small amount of the diolefin.[31] The oil was indistinguishable from a synthetic sample of the monoolefinic component[11] by spectral and refractive index data. Catalytic reduction yielded pure hydrourushiol.

3-Pentadecatrienyl-8′,11′,14′-catechol.—A sample of the dibenzyl ether of the triolefinic component (n^{25}D 1.5557, d.b.v. 2.5)[32] was debenzylated and molecularly distilled in 75% yield. The product (mainly 3-pentadecatrienyl-8′,11′,14′-catechol) was a nearly colorless oil (n^{25}D 1.5250)[31] that showed no evidence of conjugation or transisomers on ultraviolet and infrared analysis. Catalytic hydrogenation revealed a d.b.v. of 2.65 and resulted in pure hydrourushiol.

Cleavage of Benzylhydrocardanol.—In order to examine all of the products of the reductive cleavage reaction, a sample of benzylhydrocardanol (the benzyl ether of 3-pentadecylphenol) was debenzylated. The reaction mixture was put immediately onto a column of grade I alumina and washed with pentane. The washings, upon spectrophotometric assay, were found to contain only toluene in an amount corresponding to a quantitative cleavage of the benzyl ether.

The column was thereupon eluted with 95% ethanol and the eluate was assayed in the same manner. It contained only the theoretical amount of 3-pentadecylphenol.

(31) The unsuspected presence of the saturated component resulted in the taking of too large a chromatographic cut for the debenzylation of the "monoolefinic" component. As a consequence this cut contained a small amount of diolefinic material. The refractive index–double bond relationship is linear.[15] Consequently, on the basis of pure hydrourushiol (n^{25}D 1.4990) and the pure diolefin (n^{25}D 1.5178) the pure monoolefin and the triolefin have, respectively, the following n^{25}D values: 1.5084 and 1.5272.

(32) The amount of chromatographically pure triolefin (dibenzyl ether) available was so limited that it was necessary to include some fractions contaminated with diolefinic material for the cleavage experiment.

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An Aldotetraouronic Acid from the Hydrolysis of a Paper Birch 4-*O*-Methylglucuronoxylan[1]

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Partial hydrolysis of a paper birch 4-*O*-methylglucuronoxylan afforded a mixture of oligosaccharides and oligouronides. An aldotetraouronic acid fraction was isolated by preparative paper chromatography and was separated into three components by paper electrophoresis. The structure of the principal component (I) appeared to be *O*-β-D-xylopyranosyl-(1→4)-*O*-[4-*O*-methyl-α-D-glucuronopyranosyl-(1→2)]-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose, based on the isolation of 3-*O*-methyl-D-xylose, 2,3,4-tri-*O*-methyl-D-xylose, 1,2,3,5-tetra-*O*-methylxylitol, and 2,3,4-tri-*O*-methyl-D-glucose in approximately equal molar amounts. This was accomplished through methylation, reduction, and methanolysis of the parent material followed by gas chromatographic analysis. Hypotheses of conformational resistance and steric interference with protonation were used to explain the occurrence of component I in the hydrolysate.

The 4-*O*-methylglucuronoxylans have been isolated from several hardwoods and all examples of this hemicellulose have essentially the same structure.[2] As illustrated in Figure 1, this polysaccharide consists of a β-1,4-linked xylan chain, substituted randomly at C-2 of the xylose units with 4-*O*-methyl-α-D-glucuronic acid.

Hydrolysis of this hemicellulose in dilute mineral acid yields two series of oligosaccharides: one of β-1,4-linked xylodextrins and a second closely related acidic series. One of the principal acidic oligosaccharides is the crystalline aldotriouronic acid [C(F)D]. All efforts to isolate the isomeric aldotriouronic acid [BC(F)] have failed.[3,4] Two hypotheses have been advanced to account for these observations.

