769. *The Constitution of a Xylan from Cocksfoot Grass* (Dactylis glomerata).

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Alkaline extraction of cocksfoot grass gave a polysaccharide of the xylan group. Hydrolysis of the methylated xylan afforded 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-, 2,3-di-, 2- and 3-*O*-methyl-D-xylose, and (3-*O*-methyl-2-D-xylose 2,3,4-tri-*O*-methyl-D-glucopyranosid)uronic acid in the approximate molar ratio of 8 : 3 : 54 : 12 : 1 : 3. It is concluded from these and other experiments that the polysaccharide is composed of chains of 1,4-linked β-D-xylopyranose residues to which are attached side-chains of L-arabinofuranose and 4-*O*-methyl-D-glucuronic acid residues through positions 3 and 2 respectively. A small degree of branching in the backbone of D-xylose residues is indicated.

Analytical studies [1] have shown that grasses, like other lignified tissues, contain substantial proportions of xylose residues. In order to understand more fully the complex biochemical changes which occur during the wilting and ensilage of grasses and may involve structural polysaccharides in addition to the fructans and soluble sugars,[1] a knowledge of the detailed chemistry of the cell wall polysaccharides is important, and this paper describes the structure of the major polysaccharide component of the hemicellulose fraction.

Cocksfoot grass was extracted successively with boiling ethanol–water (4 : 1) to remove colouring matter and soluble sugars, and with cold water to remove water-soluble polysaccharides, and the residue was delignified with acidified sodium chlorite solution. The hemicellulose component of the grass was isolated by alkaline extraction of the holocellulose and after reprecipitation the isolated polysaccharide had 8·5% of uronic anhydride, and gave on hydrolysis xylose (50%), arabinose (22%), glucose (8%), and galactose (7%). Attempts to fractionate the polysaccharide by precipitation from aqueous solution with cetyltrimethylammonium bromide failed to give a polysaccharide devoid of glucose and

[1] Mackenzie and Wylam, *J. Sci. Food Agric.*, 1957, **8**, 38.

galactose residues. However, since subsequent hydrolysis of the methylated polysaccharide afforded no methyl ethers of glucose or galactose, it is probable that these sugars were present as constituents of contaminating polysaccharides.

Mild acid-hydrolysis of the polysaccharide resulted in the preferential removal of arabinose with only traces of xylose, glucose, and galactose, suggesting that the arabinose residues were probably present in the furanose form. Since no arabinose-containing oligosaccharides could be detected under these conditions, it appeared likely that the arabinose units were present mainly as single-unit non-reducing end groups in the polysaccharide. Under more drastic conditions of hydrolysis two acidic components (probably aldobiouronic acids) were isolated and separated chromatographically. The major component, after reduction of the methyl ester methyl glycosides with potassium borohydride and hydrolysis, gave 4-*O*-methylglucose and xylose. The minor component similarly afforded glucose and xylose. It follows that the glucuronic acid residues in the polysaccharide were mainly present as the 4-methyl ether.

The polysaccharide was converted into its fully methylated derivative, which on hydrolysis gave 2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-, 2,3-di-, 2- and 3-*O*-methyl-D-xylose, and a partially methylated aldobiouronic acid in the approximate molar ratio of 8 : 3 : 54 : 12 : 1 : 3. The following experiments showed the acidic disaccharide to be (3-*O*-methyl-2-D-xylose 2,3,4-tri-*O*-methyl-D-glucopyranosid)uronic acid. The methylated aldobiouronic acid was converted into the methyl ester methyl glycosides which were reduced with potassium borohydride. Hydrolysis of a portion of the partially methylated neutral disaccharide gave 2,3,4-tri-*O*-methylglucose and 3-*O*-methylxylose, identified by chromatography and ionophoresis. The remaining material was remethylated, and hydrolysis of the fully methylated disaccharide furnished 2,3,4,6-tetra-*O*-methyl-D-glucose and 3,4,-di-*O*-methyl-D-xylose.

