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STRUCTURAL FEATURES OF THE MUCILAGE FROM THE STEM PITH OF KIWIFRUIT (*Actinidia deliciosa*): PART I, STRUCTURE OF THE INNER CORE

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ABSTRACT

The mucilage found in the stem pith of *Actinidia deliciosa* contains D-glucuronic acid, D-mannose, L-fucose, L-arabinose, and D-galactose in the molar ratios 1.0:1.0:1.5:2.0:4.0. The native, carboxyl-reduced, and partially acid-hydrolysed polysaccharides were subjected to methylation analysis. Partial acid hydrolysis of the methylated, carboxyl-reduced glucuronomannan core produced a series of methylated oligosaccharides which, as their alditol derivatives, were isolated by reverse-phase h.p.l.c. and characterised by e.i.- and f.a.b.-m.s. The data suggest that the polysaccharide contains a →4)-β-D-Glc*p*A-(1→2)-α-D-Man*p*-(1→ backbone with most of the D-mannosyl and approximately half of the D-glucosyluronic acid residues substituted through positions 3 with oligosaccharides containing L-arabinose, α-L-fucose, and β-D-galactose.

INTRODUCTION

The mucilages from several species of *Actinidia* are acidic polysaccharides containing a glucuronomannan backbone[1]. The polysaccharide from the stem pith of *A. deliciosa* [(A. Chev.) C. F. Liang *et* A. R. Ferguson (previously *A. chinensis* Planch var. *hispida*, C. F. Liang)] has been shown[1] to be homogeneous by ion-exchange chromatography, sedimentation analysis, and electrophoresis, and we now report on its major structural features.

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RESULTS

Monosaccharide composition. — The mucilage from *A. deliciosa* contained glucuronic acid, mannose, fucose, arabinose, and galactose in the molar ratios 1.0:1.0:1.5:2.0:4.0. A trace (0.37%) of xylose was also present. The optical rotations of the purified monosaccharides, isolated after acid hydrolysis, showed the fucose and arabinose to be L and the galactose, mannose, and glucuronic acid to be D.

Methylation analyses. — G.l.c.–m.s. of the methylated alditol acetates obtained from the methylated, the methylated and carboxyl-reduced, and the methylated, carboxyl-reduced, and remethylated mucilage indicated a glucurono-mannan backbone substituted by oligosaccharides containing arabinose, fucose, and galactose (Table I, columns I–III). The recoveries of methylated alditol acetates from the methylated carboxyl-reduced polymer were in good agreement with the monosaccharide values obtained by direct analysis. Fucose occurred exclusively as non-reducing terminal residues. Most of the arabinose (both furanoid and pyranoid) residues were also non-reducing terminals although small but significant amounts of (1→5)-Ara*f* were detected. In contrast, the major proportion of galactose was accounted for by Gal*p*, (1→3)-, (1→2,3)-, and (1→2,3,6)-Gal*p*, with a small proportion of (1→3,6)-Gal*p*. The bulk of the mannose occurred as (1→2,3)-linked residues, although the small proportion of 3,4,6-Me_3-Man*p* detected showed that ~1 in 20 of the mannosyl residues was not branched. Carboxyl-reduction of the methylated mucilage (Table I, columns II and III) demonstrated the presence of (1→4)-Glc*p*A residues of which ~50% were substituted through positions 3.

Methylation analysis of a sample of mucilage (Table I, column IV), prepared at a later date, gave a higher proportion of (1→3,6)-Gal*p* and a lower proportion of Ara*f*, indicating that the fine structure may be subject to slight seasonal variation.

Graded acid hydrolysis of the mucilage with oxalic and trifluoroacetic acid (Scheme 1) produced three degraded polysaccharides (DKM1–3). Analysis of the alditol acetates obtained after methylation and carboxyl-reduction of DKM1–3 (Table II) demonstrated the points of attachment of the acid-labile sugars.

