Phytochemistry, 1973, Vol. 12, pp. 2901 to 2907. Pergamon Press. Printed in England.

MUCILAGE FROM A FRESH-WATER RED ALGA OF THE GENUS *BATRACHOSPERMUM*

JAMES R. TURVEY and LEONARD M. GRIFFITHS✠
School of Physical and Molecular Sciences, University College of North Wales, Bangor, Wales LL57 2UW

(*Received* 21 *May* 1973. *Accepted* 1 *July* 1973)

Key Word Index—*Batrachospermum* sp.; Rhodophyceae; alga; mucilage; polysaccharide composition; 3-*O*-methyl-L-rhamnose.

Abstract—The gelatinous polysaccharides of a *Batrachospermum* species have been extracted from the alga. The major polysaccharide is acidic and has been separated from neutral polysaccharides by chromatography on DEAE-cellulose. The constituent sugars of the acidic polysaccharide include D- and L-galactose, D-mannose, D-xylose, L-rhamnose, D-glucuronic acid, and two *O*-methyl sugars, which have been characterized as 3-*O*-methyl-L-rhamnose (L-acofriose) and 3-*O*-methyl-D-galactose. Partial acid hydrolysis of this polysaccharide has given a complex mixture of neutral and acidic oligosaccharides. The two preponderant acidic oligosaccharides contained galactose and glucuronic acid in 1:1 ratio, suggesting the presence of a repeating sequence of these two residues as a major structural feature of the polysaccharide.

INTRODUCTION

THE WATER-SOLUBLE polysaccharides of marine red algae (Rhodophyta) have been extensively studied, since they are of commercial importance. Many of them have a structure based on either D-galactose and its derivatives (carrageenans), or on both D- and L-galactose and their derivatives (agars), but they have a common structural feature in the presence of sulphate hemi-ester groups. In contrast, fresh-water red algae have received little attention. One unicellular species, *Porphyridium cruentum* (Class, Bangiophyceae; Order, Porphyridiales), produces a protein-polysaccharide complex which is exuded into the growth medium. On hydrolysis, this complex gives D-glucose, D-galactose, D-xylose, a uronic acid and peptides.[1] The genus *Batrachospermum* (Class, Rhodophyceae; Order, Nemalionales) contains macroscopic species found only in fresh water, and these are characterized by a gelatinous texture when handled. We have now carried out a preliminary examination of the polysaccharide material responsible for this gel.

The specimen used in this investigation was obtained mainly from fresh-water lakes near Halifax, Nova Scotia, through the kindness of Drs. J. S. Craigie and J. McLachlan. A similar species, indistinguishable under the microscope, was obtained from streams in Snowdonia, North Wales. The two specimens gave almost identical results as far as the type of polysaccharide was concerned, but minor differences in the content of individual sugars were noted, pointing perhaps to a species difference. The majority of the results reported here were obtained from the Canadian specimen, which had been plunged into boiling ethanol when fresh, filtered, and air dried for transport.

[1] JONES, R. F. (1962) *J. Cell. Comp. Physiol.* **60**, 61.

