THE KINETICS OF THE REACTION WHICH TAKES PLACE BETWEEN IODOACETIC ACID AND GLYCINE*

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(Accepted for publication, October 2, 1935)

In a recent communication, Michaelis and Schubert (1) called attention to the fact that iodoacetic acid reacts quite readily with the amino group according to the equation,

Since a similar but relatively faster reaction also takes place between the SH group and iodoacetic acid which probably accounts for the inhibitory action of iodoacetic acid on certain physiological processes (2), the question arises whether the rate of the reaction between the amino group and iodoacetic acid is sufficiently rapid over the physiological pH range to have some practical significance.

In order to decide this question, the velocity constants of the reaction between the amino group and iodoacetic acid must be known. Since the pH of the medium in which the reaction takes place influences the rate considerably, buffer solutions having a large reserve must be used. This involves the difficulty of following the change in concentrations of the reactants with time by ordinary analytical methods. An attempt to apply Warburg's manometric method for the determination of the reaction rate between halogen acetates and the SH group was made by Dickens (2). He assumed that the amount of CO_2 which is produced when the reaction takes place in a CO_2 -bicarbonate buffer solution is equivalent to the amount of HI which is liberated during the reaction and, therefore, to the decrease of the reacting components. In such buffer solutions, however, carbamates

*Aided by a grant from The Chemical Foundation, Inc., and the Research Board of the University to Professor Carl L. A. Schmidt.

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are formed from amino acids (3). A considerable influence as a result of this factor was observed when the reaction between alanine or glycine and iodoacetic acid was followed in a sodium carbonate buffer solution (4). The use of borate or phosphate buffers eliminates the manometric method for the estimation of reaction rates.

The polarographic technique which has been developed by Heyrovský (5) was employed for the study of the present problem. It is a special electrolytic method which employs a mercury dropping electrode as the cathode and a mercury electrode of the second order as the anode. The course of the electrolysis is recorded automatically on photographic paper as current voltage curves. On the basis of these curves, both the qualitative and the quantitative composition of the solution under investigation can be determined. When several electro-reducible compounds are present in the solution, they can be distinguished from each other according to the differences in their characteristic reduction potentials, and their individual concentrations can be estimated from the values of the limiting current intensities which correspond to the horizontal portions of the curve. The current-voltage curves of the solutions which contain iodoacetic acid show a distinct reduction which corresponds most probably to the reaction,

The limiting current intensities of this reaction measured in millimeters (see Fig. 1) are strictly proportional to the concentration of iodoacetic acid. The proportionality constants expressed by the ratio
$$\frac{\text{Limiting current}}{\text{Concentration of iodoacetic acid}}$$
 are given in Table I. Quantitative estimations of iodoacetic acid in buffer solutions can be carried out in 2 minutes with an accuracy of ± 1 per cent.

EXPERIMENTAL

The current-voltage curves were recorded by means of a Type VII polarograph.¹ The sensitivity of the galvanometer was 1.2×10^{-9} amp./mm., and its half-time period was 4.53 seconds. The sensitivity could be decreased by means of a special shunt. The present measurements were made at $\frac{1}{2500}$ or $\frac{1}{5000}$ of the above sensi-

¹ Manufactured by Drs. V. and J. Nejedlý, Prague, Czechoslovakia.

FIG. 1. Current-voltage curves (sensitivity of galvanometer $\frac{1}{500}$).

No.	Concentration	Substance
1	$(9.92 \times 10^{-3} \text{ N})$	sodium iodoacetate in 0.08 N sodium carbonate solution.
2	$(7.92 \times 10^{-3} \text{ N})$	
3	$(5.95 \times 10^{-3} \text{ N})$	
4	$(3.96 \times 10^{-3} \text{ N})$	
5	$(2.00 \times 10^{-3} \text{ N})$	Atmospheric oxygen dissolved in 0.08 N sodium carbonate solution.
6		

tivity value. This is most convenient for the $4 \times 10^{-3} - 4 \times 10^{-4}$ equivalent concentration of iodoacetic acid range to which the solutions were diluted.

The glycine and iodoacetic acid were checked as to their purity. The usual standardization of the hydrogen electrode against buffer mixtures was carried out. The sodium salt of iodoacetic acid and glycine in a sodium borate buffer solution were used chiefly for the rate measurements at different pH values. In order to determine the reaction rates when glycine in a borate buffer solution is treated with iodoacetic acid, it is necessary that the pH values of the solutions of the buffer components be known. The titration curves are shown in Fig. 2.

