## SUMMARY

The rate of reaction between 2-dimethylaminoethanethiol and propionic anhydride was found to be dependent upon pH. This was explained by assuming the reaction to proceed *via* attack by the anionic species of the thiol on the anhydride. This effect was observed even at low pH values where the concentration of anionic form might be considered negligible. The reaction rate was too rapid to measure conveniently at pH's above six. Thus, it would appear from this study that the reaction between thiols and anhydrides would proceed extremely fast under the conditions existing in the body.


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# Thiol Esters II: A Kinetic Study of Hydrolysis and Aminolysis of Propionyl Thiocholine Iodide and 2-Dimethylaminoethanethiol Propionate


**ANWAR HUSSAIN and P. SCHURMAN**

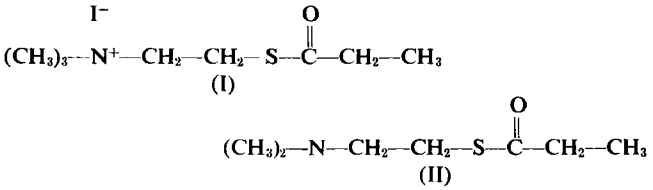


**Abstract** □ The kinetics of hydrolysis of propionyl thiocholine iodide (I) and 2-dimethylaminoethanethiol propionate (II) in aqueous buffers were studied over a pH range 0.3–13.5. The data indicate that the rate of hydronium-ion attack on both compounds is essentially the same. In the pH 4–10 range, (II) hydrolyzes much faster than (I), while above pH of 10 the quaternary compound (I) hydrolyzes faster than (II). This difference, between the rates of hydrolysis of the two compounds, can be attributed to the stabilization of the hydrolysis transition state by a protonated nitrogen at the lower pH and by the quaternary nitrogen atom at a higher pH. The effect of ionic strength, different buffer species, and temperature on the rates of hydrolysis have also been investigated. The rate of reaction between glycine and the above thiol esters has also been determined. The pH rate profile of the aminolysis reaction suggests that the attacking species is the nonprotonated form of glycine.



**Keyphrases** □ Propionyl thiocholine iodide—hydrolysis, aminolysis rates □ 2-Dimethylaminoethanethiol propionate—hydrolysis, aminolysis rates □ Kinetic equations—thiol esters, hydrolysis, aminolysis □ pH effect—thiol ester hydrolysis □ Buffer concentration effect—thiol ester hydrolysis


Smith *et al.* (1) have stated "enzyme reactions may be differentiated from normal catalytic processes occurring in chemical systems such as hydrolysis or oxidation by two different features, substrate specificity and the speed of the reaction which occurs in the pH range 2–10. These differences can be explained by the fact that enzyme reactions proceed by intramolecular mechanisms where a correct fit of a substrate at the enzyme surface will invoke the tremendous power of such reactions." Because acetyl coenzyme A, an intermediate in many biochemical reactions, is a thiol ester (2), this study was undertaken to investigate the kinetics of hydrolysis and aminolysis of propionyl thiocholine iodide (I) and 2-dimethylaminoethanethiol propionate (II) in order to obtain data which would aid in understanding biochemical mechanisms involving compounds of this type.

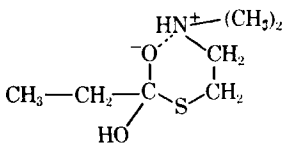


(I)



(II)

Most of the past studies have dealt with the effects of variation in thiol ester structures upon rate of hydrolysis in strongly acidic and alkaline media (3–5). Aside from the limited work of Hansen (6), little information is available regarding the nature of the hydrolytic steps for a thiol ester existing in different forms depending on the pH of the solution. In his investigation for a method to study the hydrolytic cleavage of organic protolytes, Hansen reported that the protonated tertiary amine salt is hydrolyzed 240 times faster than the corresponding quaternary salt. This difference between the two rates of hydrolysis was attributed to more efficient stabilization of the hydrolysis transition state by a labile proton, as shown (III) (7).



III

Because of the importance of these systems from the biochemical standpoint, it was felt that these reactions deserved a more detailed examination. Consequently, the rate of hydrolysis over a pH range of 0.3–13.5 and the rate of aminolysis of the above thiol esters has been determined. The effect of buffer concentrations, ionic strength, and temperature has also been examined.

## EXPERIMENTAL[1]

**Reagents and Apparatus**—2-Dimethylaminoethanethiol propionate was prepared by the reaction of propionyl chloride and 2-dimethylaminoethanethiol in benzene (3). The hydrochloride salt m.p. 105–107° was assayed iodometrically and found to be 98% pure.

The quaternary compound, propionyl thiocholine iodide was prepared according to the following procedure: 13.7 g. of sodium hydride (0.285 mole) was suspended in 200 ml. of benzene in a 500-ml. round-bottom flask. Thirty grams of distilled 2-dimethylaminoethanethiol in 100 ml. benzene was added dropwise with continuous stirring and cooling. After the addition was completed, the flask was warmed until no further hydrogen evolved. The mixture was cooled, and 26.4 g. of propionyl chloride was added slowly with continuous stirring and cooling. After the additon was completed, the mixture was left for about 1 hr. at room temperature. The clear yellow liquid was filtered and 45 g. of methyl iodide was added. The solution was kept at room temperature for 72 hr. The crystals were collected on a filter paper and recrystallized from a methanol–isopropanol–diethylether mixture. The crystalline compound, m.p. 204–206°, was assayed iodometrically and found to be 99.1% pure.

Buffers, sodium hydroxide, hydrochloric acid, and iodine solutions were prepared using reagent grade materials.

**Kinetic Procedures**—The hydroxyl and hydrogen-ion concentrations of the system were maintained constant by using buffer solutions or excess sodium hydroxide or hydrochloric acid. Buffer systems used were: hydrochloric acid, pH 0.3–1.0, phosphate, pH 1.5–2.4, 6.4–8.0, acetate, pH 3.5–4.6, dilute NaOH (carbonate free) for pH 12.0–13.5. The ionic strength of the solutions was maintained at 1.0 by the addition of sodium chloride.

