Carbohydrate Research, 124 (1983) 123–133
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

STRUCTURE OF THE ACIDIC EXTRACELLULAR GELLING POLYSACCHARIDE PRODUCED BY *Pseudomonas elodea*

MALCOLM A. O'NEILL, ROBERT R. SELVENDRAN, AND VICTOR J. MORRIS
A.R.C. Food Research Institute, Colney Lane, Norwich NR4 7UA (Great Britain)

(Received January 31st, 1983; accepted for publication, May 26th, 1983)

ABSTRACT

The gelling polysaccharide secreted by the bacterium *Pseudomonas elodea* contains L-rhamnose, D-glucose, and D-glucuronic acid in the molar ratios 1:2:1. Methylation analysis of native and carboxyl-reduced polysaccharide indicated (1→4)-Rha*p*, (1→3)-Glc*p*, (1→4)-Glc*p*, and (1→4)-Glc*p*A to be present in the ratios 1:1:1:1. Graded acid hydrolysis gave a series of acidic oligosaccharides that were isolated by ion-exchange chromatography and fractionated by gel-filtration. The purified oligosaccharides were analysed and characterised as their methylated alditol derivatives by e.i.-m.s. and c.i.-m.s., and also examined by fast-atom-bombardment (f.a.b.)-m.s. The tetrasaccharide repeating-unit **1**, excluding acetyl groups, is proposed.

→3)-β-D-Glc*p*-(1→4)-β-D-Glc*p*A-(1→4)-β-D-Glc*p*-(1→4)-α-L-Rha*p*-(1→

1

INTRODUCTION

The bacterium *Pseudomonas elodea* produces an extracellular anionic heteropolysaccharide that has rheological properties of potential industrial value[1,2]. Limited chemical analysis[1] showed it to be a partially *O*-acetylated linear polymer composed of rhamnose, glucose, and uronic acid residues.

In order to relate rheological properties and X-ray diffraction studies[2,3] to structure, a detailed chemical analysis has been carried out. The resulting data are inconsistent with the earlier analysis[1] and suggest that the polymer has a tetrasaccharide repeating-unit.

EXPERIMENTAL

The crude polysaccharide, commercially known as PS60 or gellan gum, was a gift from Mr. A. N. Bennet and Mr. A. P. Imeson (Alginate Industries Ltd.), and was purified and deacetylated as previously described[2].

Monosaccharide analysis. — Neutral sugars, released by hydrolysis with M H_2SO_4 (100° for 2.5 h), were determined[4] by g.l.c. of their alditol acetates. Uronic acid was determined colorimetrically[5], using D-glucuronic acid as the standard.

Carboxyl reduction. — A solution of the polysaccharide (50 mg) in distilled water (20 mL) at 60° was treated[6] with mM 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulphonate. The reduced material was isolated, after dialysis, by freeze-drying.

Chromium trioxide oxidation. — Carboxyl-reduced polysaccharide (10 mg) was acetylated[7] with pyridine–acetic anhydride (1 mL, 1:1), and the acetylated material was extracted into chloroform. The acetylated polysaccharide (~5 mg) and *myo*-inositol hexa-acetate (3 mg) were dissolved in glacial acetic acid (2 mL), powdered chromium trioxide (15 mg) was added, and the mixture was heated for 2 h at 50° in an ultrasonic bath[8]. The reaction was terminated by the addition of water (2 mL), and the products were extracted with chloroform (3 × 2 mL). The combined extracts were dried (Na_2SO_4) and concentrated to dryness. Sugars were analysed, after acid hydrolysis[9], as their alditol acetates[4] by g.l.c. on a column (3 m × 2.2 mm) containing 3% of SP2330 at 210°.

Partial, acid hydrolysis. — (*a*) The polysaccharide (30 mg) was hydrolysed at 100° for 4 h with 0.5M H_2SO_4 (5 mL). The hydrolysate was adjusted to pH 7.5 with $Ba(OH)_2$, filtered, concentrated (to ~2 mL), and placed on a column (10 × 1 cm) of Dowex AG1-X2 (AcO^-) resin. Neutral material was eluted with water (10 bed vol.), and the acidic fraction with 2M acetic acid (15 bed vol.). Each fraction was reduced in volume and then freeze-dried, to yield neutral (17.7 mg) and acidic materials (8.0 mg).

