

Thiol Esters I: Kinetic Study of the Formation of 2-Dimethylaminoethanethiol Propionate

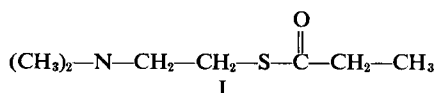
ANWAR HUSSAIN and P. SCHURMAN

Abstract □ The kinetics of formation of 2-dimethylaminoethanethiol propionate from propionic anhydride and 2-dimethylaminoethanethiol in aqueous buffer solutions has been determined. The reaction was followed spectrophotometrically at 235 m μ and by iodometric titration. Kinetic equations for the formation of the ester, taking into consideration the hydrolysis of the anhydride, have been derived. The rate of formation of the ester exhibited a pH dependency which could be explained by assuming the thiol anion to be the attacking species. On this basis, the second-order rate constant, k_d' , is given by: $k_d' = k_d \text{ obs. } \{[(H^+) + K_a]/K_a\}$ where K_a is the dissociation constant of the thiol (3.2×10^{-8}), the value of k_d' was calculated to be $1.41 \times 10^3 \text{ l. mole}^{-1} \text{ sec.}^{-1}$.

Keyphrases □ Thiol ester formation—kinetics □ 2-Dimethylaminoethanethiol propionate formation—aqueous buffers □ Kinetic equations—2-dimethylaminoethanethiol propionate formation □ pH effect—2-dimethylaminoethanethiol formation □ UV spectrophotometry—reaction monitoring

Anhydrides have been found to be an intermediate in many reactions involving the formation of amides. Kinetic studies of the rate of reactions between anhydrides and amines have been reported (1–4). However, very little work has been reported concerning reaction of thiols and anhydrides to form thiol esters.

Thiol esters have been recognized for their important role in many biological systems since the discovery that acetylated coenzyme A, an intermediate in many biological reactions is a thiol ester. Racker (5), for example, has shown that the glyoxalase enzymes act by the intermediate formation of a thiol ester in which the coenzyme glutathione participates. An understanding of the physical and chemical properties of the thiol esters should aid in a better understanding of biochemical mechanisms involving such compounds. It was the purpose of this study to obtain data on the kinetics of thiol ester formation employing 2-dimethylaminoethanethiol and propionic anhydride to form the ester (I) as the model.



EXPERIMENTAL¹

Reagents and Apparatus—2-Dimethylaminoethanethiol was furnished by Dr. C. Orzech, Ayerst Laboratories, Rouses Point, N. Y. The hydrochloride salt of 2-dimethylaminoethanethiol was obtained from the Research Department, Ayerst Laboratories, Division of Ayerst, McKenna and Harrison, Ltd., Montreal, Canada. Propionic anhydride (reagent grade, Eastman) was further purified by distillation. 2-Dimethylaminoethanethiol propionate was prepared according to the following procedure. Sodium hydride 13.7 g. (0.285 mole) was suspended into 200 ml. benzene in a

500-ml. round-bottom flask. Distilled 2-dimethylaminoethanethiol 30 g. in 100 ml. of 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. propionyl chloride was slowly added with continuous stirring and cooling. After the addition was completed the mixture was left for about 1 hr. at room temperature. The clear yellow liquid was filtered and HCl gas was bubbled through. The hydrochloride salt of the ester was filtered and washed several times with dry ether and was found to be 97% pure as determined iodometrically.

Buffer and standard iodine solutions were prepared using reagent grade materials.

Kinetic Procedures—The hydroxyl and hydrogen-ion concentrations of the system were maintained by using phosphate and acetate buffer solutions. The ionic strength of the system was adjusted to the desired values by the addition of sodium chloride. Most of the determinations were run at a total ionic strength of 0.5.

Rate of Formation of the Thiol Ester—The reaction between the anhydride and the thiol can be readily followed spectrophotometrically at 235 m μ , since the thiol ester absorbs strongly at this wavelength. Figure 1 shows the UV spectra of equimolar quantities of the thiol and of the thiol ester at pH 5.2.

Fresh 0.12 M propionic anhydride in spectro grade dioxane was prepared. Fresh solutions of the thiol (2.5×10^{-2} to 1×10^{-1} M) in the desired buffer were prepared from the hydrochloride salt. The solutions were kept under nitrogen in order to minimize oxidation.

