10f). In neither case does the area under the slow peak exceed that predicted for 100% aggregation. The hypothesis of interaction with toluene was apparently eliminated in an experiment on a preparation that had been stored unopened for 6 years under toluene. As is evident from Figure 10e, again there is no evidence of inert material under the slow peak. Thus, it would seem that, in some preparations of β-lactoglobulin, a trace amount of proteolytic enzyme has become introduced, with a resulting slow degradation of the protein and its inertness toward the low-temperature tetramerization.

As a final check on the validity of analysis of the previously published data[4] in terms of 10% nonaggregable β-A, these data were analyzed with the present stoichiometry. In all cases, the values of $k_1$, $k_2$, and $k_3$ reported in the present paper, along with an assumption that only 90% of β-A but all of β-B can participate in this reaction, were found to describe the data quantitatively, validating the conclusion that the earlier preparations of β-A were not completely homogeneous.

## Conclusion

The data and the analysis presented here establish a new, simpler, and more reasonable stoichiometry for the low-temperature tetramerization of β-A and β-B, as well as mixtures of the two lactoglobulins from isoelectric dimers to the low-temperature octamer. The old stoichiometry,[4,6] which consisted of constant bond strengths and certain proportions of unaggregable material, is hereby withdrawn as unrealistic. The new interpretation proposes bonds of three different strengths, namely, an A–A, a B–B, and an A–B bond, with all the two-chain molecules of β-A and β-B being capable of participating in the reaction. The difference in the free energies of these bonds is due to a difference in entropy which, in turn, may reflect the substitution of an aspartic acid residue in β-A for a glycine in β-B.[9] The full significance of the thermodynamic parameters reported here as well as previously will be discussed in a subsequent publication.

**Acknowledgment.** The authors wish to thank Dr. Robert Townend for many interesting discussions and valuable suggestions in the course of this work.

---

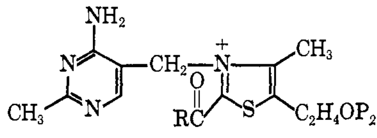
# Kinetics and Mechanism of the Hydrolysis of 2-Acetyl-3,4-dimethylthiazolium Ion[1]

**Gustav E. Lienhard**

*Contribution from the James Bryant Conant Laboratory, Harvard University, Cambridge, Massachusetts 02138.* *Received July 27, 1966*

**Abstract:** 2-Acetyl-3,4-dimethylthiazolium ion in aqueous solution undergoes rapid, general base catalyzed hydration to yield 2-(1,1-dihydroxyethyl)-3,4-dimethylthiazolium ion. At 25° at equilibrium there are approximately equal amounts of the unhydrated and hydrated compounds. 2-(1,1-Dihydroxyethyl)-3,4-dimethylthiazolium ion is stable in acid, but under more basic conditions cleaves to acetic acid and dimethylthiazolium ion in a reaction that occurs by specific hydroxide ion catalysis. Thiols and phosphate dianion, but not imidazole, appear to form carbonyl adducts with 2-acetyl-3,4-dimethylthiazolium ion. Transfer of the acetyl group from 2-acetyl-3,4-dimethylthiazolium ion to thiols, phosphate, and imidazole does not occur to any significant extent in water. The relevance of these findings to the role of thiamine in enzymatic reactions is discussed.

The 2-acylthiamine pyrophosphates (I) have been proposed as intermediates in several thiamine pyrophosphate-dependent enzymatic reactions. These include the phosphoketolase reaction, in which 2-acetylthiamine pyrophosphate may form from several hydroxymethyl ketone substrates and undergo phosphorolysis to yield acetyl phosphate,[2-4] and the reactions catalyzed by the pyruvate and α-ketoglutarate dehydrogenation complexes, in which 2-acetyl- and 2-succinylthiamine pyrophosphate or at least their corresponding hemithioketals are probably intermediates in the formation of S-acetyl- and S-succinyldihydrolipoic acid from 2-(1-hydroxyethyl)- and 2-(1-hydroxy-3-carboxypropyl)thiamine pyrophosphate and oxidized lipoic acid.[3,5,6] In addition, the oxidation of several 2-(1-hydroxyalkyl)thiamine pyrophosphate compounds by artificial electron acceptors such as 2,6-dichlorophenol, indophenol, and ferricyanide is catalyzed by phosphoketolase,[7] the pyruvate and α-ketoglutarate dehydrogenation complexes,[5,8] an acetoin-



I

---

[1] Supported by a grant (GB 4848) from the National Science Foundation.

[2] R. Breslow, *J. Cellular Comp. Physiol.*, **54** (Suppl 1), 100 (1959).

[3] F. G. White and L. L. Ingraham, *J. Am. Chem. Soc.*, **84**, 3109 (1962).

[4] M. L. Goldberg and E. Racker, *J. Biol. Chem.*, **237**, PC 3841 (1962).

[5] M. L. Das, M. Koike, and L. J. Reed, *Proc. Natl. Acad. Sci., U. S.*, **47**, 753 (1961).