DKM1 showed a three-fold increase in the proportion of (1→4)-Glc*p*A with the concomitant disappearance of the branched glucosyluronic acid residues (Table II, column II). Of the Fuc*p* residues, ~25% were still present whereas <5% of the Ara*f* residues remained, indicating that some of the arabinose residues were attached to position 3 of (1→4)-Glc*p*A. This result, in conjunction with the identification of an arabinosylarabinoside[1] (see following paper[2]), was consistent with a proportion of the (1→4)-Glc*p*A being substituted through positions 3 with either Ara*p*-(1→?)-Ara*f* or Ara*f*.

A decrease in (1→2,3)-Gal*p* and an increase in Gal*p* and (1→3)-Gal*p* demonstrated that the branched galactosyl residues of the native mucilage were substituted with both Ara*f* and Fuc*p*. A small increase in (1→2)-Man*p* was

TABLE I

METHYLATION ANALYSIS DATA FOR THE MUCILAGE FROM *A. deliciosa*

Methylated alditol[a]	T[b]	*Molar proportions (mol%)*[c]				*Linkage*
		I[e]	*II*	*III*	*IV*	
2,3,5-Me_3-Ara	0.46	16.1	13.4	12.5	12.3	Ara*f*
2,3,4-Me_3-Ara	0.55	4.0	3.5	3.2	5.1	Ara*p*
2,3-Me_2-Ara	1.06	5.4	4.3	4.4	6.1	(1→5)-Ara*f*
2,3,4-Me_3-Fuc	0.58	14.8	14.2	14.5	15.0	Fuc*p*
2,3,4,6-Me_4-Gal	1.18	13.6	11.8	12.0	12.4	Gal*p*
2,4,6-Me_3-Gal	2.03	14.4	11.4	11.8	12.6	(1→3)-Gal*p*
4,6-Me_2-Gal	3.20	12.0	9.8	10.2	11.5	(1→2,3)-Gal*p*
2,4-Me_2-Gal	4.85	2.8	2.8	2.2	6.0	(1→3,6)-Gal*p*
4-Me-Gal	8.0	11.5	9.5	9.8	13.6	(1→2,3,6)-Gal*p*
3,4,6-Me_3-Man	1.81	0.2	0.5	0.3	0.2	(1→2)-Man*p*
4,6-Me_2-Man	2.91	5.1	9.5	9.6	5.2	(1→2,3)-Man*p*
2,3,6-Me_3-Glc[d]	3.25	—	—	4.4	—	(1→4)-Glc*p*A
2,6-Me_2-Glc[d]	3.28	—	—	5.1	—	(1→3,4)-Glc*p*A
2,3-Me_2-Glc[d]	4.28	—	4.2	—	—	(1→4)-Glc*p*A
2-Me-Glc[d]	6.22	—	5.2	—	—	(1→3,4)-Glc*p*A

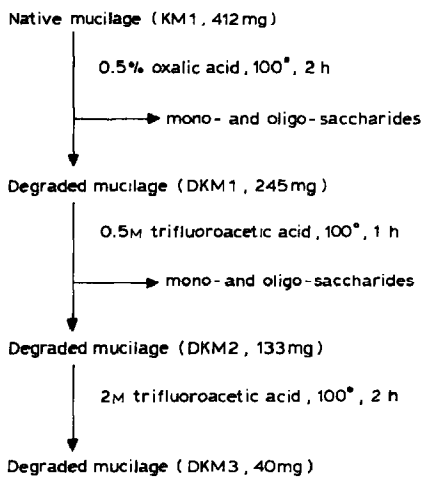
[a]2,3,5-Me_3-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, *etc.* [b]Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on 3% OV-225. [c]Values corrected using the molar response values given by Sweet *et al.*[21]. [d]Corresponds to the 6,6′-dideuterio derivatives. [e]I, Methylated mucilage; II, methylated and carboxyl-reduced mucilage; III, methylated, carboxyl-reduced, and remethylated mucilage; IV, methylated mucilage prepared from a different batch of the polysaccharide.

observed, suggesting that acid-labile sugars are not directly attached to mannose.

Further degradation of DKM1 with 0.5M trifluoroacetic acid gave DKM2 in which 70% of the residues attached to positions 3 of (1→2)-Man*p* had been hydrolysed, as determined by methylation and carboxyl-reduction (Table II, column III). Approximately equimolar ratios of Gal*p*, (1→3)-Gal*p*, and (1→2,3)-Man*p* indicated that the mannose residues were substituted through positions 3 with short oligosaccharides containing (1→3)-Gal*p* residues.

