831. *Catalytic Oxidation of Carbohydrates. Some Properties of Potassium α-D-Glucopyranuronate 1-(Dipotassium Phosphate).*

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The actions of alkaline intestinal phosphatase and potato phosphorylase on potassium α-D-glucopyranuronate 1-(dipotassium phosphate) have been investigated. Acid-hydrolysis constants have been determined of both this sugar phosphate and α-D-glucopyranose 1-(dipotassium phosphate). The platinum–O_2 method of oxidation is shown to be inhibited by ammonium carbonate and glycine and to be inapplicable to certain nitrogen-containing carbohydrates.

Gaseous oxygen in the presence of platinum black suspended in a neutral or slightly alkaline solution has been widely applied to the oxidation of carbohydrates.[1-5] The use of a platinum catalyst in the synthesis of potassium α-D-glucopyranuronate 1-(dipotassium phosphate) [GA-1-P], a compound of potential interest for biosynthesis, was described independently by Barker, Bourne, and Stacey [4] and by Marsh.[5] We now wish to report some properties of this phosphate and the inhibition of platinum–oxygen oxidation by certain nitrogenous compounds.

Crystalline potassium α-D-glucopyranuronate 1-(dipotassium phosphate) dihydrate was isolated in 45% yield from α-D-glucopyranose 1-(dipotassium phosphate) and showed optimum stability at pH 8. Removal of potassium ions on an ion-exchange resin, and hydrolysis of the free acid with formic acid, readily gave α-D-glucurono-γ-lactone. Alkaline intestinal phosphatase [6] completely hydrolysed both this phosphate and α-D-glucose 1-(dipotassium phosphate) [G-1-P]. The addition of disodium hydrogen phoshate retarded hydrolysis but did not prevent its going to completion (Table 1). By hydrolysis of the uronate phosphate GA-1-P with phosphatase, glucurone was obtained in 81% yield corresponding to an overall yield of 36% from glucose 1-phosphate.

The fact that potato phosphorylase [7] synthesised amylose from the glucose phosphate G-1-P prompted us to investigate the possibility of synthesising a polyuronide by the action of phosphorylase on the uronate phosphate GA-1-P. This enzyme, to which ammonium molybdate was added to inhibit the phosphatase impurity, had however no action on the uronate phosphate but from the glucose phosphate 82% of the theoretical amount of phosphorus was liberated, and a substance which gave a blue stain with iodine was formed in the digest.

The stabilities of the two phosphates in acid solution were compared. A pH of 1·5 or 2·0 was found to give a convenient rate of hydrolysis at 61°. Log $(a - x)$ was linear with respect to t (where a = initial concentration and x = amount of substance hydrolysed at time t) in each case, showing that the reactions were of the first order. From the equation for a first-order reaction $t = \frac{1}{k}\ln a - \frac{1}{k}\ln (a - x)$ the constant k was calculated:

$$\text{G-1-P (pH 1·5)}, k = 1{\cdot}35 \times 10^{-4}\ \text{sec.}^{-1}$$
$$\text{G-1-P (pH 2·0)}, k = 3{\cdot}65 \times 10^{-5}\ \text{sec.}^{-1}$$
$$\text{GA-1-P (pH 1·5)}, k = 6{\cdot}03 \times 10^{-6}\ \text{sec.}^{-1}$$
$$\text{GA-1-P (pH 2·0)}, k = 3{\cdot}86 \times 10^{-6}\ \text{sec.}^{-1}$$

[1] Mehltretter, Rist, and Alexander, U.S.P. 2,472,168.
[2] Mehltretter, Alexander, Mellies, and Rist, *J. Amer. Chem. Soc.*, 1951, **73**, 2424.
[3] Overend, Shafizadeh, Stacey, and Vaughan, *J.*, 1954, 3633.
[4] Barker, Bourne, and Stacey, *Chem. and Ind.*, 1951, 970.
[5] Marsh, *J.*, 1952, 1578.
[6] Schmidt and Thännhauser, *J. Biol. Chem.*, 1943, **149**, 369.
[7] Barker, Bourne, Wilkinson, and Peat, *J.*, 1950, 84.

