Cactaceae Mucilage Composition

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Determination of the main structural features of the mucilages from *Opuntia monacantha*, *Opuntia nopalea-coccinillifera*, *Cereus peruvianus* and *Wigginsia erinacea* by hydrolysis and methylation indicated that these polysaccharides have some similarity to both pectic polysaccharides and gum exudates, and, consequently, may provide an alternative source of industrial raw materials. The chemical composition of the mucilages is consistent with a role in the storage of moisture in Cactaceae and may be used to provide a useful method of taxonomy within the family.

1. Introduction

Cactaceae, a family of the order Centrospermales, are well adapted to the dry regions of the new world, where the plants have a marked capacity to withstand prolonged dry spells without collapsing. The polysaccharide mucilages present in the parenchyma of these plants have had limited industrial application to date and with the exception of cholla gum, the mucilage from *Opuntia fulgida*, structural studies have been few, although these materials have on occasion been referred to as pectins. The present study is primarily directed at obtaining further information on the main structural features of these mucilages.

Since plant polysaccharides of certain structural types can form macroscopic three-dimensional networks capable of imbibing large proportions of water, the ability of Cactaceae to retain moisture under unfavourable climatic conditions in which the polysaccharide mucilages play a part might involve such structures. In addition, since taxonomy within the family is rather difficult, there being differences in the number of species, varieties, etc., described by different authors, an approach based on the chemical structures of the mucilages could prove valuable.

In the present instance, the mucilages studied were those from *Opuntia monacantha* (Opuntia monacantha Haworth. Cerro, Montevideo. Mr Miguel A. Muriel (MAM) Collection No. 1827), *Opuntia nopalea-coccinillifera* (Opuntia nopalea-coccinillifera Mill. S. Dyck. MAM Collection No. 760), *Cereus peruvianus* (Cereus peruvianus Mill. La Totora, Colonia. MAM Collection No. 106), and *Wigginsia erinacea* (Wigginsia erinacea Br. and Rose. La Totora, Colonia. MAM Collection No. 069).
2. Experimental

2.1. General methods

*Paper chromatography* was carried out on Whatman No. 1 paper using the following solvent systems: (a) EtOAc-Pyr-H₂O (10 : 4 : 3); (b) EtOAc-HOAc-HCOOH-H₂O (18 : 3 : 1 : 4); (c) n-BuOH-EtOH-H₂O (4 : 1 : 5, upper); and (d) MeEtCO-H₂O-NH₃ (200 : 17 : 1). Sugars were detected by spraying the dried chromatograms with a saturated alcoholic solution of aniline oxalate and heating in an oven at 120–130 °C.

*Liquid chromatography* was performed on a Pye Liquid Chromatograph using a column (50 × 0.4 cm) of strongly acidic cation exchange resin (Aminex A-6, Bio-Rad Labs.) in the lithium form equilibrated with 85% ethanol/water at 75 °C.¹⁷ The column effluent was continuously monitored using a modified moving wire detector.¹⁸ The determinations were kindly carried out by Mr J. S. Hobbs.

*Gas chromatography* (g.l.c.) was performed on a Pye “104” Chromatograph using a column (150 × 0.3 cm) of 5% by weight of polyethylene glycol 20M (5% PEG 20M) on 100–120 mesh acid washed silanised celite at 185 °C unless otherwise stated. Retention times (T) were measured relative to methyl 2,3,4,6-tetra-O-methyl-β-glucoside.

*Gas chromatography–mass spectrometry* (g.l.c.–m.s.) was performed on a Pye “104” Chromatograph linked to an “AEI MS9” mass spectrometer. The collaboration of Dr T. A. Bryce on this part of the work is acknowledged.

*Infrared spectra* of methylated polysaccharides were recorded on films cast from chloroform by Mr D. Welti.

*DEAE-Sephadex columns* were prepared as follows: DEAE-Sephadex (A25) was allowed to swell overnight in water and then washed three times alternatively with 0.5 N sodium hydroxide and 0.5 N hydrochloric acid before being generated in the formate form by stirring with 15% formic acid. The resin was washed with water to remove excess formic acid and packed in a column under gravity.

