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# AN ACIDIC GALACTOARABINOXYLAN FROM THE STEM OF *AVENA SATIVA*

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**Key Word Index**—*Avena sativa*; Gramineae; acidic galactoarabinoxylan; structural analysis; plant polysaccharide; xylan; oats.

**Abstract**—An L-arabino-D(L?)-galacto-D-glucurono-(4-*O*-methyl)-D-glucurono-D-xylan has been isolated from the stem tissues of the oat plant. The hemicellulose has D.P. 80 and the percentages of arabinose, galactose, xylose and uronic acid residues are 22, 6·7, 63 and 8·5 respectively. It has *ca.* 1 in every 1·8 residues on the main xylan chain carrying substitutents, namely, D-glucuronic acid and 4-*O*-methyl-D-glucuronic acid on C2 positions, D-glucopyranuronosyl-(1 → 4)-D-xylopyranosyl-(1 → 4)-D-galactopyranose on C2 or C3 positions, and, on C3 positions, α-L-arabinofuranosyl, 5-*O*-β?-D-galactopyranosyl-L-arabinofuranosyl, 4?-*O*-β?-L?-galactopyranosyl-2-*O*-β?-D-xylopyranosyl-L-arabinofuranosyl, and β?-D-xylopyranosyl-(1 → 2)-L-arabinofuranosyl residues or side-chains.

## INTRODUCTION

THE RELATIONSHIP between the maturity of the oat plant, *Avena sativa,* and the composition of the *total* hemicelluloses has been studied.[1,2] Studies have also been carried out on other members of the Gramineae, namely on wheat, barley, and rye,[3,4] which indicate that the oat plant is probably typical in its hemicellulose composition of all members of the temperate Gramineae.

Since it was impossible to investigate compositional differences in tissues having different maturities by isolating and studying *pure* hemicelluloses,[2] it was decided to account for all the sugar residues present in each total hemicellulose and as far as possible to interpret these residues in terms of the knowledge of hemicellulosic structure gained by study of *pure* hemicelluloses. The structure of a pure acidic arabinoxylan from mature oat straw (leaf and stem) was established by Aspinall and Wilkie.[5] The average molecule was found to have a chain of 40–45 β(1→4) interlinked D-xylopyranosyl residues with one residue carrying a L-arabinofuranosyl residue on a C-3 position of the main xylan chain and a D-glucopyranuronosyl or 4-*O*-methyl-D-glucopyranuronosyl residue carried on a C-2 position of the chain. Recently Sturgeon has determined that the D.P. of an oat straw acidic arabinoxylan was *ca.* 90 (private communication). The acidic arabinoxylan studied by Aspinall and Wilkie[5] has a much lower proportion of arabinose residues than in any

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[1] A. J. BUCHALA and K. C. B. WILKIE, *Phytochem.* **10,** 2287 (1971); and Refs. 1–4 and 6–9 therein.
[2] J. S. G. REID and K. C. B. WILKIE, *Phytochem.* **8,** 2045 (1969).
[3] A. J. BUCHALA and K. C. B. WILKIE, *Naturwissenschaften* **57,** 496 (1970).
[4] A. J. BUCHALA and K. C. B. WILKIE, to be published.
[5] G. O. ASPINALL and K. C. B. WILKIE, *J. Chem. Soc.* 1072 (1956).

oat tissue total hemicellulose and it fails to account structurally for the presence of the D-galactose residues. Norman[6] has reported the presence of galactose residues in oat straw but the identity of the parent hemicellulose was not determined. Reid and Wilkie isolated, and partially characterised, an acidic galactoarabinoxylan from oat leaf[7] and the present studies report in greater detail the structure of a pure acidic galactoarabinoxylan more systematically named as a L-arabino-D(L ?)-galacto-D-glucurono-(4-*O*-methyl)-D-glucurono-D-xylan from oat stem.

## RESULTS

The oat plants were harvested a month before normal harvest time and the stem between the inflorescence and the first node excised. This plant material was milled and then delignified by treatment with acid chlorite and the total hemicellulose isolated as described earlier.[2] After hydrolysis of a sample, derived glycitol acetates were determined by GLC. The molar ratios of arabinose–galactose–glucose–xylose were 14:5:13:68. The total hemicellulose was separated into water-soluble (65%) and water-insoluble (35%) fractions and the former was fractionated by chromatography on a column of DEAE-cellulose in the acetate form using water then 0·1, 0·5 and 5 M KOAc as irrigants.[7] The material eluted by 0·5 M KOAc accounted for *ca.* 16% of the water-soluble hemicellulose and it satisfied earlier criteria of purity[2] and was concluded to be a pure acidic galactoarabinoxylan. It is referred to hereafter as the galactoarabinoxylan. It is of interest that the acidic arabinoxylan isolated by Aspinall and Wilkie[5] from oat straw formed an insoluble copper complex whereas neither the galactoarabinoxylan from oat leaf[7] nor from stem did so. The hemicellulosic materials eluted by the irrigants before and after the 0·5 M KOAc, on the other hand, formed insoluble complexes. On hydrolysis the galactoarabinoxylan yielded arabinose, galactose and xylose in the molar ratios of 10:3:25. Acidic sugars and a trace of rhamnose were also noted. The pure hemicellulose had $[\alpha]_D^{25}$ −59·4°, OMe 1·8 ± 1%, and uronic anhydride 8·2–8·6%. The galactoarabinoxylan travelled discretely on zone electrophoresis on silica gel and a Procion dyed derivative travelled likewise on cellulose acetate films.[8] The single symmetrical Schlieren peak obtained on ultracentrifugation of a solution of the galactoarabinoxylan was compatible with the material being pure but having a high degree of polymolecularity or of polydispersity, or of both. The galactoarabinoxylan was examined by free-boundary electrophoresis in a Tiselius–Svensson type apparatus. When a phosphate buffer was used, a single symmetrical boundary was obtained and the polysaccharide appeared homogeneous with respect to charge, the charge presumably being due to the acidic sugars. When a borate buffer was used, the boundary exhibited maxima corresponding to two components moving at nearly the same rate. The stationary boundaries formed on each occasion were pronounced. With a borate buffer, complex formation occurred and the migration depended both on the extent of complex formation and on the uronic acid residues.

