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Seed Polysaccharides and their Role in Germination

A SURVEY OF THE POLYSACCHARIDE COMPONENTS OF MUSTARD SEEDS WITH SPECIAL REFERENCE TO THE EMBRYOS

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1. Methods were developed for the extraction, fractionation and purification of the more soluble polysaccharides of mustard-seed embryos. 2. One of these components was a pure homopolysaccharide, an araban, which was characterized by analysis, optical rotation, chromatography on diethylaminoethylcellulose and electrophoresis; the hydrolysis products of the methylated polysaccharide were isolated and characterized by the formation of crystalline derivatives. From these studies it emerges that mustard-seed araban is very similar to the family of pectic arabans, except that it is more highly branched than usual and contains a proportion of 1→2-linkages. 3. A survey of the other polysaccharides of mustard seed, both in the embryos and in the seed coats, suggests a predominance of pectic-type polysaccharides.

The systematic exploration of plant polysaccharides by methylation and other methods has been proceeding for over 30 years and many structural types are now recognized (Aspinall, 1962). In addition, some aspects of the biosynthesis and biological functions of the simpler polysaccharides are understood (Leloir, 1961). One of the next aims in the study of complex polysaccharides must be to understand in chemical terms their biological importance and their biosynthetic relations, one molecule with another. Perhaps the richest and most varied source of polysaccharides in the plant is the cell wall; in view of the synthesis and development of cell walls that occurs in germinating seeds, we have chosen this system for a study of polysaccharide degradation, synthesis and transformation in plants. As a start to this programme we are investigating the polysaccharides of white mustard (*Sinapis alba*). This seed was chosen because the plant can be conveniently grown and handled in the laboratory, because it is a representative of a family of important vegetables (e.g. cabbage, turnip) about whose polysaccharides remarkably little is known, and because it is a fatty seed in which the extraction and fractionation of cell-wall polysaccharides would presumably not be complicated by the presence of reserve polysaccharides.

As a preliminary to more detailed work, we have made a survey in broad outline of the polysaccharides that are present in the seed. This forms the subject of the present paper.

MATERIALS AND METHODS

Materials. Agricultural white mustard (*Brassica sinapis alba*) was purchased from a local seed merchant.

Protein contents of freeze-dried extracts. These were calculated by multiplying the percentage content of N (Kjeldahl) by 6.

Carbohydrate contents. These were determined, with arabinose as standard, with the phenol–sulphuric acid reagents (Dubois, Gilles, Hamilton, Rebers & Smith, 1956).

Paper chromatography. The solvents used were: for neutral sugars, ethyl acetate–pyridine–water (8:2:1, by vol.); for uronic acids, ethyl acetate–pyridine–acetic acid–water (5:5:1:3, by vol.); for methylated sugars, butan-1-ol–ethanol–water (4:1:5, by vol.). Spots were located by spraying the paper with *p*-anisidine hydrochloride in butanol, or aniline oxalate in ethanol, followed by heating in an oven at 110–120°.

Paper and glass-fibre electrophoresis. Several buffers were used, as mentioned in the text. The paper was held between two pieces of polythene, which were clamped between two cooled copper blocks. The potential gradient was normally 100v/cm. and the running time 1hr. When a filter-paper support was used, the spots were located with potassium periodocuprate spray, followed by rosaniline (Bonner, 1960). With glass fibre, the spray was anisaldehyde and sulphuric acid in ethanol (Stahl & Kaltenbach, 1961); it was important in this case to spray the electrophoretogram lightly, otherwise a pronounced background colour was obtained. In all cases caffeine (visible under an ultraviolet lamp) was used as the endosmotic marker.

Gas-liquid chromatography of methylated sugars as their methyl glycosides. This was carried out with a Pye Argon chromatograph fitted with a ^{90}Sr detector, essentially as described by Aspinall (1963).

Column chromatography of the araban on DEAE-cellulose. The column was prepared in the borate form by the method of Neukom, Deuel, Heri & Kündig (1960), and tested by adsorption of methyl red indicator. The dye was retained at the top of the column for as long as washing was continued with water. When the eluent was changed to 0·2M-sodium metaborate, the dye moved down as a discrete band, this being taken as an indication that the column was satisfactorily packed.

