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STRUCTURAL DATA FOR THE CARBOHYDRATE OF ISPAGHULA HUSK *ex Plantago ovata* Forsk

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ABSTRACT

The husk from the seeds of *Plantago ovata* Forsk yielded two fractions when exposed to mild aikali, namely, the mucilage polysaccharide (85%, apparently a single species) and the non-polysaccharide component (15%). Methylation analysis and partial hydrolysis with acid showed the mucilage polysaccharide to be a highly branched, acidic arabinoxylan, the xylan backbone having both (1→4) and (1→3) linkages. The majority of the residues in the xylan backbone are variously substituted at O-2 and O-3 with arabinose, xylose, and an aldobiouronic acid identified as 2-*O*-(galactopyranosyluronic acid)-rhamnose. A structure incorporating these features for the husk polysaccharide is proposed.

INTRODUCTION

Ispaghula husk (the husk of the seeds of *Plantago ovata* Forsk) is widely used as a prophylactic in the treatment of large-bowel disorders. The husk has a very high content of mucilage polysaccharide that gels over a wide range of concentrations.

Percival and Laidlaw[1] presented a preliminary analysis of the mucilage in 1949, and reported[2] some structural detail in 1950. We now report on a detailed analysis of both the gross composition and the main structural features of the mucilage.

EXPERIMENTAL

General methods and instrumentation. — G.l.c. was performed at 160° on a Hewlett–Packard F & M Scientific chromatograph, with argon as the carrier gas at a flow rate of 40 ml/min. Columns (8 m × 2 mm i.d.) were packed with (*A*) 3% of ECNSS-M on Gas Chrom Q (100–200 mesh) (Field Instruments), and (*B*) 3% of OV-225 (Pye Unicam). Retention times (*T*) are given relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. G.l.c.–m.s. (70 eV) was performed with a Perkin–Elmer F11 chromatograph attached to an Edwards 606 mass spectrometer. High-

performance liquid chromatography (h.p.l.c.) was performed on a Waters Associates liquid chromatograph with a column (30 cm × 4 mm i.d.) of μ-Bondapak/carbohydrate eluted with H_2O/CH_3CN (35:65) at a flow-rate of 0.5 ml/min. Hydrolyses were performed with 2M trifluoroacetic acid (TFA) at 121° for 1 h, unless otherwise stated. Methylation was performed by the Hakomori[3] method, as described by Sandford and Conrad[4]. The degree of methylation of the mucilage was determined by i.r. spectroscopy and by the use[4] of CD_3I. The methylated polysaccharides were hydrolysed[5] with 2M TFA at 121° for 1 h, and the products were converted into alditol acetates[6] and identified by g.l.c. (column *A*) (sugar identification). Diborane reduction was effected by literature methods[5,7]. Prior to diborane reduction, methylated samples were de-esterified by treatment with 0.01M sodium hydroxide for 3 h at 2°, added to 0.1M acetic acid, dialysed against distilled water at 4°, and lyophilised.

Investigation of Ispaghula husk. — (*a*) *Compositional analysis.* A sample (10 mg) of husk (powdered form, kindly supplied by Reckitt and Colman) was hydrolysed (1 ml of TFA), and the residue was collected by centrifugation (1300 *g* for 15 min) and washed with water (1 ml). The washings and the supernatant solution were combined, evaporated to dryness with filtered air, and subjected to sugar identification.

Husk (20 mg) was hydrolysed with conc. sulphuric acid (20 ml) for 30 min at 100°, and the hydrolysate (1 ml) was analysed by the carbazole procedure[8].

(*b*) *Enzymic hydrolysis.* Husk (20 mg) was added to 50 ml of 10mM sodium acetate (pH 4) containing 0.1% of pectinase (polygalacturonase; EC 3.2.1.15; Sigma, lyophilised). After hydrolysis for 9 h at 25°, the mixture was centrifuged (1300 *g* for 20 min), and the residue was washed twice with water (5 ml). The supernatant solution and the washings were combined, concentrated to 5 ml under reduced pressure at 30°, and analysed for hexuronic acid content by the carbazole procedure[8] and by g.l.c. (column *A*) by the method outlined by Jones and Albersheim[9].