RESULTS

Extraction of the dried alga with hot water readily gave a sticky solution from which polysaccharide was obtained as a fibrous material by ethanol precipitation in 42% yield (Found: N, 0·28%; SO_4, nil). Acid hydrolysis and paper chromatography of the hydrolysate indicated the presence of uronic acid, galactose, glucose (trace), mannose, xylose, rhamnose and two unidentified sugars, *A* and *B*, the latter having a chromatographic mobility greater than that of rhamnose. The component sugars in a hydrolysate were separated by paper chromatography and characterized (see Experimental) as D- and L-galactose (ratio 1·6 D: 1·0 L), D-glucose, D-mannose, D-xylose, L-rhamnose and D-glucuronic acid. In addition, sugars *A* and *B* were identified as *O*-methyl hexoses and characterized as follows: *Sugar A.* The sugar had an R_f value similar to but not identical with that of 6-*O*-methylgalactose, and gave galactose on demethylation. In borate buffer it had M_g 0·67 (lit.[2] for 3-*O*-methyl-D-galactose, 0·63) and the sugar had $[\alpha]_D^{20}$ + 107·5° (in water) (lit.[3] for 3-*O*-methyl-D-galactose, + 108·6°). Reduction of the sugar to the glycitol, acetylation, and GLC of the product gave a peak identical in retention time with that of authentic 3-*O*-methyl-D-galactitol penta-acetate. Sugar *A* is therefore 3-*O*-methyl-D-galactose. *Sugar B.* Demethylation of the sugar gave rhamnose among the products, and the NMR spectrum indicated the presence of one *O*-methyl group (τ 5·93, *s*), in addition to the C_6 methyl (τ 8·16, *d*). The sugar was crystallized with difficulty and it had m.p. 110–112·5°, $[\alpha]_D^{20}$ + 32° (in water). The literature values for 2-*O*-methyl-L-rhamnose are m.p. 113–114°, $[\alpha]_D$ + 31°, + 24°,[4] and for 3-*O*-methyl-L-rhamnose they are m.p. 113°, 115°, $[\alpha]_D$ + 35°.[5] On PCs, sugar *B* was detected with the triphenyltetrazolium spray,[6] indicative of a free hydroxyl group on C_2, and with the *p*-anisidine hydrochloride spray it gave a yellow–green colouration, characteristic of the 3-*O*-methyl ether.[7] On electrophoresis in borate buffer, sugar *B* migrated at the same rate as authentic 3-*O*-methyl-L-rhamnose, whereas the 2-*O*-methyl ether does not migrate.

TABLE 1. MOLAR RATIO OF SUGARS IN THE POLYSACCHARIDE

Sugar	Molar ratio	Sugar	Molar ratio
D/L-Galactose	1·75	Arabinose	0·29
D-Glucose	0·45	3-*O*-Methyl-L-rhamnose	0·03
D-Mannose	1·18	3-*O*-Methyl-D-galactose	0·01
D-Xylose	1·00	D-Glucuronic acid	0·76
L-Rhamnose	0·06		

Periodate oxidation of the sugar showed a rapid consumption of 1 mol of oxidant per mol of sugar, followed by a slower rise to 3 mol; the derived methyl glycoside consumed no periodate over a period of 1 week, as expected for a 3-*O*-substituted sugar. Finally an osazone of the sugar was prepared with m.p. 118–119° (lit.[8] for 3-*O*-methyl-L-rhamnosazone hydrate, m.p. 118°). Sugar *B* is thus 3-*O*-methyl-L-rhamnose (L-acofriose).

[2] BOUVENG, H. and LINDBERG, B. (1956) *Acta Chem. Scand.* **10,** 1283.
[3] REBER, F. and REICHSTEIN, T. (1945) *Helv. Chim. Acta* **28,** 1164.
[4] YOUNG, F. G. and ELDERFIELD, R. C. (1942) *J. Org. Chem.* **7,** 241; ANDREWS, P., HOUGH, L. and JONES, J. K. N. (1955) *J. Am. Chem. Soc.* **77,** 125.
[5] HIRST, E. L. and DUNSTAN, S. (1953) *J. Chem. Soc.* 2332; GORROD, A. R. N. and JONES, J. K. N. (1954) *J. Chem. Soc.* 2522.
[6] BELL, D. J. (1955) *Modern Methods of Plant Analysis* (PAECH, K. and TRACEY, M. V., eds.), Vol. 2, p. 1, Springer, Berlin.
[7] MACLENNAN, A. P. (1962) *Biochem. J.* **82,** 394.
[8] SCHMIDT, O. T., PLANKENHORN, E. and KÜBLER, F. (1942) *Ber.* **75,** 579.

Quantitative analysis of the sugars present in the polysaccharide gave the results shown in Table 1. The figure for 3-*O*-methylgalactose is an estimate only since the proportion was too small for accurate measurement. Arabinose was detected in the hydrolysate by GLC but was not rigorously characterized.

