Anal. Calcd. for $C_{10}H_7BrF_3NO_4$ (342.09): C, 35.11; H, 2.06; Br, 23.37; N, 4.10. Found: C, 35.21; H, 2.20; Br, 23.23; N, 4.40.

1-Carbobenzyloxyamino-3-methoxy-4,5-methylenedioxybenzene (XLV).—Carbobenzyloxy chloride (3.2 g.) was added to a cooled solution of 510 mg. (3.1 mmoles) of 3-methoxy-4,5-methylenedioxyaniline (V) in 8 ml. of pyridine. The reaction mixture, after being kept overnight at room temperature, was poured onto ice, and the product was filtered. After one recrystallization of the product from ethanol, 1-carbobenzyloxyamino-3-methoxy-4,5-methylenedioxybenzene (820 mg.), m.p. 90–93°, was obtained.

Anal. Calcd. for $C_{16}H_{15}NO_5$ (301.29): C, 63.78; H, 5.02; N, 4.65. Found: C, 64.24; H, 5.12; N, 4.63.

2-Bromo-1-carbobenzyloxyamino-3-methoxy-4,5-methylenedioxybenzene (XLVI).—A solution of 304 mg. of bromine in 2 ml. of acetic acid was added slowly to a solution of 580 mg. (1.9 mmoles) of 1-carbobenzyloxyamino-3-methoxy-4,5-methylenedioxybenzene (XLV) in 4 ml. of acetic acid. The reaction mixture was diluted with water, and the product was filtered. Two recrystallizations from ethanol gave 300 mg. of 2-bromo-1-carbobenzyloxyamino-3-methoxy-4,5-methylenedioxybenzene, m.p. 93–97°.

Anal. Calcd. for $C_{16}H_{14}BrNO_5$ (380.20): C, 50.55; H, 3.71; Br, 21.03; N, 3.68. Found: C, 50.98; H, 3.37; Br, 20.01; N, 3.95.

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[CONTRIBUTION FROM THE DIVISION OF INDUSTRIAL AND CELLULOSE CHEMISTRY, MCGILL UNIVERSITY, AND THE WOOD CHEMISTRY DIVISION, PULP AND PAPER RESEARCH INSTITUTE OF CANADA]

The Polysaccharides of Yellow Birch (*Betula lutea*). II. The Constitution of the Main Hemicellulose[1]

BY T. E. TIMELL

RECEIVED FEBRUARY 25, 1959

The general chemical composition of the wood of yellow birch (*Betula lutea*) has been determined. Alkaline extraction of the wood yielded a hemicellulose, which on partial hydrolysis gave D-galacturonic acid, glucuronic acid, 4-*O*-methyl-D-glucuronic acid, 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose and an aldotriouronic acid. A crystalline tetraacetate of the methyl ester methyl glycoside of the aldobiouronic acid was prepared and fully characterized. Hydrolysis of two fully methylated hemicellulose samples yielded a mixture of 2-*O*- and 3-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2,3,4-tri-*O*-methyl-D-xylose and 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyluronic acid)-3-*O*-methyl-D-xylopyranose in molar ratios of 3:109:1:11 and 3:89:1:9. The number-average degrees of polymerization of the methylated hemicelluloses were 102 and 81 and that of the original polysaccharide 192. On the basis of this and other evidence it is suggested that this 4-*O*-methylglucuronoxylan consists of a linear framework of approximately 190 1,4-linked β-D-xylopyranose residues, with, on the average, every tenth residue carrying a single, terminal side chain of 4-*O*-methyl-α-D-glucuronic acid attached glycosidically to the 2-position of the xylose.

Yellow birch is the most important hardwood species in the northeastern part of this continent, although it has lately suffered severely from attack of birch dieback.[2] In a previous investigation[3] the molecular properties of its cellulose component were dealt with. This paper is concerned with an identification of the uronic acids obtained on partial hydrolysis of the main hemicellulose constituent and with its constitution and molecular weight.

Alkaline extraction of the wood gave a hemicellulose in a yield of 17.5%, somewhat lower than the xylan content of the wood, 20.1%. The sugar mixture obtained on hydrolysis of the hemicellulose was resolved on a column of anion exchange resin[4] to yield D-xylose, which crystallized, two monouronic acids, a mono-*O*-methyl uronic acid, an aldobiouronic acid and an aldotriouronic acid.

