Carbohydrate Research, 69 (1979) 171–179

THE MUCILAGE OF *Opuntia ficus-indica**

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(Received January 26th, 1978; accepted for publication, March 6th, 1978)

ABSTRACT

The mucilage extracted from the cladodes (modified stems) of *Opuntia ficus-indica* contains residues of D-galactose, D-xylose, L-arabinose, L-rhamnose, and D-galacturonic acid. Seasonal variation in the sugar composition of the mucilage has been investigated. Fractionation studies indicate that the mucilage is essentially homogeneous. The rate of release of the constituent sugars and the change in optical rotation on mild hydrolysis coupled with the results of chromic acid oxidation suggest that the mucilage contains α-arabinofuranosyl, β-xylopyranosyl, β-rhamnopyranosyl, β-galactopyranosyl, and α-galactopyranosyluronic acid residues. The results also suggest a core containing galacturonic acid, rhamnose, and galactose, to which xylose and arabinose are attached in peripheral positions.

INTRODUCTION

Plants belonging to the genus *Opuntia*[1], although natives of North and South America, are fairly widespread throughout the world. Several species have been recorded as pests in many countries. In South Africa, the most serious *Opuntia* infestation is found in the eastern Cape where *O. ficus-indica* and *O. aurantiaca* have become uncontrollable. Several reports have appeared in the literature concerning the structure of polysaccharides obtained from *Opuntia* species. Jones *et al.*[2–4] reported on the gum (cholla gum) exuded by *O. fulgida*. The gum is produced mainly by old plants, particularly during prolonged dry spells. Stephen *et al.*[5] investigated the polysaccharide present in the green, jelly-like material produced as a result of insect (*Cactoblastis* species) attack on the cladodes of *O. megacantha*. The larvae, which hatch from eggs deposited on the cladodes by insects, burrow into and devour the soft, spongy tissue of the cladode, excreting undigested material in the form of a green jelly. Srivastava *et al.*[6] reported on the polysaccharide extracted from the fruit of *O. dillenii*, and Egyptian workers[7] have carried out a preliminary investigation of the mucilage extracted from the "leaves" (cladodes) of *O. ficus-indica*. We now

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TABLE I

ANALYSIS OF WHOLE AND FRACTIONATED *Opuntia* MUCILAGE

Polysaccharide	A	A_1	A_2	A_3	A_4
$[\alpha]_D$ (degrees)	−62	−49	−55.5	−54	−58
N (%)	0.87	0.34	0.90	0.0	0.0
Constituent sugar units (mole %)					
Galactose	18.4	17.8	23.8	20.6	21.6
Arabinose	42.4	46.0	42.8	44.9	44.2
Xylose	24.5	23.3	22.3	20.7	19.9
Rhamnose	6.4	6.0	5.1	8.4	9.2
Galacturonic acid	8.4	6.9	6.0	5.4	5.2

report on the first part of the results of a detailed investigation of the mucilage isolated from the cladodes of *O. ficus-indica*.

RESULTS AND DISCUSSION

Extraction of the cladodes (modified stems) of *O. ficus-indica*, collected in June 1974, followed by acetone precipitation of the solution, afforded a pale-brown, friable solid in 1.67% yield. The solid gave a positive test for protein and carbohydrate. Dissolution of the material in water, followed by centrifugation, exhaustive dialysis, and freeze-drying of the solution, afforded polysaccharide *A*, $[\alpha]_D$ −62°, in 0.13% yield. Polysaccharide *A* was partially acetylated (OAc, 5.06%), and by hydrolysis was shown (paper chromatography) to contain galactose, arabinose, xylose, and a trace of rhamnose. When the time of hydrolysis was increased, uronic acid was detected and tentatively identified as galacturonic acid by paper chromatography; the concentration of rhamnose in the hydrolysate also increased considerably. Titration of the acid form of the polysaccharide indicated the presence of ~10% of uronic acid. The concentrations of the neutral sugars present in polysaccharide *A* were determined by g.l.c. of the derived alditol acetates, and are given in Table I.

