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Polysaccharides in Fungi. XIII.[1] Antitumor Activity of Various Polysaccharides isolated from *Dictyophora indusiata, Ganoderma japonicum, Cordyceps cicadae, Auricularia auricula-judae,* and *Auricularia* Species

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A mannan and water-soluble glucans from *Dictyophora indusiata,* a water-insoluble glucan from *Ganoderma japonicum,* a galactomannan from *Cordyceps cicadae,* and acidic heteroglycans from *Auricularia auricula-judae* and *Auricularia* species (Yu er), whose structural features have been reported in our previous papers of this series, were tested for antitumor activity against subcutaneously implanted sarcoma 180 in mice by intraperitoneal administration. Considerable antitumor activity was observed with partially *O*-acetylated (1→3)-α-D-mannan (T-2-HN) at doses of 10 $mg \cdot kg^{-1} \cdot d^{-1} \times 10$, water-soluble (1→3)-β-D-glucans having β-1→6 linked D-glucosyl side chains (T-4-N and T-5-N) at doses 5 or 10 $mg \cdot kg^{-1} \cdot d^{-1} \times 10$, and a glucuronoxyloglucomannan (U-3-A) at doses of 25 $mg \cdot kg^{-1} \cdot d^{-1} \times 10$.

Keywords——polysaccharide; antitumor activity; sarcoma 180; *Dictyophora indusiata*; *Ganoderma japonicum*; *Cordyceps cicadae*; *Auricularia auricula-judae*; *Auricularia* species (Yū ěr)

Non-cytotoxic and host-mediated antitumor polysaccharides have been obtained from various sources.[2,3] The glucans composed mainly of β-1→3 linkages have been well studied[3] as regards the relationship between structure and antitumor activity. In particular, a glucan such as lentinan[4,5] from the fruit bodies of *Lentinus edodes* has been reported to exhibit high activity against sarcoma 180 implanted in mice. Moreover, a protein-bound glucan (PS-K)[6,7]

TABLE I. List of the Purified Polysaccharides

Polysaccharide	Specific rotation $[\alpha]_D^{20}$	Species of fungus	Japanese name (Chinese name)
T-2-HN (mannan)	+116° in H_2O	*Dictyophora indusiata* (PERS.) FISCH.	Kinugasatake (Zhú sūn)
T-4-N (glucan)	+19° in H_2O	*Dictyophora indusiata* (PERS.) FISCH.	Kinugasatake (Zhú sūn)
T-5-N (glucan)	+28.7° in H_2O	*Dictyophora indusiata* (PERS.) FISCH.	Kinugasakake (Zhú sūn)
G-A (glucan)	+12.8° in 1N NaOH	*Ganoderma japonicum* (FR.) LLOYD	Mannentake (Ling zhū cao)
C-3 (galactomannan)	+30° in H_2O	*Cordyceps cicadae* SHING	Senka (Chán huā)
MEA (glucuronoxylomannan)	+31° in H_2O	*Auricularia auricula-judae* (FR.) QUEL.	Kikurage (Mū ěr)
MHA (glucuronoxylomannan)	+33° in H_2O	*Auricularia auricula-judae* (FR.) QUEL.	Kikurage (Mū ěr)
U-3-A (glucuronoxyloglucomannan)	—8.7° in 0.05N NaOH	*Auricularia* species	Yu ji (Yū ěr)

isolated from the mycelium of *Coriolus versicolor* has been reported to show strong antitumor activity against sarcoma 180 and is used clinically as an antitumor drug. On the other hand, there have been few studies on the biological activity of the structurally characterized, pure heteropolysaccharides from fungi,[2] though we have previously studied the antitumor activity of the acidic heteropolysaccharides from the fruit bodies of *Tremella fuciformis*.[8] The present paper deals with antitumor effects of a variety of pure, hetero- and homopolysaccharides isolated from the fruit bodies of four kinds of Basidiomycetes and from the ascocarps of an Ascomycetes in the previous studies of this series.[1]

The antitumor activity of various fungal polysaccharides against subcutaneously implanted sarcoma 180 in mice was tested, *i.e.*, a mannan (T-2-HN),[1,9] glucans (T-4-N,[10] T-5-N[11,12] and G-A[13]), a galactomannan (C-3)[14] and several acidic heteropolysaccharides (MEA,[15] MHA,[15] and U-3-A[16]). Some properties of the samples are summarized in Table I. The structural features of these polysaccharides have been previously investigated in this series of studies. The results of bioassay of the samples at the 4th week by *i.p.* injection once daily for 10 d against subcutaneously transplanted sarcoma 180 in mice are listed in Table II.

