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STRUCTURAL STUDIES ON THE HEMICELLULOSES OF THE ROOTS OF THE SUGAR MAPLE (*Acer saccharum* Marsh)

PART 2.* TWO (4-*O*-METHYL-D-GLUCURONO)GLUCOXYLANS FROM THE SAPWOOD OF MATURE LATERAL ROOTS

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ABSTRACT

Two electrophoretically homogeneous (4-*O*-methyl-D-glucurono)glucoxylans, structurally distinct from those previously reported in the mature shoot and the whole roots of immature sugar maples, were isolated from the sapwood chlorite holocellulose of mature lateral roots. On the basis of methylation, periodate oxidation, and selective degradation studies, each of the two hemicelluloses (designated P-1 and P-4) were found to consist of a chain of (1→4)-linked D-glucose and D-xylose residues with branching through O-2 of certain xylose residues. The branches were terminated with either a D-xylose or a 4-*O*-methyl-D-glucuronic acid group. The polysaccharides differed both in the amount of branching (ratio of terminal to non-terminal units: P-1, 1:7; P-4, 1:21) and in the molar ratio of D-glucose, D-xylose, and 4-*O*-methyl-D-glucuronic acid (P-1, 5:36:1; and P-4, 1.5:25:1, respectively).

INTRODUCTION

The numerous structural studies reported on the polysaccharides of woody plants[1–3] provide few examples of comparisons of polysaccharides from different locations in a plant[4,5], or from the same location at different stages of development[5,6]. Prior to the work of Scott and Hay[7] the polysaccharides of the roots of trees had not been the subject of structural investigation.

From the roots of immature sugar maples (*Acer saccharum* Marsh) two acidic glucoxylans were isolated which were structurally distinct from the polysaccharides of the shoot[7]. Both glucoxylans of the sugar maple root were primarily β-(1→4)-linked, but one, having a mean degree of polymerization (d.p.) of 96, was branched only through O-3 of certain D-xylose residues, whereas the second (d.p. 144) was

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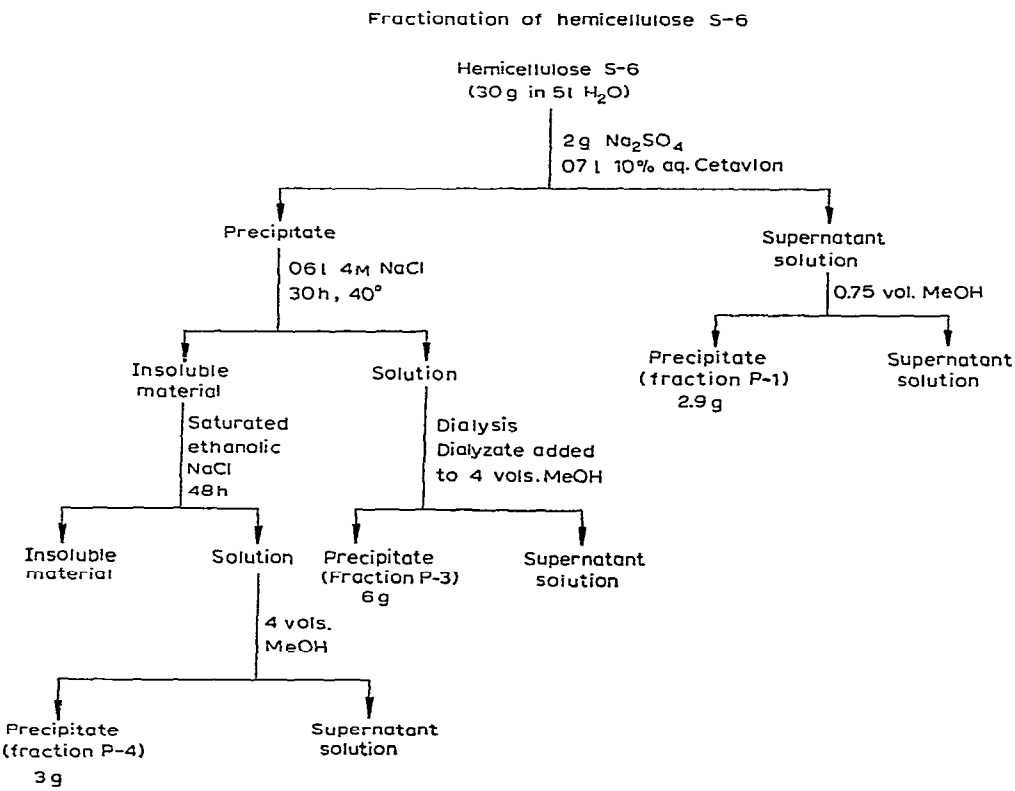
branched through O-3 of both D-xylose and D-glucose residues. The branches terminated in either a D-xylose or an unidentified acidic sugar residue. This preliminary investigation, and the prior studies on the carbohydrates of the stem[8,9], sapwood[10,11], heartwood[11], inner bark[11], and sap[12] of the sugar maple, prompted the present detailed structural investigation of the polysaccharides from the lateral roots of a mature specimen of *Acer saccharum* (Marsh). The structural features of two acidic polysaccharides from the sapwood of sugar maple roots are described herein.

