## 162. *The Oxidation of Some Aldoses by Alkaline Solutions of Iodine.*

By O. G. Ingles and G. C. Israel.

The kinetics of the oxidation of two aldopentoses and three aldohexoses by alkaline solutions of iodine of pH varying from 10·0 to 13·5 have been investigated, and the results demonstrate conclusively that the active oxidising agent is hypoiodous acid. The optimum pH for the use of this reaction in the determination of aldoses in solution is shown to be 11·35 at 25°. The configurations of the aldoses examined have some bearing on the rates at which they are oxidised by hypoiodous acid, but the exact relationship has not yet been established.

The method for the determination of aldoses in solution by quantitative oxidation to aldonic acids by alkaline solutions of iodine has been described on many occasions (Romijns, *J. Soc. Chem. Ind.*, 1897, **16**; 765; Bland and Lloyd, *ibid.*, 1914, **33**, 948; Bougault, *Compt. rend.*, 1917, **164**, 1008; Willstätter and Schüdel, *Ber.*, 1918, **51**, 780; Judd, *Biochem. J.*, 1920, **14**, 255; Kolthoff, *Analyst*, 1923, **48**, 386; Hinton and Macara, *ibid.*, 1924, **49**, 2; 1927, **52**, 668; Kline and Acree, *Ind. Eng. Chem. Anal.*, 1930, **2**, 413; Macleod and Robison, *Biochem. J.*, 1929, **23**, 517). It seems to have been assumed always that the iodine first reacts with alkali to form iodide and hypoiodite, and that the latter is the effective oxidising agent. At the same time, varying degrees of alkalinity have been recommended by the different authors and varying conditions have been specified for the oxidation.

In order to obtain more precise data on the optimum conditions for such oxidations, the kinetics of the reaction have been examined. Preliminary experiments had indicated that the oxidising agent effective in the reaction between glucose and an alkaline solution of iodine is free hypoiodous acid. This conclusion has been confirmed in the case of five aldoses—glucose, mannose, galactose, arabinose and xylose—by comparing the rate of oxidation, using buffered solutions of pH ranging from 9·0 to 14·0, with the concentrations of free hypoiodous acid and of hypoiodite ion in those solutions at the start of the reaction. Instead of determining the velocity constant for each reaction studied, the time of quarter-change, $t_{1/4}$, was measured. The reciprocal of this time, which is directly proportional to the velocity constant, was used as a measure of the rate of the reaction.

When the values of $1/t_{1/4}$ were plotted against the pH of the reaction mixtures, the curves obtained were of the same shape as the [HIO]–pH curve but differed markedly from the shape of the [$IO^-$]–pH curve. By choosing suitable scales, the rate curve could be made to coincide almost exactly with the [HIO]–pH curve, as may be seen in Figs. 1, 2, and 3. This leads to the conclusion that the active oxidising agent in each case is un-ionised hypoiodous acid.

For each aldose investigated, the reaction rate conformed to a second-order equation. Further, with glucose as the sugar in the reaction, the effect of adding varying amounts of sodium chloride to the reaction mixture was examined, and no appreciable salt effect was detected. The absence of a salt effect indicates that the reaction does not take place between ionic substances and that at least one of the reactants must be non-ionic. This adds further confirmation to the hypothesis that the reaction takes place directly between the aldose and hypoiodous acid.

Calculations of the velocity constants for the oxidation of each aldose were also made. The values of $k$ so obtained showed a slight drift; the cause of this is under investigation, and it is hoped to discuss the phenomenon in a subsequent paper.

