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Analysis of polysaccharides and monosaccharides in the root mucilage of maize (*Zea mays* L.) by gas chromatography

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Abstract

Root mucilage of maize (*Zea mays* L.) was purified using Sephadex size-exclusion chromatography to allow subsequent analysis of the polysaccharides derived from this mucilage. Hydrolysis of the polysaccharides to their constituent monosaccharides and conversion of these monomers to volatile peracetate derivatives allowed analysis of these derivatives using gas chromatography. This permitted identification and broad quantification of the major components of the polysaccharides. Two methods have been developed: (1) a two-step hydrolysis/acetylation procedure and (2) a one-step acetolysis. Gas chromatograms obtained using the latter procedure are far simpler due to the formation of predominantly one anomer for each monomer component. In both cases, the major monosaccharide components of the polysaccharides were identified as fucose, arabinose, galactose and glucose. Analysis of the crude maize mucilage demonstrated that monosaccharides co-exist with polysaccharides. The monosaccharides were again converted to their peracetates and gas chromatography identified the major monosaccharide component as glucose.

Keywords: *Zea mays*; Polysaccharides; Monosaccharides

1. Introduction

Plant roots actively modify the rhizosphere (the region of soil influenced by the root) through the production of mucilage—a complex polysaccharide gel. Understanding the chemical composition of root mucilages is essential to quantify the level and nature of carbon deposition from roots into soils and identify the mechanisms of recognition and interaction between plant roots and other organisms—both beneficial and pathogenic. However, the elucidation of the structures of such polysaccharides is complex and typically requires multi-step strategies (e.g., maize mucilage [1], rice mucilage [2] and cress mucilage [3]). The aim of this work is to develop a practical method for the rapid identification and quantification of monosaccharide constituents of polysaccharides derived from maize mucilage.

Although several methods are available for the elucidation of polysaccharide structures, commonly employed strategies often commence with enzymatic or total acid hydrolyses [4]. The aims of such strategies are to efficiently release the constituent monosaccharide building blocks from the parent polysaccharide, with minimum decomposition, and to derivatise these fragments to enable their subsequent identification and quantification. Typical derivatisation strategies include formation of volatile

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silyl ethers, methyl ethers and alditol acetates which are easily identifiable by analytical methods including high-performance liquid chromatography (HPLC) and gas chromatography (GC) [5–10]. In this paper we describe our development and application of two derivatisation and analytical methods which have provided useful results for the identification and broad quantification of the constituent monosaccharides of maize mucilage polysaccharides. We have analysed the mucilage in both its crude form and a purified form (using Sephadex size-exclusion chromatography) to allow analysis of both free monosaccharides present in the mucilage and monosaccharide constituents of the parent polysaccharides.

2. Experimental

The two analytical methods which we have applied both involve the production of peracetylated sugar monomers following acid hydrolysis of the polysaccharide components. Therefore peracetylated standards were required to allow comparison of GC retention times.

2.1. Preparation of standards

Some acetylated sugars were commercially available (Aldrich), for instance α-D-glucose pentaacetate and β-D-galactose pentaacetate. For this study, most of the peracetylated standards were prepared from the corresponding monosaccharides—glucose, galactose, mannose, rhamnose, fucose, arabinose, xylose and ribose (Aldrich)—together with the amines and uronic acids of glucose and galactose. Where the starting material was anomerically pure, interconversion to produce a mixture of anomers was achieved by freeze drying an aqueous solution of the monosaccharide prior to acetylation.

For the acetylation, free monosaccharide (0.5 g) was stirred at room temperature under nitrogen with anhydrous pyridine (15 ml) and acetic anhydride (3 ml, excess). When the reaction was complete (usually 24 h, as indicated by TLC) the mixture was cooled in an ice bath and 37% hydrochloric acid (15 ml) was added dropwise. The resulting aqueous phase was extracted with ethyl acetate (4×30 ml). The organic phases were washed with aqueous copper(II) sulphate solution, then twice with distilled water. The organic phases were then dried over magnesium sulphate, filtered and evaporated under reduced pressure at room temperature to give an oily product. This was purified by flash column chromatography on silica (eluting with diethyl ether–light petroleum ether, 70:30) and recrystallisation from diethyl ether–pentane or diethyl ether–light petroleum mixtures.

