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# HEMICELLULOSES OF *ARUNDO DONAX* AT DIFFERENT STAGES OF MATURITY

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**Key Word Index**—*Arundo donax*; Gramineae; reed; hemicelluloses; 4-*O*-methyl glucuronoxylan; structural analysis.

**Abstract**—The hemicellulosic composition of the internodes of *Arundo donax* depends on the maturity of the tissue. The percentage of xylose in the total hemicellulose increases with increasing plant maturity. The main hemicellulose is an arabino-4-*O*-methyl glucurono xylan which is already present in the youngest tissues and has the same structural features regardless of the age of the tissues. The average D.P. of this polysaccharide increases from about 60 to 150 with maturation of the plant tissue.

## INTRODUCTION

In the stem of the reed *Arundo donax*, as in the other Gramineae, the most abundant hemicellulose is an arabino-4-*O*-methyl glucurono xylan whose structure was previously established in the case of mature tissues [1]. In the case of other Gramineae, Wilkie *et al.* [2–4] pointed out variations in the proportions of sugar residues at different stages of growth, first in terms of total hemicelluloses [5], and also in terms of pure hemicelluloses.

In an attempt to establish at what stage of development a xylan first appears in the stem of *A. donax*, we showed that such a polysaccharide is already present in the youngest internodes [6]. At this stage the fibres are in an active stage of differentiation and elongation. In many monocotyledons, elongation of the stem is mainly due to the presence of an intercalary growing zone which remains meristematic for a long time at the base of each internode [7]. It is therefore clear that in a growing internode all the stages of maturity are represented. The evolution of the fibres, in number and diameter, has been studied [8] in relation to the total cell wall carbohydrates distribution along an internode and at different stages of maturity.

The present study deals with the variation in the total hemicelluloses of three sections of the stem of 1-month-old reeds, representing three stages of maturity, and describes the isolation of hemicellulosic material in conjunction with criteria of purity. A comparative structural study of the main xylan is reported.

## RESULTS AND DISCUSSION

The plants used were field-grown reeds of the species *A. donax*. The 1-month-old plants were about 1·2 m high and were divided into 8 distinct internodes ranging from 0·5 to 21 cm long from the apex to the bottom of the stem. Three sections were excised from the stem: Section 1 corresponding to the first and second internodes (about 1 and 3 cm long respectively), Section 2 corresponding to the 4th and 5th internodes (9 and 15 cm long) and Section 3 corresponding to the 7th and 8th internodes (both measuring 21 cm in length). Although an internode is a mixture of younger and older cells, the selection is representative of distinct stages of maturity because, as demonstrated elsewhere [8], the relative proportion of mature tissues within an internode increases greatly from the apex to the bottom of the stem. The maturity thus increases from Section 1 to Section 3.

Another general problem in the study of hemicelluloses, is the choice of a suitable extraction method. In agreement with Wilkie *et al.* [5], we considered that total hemicellulose would be more representative of the relationship between polysaccharides and growth. However, we preferred a stepwise extraction of the hemicellulo-

Table 1. Stepwise extraction of polysaccharides from three sections of the stem of *Arundo donax*

| Mode of extraction | Fraction§ | Yield* (%) | Ash (%) | Nitrogen† (%) | $[\alpha]_D$ (°) |
|---|---|---|---|---|---|
| 1% $KBH_4$ | 1 $P_A$—$KBH_4$ | 2 | 3·4 | 11·6 | |
| | 2 $P_A$—$KBH_4$ | 1·1 | 3·0 | 7·9 | |
| | 3 $P_A$—$KBH_4$ | 0·9 | 4·0 | | |
| | 1 $P_S$—$KBH_4$ | 0·5 | 4·1 | 4·3 | |
| | 2 $P_S$—$KBH_4$ | 4·3 | 1·8 | 0·3 | |
| | 3 $P_S$—$KBH_4$ | 1·3 | 2·5 | | |
| 0·2 M KOH–1% $KBH_4$ | 1 $P_A$—0·2 M | 3·4 | 2·5 | 10·3 | |
| | 2 $P_A$—0·2 M | — | | | |
| | 3 $P_A$—0·2 M | — | | | |
| | 1 $P_S$—0·2 M | 4·6 | 1·5 | 2·5 | −64 |
| | 2 $P_S$—0·2 M | 6·7 | 1·0 | 0·2 | −77 |
| | 3 $P_S$—0·2 M | 4·2 | 1·1 | 0·25 | −59 |
| 1·0 M KOH–0·5% $KBH_4$ | 1 $P_A$—1·0 M | 1·1 | 3·9 | 4·9 | |
| | 2 $P_A$—1·0 M | 2·6 | 7·8 | 0·1 | |
| | 3 $P_A$—1·0 M | 4·1 | 4·2 | 0·1 | |
| | 1 $P_S$—1·0 M | 12·0 | 3·8 | 0·3 | −52 |
| | 2 $P_S$—1·0 M | 17·5 | 2·7 | 0·1 | −70 |
| | 3 $P_S$—1·0 M | 8·5 | 0·9 | 0·1 | −72 |
| 2·5 M KOH–0·5% $KBH_4$ | 1 $P_S$—2·5 M | 9·3 | 0·8 | — | −42 |
| | 2 $P_S$—2·5 M | 12·5 | 4·5 | — | −54 |
| | 3 $P_S$—2·5 M | 6 | 1·5 | — | −66 |
| 4·3 M KOH | 1 $P_S$—4·3 M | 2·3 | 3·8 | — | |
| | 2 $P_S$—4·3 M | 1·1 | 5·1 | — | |
| | 3 $P_S$—4·3 M | 0·4 | 10·8 | | |

