Hemicellulose A of Beechwood (Fagus sylvatica).

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Beech hemicellulose A has been shown to contain residues of D-xylose and 4-*O*-methyl-D-glucuronic acid (by conversion into 4-*O*-methyl-D-glucose). The methylated polysaccharide gave on hydrolysis 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-*O*-(2 : 3 : 4-tri-*O*-methyl-D-glucopyruronosyl)-D-xylose in the molar ratio of 1 : 60 : 7 : 7. A structure is proposed for the hemicellulose which has a straight chain of *ca.* 70 1 : 4-linked β-D-xylopyranose residues with every tenth residue carrying a terminal 4-*O*-methyl-D-glucopyruronic acid residue linked through position 2.

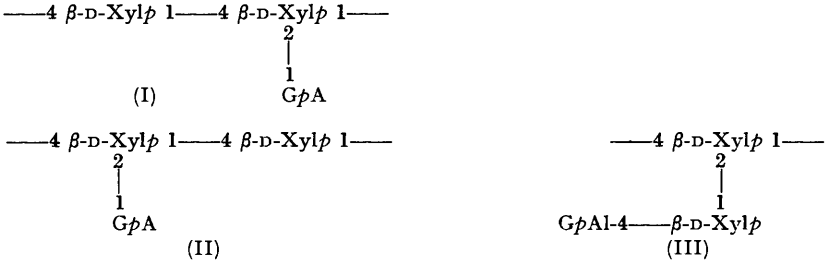
Analytical studies of the hemicelluloses of hard woods have shown that these polysaccharides consist mainly of xylose residues, but, in addition, evidence for the presence of a monomethylhexuronic acid has been obtained (cf. O'Dwyer, *Biochem. J.*, 1939, **33**, 713; 1940, **34**, 149). It was of interest, therefore, to investigate the fine structure of a polysaccharide of this type, particularly in the light of structural studies on the glucuronic acid-containing xylans from the cell-wall of ripe pears (Chanda, Hirst, and Percival, *J.*, 1951, 1240) and wheat straw (Aspinall and Mahomed, preceding paper).

For the present investigation a quantity of the hemicellulose A isolated from European beech wood (*Fagus sylvatica*) was kindly placed at our disposal for structural investigations by Mr. I. R. C. McDonald of the Forest Products Research Laboratory, Princes Risborough (for details of the isolation see McDonald, *J.*, 1952, 3183). It may be recalled that the hemicellulose was obtained by direct alkaline extraction of the extractive-free wood instead of from the "holocellulose" fraction obtained after delignification of the wood. In view of the work of Timell and Jahn (*Svensk Papperstidning*, 1951, **24**, 831), who showed that the polysaccharides of paper birch were degraded during chlorite delignification, and of Jayme and Hank (*Cellulose-chem.*, 1943, **21**, 127) and Bublitz (*Tech. Assoc. Pulp Paper*

Ind., 1951, **34**, 427), who showed that polysaccharides are present in the chlorite delignification liquors of spruce wood, it was felt advisable to carry out these structural investigations on material prepared without delignification.

The beech hemicellulose A, $[\alpha]_D^{20}$ −89·4° (*c*, 0·35 in N-sodium hydroxide), gave on hydrolysis xylose (81·6%) and a trace of rhamnose (*ca.* 0·4%). In addition, the presence of uronic anhydride (9·5%) and of a significant methoxyl content (1·7%) suggested that *O*-methylhexuronic acid residues might also be present. Methylation of the polysaccharide and fractionation of the product gave methylated beech hemicellulose A {$[\alpha]_D^{19}$ −75° (*c*, 0·23 in $CHCl_3$)}. Separation of the products of hydrolysis of the methylated polysaccharide on cellulose gave tri-, di-, and mono-*O*-methylxylose and a tetra-*O*-methylaldobiuronic acid in the molar ratio of 1 : 60 : 7 : 7. The neutral sugars were identified by the formation of crystalline derivatives as 2 : 3 : 4-tri-*O*-methyl-, 2 : 3-di-*O*-methyl-, and 3-*O*-methyl-D-xylose respectively.

