ACTA CHEMICA SCANDINAVICA 20 (1966) 206–210

The Alkaline Decomposition of 2,3,4-Tri-*O*-Methyl-D-glucose and 2-*O*-Methyl-cellobiose

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The synthesis of 4-*O*-(β-D-glucopyranosyl)-2-*O*-methyl-D-glucose (2-*O*-methyl-cellobiose) is described. This substance and 2,3,4-tri-*O*-methyl-D-glucose were found to readily decompose to acidic material in oxygen-free alkali at 100°. It was concluded, from the nature of the acids formed, that the 4-*O*-substituent is eliminated during the decomposition along with accompanying fragmentation.

Aldoses, substituted in the 2-position, are more stable in alkaline solutions than the corresponding sugars unsubstituted in this position.[1,2] The formation of saccharinic acids is inhibited, but enol ethers [3] are formed under mild conditions. For this reason the successive elimination, in alkaline solution at room temperature, of reducing residues from (1→4)-linked polysaccharides is stopped when it reaches a 2-*O*-substituted residue. At higher temperatures, however, the 2-*O*-substituent offers no protection, as demonstrated for wood xylans carrying 4-*O*-methyl-α-D-glucopyranosyluronic acid residues in 2-positions,[4] and for 2-*O*-D-xylopyranosyl-L-arabinose [5] and 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose.[6] This paper reports the synthesis of 4-*O*-(β-D-glucopyranosyl)-2-*O*-methyl-D-glucose (2-*O*-methyl-cellobiose) and some observations concerning the decomposition of this substance and 2,3,4-tri-*O*-methyl-D-glucose in oxygen-free alkali at 100°.

The cellobiose derivative was synthesised by a method, analogous to that used by Hodge and Rist [7] for the synthesis of 2-*O*-methyl-D-glucose, but in considerably lower yield. Cellobiose octa-acetate was reacted with piperidine to give N-[*O*-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl (1→4)-3,6-di-*O*-acetyl-β-D-glucopyranosyl] piperidine (I). I was methylated to give a crystalline methyl ether which, after removal of the blocking groups and chromato-

graphic purification, yielded pure but amorphous 2-*O*-methyl-cellobiose. This gave on hydrolysis 2-*O*-methyl-D-glucose and D-glucose.

I on acetylation yielded the hepta-acetate, m.p. 245—246°, $[\alpha]_D$ −19° (chloroform). Zemplén and Bruckner [8] prepared the same substance from hepta-*O*-acetyl-α-cellobiosyl bromide and piperidine. The optical rotations of the two preparations agree, but the latter authors report a melting point of 215—220°. When the substance was prepared, following their directions, a product of m.p. 245—246° was obtained, indistinguishable from that obtained by acetylation of I.

2,3,4-Tri-*O*-methyl-D-glucose, in oxygen-free M sodium hydroxide solution at 100°, decomposed completely after 5 h. During the reaction the appearance and disappearance of a second reducing substance, presumably 3-deoxy-2,4-di-*O*-methyl-D-*erythro*-hexopyranos-2-ene,[9] was observed. The reaction products were insoluble tars and acids (Table 1). The dark, water insoluble tar contained 2.9 % methoxyl. The volatile acids, formic and acetic acids, constituted only a small part of the total acids. Among the non-volatile acids lactic acid (predominating) and glycollic acid were characterised as their crystalline *p*-bromo-phenacyl esters. The presence of glyceric acid and small amounts of non-identified acids was demonstrated by paper and gas-liquid chromatography. No evidence could be found for the presence of erythronic or 2-deoxy-erythronic acid or of saccharinic acids.

2-*O*-Methyl-cellobiose, under identical reaction conditions, reacted completely in 4 h. Again the appearance and disappearance of a second reducing compound having a higher R_F-value than the starting material was observed. This intermediate was shown to be acid-labile, being readily converted to glucose and 5-(hydroxymethyl)-2-furaldehyde, and is probably 3-deoxy-4-*O*-(β-D-glucopyranosyl)-2-*O*-methyl-D-*erythro*-hexopyranos-2-ene. Similar insolubles and acid components were produced (Table 1). In addition to the products formed from 2,3,4-tri-*O*-methyl-D-glucose, glucose and saccharinic acids, derived from this sugar, were observed. It is obvious that these components derive from the non-reducing glucose residue in the starting material and that they account for the increase in acid production from this substance relative to 2,3,4-tri-*O*-methyl-D-glucose. The yield of acids from 2-*O*-methyl-cellobiose is also nearly the same as that observed by Whistler and Corbett after similar treatment of 2-*O*-D-xylopyranosyl-D-arabinose. The results suggest that the elimination of the 4-*O*-substituent in 2-*O*-methyl-cellobiose and 2,3,4-tri-*O*-methyl-D-glucose is accompanied by fragmentation of the reducing sugar residue but do not allow an interpretation of the reaction mechanism.