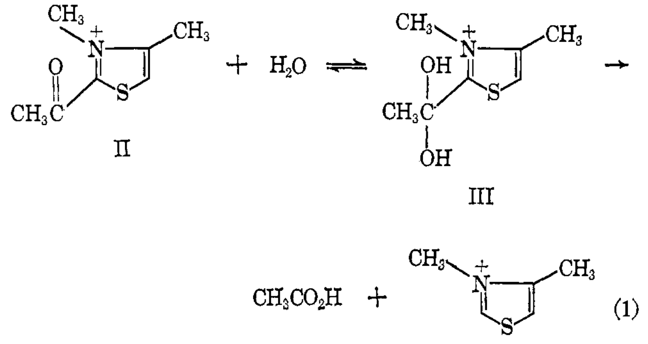
[6] D. R. Sanadi, *Enzymes*, **7**, 307 (1963).

[7] W. Schröter and H. Holzer. *Biochem. Biophys. Acta*, **77**, 474 (1963).

[8] F. da Fonseca-Wollheim, K. W. Bock, and H. Holzer, *Biophys. Biochem. Res. Commun.*, **9**, 466 (1962).

forming enzyme,[9] and pyruvate decarboxylase.[10,11] The products of the oxidations are the corresponding carboxylic acids and in some cases with high phosphate concentrations the acyl phosphates; these are presumably formed by the hydrolysis and phosphorolysis of 2-acylthiamine pyrophosphate intermediates.

Several studies with model compounds which show that the properties of 2-acylthiazolium salts are consistent with those postulated for the enzymatic reactions have been carried out. It has been found that 2-acetyl- and 2-benzoyl-3,4-dimethylthiazolium salts undergo rapid solvolysis in neutral water or methanol to yield thiazolium salt and the carboxylic acid or ester,[3,12,13] that 2-acetyl-3,4-dimethylthiazolium iodide will acetylate thiols in a solvent of low water content,[13] and that the heat of methanolysis of 2-benzoyl-3,4-dimethylthiazolium iodide indicates a large negative free energy of hydrolysis for this compound.[14] However, no detailed study of the kinetics and mechanism of the reaction of nucleophiles with 2-acylthiazolium salts has been reported. This paper describes the kinetics of the hydrolysis (eq 1) of 2-acetyl-3,4-dimethylthiazolium ion (II) and studies on the reactions of this compound with thiols, phosphate, and imidazole in water.



(1)

## Experimental Section

**Materials.** 4-Methylthiazole was prepared by the method of McNelis;[15] bp 130–132° (760 mm), lit. 133–134° (760 mm). 3,4-Dimethylthiazolium iodide was prepared from 4-methylthiazole and methyl iodide by the procedure of White and Ingraham;[8] mp 119–121°, lit. 117–118°[8] and 119–120°.[13]

2-(1-Hydroxyethyl)-4-methylthiazole was synthesized by the following procedure, which is based upon the method of Breslow and McNelis.[16] 4-Methylthiazole (33 g) was added with stirring to *n*-butyllithium (0.5 mole) in anhydrous hexane (310 ml)–ether (270 ml) at −75° under nitrogen over a period of 1 hr. Freshly distilled acetaldehyde (20 g) in ether (50 ml) was then added with stirring in 15-ml portions over the course of 1 hr. The reaction mixture was treated with water (50 ml), gradually warmed to room temperature, and adjusted to pH 4.5 by the addition of about 200 ml of 2.5 *N* sulfuric acid. The hexane–ether solution was separated; the aqueous solution was extracted with ether. The combined organic extracts were dried with sodium sulfate, and the hexane–ether was removed on a rotary evaporator. Distillation of the residue *in vacuo* yielded 30 g of 2-(1-hydroxyethyl)-4-methylthiazole, bp 95–97° (2 mm), $\lambda_{max}$ 248 m$\mu$ ($\epsilon$ 4200) in water; lit.[13] bp 91–93° (3 mm), $\lambda_{max}$ 247 m$\mu$ ($\epsilon$ 4410) in 95% ethanol; lit.[15] bp 105–107° (4.5 mm), $\lambda_{max}$ 247 m$\mu$ ($\epsilon$ 3900) in acetonitrile. 2-(1-Hydroxyethyl)-4-methylthiazole upon quaternization with methyl iodide[15] and treatment with silver chloride[17] yielded 2-(1-hydroxyethyl)-3,4-dimethylthiazolium chloride, mp 172–174° and $\lambda_{max}$ 255 m$\mu$ ($\epsilon$ 5600) in water; lit.[15] mp 171–172°.

2-Acetyl-4-methylthiazole, mp 33–35°, lit. 34–35°, was prepared from 2-(1-hydroxyethyl)-4-methylthiazole by the procedure of McNelis[15] and was converted with methyl iodide to 2-acetyl-3,4-dimethylthiazolium iodide by the method of Diago and Reed.[13] The 2-acetyl-3,4-dimethylthiazolium iodide had mp 138–139° and $\lambda_{max}$ 246 m$\mu$ ($\epsilon$ 15,900) and 292 (7700) in acetonitrile which had been dried by distillation from phosphorus pentoxide; lit.[13] mp 137–138°, $\lambda_{max}$ 246 m$\mu$ ($\epsilon$ 16,500) and 292 (6400) in acetonitrile. 2-Acetyl-3,4-dimethylthiazolium iodide was converted to the chloride by putting 0.5 ml of $2 \times 10^{-3}$ *M* solution of the iodide in $10^{-2}$ *N* HCl on a small column (0.2 ml wet resin) of Bio Rad AG1-X8, 200–400 mesh, Cl$^-$ form, which had been washed thoroughly with $10^{-2}$ *N* HCl, and eluting three times with 0.3 ml of $10^{-2}$ *N* HCl. Under these conditions 95% of the 2-acetyl-3,4-dimethylthiazolium ion and none of the iodide ion is present in the breakthrough and eluate fractions. This conversion was necessary for certain spectrophotometric measurements because iodide ion in aqueous solution absorbs strongly below 260 m$\mu$.[18]

