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# Antitumor Constituents of *Polyporus giganteus*

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**Abstract**□To investigate antitumor constituents of higher fungi, the carpophores of *Polyporus giganteus* Pers. ex. Fr. (81 g, dry weight) which were collected in Indiana, U.S.A. were examined for antitumor activity. Two protein-bound polysaccharide fractions (I and II) were prepared from the hot water extract and one fraction (III) from the 0.1 N NaOH extract of the carpophores. The antitumor effect of each fraction was tested against sarcoma 180 implanted subcutaneously in female ICR mice. Of three fractions, Fraction II showed 85.2% inhibition ratio at the dose of 20 mg/kg/day for 10 days and was named gigantan. Gigantan was found to contain 59% polysaccharide and 27% protein. Its polysaccharide moiety was a heteroglycan that consisted of mainly glucose (89.3%), galactose (7.7%), mannose (2.0%) and fructose (1.0%).

**Keywords**□ *Polyporus giganteus, Polyporaceae,* Basidiomycete, Gigantan, Antitumor activity, Heteroglycan.

Several taxa of the family *Polyporaceae* of Basidiomycetes were reported to exhibit antitumor activities. *Coriolus versicolor* (L. ex Fr.) Quelet was proved to contain a relatively strong antitumor protein-bound polysaccharide by Tsugagoshi and Ohashi[1], Kim *et al.*[2], and Shim[3]. Lentinan, an antitumor polysaccharide from *Lentinus edodes* (Berk.) Singer was isolated by Chihara *et al.*[4] *Pleurotus ostreatus* (Jacq. et Fr.) Kummer was found to exert a mild antitumor activity by Ikekawa *et al.*[5] and Kim *et al.*[2] *Ganoderma lucidum* (Fr.) Karsten was proved to exhibit antitumor activities by the author *et al.*[6-7] and Miyazaki and Nishijima[8]. The author *et al.* also found that *Pleurotus pulmonarius* showed mild antitumor activities against sarcoma 180 in mice[9]. *Cryptoporus volvatus* (Pk.) Hubb. was shown to exert antitumor activities by the author *et al.*[10] *Trametes sanguinea* (L. ex Fr.) Lloyd was reported to contain an antitumor component to sarcoma 180 by the auther *et al.*[11] *Laetiporus sulphureus* was found to have an antitumor component and to increase the number of plaque-forming cells in mice by the author *et al.*[12] *Microporus affinis* (Blume et Nees) Kuntze was prove to contain a fairly strong antitumor component by the author *et al.*[13]

Although nine taxa of the family *Polyporaceae* were reported to exhibit antitumor activities, none of the genus *Polyporus* has been investigated for that activity[14]. In a course of screening the carpophores of that genus, the authors found that *Polyporus giganteus* showed an antitumor activity against sarcoma 180 in mice and here reports the results of its activity.

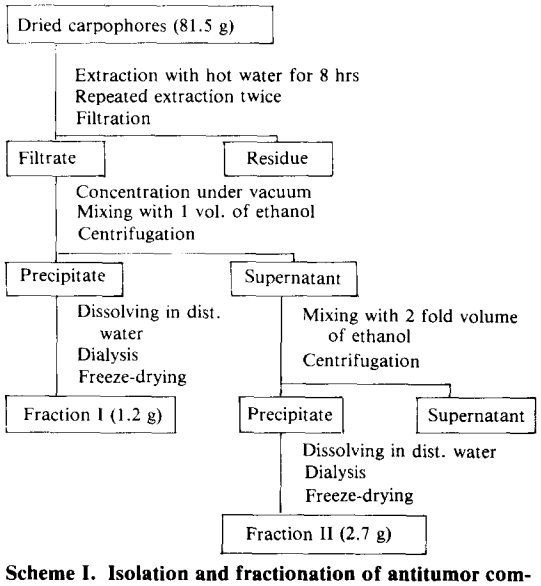
## EXPERIMENTAL METHODS

### *Fungal Material*

The carpophores of *Polyporus giganteus* Pers. ex Fr. which belongs to the family *Polyporaceae* were collected in the Shades State Park, Indiana, U.S.A., on September 3, 1981. It was later identified by Dr. Alexander H. Smith, Professor Emeritus at the Department of Botany, University of Michigan, Ann Arbor, Michigan, U.S.A. The specimen was stored at the Herbarium of the University of Michigan.

