A Polysaccharide from *Pseudomonas aeruginosa*

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From the slimy deposits occurring in a paper machine a polysaccharide fraction was obtained in yields of up to 14 %, the main component of which was isolated by chromatography on a DEAE cellulose column. The polysaccharide had [α] + 99° in water and contained 13 % uronic acid. Its solutions were very viscous; attempts to transfer its potassium salt into the free acid resulted in gelatinisation. The neutral sugars in a hydrolysate were D-galactose, D-glucose, D-mannose and L-rhamnose, the proportions being 55, 24, 3, and 17 (weight %), respectively. A strain of *Pseudomonas aeruginosa* was also isolated from the slime. When grown on a medium containing potassium D-gluconate, the organism produced an extracellular polysaccharide. The main polysaccharide, isolated as above, had almost identical properties ([α] + 94°, uronic acid content 14 %; galactose:glucose:mannose:rhamnose = 56:22:4:19) to the above material. There seems to be no doubt that the two polysaccharides are synthesised by the isolated *Ps. aeruginosa* strain. In laboratory culture, however, the organism soon ceased to synthesise the polysaccharide.

On partial hydrolysis only traces of neutral sugars other than monomers were formed. Such a result would be expected from a highly branched polysaccharide with considerable differences in the rate of hydrolysis of different linkages. An aldobiouronic acid, composed of D-mannose and D-glucuronic acid was isolated together with small amounts of glucuronic acid after more extensive hydrolysis. Reduction of the aldobiouronic acid methyl ester methyl glycoside, followed by methylation and acid hydrolysis, afforded 2,4,6-tri-*O*-methyl-D-mannose and 2,3,4,6-tetra-*O*-methyl-D-glucose. This, and the high optical rotation of the original acid, $[\alpha]_D$ + 71° in water, shows it to be 3-*O*-α-D-glucopyranuronosyl-D-mannose. The aldobiouronic acid thus represents a structural element which does not seem to have been observed previously.

In the polysaccharide, D-glucuronic acid and D-mannose appear to be present in equimolecular proportions as the majority of these sugars are found only in the above aldobiouronic acid. The small amounts of the free sugars, found in hydrolysates, can be reasonably accounted for by a slight hydrolysis of the aldobiouronic acid. As this acid is the only grouping found that could cause absorption of the polysaccharide on DEAE, it follows that D-galactose, D-glucose, and L-rhamnose most probably all occur in one polysaccharide together with D-mannose and D-glucuronic acid. The sugars would than be present in the approximate relative proportions 4:1.5:1.5:1.1.

Experimental. Unless otherwise stated, procedures similar to those used for the *Scarcina* mannan [1] were employed.

Isolation of polysaccharides. Slime deposits were extracted with hot water, the extract was clarified by centrifugation and poured into ethanol when the polysaccharide was precipitated. The cultivation of the isolated organism and the isolation of the polysaccharides synthesised were as described in Ref. 1. About 1 g of polysaccharide per litre of culture solution was obtained. The polysaccharide materials were added to columns of DEAE cellulose and eluted with aqueous potassium acetate, the concentration of which was increased stepwise. The main component both from the slime deposits and from the cultures, was eluted at 0.08 – 0.20 M potassium acetate.

Hydrolysis. The polysaccharide was hydrolysed first with 90 % formic acid at 100° for 2 h and then with 0.25 M sulphuric acid at 100° for 16 h. The acidic components were separated by means of Dowex 2 in the acetate form.

Of the neutral sugars D-galactose, D-glucose and L-rhamnose were isolated in pure, crystalline form by chromatography on thick filter papers and compared with authentic samples.

The acidic sugars were separated by chromatography on Sephadex G-25 and thick filter papers to give D-glucuronic acid, its γ-lactone, an aldobiouronic acid and its lactone. The first two were identified by chromatographic methods only.