2.2. Preparation of plant mucilages

Seeds of *Zea mays* L. cv. Freya were imbibed for 1 h in deionised water, surface-sterilised in sodium hypochlorite solution then rinsed in sterile deionised water. The seeds were germinated on moist filter papers in Petri dishes, in the dark at 26°C. After 3–4 days, mucilage was collected from the tips of the germinating roots (3–4 cm long by this stage) using a drawn glass Pasteur pipette, in a laminar flow cabinet. The mucilage was centrifuged at 12 000 rpm for 30 min, decanted and passed through a 0.45-μm syringe filter to remove all solid plant material.

Separation of the large polysaccharides from smaller monosaccharide and oligosaccharide components in the crude maize mucilage was achieved using Sephadex size-exclusion chromatography. Maize mucilage was diluted to twice its volume with distilled water (to reduce the mucilage viscosity) and loaded onto columns containing Sephadex G-25 gel (9 ml, bed height 5 cm). The samples were then eluted with distilled water and small fractions (1.5 ml) of eluent collected. Each fraction was analysed by spotting small volumes of sample on silica plates and staining with an orcinol dip [prepared by mixing ethanol (150 ml), sulphuric acid (3.38 ml) and 3,5-dihydroxytoluene monohydrate (3 g) [4]] with heating to detect the presence of carbohydrate components. Carbohydrates were typically present in the initial six fractions and these were freeze-dried separately prior to hydrolysis and analysis.

2.3. Hydrolysis/acetylation procedures

Two methods of hydrolysis and acetylation were used in this study: Method A, a two-step process involving hydrolysis of polysaccharides with aque-

ous 2 *M* trifluoroacetic acid (TFA) followed by acetylation with acetic anhydride in pyridine; Method B, a one-step acetolysis involving simultaneous hydrolysis and acetylation of the mucilage polysaccharides in a mixture of acetic anhydride, glacial acetic acid and conc. sulphuric acid (10:10:1). It was more convenient to use TFA than mineral acids in the first method due to the greater volatility of TFA which aids its removal after hydrolysis.

2.3.1. Method A: two-step hydrolysis/acetylation

2.3.1.1. Hydrolysis

The saccharides were refluxed in 2 *M* TFA at 100°C for 2 h in a round-bottomed flask equipped with a reflux condenser and a magnetic stirrer bar. The TFA was then removed in vacuo and the water was removed by freeze drying overnight (10^{-1} mbar at -40°C).

2.3.1.2. Acetylation

Anhydrous pyridine and acetic anhydride (excess) were added to the resulting mixture, under nitrogen. After stirring at room temperature overnight, the reaction mixture was cooled in an ice bath. HCl was added dropwise and the aqueous phase was then extracted with ethyl acetate. The organic layer phases were dried over magnesium sulphate, filtered and evaporated under reduced pressure at 60°C to give an oily product which was analysed by GC without further purification.

2.3.2. Method B: one-step acetolysis

Acetic anhydride–glacial acetic acid–sulphuric acid (10:10:1) were refluxed with the mixture of saccharides at 40°C for 3 h in a round-bottomed flask equipped with a reflux condenser and a magnetic stirrer bar. The reaction mixture was cooled in an ice bath, water was added dropwise and the resulting aqueous phase was extracted with diethyl ether. The organic layer phases were then washed with saturated potassium carbonate solution and dried over magnesium sulphate, filtered and evaporated under reduced pressure at 60°C to give an oily product.

2.4. GC and NMR analysis

The peracetylated standards were characterised by ^{1}H NMR and GC. All NMR analyses were performed on a Bruker 250 MHz Fourier transform (FT) NMR or a JEOL 400 FT-NMR spectrometer using tetramethylsilane (TMS) as an internal reference. Assignments were made by ^{1}H, ^{1}H COSY or by comparison with the literature [11,12]. All GC analyses were carried out on a Varian 3400 fitted with a Restek Rtx-225 column (15 m×0.25 mm I.D., 0.25-μm coating, 190°C isothermal elution). Hydrogen produced by a Whatman 75-32 hydrogen generator was used as the carrier gas (2 ml/min) and as the fuel for the flame ionisation detector. For plant mucilage samples, peaks were identified by comparison of retention times to derivatised standards and quantification was achieved by comparison of peak areas to those of standard solutions of each sugar.

3. Results and discussion

3.1. Standards

Table 1 shows the ^{1}H NMR results and GC retention times for each anomer of the eight peracetylated neutral monosaccharides and some of the amines of glucose and galactose. Anomeric assignments were made on the basis of coupling constants for the protons at C_1 and C_2. All the neutral sugars and amines gave stable peracetates. In contrast, peracetylated uronic acid derivatives were unstable at room temperature. Acetylation of galacturonic acid gave a product with GC peaks at 8.2 and 15.2 min, and acetylated glucuronic acid showed peaks at 8.4 and 19.2 min, but the intensity of these peaks gradually diminished, the solutions turned dark brown and various new peaks appeared on the chromatograms. Acetylation of simple organic acids such as citric acid, produced the same unstable behaviour.

