

Short Communications

Polysaccharides Elaborated by
Polyporus ovinus (Schaeff.)1. Structure of a Fucogalactan from
the Water Extracts of the Fruit BodiesKERSTIN AXELSSON and HÅKAN
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In previous publications¹⁻⁶ we have reported structural studies on different polysaccharides elaborated by fungi belonging to the Basidiomycetes group. In the present paper, studies on the polysaccharides from *Polyporus ovinus* are reported.

Fruit bodies of *P. ovinus* were harvested locally. The disintegrated material was extracted, first with hot water and then with 0.3 M potassium hydroxide under nitrogen at room temperature and the extracts processed to give polysaccharide fractions. Both fractions on hydrolysis gave D-glucose as the predominant sugar, together with smaller amounts of D-galactose, D-mannose, L-fucose, and D-xylose (traces only from the material extracted with hot water). Studies on the alkali extracted material will be reported separately.

Most of the glucan present in the water extracted material was removed by precipitation with cetyltrimethylammonium hydroxide⁷ (CTA-OH). A polysaccharide fraction almost free from glucan was obtained by precipitation from boric acid with CTA-OH. The polysaccharide $[\alpha]_{578}^{20} + 85^\circ$, on acid hydrolysis yielded L-fucose and D-galactose in the relative molar proportions 1:3.6 and traces of D-mannose and D-glucose. The fucogalactan was methylated by treatment with methyl-

sulphonyl sodium—methyl iodide in methyl sulphoxide, following the procedure devised by Hakomori.⁸ The fully methylated polysaccharide was hydrolysed and the mixture of methylated sugars converted into their alditol acetates, which were analysed by GLC⁹-mass spectrometry.¹⁰ The results are given in Table I, column A.

Table I. Methyl ethers from the hydrolysate of: A. methylated fucogalactan, B. methylated partially hydrolysed fucogalactan.

Sugars	T^a	mole %	
		A	B
2,3,4-Tri- <i>O</i> -methyl-L-fucose	0.65	20.0	1.5
2,3,4,6-Tetra- <i>O</i> -methyl-D-galactose	1.25	1.5	10.5
2,3,4-Tri- <i>O</i> -methyl-D-galactose	3.41	55.0	84.0
3,4-Di- <i>O</i> -methyl-D-galactose	7.00	23.5	4.0

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

Four well separated peaks were obtained on GLC, and mass spectrometry revealed that each peak contained a single component. From their retention times (T -values), which were the same as for the corresponding authentic substances, and their mass spectra the components were identified as alditol acetates of 2,3,4-tri-*O*-methyl-L-fucose, 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,4-tri-*O*-methyl-D-galactose, and 3,4-di-*O*-methyl-D-galactose. The molar proportions of L-fucose and D-galactose, as calculated from the methylation analysis, are 1:3.9, in good agreement with the sugar analysis.

Part of the fucogalactan was subjected to a mild acid hydrolysis, in order to cleave most of the L-fucosidic linkages. The polymeric material, $[\alpha]_{178}^{20} + 140^\circ$, was recovered and subjected to methylation analysis as above. The results are given in Table I, column B.

The methylation analysis of the fucogalactan shows that it consists of chains of (1→6)-linked D-galactopyranose residues, about 25% of which are substituted in the 2-position. All branches are terminated by L-fucopyranose residues. From the methylation analysis of the partially hydrolysed material, it is evident that the side chains consist of single L-fucopyranose residues. The high percentage of 2,3,4,6-tetra-*O*-methyl-D-galactose indicates that some α -(1→6)-linkages also have been hydrolysed. From the optical rotations of the undegraded and degraded polysaccharides it may be inferred that both the D-galactopyranose residues and the L-fucopyranose residues are α -linked.

(25 ml) was added. The precipitate formed was separated by centrifugation and treated with acetic acid-ethanol to give a water soluble fraction (1.3 g), which on hydrolysis yielded D-glucose only. The supernatant solution was deionised, concentrated and precipitated with ethanol. The latter fraction was dissolved in 0.1 M aqueous boric acid (50 ml) and precipitated with 0.25 M CTA-OH (5 ml) and 1 M sodium hydroxide (0.5 ml). The precipitate was processed to give a fraction which on hydrolysis yielded L-fucose and D-galactose and only traces of D-glucose and D-mannose. The final fraction weighed 0.3 g.

