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POLYSACCHARIDES FROM THE CORK OF *QUERCUS SUBER*, I. HOLOCELLULOSE AND CELLULOSE

AMPARO ASENSIO* and ELISEO SEOANE

Department of Organic Chemistry, University of Valencia, Burjassot, Valencia, Spain

ABSTRACT.—Investigations on the holocellulose released from extractive-free, delignified cork (*Quercus suber*) revealed that it contains glucose (68.45%), xylose (20.67%), arabinose (5.52%), mannose (3.52%), galactose (1.83%), and traces of rhamnose. Methylation studies showed that it consists essentially of a linear chain of (1→4)-linked glucopyranosyl residues and a main chain of (1→4)-linked xylopyranosyl residues.

As part of our continuing study of the cork of *Quercus suber* L. (Fagaceae), which grows in Valencia, Spain, we have studied the polysaccharides. In previous communications (1,2) we have extensively investigated the suberin of the cork, and we now report on the first study of the polysaccharides. The presence of cellulose in the cork of *Q. suber* has long been discussed, and estimates have varied from 1.5% (3) to 24.4% (4). Perhaps the most important finding in this field was obtained by Zetzsche and Rosenthal (5), who converted by acetolysis the cellulose of cork into octaacetylcellobiose and inferred a content of 2-2.5% (6). In spite of that, Guillemonat (6) and Fierz-David (7) stated that the presence of cellulose in the cork was uncertain. They suggested a carbohydrate similar to but distinct from cellulose. We have now studied the holocellulose isolated from the cork, and the insoluble material obtained by removal of some of the hemicellulose.

RESULTS AND DISCUSSION

Monomeric material of cork was removed by exhaustive extraction with C_6H_6, H_2O, and MeOH. The suberin, a polymer of hydroxy and epoxy acids, was eliminated by alkaline methanolysis (8,9), and lignin, an aromatic polymer, by treatment with sodium chlorite (10); the remaining residue was the holocellulose. The extracts represented ca. 22%, suberin ca. 37%, lignin ca. 28%, and holocellulose ca. 13% on the basis of the crude cork.

On acid hydrolysis, the holocellulose yielded neutral sugars identified as glucose (68.45%), xylose (20.67%), arabinose (5.52%), mannose (3.52%), galactose (1.83%), and traces of rhamnose. On enzymic hydrolysis with cellulase (11) the holocellulose gave the same sugars but in different proportions, yielding glucose (63.86%), xylose (7.72%), arabinose (3.10%), mannose (8.27%), galactose (17.05%), and traces of rhamnose. Hakomori methylation (12) of the holocellulose gave a product with an $[\alpha]$ value of $-12.54°$ indicative of β-linkages, which was confirmed by the ^1H nmr spectra (13) (δ 4.3 for H-1). The methylated polysaccharide was hydrolyzed, and the sugars were analyzed, as the partially methylated alditol acetates, by gc (14) and gc/ms (15). The results are summarized in Table 1. The formation of 2,3-di-*O*-methyl-D-xylose and 2,3,6-tri-*O*-methyl-D-glucose in high proportion indicates that the holocellulose was essentially constituted by (1→4)-linked D-xylan(s) and (1→4)-linked D-glucan, as was confirmed by further studies.

The hemicelluloses were extracted from the holocellulose with (a) methyl sulfoxide (16), (b) aqueous 4% NaOH (17), and (c) aqueous 10% NaOH (18), yielding insoluble residues of 69.70, 61.24, and 42.16%, respectively, on the basis of the dry weight of the holocellulose and ca. 10% on the crude cork. The residue, after extraction with aqueous 4% NaOH, was studied in detail. It was purified by two extractions with aqueous 4% NaOH. On acid hydrolysis it gave glucose (97.67%) and xylose (2.32%). The polymer containing the xylose residues could not be removed by 4% NaOH.

TABLE 1. Methyl Ethers Identified after Hydrolysis of the Methylated Holocellulose

Sugars[a]	T[b]	Area %
2,3,4,6-Glc	1.00	0.45
2,3-Xyl	1.47	31.81
2,3,6-Glc	2.44	62.50
3-Xyl	2.78	3.97
2,3-Glc	5.18	1.26

[a]2,3,4,6-Glc=1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, 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 an ECNSS-M column at 175°.