EXPERIMENTAL

General methods. — Descending partition chromatography on Whatman No. 1 or 3MM paper employed the following solvent systems: (*1*) 2:5:7 pyridine–ethyl acetate–water; (*2*) 5:5:3:1 pyridine–ethyl acetate–water–acetic acid; (*3*) butanone–water azeotrope; (*4*) 18:4:1:3 ethyl acetate–acetic acid–formic acid–water; (*5*) 4:1:5 1-butanol–ethanol–water; or (*6*) 4:4:1:4 1-butanol–ethanol–ammonium hydroxide (concd.)–water. The components were detected[13] with alkaline silver nitrate, *p*-anisidine hydrochloride, or an acetone solution containing trifluoroacetic acid and aniline in a 2:1 molar ratio (dipped, or sprayed, paper heated for 2–5 min at 125°). Gas–liquid partition chromatography (g.l.p.c.) was performed on a Pye-Argon chromatograph (Pye & Co., Cambridge, England) with a 20-mCi ^{90}Sr ionization detector, and argon as carrier gas at a flow rate of 100 ml/min and straight, Pyrex-glass columns [137 × 0.4 (i.d.) cm], or with an F and M Model 402 chromatograph with a flame-ionization detector and helium as carrier gas at a flow rate of 100 ml/min, and bent, Pyrex-glass columns [122 × 0.4 cm (i.d.)]. The columns were packed with (*1*) 3% SE-52 silicone gum rubber on Chromosorb W, 100–120 mesh; (*2*) 3% ECNSS-M (organosilicone polymer) on Gas-Chrom Q, 100–120 mesh; (*3*) 10% LAC 4R-886 (polyester) on Chromosorb W, 80–100 mesh; (*4*) as (*3*) except at a concentration of 5%; (*5*) 20% SF-96 (GE methylsilicone) on Chromosorb W, 60–80 mesh; (*6*) 15% butane-1,4-diol succinate polyester on Chromosorb W, 60–80 mesh; or (*7*) 10% OS-124 (polyphenyl ether, 5 rings) on Chromosorb W, 80–100 mesh. Optical rotations were measured with a Perkin–Elmer Model 141 or a Bendix Type 143A polarimeter at 23 ±3°. Infrared spectra were obtained with a Perkin–Elmer Model 21 or a Beckman IR-5A spectrometer. Electrophoresis on Whatman Chromedia GF-81 glass-fiber paper was carried out in 0.1M sodium borate (pH 9.3) at 20 mA and 1500 V (d.c.); components were detected by spraying with 10% sulfuric acid in ethanol, or with conc. sulfuric acid, and heating at 130°. Moving free-boundary electrophoresis was conducted in a Perkin–Elmer Model 8A apparatus using 0.1M sodium borate (pH 9.3) at 20 mA and 150 V (d.c.). Melting points were determined with a Fisher–Johns block and are uncorrected. Evaporations were carried out under diminished pressure at 35–45°. Polarographic analyses were obtained with a Sargent Model XVI polarograph.

Preparation of wood samples. — Samples of the major lateral roots of a dormant, mature sugar maple (*Acer saccharum* Marsh) were sawn into discs and debarked.

The sapwood was separated from the sharply defined, dark heartwood and the samples were chipped, air-dried, and ground to 80–100 mesh in a Wiley mill. The wood meal was exhaustively extracted in a Soxhlet apparatus with 1:1 chloroform–petroleum ether (b.p. 65–110°). The extractive-free sapwood meal was exhaustively extracted with hot (80°) water and an alcohol-precipitable glucan isolated (yield: 2.4%, extractive-free wood-meal basis). Chlorite delignification (4 additions of 147 g of sodium chlorite and 49 ml of acetic acid)[14] of 490 g of residue in sodium lactate–lactic acid buffer (pH 3.5) at 60° afforded 360 g of polysaccharide that was completely soluble in cold, 72% (w/w) sulfuric acid. The sapwood chlorite holocellulose (250 g) was extracted twice with 2.5 l of deaerated 1% sodium hydroxide for 6 h at room temperature under nitrogen. The residue (A) was removed by filtration and fraction S-1 (0.5 g) precipitated from the filtrate by acidification to pH 4 with 50% (v/v) acetic acid. Fractions S-2 (2 g), S-3 (1 g), and S-4 (trace) were precipitated from the acidic supernatant solution by the addition of methanol to 30, 50, and 80% (v/v), respectively. Residue A was extracted with 2 l of sodium hydroxide for 3 h at 70° under nitrogen. The residue (B) was removed by filtration. The filtrate was acidified (pH 4) as before and added rapidly to 4.5 volumes of methanol to precipitate fraction S-5



SCHEME 1

(8 g). Residue B was extracted with deaerated 15% sodium hydroxide (2 l, 6 h, 21°, under nitrogen) and the insoluble material washed with 15% sodium hydroxide. The combined supernatant and wash solutions were acidified as before and poured rapidly into 5 volumes of methanol to precipitate fraction S-6 (49 g). Fraction S-6 was treated according to Scheme 1. Fraction P-2FA was obtained from fraction S-5 by successive complex formation with Cetavlon[15] and Fehling's solution.

Electrophoretic studies of selected fractions. — Glass-fiber paper electrophoresis[16] of fractions P-1 and P-2FA revealed only one component in each. Moving free-boundary (Tiselius) electrophoresis[17] of each of fractions P-1, P-3, P-4, and P-2FA gave a single, symmetrical peak without discernible skew or shoulders; the mixed fractions were cleanly resolvable.

