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# Structural investigation of hemicellulosic polysaccharides from *Argania spinosa*: characterisation of a novel xyloglucan motif

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**Abstract**—Hemicellulose polymers were isolated from *Argania spinosa* leaf cell walls by sequential extractions with alkali. The structure of the two main polymers, xylan and xyloglucan, was investigated by enzyme degradation with specific endoglycosidases followed by analysis of the resulting fragments by high performance anion exchange chromatography (HPAEC) and matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS). The results show that *A. spinosa* xylan is composed of a β-(1→4)-linked-D-xylopyranose backbone substituted with 4-*O*-methyl-D-glucuronic acid residues. Xyloglucan oligosaccharide subunits were generated by treatment with an *endo*-(1→4)-β-D-glucanase of the xyloglucan-rich hemicellulosic fractions. MALDI-TOF mass spectra and HPAE-PAD chromatography of the pool of endoglucanase-generated xyloglucan oligomers indicated that *A. spinosa* cell wall contains a XXXG-type xyloglucan. In addition to XXXG, XXFG, XLXG/XXLG, XLFG fragments previously characterised in various plants, a second group of XXXG-type fragments was detected. The primary structure of the major subunit was determined by a combination of sugar analysis, methylation analysis, post-source decay (PSD) fragment analysis of MALDI-TOF MS and $^1$H NMR spectroscopy. This fragment, termed XUFG, contains a novel β-D-Xyl*p*-(1→2)-α-D-Xyl*p* side chain linked to C-6 of the second glucose unit from the nonreducing end of the cellotetraose sequence.


*Keywords:* *Argania spinosa*; Xyloglucan; Xylan

## 1. Introduction

Argan tree (*Argania spinosa* (L.) Skeels) belongs to the sapotaeceae family and is endemic to south western Morocco. It is adapted to grow in a harsh environment, surviving extreme drought and poor soil. *A. spinosa* is a thorny and slow growing tree that plays vital roles in the food chain. As to environment protection, argan tree helps slowing down desert progression because of its ability to grow in arid areas and of its deep-growing roots. The fruit has a green and fleshly exterior, and contains a nut inside with a hard shell, which in turn contains one or two almonds. *A. spinosa* is important for local economy since almonds are used to produce argan oil, which is largely used for cooking and believed to have various medicinal properties (e.g., decreasing cholesterol level, stimulation of vascular circulation). Argan oil is also widely incorporated in many cosmetic products.[1,2] Argan oil is mostly composed of glycerides (~99%) with oleic and linoleic acids as the major fatty acids (representing about 80%).[1] Other components are carotene, sterols, triterpens, alcohols and tocopherols.[3] Apart from lipids, essentially no studies are devoted to other macromolecules of argan tree such as polysaccharides or glycoproteins.[1,4]

In the course of our studies on the structure, function and potential industrial application of polymers from plant cell walls, we have investigated the structure of

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hemicellulosic polysaccharides from argan leaves. We found that *A. spinosa* hemicellulose fraction mainly consists of a xylan composed of a β-(1→4)-linked-D-xylopyranose backbone substituted with 4-*O*-MeGlcA residues, and a XXXG-type xyloglucan. In the latter, the cellotetraose backbone is substituted at C-6 of Glc (G) residues by terminal xylopyranose residue (X), or β-D-Gal*p*-(1→2)-α-D-Xyl*p* (L) or α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→2)-α-D-Xyl*p* (F) side chains leading to XXXG, XXFG, XLXG/XXLG and XLFG xyloglucan fragments. In addition to these oligosaccharides previously characterised in various plants, novel xyloglucan fragments containing a β-Xyl*p*-(1→2)-α-Xyl*p* side chain, linked to C-6 of the second β-(1,4)-linked Glc unit from the nonreducing end of the cellotetraose sequence, were structurally characterised.

## 2. Experimental

### 2.1. Plant materials

*A. spinosa* leaves were collected in the south of Morocco near Aoulouze village (in the Souss region). Leaves were dried and used to extract cell walls.

### 2.2. Nomenclature

Xyloglucan derived oligosaccharides are named according to Fry et al.[5] Residues of xyloglucan derived oligoglycosyl alditol are indicated by a lowercase superscript a, b, or c indicating the position of each residue onto the cellotetraose backbone ($Glc^c \rightarrow Glc^b \rightarrow Glc^a \rightarrow Glcol$). For instance, $Xyl^b$ corresponds to the xylose residue attached to $Glc^b$.

