The Constitution of a Wheat-straw Xylan.

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Fractionation of wheat-straw hemicellulose yielded a xylan, devoid of arabinose residues, but containing uronic acid residues (*ca.* 3%). Hydrolysis of the methylated polysaccharide gave 2 : 3 : 4-tri-*O*-methyl-D-xylose (2·4%), 2 : 3-di-*O*-methyl-D-xylose (93%) and 2-*O*-methyl-D-xylose (3·4%), together with some 2-*O*-methyl-3-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucuronosyl)-D-xylose. It is concluded that this xylan has a straight chain of 40—45 D-xylopyranose residues with a single D-glucopyranuronic acid residue linked as a side-chain through position 3.

The lignified tissues of grasses and straws yield, on alkaline extraction after delignification, hemicelluloses containing D-xylose residues together with 5—10% of L-arabinose residues. In some hemicelluloses D-glucuronic acid residues are also present. Recent work in this laboratory has shown that at least two molecular types are present in esparto grass: a xylan, devoid of arabinose residues, consisting of a singly branched chain of 75 (±5) D-xylopyranose units (Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289); and an araboxylan in which at least the majority of L-arabinose residues are present as side-chains attached to a main chain of 1 : 4-linked D-xylopyranose residues (Aspinall, Hirst, Moody, and Percival, *J.*, 1953, 1631). Another xylan containing no arabinose residues has been isolated from the cell-wall of ripe pears. This polysaccharide was shown to contain 115 (±5) D-xylopyranose units in a singly branched chain but in addition to carry a terminal D-glucopyruronic acid unit at one point (Chanda, Hirst, and Percival, *J.*, 1951, 1240). The present investigation was undertaken to determine the structure of the xylan from wheat straw, a material known to be rich in pentosan.

Wheat-straw hemicellulose was extracted from the delignified straw with cold aqueous sodium hydroxide. Hydrolysis of the polysaccharide indicated the presence of arabinose (6·2%) in addition to xylose residues. After repeated precipitations, as the copper complex, with Fehling's solution, had failed to give a xylan devoid of arabinose residues, the arabinose-rich fraction was removed by extraction of the hemicellulose with hot 70% aqueous alcohol, leaving behind a xylan which gave only a trace of arabinose (*ca.* 0·5%) on hydrolysis.

The xylan was methylated under nitrogen with sodium hydroxide and methyl sulphate, and subsequently with methyl iodide and silver oxide, to give a product which was fractionated in boiling chloroform–light petroleum to give a methylated xylan. This was hydrolysed successively with methanolic and with aqueous hydrochloric acid, and the resulting sugars were separated on cellulose. The following sugars were isolated and characterised as crystalline derivatives: (1) 2 : 3 : 4-tri-*O*-methyl-D-xylose (2·4%); (2) 2 : 3-di-*O*-methyl-D-xylose (93%); and (3) 2-*O*-methyl-D-xylose (3·4%). In addition a tetramethyl aldobiuronic acid (*ca.* 1%) was isolated, which after reduction with lithium aluminium hydride (Lythgoe and Trippett, *J.*, 1950, 1983) followed by hydrolysis and chromatographic separation gave 2-*O*-methylxylose and 2 : 3 : 4-tri-*O*-methylglucose. The tetramethylaldobiuronic acid, which had a high rotation ($[\alpha]_D^{20}$ +97·5°) suggestive of an α-glycosidic linkage, was attacked by sodium metaperiodate (consumption, 0·8 mol. of periodate in 26 hr.) but no formic acid was produced. It seems probable, therefore, that the aldobiuronic acid was the same as that isolated from methylated pear cell-wall xylan (Chanda, Hirst, and Percival, *loc. cit.*), namely, 2-*O*-methyl-3-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucopyruronosyl)-α-D-xylose. Quantitative chromatography indicated that tri-, di-, and mono-methylxylose were present in the ratio of 1 : 34 : 1.