The acidic fraction was identified as 3-*O*-methyl-2-*O*-(2 : 3 : 4-tri-*O*-methyl-α-D-glucopyruronosyl)-D-xylose in the following way. Reduction of the methyl ester glycoside with lithium aluminium hydride (Lythgoe and Trippett, *J.*, 1950, 1983) followed by hydrolysis and chromatographic separation gave 3-*O*-methyl-D-xylose and 2 : 3 : 4-tri-*O*-methyl-D-glucose in the molar ratio of 1·15 : 1·0. The sugars were identified by conversion into 3-*O*-methyl-D-xylosazone and methyl 2 : 3 : 4-tri-*O*-methyl-β-D-glucopyranoside respectively. That the glucuronic acid residue was linked through position 2 and not position 4 of the xylose residue was shown by the following observations: (*a*) further methylation of the aldobiuronic acid followed by reduction with lithium aluminium hydride and hydrolysis gave 2 : 3 : 4-tri-*O*-methyl-D-glucose, 3 : 4-di-*O*-methyl-D-xylose (chromatographically separable from 2 : 3-di-*O*-methyl-D-xylose), and 3-*O*-methyl-D-xylose together with a trace of 2 : 3 : 4-tri-*O*-methyl-D-xylose; (*b*) periodate oxidation of the derived 3 : 4-di-*O*-methylxylonic acid gave no formaldehyde, whereas authentic 2 : 3-di-*O*-methyl-D-xylonic acid yielded formaldehyde under similar conditions. The chromatographic identification of a trace of 2 : 3 : 4-tri-*O*-methyl-D-xylose indicates that some aldotriuronic acid was also present in the acidic fraction. The trimethylxylose could only have arisen from a trisaccharide unit derived from the polysaccharide as in (I).



Trisaccharide units (II) and (III) would have given rise to 2 : 3-di-*O*-methylxylose on further methylation followed by hydrolysis. This observation provides further evidence that the uronic acid residues are linked directly to the main chain of xylose residues as in (I) and are not linked through a side-chain as in (III).

The acidic components in beech hemicellulose A were shown to be 4-*O*-methyl-D-glucuronic acid residues by conversion into 4-*O*-methyl-D-glucose. From hydrolysis of the polysaccharide a crude aldobiuronic acid having a significant methoxyl content was isolated. Chromatographic examination showed that vigorous hydrolysis of the aldobiuronic acid yielded xylose, glucuronic acid, and 4-*O*-methylglucuronic acid. Conversion of the aldobiuronic acid into its methyl ester glycoside, followed by reduction with sodium borohydride and hydrolysis of the product gave xylose and 4-*O*-methyl-D-glucose, the latter sugar being identified as the corresponding osazone. 4-*O*-Methyl-D-glucuronic acid has also been isolated from the hydrolysis of aspen wood (*Populus tremuloides*) by Jones and Wise (*J.*, 1952, 2750) and of *Eucalyptus regnans* wood by Stewart and Foster (*Nature*, 1953, **171**, 792). It appears therefore to be a common constituent of wood hemicelluloses.

These results indicate that beech hemicellulose A consists of a linear chain of *ca.* 70 1 : 4-linked D-xylopyranose units with approximately every tenth xylose unit carrying a 4-*O*-methyl-D-glucuronic acid unit linked as a side-chain through position 2. This conclusion is supported by a molecular-weight determination by the isothermal distillation method (by the courtesy of Dr. C. T. Greenwood and Mrs. H. Zinkiewicz) which gave a value of 11,100 ± 500 (degree of polymerisation, 66—72) for the molecular weight of the methylated hemicellulose. It is difficult to explain the isolation of a large quantity (*ca.* 10%) of monomethyl xylose. Ionophoretic examination of this fraction showed it to consist almost entirely of the 3-*O*-methyl isomer whereas random undermethylation of the polysaccharide and demethylation during hydrolysis would be expected to yield more equal quantities of the two possible isomers. It is interesting that, in the case of wheat-straw xylan (Aspinall and Mahomed, *loc. cit.*) where branching of the glucuronic acid residue occurs through position 3 of the xylose residue, the monomethyl xylose isolated on hydrolysis of the methylated polysaccharide, consisted almost entirely of the 2-*O*-methyl isomer. No methyl ethers of rhamnose were isolated, so it would appear that the rhamnose obtained from hydrolysis of the polysaccharide arose either from a contaminating glycoside or from an associated polysaccharide. It will be recalled that L-rhamnose was isolated by Jones and Wise (*loc. cit.*) from the hydrolysis of aspen wood.

