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The Oxidation of Glycosides

IX. Acidic and Alkaline Degradation of Methyl β-D-2-Oxo-glucopyranoside and Methyl β-D-3-Oxo-glucopyranoside

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An investigation has been made of the acidic and alkaline degradation of methyl β-D-2-oxo-glucopyranoside (I) and methyl β-D-3-oxo-glucopyranoside (II). Under acidic conditions both gave reductic acid while I also gave D-glucosone and II gave D-ribulose. The degradation of I and II in aqueous solution at pH 4 and pH 7 at 100° was rapid and followed the alkaline type of degradation with elimination of the methoxyl group and formation of the dicarbonyl intermediate, 1,5-anhydro-2,3-dioxo-D-glucitol. The reaction products of I also contained II and D-ribulose. No fundamental differences in the type and pattern of the reaction products from those obtained by degradation of II with either lime-water or sodium hydroxide were detected but the rate of reaction of the dicarbonyl intermediate was much slower when sodium hydroxide was used.

Part VIII [1] described the degradation of some oxo-glucosides by lime-water. The present investigation was undertaken to study the degradation of methyl β-D-2-oxo-glucopyranoside (I) and of methyl β-D-3-oxo-glucopyranoside (II) at other pH values and to compare the effect of using sodium hydroxide as the base instead of lime-water.

When the oxo-glucosides were treated with sulphuric acid (0.5 N at 100°) the solution rapidly developed a yellow coloration, then gradually turned brownish and formed a dark coloured precipitate. The hydrolysate had a smell similar to that of furfural. Under similar conditions methyl β-D-glucopyranoside turned only slightly yellow. A semi-quantitative determination (Fig. 1) showed that the rates of hydrolysis of I, II and the unsubstituted methyl glucoside were rather similar and followed the sequence: methyl 3-oxo-glucoside > methyl 2-oxo-glucoside > methyl glucoside. The dioxo sugars formed by hydrolysis of I and II would be expected to be much more reactive than glucose. The oxo-glucosides were not hydrolysable with β-glucosidase.

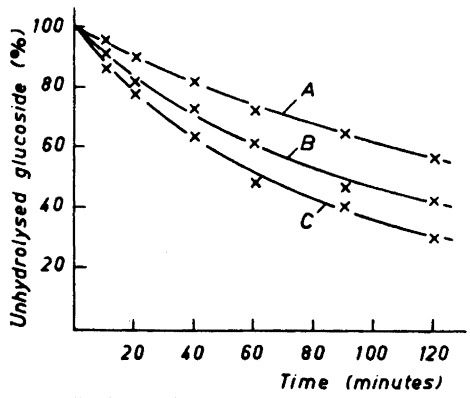
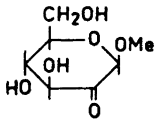


Fig. 1. Treatment of methyl β-D-glucopyranoside (A), methyl β-D-2-oxo-glucopyranoside (B) and methyl β-D-3-oxo-glucopyranoside (C) with 0.5 N sulphuric acid at 100°.

Under hydrolytic conditions that gave mostly glucose and only small amounts of further degradation products from methyl β-glucoside, there was formed from the oxo-glucosides a very complex mixture of compounds that gave positive and very characteristic reactions with silver nitrate-sodium hydroxide, *p*-anisidine hydrochloride, resorcinol-hydrochloric acid and 2,4-dinitrophenylhydrazine reagents. Electrophoresis in hydrogen sulphite buffer [2] was found to be particularly useful in studying the products and gave good separations and round, well-defined spots. Some compounds were present in the hydrolysates from both I and II, some were present only when shorter reaction times were used.

