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Note

Isolation and structure of D-xylans from pericarp seeds of *Opuntia ficus-indica* prickly pear fruits

Youssef Habibi,[a,b] Mostafa Mahrouz,[b] Michel R. Vignon[a,*]

[a]*Centre de Recherches sur les Macromolécules Végétales, CNRS, and Université Joseph Fourrier, BP 53, F-38041 Grenoble cedex 9, France*
[b]*Faculté des Sciences Semlalia (Université Cadi Ayyad), BP 2390, Marrakech, Morocco*

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Abstract

Xylans were isolated from the pericarp of prickly pear seeds of *Opuntia ficus-indica* (OFI) by alkaline extraction, fractionated by precipitation and purified. Six fractions were obtained and characterized by sugar analysis and NMR spectroscopy. They were assumed to be (4-*O*-methyl-D-glucurono)-D-xylans, with 4-*O*-α-D-glucopyranosyluronic acid groups linked at C-2 of a (1→4)-β-D-xylan. The sugar composition and the ^{1}H and ^{13}C NMR spectra showed that their chemical structures were very similar, but with different proportions of D-Xyl and 4-*O*-Me-D-GlcA. Our results showed that, on average, the water soluble xylans have one nonreducing terminal residue of 4-*O*-methyl-D-glucuronic acid for every 11 to 14 xylose units, whereas in the water non-soluble xylans, xylose units can varied from 18 to 65 residues for one nonreducing terminal residue of 4-*O*-methyl-D-glucuronic acid.

Keywords: Cactus; *Opuntia ficus-indica*; Prickly pear seeds; 4-*O*-Methyl-glucuronoxylan; ^{1}H and ^{13}C NMR spectroscopy

Opuntia ficus-indica (OFI) originates from the American continent and is mainly used for fruit production.[1] Young shoots are also eaten as a vegetable (Nopalitos) in Mexico and south of the USA. In North Africa, the cultivation of cactus OFI is also used to prevent soil erosion in arid areas and as forage substitute during periods of dryness. There is a growing interest in the non-food usage of OFI, mainly in medical applications. Ethanol extract of OFI cladodes shows an analgesic and anti-inflammatory effect.[2] Ingestion of raw and cooked OFI extracts of cladodes presents beneficial effects on growth and total cholesterol, without any secondary effect on glucose and lipoproteins levels in blood.[3] The fruit of the cactus *O. ficus-indica* or prickly pear is an important raw material for Morocco's food industry. The amount of seeds is important as it varies from 20 to 40% per dry weight of the whole fruit, depending on the cultivars. Analysis of the main constituents of prickly pear seeds showed a significant amount of polysaccharides, cellulose and hemicelluloses.[4] The present study deals with the structure of their glucuronoxylans.

Previous studies have shown that plant xylans form a family of polysaccharides which consist of a backbone

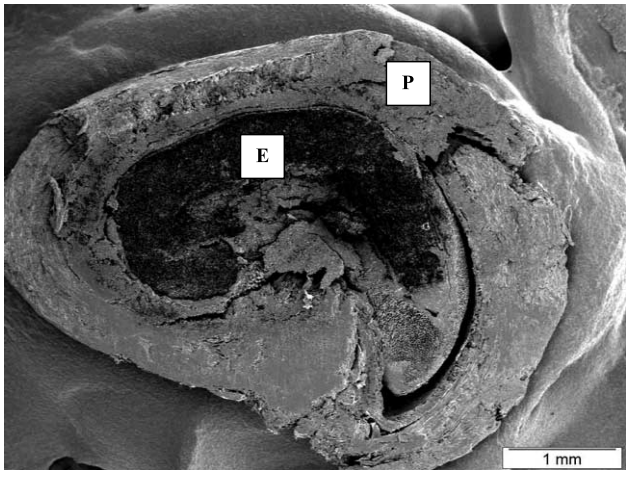

Fig. 1. SEM micrograph of a sectioned seed of *O. Ficus Indica*. P, pericarp; and E, endosperm.

* Corresponding author. Tel.: +33-4-76037614; fax: +33-4-76547203
E-mail address: michel.vignon@cermav.cnrs.fr (M.R. Vignon).

