

acid, 80:20:2). Yellow spots corresponding to DNP-valine and DNP-alloisoleucine were detected. The aqueous layer after extraction of DNP-amino acids contained valine and alloisoleucine, as shown by silica gel thin layer chromatography (butanol-acetic acid-water, 4:1:1).

Treatment of L-Valyl-L-valine with Hot Hydrochloric Acid. The dipeptide (11.8 mg) was dissolved in 6 N hydrochloric acid (1 ml) and treated as described for valylalloisoleucine. Thin layer chromatography (silica gel, butanol-acetic acid-water (4:1:1)) showed that the hydrolysate contained valine and homovalylvaline but no heterovalylvaline; hence no detectable racemization occurred. Thus the heterovalylvaline in the hydrolysate of stendomycin does not originate from homovalylvaline.

Acknowledgments. This work was supported by a Public Health Service grant (NIH AI 07515). The authors are also indebted to Drs. M. Gorman, R. L. Hamill, and G. L. Mallett of the Lilly Research Laboratories, Indianapolis, Ind., for the generous samples of stendomycin, for a sample of capreomycin, and for stimulating discussions. The help of Mr. Gary G. Marconi in the nmr studies and the assistance of Mr. Joseph Alicino in the analytical work and of Mrs. Delores J. Gaut in the amino acid analyses are greatly appreciated.

Diffusion-Controlled Proton Transfer in Intramolecular Thiol Ester Aminolysis and Thiazoline Hydrolysis¹

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Received December 6, 1968

Abstract: The following evidence supports the conclusion that a simple proton transfer step is rate determining in the intramolecular aminolysis of S-acetylmercaptoethylamine above pH 2.3 and determines the products of thiazoline hydrolysis in this pH region. (1) The value of α for general-acid catalysis by carboxylic acids and phosphate monoanion of the formation of amide from the uncharged thiol ester is experimentally indistinguishable from zero, as expected for a diffusion-controlled proton transfer. (2) The catalytic constant for the solvated proton is 24 times higher than for carboxylic acids and phosphate monoanion, corresponding to the known rapid rate of proton transfer from this species. (3) Catalytic constants for acids with pK values larger than 7.4 approach the expected Brønsted slope of $\alpha = 1.0$ expected for weak acids in a simple proton transfer reaction in the thermodynamically unfavorable direction. (4) The break in the corresponding Brønsted plot for S-chloroacetylmercaptoethylamine occurs 1.5 pH units lower than that for the acetyl compound, as predicted from the reduced basicity of the addition intermediate in this reaction. (5) Water and bicarbonate ion, which can act as bifunctional acid-base catalysts, exhibit enhanced catalytic activity, analogous to the known behavior of water in simple intramolecular proton transfer reactions. (6) The yield of thiol ester in the hydrolysis of thiazoline decreases with increasing pH, with a half-maximum yield at pH 2.37 corresponding to the change in the rate-determining step of the S to N transfer reaction at pH 2.30. (7) The proposed mechanism provides a satisfactory solution to the "thiazoline dilemma." Rate and equilibrium constants have been estimated for all of the steps of the reaction and appear to be within reasonable limits.

General acid-base catalyzed carbonyl addition and acyl transfer reactions differ from simple proton transfer reactions, such as ketone enolization, in that bonds between carbon and oxygen, nitrogen, or sulfur must be formed and broken in the former class of reactions; in fact, it is the formation or cleavage of such bonds rather than the proton transfer which is generally thought to provide the principal energy barrier in these reactions. It has been suggested that the proton transfer is the rate-determining step in reactions of this kind, by analogy with simple proton transfer reactions of carbon acids.²⁻⁵ Recently, however, the view has become increasingly accepted that many such reactions are "concerted" or "coupled," in

the sense that formation or cleavage of bonds to carbon takes place in the rate-determining step; the exact role of the associated proton transfer is not yet clear.⁶⁻¹⁰ The strongest experimental evidence for this conclusion is (1) the fact that such reactions frequently exhibit Brønsted slopes other than 0 or 1 over considerable ranges of catalyst acidity or basicity, whereas if proton transfer alone were involved the Brønsted slopes should approach the values of 0 or 1.0 characteristic of diffusion-controlled proton transfer between electronegative atoms,⁸ (2) the occurrence of heavy atom isotope effects in reactions of this kind,¹¹ and (3) the require-

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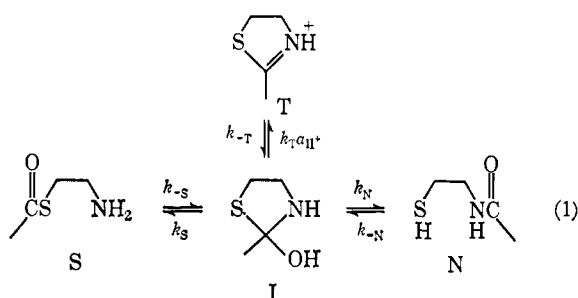
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ment for rate constants equal to or greater than those for diffusion-controlled reactions, accompanied by curvature of Brønsted plots, for the ordinary stepwise mechanism of acetaldehyde hydration.^{12,13} Although stepwise mechanisms with a rate-determining proton transfer step have been suggested even quite recently for reactions of this kind,^{4,5,14-20} there is no compelling evidence in support of such mechanisms, with the possible exception of ¹⁸O exchange accompanying the neutral hydrolysis of ethyl trifluoroacetate,^{21,22} and objections can be raised against such proposed mechanisms in a number of cases.^{23,24} We describe here evidence that the S to N acyl transfer reaction of S-acetylmercaptoethylamine (and the breakdown of 2-methyl- Δ^2 -thiazoline to this thiol ester) *does* involve a stepwise mechanism with rate-determining diffusion-controlled proton transfer under most experimental conditions. Some of this evidence has been described in a communication,²⁵ and Chaturvedi and Schmir have recently obtained evidence supporting a similar mechanism for thiol ester formation in the hydrolysis of acyclic thioimidates.^{9b}

The hydrolysis of 2-methyl- Δ^2 -thiazoline (T) to S-acetylmercaptoethylamine (S) and N-acetylmercaptoethylamine (N) is the first reaction at the acyl group level of oxidation which was shown to proceed through an intermediate, by the demonstration that it proceeds with a bell-shaped pH-rate profile and undergoes a change in rate-determining step with changing pH.²⁶ This reaction was explained by Martin and coworkers^{26-29a} according to the mechanism of eq 1, with



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(13) This objection to the usual stepwise mechanism can also be accommodated by a "one-encounter" mechanism, in which the reaction takes place in a stepwise manner within a solvent "cage" more rapidly than the catalyst diffuses away from the "cage."

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rate-determining attack of water on protonated thiazoline above pH 3 and rate-determining breakdown of the tetrahedral addition intermediate I to approximately equal amounts of thiol ester S and amide N below pH 2. The relative yields of thiol ester and amide were found to be independent of pH below pH 2, and a decrease in the observed yield of thiol ester above pH 2 was ascribed to a secondary S to N transfer reaction.^{26,27}

The S to N acyl transfer reaction shown in the lower part of eq 1 has been studied directly^{28,29} and was shown to undergo a change in rate-determining step with changing buffer concentration and with changing pH at about pH 3. Martin and coworkers recognized that this fact could not readily be accommodated by the scheme of eq 1 and the experimental data for thiazoline decomposition and first suggested that an additional proton transfer step might account for the observed behavior of both reactions.²⁸ It was suggested that this step was interposed on the amide limb of the scheme of eq 1 between I and N and that it involved proton removal from the oxygen atom of an addition compound which also was protonated on sulfur; however, this suggestion was later withdrawn and the "thiazoline dilemma" was left unresolved.²⁹

Experimental Section

Materials. Organic reagents were redistilled or recrystallized. S-Acetylmercaptoethylamine (S-acetyl-2-aminoethanethiol) hydrochloride was kindly provided by Dr. G. M. Blackburn. Solutions of N-acetylmercaptoethylamine (N-acetyl-2-aminoethanethiol) were prepared by saponification of N,S-diacetylmercaptoethylamine (N,S-diacetyl-2-aminoethanethiol) under argon followed by neutralization to pH 5.8;³⁰ the product was shown to contain a 96% yield of free sulfhydryl groups.³¹

S-Chloroacetylmercaptoethylamine (S-chloroacetyl-2-aminoethanethiol) hydrochloride was prepared by a modification of the procedure of Weiland and Bokelmann.³² To 15 g of chloroacetic anhydride in 12 ml of dioxane was added 8.3 g of mercaptoethylamine hydrochloride. After refluxing for 5 min the reaction mixture was allowed to cool to room temperature and was diluted to 75 ml with acetonitrile. Then 30 ml of anhydrous ether was added slowly. After standing overnight at 10°, the solid material was collected and recrystallized twice from dry dimethylformamide containing one drop of concentrated hydrochloric acid solution per 8 ml and dry ether; yield ca. 10%; mp 127-128°; λ_{\max} 234 m μ (ϵ 4580) in 0.1 M hydrochloric acid.

Anal. Calcd for C₄H₉NOSCl₂: C, 25.28; H, 4.77; N, 7.37; S, 16.68; Cl, 37.30. Found: C, 25.38; H, 4.85; N, 7.56; S, 17.00; Cl, 37.55.

N-Chloroacetylmercaptoethylamine (N-chloroacetyl-2-aminoethanethiol, ϵ_{234} 470) was prepared in solution by incubating ca. 10⁻² M S-chloroacetylmercaptoethylamine hydrochloride in 0.1 M potassium hydroxide under argon for 10 min, followed by neutralization with hydrochloric acid.