From the degradation of both I and II there was isolated an acidic, strongly reducing crystalline compound that was shown to be reductic acid (III). This substance was obtained by Reichstein and Oppenauer [3] by acid treatment of monomeric uronic acids or polyuronides in a yield of 7 % and in small amounts from xylose. Sohn [4] has recorded its formation in small amount on acid treatment of oxidised cellulose. The acid treatment of oxo-glucosides in the present investigation seems to give a higher yield of this substance than has been obtained previously from other carbohydrates and in this case it is probably an intermediate in the main path of degradation. It was isolated in 22 % yield from the degradation of methyl 3-oxo-glucoside. Other compounds that were obviously associated with the occurrence of reductic acid were also present such as furfural which was isolated in small amounts and has previously[3] been shown to be formed by acid treatment of III, oxidation products of the easily oxidisable III and a product that did not migrate an electrophoresis in hydrogen sulphite but gave the same characteristic reactions as III with several different reagents.

A liquid compound was formed from both I and II (ca. 5 %) which had a relatively high mobility in electrophoresis in hydrogen sulphite ($M_{Vanillin}$ = 1.70), did not reduce a neutral silver solution, gave a characteristic colour reaction with *p*-anisidine hydrochloride and with resorcinol-hydrochloric acid and rapidly gave a precipitate with 2,4-dinitrophenylhydrazine. In addition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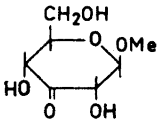
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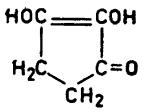
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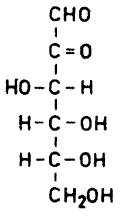
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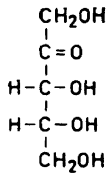
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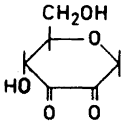
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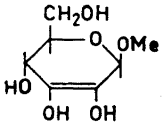
VII

to these compounds there were a few other very minor components that were present in the products from both I and II.

D-Glucosone (IV) was isolated in about 20 % yield from the product obtained from I*. Attempts to isolate a corresponding 3-oxo-glucose from the product from II were unsuccessful. This sugar is probably more reactive than IV. However a small amount of D-ribulose (V, 1.5 %) which might have arisen from cleavage of 3-oxo-glucose was isolated from a hydrolysate of II. Ribulose could not be detected in the hydrolysate from I.

In the preceding paper [1] it was shown that both I and II are degraded very rapidly at room temperature by lime-water and that the principal course of the degradation involves elimination of the methoxyl group and the formation of 1,5-anhydro-2,3-dioxo-D-glucitol (VI) which then gives rise to acids, probably by a benzilic acid type rearrangement. Blears, Machell and Richards [5] have reported that the alkaline degradation of 4-*O*-substituted glucose derivatives gives greater amounts of the dicarbonyl intermediate and that the fragmentation of this intermediate was favoured at the expense of saccharinic acid formation when dilute sodium hydroxide solution was used instead of lime-water. In the present investigation a comparison was made of the results of treatment of methyl β-3-oxo-glucoside (II) with lime water and with sodium

* *Addeed in proof:* No reductic acid was detected on treatment of D-glucosone under acid conditions in the same way as methyl 2-oxo-glucoside (I). This indicates that formation of the five-membered ring might not be preceded by hydrolysis of I.

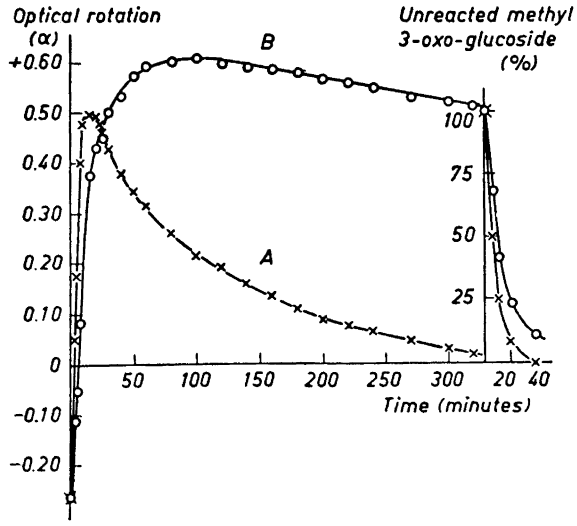

Fig. 2. Treatment of methyl β-D-3-oxo-glucopyranoside with A. lime water (— × — × —) and B sodium hydroxide (—O—O—), 0.018 N at 23°.

