744. *The Constitution of a Wheat-straw Hemicellulose.*

By G. O. Aspinall and Eric G. Meek.

A wheat-straw hemicellulose, containing uronic acid residues (*ca.* 3%), gave on hydrolysis xylose and arabinose in the ratio of 11 : 1. Hydrolysis of the methylated polysaccahride yielded 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 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid, together with some 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose. The structure of the polysaccharide is discussed in the light of these and other results.

Previous structural investigations have indicated that a variety of closely related polysaccharides constitute wheat-straw hemicellulose. Adams [1] and Bishop [2] showed that the hemicellulose contains a backbone of 1 : 4-linked β-D-xylopyranose residues, to which are attached through position 3 a number of L-arabofuranose and D-glucopyruronic acid residues as single-unit side-chains. Earlier studies in these laboratories [3] showed that it was possible to fractionate the hemicellulose and to isolate a xylan, devoid of arabinose residues, but still containing uronic acid residues as an integral part of the molecular structure. These investigations were carried out on hemicellulose fractions isolated by alkaline extraction from straw which had previously been treated with acidified sodium chlorite solution to remove lignin. In view of the possibility of degradation of polysaccharides during chlorite delignification,[4] we have now examined the hemicellulose extracted directly with alkali from wheat straw rather than from the holocellulose.

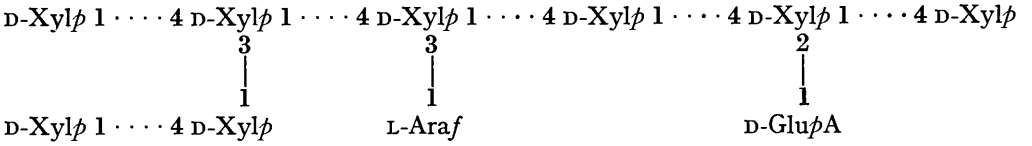
The hemicellulose was extracted from straw, previously extracted with benzene and methanol, by the procedure used in the extraction of beechwood hemicellulose A,[5] the structure of which we have previously studied.[6] The polysaccharide contained uronic acid residues (*ca.* 3%) and yielded on hydrolysis xylose and arabinose in the ratio of 11 : 1. Graded hydrolysis liberated arabinose preferentially, suggesting that this sugar occurred in the furanose form, but selective removal of these labile residues to yield a xylan devoid of arabinose residues could not be achieved without extensive degradation of the molecule, as appreciable quantities of xylose and xylose-containing oligosaccharides were released before all the arabinose residues had been removed. On more vigorous hydrolysis, a resistant acidic fraction was isolated; reduction of the derived methyl ester methyl glycoside with potassium borohydride, followed by hydrolysis, yielded 4-*O*-methylglucose, glucose, and xylose, indicating the presence in the polysaccharide of residues of glucuronic acid (partly as the 4-methyl ether).

The xylan was converted into its fully methylated derivative, hydrolysis of which yielded the following methylated sugars, 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 (in the approximate ratio of 3 : 1 : 41 : 4). Small quantities of 3-*O*-methyl-D-xylose were also present; these probably arose from hydrolysis of some of the methylated aldobiouronic acid. In addition, 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid was characterised and an acidic fraction, consisting mainly of a tetra-*O*-methylaldobiouronic acid, was isolated. Insufficient of the latter fraction was isolated for a complete identification, but the following chromatographic studies leave little doubt concerning the structure of the main component. Reduction of the derived methyl ester methyl glycoside with lithium aluminium hydride, followed by hydrolysis, gave 2 : 3 : 4-tri-*O*-methylglucose and 3-*O*-methylxylose (clearly distinguished from the 2-methyl isomer by paper ionophoresis). Further methylation of the reduced acidic fraction, followed by hydrolysis,

[1] Adams, *Canad. J. Chem.*, 1952, **30**, 698.
[2] Bishop, *ibid.*, 1953, **31**, 134.
[3] Aspinall and Mahomed, *J.*, 1954, 1731.
[4] Timell and Jahn, *Svensk Papperstidning*, 1951, **24**, 831.
[5] McDonald, *J.*, 1952, 3183.
[6] Aspinall, Hirst, and Mahomed, *J.*, 1954, 1734.

yielded 2 : 3 : 4 : 6-tetra-*O*-methylglucose and 3 : 4-di-*O*-methylxylose, together with smaller amounts of 2 : 3 : 4-tri- and a mono-*O*-methylxylose. It follows that the tetra-*O*-methylaldobiouronic acid was 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methylglucuronosyl)xylose. It is probable that the acidic fraction contained traces of a methylated aldotriouronic acid in addition to the tetra-*O*-methylaldobiouronic acid.

