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Note

An arabinogalactan from the skin of *Opuntia ficus-indica* prickly pear fruits

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Abstract—The cold-water extract from the skin of *Opuntia ficus-indica* fruits was fractionated by anion-exchange chromatography. The major fraction, which was purified by size exclusion chromatography, consisted of a polysaccharide composed of galactose and arabinose residues in the ratio 6.3:3.3, with traces of rhamnose, xylose and glucose, but no uronic acid. The results of methylation analysis, supported by ^{13}C NMR spectroscopy, indicated that this polysaccharide corresponded to an arabinogalactan having a backbone of (1→4)-linked β-D-galactopyranosyl residues with 39.5% of these units branched at O-3. The side-groups consisted either of single L-arabinofuranosyl units or L-arabinofuranosyl α-(1→5)-linked disaccharides. This polysaccharide is thus an arabinogalactan that can be classified in the type I of the arabinogalactan family.

Keywords: *Opuntia ficus-indica*; Prickly pear; Arabinogalactan; NMR

Opuntia ficus-indica (OFI) is a tropical or subtropical plant, which belongs to the Cactaceae family. Its fruits or 'prickly pears' have become an important fruit crop in many semi-arid lands of the world. These fruits as well as the young cactus shoots (nopalitos) are generally consumed fresh. However in the last decade, research studies on cactus processing have produced other alternatives leading to added value to such crops. Indeed, the stems of the plant or cladodes are a good source of fibre, an important element for the human diet, and thus present potential for medical use.[1–5]

The major studies concerning the chemical compositions of the *Opuntia* mucilages have focused on the mucilage of the cladodes, with significant contradictions in the results. For some authors, this mucilage has been considered as being neutral, consisting mainly of D-galactosyl and L-arabinosyl residues.[6] Others reports have suggested that this mucilage was acidic and contained L-arabinose, D-galactose, L-rhamnose and D-galacturonic acid.[7,8] Parikh and Jones reported that the mucilage of *O. fulgida* consisted of a backbone of β-(1→3)-linked galactose units with side chains linked at O-6, containing D-galacturonic acid, D-galactosyl, D-xylosyl, L-rhamnosyl and L-arabinosyl units.[9–11] The mucilage of OFI was analyzed by Amin et al. who found that it was neutral and contained arabinosyl, galactosyl, rhamnosyl and xylosyl residues.[12] More recently, Srivastava and Pande have purified an arabinogalactan from the mucilage of *O. dillenii*.[13] They reported that it consisted of a backbone of β-(1→4)-linked galactosyl units substituted at position O-3 with L-arabinosyl and D-galactosyl residues.

For a few years, our group has been involved with the characterization of the various polysaccharides from prickly pear fruits. From the fruit seeds, we were able to isolate several glucuronoxylans with a molar ratio of xylose to 4-*O*-Me-glucuronic acid varying from 12:1 to 65:1.[14] From the alkaline extract of the fruit skin, we have also isolated and characterized a 4-*O*-Me-glucuronoxylan,[15] and several pectic polysaccharides (to be

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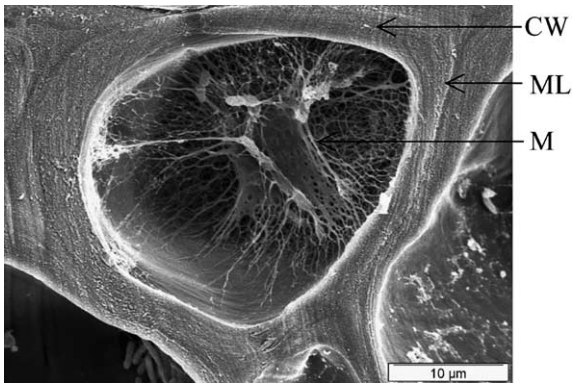


Figure 1. SEM of a cross-section of collenchyma cells from the skin of *Opuntia ficus-indica* fruit. CW: cell walls, ML: middle lamella, M: mucilage.

published). In this paper, we report on the isolation, purification and structural elucidation of an arabinogalactan.

