (data not shown), suggesting the involvement of 6-branched β-1,3-glucan in the antitumor activity. (4) A partially purified glucan fraction from the hot water extract, called GF-1, which was prepared by the usual copper complex method[3] and contained more than 90% carbohydrate, showed similar activity to the hot water extract (manuscript in preparation).

From the methylation and ^{13}C-NMR spectral analyses, it is suggested that the 6-branched β-1,3-glucans in the water-soluble glucan fractions extracted with hot water and cold and hot alkali have essentially the same primary structure. However, the average molecular weight of the hot alkali extract was smaller than that of the cold alkali extract (Fig. 1). Further, it should be noted that a β-1,3-glucan possessing no antitumor activity (pachyman) shows antitumor activity after being heated with urea (U-pachyman).[10]

Recently, Kuroda *et al.*[4] reported that the hot water extract of *G. frondosa* showed potent antitumor activity, but suggested that the antitumor-active structure is a 3-branched β-1,6-glucan. White the findings presented in this paper is limitted and we can not find 3-branched β-1,6-glucan from the hot water extract until now, it would be interest that two types of glucans, β-1,3 and β-1,6 are present in the fruit body of *G. frondosa.*

A detailed examination on the antitumor activity and the chemical structure of the glucans isolated from cultured fruit body of *G. frondosa* is in progress. Since *G. frondosa* is used as a food in Japan, as is *Lentinus edodes* (from which lentinan was isolated), the isolation of antitumor-active component(s) from the fruit body is of considerable interest.

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