Kinetics and Mechanism of the Reaction of 2,2′-Dichlorodiethylamine with Aqueous Carbon Dioxide

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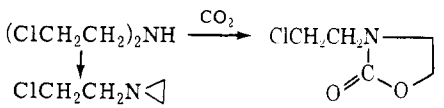
Abstract: Aqueous carbon dioxide with 2,2′-dichlorodiethylamine gives 3-(2-chloroethyl)-2-oxazolidinone. At 37° over the pH range of 6.3–7.5 and buffered with 5×10^{-4} to 170×10^{-4} *M* carbon dioxide, the half-lives of the pseudo-first-order reactions vary from 1.5 to 35 min. Rate and equilibrium constants bearing on the formation of the oxazolidinone have been determined. Ring closure from the carbamate anion, $(ClCH_2CH_2)_2NCO_2^-$, is the rate-controlling step, $k_s = 10^{-1.6 \pm 0.8}$ sec^{-1}; closure from the zwitterion, $(ClCH_2CH_2)_2NH^+CO_2^-$, and reactions involving the aziridine are kinetically unimportant. The pK_a of 2,2′-dichlorodiethylamine hydrochloride, measured by pH titration and confirmed by the kinetic results, is 6.41 at 37°.

The secondary nitrogen mustard 2,2′-dichlorodiethylamine (NSC-10873) reacts with aqueous solutions of carbon dioxide to form 3-(2-chloroethyl)-2-oxazolidinone.[2-4] Since secondary nitrogen mustards are not effective as biological alkylating agents[5,6] and since the reaction of 2,2′-dichlorodiethylamine with aqueous carbon dioxide is extremely rapid, we have examined the mechanism of the reaction in order to further our understanding of the behavior of these compounds in biological systems. Rauen[2b,c] and Williamson[7] and their coworkers have measured the rate of oxazolidinone formation, but have made no detailed mechanistic studies; our work was directed toward a determination of the mechanism.

Golumbic and Bergman and their coworkers[8] demonstrated that β-chloroethyl tertiary amines behave as alkylating agents through the intermediate formation of quaternary aziridinium ions, but a tertiary aziridine intermediate in the formation of 3-(2-chloroethyl)-2-oxazolidinone from 2,2′-dichlorodiethylamine is not possible since even at low carbon dioxide concentrations the oxazolidinone is formed remarkably faster than is the aziridine.[2b,c,4]



Experimental Section

3-(2-Chloroethyl)-2-oxazolidinone was isolated in 86% yield, distilled, from an aqueous solution 0.28 *M* in 2,2′-dichlorodiethylamine and 0.60 *M* in sodium bicarbonate after 3 hr at 31°.[4]

The 2,2′-dichlorodiethylamine used was made by the reaction of diethanolamine with thionyl chloride in chloroform according to the procedure of Ward.[9] It was recrystallized from acetone four times to a constant mp 212–213° (uncorrected) (lit.[8] mp 216°). The material was stored under vacuum over Drierite in the dark. Over long periods of time no decomposition was ever detected.

All solutions were made with degassed water. Phosphate buffer solutions were prepared in liter quantities to reduce aging effects. Sodium carbonate solutions were freshly made for each kinetic run to reduce loss of carbon dioxide. Standard sodium hydroxide solutions were stored in polyethylene bottles under Ascarite. No 2,2′-dichlorodiethylamine hydrochloride stock solutions were used; the solutions were freshly prepared from weighed amounts of mustard before each kinetic run.

Apparatus. The analytical method for following chloride ion evolution was essentially that of Swain and Ross.[10] Modifications involved the use of closed, four-necked flasks, with the stirrer introduced through a rubber sleeve in one of the necks. The use of closed cells with volumes only slightly greater than that of the solutions minimized the loss of gaseous carbon dioxide.

The electrodes were 55 cm of no. 18 silver wire, 40–45 cm of which was wound into two concentric coils as the electrode surface; the remainder was coated with polystyrene to serve as the electrode shank. The electrodes were made as pairs from adjacent pieces of wire and were anodized in 2.0 *N* hydrochloric acid for 45 min and shorted together as a pair overnight in 0.15 *N* sodium chloride to reduce small differences in potential. An electrode pair was used only if the potential difference between the electrodes was less than 0.1 mV. No attempt was made to produce standard silver–silver chloride reference electrodes of definite potential.