### 2.3. Isolation of hemicellulosic polymers

Dry *A. spinosa* leaves (15 g) were ground to a fine powder (under liquid $N_2$) and then extracted sequentially twice with 500 mL of aq 80% (v/v) EtOH, 95% EtOH and acetone with continuous stirring for 14 h. The alcohol insoluble residue (8 g) was depectinated by treatment with cold (3×300 mL) and hot water (3×100 mL), and CDTA (*trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid) (pH 6.8, 2×300 mL). The residue was then extracted sequentially twice with 500 mL of 1 and 4 M KOH solns containing 20 mM $NaBH_4$ for 14 h. Extracts were acidified with AcOH to pH 6 and dialysed extensively against water. The precipitates were removed by centrifugation to yield A1OH (400 mg) and A4OH (300 mg) fractions from 1 and 4 M KOH extracts, respectively. The soluble fractions from the 1 and 4 M KOH extracts have been designated as B1OH (300 mg) and B4OH (600 mg), respectively.

### 2.4. Sugar analysis

For the determination of monosaccharide composition, the samples were hydrolysed using trifluoroacetic acid (2 M, 2 h at 110 °C), followed by a 18 h methanolysis at 80 °C with dry 2 M methanolic-HCl. The generated methyl glycosides were converted into their TMS derivatives and separated by gas chromatography (GC).[6] The gas chromatograph was equipped with a flame ionisation detector, a WCOT (wall coated open tubular) fused silica capillary column (length 25 m, i.d. 0.25 mm) with CP-Sil 5 CP as stationary phase and helium as gas vector. The oven temperature programme was 2 min at 120 °C, 10 °C/min to 160 °C, and 1.5 °C/min to 220 °C and then 20 °C/min to 280 °C.

### 2.5. Permethylation of oligosaccharides

The pool of oligosaccharides generated from *A. spinosa* xyloglucan were reduced and permethylated according to Ciucanu and Kerek.[7] Permethylated samples were extracted, dried, hydrolysed, converted into their partially methylated alditol acetates and were separated by GC (HP6890 series) on a capillary column (length 30 m, i.d. 0.25 mm) with optima 5-MS as stationary phase of (Macharey–Nagel) and analysed by electron impact mass spectrometry (EI-MS) using an Autospec GC–MS mass spectrometer (Micromass, Manchester, UK).

### 2.6. Preparation of xylan oligosaccharides

Hydrolysis of 10 mg of the xylan-rich fractions (A1OH and A4OH) in 3 mL of 10 mM NaOAc pH 5.0 was performed using 40 units of *endo*-(1→4)-β-D-xylanase (Megazyme International Ireland, xylanase M6) at 37 °C for 24 h. To remove polymeric materials, hydrolysates were precipitated with 4 vol of cold EtOH. After centrifugation, the oligomers present in the supernatants were concentrated yielding A1Xose and A4Xose. The enzyme-resistant materials isolated in the pellets were designated as A1X-RF and A4X-RF.

### 2.7. Preparation of xyloglucan oligomers

To 5 mL solns of samples (15 mg each; B4OH/B1OH) in 50 mM NaOAc buffer pH 5.0 were added 30 units of *endo*-(1→4)-β-D-glucanase (Megazyme International Ireland, EC 3.2.1.4, cat. no. E-CELTR) and the solns were incubated for 24 h at 37 °C. Glucanase-resistant materials were removed by diluting the digests with EtOH to a final concentration of 80%. The EtOH soluble oligosaccharides were concentrated under a stream of nitrogen to yield xyloglucan oligosaccharides B4XGose and B1XGose from B4OH and B1OH, respectively. Endoglucanase-resistant fractions designated as B4G-RF and B1G-RF were obtained after lyophili-

sation of the EtOH insoluble residues. Xyloglucan oligosaccharides were reduced in 10 mL 1 M $NH_4OH$ with 100 mg sodium borohydride. Excess of borohydride was quenched with AcOH (5 M) and the soln was desalted on a column of Sephadex G-15.

### 2.8. High performance anion exchange-pulse amperometric detection-chromatography (HPAE-PAD-chromatography)

Endoglucanase-generated and oligoglycosyl alditol xyloglucan fragments were analysed by high performance anion exchange (HPAE) chromatography equipped with a CarboPac PA-1 column (Dionex) combined with pulse amperometric detection (PAD). Samples (10–50 μL) were eluted at 1 mL/min with the following NaOAc gradient in 100 mM NaOH: $0 \rightarrow 5$ min, linear gradient of $0 \rightarrow 5$ mM NaOAc; $5 \rightarrow 30$ min, linear gradient of $5 \rightarrow 8$ mM NaOAc; $30 \rightarrow 35$ min, linear gradient of $8 \rightarrow 13$ mM NaOAc; $35 \rightarrow 40$ min, linear gradient of $13 \rightarrow 15$ mM NaOAc; $40 \rightarrow 42$ min, linear gradient of $15 \rightarrow 100$ mM NaOAc. Each elution was followed by a wash with 1 M NaOAc in 100 mM NaOH, and subsequent equilibration for 5 min with 100 mM NaOH. For preparative isolation, oligoglycosyl alditols from B4XGol were separated by repeated injections on HPAEC-PAD. Fractions were collected and immediately chilled. To remove cations, the pooled fractions were passed through AG 50W-XG ($H^+$, Bio-Rad) cation exchange resin at 4 °C. The effluents were adjusted to pH 6.8 with $NH_4OH$, lyophilised, then dissolved in water and passed through DEAE-Sepharose column (5–7 mL, Bio-Rad, $AcO^-$). Fractions eluted with water were collected and lyophilised. The purity of each isolated pools was checked by HPAE-PAD chromatography and MALDI-TOF MS.

