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WATER-SOLUBLE POLYSACCHARIDES OF *OPUNTIA FICUS-INDICA* CV "BURBANK'S SPINELESS"

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Key Word Index—*Opuntia ficus-indica*; Cactaceae; polysaccharide; glycoprotein; pectin; cell wall structure.

Abstract—Carbohydrate-containing polymers have been extracted with water from the fleshy, lobed stems of *Opuntia ficus-indica* cv "Burbank's Spineless". By ion exchange chromatography, the material was separated into one neutral and two acidic fractions. Each fraction was separated in two by gel filtration. The neutral fractions consisted of two glucans and a glycoprotein, containing arabinose and galactose. All four acidic fractions contained galacturonic acid, arabinose, rhamnose, galactose and xylose in different proportions. The cell wall structure of *O. ficus-indica* is discussed.

INTRODUCTION

Carbohydrate-containing polymers isolated from various *Opuntia* spp. have been studied by various research groups. Amin *et al.* [1] isolated a neutral polymer from *Opuntia ficus-indica* M. consisting of arabinose, galactose, xylose and rhamnose. Methylation studies showed that arabinofuranosyl units constitute the non-reducing end groups, galactopyranosyl units are mainly (1→4)-linked and rhamnopyranosyl (1 → 2)-linked. Some branching occurs on the galactopyranosyl units. $[\alpha]_D$ was − 170°.

Mindt *et al.* [2] investigated the mucilages from *O. monacanta* H. and *O. nopalea-coccinillifera* M. and found that both consisted of acidic polymers. No neutral fractions were found. The content of galacturonic acid was *ca* 20%, the neutral sugars present being rhamnose, arabinose, galactose and xylose. Methylation studies showed that galactose and xylose constituted the main part of the non-reducing end groups. Arabinose was mainly present as (1→4/5)-linked units.

The mucilage from the pods of *O. dillenii* was found to be an arabinogalactan [3] with $[\alpha]_D$ + 266.8°. The main chain consists of (1→4)-linked galactose units with side chains of (1→4/5)-linked arabinose units linked through C-3 of galactose.

'Cholla gum' [4], an exudate from *O. fulgida*, has a complex structure with β(1→3)-linked galactose units as the main chain with branching on C-6. The branches consist of D-galacturonic acid, D-galactose, D-xylose and L-rhamnose in pyranose form and L-arabinofuranose units. The chains terminate in xylopyranose or arabinofuranose units.

The 'gum' from *O. megacantha* L. [5] has also a complex structure. The quantitatively most important components are arabinofuranose and galactopyranose as end groups, while the main chain consists of (1 → 4)-, (1→6)- and (1→3)-linked galactopyranose units. Branches are mainly located on C-3 and C-4, with some on C-3 and C-6. Rhamnose and glucuronic acid are present throughout the molecule. Methylation studies indicate a complex structure of the 'gum' from *O. megacantha* L.

Comparison of the mucilage present in members of the family Cactaceae and of other succulent plants [6] shows that these polysaccharides often show great similarities with polysaccharides of the pectin type. This paper reports on the polysaccharides present in *Opuntia ficus-indica* Miller cv "Burbank's Spineless".

RESULTS AND DISCUSSION

Water extraction of the ethanol-treated fleshy, lobed stems of *Opuntia ficus-indica* M. cv "Burbank's Spineless", gave after dialysis and freeze-drying a carbohydrate-containing polymer in 0.3% yield. This polymer was separated into three fractions, A, B and C by chromatography on DEAE-Sephadex. Fraction A passed unretarded through the column whereas fractions B and C were eluted with buffer containing 0.25 M NaCl and 0.5 NaCl, respectively. The yields after dialysis of the freeze-dried fractions A, B and C were 4.7, 35.9 and 10.7%, respectively. Carbohydrate, uronic acid and protein contents, and optical rotations are given in Table 1.

Gel filtration of fraction A on Sephadex 4B revealed two sub-fractions A_1 and A_2. By gel filtration on a column calibrated with dextran samples, the MW of A_1 was determined as 10^6, and the polymer consisted of glucose only. Due to small amounts available, further studies have not been carried out. A_2, the main fraction of A, had MW 5600 with reference to dextran, determined on a Sephadex G-100 column.