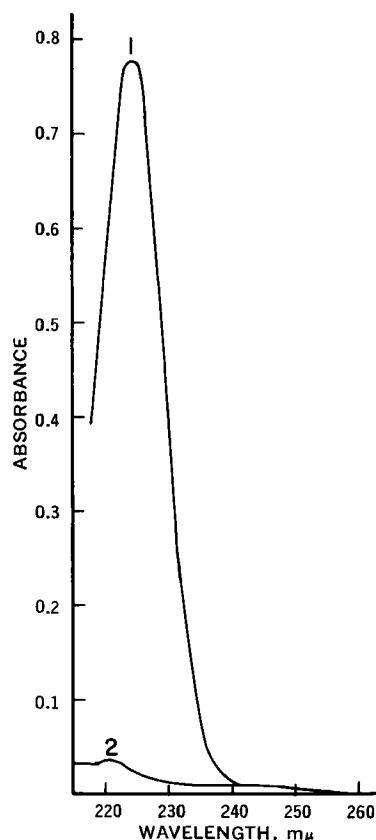


Figure 1—UV absorption spectra of equimolar quantities (2×10^{-4} M) of (1) 2-dimethylaminoethanethiol propionate and (2) 2-dimethylaminoethanethiol at pH 5.2.

¹ The instruments used in this study were a Cary model 14 spectrophotometer and Beckman Expandomatic pH meter.

An accurately measured volume of the anhydride solution, to obtain the desired concentration (range 2×10^{-3} to 8×10^{-4} M), was transferred into a 1-cm. light path silica cell. Exactly 3 ml. of the thiol solution was injected into the cell, and the change in absorbance with time was followed at 235 m μ .

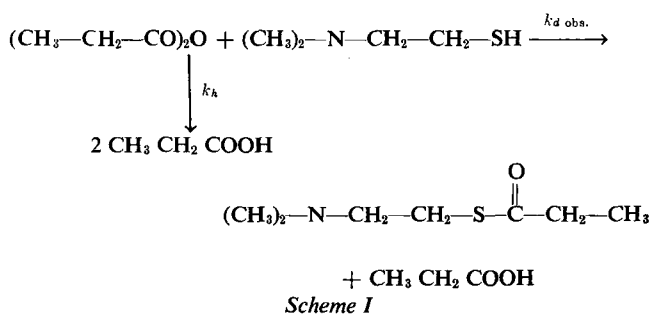
When the rate was determined iodometrically, 0.5 ml. of 0.2×10^{-1} M propionic anhydride in dioxane was added to the solution of the thiol in phosphate buffer at pH 3.3. Periodically, 5-ml. samples were quickly discharged into a 50-ml. conical flask containing 2 ml. of 1 N HCl to quench the reaction. The residual thiol was then titrated with 5×10^{-3} M iodine solution under a blanket of nitrogen using starch as an indicator. The experiment was repeated at several thiol concentrations from 1×10^{-2} M to 7.6×10^{-2} M but at a constant anhydride concentration of 1.48×10^{-2} M.

Determination of the Rate of Hydrolysis of Propionic Anhydride—The rate of hydrolysis of propionic anhydride was determined spectrophotometrically at 228 m μ . The reaction rate was determined at different pH's and an initial anhydride concentration of 5.0×10^{-3} M.

A solution of propionic anhydride (0.1 ml. of 1.5×10^{-1} M) in dioxane was placed in a 1-cm. light path silica cell. Exactly 3 ml. of the buffer solution at the desired pH was added and the change in absorbance with time at 228 m μ was followed using a spectrophotometer. After the run was completed, the pH of the reaction mixture was determined to be unchanged.

RESULTS AND DISCUSSIONS

Rate of Formation of 2-Dimethylaminoethanethiol Propionate from Propionic Anhydride and 2-Dimethylaminoethanethiol—The reaction between propionic anhydride and the thiol can be represented as:



The rate equations implicit in Scheme I can be readily derived for certain fixed conditions. If the thiol concentration is in large excess over the anhydride, the following integrated equation is valid,

$$\log (E_e - E_t) = \log \frac{k_d \text{ obs. (thiol) (anhydride)}}{k_h + k_d \text{ obs. (thiol)}} - \left(\frac{k_h + k_d \text{ obs. (thiol)}}{2.303} \right) t \quad (\text{Eq. 1})$$

where E_e is the equilibrium concentration of the ester, E_t the concentration at time t of the ester, $k_d \text{ obs.}$ the observed second-order rate constant for the direct reaction of the thiol with the anhydride, k_h the first-order hydrolytic rate constant of the anhydride.

