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Note

Isolation and characterization of xylans from seed pericarp of *Argania spinosa* fruit

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Abstract—Hemicellulose-type polysaccharides were isolated from the pericarp of seeds of *Argania spinosa* (L.) Skeels fruit by sequential alkaline extractions and fractionated by precipitation. Water soluble and water insoluble fractions were obtained, purified and characterized by sugar analysis and ^{1}H and ^{13}C NMR spectroscopy. The water soluble fractions were assumed to be (4-*O*-methyl-D-glucurono)-D-xylans, with 4-*O*-methyl-D-glucopyranosyluronic acid groups linked to C-2 of a (1→4)-β-D-xylan. The ^{1}H NMR spectrum showed that the water soluble xylans have, on average, one non-reducing terminal residue of 4-*O*-methyl-D-glucuronic acid for every seven xylose units. The water insoluble fractions consisted of a neutral xylan with linear (1→4)-β-D-xylopyranosyl units.

Keywords: *Argania spinosa*; Seed; (1→4)-β-D-Xylan; ^{1}H and ^{13}C NMR spectroscopy

The argan tree (*Argania spinosa* (L.) Skeels) is a tropical plant, which belongs to the Sapotaceae family and is endemic in south western Morocco, where it grows over about 320,000 square miles. This forest species is adapted to the dryness of the climate and the severity of the natural conditions. It has been extremely useful in controlling desertification, improving depleted natural rangelands by preventing long-term degradation of ecologically weak environments. Thus, argania preservation and cultivation has become an important crop for the maintenance of agriculture activity in this part of Morocco. Several bilateral projects and local programmes have promoted the establishment of argania to orient the utilization of the plant for multiple purposes.[1]

The argania tree is exploited essentially for its fruits. The endosperm seed of fruit constitutes a good potential source of edible oil for human consumption and endowed with important medicinal properties (e.g., decreasing cholesterol level, stimulation of vascular circulation).[2] Argan oil is also widely incorporated in many cosmetic products such as anti-acne agents.[3–6] For these reasons, numerous studies have been engaged on the chemical composition of argan oil in the last decade. The results of physical and chemical analysis showed the original fatty acid composition, with oleic acid (47%), linoleic acid (32%) and palmitic acid (14%). Other components are also present such as saponins, carotene, sterols, triterpens, alcohols and tocopherols.[7–11] After extraction of the oil the cattle cake was used to complement the leaves and pulp fruit residue, already used as forage. However, the woods of *A. spinosa* as well as the seed pericarp, recovered after extraction of the oil from endosperm seed, were used traditionally for fuel.[1]

For a few years, our group has been involved with the study of plant cell wall polymers of some xerophyte species of the Moroccan flora such as *Opuntia ficus-indica* prickly pear cactus and argan tree. Indeed in previous works, we studied the cell wall polysaccharides isolated from different parts of plant and fruit of *Opuntia ficus-indica*.[12–15] The data on the argan polysaccharides

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are very rare, only Ray et al. achieved a study about hemicelluloses isolated from the leaves of the tree.[16] In this work, we investigated the isolation and structural characterization of the hemicellulose-type polysaccharides from pericarp seed of *A. spinosa*.

Xylans are the most abundant hemicellulose-type polysaccharides constituent in the plant. They are known to occur in several structural varieties in terrestrial plants and even in different plant tissues within one plant. Based on the hitherto published results it can be deduced that the structural diversity of xylans is related to their functionality in plants and may explain the distribution of certain xylan structures in the plant kingdom.[17] From a structural point of view, xylans of all higher plants possess β-(1→4) linked xylopyranose units as the backbone. Depending on their origin, that is, Gramineae (grasses and cereals), Gymnosperms (softwoods) or Angiosperms (hardwoods), the backbone is usually substituted with α-D-glucuronic acid, 4-*O*-methyl-α-D-glucuronic acid and some neutral sugar units such α-L-arabinose, α-D-xylose and α-D-galactose. Among the usual side groups we can found acetyl groups, phenolic acids, ferulic and coumaric acids.[17–20]

The hemicellulose-type polysaccharides were extracted by sequential alkaline treatments from pericarp powder of seed of *A. spinosa* fruit after a bleaching treatment with sodium chlorite following the method of Wise et al.[21] The extractions were performed at 80 °C with 0.5, 1 and 2 M alkaline solution, respectively. All the extracts were acidified (pH ≈ 5–6) and the precipitates recovered by centrifugation to give the crude water insoluble fractions (CWIF-I, CWIF-II and CWIF-III, respectively). The resulting supernatants for every extract were precipitated with EtOH (4 volumes) to give crude water soluble fractions CWSF-I, CWSF-II and CWSF-III, respectively. The extraction scheme is given in Figure 1 and showed a similarity to the extraction scheme used in our previous work with minor modifications.[14] The yields of all recovered fractions are reported in Table 1.