One serious overlap in retention times occurs (at 5.3 min, between β-rhamnose and α-xylose), otherwise the GC method is able to separate all the neutral anomers successfully. Detection of all the peracetylated standards was possible at (individual) concentrations $<10^{-3}$ *M*.

Fig. 1a shows the gas chromatogram resulting from acetylation of a freeze-dried mixture of the

Table 1
^{1}H NMR chemical shifts (ppm) for peracetylated monosaccharides in $C^{2}HCl_{3}$ and GC retention times

	H-1	H-2	H-3	H-4	H-5	H-5′	H6	H6′	H (acetyl groups)	GC retention time (min)	GC peak no.
1,2,3,4-Tetra-*O*-acetyl-α-L-rhamnopyranose	5.99 d (1.5)	5.04–5.36 m	5.04–5.36 m	5.04–5.36 m	3.85–3.97 m	–	1.21 d (6.0)	–	1.71–2.41 m	4.77	1
1,2,3,4-Tetra-*O*-acetyl-α-L-fucopyranose	6.31 d (1.5)	5.01–5.58 m	5.01–5.58 m	5.01–5.58 m	4.25 q (6.5)	–	1.14 d (6.5)	–	1.86–2.55 m	5.15	2
1,2,3,4-Tetra-*O*-acetyl-β-L-rhamnopyranose	5.81 d (1.0)	5.04–5.36 m	5.04–5.36 m	5.04–5.36 m	3.60–3.70 m	–	1.26 d (6.0)	–	1.71–2.41 m	5.29	3a
1,2,3,4-Tetra-*O*-acetyl-α-D-xylopyranose	6.23 d (3.5)	4.94–5.05 m	5.44 t (10.0)	4.94–5.05 m	3.91 dd (6.0,11)	3.68 t (11)	–	–	1.73–2.41 m	5.32	3b
1,2,3,4-Tetra-*O*-acetyl-β-L-fucopyranose	5.66 d (8.0)	5.01–5.58 m	5.01–5.58 m	5.01–5.58 m	4.21–4.29 m	–	1.28 d (6.5)	–	1.86–2.55 m	5.72	4
1,2,3,4-Tetra-*O*-acetyl-β-D-arabinopyranose	6.31 d (2.5)	5.17–5.38 m	5.17–5.38 m	5.17–5.38 m	4.04 dd (13.0)	3.8 dd (1.5,13.0)	–	–	1.73–2.39 m	6.02	5
1,2,3,4-Tetra-*O*-acetyl-β-D-xylopyranose	5.69 d (7.0)	4.94–5.05 m	5.18 t (8.0)	4.94–5.05 m	4.12 dd (5.0,12.0)	3.63-3.73	–	–	1.73–2.41 m	6.27	6
1,2,3,4-Tetra-*O*-acetyl-α-D-arabinopyranose	5.63 d (7.0)	5.17–5.38 m	5.08 dd (3.5,9.0)	5.17–5.38 m	4.37 dd (9.5)	4.19 dd (9.5)	–	–	1.73–2.39 m	7.45	7
1,2,3,4-Tetra-*O*-acetyl-β-D-riboopyranose	6.00 d (5.0)	5.20–4.94 m	5.47 app t (3.0)	4.00–3.75 m	4.02 dd (12.0,3.5)	3.90 dd (12.0,5.5)	–	–	2.00–2.20 m	7.59	8
1,2,3,4-Tetra-*O*-acetyl-α-D-riboopyranose	6.10 d (1.0)	4.80–5.20 m	5.55 app t (3.0)	4.80–5.20 m	4.02 dd (12.0,3.5)	3.90 dd (12.0,5.5)	–	–	2.00–2.20 m	8.16	9
2-Acetamido-1,3,4,6-tetra-*O*- acetyl-2-deoxy-α-D-glucopyranose	6.13 d (3.5)	4.45 ddd (10.5, 9.0, 3.5)	5.00–5.27 m	5.00–5.27 m	3.96 ddd (2.5,4.0,9.5)	–	4.21 dd (4.0,12.5)	4.03 dd (2.5,12.5)	1.75–2.05 m	10.06	
2-Acetamido-1,3,4,6-tetra-*O*- acetyl-2-deoxy-β-D-glucopyranose	5.68 d (9.0)	4.20–4.40 m	5.00–5.27 m	5.00–5.27 m	3.85 ddd (2.0,4.0,9.5)	–	4.28 dd (4.0,12.5)	4.11 dd (2.0,12.5)	1.75–2.05 m	12.75	
2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-β-D-galactopyranose	5.67 d (9.0)	4.30–4.49 m	5.09 dd (11.0,3.0)	5.30 d (3.0)	3.90–4.10 m	–	3.90–4.10 m	3.90–4.10 m	1.75–2.07 m	13.80	
1,2,3,4,6-Penta-*O*-acetyl-α-D-galactopyranose	6.37 d (1.5)	5.22–5.32 m	5.22–5.32 m	5.50 d (1.5)	4.34 dt (1.0,6.5)	–	4.11 d (6.5)	4.08 d (6.5)	2.00–2.20 m	17.75	13
1,2,3,4,6-Penta-*O*-acetyl-α-D-glucopyranose	6.33 d (3.5)	5.00–5.20 m	5.47 app t (9.5)	5.00–5.20 m	4.05–4.19 m	–	4.05–4.19 m	4.26 dd (12.0,3.5)	2.00–2.20 m	18.48	14
1,2,3,4,6-Penta-*O*-acetyl-β-D-glucopyranose	5.65 d (8.0)	5.00–5.25 m	5.00–5.25 m	5.00–5.25 m	3.85 ddd (2.0,4.0,9.5)	–	4.25 dd (4.0,12.5)	4.05 dd (2.0,12.5)	2.00–2.20 m	18.72	15
1,2,3,4,6-Penta-*O*-acetyl-α-D-mannopyranose	6.06 d (2.0)	5.23–5.38 m	5.23–5.38 m	5.23–5.38 m	3.99–4.15 m	–	4.22–4.33 m	3.99–4.15 m	1.72–2.20 m	19.78	16
1,2,3,4,6-Penta-*O*-acetyl-β-D-galactopyranose	5.70 d (8.0)	5.30 dd (8.0, 10.5)	5.11 dd (3.5,10.5)	5.43 dd (3.5,1.0)	4.00–4.25 m	–	4.00–4.25 m	4.00–4.25 m	1.95–2.20 m	20.19	17
1,2,3,4,6-Penta-*O*-acetyl-β-D-mannopyranose	5.84 d (1.0)	5.46 dd (1.0, 3.0)	5.1 dd (3.0,10.0)	5.23–5.38 m	3.78 ddd (2.0,5.0,7.5)	–	4.22–4.33 m	3.99–4.15 m	1.72–2.20 m	21.91	18