Vigorous hydrolysis of the wheat-straw xylan gave only one acidic substance, which travelled on the chromatogram at the same rate as glucuronic acid. Under similar conditions of hydrolysis (see Aspinall, Hirst, and Mahomed, succeeding paper) residues of 4-*O*-methyl-D-glucuronic acid are partly demethylated to D-glucuronic acid but the methyl-glucuronic acid may still be detected chromatographically. It is probable therefore that

only D-glucuronic acid residues are present in the xylan. Furthermore the low methoxyl content of the polysaccharide suggested that few, if any, uronic acid residues were present as the methyl ether. The discrepancy between the determined uronic anhydride (3·2%) in the xylan and the amount of tetramethylaldobiuronic acid (mol. % *ca.* 1) isolated from the hydrolysis of the methylated xylan may arise from decomposition of uronic acid residues during hydrolysis or from loss of the acidic components during deionisation of the hydrolysate.

A molecular-weight determination by the isothermal-distillation method (by the courtesy of Dr. C. T. Greenwood) gave a value of 8000 ± 500 (degree of polymerisation 47—53) for the methylated xylan. This value, taken together with the value of one non-reducing terminal group per 40—45 xylose residues obtained from the methylation data, suggests that wheat-straw xylan comprises an unbranched chain of xylose residues. Although the presence of some monomethylxylose would be expected from under-methylation of the polysaccharide and/or demethylation during hydrolysis of the methylated polysaccharide, it is interesting that the monomethylxylose consisted almost entirely of the 2-*O*-methyl isomer. Hydrolysis of some aldobiuronic acid units would be expected and it is probable that some of the 2-*O*-methyl-D-xylose was produced in this way.

During the present investigation the results of structural investigations of wheat-straw hemicellulose have been published elsewhere. Without attempting the isolation of a xylan containing no arabinose residues, Adams (*Canad. J. Chem.*, 1952, **30**, 698) studied the structure of a wheat-straw hemicellulose and on the basis of methylation data put forward a structure consisting of a chain of 32 1 : 4-linked D-xylopyranose residues to which are attached 5 L-arabofuranose and 3 D-glucopyruronic acid residues linked through position 3. In addition to the higher proportion of uronic acid residues, it was later suggested by Bishop (*ibid.*, 1953, **31**, 134), investigating the aldobiuronic acid 3-*O*-D-glucopyruronosyl-D-xylose isolated on hydrolysis of the same hemicellulose, that every third uronic acid residue was present as the monomethyl ether. In other respects this hemicelluose appears similar to our wheat-straw xylan: (*a*) the molecular dimensions are of the same order; and (*b*) the side-chains are linked to the main chain through position 3 of the xylose residues. In the light of our present work and of the previous structural investigations on esparto hemicelluloses it seems probable that Adams and Bishop's wheat-straw hemicellulose consists of a mixture of molecular species, ranging from a xylan carrying glucuronic acid but no arabinose side-chains to a highly branched araboxylan.

The present investigation indicates that this xylan, which is only one component of wheat-straw hemicellulose, contains a straight chain of 1 : 4-linked β-D-xylopyranose residues, to one of the non-terminal residues of which a D-glucopyruronic acid residue is linked through position 3. This xylan, therefore, differs in its fine structure from both esparto and pear cell-wall xylans; it resembles pear cell-wall xylan in containing a D-glucuronic acid residue linked to the main chain through position 3, but differs in molecular size and in having an unbranched chain of xylose residues.

Experimental

The following solvents (v/v) were used to separate the sugars and their derivatives: (A) butanol–benzene–pyridine–water (5 : 1 : 3 : 3, top layer), (B) butanol–ethanol–water (4 : 1 : 5, top layer), and (C) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4).

Isolation of Wheat-straw Xylan.—Wheat straw (variety "White Victor," cut in September 1950; 500 g.) was extracted successively with benzene and methanol, and was then delignified by Wise's method (*Ind. Eng. Chem. Anal.*, 1945, **17**, 63). The holocellulose (318 g.) was extracted with sodium hydroxide solution (4%), the extract acidified with glacial acetic acid, and the crude xylan precipitated by addition of an equal volume of ethanol. The polysaccharide was purified by five successive precipitations of the copper complex formed on addition of Fehling's solution to a solution in aqueous sodium hydroxide (4%). Further purification was effected by two extractions with boiling aqueous ethanol (70%; v/v). The purified xylan (29·6 g.) had $[\alpha]_D^{20}$ −93° (*c*, 0·21 in N-sodium hydroxide) [ash (as sulphate), 0·74; lignin 0·4; uronic anhydride, 3·2; OMe, 0·4%]. Chromatographic examination of the

hydrolysate (Hirst and Jones, *J.*, 1949, 1659) in solvent A showed the presence of xylose (88%) and arabinose (0·5%).

