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ISOLATION, PURIFICATION, AND STRUCTURE OF COMPONENTS FROM ACIDIC POLYSACCHARIDES OF *Pleurotus ostreatus* (Fr.) Quél.*

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ABSTRACT

Isolation of an antitumor component from polysaccharide fraction A_5 of some Basidiomyces was achieved by column chromatography on Sephadex G-200. A detection method based on the specific rotatory characteristics of the polysaccharide was applied to estimate components in effluent fractions from the chromatography, and it was confirmed that a series of eluates having similar specific rotation was made up of homogeneous polysaccharide. Three components (H_{51}, H_{52}, and H_{53}) were isolated, in chromatographically pure state, from fraction A_5. Component H_{51} consisted of a skeleton of β-(1→3)-linked glucose residues, probably having branches of galactose and mannose residues, and also containing acidic sugars. Component H_{53} had a main structure similarly consisting of β-(1→3)-linked glucose residues and a larger proportion of acidic sugar than H_{51}. Component H_{52} was a hetero-polysaccharide made up of α-linked galactose and mannose residues. Components H_{51} and H_{53} had a higher and a lower molecular weight, respectively, than H_{52}. The only antitumor-active component was H_{51}.

INTRODUCTION

We have been attempting to find a chemotherapeutic method for selectively destroying tumors within the host by chemical agents. Our attention was called to some polysaccharide fractions, prepared from natural sources[2–9], that, although not cytocidal, have some antitumor properties. When a mouse bearing a solid-tumor transplant is treated with an antitumor-active polysaccharide fraction, growth of the tumor is inhibited, followed by regression. It has been considered that this therapeutic effect is due to a host-mediated action[10–14].

In our previous study[9], a water-soluble extract having antitumor activity was obtained from a fungus of the Basidiomycetes group, *Pleurotus ostreatus* (Fr.) Quél., the edible mushroom popularly named "Hiratake" in Japanese. Two antitumor-active fractions (A_5 and A_3) were obtained from the extract by several fractionation

*Antitumor Activity of Some Fractions from Basidiomyces. II. For part I, see ref. 1.

methods[1]. The two active fractions, A_5 and A_3, were found to consist of macromolecules, made up of a polysaccharide mainly composed of glucose, and constituents other than polysaccharide were negligible. Fraction A_5 differed from fraction A_3 in some of its chemical properties. Fraction A_5 was found to be an acidic polysaccharide that had a low, positive specific rotation, but fraction A_3 was a neutral polysaccharide that had a high, positive specific rotation.

Fraction A_5 was fairly pure, because it gave a single spot on electrophoresis in 0.1M borate buffer[1]. However, in a study on the relationship between some kind of biological activity and a product, prepared from natural sources, that has this activity, purity of the sample is of importance. When this sample is a polysaccharide preparation, attention should be given to the range of its molecular weight. In the case of fraction A_5, the possibility that a few kinds of components were present in it was not excluded. Its further purification was, therefore, attempted.

This paper describes some useful detection methods by which the chromatographic purity of the polysaccharide fractions was estimated. It also describes fractionation of fraction A_5 by gel chromatography; thereby, three pure components were obtained, as revealed by the aforementioned detection methods, and their chemical structure was elucidated. The relationship between the chemical structure and the antitumor activity of each pure component will also be discussed.

EXPERIMENTAL

General. — Thin-layer chromatography (t.l.c.) of hydrolyzates was performed on crystalline cellulose (Avicel SF) with a solvent system of 6:4:3 (v/v) 1-butanol–pyridine–water[15]. Development was repeated 4 times in a rectangular tank. The plate was sprayed with diphenylamine–aniline reagent[16], and heated at 110°. Another t.l.c. analysis of acetolyzates was performed on Silica Gel G with 1:24 (v/v) methanol–benzene[17]; the chromatogram was developed in a sandwich chamber (Toyo Roshi Co., Model CF-TLC), the top of which is not covered, so that the solvent continually ascends and evaporates from the uncovered area. The unidimensional, multiple development of the acetolyzates was conducted for ~2 h. After the development, the plate was heated at 110°, and then sprayed with ammonium vanadate–sulfuric acid reagent, and further heated, if necessary. The reagent was prepared by adding 50% sulfuric acid (50 ml) to ammonium vanadate (2 g) suspended in water (5 ml). Infrared (i.r.) spectra were recorded with a Japan Spectroscopic Co. Model DS-402G spectrometer. Optical rotations were measured with a Japan Spectroscopic Co. Model DIP-S automatic polarimeter. Gas–liquid chromatography (g.l.c.) was performed with a Shimadzu Model GC-4A (PF) gas chromatograph, fitted with a hydrogen-flame detector and a glass column (3 m × 4 mm) packed with 2% of OV-17 on Chromosorb W. Samples were converted into the corresponding trimethylsilyl ethers[18], and were separated by performing linear, temperature-programmed analyses (100–200°, at $4°.min^{-1}$).