The crude water soluble fractions (CWSF-I, CWSF-II and CWSF-III) were purified by size-exclusion chromatography to give purified water soluble fractions (PWSF-I, PWSF-II and PWSF-III). A typical chromatogram corresponding to the purification of CWSF-I is shown in Figure 2. The crude water insoluble fractions (CWIF-I, CWIF-II and CWIF-III) were purified by copper precipitation to give the purified water insoluble fractions (PWIF-I, PWIF-II and PWIF-III).

The sugar content of pericarp seed of *A. spinosa* fruit and of the crude and purified fractions are given in Table 1. The three purified water soluble fractions have a similar composition and contained in average, 5.8 xylose residues for 1 uronic acid unit (Xyl/UA, 5.8:1). All purified water insoluble fractions are composed exclusively of xylose.

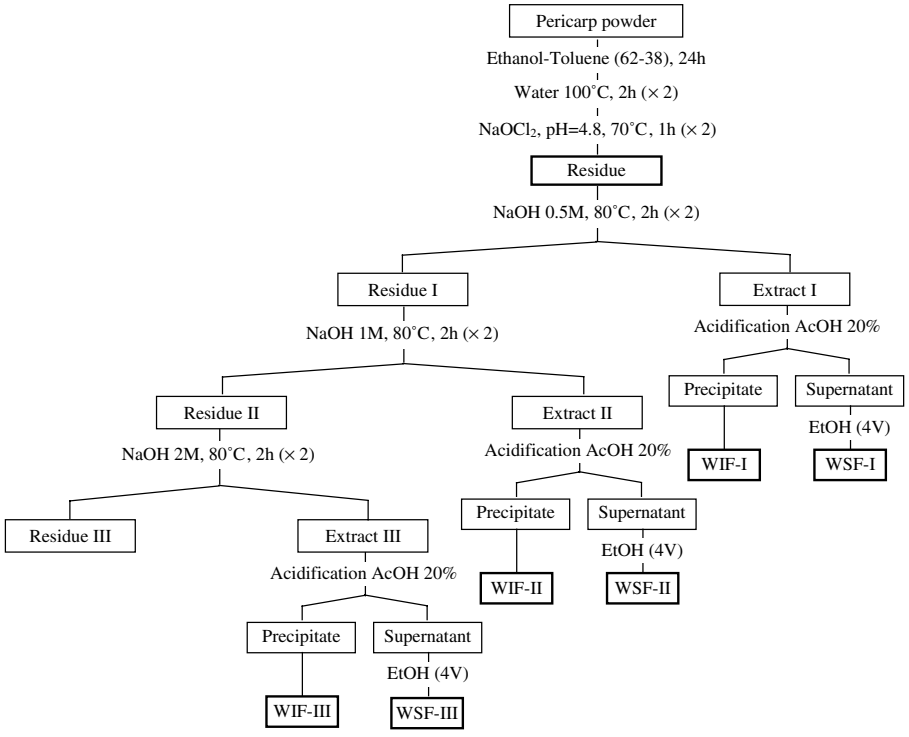

Figure 1. Scheme of fractionation of hemicelluloses from pericarp seed of *A. spinosa*.

Table 1. Sugar composition of pericarp and of xylan fractions from seeds of *A. spinosa*

Fraction	Yield[a]	Sugar composition[b] (% wt/wt)				
		Uronic acid	Arabinose	Xylose	Galactose	Glucose
Pericarp	—	9.0	2.2	51.7	1.1	36.0
Water insoluble fractions						
CWIF-I	9.5	0	0.7	97.6	0.2	1.1
CWIF-II	13.3	0	0.6	98.0	0.1	1.3
CWIF-III	9.3	0	Traces	98.3	—	1.2
PWIF-I	—	0	—	100	—	—
PWIF-II	—	0	—	100	—	—
PWIF-III	—	0	—	100	—	—
Water soluble fractions						
CWSF-I	2.8	21.5	1.3	76.1	0.5	0.3
CWSF-II	2.9	21.7	1.2	76.2	0.3	0.1
CWSF-III	1.4	21.7	0.9	76.5	0.4	Traces
PWSF-I	—	22.6	Traces	76.4	—	—
PWSF-II	—	22.0	—	77.5	—	—
PWSF-III	—	22.5	—	77.3	—	—

[a] As % of pericarp dry matter.
[b] Expressed in relative weight percentages.

