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Note

Structural studies of a hemicellulose B fraction from the cork of *Quercus suber**

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Hemicellulose A, isolated[1] from the cork of *Quercus suber*, is a xylan having β-(1→4)-glycosidic linkages. Hemicellulose B has now been fractionated, using Fehling's solution[2], into B-1 and B-2, and the structure of the pure hemicellulose B-1 is now reported.

B-1, which contained xylose, 4-*O*-methylglucuronic acid, arabinose, galactose, mannose, and glucose in the molar ratios 135:12:7:11:2:30 together with traces of rhamnose, was purified by complexing with Fehling's solution. Purified B-1 appeared to be homogeneous on gel-filtration on Sephacryl S-400 and had $[\alpha]_D$ −61.5° (*c* 1, aqueous 1% sodium hydroxide). The molar ratios of xylose, 4-*O*-methylglucuronic acid, and arabinose were then 170:13:3, and there was some arabinose and traces of hexoses.

Hakomori methylation[3] of B-1 gave a product with $[\alpha]_D$ −32.5° (chloroform) indicative of β linkages, which was confirmed by the n.m.r. spectra[4] (δ 4.3 for H-1 and δ 102.55 for C-1). The methylated polysaccharide was reduced with lithium aluminium hydride and then hydrolysed, and the sugars were analysed, as the partially methylated alditol acetates, by g.l.c. and g.l.c.[5]–m.s.[6]. The results are summarised in Table I. The formation of a small proportion of 2,3,5-tri-*O*-methylarabinose indicated the existence of 1 terminal arabinofuranosyl group per 56 xylose residues. The formation of 2,3,4-tri-*O*-methylxylose and 2,3,4-tri-*O*-methylglucose indicated that xylopyranosyl and 4-*O*-methylglucopyranosyluronic acid groups were also present as terminal units. That the backbone consisted of (1→4)-linked β-D-xylosyl residues was indicated by the formation of a large proportion of 2,3-di-*O*-methylxylose. Side-chains were attached to positions 2 of the xylosyl residues, as indicated by the formation of 3-*O*-methylxylose.

Identification of the 3-*O*-methyl-D-xylose was not possible by g.l.c. on

**Quercus suber* Polysaccharides, Part II. For Part I, see ref. 1.

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TABLE I

PRODUCTS OBTAINED BY HYDROLYSIS OF THE METHYLATED (A) AND METHYLATED CARBOXYL-REDUCED B-1 (B)

Sugars[a]	T[b]		*Mole %*	
	(a)	(b)	*A*	*B*
2,3,5-Me_3-Ara	0.51	0.69	4.3	1.5
2,3,4-Me_3-Xyl	0.63	0.75	2.5	7.0
2,3-Me_2-Xyl	1.43	0.91	83.8	73.4
3-Me-Xyl	2.70	1.08	9.4	11.5
2,3,4-Me_3-Glc	2.35	1.20	—	6.6

[a]2,3,5-Me_3-Ara = 2,3,5-tri-*O*-methyl-L-arabinose, *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 (*a*) ECNSS-M at 175°, and (*b*) OV-1 at 120–220° at 4°/min.

ECNSS-M, but it was identified by the fragments obtained by g.l.c.–m.s. of the partially methylated alditol acetates[6,7]; the fragments with *m/z* 43, 87, 129, and 189 were the same as those of 3-*O*-methyl-D-xylose obtained in previous work[1]. Mixtures of 2- and 3-*O*-methyl-D-xylose gave[8] fragments with *m/z* 117, 129, 189, and 261. Fragments with *m/z* 117 and 261 are characteristic[6,7] of 2-*O*-methylpentoses; hence, it is concluded that only 3-*O*-methyl-D-xylose was formed from B-1.

That the 4-*O*-methyl-D-glucuronic acid was α was established by the $[\alpha]_D$ value (+98°) of the aldobiouronic acid obtained by partial hydrolysis of the pure B-1 and which was identified as 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose by g.l.c.–m.s.[9]. Thus, hemicellulose B-1 was shown to be essentially a (1→4)-linked β-D-xylan with 4-*O*-methyl-α-D-glucopyranosyluronic acid, D-xylopyranosyl and L-arabinofuranosyl groups attached at positions 2. For every 15 D-xylopyranosyl residues in the main chain, there was one uronic acid unit. For 13 such D-xylopyranosyl residues, there was one D-xylopyranosyl group, and for ~56 such D-xylopyranosyl residues, there was one L-arabinofuranosyl group.

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[10]. Optical rotations were recorded with a Perkin–Elmer 141 polarimeter and i.r. spectra with a Perkin–Elmer Model 281 spectrophotometer. ^1H- and ^{13}C-n.m.r. spectra (internal Me_4Si) were recorded with a Bruker AC-200 (200 MHz) spectrophotometer. 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 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.