Degradation of DKM2 with 2M trifluoroacetic acid gave DKM3 that was composed almost entirely of mannose and glucuronic acid. Analysis of the methylated and carboxyl-reduced material (Table II, column IV) showed it to contain approximately equal proportions of (1→4)-Glc*p*A and (1→2)-Man*p*. From the ratios of the derivatives 1,3,4,5,6-Me_5-Man, 3,4,6-Me_3-Man, and 2,3-Me_2-Glc (1.0:9.7:8.8, respectively), a minimum d.p. for the degraded core polymer would be ~20.

Chromium trioxide oxidation. — Chromium trioxide oxidation[3] of the carboxyl-reduced and acetylated DKM1 oxidised 90% of the D-glucose and 80% of the D-galactose, whereas L-fucose and D-mannose were resistant. This result



Scheme 1.

indicated that most of the D-galactose and all of the D-glucuronic acid were β-linked with L-fucose and D-mannose α-linked.

DKM1 was used because 90% of the glucosyluronic acid residues could be reduced after two treatments with carbodi-imide[4]. Only 70% of the carboxyl groups in the mucilage were reduced after seven treatments.

Partial acid hydrolysis. — The mass spectra of the methylated oligosaccharide-alditols were interpreted according to established principles[5–8], except that the alditol fragment was designated *ald*J_2 and is produced by loss of 60 m.u. from the corresponding J_1 fragment[9].

Hydrolysis of the mucilage with 2M trifluoroacetic acid followed by ion-exchange chromatography gave an aldobiouronic acid. F.a.b.-m.s. of the methylated oligosaccharide-alditol methyl ester gave an ion at *m/z* 486, corresponding to $(M + 1)^+$ from a derivative containing hexuronic acid and hexitol residues. E.i.-m.s. gave intense ions of the aA series [*m/z* 233 (10.8%), 201 (100.0), and 169 (7.0)] with ions at *m/z* 236 (*ald*J_2, 27.0%) and 296 (*ald*J_1, 0.4) confirming the disaccharide nature of the derivative. Ions at *m/z* 133 (8.2%) and 177 (5.7) produced by cleavage of C–C bonds in the alditol moiety[5] established that the hexitol was substituted through position 2.

The ^{1}H-n.m.r. spectrum (D_2O) of the oligosaccharide-alditol contained a single resonance in the anomeric region at δ 4.65 ($J_{1,2}$ 7.3 Hz) indicating a β-glucuronic acid residue. These data established the aldobiouronic acid to be β-D-Glc*p*A-(1→2)-D-Man*p* (**1**). The yield (13.5%) of the aldobiouronic acid was close to the theoretical value (15.0%) expected from the mucilage if it contained **1** as the sole chemical repeating-unit of the backbone.

Reverse-phase h.p.l.c. (Fig. 1) of the methylated oligosaccharide-alditols obtained after partial hydrolysis of methylated, carboxyl-reduced DKM3 revealed