**Kinetic and Equilibrium Measurements.** The rates of hydration of the 2-acetyl-3,4-dimethylthiazolium ion were measured by following the decreases in absorbance at 295 m$\mu$, using a Ziess PMQII spectrophotometer equipped with a thermostated cell holder. Reaction mixtures containing all component except the 2-acetyl-3,4-dimethylthiazolium iodide in a total volume of 2.92 ml were prepared in 3.5-ml, 1-cm cuvettes and temperature equilibrated. Reaction was initiated by the addition of 0.075 ml of a temperature-equilibrated solution of $4 \times 10^{-3}$ *M* 2-acetyl-3,4-dimethylthiazolium iodide in dry acetonitrile from a blowout pipet, followed by rapid mixing with a Teflon stirring rod. The first reading of absorbance was taken about 5 sec after the addition, and thereafter readings were taken at 2.5-sec intervals. Observed first-order rate constants were obtained from plots of log $(A_t - A_\infty)$ against time, where $A_t$ is the absorbance at time *t* and $A_\infty$ is the final absorbance, and the equation $k_{obsd} = 0.693/t_{0.5}$. Above about pH 6 recognizably stable end absorbances were not obtained in the hydration reaction. Instead, at the end of the reaction there was a relatively slow decrease in the absorbance, which was linear with time. This decrease represents the early stages of the cleavage of the hydrate, a reaction which even at the highest pH values at which the rates of hydration were measured was only 0.025 times as fast as hydration. Suitable correction was made for this slow decrease by plotting absorbance against time, extrapolating the final linear portion to zero time, and taking $A_t - A_\infty$ as equal to the difference between the measured absorbance and the extrapolated line. All the plots of log $(A_t - A_\infty)$ against time were linear for at least two half-times, and even with a half-time of 5 sec rate constants were reproducible within ±3% of the average. The extinction coefficient of unhydrated 2-acetyl-3,4-dimethylthiazolium iodide at 295 m$\mu$ in $10^{-2}$ *N* HCl at 5° was obtained by extrapolating of the plot of log $(A_t - A_\infty)$ against time to the moment at which the unhydrated compound in acetonitrile was added to the $10^{-2}$ *N* HCl. Five determinations gave values between 6800 and 7200 $M^{-1}$ cm$^{-1}$, with an average value of 6980 $M^{-1}$ cm$^{-1}$.

The pseudo-first-order rate constants for the cleavage of the hydrate were derived from spectrophotometric measurements at 295 m$\mu$ which were performed and analyzed in a way similar to that described above for the hydration reaction. The only significant change in procedure was that the cleavage reaction was generally initiated by addition to the remainder of the reaction mixture of an aliquot of a freshly prepared solution of 2-acetyl-3,4-dimethylthiazolium iodide (equilibrated with its hydrate) in $10^{-2}$ *N* HCl. This method of initiation was also used in measurements of the absorbance at 295 m$\mu$ after the equilibration of additions to the carbonyl group but before any cleavage had occurred. Below pH 5.5, the cleavage reaction is so slow that stable equilibrium absorbances were obtained in the studies of the addition of water, phosphate monoanion, and thiols. Above pH 5.5 the absorbances at equilibrium for the addition of water, imidazole, and phosphate dianion were assumed to be the absorbances given by extrapolation to zero time of the first-order plots for cleavage. For these and all other determination of extinction coefficients or ultraviolet spectra the contents of the blank in the spectrophotometer were identical

---

[9] L. O. Krampitz, I. Suzuki, and G. Greull, *Federation Proc.*, 20, 971 (1961).

[10] H. Holzer and R. M. M. Crawford, *Nature*, 188, 410 (1960).

[11] H. Holzer and K. Beaucamp, *Biochem. Biophys. Acta*, 46, 225 (1961).

[12] R. Breslow and E. McNelis, *J. Am. Chem. Soc.*, 82, 2394 (1960).

[13] K. Diago and L. J. Reed, *ibid.*, 84, 659 (1962).

[14] C. P. Nash, C. W. Olsen, F. G. White, and L. L. Ingraham, *ibid.*, 83, 4106 (1961).

[15] E. J. McNelis, Thesis, Columbia University, 1960.

[16] R. Breslow and E. McNelis, *J. Am. Chem. Soc.*, 81, 3080 (1959).

[17] G. D. Maier and D. E. Metzler, *ibid.*, 79, 4386 (1957).

[18] F. S. H. Head and H. A. Standing, *J. Chem. Soc.*, 1459 (1952).

