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STRUCTURAL FEATURES OF THE SULPHATED POLYSACCHARIDE FROM A GREEN SEAWEED, *CAULERPA TAXIFOLIA**

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Key Word Index—*Caulerpa taxifolia*; Chlorophyceae; green seaweed; sulphated heteropolysaccharide.

Abstract—A sulphated heteropolysaccharide was isolated from a green seaweed, *Caulerpa taxifolia*, by extraction with acid and purified *via* its copper complex. Methylation analysis of both the sulphated and desulphated polysaccharides revealed the presence of 1,4-linked xylose, 1,6-linked galactose, 1,4,6-linked mannose and non-reducing galactose end group units which are all devoid of sulphate groups. In addition 1,4-linked galactose units sulphated at C-3 are also present. Quantitative periodate oxidation showed a consumption of 1.30 and 1.60 moles of oxidant per anhydrosugar unit in the sulphated and desulphated polysaccharides respectively. The oxo-polysaccharides after reduction and hydrolysis revealed the presence of glycerol, erythritol and unoxidized galactose in the mol ratio 11.6:5.1:4.9 and 11.2:5.0:1.0 respectively, besides threitol (3.9 mol) in the desulphated polysaccharide.

INTRODUCTION

Although the constituent sugars of heteropolysaccharides present in a number of species of the Chlorophyceae have been determined, only a few structural studies have been reported. Studies [2–4] on *Caulerpa* (order Siphonales) spp. by several workers revealed that the major polysaccharide synthesized by this green seaweed is a β-1,3-xylan. In addition, a water-soluble mixture of polysaccharides was extracted [5] from *C. filiformis* and fractionated into amylopectin type glucan and a sulphated polysaccharide containing galactose, mannose and xylose. A soluble β-D-glucan was reported [6, 7] from *C. simpliciuscula* which Is not commonly found in members of the Chlorophyceae. As far as we are aware no sulphated heteropolysaccharide from *Caulerpa* spp. has been investigated chemically. This paper deals with the isolation, purification and characterization of a sulphated heteropolysaccharide from a local green alga, *Caulerpa taxifolia*.

RESULTS AND DISCUSSION

The acid-extractable polysaccharide from *C. taxifolia*, isolated as light brown powder, contained *ca* 65% carbohydrates and purified *via* its copper complex. Two fractions **A** and **B** were obtained. Fraction **A** mainly consisted of galactose, mannose and xylose while fraction **B** contained glucose. Hence fraction **B** was thought to be a glucan. A similar glucose-rich fraction, obtained from a mixture of acetylated polysaccharides of *Spongomorpha arcta*, was reported by O'Donnell and Percival [8]. Amylopectin type of glucans were separated from the mixture of water-soluble polysaccharides by Mackie and Percival [9] from *C. filiformis*, *C. racemosa* and *C. setularoides*. It is possible that the glucan separated during fractionation From *C. taxifolia* might be of an amylopectin type. Further work on fraction **B** was not carried out due to very low yield.

Fraction **A** was further purified by dialysis and its homogeneity was checked by column chromatography on Sephadex G-100 and high voltage paper electrophoresis. The purified polysaccharide had $[\alpha]_D + 88.5°$ (H_2O) and contained 75.5% carbohydrates and 11.6% half-ester sulphate. Acid hydrolysis of the polysaccharide gave galactose, mannose and xylose in mol proportion of about 16.4 : 5.0:1.0. The sulphated polysaccharide (S-PS) was desulphated using methanol–HCl which resulted in the removal of 73% of the total sulphate. The desulphated polysaccharide (DS–PS) contained 75.1% carbohydrates and 3% sulphate, and showed galactose, mannose and xylose in the mol ratio 15.1:5.0:1.0 on acid hydrolysis.