The following procedure, in general, was employed for the rate determination: to a known volume of the glycine-containing buffer mixture, a certain amount of 0.5 N sodium iodoacetate was added. The mixture was incubated at $25^\circ \pm 0.01^\circ$. 5 cc. portions were withdrawn at certain time intervals and added to an amount of HCl which brought the mixture approximately to neutrality in order to stop the reaction. Since the iodide ion, which is one of the products of the reaction, affects the potential of the anode, giving to it a more negative value, the HCl was mixed

TABLE I

Curve.....	1	2	3	4	5
Limiting current (mm. on scale).....	77.5	62.5	47.5	31.6	15.8
Concentration of iodoacetic acid (equiv's. $\times 10^3$).....	9.92	7.92	5.95	3.96	2.0
Ratio.....	7813	7891	7983	7980	7900

with 0.04 N KI in order to maintain constant conditions at the anode during the polarographic estimations.

A mercury cathode dropping at a 3 seconds rate was used. The electromotive polarizing force was supplied from a 4 volt lead battery. The vertical lines on the polarograms (Figs. 1 and 3) correspond to 200 mv. It was necessary to record only a part of the horizontal portions of the current-voltage curves. From these curves the concentrations of iodoacetate in the solutions were determined. The horizontal portions were obtained by using a polarizing E.M.F. of 1-1.6 volts (see Fig. 3).

Besides the reduction of the iodoacetate, the current-voltage curves include the reduction of the atmospheric oxygen which is present in the aqueous solutions in about 10^{-3} equivalent concentration. This reduction takes place in 2 steps (Fig. 1, Curve 6), the first corresponding to the reaction

and the second to

It is possible to remove the oxygen by blowing hydrogen or nitrogen through the solution. This procedure was not followed since the current due to the oxygen reduction can be determined in the buffer solu-

FIG. 2. Electrometric titration curves

No.

- 1 0.204 N glycine + 0.247 N boric acid in 0.1 N KCl
- 2 0.247 N boric acid in 0.1 N KCl
- 3 0.204 N glycine
- 4 Water blank.

tions and subtracted from that of the corresponding solutions which contained iodoacetate. The horizontal line recorded on each set of estimations (Fig. 3) accounts for the current intensity due to the reduction of oxygen so that the distance between this and the horizontal

FIG. 3. Limiting current intensities due to the electro-reduction of iodoacetates. These are proportional to the values of $(a-X)$, recorded at different times t (in minutes) during the course of the reaction of iodoacetate with glycine in a borate buffer solution (a) at pH 9.76 (decreasing curves from left to right), (b) at pH 9.46 (decreasing curves from right to left). (Sensitivity of galvanometer $\frac{1}{2} \frac{1}{5} 0$).

portion of the iodoacetate-current-voltage curve corresponds to its actual concentration.

In studying the reaction which takes place between glycine and iodoacetic acid in an alkaline buffer solution, consideration must be given to the fact that a single reaction does not take place. Iodoacetic acid is saponified by hydroxyl ions yielding glycollic acid (6). The total decrease in concentration of iodoacetate which is observed experimentally therefore involves two independent reactions. Allowance was made for the effect of the saponification reaction by parallel experiments in which the glycine was eliminated. The determination of the velocity constants of the saponification reaction will be reported in another paper (7).

Since there is an ample reserve of OH^- ions in the buffer mixtures, the rate of saponification of iodoacetic acid can be expressed by an equation of the first order in which the monomolecular velocity constant, k_s , is related to pH.

When the saponification takes place simultaneously with the glycine-iodoacetic acid reaction, the equation for the saponification must be written in the form,

$$\frac{dz}{dt} = k_s(a - X - z) \quad (1)$$

where a is the initial equivalent concentration of the iodoacetic acid, z its decrease due to saponification during the time t , and X , the decrease of iodoacetic acid resulting from its reaction with glycine.

Even when the value for X as a function of t is not known, the value for z can be obtained from the value for k_s , which was determined in the study of the saponification reaction, and the value for $(a - X - z)$, which denotes the estimated concentration of iodoacetic acid at time t in the glycine-iodoacetate mixture. If the values for $(a - X - z)$ are plotted against t , then for any value of t the area below the plotted curve, multiplied by the value for k_s , determines the value for z (see Table II and Fig. 4). Knowing the value for z , it is possible to calculate values for $(a - X)$. The curve which is obtained by plotting $(a - X)$ against t (Fig. 4) shows the rate of decrease of iodoacetate as a result of its reaction with glycine.

It would be expected that the reaction rate between glycine and iodo-

acetic acid should be trimolecular. The experimental data, however, do not bear this out. They conform more closely to the bimolecular type of reaction. The explanation that only one hydrogen of the amino group is replaced is not correct, since chemical findings show that the disubstituted product is formed (1). These views can be reconciled by the assumption that the reaction rate is only apparently bimolecular, the kinetics being different from that which would be expected from the chemical equation.

