

Preliminary communication

The isolation of a sulphated mannan and a neutral xylan from the red seaweed *Nemalion vermiculare* Sur.

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The polysaccharides of the red algae belonging to the order Nemalionales have been little investigated. It has recently been shown that *Rhodochorton floridulum*¹ and *Chaetangium fastigiatum*^{2,3} contain xylans with mixed β -(1→3) and β -(1→4) linkages. There is evidence that polysaccharide fractions extracted from several representatives of Nemalionales gave mannose, in admixture with other sugars, after acid hydrolysis^{2,4,5}, but, as far as we are aware, the corresponding mannan has not been isolated and studied.

We now report on the polysaccharides of the red seaweed *Nemalion vermiculare* Sur. The sample of seaweed was collected from the Japan Sea in the late summer of 1967, extracted with methanol, and dried. Treatment of the milled alga with hot water, followed by ethanol precipitation, afforded a water-soluble polysaccharide preparation (*A*, 62.5% of dry seaweed). Acid hydrolysis of *A* gave mannose as the main component, xylose, and traces of galactose and glucose. The residual weed was extracted with M sodium hydroxide, and the alkali-soluble polysaccharide (*B*, 9.5%) was recovered from the neutralized solution by precipitation with ethanol. Hydrolysis of this fraction gave xylose, together with small proportions of mannose, galactose, and glucose.

Fractional precipitation of *A* with ethanol and then with Cetavlon gave a sulphated mannan. The fractionation was monitored by quantitative determination of mannose with the *o*-toluidine reagent⁶, using the absorbance at 630 nm since the xylose-*o*-toluidine reaction product does not absorb significantly in this region. The xylose content could be calculated from the absorbance at 385 nm. It was thus shown that the xylan was precipitated first during ethanol fractionation. Subsequent precipitation of the mannan as the cetyltrimethylammonium salt, dissolution of the precipitate in concentrated, aqueous sodium chloride, and precipitation with ethanol gave the sodium salt of a sulphated mannan (> 30% of the dry seaweed), $[\alpha]_D^{20} +58.8^\circ$ (c 0.05, water). In addition to mannose, the polysaccharide contained xylose (3.1%) and sulphate (15.5% as SO₃Na), and had i.r. bands at 815–830, 860, and 1220–1270 cm⁻¹ (sulphate). D-Mannose was identified as the

crystalline phenylhydrazone and as methyl α -D-mannopyranoside. The sulphate groups in the polysaccharide are stable to alkali under conditions recommended by Rees⁷ for the elimination of such groups from sulphated galactans. The cetyltrimethylammonium salt of the polysaccharide was completely desulphated after four treatments with 0.1M methanolic hydrogen chloride, and the resulting neutral mannan was almost insoluble in water and aqueous alkali. The desulphated product gave essentially only mannose upon acid hydrolysis. It was methylated initially by the Haworth and Hakomori procedures and finally by the action of methyl sulphate and powdered sodium hydroxide in tetrahydrofuran⁸ to give the methylated mannan, $[\alpha]_D +93^\circ$ (c 0.54, chloroform) (Found: OMe, 41.9%). The mixture of methylated methyl mannosides, obtained from the methylated polysaccharide after formolysis followed by methanolysis, was analysed by g.l.c.⁹. Only the methyl 2,4,6-tri-*O*-methyl-D-mannosides and traces of the methyl 2,3,4,6-tetra-*O*-methyl-D-mannosides were detected. Thus, the sulphated mannan has an essentially linear chain of α -(1 \rightarrow 3)-linked D-mannopyranose residues.

The purification of the xylan was accomplished as follows. Fraction *B* was treated with 0.1M sodium hydroxide and, after hydrolysis of the insoluble residue, D-xylose was obtained in crystalline form. The alkaline solution was neutralized and treated with Cetavlon, and the soluble xylan was then purified *via* the copper complex (*cf.* ref. 10). The resulting xylan, $[\alpha]_D -83^\circ$ (c 0.2, 0.5M sodium hydroxide), was methylated by the Haworth and then the Hakomori procedures. After methanolysis of the methylated polysaccharide, the methyl glycosides of 2,3- and 2,4-di-*O*-methyl-D-xylose (ratio \sim 4:1) and small proportions of the methyl 2,3,4-tri-*O*-methyl-D-xylopyranosides were identified by g.l.c.¹. Thus, the xylan is a linear (or slightly branched) polysaccharide with β -(1 \rightarrow 4) and β -(1 \rightarrow 3) linkages, and is probably similar to the other water-soluble xylans from the red algae^{1-3,10}.

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