Fourteen sugars were detected on paper chromatographic examination of a partial acid hydrolysate of the galactoarabinoxylan. Nine neutral and eight acidic components were isolated by a combination of PC and anion-exchange chromatography. In addition to xylose, arabinose, and galactose, the $\beta(1 \rightarrow 4)$ di, tri-, tetra-, and pentasaccharides of D-xylose

[6] A. G. NORMAN, *Biochem. J.* **23**, 1353 (1929).
[7] J. S. G. REID and K. C. B. WILKIE, *Phytochem.* **8**, 2053 (1969).
[8] W. F. DUDMAN and C. T. BISHOP, *Can. J. Chem.* **46**, 3079 (1968).

were present in the ratio of 6:3:2:- and a galactosylarabinose and either a xylosylgalactosylarabinose or a galactosylxylosylarabinose in the ratio to those earlier of 1:trace; the trisaccharide was probably the latter. The galactosylarabinose gave a positive reaction with triphenyltetrazolium chloride indicative of the glycosidic linkage not being (1→2).[9] It is probable that the disaccharide is 5-*O*-β-D-galactopyranosyl-L-arabinose, a disaccharide isolated from the partial hydrolysate of a maize hull acidic galactoarabinoxylan.[10] This would be compatible with the results of methylation analysis. The triheterosaccharide containing galactose, xylose and arabinose residues gave no reaction with triphenyltetrazolium chloride[9] and it gave arabinitol on reduction and hydrolysis. A separate partial acid hydrolysis of a pure acidic galactoarabinoxylan from the same oat plantstuff was carried out and a xylosylarabinose was found. The various facts indicate that the triheterosaccharide was a galactosylxylosyl-(1→2)-arabinose, possibly L-galactosyl-(1→4)-D-xylopyranosyl-(1→2)-L-arabinose which has been isolated from maize hull fibre[11] or the D-galactosyl diastereoisomer which has been isolated from perennial rye grass roots.[12] The isolation of the disaccharide and of the trisaccharide both proved the presence of non-terminal L-arabinose residues. The isolation of the trisaccharide showed that all three sugar residues were present in the same hemicellulosic molecules. Further studies are being carried out to determine whether or not L-galactosyl residues are present.

The eight compounds isolated were thus D-glucuronic acid, 4-*O*-methyl-D-glucuronic acid, 4-*O*-methyl-D-glucopyranuronosyl-(1→2)-D-xylose, 4-*O*-methyl-D-glucopyranuronosyl-(1→2)-D-xylopyranosyl-(1→4)-D-xylopyranose, D-glucopyranuronosyl-(1→2)-D-xylopyranosyl-(1→4)-D-xylose, D-glucopyranuronosyl-(1→2)-D-xylose, and D-glucopyranuronosyl-(1→4)-D-xylose. The last two disaccharides were not successfully separated. D-Glucopyranuronosyl-(1→4)-D-xylose has not been isolated from a hydrolysate although its presence has been deduced.[13] Another aldotriouronic acid was also isolated. It contained galactose, xylose, and glucuronic acid residues. This trisaccharide was probably the same as that isolated by Falconer and Adams[14] from oat hulls and by Reid and Wilkie[7] from oat leaf acidic galactoarabinoxylan.

The oat stem galactoarabinoxylan was methylated successively by the methods of Hakomori,[15] Haworth,[16] Kuhn,[17] and Purdie.[18] From 1 g of hemicellulose 850 mg of a methylated product was obtained which had OMe 33·6% and exhibited slight absorption in its IR spectrum attributable to free hydroxyl groups. The product was sub-fractionated by dissolution in mixtures of chloroform and light petroleum. The material soluble in 20% chloroform exhibited no peak in its IR spectrum of the above type and it had OMe 39·0%. This material accounted for 50% of that subjected to fractionation and it is hereafter referred to as the methylated galactoarabinoxylan. Ultracentrifugal examination indicated that the material was pure and not polydiverse.[2] The methylated galactoarabinoxylan was

[9] D. S. Feingold, G. Avigad and S. Hestrin, *Biochem. J.* **64**, 351 (1956).
[10] H. C. Srivastava and F. Smith, *J. Am. Chem. Soc.* **79**, 982 (1957); I. J. Goldstein, F. Smith and H. C. Srivastava, *J. Am. Chem. Soc.* **79**, 3858 (1957).
[11] R. L. Whistler and W. M. Corbett, *J. Am. Chem. Soc.* **77**, 6328 (1955).
[12] G. O. Aspinall, I. M. Cairncross and K. M. Ross, *J. Chem. Soc.* 1721 (1963).
[13] R. L. Whistler and L. Hough, *J. Am. Chem. Soc.* **75**, 4918 (1953).
[14] E. L. Falconer and G. A. Adams, *Can. J. Chem.* **34**, 338 (1956).
[15] S. Hakomori, *J. Biochem. Tokyo* **55**, 205 (1964).
[16] W. N. Haworth, *J. Chem. Soc.* **107**, 8 (1915).
[17] R. Kuhn, H. Trischmann and I. Löw, *Angew Chem.* **67**, 32 (1955).
[18] T. Purdie and J. C. Irvine, *J. Chem. Soc.* **83**, 1021 (1903).

found by osmometry to have $M_n$ 18 100 equivalent to $M_n$ 14 570 for the parent unmethylated material which must accordingly have had D.P. *ca.* 106.

The components in an acid hydrolysate of the methylated galactoarabinoxylan were separated by a combination of TLC and PC. The components were identified as 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3,4,6-tetra-*O*-methyl-D-galactose, 3,5-di-*O*-methyl-L-arabinose, 2,3-di-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-L-arabinose, 2-*O*-methyl-D-xylose, 3-*O*-methyl-D-xylose, 2,3,4-tri-*O*-methyl-D-glucuronic acid. Quantitative determinations were carried out by GLC of the acetates of the derived glycitols by measurement of peak areas; by this method, each component gives only one peak. The method of Björndal *et al.*[19] was modified to minimize losses of volatile compounds. The first six of the above methylated and partly methylated sugars were present in the peak area ratios of 2·7:1:0·36:0·20:5·4:0·13. A mixture of the mono-*O*-methyl-D-xyloses was present in ratio to these values of 2·2. Four unidentified components were detected by GLC. Together they accounted for less than 1% of the material. A sample of the methylated galactoarabinoxylan was methanolysed and the products quantitatively determined by GLC. The first five of the above neutral sugars were present in the peak area ratios of 2·6:1:0·2:0·1:5·0 and 2-*O*-methyl-D-xylose was present in ratio to these values of 2·3. For neither set of GLC quantitative determinations were molar response coefficients determined. In the case of the methanolysate, this would have led to the underestimation of the less volatile derivatives with lower degrees of substitution. The assertion[20] that it is unnecessary to calibrate the detector for *relative* quantitative results is not necessarily true.[21] Björndal *et al.*[22] have concluded that the response coefficients for the partially methylated glycitol acetates are similar and have assumed that the errors introduced by ignoring these factors are probably much smaller than errors due to degradation during hydrolysis and losses due to volatility.

During the methylation of any polysaccharide, there are invariably losses and it is highly unlikely that the material finally isolated represents the parent material in all aspects of polymolecularity and polydispersity.[2] However, all the structural features of pure parent polysaccharides will be represented in methylated products, although the proportions may be distorted by the fractionation that incidentally accompanies methylation. The parent oat galactoarabinoxylan had a slightly lower D.P. than did the methylated derivative as would be expected if material of lower molecular weight or of a higher degree of branching were preferentially lost during the methylation procedures because of the relatively higher solubility of such materials.

