263. *The Oxidation of Aldoses by Hypoiodous Acid. Part II.*

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It was recently shown that the active oxidising agent in the reaction between aldoses and alkaline solutions of iodine is hypoiodous acid. This reaction has now been further investigated, and the complete mechanism in alkaline solution is shown to be the oxidation of aldopyranose by hypoiodous acid to aldonic acid. The reaction is shown to be of second order. It is shown that glucono-δ-lactone is produced as an intermediate when the reaction is carried out below pH 7, but this is not necessarily the case in alkaline solution.

Some further steric effects on the rate of oxidation are reported and discussed.

We have shown (*J.*, 1948, 810) that when a number of aldopentoses and aldohexoses are oxidised by alkaline solutions of iodine, the active oxidising agent is un-ionised hypoiodous acid. In the course of the oxidation of aldoses by hypoiodous acid, two reactions occur simultaneously, viz.,

$$3HIO \longrightarrow IO_3^- + 2I^- + 3H^+ \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (A)$$

$$C_6H_{12}O_6 + HIO \longrightarrow C_6H_{12}O_7 + I^- + H^+ \quad . \quad . \quad . \quad . \quad . \quad . \quad (B)$$

Reaction (A) has been investigated by several workers who have shown that it is of second order (Schwicker, *Chem.-Ztg.*, 1891, **15**, 630; Giuseppe d'Este, *Boll. Chim. Farm.*, 1939, **78**, 117; Skrabal, *Monatsh.*, 1907, **28**, 319; 1909, **30**, 51; *Chem.-Ztg.*, 1905, **29**, 550). We have investigated the order of reaction (B) by determining the relative rates of oxidation of glucose at two different initial concentrations, account being taken of the simultaneous formation of iodate. Starting with equal concentrations (a) of glucose and of hypoiodous acid in the reaction mixture, the time (t) for a given fraction of the oxidation to occur was determined. The apparent order of the reaction was then calculated from the formula $n = 1 + (\log t_1/t_2)/\log (a_2/a_1)$, where t_1 and t_2 are the times for a given fraction of the reaction to occur for initial concentrations a_1 and a_2, respectively. The results are summarised in Table I.

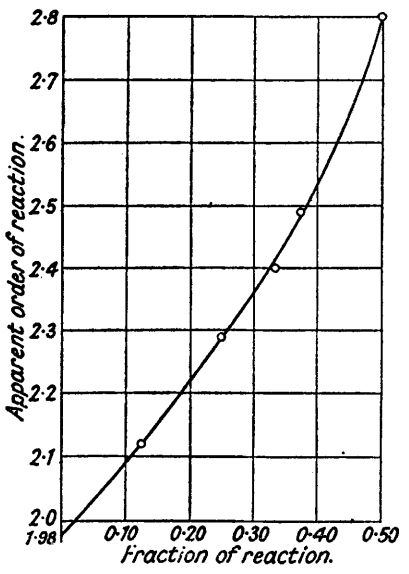
TABLE I.

Times of fractional change and apparent order of reaction.

Initial concns.: $a_1 = 0{\cdot}1667$M; $a_2 = 0{\cdot}1000$M. pH = 11·15.

Fraction of reaction (x)	0·125	0·250	0·333	0·375	0·500
t_1 (secs.)	2·6	5·0	6·7	7·9	14·0
t_2 (secs.)	4·6	9·7	13·8	17·0	35·0
n	2·12	2·29	2·40	2·49	2·80

The marked drift in the values of n so obtained is due to the fact that the formula given above is not strictly applicable. This formula applies in the case where both reactants are at equal concentrations at each stage during the reaction. However, in this particular case, the simultaneous formation of iodic acid by reaction (A) results in the concentration of hypoiodous acid becoming progressively lower than that of glucose at any given time during the reaction. To eliminate this factor values of n were plotted against values of x (see fig.), and the plot extrapolated to $x = 0$, which gives the value of n as 1·98. Hence it is clear that the reaction is of second order within the limits of experimental error.



The product of the oxidation of glucose in alkaline solution has been investigated by an ion-exchange method, and shown to be gluconic acid. The possibility that oxidation proceeds further than this stage to give saccharic acid has been eliminated, and it has been found that no degradation of the glucose molecule occurs. Experiments made on the rate of oxidation of both mannitol and gluconic acid under similar conditions to those employed in the oxidation of aldoses have been performed and in neither case was oxidation observed. Thus it is clear that hypoiodous acid does not attack either primary or secondary alcohol groups in a normal aldopyranose.

Since Isbell (*J. Res. Nat. Bur. Stand.*, 1932, R.P. 441; 1933, R.P. 534) has shown that the oxidation of glucose by bromine at pH 6·4 leads directly to glucono-δ-lactone, it is possible that oxidation by hypoiodous acid proceeds through a similar intermediate. We have found that the oxidation of glucose by hypoiodous acid at the same pH (6·4) follows a similar course. However, it does not necessarily follow that when the oxidation is carried out in alkaline solution, this same mechanism is operative, and attempts to detect any intermediate lactone at the normal pH of oxidation, *i.e.*, pH 11·35, have so far been unsuccessful. At such a pH, any lactone formed would be immediately saponified.

