The OXIDATION

of some ALDOSE SUGARS and sugar derivatives, by ALKALINE solutions of IODINE.

Thesis

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C O N T E N T S.

FOREWORD.

The following work was carried out at the Chemistry Department of the University of Tasmania during 1946-47. It is submitted in the Honours School of Chemistry for consideration for the degree of Master of Science.

The author wishes to express his thanks to Mr. G. C. Israel M.Sc., for valuable guidance and suggestions during the course of the investigation, to Dr. J. B. Polya for pure samples of chitosamine hydrochloride and to Mr. MacMahon for photographic reproductions of the graphs incorporated in this thesis.

Some sections of the following work have already been published in the Journal of the Chemical Society, under the title of the thesis. (J.C.S., 1948, 810) [redacted].

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November 1947.

INTRODUCTORY.

An analytical method for the estimation of aldoses in solution by a quantitative oxidation to the corresponding aldonic acids, using alkaline solutions of iodine as the oxidising agent, has been described in a number of papers (1). In all cases, it seems to have been assumed that the iodine first reacts with the alkali to form hypoiodite, and that this latter is the active oxidising agent. No definite evidence, however, has been brought forward in support of this assumption. At the same time, varying degrees of alkalinity and other conditions have been recommended by the several authors for carrying out the oxidation.

Accordingly, the project undertaken was an attempt to clarify the position by a study of the mechanism of aldose oxidation in alkaline solutions of iodine. In the course of the work, however, it became apparent that configurations of the sugars exercised a considerable effect on the rate of oxidation, and this effect was also studied.

These investigations have shown a need for much more work in various closely related directions which promise to yield information of value; hence a section has been included wherein they are outlined, together with some preliminary work in these directions which the author has carried out, but which lack of time has forced temporarily to be abandoned.

MATERIALS.

Materials used throughout this investigation were all B.D.H. Analar reagents with the exception of the sugars and sugar derivatives. These were either pure samples supplied by B.D.H. or samples prepared and carefully purified in these laboratories. All sugars and sugar derivatives were checked for purity by moisture determinations, measurement of their specific rotations and by other means wherever possible. The values observed are as set out in table I below:-

TABLE I.

SUGAR	a_D^{20} (obs)	a_D^{20}	n_D^{20} (obs)	n_D^{20}
d-glucose	+ 52.70	+ 52.70 (1)	-	-
d-galactose	+ 78.80	+ 79.25 (2)	1.3367	1.3366 (2)
d-xylose	+ 19.00	+ 18.80 (1)	-	-
l-arabinose	+104.5	+104.5 (1)	-	-
d-mannose	+ 12.60	+ 14.20 (1)	-	-
l-rhamnose (hydrate)	+ 8.13	+ 8.20 (1)	-	1.534 (2)
2,3,4,6 tetramethyl glucose	+ 83.4	+ 83.3 (1)	-	-
Glucosamine hydrochlor.	+ 72.5	+ 72.4 (1)	-	-

(1) Bates: Polarimetry and Saccharimetry of the Sugars.
(2) International Critical Tables.

SECTION A.

Establishment of the active oxidising agent.

In alkaline solutions of iodine, there will be hypoiodous acid and hypoiodite, also iodine and iodate formed by the reaction $3KOI \longrightarrow 2KI + KIO_3$ (Lenssen and Löwenthal (2)). Iodate is known to be inactive as an oxidising agent for aldoses, and hence the effective oxidising agent must be either hypoiodous acid or hypoiodite ion. It should be possible to determine which of these two is effective in the oxidation process by a determination of the rate of aldose oxidation at various hydrogen ion concentrations, and by comparison of these results with the amounts of hypoiodous acid and hypoiodite ion theoretically present in the solutions. This is the method which has been adopted here. In order to evaluate the concentrations of hypoiodite ion and unionised hypoiodous acid present in iodine solutions of various hydroxyl ion concentrations, the following procedure was used.

Calculation of (HIO) and (IO^-) in Alkaline Iodine Solutions.

Furth (3) has given a preliminary value of the ionisation constant of hypoiodous acid as 10^{-11}.

$$\text{Hence } \frac{[H^+]\,[IO^-]}{[HIO]} = 10^{-11} \qquad \ldots.1$$

Further, the equilibrium constant for the hydrolysis of iodine at 25°C. is known to be 3×10^{-13} (4), i.e.

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

For the reaction between iodine and iodide ion, to form triiodide, the equilibrium constant at 25°C is given by (5)

$$\frac{[I_2]\ [I^-]}{[I_3^-]} = 1.36 \times 10^{-3} \qquad \ldots.3$$

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 concentrations of hypoiodous acid and of hypoiodite ion as follows:- In all subsequent experiments, the total iodine concentration was 4.0×10^{-3} mols per litre and this value is now used in the calculations. In contact with hydroxyl ions and iodide ions, some of this iodine present is converted to hypoiodous acid, hypoiodite ion and tri-iodide ion. Iodate is not present initially since it is formed from hypoiodite. Thus

$$[I_2] + [HIO] + [IO^-] + [I_3^-] = 4.0 \times 10^{-3} \quad ..4$$

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, $[I^-] = 1.6 \times 10^{-2}$. Thus for any given concentration of hydrogen ion a value of $\frac{[IO^-]}{[HIO]}$ is obtained from eqn. 1, and of $\frac{[HIO]}{[I_2]}$ from eqn. 2, and of $\frac{[I_3^-]}{[I_2]}$ from eqn. 3. By substituting these values in 4, values of $[I_2]$ and $[I_3^-]$ 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. Successive approximations can be made in this manner until the values for $[I^-]$, $[HIO]$ and $[IO^-]$ become constant. The appropriate values, for each pH, calculated in this manner, are as shown in table II.

TABLE II.

pH	[HIO]	[IO⁻]
9.00	9.6×10^{-6}	9.6×10^{-8}
9.40	2.40×10^{-5}	0.60×10^{-6}
10.00	9.38×10^{-5}	9.38×10^{-6}
10.40	2.20×10^{-4}	0.55×10^{-4}
10.80	4.47×10^{-4}	2.82×10^{-4}
11.00	5.83×10^{-4}	5.83×10^{-4}
11.20	6.80×10^{-4}	1.08×10^{-3}
11.30	6.97×10^{-4}	1.39×10^{-3}
11.40	6.93×10^{-4}	1.73×10^{-3}
11.50	6.66×10^{-4}	2.10×10^{-3}
11.60	6.15×10^{-4}	2.45×10^{-3}
11.80	4.78×10^{-4}	3.02×10^{-3}
12.00	3.43×10^{-4}	3.43×10^{-3}
12.40	1.52×10^{-4}	3.80×10^{-3}
13.00	3.95×10^{-5}	3.95×10^{-3}
13.40	1.59×10^{-5}	3.98×10^{-3}
14.00	3.99×10^{-6}	3.99×10^{-3}

Although the preceding table gives the concentrations of hypoiodite and unionised hypoiodous acid in solutions of different alkalinities, it must be remembered that during the course of oxidation of a sugar by alkaline iodine solutions, iodate will be formed from the hypoiodite present: hence the values of table II show concentrations at the <u>initial moment of reaction only</u>.

However, the formation of iodate from hypoiodite (by the reaction $3IO^- \longrightarrow IO_3^- + 2I^-$) will not affect the titre of total iodine by which the reaction is followed, since on acidification as much iodine is obtained from the iodate formed as would have been obtained from the hypoiodite consumed. Iodate moreover, will not affect the oxidation rates of the sugars, since it is inactive as an oxidising agent for the aldoses.

DETERMINATION OF REACTION VELOCITY.

The velocity of oxidation of glucose by alkaline solutions of iodine was investigated by the following method. 3% solutions of each aldose or aldose derivative, an $^M/_{40}$ solution of iodine in $^M/_{10}$ potassium iodide and a buffer solution were immersed in a constant temperature bath (25.00 $\pm$ 0.05°C) for at least one half-hour, so that they might reach the required temperature of 25°C.

250 ml. conical flasks were immersed in the bath as reaction vessels, and into these were pipetted in order 25 mls of the buffer solution, 1.25 mls. of the sugar solution, and finally 5 mls. of the $^M/_{40}$ iodine solution, the flask being swirled after each addition to ensure proper mixing.

The 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 iodine. After the required interval of time had elapsed, the reaction was stopped by addition of 50 mls.

of cold 3% sulphuric acid. The iodine liberated was titrated immediately against $^{N}/100$ (or, in some cases, against $^{N}/50$) sodium thiosulphate, using a microburette, the titration being carried out with a stream of carbon dioxide bubbling through the solution.

This procedure permitted accurate determinations of the amounts of unused iodine in the reaction mixture as early as 4 seconds after the commencement of the reaction.

However, care must be exercised in the choice of buffer solutions in which to carry out the reaction. Those used are listed below in table III.