The homogeneity of polysaccharide *A* was investigated by precipitation with cetyltrimethylammonium bromide (CTAB) and by column chromatography on DEAE-cellulose. Polysaccharide *A* failed to precipitate from its solution when treated with CTAB, even after standing at room temperature for 48 h. However, on dialysis of the CTAB-polysaccharide solution, a precipitate formed slowly. After dialysis for 7 days against frequently changed distilled water, the precipitate was collected and afforded, after dissolution in aqueous potassium chloride and precipitation in ethanol followed by purification of the precipitate by dialysis, an acidic polysaccharide A_1 in 75% yield. The solution remaining after removal of the CTAB-polysaccharide complex afforded, after processing, further polysaccharide material in 11% yield. The latter contained the same sugars (in similar proportions) as poly-

TABLE II

SEASONAL ANALYSIS OF *O. ficus-indica* MUCILAGES

Polysaccharide	A	B	C	D
$[\alpha]_D$ (degrees)	−62	−67	−50	−54
N (%)	0.87	1.10	1.56	1.12
Constituent sugar units (mole %)				
Galactose	18.4	22.8	25.2	21.7
Arabinose	42.4	41.3	40.0	42.8
Xylose	24.5	22.1	20.0	20.5
Rhamnose	6.4	7.0	6.9	7.3
Galacturonic acid	8.4	6.8	8.0	7.8

saccharide A_1 and probably consists of polymeric material of lower molecular weight whose CTAB complex is soluble in water. Polysaccharide *A* was not eluted from DEAE-cellulose (acetate form) by water or 5% acetic acid, thereby confirming the absence of a neutral polysaccharide in the mucilage. Elution with 5% formic acid afforded three polysaccharide fractions (A_2, A_3, and A_4) that are compared with polysaccharides *A* and A_1 in Table I.

The fractionation results show that each polysaccharide fraction contains the same sugars, and in similar proportions, as those present in the unfractionated mucilage. Polysaccharides A_3 and A_4, which account for 21 and 23%, respectively, of the total polysaccharide recovered from the fractionation study on DEAE-cellulose, show slightly higher concentrations of rhamnose compared with polysaccharide A_2, suggesting that polysaccharide *A* may be slightly heterogeneous. However, the almost identical optical rotations of polysaccharides A_2, A_3, and A_4 suggest that they have very similar structures. The whole mucilage probably consists of a group of structurally very similar, acidic heteropolysaccharides differing mainly in molecular weight. All structural work was therefore performed on the unfractionated mucilage.

In a preliminary investigation[7], the whole mucilage of *O. ficus-indica* was shown to contain arabinose, galactose, xylose, and rhamnose in the molar ratios 3.6:2.8:1.5:1.0, but no uronic acid. In view of the presence of uronic acid in polysaccharide *A*, possible seasonal variation in the sugar composition and structure of the mucilage was investigated. Samples of mucilage were accordingly prepared from the same plant in March (polysaccharide *B*), June (polysaccharide *C*), and October (polysaccharide *D*) 1976, and analysed (Table II).

The results show the presence of uronic acid in similar concentration in each polysaccharide and only minor variation in the ratios of the neutral sugars. It is interesting to note the ~2:1 molar ratio of arabinose and xylose, and the approximately equimolar proportions of rhamnose and galacturonic acid in each of the polysaccharide samples.

The neutral sugars from polysaccharide *A* were separated by paper chromatog-

TABLE III

ANALYSIS OF DEGRADED POLYSACCHARIDE *A*

Polysaccharide	A	AD_2	AD_3
$[\alpha]_D$ (degrees)	−62	− 0.8	+45.5
Constituent sugar units (mol. ratio)			
Galactose	22.1	26.9	15.0
Arabinose	50.8	14.7	0.9
Xylose	29.3	8.8	0.6
Rhamnose	7.6	7.4	9.9
Galacturonic acid	10.0	10.0	10.0

raphy and characterized as D-galactose, D-xylose, and L-arabinose. Only traces of rhamnose and uronic acid were detected in the hydrolysate.

