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HEMICELLULOSES OF YOUNG INTERNODES OF *ARUNDO DONAX*

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Key Word Index—(*Arundo donax*); Gramineae; 4-*O*-methyl glucurono xylan; structural analysis; immature tissues.

Abstract—An arabino 4-*O*-methyl glucurono xylan has been isolated as the dominant form of the hemicellulosic material of the youngest internodes of *Arundo donax*. The polysaccharide contained D-glucose, D-xylose and L-arabinose in the molar ratios of 0·1:9·2:0·66, respectively, with a uronic acid content of 4·5%. Methylation and periodate-oxidation showed that the xylan has a β-1 → 4-linked xylose backbone and a degree of polymerization *ca.* 63. The xylan is very similar to that found in the mature tissues.

INTRODUCTION

CHANGES in the polysaccharide composition of the cell wall of different plant species during growth have been recorded.[1,2] In the case of the stems of cereals, Reid and Wilkie[3] followed these changes in relation to plant maturity on the basis of the total hemicellulose composition. We have attempted to establish at which stage in the succession of the internodes a typical xylan appears in the stem of the reed *Arundo donax*. Molecular characteristics of the xylan have been compared with those of the same polysaccharide isolated from the corresponding mature tissue, namely an arabino 4-*O*-methyl glucurono xylan.[4]

RESULTS AND DISCUSSION

The total hemicellulosic material extracted with alkaline solutions from the chlorite holocellulose amounted to 27·7% and gave, on acid hydrolysis arabinose, xylose, glucose and galactose in the molar proportions 9·8:68:11:2·7 with traces of rhamnose and fucose.

Tables 1 and 2 show the analytical data obtained from the various fractions obtained by stepwise extraction of the hemicellulosic material. The holocellulose was first extracted with 1% KBH_4 which had the double advantage of reducing the polysaccharides *in situ* thus protecting them from possible degradation[5,6] and of completing the extraction of H_2O-soluble acidic polysaccharides.[7] The most abundant fraction, representing 41% of the total hemicelluloses and containing 1·7% protein, was obtained from the 1 M KOH extract precipitated in alcohol (fraction PS–1·0 M). The sugar composition of this fraction (Table 2) showed arabinose, xylose, glucose and galactose in the molar proportions

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11·7:70·4:14·3:3·6. Purification by Fehling's solution gave two insoluble copper complexes and a soluble one which, after decomposition, gave three fractions F_1, F_2 and F_3 (Table 3).

TABLE 1. YIELDS OF HEMICELLULOSES OBTAINED FROM THE TWO YOUNGEST INTERNODES OF *Arundo donax* BY SUCCESSIVE EXTRACTION WITH KBH_4 AND KOH SOLUTIONS

Method of extraction	Designation of fractions*	Yield %†
1% KBH_4	P_A–KBH_4	0·52
	P_S–KBH_4	0·35
1% KBH_4 + 0·2 M KOH	P_A 0·2 M	1·18
	P_S 0·2 M	2·10
0·5% KBH_4 + 1·0 M KOH	P_A–1·0 M	0·73
	P_S–1·0 M	11·36
0·5% KBH_4 + 2·5 M KOH	P_A–2·5 M	—
	P_S–2·5 M	9·15
4·3 M KOH	P_A–4·3 M	—
	P_S–4·3 M	2·3

* P_A—precipitate obtained by direct neutralization with acetic acid. P_S—precipitate obtained by EtOH from the supernatant of P_A.

† Calculated as ash-free and protein-free polysaccharides on a dry weight basis after 80% EtOH treatment of the tissues.