The salt bridge was made from a length of polyethylene tubing sealed at each end with a porous glass plug; the salt-bridge solutions, containing phosphate, carbonate, and nitrate, duplicated the kinetic run solutions in buffer, carbonate, and ionic strength.

The chloride ion concentrations, measured by the concentration cell technique, were subject to the usual errors in electrical methods: liquid junctions of the salt bridge, changing electrode response, and changes in the stirring rate. In order to reduce the effect of these errors the kinetic data were treated by the Guggenheim method.[11]

The kinetic cell assembly was mounted in a thermostat maintained at 36.7 ± 0.05° measured with a thermometer calibrated against an N.B.S. thermometer. During the kinetic runs the pH was found never to vary more than ±0.01 pH unit as measured on a Beckman Research pH Meter capable of reading to ±0.002 pH unit.

Kinetic Procedure. 3-(2-Chloroethyl)-2-oxazolidinone Formation. In a typical run, sodium carbonate necessary ultimately to produce solutions 0.009–0.08 *M* was dissolved in 0.24 *M* phosphate buffer. Fifty milliliters of this solution was pipetted into each thermostated cell. Sodium nitrate necessary to give the desired ionic strength (0.26–0.36) was added. Equal amounts of water were added to each cell in order to make about 100 ml of total solution. The electrodes were mounted and the cells closed. Nitric acid, 1.0 *N*,

(1) Support by a Sterling-Winthrop Research Fellowship and a Stauffer Chemical Fellowship is gratefully acknowledged.

(2) (a) H. Arnold and H. Bekel, *Arzneim.-Forsch.*, **14**, 750 (1964); (b) H. M. Rauen, *ibid.*, **14**, 855 (1964); (c) H. M. Rauen and H. Palla, *ibid.*, **16**, 40 (1966).

(3) R. H. Wood, Ph.D. Thesis, Rensselaer Polytechnic Institute, Troy, N. Y., 1963.

(4) P. Henkart, B.S. Thesis, Rensselaer Polytechnic Institute, Troy, N. Y., 1963.

(5) T. J. Bardos, N. Datta-Gupta, P. Hebborn, and D. J. Triggle, *J. Med. Chem.*, **8**, 167 (1965).

(6) L. H. Schmidt, R. Fradkin, R. Sullivan, and A. Flowers, "Comparative Pharmacology of Alkylating Agents," Cancer Chemotherapy Reports, Bethesda, Md., 1965, pp 1017–1040.

(7) C. E. Williamson, J. G. Kirby, J. I. Miller, S. Sass, S. P. Kramer, A. M. Seligman, and B. Witten, *Cancer Chemother. Rep.*, **41**, 47 (1964); *Cancer Res.*, **26**, 323 (1966).

(8) M. A. Stahmann and M. Bergman, *J. Org. Chem.*, **11**, 586 (1946), and previous papers.

(9) K. Ward, Jr., *J. Amer. Chem. Soc.*, **57**, 914 (1935).

(10) C. G. Swain and S. D. Ross, *ibid.*, **68**, 658 (1946).

(11) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, Wiley, New York, N. Y., 1961, pp 49–50.

Table I. K_a of 2,2′-Dichlorodiethylamine Hydrochloride (K_{a1}) at 36.7 ± 0.05°

10^7K_a, mol l.$^{-1}$	Ref
3.9 ± 0.4[a]	This work
3.2	7
0.630[b]	c

[a] Average of four titrations of 2,2′-dichlorodiethylamine hydrochloride; 95% confidence limits. [b] At 22°. [c] N. Brock and H. J. Hohorst, *Arzneim.-Forsch.*, **11**, 164 (1961).

the approximate range found for a 95% confidence level in determinations where there were several measurements.

Results and Discussion

Table II summarizes the pseudo-first-order rate constants for formation of chloride ion in aqueous solutions of 2,2′-dichlorodiethylamine buffered for pH and carbon dioxide at controlled ionic strength. No systematic effects, within the limits of error of the measurements, were found when the ionic strength of the solutions was varied from 0.26 to 0.36. The carbon dioxide concentrations were calculated from the stoichiometric concentrations of sodium carbonate using published values for the equilibrium constants[12] which relate carbonate and bicarbonate to carbon dioxide.