A partially degraded polysaccharide (AD_1) was prepared by heating polysaccharide *A* in 0.05M sulphuric acid for 10 h, and the release of sugars was followed by paper chromatography. After 0.5 h, an oligosaccharide corresponding to 5-*O*-β-D-xylopyranosyl-L-arabinose[4] appeared. Arabinose appeared after 1 h and was followed by a second oligosaccharide. Xylose was only detected after 8 h. Polysaccharide AD_1 was isolated by dialysis and freeze-drying. The neutralised diffusate was freeze-dried; hydrolysis showed the presence of xylose, arabinose, and a small proportion of galactose. Partially degraded polysaccharide AD_1 was hydrolysed for a further 10 h at 80°, to afford polysaccharide AD_2 and a second diffusate which was shown by paper chromatography to contain xylose, arabinose, and several oligosaccharides. A hydrolysate of the diffusate revealed the same sugars (in similar concentrations) as observed in the hydrolysate of the first diffusate. Polysaccharide AD_2 was heated with sulphuric acid for a further 10 h, but at 100°, to afford degraded polysaccharide AD_3. Larger amounts of galactose and two galactose oligosaccharides then appeared in the hydrolysate. The properties of polysaccharides *A*, AD_2, and AD_3 are shown in Table III.

The results show that, after hydrolysis for 20 h, a major proportion of both xylose and arabinose had been cleaved, while the uronic acid–rhamnose–galactose ratios were virtually the same as in polysaccharide *A*. Nearly all of the arabinose and xylose and a substantial proportion of the galactose residues are removed after hydrolysis for 30 h. The ease with which the arabinosyl residues are released during the hydrolysis strongly suggests that the majority are present in the furanoid form. Xylose, although readily removed from the polysaccharide, did not appear in the hydrolysate until after 8 h. This implies that xylose is cleaved from the polysaccharide as a component of one or more arabinofuranose-containing oligosaccharides, and that it is present in the pyranoid form. Cleavage of such arabinofuranosyl linkages would result in the removal of xylose from the polymer, but because of the greater resistance to hydrolysis of the xylopyranosyl linkage compared to the arabinofurano-

TABLE IV

ANALYSIS OF DEGRADED AND REDUCED, DEGRADED POLYSACCHARIDE *B*

Polysaccharide	B	BD	*Reduced* BD
$[\alpha]_D$ (degrees)	−67	+31	
Constituent sugar units (mole %)			
Galactose	22.8	41.3	68.5
Arabinose	41.3	trace	trace
Xylose	22.1	trace	trace
Rhamnose	7.0	34.1	31.5
Galacturonic acid	6.8	24.5	—

syl linkage, no free xylose is detected during the early stages of hydrolysis. The release of only small proportions of galactose from the polymer during the first and second periods of hydrolysis tends to indicate that very little of this sugar is situated at the periphery of the molecule.

A second, degraded polysaccharide (*BD*) was prepared from polysaccharide *B*. The molar ratios of the component sugars of polysaccharide *B*, *BD*, and carboxyl-reduced *BD* are shown in Table IV. These data show that reduction of the uronic acid in polysaccharide *BD* results in the formation of an equivalent amount of galactose, thereby confirming the identity of the uronic acid. Hydrolysis of polysaccharide *BD*, followed by fractionation of the hydrolysate on Amberlite IRA-400 (acetate) resin, afforded a neutral and two acid fractions. The neutral fraction contained L-rhamnose and D-galactose, and the two acid fractions afforded D-galacturonic acid and a biouronic acid, respectively. The biouronic acid gave rhamnose and galacturonic acid on hydrolysis, and was tentatively identified as 2-*O*-(α-D-galactopyranosyluronic acid)-L-rhamnose[8].

Chromic acid oxidation[9] of acetylated polysaccharide *A* revealed that 75, 72, 60, and 42% of the galactose, xylose, rhamnose, and arabinose residues, respectively, were consumed after 4 h. This finding indicates that the rhamnose, galactose, and xylose residues are β-linked. The oxidation does not provide information on the configuration of the arabinose linkages, as the acetylated α- and β-glycofuranosides are both readily oxidized.

