Phytochemistry, 1973, Vol. 12, pp. 1373 to 1376. Pergamon Press. Printed in England.

AN ARABINOGALACTO(4-*O*-METHYLGLUCURONO)XYLAN FROM THE LEAVES OF *HORDEUM VULGARE**

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(*Received* 18 *October* 1972. *Accepted* 22 *January* 1973)

Key Word Index—*Hordeum vulgare*; Gramineae; barley; arabinogalacto(4-*O*-methylglucurono)xylan; hemicellulose; xylan.

Abstract—An arabinogalacto(4-*O*-methylglucurono)xylan with a $\overline{DP_n}$ of *ca.* 96 has been isolated from the leaves of barley. Based on structural studies it is proposed that the hemicellulose consists of a main chain of $\beta(1 \rightarrow 4)$-linked D-xylopyranosyl residues to which are attached an average of 8·1 L-arabinofuranosyl residues, 3·8 galactopyranosyl-$(1 \rightarrow 4)$-D-xylopyranosyl-$(1 \rightarrow 2)$-L-arabinofuranosyl residues and 4·4 4-*O*-methyl-D-glucopyranuronosyl residues.

INTRODUCTION

RECENTLY, a series of detailed studies has been carried out on the relationship between the maturity of the oat plant and the composition of the *total hemicellulose* contained in the plant tissues.[1] These studies have also been extended to other members of the *Gramineae*, namely barley, rye and wheat.[2,3] In studies on the oat plant[1,4] the object was to account for all of the sugar residues in the hemicelluloses in each plant tissue and to interpret the results in terms of structures of *pure* hemicelluloses.

Structural studies have been carried out on the hemicelluloses from barley husks[5] but there have been none on the hemicelluloses from leaf or stem tissues. The acidic arabinoxylan isolated from barley husks[5] has structural features not present in most of the xylans so far examined[6] and so structural studies of the xylans from barley leaf or stem were considered desirable extensions to the earlier work.[3] The present studies report the structure of an arabinogalacto(4-*O*-methylglucurono)xylan isolated from barley leaves.

RESULTS AND DISCUSSION

The total hemicellulose isolated from the leaves of mature barley plants contained arabinose, galactose, glucose and xylose residues (in the molar ratio 1·0:0·5:0·5:3·1), traces

* The studies reported in this paper do not permit an unambiguous assignment of D or L configuration to the products described, but the D and L configurations for xylose and arabinose respectively have been inferred on the basis of well-established precedents.

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of rhamnose and both glucuronic and 4-*O*-methylglucuronic acids. This hemicellulosic material was separated into water-insoluble and water-soluble fractions and the latter was the source of the arabinogalacto(4-*O*-methylglucurono)xylan which was purified via its copper complex. After purification a small proportion of glucose residues still remained. These were considered to indicate the presence of a contaminating β-glucan of the type isolated from oat leaf[7] and maize stem.[8] The hemicellulose was treated with an enzyme preparation from *Cytophaga*[9,10] to degrade the glucan and the xylan then recovered was free of glucose residues. This xylan, after esterification with aqueous propylene oxide and reduction with $NaBH_4$,[11] released on acid hydrolysis, arabinose, galactose, 4-*O*-methylglucose and xylose in the molar ratio 1·0:0·32·0·37:6·3, corresponding to a 4-*O*-methylglucuronic acid content of 4·6%.

Partial acid hydrolysis of the xylan gave *inter alia* the $\beta(1 \rightarrow 4)$-linked di-, tri-, tetra-, and penta-saccharides 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.

After methylation by the methods of Haworth[12] and Hakomori[13] the xylan yielded 74% of a product which displayed no absorption in its IR spectrum attributable to hydroxyl. Analysis of the glycosides in a methanolysate of the methylated material by GLC enabled the following sugars to be identified: 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4,6-tetra-*O*-methylgalactose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose, 3-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucuronic acid as its methyl ester. Hydrolysis of the methylated xylan gave 3,5-di-*O*-methyl-L-arabinose in addition to the above sugars. A sample of the hydrolysate was reduced with $NaBH_4$, acetylated and quantitative estimation of the products was carried out by GLC. The following sugars were identified as their derived glycitol peracetates: 2,3,5-tri-*O*-methyl-L-arabinose, 3,5-di-*O*-methyl-L-arabinose, 2,3,4,6-tetra-*O*-methylgalactose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, and 2-*O*-, and 3-*O*-, methyl-D-xyloses in the peak area ratio of 6·7:3·6·5·2:1·0:56·9:19·0.

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, arabinose and xylose in the molar ratio 0·04:4·67.0·27.1·0. A sample of the xylan was reduced with $NaBH_4$ and oxidised with $NaIO_4$. The $\overline{DP_n}$ of the xylan was estimated as *ca.* 96 based on the HCHO produced.