Although no unique structure can be advanced for this xylan, the results are consistent with the general type of structure shown :



In agreement with other workers,[1, 7, 8] we are of the opinion that the L-arabinose residues are integral parts of the molecule, occurring exclusively as end-groups in the furanose form. It is probable that these residues occur as single-unit side-chains linked to the backbone of β-1 : 4-D-xylopyranose residues through position 3 of xylose; direct proof for this mode of attachment has been provided by Bishop,[9] who isolated the trisaccharide, *O*-L-arabofuranosyl-(1→3)-*O*-D-xylopyranosyl-(1→4)-D-xylopyranose, from the enzymic hydrolysis of wheat-straw hemicellulose. The methylated aldobiouronic acid isolated from the hydrolysis of the methylated xylan shows that the D-glucuronic acid residues (partially present as the 4-methyl ether) are linked directly to the main chain through position 2 of xylose; previously, 1 : 3-linkages were reported for the aldobiouronic acid units in wheat-straw xylans.[2, 3] Although the evidence for a 1 : 3-linked aldobiouronic acid unit in the arabinose-free xylan from wheat straw [3] rested solely on the chromatographic distinction between 2- and 3-*O*-methylxylose, it will be recalled that Bishop [2] provided conclusive proof for the structure of the aldobiouronic acid which he isolated from the hydrolysis of wheat straw. It seems probable, therefore, that the several wheat-straw xylans differ amongst themselves as to the mode of attachment of glucuronic acid to xylose residues, and, indeed, in some fractions [7] glucuronic acid residues appear to be absent. The 1 : 2-linked aldobiouronic acid unit found in this xylan is a common structural feature of wood hemicelluloses [6, 10, 11] and has also been encountered in oat-straw xylan.[12]

A molecular-weight determination by the isothermal-distillation method (by the courtesy of Dr. C. T. Greenwood and Mr. W. N. Broatch) gave a value of 11,800 ± 400 (degree of polymerisation, 71—76) for the methylated xylan. This value, taken together with the value of one non-reducing end-group per *ca.* 50 residues, suggests that some of the xylan chains are branched, probably through position 3. In this respect, this xylan differs from the arabinose-free xylan,[3] also isolated from wheat straw but after chlorite delignification, in which no evidence for branching was obtained. However, Bishop [13] has obtained evidence for branching in some wheat-straw hemicullulose fractions by the isolation of non-linear xylose-containing oligosaccharides on partial acid hydrolysis.

It is clear from these and previous results that many closely related polysaccharides may be derived from wheat straw; it is not certain, however, that all these different molecular species exist side by side in the same plant. Although it is possible that some of these may have arisen from the use of different methods of extraction, our earlier studies [3] have shown that xylans, differing significantly in the proportion of arabinose residues present, may be obtained by the fractionation of a particular wheat-straw hemicellulose. The present situation with regard to the detailed structure of wheat-straw xylan re-emphasises

[7] Ehrenthal, Montgomery, and Smith, *J. Amer. Chem. Soc.*, 1954, **76**, 5509.
[8] Roudier, *Compt. rend.*, 1953, **237**, 840; *Assoc. tech. ind. papetiere Bull.*, 1954, 53.
[9] Bishop, Amer. Chem. Soc. Meeting, Minneapolis, Sept., 1955, Abs. Papers 7E.
[10] Jones and Wise, *J.*, 1952, 3389.
[11] Gorrod and Jones, *J.*, 1954, 2522.
[12] Aspinall and Wilkie, *J.*, 1956, 1072.
[13] Bishop, *Canad. J. Chem.*, 1955, **33**, 1073.

the need for using the mildest possible methods for the extraction of polysaccharides from plant cell wall and for developing more selective methods for the fractionation of mixtures of polysaccharides.

Experimental

Unless otherwise stated paper chromatography was carried out on Whatman No. 1 filter paper with the upper layers of the following solvent systems (v/v) : (A) butan-1-ol–ethanol–water (4 : 1 : 5); (B) benzene–ethanol–water (167 : 47 : 15); (C) butan-1-ol–acetic acid–water (4 : 1 : 5); (D) butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3); (E) pentan-1-ol–ethanol–water (2 : 1 : 2). Extractions and reactions involving the use of alkali were performed, as far as possible, under nitrogen.

