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Note

An alkali-soluble α-D-glucan from the fruiting body of *Grifola frondosa*

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Previously, we reported studies of the polysaccharides extracted from the fruiting body of the basidiomycete *Grifola frondosa* with 10% aqueous zinc chloride (designated F4)[1] and 10% aqueous sodium hydroxide (designated F7 and F8)[2]. In continuation of this work, we now describe the isolation of an alkali-soluble α-D-glucan present in the fruiting body. The structure of the glucan was deduced from methylation analysis, and a study of the fragmentation by acid.

The defatted fruiting bodies (100 g) were successively extracted with water, hot water, and 10% aqueous zinc chloride[1]. The residual material was then extracted with 2% sodium hydroxide for 1 h at 4°. Neutralization of the base in the extract with 3M hydrochloric acid, under cooling, gave a precipitate which was collected by centrifugation, desalted by dialysis, and lyophilized (F5, yield 2.2 g). Fraction F5 had $[\alpha]_D$ +148° (*c* 0.3, 0.5M sodium hydroxide) and gave, on complete acid hydrolysis, D-glucose, D-mannose, and D-xylose in the ratios of 100:20:11. Because F5 was presumed to be a mixture of α-D-glucan (F5-αG) and xylomannan (F5-XM), fractional precipitation with methanol was attempted. A sample of F5 (1 g) was dissolved in 0.5M sodium hydroxide (100 mL), and methanol (50 mL) was added dropwise to the solution with stirring. The precipitate deposited was collected by centrifugation and successively washed with methanol and ether (p-1; yield 500 mg). To the supernatant liquor was added methanol (50 mL) until precipitation was complete (p-2; yield 210 mg). On complete acid hydrolysis, p-1 gave D-glucose, D-mannose, and D-xylose, while p-2, $[\alpha]_D$ +164° (*c* 0.3, 0.5M sodium hydroxide), gave D-glucose with only a trace of D-mannose. As the relatively low $[\alpha]_D$ value of p-2, when compared to values of other (1→3)-α-D-glucans[3,4], suggested a contamination of β-D-glucan[1,2], digestion with β-D-glucanase was attempted. Exo-(1→3)-β-D-glucanase (E.C. 3.2.1.6) was prepared from a culture of *Basidiomycete* QM806 (refs. 5, 6) according to the method described by Reese and Mandels[5]. Fraction p-2 (100 mg) was incubated with the enzyme in 150 mL of 0.05M acetate buffer, pH 4.8, at 37°, and the reducing power of the reaction mixture was monitored at intervals.

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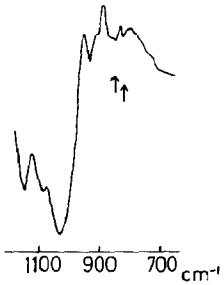

Fig. 1. Infrared spectrum of F5-αG in a KBr disc. Arrows show absorptions at 820 and 850 cm^{-1}, suggesting that F5-αG is a (1→3)-α-D-glucan.

The reducing power increased with the incubation time, and reached a maximum of 15.8% of the total sugar. After inactivation of the enzyme by heating for 10 min at 100°, the supernatant liquor was added to an equal amount of methanol. The precipitate deposited was collected, and washed first with methanol, and then with ether (F5-αG, yield, 61 mg). F5-αG had $[\alpha]_D$ +225° (*c* 0.3, 0.5M sodium hydroxide) and gave only D-glucose on acid hydrolysis. The i.r. spectrum (Fig. 1) showed absorption at 850 cm^{-1} but not at 890 cm^{-1}, suggesting the removal of β-D-glucan. Moreover, the presence of absorption at 820 as well as at 850 cm^{-1} suggested that F5-αG is a (1→3)-α-D-glucan[7].