Equilibrium Constants. The equilibrium of S-acetylmercaptoethylamine, N-acetylmercaptoethylamine, and 2-methyl- Δ^2 -thiazoline was followed by measuring the absorption of the thiol ester at 230 m μ and the absorption of thiazoline at 260 m μ . The concentration of N-acetylmercaptoethylamine was taken as the difference between the total concentration of substrates ($3-9 \times 10^{-4}$ M) and the concentrations of thiazoline and thiol ester. The extinction coefficients used in the calculations are shown in Table I. Experiments were carried out at pH 1.6, 1.8, and 2.1. The approach to equilibrium was found to be first order for about 2 half-lives and was followed for at least 14 half-lives at 230 m μ . Equilibration between thiazoline and thiol ester was 93, 97, and 98% complete at pH 1.6, 1.8, and 2.1. The absorbance at equilibrium slowly de-

step on the thiol ester limb at the time that his alternative suggestion was published.

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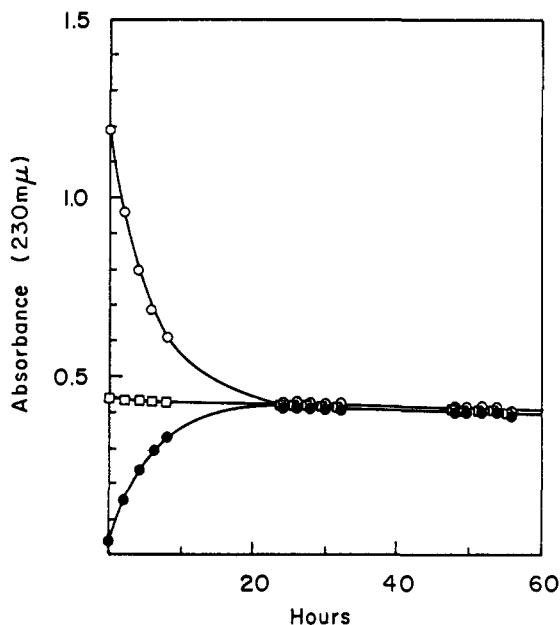


Figure 1. Approach to equilibrium for mixtures of S-acetylmercaptoethylamine and N-acetylmercaptoethylamine at pH 1.62, 50°, and ionic strength 1.0 *M*: starting with 0.28 mM thiol ester, O; starting with 0.10 mM thiol ester and 0.18 mM amide, □; and starting with 0.28 mM amide, ●.

creased, presumably because of hydrolysis of the thiol ester, and the absorbance at equilibrium was obtained by extrapolation to zero time. The correction is small, the decrease in absorbance being about 5% in 30 hr. Representative data at pH 1.6 are shown in Figure 1.

Table I. Extinction Coefficients for 2-Methyl- Δ^2 -thiazoline, S-Acetylmercaptoethylamine, and N-Acetylmercaptoethylamine^a

	ϵ_{260}	ϵ_{230}
2-Methyl- Δ^2 -thiazoline (protonated)	5300	802
S-Acetylmercaptoethylamine (protonated)	60	4300
N-Acetylmercaptoethylamine	65	122

^a Average of ref 27 and unpublished results of Dr. G. M. Blackburn.

The apparent equilibrium constant at a given pH value for the formation of N-acetylmercaptoethylamine from protonated S-acetylmercaptoethylamine, K_{SN}' , is defined by eq 2, in which N is

$$K_{SN}' = \frac{\epsilon_S C}{A_{230} - A_{230}'} - (1 + 1/K_{TS}) = \frac{[N]}{[SH^+]} \quad (2)$$

amide, *C* is the total concentration of thiol ester, amide, and thiazoline, A_{230} is the absorption at 230 $m\mu$ at equilibrium, ϵ_S is the extinction coefficient of the protonated thiol ester, and A_{230}' is the estimated absorption of amide at 230 $m\mu$. The value of A_{230}' was calculated from a preliminary estimate of amide concentration, based on the difference between *C* and the thiol ester concentration and neglecting thiazoline formation. This approximation does not introduce a significant error, because A_{230}' is generally less than 7% of A_{230} . The pH-independent equilibrium constant $K_{SN}' = [N]a_{H^+}/[SH^+]$ is obtained by multiplying K_{SN}' by the hydrogen ion activity.

The equilibrium constant for the formation of N-chloroacetylmercaptoethylamine from S-chloroacetylmercaptoethylamine was obtained at pH 1.6 by starting either with 5×10^{-4} *M* amide or thiol ester and following the formation or disappearance of thiol ester at 234 $m\mu$. No increase in absorbance at 260 $m\mu$ indicative of 2-chloromethyl- Δ^2 -thiazoline formation was observed.

The pK' of 2-methyl- Δ^2 -thiazoline at 50° and ionic strength 1.0

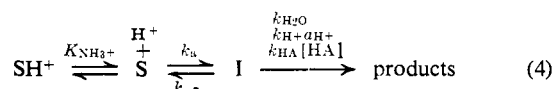
M was found to be 5.35 by spectrophotometric examination at 260 $m\mu$ of 3×10^{-4} *M* solutions of thiazoline in 0.01 *M* acetate and phosphate buffers.

Kinetics. The kinetics of the intramolecular conversion of S-acetylmercaptoethylamine and S-chloroacetylmercaptoethylamine to the corresponding amides was studied at 50° and ionic strength 1.0 *M* using a Zeiss PMQ spectrophotometer with a thermostated brass cell holder and Teflon-stoppered cuvettes by following the disappearance of thiol ester at 230 or 234 $m\mu$. The buffer was incubated for 5–10 min in the thermostated brass block before the substrate was added, except when catalysis by trifluoroethylamine buffers was studied. In the case of trifluoroethylamine buffers the substrate was added at room temperature and the cuvette was tightly stopped before putting it in the 50° thermostated block because of the volatility of this amine; absorbance measurements were made after the cuvette had been in the block for at least 5 min. The reaction was initiated by adding 0.02 ml of thiol ester in 0.01 *M* hydrochloric acid to 2.5–3 ml of buffer or hydrochloric acid solution. The initial concentration of substrate was $2-3 \times 10^{-4}$ *M*. Below pH 2.5–3 the S to N acetyl transfer reactions do not go to completion and the observed pseudo-first-order rate constants were corrected for reversal of the reaction according to eq 3, in which k_0

$$k_0 = k_{obsd}/(1 + 1/K_{SN}''') \quad (3)$$

is the pseudo-first-order rate constant for S to N transfer and k_{obsd} is the observed first-order rate constant. The reaction was found to follow good first-order kinetics for at least three half-lives. Although some thiazoline is formed below pH 2.5, the amount of thiazoline formed was never more than 5% of the total products. Reactions in carbonate buffer solutions were studied in a stopped-flow apparatus. The reaction mixtures were prepared with potassium carbonate in one syringe and the substrate in hydrochloric acid solutions in the other syringe. The concentrations of the buffers used to determine catalytic constants were generally in the range 0.01–0.10 *M*. In some cases buffer concentrations of up to 1.0 *M* were used in order to determine the rate constant for the step that is rate determining at high buffer concentrations.

The rate constants for the S to N transfer reaction of S-acetylmercaptoethylamine exhibit a nonlinear dependence upon buffer concentration because the reaction undergoes a change from a buffer-catalyzed to an uncatalyzed rate-determining step as the buffer concentration is increased.^{29a} For the evaluation of kinetic constants, this system may be described by the mechanism of eq 4, from which the steady-state rate law of eq 5 may be derived. Equation 5 may be simplified by setting $(K_{NH_3} + k_a/k_{-a}a_{H^+})k_{H_2O} =$



$$k_{obsd} = \frac{(K_{NH_3} + k_a/a_{H^+})(k_{H_2O} + k_{H^+}a_{H^+} + k_{HA}[HA])}{k_{-a} + k_{H_2O} + k_{H^+}a_{H^+} + k_{HA}[HA]} \quad (5)$$

k_p'/a_{H^+} ; $(K_{NH_3} + k_a/k_{-a})k_{H^+} = k_p$; $(K_{NH_3} + k_a/k_{-a}a_{H^+})k_{HA}[HA] = k_B[A^-]$; and $K_{NH_3} + k_a = k_c$. This gives the rate law shown in eq 6.

$$k_{obsd} = \frac{(k_c/a_{H^+})(k_p + k_p'/a_{H^+} + k_B[A^-])}{k_c/a_{H^+} + k_p + k_p'/a_{H^+} + k_B[A^-]} \quad (6)$$

A rate law of the same form as eq 6 is obtained if the assignment of which step is catalyzed in the mechanism of eq 4 is reversed. Rearrangement of eq 6 gives eq 7. In acid solutions or in con-

$$k_p + k_p'/a_{H^+} + k_B[A^-] = k_{cor} = \frac{k_{obsd}(k_c/a_{H^+})}{(k_c/a_{H^+}) - k_{obsd}} \quad (7)$$

centrated buffer solutions the rate law simplifies to $k_{obsd} = k_c/a_{H^+}$, while in dilute buffer solutions of low hydrogen ion activity the limiting rate is $k_{cor} = k_{obsd} = k_p + k_p'/a_{H^+} + k_B[A^-]$.

As defined above, k_c is the rate constant for the S to N acetyl transfer reaction below pH 2.3 and in concentrated buffer solutions, *i.e.*, under conditions in which the uncatalyzed step is rate determining. Division of k_c by the hydrogen ion activity gives k_c' , the observed pseudo-first-order rate constant at a given pH below pH 2.3 and in concentrated buffer solutions. The value of k_c (or k_c') may be obtained: (a) from rate measurements in solutions

sufficiently acidic that the first step (k_a) of eq 4 is entirely rate determining, (b) by measurement or extrapolation of the rate constants at high buffer concentrations, at which this uncatalyzed step is rate determining, or (c) by successive approximations from rate measurements at a series of buffer concentrations at which both steps are partially rate determining.