Isolation of Wheat-straw Xylan.—Wheat straw, previously extracted with benzene and methanol, was ground in a " Raymond " laboratory mill, and the finely ground straw (150 g.; moisture content, 9·4%) was extracted successively with aqueous sodium hydroxide of increasing concentration (0·01N, 2 l. + 2 × 1·5 l.; 0·1N, 1·5 l.; N, 3 × 1·5 l.). The extracts with N-sodium hydroxide were acidified with glacial acetic acid to pH 4, and the precipitates (A) thus obtained were separated at the centrifuge. Further precipitates (B) were obtained on addition of an equal volume of ethanol to the supernatant liquors. A qualitative chromatographic examination of the sugars given on hydrolysis of the precipitates A and B indicated that the same sugars were present, so the polysaccharides were combined. The polysaccharide (37·3 g.) was purified by three precipitations from alkaline solution by the addition of acetic acid and ethanol. The purified polysaccharide had $[\alpha]_D^{19}$ −91·7° (*c*, 0·5 in N-sodium hydroxide) [Found : lignin, 7·2%; ash, 0·87%; uronic anhydride, 2·95% (by decarboxylation); OMe, 1·5%]. Chromatographic examination of the hydrolysate by Hirst and Jones's [14] method indicated the presence of xylose (90·6%) and arabinose (8·4%).

Acid Hydrolysis of Xylan.—(a) *Graded hydrolysis.* A suspension of xylan in 0·02N-oxalic acid was heated at 100°; samples were withdrawn at intervals, an equal volume of ethanol was added, and the precipitate was removed at the centrifuge. The precipitate was hydrolysed with 2N-sulphuric acid, and the hydrolysate was examined on the chromatogram with solvent A. The supernatant liquid was neutralised with barium carbonate and examined directly on the chromatogram. The results indicated that arabinose was rapidly released but that before all the arabinose residues were removed from the remaining polysaccharide (2 hr.) appreciable quantities of xylose and xylose-containing oligosaccharides could be detected in the supernatant liquid.

(b) *Examination of the acidic components in the hydrolysate.* Xylan (2·3 g.) was treated with 2N-sulphuric acid (50 ml.) at room temperature for 22 hr. and then heated at 100° for 4·5 hr.; the insoluble residue which remained was treated with 2N-sulphuric acid (50 ml.) at 100° for a further 5 hr. The combined hydrolysates were neutralised with barium carbonate, and the filtrate was de-ionised by treatment with Amberlite resin IR-120(H) and concentrated to a syrup (2·0 g.), which contained xylose, arabinose, glucose (trace), and acidic oligosaccharides. The syrup was fractionated on acid-washed charcoal–celite (1 : 1, w/w) (42 × 1·8 cm.), elution with water yielding monosaccharides together with a small quantity of acidic material (fraction 1), and elution with ethyl methyl ketone–water (5 : 95, v/v) yielding acidic oligosaccharides together with a trace of xylose (fraction 2). The acidic components from fraction 1 were separated on filter sheets with solvent D and combined with fraction 2. The acids were converted into the methyl ester methyl glycosides, reduced with potassium borohydride, and hydrolysed, chromatography showing 4-*O*-methylglucose, glucose, and xylose.

Methylation of Xylan.—Xylan (20·5 g.) was methylated fifteen times with methyl sulphate and sodium hydroxide. The fraction (5·1 g.) soluble in boiling chloroform–light petroleum (b. p. 60—70°) (9 : 11, v/v) but insoluble in boiling chloroform–light petroleum (1 : 4, v/v) was further methylated with methyl iodide and silver oxide to give a brown glass (4·77 g.) (Found : OMe, 36·0%). This material was fractionally precipitated from chloroform solution by the addition of light petroleum (b. p. 60—70°), and the fraction precipitated in light petroleum–chloroform (4 : 1) but not by light petroleum–chloroform (3 : 1 v/v) was used in subsequent experiments (Found : OMe, 38·3%).

Hydrolysis of Methylated Xylan and Separation of Methylated Sugars.—Methylated xylan (3·54 g.) was hydrolysed successively with boiling methanolic 1% hydrogen chloride (400 ml.)

[14] Hirst and Jones, *J.*, 1949, 1649.

for 6 hr. ($[\alpha]_D$ +58·7°, constant) and 0·5N-hydrochloric acid (200 ml.) for 7·5 hr. ($[\alpha]_D$ +18·6°, constant). After neutralisation with silver carbonate, the hydrolysate was taken to dryness to yield a syrup (3·75 g.). Quantitative paper chromatography [15] in solvent A showed the presence of tri-*O*-methylpentose (tri-*O*-methylarabinose and tri-*O*-methylxylose) (10·8%), di-*O*-methylxylose (75·5%), and mono-*O*-methylxylose (10·8%), together with small quantities of acidic substances. Quantitative chromatography in solvent B showed that tri-*O*-methylarabinose and tri-*O*-methylxylose were present in the ratio of 3 : 1. The syrup (3·5 g.) was fractionated on cellulose [16] (93 × 2·7 cm.) by elution with solvent B to yield seven fractions, and two further fractions were obtained by elution with butan-1-ol 50% saturated with water, and with water.

Fraction 1. The syrup (1 mg.) travelled on the chromatogram in solvent B at the same rate as 2 : 3 : 5-tri-*O*-methyl-L-arabinose and was combined with fraction 2*a*.

