724. *The Constitution of Jute Hemicellulose I.*

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Partial acid hydrolysis of jute hemicellulose I yields the aldobiouronic acid (2-D-xylose 4-*O*-methyl-α-D-glucopyranosid)uronic acid. Hydrolysis of the methylated polysaccharide affords 2 : 3 : 4-tri-*O*-methyl-D-xylose, 2 : 3-di-*O*-methyl-D-xylose, 3-*O*-methyl-D-xylose, and (3-*O*-methyl-2-D-xylose 2 : 3 : 4-tri-*O*-methyl-α-D-glucopyranosid)uronic acid in the approximate molar ratio 3 : 79 : 4 : 14. The methylated polysaccharide has a molecular weight of 21,000 ± 500 (degree of polymerisation, 123 ± 3). It is concluded that the polysaccharide is composed of chains of *ca.* 108 1 : 4-linked β-D-xylopyranose residues with approximately every seventh residue carrying a terminal 4-*O*-methyl-α-D-glucopyranosiduronic acid residue linked through position 2. A small degree of branching in the backbone of D-xylose residues is indicated.

The various xylans from land plants contain backbones of 1 : 4-linked β-D-xylopyranose residues but differ amongst themselves in molecular size, and in the nature, number, and mode of attachment of other sugar residues linked as side-chains.[1] The xylans from cereals [1] are generally characterised by the presence of L-arabofuranose residues linked to position 3 of xylose, although in several cases uronic acid residues are also present. The xylans from both hard and soft woods [2] all contain 4-*O*-methyl-D-glucuronic acid residues attached as side-chains by 1 : 2-linkages, but some also contain a small proportion of L-arabofuranose residues. The hemicelluloses of the bast fibres have been less extensively studied and this paper describes the structure of a xylan from jute. Some preliminary investigations on this material have been reported.[3,4]

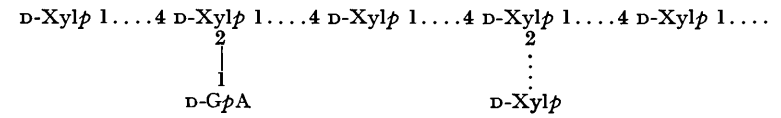
Partial acid hydrolysis of jute hemicellulose I yields xylose and an acidic fraction. On

[1] Hirst, *J.*, 1955, 2974.
[2] Aspinall and McKay, *J.*, 1958, 1059, and references there cited.
[3] Sarkar, Mazumder, and Pal, *Textile Res. J.*, 1952, **22**, 529.
[4] Das Gupta and Sarkar, *ibid.*, 1954, **24**, 705.

the basis of its reaction with periodate the structure (3-D-xylose 3-*O*-methyl-D-glucopyranosid)uronic acid was proposed for the acidic material.[4] Chromatography of this material, however, showed a main component together with three minor components, and a pure sample of the aldobiouronic acid was isolated after separation of the mixture on cellulose.[5] The acidic disaccharide contained residues of 4-*O*-methyl-D-glucuronic acid and D-xylose since conversion into the methyl ester methyl glycoside followed by reduction with lithium aluminium hydride and hydrolysis afforded 4-*O*-methyl-D-glucose and D-xylose. The aldobiouronic acid was established to be (2-D-xylose 4-*O*-methyl-α-D-glucopyranosid)uronic acid since reduction of the methylated derivative with lithium aluminium hydride followed by re-methylation and hydrolysis gave 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and 3 : 4-di-*O*-methyl-D-xylose. The large positive optical rotations of the aldobiouronic acid and its methylated derivative indicate the presence of an α-glycosidic linkage. The minor acidic components were not examined in detail but it is probable that 4-*O*-methylglucuronic acid and an aldotriouronic acid were present. In addition, reduction and hydrolysis of one fraction afforded galactose and rhamnose, the former probably arising from galacturonic acid. It is likely that these sugars are formed from a small amount of a polysaccharide contaminant since subsequent methylations gave no evidence that they were constituents of the xylan.