Hemicellulose B-1. — Holocellulose (34 g), isolated[1] from the cork of *Quercus suber*, was extracted[11] with aqueous 10% sodium hydroxide. Adjustment of the pH of the extracts to 5 with glacial acetic acid precipitated hemicellulose A. The supernatant solution was dialysed for 24 h against distilled water and the precipitate, which was formed by the addition of ethanol (4 vol.) at 0°, was collected by centrifugation (14,000 r.p.m. at 5°), washed with ethanol, and dried over phosphorus pentaoxide *in vacuo*, to give hemicellulose B (4.27 g), $[\alpha]_D$ −8° (*c* 5, aqueous 1% sodium hydroxide).

To a solution of hemicellulose B (4 g) in aqueous 5% potassium hydroxide (400 mL) was added Fehling's solution until precipitation was complete. The copper complex was collected by centrifugation, washed with aqueous 5% potassium hydroxide, and precipitated from a solution in water at 0° by the addition of ethanol (4 vol.). After storage at 0° for 1 h, the precipitate was collected by centrifugation, macerated at 0° with ethanolic 5% hydrogen chloride (1 min), then washed with ethanol, and dried over phosphorus pentaoxide *in vacuo*, to give B-1 (1.32 g), $[\alpha]_D$ −21° (*c* 6.6, aqueous 1% sodium hydroxide). The process was repeated to give purified B-1 (719 mg), which had $[\alpha]_D$ −61° (*c* 10, aqueous 1% sodium hydroxide).

Hemicellulose B-2 was isolated from the supernatant alkaline solution by precipitation with ethanol.

Sugar analysis — B-1 and purified B-1 (16 mg of each) were separately treated with aqueous 72% sulphuric acid (0.32 mL) for 1 h at 30°. The solutions were diluted with water (8.1 mL) and then heated for 3 h at ~100°. *myo*-Inositol (2 mg) was added as internal standard, and each hydrolysate was neutralised ($BaCO_3$) and decationised with Amberlite IR-120 (H^+) resin. Monosaccharides were detected by p.c. (solvents *A*–*C*) and quantified as their alditol acetates by g.l.c.[12]. The contents of glucuronic acid in B-1 and purified B-1, determined by the carbazole method[13] (using D-glucuronic acid as the standard), were 6.36 and 6.78%, respectively.

Hemicellulose B-1. — (*a*) *Homogeneity.* Gel-filtration chromatography was performed on a column (46 × 1.6 cm) of Sephacryl S-400 by elution 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[14]. A single band was obtained.

(*b*) *Methylation analysis.* To a stirred solution of sodium methylsulfinylmethanide (prepared under nitrogen from 1.5 g of sodium hydride and 31 mL of methyl sulfoxide) at room temperature was added a solution of B-1 (200 mg) in methyl sulfoxide (5 mL). After stirring for 12 h, methyl iodide (5 mL) was added with external cooling. Stirring was continued for 7 h, water (100 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 (b.p. 30–60°) to precipitate the methylated polysaccharide (149 mg), $[\alpha]_D$ −32.5° (*c* 16, chloroform). A portion (10 mg) of this material was hydrolysed conventionally and the resulting sugars were converted into alditol acetates[15] and analysed by g.l.c. and g.l.c.[5]–m.s.[6].

To a solution of another portion (31 mg) in dry tetrahydrofuran (15 mL) was added lithium aluminium hydride (150 mg), the mixture was boiled under reflux for 24 h and then worked-up in the usual way, and the reduced product was extracted into chloroform. The product had ν_{max} at 3600 cm^{-1} (OH) but not at 1735 cm^{-1} (ester C=O). The product was hydrolysed as described above, and the resulting methylated sugars were converted into alditol acetates, and analysed by g.l.c. and g.l.c.–m.s.

(*c*) *Partial hydrolysis.* B-1 (0.5 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. P.c. (solvents *A*–*C*) of the acidic sugars revealed an aldobiouronic acid in addition to 4-*O*-methyl-D-glucuronic acid. Preparative p.c. (solvent *A*) gave the aldobiouronic acid (11 mg), $[\alpha]_D$ +98° (*c* 1, water), which was converted into the methyl ester methyl glycoside[16] by treatment with boiling methanolic 3% hydrogen chloride (5 mL) for 20 h, and the product was acetylated with acetic anhydride–pyridine (1:1) for 24 h at room temperature. Conventional work-up gave a product which, when subjected to g.l.c.–m.s.[9], was shown to be methyl 3,4-di-*O*-acetyl-2-*O*-(methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyluronate)-α-D-xylopyranoside.

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