One of the monouronic acids corresponded on the paper chromatogram to glucuronic acid-glucuronolactone. The other acid was converted to D-galactose, identified through its crystalline 1-methyl-1-phenylhydrazone derivative, and was therefore D-galacturonic acid. The third monouronic acid was identified by reduction if its ester glycoside with lithium aluminum hydride[5,6] to 4-*O*-methyl-D-glucose, characterized as its phenylosazone derivative,[7,8] and was therefore 4-*O*-methyl-D-glucuronic acid. The aldotriouronic acid was chromatographically identical to similar compounds obtained from milkweed floss[9] and white elm wood[10] and yielded the same hydrolysis products. Glucuronic acid has previously been encountered in hydrolyzates from the woods of trembling aspen[11] and black spruce.[12] Aldotriouronic acids composed of 4-*O*-methyl-D-glucuronic acid and two xylose residues have been isolated from several wood species.[10,13]

The methoxyl content and equivalent weight of the aldobiouronic acid, $[\alpha]^{20}D$ + 104°, corresponded to that of a mono-*O*-methylated aldobiouronic acid containing a pentose and a hexuronic acid residue. Its infrared spectrum was identical to that of an authentic sample of 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose.[14] Reduction of its ester glycoside and hydrolysis yielded 4-*O*-methyl-D-glucose and D-xylose. When the ester glycoside of the fully methylated aldobiouronic acid was similarly reduced and hydrolyzed, 3,4-di-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose were obtained, both of which

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were identified as their aniline derivatives.[15,16] This evidence shows that the aldobiouronic acid was 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylopyranose.[17] The same acid has previously been isolated from many natural sources, including hardwoods such as trembling aspen,[11] American beech,[18] European beech,[19] Finnish birch,[20] white birch,[14] white elm[4] and balsam poplar.[21]

With a view to simplifying the identification of this ubiquitous aldobiouronic acid, an attempt was made to convert it into a crystalline derivative. Both the tetrabenzoate and the tetra-*p*-nitrobenzoate of the derived methyl ester methyl glycoside were amorphous and could not be induced to crystallize from any solvent. A portion of the corresponding tetraacetate, however, crystallized easily from alcohol or ether. The methyl 2-*O*-[methyl (2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyl) uronate]-3,4-di-*O*-acetyl-D-xylopyranoside had m.p. 200–201°, $[\alpha]^{20}D$ + 100° in chloroform, and was further characterized through its infrared spectrum and X-ray diagram. The configuration of the xyloside moiety was unknown. The residual sirup had almost the same specific rotation as the crystalline portion and gave an infrared spectrum which was similar, albeit not identical, to that of the latter. The pentaacetate ester glycoside of a related aldobiouronic acid[22] has been successfully resolved into its two anomers. Recrystallizations effected no such resolution in the present case.

While the galacturonic acid was considered to be derived from a pectic contaminant, the presence of the other two uronic acids and the xylose suggested that the hemicellulose was a 4-*O*-methylglucuronoxylan. On this assumption, the uronic anhydride and methoxyl contents of the polysaccharide both corresponded to 10.5 anhydroxylose units per acid group, while the equivalent weight of the acidic hemicellulose indicated a value of 10.8. Oxidation with trisodium paraperiodate and with sodium metaperiodate gave values of 10.5 and 9.5, respectively.

A portion of the hemicellulose, which appeared to be electrophoretically homogeneous, was methylated eleven times with alkali and dimethyl sulfate.[23] Another part was subjected to a similar series of treatments followed by a final methylation according to Kuhn.[24] Both products (A and B) had methoxyl contents only slightly lower than those required by theory and exhibited infrared spectra indicative of the absence of hydroxyl groups. Preparation B was more depolymerized than A.

Each of the two methylated hemicelluloses were subjected to methanolysis and the glycosides formed were quantitatively separated into acidic and neutral components on an anion exchange resin. The acid fraction in both cases contained only one compound. Reduction of its ester glycoside and hydrolysis (A) gave 3-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose, both of which were identified through their crystalline aniline derivatives. In the second case (B), the reduced glycoside crystallized and was identified through its melting point, specific rotation, infrared spectrum and X-ray powder diagram as methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-3-*O*-methyl-α,β-D-xylopyranoside,[25–27] in accordance with the above evidence.

The neutral glycosides were hydrolyzed to the corresponding mixture of reducing sugars, the aqueous solution of which was fractionally extracted with chloroform. The first compound to be removed was identified as 2,3,4-tri-*O*-methyl-D-xylose through its crystalline aniline derivative. The second and main constituent, which crystallized, was similarly characterized as 2,3-di-*O*-methyl-D-xylose. The residual aqueous solution contained approximately equal quantities of 2-*O*- and 3-*O*-methyl-D-xylose, resolvable by paper ionophoresis.[28] A minor portion of the methylated xyloses was also resolved on the paper chromatogram and the amounts of sugars present were quantitatively determined.[29] The values thus obtained, in conjunction with the weight of the acidic fraction, gave the molar ratios presented in Table I.

TABLE I

MOLE RATIOS OF THE HYDROLYSIS PRODUCTS AND $\overline{P}_n$[a] OF THE METHYLATED HEMICELLULOSES

	A	B
2-*O*- and 3-*O*-methyl-D-xylose	3	3
2,3-Di-*O*-methyl-D-xylose	109	89
2,3,4-Tri-*O*-methyl-D-xylose	1	1
2-*O*-(2,3,4-Tri-*O*-methyl-α-D-glucopyranosyluronic acid)-3-*O*-methyl-D-xylopyranose	11	9
$\overline{P}_n$	124	102

[a] Number-average degree of polymerization based on number of non-reducing end groups.