(*c*) *Alkaline fractionation.* Husk (5 g) was treated under nitrogen with 1.2M NaOH (1 litre). The insoluble fraction (0.70 g) was removed by centrifugation (1300 *g* for 20 min), washed twice with water (20 ml), and dried over P_2O_5 at 60° under reduced pressure (Found: N, $<0.3\%$). The washings and the supernatant solution (the polysaccharide content of which constitutes the mucilage) were combined, and neutralised with acetic acid. Attempts to fractionate the mucilage in this solution (at concentrations of 0.01–0.25%) by addition of ethanol (to 90%) or aqueous 7% cupric acetate (to 0.5%) were unsuccessful, as all of the mucilage was precipitated at concentrations of 75 and 0.41%, respectively.

The insoluble residue (50 mg) was hydrolysed (1 ml of TFA); after centrifugation (1300 *g* for 15 min), the residue (42 mg) was washed twice with water (3 ml). The washings and the supernatant solution were combined and evaporated to dryness, and the sugars analysed as alditol acetates by g.l.c. (column *A*). The residue was dried over P_2O_5 for 24 h at 60°, and a portion (10 mg) hydrolysed with 72% H_2SO_4 (1 ml) at 4° for 16 h, by which time dissolution had occurred. The solution was diluted to 5 ml with water and neutralised with $Ba(OH)_2$; hexose was then determined by an anthrone procedure[10], to give an estimate of the content of cellulose[11].

The solution was evaporated to dryness at 30° under reduced pressure, and the residue was subjected to sugar identification.

A solution of the alkali-soluble fraction (10 mg) in 5% aqueous NaOH (100 μl) was applied to columns (100 × 2.5 cm) of Sephadex G75 and G50 which had been equilibrated with 5% aqueous sodium hydroxide (alkali necessary to maintain solubility). Each column was eluted with 5% aqueous sodium hydroxide, and the eluate was monitored with a continuous-flow, sugar-analysis system involving reaction with orcinol in hydrochloric acid[12].

(*d*) *Methylation analysis.* Husk (1 g) was methylated by the Hakomori method. The methylated product (0.8 g) was extracted with chloroform, and the residue (120 mg; chloroform-insoluble fraction, corresponding to the residue from the alkali treatment) was removed by centrifugation at 1300 *g* for 15 min; this fraction remained insoluble in chloroform after remethylation. The supernatant solution (chloroform-soluble fraction, corresponding to husk mucilage) was evaporated to dryness under reduced pressure at 30°; i.r. spectroscopy of the residue showed no hydroxyl absorption. The CD_3I procedure[4] and the Zeisel[13] method showed the degree of methylation to be 96.0 and 95.5%, respectively (based on methoxyl contents of 36.8 and 36.5%, respectively; calc., 38.2%) see Table I.

The chloroform-soluble fraction (50 mg) was reduced with diborane, and the product (45 mg) and the chloroform-insoluble fraction (50 mg) were hydrolysed (1 ml of TFA). The hydrolysates were subjected to sugar identification, including g.l.c. (columns *A* and *B*)–m.s.

(*e*) *Partial hydrolysis.* Samples (0.5 g) of husk powder were heated at 95° in 5mM H_2SO_4 (50 ml) for 3, 6, 9, and 12 h. After centrifugation of each hydrolysate at 1300 *g* for 15 min, the residue was washed with water (5 ml), the washings and the supernatant solution were combined and neutralised with $Ba(OH)_2$, and the $BaSO_4$ precipitate was removed by centrifugation at 1300 *g* for 45 min. The supernatant solution and the husk residue were combined, dialysed against water for 48 h, and re-separated. Sugar identification of the hydrolysate of the supernatant solution showed that the arabinose content was virtually zero after partial hydrolysis for 9 h, although there had been no significant cleavage of the xylopyranosyl linkages. Hydrolysis of the husk for more than 9 h resulted in a large increase in the xylose content of the dialysate. Hydrolysis of the residue from the 9-h partial hydrolysis revealed it to contain 40% of carbohydrate, consisting of xylose 97.8%, arabinose 2.2%, and a trace of galactose (the latter is present in the non-polysaccharide components).

Husk (2 g) was hydrolysed (300 ml of 5mM H_2SO_4 for 9 h at 95°), centrifuged (1300 g for 20 min), and the residue washed (water, 20 ml). The combined washings and supernatant were neutralised as before, concentrated to 100 ml, recombined with the residue (to ensure that all non-arabinose material was analysed), dialysed, and subjected to methylation analysis as in (*d*) after lyophilisation (see Table II).

