The Hemicelluloses of Scots Pine (Pinus sylvestris) *and Black Spruce* (Picea nigra) *Woods.**

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Acid hydrolysis of extractive-free wood of Scots Pine and Black Spruce has yielded 2-*O*-(4-*O*-methyl-D-glucosidurono)-D-xylose, 4-*O*-methyl-D-glucuronic acid, and D-galacturonic acid. The neutral sugars were D-xylose, L-arabinose, D-mannose, D-galactose, L-rhamnose, and L-fucose. Small amounts of 3-*O*-methyl-L-rhamnose and glucurone were isolated from the hydrolysate from Black Spruce wood.

Extractive-free woods of the two species, Scots Pine (*Pinus sylvestris*) and Black Spruce (*Picea nigra*), have been partially hydrolysed with dilute sulphuric acid, and the products investigated. It was of interest to compare the results obtained from the hydrolysis of these two soft woods with those obtained from the hydrolysis of Aspen wood (Jones and

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Wise, *J.*, 1952, 2750). It seems that the apparent difference between these three woods is that 3-*O*-methyl-L-rhamnose and D-glucurone are found in the monosaccharide fraction from Black Spruce wood, albeit in minute quantities, and that L-fucose has not been isolated from aspen wood. L-Fucose occurs in small amounts in many gums and mucilages, notably gum tragacanth (cf. James and Smith, *J.*, 1945, 739). The neutral and acidic fractions were separated and identified as described by Jones and Wise (*loc. cit.*). The 3-*O*-methyl-L-rhamnose was isolated as the crystalline sugar and identified by its rate of movement on the chromatogram (Dunstan and Hirst, *J.*, 1953, 2332) and by the identity of its *X*-ray diffraction pattern with that of an authentic specimen. Black Spruce wood contains quantities of materials which are soluble in benzene and ethanol. Samples of alcohol- and benzene-soluble material were isolated and hydrolysed; 3-*O*-methyl-L-rhamnose was not detected in the hydrolysate. It is inferred that this sugar is not a component of the extractives of wood but that it is probably a component of a polysaccharide. This is the first time this derivative of L-rhamnose has been encountered as a component of a natural product. It is noteworthy that the methoxyl group is on $C_{(3)}$ and that every methylated sugar so far encountered in Nature, with the exception of 4-*O*-methyl-D-glucuronic acid, has its methoxyl on $C_{(3)}$ (Hough and Jones, *Nature*, 1951, **167**, 180). It is possible that D-glucuronic acid may arise from D-glucose by an inversion of hydroxyl groups in which $C_{(1)}$ of the glucose becomes $C_{(6)}$ of the glucuronic acid and that 4-*O*-methyl-D-glucuronic acid arises from 3-*O*-methyl-D-glucose by a similar inversion (cf. the origin of vitamin C; Isherwood *et al.*, *Biochem. J.*, 1954, **561**). This possibility is being investigated.

Other sugars and sugar acids remain to be identified in the mixture of sugars prepared from these woods and it may be that such identification will throw some light on the reason for the different physical and chemical properties of these woods.

Experimental

Paper chromatography was carried out by the descending method (Partridge, *Biochem. J.*, 1948, **42**, 238) on Whatman No. 1 filter paper, the following solvent systems being used: (*a*) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), (*b*) *n*-butanol–pyridine–water (10 : 3 : 3), (*c*) *n*-butanol–ethanol–water (40 : 11 : 19); all v/v. The positions of the sugars on the chromatograms were determined by the use of ammoniacal silver nitrate spray (Partridge, *loc. cit.*) or *p*-anisidine hydrochloride spray (Hough, Jones, and Wadman, *J.*, 1950, 1702). The rates of movement of the sugars are approximate only and are quoted relative to that of a sugar of about the same mobility. Thus R_G, R_{RH}, R_X, or R_{gal} is the rate of movement of the sugar relative to **2 : 3 : 4** : 6-tetra-*O*-methyl-D-glucose, rhamnose, xylose, or galactose, respectively. Optical rotations were determined at 18° ± 2° in water and are equilibrium values unless otherwise stated. Solvents were removed under reduced pressure.