It is apparent from the form of Eq. 1 that a plot of $\log (E_e - E_t)$, or equivalently in terms of the change in absorbance $\log (A_e - A_t)$, against time will yield a straight line with slope of $[k_h + k_d \text{ obs. (thiol)}]/2.303$, as shown in Fig. 2. Since,

$$2.303 \text{ slope} = k_h + k_d \text{ obs. (thiol)} = k_{\text{app.}} \quad (\text{Eq. 2})$$

a plot of $k_{\text{app.}}$, obtained from plots such as Fig. 2, versus thiol

Table I—Hydrolytic Rate Constant of Propionic Anhydride at 25°

pH	$k_h \text{ sec.}^{-1}$
2.4	1.43×10^{-3}
4.5	1.43×10^{-3}
5.5	1.20×10^{-3}

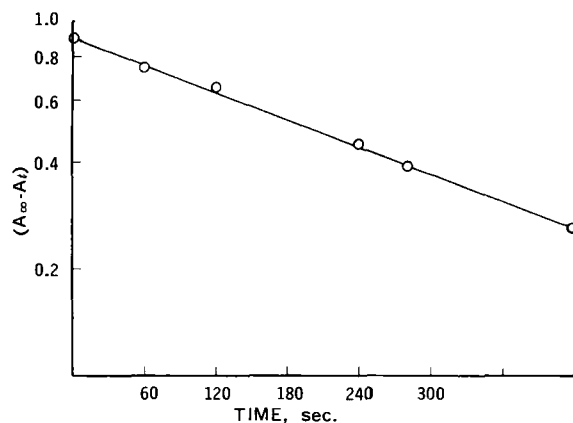


Figure 2—A plot showing apparent pseudo first-order reaction between propionic anhydride and the thiol at pH 2.9 [2-dimethylaminoethanethiol] = 5×10^{-2} M, [propionic anhydride] = 2×10^{-3} M.

concentration should be linear with a slope of $k_d \text{ obs.}$ and an intercept of k_h/k_h , the hydrolytic rate constant for the anhydride, also can be determined directly.

Table I shows the observed first-order hydrolytic rate constants of propionic anhydride determined spectrophotometrically at different pH values and 25°, which are in good agreement with the reported values (6).

It is apparent from Table I that the rate of the anhydride hydrolysis is fairly constant over the pH range studied. However, it was found that the rate of the ester formation, $k_d \text{ obs.}$, was not only dependent on the thiol concentration, but was markedly influenced by the hydrogen ion concentration. In Fig. 3 are shown results of two series of experiments run at pH 2.8 and 4.0 according to Eq. 2. k_h values indicated by the intercepts are in good agreement with those determined directly for the anhydride alone. The second-order rate constant $k_d \text{ obs.}$ for the direct reaction was calculated from the slopes of the lines and was found to be 3.01×10^{-2} l. mole $^{-1}$ sec. $^{-1}$ at pH 2.8 and 4.95×10^{-1} l. mole $^{-1}$ sec. $^{-1}$ at pH 4.0. It would appear from the indicated magnitude obtained for $k_d \text{ obs.}$ at these two pH values that the rate of formation of the thiol ester is directly proportional to the hydroxyl-ion concentration. Therefore, at higher hydroxyl ion concentrations k_h will be negligible compared to $k_d \text{ obs.}$ and the reaction will exhibit simple second-order kinetics.

