even though both possess an asymmetric center at C_5 in addition to that at C_1.

It is also of interest to note that the aldehyde, obtained by periodate oxidation of 1,2-*O*-isopropylidene-D-glucofuranose, and its reduction product 1,2-*O*-isopropylidene-D-xylofuranose display little difference in rotation[13] since there is no chance for either compound to undergo cyclization.

The phenomenon of cyclization established herein for the dialdehyde V from methyl α-L-rhamnopyranoside (IV) and postulated previously in a number of instances[14–21] is a general one for the products of periodate oxidation of the simple and complex carbohydrates.

Experimental

Oxidation of Methyl α-L-Rhamnopyranoside with Periodic Acid.—Methyl α-L-rhamnopyranoside was oxidized with periodic acid in the usual way[5,6] to give the crystalline monohydrate of L′-methoxy-L-methyldiglycolic aldehyde, m.p. 102° and $[\alpha]^{23}\text{D}$ −144° in water (*c* 1); lit.[6] m.p. 101–102°, $[\alpha]^{20}\text{D}$ −143° in water. The product showed a strong hydroxyl absorption but no carbonyl absorption in the infrared region of the spectrum. The molecular weight determined in camphor by the Rast method using $K = 26$ was 160 (calcd. for $C_6H_{12}O_5$ 164). The *K*-value was ascertained with methyl β-L-arabinopyranoside.

Methylation of L′-Methoxy-L-methyldiglycolic Aldehyde Monohydrate (Va) with Silver Oxide and Methyl Iodide.—Silver oxide (3 g.) was added to a suspension of the crystalline dialdehyde monohydrate (0.797 g.) in methyl iodide (20 ml.). After standing at room temperature for 1 hr. when no visible reaction occurred, the reaction mixture was boiled under reflux for 24 hr. Evaporation of the solvent at room temperature gave a colorless crystalline residue in almost quantitative yield, which after two recrystallizations from light petroleum ether yielded 2-L″,3,5-trimethoxy-6-D″-methyl-1,4-dioxane (VI) as fine, flat needles (0.300 g.), m.p. 73–74.5° with sublimation and $[\alpha]^{22}\text{D}$ −142° in ethanol (*c* 1). In the infrared, the compound showed a strong ether absorption but no carbonyl absorption.

Anal. Calcd. for $C_8H_{16}O_5$: C, 50.0; H, 8.4; OCH_3, 48.5. Found: C, 50.0; H, 8.3; OCH_3, 48.7.

Treatment of 2-L″,3,5-Trimethoxy-6-D″-methyl-1,4-dioxane (VI) with Sodium Borohydride.[8]—Sodium borohydride (0.200 g.) was added to a solution of 2-L″-3,5-trimethoxy-6D″-methyl-1,4-dioxane (0.100 g.) in water (5 ml.). After 5 hr. the reaction mixture was extracted with diethyl ether (4 × 30 ml.). Concentration of the combined ethereal extracts gave the starting material, m.p. and mixed m.p. 73–74° and $[\alpha]^{22}\text{D}$ −141° in ethanol (*c* 1), after recrystallization from light petroleum ether.

Reaction of L′-Methoxy-L-methyldiglycolic Aldehyde with *p*-Nitrobenzoyl Chloride.—A solution of the dialdehyde, L′-methoxy-L-methyldiglycolic aldehyde (0.062 g.), in dry pyridine (3 ml.) was treated with *p*-nitrobenzoyl chloride (0.230 g.) at 80° for 0.5 hr. Upon pouring the cooled reaction mixture into a saturated aqueous solution of sodium bicarbonate, the product crystallized immediately. After being filtered, the product was washed with water and dried (crude yield 0.180 g.). Recrystallization from a mixture of ethanol, chloroform and light petroleum ether gave the di-*p*-nitrobenzoate of L′-methoxy-L-methyldiglycolic aldehyde, m.p. 170.5° and $[\alpha]^{22}\text{D}$ +39° in chloroform (*c* 0.8).

Anal. Calcd. for $C_{20}H_{18}O_{11}N_2$: C, 52.0; H, 3.9; N, 6.1. Found: C, 52.2; H, 3.8; N, 6.1.

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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 White Birch (Betula papyrifera). III. Determination of Composition and Identification of 2-*O*-(4-*O*-Methyl-D-glucopyranosyluronic acid)-D-xylopyranose[1]

BY C. P. J. GLAUDEMANS AND T. E. TIMELL

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Alkaline extraction of a holocellulose from white birch (*Betula papyrifera*) has yielded a hemicellulose in an amount closely corresponding to the anhydroxylose and uronic anhydride content of the wood. Hydrolysis of the hemicellulose gave xylose, galacturonic acid, 4-*O*-methyl-D-glucuronic acid and 2-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-xylopyranose, together with traces of galactose, glucose, arabinose and rhamnose.