Since antitumor β-D-glucans have been reported to inhibit sarcoma 180 in mice at low dose (below 10 mg/kg/d) *i.p.*,[2,3] the primary screening test of two water-soluble glucans (T-4-N and

TABLE II. Antitumor Activity of the Polysaccharides against Sarcoma 180

Sample	Dose ($mg \cdot kg^{-1} \cdot d^{-1} \times 10$)	Mean tumor wt. ±S. D.[a] (g)	Inhibition ratio (%)	Mortality at 4 weeks[b]	Complete regression
Exp-1					
T-4-N	10	7.60±6.24	25	0/9	2/9
T-5-N	10	2.36±3.46**	77	0/9	3/9
G-A	25	10.33±4.50	−2	0/9	0/9
C-3	20	5.43±3.85	47	2/9	0/7
T-2-HN	25	2.36±1.43**	77	1/9	0/8
MEA	25	5.93±2.63*	42	0/9	0/9
MHA	25	7.23±4.34	29	0/9	0/9
U-3-A	25	1.47±1.50**	86	0/9	2/9
Control	—	10.17±6.19	—	1/18	0/17
Exp-2					
T-4-N	5	3.99±5.96*	58	0/9	2/9
	1	5.19±4.82	38	0/9	0/9
T-5-N	5	6.55±6.35	30	0/9	0/9
	1	5.19±4.82	45	1/9	1/8
T-2-HN	10	9.49±3.54	−1	1/9	0/8
	2	8.83±4.17	6	0/9	0/9
Control	—	9.40±5.98	—	0/18	0/18
Exp-3					
G-A	5	11.85±5.84	−18	0/9	0/9
U-3-A	10	7.87±5.82	22	0/9	0/9
	2	10.35±4.54	−3	0/9	0/9
Control	—	10.07±5.97	—	3/17	0/14
Exp-4					
MEA	10	9.57±7.28	24	1/9	0/8
Control	—	12.59±4.54	—	0/18	0/18
Exp-5					
PS-K	250	2.44±3.25**	77	0/8	3/8
	50	5.25±1.85**	50	0/8	2/8
	10	9.30±3.65	12	0/8	0/8
Control	—	10.56±4.92	—	0/17	0/17

a) Significant difference from control (*, $p<0.05$; **, $p<0.01$).
b) All the deaths from tumors occurred after the 3rd week of the tumor transplantation.

T-5-N) was carried out at doses of 10 mg·kg^{-1}·d^{-1}×10. A water-insoluble glucan (G-A) was tested at a dose of 25 mg/kg. On the other hand, the activities of polysaccharides other than glucans have been found at relatively higher doses (*e.g.*, 50—200 mg/kg) against sarcoma 180 implanted in mice.[2] Such dose levels were not tested in this experiment, since our purpose was to find polysaccharides possessing marked antitumor activity at relatively low dose. Mannan (T-2-HN) and heteroglycans (MEA, MHA, C-3 and U-3-A) were administrated at 20 or 25 mg·kg^{-1}·d.$^{-1}$

Glucans (T-4-N, T-5-N and G-A) consist of a main chain of β-1→3 linked D-glucosyl residues, and have side chains of single D-glucosyl units attached by β-1→6 linkage to the main chain. T-4-N has two side chains attached, on average, to every 5th residue of the main chain, and T-5-N has two side chains attached, on average, to every 7th residue of the main chain. The chemical structures of both glucans obtained from the fruit bodies of *Dictyophora indusiata* are similar to that of sclerotan isolated from the sclerotia of *Sclerotinia libertiana*[17] and schizophyllan produced by *Schizophyllum commune*,[18] which have been reported to show strong antitumor activity, though there are differences in molecular weight (T-4-N,[10] 5.5×10^6; T-5-N,[12] 1.0×10^6; sclerotan,[17] degree of polymerization 10; schizophyllan,[18] 4.3×10^6) among these glucans. In exp-1, T-5-N showed a marked antitumor activity at 10 mg/kg with complete regression in 3 out of 9 mice, but T-4-N was hardly effective at the same dose. PS-K as a positive control showed considerable activity with complete regression in 3 and 2 out of 8 mice at doses of 250 and 50 mg·kg^{-1}·d^{-1}×10, respectively. A water-insoluble glucan having fewer side chains (G-A) (one for every 30th residue of the main chain) was inactive at a dose of 25 mg/kg. In exp-2, T-4-N showed inhibition of 58% at a dose of 5 mg/kg with complete regression in 2 out of 9 mice, but the inhibition by T-5-N at doses of 5 mg/kg and 1 mg/kg was weaker than that by T-5-N in exp-1. The antitumor activities of T-4-N and T-5-N were not higher than might have been predicted from the structural features. The differences of antitumor activity in the glucans seems to be related to their high-order structure.[19]

A galactomannan (C-3) obtained from the ascocarps of *Cordyceps cicadae* did not show significant inhibition at a dose of 20 mg/kg in exp-1.

