Phytochemistry, 1974, Vol. 13, pp. 2185 to 2188. Pergamon Press. Printed in England.

XYLANS FROM THE TROPICAL GRASS *PANICUM MAXIMUM*

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(*Revised received* 6 *February* 1974)

Key Word Index—*Panicum maximum*; Guinea grass; Gramineae; xylan; galactoarabinoxylan; β-glucan; hemicellulose.

Abstract—Two xylans have been isolated from the mature tissues of the tropical grass *Panicum maximum*—an arabino(4-*O*-methylglucurono)xylan and an acidic galactoarabinoxylan. Both consist of a main chain of β(1 → 4) linked D-xylopyranosyl residues. The former has average of *ca* 46 such residues to which are attached *ca* 7 L-arabinofuranosyl and *ca* 2 4-*O*-methyl-D-glucopyranuronosyl residues at C3 and C2 positions respectively. The acidic galactoarabinoxylan has a $\overline{DP}_n$ of *ca* 90 and contains arabinose, galactose, xylose and uronic acid residues in the molar ratio 10:5:22:4. Methylation analysis and periodate oxidation indicated the highly branched nature of this polysaccharide.

INTRODUCTION

HEMICELLULOSES from the Gramineae have been the subject of intensive studies[1] but only more recently have a few tropical species been examined.[2–4] From each of these tropical grasses a xylan was isolated and structural studies showed that they were similar to the xylans isolated from temperate grasses.[1] More highly branched acidic galactoarabinoxylans have been isolated from oat tissues,[5,6] maize hulls[7] and bamboo stalks[8] and β-glucans have been isolated from oat leaf[9] and maize stem.[10] The hemicelluloses from the tropical grass *Panicum maximum* (Guinea grass) were examined in order to establish the presence of the above types of polysaccharide.

RESULTS AND DISCUSSION

The mature plants were harvested before they had begun to become yellow. The aerial tissues were boiled in EtOH, milled and successively extracted with 80% EtOH, hot H_2O and 2% EDTA in order to remove low molecular weight sugars, starch and pectic substances. The residual plant material was delignified by treatment with sodium chlorite and the resultant hollocellulose was treated successively with 5 and 24% KOH. The total hemicellulose so obtained released on acid hydrolysis, arabinose, galactose, glucose, xylose (in

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the molar ratio 1·0:0·3:0·4:5·5), rhamnose, both glucuronic and 4-*O*-methylglucuronic acids and other acidic material.

The total hemicellulose was separated into a water-soluble and a water-insoluble fraction and the latter was subjected to successive precipitation of the copper complex formed on addition of Fehling's solution to an alkaline solution of the hemicellulosic material. The acidic arabinoxylan so obtained (2·5% of the plant material) released on acid hydrolysis arabinose, xylose, acidic sugars and traces of galactose. A sample of the xylan was esterified with aqueous propylene oxide and reduced with $NaBH_4$.[11] This material on hydrolysis yielded arabinose, 4-*O*-methylglucose and xylose in the molar ratio 1·0:0·3:9·7 corresponding to a uronic acid content of 2·7%.

Partial acid hydrolysis of the xylan gave *inter alia* the $\beta(1 \rightarrow 4)$ linked di-, tri-, tetra- and pentasaccharides of D-xylose and a series of oligouronic acids containing 4-*O*-methylglucuronic acid and xylose. The oligouronic acids 2-*O*-(4-*O*-methyl-α-D-glucopyranuronosyl)-D-xylose and *O*-α-4-*O*-methyl-D-glucopyranuronosyl-$(1 \rightarrow 2)$-*O*-β-D-xylopyranosyl-$(1 \rightarrow 4)$-D-xylose were identified.

Methylation[12,13] of the xylan gave a product (78%) which displayed no hydroxyl absorption in its IR spectrum. The $\overline{DP}_n$ determined by vapour phase osmometry was *ca* 55. Analysis of the glycosides in a methanolysate of this material by GLC enabled the following sugars to be identified: 2,3,5-tri-*O*-methylarabinose, 2,3,4,-tri-*O*-methylxylose, 2,3-di-*O*-methylxylose, 2-*O*-methylxylose and traces of 3-*O*-methylxylose and methyl 2,3,4-tri-*O*-methylglucuronate. A sample of the methylated xylan was reduced with $LiAlH_4$, hydrolysed,[14] reduced with $NaBH_4$ and acetylated. The following sugars were identified by GLC of their derived glycitol acetates: 2,3,5-tri-*O*-methylarabinose, 2,3,4-tri-*O*-methylglucose, 2,3,4-tri-*O*-methylxylose, 2,3-di-*O*-methylxylose and 2-*O*-, and 3-*O*-methylxyloses in the peak area ratio of 4·6:1·1:1·0:48:4·8.