Thus the introduction of a carboxylic acid group at $C_{(6)}$ increases the stability towards acid by a factor of 10. This feature is probably related to the well-known acid stability of aldobiuronic acids.[8]

Two attempts to oxidise α-D-glucosylamine by oxygen and a platinum catalyst were unsuccessful. In one attempt, in which the pH value of the reaction mixture fell to 4·83, the α-D-glucosylamine was completely hydrolysed to glucose. This was prevented by first passing the oxygen through a wash-bottle containing dilute ammonia. Although the pH was thus maintained between 8 and 9 and only very slight hydrolysis of the glucosylamine occurred, no oxidation products could be detected. This was not due to the high pH value since it has been found [9] that the catalyst functioned efficiently over the range pH 3—10. Also in the previous experiment, in which glucose had been formed by hydrolysis of the glucosylamine, no saccharic acid could be detected by paper chromatographic analysis. D. J. C. Wood (personal communication) also failed to oxidise methyl *N*-benzyloxycarbonyl-α-D-glucosaminide and methyl *N*-acetyl-α-D-glucosaminide by the same method and it seemed probable that nitrogenous substances in some way inhibited the oxidation.

To investigate this, attempts were made to oxidise methyl α-D-glucopyranoside in the presence and absence of ammonium carbonate or glycine. No oxidation was observed in the presence of either of these substances, and when ammonium carbonate was added to partly oxidised methyl α-D-glucopyranoside there was no further change in the optical rotation of the solution (Table 4). The catalyst which had been used for the attempted oxidation in the presence of ammonium carbonate was recovered and washed with hot water, before being incorporated into a fresh solution of methyl α-D-glucopyranoside. Initially (first 1·5 hr.) little reaction occurred but after 6 hr. the Tollens test [10] became positive and there was a definite decrease in optical rotation and pH. These results indicated that nitrogenous compounds were in some way adsorbed on to the catalyst and rendered it inactive, and that the adsorbed substance was only removed slowly. This method of oxidation therefore seems to be inapplicable to nitrogen-containing carbohydrates.

Finally to confirm that this method results in oxidation of primary, and not secondary, alcohol groups dulcitol was oxidised to mucic acid (yield 57·8%) which was further characterised by conversion into dimethyl 2 : 3-4 : 5-di-*O*-methylenemucate.

Experimental

Potassium α-D-Glucopyranuronate 1-(Dipotassium Phosphate).—α-D-Glucose 1-(dipotassium phosphate) dihydrate (5·0 g.) was oxidised with a stream of purified oxygen in the presence of a platinum–charcoal catalyst [9] (1·6 g.) suspended in water (200 c.c.) at 61° for 92 hr. After removal of the catalyst, the solution (pH 5·60) was made slightly alkaline with potassium hydroxide and concentrated to *ca.* 30 c.c., and a syrup was precipitated by addition of methanol (150 c.c.). After purification by eight reprecipitations from aqueous methanol, potassium α-D-glucopyranuronate 1-(dipotassium phosphate) dihydrate (2·6 g.) crystallised in rosettes of fine needles, $[\alpha]_D^{19}$ +53·6° (*c* 1·54 in water) (Found: C, 16·5; H, 3·0; P, 7·3; K, 27·9. Calc. for $C_6H_{12}O_{12}PK_3$: C, 17·0; H, 2·8; P, 7·3; K, 27·6%).

Conversion into Glucurone by Acid-hydrolysis.—Potassium α-D-glucopyranuronate 1-(dipotassium phosphate) dihydrate (0·55 g.) in water (5 c.c.) was passed through a column of ion-exchange resin (Zeo-Karb 215) to give α-D-glucopyranuronic acid 1-(dihydrogen phosphate) which, after concentration to a syrup (0·37 g.), was hydrolysed with 90% formic acid for 4 hr. at 100°. Removal of formic acid by distillation *in vacuo*, decolorisation with charcoal, and crystallisation from methanol of the syrup obtained after concentration gave α-D-glucurono-γ-lactone (0·21 g.), m. p. and mixed m. p. 170—172°.

[8] O'Dwyer, *Biochem. J.*, 1934, **28**, 2116.
[9] Merck and Co., U.S.P. 2,483,251.
[10] Tollens, *Ber.*, 1908, **41**, 1788.

