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Structure of the Fucoxylomannan from *Polyporus pinicola* (Fr)

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The structure of the fucoxylomannan from *Polyporus pinicola* has been reinvestigated, essentially by methylation analysis of the original polysaccharide and of a partially hydrolysed product. As a result of these studies, a detailed structure of the fucoxylomannan is presented.

In a previous publication,[1] studies on the polysaccharides elaborated by the fungus *Polyporus pinicola* were reported. Two heteropolysaccharides, a fucomannogalactan and a fucoxylomannan, were isolated. The structure of the former was determined but the results did not allow a detailed structural assignment of the latter. The present paper reports further structural studies on this heteropolysaccharide.

The water-soluble fucoxylomannan,[1] $[\alpha]_{578}^{20}$ −42°, on acid hydrolysis yielded L-fucose, D-xylose, and D-mannose in the relative proportions 0.8:0.7:1, together with traces of D-galactose and D-glucose. The polysaccharide was methylated by treatment with dimethylsulphinyl sodium-methyl iodide in dimethyl sulphoxide, following the procedure devised by Sandford and Conrad.[2] The methylated polysaccharide was hydrolysed and the mixture of methylated sugars converted into their alditol acetates, which were analysed by GLC[3]-mass spectrometry.[4] The results are given in Table 1. Four well

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

Sugars	T^a	mole %
2,3,4-Tri-*O*-methyl-L-fucose	0.65	30.2
3,4-Di-*O*-methyl-D-xylose	1.54	29.5
2,4,6-Tri-*O*-methyl-D-mannose	2.10	9.7
2,6-Di-*O*-methyl-D-mannose	3.35	30.6

[a] Retention times of the corresponding alditol acetates on an ECNSS–M column at 175° relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

separated peaks were obtained on GLC and mass spectrometry revealed that each peak represented a single component. Three components were identified by their mass spectra as alditol acetates of 2,3,4-tri-*O*-methyl-L-fucose, 2,4,6-tri-*O*-methyl-D-mannose, and 2,6-di-*O*-methyl-D-mannose. The retention times (*T*-values) for the two tri-*O*-methyl ethers were the same as for the authentic samples. No reference sample of 2,6-di-*O*-methyl-D-mannose was available. As D-mannose is the only hexose sugar component in the polysaccharide, the mass spectrometric identification of the 2,6-di-*O*-methyl-D-mannose is, however, unambiguous. From the mass spectrometrical evidence alone it was not possible to establish whether the fourth peak corresponded to a 2,3- or 3,4-di-*O*-methyl-D-xylose derivative, as these on reduction both yield 2,3-di-*O*-methyl-xylitol (D- and L-form, respectively). In the earlier investigation,[1] it was, however, demonstrated that the di-*O*-methyl-D-xylose was the 3,4-derivative. The molar proportions of L-fucose, D-xylose, and D-mannose, as calculated from the methylation analysis, are 0.7:0.7:1, in good agreement with the sugar analysis.

Part of the fucoxylomannan was then subjected to a mild acid hydrolysis, in order to remove most of the L-fucopyranosidic residues. The polymeric material, $[\alpha]_{578}^{20}$ −17°, was recovered and subjected to methylation analysis as above. The results are given in Table 2. The peak with the lowest retention time (T=0.68), from its mass spectrum, contained only traces of 2,3,4-tri-*O*-methyl-L-fucose (T=0.65), the main component being 2,3,4-tri-*O*-methyl-D-xylose (T=0.68).

Table 2. Methyl ethers from the hydrolysate of the methylated partially hydrolysed fucoxylomannan.

Sugars	T [a]	mole %
2,3,4-Tri-*O*-methyl-D-xylose + traces of 2,3,4-tri-*O*-methyl-L-fucose	0.68	38.8
3,4-Di-*O*-methyl-D-xylose	1.54	3.4
2,4,6-Tri-*O*-methyl-D-mannose	2.10	19.4
2,6-Di-*O*-methyl-D-mannose	3.35	38.4

[a] See Table 1.