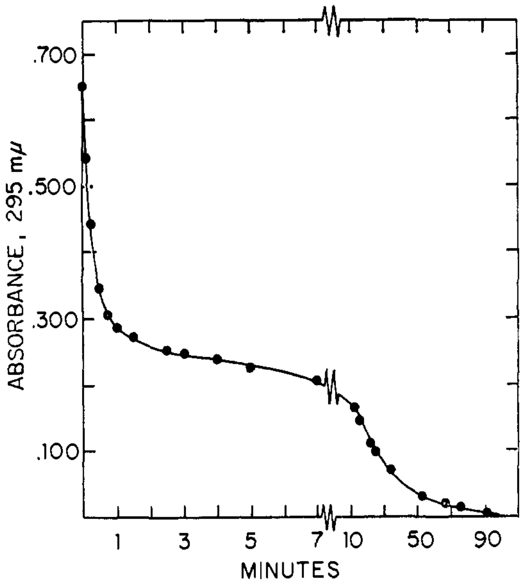


Figure 1. Disappearance of 2-acetyl-3,4-dimethylthiazolium iodide at 5°, 0.5 *M* ionic strength, and pH 7.18. The reaction was initiated by the rapid mixing (complete within 3 sec) of 0.10 ml of 0.003 *M* 2-acetyl-3,4-dimethylthiazolium iodide in acetonitrile with 2.9 ml of 0.005 *M* phosphate buffer–0.495 *M* KCl. Timing of the reaction was started at the moment of addition of the acetonitrile solution. The absorbance at zero time was obtained by extrapolation of the first-order plot for the initial reaction.

with that of the reaction mixture except for the omission of the compound whose absorbance was being measured.

Measurements of pH values were made at the end of the reactions at 25° with a Radiometer 25SE pH meter. When reactions were carried out at 5° in dilute phosphate buffer at 0.5 *M* ionic strength, the pH of the reaction mixture at 5° was calculated from measured values for $pK'_a$ of 0.05 *M* potassium dihydrogen phosphate in 0.5 *M* KCl at 5 and 25° and the pH at 25°. These calculated values are the ones given in this paper. Hydroxide ion activities were calculated using $K_w$ values[19] of $1.86 \times 10^{-15}$ at 5° and $10^{-14}$ at 25°. Deuteroxide ion activities were calculated from the "pH" values measured with the pH meter by use of the relationship,[20] pD = pH reading + 0.40, and the value[21] of $1.54 \times 10^{-15}$ for the ion product of $D_2O$ at 25°. The second-order rate constants for the reactions which are first-order with respect to hydroxide (deuteroxide) ion have been calculated by use of the hydroxide (deuteroxide) ion activities rather than concentrations.

The ionic strength was always adjusted with potassium chloride.

**Product Identification.** The ultraviolet spectrum of a solution that initially contained $10^{-4}$ *M* 2-acetyl-3,4-dimethylthiazolium iodide in 0.05 *M* potassium phosphate buffer–0.4 *M* KCl, pH 6.6, showed after complete disappearance of the absorbance at 295 mμ, a band with $\lambda_{max}$ 250 ($\epsilon$ 3500) when taken against $10^{-4}$ *M* potassium iodide in the same solvent. Under the same conditions the spectrum of authentic 3,4-dimethylthiazolium iodide taken against potassium iodide had $\lambda_{max}$ 250 ($\epsilon$ 3700).

## Results

**Products of the Reaction of 2-Acetyl-3,4-dimethylthiazolium Ion with Water.** 2-Acetyl-3,4-dimethylthiazolium salts have an ultraviolet absorption maximum near 295 mμ, which is not present in simple thiazolium salts and which results from the carbonyl group conjugated with the thiazolium ring.[13,15] Upon the addition of 2-acetyl-3,4-dimethylthiazolium iodide in acetonitrile to water there is a rapid decrease in the absorbance of the 295-mμ band to an intermediate value, a decrease which is followed by the slower complete disappearance of the band. Figure 1 shows an example of this behavior at pH 7.18. The initial rapid decrease in absorbance is attributed to the partial hydration of 2-acetyl-3,4-dimethylthiazolium ion to its hydrate, 2-(1,1-dihydroxyethyl)-3,4-dimethylthiazolium ion (III), while the slower, complete disappearance of the band is the result of the cleavage of this hydrate to acetate and the 3,4-dimethylthiazolium ion (eq 1). The identification of the final products of the reactions as acetate and the 3,4-dimethylthiazolium ion is based upon the observation that the ultraviolet spectrum of the product is identical with that of the 3,4-dimethylthiazolium ion (see Experimental Section) and upon the finding of Diago and Reed[13] that acetic acid is formed in nearly theoretical yield upon dissolution of 2-acetyl-3,4-dimethylthiazolium iodide in phosphate buffer, pH 7.5.