Marchessault and Rånby[5] have suggested that linkage C–D, in Figure 1, has been stabilized through the inductive influence of the uronic acid carboxyl groups. These workers have further suggested simultaneous activation of linkage B–C through the same mechanism. McKee[6] has shown, through study of a 4-*O*-methylglucoxylan, that stabilization of linkage C–D is not dependent on the carboxyl group of unit F. McKee has attributed the resistance to hydrolysis of C–D to the conformational stability of unit C. This stability, induced by the size of the acidic substituent at C-2 of this xylose unit, hinders adoption of a necessary hydrolysis intermediate and in turn stabilizes C–D. McKee's work did not indicate whether or not linkage B–C had been activated, as suggested by Marchessault and Rånby.

In order to test these hypotheses, a 4-*O*-methylglucuronoxylan, isolated from paper birch, was partially hydrolyzed in dilute sulfuric acid. Paper chromatography indicated the usual spectrum of oligosaccharides. The aldotetraouronic acid was isolated by preparative paper chromatography and electrophoresis and was characterized by $[\alpha]^{25}$D 24°, equiv. wt. 604, R_x 0.14 (solvent A), and M_g (mobility) 0.56 (0.1 *M* sodium borate). These data are in general agreement with those of other workers.[3,7]

Three possible tetrasaccharide acid isomers may be expected to occur in the hydrolysate of a 4-*O*-methylglucuronoxylan, on the basis of the supposed structure of this hemicellulose: the linear isomer I [C(F)DE] previously isolated by Timell,[8] the branched isomer II [BC(F)D], and the linear isomer III [ABC(F)]. A reaction sequence involving sodium borohydride reduction, methylation, lithium aluminum hydride reduction, and cleavage of the glycosidic linkages would produce the sugars and sugar alcohols shown in Table I. Anal-

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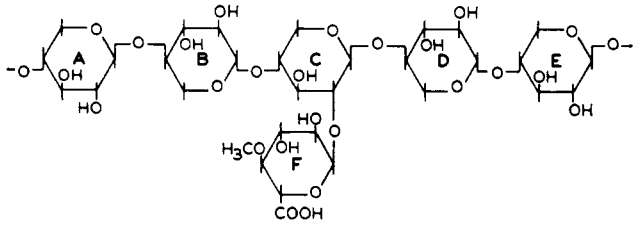

Figure 1.—Structure of 4-*O*-methylglucuronoxylan.

TABLE I

METHYLATED COMPONENTS FROM A MIXTURE OF ALDOTETRAOURONIC ACIDS

Methylated components	Isomers I	II	III	Mole ratio (exptl. found)
2,3-Di-*O*-methyl-D-xylose	X		X	0.06
3,4-Di-*O*-methyl-D-xylose	X			0.06
3-*O*-Methyl-D-xylose		X		0.88
2,3,4-Tri-*O*-methyl-D-xylose		X	X	0.93
1,3,5-Tri-*O*-methylxylitol			X	0.00
1,2,3,5-Tetra-*O*-methylxylitol	X	X		0.97
2,3,4-Tri-*O*-methyl-D-glucose	X	X	X	1.00

ysis of these methylated components would identify each of the three isomers, even in an isomeric mixture.

The purified aldotetraouronic acid fraction was studied by this reaction sequence. Cleavage of the glycosidic linkages was accomplished by methanolysis, converting the sugars to the analogous methyl glycosides. The methanolysis mixture was fractionated by gas chromatography, and the methylated components, in the molar ratios shown in Table I, were isolated. These data support isomer II as the principal component (over 90 mole %) and possible isomer I (6 mole %) as a minor component of the tetrasaccharide acid. These proportions of the isomers may be incorrect, however, since only 54% of the starting material was recovered in the methylated components. All product losses were assumed to be nonselective, however, and probably occurred during lithium aluminum hydride reduction. An attempted quantitative analysis of the methanolysis mixture by gas chromatography, utilizing peaks areas, was inconclusive.

The identification of isomer II as the principal tetrasaccharide acid supports the structure of the 4-*O*-methylglucuronoxylan, by confirming the presence of linkage B–C. The isolation of isomer II is not, however, compatible with the induction stabilization theory of Marchessault and Rånby.[5] From the predominance of isomer II, it appears likely that linkage B–C has been stabilized by the acidic substituent at position 2 of unit C, rather than activated as predicted by the induction theory. The small quantity of isomer I suggests that B–C is more stable than D–E, since these two linkages are in direct competition in the production of these two isomers.