TABLE II

METHYLATION ANALYSIS DATA FOR THE PARTIALLY ACID-HYDROLYSED MUCILAGE FROM *A. deliciosa*

Methylated alditol[a]	T[b]	*Molar proportions (mol%)*[c]				*Linkage*
		I[e]	*II*	*III*	*IV*	
2,3,5-Me_3-Ara	0.46	13.4	1.1	—	—	Ara*f*
2,3,4-Me_3-Ara	0.55	3.5	—	—	—	Ara*p*
2,3-Me_2-Ara	1.06	4.3	—	—	—	(1→5)-Ara*f*
2,3,4-Me_3-Fuc	0.58	14.2	3.4	—	—	Fuc*p*
2,3,4,6-Me_4-Gal	1.18	11.8	24.0	12.0	0.9	Gal*p*
2,4,6-Me_3-Gal	2.03	11.4	28.0	10.0	1.1	(1→3)-Gal*p*
4,6-Me_2-Gal	3.20	9.8	3.6	—	—	(1→2,3)-Gal*p*
2,4-Me_2-Gal	4.85	2.8	7.5	—	—	(1→3,6)-Gal*p*
4-Me-Gal	8.00	9.5	2.8	—	—	(1→2,3,6)-Gal*p*
1,3,4,5,6-Me_5-Man	0.38	—	—	—	4.6	2-Mannitol
3,4,6-Me_3-Man	1.81	0.5	1.4	30.4	44.7	(1→2)-Man*p*
4,6-Me_2-Man	2.91	9.5	15.2	12.5	1.5	(1→2,3)-Man*p*
2,3,4-Me_3-Glc[d]	2.20	—	—	—	6.4	Glc*p*A
2,3-Me_2-Glc[d]	4.25	4.5	13.0	35.1	40.7	(1→4)-Glc*p*A
2-Me-Glc[d]	6.22	5.2	—	—	—	(1→3,4)-Glc*p*A

[a]2,3,5-Me_3-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, *etc.* [b]Retention time relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol. [c]Values corrected using the molar response values of Sweet *et al.*[21]. [d]Corresponds to the 6,6'-dideuterio derivatives. [e]I, Methylated and carboxyl-reduced mucilage; II, 0.5% oxalic-acid treated, methylated, and carboxyl-reduced mucilage; III, 0.5M trifluoroacetic acid-treated, methylated, and carboxyl-reduced mucilage; IV, 2M trifluoroacetic acid-treated, methylated, and carboxyl-reduced mucilage.

di- to penta-saccharide-alditol derivatives.

Each of the four major products (Fig. 1, 1–4) was characterised on the basis of its retention time (relative to that of methylated maltopentaose-alditol) by g.l.c. on 1% OV-1, direct-insertion e.i.-m.s. and f.a.b.-m.s., and linkage analysis.

The derivative in peak 1 was eluted (*T* 0.2) in the region for methylated disaccharide-alditols. F.a.b.-m.s. gave an ion at *m/z* 474, corresponding to $(M + 1)^+$ from a derivative containing hexose and hexitol residues with one residue containing two deuterium atoms. E.i.-m.s. gave ions of the aA series [*m/z* 221 (8.0%), 189 (49.9), and 157 (8.2)] with the ions at *m/z* 236 (*ald*J_2, 21.8%) and 296 (*ald*J_1, 0.5) confirming the disaccharide nature of the derivative (Fig. 2a) and the presence of a dideuterated, non-reducing, terminal hexose residue. A relatively intense ion at *m/z* 133 (8.0%) showed that the hexitol was linked through position 2. Linkage analysis gave approximately equal proportions of Glc*p* and (1→2)-Man-ol. These data demonstrated that the product in peak 1 was derived from D-Glc*p*A-(1→2)-D-Man*p* (**2**).

The component in peak 2 on g.l.c. was eluted (*T* 0.6) in the region for a

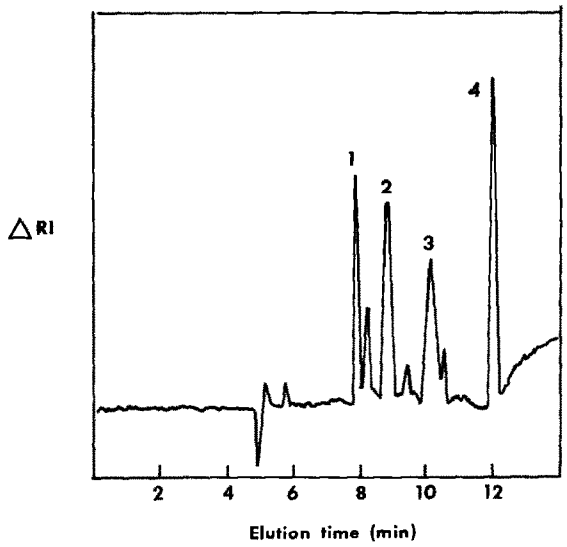


Fig. 1. Reverse-phase h.p.l.c. of the methylated oligosaccharide-alditols obtained by partial acid hydrolysis of methylated, carboxyl-reduced DKM3.