Jute hemicellulose was converted into its fully methylated derivative, two main fractions of which were isolated. These fractions had similar physical properties and hydrolysis gave the same sugars but in slightly different proportions. One fraction was examined in greater detail; hydrolysis gave 2 : 3 : 4-tri-*O*-methyl-, 2 : 3-di-*O*-methyl-, and 3-*O*-methyl-D-xylose, and a partially methylated aldobiouronic acid in the approximate molar ratio of 3 : 79 : 4 : 14. The following experiments showed the acidic disaccharide to be (3-*O*-methyl-2-D-xylose 2 : 3 : 4-tri-*O*-methyl-α-D-glucopyranosid)uronic acid. The methylated aldobiouronic acid was converted into the methyl ester methyl glycoside which was reduced with lithium aluminium hydride. Hydrolysis of a portion of the partially methylated disaccharide gave 2 : 3 : 4-tri-*O*-methylglucose and 3-*O*-methylxylose, identified by chromatography and ionophoresis. The remaining material was re-methylated; 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 chains of 1 : 4-linked β-D-xylopyranose residues with approximately every seventh xylose residue carrying a terminal 4-*O*-methyl-D-glucuronic acid residue attached as a side-chain to position 2.

Determination of molecular weight by isothermal distillation (by the courtesy of Dr. C. T. Greenwood) gave a value of 21,000 ± 500 (degree of polymerisation, 123 ± 3) for the methylated polysaccharide. Since the methylation analysis indicated the presence of three non-reducing xylose end groups for a molecule of this size there will be on the average two branches in the main chain of xylose residues per molecule. It follows also that the branch points must involve 1 : 2-linkages, but at present there is no indication of the length of the side-chains. The accompanying partial structure for the xylan indicates the main features of the molecule.



(D-Xyl*p* = D-xylopyranose, D-G*p*A = 4-*O*-methyl-D-glucuronic acid.)

Probably, in the case of jute hemicellulose, as with other xylans, a range is present of closely-related molecular species of the same general type, which differ in their more detailed structures. This is suggested by the isolation of two fractions of methylated jute

[5] Hough, Jones, and Wadman, *J.*, 1949, 2511; Whistler, Conrad, and Hough, *J. Amer. Chem. Soc.*, 1954, **76**, 1663.

hemicellulose, hydrolysis of which gave slightly different porportions of tri-, di-, and mono-*O*-methyl sugars, and by the fact that the detailed methylation analysis indicated the presence of one uronic acid residue per seven xylose residues, whereas the uronic acid content of the original polysaccharide [3] showed one acid residue per six xylose residues.

This jute xylan then resembles most closely the wood xylans in carrying 4-*O*-methyl-D-glucuronic acid residues as single-unit side-chains attached to position 2 of xylose residues in the main chain. It differs, however, from the wood xylans in that there is clear evidence of branching in the backbone of xylose residues.

[Added, *August 22nd*, 1958.—Since the submission of this paper Srivastava and Adams (*Chem. and Ind.*, 1958, 920) reported conclusions, similar to those described here, regarding the structure of the aldobiouronic acid from jute hemicellulose. From the partial acid hydrolysis of jute fibres they isolated and characterised the same aldobiouronic acid (2-D-xylose 4-*O*-methyl-α-D-glucosid)uronic acid, and identified 4-*O*-methyl-D-glucuronic acid and the acidic trisaccharide, D-Xyl 4 ⟶ 1 β-D-Xyl*p* 2 ⟶ 1 α-D-G*p*A].

Experimental

Jute hemicellulose I was prepared as described previously.[3,4] Paper partition chromatography was carried out on Whatman No. 1 filter paper with the following solvent systems (v/v): (A) butanol–ethanol–water (4 : 1 : 5; upper layer); (B) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4); (C) ethyl acetate–acetic acid–water (9 : 2 : 2); (D) butan-2-one, two-thirds saturated with water; (E) light petroleum (b. p. 100—120°)–butanol (7 : 3), saturated with water; (F) benzene–ethanol–water (169 : 47 : 15; upper layer). Paper ionophoresis was in borate buffer at pH 10.

