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HEMICELLULOSES FROM THE STALK OF *CYPERUS PAPYRUS*

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Abstract—The alkali soluble hemicelluloses of *Cyperus papyrus* have been examined and a pure arabinoglucuronoxylan has been isolated. Structural studies showed that the xylan has a $\overline{DP}_n$ of *ca.* 57 and consists of a main chain of β(1 → 4) linked D-xylopyranosyl residues to which are attached an average of 3·2 L-arabinofuranosyl residues and 1·7 D-glucopyranuronosyl residues.

INTRODUCTION

HEMICELLULOSES from the Gramineae have been the subject of intensive studies.[1] In each case the dominant hemicellulose has been found to be of the xylan type. Typically such molecules are composed of a backbone of β(1 → 4) linked D-xylopyranosyl residues to which are attached L-arabinofuranosyl residues and D-glucopyranuronosyl or 4-*O*-methyl-D-glucopyranuronosyl residues. In most of these xylans the arabinose residues and the uronic acid residues are linked to C3 and C2 positions respectively of the main xylan chain. In more complex xylans, such as those from maize hulls,[1] oat stem[2] and oat leaf[3] there are also non-terminal L-arabinofuranose residues and terminal and non-terminal galactose residues. More recently non-cellulosic linear β-glucans possessing β(1 → 3) and β(1 → 4) glucosidic linkages have been isolated.[4,5] The hemicelluloses from other herbaceous monocotyledons have not been closely examined. The present work describes studies carried out on the hemicelluloses from *Cyperus papyrus*, a member of the *Cyperaceae* which is closely related to the *Gramineae*. A preliminary study of this plant was carried out by Votocek in 1937.[6]

RESULTS AND DISCUSSION

Initial extracts of the plant material with EtOH and with water gave fructose, sucrose and higher fructose-containing oligosaccharides which were not further studied. Only traces of polysaccharide were obtained on subsequent extraction with 2% oxalic acid and it can be concluded that 'pectic substances' are not present. The residual plant material was delignified by treatment with sodium chlorite[7] and the resultant holocellulose was treated

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successively with 5 and 24% KOH. The total hemicellulose[8] so obtained released, on acid hydrolysis, arabinose, galactose, glucose and xylose (in the molar ratio 1·0:0·5:1·1:5·2), traces of rhamnose, both glucuronic and 4-*O*-methylglucuronic acids and other acidic material. The total hemicellulose composition is similar to those from the mature tissues of members of the Gramineae.[9]

A variety of fractionation methods were attempted but only an acidic arabinoxylan, purified via its copper complex, was obtained sufficiently pure to warrant structural studies. A sample of this xylan which contained no glucose residues, was esterified with aqueous propylene oxide and reduced with $NaBH_4$.[10] This material, on acid hydrolysis, yielded arabinose, glucose and xylose in the molar ratio 1·0:0·5:16 corresponding to a glucuronic acid content of 3%. Partial acid hydrolysis gave, in addition to arabinose and xylose, the $\beta(1 \rightarrow 4)$ linked di-, tri-, tetra- and pentasaccharides of D-xylose and an aldobiouronic acid which was probably 2-*O*-α-D-glucopyranuronosyl-D-xylose. Methanolysis of the reduced and methylated aldobiouronic acid gave the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose.

The xylan was methylated successively by the methods of Haworth,[11] Hakomori[12] and Purdie[13] and the product, which was not sub-fractionated, showed only trace hydroxyl absorption in its IR spectrum. The $\overline{DP_n}$ determined by vapour phase osmometry was *ca.* 45. The methylated material was reduced with $LiAlH_4$ and a sample of the reduced material was methanolysed and examined by GLC. The methyl glycosides of the following sugars were identified: 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose and 2-*O*-, and 3-*O*-, methyl-D-xyloses. A second sample of the methylated and reduced xylan was hydrolysed by the HCO_2H–H_2SO_4 method[14] and quantitative estimation of the products was carried out by GLC of the derived glycitol acetates. The above methylated sugars were present in the peak area ratio of 2·7:1·7:1·0:47:4·5, but this method did not distinguish between the two mono-*O*-methyl-D-xyloses. Although the methylated xylan was obtained in only 61% yield its composition, on acid hydrolysis, indicated that it was representative of the parent xylan.

The methylation results were confirmed by the periodate oxidation study. The xylan consumed 0·90 mol of periodate per anhydro sugar residue and the reduced oxopolysaccharide, on acid hydrolysis, gave ethylene glycol, glycerol and xylose in the molar ratio 1·0:50:4·9. Neither the methylation analysis nor the periodate oxidation results indicated the presence of main chain branching of the xylan which is concluded to have an average of *ca.* 52 contiguous $\beta(1 \rightarrow 4)$ linked D-xylopyranosyl residues to which are attached 3·2 L-arabinofuranosyl residues and 1·7 D-glucopyranuronosyl residues at C3 and C2 positions respectively. These values are based on the molar proportions of the sugars in the hydrolysate of the reduced xylan.