* Expressed on dry wt after 80% EtOH.
† Micro-Kjeldahl procedure and colorimetric determination with a Technicon Auto-Analyser [20].
§ 1, 2 and 3 refer to sections of stem: PA + PS to HOAc and EtOH precipitates respectively.

sic material from the chlorite holocellulose with increasing concentrations of alkali which allows a better accessibility to the polysaccharides and an exhaustive penetration of the solvents. To prevent the polysaccharides from alkaline peeling [9–10], all extractions were carried out in the presence of potassium borohydride. The yields of hemicelluloses extracted at each step are given in Table 1. Each alkaline extract was neutralized with HOAc to give a precipitate $P_A$, and after centrifugation the supernatant was poured into EtOH to give a precipitate $P_S$. For the three sections, the highest yields were obtained in the 1·0 and 2·5 M KOH extracts. The total hemicellulose content is 34% in the youngest tissues, is as much as 44% in Section 2 but is only 25% in the more mature part. The xylose content, as determined by acid hydrolysis, increased from 68% (Section 1) to 89% (Section 2) and then decreased to 81% (Section 3). The percentage of arabinose decreased with increasing plant maturity and this phenomenon could be evidence of secondary thickening of the cells since secondary walls invariably contain xylans [11]. Similar results were obtained by Meier [12] in birch fibres where the xylose content increased at first, reached a maximum and then decreased in the fully developed fibres.

The carbohydrate analysis of the individual fractions obtained by the successive alkaline extractions (Table 2) showed that the fractions extracted with 0·2, 1·0 and 2·5 M KOH respectively were xylan-type polysaccharides. The fractions obtained from Sections 2 and 3 in the 1·0 M KOH extracts, which were the most abundant, thus the most representative, also had less than 5% of sugars other than xylose or arabinose in their components.

The purity of the polysaccharides was checked by fractionation on a DEAE Sephadex A-25 column in phosphate buffer (0·01 M, pH 6·5) and the major fraction (about 70% yield) showed no noticeable change in composition by comparison with the starting polysaccharide. Further attempts at purification of this fraction was carried out after

