405. *Heats of Activation in the Mutarotation of Glucose. Part I. Catalysis in Sodium Hydroxide Solutions.*

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The determination of the catalytic coefficient for the hydroxyl ion in the mutarotation of glucose presents considerable difficulty, mainly because of its extraordinary magnitude, which necessitates measurements in solutions of extremely small hydroxyl-ion concentration. If these are obtained by means of a buffer or carbonates, it is necessary, as pointed out by Brönsted and Guggenheim (*J. Amer. Chem. Soc.*, 1927, **49**, 2554), to allow for the contribution to the total catalytic effect made by these catalytically active substances. This is not easy to assess precisely and it is preferable to eliminate it entirely, as was first done successfully by Lowry and Wilson (*Trans. Faraday Soc.*, 1928, **24**, 683), by the use of faintly alkaline unbuffered solutions. The interpretation of the results is then much simpler, but the assumption that basic catalysis is due solely to hydroxyl ions is not necessarily correct, since it neglects possible effects due to glucosate ions, which must be present in perceptible amounts in alkaline solutions. The strongly basic character of these ions suggests, indeed, a very considerable catalytic activity, which should be taken into account in the evaluation of k_{OH}. This has been attempted in the present experiments, in which mutarotation has been studied in dilute solutions of sodium hydroxide.

In such a solution, the observed velocity is the sum of partial velocities, *viz.*,

$$V_{\mathrm{obs.}} = V_{\mathrm{H_2O}} + V_{\mathrm{OH}} + V_{\mathrm{G}} = V_{\mathrm{H_2O}} + k_{\mathrm{OH}} \cdot C_{\mathrm{OH}} + k_{\mathrm{G}} C_{\mathrm{G}}$$

where the subscript G relates to the glucosate ion, or, writing $V_{\mathrm{obs.}} - V_{\mathrm{H_2O}} = V_{\mathrm{corr.}}$, then $V_{\mathrm{corr.}}/C_{\mathrm{G}} = k_{\mathrm{G}} + k_{\mathrm{OH}} \cdot C_{\mathrm{OH}}/C_{\mathrm{G}}$, and since C_{OH} is defined by the relation $C_{\mathrm{GH}} \cdot C_{\mathrm{OH}}/C_{\mathrm{G}} = K_w/K_a = K_h$, where K_a refers to the equilibrium $\mathrm{GH} \rightleftarrows \mathrm{G}' + \mathrm{H}^{\bullet}$, the above expression may be rewritten as

$$V_{\mathrm{corr.}}/C_{\mathrm{G}} = k_{\mathrm{G}} + K_h \cdot k_{\mathrm{OH}}/C_{\mathrm{GH}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

It follows that in a series of solutions in which both C_{G} and C_{GH} are varied, the plot of $V_{\mathrm{corr.}}/C_{\mathrm{G}}$ against $1/C_{\mathrm{GH}}$ will be a straight line, the intercept of which gives k_{G} and the slope of which is $K_h \cdot k_{\mathrm{OH}}$. This will be valid only if no medium effect accompanies the alteration in the glucose concentration. The figures given below show this to be the case, since the velocity coefficient for mutarotation in water at 15° is independent of the concentration of glucose :

C_{GH}, moles/l.	0·759	0·512	0·410	0·327
$10^5 V_{\mathrm{H_2O}}$	898	894	895	900

The applicability of equation (1) has been tested by measurements at 0°, 5°, 10°, and 15°. Similar results were obtained at each temperature, those for 0° and 10° being given in Table I. At 0° the glucose concentration ranged from 0·774*M* to 0·257*M*, and that of sodium hydroxide from $7{\cdot}78 \times 10^{-3}$ to $2{\cdot}09 \times 10^{-3}M$. At 10° the concentrations were restricted to a narrower range by the necessity of keeping the velocity at a reasonably slow value, susceptible of accurate measurement.

TABLE I.

Mutarotation of glucose in sodium hydroxide solutions at 0°.