### 2.9. Matrix-assisted laser desorption ionisation-time of flight mass spectra (MALDI-TOF MS)

MALDI-TOF mass spectrometry was performed on a Micromass (Manchester, UK) TOF spec E mass spectrometer equipped with a nitrogen laser operating at 337 nm. Mass spectra were recorded in reflectron mode and in positive ion detection using 2,5-dihydroxybenzoic acid (10 mg/mL) as matrix. Each of the samples (2 μL) was mixed with 2 μL of the matrix soln in 1.75:0.75 TFA–MeCN. A 2 μL portion of this soln was applied on a stainless steel sample plate and allowed to dry under diminished pressure. Spectra were acquired in the reflectron mode. For collision induced dissociation (CID) and post-source decay (PSD) experiments, selection of the precursor ion was carried out using a Bradbury–Nielsen ion gate with a $m/\Delta m = 100$ resolution. CID was carried out by collision with helium under diminished pressure of $10^{-6}$ mbar. Extraction with a delay time of 600 ns was used. To record the full PSD spectra, reflector voltage was decreased into successive 25% steps leading to 10 spectral segments using 30 laser shots per segment. PSD spectra were calibrated using ACTH (fragment 18–39).

### 2.10. NMR spectrometry

The NMR spectrum of the oligoglycosyl alditol was recorded on a Bruker Avance DMX 600 spectrometer equipped with a 5 mm TXI probe and operating at 600.13 MHz for $^1H$. The sample was dissolved in 99.95 $D_2O$ (0.6 mL) and the $^1H$ NMR spectrum was recorded at 300 K.

## 3. Results

### 3.1. Isolation and composition of hemicellulosic polysaccharides

Hemicellulosic polysaccharides were isolated from the depectinated cell wall of *A. spinosa* leaves by sequential extraction with 1 and 4 M KOH. These extracts, which form precipitates during neutralisation, were then separated into two fractions: the hemicellulose fractions A1OH and A4OH corresponding to the precipitates and the hemicellulose fractions B1OH and B4OH corresponding to the soluble polymers. Sugar analysis of the latter fractions mainly revealed the presence of xylose, glucose and galactose suggesting that they are composed of xyloglucan (Table 1). In contrast, xylose was the main monosaccharide of the hemicellulose A1OH and A4OH fractions indicating the presence of xylan. Other monosaccharides, such as arabinose, galacturonic acid and rhamnose, probably arose from pectic material co-extracted with hemicelluloses.

### 3.2. Structural analysis of xylan

To gain information on the structure of xylan present in the A1OH and A4OH fractions, the latter was treated with an endoxylanase. The enzyme converted the cell wall extracts into soluble xylan oligomers, A1Xose and A4Xose, and endoxylanase-resistant fractions, A1X-RF and A4X-RF. Similar results were obtained for both extracts. Table 1 shows results obtained from the digestion of A4OH. The A4Xose fraction consisted mainly of xylose together with small amount of glucose and traces of other sugars including 4-*O*-methyl glucuronic acid (4-*O*-MeGlcA). Arabinose, galactose and galacturonic acid detected in the enzyme-resistant fractions are indicative of pectic material co-extracted with xylans. The remaining xylose detected in the enzyme-resistant fractions probably arised from xyloglucan or from endoxylanase-resistant xylans.

**Table 1.** Sugar composition of hemicellulosic fractions isolated from *A. spinosa* and of oligosaccharides generated by digestion with endoglucanase and endoxylanase (see text for identification of fractions)

| | A1OH | B1OH | A4OH | B4OH | Residue | A4X-RF | A4Xose | B4G-RF | B4XGose | F6 |
|---|---|---|---|---|---|---|---|---|---|---|
| Ara[a] | 14 | 14 | 9 | 9 | 13 (tr[c]) | 21 | nd | 26 | 4 | nd |
| Rha[a] | 3 | 2 | 2 | 1 | 6 (tr[c]) | 4 | 1 | 3 | tr | nd |
| Fuc[a] | tr | 1 | tr | 4 | tr (tr[c]) | tr | nd | tr | 4 | 9 |
| Xyl[a] | 64 | 32 | 80 | 41 | 31 (7[c]) | 9 | 94 | 22 | 45 | 35 |
| GlcA[a,b] | 3 | 2 | 2 | 1 | tr (2[c]) | 0 | tr | tr | tr | nd |
| GalA[a] | 6 | 6 | 3 | 2 | 24 (tr[c]) | 19 | tr | 7 | tr | nd |
| Man[a] | tr | tr | tr | 6 | 3 (tr[c]) | 5 | nd | 6 | 6 | nd |
| Gal[a] | 6 | 11 | 2 | 10 | 11 (tr[c]) | 19 | nd | 19 | 8 | 12 |
| Glc[a] | 3 | 31 | 2 | 25 | 12 (85[c]) | 23 | 5 | 17 | 32 | 44 |

tr, trace; nd, not detected.
[a]Mole percent.
[b]Glucuronic acid including 4-*O*-Me derivative.
[c]Mole percent after Seamen hydrolysis.

