35. *The Oxidation of Aldoses by Hypoiodous Acid. Part III. Comparison of the Rates of Oxidation of α- and β-D-Glucose.*

By K. D. Reeve.

β-D-Glucose is shown to be oxidised by hypiodous acid, under certain conditions, at least 25 times as fast as is its α-isomer. The optimum pH for the observance of this difference in rates is 9·8 but the simultaneous mutarotation tends to equalise the two rates as oxidation proceeds. Outside the pH range 7·6—11·8, the α- and the β-form of D-glucose are oxidised at identical rates since under these conditions mutarotation is much faster than oxidation. The greater reactivity of β-D-glucose is in agreement with expectation.

Of the two pyranose forms of D-glucose, the β-form is generally the more reactive towards oxidising agents. The ratio v_β/v_α ($= r$) has been reported as 1·7 for oxidation by potassium permanganate (Kuhn and Wagner-Jauregg, *Ber.*, 1925, **58**, 1441) and 35 when bromine water is the oxidising agent (Isbell and Pigman, *Bur. Stand. J. Res.*, 1933, **10**, 337). For a wide variety of other aldoses values of r are greater than 1 for bromine-water oxidation (*idem, ibid.*, 1937, **18**, 141). Chlorous acid as an oxidising agent is anomalous in this respect, since r is less than 1 for hexoses (Jeanes and Isbell, *ibid.*, 1941, **27**, 125).

Since oxidations by iodine and bromine in aqueous solution are closely related it would be expected that r would be greater than 1 for both oxidation reactions. This has been assumed by Myrbäck (*Svensk Kem. Tidskr.*, 1940, **52**, 293) but since it has not hitherto been confirmed it was deemed desirable to test the validity of the assumption.

When either α- or β-D-glucose is oxidised in aqueous solution, a simultaneous mutarotation occurs, whereby an equilibrium solution containing 67% of the β- and 33% of the α-form is finally produced. If mutarotation is slow compared with oxidation a true measure of the ratio r can be obtained; otherwise the value of r will change during the reaction and its true initial value r^0 can be obtained only by extrapolation. The former conditions apply to bromine oxidation (Isbell and Pigman, *loc. cit.*, 1933), since the reaction is carried out at a pH (~6) where the mutarotation is slowest, while each oxidation reaction is conveniently fast. However, since the oxidising agent in aqueous iodine solutions is hypoiodous acid (Ingles and Israel, *J.*, 1948, 810), a higher pH must be employed in order to obtain a sufficient concentration of hypiodous acid for convenient oxidation rates. At these higher pH's, mutarotation rate is faster.

The variation of mutarotation rate with pH can be determined as follows. Mutarotation of D-glucose is catalysed by all Brønsted acids and bases. As the exact concentrations of all species in buffer solutions are not easy to assess, mutarotation constants have been calculated by taking into account the catalytic effects of H_2O, H_3O^+, and OH^- only. However, it has been shown that the contributions of bases such as pyrophosphate and carbonate ions are not negligible, by polarimetric measurements at 17·5° on a 5% solution of α-D-glucose at pH 8·8. The value of k_m was found to be 0·157, compared with the calculated value of 0·05 (see Table V). Thus minimum values are given in the tables.

The specific rate (k_m) of mutarotation under various conditions has been calculated from the equation

$$k_m = k_{H_2O} + k_{H_3O^+}[H_3O^+] + k_{OH^-}[OH^-]$$

data given by Smith and Smith (*J.*, 1937, 1413) being used. Table I gives some results of these calculations; others are in Table V.

TABLE I.

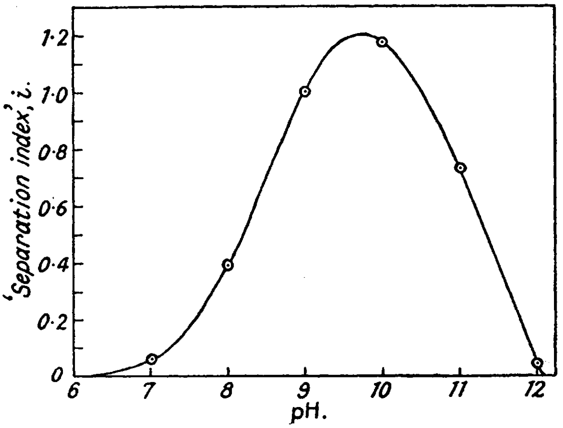
pH.	k_m. 0°.	k_m. 18°.	k_m. 25°.	pH.	k_m. 0°.	k_m. 18°.	k_m. 25°.
5	0·0017	0·0122	0·0240	10	0·0947	0·7167	1·160
6	0·0017	0·0123	0·0241	11	0·9317	7·057	11·38
7	0·0018	0·0129	0·0251	12	9·302	70·46	113·6
8	0·0026	0·0192	0·0354	13	93·00	704·5	1136·0
9	0·0110	0·0827	0·1376				