The washed column (24cm. × 1·5cm. diam.) was loaded with polysaccharide (0·2g.) and eluted in turn with (i) water (900ml.), (ii) 0·01M-sodium metaborate (300ml.), (iii) a linear gradient from 0·01M- to 0·10M-sodium metaborate (800ml.) and (iv) 0·5M-sodium metaborate. The flow rate was 0·5ml./min., and 10ml. fractions were collected automatically for analysis with the phenol–sulphuric acid reagents (Dubois *et al.* 1956).

Separation into seed coats and embryos. Whole seeds were passed through a small hand mill into boiling aq. 80% (v/v) ethanol. After 5–20min. in this solution they were drained, partly separated in a draught of air and then extracted in the Soxhlet extractor, first with ethanol and then with acetone. No further material was removed from the embryos with chloroform or with light petroleum. After further purification by sieving and in the air draught, the fractions were obtained by hand sorting.

RESULTS

Preliminary survey of the polysaccharides in the embryos and seed coats

For these experiments, the parts were separated by hand and were not extracted with organic solvents before use. The seed coats (1g.) and the embryos (3g.) were each treated with the following series of reagents: (*a*) boiling 80% (v/v) ethanol (100ml.); (*b*) cold water (2 × 500ml. at room temperature for 24hr.); (*c*) boiling water (2 × 500ml. for 4hr.); (*d*) aq. 0·5% ammonium oxalate (2 × 400ml. for 3hr. at 65–70°); (*e*) delignification by the chlorite method (Jermyn, 1955) followed by hot-water extraction (2 × 400ml. for 3hr.); (*f*) aq. 5% (w/v) potassium hydroxide under nitrogen (400ml. for 16hr. at room temperature); (*g*) aq. 20% (w/v) potassium hydroxide under nitrogen (400ml. for 16hr. at room temperature). The insoluble material was separated on a filter, washed with water and dried in a desiccator.

All fractions except those isolated with reagent (*a*) were purified by dialysis and isolated as freeze-dried solids. Part of each product was suspended in 72% (w/v) sulphuric acid and shaken until it dissolved. After dilution to 1·5N-sulphuric acid concentration, the solution was heated on a boiling-water bath for 4hr., neutralized with calcium carbonate, filtered, concentrated under diminished pressure and examined by paper chromatography. The results are given in Table 1. Some additional results, given in Table 2, were obtained with fractions that were isolated in a separate series of extractions on a larger scale. In this series, the fractions obtained by cold- and by hot-water extraction were not dialysed before being freeze-dried, but the procedure was otherwise the same as for the small-scale experiment. The fraction isolated with reagent (*a*) contained the low-molecular-weight carbohydrates

Table 1. *Sugars detected after hydrolysis of the polysaccharide fractions from mustard seed*

Experimental details are given in the text. For each fraction, sugars are listed in approximately descending order of concentration; those in parentheses are relatively minor components.

	Seed coats		Embryos	
Solvent used for extraction	Yield (%)	Sugars detected after hydrolysis	Yield (%)	Sugars detected after hydrolysis
Cold water	6·4	Galactose, arabinose, galacturonic acid (xylose, glucose, mannose)	2·4	Arabinose, glucose (xylose, galactose, galacturonic acid)
Hot water	8·6	Arabinose, galactose, galacturonic acid (xylose, rhamnose, glucose, mannose)	3·4	Arabinose (xylose, galactose, galacturonic acid)
Ammonium oxalate	16·1	Galacturonic acid, xylose (arabinose, mannose, galactose)	1·7	Arabinose (xylose, galactose, galacturonic acid, glucose)
Hot water (after delignification)	14·0	Arabinose, galacturonic acid (xylose, galactose)	8·5	Arabinose, galacturonic acid (xylose, galactose, rhamnose)
5% KOH	6·4	Xylose, galacturonic acid (glucose, galactose, mannose, arabinose)	9·8	Xylose, arabinose (glucose, galacturonic acid, mannose)
20% KOH	5·4	Xylose, mannose, glucose, galacturonic acid (galactose, arabinose)	1·7	Xylose, glucose (mannose, arabinose)
Residue	16·0	Glucose (galacturonic acid)	2·4	Glucose (arabinose, galacturonic acid)

Table 2. *Yield and analyses of the fractions obtained by large-scale extraction of mustard-seed embryos*

Experimental details are given in the text. Reasons for the differences between the yields in this experiment and corresponding ones in Table 1 are: (i) some of the fractions in this experiment were isolated without dialysis; (ii) the starting material in this experiment was more thoroughly extracted with organic solvents before use.