Periodate oxidation of the S-PS resulted in the reduction of 0.4 mol of the oxidant, liberating 0.54 mol of formic acid per anhydrosugar unit in 72 hr. The oxo-polysaccharide, isolated in 60% yield, on reduction and hydrolysis yielded glycerol, erythritol and unoxidized galactose in the mol ratio 11.6:5.1:4.9. Glycerol is derived from hexose units which are 1,6-linked and/or non-reducing end units, and from 1,4-linked pentopyranose units. The formation of erythritol and its mol yield indicated the presence of 1,4-linked mannopyranose units and no unit has escaped periodate attack. Similar studies on the DS-PS showed an increase in the periodate consumption (1.60 mol), but with unchanged release of formic acid (0.51 mol) per anhydrosugar unit. The hydrolysate of reduced oxo-polysaccharide yielded glycerol, erythritol, threitol and unoxidized galactose in the mol ratio 11.6:5.0:3.9:1.0. Threitol is derived from 1,4-linked galactopyranose residues previously sulphated at position C-2 or C-3.

Partial hydrolysis of the S-PS with 0.1 M H_2SO_4 yielded mainly one oligosaccharide. The oligosaccharide, $[\alpha]_D + 44.5°$ (H_2O), on hydrolysis gave galactose and mannose in the mol proportion 1.0:2.1. Reduction of the oligomer with sodium borohydride revealed that mannose is occupying the reducing end. Analysis of the

*Part 3 in the series "Studies on Indian Seaweed Polysaccharides." For Part 2 see ref. [1].

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Table 1. Methylation analysis of the polysaccharides from *C. taxifolia*

Methyl sugar	RR_1*	Mole proportion in S-PS	DS-PS	Mode of linkage
2,3,4,6-Me_4-D-Gal	1.25	5.0	5.1	Gal*p*-(1→
2,3-Me_2-D-Xyl	1.50	1.2	1.1	→4)-Xyl*p*-(1→
2,3,6-Me_3-D-Gal	2.40	—	4.3	→4)-Gal*p*-(1→
2,3,4-Me_3-D-Gal	3.38	5.8	6.2	→6)-Gal*p*-(1→
2,6-Me_2-D-Gal	3.66	5.2	1.1	→3,4)-Gal*p*-(1→
2,3-Me_2-D-Man	4.84	4.9	5.1	→4,6)-Man*p*-(1→

*Retention times of the corresponding alditol acetates relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on a 3% ECNSS column at 170°.

hydrolysate of the methylated oligosaccharide gave 2,3,4-tri-*O*-methyl-D-galactopyranose, 2,3,6-tri-*O*-methyl-D-mannopyranose and 2,3,4,6-tetra-*O*-methyl-D-mannopyranose in equal mol ratio and its structure was deduced as 4-*O*-(D-mannopyranosyl-4-*O*-D-galactopyranosyl)-D-mannopyranose.

The S-PS and DS-PS were methylated, hydrolysed and the methyl sugars separated were characterized as their *N*-phenylglycosylamine derivatives. They were also identified by GC (Table 1).

The formation of 2,3,4,6-tetra-*O*-methyl-D-galactopyranose indicates that *ca* 31% of galactose residues form the non-reducing end groups. The analysis also showed the presence of 1,6-linked galactopyranose, 1,4-linked galactopyranose branched at C-3 or 1,3-linked galactopyranose branched at C-4, 1,4,6-linked mannopyranose and 1,4-linked xylopyranose units. The formation of 2,3,6-tri-*O*-methyl-D-galactopyranose from the methylated DS-PS and a simultaneous decrease in the amount of 2,6-di-*O*-methyl-D-galactopyranose is evidence for the presence of 1,4-linked galactose sulphated at C-3 which corresponds to the sulphation of every fourth unit in the native polysaccharide.

The S-PS, after acetylation, was oxidized with CrO_3 and the sugars surviving after oxidation were estimated by GC (Table 2). The results suggest that the majority of the linkages in the native polysaccharide might be of α-configuration.

The sulphated polysaccharide from *C. taxifolia* appeared to be similar to the polysaccharides from *C. filiformis*, *C. racemosa* and *C. sertularoides*, but differs from the polysaccharide of another sample of *C. filiformis* (collected in a different environment) which is reported [9] to contain arabinose, besides galactose, mannose and xylose. It also differs from other green seaweed polysaccharides in the absence of rhamnose and uronic acid [8, 10, 11].

Table 2. Analysis of CrO_3 oxidation products of the S-PS

Time (hr)	Inositol	Galactose	Mannose	Xylose
0	100	26.9	8.5	1.9
1	100	25.0	7.1	1.8
2	100	24.9	6.9	1.8

EXPERIMENTAL

Plant material. *Caulerpa taxifolia*, a local green alga, was collected in March, 1982, from station III of Visakhapatnam coast. The alga was washed with H_2O, air-dried and milled.