The tissues constituting the skin of prickly pear fruits consist essentially of collenchyma and parenchyma cells. Figure 1 corresponds to the cross-section of a collenchyma cell showing the presence of mucilage inside the cell.

The dry skin was first refluxed in a toluene–EtOH mixture to produce the skin defatted residue (SDR). Then the mucilaginous crude polysaccharide (CP) was solubilized with de-ionized water at 20 °C and fractionated by anion-exchange chromatography into five fractions (F_I to F_V) eluted sequentially with 0.01, 0.1, 0.3, 0.5 and 1 M acetate buffer. The major fraction (F_I) was subsequently purified by gel permeation chromatography on a Biogel P6 column. We eliminated the low molecular weight oligomers and kept only the excluded fraction. The homogeneity of purified fraction was checked by high-performance size exclusion chromatography (HPSEC). Its molecular weight was estimated to be 8.5×10^4 by HPSEC, using a dextran calibrated column. The specific rotation was $[\alpha]_D^{20}$ −44.8 (*c* 0.5, water). It did not contain protein according to the Lowry method.[16]

The sugar compositions of SDR, CP and F_I are reported in Table 1. The CP and purified fraction F_I showed a high content of galactose and arabinose, suggesting the presence of an arabinogalactan.

The purified fraction F_I was characterized by sugar and methylation analysis and by ^{13}C NMR spectroscopy. A hydrolysate of F_I revealed that galactose and arabinose were the major component with a small amount of rhamnose, xylose and glucose. The molar ratios of galactose and arabinose content were 6.3:3.3, respectively.

Table 2. Methylation analysis data of arabinogalactan F_I

Sugar derivatives	Molar ratio[a]	Mode of linkage
2,3,6-Me_3-Gal[b]	38.4	→4)-β-Gal*p*-(1→
2,6-Me_2-Gal	25.1	→3,4)-β-Gal*p*-(1→
2,3-Me_2-Ara	10.5	→5)-α-Ara*f*-(1→
2,3,5-Me_3-Ara	26.2	T-Ara*f*
2,3,4-Me_3-Rha	Tr[c]	T-Rha*p*
2,3,4-Me_3-Xyl	Tr[c]	T-Xyl*p*
2,3,4,6-Me_4-Glc	Tr[c]	T-Glc*p*

[a] Expressed as area percentages of the total methylated alditol acetates.
[b] 2,3,6-Me_3-Gal=1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-galactitol, etc.
[c] Traces.

The methylation analysis (Table 2) revealed the presence of 2,3,6-tri-*O*-methyl galactitol and 2,6-di-*O*-methyl galactitol, suggesting that the backbone was constituted with (1→4)-linked galactose residues with 39.5% of galactose units being substituted at the O-3 position.

The proportion of 2,3,5-tri-*O*-methyl arabinitol and 2,3-di-*O*-methyl arabinitol was 2.1:1, suggesting that the side-chains consisted of oligoarabinan linked (1→5). The relatively high proportion of terminal arabinofuranosyl residues (71%) indicated that the side-chain oligosaccharides have an average DP of 1.5. The proportion of 2,6-di-*O*-methyl galactitol, 2,3,5-tri-*O*-methyl arabinitol and 2,3-di-*O*-methyl arabinitol suggested that among seven galactose units, two are substituted by only one arabinofuranosyl unit, one carry two arabinofuranosyl residues that are (1→5) linked and four are free.

The ^{13}C NMR spectrum of the arabinogalactan contained 19 signals. The six major signals at δ 105.26 (C-1), 72.86 (C-2), 74.30 (C-3), 78.49 (C-4), 75.43 (C-5) and 61.73 (C-6) ppm, can be assigned to (1→4) linked β-D-Gal*p* residues. Other less intense signals are observed at δ 104.11, 73.86, 82.22 ppm, corresponding, respectively, to C-1, C-2 and C-3 of O-3 substituted β-D-Gal*p* residues. The resonances of the arabinose residues were assigned by reference to previous results.[17] The signals at 108.36, 81.01, 84.95 and 69.70 ppm corresponded, respectively, to C-1, C-2, C-4 and C-5 of (1→5) linked α-L-Ara*f* residues. The terminal arabinose residues are easily identified by signals at 110.40, 81.34, 77.39, 85.26 and

