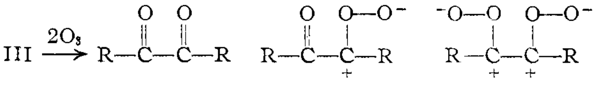
Because of its unfavorable charge distribution VI seems unlikely as a reaction intermediate. Yet IV cannot be formed unless VI also is formed, and evidence is presented in the Experimental section that biacetyl can be produced directly by ozonization of hexamethylbenzene (either in carbon tetrachloride or acetic acid) without hydrolysis of the reaction products. Products V and VI may be presumed to undergo polymerization to form polymeric peroxides or, in acetic acid, to form hydroperoxides by reaction with the solvent. On hydrolysis under reducing conditions these compounds should break down to diketone IV. In one variation of the suggestions concerning the reaction mechanism intermediate III may be pictured as undergoing polymerization or reaction with hydroxylic solvent before its double bonds are attacked by ozone.



$$\mathrm{III} \xrightarrow{2O_3} R\text{—}\overset{\overset{\displaystyle O}{\|}}{C}\text{—}\overset{\overset{\displaystyle O}{\|}}{C}\text{—}R \qquad R\text{—}\overset{\overset{\displaystyle O}{\|}}{C}\text{—}\underset{+}{\overset{\overset{\displaystyle O\text{—}O^-}{|}}{C}}\text{—}R \qquad R\text{—}\underset{+}{\overset{\overset{\displaystyle ^-O\text{—}O}{|}}{C}}\text{—}\underset{+}{\overset{\overset{\displaystyle O\text{—}O^-}{|}}{C}}\text{—}R$$

IV V VI

Product V is very likely susceptible to rearrangement to the isomeric anhydride,[28] IV. It is therefore reasonable that, as is the case, acetic acid should be obtained by hydrolysis of the ozonization products of hexamethylbenzene and mesitylene in carbon tetrachloride and that propionic acid should be obtained similarly from hexaethylbenzene. It is noteworthy, however, that acetic acid has been detected, by gas–liquid chromatography, as an immediate product of reaction of mesitylene and ozone in carbon tetrachloride. It is conceivable that this acid was formed by thermal decomposition of a reaction product at the relatively high temperature (~100°) of the chromatograph column.

Acknowledgment.—The authors are indebted to the National Science Foundation for a grant in support of this research.

[28] R. Criegee and M. Lederer, *Ann.*, **583**, 29 (1953).

DAVIS, CALIFORNIA

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HEBREW UNIVERSITY]

The Kinetics of the Oxidation of D-Glucose by Bromine and by Hypobromous Acid

BY B. PERLMUTTER-HAYMAN AND A. PERSKY

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The kinetics of the oxidation of D-glucose by aqueous bromine solutions has been reinvestigated. From the dependence of the rate of reaction on bromide concentration it was concluded that molecular bromine is the oxidizing agent, tribromide and hypobromous acid making only negligible contributions. This was confirmed by the slowness of the reaction of hypobromous acid with glucose. The dependence of the reaction rate on *p*H is consistent with the assumption that the anionic form of glucose is oxidized much faster than glucose itself.

The oxidation of D-glucose by bromine has been the subject of several investigations.[1–4] Bunzel and Mathews[2] studied the kinetics in dilute acid solutions and interpreted their results on the assumption that OH^- takes part in the rate-determining step. Isbell and Pigman[3] carried out an extensive study at the *p*H of a $BaCO_3$–CO_2 mixture. The dependence of the rate on bromide concentration pointed to free bromine as the only oxidizing agent. The primary product was the δ-lactone of gluconic acid. Furthermore, β-D-glucose was found to react about 35 times faster than the α-form. More recently[4] it was concluded from the strong increase of the rate with increasing *p*H that hypobromous acid is the oxidizing agent in bromine water.

In view of these discrepancies and as a continuation of our program[5–7] to investigate the specific oxidizing properties of bromine and of hypobromous acid it seemed interesting to reinvestigate the oxidation of D-glucose by bromine water, measuring *both* the dependence on bromide concentration and on *p*H. A direct measurement of the rate of reaction between hypobromous acid and glucose also seemed desirable.

Experimental

The experiments were carried out at 0°, in glass-stoppered erlenmeyer flasks immersed in a Dewar bottle containing ice and water. The progress of the reaction was followed by iodometric titration, using 0.01 *N* thiosulfate solution.