TABLE III.

pH	Composition of Buffer	Reference
10.17	50 ml 0.1 M Na_2CO_3 + 20 ml. 0.1 N HCl made up to 100 ml	6
10.35	" " 15 " "	6
10.55	" " 10 " "	6
10.86	" " 5 " "	6
11.00	25 ml 0.1 N Na_2HPO_4 + 4.13 ml. 0.1 N NaOH made up to 50 ml.	7
11.20	" " 6.00 " "	7
11.40	" " 8.67 " "	7
11.60	" " 12.25 " "	7
11.80	" " 16.65 " "	7
12.00	" " 21.60 " "	7
12.50	0.05 N NaOH	-
12.80	0.10 N NaOH	-
13.10	0.20 N NaOH	-
13.45	0.50 N NaOH	-

Since these buffers were used at a temperature (25°C) often differing slightly from that specified, and in view of possible inaccuracies entailed in the fine measurement of volumes

required for making up small quantities of the buffers, the pH of each solution (up to pH 12.00) was measured by means of a Coleman pH-meter (glass electrode) standardised against the standard buffer proposed by Bates, Hamer, Manov and Acree (8), the pH of which is 11.68 at 25°C. The use of sodium hydroxide alone as the buffer substance for the pH range 12.20 - 13.50 is justified, since in such reaction mixtures there is a sufficiently large excess of sodium hydroxide for the pH to remain almost unchanged during the reaction. The pH values of such solutions were calculated from the concentrations of sodium hydroxide, using the values of the activity coefficients given by MacInnes (9).

A number of high pH buffers are unsatisfactory for this work since they lead to formation of 1:2 addition products with glucose; the significance of this addition will be discussed later. Such buffers are those containing boric acid or borates (formation of 1:2-α-d-glucose pyroborate (10)) and carbonate buffers under certain conditions (formation of 1:2-α-d-glucose carbonate (11)). Carbonate buffers containing hydrochloric acid, however, are suitable for the work since ionisation of the carbonic acid is repressed by the presence of hydrochloric acid, and the effect of 1:2 addition is avoided. Buffers containing glycine or amino-acids are also objectionable due to the formation of condensation products with the sugar.

EXPERIMENTAL RESULTS.

The experimental results are shown graphically in figs. I-IV. The plot is of thiosulphate titres against time. The time of quarter change ($t_{\frac{1}{4}}$) could be read from the graphs with a possible error of $\pm \frac{1}{3}$ sec. All results are for the oxidation of glucose, the value at pH 9.96 being doubtful, since the buffer used for this one pH contained some glycine, which may be objectionable. Table IV summarises the results:-

TABLE IV.

pH	9.96	10.20	10.60	10.85	10.95	11.15	11.35
$t_{\frac{1}{4}}$ (sec)	70	13	6.5	5	4.5	4	3.5
$1/t_{\frac{1}{4}}$ (mins^{-1})	0.86	4.6	9.24	12.0	13.4	15.0	17.2

pH	11.55	11.75	11.95	12.50	12.80	13.10	13.45
$t_{\frac{1}{4}}$ (sec)	3.75	4.5	5.5	14.5	32	64	92
$1/t_{\frac{1}{4}}$ (mins^{-1})	16.0	13.4	10.8	4.15	1.88	0.94	0.65

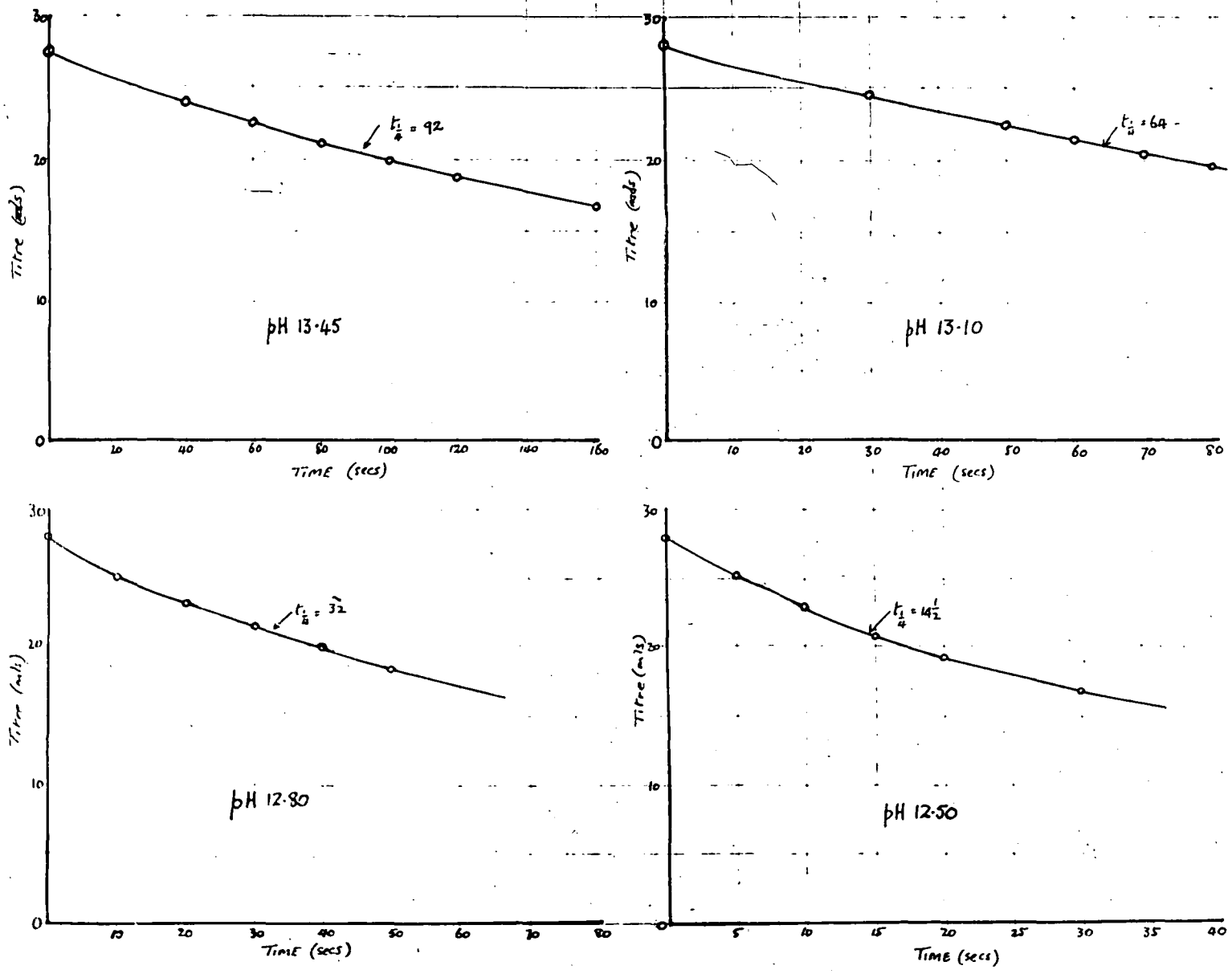

Titre (mls)
TIME (secs)
pH 13·45
$t_{\frac{1}{4}} = 92$
pH 13·10
$t_{\frac{1}{4}} = 64$
pH 12·80
$t_{\frac{1}{4}} = 32$
pH 12·50
$t_{\frac{1}{4}} = 14\frac{1}{2}$