Table 2. Sugar composition of the hemicellulosic fractions*

| Fraction† | Gal | Glc | Man | Ara | Xyl | Uronic acid (mol %)§ |
|---|---|---|---|---|---|---|
| 1 $P_A$—$KBH_4$ | 7·5 | 3·8 | 3·4 | 10·8 | 43·6 | x |
| 2 $P_A$—$KBH_4$ | t | t | — | 7·7 | 92 | x |
| 3 $P_A$—$KBH_4$ | 1·2 | 0·6 | t | 5·5 | 92·3 | x |
| 1 $P_S$—$KBH_4$ | 8·9 | 15·9 | 3·2 | 10 | 42·4 | x |
| 2 $P_S$—$KBH_4$ | 6·4 | 3·3 | — | 13 | 77·2 | 3·1 |
| 3 $P_S$—$KBH_4$ | 1·5 | 1·1 | 0·3 | 5·1 | 91·8 | x |
| 1 $P_A$—0·2 M | 11·9 | 15·2 | 3·6 | 10·5 | 58·8 | 1·0 |
| 1 $P_S$—0·2 M | 3·6 | 4·9 | t | 22 | 69·5 | 3·7 |
| 2 $P_S$—0·2 M | 1·8 | 2·2 | — | 11·3 | 85·2 | 1·4 |
| 3 $P_S$—0·2 M | 1·8 | 2·2 | — | 10·1 | 85·9 | 1·7 |
| 1 $P_A$—1·0 M | 1·5 | 1 | t | 5·7 | 91·8 | x |
| 2 $P_A$—1·0 M | t | t | — | 4·4 | 94 | x |
| 3 $P_A$—1·0 M | 0·9 | 0·6 | — | 5·1 | 93·4 | x |
| 1 $P_S$—1·0 M | 3·6 | 14·3 | — | 11·7 | 70·4 | 2·8 |
| 2 $P_S$—1·0 M | 1·1 | 2·0 | — | 5·9 | 91 | 1·3 |
| 3 $P_S$—1·0 M | 1·4 | 3·3 | — | 5·7 | 89·6 | 1·3 |
| 1 $P_S$—2·5 M | 1·9 | 9·3 | — | 5·3 | 83·5 | 2·6 |
| 2 $P_S$—2·5 M | 1·2 | 5·6 | — | 4·9 | 88·3 | 0·8 |
| 3 $P_S$—2·5 M | 4·1 | 32·5 | — | 12·5 | 50·9 | 1·2 |
| 1 $P_S$—4·3 M | 5·2 | 37·5 | 0·7 | 15 | 41·2 | 3·5 |
| 2 $P_S$—4·3 M | 1·3 | 18·4 | 0·5 | 4·5 | 71·6 | 1·4 |
| 3 $P_S$—4·3 M | 1·4 | 15·6 | 0·6 | 5·4 | 77 | 1·2 |

* Neutral sugars are expressed in molar proportions and were determined by GLC. Uronic acids are expressed in mol % and were determined by decarboxylation.

† See Table 1.

§ x Fraction too small for analysis.

complex formation with a Red Procion dye [13] and successive gel filtrations on a Biogel $P_2$ column and an Agarose A-0·5 M column eluted with 1·0 M NaCl solution. Only one band was obtained which was identical to the starting material, and gave a single band on electrophoresis on a cellulose acetate strip. The xylans from stem Sections 2 and 3 were thus concluded to be homogeneous and pure polysaccharides.

Structural studies of the xylans from Sections 1–3 were carried out on the 1·0 M KOH extracts by permethylation according to Hakomori [14] and completed according to Purdie [15]. In each case, a fractionation of the permethylated polysaccharides with $CHCl_3$–light petroleum mixtures gave the principal fraction for the 25:75 extract. Hydrolysis of the methylated products gave for all the polysaccharides the following sugars: 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose and a mixture of 2-*O*- and 3-*O*-methyl-D-xylose in the relative molar proportion indicated in Table 3. The sugar derivatives were identified by GLC or their alditol acetate derivatives [16] and their identification confirmed by MS [17]. From the data in Table 3 the degree of polymerization for the arabino-glucurono xylan of

Table 3. Quantitative analysis of methylated sugars of xylans from 1·0 M KOH extracts

| Compound | Mol ratio in stem sections* 1 | 2 | 3 |
|---|---|---|---|
| 2,3,5-Tri-*O*-methyl-L-arabinose | 1·9 | 3·5 | 5·2 |
| 2,3,4-Tri-*O*-methyl-D-xylose | 1·0 | 1·0 | 1·0 |
| 2,3-Di-*O*-methyl-D-xylose | 58·5 | 84·0 | 140·5 |
| (2-*O*- + 3-*O*-)methyl-D-xylose | 3·3 | 6·0 | 10 |

* Mol ratio from GLC.