Previous studies in this series dealt with methods for isolating an unchanged holocellulose from white birch (*Betula papyrifera*)[2] and with the molecular properties of its cellulose component.[3] This paper is concerned with the general chemical composition of the wood and with the isolation and characterization of an aldobiouronic acid obtained on partial hydrolysis of its hemicellulose portion.

Delignification by a chlorination procedure[2] of the wood followed by extraction with alkali, afforded a hemicellulose which upon acid hydrolysis yielded galacturonic acid, 4-*O*-methyl-D-glucuronic acid, 2-*O*-(-4-*O*-methyl-D-glucopyranosyluronic acid)-D-xylopyranose, and a mixture of sugars. Chromatographic analysis indicated that this mix-

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[1] Paper presented before the Division of Cellulose Chemistry at the 132nd Meeting of the American Chemical Society in New York, N. Y., September, 1957.

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ture contained xylose together with small amounts of galactose, glucose, arabinose and rhamnose. The galacturonic acid, which is believed to arise from pectin in the wood, was identified chromatographically after transforming it into galactose. The 4-*O*-methyl-D-glucuronic acid was characterized after reduction of its ester glycoside with lithium aluminum hydride[4,5] followed by hydrolysis, as 4-*O*-methyl-D-glucose phenylosazone.[5,6]

Designation of the aldobiouronic acid (I) as 2-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-xylopyranose was based on the following evidence[7]: the methoxyl content and equivalent weight corresponded to that of an aldobiouronic acid composed of a pentose and a methoxyhexuronic acid. Reduction of the ester glycoside of I with lithium aluminum hydride followed by hydrolysis[4] yielded 4-*O*-methyl-D-glucose, recognized as its osazone,[5,6] and crystalline D-xylose. Methylation of I with subsequent reduction and hydrolysis yielded 2,3,4-tri-*O*-methyl-D-glucose[8] and 3,4-di-*O*-methyl-D-xylose,[9,10] both of which were characterized as the corresponding aniline derivatives. This evidence proved that the biose link in I engaged C_2 of the D-xylose residue.

Confirmation of the structure of I was obtained by demonstrating that lithium aluminum hydride reduction of the ester glycoside of I, followed by methylation, gave methyl 2-*O*-(2,3,4,6-tetra-*O*-methyl-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside. Hydrolysis of the latter gave 2,3,4,6-tetra-*O*-methyl-D-glucose[11] and 3,4-di-*O*-methyl-D-xylose.[9,10] Support for the structure assigned to I also was obtained from lead tetraacetate oxidation which showed that compound I yielded 0.8 mole of formaldehyde, a result to be expected[12] from a 1,2-linked disaccharide.

Further proof of the structure of I, which is believed[13] to possess an α-biose linkage, is being sought by synthesis.[14]

The aldobiouronic acid isolated here is identical to that obtained by partial hydrolysis of a large number of wood hemicelluloses, including those from trembling aspen,[15–17] American beech,[18] European beech,[19] western hemlock,[7] loblolly pine,[20,21] Monterey pine,[22] Scots pine,[23] black spruce[23] and Norway spruce.[24,25] It also has been tentatively identified among the hydrolysis products of hemicelluloses from Finnish birch,[26] *Eucalyptus regnans*[27,28] and maritime pine[29] as well as from yellow birch[30] and white elm.[30] The same aldobiouronic acid has been obtained also from a few non-woody plants such as corncob,[31] flax straw,[32] oat hulls,[33] kapok[30] and milkweed floss.[30]

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 neutral and acidic sugars were (A) ethyl acetate–acetic acid–water (9:2:2), (B) 1-butanol–pyridine–water (10:3:3) and (C) 1-butanol–ethanol–water (40:11:19). Separations were carried out on Whatman No. 1 filter papers by the descending technique. *o*-Aminodiphenyl was used as a spray reagent.[34] R_G values are mobilities relative to 2,3,4,6-tetra-*O*-methyl-D-glucose.

Composition of the Wood.—Analysis showed that the wood contained α-cellulose[35] (41.0), pentosan[36] (25.5), lignin[36] (18.9), acetyl[37] (4.4), ash[36] (0.2), uronic anhydride[38] (4.6), galactan (0.6), glucan (44.7), mannan (1.5), araban (0.5) and xylan[34] (24.6%).

