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STRUCTURAL INVESTIGATION OF OXALATE-SOLUBLE RAPESEED (*Brassica campestris*) POLYSACCHARIDES

PART IV. PECTIC POLYSACCHARIDES*

IQBAL R. SIDDIQUI AND PETER J. WOOD
Food Research Institute, Agriculture Canada, Ottawa K1A 0C6 (Canada)
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ABSTRACT

A pectin isolated from rapeseed cotyledon meal yielded two, closely related, pectic polysaccharides which were homogeneous on the ultracentrifuge. The major pectinic-acid fraction (*H*) contained L-arabinose, D-xylose, D-galactose, and L-rhamnose residues in molar proportions of 5.3:3.2:1.0:0.57, and 32.9% of galacturonic acid residues having a degree of esterification of 36%. The polysaccharide was subjected to methylation analysis and its general structural features are discussed.

INTRODUCTION

In previous papers[1–3], the isolation and characterization of an amyloid, an acidic arabinogalactan, and an arabinan from rapeseed cotyledon meal were described. Further work on the oxalate-soluble fraction of the meal has led to the fractionation of the pectic polysaccharides, and we now report on their main structural features. The structural features of the pectic polysaccharide from rapeseed hull have recently been reported by Aspinall[4].

RESULTS AND DISCUSSION

On sedimentation analysis[5], the acidic polysaccharide material [62.3%, isolated by fractionation on DEAE-Cellulose (PO_4^{3-})[3]] showed a minor and a major peak, the components of which were separated by prolonged sedimentation (ratio, 1:3.58). Further fractionation on Sephadex G-200 confirmed this finding and showed a ratio of 1:3.57. Multiple separation on Sephadex G-200 provided the two pectic-polysaccharide fractions (*A* and *H*), each of which, on hydrolysis, gave arabinose, xylose, galactose, glucose, rhamnose, fucose (trace), galacturonic acid, and an unidentified component (trace). The minor fraction (*A*) had general features which were similar

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to those of fraction *H*, but showed an extremely poor correspondence between the ratios of the sugar components determined on the unmethylated polysaccharide and those calculated after methylation and hydrolysis (See Experimental); hence, the results must be regarded as qualitative.

Since glucose is not a known constituent of pectic substances, and fucose and the unidentified component were present in amounts too small to allow unambiguous identification, the significance of these components is doubtful. However, the unidentified component does not appear to be an *O*-methyl sugar, as reported to be present in the acidic polysaccharide complex from soybean.

Fraction *H* had $[\alpha]_D^{24}$ −69° and showed a single, symmetrical peak on sedimentation analysis[5]. Analysis revealed the major constituents to be arabinose, xylose, galactose, and rhamnose in the molar ratios 5.3:3.2:1.0:0.57, and the uronic acid content to be 33%. The methyl ester content[8] (2.41%) of *H* indicated a degree of esterification of the uronate residues of 36%.

Hydrolysis of *H*, with fractionation of the resulting sugars on an anion-exchange resin and subsequently on paper chromatograms, gave crystalline D-galactose, D-xylose, and L-arabinose. That the rhamnose was the L isomer was inferred from its positive optical rotation. The major acidic component was tentatively identified as the commonly occurring (1→2)-linked (galactosyluronic acid)-rhamnose.

The polysaccharide was methylated[9,10], and the fully methylated product was subjected to methanolysis and hydrolysis. After fractionation of the hydrolysis products on an anion-exchange resin, the neutral sugars were resolved into ten fractions (p.c.). The identities of the sugars in each fraction were determined by g.l.c.–m.s.[11,12] of the derived alditol acetates. The molar proportions of the neutral sugars (determined by g.l.c.) were as follows: 2,3,5-tri-*O*-methyl-L-arabinose, 21; 2,3,4-tri-*O*-methyl-D-xylose, 20; 3,4-di-*O*-methyl-L-rhamnose, 5; 2,3-di-*O*-methyl-L-arabinose, 10; 2,3,4,6-tetra-*O*-methyl-D-galactose, 4; 2-*O*-methyl-L-arabinose, 14; 3-*O*-methyl-L-arabinose, 3; 2,3,6-tri-*O*-methyl-D-galactose, 3; L-arabinose, 3; and 2,4-di-*O*-methyl-D-galactose, 1.