Several attempts were made to separate the algal polysaccharide into different fractions. Gel filtration through a variety of media were all unsuccessful, but column chromatography on DEAE-cellulose (Cl^- form) gave four fractions on elution with; (a) H_2O (E1 and E2), (b) 0·1 M (E3) and (c) 0·2 M KCl (E4). The molar ratios of selected sugars, and the content of uronic acid in the four fractions are given in Table 2. Fraction E1, which was non-acidic and had a relatively high content of glucose was tested with iodine solution. It gave a red-brown stain (λ_{max} 510 nm), similar to that given by floridean starches.[9] Fractions E3 and E4 had similar compositions, and on rechromatographing on the above column, each was apparently resolved into two components with retention times identical to those given by E3 and E4 on the original column. This suggests that they are the same polysaccharide and the apparent separation into two fractions is an artefact of the procedure used.

TABLE 2. PROPERTIES OF FRACTIONS FROM DEAE-CELLULOSE COLUMN

Fraction	Yield (mg)*	Molar ratio of sugars†				Uronic acid (%)
		Man	Glc	Gal	Xyl	
E1	14	0·16	0·82	0·17	1·0	0
E2	4·5	1·01	0·35	0·53	1·0	4·05
E3	19·5	1·42	0	1·83	1·0	15·4
E4	19·5	0·87	0	2·02	1·0	16·3

* From 65 mg polysaccharide.
† Man—mannose; Glc—glucose, Gal—galactose, Xyl—xylose.

Graded acid hydrolysis of the total polysaccharide was used to give some preliminary information on the structure of the polysaccharide. In 25 mM H_2SO_4 at 100°, both xylose and galactose were being liberated in less than 1 hr. In 0·25 M H_2SO_4 at 100°, the order of detection of liberated monosaccharides was xylose (15 min), galactose (30 min), glucose and mannose (90 min), but no uronic acid was liberated in 3 hr. Hydrolysis of 10 g of polysaccharide with 0·25 M oxalic acid at 100° for 3 hr gave neutral mono- and oligo-saccharides together with 8 g of degraded polysaccharide (precipitated in 75% ethanol). Two sequential hydrolyses of this degraded polysaccharide with 0·5 M oxalic acid, each at 100° for 7 hr, gave yields of 2·5 and 1·0 g, respectively, of residual polysaccharide. The final residue was completely fragmented by treatment with M H_2SO_4 at 100° for 5 hr to give mainly acidic oligosaccharides. The combined hydrolysates were separated into neutral and acidic oligosaccharides on an ion-exchange column and the acidic oligosaccharides were further resolved into six fractions (C to H) by a combination of PC and electrophoresis in a neutral buffer. Each of the six fractions appeared to be chromatographically and electrophoretically homogeneous but some of them are probably mixtures of closely related oligosaccharides. Some properties of the fractions are listed in Table 3.

[9] PEAT, S., TURVEY, J. R. and EVANS, J. M. (1959) *J. Chem. Soc.* 3341.

TABLE 3. PROPERTIES OF ACIDIC OLIGOSACCHARIDES

Oligosaccharide	Yield (mg)	$M_{G\cdot A}$.*	Molar ratio of sugars Glucuronic acid†	Galactose	Mannose
C	27·7	0·72	1·0	1·03	0
D	26·0	0·59	1·0	1·29	0
E	8·7	0·95	1·0	0·37	0·20
F	5·9	0·79	1·0	0·19	0·12
G	9·5	0·70	1·0	0·75	0·14
H	5·2	0·59	1·0	0·95	0·14

* Electrophoretic mobility compared with that of glucuronic acid in neutral buffer.
† Determined, after reduction, as glucose.

DISCUSSION

The major gelatinous polysaccharide of the *Batrachospermum* sp. is a complex acidic polysaccharide containing glucuronic acid and several neutral sugar residues. In addition to the uronic acid, the pattern of sugars found is similar to that found in many gums and mucilages of land plants, rather than that found in typical red algae. One important difference from the plant polysaccharides is, however, the presence of both D- and L-enantiomers of galactose, in which respect there is an affinity with the galactans of some red algae.[10] The lack of ester sulphate in the polysaccharide distinguishes this alga from marine red and green algae, most of which contain sulphated polysaccharides,[10] and the absence of peptides distinguishes it from the polysaccharide complex extruded by *Porphyridium cruentum*.[1] The occurrence of residues of 3-*O*-methyl-D-galactose and 3-*O*-methyl-L-rhamnose is also interesting. While *O*-methyl ethers of galactose are found in several galactans of red algae, the 3-*O*-methyl ether occurs only in certain leaves and in grasses among terrestial plants.[11] 3-*O*-Methyl-L-rhamnose is found in cardiac glycosides,[12] in glycolipids of microorganisms,[7,13] and as a component of polysaccharides from gymnosperms.[14] In the algae, the only reported occurrence is from the green alga, *Enteromorpha* sp.[15]