A sample of the galactoarabinoxylan was oxidized with aqueous $NaIO_4$ and after dialysis, the non-diffusible material was reduced with $NaBH_4$ and the derived polyalcohol hydrolysed. Arabinose, galactose, glucose, xylose, threitol, glycerol, and a trace of glycoaldehyde were detected on paper chromatograms. Derived peracetates of ethylene glycol, glycerol, threitol, rhamnitol, arabinitol, xylitol, and galactitol were detected by GLC in the molar ratios of 0·7:10:0·4:trace:1·3:7·6:0·7. Dutton *et al.*[23] have shown that the glycolaldehyde, derived from C1 and C2 of most residues of the polyalcohol, is almost entirely destroyed during hydrolysis of the polyalcohol. Glyceric acid and 3-*O*-methylerythronic

[19] H. BJÖRNDAL, B. LINDBERG and S. SVENSSON, *Acta Chem. Scand.* **21**, 1801 (1967).
[20] C. T. BISHOP, *Adv. Carbohyd. Chem.* **19**, 95 (1964).
[21] R. J. YU, C. T. BISHOP, F. P. COOPER and H. J. HASENCLEVER, *Can. J. Chem.* **45**, 2206 (1967).
[22] H. BJÖRNDAL, C. G. HELLERQVIST, B. LINDBERG and S. SVENSSON, *Angew. Chem. Intern. Edn.* **9**, 610 (1970).
[23] G. G. S. DUTTON, K. B. GIBNEY, G. D. JENSEN and P. E. REID, *J. Chromat.* **36**, 152 (1968).

acid were the expected products from the glucuronic acid and 4-*O*-methylglucuronic acid residues respectively. Attempts to acetylate glyceric acid, under the conditions employed in the above analysis, failed to give any recognisable products. It appeared that the above procedure resulted in the degradation of the acidic material. There was no evidence of the formation of hemiacetal linkages of the type proposed by Painter and Larsen[24] in studies on maize cob xylan. Precautions were taken to avoid losses of volatile acetates such as ethylene glycol diacetate.

A sample of reduced galactoarabinoxylan was hydrolysed and examined on paper chromatograms. The only glycitol detected was xylitol. On periodate oxidation of the reduced galactoarabinoxylan the amount of HCHO released by the terminal xylitol residues indicated the hemicelluloses had D.P. *ca.* 93.

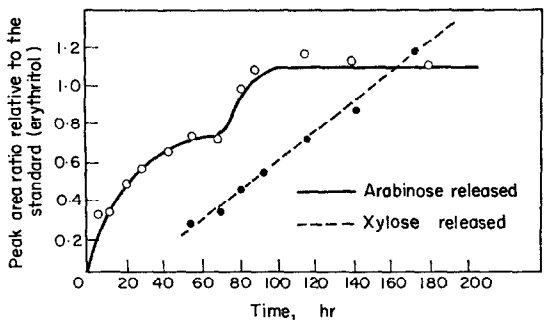



FIG. 1. RELEASE OF L-ARABINOSE AND D-XYLOSE ON CONTROLLED ACID HYDROLYSIS OF THE GALACTO-ARABINOXYLAN FROM OAT STEM.

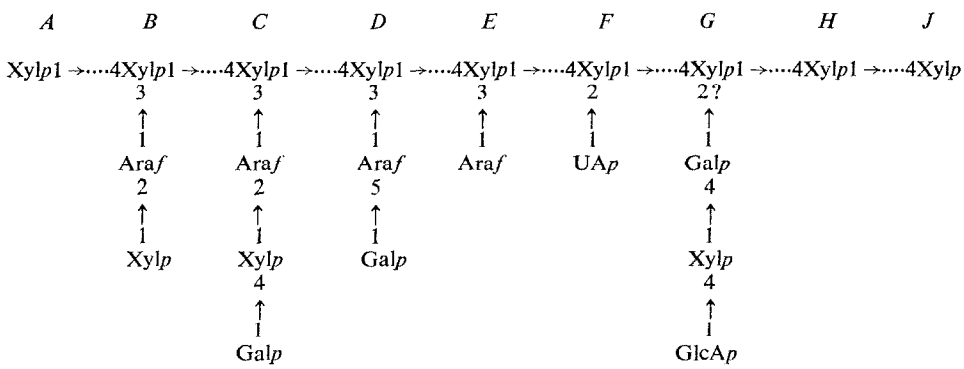
A sample of the galactoarabinoxylan was subjected to controlled acid hydrolysis and the trimethylsilyl derivatives of the arabinose and xylose released were quantitatively determined by GLC. The clear discontinuity in the arabinose curve (Fig. 1) indicated that the arabinose residues were linked in more than one way; a repeat determination gave the same results.

## DISCUSSION

From the above results, it is clear that the oat galactoarabinoxylan has contiguous $\beta(1\rightarrow4)$ linked xylopyranosyl residues. The majority of the xylose residues carry one or other of the following side-residues: (i) glucuronic acid and to a lesser extent 4-*O*-methyl-glucuronic acid directly attached to C2 positions; (ii) an aldotriouronic acid residue probably a glucopyranuronosyl-$(1\rightarrow4)$-xylopyranosyl-$(1\rightarrow4)$-galactopyranose,[14] attached to either the C2 or C3 position (more probably the former) through the terminal galactopyranose residue; and (iii) four groups of residues involving attachment of L-arabinofuranosyl residues directly to the main xylan chain. Side-residues in this last group are attached to C3 positions and are of four types: $\alpha$-L-arabinofuranosyl, galactopyranosyl-2-*O*-D-xylo-pyranosyl-L-arabinofuranosyl (possibly 4-*O*-$\beta$-L-galactopyranosyl-2-*O*-$\beta$-D-xylopyranosyl-L-arabinofuranosyl[11]), 5-*O*-$\beta$?-D-galactopyranosyl-L-arabinofuranosyl,[10] and 2-*O*-$\beta$?-D-xylo-pyranosyl-L-arabinofuranosyl residues.

[24] T. J. PAINTER and B. LARSEN, *Acta Chem. Scand.* **24**, 2366 (1970).

These structural features are shown in Fig. 2 and are denoted *A-J*. The proportions of arabinose, galactose, and xylose residues in the galactoarabinoxylan were found to be 26·5, 8·1, and 65·6 mol% respectively and the uronic anhydride was 8·5 mol%. Assuming that all of the uronic acid was present as aldobiouronic acid in hydrolysates the recalculated composition of the galactoarabinoxylan was arabinose (22%), galactose (6·7%), uronic acid (8·5%), and xylose (63%). The D.P. calculated on the basis of the HCHO released on $IO_4^-$ oxidation was 93 but substitution at position C2 of the terminal reducing end-residue rendered it stable to oxidation. If it is assumed that all of the uronic acid residues were randomly distributed along the xylan chain, then 1 in 7·4 of the xylose residues would




Residues—Ara, L-arabinosyl; Gal, D-galactosyl + possibly L-galactosyl at feature C; Glc, D-glucosyl; GlcA, D-glucuronosyl; U, 4-*O*-methyl-D-glucuronosyl and D-glucuronosyl; Xyl, D-xylosyl; *f*, furanose ring; *p*, pyranose ring.

FIG. 2. STRUCTURAL FEATURES OF THE GALACTOARABINOXYLAN MOLECULES FROM OAT STEM.

carry a uronic acid residue at C2, either directly or indirectly. The D.P., on the basis of this assumption, was re-evaluated as 80. Not all the xylose residues were present in the main chain. Some were in structural features *B*, *C* and *G* and this fact is taken into account in the calculation presented later. For a molecule having D.P. 80 the number of arabinose, galactose, uronic acid, and xylose residues is 17·5, 5·4, 6·8 and 50·3 respectively.