Fig. 1

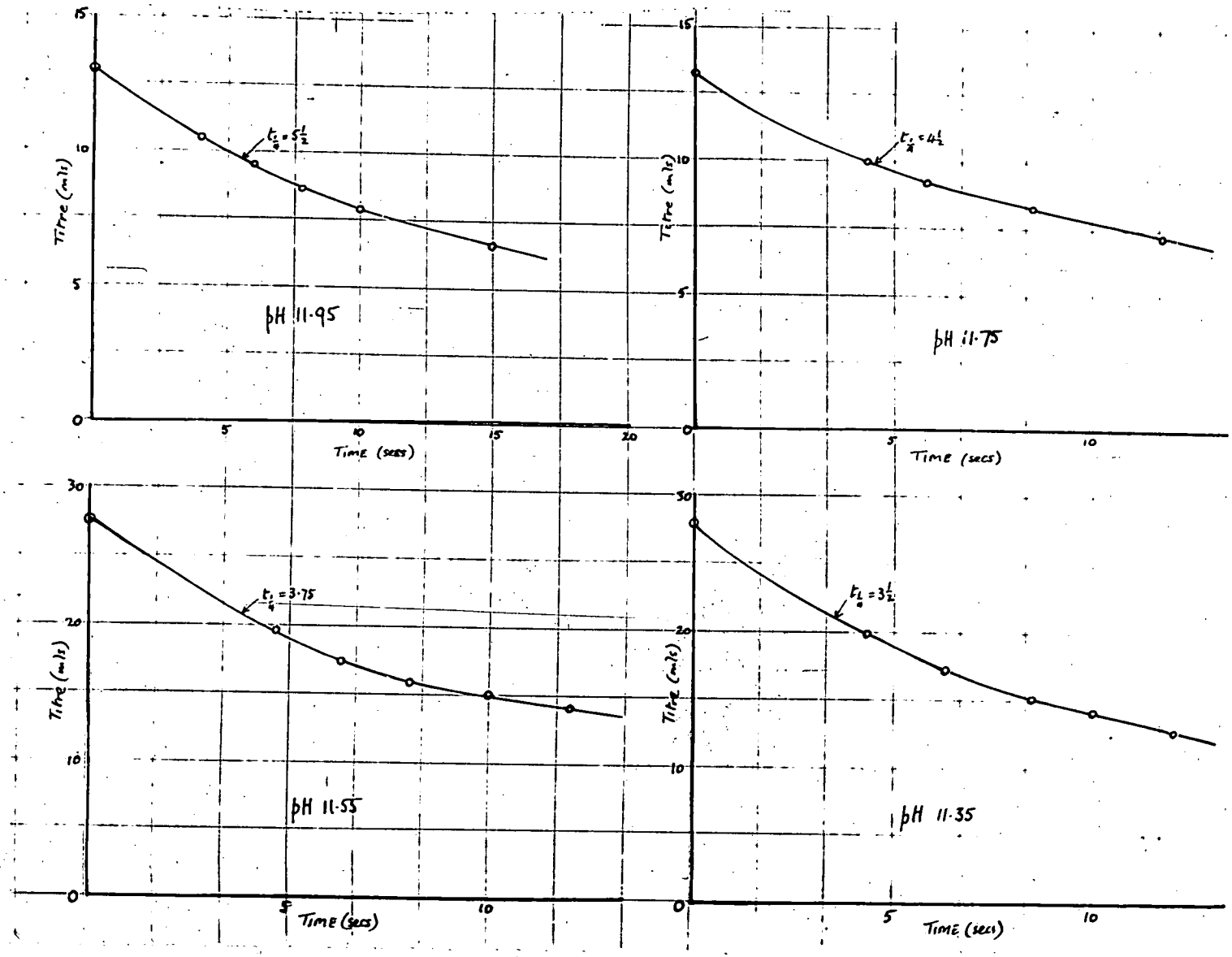

pH 11·95
pH 11·75
pH 11·55
pH 11·35
Time (secs)
Time (secs)
Time (secs)
Time (secs)

FIG. II

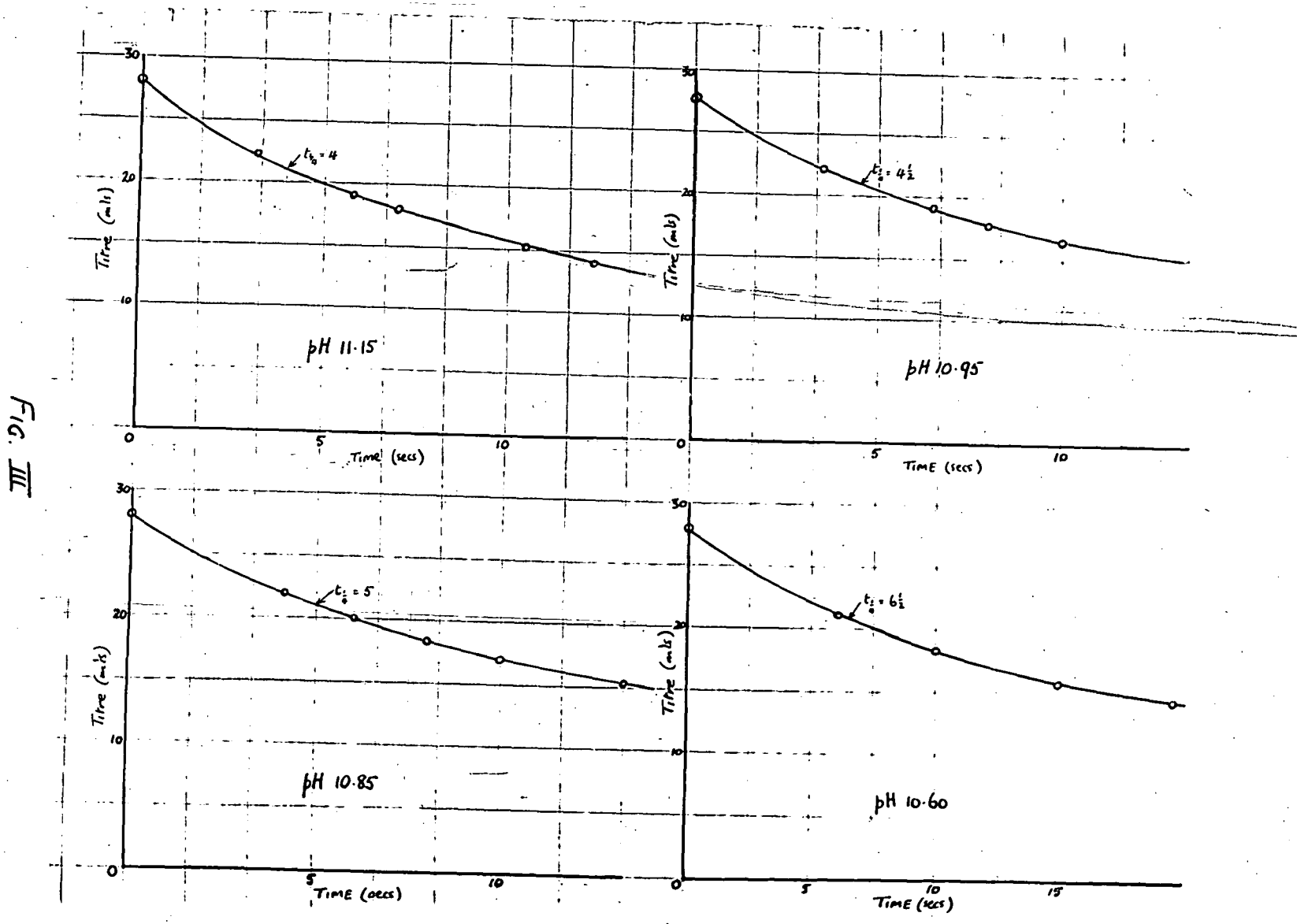
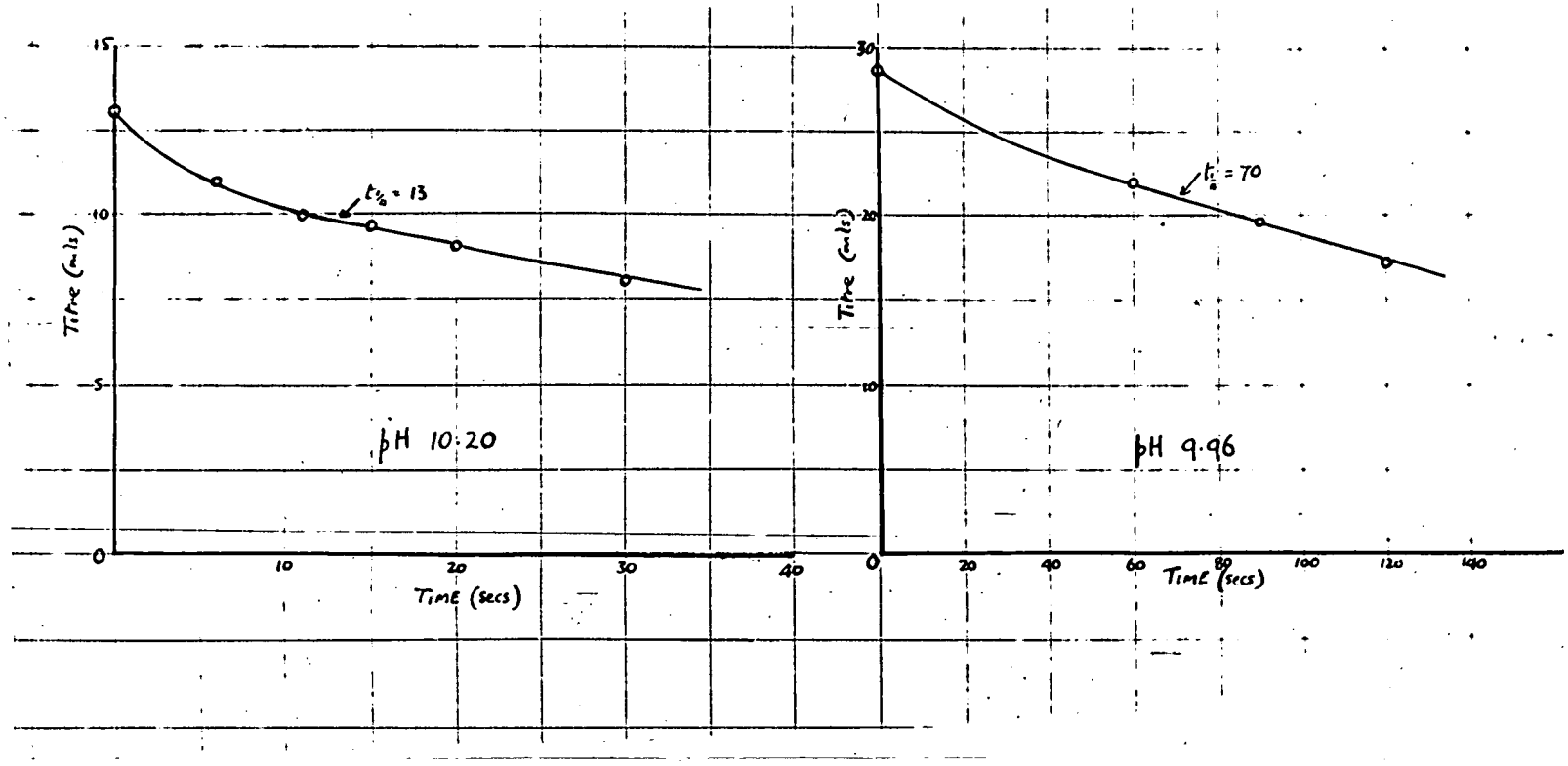

pH 11·15
pH 10·95
pH 10·85
pH 10·60
Time (secs)
Time (secs)
Time (secs)
Time (secs)
Titre (ml)
Titre (ml)
Titre (ml)
Titre (ml)
0
5
10
15
20
30