(*b*) A suspension of the polysaccharide (100 mg) in 0.2M trifluoroacetic acid (20 mL) was heated for 2 h at 100°, filtered, and concentrated to dryness. Residual acid was removed by co-distillation with water (3 × 5 mL). A solution of the residue in water (1 mL) was adjusted to pH 7.5, applied to a column (15 × 1 cm) of Dowex AG1-X2 (AcO^-) resin, and eluted with water (10 bed vol.) to give the neutral fraction (35 mg), and then with 2M acetic acid (15 bed vol.) to give the acid fraction (56 mg). The acid fraction was concentrated to dryness, and a solution of the residue in 0.1M acetic acid (2 mL) was eluted from a column (150 × 1 cm) of Bio-Gel P-2 (−400 mesh) at 50° with 0.1M acetic acid. Fractions (1 mL) were collected and portions (30 μL) were assayed for total sugar with phenol–sulphuric acid[9]. Appropriate fractions were combined and freeze-dried.

Methylation analysis. — (*a*) *Polymeric material.* Polysaccharide (~2 mg) was dissolved in methyl sulphoxide, methylated as previously described[10], and analysed[11] as the alditol acetates by g.l.c.–m.s.[11].

(*b*) *Oligomeric material.* Oligosaccharide fractions (~1 mg) were reduced with NaB^2H_4 and desalted as previously described[7]. The oligosaccharide alditols were methylated, extracted with dichloromethane[12], and analysed by g.l.c.–m.s.[7] or direct-probe insertion[13] using e.i.-m.s. or c.i.-m.s. (NH_3 as the reagent gas).

Fast-atom-bombardment (f.a.b.)-m.s. — An AEI MS902 mass spectrometer[14] was used with xenon as the primary bombarding-gas beam.

RESULTS AND DISCUSSION

Monosaccharide composition. — Analysis of the polysaccharide produced by *P. elodea* showed it to contain rhamnose, glucose, and uronic acid in the molar ratios 1:1.5:1. After carboxyl-reduction, rhamnose and glucose were found in the ratio 1:2.8 and the content of uronic acid was <5%. Hence, the uronic acid had the *gluco* configuration. The configuration of the sugar residues was not determined but, by analogy with related polymers[15], glucose and glucuronic acid were assumed to be D, and rhamnose to be L.

Linkage analysis. — It was reported earlier[1] that glucose and rhamnose were present as (1→4)-linked residues. However, our data showed that glucose was also (1→3)-linked and that the three linkage types were present in equal amounts (Table I, column A). The possibility that (1→3)-linked glucose was derived from a contaminating neutral glucan was examined by ion-exchange chromatography [DEAE-Sephadex (Cl^- form)]. Most of the material (~95%) was bound to the column and could only be eluted with salt, indicating that a neutral (1→3)-linked glucan was unlikely to be present. The native polysaccharide also gave a single symmetrical peak in both ascending and descending limbs of a Tiselius electrophoresis apparatus. Therefore, the polysaccharide was probably a single polymer.

The neutral-sugar ratios obtained by direct analysis reflect an underestimate of glucose. This is attributed to the stability of the glucosyluronic acid→glucose linkage (see below), which is diminished on methylation. Methylation analysis of the carboxyl-reduced polysaccharide gave a two-fold increase in (1→4)-linked glucose (Table I, column B), showing the presence of (1→4)-linked glucuronic acid.

Anomeric configurations. — In order to determine the anomeric configuration of the glycosyl residues, the carboxyl-reduced and acetylated polysaccharide was oxidised with chromium trioxide[8]. Glycopyranosides having an equatorial ag-

TABLE I

METHYLATED ALDITOL ACETATES FROM METHYLATED NATIVE AND CARBOXYL-REDUCED *P. elodea* POLYSACCHARIDE

Partially methylated alditol acetate[a]	*Native polysaccharide (A)*	*Carboxyl-reduced polysaccharide (B)*	*Deduced linkage (C)*
2,3-Me_2-Rha[b]	1.00	1.00	→4)-Rha*p*-(1→
2,4,6-Me_3-Glc	0.94	1.11	→3)-Glc*p*-(1→
2,3,6-Me_3-Glc	0.90	1.86	→4)-Glc*p*-(1→

[a] Expressed as peak area relative to that of the rhamnose derivative. [b] 2,3-Me_2-Rha = 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-rhamnitol, *etc.*

