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Polysaccharides Elaborated by *Fomes annosus* (Fr.) Cooke

I. A Water-soluble Acidic Polysaccharide from the Fruit Bodies

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In a recent publication,[1] studies on the structure of two acidic polysaccharides isolated from *Polyporus fomentarius* and *Polyporus igniarius*, were reported. In the present communication, studies are reported on a similar polysaccharide isolated from *Fomes annosus (Polyporus annosus)*, another species which also causes severe wood destruction.

Fruit bodies of *F. annosus* were harvested locally. The isolation of water-soluble polysaccharides and their separation into neutral and acidic polysaccharides was performed as previously described.[2] Studies on the neutral polysaccharides will be reported separately. The acidic material was further purified by column chromatography on DEAE-Sephadex in the acetate form.[1] The purified acidic polysaccharide, $[\alpha]_{578}^{20} - 37°$ (*c* 0.13, H_2O), yielded on hydrolysis D-glucose and D-glucuronic acid. This glucuronoglucan contained *ca.* 35 % uronic acid residues, as determined by the carbazole method,[3] with correction for the colour reaction given by the glucose residues. The following method was also applied for determining glucuronic acid content. The glucuronoglucan was partially hydrolysed and the mixture of mono- and acidic oligosaccharides was treated with methanolic hydrogen chloride and then converted to ether-soluble trimethylsilyl-derivatives. Finally, the esterified glucuronic acid residues were reduced with lithium aluminiumdeuteride.[4] By this procedure, the D-glucuronic acid residues were transformed into D-glucose residues deuterated at C-6 ($-CD_2OH$). The mixture of deuterated and unlabelled glucose, obtained on subsequent hydrolysis, was reduced with sodium borohydride, acetylated and analysed by GLC[5]—mass spectrometry.[6] Fission of unlabelled D-glucitol hexaacetate between C-3 and C-4, yields the primary fragment, *m/e* 217, from both halves of the molecule; whereas, the deuterated compound, by the same fission should give equal amounts of an *m/e* 217 fragment from C-1 to C-3 and an *m/e* 219 fragment from C-4 to C-6. From the relative intensities of the peaks with *m/e* 219 and *m/e* 217, the molar percentage of deuterated glucitol acetate (*i.e.* glucuronic acid) was estimated as being approximately 30 %.

The polysaccharide was methylated as previously described.[1] Part of the methylated polysaccharide was hydrolysed, the

Table 1. Methyl ethers obtained from the hydrolysate of A: methylated glucuronoglucan, B: methylated-reduced glucuronoglucan.

Sugars	T^a	mole %	
		A	B
2,3,4,6-Tetra-*O*-Me-D-G	1.00	17	12
2,4,6-Tri-*O*-Me-D-G	1.95	18	17
2,3,4-Tri-*O*-Me-D-G	2.48	41	31 (6)[b]
2,4-Di-*O*-Me-D-G	5.10	24	17
2,3-Di-*O*-Me-D-G	5.40	—	23

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

[b] The figure in the bracket represents the 2,3,4-tri-O-methyl-D-glucose, derived from D-glucuronic acid residues.

mixture of partially methylated sugars in the hydrolysate converted into alditol acetates and analysed by GLC[7] —mass spectrometry[8] (Table 1, column A). The other part was treated with methanolic hydrogen chloride and reduced by lithium aluminiumdeuteride in order to transform the D-glucuronic acid residues to deuterated D-glucose residues. This product was then hydrolysed and the hydrolysate investigated as above (Table 1, column B).

The identification of the methyl ethers by mass spectrometry was facilitated by the fact that only D-glucose derivatives were present in the final hydrolysate. These were unambiguously characterised as derivatives of 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,4,6-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-glucose, 2,4-di-*O*-methyl-D-glucose and 2,3-di-*O*-methyl-D-glucose. The *T*-values of these substances agreed with those given by authentic substances.

Of the different methyl ethers found, only 2,3-di-*O*-methyl-D-glucose, 2,4-di-*O*-methyl-D-glucose, and 2,3,4-tri-*O*-methyl-D-glucose could have been derived from D-glucuronic acid residues. Since uronic acid residues had been reduced with deuteride, these would be labelled at C-6. Mass spectra revealed that all of the 2,3-di-*O*-methyl-D-glucose and only part of the 2,3,4-tri-*O*-methyl-D-glucose were deuterated. The 2,4-di-*O*-methyl-D-glucose was not deuterated. The molar percentage of labelled 2,3,4-tri-*O*-methyl-D-glucose, representing terminal D-glucuronic acid residues, was estimated to be approximately 6 %. The percentage of total terminal residues is in good agreement with the percentage of branching points (2,4-di-*O*-methyl-D-glucose). The determinations of uronic acid content correspond fairly well with the proportion of uronic acid residues found by methylation analysis.

The glucuronoglucan on graded acid hydrolysis yielded several acidic oligosaccharides, which were fractionated on DEAE-Sephadex (formate form) as previously described.[1] Three of the main components were further purified by paper chromatography. Their low optical rotations, $A=[\alpha]_{578}^{20}+4°$, $B=[\alpha]_{578}^{20}-20°$ and $C=[\alpha]_{578}^{20}-2°$, indicated that they were β-linked. A, B, and C were chromatographically and electrophoretically indistinguishable from *O*-β-D-glucuronosyl-(1 → 3)-D-glucose, $[\alpha]_{578}^{20}$ +5°, *O*-β-D-glucuronosyl-(1 → 4)-*O*-β-D-glucuronosyl (1 → 3)-D-glucose, $[\alpha]_{578}^{20}-12°$, and *O*-β-D-glucuronosyl-(1 → 4-)D-glucuronic acid, $[\alpha]_{578}$ −3°, respectively. These oligosaccharides had been characterised in a previous investigation.[1]

Other acidic oligosaccharides, obtained on partial acid hydrolysis of the glucuronoglucan were probably higher oligosaccharides containing several D-glucuronic acid residues as deduced from their paper chromatographic and paper electrophoretic mobilities.

In conclusion, the glucuronoglucan from *Fomes annosus* is similar to the glucuronoglucans from *Polyporus fomentarius* and *P. igniarius*. They all seem to contain a β-glucan backbone. Chains, composed on average of five β-(1 → 4)-linked D-glucuronic acid residues, are attached to D-glucose residues through C-3. It is not yet known if the latter form part of the backbone or the side chains. Branched β-glucans, in which the D-glucose residues are substituted at the 3-, 6-, or 3,6-positions, are common in fungi.[9]

Assignment of the structure of the backbone must await further studies.

Experimental. The methods used were described before.[1] Trimethylsilyl derivatives were prepared and reduced as described by Aspinall *et al.*[4]

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