Partial Acid Hydrolysis of Jute Hemicellulose I and Separation of Acidic Oligosaccharides.—Hemicellulose (40 g.) was heated with N-sulphuric acid (2 l.) at 100° for 7 hr. The cooled solution was neutralised with barium hydroxide and barium carbonate, the filtrate was concentrated to small volume, and the solution was poured into ethanol. The precipitated barium salts were extracted with boiling ethanol to remove adhering xylose, and redissolved in water, and the solution was treated with Amberlite resin IR-120(H) to remove barium ions, and concentrated to a syrup. Chromatography in solvent B showed a main component with R_{xylose} 0·85 and others with R_{xylose} 1·14, 0·58, 0·41, and 0·22, the first of these moving at the same rate as 4-*O*-methyl-D-glucuronic acid.

The acidic syrup (3·2 g.) was dissolved in a little water, absorbed on cellulose powder, and freeze-dried, and the mixture was placed on a cellulose column (48 × 4·5 cm.).[5] After elution with solvent C three fractions were isolated and examined further. The main component, fraction 1 (1·2 g.), had R_{xylose} 0·85, $[\alpha]_D^{19}$ +84·7° (*c* 1·5 in H_2O) (Found: OMe, 8·5%; equiv., 368. Calc. for $C_{12}H_{22}O_{11}$: OMe, 9·1%; equiv., 342). Fraction 2 (140 mg.) had R_{xylose} 0·58, $[\alpha]_D^{19}$ +45° (*c* 1·0 in H_2O), and gave xylose and rhamnose as neutral components on hydrolysis; conversion into the methyl ester methyl glycoside, followed by reduction with lithium aluminium hydride and hydrolysis, gave xylose, rhamnose, galactose, 4-*O*-methylglucose, and a trace of glucose. Fraction 3 (260 mg.) had R_{xylose} 0·41, $[\alpha]_D^{19}$ +46° (*c* 2·0 in H_2O), and gave xylose as the only neutral product of hydrolysis; reduction of the methyl ester methyl glycoside and hydrolysis gave xylose, 4-*O*-methylglucose, and a trace of glucose.

Characterisation of Fraction 1 *as* (2-D-*Xylose* 4-O-*Methyl*-α-D-*glucopyranosid*)*uronic Acid.*—The aldobiouronic acid (0·4 g.) was refluxed with methanolic 2% hydrogen chloride, and the resulting methyl ester methyl glycoside was reduced with lithium aluminium hydride in tetrahydrofuran.[6] The product was hydrolysed with N-sulphuric acid at 100° for 7 hr., and after neutralisation with barium carbonate the hydrolysate was separated on filter sheets, solvent A being used, giving fractions *a* and *b*. Chromatography of the sugar and the products of periodate oxidation [7] indicated that fraction *a* was 4-*O*-methylglucose [4-*O*-methyl-D-glucose phenylosazone, identified by m. p. 158—159° and *X*-ray powder photograph (Found: OMe, 8·5; N, 15·4. Calc. for $C_{19}H_{26}O_4N_4$: OMe, 8·3; N, 15·0%)]. Fraction *b* had m. p. and mixed m. p. (with D-xylose) 143°, $[\alpha]_D^{19}$ +18° (*c* 1·0 in H_2O), and was further characterised by conversion into the di-*O*-benzylidene dimethyl acetal, m. p. 211°.

[6] Lythgoe and Trippett, *J.*, 1950, 1983.
[7] Lemieux and Bauer, *Canad. J. Chem.*, 1953, **31**, 814.