The *p*H was measured at the beginning and at the end of each reaction, using a Beckman Model G *p*H-meter. Unless otherwise stated, it changed by less than 0.1 unit. The buffers used were Na_2HPO_4–NaH_2PO_4, sodium acetate–acetic acid, NaH_2PO_4–phosphoric acid, and appropriate concentrations of sulfuric acid, thus covering the desired *p*H range.

Hypobromous acid solutions were prepared as usual.[6] All the reagents were "Analar"-grade. The α-D-glucose was Hopkin and Williams, and the β-D-glucose Nutritional Biochemical Corporation.

The concentration of the oxidizing agent was between (5 to 10) × 10^{-3} *M*, and that of D-glucose was always in excess and was varied between 0.025 and 0.500 *M* according to conditions, in order to get convenient reaction rates.

When experiments were repeated, their rate constants agreed within less than ±3%.

Rate constants are expressed in $\text{mole}^{-1}\ \text{l.}\ \text{min.}^{-1}$.

Results

1. The Oxidation of α- and of β-D-Glucose, and the Influence of Anomerization.

—At *p*H 4.95,

[1] J. W. Green. *Advances in Carbohydrate Chem.*, **3**, 129 (1948).

[2] H. H. Bunzel and A. P. Mathews, THIS JOURNAL, **31**, 464 (1909).

[3] H. S. Isbell and W. Pigman, *J. Research Natl. Bur. Standards*, **10**, 337 (1933); **18**, 141 (1937).

[4] K. C. Grover and R. C. Mehrotra, *Z. physik. Chem. (Frankfurt)*, **14**, 345 (1958).

[5] L. Farkas, B. Perlmutter and O. Schächter, THIS JOURNAL, **71**, 2829 (1949).

[6] Y. Knoller and B. Perlmutter-Hayman, *ibid.*, **77**, 3212 (1955).

[7] L. Binoun and B. Perlmutter-Hayman, *Bull. Research Council Israel*, **A5**, 52 (1955).

a freshly prepared solution of α-D-glucose, and a freshly prepared solution of β-D-glucose were allowed to react with bromine. A plot of log $(b - x)/(a - x)$ (b and a are the initial molarities of glucose and of oxidizing agent, respectively, and x that of the product) against time yielded a curve whose slope increased with time in the first case, but decreased in the second. This confirms the greater reactivity of the β-form, and at the same time shows that the rate of anomerization is comparable to that of oxidation of β-D-glucose.

We can assume that within experimental accuracy the rate equation may be written

$$dx/dt = k^{\beta}[\beta\text{-D-glucose}](a - x) \qquad (1)$$

It would be difficult, however, to calculate the value of [β-D-glucose] at every stage of a given run, since oxidation and anomerization follow different orders, and both are affected by a change in pH, but in different ways.[8a] Instead, when starting with an anomerically equilibrated solution (containing the α- and β-forms[3] in the ratio 0.37 to 0.63), we considered the two extremes (a) No transformation from α to β-form takes place while the oxidation is measured and equation 1 becomes

$$dx/dt = k^{\beta}(0.63b - x)(a - x) \qquad (2)$$

(b) Anomerization is fast in comparison with oxidation, when equation 1 becomes

$$dx/dt = 0.63k^{\beta}(b - x)(a - x) \qquad (3)$$

According to conditions (pH, nature of oxidant) we used equation (2) or (3). When in doubt, we calculated our results according to both. Since the ratio of the two constants is $(b - 1.6x)/(b - x)$ and b is always in large excess, the difference between the results obtained from the two assumptions is at most 10%, and usually much smaller, and in no case was found to obscure the conclusions which can be drawn from our results.

2. Dependence of the Rate of Reaction on Bromide Concentration.—If we consider the three oxidizing species to be Br_2, Br_3^- and HOBr, the meaning of k^{β} in equation 1 becomes

$$k^{\beta} = k_{Br_2}K_3/(K_3 + [Br^-]) + k_{Br_3^-}[Br^-]/(K_3 + [Br^-]) + k_{HOBr}K_3K_h/(K_3 + [Br^-])[Br^-][H^+] \qquad (4)$$

where K_3 is the dissociation constant of tribromide, K_h the hydrolysis constant of bromine, and use is made of the fact that under our experimental conditions