The 4% NaOH-insoluble material was successively permethylated first by the Kuhn method (19) and then by the Hakomori method (12), until the OH absorption band was absent from the ir spectrum and gave a product with an [α] value of −49.6° indicative of β-linkages. The nmr spectra were consistent with the presence therein of β-linkages, showing signals at δ 4.4 in the ^{1}H spectrum assignable to H-1 and δ 103.14 in the ^{13}C spectrum assignable to the anomeric carbon (C-1). The methylated polysaccharide was hydrolyzed, and the sugars were converted into their alditol acetates and then analyzed by gc and gc/ms. The results are shown in Table 2. The formation essentially of 2,3,6-tri-*O*-methyl-D-glucose indicated that the polysaccharide was a (1⟼4)-linked D-glucan.

Controlled acetolysis of the 4% NaOH-insoluble residue (20) yielded α-cellobiose octaacetate (α_D+37.46°). The crystalline free sugars were obtained by transesterification with methanolic sodium methoxide. Pc of the sugars revealed a component having R_{XYL} 0.25, 0.75, and 0.55 in solvents *A-C*, respectively, the same mobilities as authentic cellobiose.

From the results of these experiments, it is concluded that the holocellulose is essentially constituted by cellulose [a polysaccharide composed of a linear chain of (1⟼4)-linked β-D-glucopyranosyl residues] and hemicellulose [polysaccharides composed esentially of a main chain of (1⟼4)-linked β-D-xylopyranosyl residues].

EXPERIMENTAL

GENERAL METHODS.—Descending pc was performed on Whatman Nos. 1 and 3 MM papers with solvents *A*, EtOAc-HOAc-HCO_2H-H_2O (18:3:1:4); *B*, *n*-BuOH-EtOH-H_2O (2:1:1); and *C*,*n*-BuOH-pyridine-H_2O (6:4:3), and detection with diphenylamine aniline (21). Optical rotations were measured on a Perkin-Elmer 141 polarimeter and ir spectra on a Perkin-Elmer 281 spectrophotometer. The melting

TABLE 2. Methyl Ethers Identified after Hydrolysis of the Methylated Polysaccharide

Sugars[a]	T[b]		Area %
	a	b	
2,3,4,6-Glc	1.00	1.00	0.85
2,3,6-Glc	2.42	1.21	97.08
2,3-Xyl	1.45	0.92	2.06

[a]2,3,4,6-Glc=1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, 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.

point was determined on a Büchi apparatus and is uncorrected. ^{1}H-nmr ($CDCl_3$) spectra were recorded on a Perkin-Elmer R-12B (60 MHz) and ^{13}C-nmr ($CDCl_3$) spectra on a Varian XL-200 (200 MHz) spectrometer. TMS was used as internal standard. 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). For gc/ms a Hewlett-Packard 5995B instrument was used fitted with a capillary column (12 m×0.2 mm) containing OV-1. Ei mass spectra were recorded at 70 eV with temperature programmed to rise from 100° to 220° at 4°/min.

ISOLATION AND PURIFICATION OF THE HOLOCELLULOSE.—Finely powdered cork of *Q. suber* (1320 g) was exhaustively extracted (Soxhlet) with C_6H_6 (90 h), H_2O (60 h), and MeOH (20 h) to remove the nonpolymeric material and then air-dried. The residue (278 g) was treated with NaOMe (6.87 g) in dry MeOH (4726 ml) 5 h under reflux to remove the monomers of suberin as methyl esters. The residual material was extracted (Soxhlet) with Et_2O and then with MeOH.

The extractive-free material (178 g) was stirred with H_2O (5700 ml) and delignified with $NaClO_2$ (54 g) in the presence of HOAc (21 ml) for 1 h at 70°, and the fibrous material was recovered by filtration and washed with H_2O. The whole process was repeated twice. After the last treatment, the residue was washed with EtOH, stirred with boiling ethanolic 3% $EtNH_2$ for 5 min, then collected and washed with H_2O. The chlorite-ethanolamine treatment was repeated, and the final residue was washed with EtOH and Et_2O and then air-dried.

The resulting holocellulose (53 g) was extracted with (a) DMSO, (b) aqueous 4% NaOH, and (c) aqueous 10% NaOH, yielding residues comprising 69.70, 61.24, and 42.16%, respectively, of the dry weight of the holocellulose. The residue (32.45 g) remaining after extraction with 4% aqueous NaOH was studied in detail.