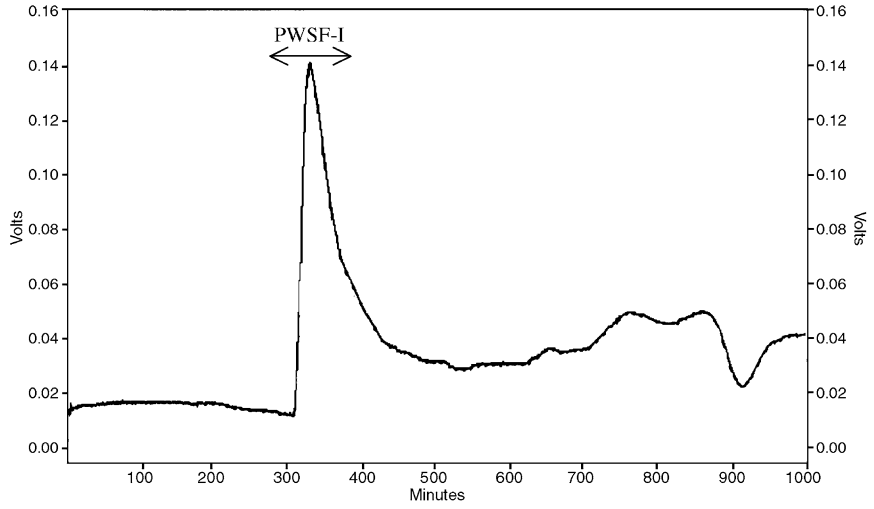

Figure 2. Size-exclusion chromatogram of CWSF-I.

The purified xylan fractions extracted from seed pericarp of *A. spinosa* were also investigated by NMR spectroscopy. The ^{1}H and ^{13}C NMR spectra of purified water soluble fractions were recorded in D_2O; all fractions showed very similar spectra and we shown in Figure 3 only the ^{1}H and ^{13}C spectra of PWSF-II. The ^{13}C NMR spectrum (Fig. 3a) showed five main signals at δ 102.46 (C-1), 73.52 (C-2), 74.54 (C-3), 77.23 (C-4) and 63.81 (C-5) ppm, corresponding to (1→4) linked β-D-xylose residues. Other less intense signals at δ 177.24, 98.36, 73.03, 72.18, 83.12, 73.17 and 60.41 ppm, are characteristic, respectively, of C-6, C-1, C-2, C-3, C-4, C-5 and of methoxyl group of 4-*O*-methyl-α-D-glucuronic acid residue. Signals at δ 102.04, 77.46, 77.56, 76.48 and 63.61 ppm are characteristic of C-1, C-2, C-3, C-4, C-5, respectively, of β-D-xylose units substituted in position O-2 with 4-*O*-methyl-α-D-glucuronic acid.

Examination of the proton spectrum of PWSF-II (Fig. 3b) showed the relative simplicity of the structure as exhibited by (i) major signals at δ 4.38 (H-1), 3.98 (H-5eq), 3.65 (H-4), 3.46 (H-3), 3.25 (H-5ax) and 3.20 ppm (H-2), corresponding to non-substituted β-D-Xyl residues; (ii) minor signals at δ 5.19 (H-1), 4.23 (H-5), 3.64 (H-3), 3.47 (H-2), 3.37 (OCH_3), 3.17 ppm (H-4), corresponding to 4-*O*-methyl-α-D-GlcA acid residues, and at δ 4.53 (H-1), 4.02 (H-5eq), 3.63 (H-4), 3.56 (H-3), 3.38 (H-2) and 3.22 ppm (H-5ax) assigned to β-D-Xyl units substituted in position O-2 with 4-*O*-methyl-α-D-glucuronic acid.

The proton of purified water soluble fractions spectra showed three doublets in the anomeric region assigned to (1→4)-β-D-Xyl*p*, (1→4)-β-D-Xyl*p*-2-*O*-Glc*p*A and 4-*O*-methyl-β-D-Glc*p*A residues. The average integration of these anomeric signals for the three water soluble