The molar ratios (6.4:2.5:1.0:0.62) for arabinose, xylose, galactose, and rhamnose, calculated from the proportion of methylated sugars, were in fair agreement with that found for the original polysaccharide *H*.

The methylation-analysis data show that *H* has a highly branched structure. From the above molar proportions, it will be seen that, on average, for every 84 neutral sugar residues, there are 21 terminal, non-reducing end-groups of L-arabinose, 20 terminal, non-reducing end-groups of D-xylose, and 4 terminal, non-reducing end-groups of D-galactose. There are 20 residues of L-arabinose involved in branching, including 14 through positions 3 and 5, 3 through positions 2 and 5, and 3 through positions 2, 3. and 5. There is also one D-galactose residue involved in branching through positions 3 and 6. The remaining 18 non-terminal residues consist of five (1→2)-linked L-rhamnose units, ten (1→5)-linked L-arabinose units, and three (1→4)-linked D-galactose units.

The polysaccharide contains 33% of uronic acid, corresponding to 28 uronic

acid residues in an average unit of 112 residues. In order to achieve a balance between the end-groups and branch-points, 21 (*i.e.*, 75%) of the galacturonic acid residues ought to be branched. Repeated attempts to reduce fraction *H* by the procedure of Taylor and Conrad[13] gave a partially reduced product containing ~40% of the uronic acid residues still intact. Depolymerization of the methylated[9], carboxyl-reduced polysaccharide and g.l.c. of the resulting sugars showed that 2,3,6-tri- and 2,6-di-*O*-methyl-D-galactose, derived from 2,3-di-*O*-methyl-D-galacturonic and 2-*O*-methyl-D-galacturonic acids, were present in a ratio of 1.4:1. The results showed that the major branch points were through O-3 of D-galacturonic acid and, in order to provide a reasonable balance between end-groups and branch-points, it was assumed that all the remaining, unreduced, uronic acid residues were also branched at position 3.

The foregoing results for fraction *H* are consistent with a (1→4)-linked galacturonan chain, containing 2-linked rhamnose residues, commonly found in pectic polysaccharides. About 75% of the galacturonic acid residues and a negligible amount of the rhamnose units carry side chains made up mainly of arabinose residues and similar in structure to those of the arabinan previously characterized[3]. Rapeseed, like the mustard pectin[15], does not contain chains of (1→4)-linked β-D-galactopyranosyl units as have been reported in soybean[14] and a commercial citrus pectin[14]. Most of the xylose is attached as single units to the galacturonan chain, as in tragacanthic acid and pollen galacturonan[16].

A major fraction of the soluble polysaccharides from rapeseed cotyledon meal is composed of polysaccharides of the pectic type characterized by the presence of galacturonic acid, arabinose, galactose, rhamnose, and xylose[6,14–22]. The rapeseed-meal pectin polysaccharides have structural features in common with the pectinic acids from soybean and mustard cotyledon meal, and constitute a group which is characterized by the presence of an unusually high proportion of neutral sugars. The results are also generally consistent with the structure proposed for the sycamore-cell pectic polymer[23], which is suggested to form, within the primary cell-wall, a covalent connection between xyloglucans (amyloid) and the structural protein of the cell-wall. The small proportion of glucose found by Rees *et al.*[15] in the course of methylation analysis of mustard-seed pectic polymer, and recovered as 2,3,6-tri- and 2,3-di-*O*-methylglucose, has been interpreted as evidence in support of a covalent linkage between the amyloid and the pectic materials[24,25]. In the investigation reported here, this possibility seems unlikely, as the glucose was converted almost exclusively into the 2,3,4,6-tetramethyl ether after methylation of *H*.

EXPERIMENTAL

The general experimental methods have been reported previously[1].

