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Polysaccharides Elaborated by *Polyporus borealis* (Wahlenb.)

1. Water-soluble Neutral Polysaccharides from the Fruit Bodies

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Two neutral, water-soluble polysaccharides, a fucogalactan and a mannan, have been isolated from fruit bodies of *Polyporus borealis*. The fucogalactan consists of chains of (1→6)-linked α-D-galactopyranose residues, about 35 % of which are substituted with an α-L-fucopyranose residue in the 2-position (Fig. 3). The mannan is essentially α-linked and contains (1→2)-, (1→3)-, and (1→6)-linked chain residues, of which the (1→2)- and (1→3)-linked residues may be substituted in the 6-position. A regular structure (Fig. 4) for this polysaccharide is tentatively proposed.

In previous publications we have reported studies on different polysaccharides isolated from wood-destroying fungi of the Basidiomycetes group, *Polyporus giganteus*,[1] *P. pinicola*,[2] *Armillaria mellea*,[3] *P. fomentarius*, and *P. igniarius*.[4] The present communication reports similar studies on polysaccharides from *P. borealis*.

Fruit bodies of *P. borealis* were harvested locally. The disintegrated material was extracted, first with hot water and then with 0.3 M potassium hydroxide at room temperature and the extracts processed to give the polysaccharide fractions. Hydrolysates of the two extracts contained D-glucose as the dominant sugar, and smaller amounts of D-galactose, D-mannose, and L-fucose. In addition to these sugars, D-xylose and D-glucuronic acid were present in the hydrolysate from the potassium hydroxide extract. Studies on the alkali-soluble polysaccharides will be reported separately.

The fraction from the hot water extract contained considerable amounts of glucans, probably both α-glucans and β-glucans. Part of the latter was removed by precipitation with cetyltrimethylammonium hydroxide [5] (CTA—OH). The heteropolysaccharide fraction was then enriched by precipitation with boric acid and CTA—OH and obtained free from glucan by two further precipitations with this reagent. A hydrolysate of the fraction contained

D-galactose, D-mannose, and L-fucose. Glass fiber sheet electrophoresis in borate buffer revealed two distinct spots, indicating that the fraction contained two different polysaccharides. An analytical separation on a Sepharose 6 B column yielded two fractions, one (20 %) of $\overline{M}_w$ 350 000, and another (80 %) of $\overline{M}_w$ 17 900. The relation between molecular weight and elution volume was determined with dextran samples of known molecular weights, and the $\overline{M}_w$ values for other polymers must be regarded as approximate. A preparative separation was obtained on a Sephadex G-150 column. The material eluted first, $[\alpha]_{578}+82°$, on hydrolysis yielded D-mannose and small amounts of D-galactose and L-fucose, indicating it to be an essentially pure mannan. On electrophoresis, it gave a single spot corresponding to the slower of the two spots in the electrophoretogram of the main fraction. The more slowly eluted material, $[\alpha]_{578}+92°$, on hydrolysis yielded D-galactose and L-fucose in the relative proportions 2.6:1 and only traces of D-mannose. It gave a single spot on electrophoresis, corresponding to the faster of the two spots in the electrophoretogram of the main fraction. This component is consequently a fucogalactan.

The polysaccharides were methylated by two treatments with dimethylsulphinyl sodium-methyl iodide in dimethyl sulphoxide, following the procedure devised by Sandford and Conrad.[6] The fully methylated polysaccharides were hydrolysed and the resulting sugars reduced to alditols with sodium borohydride and then acetylated. The mixtures of partially methylated alditol acetates were analysed by GLC [7]-mass spectrometry.[8] The results are summarised in Tables 1 and 2. It has been demonstrated that for partially

Table 1. Methyl ethers from the hydrolysate of methylated heterogalactan.

Sugars	T^a	mole %
2,3,4-Tri-*O*-methyl-L-fucose	0.64	27.2
2,3,4,6-Tetra-*O*-methyl-D-galactose	1.26	1.3
2,3,4-Tri-*O*-methyl-D-galactose	3.41	44.5
3,4-Di-*O*-methyl-D-galactose	6.90	27.0

[a] Retention times of the corresponding alditol acetates on the ECNSS—M column relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

Table 2. Methyl ethers from the hydrolysate of methylated mannan.

