221. *The Constitution of an Oat-straw Xylan.*

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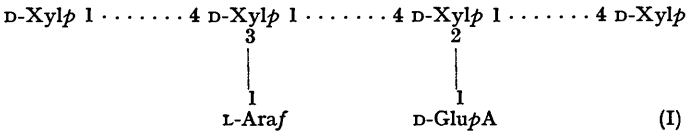
Fractionation of oat-straw hemicellulose yielded a xylan containing small quantities of arabinose (*ca.* 3%) and uronic acid (*ca.* 3·5%) residues. Hydrolysis of the methylated polysaccharide gave 2 : 3 : 5-tri-*O*-methyl-L-arabinose, 2 : 3 : 4-tri-*O*-methyl-D-xylose, 2 : 3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose, and 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose in the approximate molar ratios 1 : 1 : 41 : 1 : 1. It is concluded from these and other experiments that the xylan molecule is composed of 40—45 β-D-xylopyranose residues, the main chain carrying two side-chains linked through positions 3 and 2 of the D-xylose residues, and terminated by L-arabofuranose and 4-*O*-methyl-D-glucopyranuronic acid residues respectively. Possible structures for the polysaccharide are discussed.

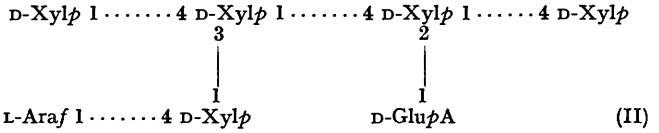
In continuation of our structural studies of the hemicelluloses of lignified tissues, this paper describes the results of an examination of a xylan from oat straw. The hemicellulose was extracted from the delignified straw with cold aqueous sodium hydroxide, and hydrolysis of the polysaccharide indicated the presence of arabinose (5·6%) in addition to xylose residues. Repeated fractionations of the hemicellulose *via* the copper complex gave a xylan in which the proportion of arabinose residues had been reduced, but no xylan devoid of arabinose residues could be isolated. The xylan, thus obtained, gave on hydrolysis xylose and arabinose in the ratio 32 : 1 and had a uronic anhydride content of 3·5%. The acidic fraction obtained on hydrolysis of the xylan was converted into the methyl ester methyl glycoside, reduction of which with lithium aluminium hydride followed by hydrolysis of the reduction product yielded xylose and 4-*O*-methylglucose together with a trace of glucose. The acidic residues present in the polysaccharide, therefore, were those of glucuronic acid present for the most part as the 4-methyl ether.

The xylan was converted into the methylated derivative and the sugars obtained on hydrolysis of the methylated polysaccharide were partitioned on cellulose. The following sugars, which were isolated and characterised by the formation of crystalline derivatives—2 : 3 : 5-tri-*O*-methyl-L-arabinose, 2 : 3 : 4-tri-*O*-methyl-D-xylose, 2 : 3-di-*O*-methyl-D-xylose and 2-*O*-methyl-D-xylose—were present in the molar ratios 0·8 : 1·0 : 41 : 1·2. In addition a tetra-*O*-methylaldobiouronic acid was isolated and shown to be 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose as reduction of its methyl ester methyl glycoside with lithium aluminium hydride followed by hydrolysis gave 2 : 3 : 4-tri-*O*-methyl-D-glucose and 3-*O*-methyl-D-xylose.

The main structural features of this oat-straw xylan are clear from these results. The isolation of the above aldobiouronic acid shows that each xylan chain contains a single D-glucuronic acid residue (probably as the 4-methyl ether) linked directly to the main chain through position 2 of a D-xylose residue. The isolation of 2 : 3 : 5-tri-*O*-methyl-L-arabinose accounts for all the arabinose residues present in the polysaccharide. The quantity of this compound isolated, together with that of 2-*O*-methyl-D-xylose, indicates that on the average each xylan carries a second side-chain terminated by an L-arabofuranose unit. It is not possible on the present evidence to distinguish between structures in which the L-arabofuranose residue is linked directly to the main chain (I) and those in which a side-chain of D-xylose residues is terminated by an arabinose residue (II), nor is it possible to indicate the relative positions in the main chain of the two branch points. The quantity of monomethylxylose isolated was only slightly in excess of that required by the branching point to the L-arabofuranose residue. The sugar, therefore, was not present in sufficient amount to accommodate a side-chain terminated by a D-xylopyranose residue, and it is probable that the excess arose from undermethylation of the polysaccharide and/or demethylation during hydrolysis. The results of periodate oxidation of the xylan were consistent with the picture of a xylan of 40—45 residues with side-chains terminated by an L-arabofuranose residue and a 4-*O*-methyl-D-glucuronic acid residue, in that 1·0 mole of periodate was consumed per pentose residue and 1 mole of formic acid was released per 15·7 residues, corresponding to a molecule with one non-reducing D-xylopyranose residue per chain of 47 residues.