Procedure a requires correction of the observed rate constants for the back-reaction, since the reaction does not proceed to completion in acid solution. Extrapolation of the observed rate constants to infinite buffer concentration (procedure b) can be carried out as follows. If $k_p + k_p'/a_{H^+}$ (which is equal to k_{cor}^0) is much less than k_c' then eq 7 can be simplified and rearranged to give eq 8, in which k_0 is the value of k_{obsd} at zero buffer concentra-

$$\frac{1}{k_{obsd} - k_0} = \frac{1}{k_c'} + \frac{1}{[A^-]} \quad (8)$$

tion. A plot of $1/(k_{obsd} - k_0)$ vs. $1/[A^-]$ gives a straight line with an intercept of $1/k_c'$. However, k_0 is not known exactly and k_{cor}^0 is of comparable magnitude to k_c' at some pH values. At pH 4.29 using acetate buffers (30% base) of up to 1.0 M, k_{cor}^0 is negligible and k_0 is small compared to k_{obsd} for the buffer concentrations used. A value of k_c of $7.6 \times 10^{-7} M \text{ sec}^{-1}$ was obtained from this procedure under these conditions.

The procedure used routinely for obtaining rate constants is based on a series of successive approximations for k_0 with the aid of a computer. The value of k_0 was first estimated from an extrapolation of the observed rate constants in dilute buffer solutions to zero buffer concentration. A computer program³³ was then used to estimate the constants k_c , k_p , and k_p' of eq 6 as a first approximation. Equation 7 was then used to calculate k_{cor} , and the corrected pseudo-first-order rate constants were plotted against buffer concentration. A second approximation for k_0 was then obtained from eq 9. The new values of k_0 were then used in the

$$k_0 = \frac{k_c' k_{cor}^0}{k_c' + k_{cor}^0} \quad (9)$$

computer program as before and the calculations of eq 7 and 9 were repeated. After two repetitions of this procedure, no further changes occurred in the values of k_B and k_0 . A typical example of the magnitude of the corrections is given by experiments in a series of acetate buffers (30% base) from 0.01 to 0.05 M at pH 4.26. Extrapolation of the uncorrected rate constants to zero buffer concentration gives a value of k_0 of 0.042 min^{-1} , and the slope of the plot of observed rate constants against buffer concentration gives a catalytic constant, k_B , of $11.8 M^{-1} \text{ min}^{-1}$. After correction, the value of k_0 is 0.031 min^{-1} and the value of k_B is $15.5 M^{-1} \text{ min}^{-1}$. It is noteworthy that both the uncorrected and corrected data may give apparently linear plots of rate constants against buffer concentration, so that erroneous rate constants would be obtained if the correction for the contribution to the observed rate constants of the step which is not subject to buffer catalysis were not made. The value of k_c obtained by the iterative procedure is $8.06 \times 10^{-7} M \text{ sec}^{-1}$.

For the S to N transfer reaction of S-chloroacetylmercaptoethylamine the change in rate-determining step occurs at a higher pH and the value of k_c of $1.25 \times 10^{-5} M \text{ sec}^{-1}$ was obtained directly from rate measurements near pH 2, at which reversibility of the reaction does not significantly affect the observed rate constants. However, at higher pH values the differences between the rate constants for the two steps of the reaction are smaller for this compound than for the acetyl compound, so that the plots of k_{obsd} against buffer concentration show greater curvature and the correction of the rate constants for the contribution of k_c is larger. The largest correction was necessary for the rate data obtained in acetate buffers, 50% base. The observed rate constants in these buffers (Figure 2) exhibit a leveling off with increasing buffer concentration as the uncatalyzed step becomes rate determining; after correction the rate constants increase linearly with increasing buffer concentration. Because of this correction, the catalytic constants for this compound are estimated to be accurate to within 10–15%, compared to about 5% for the acetyl compound.

The kinetics of the hydrolysis of 2-methyl- Δ^2 -thiazoline was followed at 260 m μ . The reaction was initiated by adding 0.02 ml of thiazoline in either 0.1 M hydrochloric acid or 1.0 M potassium chloride to 2.5–3 ml of buffer solution or hydrochloric

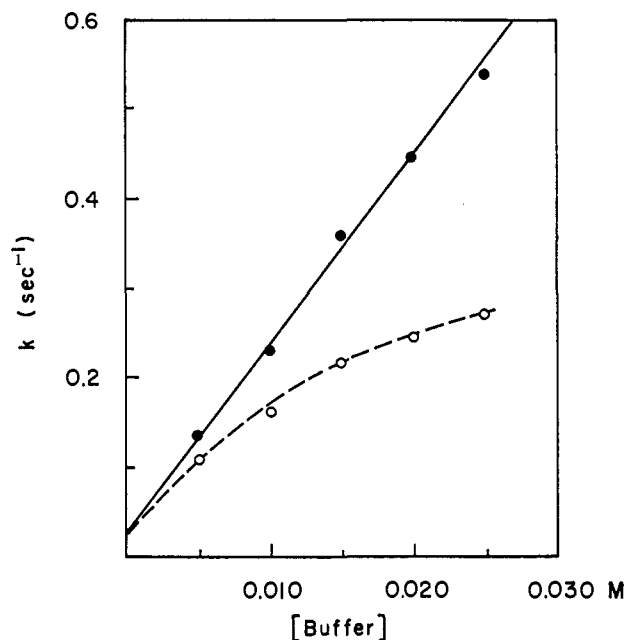


Figure 2. Effect of acetate buffer concentration on the rate of the S to N acetyl transfer reaction of S-chloroacetylmercaptoethylamine at pH 4.60, ionic strength 1.0 M, and 50°: observed rate constants, O; rate constants corrected for the change in rate-determining step with increasing buffer concentration, ●.

acid solution. The initial concentration of substrate was $2-6 \times 10^{-4} M$. For reactions at pH values below 3 the appearance of S-acetylmercaptoethylamine was followed at 230 m μ . The hydrolysis reaction was found to follow first-order kinetics for at least three half-lives and the reaction goes to completion above pH 1.4; at the lower pH values the reaction proceeded at least 90% to completion and corrections were not made for reversal of the reaction.

The fraction of thiol ester formed as a product of the hydrolysis of thiazoline was determined by measuring the appearance of thiol ester absorption at 230 m μ and the disappearance of thiazoline absorption at 260 m μ . The fraction of the total product which is thiol ester, f , was calculated from eq 10, in which A_{230} is the ob-

$$f = \frac{(A_{230} - A_{230}')/\epsilon_S}{(A_{260}^0 - A_{260})/\epsilon_T} = \frac{[\text{SH}^+]}{[\text{N}] + [\text{SH}^+]} \quad (10)$$

served absorbance at 230 m μ , A_{230}' is the absorption of thiazoline at 230 m μ , A_{260} is the absorption at 260 m μ , A_{260}^0 is the initial absorption at 260 m μ , ϵ_S is the extinction coefficient of the protonated thiol ester at 230 m μ , and ϵ_T is the extinction coefficient of protonated thiazoline at 260 m μ .

Results

Equilibrium Constants. The equilibrium constants for the interconversion of N-acetylmercaptoethylamine, S-acetylmercaptoethylamine, and 2-methyl- Δ^2 -thiazoline are summarized in Table II. A representative experiment for the approach to equilibrium of mixtures of S-acetylmercaptoethylamine and N-acetylmercaptoethylamine is shown in Figure 1. The amount of thiazoline formed under the experimental conditions is small (15–17% of the concentration of thiol ester at equilibrium), so that the equilibrium constant, K_{TS} , for the formation of protonated S-acetylmercaptoethylamine from protonated thiazoline is less accurate than K_{SN}' . Starting with $8.9 \times 10^{-4} M$ N-acetylmercaptoethylamine at pH 1.8, the total concentrations of thiol ester, amide, and thiazoline at equilibrium were found to be 2.2×10^{-4} , 6.2×10^{-4} , and $3.8 \times 10^{-5} M$, respectively; at pH 1.6 the corresponding values

(33) The computer program is given in ref 24.

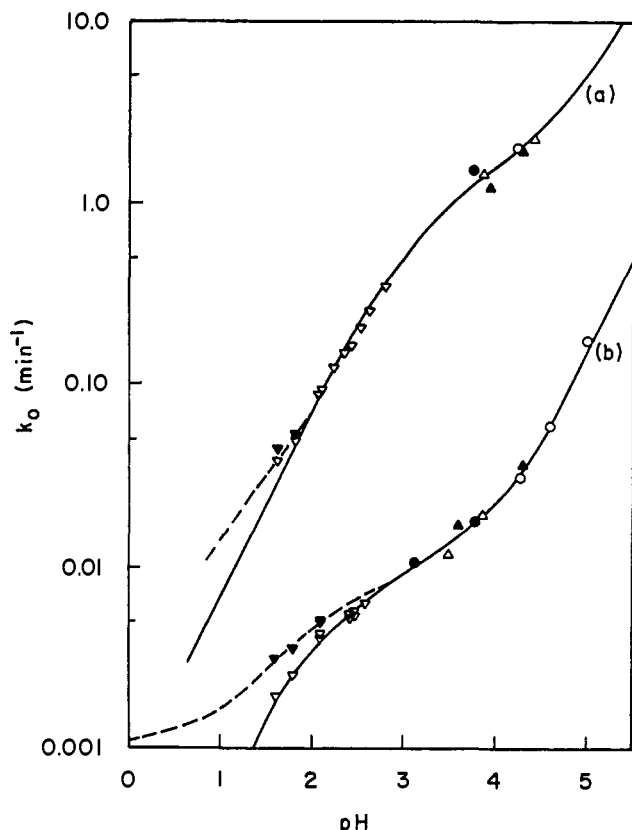


Figure 3. Dependence on pH of the rate of S to N transfer of (protonated) S-chloroacetylmercaptoethylamine (curve a) and S-acetylmercaptoethylamine (curve b) at 50° and ionic strength 1.0 M, extrapolated to zero buffer concentration. The solid line is calculated according to eq 6. Buffers: acetate, O; β -chloropropionate, \blacktriangle ; methoxyacetate, Δ ; chloroacetate, \bullet ; hydrochloric acid, ∇ ; hydrochloric acid uncorrected for reversal of the reaction, \blacktriangledown . The dashed line represents the rate of the reaction uncorrected for reversal of the reaction at low pH.

are 2.9×10^{-4} , 5.4×10^{-4} , and 4.9×10^{-5} M. The value obtained for K_{TS} of 5.85 may be compared with the value of 11 found by Martin, *et al.*,² at 25° and ionic strength 0.2 M. The equilibrium constant for the conversion of neutral thiol ester to amide, $K_{SN} = [N]/[S] = K_{SN}'/K_{NH_3^+}$, in which S represents the un-

Table II. Equilibrium Constants at 50° and Ionic Strength 1.0 M^a

	No. of runs	Equilibrium constant
K_{SN}' , M	9	4.18×10^{-2}
K_{SN}		5.2×10^7
K_{TS}	2	5.85 ^b
		6.69 ^c
$K_{SN(Cl)}'$, M	2	1.4×10^{-1}
$K_{SN(Cl)}$		1.7×10^8

^a $K_{SN}' = [N]_{aH^+}/[SH^+]$, $K_{SN} = [N]/[S]$, and $K_{TS} = [SH^+]/[TH^+]$, in which N represents amide, S neutral thiol ester, SH^+ protonated thiol ester, and TH^+ protonated thiazoline. The subscript (Cl) refers to the chloro-substituted compound. ^b From equilibrium measurements. ^c Kinetic value (see text).

protonated thiol ester and $K_{NH_3^+}$ is the dissociation constant of the protonated thiol ester, was calculated from an estimated value of $pK_{NH_3^+} = 9.1$.