hydroxide. The degradation was followed by the optical rotation, by semi-quantitative determination of II (Fig. 2) and by paper chromatographic examination. The dicarbonyl intermediate (VI) reacted much more slowly in sodium hydroxide solutions than in lime-water; II was also degraded somewhat more slowly by sodium hydroxide. The only difference which was detected between samples taken from the two reaction mixtures when the rotation was a maximum was a somewhat lower amount of II and a larger amount of VI in the sodium hydroxide product. No other difference was detected in either the neutral or the acid products which could indicate that the reactions with sodium hydroxide were any different from those with lime-water.

The stability of the oxo-glucosides at the neutral point and at a pH value (pH 4) near that at which carbohydrates are known to be most stable was also of interest.

On treatment of either I or II at pH 7 (at 100°) the solution turned yellow in a few minutes just as in alkaline treatment at room temperature paper chromatographic examination showed that they underwent the same degradation *via* the dicarbonyl intermediate VI as when treated with alkali. In a large scale experiment with the methyl 2-oxo-glucoside (I) at pH 7 for 10 min, methyl 3-oxo-glucoside (18 %) and D-ribulose (3 %) were isolated; most of the remainder of the product was made up of compounds similar to those formed in the alkaline degradation. No methyl 3-oxo-mannoside (an epimerisation product that would be expected together with II) could be isolated and if present it was probably only in much smaller amount than II.

In a large scale experiment with the methyl 3-oxo-glucoside (II) at pH 7 the dicarbonyl intermediate VI and the neutral, unidentified crystalline com-

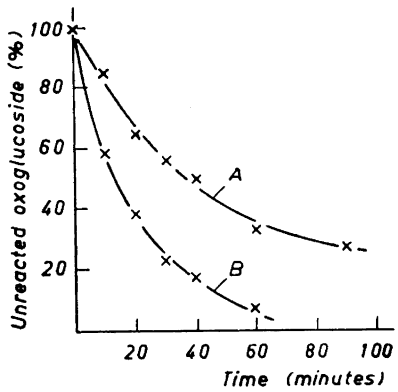

Fig. 3. Treatment of methyl β-D-2-oxo-glucopyranoside and methyl β-D-3-oxo-glucopyranoside at pH 4 and 100°. A. Methyl 3-oxo-glucoside. B. Methyl 2-oxo-glucoside.

pound that had been obtained by alkaline treatment were isolated; traces of I were also detected. The acids obtained showed a similar paper electrophoretic pattern to those from the product of alkaline treatment.

The principal neutral products obtained at pH 4 were the same as those obtained at pH 7 but the rate of degradation was slower. Semi-quantitative determinations of I and II were made throughout the course of the reaction (Fig. 3). The 2-oxo-glucoside was rapidly converted to the 3-oxo-glucoside which then underwent a further change *via* VI. This compound was isolated and identified chromatographically and was shown electrophoretically to give the expected products on reduction with Raney nickel and with borohydride. These results show that the mechanism of the alkaline degradation of oxo-glucosides involving epimerisation, the elimination of the methoxyl group and the formation of a dicarbonyl intermediate is also operative under slightly acid conditions. Whether the further transformation of the dicarbonyl intermediate follows the benzilic acid type rearrangement at this low pH value is, however, uncertain since preliminary results have shown [1] that VI is transformed in different ways at low and high pH values.

Among the products obtained by treatment of I and II at pH 4 there was found in small amount a compound with the same chromatographic behaviour and giving the same reactions with the reagents used as the possible enediol VII, that was suggested [1] as an intermediate in the formation of II on alkaline treatmetnt of I.