Table II. Pseudo-First-Order Rate Constants for the Formation of 3-(2-Chloroethyl)-2-oxazolidinone from 0.0008 *M* Aqueous Solutions of 2,2′-Dichlorodiethylamine at 36.7 ± 0.05°

pH	10^2[Na_2CO_3],[b] *M*	CO_2, mol %	10^4[CO_2], *M*	Ionic strength	$10^3k'$,[a] sec^{-1}	No. of runs in av
7.48 ± 0.01	1.778 ± 0.018	2.91	5.17 ± 0.05	0.258 ± 0.001	3.2 ± 0.4	5
7.49 ± 0.01	3.531 ± 0.021	2.81	9.92 ± 0.06	0.281 ± 0.001	5.3 ± 0.3	8
7.50 ± 0.01	5.138 ± 0.029	2.71	13.92 ± 0.08	0.301 ± 0.001	7.1 ± 0.3	5
7.49 ± 0.01	6.79 ± 0.16	2.72	18.47 ± 0.45	0.321 ± 0.001	8.8 ± 0.8	3
6.62 ± 0.01	1.809 ± 0.081	14.2	25.7 ± 1.2	0.291 ± 0.001	1.0 ± 0.2	2
6.63 ± 0.01	3.47 ± 0.40	13.8	47.9 ± 5.5	0.297 ± 0.001	1.9 ± 0.5	2
6.63 ± 0.01	5.249 ± 0.057	13.6	71.4 ± 0.8	0.321 ± 0.001	2.6 ± 0.6	3
6.64 ± 0.01	6.263 ± 0.011	13.5	84.6 ± 0.1	0.326	3.1 ± 0.6	1
6.63 ± 0.01	7.047 ± 0.012	13.6	95.8 ± 0.2	0.330	3.9 ± 0.8	1
6.64 ± 0.01	8.628 ± 0.014	13.6	117 ± 2	0.313	4.4 ± 0.9	1
6.33 ± 0.01	0.976 ± 0.016	20.4	19.9 ± 0.3	0.289	0.33 ± 0.06	1
6.32 ± 0.01	1.823 ± 0.031	20.4	37.2 ± 0.6	0.307	0.67 ± 0.13	1
6.34 ± 0.01	5.137 ± 0.087	19.6	101 ± 2	0.342	1.10 ± 0.20	1
6.32 ± 0.01	8.48 ± 0.14	19.9	169 ± 3	0.364	1.76 ± 0.40	1

[a] All errors are for a 95% confidence level. Rate constants from a single determination are arbitrarily assigned a ±20% deviation. [b] Average stoichiometric concentrations. Where only a single value is used to represent this average concentration, a deviation of ±1.7% was assumed by averaging the 95% confidence limits of average concentrations having multiple values.

The effects of pH and carbon dioxide concentrations on the rate of oxazolidinone and chloride ion formation can be expressed in terms of eq 1–8. From these equations, the rate of formation of chloride ion in

$$\underset{MH^+}{(ClCH_2CH_2)_2NH_2^+} \overset{K_{a1}}{\rightleftharpoons} \underset{M}{(ClCH_2CH_2)_2NH} + H^+ \quad (1)$$

$$\underset{M}{(ClCH_2CH_2)_2NH} \xrightarrow{k_2} ClCH_2CH_2\text{-}\overset{+}{N}\text{(ring)}\text{-}H + Cl^- \quad (2)$$

$$\underset{M}{(ClCH_2CH_2)_2NH} + CO_2 \overset{K_3}{\rightleftharpoons} \underset{M^+CO_2^-}{(ClCH_2CH_2)_2NH^+CO_2^-} \quad (3)$$

$$\underset{M^+CO_2^-}{(ClCH_2CH_2)_2NH^+CO_2^-} \xrightarrow{k_4} ClCH_2CH_2\text{-}\overset{+}{N}(H)\text{-}C(=O)\text{-}O\text{-ring} + Cl^- \quad (4)$$

(12) H. S. Harned and R. Davis, Jr., *J. Amer. Chem. Soc.*, **65**, 2030 (1943); H. S. Harned and F. T. Bonner, *ibid.*, **67**, 1026 (1945); H. S. Harned and S. R. Scholes, Jr., *ibid.*, **63**, 1706 (1941); D. Berg and A. Patterson, Jr., *ibid.*, **75**, 5197 (1953); K. F. Wissbrun, D. M. French, and A. Patterson, Jr., *J. Phys. Chem.*, **58**, 693 (1954).