Autoanalysis of neutral sugars. — Liquid-chromatographic autoanalyses were

performed with a Japan Electron Optics Lab. liquid-chromatographic autoanalyzer Model JLC-3BC, fitted with an anion-exchange column (13 cm × 8 mm). The anion-exchanger, JEOL Resin LC-R-3, was transformed into the borate form and was equilibrated with 0.13M borate buffer of pH 7.5. Samples were dissolved in the 0.13M borate buffer, the solutions were applied to the column (which was water-jacketed at 55°), and the column was eluted stepwise with three different borate buffers at a flow-rate of 0.51 ml.min^{-1}. The first buffer (0.13M borate of pH 7.5) was run during 100 min, the second (0.25M of pH 9.0) during 100 min, and the neutral sugars retained were finally eluted with 0.35M borate of pH 9.6. (Under these conditions, acidic components were still retained.) Color was developed with 0.15% orcinol in 90% sulfuric acid at 95°, and measured at 423 and 510 nm. For quantitative analysis, the integrated area of the peak for each sugar was measured in the spectrum, and the quantity of the sugar was calculated by comparing with that of the corresponding peak in a standard spectrum. The content of each sugar in the sample is expressed as the ratio of that sugar to the total content of glucose, galactose, and mannose.

Isolation. — Extraction of the antitumor-active fraction from the fruit bodies of *P. ostreatus* (Fr.) Quél. with water, and separation into the acidic polysaccharide fraction A_5 and the neutral polysaccharide fraction A_3, were conducted as described previously[1].

Fractionation. — (*a*) *Preparation of samples for estimation of molecular weight.* Sephadex G-200 was packed into a column (36 × 2.5 cm) with de-ionized water. A solution of fraction A_5 (200 mg) in water (2 ml) was applied to the Sephadex gel. Fraction A_5 was eluted with water at a flow-rate of 24 ml.h^{-1}, and fractions of 8 ml each were collected; the optical rotation was measured, and the solution was lyophilized, and the residue weighed. The specific rotation was calculated from these data, and recorded as shown in Fig. 1. Fractions having the higher specific rotation and those with the lower specific rotation were respectively combined (A_{52} and A_{53}). This method of preparation was duplicated.

(*b*) *Preparation of samples for bioassay, and chemical analysis.* Fraction A_5 (200 mg) in water (1 ml) was eluted with water at a flow-rate of 8–12 ml.h^{-1} from a column (96 × 2.5 cm) packed with Sephadex G-200; fractions of 10.3 ml each were collected, and the specific rotation was measured; the elution pattern was charted by use of these values. One sample was obtained from one effluent fraction, or a few fractions were united to give enough sample for analyses, as shown in Fig. 2.

(*c*) *Preparation of pure components by gel chromatography on Sephadex G-200.* Fraction A_5 (200 mg) in water (2.4 ml) was applied to the Sephadex G-200 column (97 × 2.5 cm), and eluted with water at a flow-rate of 18 ml.h^{-1}; fractions of 10.3 ml each were collected, and the specific rotations of these fractions were measured, and plotted as shown in Fig. 5. Three components (HA_{51}, HA_{52}, and HA_{53}) were recognized on the basis of the difference in their specific rotations. This method of preparation was duplicated. Component HA_{51} (99 mg) in water (3.5 ml) was rechromatographed under the same conditions. Pure component H_{51} was obtained by collecting the eluates ranging in specific rotation from +8 to +25° ($[\bar{\alpha}]_D^{20}$ +16°), as shown in

Fig. 6. Component HA_{52} (113 mg) in water (2.5 ml) and HA_{53} (272 mg) in water (3 ml) were rechromatographed at a flow-rate of 12 ml.h^{-1}, and H_{52} and H_{53} were respectively obtained from the eluates ranging in specific rotation from +105 to 159° ($[\bar{\alpha}]_D^{20}$ +128°) and from −28 to −18° ($[\bar{\alpha}]_D^{20}$ −24°), as shown in Figs. 7 and 8.

Complete hydrolysis with acid. — Each sample (4.5–10 mg) was hydrolyzed in 0.5M sulfuric acid (5 ml) for 4 h on a boiling-water bath. The acid was neutralized with barium carbonate to a final pH of 7, the barium sulfate was filtered off (Millipore membrane), and the remaining traces of barium ions were removed by passing the solution through a column of Amberlite CG-120 (H^+) ion-exchange resin. The column was washed with water (150 ml) to recover all sugars, and the eluate and washings were combined, and lyophilized to give the material in the hydrolyzate.

Partial hydrolysis with acid. — Each sample (10–11 mg) was heated in 125mM sulfuric acid (5 ml) for 2 h on a boiling water bath, except H_{51}, which was hydrolyzed for 255 min. The acid was neutralized with barium carbonate, the barium sulfate was filtered off (Millipore membrane), and the filtrate and washings were passed through a column of Amberlite CG-120 (H^+) ion-exchange resin to remove all barium ions. The effluent was lyophilized.

Partial acetolysis. — Each sample (10–11 mg) was dissolved by mechanical stirring in 1 ml of 10:10:1 (v/v) acetic anhydride–glacial acetic acid–98% sulfuric acid while the reaction mixture was externally cooled with ice–water. The mixture was allowed to react for 96 h at room temperature, except for H_{53}, which was kept for 48 h. The resulting solution was poured into ice–water, made neutral with sodium carbonate to a final pH of 7, and extracted with chloroform. The extract was dried (anhydrous sodium sulfate), and evaporated to dryness under diminished pressure.

Method for assay of antitumor activity. — The mice used were females of the ICR strain, and initially weighed ~20 g. Ascites of sarcoma-180 (0.05 ml; $\sim 8 \times 10^6$ cells) was subcutaneously injected into the right groin of each mouse. At elapse of 24 h after tumor implantation, a solution of the sample in distilled water was intraperitoneally injected daily for 10 days. The growth of the solid tumor was charted weekly, and the tumor was weighed at the end of 5 weeks. The inhibition ratio was calculated by comparing the average weight of the tumors of treated mice with that of those of untreated controls.