Signal multiplicities: d, doublet; dd, doublet of doublets; dt, doublet of triplets; ddd, doublet of doublet of doublets; t, triplet; app t, apparent triplet; q, quadruplet; m, multiplet. Numbers in parentheses indicate coupling constants (Hz).

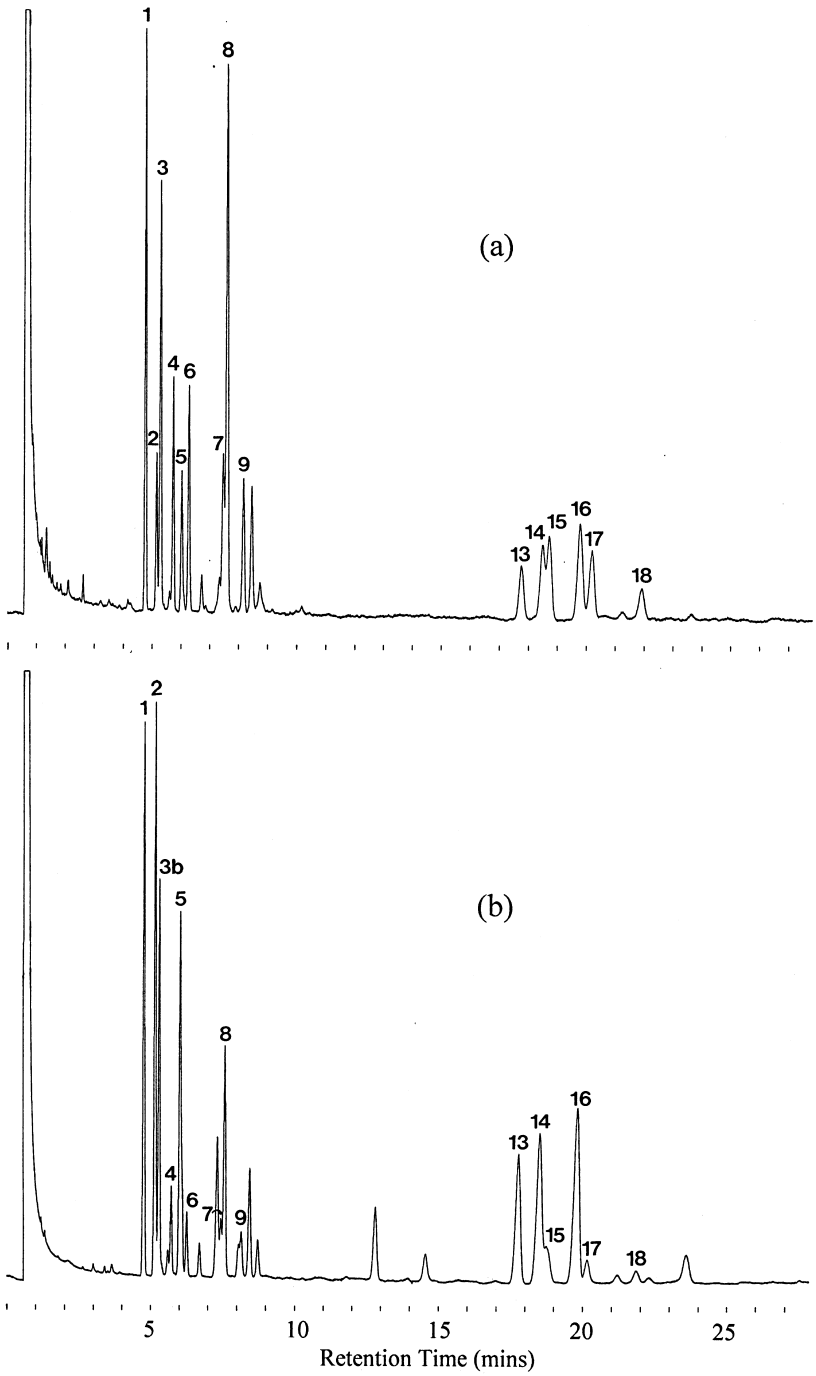

Fig. 1. Gas chromatograms resulting from the acetylation of a freeze-dried mixture of eight neutral monosaccharides: (a) acetylation with acetic anhydride in pyridine produces peaks corresponding to the α- and β-anomers of each sugar; (b) acetylation with acetic anhydride–glacial acetic acid–sulphuric acid (ratio 10:10:1) gives predominantly one peak per sugar, corresponding to the more stable anomer in each case. Peak assignments are listed in Table 1.

eight neutral monomers. Two peaks per sugar can be assigned to the α- and β-anomers, respectively (with the exception of the one overlap). If a mixture of the same monosaccharides is acetylated under one-step conditions, predominantly one peak per sugar is observed in GC—six corresponding to α-peracetates and two (arabinose and ribose) corresponding to β-peracetates (Fig. 1b), presumably the more stable anomers in each case. It should be noted that different quantities of monosaccharides were used in the two derivatisations although, in both cases, the sugars commonly found in mucilages (glucose, galactose, fucose and arabinose) were mixed in equal amounts by weight. The main purpose of Fig. 1, however, is to show (1) that we can separate almost all of the isomers on the basis of their retention times and (2) that the peak ratio for the isomers of each sugar is different according to the derivatisation procedure.

In addition to the major peaks corresponding to the α- and β-pyranose forms of each sugar, acetylation of the pentoses and deoxyhexoses usually produced one or two minor peaks (in the region 5–9 min) which may be due to furanose forms. The origin of the peaks at 12–15 and 23–24 min is unknown; however, these generally occur under one-step acetylation conditions only.

To test and compare the hydrolysis/acetylation methods prior to analysis of the more complex maize mucilage, a commercial sample of a disaccharide, β-D-lactose, was subjected to both one- and two-step procedures. Two-step hydrolysis/acetylation of lactose gave four peaks on subsequent GC analysis, corresponding to the α- and β-anomers of glucose and galactose pentaacetate. Under one-step acetolysis conditions, however, just two peaks were observed when the product was analysed by GC, corresponding to only the α-anomers of glucose and galactose pentaacetate. To understand this mechanism further, a sample of β-glucose peracetate was subjected to the same acetolysis conditions. Since it was converted entirely to α-glucose peracetate, interconversion of the anomers must occur under the reaction conditions to produce the thermodynamically favoured anomer. Furthermore, when a sample of commercially available β-D-lactose octaacetate was subjected to these reaction conditions, the α-anomers of glucose and galactose pentaacetates were formed exclusively. Hence hydrolysis of the saccharides to produce monomer units need not necessarily be the first step of the reaction process.