Table 1. Optical rotation and % composition of the high molecular weight carbohydrate containing fractions A, B and C

	A	B	C
$[\alpha]_D^{20}$	+59°	+80°	+90°
Total carbohydrate	90.7	88.0	94.1
Protein	9.9	2.9	1.9
Uronic acid	0	30.2	50.8

Table 2. Optical rotation and relative sugar composition of fraction A_2 after different periods of dialysis

	4 hr	24 hr	5 days
$[\alpha]_D^{20}$	+95°	+59°	+59°
Galactose	2.1	1.1	0.7
Arabinose	1.0	1.0	1.0
Glucose	15.7	1.6	0.2

The yield of fraction A, which appeared at first to be higher than that of fraction C, was surprisingly low. Fraction A_2 passed partly through the dialysis bag, and depending on dialysis time, the yield and especially the composition of the retentate varied (Table 2). Analyses of the retentate after 4 hr, 24 hr and 5 days, showed that a glucose-containing polymer had the greater ability to penetrate the dialysis bag, and by dialysing for a sufficient period this polymer would probably disappear. A polymer with higher content of galactose than arabinose, was also able to pass through the dialysis membrane. These observations show that dialysis of fraction A should be avoided. Fraction A_2 was incubated with α-amylase; both glucose and maltose were detected when analysing the reaction mixture by PC, indicating a (1→4)-linked glucan.

A_2 after dialysis for 4 hr, 24 hr and 5 days were subjected to methylation by the Hakomori method [7]. Analysis of the derived alditol acetates by GC–MS showed the same components to be present, but in different proportions (Table 3). The main quantitative changes were in the glucose derivatives. The presence of non-reducing end groups, (1→4)-linked units carrying branches on C-4 and C-6, compatible with the results from the enzyme digestion, suggests that the glucan present is an amylopectin-type glucan, but with an unusually low MW. The high positive $[\alpha]_D$ obtained after short dialysis time of A_2 is probably mainly due to the glucan.

Table 3. GC–MS data for the methylated alditol acetates derived from fraction A_2 of *Opuntia ficus-indica*

R_{TMG}	Primary fragments MS *m/e*	Alditol acetate
0.47	45, 117, 161	2,3,5-Tri-*O*-methylarabinitol
0.59	117, 161	2,3,4-Tri-*O*-methylarabinitol
0.86	45, 117, 233	2,5-Di-*O*-methylarabinitol
1.04	45, 117, 161, 205	2,3,4,6-Tetra-*O*-methylglucitol
1.17	45, 117, 161, 205	2,3,4,6-Tetra-*O*-methylgalactitol
1.50	45	5-*O*-methylarabinitol
1.88	45, 117, 161, 233	2,4,6-Tri-*O*-methylgalactitol
2.15	45, 117, 233	2,3,6-Tri-*O*-methylglucitol
2.62	117, 161, 189, 233	2,3,4-Tri-*O*-methylgalactitol
2.93	45, 117	2,6-Di-*O*-methylgalactitol
3.94	117, 261	2,3-Di-*O*-methylglucitol
4.50	45, 117, 189	2,4-Di-*O*-methylgalactitol

R_{TMG}: R_t relative to 1,5-Di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol.

The polymer containing arabinose and galactose is probably a glycoprotein. The protein content of one preparation was 3.5%, another 9.9%; some disappeared on dialysis. The methylation results show that arabinose is present both as furanose and pyranose end groups, as (1→3)-linked furanose units and as furanose at branch points. Galactopyranose is present as end groups, (1→3)- and (1→4)-linked units, at branch pojnts with branches on C-3 and C-4 and on C-3 and C-6.

Fraction B was eluted from the DEAE-Sephadex column with 0.25 M KCl. Gel filtration on a Sepharose 4B column gave two peaks, B_1 and B_2. MWs and sugar compositions for B_1 and B_2 are given in Table 4. Fraction C, eluted with 0.5 M KCl from the DEAE-Sephadex column also gave two peaks (C_1 and C_2) when subjected to gel filtration on a Sepharose 4B column. MW and sugar compositions are given in Table 4.

To achieve complete methanolysis, 6 M HCl in MeOH had to be used for fractions C_1 and C_2. It is well known that glycosidic linkages involving uronic acids are more difficult to break than those involving only neutral sugars, but this cannot be the only explanation, as B_1 and B_2 would methanolyse completely with 1 M HCl in MeOH.

