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1. Carter, H. E., Johnson, P. and Weber, E. J. *Ann. Rev. Biochem.* **34** (1965) 109.
2. Mårtensson, E. *Thesis*, Göteborg 1966.
3. Makita, A., Suzuki, C. and Yosizawa, Z. *J. Biochem.* **60** (1966) 502.
4. Karlsson, K.-A. and Mårtensson, E. *Biochim. Biophys. Acta* **152** (1968) 230.
5. Karlsson, K.-A. and Holm, G. A. L. *Acta Chem. Scand.* **19** (1965) 2423.
6. Karlsson, K.-A. *Acta Chem. Scand.* **18** (1964) 565.
7. Karlsson, K.-A. *Acta Chem. Scand.* **18** (1964) 2395.
8. Karlsson, K.-A. *Acta Chem. Scand.* **18** (1964) 2397.
9. Karlsson, K.-A. *Acta Chem. Scand.* **20** (1966) 2884.
10. Karlsson, K.-A. *Acta Chem. Scand.* **21** (1967) 2577.
11. Carter, H. E., Gaver, R. C. and Yu, R. K. *Biochem. Biophys. Res. Commun.* **22** (1966) 316.
12. Carter, H. E. and Gaver, R. C. *Biochem. Biophys. Res. Commun.* **29** (1967) 886.

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An Extracellular Glucan Produced by the Rot Fungus *Stereum sanguinolentum*

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The enzymes produced by the rot fungus *Stereum sanguinolentum* (Basidiomycetes) have been studied by Eriksson *et al.*[1-3] When the fungus was cultivated in a medium containing cellulose as the carbon source, an extracellular, slimy polysaccharide was formed. The same polysaccharide was produced in a higher yield when cellobiose was used as the carbon source, and the present paper reports structural studies on this polysaccharide.

The polysaccharide, which caused the culture solution to become viscous, was isolated by precipitation as a fibrous material which was insoluble in water. It gave $[\alpha]_{578}$ + 10° (1 M KOH) and on acid hydrolysis yielded D-glucose only, showing that it was a β-glucan. Two methylations with dimethylsulphinyl sodium-methyl iodide, following the procedure devised by Sandford and Conrad,[4] yielded the fully methylated polysaccharide. The mixture of methylated sugars obtained on acid hydrolysis of this material was converted into the alditol acetates and analyzed by GLC [5] — mass spectrometry.[6] A unique mass spectrum is obtained for each substitution pattern in the partially methylated alditol acetates. As all the methyl ethers are derived from D-glucose, the components have been fully identified by their mass spectra. The identifications were further confirmed by the *T*-values, which were the same as those given by the corresponding authentic substances.

The methylation analysis, summarized in Table 1, shows that the polysaccharide consists of a backbone of β-(1→3)-linked D-glucose residues, with a branch in the 6-position on approximately every third residue. The insolubility of the polysaccharide prevented the application of periodate oxidation studies, enzymic hydrolysis or other methods, which might have given information about the length

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

Sugars	T [a]	mole %
2,3,4,6-Tetra-*O*-methyl-D-glucose	1.00	27.5
2,4,6-Tri-*O*-methyl-D-glucose	1.95	46.0
2,4-Di-*O*-methyl-D-glucose	5.10	26.5

[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.

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of the side chains. From the physical properties of the polysaccharide it may be inferred, however, that the branches are short, possibly consisting of single D-glucose residues.

β-Glucans containing (1→3)- and (1→6)-linkages often occur in fungi,[7] and closely related, extracellular glucans are produced by *Claviceps purpurea*,[8] *Pullularia pullulans*,[9] and an unidentified species of *Fungi imperfecti*.[10]

Experimental. Concentrations were carried out under reduced pressure at bath temperatures below 40°. GLC and mass spectrometry were performed as described in Refs. 5 and 6, respectively. Optical rotations were determined with a Perkin Elmer 141 photoelectrical polarimeter. Paper chromatograms were run on Whatman No. 1 papers, using ethyl acetate-pyridine-water, 8:2:1, as solvent and anisidine hydrochloride as spray reagent.

Isolation of the β-glucan. Stereum sanguinolentum mycelium was cultivated in aerated submersed cultures on a modified Norkrans' medium,[11] using cellobiose (5 g/l) as the only carbon source. The cultivation was carried out for 4—5 days at 25°. The mycelium was removed by filtration and the polysaccharide precipitated by the addition of an equal amount of ethanol to the solution. The precipitate was filtered off, washed with 50 % ethanol and dried. Approximately 400 mg polysaccharide per liter of culture solution was obtained. The polysaccharide gave $[\alpha]_{578}$ + 10° (*c* 0.5, 1 M KOH). A hydrolysate of the polysaccharide contained D-glucose only, as revealed by paper chromatography and GLC of the derived alditol acetate. D-Glucose was further characterized as penta-*O*-acetyl-β-D-gluco-pyranose, m.p. 132—133°, $[\alpha]_{578}$ + 5° (*c* 0.1, chloroform).

Methylation analysis. The β-glucan (5 mg), in a 5 ml serum bottle, was dissolved in dry dimethyl sulphoxide (1 ml). Nitrogen was flushed through the bottle and a solution of 2 M dimethyl sulphinyl sodium [4] in dimethyl sulphoxide (1 ml) was added dropwise, using a syringe. The resulting, gelatinous solution was agitated in an ultrasonic bath (40 kc/s) for 30 min and kept at room temperature for 8 h. Methyl iodide (0.13 ml) was then added dropwise, with external cooling, and the resulting turbid solution was agitated for 30 min in the ultrasonic bath, when a clear solution was obtained. This solution was subjected to a second methylation, using the same procedure. The mixture was then poured into water (50 ml), dialyzed and evaporated to dryness.

The methylated glucan was dissolved in 90 % formic acid (5 ml), kept at 100° for 2 h, evaporated to dryness, dissolved in 0.13 M sulphuric acid and kept at 100° for 18 h. The hydrolysate was neutralized with barium carbonate and filtered. The sugars were reduced to alditols by sodium borohydride and then acetylated.[5] The resulting mixture of partially methylated alditol acetates was analyzed by GLC-mass spectrometry.

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1. Bucht, B. and Eriksson, K.-E. *Arch. Biochem. Biophys. In press.*
2. Eriksson, K.-E. and Pettersson, B. *Arch. Biochem. Biophys. In press.*
3. Björndal, H. and Eriksson, K.-E. *Arch. Biochem. Biophys. In press.*
4. Sandford, P. A. and Conrad, H. E. *Biochemistry* **5** (1966) 1508.
5. Björndal, H., Lindberg, B. and Svensson, S. *Acta Chem. Scand.* **21** (1967) 1801.
6. Björndal, H., Lindberg, B. and Svensson, S. *Carbohydrate Res.* **5** (1967) 433.
7. Bull, A. T. and Chesters, C. G. C. *Advan. Enzymol.* **28** (1966) 325.
8. Perlin, A. S. and Taber, W. A. *Can. J. Chem.* **41** (1963) 2278.
9. Bouveng, H. O., Kiessling, H., Lindberg, B. and McKay, J. *Acta Chem. Scand.* **17** (1963) 1351.
10. Johnson, J., Kirkwood, S., Misaki, A., Nelson, T. E., Scaletti, J. V. and Smith, F. *Chem. Ind. (London)* **1963** 820.
11. Ahlgren, E. and Eriksson, K.-E. *Acta Chem. Scand.* **21** (1967) 1193.

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