3.2. Analysis of maize mucilage

Fig. 2 shows the various preparative and analytical procedures followed in our study of mucilage composition, and Table 2 summarises the results of all the GC analyses. When the mucilage was analysed in crude form, the dominant component identified by GC was glucose (nearly 80%, Fig. 3a), with the two-step hydrolysis/acetylation in good agreement with the one-step acetolysis procedure. After separation of the polysaccharides from the oligosaccharide components, a clear contrast was apparent between the compositions of the two fractions. Both two- and one-step acetylation procedures showed the major component of the polysaccharide to be fucose (>60%, Fig. 3c and d). In the oligosaccharide fraction, however, <1% fucose was present and the analysis was again dominated by glucose (~95%, Table 2). Furthermore, when the oligosaccharide fraction was acetylated *without* hydrolysis, a very similar result was obtained (Fig. 3b), demonstrating that the glucose was originally present in the mucilage in monosaccharide form. The slight variation in the analyses when the oligosaccharide fraction is acetylated with and without prior hydrolysis may indicate the presence of some small polymers.

There is no clear evidence in these analyses that amines or uronic acids are present in addition to the neutral sugars. Small peaks do occasionally appear on the chromatograms at the expected retention times for the amines but no consistent pattern is observed. Also, detection of uronic acids is unlikely in view of the instability of these acetylated monosaccharides.

In agreement with the results obtained with our simple disaccharide study, each monosaccharide unit released from the maize polysaccharide during the two-step hydrolysis/acetylation protocol (Method A) displayed at least two peaks in the GC corresponding to the α- and β-anomers of the pyranose units. Smaller peaks, presumably corresponding to the furanose form of the monosaccharides, were sometimes observed. As the formation of multiple peaks for each monosaccharide unit is a result of mutarotation, it is not possible to obtain information con-