$$[HOBr] \ll [Br_2] \qquad (5)$$

A series of 6 experiments was carried out at pH 4.45, where sodium bromide was added to the reaction mixture in concentrations between 0.035 to 0.365 M. The values of k^{β} were found to decrease with increasing bromide concentration. A plot of these values against $[1/(K_3 + [Br^-])]$ is shown in Fig. 1. (For K_3 we took the value[3] of 0.051, and for $[Br^-]$ its mean value during each reaction, taking tribromide formation into account.) From the fact that a straight line is obtained we conclude the contribution of the third member in equation 4 to be negligible; furthermore, the fact that this straight line passes through the origin or very near to it shows that as $[Br^-] \rightarrow \infty$ and therefore $[Br_3^-] \rightarrow (a - x)$, $k^{\beta} \rightarrow 0$. Hence, $k_{Br_3^-} \ll k_{Br_2}$.

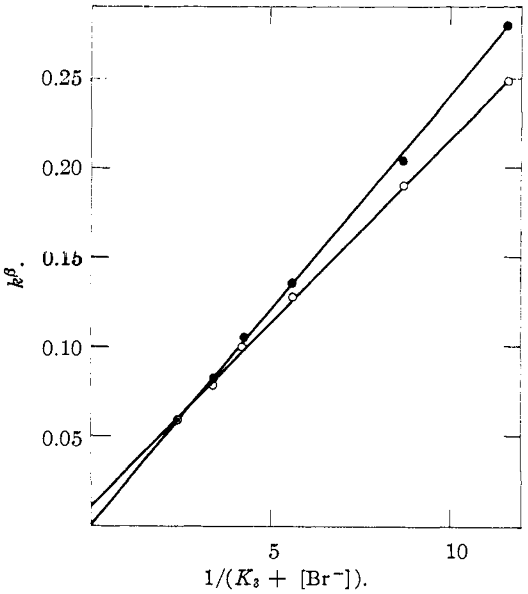

Fig. 1.—The dependence of k^{β} on $1/(K_3 + [Br^-])$: open circles, calculated on the assymption of anomeric equilibrium; closed circles, calculated on the assumption that the rate of anomerization is negligible in comparison with the rate of oxidation.

We thus confirm the view that *in bromine water, molecular bromine is the oxidizing agent*, and equation 4 reduces to

$$k^{\beta} = k_{Br_2}K_3/(K_3 + [Br^-]) \qquad (6)$$

From this, or from the slope of the straight line in Fig. 1 which is equal to $k_{Br_2}K_3$ we calculate a mean value of

$$k_{Br_2} = 0.47_4 \text{ at } p\text{H } 4.45$$

3. Oxidation by Hypobromous Acid.—In view of relation 5, the findings of the previous section yield no information about k_{HOBr} (except that it can*not* be by many orders of ten *larger* than k_{Br_2}). However when log $(b - x)/(a - x)$ was plotted against time for a reaction which initially contained pure hypobromous acid as an oxidizing agent, the curve started very flat and became steeper and steeper as the reaction proceeded. Now, during the oxidation, bromide is formed which reacts with unchanged hypobromous acid liberating bromine which in turn attacks glucose.[7] We can therefore conclude from our experimental finding that the bromine thus liberated reacts at a higher specific rate than the hypobromous acid.

For quantitative conclusions to be drawn, bromine must therefore be absent. This was again[5,7] achieved by the addition of silver nitrate. Again[5,7] this was found to catalyze the reaction, but when a series of experiments was carried out at $[AgNO_3] \cong (0.5 \text{ to } 4) \times 10^{-3}$ M, the plot of k^{β} *vs.* $[AgNO_3]$ lent itself very well to extrapolation to $[AgNO_3] = 0$. The results at three values of pH are shown in Table I. We see that in fact $k_{HOBr} \ll k_{Br_2}$.

4. Oxidation of Glucose by a Mixture of Bromine and Hypobromous Acid.—When the reaction

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[8] See, *e.g.*, W. Pigman, "The Carbohydrates," Academic Press, Inc., New York, N. Y., 1957, (a) p. 55; (b) p. 61.