methylated trisaccharide-alditol. F.a.b.-m.s. gave an intense ion at *m/z* 678 corresponding to $(M + 1)^+$ from a derivative containing one hexitol and two hexose residues, with one residue containing two deuterium atoms. E.i.-m.s. gave ions of the aA series [*m/z* 219 (82.8%), 187 (78.1), and 155 (14.0)] and baA series [*m/z* 425 (1.7%), 393 (1.3), and 361 (0.7)] showing that residue b (Fig. 2b) contained two deuterium atoms. Ions at *m/z* 236 (*ald*J_2, 100.0%), 296 (*ald*J_1, 1.8), 442 (b*ald*J_2, 1.4), and 502 (b*ald*J_1, 3.8) confirmed the trisaccharide nature of the derivative. The occurrence of an intense ion at *m/z* 133 (18.8%) showed that the hexitol residue was substituted through position 2.

Linkage analysis of the trisaccharide-alditol gave approximately equal amounts of the derivatives from Man*p*, (1→4)-Glc*p*A, and (1→2)-Man-ol. These data established that the product in peak 2 was derived from D-Man*p*-(1→4)-D-Glc*p*A-(1→2)-D-Man*p* (**3**).

The derivative contained in peak 3 was eluted (*T* 0.8) in the region for a methylated tetrasaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 884 corresponding to $(M + 1)^+$ from a derivative containing one hexitol and three hexose residues with four deuterium atoms. E.i.-m.s. gave ions at *m/z* 221 (aA, 16.2%), 236 (*ald*J_2, 100.0), 296 (*ald*J_1, 0.7), 25 (baA$_1$, 3.5), 442 (*cald*J_2, 0.5), 502 (*cald*J_1, 1.0), 614 (bc*ald*J_2 − 32, 0.7), 631 (cbaA$_1$, 0.1), 750 (M − 133, 0.3), and 794 (M − 89, 0.3) confirming the tetrasaccharide nature of the derivative and di-deuteration of residues a and c (Fig. 2c). The presence of the ion at *m/z* 133 (9.9%) suggests that the hexitol was substituted through position 2.