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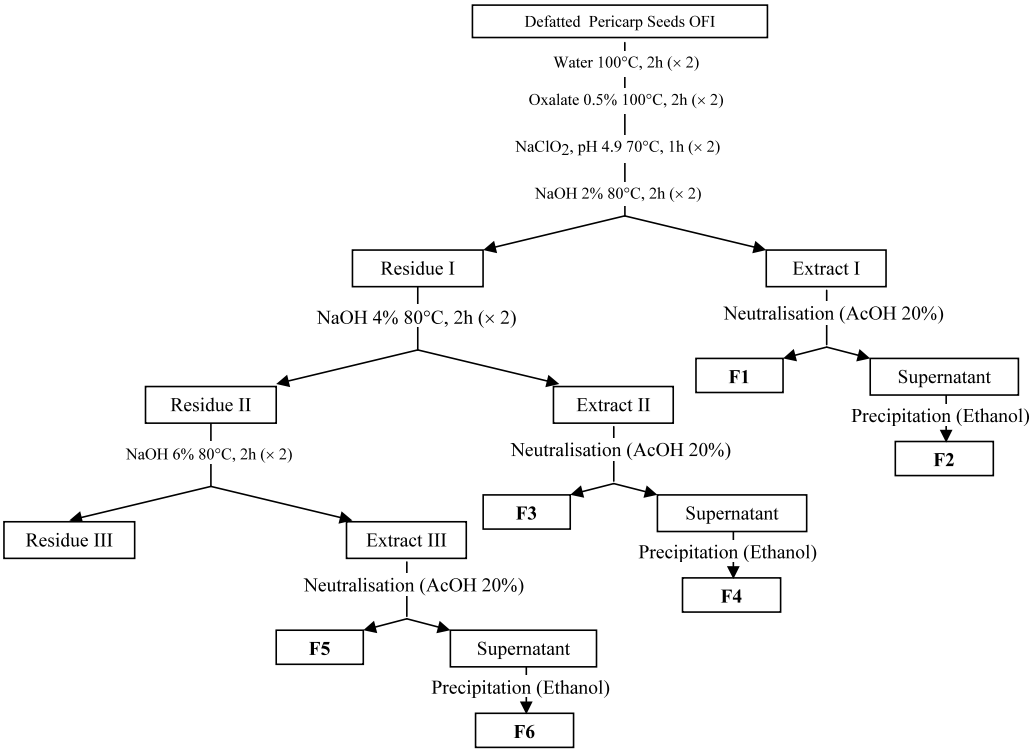

Fig. 2. Fractionation scheme of xylans from pericarp seeds of prickly pears.

Table 1
Yield and sugar composition of pericarp and D-xylans from seeds of OFI

Extract	Yield (%) [a]	Sugar composition [b] (% wt/wt)			
		Arabinose	Xylose	Glucose	Uronic acid
Pericarp	90	1.3	61.7	37	nd [c]
F1	5.1		88.3	tr [d]	11.5
F2	1.0	2.2	80.7	3.9	13.2
F3	2.2		93.0	1.1	5.9
F4	1.7		87.5	2.3	10.1
F5	7.0		98.2	tr [d]	2.5
F6	2.0		89.8	tr [d]	10.2

[a] Expressed as percentage dry-weight of OFI seeds.
[b] Expressed as percentage dry-weight of samples.
[c] nd, not determined.
[d] tr, traces.

of β-(1→4)-D-xylopyranose residues. In seaweed xylans, the D-xylopyranose residues can be either completely β-(1→4) linked as in *Chetangium fastigiatum*[5] (red algae) and *Rhodochorton floridulum*,[6] or completely β-(1→3)-linked as in *Porphyra umbilicalis* and certain siphonaceous green algae[7–9] or of the "mixed linkage" type containing both β-(1→4) and β-(1→3) linkages in *Rhodymenia palmata*,[10] *P. umbilicalis*,[10] *Laurencia pinnatifida*,[10] *Nemalion vermiculare*,[11] *Chaetangium erinaceum*[12] and *C. fastigiatum*.[13]

In plants, the xylopyranosyl residues can be substituted in C-2 and/or C-3 by short and flexible side chains. These chains are constituted mainly of units of α-D-glucuronic acid, or 4-*O*-methyl-α-D-glucuronic acid and occasional units of α-L-arabinofuranose, α-D-xylopyranose or α-D-galactopyranose.

Neutral homoxylans contained xylose residues only, and can be either linear, such as the (1→4)-β-D-xylan of guar seed husk, tobacco stalk, esparto grass, or branched, such as the xylans of groundnut seed en-

Table 2
Molar ratios of xylose to 4-*O*-methyl-glucuronic acid in the fractions

	Xylose/4-*O*-Me-glucuronic acid [a]	Xylose/4-*O*-Me-glucuronic acid [b]
F1	10.6	18.4
F2	8.4	11.2
F3	21.9	26.0
F4	11.9	12.0
F5	54.5	65.0
F6	12.2	14.2

[a] Determined by neutral sugar analysis and colorimetric method.
[b] Determined by ^{1}H NMR.

dosperm, or angiosperm xylans.[14] Neutral heteroxylans or arabinoxylans contained either single α-L-arabinofuranose residues, in general attached by (1→3) linkages, but with double branching (1→3) and (1→2) on xylose units in the more substituted arabinoxylans, such as these from cereal endosperms, or more extended side chains with L-arabinofuranose residues carrying additional substituents.[14,15] In general, most of the acidic xylans from different plant sources contained only 4-*O*-methyl-α-D-glucuronic acid.