The equilibrium constants for the conversion of S-chloroacetylmercaptoethylamine to amide, $K_{SN(Cl)}'$

and $K_{SN(Cl)}$ were calculated in the same manner, taking the pK_a of the amino group to be the same as that of S-acetylmercaptoethylamine.

Kinetics. The rate constants for the S to N acetyl transfer reaction of S-acetylmercaptoethylamine at 50° show a nonlinear dependence on buffer concentration and a stepwise dependence on pH similar to that previously observed by Martin and coworkers.^{28,29} This behavior is caused by a change in the step which is predominantly rate determining from a step which is subject to general base catalysis and includes water and hydroxide ion catalyzed terms above pH 2.3 and at low buffer concentrations to a step which is not subject to general acid-base catalysis and which decreases in rate with increasing acidity at low pH and high buffer concentrations. Since neither step is completely rate determining over most of the range of pH and buffer concentrations examined, the observed rate constants were corrected according to the steady-state rate equations 5-7 to obtain the rate constants for individual catalyzed and uncatalyzed steps. The methods by which these corrections were made are described in the Experimental Section and gave good agreement in several cases in which the results of the different methods were compared.

In Figure 3 are shown the pH-rate profiles for the S to N transfer reactions of protonated S-acetylmercaptoethylamine (lower curve) and S-chloroacetylmercaptoethylamine (upper curve), extrapolated to zero buffer concentration as described in the Experimental Section. The closed triangles for experiments in acid solution represent observed pseudo-first-order rate constants for reactions which proceed to equilibrium, rather than to completion; the open inverted triangles are the rate constants for the reaction in the forward direction. The changes in rate-determining step are apparent as decreases in slope with increasing pH near pH 2 for the acetyl compound and above pH 3 for the chloroacetyl compound. The experimental points show satisfactory agreement with the solid lines calculated from eq 6; in particular, there is good agreement of the rate constants obtained in the acid region, in which the uncatalyzed step is almost completely rate determining, with the calculated line based on the rate constants which were calculated from the results obtained at higher pH, as described in the Experimental Section. The catalytic rate constants, k_B , for catalysis by the conjugate bases of a series of acids are summarized in Tables III and IV.

Some effects of varying salts and solvents on the rate of the S to N transfer reaction of S-acetylmercaptoethylamine in acetate buffer are shown in Table V. Addition of *n*-butyl alcohol up to 0.5 M has no effect on the rate, and substitution of up to 0.5 M tetramethylammonium chloride for potassium chloride causes a small rate increase. Under similar conditions, solutions of methylammonium and *n*-butylammonium chlorides up to 0.5 M caused comparable rate increases of less than 11%. Upper limits for the catalytic constants for methylamine and butylamine were estimated assuming that a 5% rate increase which is not caused by a salt effect could be detected; limits for the rate constants for the reaction of S-chloroacetylmercaptoethylamine were calculated in the same manner.

Table III. Rate Constants for Catalysis of the Acetyl Transfer Reaction of S-Acetylmercaptoethylamine at 50° and Ionic Strength 1.0 M

Acid	% base	pH	No. of runs	pK _a ^a	k _B , M ⁻¹ sec ⁻¹	k _{HA} , ^b M ⁻¹ sec ⁻¹
H ₃ O ⁺ ^c				-1.74	2.92 × 10 ⁻⁶	6.5 × 10 ¹⁰
ClCH ₂ COOH	70	3.12	10	2.79	5.61 × 10 ⁻³	3.13 × 10 ⁹
	90	3.79	10		4.80 × 10 ⁻³	
CH ₃ OCH ₂ COOH	70	3.86	5	3.50	1.87 × 10 ⁻²	2.58 × 10 ⁹
	50	3.49	5		2.17 × 10 ⁻²	
ClCH ₂ CH ₂ COOH	70	4.30	5	3.95	5.78 × 10 ⁻²	2.64 × 10 ⁹
	30	3.59	5		5.88 × 10 ⁻³	
CH ₃ COOH	70	5.02	5	4.64	2.79 × 10 ⁻¹	2.44 × 10 ⁹
	50	4.61	5		2.50 × 10 ⁻¹	
	30	4.29	5		2.59 × 10 ⁻¹	
H ₂ PO ₄ ⁻	0.55 ^d	4.26	5	6.52	2.34 × 10	2.92 × 10 ⁹
	0.27 ^e	3.96	5		2.44 × 10	
C ₂ H ₅ OCOCH ₂ NH ₃ ⁺	0.089 ^d	4.26	5	7.31	2.76 × 10	5.87 × 10 ⁹
	0.032 ^e	3.81	5		3.14 × 10	
HOCH ₂ CH ₂ NH ₃ ⁺	1.2 × 10 ⁻³ ^d	4.26	5	9.13	1.18 × 10 ²	3.53 × 10 ⁷
	2.5 × 10 ⁻³ ^d	4.54	5		1.16 × 10 ²	
CH ₃ NH ₃ ⁺	1.2 × 10 ⁻⁴ ^d	4.26	5	10.16	<1.5 × 10 ² ^f	<5 × 10 ⁶ ^f
	2.4 × 10 ⁻⁴ ^d	4.54	5		<2.5 × 10 ² ^f	
CH ₃ (CH ₂) ₃ NH ₃ ⁺	9.5 × 10 ⁻⁵ ^d	4.26	5	10.26	<2.9 × 10 ² ^f	<6 × 10 ⁶ ^f
	2.4 × 10 ⁻⁴ ^d	4.54	5		<2.1 × 10 ² ^f	
CF ₃ CH ₂ NH ₃ ⁺	0.67 ^e	3.11	5	5.28	1.48	3.10 × 10 ⁹
	1.39 ^e	3.43	5		1.42	
HCO ₃ ⁻	50	9.30	5	9.30	6.06 × 10 ³	1.2 × 10 ⁹
H ₂ O ^c				15.00	3.92 × 10 ⁶	1.59 × 10 ⁶

^a Except for hydronium ion, water, and the amines, determined from the pH of the reaction mixtures. The pK_a's of the amines were determined at 50°, ionic strength 1.0 M, from the pH of a 50% neutralized solution. ^b Based on a catalytic constant of 6.5 × 10¹⁰ for the solvated proton (see text). ^c Determined from pH-rate profile (Figure 3). ^d In 0.01 M acetate buffer. ^e In 0.01 M methoxyacetate buffer. ^f Upper limit (see text).

Table IV. Rate Constants for Catalysis of the Acetyl Transfer Reaction of S-Chloroacetylmercaptoethylamine at 50° and Ionic Strength 1.0 M

Acid	% base	pH	No. of runs	pK _a ^a	k _B , M ⁻¹ sec ⁻¹	k _{HA} , ^b M ⁻¹ sec ⁻¹
H ₃ O ⁺ ^c				-1.74	4.6 × 10 ⁻⁴	6.5 × 10 ¹⁰
ClCH ₂ COOH	90	3.78	5	2.83	5.42 × 10 ⁻¹	2.56 × 10 ⁹
CH ₃ OCH ₂ COOH	90	4.42	10	3.48	2.57	2.23 × 10 ⁹
	70	3.87	5		3.03	
ClCH ₂ CH ₂ XOOH	50	3.93	5	3.93	1.09 × 10	3.05 × 10 ⁹
	70	4.30	5		1.05 × 10	
CH ₃ COOH	50	4.62	10	4.62	4.14 × 10	2.42 × 10 ⁹
	30	4.26	5		3.80 × 10	
C ₂ H ₅ OCOCH ₂ NH ₃ ⁺	2.9 × 10 ⁻² ^d	3.78	5	7.31	4.65 × 10 ²	5.85 × 10 ⁷
	4.4 × 10 ⁻² ^e	3.95	5		4.75 × 10 ²	
HOCH ₂ CH ₂ NH ₃ ⁺	3.8 × 10 ⁻⁴ ^e	3.71	5	9.13	<2 × 10 ³	<3 × 10 ⁶ ^f
CH ₃ (CH ₂) ₃ NH ₃ ⁺	2.8 × 10 ⁻⁵ ^e	3.71	5	10.26	<2 × 10 ⁴	<3 × 10 ⁷ ^f
H ₂ O ^c				15.00	3.1 × 10 ⁷	7.9 × 10 ⁴

^a Except for hydronium ion, water, and the amines, determined from the pH of the reaction mixtures. The pK_a's of the amines were determined at 50°, ionic strength 1.0 M, from the pH of a 50% neutralized solution. ^b Based on a catalytic constant of 6.5 × 10¹⁰ for the solvated proton (see text). ^c Determined from the pH-rate profile (Figure 3). ^d In 0.01 M methoxyacetate buffer. ^e In 0.01 M β-chloropropionate buffer. ^f Upper limit (see text).

