solution was washed with water, dried with anhydrous sodium sulfate, and evaporated to a sirup which was crystallized from ether–petroleum ether; yield 1.1 g., m.p. 115–116°. Pure material was obtained on recrystallization from ethanol; m.p. 120–121°, $[\alpha]^{25}D$ +44° (*c* 1, ethanol), X-ray powder diffraction data[20]: 9.71m, 7.76w, 6.02m, 5.29s(1), 4.92 m(2), 4.37 m, 3.32m(3), 3.56 w, 3.31 m, 2.98 m, 2.84 vw, 2.70 vw.

Anal. Calcd. for $C_{26}H_{30}N_2O_4$: C, 71.98; H, 6.93; N, 6.44. Found: C, 71.86; H, 7.05; N, 6.51.

3-*O*-Benzyl-6-deoxy-L-*xylo*-hexose phenylosazone.—3-*O*-Benzyl-6-deoxy-L-idose (XI, 0.5 g.) was heated with 1.5 g. of sodium acetate and 1.0 g. of phenylhydrazine hydrochloride in 10 ml. of water in a boiling water-bath for 2 hr. The solvent was decanted and the residue was dissolved in benzene which, upon dilution with petroleum ether, deposited crystals after a few hours. Pure material was obtained on recrystallization from ethanol–water; yield 0.6 g., m.p. 95–96°, $[\alpha]^{25}D$ +36° (*c* 0.3, pyridine–ethanol, 2:3 by vol.).

Anal. Calcd. for $C_{25}H_{28}N_4O_3$: C, 69.42; H, 6.53; N, 12.95. Found: C, 68.60; H, 6.46; N, 12.46.

6-Deoxy-L-idose (XII).—6-Deoxy-1,2-*O*-isopropylidene-L-idofuranose[11] (VIII, 0.13 g.) was dissolved in 1.4 ml. of 0.5% sulfuric acid and heated at 70° for 2 hr. The solution was neutralized with powdered barium carbonate, filtered with decolorizing carbon and evaporated, under reduced pressure, to a sirup. The material was chromatographed on paper using 1-butanol–ethanol–water (4:1:5 by vol.) as developer and ammoniacal silver nitrate[23] indicator. Three spots A, B, C, with R_f values 0.22, 0.32, and 0.44, respectively, were obtained.

Four chromatographs, each with 50 mg. of the sirupy hydrolyzate spread evenly on a line 8 cm. long on 12 × 42 cm. Whatman No. 1 paper, were developed for 42 hr. with 1-butanol–ethanol–water (4:1:5 by vol.) at room temperature. Guide strips were then cut from the ends of the lines and sprayed with ammonical silver nitrate indicator.[23] Three zones, A, B, and C, with R_f values 0.22, 0.32, and 0.44, respectively, appeared. The order of intensities was C>B>A. The zone materials were eluted with water and the solutions were freeze-dried. Zone A: yield 4.9 mg., dec. 60°, was not further studied; Zone B: yield 12.8 mg., very hygroscopic; Zone C: yield 70 mg., $[\alpha]^{25}D$ −24° (*c* 0.7, water) recorded value[16] for 6-deoxy-L-sorbose (XIII), $[\alpha]^{25}D$ −27.7° (water).

A preparation of 6-deoxy-L-idose (XII) was also made, under neutral conditions, by the reductive debenzylation of 0.1 g. of 3-*O*-benzyl-6-deoxy-L-idose (XI) in 25 ml. of absolute ethanol containing 10 mg. of palladium-on-carbon catalyst by hydrogenation at 500 p.s.i. and 65° for 4.5 hr. Filtration and concentration, under reduced pressure, gave a colorless sirup; yield 80 mg., $[\alpha]^{25}D$ −1.9° (*c* 2.5, water). This material showed, on paper chromatography, one distinct spot corresponding to Zone B, R_f 0.32, and a very faint spot at Zone C, R_f 0.44. An authentic sample of 6-deoxy-L-sorbose,[16] kindly furnished by Professor Reichstein, produced a spot identical with spot C.

Material from Zone C of the hydrolysis product of 6-deoxy-1,2,-*O*-isopropylidene-β-L-ido(L-*glycero*-α-D-*xylo*-hexo)furanose and from the hydrogenolysis of 3-*O*-benzyl-L-idose produced 6-deoxy-L-*xylo*-hexose phenylosazone by the conventional method of preparation: phenylosazone from Zone C of the hydrolyzate, dec. 182–184°; from the hydrogenolysis of XI, dec. 160–166°; (literature values: 6-deoxy-L-*xylo*-hexose phenylosazone from 6-deoxy sorbose,[16] dec. 184–185°; from 6-deoxy-L-gulose,[24] dec. 183–184°; from 6-deoxy-L-idose, dec. 184–185°,[11] 168–172°[25]); the products gave identical X-ray powder diffraction data[20]: 10.05w, 7.68vw, 4.98m(3), 4.67s(1), 4.10m(2), 3.80vw, 3.56vw, 3.47w, 3.19w, 3.05vw, 2.85w; the infrared absorption spectra of the phenylosazones were also identical.