RESULTS AND DISCUSSION

The polysaccharide fraction A_5 had antitumor activity, and was relatively soluble in water; the aqueous solution was not so viscous that it could not be applied in molecular-sieve chromatography. Purification of fraction A_5 was attempted by chromatography on Sephadex G-200. In a preliminary experiment, an aqueous solution (2 ml) of fraction A_5 (200 mg) was applied to a column (36 × 2.5 cm) packed with Sephadex G-200. Components were eluted with water at a flow-rate of 24 ml.h^{-1}, and the eluates were collected on a volume basis.

In chromatography of polysaccharides, it is difficult to detect a series of

effluent fractions in which one component is eluted, as it is difficult to analyze the secondary structure of polysaccharides by using a small quantity of a sample. The specific rotation of a polysaccharide is characteristic of this chemical structure, and the sample used is not lost. In chromatography, if solutes in adjacent effluent fractions have the same specific rotation, they should have the same components. A series of effluent fractions having the same specific rotation consists of homogeneous fractions; it indicates one peak on the chromatogram. Specific rotation is useful for the detection of polysaccharide species in chromatography.

In the chromatography of fraction A_5, the optical rotation of each effluent fraction was measured, the eluate was lyophilized, and the dry product was weighed. The specific rotation of each effluent fraction was calculated from the equation: $[\alpha]_D = (100\alpha \times 100v)/(w \times l)$, where α is the value of the optical rotation of the effluent fraction, w is the weight (in mg) of the dry product, v is the volume (in ml) of the eluate in a fraction tube, and l is the length (in cm) of the light path. An elution pattern was charted by plotting these values of specific rotation against the number of the effluent fraction. The calibration curve indicating the difference between the positive and negative values of specific rotation is shown in Fig. 1. It seemed possible

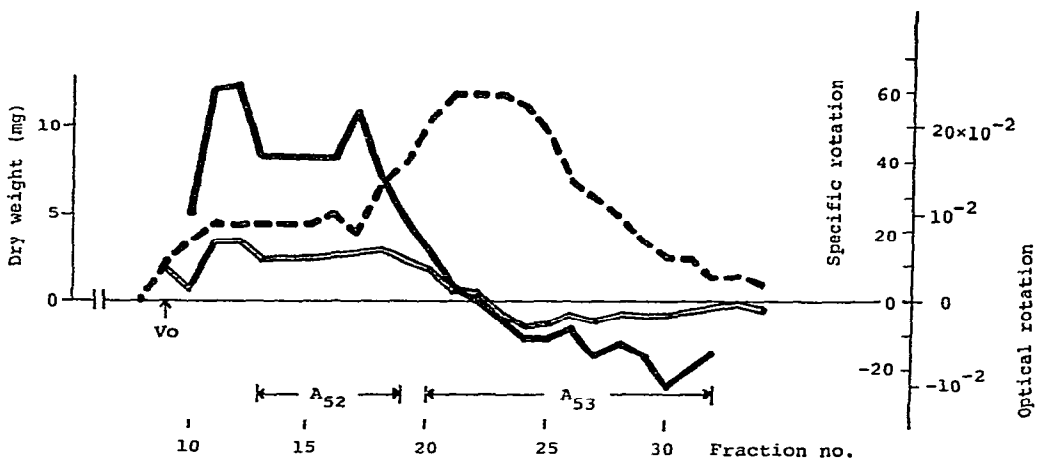

Fig. 1. Elution pattern of fraction A_5 from Sephadex G-200 column (36 × 2.5 cm). (Key: ▬ ▬ ▬ W, dry weight; ═══ α, optical rotation in 2-cm cell; and ——— $[\alpha]_D^{20}$, calculated specific rotation.)

that fraction A_5 contained at least two components, namely, A_{52} with the positive, and A_{53} with the negative, specific rotation. Component A_{52} had a molecular weight larger than that of A_{53}. The two components were subdivided by chromatography. The molecular weight of each subdivided sample was estimated by light-scattering experiments; the molecular weight of A_{52} was 16×10^5, and that of A_{53} was 2×10^5.

The following experiments were performed to find the relationship between the antitumor activity and the chemical properties of the component obtained from

fraction A_5. Samples for bioassay and chemical analysis were prepared by chromatographic fractionation. Fraction A_5 was separated in a column (96 × 2.5 cm) packed with Sephadex G-200, as shown in Fig. 2. The samples obtained by the fractionation were hydrolyzed, and the constituent neutral sugars were analyzed by liquid chromatography on an anion-exchange resin (borate form). Glucose, galactose, and mannose were detected in the samples, and traces of unidentified sugars were found in some

