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POLYSACCHARIDES FROM THE CORK OF *QUERCUS SUBER*, II. HEMICELLULOSE[1]

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ABSTRACT.—Hemicellulose isolated from the cork of *Quercus suber* contains D-xylose and 4-*O*-methylglucuronic acid in the molar ratio of 100:2; traces of glucose, galactose, mannose, rhamnose, and arabinose were also present. Investigations on the hemicellulose revealed that it consists of a backbone of (1→4)-linked β-D-xylopyranosyl residues with side chains consisting of single units of 4-*O*-methyl-α-D-glucopyranosyluronic acid and D-xylopyranosyl residues attached at some 2 positions of β-D-xylopyranosyl. For every 44 D-xylopyranosyl residues in the main chain, there is one uronic acid group branch; for 96 such D-xylopyranosyl residues, there is one D-xylopyranosyl group branch.

In continuing studies of the polysaccharides from the cork of *Quercus suber* L. (Fagaceae), the structure of a hemicellulose, inferred from the results of complete and partial hydrolysis with acid and methylation analysis, is now discussed. The anomeric configuration of the xylosyl groups was determined by nmr spectroscopy of the methylated polysaccharide. In previous publications suberin and holocellulose have been investigated in detail (1,2). Similar hemicelluloses have been reported from other sources (3–5). Hirst (6) and Wilkie and Woo (7), in early studies of the xylans from esparto grass, laid the modern foundation for the hemicellulose chemistry by establishing that xylans have (1→4)-linked β-D-xylopyranosyl residues.

RESULTS AND DISCUSSION

Powdered cork of *Q. suber* was extracted sequentially with C_6H_6, H_2O, and MeOH. The residue was treated with methanolic NaOMe to eliminate suberin (1), and lignin was removed by three successive treatments of the material with sodium chlorite/HOAc (8). The resulting holocellulose was extracted with DMSO and aqueous 4% NaOH to yield the hemicellulose preparation I and hemicellulose preparation II, respectively. The two preparations were obtained in yields of 19.9 and 17.5%, respectively, based on the dry wt of the holocellulose.

Preparation I contained xylose (92.4%), 4-*O*-methylglucuronic acid (2.5%), rhamnose (0.6%), arabinose (0.8%), galactose (0.9%), and glucose (2.7%). Preparation II was studied in detail after purification using Fehling solution (9). It contained xylose (83.8%), 4-*O*-methylglucuronic acid (2.8%), arabinose (3.4%), rhamnose (0.6%), mannose (0.8%), galactose (3.6%), and glucose (4.9%). The minor neutral sugar components were not removed by the purification step; however, they could not be detected after methylation of the hemicellulose.

The hemicellulose was methylated by the Hakomori method (10); that it was fully methylated was shown by the absence of the OH bands from the ir spectrum. The $[\alpha]$ value of $-74°$ of the methylated polysaccharide is indicative of β linkages. The nmr spectra were consistent with the presence therein of β linkages showing signals (11,12) at δ 4.3 in the ^1H spectrum assignable to H-1 and δ 102.67 in the ^{13}C spectrum assignable to the anomeric carbon atom (C-1) in the β configuration (13,14).

A part of the methylated polysaccharide was reduced with $LiAlH_4$ (15). The two samples were hydrolyzed, and the sugars were converted into their alditol acetates and analyzed by gc (16) and by gc-ms (17). The results are summarized in Table 1.

[1]For Part I, see Asensio and Seoane (1).

TABLE 1. Gc and Gc-ms of Methyl Ethers Identified after Hydrolysis of the Methylated (A) and Methylated, Carboxyl-Reduced (B) Polysaccharide.

Sugar[a]	T[b]		Area (%)		Characteristic fragments (*m/z*)
	a	b	A	B	
2,3,4-Me_3-Xyl	0.66	0.76	0.6	1.0	101, 117, 161
2,3-Me_2-Xyl	1.43	0.93	93.7	93.1	101, 117, 129, 189
2,3,4-Me_3-Glc	2.35	1.20	—	2.2	101, 117, 161, 189, 233
3-Me-Xyl	2.71	1.08	5.6	3.7	87, 129, 189

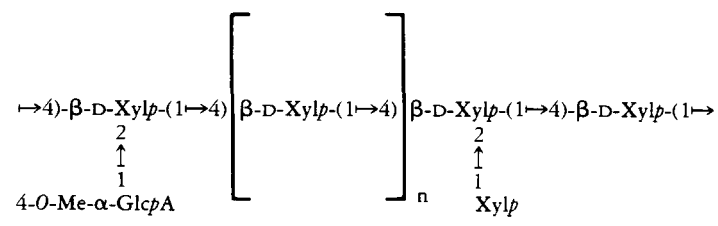
[a]2,3,4-Me_3-Xyl = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylxylitol, etc.