TABLE II

	$a-X-z$	$\int_0^t (a-X-z)dt$	z	X	$\frac{dX}{dt}$	$\frac{1}{a-X} \cdot \frac{dX}{dt}$	$\frac{k'_1}{2} (2c-X)^*$
0	56.8	0	0	0	0.244	0.00429	0.00429
50	46	2570	0.95	9.8	0.162	0.0034	0.0032
180	32	7545	2.8	22	0.070	0.0020	0.0018
270	26.1	10160	3.8	26.9	0.042	0.0014	0.0012
360	22.6	12351	4.6	29.6	0.028	0.0010	0.0009
480	19.1	14853	5.5	32.2	0.019	0.0007	0.0006
800	14.8	20245	7.5	34.5			
1200	11	25405	9.4	36.4			

$$a = 0.10 \text{ N}$$

$$k_s = 3.7 \times 10^{-4}$$

$$c = 0.033 \text{ N}$$

$$\text{pH} = 12.96$$

$$k'_1 = \left(\frac{dX}{dt} \right)_0 \cdot \frac{1}{ac} = 0.130$$

* Since X is expressed in arbitrary units, its value has to be transformed into equivalent concentration. If the concentration of a ($a = 0.01 \text{ N}$) corresponds to 56.8 mm. units, then in order to express X in equivalent concentrations, it has to be multiplied by the factor, $\frac{0.1}{56.8}$.

There are many examples in chemical kinetics which show that two or more substitutions in the same molecule usually proceed in steps and that each substitution reaction possesses its own velocity constant (8). If this be the case when iodoacetic acid and glycine react, the following reactions must take place:

Since both of these reactions are bimolecular, the following kinetic equations express the relations:

$$\frac{dx_1}{dt} = k'_1(a - x_1 - x_2)(c - x_1) \quad (2)$$

$$\frac{dx_2}{dt} = k'_2(a - x_1 - x_2)(x_1 - x_2) \quad (3)$$

FIG. 4. Curves showing the changes of $(a - X - z)$, $(a - X)$, X , $(x_1 - x_2)$, and x_2 , with time, during the reaction between 0.01 N sodium iodoacetate and 0.033 N glycine in a borate buffer of pH 12.96. The ordinates are represented in arbitrary units which are proportional to the equivalent concentrations of the reacting components.

where a and c represent the initial equivalent concentrations of iodoacetate and glycine, respectively, x_1 , the total decrease in the concentration of glycine at the time t , and x_2 , the decrease in the concentration

of monosubstituted glycine which is equal to the concentration of the disubstituted product.

The concentration of iodoacetate at any time is equal to $(a - x_1 - x_2)$, that of glycine to $(c - x_1)$, and that of the monosubstituted form to $(x_1 - x_2)$. The apparent velocity constants for each step in the reaction at the given pH are designated by k'_1 and k'_2 .

In general, these differential equations cannot be solved for k'_1 and k'_2 . However, by eliminating t , the following relationship can be obtained:

$$\frac{dx_1}{dx_2} = \frac{k'_1 (c - x_1)}{k'_2 (x_1 - x_2)} \quad (4)$$

which, when solved, gives

$$k'_1 c - k'_2 (x_1 - x_2) - k'_1 x_2 = k'_1 c \left(1 - \frac{x_1}{c}\right)^{\frac{k'_2}{k'_1}} \quad (4a)$$

(when $x_1 = 0$, it is assumed that $x_2 = 0$).

The sum $(x_1 + x_2)$ only can be calculated from the experimental values of the concentration of iodoacetate at certain time intervals. This value is identical with the previously mentioned value for X .

The addition of equations (2) and (3) gives

$$\frac{dX}{dt} \cdot \frac{1}{a - X} = k'_1 c - x_1 (k'_1 - k'_2) - k'_2 x_2 \quad (5)$$

By combining equations (5) and (4a), a further relationship is obtained:

$$\frac{1}{a - X} \cdot \frac{dX}{dt} = k'_1 (2c - X) - k'_1 c \left(1 - \frac{x_1}{c}\right)^{\frac{k'_2}{k'_1}} \quad (5a)$$

Values for the left side of this equation can be determined experimentally as $\frac{dX}{dt}$ represents the tangent to the curve when X is plotted against t (see Fig. 4). This tangent for zero time when $X = 0$ determines the value for k'_1 according to the relationship

$$\frac{dX}{dt} = a k'_1 c \quad (5b)$$

The experimental values for other tangents show that the following relationship is satisfied (see Table II)

$$\frac{1}{a-X} \cdot \frac{dX}{dt} = \frac{k_1'}{2} (2c-X) \quad (6)$$

From equation (5a) the following relationship must also hold

$$\frac{k_1'}{2} (2c-X) = k_1' c \left(1 - \frac{x_1}{c}\right)^{\frac{k_2'}{k_1'}} \quad (6a)$$