Isolation of the Hemicellulose.—Extractive-free birch wood meal (1.2 kg., 40–60 mesh) was delignified by three chlorinations[2] to give the holocellulose (985 g.) which contained lignin, 0.41% (Klason), and pentosan, 30.2%. The holocellulose (400 g.) was extracted (nitrogen atmosphere) at room temperature with 24% potassium hydroxide (4 l.). The filtrate (sintered glass) was poured into a cooled (−16°) mixture of acetic acid (1.5 l.) and ethanol (12 l.). The hemicellulose was centrifuged at −20°, washed successively with 80% aqueous ethanol, ethanol, benzene, and dried *in vacuo*; yield 132 g. (33% of the holocellulose or 27.1% of the original wood).

Examination by paper chromatography of the sugars formed on hydrolysis of the hemicellulose revealed the presence of xylose and various uronic acids together with traces of galactose, glucose, arabinose and rhamnose.

Anal. Uronic anhydride (ash-free material), 10.88; OMe, 2.00; $[\alpha]^{20}_{D}$ −83° (*c* 1.0 in 5% sodium hydroxide), −60° (*c* 1.0 in anhydrous dimethyl sulfoxide).

Separation of Sugar Acids.—Hemicellulose (60 g.) was partially hydrolyzed with sulfuric acid.[39] After neutralization (barium hydroxide) and filtration through Celite[40] chromatographic examination using solvents A and B revealed the presence of xylose and three acids which corresponded to D-galacturonic acid, 4-*O*-methyl-D-glucuronic acid and 2-*O*-(4-*O*-methyl-D-glucopyranosyluronic acid)-D-xylose. The filtrate was treated with Amberlite IR 120 exchange resin[41] and then passed through a column of Am-

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berlite IR 45 resin (acetate form). The column was washed with water until the washings gave a negative Molisch test (20 l.). The acid fraction was eluted with *N* sulfuric acid (2 l.) and the column was washed with water (3 l.). The eluate was neutralized (barium hydroxide) and the solution was evaporated to yield a pale yellow sirup (4.20 g.). A part of the material (500 mg.) was applied to the top of a cellulose column (90 × 5 cm.) and resolved with solvent A. The remainder of the sugar acids was separated on large sheets of filter paper (solvent A). Four fractions were obtained corresponding to xylose, a monouronic acid (50 mg.), a methoxymonouronic acid (200 mg.) and an aldobiouronic acid (2.80 g.). The xylose crystallized, m.p. and mixed m.p. 144°, $[\alpha]^{20}_{D}$ +18° (*c* 1.0 in water).

Anal. Calcd. for $C_{12}H_{20}O_{11}$: OMe, 9.1; equiv. wt., 340. Found: OMe, 8.6; equiv. wt., 350. Calcd. for $C_7H_{12}O_7$: equiv. wt., 208. Found: equiv. wt., 220.

Chromatographic Identification of Galacturonic Acid.—A portion of the monouronic acid (40 mg.) was dissolved in anhydrous methanol containing 2% hydrogen chloride (15 ml.), and was refluxed for 6 hr. The solution was cooled and the acid was neutralized with silver carbonate. Purification and concentration yielded a brown sirup which was reduced with lithium aluminum hydride.[4,5] Hydrolysis (sulfuric acid) and neutralization (barium hydroxide), and then concentration of the filtrate, yielded a sirup. Examination by paper chromatography (solvent B) indicated the presence of galactose only.

Identification of 4-*O*-Methyl-D-glucuronic Acid.—The methylmonouronic acid (190 mg.) was converted to the ester glycoside, reduced with lithium aluminum hydride and hydrolyzed. Evaporation yielded a clear sirup (80 mg.) which was chromatographically identical with 4-*O*-methyl-D-glucose (solvent B).

Anal. Calcd. for $C_7H_{12}O_6$: OMe, 16.0. Found: OMe, 15.1; $[\alpha]^{20}_{D}$ +52° (*c* 1.6 in water).

Recrystallization of the osazone[5,6] from ethanol gave needles, m.p. and mixed m.p. 157°. An X-ray diffraction pattern was identical with that of an authentic specimen.

Reduction and Hydrolysis of the Aldobiouronic Acid.—The aldobiouronic acid (620 mg.) was converted to its methyl ester methyl glycoside and reduced with lithium aluminum hydride[4,5] after which it was hydrolyzed with *N* sulfuric acid. Concentration yielded a sirup which showed two spots of equal intensity on the paper chromatogram with solvent B, corresponding to xylose and 4-*O*-methyl-D-glucose.