Table V. Solvent and Salt Effects on the Acetyl Transfer Reaction of S-Acetylmercaptoethylamine in 0.01 M Acetate Buffer, 50% Base, Ionic Strength Maintained at 1.0 M with Potassium Chloride at 50°

Conditions	pH	k _{obsd} , min ⁻¹
Control	4.57	0.121
0.2 M <i>n</i> -butyl alcohol	4.58	0.124
0.5 M <i>n</i> -butyl alcohol	4.60	0.121
0.2 M tetramethylammonium chloride	4.55	0.124
0.5 M tetramethylammonium chloride	4.57	0.130

Catalysis of the S to N transfer reaction of S-acetylmercaptoethylamine by carbonate buffers was studied at high pH by the stopped-flow technique. The rate

constants extrapolated to zero buffer concentration are smaller than those predicted by a linear extrapolation of the data obtained at lower pH, because of conversion of the amine to the free base form at high pH. The dependence of the observed rate constants on acidity and the ionization constant of the amine hydrochloride are given by eq 11, in which k₀' is the rate constant ex-

$$K_{NH_3^+} = a_{H^+}(k_0'/k_{obsd} - 1) \quad (11)$$

pected in the absence of conversion to the free amine. The observed rate constants (extrapolated to zero buffer concentration) of 16 and 26 sec⁻¹ at pH 9.30 and 9.98, compared to values of 43 and 210 sec⁻¹, respectively, for k₀' at these pH values, show the leveling off which is expected from conversion to the free base form and

give a pK value of 9.1 for the amine hydrochloride at 50°. Martin, *et al.*,²⁶ have estimated a value of 9.1 for this pK at 25° by comparison with structurally related compounds.

The hydrolysis of 2-methyl- Δ^2 -thiazoline at 50° follows a bell-shaped pH-rate profile similar to that observed at 25°. The pH dependence of the observed rate constants, extrapolated to zero buffer concentration, follows eq 12, in which K_{TH^+} is the dissociation

$$k_{\text{obsd}} = \frac{PQa_{H^+}}{(a_{H^+} + K_{TH^+})(a_{H^+} + P)} \quad (12)$$

constant of protonated thiazoline, $P = 0.143 M$, and $Q = k_{-T} = 1.4 \times 10^{-4} \text{ sec}^{-1}$.²⁴ The catalytic constants for general base catalysis (in terms of protonated thiazoline), obtained from plots of k_{obsd} against buffer concentration, are summarized in Table VI and follow a Brønsted relationship with a slope of approximately 0.3.

Table VI. Rate Constants for General Base Catalysis of 2-Methyl- Δ^2 -thiazoline Hydrolysis at 50° and Ionic Strength 1.0 M

Base	% base	pH	No. of runs	pK_a^a	$k_{-T(B)} \times 10^4, M^{-1} \text{ sec}^{-1}$
CH ₃ OCH ₂ COO ⁻	70	3.86	5	3.50	2.34
	90	4.46	5		2.12
ClCH ₂ CH ₂ COO ⁻	70	4.28	5	3.91	2.02
	82	4.62	5		2.05
CH ₃ COO ⁻	70	5.01	5	4.64	2.88
	90	5.59	5		2.92
CH ₃ CH ₂ COO ⁻	30	4.38	5	4.80	4.0
	70	5.22	5		3.6
	90	5.75	5		3.0
H ₂ O				-1.74	0.027

^a Except for water, the pK_a values are based on the observed pH of the reaction mixtures.

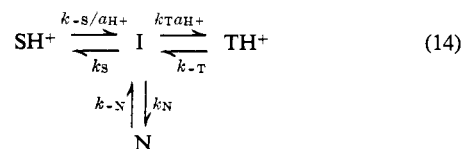
The hydrolysis of thiazoline gives both thiol ester and amide as products at low pH, with a relative yield which is independent of the pH, and only amide at higher pH values.^{26,27} The yield of thiol ester at 50° as a function of pH (Figure 4) follows a sigmoid curve and approaches a pH-independent value of 0.5 in acid solution, similar to that observed at 25°. The small change in yield between pH 0.5 and 1.5 over a tenfold change in hydrogen ion concentration makes it certain that the yield is not proportional to hydrogen ion concentration at low pH. Each point in Figure 4 was obtained by extrapolating the yield of thiol ester to zero time, so that the decrease in yield at higher pH values is a direct reflection of the mechanism of thiazoline decomposition and is not a consequence of a secondary reaction.²⁶ The solid line of Figure 4 is calculated from eq 13, in which f^0 is the limiting value

$$f = \frac{f^0 a_{H^+}}{a_{H^+} + K_p} \quad (13)$$

of f at low pH and K_p is the hydrogen ion activity at which $f = \frac{1}{2}f^0$, and is based on values of f^0 and K_p of 0.50 and 4.27×10^{-3} , respectively.

The over-all equilibrium constant K_{TS} for the formation of protonated thiol ester SH^+ from protonated thiazoline TH^+ may be calculated from the observed

rate constants for thiazoline hydrolysis and the S to N transfer reaction and the relative yields of products from thiazoline hydrolysis, based on the scheme of eq 14. Consider the reaction at low pH values, at

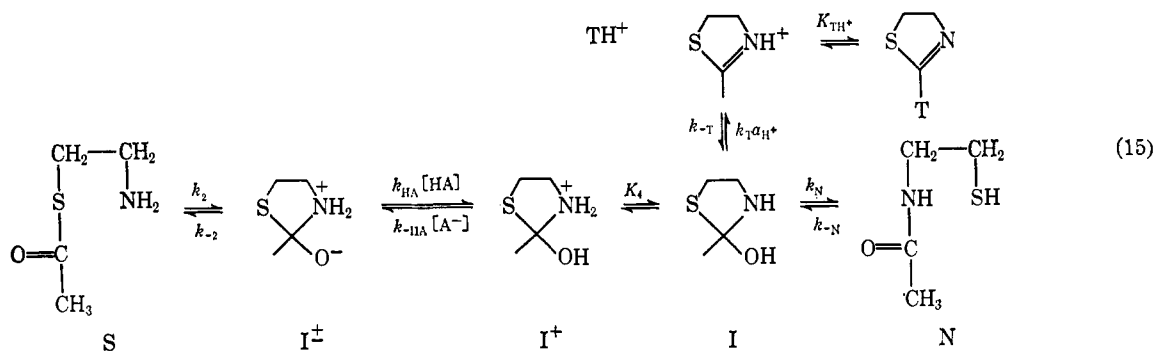


which the thiol ester and thiazoline are completely protonated and the transition states for the formation of intermediate from thiol ester and amide have no net charge. The equilibrium constant for the formation of protonated thiol ester from protonated thiazoline is $k_{-T}k_S/k_Tk_{-S}$. The value of k_{-T} is known (Table VI) and $k_{-S} = k_c/(1 - f^0)$. The ratio of the rate constants k_S/k_T is equal to fP where P is the hydrogen ion activity at which both the formation and breakdown of the intermediate formed in thiazoline hydrolysis are equally rate determining. The value for K_{TS} of 6.69 obtained by this method may be compared with the value of 5.85 that was obtained by direct measurements of the equilibrium.

Discussion

The experiments reported here confirm the main features of the basic scheme shown in eq 1 for the hydrolysis of 2-methyl- Δ^2 -thiazoline.²⁶⁻²⁹ The observed rate of hydrolysis follows the ionization curve of protonated thiazoline down to a pH of about 2. In acid solution there is a change in rate-determining step with a midpoint at pH 0.85 and the hydrolysis reaction occurs through a transition state(s) of zero net charge. Between pH 2 and 4 the fraction of thiol ester formed as a product of the hydrolysis reaction at 50° decreases from 0.37 to 0.01, but there is little change in the rate of thiazoline hydrolysis. This means that the rate-determining step is different from the product-determining step and shows that the rate-determining step under these conditions is the attack of water on protonated thiazoline, rather than the breakdown of the intermediate.

The "Thiazoline Dilemma." Of the several ways in which the "thiazoline dilemma"^{29a} may be formulated, we find the following easiest to understand. The hydrolysis of 2-methyl- Δ^2 -thiazoline gives approximately equal amounts of thiol ester S and amide N as products in acid solution. The fact that the ratio of these products approaches a limiting value which is independent of pH means that the transition states for the formation of these two products from the intermediate addition compound I in acid solution must have the same charge; *i.e.*, the stabilities of these two transition states must have an identical dependence upon the acidity of the solution. The "dilemma" arises from the facts that (a) there is a change in rate-determining step in the S to N transfer reaction with increasing pH, which corresponds to a change from a transition state of zero charge to one with a charge of +1, and (b) there is a decrease in the yield of thiol ester from thiazoline hydrolysis with increasing pH, which is not caused by a secondary S to N acyl transfer reaction. If the steps for the formation of the thiol ester and amide from the intermediate I have the same



charge, there is no reason why there should be either a change in rate-determining step or a decrease in the yield of thiol ester with increasing pH. The results demand that a new barrier appear between S and I on the thiol ester limb of the scheme of eq 1 as the pH is increased above pH 2.3, *i.e.*, that a new step become rate-determining at this point. Since all steps which involve the making and breaking of bonds to carbon are already included in the scheme of eq 1, this new step must be a simple proton transfer step. The results are explained if this step involves proton transfer by an acid to the dipolar intermediate I^\pm which is initially formed in the S to N transfer reaction (k_{HA} , eq 15) and, in the reverse direction, the removal of a proton from the oxygen atom of a protonated tetrahedral addition intermediate I^+ (k_{-HA} , eq 15). According to this mechanism the rate-determining step for the forma-

served rate of S to N transfer, expressed in terms of protonated starting material, becomes independent of pH. At still higher pH the protonation of I^\pm occurs through a water-catalyzed reaction and the kinetics again reflect a transition state of zero net charge (an increase in rate with increasing pH in terms of protonated starting material). Under conditions in which either of these proton transfer steps are rate determining the barrier for thiol ester formation from I is larger than that for amide formation and no thiol ester is formed as a product of thiazoline hydrolysis.