If the right side of this equation is replaced by the left side of equation (4a), equation (6a) assumes the form

$$\frac{k_1'}{2} (2c-X) = k_1' c - k_2' (x_1 - x_2) - k_1' x_2 \quad (6b)$$

Both sides of this equation will be identical in only one case; *viz.*, when $k_1' = 2 k_2'$. Using this relationship in equations (2) and (3), substituting X for $(x_1 + x_2)$, and integrating, the following equation is obtained:

$$k_1' = \frac{2}{t(2c-a)} \cdot \ln \frac{a}{2c} \cdot \frac{(2c-X)}{(a-X)} \quad (7)$$

This is similar to the equation for the bimolecular reaction.

It has been pointed out by Abel (9) that some reactions which show a lower order of rate than would be expected from the chemical equation can be explained as consecutive reactions when the ratio, 1:2:---, is assumed for the velocity constants. He interprets (10) the results of Kremann dealing with the saponification of glycodiacetate, which show that the course of the reaction is apparently bimolecular instead of trimolecular, on this basis (11). If the steric effect is disregarded, the physical basis for the simple ratio of the velocity constants accounts for the probability of an effective collision. Thus, for example, a molecule in which equivalent substitutions can take place will have a twofold greater chance for such a collision than when one group is already substituted. The correctness of this principle was confirmed in the investigations on the hydrolysis of polybasic esters (12).

Disregarding the secondary effects which will be discussed later, the consecutive character of the glycine-iodoacetate reaction having

velocity constants in the ratio 2:1 appears to be supported amply by the present experiments. The ratio of the velocity constants involves in the first place a solution of the relationships between the concentrations of the mono- and the disubstituted form of glycine when the total decrease X of iodoacetate has been determined experimentally.

These relationships follow from equation (4a) under the condition when $k'_1:k'_2 = 2:1$, and are expressed by the following equations:

$$x_1 = X - \frac{X^2}{4c} \quad (8)$$

$$(x_1 - x_2) = X - \frac{X^2}{2c} \quad (9)$$

$$x_2 = \frac{X^2}{4c} \quad (10)$$

The relationship between t and the components is represented graphically in Fig. 4 for the case: $a = 0.01$ N, $c = 0.033$ N, and pH = 12.96.

According to equation (9), if $a > c$, then the concentration of monosubstituted glycine ($x_1 - x_2$) will reach a maximum when $X = c$. The corresponding time can be expressed by equation (7) in which $X = c$

$$t_{\max.} = \frac{2}{k'_1(a - 2c)} \ln 2 \left(1 - \frac{c}{a} \right) \quad (11)$$

For the same time, x_2 shows an inflection point. The monosubstituted glycine will disappear entirely at the end of the reaction only in the case in which $a \geq 2c$, *i.e.* when the initial concentrations are in accordance with the stoichiometric chemical equation or, if the amount of iodoacetic acid is in excess of the stoichiometric ratio. In every other case a mixture of the mono- and disubstituted form will be obtained, the ratio of which is given by equations (9) and (10). When $c > a$, the concentration of the monosubstituted form will not reach a maximum. The yield of the mono- will be greater than the yield of the disubstituted form.

It is more convenient for purposes of further study to assume the condition when the initial concentration of c is greater than that of a in order to show the relationship between the velocity constants and

pH. The buffering action of glycine itself helps to maintain in part the constancy of the pH during the reaction and, in part, to increase the reaction rate. If an excess of glycine over iodoacetate is used such that the reaction rate can be treated as being approximately monomolecular, the calculated value for the velocity constant is in reality the sum of k'_1c and k_s (velocity constant of saponification). Experience shows (7) that k_s , being of the order of 10^{-4} — 10^{-6} over the pH range investigated, can be neglected when the initial concentrations of glycine and iodoacetate respectively are about 0.2 and 0.02 N.

TABLE III

(a) pH = 9.76				(b) pH = 9.46		
Time	(a-X)	(2c-X)*	$k'_1 \times 10^3$	(a-X)	(2c-X)*	$k'_1 \times 10^3$
<i>min.</i>						
0	63	1305		63	1305	
30	47	1289	4.80	52	1294	3.14
60	35	1277	4.84	42.8	1284	3.17
90	26.3	1270	4.82	35.3	1277	3.28
150	15.3	1257	4.71	23.5	1265	3.26
200	9.5	1251	4.75	16.7	1258	3.31
340	2.5	1244	4.80	6.3	1248	3.34

* (2c-X) must be expressed in the same arbitrary units as (a-X). Since for $a = 0.0198$ N the value in the arbitrary units equals 63, for c the value of 0.205 $\times \frac{63}{0.0198} = 1305$ must be used. It is immaterial which units in the term behind the logarithms in equation (7) are used. The term (2c-a) before the logarithms, however, must be expressed in equivalent concentrations.

