In a separate experiment the polysaccharide (100 mg.) was oxidized with periodate[20] for 48 hr. as before. The excess periodate was destroyed by the addition of ethylene glycol and the solution was dialyzed against tap water for 72 hr. The nondialyzable material was recovered by concentration and hydrolyzed with N sulfuric acid. The reaction mixture was neutralized with barium carbonate, filtered, and concentrated. On chromatographic examination neither glucose nor arabinose was detected.

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An Investigation of the Hydrolysis of a Reduced 4-*O*-Methylglucuronoxylan[1]

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Uronic acid groups in an elm 4-*O*-methylglucuronoxylan were reduced by diborane without a decrease in degree of polymerization of the polymer. Partial hydrolysis of the reduced polymer gave a neutral hetero-trisaccharide fraction from which a new trisaccharide, *O*-α-4-*O*-methyl-D-glucopyranosyl(1 → 2)-*O*-β-D-xylopyranosyl(1 → 4)-D-xylopryanose, was isolated, and its crystalline phenylosazone was prepared and characterized. An authentic specimen of the new sugar was prepared from the ubiquitous aldotriouronic acid by an adaptation of the diborane procedure. An hypothesis based on conformational resistance was presented to account for the formation of the new trisaccharide and the aldotriouronic acid during partial hydrolysis of the reduced and unreduced 4-*O*-methylglucuronoxylan, respectively.

The 4-*O*-methylglucuronoxylans obtainable from most hardwoods consist of chains of 1 → 4 linked β-D-xylopyranose units with single 4-*O*-methyl-D-glucopyranosiduronic acid units attached as side chains on C-2 of the xylose units[4] as shown in Fig. 1.

Partial hydrolysis of such hemicelluloses in aqueous acid results in the formation of a polymer-homologous series of β-1–4 xylodextrins[5] and a closely related acidic series (Fig. 2). The linkage (α-1–2) between E and B, Fig. 1, is especially resistant, and the amorphous aldobiouronic acid, therefore, is the chief acidic product of the acid hydrolysis of these polymers. The crystalline aldotriouronic acid (EBC)[6,7] is the second most abundant product in the acidic series, but all efforts to find the isomeric acid (ABE) have failed. To account for these facts Hamilton and Thompson[6] suggested that the uronic acid carboxyl "stabilized" the linkages B–E and B–C through an inductive effect. Marchessault and Rånby[8] supported the stabilization hypothesis,[9,10] and further suggested that, simultaneously, the linkage A–B was "activated."

In order to test these hypotheses, the carboxyl groups in a 4-*O*-methylglucuronoxylan, isolated from American elm sapwood (*Ulmus americana*), were reduced to primary hydroxyl groups.[11] Then upon partial hydrolysis of the 4-*O*-methylglucoxylan in dilute aqueous acid, the products of the reduced and the unreduced polymers were compared. As expected, the 4-*O*-methylglucoxylan afforded two series of neutral, reducing oligosaccharides as shown in Fig. 2.

The hetero-trisaccharide component of the hydrolyzate was isolated by a gradient elution technique on a carbon–Celite column[12] followed by preparative paper chromatography. Although the trisaccharide

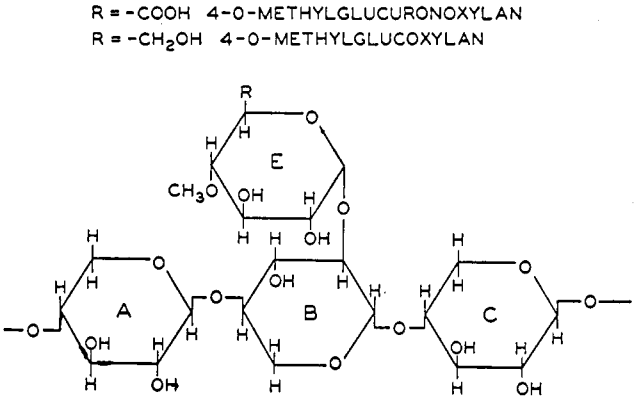

Fig. 1.—Principal linkages in 4-*O*-methylglucuronoxylans.