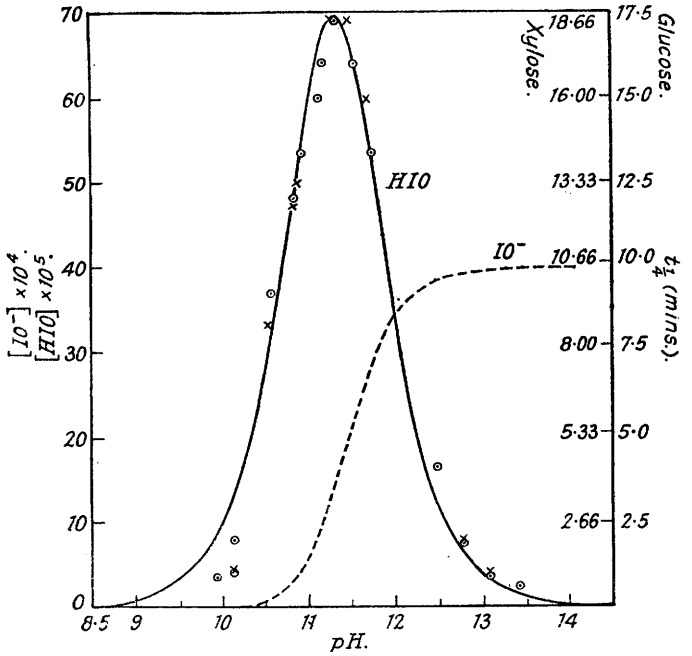
Finally, one reaction mixture in which glucose was used was allowed to come to equilibrium, and the resulting solution was analysed. Gluconic acid was identified as the main constituent of the solution obtained when reaction is complete.

From these observations, it follows that the probable mechanism of the oxidation of aldoses by iodine in alkaline solution follows the equation :

$$R{\cdot}CHO + HIO \longrightarrow R{\cdot}CO_2H + H^+ + I^-,$$

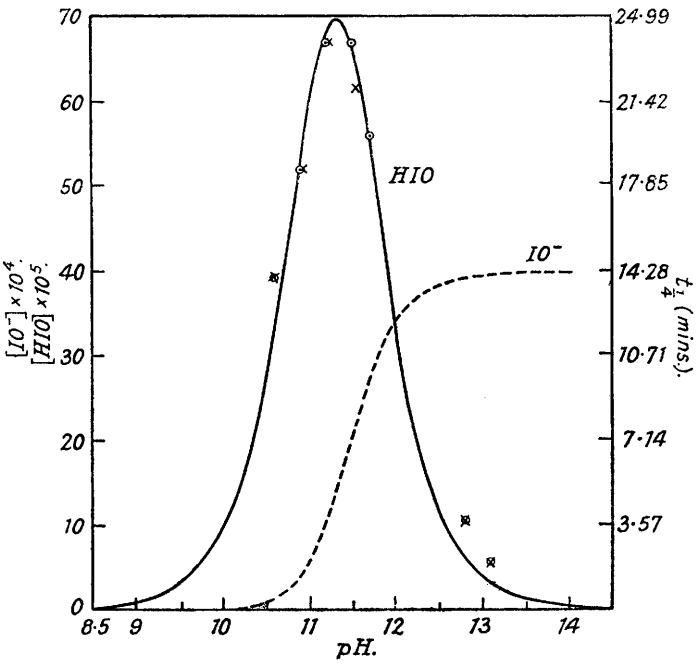
where R is the carbohydrate chain.

FIG. 1.




——— *Theoretical curve for* [HIO]–pH.
- - - - - *Theoretical curve for* [IO⁻]–pH.
⊙ $t_{\frac{1}{4}}$ *(time of quarter change) for glucose.*
× $t_{\frac{1}{4}}$ *(time of quarter change) for xylose.*