**Rate of Hydrolysis of 2-Dimethylaminoethanethiol Propionate**—The rate of hydrolysis of 2-dimethylaminoethanethiol propionate was determined by measuring the rate of formation of 2-dimethylaminoethanethiol iodometrically and consumption of hydroxide ion employing a pH stat.

When the reaction was followed iodometrically, solutions of 0.3 g. of the compound in 100 ml. of the buffer at the desired pH and ionic strength were prepared. The solutions were filled into hard glass ampuls and saturated with nitrogen before sealing. The ampuls were then placed in a constant-temperature bath which was maintained at the desired temperature ± 0.05°. After allowing 15 min. for temperature equilibration, the 0-hr. samples were removed and further samples were withdrawn at time intervals suitable to the nature of the system. The reaction was quenched by immersion of the samples in ice water. Five-milliliter samples containing 15 mg. of the compound were titrated under a blanket of nitrogen with $5 \times 10^{-3}$ *M* iodine solution using starch as an indicator. At higher pH's, where the reaction was more rapid studies were carried out in stoppered conical flasks. Periodically, 5-ml. samples were withdrawn and quickly discharged into a 50-ml. conical flask containing 2 ml. of 1.0 *M* phosphoric acid in order to quench the reaction. The thiol was then titrated as described above.

The rates of hydrolysis at pH values greater than 7 were also determined from data obtained on a pH stat. Fifty milligrams of the compound was dissolved in 50 ml. of water and the concentration of hydroxyl ion was followed at constant temperature and fixed pH. Not more than 0.5 ml. of 1.0 *N* sodium hydroxide was added totally to maintain the pH throughout the reaction, so that dilution effect was negligible.

The effect of buffer concentration and ionic strength, upon the rate of hydrolysis, was studied iodometrically. The effect of temperature was determined at several pH values. 0.1 *N* HCl and 0.1 *N* NaOH were used in the acidic and alkaline pH range, respectively, and the reaction was determined iodometrically. In the intermediate pH range, the effect of temperature was determined using both the pH stat and iodometric titrations. When the reaction was determined iodometrically in this pH range, several buffer concentrations were used at each temperature. The activation energies were calculated from the observed rate constants at zero buffer concentration.

**Rate of Hydrolysis of Propionyl Thiocholine Iodide**—In the acidic and alkaline pH range, the rate of hydrolysis of the quaternary compound was determined as described above.

In the intermediate pH range, however, where the reaction is very slow even at elevated temperatures, the rate constants were determined from initial rate measurements. This was found to be necessary since the quaternary thiol is unstable on prolonged heating in this pH range.

It was also possible to determine these rate constants from the rate of loss of the thiol ester. Periodically 5-ml. samples were hydrolyzed under nitrogen with 5 ml. of 1 *N* sodium hydroxide for 10 min. Two milliliters of 1 *M* phosphoric acid was then added and the liberated thiol was titrated with $5 \times 10^{-3}$ moles/l. standard iodine solution using starch as the indicator. A residual blank titration for the thiol in solution was performed using the same procedure except that the alkaline hydrolysis was omitted. The difference between the volume of iodine solution consumed in the two determinations is equivalent to the amount of thiol liberated from the intact molecule.

**Rate of Aminolysis of (I) and (II) by Glycine**—The rate of aminolysis of I and II by glycine was measured by following the formation of the liberated thiols. Solutions containing different concentrations of glycine were prepared at several pH's and were saturated with nitrogen. Fresh solutions of the thiol esters containing 1.2 mg./ml. were prepared and stored under nitrogen. Glycine buffer (25 ml.) at the desired pH and concentration was quickly mixed with 25 ml. of the thiol ester solution in a conical flask. The solution was then saturated with nitrogen. Five-milliliter samples were periodically withdrawn and quickly discharged into a conical flask containing 3 ml. of 1 *N* HCl to quench the reaction. The liberated thiol was then titrated under a blanket of nitrogen with $1 \times 10^{-3}$ *M* iodide using starch as an indicator.

## RESULTS

**Order of the Hydrolytic Reaction**—The results of this study indicate that in aqueous buffered solutions, the hydrolysis of the above thiol esters was first order with respect to the compounds over a broad pH and temperature range.

**Effect of Buffer Concentration on the Rate of Hydrolysis of I**—Typical plots showing the effect of buffer species employed in this study on the rate of hydrolysis of the quaternary thiol ester is shown in Figs. 1 and 2. It is evident from the plots that both phos-

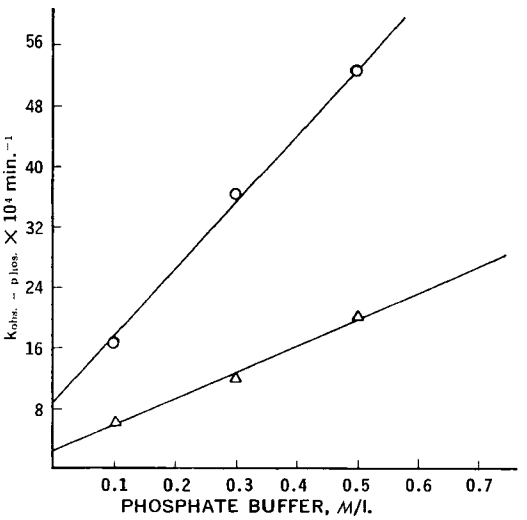



**Figure 1**—*Plot showing the effect of phosphate buffer concentration on the rate of hydrolysis of I at 63°. Key: ○, pH 7.2; △, pH 6.4.*

[1] The instruments used in this study were a Sargent pH stat, catalog No. S-30240, and a Beckman Expandomatic pH meter.

phate and acetate have a significant catalytic effect on the rate of hydrolysis of I.