Composition of fraction P-1. — The neutral and acidic components of the polysaccharide hydrolyzate (M trifluoroacetic acid, 14 h, reflux) were separated by passage through a column of Dowex-1X (acetate form) ion-exchange resin. The neutral moiety was resolved (Whatman 3MM, solvent *5*) into D-glucose (5 mg, $[\alpha]^{23}$ +50° (*c* 0.4, water); identified as *N*-*p*-nitrophenyl-α-D-glucosylamine, m.p.[18] and mixed m.p. 183–185°), and D-xylose (55 mg, $[\alpha]_D^{23}$ +19.2° (*c* 1, water); identified as di-*O*-benzylidene-D-xylose dimethyl acetal, m.p.[19] and mixed m.p. 209–210°). The neutralization equivalent of the polysaccharide[20] was found to be 6600. The proportions of the neutral sugars, as determined by the phenol–sulfuric acid method[21], are reported in Table I.

TABLE I

SELECTED PROPERTIES OF HEMICELLULOSE FRACTIONS P-1, P-2FA, P-3, AND P-4

Property	*Hemicellulose Fraction*			
	P-1	*P-2FA*	*P-3*	*P-4*
D.p.	188	290	300	180
$[\alpha]_D$ in M NaOH	−33.5°[a]	−55°[b]	−14.3°[c]	−61°[d]
Composition (mole ratio):				
D-Glucose	5	1	1	1.5
D-Xylose	36	7.5	10	25
Uronic acid	1		1	1

[a]Concentration: 0.4 g/100 ml; temperature, 24°. [b]Concentration: 0.4 g/100 ml; temperature, 24.5°. [c]Concentration: 0.98 g/100 ml; temperature, 23°. [d]Concentration: 0.13 g/100 ml; temperature, 23°.

Identification of 2-O-(*4-O-methyl-α-D-glucopyranosyluronic acid*)-D-*xylose* (**1**). — Paper chromatography (solvent *4*) indicated the acidic moiety of the P-1 hydrolyzate to be a single compound indistinguishable from an authentic sample of **1**. This component (4 mg; $[\alpha]_D^{23}$ +87.5° (*c* 0.51, water); lit.[22] $[\alpha]_D$ +84.7°) was refluxed in 2% methanolic hydrogen chloride. A portion of the product was reduced with lithium aluminum hydride in tetrahydrofuran[23] and hydrolyzed (M trifluoroacetic

acid, reflux) to give xylose and 4-*O*-methyl-D-glucose (paper chromatography, solvent *5*) in a 1:1 molar ratio[21] (calculated as pentose and monomethylhexose). Analysis by g.l.p.c. (column *1*, 150°) of the trimethylsilyl ethers of these components, and co-chromatography with authentic reference standards, corroborated these results. The 4-*O*-methyl-D-glucose was converted into its alditol acetate and found to be indistinguishable from an authentic sample by g.l.p.c. (column *3*, 175°). The remainder of the **1** methyl ester methyl glycoside was converted into its methyl ether by the modified Hakomori method[25,26] under nitrogen, prior to reduction and methanolysis as before. G.l.p.c. (column *6*, 160°) of the products indicated the presence of equimolar amounts of methyl 2,3,4-tri-*O*-methyl-D-glucoside and methyl 3,4-di-*O*-methyl-D-xyloside.

Partial acid hydrolysis of fraction P-1. — The hydrolyzate (0.02M trifluoroacetic acid, 8 h, reflux) from a sample of P-1 (150 mg) was separated into a neutral and an acidic fraction on Dowex-1X (OAc^- form) as before. Preparative paper chromatography (solvent *1*) of the neutral sugars afforded D-xylose (20 mg; R_{Xyl} 1.00) and D-glucose (8 mg; R_{Xyl} 0.77), identified as already described, and 3 other components having R_{Xyl} 0.70, 0.55, and 0.31, respectively.

Identification of 4-O-β-D-xylopyranosyl-D-xylose (**2**). — A 4-mg sample of this component (16 mg; $[\alpha]_D^{23}$ −28° (*c*, 0.3 water); lit.[27] $[\alpha]_D^{22}$ −25.5°; R_{Xyl} 0.70 in solvent *1*) was oxidized in 0.02M sodium periodate at 20–23° in the dark. Samples (2.0 ml) were mixed with 2 ml of acetate buffer (pH 4.2) as supporting electrolyte and 6 drops of 0.005% polystyrenesulfonate[28] as a maximum supressor (and diluted, if necessary, to give measurable diffusion currents), and the periodate content was measured polarographically[29,30]. In this experiment, 1 mole of carbohydrate reduced 3.9 moles of periodate. The disaccharide (5 mg) was permethylated[25,26] and hydrolyzed (M trifluoroacetic acid) to give 2,3-di- and 2,3,4-tri-*O*-methyl-D-xylose only (paper chromatography, solvent *5*). G.l.p.c. (column *2*, 150°) of the alditol acetates[31] prepared from this hydrolyzate corroborated the identification and indicated the presence of equimolar amounts of the compounds.

Composition of the minor components. — The minute amounts of compounds having R_{Xyl} 0.55 and 0.31 (solvent *1*) from the hydrolyzate of P-1 precluded structural studies. Hydrolysis of each afforded only xylose (g.l.p.c. of Me_3Si derivatives, column *1*, 150°). The acidic moiety contained 2 components: one (R_{Xyl} 0.90, solvent *4*) was chromatographically indistinguishable from **1** and, on complete acid hydrolysis afforded only two products, provisionally identified as xylose and 4-*O*-methyl-glucuronic acid (solvent *5*); the second (R_{Xyl} 0.60, solvent *4*) was hydrolyzed to give xylose and **1** (solvent *5*).

