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A NEW ANTITUMOR POLYSACCHARIDE FROM THE MYCELIA OF *PORIA COCOS* WOLF

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A new antitumor polysaccharide, H_{11}, was isolated from the mycelia of *Poria cocos* Wolf. The structure of H_{11} is a (1, 3)-(1, 6)-β-D-glucan having a molecular weight of 5×10^6.

KEYWORDS——(1, 3)-(1, 6)-β-D-glucan; *Poria cocos*; Polyporaceae; antitumor effect; sarcoma 180; mycelium;

Polysaccharides of various Basidiomycetes have been studied extensively from the viewpoint of antitumor effect.[1] Pachyman, (1, 3)-(1, 6)-β-D-glucan with no-antitumor effect, was isolated from *Poria cocos* Wolf which has been used in Chinese medicine as a diuretic.[2] Chihara et al. reported that some derivatives from pachyman, e.g., pachymaran and carboxymethyl pachymaran had a strong antitumor effect against sarcoma 180.[3] Recently Shibata et al. have reported the isolation of the polysaccharide, U-pachyman f, from the hyphae of *P. cocos* but they did not mention any antitumor activity.[4] We wish to report the first isolation of the antitumor polysaccharide, H_{11}, from these mycelia. H_{11}, a new (1, 3)-(1, 6)-β-D-glucan, obtained by affinity chromatography, showed a remarkable antitumor effect against sarcoma 180.

A piece of internal tissue of the sclerotium of *P. cocos*, gathered in Ishikawa prefecture in Japan, was incubated for 10 d at 25°C on a potato-dextrose-agar medium. The obtained hyphae (0.5 g) were inoculated in a culture medium [D-glucose (25 g), corn steep liquor (7 ml), yeast extract (3.2 g), KH_2PO_4 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), $CaCl_2 \cdot 2H_2O$ (0.06 g), Ferric citrate (5 mg), $MnCl_2 \cdot 4H_2O$ (5 mg), $ZnCl_2$ (4 mg), vitamin B_1 (0.1 mg) and distilled water (1 l)] and cultivated under an aerobic condition for 7 d. The brownish mycelia were separated by filtration and lyophilized. (Yield of mycelia: 10 g dried weight/medium 1 l).

Fig. 1 shows the fractionation of three polysaccharide fractions, H_{11}, H_{12} and H_2, from these mycelia. Fraction A, a crude extract from the mycelia, showed a strong antitumor effect against sarcoma 180 at 200 and 500 mg/kg x 10 doses as shown in Table I. Fraction A was fractionated by salting out with ammonium sulfate and subsequently by column chromatography. Fraction ASP (6.3% yield from the mycelia), precipitated with 70% saturated ammonium sulfate, was applied to a column of DEAE-Sephadex A-25 to give two fractions F_1 ($[\alpha]_D^{26}$ +120°) and F_2 ($[\alpha]_D^{26}$ +170°) in a ratio of 3.8:1.0. Fraction F_1 showed high carbohydrate con-