A mannan (T-2-HN) obtained from the fruit bodies of *D. indusiata* is composed of α-1→3 linked D-mannopyranosyl residues and contains *O*-acetyl groups in the molecule. T-2-HN showed high antitumor activity at a dose of 25 mg/kg in exp-1, but did not show activity at the lower doses of 10 mg/kg and 2 mg/kg in exp-2.

Glucuronoxylomannans (MEA and MHA) obtained from the fruit bodies of *Auricularia auricula-judae* were less effective than the mannan (T-2-HN) in exp-1, though both MEA and MHA have a core of partially *O*-acetylated α-1→3 linked D-mannosyl residues, like T-2-HN. A similar acidic heteroglycan, glucuronoxyloglucomannan (U-3-A), obtained from the fruit bodies of *Auricularia* species (Yu er) showed strong activity with complete regression in 2 out of 9 mice at a dose of 25 mg/kg in exp-1; its activity was higher than that of PS-K in exp-5, but no antitumor activity was observed at doses of 10 mg/kg and 2 mg/kg in exp-4. The chemical structure of U-3-A has not yet been well characterized, and further structural analysis is in progress.

The results of antitumor assay provide useful information on the relationship between activity and structure of the polysaccharides. Water-soluble glucans (T-4-N and T-5-N) showed considerable activity at 5 mg/kg or 10 mg/kg daily doses, but such strong activity as reported in other antitumor β-1→3 linked D-glucans[2,3,20] was not observed here. On the other hand, it is of interest that partially *O*-acetylated α-1→3 linked D-mannan (T-2-HN) and glucuronoxyloglucomannan (U-3-A) exhibited significant antitumor activity at 25 mg·kg^{-1}·d^{-1}×10 in the present experiments. The antitumor activity should be affected by the dosage, route of administration, and species of animals used in the bioassay method,[3] and by chemical modification of the polysaccharides.[21-23] Therefore, further chemical and biological studies should provide interesting results.

Experimental

Materials——The polysaccharides tested are listed in Table I. The dried, fruit bodies of Basidiomycetes and the dried ascocarps of Ascomycetes (*Cordyceps cicadae* SHING) are commercially available in Hong Kong, except for the fruit body of *Ganoderma japonicum* (FR.) LLOYD, which was harvested in Gifu. A mannan (T-2-HN)[1,9] and water-soluble glucans (T-4-N[10] and T-5-N[11,12]) have been isolated from the aqueous EtOH and alkaline solution extracts of *Dictyophora indusiata* (PERS.) FISCH., respectively. A water-insoluble glucan (G-A)[13] has been isolated from the alkaline solution extract of *G. japonicum*, a galactomannan (C-3)[14] from the water extract of *C. cicadae*, glucuronoxylomannans (MEA and MHA)[15] from the aqueous EtOH or hot water extracts of *Auricularia auricula-judae* (FR.) QUEL., and a glucuronoxyloglucomannan (U-3-A)[16] from the hot water extract of *Auricularia* species (Yūěr).

Assay Method for Antitumor Activity——The animals used in the experiment were ddY male mice, weighing *ca.* 20 g. They were obtained from an animal farm in Shizuoka Prefecture and kept on standard diet F-2 (Funabashi Nojo Inc.) with water *ad libitum*. Sarcoma 180 ascites cells, maintained by serial *i.p.* transplantation into ddY male mice, were used. Tumor cells (7 d old) were employed for the experiment. The cells were transplanted at a dose of 0.2 ml (2×10^6 cells) subcutaneously into the right groin of mice. One day after the transplantation, 0.2 ml of physiological saline solution or a suspension of the test materials was given *i.p.* once a day for 10 d. The rate of tumor growth was measured by use of calipers applied to the external surface of the animal and the results were recorded weekly for 4 weeks. At the end of the 4th week after the transplantation, any remaining tumors were dissected out and weighed, then the inhibition ratios were calculated by use of the formula: inhibition ratio (%)$=[(A-B)/A]\times100$, where A is the average tumor weight of the control group, and B is that of the tested group.

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