Table III gives the data for the calculation of the velocity constant, k'_1 , according to equation (7) for two sets of experiments which were carried out in borate buffer solutions at pH 9.76 and 9.46, respectively (see also Fig. 3). Table IV contains the average values of the velocity constant, k'_1 , over the investigated range of pH, calculated with the aid of equation (7). This is the same method as was used in calculating the k'_1 values which are given in Table III. The pH values of the reacting solutions for any given concentration of NaOH were estimated from the titration curves which are given in Fig. 2.

The titration curve of the glycine-borate buffer mixture (Curve 1,

Fig. 2) shows the pH change which must take place during the reaction between glycine and iodoacetate as a result of the neutralization of a part of the NaOH by HI. From the data which are given in Table IV, it can be seen to what extent the reaction rate depends on the pH. A decrease in the value of the velocity constants with decrease of pH during the reaction should be expected. Such an effect, however, was

TABLE IV

pH	NaOH	$k'_1 \times 10^3$	b	$\frac{(k'_1 \times 10^3)c}{b} = k_1$	μ
8.18	0.062	0.21	0.0062	6.91	0.162
8.63	0.104	0.60	0.017	7.20	0.204
8.89	0.145	1.18	0.029	8.30	0.245
9.13	0.186	1.90	0.047	8.34	0.286
9.46	0.249	3.40	0.083	8.36	0.349
9.66	0.293	4.35	0.106	8.37	0.393
9.76	0.312	4.80	0.117	8.37	0.412
9.85	0.331	5.40	0.130	8.47	0.431
9.96	0.350	5.95	0.143	8.49	0.450
9.98	0.353	6.00	0.144	8.50	0.453
10.02	0.358	6.20	0.148	8.55	0.458
10.14	0.373	6.65	0.159	8.53	0.473
10.29	0.395	7.30	0.173	8.61	0.495
10.56	0.416	8.05	0.191	8.60	0.516
11.22	0.455	9.30	0.204	9.30	0.555
11.52	0.466	9.60	0.204	9.60	0.566
12.16	0.482	10.0	0.204	10.0	0.582
12.38	0.500	10.1	0.204	10.1	0.600
12.64	0.545	10.4	0.204	10.4	0.645
12.94	0.635	10.8	0.204	10.8	0.735
13.17	0.725	11.2	0.204	11.2	0.825

Initial concentration of (a) iodoacetate, 0.0198 N; (b) glycine, 0.204 N; (c) boric acid, 0.297 N; (d) KCl, 0.1 N.

Maximum deviation of $k'_1 = \pm 4$ per cent.

not observed. Only one explanation remains; *viz.*, that the decrease in the values of the velocity constants is balanced by another effect. This will be discussed in the paragraph dealing with salt effects.

If the values for the velocity constants, k'_1 , are plotted against pH, a sigmoid curve is obtained which is very similar to the titration curve of glycine (see Fig. 5). It can be shown that the velocity constants,

k'_1 , for any pH value are nearly proportional to the concentration of glycinate anion. The latter can be determined from Curve 3 of Fig. 2 at the corresponding pH. This fact indicates that the rôle played by pH in the glycine-iodoacetate reaction consists in shifting the equi-

FIG. 5. The relation of the constants, k'_1 , calculated on the basis of equation (7) to (a) pH and (b) the concentration of NaOH.

librium between the two forms of glycine of which only the anionic form is reactive.

In order to obtain the velocity constants independent of pH, the degree of dissociation of glycine has to be taken into account. In an

alkaline solution an equilibrium exists between the two forms of glycine which, in terms of the zwitter ion theory, is expressed by

and is defined by the apparent zwitter ionic constant, K'_B .

Denoting the analytical concentration of glycine by c , and the concentration of its anionic form by b , the following relationship must hold:

$$K'_B = \frac{(c-b)(\text{OH}^-)}{b} \quad (12)$$

If the anionic form only is able to react, the decrease in glycinate concentration, x_1 , must be balanced by the formation of anionic glycine from the zwitter ionic form in order to maintain the equilibrium. Denoting the amount of zwitter ionic glycine which is transformed into the anionic form by y , then

$$K'_B = \frac{(c-b-y)(\text{OH}^-)}{(b-x_1+y)} \quad (12a)$$

The term, $(b-x_1+y)$, must be used in equation (2) in place of $(c-x_1)$. By assuming a constant value for pH and combining equations (12) and (12a), the equation,

$$(b-x_1+y) = (c-x_1) \frac{(\text{OH}^-)}{K'_B + (\text{OH}^-)}, \quad (12b)$$

is obtained.