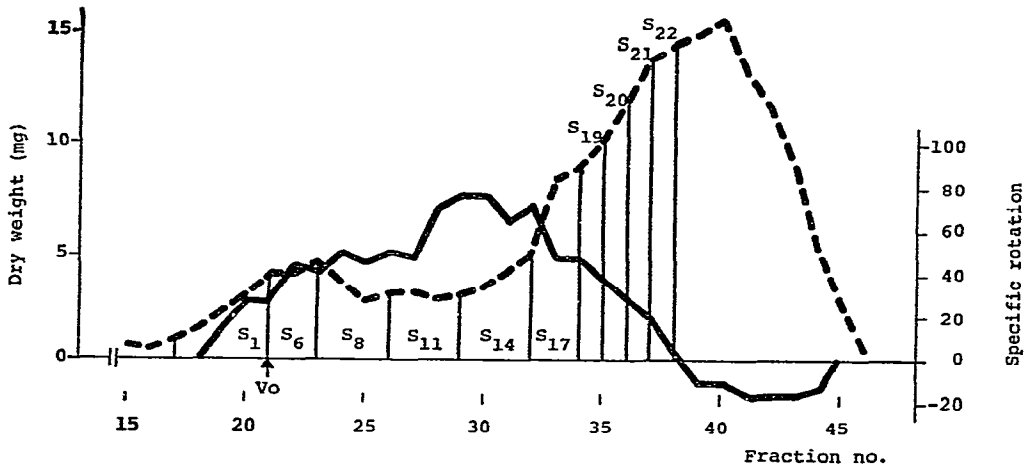

Fig. 2. Fractionation of fraction A_5 for preparation of samples on Sephadex G-200 column (96 × 2.5 cm). (Key: — — — w, dry weight; and ——— $[\alpha]_D^{20}$, specific rotation.)

TABLE I

ANTITUMOR ACTIVITIES AND NEUTRAL SUGAR COMPOSITION OF SAMPLES PREPARED FROM FRACTION A_5 BY GEL CHROMATOGRAPHY

Sample	*Antitumor activity*[a]		*Proportion of sugars*		
	Inhibition ratio (%)	*Grading*[b]	*Glucose* (%)	*Galactose* (%)	*Mannose* (%)
S_1	91	+++	75	18	8
S_6	91	+++	76	18	6
S_8	85	+++	61	26	13
S_{11}	55	++	44	31	25
S_{14}	17	−	36	36	28
S_{17}	27	−	41	39	19
S_{19}	23	−	51	30	19
S_{20}	24	−	59	27	14
S_{21}	−9	−	66	22	13
S_{22}	17	−	70	19	11

[a]Bioassay at a dose of 1 mg/kg/day × 10. [b]Key: −, 0–30%; +, 30–50%; ++, 50–70%; and +++, 70–100%.

samples. The proportion of glucose, galactose, and mannose in a sample is expressed as a value relative to the sum of their analytical values. The results are summarized in Table I. The proportions of sugars in this Table suggested that fraction A_5 is a mixture of polysaccharides containing a variety of constituent sugars, and that fraction A_5 is fractionated into three component parts by gel chromatography. The first component was the largest molecular species and was mainly constituted of glucose residues. The next part differed from the first in that it was rich in galactose and mannose. The last part contained glucose as the preponderant sugar, as for the first, but this component had a smaller molecular size. The component parts consisting of glucose corresponded approximately to the eluate fractions having the lower specific rotation, but that containing galactose and mannose was distributed in the eluate fractions having the higher specific rotation. The proportions of galactose and mannose ran parallel to the values of the specific rotation, as shown in Fig. 3. From these correlations between the values of the specific rotation and the proportions of sugars in the samples, it was speculated that the first largest molecule was made up mainly of a β-glucan, the second part largely contained α-linked galactose and mannose residues, and the last part was also made up of a β-glucan.

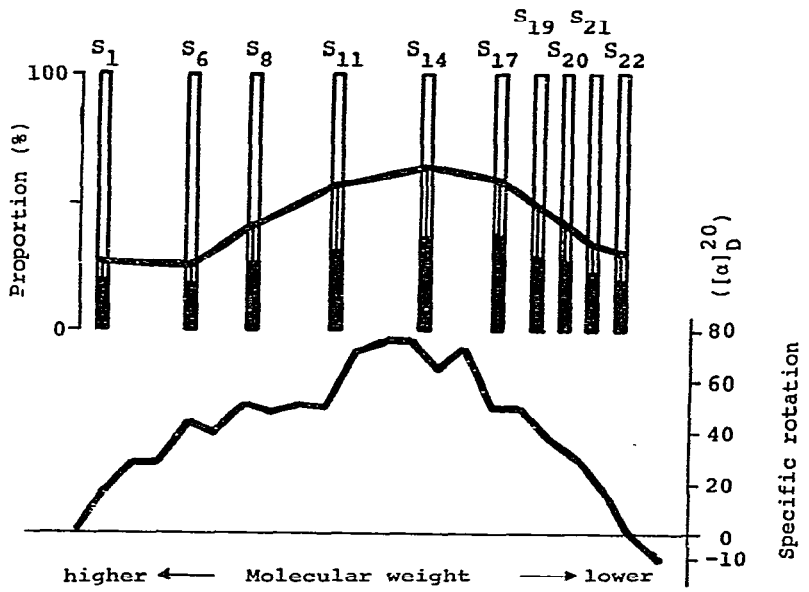

Fig. 3. Correlation of specific rotation of fractions to proportions of galactose–mannose of samples obtained from fraction A_5 by gel chromatography. (Key: proportion of glucose, ═══; mannose, ═══; and galactose, ▬▬.)

Each of the samples was administered intraperitoneally in a dose of 1 mg/kg/day × 10 to tumor-bearing mice. The antitumor activity of various samples is shown in Table I. The activity was maximal for the samples having the largest

molecules, and samples containing smaller molecules were inactive, although a tailing of the activity was observed. The active samples (S_1, S_6, and S_8) consisted of β-glucan, and the inactive samples (S_{21} and S_{22}) were also β-glucan, suggesting that the structure of the β-glucan is not essential for the activity. Correlation of the activities to the proportion of glucose and to the molecular weight of the samples is shown in Fig. 4. The activity had some correlation to the molecular size of the samples, but the kind of constituent sugars did not have any direct correlation with the activity.