Examination of the Products of Hydrolysis of Pine Wood (Pinus sylvestris).—Sawdust (500 g.; extractive free; cf. Jones and Wise, *J.*, 1952, 2759) was washed with hot water (6 l.) during 2 hr., the washings were then colourless and gave only a faint Molisch test. The aqueous extract, which contained water-soluble polysaccharides, was examined separately (further details will be published later). The sawdust was pressed to remove as much water as possible and suspended in 2N-sulphuric acid (2 l.), and the slurry heated on the steam-bath for 10 hr; the bulk of the material was thus maintained at a temperature of about 60° during this time. The slurry was filtered and the residue washed with more hot water (2·5 l.). The combined filtrate and washings were brought to pH 3 (barium hydroxide), and the solution was filtered. The filtrate was passed through Amberlite resin 1R120, and the acidic effluent evaporated. The concentrate (500 c.c.) was passed through Amberlite resin 1R4B to remove acidic material, and the effluent, which contained the neutral sugars, was evaporated to a syrup (*A*; 10 g.). The acids were displaced from the Amberlite resin 1R4B with N-sulphuric acid, and the effluent solution neutralised (barium hydroxide) and filtered, and the filtrate passed through Amberlite resin 1R120. Concentration of this acidic effluent furnished a syrupy mixture of uronic acids (*B*; 2 g.).

Examination of syrup A. The syrup (10 g.) was fractionated on charcoal (Darco G60)–Celite (1 : 1; w/w) by elution first with water and then with aqueous ethanol (Whistler and Durso, *J. Amer. Chem. Soc.*, 1950, **72**, 677). The major fraction (6·5 g.), which was eluted with water, consisted of monosaccharides. Later fractions contained oligosaccharides of which xylobiose (30 mg.) was isolated after further fractionation on sheets of paper (solvent *c*). This substance had m. p. and mixed m. p. 183°, $[\alpha]_D$ −20°, and yielded xylose only on hydrolysis. A portion

(3·8 g.) of fraction *A* was refractionated on hydrocellulose (30 × 3·5 cm.), *n*-butanol half saturated with water being used as the mobile phase. Concentration of the appropriate portions of the effluent gave the following crystalline sugars: L-rhamnose, m. p. 95°, $[\alpha]_D$ +8°; D-xylose, m. p. 144°, $[\alpha]_D$ +18°; L-arabinose, m. p. 158°, $[\alpha]_D$ +100°; and D-galactose, m. p. 167°, $[\alpha]_D$ +80°. In addition, D-mannose was isolated and characterised as its phenylhydrazone, m. p. 189°, and L-fucose, $[\alpha]_D$ −70°, was characterised as its toluene-*p*-sulphonhydrazone, m. p. 172° (Easterby, Hough, and Jones, *J.*, 1951, 3416) (Found: C, 46·7; H, 6·0. $C_{13}H_{20}O_6N_2S$ requires C, 47·0; H, 6·0%). This substance was indistinguishable from an authentic specimen on *X*-ray crystallographic examination.

Examination of uronic acid fraction B. The uronic acids (2 g.) were placed on charcoal (Darco G60)–Celite (1 : 1; w/w) and eluted successively with water, 5% and then 10% ethanol, and finally with 20% acetic acid, and thus yielded fractions B_1 (0·13 g.), B_2 (0·79 g.), B_3 (0·50 g.), and B_4 (0·47 g.). Paper-chromatographic examination of these fractions (solvent *a*) indicated that B_1 contained 4-*O*-methylglucuronic acid and galacturonic acid, that fractions B_2 and B_3 consisted of these two acids, a uronic acid with R_X 0·73, and slower moving acids, and that fraction B_4 contained acidic oligosaccharides.

Fraction B_1 was freed from ash (Amberlite resin 1R120) and fractionated by chromatography on a sheet of filter paper. Elution of the appropriate sections of the paper gave D-galacturonic acid, m. p. 157°, $[\alpha]_D$ +110° ⟶ +40° (equilibrium value) [characterised after oxidation with bromine water as mucic acid, m. p. 215°, and as its 2 : 5-dichlorophenylhydrazone, m. p. and mixed m. p. 179° (Mandl and Neuberg, *Arch. Biochem. Biophysics*, 1952, **35**, 326)]. Fractions B_2 and B_3 were combined and fractionated on cellulose, *n*-butanol–formic acid (50 : 1, v/v) being used as eluant. The fastest-moving component (80 mg.) was 4-*O*-methyl-D-glucuronic acid, $[\alpha]_D$ +45°, R_{BH} 0·98, R_X 1·37 (solvent *a*), and was characterised as the amide of methyl 4-*O*-methyl-α-D-glucosiduronate, m. p. and mixed m. p. 230—235°. The next component to be eluted was 2-*O*-(4-*O*-methyl-α-D-glucosidurono)-D-xylose (*C*; 1·1 g.). Thereafter galacturonic acid and oligosaccharides were eluted (cf. Jones and Wise, *loc. cit.*).