The large quantity of 2,3-di-*O*-methyl-D-xylose obtained, together with the high negative rotation of the polysaccharide (−81°), showed that the xylose residues forming the backbone of the macromolecule were linked together by 1,4-β-glycosidic bonds. The relative amounts of partly methylated aldobiouronic acid obtained on hydrolysis in both cases corresponded to 10 xylose residues per acid group in accordance with previous results, thus corroborating the assumption[30] that the methanolysis had produced no monouronic acids. The partly methylated aldobiouronic acid isolated from the methylated hemicellulose contained no substituent at C_3 in the xylose moiety, whereas methylation of the free aldobiouronic acid itself produced a methyl group in this position. This evidence, together with the absence of any 3,4-di-*O*-methyl-D-xylose in the hydrolyzate from the methylated product, indi-

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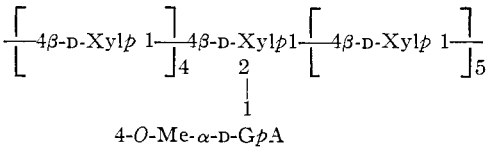
cated that the acid groups were attached *directly* to the xylan framework. The constitution of the aldobiouronic acid showed that these side chains consisted of 1,2-linked 4-*O*-methyl-α-D-glucuronic acid residues.

The 2,3,4-tri-*O*-methyl-D-xylose was obviously derived from the non-reducing end groups of the hemicellulose. Since the mono-*O*-methyl xyloses could not have been formed by a removal of acid side groups, their presence could be accounted for only as a result of incomplete methylation, demethylation or branching. The fact, however, that both the 2-*O*- and the 3-*O*-methyl derivatives. were obtained and that their amount exceeded that of the tri-*O*-methyl xylose strongly suggested that branching could not be the only factor involved. When the number-average molecular weights of the two methylated hemicelluloses were determined by osmometry,[31] values of 18,500 (A) and 14,800 (B) were obtained, corresponding to degrees of polymerization ($\bar{P}_n$) of 102 and 81, respectively. These values were somewhat lower than those indicated by the number of non-reducing end groups, 124 and 102, respectively, and definitely precluded the possibility of any branching of the xylan backbone.[14] The mono-*O*-methylxyloses were thus artifacts of no structural significance, most probably caused by random demethylation during the degradation of the methylated polysaccharide. Such a demethylation has been observed on numerous occasions with similar materials[9,10,14,18,19,32] and tends to render the classical methylation technique of limited usefulness of this particular point.

A portion of the hemicellulose was converted to the fully substituted acetate derivative.[31,33] Its number-average molecular weight, as determined by osmometry, was 45,800, corresponding to a $\bar{P}_n$ (number of xylan residues in the xylan framework) of 192. The intrinsic viscosities of the unsubstituted hemicellulose in cupriethylenediamine and in 10% potassium hydroxide were 0.93 and 0.67, respectively, corresponding to $\bar{P}_n$ values of 197 and 216, in reasonable accordance with the above results.

From the above evidence it is now possible to suggest a tentative structure for the 4-*O*-methylglucuronoxylan present in yellow birch wood. This polysaccharide contains an average number of approximately 190 β-D-xylopyranose residues linked together by 1,4-glycosidic bonds to a linear framework, where, on the average, every tenth anhydroxylose unit carries a single, terminal side chain of 4-*O*-methyl-α-D-glucuronic acid, attached glycosidically through C_2 of the xylose, these side groups probably being distributed at random along the macromolecule. In analogy with the similar hemicellulose present in white birch wood,[31] the present polysaccharide is undoubtedly polymolecular.



4-*O*-Me-α-D-G*p*A

In the native polymer, as it occurs in the wood, a large portion of the hydroxyl groups are evidently esterified with acetic acid[34,35] and the carboxyl groups might take part in the formation of ester linkages,[36] connecting the polysaccharide to other wood constituents, such as lignin.[37] From results obtained with a white birch hemicellulose,[38] it appears probable that the portion of the present polysaccharide which could be extracted from the wood with alkali was identical to the remainder, the latter probably being located near the lumen[39,40] and thus less accessible. Very little is known at present concerning the exact location of the hemicelluloses in the cell wall of wood, except that the concentration is higher in the outer than in the inner sections of the secondary wall.[39] Indirect evidence recently obtained with white birch wood[41] indicates that little, if any, of the methyl glucuronoxylan in this species is extracellular.

The constitution of the yellow birch xylan is apparently almost exactly the same as that established for the main hemicellulose component of European beech,[19] white birch[14,31] and sugar maple.[42] Actually, all hardwood xylans so far studied appear to be of this type[43] although variations frequently occur, such as the considerably higher number of acid side groups typical of the xylan present in white elm wood,[10] the arabofuranose residues found in a xylan isolated from trembling aspen[11] and the branched framework reported for a beech xylan.[18]

The molecular weight of the present hemicellulose is of the same order of magnitude as that found earlier for other native 4-*O*-methyl glucuronoxylans, such as those present in white birch,[31] white elm,[10] sugar maple,[42] milkweed floss[9] and kapok.[27] These polysaccharides were all isolated by a direct alkaline extraction of the natural products. This treatment, albeit mild,[44,45] might conceivably entail some depolymerization of the original hemicellulose. Substitution of dimethyl sulfoxide for the alkali could be expected to eliminate this effect.