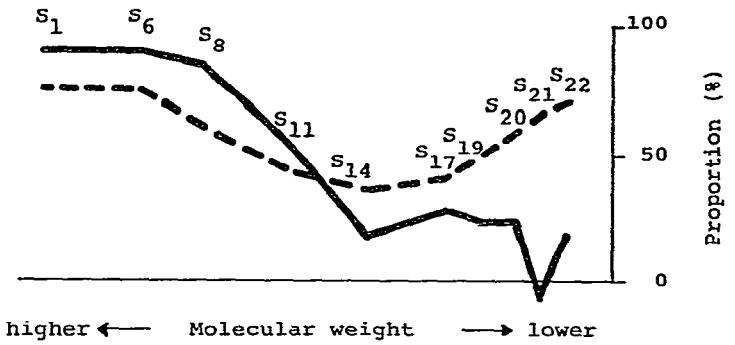

Fig. 4. Correlation of antitumor activities to proportion of glucose or molecular weight for samples prepared from fraction A_5 by gel chromatography. (Solid line, antitumor activity; dashed line, proportion of glucose.)

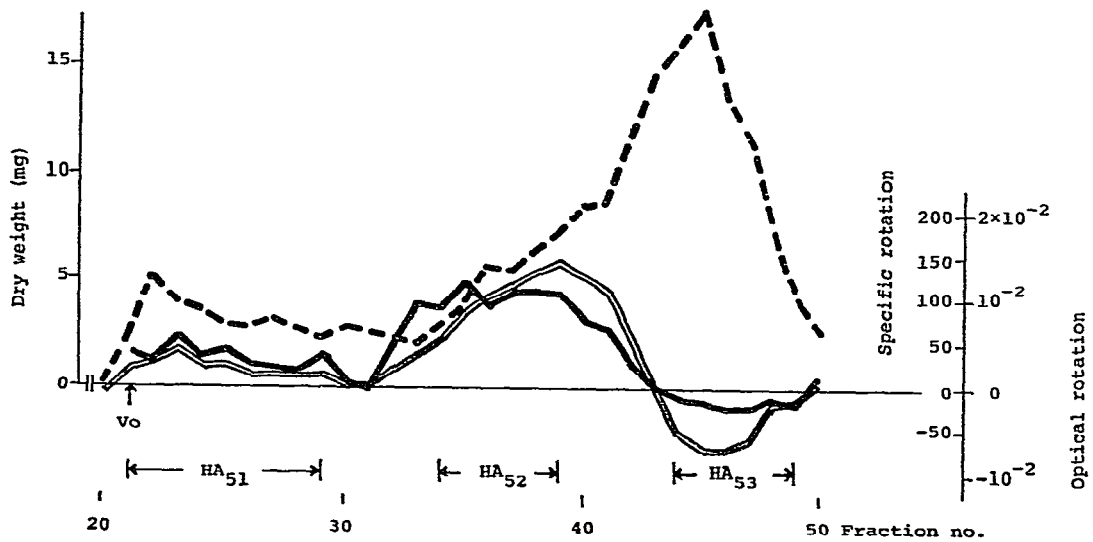

Fig. 5. Gel chromatography on Sephadex G-200 column (97 × 2.5 cm) for fractionation of fraction A_5 into three components. (Key: ▬▬ w, dry weight; ═══ α, optical rotation in 2-cm cell; and ▬▬ $[\alpha]_D^{20}$, specific rotation.)

Because of these results, an attempt was made to (*a*) obtain three pure components from fraction A_5, (*b*) confirm their purities by the detection method using measurement of the specific rotation of fractions obtained in Sephadex G-200 chromatography, and (*c*) establish their chemical structure and their antitumor activity. Chromatography of fraction A_5 was conducted on a column (97 × 2.5 cm) packed with Sephadex G-200, and the specific rotations of the fractions were measured and plotted (see Fig. 5). The curve of values of specific rotation was found to consist of three distinct regions, in which these values were approximately constant. Component HA_{51} was found in the first region (with the low, positive value), HA_{52} in the second region (with the high, positive value), and HA_{53} in the last region (with the negative value).

For purification of these components, each component was rechromatographed under the same conditions. The resulting "rechromatograms" of HA_{51}, HA_{52}, and HA_{53} are shown in Figs. 6, 7, and 8, respectively. In the rechromatogram of HA_{51}, almost all of the fractions showed a constant value of specific rotation ($[\alpha]_D^{20}$ +16°). In the rechromatogram of HA_{52}, the main fractions showed an almost constant value of $[\alpha]_D^{20}$ +128°. The rechromatogram of HA_{53} also showed a region having a constant value, namely, $[\alpha]_D^{20}$ −24°, except for the fractions in the first half of the curve. It was assumed that, in these chromatograms, the regions having a constant specific rotation were homogeneous fractions, and that the other regions were mixtures of components. This supposition was confirmed from the results of the following experiment. On rechromatography of HA_{53}, sample No. 39 was obtained from the first effluent fraction in the region of constant specific rotation, and another sample, No. 47, was obtained from the last fraction in the same region, as shown in Fig. 9. Both samples were hydrolyzed, and the proportions of constituent sugars were determined by liquid chromatography. These analytical data gave the values listed in Table II. This confirmed that the eluates were made up of a homogeneous component from fractions No. 39 to 47, and that the component was chromatographically