Extraction of the alga with hot water also solubilizes some neutral polysaccharides, in addition to the major acidic polysaccharide. Two neutral fractions (E1 and E2) obtained by chromatography of the extract on ion-exchange cellulose, probably contain a glucan, similar to floridean starch in iodine staining properties, and a xylan (in E1), and a heteropolysaccharide (in E2). Acidic fractions E3 and E4 are probably parts of the same spectrum of acidic polysaccharides best regarded as one molecular species (see Table 2).

Although only of a preliminary nature, the results obtained by graded acid hydrolysis of the polysaccharide indicate certain structural features. The extreme ease with which some of the galactose and xylose is liberated (25 mM H_2SO_4 at 100°) suggests that some of these residues may be present in furanose form. As expected, the portions of the molecule containing uronic acid are difficult to hydrolyse. After hydrolysis for 14 hr at 100° in 0·5 M oxalic acid, a portion of the molecule was still large enough to be readily precipitated by

[10] PERCIVAL, E. E. and MCDOWELL, R. H. (1967) *Chemistry and Enzymology of Marine Algal Polysaccharides*, Academic Press, London.
[11] BACON, J. S. D. and CHESHIRE, M. V. (1971) *Biochem. J.* **124,** 555.
[12] REICHSTEIN, T. and WEISS, E. (1962) *Adv. Carbohyd. Chem.* **17,** 65.
[13] BJORNDAL, H., LINDBERG, B. and NIMMICH, W. (1970) *Acta Chem. Scand.* **24,** 3414.
[14] ANDERSON, D. M. W. and MUNRO, A. C. (1969) *Phytochemistry* **8,** 633.
[15] LOWE, J. (1965) Ph. D. Thesis, University of London.

ethanol. This residual material was completely fragmented into acidic oligosaccharides only after prolonged hydrolysis, a characteristic of polysaccharides with a high content of uronic acids. The acidic oligosaccharides were separated from the hydrolysate and, of these, the two most abundant (*C* and *D*, Table 3) consisted of glucuronic acid and galactose only in *ca.* 1:1 ratio. It is suggested that these two oligosaccharides are derived from a backbone in the molecule which consists of alternating glucuronic acid and galactose residues. To this backbone are then attached chains of mainly neutral sugar residues, some of which give rise to the neutral oligosaccharides released earlier in the hydrolysis. Further information on the structure of the molecule must await a detailed structural analysis of the oligosaccharides.

EXPERIMENTAL

General. The alga obtained in Nova Scotia was hand sorted and then plunged into boiling EtOH. After 10 min the alga was filtered and air dried at 20°. Alga from Snowdonia was hand sorted and was then used in the fresh state. Hydrolysis of the polysaccharide was achieved with M H_2SO_4 at 100° for 3 hr. The hydrolysate was neutralized ($BaCO_3$), filtered, and the filtrate concentrated at 35° to a syrup. PC was performed on Whatman No. 54 or 3 MM paper with the solvents: (a) BuOH–pyridine–C_6H_6–H_2O (5:3:1:3, upper layer); (b) EtOAc–pyridine–AcOH–H_2O (5:5:3:1); (c) EtOAc–formic acid–AcOH–H_2O (18:1:4:5). Paper electrophoresis employed Whatman 3 MM paper and a potential gradient of 50 v/cm with either borate buffer (0·2 M, pH 9) or 0·1 M AcOH buffered to pH 6·0 with pyridine (neutral buffer). Sugar zones on paper were detected with either the *p*-anisidine hydrochloride spray[16] or the aniline hydrogen phthalate spray.[17]

