Carbohydrate Research, 93 (1981) 247–259
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

THE GEL NATURE AND STRUCTURE OF THE CARBOHYDRATE OF ISPAGHULA HUSK *ex Plantago ovata* FORSK

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(Received November 3rd 1980; accepted for publication, January 16th, 1981)

ABSTRACT

Mucilage from the seed husk of *Plantago ovata* Forsk is an arabinosyl(galactosyluronic acid)rhamnosylxylan. A wide range of molecular sizes was revealed by chromatography of the methyiated mucilage on Sephadex LH-60, and the resulting 12 fractions were homologous. Partial hydrolysis with acid and enzymic hydrolysis variously of the mucilage and carboxyl-reduced mucilage led to identification of the β-D-xylosyl residues forming the xylan backbone of the polymer. Similarly. the arabinose, rhamnose, and galacturonic acid residues were shown to be α-L, α-L, and α-D, respectively. A structure for the polysaccharide is proposed and related to gel formation. Electron microscopy of the gel revealed a co-linear association. This association is reversible, and therefore not the result of covalent bonding.

INTRODUCTION

Ispaghula husk, obtained by milling the seeds of *Plantago ovata* Forsk, has important physiological effects on large-bowel action and is in wide use as a prophylactic in the treatment of such large-bowel disorders as diverticular disease. The husk has a high content of polysaccharide. This fraction forms a gel in water, and is classified as a mucilage. It is this ability to form a gel, which retains many times its own weight of water, that is responsible for the laxative action.

The composition and main glycosidic linkages of the components of the Ispaghula husk have been reported[1]. In an attempt to understand the gelling mechanism of the polysaccharide, further structural studies have been undertaken.

EXPERIMENTAL AND RESULTS

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 (40 ml/min) and a column (8 m × 2 mm i.d.) of 3% of ECNSS-M on Gas Chrom Q

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(100–200 mesh). Reduction and acetylation of carbohydrates were performed as described previously[1]. Retention times (*T*) are given relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. G.l.c.–m.s. was performed with a Perkin–Elmer F11 chromatograph attached to an Edwards 606 mass spectrometer.

Methylation analysis. — A sample (1 g) of husk polysaccharide[1] was methylated[2] by the Hakomori procedure[3]. The product (0.85 g) was extracted with chloroform, and the residue (138 mg; chloroform-insoluble fraction) was removed by centrifugation at 1300*g* for 15 min. It remained chloroform-insoluble after a second methylation. The extract (chloroform-soluble fraction), which contained the mucilage fraction, was concentrated to dryness under diminished pressure at 30°.

A solution of a sample (0.5 g) of methylated mucilage in 1,4-dioxane (2 ml) was applied to a column (90 × 2.5 cm) of Sephadex LH-60 pre-equilibrated with 1,4-dioxane. Elution with 1,4-dioxane at 1 ml/min gave optimal resolution. Part of this eluate (0.04 ml/min) was diverted, and monitored by the orcinol procedure[4]; the remainder was collected as 1-ml fractions. The elution profile (Fig. 1) showed 12 peaks.

Fractions corresponding to each of the 12 peaks were combined, concentrated to dryness at 30° with filtered air, and reduced[5] with diborane[6]. The reduced samples were hydrolysed with 2M trifluoroacetic acid (1 ml, 1 h, 121°) in a sealed tube, and the products were analysed as partially methylated alditol acetates by g.l.c. and m.s. (Table I).

Linkage analyses. — α-D-Galactosidase (EC 3.2.1.22, Sigma), α-L-rhamnosidase [EC 3.2.1.40, provided by Dr. K. M. Agrawal (Federal Institute of Technology, Zurich, Switzerland)], β-D-xylosidase (EC 3.2.1.37, Sigma), and α-L-arabinofuranosidase [EC 3.2.1.55, provided by Dr. A. Kaji (Kagawa University, Japan)] were used. The α-L-rhamnosidase (from *Klebsiella aerogenes*) and α-L-arabinofuranosidase (from *Aspergillus niger*) were obtained as purified enzymes, but the α-D-galactosidase and β-D-xylosidase were purified further[7]. For each enzyme, 1 unit of enzyme activity is defined as the amount of enzyme that liberates 1 μmol of 2-nitrophenol in 1 min from the 2-nitrophenyl glycoside (at the pH and temperature stated). The specific activity of the enzyme is expressed as the number of units per mg of protein.

Optical rotations were determined, after equilibration for >6 h, with a Perkin–Elmer 241 polarimeter (1-dm tube).

Separations on Bio-Gel P-2 were performed[8] at 65° using deionised water and columns (*1*) 2.5 × 150 cm at 28 ml/h (9-ml fractions) and (*2*) 0.9 × 150 cm at 14 ml/h (5-ml fractions). The eluate was monitored with a Waters Associates refractometer 401. Each column was calibrated for retention times by using starch oligosaccharides[9–11] having d.p. 1–13.

(*a*) *Xylose.* Husk polysaccharide (8 g) was hydrolysed with 5mM H_2SO_4 (400 ml, 9 h, 95°). The hydrolysate was centrifuged for 15 min at 1300*g*, and the residue was washed with water (40 ml). The washings and the supernatant solution were combined, neutralised with $Ba(OH)_2$, centrifuged at 1300*g* for 45 min, concentrated under diminished pressure at 40° to 200 ml, dialysed for 48 h against water,

TABLE I

METHYL ETHERS FROM THE HYDROLYSATE OF THE METHYLATED POLYSACCHARIDE OF ISPAGHULA HUSK AFTER FRACTIONATION ON SEPHADEX LH-60