The colorless sirupy hydrogenolysis product (15 mg., exhibiting essentially only one spot at Zone B), dissolved in water containing Amberlite IR-120 (H^+),[17] was heated on a steam bath for 3 hr. Samples were spotted on paper at intervals. Upon development and spraying the paper the second spot at Zone C had appeared with an intensity nearly equal to that in Zone B with as little as 5 min. of heating.

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Constitution of an Aldohexaouronic Acid Formed on Enzymatic Hydrolysis of a 4-*O*-Methylglucuronoxylan

T. E. TIMELL

The Pulp and Paper Research Institute of Canada, and the Department of Chemistry, McGill University, Montreal, Que.

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An aldohexaouronic acid has been isolated after enzymatic hydrolysis of a 4-*O*-methylglucuronoxylan from white birchwood. It has been identified as *O*-4-*O*-methyl-α-D-glucosyluronic acid-(1 → 2)-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-β-D-xylosyl (1 → 4)-*O*-β-D-xylosyl-(1 → 4)-*O*-β-D-xylosyl-(1 → 4)-D-xylose.

When a 4-*O*-methylglucuronoxylan from white birch wood was treated with a commercial pectinase preparation, a series of oligosaccharides was obtained.[1,2] The xylan had previously been shown to contain a linear framework of (1→4)-linked β-D-xylose residues, every tenth of which, on the average, carried a (1→2)-linked 4-*O*-methyl-α-D-glucuronic acid residue.[3] The enzymic hydrolysis was carried out inside a semipermeable membrane surrounded by a large volume of water so that the sugars formed could diffuse rapidly through the membrane, thus escaping further hydrolysis.[4] High yields were obtained of a neutral and an acidic series of polysaccharides. This paper is concerned

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(3) C. P. J. Glaudemans and T. E. Timell, *J. Am. Chem. Soc.*, **80**, 941, 1209 (1958).

(4) T. J. Painter, *Can. J. Chem.*, **37**, 497 (1959).

with the constitution of one of the sugar acids, an aldohexaouronic acid.

The acid and neutral sugars obtained after the enzymatic hydrolysis were separated with an anion exchange resin. The acid fraction was resolved by preparative paper chromatography. One of the acids had a rate of movement on the paper chromatogram indicative of an aldehexaouronic acid.[5] This compound had $[\alpha]^{20}D$ −11.8° and was obtained in a yield of 9% of the original sugar mixture. Its methoxyl content, equivalent weight, and behavior on partial hydrolysis suggested the occurrence of one *O*-methylglucuronic acid and five xylose residues.

The aldohexaouronic acid was methylated to completion and subjected to methanolysis under conditions known to leave glycuronosidic linkages intact.[6] The acid fraction contained as only constituent a methylated aldobiouronic acid which was esterified and then reduced. The partly methylated disaccharide on hydrolysis gave a mixture containing equimolar portions of a di-*O*-methylxylose and a tri-*O*-methylglucose. After separation by paper chromatography, these compounds were identified as 3,4-di-*O*-methyl-D-xylose and 2,3,4-tri-*O*-methyl-D-glucose. The methylated aldobiouronic acid was therefore methyl 2-*O*-[methyl (2,3,4-tri-*O*-methyl-α-D-glucosyl)uronate]-3,4-di-*O*-methyl-D-xyloside. The neutral glycoside fraction after hydrolysis contained as only constituent 2,3-di-*O*-methyl-D-xylose, four moles being obtained per mole of tri-*O*-methylglucose.

Five alternative structures are possible for an aldohexaouronic acid of the present type. Four of these on methanolysis and subsequent hydrolysis would have produced one mole of 3-*O*-methylxylose in the acid fraction, while the neutral portion would have consisted of one mole of 2,3,4-tri-*O*-methylxylose and three moles of 2,3-di-*O*-methylxylose. The fifth alternative alone, where the acid group is attached to the nonreducing end group of a xylopentaose, is compatible with the present data.

Oxidation of the hexaouronic acid with periodate confirmed this conclusion, each molecule consuming seven moles of oxidant with concomitant formation of two moles of formic acid. These results could have been obtained only with a compound of the type referred to above. The combined evidence shows that the aldohexaouronic acid was *O*-4-*O*-methyl-α-D-glucosyluronic acid-(1→2)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylosyl(1→4)-*O*-β-D-xylosyl-(1→4)-*O*-β-D-xylosyl-(1→4)-D-xylose.