Mild acid hydrolysis of the aldotetraouronic acid fraction yielded a 1:1 ratio of xylose and an aldotriouronic acid [presumably C(F)D]. Similar treatment of the nonreducing analog of the aldotetraouronic acid yielded xylose and the nonreducing analog of the aldotriouronic acid. These observations indicated that glycosidic linkage C–D was more stable than B–C. This difference in stability would account for the structure [C(F)D] of the aldotriouronic acids isolated.

The conformation resistance hypothesis of McKee[6] appears to explain the stability of linkage C–D, but it does not account for the stabilization of B–C. It is suggested, therefore, that the acidic substituent F interferes with protonation of the glycosidic oxygen in B–C and thus increases the stability of this linkage. Thus, both B–C and C–D have been stabilized by the acidic side chain.

Experimental

Paper Chromatography.—Sugars were separated by descending paper chromatography on Whatman No. 1 paper or for preparative purposes on Whatman No. 17 paper. Solvents employed in the separation of uronic acids, sugars, and oligosaccharides were (A) ethyl acetate–acetic acid–formic acid–water (18:3:1:4) and (B) ethyl acetate–acetic acid–water (6:3:2). The solvent for separation of neutral sugars, sugar alcohols, and oligosaccharides was (C) ethyl acetate–pyridine–water (8:2:1). The solvent for separation of methylated sugars was (D) 2-butanone–water azeotrope. Qualitative paper electrophoresis was carried out in 0.1 *M* sodium borate solution at 500 v. for 3 hr. on Whatman No. 1 paper. Preparative paper electrophoresis was carried out in 0.1 *M* sodium borate solution at 360 v. for 5 hr. on Whatman No. 17 paper. Reducing sugars were detected with *p*-anisidine hydrochloride.[9] Trichloroacetic acid was added for electrophoretograms. Nonreducing substances were detected by alkaline silver nitrate.[10]

Isolation of 4-*O*-Methylglucuronoxylan.—An air-dried sample of paper birchwood meal, 2657 g. (oven dried), was extracted continuously, on a table-top Büchner funnel, with 25 l. of 0.1 *N* sodium hydroxide for 4 hr. The residual solution was expressed, under vacuum, with a rubber dam. The birch meal residue was then extracted for 2 hr. with 25 l. of 10% potassium hydroxide. The solution was neutralized with acetic acid and poured into 10 gallons of 95% ethanol. The precipitated hemicellulose was washed with absolute ethanol and isolated by filtration. The sample was retained as the moist filter cake; yield 345 g. (o.d.), corrected for 12.2% ash.

A sample of the 4-*O*-methylglucuronoxylan, 13.2 g. (o.d.), was dialyzed against deionized and then distilled water. The product was concentrated and freeze dried; yield 12.0 g. Analysis revealed 3.97% moisture, 1.55% ash as K, 10.40% uronic anhydride, 1.78% methoxyl, intrinsic viscosity $[\eta]$ = 0.50 in dimethyl sulfoxide.

Partial Hydrolysis of 4-*O*-Methylglucuronoxylan.—A sample of the crude hemicellulose, 100 g. (o.d.), was stirred overnight at room temperature in 1 l. of distilled water. The suspension was then heated to 70° and 1 l. of 2 *N* sulfuric acid, at 70°, was added. The reaction was maintained at 70 ± 1°, with stirring, for 14 hr. After cooling in a cold-water bath, the hydrolysate was neutralized to pH 6 with barium hydroxide solution. The reaction mixture was then centrifuged and the clear supernatant was concentrated to a heavy sirup; approximate yield 40 g.

Isolation of Aldotetraouronic Acid.—The hydrolysate was deionized with Amberlite IR-120(H^+), and the crude aldotetraouronic acid fraction was isolated by preparative paper chromatography on twenty Whatman No. 17 sheets[11] in solvent B. The crude acid fraction was purified further on water-washed Whatman No. 17 sheets, eluted successively with solvents B and C, 3 days with each; yield 1.01 g. of a dried sirup; specific rotation $[\alpha]^{25}D$ 23° (*c* 0.667, water); 4.8% methoxyl; R_x 0.14 in solvent A.