TABLE II.

pH	6	7	8	9	10	11	12
[HOI]	0·001*C*	0·01*C*	0·1*C*	*C*	9·8*C*	60·7*C*	35·7*C*
i	0·006	0·06	0·39	1·0	1·17	0·73	0·04

The optimum pH may be determined from comparative values of the " separation index " *i*. This is defined as proportional to the ratio of the initial velocities of oxidation of β-D-glucose and of mutarotation. For a constant concentration of sugar the latter is proportional to k_m and the former to the concentration of hypoiodous acid. The value of *i* is arbitrarily taken as 1 at pH 9 at 25°. Values of *i* at 25° at other pH can now be obtained from the equation $i = 0{\cdot}138[HOI]/k_mC$, where *C* is the concentration of hypoiodous acid at pH 9. The necessary comparative concentrations of hypoiodous acid in iodine–iodide solutions of constant concentration at various pH have been given by Ingles and Israel (*loc. cit.*). These and values of *i* are given in Table II.

FIG. 1.



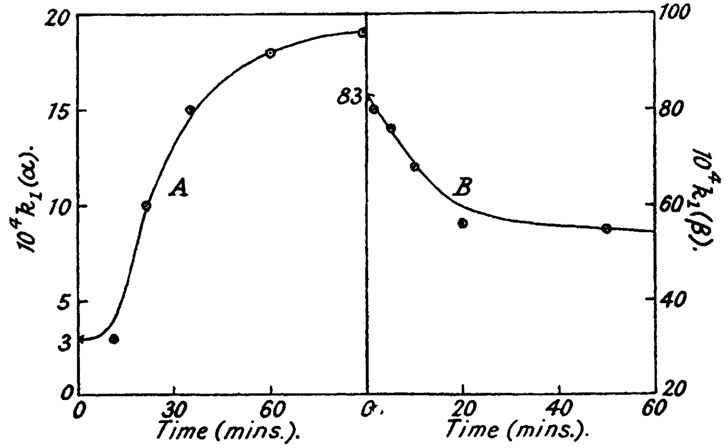
The plot of *i* against pH (Fig. 1) shows that *i* is a maximum at pH 9·8 and that outside the pH range 7·6—11·8 is less than 20% of this value. Under the latter conditions the two oxidation rates should be practically identical. Accordingly, oxidation experiments were carried out in solutions of pH as close as possible to 9·8.

The oxidation of D-glucose has been shown to be initially of the second order at pH 11·15 (Ingles and Israel, *J.*, 1949, 1249). At lower pH, iodate formation is neglible, so that the oxidation of each form should obey a second-order equation. Second-order velocity constants are calculated from the equation

$$k_2 = \frac{1}{T_\infty t}\log_e\frac{100 \times T_t}{U \times T_0}$$

where $U = \%$ of glucose unchanged and T is the sodium thiosulphate titre (see Experimental) at the time t. They decrease markedly during the oxidation of β-D-glucose and increase during the oxidation of α-D-glucose. The ratio r [$= k_2(\beta)/k_2(\alpha)$] is greater than unity at various times after reaction. The latter results show that β-D-glucose is the more readily oxidised form, and the former that mutarotation is interfering, as predicted, to cause the drifts in the values of k_2.

FIG. 2.



Extrapolations of velocity constants for Experiment 1, *Table* II.
A, *α-D-Glucose*. *B*, *β-D-Glucose*.

The following extrapolation procedure was used to obtain r^0. First-order constants were calculated for the oxidation reaction in order to be compared with the first-order mutarotation constants, the equation used being $k_1 = (1/t)\log_e(100/U)$. Here also drifts similar to those described above were observed in each set of constants. The approximate determination of r^0 depends on obtaining a series of values for $k_1(\alpha)$ and $k_1(\beta)$ and extrapolating each set to zero time. The extrapolation is easy in the case of $k_1(\beta)$, but for $k_1(\alpha)$ it is not as definite and in many cases an extrapolation to 0 is indicated. However this is not justified and where values of $k_1^0(\alpha)$ are quoted they are maximum values. A typical set of extrapolations is shown in Fig. 2.