In our previous paper (*loc. cit.*) some effects of configuration of the carbohydrate chain in several aldohexoses and aldopentoses on the rate of oxidation by hypoiodous acid were reported. Experiments on the oxidation of further aldose derivatives, namely L-rhamnose, 2 : 3 : 4 : 6-tetramethyl glucose, α-methylglucoside, lactose, cellobiose, and melibiose have provided further information on these effects. Rhamnose is oxidised at the same rate as mannose; 2 : 3 : 4 : 6-tetramethyl glucose is oxidised at almost the same rate as glucose; α-methylglucoside is not attacked, and the disaccharides of aldopyranoside–glucose type are oxidised at a rate distinct from that of any monosaccharide, and more slowly than glucose. The active oxidising agent

in all cases was shown to be hypoiodous acid. The relative rates of oxidation are shown in Table II, those found by Myrbäck (see Ingles and Israel, *loc. cit.*) being added for comparison.

TABLE II.

Sugar.	Relative rate.	Relative rate (Myrbäck).	Sugar.	Relative rate.	Relative rate (Myrbäck).
D-Glucose	1·00	1·00	Cellobiose	0·82	0·76
2 : 3 : 4 : 6-Tetramethyl glucose	0·95	0·68	Lactose	0·81	0·76
D-Mannose	0·24	0·24	Melibiose	0·78	0·78
L-Rhamnose	0·26	0·20	Maltose	—	0·79

(α-Methylglucoside is not attacked.)

It is apparent from these and from our previous results that: (i) Similar configurations on carbon atoms 2, 3, and 4 give rise to the same rate of oxidation. (ii) Replacement of $CH_2 \cdot OH$ in an aldose by H or by CH_3 has little or no influence on the rate of oxidation. (iii) Methylation of the hydroxyl groups on carbon atoms 2, 3, 4, and 6 of glucose has only slight effect on the rate of oxidation of this sugar (this conflicts with Myrbäck's finding). (iv) The presence of an aldopyranoside linkage depresses the rate of oxidation of glucose, but this rate remains independent of the nature of the aldopyranoside present. (v) Methylation on carbon atom 1 protects an aldose from attack by hypoiodous acid. This finding is in agreement with (iv) above, that pyranosidic groups are not attacked.

EXPERIMENTAL.

Materials.—All sugars used were checked for purity by measurement of their specific rotations, the values found being as follows: L-rhamnose (hydrate), $[\alpha]_D^{20°}$ + 8·13° (Bates, "Polarimetry and Saccharimetry of the Sugars," U. S. Dept. of Commerce, 1942, + 8·20°); 2 : 3 : 4 : 6-tetramethyl glucose, $[\alpha]_D^{20°}$ + 83·4° (Bates, + 83·3°); lactose (hydrate), $[\alpha]_D^{20°}$ + 52·5° (Bates, + 52·6°); cellobiose, $[\alpha]_D^{20°}$ + 33·8° (Bates, + 34·6°); melibiose, $[\alpha]_D^{20°}$ + 129·0° (Bates, + 129·5°); α-methylglucoside, $[\alpha]_D^{20°}$ + 157·5°, m. p. 166° (Bates, + 158·0°, m. p. 166°). All other materials used were B.D.H. "AnalaR" reagents.

Kinetic Methods and Buffer Solutions.—These have been described in our previous paper (*loc. cit.*).

Determination of the Order of Reaction.—The experimental technique was similar to that previously employed, the initial concentration of iodine, however, being equimolecular with that of glucose.

Direct acidification of a reaction mixture liberates iodine proportional to iodate and hypoiodite, and titration with sodium thiosulphate then gives the sum of these, together with free iodine and tri-iodide (T).

The amount of hypoiodous acid removed by reaction with glucose at any instant is thus given by $T_0 - T_t$, where T_0 is the titre at zero time, and this value is also proportional to the amount of glucose oxidised at any instant. Experimental results are given in Table III. Plotting of these data enables

TABLE III.

Amount of glucose oxidised ($T_0 - T_t$) (titres against 0·01N-$Na_2S_2O_3$).

	Time (secs.).									
Concn. ...	0	3	4	5	7	9	10	15	20	25	30
M/10	0·00	2·20	3·00	3·54	—	6·16	6·74	9·20	10·50	—	12·44
M/6	0·00	4·00	—	—	9·60	—	·12·20	14·25	15·05	16·80	17·25

times of fractional change to be read, and these have been shown in Table I. Hence, as shown in the discussion, the order of reaction was determined.

Determination of Gluconic Acid.—A solution of glucose in 0·1M-sodium carbonate was treated with an amount of solution of iodine in potassium iodide in excess of that theoretically required for the complete oxidation of the glucose, and the reaction was allowed to proceed to completion. The resultant solution was then passed through a column containing a cation exchange resin (Zeo-Karb 215) whereby the cations other than hydrogen ion were removed, and subsequently through a column containing an anion exchange resin (De-Acidite B). In the latter column, iodide ions were adsorbed preferentially to the anions of any organic acids present or to carbonate ion. The resultant dilute solution had a pH in the region 5·3—5·6. This solution was then evaporated to small volume (whereby any carbonic acid present was removed), and was then treated with a large excess of absolute alcohol, whereupon a semi-colloidal suspension separated. This suspension was separated by centrifuging and was shown to be gluconic acid by the method of Bennet-Clark (*Biochem. J.*, 1934, **28**, 45).