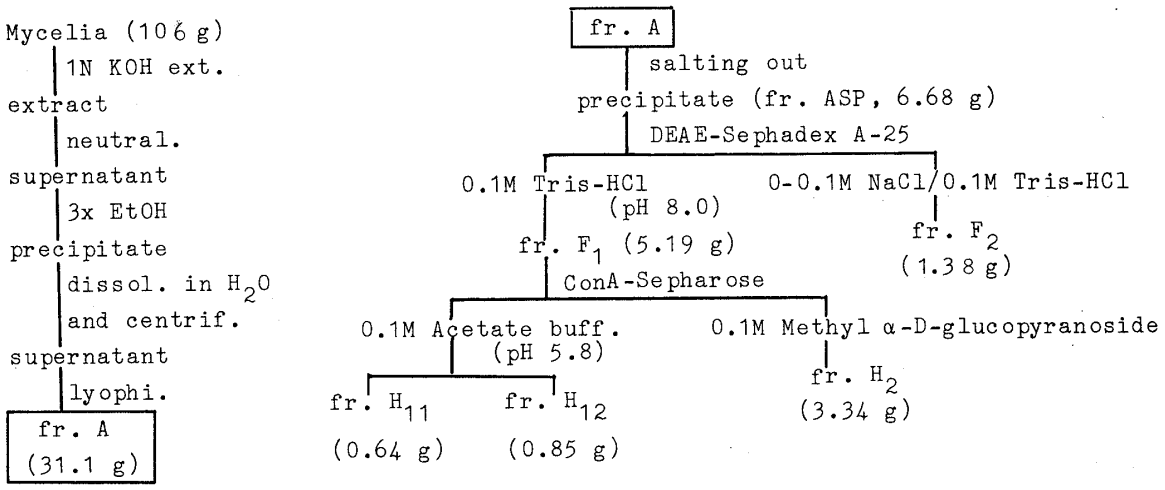

Fig. 1. Fractionation of Polysaccharide Fractions from *P. cocos*

tent, whereas that of fraction F_2 was very low. Furthermore, antitumor active fraction F_1 was separated into three fractions by affinity chromatography using ConA-Sepharose as shown in Fig. 2; H_{11} (0.6% yield from the mycelia), H_{12} (0.8% yield) and H_2 (3.2% yield) were obtained. Of these three fractions, only H_{11}, homogeneous on electrophoresis, showed a strong antitumor effect against sarcoma 180. Therefore, the structure of H_{11} has been studied intensively. On complete hydrolysis (1N H_2SO_4, reflux for 8 h), H_{11} gave D-glucose ($[\alpha]_D^{26}$ +50°) as a sole product. The total carbohydrate content was 98.2% by the phenol-sulfuric acid method. It is suggested that the glucosidic linkage in H_{11} is the β-configuration from the negative low specific rotation ($[\alpha]_D^{26}$ -28.2°, C=0.15 in H_2O) and absorption at 890 cm^{-1} in the infrared spectrum. In the ^{13}C-NMR spectrum of H_{11} (40 mg/ml D_2O) signals at 102.9 and 84.8 ppm were observed, and assigned to C-1 of the β-linkage and C-3 of the β-D-(1, 3)-linked D-glucosyl residues, respectively.[5] On addition of NaOD solution, the C-1 signal was separated into two signals at 104.2 and 104.8 ppm which can be assigned to the C-1 signal of the β-D-(1, 3)-linked and the β-D-(1, 6)-linked D-glucosyl residues, respectively.[6] After H_{11} was methylated by the method of Hakomori,[7] hydrolysis was carried out with 1N sulfuric acid. By gas chromatogram (3% ECNSS-M, column tem.: 180°C) of the O-methyl-monosaccharides formed as alditol acetates, 2, 4, 6-tri-, 2, 3, 4-tri-, 2, 4-di- and 2, 3, 4, 6-tetra-O-methyl-glucose were identified in a molar ratio of 3.96:0.95:1.05:1.00. Complete Smith degradation of H_{11} gave glycerol and oxidation-resistant glucose in a molar of approximately 0.5:1.0. H_{11} was decomposed to glucose in 70% yield by lysing enzymes (yeast glucanase, 0.1M acetate buffer, pH 5.0) for 24 h at 37°C. The molecular weight of H_{11} was calculated as ca. 5×10^6 by the gel filtration method using CPG-1000 Å. From these results, it has been revealed that H_{11} is a branched (1, 3)-(1, 6)-β-D-glucan containing each component in 4:1 ratio. H_{11} is a new antitumor polysaccharide and differs from other antitumor polysaccharides such as lentinan (1, 3)-(1, 6)-β-D-glucan, (1, 3)/(1, 6)=17:1, Mw. 1×10^6,[1),8)] sclero-glucan[9] and shizophyllan[10] which has a main chain of (1, 3)-β-D-glucosyl residues, with every third or fourth residue carrying a (1, 6)-β-D-glucopyranosyl