### *Extraction and Isolation*

The dried carpophores of *Polyporus giganteus* were homogenized with distilled water in a blender for five minutes and extracted on a water bath by refluxing for eight hours. The extraction was then repeated twice. The combined extractive was filtered and concentrated under reduced pressure. After it was mixed with an equal volume of eth-




**Scheme I. Isolation and fractionation of antitumor components from the carpophores of *Polyporus giganteus*.**

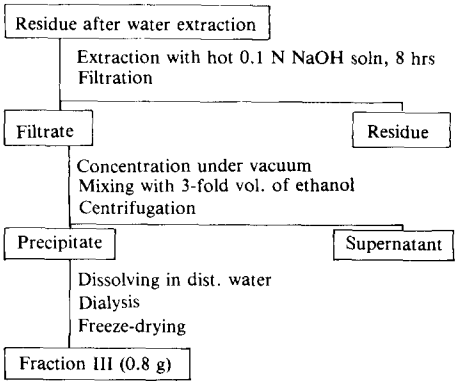
anol, precipitation occurred and the precipitate (Fraction I) was separated by centrifugation.

By adding two-fold volume of ethanol to the supernatant, precipitation was caused and its precipitate was separated in the same manner (Fraction II).

The hot water extracted residue was extracted with 0.1 N NaOH solution by refluxing for eight hours and then filtered. Three-fold volume of ethanol was added to the filtrate which was concentrated by rotary vacuum evaporator. The resulting precipitate was centrifugated and dissolved in distilled water and subjected to dialysis with Visking tube at 5 °C for 72 hours until the colored low molecular weight material disappeared. The dialysate was lyophilized in a freeze dryer to obtain 0.8 g (Fraction III). These are shown in Schemes I and II.

### *Antitumor Test in Mice*

The cells of sarcoma 180 were intraperitoneally transplanted in five ICR mice weekly. The sarcoma 180 cells were obtained aseptically from the ascite of the transplanted mice and implanted into 40 ICR mice at their right groin to induce solid tumor. Each mouse was injected $1 \times 10^6$ tumor cells in a volume of 0.1 m*l*. Four groups of 8 female mice were divied, one group as control and the others were treated groups. The former was intraperitoneally injected with physiological saline and the latters




**Scheme II. Isolation and fractionation of antitumor components from the carpophores of *Polyporus giganteus*.**

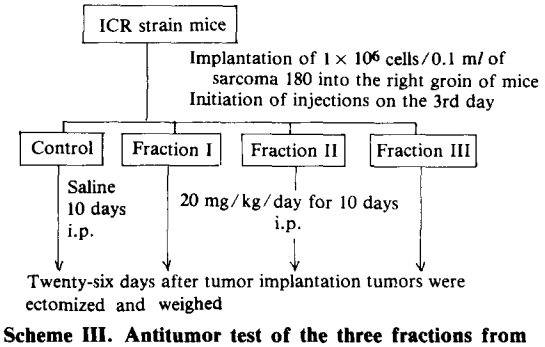
were intraperitoneally received 20 mg/kg of Fraction I, II and III. On the third day of the tumor implantation, sarcoma 180 bearing mice were injected *i.p.* with the above fractions everyday for 10 consequtive days. Twenty six days after tumor implantation, mice were killed and solid tumor was ectomized to be weighed (Scheme III). Mean values of the tumor weights of each group were calculated. Inhibition ratio (I.R.) as index of anticancer activity was calculated as follows:

$$\text{I.R. } (\%) = \frac{\text{Cw-Tw}}{\text{Cw}} \times 100$$

where, Cw was a mean value of the tumor weights of the control group and Tw was that of the treated groups.

### *Color Reactions*

Various color tests were done to confirm the nature of the high molecular weight fractions. These were Molish test, anthrone test, iodine test, ninhydrine test after acid hydrolysis, Lowry-Folin test and Biuret test.