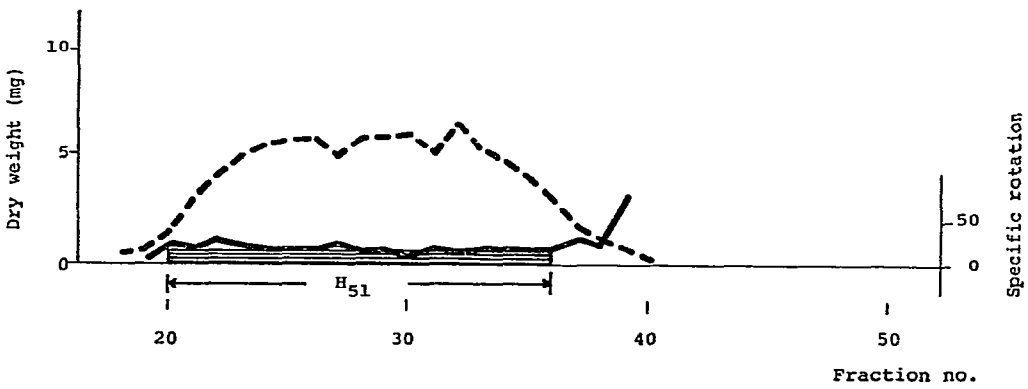

Fig. 6. Rechromatography on Sephadex G-200, to obtain the pure component H_{51} from HA_{51}. (Key: ▬▬ w, dry weight; and ——— $[\alpha]_D^{20}$, specific rotation.)

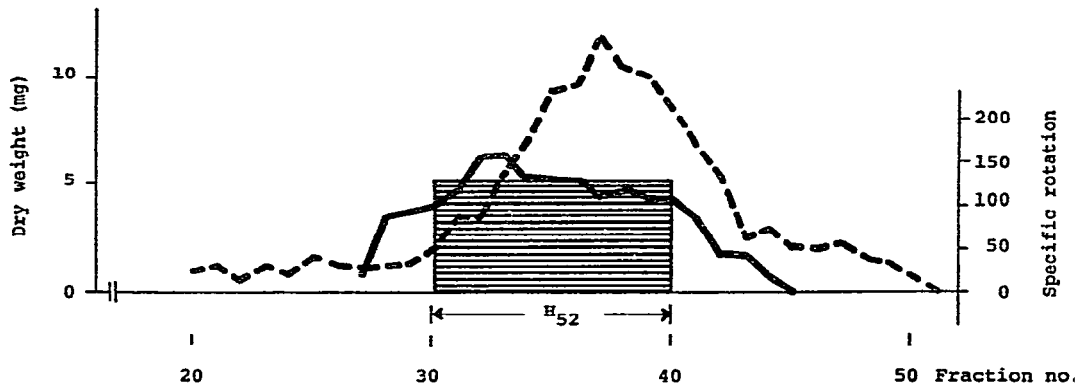

Fig. 7. Rechromatography on Sephadex G-200, to obtain the pure component H_{52} from HA_{52}. (Key: ▬▬ w, dry weight; and ——— $[\alpha]_D^{20}$, specific rotation.)

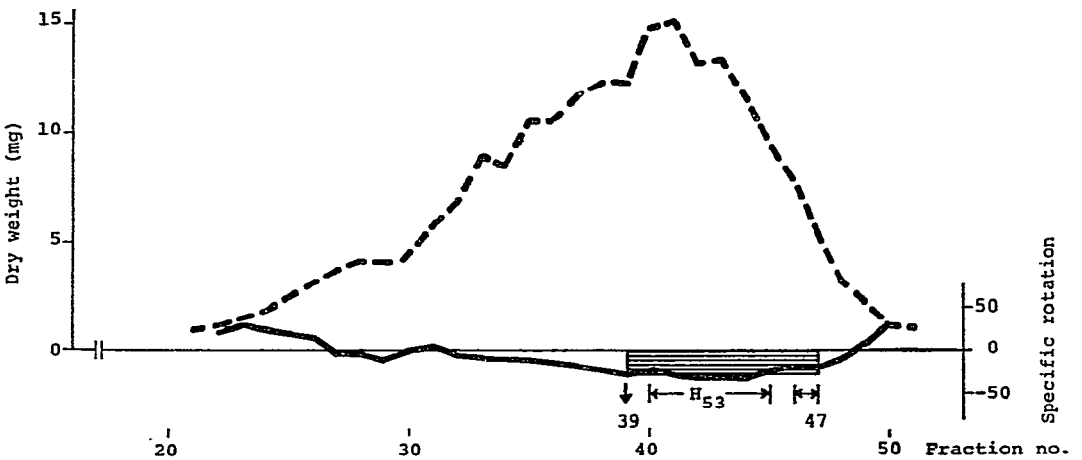

Fig. 8. Rechromatography on Sephadex G-200, to obtain the pure component H_{53} from HA_{53}. (Key: ▬▬ w, dry weight; and ——— $[\alpha]_D^{20}$, specific rotation.)

pure. The three pure components (H_{51}, H_{52}, and H_{53}) were obtained on the basis of the constancy of the respective specific rotation, as shown in Figs. 6, 7, and 8.