The high performance anion exchange-pulse amperometric detection-chromatography (HPAEC-PAD) elution profile of A4Xose indicated the presence of xylose and xylobiose, as well as peaks eluted with high concentration of sodium acetate arising from xylan-derived acidic oligosaccharides (Fig. 1A). The xylanase-generated oligomers were analysed by matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry (Fig. 1B). The mass spectrum also showed the presence of various acidic fragments. The main peak at $m/z$ 759 is in agreement with the $[M+Na]^+$ adduct of a 4-*O*-MeGlcA residue linked to four pentose residues. Considering that xylose is the unique pentose detected in the fraction (Table 1), this ion was assigned to $Xyl_4$-4-*O*-MeGlcA. In the same way, ions at $m/z$ 891 and 1023 were assigned to $[M+Na]^+$ of $Xyl_5$-4-*O*-MeGlcA and $Xyl_6$-4-*O*-MeGlcA, respectively. Another group of ions at $m/z$ 1213 and 1345 was assigned to fragments containing two 4-*O*-MeGlcA units linked to six and seven xylose residues, respectively.

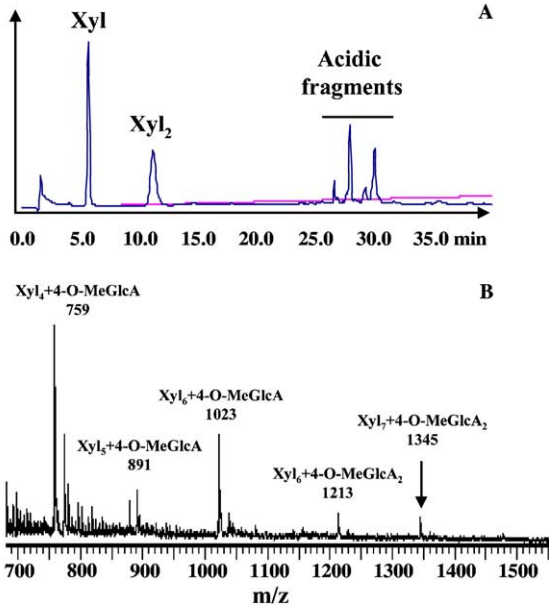


**Figure 1.** Structural analysis of the xylan-rich A4OH fraction isolated from the cell wall of *A. spinosa* leaves. HPAEC-PAD profile (A) and MALDI-TOF mass spectrum (B) of A4Xose xylan fragments generated by treatment of A4OH with an endoxylanase.

### 3.3. Structural analysis of the xyloglucan

Information on the structure of polymers present in xyloglucan-rich fractions was obtained by enzymatic degradation and structural elucidation of the resulting fragments. B1OH and B4OH were submitted to a depolymerisation with an *endo*-(1→4)-β-D-glucanase. This enzyme can cleave β-(1→4)-glucosidic linkages of xyloglucan backbone next to an unbranched glucose residue. The endoglucanase-resistant fractions, B1G-RF and B4G-RF isolated from B4OH and B1OH, respectively, were removed from the corresponding digests by precipitation with 80% (v/v) EtOH. Endoglucanase-generated xyloglucan oligomers namely, B4XGose and B1XGose, were recovered from the supernatant of the digests. It is worth noting that the structural analysis obtained from B4OH and B1OH-derived materials were qualitatively the same. Therefore, only the results obtained for B4OH fraction are presented. Table 1 shows that xylose and glucose are the major monosaccharides of B4XGose. The ratio of xylose to glucose for this fraction is 1.4. It is therefore likely that the xylan present in B4OH was partially hydrolysed by endoxylanase present as contaminant in the commercial endoglucanase preparation used in this study. Similarly, mannose detected in B4OH was assigned to partially hydrolysed mannans by contaminating mannanases. The sugar composition of the B4G-RF fraction indicates that it mainly consists of pectic materials, mannans and xylans. B4XGose was reduced into the corresponding oligoglycosyl alditols (B4XGol) and then permethylated. Table 2 shows that B4XGol yielded partially methylated alditol acetates corresponding to T-Xyl*p* (terminal xylopyranose), T-Fuc*p*, T-Gal*p*, 1,2-Xyl*p* (2-linked xylo-