TABLE II

MONOSACCHARIDE COMPOSITION OF ACIDIC OLIGOSACCHARIDES ISOLATED AFTER HYDROLYSIS OF *P. elodea* POLYSACCHARIDE

Component[a]	*Hydrolysis agent*		
	*0.5*M *H_2SO_4 (A)*	*0.2*M *CF_3COOH*	
		Bio-Gel-I (B)	*Bio-Gel-II (C)*
Rha	1.0	1.0	1.0
Glc	6.1	1.7	1.8
GlcA	7.4	1.2	1.4
Recovery (% of acidic fraction)	90.0	37.1	50.1

[a]Calculated as molar ratios with rhamnose = 1.0.

lycon are oxidised, whereas those with an axial aglycon are not reactive[16]. The rhamnose–glucose ratio was 1:2.8 before oxidation and 1:1 after oxidation. This is consistent with two of the glucosyl residues being β and the rhamnose being α. The third glucosyl residue apparently was α, and presumably was formed from α-D-glucosyluronic acid residues in the native polysaccharide*.

Isolation and characterisation of an aldobiouronic acid. — High yields of aldobiouronic acid can be obtained[17] by selective acid hydrolysis of the anionic polysaccharide from *Acetobacter aerogenes*. The polysaccharide from *P. elodea* was therefore hydrolysed for 4 h with 0.5M H_2SO_4, and the acidic fraction subsequently isolated by ion-exchange chromatography was rich in glucose and glucuronic acid (Table II, column A). This material was reduced with NaB^2H_4, and then methylated, and the product was analysed by direct-insertion m.s. and g.l.c.–m.s. The mass-spectral data were interpreted using the principles of Kovacik *et al.*[18,19] as applied to acidic oligosaccharides obtained from plant cell-walls[20].

G.l.c. of the methylated fraction gave one major peak in the region for a methylated disaccharide-alditol methyl ester (*T* 1.21; *T* 1.00 for methylated cellobiitol). Ammonia c.i.-m.s. gave ions at *m/z* 486 (1.3%) and 503 (4.4%), corresponding to $(M + 1)^+$ and $(M + 18)^+$, respectively, from a disaccharide derivative containing hexosyl and hexosyluronic acid residues. G.l.c.–e.i.-m.s. gave intense ions of the aA series [*m/z* 233 (22.3%), 201 (100.0%), and 169 (10.2%)] and bA

---

*After this paper had been submitted, we were informed (Dr. P. A. Sandford, personal communication) that the repeating unit 1 had also been found by Jansson, Lindberg, and Sandford (see following paper) and that, from the n.m.r. data, they concluded the D-glucopyranosyluronic acid residue to be β. We have now found that (*1*) a 300-MHz ^{1}H-n.m.r. spectrum ($CDCl_3$, internal $CHCl_3$) of the methylated polymer contained signals for anomeric protons at δ 4.22 ($J_{1,2}$ 7.67 Hz), 4.42 ($J_{1,2}$ 7.67 Hz), 4.63 ($J_{1,2}$ 7.68 Hz), and 5.31 (not resolved); and (*2*) the $[\alpha]^{20}_{580}$ values (water) of the aldobiouronic acid, aldotriouronic acid, and aldotetraouronic acid were +6°, −1°, and −20°, respectively. These data are consistent with one α-rhamnopyranosyl and three β-pyranoid glycosyl residues in the repeating unit. The discrepancy between the above data and those for the chromium trioxide oxidation may be due to insufficient reaction time with the chemical procedure.