Enzymic hydrolysis of fraction P-1. — Paper chromatography of crude fungal pectinase (Nutritional Biochemical Corp.) revealed no oligosaccharides or simple sugars in the preparation. The enzyme was incubated with 5 times its weight of each of D-glucose, D-xylose, D-glucuronic acid, maltose, xylobiose, and cellobiose, in aqueous solution for 24 h at 40°. No change in the substrates was detectable by paper chromatography (solvents *1*, *2*, and *5*)[30].

An aqueous solution of fraction P-1 (1 g) and the pectinase (0.2 g) was incubated in dialysis tubing (Visking Corp.) suspended in 2 l of water for 12 days at 30–40°. The dialyzate was removed daily and evaporated to dryness (total weight, 0.8 g). Chromatographic analysis (solvents *1*, *2*, and *5*) revealed the presence of **2**, and xylotriose as major components, with small amounts of D-xylose, D-glucose, **1**, and 4-*O*-α-D-xylopyranosyl-D-glucose (**3**), and traces of at least 2 other components. The monosaccharides, and compounds **1** and **2** were identified as already described above.

Identification of O-β-D-*xylopyranosyl*-(*1*→*4*)-O-β-D-*xylopyranosyl*-(*1*→*4*)-D-*xylose* (**4**). — This compound (R_{Xyl} 0.60 in solvent *2*, $[\alpha]_D^{22}$ −50° (*c* 0.3, water); lit.[27] $[\alpha]_D^{25}$ −47.0°) was a syrup that consumed 5.10 moles of periodate per mole of trisaccharide[29,30]. A sample (7 mg) was permethylated[26] and the methanolysis products were determined by g.l.p.c. (column *6*, 160°) to be methyl 2,3,4-tri-*O*-methyl-D-xyloside and methyl 2,3-di-*O*-methyl-D-xylopyranoside in a molar ratio of 0.9:2.0, respectively.

Identification of **3**. — This component ($[\alpha]_D^{23}$ +85° (*c* 0.2, water), lit.[30] $[\alpha]_D$ +80° (*c* 1, water); R_{Xyl} 0.50 in solvent *1*) reduced 5.2 moles of periodate per mole[29]. The hydrolyzate (M trifluoroacetic acid, sealed tube, 12 h, 100°) consisted of an equimolar mixture of xylose and glucose as determined chromatographically (paper, solvents *1* and *5*; g.l.p.c. of Me_3Si derivatives, column[32] *5*, 150°, with authentic reference standards and co-chromatography to establish quantitation). Methanolysis of a permethylated[26] sample afforded an equimolar mixture of methyl 2,3,4-tri-*O*-methyl-D-xyloside and methyl 2,3,6-tri-*O*-methyl-D-glucopyranoside (g.l.p.c.[33], column *6* at 170°, column *7* at 160°; co-chromatography with standards as before).

Methylation of fraction P-1. — A 500-mg sample was converted into the methyl ether by repeated treatment in the usual manner[25,26] until permethylated[34]; the i.r. spectrum of the product showed negligible absorbance in the characteristic OH region (3500–3600 cm^{-1}).

Examination of the methyl ether derivative of fraction P-1. — A portion (200 mg) of the ether derivative was subjected to formolysis (90% aq. formic acid, 2 h, 100°)[35], and the residual syrup hydrolyzed (0.25M trifluoroacetic acid, 12 h, 100°), evaporated to dryness to remove the acid, and separated into a neutral and an acidic fraction by ion exchange as before.

The acidic fraction migrated as a single component upon glass-fiber paper electrophoresis. The compound was esterified in methanol with excess ethereal diazomethane[36] and reduced with lithium aluminum hydride[23]. The product was methanolyzed. G.l.p.c.[33] (column *6*, 150°) demonstrated the presence of equimolar amounts of methyl 2,3,4-tri-*O*-methylglucoside and methyl 3-*O*-methylxylopyranoside as the only products.

Quantitative paper-chromatographic analysis[21] of the neutral fraction with solvents *3* and *5* afforded the results summarized in Table II. The constituents were resolved (Whatman 3MM, solvent *5*) and identified as: 2,3,4-tri-*O*-methyl-D-xylose [5 mg, $[\alpha]_D^{23}$ +19° (*c* 0.5, water)][37]; converted into the aniline derivative[38], m.p. and mixed m.p. 99–101° (from ethyl acetate–petroleum ether); 2,3-di-*O*-methyl-D-xylose

(25 mg, $[\alpha]_D^{23}$ +23° (*c* 1.53, water)[39], converted into *N*-phenyl-2,3-di-*O*-methyl-D-xylopyranosylamine, m.p.[40] and mixed m.p. 146–147°); 3-*O*-methyl-D-xylose (7 mg, $[\alpha]_D$ +20° (*c* 0.53, water)[9], converted into *N*-phenyl-3-*O*-methyl-D-xylopyranosylamine, m.p.[9] and mixed m.p. 130–132°); and 2,3,6-tri-*O*-methyl-D-glucopyranose [5 mg, $[\alpha]_D$ +66° (*c* 0.4, water)[41]], identified as methyl 4-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucoside (after derivatization[42,43]) by g.l.p.c.[44] (column *4*, 170°).