A sample of F5-αG (3 mg) was dissolved in 0.5M sodium hydroxide (0.5 mL), and the solution applied to a column (1.6 × 83 cm) of Sepharose CL-6B equilibrated with 0.5M sodium hydroxide. The column was eluted with the same solvent (0.5 mL/min), and the effluent was collected in 1.5-mL fractions. The carbohydrate content of each fraction was determined by the phenol–sulfuric acid method. The column was calibrated with dextrans T-70 (mol. wt. 70 000), T-40 (43 500), T-20 (20 400), and T-10 (10 500) from Pharmacia Fine Chemicals. From the elution volume, the molecular weight was estimated to be ~35 000 (Fig. 2).

A sample of F5-αG (5 mg) was methylated by the Hakomori procedure[8]. The

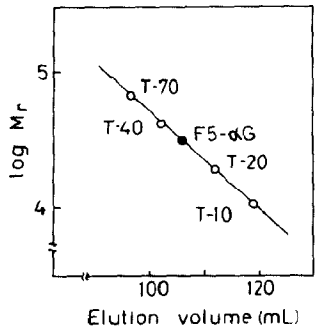

Fig. 2. Estimation of the molecular weight of F5-αG on Sepharose CL-6B gel. The column (1.6 × 83 cm) was standardized with dextrans as noted on the plot.

TABLE I

^{13}C-N.M.R. SIGNAL ASSIGNMENTS FOR α-(1→3)-D-GLUCANS

	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
F5-αG	102.0	72.7	84.5	72.2	74.4	62.7
Ref[a]	101.3	72.2	83.2	71.7	73.7	62.2

[a](1→3)-α-D-glucan from *Penicillium patulum*.

reaction mixture was dialyzed against running water for 3 days, and the non-dialyzable fraction was dried. The methylation procedure was repeated until the product showed no absorption for free hydroxyl groups in its i.r. spectrum. The methylated polysaccharide was then hydrolyzed with 90% formic acid (1 mL) for 3 h at 100°, and then with 0.25M sulfuric acid (1 mL) for 14 h at 100°. The acid was neutralized with barium carbonate, and the neutral hydrolyzate was evaporated to dryness. The sugars thus obtained were converted into their alditol acetates[9] for g.l.c.–m.s. analysis. G.l.c.–m.s. was conducted with a Hitachi M-52 apparatus equipped with a glass column (0.3 × 100 cm) packed with 3% of Silar 10C on Uniport B (60–80 mesh) and programmed from 200 to 230° at 2°/min. Mass spectra were recorded at an ionizing potential of 20 eV. Only one peak, corresponding to alditol acetate from 2,4,6-tri-*O*-methylglucose, was detected.

A sample of F5-αG (100 mg) was dissolved in 90% formic acid (1.5 mL) and heated for 1 h at 100°. After evaporation of the formic acid, the hydrolysis was continued in 0.25M sulfuric acid (15 mL) for 1.5 h at 100°. After neutralization of the acid with barium carbonate, the solution was concentrated, and the resulting sugar mixture was resolved by chromatography on BioGel P-2 (2.6 × 88 cm). The column was eluted with water (0.45 mL/min), and the effluent was collected in 4-mL fractions. The fractions corresponding to disaccharide were combined, and analyzed by paper chromatography using 6:4:3 (v/v) 1-butanol–pyridine–water as the irrigant. Only one spot, corresponding to nigerose, was detected. Moreover, the values of log $R_F/(1 - R_F)$ for D-glucose and the oligosaccharides up to tetrasaccharide, when plotted against the degree of polymerization, gave a straight-line graph[10], indicating that F5-αG is a linear (1→3)-α-D-glucan. This was confirmed by ^{13}C-n.m.r.-spectral analysis. The spectrum was recorded at 67.8 MHz with a JEOL JNM GX-270 spectrometer for a solution in 2% NaOD–D_2O. Dimethyl sulfoxide-d_6 (39.5 p.p.m.) was used as the internal standard. The sample temperature was 25°. The result and published data[11] are summarized in Table I.

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