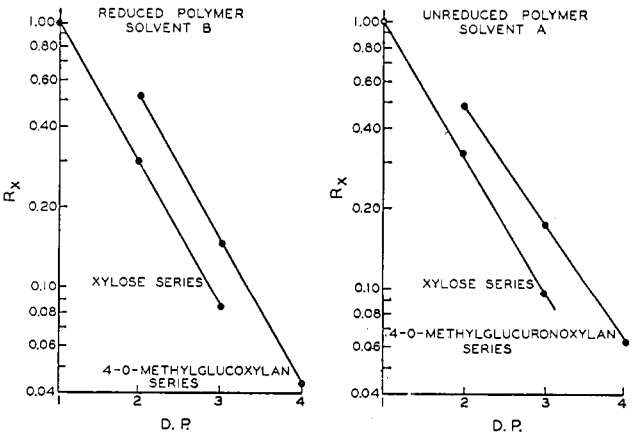

Fig. 2.—log R_x *vs.* D.P. for saccharides from the partial acid hydrolysis of reduced and unreduced 4-*O*-methylglucuronoxylan.

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component was chromatographically uniform, electrophoresis on paper in a sodium borate buffer revealed two distinct components. The major component (about 95% by visual inspection) had the greater mobility which corresponded closely with the mobility of the authentic hetero-trisaccharide (EBC); the minor component was presumed to be the isomer (ABE).[13] Proximate analyses by paper chromatography of partial hydrolyzates of the reduced and unreduced polymers indicated that the yield of the hetero-trisaccharide was 4% and the aldotriouronic acid, 7%. Furthermore, about 20% of the 4-*O*-methylglucose units were recovered as the monosaccharide from the reduced polymer, but no 4-*O*-methylglucuronic acid was detected for the unreduced sample.

These observations illustrate the well known stability toward acid hydrolysis of glycuronides, in contrast to most glycosides, and are compatible with the hypothetical inductive effect of the uronic acid carboxyl. However, the fact that the new trisaccharide predominated about 20:1 over the substance believed to be its isomer suggests that the inductive effect of the carboxyl probably does not extend to the glycosidic linkages of the xylan chain, and that the hypotheses of Hamilton and Thompson[6] and Marchessault and Rånby[8] may require modification. Thus, an alternate hypothesis, based on conformational resistance, suggested itself.

Assuming that glycosidic hydrolysis proceeds *via* the cyclic mechanism[9,10] and that the formation of a carbonium ion necessitates a transformation from the puckered chair form to a planar half-chair form, as suggested by Edward,[14] the large bulky substituent on C-2 of the xylose moiety will diminish its tendency toward the half-chair conformation. This resistance could be sufficient to cause a lower rate of hydrolysis for glycosidic bond B–C relative to A–B in the reduced and unreduced polymers, and in turn would account for the predominance of isomer EBC in hydrolyzates of both polymers.

An authentic specimen of the new trisaccharide, *O*-α-4-*O*-methyl-D-glucopyranosyl-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylopyranose, was prepared in a metastable crystalline form from the aldotriouronic acid (EBC), and was identical with the main hetero-trisaccharide obtained from the 4-*O*-methylglucoxylan; the phenylosazone was prepared and characterized.

Experimental

Paper Chromatography.—The following solvents were used in paper chromatography: (A) for uronic acids, ethyl acetate–acetic acid–water (9:2:2); (B) for neutral sugars and oligosaccharides, ethyl acetate–pyridine–water (8:2:1); (C) for galacturonic and glucuronic acids, ethyl acetate–pyridine–water–acetic acid (5:5:3:1); (D) for the separation of xylose and xylitol, butanol–pyridine–water (10:3:3); (E) for quantitative analysis, ethyl acetate–pyridine–water (8:2:1) and 0.15 *N* in silver nitrate.