Galactosyl-$(1 \rightarrow 4)$-xylosyl-$(1 \rightarrow 2)$-arabinosyl residues have been concluded to be present in the xylans from perennial ryegrass roots,[14] maize hulls[15] and oat stem.[16] The presence of 3,5-di-*O*-methylarabinose and 2,3,4,6-tetra-*O*-methylgalactose in approximately equal proportions in the hydrolysate of the methylated xylan is compatible with the presence of

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this structural feature. On this basis the xylan is concluded on average to consist of *ca.* 72 contiguous $\beta(1 \rightarrow 4)$-linked D-xylopyranosyl residues to which are attached 8·1 L-arabinofuranosyl residues and 3·8 galactosyl-$(1 \rightarrow 4)$-xylosyl-$(1 \rightarrow 2)$-arabinosyl residues at C3 positions and 4·4 4-*O*-methyl-D-glucopyranuronosyl residues at C2 positions. The possible presence of single non-reducing galactopyranosyl residues can not be excluded but this structural feature is not known in the xylans from the *Gramineae*. The configuration of the galactose residues is not known but both the D and the L forms have been reported in land plants.[14,15] Galactose occurs in the structural features of some xylans isolated[14–17] but is absent from many others.[6] A detailed examination of the literature reveals that in many cases xylans, even after purification, often retain galactose residues to which no structural role has been assigned. It is of interest to note that this xylan, in common with most xylans, forms an insoluble copper complex whereas the galactoarabinoxylans from oat tissues do not.[16] The xylan isolated in this study of barley leaf tissue represents a portion of the polydisperse spectrum of the xylans in the cell wall.

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–HCOOH–H_2O (18:3:1:4); (*D*), *n*-BuOH–EtOH–H_2O–NH_3 (4:1:5:trace); (*E*) MeCOEt–H_2O–NH_3 (10:1:trace); (*F*)C_6H_6–EtOH–H_2O–HOAc (200:47:15:1) and (*G*) *n*-BuOH–HOAc–H_2O (5:1:4). 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); (*b*) 10% *m*-bis (*m*-phenoxyphenoxy)benzene on AW DMCS Chromosorb W (100–120 mesh) and (*c*) 3% OV 225 on AW DMCS Chromosorb G (100–120 mesh) Hemicellulosic samples were hydrolysed with 0·5 M H_2SO_4 in sealed tubes at 100° for 12–16 hr and the hydrolysates were neutralized with $BaCO_3$ and, where appropriate, Dowex 50 (H^+) was used prior to examination for acidic sugars. The neutral sugars in hydrolysates were estimated by GLC of their glycitol acetates (column *a*).

Isolation of the hemicellulosic material. Barley plants (var. Ymer) were harvested at Tillycorthie Farm, Aberdeenshire in late September 1969. Leaves and stems were separated, boiled in EtOH for 20 min and air-dried. A sample (40 g) of milled leaf tissue was delignified by the method of Wise *et al.*[18] and the total hemicellulose (13 g) was isolated by the method of Reid and Wilkie.[4]

Fractionation of the total hemicellulose. Total hemicellulose (13 g) was dispersed in H_2O and the water-insoluble fraction (8·1 g) and the water-soluble fraction (4·8 g) were obtained after centrifugation and freeze-drying. The water-soluble fraction was dissolved in 4% NaOH and was subjected to four successive precipitations of the copper complex formed on addition of Fehling's solution. The hemicellulosic material precipitated released, on acid hydrolysis, arabinose, galactose, glucose and xylose in the molar ratio (1·0·0 3: 0·1:6·0) and acidic sugars. This material was suspended in 0·1 M phosphate—citrate buffer (50 ml; pH 5·5) and the enzyme mixture from *Cytophaga* (100 mg) was added. The suspension, in a dialysis sac, was incubated at 40° in the buffer solution for 1 day. The non-diffusible material was heated at 100° for 5 min, cooled, centrifuged at 1000 g to remove the insoluble enzyme preparation and redialysed against H_2O. The hemicellulosic material recovered (700 mg) gave no glucose on hydrolysis.

Examination of the xylan. The above material, referred to as the xylan, had $[\alpha]_D^{23}$ −62·1° (*c* 1·3, 1 M NaOH). A sample (20 mg) of the xylan was suspended in H_2O saturated with propylene oxide (50 ml) for 5 days. The propylene oxide was removed under reduced pressure and $NaBH_4$ (20 mg) was added. After 2 days the excess of borohydride was destroyed with HOAc and the solution was dialysed. The esterification and reduction were repeated. The product, on acid hydrolysis, gave arabinose, galactose, 4-*O*-methylglucose and xylose in the molar ratio 1·0:0·32.0·37:6·3. No acidic material was obtained.

Partial acid hydrolysis of the xylan. A sample (200 mg) of the xylan was suspended in 0·05 M H_2SO_4 (30 ml) and heated for 3 hr at 100°. The cooled hydrolysate was neutralized ($BaCO_3$), deionized (Dowex 50 H^+) and reduced in volume. The hydrolysate was fractionated into neutral components (eluted with H_2O) and acidic components (eluted with 30% HOAc) on a column of DEAE Sephadex A25 (acetate form).