Husk (4 g) was then hydrolysed on a larger scale (600 ml of 5mM H_2SO_4 for 9 h at 95°), and the residue (Fraction 1, 1.02 g) was collected by centrifugation

(1300 *g* for 20 min) and washed with water (20 ml). The washings and the supernatant solution were combined, neutralised as before, and concentrated to ~100 ml prior to dialysis. The solution outside the dialysis membrane was lyophilised, and a portion (10 mg) of the residue was hydrolysed (2 ml of TFA). The solution inside the membrane was evaporated under reduced pressure at 30° to dryness (Fraction 2).

Fraction 1 (100 mg) was dried over P_2O_5 under reduced pressure at 60° for 24 h and then methylated. The product (80 mg) was extracted with chloroform, leaving a residue (48 mg) that was removed by centrifugation. The chloroform-soluble material (32 mg), which showed no i.r. hydroxyl absorption, was hydrolysed (3 ml of TFA) and then subjected to sugar identification (columns *A* and *B*) (see Table V).

A solution of Fraction 2 (2.29 g) in water (40 ml) was adjusted to pH 4 with NaOH and applied to a column (100 × 2 cm) of Amberlite 401 (AcO^-) resin. The neutral fraction was eluted with water (150 ml), and then the acidic fraction was eluted with 20% aqueous acetic acid (100 ml). The neutral fraction was lyophilised, and the acidic fraction was evaporated to dryness under reduced pressure at 30°. Both products were dried over P_2O_5 at 60° under reduced pressure for 24 h.

Samples (15 μl of 10 mg/ml in water) of these products were analysed by h.p.l.c. on a μ-Bondapak column that had been calibrated by using malto-oligosaccharides having d.p. from 1 to 10. It is recognised that linkage type and monosaccharide structure affect[14,15] the precise position of elution of oligosaccharides having the same d.p. The acidic fraction (from Fraction 2) yielded two peaks, of approximately equal area, corresponding to the tetra- and penta-saccharide positions of the standard. The neutral fraction (from Fraction 2) yielded two major peaks corresponding to the hexa- and hepta-saccharides, and two minor peaks corresponding to the tetra- and penta-saccharides.

The acidic and neutral fractions were also methylated, and the product from

TABLE I

METHYL ETHERS PRODUCED BY HYDROLYSIS OF METHYLATED ISPAGHULA HUSK (CHLOROFORM-SOLUBLE FRACTION)

Components	T[a]	T[b]	*Molar response factor*[c]	*Corrected peak area*[d,e]	*Percent of total carbohydrate*[f]	*Molar ratio*[g]
2,3,5-Tri-*O*-methyl-L-arabinose	0.48	0.51	0.6C	9.2	20.8	1.63
2,3,4-Tri-*O*-methyl-D-xylose	0.66	0.64	0.61	6.6	15.1	1.18
2,3-Di-*O*-methyl-D-xylose	1.24	1.00	0.66	0.5	1.1	0.08
2,4-Di-*O*-methyl-D-xylose	1.20	1.10	0.66	5.6	12.7	1.00
2-*O*-Methyl-D-xylose	1.85	1.55	0.70	11.0	25.2	1.98
3-*O*-Methyl-D-xylose	1.75	1.55	0.70	1.8	4.0	0.31
D-Xylose	2.59	2.50	0.75	1.4	3.2	0.25
3,4-Di-*O*-methyl-L-rhamnose	0.88	0.87	0.75	3.9	8.8	0.69
2,3,4-Tri-*O*-methyl-D-galactose	1.95	—	0.75	4.0	9.1	0.71

[a]Column *A*. [b]Column *B*. [c]As determined by Albersheim *et al.*[16]. [d]Normalised peak area for this case. [e]Corrected peak area = peak area/molar response factor. [f]Based on corrected peak area. [g]With respect to 2,4-di-*O*-methyl-D-xylose.