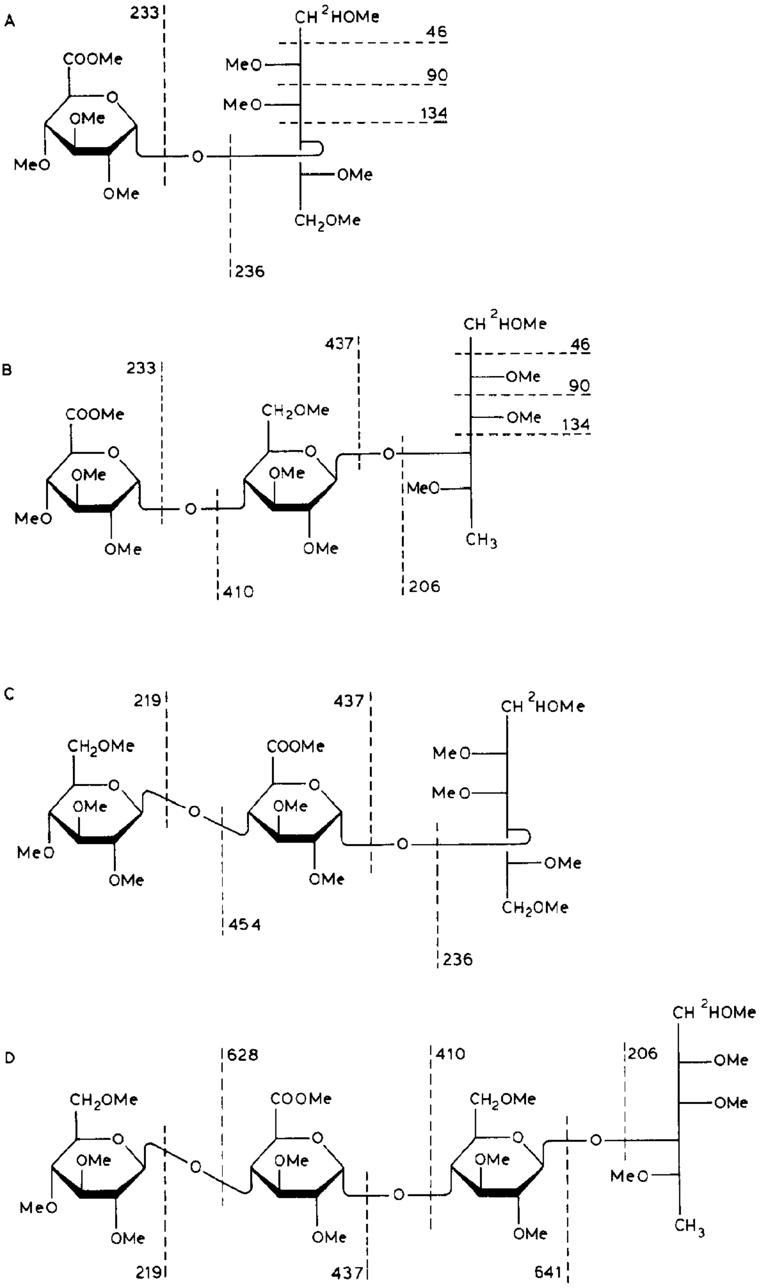

Fig. 1. Mass-spectral fragmentation patterns of methylated oligosaccharide-alditol methyl esters.

series [*m/z* 236 (17.3%), 204 (2.2%), and 172 (13.7%)] derived from methylated hexosyluronic acid groups and hexitol residues, respectively (Fig. 1A). The disaccharide nature of the component was confirmed by the presence of the abJ$_1$ ion at *m/z* 296 (1.4%) and ions at *m/z* 440 (0.4%), 439 (0.3%), 395 (2.6%), and 363 (1.5%), corresponding to $(M - 45)^+$, $(M - 46)^+$, $(M - 90)^+$, and $(M - 122)^+$, respectively.

The nature of the linkage to the hexitol moiety can be deduced from cleavage of C–C bonds of the methylated hexitol. Characteristic fragments are obtained[7,21] from (1→2), (1→3), and (1→4) linkages after incorporation of deuterium at C-1 of the hexitol. The relative abundance of the ion *m/z* 134 (23.5%) is consistent with the occurrence of a (1→4) linkage.

Sugar and methylation analysis indicated the presence of glucuronic acid and further evidence was provided by mass spectrometry. Kovacik *et al.*[18] showed the ion intensity ratios $aA_1 \ll aA_2$ for glucuronic acid and $aA_1 \simeq aA_2$ for galacturonic acid; the ratio for the methylated aldobiouronic acid was 1.0:4.5. The aldobiouronic acid Glc*p*A-(1→2)-Xyl*p* from wheat bran[20] gave a ratio of 1.0:4.3, and Gal*p*A-(1→4)-Rha*p* from runner-bean pectin[22] gave a ratio of 1.0:1.2. Therefore, the aldobiouronic acid from *P. elodea* polysaccharide has the structure Glc*p*A-(1→4)-Glc*p*. G.l.c.–e.i.-m.s. of the trailing edge of the major peak showed that it contained small proportions of the derivative from the pseudoaldobiouronic acid Glc*p*-(1→4)-Glc*p*A. This is inferred from the occurrence of the ions of the aA series [*m/z* 219 (3.8%), 187 (21.4%), and 155 (8.9%)] and bA series [*m/z* 250 (1.1%), 218 (0.5%), and 186 (0.3%)] and the ion at *m/z* 310 (0.6%) corresponding to abJ$_1$. The ratio of Glc*p*A-(1→4)-Glc*p* and Glc*p*-(1→4)-Glc*p*A was ~20:1.