The accuracy of such extrapolations depends on obtaining values of the specific rates of oxidation at times when the amount of mutarotation is negligible, and is improved by measuring velocities during the very early stages of the reaction. Experiments at 1·5° have been useful, since both oxidation and mutarotation proceed slowly. However, this temperature is clearly about the lowest available.

A second possibility of obtaining more accurate values of r^0 is to increase the oxidation rates without increasing the mutarotation rate. Increasing the temperature was found to be in no way advantageous since any possible advantage obtained by increasing the oxidation rates in a greater ratio than the mutarotation rate is offset by decreased accuracy of measurement of the faster oxidation rates. Increasing the iodine concentration gives a slightly improved accuracy, but the necessary use of more iodide in concentrated iodine solutions results in the depression of iodine hydrolysis and the concentration of hypoiodous acid is not increased; there is thus little resultant advantage.

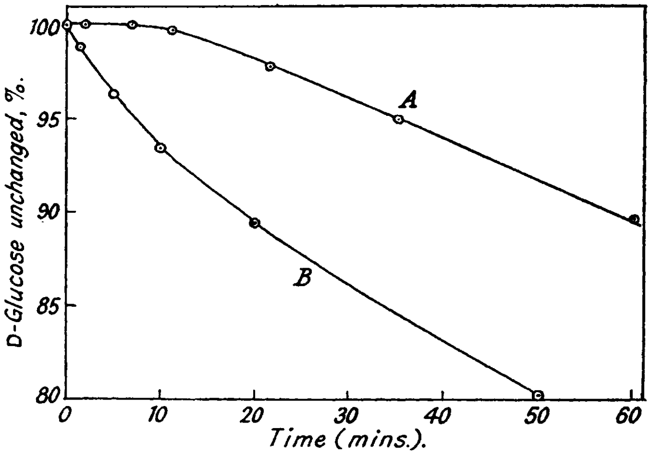
Results for seven experiments are summarised in Table III.

TABLE III.

Exp. no.	pH.	Temp.	$10^4k_1^0$ (min.$^{-1}$)		r^0.
			α-.	β-.	
1	9·2	1·5°	3	83	28
2	9·2	1·5	5	137	28
3	8·3	17·0	28	220	8
4	8·8	17·0	90	1660	18
5	10·1	17·0°	800	10,000	13
6	8·4	35·0	1800	10,000	6
7	9·4	35·0	4000	16,000	4

Parts of the oxidation curves for experiment 1 are shown in Fig. 3. For reasons mentioned above and below (see " Materials " in the Experimental section) these values of r^0 cannot be regarded as accurate but are minimum values. It is in just those experiments (3, 6, 7) where measurements are least accurate that low values of r^0 are obtained.

FIG. 3.



Oxidation of α- (*A*) *and* β-D-*glucose* (*B*) *separately* (*Expt.* 1, *Table* III).

Thus as a first approximation it can be taken that D-glucose solution is oxidised by hypoiodous acid through the β-form and that α-D-glucose is istelf not oxidised. From this it follows that the initial velocity constant k^0 (obtained by extrapolation) for various solutions should be proportional to the percentage of β-D-glucose in the solution. To test this, a mixture of equal weights of D-glucose samples containing 86% and 0% of β-D-glucose respectively was subjected to oxidation, and the initial velocity constant compared with that of the sample containing 86% of β-D-glucose. k^0 for the latter was 0·00360; for the mixture it should thus be 0·00180, in good agreement with the observed value of 0·00185.

It should also be possible to calculate approximate percentages of β-D-glucose in solution at various times during oxidation by extrapolating velocity constants to times other than $t = 0$. An experiment on α- or β-D-glucose included determination of the titre T and the percentage (U) of unchanged glucose, at about four time intervals. The percentage of β-D-glucose at time t_1 was found by using the equation

$$k_2 = \frac{1}{(t - t_1)T} \log_e \frac{U_1 T_t}{U_t T_1}, \quad \text{where } t = t_2, t_3, t_4.$$

The constants so obtained were extrapolated to give k_2 at time t_1.