Table 1. Yields and sugar composition of skin defatted residue SDR, crude polysaccharide CP and purified arabinogalactan F_I

Fraction	Yield (%)[a]	Sugar composition						
		GalA	Rha	Glc	Gal	Ara	Xyl	Man
SDR	89	35.6	3.3	29.3	8.4	12.3	4.5	1.6
CP	4.1	14.2	4.2	1.5	23.5	32.7	4.5	—
F_I	2.2	—	1.1	1.9	65.5	30.1	1.3	—

[a] Expressed as weight percent (wt%) of skin dry matter.

Table 3. Carbon-13 NMR data[a] (333 K) of arabinogalactan F_I

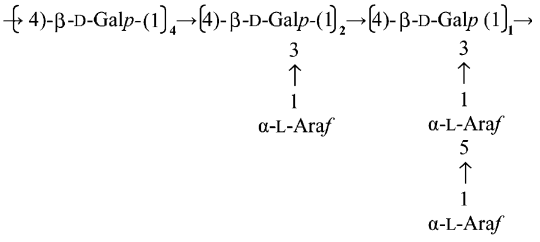
Residue and linkage assignment	Chemical shifts					
	1	2	3	4	5	6
Galactosyl residue						
→4)-β-D-Gal*p*-(1→	105.26	72.86	74.30	78.49	75.43	61.73
→3,4)-β-D-Gal*p*-(1→	104.11	73.86	82.22	79.12	75.43	61.73
Arabinosyl residue						
→5)-α-L-Ara*f*-(1→	108.36	81.01	77.86	84.95	69.70	—
T-α-L-Ara*f*-(1→	110.40	81.34	77.39	85.26	62.44	—

[a]In ppm relative to the signal of internal acetone in deuterium oxide at 31.5 ppm.

62.44 ppm, which are characteristics of terminal arabinose units. All the NMR data are reported in Table 3 and are in good agreement with already published NMR data for galactan, arabinan and arabinogalactan.[17–24]

The comparison of integrated intensities of anomeric signals for the different sugar residues, in ^{13}C quantitative spectrum, provided important data on the relative proportions of basic units in the arabinogalactan. These data indicated that the molar ratio of galactose residues branched at O-3 to free units was 3:4 and the ratio of the arabinose units linked through O-5 to terminal arabinose was 1:2, respectively.

These ^{13}C NMR results corroborated the methylation data and finally we can propose for the structure of the arabinogalactan the following repeating unit, but other variations are possible:



The structure of this arabinogalactan consists of a main chain of (1→4)-linked β-D-galactopyranosyl residues with 60.5% of free galactose units and 39.5% of galactose units being branched at position 3. These branched galactose units hold either single L-arabinofuranosyl units or L-arabinofuranosyl α-(1→5) L-arabinose linked disaccharide.

1. Experimental

1.1. Materials

Fresh prickly pear fruits of *O. ficus-indica* (OFI) from an experimental plantation in the vicinity of Marrakech (Morocco) were collected in November 2000. The fruits were carefully hand-peeled, and the peels (with a thickness of about 3–4 mm) were cut into small pieces and dried in a ventilated oven adjusted at 50 °C. After drying, the peel fragments were ground for a few minutes in a domestic coffee grinder and sieved.

1.2. Microscopy

For scanning electron microscopy (SEM) analysis, small cubes were cut out from fresh skin of OFI, fixed with glutaraldehyde and dried under critical point conditions in a Polaron critical point dryer operated with liquid CO_2. Before observation, the samples were sputtered with gold palladium alloy in a JEOL JFC sputterer. The observations were made with a JEOL JMS-6100 SEM operated at an accelerating voltage ranging from 5 to 8 kV and under secondary electron mode.