General methods. The homogeneity of the polysaccharide was tested by paper electrophoresis (7.2 V/cm) using borate buffer (0.2 M) at pH 10.8. PC on Whatman No. 1 and 3 MM papers in the solvent systems (A) *n*-BuOH–C_5H_5N–H_2O, 6:4:3, (B) upper layer of *n*-BuOH–EtOH–H_2O, 4:1:5, (C) EtOAc–C_5H_5N–H_2O, 10:4:3, and (D) butanone–H_2O azeotrope. Detection was effected with (1) alkaline $AgNO_3$ and (2) *p*-anisidine HCl. Total carbohydrate content and the sugars in the acid hydrolysate were determined by the PhOH–H_2SO_4 method [12]. Neutral sugars in the hydrolysate were converted into their corresponding alditol acetates and analysed by GC using a column of 3% ECNSS on Gas Chrom Q (100–200 mesh) at temps ranging from 170 to 190°. Sulphate was estimated by the method using barium chloranilate [13]. Reductions were carried out with $NaBH_4$ in aq. soln. Excess borohydride was destroyed, sodium ions were removed with Amberlite IR-120 (H^+) resin, and boric acid by distillation with MeOH. Hydrolysis of methylated polysaccharides was carried out in sealed tubes with 90% (w/w) HCO_2H [14] and 72% (w/w) H_2SO_4 for 10 hr at 100°. R_g values were measured in solvent B and R_f values in solvent D.

Isolation of the polysaccharide. The alga (100 g) was extracted with dil. HCl (pH 3.0–4.0, 2 × 1 l.) with stirring for 1 hr at 70°. After filtration the combined extracts were neutralized with 0.1% Na_2CO_3 soln and poured into 1 vol. of EtOH. The ppt was collected at the centrifuge, redissolved in H_2O (50 ml), dialysed and freeze-dried (9.1 g).

Fractionation of the polysaccharide. The acid-extractable polysaccharide (5 g) was dissolved in H_2O (200 ml) and 10% $Cu(OAc)_2$ soln (20 ml) added dropwise to the soln. EtOH (100 ml) was then added until the precipitation was complete. The ppt (**A**, 4.1 g) formed was collected by centrifugation and a second vol. of the reagent added to the centrifugate followed by sufficient EtOH (200 ml) to obtain a ppt (**B**, 10 mg). The fractions **A** and **B** were washed with cold EtOH containing 5% (v/v) conc HCl separately, dialysed after redissolving in minimal amount of H_2O and freeze-dried. A soln of **A** (1.1 g) in H_2O (25 ml) was added to a column (3 × 35 cm) of Sephadex G-100. After allowing the polysaccharide soln to percolate in, the column was eluted with H_2O. The carbohydrate content in each fraction was monitored by PhOH–H_2SO_4 reaction.

Desulphation of the S-PS. The S-PS (950 mg) was suspended in 0.05 M dry MeOH–HCl (250 ml) in a stoppered bottle and agitated on an automatic shaker at room temp. for 20 hr. The insoluble material was collected by centrifugation, dissolved in H_2O, dialysed and freeze-dried (550 mg).

Periodate oxidation and reduction of the derived polyaldehyde. Both the S-PS (25.1 mg) and DS-PS (20.2 mg) were oxidized with 0.01 M $NaIO_4$ in H_2O (10 ml) in the dark. Aliquots (0.1 ml) were withdrawn at intervals and the extent of oxidation was measured [15]. The reaction was complete in 48 hr in both cases and the excess periodate was destroyed with ethylene glycol. The polyaldehydes were reduced to the polyalcohol with $NaBH_4$ (yield 75%). Aliquots of the polyalcohol were hydrolysed with 2 M HCl and the hydrolysates analysed by PC (solvents A and B) and by GC of the derived alditol acetates.