The proportions of the various components present in the reduced hydrolysate obtained after the Smith degradation of the galactoarabinoxylan were adjusted to allow for the undetected products derived from the 6·8 uronic acid residues. The average number of molecules of ethylene glycol, glycerol, threitol, arabinitol, xylitol, and galactitol was 2·6, 36·0, 1·6, 4·6, 26·1, and 2·5 respectively for a molecule of D.P. 80.

From the various results obtained by the study of unmethylated material, it is possible to deduce (see Experimental) the average values for the various structural features shown in Fig. 2. On average, the molecules of the acidic galactoarabinoxylan are of D.P. 80. They have 12·6 L-arabinofuranosyl residues directly attached to the xylan chain and, of the remaining 4·9 arabinose residues, 2·0 are contained in 2-*O*-D-xylopyranosyl-L-arabinofuranosyl residues, 2·6 in 4-*O*-β-L ?-galactopyranosyl-2-*O*-β-D-xylopyranosyl-L-arabinofuranosyl residues, and 0·3 in 5-*O*-β-D-galactopyranosyl-L-arabinofuranosyl residues. There is no proof of the presence of any L-galactose residues in this galactoarabinoxylan but by analogy with the acidic galactoarabinoxylan from maize hulls,[11] it is possible that the

galactose residues in the galactosylxylosylarabinosyl side chain are of the L enantiomer. Further evidence of the way in which the arabinose residues are linked was obtained by the isolation of 3,5-di-*O*-methyl-L-arabinose and 2,3-di-*O*-methyl-L-arabinose. Furthermore, approximately two thirds of the L-arabinofuranosyl residues are readily released on acid hydrolysis (Fig. 1); results which accord very well with the proposed structure. In the case of the uronic acid residues, 4·3 are directly attached to the xylan chain, and the remaining 2·5 residues are in D-glucopyranuronosyl-(1→4)-D-xylopyranosyl-(1→4)-D-galactopyranosyl residues. The nature of the glycosiduronic linkage was obtained from results of the methylation analysis of the aldobiouronic acids produced on partial acid hydrolysis. The nature of the galactose linkage was based on the tentative identification of 2,3,6-tri-*O*-methyl-D-galactose in methanolysates of the methylated galactoarabinoxylan. There was no evidence of the structural role of the rhamnose residues although it was remarked that some of these residues survived periodate oxidation. There was no evidence of the presence of doubly substituted xylose residues. The galactoarabinoxylan is obviously very highly branched: 1 in every 1·8 residues on the main chain carry substituent residues or chains. This conclusion is supported by the observation that the galactoarabinoxylan does not form an iodine complex in aqueous calcium chloride whereas the less substituted acidic arabinoxylan isolated by Aspinall and Wilkie[5] from oat straw does. There is little evidence on the distribution of side residues or side chains. After partial acid hydrolysis, $\beta(1\rightarrow4)$ linked oligomers of D-xylopyranose were isolated. This is compatible both with the uronic acid residues being spaced well apart and with them being grouped together.

All higher land plants have D-xylans in their non-endospermic cell-walls and all those have backbones of $\beta(1\rightarrow4)$ linked D-xylopyranosyl residues.[25] Only one homoxylan, from esparto grass,[26] has been isolated. Normally the xylans isolated from the Gramineae are heteroglycans having L-arabinofuranosyl residues attached to C3 positions of the D-xylan chains and 4-*O*-methyl-D-glucuronosyl, and D-glucuronosyl, residues on C2 positions. Early reports that the acidic residues are attached to C3 positions have not been substantiated in later studies.[5] Heteroxylans isolated from wheat straw display an interesting diversity.[27–33] Studies on the total hemicelluloses from the non-endospermic tissues of the oat plant make it clear that the degree of plant maturity is markedly associated with differences in hemicellulosic composition.[1] Normally there is a much higher proportion of L-arabinosyl residues in the parent hemicellulosic material than there is in the pure heteroxylan finally isolated. There is no evidence in mature tissues of an arabinan of pectic or other type. Giant star grass,[34] perennial rye grass,[35] and cocksfoot grass,[36] are of interest as the proportion of L-arabinosyl residues is high.

D-Galactosyl residues are integral parts of a few heteroxylans isolated. These residues

[25] G. O. Aspinall, E. E. Percival, D. A. Rees and M. Rennie, in *Rodd's Chemistry of Carbon Compounds* (edited by S. Coffey), Vol. 1F, p. 670, Elsevier, Amsterdam (1967).
[26] S. K. Chanda, E. L. Hirst, J. K. N. Jones and E. G. V. Percival, *J. Chem. Soc.* 1289 (1950).
[27] I. Ehrenthal, R. Montgomery and F. Smith, *J. Am. Chem. Soc.* **76**, 5509 (1954).
[28] G. O. Aspinall and R. S. Mahomed, *J. Chem. Soc.* 1731 (1954).
[29] A. Roudier, *Compt. Rend.* **237**, 840 (1953).
[30] A. Roudier, *Compt. Rend.* **248**, 1432 (1959).
[31] G. O. Aspinall and E. G. Meek, *J. Chem. Soc.* 3830 (1956).
[32] G. A. Adams, *Can. J. Chem.* **30**, 698 (1952).
[33] C. T. Bishop, *Can. J. Chem.* **31**, 134 (1953).
[34] G. O. Aspinall and I. M. Cairncross, *J. Chem. Soc.* 3877 (1960).
[35] M. Alam and R. J. McIlroy, *J. Chem. Soc.* **C**, 1577 (1967).
[36] G. O. Aspinall and R. J. Sturgeon, *J. Chem. Soc.* 4469 (1957).

have been noted in heteroxylans from perennial rye grass roots,[12] and maize stalk,[37] cobs,[38] gum[39] and hulls.[10] They are also present in the heteroxylan isolated from oat leaf.[7] Features *D* and *C* in Fig. 2 were earlier noted in heteroxylans from maize hulls isolated by Whistler *et al.*[11] and by F. Smith *et al.*[10] Non-terminal L-arabinofuranosyl residues are present not only in the galactoarabinoxylans from oat stem but in the heteroxylans from barley husks,[40,41] perennial rye grass roots,[12] wheat bran,[42–44] esparto grass,[45,46] and many maize tissues.[38,10] Non-terminal galactosyl residues occur not only in oat stem and leaf galactoarabinoxylans but in the heteroxylans from oat hull[47] and brome grass.[48]

TABLE 1. HEMICELLULOSIC MATERIALS DERIVED FROM THE WATER-SOLUBLE FRACTION OF THE OAT STEM TOTAL HEMICELLULOSE BY FRACTIONATION ON A DEAE-CELLULOSE COLUMN

| Irrigant | Wt (g) | Acidic sugars | Galactose | Glucose | Xylose | Rhamnose |
|---|---|---|---|---|---|---|
| | | | molar ratios (arabinose = 1) | | | |
| $H_2O$ | 0·89 | + | 0·5 | 1·5 | 2·1 | trace |
| 0·1 M KOAc | 1·23 | + | 0·2 | 0·05 | 1·5 | trace |
| 0·5 M KOAc | 2·60 | + | 0·3 | trace | 2·6 | trace |
| 5·0 M KOAc | 1·30 | + | 0·3 | 0·1 | 3·0 | trace |

As has already been stated,[2,49,50] the methods of isolating hemicelluloses are so varied that comparative studies based on present data are of very doubtful value. In earlier studies on oats by Aspinall and Wilkie,[5] no hemicellulose rich in L-arabinosyl residues was isolated nor was any hemicellulose having galactosyl residues detected. It is therefore desirable to re-examine many plants, not only members of the Gramineae, to determine how widespread are galactoarabinoxylans of the type now reported.

