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Note

Structural studies of the hemicellulose A from the cork of *Quercus suber**

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Hemicellulose A, which has been isolated from various species of woody angiosperms (hardwoods), is a 4-*O*-methylglucuronoxylan. Structural studies of the hemicellulose A isolated from *Quercus suber*, an arborescent angiosperm that grows in north Valencia, are now reported.

The powdered cork of *Quercus suber* was extracted sequentially with benzene, water, and methanol, treated with methanolic sodium methoxide, and then extracted with ether and methanol. The product was delignified with sodium chlorite–acetic acid and then treated with aqueous 10% sodium hydroxide under nitrogen, and the hemicellulose A was precipitated by acidification to pH 5. The product, after purification using Fehling's solution, was homogeneous by gel filtration[1]. On acid hydrolysis, the polysaccharide yielded xylose (95.22%) and 4-*O*-methylglucuronic acid (5.90%). Methylation gave a product with an $[\alpha]_D$ value of −53° indicative of β linkages, which was confirmed by the n.m.r. spectra[2] (δ 4.3 for H-1, and 102.64 for C-1).

TABLE I

METHYL ETHERS IDENTIFIED AFTER HYDROLYSIS OF THE METHYLATED (A) AND METHYLATED, CARBOXYL-REDUCED POLYSACCHARIDE (B)

Sugars[a]	T[b]	*Mole %*	
		A	*B*
2,3-Xyl	1.42	93.1	78.2
3-Xyl	2.71	6.9	6.4
2,3,4-Glc	2.35		15.4

[a]2,3-Xyl = 2,3-di-*O*-methyl-D-xylose, *etc.* [b]Retention times of the corresponding alditol acetates, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on ECNSS-M at 175°.

**Quercus suber* Polysaccharides, Part I.

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The methylated polysaccharide was reduced with lithium aluminium hydride and then hydrolysed, and the sugars were analysed, as their alditol acetates, by g.l.c. and g.l.c.–m.s. The results are summarised in Table I.

The foregoing data indicate the hemicellulose to contain (1→4)-linked β-D-xylopyranosyl residues with single 4-*O*-methyl-D-glucuronic acid residues attached at some positions 2. That the 4-*O*-methyl-D-glucuronic acid was α was etablished by the $[\alpha]_D$ value (+81°) of the aldobiouronic acid obtained by partial hydrolysis of the hemicellulose. The constituent sugars were determined conventionally by acid hydrolysis, followed by borohydride reduction, lactonisation, borohydride reduction, acetylation, and g.l.c.–m.s., which revealed equimolecular amounts of xylitol and 4-*O*-methylglucitol.

EXPERIMENTAL

General methods. — Descending p.c. was performed on Whatman Nos. 1 and 3MM papers with *A*, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); *B*, 1-butanol–ethanol–water (2:1:1); and *C*, 1-butanol–pyridine–water (6:4:3); and detection with diphenylamine–aniline[3]. Optical rotations were recorded with a Perkin–Elmer 141 polarimeter and i.r. spectra with a Perkin–Elmer Model 281 spectrophotometer. ^{1}H-N.m.r. and ^{13}C-n.m.r. spectra (internal Me_4Si) were recorded with a Bruker AC-200 (200 MHz) spectrophotometer. Light petroleum refers to the fraction of b.p. 30–60°. G.l.c. was performed with a Hewlett-Packard model 5710A chromatograph fitted with a flame-ionisation detector and a glass column (200 × 0.6 cm) containing 3% of ECNSS-M on Gas Chrom Q (100–200 mesh) at 190° (alditol acetates) or at 175° (partially methylated alditol acetates). For g.l.c.–m.s., a Hewlett-Packard 5995B instrument fitted with a capillary column (12 m × 0.2 mm) containing OV-1 was used. E.i.-mass spectra were recorded at 70 eV with a temperature programme of 100→220° at 4°/min. H.p.l.c. was performed with a Hewlett-Packard Model 1081B fitted with a refractive index detector, using[4] a LiChrosorb NH_2 column and acetonitrile–water (75:25).

Hemicellulose A. — *(a) Isolation.* Finely powdered cork of *Quercus suber* (848 g) was exhaustively extracted (Soxhlet) with benzene, water, and methanol, and then air-dried. The residue (100 g) was treated with methanolic 0.1M sodium methoxide in methanol (5 h, reflux). The residue was collected and extracted (Soxhlet) with dry ether (to remove the monomers of suberine as methyl esters[5]) and then with methanol. The insoluble material (114.3 g) was stirred with water and delignified with sodium chlorite in the presence of acetic acid for 1 h at 70°. The resulting fibrous material was collected, and washed with water, and the whole process was repeated. The product was washed with ethanol, stirred with boiling ethanolic 3% ethanolamine for 5 min, then collected, and washed with water. The treatment was repeated, and the final residue was washed with ethanol and ether and then air-dried. The resulting holocellulose (34 g) was extracted[6] with aqueous 10% sodium hydroxide, the extract was vacuum-filtered through cloth, and the hemicellulose A was precipitated by acidification to pH 5 with 50% acetic acid. After storage for 3 h at 5°, the precipitate was collected by centrifugation, washed with water and ethanol, and then dried to yield the crude polysaccharide (11.78 g).