The present communication describes the isolation and characterization of xylans from pericarp of seeds of prickly pear fruits of cactus *O. ficus-indica*. A scanning electron microscopy (SEM) shows that the seed consists of two different tissues, the endosperm and the pericarp in a relative proportion of 1:9, respectively (Fig. 1).

The pericarp of seeds of prickly pears was grounded, defatted and extracted according to the procedure presented in Fig. 2. The recovered fractions thus denoted F1, F3, F5 (water non-soluble fractions) and F2, F4, F6 (water-soluble fractions) were obtained in yields of 5.1, 2.2, 7.0, 1.0, 1.7 and 2.0%, respectively, on the basis of the dry weight of starting prickly pear seeds (Table

Table 3
NMR data [a] for glycosyl residues of water soluble xylans from pericarp seeds of *O. ficus indica* (D_2O, 333 K)

Glycosyl residues		Assignment				
		1	2	3	4	5
F2						
(1→4)-β-D-Xyl*p*	^{1}H	4.31	3.14	3.40	3.60	H_{eq}, 3.97; H_{ax}, 3.23
	^{13}C	102.41	73.44	74.47	77.17	63.74
(1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A	^{1}H	4.47	3.38	3.50	3.60	H_{eq}, 3.92; H_{ax}, 3.22
	^{13}C	101.97	77.41	72.12	75.95	62.57
4-*O*-Me-α-D-Glc*p*A	^{1}H	5.09	3.46	3.58	3.12	4.16
	^{13}C	98.29	72.95	77.56	83.05	73.15 (3.32/60.3, OCH_3) (C-6, 176.84)
F4						
(1→4)-β-D-Xyl*p*	^{1}H	4.30	3.11	3.37	3.60	H_{eq}, 3.91; H_{ax}, 3.19
	^{13}C	102.00	73.05	74.01	76.71	63.30
(1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A	^{1}H	4.45	3.35	3.40	3.62	H_{eq}, 3.93; H_{ax}, 3.22
	^{13}C	101.61	77.15	72.56	75.95	62.88
4-*O*-Me-α-D-Glc*p*A	^{1}H	5.10	3.46	3.58	3.13	4.15
	^{13}C	97.83	71.73	78.25	82.81	na [b] (3.28/60.2, OCH_3) (C-6, 177.07)
F6						
(1→4)-β-D-Xyl*p*	^{1}H	4.31	3.14	3.40	3.60	H_{eq}, 3.92; H_{ax}, 3.17
	^{13}C	102.39	73.47	74.52	77.2	63.76
(1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A	^{1}H	4.46	3.42	3.52	3.62	H_{eq}, 3.94; H_{ax}, na
	^{13}C	102.61	76.44	71.78	74.74	62.90
4-*O*-Me-α-D-Glc*p*A	^{1}H	5.08	3.42	3.57	3.11	4.25
	^{13}C	97.29	72.22	78.00	82.12	73.05 (3.30/66.0, OCH_3) (C-6, 177.29)

[a] In ppm relative to the signal of internal acetone in D_2O, at 2.1 ppm (^{1}H) or at 31.5 ppm (^{13}C).
[b] na, not assigned.

Table 4
NMR data [a] for glycosyl residues of water insoluble xylans from pericarp seeds of *O. ficus indica* (Me_2SO-d_6, 333 K)