The structural study shows that the arabinoglucuronoxylan from *Cyperus papyrus* is very similar to the simpler xylans which have been isolated from many Gramineae except that in the latter the D-glucuronic acid is often present as the 4-*O*-methyl ether. The preliminary fractionations carried out did not indicate the presence of an arabinogalactan and

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it seems possible that the galactose residues are present in more complex acidic galacto-arabinoxylans similar to those found in oat stem[2] and leaf[3] tissues. The xylan isolated probably represents a portion of the polydisperse spectrum of the xylans in the cell wall.

Ethanol precipitation of the water-soluble fraction of the alkali-extracted hemicellulose gave material enriched in glucose residues. Periodate oxidation studies showed that some of the glucose residues were susceptible to oxidation, yielding erythritol, and were (1 → 4) linked. Some of the glucose residues were resistant to oxidation and could be (1 → 3) linked. The hemicellulose was treated with the enzyme mixture from *Cytophaga*, which is known to contain β-1,3-glucanase activity,[15] and glucose, laminaribiose, cellobiose, cellotriose, and cellotetraose were obtained. Starch was not detected and so these results indicate that it is likely that the glucose residues in the total hemicellulose are present in a β-glucan containing β(1 → 3) and β(1 → 4) linkages such as those already isolated from oat leaf[4] and maize stalks.[5]

EXPERIMENTAL

General methods. PC was on Schleicher and Schuell No. 2043b paper and TLC on Kieselgel G (Merck) using the following irrigants: A, EtOAc–pyridine–H_2O (8:2:1); B, EtOAc–pyridine–H_2O (2:1:2); C, EtOAc–HOAc–HCO_2H–H_2O (18:3:1:4); D, EtOAc–HOAc–H_2O (3:1:3); E, *n*-BuOH–EtOH–H_2O–NH_3 (4:1:5:trace); F, benzene–EtOH–H_2O–HOAc (200:47:15:1). Chromatographic detection reagents were alkaline $AgNO_3$, *p*-anisidine–HCl, alkaline triphenyltetrazolium chloride or naphth-1-ol/conc. H_2SO_4. A Perkin–Elmer F 30 chromatograph was used for GLC with glass columns (2 m × 2 mm i.d.) containing *a*: 3% ECNSS-M on Gas Chrom Q (100–120 mesh) or *b*: *m*-bis(*m*-phenoxyphenoxy)benzene on AW DMCS Chromosorb W (100–120 mesh). Hemicellulosic samples were hydrolysed in 0·5 M H_2SO_4 by heating for 1 hr at 120° in an autoclave or by the 72%/3% H_2SO_4 procedure. The hydrolysates were neutralized with $BaCO_3$ and, where appropriate, Dowex 50 (H^+) was used before examination for acidic sugars. The neutral sugars in the hydrolysates were determined by GLC of their derived glycitol acetates (column *a*).

The plant material. The plants were grown in a green-house and a stem *ca.* 4 m high was chosen for study. Only the inner white stem tissue was studied. This was boiled in EtOH immediately after the plant was dissected and then the air dried tissue was ground in a hammer mill.

Isolation of the total hemicellulose. Aqueous and 2% ammonium oxalate extracts of the plant material contained fructose, glucose, sucrose and other oligosaccharides but only negligible quantities of polysaccharide. The residual material (22 g) was delignified and the resulting holocellulose was treated successively with 5 and 24% KOH (200 ml). The total hemicellulose (5·4 g) was isolated by the method of Reid and Wilkie.[8]

Fractionation of the total hemicellulose. The total hemicellulose was dispersed in H_2O and a water-soluble fraction (2·7 g) and a water-soluble fraction (1·7 g) were isolated. The water-insoluble material was fractionated by precipitating the copper complex formed on addition of Fehling's solution to a solution in 10% NaOH (50 ml). The ratio of arabinose to xylose remained constant after three such precipitations and the material (490 mg) hereafter referred to as the xylan, could not be further fractionated by use of $Ba(OH)_2$ or cetavlon.

Examination of the xylan. The xylan had $[\alpha]_D^{23}$ −65·4° (*c*, in M NaOH) and on acid hydrolysis released arabinose, xylose, glucuronic acid and traces of galactose, 4-*O*-methylglucuronic acid and other acidic material. A sample (20 mg) of the xylan was suspended in 40% aq. propylene oxide (5 ml)[10] for 7 days and, after the propylene oxide had been removed under reduced pressure, H_2O (5 ml) and $NaBH_4$ (20 mg) were added. After 24 hr the excess of $NaBH_4$ was destroyed with HOAc and the product was dialysed until free of inorganic material. The esterification and reduction were repeated and the material on hydrolysis released arabinose, xylose and glucose in the molar ratio (1·0:16:0·5).

Partial acid hydrolysis of the xylan. A sample (100 mg) of the xylan was suspended in 0·1 M H_2SO_4 (20 ml) and heated at 100° for 3 hr. After cooling the insoluble material was removed and the neutralized and deionized hydrolysate was reduced in volume. The hydrolysate was fractionated into neutral (eluted with H_2O) and acidic components (eluted with 30% HOAc) on a column of Dowex 1 (acetate form).