## EXPERIMENTAL

*General methods.* PC was on Whatman No. 1 and 3MM papers, and TLC on silica gel (Kieselgel G or GF). The irrigants (v/v) were: *A*, EtOAc–pyridine–$H_2O$ (72 : 20 : 23); *B*, EtOAc–HOAc–$HCO_2H$–$H_2O$ (18 : 3 : 1 : 4); *C*, EtOAc–HOAc–$H_2O$ (3 : 1 : 3); *D*, *n*-BuOH–EtOH–$H_2O$–$NH_4OH$ (4 : 1 : 5 : trace); *E*, MeCOEt–$H_2O$–$NH_4OH$ (10 : 1 : trace); *F*, benzene–EtOH–$H_2O$–HOAc (200 : 47 : 15 : 1); *G*, n-BuOH–pyridine–benzene–$H_2O$ (5 : 3 : 1 : 3); *H*, *n*-BuOH–HOAc–$H_2O$ (5 : 1 : 4). Spray reagents were *p*-anisidine HCl, alkaline $AgNO_3$, 2,3,5-triphenyltetrazolium chloride, or 1-naphthol–$H_2SO_4$. A Perkin–Elmer F11 gas chromatograph was used for GLC. The columns (2 m × 3 mm i.d.) contained *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); *C*, 3% E301 on AW-HMDS celite (80–100 mesh); and *D*, 8% butan-1,4-diol succinate on AW-HMDS Chromosorb W (80–100 mesh). The nitrogen carrier gas flow-rate was 50–80 ml/min. Electrophoretic examination of polysaccharides was carried out by the method of Reid and Wilkie[7] on TLC plates

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coated with Kieselgel G sprayed with 0·05 M borate or on cellulose acetate using polysaccharides which had been dyed with Procion Red M-GS.[8] Free-boundary electrophoresis was carried out using a Tiselius–Svensson type apparatus (supplied by LKB) at 4°. Sedimentation analyses were carried out in a Beckman Model E analytical ultracentrifuge using a titanium rotor with 12 mm sector-shaped cells at speeds of up to 48 000 rpm. Hemicellulosic samples were hydrolysed in sealed tubes by heating with 0·5 M $H_2SO_4$ (12–16 hr at 100°); the hydrolysates were neutralised with $BaCO_3$ and, where appropriate, Zeo-Karb 325 ($H^+$) was used prior to examination for acidic sugars.

*Isolation of the total hemicellulose.* Oat plants (var. Blenda) were harvested at Sunnybrae Farm, Aberdeenshire, in mid-August 1968. The aerial tissues were immersed in boiling EtOH, air-dried, and stored below 0° until required. The stem tissue between the inflorescence and the first node was separated and the total hemicellulose isolated by the method of Reid and Wilkie;[2] 36 g of total hemicellulose was isolated from 170 g of air-dried plant tissue.

*Fractionation of the total hemicellulose.* An acidic galactoarabinoxylan was isolated by the method of Reid and Wilkie.[7] Total hemicellulose (26 g) was dispersed in $H_2O$ and the water-soluble fraction (*S*; 16·0 g) and a water-insoluble fraction (*I*; 9·1 g) were obtained after centrifugation and freeze-drying. Fraction *S* was fractionated on a column of DEAE-cellulose (Whatman DE 11-acetate form; 250 g) using $H_2O$ (4 l.) and then 0·1, 0·5, and 5 M KOAc (3 l. of each) as eluents; hemicellulosic materials *a*, *b*, *c* and *d*, were isolated respectively. Fraction *I* on hydrolysis yielded arabinose, xylose and glucose in the molar ratios of 2 : 6 : 1 and traces of galactose and rhamnose.

*Examination of hemicellulosic material* c. Previous work[7] has shown that fractions of similar composition, isolated in the way described above, cannot normally be sub-fractionated. On treatment with Fehling's solution no precipitate was formed and the material did not form an insoluble iodine complex in saturated calcium chloride solution.[51] On ultracentrifugal examination the material sedimented as a single symmetrical peak. Zone electrophoresis of the material on thin-layers of silica gel[7] and of a Procion-dyed derivative on cellulose acetate each showed that the materials migrated as single compact zones. On free-boundary electrophoresis using a phosphate buffer (0·008 M $NaH_2PO_4$–0·06 M $Na_2HPO_4$; pH 7·6), a single symmetrical boundary (mobility $-3{\cdot}45\ 10^5 \times cm^2V^{-1}\ sec^{-1}$) was obtained. Using a 0·05 M borate buffer (pH 9·3) it was possible to distinguish two peaks of very similar mobility ($-5{\cdot}50$ and $-4{\cdot}30\ 10^5 \times cm^2V^{-1}\ sec^{-1}$) after prolonged electrophoresis. The molar ratio of arabinose, galactose, and xylose (10 : 3 : 25) was determined by GLC (column *A*) of the derived glycitol acetates[52] and the uronic anhydride[53] was 8·2–8·6% (depending on the proportion of glucuronic acid and 4-*O*-methylglucuronic acid). The hemicellulosic material had $[\alpha]_D^{25}$ −59·4° (*c*. 1·67 in 2·5 M NaOH).

*Partial acid hydrolysis of the acidic galactoarabinoxylan.* A sample (500 mg) of the acidic galactoarabinoxylan was heated with 0·05 M $H_2SO_4$ (20 ml; 100° for 45 min). The neutralised ($BaCO_3$), deionized (Zeo-Karb 325) hydrolysate was examined by paper chromatography (irrigant *B*). In addition to arabinose, galactose, and xylose there were at least ten other components. The hydrolysate was fractionated into neutral components (eluted with $H_2O$) and acidic components (eluted with 30% HOAc) on a column of Deacidite FF-IP (acetate form).