[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) an ECNSS-M column at 175°, and (b) an OV-1 (12 m) column at 120↦220° at 4°/min.

The formation of 2,3-di-*O*-methylxylose in high proportion and 3-*O*-methyl-D-xylose in relatively small proportion indicated that the polysaccharide was essentially a (1↦4)-linked D-xylan having a few branches at O-2. The formation of a very small amount of 2,3,4-tri-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose in the molar ratio 1:1.6 suggested that the branch points consisted of D-xylopyranosyl and 4-*O*-methylglucopyranosyluronic acid groups.

Partial hydrolysis of the hemicellulose yielded several oligosaccharides from which an acidic oligosaccharide was isolated by resolution on paper. On hydrolysis this oligosaccharide ([α]D + 101°) gave products that in pc showed the same mobilities (R_f 0.11, 0.28, and 0.32, respectively) as the original oligosaccharide, 4-*O*-methylglucuronic acid, and xylose, indicating that the oligosaccharide was an aldobiouronic acid of xylose and 4-*O*-methylglucuronic acid. Hydrolysis of the oligosaccharide, borohydride reduction, lactonization, borohydride reduction (18), and acetylation with Ac_2O/pyridine gave equimolecular amounts of xylitol and 4-*O*-methylglucitol when analyzed by gc-ms (19). These results indicated that the aldobiouronic acid might be 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose. To check this, the aldobiouronic acid was converted into its methyl ester, methyl glycoside (20), and this was acetylated and subjected to gc-ms (21), which revealed methyl 3,4-di-*O*-acetyl-2-*O*-(methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyluronate)-D-xylopyranoside. This result proved that the aldobiouronic acid was, indeed, 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose, a component found in fibers of pineapple, jute, roselle, mesta, agave, sisal, and sansevieria (22).

Thus, it is concluded that the hemicellulose had a structure composed of a main chain of (1↦4)-linked β-D-xylopyranosyl residues, with (1↦2)-linked 4-*O*-methylglucopyranosyluronic acid and D-xylopyranosyl residues at the branch points. The general structure **1** may be assigned to the hemicellulose. For every 44 D-xylopyranosyl residues in the main chain, there is one uronic acid group branch; for 96 such D-xylopyranosyl residues, there is one D-xylopyranosyl group branch.



EXPERIMENTAL

GENERAL METHODS.—Descending pc was performed on Whatman No. 1 and No. 3 MM papers with EtOAc-HOAc-HCO_2H-H_2O (18:3:1:4) with detection by reaction with diphenylamine aniline (23). Optical rotations were measured on a Perkin-Elmer 141 polarimeter and ir spectra with a Perkin-Elmer 281 spectrophotometer. ^{1}H-nmr ($CDCl_3$, internal TMS) spectra were recorded with a Perkin-Elmer R-12B (60 MHz) and ^{13}C-nmr ($CDCl_3$, internal TMS) spectra on a Varian XL-200 (200 MHz) spectrometer. Gc was performed with a Hewlett-Packard model 5710A chromatograph fitted with FID and a glass column (200 × 0.6 cm) containing 3% ECNSS-M on Gas Chrom Q (100–200 mesh) at 190° (alditol acetates) or at 175° (partially methylated alditol acetates). Alditol acetates were characterized by matching gc peaks against those of standard alditol acetates produced from mixtures (Sigma) of D-glucose, D-galactose, D-mannose, D-xylose, L-arabinose, L-rhamnose, and *myo*-inositol (internal standard). For comparative studies involving partial hydrolysis and methylation analysis, we used a standard xylan (Fluka) with a structure composed of a main chain of (1→4)-linked β-D-xylopyranosyl residues with (1→2)-α-linked 4-*O*-methylglucopyranosyluronic acid at the branch points. For gc-ms we used a Hewlett-Packard 5995B instrument fitted with a capillary column (12 m × 0.2 mm) containing OV-1. Eims was recorded at 70 eV with temperature programmed to rise from 100° to 220° at 4°/min.

ISOLATION AND PURIFICATION OF THE HEMICELLULOSE.—Extraction of the polysaccharides from the cork of *Q. suber* has been reported (1). The resulting holocellulose (53 g) was extracted with DMSO (24) and aqueous 4% NaOH.

Extraction with DMSO.—The holocellulose (1 g) was stirred in freshly distilled DMSO (12 ml) at room temperature for 4 days, and the extract was vacuum-filtered through a glass filter and washed with H_2O. The hemicellulose was precipitated by adding EtOH (4 vol) and HOAc (4 ml). The polysaccharide was collected by centrifugation, washed with EtOH and Me_2CO, and dried to give hemicellulose preparation I (200 mg).

