An Alkali-Soluble Polysaccharide from the Cell Walls of *Coprinus lagopus*

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Abstract. An alkali-soluble polysaccharide was isolated from the purified mycelial walls of *Coprinus lagopus*. The hydrolysis products, optical rotation, and infrared spectrum indicate a β-glucan. Hydrolysis of the glucan after permethylation gave only 2,3,4,6-tetra-, 2,4,6-tri-, and 2,4-di-O-methyl-D-glucose. These methylated sugars and their relative quantities reveal that the glucan is a polysaccharide containing β-1,3-linked glucose units with about 14% of the sugars having 1,6-linked branch points. Partial hydrolysis of the product derived from Smith degradation of the glucan released laminaribiose and gentiobiose suggesting that the branches are generally longer than a single glucose unit.

Key words: *Coprinus* — Polysaccharide — Glucan — Cell wall.

The characteristic wall structure of the higher Basidiomycetes apparently consists of a microfibrillar network of chitin set in a matrix of more amorphous glucan (Bartnicki-Garcia, 1968). This type of chitin-glucan system with its surrounding polysaccharide has been described through ultrastructural studies in several Basidiomycetes including *Schizophyllum commune* (Hunsley and Burnett, 1970; Wessels et al., 1972), *Polystictus versicolor* (Strunk, 1963, 1969), and *Agaricus bisporus* (Michalenko et al., 1976). Although the presence of chitin has been confirmed for many Basidiomycetes both by chemical and X-ray techniques, the nature of the glucans is well known in few of them (Arionson, 1965; Gorin and Spencer, 1968). The most extensively studied Basidiomycete has been *S. commune* where both an alkali-soluble α-1,3-glucan and alkali-resistant and water-soluble β-1,3-glucans with 1,6-linked branches have been described and related to the ultrastructural architecture of the walls (Wessels et al., 1972; Siehr, 1976). In *Coprinus*, although the presence of chitin has been shown by X-ray analysis (Frey, 1950), nothing is known of the nature of the other polysaccharides. This paper describes an alkali-soluble glucan isolated from the mycelial walls of *Coprinus lagopus* (sensu Buller).

MATERIALS AND METHODS

The Organism and Its Culture. The strain of *Coprinus lagopus* used in this research was Hx H9 obtained from Dr. P. R. Day of the Connecticut Agricultural Experiment Station. Inoculum was prepared by transferring a small amount of mycelial growth from a slant culture into a 125 ml flask containing 25 ml of the yeast extract-glucose broth described by Madelin (1956). The cultures were incubated at 37°C for 4 days. After washing with sterile water, the mycelial mats from two flasks were blended with 70 ml of water for 15 s in a Waring blender. One ml of the resulting suspension was added to each 250 ml Erlenmeyer flasks containing 30 ml of 4 mm diam. glass beads and 50 ml of medium. The medium, a modification of the defined medium of Madelin (1956), contained 1000 ml of water, 10 g glucose, 1 g D,L-α-alanine, 0.2 g MgSO₄·7H₂O, 0.2 g K₃HPO₄, 500 μg thiamine·HCl, 1.4 mg Fe(NO₃)₃, and 0.88 mg ZnSO₄·7H₂O. The cultures were incubated for 4 days in the dark at 35°C and then at 25°C under constant fluorescent illumination until harvest.

Isolation of the Cell Walls and Glucan. Eight-day-old mycelial mats were blended in 0.2 M pH 7 phosphate buffer at maximum speed with a Sorvall Omni-mixer. The macerated mycelium was collected by centrifugation, washed with buffer, and further fragmented with 0.5 mm diam. glass beads in a Braunwill MSK homogenizer for a total of 8 min. Microscopic examination of the fragments mounted in methylene blue showed nearly complete breakage of the cells. The broken mycelial walls were washed repeatedly first with buffer...
and then with water well beyond the point where the supernatant showed no UV absorption at 260 nm.

The polysaccharide was isolated by suspending the wall fragments in N KOH at 25°C for 11 h with constant stirring. After the insoluble material was separated by centrifugation and washed with water, the combined extract and wash was acidified with glacial acetic acid, and the polysaccharide was precipitated by the addition of three volumes of ethanol. The resulting fibrous precipitate was collected on a bent glass rod, dissolved in N KOH, filtered through glass wool, and reisolated. After washing the product with ethanol, it was suspended in a small amount of water and lyophilized.