Components	T[a]	*Normalised peak-area*[b]											
		1[c]	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
2,3,5-Tri-*O*-methyl-L-arabinose	0.45	9.2	9.3	9.1	9.5	8.9	9.4	9.1	8.9	8.8	9.1	9.0	8.9
2,3,4-Tri-*O*-methyl-D-xylose	0.68	3.2	3.4	3.6	4.5	5.3	6.8	7.1	8.2	8.9	9.2	9.6	10.0
2,3-Di-*O*-methyl-D-xylose	1.24	3.9	4.1	3.6	3.2	2.0	1.0	0.5	0.4	0.3	0.5	0.6	0.4
2,4-Di-*O*-methyl-D-xylose	1.19	5.9	6.0	5.8	5.5	5.2	5.5	5.6	5.3	5.1	4.9	5.0	4.7
2-*O*-Methyl-D-xylose	1.87	11.5	11.0	11.5	11.2	10.7	11.1	10.8	9.9	9.6	9.3	8.9	8.5
3-*O*-Methyl-D-xylose	1.74	1.7	1.6	1.8	1.7	1.5	1.8	1.6	1.5	1.7	1.6	1.5	1.8
D-Xylose	2.59	0.8	0.9	0.7	1.0	1.2	1.4	1.3	1.1	1.2	1.4	1.3	1.3
3,4-Di-*O*-methyl-L-rhamnose	0.86	3.9	4.1	4.0	3.9	4.1	3.8	4.2	4.0	3.9	4.1	4.0	3.8
2,3,4-Tri-*O*-methyl-D-galactose	1.94	4.0	4.3	4.2	4.0	4.2	3.9	3.7	4.1	3.8	3.9	4.1	4.0

[a]Retention time of alditol acetate relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

[b]Normalised peak-area $= \left[\frac{\text{peak area}}{\text{molar response factor}} \right] \times$ xylose correction-factor[1].

[c]Numbers 1–12 correspond to the peaks (1–12) in Fig. 1.

and freeze-dried. A solution of the product (4.80 g) in water (40 ml) was adjusted to pH 4 with NaOH, and loaded on a column (100 × 2.5 cm) of Amberlite 401 (AcO^-) resin. The neutral fraction was eluted with water (178 ml), and the acidic fraction with 20% aqueous acetic acid (145 ml). The neutral fraction was freeze-dried, and the acidic fraction was concentrated to dryness under diminished pressure at 30°.

A portion (30 mg) of the neutral-carbohydrate fraction, $[\alpha]_D^{25}$ −96.5° (*c* 1.5, water), was hydrolysed with 2M trifluoroacetic acid (2 ml, 1 h, 121°), and the products were analysed as alditol acetates by g.l.c.

A further portion (1.2 g) was hydrolysed with 40% aqueous H_2SO_4 (20 ml, 4°, 8 h). The hydrolysate was then diluted to 100 ml, neutralised with $Ba(OH)_2$, centrifuged, and freeze-dried. Chromatography of the residue on Bio-Gel P-2 (column *1*) yielded three peaks. Peak I (major) corresponded to a monosaccharide, peak II to a disaccharide, and peak III approximately to a trisaccharide. The material in these peaks was isolated by freeze-drying. Peak I had $[\alpha]_D^{25}$ +19° (*c* 2, water), peak II had $[\alpha]_D^{25}$ −27° (*c* 2.1), and peak III had $[\alpha]_D^{25}$ −49° (*c* 2.6); *cf.* $[\alpha]_D^{25}$ −25.6° for the (1→4)-β-D-linked xylobiose, and $[\alpha]_D^{25}$ −48.1° for (1→4)-β-D-linked xylotriose[14].

Material (40 mg) from peak II was added to a solution (2 ml) of 3.5M $(NH_4)_2SO_4$ and 50mM CH_3COONa (pH 5.2) containing β-D-xylosidase (16 units), and the mixture was incubated for 24 h at 25°, and then at ~100° for 3 min to inactivate the enzyme. Gel filtration on Bio-Gel P-2 (column *2*) gave one peak corresponding to a monosaccharide. The appropriate fractions were combined and freeze-dried, and the residue was analysed as alditol acetates by g.l.c. The identification of xylose showed that β-D-xylosidase hydrolyses the xylobiose to the monosaccharide, thus providing further evidence that the xylosyl residues in the mucilage polysaccharide are predominantly β-D.

(*b*) *Galacturonic acid and rhamnose.* A sample (12 g) of Ispaghula husk was hydrolysed for 1 h at 121° in 0.1M trifluoroacetic acid (600 ml). Ethanol (600 ml) was added and the resulting suspension was centrifuged at 1300*g* for 20 min. The precipitate was washed with 75% aqueous ethanol (60 ml), and the washings and the supernatant solution were combined and concentrated to dryness under diminished pressure at 30°. A solution of the dried material in water (30 ml) was adjusted to pH 4 with NaOH, applied to a column (2.5 × 100 cm) of Amberlite 401 (AcO^-) resin, and eluted with water (165 ml) to remove the neutral oligosaccharide. The acidic fraction was eluted with 20% aqueous acetic acid (120 ml). The eluate was evaporated to dryness under diminished pressure at 30°, and a solution of the residue (540 mg) in water (1.5 ml) was subjected to gel filtration on Bio-Gel P-2 (column *1*).

Three peaks were eluted, corresponding to di- (I), tetra- (II), and hexa-saccharide (III). The material isolated by freeze-drying from peak I had $[\alpha]_D^{22}$ +102° (*c* 1, water) [*cf.* +101° for 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose[15]] and that from peak II had $[\alpha]_D^{22}$ +48° (*c* 1.2, water). The materials from peaks I and II were acetylated separately in sealed tubes with acetic anhydride (2 ml) and

pyridine (2 ml) for 3 h at 121°. The mixtures were concentrated at 30° under diminished pressure. Each syrupy residue was mixed with water (1 ml), the solution was concentrated to dryness under diminished pressure at 30°, and the residue was reduced with diborane[5,6] and then de-acetylated[5].

Portions of these products (28 mg from I, 25 mg from II) were hydrolysed with 2M trifluoroacetic acid for 1 h at 121°. The products of hydrolysis were analysed as alditol acetates by g.l.c. Analysis of peak I revealed D-galactose (derived from reduced D-galacturonic acid) and L-rhamnose in the molar ratio 1:0.9. The material from peak II was composed of D-galactose (from D-galacturonic acid), L-rhamnose, and D-xylose in the molar ratios 1:1:2.