From the equations

$$k_2 = \frac{1}{(t - t_2)T} \log_e \frac{U_2 T_t}{U_t T_2}, \quad \text{where } t = t_3, \text{t}_4$$

and

$$k_2 = \frac{1}{(t - t_3)T} \log_e \frac{U_3 T_t}{U_t T_3}, \quad \text{where } t = t_4$$

the values of k_2 at times t_2 and t_3 were obtained in a similar manner. These values of k_2 at 0, t_1, t_2, t_3 were then each taken as proportional to the percentage of β-D-glucose in each solution of experiment 1 (Table III) at the respective times, to give the values in Table IV.

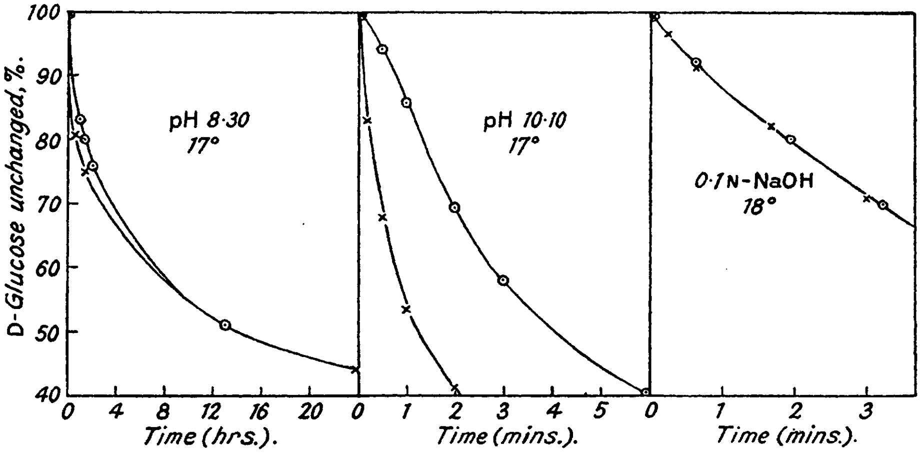
TABLE IV.

(*a*) β-D-Glucose, 86%.			(*b*) α-D-Glucose, 100%.		
t (mins.).	10^3k_2.	β-, %.	t (mins.).	10^3k_2.	β-, %.
0	3·6	86	0	0·10	2·5
5	3·4	81	11	0·80	20
10	2·2	55	21·5	0·96	24
20	2·2	55	35	1·13	28

These values show that the normal equilibrium solution containing 67% of β-D-glucose is not attained during reaction, but since β-D-glucose is oxidised preferentially a dynamic equilibrium is reached in which the percentage of this isomer is much less than that in the normal equilibrium solution. In case (*b*) β-D-glucose is oxidised almost as soon as it is formed through mutarotation, and hence the increase in the percentage of this isomer is slower than its decrease in case (*a*) where both mutarotation and oxidation reactions combine in impoverishing the solution in the more reactive form.

At a sufficiently high pH, mutarotation should be fast enough to maintain the normal equilibrium percentages in the solution. This is shown to be true by the identity of the rates of oxidation of α- and β-D-glucose in 0·1N-sodium hydroxide at 18°. Similarly, the normal equilibrium would be maintained in neutral or slightly acid solution owing to the very slow oxidation rates. Fig. 4 summarises the effect of pH on the relative rates of oxidation.

FIG. 4.



Oxidation of α- and β-D-glucose separately.
○ *α-D-Glucose.* × *β-D-Glucose.*

Further evidence may be obtained by considering the figures of Table V (expt. numbers refer to Table III).

TABLE V.

Expt. no.	k_m.	k_1^0 (β).	k_1^0 (α).	k_m/k_1^0 (α).	Expt. no.	k_m.	k_1^0 (β).	k_1^0 (α).	k_m/k_1^0 (α).
1	0·01	0·014	0·0005	20	5	0·90	1·0	0·08	11
2	0·01	0·008	0·0003	33	6	0·14	1·0	0·18	0·8
3	0·03	0·022	0·0028	11	7	0·60	1·6	0·40	1·5
4	0·05	0·165	0·009	6					

It may be noted that in all cases but one the oxidation rate of α-D-glucose is less than the mutarotation rate. This is in agreement with the hypothesis that α-D-glucose must await mutarotation to β-D-glucose before being oxidised, since $k_1^0(\alpha)$ must not exceed k_m. On the other hand, k_m is never negligible in comparison with $k_1^0(\beta)$. The case where $k_1^0(\alpha) > k_m$ is probably caused by the calculated value of k_m being much too low.