C_{GH}	0·774	0·724	0·7365	0·739	0·495	0·403	0·336	0·2574
$C_{\mathrm{N_2OH}} \times 10^3$	7·88	5·95	3·035	1·520	4·075	3·020	2·093	2·115
$C_{\mathrm{G}} \times 10^3$	7·82	5·90	3·01	1·507	4·02	2·97	2·055	2·065
$V_{\mathrm{obs.}}$	0·100	0·0788	0·0404	0·0213	0·0691	0·0595	0·0466	0·0570
$V_{\mathrm{corr.}}$	0·0983	0·0771	0·0387	0·0196	0·0674	0·0578	0·0449	0·0553
$V_{\mathrm{corr.}}/C_{\mathrm{G}}$	12·6	13·1	12·9	13·0	16·8	19·4	21·8	26·8
$V_{\mathrm{corr.}}/C_{\mathrm{G}}$, calc. *	12·6	13·1	12·9	12·9	16·7	19·4	22·1	27·3
V_{G}	0·0410	0·0309	0·0154	0·0079	0·0212	0·0157	0·0109	0·0110

* Calculated from $V_{\mathrm{corr.}}/C_{\mathrm{G}} = 5{\cdot}2 + 5{\cdot}7/C_{\mathrm{GH}}$.

Mutarotation of glucose in sodium hydroxide solutions at 10°.

C_{GH}	1·117	1·107	0·946	0·724	0·718	0·617	0·512	0·503
$C_{\mathrm{NaOH}} \times 10^3$	1·810	1·816	1·845	1·683	1·412	1·363	1·229	1·223
$C_{\mathrm{G}} \times 10^3$	1·797	1·803	1·829	1·664	1·396	1·340	1·209	1·203
$V_{\mathrm{obs.}}$	0·0712	0·0710	0·0805	0·0875	0·0730	0·0786	0·0810	0·0808
$V_{\mathrm{corr.}}$	0·0659	0·0657	0·0752	0·0822	0·0677	0·0733	0·0757	0·0755
$V_{\mathrm{corr.}}/C_{\mathrm{G}}$	36·7	36·4	41·1	49·4	48·5	54·7	62·6	62·7
$V_{\mathrm{corr.}}/C_{\mathrm{G}}$, calc. †	37·0	37·1	40·8	48·4	48·7	54·1	62·0	62·7
V_{G}	0·0284	0·0285	0·0290	0·0264	0·0222	0·0214	0·0193	0·0192

† Calculated from $V_{\mathrm{corr.}}/C_{\mathrm{G}} = 15{\cdot}7 + 23{\cdot}7/C_{\mathrm{GH}}$.

In rows 1 and 2 respectively are given the concentrations of free glucose and of titratable sodium hydroxide. The concentrations of the glucosate ion given in the third row have been calculated from the figures in rows 1 and 2, K_h being taken as 6×10^{-3} at 0° and 8×10^{-3} at 10° (see p. 1826). A comparison of the figures in rows 2 and 3 shows that the hydrolysis of the glucose salt does not exceed about 2%, and that consequently a considerable error in the values assigned to K_h would cause no appreciable alteration to the values of C_{G}. The figures given in row 5 for $V_{\mathrm{corr.}}$ are obtained by subtracting from the observed velocities (row 4) that due to water, which is 0·001715 at 0° and 0·00532 at 10°, all velocities being expressed in the units l./g.-mol.-min. The resulting values of $V_{\mathrm{corr.}}/C_{\mathrm{G}}$ are given in row 6, and in row 7 those calculated by means of the equation at the foot of each table. It will be seen that these equations reproduce the experimental results with considerable accuracy, the maximum divergence being about 2%, which is not greater than the possible experimental error. The values obtained for the catalytic coefficient for the glucosate ion are 5·2 at 0°, and 15·7 at 10°, which indicate a catalytic activity over 100 times greater than that for the hydrion at the same temperature. The partial velocities due to the glucosate ion given in the last rows of the tables show that in all the solutions investigated it accounts for a considerable proportion of the total velocity, this proportion varying from about 18% to 41%.

The results obtained for all four temperatures are summarised in Table II. The heat of activation for catalysis by the glucosate ion derived from the figures in col. 2 is 17,000 cals. The reaction conforms closely to the Arrhenius equation, as is shown by the numbers in col. 3, which are calculated from the expression $\log_{10} k_G = 14{\cdot}336 - 17{,}000/2{\cdot}3026RT$.

TABLE II.