TABLE II

COMPOSITION OF HYDROLYZATE OF PERMETHYLATED FRACTIONS P-1 AND P-4

Component	*Mole ratio*	
	P-1	*P-4*
2,3,4-Tri-*O*-methyl-D-xylose	5	1.7
2,3-Di-*O*-methyl-D-xylose	29	52
3-*O*-Methyl-D-xylose	4.5	1.5
2,3,6-Tri-*O*-methyl-D-glucose	6	1.7
Uronic acid	1	1

Determination of $\overline{d.p.}$ of fraction P-1. — A 50-mg sample of polysaccharide was treated according to the method of Unrau and Smith[45]. A blank experiment was carried out concurrently. The polysaccharide produced 0.44 μg of formaldehyde per ml, corresponding to 1.14 μg of formaldehyde per mg of polysaccharide. Based on a molecular weight of 140 for the average sugar residue, and the producion of 1 mole of formaldehyde per mole of polysaccharide the $\overline{d.p.}$ was calculated to be 188.

Periodate oxidation of fraction P-1. — The polysaccharide (20 mg) was suspended in 15 ml of distilled water and oxidized by the addition of 20 ml of 0.1M sodium periodate in acetate buffer[46,47], pH 4.2, at 4–5°, in the dark. A blank experiment was conducted concurrently. Polarographic analysis[28,29] indicated the reduction of 0.98 mole of periodate per average sugar unit. In a parallel, unbuffered reaction excess periodate was reacted with ethanediol. Titration with 1 mM NaOH under nitrogen to a phenolphthalein endpoint[48] indicated the formation of 0.10 mole of formic acid per average sugar unit.

Smith degradation[49,50] of fraction P-1. — The oxidation of 500 mg of P-1 (dispersed in 25 ml of water) with 40 ml of 0.5M sodium periodate at 5° in the dark was complete after 5 days, as monitored polarographically[28,29]. The reduced polyaldehyde was partially hydrolyzed with 0.2M trifluoroacetic acid, 10 h, 22°). Acidic and neutral components were separated as before. Paper-chromatographic analysis and resolution (solvents *1*, *4* and *5*) of the hydrolyzate afforded the following compounds: (*a*) *2-O-β-D-xylopyranosylglycerol*, $[\alpha]_D^{23}$ −38.7° (*c* 0.2, water); lit.[51], $[\alpha]_D$ −34.4° (*c* 2, water), which was hydrolyzed (M trifluoroacetic acid, 4 h, 100°, sealed tube) and the Me_3Si derivatives of the hydrolyzate identified by g.l.p.c.[31] (column *5*, 150°) as glycerol and xylose in a molar ratio of 1.0:1.06; (*b*) *erythritol*,

glycerol, and *ethylene glycol*, further identified by g.l.p.c. of Me_3Si (column *5*, 100–150° at $\Delta t = 3°$/min) and acetyl (column *3*, 80–150°, $\Delta t = 3°$/min) derivatives, which gave explicit enhancement of signals upon cochromatography with authentic standards; and (*c*) D-*xylose*, $[\alpha]^{23}$ +17° (*c* 0.2, water)[52], corroborated by g.l.p.c. of Me_3Si derivatives (column *1*, 140°)[24].

Paper chromatography (solvent *6*) of the acidic moiety of the hydrolyzate revealed only 3-*O*-methyl-D-erythronic acid. This compound was esterified (1% hydrogen chloride in methanol), and reduced (lithium aluminum hydride in tetrahydrofuran) to 2-*O*-methyl-D-erythritol ($[\alpha]_D^{23}$ +30° (*c* 0.10, methanol); lit.[53] $[\alpha]_D^{22}$ +28° (*c* 3.2, methanol); which reduced 1.03 moles of periodate/mole[28]) and was chromatographically indistinguishable from an authentic sample (solvent *5*). The identity was supported by g.l.p.c. of the alditol Me_3Si[24] (column *5*, 160°) and acetyl[43] (column *4*, 175°) derivatives.

Complete acid hydrolysis of the reduced polyaldehyde from fraction P-1. — The remainder of the reduced polyaldehyde was hydrolyzed (M trifluoroacetic acid, 8 h, reflux) and separated into a neutral and an acidic fraction as before. Paper chromatography (solvents *1*, *4*, and *5*) and g.l.p.c. (Me_3Si derivatives, column *5*, 100–150°, $\Delta t = 3°$ per min; acetates, column *3*, 80–150°, $\Delta t = 3°$ per min) of the neutral components indicated the presence of ethylene glycol, glycerol, erythritol, and xylose in a molar ratio (Me_3Si derivatives, column *5*, 100–220°, $\Delta t = 3°$/min), of 1.0:5.7:1.1:1.0, respectively.

Structural investigation of fraction P-4. — Fraction P-4 reduced 0.96 mole of sodium periodate (20-mg samples)[29,46,47] and formed 0.025 mole of formic acid (30-mg samples)[48] per average sugar residue. Periodate oxidation[46] of the reduced[54] polysaccharide P-4 (85-mg samples) formed 1.19 μg formaldehyde/mg of polysaccharide[45] (see Table I).

Chromatographic analysis (solvents *1*, *4*, and *5*) and preparative resolution (solvent *5*) of the complete acid hydrolyzate (M trifluoroacetic acid, 10 h, 100°, sealed tube) yielded D-glucose, D-xylose, and **1**. Similarly, partial acid hydrolysis (20 mM trifluoroacetic acid, 6 h, reflux) afforded D-xylose (50 mg), D-glucose (7 mg), **2** (20 mg) and **1** (wt. uncertain). Enzymolysis of the P-4 polysaccharide with fungal pectinase, in the manner described, gave **1**, **2**, **3**, **4** and trace amounts of 3 other components (R_{Xyl} 0.44, 0.32, and 0.25; solvent *2*). The identity of each of these components (except for those obtained in trace amounts) was established by g.l.p.c. of Me_3Si and acetyl derivatives, and by conversion into characteristic crystalline compounds, as described previously. Each of the trace components yielded only xylose on acid hydrolysis.