Solvent used for extraction	Yield (%)	Protein content (%)	Carbohydrate content (%)
Cold water	6·3	47	26
Hot water	6·1	60	16·1
Ammonium oxalate	3·6	19	12·5

of the seed. These were characterized as sucrose and stachyose, together with traces of raffinose.

Large-scale extraction and purification of the araban

The unground solvent-extracted embryos (1·5kg.) were extracted with aq. 2% (w/v) EDTA solution (adjusted to pH 7·5 with sodium hydroxide) (3 × 5l. at 90°) and then with water (3 × 5l. at 90°), since it was found that after the initial treatments with EDTA the carbohydrate material could be extracted with water alone. After each extraction, the suspension was filtered through muslin, or, in the later extractions, by which time the embryos had disintegrated into a flour, through glass paper (residue 665g.). When the combined extracts had been concentrated under diminished pressure to about 5l., the viscous solution was passed through the Sharples super-centrifuge (twice) and then phenol added to give a concentration of 45% (w/v). After shaking for 30min. the emulsion was set aside at 2° (Westphal, Luderitz & Bister, 1952), but the layers did not separate. It was eventually found that the emulsion could be broken by warming to 70° and adding *n*-butyric acid until the milkiness disappeared (approx. 500ml.). The aqueous layer that separated on cooling was extracted twice with ether to remove residual phenol, whereupon similar difficulties were encountered as a result of emulsion formation (broken in this case by dilution and the addition of about 3g. of Cetavlon). In view of these difficulties, further phenol extractions were postponed until a later stage.

After concentration *in vacuo* to 4l., the solution was adjusted to 75% (v/v) ethanol concentration. The precipitate was removed on a filter, redissolved in water and reprecipitated at the same ethanol concentration. After redissolution, dialysis (trial experiments had shown that there was no loss of carbohydrate through the membrane), phenol extraction (twice) and removal of the residual phenol by dialysis, the polysaccharide was isolated by freeze-drying (yield, 75g.) (Found: N, 1·41%). After complete acid hydrolysis (N-sulphuric acid at 100° for 16hr.) and neutralization (calcium carbonate) paper chromatography showed the presence of arabinose as the major component, together with substantial amounts of galacturonic acid and xylose and smaller quantities of galactose, glucose and rhamnose.

The combined filtrates from the ethanol precipitation were concentrated *in vacuo* (1·5l.) and acetone (9vol.) was added with stirring. On leaving the mixture overnight at 2°, an oil separated that was dissolved in water (500ml.), concentrated *in vacuo* (200ml.) and reprecipitated with acetone. The oil was dissolved in water and passed through a mixed-bed ion-exchange column (1200ml. of Amberlite IRA-120 and 1800ml. of Amberlite IR-45). The effluent and washings (which were neutral and gave a negative flame test for sodium) were concentrated and freeze-dried (Found: N, 2·5%; carbohydrate, 85%).

The freeze-dried material was dissolved in water and deproteinized with phenol. After removal of the residual phenol with ether, the aqueous layer was concentrated and freeze-dried (yield, 11·5g.) (Found N, 0·33%). Hydrolysis and paper chromatography showed the presence of arabinose as the major component, with small amounts of other sugars (galactose, glucose and a trace of ribose), which were readily removed by further purification (see below).

Chromatography on DEAE-cellulose. Part of the impure araban from the 75%-ethanol-soluble fraction was purified by a procedure similar to that described in the Materials and Methods section, but on a larger scale (1·5g. of polysaccharide). Although this technique is very useful as an analytical tool (Fig. 1), it is cumbersome and inconvenient for preparative use; for these purposes, purification with Cetavlon (see below) is now preferred. Most of the arabinose was contained in a single band, which was discrete and well-defined, and separated from the other polysaccharide components. The pooled fractions containing the araban were passed through cation-exchange resin, and the boric acid was removed as volatile methyl borate. The product thus isolated gave no other sugar on hydrolysis and paper chromatography, and had $[\alpha]_D$ −157° (water).