Although the effect of these buffers was determined at only two hydrogen-ion concentrations, it can be assumed that the curves representing the effect of phosphate buffer at pH's 6.4 and 7.2 give a picture of the catalytic effect of $HPO_4^-$, and the curves representing the effect of acetate buffer at pH's 3.9 and 4.7 give a picture of the catalytic effect of $CH_3COO^-$. If this assumption is valid, the catalytic constants $k_{HPO_4^-}$ and $k_{CH_3COO^-}$ can be calculated from the observed rate constant $k_{obs.}$ at any given pH according to the following relationship:

$$k_{obs.\ -\ phos.} = k_0 + k_{HPO_4^-}(HPO_4^-) \qquad \text{(Eq. 1}a\text{)}$$

$$k_{obs.\ -\ acet.} = k_0 + k_{CH_3COO^-}(CH_3COO^-) \qquad \text{(Eq. 1}b\text{)}$$

where $k_{obs.\ -\ phos.}$ and $k_{obs.\ -\ acet.}$ are the observed first-order rate constants and $k_0$ is the rate constant at zero buffer concentration. The concentration of $HPO_4^-$ or $CH_3COO^-$ can be calculated from the total buffer concentration at any pH. On this basis, $k_{HPO_4^-}$ calculated at 25° for two pH's was found to be $6.1 \times 10^{-4}\ M^{-1}$ min.$^{-1}$ and $8.2 \times 10^{-4}\ M^{-1}$ min.$^{-1}$ and $k_{CH_3COO^-}$ was calculated in the same way and found to be $3.1 \times 10^{-5}\ M^{-1}$ min.$^{-1}$ at pH 3.9 and $3.0 \times 10^{-5}\ M^{-1}$ min.$^{-1}$ at pH 4.7. Thus, within experimental error, it would appear that $HPO_4^-$ and $CH_3COO^-$ are the primary catalytic species.

**pH Rate Profile of the Hydrolysis of I**—The observed first-order rate constants extrapolated to zero buffer concentration and calculated for 25° using the apparent activation energies are plotted as a function of pH in Fig. 3.

The pH dependency of the quaternary thiol ester, I, suggests that the overall degradative rate represents a summation of the following separate reactions:

$$I + H^+ \xrightarrow{k_1} \text{product} \qquad \text{Reaction 1}$$

$$I + H_2O \xrightarrow{k_2} \text{product} \qquad \text{Reaction 2}$$

$$I + OH^- \xrightarrow{k_3} \text{product} \qquad \text{Reaction 3}$$

The overall rate is equal to the sum of all these reactions.

$$-\frac{d(I)}{dt} = k_1\,I(H^+) + k_2(I)(H_2O) + k_3(I)(OH^-) \qquad \text{(Eq. 2)}$$

Since water is in great excess it can be simply incorporated into $k_2$.

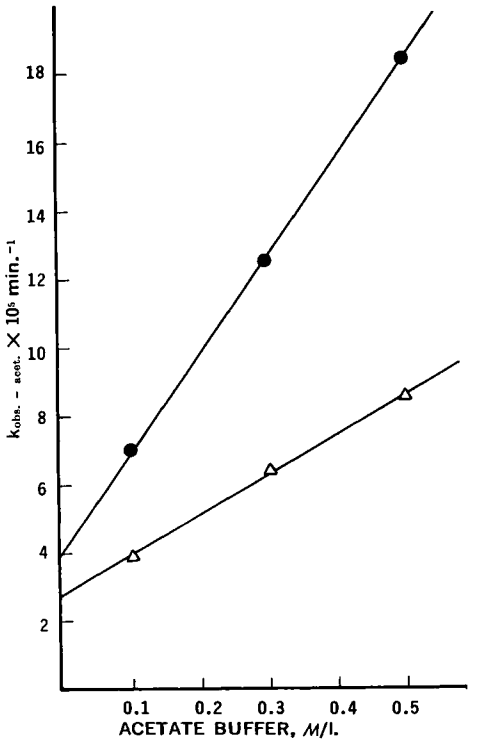



**Figure 2**—*Plot showing the effect of acetate buffer concentration on the rate of hydrolysis of I at 63°. Key: ●, pH 4.7; △, pH 3.9.*

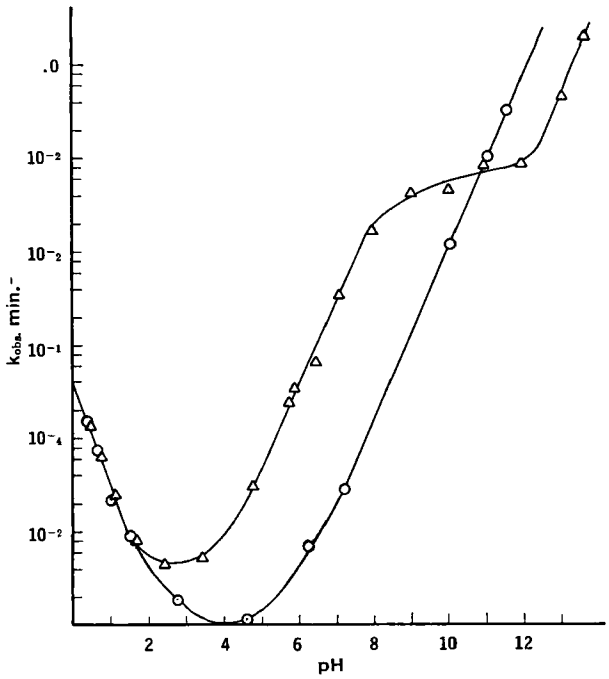



**Figure 3**—*Curves relating the observed first-order rate constant with pH at 25° for I (○), and II (△).*

Because of the pseudo-first-order character of the reaction

$$-\frac{d(I)}{dt} = k_{obs.}(I) \qquad \text{(Eq. 3)}$$

Combining Eqs. 2 and 3 gives:

$$k_{obs.} = k_1(H^+) + k_2 + k_3(OH^-) \qquad \text{(Eq. 4)}$$

From the experimental results the following $k$ values have been calculated at 25°.

$$k_1 = 3.7 \times 10^{-4}\ M^{-1}\ \text{min.}^{-1}$$
$$k_2 = 1.1 \times 10^{-6}\ \text{min.}^{-1}$$
$$k_3 = 9.9 \times 10\ M^{-1}\ \text{min.}^{-1}$$

The theoretical line in Fig. 3 has been calculated by substituting the above $k$ values into Eq. 4.