Analytical. The neutral sugars in a polysaccharide were determined, after hydrolysis, by quantitative GLC of the derived alditol acetates,[18] on a column 150 cm of 3%—ECNSS-M on Chromosorb W at an oven temp. of 180°.[19] Uronic acids were determined by the carbazole method of Dische.[20] The concentration of a sugar in solution was measured with the phenol–H_2SO_4 reagent,[21] and the content of D-galactose in a solution by D-galactose oxidase using method 1 of Roth *et al.*[22]

Extraction of the polysaccharide. The air-dried alga (50 g) was stirred in H_2O (3 l.) at 95° for 24 hr. and the insoluble material was then removed on the centrifuge. The residue was re-extracted with H_2O (3 l.) at 95° for a further 24 hr and the final residue was rejected. The combined extracts were evaporated (35°) to 1 l. and poured into EtOH (2 l.). The precipitated polysaccharide was recovered on the centrifuge, redissolved in warm H_2O (600 ml) and the solution clarified on the centrifuge. The solution was dialysed against tap H_2O for 48 hr and the polysaccharide was recovered by EtOH precipitation, washed with EtOH, then with Et_2O and dried at 40° in a vacuum oven (yield 21 g; N, 0·28%; SO_4 0·0%). Complete hydrolysis of the polysaccharide (100 mg) and examination by PC (solvents 1 and 2) revealed the presence of a complex mixture of sugars (see Results).

Characterization of constituent sugars. The polysaccharide (14 g) was hydrolysed and the hydrolysate separated into its constituent sugars by preparative PC on Whatman 17 filter paper using solvent 1. Guide strips on the paper were cut out and sprayed with *p*-anisidine hydrochloride, the relevant zones were then eluted with water. Where necessary, the eluted sugars were rechromatographed using solvent 3 to effect complete separation. All the sugars were chromatographically homogeneous in all three solvent systems. The sugars were characterized as follows: *Galactose.* The syrup (270 mg) was dissolved in H_2O and its concentration determined with the phenol–H_2SO_4 reagent. The solution had $[\alpha]_D^{20}$ + 20°, indicating a ratio of D- to L- isomer of 1·6:1·0. The content of D-galactose was then measured by the D-galactose oxidase method, which indicated a ratio of 1·4 D:1·0 L. The derived phenylosazone had m.p. 190–191° (lit.[23] m.p. 190°).

D-*Glucose.* The syrup (77 mg) had $[\alpha]_D^{20}$ + 52° (in H_2O) and the derived β-D-glucopyranose pentaacetate had m.p. and m.m.p. 130°, $[\alpha]_D^{20}$ + 3·9° (lit.[24] $[\alpha]_D^{20}$ + 3·8°).

[16] PRIDHAM, J. B. (1956) *Anal. Chem.* **28,** 1967.
[17] WILSON, C. M. (1959) *Anal. Chem.* **31,** 1199.
[18] BOWKER, D. M. and TURVEY, J. R. (1968) *J. Chem. Soc.* **C,** 983.
[19] SAWARDEKER, J. S. and SLONEKER, J. H. (1965) *Anal. Chem.* **37,** 945.
[20] DISCHE, Z. (1962) *Methods in Carbohydrate Chemistry* (WHISTLER, R. L. and WOLFROM, M. L., eds.), Vol. 1, p. 497, Academic Press, New York.
[21] DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. and SMITH, F. (1956) *Anal. Chem.* **28,** 350.
[22] ROTH, H., SEGAL, S. and BERTOLI, D. (1965) *Anal. Biochem.* **10,** 32.
[23] HENSEKE, G. and KÖHLER, H. (1958) *Ann. Chem.* **614,** 105.
[24] *Polarimetry, Saccharimetry and the Sugars, Circular C440* (1942) National Bureau of Standards, Washington.

D-*Mannose*. The sugar (164 mg) had $[\alpha]_D^{20}$ + 11·3° (lit.[24] + 14·2°) and the derived phenylhydrazone had m.p. and m.m.p. 196°.