A sample of the crude acid, 460 mg., was purified by preparative paper electrophoresis on Whatman No. 17 paper in 0.1 *M* sodium borate. Three fractions, as shown in Table II, were located and isolated from the electrophoretogram. The equivalent weight values, also shown in Table II, were determined by potentiometric titration of each sample with 0.0962 *N* sodium hydroxide. The theoretical equivalent weight for an aldotetraouronic acid is 604.5 atomic weight units. Fraction B was, therefore, assumed to be the purified aldotetraouronic acid; $[\alpha]^{25}D$ 24° (*c* 0.800, water).

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TABLE II

PREPARATIVE ELECTROPHORESIS FRACTIONS

Fraction	M_g[a]	Yield, mg.	Equiv. wt., atomic wt. units
A	0.67	49	560
B	0.56	386	604
C	0.13	13	1370

[a] M_g = electrophoretic migration with respect to glucose.

Sodium Borohydride Reduction.—A sample of fraction B, 150 mg., was converted to the nonreducing tetrasaccharide acid by reduction in 25 ml. of 4% sodium borohydride–0.1 *N* sodium hydroxide solution. After 2 hr. the reaction mixture was neutralized with dilute acetic acid, deionized on IR-120(H^+), and concentrated under reduced pressure to dryness several times with methanol (yield not determined).

Methylation.[6]—The sirupy, nonreducing tetrasaccharide acid was dissolved in 6.25 ml. of distilled water containing 300 mg. of sodium bicarbonate. After flushing the reaction vessel thoroughly with nitrogen, 3.25 ml. of dimethyl sulfate and 3.25 ml. of 40% sodium hydroxide solution were added, dropwise and alternately, over a 4-hr. period. The reaction vessel was maintained under a slight positive nitrogen pressure. After stirring for 1 hr., 1.8 g. of solid sodium hydroxide was added and 3.25 ml. of dimethyl sulfate was added dropwise over 18 hr. A second addition of solid sodium hydroxide and dimethyl sulfate was completed in the same manner. The reaction mixture was stirred overnight, then neutralized to pH 5 with 1 *N* sulfuric acid, and extracted with chloroform. The chloroform extract was dried over anhydrous sodium sulfate and evaporated to a heavy sirup. The entire sample was dissolved in 5 ml. of dry dimethylformamide. Methyl iodide (2 ml.) and 250 mg. of anhydrous calcium sulfate were added. After stirring for 4 hr., 2 g. of silver oxide was added in small portions over a 3-hr. period. A second addition of methyl iodide and silver oxide was made in the same manner, and the reaction mixture was allowed to stir in the dark for 3 days. The solids were removed by filtration on a Celite pad and the residue was washed extensively with chloroform. The combined filtrates were evaporated to 5 ml. and added to 50 ml. of chloroform. The chloroform solution was then extracted with 25 ml. of 10% potassium cyanide solution, followed successively by two water washings. The chloroform solution was dried over anhydrous calcium sulfate and concentrated to a dried sirup; yield 176 mg. Infrared spectra revealed no peak in the hydroxyl stretching region at 2.85 μ.

Anal. Calcd.: methoxyl, 49.7. Found: methoxyl, 46.

Reduction.[6]—A sample of the methylated, nonreducing methyl ester, 160 mg., was dissolved in 9.5 ml. of dry tetrahydrofuran. Lithium aluminum hydride, 160 mg., was added slowly. After stirring for 2 hr., the excess lithium aluminum hydride was destroyed by slow addition of ethyl acetate to the cold reaction mixture, followed by distilled water. The reduced product was filtered, deionized, and evaporated to dryness under reduced pressure (yield not determined).