Linkage analysis revealed Glc*p*, (1→4)-Glc*p*, (1→2)-Man*p*, and (1→2)-

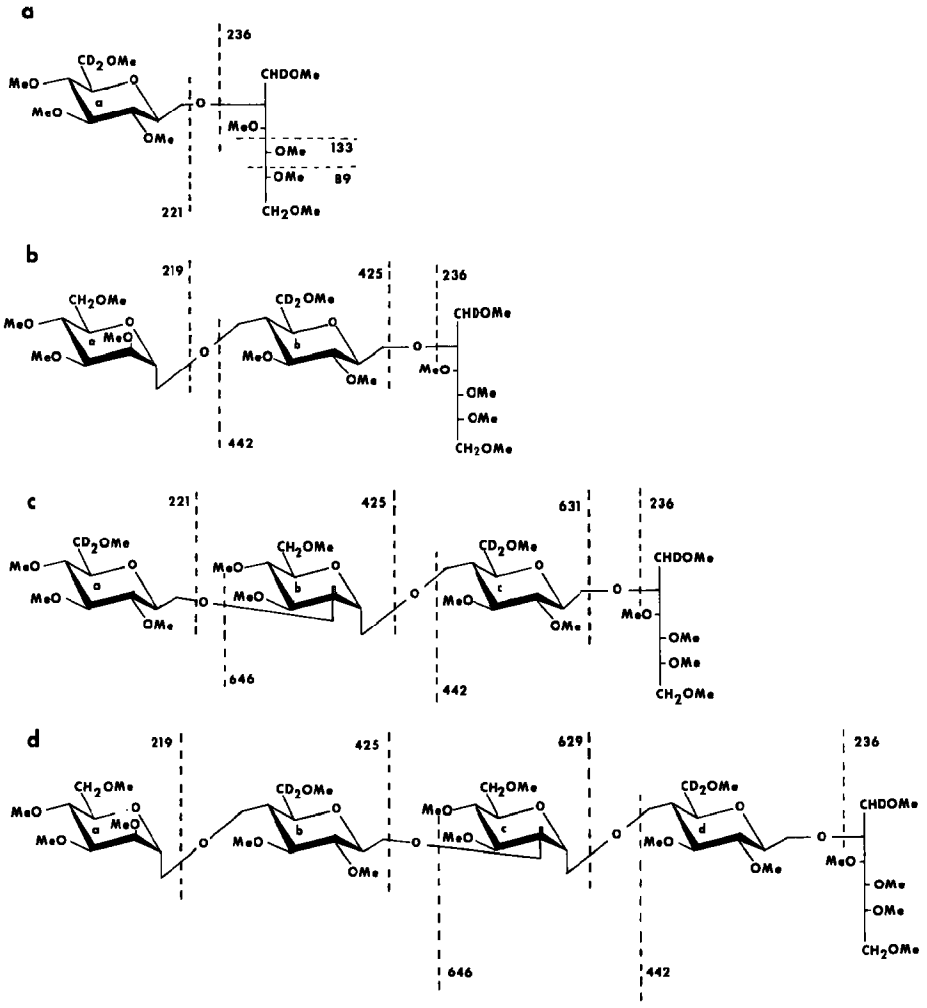


Fig. 2. Mass-spectral fragmentation patterns of the methylated oligosaccharide-alditols obtained by partial acid hydrolysis of methylated, carboxyl-reduced DKM3.

Man-ol in approximately equal proportions. These data demonstrated that the product in peak 3 was derived from tetrasaccharide **4**.

D-Glc*p*A-(1→2)-D-Man*p*-(1→4)-D-Glc*p*A-(1→2)-D-Man*p* (**4**)

The methylated derivative in peak 4 was eluted (*T* 1.1) in the region for a methylated pentasaccharide-alditol. F.a.b.-m.s. gave an ion at *m/z* 1088 corresponding to $(M + 1)^+$ from a derivative containing the hexitol and four hexose residues. E.i.-m.s. gave ions at *m/z* 219 (aA_1, 11.7%), 236 (*ald*J_2, 27.9), 296 (*ald*J_1, 0.1), 393 (baA_2, 1.3), 425 (baA_1, 0.3), 442 (d*ald*J_2, 0.1), 502 (d*ald*J_1, 0.2), 597

(cbaA_2, 2.7), and 614 (cd*ald*J_2 − 32, 0.1) confirming the pentasaccharide nature of the derivative and the presence of dideuterated residues b and d (Fig. 2d).

Linkage analysis revealed Man*p*, (1→4)-Glc*p*, (1→2)-Man*p*, and (1→2)-Man-ol in the ratios ~1:2:1:1. These confirmed that the product in peak 4 was derived from pentasaccharide **5**.

D-Man*p*-(1→4)-D-Glc*p*A-(1→2)-D-Man*p*-(1→4)-D-Glc*p*A-(1→2)-D-Man*p* (**5**)

DISCUSSION

The foregoing results demonstrated the mucilage from *A. deliciosa* to be a glucuronomannan with a backbone composed of a repeating unit, →4)-β-D-Glc*p*A-(1→2)-α-D-Man*p*-(1→, which occurs in a range of plant tissues[10–12]. In these related polysaccharides, the backbone accounts for >50% of the polymer, whereas in *A. deliciosa* it represents only 20%. The major proportion of the mucilage contained neutral side-chains attached to positions 3 of ~95% of the D-mannosyl and ~50% of the D-glucosyluronic acid residues. The results of methylation analysis indicated that the bulk of the side-chains contained (1→3)-linked galactosyl residues terminated with galactosyl, fucosyl, and arabinosyl groups. The nature of these side-chains is reported in the following paper[2].

EXPERIMENTAL

Monosaccharide analysis. — Neutral sugars were released by hydrolysis with M H_2SO_4 (100°, 2.5 h) and analysed[13] as their alditol acetates by g.l.c. Uronic acid was determined colorimetrically[14], using D-glucuronic acid as the standard.

The absolute configuration of the monosaccharides was determined by optical rotation (*c* 1, water). The monosaccharides, released by acid hydrolysis, were purified by preparative p.c. on acid-washed Whatman 3MM paper, using propyl acetate–formic acid–water (11:5:3) and detection with *p*-anisidine[15].