(I)



(II)

These results indicate that this xylan is of a similar molecular size to the wheat-straw xylan [1] previously examined in these laboratories. The occurrence of side-chains terminated by L-arabofuranose residues and linked to the main chain through position 3 of the D-xylose residue is a structural feature also found in the hemicelluloses of esparto grass [2] and of wheat-straw.[3–5] On the other hand, the linking of 4-*O*-methyl-D-glucuronic acid residues to position 2 of the xylose residues recalls a linkage characteristic of the wood hemicelluloses [6–8] rather than that of wheat straw.[9] It is of interest that in this investigation it was not possible by the fractionation of oat-straw hemicellulose to isolate a xylan

[1] Aspinall and Mahomed, *J.*, 1954, 1731.
[2] Aspinall, Hirst, Moody, and Percival, *J.*, 1953, 1631.
[3] Adams, *Canad. J. Chem.*, 1952, **30**, 698.
[4] Ehrenthal, Montgomery, and Smith, *J. Amer. Chem. Soc.*, 1954, **76**, 5509.
[5] Roudier, *Compt. rend.*, 1953, **237**, 840.
[6] Jones and Wise, *J.*, 1952, 3389.
[7] Aspinall, Hirst, and Mahomed, *J.*, 1954, 1734.
[8] Gorrod and Jones, *J.*, 1954, 2522.
[9] Bishop, *Canad. J. Chem.*, 1953, **31**, 134.

devoid of arabinose residues, although it is still possible that such xylans may be present in oat straw. This fact, however, confirms the view that the xylans devoid of arabinose residues isolated from esparto grass [10] and wheat straw [1] by similar methods were true xylans and not artefacts from which the relatively labile arabofuranose residues had been removed during their isolation.

It is probable that the xylan studied in this investigation is only one of many xylans present in oat straw. In order, however, to decide whether the xylans from a particular source differ only in molecular size and in the number rather than in the nature of the residues linked as side-chains, or whether different structural features are present in these several xylans, it will be necessary for much more selective methods for the fractionation of such closely related polysaccharides to be developed.

Experimental

Paper partition chromatography was carried out on Whatman No. 1 filter paper with the upper layers of the following solvents system (v/v) : (A) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (B) butan-1-ol–ethanol–water (5 : 1 : 4); (C) butan-1-ol–acetic acid–water (4 : 1 : 5); and (D) benzene–ethanol–water (167 : 47 : 15).

Isolation and Fractionation of Oat-straw Hemicellulose.—Oat straw (variety Sun II, cut in September 1952; 365 g.) was delignified by Wise's method.[11] The holocellulose (*ca.* 220 g.) was extracted with sodium hydroxide solution (4%), the extract was acidified with glacial acetic acid, and the oat-straw hemicellulose (46 g.) was precipitated by the addition of an equal volume of acetone. The crude hemicellulose had $[\alpha]_D^{18}$ −94·7° (*c* 0·6 in N-sodium hydroxide) and chromatographic examination of the hydrolysate by Hirst and Jones's method [12] in solvent A showed the presence of xylose (94·4%) and arabinose (5·6%) (calc. as 100% pentose). The hemicellulose was fractionated by five successive precipitations of the copper complex formed on addition of Fehling's solution to a solution in sodium hydroxide (4%). The oat-straw xylan (19 g.) thus obtained had $[\alpha]_D^{19}$ −95·0° (*c* 0·5 in N-sodium hydroxide) [Found : ash (as sulphate), 0·8%; lignin, 3·3; uronic anhydride (by decarboxylation), 3·5; OMe, 0·5%] and was used in all subsequent investigations. Chromatographic examination of the hydrolysate showed the presence of xylose (97·0%) and arabinose (3·0%) (calc. as 100% pentose).

