

An Extracellular Polysaccharide from *Beijerinckia indica*, Containing L-Guluronic Acid Residues

ARNE HAUG and BJØRN LARSEN

Norwegian Institute of Seaweed Research,
7034 Trondheim-NTH, Norway

The extracellular polysaccharide produced by *Beijerinckia indica* Starket and De (syn. *Azotobacter indicum*) was described by Quinnell, Knight and Wilson¹ as consisting of D-glucose, D-glucuronic acid, and an aldoheptose in the proportion 3:2:1. Jones, Perry and Sowa² identified the aldoheptose as D-glycero-D-manno-heptose and also reported the presence of D-glucose, D-mannose, D-glucuronic acid, and traces of D-galacturonic and L-guluronic acid. Parikh and Jones³ subjected the polysaccharide to periodate oxidation and methylation and concluded that the polysaccharide was a linear polymer containing repeating units of a trisaccharide composed of D-glucuronic acid, D-glucose, and D-glycero-D-manno-heptose. Only traces of mannose was reported in this preparation.

In the cause of an investigation of extracellular polysaccharides from *Azotobacter* species and related organisms we isolated a polysaccharide from 1 l of a 6 days old liquid culture of *B. indica* (composition of medium described in Ref. 4) by removal of cells by centrifugation, dialysis and precipitation of the polysaccharides by addition of equal volumes of ethanol and potassium chloride to 0.02 N. The yield of polysaccharide was 2 g. Free-boundary electrophoresis in phosphate buffer pH 7, revealed one peak with a mobility of $0.95 \times 10^{-4} \text{ cm}^2 \text{ sec}^{-1} \text{ V}^{-1}$. The equivalent weight was determined by titration with cetylpyridinium chloride⁵ and found to be 560. Acetyl groups were determined according to Kunz⁶ and found to correspond to a degree of acetylation of 0.5. Hydrolysis (5 h, 2 N sulphuric acid, 100°) and paper chromatography (A: pyridine, ethyl acetate, acetic acid, water 5:5:1:3, B: butan-1-ol, ethanol, water 3:1:1 and C: ethyl acetate, acetic acid, formic acid, water 18:3:1:4) revealed the presence of three components; one with the mobility of glucose, which could be removed by glucose oxidase, one with a mobility

identical to that of an authentic sample of the heptose described by Jones *et al.*,² and one with a mobility of an uronic acid. The hydrolysate was separated into neutral and acidic components on an anion exchange column. The fraction containing the acidic components was investigated by chromatography in medium A, by paper electrophoresis in borate buffer containing calcium ions⁷ and by lactonization in 50 % formic acid at 100° for 30 min followed by paper chromatography in pyridine, ethyl acetate, water (11:40:6). In all cases only one component was indicated, with a mobility identical to that of L-guluronic acid (or its lactone) isolated from alginates from brown algae. No spot corresponding to D-glucuronic acid, D-mannuronic acid or their lactones could be observed. On column chromatography on anion exchange resin in an acetic acid gradient the elution pattern indicated only one component, eluted at the acid strength expected for guluronic acid.⁸ Knutson and Jeanes⁹ have recently published a study of the carbazol reaction of uronic acids, where they found that the four most common uronic acids could easily be distinguished by comparing the results of carbazol reactions with and without borate present, and at 100 and 55°. The results of the acidic component from *B. indica* agreed well with those for guluronic acid, and clearly demonstrated that significant amounts of mannuronic or glucuronic acid were not present. The optical rotation of the component was $[\alpha]_D = +36.3$ ($c = 0.77$ in H₂O), while that of L-guluronic acid prepared from marine algae was +37. Identical results were obtained for several different batches of polysaccharide, including batches produced with ammonium acetate as a nitrogen source. It seems therefore safe to conclude that the extracellular polysaccharide from the strain of *B. indica* investigated by us contained L-guluronic acid as the only acidic component occurring in significant quantities.

Smidsrød and Haug¹⁰ have shown that the selectivity of alginates in ion exchange processes involving strontium, calcium and magnesium ions is associated solely with the L-guluronic acid residues in the alginate, and that the highest selectivities, e.g. for strontium ions compared to magnesium¹⁰ or calcium ions¹¹ were found for alginate fragments with a composition approaching poly-L-guluronic acid. The ion exchange properties of the deacetylated polysaccharide from *B. indica* were inves-

Table 1.

	k_{Mg}^{Ca}	k_{Mg}^{Sr}	k_{Ca}^{Cu}
<i>B. indica</i> polysaccharide, deacetylated	1.1	1.0	14
Alginate fragment, 90 % L-guluronic acid	35	120	8

tigated and the results given in Table 1 show that no selectivity in exchange reactions involving Mg^{2+} , Ca^{2+} , and Sr^{2+} was observed, while a selectivity for Cu^{2+} compared to Ca^{2+} was found. This latter selectivity has previously been shown to be characteristic of polymers containing carboxyl groups.¹² The remarkable lack of selectivity of the L-guluronic acid residues of *B. indica* polysaccharide for alkaline earth ions is most probably caused by these residues occurring as isolated units as indicated by Parikh and Jones.³ This is in agreement with observations by Kohn and Furda¹³ who found that the selectivity of pectins in the potassium-calcium ion exchange rapidly decreased with increasing degree of esterification.

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Crystallographic and Other Studies of Bis(*N*-methylethylenediamine)-copper(II) Complexes

III. Bis(*N*-methylethylenediamine)-copper(II) Chloride

REIJO HÄMÄLÄINEN

*Department of Inorganic Chemistry,
University of Helsinki,
Helsinki 17, Finland*

Bis(*N*-methylethylenediamine)copper(II) bromide and iodide, both of which are triclinic have been prepared previously.¹ The bis(*N*-methylethylenediamine)copper(II) chloride described below is triclinic also and its other properties are similar to those of the bromide and iodide.

Experimental. *N*-Methylethylenediamine (*N*-Meen) of practicum grade from Fluka AG was purified by distillation. The other chemicals were analytical reagents.

Preparation and analyses. 0.071 mole of copper(II) chloride dihydrate in 80 ml of methanol was added dropwise to 80 ml of mechanically stirred methanol containing 0.142 mole of *N*-Meen. A crude product was precipitated with ether and dried at room temperature. Recrystallizations were performed from ethanol containing about 10 % water. Copper was determined electroanalytically, chloride as silver chloride and *N*-methylethylenediamine by potentiometric titration. (Found: Cu 22.48; Cl 25.02; *N*-Meen 52.92. Calc.: Cu 22.48; Cl 25.08; *N*-Meen 52.44).

Measurements. Weissenberg photographs were taken around two axes with a Nonius