The Proton Transfer Step. The activation energy for simple proton transfers between electronegative atoms, such as the k_{HA} step of eq 15, is so small that the observed reaction rate in one or the other direction is usually controlled by the rate of diffusion together of the reactants. Reactions of this kind have several characteristics which permit their identification, even when a rate constant corresponding to that of a diffusion-controlled reaction cannot be observed directly.⁸ (1) The rate of proton transfer from a series of structurally similar acids to a base in the thermodynamically favored direction will be diffusion controlled, so that it is independent of the strength of the acid; *i.e.*, the Brønsted α value for the reaction is zero. (2) The rate constant for proton transfer from the solvated proton is some 20-fold faster than that for other acids, presumably because this proton transfer can occur rapidly through adjacent water molecules by a "proton-jump" mechanism. (3) As the strength of the acid is decreased and the proton transfer becomes thermodynamically unfavorable, the observed rate of proton transfer will decrease and will eventually become directly proportional to the strength of the acid; *i.e.*, the value of α will approach 1.0. The pH value at which the slope of the Brønsted plot changes from 0 to 1.0 depends on the relative acid-base strengths of the proton donor and acceptor molecules. (4) In favorable cases, such as the transfer of a proton from one oxygen atom of a carboxylic acid to the other, proton transfer may occur by a concerted, intramolecular, cyclic mechanism through a water molecule or some other bifunctional proton donor and acceptor.^{34,35} All of these characteristics are observed for the reaction described here under conditions in which the postulated proton transfer step is rate determining.

(1) The observed rate constants k_B for catalysis of the S to N transfer reaction of protonated thiol ester by general bases correspond to rate constants k_{HA}'

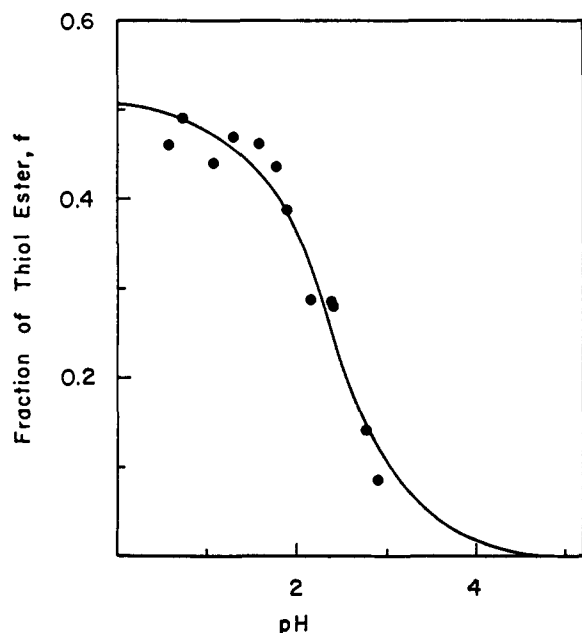


Figure 4. Variation in the fraction of S-acetylmercaptoethylamine, f , produced in the hydrolysis of 2-methyl- Δ^2 -thiazoline, with respect to pH at 50° and ionic strength 1.0 M .

tion of intermediate from thiol ester in acid solution is the attack of the free amino group on the thiol ester; the breakdown of the tetrahedral intermediate I to amide occurs through a transition state of zero charge and of similar free energy. As the pH is increased the dipolar intermediate I^\pm reverts to thiol ester (k_{-2}) more rapidly than it is protonated (k_{HA}), protonation of the intermediate becomes rate determining, and the ob-

(34) E. Grunwald, C. F. Jumper, and S. Meiboom, *J. Am. Chem. Soc.*, **85**, 522 (1963); E. Grunwald and S. Meiboom, *ibid.*, **85**, 2047 (1963).

(35) Z. Luz and S. Meiboom, *ibid.*, **85**, 3923 (1963).

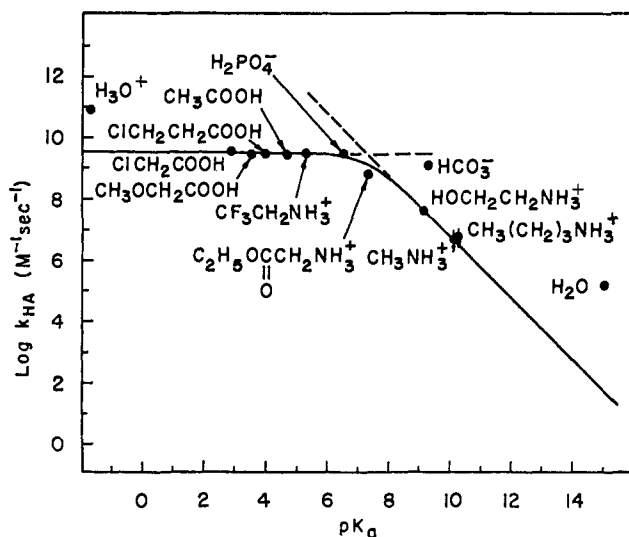


Figure 5. Brønsted plot for general acid catalysis of the acetyl transfer reaction of S-acetylmercaptoethylamine at 50° and ionic strength 1.0 M, based on a value of $k_{H_3O^+} = 6.5 \times 10^{10} M^{-1} \text{sec}^{-1}$ (see text).

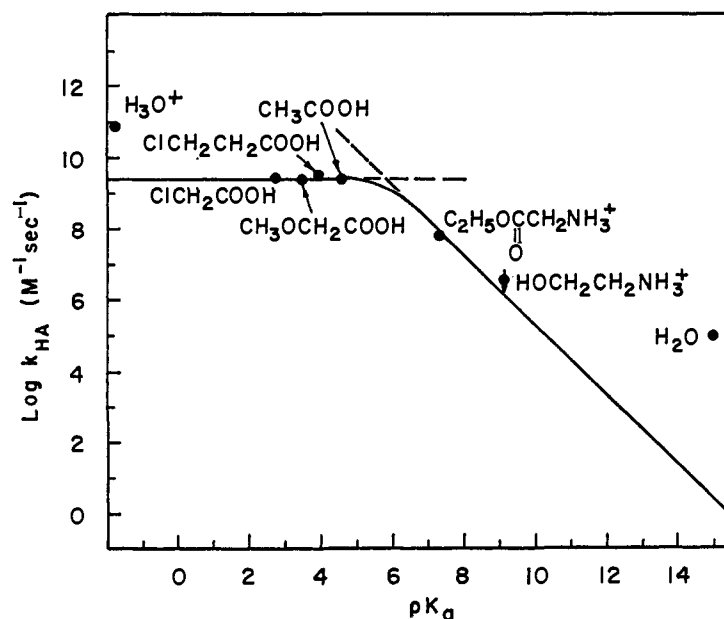


Figure 6. Brønsted plot for general acid catalysis of the acetyl transfer reaction of S-chloroacetylmercaptoethylamine at 50° and ionic strength 1.0 M, based on a value of $k_{H_3O^+} = 6.5 \times 10^{10} M^{-1} \text{sec}^{-1}$ (see text).

for catalysis of a reaction of neutral thiol ester or intermediate by general acids, as shown in eq 16. The



rate constants k_B for catalysis by carboxylate ions and phosphate dianion exhibit a dependence on base strength β of 0.97; when expressed in terms of acid catalysis of a neutral substrate they exhibit no dependence on acid strength; *i.e.*, the value of α is experimentally indistinguishable from the value of zero expected for the diffusion-controlled rate-determining proton transfer step of eq 15. The k_{HA} of eq 15 differs from k_{HA}' by the (unknown) equilibrium constant for the formation of I^\ddagger from S. However, absolute values of k_{HA} may be estimated, based upon the assumption that catalysis by the solvated proton also occurs

through a diffusion-controlled step and that the rate constant for this step is $6.5 \times 10^{10} M^{-1} \text{sec}^{-1}$ at 50°. These values are shown in Tables III and IV and are plotted in Figures 5 and 6.

(2) The rate constants for catalysis by the solvated proton are 23 and 25 times larger than the average of the rate constants for catalysis by carboxylic acids and phosphate of the S to N transfer reactions of the acetyl and chloroacetyl thiol esters, respectively. This ratio may be compared with the rate constants for the directly measured proton transfer from the solvated proton to acetate, β -naphthoxide, and ammonia in the range $4.3\text{--}5.1 \times 10^{10} M^{-1} \text{sec}^{-1}$ and for proton transfer from carboxylic acids to strong nitrogen bases of $1.5\text{--}3 \times 10^9 M^{-1} \text{sec}^{-1}$.

(3) Although the rate constant for catalysis of the S to N transfer reaction of the acetyl thiol ester by trifluoroethylammonium ion is the same as that for carboxylic acids and presumably represents a step which takes place at a diffusion-controlled rate, the rate constants for catalysis by more weakly acidic ammonium ions are smaller. No definite evidence for catalysis

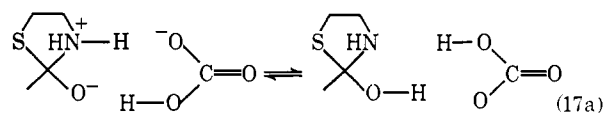
by methylammonium and butylammonium ions was obtained and the rate constants for these acids are given in Figure 5 as upper limits; the value for hydroxyethylammonium ion in Figure 6 is also an upper limit. This falling off of catalytic rate constants would be expected if the ionization constant of the group in I^\ddagger which is being protonated is in the same range as the ionization constants of the catalyzing acids. The decrease in rate constant with decreasing acidity approaches or follows the line of slope 1.0 expected for proton transfer from a weak acid to a weak base (Figure 5). A similar conclusion may be reached from the less extensive data for the chloroacetyl compound (Figure 6).

(36) This rate constant is based upon the assumptions that $E^\ddagger = 2$ kcal/mol and that ionic strength has little effect on diffusion coefficients.