Sugars	T^a	mole %
2,3,4,6-Tetra-*O*-methyl-D-mannose	1.00	11.2
3,4,6-Tri-*O*-methyl-D-mannose	1.95	32.5
2,4,6-Tri-*O*-methyl-D-mannose	2.08	33.7
2,3,4-Tri-*O*-methyl-D-mannose	2.48	11.2
3,4-Di-*O*-methyl-D-mannose	5.28	7.5
2,4-Di-*O*-methyl-D-mannose	5.44	3.9

[a] See Table 1.

methylated alditol acetates, a unique mass spectrum is obtained for each substitution pattern. Thus, for the actual mixtures, the components could be unambiguously identified by their mass spectra. For all of the components, except the 2,4-di-*O*-methyl-D-mannose derivative, the retention times on GLC (*T*-values) had been determined previously, [7,4] and there was good agreement between these values and those obtained in the present investigation.

The GLC for the alditol acetate mixture obtained from the fucogalactan is given in Fig. 1. The component in peak A had the same *T*-value and mass spectrum as the alditol acetate from 2,3,4-tri-*O*-methyl-L-fucose. Peak B from its *T*-value and mass spectrum was identified as the 2,3,4,6-tetra-*O*-methyl-D-galactose derivative, peak C as the 2,3,4-tri-*O*-methyl-D-galactose derivative, and peak D as the 3,4-di-*O*-methyl-D-galactose derivative. From the results of the methylation analysis (Table 1), the molar proportions of L-fucose and D-galactose can be estimated as 1:2.7 in good agreement with the sugar analysis.

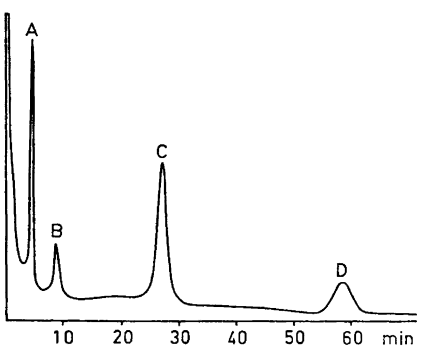

Fig. 1. GLC separation, on the ECNSS—M column at 175°, of the alditol acetates of the methylated sugars, obtained from the hydrolysate of the fully methylated heterogalactan.

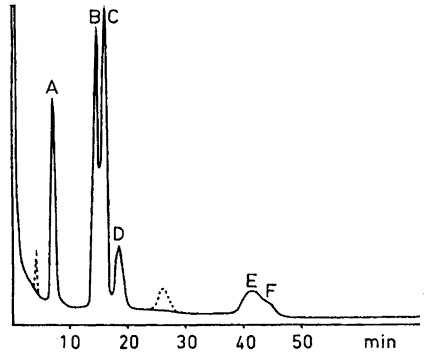

Fig. 2. GLC separation, on the ECNSS—M column at 175°, of the alditol acetates of the methylated sugars, obtained from the hydrolysate of the fully methylated mannan.

The GLC for the alditol acetate mixture obtained from the mannan is given in Fig. 2. The component in peak A had the same *T*-value and mass spectrum as the alditol acetate derived from 2,3,4,6-tetra-*O*-methyl-D-mannose. Peak B and C were not well resolved but were shown by their mass spectra to be the 3,4,6- and 2,4,6-tri-*O*-methyl-hexose derivatives, respectively. The *T*-values for the alditol acetates from 3,4,6- and 2,4,6-tri-*O*-methyl-D-mannose, 1.95 resp. 2.09, agree with the *T*-values for the components in these peaks (1.95 resp. 2.08). The component in peak D had the same *T*-value and mass spectrum as the alditol acetate derived from 2,3,4-tri-*O*-methyl-D-mannose. Peaks E and F were identified by their mass spectra as the 3,4- and 2,4-di-*O*-methyl-hexose derivatives, respectively. *T*-Values for the alditol

acetates from 3,4-di-*O*-methyl-D-mannose and 2,4-di-*O*-methyl-D-mannose were 5.37 and 5.44, respectively. The peaks E and F showed the *T*-values 5.28 and 5.44, respectively. The results are summarised in Table 2. Some minor peaks in the chromatogram (dotted) may be attributed to the contaminating fucogalactan.

Part of the fucogalactan was subjected to a mild acid hydrolysis, by which fucosidic linkages should be cleaved. The resulting polymeric material ($[\alpha]_{578}+148°$) was then recovered. Methylation analysis of this material (Table 3) showed a decrease in 2,3,4-tri-*O*-methyl-L-fucose and 3,4-di-*O*-methyl-D-galactose, compared to the values obtained from the undegraded polysaccharide, and a corresponding increase of 2,3,4-tri-*O*-methyl-D-galactose.