Examination of the neutral fraction. PC (irrigants *A*, *C* and *D*) showed the presence of arabinose, xylose and at least four oligosaccharides. They had R_{xylose} (irrigant *B*) 0·57, 0·27, 0·11, 0·03 and the relative proportions (estimated by the method of Nelson and Somogyi[16]) were 5:3:2:– respectively. The oligosaccharides

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were purified by PC (irrigant *B*). A sample of each oligosaccharide released only xylose on acid hydrolysis and they were indistinguishable by PC (irrigants *A*, *C* and D) from authentic samples of xylobiose, xylotriose, xylotetraose and xylopentaose. A sample of each oligosaccharide was reduced with $NaBH_4$, hydrolysed and acetylated ($NaOAc$–Ac_2O). The products were indistinguishable (GLC; column *a*) from those prepared by acetylating a mixture of xylose and xylitol. The molar ratios of xylose to xylitol (= 1·0) were 1·05, 1·97, 3·05 and 4·10 respectively.

Examination of the acidic fraction. PC (irrigant *A*) showed that there were no neutral components in this fraction. Further examination (irrigants *C* and *D*) showed the presence of traces of components indistinguishable from D-glucuronic acid and 4-*O*-methyl-D-glucuronic acid and a major component (R_{xylose} 0·28; irrigant *C*) which was probably an aldobiouronic acid. The purified material (PC irrigant *C*) released glucuronic acid and xylose on acid hydrolysis. The oligosaccharide was treated with 1% MeOH–HCl for 12 hr at room temp. and the neutralized (Ag_2CO_3) solution was taken to dryness. The product was dissolved in dry tetrahydrofuran, heated under reflux with $LiAlH_4$ for 1 hr and the isolated product was methylated successively by the methods of Hakomori[12] and Purdie.[13] This material was treated with 4% MeOH–HCl in a sealed tube (100°; 16 hr) and after cooling was examined directly by GLC (columns *a* and *b*). Peaks corresponding in relative retention time to those produced by the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-di-*O*-methyl-D-xylose were obtained.

Methylation of the xylan. A sample (200 mg) of the xylan was methylated successively by the methods of Haworth,[11] Hakomori[12] and Purdie.[13] The methylated material was extracted with light petrol. and the residue (150 mg) which was soluble in $CHCl_3$ showed only trace absorption, attributable to hydroxyl, in its IR spectrum. The $\overline{DP_n}$, determined using a Knauer vapour phase osmometer, was found to be *ca.* 45.

The methylated xylan was dissolved in dry tetrahydrofuran (20 ml) and heated under reflux with $LiAlH_4$ (50 mg) for 1 hr. The excess of $LiAlH_4$ was destroyed by the addition of EtOAc and H_2O and the reduced xylan was isolated. A sample (5 mg) was treated with 4% MeOH–HCl in a sealed tube (100°; 16 hr) and the products were examined by GLC (columns *a* and *b*). The methyl glycosides of the following sugars were were identified by comparison with authentic compounds: 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose and 2-*O*-, and 3-*O*-methyl-D-xyloses.

The remainder of the methylated xylan was hydrolysed by the $HCOOH$–H_2SO_4 method[14] and the neutralised ($BaCO_3$) and deionised (Dowex 50 H^+) hydrolysate was evaporated to dryness. A sample of the hydrolysate was reduced with $NaBH_4$ and acetylated with Ac_2O–pyridine (1:1). The following sugars were identified (GLC; column *a*), as the glycitol peracetate derivatives, by comparison with authentic compounds: 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-glucose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3,di-*O*-methyl-D-xylose and 2-*O*- and 3-*O*-methyl-D-xyloses (unresolved). Samples of the hydrolysate were examined by paper chromatography and by TLC (irrigants *E* and *F*) and the identities of the above methylated sugars were further confirmed.

Periodate oxidation of the xylan. A sample (15 mg) of the xylan was oxidized with 0·015 M $NaIO_4$ at 35° in the dark. The amount of periodate reduced was determined at intervals by the method of Aspinall and Ferrier[17] and a value of 0·90 mol per anhydro sugar residue was obtained by extrapolation to zero time. Another sample (50 mg) was oxidized with 0·05 M $NaIO_4$ at 5° in the dark for 28 days. The periodate was removed by dialysis and the oxopolysaccharide was reduced with $NaBH_4$. The product was hydrolysed and PC (irrigant *A*) showed the presence of glycerol, arabinose and xylose. The hydrolysate was reduced with $NaBH_4$ and acetylated with Ac_2O–NaOAc. The glycitol acetate mixture was examined by GLC (column *a*) and the following compounds were identified by comparison with standard compounds: ethylene glycol diacetate, glycerol triacetate, arabinitol pentaacetate and xylitol pentaacetate in the molar rato 1·0:50:tr:4·9.

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