The sugar compositions of the four acidic fractions indicate pectin-type polysaccharides. The polysaccharide fraction isolated from *O. ficus-indica* M. by Amin *et al.* [1] did not contain uronic acid. This sample was composed of arabinose, galactose, xylose and rhamnose in the ratio 2.4:2.3:1:0.7, being quite different from the fractions obtained from *O. ficus-indica* cv "Burbank's Spineless". The present work and that of Amin *et al.* [1] indicate arabinofuranose as non-reducing end groups, but other features seem to be different.

Fraction A_2 is probably a glycoprotein containing arabinose and galactose, fraction C_2 is mainly a rhamnogalacturonan containing traces of galactose and arabinose, and the other fractions are in between these extremes. The polymers being present in *O. ficus-indica* cv "Burbank's Spineless" resemble those Albersheim *et al.* [8] found in the cell wall of *Acer pseudoplatanus* and a similar composition of the cell wall might be present.

EXPERIMENTAL

Materials. Opuntia ficus-indica Miller, cv "Burbank's Spineless" was collected in Morocco by Prof. Dr. A. Nordal, Oslo, March 1976. The identification was carried out by Prof. Nordal and Dr. P. Sunding, The Botanical Garden, University of Oslo. The plant material was cut in pieces, boiled with 80% EtOH to inactivate enzyme activity and kept in EtOH until required.

Extraction of the material. 3.375 kg of the pre-treated material was extracted by H_2O × 3 with 9 l. at 60–65°. After centrifuga-

Table 4. Molecular weight and relative sugar composition of fractions B_1, B_2, C_1 and C_2

	B_1	B_2	C_1	C_2
MW	10^6	6×10^4	1.1×10^6	35000
Galacturonic acid	0.4	2.3	1.1	8.7
Galactose	1.0	1.0	1.0	1.0
Arabinose	0.9	1.2	0.9	1.3
Rhamnose	0.4	0.9	1.1	7.3
Xylose	0.3	0.2	0.5	trace

tion, filtration and dialysis the raw extract was lyophilized. Yield 10 g.

Fractionation. The lyophilized material (920 mg) was applied on top of a DEAE-Sephadex A-25 column (2.5 × 55 cm) in chloride form and successively eluted with 0.05 M Tris–HCl buffer (pH 7.3), buffer containing 0.25 M NaCl and buffer containing 0.5 M NaCl. Yield of neutral fraction (A) 45 mg, acidic fraction (B) 330 mg and acidic fraction (C) 99 mg.

General methods. Concentrations were performed with red. pres. below 40°. Dialysis was carried out against distilled H_2O. Optical rotation was measured in a Perkin-Elmer 141 Polarimeter using 0.1% solns in H_2O. The carbohydrate contents were estimated with the PhOH–H_2SO_4 method [9], uronic acid contents by the cpc-method [10], the sugar composition was determined by GLC as the TMSi-derivatives of the methylglycosides [11]. Protein was estimated by Lowry's method [12]. MWs were determined by gel filtration on a column of Sepharose 4B (2.5 × 36 cm) or of Sephadex G-100 (0.9 × 27.5 cm). The columns were calibrated against the Dextran T series from Pharmacia and eluted with 0.05 M Tris–HCl buffer pH 7.3, fractions of 3 ml and 0.3 ml respectively were collected. All GLC analyses were performed on a Varian 1400 (FID) using N_2 as carrier gas. For GC–MS the column was coupled with a Varian CH-7 mass spectrometer. Incubation with α-amylase (EC 3.2.1.1) was carried out with the sample (2 mg) in phosphate buffer pH 6.9 (1 ml) at 37° for 24 hr. One drop of toluene was added to prevent microbial growth. After boiling for 2 min, cooling and deionization, the mixture was subjected to PC in EtOAc–HOAc–HCOOH–H_2O (18:3:1:4).

Methanolysis. The dried sample (2–4 mg) was heated with M or 6 M HCl in MeOH with mannitol as internal standard at 80° for 24 hr followed by TMS derivatization [13] and analysed by GLC on a 3% SE-52 column (390 × 0.2 cm) on Varaport 30. The temp. programme used started at 130° with an increase of 1°/min for 10 min, followed by an increase of 2°/min to 160°, and finally 4°/min to 235°.

Methylation analysis. The methylation was performed using the Hakomori method as previously described [7]. The methylated product was hydrolysed with HCOOH, reduced with $NaBH_4$ and acetylated. The partly methylated alditol acetates were analysed by GC–MS on a 3% OV-225 column (200 × 0.2 cm) at 190°.

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