A solution of the reduced and de-acetylated material (30 mg) from peak I in 0.01M sodium citrate buffer (pH 4.0, 2 ml) was incubated with α-D-galactosidase (14 units) at 37° for 24 h under toluene. After enzyme inactivation at ~100° for 3 min, the solution was subjected to gel filtration on Bio-Gel P-2 (column *2*). A single peak was eluted, corresponding to monosaccharide, which was isolated by freeze-drying and analysed as the alditol acetates by g.l.c. Identification of galactose and rhamnose in the molar ratio 0.9:1 showed that the α-D-galactosidase hydrolyses the disaccharide arising from carboxyl-reduction, thus providing further evidence that the galactose residues (derived from galacturonic acid) are α-D.

A solution of reduced, de-acetylated material (80 mg) from peak II in 0.01M sodium citrate (pH 4, 2 ml) was incubated with α-D-galactosidase (20 units) at 37° for 24 h under toluene. Enzyme inactivation and gel filtration (column *1*) were performed as described above. Two peaks were detected, corresponding to mono- (peak A) and (approximately) tri-saccharide (peak B).

G.l.c. of the contents of peak A (as alditol acetates) indicated the presence of D-galactose only. The material (55 mg) in peak B was dissolved in 0.05M sodium citrate buffer (pH 6.8, 2 ml) at 37° and incubated with α-L-rhamnosidase (10 units) for 12 h, followed by gel filtration (column *2*). Two peaks were obtained, corresponding to mono- (peak A) and di-saccharide (peak B).

G.l.c. of the material in peak A (as alditol acetates) indicated the presence of L-rhamnose only. The material in peak B was hydrolysed with 2M trifluoroacetic acid for 1 h at 121°. G.l.c. of the product (as alditol acetates) showed the presence of D-xylose only.

Identification of galactose after application of α-D-galactosidase to the tetrasaccharide (from peak II) composed of D-xylose, L-rhamnose, and D-galactose residues in the molar ratios 1:1:2 indicated that the galactose residues (originally galacturonic acid) were α-D. Subsequent release of rhamnose from the resulting trisaccharide by α-L-rhamnosidase shows that the rhamnosyl residues were α-L, and linked to the xylan backbone.

(*c*) *Chromium trioxide oxidation.* The diborane-reduced material (50 mg) from peak II [(*b*) above] was further reduced with sodium borohydride and acetylated with acetic anhydride[5], *myo*-inositol hexa-acetate (4 mg) was added as the internal standard, and the mixture (in part) was subjected to quantitative g.l.c. The remainder

TABLE II

CHROMIUM TRIOXIDE OXIDATION OF THE OLIGOSACCHARIDE PRODUCED BY PARTIAL HYDROLYSIS OF ISPAGHULA HUSK WITH ACID

Components[a]	*Time (h)*			
	0	*1*	*3*	*6*
D-Xylose	2.00	1.73	1.47	0.97
L-Rhamnose	1.00	0.99	0.96	1.00
D-Galactose[b]	1.00	1.00	1.00	1.00

[a]Determined by g.l.c. of alditol acetates. All of the results are given as molar ratios (normalised to D-galactose). [b]Derived from D-galacturonic acid.

was treated with chromium trioxide (150 mg) in acetic acid (5 ml) at 50°. Samples (1.5 ml) were removed at intervals (1, 3, and 6 h), poured into water (6 ml), and extracted into chloroform (3 × 6 ml). The extracts were concentrated to dryness, the residues were hydrolysed with 2M trifluoroacetic acid (2 ml) for 1 h at 121°, and the products were analysed as alditol acetates by g.l.c. (Table II).

Chromium trioxide oxidation of the diborane-reduced oligosaccharide (composed of D-xylose, L-rhamnose, and D-galactose) indicated that D-galacturonic acid and L-rhamnose have α linkages, since the recovery of these two components was similar during the oxidation period (see Table II); the level of D-xylose declined during the oxidation reaction, suggesting the presence of a β linkage (see Table II).

(*d*) *The arabinofuranose component*. Ispaghula husk (4 g) was hydrolysed in 5mM H_2SO_4 (600 ml, 3 h, 95°). The hydrolysate was centrifuged for 15 min at 1300*g* and the residue (1.04 g) was washed with water (20 ml). The washings and the supernatant solution were combined, neutralised with $Ba(OH)_2$, and centrifuged, and the supernatant solution was concentrated under diminished pressure at 40° to ~100 ml and then dialysed for 48 h against water. The solution surrounding the dialysis bag was freeze-dried (→fraction 1). The liquid inside the dialysis bag (→fraction 2) was concentrated to dryness (residue, 2.56 g) under diminished pressure at 40°.

Fraction 1 (20 mg) and a portion of fraction 2 (28 mg) were separately hydrolysed with 2M trifluoroacetic acid for 1 h at 121°. The products were converted into alditol acetates and analysed by g.l.c. The uronic acid content of each fraction was determined by the carbazole procedure[1] (Table III).

To a solution of a portion (100 mg) of fraction 2 in water (10 ml) was added α-L-arabinofuranosidase (12 units/ml) in 0.1M sodium citrate–sodium phosphate buffer (pH 4.0, 5 ml). A few drops of toluene were added, and the mixture was incubated[12] at 40° for 24 h and then dialysed against water for 24 h at room temperature. The solutions surrounding (→fraction 3) and inside the dialysis bag (→fraction 4) were freeze-dried.

Material from fraction 3 and a portion of the material from fraction 4 (20 mg)

TABLE III

COMPOSITION[a] OF ISPAGHULA HUSK MUCILAGE AFTER SEQUENTIAL DEGRADATION BY SULPHURIC ACID AND α-L-ARABINOFURANOSIDASE

Components	*Fraction*[b]			
	1	*2*	*3*	*4*
D-Xylose[c]	0.00	5.57	0.00	5.59
L-Arabinose[c]	0.30	1.82	1.44	0.28
L-Rhamnose[c]	0.00	0.68	0.00	0.71
Uronic acid[d]	0.00	1.00	0.00	1.00

[a]All of the results are given as molar ratios with respect to the uronic acid (the results have been normalised for the neutral sugars by using the internal standard *myo*-inositol). [b]Fractions: 1, dialysable material after hydrolysis of Isphaghula husk with 5mM H_2SO_4; 2, non-dialysable material; 3, dialysable material after incubation of 2 with α-L-arabinofuranosidase; and 4, non-dialysable material. [c]Determined by g.l.c. of alditol acetates. [d]Determined by the carbazole procedure[1].

were hydrolysed with 2M trifluoroacetic acid for 1 h at 121°, and the products were converted into alditol acetates and analysed by g.l.c. The uronic acid content of each fraction was determined by the carbazole procedure[1] (see Table III).