FIG. III

Fig. II


15
10
5
0
Time (mins)
$t_{\frac{1}{2}}$ = 13
pH 10·20
10
20
30
40
Time (secs)
30
20
10
0
Time (mins)
$t_{\frac{1}{2}}$ = 70
pH 9·96
0
20
40
60
80
100
120
140
Time (secs)

It will be shown presently that the oxidation reaction is bimolecular; and for a bimolecular reaction the velocity of reaction varies inversely as the time taken for any given fraction of the reactants to undergo change. As the initial concentration of glucose is the same in each case, then the time of quarter change, $t_{\frac{1}{4}}$, will vary inversely as the concentration of the active oxidising agent. In figure V the values of $1/t_{\frac{1}{4}}$ from table IV (indicated by points - o), are plotted against pH. Agreement with the theoretically calculated curve for $[HIO]$ (the full line in fig. V) is very close, with the exception of two points near pH 11.3, where however, an error of $\frac{1}{2}$ second in measurement of the time of quarter change would be sufficient to account for the slight divergence. As previously remarked, this is within the experimental error. Again as previously noted, the value at pH 9.96 is doubtful, since this buffer contained glycine. The marked dissimilarity of the experimental results to the calculated concentrations of hypoiodite ion, together with the marked similarity to the curve calculated for unionised hypoiodous acid, affords strong evidence that the active oxidising agent is, in fact, unionised hypoiodous acid. The pH value for optimum reaction velocity is seen to be near 11.35.

Further evidence that the reaction is non-ionic was provided by the addition of varying amounts of sodium chloride to the reaction mixture. No appreciable salt effect was detected and the reaction rate remained unchanged.

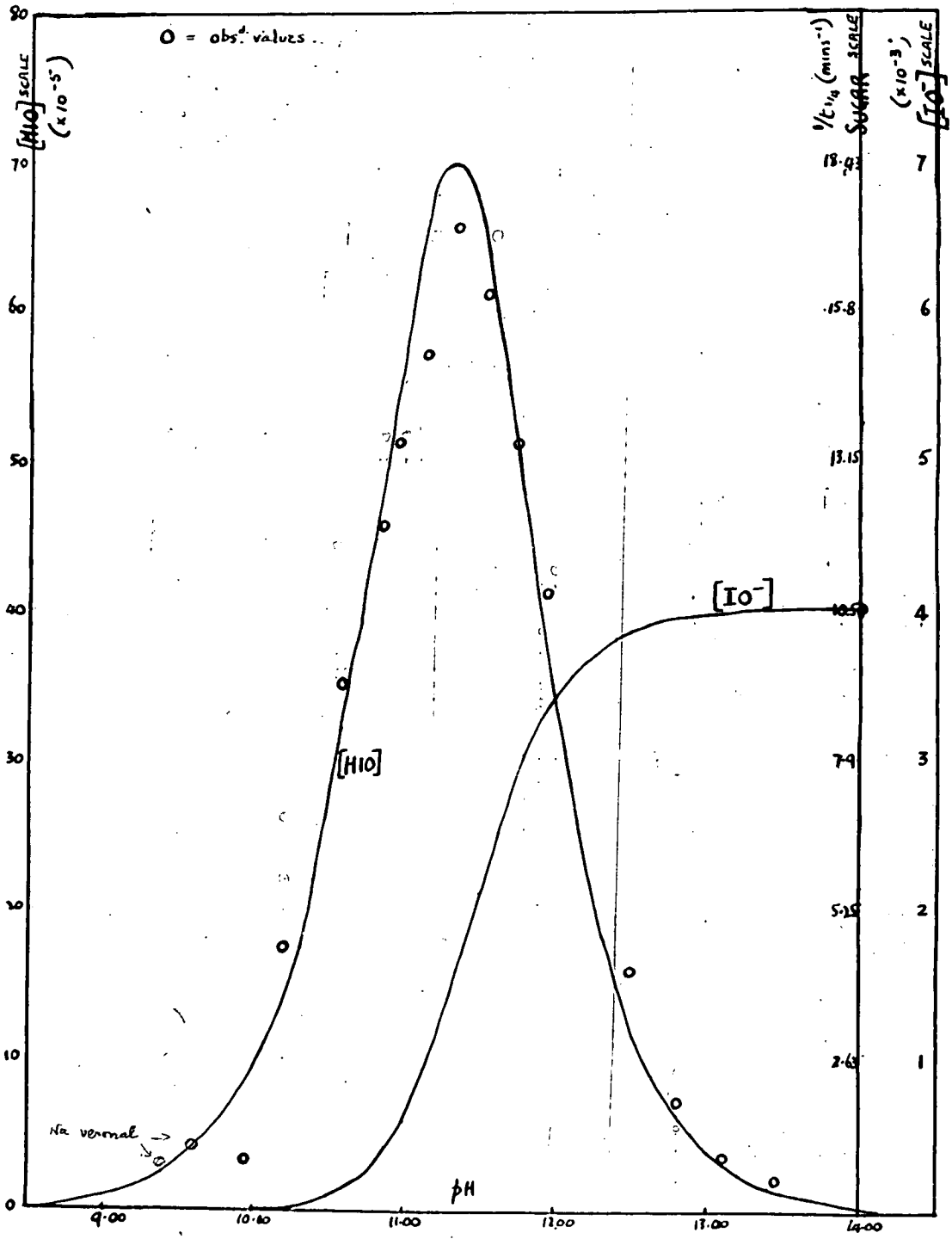

O = obsd. values
[HIO] SCALE (x10⁻⁵)
1/t½ (mins⁻¹) SUGAR SCALE
(x10⁻³) [IO⁻] SCALE
[HIO]
[IO⁻]
Na vernal
pH
9·00
10·00
11·00
12·00
13·00
14·00
80
70
60
50
40
30
20
10
0
18·9
15·8
13·15
10·5
7·9
5·25
2·63
7
6
5
4
3
2
1

FIG. V

ORDER OF REACTION.

The order of the reaction was established by carrying out the oxidation using a glucose solution of strength $^{M}/_{10}$, so that the initial concentrations of glucose and iodine in the reaction mixture are identical. The pH of the buffer was 11.15. There are two reactions occurring simultaneously in the solution, viz:

$$3HIO \longrightarrow HIO_3 + 2HI \quad .....1$$

and $$C_6H_{12}O_6 + HIO \longrightarrow C_6H_{12}O_7 + HI \quad .....2$$

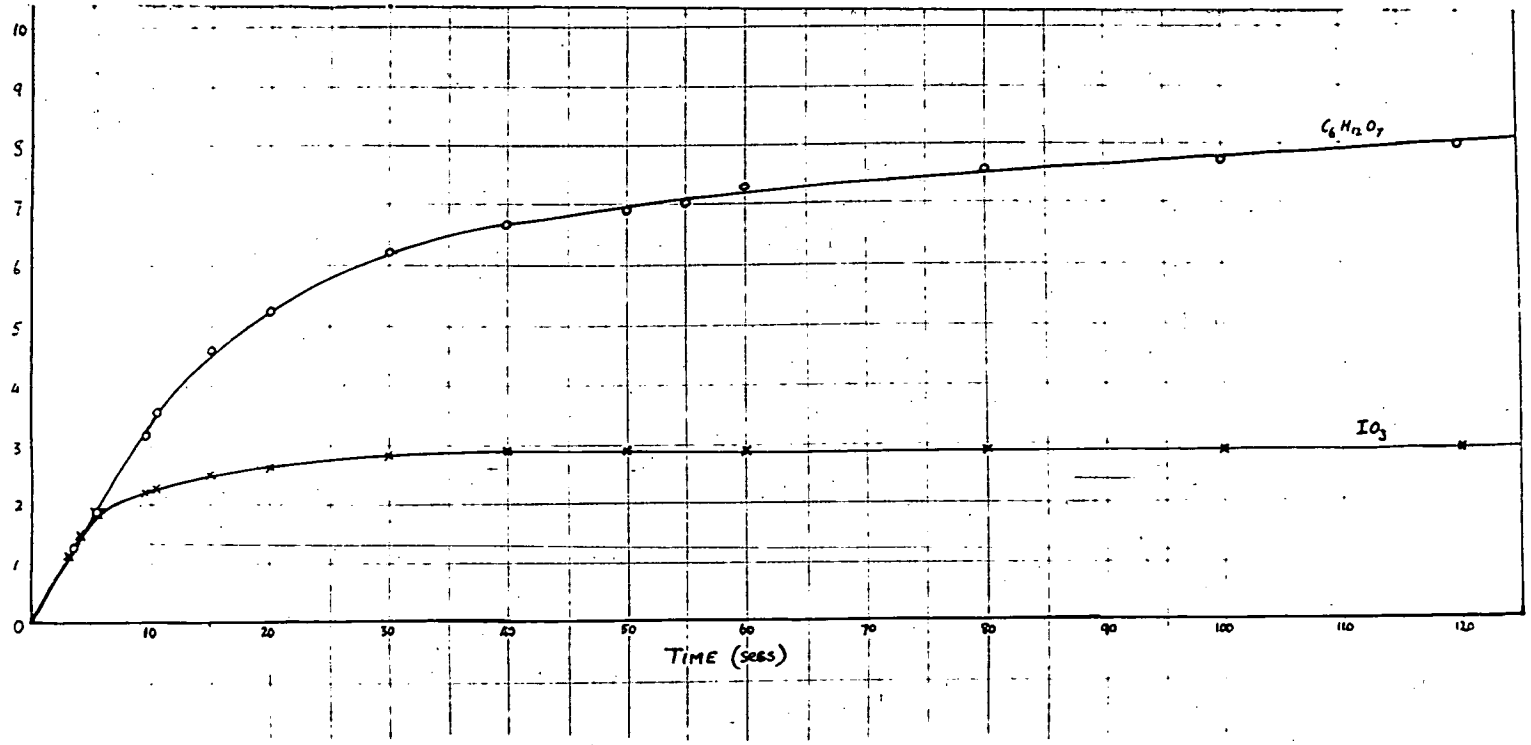
Reaction 1 has been investigated by several workers (12) who agree that the reaction is of second order. Hence if reaction 2 is also of second order, a Wegscheider test should show constancy.

In the experiment performed to test this, the oxidation was carried out as described before, and iodate was determined by stopping the reaction by the addition of excess 5% phenol, which removes hypoiodite and, in the presence of free alkali, free iodine and triiodide also. Acidification of the solution then liberates iodine corresponding to the amount of iodate present. This was titrated with thiosulphate as before. Fig. VI shows graphically the amounts of iodate formed, and of sugar oxidised, against time. The graph shows that, in the initial stages of the reaction (for the first 6 seconds) the amounts of the two products formed are virtually identical and the Wegescheider test must apply, as in fact it does. Table V shows the results of application of this test. Beyond six seconds, the constancy no longer holds, and apparently the order of reaction has changed.

TABLE V.

Time (secs.)	$C_6H_{12}O_7$	IO_3^-	$\frac{IO_3^-}{C_6H_{12}O_7}$ (= R)		a - x	$\frac{R}{(a-x)^{\frac{1}{2}}}$	
0	0.00	0.00	-		13.20	-	
3	1.10	1.10	1.00	Av. 1.00	11.00	0.332	
$3\frac{1}{2}$	1.27	1.27	1.00		10.74	0.327	
4	1.50	1.50	1.00		10.20	0.319	
$5\frac{1}{2}$	1.87	1.85	0.99		9.48	0.319	
$9\frac{1}{2}$	3.17	2.20	0.70		7.83	0.249	
$10\frac{1}{2}$	3.50	2.25	0.64		7.45	0.233	
15	4.60	2.50	0.54		6.10	0.218	
20	5.24	2.62	0.50		5.34	0.217	
30	6.22	2.85	0.46		4.13	0.225	
40	6.70	2.88	0.43		3.62	0.225	
50	6.88	2.88	0.42		3.44	0.226	
55	7.00	2.88	0.41		3.32	0.225	
60	7.30	2.88	0.395		3.02	0.227	Av. 0.227
80	7.60	2.88	0.38		2.72	0.230	
100	7.73	2.88	0.37		2.59	0.228	
120	7.97	2.88	0.36		2.35	0.230	
	10.25	2.95	0.29		0.00	-	

Fig. VI


10
9
8
7
6
5
4
3
2
1
0
10
20
30
40
50
60
70
80
90
100
110
120
TIME (secs)
$C_6H_{12}O_7$
IO_3

If, however, the ratios obtained in this test be divided by $(a - x)^{\frac{1}{2}}$ where a is the initial concentration of iodine, and x the amount changed by the two reactions at time t,* then good constancy is seen to be obtained beyond a reaction time of 30 seconds. This suggests that the rate of reaction in the second stage conforms fairly closely to an order of 1.5, since for such a reaction, the ratio

$$\frac{[IO_3^-]}{[C_6H_{12}O_7]} = \frac{k_1(a - x)^{\frac{1}{2}}}{k_2}$$ where k_1 and k_2 are the specific rate constants for the formation of iodate and sugar oxidation product respectively.

These results therefore, show that the reaction occurs in two stages, namely:-

(a) a rapid bimolecular, second order reaction.
(b) a slower reaction of order about 1.5.

If, as Isbell (13) reports, the β-forms of the aldoses oxidise much more rapidly than the α-, it is to be expected that either 1) the initial rapid oxidation represents that of the β-form, ~~and the final slower oxidation that of the α-form,~~ and the final slower oxidation that of the α-form, or 2) the final slower oxidation might measure the rate of mutarotation α→β (as the slowest stage) followed immediately by the rapid oxidation of the β-sugar formed. If 1) be the case then it seems reasonable to assume that the

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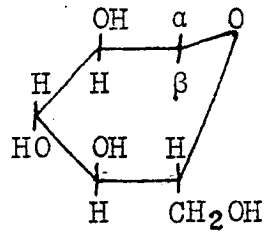
* The use of the initial concentration of iodine in place of that of hypoiodous acid is justified since the equilibrium
$I_2 + 2OH^- \rightleftharpoons I^- + IO^- + H_2O$ (Lenssen and Löwenthal)
is established very rapidly, and removal of hypoiodite is immediately compensated by its further formation from iodine and hydroxyl ions.

oxidation of the α-form (slow stage) should be of the second order similar to that of the β-form. If 2, then the slow stage, measuring the mutarotation, should be a first order reaction, since this is the case for the mutarotation of glucose (14). The observed order of 1.5 suggests that both these mechanisms may be operative simultaneously.

DEPRESSION of the OXIDATION RATE by CERTAIN SUBSTANCES.

As previously mentioned (page 12), the presence of carbonate or borate in the buffer solutions used in the oxidation experiments on glucose, leads to a marked depression in the rate of oxidation. These substances are known to add to the glucose molecule across carbon atoms 1 and 2 (10,11). In order for this addition to be possible, the hydroxyl groups on these carbon atoms must be in cis position. If they are in cis position in d-glucose the hydroxyl group on carbon atom 1 will be fixed in α-position (15,19). Thus, we can write ~~writing~~ the configuration of d-glucose according to Isbell (19), where α and β represent the positions of the hydroxyl group on C_1 for α- and β-forms respectively.



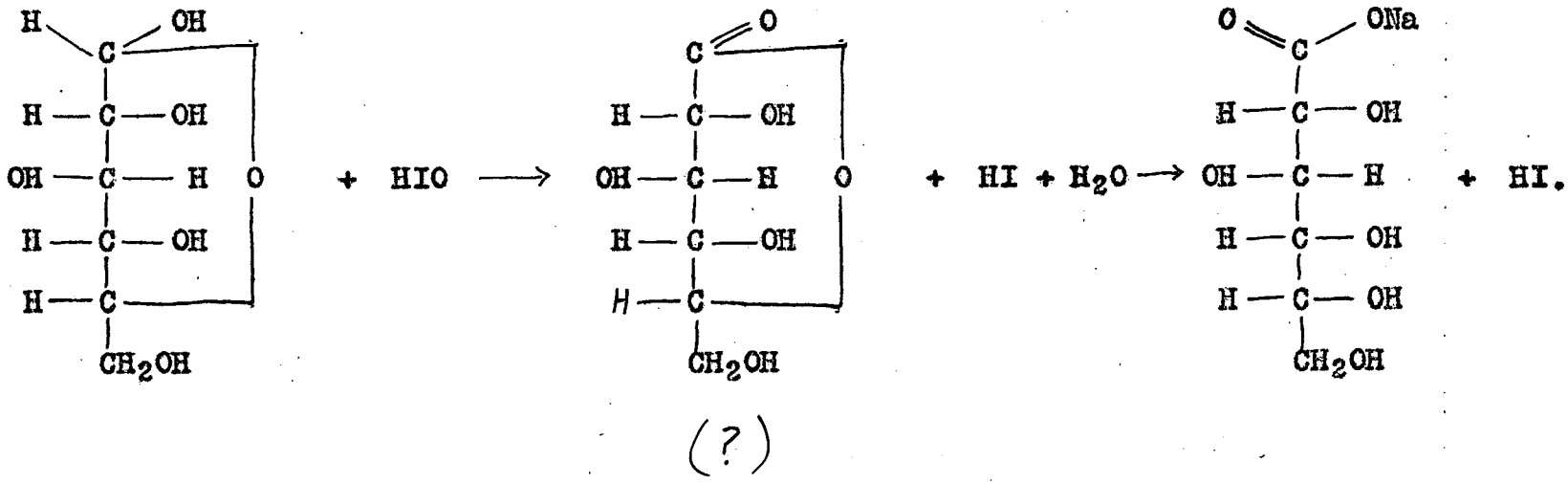
This, then, would account for observed depression of the oxidation rate of d-glucose in the presence of borates and carbonates.