Methylation analysis. — (*a*) *Polymeric material.* The polysaccharides (pre-reduced with NaB^2H_4) in methyl sulphoxide were methylated as previously described[16]. A portion of the methylated material was analysed[16] as the methylated alditol acetates by g.l.c.–m.s. The remaining portion in dichloromethane–ether (1:4) was carboxyl-reduced with $LiAl^2H_4$ (50 mg) under reflux[17], and analysed[16] as the methylated alditol acetates by g.l.c.–m.s.

(*b*) *Oligomeric material.* Oligosaccharides were reduced to their corresponding alditols with NaB^2H_4 and methylated as previously described[18]. The methylated oligosaccharide-alditols were isolated using Sep-Pak C_{18} cartridges (Waters) as described in the following paper[2]. The derivatives were analysed by g.l.c.–m.s.[19] or further purified by reverse-phase h.p.l.c.[2] prior to probe e.i.-m.s. and f.a.b.-m.s.[20].

Graded acid hydrolysis. — This was performed on native mucilage (KM1) as

shown in Scheme 1. The polymeric material was separated from the mono- and oligo-saccharides by dialysis against distilled water (2 × 600 mL) over 24 h. The details of the structural analysis of the oligosaccharides released by acid hydrolysis are reported in the following paper[2]. The degraded polysaccharides were dialysed against distilled water and freeze-dried.

Chromium trioxide oxidation. — The procedure of Hoffman *et al.*[3] was used to oxidise carboxyl-reduced[4] DKM1 (20 mg). The oxidised product was hydrolysed with M H_2SO_4 (2 mL, 100°, 2.5 h) and the released monosaccharides were analysed[13] as alditol acetates by g.l.c.

Preparation of the aldobiouronic acid. — The mucilage (KM1, 600 mg) was treated with 2M trifluoroacetic acid (100 mL) for 6 h at 100°. The cooled solution was filtered and concentrated to dryness, and the residual acid was removed by co-distillation with water. A solution of the residue in 0.01M NH_4OH (5 mL) was placed on a column (10 × 2 cm) of Dowex AG1-X2 (AcO^-) resin, neutral material was eluted with water (200 mL), and acidic material with 2M acetic acid (200 mL). A solution of the acidic fraction in 0.01M NH_4OH (3 mL) was reduced with NaB^2H_4 (80 mg) for 5 h at 20° and then acidified by the addition of Dowex AG50W-X8 (H^+) resin, and boric acid was removed by co-distillation with methanol. A solution of the residue in water (5 mL) was filtered and freeze-dried (yield, 80 mg).

Preparation of oligosaccharides. — The mucilage (KM1, 600 mg) was treated with 2M trifluoroacetic acid (100 mL) for 2 h at 100°. The cooled hydrolysate was filtered, concentrated (~20 mL), dialysed exhaustively against distilled water, and freeze-dried (yield, 65 mg). A portion of the degraded material (60 mg) was reduced with NaB^2H_4, methylated, and carboxyl-reduced[17] with $LiAl^2H_4$. The partially methylated material was treated with aqueous 90% formic acid (6 mL, 70°, 50 min), and the hydrolysate was filtered and concentrated to dryness. A solution of the residue in aqueous 50% ethanol (5 mL) containing M NH_4OH was reduced with NaB^2H_4 and remethylated. The mixture of methylated oligosaccharide-alditols was isolated from Sep-Pak C_{18} cartridges and fractionated by reverse-phase h.p.l.c.[2].

^1H-N.m.r. spectroscopy. — A Bruker CXP-300 spectrometer was used. The aldobiouronic acid alditol (20 mg) was freeze-dried from D_2O (4 × 2 mL), and spectra were obtained on solutions in D_2O containing 0.1% of acetonitrile at 300 MHz under non-saturating conditions. Data were acquired in 32k data points with zero-filling to 64k data points prior to Fourier transformation. Chemical shifts (δ) are reported in p.p.m. downfield from internal acetonitrile (δ 2.09 relative to Me_4Si).

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