The approximately equimolar proportions of rhamnose and galacturonic acid in the native and degraded mucilages, the increase in concentrations of both rhamnose and galacturonic acid with increasing time of hydrolysis, the isolation of the biouronic acid, and the failure to detect the release of either rhamnose or galacturonic acid from the polymer during partial hydrolysis strongly indicate the presence of all of the rhamnose and galacturonic acid in the core of the mucilage. The upward change in the optical rotation, with the concomitant removal of xylose and arabinose, confirms the β-linkage of xylose and indicates the presence of α-arabinofuranosyl linkages, while the values of the specific rotation for each of the degraded poly-

TABLE V

POLYSACCHARIDES FROM *Opuntia* SPECIES

	O. ficus-indica *mucilage (South Africa)*	O. ficus-indica *mucilage (Egypt)*	O. fulgida *gum*	O. megacantha *gum*	O. dillenii *mucilage*
$[\alpha]_D$ (degrees)	−62	−170	−86	+28	+267
Equivalent weight	1735		1734	1250	
Constituent sugar units (mole %)					
Galactose	18.4	31.5	25.2	46	75
Arabinose	42.4	40.6	50.4	30	25
Xylose	24.5	16.8	14.6	tr	
Rhamnose	6.4	11.3	1.5	11	
Galacturonic acid	8.4		8.3		
Glucuronic acid				13	

saccharides indicate that probably all of the galacturonic acid residues are α-linked.

The properties and composition of the mucilage of *O. ficus-indica* used in the present investigation are compared in Table V with those for gums and mucilages obtained from other *Opuntia* species[2-7]. All of the polysaccharides, with the exception of the mucilage of *O. dillenii*, were obtained from the modified stems of the plants. *O. megacantha* gum is unique among the stem polysaccharides in having a positive optical rotation. It also differs from the two other acid polysaccharides in the nature of the uronic acid component. The properties and composition of the gum of *O. fulgida* closely resemble those of the mucilage of *O. ficus-indica* used in the present investigation. The main difference lies in the variation in the relative molar proportions of the neutral sugars.

EXPERIMENTAL

General. — Solutions were concentrated at 40° under reduced pressure, and specific rotations were determined in water with a Perkin–Elmer 141 Polarimeter. Paper chromatography was performed on Whatman No. 1 paper with (*1*) ethyl acetate–acetic acid–formic acid–water (18:3:1:4), (*2*) ethyl acetate–pyridine–water (8:2:1), and (*3*) ethyl acetate–pyridine–water (10:4:3). *p*-Anisidine hydrochloride[10] (2%) was used as the spray reagent. R_{Gal} values refer to solvent *2*. G.l.c. was performed with a Beckman GC-4 chromatograph equipped with dual f.i.d.; nitrogen was used as the carrier gas. Separation of alditol acetates was effected on columns[11] containing 15% of Apiezon T on Gas Chrom Q (100–120 mesh) at 180° (column *1*), 3% of ECNSS-M on Gas Chrom Q (100–120 mesh) at 175° (column *2*), and 3% of OV-225 on Gas Chrom Q (100–120 mesh) at 190° (column *3*), at flow rates of 60–80 ml/min.

Analytical determinations. (*a*) *Component sugars.* Polysaccharide (~7 mg) and internal standard were heated at 100° for 16 h with 0.5M sulphuric acid (2 ml), and the neutralised solution was reduced (4 h) with sodium borohydride, treated with Amberlite IR-120 (H^+) resin, evaporated to dryness, and distilled with methanol to remove borate. The residue was then treated with acetic anhydride–pyridine (1 : 1) for 1 h at 100° in a sealed tube, after which samples were directly analysed by g.l.c. on column *3*.

(*b*) *Uronic acid.* A solution of the polysaccharide in water was passed through Amberlite IR-120 (H^+) resin, centrifuged, and freeze-dried. The free-acid form of the polysaccharide was titrated with standard sodium hydroxide solution.

(*c*) *Acetate.* Polysaccharide (~10 mg) was heated in a sealed tube with M methanolic hydrogen chloride [which contained toluene (3.57 mg/ml) as internal standard] at 100° for 1.5 h, and the methyl acetate was determined by g.l.c. on a column of 20% of Carbowax 1000 on Chromosorb W (80–100 mesh) at 50°. D-Glucose penta-acetate was used as standard and treated similarly.