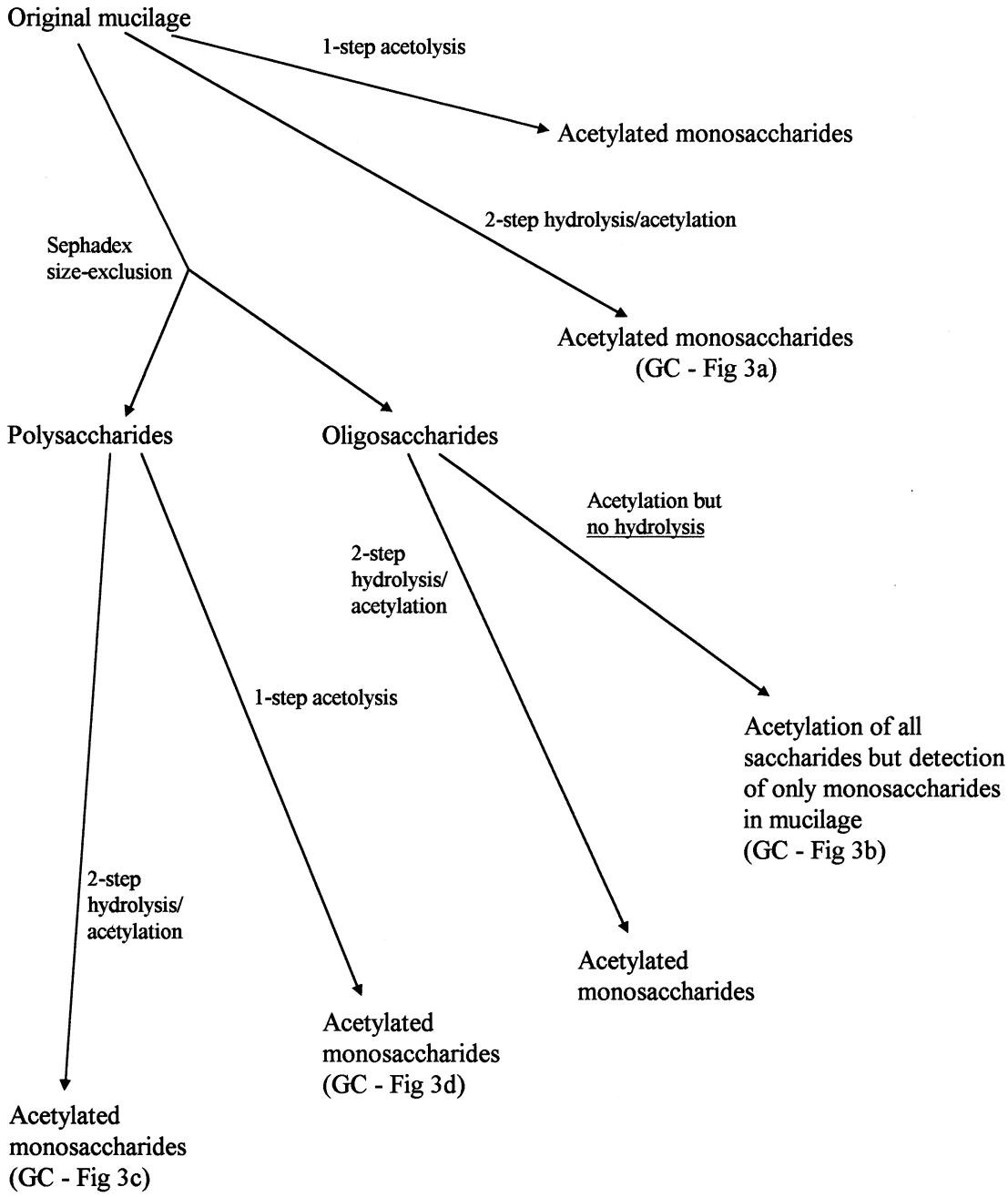

Fig. 2. Summary of the preparative and analytical procedures used to study maize root mucilage composition.

cerning the nature of glycosidic linkages within the polysaccharide using this protocol. However, as the individual peaks in the GCs were almost always sufficiently resolved, identification of the major monosaccharide constituents of the parent polysaccharide by comparison with known standards was easily possible.

Our second strategy, namely the one-step hydrol-

Table 2
Neutral monosaccharide analysis of maize mucilage (concentrations in mol%), listed in order of increasing GC retention time of the first peak for each sugar

	Original mucilage		After Sephadex size-exclusion chromatography			
	Two-step, Method A (Fig. 3a)	One-step, Method B	Polysaccharide fraction		Oligosaccharide fraction	
			Two-step, Method A (Fig. 3c)	One-step, Method B (Fig. 3d)	Two-step, Method A	Acetylated, *not hydrolysed* (Fig. 3b)
Rhamnose	0	0	2.2	0	0	0.1
Fucose	7.1	10.1	66.2	61.0	0.3	0.2
Xylose	2.4	0	1.8	0	0	0
Arabinose	4.7	3.2	14.2	4.4	1.4	0.2
Galactose	5.0	7.3	7.1	3.2	2.1	5.1
Glucose	79.9	79.1	8.4	31.4	95.8	87.5
Mannose	1.0	0.3	0	0	0.3	6.9

ysis-derivatisation protocol (Method B), also allowed identification of the major monosaccharide constituents of the parent polysaccharide present in maize mucilage. The gas chromatograms obtained in this manner were again simplified compared with the two-step protocol. However, an interesting anomaly is apparent in the case of fucose, where the retention time of the major peak is consistent with the presence of β-fucose