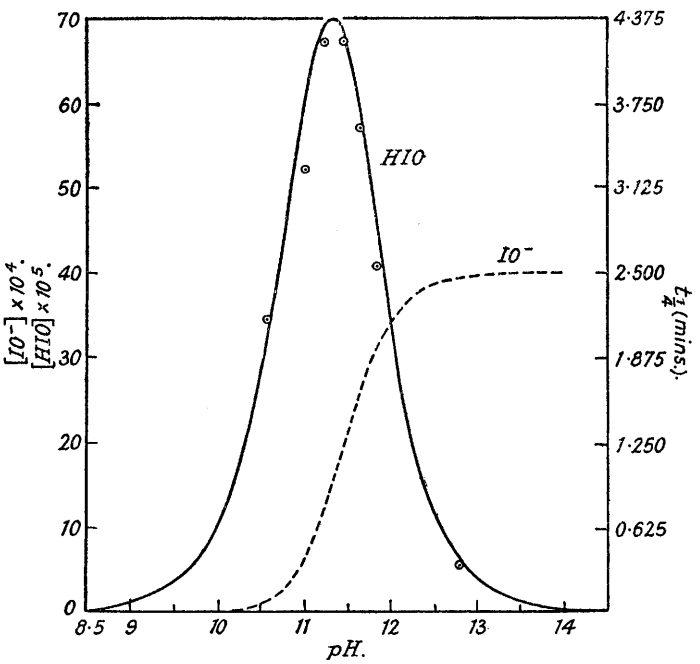
FIG. 2.




——— *Theoretical curve for* [HIO]–pH.
- - - - - *Theoretical curve for* [IO⁻]–pH.
⊙ $t_{\frac{1}{4}}$ *(time of quarter change) for arabinose.*
× $t_{\frac{1}{4}}$ *(time of quarter change) for galactose.*

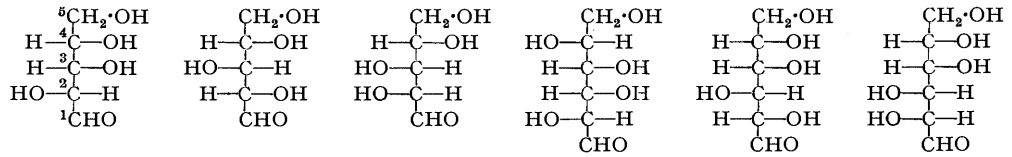
An interesting feature was noticed in regard to the rates at which the various aldoses are oxidised. It was found that xylose and glucose react with hypoiodous acid at almost the same rate, and that the rate of oxidation of galactose or arabinose is about 33⅓% greater. The oxidation rate for mannose was much smaller than that for glucose. Thus, it appears that the configurations of the carbohydrate chains affect the rate at which the sugar is oxidised. Arabinose (I) and galactose (IV) are identical in configuration about carbon atoms 2, 3, and 4.

FIG. 3.




——— *Theoretical curve for* [HIO]–pH.
- - - - - *Theoretical curve for* [IO⁻]–pH.
⊙ $t_{\frac{1}{4}}$ *(time of quarter change) for mannose.*

Xylose (II) and glucose (V) are similarly related, but mannose (VI) corresponds to lyxose (III) which has not yet been investigated.




Our observations confirm certain of the results obtained by Myrbäck (*Svensk Kem. Tids.*, 1939, **51**, 7, 74, 149, 179, 206, 225; 1940, **52**, 21, 200, 293; 1942, **54**, 17), who estimated that the maximum rate of oxidation of aldoses by alkaline solutions of iodine should occur in the neighbourhood of pH 11. Myrbäck calculated the relative rates of oxidation of several aldoses on the basis of glucose = 1. Table I provides a comparison between these values and those obtained during this investigation.

TABLE I.

*Relative Rates of Oxidation of Aldoses.*

| Aldose. | Myrbäck. | I. and I. |
|---|---|---|
| Glucose | 1·00 | 1·00 |
| Xylose | 1·03 | 1·02 |
| Arabinose | 1·30 | 1·36 |
| Galactose | 1·22 | 1·36 |
| Mannose | 0·24 | 0·24 |

These values show a very good agreement in view of the method used by Myrbäck to obtain his results, a method which would not give results of the same degree of accuracy as that employed in this investigation.