The designation of the initial reaction as partial hydration is based upon the following considerations. (1) Below about pH 4 the hydrolysis of the 2-acetyl-3,4-dimethylthiazolium ion to acetic acid and 3,4-dimethylthiazolium ion occurs very slowly, whereas the initial decrease in absorbance at 295 mμ is still rapid (see the kinetic studies). Consequently, the ultraviolet spectrum of reaction mixtures after the initial reaction could be obtained in acidic solution without the complications of the hydrolytic cleavage. Figure 2 shows the ultraviolet spectrum of an acidic solution of 2-acetyl-3,4-dimethylthiazolium chloride at 45° (solid circles) and 5° (open circles), recorded after the initial equilibrium had been established. Upon a decrease in temperature there is a decrease in the absorbance of the 295-mμ band, with the ratio of the absorbance at 5° to that at 45° approximately constant for wavelengths longer than 285 mμ. Also at the lower temperature there is an increase in the absorbance near 250 mμ. These findings are consistent with partial hydration of the carbonyl group to form the 2-(1,1-dihydroxyethyl)-3,4-dimethylthiazolium ion, since the extent of hydration of ketones in water increases as the temperature is lowered[22] and since unconjugated thiazolium ions have an absorption maximum near 250 mμ and do not absorb significantly at wavelengths longer than 285 mμ.[15]

The spectrum of the product of the initial reaction can, in fact, be calculated from the composite spectra at 5 and 45°. The equation which describes the equilibrium constant for the formation of the product (hydrate) is

$$K_{\text{hyd}} = (\text{hydrate})/(\text{carbonyl}) = \frac{\epsilon_{\text{C=O},295} - \epsilon'_{295}}{\epsilon'_{295}} \qquad (2)$$

where $\epsilon_{\text{C=O},295}$ is the extinction coefficient at 295 mμ of the unhydrated 2-acetyl-3,4-dimethylthiazolium ion in water and $\epsilon'_{295}$ is an apparent extinction coefficient which is obtained by dividing the equilibrium absorbance at 295 mμ (1-cm path) by the total concentration (hydrated and unhydrated) of 2-acetyl-3,4-dimethylthiazolium ion. In addition, at any wavelength and temperature the measured absorbance (*A*) is equal to the extinction coefficient of the carbonyl compound at that wavelength ($\epsilon_{\text{C=O}}$) times its concentration (*C*)

---

(19) V. Gold, "pH Measurements," Methuen and Co., London, 1956, p 120.

(20) P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).

(21) R. W. Kingerley and V. K. LaMer, *J. Am. Chem. Soc.*, **63**, 3256 (1941).

(22) R. P. Bell and A. O. McDougall, *Trans. Faraday Soc.*, **56**, 1281 (1960).

plus the extinction coefficient of the hydrate at that wavelength ($\epsilon_{C(OH)_2}$) times its concentration ($H$).

$$A = \epsilon_{C=O}C + \epsilon_{C(OH_2)}H \qquad (3)$$

From these equations the following expression for $\epsilon_{C(OH)_2}$ can be derived.

$$\epsilon_{C(OH)_2}(C + H) = \frac{A_{5°}\epsilon'_{295,45°} - A_{45°}\epsilon'_{295,5°}}{\epsilon'_{295,45°} - \epsilon'_{295,5°}} \qquad (4)$$

The spectrum of the initial reaction product calculated with eq 4 shows $\lambda_{max}$ 255–256 m$\mu$ ($\epsilon$ 6000) (Figure 2, ▲), which is similar to $\lambda_{max}$ 255 m$\mu$ ($\epsilon$ 5600) found for the related 2-(1-hydroxyethyl)-3,4-dimethylthiazolium ion (see Experimental Section). The derivation of eq 4 assumes that the extinction coefficients of the pure compounds in water at each wavelength, $\epsilon_{C=O}$ and $\epsilon_{C(OH)_2}$, are temperature independent between 5 and 45°. This assumption seems legitimate, since the values of $\epsilon$ for the 2-(1-hydroxyethyl)-3,4-dimethylthiazolium ion at the wavelengths between 230 and 270 m$\mu$ were found to change by less than 11% on going from 5 to 45°.

(2) The extent of initial, rapid disappearance of 2-acetyl-3,4-dimethylthiazolium ion upon solution in water, as measured by the absorbance at 295 m$\mu$ before any cleavage had occurred, was determined over the pH range 2.1 to 6.8 (Table I). The equilibrium is pH independent, a property which is also consistent with hydration. Table I summarizes the equilibrium constants for hydration (eq 2) under a number of different conditions.

Table I. Equilibrium Constants for the Hydration of the 2-Acetyl-3,4-dimethylthiazolium Ion

| Solvent | pH | Temp, °C | Apparent extinction coefficient at 295 m$\mu$, $\epsilon'_{295}$, $M^{-1}$ cm$^{-1}$ | $K_{hyd}$[a] |
|---|---|---|---|---|
| 0.01 $N$ HCl–0.49 $N$ KCl | 2.1 | 25 | 3800 | 0.83 |
| 0.025 $N$ acetate buffer–0.49 $N$ KCl | 4.6 | 25 | 3880 | 0.79 |
| 0.025 $M$ phosphate buffer–0.45 $N$ KCl | 6.8 | 25 | 3800 | 0.83 |
| 0.002 $N$ HCl–0.5 $N$ KCl | | 5 | 2750 | 1.53 |
| 0.01 $N$ HCl | | 5 | 2700 | 1.58 |
| 0.01 $N$ HCl | | 45 | 4980 | 0.40 |
| 0.01 $N$ HCl–0.49 $N$ KCl in $D_2O$ | | 25 | 3770 | 0.85 |

[a] Calculated from eq 2 in the text, using the value of 6980 $M^{-1}$ cm$^{-1}$ for $\epsilon_{C=O,295}$.