Identification of the aldobiuronic acid, C (cf. Jones and Wise, *loc. cit.*). A portion (10 mg.) of this acid (*C*) was boiled with N-sulphuric acid (2 ml.) for 18 hr. (much decomposition occurred). The solution was neutralised (barium hydroxide) and filtered, and the filtrate examined on the paper chromatogram (solvent *a*). Xylose, 4-*O*-methyl-D-glucuronic acid, and the original disaccharide were identified.

The acid (*C*; 1·0 g.) was neutralised (barium hydroxide) and the solution evaporated to dryness. The barium salt showed $[\alpha]_D$ +80° [Found: Ba (as sulphated ash), 17·9; OMe, 6·7. Calc. for $(C_{12}H_{19}O_{11})Ba$: Ba (as sulphated ash), 16·8; OMe, 7·6%]. The barium salt (1·1 g.) was methylated with methyl sulphate and sodium hydroxide, and the methylated acid isolated as described by Jones and Wise (*loc. cit.*). This acid was further methylated with Purdie's reagent, and the product (500 mg.), $[\alpha]_D$ +105° ($CHCl_3$) (Found: OMe, 45·7. Calc. for $C_{18}H_{32}O_{11}$: OMe, 51·3%), was reduced with lithium aluminium hydride. The methylated disaccharide, which was isolated in the usual manner, was further methylated (sodium hydroxide and methyl sulphate) giving the fully methylated glucosidylxylose derivative (300 mg.), n_D^{18} 1·4644 (Found: OMe, 50·4. Calc. for $C_{18}H_{34}O_{10}$: OMe, 52·9%). The methylated disaccharide was boiled with N-sulphuric acid until the optical rotation of the solution had become constant {$[\alpha]_D$ +44° (*c*, 3·0) (18 hr.)}. The cooled solution was neutralised and extracted exhaustively with chloroform. Concentration of the extract yielded a syrup which contained two sugars, indistinguishable on paper chromatograms, in solvent *a*, *b*, or *c*, from 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose and 3 : 4-di-*O*-methyl-D-xylose. These sugars were separated on sheet-paper chromatograms (solvent *c*) and identified as: (*a*) 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose (155 mg.), m. p. 90°, $[\alpha]_D$ +85° ($CHCl_3$) {the derived *N*-phenylglucosylamine 2 : 3 : 4 : 6-tetra-*O*-methyl ether had m. p. and mixed m. p. 137°, $[\alpha]_D$ +250° (Me_2CO)}, and (*b*) 3 : 4-di-*O*-methyl-D-xylose (61 mg.), $[\alpha]_D$ +30° ($CHCl_3$) (Found: OMe, 32·2. Calc. for $C_7H_{14}O_5$: OMe, 34·8%). The latter was converted by oxidation with bromine water into 3 : 4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 64° (from water).

Examination of the Products of Hydrolysis of Spruce Wood (Picea nigra).—Sawdust (700 g.; extractive free; cf. Jones and Wise, *loc. cit.*) was hydrolysed with 2N-sulphuric acid (3 l.) for 6 days on the steam-bath. The resultant solution was separated into a neutral fraction (*C*; 50 g.) and an acidic fraction (*D*; 12 g.) by the procedure described above (cf. Pine Wood hydrolysis).

The neutral sugars (*C*; 50 g.) were fractionated on cellulose, *n*-butanol half saturated with water being used as the mobile phase, and yielded the following crystalline sugars: L-rhamnose (trace), $[\alpha]_D$ 5°, m. p. 96°, m. p. 100° on admixture with an authentic specimen; D-xylose, $[\alpha]_D$