In accordance with these proposed reactions, a positive salt effect was observed in the acidic media, and a negative salt effect was observed in the alkaline media. This is shown in Figs. 4 and 5 where a logarithmic plot of the pseudo-first-order rate constants *versus* $\sqrt{\mu}$ yields a straight line of positive slopes for the acidic catalyzed hydrolysis, and a negative slope for the alkaline catalyzed hydrolysis.

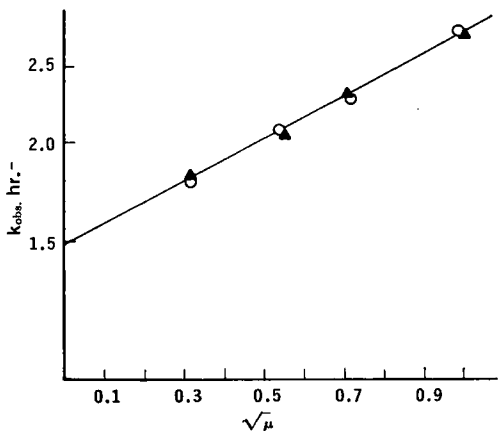



**Figure 4**—*Plot showing the effect of ionic strength on the rate of hydrolysis of I (○) and II (▲) in 0.1 N HCl at 84°.*

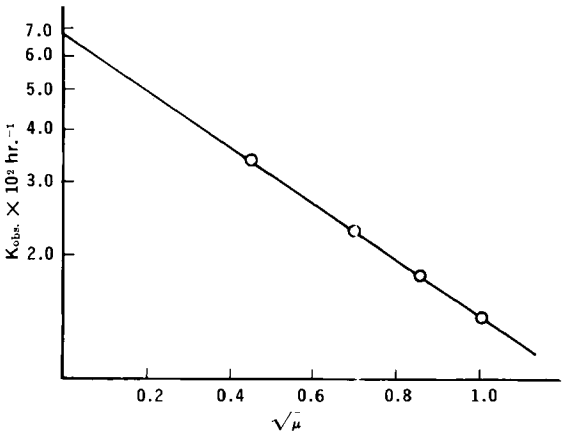



**Figure 5**—*Plot showing the effect of ionic strength on the rate of hydrolysis of I at pH 7.2 and 63°.*

**Effect of Buffer Concentration on the Rate of Hydrolysis of II**—The effect of phosphate and acetate buffer on the rate of hydrolysis of 2-dimethylaminoethanethiol propionate (II) is shown in Figs. 6–8. Phosphate buffer at pH 2.4 has some catalytic effect while at pH's 6.2 and 7.2 the effect is relatively small. Acetate buffers at pH's 3.9 and 4.3 have an effect although this is much less than that observed for the quaternary thiol ester.

**pH Rate Profile of the Hydrolysis of (II)**—In Fig. 3 the observed first-order rate constants extrapolated to zero buffer concentration and 25° are plotted as a function of pH. The shape of the pH rate profile may be explained by assuming the following reactions taking place at zero buffer concentration.

$$IIH^+ + H^+ \xrightarrow{k_4} \text{product} \qquad \text{Reaction 4}$$

$$IIH^+ + H_2O \xrightarrow{k_5} \text{product} \qquad \text{Reaction 5}$$

$$IIH^+ + OH^- \xrightarrow{k_{6a}} \text{product} \qquad \text{Reaction 6}a$$

or

$$II + H_2O \xrightarrow{k_{6b}} \text{product} \qquad \text{Reaction 6}b$$

and

$$II + OH^- \xrightarrow{k_7} \text{product} \qquad \text{Reaction 7}$$

Reactions 6*a* and 6*b* are kinetically equivalent. In these equations $IIH^+$ = the protonated form of the thiol ester and II = the free form of the thiol ester.

Two rate equations can be derived to describe the above proposed reactions:

$$-\frac{d(II_T)}{dt} = k_4(IIH^+)(H^+) + k_5(IIH^+)H_2O + k_{6a}(IIH^+)(OH^-) + k_7(II)(OH^-) \quad \text{(Eq. 5)}$$

or

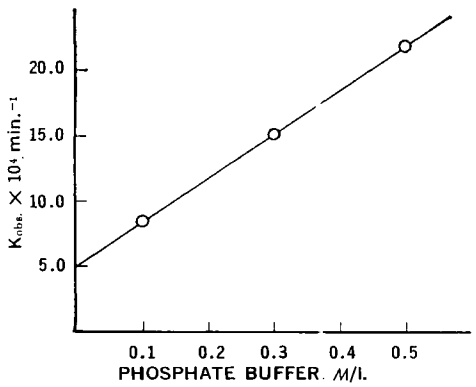



**Figure 6**—*Plot showing the effect of phosphate buffer concentration on the rate of hydrolysis of II at pH 2.4 and 30°.*