Temp.	k_G.	k_G, calc.	$K_h . k_{OH}$.	$K_h . k_{OH}$, calc.	k_{OH}.
0°	5·2	5·2	5·7	5·7	930
5	9·2	9·1	12·0	11·8	1700
10	15·7	15·6	23·7	23·9	2950
15	26·3	26·4	47·2	47·3	5100

In order to obtain the heat activation for hydroxyl-ion catalysis, E_{OH}, it is preferable to use as a basis the reasonably accurate values of $K_h . k_{OH}$ (Table II, col. 4), rather than those of k_{OH} itself, the accuracy of which is limited by the uncertainty as to the values of K_h. The figures given in col. 5, which are calculated from the equation $\log_{10} K_h . k_{OH} = 18{\cdot}387 - 22{,}000/2{\cdot}3026RT$, show that the effect of temperature on $K_h . k_{OH}$ can be represented by an Arrhenius equation, with an apparent heat of activation, E', of 22,000 cals. This is not E_{OH}, which, as shown below, is less by an amount Q_h, representing the heat of hydrolysis of sodium glucosate. Since, from the above, $\log K_h + \log k_{OH} = \text{const.} - E'/RT$, then

$$d \log K_h/dT + d \log k_{OH}/dT = E'/RT^2$$

and hence

$$Q_h/RT^2 + E_{OH}/RT^2 = E'/RT^2$$

or

$$E_{OH} = E' - Q_h$$

To evaluate E_{OH} it was necessary to undertake the calorimetric determination of Q_h, since no value was available. This work is not yet completed, but the preliminary measurements of the heat of neutralisation of glucose by sodium hydroxide show that it is approximately 4300 cals. at 20°; E_{OH} is thus $22{,}000 - 4300 = 17{,}700$ cals. This can be regarded as substantially correct, although it may be necessary to amend it somewhat if the variation of Q_h with temperature proves to be considerable.

In the last col. of Table II are given values for k_{OH}, calculated from the corresponding values of $K_h . k_{OH}$ and K_h. The latter are derived from ${}_{20}K_h$, Q_h being assumed to be 4300 cals. The value adopted for ${}_{20}K_h$ is $1{\cdot}05 \times 10^{-2}$, obtained by taking ${}_{20}K_a = 6{\cdot}5 \times 10^{-13}$ (Ölander, *Z. anorg. Chem.*, 1925, **136**, 61) and ${}_{20}K_w = 6{\cdot}8 \times 10^{-15}$ (Harned and Hamer, *J. Amer. Chem. Soc.*, 1933, **55**, 2194). This value of K_w refers to pure water, but its use in this case is probably justifiable, for it may be assumed that the effect of the presence of glucose will be similar to that of glycerol, which produces no noticeable alteration in K_w (Colvin, J., 1925, **127**, 2788). The only trustworthy previous determination of k_{OH} is that of Lowry and Wilson (*loc. cit.*), who for 20° obtained $k_{OH} = 8000$. An extrapolation of the present results to this temperature, with $E_{OH} = 17{,}700$ cals., gives ${}_{20}k_{OH} = 8800$. This close agreement must, to some extent, be fortuitous, for, since Lowry and Wilson's value includes glucosate-ion catalysis, the true value of k_{OH} will be less. The real discrepancy is probably of the order of 30%, which is not excessive in view of the difficulties involved in the determinations. It is possibly due to the difference in the values assigned to K_w in the two investigations.

It is proposed to discuss in a later paper the bearing of these results and of others on current views of reaction mechanism. One point may, however, be referred to here. This is in connexion with the theory of acid and basic catalysis advocated principally by Euler, according to which an instantaneous reaction takes place between the acid or basic catalyst and the substrate with the formation of a highly reactive ion, which then undergoes a spontaneous change at the measured reaction rate. In an alkaline glucose solution, this reactive ion is identified with the glucosate ion (Euler and Ölander, *Z. anorg. Chem.*, 1926, **152**, 113), and the catalytic effect of the hydroxyl ion is attributed solely to its ability to increase the concentration of this ion. The experimental basis for this view was found in the apparent proportionality between the acceleration produced and the concentration of glucosate ions in the solution (*Trans. Faraday Soc.*, 1928, **24**, 651) as represented by the relation $V_{\text{corr.}} =$ const. $\times C_G$. This is seen to be incompatible with the equation (1), except in the special case when C_{GH} is maintained constant. In general, no proportionality exists between $V_{\text{corr.}}$ and C_G, and the basic catalysis of mutarotation cannot, therefore, be explained in terms of the glucosate ion in the manner Euler has suggested.