However, it also suggests that with such sugars as mannose and talose, an elevation of the oxidation rate will be found, since in these two cases borate and carbonate would fix the hydroxyl group on C_1 in the β- (rapidly oxidised) position.

OXIDATION PRODUCTS.

The oxidation of glucose by alkaline solutions of iodine has variously been stated to lead to gluconic acid (as the sodium salt) and even beyond this stage. Isbell (16) has shown that bromine oxidation leads directly to a δ-lactone. The actual oxidation product formed in the reaction here investigated has not been isolated; indeed, if a δ-lactone is formed this would prove difficult to isolate and identify working solely under the present oxidation conditions. However, an approach to the problem was made by attempting the oxidation of a) gluconic acid and b) mannitol by the previous methods. No oxidation whatsoever was observed in either case, and hence a $-CH_2OH$ grouping is not attacked by alkaline iodine oxidation (as shown by the failure further to oxidise mannitol) and similarly a -COOH grouping is unattacked as shown by the failure to oxidise further gluconic acid. This suggests that the final product of the alkaline iodine oxidation of glucose is either gluconic acid or a lactone thereof. Attempts have been made to detect whether a δ-lactone is formed by the iodine oxidation of glucose, as is the case with the bromine oxidation (Isbell). Time has not permitted this work to be carried to completion, although some preliminary work has been done, and is outlined in a later section.

If a δ-lactone is formed as the primary oxidation product, then in alkaline solution it will be saponified rapidly to the sodium salt of the sugar acid. Thus the reaction, when glucose is oxidised by alkaline solutions of iodine may be written



(?)

Good evidence has been shown by the foregoing work for the initial and final substances partaking in the reaction; but a possible intermediate stage (as indicated by the query) has yet to be proven or disproven. It must be remembered however, that the evidence adduced here for the final oxidation product is of a negative kind, although positive evidence may be obtained readily as outlined in section C.

SECTION B.

The oxidation of various aldohexoses and aldopentoses, and their derivatives.

The alkaline iodine method of sugar oxidation was extended to various other aldohexoses and aldopentoses. The method of oxidation used was similar in all respects to that previously described. Experimental results are tabulated in Table VI.

TABLE VI.

Times of quarter-change, $t_{\frac{1}{4}}$, in seconds.

pH	Mannose	Rhamnose	Galactose	Arabinose	Xylose	2,3,4,6, Tetra-methyl Glucose
10.15	-	-	-	-	51	-
10.35	-	-	-	-	-	8.75
10.55	-	24	-	-	6.75	7
10.60	28	-	4.25	4.25	-	-
10.85	-	-	-	-	4.75	-
10.90	-	-	-	3.75	4.5	-
10.95	-	17	3.25	-	-	-
11.00	18.5	-	-	-	-	4.25
11.25	14.25	-	-	-	-	-
11.30	-	-	-	2.5	3.25	-
11.35	-	12.5	2.5	-	-	-
11.40	-	-	-	-	-	3.50
11.45	14.25	-	-	-	-	-
11.50	-	-	-	2.5	3.25	-
11.55	-	-	2.75	-	-	-
11.65	17	-	-	-	-	-
11.70	-	-	-	3	3.75	-
11.80	-	-	-	-	-	4.5
11.85	23.5	-	-	-	-	-
12.00	-	25	-	-	-	5.75
12.80	175	147	16.25	16.0	28.0	41
13.10	-	-	31.5	29.5	55	-

These results are shown graphically in figs VII, VIII, IX. Again, in each case, very close similarity to the

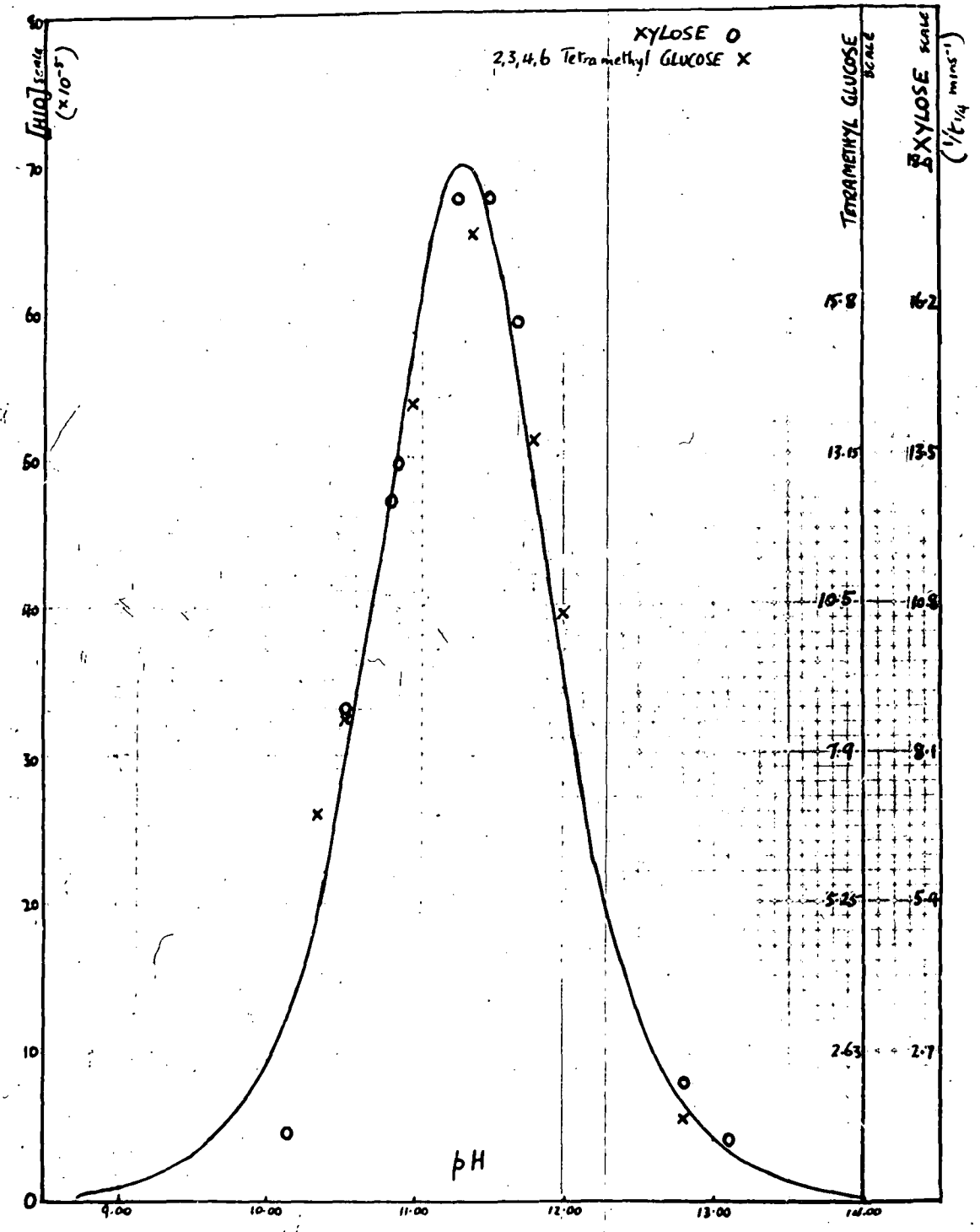

XYLOSE O
2,3,4,6 Tetramethyl GLUCOSE X
[HIO] scale (×10⁻⁵)
TETRAMETHYL GLUCOSE SCALE
D-XYLOSE scale (½t½ mins⁻¹)
pH
9.00
10.00
11.00
12.00
13.00
14.00
0
10
20
30
40
50
60
70
80
7·8
13·15
10·5
7·9
5·25
2·63
16·2
13·5
10·8
8·1
5·4
2·7