Examination of gel structure. — (*a*) *By thin-sectioning.* The gel was prepared by adding Ispaghula-husk powder (3%) to water and stirring for 1 h at room temperature. The alkali-soluble mucilage was prepared by placing Isphagula husk (4%) in 1.2M sodium hydroxide, under nitrogen for 1 h at room temperature. The solution, still under nitrogen, was centrifuged at 1300*g* for 20 min. The supernatant solution was removed and neutralised with acetic acid, and then ethanol was added to precipitate the polysaccharide material. The precipitate was collected, washed with ethanol, and dried over P_2O_5 at 30°.

Samples (3 mm^3) of the gels were placed in 1% periodic acid in 70% aqueous ethanol for 1 h at room temperature. The oxidised gel was twice washed thoroughly for 30 min, and then immersed[13] in 1% thiocarbohydrazide in 5% aqueous acetic acid for 2 h at 60°. The sample was washed twice with water to remove any thiocarbohydrazide not bound to the polysaccharide, and then treated with 1% aqueous osmium tetraoxide for 3 h at room temperature. The sample was twice washed thoroughly with water, and then successively dehydrated with 70%, 80%, 90%, and absolute ethanol.

Samples were embedded in a resin mixture: 50.1% of Epon (812)/29.9% of DDSA (dodecenyl succinic anhydride)/20% of MNA (methyl nadic anhydride) by volume, with 1.5% of DMP-30 as the accelerator. Sections (500–700 Å thick) were cut with a Cambridge–Huxley Mark I (hand-operated) ultramicrotome, collected on collodion-covered copper grids, and examined in a Hitachi HS-7S electron microscope. Representative fields are shown in Figs. 3 and 4.

(*b*) *By freeze-etching.* 1,4-Dioxane–glycerol–water (49.5:1:49.5) was used as cryoprotectant. A gel was prepared in this mixture (100 ml) by adding Ispaghula

husk [see (*a*)] to 3% and stirring for 1 h at room temperature. A small sample of Ispaghula-husk gel was placed on a gold disc, frozen rapidly (2940°/sec) in liquid Freon 12 to −150°, and stored in liquid nitrogen. The samples were transferred to the cold stage of the freeze-etching unit (NGN FE 600) set at −150°. A vacuum was produced around the sample, and the stage temperature was adjusted to −90°. The microtome knife was cooled to −190° and, at a vacuum of 3×10^{-6} Torr, the sample was fractured, and the knife positioned just above the specimen to cause sublimation of ice away from the specimen surface.

A replica was made by evaporating a layer of carbon/platinum, at an angle of 30°, onto the specimen surface, and then a layer of carbon at an angle of 90° to the specimen surface. The sample was removed from the freeze-etcher and allowed to thaw in 70% ethanol. During this stage, the replica became detached from the specimen. The replica was first washed with distilled water for 15 min, and then placed in 1.2M NaOH for 10 min. After a second wash with distilled water, the replica was placed in 1.2M $NaClO_3$ for 30 min and, finally, washed with distilled water. The cleaned replica was picked up on a copper grid, and dried by touching to the edge of a piece of filter paper before examination in the electron microscope. Representative fields are shown in Figs. 5–7.

DISCUSSION

Structural analysis. The methylated mucilage displayed a wide range of molecular sizes when subjected to chromatography on Sephadex LH-60. The elution profile showed 12 discrete peaks (Fig. 1), and methylation analysis of the contents of each