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Examination of the neutral fraction. Examination by PC (*A*, *B* and *C*) showed the presence of arabinose, galactose, xylose and four other components. They had R_{xylose} (irrigant *B*) 0·55, 0·27, 0·11 and 0·02 and the relative proportions (estimated by the Nelson-Somogyi method[19]) were 10:7:10:-respectively. The oligosaccharides 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*, *B* and *C*) 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 examined by GLC (column *a*) and they were indistinguishable from those prepared by acetylating a mixture of xylose and xylitol. The molar ratios of xylose to xylitol (1·0) were 1·01, 2·07, 3·10 and 4·08 respectively.

Examination of the acidic fraction. PC (irrigant *A*) showed that there were no neutral components in this fraction Further examination (irrigants *C* and *G*) showed the presence of five components R_{xylose} (irrigant *C*) 1 28, 0·76, 0·31, 0 13 and 0 05 in the relative proportions (estimated by the method of Nelson and Somogyi[19]) trace:1:3:9:6 respectively. These components were purified by PC (irrigant *C*) *Component* 1 was indistinguishable from 4-*O*-methyl-D-glucuronic acid (PC, irrigants *C* and *G*). *Component 2* was indistinguishable from an authentic sample of 2-*O*-(4-*O*-methyl-α-D-glucopyranuronosyl)-D-xylose (PC; irrigants *C* and *G*) Acid hydrolysis gave xylose and 4-*O*-methyl-D-glucuronic acid. *Component* 3 was indistinguishable from an authentic sample of *O*-α-4-*O*-methyl-D-glucopyranuronosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylose (PC; irrigants *C* and *G*) and on acid hydrolysis released xylose, 4-*O*-methyl-D-glucuronic acid and traces of component 2 *Components* 4 *and* 5, on acid hydrolysis, gave xylose, 4-*O*-methyl-D-glucuronic acid and components with the same mobilities as those described above They were probably the corresponding aldotetrao-, and aldopentao-uronic acids.

Methylation of the xylan. A sample (100 mg) of the xylan was methylated successively by the methods of Haworth[12] and Hakomori [13] The methylated material was extracted with light petrol. and the residue (90 mg) which was soluble in $CHCl_3$ showed no absorption attributable to hydroxyl in its IR spectrum. The methoxyl content was 37·9%. A sample of the methylated xylan 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 methy lglycosides of the following sugars were identified by comparison with authentic compounds: 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose, 3-*O*-methyl-D-xylose, 2,3,4,6-tetra-*O*-methylgalactose and 2,3,4-tri-*O*-methyl-D-glucuronic acid as its methyl ester.

The remainder of the methylated xylan was hydrolysed by the method of Bouveng *et al* [20] and the neutralized ($BaCO_3$) and deionized (Dowex 50 H^+) hydrolysate was taken to dryness. The hydrolysate was examined by PC and TLC (irrigants *D*, *E*, *F* and *G*) and the identities of the above sugars were further confirmed. In addition a compound which was chromatographically identical to 3,5-di-*O*-methyl-L-arabinose and which gave a positive reaction with triphenyltetrazolium chloride was found A sample of the hydrolysate was reduced with $NaBH_4$ and acetylated with Ac_2O–pyridine (1·1) The following sugars were identified by GLC (columns *a* and *c*) as their glycitol peracetate derivatives by comparison with authentic compounds. 2,3,5-tri-*O*-methyl-L-arabinose, 3,5-di-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2-*O*-, and 3-*O*-methyl-D-xyloses (unresolved) and 2,3,4,6-tetra-*O*-methylgalactose. There were minor unidentified peaks corresponding to *ca.* 0·5% of the total peak area.

Periodate oxidation of the xylan. A sample (20 mg) of the xylan was oxidized with 0·05 M $NaIO_4$ at 5° in the dark for 25 days. The amount of periodate reduced was determined spectrophotometrically at intervals. A value of 0·95 mol per anhydrosugar residue was obtained by extrapolation to zero time The residual material was dialysed and the oxopolysaccharide was reduced with $NaBH_4$. The product, on acid hydrolysis, gave (PC: irrigant *A*) glycerol, arabinose and xylose. The hydrolysate was reduced with $NaBH_4$ and acetylated with $NaOAc–Ac_2O$. The glycitol acetate mixture was examined by GLC (column *a*) and the following compounds were identified by comparison with authentic samples: ethylene glycol diacetate, glycerol triacetate, arabinitol pentaacetate and xylitol pentaacetate.

Determination of $\overline{DP_n}$ of the xylan.[21] A sample of the xylan (67·6 mg) was reduced with $NaBH_4$ and the excess of borohydride was destroyed with HOAc to give a final pH of 5·6. The solution was made 0 5 M with respect to $NaIO_4$ and was incubated in the dark at 18°. At intervals aliquots were withdrawn and the HCHO produced was determined by the chromatropic acid method. The $\overline{DP_n}$ calculated on the basis of the HCHO released was *ca* 103 and this was re-evaluated as *ca.* 96 when random substitution of the xylan chain at C2 positions was taken into consideration.

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. A sample of 3,5-di-*O*-methyl-L-arabinose was kindly supplied by Professor J. K. N. Jones.

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