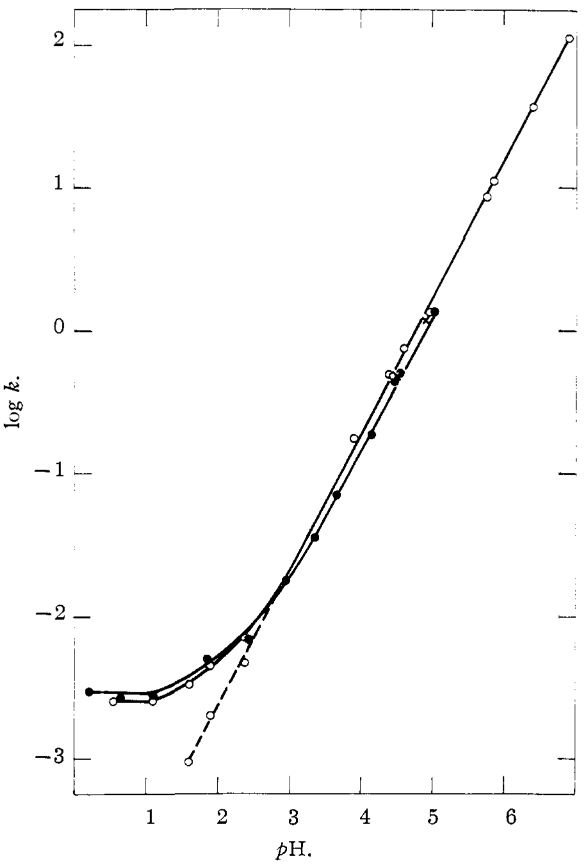

Fig. 2.—The dependence of log k_{Br_2} on *p*H: open circles, in the presence of bromide; closed circles, in the presence of hypobromous acid; crosses, in pure bromine water. The dashed line is obtained by subtracting $k_{C_6H_{12}O_6}$ from the experimental value of k_{Br_2}.

mixture initially contains both bromine and hypobromous acid, the reaction will thus chiefly proceed according to the scheme

$$\beta\text{-D-glucose} + Br_2 \longrightarrow \text{gluconic acid lactone} + 2HBr \quad \text{(I)}$$

$$2HBr + 2HOBr \longrightarrow 2Br_2 + 2H_2O \text{ (fast)} \quad \text{(II)}$$

$$\beta\text{-D-glucose} + 2HOBr \longrightarrow \text{gluconic acid lactone} + Br_2 + 2H_2O \quad \text{(III)}$$

only a small percentage of glucose being oxidized by hypobromous acid directly. Experiments carried out under such conditions have the advantage that—as long as hypobromous acid is still present—no bromide accumulates during the reaction. This simplifies the calculations and makes the result independent of the accuracy of K_3. Nor do hydrogen ions accumulate and therefore an extremely small amount of buffer should suffice to keep the *p*H constant.

TABLE I

THE RATE CONSTANT OF THE REACTION BETWEEN β-D-GLUCOSE AND HYPOBROMOUS ACID

*p*H	$k_{HOBr} \times 10^3$	k_{Br_2}/k_{HOBr} (*cf.* Sect. 5)
1.25	0.34	7.2
4.08	2.70	85
4.55	9.53	72

If c is the initial molarity of bromine, and e that of hypobromous acid ($c + e = a$), the rate equation is

$$dx/dt = k_{Br_2}[(c + x) + (k_{HOBr}/k_{Br_2})(e - 2x)] \times [\beta\text{-D-glucose}] \quad (7)$$

5. The Dependence of the Reaction Rate on *p*H.—Two series of experiments were carried out, the first in the presence of excess bromide, the second in the presence of hypobromous acid. In the first series, the *p*H was varied from 0.55 to 6.90; the initial stoichiometric concentration of bromide was 0.187 *N*. Equation 6 served to calculate k_{Br_2}; the results are shown in Fig. 2 where log k_{Br_2} is plotted *vs.* *p*H (open circles). It is seen that, in strongly acid solution, the rate constant is independent of *p*H, but at *p*H $\cong$ 1 the line begins to rise and soon reaches a constant slope of almost exactly unity. Our value at *p*H $\sim$ 5.0 is equal to that given by Isbell and Pigman.[1,3]

Interpolating for the appropriate values of *p*H we find the ratio of k_{Br_2}/k_{HOBr} shown in the last row of Table I. Thus, when both bromine and hypobromous acid are present at the beginning of the reaction, the second member in the square bracket in equation 7 will be negligible except at the lowest values at *p*H where we took it into account.