The most abundant fraction, F_1, on acid hydrolysis, yielded arabinose, xylose, glucose and galactose in the molar proportions 6·6:92:1·1:0·4. The uronic acid content estimated by decarboxylation[8] was 4·5%. The uronic acid was tentatively identified as 4-*O*-methyl glucuronic acid; PC of the acidic part of the polysaccharide hydrolysate gave a spot corresponding in R_f with an authentic sample of 2-*O*-(4-*O*-methyl-α-D-glucopyranosiduronic acid)-D-xylose. Fraction F_1 was also exhaustively methylated[9,10] and hydrolysis of the methylated xylan produced gave the following partially methylated compounds: 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- and 3-*O*-methyl-D-xylose, identified by PC, GLC and MS of the alditol acetate derivatives.[11,12] The methylated sugars were present in the molar proportions 1·9:1:58·5:3·3, respectively, corresponding to a degree of polymerization ($\overline{DP}$) for a linear polymer of 63. This result agreed well with the result obtained by periodate oxidation of the fraction followed by $NaBH_4$ reduction and hydrolysis. Ethylene–glycol, glycerol and xylose were released in the molar proportions 1:61·4:1·8 respectively, corresponding to a degree of polymerization of 64. An unidentified GLC peak with a retention time between that of glycerol and arabinose, but which was not erythritol, was also present and accounted for 7% of the total.

The macromolecular structure of xylans has been established mainly on polysaccharides isolated from fully differentiated fibrous tissues of Dicotyledons[13] as well as from the lignified fibres of Monocotyledons.[14] Recently, a galactoarabinoxylan has been characterized

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TABLE 2. SUGAR COMPOSITION OF THE VARIOUS HEMICELLULOSE FRACTIONS ISOLATED FROM THE TWO YOUNGEST INTERNODES OF *Arundo donax*

Fraction	Neutral sugars* Galactose	Glucose	Arabinose	Xylose	Uronic acid content (%)†
P_A–KBH_4	7·5	3·8	10·8	43·6	‡
P_S–KBH_4	8·9	15·9	10	42·4	4·5
P_A–0·2 M	11·9	15·2	10·5	58	1·6
P_S–0·2 M	3·6	4·9	22	69·5	6·0
P_A–1·0 M	1·5	1	5·7	91·8	‡
P_S–1·0 M	3·6	14·3	11·7	70·4	4·5
P_A–2·5 M	—	—	—	—	
P_S–2·5 M	1·9	9·3	5·3	83·5	4·2
P_A–4·3 M	—	—	—	—	
P_S–4·3 M	5·2	37·5	15	41·2	5·7

* Determined by GLC of the alditol acetate derivatives and expressed in molar proportions.
† Determined by decarboxylation.
‡ Fraction too small for analysis.

in the upper part of the stem tissues of the oat plant.[15] In the case of *Arundo donax*, our interest was to establish at what stage of the development of the internodes a polysaccharide from the xylan group appeared. The presence of immature fibres in the tissues of the internodes used for our study were shown by microscopic examinations. At this stage of development, the fibres constitute a distinct sheath around each vascular bundle but have a very thin cell wall. The absence of the characteristic birefringence of the secondary walls under polarized light is an indication that the fibres have not reached the stage of fully differentiated secondary walls. We have also observed that, except for the wood vessels, no lignin is present in the walls of the tissues.

TABLE 3. SUGAR COMPOSITION OF THE FRACTIONS OBTAINED BY PURIFICATION OF FRACTION P_S–1·0 M WITH FEHLING'S SOLUTION

Fraction	Yield*	Neutral sugars† Galactose	Glucose	Arabinose	Xylose	Uronic acid content (%)†
F_1	38·5	0·4	1·1	6·6	92	4·5
F_2	12	2·6	—	10	80·7	‡
F_3	14	6·7	36·6	19·7	36	‡

* In % of fraction P_S–1·0 M.
†‡ Determination as in Table 2.

We have identified therefore, a xylan very similar to that present in mature tissues.[4] However, the xylan from young tissues is less substituted by arabinose and uronic acid than that of the mature tissues and its $\overline{DP}$ is slightly lower. The $\overline{DP}$ of 63 (immature tissues) and 80 (mature tissues)[4] were established using methylation and confirmed by periodate oxidation. The presence of such a polysaccharide in very young tissues indicates that biosynthesis of the arabinoglucuronoxylan takes place at a very early stage during the organization of the fibres.