Beech hemicellulose A resembles most closely the polyuronide hemicellulose of New Zealand flax (*Phormium tenax*) (McIlroy, Holmes, and Mauger, *J.*, 1945, 796; McIlroy, *J.*, 1949, 121) in that an acid residue is linked to every tenth residue of the xylan chain. It is not known, however, whether the acidic residue in the latter case are of D-glucuronic acid or of 4-*O*-methyl-D-glucuronic acid. Beech hemicellulose differs from hemicelluloses of the xylan type previously examined in these laboratories in that branching to the main chain of xylose units occurs through position 2. In the araboxylan from esparto grass (Aspinall, Hirst, Moody, and Percival, *J.*, 1953, 1631) the L-arabofuranose units and in pear cell-wall xylan (Chanda, Hirst, and Percival, *loc. cit.*) and wheat-straw xylan (Aspinall and Mahomed, *loc. cit.*) the D-glucopyruronic acid units are linked to the xylan chain through position 3. The linkage through position 2 has also been found in the hemicelluloses of aspen wood by Jones and Wise (*J.*, 1952, 3389), who isolated 2-*O*-(4-*O*-methyl-D-glucopyruronosyl)-α-D-xylose from the products of hydrolysis. It is clear that hemicelluloses of the xylan type differ markedly amongst themselves in their fine structure and that further investigations are required to unravel their complex relations.

Experimental

The solvents (A, B, and C) used to separate the sugars and their derivatives were those detailed in the preceding paper.

The polysaccharide was prepared, and kindly made available, by Mr. I. R. C. MacDonald of the Forest Products Research Laboratory, Princes Risborough (see *J.*, 1952, 3183). It was received as a fine white powder, $[\alpha]_D^{20}$ −89·4° (*c*, 0·35 in N-NaOH) (Found: OMe, 1·7; uronic anhydride, 9·5; lignin, 1·9%). Chromatographic examination of the hydrolysate (Hirst and Jones, *J.*, 1949, 1659) in solvent A showed the presence of xylose (81·6%) and (*ca.* 0·4%) of rhamnose.

Methylation of Beech Hemicellulose A.—Hemicellulose A (20 g.) was methylated twelve times with methyl sulphate and sodium hydroxide and four times with methyl iodide and silver oxide. The product (14·6 g.; OMe, 38·2%) was fractionated in boiling chloroform–light petroleum (b. p. 60—65°) mixtures to give a main fraction (12·7 g.) {OMe, 38·6%; $[\alpha]_D^{19}$ −75° (*c*, 0·23 in $CHCl_3$), $[\alpha]_D^{18}$ −113° (*c*, 1·25 in *m*-cresol)}.

Hydrolysis of Methylated Hemicellulose A and Separation of Methylated Sugars.—Methylated hemicellulose A (5·0 g.) was hydrolysed successively with 1·5% methanolic hydrogen chloride (500 c.c.) for 14 hr. and N-hydrochloric acid (200 c.c.) at 100° for 12 hr. (constant rotation). Evaporation after neutralisation with silver carbonate yielded a syrup (4·75 g.). The syrup (3·9 g.) was fractionated on cellulose (90 × 4 cm.) (Hough, Jones, and Wadman, *J.*, 1949, 2511) with light petroleum (b. p. 100—120°)–*n*-butanol (65 : 35), saturated with water as eluant. Three discrete fractions, 1 (22 mg.), 2 (46 mg.), and 3 (56 mg.), were obtained, but thereafter all fractions were contaminated by the acidic component and the column was eluted with water

to give three further fractions, 4 (0·121 g.), 5 (2·988 g.), and 6 (0·321 g.) (recovery, 91%). Partial separation of fraction 4 was effected by dissolving it in water (10 ml.), neutralising the solution with barium carbonate, and exhaustively extracting it with chloroform. The aqueous solution was deionised with Amberlite resin IR-100 to give an acidic fraction 4*a* (63 mg.). The chloroform extract gave fraction 4*b* (35 mg.), which consisted mainly of dimethylxylose but also contained some of the acidic component. Fractions 4*b* and 5 were combined and refractionated on cellulose, with solvent B as eluant, to give fractions 7 (2·108 g.) and 8 (0·390 g.); elution with water gave fraction 9 (0·242 g.) (recovery, 91%).

Examination of the Neutral Fractions.—*Fraction* 1. The syrup crystallised and had m. p. 63°, $[\alpha]_D^{16}$ −47·3° (*c*, 0·15 in H_2O). The substance was non-reducing and after hydrolysis of a sample with N-hydrochloric acid at 100° for 3 hr., chromatographic examination of the hydrolysate showed only 2 : 3-di-*O*-methylxylose. It is concluded that the substance was methyl 2 : 3-di-*O*-methyl-β-D-xylopyranoside.

Fraction 2. The syrup crystallised completely when seeded with 2 : 3 : 4-tri-*O*-methyl-D-xylose and after recrystallisation from ether had m. p. and mixed m. p. 89°, $[\alpha]_D^{20}$ +22° (*c*, 0·9 in H_2O) (Found: OMe, 48·2. Calc. for $C_8H_{16}O_5$: OMe, 48·4%). Hypoiodite oxidation indicated 99% purity and the derived 2 : 3 : 4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 98—99°.