The break in the curve of Figure 5 occurs at pH 7.4 and suggests that the group which is being protonated has a pK in the vicinity of 7.4. A pK value of 8.9 is predicted for this group from a σ_1 - pK correlation for substituted alcohols.^{24,37} In view of the uncertainties of this correlation, especially for charged substituents, and the fact that the breaks in pK -rate plots for diffusion-controlled proton transfers do not occur exactly at $\Delta pK = 0$, especially when the reacting groups are of different charge types,⁸ these pK estimates appear to be in satisfactory agreement.

A critical test of the proposed mechanism is that the position of this break should shift as the basicity of I^\pm is changed; for this reason the catalytic constants for the chloroacetyl compound were examined (Figure 6). The σ_1 - pK correlation suggests that the pK of I^\pm should be decreased by 1.4 to 1.6 pK units for the chloroacetyl compared to the acetyl compound. The observed break for this compound is at pH 5.9, 1.5 units below that for the acetyl compound. Such a change in the position of the break would not be expected for the usual "coupled" mechanism of general acid-base catalysis.

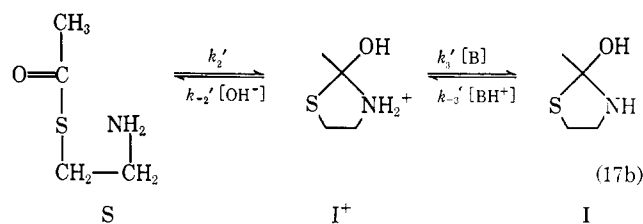
(4) According to the mechanism of eq 15, the two proton transfers which are required for the conversion of I^\pm to I occur in a stepwise manner. It might be expected that in the presence of suitable bifunctional catalysts these proton transfers could take place in a concerted fashion. Such a concerted mechanism should be most important for weak acids which protonate the oxygen atom of I^\pm at a relatively slow rate. The rate constants for catalysis by bicarbonate ion and by water exhibit positive deviations of two to four orders of magnitude from the Brønsted plots of Figures 5 and 6, which may be ascribed to bifunctional catalysis; these catalysts, in contrast to ammonium ions, contain both proton-donating and -accepting groups. A positive deviation is not observed for phosphate monoanion and carboxylic acids, which may also act through a concerted mechanism, because these catalysts already react at a diffusion-controlled rate. Although the positive deviations of bicarbonate and water suggest that the concerted mechanism can provide a lower energy pathway for the reaction than the stepwise mechanism, the fact that the rate constant for bicarbonate catalysis is larger than that for water suggests that proton donation is of primary importance even for the concerted mechanism. A mechanism for concerted catalysis by bicarbonate ion is shown in eq 17a.



The rate constants for the water reactions (expressed as pseudo-first-order rather than as catalytic constants) are 8.8×10^6 and $4.3 \times 10^6 \text{ sec}^{-1}$ for the acetyl and chloroacetyl compounds, respectively. These are in the same range as the rate constants of 1.3×10^5 to $1.9 \times 10^6 \text{ sec}^{-1}$ for the intramolecular transfer of a proton between the oxygen atoms of substituted benzoic acids in methanol³⁴ and $4.7 \times 10^7 \text{ sec}^{-1}$ for the same process in water.³⁵

(37) R. W. Taft, Jr., and I. C. Lewis, *J. Am. Chem. Soc.*, **81**, 5343 (1959); P. R. Wells, *Chem. Rev.*, **63**, 171 (1963); M. Charton, *J. Org. Chem.*, **29**, 1222 (1964).

An alternative mechanism involves a concerted addition and protonation to give I^+ directly, followed by proton abstraction from I^+ by a base (eq 17b). This



mechanism may be ruled out because it requires values of k_{-2}' and k_3' for reactions with hydroxide ion which are larger than the diffusion-controlled limit in order to account for the observed reaction rates. A pK_a value of 6 for I^+ was estimated from a σ_1 - pK correlation for amines.^{24,37} The rate of formation of I from I^+ by donation of a proton to water is then about 10^4 sec^{-1} , assuming that k_{-3}' is diffusion controlled for the reaction of the solvated proton. In order for k_{-2}' to be larger than 10^4 sec^{-1} under conditions in which k_3' is rate determining, the value of k_{-2}' would have to be ca. $10^{16} \text{ M}^{-1} \text{ sec}^{-1}$, an unreasonably large value. Furthermore, the appearance of an upward deflection in the pH-rate profile near pH 4 requires that the k_3' term for hydroxide ion become comparable in magnitude to the water reaction at this pH value. This would require a value of $k_3' = 10^{14} \text{ M}^{-1} \text{ sec}^{-1}$, which is also above the diffusion-controlled limit. A mechanism in which the proton transfer step is on the amide limb of the scheme does not account for the decrease in the yield of thiol ester from the hydrolysis of thiazoline as the pH is increased.

Martin, *et al.*,²⁹ have reported a β value of 0.6, equivalent to an α value of 0.4, for the S to N transfer reaction at 25°, based on catalytic constants for the solvated proton, formic acid, phenol, boric acid, and water. These results are consistent with those reported here; the characteristic shape of the Brønsted plot of Figure 5 was not observed because of the limited number of catalysts examined.

The steady-state rate law for the S to N transfer reaction of eq 15 under conditions in which the thiol ester is fully protonated and thiazoline formation may be neglected is given in eq 18, in which $k_S = (k_{H^+}/K_4) \cdot (\Sigma k_{-HA}[A^-]k_{-2})/(\Sigma k_{HA}[HA] + k_{-2})$. Setting $k_c = K_{NH_3} \cdot k_2(1 - f^0)$ and $\Sigma k_B[A^-] = K_{NH_3} \cdot k_2 \Sigma k_{HA}[HA]/k_{H^+} \cdot k_{-2}$ gives eq 19. Below pH 2.3 or at high buffer

$$k_{\text{obsd}} = \frac{(K_{NH_3} \cdot \Sigma k_{HA}[HA]k_2)/(\Sigma k_{HA}[HA] + k_{-2})}{a_{H^+}(1 + k_S/k_N)} \quad (18)$$

$$k_{\text{obsd}} = \frac{(k_c/a_{H^+})\Sigma k_B[A^-]}{\Sigma k_B[A^-] + k_c/a_{H^+}} \quad (19)$$

concentration this rate law reduces to $k_{\text{obsd}} = k_c/a_{H^+}$. When the rate of the attack step is fast relative to $k_{HA}[HA]$, a condition which is approached at low buffer concentration and higher pH values, it reduces to $k_{\text{obsd}} = \Sigma k_B[A^-]$.

The equilibrium constant for the formation of I from I^\pm is estimated to be approximately 2.5×10^3 from σ_1 - pK correlations for the ionization of amines and alcohols.^{24,37} Based on this value and a rate constant of $6.5 \times 10^{10} \text{ M}^{-1} \text{ sec}^{-1}$ for the diffusion-controlled reaction of the solvated proton, absolute values of all

of the rate and equilibrium constants in the mechanism of eq 15 may be calculated. The results are given in Table VII. Although these rate constants are probably not of high precision, they do serve to indicate that the individual rate constants which are required for this mechanism are of reasonable magnitude.

Table VII. Rate Constants for the Mechanism of Eq 15^a

k_2, sec^{-1}	4.1×10^8
k_{-2}, sec^{-1}	6.6×10^8
$k_{\text{H}_3\text{O}^+}, M^{-1} \text{sec}^{-1}$	6.5×10^{10}
$k_{\text{H}_2\text{O}}, \text{sec}^{-1}$	8.8×10^6
$k_{\text{N}}, \text{sec}^{-1}$	2.6×10^5
$k_{-\text{N}}, \text{sec}^{-1}$	3.8×10^{-5}
$k_{\text{T}}, M^{-1} \text{sec}^{-1}$	3.6×10^6
$k_{-\text{T}}, \text{sec}^{-1}$	1.4×10^{-4}
$k_{\text{S}}^0/k_{\text{N}}^b$	1.02
$k_{\text{S}}^0/k_{\text{T}}, M^b$	7.6×10^{-2}
K_4, M	$\sim 10^{-6}$
k_4, sec^{-1}	$\sim 6.5 \times 10^4$
$k_{-4}, M^{-1} \text{sec}^{-1}$	6.5×10^{10}

^a See text for details and definitions of symbols. ^b k_{S}^0 is the value of k_{S} at low pH.

The most critical rate constant for the proposed mechanism is k_{-2} . A diffusion-controlled protonation of the intermediate I^\pm can become rate determining only if this step, the expulsion of amine from the zwitterion I^\pm , occurs more rapidly than the protonation step. The value of $6.6 \times 10^8 M^{-1} \text{sec}^{-1}$ for this rate constant is large, but is not unreasonable. Precedent for rate constants of this magnitude is found in the hydroxide ion catalyzed expulsion of thiol anion from the hemithioacetal of benzenethiol and acetaldehyde, which occurs at a rate comparable to that of the diffusion-controlled encounter of the hemithioacetal with hydroxide ion ($k = 6.8 \times 10^9 M^{-1} \text{sec}^{-1}$).³⁸ Similarly, the observed rate constants for the addition of hydroxylamine to *p*-chlorobenzaldehyde require that any zwitterionic addition intermediate which is initially formed in this reaction decompose to starting materials with a rate constant of about 10^9sec^{-1} .³⁹

The rate constants for hemithioacetal decomposition³⁸ also serve as a precedent for the rate constant k_{N} of $2.5 \times 10^5 \text{sec}^{-1}$ for the expulsion of thiol anion from the neutral intermediate I of eq 15 to give amide. The rate constant for the water-catalyzed breakdown of the hemithioacetal is 0.01sec^{-1} , while that for the hydroxide ion catalyzed reaction is close to the diffusion-controlled limit of about $10^{10} M^{-1} \text{sec}^{-1}$. Since the basicity and driving force provided by the free electron pair of the nitrogen atom of I would be expected to be intermediate between those of OH and O^- , it is reasonable that the value of k_{N} is between these values.

