The Structure of an Aldopentaouronic Acid from a Birch Wood Xylan

TORE E. TIMELL

Pulp and Paper Research Institute of Canada and McGill University, Montreal, Canada

An aldopentaouronic acid has been isolated after enzymatic hydrolysis of a 4-*O*-methylglucuronoxylan from white birch wood. The compound has been identified as *O*-(4-*O*-methyl- α -D-glucosyluronic acid)-(1 $\rightarrow$ 2)-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)-*O*- β -D-xylosyl-(1 $\rightarrow$ 4)-*O*- β -D-xylosyl-(1 $\rightarrow$ 4)-D-xylose.

In previous communications ^{1,2} a brief account was given of preliminary results obtained on treating a 4-*O*-methylglucuronoxylan from white birch wood with a commercial pectinase enzyme. The xylan had previously been shown to contain a linear framework of (1 $\rightarrow$ 4)-linked β -D-xylose residues, every tenth of which, on the average, carried a (1 $\rightarrow$ 2)-linked 4-*O*-methyl- α -D-glucuronic acid residue ³. The enzymic hydrolysis was carried out inside a semipermeable membrane, suspended in a large volume of water as suggested by Painter ⁴. Oligosaccharides formed rapidly diffused through the membrane into the outside water, thus escaping further hydrolysis. Considerable improvement in yield of oligomers was achieved as could also be expected from the theoretical treatment of Kuhn ⁵. Two series of oligosaccharides were obtained, one neutral and the other acidic. This paper deals with the constitution of one of the sugar acids, an aldopentaouronic acid.

The enzymatic hydrolysis yielded a mixture of sugars which was resolved into an acid and a neutral portion with an anion exchange resin. The acids were separated by gradient elution from charcoal-Celite. One of the acids with $[\alpha]_D + 0.63^\circ$ had a rate of movement on the paper chromatogram typical of a pentaouronic acid ⁶. It was obtained in a yield of 11 % of the original sugar mixture. Its methoxyl content, equivalent weight, and behaviour on partial hydrolysis suggested that it consisted of one *O*-methylglucuronic acid and four xylose residues.

The pentaouronic acid was methylated, first according to Haworth and subsequently by the method of Kuhn *et al.* ⁷ The fully methylated acid was subjected to methanolysis under conditions known to leave glycuronosidic linkages intact. The methyl ester-methyl glycoside component was saponified

and acidified and the acid and neutral glycosides were separated with an anion exchange resin. The acid fraction contained only one compound, a fully methylated aldobiouronic acid, which was esterified and reduced with lithium aluminum hydride. The partly methylated disaccharide thus obtained could not be induced to crystallize. Hydrolysis gave equimolar amounts of a di-*O*-methylxylose and a tri-*O*-methylglucose, which were separated by paper chromatography. The xylose derivative was identified as 3,4-di-*O*-methyl-D-xylose and the glucose as 2,3,4-tri-*O*-methyl-D-glucose. The methylated aldobiouronic acid was accordingly 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 was hydrolysed. It contained only one compound, characterized as 2,3-di-*O*-methyl-D-xylose, three moles being obtained per mole of tri-*O*-methylglucose.

Four alternative structures are possible for an aldopentaouronic acid of the present type. Of these, three on methanolysis and subsequent hydrolysis would give rise to one mole of 3-*O*-methylxylose in the acid fraction while the neutral portion would consist of one mole of 2,3,4-tri-*O*-methylxylose and 2 moles of 2,3-di-*O*-methylxylose. The fourth alternative alone, where the acid group is attached to the non-reducing end group of a xylotetraose, is consistent with the present data.

This conclusion was corroborated by the behaviour of the pentaouronic acid on oxidation with periodate, when 6 moles of oxidant were consumed per molecule, with concomitant formation of 2 moles of formic acid. These results could only have been obtained with a compound of the type referred to above. The combined evidence shows that the aldopentaouronic acid was *O*-(4-*O*-methyl- α -D-glucosyluronic acid)-(1 $\rightarrow$ 2)-*O*- β -D-xylopyranosyl-(1 $\leftarrow$ 4)-*O*- β -D-xyl-*osyl*-(1 $\rightarrow$ 4)-*O*- β -D-xyl-*osyl*-(1 $\rightarrow$ 4)-D-xylose.

The nature of the other oligosaccharides formed during the enzymatic hydrolysis and the possible mechanism of the latter will be discussed elsewhere⁸.

EXPERIMENTAL

Specific rotations were determined at 20°C and melting points are corrected. Evaporations were carried out *in vacuo* at 40–50°. Sugars were separated on Whatman No. 1 filter paper and, for preparative purposes, on No. 3MM papers provided with a wick of paper No. 50. Solvents (v/v) 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). The spray reagent was *o*-aminodiphenyl.

Isolation of the aldopentaouronic acid. The enzymic hydrolysis was carried out with a 4-*O*-methylglucuronoxylan from birch wood⁹ and a 0.2 % aqueous solution of a commercial enzyme preparation ("Pectinase", Nutritional Biochemical Corporation, Cleveland, Ohio). Both were kept inside a cellophane dialysing tubing, surrounded by a large quantity of water, as described previously^{1,2}. In a typical experiment, neutral and acidic sugars were separated with a Dowex 1-X4 exchange resin (acetate form). The acid mixture (15 g, 40 % of the total) was resolved by gradient elution with aqueous ethanol on a charcoal-Celite column to give a series of uronic acids.