Examination of the Acidic Fraction from Xylan Hydrolysis.—Xylan (14 g.) was hydrolysed with 0·5N-sulphuric acid (100 c.c.) for 6 hr. at 100° and the hydrolysate was neutralised by passage through a column of Amberlite resin IR-4B. The resin was washed with 2N-sulphuric acid, the washings were neutralised with barium carbonate, and the filtrate was deionised with Amberlite resin IR-120 and examined on the chromatogram. As large quantities of xylose were present the acidic sugars were re-adsorbed on Amberlite resin IR-4B, the neutral sugars were removed by elution with water, and the acidic sugars were isolated by the procedure previously described and freeze-dried, to give a solid (200 mg.). A portion (25 mg.) of the solid was converted into the methyl ester methyl glycoside, which was reduced with lithium aluminium hydride. The reduction product was hydrolysed with N-sulphuric acid and chromatographic examination of the hydrolysate showed the presence of 4-*O*-methylglucose, xylose, and glucose (trace).

Methylation of Oat-straw Xylan.—Xylan (17 g.) was methylated ten times with methyl sulphate and sodium hydroxide, and once with methyl iodide and silver oxide. The product (12·6 g.) was fractionated by dissolution in boiling chloroform–light petroleum (b. p. 60—65°) to give a main fraction, soluble in boiling chloroform–light petroleum (30 : 70) (7·4 g.), which had $[\alpha]_D^{17}$ −87·0° (*c* 0·91 in $CHCl_3$) and was used in subsequent experiments (Found : OMe, 38·2%).

Hydrolysis of Methylated Xylan and Separation of Methylated Sugars.—The methylated xylan (5·49 g.) was hydrolysed successively with boiling methanolic 1% hydrogen chloride (600 c.c.) for 30 hr. and with 0·5N-hydrochloric acid (300 c.c.) at 100° for 15 hr. (constant rotation). Evaporation after neutralisation with silver carbonate yielded a syrup (5·99 g.). The syrup (5·93 g.) was fractionated on cellulose (88 × 4 cm.) [13] with light petroleum (b. p. 100—120°)–butan-1-ol (70 : 30), saturated with water, as eluant to give four fractions.

[10] Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289.
[11] Wise, *Ind. Eng. Chem. Anal.*, 1945, **17**, 63.
[12] Hirst and Jones, *J.*, 1949, 1659.
[13] Hough, Jones, and Wadman, *J.*, 1949, 2511.

Fraction 1. Hypoiodite oxidation indicated 95·3% aldopentose, but chromatographic examination in solvent D showed the presence of 2 : 3 : 5-tri-*O*-methylarabinose, 2 : 3 : 4-tri-*O*-methylxylose, and 2 : 3-di-*O*-methylxylose. Although complete separation of the three sugars on filter sheets (Whatman 3MM) with solvent D was not always possible, in one case the three components were completely separated and hypoiodite oxidation showed tri-*O*-methylarabinose and tri- and di-*O*-methylxyloses to be present in the ratios 0·78 : 1·0 : 0·47, corresponding to 96, 124, and 58 mg. respectively of each sugar present in the fraction. Separation of the major part of fraction 1 gave chromatographically pure samples of the two tri-*O*-methylpentoses [fractions 1*a* (36 mg.) and 1*b* (42 mg.)] together with two fractions, containing respectively a mixture of the two tri-*O*-methylpentoses and di-*O*-methylxylose, which were not examined further. Fraction 1*a* had $[\alpha]_D^{19}$ +34·5° (*c* 0·6 in H_2O) and was identified 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. 136—137°, $[\alpha]_D^{20}$ −16·0° (*c* 1·0 in H_2O), which gave an identical *X*-ray powder photograph (by courtesy of Dr. C. A. Beevers) with that of the authentic amide. Fraction 1*b* crystallised on nucleation with 2 : 3 : 4-tri-*O*-methyl-D-xylose. After recrystallisation from dry ether the sugar had m. p. and mixed m. p. 90—91° and $[\alpha]_D^{18}$ +20° (equil.) (*c* 0·75 in H_2O), and gave an identical *X*-ray powder photograph with that of an authentic specimen. The derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 95° and $[\alpha]_D^{18}$ +40° (*c* 0·1 in EtOH).