Isolation and characterisation of the higher oligosaccharides. — The polysaccharide was partially hydrolysed with 0.2M trifluoroacetic acid, and the acidic fraction was isolated by ion-exchange chromatography and subjected to gel-filtration (Fig. 2). The two major components (I and II), eluted in the tetra- and tri-saccharide regions, respectively, accounted for 87% of the acidic fraction and were found to contain rhamnose, glucose, and glucuronic acid (Table II, columns B and C).

G.l.c. of the reduced and methylated fraction II gave a major peak in the region for methylated trisaccharide-alditol methyl ester (*T* 2.91). Selective ion monitoring (s.i.m.) over the area of this peak (Fig. 3) at *m/z* 206 (6-deoxyhexitol), 219 (non-reducing terminal hexose), 233 (non-reducing terminal hexuronic acid), and 236 (hexitol) revealed two partially separated components II-X and II-Y in the ratio 1.0:1.7. The identities of the components were established by c.i.-m.s. (ammonia reagent gas, direct insertion) and e.i.-m.s. The faster moving component, II-X, in c.i.-m.s. gave ions at *m/z* 660 (0.1%) and 677 (8.4%), corresponding to $(M + 1)^+$ and $(M + 18)^+$, respectively, of a methylated trisaccharide-alditol methyl ester containing hexosyluronic acid, hexosyl, and 6-deoxyhexitol residues. G.l.c.–e.i.-m.s. gave intense ions of the aA series [*m/z* 233 (6.6%), 201 (39.6%), and 169 (24.9%)] and cA series [*m/z* 206 (22.9%), 174 (7.4%), and 142 (8.1%)], showing

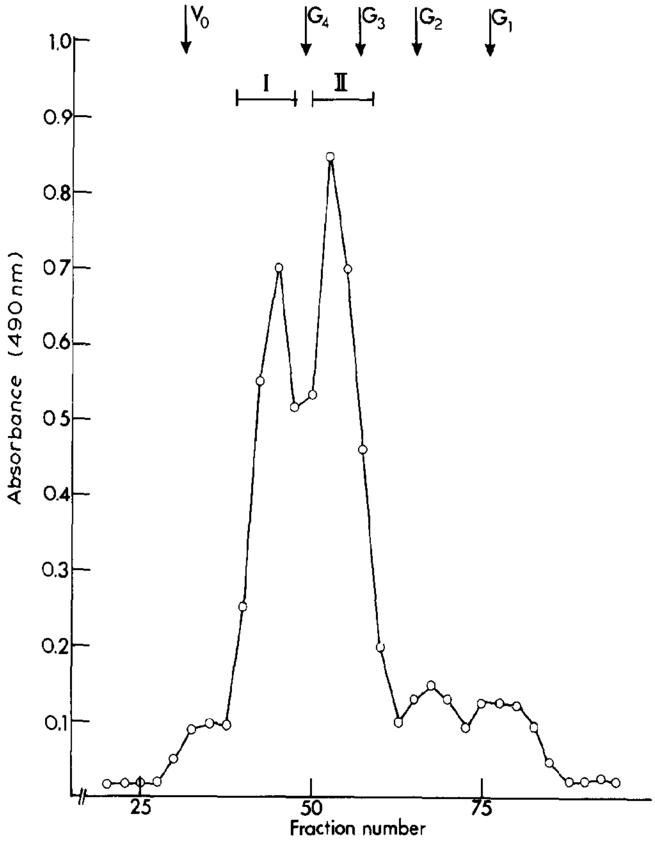

Fig. 2. Fractionation of acidic oligosaccharides derived from *P. elodea* polysaccharide on Bio-Gel P-2. Void volume (V_0) determined with Blue Dextran; G_1–G_4, D-glucose, cellobiose, raffinose, and stachyose, respectively.

the presence of terminal non-reducing hexosyluronic acid and 6-deoxyhexitol residues (Fig. 1B). Confirmation of the trisaccharide nature of II-X was obtained from the presence of the J series of ions [*m/z* 470 (abcJ_1, 0.2%) and 266 (bcJ_1, 6.3%)] and also from the ions at *m/z* 437 (baA_1, 0.9%) and 410 (bcA_1, 0.6%). The occurrence of a relatively large amount of the bcJ_1 ion shows that O-4 and not O-3 of the internal residue carries the terminal uronic acid[23]. Methylation analysis has already shown rhamnose to be 4-linked, and confirmation that the 6-deoxyhexitol was 4-substituted was derived from the relative abundance of the ion at *m/z* 134 (18.3%). Since the aldobiouronic acid described earlier contains 4-substituted glucose, the sequence of the trisaccharide II-X is Glc*p*A-(1→4)-Glc*p*-(1→4)-Rha*p*.