## Experimental.

*Materials.*—The materials used were B.D.H. " AnalaR " reagents, with the exception of galactose, xylose, arabinose, and mannose, which were pure samples. All sugars were checked for purity by measurement of their specific rotations, the values found for these substances being glucose, $[\alpha]_D^{20°}$ + 52·70° (International Critical Tables give + 52·83°); galactose, $[\alpha]_D^{20°}$ + 78·80° (I.C.T. + 79·25°); xylose, $[\alpha]_D^{20°}$ + 19·00° (I.C.T. + 19·13°); arabinose, $[\alpha]_D^{20°}$ + 104·5° (I.C.T. + 105·0°); mannose, $[\alpha]_D^{20°}$ + 12·60° (I.C.T. + 14·40°). In addition, the refractive index of a solution of galactose containing 2·3 grams of galactose per 100 ml. of solution was determined and found to be $n_D^{20°}$ 1·3367 (International Critical Tables give 1·3366).

Buffer solutions employed are listed in Table II. Since these buffers were used at a temperature

Table II.

| pH. | Composition of buffer. | Ref. |
|---|---|---|
| 10·17 | 50 ml. 0·1M-$Na_2CO_3$ + 20 ml. 0·1N-HCl made up to 100 ml. | 1 |
| 10·35 | 50 ml. ,, + 15 ml. ,, ,, ,, | 1 |
| 10·55 | 50 ml. ,, + 10 ml. ,, ,, ,, | 1 |
| 10·86 | 50 ml. ,, + 5 ml. ,, ,, ,, | 1 |
| 11·00 | 25 ml. 0·1N-$Na_2HPO_4$ + 4·13 ml. 0·1N-NaOH made to 50 ml. | 2 |
| 11·20 | 25 ml. ,, + 6·00 ml. ,, ,, ,, | 2 |
| 11·40 | 25 ml. ,, + 8·67 ml. ,, ,, ,, | 2 |
| 11·60 | 25 ml. ,, + 12·25 ml. ,, ,, ,, | 2 |
| 11·80 | 25 ml. ,, + 16·65 ml. ,, ,, ,, | 2 |
| 12·50 | 0·05N-NaOH | — |
| 12·80 | 0·1N-NaOH | — |
| 13·10 | 0·2N-NaOH | — |
| 13·45 | 0·5N-NaOH | — |

1. Kolthoff, *J. Biol. Chem.*, 1925, **63**, 135.
2. Kolthoff and Vleeschhouwer, *Biochem. Z.*, 1927, **189**, 191; *Chem. Weekblad*, 1927, **24**, 526.

(25°) differing from that which was specified by Kolthoff, and in view of the inaccuracies entailed in the fine measurement of the volumes required for making up these buffers, the pH of each solution (up to 11·85) was measured by means of a Coleman pH-meter standardised against the standard buffer recommended by Bates, Hamer, Manov, and Acree (*J. Res. Nat. Bur. Stand.*, 1942, R.P. 1495), the pH of which is 11·68 at 25°. The use of sodium hydroxide alone as the buffer substance for the pH range from 12·40 to 13·50 is justified since, in such reaction mixtures, there is such a large excess of sodium hydroxide that the pH remains almost unchanged during the reaction. The pH values of such solutions were calculated from the concentrations of sodium hydroxide using values of the activity coefficients given by MacInnes (" The Principles of Electrochemistry ", New York, 1939, p. 167).

The sodium carbonate–hydrogen carbonate buffer employed gave low values for the rate of oxidation of glucose in the region pH 10·95—11·35. Such low values are probably due to the formation of 1 : 2-α-D-glucose carbonate in such reaction mixtures (cf. Degering, " Outline of the Chemistry of the Carbohydrates ", Cincinatti, 1943, p. 377). When ionisation of the carbonic acid is repressed by lowering the pH, this effect is avoided. For a similar reason an alkaline boric acid buffer (Clark and Lubs, *J. Biol. Chem.*, 1916, **25**, 479) also gave low values for the oxidation rate of glucose, an effect probably due to the formation of 1 : 2-α-D-glucose pyroborate (Myrbäck and Gyllensvärd, *Svensk Kem. Tids.*, 1942, **54**, 17).

*Kinetic Methods.*—For the purpose of these experiments, 3% solutions by weight of each aldose and a M/40-solution of iodine in M/10-potassium iodide were used. These solutions together with the various buffer solutions were immersed in a constant-temperature bath (25·00° ± 0·05°) for at least ½ hour to allow them to reach the required temperature.