The steady-state rate law for thiazoline hydrolysis is given in eq 20, in which K_{TH^+} is the ionization constant for the conjugate acid of 2-methyl- Δ^2 -thiazoline. Be-

$$k_{\text{obsd}} = \frac{k_{-\text{T}}a_{\text{H}^+}(k_{\text{S}} + k_{\text{N}})}{k_{\text{T}}(a_{\text{H}^+} + K_{\text{TH}^+})[a_{\text{H}^+} + (k_{\text{S}} + k_{\text{N}})/k_{\text{T}}]} \quad (20)$$

low pH 0.8, at which a change in the rate-determining step of this reaction occurs, the rate law reduces to

(38) R. Barnett and W. P. Jencks, *J. Am. Chem. Soc.*, **89**, 5963 (1967).

(39) J. E. Reimann and W. P. Jencks, *ibid.*, **88**, 3973 (1966).

$k_{\text{obsd}} = k_{-\text{T}}(k_{\text{S}} + k_{\text{N}})/k_{\text{T}}a_{\text{H}^+}$. Under these conditions the reaction proceeds through neutral transition states and the breakdown of the intermediate to thiol ester (k_{-2}) and amide (k_{N}) is rate determining. At higher pH values $k_{\text{obsd}} = k_{-\text{T}}a_{\text{H}^+}/(a_{\text{H}^+} + K_{\text{TH}^+})$ and the attack of water on protonated thiazoline is rate determining. This step is also general base catalyzed. The rate constant k_{T} for the acid-catalyzed breakdown of I to protonated thiazoline is estimated to be $3.6 \times 10^6 M^{-1} \text{sec}^{-1}$. This may be compared to the rate constants of $5.6 \times 10^4 M^{-1} \text{sec}^{-1}$ for the acid-catalyzed dehydration of the addition compound of *p*-chlorobenzaldehyde and hydroxylamine to the oxime³⁹ and $1.4 \times 10^8 M^{-1} \text{sec}^{-1}$ for the dehydration of the addition compound of pyridine-4-carboxaldehyde and glycine to the Schiff base.⁴⁰

The change in rate-determining step of the S to N transfer reaction at pH 2.30 accounts for the change in the products of thiazoline hydrolysis, which has a midpoint at pH 2.37 (Figure 4). As the proton transfer step ($k_{-\text{HA}}$) becomes slow the barrier to thiol ester formation increases and the yield of this product decreases. It is intuitively reasonable and can be shown mathematically²⁴ that the pH corresponding to the midpoint of the change in the rate-determining step corresponds to the pH at which the yield of thiol ester is reduced to half of its maximum value.

The hydrolysis of thiazoline in acid solutions follows a Bunnett plot⁴¹ with a value of $w = 8.2$,^{29a} which is thought to be an indication of the presence of several water molecules in the transition state. Since the breakdown of I is rate determining under these conditions, the transition state contains at least one more water molecule than the starting material. If activity coefficient effects on the protonated substrate are not the primary cause of this large value of w , it may reflect solvation of the transition state(s) for the breakdown of I by several water molecules.

The interrelationships of the different steps of thiazoline hydrolysis and the S to N transfer reaction are summarized by the reaction profiles shown in Figure 7. The free energies of activation are calculated from the first-order rate constants for each step and the relative free energies of the different reactants from the equilibria at each pH value. At pH 0 the covalent steps for thiol ester and amide formation and breakdown present the largest energy barriers, the formation of the intermediate from thiazoline is easier, and the proton transfer step is not significant. At pH 5 the largest energy barrier is for the formation of intermediate from thiazoline. The next highest barrier is the proton transfer step, which determines the products formed in thiazoline hydrolysis and is the rate-determining step in the S to N transfer reaction. This is the first acyl transfer reaction for which a complete, quantitative energy profile of this kind has been constructed.

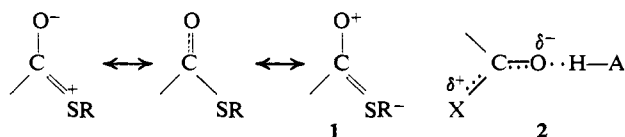
It is not yet clear how widespread this stepwise mechanism of diffusion-controlled proton transfer may be in general acid-base catalyzed reactions. The dependencies on pH and buffer concentration of the kinetics of the intermolecular reaction of thiol esters with hydroxylamine⁴² and of the products formed in the hydrolysis of acyclic thioimides under conditions in

(40) T. C. French and T. C. Bruice, *Biochemistry*, **3**, 1589 (1964).

(41) J. F. Bunnett, *J. Am. Chem. Soc.*, **83**, 4968 (1961).

(42) T. C. Bruice and L. R. Fedor, *ibid.*, **86**, 4886 (1964).

which there is little effect on the rate of hydrolysis establish that intermediates are formed in the course of these reactions.⁴³ The pH dependence of the product yield from thioimide hydrolysis may be interpreted in terms of anionic and neutral or neutral and cationic transition states for breakdown of the addition intermediate to the two products.⁴³ There is an indication that the yield of thiol ester product in some of these reactions levels off at a limiting value of less than 100% in acid solution; this is analogous to the partitioning of thiazoline to both products in acid solution and suggests that the same mechanism may apply in both the cyclic and acyclic reactions. Recently it has been shown^{9b} that there are at least two changes in product yields with changing pH, which implicates at least three transition states in the reaction, and that the value of β for general base catalysis of the step which gives thiol ester as product is 0.95. This value of β is similar to that for the observed catalytic constants in the intramolecular S to N acyl transfer reaction and suggests that a diffusion-controlled proton transfer step is also important in the corresponding intermolecular reaction. General acid catalysis of the methoxyaminolysis of phenyl acetate by carboxylic acids has been reported to exhibit an α value of zero,⁴⁴ which might reflect a rate-determining diffusion-controlled proton transfer. Caplow^{45b} has presented evidence for a diffusion-controlled proton transfer in carbamate breakdown. On the other hand, there are many reactions of acyl and carbonyl compounds which occur with Brønsted α and β values clearly different from 1.0 or 0 over a large range of acidity or basicity of the catalyst, so that it appears that these reactions do *not* involve only proton transfer in the rate-determining step. It is not yet apparent what factors determine whether the proton transfer step in this type of reaction is "concerted" or "coupled" with the making and breaking of bonds to carbon or occurs in a stepwise manner. The absence of general acid catalysis concerted with the attack of an amino group on a thiol ester may be related to the low basicity of the carbonyl group of thiol esters caused by the contribution of the resonance form 1;⁴⁵ this low basicity would decrease



the hydrogen bonding of a general acid to the carbonyl group (2) which presumably occurs at an early stage of a concerted reaction.

(43) R. K. Chaturvedi, A. E. MacMahon, and G. L. Schmir, *J. Am. Chem. Soc.*, **89**, 6984 (1967); G. L. Schmir, *ibid.*, **90**, 3478 (1968).

(44) (a) L. do Amaral, K. Koehler, D. Bartenbach, T. Pletcher, and E. H. Cordes, *ibid.*, **89**, 3537 (1967); (b) M. Caplow, *ibid.*, **90**, 6795 (1968).

(45) A. W. Baker and G. H. Harris, *ibid.*, **82**, 1923 (1960).

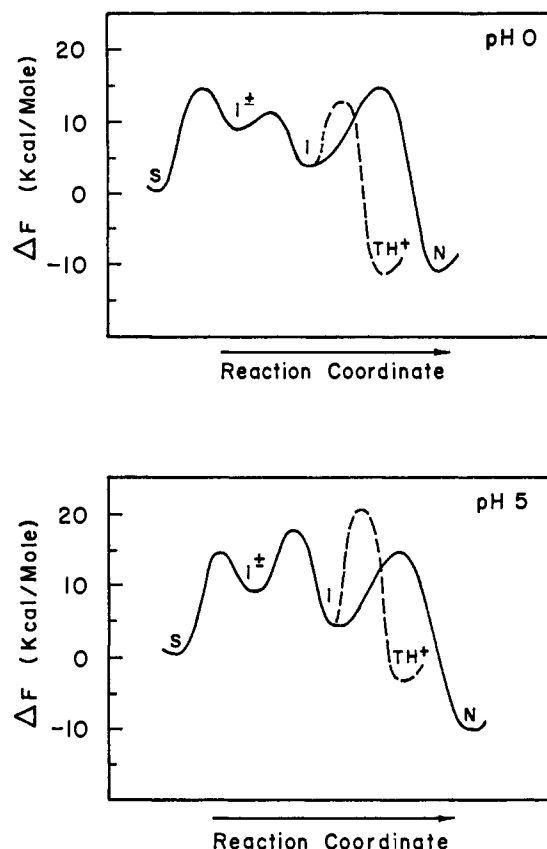


Figure 7. Free-energy reaction coordinate diagrams for the hydrolysis of 2-methyl- Δ^2 -thiazoline and the associated S to N transfer reaction at 50° and ionic strength 1.0 M. The symbols are defined in the text.

Two generalizations are supported by the results of the experiments described here. First, tetrahedral addition intermediates certainly exist in some acyl transfer reactions, but their lifetime is sufficiently short that they may decompose to starting materials or products at rates comparable to the rates of diffusion-controlled proton transfers. This is the case for the intermediate I^\pm , which decomposes to thiol ester unless it is trapped by protonation on its oxygen atom, and the intermediate I^+ , which decomposes to amide unless a proton is removed from it to give I^\pm . This suggests that intermediates in other reactions may have even shorter lifetimes and that some such reactions may be, in some sense, concerted. Second, diffusion-controlled proton transfer may be the rate-determining step of even very slow reactions, if this step is preceded by an unfavorable equilibrium step. The S to N acetyl transfer reaction has a half-time of about 1 hr under conditions in which diffusion-controlled protonation of I^\pm is rate determining, because this step is preceded by the unfavorable equilibrium steps for the conversion of S-acetylmercaptoethylammonium ion to the free amine and for the formation of the dipolar addition intermediate I^\pm from the free amine.