peracetate. Acetylation of monosaccharides under one-step acetolysis conditions favoured the formation of peracetylated derivatives in their thermodynamically stable forms—presumably as a result of the anomerisation mechanism outlined by Kaczmarek et al. [13]. However, acetolysis of the mucilage polysaccharide produces the β-derivative of fucose almost exclusively and not the α-anomer as predicted. The abundance of the β-anomer may suggest that fucose residues are released late in the hydrolysis process. If this is the case, the kinetically favoured β-anomer may have insufficient time to convert to the thermodynamically stable α-form.

The one- and two-step protocols employed by us are complementary procedures. Thus, in the former procedure, 1→6 glycosidic linkages are the most susceptible to attack, whereas in the latter they are less easily ruptured [14]. The procedures we have used are based on methods previously optimised to balance hydrolysis efficiency with polysaccharide/monosaccharide decomposition [4].

As predominantly one anomer was produced for each monosaccharide using the one-step acetolysis protocol, the gas chromatograms were considerably simplified compared with the two-step protocol previously employed. Furthermore, this simplification allows more sensitive identification of monosaccharide constituents and even allows for time-dependent analysis by removal of small aliquots of material at timed intervals. In this way, tentative identification of linkages may be obtained, and sensitive carbohydrate monomers which may be decomposed under the reaction conditions may be observed.