In an aqueous solution of methyl β-2-oxo-glucoside in contact with an excess of a weakly basic resin (Amberlite IR-4B: free base) for 4 h at room temperature there was formed about 10 % of methyl β-3-oxo-glucoside. However, when the latter was treated in the same way only traces of transformation products were detected. The 3-oxo-glucoside as well as the possible enediol (VII) mentioned above were detected in a solution of the 2-oxo-glucoside in distilled water after keeping at 4° for a few months but no transformation products were found in a solution of the 3-oxo-glucoside kept under similar conditions.

EXPERIMENTAL

Melting points are corrected. All solvents were removed either by distillation under reduced pressure or by lyophilisation. Paper chromatograms were normally run on Schleicher and Schüll No. 2043 a paper; preparative chromatographic separations were done on Whatman 3MM (after thorough washing) or 31 papers; paper electrophoreses were run on Whatman 3MM paper.

The spray reagents used were: (a) silver nitrate-sodium hydroxide, (b) *p*-anisidine hydrochloride, (c) resorcinol-hydrochloric acid, (d) 2,4-dinitrophenylhydrazine-hydrochloric acid and (e) ferric chloride.

The eluting solvents and buffers used were:

A Ethyl acetate-acetic acid-water, 3:1:3 (upper phase)
B Butanol-ethanol-water, 10:3:5
C Hydrogen sulphite buffer pH 4.7, 0.1 M[2] (used at 50°, M_V = migration relative to that of vanillin)
D Borate buffer, pH 10.0, 0.1 M
E Acetate buffer pH 4.0, 0.1 M
F Phosphate buffer pH 7.0, 0.1 M

Reaction of methyl β-2-oxo-glucopyranoside (I) and methyl β-3-oxo-glucopyranoside (II) with sulphuric acid

Solutions of I, II (prepared as previously [6] described) and methyl β-D-glucopyranoside (0.5 ml, 1 %) in 0.5 N sulphuric acid in sealed glass tubes were kept in a water bath at 100° for different times. After cooling, water (1 ml) was added and the pH was adjusted to 5 by the addition of dry Amberlite resin IR 4B (free base). Aliquots of these solutions were paper chromatographed using solvent A and semi-quantitative determinations were made of the components present by visual estimation on three runs as described previously [1]. For comparison, the amounts of unhydrolysed glucoside in the products from the methyl glucoside were calculated from determinations of glucose by the method of Saeman [7]; the two methods gave good agreement. The kinetic results are shown in Fig. 1.

Paper chromatographic and electrophoretic (buffer C) studies showed that the products obtained from I and II were quite complicated mixtures but there were only a few main components.

Separation of the degradation products obtained from II. In a preliminary large scale experiment, a solution of II (1.00 g) in sulphuric acid (100 ml, 0.5 N) was kept in a water bath at 100° for 2 h. The brownish hydrolysate after cooling was filtered to remove the small amounts of brown precipitate and then extracted continuously with ether for 24 h. The semi-solid dark brown product obtained on evaporation of the ethereal solution contained two main components, A and B, with M_V-values of 1.07 and 1.70 respectively (buffer C) and a considerable amount of material which did not migrate in this buffer. Some minor products were also present which had high migration values in buffers E and F. The product was distilled in a vacuum (ca. 0.06 mm of Hg). A colourless oil distilled at 85° which contained largely B together with traces of other compounds. At 125° a colourless product sublimed which was found to be pure A. It was recrystallised from methanol-methyl ethyl ketone and identified as reductic acid (III). The principal component remaining in the ether-extracted aqueous solution was unchanged II (0.228 g).

Since the hydrolysates from the kinetic investigation showed that some components of the products of the degradation were more rapidly destroyed than others, a solution of II (0.50 g) in 0.5 N sulphuric acid was heated in a water bath at 100° for only 30 min. Chromatography on thick filter paper gave about 60 per cent of II together with a mixture containing principally A and B and, in addition, small amounts (1.5 %) of a compound which was isolated and shown to be D-ribulose (V).