Isolation and purification of the mucilage. — Six cladodes (6,606 g), collected in July 1974, were macerated in a Waring blender, and the resulting mucilaginous pulp was centrifuged, filtered (sintered glass), and precipitated in acetone (5 vol.). The precipitate was collected by decantation and centrifugation, washed with ether, and dried *in vacuo* at 50°. The crude polysaccharide (110 g) was dissolved in distilled water (550 ml) and dialysed against distilled water (7 days), and the retentate was filtered and freeze-dried to afford polysaccharide *A* (8.34 g), $[\alpha]_D$ −62° (*c* 0.84) (Found: N, 0.87; OAc, 5.06%). Further samples of mucilage were isolated from the same plant in March (polysaccharide *B*), June (polysaccharide *C*), and October 1976 (polysaccharide *D*) (see Table II).

Fractionation of polysaccharide A. (*a*) *With cetyltrimethylammonium bromide* (*CTAB*). A 10% aqueous solution of CTAB (15 ml) was added with stirring to a 2% solution of polysaccharide *A* (50 ml). No precipitate had formed after 48 h at room temperature. The solution was therefore dialysed against distilled water for 7 days, during which time the CTAB–polysaccharide complex precipitated from solution. The precipitate was removed by centrifugation, washed with water, and dissolved in 2M potassium chloride (125 ml). The solution was poured into ethanol (700 ml), the precipitate was collected and dissolved in distilled water, the solution was dialysed against deionized water (16 h), and polysaccharide A_1 was isolated by freeze-drying (735 mg). The supernatant solution remaining after removal of the CTAB–polysaccharide complex was freeze-dried, to afford solid material (109 mg).

(*b*) *On DEAE-cellulose.* Polysaccharide (250 mg), in the minimal amount of water, was applied to a column (34 × 3 cm) of DEAE-cellulose (acetate form). The column was eluted sequentially with distilled water (2 litres), 5% acetic acid (2 litres), and 5% formic acid (2 litres). Fractions (20 ml) were collected, tested for carbohydrate material, and combined into three fractions which were dialysed; the polysaccharides A_2 (114 mg), A_3 (43 mg), and A_4 (46 mg) were isolated by freeze-drying (see Table I).

Isolation and characterization of the component sugars. — Polysaccharide *A* (433 mg) was hydrolysed in 0.25M sulphuric acid (5 ml) on a boiling water-bath (8 h), the acid was neutralised ($BaCO_3$), the filtered solution was concentrated, and the syrup was subjected to paper chromatography (solvent *2*, 18 h) to afford three fractions. Fraction 1, which had R_{Gal} 1.0 and crystallised from methanol–ethanol, was D-galactose, m.p. 163–164°, mixture m.p. 164–165°, $[\alpha]_D$ +77° (*c* 0.39). Fraction 2, which had R_{Gal} 1.76 and crystallised from methanol–ethanol after standing at room temperature for some time, was L-arabinose, m.p. 152–153°, mixture m.p. 153–154°, $[\alpha]_D$ +85° (*c* 0.8). Fraction 3, which had R_{Gal} 2.26 and crystallised from ethanol, was D-xylose, m.p. 143–144°, mixture m.p. 142–143°, $[\alpha]_D$ +22° (*c* 0.36).

Preparation of degraded polysaccharides. — Polysaccharide *A* (4.0 g) in 0.05M sulphuric acid (80 ml) was heated at 80° for 10 h, and the release of sugars was followed by paper chromatography. The hydrolysate was dialysed against two changes of an aqueous suspension of barium carbonate. The combined diffusates were filtered and freeze-dried (930 mg). A partially degraded polysaccharide (AD_1, 2.6 g), $[\alpha]_D$ −52° (*c* 0.96), was isolated from the retentate by freeze-drying. The diffusate, hydrolysed diffusate, and hydrolysed AD_1 (0.25M sulphuric acid, 4.5 h, boiling water-bath) were examined by paper chromatography (solvent *2*). The hydrolysed diffusate contained arabinose, xylose, and a small proportion of galactose, whereas the unhydrolysed diffusate contained xylose, arabinose, galactose, and two oligosaccharides. The hydrolysed, degraded polysaccharide contained galactose, arabinose, xylose, rhamnose, and galacturonic acid.