Fraction P-4 (300 mg) was methylated[25,26] until the infrared OH absorbance at 3500–3600 cm^{-1} became negligible. Anal.[34]: Found: OMe, 38.6%; calc. OMe, 39.0%. Formolysis, hydrolysis, and fractionation of the hydrolyzate as described previously afforded the components listed in Table II.

A Smith degradation[49,50] of fraction P-4 was performed as already described. The products (identified as before) were ethylene glycol, glycerol, erythritol, 3-*O*-

methylerythronic acid, D-xylose, and 2-*O*-(β-D-xylopyranosyl)glycerol. Complete acid hydrolysis of the reduced polyaldehyde of fraction P-4 gave, in addition to 3-*O*-methyl-D-erythronic acid, a mixture of ethylene glycol, glycerol, erythritol, and D-xylose in a molar ratio of 1:29.2:0.90:0.84, respectively. These components were identified in the manner described earlier.

DISCUSSION

Purification of the root polysaccharides afforded four electrophoretically homogeneous hemicelluloses: fractions P-1, P-3, P-4, and P-2FA (Table I). Fractions P-1 and P-4 are similar (4-*O*-methylglucurono)glucoxylans and will be discussed concurrently, the differences being noted as the pertinent evidence is considered.

The specific optical rotations of fractions P-1 and P-4 were negative, indicating that most glycosidic linkages were of the β-D configuration. The neutral moiety of the acid hydrolyzate contained only D-glucose and D-xylose, whereas the acidic moiety was constituted of a single aldobiouronic acid. The fact that, upon reduction and methanolysis, the permethylated methyl ester of this acidic component yielded equimolar quantities of methyl 3,4-di-*O*-methyl-D-xyloside and methyl 2,3,4-tri-*O*-methyl-D-glucoside, whereas upon reduction and hydrolysis the methyl ester gave equimolar amounts of D-xylose and 4-*O*-methyl-D-glucose, established that the acidic component is **1**. This aldobiouronic acid occurs in the hemicelluloses of several hardwood species, including *Populus tremuloides* Michx.[55], *Betula papyrifera*[56], *Populus monilifera* H.[57], *Salix alba* L.[22,58], and the stem of *Acer saccharum* Marsh[8].

The results of partial hydrolysis of fractions P-1 and P-4 indicated the presence of uninterrupted sequences of β-(1→4)-linked D-xylose residues extending for 3, and probably as many as 6, sugar residues, as evidenced by the recovery of **4** and the chromatographic analyses of the small amounts of higher homologs. These data also revealed that the uronic acid was bonded exclusively to D-xylose. These structural features were corroborated by the results of enzymolysis with fungal pectinase but, in addition, this selective degradation afforded **3**. This compound revealed both that D-glucose was an integral part of the hemicellulose molecule, and that at least some of the glycosidic linkages of xylose were of the α-type. The anomeric configuration of the D-glucose residues could not be deduced and there was no evidence of sequences of contiguous glucose residues.

Formolysis and hydrolysis of the permethylated P-1 and P-4 hemicelluloses gave the products listed in Table II. From these data it can be concluded that these hemicelluloses consist of a primary chain of (1→4)-linked D-xylose and D-glucose residues, with branching only through O-2 of certain (1→4)-linked D-xylose residues. The branches terminate in either a D-xylose or a 4-*O*-methyl-D-glucuronic acid residue. The isolation of methyl 3-*O*-methyl-D-xylopyranoside from the methanolysis of the reduced aldobiouronic acid methyl glycoside revealed that the uronic acid moiety is bonded through O-2 to a xylopyranosyl residue, which is itself a part of the β-(1→4)-linked chain of sugar residues. Thus, the 4-*O*-methyl-D-glucuronic acid

forms a single-unit side-chain of this hemicellulose. Since there are 4–5 times as many branch points in P-1 (and twice as many in P-4) as there are uronic acid residues, it is evident that many of the branches do not involve a uronic acid residue and hence cannot be defined on the basis of the present evidence. The methylation data show clearly that P-1, having a ratio of branched to unbranched, internal, sugar residues of approximately 1:8 is much more highly branched than P-4, where the corresponding ratio is 1:36. Similarly, the proportion of uronic acid is found to be significantly higher in P-1 with one uronic acid residue in an average repeating unit of approximately 46, as compared to P-4 with one uronic acid residue in an average repeating unit of 58. The $\overline{\text{d.p.}}$ of fraction P-1 was found to be 188, which suggested that it consisted of four average repeating units; the $\overline{\text{d.p.}}$ 180 for fraction P-4 indicated the presence of three average repeating units per molecule.

A hemicellulose having the structural features indicated by the methylation data for fraction P-1 would be expected to consume 1.01 mole of periodate and produce 0.11 mole of formic acid per average sugar residue. The experimental results—0.98 mole of periodate and 0.10 mole of formic acid—were in close agreement and support the general conclusions based on the methylation study. By the same reasoning, the methylation data for fraction P-4 predicted the consumption of 0.96 mole of periodate and formation of 0.025 mole of formic acid per average sugar residue. Again the experimental results—1.03 mole of periodate and 0.03 mole of formic acid—were fully consistent with these conclusions.