The structure of the other oligosaccharides formed during the enzymatic hydrolysis and a possible mechanism of the latter will be discussed elsewhere.[7]

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Experimental

Specific rotations were equilibrium values and determined at 20°. Melting points are corrected. Evaporations were carried out *in vacuo* at 40–50°. Infrared spectra were measured on a Perkin-Elmer Infracord spectrophotometer.

Paper Chromatography.—Sugars were separated on Whatman No. 3 MM filter paper by the descending technique. Solvents (v./v.) used were (A) ethyl acetate–acetic acid–water (18:7:8), (B) butanone saturated with water and containing 2% of ammonia, and (C) ethanol–benzene–water (47:200:15). A wick of Whatman No. 50 paper was used with solvent B. Paper electrophoresis was carried out in 0.05 *M* borate solution with 3 MM paper at 700 volts for 5 hr. *o*-Aminodiphenyl was used as a spray reagent and for quantitative analyses.[8]

Isolation of the Aldohexaouronic Acid.—The enzymic hydrolysis was carried out with a 4-*O*-methylglucuronoxylan isolated from white birch wood[3] and with a 0.2% aqueous solution of a commercial pectinase preparation.[9] Polysaccharide and enzyme were kept inside a cellophane membrane, surrounded by a large quantity of water as described previously.[1,2] Neutral and acidic sugars were separated with Dowex 1-X4 exchange resin[10] (acetate form). The acid mixture (22 g., 40% of the total) was resolved on strips of filter paper (18 cm. wide, 4–5 days at 25°, solvent A).

The chromatographically pure hexaouronic acid was dissolved in water and treated with Amberlite IR-120 exchange resin[11] (acid form). The acidic solution was evaporated to near dryness *in vacuo* at 25°. Repeated evaporations from acetone gave a white, amorphous solid (4.9 g.), $[\alpha]D$ −11.8° (*c* 2.3 in water).

Anal. Calcd. for $C_{32}H_{52}O_{27}$: OMe, 3.57; equiv. wt.[12] 868.4. Found: OMe, 3.45; Equiv. wt. 850.

Partial hydrolysis with 0.22 *N* sulfuric acid at 100° for 30 min. gave a series of uronic acids, from the mono- to the hexaouronic acid, and xylose, xylobiose, xylotriose, xylotetraose, and xylopentaose, as indicated by paper chromatography (solvent A).

Methylation of the Aldohexaouronic Acid.—The sugar acid (3.0 g.) was dissolved in water (100 ml.) containing sodium bicarbonate (5.0 g.). Dimethyl sulfate (50 ml.) and 40% (w./w.) aqueous sodium hydroxide (50 ml.) were added dropwise at 10° over a period of 5 hr. Solid sodium hydroxide (30 g.) was added, followed by dropwise addition of dimethyl sulfate (50 ml.) at room temperature. This process was repeated once. The *p*H of the reaction mixture was adjusted to 2.0 with sulfuric acid and the partly methylated uronic acid was removed with chloroform. The product (3.3 g.) was dissolved in dry dimethylformamide (100 ml.)[13] and the solution was shaken for five days with silver oxide (100 g.) and methyl iodide (100 ml.) in the presence of Drierite.[14] Chloroform (300 ml.) was added, insoluble material was removed by filtration through Celite,[15] and the chloroform solution was washed with 10% aqueous potassium cyanide (500 ml.) and with water (2 × 500 ml.). After drying with anhydrous sodium sulfate and evaporation to dryness, a semisolid material (3.0 g.) was obtained. Its infrared diagram indicated the absence of hydroxyl groups.

Separation of Acidic and Neutral Sugars.—A portion of the methylated uronic acid (2.5 g.) was boiled under reflux with *N*-methanolic hydrogen chloride for 8 hr. The sirup (2.6 g.) was heated to 60° for 2 hr. with 5% aqueous barium hydroxide. Excess carbon dioxide (solid)

(8) T. E. Timell, C. P. J. Glaudemans, and A. L. Currie, *Anal. Chem.*, **28**, 1916 (1956).

(9) A product of Nutritional Biochemical Corp., Cleveland, Ohio.

(10) A product of The Dow Chemical Co., Midland, Mich.

(11) A product of Rohm & Haas Co., Philadelphia, Pa.

(12) E. L. Hirst, L. Hough, and J. K. N. Jones, *J. Chem. Soc.*, 928 (1949).

(13) R. Kuhn, H. Trischmann, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).

(14) A product of the W. A. Hammond Drierite Co., Xenia, Ohio.

(15) A product of Johns-Manville Co., New York, N. Y.

was added, barium carbonate was removed by filtration through Celite, and the solution was treated with Amberlite IR-120 exchange resin (acid form). After concentration, the solution was added to the top of a column with Dowex 1-X4 exchange resin (acetate form). A neutral fraction (1.35 g.) was eluted with water after which an acid fraction (0.80 g.) was removed with 30% aqueous acetic acid.