To give a better idea of the proportions of the different components present a further large scale run was made omitting the somewhat drastic distillation step. II (1.00 g) was heated in 0.5 N sulphuric acid (100 ml) for 3 h in a water bath at 100°. The hydrolysate (which had a smell of furfural) was diluted with water (100 ml) and distilled at ordinary pressure until 100 ml had been distilled off. Furfural only was detected in this fraction (by electrophoresis using buffer C); estimation by colorometric analysis showed that 15 mg was present. The hydrolysate, which was now free of the smell of furfural, was continuously extracted with ether for 4 days. The aqueous phase was treated with barium carbonate, deionised on Amberlite IR-120 (H^+) and taken to dryness (0.24 g). The ether extract was evaporated (0.43 g) and on crystallisation gave reductic acid (85 mg) in an electrophoretically pure state; the mother liquor was fractionated by electrophoresis on thick filter paper using buffer C. The strips containing separate fractions were eluted with water and the resultant solutions treated with barium carbonate to a pH 5—6, filtered, passed through an Amberlite IR-4B (free base) column and taken to dryness by lyophilisation. Before evaporation the fraction containing reductic acid was given a short treatment with hydrogen sulphide to reduce any tri-oxo-pentane which might have been present [8]. The material not extracted by ether contained some B but only small amounts of reductic acid; it was fractionated by paper chromatography on thick filter paper using solvent A. The two fractionations gave unreacted II (165 mg) reductic acid (90 mg) and B (45 mg).

Separation of the degradation products from I. Methyl β-2-oxo-glucoside (I, 200 mg) was heated in sulphuric acid solution (0.5 N, 20 ml) for 3 h in a water bath at 100°. Chromatography on thick filter paper (solvent A) gave: unreacted I (35 mg), D-glucosone (30 mg) and reductic acid (20 mg). The substance B that had been isolated from the product of acid treatment of II was present but no trace of ribulose could be detected.

Reductic acid (II). Recrystallisation from a methanol-methyl ethyl ketone mixture gave reductic acid, m.p. 211—212°, undepressed on admixture with an authentic sample, $R_F = 0.65$ (solvent A), $M_V = 1.07$ (buffer C). The infrared spectrum was identical with that of authentic material. It reduced a neutral silver solution immediately at room temperature and gave a characteristic green-yellow coloration with spray reagent *b*. These two characteristic spray reactions were also given by small amounts of material with $M_V = 0$ on an electrophoretogram (buffer C) of the ether extract from II. The product of oxidation of the reductic acid with iodine, as described by Reichstein and Oppenauer [8] in their preparation of trioxo-pentane from reductic acid, gave two spots on electrophoretograms (buffer C) with M_V-values 2.03 and 2.12, respectively, and a single elongated spot $R_F = 0.31$ on paper chromatograms (solvent A). These spots were detected by reduction of a neutral silver solution at room temperature or by a red brown coloration with spray reagent *b*. It was found that the same substances were also present in smaller amounts in the ether extract from II and were formed in a few days when the easily oxidisable reductic acid was kept in aqueous solution at room temperature.

Furfural. It was electrophoretically shown to be indistinguishable from an authentic sample and characterised as its 2,4-dinitro-phenylhydrazone, m.p. and mixed m.p. 228°.

Compound B. This was not identified. It distilled at 85—90°/ca 0.06 mm; $R_F = 0.56$ (solvent A), $M_V = 1.70$ (buffer C), $M_V = 0$ (buffer F). It reduced alkaline silver solution immediately, gave on orange colour with spray reagent *b* and pink colour with spray reagent *c*. It gave an orange precipitate immediately with 2,4-dihitrophenylhydrazine. It did not give reductic acid on acid treatment.

D-*Ribulose* (V). The amorphous compound, $[\alpha]_D^{24}$ ca. −15°, (*c*, 0.5 in water), paper chromatographically and electrophoretically indinstinguishable from authentic material. The gray colour with spray reagent *c* was particularly characteristic and sensitive. Part of the product was converted to the *o*-nitrophenylhydrazone, m.p. and mixed m.p. 167—168°. Part of the product was reduced with borohydride, deionised and acetylated (acetic anhydride-pyridine). The two acetates obtained were indistinguishable from the authentic acetates of ribitol and D-arabitol by paper chromatography using the method of Wickberg [9] with formamide + dimethyl formamide as stationary phase and eluting with light petroleum (b.p. 60—70°).