Table 4. Quantitative analysis of methylated sugars of xylans from Stem Section 2

| Compound | Fractions (mol ratios*) $2P_S$—0·2 M | $2P_A$—1·0 M | $2P_S$—1·0 M |
|---|---|---|---|
| 2,3,5-Tri-*O*-methyl-L-arabinose | 7·4 | 4·1 | 5·2 |
| 2,3,4-Tri-*O*-methyl-D-xylose | 1·0 | 1·0 | 1·0 |
| 2,3-Di-*O*-methyl-D-xylose | 83·5 | 82·5 | 84·0 |
| (2-*O*- + 3-*O*-)methyl-D-xylose | 13·3 | 5·9 | 6·0 |

* As in Table 3.

the Sections 1, 2 and 3 were respectively 63, 91 and 151. Since the three sections corresponded to a different degree of maturation of the fibrous tissues, it appears that there is a marked increase of the $DP_n$ of the xylans with an increasing maturation of the tissues.* In order to check the homogeneity of the xylans extracted from one section by different reagent concentrations, we carried out a similar methylation study on the fraction extracted from Section 2 with 0·2 M KOH and precipitated with EtOH (fraction $2P_S$ 0·2 M) and with 1·0 M KOH and obtained by neutralization with HoAc (fraction $2P_A$—1·0 M), respectively. The results of the analysis of the permethylated products are given in Table 4 and correspond to $DP_n$ of 97 and 90 respectively showing no significant difference from the value of 91 found for the most abundant fraction $2P_S$—1·0 M. This relatively constant value of DP suggests good homogeneity of the xylans extracted at a definite stage of maturity of the corresponding fibrous tissues.

All the xylans were similar in composition as shown by hydrolysis, and in their structural constitution as shown by methylation studies. All are arabino-4-*O*-methyl-D-glucurono xylans with terminal units of arabinose linked on position 3 of the xylose and 4-*O*-methyl glucuronic acid residues linked on position 2 of the xylan backbone. It is noteworthy that xylans from younger tissues have about twice as much uronic acid residues than those from more mature tissues. The interpretation of these data is difficult since the polysaccharides have been reduced *in situ* prior to extraction [18, 19] and since the lignin content increases with increasing maturation of the tissues [8].

* These DP are not to be compared with the DP given in a previous paper [1] where a mixture of all the tissues of a culm were used for the study.

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## EXPERIMENTAL

*General methods.* PC was run on Whatman No. 1 paper using the following solvent systems: (i) EtOAC–$C_5H_5N$–$H_2O$ (4:1:1); EtOAC–HOAC–$HCO_2H$–$H_2O$ (18:3:1:4). Spray reagents were aniline phthalate or oxalate. GLC analyses were carried out on a dual column FID instrument, fitted with a digital integrator. Glass columns (2 m × 3 mm) were packed with 3% ECNSS-M on Gas-Chrom. Q. MA were recorded using a GC–MS system.

Plant tissues preparation, isolation of hemicellulosic material and hydrolysis of the different fractions have been performed as described previously [6] (unless otherwise stated).

*Purity test on fractions $2P_S$—1·0 m and $3P_S$—1·0 m.* The polysaccharide was solubilized in 1·0 N KOH and dialyzed until neutral. The dialyzed soln was concentrated to a small vol and placed on a DEAE–Sephadex A-25 column equilibrated in phosphate buffer (0·01 M, pH 6·5). The material on the column was eluted with 0·1, 1 and 2 M solns of NaCl. The main fraction (about 70%) was hydrolysed and found to be very similar in composition to the starting material.

*Gel filtration and electrophoresis of dyed polysaccharides.* DEAE-Sephadex purified fractions of the xylans were dyed according to Dudman and Bishop [13] with an aq. soln of Procion red MX-G.† Excess dye was removed on a Biogel $P_2$ column and the band containing the dyed polysaccharide eluted on an Agarose A—0·5 M column with 1·0 M NaCl soln. A single band was obtained. NaCl was dialyzed out and the dyed xylan freeze-dried. Hydrolysates of fractions obtained from $2P_S$—1·0 M had a similar composition to the starting polysaccharide (Table 5) and showed a single band by electrophoresis on cellulose acetate strips (2·5 × 17 cm) in 0·1 M sodium tetraborate–NaCl buffer (250–350 V, 7 mA).

Table 5. Tests of purity on fraction $2P_S$—1·0 M

| Fraction | Mol ratio Gal | Glc | Ara | Xyl |
|---|---|---|---|---|
| Starting xylan | 1·1 | 2 | 6 | 90 |
| DEAE-Sephadex | 1·3 | 3·5 | 6·4 | 89 |
| Agarose A —0·5 M | 1·3 | 3·4 | 5·2 | 90 |

*Methylation.* All methylations were carried out with a single treatment according to the Hakomori [14] procedure (NaH, DMSO, $CH_3I$) followed by 2 or 3 methylations according to Purdie [15]. The permethylated products were fractionated by $CHCl_3$–light petroleum mixtures. All operations were as described previously [6].

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