group. Though H_{11} showed an inhibition ratio of ∿100% at 4 or 8 mg/kg x 10 doses against subcutaneous sarcoma 180, it had no effect on the ascites sarcoma 180 at 2-20 mg/kg x 10 doses. Accordingly, it is suggested that H_{11} does not seem to act directly on tumor cells but through a host-mediated reaction as other antitumor polysaccharides do.[1),11),12)] On the other hand, H_{12}, $[\alpha]_D^{26}$ -6°, (1, 3)-(1, 4)-(1, 6)-β-D-glucan and H_2, $[\alpha]_D^{26}$ +184°, (1, 4)-(1, 6)-α-D-glucan, showed a weak antitumor effect.

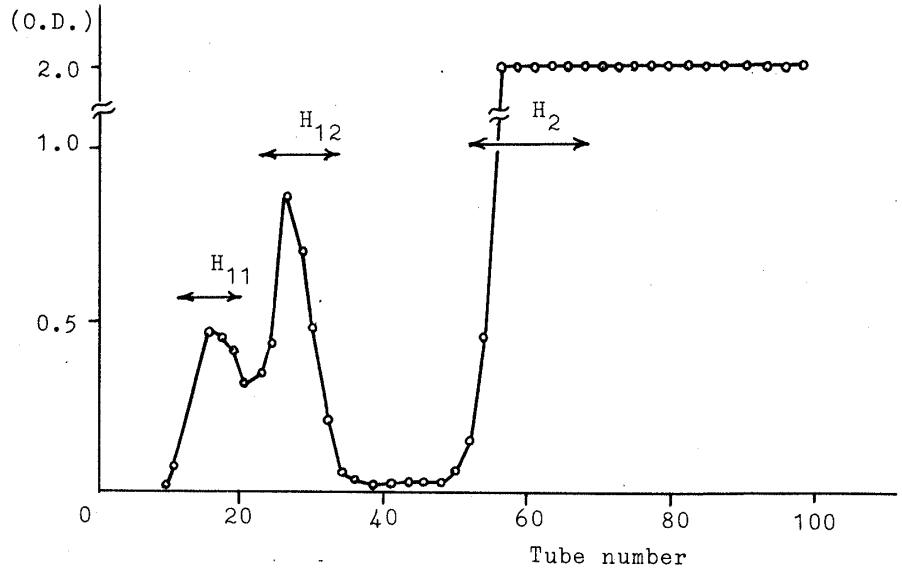

Fig. 2. Separation of Fraction F_1 by ConA-Sepharose

F_1 (150 mg) was dissolved in 15 ml of 0.1M acetate buffer (pH 5.8) and eluted with the same solution (fr. 1-40), and then eluted with 0.1M methyl α-D-glucopyranoside solution (fr. 41-100). Sugar (—o—) was determined by the phenol-sulfuric acid method. The sugar positive fractions were collected, dialyzed and lyophilyzed.

Table I. Antitumor Activities of Polysaccharide Fractions from *P. cocos* Wolf against Sarcoma 180[13)]

Fraction	Dose (mg/kg)	Average tumor wt.(g)	Inhibition ratio (%)	Complete regression
A	control	9.3	-	0/10
	200	1.9	80	1/10
	500	0.4	96	3/10
ASP	control	9.7	-	0/10
	30	3.9	60	0/6
	100	0.4	96	3/6
F_1	control	8.0	-	0/9
	15	3.0	62	1/9
	30	0.9	89	3/10
	60	0.1	99	4/10
H_{11}	control	6.6	-	0/6
	4	0.4	94	3/6
	8	0.3	96	1/6
H_{12}	control	6.6	-	0/6
	4	6.9	-5	0/6
	8	4.3	35	1/6
H_2	control	9.6	-	0/10
	30	5.8	40	0/8
	60	6.7	30	0/9

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13) Samples, dissolved in 0.85% sodium chloride solution, were administered by intraperitoneal injection once daily for 10 d, beginning 24 h after implantation. After 5 weeks, the tumor weights of treated mice were compared with those of untreated mice and the inhibition ratios were calculated.

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