The slower-eluting component, II-Y, gave (c.i.-m.s.) ions at *m/z* 690 (0.1%) and 707 (24.4%) corresponding to $(M + 1)^+$ and $(M + 18)^+$, respectively, of a methylated trisaccharide-alditol methyl ester containing hexosyluronic acid, hexosyl, and hexitol residues. G.l.c.–e.i.-m.s. gave intense ions of the aA series

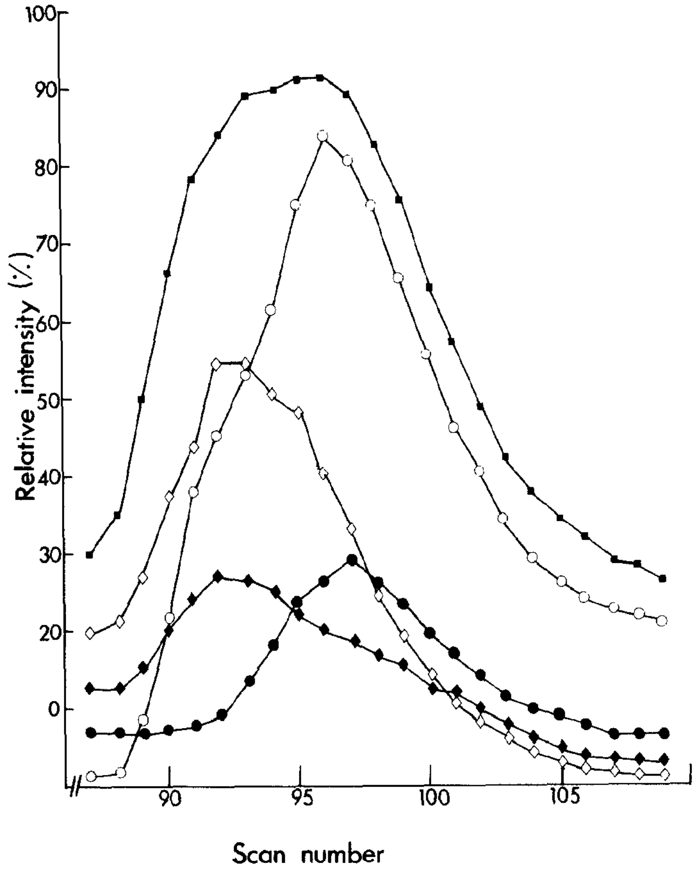

Fig. 3. G.l.c. of methylated oligosaccharide-alditol methyl esters from fraction II with s.i.m. profile, using a column (45 × 0.44 cm) containing 1% of OV-1 held at 150° for 5 min and then 4°/min to 320°: —■—, flame-ionisation detector response; —◇—, *m/z* 206; —●—, *m/z* 219; —◆—, *m/z* 233; —○—, *m/z* 236. See text for details of abundance and origin of the ions.

[*m/z* 219 (10.0%), 187 (57.5%), and 155 (13.1%)] and cA series [*m/z* 236 (26.8%), 204 (2.7%), and 172 (12.6%)], showing the presence of terminal non-reducing hexosyl and hexitol residues (Fig. 1C). Confirmation of the trisaccharide nature of II-Y was obtained from the J series of ions [*m/z* 514 (abcJ_1, 0.5%) and 296 (bcJ_1, 1.8%)] and also ions at *m/z* 437 (baA_1, 0.9%) and 454 (bcA_1, 0.3%). The presence of the bcJ_1 ion showed that the internal hexosyluronic acid residue was not 3-linked. The relative abundance of the ion at *m/z* 134 (13.4%) showed that the terminal hexitol was substituted through O-4. Methylation analysis of the carboxyl-reduced polysaccharide showed that the glucuronic acid residues were 4-linked. This information, coupled with the structure of the aldobiouronic acid and pseudoaldobiouronic acid, suggest the structure of the parent oligosaccharide of II-Y to be Glc*p*-(1→4)-Glc*p*A-(1→4)-Glc*p*.