Fractions 7 *and* 8*a*. Fraction 7 and fraction 8*a* (see below) were shown to be chromatographically identical and were combined. The syrup had $[\alpha]_D^{15}$ +22·7° (*c*, 1·1 in H_2O), and hypoiodite oxidation indicated 97—98% purity (Found: OMe, 34·5. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The syrup crystallised slowly when seeded with 2 : 3-di-*O*-methyl-D-xylose and, separated on a porous tile, then had m. p. and mixed m. p. 78°. The derived 2 : 3-di-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. and mixed m. p. 123°.

Fraction 8. Chromatographic examination of the syrup showed the presence of two sugars and separation on cellulose (50 × 3 cm.) with light petroleum (b. p. 100—120°)–*n*-butanol (7 : 3), saturated with water as eluant, gave fractions 8*a* (112 mg.) and 8*b* (252 mg.). Fraction 8*a* was combined with fraction 7 (see above). Fraction 8*b*, a syrup which did not crystallise when seeded with 3-*O*-methyl-D-xylose, had $[\alpha]_D^{20}$ +17° (*c*, 0·8 in H_2O), and hypoiodite oxidation indicated a purity of 97% (Found: OMe, 18·5. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). Paper ionophoresis (Consden and Stanier, *Nature*, 1952, **170**, 1069) showed the presence of 3-*O*-methyl-D-xylose and of a trace of 2-*O*-methyl-D-xylose. The derived 3-*O*-methyl-*N*-phenyl-D-xylosylamine had m. p. 136°.

Examination of the Acidic Fractions.—Fractions 4*a*, 6, and 9 were chromatographically similar and were combined. Fraction 3 behaved differently on the chromatogram and was non-acidic (Found: OMe, 34·9. Calc. for $C_{15}H_{26}O_{11}$: OMe, 32·5%), but after hydrolysis with 0·5N-hydrochloric acid (50 c.c.) at 100° for 3 hr. was chromatographically identical with the other acidic fractions, and all the acidic fractions were combined.

The combined acidic fractions (0·682 g.) had $[\alpha]_D^{19}$ +51·5° (*c*, 0·78 in H_2O) (Found: OMe, 32·3%; equiv., 399. $C_{15}H_{26}O_{11}$ requires OMe, 32·5%; equiv., 382).

Reduction with Lithium Aluminium Hydride.—The acid (210 mg.) was refluxed with methanolic hydrogen chloride for 6 hr., neutralised, and taken to dryness. The resulting syrup was dissolved in dry ether (75 c.c.), and lithium aluminium hydride (200 mg.) was added during 3 hr. to the refluxing solution. Excess of hydride was destroyed by addition of water, and the solution was acidified with 2N-sulphuric acid and extracted with chloroform (3 × 50 c.c.). The chloroform extract was taken to dryness and the syrup was hydrolysed with 0·5N-hydrochloric acid (50 c.c.) for 7 hr. at 100°. After neutralisation with silver carbonate, the hydrolysate was shown chromatographically to contain sugars travelling at the same rate as 3-*O*-methyl-D-xylose and 2 : 3 : 4-tri-*O*-methyl-D-glucose. The aqueous extract from the reduction was taken to small volume, hydrolysed with N-sulphuric acid (10 c.c.) for 5 hr. at 100°, neutralised with barium carbonate, and taken to a syrup. The syrup was dissolved in methanol, filtered from inorganic material, taken to dryness, and deionised in aqueous solution with Amberlite resins IR-120 and IR-4B. Chromatographic examination of the resulting syrup showed the same two sugars as from the chloroform extract; the sugars from both chloroform and aqueous extracts were therefore combined to give a syrup (163 mg.).

This was fractionated on filter sheets with solvent B, to give fractions *a* (67 mg.) and *b* (79 mg.). Chromatographic and ionophoretic examination of fraction *a* showed only 3-*O*-methylxylose, and the syrup crystallised partly when seeded with 3-*O*-methyl-D-xylose. The crystals had m. p. and mixed m. p. 86—88°, $[\alpha]_D^{20}$ +19·5° (*c*, 0·51 in H_2O) (Found: OMe, 18·5. Calc. for $C_6H_{12}O_5$: OMe, 18·9%). The derived 3-*O*-methyl-D-xylosazone had m. p. and

mixed m. p. 172°. Fraction *b* 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. 92—93°.