In carrying out the experiments, into a 250 ml. conical flask, also immersed in the bath, were pipetted in order, 25 ml. of the buffer solution, 1·25 ml. of the 3% sugar solution, and finally 5 ml. of the M/40-iodine. The total draining time of the 5 ml. pipette used for adding the iodine solution was found to be 4 seconds and hence the time for the commencement of the reaction was taken as 2 seconds after the initial addition of the iodine. The mixture was shaken immediately and reaction was stopped after the required time by adding 50 ml. of cold 3% sulphuric acid. The iodine liberated was titrated immediately against N/100-sodium thiosulphate using a microburette, the titration being carried out with a stream of carbon dioxide bubbling through the solution. No account was taken of the amount of iodate formed during the reaction, since it is known that iodate is completely inactive as an oxidising agent for aldoses. On acidification, the iodate is decomposed in the presence of iodide to regenerate the equivalent amount of iodine.

This procedure permitted accurate determinations of the amounts of unused iodine in the mixture as early as 4 seconds after the start of reaction. For each mixture, a graph was obtained by plotting the titre of thiosulphate against the time, and from the graphs, the time of quarter-change, $t_{1\,4}$, was read.

The results of these experiments are recorded in Table III.

*Calculation of the Initial Concentrations of Hypoiodous acid and Hypoiodite Ion in the Reaction Mixtures.*—A preliminary value of the ionisation constant of hypoiodous acid has been given by Fürth (*Z. Elektrochem.*, 1922, **28**, 57) as :

$$\frac{[H^+]\,[IO^-]}{[HIO]} = 10^{-11} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

TABLE III.

*Times of Quarter-change, $t_{1/4}$, in Seconds.*

| pH. | Mannose. | Galactose. | Arabinose. | Glucose. | Xylose. |
|---|---|---|---|---|---|
| 10·15 | — | — | — | 60 | 51 |
| 10·55 | — | — | — | — | 6·75 |
| 10·60 | 28 | 4·25 | 4·25 | 6·5 | — |
| 10·85 | — | — | — | 5 | 4·75 |
| 10·90 | — | — | 3·75 | — | 4·5 |
| 10·95 | — | 3·25 | — | 4·5 | — |
| 11·02 | 18·5 | — | — | — | — |
| 11·15 | — | — | — | 4 | — |
| 11·20 | — | — | — | 3·75 | — |
| 11·25 | 14·25 | — | — | — | — |
| 11·30 | — | — | 2·5 | — | 3·25 |
| 11·35 | — | 2·5 | — | 3·5 | — |
| 11·45 | 14·25 | — | — | — | — |
| 11·50 | — | — | 2·5 | — | 3·25 |
| 11·55 | — | 2·75 | — | 3·75 | — |
| 11·65 | 17 | — | — | — | — |
| 11·70 | — | — | 3 | — | 3·75 |
| 11·75 | — | — | — | 4·5 | — |
| 11·85 | 23·5 | — | — | — | — |
| 12·50 | — | — | — | 14·5 | — |
| 12·80 | 175 | 16·25 | 16 | 32 | 28 |
| 13·10 | — | 31·5 | 29·5 | 64 | 55 |
| 13·45 | — | — | — | 90 | — |

Further, the equilibrium constant for the hydrolysis of iodine at 25° : $I_2 + H_2O \rightleftharpoons HIO + H^+ + I^-$, is known to be

$$\frac{[H^+]\,[I^-]\,[HIO]}{[I_2]} = 3 \times 10^{-13} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

(Bray, *J. Amer. Chem. Soc.*, 1911, **33**, 932; Bray and Connolly, *ibid.*, p. 1485), and for the reaction between iodine and iodide ion to form tri-iodide at 25° the equilibrium constant is given by

$$\frac{[I_2]\,[I^-]}{[I_3^-]} = 1{\cdot}36 \times 10^{-3} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

(Jakowkin, *Z. physikal. Chem.*, 1894, **13**, 539; 1896, **20**, 19; Bray and McKay, *J. Amer. Chem. Soc.*, 1910, **32**, 914, 1207).