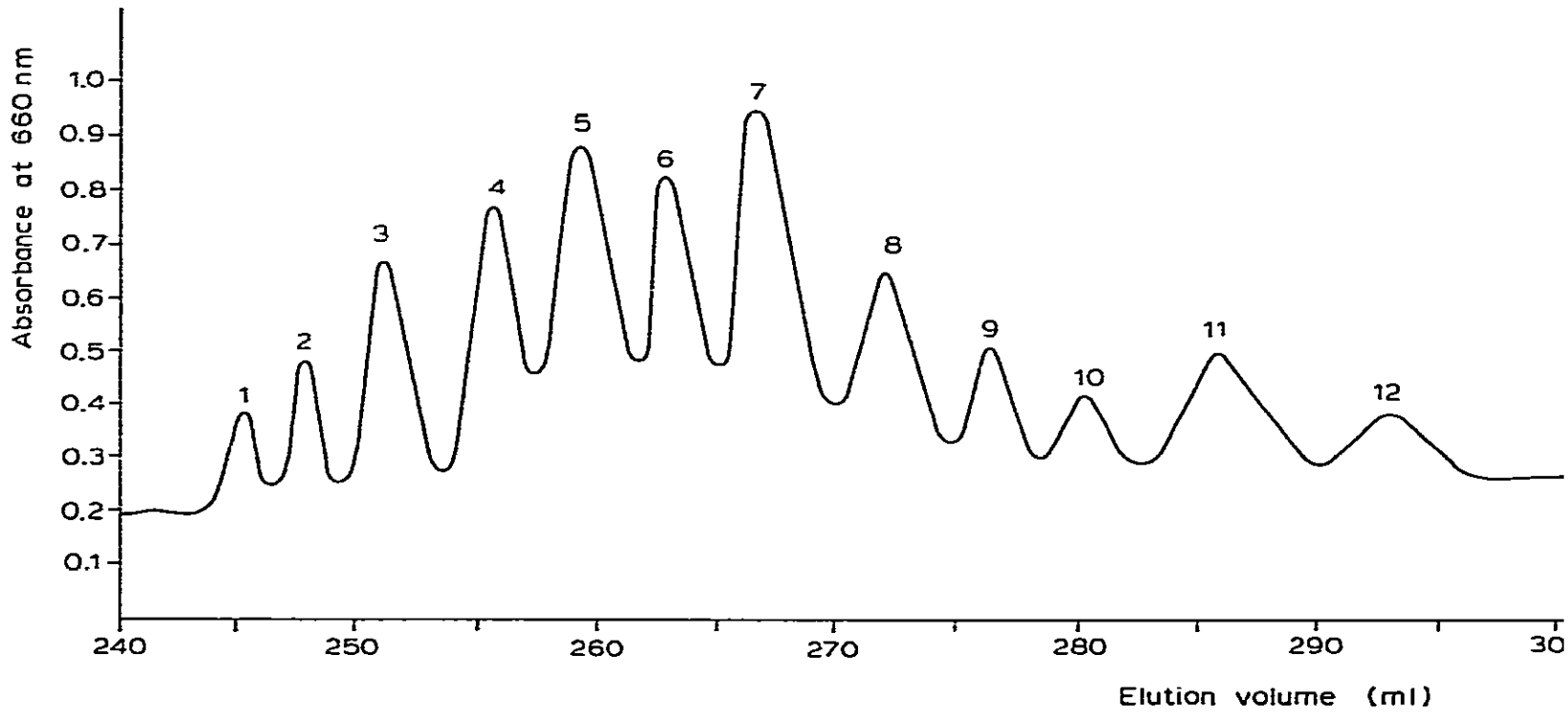


Fig. 1. Gel chromatography of the methylated polysaccharide of Ispaghula husk on Sephadex LH-60 in 1,4-dioxane: column, 2.5 × 90 cm; flow rate, 1 ml/min; continuous-flow cell (light path of 20 mm).