Treatment of the aldobiouronic acid (0·7 g.), first with methyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide gave the methylated derivative (627 mg.), $[\alpha]_D^{18}$ +89° (*c* 1·5 in $CHCl_3$) (Found: OMe, 49·8. Calc. for $C_{18}H_{32}O_{11}$: OMe, 51·2%). Reduction of the methylated aldobiouronic acid with lithium aluminium hydride [6] in ether, followed by methylation with methyl iodide and silver oxide, furnished the fully methylated disaccharide (450 mg.), $[\alpha]_D^{19}$ +93·2° (*c* 1·4 in $CHCl_3$) (Found: OMe, 52·2. Calc. for $C_{18}H_{34}O_{10}$: OMe, 52·9%). Hydrolysis of this (400 mg.) with N-sulphuric acid at 100° for 6 hr. afforded syrupy sugars (300 mg.) which were separated on cellulose (60 × 1·7 cm.) by solvent E into two fractions. Fraction (i) (105 mg.) was 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, m. p. and mixed m. p. 78—84°, $[\alpha]_D^{18}$ +84° (equil.) (*c* 1·0 in H_2O) (Found: OMe, 52·0. Calc. for $C_{10}H_{20}O_6$: OMe, 52·5%). The aniline derivative (from light petroleum) had m. p. and mixed m. p. 126—128°, $[\alpha]_D^{17}$ +206° (*c* 0·5 in $COMe_2$). Chromatography and ionophoresis of fraction (ii) (75 mg.) showed only 3 : 4-di-*O*-methylxylose, and the sugar was characterised by conversion into 3 : 4-di-*O*-methyl-D-xylonolactone, m. p. 66—67°, $[\alpha]_D^{17}$ −20·5° (equil.) (*c* 1·0 in H_2O) (Found: OMe, 34·9. Calc. for $C_7H_{12}O_5$: OMe, 35·2%). The derived aldonamide, after treatment with sodium hypochlorite and addition of semicarbazide, afforded hydrazodicarbonamide, m. p. 256°.

Methylation of Jute Hemicellulose.—The polysaccharide (25 g.) was methylated by successive additions of methyl sulphate and sodium hydroxide, and then with methyl iodide and silver oxide to give methylated jute hemicellulose (27 g.), $[\alpha]_D^{23}$ −32·6° (*c* 0·8 in $CHCl_3$) (Found: OMe, 38·6%). This (20 g.) was fractionated by dissolution in boiling chloroform–light petroleum (b. p. 40—60°), and two main fractions were obtained: (1) {7·5 g., soluble in chloroform–light petroleum (3 : 7), $[\alpha]_D^{25}$ −33·2° (*c* 1·0 in $CHCl_3$); OMe, 38·9%} and (2) {11·8 g., soluble in chloroform–light petroleum (4 : 6), $[\alpha]_D^{25}$ −36·1° (*c* 1·0 in $CHCl_3$); OMe, 38·9%}. Hydrolysis of fraction (1) [*M* (isothermal distillation in benzene), 20,700 ± 500 (degree of polymerisation, 121 ± 3)], and quantitative chromatography [8] of the resulting neutral methylated sugars showed tri-, di-, and mono-*O*-methylxylose in the molar percentages 2·5, 94·2, and 3·3. Hydrolysis of fraction (2) [*M*, 21,000 ± 500 (degree of polymerisation, 123 ± 3)] gave tri-, di-, and mono-*O*-methylxylose in the molar percentages 3·3, 92·1, and 4·6. Fraction (2) was used in subsequent experiments.

Hydrolysis of Methylated Hemicellulose and Separation of Methylated Sugars.—The methylated polysaccharide (6 g.) was hydrolysed successively with boiling methanolic 1% hydrogen chloride (500 ml.) for 9 hr. and with 0·5N-hydrochloric acid (360 ml.) at 100° for 7 hr. Evaporation after neutralisation with silver carbonate yielded a syrup (7 g.), which was treated (in aqueous solution) with barium carbonate. The mixture of methylated sugars was fractionated on cellulose (60 × 3·5 cm.) by solvent D into three fractions, and elution with water gave a fourth fraction, containing barium salts, which was treated with Amberlite resin IR-120(H) to remove barium ions and concentrated to a syrup.