The necessary conditions for accurate assessment of $k_1^0(\beta)$ and $k_1^0(\alpha)$ would be $k_m < k_1^0(\alpha) < k_1^0(\beta)$. $k_1^0(\alpha)$ could not be shown to be zero unless mutarotation could be reduced to zero.

The great difference in the rates of oxidation of α- and β-D-glucose by bromine water has been traced to a steric factor by Isbell and Pigman (*loc. cit.*, 1933). A similar mechanism may also apply to hypoiodous acid oxidations.

EXPERIMENTAL.

Buffers.—Buffers used included those described in Part I of this series, sodium pyrophosphate–hydrochloric acid buffers (Israel and Reeve, *Australian Chem. Inst. J. and Proc.*, 1949, **16**, 97) and a phosphate buffer (Sørensen, cf. Britton, " Hydrogen Ions," Chapman and Hall, 1932, p. 241). All solutions were checked for pH after addition of the iodine to the buffer at the temperature of reaction, by using a pH electrometer (N. L. Jones, Melbourne) standardised against 0·05M-sodium borate (pH 9·25 at 20°). The interference in the reaction of the usual buffers for the pH range 8·8—10·1 (Ingles and Israel, *J.*, 1948, 810) prevented measurements in this range.

Materials.—Materials used were of Analytical Reagent grade, except α- and β-D-glucose. α-D-Glucose used was a B.D.H. sample and when checked polarimetrically gave an initial value of 112·0° for $[\alpha]^{20}_{\mathrm{D}}$ (Bates, " Polarimetry and Saccharimetry of Sugars," U.S. Dept. of Commerce, gives 112·2° for α-D-glucose), indicating that the sample was pure. β-D-Glucose was prepared by treating D-glucose for several minutes with boiling pyridine, cooling the mixture, and filtering off the precipitated β-D-glucose–pyridine complex. The pyridine was driven off in an air-oven at 105° and the sample placed in a vacuum-desiccator for several hours (Beilstein, Vol. XXXI, p. 87). When checked polarimetrically an initial specific rotation of 31·8° was obtained (Bates, *op. cit.*, gives 18·7°), indicating an 86% content of β-D-glucose.

The $[\alpha]^{20}_{\mathrm{D}}$ of equilibrium solutions of both sugars was 52·6° (Bates gives 52·7°).

The impurity of the β-D-glucose used would indicate a slight correction (+16%) to r^0 but the accuracy of values in Table III is not high enough for such a correction to have much significance. Thus r^0 in Table III is uncorrected.

Kinetic Methods.—Two general methods were used.

(*a*) Where the oxidation was slow enough, solid glucose was added to a mixture of buffer and iodine solution kept within 0·1° of the temperature stated. For 1·5°, an ice-water bath was used. A required amount of this solution was withdrawn at various times and run into 3% sulphuric acid (50 ml.). The liberated iodine was titrated against 0·02N-sodium thiosulphate (micro-burette).

(*b*) The above technique was not satisfactory when the oxidation rate was fast, since the glucose takes a finite time to dissolve. In these cases a separate flask was used for each determination. The glucose was dissolved in a standard amount of distilled water and as soon as possible added to the reaction flask. A stock solution of glucose could not be used since a complete experiment occupied about half an hour.

In each case the residual iodine titre at the end of the reaction was determined by making the solution alkaline with 0·1N-sodium hydroxide, the reaction then proceeding to completion within a few minutes.

The following is a summary of the techniques used for each experiment of Table III :

Expt. no.	Buffer type.	Tech-nique.	Buffer, ml.	I_2 soln., ml.	Sample withdrawn, ml.	I_2 soln., M.	Glucose, g.
1	Pyrophosphate	(*a*)	150	50	25	0·050	0·2
2	,,	(*a*)	150	50	25	0·100	0·2
3	Phosphate	(*a*)	50	55	10	0·037	0·2
4	Pyrophosphate	(*a*)	150	50	25	0·037	0·2
5	Carbonate	(*b*)	15	5	—	0·037	0·02
6	Pyrophosphate	(*b*)	15	5	—	0·037	0·02
7	Carbonate	(*b*)	15	5	—	0·037	0·02

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