(3) At 25° in aqueous solution, 1,1-dichloroacetone is approximately 75% in the form of the carbonyl hydrate and chloroacetone is about 40% in the form of the hydrate.[22] Since the inductive effect of the $(CH_3)_3\overset{+}{N}CH_2$ group ($\sigma^*$ 1.90) lies between that of the $Cl_2CH$ ($\sigma^*$ 1.94) and $ClCH_2$ ($\sigma^*$ 1.05) groups,[23] partial hydration of the 2-acetyl-3,4-dimethylthiazolium ion is, by analogy, reasonable.

Since nucleophiles are known to add to the 2 position of thiazolium compounds,[17] it is possible that the product of the initial rapid reaction is IV or V, rather than the carbonyl hydrate.

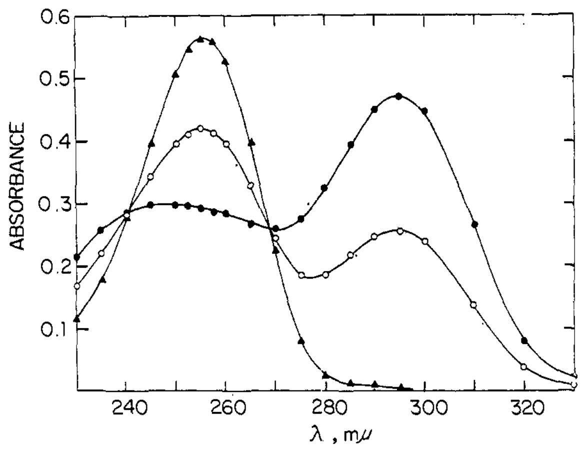
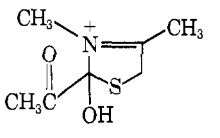
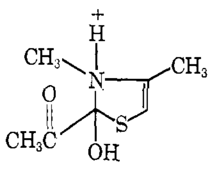


Figure 2. Spectrum of 2-acetyl-3,4-dimethylthiazolium chloride and its carbonyl hydrate at 5 (○) and 45° (●) and of the hydrate alone (▲). The spectra of 2-acetyl-3,4-dimethylthiazolium chloride and its hydrate were obtained by measuring the absorbances of a single solution of 9.5 × $10^{-4}$ $M$ compound in 0.01 $N$ HCl at 5, 45, and then again at 5° over a period of 2 hr, against a blank of 0.01 $N$ HCl. The second spectrum at 5° was identical with the first. The spectrum of the hydrate alone was calculated by the procedure described in the text.



IV



V

Structure IV seems unlikely because simple tertiary immonium salts have ultraviolet absorption maxima in the region 220–230 m$\mu$.[24] Structure V would be expected to have a p$K$ of considerably less than 6.3, the p$K$ of thiazolidine,[25] because the amine function is $\alpha$ to a hydroxyl group and a double bond, each of which are known to lower the p$K$ of an amine relative to the saturated analog by about two units.[26,27] It is therefore inconsistent with the equilibrium being pH independent below pH 7.

**Rates of Hydration and Cleavage of the 2-Acetyl-3,4-dimethylthiazolium Ion.** The rates of hydration of 2-acetyl-3,4-dimethylthiazolium ion and of the cleavage of the hydrate intermediate 2-(1,1-dihydroxyethyl)-3,4-dimethylthiazolium ion were measured by the successive changes in absorbance at 295 m$\mu$. The two rates could easily be separated, since under all the conditions of temperature, pH, and buffer concentration that were used, the observed rate constant for the hydration reaction was at least 30 times greater than that for the cleavage. The pH–rate profile for the establishment of the hydration equilibrium at 5° is shown in Figure 3 (solid circles). These rate constants were obtained at buffer concentrations sufficiently low so that general base catalysis was negligible (see Figure 4 and legend of Figure 3). It is evident that

---

[23] R. W. Taft, Jr., in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, p 556.

[24] G. Opitz, H. Hellmann, and H. W. Schubert, *Ann. Chem.*, 623, 117 (1959).

[25] S. Ratner and H. T. Clarke, *J. Am. Chem. Soc.*, 59, 200 (1937).

[26] R. Kallen and W. P. Jencks, personal communication.

[27] E. J. Stamhuis, W. Maas, and H. Wynberg, *J. Org. Chem.*, 30, 2160 (1965).