**Table 2.** Methylation analysis of B4XGol and F6 fractions generated from *A. spinosa* leaves (see text for the identification of fraction)

| Monomer[a] | B4XGol[b] | F6[b] |
|---|---|---|
| T-Gal*p*[a] | 5 | nd |
| 1,2-Gal*p* | 2.3 | 8.5 |
| T-Fuc*p* | 7.3 | 8 |
| T-Ara*f* | 2 | nd |
| 1,3-Ara*f* | 5 | nd |
| T-Xyl*p* | 33.6 | 27 |
| 1,4-Xyl*p* | 7 | nd |
| 1,2-Xyl*p* | 12.1 | 26 |
| 1,6-Glc*p* | 7.7 | 12 |
| 1,4,6-Glc*p* | 13.5 | 22 |
| T-Man*p* | tr | nd |
| 1,4-Man*p* | 3 | nd |

nd, not detected; tr, traces.
[a]Linkage of monosaccharides. T-Gal denotes 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactitol, etc.
[b]Percentage of total area of the identified peaks.

pyranose), 1,2-Gal*p*, 1,6-Glc*p* and 1,4,6-Glc*p* residues, consistent with the presence of xyloglucan fragments. The 1,4-Xyl*p* probably arose from xylan fragments. Similarly, terminal mannose and (1→4)-Man*p* residues are likely due to result from the permethylation of the contaminating mannan fragments.

MALDI-TOF mass spectrum (Fig. 2) of B4XGose revealed the presence of numerous oligosaccharide fragments. On the basis of their molecular weight, ions at $m/z$ 1085, 1247, 1393, 1409 and 1555, corresponding respectively to $Hex_4Pent_3$ (four hexose and three pentose residues), $Hex_5Pent_3$, $Hex_5Pent_3dHex$ (dHex, deoxyhexose), $Hex_6Pent_3$ and $Hex_6Pent_3dHex$ can be assigned to $[M+Na]^+$ of partially fucosylated XXXG-type fragments ranging from XXXG to XLFG. Ions at 1217, 1379 and 1525, corresponding to $Hex_4Pent_4$, $Hex_5Pent_4$ and $Hex_5Pent_4dHex$, respectively, suggested the presence of another set of fragments with substitution of an hexose by a pentose residue.

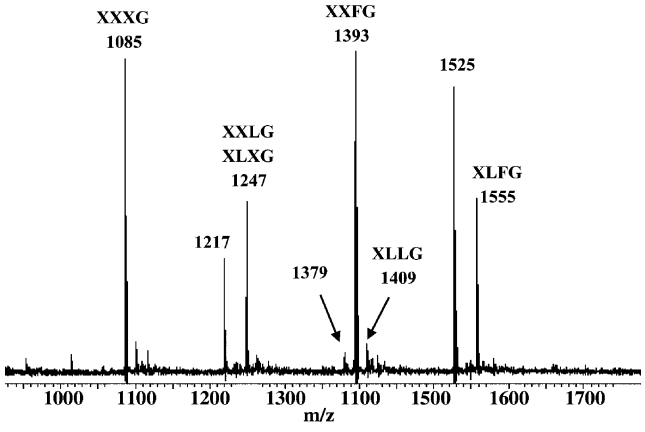


**Figure 2.** MALDI-TOF mass spectrum of the B4XGose fraction generated by endoglucanase treatment of the xyloglucan-rich extract isolated from the cell wall of *A. spinosa* leaves.

### 3.4. Isolation of a novel xyloglucan fragment

Endoglucanase-generated fragments were isolated for their structural elucidation. It is well known that oligoglycosyl alditols afford simpler proton spectra than the parent glycan because of the absence of reducing-end effect. Moreover, compared to glycan, the corresponding oligoglycosyl alditols are more stable towards alkali conditions used for HPAE-PAD chromatography. The elution pattern of oligoglycosyl alditol B4XGol, resulting from reduction of B4Gose, showed that it was resolved into six major fractions by HPAEC (Fig. 3A). By comparison with reduced standard xyloglucan fragments isolated from *Arabidopsis thaliana*[8] fractions F1, F2 and F3 were assigned to oligoglycosyl alditols XXXGol, XXFGol + XXLGol and XLFGol, respectively. All fractions were collected manually and desalted. MALDI-TOF MS of the purified fractions F1 to F3 showed the presence of ions at $m/z$ 1087 (F1), 1249 + 1395 (F2) and 1557 (F3) (data not shown), consistent with the assignments deduced by comparison of HPAEC-PAD retention times with oligoglycosyl alditol standards (Fig. 3A). The mass spectrum of F4 consisted only of the $Hex_4Pent_4$ fragment. Figure 3B and C shows that F6 fraction contains a single component. The structure of fraction F5, containing low amount of material, was not characterised.