The Smith degradation of each of fractions P-1 and P-4 gave 2-*O*-(β-D-xylopyranosyl)glycerol, in conformity with the presence of branching only at O-2 of (1→4)-linked D-xylose residues. The absence of any xylopyranosyl-erythritol in the product of this reaction sequence argues for the location of branch points at least one xylose residue away from a D-glucose residue. The detection of erythritol but not glucose in the product was consistent with the foregoing evidence that every glucose residue should be susceptible to periodate oxidative cleavage between C-2 and C-3. The detection of 3-*O*-methyl-D-erythronic acid, the identity of which was established by reduction of the corresponding methyl ester to 2-*O*-methyl-D-erythritol, added to prior evidence for the presence of a periodate-oxidizable 4-*O*-methyl-D-glucuronic acid unit in the hemicelluloses.

Complete hydrolysis of the reduced polyaldehyde afforded ethylene glycol, glycerol, erythritol, and D-xylose in the molar ratios of 1.0:5.7:1.1:1.0, respectively, for fraction P-1, and 1.0:29.2:0.90:0.84, respectively, for P-4. These data were judged to agree well, within the limits of experimental accuracy, with the ratios predicted on the basis of methylation data, namely 1:5.8:0.9:1.2 for P-1, and 1:31:1:0.9 for P-4. The slight discrepancy is probably due to some loss of the relatively volatile tri-*O*-methyl-D-xylose and/or ethylene glycol during the isolation from these experiments.

Neubauer and Purves[8] reported the presence of an acidic xylan in the stem of the sugar maple. The polysaccharide was demonstrated[8,9] to have a branched backbone of β-(1→4)-linked D-xylose residues, some of which carried terminal, 4-*O*-methyl-D-glucuronic acid[59] residues linked through O-2 of xylose. Adams[11]

reported a water-soluble 4-*O*-methylglucuronoxylan from the stem sapwood of *Acer saccharum* Marsh, but, prior to the report of Scott and Hay[7], no acidic glucoxylan had been described in these wood hemicelluloses. The latter work[7] disclosed the presence of polysaccharides similar to P-1 and P-4 in the roots of immature sugar maples but having branches through O-3 of (1→4)-linked xylose or xylose and glucose residues. The relationship between the location of branching and the development of the tree roots is not known. The results reported in this paper establish that the hemicelluloses, P-1 and P-4, represent hitherto unreported constituents of the wood of *Acer saccharum* Marsh, and add to the previous evidence[7] that the roots of this species contain an array of hemicelluloses distinct from those of other parts of the tree.

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REFERENCES

1 T. E. Timell, *Wood Sci. Technol.*, 1 (1967) 45.
2 H. F. J. Wenzl, *The Chemical Technology of Wood*, Academic Press, New York, 1970.
3 T. E. Timell, *Advan. Carbohyd. Chem.*, 19 (1964) 247; 20 (1965) 409.
4 G. C. Hoffman and T. E. Timell, *Svensk Papperstidn.*, 75 (1972) 4, 135, 241, 297; T. E. Timell, *ibid.*, 72 (1969) 6, 173.
5 C. M. Stewart, *The Chemistry of Secondary Growth in Trees*, Division of Forest Products Technological Paper No. 43, C.S.I.R.O., Australia, 1966, and references therein; H. Meier, in M. H. Zimmerman (ed.), *The Formation of Wood in Forest Trees*, Academic Press, New York, 1964, p. 137.
6 D. J. Nevins, P. D. English, and P. Albersheim, *Plant Physiol.*, 43 (1968) 914; V. D. Harwood, *Holzforsch.*, 25 (1971) 73.
7 S. J. Scott, M.Sc. Thesis, Queen's University, Kingston, Canada, 1966; S. J. Scott and G. W. Hay, *Can. J. Chem.*, 45 (1967) 2217.
8 L. G. Neubauer and C. B. Purves, *Can. J. Chem.*, 35 (1957) 388.
9 T. E. Timell, *Can. J. Chem.*, 37 (1959) 893.
10 G. A. Adams, *Can. J. Chem.*, 39 (1961) 2423.
11 G. A. Adams, *Svensk Papperstidn.*, 67 (1964) 82.
12 W. L. Porter, N. Hoban, and C. O. Willits, *Food Research*, 19 (1954) 597; S. Haq and G. A. Adams, *Can. J. Chem.*, 39 (1961) 1165; G. A. Adams and C. T. Bishop, *ibid.*, 38 (1960) 2380.
13 W. E. Trevelyan, D. P. Procter, and J. S. Harrison, *Nature*, 166 (1950) 444; L. Hough, J. K. N. Jones, and W. H. Wadman, *J. Chem. Soc.*, (1950) 1702.
14 L. E. Wise, M. Murphy, and A. A. D'Addieco, *Paper Trade J.* 122 (1946) 35.
15 J. E. Scott, *Methods Carbohyd. Chem.*, 5 (1965) 38.
16 B. A. Lewis and F. Smith, *J. Amer. Chem. Soc.*, 79 (1957) 3929; N. J. O'Leary, R. B. Hobbs, J. K. Missimer, and J. J. Erving, *Tappi*, 37 (1954) 446.
17 R. L. Whistler and C. S. Campbell, *Methods Carbohyd. Chem.*, 5 (1965) 201.
18 G. Weygand, W. Perkow, and P. Kuhner, *Chem. Ber.*, 84 (1951) 594.
19 L. J. Breddy and J. K. N. Jones, *J. Chem. Soc.*, (1945) 738.