*Uronic acid anhydride content* was determined using the carbazole reagent¹⁹ with glucuronic acid as standard.

*Total sugar content* was determined by the phenol-sulphuric acid method.²⁰

*Dialysis* was performed in a closed system against distilled water, which was changed daily.

*Optical rotations* were measured with a Perkin-Elmer “141” Polarimeter at room temperature in a 1 dm cell using the appropriate solvent.

*Hydrolysis:* (a) Polysaccharide (5 mg) was hydrolysed overnight in 2 N sulphuric acid (0.5 ml) in a sealed tube at 100 °C; the solution was neutralised with barium carbonate, filtered, treated with Amberlite IR120 (H⁺) resin and concentrated to a syrup; and (b) methylated polysaccharide (2 mg) was hydrolysed overnight in 49% formic acid (1.0 ml) in a sealed tube at 100 °C. Formic acid was removed by co-distillation with water and the product was allowed to stand in water (0.5 ml) at RT for 3 h to hydrolyse formate esters before concentration to a syrup.

2.2. Purification of samples

Cactus tissue was pulped with water, left overnight in a refrigerator and filtered. The aqueous filtrate was concentrated in a rotary evaporator and the polysaccharide
precipitated with ethanol. The precipitate was redissolved in water and the solution dialysed against distilled water for 5 days, purified by "millipore" filtration and freeze-dried to yield the mucilages from *C. peruvianus* (105 mg), *W. erinacea* (716 mg), *O. monacantha* (44 mg), and *O. nopalea-coccinillifera* (116 mg).

Polysaccharide yields after ethanol precipitation, expressed as a percentage of the total weight of fresh plant used, were as follows: *C. peruvianus* (1.6%), *W. erinacea* (0.31%), *O. monacantha* (0.53%), and *O. nopalea-coccinillifera* (0.48%). These yields are dependent on climatic conditions and time of harvesting.

### 2.3. Determination of polysaccharide composition

Polysaccharide (10 mg) was dissolved in water and the solution applied to a column (1 × 7 cm) of DEAE-Sephadex. In all cases, elution with water yielded no neutral fraction.

The uronic acid content of each polysaccharide, determined by the carbazole reagent, was as follows: *C. peruvianus* (44%), *O. monacantha* (25%), *W. erinacea* (51%), *O. nopalea-coccinillifera* (20%). (These values were obtained using glucuronic acid as standard, and the uronic acid subsequently identified in the polysaccharide was galacturonic acid. However, examination of *W. erinacea* mucilage by infrared spectroscopy using galacturonic acid as standard gave a uronic acid content of 48% which was close to the value of 51% obtained with the carbazole reagent using glucuronic acid. The infrared method also showed that 40% of the uronic acid residues were esterified and no methanol was detected in the polysaccharide hydrolysate by mass spectrometry indicating the absence of methyl esterification.)

Polysaccharide (5 mg) was hydrolysed in 2 N sulphuric acid and the final syrup examined by paper chromatography in solvents A and B. The following neutral sugars were detected: *C. peruvianus*: rhamnose, arabinose, galactose (1 : 1 : 2); *O. monacantha*: rhamnose, arabinose, galactose, xylose (1 : 3 : 3.5 : 1.5); *W. erinacea*: rhamnose, arabinose, galactose (3.7 : 1 : 2.7); *O. nopalea-coccinillifera*: rhamnose, arabinose, galactose, xylose (1 : 4.7 : 2.1 : 1.8).

The relative proportions of each sugar, determined by liquid chromatography, are shown in brackets. No uronic acids were detected in the hydrolysates, except in the case of *W. erinacea* mucilage, when small amounts of a component with chromatographic behaviour similar to galacturonic acid were detected.

Polysaccharide (10 mg) was hydrolysed in 2 N sulphuric acid and the hydrolysate was applied to a DEAE-Sephadex column. Neutral sugars were eluted with water and acidic sugars eluted with 0.4 M formic acid. The formic acid eluate was concentrated to a syrup which was examined by paper chromatography in solvent B. A component chromatographically identical to galacturonic acid was detected in each hydrolysate. No glucuronic acid was detected.