### 3.5. Characterisation of the novel xyloglucan fragment

After collection, fraction F6 appeared as a unique well-resolved peak consisting of a $Hex_5Pent_4dHex$ decasaccharide alditol (Fig. 3B and C). The sugar composition showed that it is composed of glucose, xylose, galactose and fucose in the 4:4:1:1 molar ratio, respectively (Table 1). After permethylation, analysis of the resulting partially methylated alditol acetates showed the presence of T-Xyl*p*, T-Fuc*p*, 1,2-Xyl*p*, 1,2-Gal*p*, 1,6-Glc and 1,4,6-Glc*p* residues (Table 2). It should be noted that the 1:1 ratio of terminal and 1,2-Xyl*p* indicated the presence of two terminal xylose residues and two 2-linked xylose residues in F6.

Post-source decay (PSD) method of MALDI-TOF mass spectrometry is a convenient tool for structural analysis of highly branched oligosaccharides.[9] In order to determine the sequence and branching of sugars present in the F6 xyloglucan oligosaccharide isolated from *A. spinosa*. The $[M+Na]^+$ ion (precursor ion) was fragmented by collision induced dissociation (CID) and the resulting fragment ions were analysed by MALDI-PSD mass spectrometry (Fig. 4A). As previously reported,[10] the preferential Y-type fragmentation results in the sequential loss of neutral nonreducing moieties, which facilitates the assignment of the oligoglycosyl sequence of the xyloglucan fragment. For instance, the fragment ions at $m/z$ 1381, 1219 and 1087 arose from the

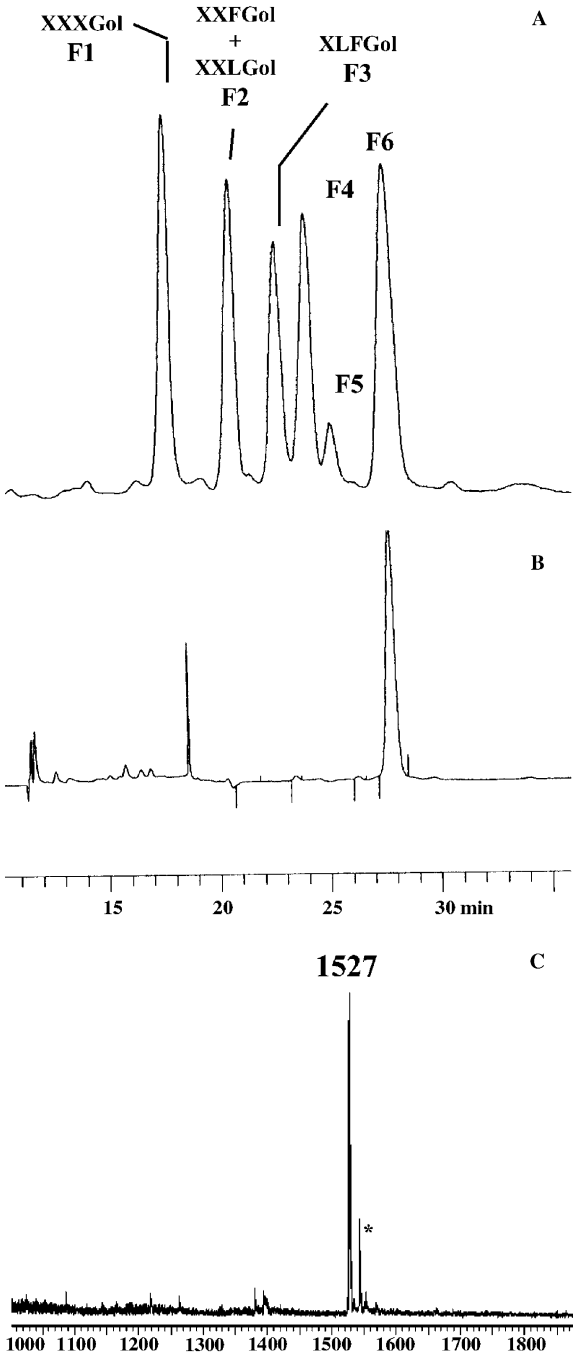


**Figure 3.** HPAEC-PAD profiles of (A) the reduced endoglucanase-generated B4XGol fraction and of (B) the purified and reduced F6 fragment. (C) MALDI-TOF mass spectrum of the reduced F6 fragment. *: $[M+K]^+$ adduct.

loss of dHex, dHex-Hex and dHex-Hex-Pent sequences, respectively, from the 1527 parent molecule by a Y-type cleavage. In the same way, diagnostic ions at $m/z$ 743 and 807 indicated that the Fuc-Gal-Xyl side chain is located on the Glc[a] residue of cellotetraose backbone. However, the fragmentation pattern did not allow the location of the third xylose on the side chains. For comparison, the fragmentation pattern of the oligoglycosyl alditol XLFG was determined by MALDI-PSD mass spectrometry (Fig. 4B). We observed Y-type diagnostic ions resulting from a fragmentation pattern similar to the one of F6 xyloglucan fragment with ions containing Gal[b] differing by 30 Da.