The kinetic equation which contains the velocity constant independent of the pH assumes the form

$$\frac{dx_1}{dt} = k_1 \frac{(\text{OH}^-)}{K'_B + (\text{OH}^-)} \cdot (c-x_1)(a-x_1-x_2) \quad (2a)$$

The same reasoning can be applied to the second step of the reaction. The assumption has to be made that the same zwitter ionic equilibrium exists for the monosubstituted form of glycine as for glycine itself. This assumption appears to be affirmed by the titration curve of trimethyl carboxylamine (1). In this case a certain amount of mono-

substituted glycine which is formed from glycine anions must be converted to the zwitter ionic form according to the equilibrium,

$$K'_B = \frac{y_1(\text{OH}^-)}{x_1 - y_1}, \quad (13)$$

where y_1 represents the concentration of the zwitter ionic form of monosubstituted glycine.

The decrease x_2 of monosubstituted glycine in the second step of this reaction is balanced by the formation of monosubstituted glycine anion from the zwitter ionic form. Designating the amount of the zwitter ionic form thus transformed by y_2 , then

$$K'_B = \frac{(y_1 - y_2)(\text{OH}^-)}{x_1 - y_1 - x_2 + y_2} \quad (13a)$$

The term, $(x_1 - x_2)$, must be replaced by $(x_1 - x_2 - y_1 + y_2)$ in equation (3). By combining equations (13) and (13a), the equation

$$(x_1 - x_2 - y_1 + y_2) = \frac{(\text{OH}^-)}{K'_B + (\text{OH}^-)} (x_1 - x_2) \quad (13b)$$

is obtained.

The second kinetic equation which is defined by velocity constants which are independent of pH assumes the form

$$\frac{dx_2}{dt} = k_2 \frac{(\text{OH}^-)}{K'_B + (\text{OH}^-)} (x_1 - x_2)(a - x_1 - x_2) \quad (3a)$$

The integration of the sum of differential equations (2a) and (3a) gives

$$k_1 = \frac{K'_B + (\text{OH}^-)}{(\text{OH}^-)} \cdot \frac{2}{t(2c - a)} \cdot \ln \frac{a}{2c} \cdot \frac{(2c - X)}{(a - X)} \quad (7a)$$

It follows from equation (12) that

$$\frac{K'_B + (\text{OH}^-)}{(\text{OH}^-)} = \frac{c}{b}$$

The value for the velocity constant, k_1 , can be obtained from any of the values for k'_1 (Table IV) by multiplying them by the value for the ratio $\frac{c}{b}$. The value for b at a given pH can be obtained from the titration curve of glycine (Curve 3, Fig. 2).

These velocity constants which have to be regarded as being independent of pH are given in the 5th column of Table IV.

The conclusion that only the anionic form of glycine reacts with iodoacetate indicates that this reaction is of the ionic type. Since one of the reacting components is supplied by a weak electrolyte, both primary and secondary salt effects must take place according to Brönsted's hypothesis (13). In accordance with this hypothesis, the velocity constants which were calculated on the basis of the above equations are functions of the salt concentrations in the reacting medium.

Under the present experimental conditions, the first step of the consecutive reactions predominates. The second step may, therefore, be disregarded.² The first step corresponds to the reaction type which is given by Brönsted, *viz.*

where A^- denotes the glycine anion, B^- the iodoacetate anion, and X^- an unstable "critical" complex which represents an addition compound between the two reacting ions. In such a case a positive catalytic salt effect is to be expected. It is characterized generally by the factor F which depends on the salt concentration in the reacting medium. The measured velocity constants are equal to Fk_0 where k_0 represents a constant which is independent of the salt concentration.

The factor F is determined by the activity coefficients of the reacting ions, f_{A^-} , f_{B^-} , and of the critical complex, f_{X^-} :

$$F = \frac{f_{A^-} \cdot f_{B^-}}{f_{X^-}}$$

In the extreme dilution where the Debye-Hückel limiting law for activity coefficients can be applied, the value for F can be calculated from the ionic strength μ . For the type of reaction in question, its value should be equal to $10^{\mu^{1/2}}$.