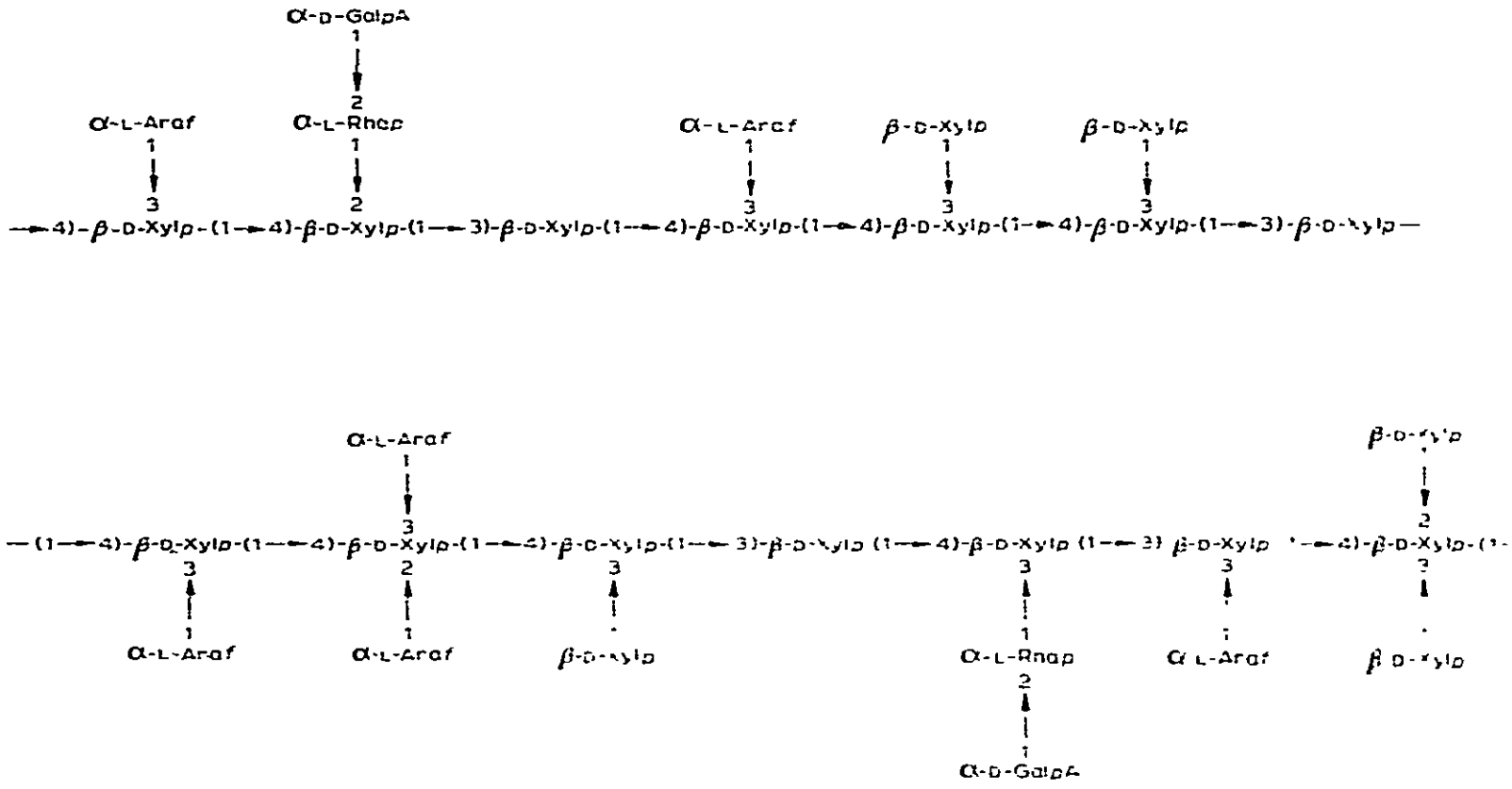


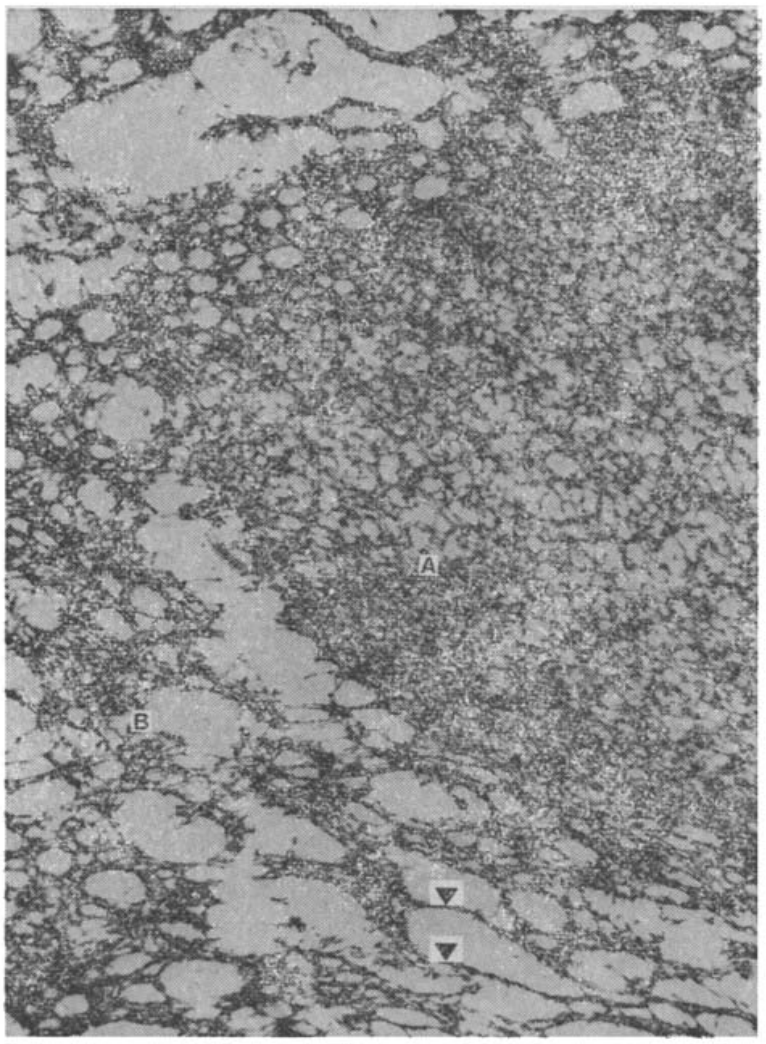
Fig. 2. Representation of the main structural features of the mucilage from *Plantago ovata* Forsk.

(Table I) gave similar amounts of 2,3,5-tri-*O*-methyl-L-arabinose, 3,4-di-*O*-methyl-L-rhamnose, and 2,3,4-tri-*O*-methyl-D-galactose. However, the content of 2,3,4-tri-*O*-methyl-D-xylose decreased from 10 units in peak 12 to 3.2 units in peak 1, whereas 2,3-di-*O*-methyl-D-xylose increased from 0.4 unit in peak 12 to 3.9 units in peak 1, suggesting a gradual decrease in molecular size from peak 1 to peak 12. Thus, the mucilage contains a population of molecules of similar composition, but a range of molecular sizes. Further evidence for heterogeneity of molecular size was obtained on ultracentrifugation of the methylated mucilage, which gave six discrete components.

Enzymic degradation, chromium trioxide oxidation, and optical rotation studies were performed to determine the anomeric linkages and absolute configurations of the monosaccharide residues present in the mucilage (see Fig. 2). The xylan backbone was shown to have both (1→3)- and (1→4)-β-D linkages, and to be substituted by 2-*O*-(α-D-galactopyranosyluronic acid)-α-L-rhamnosyl groups. A small proportion of the L-arabinofuranose residues present in the mucilage was not released by α-L-arabinofuranosidase (see Table III) and may be β-linked, or α-linked and sterically hindered.

Appearance in the electron microscope. The possible effects on the network structure of the preparation of samples of the mucilage gel for electron microscopy by thin-sectioning and by freeze-etching have been discussed elsewhere[16]. Examination of samples prepared by each technique revealed that the network density is variable (Figs. 3–5). The thickness of the fibres was 100–200 Å in thin sections and 400 Å in freeze-etched samples, suggesting that the polysaccharide chains are aligned.

Examination by freeze-etching of the gel dissolved in alkali showed that a small fraction of the network remained associated (Fig. 6, arrow A). The network



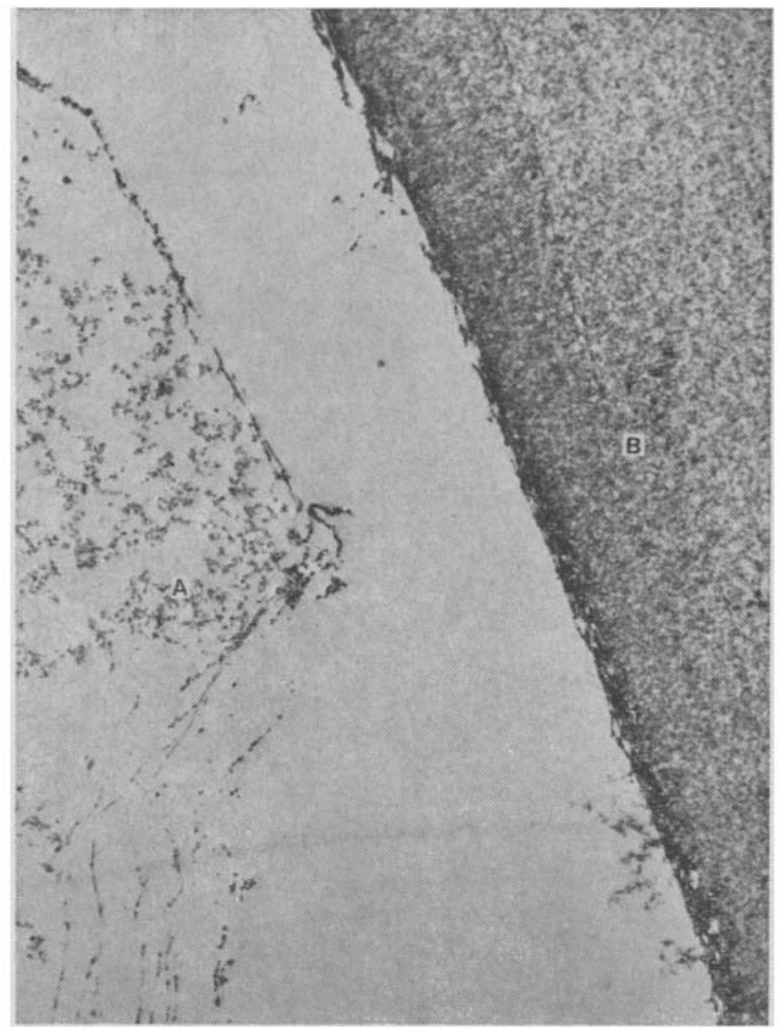


Fig. 3. Electron micrograph of Ispaghula husk in water (14,100 ×, thin-sectioning). The photograph shows variation in the density of the gel network (areas A and B). The arrows indicate where the fine fibres of the gel have associated into thick strands.

