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Note

Structural studies of the extracellular polysaccharides elaborated by *Rhinocladiella elatior* and *Rhinocladiella mansonii*

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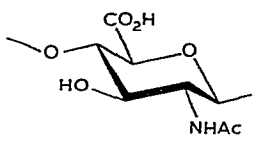
The black, yeast-like fungi NRRL YB-4163 and NRRL Y-6272 produce extracellular polysaccharides which give highly viscous solutions[1,2]. The fungi have been identified as *Rhinocladiella elatior* and *R. mansonii*, respectively[3]. The polysaccharide from *R. elatior* is a homopolysaccharide composed of 2-acetamido-2-deoxy-D-glucuronic acid residues, and has[1] $[\alpha]_D$ −75°. The polysaccharide from *R. mansonii* is composed of 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucuronic acid residues in the molar ratio 2:1, and has[4] $[\alpha]_D$ −6°. We now report structural studies of these polysaccharides.

Solutions of the *R. elatior* polysaccharide in water or dimethyl sulfoxide were so viscous that they were difficult to work with. A partially degraded, but still polymeric, product was obtained on hydrolysis in fuming hydrochloric acid for 18 h at room temperature.

A sample of the partially degraded polysaccharide was acetylated, in order to increase its solubility in dimethyl sulfoxide, and methylated according to Hakomori[5]. The methylated polysaccharide was subjected, in sequence, to carboxyl-reduction and hydrolysis with acid. Analysis of the product as the alditol acetates by g.l.c.–m.s.[6] gave a single component, derived from 2-deoxy-3-*O*-methyl-2-*N*-methylacetamido-D-glucose. The *R. elatior* polysaccharide is consequently composed of (1→4)-linked 2-acetamido-2-deoxy-D-glucopyranosyluronic acid residues, which, from the negative optical rotation of the polysaccharide, should be β-linked, as in **1**. Carboxyl-reduction of this polysaccharide should give chitin, and an insoluble product was produced on attempted carboxyl-reduction.



1

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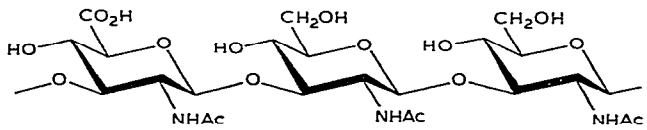
The *R. mansonii* polysaccharide was partially degraded by treatment with fuming hydrochloric acid for 6 h at room temperature. The still-polymeric material had $[\alpha]_{578}$ −5°, indicating that the sugar residues are β-D-linked. Methylation analysis, with carboxyl-reduction after the methylation, gave a mixture of 2-deoxy-4,6-di-*O*-methyl-2-*N*-methylacetamido-D-glucose and 2-deoxy-4-*O*-methyl-2-*N*-methylacetamido-D-glucose. Only the former component was obtained when the carboxyl-reduction was either omitted or performed before the methylation step. The polysaccharide is consequently linear, and both the 2-acetamido-2-deoxy-β-D-glucopyranosyl and the 2-acetamido-2-deoxy-β-D-glucopyranosyluronic acid residues are linked through O-3. The carboxyl-reduced polysaccharide should be a (1→3)-linked 2-acetamido-2-deoxy-D-glucan, and, in agreement with this view, it gave a simple ^{13}C-n.m.r. spectrum.

Polysaccharides from micro-organisms are often composed of oligosaccharide repeating-units. It is possible that the *R. mansonii* polysaccharide is composed of trisaccharide repeating-units with the structure **2**, although experimental evidence for this assumption has not been obtained.



2

EXPERIMENTAL

General methods. — ^{13}C-N.m.r. spectra were recorded with a JEOL FX-100 instrument for solutions in deuterium oxide at 70°, with tetramethylsilane as external reference. Carboxyl-reduction of methylated polysaccharides was performed with sodium borohydride in 1,4-dioxane–ethanol (3:1). Methylation analyses were performed as previously described[7], using OV-17 columns for g.l.c.

Partial, acid hydrolysis of the polysaccharides. — A solution of the polysaccharide (200 mg) in 10 ml of fuming hydrochloric acid (d, 1.19) was kept at room temperature. After 18 h (*R.e.*) or 6 h (*R.m.*), the solution was diluted with water and freeze-dried. The polysaccharide was recovered by gel filtration on a column of Sephadex G-50 and freeze-drying. The yield of degraded polysaccharide was almost quantitative. The ^{13}C-n.m.r. spectrum of the *R. elatior* polysaccharide, as the sodium salt, showed the following signals: δ 176.1, 175.6, 102.5, 82.3, 78.4, 74.0, 56.8, and 24.3.

Carboxyl-reduction. — Carboxyl-reduction of the *R. mansonii* polysaccharide was performed as devised by Taylor *et al*[8]. The ^{13}C-n.m.r. spectrum showed the following signals: δ 176.2, 102.3, 82.4, 77.5, 70.7, 63.0, 57.0, and 24.6.

ACKNOWLEDGMENTS

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