Methylation and Reduction with Lithium Aluminium Hydride.—The acid (111 mg.) was converted into the methyl ester glycoside which was methylated twice with methyl iodide and silver oxide. The product was reduced with lithium aluminium hydride as described previously and the resulting syrup was hydrolysed with 0·5N-hydrochloric acid (25 c.c.) for 7 hr. at 100°. After neutralisation with silver carbonate the hydrolysate was taken to dryness, to give a syrup (56 mg.), chromatographic examination of which showed the presence of 2 : 3 : 4-tri-*O*-methylglucose, 3 : 4-di-*O*-methylxylose, 3-*O*-methylxylose, and a trace of 2 : 3 : 4-tri-*O*-methylxylose. Chromatographic separation of the sugars with solvent B, followed by hypoiodite oxidation (Chanda, Hirst, Jones, and Percival, *J.*, 1950, 1289), showed 2 : 3 : 4-tri-*O*-methylglucose, 3 : 4-di-*O*-methylxylose, and 3-*O*-methylxylose to be present in the molar ratio 1 : 0·95 : 0·48. The solutions resulting from the hypoiodite oxidation of 2 : 3 : 4-tri-*O*-methylglucose, 3 : 4-di-*O*-methylxylose, and an authentic sample of 2 : 3-di-*O*-methyl-D-xylose were each oxidised with sodium metaperiodate solution for 48 hr., the excess of periodate was destroyed with sodium arsenite solution, and the resulting solutions were tested for formaldehyde with phenylhydrazine hydrochloride and potassium ferricyanide (cf. Chanda, Hirst, Percival, and Ross, *J.*, 1952, 1833). Formaldehyde was produced from 2 : 3 : 4-tri-*O*-methylglucose and 2 : 3-di-*O*-methyl-D-xylose but not from 3 : 4-di-*O*-methylxylose.

The Acidic Fraction from the Hydrolysis of Beech Hemicellulose A.—Hemicellulose A (10 g.) was heated with N-sulphuric acid (100 c.c.) at 100° for 6 hr. and, after cooling, the supernatant liquid was neutralised with barium carbonate, filtered, and set aside (I). The residue was heated with N-sulphuric acid (100 c.c.) for a further 4 hr. and the resulting solution was neutralised with barium carbonate, filtered, and combined with (I). The combined solutions were concentrated to a syrup which was poured into methanol to give a supernatant liquid (II) and a brown solid (III). The solid (III) was reprecipitated from aqueous solution with excess of methanol, washed with methanol, and dried; the washings were added to (II). Chromatographic examination of the reprecipitated solid (III) after removal of barium ions with Amberlite resin IR-120 showed an aldobiuronic acid and xylose to be present.

Further precipitates were obtained from the liquid (II) by the addition of ethanol and concentration of the resulting supernatant liquor to a syrup which was poured into ethanol. These solids were chromatographically similar to solid (III). The combined solids were reprecipitated several times from aqueous solution with methanol and dried (yield, 1·1 g.). A small sample was dried from aqueous solution for analysis (Found : OMe, 4·9. Calc. for barium monomethylaldobiuronate : OMe, 7·6%. Equiv. of resulting acid, 384. Calc. for $C_{12}H_{20}O_{11}$: equiv., 340). The crude barium aldobiuronate (70 mg.) was heated with methanolic 16% hydrogen chloride (5 c.c.) for 20 hr. at 70—80°, neutralised with silver carbonate, and taken to a syrup, which was hydrolysed with 0·5N-hydrochloric acid (2 c.c.) and isolated in the usual manner. Chromatographic examination of the hydrolysate in solvents B and C showed xylose, 4-*O*-methylglucuronic acid, and glucuronic acid to be present.

Reduction with Sodium Borohydride.—Crude barium aldobiuronate (2·3 g.) was converted into the methyl ester glycoside by refluxing methanolic 2% hydrogen chloride (60 c.c.) during 5 hr. After neutralisation with silver carbonate the resulting syrup was dissolved in water (150 c.c.), and sodium borohydride was (1·5 g.) added. The mixture was shaken for 16 hr. at room temperature, acidified with glacial acetic acid to pH 4, and was filtered. The filtrate was made 1·5N with respect to hydrochloric acid and heated for 6 hr. at 100°. After neutralisation with silver carbonate the hydrolysate was deionised with Amberlite resins IR-120 and IR-4B and taken to a syrup. Chromatographic examination showed the presence of xylose, 4-*O*-methylglucose, and a trace of glucose. Part of the syrup (200 mg.) was separated on filter sheets with solvent B, and the 4-*O*-methyl-D-glucose was identified by conversion into 4-*O*-methyl-D-glucosazone, m. p. and mixed m. p. 153—154°. Examination by the *X*-ray powder photograph method, by the kindness of Dr. C. A. Beevers, confirmed the identity of the osazone.

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