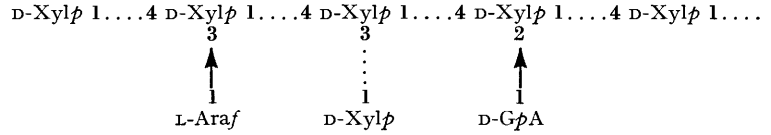
These results show that the polysaccharide contains main chains of 1,4-linked β-D-xylopyranose residues to which are attached as side-chains end groups of L-arabinofuranose and 4-*O*-methyl-D-glucuronic acid residues. The isolation from the methylated polysaccharide of 2-*O*-methyl-D-xylose, which represents the main branching point in the molecule, shows that the arabinose units are joined to xylose units in the main chains by 1,3-linkages. Evidence that these L-arabinofuranose end groups in the polysaccharides are present as single-unit side-chains and are attached directly to the main chains is provided by the isolation of the trisaccharide, *O*-L-arabinofuranosyl-(1 ⟶ 3)-*O*-β-D-xylopyranosyl-(1 ⟶ 4)-D-xylose, as a product of partial enzymic hydrolysis of the polysaccharide.[2] The isolation of the partially etherified aldobiouronic acid, (3-*O*-methyl-2-D-xylose 2,3,4-tri-*O*-methyl-D-glucopyranosid)uronic acid, from the methylated polysaccharide shows that the 4-*O*-methyl-D-glucuronic acid end groups are present as single-unit side-chains joined to xylose residues in the main chain by 1,2-linkages.

Determination of molecular weight by isothermal distillation (by courtesy of Dr. C. T. Greenwood) gave a value of 22,100 ± 1000 (degree of polymerisation, 138 ± 6) for the methylated polysaccharide. Since the methylation analysis indicated the presence of approximately five non-reducing xylose end groups for a molecule of this size there will be on the average four branches in the main chain of xylose residues per molecule. The majority of such branch points must be through position 3, but the possibility of some branching through position 2 also cannot be excluded. In this connection it may be noted that the small amount of 3-*O*-methyl-D-xylose isolated from the methylated polysaccharide could arise from (*a*) branching in the main chain, (*b*) partial hydrolysis of the partially methylated aldobiouronic acid, or (*c*) incomplete methylation, or demethylation during the hydrolysis of the methylated polysaccharide.

On the basis of these results the annexed partial structure for the xylan from cocksfoot grass indicates the main features of the molecule. Although the proportions of the

[2] Aspinall, Cairncross, Sturgeon, and Wilkie, *J.*, 1960, following paper.

constituent sugar residues in the methylated polysaccharide were different from those in the original polysaccharide, it is probable that the methylated xylan which was obtained is a genuine subfraction resulting from inadvertent fractionation of a series of structurally related polysaccharides containing the same sugar units linked in the same way but in different proportions. The xylan is clearly very similar to other xylans from lignified tissues,[3] and most closely resembles the xylans from cereal straws in the proportions of arabinose and glucuronic acid and/or 4-*O*-methylglucuronic acid side-chains present. The modes of attachment of these side-chains to positions 3 and 2 of xylose residues respectively are those most commonly encountered amongst polysaccharides of this group.



[D-Xyl*p* = D-xylopyranose, L-Ara*f* = L-arabinofuranose, D-G*p*A = D-glucuronic acid (mainly as the 4-methyl ether).]

Experimental

Paper partition chromatography was carried out on Whatman Nos. 1 and 3MM filter papers with the following solvent systems (v/v): (A) ethyl acetate–acetic acid–water (3 : 1 : 3, upper layer); (B) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3, upper layer); (C) butan-1-ol–ethanol–water (4 : 1 : 5, upper layer); (D) benzene–ethanol–water (169 : 47 : 15, upper layer); (E) butan-2-one, half saturated with water; (F) ethyl acetate–pyridine–water (10 : 4 : 3). Methylated sugars were demethylated with hydrobromic acid.[4] Optical rotations were observed at 18° ± 2°. Extractions and reactions involving the use of alkali were performed, as far as possible, under nitrogen. Paper ionophoresis was in borate buffer at pH 10.