Fraction 2. Chromatographic examination of the syrup (282 mg.) in solvent B showed the presence of two components, which were separated on filter sheets (Whatman 3MM) with solvent B, to give fractions 2*a* and 2*b*. Fraction 2*a* had n_D^{15} 1·4516 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. 134°. Fraction 2*b* crystallised and had m. p. and mixed m. p. (with 2 : 3 : 4-tri-*O*-methyl-D-xylose) 91—92° and $[\alpha]_D^{16}$ +18·2° (equil.) (*c*, 0·55 in H_2O). The derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine did not crystallise, but chromatographic examination of the syrup in butan-1-ol–pyridine–water (10 : 3 : 3) showed two components (corresponding to the glycosylamine and the parent sugar [17]) having the same rates of movement as those derived from an authentic sample

Fraction 3. The syrup (4 mg.) travelled on the chromatogram in solvent B at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-xylose and was combined with fraction 2*b*.

Fraction 4. The syrup (2·8 g.), when seeded with 2 : 3-di-*O*-methyl-α-D-xylose, crystallised as the β-anomer (full details have been published previously [18]). The identity of the sugar was confirmed by conversion into the aniline derivative, m. p. and mixed m. p. 123°, and into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 134°.

Fractions 5 *and* 7. Chromatographic examination of the syrup (105 mg.) indicated the presence of a major component having $R_{rhamnose}$ 1·64—1·78 in solvent C and giving a dipolar spot similar to that given by the acidic component isolated from the hydrolysis of methylated pear cell wall xylan,[19] together with small quantities of di-*O*-methylxylose. Reduction of the derived methyl glycoside methyl ester with lithium aluminium hydride, followed by hydrolysis, yielded sugars identified chromatographically as mono- and di-*O*-methylxylose and 2 : 3 : 4-tri-*O*-methylglucose.

Fraction 6. Chromatographic examination of the syrup (22 mg.) gave the same dipolar spot, with characteristic orange fluorescence in ultraviolet light, as that given by fractions 5 and 7. On hydrolysis with 2N-sulphuric acid at 100° for 44 hr., the slower-moving component decreased in amount, whilst the faster-moving component, which travelled at the same rate as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid, increased, and mono-*O*-methylxylose was liberated. Complete hydrolysis was achieved by 2N-sulphuric acid at 100° in a sealed tube in 66 hr.; the products were separated on filter sheets with solvent C. The first component was identified as 2 : 3 : 4-tri-*O*-methyl-D-glucuronic acid by conversion into the β-methyl glycoside, m. p. 130—132°. Ionophoretic examination of the mono-*O*-methylxylose showed it to be mainly the 3-methyl ether with small quantities of the 2-methyl ether.

Fraction 8. The fraction (227 mg.) partially crystallised. The crystalline portion was ionophoretically homogeneous and had m. p. and mixed m. p. (with 2-*O*-methyl-D-xylose) 131—132° and $[\alpha]_D^{15}$ +28·9° (equil.) (*c*, 2·4 in H_2O). The identity of the sugar was confirmed by conversion into 2-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 128—129°. The syrupy portion was shown ionophoretically to consist mainly of the 2-methyl ether with small amounts of the 3-methyl ether.

Fraction 9. The syrup (118 mg.) was chromatographically similar to fractions 5—7, but also contained traces of slow-moving components. Chromatography on filter sheets in solvent C yielded a purified acidic fraction, having $[\alpha]_D^{18}$ +10·2° (*c*, 0·1 in H_2O). The derived methyl ester methyl glycoside was reduced with lithium aluminium hydride, and the product was divided into two portions. The first portion was hydrolysed with 0·5N-hydrochloric acid at 100° for 8 hr., and quantitative chromatography [15] showed 2 : 3 : 4-tri-*O*-methylglucose and

[15] Hirst, Hough, and Jones, *J.*, 1949, 928.
[16] Hough, Jones, and Wadman, *J.*, 1949, 2511.
[17] Barclay, Foster, and Overend, *J.*, 1955, 1541.
[18] Meek, *J.*, 1956, 219.
[19] Chanda, Hirst, and Jones, *J.*, 1951, 1240.

mono-*O*-methylxylose to be present in the ratio of 1 : 1. The second portion was methylated twice with silver oxide and methyl iodide, and chromatography of the hydrolysate in solvents B and E showed 2 : 3 : 4 : 6-tetra-*O*-methylglucose, 2 : 3 : 4-tri-*O*-methylxylose, 3 : 4-di-*O*-methylxylose, and mono-*O*-methylxylose.

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DEPARTMENT OF CHEMISTRY, UNIVERSITY OF EDINBURGH. [*Received, May 28th*, 1956.]