*Examination of the neutral fraction.* Examination by PC (irrigants *A*, *B*, and *C*) showed the presence of arabinose, galactose, xylose, and at least six other components. They had $R_{xylose}$ (irrigant *B*) 0·36,0·30, 0·26, 0·14, 0·06 and 0·01; relative proportions (visual estimation with alkaline $AgNO_3$) were 6 : 1 : trace : 3 : 2 : - respectively. The material was separated into six components by PC (irrigant *B*). *Component* 1 showed no evidence of heterogeneity on PCs (irrigants *A*, *B* and *C*) and was indistinguishable from an authentic sample of xylobiose. On hydrolysis xylose alone was released. A sample was reduced with $NaBH_4$ and after 12 hr the excess of $NaBH_4$ was destroyed by the addition of HOAc. The $H_3BO_4$ produced was removed by codistillation with successive vols. of MeOH. Hydrolysis of the product yielded xylose and xylitol. The hydrolysate was acetylated ($Ac_2O$–NaOAc) in a sealed tube (120°; 4 hr) and the products on examination by GLC (column *A*) gave components, indistinguishable from those prepared by acetylating a mixture of xylose, and xylitol, in the molar ratio of 1 : 1. *Component* 2 was homogeneous on PCs (irrigants *B* and *C*). Arabinose and galactose were released on hydrolysis and a sample of the oligosaccharide, on reduction with $NaBH_4$ and hydrolysis, gave galactose and arabinitol. GLC of the derived acetates gave products, indistinguishable from those prepared from galactose and arabinitol, in the molar ratio *ca.* 1 : 1. *Component* 3 was homogeneous when examined by PC (irrigants *B* and *C*). Acid hydrolysis gave arabinose, galactose, and xylose and GLC of the derived TMS ethers showed that the sugars were present in *ca.* equimolar amounts. A sample of the oligosaccharide on reduction with $NaBH_4$ and hydrolysis gave galactose, xylose, and arabinitol. GLC of the derived peracetates gave products indistinguishable from those prepared from galactose, xylose, and arabinitol, in the molar ratio *ca.* 1 : 1 : 1. Component 3 did not form a formazan derivative with 2,3,5-triphenyltetrazolium chloride. *Component* 4 was homogeneous on PCs (irrigants *A*, *B* and *C*) and was indistinguishable from xylotriose. Acid hydrolysis gave xylose alone. A sample, after

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reduction with $NaBH_4$ and hydrolysis, gave xylose and xylitol. GLC of the derived acetates showed that the molar ratio of xylose to xylitol was 2·01 : 1. *Component* 5 was homogeneous on PCs (irrigants *A*, *B* and *C*) and was indistinguishable from xylotetraose. Acid hydrolysis gave xylose alone. A sample, after reduction with $NaBH_4$ and hydrolysis, gave xylose and xylitol and GLC of the derived acetates showed that these sugars were present in the molar ratio of 3·07 : 1 respectively. *Component* 6 was present only in trace amount and was chromatographically similar to xylopentaose (irrigants *B* and *C*). Xylose alone was released on hydrolysis. A sample, after reduction with $NaBH_4$ and hydrolysis, was acetylated. GLC showed the presence of the acetates of xylose and xylitol in the molar ratio of 4·5 : 1. Evidently component 6 was xylopentaose contaminated with oligomer homologues of higher D.P.

*Examination of the acidic fraction.* PC showed the presence of seven clearly separated components and some material of low mobility. The components had $R_{xylose}$ (irrigant *B*) 1·21, 0·67, 0·64, 0·37, 0·27, 0·15 and 0·10, in the relative proportions (estimated visually with alkaline $AgNO_3$), trace : 10 : 1 : trace : 10 : 5 : 1 respectively. The material of $R_{xylose}$ 0·27 was later found to be a mixture of two components. The acidic fraction was separated by PC (irrigant *B*). A sample of each component was hydrolysed with 1 M $H_2SO_4$ at 100° for 16 hr and the monosaccharides estimated visually on PCs treated with alkaline $AgNO_3$. *Component* 1 was indistinguishable from 4-*O*-methyl-D-glucuronic acid (PC; irrigants *B* and *C*). *Component* 2 was indistinguishable from D-glucuronic acid (PC; irrigants *B* and *C*). *Component* 3 was homogeneous on PCs (irrigants *B* and *C*) and was indistinguishable from an authentic sample of 2-*O*-(4-*O*-methyl-α-D-glucopyranuronosyl)-D-xylose. Acid hydrolysis gave xylose and 4-*O*-methylglucuronic acid in the ratio *ca.* 1 : 1. *Component* 4 was homogeneous on PC (irrigants *B* and *C*) and was indistinguishable from an authentic sample of *O*-α-4-*O*-methyl-D-glucopyranuronsyl-(1 → 2)-*O*-β-D-xylo-pyranosyl-(1 → 4)-D-xylose. Acid hydrolysis gave xylose and 4-*O*-methylglucuronic acid in the ratio *ca.* 2 : 1. *Components* 5 *and* 6 travelled together on PC (irrigants *B* and *C*). A sample, on hydrolysis, released xylose and glucuronic acid in the ratio *ca.* 1 : 1. The remaining material was boiled under reflux with 4% MeOH–HCl for 12 hr, neutralized ($Ag_2CO_3$) and the filtrate treated with $NaBH_4$ for 12 hr. The excess of $NaBH_4$ was destroyed by the addition of Zeo-Karb 325 ($H^+$) and the borate was removed as described previously. A sample, on hydrolysis, released xylose and glucose in the ratio *ca.* 1 : 1. The remainder of the reduced material was methylated successively by the methods of Kuhn[17] and Purdie.[18] The IR spectrum of the product showed only trace absorption at *ca.* 3500 $cm^{-1}$. The material was treated with 4% MeOH–HCl (100°; 16 hr) and the products examined by GLC (columns *A* and *B*). The methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4-, and 2,3-, di-*O*-methyl-D-xyloses were detected in the ratio 1·01 : 1. The glycosides were hydrolysed and the products examined by PC (irrigant *F*); 2,3,4,6-tetra-*O*-methyl-D-glucose, 3,4-, and 2,3-, di-*O*-methyl-D-xyloses were detected. *Component* 7 was homogeneous when examined by PC (irrigants *B* and *C*). A sample, on hydrolysis, released xylose and glucuronic acid in the ratio *ca.* 2 : 1. The remainder of the material was treated with an excess of $CH_2N_2$ in ether and the product was reduced with $NaBH_4$ and methylated as described above. GLC of the methanolysate (columns *A*, *B* and *D*) showed the presence of methyl 2,3,4,6-tetra-*O*-methyl-D-glucosides, and methyl 2,3-, and methyl 3,4-, di-*O*-methyl-D-xylosides in the ratio *ca.* 1 : 2. The methyl glycosides were hydrolysed and the products examined by PC (irrigant *F*); 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3-, and 3,4-, di-*O*-methyl-D-xyloses were detected. *Component* 8 was homogeneous on PCs (irrigants *B* and *C*). A sample, on acid hydrolysis, released galactose, xylose, and glucuronic acid in the ratio *ca.* 1 : 1 : 1. The attempted methylation of the reduced aldotriouronic acid was unsuccessful. Material of low chromatographic mobility was noted on PCs. Xylose and glucuronic acid were released on hydrolysis. The material was most probably a mixture of oligouronic acids of higher D.P.