D-*Xylose*. The sugar (800 mg) crystallized from EtOH with m.p. 145–146°, $[\alpha]_D^{20}$ + 17° (lit.[24] m.p. 146°, $[\alpha]_D$ + 18·8°). The derivative with *p*-tolylsulphonyl hydrazine had m.p. 148–149°, $[\alpha]_D^{20}$ − 37° (lit.[25] m.p. 149°, $[\alpha]_D$ − 36°).

L-*Rhamnose*. The crystalline sugar (119 mg) had m.p. 92°, $[\alpha]_D^{20}$ + 7·5° (lit.[24] for L-rhamnose monohydrate, m.p. 93–94°, $[\alpha]_D$ + 8·2°). The derived tetra-*O*-acetyl-L-rhamnose diethyldithioacetal had m.p. 57–58°, $[\alpha]_D^{20}$ − 40° (lit.[24] m.p. 59–61°, $[\alpha]_D$ − 42°).

Sugar A (66 mg). A sample (3 mg) of the syrup was heated at 100° in a sealed tube with 50% HBr (0·5 ml) for 10 min. The tube was cooled, opened, and the contents diluted with H_2O (10 ml) before being treated with Biodeminrolit resin (CO_3^{2-} form). The deionized solution was concentrated and examined by PC in solvent 1. Galactose was detected as the main product, suggesting that sugar *A* was a galactose derivative. Its R_f in solvent 1 was similar to that of 6-*O*-methyl-D-galactose, and in borate buffer it had electrophoretic mobility, M_g, 0·67. The syrup (5 mg) was reduced in H_2O (0·5 ml) with $NaBH_4$ (5 mg) and the product acetylated and examined by GLC.[18]

Sugar B. The syrup (109 mg) crystallized poorly from EtOH–C_6H_6 with m.p. 110–112·5°, $[\alpha]_D^{20}$ + 32°. Demethylation with HBr as for sugar *A* gave rhamnose and degradation products. On PCs, the sugar was detected as a pink spot with the triphenyltetrazolium spray,[6] and as a yellowish-green spot with *p*-anisidine hydrochloride spray. It had R_f in solvents 1 and 2 identical with that of 3-*O*-methyl-L-rhamnose. In borate buffer it migrated at the same rate as the 3-*O*-methyl ether, whereas authentic 2-*O*-methyl-L-rhamnose did not migrate. The sugar (6·3 mg) was oxidized in 15 mM sodium metaperiodate (9 ml) at 35° in the dark. The consumption of periodate was followed by the spectrophotometric method.[26] The sugar consumed one molecular equivalent of periodate in 1 hr and a slow consumption of 2 mol occurred in 24 hr. The sugar (10 mg) was converted to the methyl glycosides by heating at 100° for 6 hr in a sealed tube with 3% methanolic HCl. The product was evaporated to dryness with several additions of dry MeOH to ensure removal of HCl. The product was treated with sodium metaperiodate as above but showed no consumption of oxidant after 1 week. An osazone was prepared by the standard method, and the product recrystallized 2× from EtOH–H_2O and finally from MeOH had m.p. 118–119°.

Glucuronic acid. The mucilage (0·5 g) was heated at 100° with M H_2SO_4 (50 ml) for 7 hr, cooled and neutralized with $Ba(OH)_2$. The precipitate was removed and the filtrate concentrated to a syrup, which was separated into acidic and neutral products by preparative paper electrophoresis in neutral buffer. A band with the mobility of glucuronic acid was detected but the major migrating band was an oligosaccharide with a mobility 0·67 of that of the glucuronic acid. The oligosaccharide was eluted from the paper and concentrated to a syrup. The syrup (12 mg) was treated with 3% methanolic HCl (2 ml) under reflux for 6 hr and the product reduced to dryness *in vacuo*. The product in dry tetrahydrofuran (2 ml) was treated with a satd soln of $LiAlH_4$ in tetrahydrofuran (0·5 ml) under reflux for 1 hr and then cooled. H_2O (5 ml) was added dropwise, the ppt filtered off and the soln deionized with Amberlite resin IR-120 (H^+ form). The product was then hydrolysed in 0·75 M H_2SO_4 at 100° for 3 hr, neutralized, concentrated and examined by PC. The only products detected were galactose and glucose, whereas similar hydrolysis of the original oligosaccharide gave galactose and a uronic acid. The glucose thus arose by reduction of glucuronic acid.