Purification by precipitation with cationic detergents. The two reagents investigated were cetylpyridinium hydroxide and cetyltrimethylammonium hydroxide, in each case the method of Barker, Stacey & Zweifel (1957) being used. No precipitation of polysaccharide occurred in the presence

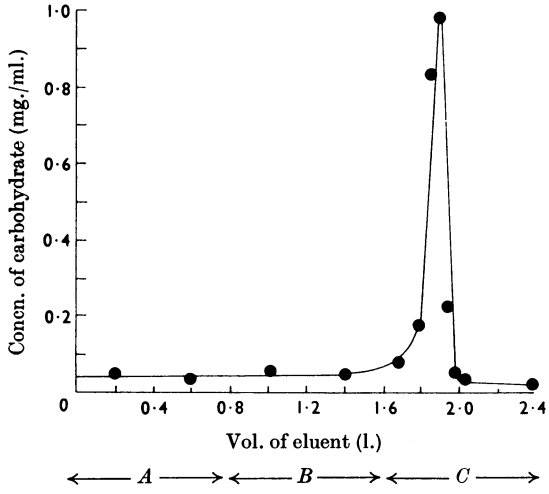

Fig. 1. Typical chromatogram of araban on DEAE-cellulose (material purified from hot-water extract of embryos). Experimental details are given in the text. Eluents: *A*, water; *B*, 0·01M-borate; *C*, 0·01–0·1M-borate (linear gradient).

of either of the reagents alone or when boric acid was added. Part of the polysaccharide was, however, precipitated when either of the reagents was added together with sodium hydroxide, and this formed the basis of an excellent method for fractionation. Both reagents gave the same fractionation, but cetyltrimethylammonium hydroxide is preferred because it is much the more stable. Cetylpyridinium hydroxide is very difficult to work with, because immediately it is prepared from the chloride it begins to decompose to coloured materials that are difficult to remove from the product.

The fractionation was performed as follows: an aqueous 10% (w/v) solution of cetyltrimethylammonium bromide (Cetavlon, purchased from British Drug Houses Ltd., Poole, Dorset) was passed through a column of Amberlite IRA-400 resin (OH^- form). A part of this solution (2ml.) was added to the crude araban (0·250g.) in water (30ml.). On adding N-sodium hydroxide solution (2ml.) a flocculent white precipitate was formed that was removed on the centrifuge after 20 min. No further precipitate was obtained by the addition of more detergent and alkali to the supernatant solution. The supernatant solution was neutralized with acetic acid, concentrated and freeze-dried. The precipitate was dissolved in 30ml. of water (containing sufficient acetic acid to give a neutral solution) and then reprecipitated by the addition of alkali and centrifuged. After redissolving in very dilute acetic acid, the polysaccharide was obtained by the addition of 9vol. of butan-2-one. It was dissolved in water and passed slowly through a column of mixed Amberlite IR-120 resin (H^+ form) and IRA-400 (CO_3^{2-} form). The solution was concentrated and freeze-dried (yield, 0·113g.) (Found: anhydroarabinose, 98·4%); it had $[\alpha]_D$ −176° (water).

Deionization of a part of the supernatant solution, followed by hydrolysis and paper chromatography, showed approximately equal amounts of galactose, glucose and arabinose.

Electrophoresis of the araban. The araban, whether purified by either technique, travelled as a single compact zone on electrophoresis under all conditions. In 0·05M-sodium tetraborate, pH9·2, it had $M_{\text{picric acid}}$ 0·16. That this movement was genuinely due to complex-formation and not to the presence of inherent acidic functions was shown by the fact that there was no movement ($M_{\text{picric acid}}$ 0·03) in 0·05M-sodium bicarbonate–sodium carbonate buffer, pH9·3. The weakly acidic groups that are indicated by the movement at higher pH ($M_{\text{picric acid}}$ 0·10 in 0·05M-sodium carbonate, pH11·4) are probably hydroxyl groups.

Methylation. The procedure is based on that of Kuhn & Trischmann (1963). Polysaccharide purified by the chromatographic method (0·250g.) was dissolved in dimethyl sulphoxide (20ml.), and dimethylformamide (20ml.) was added. After cooling in an ice bath, barium hydroxide octahydrate (20g.) followed by dimethyl sulphate (14ml.; in four portions over 30min.) was added to the efficiently stirred mixture at 0°, and stirring continued for a further 90min. At this point, the ice bath was removed and stirring continued for a further 48hr. Ammonia (sp.gr.0·88; 10ml.) was added, followed by stirring for 1hr., and the mixture was transferred to a separating funnel with chloroform (250ml.). After the chloroform layer had been washed several times with water (the stable emulsion that forms may be broken by the addition of EDTA), it was dried over sodium sulphate and concentrated to a pale-yellow glass [yield, 0·225g. (73%)] (Found: OMe, 38·3. Calc.: OMe, 38·7%).