D-*Glucosone* (IV). The amorphous compound was paper chromatographically and electrophoretically (buffer C; M_V = 1.24) indistinguishable from authentic D-glucosone. It was characterised by the formation at room temperature of D-glucose phenylosazone, m.p. and mixed m.p. 205–207°.

Reaction of methyl β-D-3-oxo-glucopyranoside (II) with lime-water and with sodium hydroxide

Two series of experiments were made on the reactions of II (50 mg) in lime-water (25 ml, 0.018 N) and in aqueous sodium hydroxide solution (25 ml, 0.018 N) at 23°. At intervals the change in optical rotation was determined and samples were withdrawn, acidified with Amberlite IR-120 and examined by paper chromatography and electrophoresis; a semi-quantitative estimation of the amount of unreacted II present was also made using the method previously[1] described. The results are shown in Fig. 2. Oxygen was removed from the basic solutions by bubbling nitrogen through the solutions, but later experiments showed that degradation proceeded at the same rate without this treatment.

Reaction of methyl β-D-2-oxo-glucopyranoside (I) and methyl β-D-3-oxo-glucopyranoside (II) at pH 7

Methyl β-3-oxo-glucoside (II, 500 mg) in 0.2 N phosphate buffer (50 ml) was kept in a water bath at 100° for 30 min. A yellow colour appeared after a few minutes and gradually increased in strength. After cooling the solution was stirred with Amberlite IR-120 (H+) and then with Amberlite IR-4B (free base), the pH was adjusted to 5 and the solution was concentrated to a small volume and separated on Whatman No. 31 paper (solvent A). The neutral product (240 mg) gave unreacted II (85 mg), 1,5-anhydro-2,3-dioxo-D-glucitol (VI) (65 mg) and the unidentified crystalline compound previously[1] obtained from the alkaline treatment of II (5 mg), m.p. and mixed m.p. 156–158°. Traces of I were detected but no ribulose was present. The acids were recovered as the ammonium salts from the Amberlite IR-4B resin; electrophoresis showed a similar pattern to that given by the corresponding product from the alkaline treatment.

Methyl β-2-oxo-glucoside (I, 250 mg) was treated in the same way as above for 10 min. After deionisation the product was fractionated on a carbon-Celite column (2.5 × 22 cm) using gradient elution and gave unreacted I (20 mg), D-ribulose (15 mg) and II (70 mg); other minor compounds were also present. D-Ribulose was characterised as above. The methyl β-3-oxo-glucoside (II), m.p. and mixed m.p. 130–131°, $[\alpha]_D^{22}$ −61° (*c*, 2 in water) was chromatographically and electrophoretically identical with an authentic sample.

Treatment of methyl β-D-2-oxo-glucopyranoside (I) and methyl β-D-3-oxo-glucopyranoside (II) at pH 4

Two series of experiments were made with I and II, respectively. Solutions of the substance in 0.2 N acetate buffer (0.5 ml, 1 %) in sealed glass tubes were kept in a water bath at 100° for different times. After cooling. the samples were diluted with water (1 ml), excess Amberlite IR-120 (H+) was added and semi-quantitative determinations were made of unreacted I and II present as described previously. The results are shown in Fig. 2.

In one experiment, II (250 mg) in 0.2 N acetate buffer (25 ml) was kept in a water bath at 100° for 45 min. After cooling and deionisation the product was separated on Whatman No. 31 paper (solvent A) giving: unreacted II (90 mg), VI (15 mg); the unidentified crystalline product (m.p. 156–158°) mentioned above was detected by paper chromatography. A part of VI was reduced with borohydride and with Raney nickel and the four 1,5-anhydroglycitols obtained were characterised by paper electrophoresis (buffer D) as described previously[1].

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