Identification of Hydrolysis Products.—The above sugar mixture was resolved on sheets of filter paper (solvent B) to yield xylose (40 mg.) and 4-*O*-methyl-D-glucose (55 mg.). The xylose crystallized, m.p. and mixed m.p. 144°, $[\alpha]^{20}_{D}$ +18.1° (*c* 1.0 in water). The 4-*O*-methyl-D-glucose, $[\alpha]^{22}_{D}$ +52° (*c* 1.2 in water), was converted to the osazone, m.p. and mixed m.p. 157°. It was distinguished from 4-*O*-methyl-D-mannose by treatment with hydrogen bromide,[42] glucose being the only sugar detectable on the paper chromatogram.

Direct Methylation of the Aldobiouronic Acid.—The aldobiouronic acid (500 mg.) was methylated cautiously in water solution (10 ml.) with dimethyl sulfate (10 ml.) and 10% sodium hydroxide (10 ml.). Further methylations were carried out with powdered sodium hydroxide (45 g.) and dimethyl sulfate (20 ml.), after which the material was recovered and methylated three times with methyl iodide (7 ml.) and silver oxide (5 g.). The product finally was methylated once with methyl iodide (7 ml.), silver oxide (7 g.) and dimethylformamide (25 ml.).[43] The colorless sirup obtained[43] (597 mg.) had an infrared spectrum which indicated the absence of any hydroxyl groups.

Reduction and Hydrolysis of the Methylated Aldobiouronic Acid.—The sirup was dissolved in anhydrous ethyl ether and reduced with lithium aluminum hydride in the same solvent. The chloroform extract was dried and concentrated to give methyl 2-*O*-(2,3,4-tri-*O*-methyl-D-glucopyranosyl)-3,4-di-*O*-methyl-D-xylopyranoside. The reduced material was subjected to methanolysis and then hydrolyzed and the sirup obtained was decolorized with charcoal. Examination by paper chromatography (solvent C) indicated the presence of 2,3,4-tri-*O*-methyl-D-glucose (R_G 0.89) and 3,4-di-*O*-methyl-D-xylose (R_G 0.78).

Identification of 2,3,4-Tri-*O*-methyl-D-glucose and 3,4-Di-*O*-methyl-D-xylose.—The sugar mixture was resolved on large sheets of filter paper (solvent C) and the appropriate sugars were located, eluted, and purified to yield clear sirups.

Anal. Calcd. for $C_9H_{18}O_6$: OMe, 41.9. Found: OMe, 40.8.

The trimethylglucose was converted to 2,3,4-tri-*O*-methyl-N-phenyl-D-glucosylamine, m.p. and mixed m.p. 144°, $[\alpha]^{20}_{D}$ −21.6° (*c* 1.2 in ethyl acetate).

Anal. Calcd. for $C_7H_{14}O_5$: OMe, 34.8. Found: OMe, 33.0; $[\alpha]^{20}_{D}$ +17° (*c* 1.0 in water).

The 3,4-di-*O*-methyl-N-phenyl-D-xylosylamine had m.p. 119°.

Methylation of the Reduced Aldobiouronic Acid.—The acid (650 mg.) was converted to the ester glycoside, reduced with lithium aluminum hydride, and then methylated twice with 40% sodium hydroxide (40 ml.) and dimethyl sulfate (30 ml.). The sirup obtained was dissolved in anhydrous tetrahydrofuran,[33] and powdered sodium hydroxide (10 g.) was added followed by dimethyl sulfate (8 ml.). This procedure was repeated once more after which the chloroform extract yielded a clear sirup (415 mg.).

Anal. Calcd. for $C_{18}H_{34}O_{10}$: OMe, 52.9. Found: OMe, 51.5.

Hydrolysis of the Disaccharide and Identification of 2,3,4,6-Tetra-*O*-methyl-D-glucose and 3,4-Di-*O*-methyl-D-xylose.—The methylated disaccharide (400 mg.) was hydrolyzed and the sirupy product was resolved by sheet-paper chromatography in the usual way. The 2,3,4,6-tetra-*O*-methyl-D-glucose had m.p. 89–92°, $[\alpha]^{22}_{D}$ +83° (*c* 0.4 in water). Its infrared spectrum was identical with that of an authentic sample. The 3,4-di-*O*-methyl-D-xylose was identified as described previously.

Oxidation of the Aldobiouronic Acid with Lead Tetraacetate.—The oxidation of the aldobiouronic acid was carried out by the method of Perlin[44] as described previously.[12,45]

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