TABLE II

METHYL ETHERS FROM THE HYDROLYSATE OF METHYLATED, PARTIALLY DEGRADED, ISPAGHULA HUSK

Components	*Corrected peak area*[e,h]	*Normalised peak area*[i]	*Percent of total carbohydrate*[f]	*Molar ratio*[g]
2,3,5-Tri-*O*-methyl-L-arabinose	0.30	0.09	0.3	0.03
2,3,4-Tri-*O*-methyl-D-xylose	30.66	9.35	26.7	3.34
2,3-Di-*O*-methyl-D-xylose	28.98	8.84	25.3	3.16
2,4-Di-*O*-methyl-D-xylose	9.18	2.80	8.0	1.00
2-*O*-Methyl-D-xylose	11.08	3.38	9.7	1.21
3-*O*-Methyl-D-xylose	6.49	1.90	5.4	0.68
D-Xylose	2.32	0.71	2.0	0.25
3,4-Di-*O*-methyl-L-rhamnose	13.21	4.03	11.5	1.44
2,3,4-Tri-*O*-methyl-D-galactose	12.69	3.87	11.1	1.38

[e–g]See Table I. [h]For *T* values (columns *A* and *B*) and values for molar response factor, see Table I. [i]Normalised peak area = corrected peak area × xylose correction factor.

TABLE III

METHYL ETHERS FROM THE HYDROLYSATE OF THE METHYLATED ACID FRACTION (FROM FRACTION 2) OF PARTIALLY DEGRADED, ISPAGHULA HUSK

Components	*Corrected peak area*[e,h]	*Normalised peak area*[i]	*Percent of total carbohydrate*[f]	*Molar ratio*[g]
2,3,5-Tri-*O*-methyl-L-arabinose	0.0	0.00	0.0	0.00
2,3,4-Tri-*O*-methyl-D-xylose	23.4	9.42	24.4	3.02
2,3-Di-*O*-methyl-D-xylose	19.1	7.69	19.9	2.47
2,4-Di-*O*-methyl-D-xylose	7.7	3.10	8.0	1.00
2-*O*-Methyl-D-xylose	9.3	3.75	9.7	1.20
3-*O*-Methyl-D-xylose	7.3	2.94	7.6	0.94
D-Xylose	0.0	0.00	0.0	0.00
3,4-Di-*O*-methyl-L-rhamnose	14.9	6.00	15.5	1.98
2,3,4-Tri-*O*-methyl-D-galactose	14.3	5.75	14.8	1.92

For footnotes, see Table II.

the acidic fraction was reduced with diborane as before, hydrolysed, and subjected to sugar identification (see Tables III and IV, respectively).

(*f*) *Isolation of an aldobiouronic acid.* Husk powder (1 g) was hydrolysed for 1 h at 121° in 0.1M TFA (100 ml). Ethanol (100 ml) was added and the resulting suspension was centrifuged at 1300 *g* for 20 min. The precipitate was washed with 75% aqueous ethanol (50 ml), and the supernatant solution and washings were combined, and evaporated to dryness under reduced pressure at 30°.

A solution of the residue in water (30 ml) was adjusted to pH 4 with NaOH,

TABLE IV

METHYL ETHERS FROM THE HYDROLYSATE OF THE METHYLATED NEUTRAL FRACTION (FROM FRACTION 2) OF PARTIALLY DEGRADED, ISPAGHULA HUSK

Components	*Corrected peak area*[e,h]	*Normalised peak area*[i]	*Percent of total carbohydrate*[f]	*Molar ratio*[g]
2,3,5-Tri-*O*-methyl-L-arabinose	0.0	0.00	0.0	0.00
2,3,4-Tri-*O*-methyl-D-xylose	30.3	10.70	39.8	4.03
2,3-Di-*O*-methyl-D-xylose	24.3	8.57	31.8	3.24
2,4-Di-*O*-methyl-D-xylose	7.5	2.64	9.8	1.00
2-*O*-Methyl-D-xylose	8.5	2.99	11.1	1.13
3-*O*-Methyl-D-xylose	0.0	0.00	0.0	0.00
D-Xylose	5.7	2.00	7.5	0.76
3,4-Di-*O*-methyl-L-rhamnose	0.0	0.00	0.0	0.00
2,3,4-Tri-*O*-methyl-D-galactose	0.0	0.00	0.0	0.00

For footnotes, see Table II.