20 D. V. MYHRE AND F. SMITH, *J. Agr. Food Chem.*, 8 (1960) 359.
21 M. DUBOIS, K. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350.
22 S. KARACSONYI, M. KUBACKOVA, AND J. HRIVNAK, *Coll. Czech. Chem. Commun.*, 32 (1967) 3597; H. C. SRIVASTAVA AND G. A. ADAMS, *J. Amer. Chem. Soc.*, 81 (1959) 2409.
23 G. O. ASPINALL, E. L. HIRST, AND N. K. MATHESON, *J. Chem. Soc.*, (1956) 989; G. A. ADAMS, *Can. J. Chem.*, 37 (1959) 29; M. ABDEL-AKHER AND F. SMITH, *Nature*, 166 (1950) 1037.
24 C. C. SWEELEY, R. BENTLEY, M. MAKITA, AND W. W. WELLS, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
25 S. HAKOMORI, *J. Biochem.* (Tokyo), 55 (1964) 205.
26 P. A. SANFORD AND H. E. CONRAD, *Biochemistry*, 5 (1966) 1508.
27 R. L. WHISTLER AND C. TU, *J. Amer. Chem. Soc.*, 74 (1952) 3609.
28 R. D. CORLETT, W. G. BRECK, AND G. W. HAY, *Anal. Chem.*, 42 (1970) 792.
29 R. D. CORLETT, W. G. BRECK, AND G. W. HAY, *Can. J. Chem.*, 48 (1970) 2474.
30 G. A. HENDERSON AND G. W. HAY, *Carbohyd. Res.*, 23 (1972) 379.
31 H. BJÖRNDAL, B. LINDBERG, AND S. SVENSSON, *Acta Chem. Scand.*, 21 (1967) 1801.
32 G. G. S. DUTTON, K. B. GIBNEY, G. D. JENSEN, AND P. E. REID, *J. Chromatogr.*, 36 (1968) 152.
33 G. O. ASPINALL, *J. Chem. Soc.*, (1963) 1676.
34 F. J. BATES AND ASSOCIATES, *Polarimetry, Saccharimetry and the Sugars*, Circular 440, U.S. National Bureau of Standards, Washington, D.C., 1942, p. 510; E. P. CLARK, *J. Assn. Official Agr. Chem.*, 15 (1932) 136.
35 H. O. BOUVENG, H. KIESSLING, B. LINDBERG, AND J. MCKAY, *Acta Chem. Scand.*, 16 (1962) 615.
36 T. J. DE BOER AND H. J. BACKER, *Rec. Trav. Chim.*, 73 (1954) 229.
37 R. A. S. BYWATER, W. N. HAWORTH, E. L. HIRST, AND S. PEAT, *J. Chem. Soc.*, (1937) 1983; S. K. CHANDA, E. L. HIRST, J. K. N. JONES, AND E. G. V. PERCIVAL, *ibid.*, (1950) 1289.
38 R. A. LAIDLAW AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1949) 1600; (1950) 528.
39 S. K. CHANDA, E. E. PERCIVAL, AND E. G. V. PERCIVAL, *J. Chem. Soc.*, (1952) 260.
40 I. EHRENTHAL, M. C. RAFIQUE, AND F. SMITH, *J. Amer. Chem. Soc.*, 74 (1952) 1341.
41 H. C. CARRINGTON, W. N. HAWORTH, AND E. L. HIRST, *J. Amer. Chem. Soc.*, 55 (1933) 1084.
42 C. S. HUDSON, *J. Amer. Chem. Soc.*, 47 (1925) 267.
43 M. L. WOLFROM AND A. THOMPSON, *Methods Carbohyd. Chem.*, 2 (1963) 211.
44 H. G. JONES, Ph. D. Thesis, Queen's University, Kingston, Canada, 1964.
45 G. W. HAY, B. A. LEWIS, F. SMITH, AND A. M. UNRAU, *Methods Carbohyd. Chem.*, 5 (1965) 251.
46 G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohyd. Chem.*, 5 (1965) 357.
47 K. H. MEYER AND P. RATHGEB, *Helv. Chim. Acta*, 32 (1949) 1102.
48 F. BROWN, T. G. HALSALL, E. L. HIRST, AND J. K. N. JONES, *J. Chem. Soc.*, (1948) 27.
49 I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS, AND F. SMITH, *Abstr. Papers Amer. Chem. Soc. Meeting*, 135 (1959) 3D.
50 I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohyd. Chem.*, 5 (1965) 367,
51 G. O. ASPINALL AND K. M. ROSS, *J. Chem. Soc.*, (1963) 1681.
52 H. S. ISBELL AND W. W. PIGMAN, *J. Res. Nat. Bur. Stand.*, 18 (1937) 141.
53 G. G. S. DUTTON AND A. M. UNRAU, *Can. J. Chem.*, 40 (1962) 348.
54 M. ABDEL-AKHER, J. K. HAMILTON ,AND F. SMITH, *J. Amer. Chem. Soc.*, 73 (1951) 4691.
55 J. K. N. JONES, C. B. PURVES, AND T. E. TIMELL, *Can. J. Chem.*, 39 (1961) 1059; J. K. N. JONES AND L. E. WISE, *J. Chem. Soc.*, (1952) 3389.
56 C. P. J. GLAUDEMANS AND T. E. TIMELL, *J. Amer. Chem. Soc.*, 80 (1958) 941.
57 S. KARACSONYI AND M. KUBACKOVA, *Coll. Czech. Chem. Commun.*, 34 (1969) 2002.
58 M. KUBACKOVA, S. KARACSONYI, AND J. HRIVNAK, *Coll. Czech. Chem. Commun.*, 33 (1968) 2518.
59 R. NELSON AND C. SCHUERCH, *Tappi*, 40 (1957) 419.