Sugars were detected in qualitative chromatography with *p*-anisidine hydrochloride[15] for reducing sugars (for electrophoretograms monochloroacetic acid was added), and silver nitrate for nonreducing substances.[16] Aniline–monochloroacetic acid in ether was used for quantitative chromatography.[17]

Isolation of 4-*O*-Methylglucuronoxylan.—Five American elm (*Ulmus americana*) trees, averaging 3.5-in. d.b.h. (diameter at breast height: 4.5 ft.) were cut, peeled, and chipped, and the sapwood portion ground in a no. 1 Wiley mill. The isolation of 4-*O*-methylglucuronoxylan was accomplished in an inverted 5-gal. polyethylene bottle with the bottom removed. About 1 kg. of the undried wood meal (54% oven dry) was extracted twice with 5.5 l. of 70% ethanol for 12 hr. at room temperature, washed with deionized water, and then leached with 8 l. of 0.1 *N* sodium hydroxide for 4 hr. at room temperature; the extract was discarded. The wood meal was washed thoroughly with deionized water, pressed to 25% moisture (oven-dry basis), extracted with 5.5 l. of 10% potassium hydroxide at room temperature for 2 hr., and the extract was poured into two volumes of cold 95% ethanol containing sufficient acetic acid to bring the pH to about 6. The precipitated hemicellulose was washed and solvent-exchanged with 80% ethanol, 95% ethanol, absolute ethanol, and petroleum ether (b.p. 30–60°), and dried *in vacuo* over calcium chloride; yield, 98 g. Analytical data for the large scale preparation and for three small scale experiments using different concentrations of potassium hydroxide are summarized in Table I.

Table I

Effect of Potassium Hydroxide Concentration on Hemicellulose

Sample	1	2	3	4 (Large scale)
70% ethanol extracts, % oven-dry wood		1.5		
0.1 *N* NaOH extract (pptd. by EtOH), oven-dry wood			0.2	
Concentration of KOH soln., % by wt.	5	10	24	10
Yield of hemicellulose, % oven-dry wood	7.4	13.0	13.8	~10
Analysis of hemicellulose				
Moisture, %	8.45	7.94	7.56	9.22
Sulfated ash as K, %	4.17	6.95	7.77	2.85
CO_2,[a] %	3.16	2.77	2.69	3.07
$D.P._n$				162
Yield, of hemicellulose (ash- and moisture-free), % oven-dry wood	6.32	10.1	10.5	~9
CO_2,[a] % on ash- and moisture-free hemicellulose	3.70	3.54	3.51	3.51
Xylose/4-*O*-methylglucuronic acid	~7.5	~8	~8	~8

[a] See ref. 18.

Reduction of 4-*O*-Methylglucuronoxylan.[19]—The 4-*O*-methylglucuronoxylan, 36.0 g., was acetylated according to the method of Carson and Maclay[20]; yield, 90.3%.

Anal. of the acetylated hemicellulose: sulfated ash, 0.03; moisture, 2.38; acetyl,[21] 37.2. The theoretical acetyl content was 37.4% for a polymer of $D.P._n$ = 162 and a xylose/uronic acid ratio of 8.