TABLE V

METHYL ETHERS FROM THE HYDROLYSATE OF THE METHYLATED RESIDUE (FRACTION 1) OF PARTIALLY DEGRADED, ISPAGHULA HUSK

Components	*Corrected peak area*[e,h]	*Normalised peak area*[i]	*Percent of total carbohydrate*[f]	*Molar ratio*[g]
2,3,5-Tri-*O*-methyl-L-arabinose	0.21	0.03	0.1	0.18
2,3,4-Tri-*O*-methyl-D-xylose	47.2	6.80	25.6	4.74
2,3-Di-*O*-methyl-D-xylose	108.2	15.28	57.5	10.64
2,4-Di-*O*-methyl-D-xylose	10.3	1.48	5.4	1.00
2-*O*-Methyl-D-xylose	20.6	2.96	11.2	2.07
3-*O*-Methyl-D-xylose	0.0	0.00	0.0	0.00
D-Xylose	0.0	0.00	0.0	0.00
3,4-Di-*O*-methyl-L-rhamnose	0.0	0.00	0.0	0.00
2,3,4-Tri-*O*-methyl-D-galactose	0.0	0.00	0.0	0.00

For footnotes, see Table II.

applied to a column (100 × 2 cm) of Amberlite 401 (AcO^-) resin, and eluted with water (150 ml) to remove the neutral fraction. The acidic fraction was eluted with 20% aqueous acetic acid (100 ml), and the eluate was evaporated to dryness under reduced pressure at 30°. The residue (10 mg) was dissolved in water (1 ml), and an aliquot (15 μl) was subjected to h.p.l.c. Two minor peaks corresponding to the tetra- and penta-saccharides were detected, together with a major peak corresponding to disaccharide. The combined fractions containing the disaccharide peak were concentrated to near dryness, and then dried over P_2O_5 at 60° for 24 h under reduced pressure. The residue was subjected to methylation analysis.

Correlation of methylation analysis data. — The results given in Tables I–V correspond to different samples and to different fractions in a given sample. The results from the various methylations are not directly comparable from one Table to another, since (*a*) the samples were of different concentration, and (*b*) the quantity of sample injected on to the g.l.c. column was different. The differences are eliminated by applying a "xylose correction factor" calculated from (total peak area of xylose components in Table I)/(total peak area of xylose components in Table Y). Normalised peak-areas for Tables II–V are obtained from (corrected peak area) × (xylose correction factor; 0.305, 0.402, 0.352 and 0.144, for Tables II–V, respectively).

RESULTS AND DISCUSSION

Isphaghula husk is composed of two fractions. The water-soluble fraction (85%) forms a gel at all concentrations tested, and is also soluble in 5% aqueous sodium hydroxide. The major part of this work is concerned with this fraction, which is referred to as the mucilage.

The alkali-insoluble fraction (15%) of the husk was found to contain 0.3% of nitrogen (equivalent to 0.3% of protein in the husk, assuming a protein-nitrogen content of 16%). This fraction is insoluble in methyl sulphoxide and therefore could not be methylated. Sugar analysis by g.l.c. revealed only traces of arabinose and galactose, and no other carbohydrate; a specific analysis for cellulose gave negative results. This fraction clearly contains no polysaccharide material and was not further investigated.

The husk contained (g.l.c.) 54.1% of D-xylose, 17.3% of L-arabinose, and 5.4% of L-rhamnose; D-galactose and D-glucose were absent. The absolute configurations of these monosaccharides and the D configuration of the galacturonic acid residues were established earlier[1,2]. The carbazole procedure gave a value of 8.2% for uronic acid. When the husk was treated with polygalacturonase, no uronic acid-containing materials were released into the supernatant, suggesting the absence of pectic polymers.

The mucilage could not be fractionated by precipitation from neutral solution with ethanol or cupric acetate, and was eluted as a single peak from Sephadex G75 and G50 (96% recovery). The mucilage was totally included in G75 (fractionation range, 5/6–40 × 10^3), whereas it was partially included in G50, corresponding to a molecular weight of 5–6 × 10^3. Thus, the polysaccharide component of Ispaghula husk appears to be a single species (even after any occurrence of alkaline degradation).

Methylation of the husk by the Hakomori method gave chloroform-soluble and chloroform-insoluble materials, the latter being the non-polysaccharide component of the husk. The chloroform-soluble fraction was reduced with diborane to facilitate analysis of the uronic acid component, and hydrolysed. Diborane was used in preference to lithium aluminium hydride, which was less effective. The results of the methylation analysis are given in Table I.