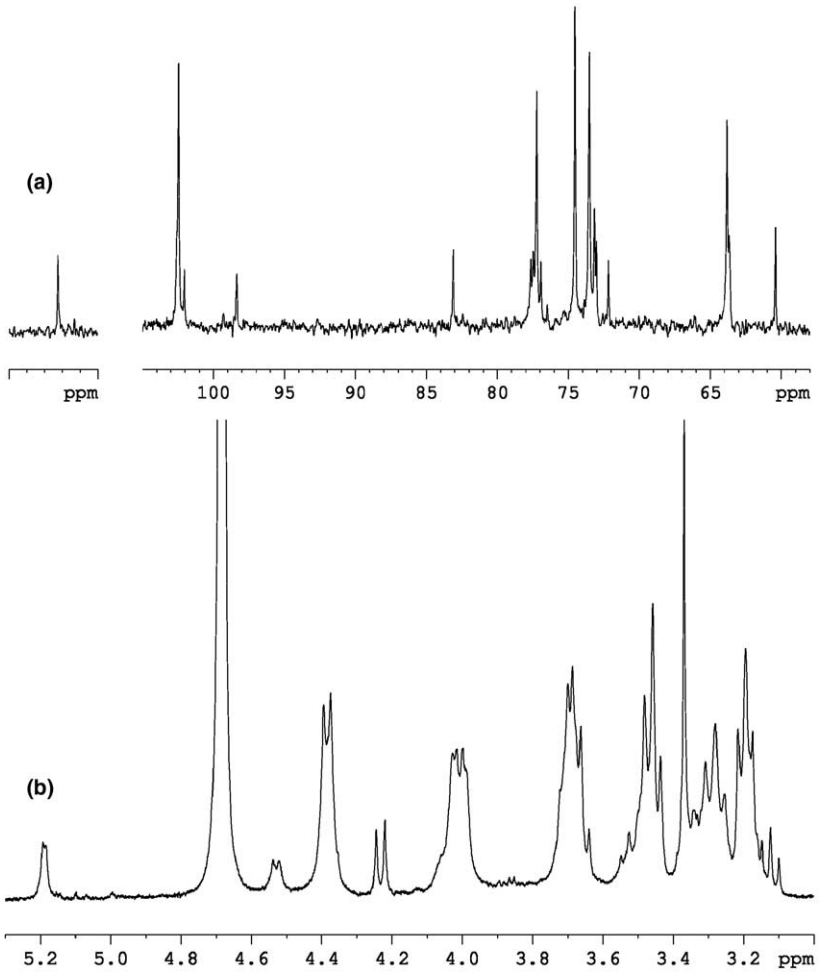

Figure 3. ^{13}C and ^{1}H NMR spectra of PWSF-II (D_2O, 323 K, 400.13 MHz).

fractions presented a molar ratio of non-substituted xylose units, substituted xylose units and 4-*O*-methyl-α-D-glucuronic acid estimated to be 6:1:1, respectively. Thus the proton NMR data indicated the presence of seven xylose units per uronic acid residue. These data are slightly different with sugar composition data presented above (Xyl/UA, 5.8:1), but it is well known that incomplete hydrolysis of the Glc*p*A→Xyl*p* linkage can induce an under-estimation of the xylose.

All the data reported are in good agreement with the structures of (4-*O*-methyl-α-D-glucurono)-β-D-xylans already found in a number of plants.[14,15,22,23]

The ^{1}H and ^{13}C NMR spectra of water insoluble fractions were recorded in DMSO-d_6 and all the fractions showed similar spectra. Figure 4a showed the ^{13}C NMR spectrum of PWIF-II in DMSO-d_6. We can observe only five signals at δ 102.64 (C-1), 73.53 (C-2), 74.89 (C-3), 76.47 (C-4) and 64.13 (C-5) ppm, corresponding to (1→4) linked β-D-Xyl residues. The ^{1}H spectrum (Fig. 4b) showed six signals at δ 4.26 (H-1), 3.05 (H-2) 3.28 (H-3), 3.53 (H-4), 3.18 (H-5_{ax}) and 3.88 (H-5_{eq}).

1. Experimental

1.1. Materials

Argania fruits were collected in July 2003 from the station plantation located in the vicinity of Essaouira (Morocco). The fruit seeds were hand crushed to eliminate the endosperm. Finally the pericarp was ground for a few minutes in a mixer grinder and sieved to produce a homogeneous powder.