Table 1. Acids produced by the 2-*O*-substituted sugars. Values calculated as moles of acid/mole of sugar.

Compound	Total acids	Volatile acids	Formic acid
2-*O*-Methyl-cellobiose	2.4	0.3	0.18
2,3,4-Tri-*O*-methyl-D-glucose	1.0	0.2	0.05

EXPERIMENTAL

Concentrations were carried out under reduced pressure below 45°. Melting points are corrected. The solvent systems used for paper chromatography (on Whatman No. 1 papers) were: A. butanol-ethanol-water, 10:3:5, B. butanone, saturated with water, C. ethyl acetate-acetic acid-water, 3:1:1 and D. amyl alcohol-5 M formic acid, upper phase. Conventional spraying agents for reducing sugars, polyols, acids, and lactones were used.

N-[O-2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosyl* (1→4)-3,6-*di*-O-*acetyl*-β-D-*glucopyranosyl*] *piperidine* (*I*). β-Cellobiose octa-acetate [120 g, m.p. 195—196°, $[\alpha]_D^{25}$ —10° (in chloroform) was slurried with tetrahydrofuran (400 ml) and piperidine (80 ml) was added. The slurry was stirred at 25° for 36 h, solution being attained after about 3 h. The solution was then diluted with chloroform (400 ml) and poured into a separating funnel containing ice-water. Acetic acid was added with occasional shaking until unreacted piperidine was neutralised, the chloroform phase was washed with water, dried over anhydrous sodium sulphate and concentrated to a syrup (40 g). This readily crystallised and was recrystallised several times from methanol, m.p. 189° $[\alpha]_D^{25}$ —2° (*c* 1.0, chloroform). [Found: C 53.0, H 6.68; N 2.24. $C_{29}H_{43}O_{16}N$ requires: C 52.6; H 6.55; N 2.12].

Part of this substance was acetylated with acetic anhydride and piperidine and the product was crystallised from chloroform-ethanol, m.p. 245—246°, $[\alpha]_D^{20}$ —19° (*c* 2.1, chloroform). Hepta-*O*-acetyl-α-cellobiosyl bromide was reacted with piperidine and the product was isolated as described by Zemplén and Bruckner. [8] It had m.p. 245—246°, $[\alpha]_D^{25}$ —17° and was indistinguishable from the substance obtained by acetylation of I (mixed m.p., IR).

N-[O-*2,3,4,6-Tetra*-O-*acetyl*-β-D-*glucopyranosyl* (*1→4*)-*3,6-di*-O-*acetyl*-*2*-O-*methyl*-β-D-*glucopyranosyl*] *piperidine*. Compound I (22 g) was dissolved in dimethyl formamide (100 ml), and methyl iodide (35 ml) and silver oxide (40 g) were added with stirring. After 12 h the suspension was filtered, the salts were washed with acetone and the solution was worked up in the usual manner.[10] The reaction product (14 g) crystallised spontaneously and was recrystallised from methanol, m.p. 233°, $[\alpha]_D^{25}$ —6.0° (*c* 1.3, chloroform). [Found: C 53.4; H 6.85; N 2.24; OCH_3 4.62. $C_{30}H_{45}O_{16}N$ requires: C 53.3; H 6.71; N 2.07; OCH_3 4.59].

2-O-*Methyl-cellobiose*. The above substance (8.9 g) was dissolved in anhydrous methanol (100 ml) and a catalytic amount of sodium methoxide was added. After 12 h the solution was concentrated to dryness, the product was dissolved in 0.5 M hydrochloric acid (10 ml) and the solution was kept at room temperature. The progress of the hydrolysis was followed by paper chromatography (solvent system A) and reached an optimum yield after about 20 h, when the solution was deionised and concentrated to a syrup (3.0 g). Paper chromatography (solvent system A and C) revealed the presence of the desired substance ($R_{glucose}$ 0.87 in solvent system A) together with glucose, 2-*O*-methyl-glucose and small amounts of cellobiose. 2-*O*-Methyl-cellobiose (1.8 g) was obtained chromatographically pure by carbon column chromatography, using aqueous ethanol as irrigant. The substance, which did not crystallise, had $[\alpha]_D^{25}$ +33° (*c* 1.9, water). [Found: OCH_3 8.2. $C_{13}H_{24}O_{11}$ requires: OCH_3 8.7]. Acid hydrolysis of this substance in N sulphuric acid at 100° for 2 h, yielded equal amounts of glucose and 2-*O*-methyl-glucose, as indicated by paper chromatography and paper electrophoresis in borate and germanate buffers.