Methylation of the polysaccharides. Dried samples of the S-PS (1.2 g) and DS-PS (750 mg) were methylated by Hakomori method [16] followed by Kuhn [17] and Purdie [18] methods. The methylated polysaccharides were extracted into $CHCl_3$ (3 × 150 ml) from the reaction mixture, dried (Na_2SO_4) and evapd to dryness. The methylated polysaccharides were hydrolysed, and the methyl sugars so obtained were resolved by prep. PC (solvents A and B) on Whatman No. 3 MM sheets. Five fractions (I–V) were thus obtained.

Preparation of N-*phenylglycosylamines* [19]. Methyl sugar fraction (10 mg) was dissolved in 0.5 ml of MeOH–H_2O (8:1). *p*-Nitroaniline (15 mg) and glacial HOAc (0.1 ml) were added to the soln and boiled under reflux for 5 min. The reaction mixture was stored at 0° overnight and the crystalline product collected, washed with EtOH, Et_2O and dried.

Characterization of methyl ether fractions. Fraction I. 2,3,4,6-Tetra-*O*-methyl-D-galactose. Syrup (30 mg), R_g 0.88, R_f 0.70, $[\alpha]_D^{30}$ + 109° (*c* 0.5; H_2O); 2,3,4,6-tetra-*O*-methyl-*N*-phenyl-D-galactopyranosylamine: mp 190–191° [20].

Fraction II. 2,3-Di-*O*-methyl-D-xylose. Syrup (10 mg), R_g 0.74, R_f 0.58, $[\alpha]_D^{30}$ + 28.1° (*c* 0.5; H_2O); 2,3-di-*O*-methyl-*N*-phenyl-D-xylopyranosylamine: mp 125° [21].

Fraction III. 2,3,4-Tri-*O*-methyl-D-galactose. Crystalline product (40 mg), mp 84–85°, R_g 0.64, R_f 0.36, $[\alpha]_D^{30}$ + 154° → 117° (*c* 1.0; H_2O). The anilide had mp 169° [22].

Fraction IV. 2,3-Di-*O*-methyl-D-mannose. Syrup (31.5 mg), R_g 0.54, R_f 0.22, $[\alpha]_D^{30}$ − 16° (*c* 1.0; H_2O), $[\alpha]_D^{30}$ + 5.6° (*c* 1.0; MeOH). The anilide had mp 134–135° [23].

Fraction V. 2,6-Di-*O*-methyl-D-galactose. Crystalline product (36.5 mg), mp 129°, R_g 0.44, R_f 0.13, $[\alpha]_D^{30}$ + 49° → + 87° (*c* 0.5; H_2O). The anilide had mp 120°[24].

Besides the above fractions, one more fraction was obtained from the DS-PS. It was characterized as 2,3,6-tri-*O*-methyl-D-galactose, syrup (15.3 mg), R_g 0.71, R_f 0.48, $[\alpha]_D^{30}$ + 77° → + 96° (*c* 0.5; H_2O) [25]. The identity of the methyl sugars obtained from both the S-PS and DS-PS was further confirmed by GC analysis after converting them into the corresponding alditol acetates.

Partial hydrolysis. The S-PS (1.5 g) was stirred with 0.1 M H_2SO_4 (175 ml) for 1 hr at 60°. The hydrolysate was neutralized ($BaCO_3$), filtered, deionized and concd *in vacuo*. Prep. PC on Whatman No. 3 MM sheets (solvent A) yielded mainly one oligosaccharide in addition to component sugars.

The oligosaccharide was a syrup (30 mg, R_{gal} 0.58 in solvent A), $[\alpha]_D^{30}$ + 44.5° (*c* 1.0; H_2O). It was reduced with $NaBH_4$, hydrolysed and the products identified by PC. Methylation of the oligomer was performed using Kuhn's method [17] and the fully methylated product, $[\alpha]_D^{30}$ + 16.7° (*c* 1.0; $CHCl_3$) was hydrolysed as usual. The hydrolysate was analysed by PC (solvents B and D) and by GC of derived alditol acetates.

CrO_3 *oxidation of the S-PS.* The S-PS (100 mg) dispersed in DMF (8 ml) and C_5H_5N (15 ml) by stirring vigorously for 1 hr. Ac_2O (15 ml) was added and stirred for 70 hr at room temp. The reaction mixture was added to an equal vol. of H_2O, dialysed and the polysaccharide isolated by freeze-drying. CrO_3 oxidation was carried out using the method of ref. [26] with *myo*-inositol as internal standard.

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