Characterization.—The methylated, nonreducing, neutral tetrasaccharide was dissolved in 25 ml. of 0.5 *N* methanolic hydrogen chloride. The solution was refluxed for 8 hr., neutralized with silver carbonate, deionized, and filtered. The filtrate was concentrated to dryness under reduced pressure (yield not determined) and dissolved in 1 ml. of ethanol. A 10-μl. sample of this solution was analyzed by gas chromatography, on the Aerograph Hi Fi Model A 600-B, using a 4-ft. column packed with butane-1,4-diol succinate polyester at 160°. Approximately equimolar quantities of 2,3,4-tri-*O*-methylxylose, 3-*O*-methylxylose, 2,3,4-tri-*O*-methylglucose (all as the respective methyl glycosides), and 1,2,3,5-tetra-*O*-methylxylitol were noted. Materials having similar retention times as 2,3- and 3,4-di-*O*-methylxylose (also as the respective methyl glycosides) were also present. Neither sugar could be isolated from these peaks, however. These peaks may have been caused by some thermally degradable material which was destroyed on the column. Since these data were apparently not internally consistent, no conclusive interpretation could be made.

The methanolysis mixture was separated into five fractions by preparative gas chromatography, using a 4-ft. column packed with butanediol succinate polyester. The Aerograph Model A 90-S gas chromatograph, operating at 190°, was utilized for these isolations. All five fractions were dissolved in ethyl ether, filtered, dried, and weighed. Each fraction was then dissolved in 2 ml. of 1 *N* sulfuric acid and sealed in a 4-ml. vial. Following hydrolysis for 1.5 hr. at 105°, the solutions were neutralized with barium carbonate and centrifuged. The clear supernatant was extracted three times with chloroform and these latter solutions were evaporated to dryness.

Identifications.—All melting points were corrected; the D designations of the sugars were assumed on the basis of previous work on this paper birch hemicellulose.[7]

2,3,4-Tri-*O*-methyl-D-xylose.—The clear sirup crystallized on standing and was recrystallized from ethyl ether, m.p. 89–90° (lit.[12] m.p. 91–92°). The infrared spectrum, gas chromatographic mobility (as the methyl glycoside), and paper chromatographic mobility (in solvent D) were identical with authentic samples; yield 16.5 mg. (as the methyl glycosides).

1,2,3,5-Tetra-*O*-methylxylitol.—The mobile, nonreducing sirup was converted to the *p*-nitrobenzoate,[6] m.p. 185–186° (lit.[4] m.p. 187–189°). The gas chromatographic mobility of this sugar alcohol was identical with that of a sample synthesized from xylobiose[4]; yield 17.5 mg.

3-*O*-Methyl-D-xylose.—The clear sirup crystallized on standing and was recrystallized from ethyl acetate, m.p. 101–102° (lit.[12] m.p. 102–103°). The infrared spectrum, gas chromatographic mobility (as the methyl glycoside), and paper chromatographic mobility (in solvent D) were identical with authentic samples; yield 13.5 mg. (as the methyl glycosides).

2,3,4-Tri-*O*-methyl-D-glucose.—The mobile reducing sirup could not be induced to crystallize. The infrared spectrum, gas chromatographic mobility (as the methyl glycoside), and paper chromatographic mobility (in solvent D) were identical with authentic samples; yield 20.5 mg. (as the methyl glycosides).

2,3- and 3,4-Di-*O*-methyl-D-xylose.—Gas chromatographic mobilities (as the methyl glycosides) and paper chromatographic mobilities (in solvent D) suggested that these two sugars were present, presumably in equimolar amounts. Insufficient quantities were obtained to permit more positive identification; combined yield 2.0 mg. (as the methyl glycosides).

Mild Acid Hydrolysis of Fraction B.—A 50-mg. sample of the purified aldotetraouronic acid, fraction B, 150 mg., was hydrolyzed for 1 hr. at 70° in 25 ml. of 0.5 *N* sulfuric acid. The reaction mixture was then neutralized with barium carbonate and centrifuged; the clear supernatant was concentrated to a thin sirup. Quantitative paper chromatography[13] (in solvent A) revealed a 1:1 ratio of xylose and the aldotriouronic acid, in addition to residual aldotetraouronic acid. A 50-mg. sample of the purified aldotetraouronic acid, fraction B, was converted to the nonreducing analog, as previously described, with sodium borohydride. The nonreducing acid was hydrolyzed as above, and the hydrolysate was examined by paper chromatography. No evidence of xylitol was found, although xylose, the nonreducing analog of the aldotriouronic acid, and unchanged starting material were present.

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