Fractionation of the crude, acidic polysaccharide complex. — (*a*) The middle fraction[3] (3.76 g), eluted from DEAE-Cellulose (PO_4^{3-}) with 0.25–0.5M phosphate buffer, showed two peaks on sedimentation analysis (Fig. 1) of a 1% solution in

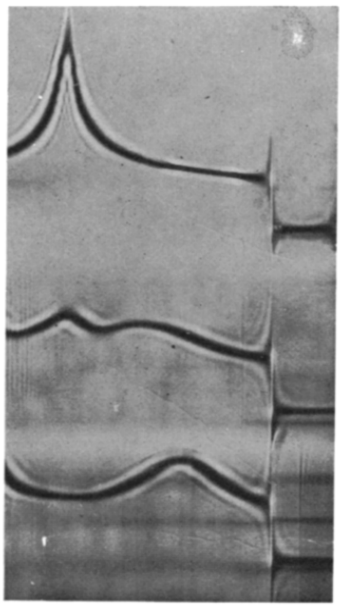

Fig. 1. Sedimentation analysis in acetate buffer (pH 5.03).

acetate buffer (pH 5.03) at 44,770 rev./min. The areas under the faster- and slower-sedimenting peaks showed a ratio of 1:3.58. A portion (376 mg) of the acidic polysaccharide complex was fractionated on a column (1 × 25 in.) of Sephadex G-200. Elution with water (5-ml fractions) gave the profile shown in Fig. 2. The areas under the peaks corresponding to the faster-(*A*) and slower-moving (*H*) components showed a ratio of 1:3.57. The minor and major fractions were recovered by freeze-drying (59 and 233 mg, respectively; ratio, 1:3.9).

P.c. and electrophoresis (borate and acetate buffers; pH 10 and 5, respectively) of the hydrolysates of the minor (*A*) and major (*H*) fractions showed mainly galactose, arabinose, xylose, and galacturonic acid, together with minor amounts of glucose and rhamnose and traces of fucose and an unidentified component [R_{Rha} 1.36 (solvent *A*), 1.26 (1-butanol–acetic acid–water, 4:1:5)].

(*b*) A solution of the complex (3 g) in water (100 ml) was eluted from a column (2 × 35 in.) of Sephadex G-200 with water (15-ml fractions, spot-tested with anthrone–H_2SO_4) to give: *A* (tubes 48–80; content, 875 mg), which showed a single, sharp, symmetrical peak on sedimentation analysis (Fig. 1); *B* (81–115), which showed two peaks; and *C* (116–150; content, 580 mg), which showed a major, symmetrical peak with a minor peak.

Fraction *B* was concentrated (to 100 ml) and refractionated as described above to give *D* (31–60; content, 360 mg) and *E* (64–85; content, 490 mg), each of which showed two peaks on sedimentation analysis and was discarded; and *F* (86–120; content, 340 mg), which showed[5] a single, symmetrical peak.

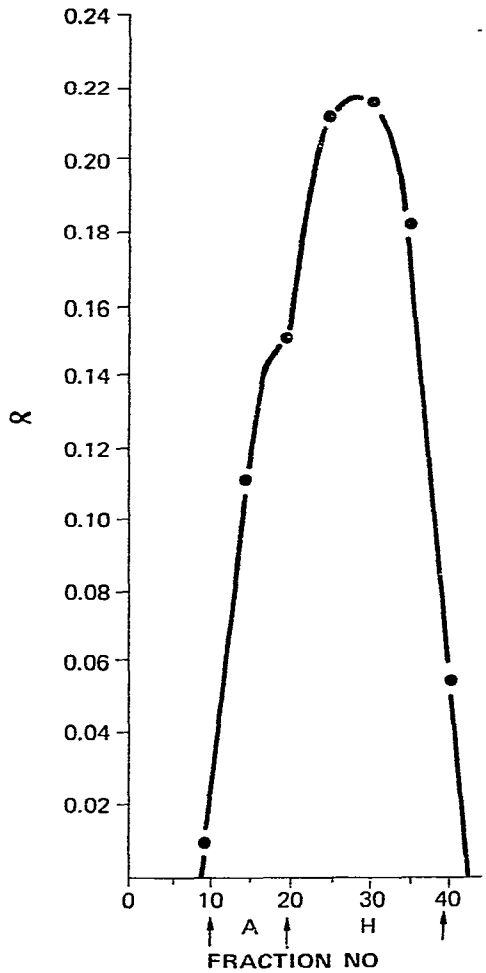

Fig. 2. Fractionation profile on Sephadex G-200.