Experimental

All specific rotations are equilibrium values and melting points are corrected. Evaporations were carried out *in vacuo* at 40–50°.

Paper Chromatography.—Solvents (v./v.) used for separating the sugars were (A) ethyl acetate–acetic acid–water (9:2:2), (B) butan-1-ol-pyridine–water (10:3:3), (C) butan-

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1-ol–ethanol–water (40:11:19) and (D) butan-2-one–water (89:11). Separations were carried out by the descending technique on Whatman No. 1 and (for preparative purposes) on No. 3MM filter paper. *o*-Aminobiphenyl was the preferred spray reagent.[29]

Composition of the Wood.—Analyses according to standard methods[9,14] gave the following composition of the wood: α-cellulose (40.6), pentosan (23.0), lignin (21.5), acetyl (3.3), ash (0.3), uronic anhydride (4.5), galactan (0.9), glucan (46.7), mannan (3.6), araban (0.6) and xylan (20.1%). The xylan content was lower than that of *Betula papyrifera*[14] and *Betula verrucosa*.[46]

Isolation of the Hemicellulose.—Extractive-free yellow birch wood meal from a sound, 100 years old specimen[47] (1.5 kg., 40–60 mesh) was extracted directly with 24% (w./w.) potassium hydroxide (5 l.) in an atmosphere of nitrogen at room temperature for 2 hr. After filtration through sintered glass, the filtrate and washings were poured with stirring into a cooled (−16°) mixture of ethanol (25 l.) and acetic acid (3 l.). The precipitate was washed on the centrifuge with 80% ethanol, anhydrous methanol and petroleum ether (b.p. 30–60°), and finally dried *in vacuo* to yield 262 g. (17.5%). Paper chromatography (solvents A and B) indicated the presence of xylose and uronic acids only.

Anal. (acid form): Uronic anhydride, 11.24; OMe, 1.97; equiv. wt., 1610; $[\alpha]^{23}D$ −81° (*c* 1.1 in 2.5% sodium hydroxide).

Hydrolysis of the Hemicellulose and Preliminary Resolution of the Hydrolyzate.—Hemicellulose (113 g.) was dissolved in 72% sulfuric acid (100 ml.) and, after dilution to 2 l., boiled under reflux for 6 hr. After neutralization with barium hydroxide to *p*H 5 and treatment with Amberlite IR-120 cation exchange resin,[48] evaporation yielded a pale yellow sirup. An aqueous solution of this sirup (2 l.) was added to the top of a column (6.5 × 160 cm.) containing Dowex 1-X4 anion exchange resin[49] (acetate form). Washing with water (30 l.) was carried out until no more sugar was removed (negative Molisch test). Evaporation of a portion of the eluate gave crystalline D-xylose, m.p. and mixed m.p. +144.5° after recrystallization from absolute ethanol; $[\alpha]^{20}D$ +18° (*c* 1.0 in water). The dibenzylidene dimethyl acetal derivative[50] had m.p. and mixed m.p. 210° after recrystallization from chloroform–ligroin.

Separation of Sugar Acids.—The exchange resin column was eluted with 2 *N* acetic acid.[4] Fractions (25 ml. each) were collected mechanically, every 15th of which was examined by paper chromatography (solvent A). The first 200 fractions contained a mixture of uronic acids. Resolution on sheets of filter paper (solvent A) yielded a pure hexuronic acid from a portion of this mixture, chromatographically identical to a galacturonic acid (300 mg.).

Fractions 201–265 contained approximately equal amounts of an aldotrio- and an aldobiouronic acid (4.0 g.). Resolution of this mixture on a coconut charcoal[51] column by gradient elution with aqueous ethanol[52,53] gave a pure triouronic acid (0.5 g., 4–6% alcohol), a mixture of both acids (1.5 g., 10–20%) and a pure aldobiouronic acid (1.5 g., 10–20%). Fractions 266–325 gave a white, amorphous powder of chromatographically pure aldobiouronic acid (3.4 g.). The subsequent 350 fractions contained mixtures of uronic acids. When a portion of this material was resolved on strips of filter paper (solvent A), a compound (50 mg.) was obtained which gave only two spots on the paper chromatogram, corresponding to glucuronic acid and glucuronolactone. Elution with 3 *N* acetic acid, finally, gave fractions 680–780, containing a chromatographically pure mono-*O*-methylhexuronic acid (1.6 g.).