Components H_{51}, H_{52}, and H_{53} were completely hydrolyzed, and the constituent sugars in these components were qualitatively determined by g.l.c. and t.l.c., the neutral sugars were quantitatively determined by liquid chromatography, and the acidic sugars were estimated by the carbazole method. These data and the physicochemical properties of these pure components are summarized in Table II. The chemical analyses indicated that H_{51} and H_{53} are mainly constituted of glucose residues, with a trace of galactose and mannose residues, and that H_{52} is a heteroglycan constituted of galactose and mannose residues. The specific rotation of H_{51}

TABLE II

PHYSICOCHEMICAL PROPERTIES AND SUGAR COMPOSITION OF THE PURE COMPONENTS OBTAINED FROM FRACTION A_5 BY GEL CHROMATOGRAPHY

Component	$[\alpha]_D^{20}$ (*degrees*)[a]	*Infrared absorption*[b] (cm^{-1})		*Proportion of neutral sugars*			*Content of uronic acid*[c] (%)
		Characteristic absorption	*Shift of band from salt form to acid form*	*Glucose* (%)	*Galactose* (%)	*Mannose* (%)	
H_{51}	+16	885(w)	1610 → ~1710	79	15	6	13
H_{52}	+128	870(w), 790(m)	→ 1710(vw)	15	52	34	12
H_{53}	−24	—	→ 1715(s)				44
No. 39				89	8	3	
No. 47				94	5	1	

[a]Arithmetic mean of the values for fractions from the gel chromatography. [b]Key: m = moderate, s = strong, v = very, and w = weak. [c]Carbazole method, with D-glucuronic acid as standard. These values are not corrected for the kind of acidic sugar.

was low (+16°), and its i.r. spectrum had an absorption band of type 2b at 885 cm^{-1}. These properties suggested that the glucosidic linkages in H_{51} have mainly the β configuration. It seemed probable that the structure of H_{53} was mainly of the β-glucan type on the basis of the negative value of its specific rotation (−24°). The high value of the specific rotation (+128°) of H_{52} indicated a preponderance of α-glycan.

Examination was made of the position through which the glucose residues were linked. The samples were partially hydrolyzed, and the oligosaccharides in each hydrolyzate were analyzed by t.l.c. on crystalline cellulose. In both H_{51} and H_{53}, the R_F value of the main spot from each hydrolyzate agreed with that of laminarabiose, and gentiobiose and cellobiose were not detected. Acetolyzates of H_{51}, H_{52}, and H_{53} were obtained by controlled acetolysis, and the resulting sugar acetates were characterized by t.l.c. on silica gel G, by unidimensional, multiple development. In the acetolyzate of H_{51}, most of the spots on the chromatogram agreed in position with those for the acetolyzate of pachyman, but not with those from amylose and dextran. When a series of log R_F values of these spots obtained by the chromato-

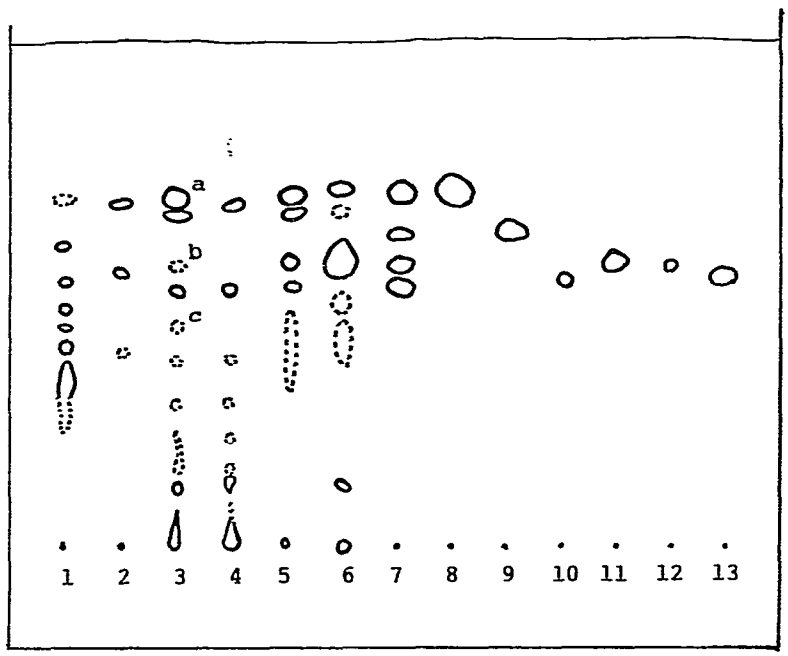

Fig. 9. Thin-layer chromatogram of carbohydrate acetates obtained by partial acetolysis of the components. (Apparatus, Toyo Roshi Co. Model CF-TLC; adsorbent, silica gel G; solvent, 1:24 (v/v) methanol–benzene; development, continuous for 125 min; detection, ammonium vanadate–H_2SO_4 reagent. Key: 1, amylose acetolyzate; 2, dextran acetolyzate; 3, H_{51} acetolyzate; 4, pachyman acetolyzate; 5, H_{53} acetolyzate; 6, H_{52} acetolyzate; 7, mixture of sugar acetates; 8, glucose acetate; 9, maltose acetate; 10, cellobiose acetate; 11, gentibiose acetate; 12, nigerose acetate; and 13, laminarabiose acetate.)

TABLE III

RELATIVE MOBILITIES[a] OF MAIN OLIGOSACCHARIDE ACETATES OBTAINED BY PARTIAL ACETOLYSIS OF THE COMPONENTS FROM THIN-LAYER CHROMATOGRAMS

Standard acetate	R_F	R_F *of acetolyzate from*					
		H_{S1}	H_{S2}	H_{S3}	*pachyman*	*amylose*	*dextran*
D-Glucose	0.69	0.69	0.70	0.69	0.67	0.68	0.67
		0.65	(0.65)[b]	0.65			
Maltose	0.62	—	—	—		0.59	
		0.55	0.56	0.56			
Gentiobiose	0.56						
Nigerose	0.54						
Isomaltose		—	—	—			0.53
Laminarabiose	0.52	0.50	—	0.51	0.50		
Cellobiose	0.51		—				

[a]Apparatus, Toyo Roshi Co. Model CF-TLC; adsorbent, silica gel G; solvent, 1:24 (v/v) methanol–benzene; development, continuous for 125 min; detection, ammonium vanadate–H_2SO_4 reagent. [b]Trace quantity.