Fig. 4. Electron micrograph of Ispaghula husk in water (3.500 , thin-sectioning).

was re-established when the mucilage was removed from alkali and placed in water (Fig. 7).

The gelling mechanism. Most plant xylans are not extractable with water, and the backbone consists solely of (1→4)-linked β-D-xylosyl residues. Ispaghula-husk mucilage has a xylan backbone with both (1→3)- and (1→4)-β-D linkages, and is extractable with water[17], in which it forms a gel. Since the D-xylan displays junction zones, the distribution of linkages and substituents is probably non-random. It is unlikely that the (1→3)-β-D linkages occur as contiguous blocks, since such regions would be capable of forming a double-helical structure[18]. No such structure was detectable by X-ray diffraction studies of the Ispaghula-husk powder or of stretched fibres.

The strands of the gel network were shown by electron microscopy to be the result of parallel alignment of the D-xylan polymers, although the details of the alignment are not clear. However, it seems likely that the junction zones also associate linearly. The association cannot be through covalent bonds, since the gel dissociates almost completely in the presence of sodium hydroxide (see Fig. 6).

It has been proposed[19] that the (1→4)-β-D-xylan isolated from white birch

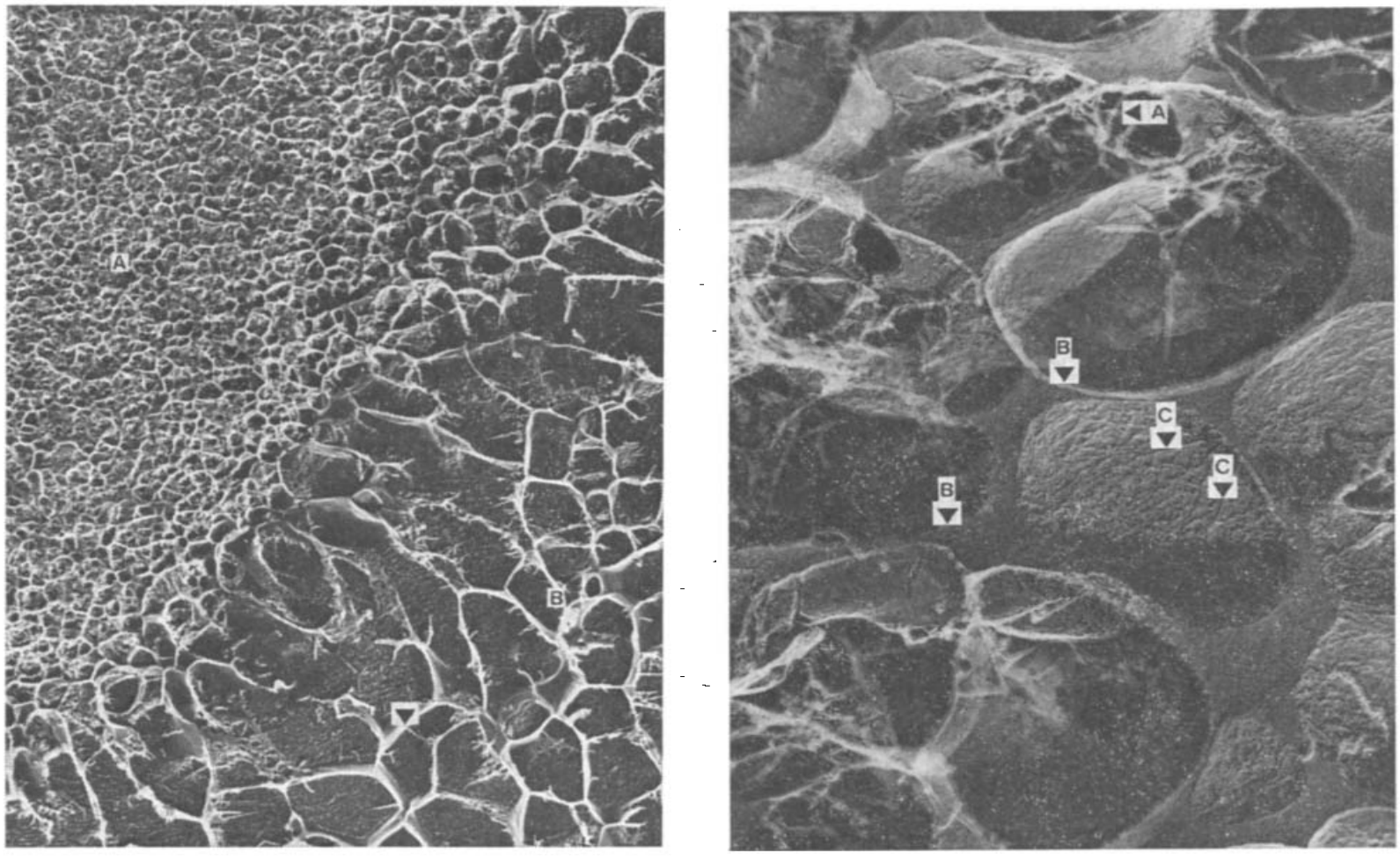


Fig. 5. Electron micrograph of Ispaghula husk in water (3,500×, freeze-etching). The photograph shows regions of (B) low and (A) high density of the gel network. The arrow indicates a region of extensive phase separation. The diameter of the network fibre in this area is greater than in region A.