Fractions *C* and *F* were combined and a solution of a portion (900 mg) in water (30 ml) was refractionated as described above (10-ml fractions) to give *G* (tubes 53–85; content, 209 mg), which showed two peaks on sedimentation analysis and was discarded; and *H* (86–150; content, 660 mg), which showed a single, symmetrical peak (Fig. 1).

Analysis of pectic polysaccharides H *and* A. — Fraction *H* had $[\alpha]_D^{24}$ +69° (*c* 0.54, water) (Found: N, 0.39; ash, 5.11; uronic anhydride[7], 32.9; OMe[8], 2.41%). Sedimentation analysis[5] of a 1% solution in acetate buffer (pH 5.03) at 44,770 rev./min showed a single, symmetrical peak. A portion (5 mg) of the mixture of neutral sugars, prepared by heating the polysaccharide with M sulphuric acid for 3 h at 100°, was reduced with sodium borohydride, and the resulting mixture of alditols was acetylated, and examined on a Pye 104 Gas Chromatograph using dual columns

(5 ft) of 3% of OV-225 on Chromosorb W-HP (80–100 mesh). with temperature programming at 2°/min from 180→250°, and a nitrogen flow-rate of 45 ml/min. Peak areas, evaluated by a CSI digital integrator (model 208), gave molar ratios of arabinose, xylose, galactose, rhamnose (containing traces of fucose), and glucose of 5.3:3.2:1.0:0.57:0.24.

Fraction *A* had $[\alpha]_D^{24}$ +58° (*c* 0.2, water) (Found: N, 0.40; ash, 4.06; uronic anhydride, 33.5; OMe, 1.48%) and gave a single, symmetrical peak on sedimentation analysis. The molar ratios for the above-mentioned, neutral, sugar components were 4.6:2.4:1.0:0.56:0.34.

Acid hydrolysis of fractions H *and* A. — Fraction *H* (195 mg) was hydrolysed with M sulphuric acid (10 ml) for 3 h at 100°. After neutralization ($BaCO_3$), filtration, deionization with Rexyn-101 (H^+) resin, and concentration, the mixture of acidic and neutral sugars was fractionated on a column of Dowex 1 X2 (CO_3^{2-}) resin. Washing with water (150 ml) removed the neutral sugars (97 mg), and elution with 0.25M ammonium carbonate (150 ml) yielded the uronic acid material. The uronic acid fraction (32 mg) was recovered by removing the bulk of ammonium carbonate *in vacuo* at 65–70° and the remainder with Rexyn 101 (H^+) resin.

Fraction *A* (200 mg) was similarly hydrolysed, and the product mixture fractionated to yield neutral sugars (99 mg) and acidic sugars (30 mg).

(*a*) *Neutral sugars.* The mixture of neutral sugars (from fraction *H*, 97 mg) was separated on eight sheets (9 × 22 in.) of Whatman No. 1 paper by using 1-butanol–acetic acid–water (4:1:5). Some of the fractions thus isolated were rechromatographed in solvent *B*. The approximate yields (mg) of the seven fractions isolated were as follows: **1**, 13; **2**, 3; **3**, 40; **4**, 27; **5**, traces; **6**, 8; and **7**, traces. Fractions **1**, **3**, and **4**, on crystallization and recrystallization from 90–95% ethanol in the cold, gave, respectively, D-galactose, m.p. and mixture m.p. 164–166°, $[\alpha]_D^{26}$ +114 (5 min) →75° (equil.) (*c* 0.28, water); L-arabinose, m.p. and mixture m.p. 152–153°, $[\alpha]_D^{26}$ +137 (6 min) →105° (equil.) (*c* 1.16, water); and D-xylose, m.p. and mixture m.p. 145–146°, $[\alpha]_D^{26}$ +60.5→18.5° (equil.) (*c* 0.625, water). Fractions **2** (glucose) and **5** (fucose) were present in amounts too small to permit further identification. Fraction **6**, which was identical with rhamnose in p.c. and electrophoresis, showed $[\alpha]_D^{24}$ +4.6 ±1° (*c* 0.4, water) (*cf.* $[\alpha]_D^{20}$ +8° (*c* 4, water) for authentic L-rhamnose). Fraction **7** (unidentified component) showed R_{Rha} 1.13 (1-butanol–pyridine–water, 4:6:3), 1.32 (solvent *A*), 1.25 (1-butanol–acetic acid–water, 4:1:5), and 0.15 (elongated spot, solvent (*c*), and M_G 0.37 in borate buffer (pH 10). Fraction **7** did not respond to demethylation.