4. Conclusion

Analysis of the results obtained with crude and purified mucilage has provided data on both the monosaccharide and polysaccharide components of the mucilage. The results obtained with polysaccharides obtained by Sephadex purification of the mucilage illustrate that these components are mainly derived from fucose, arabinose, galactose and glucose. In contrast, acetylation of crude mucilage reveals data on the identity of monosaccharide components of mucilage and in this case it is found that glucose is by far the principal component. Indeed, the majority of the neutral sugars found in the crude mucilage are present as free monosaccharides and not bound within polysaccharides. This result contrasts with previous analyses of maize mucilage [1,15], and the reason for this discrepancy is not yet clear. However, high glucose contents (88.8%) have been reported in unfractionated rice

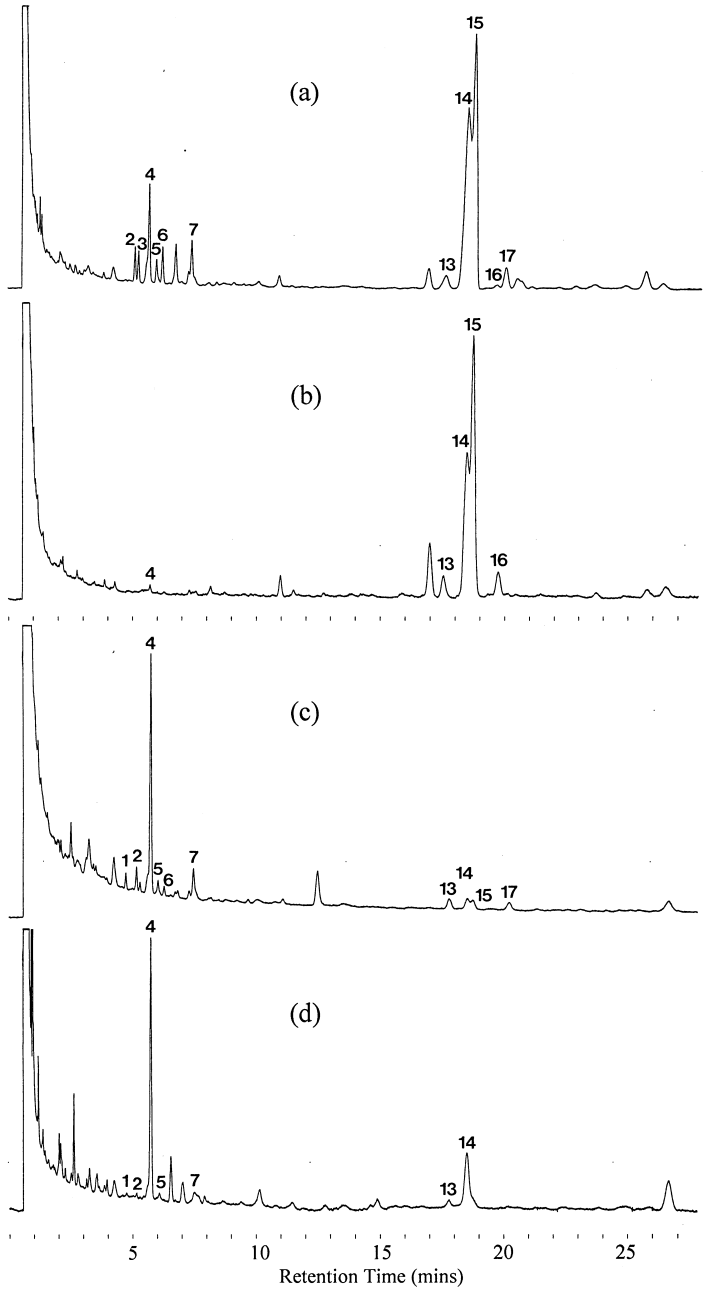

Fig. 3. Gas chromatograms from the analyses of maize root mucilage: (a) mucilage analysed in crude form using the two-step hydrolysis/acetylation procedure; (b) analysis of the free monosaccharide components of the mucilage showing glucose to be the main component; (c) two-step hydrolysis/acetylation of the polysaccharide fraction of the mucilage showing predominantly fucose; (d) one-step acetolysis of the polysaccharide fraction confirming fucose as the major polysaccharide component.

mucilage [2], and even after dialysis to remove diffusible small sugar molecules, the rice mucilage still contained 37.9% glucose.

Soil in the vicinity of the root supports higher numbers of organisms than soil more distant from the plant and this 'rhizosphere effect' is evident, not only in total numbers, but in the preferential stimulation of certain groups of microorganisms. The high level of monosaccharide glucose found in this study has significant implications for the rate of carbon turnover around roots, as monosaccharides are rapidly broken down by soil microbes [16]. At this stage, there is no evidence that the plant is able to control the composition of the mucilage it secretes. However, the simple, relatively rapid analytical methods described here will facilitate comparison of mucilages collected at different stages of the plant's development, before and after photosynthesis has commenced.

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