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The acetylated hemicellulose, was reduced with diborane (13.3 moles/mole of carboxyl) according to the general procedure of Smith and Stephen.[11] The diborane was generated *in situ* over a period of 4 hr. under mechanical stirring by the dropwise addition of 75.3 ml. of boron trifluoride etherate, diluted with 50 ml. of purified diglyme [bis(2-methoxyethyl) ether],[22] to a solution of 17.0 g. of sodium borohydride dissolved in 440 ml. of diglyme in which 45.5 g. of the hemicellulose acetate was suspended. Stirring was continued for an additional 3 hr., the reaction mixture was allowed to stand overnight, and was decomposed by addition of ice–water. When the evolution of hydrogen ceased, the mixture was neutralized with 0.5 *N* sodium hydroxide to pH 7 and poured into two volumes of 95% ethanol. The precipitate was dissolved in 5% potassium hydroxide, heated for 45 min. at 55°, and the 4-*O*-methylglucoxylan recovered in the usual way (see above isolation procedure); yield, 81.4% of the original hemicellulose, corrected for ash and moisture. Chromatographic examination (in solvents A and B) of the total hydrolyzate from the 4-*O*-methylglucoxylan showed that xylose and 4-*O*-methylglucose were the predominant constituents but that a trace of galactose remained.

Anal. moisture, 2.4; sulfated ash calcd. as potassium acetate, 1.3; uronic acid CO_2,[18] 0.84; D.P.$_n$, 171.

Partial Hydrolysis of 4-*O*-Methylglucoxylan.—4-*O*-Methylglucoxylan, 20.5 g., was dissolved in 420 ml. of 1.0 *N* sulfuric acid, and the solution was heated at 70° (bath temperature) for 8 hr. After cooling and centrifuging, the supernatant hydrolyzate was neutralized with barium hydroxide to pH 5.5, and the barium sulfate was removed by filtration. The hydrolyzate was sorbed on a charcoal–Celite column (5 cm. in diameter by 90 cm. long) which was packed with a mixture of 400 g. of Darco G-60[23] and 400 g. of Celite[24] and treated with stearic acid.[12] The column was washed with 2 l. of distilled water and with aqueous ethanol by the gradient elution technique as described by Alm and coworkers.[12] Fractions were collected automatically and were monitored by paper chromatography. The hetero-oligosaccharides were recovered and further purified on Whatman 3MM paper in solvent B. Upon complete hydrolysis and chromatographic analysis each hetero-oligosaccharide yielded xylose and 4-*O*-methylglucose.

Preparation of *O*-α-4-*O*-Methyl-D-glucopyranosyl-(1→2)-*O*-β-D-xylopyranosyl-(1 → 4)-D-xylopyranose.—Aldotriouronic acid, 0.51 g., was refluxed with 30 ml. of acetic anhydride and 0.35 g. of anhydrous sodium acetate. The reaction mixture was poured into ice–water, extracted with chloroform, and the solvent was evaporated *in vacuo;* yield of aldotriouronic acid acetate, 0.85 g. The acetate was dissolved in purified tetrahydrofuran[25] in a gas washing bottle supplied with a sintered glass gas disperser and diborane (15 moles/mole of carboxyl) was swept from the generator into the solution by a stream of dry nitrogen over a 45-min. period. After a total reaction time of 3.8 hr., the reaction was terminated by the addition of methanol and allowed to stand overnight. The solvents were removed by evaporation *in vacuo* at 35° followed by three successive 30-ml. portions of methanol to remove boric acid as methyl borate; yield, 0.66 g. Deacetylation with barium methylate,[26] followed by neutralization with sulfuric acid and removal of barium sulfate, and concentration *in vacuo* gave a sirup; yield, 0.51 g.

Resolution of the crude sirup on Whatman 3MM paper in solvent B gave a sirup, 0.30 g., which became a slush of hygroscopic, metastable crystals of a new trisaccharide which was assumed to be *O*-α-4-*O*-methyl-D-glucopyranosyl (1 → 2)-*O*-β-D-xylopyranosyl(1 → 4)-D-xylopyranose based on the previously determined structure of the starting material.[6,7] The chromatogram indicated that unreduced acid, xylobiose, xylose, and 4-*O*-methylglucose were also present in the product.