Anal. Calcd. for $C_{12}H_{20}O_{11}$: OMe, 9.1; equiv. wt., 340. Found: OMe, 9.2; equiv. wt., 348; $[\alpha]^{21}D$ +104° (*c* 2.0 in water). Calcd. for $C_7H_{12}O_7$: OMe, 14.9; equiv. wt., 208. Found: OMe, 14.8; equiv. wt., 216; $[\alpha]^{21}D$ +41° (*c* 1.1 in water).[12]

Identification of D-Galacturonic Acid.—A portion of the hexuronic acid first eluted from the exchange resin column (100 mg.) was boiled under reflux with 2.5% methanolic hydrogen chloride (75 ml.) for 8 hr. After neutralization with silver carbonate, filtration through Celite[54] and evaporation, a sirup was obtained which was dissolved in dry tetrahydrofuran (50 ml.) and reduced with lithium aluminum hydride.[5,6] The reduced glycoside was hydrolyzed with *N* sulfuric acid to give a sirup (70 mg.), chromatographic examination of which (solvent B) indicated the presence of galactose only, $[\alpha]^{21}D$ +75° (*c* 1.0 in water). The 1-methyl-1-phenylhydrazine derivative had m.p. and mixed m.p. 182–183°.

Identification of 4-*O*-Methyl-D-glucuronic Acid.—A portion of the mono-*O*-methylhexuronic acid (300 mg.) was converted to the ester glycoside, reduced with lithium aluminum hydride and hydrolyzed. The sirupy sugar obtained (150 mg.) was chromatographically indistinguishable from 4-*O*-methyl-D-glucose (solvents A and B). The crude osazone derivative,[6,8] when lixiviated with benzene, yielded pure 4-*O*-methyl-D-glucosazone, m.p. and mixed m.p. 159°. Its infrared spectrum was identical to that of an authentic, synthetic specimen.

Reduction and Hydrolysis of the Aldobiouronic Acid and Identification of Hydrolysis Products.—The aldobiouronic acid (1.00 g.) was converted to its ester glycoside (1.05 g.), dissolved in dry tetrahydrofuran (100 ml.) and reduced with pulverized lithium aluminum hydride (2 g.). The reduced material was hydrolyzed by refluxing for 8 hr. with *N* sulfuric acid to give a sirup (1.00 g.), giving two spots of equal intensity on the paper chromatogram (solvent B), corresponding in rate of movement and color to xylose and 4-*O*-methylglucose. The sugar mixture was dissolved in water and added to the top of a coconut charcoal column (4.5 × 18 cm.). Elution with 2 and 10% aqueous ethanol (1 l. each) yielded chromatographically pure D-xylose, $[\alpha]^{20}D$ +18° (*c* 2.0 in water), and 4-*O*-methyl-D-glucose, $[\alpha]^{20}D$ +57° (*c* 2.0 in water).

Anal. Calcd. for $C_7H_{14}O_6$: OMe, 16.0. Found: OMe, 16.0.

Recrystallization from benzene[55] of the 4-*O*-methyl-D-glucosazone gave yellow crystals, m.p. and mixed m.p. 159°. The infrared spectrum was identical to that of an authentic sample.

Methylation, Reduction and Hydrolysis of the Aldobiouronic Acid.—Aldobiouronic acid (1.00 g.) was methylated cautiously in water solution (20 ml.) with dimethyl sulfate (20 ml.) and 10% sodium hydroxide (20 ml.). After two more methylations with dimethyl sulfate and 40% alkali, the recovered product was dissolved in dry dimethylformamide (25 ml.) and shaken with methyl iodide (5 ml.) and silver oxide (5 mg.) for 48 hr. The infrared diagram of the recovered[24] product indicated the absence of any hydroxyl groups and was identical to that of an authentic sample of methyl 2-*O*-[methyl (2,3,4-tri-*O*-methyl-α-D-glucopyranosyl) uronate]-3,4-di-*O*-methyl-D-xylopyranoside.[14] Reduction with lithium aluminum hydride (2 g.) in dry ethyl ether (50 ml.) yielded a clear sirup (500 mg.) of a sugar mixture, a portion of which was resolved by paper chromatography (solvent C) into two components, chromatographically identical to a di-*O*-methylxylose and a tri-*O*-methylglucose.

Identification of 3,4-Di-*O*-methyl-D-xylose and 2,3,4-Tri-*O*-methyl-D-glucose.—Extraction of the appropriate paper strips with aqueous ethanol (50%), concentration of the eluate, treatment with Darco G-60 charcoal[56] and Amberlite IR-120 exchange resin and then filtration through Celite and evaporation to dryness gave a chromatographically pure 3,4-di-*O*-methyl-D-xylose (80 mg.), $[\alpha]^{20}D$ +20° (*c* 0.5 in water). The compound had the same ionophoretic mobility and infrared spectrum as an authentic specimen. The 3,4-di-*O*-methyl-*N*-phenyl-D-xylosylamine had m.p. 116° and an infrared spectrum indistinguishable from that of an authentic sample.[14]

Anal. Calcd. for $C_9H_{18}O_6$: OMe, 41.9. Found: OMe, 41.6; $[\alpha]^{20}D$ +76° (*c* 1.0 in water).

The tri-*O*-methylglucose (120 mg.) was converted to 2,3,4-tri-*O*-methyl-*N*-phenyl-D-glucosylamine, m.p. 146–147°. The infrared spectra of the 2,3,4-tri-*O*-methyl-D-glucose

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and its aniline derivative were both indistinguishable from those of authentic specimens.