Tentative identification of the methylated sugars formed on hydrolysis. A sample of the methylated product (1–2mg.) was sealed in a Pyrex test tube with methanolic 3% (w/v) hydrogen chloride (0·5ml.) and heated on a boiling-water bath for 6hr. After neutralization with silver carbonate, filtration and evaporation to dryness at room temperature, the residue was dissolved in a drop of chloroform and examined by gas–liquid chromatography. The pattern of peaks corresponded to that expected for a mixture of the methyl glycosides of 2,3,5-tri-*O*-methyl-, 2,3-di-*O*-methyl- and 2-*O*-methyl-arabinose. There was also some indication of traces of methyl 2,3,4-tri-*O*-methylarabinoside.

A further small portion of the polysaccharide

(1–2mg.) was hydrolysed in aq. 45% (v/v) formic acid (0·05ml.) at 100° for 3hr. Paper chromatography of the hydrolysate indicated the presence of the same methylated sugars as had gas–liquid chromatography, and in addition there was evidence for a small amount of arabinose and traces of a second monomethyl ether.

Hydrolysis and separation of the methylated sugars on a cellulose column. Methylated polysaccharide (0·5g.) was dissolved in 45% formic acid (100ml.) and heated at 100° for 3hr. The solution was carefully concentrated to dryness in a rotary evaporator, a bath temperature less than 40° being used to prevent loss of the volatile 2,3,5-tri-*O*-methylarabinose. The syrup was left in an evacuated desiccator over sodium hydroxide pellets for 24hr., redissolved in water and allowed to stand at room temperature for 24hr., before once more being concentrated to a syrup (0·470g.). This syrup was separated on a cellulose column (50cm. × 3·7cm. diam.) by using the sequence of solvents: (i) water-saturated butan-1-ol–light petroleum (b.p. 100–120°) (1:3, v/v; upper layer); (ii) the same solvents but mixed in the proportions 1:1 (upper layer); (iii) the same solvents but mixed in the proportions 3:1 (upper layer); (iv) butan-1-ol half saturated with water. About 800ml. of each solvent was used. Fractions (25ml.) were collected automatically and examined by paper chromatography. The first solvent eluted pure 2,3,5-tri-*O*-methyl-L-arabinose (0·180g.), identified as the amide of the derived aldonic acid (m.p. 136–137°, not depressed on mixing with authentic material; cf. Laidlaw & Percival, 1952), followed by a mixed fraction (0·007g.) containing 2,3,5-tri-*O*-methylarabinose and 2,3-di-*O*-methylarabinose. The second solvent eluted pure 2,3-di-*O*-methyl-L-arabinose (0·083g.), identified as the amide of the derived aldonic acid, m.p. 158–159° (cf. Laidlaw & Percival, 1952). Unfortunately, the sample available of the authentic derivative was rather impure (m.p. 149–150°), but the mixed m.p. indicated the identity of the two compounds (mixed m.p. 151–152°), and the X-ray powder photographs were identical. A small mixed fraction (0·010g.) was obtained by continued elution with the second solvent; this contained 2,3-di-*O*-methylarabinose, 2,3,4-tri-*O*-methylarabinose and traces of another component that might have been 2,4- or 2,5-di-*O*-methylarabinose. The third solvent gave 2-*O*-methyl-L-arabinose (0·150g.), identified as the crystalline toluene-*p*-sulphonylhydrazone (m.p. and mixed m.p. 146°; cf. Aspinall & Baillie, 1963), and a mixture (0·020g.) containing in addition a small amount of a component that might have been a second mono-*O*-methyl ether. The fourth solvent eluted L-arabinose (0·025g.), which was identified as the toluene-*p*-sulphonylhydrazone (m.p. and mixed m.p. 153°; cf. Aspinall & Baillie, 1963).

DISCUSSION

So far as we are aware, the only other published investigations of mustard-seed polysaccharides are those of Bailey & Norris (1932) and Bailey (1935), who isolated a mucilage in 2% yield by extraction of the whole seed with cold water. This was fractionated into cellulose and two acidic polysaccharides, each of which contained galactose, galacturonic acid and arabinose. This agrees well with our own results for the composition of the cold-water extract of the seed coats (Table 1), although we have not found rhamnose and glucuronic acid (which were also reported by the early workers) in this fraction.

The most striking feature of the results of the survey (Table 1) is the preponderance of those sugars that are thought of as major constituents of pectic materials, i.e. galactose, arabinose and galacturonic acid. Indeed, with the exception of some of the less-soluble 'hemicellulosic' and cellulosic fractions giving xylose or glucose as the main sugar on hydrolysis, all fractions contained these three sugars with one of them as the main sugar.