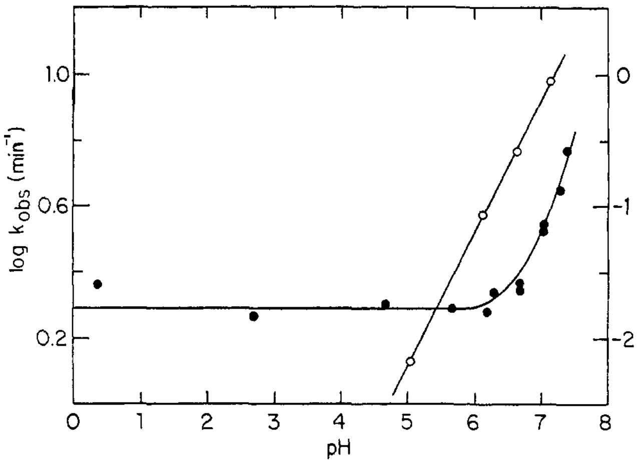


Figure 3. pH-rate profiles for the hydration of the 2-acetyl-3,4-dimethylthiazolium ion at 5° and 0.5 $M$ ionic strength (●, left ordinate) and for the cleavage of this hydrate at 25° and 0.5 $M$ ionic strength (○, right ordinate). The buffers used to study the hydration were: pH 0–3, HCl; pH 3–5, 0.002 $M$ potassium acetate; pH 5–8, 0.002–0.008 $M$ potassium phosphate. At constant pH the rate of hydration did not change over this range of concentration in phosphate buffer. The buffers used to study the cleavage were: pH 5.1, 0.005 $M$ potassium acetate; pH 6–8, 0.05 $M$ potassium phosphate. All reaction mixtures contained 2.5% acetonitrile. The lines are calculated from the derived rate constants.

the hydration reaction occurs by pH-independent and hydroxide ion catalyzed pathways, with no significant hydronium ion catalysis. The rate law is

$$-\frac{dC}{dt} = +\frac{dH}{dt} = k_f C - k_r H + k_{2f}(\mathrm{OH}^-)C - k_{2r}(\mathrm{OH}^-)H \quad (5)$$

in which the subscripts f and r designate hydration and dehydration, respectively. The values for the rate constants in eq 5, derived from the data in Figure 3 and that in a similar pH–rate profile at 25°, are given in Table II. The curve in Figure 3, calculated using these derived constants, shows satisfactory agreement with the experimental points.

**Table II.** Rate Constants for the Hydration of the 2-Acetyl-3,4-dimethylthiazolium Ion at 0.5 $M$ Ionic Strength

| Catalyst | Temp, °C | $k_f$[a] | $k_r$[b] |
|---|---|---|---|
| pH-independent (water)[c] | 5 | 1.17 min$^{-1}$ | 0.77 min$^{-1}$ |
| | 25 | 3.7 | 4.5 |
| Hydroxide ion[d] | 5 | 4.65 × 10$^7$ $M^{-1}$ min$^{-1}$ | 3.05 × 10$^7$ $M^{-1}$ min$^{-1}$ |
| | 25 | 8.9 × 10$^7$[e] | 10.7 × 10$^7$[e] |
| Acetate ion[f] | 5 | 4.1 | 2.7 |
| Pyridine[f] | 5 | 3.4 | 2.4 |

[a] For hydration. [b] For dehydration. [c] These rate constants were calculated from the average values of $k_{\mathrm{obsd}}$ in the pH-independent region of the pH–rate profile (Figure 3), the values for $K_{\mathrm{hyd}}$ given in Table II, and the equations $k_f = k_{\mathrm{obsd}}K_{\mathrm{hyd}}/(1 + K_{\mathrm{hyd}})$ and $k_r = k_{\mathrm{obsd}} - k_f$. [d] These rate constants were obtained from the slopes of the plots of $k_{\mathrm{obsd}}$ against hydroxide ion activity and the equations $k_{2f}$ = slope × $K_{\mathrm{hyd}}/1 + K_{\mathrm{hyd}}$ and $k_{2r}$ = slope − $k_{2f}$. [e] These values are only estimates because they were derived from fast reaction rates ($t_{0.5}$ 2.5 to 5 sec). [f] These constants were obtained from the data in Figure 4 by the method used for hydroxide ion.

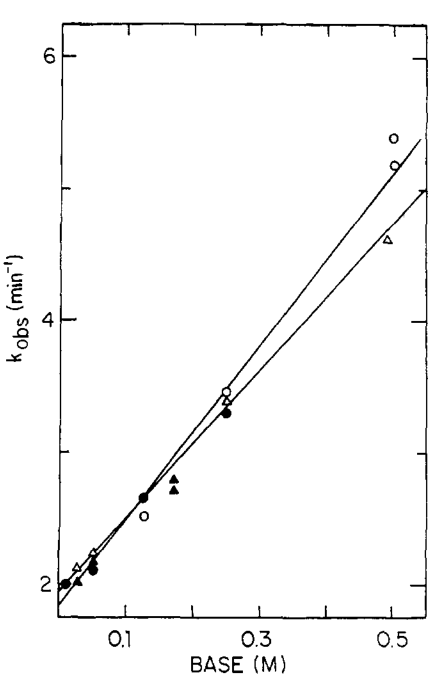


Figure 4. Catalysis of the hydration of the 2-acetyl-3,4-dimethylthiazolium ion by acetate ion and pyridine, at 5° and 0.5 $M$ ionic strength. The buffer ratios were acetic acid–potassium acetate, 3:1 (●) and 1:3 (○); pyridine hydrochloride–pyridine, 3:1 (▲) and 1:1 (△).