Glycosyl residues		Assignment				
		1	2	3	4	5
F1						
(1→4)-β-D-Xyl*p*	^1H	4.16	2.93	3.14	3.40	H_{eq}, 3.76; H_{ax}, 3.12
	^{13}C	101.79	72.63	73.93	75.79	63.24
(1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A	^1H	4.37	3.19	3.26	3.41	H_{eq}, 3.87; H_{ax}, 3.18
	^{13}C	100.81	76.52	71.9	73.31	62.60
4-*O*-Me-α-D-Glc*p*A	^1H	4.74	3.37	3.51	3.14	na [b]
	^{13}C	97.55	71.70	78.0	81.57	na [b] (3.16/59.1, OCH_3) (C-6, na [b])
F3						
(1→4)-β-D-Xyl*p*	^1H	4.24	3.02	3.26	3.48	H_{eq}, 3.86; H_{ax}, 3.14
	^{13}C	101.87	72.73	74.10	75.54	63.36
(1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A	^1H	4.46	3.19	3.31	3.49	H_{eq}, 3.87; H_{ax}, 3.18
	^{13}C	100.96	77.82	71.70	74.90	62.02
4-*O*-Me-α-D-Glc*p*A	^1H	5.00	3.37	3.51	3.14	na [b]
	^{13}C	97.65	71.13	78.19	81.99	na [b] (3.16/59.0, OCH_3) (C-6, na [b])
F5						
(1→4)-β-D-Xyl*p*	^1H	4.15	2.92	3.15	3.38	H_{eq}, 3.75; H_{ax}, 3.08
	^{13}C	101.91	72.70	74.07	75.67	63.41
(1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A	^1H	4.46	3.19	3.31	3.49	H_{eq}, 3.87; H_{ax}, 3.18
	^{13}C	102.11	na [b]	na [b]	74.74	63.05
4-*O*-Me-α-D-Glc*p*A	^1H	4.97	3.37	3.51	3.14	na [b]
	^{13}C	97.65	na [b]	na [b]	82.58	na [b] (3.16/59.1, OCH_3) (C-6, na [b])

[a] In ppm relative to the signal of internal acetone in Me_2SO-d_6, at 2.1 ppm (^1H) or at 31.5 ppm (^{13}C).
[b] na, not assigned.

1). The water-soluble fractions were purified by size-exclusion chromatography on a polyacrylamide Biogel P6 column, and the water insoluble fractions by precipitation with Fehling's solution. Each purified fraction was subsequently characterized by sugar analysis, ^1H and ^{13}C NMR spectroscopy.

A hydrolyzate of the different fractions revealed that they were composed essentially of Xyl and small amounts of Glc and Ara. Uronic acid was estimated by colorimetric method, and can varied from 2.5% (w/w) to 11.5% (w/w) in the water non-soluble xylans, and from 10.1 (w/w) to 13.2% (w/w) in the water-soluble fractions (Table 1). We reported in Table 2 the corresponding molar ratios of xylose to uronic acid in the different fractions.

The ^1H and ^{13}C spectra of water-soluble fractions were performed in D_2O, whereas the spectra of water insoluble fractions were performed in Me_2SO-d_6. Examination of each spectrum shows the relative simplicity of the structure exhibited by: (i) major signals corresponding to non-substituted β-D-Xyl residues; (ii) minor signals assigned to 4-*O*-methyl-β-D-GlcA acid residues, and to β-D-Xyl units substituted with 4-*O*-methyl-α-D-GlcA. We reported all the NMR data in Tables 3 and 4, and they are in good agreement with the structures of (4-*O*-methyl-α-D-glucurono)-β-D-xylans already described in other plants.[16–20]

The proton spectra for the different substrates showed three doublets in the anomeric region assigned to H-1 of [(1→4)-β-D-Xyl*p*], H-1 of [(1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A] and H-1 of [(4-*O*-methyl-α-D-Glc*p*A)] with variable intensity. The molar proportions of D-Xyl and 4-*O*-methyl-α-D-GlcA were determined by integration of corresponding anomeric protons, and were found respectively to 18:1 for F1, 11:1 for F2, 26:1 for F3, 12:1 for F4, 65:1 for F5, and 14:1 in the case of F6. These NMR data suggested the presence, in average, 12 xylose residues per uronic acid residue in the water-soluble glucuronoxylans. On the other hand, the water non-soluble fractions presented much lower acid con-

tent, and the xylose residues can varying from 18 to 65 per uronic acid residue.

The hemicelluloses of the seeds of OFI were therefore 4-*O*-methylglucuronoxylans, the main difference in their structures being the relative amounts of 4-*O*-methyl-α-D-GlcA residues, as observed by Bazus et al.[17] in glucuronoxylans isolated from sunflowers hulls.

We presented in Table 5 the xylose to 4-*O*-methyl-α-D-GlcA molar ratios found in the literature for different xylans. The xylose–4-*O*-methyl-α-D-GlcA molar ratios can vary from 2:1 in quince tree seeds xylan to 65:1 in prickly pears seeds, but with most average values from 6:1 to 12:1. Typical xylans from softwoods contain more acidic groups, 4–6 xylose residues per acid group.[22] Most of the hardwood xylans contain 9–11 xylose residues per acid side group,[23–25,27] with some exception like beech wood (*Fagus sylvatica* L.).[23]

Our results showed that the purified xylans fractions extracted from cactus pericarp seeds presented large discrepancies in the molar proportion of D-xylose and 4-*O*-methyl-α-D-GlcA, which can vary from 11:1 to 65:1.

