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Isolations and Characterizations of Polysaccharides from Tremella fuciformis Berk¹⁾

The polysaccharides of basidiomycetes are studied widely,²⁾ but no report on *Tremella fuciformis* Berk (Shiro-kikurage) has been published until present time. We have now isolated the polysaccharides (A, B, and C) from the fruit bodies of this fungus. Their properties are described in this paper, with the aim of examining the nutritive value and the antitumor activity of these carbohydrates.

The polysaccharides were fractionated by the scheme in Chart 1. The aqueous solutions of fractions were lyophilized to yield A- and B-polysaccharides as colorless flakes and C-

Tremella fuciformis extracted with hot H2O extract residue dialyzed against running H2O extracted with 2n NaOH inner solution residue extract deproteinized by Sevag method, neutralized with HCl centrifuged dialyzed against running H₂O aqueous phase inner solution concentrated under reduced pressure deproteinized by Sevag method, added with 3 volumes of EtOH centrifuged supernatant aqueous phase precipitate concentrated under reduced pressure A-polysaccharide B-polysaccharide added with 3 volumes of EtOH precipitate supernatant C-polysaccharide Chart 1

¹⁾ Presented at the 91st (Fukuoka, April 1971) and the 92nd (Osaka, April 1972) Annual Meeting of the Pharmaceutical Society of Japan.

²⁾ P.A.J. Gorin and J.F.T. Spencer, Adv. Carbohyd. Chem., 23, 367 (1969); G. Chihara, Y. Maeda, J. Hamuro, T. Sasaki and F. Fukuoka, Nature, 222, 687 (1969); M. Shida, T. Mase, Y. Sasakawa and K. Matsudo, Nippon Nogeikagaku Zasshi, 45, 454 (1971).

polysaccharide as slightly yellowish white flakes, respectively.

Each polysaccharide was homogeneous as judged by gelfiltration on Sephadex G-200 and electrophoresis (Tiselius and glassfiber paper) in an alkaline borate buffer of pH 12. Also these compounds were found to be pure by ultracentrifugal analysis and did not contain nitrogen and phosphorus.

The B-polysaccharide showed a specific rotation of $[\alpha]_D^{20}+11$ (c=1, H₂O), an intrinsic viscosity value of 4.3 dl/g, a partial specific volume value of 0.55 ml/g, a sedimentation constant of 6.7 S, and a molecular weight of about 4.7×10^5 by ultracentrifugal analysis. These physical data, except that of optical rotation, were determined in a 0.3 m sodium chloride-0.001 m sodium phosphate buffer of pH 7 at 20° by standard means.

The infrared spectra of the A- and B-polysaccharides had absorption bands due to acetoxyl group at 1725 and 1250 cm⁻¹. The acid liberated after saponification of each polysaccharide (A and B) has been identified to be acetic acid by comparison of the p-bromophenacyl acetate with the corresponding derivative of the authentic sample, thus the presence of O-acetyl groups was confirmed. The total acetyl contents of the two polysaccharides were determined to be approximately 5.9% in A, and 10.2% in B, respectively, by the procedure of Hestrin³⁾ and Liu, et al.⁴⁾

Thin-layer (Avicel SF cellulose and Kieselguhr G) chromatography of hydrolysate and gas liquid chromatography⁵⁾ of the trifluoroacetate of reduction product of the hydrolysate indicated that the component sugars of each polysaccharide (A, B, and C) were xylose, glucuronic acid and mannose in addition to a small amount of glucose and a trace of fucose. The hydrolysate above mentioned was prepared by heating each polysaccharide with 2n sulfuric acid at 120° in a sealed tube for 1 hr. Quantitative determinations⁶⁾ of the main suger components of the B-polysaccharide showed that the molar ratio of them was as follows; xylose: glucuronic acid: mannose (containing a small amount of glucose) was 1.5:1:3.7.

The results of the experiments described here demonstrate that these polysaccharides (A, B, and C) are acidic heteroglycans composed mainly of xylose, glucuronic acid and mannose, and that A and B contain acetoxyl groups in their molecules.

It is of interest that acidic heteroglycans were isolated in good yields from *Tremella fuciformis* classified into heterobasidiae. Polysaccharides so far obtained from mushrooms belonging to homobasidiae were mostly glucans.²⁾ Further the characterization, the structural elucidation and the antitumor activity of these polysaccharides will be discussed in following papers.

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³⁾ S. Hestrin, J. Biol. Chem., 180, 249 (1949).

⁴⁾ T.Y. Liu, E.C. Gotschlich, F.T. Dunne and E.K. Jonssen, J. Biol. Chem., 246, 4703 (1971).

⁵⁾ T. Imanari, Y. Arakawa and Z. Tamura, Chem. Pharm. Bull. (Tokyo), 17, 1967 (1969).

⁶⁾ Z. Dische, J. Biol. Chem., 181, 379 (1949); T. Bitter and H.M. Muir, Anal. Biochem., 4, 330 (1962).