was added to adjust the pH to the value desired for the run. The circuit was closed and allowed to equilibrate for about 5 min; then 0.01 *M* sodium chloride was added as necessary to either cell to balance one cell against the other. With the circuit open, sodium chloride equivalent to the chloride ion expected from the mustard hydrochloride counterion and enough water were added to the reference cell to bring the solution to a known total volume of about 125 ml. At time zero a 20-ml aliquot of 2,2′-dichlorodiethylamine hydrochloride was blown from a pipet into the reaction cell to start the reaction quickly. This gave a mustard concentration of about 0.0008 *M* and a total volume of about 125 ml in the reaction cell. The chloride ion concentration at t_0 was the same in both the reaction and reference cells; it was equal to about 0.0008 *M* plus a small amount of chloride ion that was added to balance the cells. At intervals thereafter the circuit was closed and excess sodium chloride solution was added to the reference cell, displacing the galvanometer from zero. The production of chloride ion in the reaction cell caused the galvanometer to move back to zero. When zeroed, the chloride ion concentration in both cells was taken to be the same. At the end of the reaction the chloride ion concentration had approximately doubled.

1-(2-Chloroethyl)aziridine Formation. Conditions for measurement of the kinetics of aziridine formation were the same as those for oxazolidinone formation except that no sodium carbonate was added. All solutions were degassed in the cells before aziridine runs were started, and the runs were conducted under nitrogen.

pK_a of 2,2′-Dichlorodiethylamine Hydrochloride. The pK_a of 2,2′-dichlorodiethylamine hydrochloride, pK_{a1} of eq 1, was determined by a pH titration. Each pH titration of the four determinations was completed in less than 5 min. A comparison of the stoichiometric equivalence point with the experimental equivalence point showed no excess acid within the pH error limits; if the determination were carried out more slowly, a correction would have been necessary because of additional acid formed from the reaction of free mustard to aziridine (eq 2). All results are summarized in Table I.

Error Treatment. Where enough measurements of a value were available, deviations from the arithmetic mean have been expressed to a 95% confidence level. Where there was only one measurement of a value, a 20% range has been assumed corresponding to

Table III. Slope and Intercept of Least-Squares Plots of $1/k'$ *vs.* $1/[CO_2]$ from Equation 13[a]

pH	$10^7[H^+]$, *M*	Slope, sec^{-1}	Intercept, mol $l.^{-1}$ sec^{-1}	$10^6/[H^+]$, l. mol^{-1}	10^{-6}slope/$[H^+]$, l. mol^{-1} sec^{-1}
7.49 ± 0.01	0.324 ± 0.008	0.14 ± 0.05	42 ± 20	31 ± 1	4.4 ± 1.5
6.63 ± 0.01	2.34 ± 0.06	2.3 ± 0.6	48 ± 41	4.3 ± 0.1	9.8 ± 2.5
6.33 ± 0.01	4.7 ± 0.1	5.3 ± 2.2	267 ± 644	2.14 ± 0.05	11.3 ± 4.7

[a] All errors are for a 95% confidence level.

$$(ClCH_2CH_2)_2NH^+CO_2^- \underset{}{\overset{K_{a5}}{\rightleftharpoons}} (ClCH_2CH_2)_2NCO_2^- + H^+ \quad (5)$$
$$M^+CO_2^- \qquad\qquad MCO_2^-$$

$$(ClCH_2CH_2)_2NCO_2H \overset{K_{a6}}{\rightleftharpoons} (ClCH_2CH_2)_2NCO_2^- + H^+ \quad (6)$$
$$MCO_2H \qquad\qquad MCO_2^-$$

$$(ClCH_2CH_2)_2NH^+CO_2H \overset{K_{a7}}{\rightleftharpoons} (ClCH_2CH_2)_2NH^+CO_2^- + H^+ \quad (7)$$
$$M^+CO_2H \qquad\qquad M^+CO_2^-$$

$$(ClCH_2CH_2)_2NCO_2^- \xrightarrow{k_8} ClCH_2CH_2\text{–}N\text{–}C(=O)\text{–}O + Cl^- \quad (8)$$
$$MCO_2^-$$

terms of free mustard results (eq 9). The mustard

$$\frac{d[Cl^-]}{dt} = \left(k_2 + k_4K_3[CO_2] + \frac{k_8K_{a5}K_3[CO_2]}{[H^+]}\right)[M] \quad (9)$$