Preparation and Characterization of Methyl 2-*O*-[Methyl (2,3-di-*O*-acetyl-4-*O*-methyl-α-D-glucopyranosyl) uronate]-3,4-di-*O*-acetyl-D-xylopyranoside.—A portion of the aldobiouronic acid (1.00 g.) was converted to its methyl ester methyl glycoside (1.10 g.), which was dissolved in pyridine (75 ml.), freshly distilled over barium oxide, after which redistilled acetic anhydride (25 ml.) was added. The solution was kept at 25° for 20 hr. and then poured into a mixture of ice and water (600 ml.). The aqueous solution was extracted three times with chloroform. The extract was washed thrice with 10% ice-cold hydrochloric acid, followed by three extractions with saturated aqueous sodium bicarbonate. The chloroform solution was dried over anhydrous sodium sulfate and evaporated to yield a yellow sirup (1.50 g., 95%) which was dissolved in boiling ethyl ether (25 ml.). On cooling, crystals immediately formed. Crystallization was allowed to proceed at −4° for 24 hr. after which the crystals were collected on sintered glass, washed with cold ethyl ether and dried (280 mg., 17.8%), m.p. 193–197°, $[\alpha]^{20}D$ +100° (*c* 2.6 in chloroform). Recrystallization from ethanol–ether (1:1 v./v.) yielded crystals (160 mg.), m.p. 200–201.5°, $[\alpha]^{20}D$ +100° (*c* 1.2 in chloroform).

Anal. Calcd. for $C_{22}H_{32}O_{15}$: C, 49.2; H, 6.02; OMe, 17.3; ester (as acetyl), 40.6. Found: C, 49.5; H, 5.91; OMe, 16.7; ester, 40.1.

The remaining sirup had $[\alpha]^{20}D$ +95° (*c* 1.7 in chloroform).

The infrared spectrum of the compound exhibited absorption maxima at: 2940, 1745, 1440, 1370, 1225, 1055, 965 925, 885, 790, 745, 655 cm.$^{-1}$. The X-ray powder diagram had these interplanar lattice spacings in Å.: 8.92(vs), 6.69(s), 6.02(s), 5.29(s), 4.69(vs). 4.07(vs), 3.36(s), 3.01(s), 2.62(m), 2.34(m), 2.23(m), 2.10(m), 1.98(m), 1.85(m), 1.75(w).

Methylation of the Hemicellulose.—The hemicellulose (20 g.) was dissolved in 40% (w./w.) aqueous sodium hydroxide (300 ml.) in an atmosphere of nitrogen, and dimethyl sulfate (300 ml.) was added dropwise at 0° over a period of 10 hr. This treatment was repeated five times at room temperature. The recovered product was suspended in redistilled tetrahydrofuran (2 l.) together with powdered sodium hydroxide (200 g.), and dimethyl sulfate (300 ml.) was added dropwise as before. After four similar treatments, the solvent was removed by distillation and the residue was extracted with chloroform, which was filtered, dried and poured into petroleum ether to yield a white, fluffy precipitate (13 g., A).

Anal. Calcd. for the sodium salt of the completely methylated glucuronoxylan: OMe, 37.3. Found: OMe, 36.8.

Another portion of the hemicellulose (30 g.) was methylated in the same way to yield a product (22 g.), OMe, 34.1%. It was dissolved in dry dimethylformamide (400 ml.) and shaken at room temperature with methyl iodide (20 ml.) and silver oxide (20 g.) for 48 hr.[24] The recovered product was precipitated from its chloroform solution into petroleum ether to give a white powder (18 g., B).

Anal. Calcd. for the methyl ester of the fully methylated glucuronoxylan: OMe, 39.2. Found: OMe, 38.6.

The infrared spectra of both preparations indicated the absence of any hydroxyl groups.

Methanolysis of the Methylated Hemicelluloses and Separation of the Acidic Component.—Methylated hemicellulose (B, 10.0 g.) was boiled under reflux with 2.5% methanolic hydrogen chloride in the presence of Drierite for 12 hr. After neutralization (silver carbonate) and purification (hydrogen sulfide), the sirup obtained (11.1 g.) was heated at 60° in aqueous barium hydroxide for 2 hr.[25] After removal of barium hydroxide with carbon dioxide and filtration through Celite, the solution was treated with Amberlite IR-120 exchange resin and evaporated to dryness to give a clear sirup (11.0 g.). The acidic glycoside mixture was added to the top of a column (8 × 20 cm.) of Dowex 1-X4 anion exchange resin (acetate form), which was washed with water (6 l.) for removal of the neutral glycosides (8.20 g.). Elution with 30% (v./v.) aqueous acetic acid yielded an acidic sirup (1.80 g.). Preparation A (5.08 g.) similarly gave a mixture of neutral glycosides (3.70 g.) and an acidic fraction (0.81 g.).