The results of the second series in the *p*H range of 0.22 to 5.05 are also shown in Fig. 2 (closed circles). Preliminary experiments had shown the existence of a positive salt effect. Therefore, in the *p*H-range where dilute sulfuric acid was employed instead of buffer (*p*H $\leqslant$ 3.6), sodium nitrate at an appropriate concentration was added to ensure equal salt concentration in the two series. The two curves are seen to be closely parallel, but the distance between them—though small—is larger than our experimental error in measuring k^{β} or *p*H.

The difference in the two series at *p*H $\leqslant$ 3.6 might be due to the different influence of the nitrate and $H_2PO_4^-$-ions. In order to check this point, two experiments at *p*H 2.47 were carried out, one in the presence of 0.375 *N* sodium nitrate and one in the presence of 0.3 *M* NaH_2PO_4 and 0.1 *M* phosphoric acid; identical results were obtained. *There is therefore no specific influence of the buffer ion.*[9]

6. Oxidation in Pure Bromine Water.—We suspected that the discrepancy between the two curves in Fig. 2 might be due to our value of K_3 being not sufficiently accurate. When no bromide is added initially, the value of $K_3/(K_3 + [Br^-])$ is very nearly unity, and quite insensitive to errors in K_3. However, the percentage change in $[Br^-]$ during a reaction is very great, and the usual

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(9) The constancy of the *p*H in the absence of buffer supports the view that the lactone, and not gluconic acid, is the primary product. Only above *p*H 4 was there a decrease in *p*H ($\sim$0.35 unit at *p*H 4.1) and hence also in k, as the reaction proceeded. Since the dissociation constant of gluconic acid is $\sim 10^{-4}$ at 0° (see "International Critical Tables," McGraw-Hill Book Co., Inc., Vol. V1, p. 277), such a decrease would be caused by the hydrolysis of about *one fifth* of the lactone formed while the reaction was observed. The experiments at *p*H $\geqslant$ 4 in the absence of buffer were not included in Fig. 2. However, the approximate values of k^{β} calculated from the initial rate were in good agreement with those obtained in buffered solution; this again shows that the buffer has no special influence.

second-order plot becomes somewhat flatter as the reaction proceeds, so that equation 6 cannot give accurate results. On the other hand, an *exact* calculation of $[Br_2]$ at every stage yields a very cumbersome result after integration (even for pseudo first-order conditions).[5] In the initial absence of bromide we can write to a good approximation

$$[\mathrm{Br_2}] = (a - x)[1 - 2x/(a + x + K_3)] \qquad (8)$$

and hence get the rate equation

$$\frac{\mathrm{d}x}{\mathrm{d}t} = k_{\mathrm{Br_2}}(a-x)[\beta\text{-D-glucose}]\,\frac{a - x + K_3}{a + x + K_3} \qquad (9)$$

Since $a + K_3$ is usually more than 20 times x, this expression is rather insensitive to variations in K_3. Values of $k_{\mathrm{Br_2}}$ obtained from integration of equation 9 also are shown in Fig. 2 (crosses). They are seen to fit exactly between the two sets of data, thus showing the reliability of both methods without explaining the small difference between them.

Discussion

We thus confirm all the conclusions drawn by Isbell and Pigman[3] from their data at a single value of *p*H. In particular, by direct measurements in pure hypobromous acid solutions, molecular bromine was proved conclusively to be the oxidizing agent in bromine–water. Yet we *also* confirm the dependence of the rate constant on *p*H which has led other authors[4] to the erroneous assumption that hypobromous acid is the active agent.

As an explanation of the dependence on *p*H we propose that apart from β-D-glucose, the anion of β-D-glucose also is attacked, and at a much higher specific rate. The rate constant is thus composite, *viz.*

$$k_{\mathrm{Br_2}} = k_{\mathrm{C6H12O6}} + k_{\mathrm{C6H11O6}}\,K_{\mathrm{G}}/(K_{\mathrm{G}} + [\mathrm{H^+}]) \qquad (10)$$

(where K_G is the acid dissociation constant of glucose) with an analogous expression for k_{HOBr}.

In strongly acid solution, the first member predominates. We found

$$k_{\mathrm{C_6H_{12}O_6}} = 2.46 \times 10^{-3} \text{ and } 0.31 \times 10^{-3}$$

for bromine and hypobromous acid, respectively.