Comparison of the Trisaccharides.—The hetero-trisaccharide ("unknown") from the hydrolysis of reduced 4-*O*-methylglucuronoxylan and the new trisaccharide ("known") prepared from the aldotriouronic acid were indistinguishable by paper chromatography. Paper electrophoresis in 0.1 *M* borate gave the following results: xylotriose (M_G = 0.145); "unknown" mixed trisaccharide, major component (M_G = 0.147), minor component (M_G = 0.058); "known" trisaccharide (M_G = 0.145). Optical rotations of the "known" and "unknown" trisaccharides were measured after first drying the sirups *in vacuo* over calcium chloride; "known" trisaccharide, $[\alpha]^{25}D$ +45.7 (*c* 6.7, water); "unknown" trisaccharide, $[\alpha]^{25}D$ +34.0 (*c* 7.2, water).

Phenylosazones were prepared as follows: a mixture of 0.16 g. of the "known" trisaccharide, 0.45 g. of sodium acetate trihydrate, 0.30 g. of phenylhydrazine, and 3.5 ml. of water were heated in a boiling water bath for 30 min.; osazone formation began at about 14 min.; yield, 0.057 g., after recrystallization from 60% aqueous ethanol, 0.039 g., m.p. 240–241°. The phenylosazone of the "unknown" trisaccharide was prepared in a similar manner from 0.22 g. of the sugar and corresponding quantities of reagents; yield, 0.052 g., after recrystallization, 0.047 g., m.p. 240–241°. The low yields of osazones were due primarily to large mechanical losses. The osazones were dried *in vacuo* over phosphorus pentoxide at 56° for 1.5 hr. prior to analysis.[27]

Anal. Calcd. for $C_{29}H_{40}O_{12}N_4$: C, 54.71; H, 6.33; N, 8.80; OCH_3, 4.88. Found: "known," C, 54.9; H, 6.2; N, 8.9; OCH_3, 4.0. "Unknown," C, 53.9; H, 6.2; N, 8.8; OCH_3, 3.9.

Infrared absorption spectra and X-ray diffraction patterns were identical.

Methyl-4-*O*-methyl-α-D-glucopyranoside, xylose, and the "known" and "unknown" trisaccharides were dissolved separately in 0.5 *N* hydrochloric acid, heated on a boiling water bath for 3 hr., neutralized with silver carbonate, and spotted for quantitative chromatography[28] in solvent E. The xylose/4-*O*-methylglucose ratios were as follows: "known," 1.45; and "unknown," 1.67; theoretical ratio, 2.0.

Comparison of the Partial Hydrolyzates of Reduced and Unreduced 4-*O*-Methylglucuronoxylan.—Approximately 0.1-g. samples of reduced and unreduced 4-*O*-methylglucuronoxylan were hydrolyzed with 2.0 ml. of *N* sulfuric acid at 70° for 8 hr. After neutralization with barium acetate, the hydrolyzates were analyzed by quantitative paper chromatography in solvent A for the unreduced sample and solvent B for the reduced sample. Known quantities of aldotriouronic acid (for unreduced sample) and "known" trisaccharide (for reduced sample) were also spotted and served as controls. An aniline–monochloroacetic acid dip was used for spot development, and the quantity of trisaccharide present was estimated by visual comparison with standards using transmitted light; yield of aldotriouronic acid, approximately 7% and of neutral hetero-trisaccharide, about 4%. Considerable 4-*O*-methylglucose was observed in the 4-*O*-methylglucoxylan hydrolyzate but no 4-*O*-methylglucuronic acid was detected in the unreduced polymer hydrolyzate. Movement on paper chromatograms of oligosaccharides from the hydrolyzates relative to xylose are shown in Fig. 2.

Viscosity Measurements.—Viscosity of the polymers was measured in molar cupriethylenediamine utilizing an Ubbelohde-type viscometer manufactured by the Cannon Instrument Co. The degree of polymerization (D.P.$_n$) was calculated from the relationship D.P.$_n$ = $K[\eta]$, where K = 166 (calculated from the data of Gillham and Timell[29]) and $[\eta]$ = intrinsic viscosity. The results are listed in Table I.

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