initially added, however, exists not only as free mustard and as product but also as those compounds identified with the equilibria represented by eq 3,[13] 5, 6, and 7. Consequently, the concentration of free mustard is given by eq 10 and the rate of chloride ion formation

$$[M] = ([M_0] - [Cl^-])\Big/\left(1 + \frac{[H^+]}{K_{a1}} + K_3[CO_2] + \frac{K_{a5}K_3[CO_2]}{[H^+]} + \frac{K_{a5}K_3[CO_2]}{K_{a6}} + \frac{K_3[H^+][CO_2]}{K_{a7}}\right) \quad (10)$$

by eq 11. If the pH and carbon dioxide concentrations

$$\frac{d[Cl^-]}{dt} = \left[\left(k_2 + k_4K_3[CO_2] + \frac{k_8K_{a5}K_3[CO_2]}{[H^+]}\right)\Big/\left(1 + \frac{[H^+]}{K_{a1}} + K_3[CO_2] + \frac{K_{a5}K_3[CO_2]}{[H^+]} + \frac{k_{a5}K_3[CO_2]}{K_{a6}} + \frac{K_3[H^+][CO_2]}{K_{a7}}\right)\right]([M_0] - [Cl^-]) \quad (11)$$

are held constant, the pseudo-first-order rate constant for chloride ion formation is then given by eq 12.

$$k' = \left(k_2 + k_4K_3[CO_2] + \frac{k_8K_{a5}K_3[CO_2]}{[H^+]}\right)\Big/\left(1 + \frac{[H^+]}{K_{a1}} + K_3[CO_2] + \frac{K_{a5}K_3[CO_2]}{[H^+]} + \frac{K_{a5}K_3[CO_2]}{K_{a6}} + \frac{K_3[H^+][CO_2]}{K_{a7}}\right) \quad (12)$$

3-(2-Chloroethyl)-2-oxazolidinone Formation. Four possible kinetic pathways for the formation of oxazolidinone can be envisioned: (1) formation of aziridinium ion with subsequent production of oxazolidinone, eq 2, (2) carbon dioxide attack on the amine as the slow step in oxazolidinone formation, eq 3, (3) ring closure to oxazolidinone from the carbamate zwitterion as the slow step, eq 4, and (4) ring closure to oxazolidinone from the carbamate anion as the slow step, eq 8.

Since oxazolidinone is formed 50 times faster than is aziridine,[2,4] *vide infra*, the aziridinium ion (eq 2) cannot play any role in oxazolidinone formation.

Carbon dioxide attack on the amine (eq 3) cannot be the slow step since Faurholt, Jensen, and coworkers[14,15] have shown that carbon dioxide in excess reacts with amines to give carbamates at pseudo-first-order rates about 10^6 times greater than those we have observed for oxazolidinone formation.

If the slow step were that represented by eq 4, the second term in the numerator of eq 12 would be larger than the other two which, consequently, can be deleted; the reciprocal of the resultant equation is then given by eq 13. A plot of $1/k'$ *vs.* $1/[CO_2]$ at constant pH gave

$$1/k' = \frac{1}{k_4K_3[CO_2]} + \frac{[H^+]}{K_{a1}K_3k_4[CO_2]} + \frac{1}{k_4} + \frac{K_{a5}}{k_4[H^+]} + \frac{K_{a5}}{k_4K_{a6}} + \frac{[H^+]}{k_4K_{a7}} \quad (13)$$

a line whose slope divided by the hydrogen ion concentration is given by eq 14. A plot of values (Table

$$\frac{\text{slope}}{[H^+]} = \frac{1}{k_4K_3[H^+]} + \frac{1}{K_{a1}K_3k_4} \quad (14)$$

III) for slope/$[H^+]$ *vs.* $1/[H^+]$ resulted in a line of negative slope, $1/k_4K_3 = -0.234$ sec, with an intercept, $1/K_{a1}K_3k_4$, of 11.4×10^6 sec. Such a result has no chemical significance, since it would require negative values for two rate or equilibrium constants; consequently the slow step in the reaction cannot be ring closure through the carbamate zwitterion.