Preparation and Reduction of the Ester Glycoside of the Partially Methylated Aldobiouronic Acid.—The acidic glycoside fraction (B, 1.80 g.) was treated with 2.5% methanolic hydrogen chloride and the ester formed (1.90 g.) was reduced with lithium aluminum hydride[5,6] (3 g.) in dry ethyl ether (100 ml.) to yield a pale yellow sirup (1.40 g.) of the reduced disaccharide. The second acid fraction (0.81 g.) gave, when treated similarly, the same disaccharide (0.62 g.).

Identification of Methyl 2-*O*-(2,3,4-Tri-*O*-methyl-α-D-glucopyranosyl)-3-*O*-methyl-α,β-D-xylopyranoside.—One of the above sirups (B) crystallized on standing. After two recrystallizations from ethyl acetate, the compound had m.p. 165–166°, undepressed on admixture with an authentic sample of methyl 2-*O*-(2,3,4-tri-*O*-methyl-α-D-glucopyranosyl)-3-*O*-methyl-β-D-xylopyranoside,[25,26] $[\alpha]^{20}D$ +91° (*c* 2.0 in water).

Anal. Calcd. for $C_{16}H_{30}O_{10}$: C, 50.3; H, 7.92; OMe, 40.6. Found: C, 50.7; H, 7.85; OMe, 40.1.

A second crop of crystals from the ethyl acetate solution had $[\alpha]^{20}D$ +98°, a third + 121° and the residual sirup + 124° (*c* 2.0 in water throughout). The β-xylopyranoside is reported to have $[\alpha]D$ +79°[25] and +85°[26] in water. The first crystals obtained therefore probably constituted a mixture of the α- and the β-anomers, albeit with the latter predominating.

The infrared spectrum, which was identical to that of an authentic specimen,[27] exhibited absorption maxima at: 3450, 2930, 1460, 1375, 1320, 1270, 1235, 1180, 1025, 1000, 980, 940, 880, 845, 765, 710, 650 cm.$^{-1}$. The X-ray powder diagram, which was also indistinguishable from that of an authentic specimen,[27] had the following interplanar distances (Å.) and relative intensities (%): 6.5–100, 4.82–62, 4.18–57, 3.77–20, 3.50–5, 3.21–47, 3.02–47, 2.84–2, 2.58–2, 2.47–7, 2.30–5, 2.17–2, 2.06–20, 1.93–7, 1.79–25, 1.68–12, 1.60–7.

Hydrolysis of the Partially Methylated Disaccharide and Identification of 3-*O*-Methyl-D-xylose and 2,3,4-Tri-*O*-methyl-D-glucose.—The partially methylated disaccharide (A, 0.62 g.) was hydrolyzed with *N* sulfuric acid. The sugar mixture obtained, when examined by paper chromatography (solvents C and D), contained equal quantities of a mono-*O*-methylxylose and a tri-*O*-methylglucose. The mixture was resolved on large sheets of filter paper (solvent D). After treatment of the eluates from the appropriate strips with Amberlite IR-120 exchange resin and with Darco G-60 charcoal, filtration and evaporation yielded clear sirups (200 mg. each).

The slower moving component was identical with 3-*O*-methyl-D-xylose on ionophoresis in a borate buffer solution.

Anal. Calcd. for $C_6H_{12}O_5$: OMe, 18.9. Found: OMe, 19.2; $[\alpha]^{20}D$ +14° (*c* 1.0 in water).

The 3-*O*-methyl-*N*-phenyl-D-xylosylamine had m.p. and mixed m.p. 134°. The infrared spectra of the methylxylose and its aniline derivative were indistinguishable from those of authentic specimens.

The 2,3,4-tri-*O*-methyl-D-glucose, $[\alpha]^{20}D$ +75° (*c* 1.0 in water), gave an aniline derivative, m.p. and mixed m.p. 143–144°. Its infrared spectrum, as well as that of the sugar itself, was identical with that of an authentic sample.

Separation of the Neutral Components of the Methylated Hemicellulose.—A portion of the mixture of neutral glycosides (B, 7.5 g.) was dissolved in water and extracted continuously with chloroform. After 24 hr. a mixture of di-*O*- and tri-*O*-methylxyloses had been removed which was resolved by paper chromatography (solvent D) to yield chromatographically pure tri-*O*-methylxylose (50 mg.). Further extraction for one month gave a chromatographically pure, colorless sirup of di-*O*-methylxylose (4.0 g.). The remaining aqueous solution, when evaporated to dryness, gave a mixture of mono-*O*-methylxyloses (150 mg.), which was further purified by paper chromatography (solvent D).

Spectrophotometric Analysis of the Neutral Glycosides.—A small portion of the mixture of neutral glycosides was resolved on paper strips (solvent C) and the concentration of the sugars in the aqueous solutions obtained was determined by the *o*-aminobiphenyl method.[14,29] The data in Table I are based on six determinations each. Use of solvent D resulted in high blanks.

Preliminary Characterization of Mono-*O*-methylxyloses.—The specific rotation of this fraction, $[\alpha]^{20}D$ +27° (*c* 1.0 in water), suggested the presence of approximately equal

quantities of 2-*O*- and 3-*O*-methyl-D-xylose. Paper chromatography (solvent D) as well as ionophoresis in a borate buffer corroborated this conclusion.