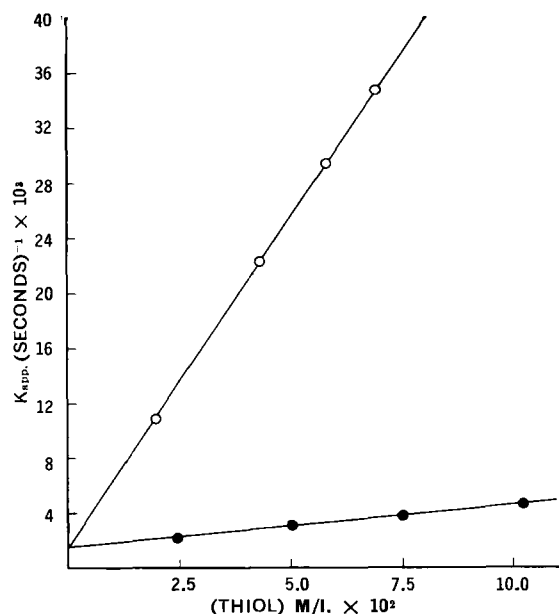


Figure 3—Observed apparent first-order rate constant $k_{\text{app.}}$ of reaction between 2-dimethylaminoethanethiol and propionic anhydride as a function of (thiol) at 25° and pH 2.8 (●) and pH 4.0 (○). These results were plotted according to Eq. 2.

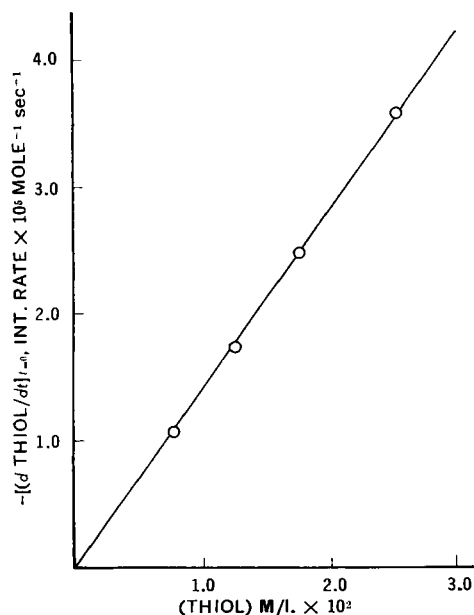


Figure 4—Initial rate of disappearance of 2-dimethylaminoethanethiol versus the initial concentration of the thiol at pH 3.3 and fixed (anhydride) of 1.48×10^{-2} M. These data were plotted according to Eq. 4.

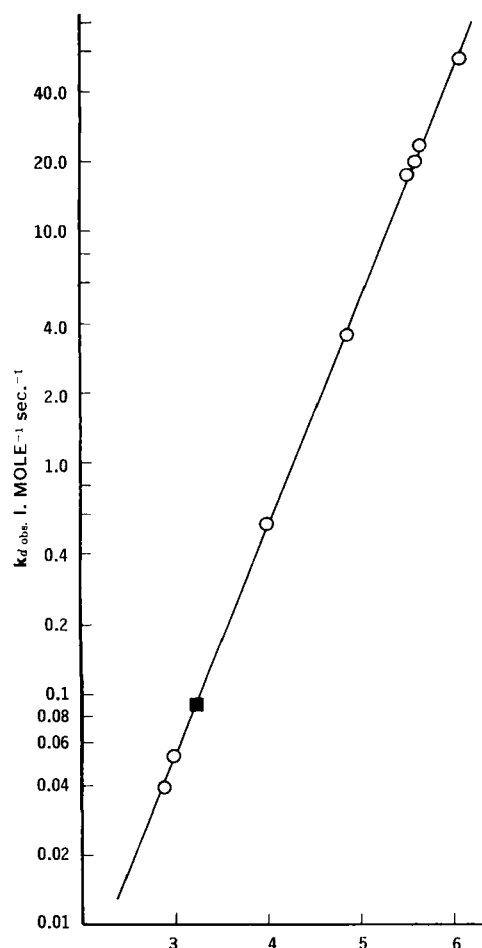


Figure 5—pH rate profile of the direct reaction of propionic anhydride and 2-dimethylaminoethanethiol at 25°. Key: ○, determined spectrophotometrically by following the appearance of thiol ester at 235 mμ; ■, determined iodometrically by following the disappearance of the thiol.