On oxidation with $NaIO_4$ the xylan consumed 0·95 mol of periodate per sugar residue and the reduced oxopolysaccharide, on acid hydrolysis, gave ethylene glycol, glycerol and xylose in the molar ratio 1·0:55:9·0. Neither the methylation analysis nor the periodate oxidation study indicated the presence of main chain branching of the xylan which is concluded to have an average of *ca* 46 contiguous $\beta(1 \rightarrow 4)$ linked D-xylopyranosyl residues to which are attached *ca.* 7 L-arabinofuranosyl residues and *ca* 2 4-*O*-methyl-D-glucopyranuronosyl residues at C3 and C2 positions respectively.

The water-soluble fraction of the total hemicellulose was fractionated by precipitation with EtOH to give glucan enriched material (insoluble in 50% EtOH) and material which contained little glucan (soluble in 50% EtOH). The latter was further fractionated on a column of DEAE-cellulose in the acetate form. The column was irrigated with H_2O and then the major fraction was eluted as a single narrow component in a gradient of aqueous KOAc. Sub-division of this material into early and late fractions did not give fractions which differed significantly in their composition.

This major fraction, $[\alpha]_D^{24} - 64°$, released on acid hydrolysis arabinose, galactose, rhamnose, xylose and acidic sugars in the molar ratio 10:5:trace:22:—. The Procion dyed derivative was electrophoretically homogeneous on cellulose acetate and the polysaccharide, hereafter referrred to as the acidic galactoarabinoxylan, could not be further subfractionated. A value of *ca* 7% was obtained for the uronic anhydride.

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Partial acid hydrolysis gave twelve identifiable oligosaccharides. In addition to arabinose, galactose and xylose, six neutral and eight acidic components were isolated by a combination of PC and anion-exchange chromatography. The expected $\beta(1 \rightarrow 4)$ di-, tri-, tetra- and pentasaccharides of D-xylose were obtained and in addition a xylosylarabinose, a galactosylarabinose and a trisaccharide containing arabinose, galactose and xylose. The latter three oligosaccharides were probably 2-*O*-β-D-xylopyranosyl-L-arabinose, 5-*O*-β-D-galactopyranosyl-L-arabinose and galactopyranosyl-(1 → 4)-D-xylopyranosyl-(1 → 2)-L-arabinose respectively. The acidic components were D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, 4-*O*-methyl-D-glucopyranuronosyl-(1 → 2)-D-xylose, D-glucopyranuronosyl-(1 → 2)-D-xylose, 4-*O*-methyl-D-glucopyranuronosyl-(1 → 2)-D-xylopyranosyl-(1 → 4)-D-xylose, D-glucopyranuronosyl-(1 → 2)-D-xylopyranosyl-(1 → 4)-D-xylose and two other higher oligouronic acids. The aldotriuronic acid containing xylose, galactose and glucuronic acid reported present in hydrolysates of oat xylans[5,6] was not found.

The galactoarabinoxylan was methylated successively by the methods of Haworth[12] and Hakomori[13] to give a product (65%) which had no hydroxyl absorption in its IR spectrum. The $\overline{DP}_n$ determined by vapour phase osmometry was *ca* 90. A methanolysate of the polysaccharide was examined by GLC and TLC and PC of a hydrolysate of the methylated material gave the following sugars: 2,3,5-tri-*O*-methylarabinose, 2,3-di-*O*-methylarabinose, 3,5-di-*O*-methylarabinose, 2,3,4,6-tetra-*O*-methylgalactose, 2,3,4,-tri-*O*-methylxylose, 2,3-di-*O*-methylxylose, 2-*O*-methylxylose, 3-*O*-methylxylose and 2,3,4-tri-*O*-methylglucuronic acid. A sample of the hydrolysate was reduced with $NaBH_4$, acetylated and examined by GLC. The above sugars were present in the peak area ratio of 5·5:1·7:1·1:2·1:1·0:10:14·5:— (the mono-*O*-methylxyloses were unresolved).

A sample of the galactoarabinoxylan was oxidized with $NaIO_4$ and after dialysis, the non-diffusible material was reduced with $NaBH_4$ and the derived polyalcohol hydrolysed. Ethylene glycol, glycerol, threitol, rhamnose, arabinose, xylose and galactose were released in the molar ratio 0·5:100:0·1:trace:67:9.