Table 3. Methyl ethers from the hydrolysate of methylated partially hydrolysed heterogalactan.

Sugars	T^a	mole %
2,3,4-Tri-*O*-methyl-L-fucose	0.64	11.4
2,3,4,6-Tetra-*O*-methyl-D-galactose	1.26	3.6
2,3,4-Tri-*O*-methyl-D-galactose	3.41	74.0
3,4-Di-*O*-methyl-D-galactose	6.92	11.0

[a] See Table 1.

DISCUSSION

The methylation analysis of the fucogalactan shows that it consists of chains of (1→6)-linked D-galactopyranose residues, about 35 % of which are substituted in the 2-position. All branches are terminated by L-fucopyranose residues and, from the methylation analysis of the partially hydrolysed material, it is evident that they consist of single L-fucopyranose residues. The high optical rotations of the undegraded and degraded polysaccharide indicate that the D-galactopyranose residues are α-linked and the increase in rotation during partial hydrolysis, suggested that the L-fucopyranose residues are α-linked.

From the results of this and previous studies,[1-4] it seems probable that (1→6)-linked galactans with branches in the 2-position are typical for the Basidiomycetes group of fungi. In one of the previously studied polysaccharides, the main chain is β-linked, in the others it is α-linked. There is some variation in the structure of the side chains, which may consist of 3-*O*-α-D-

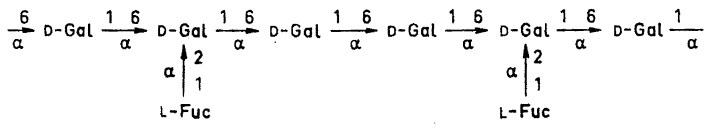

Fig. 3. Proposed structure for the heterogalactan from *P. borealis*. (All sugars are in the pyranosidic form.)

mannopyranosyl-α-L-fucopyranose residues, L-fucopyranose residues, or D-galactopyranose residues. The heterogalactan (Fig. 3) from *P. borealis* is the first member of this group of polysaccharides, having exclusively α-L-fucopyranose side chains.

The mannan, from its high optical rotation, must be essentially α-linked. The approximately equivalent proportions of (1→2)- and (1→3)-linkages suggest that it is composed of chains of alternating (1→2)- and (1→3)-linked α-D-mannopyranose residues with branches in the 6-position. The percentage of terminal (11.2) and of branched residues (11.4) should be the same, and the close agreement between these values and the percentage of the (1→6)-linked D-mannopyranose residues (11.2) may indicate that all branches consist of 6-*O*-D-mannopyranosyl-D-mannopyranose residues. The proposed structure (Fig. 4) is, however, hypothetical, and the present results do not exclude less regular structures.

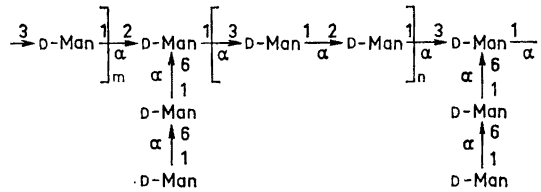

Fig. 4. Proposed structure for the mannan from *P. borealis*. (All sugars are in the pyranosidic form.)

EXPERIMENTAL

General methods. Paper chromatograms were run on Whatman No. 1 and 3 MM papers using the following solvent systems (v/v): a) Ethyl acetate-acetic acid-water, 3:1:1, b) Ethyl acetate-pyridine-water, 8:2:1. The components were detected with *p*-anisidine hydrochloride. Paper electrophoresis was performed on Schleicher und Schüll No. 8 glass fiber sheets (0.27—0.33 mm) using borate buffer at pH 10. The polysaccharides were detected with α-naphthol-sulphuric acid in butanol. Other general methods were the same as in a previous investigation.[9]

Isolation of the polysaccharides. The fungus was cut in small pieces, disintegrated in a Turmix blender and washed with acetone. The dried material (415 g) was extracted with boiling water (3 l) for 1 h and the procedure repeated twice. The combined extracts were concentrated and the polysaccharide material (9 g) isolated by precipitation with ethanol.

The fungal material was then extracted, under nitrogen, for 17 h with 0.3 M aqueous potassium hydroxide (3 l) and this extraction repeated once. The extract was neutralised with acetic acid, concentrated and precipitated with ethanol. The fraction weighed 15 g.