**Scheme III. Antitumor test of the three fractions from the carpophores of *Polyporus giganteus*.**

*Chemical Analysis*

Total polysaccharide contents of Fractions I, II and III were determined by anthrone test. The polysaccharide contents were calculated from absorbance at 625 nm using glucose as standard sugar. Total protein contents were determined by absorbance at 540 nm according to Lowry-Folin method using bovine serum albumin (BSA) as a standard protein.

Gas chromatography was used for monosaccharide analysis of each fraction. Twenty mg of each sample and 10 mg of each standard monosaccharide were dissolved in two m*l* of 3% HCl-MeOH and methanolyzed at 100 ± 5 °C for 20 hours in duplicate screw capped test tubes filled with nitrogen gas. After filtration, the filtrate was evaporated in vacuum pressure and redissolved in absolute methanol. The solution was again evaporated in vacuum and dissolved in one m*l* pyridine. Trimethylsilylation was carried out with 0.2 m*l* hexamethyldisilazane (HMDS) and 0.1 m*l* trimethylchlorosilane (TMCS). Thirty seconds made the reaction complete and gas chromatography was performed. To identify each monosaccharide, retention times of each peak were compared with those of standard monosaccharide. The content of each monosaccharide was calculated from the chromatograms by measuring the peak area.

## RESULTS

*Identification of the Fungus*

The morphology of the collected mushroom suggested that it is very similar to *Grifola frondosa*. The carpophore of the sample was beige-white and fully grown when collected on a rainy day. It, however, did not appear to be *Grifola umbellata*, since the sample had more compact branching and fleshier framework than the latter. The morphological characteristics of the spore of the sample gave its important criteria of *Polyporus giganteus* (Fig. 1).

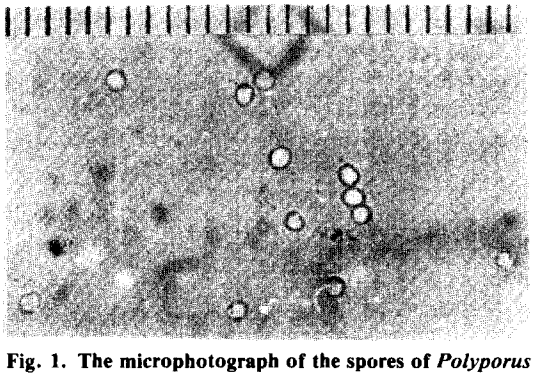


**Fig. 1. The microphotograph of the spores of *Polyporus giganteus* (× 1000).**

**Table I. Antitumor effect of the Fraction II that were obtained from the carpophores of *Polyporus giganteus* on ICR mice with sarcoma 180 tumor cell line.**

| Fraction | Dose (mg/kg/day) | Tumor weight* (g) | I.R.** (%) | Complete regression |
|---|---|---|---|---|
| Control | Saline | 2.25 ± 0.28 | | 0/8 |
| I | 20 | 1.32 ± 0.18 | 41.3 | 0/8 |
| II | 20 | 0.32 ± 0.08 | 85.5 | 2/8 |
| III | 20 | 2.05 ± 0.32 | 8.9 | 0/8 |

* Mean ± standard deviation
** I.R. = Inhibition ratio

*Yield of the Fractions*

Fraction I that could be thought as the highest molecular weight compounds of the water extract was obtained at the amount of 1.2 g. Fraction II, a medium molecular weight compound, was named gigantan and obtained at the amount of 2.7 g. Fraction III, an alkali soluble compound, was obtained at the amount of 0.8 g.

*Antitumor Activity*

The fractions examined in this paper are Fraction I, II and III. The antitumor effect of each fraction was assayed by comparing the growth of solid-form sarcoma 180 cells in ICR mice (Table I). Fraction II, gigantan, showed the highest antitumor activity, resulting in 85.8% and complete regressing in two of eight mice at 20 mg/kg/day. The inhibition ratios of Fraction I and III were 41.3% and 8.9%

**Table II. Various color reactions on Fraction II that was obtained from the carpophores of *Polypores giganteus*.**

| Method | Color | Result |
|---|---|---|
| Molish test | Purple | ++ |
| Anthrone test | Dark green | ++ |
| Iodine test | Negative | − |
| Ninhydrin test | Blue violet | + |
| Ninhydrin test after acid hydrolysis | Violet | ++ |
| Lowry-Folin test | Dark blue | ++ |
| Biuret test | Purple blue | + |