*W. erinacea* mucilage (10 mg) was hydrolysed in 1 N sulphuric acid (0.5 ml) for 4 h at 100 °C. The hydrolysate was processed as described in the "general methods" and examined by chromatography in solvent B. In addition to galactose, a major product ($R_{Gal} 0.80$) and a minor product ($R_{Gal} 0.19$) were detected. The major component had identical mobility to galacturonosyl rhamnose and gave the same characteristic
fluorescence under u.v. as did the minor component with $R_{G_{at}}$ 0.19. The main component and galactose were present in the partial hydrolysate in similar amounts.

2.4. Methylation analysis of the polysaccharides

Methylation was carried out using the Hakomori procedure\footnote{22} as described below. Sodium hydride (1.0 g) was washed with dry light petroleum (b.p. 40–60 °C) and stirred with dimethylsulphoxide (10 ml) under nitrogen overnight. The blue solution was centrifuged and added to a solution of the polysaccharide (20 mg) in dimethylsulphoxide (20 ml) until a slight blue colour persisted. Stirring was continued for 5 h and excess methyl iodide was added. After stirring overnight, the solution was dialysed and freeze-dried, and the solid extracted with chloroform. The chloroform extracts were dried over anhydrous sodium sulphate, filtered, and concentrated to yield the methylated polysaccharide as a straw-coloured syrup. Yields and optical rotations of the methylated polysaccharides are recorded below.

<table>
<thead>
<tr>
<th></th>
<th>19 mg, $[\alpha]_D$</th>
<th>$-24^\circ$ (c 0.76, CHCl$_3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. peruvianus:</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>O. monacantha:</em></td>
<td>17 mg, $[\alpha]_D$</td>
<td>$-28^\circ$ (c 0.68, CHCl$_3$)</td>
</tr>
<tr>
<td><em>W. erinacea:</em></td>
<td>13 mg, $[\alpha]_D$</td>
<td>$-58^\circ$ (c 0.44, CHCl$_3$)</td>
</tr>
<tr>
<td><em>O. nopalea-coccinillifera:</em></td>
<td>22 mg, $[\alpha]_D$</td>
<td>$-116^\circ$ (c 0.73, CHCl$_3$)</td>
</tr>
</tbody>
</table>

The presence of only a very weak hydroxyl absorption in the infrared indicated that each sample was essentially fully methylated.

Methylated polysaccharide (5 mg) was heated in methanolic hydrogen chloride (4%, 1.0 ml) at 100 °C in a sealed tube overnight. The solution was neutralised with silver carbonate, filtered and concentrated to a syrup which was examined by gas chromatography. The retention times of the main peaks and sugars to which they correspond are shown in Table 1. Assignments were facilitated by g.l.c.-m.s., with reference to previous work\footnote{23} and by paper chromatography (see below) with reference to the appropriate standard compounds.

Methylated polysaccharide (3 mg) was hydrolysed in formic acid and the resulting syrup examined by paper chromatography in solvents C and D. The following sugars were detected.

<table>
<thead>
<tr>
<th></th>
<th>2,3,5-tri-O-methyl-arabinose</th>
<th>2,3,4-tri-O-methyl-xylose</th>
<th>2,3,4,6-tetra-O-methyl-galactose</th>
<th>2,3,6-tri-O-methyl-galactose</th>
<th>2,4,6-tri-O-methyl-galactose</th>
<th>2,3-di-O-methyl-arabinose</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. peruvianus</em> mucilage</td>
<td>+</td>
<td>tr</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>O. monacantha</em> mucilage</td>
<td>+</td>
<td>tr</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>W. erinacea</em> mucilage</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>O. nopalea-coccinillifera</em> mucilage</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 1. Assignment of the main peaks obtained on gas chromatography of the methanolysates from methylated cactus polysaccharides