If the mechanism were to proceed through the reaction represented by eq 8, ring closure to oxazolidinone from the carbamate anion as the rate-controlling step, the last term in the numerator of eq 12 would be larger than the other terms in the numerator; the reciprocal of the resultant equation if the first two terms in the numer-

(13) Justification for the inclusion of the reaction represented by eq 3 as an equilibrium rather than as the slow step in the formation of oxazolidinone results from a comparison of our results with the rates of reaction of carbon dioxide with secondary amines as measured by Faurholt,[14,15] *vide infra*.

(14) C. Faurholt, *J. Chim. Phys.*, **22**, 1 (1925); V. Lund and C. Faurholt, *Dan. Tidsskr. Farm.*, **22**, 109 (1948).

(15) A. Jensen, M. B. Jensen, and C. Faurholt, *Acta Chem. Scand.*, **8**, 1129 (1954); M. B. Jensen, *ibid.*, **11**, 499 (1957).

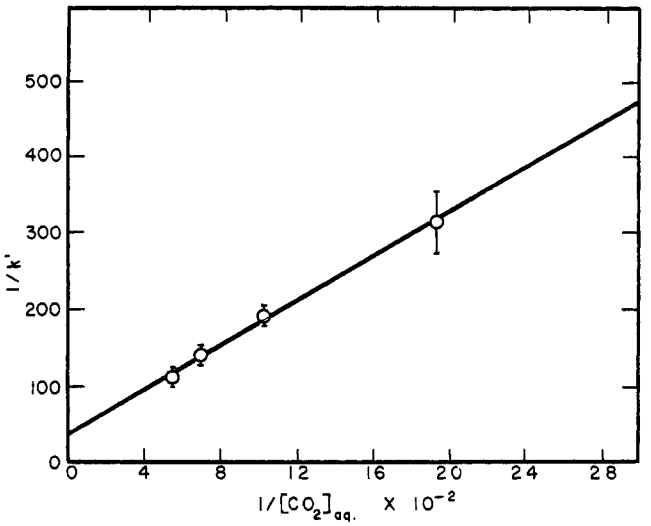


Figure 1. $1/k'$ *vs.* $1/[CO_2]_{aq}$ at pH 7.49 ± 0.01 and 36.7°; eq 15.

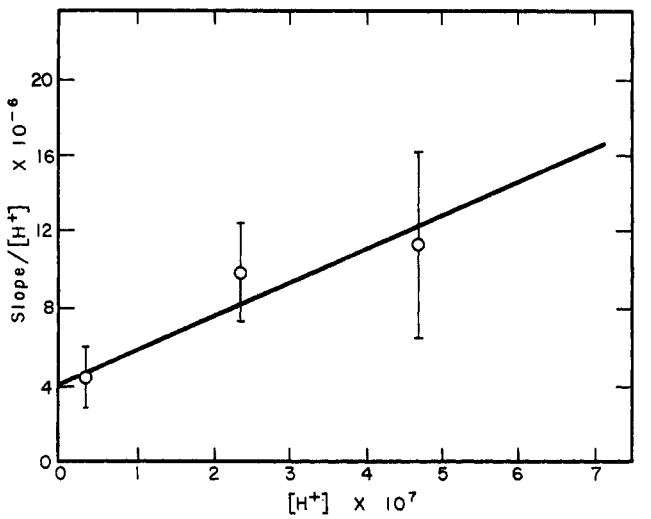


Figure 2. Slope/$[H^+]$ *vs.* $[H^+]$ for ring closure from carbamate anion; eq 16.

ator are deleted is eq 15. A plot, Figure 1 is typical, of $1/k'$ *vs.* $1/[CO_2]$ at constant pH gives a line whose slope

$$1/k' = \frac{[H^+]}{k_8K_{a5}K_3[CO_2]} + \frac{[H^+]^2}{k_8K_{a5}K_3K_{a1}[CO_2]} + \frac{[H^+]}{k_8K_{a5}} + \frac{1}{k_8} + \frac{[H^+]}{k_8K_{a6}} + \frac{[H^+]^2}{k_8K_{a5}K_{a7}} \quad (15)$$

divided by the hydrogen ion concentration is represented by eq 16. From this a plot of values (Table III) of slope/

$$\frac{\text{slope}}{[H^+]} = \frac{1}{k_8K_{a5}K_3} + \frac{[H^+]}{k_8K_{a5}K_3K_{a1}} \quad (16)$$