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EXPERIMENTAL

General methods. PC was run on Whatman No. 1 paper using (i) EtOAc–pyridine–H_2O (4:1:1); (ii) EtOAc–HOAc–HCO_2H–H_2O (18:3:1:4); (iii) MeCOEt–H_2O (azeotrope). GLC analyses were carried out using FID and 2 m × 3 mm i.d. columns packed with 3% ECNSS-M on Gas-chrom Q and 10% GE–SF 96 on Diatoport S; quantitative analysis was performed using an electronic integrator. MS were recorded on a LKB 9000-S.

Preparation of tissues. Field-grown reeds (*Arundo donax* L.) about 1-month-old were harvested on 10 May 1972. The stems measured 1–1·2 m and had an average of 8 distinct internodes ranging 0·5–21 cm long. The first two internodes beneath the apex (0·5–3 cm long) were excised. The material was frozen at −15°, immediately disintegrated and immersed for 5 min in boiling EtOH. Waxes and pigments were removed in a Soxhlet using EtOH–C_6H_6 (1:2). The H_2O-soluble polysaccharides were extracted successively with cold H_2O, hot H_2O (80°) and finally with ammonium oxalate (0·5% at 90°). Chlorite delignification was carried out at room temp. using the method of Wise *et al.*[16]

Isolation of hemicelluloses. Successive 24 hr extractions were carried out with 1% aq. KBH_4 and then with KOH soln of increasing concn: 0·2 M KOH containing 1% KBH_4, 1 M KOH–0·5% KBH_4, 2·5 M KOH–0·5% KBH_4, and finally 4·3 M KOH. Neutralization of the extracts below 5° with HOAc gave a ppt. P_4 collected by centrifugation. The supernatant was dialysed, concn to about 100 ml and poured into 3 vol. EtOH, giving a ppt. P_S. All the ppts were washed with EtOH, Me_2CO and Et_2O, and finally air dried (yields are shown in Table 1).

Analysis of sugars. All fractions were hydrolysed for 30 min at 25° in 72% H_2SO_4 and then in 1 N H_2SO_4 for 5 hr at 100° in a sealed tube. The hydrolysates were neutralized with $BaCO_3$ and sugars were collected after passage through ion-exchange columns of IR-45 and IR-120. The sugars were identified by PC and estimated by GLC of the alditol acetate drivatives of the neutral sugars, on ECNSS. Uronic acid content was determined by decarboxylation according to Bylund and Donetzhuber.[8]

Purification of Fraction P_S–1·0 *M.* 2 g was dissolved in 1 M NaOH (100 ml) and Fehling's soln was added in 10 ml portions. The insoluble fractions were collected by centrifugation. Complexing was complete after 2 additions. 3 more additions of reactant did not give any further precipitation. The 2 insoluble fractions F_1 (0·77 g) and F_2 (0·24 g) respectively, were treated with EtOH–HCl 5% at 0° and gave 2 white ppts which were dried. The soluble fraction was treated with glacial HOAc and dialysed. Reprecipitation in EtOH gave fraction F_3 (0·28 g). The results of hydrolysis are given in Table 3.

Methylation of Fraction F_1. The polysaccharide (245 mg) was methylated once by the method of Hakomori[9,17] and 4× by the method of Purdie.[10] The methylated product (227 mg) showed no IR absorption at *ca.* 3·450 cm^{-1}. A sample (*ca.* 10 mg) was hydrolyzed in 1 N H_2SO_4 at 100° for 5 hr, and neutralized with $BaCO_3$. The hydrolyzate was reduced with $NaBH_4$ and acetylated.[11] The mixture of alditols was examined by GLC (ECNSS column) and the components identified by comparison with authentic standards and by MS.[12]

Periodate oxidation of Fraction F_1. A sample (50 mg) was oxidized in the dark with 0·05 M sodium periodate (50 ml) for 15 days. The periodate consumed was determined at intervals by the method of Aspinall and Ferrier[18] and a value of 0·75 mol per anhydroxylose unit was obtained by extrapolation to zero time. An aliquot of the soln was dialysed and the oxidized polysaccharide was reduced with $NaBH_4$. The product was hydrolysed and after neutralization examined by GLC (GE–SF column) using the trimethylsilyl ether derivatives.[19]

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