Identification of 2,3-Di-*O*-methyl-D-xylose.—The sirup of di-*O*-methylxylose crystallized rapidly and completely when seeded with an authentic crystal[10] to give large crystals with a distinct X-ray pattern but with a somewhat unsharp m.p. (77–80°), $[\alpha]^{20}D$ +22.6° (*c* 3.0 in water).

Anal. Calcd. for $C_7H_{14}O_5$: OMe, 34.8. Found: OMe, 34.9.

The dimorphic[57] 2,3-di-*O*-methyl-*N*-phenyl-D-xylosylamine had m.p. 126–127°, $[\alpha]^{20}D$ +177° (*c* 3.0 in ethyl acetate). Two similar samples, both with an original m.p. of 125°,[9,14] had m.p. 130° after one year, the second m.p. of this compound being 146°.[58] The infrared spectrum of the aniline derivative was identical to that of an authentic specimen.

Identification of 2,3,4-Tri-*O*-methyl-D-xylose.—The sirup of tri-*O*-methyl xylose, $[\alpha]^{20}D$ +20° (*c* 0.5 in water), could not be induced to crystallize. The 2,3,4-tri-*O*-methyl-*N*-phenyl-D-xylosylamine had m.p. and mixed m.p. 97°. The infrared spectra of both the sugar and its aniline derivative corresponded to those of authentic specimens.

Anal. Calcd. for $C_{14}H_{21}O_4N$: OMe, 34.9. Found: OMe, 33.6.

Periodate Oxidation of the Hemicellulose.—Portions of the hemicellulose (100 mg.) were oxidized with 0.05 *M* trisodium paraperiodate, buffered to *p*H 4.1 with acetic acid, or with 0.05 *M* sodium metaperiodate. The reaction was allowed to proceed in the dark for 7 days, the consumption of periodate being determined every day by the arsenite method. Extrapolation to zero time gave the amount of periodate consumed, 10.5 and 9.5 moles per repeating unit, respectively.

Paper Electrophoresis of the Hemicellulose.—This was carried out in a borate buffer solution as described elsewhere.[14] One single band or spot only was observed on spraying with a modified *o*-aminobiphenyl spray reagent.[14]

Determination of the Molecular Weight of the Methylated Hemicellulose.—The five osmometers used were of the Zimm–Myerson[59] type, later improved by Stabin and Immergut.[60] Gel cellophane membranes, the static method of measuring the osmotic height and a temperature of 30° were used.[14] The solvent was a mixture of chloroform and ethanol (9:1 v./v.). The osmotic pressure was determined at six different concentrations for both methylated hemicelluloses (Table II).

Preparation of Acetylated Hemicellulose.—Dry hemicellulose (2.0 g.) was swollen or partly dissolved in formamide (40 ml.).[33] Pyridine (80 ml.) and redistilled acetic anhydride (60 ml.) were added. After 3 days at −4° the gel formed was poured into ice-water with vigorous stirring (3 l.) and washed on the filter, first with cold 2% hydrochloric acid and then with water until neutral. Water was replaced by ethanol and ethanol by petroleum ether. The dried product was subjected to a second, similar acetylation, although without the addition of formamide.

TABLE II

OSMOMETRY DATA FOR THE METHYLATED HEMICELLULOSES

A			B		
w[a]	h[b]	h/w	w	h	h/w
3.982	9.120	2.290	3.644	6.655	1.826
3.243	6.537	2.016	2.883	4.949	1.717
3.026	6.538	2.160	2.145	3.870	1.804
2.225	4.305	1.935	1.920	3.538	1.843
2.014	3.753	1.868	1.593	2.980	1.870
1.651	2.807	1.700	1.368	2.440	1.784
0	...	1.38	0	...	1.81

[a] Concentration in g./kg. solution. [b] Osmotic height in cm. solvent.

Anal. Calcd. for a fully acetylated hemicellulose: acetyl, 37.8. Found: acetyl, 38.3.

Determination of the Molecular Weight of the Hemicellulose Acetate.—The molecular weight of the acetylated hemicellulose was determined as described above for the methyl derivative. The results are given in Table III.

TABLE III

OSMOMETRY DATA FOR THE ACETYLATED HEMICELLULOSE

w	h	h/w
3.838	2.308	0.603
3.449	2.041	.592
2.989	1.751	.586
2.028	1.178	.581
0	...	.56

Estimation of the Intrinsic Viscosity of the Hemicellulose.—Intrinsic viscosities ($[\eta]$) were determined with a Craig–Henderson viscometer[61] in *M* cupriethylenediamine and in 10% aqueous potassium hydroxide as described elsewhere.[31] The relationship $\bar{P}_n = K[\eta]$, where K was 212 and 323, respectively, was used for estimating the degree of polymerization.

Acknowledgment.—The author is grateful to Dr. C. P. J. Glaudemans for assistance in isolating the uronic acids and to Dr. R. St. J. Manley for providing the X-ray data. He also wishes to express his gratitude to Professor Fred Smith, University of Minnesota, for the gift of several sugar samples.

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