A relatively small proportion of the trisaccharide derivative corresponding to Rha*p*-(1→3)-Glc*p*-(1→4)-Glc*p*A was detected. This is inferred from the presence of the ions of the aA series [*m/z* 189 (4.3%), 157 (8.1%), and 125 (9.2%)] and cA series [*m/z* 250 (0.9%), 218 (3.0%), and 186 (0.4%)]. The absence of the ion at *m/z* 310 (cJ$_1$) implies that the internal sugar residue was substituted through O-3. Presumably, the small proportion of this trisaccharide derivative is due to the ease with which the terminal 6-deoxyhexose can be hydrolysed[17].

The methylated trisaccharide-alditol methyl esters accounted for ~90% of the oligosaccharides detected by g.l.c. The remaining 10% were eluted in the region for methylated tetrasaccharides and are discussed under fraction I.

To confirm that the oligosaccharides analysed by g.l.c.–m.s. were not formed by β-elimination of uronic acid residues or carboxyl-reduction, fraction II was subjected to f.a.b.-m.s. Negative-ion f.a.b.-m.s. gave ions at *m/z* 501 and 517 corresponding to $(M - 1)^-$ for trisaccharides II-X and II-Y, respectively. Poor spectra were obtained in the positive-ion mode. However, after borohydride reduction of Fraction II, positive-ion f.a.b.-m.s. gave ions at *m/z* 505 and 521 corresponding to $(M + 1)^+$ for II-X and II-Y, respectively. Cationised pseudomolecular ions at *m/z* 527 and 543, corresponding to $(M + 23)^+$, were also detected. Therefore, the oligosaccharides II-X and II-Y, characterised as their methylated oligosaccharide-alditol methyl esters, are authentic.

Fraction I. — After reduction (NaB^2H_4) and methylation, g.l.c. revealed a major peak in the region for methylated tetrasaccharide-alditol methyl esters (*T* 3.92). Direct-insertion c.i.-m.s. gave ions at *m/z* 864 (0.2%) and 881 (6.4%) corresponding to $(M + 1)^+$ and $(M + 18)^+$, respectively, derived from a methylated tetrasaccharide derivative containing 2 hexosyl, hexosyluronic acid, and 6-deoxyhexitol residues. G.l.c.–e.i.-m.s. gave ions of the aA series [*m/z* 219 (16.9%), 187 (96.0%), and 155 (25.5%)] and dA series [*m/z* 206 (69.4%), 174 (14.1%), and 142 (9.0%)], indicating the presence of terminal non-reducing hexosyl and 6-deoxyhexitol residues. Supporting evidence for the tetrasaccharide nature of the component was derived from the presence of ions at *m/z* 296 (cdJ$_1$, 23.4%) and 688 (abcdJ$_1$, 1.5%). The sequence of the internal glycosyl and glycosyluronic acid residues was determined from the ions of the series baA [*m/z* 437 (1.3%), 405 (0.8%), and 373 (1.8%)] and cdA series [*m/z* 410 (2.1%), 378 (2.6%), and 346 (0.5%)] (Fig. 1D). The data are consistent with the structure Glc*p*-(1→4)-Glc*p*A-(1→4)-Glc*p*-(1→4)-Rha*p*.

The above tetrasaccharide accounted for ~90% of the g.l.c. peak. However, the occurrence of ions at *m/z* 233 (2.6%), 201 (14.0%), and 169 (24.0%) of the aA series and ions at *m/z* 236 (1.8%), 204 (2.3%), and 172 (2.4%) of the dA series indicated the presence of a tetrasaccharide containing terminal non-reducing hexosyluronic acid and hexitol residues. Further, examination of the g.l.c.–e.i.-m.s. data showed that, at the leading edge of the peak, the ratio of ions of *m/z* 133 and 134 was 1:2.2 compared to 1:5.7 at the peak maximum. This finding is consistent with the presence of a hexitol substituted through O-3. Thus, a small proportion of fraction I may be inferred to be Glc*p*A-(1→4)-Glc*p*-(1→4)-Rha*p*-(1→3)-Glc*p*.

The negative-ion f.a.b. mass spectrum contained an ion at *m/z* 663 corresponding to $(M - 1)^-$ of the tetrasaccharide. Positive-ion f.a.b.-m.s. of reduced ($NaBH_4$) fraction I gave ions at *m/z* 667 $(M + 1)^+$ and 689 $(M + 23)^+$, confirming that the methylated derivative corresponded to the native tetrasaccharides.