Hydrolysis and Sugar Identification. The component sugars of the polysaccharide were determined after hydrolysis at 100°C in sealed tubes with 5 N HCl for 5 h or with N H2SO4 for 20 h and neutralized with 10% di-N-octylmethylamine in CHCl3 and BaCO3 respectively. The hydrolysates were chromatographed on thin layers of silica-gel G made with 0.02 M pH 8 borate buffer and developed with n-butanol-acetic acid-water (5:4:1). The sugars were visualized with aniline pthalate. The hydrolysates were also analysed by gas-liquid chromatography (glc) using a flame ionization detector and a 6 ft column of 12% ECNSS-M (ethylenesuccinate-cyanoethylsilicone copolymer) on acid washed chromosorb W DMCS after converting the sugars to their alditol acetates using the technique as outlined by Knaak et al. (1969).

Periodate Oxidation and Smith Degradation. Periodate oxidation of the polysaccharide was carried out in 0.4 M NaIO4 at 6°C in the dark. Samples were removed at 24, 48 and 73 h and the periodate consumption and formic acid release were determined by the iodometric techniques described by Hay et al. (1965). Smith degradation of the oxidized polysaccharide was carried out after first decomposing the excess periodate with ethylene glycol followed by dialysis of the sample and concentration of the material by pervaporation. The oxidized polysaccharide was recovered by precipitation in ethanol, reduced with NaBH4, and reisolated. After mild hydrolysis at room temperature in H2SO4 at pH 4 for 48 h, the polysaccharide was reisolated and subjected to a second periodate oxidation. Following partial hydrolysis with concentrated HCl for 1 h at room temperature, the neutralized products were separated by descending paper chromatography with n-propanol-ethyl acetate-water (7:1:2) and visualized with alkaline AgNO3.

Methylation Analysis. The purified polysaccharide, after borohydride reduction, was methylated once following the Haworth procedure (Haworth, 1915) and a second time using the procedure of Kuhn and Trischmann (1963). The fully methylated product was hydrolyzed with 90% formic acid for 1 h at 100°C followed by 0.5 N H2SO4 for 14 h at 100°C. The methylated sugars were separated by descending paper chromatography on Whatman 3MM paper developed with water-saturated methyl ethyl ketone. The areas containing the methylated sugars were cut from the chromatograms and the sugars were extracted with methanol. Methyl glucosides of the pure methyl sugars or the mixed products of the hydrolysis were prepared by refluxing the samples for 6 h in 2% HCl in methanol followed by removing the acid with AgCO3. Mixtures of the O-methyl glucosides were separated by glc on a 6 ft column of 10% DEGS (Diethylenglycol succinate) on Chromosorb W at 200°C. Pure samples of the methyl glucosides of the methylated sugars were collected at the glc exit port in glass capillary tubes. The identity of the methylated sugars separated by paper chromatography or the methyl glucosides collected by glc was determined by melting points and optical rotations. The relative quantity of each methylated sugar in the hydrolysis mixture was determined from the peak areas obtained by glc of the mixture. The glc peak areas were determined by multiplying the peak height by the width at one-half the height (Gough and Walker, 1969).

RESULTS

The polysaccharide isolated from the mycelial walls of Coprinus lagopus formed a viscous solution in the KOH after extraction, a loose gel when acidified with acetic acid, and a white fibrous precipitate when the acidified solution was treated with ethanol. The isolated material appeared as a single spot after electrophoresis on glass fiber paper in 0.05 M NaBrO3 buffer at 300 volts for about 1 h (Kensco Model 50 electrophoresis apparatus, Kensington Scientific Corporation). The polysaccharide showed no electrophoretic mobility in the presence of the borate buffer indicating that it is a non-charged compound with no adjacent cis hydroxyl groups that would be able to complex the borate buffer.

Chromatographic examination of the hydrolyzed polysaccharide by thin-layer chromatography showed the presence of only glucose, but glc indicated a trace of mannose. Since the relative amount of mannose was quite variable among samples isolated at different times, it was assumed that the mannose was contaminant.

An infrared spectrum of the glucan (Fig. 1) shows absorption characteristic of β-glucoside bonds at about 890 cm⁻¹ (Barker et al., 1954) but no absorption characteristic of α-glucoside bonds at 844 ± 8 cm⁻¹. The optical rotation of the glucan of [α]D₂₀ = −7 (ca. 0.1% in 1% NaOH) further suggests that the polysaccharide is a β-glucan. The polysaccharide consumed 0.33 moles of periodate per mole of anhydrous glucose upon oxidation and released 0.15 moles of formic acid per mole of anhydrous glucose. This approximates a 2:1 ratio of periodate consumed to formic acid released and is consistent with the methylation data indicating a 1,3-linked glucan. Such a glucan would show no electrophoretic mobility in borate buffer. A sample of the oxidized glucan, reduced with NaBH₄ and hydrolyzed with N HCl, was chromatographed on thin-layers of silica-gel G-aluminum oxide G (1:1) developed with butanol-acetic acid-water (7:3:1). When visualized with 1% NaIO₄ followed by benzidine, only glyceral and