HOLOCELLULOSE AND CELLULOSE.—*Sugar analysis.*—Holocellulose and the 4% NaOH-insoluble material (40 mg of each) were hydrolyzed by a two-step procedure 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°). *myo*-Inositol was added as internal standard. The acid was neutralized with $BaCO_3$, and the solution was decationized with Amberlite IR-120 (H^+) resin. The sugars were converted into alditol acetates and analyzed by gc (22) and gc/ms (23).

Methylation analysis.—To a stirred solution of $DMSO^-$ [prepared (24) under N_2 from 14.8 g of NaH and 300 ml of DMSO] at room temperature, was added a suspension of holocellulose (2 g) in DMSO (40 ml). After stirring for 3 h, MeI (30 ml) was added with external cooling. Stirring was continued for 12 h, H_2O (800 ml) was then added, and the mixture was extracted with $CHCl_3$. The combined extracts were washed thrice 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. The residue (2.32 g) was purified by column chromatography [Sephadex LH 20, using $CHCl_3$-Me_2CO (2:1) as eluent] to afford a yellow product (1.69 g) $[\alpha]_D$ −12.54° (*c* 3.1, $CHCl_3$) showing no OH band in its ir spectrum. A portion (60 mg) of the material was hydrolyzed conventionally, and the resulting sugars were converted into alditol acetates and analyzed by gc and gc/ms.

The 4% NaOH-insoluble material was first methylated by the Kuhn method (18). To a suspension of the polysaccharide (2 g) in H_2O (20 ml) containing 38% aqueous NaOH (67 ml) was added dropwise dimethyl sulfate (30 ml) with stirring and under N_2, over a period of 5 h at room temperature. After the addition was completed, the reaction mixture was heated at 40° for 8 h, then at 100° for 20 min, and finally was set aside for 1 day. The mixture was then extracted with $CHCl_3$, and the solvent was removed in vacuo. This product was further methylated by the Hakomori method (12) twice as above, whereupon a fully methylated product was obtained (733 mg). The yellow material, after 2 days drying at 40° with P_2O_5 in vacuo, was dissolved in C_6H_6 and precipitated by addition of petroleum ether (bp 30-60°). The methylated product (124 mg) $[\alpha]_D$ −49.6° (*c* 1.26, $CHCl_3$), which showed no OH bands in its ir spectrum, was hydrolyzed conventionally, and the resulting methyl-sugars were converted into alditol acetates and analyzed by gc and gc/ms.

CELLULOSE.—*Acetolysis.*—Cellulose (1 g) was dissolved with stirring in a mixture (20 ml) of 17 ml glacial HOAc, 17 ml Ac_2O, and 1.8 ml of H_2SO_4 and was allowed to react 80 h at 30°. Then the mixture was filtered, and the filtrate was poured into a mixture of ice and H_2O (60 ml), neutralized with $NaHCO_3$, and then kept for 18 h at 0°. The white precipitate was recovered by filtration, washed with H_2O, and dried in vacuo over solid NaOH, yielded 1.1 g. A portion of this product (452 mg) was recrystallized (MeOH) to afford white crystals of α-cellobiose octaacetate (119 mg) mp 227°; $[\alpha]_D$ +37.46° (*c* 23.86, $CHCl_3$). The free sugars were obtained by transesterification of the product (452 mg) with methanolic NaOMe for 48 h at 0°. The solution was deionized with Amberlite IR-120 (H^+) resin, and the sugars were concentrated and analyzed by pc. (Solvents *A*, *B* on Whatman no. 1 and solvent *C* on Whatman no. 3).

HOLOCELLULOSE.—*Enzymic hydrolysis.*—Holocellulose (2 g) was added to a solution of "cellulase" (0.2 g) from *Aspergillus niger* (Serva; Feinbiochemica, Heidelberg) in 0.05 M acetate buffer (100 ml; pH 4.5) and 1 mg of phenylmercuric acetate. After stirring the mixture for 4 h at 45°, the insoluble residue was filtered off, and the filtrate was boiled for 5 min. The precipitate was removed by centrifugation, and the supernatant was concentrated in vacuo to a brown solid (862 mg). The hydrolyzed products were analyzed, as their alditol acetates, by gc.

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