Alkaline decomposition of 2,3,4-tri-O-*methyl*-D-*glucose*. This substance was prepared by acid hydrolysis of 1,6-anhydro-β-D-glucopyranose trimethyl ether, as described previously.[11]

The sugar (50 mg) was dissolved in oxygen-free N sodium hydroxide (4 ml) and the solution was heated on a steam bath. Samples were removed at intervals, treated with excess of Dowex 50 (H^+) and the resulting solutions were examined by paper chromatography (solvent systems A and B). After 0.5 h small amounts of a new reducing compound were observed, having a higher mobility than the starting material. After 2 h both compounds appeared to be present in equal amounts and after 5 h they had both completely disappeared. Similar results were obtained with saturated lime-water, complete decomposition requiring about 8 h.

Tri-*O*-methyl-D-glucose (100 mg) was treated with sodium hydroxide (5 ml) as above for 6 h, passed through a column of Dowex 50 (H^+) and the effluent made up to 50 ml. Aliquot portions of this solution were titrated with 0.2 M sodium hydroxide. Further

aliquot portions were distilled and volatile acids were determined by titration. Formic acid was determined in a further aliquot portion using the procedure of Richards and Sephton.[12]

A larger sample (1.0 g) of 2,3,4-tri-*O*-methyl-D-glucose in M sodium hydroxide (50 ml) was heated on a steam bath for 6 h, passed through a Dowex 50 (H^+) column and concentrated. Some insoluble material separated, and this was collected by centrifugation, washed with water and dried, to give a brown powder (150 mg, OCH_3 2.9). The yellow solution was stirred with Dowex 1 (carbonate form) overnight, and the resin was filtered off. The solution, which contained no glucose, acids or lactones, contained 120 mg of dry material. The acids were displaced from the resin with M sulphuric acid, the solution was neutralised with barium carbonate, barium sulphate was removed by filtration and the solution was passed through a column of Dowex 50 (H^+) and concentrated to a syrup (0.57 g). Paper chromatographic examination in solvent systems A, B, and C indicated the presence of a number of acidic components of which the three predominant ones were chromatographically indistinguishable from glycollic, glyceric, and lactic acid. Of these, lactic acid was the predominant. No spots resulting from saccharino-lactones were observed and the absence of these was further substantiated by subjecting part of the syrup to trimethylsilylation and then gas-liquid chromatography,[13,14] whereupon no peaks corresponding to any of the glucosaccharino-lactones were observed.

Part of the syrup (400 mg) was fractionated on Whatman 3MM filter paper (solvent system D) and after steaming the papers to completely remove the irrigant, the zones corresponding to the three acids were excised and the acids were isolated by elution.

The glycollic and lactic acids were characterised as their crystalline *p*-bromophenacyl esters, melting at 135—137° and 110—112°, respectively, and found to be indistinguishable from the corresponding authentic materials. The glyceric acid was methylated with diazomethane. The methyl ester was indistinguishable from an authentic sample on gas-liquid chromatography, using a diallylglycol-succinate column at 150°.

The volatile acids from another alkaline decomposition of 2,3,4-tri-*O*-methyl-D-glucose were esterified with diazomethane and the presence of methyl acetate in the product was demonstrated by gas-liquid chromatography (diallylglycol-succinate column, 25°).

Alkaline decomposition of 2-O-methyl-cellobiose. The rate of decomposition of this sugar in oxygen-free M sodium hydroxide was followed in the same manner as for 2,3,4-tri-*O*-methyl-D-glucose. Here also a new reducing compound appeared ($R_{glucose}$ 0.92) and both compounds disappeared completely after 4 h reaction. On acid hydrolysis (0.01 M hydrochloric acid at 50°) glucose and 5-(hydroxymethyl)-2-furaldehyde were rapidly formed as demonstrated by paper chromatography. Quantitative analysis of volatile and non-volatile acids and formic acid (Table 1) were performed as above.

A sample (0.50 g) was decomposed in M sodium hydroxide at 100° for 4 h, the solution was neutralised with Dowex 50 (H^+) and concentrated to a syrup (0.35 g). Paper chromatograms indicated the presence of the same acids as obtained from 2,3,4-tri-*O*-methyl-D-glucose and, in addition to these substances, glucose and saccharinic acids and their lactones. The presence of glucose was further verified by paper electrophoresis. The compunds having mobilities similar to the saccharino-lactones were isolated by paper chromatography (solvent system A) and examined by gas-liquid chromatography as their trimethylsilyl ethers.[14] Five peaks were obtained, with the same retention times as observed for the trimethylsilyl ethers "α"- and "β"-D-glucometa-, "α"- and "β"-D-glucoiso- and "α"-D-gluco-saccharino-1,4-lactones.

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Received September 29, 1965.