Fraction 1. Chromatography of the syrup (112 mg.) showed 2 : 3 : 4-tri-*O*-methylxylose, but hydrolysis of a portion gave some 2 : 3-di-*O*-methylxylose indicating the presence of unhydrolysed methyl glycoside. After hydrolysis of the syrup with N-hydrochloric acid at 100° for 4 hr., the hydrolysate was separated on filter sheets with solvent D, and 2 : 3 : 4-tri-*O*-methyl-D-xylose (80 mg.) was isolated, m. p. and mixed m. p. 86—87°, $[\alpha]_D^{20}$ +19·9° (equil.) (*c* 0·5 in H_2O) (Found: OMe, 48·0. Calc. for $C_8H_{16}O_5$: OMe, 48·4%).

Fraction 2. The syrup (2·276 g.) crystallised when seeded with 2 : 3-di-*O*-methyl-β-D-xylose and had m. p. and mixed m. p. 78°, $[\alpha]_D^{21}$ −27·9° (2 min.) ⟶ +24° (77 min., const.) (*c* 2·0 in H_2O) (Found: OMe, 34·3. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The identity was confirmed by conversion of the sugar into 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine, m. p. and mixed m. p. 123°, $[\alpha]_D^{20}$ +183° (*c* 0·7 in ethyl acetate) (Found: OMe, 24·2; N, 5·6. Calc. for $C_{13}H_{19}O_4N$: OMe, 24·5; N, 5·5%), and 2 : 3-di-*O*-methyl-D-xylonamide, m. p. 132°, $[\alpha]_D^{20}$ +50° (*c* 1·0 in H_2O) (Found: OMe, 31·9; N, 7·4. Calc. for $C_7H_{15}O_5N$: OMe, 32·1; N, 7·25%).

Fraction 3. Chromatography and ionophoresis of the syrup (111 mg.), $[\alpha]_D^{17}$ +15·6° (*c* 1·0 in H_2O), showed 3-*O*-methylxylose and a trace of the 2-methyl ether.

Fraction 4. Chromatography of the syrup (855 mg.) in solvent B showed a main component and small amounts of 2 : 3 : 4-tri-*O*-methylglucuronic acid. The syrup (0·5 g.) was converted into the methyl ester methyl glycoside by refluxing with methanolic 1% hydrogen chloride (100 ml.) for 7 hr. Lithium aluminium hydride in methylal was added slowly to a boiling solution of the ester glycoside in methylal and the mixture was refluxed for 2 hr. Excess

[8] Hirst, Hough, and Jones, *J.*, 1949, 928; Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289.

of hydride was destroyed by water, the solution was filtered, methylal was removed under reduced pressure, and the aqueous solution was de-ionised with Amberlite resins IR-120(H) and IR-4B(OH), and concentrated to a syrup (415 mg.). A portion (100 mg.) of the syrup was hydrolysed with N-sulphuric acid at 100° for 6 hr., and chromatography and ionophoresis of the hydrolysate showed 2 : 3 : 4-tri-*O*-methylglucose, 3-*O*-methylxylose, and traces of 2 : 3-di-*O*-methylxylose. Another portion of the syrup was methylated with methyl iodide and silver oxide; hydrolysis of the fully methylated disaccharide with N-sulphuric acid at 100° for 6 hr. gave a mixture which was separated on cellulose (60 × 1·7 cm.) with solvent E into two main fractions *a* and *b*. The sugar (90 mg.) was identified as 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose, m. p. and mixed m. p. 78—84°, $[\alpha]_D^{17}$ +96° ⟶ +83·8° (*c* 1·0 in H_2O) {aniline derivative, m. p. 126—128°, $[\alpha]_D^{17}$ +206° (*c* 0·5 in $COMe_2$)}. Fraction *b* (65 mg.) was shown by chromatography and ionophoresis to be 3 : 4-di-*O*-methylxylose and was identified by conversion into 3 : 4-di-*O*-methyl-D-xylonolactone, m. p. 66—67°, $[\alpha]_D^{17}$ −54° ⟶ −20·5° (66 hr., const.) (*c* 0·8 in H_2O).

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