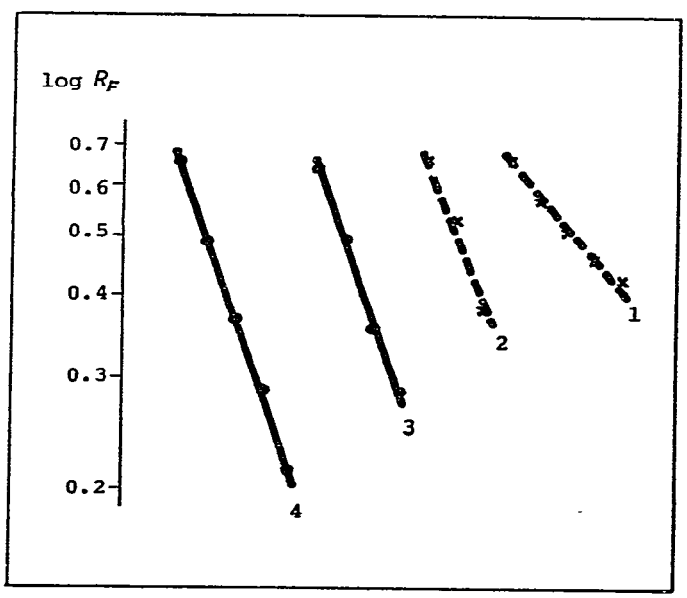

Fig. 10. Comparison of a series of log R_F values of sugar acetates from H_{S1} with that of sugar acetates from standard polysaccharides on thin-layer chromatograms. (Apparatus, Toyo Roshi Co. Model CF-TLC; adsorbent, silica gel G; solvent, 1:24 (v/v) methanol–benzene; development, continuous for 125 min; detection, ammonium vanadate–H_2SO_4 reagent. Key: 1, amylose acetolyzate; 2, dextran acetolyzate; 3, H_{S1} acetolyzate; and 4, pachyman acetolyzate.)

graphy of H_{51} were plotted against the degree of polymerization, the plot was found to be linear, and the line was symmetrical with that of pachyman, except for a few spots (a, b, and c in Fig. 9) which agreed in position with spots of unidentified constituents in H_{52}. In the acetolyzate of H_{53}, one spot was in agreement with that for laminarabiose acetate. The chromatogram of these acetolyzates is shown in Fig. 9. The R_F values of these acetates and those of the reference standards are summarized in Table III, and the log R_F values are plotted in Fig. 10.

These results suggested that a large part of H_{51} consists of laminarabiose units, that is, H_{51} is constituted mainly of (1→3)-β-glucan and it probably contains a small proportion of an α-glycan containing galactose and mannose residues as side chains. For H_{53}, as for H_{51}, it seemed likely that a (1→3)-β-glucan having branching galactose and mannose residues is the main structure, but this higher-order structure should be influenced by the rather acidic sugar component, because an absorption band of type 2b could not be detected in its i.r. spectrum. Component H_{52} is an α-glycan composed of galactose and mannose residues, and it contains almost no β-glucan. The antitumor activities of H_{51}, H_{52}, and H_{53} were assayed, and these data showed that only H_{51} is the active component, as shown in Table IV.

TABLE IV

ANTITUMOR ACTIVITIES[a] OF THE COMPONENTS

Component	*Experiment 1*		*Experiment 2*			
	Dose of 1 mg/kg/day × 10		*Dose of 1 mg/kg/day × 10*		*Dose of 5 mg/kg/day × 10*	
	Inhibition ratio (%)	*Activity*	*Inhibition ratio* (%)	*Activity*	*Inhibition ratio* (%)	*Activity*
H_{51}	90	+++	91	+++		
H_{52}	44	+	14	−	5	−
H_{53}					10	−

[a]Tumor, Sarcoma-180 transplanted subcutaneously (cell number, 6×10^6/mouse); administration of samples, intraperitoneal.

In an antitumor-active component, the relationship between the activity and the structure of the polysaccharide is not simple. A common structure has not been found among the many active polysaccharides obtained from various sources[1–3,6–8,19–26]. In the present report, the relationship between the biological and chemical properties of the components separated from one material can be considered. The active component, H_{51}, is the (1→3)-β-glucan, and the α-mannogalactan, H_{52}, is not active; however, the chemical structure of a (1→3)-β-glucan does not seem to be responsible for the activity of the component, because H_{53} is not active, although it is constituted mainly of a (1→3)-β-glucan, like H_{51}. This fact seems to be closely related to the finding that the (1→3)-β-glucan obtained by removal of branches from scleroglucan is an inactive glucan, as shown by Whistler *et al.*[27].

The antitumor activity does not depend directly on the kind of constituent sugars or on the mode of glycosidic linkage in the polysaccharide. Component H_{51} differs from H_{53} in molecular weight and in high-order structure. For possession of activity, it may be necessary that the active component have a large molecule or a high-ordered form, or both. This matter is of interest in connection with immune response in animals[11,12,14,28–30].

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