Thus, it is concluded that the polysacharide secreted by *P. elodea* has the repeating structure **1**. Such a structure accounts for all of the oligosaccharides isolated and also explains the absence, or formation of small proportions, of oligosaccharides containing the sequence Rha*p*-(1→3)-Glc*p*, as this linkage would be relatively unstable to acid hydrolysis.

The acetylated polymer has been shown[2] from X-ray diffraction data to form a 3-fold helical structure with an axial repeat of 0.94 nm. Deacetylation changes the rheology of the gels from weakly elastic to strongly brittle. X-Ray studies show that deacetylation enhances crystallinity, suggesting that *O*-acetyl groups hinder packing of the helices. Further work is therefore required to determine the positions of *O*-acetyl groups. Physico-chemical studies suggest that the helical structure is retained in solution, with the polymer behaving as an extended rod[3].

The range of data now available makes computer-aided model building possible. With a knowledge of the three-dimensional structure of the polysaccharide, the relationship between structure and rheological properties may be determined.

ACKNOWLEDGMENTS

The authors thank Ms. S. B. Ward and Mr. V. Carroll for technical assistance, Mr. J. Eagles and Dr. F. Mellon for performing the mass spectrometry, Dr. R. G. Radcliffe for n.m.r. spectroscopy, and Drs. M. J. Miles and S. G. Ring for advice.

REFERENCES

1 R. Moorhouse, G. T. Colegrave, P. A. Sandford, J. K. Baird, and K. S. Kang, *ACS Symp. Ser.*, 150 (1981) 111–124.
2 V. Carroll, M. J. Miles, and V. J. Morris, *Int. J. Biol. Macromolecules*, 4 (1982) 432–433.
3 V. Carroll, G. R. Chilvers, D. Franklin, M. J. Miles, V. J. Morris, and S. G. Ring, *Carbohydr. Res.*, 114 (1983) 181–191.
4 R. R. Selvendran, J. F. March, and S. G. Ring, *Anal. Biochem.*, 96 (1979) 282–292.
5 N. Blumenkrantz and G. Asboe-Hansen, *Anal. Biochem.*, 54 (1973) 484–489.
6 R. L. Taylor and H. E. Conrad, *Biochemistry*, 11 (1972) 1383–1388.
7 M. A. O'Neill and R. R. Selvendran, *Carbohydr. Res.*, 111 (1983) 239–255.
8 J. Hoffman, B. Lindberg, and S. Svensson, *Acta Chem. Scand.*, 26 (1972) 661–666.
9 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F Smith, *Anal. Chem.*, 28 (1956) 350–356.
10 M. A. O'Neill and R. R. Selvendran, *Carbohydr. Res*, 79 (1980) 115–124.
11 S. G. Ring and R. R. Selvendran, *Phytochemistry*, 17 (1978) 745–752.
12 P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, and J. Lonngren, *Chem. Commun. Univ. Stockholm*, 8 (1976) 1–76.

13 D. ASHFORD, N. N. DESAI, A. K. ALLEN, A. NEUBERGER, M. A. O'NEILL, AND R. R. SELVENDRAN, *Biochem. J.*, 201 (1982) 199–208.

14 G. R. FENWICK, J. EAGLES, AND R. SELF, *Org. Mass Spectrom.*, 17 (1982) 544–546.

15 D. A. POWELL, in R. C. W. BERKELEY, G. W. GOODAY, AND D. C. ELLWOOD (Eds.), *Microbial Polysaccharides and Polysaccharases*, Academic Press, New York, 1979, pp. 117–160.

16 S. J. ANGYAL AND K. JAMES, *Carbohydr. Res.*, 12 (1970) 147–153.

17 H. E. CONRAD, J. R. BAMBURG, J. D. EPLEY, AND J. J. KINDT, *Biochemistry*, 5 (1966) 2808–2817.

18 V. KOVACIK, S. BAUER, J. ROSIK, AND P. KOVAC, *Carbohydr. Res.*, 8 (1968) 282–290.

19 V. KOVACIK, V. MIHALOV, J. HIRSCH, AND P. KOVAC, *Biomed. Mass Spectrom.*, 5 (1978) 136–145.

20 S. G. RING AND R. R. SELVENDRAN, *Phytochemistry*, 19 (1980) 1723–1730.

21 J. KARKKÄINEN, *Carbohydr. Res.*, 14 (1970) 27–33.

22 M. A. O'NEILL AND R. R. SELVENDRAN, unpublished result.

23 N. K. KOCHETKOV AND O. S. CHIZHOV, *Adv. Carbohydr. Chem.*, 21 (1966) 39–93.