Fractionation of the polysaccharide. DEAE-cellulose (Whatman D.E.32) was equilibrated in 3 M HCl, washed with water and then packed into a column (300 × 23 mm). The polysaccharide (65 mg in 2 ml H_2O) was absorbed on the column which was then eluted sequentially with H_2O (200 ml), 0·1 M KCl (250 ml), 0·2 M KCl (250 ml) and increasing concentrations of KCl to Molar. Use of concentrations greater than 0·2 M KCl did not elute any further polysaccharide. Fractions (5 ml each) were collected and analysed with the phenol–H_2SO_4 reagent. Fractions were combined (E1–E4, see Results), dialysed, concentrated and the polysaccharides recovered by evaporation. A portion of each polysaccharide was hydrolysed and selected sugars in the hydrolysates estimated (Table 2). Fractions E3 and E4 (15 mg) were both separately rechromatographed on the above column using identical eluting solvents. In each case, the fraction was split into two, one portion being eluted with 0·1 M and the other with 0·2 M KCl. Solutions of fractions E1 and E2 (1 mg each) in H_2O (20 ml) were tested with I_2 soln in KI. Fraction E1 gave a red-brown stain with λ_{max} 510 nm but fraction E2 did not stain.

Graded hydrolysis of the polysaccharide. The polysaccharide (100 mg) was heated at 100° in 25 mM H_2SO_4 (20 ml) and portions (2 ml) were removed at intervals, neutralized and concentrated for examination by PC. After 30 min both xylose and galactose were detected but glucose and mannose were only detected after 1·5 hr. No acidic oligosaccharides were detected within 3 hr. In 0·25 M H_2SO_4, the order of detection of monosaccharides was xylose (15 min), galactose (30 min), glucose and mannose (90 min); after 3 hr no rhamnose or acidic oligosaccharides were detected. In a large scale hydrolysis, the polysaccharide (10 g) was heated with 0·25 M oxalic acid (250 ml) at 100° for 3 hr. The mixture was neutralized ($CaCO_3$), insoluble

[25] EASTERLY, D. G., HOUGH, L. and JONES, J. K. N. (1951) *J. Chem. Soc.* 3416.
[26] ASPINALL, G. and FERRIER, R. J. (1957) *Chem. Ind. (London)* 1216.

salts filtered off, and the solution poured into EtOH (4 vol.). The precipitated polysaccharide was collected, washed with EtOH and dried (8 g), and the ethanolic solns retained. Further hydrolysis of the ppt in 0·5 M oxalic acid at 100° for 7 hr, and treatment as above, gave a ppt (2·5 g), which was again hydrolysed to give residual polysaccharide (1·0 g). This material was hydrolysed with M H_2SO_4 at 100° for 5 hr, the hydrolysate neutralized ($BaCO_3$) and the filtrate combined with the ethanolic solutions obtained from the previous oxalic acid hydrolyses. The combined solutions were concentrated, treated with cation-exchange resin (ZeoKarb 225, H^+ form) and evaporated to a syrup (5·0 g). The syrup, in H_2O, was percolated through a column (27 × 5 cm) of Amberlite IR-4B (OH^- form), and the neutral sugars eluted with H_2O (2 l.) and examined. No acidic sugars were detected. The column was then eluted with 0·5 M H_2SO_4 until all the acidic sugars had been recovered. The combined fractions were neutralized ($BaCO_3$), cations were removed on Zeo-Karb 225 (H^+) form, and the acidic sugars obtained by concentration. By a combination of chromatography on thick paper (solvent 3), and preparative paper electrophoresis in neutral buffer, 6 chromatographically and electrophoretically homogeneous fractions (C–H, Table 3) were obtained. Portions (1·5 mg) of each fraction were esterified with methanolic HCl, reduced with $LiAlH_4$ and the reduced oligosaccharide methyl glycosides recovered, as above. The neutral sugars in each fraction were then determined by quantitative GLC.

Acknowledgement—The gift of alga from Drs. J. S. Craigie and J. McLachlan is gratefully acknowledged.