Fig. VII

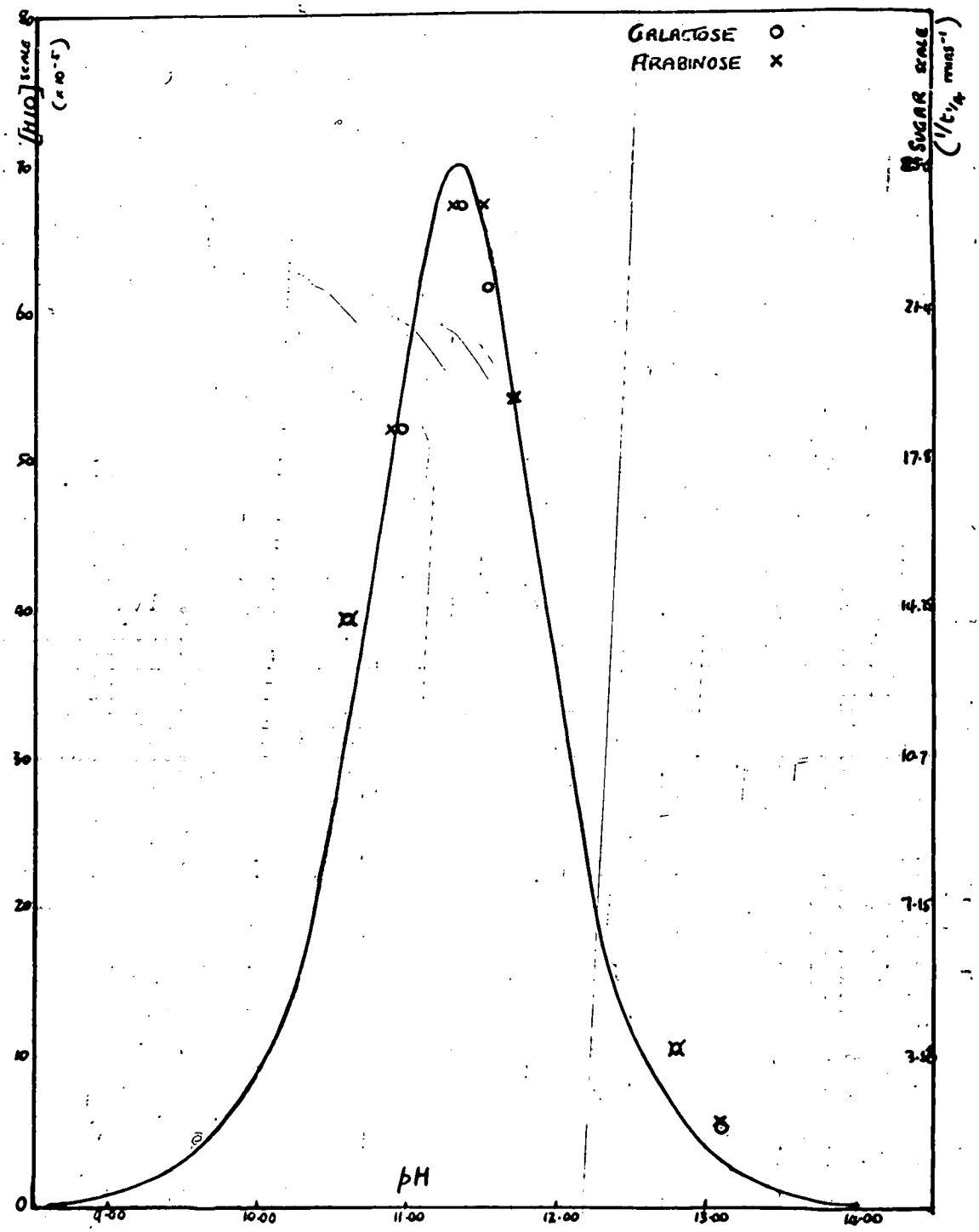


FIG. VIII

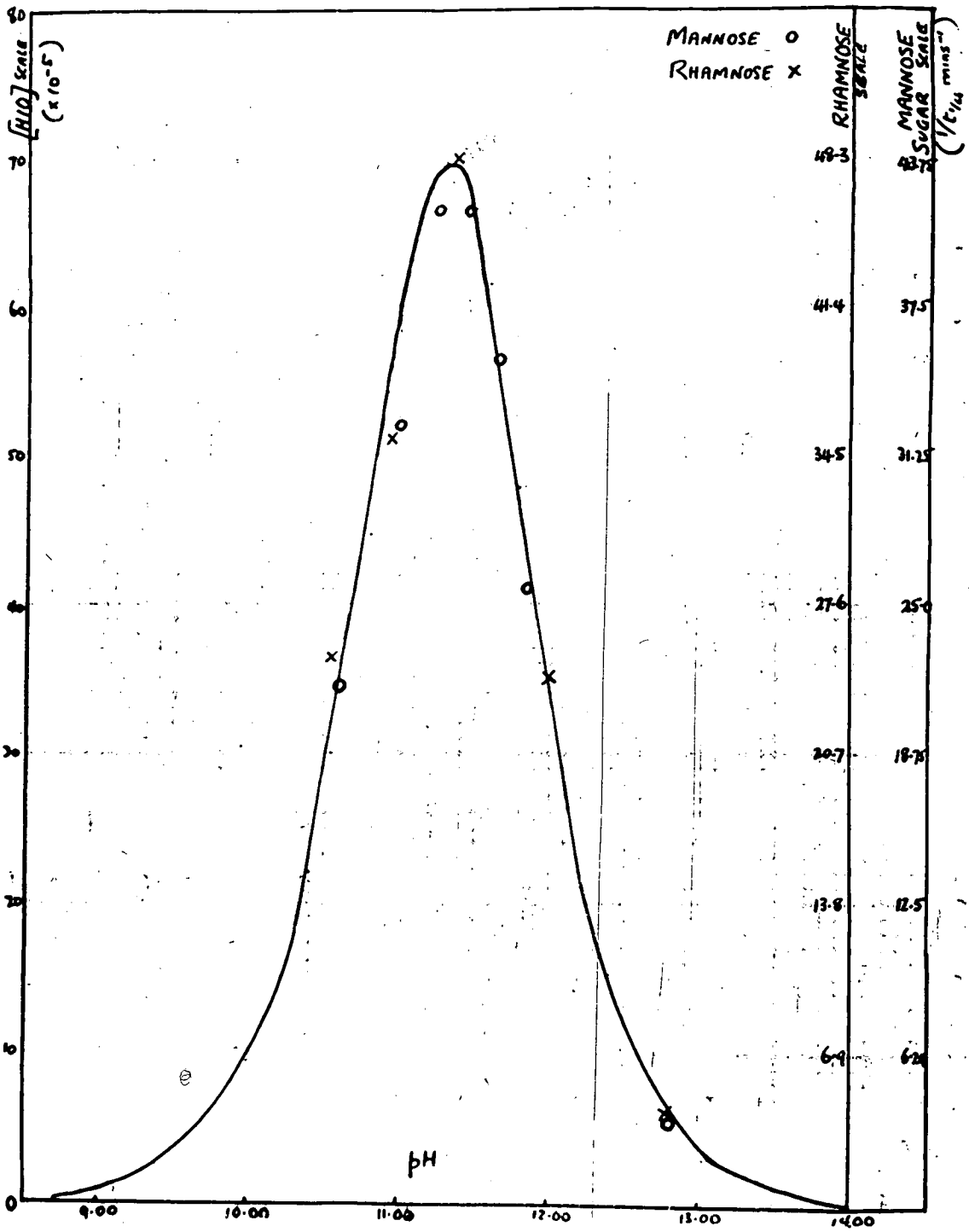

MANNOSE o
RHAMNOSE x
[HIO] SCALE (x10⁻⁵)
RHAMNOSE SCALE
MANNOSE SUGAR SCALE (1/t½ mins⁻¹)
80
70
60
50
40
30
20
10
0
48·3
41·4
34·5
27·6
20·7
13·8
6·9
43·75
37·5
31·25
25·0
18·75
12·5
6·25
9·00
10·00
11·00
12·00
13·00
14·00
pH

FIG. IX

theoretical curve for unionised hypoiodous acid is observed, which shows this to be the active oxidising agent in all cases. However, it is also apparent that some sugars oxidise much more rapidly than others. These rates of oxidation can be compared on the basis of glucose = 1.00 by comparing the factors by which the reciprocals of the times of quarter change (in minutes) must be multiplied, if the plots of pH against $^{1}/t_{\frac{1}{4}}$ are to be identical for each sugar. This method of comparison largely eliminates any possible experimental error which might be involved in the comparison of times of quarter change at individual pH values, should these alone be used. Table VII shows the factors which were required to make the oxidation curves for the various sugars coincide, together with a comparison of the relative rates of oxidation based on glucose = 1.00. Myrbäck (17) has also reported relative rates of oxidation of various sugars in this reaction, and the values which he records are listed for comparison. There is a very good agreement between the results obtained in this investigation and those of Myrbäck, in view of the method by which Myrbäck derived his values, a method which could not be susceptible to as high a degree of accuracy as that obtained in this investigation.