1.3. Preparation of skin defatted residue (SDR)

Fats, waxes and oils (11 wt% of dry material) were removed from the skin powder sample by refluxing in a Soxhlet apparatus during 24 h with 38:62 (v:v) toluene–ethanol.

1.4. Isolation and purification of the polysaccharide

The SDR was extracted twice with de-ionized water at room temperature for 2 h. The extract, after concentration in a rotary evaporator, was diluted with ethanol to produce a white precipitate, which was recovered by centrifugation. The pellets were re-dissolved in water and ethanol was added slowly into the solution at room temperature, until a concentration of 75% in ethanol (v/v) was reached. The resulting precipitate was recovered by centrifugation (15 min, 11,000 g) and washed thoroughly with pure ethanol. The precipitate was re-dissolved in water and the solution was dialyzed against distilled water (2×24 h) and freeze-dried to give the crude polysaccharide (CP), which represented 4.1 wt% of skin dry matter.

1.5. Ion exchange chromatography

The crude polysaccharide CP (200 mg) was dissolved in 0.01 M NaOAc buffer (pH 5.6) and the solution was loaded onto a diethylaminoethyl (DEAE)-Sepharose CL-6B column (1.3 cm × 35 cm) previously equilibrated with the same buffer. The column was then eluted successively with 0.01, 0.1, 0.3, 0.5 and 1 M NaOAc buffer pH 5.6, 150 mL each and finally with 0.2 M NaOH (50 mL). Each recovered fraction was dialyzed against distilled water and freeze-dried. The amounts of sample recovered in each fraction were: 0.01 M (F_I, 55.20%), 0.1 M (F_{II}, 0.32%), 0.3 M (F_{III}, 4.96%), 0.5 M (F_{IV}, 11.80%) and 1 M (F_V, 9.60%).

1.6. Size exclusion chromatography

The major fraction F_I was purified by size-exclusion chromatography (on preparative scale) through a polyacrylamide Biogel P6 column (4–100 cm), eluted with 0.05 M sodium nitrate solution at 80 mL/h. The low molecular oligomers were discarded and the excluded fraction was desalted by dialysis and freeze-dried. HPSEC was performed on a Shodex-OHpak B-804 (7.5 × 500 mm) column eluted at 1 mL/min flow rate with 0.05 M $NaNO_3$ solution, and at room temperature. The column effluent was monitored using a refractive index detector. The column was previously calibrated by dextran samples of known molecular weight as standard (Pharmacia Co.).

1.7. Analytical methods

Uronic acid content was determined according to Blumenkrantz and Asboe-Hansen method.[25] Neutral sugars were analyzed, after H_2SO_4 hydrolysis, by GLC as their corresponding alditol acetates,[26] using a Hewlett–Packard 5890 instrument equipped with a fused-silica wide-bore column (30 m × 0.53 mm × 0.32 mm) packed with 3% SP 2380, coupled to a Hewlett–Packard 3395 A integrator.

A sample of purified arabinogalactan F_I was methylated twice by the Hakomori procedure, as modified by Jansson et al.[27] The methylated polysaccharide after hydrolysis with 2 M TFA (1 h, 120 °C) was then converted into partially methylated alditol acetates by successive treatments with $NaBH_4$ and pyridine-Ac_2O and analyzed by GLC as above. Peak identification was based on retention times using partially methylated alditol acetate standards and confirmed by GLC by using a SP 2380 capillary column coupled to a Nermag R1010C mass spectrometer. Peak areas were corrected by using the molar response factors according to Sweet et al.[28]

1.8. NMR spectroscopy

^{13}C NMR spectra were obtained with an AC 300 Bruker spectrometer operated at the frequency of 75.468 MHz. Spectra were recorded from solutions in D_2O at 333 K in 5 mm o.d. tube (internal acetone $^{13}C(CH_3)$ at 31.5 ppm relative to Me_4Si). Quantitative ^{13}C NMR spectra were recorded using the Invgate Bruker sequence, with 90° pulse length (6.5 μs), relaxation delay of 2 s, 8000 data points, 0.54 s acquisition time and 30,000 scans.

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