*Controlled acid hydrolysis of the acidic galactoarabinoxylan.* A sample (*ca.* 100 mg) of the galactoarabinoxylan was dissolved in 0·025 M $H_2SO_4$ (50 ml) and erythritol (40 mg) was added. The solution was incubated at 40° and aliquots (2·5 ml) were withdrawn at intervals. The aliquots were taken to dryness and the TMS derivatives prepared. GLC examination (column *C*) revealed components corresponding to those produced by the TMS derivatives of erythritol, L-arabinose, and D-xylose. The ratios of arabinose and of xylose to the standard were calculated for each aliquot. The arabinose release was complex; a curve of release against time possessed a reproducible step after *ca.* 40 hr. The xylose release was nearly linear in relation to time. After *ca.* 200 hr the arabinose release became constant and the polymeric material remaining was isolated by dialysis, after neutralization ($BaCO_3$) of the reaction mixture. The polymeric material, on hydrolysis, released mainly xylose.

*Methylation of the acidic galactoarabinoxylan.* A sample (1 g) of the galactoarabinoxylan was methylated successively by the methods of Hakomori,[15] Haworth,[16] Kuhn[17] and Purdie.[18] The methylated material showed only trace absorption in its IR spectrum (KBr disc), attributable to hydroxyl and had a methoxyl content of 33·6%. The methylated material (850 mg) was fractionated by extraction with mixtures of $CHCl_3$ and light petroleum progressively increasing in the proportion of $CHCl_3$. Up to 15% $CHCl_3$ a total of *ca.* 15% of the material dissolved and was not further investigated. Further treatment with 20 and 30% $CHCl_3$ extracted 48 and 27% respectively of the material. The major fraction showed negligible absorption at *ca.* 3500 $cm^{-1}$ in the IR spectrum and had a methoxyl content of 39·0%. The methylated galactoarabinoxylan had $[\alpha]_D^{24}$ −75·0° (*c.* 2·3 in $CHCl_3$).

*Examination of the methylated acidic galactoarabinoxylan.* On ultracentrifugal examination the material, in $CHCl_3$, sedimented as a single symmetric peak. The number-average MW, determined using a Hewlett–Packard membrane osmometer model 501, was found to be *ca.* 18 100 corresponding to a $D.P._n$ of 106. A sample (*ca.* 400 mg) of the methylated galactoarabinoxylan was hydrolysed by the $HCOOH–H_2SO_4$ method, the hydrolysate was neutralised ($BaCO_3$), deionized (Zeo-Karb 325) and evaporated to dryness. The mixture was separated into eight fractions (TLC; irrigant *F*) which were further purified by TLC (irrigants *E* and *F*) or PC (irrigants *D* and *E*). *Component* 1 was indistinguishable (TLC; irrigants *D*, *E* and *F*) from 2,3,5-tri-*O*-methyl-L-arabinose. The material was treated with 4% MeOH–HCl (100°; 16 hr) in a sealed tube. GLC examination (columns *A*, *B* and *D*) of the neutralised ($Ag_2CO_3$) product revealed components corresponding in retention time and relative area to those produced by the methyl glycosides of 2,3,5-tri-*O*-methyl-L-arabinose. Demethylation with $BCl_3$[54] and PC (irrigant *A*) gave arabinose alone. *Component* 2 was homogeneous on TLC (irrigants *D*, *E* and *F*) and was indistinguishable from 2,3,4-tri-*O*-methyl-D-xylose. The derived methyl glycosides, on GLC examination, were identical in retention time and relative area to those produced by the methyl glycosides of 2,3,4-tri-*O*-methyl-D-xylose. Demethylation with $BCl_3$ yielded only xylose. *Component* 3 was homogeneous on TLC (irrigants *D*, *E* and *F*) and was indistinguishable from 2,3,4,6-tetra-*O*-methyl-D-galactose. Conversion to the methyl glycosides yielded products indistinguishable by GLC from methyl 2,3,4,6-tetra-*O*-methyl-D-galactosides. *Component* 4 was homogeneous on TLC (irrigants *D*, *E* and *F*) and was indistinguishable from 3,5-di-*O*-methyl-L-arabinose. GLC examination (columns *A* and *B*) of the derived methyl glycosides showed the presence of two peaks corresponding in retention time and relative size to those produced by methyl 3,5-di-*O*-methyl-L-arabinosides. Traces of the methyl glycosides of 2,3-di-*O*-methyl-D-xylose were also detected. Demethylation of the methyl glycosides with $BCl_3$ gave only arabinose. *Component* 5 had m.p. 79–81° (lit.,[55] 81–82°), was homogeneous when examined by TLC (irrigants *D*, *E* and *F*) and was indistinguishable from 2,3-di-*O*-methyl-D-xylose. GLC examination of the derived methyl glycosides revealed products indistinguishable from methyl 2,3-di-*O*-methyl-D-xylosides. A sample (10 mg) of component 5 was boiled under reflux with freshly distilled aniline (0·5 ml) and MeOH (5 ml) for 2 hr. The reaction mixture was taken to dryness and the resultant syrup was crystallised from EtOAc. The *N*-phenyl-D-xylosylamine-2,3-di-*O*-methyl ether had m.p. 122–124° (lit.,[55] 126°). A sample, on demethylation with $BCl_3$, gave only xylose. *Component* 6 was present only in trace amount and was contaminated with 2,3-di-*O*-methyl-D-xylose. On TLC examination (irrigants *D*, *E* and *F*), a component corresponding to 2,3-di-*O*-methyl-L-arabinose was detected. GLC examination of the derived methyl glycosides revealed *inter alia* two peaks corresponding in retention times to those produced by an authentic sample of 2,3-di-*O*-methyl-L-arabinosides. *Components* 7 *and* 8 were present in a mixture which was homogeneous on TLC (irrigants *D* and *E*) and on PCs (irrigant *H*) but was found to be a mixture by TLC (irrigant *E*). The main component was identical to 2-*O*-methyl-D-xylose and the minor component was identical to 3-*O*-methyl-D-xylose. GLC examination (column *B*) of the derived methyl glycosides showed that components corresponding in retention time and relative area to methyl 2-*O*-methyl-D-xylosides and methyl 3-*O*-methyl-D-xylosides were present in the ratio of *ca.* 15 : 1. A sample, on demethylation, with $BCl_3$, gave only xylose. *Components* 9 *and* 10 were present in a mixture which was readily resolved by PC (irrigants *D* and *H*). *Component* 9 was homogeneous on PCs (irrigants *D* and *H*) and was indistinguishable from 2,3,4-tri-*O*-methyl-D-glucuronic acid. A sample was treated with MeOH–HCl as described above and the products, on GLC examination (column *B*) were identical in retention time and relative area to those produced by methyl methyl 2,3,4-tri-*O*-methyl-D-glucopyranosiduronates. The methyl glycosides were reduced by the addition of $NaBH_4$ and further GLC examination revealed two components corresponding in retention time to methyl 2,3,4-tri-*O*-methyl-D-glucosides. *Component* 10 was homogeneous on PCs (irrigant *A*) and was indistinguishabe from D-xylose.