The mixture of neutral sugars (from fraction *A*, 99 mg) was similarly fractionated to yield **1**, 15 mg; **2**, 3 mg; **3**, 33 mg; **4**, 23 mg; **5**, traces; **6**, 8 mg; and **7**, traces. Fractions **1**, **3**, and **4** were characterized, as described above, as D-galactose, L-arabinose, and D-xylose, respectively. Fractions **2** and **5** were present in amounts too small to permit further identification. Fraction **6** was identified as L-rhamnose (p.c. and electrophoresis) and had $[\alpha]_D^{24}$ +4° (*c* 0.45, water). Fraction **7** was identical to the corresponding fraction described above.

(*b*) *Uronic acid fraction.* The acidic sugars (30 mg) from fraction *H* were separated on 4 sheets (9 × 22 in.) of Whatman No. 1 paper by using 1-butanol–acetic acid–water (4:1:5). Three components (**1**, 16 mg; **2**, 5 mg; and **3**, 4 mg) were recovered with R_{GalA} values of 1.0, 0.74, and 0.40, respectively. Paper electrophoresis (borate buffer pH 10) showed **1** to contain two components (M_G 0.14 and 0.69), **2** to contain three components (M_G 1.15, 1.05, and 0.70), and **3** to contain two components (M_G 1.15 and 1.07). Hydrolysis of the major acidic component (2 mg) with M sulphuric acid for 3 h at 100° gave (p.c. and electrophoresis) unhydrolysed compound, galacturonic acid, and rhamnose. A portion (10 mg) was treated with boiling, 2% methanolic hydrogen chloride for 10 h and the resulting methyl ester methyl glycoside was reduced with lithium aluminum hydride[26] in tetrahydrofuran. The product was hydrolysed with M sulphuric acid for 3 h. P.c. (solvent *A*) then revealed mainly rhamnose together with small amounts of galactose.

The acidic sugars (32 mg) from fraction *A* similarly yielded the components **1**, 16 mg; **2**, 3 mg; and **3**, 5 mg; which were identical with the corresponding components from fraction *H*.

Methylation analysis of fractions H *and* A. — Fraction *H* (195 mg) was dissolved in dry methyl sulphoxide (15 ml) by stirring for 2 h at 60° and 24 h at room temperature. The solution was flushed with nitrogen, and a 2M solution (2 ml) of methylsulphinyl carbanion[9] was added dropwise. The resulting, viscous solution was stirred for 8 h at room temperature. Methyl iodide (2 ml) was added dropwise with external cooling (ice–water), and the mixture was stirred at room temperature overnight, then poured into water (100 ml), dialysed for 24 h against running tap-water, concentrated, and extracted continuously with chloroform. The extract was dried (Na_2SO_4) and concentrated. The solid residue (182 mg) showed a weak i.r. absorption for hydroxyl. A portion (168 mg) was dissolved in methyl iodide (20 ml), and silver oxide (1 g) was added in 4 equal portions during 8 h. After two more such methylations, a product (148 mg) was recovered that had $[\alpha]_D^{25}$ +14° (*c* 1.1, chloroform) and showed no i.r. absorption for hydroxyl.

Fraction *A* (200 mg), when similarly methylated, yielded a product (160 mg), $[\alpha]_D^{25}$ 0 ±0.5° (*c* 0.88, chloroform).