Isolation of Cocksfoot Xylan.—Cocksfoot grass was extracted with boiling ethanol–water (4 : 1) to remove colouring material and soluble sugars. The residual grass was delignified with acidified sodium chlorite solution according to the procedure of Wise *et al.*[5] The resulting holocellulose (200 g.) was extracted twice with N-sodium hydroxide (2·5 l.) for periods of 48 hr. The extracts were acidified with glacial acetic acid to pH 4—5 and the crude polysaccharide (20 g.; ash, 7·7%) was precipitated by the addition of acetone (1·5 vol.). The polysaccharide was dissolved in water at 60°, and the solution was centrifuged to remove a small amount of insoluble material and poured into acetone (2 vol.) to give cocksfoot xylan (18 g.). The xylan had $[\alpha]_D$ −66·5° (*c* 0·5 in 0·5N-sodium hydroxide), and chromatographic examination of the hydrolysate in solvents A and B followed by quantitative estimation [6] showed the presence of xylose (50%), arabinose (22%), glucose (8%), and galactose (7%) [Found: ash, 1·5%; uronic anhydride (by decarboxylation), 8%]. Attempts to fractionate the polysaccharide by precipitation from aqueous solution with cetyltrimethylammonium bromide in the absence and the presence of borate buffer [7] failed to give fractions differing significantly in composition.

Xylan (25 mg.) was heated in 0·02N-oxalic acid (25 ml.) at 100°. At hourly intervals samples were withdrawn for chromatographic examination in solvents A and B. The results indicated that arabinose was rapidly released together with traces of xylose, glucose, and galactose, but that no arabinose-containing oligosaccharides could be detected.

Xylan (5 g.) was heated with N-sulphuric acid (250 ml.) at 100° for 4 hr. The cooled solution was neutralised with barium carbonate, and the filtrate was concentrated to *ca.* 50 ml., treated with Amberlite resin IR-120(H) to remove barium ions, and poured on a column of charcoal–Celite (1 : 1; 100 g.). Elution with water gave monosaccharides with traces of aldobiouronic acids, and elution with water containing 5% of butan-2-one gave aldobiouronic acids together with some monosaccharide. Chromatographic separation of the second fraction on filter sheets with solvent F gave two chromatographically pure aldobiouronic acids I (200 mg.) and II (25 mg.). Aldobiouronic acid I had R_{xylose} 0·083 in solvent F, and reduction of

[3] Aspinall, *Adv. Carbohydrate Chem.*, 1959, **14**, 429.
[4] Hough, Jones, and Wadman, *J.*, 1950, 1705.
[5] Wise, Murphy, and D'Addieco, *Paper Trade J.*, 1946, **122**, 35.
[6] Flood, Hirst, and Jones, *J.*, 1948, 1679.
[7] Barker, Stacey, and Zweifel, *Chem. and Ind.*, 1957, 330.

the methyl ester methyl glycosides with potassium borohydride followed by hydrolysis gave xylose and 4-*O*-methylglucose (identified by chromatography of the sugar and of its periodate oxidation products [8]). Aldobiouronic acid II had R_{xylose} 0·05 in solvent F, and reduction of the methyl ester methyl glycosides followed by hydrolysis gave xylose and glucose.

Preparation and Hydrolysis of Methylated Xylan.—The xylan (10 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide. The product (3 g.) had $[\alpha]_D$ −70° (*c* 0·49 in $CHCl_3$) (Found: OMe, 38·7. Calc. for $C_7H_{12}O_4$: OMe, 38·7%).

The methylated xylan (2 g.) was shaken overnight with methanolic 4% hydrogen chloride (500 ml.), and the resulting solution was refluxed for 5 hr. (to constant rotation). After removal of methanol under reduced pressure, the resulting syrup was heated with N-hydrochloric acid (350 ml.) at 100° for 5 hr. (to constant rotation). Evaporation after neutralisation with silver carbonate yielded a syrup (1·7 g.), which was treated (in aqueous solution) with barium carbonate. The mixture (1·6 g.) of methylated sugars was extracted with butan-2-one, and the insoluble residue (0·161 g.) was separated. The soluble sugars were fractionated on cellulose (70 × 3 cm.) with solvent E to give five fractions, and a further fraction was obtained by elution of the cellulose with water. Chromatography showed the fractions to contain the following sugars: fraction 1 (180 mg.), tri-*O*-methylarabinose and tri-*O*-methylxylose; fraction 2 (210 mg.), tri-*O*-methylarabinose and tri- and di-*O*-methylxylose; fraction 3 (740 mg.), di-*O*-methylxylose; fraction 4 (15 mg.), 3-*O*-methylxylose; fraction 5 (182 mg.), 2-*O*-methylxylose; and fraction 6 (47 mg.), barium salts of methylated aldobiouronic acids. Chromatography of the residue, insoluble in butan-2-one, showed methylated aldobiouronic acids with traces of tri-*O*-methylarabinose and di-*O*-methylxylose. This material was combined with fraction 2, and the mixture was separated on cellulose (50 × 1·5 cm.) with light petroleum (b. p. 100—120°)–butan-1-ol (7 : 3) saturated with water, as eluant, to give fractions 7 (16 mg.) and 8 (130 mg.), and elution of the cellulose with water gave fraction 9 (56 mg.). Fractions containing the same sugars were combined for further identification.