Aldobiouronic acid. The substance had R_{Glu} 0.52 in ethyl acetate, acetic acid, water, 3:1:1 and yielded on hydrolysis mannose, glucuronic acid, and its γ-lactone. A sample was refluxed with 3 % methanolic hydrogen chloride for 16 h and then treated with sodium hydridoborate. The product was methylated and hydrolysed to give, after separation on thick filter paper, *2,4,6-tri*-O-*methyl*-D-*mannose*, $[\alpha]_D$

+ 16.5°, m.p. 61–62°, undepressed on admixture with a sample obtained from the methylated *Scarcina* mannan,[1] and 2,3,4,6-tetra-*O*-methyl-D-glucose, $[\alpha]_D$ + 80°, m.p. and mixed m.p. 93–95°. Both ethers behaved similarly to reference samples on gas-liquid, paper, and thin layer chromatography.

A lactone of the aldobiouronic acid (R_{Glu} 1.20) was also obtained. It yielded mannose, glucuronic acid and its γ-lactone on acid hydrolysis. When a solution of either the aldobiouronic acid or its lactone was heated in aqueous acetic acid, the same equilibrium mixture was obtained.

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Polysaccharides in Pollen

III. The Acidic Arabinogalactan in Mountain Pine Pollen

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The acidic arabinogalactan from Mountain Pine pollen has previously been shown to be made up from L-arabinose, D-galactose, L-rhamnose and glucuronic acid,[1] and a brief examination by methylation and by partial hydrolysis is now reported. The properties of two samples obtained in the fractionation series described in Part II[2] are given in Table 1. Sample A was eluted by 0.05–0.16 M and sample B by 0.13–0.34 M potassium acetate. Mild acid hydrolysis gave, besides monomeric sugars and a polymeric fraction, a disaccharide with $[\alpha]$ + 139° (*c*, 1.2) and R_{Gal} 0.67 (solvent B, Ref. 2), giving on hydrolysis galactose and arabinose and after reduction only galactose. Its mobilities on electrophoresis (M_G) in borate (pH 10), germanate (pH 10.5)[3] and sulphonated benzene boronic acid (pH 6.8)[4] buffers were, respectively, 0.86, 1.61, and 3.8, whilst those of 3-*O*-β-L-arabinopyranosyl-L-arabinose, obtained from larch arabinogalactan, were 0.87, 1.61, and 3.5, respectively. It is therefore 3-*O*-α-D-galactopyranosyl-β-arabinose which has previously been isolated, *e.g.* by Smith from gum arabic.[5] The ease of formation of the disaccharide shows the arabinose to be present in the furanosidic form in the polysaccharide. A mixture of two acid-labile arabinobioses with R_{Gal} 1.08 and 1.18 and $[\alpha]$ + 55° was also obtained. The electrophoretic mobilities of the two were, in borate 0.28 and 0.52, in germanate 0.53 and 1.34 and in sulphonated benzeneboronic acid 0.3 and 3.3. This indicates one to have a (1→2)- and the other to have a (1→3)-linkage. The optical rotation of the mixture gives no clear indication of the configuration of these linkages. Hydrolysis of the above polymeric fraction gave galactose and rhamnose as the main products, and an aldobiouronic acid with $[\alpha]$ + 7° (*c*, 1.7), shown by methylation to be 6-*O*-β-D-glucopyranuronosyl-D-galactose, which is commonly obtained from plant gums of the present type.

For methylation a fraction isolated by ethanolamine-extraction of pollen was used. The resulting product, which showed no hydroxyl band on IR, still contained some unmethylated arabinose residues which did not, by chromatography of hydrolysed samples, decrease on further methylation. The hydrolysed methylated polysaccharide was fractionated on a carbon-Celite column and on thick filter papers to give the ethers shown in the experimental section. The complexity of the mixture allowed only a semi-quantitative determination of its composition.

The results indicate a highly branched structure where D-galactose, L-rhamnose and D-glucuronic acid[1] make up a backbone that represents about 20 % of the molecule. To this chains of L-arabinose residues are joined, the linkages combining the arabinose residues being (1→2), (1→3), as well as (1→5). The significant fraction of unmethylated arabinose indicates triple branching. D-Galactose occurs also to a large extent in the exterior part of the molecule as non-reducing terminal groups