The formation of 2-*O*-methyl-D-xylose and 3-*O*-methyl-D-xylose indicates branching through O-3 and O-2, respectively, and the presence of D-xylose in the

hydrolysate indicates that some D-xylose residues are branched through both O-2 and O-3. The formation of 2,3,5-tri-*O*-methyl-L-arabinose indicates terminal arabinofuranose residues in the mucilage, which must be present in/as branch-chains. Since the only L-rhamnose derivative detected in the hydrolysate was 3,4-di-*O*-methyl-L-rhamnose, the L-rhamnose residues must occupy solely internal positions. D-Galactose was absent from the hydrolysate of the mucilage, and therefore the 2,3,4-tri-*O*-methyl-D-galactose identified in the methylation analysis must have originated from terminal D-galacturonic acid residues. The equimolar amounts of 2,3,4-tri-*O*-methyl-D-galactose and 3,4-di-*O*-methyl-L-rhamnose suggest an association between the two parent monosaccharide residues in the polysaccharide structure.

An aldobiouronic acid was isolated after partial hydrolysis of the mucilage with acid. Methylation, followed by reduction and hydrolysis, gave methyl 3,4-di-*O*-methyl-L-rhamnoside and 2,3,4-tri-*O*-methyl-D-galactose. Therefore the aldobiouronic acid is 2-*O*-(D-galactopyranosyluronic acid)-L-rhamnopyranose. The formation of 3,4-di-*O*-methyl-L-rhamnose (Table I) indicates that the aldobiouronic acid is linked directly or indirectly to the polysaccharide backbone through O-1 of L-rhamnose. The data in Table I show that the xylan component of the mucilage polysaccharide is highly branched. Of the total carbohydrate in the mucilage, 61.3% is xylose, and 52.9% of these xylose residues are branch points, *i.e.*, 32.4% of all the carbohydrate residues are branch points. These branch-point xylose residues may be substituted by a residue of arabinose, aldobiouronic acid, or xylose. Controlled, acid hydrolysis of the husk, under conditions (5mM H_2SO_4, 95°, 9 h) chosen to hydrolyse L-arabinofuranosyl linkages with minimum degradation of the xylan backbone, released mainly arabinose together with a trace of xylose (<1% of total carbohydrate), but no dialysable hexuronic acid-containing material. Comparison of the methylation-analysis data for the non-dialysable fraction (Table II) and for the intact polysaccharide (Table I) reveals similar levels of 3,4-di-*O*-methyl-L-rhamnose and 2,3,4-tri-*O*-methyl-D-galactose, but that the arabinose content has fallen on partial hydrolysis from 9.2 to 0.09 (peak-area) units. There is also a change in the pattern of methylated xylose components (*cf.* Tables I and II), indicating that the arabinose residues are linked to xylose residues. The linkages are principally to the majority of the 1,3,4-substituted xylose residues at O-3, but also to some of the 1,2,3,4-substituted xylose residues at either or both O-2 and O-3 in particular D-xylose residues. The levels of D-xylose, 2-*O*-methyl-D-xylose, and 2,4-di-*O*-methyl-D-xylose fall in total by 11.1 units compared with a gain of 11.0 for 2,3-di-*O*-methyl-D-xylose plus 2,3,4-tri-*O*-methyl-D-xylose and a loss of 9.01 units for arabinose.

Methylation analysis of the acid fraction of partially hydrolysed husk (*i.e.*, from Fraction 2) showed (Table III) that the arabinose had been removed completely. Clearly, the majority of branches on the xylan must be aldobiouronic acid residues; since D-xylose is absent from this fraction, they are linked to O-3 and O-2 (shown by the presence of 2-*O*- and 3-*O*-methyl-D-xylose, respectively). Methylation analysis of the neutral fraction of partially hydrolysed husk (*i.e.*, from Fraction 2) showed (Table IV) the absence of arabinose or aldobiouronic acid, and that the branch

points are occupied by xylose. The formation of 2-*O*-methyl-D-xylose shows that xylose residues are linked to O-3, and the presence of D-xylose shows that xylose residues are also linked to the xylan backbone at both O-2 and O-3 of some residues. This finding indicates, in turn, that the other 1,2,3,4-substituted xylose residues are di-substituted with arabinose. 3-*O*-Methyl-D-xylose was absent from the neutral fraction, the removal of arabinose alone (Table II) did not change its value, and it was present in the acid fraction (Table III); this indicates that only aldobiouronic acids are present in this position on the xylan backbone. The material remaining insoluble after partial hydrolysis with acid was mainly xylan (Table V).