Table II—Second-Order Rate Constants k_d' Calculated for Several pH Values at 25°

pH	Rate Constant $\times 10^{-3}$ l. mole ⁻¹ sec. ⁻¹
2.85	1.38
3.0	1.42
4.0	1.43
4.85	1.41
5.5	1.44
5.65	1.41
6.1	1.37

In order to obtain an independent check on the spectrophotometric data, the reaction between the anhydride and the thiol was also determined iodometrically by measuring the initial loss of thiol. Initial rate measurements were obtained within 40 sec. of the beginning of the reaction, during which time only insignificant amounts of the anhydride were lost through the hydrolytic route. Therefore:

$$d(E) \frac{d}{dt} = -d \frac{(\text{thiol})}{dt} = k_{d \text{ obs.}} (\text{thiol}) (\text{anhydride}) \quad (\text{Eq. 3})$$

In Fig. 4, the initial rates of the disappearance of the thiol were plotted as a function of initial thiol concentrations at a constant anhydride concentration of 1.48×10^{-2} and pH 3.3. The observed second-order rate constant $k_{d \text{ obs.}}$ was calculated from the slope of the straight line and is shown as a solid square in Fig. 5. It is evident, therefore, that the iodometric and spectrophotometric data are in good agreement.

Variation of $k_{d \text{ obs.}}$ with pH—In Fig. 5, the experimentally determined values of $k_{d \text{ obs.}}$ are plotted against the pH of the solutions.

The straight line drawn through the points has a slope of one over the measured pH range. The pH dependency suggests that the reaction probably proceeds *via* attack on anhydride by the anionic form of the thiol. On this basis the relationship between the specific second-order rate constant, k_d' , and $k_{d \text{ obs.}}$ at any given pH can be derived as follows:

$$\begin{aligned} d(E) \frac{d}{dt} &= -d \frac{(\text{thiol})}{dt} = k_d' (\text{thiol}^-) (\text{anhydride}) \\ &= k_{d \text{ obs.}} (\text{thiol}) (\text{anhydride}) \quad (\text{Eq. 4}) \end{aligned}$$

where (thiol^-) is the concentration of the anionic species and (thiol) is the total concentration of the thio, (thiol^-) can be expressed in terms of the total thiol concentration, the hydrogen ion concentration, and the dissociation constant of the thiol, $K_a = 3.2 \times 10^{-8}$.

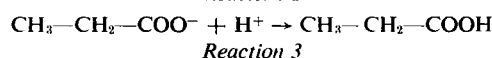
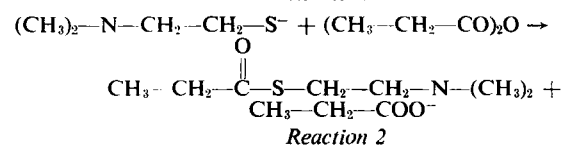
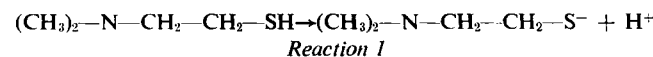
$$(\text{thiol}^-) = (\text{thiol}) \left[\frac{K_a}{K_a + (\text{H}^+)} \right] \quad (\text{Eq. 5})$$

Equation 5 can be combined with Equation 4 to give,

$$k_d' = k_{d \text{ obs.}} \left[\frac{K_a + (\text{H}^+)}{K_a} \right] \quad (\text{Eq. 6})$$

The specific second-order rate constants calculated for several pH values at 25° using Eq. 6 are given in Table II.

The mechanism proposed for the direct reaction consistent with the data is given in the sequence:



where Reaction 2 is the rate-determining step. A similar mechanism was reported for the reaction of ethylene oxide and mercaptan in aqueous systems (7).

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

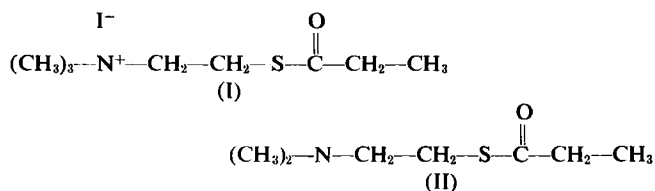
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Abstract □ The kinetics of hydrolysis of propionyl thiocholine iodide (I) and 2-dimethylaminoethanethiol propionate (II) in aqueous buffers were studied over a pH range 0.3–13.5. The data indicate that the rate of hydronium-ion attack on both compounds 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.



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).