Sugar analysis. The fucogalactan, $[\alpha]_{178}^{20} + 85^\circ$ (c 0.2, H₂O), (5 mg) was dissolved in 0.13 M sulphuric acid (5 ml), kept at 100° for 16 h and then neutralised with Dowex 3 (free base). The sugars in the hydrolysate were converted into alditol acetates and analysed by GLC.¹² Another part of the polysaccharide was hydrolysed and neutralised as above, and the resulting sugars fractionated on thick filter paper, using solvent system (a). The

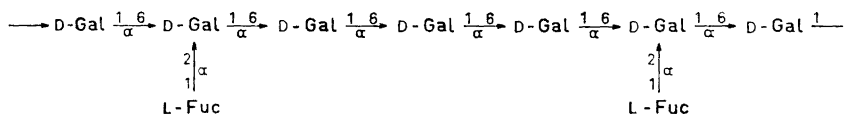


Fig. 1. Proposed structure for the fucogalactan from *Polyporus ovinus*.

The heterogalactan (Fig. 1) from *P. ovinus* seems to have a very similar structure to the fucogalactan from *P. borealis*.⁵ The fucose content is, however, slightly lower.

Experimental. Paper chromatograms were run on Whatman Nos. 1 and 3 MM papers using the following solvent system (v/v): a) Ethyl acetate-pyridine-water, 8:2:1. The components were detected with *p*-anisidine hydrochloride. Other general methods were the same as in a previous investigation.¹¹

Isolation of the polysaccharide. The fungus was washed with ethanol and acetone, disintegrated in a Turmix blender in the presence of ethanol and then dried. The fungal material (50 g) was extracted three times with boiling water for one hour. The combined extracts were concentrated and the polysaccharides (4.5 g) precipitated with ethanol. The polymeric material, a hydrolysate of which contained no acidic sugars, was dissolved in water (400 ml) and 0.25 M aqueous CTA-OH

following components were isolated as syrups: D-galactose, $[\alpha]_{178}^{20} + 60^\circ$ and L-fucose, $[\alpha]_{178}^{20} - 55^\circ$.

Methylation analysis. The polysaccharide (5 mg) in a 5 ml serum bottle sealed with a rubber cap, was dissolved in dry methyl sulphoxide (1 ml). Nitrogen was flushed through the bottle and a solution of 2 M methylsulphonyl sodium in methyl sulphoxide (1 ml) was added by means of a syringe. The gelatinous solution was agitated in an ultrasonic bath (40 kc/s) for 1 h and kept at room temperature for 5 h. Methyl iodide (1 ml) was added dropwise with external cooling. The resulting solution was agitated for 30 min in the ultrasonic bath, poured out into water (50 ml), dialysed overnight against tap-water and concentrated to dryness. The fully methylated polysaccharide was hydrolysed overnight in 0.13 M sulphuric acid (5 ml) at 100°. The hydrolysate was neutralised with Dowex 3 (free base) and the mixture of partially methylated sugars was converted into alditol acetates and analysed by GLC⁹-mass spec-

trometry.¹⁰ The results are summarised in Table 1, column A.

Partial hydrolysis. The fucogalactan (20 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 concentrated to dryness. The polymeric residue, $[\alpha]_{578}^{20} + 140^\circ$ (c 0.1, H₂O), was subjected to methylation analysis as described above. The results are summarised in Table 1, column B.

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Mass Spectrometric Studies on Organic Selenium-Oxygen Compounds

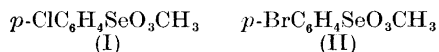
1. Mass Spectra of Methyl *p*-Chlorobenzene Selenonate and Methyl *p*-Bromobenzene Selenonate

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Recently it has been shown by several workers that a variety of sulphur-oxygen compounds, such as sulphoxides,^{1,2} sulphones,¹⁻⁶ sulphonamides,^{7,8} sulphonyl chlorides,⁷ sulphonyl hydrazones⁹ and sulphonylureas,¹⁰ in most of the cases studied with the sulphur atom bonded to an aromatic carbon, are subjected to skeletal rearrangements upon electron impact with formation of C—O bonds.

No reports are, however, found in the literature on mass spectrometric studies in the domain of selenium-oxygen compounds. An investigation of the behaviour of different types of compounds in this field upon electron impact has been started at this Institute and in this first paper the mass spectra of the methyl esters of *p*-chlorobenzene and *p*-bromobenzene selenonic acids, I and II, are briefly discussed.



In the following discussion only fragment ions of higher masses are mentioned because the final fragmentation of the non-selenious ions intermediately obtained, gives peaks well-known for aromatic compounds.¹¹ Moreover, only peaks corresponding to the main isotopes of selenium, ⁷⁸Se, and of the halogens in question, ³⁵Cl and ⁷⁹Br, are considered. Transitions supported by appropriate metastable peaks are indicated in the scheme by asterisks.

The spectra of I and II, Figs. 1 and 2, show a series of very characteristic groups of peaks. Generally speaking, the principal ions are derived from cleavages around the selenium containing group. Alternative routes possible for the formation of the non-selenious ions are summarized in the scheme below.