*Quantitative estimation of the products on hydrolysis of the methylated galactoarabinoxylan.* A sample of the hydrolysate (*ca.* 20 mg) was dissolved in aqueous MeOH (10 ml) and $NaBH_4$ (50 mg) added. After 12 hr the excess of $NaBH_4$ was destroyed by the addition of HOAc and the borate was removed by passing the solution through a column of Borasorb (a borate specific ion-exchange resin supplied by Calbiochem Ltd.). The eluate was taken to dryness under reduced pressure at room temp. and heated with $Ac_2O$ (1 ml) and pyridine (1 ml) in a sealed tube (120°; 30 min). The excess of $Ac_2O$ was hydrolysed by the addition of $H_2O$ (2 ml) and the reaction-mixture extracted with $CHCl_3$. The products were examined by GLC (column *A*) and the identities of the components established by comparison with authentic compounds. The following compounds were identified as the methylated glycitol peracetates (relative peak areas in parentheses), 2,3,5-tri-*O*-methyl-L-arabinose (2·65), 2,3,4-tri-*O*-methyl-D-xylose (1·00), 3,5-di-*O*-methyl-L-arabinose (0·20), 2,3-di-*O*-methyl-L-arabinose (0·13), 2,3,4,6-tetra-*O*-methyl-D-galactose (0·36), 2,3-di-*O*-methyl-D-xylose (5·39), and 2-*O*-, and 3-*O*-, methyl-D-xyloses (2·16).

*Methanolysis of the methylated galactoarabinoxylan.* A sample (*ca.* 5 mg) was heated in a sealed tube (100°; 16 hr) with 4% MeOH–HCl. On cooling, the contents were examined directly by GLC (columns *A* and *B*) but it was necessary to concentrate the solution, at a later stage, to facilitate the identification

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of minor components. The following compounds were identified as the methyl glycosides (relative peak areas in parenthesis where resolution permitted estimation), 2,3,5-tri-*O*-methyl-L-arabinose (2·6), 2,3,4-tri-*O*-methyl-D-xylose (1·0), 3,5-di-*O*-methyl-L-arabinose (0·1), 2,3,4,6-tetra-*O*-methyl-D-galactose (0·2), 2,3-di-*O*-methyl-D-xylose (5·0), 2-*O*-methyl-D-xylose (2·3), 3-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-L-arabinose, 2,3,6-tri-*O*-methyl-D-galactose, and 2,3,4-tri-*O*-methyl-D-glucuronic acid (as the methyl ester).

*Periodate oxidation of the acidic galactoarabinoxylan.* A sample (50 mg) of the acidic galactoarabinoxylan was oxidised with 0·05 M $NaIO_4$ at 5° in the dark for 28 days. The solution was dialysed and $NaBH_4$ (100 mg) added. After 24 hr the excess of $NaBH_4$ was destroyed by the addition of Zeo-Karb 325 ($H^+$) and the borate removed by co-distillation with MeOH. The product was hydrolysed with 0·05 M $H_2SO_4$ (100°; 12 hr), neutralised ($BaCO_3$) and the products examined by PC (irrigants *A*, *B* and *C*). Glycerol, threitol, arabinose, galactose, and xylose were detected. A sample (20 mg) was reduced with $NaBH_4$ (50 mg) and the excess $NaBH_4$ was destroyed after 24 hr by the addition of HOAc. The borate was removed as before and the products were acetylated ($Ac_2O$–NaOAc) in a sealed tube (120°; 4 hr). A sample of the glycitol acetate mixture was examined by GLC (column *A*) and the following compounds were identified by comparison with standard compounds (molar ratios in parenthesis), ethylene glycol diacetate (3·3), glycerol triacetate (49·2), threitol tetraacetate (2·2), arabinitol pentaacetate (6·2), galactitol hexaacetate (3·4), and xylitol pentaacetate (35·6).

*Determination of the $D.P._n$ by formaldehyde release.* A sample (80·1 mg) of the acidic galactoarabinoxylan was reduced with $NaBH_4$ (100 mg) and after 24 hr the excess $NaBH_4$ was destroyed by the addition of HOAc. 0·5 M $NaIO_4$ (5 ml) was added and the volume adjusted to 25 ml. The solution was incubated at 20° in the dark and, at intervals, aliquots were withdrawn. The HCHO released, determined by the chromotropic acid method, was (mol $\times 10^6$) 3·8 (1 hr), 4·4 (2 hr) 5·75 (5 hr), 6·2 (20 hr), 6·2 (44 hr), and 5·8 (68 hr). The average value for the HCHO released, extrapolated to 0 time, was $6{\cdot}0 \times 10^{-6}$ mol. The HCHO released from the unreduced galactoarabinoxylan was negligible under the above conditions.

*Method of deducing average values of sugar residues.* The average number of side-residues containing arabinose would be $B + C + D + E = 17{\cdot}5$ (*1*), the number of side-residues containing galactose would be $C + D + G = 5{\cdot}4$ (*2*), and the number of side-residues containing uronic acid residues would be $F + G = 6{\cdot}8$ (*3*); Since 4·6 arabinose residues survived oxidation then $B + C = 4{\cdot}6$ (*4*) and therefore $D + E = 12{\cdot}9$, i.e. $1 - 4$, (*5*). After prolonged oxidation, an average of 2·5 galactose residues remained unattacked. It is suggested that their survival is not due to the absence of vicinal diol groups but to steric hindrance impeding access by the oxidant to the non-terminal galactose residues. Such survival would not be surprising in a molecule having a very high degree of branching, but occasional oxidation would be expected were the substitution random. As $G = 2{\cdot}5$ (*6*) therefore $F = 4{\cdot}3$, $3 - 6$, (*7*) that is there were 4·3 uronic acid residues directly attached to the xylan chain at position C2. There were 2·9 terminal galactose residues, that is, $C + D = 2{\cdot}9$, $2 - 6$, (*8*). Ethylene glycol would be produced by terminal non-reducing xylose residues. One of these residues was known to be present in the main chain and the quantity of ethylene glycol produced by this residue depended on the extent of its substitution at position C3. The conclusion was reached that 7·1 xylose residues ($4 + 6$) were not present in the main chain and therefore 17·5 side residues were randomly distributed over 43·2 xylose residues. There was a likelihood that 1 in every 2·5 of the terminal non-reducing xylose residues carried a substituent at position C3, that is $0{\cdot}4A + B = 2{\cdot}4$ (*9*) and as $A = 1$, therefore $B = 2{\cdot}0$ and so $C = 0{\cdot}3$ and $D = 2{\cdot}6$. Substituting these values in equation (*1*), the value for $E$ was found to be 12·6, that is, there were 12·6 arabinofuranosyl residues directly attached to the main chain. If the basis of this calculation is correct then the implication is that 24·3 xylose residues ($= B + C + D + E + F + G$) would have survived the periodate oxidation. In fact a value of 26·1 was obtained. Of the 43·2 xylose residues in the main chain, 17·5 were concluded to be substituted at C3, 6·8 at C2, and 2 were present as terminal residues. This indicated that 16·9 of these residues must be non-terminal and unbranched. A second indication that the calculation was sound was that the theoretical value for the amount of glycerol produced during Smith degradation was 38·4 ($= 2C + 2D + E + G + H$) molecules and in fact the determined value was 36·0. The terminal residues substituted at position C3 should have given rise to $0{\cdot}4 \times 2 = 0{\cdot}8$ residues of threitol but in fact 1·6 residues were obtained. This slightly high value may be attributed to some of the galactose residues in structural feature G being subject to attack by periodate.

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