Fig. 6. Electron micrograph of Ispaghula husk in 1.2M sodium hydroxide (23,300×, freeze-etching). Arrows indicate phase separation mainly due to sodium hydroxide (A), the non-solubilised fraction of gel network (B), and the dissociated fibres of the gel network (C).

forms a hexagonal unit-cell with a columnar lattice along the fibre axis that can accommodate side chains or a column of water molecules. The same packing mode was proposed[20] for the (1→4)-β-D-xylan isolated from oat hulls. Many studies of the gelling polysaccharides have illustrated the dependence of the gelling mechanism on fine structure. For example, changes in the gelling properties of ι-carrageenan parallel changes in the sequence of the 3,6-anhydro-D-galactosyl and β-D-galactosyl 4-sulphate substituents of the backbone polymer. Therefore, it seems likely that the polymer from Ispaghula mucilage will have an ordered arrangement of (1→3)- and (1→4)-β-D linkages, and of the substituent side-chains.

Unsubstituted xylan polymers are insoluble in water unless substituted by hydrophilic residues (*e.g.*, L-arabinofuranose) or ionic residues (*e.g.*, uronic acids) which bind water at the surfaces or within gels[22]. L-Arabinofuranosyl groups are not thought to contribute specific water-binding properties, but they could be important with respect to non-specifically bound water (*i.e.*, osmotic in origin), and thus result in the hydrophilic character of arabinofuranosyl homopolymers[22].

Regions of a xylan polymer that are highly substituted with arabinose or aldobiouronic acid residues are fully extended and inflexible, whereas the unsubstituted

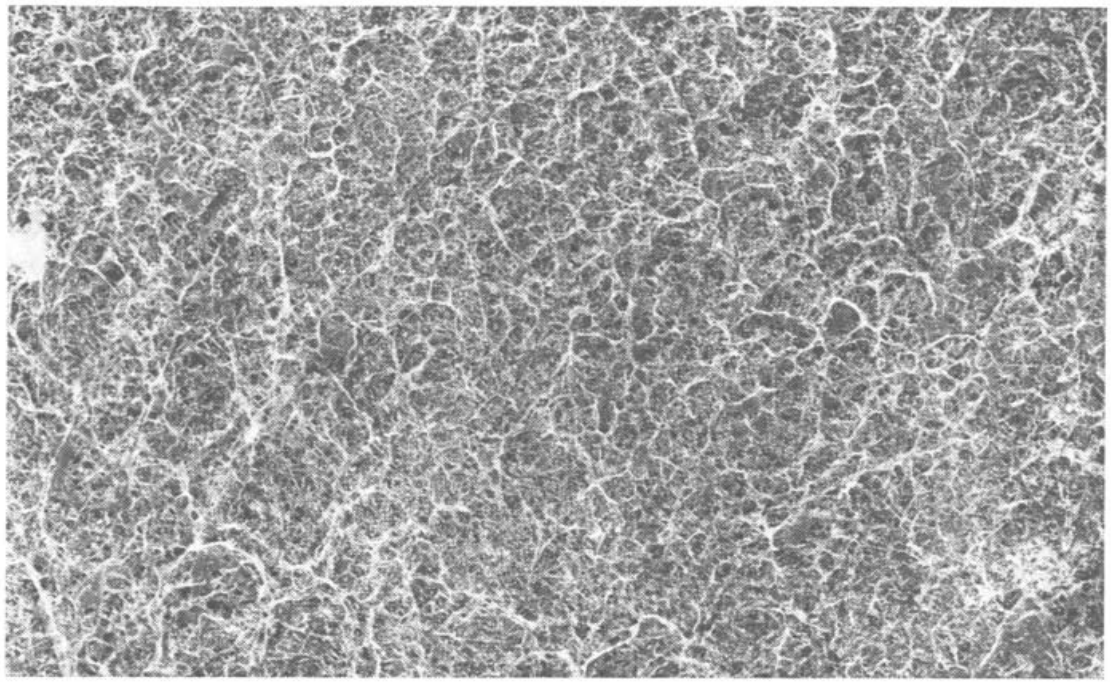


Fig. 7. Electron micrograph of alkali-soluble mucilage in water (5,300× freeze-etching).

regions appear to be quite flexible[23] and are capable of forming stable, inter-chain associations[22]. It is not clear whether only the unsubstituted regions of the Ispaghula D-xylan are involved in the formation of junction zones, or if the substituted areas are also involved. The substituted regions of the xylan isolated from sugar cane can form intermolecular associations, but the unsubstituted regions tend to associate first[24].

Cellulose and (1→4)-β-D-xylans have a similar, ribbon-like conformation with hydrogen bonding between the chains[25]. Thus, it is plausible that the (1→3)-β-D linkages in the Ispaghula D-xylan are distributed along the backbone in such a way as to delineate contiguous blocks of (1→4)-β-D-linked xylosyl residues. These blocks would then be able to associate linearly through hydrogen bonding. The conformation of the (1→3)-β-D linkages will prevent association in their immediate vicinity, and their distribution will determine the number and size of junction zones along the polymer. In order to test this hypothesis, it would be necessary to determine the precise arrangement of (1→3)- and (1→4)-β-D linkages in the D-xylan polymer, and this could be achieved by sequential, enzymic hydrolysis.

The previously published data for the methylation analysis of Ispaghula husk have been used as a basis for further structural elucidation of the mucilage polysaccharide. The main conclusions of this investigation (based on enzymic degradation, freeze-etching, and electron microscopy) are that (*1*) Ispaghula husk displays a range of molecular sizes, (*2*) the xylose, arabinose, rhamnose, and galacturonic acid residues are D, L, L, and D, respectively, (*3*) the D-xylosyl residues are β, (*4*) (1→3)-β-D-xylosyl linkages are unlikely to be contiguous, (*5*) (1→4)-β-D linkages are likely to occur in contiguous blocks of D-xylosyl residues that form junction zones, (*6*) the majority of the L-arabinosyl substituents are α, (*7*) the D-galactose residues are α, (*8*) 2-*O*-(α-D-galactopyranosyluronic acid)-α-L-rhamnosyl side-chains are linked to the xylan backbone, (*9*) substituents (*6*) and (*8*) are arranged in a non-random manner, (*10*) gel formation is likely to occur by a co-linear, non-covalent association of junction zones.

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