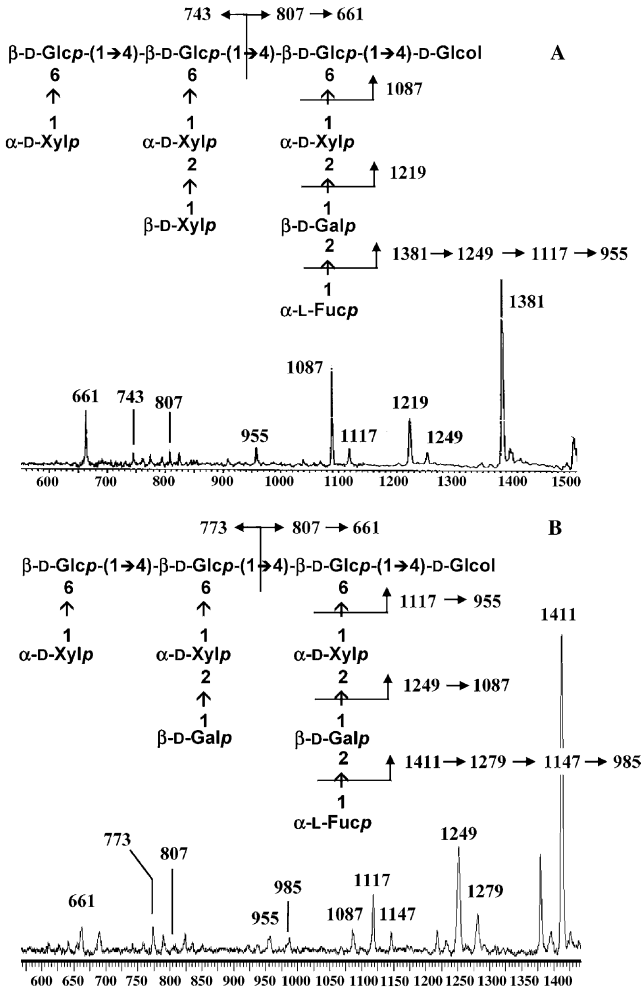


**Figure 4.** MALDI-PSD spectra of (A) oligoglycosyl alditol F6 fragment ($m/z$ 1527) and of (B) oligoglycosyl alditol XLFGol ($m/z$ 1557).

The $^1$H NMR spectrum of Fraction F6 shows the presence of nine anomeric signals, which is in agreement with a decasaccharide alditol structure. Four H-1 belong to α-linked sugars and five signals results from β anomers (Fig. 5). The assignment of the anomeric protons were done according to previously reported data on proton NMR of xyloglucan fragments.[11,12] In the α anomer region, the doublet at $\delta$ 5.260 ($J_{1,2}$ 3.9 Hz) was assigned to H-1 of α-L-Fuc residue linked to C-6 of the Gal residue. The H-1 of terminal α-D-Xyl*p* linked to C-6 of Glc[c] yielded the doublet at $\delta$ 4.951 ($J_{1,2}$ 3.6 Hz). Substitution at C-2 or other position of α-D-Xyl*p* residues results in a shift down field by 0.18–0.23 ppm relative to the corresponding resonances for the terminal α-D-Xyl*p* residue.[11] As a consequence, H-1 signals at $\delta$ 5.135 and 5.149 ppm ($J_{1,2}$ 3.6 Hz) were assigned to

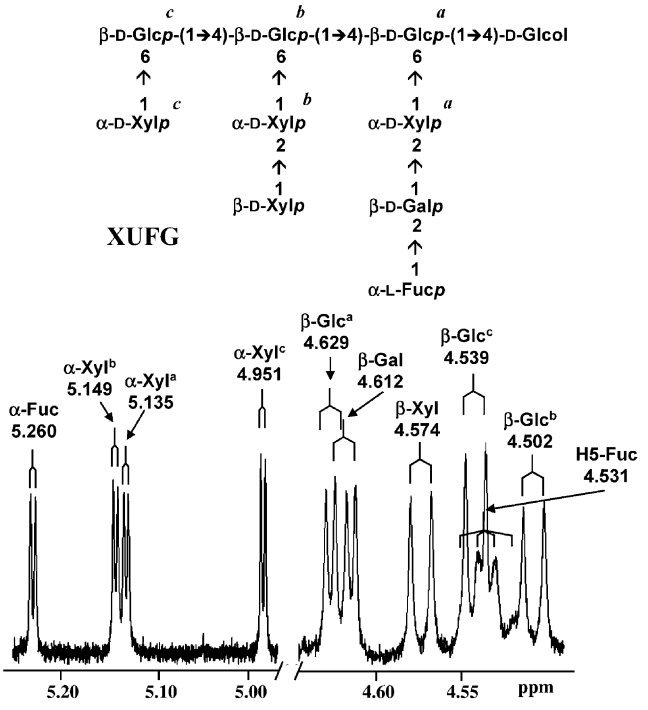


**Figure 5.** Anomeric region of the $^1$H NMR, assignment to reporter protons and proposed structure for the glycosyl alditol F6 fragment (XUFG).