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>T  average</th>
<th>Corresponding sugar (detected as the methyl glycoside)</th>
<th>T  average</th>
<th>C. peruvianus mucilage</th>
<th>O. monacantha mucilage</th>
<th>W. erinacea mucilage</th>
<th>O. nopalea-coccinillifera mucilage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,3,4-tri-O-methyl-xylose</td>
<td>0.36&lt;sup&gt;m&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+/tr</td>
</tr>
<tr>
<td>2</td>
<td>0.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,3,4-tri-O-methyl-xylose/2,3,5-tri-O-methyl-arabinose</td>
<td>0.48&lt;sup&gt;s&lt;/sup&gt;</td>
<td>/+</td>
<td>+/tr</td>
<td>+</td>
<td>+/tr</td>
</tr>
<tr>
<td>3</td>
<td>0.62&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2,3,5-tri-O-methyl-arabinose</td>
<td>0.61&lt;sup&gt;w&lt;/sup&gt;</td>
<td>+</td>
<td>tr</td>
<td>+</td>
<td>tr</td>
</tr>
<tr>
<td>4</td>
<td>1.52</td>
<td>2,3,4,6-tetra-O-methyl-galactose/2,3,di-O-methyl-arabinose</td>
<td>1.54&lt;sup&gt;/&lt;/sup&gt;</td>
<td>+/</td>
<td>+/ +</td>
<td>+</td>
<td>+/ +</td>
</tr>
<tr>
<td>5</td>
<td>1.71</td>
<td>2,3,di-O-methyl-arabinose</td>
<td>1.68&lt;sup&gt;w&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>2.62</td>
<td>2,3,6-tri-O-methyl-galactose</td>
<td>2.57&lt;sup&gt;s&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>3.02</td>
<td>2,3,6-tri-O-methyl-galactose/3-O-methyl-rhamnose</td>
<td>2.90&lt;sup&gt;/&lt;/sup&gt;</td>
<td>+/ +</td>
<td>+/ +</td>
<td>+/ +</td>
<td>+/ +</td>
</tr>
<tr>
<td>8</td>
<td>3.23</td>
<td>2,3,6-tri-O-methyl-galactose/2,4,6-tri-O-methyl-galactose</td>
<td>3.24&lt;sup&gt;/&lt;/sup&gt;</td>
<td>+/ +</td>
<td>+/ +</td>
<td>+/ +</td>
<td>+/ +</td>
</tr>
<tr>
<td>9</td>
<td>3.48</td>
<td>2,3,6-tri-O-methyl-galactose/2,4,6-tri-O-methyl-galactose</td>
<td>3.45&lt;sup&gt;m&lt;/sup&gt;</td>
<td>+/ +</td>
<td>+/ +</td>
<td>+/ +</td>
<td>+/ +</td>
</tr>
<tr>
<td>10</td>
<td>3.75</td>
<td>3-O-methyl-rhamnose</td>
<td>3.53&lt;sup&gt;s&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> Peaks recorded at 125 °C; s (strong), m (medium), and w (weak) refer to the relative intensities of the various peaks obtained from one particular sugar; tr = traces.
2.5. Fractionation of *W. erinacea* mucilage

The polysaccharide (70 mg) was applied to a DEAE-cellulose column (25 x 3 cm, phosphate form) and eluted successively with 0.025 M (500 ml), 0.05 M (500 ml), 0.1 M (500 ml), 0.25 M (500 ml), and 0.5 M (500 ml) sodium dihydrogen phosphate buffer at pH 6. Analysis of the eluate with phenol-sulphuric acid reagent showed that the polysaccharide was eluted as a number of overlapping fractions with 0.25 M and 0.5 M buffer, with one particular fraction predominating at each buffer concentration.

3. Results and discussion

Retention of the polysaccharide on the anion exchanger, DEAE-Sephadex, indicated that each contained one or more uronic acid residue which, by colorimetric estimation, was found to be a major constituent of the molecule, notably in the case of *W. erinacea* mucilage. (*C. peruvianus*, 44%; *O. monacantha*, 25%; *W. erinacea*, 51%; *O. nopalacoccinilllfera*, 20%). The uronic acid was subsequently identified as galacturonic acid by hydrolysis and paper chromatography after removal of the neutral sugars on DEAE-Sephadex. The nature and relative amounts of the neutral sugars present in the polysaccharides was determined by hydrolysis followed by paper and liquid chromatography and the *Opuntia* mucilages were found to contain the same sugars, namely rhamnose, arabinose, galactose and xylose, although in different proportions (see experimental), in addition to similar amounts of galacturonic acid. The mucilages from *C. peruvianus* and *W. erinacea* contained rhamnose, arabinose and galactose in different proportions. The galacturonic acid contents of these latter polysaccharides were similar (44% and 51% respectively) and also higher than those of the *Opuntia* polysaccharides.