A solution of methylated *H* (128 mg) in 4% methanolic hydrogen chloride (25 ml) was boiled for 18 h, then neutralised (Ag_2CO_3), and concentrated. The syrupy product was hydrolysed with 0.5M sulphuric acid (5 ml) for 32 h. The hydrolysate was neutralized ($BaCO_3$) and concentrated. To a solution of the syrupy residue (133 mg) in water (10 ml), Rexyn 101 (H^+) resin was added. The mixture was filtered and the filtrate was stirred with Dowex 1 X2 (CO_3^{2-}) resin (25 ml) for 24 h. The slurry was added to a column containing more (25 ml) Dowex 1 X2 (CO_3^{2-}) resin. Elution with water (150 ml) removed the neutral sugars (67 mg). The acidic sugars (31 mg), removed by elution with 0.5M ammonium carbonate, were recovered by removing the bulk of the ammonium carbonate *in vacuo* at 65° and the remainder by passing the solution through a column of Rexyn 101 (H^+) resin.

Fraction *A* (151 mg), when similarly hydrolysed and fractionated, yielded neutral sugars (70 mg) and acidic sugars (30 mg).

(*a*) *Neutral sugars.* Portions (30 mg) of the neutral, methylated sugars from *H* and *A*, when separately fractionated on sheets of Whatman No. 1 paper using solvent *B*, gave the same 10 fractions. Portions (1–2 mg) of fractions 1–10 were demethylated with boron trichloride[27], and the products were examined by p.c. Portions (2–3 mg) of fractions 1–10 were reduced with sodium borohydride[28], and the products were acetylated and examined by g.l.c. as described above (temperature programming from 180–200° at 1°/min). G.l.c.–m.s. was carried out on a combined Finnigan 3100D GC/MS, equipped with a U-shaped column (5 ft × 0.25 in.) of 3% of OV-225 on Chromosorb W-HP (80–100 mesh) with temperature programming from 110–250° at 10°/min. The separator temperature was 250°, analyser temperature 100°, and ionising electron energy 70 eV. The spectra were recorded as bar graphs by means of the Finnigan 6000 MS Data System. The results are summarized in Table I.

TABLE I

PAPER-CHROMATOGRAPHIC, DEMETHYLATION, AND G.L.C.–M.S. RESULTS FROM NEUTRAL, METHYLATED FRACTIONS FROM *H* OR *A*

Fraction	R_F (*Solvent* B)	*Parent sugar*[a]	*Reduced product*[b]
1	0.82	Arabinose, xylose (trace)	2,3,5-Tri-*O*-methylarabinitol, 2,3,4-tri-*O*-methylxylitol
2	0.79	Xylose, arabinose (trace)	2,3,4-Tri-*O*-methylxylitol, 2,3,5-tri-*O*-methylarabinitol
3	0.64	Galactose and rhamnose	3,4-Di-*O*-methylrhamnitol, 2,3,4,6-tetra-*O*-methylgalactitol
4	0.52	Arabinose, small amounts of galactose and xylose	3,4-Di-*O*-methylxylitol, 2,3-di-*O*-methylarabinitol, 2,3,4,6-tetra-*O*-methylgalactitol
5	0.45	Arabinose	2,3-Di-*O*-methylarabinitol
6	0.39	Arabinose, small amounts of galactose	2,3-Di-*O*-methylarabinitol, 2,3,6-tri-*O*-methylgalactitol
7	0.28	Arabinose (trace), rhamnose (trace)	2,3-Di-*O*-methylarabinitol, 3-*O*-methylrhamnitol
8	0.19	Arabinose, galactose (trace)	2-*O*-Methylarabinitol, 2,4-di-*O*-methylgalactitol
9	0.14	Arabinose, small amounts of galactose	2-*O*-Methylarabinitol, 3-*O*-methylarabinitol, 2,4-di-*O*-methylgalactitol
10	0.048	Arabinose	Arabinitol

[a]Identified by demethylation. [b]Identified by g.l.c.–m.s. of the acetate.

A second portion (5 mg) was similarly reduced, acetylated, and examined by g.l.c. The proportions (to the nearest whole number) of the methylated alditol

acetates with respect to 2,4-di-*O*-methylgalactitol tetra-acetate, and their retention times with respect to that of 2,3,4,6-tetra-*O*-methylglucitol diacetate are summarized in Table II.