1.2. Isolation of hemicelluloses fractions

Fats, waxes and oils were removed from seed pericarp powder by refluxing in a Soxhlet apparatus during 24 h with 38:62 toluene–EtOH. The resulting defatted material was treated by water (2 × 2 h at 100 °C) and two sodium chlorite treatments were performed to remove residual protein and lignin. The bleached residue was sequentially extracted at 80 °C by 0.5 M NaOH, 2 × 2 h, 1 M NaOH, 2 × 2 h, and finally by 2 M NaOH, 2 × 2 h. The extracts were acidified to pH ≈ 5–6 with

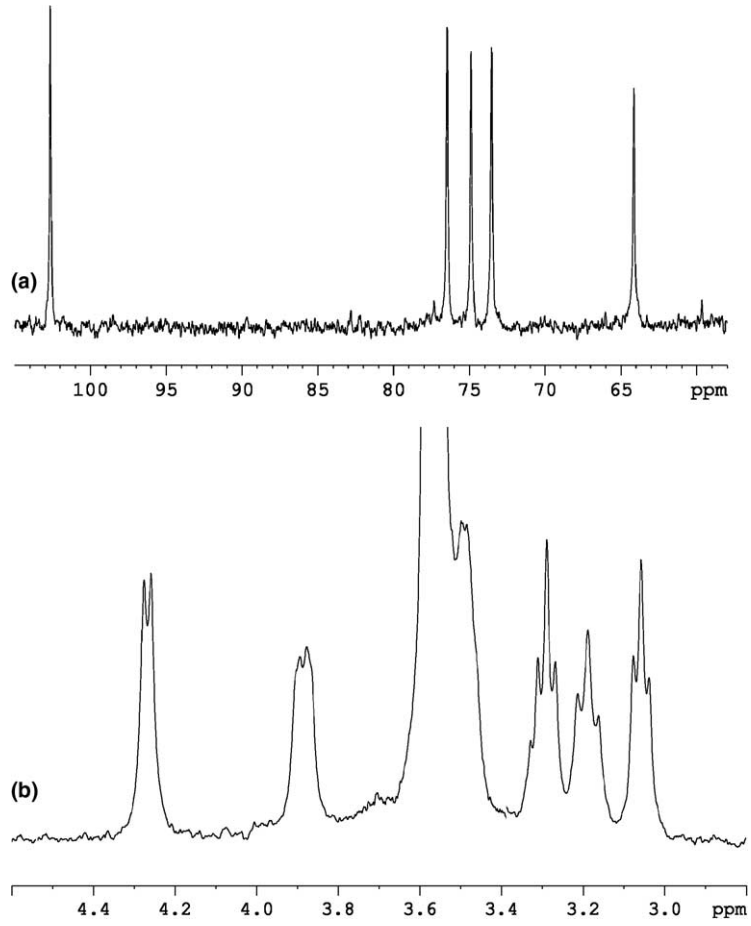

Figure 4. ^{13}C and ^{1}H NMR spectra of PWIF-II (in DMSO-d_6, 323 K, 400.13 MHz).

20% acetic acid and the precipitates were recovered by centrifugation to give the crude water insoluble fractions (CWIF-I, CWIF-II and CWIF-III). The resulting supernatants for each extract were precipitated with EtOH (4 volumes) to give crude water soluble fractions (CWSF-I, CWSF-II and CWSF-III). The extraction scheme is given in Figure 2.

1.3. Purification of hemicelluloses fractions

The water soluble fractions (CWSF-I, CWSF-II and CWSF-III) were purified by size-exclusion chromatography on a polyacrylamide Biogel P6 column (4–100 cm), eluted at 80 mL/h with 0.05 M sodium nitrate solution, and at room temperature. The column effluent was monitored using a refractive index detector. The salts were removed by dialysis and the solution freeze-dried, to give the purified water soluble fractions (PWSF-I, PWSF-II and PWSF-III).

The water non-soluble fractions (CWIF-I, CWIF-II and CWIF-III) were purified by precipitation with Fehling solution according to Jones and Stoodley.[24] The samples were dissolved in 0.5 M NaOH solution and the Fehling solution was added to the solution until precipitation of the copper complex. After 4 h the precipitate is collected by centrifugation, washed with water and decomposed by maceration for 1 min with ethanol containing 5% (v/v) of hydrochloric acid, washed exhaustively with water and freeze-dried.

1.4. Analytical methods

Neutral sugars were analyzed, after H_2SO_4 hydrolysis, by GLC as their corresponding alditol acetates, 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. Peak areas were corrected by using the molar response factors according to Sweet et al.[25] Uronic acid content was determined according to Blumenkrantz and Asboe-Hansen method.[26]

1.5. NMR spectroscopy

^{1}H and ^{13}C NMR experiments were recorded on a Bruker Avance 400 spectrometer (operating frequency of 400.13 and 100.57 MHz, respectively). Samples were

recorded as solution in D_2O for water soluble fractions or in DMSO-d_6 for water insoluble fractions at 323 K in 5 mm od. tube (internal acetone ^1H (CH_3) at 2.1 ppm and ^{13}C (CH_3) at 31.5 ppm relative to Me_4Si).

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