Conversion into Glucurone by Alkaline Phosphatase.—A digest was prepared containing potassium α-D-glucopyranuronate 1-(dipotassium phosphate) dihydrate (0·5 g.), 0·2M-borate buffer (pH 9·24; 10 c.c.), and a solution (10 c.c.) of alkaline phosphatase (20 mg.) in a total volume of 30 c.c. The alkaline phosphatase had been extracted from the intestines of ten calves by Schmidt and Thannhäuser's method [6] and liberated 217 μg. of *p*-nitrophenol per minute per mg. of nitrogen in the preparation under the standard conditions described by Axelrod.[11] The digest was incubated at 37°, and the progress of the hydrolysis followed by the removal, at intervals, of aliquot portions for the determination of inorganic phosphate.[12] After 4 hr., when the degree of hydrolysis was 99·3%, enzyme action was arrested by heating the solution at 100° for 5 min. Most of the liberated phosphate was removed by precipitation with magnesia mixture, and the filtrate concentrated *in vacuo* to a syrup which was extracted with methanol. After evaporation the methanol extract gave crystalline α-D-glucurono-γ-lactone (0·18 g.), m. p. and mixed m. p. 170—171°.

Effect of Phosphate on the Enzymic Hydrolysis of the Two Phosphates by Alkaline Phosphatase.—Three digests, containing 0·01M-α-D-glucopyranuronate 1-phosphate (2 c.c.), 0·2M-borate buffer (pH 9·26, 2 c.c.), and a solution (2 c.c.) of alkaline phosphatase (0·2 mg.) were prepared. In the second and third of these digests (total volume, 8 c.c.), 0·00603M-disodium hydrogen phosphate (1 c.c. and 2 c.c. respectively) was incorporated. Three similar digests, in which the uronate phosphate was replaced by α-D-glucose 1-phosphate, were also prepared and all were incubated at 37°. Aliquot portions were removed at intervals for the determination of the degree of hydrolysis. The results are given in Table 1.

TABLE 1. *Enzymic hydrolysis of* α-D-*glucose* 1-*phosphate* (G-1-P) *and* α-D-*glucopyruronate* 1-*phosphate* (GA-1-P) *in the presence of phosphate.*

Time (hr.)	No added phosphate	Phosphate 1 c.c.	Phosphate 2 c.c.
	Hydrolysis (%) *of* GA-1-P		
1·5	99·8	99·5	96·4
3·0	100·2	101·0	98·5
6·0	99·8	101·0	99·3
24·0	101·0	100·5	100·3
	Hydrolysis (%) *of* G-1-P		
1·5	93·5	89·8	84·2
5·0	101·0	101·0	98·4
22·0	100·2	101·0	100·2

TABLE 2. *Action of phosphorylase.*

	G-1-P		GA-1-P
Time (hr.)	A.V. (680 mμ)	Inorg. P liberated (%)	Inorg. P liberated (%)
1	0·013	5·3	—
20	0·168	78·7	—
92	0·292	82·0	0·5

TABLE 3. *Rates of hydrolysis by acid.*

	Hydrol. (%) of G-1-P at		Hydrol. (%) of GA-1-P at	
Time (hr.)	pH 1·50	pH 2·00	pH 1·50	pH 2·00
0·5	20·4	7·3	—	—
1·0	36·4	12·2	1·3	1·3
1·5	49·5	17·9	2·4	2·2
2·5	70·2	31·1	4·5	2·7
5·0	91·4	47·7	9·6	6·9
22·0	100·2	97·3	38·4	26·0
29·0	—	99·8	46·7	33·6
pH after 24 hr.	1·60	2·05	1·53	2·04

These phosphates were also hydrolysed with phosphatase in the presence of an approximately equimolecular concentration of glucose (for G-1-P) and glucurone (for GA-1-P). In both cases the substrate was hydrolysed completely at almost the same rate as in the absence of added glucose or glucurone.

[11] Axelrod, *J. Biol. Chem.*, 1947, **167**, 57.
[12] Allen, *Biochem. J.*, 1940, **34**, 858.

Action of Phosphorylase on the Two Phosphates.—A digest, containing potato phosphorylase solution (1 c.c.), 0·5M-citrate buffer (pH 6·0; 0·5 c.c.), 0·1M-α-D-glucose 1-phosphate (1 c.c.), 8% ammonium molybate solution (1 c.c.) and water (0·5 c.c.) was prepared. A similar digest containing α-D-glucopyruronate 1-phosphate in the place of the glucose derivative, and control digests, containing no phosphorylase, were also prepared. The four digests were incubated at 37°, and aliquot portions removed at intervals, for the determination of inorganic phosphate [12] and the iodine staining power, after inactivation of the phosphorylase with trichloroacetic acid. In the case of the control digests an equivalent amount of phosphorylase solution was added after the addition of trichloroacetic acid. The results are shown in Table 2.