**Table III. Polysaccharide and protein contents of the fractions that were obtained from the carpophores of *Polyporus giganteus*.**

| Fraction | Contents (%) | |
|---|---|---|
| | Polysaccharide* | Protein** |
| I | 19 | 29 |
| II | 59 | 27 |
| III | 60 | 14 |

* Determined by Anthrone test (glucose, 625 nm)
** Determined by Lowry-Folin method (BSA, 540 nm)

**Table IV. Monosaccharide contents (%) of the fractions that were obtained from the carpophores of *Polyporus giganteus*.**

| Fraction | Glucose | Galactose | Mannose | Fructose |
|---|---|---|---|---|
| I | 66.2 | 19.7 | 9.7 | 4.4 |
| II | 89.3 | 7.7 | 2.0 | 1.0 |
| III | 86.8 | 6.0 | 5.4 | 1.8 |

respectively.

*Color Reactions*

The results of various color reactions were positive except iodine test as shown in Table II.

*Chemical Analysis*

The contents of total polysaccharide and total protein of the three fractions were exhibited in Table III. All of the fractions had the polysaccharide and protein as major moieties. As shown in Table IV, the polysaccharide moiety of the fractions was composed of glucose, galactose, mannose and fructose. Among the monosaccharides, glucose was identified as a major subunit of the polysaccharide moiety of the fractions (Fig. 2).

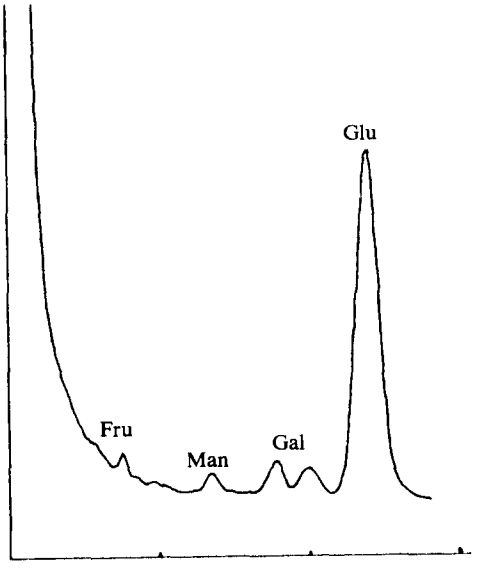



**Fig. 2. GLC chromatograph of the monosaccharides of Fraction II of *Polyporus giganteus* after methanolysis.**

## DISCUSSION

The protein-bound polysaccharide ioslated from the carpophores of *Polyporus giganteus* showed high antitumor activity against sarcoma 180 solid tumor. Among the fractions, Fraction II, gigantan, showed the highest antitumor activity. The gigantan did not show any toxicity during the experiment, and complete regression was observed in two of eight mice.

Mouse strain difference in the expression of antitumor activity can occur. In case of sarcoma 180 with the protein-bound polysaccharide, PS-K, Yoshikumi and his coworkers reported antitumor activity in ICR strain, but not in AKR mice[16]. Thus in our experiment, ICR mice was used according to the previous results. However, combination of tumor cell line and host strain is required for the determining the effectiveness of the protein-bound polysaccharide.

The nature of gigantan was determined as a protein-bound polysaccharide by various color tests. All the reactions such as Molish, anthrone, ninhydrine, Lowry-Folin and Biuret test, resulted in positive reaction, but not in iodine test. These results showed that the polysaccharide moiety of gigantan does not have similar structure to starch. The contents of polysaccharide and protein of gigantan were 59% and 27%, respectively. Moreover its monosaccharide units were identified as mainly glucose (89.3%), galactose (7.7%), mannose (2.0%) and fructose (1.0%) by gas chromatography after methanolysis with 3% HCl-MeOH. Although many protein-bound polysaccharides were reported in Basidiomycetes, we could find no report that has the same or similar properties of gigantan. Further investigations on the nature and antitumor mechanism are in progress.

## ACKNOWLEDGEMENT

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