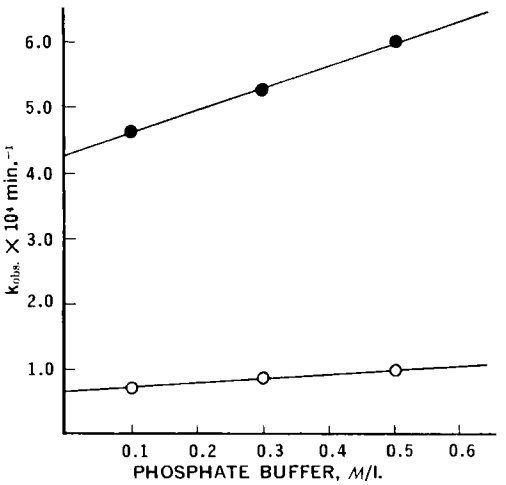



**Figure 7**—*Plot showing the effect of phosphate buffer concentration on the rate of hydrolysis of II at 37°. Key: ●, pH 7.2 and ○ pH 6.4.*

$$-\frac{d(II_T)}{dt} = k_4(IIH^+)(H^+) + k_5(IIH^+)(H_2O) + k_{6b}(II)(H_2O) + k_7(II)(OH^-) \quad \text{(Eq. 6)}$$

where $k_4$, $k_5$, $k_{6a}$, $k_{6b}$, and $k_7$ are the specific catalytic constants and $II_T$ represents the total thiol concentration. By substituting for the thiol ester species in terms of their equilibrium concentrations in the manner previously reported by Higuchi *et al.* (9), Eqs. 5 and 6 can be rewritten to give Eqs. 7 and 8, respectively.

$$k_{obs.} = \frac{1}{K_b(OH^-)}[k_4K_b(H^+) + k_5K_b + k_{6a}K_b(OH^-) + k_7(OH^-)^2] \quad \text{(Eq. 7)}$$

or

$$k_{obs.} = \frac{1}{K_b + (OH^-)}[k_4K_b(H^-) + k_5K_b + k_{6b}(OH^-) + k_7(OH^-)^2] \quad \text{(Eq. 8)}$$

The constant $K_b$ was calculated from the pH of the half neutralized solution of the thiol ester at $\mu = 0.5$ and was found to be $2.2 \times 10^{-6}$ at 25°.

From the experimental results, the following $k$ values have been calculated at 25°.

$$k_4 = 3.7 \times 10^{-4}\ M^{-1}\ \text{min.}^{-1}$$
$$k_5 = 3.8 \times 10^{-6}\ \text{min.}^{-1}$$
$$k_{6a} = 3.2 \times 10^{4}\ M^{-1}\ \text{min.}^{-1}$$
$$k_{6b} = 9.1 \times 10^{-2}\ \text{min.}^{-1}$$
$$k_7 = 4.4\ M^{-1}\ \text{min.}^{-1}$$

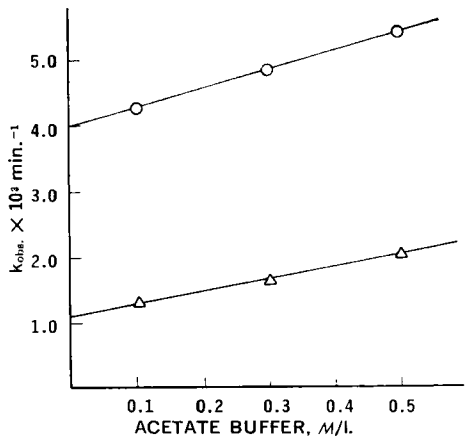



**Figure 8**—*Plot showing the effect of acetate buffer concentration on the rate of hydrolysis of II at 80°. Key: ○, pH 4.6 and △, pH 3.9.*

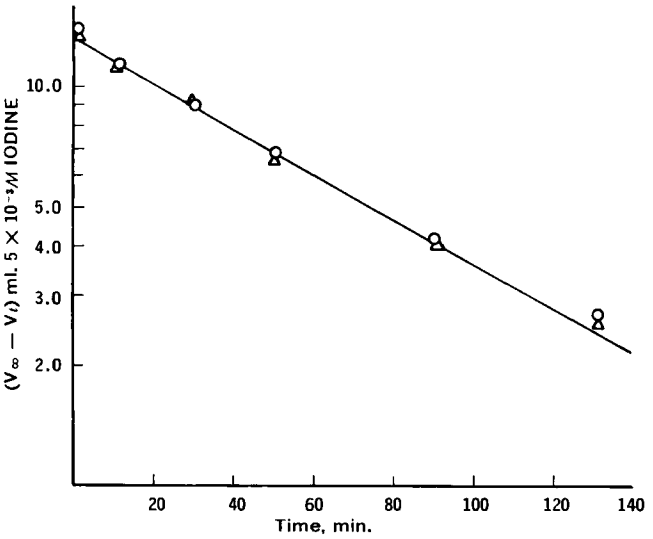



**Figure 9**—*Plot showing the rate of hydrolysis of II at pH 6.4 phosphate and 63°. Key:* ○, *0.1* M *phosphate and* △, *0.1* M *phosphate containing 0.7* M *sodium chloride.*

The effect of ionic strength on the rate of hydrolysis was examined in the acidic and neutral pH range. As expected, a positive salt effect was observed in the acidic medium (Fig. 4). However, no salt effect was detected in the neutral pH range (Fig. 9).

**Effect of Temperature on the Rate of Hydrolysis of I and II**—The effect of temperature on the rate of hydrolysis of I and II was determined at several pH values. Typical Arrhenius plots are shown in Fig. 10. The apparent activation energies at different pH's are shown in Table I for both compounds. The energy values for both compounds are comparable.

**Aminolysis of Compounds I and II**—The appearance of thiol in the reaction of glycine with the above thiol esters was followed under pseudo-first-order conditions. The following rate expression was found to be consistent with the experimental data.

$$-\frac{d(\text{I) or (II)}}{dt} = [k_8(G_f) + k_{\text{hyd.}}(\text{OH}^-)](\text{ester}) \qquad \text{(Eq. 9)}$$

$$k_{\text{obs.}} - k_{\text{hyd.}}(\text{OH}^-) = k_8(G_f) \qquad \text{(Eq. 10)}$$

where $k_8$ is the second-order rate constant for the aminolysis reaction, $k_{\text{hyd.}}(\text{OH}^-)$ is the pseudo-first-order rate constant for the hydrolytic reaction and $(G_f)$ is the concentration of the free form of glycine ($pK_{a_2}$ 9.71).