Relative Stability of the Two Phosphates to Acid-hydrolysis.—Two solutions of α-D-glucose 1-(dipotassium phosphate) (20 mg.) were adjusted with hydrochloric acid to pH 1·50 and pH 2·00 respectively, in final volumes of 20 c.c. Similar solutions containing potassium α-D-glucopyranuronate 1-(dipotassium phosphate) were also prepared and all four, in glass-stoppered tubes, placed in a thermostatically controlled water-bath held at 61°. The degree of hydrolysis, measured at intervals by the withdrawal of aliquot portions for inorganic phosphate determination, is shown in Table 3.

Attempted Oxidation of α-D-Glucopyranosylamine.—α-D-Glucopyranosylamine (3 g.) was dissolved in water (200 c.c.), sodium hydrogen carbonate (1·2 mol.), and platinum–charcoal (1·0 g.) were added, and a stream of purified oxygen was passed through the suspension at 61°. During the reaction the pH fell from 8·61 to 4·83, and paper chromatography of the products showed that the α-D-glucopyranosylamine had been hydrolysed completely to glucose. No sugar, other than glucose, could be detected and the solution gave a negative Tollens test [10] for uronic acid.

The experimental procedure was modified by passing the oxygen through a dilute solution of ammonia before its injection into the reaction mixture. The pH value then increased from 8·10 to 9·04 during the reaction, and remained at this value. After 90 hr. paper chromatography showed slight hydrolysis of α-D-glucopyranosylamine to di-α-D-glucopyranosylamine but no other sugars could be detected, and the solution gave a negative test for uronic acids.

Oxidation of Methyl α-D-Glucopyranoside in the Presence of Nitrogenous Compounds.—Four solutions, each containing methyl α-D-glucopyranoside (0·49 g.) and sodium hydrogen carbonate (0·25 g.) in water (50 c.c.), were prepared, and freshly prepared catalyst (0·35 g.) was added to three of these (A, B, and C). Ammonium carbonate (0·14 g.) was incorporated into two of these solutions as follows: (A) control, no ammonium carbonate added, (B) ammonium carbonate

TABLE 4. *Oxidation of methyl α-D-glucopyranoside in the presence of ammonium carbonate.*

Time	A		B		C		D	
(hr.)	pH	$[\alpha]_D^{17}$	pH	$[\alpha]_D^{17}$	pH	$[\alpha]_D^{17}$	pH	$[\alpha]_D^{17}$
0	8·66	+157°	8·97	+157°	8·66	+157°	8·63	+159°
0·25	7·14	122	9·33	157	7·04	118	9·02	151
0·75	6·31	104	9·52	157	6·13 *	100	9·14 †	151
1·0	—	—	—	—	8·89	90	—	—
1·5	5·57	86	9·68	151	9·08	88	9·16 †	149
3·0	5·25	75	9·74	147	9·21	90	—	—
5·0	5·08 *	73	9·75 †	151	9·24	90	—	—
6·0	—	—	—	—	—	—	8·13 *	112

* Uronic acid test positive. † Uronic acid test negative.

added at commencement of oxidation, (C) ammonium carbonate added after 1 hr. The three solutions A, B, and C were oxidised as previously described and the progress of the reaction in each case was followed by changes in pH value and optical rotation (Table 4). The catalyst from solution B was recovered, washed, and dried, and added to the fourth solution (D) which was then oxidised in the usual way.

When the experiment was repeated with glycine in place of ammonium carbonate, similar results were obtained.

Oxidation of Dulcitol.—Dulcitol (2·7 g.) was dissolved in a solution of sodium hydrogen carbonate (1·5 g.) in water (100 c.c.), and oxidised in a stream of oxygen at a platinum–charcoal catalyst [9] (1·4 g.). During the reaction the pH fell from 8·46 to 5·00; at this point a further amount (1·3 g.) of sodium hydrogen carbonate was added, the pH of the solution increasing to

7·6. The reaction was continued until a constant pH (6·4) was reached after 15 hr. The suspension was made alkaline to phenolphthalein with sodium hydroxide, and the catalyst was removed and washed with hot distilled water. The filtrate and washings were concentrated *in vacuo* and made acid with hydrochloric acid and, the precipitated solid was removed. After purification, mucic acid (1·8 g., 57·8%), m. p. and mixed m. p. 213°, was isolated. This was further characterised by conversion into dimethyl 2 : 3·4 : 5-di-*O*-methylenemucate, m. p. and mixed m. p. 101°.

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