Fractions 1 *and* 7. Chromatography of the syrup (196 mg.) in solvent D showed 2,3,5-tri-*O*-methylarabinose and 2,3,4-tri-*O*-methylxylose, and the optical rotation, $[\alpha]_D$ −23·4° (*c* 0·5 in H_2O), corresponded to that of a mixture of 2,3,5-tri-*O*-methyl-L-arabinose and 2,3,4-tri-*O*-methyl-D-xylose in the proportion of 73 : 27. Separation on filter sheets with solvent D gave two fractions. Fraction 1*a* gave only arabinose on demethylation and was characterised as 2,3,5-tri-*O*-methyl-L-arabinose by conversion into 2,3,5-tri-*O*-methyl-L-arabonamide, m. p. and mixed m. p. 135°. Fraction 1*b* gave only xylose on demethylation and was identified as 2,3,4-tri-*O*-methyl-D-xylose by conversion into the aniline derivative, m. p. and mixed m. p. 99°.

Fractions 3 *and* 8. The sugar (870 mg.) crystallised when seeded with 2,3-di-*O*-methyl-β-D-xylose and had m. p. and mixed m. p. 80°, and $[\alpha]_D$ −21° ⟶ +23° (*c* 0·65 in H_2O). The sugar was further identified by conversion into the aniline derivative, m. p. and mixed m. p. 122°, and into 2,3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 132°.

Fraction 4. The sugar (16 mg.) was indistinguishable from 3-*O*-methyl-D-xylose on chromatography in solvents C and E, and on paper ionophoresis, and was characterised as the phenylosazone, m. p. and mixed m. p. 170°.

Fraction 5. The sugar (182 mg.), $[\alpha]_D$ +34·5° (*c* 0·5 in H_2O), was chromatographically and ionophoretically indistinguishable from 2-*O*-methyl-D-xylose, and was characterised as the aniline derivative, m. p. and mixed m. p. 125°.

Fractions 6 *and* 9. The barium salt was converted into the corresponding methylated aldobiouronic acid by treatment with Amberlite resin IR-120(H), and the resulting syrup was refluxed with boiling 5% methanolic hydrogen chloride for 4 hr. The product, after neutralisation with silver carbonate, was reduced with potassium borohydride (100 mg.) in water (5 ml.) at room temperature for 3 hr. Excess of hydride was destroyed by the addition of dilute sulphuric acid and extraction with chloroform afforded the partially methylated disaccharide (33 mg.). A portion (5 mg.) of the syrup was hydrolysed with N-hydrochloric acid, and chromatography and ionophoresis showed 2,3,4-tri-*O*-methylglucose and 3-*O*-methylxylose. The remainder of the syrup was further methylated with methyl iodide and silver oxide to give a fully methylated disaccharide, hydrolysis of which with 0·8N-hydrochloric acid at 100° for

[8] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

6 hr. afforded a mixture of sugars, which were separated on filter sheets with solvent C to give two fractions *a* and *b*. Fraction *a* was characterised as 2,3,4,6-tetra-*O*-methyl-D-glucose by conversion into the aniline derivative, identified by m. p. and mixed m. p. 114° and by *X*-ray powder photograph. Fraction *b* was identified as 3,4-di-*O*-methyl-D-xylose by conversion into 3,4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 65—67°.

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