We have chosen the polysaccharides of the embryos for further study because it is presumably in this part of the seed that the more marked biochemical changes occur on germination. A particularly simple extraction pattern is to be seen here. Polysaccharides having arabinose as the major sugar component are extracted first, but after their removal and when the extraction conditions are more severe xylose becomes predominant. No evidence was found for the presence of starch (iodine-staining) or fructans (Seliwanoff test). Considerable amounts of protein were present in all fractions (e.g. Table 2). In view of the close similarity in composition between the fractions obtained from the embryos with cold water, hot water and ammonium oxalate, these were extracted together for more detailed investigation. Preliminary experiments showed that EDTA (McCready & McComb, 1952) was a more efficient solvent than ammonium oxalate for these polysaccharides, and it was therefore used for the preparative extraction. After purification, two main fractions were obtained, both giving arabinose on hydrolysis. In one case relatively small amounts of other sugars were also present, notably galacturonic acid, but the second fraction ('mustard-seed araban') gave only arabinose. The araban moved as a discrete band on electrophoresis and when chromatographed on DEAE-cellulose (Fig. 1). The physical properties suggested a similarity with pectic arabans (cf. Hirst & Jones, 1946), and this was confirmed by the results of methylation analysis.

The four major products of hydrolysis of the methylated polysaccharides were L-arabinose, 2-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-L-arabinose

and 2,3,5-tri-*O*-methyl-L-arabinose; these were isolated in pure form by column chromatography in the molar proportions 2:11:6:12 and characterized unambiguously by the formation of crystalline derivatives. From the weights of these sugars it appears that they represent at least 95% of the structural units in the araban. Although there is no direct evidence from our results about the ring size of the arabinose units in the polysaccharide other than at the non-reducing end groups, it seems likely that they are all of the same type, i.e. furanose, the units being joined by 1→5-linkages with a considerable proportion of 1→3-branch linkages. The high negative value for the optical rotation suggests that the units are α-linked. The isolation and characterization of a small amount of arabinose from the methylated polysaccharide after hydrolysis indicates that there is a second mode of branching in the polysaccharide, namely through position 2 of a relatively small proportion of units that are also linked through positions 3 and 5. It is unlikely that this arabinose arises merely from incomplete methylation of the polysaccharide, since it was repeatedly observed in about the same concentration after different methylation experiments, and its concentration was not diminished by remethylation by different techniques. The structure indicated by these results is typical of that normally associated with pectic arabans (Hirst & Jones, 1946; Hough & Powell, 1960), except that it is rather more highly branched and that it contains a proportion of 1→2-linkages.

Preliminary fractionation and methylation experiments with the other main fraction of the EDTA extract (D. A. Rees, N. G. Richardson & J. W. B. Samuel, unpublished work) suggest that the major component is a 'pectic araban' containing a significant proportion of covalently bound galacturonic acid and other sugar units. The results of both these methylation studies therefore reinforce the general impression that a high concentration of pectic-type polysaccharides is present in mustard seed. The possibility that these two polysaccharides are related chemically or biologically is under investigation. It seems unlikely that the neutral araban is an artifact that is split from a larger acidic polysaccharide by base-catalysed elimination during extraction, e.g. at uronic acid units, because extraction with EDTA at different pH values (4·9 and 7·5) gave the neutral araban in similar yields.

Purification of mustard-seed araban is readily effected by methods that make use of the ability of the molecule to form a complex with borate ions, or of the fact that the hydroxyl groups ionize in alkaline solution, or of both. A rationalization of the purification methods in these terms is consistent with the electrophoretic behaviour of the araban described in the Results section. It is surprising that the araban forms a complex with borate since model compounds (e.g. methyl α- and β-arabinofuranoside and methyl α- and β-xylopyranoside) that contain the major structural features that are consistent with the methylation results do not form complexes (Foster, 1957). Three explanations of the complex-formation may be suggested that might be separately or jointly responsible: (i) it is likely that the reducing arabinose unit in each polysaccharide molecule will be able to form a complex with borate; (ii) in the highly branched araban molecule it is possible that some strain is present arising from steric factors, and that this leads to the distortion of some sugar units, thereby altering the distance between hydroxyl groups and making complex-formation possible; (iii) borate ions might form a complex with the polysaccharide by bridging different sugar units.

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