From the values of these three equilibrium constants and the known concentration of total iodine in any given reaction mixture, it is possible to calculate the initial concentrations of hypoiodous acid and hypoiodite ion as follows. In all experiments, the total iodine concentration was $4{\cdot}0 \times 10^{-3}$ mols./l. In contact with hydroxyl ions and iodide ions, some of this iodine is converted into hypoiodous acid, hypoiodite ion, and tri-iodide ion. Thus :

$$[I_2] + [HIO] + [IO^-] + [I_3^-] = 4{\cdot}0 \times 10^{-3} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

As a first approximation, the concentration of iodide ion may be taken as equal to the amount of iodide added, *i.e.*, in this case, $[I^-] = 1{\cdot}6 \times 10^{-2}$. Thus for any given concentration of hydrogen ion a value of $\frac{[IO^-]}{[HIO]}$ is obtained from (1), of $\frac{[HIO]}{[I_2]}$ from (2), and of $\frac{[I_3^-]}{[I_2]}$ from (3). By substituting these values in (4), values of $[I_2]$ and of $[I_3^-]$ are obtained as a first approximation. From this value of $[I_3^-]$, a more accurate value of $[I^-]$ may be obtained and the whole calculation is then repeated. In this way successive approximations are taken until the values of $[I^-]$, $[HIO]$, and $[IO^-]$ become constant. The values given in Table IV were obtaned by this method of calculation.

TABLE IV.

| pH. | [HIO]. | [IO⁻]. | pH. | [HIO]. | [IO⁻] |
|---|---|---|---|---|---|
| 9·00 | $9{\cdot}60 \times 10^{-6}$ | $9{\cdot}60 \times 10^{-3}$ | 11·50 | $6{\cdot}66 \times 10^{-4}$ | $2{\cdot}10 \times 10^{-3}$ |
| 9·40 | $2{\cdot}40 \times 10^{-5}$ | $0{\cdot}60 \times 10^{-6}$ | 11·60 | $6{\cdot}15 \times 10^{-4}$ | $2{\cdot}45 \times 10^{-3}$ |
| 10·00 | $9{\cdot}38 \times 10^{-5}$ | $9{\cdot}38 \times 10^{-6}$ | 11·80 | $4{\cdot}78 \times 10^{-4}$ | $3{\cdot}02 \times 10^{-3}$ |
| 10·40 | $2{\cdot}20 \times 10^{-4}$ | $0{\cdot}55 \times 10^{-4}$ | 12·00 | $3{\cdot}43 \times 10^{-4}$ | $3{\cdot}43 \times 10^{-3}$ |
| 10·80 | $4{\cdot}47 \times 10^{-4}$ | $2{\cdot}82 \times 10^{-4}$ | 12·40 | $1{\cdot}52 \times 10^{-4}$ | $3{\cdot}80 \times 10^{-3}$ |
| 11·00 | $5{\cdot}83 \times 10^{-4}$ | $5{\cdot}83 \times 10^{-4}$ | 13·00 | $3{\cdot}95 \times 10^{-5}$ | $3{\cdot}95 \times 10^{-3}$ |
| 11·20 | $6{\cdot}80 \times 10^{-4}$ | $1{\cdot}08 \times 10^{-3}$ | 13·40 | $1{\cdot}59 \times 10^{-5}$ | $3{\cdot}98 \times 10^{-3}$ |
| 11·30 | $6{\cdot}97 \times 10^{-4}$ | $1{\cdot}39 \times 10^{-3}$ | 14·00 | $3{\cdot}99 \times 10^{-6}$ | $3{\cdot}99 \times 10^{-3}$ |
| 11·40 | $6{\cdot}93 \times 10^{-4}$ | $1{\cdot}73 \times 10^{-3}$ | | | |

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UNIVERSITY OF TASMANIA, HOBART. [*Received, June 18th*, 1947.]