Fraction 2. The chromatographically pure syrup (4·91 g.) was shown by hypoiodite oxidation to be 94% aldopentose (Found : OMe, 34·4. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). Portions of the syrup were seeded with α- and β-forms of 2 : 3-di-*O*-methyl-D-xylose but in both cases the syrup crystallised as 2 : 3-di-*O*-methyl-β-D-xylose.[14] The two crystalline samples had m. p. 78—81°, $[\alpha]_D^{18}$ −20·3° (13 min.) ⟶ +27·8° (240 min., constant) (*c* 0·64 in H_2O), and m. p. 82—84° and $[\alpha]_D^{18}$ −18·1° (12 min.) ⟶ +26·2° (200 min., constant) (*c* 0·7 in H_2O) respectively, and had mixed m. p. 78·5—83·5°. The identity of the sugar was confirmed by conversion into 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 144—145°, and into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 136·5°.

Fraction 3. The syrup (132 mg.) crystallised and had m. p. and mixed m. p. (with 2-*O*-methyl-D-xylose) 132—133° and $[\alpha]_D^{18}$ +34·0° (equil.) (*c* 1·0 in H_2O) (Found : OMe, 18·3. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). Hypoiodite oxidation indicated 93% aldopentose and paper ionophoresis [15] showed that no 3-*O*-methyl-D-xylose was present. The *X*-ray powder photograph was identical with that of an authentic sample and the derived 2-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 123·5°.

Fraction 4. The syrup (338 mg.), obtained by elution of the cellulose with water, was incompletely soluble in methanol. Purification was effected by dissolution in hot methanol, the insoluble residue was discarded, and the solution was decolorised with charcoal, to give a syrup (293 mg.), $[\alpha]_D^{19}$ +54° (*c* 0·5 in H_2O), R_G 0·12 in solvent C. A portion of the purified syrup (136 mg.) was refluxed for 6 hr. with methanolic 1·5% hydrogen chloride (50 c.c.), neutralised with silver carbonate, and taken to dryness. The resulting syrup was dissolved in dry ether (25 c.c.), and the ethereal solution was added during 3 hr. to a boiling solution of lithium aluminium hydride (250 mg.) in ether (25 c.c.). After a further 2 hr. excess of hydride was destroyed by the addition of water, the solution was acidified with 2N-sulphuric acid and extracted with chloroform, and the chloroform extract was taken to dryness. The resulting syrup (78 mg.) was hydrolysed with 0·5N-hydrochloric acid (30 c.c.) for 8 hr. at 100°, neutralised with silver carbonate, and taken to dryness, to give a syrup (60 mg.). Chromatographic examination of the syrup showed sugars travelling at the same rates as 2 : 3 : 4-tri-*O*-methyl-D-glucose and 3(and/or 2)-methyl-D-xylose, but paper ionophoresis showed that only the 3-methyl ether was present. The major portion of the syrup was fractionated on filter sheets with solvent D, to give fractions *a* (21 mg.) and *b* (28 mg.). Fraction *a* was identified as 2 : 3 : 4-tri-*O*-methyl-D-glucose by conversion into the methyl β-D-pyranoside, m. p. and mixed m. p. 89·5°. Fraction *b* was identified as 3-*O*-methyl-D-xylose by conversion into the aniline derivative, m. p. 136—137°.

Periodate Oxidation of Oat-straw Xylan.—Oxidation of xylan (50 mg. batches) with potassium periodate solution by Halsall, Hirst, and Jones's method [16] gave the following results (expressed as moles of formic acid × 10^2 released per $C_5H_8O_4$ residue) : 7·25 (73 hr.) ; 8·76 (121 hr.) ; 9·31 (168 hr.) ; 11·01 (244 hr.) ; 12·27 (312 hr.) ; 15·77 (455 hr.). As the formic acid released did not

[14] Meek, *J.*, 1956, 219.
[15] Consden and Stanier, *Nature*, 1952, **170**, 1069.
[16] Halsall, Hirst, and Jones, *J.*, 1947, 1399, 1427.

reach a constant value, extrapolation to zero time gave a value corresponding to formic acid released from α-glycol scission, namely, 1 mole per 15·7 $C_5H_8O_4$ residues. The release of two mols. of formic acid from the reducing end-group and one mol. from the non-reducing end-group being assumed, this value corresponded to a chain length of 47 residues.

Oxidation of xylan with sodium metaperiodate solution showed that the polysaccharide consumed 1·0 mole of periodate (constant after 334 hr.) per $C_5H_8O_4$ residue. Hydrolysis of the periodate-oxidised polysaccharide showed the presence of a small quantity of xylose.

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