TABLE VIII.

Sugar.	Factor.	Relative Rate	Relative Rate (Myrbäck)
d-Glucose	1.00	1.00	1.00
d-Xylose	0.97	1.02	1.03
2,3,4,6 Tetramethyl Glucose	1.00	1.00	-
l-Arabinose	0.735	1.36	1.30
d-Galactose	0.735	1.36	1.22
d-Mannose	4.2	0.24	0.24
l-Rhamnose	3.8	0.26	0.28
d-Ribose	-	-	0.70
d-Lyxose	-	-	0.24

It is apparent that the sugars can be classified in groups according to their reaction rates, each group having a characteristic rate of oxidation. Thus d-glucose, d-xylose, and 2,3,4,6-tetramethyl glucose form one group; l-arabinose and d-galactose another; and d-mannose and l-rhamnose a third. This suggests that the configuration of the sugars may have an effect upon their rate of oxidation. It has been shown by Isbell (18) and by Isbell and Pigman (13) that in the bromine oxidation of the sugars, β-forms are oxidised much more rapidly than α forms (varying between 10 and 50 times as fast). If this also applies for iodine oxidation - it has not yet been investigated - then it is significant that those sugars which oxidise most rapidly also contain, in equilibrium solution, the highest

percentages of the β-form. Isbell (13) records these percentages as

	(Expressed as Percentages.)		
	α-form	β-form	Relative Rate
d-glucose	37.4	62.6	1.00
d-mannose	68.9	31.1	0.24
d-galactose	31.4	68.6	1.36
l-arabinose	32.4	67.6	1.36
d-xylose	32.1	67.9	1.02
l-rhamnose	69.0	31.0	0.26

However, even if this does apply, there is insufficient difference between the amounts of β-d-xylose and β-d-galactose in the equilibrium solution to account for the relatively large difference in their oxidation rates.

This suggests that other structural influences are to be sought, probably in conjunction with the above mentioned α- and β-configurations. More sugars must be investigated before any inferences can be drawn in the matter: however, it appears from the results so far obtained that, in alkaline solutions of iodine,

1. Similar configurations on carbon atoms 2,3,4 simultaneously give rise to the same rate of oxidation of a sugar.
2. Replacement of $-CH_2OH$ in a hexose by $-H$ or by $-CH_3$ has little or no influence on the rate of oxidation.
3. Methylation of the hydroxyl groups on carbon atoms 2,3,4 and 6 has no effect on the rate of oxidation of glucose.

APPLICATION of OXIDATION RATES to STRUCTURAL INVESTIGATION.

When the effect of sugar configuration on the rate of oxidation by alkaline iodine solutions has been investigated fully, it should be possible to obtain evidence as to the configuration of any sugar or sugar derivative by a determination of its rate of oxidation. A

sample of chitosamine hydrochloride prepared from the carapace of Jasus lalandii was submitted to oxidation by the previous methods, and its rate of oxidation was found to be identical with those of galactose and arabinose as shown in table IX.

TABLE IX.

pH	$t_{\frac{1}{4}}$ (secs.)		
	Glucosamine hydrochlor.	Galactose	Arabinose
10.55	4.25	4.25	4.25
10.95	3.25	3.25	-
12.80	15.0	16.25	16.0

This is peculiar, since polarimetry of the chitosamine hydrochloride shows it to be identical with glucosamine hydrochloride, as also do its other properties.
(e.g. melting point, solubility, etc.)

This being so, it was to be expected that the oxidation rate would conform to that for glucose. Possibly the agreement of the oxidation rate with that for galactose may be due to the presence of a furanose ring in the chitosamine hydrochloride; Myrbäck (19) however, states that alkaline iodine oxidation of glucosamine results in a splitting of the molecule, and this may be significant. Clearly, more work on the relationship between sugar configuration and the rate of iodine oxidation is needed.

SECTION C.

FURTHER AVENUES of INVESTIGATION.

In view of the results obtained from the work detailed in sections A and B, it appears that further work in various directions is highly desirable, and may yield results of interest. The lines along which further work might be carried out are summarised, as follows:-

1. The oxidation rates of more sugars and sugar derivatives should be determined, in order to clarify the effects of configuration on the rate of oxidation. In particular, determination of oxidation rates for the following are highly desirable: (a) α- and β-d-glucose separately, (b) the d- and l- forms of a sugar, (c) an aldehydo - sugar, (d) talose or ribose, (e) glucosone. The significance of these particular sugars can be seen by an examination of their accepted structures.
2. Investigation of the primary oxidation product of the reaction, which may be a δ -lactone. Some preliminary work has been done in this direction by the author, as follows:- A solution of hypoiodous acid was prepared by the method of Koene (20), whose method is, briefly:- Iodine, dissolved in 95% alcohol, is then shaken with mercuric oxide to remove any iodine which has not been converted to hypoiodous acid. The solution is then rapidly filtered through a layer of mercuric oxide supported on asbestos. An alcoholic solution of hypoiodous acid is obtained in this manner, but must be used immediately to forestall decomposition.

2. continued.

This hypoiodous acid solution was reacted with a glucose solution buffered by means of a suspension of barium carbonate through which was bubbled carbon dioxide. This buffer maintains a pH of 6.4. After from 5 to 10 minutes any excess iodine was removed by shaking with phellandrene, which simultaneously floats off the barium carbonate (a method developed by Polya and Ingles (21)). Hydrogen iodide formed in the oxidation reaction can be removed by shaking with mercuric oxide, and filtering off the precipitated mercuric iodide. The pH of the resulting solution was followed over a period of time by means of a Coleman pH meter, and observed to fall steadily and progressively from a pH of approximately 6.1 shortly after the reaction had been stopped, to a pH of approximately 2.8 some two hours later. This suggests strongly that a δ-lactone has been formed and is slowly being hydrolysed. Time has not permitted this work to be put on a quantitative and exact basis, but results above suggest that when this is done, the primary oxidation product may be shown in this manner to be a δ-lactone.

3. Some early work was performed using a large excess of iodine in the oxidation, so that the reaction became one of the first order. The buffer used contained glycine, which, as it was later realised, interferes with the reaction by formation of a condensation

product with the aldose. However, the oxidation did occur under these conditions and the rate of oxidation continually increased from pH 9 to pH 12.8, following a curve of similar type to that theoretically calculated for hypoiodite ion, although showing an arrest between pH 10.0 to pH 11.5. These preliminary observations might be further pursued.

4. There is an apparent need for a new determination of the ionisation constant of hypoiodous acid.

$$\frac{[H^+]\ [IO^-]}{[HIO]} = k_a.$$

As previously mentioned, k_a has been given by Fürth as 10^{-11}; more recently however, Skrabal (22) has reported a value of 4.5×10^{-13}. If, in the previous calculations, Skrabal's value were to be used, then the curve of unionised hypoiodous acid would have a very similar shape to that in fig. V, but the maximum point would be transferred to pH 12.00. As there seems no doubt as to the active oxidising agent in the alkaline iodine oxidation of the sugars (witness the entire dissimilarity to the curve for hypoiodite ion, which is only very slightly affected by the use of Skrabal's value in the calculations, and is not affected in shape), then doubt must be cast on Skrabal's value, since the observed maximum oxidation rate is between pH 11.30 and pH 11.40. On this basis, Fürth's value, which gives a maximum at pH 11.38 cannot be

greatly in error. It should be noted that both Fürth and Skrabal used a colorimetric method, which can hardly be expected to yield results of a high order of accuracy, and hence an independent determination of this ionisation constant by a different method is highly desirable.

5. A positive proof that the final oxidation product of alkaline iodine oxidation of glucose is gluconic acid should be obtained readily by carrying out the oxidation with excess iodine in alkaline solution, followed by removal of the iodine and determination of the specific rotation of the resulting solution. This would permit detection and estimation of the gluconic acid (as previous evidence suggests it is) formed. Time has not yet allowed this test to be performed.

6. A precise investigation into full extent of the depression of the oxidation rate by carbonates and borates should give information of interest with bearing upon the relationship between sugar configuration and rate of oxidation. This is especially so in the case of mannose and talose, where it is predicted by the author that an elevation of the rate will be observed.

SUMMARY.

The oxidation of glucose (and other aldose sugars) by alkaline solutions of iodine, has been shown to occur by the following mechanism:-

$$C_6H_{12}O_6 + HIO \longrightarrow C_6H_{12}O_7 + HI \text{ (second order)}$$

The reaction is shown to occur in two stages: 1) a rapid initial oxidation, and 2) a subsequent slower oxidation. This is discussed. Optimum conditions for the reaction are stated.

Different rates of oxidation have been observed for various aldopentoses, aldohexoses, and their derivatives, and these may bear a close connection with the configurations of the respective sugars.

Preliminary indications have been obtained that the primary oxidation product is a δ -lactone.

Suggestions are made for further work in this field.

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