Since

$$(G_f) = (G_T)\frac{K_{a2}}{K_{a2} + (\text{H}^+)} \qquad \text{(Eq. 11)}$$

where $G_T$ is the total glycine concentration, substitution of Eq. 11 into Eq. 10 gives:

$$\frac{k_{\text{obs.}} - k_{\text{hyd.}}(\text{OH}^-)}{(G_T)} = \frac{K_{a2}}{K_{a2} + (\text{H}^+)}k_8 \qquad \text{(Eq. 12)}$$

Figure 11 shows first-order plots at two different hydroxyl ion concentrations and a total glycine concentration of 0.5 *M*. A plot

**Table I**—Apparent Activation Energies for Hydrolysis of I and II Determined Under Several Constant pH Conditions

| pH of Experiment | Ea for (I) kcal./mole | Ea for (II) kcal./mole |
|---|---|---|
| 0.3 | 18.0 | 17.9 |
| 4.6 | 17.9 | 17.9 |
| 7.2 | 18.1 | 17.8 |
| 8.0 | — | 17.9 |
| 13.0 | 14.4 | 14.6 |

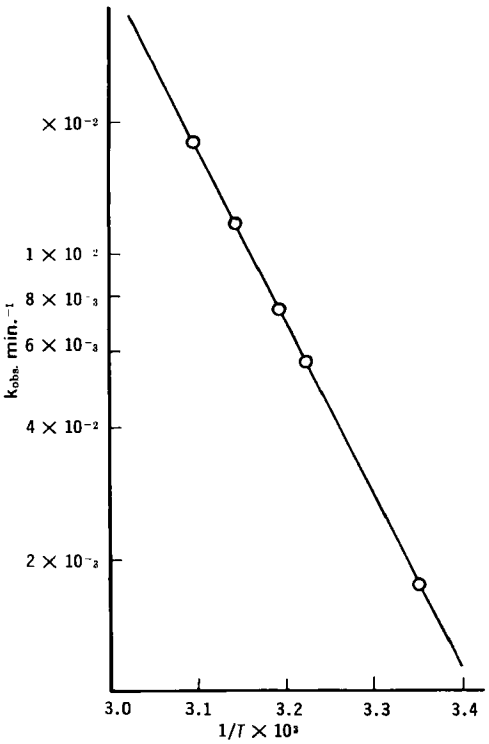



**Figure 10**—*Typical arrhenius plot showing the temperature dependency of the rate of hydrolysis of II at pH 8.*

of $k_{\text{obs.}}$, the observed rate constant for the aminolysis reaction, *versus* $(G_T)$ is shown in Fig. 12.

The linearity of the plots indicates that the reaction is first order with respect to glycine concentration. A plot of the logarithm of the left side of Eq. 12 *versus* the pH of the solution for both compounds is shown in Fig. 13. The pH independent rate constant $k_8$ calculated at several pH values using Eq. 12 is shown in Table II.

## DISCUSSION

**Hydrolysis of I and II**—The second-order rate constants for the acid-catalyzed hydrolysis of I and II are essentially the same. This is to be expected if one considers acid-catalyzed hydrolysis of thiol esters proceeds as in Scheme I (5) and considering that the

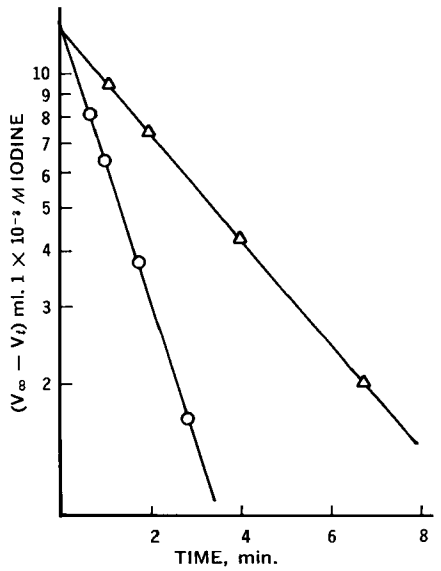



**Figure 11**—*Typical kinetic runs in the reaction of I with 0.5* M *glycine at 24°. Key:* △, *pH 8.8 and* ○, *pH 9.4.*

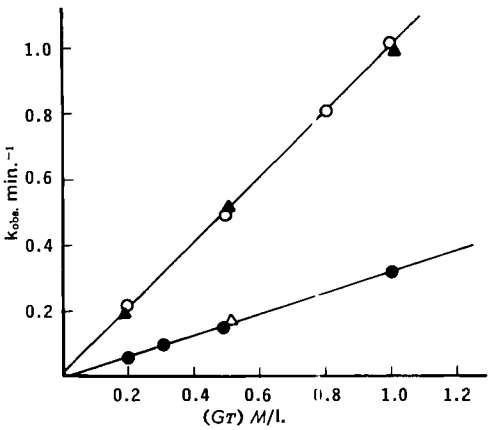
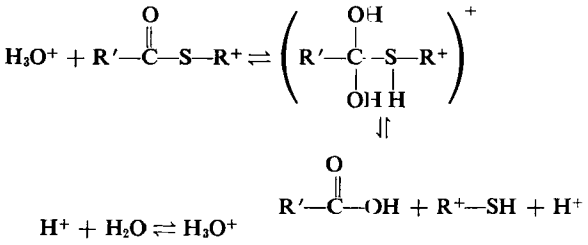



**Figure 12**—*Plot showing the dependency of the observed rate of the aminolysis reaction of I and II upon total glycine concentration at pH 8.8 and pH 9.4. Key: ○ I at pH 9.4 and ▲ II at pH 9.4; △ I at pH 8.8 and ● (II) at pH 8.8.*

nitrogen atom in both compounds is positively charged.