Characterization of the Acid Fraction.—This fraction, which contained only one compound, was esterified with methanolic hydrogen chloride in the usual way and then reduced with lithium aluminum hydride (1.5 g.) in dry tetrahydrofuran (50 ml.). The disaccharide obtained was hydrolyzed with *N*-sulfuric acid, yielding a sirup (400 mg.) which contained equimolar quantities of a di-*O*-methylxylose and a tri-*O*-methylglucose. The mixture was resolved by paper chromatography (solvent B).

The xylose derivative (110 mg.) was obtained as a colorless sirup, $[\alpha]_D$ +21° (*c* 0.5 in water). Its rates of movement on the paper chromatogram (solvents B and C) and on the electrophoretogram were the same as those of an authentic specimen of 3,4-di-*O*-methyl-D-xylose. The 3,4-di-*O*-methyl-*N*-phenyl-D-xylosylamine[16] had m.p. 118–119°. Its infrared diagram was identical with that of an authentic sample.

The glucose derivative (150 mg) had $[\alpha]_D$ +75° (*c* 0.5 in water). The 2,3,4-tri-*O*-methyl-*N*-phenyl-D-glucosylamine[17] had m.p. and mixed m.p. 143–144°. Its infrared diagram was the same as that of an authentic specimen.

Characterization of the Neutral Fraction.—The neutral fraction was hydrolyzed with *N*-sulfuric acid to give a colorless sirup (1.20 g.) which crystallized immediately, m.p. 87–90°.[18] Paper chromatography (solvents B and C) and paper electrophoresis indicated the presence of only one compound. The 2,3-di-*O*-methyl-D-xylose had $[\alpha]_D$ +22.3° (*c* 2.3 in water).

Anal. Calcd. for $C_7H_{14}O_5$: OMe, 34.8. Found: OMe, 34.5.

The 2,3-di-*O*-methyl-*N*-phenyl-D-xylopyranosylamine[19] had m.p. and mixed m.p. 124–125°. Its infrared diagram was identical to that of an authentic specimen.

Anal. Calcd. for $C_{13}H_{19}O_4N$: OMe, 24.5. Found: OMe, 24.2.

Periodate Oxidation of the Aldohexaouronic Acid.—Aliquots (30–40 mg.) of the hexaouronic acid were dissolved in 0.05 *M* sodium metaperiodate (50 ml.) and the reaction was allowed to proceed in the dark at 30° for various lengths of time. The results, given as moles per mole of hexaouronic acid, were as follows:

Time, hr.	15	24	48	72	96
Periodate consumed	5.70	6.62	7.02	7.13	7.05

For estimation of formic acid production, the same amounts of the hexaouronic acid were dissolved in water (10 ml.) and neutralized with 0.01 *N* sodium hydroxide. Periodate solution (50 ml.) was added and the oxidation was carried out as before. Excess periodate was destroyed by addition of excess ethylene glycol and formic acid was determined by titration with 0.01 *N* sodium hydroxide. The following moles of formic acid were produced per mole of hexaouronic acid:

Time, hr.	24	48	72	96
Formic acid	2.00	2.04	2.27	2.67

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Studies on the Barks of the Family Salicaceae. V.[1] Grandidentatin, a New Glucoside from the Bark of *Populus grandidentata*

IRWIN A. PEARL AND STEPHEN F. DARLING

The Institute of Paper Chemistry, Appleton, Wis.

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A new glucoside has been isolated from the bark of *Populus grandidentata*. This glucoside, which we have named grandidentatin, has been identified as *cis*-2-hydroxycyclohexyl 2-*O*-*p*-coumaroyl-β-D-glucopyranoside. Alkaline hydrolysis of grandidentatin yields *p*-coumaric acid, and the de-*p*-coumaroylated glucoside, grandidentin, which has been identified as *cis*-2-hydroxycyclohexyl β-D-glucopyranoside. Enzymatic hydrolysis of grandidentin with β-glucosidase yields glucose and *cis*-1,2-cyclohexanediol. Complete methylation of grandidentatin followed by complete hydrolysis with acid yielded 3,4,6-tri-*O*-methyl-D-glucose.

In the earlier preliminary evaluation of barks of several species of the genus *Populus*, a compound melting at 210–211°, which appeared to be a new glycoside, was obtained from the bark of *Populus grandidentata*, bigtooth aspen. This glycoside was obtained in very small yield by Craig machine fractionation of a fraction of hot water extractives which had been freed of most of its salicin, tremuloidin, and salireposide. This finding led to a more extensive investigation of the glucosides of *P. grandidentata* and an attempt to isolate a larger quantity of the new substance. The present paper reports the first studies on the reinvestigation of the bark of *P. grandidentata*. A thirty-four year old

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