The methylation analysis and the periodate oxidation results clearly indicate the highly branched nature of this polysaccharide which is essentially similar to that isolated from oat stem[6] except that it has a higher proportion of galactose residues. The acidic galactoarabinoxylan has then, contiguous $\beta(1 \rightarrow 4)$ linked D-xylopyranosyl residues the majority of which carry one of the following side-residues: glucuronic acid or 4-*O*-methylglucuronic acid directly attached to C2 positions and L-arabinofuranosyl, galactopyranosyl-D-xylopyranosyl-L-arabinofuranosyl, galactopyranosyl-L-arabinofuranosyl and D-xylopyranosyl-L-arabinofuranosyl residues attached to C3 positions.

Further attempts to purify the glucan enriched fraction (insoluble in 50% EtOH) were unsuccessful. Periodate oxidation studies showed that some of the glucose residues were susceptible to oxidation, yielding erythritol and were (1 → 4) linked. Some of the residues were resistant to oxidation and could be (1 → 3) linked. The fraction was treated with the enzyme preparation from *Cytophaga* which is known to contain β-1,3-glucanase activity[15] and glucose, laminaribiose, cellobiose, cellotriose, cellotetraose and higher molecular weight glucose-containing material were obtained. Since starch was not detected these results indicate the presence of a β-glucan containing $\beta(1 \rightarrow 3)$ and $\beta(1 \rightarrow 4)$ linkages such as has been found in oat leaf[9] and maize stem.[10]

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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 (5:1:4:trace); *F*, MeCOEt–H_2O–NH_3 (10:1:trace) and *G*, *n*-BuOH–C_6H_6–pyridine–H_2O (5:5:1:3). Chromatographic detection reagents were alkaline $AgNO_3$, *p*-anisidine–HCl, alkaline triphenyltetrazolium chloride or naphth-1-ol–conc. H_2SO_4. A Parkin–Elmer F 30 chromatograph was used for GLC with columns (2 m × 2 mm i.d.) containing *a*, 3% ECNSS-M on Gas Chrom-Q (100–200 mesh) or *b*, 10% *m*bis-(phenoxyphenoxy)benzene on Chromosorb W (100–120 mesh). Hemicellulosic samples were hydrolysed with 0·5 M H_2SO_4 by heating at 100° for 12–16 hr in sealed tubes. Hydrolysates were neutralized with $BaCO_3$ and deionised with Dowex 50 (H^+). The neutral sugars in the hydrolysates were determined by GLC of their derived glycitol acetates (column *a*). MW were determined with a Knauer vapour phase osmometer using toluene solutions of the methylated polysaccharides.

Isolation of the total hemicellulose. Mature *Panicum maximum* plants were harvested in September 1972 and immediately boiled in EtOH and milled. The plant material (56 g) was successively extracted with boiling 80% EtOH, H_2O and 2% EDTA (2 l.; 2 hr; 80°) and then delignified with sodium chlorite. The residual material (38 g) was treated successively with 5 and 24% KOH and the total hemicellulose (11·2 g) was isolated.

Fractionation of the total hemicellulose. The total hemicellulose was dispersed in H_2O to give a H_2O-soluble fraction (6 g) and a H_2O-insoluble fraction (5 g). The latter was fractionated by precipitating the copper complex formed on addition of Fehling's solution to a solution of the hemicellulose in 10% NaOH. The ratio of arabinose to xylose remained constant after four such precipitations and the material (1·4 g), hereafter referred to as the xylan, could not be further fractionated by use of $Ba(OH)_2$ or Cetavlon. EtOH was added to a 2% aq. soln of the H_2O-soluble material and the precipitate formed at 50% EtOH was isolated. This precipitate was enriched in glucan whereas the hemicellulosic material in the supernatant contained only traces of glucan. This latter material was further fractionated on a column of DEAE-cellulose (Whatman DE 11; acetate form). A neutral fraction (30%) was eluted with H_2O and the principal fraction (50%) was eluted between 0·4 and 0·5 M KOAc as a single well-defined component using a linear gradient of aqueous KOAc.

Partial acid hydrolysis of the polysaccharides and separation of the oligosaccharides was carried out as previously described.[16] All of the oligosaccharides were chromatographically homogeneous (PC irrigants *A*, *B* and *C*) and were identified by comparison with samples of established identity. Complete hydrolysis gave qualitatively and quantitatively the expected products.

Methylation of the polysaccharides was carried out by the methods of Haworth[12] and Hakomori.[13] Methanolysates were examined by GLC (columns *a* and *b*); hydrolysates were examined by PC and TLC (irrigants *E* and *F*) and the derived glycitol acetates were identified and estimated by GLC (column *a*).

Periodate oxidation was carried out as described previously.[6,16]

Acknowledgements—Thanks are expressed to the Royal Society for a European Fellowship, to the Swiss National Research Foundation for financial support and to Professor H. Meier for his interest in this work.

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