*Scheme I*

The dependency of the acid-catalyzed rates of hydrolysis of I and II on the ionic strength (Fig. 4) is consistent with the above mechanism.

The rate constants for the spontaneous hydrolysis $k_2$ and $k_5$ are also within the same order of magnitude. However, in the pH range 4–8 Compound II is hydrolyzed more than 100 times faster than Compound I. This difference in the two rates of hydrolysis may be due to either intramolecular acid catalysis by the positive charge

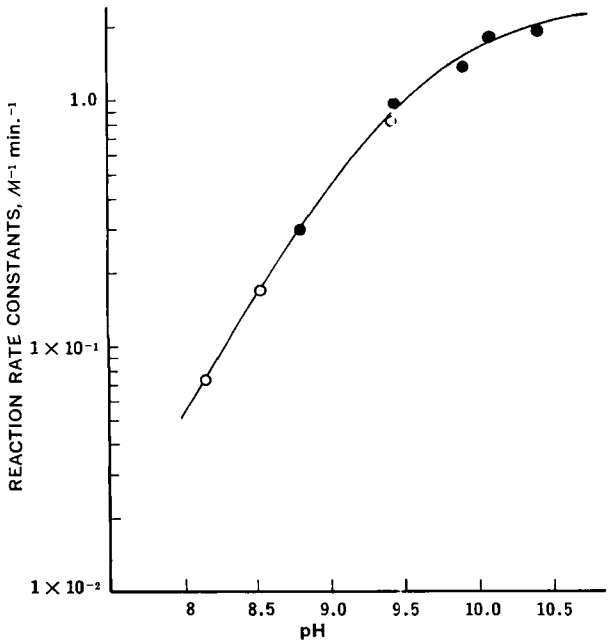


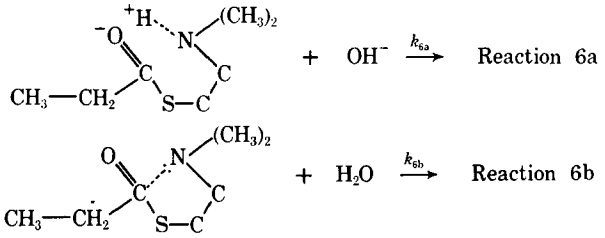

**Figure 13**—*The pH dependency of the observed second-order rate constant of the aminolysis reaction of I (○) and II (●) at 24°, plotted according to Eq. 12.*

**Table II**—Second-Order Rate Constants for Reaction of Glycine with I and II at 24°

| pH | Rate Constants $M^{-1}$ min.$^{-1}$ |
|---|---|
| 8.3 | 2.72 |
| 8.5 | 2.68 |
| 8.8 | 2.71 |
| 9.4 | 2.74 |
| 9.9 | 2.43 |
| 10.13 | 2.68 |
| 10.32 | 2.55 |
| | Mean = 2.65 $M^{-1}$ min.$^{-1}$ |

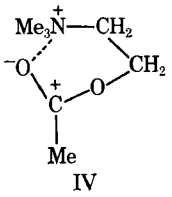
carried by the labile proton (7) or alternatively to intramolecular base catalysis by the nonprotonated nitrogen.

Therefore, the following two kinetically equivalent reactions could be postulated:




*A priori*, a primary negative salt effect is expected if the first pathway is operative, however, the lack of dependency of the rate of hydrolysis at pH 6.4 on the ionic strength may be due to the fact that the positive charge on the labile hydrogen is masked by either intramolecular hydrogen bonding with the carbonyl oxygen or intermolecular hydrogen bonding with water in the cyclic transition state. It should be mentioned that ionic strength effect also was not observed for the alkaline hydrolysis of the protonated form of procaine (9).

The choice between the above two reactions was made on the basis of the difference in rate of hydrolysis of I and II in the alkaline media above pH 11. As seen from the pH rate profile (Fig. 3) the quaternary Compound I hydrolyzes about 20 times faster than II in this region. Davis and Ross (10) found that acetyl choline hydrolyzes in basic solution about 32 times faster than dimethylaminoethyl acetate. This difference was attributed to general acid catalysis by the positively charged nitrogen atom which can increase the electrophilic nature of the carbon center as shown (IV):



IV

On the basis of the above considerations, a more pronounced effect is expected if the positive charge is carried by a labile proton. This may be attributed to more efficient stabilization of the transition state by the proton (7). Therefore, it is concluded that the most probable mechanism is described by Reaction 6*a*.

A comparison between $k_3$, $k_{6a}$, and $k_7$ reveals the significance of these intramolecular processes. The protonated nitrogen of II is about 320 times more effective as a general acid catalyst than the quaternary nitrogen of I, while an electrically neutral $\beta$-nitrogen atom is not an effective activating group.

The buffer effect observed to be relatively small in the pH range 4–7.2 on the hydrolysis of II may be due to the large reactivity of hydroxyl ion on the protonated form of II, which makes the contribution of buffer catalysis insignificant. At pH 2.4, however, when catalysis by water is significant, buffer effect was noted.