Polysaccharide AD_1 (2.4 g) in 0.05M sulphuric acid (48 ml) was heated at 80° for 10 h, and the hydrolysis was followed by paper chromatography. The hydrolysate was treated as described above, to afford a second diffusate (880 mg) and degraded polysaccharide AD_2 (1.16 g), $[\alpha]_D$ −0.8° (*c* 2.4). Paper chromatography of the second diffusate showed the presence of xylose, arabinose, and several oligosaccharides, whereas the hydrolysed diffusate contained arabinose, xylose, and a small proportion of galactose.

Polysaccharide AD_2 was heated with 0.05M sulphuric acid (14 ml) at 100° for 10 h. The hydrolysate was treated as described for AD_1, to yield a third diffusate (257 mg) and degraded polysaccharide AD_3 (169 mg), $[\alpha]_D$ +45.5° (*c* 1.23). Paper chromatography of the diffusate showed the presence of xylose, arabinose, galactose, and galactose di- and tri-saccharides. The analysis of polysaccharides AD_2 and AD_3 is shown in Table III.

Polysaccharide *B* (4 g) was similarly degraded, to afford degraded polysaccharide BD_1 (800 mg), $[\alpha]_D$ +31.4° (*c* 3.8). The analysis of the component sugars is shown in Table IV.

Reduction of polysaccharide BD_1. — Degraded polysaccharide (100 mg) was converted into the methyl ester with diazomethane and then reduced with sodium borohydride[12]. The procedure was repeated twice, to afford carboxyl-reduced polysaccharide BD_1 (98 mg). Determination of the uronic acid content of the polysaccharide showed that reduction was essentially complete (uronic acid <1%).

Hydrolysis of reduced BD_1 gave (p.c.) xylose (trace), arabinose (trace), galactose, and rhamnose only. G.l.c. analysis of the derived alditol acetates is shown in Table IV.

Hydrolysis of polysaccharide BD_1 *and isolation of the component sugars.* Polysaccharide BD_1 (150 mg) was hydrolysed with 0.5M sulphuric acid (20 ml) at 100° for 16 h. The neutralised ($BaCO_3$), filtered solution was passed through a column of Amberlite IRA-400 (AcO^-) resin, and the concentrated eluate was fractionated by p.c. (solvent *2*, 16 h) to afford syrupy L-rhamnose (14.1 mg), $[\alpha]_D$ +7° (*c* 1.4), and D-galactose (23 mg), $[\alpha]_D$ +71° (*c* 2.3).

The resin was then eluted with 0.05M zinc acetate (200 ml), and two fractions were collected. Fraction 1 was passed through Amberlite IR-120 (H^+) resin and evaporated to afford a syrup (25.4 mg). P.c. (solvents *1* and *2*) showed the syrup to be galacturonic acid. The syrup had $[\alpha]_D$ +41° (*c* 2.5) and, on oxidation with nitric acid–water (1 : 1), afforded galactaric acid, m.p. and mixture m.p. 217–218°.

Fraction 2 was similarly treated, to afford a syrup (3.15 mg), $[\alpha]_D$ +25° (*c* 0.32), which showed a single component, R_{Gal} 0.84 (solvent *1*), in p.c. Hydrolysis, monitored by p.c. (solvents *1* and *2*), gave rhamnose and galacturonic acid. 2-*O*-(α-D-Galactopyranosyluronic acid)-L-rhamnose has been reported[8,13] to have R_{Gal} 0.75 (solvent *1*), $[\alpha]_D$ +88°, and R_{Gal} 0.97 (solvent *1*), $[\alpha]_D$ +15°.

Chromic acid oxidation of polysaccharide A. — Polysaccharide *A* (46 mg) in formamide (5 ml) was treated with acetic anhydride (4 ml) and pyridine (3 ml), to afford acetylated polysaccharide *A* (66.6 mg). To acetylated polysaccharide *A* (66.6 mg) in glacial acetic acid (5 ml) were added chromium trioxide (157.4 mg), and 2-deoxy-D-*lyxo*-hexitol penta-acetate (36 mg) as the internal standard. The solution was stirred at room temperature, aliquots were withdrawn at intervals, the polymer was hydrolysed, and the derived alditol acetates were examined by g.l.c. (column *3*).

ACKNOWLEDGMENTS

The authors thank Rhodes University and the South African Council for Scientific and Industrial Research for financial support.

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