The characteristic sugar compositions of the mucilages immediately suggest a strong similarity to pectins which was confirmed by analysis of the cleavage products from the fully methylated polysaccharides. The following sugars were identified by g.l.c. and g.l.c.-m.s. of the corresponding glycosides, and by paper chromatography of the free sugars: 2,3,5-tri-O-methyl-arabinose, 2,3,4,6-tetra-O-methyl galactose, 2,3,6-tri-O-methyl-galactose and 2,4,6-tri-O-methyl-galactose from the mucilages from *C. peruvianus* and *W. erinacea*; 2,3,4-tri-O-methyl-xylose, 2,3,5-tri-O-methyl-arabinose (traces), 2,3,4,6-tetra-O-methyl-galactose and 2,3-di-O-methyl-arabinose from the mucilages from *O. monacantha* and *O. nopalacoccinilllfera*. Thus, in the former pair, the arabinose residues and some of the galactose residues occur as non-reducing end-groups, the former in the furanose ring form, while the remainder of the galactose is present as 1,4- and 1,3-linked chain units. In the latter pair, xylose and galactose are major non-reducing end-groups with arabinose present to a large extent as chain units (probably 1,5-linked) and to a minor extent as arabinofuranose end-groups. These modes of attachment of the various sugar residues in the cactus polysaccharides are typical of those found in pectins which normally contain a high proportion of galacturonic acid residues (ca. 75%) and provide a complex mixture of acidic oligosaccharides on partial hydrolysis. In view of the relatively small amounts of galacturonic acid and relatively high proportions of rhamnose in the mucilages, and on the appearance of galacturonosyl...
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rhamnose as the only significant product of partial hydrolysis of \textit{W. erinacea} mucilage, a structural similarity between these cactus polysaccharides and the gum exudates of the \textit{Sterculia} and \textit{Khaya} species\textsuperscript{25–27} which contain less galacturonic acid and more rhamnose than the pectins is suggested.

On the basis of the above results, it is perhaps not surprising that in the past Cactaceae have been referred to as a new source of pectin\textsuperscript{10,11} and uses of the mucilages such as constituents of cosmetic preparations\textsuperscript{1} or adhesives\textsuperscript{2} are typical uses of exudate gums. Clearly, the mucilages have many potential applications in light of the widespread use of pectins and exudate gums and their commercial exploitation merits further study, particularly at the present time when new sources of raw materials are becoming increasingly important. The mucilage structures may well be hygroscopic\textsuperscript{28} to help bind large quantities of water as required by the peculiar ecological niche occupied by Cactaceae and the presence of low molecular weight carbohydrates such as those found in the cortical tissue of Saguaro cactus \textit{(Carnegiea gigantea Br. and Rose)}\textsuperscript{29} would assist water-binding by reducing water activity in much the same way as sucrose used in the preparation of pectin gels \textit{in vitro}.

Although the mucilages of the present study may be composed of more than one acidic polysaccharide, as seems the case with \textit{W. erinacea}, they clearly fall into two structural types which differ in the nature of their neutral side chains, but which have certain main structural features which are closely related. The structural similarity between the mucilages from \textit{O. monacantha} and \textit{O. nopalea-coccinillifera} can be extended to other members of the species namely the mucilages from \textit{O. fulgida} (cholla gum) and \textit{O. ficus-indica} notably in terms of sugar composition, in spite of the fact that certain structural differences do appear to exist from methylation studies.\textsuperscript{4,8}

Thus, from a taxonomic point of view, establishment of broad divisions and phylogenetic relationships between the Cactaceae in terms of mucilage structure seems a distinct possibility. This type of approach has met with considerable success in the classification of complex heteropolysaccharides.\textsuperscript{25–27}

\textbf{Acknowledgements}

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\textbf{References}