In slightly less acid solution, the rates of consumption of bromine due to β-D-glucose and due to the extremely small amount of the anion are commensurate,[10] whereas, above *p*H 3, the oxidation of the anion completely swamps that of the molecular form. Since even at the highest *p*H considered $K_G \ll [H^+]$, the inverse proportionality between $k_{\mathrm{Br_2}}$ and $[H^+]$, apparent from Fig. 2, is quantitatively explained.

The acidic properties of glucose are recognized[11-13] and K_G has been measured by several methods. Adopting Thamsen's value[12] of 1.2×10^{-13} at 0° and an ionic strength of 0.03, and taking into account that at our ionic strength it should be slightly higher, we calculate

$$k_{\mathrm{C_6H_{11}O_6^-}} = 10^8 \text{ and } \sim 1.35 \times 10^6$$

for bromine and hypobromous acid, respectively.

The positive salt effect observed is also consistent with our hypothesis, as a typical case of secondary kinetic salt effect.

We feel, however, that a certain difficulty should not be left unmentioned. During the oxidation, the pyranose ring remains intact.[3] On the other hand, it usually is assumed[11,14] that the acidic properties of glucose are due to the open-chain ene–diol present in alkaline solution. If this is the case, our explanation is untenable.

We repeated some of Thamsen's[12] experiments at 20° using an ordinary (not Type *E*) Beckman electrode because of its faster response, and applying appropriate corrections for salt error in alkaline solution. Within 20 seconds of the addition of sodium hydroxide to a glucose solution—*i.e.*, as fast as it could be measured—the *p*H reached a value which remained virtually constant for several hours. On the other hand, enolization is usually not an "instantaneous" reaction. Its slowness in the present case is proved by the slowness of the Lobry de Bruyn–Alberda van Ekenstein reaction.[3b,14,15] This discrepancy already has been discussed by Hirsch and Schlags[13]; like these authors, we are led to the conclusion that glucose has acidic properties in its pyranose form.[16] It would then be reasonable to suppose that the OH group on the anomeric carbon atom is the first one to dissociate.

In a recent reinvestigation of the oxidation of glucose by chlorine, Lichtin and Saxe[17] found buffer anions to exert an accelerating influence and ascribed this to general base-catalysis. A similar effect in the case of bromine was construed by these authors from a comparison of Bunzel and Mathew's[2] and Isbell and Pigman's[3] results. Our own measurements in the presence of nitrate and phosphate at equal *p*H show no indication of such an effect.

The mechanism of the oxidation of ethanol by bromine has been reinvestigated recently by Kaplan[18] utilizing the isotope effect. Rupture of a C–H linkage was shown to be involved in the rate-determining step, the hydrogen possibly being abstracted by the bromine in the form of a hydride ion.[5] A mechanism of this kind may well be operative in the present case. Such a reaction can easily be imagined to be facilitated by the presence of a negative charge on the organic molecule. Preliminary results on the influence of *p*H on the rate of oxidation of simple alcohols point in a similar direction.

The authors are indebted to Prof. G. Stein for helpful discussion.

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[10] That the rate constant at *p*H 1.6 to 2.4 is indeed represented by equation 10 can also be seen from the dashed line in Fig. 2. This was obtained by subtracting $k_{\mathrm{C_6H_{12}O_6}}$ from the experimental values of $k_{\mathrm{Br_2}}$ in this *p*H range, and is seen to be a continuation of the straight line obtained at higher values of *p*H.

[11] J. M. Sugihara, *Advances in Carbohydrate Chem.*, **8**, 1 (1953), where literature is also quoted.

[12] J. Thamsen, *Acta Chem. Scand.*, **6**, 270 (1952).

[13] P. Hirsch and R. Schlags, *Z. physik. Chem.*, **141**, 387 (1927).

[14] See *e.g.*, E. S. West and W. R. Todd, "Textbook of Biochemistry," The Macmillan Co., New York, N. Y., 1955, p. 224.

[15] W. L. Evans, *Chem. Revs.*, **31**, 537 (1942).

[16] A further proof of this view may be seen in the fact that the optical rotation of an anomerically equilibrated glucose solution remains unchanged under conditions where we should expect 70% of the glucose to be present in the anionic form.

[17] N. N. Lichtin and M. H. Saxe, THIS JOURNAL, **77**, 1875 (1955).

[18] L. Kaplan, *ibid.*, **76**, 4645 (1954); **80**, 2639 (1958).