$[H^+]$ *vs.* $[H^+]$ (Figure 2) gave a line with a slope $1/k_8K_{a5} \cdot K_3K_{a1} = (1.7 \pm 1.2) \times 10^{13}$ l. sec mol^{-1} and an intercept $1/k_8K_{a5}K_3 = (4.1 \pm 1.9) \times 10^6$ sec. Division of the slope by the intercept gives a value for $K_{a1} = (2.5 \pm 2.0) \times 10^{-7}$ mol l.$^{-1}$. The agreement between this value and that which was determined directly by pH titrations on 2,2′-dichlorodiethylamine, $(3.9 \pm 0.4) \times 10^{-7}$ mol l.$^{-1}$, is excellent evidence that the rate-controlling step in the formation of the oxazolidinone is ring closure from the carbamate anion.

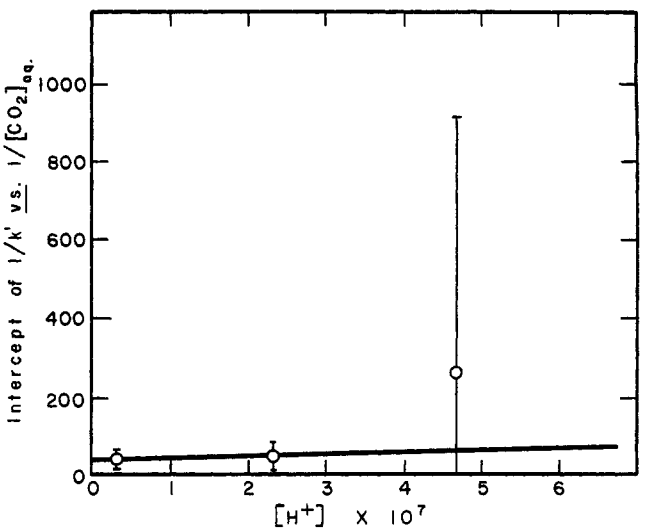


Figure 3. Intercept *vs.* $[H^+]$ for ring closure from carbamate anion; eq 17.

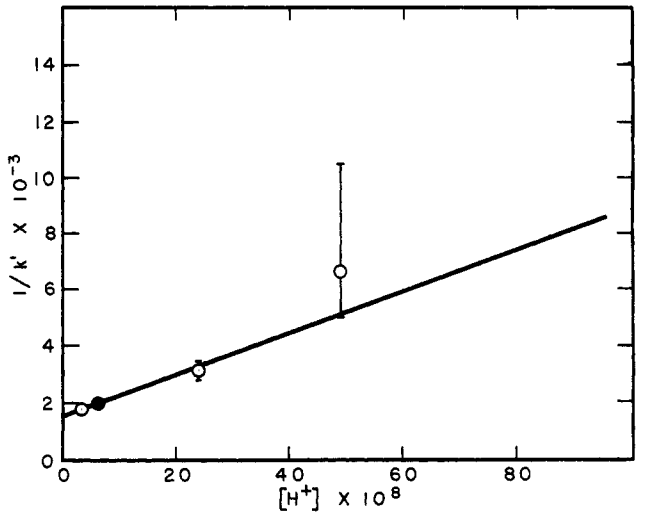


Figure 4. $1/k'$ *vs.* $[H^+]$ in the absence of carbon dioxide; eq 18: ○, this work; ●, from ref 2b,c.

Table IV. Pseudo-First-Order Rate Constants for 1-(2-Chloroethyl)aziridinium Ion Formation at 36.7 ± 0.05°

pH	Ionic strength	$10^3k'$, sec^{-1}	No. of runs in av
7.48 ± 0.02	0.283 ± 0.015	0.56 ± 0.03	4
6.62 ± 0.01	0.290	0.32 ± 0.03	3
6.31 ± 0.01	0.298 ± 0.001	0.15 ± 0.06	2
7.2		0.5[a]	
6.0		0.07[a]	

[a] Reference 2c.

The intercept of a plot of $1/k'$ *vs.* $1/[CO_2]$ (eq 15) is expressed by eq 17. When the intercept is plotted

$$\text{intercept} = \frac{1}{k_8} + \frac{[H^+]}{k_8K_{a5}} + \frac{[H^+]}{k_8K_{a6}} + \frac{[H^+]^2}{k_8K_{a5}K_7} \quad (17)$$

against the hydrogen ion concentration (Figure 3) the intercept of the resulting line provides a value for $k_8 = (2.4(+5.3, -1.0)) \times 10^{-2}$ sec^{-1}.