Hemicellulose B was isolated from the supernatant solution by precipitation with ethanol.

(b) Purification. To a solution of hemicellulose A (7.83 g) in aqueous 5% potassium hydroxide (783 mL) was added Fehling's solution until the precipitation was complete. The polysaccharide was collected by centrifugation, dispersed in water (pH 6), and dialysed for 48 h against distilled water. Ethanol (4 vol.) was added, and the precipitate was collected by centrifugation, treated at 0° with ethanolic 5% hydrogen chloride (1 min), and then centrifuged. The residue was washed with ethanol and acetone, and then dried over phosphorus pentaoxide *in vacuo,* to give the hemicellulose A (3.48 g), $[\alpha]_D$ −61° (*c* 4.8, aqueous 1% sodium hydroxide) (Found: D-xylose, 94.10; 4-*O*-methyl-D-glucuronic acid, 5.90%).

(c) Homogeneity. Gel-filtration chromatography was conducted by elution from a column (46 × 1.6 cm) of Sephacryl S-400 with 0.5M sodium chloride at 0.5 mL/min. The column was calibrated with dextrans of known molecular weight (Pharmacia). Fractions were monitored by the phenol–sulphuric acid method[1], which gave a single band.

(d) Sugar analysis. Hemicellulose A (37 mg) was treated with aqueous 72% sulphuric acid (0.4 mL) for 1 h at 30° and then with 0.5M sulphuric acid (20 mL of water) for 3 h at 100°, using *myo*-inositol as the internal standard. The excess of acid was neutralised with barium carbonate and the solution was decationised with Amberlite IR-120 (H^+) resin. The sugar was converted into the alditol acetate[7] and analysed by g.l.c.

The glucuronic acid in the hemicellulose A, determined by the carbazole method[8] (using D-glucuronic acid as the standard), was 5.9%.

(e) Methylation analysis. To a stirred solution of sodium methylsulfinylmethanide (prepared under nitrogen from 1 g of sodium hydride and 23 mL of methyl sulfoxide) at room temperature was added a solution of hemicellulose A (148 mg) in methyl sulfoxide (5 mL). After stirring for 4 h, methyl iodide (4 mL) was added with external cooling. Stirring was continued for 12 h, water (60 mL) was then added, and the mixture was extracted with chloroform. The combined extracts were washed thrice with water, dried (Na_2SO_4), and concentrated to a yellow solid which was dried over phosphorus pentaoxide *in vacuo* at 40° for 2 days. A solution of the product in benzene was diluted with light petroleum to precipitate the methylated polysaccharide (132 mg), $[\alpha]_D$ −53° (*c* 5.6, chloroform). A portion (15 mg) of the material was hydrolysed conventionally and the resulting sugars were converted into alditol acetates[9] and analysed by g.l.c. and g.l.c.[10]–m.s.[11].

To a solution of another portion (28 mg) in dry tetrahydrofuran (10 mL) was added lithium aluminium hydride (150 mg), and the mixture was boiled under reflux for 24 h. The mixture was worked-up in the usual way and the reduced product was extracted into chloroform. The product had i.r. absorption at 3600

cm^{-1} (OH) but not at 1735 cm^{-1} (ester C=O). The product was hydrolysed as in (*d*), and the methylated sugars were converted into alditol acetates and analysed by g.l.c. and g.l.c.–m.s.

(*f*) *Partial hydrolysis.* Hemicellulose A (1.5 g) was treated with 0.125M sulphuric acid for 90 min at 100°. The hydrolysate was neutralised ($BaCO_3$), basified with 0.1M potassium hydroxide, then passed through a column of Amberlite IR-120 (H^+) resin, and concentrated. The syrupy residue was eluted from a column of Amberlite IRA-400 (AcO^-) resin, first with water to yield the neutral oligosaccharides and then with aqueous 10% acetic acid to yield the acidic oligosaccharides. The neutral sugars were analysed[12] by p.c. (solvents *A*–*C*) and by h.p.l.c.[4]. P.c. (solvent *A*) of the acidic sugars revealed an aldobiouronic acid and 4-*O*-methyl-D-glucuronic acid. Preparative p.c. gave the aldobiouronic acid (8 mg), $[\alpha]_D$ +81° (*c* 1.6, water), which was hydrolysed with aqueous 72% sulphuric acid at 100° for 4 h. The products were reduced with sodium borohydride, the decationised product was treated with 1.2M hydrochloric acid, and a solution of the resulting aldonolactone in 0.4M boric acid (3 mL) at 0° was reduced[13] by addition of 0.3M sodium borohydride (6 mL) dropwise with stirring during 30 min. The mixture was stored for an additional 30 min at 0°, then basified (pH 9), and kept for 12 h at 5°. Sodium borohydride (4 mL) was added, and the mixture was kept at 5° for 3 h, then acidified with acetic acid, deionised, and freed from boric acid. The product was acetylated with acetic anhydride–pyridine (1:1), and g.l.c.–m.s.[14] of the resulting alditol acetates revealed equimolecular amounts of xylitol and 4-*O*-methylglucitol.

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