Methylation of Wheat-straw Xylan.—Xylan (18·4 g.) was methylated ten times with methyl sulphate and sodium hydroxide, and the product was fractionated by dissolution in boiling chloroform–light petroleum (b. p. 60—65°) mixtures. The two main fractions were further methylated twice with methyl iodide and silver oxide, and the products combined and fractionated as before, to give a main fraction, soluble in boiling chloroform–light petroleum (30 : 70) (9·4 g.) {OMe, 38·2%; $[\alpha]_D^{14}$ −82·7° (*c*, 0·45 in $CHCl_3$)}.

Hydrolysis of Methylated Xylan.—The methylated xylan (5·0 g.) was refluxed successively with methanolic hydrogen chloride (300 c.c.; 0·5%) for 24 hr. and with hydrochloric acid (300 c.c.; 0·5N) for 16 hr. The hydrolysate was neutralised with Amberlite resin IR-4B, and the solution concentrated to a syrup (4·9 g.). Quantitative paper chromatography (Hirst, Hough, and Jones, *J.*, 1949, 298) in solvent B showed the presence of tri-, di-, and mono-methyl xylose in the ratio 1 : 34 : 1.

Separation of Methylated Sugars.—The syrup (4·9 g.) was fractionated on cellulose (90 × 4 cm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511) with light petroleum (b. p. 100—120°)–*n*-butanol (7 : 3) saturated with water as eluant, to give four fractions.

Fraction 1. The syrup (144 mg.) did not crystallise and hypoiodite oxidation indicated 79% of aldopentose. A sample (5 mg.) was rehydrolysed and chromatographic examination of the hydrolysate in solvent B showed the presence of 2 : 3-di- and 2 : 3 : 4-tri-*O*-methylxylose. The syrup therefore contained some (*ca.* 20%) methyl 2 : 3-di-*O*-methylxyloside.

The syrup (133 mg.) was rehydrolysed with N-hydrochloric acid (20 c.c.) on the water-bath for 6 hr. {$[\alpha]_D^{20}$ + 23 ⟶ +20° (5 hr., const.)}. After neutralisation with silver carbonate the resulting syrup (130 mg.) was fractionated on cellulose (50 × 1·4 cm.) as before, to give fractions 1*a* (101 mg.) and 1*b* (25 mg.). Fraction 1*a* after recrystallisation from dry ether had m. p. and mixed m. p. (with authentic 2 : 3 : 4-tri-*O*-methyl-D-xylose) 89° and $[\alpha]_D^{16}$ +20° (*c*, 0·9 in H_2O) (Found: OMe, 48·2. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). The derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 101°. Fraction 1*b* was identified as 2 : 3-di-*O*-methyl-D-xylose by conversion into its aniline derivative, m. p. and mixed m. p. 122°.

Fraction 2. The syrup (4·18 g.) had $[\alpha]_D^{20}$ +22·9° (*c*, 1·49 in H_2O), n_D^{20} 1·4694 (Found: OMe, 33·9. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). Chromatographic examination showed only 2 : 3-di-*O*-methyl-D-xylose, and hypoiodite oxidation indicated 98·4% purity. The syrup partially crystallised when seeded and had m. p. 77—78°. The sugar was identified by conversion into 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 122—123°, and into 2 : 3-di-*O*-methyl-D-xylonamide, m. p. and mixed m. p. 132°.

Fraction 3. The syrup (139 mg.) crystallised and after recrystallisation from methanol had m. p. and mixed m. p. with authentic 2-*O*-methyl-D-xylose 135—136°, and $[\alpha]_D^{19}$ +30° (*c*, 1·6 in H_2O) (Found: OMe, 18·4. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). Chromatographic examination showed only 2-*O*-methyl-D-xylose, and hypoiodite oxidation indicated 99% purity. The identity of the sugar was confirmed by conversion into 2-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 123—124°.