According to the data in Table I, there are $1.98 + 0.31 + (0.25 \times 2) = 2.79$ mol. units of branch points. The main substituents are L-arabinose (1.64 mol. units) and the aldobiouronic acid $[(0.69 + 0.71)/2 = 0.70$ mol. units$]$, which gives a total of 2.33 mol. units. The 1.18 mol. units of terminal D-xylose are distributed between chain ends of the branch chains (possibly $2.79 - 2.33 = 0.46$ mol. units) and the main chain.

Thus, the mucilage contains D-xylose (63.6%), L-arabinose (20.4%), L-rhamnose (6.4), and D-galacturonic acid (9.4%). These percentages correlate well with those derived from methylation analysis: D-xylose (61.8%), L-arabinose (19.9%), L-rhamnose (9.3%), and D-galacturonic acid (9.0%). The amount of L-rhamnose recovered as alditol acetates is lower than that obtained by methylation analysis; this can be explained by the fact that the acid-resistant aldobiouronic acid linkage is removed in the methylated form by the diborane reduction. The main structural features of the mucilage, determined by methylation analysis, are shown in Fig. 1; the complexity suggests that a regular repeating-unit is unlikely.

The results reported here are in good qualitative agreement with those of lesser detail of Laidlaw and Percival[1,2] for analogous, but not identical, materials. These

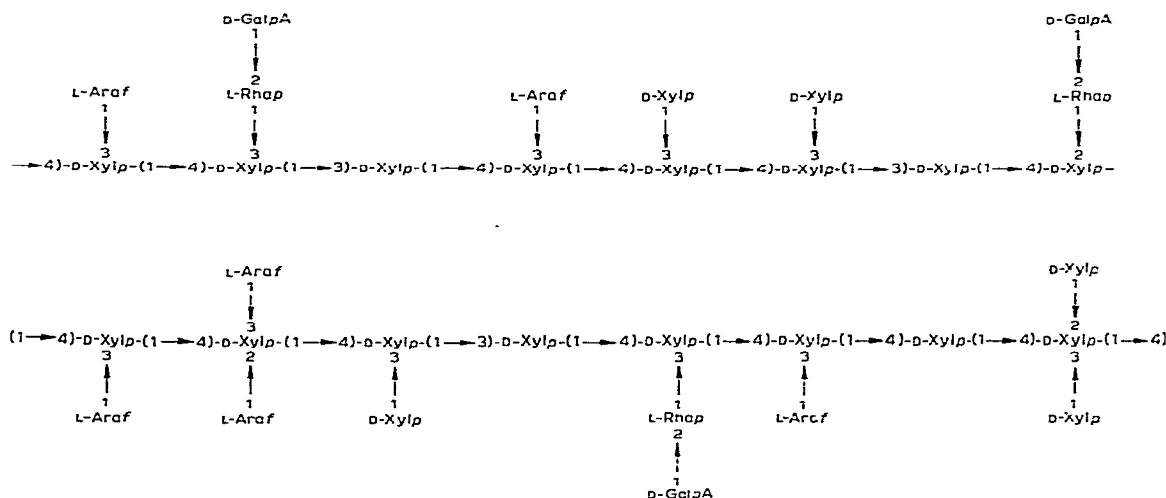

Fig. 1. Main structural features of the mucilage from *Plantago ovata* Forsk.

authors thought[1] that the aldobiouronic acid was a component of a glycuronan, rather than an integral part of a xylan polymer. However, the enzymic analysis in our study of Ispaghula husk showed the absence of pectic components.

The data in Tables I and IV show the presence of D-xylose, but it was absent from the samples for which data are given in Tables III and V, and its level in Table II was diminished. These observations show that D-xylose was not present as a result of incomplete methylation of the sample.

The work of Laidlaw and Percival[1,2] and the present study show the xylan component of the mucilage to be highly branched, the various positions being occupied by arabinose, aldobiouronic acid, and xylose residues.

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