Since the ionic strengths of the solutions which have been investi-

² It can be shown by means of equations (9) and (10) that, at the end of reaction, 97.6 per cent of the glycine which reacted with iodoacetate was transformed to monosubstituted glycine, and 2.4 per cent to the disubstituted form when the initial concentrations of glycine and iodoacetate were 0.2 N and 0.02 N, respectively.

gated are greater than 0.1μ (see Table IV, Column 6), neither this limiting law nor the Brönsted expression,

$$\ln \frac{f_{A^-} \cdot f_{B^-}}{f_{X^-}} = 2\alpha\mu^{1/2} - (\beta_A + \beta_B - \beta_X) c,$$

which holds for middle concentrations, can be used. (α is a universal constant for all ions and β depends on their character (14).)

By considering the primary salt effect only, the velocity constant, k_0 , independent of the salt concentration, can be calculated when the value for F for one ionic strength is known. In the present case, however, a further complication arises since the primary salt effect interferes with the secondary salt effect which is related to the concentration of the reacting glycine anion.

It follows from the zwitter ionic concept that the equivalent concentration of the glycine anion is given by

$$(\text{NH}_2\text{CH}_2\text{COO}^-) = \frac{f_{A^\pm}}{f_{A^-} \cdot f_{\text{H}^+}} \frac{(\text{NH}_3^+\text{CH}_2\text{COO}^-)}{(\text{H}^+)} \frac{a_w}{K_B}$$

where f_{A^-} , $f_{A^\pm}$, and f_{H^+} represent the activity coefficients of glycine anion, glycine zwitter ion, and hydrogen ion, respectively, a_w the ionic product of water, and K_B the true zwitter ionic constant of glycine.

By determining the velocity constants, k_1 , independent of pH (Column 5, Table IV), the concentrations of glycine anion were read from the glycine titration curve (Curve 3, Fig. 2) at the corresponding pH at which the reaction was carried out. This was not entirely correct, since, in the reacting medium where the ionic strength was always higher than in case of the solutions used for the titration curves, the activity coefficients f_{A^-} , on the other hand, possessed a smaller value. This fact influences, at the same time, the change in the equilibrium concentrations of glycine anion.

At a given pH the concentration of the glycine anion can be expressed as a function of its activity coefficient, *i.e.*

$$\frac{(\text{NH}_2\text{CH}_2\text{COO}^-)}{c - (\text{NH}_2\text{CH}_2\text{COO}^-)} = K \cdot \frac{1}{f_{A^-}} \quad (14a)$$

or,

$$(\text{NH}_2\text{CH}_2\text{COO}^-) = \frac{Kc}{K + f_{A^-}} \quad (14b)$$

where

$$K = \frac{a_w \cdot f_{A^+}}{K_B(H^+)f_{H^+}}$$

The value of K is approximately independent of the ionic strength since f_{A^+} is affected very little by the presence of salts (15). Since values for the thermodynamic constant, K_B and a_w are known ($K_B = 6.04 \times 10^{-6}$; $a_w = 1.01 \times 10^{-14}$) (16), the value of K can be determined for various pH values. At pH 8, $K = 1.67 \times 10^{-2}$. The value for K increases tenfold for each unit in the pH value.

When the concentration of glycine anion, $(\text{NH}_2\text{CH}_2\text{COO}^-)_1$, (obtained from the titration curve), is compared at the same pH with its concentration in the reacting medium, the following relationship is used:

$$\frac{(\text{NH}_2\text{CH}_2\text{COO}^-)_1}{(\text{NH}_2\text{CH}_2\text{COO}^-)} = \frac{K + f_{A^-}}{K + (f_{A^-})_1} \quad (15)$$

where f_{A^-} and $(f_{A^-})_1$ represent the activity coefficients of glycine anion (titration curve and reaction mixture, respectively).

For pH values < 9 , the value of K is small in comparison with those of f_{A^-} and $(f_{A^-})_1$, and can therefore be neglected. Equation (15) can then be written

$$(\text{NH}_2\text{CH}_2\text{COO}^-) = \frac{(f_{A^-})_1}{f_{A^-}} (\text{NH}_2\text{CH}_2\text{COO}^-)_1 \quad (15a)$$

When pH > 11 , the activity coefficients, f_{A^-} and $(f_{A^-})_1$, can be neglected since their values are small in comparison with that of K . Then

$$(\text{NH}_2\text{CH}_2\text{COO}^-) = (\text{NH}_2\text{CH}_2\text{COO}^-)_1 \quad (15b)$$

On the basis of equations (15a) and (15b), the factor, F , which characterizes the primary salt effect, must be multiplied by the ratio $\frac{(f_{A^-})_1}{f_{A^-}}$ (when the pH is lower than 9) in order to obtain the factor $(F)_T$ which takes into account both the primary and the secondary salt effect

$$(F)_T = \frac{(f_{A^-})_1 \cdot f_{B^-}}{f_{X^-}}$$

Above pH 11 the factor F accounts for the primary salt effect only, being given by

$$F = \frac{f_{A^-} \cdot f_{B^-}}{f_{X^{2-}}}$$

Since all of these activity coefficients are not known, the question of the salt effect cannot be solved quantitatively and, for the same reason, the velocity constants, independent of the salt concentration, cannot