+18°, m. p. 144°; L-arabinose, $[\alpha]_D$ +102°, m. p. 158°; and D-galactose, $[\alpha]_D$ +80°, m. p. 167°. L-Fucose, identified as the toluene-*p*-sulphonhydrazone, m. p. 168°, the *X*-ray diffraction picture of which was identical with that of an authentic specimen, D-glucurone, m. p. and mixed m. p. 173—175°, and xylobiose (25 mg.), m. p. and mixed m. p. 183°, were detected after further fractionation of mixtures of sugars on sheet-paper chromatograms and were present in trace amounts. A quantity (30 mg.) of a methylated sugar, $[\alpha]_D$ +35° (Found: OMe, 13·4%), was also isolated. This material moved faster than rhamnose on the paper chromatogram (R_{RH} 1·38, 1·42, and 1·51 in solvents *a*, *b*, and *c*) and was indistinguishable from 3-*O*-methyl-L-rhamnose on the chromatogram. On nucleation with a specimen of this sugar the syrup crystallised; the solid had m. p. and mixed m. p. 113° (Found: OMe, 17·4. $C_7H_{14}O_5$ requires OMe, 17·5%). An *X*-ray diffraction pattern of this material was indistinguishable from that of 3-*O*-methyl-L-rhamnose.

Benzene–ethanol extract of Spruce wood. Spruce-wood sawdust (60 g.) was extracted exhaustively first with benzene–ethanol (2 : 1; v/v) and then with ethanol, and the syrup resulting after removal of the solvents was hydrolysed with boiling N-sulphuric acid for 5 hr. Chromatographic examination of the solution showed that 3-*O*-methyl-L-rhamnose was absent, but traces of xylose and rhamnose were detected.

Examination of fraction D. Paper-chromatogram examination of this fraction showed neutral sugars as well as uronic acids. Accordingly the syrup (12 g.) was shaken with methanolic hydrogen chloride (2% w/v; 300 c.c.) until a test portion of the solution no longer reduced Fehling's solution. The solution was then filtered, after the addition of silver carbonate, and concentrated to a syrup (12 g.). An attempt was made to extract preferentially the methylated uronic acid derivatives from their solution in water with chloroform. There resulted a chloroform-soluble extract (D_1, 3 g.) and a water-soluble sugar fraction (D_2; 9 g.) but no obvious fractionation of the sugars had taken place.

The Isolation of Methylated Uronic Acids from D_1.—The chloroform-soluble extract (D_1; 3 g.) was methylated with sodium hydroxide and methyl sulphate, and the solution extracted continuously with chloroform, first while strongly alkaline and then after acidification with dilute sulphuric acid. The first extract, which consisted of neutral sugars, was discarded. The methylated sugar acids (2 g.), which were obtained on concentration of the second extract, were reduced with a solution of lithium aluminium hydride (1·5 g.) in ether, and the neutral methylated sugars resulting (1 g.) were isolated in the usual manner. When the residual aqueous solution was acidified and again extracted a quantity (0·3 g.) of acids which had escaped reduction was recovered. The neutral fraction (1·0 g.) was further methylated, first with sodium hydroxide and methyl sulphate and then with silver oxide and methyl iodide, and the product (0·75 g.) (Found: OMe, 51·6. Calc. for $C_{18}H_{34}O_{10}$: OMe, 52·9%) was distilled, giving a main fraction (0·62 g.), b. p. 170—175° (bath-temp.)/0·3 mm., n_D^{18} 1·4636, $[\alpha]_D$ +112° ($CHCl_3$).

Hydrolysis of the disaccharide. The syrup (620 mg.) was hydrolysed with boiling N-sulphuric acid (20 c.c.) for 7 hr. The cooled solution was neutralised (barium hydroxide) and filtered, and the filtrate extracted exhaustively with chloroform. Concentration of the extract gave a syrup (580 mg.) which was separated on sheets of filter paper (solvent *c*). There resulted unchanged disaccharide, 2 : 3 : 4 : 6-tetra-*O*-methyl-D-glucose (95 mg.), $[\alpha]_D$ +81° ($CHCl_3$) {the derived *N*-phenylglycosylamine derivative had m. p. 137°, $[\alpha]_D$ +260° ($COMe_2$)}, and 3 : 4-di-*O*-methyl-D-xylose (100 mg.), $[\alpha]_D$ +40° ($CHCl_3$) (Found: OMe, 36·1. Calc. for $C_7H_{14}O_5$: OMe, 34·8%) (characterised as 3 : 4-di-*O*-methyl-D-xylonolactone, m. p. and mixed m. p. 64°).

Similar examination of fraction *D* showed that it contained 4-*O*-methyl-D-glucuronic acid, D-galacturonic acid, and 2-*O*-(4-*O*-methyl-α-D-glucosidurono)-D-xylose.

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