EXPERIMENTAL.

Thermostatic Arrangements.—In order to obtain temperatures below that of the room, a refrigerator was used in conjunction with the thermostat, which consisted of a well-lagged copper bath of about 25 gallons capacity and fitted with electrical regulator, heating lamp, and stirrer. The refrigerator compressor was of the air-cooled type, methyl chloride being the refrigerant, and had an extraction capacity of about 1000 B.T.U. per hour. A special cooling unit was supplied by the makers (Messrs. Automatic Refrigerators Ltd., London) and consisted of a spiral coil of tinned copper tubing, about 12″ long and 7″ in diameter. This was fixed in a vertical position, completely immersed in the water, near one end of the bath, with the compressor mounted on a wooden stand in close proximity. The refrigerator was run continuously, and a steady temperature secured by the intermittent action of a 300-watt heating lamp. By this means, merely by adjusting the thermoregulator, any temperature between that of the room and 0° could be reached, and could be maintained to $\pm$ 0·02°. Enough ethylene glycol was added to the water in the bath to lower the freezing point to about $-$ 2°, and under these conditions, with adequate stirring, no freezing of the liquid on the cooling coil takes place. The apparatus has been in use for about 18 months and has proved entirely satisfactory.

Kinetic Measurements.—The reaction velocities were measured polarimetrically, the mercury green line, $\lambda = 5461$, being used as a source of illumination. The 2-dcm. jacketed polarimeter tube was kept at constant temperature by a rapid flow of water from the thermostat, with precautions to avoid alterations in the temperature of this water during its circulation. The short delivery tube from the thermostat, and the polarimeter tube, were both surrounded by efficiently lagged outer casings, leaving an annular space between. A rapid current of air, which had attained thermostat temperature by passage through a metal coil immersed in the water, was blown through this annular space over the entire lengths of delivery and polarimeter tubes. This insulation proved adequate, since the change in temperature of the water during circulation did not exceed $\pm$ 0·02°, even under the most adverse conditions.

The unbuffered nature of the solutions and the extreme dilution of the sodium hydroxide necessitated all the essential manipulations being carried out in an atmosphere free from carbon dioxide. Therefore, throughout each of the operations of (*a*) filling a small burette with standard sodium hydroxide, (*b*) running a measured volume of the sodium hydroxide from the burette, (*c*) dissolution of the glucose in water, (*d*) mixing of these solutions, and (*e*) transfer of the mixture to the polarimeter tube, the liquids were either in a closed vessel swept free of carbon dioxide, or were protected by a rapid stream of carbon dioxide-free air passing through or around the liquid. The velocity measurements were reproducible to about 2%, which indicates that contamination of the solutions with carbon dioxide had been practically eliminated. The concentration of glucose in the reaction mixture was obtained by comparison of the final rotatory power of the solution with that given by glucose solutions of known concentration. Since there was a known weight of glucose in the solution, this gave the final volume of the solution, which then allowed of the calculation of the concentration of sodium hydroxide from the quantity taken.

Materials.—B.D.H. AnalaR dextrose was employed and gave a velocity coefficient for mutarotation in water of 0·0147 at 20°, as compared with the standard value of 0·0146 (Richards, Faulkner, and Lowry, J., 1927, 1733). A portion was crystallised from aqueous alcohol, but the velocity of mutarotation in water and in alkaline solutions was unchanged, indicating the absence of any appreciable amount of catalytically active impurity. Sodium hydroxide was prepared free from carbonate, and stored in a silica flask at a concentration of about 0·01*N*. No change in normality was detectable during a period of one month.

SUMMARY.

1. It has been shown that the glucosate ion is an active catalyst in the mutarotation of glucose in alkaline solutions, and values have been derived for the catalytic coefficients of glucosate and hydroxyl ions at 0°, 5°, 10°, and 15°.

2. The heats of activation are 17,000 cals. and 17,700 cals. respectively for glucosate- and hydroxyl-ion catalysis.

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