Extraction with aqueous 4% NaOH.—The holocellulose (18 g) was extracted with aqueous 4% NaOH according to the method of Dorée (9). The extract was vacuum-filtered through a cloth, and the hemicellulose was precipitated by acidification to pH 4–5 with aqueous HOAc followed by addition of EtOH (1 vol). The precipitate was collected by centrifugation, washed with EtOH and Et_2O, and then dried to yield the crude polysaccharide (5.09 g). The treatment was repeated, and the resulting hemicellulose II was purified through its copper complex by treatment with Fehling's solution (9). The hemicellulose preparation II (3.15 g) had $[\alpha]^{20}D$ −100.6° (*c* = 2, 1% NaOH).

SUGAR ANALYSIS.—Hemicellulose preparations I and II (30 mg of each) were hydrolyzed, using *myo*-inositol as the internal standard, by a two-step procedure (25) that includes solubilization in 13.2 M H_2SO_4 (4 h at room temperature) followed by a secondary hydrolysis in 0.5 M H_2SO_4 (4 h at 100°). The acid was neutralized with $BaCO_3$, and the filtered solution was decationized with Amberlite IR-120 (H^+) resin. The sugars were converted into alditol acetates and analyzed by gc (26) and gc-ms (27).

The glucuronic acid in the unhydrolyzed preparations I and II was determined by the carbazole method (28) (using D-glucuronic acid as the standard) and found to be 2.5 and 2.8%, respectively.

HEMICELLULOSE II.—*Methylation analysis.*—To a stirred solution of $DMSO^-$ [prepared (29) under N_2 from 4.40 g of NaH and 180 ml of DMSO] at room temperature was added a solution of hemicellulose preparation II (1.42 g) in DMSO (25 ml). After stirring for 2 h, MeI (20 ml) was added with external cooling. Stirring was continued for 12 h, H_2O (500 ml) was then added, and the mixture was extracted with $CHCl_3$. The combined extracts were washed three times with H_2O, dried (Na_2SO_4), and concentrated to a yellow solid that was dried over P_2O_5 in vacuo at 40° for 2 days. A solution of the product in C_6H_6 was diluted with light petroleum (bp 30–60°) to precipitate the methylated polysaccharide (1.79 g), $[\alpha]D$ −74° (*c* = 5.6, $CHCl_3$). A portion (50 mg) of the material was hydrolyzed conventionally, and the resulting sugars were converted into alditol acetates and analyzed by gc and gc-ms.

To a solution of another portion (93 mg) of the methylated material in dry THF (30 ml), $LiAlH_4$ (150 mg) was added, and the mixture was boiled under reflux for 24 h. The mixture was worked up in the usual way (15), and the reduced product was extracted into $CHCl_3$. The product had ir absorption at 3600 cm^{-1} (OH) but not at 1735 cm^{-1} (ester C=O). The product was hydrolyzed as above, and the methylated sugars were converted into alditol acetates and analyzed by gc and gc-ms.

Partial analysis.—Hemicellulose preparation II (1.5 g) was treated with 0.125 M H_2SO_4 for 90 min at 100°. The hydrolyzate was neutralized ($BaCO_3$), brought to pH 8.5–9 with 0.1 M KOH, 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 H_2O to yield the neutral oligosaccharides, then with aqueous 10% HOAc to yield to acidic oligosaccharides. Pc of the acidic sugars revealed an al-

dobiouronic acid and 4-*O*-methyl-D-glucuronic acid. Preparative pc gave the aldobiouronic acid (12 mg) $[\alpha]_D + 101°$ ($c = 1$, H_2O) that was hydrolyzed with 13.2 M H_2SO_4 at 100° for 4 h. The products were reduced with $NaBH_4$. The decationized product was treated with 1.2 M HCl, and the solution was concentrated to dryness. A solution of the resulting aldonolactone in 0.4 M H_3BO_3 (3 ml) at 0° was reduced (18) by addition of 0.3 M $NaBH_4$ (6 ml) dropwise with stirring during 30 min. The mixture was stored for an additional 30 min at 0°; the solution was made alkaline (pH 9) by addition of NaOH solution and kept for 12 h at 5°. $NaBH_4$ (4 ml) was added, and the mixture was kept at 5° for 3 h, then acidified with HOAc, deionized, and freed from H_3BO_3. The product was acetylated with Ac_2O-pyridine (1:1). Gc-ms (19) of the resulting alditol acetates revealed equimolecular amounts of xylitol and 4-*O*-methylglucitol. The disaccharide was also converted into its methyl ester methyl glycoside by treatment with boiling methanolic 3% HCl (5 ml) for 20 h. The product was acetylated with Ac_2O-pyridine (1:1) and then subjected to gc-ms (21), which revealed methyl 3,4-di-*O*-acetyl-2-*O*-(methyl 2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyluronate)-D-xylopyranoside.

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