Fraction 4. Elution of the cellulose column with water gave a solid (308 mg.) incompletely soluble in ethanol, methanol, and water. Chromatographic examination in solvent B showed two spots [R_G 0·05 and 0·09—0·10 (dipolar)], and demethylation (Hough, Jones, and Wadman, *J.*, 1950, 1702) gave xylose, 2-*O*-methylxylose, and a trace of 2 : 3-di-*O*-methylxylose. A sample (10 mg.) was heated in a sealed tube at 100° for 6 hr. with methanolic hydrogen chloride, and the solution neutralised with silver carbonate and taken to dryness. An ethereal solution of the resulting syrup was treated with lithium aluminium hydride as described by Chanda, Hirst, and Percival (*loc. cit.*) and chromatographic examination of the hydrolysate showed the presence of 2-*O*-methyl-D-xylose and 2 : 3 : 4-tri-*O*-methyl-D-glucose.

The remainder of fraction 4 (270 mg.), which was contaminated with inorganic material, was purified by dissolution in hot methanol and the resulting syrup was heated on the water-bath for 5 hr. with N-hydrochloric acid (20 c.c.). After neutralisation with silver carbonate, the hydrolysate was fractionated on filter sheets with solvent B, to give fractions 4*a* (20 mg.) and 4*b* (72 mg.). Fraction 4*a* was shown chromatographically to consist of 2 : 3-di-*O*-methyl-D-xylose only. Fraction 4*b* had $[\alpha]_D^{16}$ +97·5° (*c*, 0·72 in H_2O) (Found: OMe, 32·2%; equiv. 350. Calc. for $C_{15}H_{26}O_{11}$: OMe, 32·4%; equiv., 382).

Fraction 4*b* (13 mg.) was heated with methanolic hydrogen chloride (2 c.c.; 1%) for 6 hr. and neutralised in the usual way. The resulting ester glycoside was reduced with lithium

aluminium hydride (Chanda, Hirst, and Percival, *loc. cit.*). The 2-*O*-methyl-D-xylose and 2 : 3 : 4-tri-*O*-methyl-D-glucose formed on hydrolysis of the product were separated chromatographically in solvent B, and estimation by hypoiodite oxidation (Chanda, Hirst, Jones, and Percival, *loc. cit.*) showed them to be present in the ratio of 1·1 : 1. The aldobiuronic acid (53·6 mg.) was converted into the sodium salt and oxidised with 0·25M-sodium metaperiodate solution (3 c.c.), and the periodate consumed after 26 hr. estimated by Fleury and Lange's method (*J. Pharm. Chim.*, 1933, **17**, 107, 196) (Found : 0·8 mol. per $C_{15}H_{26}O_{11}$ unit). Examination of the products of periodate oxidation by the method of Buchanan, Dekker, and Long (*J.*, 1950, 3162) showed that no formic acid was produced.

Chromatographic Examination of the Acidic Fraction from Xylan Hydrolysis.—Xylan (20 g.) was heated with N-sulphuric acid (400 c.c.) at 100° for 7 hr. and the hydrolysate neutralised by passage through a column of Amberlite resin 1R-4B. The resin was washed with 2N-sulphuric acid, and then with water until the eluate was free from sulphate ions. The eluate was neutralised with barium carbonate and the filtrate concentrated to a syrup (A), chromatographic examination of which showed the presence of xylose and an aldopolyuronic acid. Syrup (A) was poured into ethanol, the resulting precipitate was removed, and the solution taken to dryness to give a pale yellow solid (B). The solid (B) which contained xylose and the water-soluble barium salt of an aldopolyuronic acid was hydrolysed with 2N-sulphuric acid (2 c.c.) at 100° for 8 hr. The hydrolysate was partially neutralised with barium carbonate, and the filtrate examined chromatographically, showing the presence of xylose, glucuronic acid, and glucurone but the absence of 4-*O*-methylglucuronic acid.

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