substituted α-D-Xyl residues, which indicated that Xyl$^a$ and Xyl$^b$ bear additional monomers. The H-1 signals between 4.4 and 4.7 ppm arose from five β-linked anomeric signals ($J_{1,2}$ 7.8 Hz). Of these, three were assigned to the three β-D-Glc residues of the backbone and one to the β-D-Gal residue. The remaining signal was assigned to a β-D-Xyl residue, considering that the fraction F6 contains four xylose, three of them being α-linked.

Based on the results obtained from MALDI-PSD and proton NMR spectroscopy. We conclude that Fraction F6 contains three side chains, two of them are chains usually found in XXXG-type xyloglucan: a terminal xylopyranose residue linked at C-6 of Glc$^c$ and a α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→2)-α-D-Xyl*p* side chain attached to C-6 of Glc$^a$. From NMR and permethylation analysis demonstrating the presence of an additional β-Xyl unit and two 2-linked xylose residues in F6, we propose that the third side chain is a novel β-D-Xyl*p*-(1→2)-α-D-Xyl*p* side chain linked to C-6 of Glc$^b$ as represented in Figure 5.

## 4. Discussion

Most, if not all, of the biochemical studies devoted to argan tree is related to the characterisation of the edible oil produced from the seeds.[1] To the best of our knowledge, no study has been performed on the cell wall carbohydrates and, therefore, this is the first report on the chemical composition of cell wall polysaccharides from *Argania spinosa*. The central goal of this study was the structural characterisation of the hemicellulosic polymers. Hemicellulose polymers were extracted from *A. spinosa* leaves by sequential extractions with alkali. In both fractions, the precipitates obtained after dialysis contained mainly xylans. In contrast, the soluble fractions were composed mainly of xyloglucan. The structure of the two polymers was investigated by enzyme degradation with specific endoglycosidases and structural analysis of the resulting fragments. Based on HPAEC-PAD and MALDI-TOF MS analysis, it appeared that *A. spinosa* xylan is composed of a β-(1→4)-linked-D-xylopyranose backbone substituted with 4-*O*-MeGlcA residues, as previously characterised in numerous plants. The amount of 4-*O*-MeGlcA, as detected in A4OH as well as in A4Xose by gas chromatography, is very small. However, it is well known that aldobiuronic acids are very resistant to acid hydrolysis, which results in underestimation of uronic acid contents.[13,14]

Xyloglucan oligosaccharide subunits were generated by endoglucanase digestion of the xyloglucan-rich hemicellulosic fractions. MALDI-TOF mass spectra and HPAE-PAD chromatography of the pool of endoglucanase-generated xyloglucan oligomers indicated that *A. spinosa* contains a XXXG-type xyloglucan. In addition to XXXG, XXFG, XLXG/XXLG, XLFG fragments previously characterised in various plants,[10] a second group of fragments was detected. These fragments correspond to oligosaccharides homologue to XLXG, XLLG and XLFG with substitution of an hexose by a pentose residue. The primary structure of the major subunit was determined by a combination of sugar analysis, methylation analysis, MALDI-PSD mass spectrometry and $^1$H NMR spectroscopy. This fragment contains a novel β-Xyl*p*-(1→2)-α-Xyl*p* side chain linked to C-6 of Glc$^b$. So far, the main side chains characterised on xyloglucan fragments are β-D-Gal*p*-(1→2)-α-D-Xyl*p* (L), α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→2)-α-D-Xyl*p* (F), α-L-Ara*f*-(1→2)-α-D-Xyl*p* (S) and β-Ara*f*-(1→3)-α-L-Ara*f*-(1→2)-α-D-Xyl*p* (T).[5,10] We propose to term as U the novel β-Xyl*p*-(1→2)-α-Xyl*p* side chain identified in this study. Using this nomenclature, oligoglycosyl alditol F6 isolated from *A. spinosa* cell wall is defined as XUFG (Fig. 5). Similarly, although not structurally characterised, other novel endoglucanase-generated fragments detected in the MALDI-TOF mass spectra could be assigned to XUXG ($m/z$ 1217) and XULG ($m/z$ 1379). Such U side chain probably results from the action of a xyloglucan-specific β-(1→2)-xylosyltransferase. However, considering that these novel fragments only differ from XLXG, XLLG and XLFG by the substitution of the Gal by a β-(1→2)-linked xylose residue on the second side chain, we could also postulate that such oligomers result from the transfer of xylose residues from UDP-Xyl by a β-(1→2)-galactosyltransferase

exhibiting a low specificity for the nucleotide-sugars. Future works will help to investigate further these possibilities.

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