The Mucilage of *Opuntia ficus-indica*. Part 2.[1] The Degraded Polysaccharide

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The degraded polysaccharide produced on partial hydrolysis of the mucilage of *Opuntia ficus-indica* has been shown by methylation analysis and periodate oxidation to be composed of chains of alternating α-1,4-D-galactopyranosyluronic acid and β-1,2-L-rhamnopyranosyl units to which are attached chains of β-1,6-D-galactopyranosyl units at position C-3 of most of the rhamnopyranosyl residues. The analytical data support the proposed structure.

THE mucilage isolated from the modified stems of *Opuntia ficus-indica* [1] contains D-galactose, L-arabinose, D-xylose, L-rhamnose, and D-galacturonic acid. Fractionation studies [1] suggest that the mucilage is essentially homogeneous. Partial hydrolysis [1] of the mucilage results in the liberation of virtually all the xylose and arabinose units together with some of the galactose units and the production of a degraded polysaccharide composed of galactose, rhamnose, and galacturonic acid. Chromic acid oxidation, the release of sugars during partial hydrolysis, and the specific rotations of the degraded and native polysaccharides indicate that the constituent units are present as β-xylopyranose, β-galactopyranose, β-rhamnopyranose, α-galactopyranosyluronic acid, and mainly α-arabinofuranose. We now report the structure of the degraded polysaccharide.

RESULTS AND DISCUSSION

Degraded polysaccharide BD had $[\alpha]_D$ +31.4° and on hydrolysis afforded galactose, rhamnose, galacturonic acid, and trace amounts of xylose and arabinose. Periodate oxidation of polysaccharide BD was complete after 48 h (Table 1) when 1.21 mol periodate was consumed per ' average anhydro-unit '. The value of the average anhydro-unit (161.4) was calculated from the mole ratios of the sugars in the degraded polysaccharide. The periodate-oxidised degraded polysaccharide was reduced with borohydride and hydrolysed. Paper chromatography of the hydrolysate and g.l.c. of the derived alditol acetates revealed that only rhamnose survives periodate oxidation and is therefore either 1 ⟶ 3 or 1 ⟶ 2,4 linked.

TABLE 1

Periodate reduced per ' average anhydro-unit '

Time/h	4	24	48	72
Polysaccharide BD	0.85	1.09	1.21	1.21

Degraded polysaccharide BD and carboxy-reduced polysaccharide BD were each methylated by the Hakomori [2] procedure to afford in each case an undermethylated polysaccharide which was completely methylated in *NN*-dimethylformamide with methyl iodide and silver oxide. A hydrolysate of each of the methylated polysaccharides showed on paper chromatography the presence of mono- and di-*O*-methylrhamnose and 2,3,4-tri- and 2,3,4,6-tetra-*O*-methylgalactose. In addition, 2,3,6-tri-*O*-methylgalactose was observed in the hydrolysate of methylated carboxy reduced BD. The derived alditol acetates of both polysaccharides were also examined by g.l.c. and g.l.c.-m.s. The results are shown in Table 2.

TABLE 2

Methylation analysis of degraded polysaccharide BD and reduced degraded BD

	Methylated BD	Methylated reduced BD (mole %)	Significant m.s. peaks
2,3,5-Me_3-arab	+	+	
2,3,4-Me_3-xyl	+	+	
2,3,4,6-Me_4-gal	+++	23.1	45, 117, 161, 205
2,3,4-Me_3-gal	+++	17.8	117, 161, 189, 233
2,3,6-Me_3-gal	+	27.9	45, 117, 233
3,4-Me_2-rham	++	7.9	131, 189
3-Me-rham	+++	23.2	189, 203

Key: +++ = major; ++ = minor; + = trace.

The results of methylation clearly show that the 2,3,6-tri-*O*-methylgalactose, present in large amount in the hydrolysate of methylated carboxy-reduced BD and only in trace amount in the hydrolysate of methylated BD, must have been derived from galactose units produced by reduction of 1,4-linked galacturonic acid residues present in polysaccharide BD. The 3-*O*-methyl and 3,4-di-*O*-methylrhamnose indicate that the rhamnose units are 1,2-linked and branched through position 4. The isolation of a biouronic acid tentatively identified as 2-*O*-α-(D-galactopyranosyluronic acid)-L-rhamnose,[1] the observation that the rhamnose : galacturonic acid ratio is approximately unity in the native and degraded polysaccharides,[1] and the methylation results indicate the presence of a 4-galacturono-2-rhamnan backbone in polysaccharide BD. 6-Linked galactopyranose units are attached to the backbone through position 4 of the rhamnose units. The Figure shows a proposed repeating structure for degraded polysaccharide BD. The β anomeric configurations for the rhamnose and galactose residues were established by chromium trioxide oxidation of the native [1] and degraded polysaccharides, while the α-configuration for the galacturonic acid follows from the specific rotation of the degraded polysaccharide.

The structure proposed for the degraded polysaccharide would be expected to consume 1.15 mol

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periodate per average anhydro-unit. This is in fairly good agreement with the experimentally determined value of 1.21. The molar ratio of galactose : rhamnose in the carboxy-reduced degraded polysaccharide [1] is 68.5 : 31.5. This is in excellent agreement with the ratio 68.8 : 31.2 calculated from the methylation results. In addition, the molar ratios of the methylated sugars present in the carboxy-reduced polymer are in excellent agreement with the values calculated from the proposed structure. The specific rotation observed for degraded BD (+31.4°) does not agree too well with the predicted value of *ca.* +45°. This can be partly accounted for by the presence of small amounts of β-xylopyranose and α-arabinofuranose in the degraded polysaccharide. The presence of equimolar amounts of these two sugars in a combined concentration of 5% would be expected to lower the specific rotation by approximately 7°.