Table 5
4-*O*-Methyl glucuronoxylans from some land plant tissues

Source	Molar ratio	References
	Xyl/–4-*O*-MeGlcA	
Quince tree seeds (*Cydonia oblonga*)	2:1	16, 18
Sunflower hulls	3.4:1	17
	7.2:1	17
	9.1:1	17
Pineapple leaf fibre (*Ananas comosus* L. MERR.)	5:1	21
Norvegian spruce	5:1	22
Beech wood (*F. sylvatica* L.)	6:1	23
Sugar beet pulp	7:1	20
Luffa fibre	7.5:1	18
Aspen wood (*Populus tremula*)	9:1	24
European beech	10:1	25
White birch wood (*Betula papyrifera*, Marsh)	11:1	26
Aspen bark (*Populus tremuloïdes*)	12:1	27
Siberian apricot (*Armeniaca siberica* L.)	14:1	28
Finnish birch	22:1	29
Prickly pear seeds	11:1	this paper
	12:1	this paper
	14:1	this paper
	18:1	this paper
	26:1	this paper
	65:1	this paper

1. Experimental

Materials.—Fresh prickly pear fruits of *O. ficus-indica* (OFI) were gathered in a pilot plantation in Amezmiz, 30 km from Marrakech (Morocco).

General methods.—Uronic acid content was determined according to Blumenkrantz and Asboe-Hansen.[30] Neutral sugars were released by H_2SO_4 hydrolysis and analyzed by GLC as their corresponding alditol acetates[31] using a Packard and Becker 417 instrument coupled to a Hewlett–Packard 3380 A integrator. Glass columns (3 mm × 2 m) packed with 3% SP 2340 on Chromosorb W-AW DMCS (100–120 mesh), or 3% OV 17 on the same support, were used.

Isolation of (4-O-methyl-D-glucurono)-D-xylans (Fig. 2).—The peel and the pulp were removed and the seeds were cleaned by several washings in distilled water. After drying, the seeds were cracked in an analytical grinder, and the endosperm was removed by seaving (60 mesh sieve). The purified pericarp, which is very hard, was then finely grounded in a mixer, and fats, waxes and oils were solubilized in a Soxhlet by refluxing 24 h with 19:31 toluene–EtOH. The pectic polysaccharides were extracted sequentially with boiling water (2 × 4 h), aq 0.5% ammonium oxalate (2 × 4 h at 100 °C). Two sodium chlorite treatments ($NaClO_2$, pH 4.9, 1 h, 70 °C) were performed to remove residual protein and lignin. The bleached residue was then extracted at 80 °C (2 × 2 h) successively with 0.5 M, 1 M and 1.5 M NaOH solution. Each extract was neutralized with 20% AcOH solution to pH 4–5, maintained overnight at 4 °C, and the resulting precipitates were removed by centrifugation at 6000 rpm for 0.5 h. These precipitates were suspended in water, dialyzed and freeze-dried to give fractions F1, F3 and F5, respectively. Ethanol (4 vols) was added to each resulting supernatant to precipitate the remaining polysaccharide. The precipitates that formed were recovered by centrifugation, washed with 18:1.5:0.5 EtOH–water–HCl, dissolved in water, dialyzed against distilled water (2 × 24 h), and freeze-dried to give fractions F2, F4 and F6, respectively.

The soluble fractions, **F2**, **F4** and **F6**, respectively, were purified by size-exclusion chromatography on a polyacrylamide Biogel P6 column (4 × 100 cm), eluted with 0.05 M sodium nitrate solution at 80 mL/h. The salts were removed by dialysis and the solution freeze dried. The water non-soluble fractions F1, F3 and F5, respectively, were purified by precipitation with Fehling's solution according to Chanda et al.[32]

NMR spectroscopy.—^{1}H and ^{13}C NMR experiments were recorded on an AC 300 Bruker spectrometer (^{1}H frequency of 300 MHz and ^{13}C frequency of 75.468 MHz). Water soluble samples F2, F4 and F6 were studied as solution in D_2O at 333 K in a 5 mm o.d. tube (internal acetone ^{1}H (CH_3) at 2.1 ppm relative to Me_4Si and ^{13}C (CH_3) at 31.5 ppm relative to Me_4Si). Samples of water insoluble fractions were studied as solution in Me_2SO-d_6 at 333 K in a 5 mm o.d. tube (internal acetone ^{1}H (CH_3) at 2.1 ppm relative to Me_4Si and ^{13}C (CH_3) at 31.5 ppm relative to Me_4Si).

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