Following this method a 9·0 mg. sample of the alcohol-insoluble extract from the ion-exchange columns was dissolved in 8 ml. of water; 5 ml. of saturated ammonium molybdate and 2 ml. of glacial acetic acid were added, and the mixture allowed to stand for 3 hours in the dark, the rotation being then measured. The amount of gluconic acid in the sample was found to be 8·7 ± 0·1 mg. The alcohol extract was examined for organic materials with negative results.

By submitting a 280 mg. sample of glucose to oxidation by excess of hypoiodous acid, and examining the solution from the anion exchange column for gluconic acid by the same method, 273 mg. of gluconic acid were found, *i.e.*, 90% of theory.

Investigation for Possible Intermediates.—All attempts to identify an intermediate lactone when oxidation is carried out at high pH proved negative. However, working in the range of maximum lactone stability, *i.e.*, slightly on the acid side of the neutral point, an intermediate δ-lactone was detected. A comparatively concentrated solution (~ 0·15N) of hypoiodous acid was prepared by a slight modification of Koene's method (*Pogg. Ann.*, 1844, **56**, 302): 0·4 g. iodine was dissolved in 50 ml. of 95% alcohol, and the solution was shaken for 45 secs. with 0·8 g. of mercuric oxide to remove the hydriodic acid formed. The solution was rapidly filtered (30 secs.) through a layer of mercuric oxide supported on asbestos. The alcoholic solution of hypoiodous acid so obtained was straw-yellow in colour, and was used immediately to forestall major decomposition. 5 Ml. of this solution were added to 25 ml. of glucose solution containing 0·1125 g. of glucose, buffered to a pH of 6·4 by means of a suspension of barium carbonate, through which carbon dioxide was bubbled. After 10 minutes, any excess of iodine was removed by shaking with phellandrene, which simultaneously floats off the barium carbonate (Polya and Ingles, *J. Proc. Aust. Chem. Inst.*, 1947, **14**, 519). The solution was again shaken with mercuric oxide to remove any traces of hydriodic acid or iodic acid still present, and filtered. The pH of the resultant solution was followed over a period of time by means of a Coleman pH-meter, and was observed to fall progressively from approximately 6·1, immediately after the reaction had been stopped, to a constant valve of 2·8, some 2 hours later. This must indicate δ-lactone formation, and its subsequent transformation into gluconic acid (Isbell, *loc. cit.*, 1933). May, Weisberg, and Herrick (*J. Wash. Acad. Sci.*, 1929, **19**, 443) have found the acid dissociation constant of gluconic acid to be $1{\cdot}65 \times 10^{-4}$. On this basis, the pH of a solution containing 0·1225 g. of gluconic acid in 30 ml. (the theoretical amount obtained from 0·1125 g. of glucose) would be 2·70, in good agreement with the value observed.

Other Oxidation Experiments.—Mannitol, gluconic acid, and α-methylglucoside, submitted to the usual oxidation procedure, showed no change of titre after 15 minutes of reaction time, except in the case of α-methylglucoside at very high pH. This was probably due to slight hydrolysis. The results are shown :

α-Methylglucoside (titres against 0·02N-$Na_2S_2O_3$).

	pH. 11·20				pH. 12·80			
Time (mins.).	0	1	3	10	0	1	3	10
Titre.	13·20	13·18	13·18	13·18	13·20	13·05	12·95	12·75

Further confirmation that un-ionised hypoiodous acid is the active oxidising agent when aldoses are oxidised by alkaline solutions of iodine has been obtained by measurement of the oxidation rate of glucose in buffer solutions containing veronal of lower pH than those previously used. The theoretical values for the time of quarter change have been obtained from the theoretical curve of concentrations of un-ionised hypoiodous acid given in our previous paper (*loc. cit.*). The results are as follows :

Time of quarter change (secs.).

pH.	Calc.	Obs.	pH.	Calc.	Obs.
9·60	55	53	9·45	88	85

In addition, three disaccharides, 2 : 3 : 4 : 6-tetramethylglucose, and rhamnose were submitted to oxidation by hypoiodous acid. Experimental results are shown below :

Times of quarter change (seconds).

pH.	Lactose.	Melibiose.	Cellobiose.	Rhamnose.	2 : 3 : 4 : 6-Tetramethyl glucose.
9·60	—	—	60	—	—
10·60	7¾	8¼	6¾	—	6½
10·95	—	—	5	17	4¼
11·35	—	—	—	12½	3½
11·60	—	—	4⅓	—	—
11·80	—	—	—	—	4½
11·90	7½	8¼	6¾	—	—
12·00	—	—	—	25	6
12·80	45	47	48½	147	41

From these results the relative rates of oxidation of these sugars, compared with glucose as 1·00, can be readily calculated as in our previous paper.

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