1-(2-Chloroethyl)aziridine Formation. The pseudo-first-order rate of chloride ion production from aziri-

dine formation (eq 2) can be obtained from eq 12 by dropping all carbon dioxide containing terms. The reciprocal of such a reduced equation is given by eq 18.

$$\frac{1}{k'} = \frac{1}{k_2} + \frac{[H^+]}{k_2 K_{a1}} \quad (18)$$

A plot, Figure 4, of $1/k'$ *vs.* $[H^+]$, Table IV, yields a line whose intercept divided by the slope provides another value of K_{a1}, $(2.4(+0.7, -0.9)) \times 10^{-7}$, to be compared with the value of $(3.9 \pm 0.4) \times 10^{-7}$ obtained titrimetrically.

Rauen's results[2b,c] are included in Figure 4 but have not been used in the calculation of the slope or intercept. From the intercept, $k_2 = (6.3 \pm 1) \times 10^{-4}$ sec^{-1}.

Hydrolysis of Substituted Trifluoroacetanilides. Some Implications for the Mechanism of Action of Serine Proteases

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Contribution from the Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45239. *Received March 14, 1972*

Abstract: The hydrolysis of seven trifluoroacetanilides having various substituents in the aniline ring was investigated at low and high pH. The rate constants for all reactions postulated to occur were obtained separately, and Hammett ρ coefficients for each rate constant were calculated. Positive or zero ρ values were obtained for the reactions: addition of OH^- to trifluoroacetanilide to form a tetrahedral intermediate (k_1); expulsion of OH^- from the intermediate (k_{-1}); and donation of a proton to the intermediate by water (k_2) to form products. The OH^- catalyzed breakdown of the intermediate (k_3) had a negative ρ coefficient. The rate-limiting step for k_3 is considered to involve a proton transfer, while that for k_2 is concluded to be heavy-atom rearrangement. The implications of these findings for the mechanism of action of serine proteases (where hydrolysis of anilide substrates has a negative ρ value) is discussed.

The alkaline hydrolysis of substituted acetanilides has been studied by a number of workers and recently reviewed by O'Conner.[1] Biechler and Taft[2] showed that the pseudo-first-order rate constant for the hydrolysis of trifluoroacetanilide displayed a parabolic dependence on OH^- concentration. They postulated a model in which abstraction of a proton by hydroxide (*i.e.*, ionization of the neutral form of trifluoroacetanilide) produced a nonreactive species. From their data they calculated a pK_a of 11.9 in water. Mader[3] measured the pK_a of trifluoroacetanilide spectrophotometrically and showed it to be 9.5. He proposed a mechanism for the hydrolysis in which a reactive intermediate arises either by addition of hydroxide to the un-ionized form or water to the ionized form of trifluoroacetanilide. Eriksson and coworkers[4,5] studied the effect of various bases as catalysts for the hydrolysis of trifluoroacetanilide. They postulated that the tetrahedral intermediate was formed by addition of hydroxide ion to the un-ionized substrate. Ionization of the trifluoroacetanilide in their model represented a nonhydrolytic pathway. Bender and coworkers[6,7] assumed that the intermediate for the hydrolysis of acetanilide was formed by addition of OH^-. With this substrate no ionization of substrate takes place in the range of OH^- concentrations studied. Schowen and his coworkers[8-11] used a model similar to that of Bender for analyzing their data on the hydrolysis of *N*-methyltrifluoroacetanilide. Again, no ionization is possible with this substrate.

The hydrolysis of acylanilides might be thought a reasonable model reaction to compare with the enzymatic hydrolysis of amides, much as the catalyzed hydrolysis of esters has been used as a model of enzymatic hydrolysis of esters.[12-14] In fact, published data have not supported the analogy for the anilide case. Studies of the hydrolysis of *N*-acetyltyrosylanilides by chymotrypsin[15-18] have yielded negative ρ values with respect to the effect of substituents in the anilide moiety on the rate of acylation of enzyme by the substrate. In contrast, ρ values derived from studies of the hydroxide ion catalyzed hydrolysis of anilides[6,11] have been positive.

In this paper I report some studies on the hydroxide-catalyzed hydrolysis of a number of meta- and para-substituted trifluoroacetanilides. This work was under-

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