TABLE II

ANALYTICAL DATA FOR NEUTRAL, METHYLATED FRACTIONS FROM *H* AND *A*

Acetate of	*Retention time (min)*	*Molar ratio Fraction* H	*Molar ratio Fraction* A
2,3,5-Tri-*O*-methylarabinitol	0.58	21	44
2,3,4-Tri-*O*-methylxylitol	0.69	20	29
3,4-Di-*O*-methylrhamnitol	0.90	5	10
2,3-Di-*O*-methylarabinitol[a]	1.00	10	24
2,3,4,6-Tetra-*O*-methylgalactitol	1.08	4	5
2-*O*-Methylarabinitol[b]	1.39	14	28
3-*O*-Methylarabinitol	1.47	3	8
2,3,6-Tri-*O*-methylgalactitol	1.51	3	5
Arabinitol	1.74	3	6
2,4-Di-*O*-methylgalactitol	2.43	1	1

[a]Contains trace amounts of 3,4-di-*O*-methylxylitol. [b]Contains trace amounts of 3-*O*-methylrhamnitol.

A third portion (20 mg, from *H*) was fractionated on Whatman No. 3MM paper in borate buffer (pH 10) to give 3 major components M_G 0.31 (2-*O*-methylarabinose), 0.68 (3-*O*-methylarabinose), and 1.0 (arabinose). The component having M_G 0.31 was recovered after removal of sodium ions with Rexyn 101 (H^+) resin, and boric acid as methyl borate. P.c. (solvent *B*) showed mainly 2-*O*-methylarabinose (R_F 0.14). Small amounts of a second component (R_F 0.63, 3,4-di-*O*-methylrhamnose) and traces of two other components [R_F 0.22 (3-*O*-methylrhamnose) and 0.50 (3,4-di-*O*-methylxylose] were also detected.

(*b*) *Acidic sugars.* The methylated uronic acid fraction (20 mg) from *H* was fractionated on 3 sheets (7 × 22 in.) of Whatman No. 1 paper using 1-butanol–acetic acid–water (4:1:5) to give components 1–3 (R_F 0.59, 0.42, and 0.24), which were separately hydrolyzed with M sulphuric acid for 5 h. P.c. (solvent *B*) of each product showed large amounts of acidic material (R_F 0.00) and traces of neutral components (R_F 0.69 and 0.26).

The methylated uronic acid fraction from *A* gave exactly the same results when examined as described above.

Carboxyl-reduced fraction H *and its methylation analysis.* — A solution of Fraction *H* (100 mg) in water (20 ml) containing 1-cyclohexyl-3-[2-(4-methylmorpholino)ethyl]carbodiimide *p*-toluenesulphonate (1 g) was maintained at pH 4.75 for 2 h by the addition of 0.1M hydrochloric acid using a pH stat (Type SBR2/SBU1/TTA3 Titration Equimpent, Copenhagen, Denmark). 2M Sodium borohydride (15 ml) was added to the stirred reaction mixture maintained for 3–4 h at pH 7 by the automatic addition of 4M hydrochloric acid, and, when necessary, 1-octanol was

added to prevent foaming. The mixture was then dialyzed against running tap-water for 16 h and against distilled water for 4 h, and then concentrated to 20 ml. Two more such treatments yielded a carboxyl-reduced product (60 mg), a portion of which was hydrolyzed, and the derived alditol acetates were examined by g.l.c., giving molar ratios for rhamnose, arabinose, xylose, and galactose of 0.17:1.00:0.50:0.45. Three further reductions yielded a product (30 mg) having molar ratios for rhamnose, arabinose, xylose, and galactose of 0.23:1.00:0.38:0.56. When a sample of fraction *H* was subjected to hydrolysis, reduction, acetylation, and g.l.c., molar ratios for rhamnose, arabinose, xylose, and galactose of 0.16:1.00:0.56:0.17 were obtained. The difference in the amount of galactose between the original sample (~33% uronic acid) and the final reduced sample showed that only 62% of the galacturonic acid residues were reduced.

Partially carboxyl-reduced fraction *H* (10 mg) was methylated in dry methyl sulphoxide as described previously and the product was subjected to methanolysis, hydrolysis, reduction, acetylation, and g.l.c., as described above. After correction for 2,3,6-tri-*O*-methylgalactitol triacetate of non-uronic acid origin, the 2,3,6-tri-*O*-methylgalactitol triacetate and 2,6-di-*O*-methylgalactitol tetra-acetate were present in the ratio ~1.4:1.0.

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