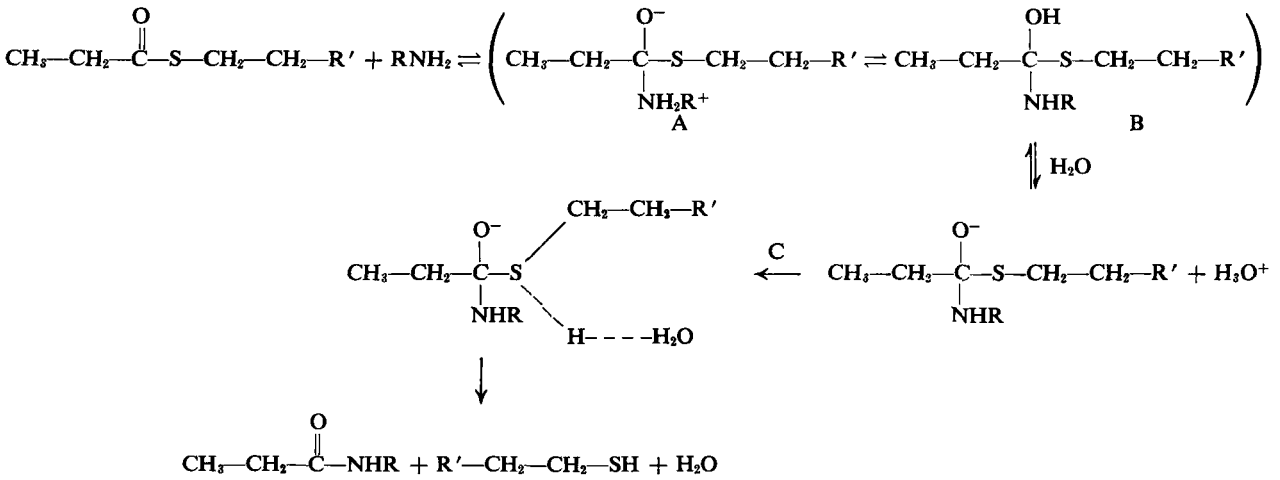
Although it is difficult to obtain a quantitative comparison between the effect of intra and intermolecular catalysis on the rate of hydrolysis of I and II, a rough estimation was made at pH 4.6. This indicated that in order for the two compounds to have equivalent rates of hydrolysis at pH 4.6, 15 *M* acetate ion would be re-

quired as a catalyst for Compound I. The hydrolysis of phenyl acetate with acetate ion was found to require about 8 $M$ acetate ion in order that the rate of hydrolysis of this intermolecular process be equivalent to the rate of intramolecular hydrolysis of a corresponding concentration of aspirin (7).

**Aminolysis of I and II**—Noda *et al.* (5), in their investigation of the kinetics of aminolysis of several thiol esters by hydroxylamine, found that the reaction is first order with respect to both hydroxylamine and thiol ester. Hawkins *et al.* (11), however, found a higher order dependency on amine concentration in their study on the kinetics of aminolysis and hydrolysis of ethyl thioacetate and β-acetaminoethyl thioacetate. The reaction between Compounds I and II and glycine was found to be first order in glycine and first order in esters. The lack of detectable higher-order terms, with respect to glycine, indicates that intermolecular general base and general acid catalysis by another glycine molecule is not significant in the glycinolysis of the above thiol esters.

A comparison of the alkaline rate of hydrolysis of Compounds I and II with their rate of glycinolysis shows that although the alkaline rate of hydrolysis of the protonated thiol ester is 320 times faster than the quaternary compound and $1 \times 10^4$ times faster than the nonprotonated form of II, their rate of reaction with glycine is identical (see Footnote 2 for the derivation of the rate of glycinolysis of the different species of II). In other words, the enhancement of electrophilic reactivity of the carbonyl carbons toward hydroxyl attack by intramolecular acid catalysis, did not facilitate then nucleophilic attack by glycine molecules.

A mechanism for thiol ester aminolysis (12), consistent with the above results, has been postulated to involve a series of pre-equilibrium steps between the amine and the thiol ester followed by a slow rate-determining step in which the thiol group is liberated, as shown in Scheme II. In the above scheme, the formation of C from A and B was considered as a fast reversible reaction.



*Scheme II*

[2] The rate of aminolysis of the different species of II could be derived as follows:

$$\frac{-d(\mathrm{II_T})}{dt} = [G_f][k_{\mathrm{IIH}^+}(\mathrm{IIH}^+) + k_{\mathrm{II}}(\mathrm{II})]$$

where $k_{\mathrm{IIH}^+}$ and $k_{\mathrm{II}}$ is the rate of aminolysis of $\mathrm{IIH}^+$ and II, respectively. By substituting for the thiol ester species and for $G_f$ in terms of their equilibrium concentration, see Eqs. 7 and 11, the above equation can be reduced to:

$$\frac{k_{obs.}}{(G_T)} = \left[k_{\mathrm{IIH}^+}\frac{k_b}{k_b + (\mathrm{OH}^-)} + k_{\mathrm{II}}\frac{(\mathrm{OH}^-)}{k_b + (\mathrm{OH}^-)}\right]\frac{k_{a2}}{k_{a2} + (\mathrm{H}^+)}$$

$k_{obs.}/(G_T)$ could be determined from slopes such as shown in Fig. 11. $k_{\mathrm{IIH}^+}$ and $k_{\mathrm{II}}$ were calculated out to be 2.71 $M^{-1}$ min.$^{-1}$ and 2.68 $M^{-1}$ min.$^{-1}$, respectively.


## SUMMARY

The hydrolysis of propionyl thiocholine iodide (I) and 2-dimethylaminoethanethiol propionate (II) in aqueous buffered solutions was found to be first order with respect to the thiol esters. The second order rate constants of the acid catalyzed hydrolysis are essentially the same for both compounds. The hydroxyl ion catalyzed hydrolysis of (I) was found to be 320 times slower than that of the protonated form of (II) and 25 times faster than that of the free form of (II). This difference is attributed to the stabilization of the transition state by a protonated nitrogen at lower pH's and by quaternary nitrogen at higher pH's.

Phosphate and acetate buffers have relatively less effect on the rates of hydrolysis of I than on the rate of hydrolysis of II in agreement with the proposed mechanism. The reaction of I and II with glycine proceeds with identical rates. This was explained on the basis of the existence of fast equilibrium between the glycine and the thiol esters followed by a slower rate-determining step.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