The aldopentaouronic acid was dissolved in water, treated with Amberlite IR-120 exchange resin (acid form), and recovered by repeated evaporations from acetone as a white, amorphous solid (3.6 g), $[\alpha]_D + 0.63^\circ$ (water, *c* 2.6). (Found: OCH₃ 4.00; equiv.wt.⁹. 737. Calc. for C₂₂H₄₄O₂₃: OCH₃ 4.21; equiv.wt. 736.3). Partial hydrolysis with 0.22 N sulfuric acid at 100° for 30 min. gave a series of uronic acids, from the mono- to the pen-

tauronic acid, and xylose, xylobiose, xylotriose, and xylotetraose, as indicated by paper chromatography (solvent A).

Methylation of the aldopentaouronic acid. The uronic acid (3.0 g) was dissolved in water (100 ml) containing sodium bicarbonate (5.0 g). Dimethyl sulphate (50 ml) and 40 % (w/w) aqueous sodium hydroxide (50 ml) were added dropwise over a period of 3 h at 10°. Solid sodium hydroxide (30 g) was added, followed by dropwise addition of dimethyl sulphate (50 ml). This process was repeated once. The mixture was acidified to pH 2.0 with sulphuric acid and then extracted with chloroform to give a partly methylated product (3.4 g, OCH_3 37.5 %). The latter was dissolved in dry dimethyl formamide (100 ml) and methylated with silver oxide (75 g) and methyl iodide (100 ml) in the presence of Drierite⁷. Chloroform (300 ml) was added, followed by filtration over Celite. The methylated oligosaccharide (3.2 g) was recovered in the usual way after washing with 5 % aqueous potassium cyanide⁷. Its infrared diagram indicated the absence of hydroxyl groups.

Separation of acidic and neutral sugars. A portion of the methylated compound (2.2 g) was boiled under reflux with N methanolic hydrogen chloride for 8 h. The syrup obtained (2.1 g) was treated with 5 % aqueous barium hydroxide (100 ml) at 60° for 2 h. Solid carbon dioxide was added, barium carbonate was removed by filtration through Celite and the solution was treated with Amberlite IR-120 exchange resin (acid form). The concentrated solution was added to the top of a column containing Dowex 1-X4 exchange resin (acetate form). Elution with water removed neutral glycosides (1.30 g), after which an acid fraction (0.95 g) was recovered by washing with 30 % aqueous acetic acid.

Acid fraction. The acid fraction which contained only one compound was subjected to methanolysis and the methyl ester-methyl glycoside was reduced with lithium aluminium hydride (2.0 g) in tetrahydrofuran (50 ml). The disaccharide obtained was hydrolysed with N-sulphuric acid to give a syrup (330 mg) which contained a di-*O*-methylxylose and a tri-*O*-methylglucose in equimolar quantities. The mixture was resolved by paper chromatography (solvent B).

3,4-Di-O-methyl-D-xylose. This sugar (75 mg) was obtained as a clear syrup with $[\alpha]_D + 20^\circ$ (water, *c* 0.5). Its rate of movement on paper electrophoresis in 0.05 M borate buffer and on paper chromatography (solvents B and C) were the same as those of an authentic specimen. The 3,4-di-*O*-methyl-*N*-phenyl-D-xylopyranosylamine had m.p. 117–118°¹⁰. Its infrared diagram was identical with that of an authentic sample.

2,3,4-Tri-O-methyl-D-glucose. This syrup (100 mg) had $[\alpha]_D + 75^\circ$ (water, *c* 0.6). The 2,3,4-tri-*O*-methyl-*N*-phenyl-D-glucosylamine had m.p. and mixed m.p. 142–143°¹¹. Its infrared spectrum was the same as that of an authentic specimen. (Found: OCH_3 30.5. Calc. for $\text{C}_{15}\text{H}_{23}\text{O}_5\text{N}$: OCH_3 31.3.)

Neutral fraction. The neutral portion was hydrolysed with N sulphuric acid. Paper electrophoresis and paper chromatography (solvent C) showed the presence of only 2,3-di-*O*-methyl-D-xylose. The colourless syrup crystallised spontaneously on standing, m.p. 80–82°¹², $[\alpha]_D + 23.4^\circ$ (water, *c* 1.7). (Found: OCH_3 34.4. Calc. for $\text{C}_7\text{H}_{14}\text{O}_5$: OCH_3 34.8). The 2,3-di-*O*-methyl-*N*-phenyl-D-xylopyranosylamine had m.p. and mixed m.p. 123–124°¹³. (Found: OCH_3 24.5. Calc. for $\text{C}_{13}\text{H}_{19}\text{O}_5\text{N}$: OCH_3 24.5).

Periodate oxidation of the aldopentaouronic acid. Aliquots (30–40 mg) of the uronic acid were oxidized in the dark with 0.05 M sodium metaperiodate (50 ml) at room temperature for various lengths of time. The results, given as moles oxidant consumed per mole of pentaouronic acid, were as follows:

Time, h	6	24	48	72	96
Periodate consumed	5.2	5.5	5.9	6.0	6.3

Samples (30–40 mg) of the acid were dissolved in water (10 ml) and neutralised with 0.01 N sodium hydroxide. Periodate oxidations were carried out in the same way as before. Oxidation was interrupted by addition of excess ethylene glycol. Formic acid was determined by titration with 0.01 N alkali. The following moles of formic acid were liberated per mole of pentaouronic acid:

Time, h	24	48	72	96
Formic acid	1.84	1.96	2.09	2.24

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