The water-soluble fraction was dissolved in water (750 ml) and 0.25 M CTA—OH (75 ml) was added. The precipitate on hydrolysis yielded D-glucose only. The supernatant solution was deionised, concentrated and precipitated with ethanol to give a polysaccharide fraction (3.6 g) which on hydrolysis yielded D-glucose, D-galactose, D-mannose, and L-fucose. This fraction, in 0.1 M boric acid (100 ml) was precipitated with 0.25 M CTA—OH (75 ml). The precipitate was treated with acetic acid-ethanol to yield a fraction (2.5 g) which on hydrolysis yielded D-galactose, D-mannose, L-fucose, and only small amounts of D-glucose. The polysaccharide material from the supernatant solution on hydrolysis yielded essentially D-glucose. The last traces of D-glucose in the hetero-

polysaccharide fraction were removed by two further precipitations with the same reagent. The final fraction weighed 1.8 g.

Distribution analysis. Gelchromatographic distribution analysis [10] on Sepharose 6 B was performed by Dr. Kirsti Granath, Pharmacia AB, Uppsala. The heteropolysaccharide fraction was composed of a mixture of two polysaccharides with $\bar{M}_w$ 350 000 and 17900, respectively. The column was calibrated by means of dextran fractions.

Separation of the polysaccharides on Sephadex. The polysaccharide mixture (100 mg) was separated on a column (40×5 cm) of Sephadex G-150. The separation was followed polarimetrically. Intermediate fractions were discarded and a fast moving material (19.5 mg) and a slow moving material (67 mg) were collected. Hydrolysis of the first fraction gave D-mannose and small amounts of D-galactose and L-fucose. Hydrolysate of the second fraction contained D-galactose and L-fucose and only traces of D-mannose. The polysaccharides showed $[\alpha]_{578}^{20}$ +82° and $[\alpha]_{578}^{20}$ +92°, (*c* 1, water), respectively.

For the sugar analysis, the polysaccharide was dissolved in 0.25 M sulphuric acid, kept at 100° for 12 h, and neutralised with barium carbonate. Part of the sugar mixture was converted into alditol acetates and analysed by GLC. The identities of the sugars were confirmed by mass spectrometry of their alditol acetates. Another part of the sugar mixture was fractionated on thick filter paper, using solvent system (b). The following components were isolated as syrups: D-galactose, $[\alpha]_{578}^{20}$ +136°, D-mannose, $[\alpha]_{578}^{20}$ +7.5°, and L-fucose, $[\alpha]_{578}^{20}$ −67°.

Methylation analysis of the polysaccharide. The polysaccharide (10 mg), in a 5 ml serum bottle sealed with a rubber cap, was dissolved in dry dimethyl sulphoxide (1.5 ml). Nitrogen gas was flushed through the bottle and a solution of 2 M dimethylsulphinyl sodium in dimethyl sulphoxide (1.0 ml) was added dropwise, using a syringe. The resulting, gelatinous solution was agitated in an ultrasonic bath (40 kc/s) for 1 h and kept at room temperature for 6 h. Methyl iodide (0.1 ml) was then added dropwise, with external cooling by tap-water, and the resulting turbid solution agitated for 20 min in the ultrasonic bath, when a clear solution was obtained. A second portion of 2 M dimethylsulphinyl sodium in dimethyl sulphoxide (1.0 ml) was added and the procedure above was repeated, except that an excess of methyl iodide (1.0 ml) was added. The solution was then poured into water (25 ml), dialysed overnight against tap-water and evaporated to dryness. The methylated polysaccharide was treated with 90 % formic acid (3 ml) at 100° for 3 h, concentrated and then hydrolysed in 0.25 M sulphuric acid (3 ml) at 100° for 12 h. The hydrolysate was neutralised with barium carbonate and the sugars were converted into alditol acetates and analysed by GLC [7]-mass spectrometry.[8] As is evident from Fig. 2, the separation of some components on the ECNSS−M column at 175° was not very good. The separation of the alditol acetates derived from the two di-*O*-methyl-D-mannoses, giving peaks E and F, was improved when the separation was performed at 160°, on the same column. A better separation of the alditol acetates derived from the two tri-*O*-methyl-D-mannoses, could, however, not be attained. The results are summarised in Tables 1 and 2.

Partial hydrolysis of the polysaccharide. The fucogalactan (30 mg) was dissolved in 0.025 M sulphuric acid (3 ml) and kept at 100° for 1.5 h. The hydrolysate was dialysed against tap-water overnight and lyophilysed. The hydrolysate (21 mg) showed $[\alpha]_{578}^{20}$ +148°. Part of this product (5 mg) was subjected to methylation analysis. The results are summarised in Table 3.

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