The structure proposed for the degraded polysaccharide of *O. ficus-indica* differs considerably from that for the degraded polysaccharide of *O. fulgida*.[3] The latter is composed of a backbone of β-1,6-linked galactopyranose units to which galactose units are attached through C-3; some of the galactose units are terminated by galacturonic acid residues.

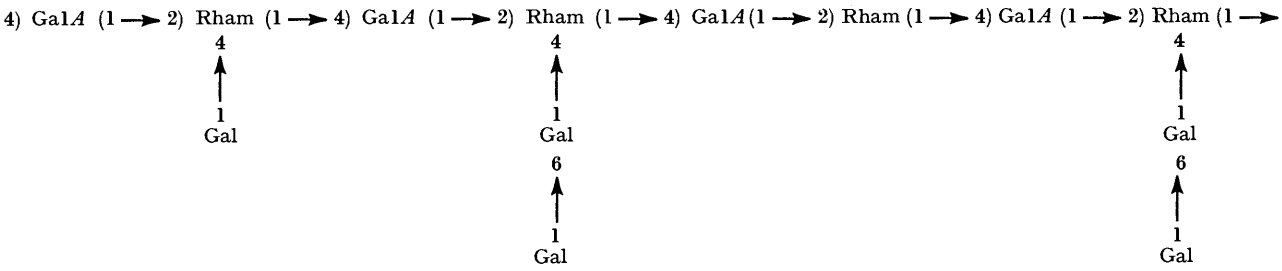

FIGURE Proposed structure for degraded *O. ficus-indica* mucilage

There are only three other polysaccharides reported in the literature in which an alternating core of β-1,2-L-rhamnopyranosyl and α-1,4-D-galactopyranosyl uronic acid units has been established. These are the mucilage from *Ulmus fulva* (slippery elm mucilage),[4] the gum from *Rhizophora mangle* (mangle gum),[5] and the mucilage of *Hibiscus ficulneus*.[6] Slippery elm mucilage differs from degraded *O. ficus-indica* mucilage in that the galactose side-chains are 1,4-linked and are attached mainly to C-3 of the rhamnose units. Degraded mangle gum has a far more complex structure than degraded *O. ficus-indica* mucilage. The side-chains which contain rhamnose, galactose, arabinose, galacturonic acid, and 4-*O*-methylglucuronic acid are attached to C-3 of both the rhamnose and galacturonic acid residues. *Hibiscus ficulneus* mucilage, like that of degraded *O. ficus-indica* mucilage, has β-1,6-linked galactose side-chains attached to rhamnose but in this case to C-3.

EXPERIMENTAL

General and analytical methods are described in Part 1.[1] R_{TMG}. Values refer to the rates of sugars on paper chromatograms relative to 2,3,4,6-tetra-*O*-methyl-D-galactose in solvent 2. G.l.c.–m.s. analyses were carried out using a Pye–Unican 104 Gas Chromatograph linked to an A.E.I. M.S. 30 mass spectrometer. The alditol acetates were separated on column 3 at 173 °C and a flow-rate of 30 ml min^{-1}, and mass spectra were determined at 70 eV and a source temperature of 230 °C.

The preparation of degraded polysaccharides BD and carboxy-reduced BD used in the present study was described in Part 1.

Periodate Oxidation of Degraded Polysaccharide BD.—The polysaccharide (48.3 mg) was dissolved in deionized distilled water (25 ml) and sodium metaperiodate (0.0307M; 5 ml) was added. The solution was set aside at room temperature in the dark, and at intervals aliquots (0.1 ml) were removed and the reduction of periodate measured spectrophotometrically.[7] After 72 h ethylene glycol was added and the oxopolysaccharide reduced with sodium borohydride. The solution was then dialysed against running deionized water and the polyalcohol isolated by freeze-drying. The polyalcohol was hydrolysed and the hydrolysate examined by paper chromatography (solvents 1 and 2). The remainder was converted to alditol acetates and examined by g.l.c.

Methylation of Degraded Polysaccharide BD and Degraded Carboxy-reduced BD.—Degraded BD (100 mg) and reduced degraded BD (85 mg) were separately dissolved in dry dimethyl sulphoxide (3 ml) and methylated with methylsulphinyl anion and methyl iodide to afford, after dialysis and freeze-drying, partially methylated BD (64 mg) and partially methylated reduced BD (45 mg). The partially methylated polysaccharides were completely methylated in dimethylformamide (1 ml) with methyl iodide (8 ml) and silver oxide (250 mg) under reflux to afford methylated BD (68 mg) and methylated reduced BD (23 mg). The i.r. spectra of both methylated polysaccharides showed no hydroxy-absorption.

Hydrolysis of Methylated Degraded BD and Methylated Reduced Degraded BD.—Methylated degraded BD (30 mg) and methylated reduced degraded BD (23 mg) were separately hydrolysed with sulphuric acid (0.5M; 3 ml) at 100 °C for 16 h. Examination of the neutralised ($BaCO_3$) hydrolysates (solvent 2) showed the presence in each of a mono-*O*-methylrhamnose (R_{TMG} 0.70), 2,3,4-tri-*O*-methylgalactose (R_{TMG} 0.76), and 2,3,4,6-tetra-*O*-methylgalactose (R_{TMG} 1.00). In addition 2,3,6-tri-*O*-methylgalactose (R_{TMG} 0.87) was observed in the hydrolysate of methylated reduced BD. The hydrolysate was then reduced and acetylated and the methylated alditol acetates were examined by g.l.c. (columns 1 and 3) and g.l.c.–m.s. (Table 2).

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