TABLE V

pH	$k_1' \times 10^3$	Concentrations of buffer components				
		Glycine	H ₂ BO ₃	K ₂ HPO ₄	NaOH	KCl
		<i>mols</i>	<i>mols</i>	<i>mols</i>	<i>mols</i>	<i>mols</i>
10.29	7.3	↑ 0.204 ↓	0.247		0.395	0.1
	8.2		0.247		0.395	0.37
10.52	8.0		0.247		0.413	0.1
	9.5			0.50	0.290	
10.89	8.8		0.247		0.437	0.1
	12.0			0.50	0.370	
11.41	9.5		0.247		0.460	0.1
	10.8			0.25	0.370	
12.10	10.0		0.247		0.480	0.1
	7.4				0.230	
12.90	10.7		0.247		0.616	0.1
	7.7				0.350	

be given. From a qualitative standpoint, however, Brönsted's postulates are satisfied. The velocity constants k_1 (see Table IV, Column 5) increase with the ionic strength, thus showing that there is positive salt catalysis. The increase in the velocity constants over the pH range of 9–11 is relatively smaller than outside of this pH range. This is due probably to the continuous disappearance of the secondary salt effect. At lower pH values, this effect resulted in an increase in the value for F .

Further evidence for the existence of the positive catalytic salt effect can be demonstrated when the values for k_1' are compared in solutions of the same pH but of different ionic strengths. The data which are given in Table V show that the increase in the concentration of KCl increases the value for k_1' . A considerable increase in the velocity constants was found when phosphate buffers were used. This is due to the high valency of HPO_4^- and PO_4^{3-} . When, on the other hand, only glycine was used as a buffer, the velocity constants were lower than in the glycine-borate buffer mixture of higher ionic strength. (See data which are given in the last four horizontal lines of Table V.)

In concluding this discussion on the salt effect, it should be mentioned that the ionic strength does not remain constant during the reaction, since divalent and, to some extent, trivalent substituted glycine anions are produced under the described experimental conditions. It can be expected, therefore, that the velocity constants will increase during the course of the reaction. On the other hand, the decrease in pH during the reaction tends to diminish the velocity constants. It appears, therefore, that these effects compensate each other, and the constants remain only apparently unchanged.

Some increase in the velocity constants can be observed at pH values which are higher and at which the decrease of pH during the reaction does not influence the concentration of glycine anion.

The experimental evidence that only the anionic form of glycine is able to react with iodoacetate finds also some theoretical support.

Olson (17) discussed recently the mechanism of certain substitution reactions of the type

in which an addition compound, ABC , represents the first step. He showed that the existence of this intermediate complex is in accordance with the quantum mechanical conception of the chemical bond. In the case of the halogen acids, where the halogen is placed in the apex of the carbon tetrahedron, the carbon halogen bond extends beyond the carbon atom on the side away from the halogen. Therefore, the carbon face opposite to the halogen is able to react with a particle carrying unbonded electron pairs, whereas the halogen ion is ejected from the molecule.

On the basis of these considerations, an explanation offers itself that iodoacetate can react with the amino group if the nitrogen possesses an unbonded electron pair. Since in the zwitter ionic form this electron pair is shared with hydrogen ion forming $-\text{NH}_3^+$, only the anionic form of glycine, where this pair is free, is able to react. The intermediate addition compound corresponds then to Brönsted's critical complex.

The author is cordially indebted to Professor Carl L. A. Schmidt for the suggestion of this problem and for his helpful interest and criticism during the work.

SUMMARY

1. The kinetics of the reaction which takes place between glycine and iodoacetic acid was studied by means of the polarographic method.
2. On the basis of kinetic equations, evidence was obtained that (a) The reaction proceeds in two steps in which the hydrogens of the amino group are consecutively replaced by the acetyl radicals, the velocity constants being in the ratio 2:1. (b) Only the anionic form of glycine is able to react since the velocity constants at any pH are proportional to the concentration of glycine anion. (c) The reaction is of the ionic type, showing a positive salt catalysis, which, according to Brönsted's hypothesis, involves the primary and the secondary salt effects.
3. The fact that only the glycine anion is able to react was explained as being due to the existence of an unbonded pair of electrons on the nitrogen in the NH_2 group. The NH_3^+ group, however, in which these electrons are shared by H^+ , must, therefore, be inactive.

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