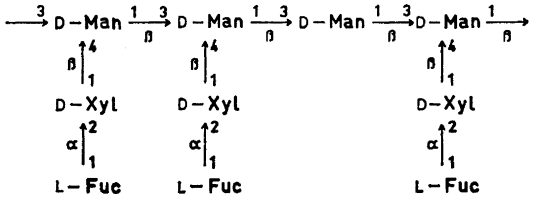

Fig. 1. Proposed structure of the fucoxylomannan from *P. pinicola*. (All sugar residues are pyranosidic.)

From these results, a detailed structure of the fucoxylomannan may be formulated (Fig. 1). The methylation analysis shows that all sugar residues are pyranosidic. The percentages of terminal L-fucose residues, monosubstituted D-xylose residues, and disubstituted D-mannose residues in the original polysaccharide are equivalent. After hydrolysis of the L-fucose residues, the percentages of terminal D-xylose residues and disubstituted mannose residues were equal. All branches therefore contain terminal 2-*O*-L-fucopyranosyl-D-xylose residues and these account for all the L-fucose and D-xylose residues in the polysaccharide. The chains consequently contain only (1→3)-linked D-mannose residues, with branch points at the 4-position. The present results do not exclude the possibility that in some branches, one or several D-mannose residues may be inserted between the chain and the L-fucosyl-D-xylose residue. It seems more plausible, however, that the polysaccharide consists of (1→3)-linked chains of D-mannose residues, about 75 % of which are substituted with a 2-*O*-L-fucosyl-D-xylose residue in the 4-position. The low specific optical rotation of the partially degraded polysaccharide, in which the molar proportion of D-xylose to D-mannose, (0.6:1), is almost the same as in the original polysaccharide, (0.7:1), indicates that both the D-xylose and the D-mannose residues are β-linked. The optical rotation of the polysaccharide increased when the L-fucose residues were removed by hydrolysis, indicating that these residues are α-linked.

EXPERIMENTAL

General methods. Concentrations were carried out under reduced pressure at bath temperatures below 40°. GLC [3] and mass spectrometry [4] were performed as previously described. Optical rotations were determined with a Perkin-Elmer 141 photoelectric polarimeter.

Sugar analysis. The fucoxylomannan, $[\alpha]_{578}^{20}$ −42° (*c* 0.65, H_2O), (5 mg) was dissolved in 0.13 M sulphuric acid (5 ml), kept at 100° for 16 h and neutralised with Dowex 3 (free base). The sugars in the hydrolysate were converted into alditol acetates and analysed by GLC.[5]

Methylation analysis. The polysaccharide (5 mg) in a 5 ml serum bottle sealed with a rubber cap, was dissolved in dry dimethyl sulphoxide (1 ml). Nitrogen was flushed through the bottle and a solution of 2 M dimethylsulphinyl sodium in dimethyl sulphoxide (1 ml) was added with the aid of a syringe. The gelatinous solution was agitated in an ultrasonic bath (40 kc/s) for 1 h and kept at room temperature for 6 h. Methyl iodide (1 ml) was added dropwise with external cooling. The resulting solution was agitated for 30 min in the ultrasonic bath, poured into water (50 ml), dialysed overnight against tap-water and concentrated to dryness.

The methylated polysaccharide was hydrolysed overnight in 0.13 M sulphuric acid (5 ml) at 100°. The hydrolysate was neutralised with Dowex 3 and the mixture of partially methylated sugars was converted into alditol acetates and analysed by GLC [3]-mass spectrometry.[4]

Partial hydrolysis. After some preliminary experiments, in order to find optimal conditions for hydrolysis of the L-fucosidic linkages without significant hydrolysis of other linkages, the fucoxylomannan (15 mg) was dissolved in 0.05 M sulphuric acid (10 ml) and kept at 100° for 1.5 h. The hydrolysate was dialysed overnight against tap-water and evaporated to dryness. The polymeric residue, $[\alpha]_{578}^{20}$ −17° (*c* 0.5, H_2O), was subjected to methylation analysis as described above.

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