The hydration reaction is also catalyzed by buffers. Figure 4 shows such catalysis by acetate and pyridine buffers, measured in the pH range where hydroxide ion catalysis is negligible. The fact that the plots of $k_{\mathrm{obsd}}$ against the concentration of the basic species describe the same straight line regardless of the buffer ratio demonstrates that only the basic component is catalytically active. The rate constants for general base catalysis by acetate ion and pyridine are given in Table II. The apparent equilibrium constants for the hydration of the 2-acetyl-3,4-dimethylthiazolium ion at 5° and 0.5 $M$ ionic strength in 1 $M$ potassium acetate buffer, 75% acid, and in 1 $M$ pyridine hydrochloride buffer, 50% acid, differed by less than 10% from $K_{\mathrm{hyd}}$ under the same conditions in dilute hydrochloric acid buffer. This finding indicates that neither acetic acid nor pyridine add to any extent to the carbonyl group.

The logarithms of the observed rate constants at 25° in dilute buffers for the cleavage of the hydrate to acetic acid and 3,4-dimethylthiazolium ion are plotted against pH in Figure 3 (open circles). Over the pH range from 5 to 7.5 the plot is a straight line with a slope of +1, and thus in this pH range the cleavage is catalyzed by hydroxide ion. The rate law is

$$-\mathrm{d}(H+C)/\mathrm{d}t = k_2(\mathrm{OH}^-)H = k_{\mathrm{obsd}}H(K_{\mathrm{hyd}} + 1)/K_{\mathrm{hyd}} \quad (6)$$

and the average values of $k_2$, calculated with this relationship between $k_{\mathrm{obsd}}$ and $k_2$ from the data in Figure 3 and similar data obtained at 5°, are 13.6 × 10$^6$ $M^{-1}$ min$^{-1}$ at 25° and 0.5 $M$ ionic strength and 2.3 × 10$^6$ $M^{-1}$ min$^{-1}$ at 5° and 0.5 $M$ ionic strength. The rate of cleavage was also examined at pH 1.1 and 2.1; at these pH values only ~5% of the 2-acetyl-3,4-dimethylthiazolium ion and its hydrate disappeared in

**Table III.** Reaction of Nucleophiles with 2-Acetyl-3,4-dimethylthiazolium Ion (25°, 0.5 $M$ Ionic Strength)

| Nucleophile | Concn, $M$ | pH | $\epsilon'_{295}$,[a] $M^{-1}$ cm$^{-1}$ | $K_{add}$,[b] $M^{-1}$ | $k_{obsd}$,[c] min$^{-1}$ | $k_{calcd}$,[d] min$^{-1}$ |
|---|---|---|---|---|---|---|
| $KH_2PO_4$–$K_2HPO_4$ | | | | | | |
| 100% monoanion | 0 | 4.5[e] | 3880 | | | |
| | 0.5 | 4.3 | 3620 | <0.5 | | |
| 40% monoanion | 0.025 | 6.75 | 3800 | | | |
| | 0.20 | 6.9 | 3300 | | | |
| | 0.20 | 6.9 | 3300 | 2.7[f] | | |
| 50% monoanion | 0.05 | 6.63 | | | 0.26 | 0.25 |
| | 0.15 | 6.69 | | | 0.31 | 0.27 |
| 25% monoanion | 0.05 | 7.14 | | | 0.88 | 0.81 |
| | 0.15 | 7.25 | | | 1.15 | 0.95 |
| Imidazole HCl | 0.075 | 6.92 | 3800 | | 0.50 | 0.52 |
| 38% free base | 0.80 | 6.85 | 3550 | | 0.55 | 0.44 |
| | 0.80 | 6.85 | 3550 | <0.5 | 0.55 | 0.44 |
| $CH_3O_2CCH_2SH$[i] | 0 | 1.5[g] | 4040 | | | |
| | 0.125 | 1.5[g] | 3800 | | | |
| | 0.25 | 1.5[g] | 3460 | 1.1 | | |
| | 0 | 5.70[h] | | | 0.030 | 0.031 |
| | 0.25 | 5.68[h] | | | 0.023 | 0.026 |
| $^{-}O_2CCH_2SH$ | 0 | 5.1[e] | 3880 | | | |
| | 0.25 | 5.5 | 2625 | | | |
| | 0.50 | 5.5 | 2060 | 3.4 | | |
| | 0 | 6.55[h] | | | 0.20 | 0.22 |
| | 0.25 | 6.55[h] | | | 0.14 | 0.19 |
| $CH_3SH$ | 0 | 1.5[g] | 3800 | | | |
| | 0.12 | 1.5[g] | 3140 | 3.2 | | |

[a] Apparent extinction coefficients at 295 m$\mu$ after equilibration of addition but before any cleavage has occurred (see Experimental Section). [b] $K_{add} = (\text{adduct})/CN = (\epsilon'_{0,295} - \epsilon'_{295})(K_{hyd} + 1)/\epsilon'_{295}N$, where $\epsilon'_{0,295}$ is the apparent extinction coefficient in the absence of nucleophile and $N$ is the concentration of nucleophile. [c] For cleavage of the hydrate. [d] The value expected if cleavage occurs only by the hydroxide ion catalyzed cleavage of the hydrate, calculated from the expression $k_{calcd} = k_2(OH^-)K_{hyd}/(1 + K_{hyd} + K_{add})N$, using the values for $k_2$, $K_{hyd}$, and $K_{add}$ given in the text, Table I, and this table, respectively. [e] With 0.025 $M$ potassium acetate buffer. [f] For the dianion, assuming monoanion does not react. [g] With HCl buffer. [h] With 0.1 $M$ potassium phosphate buffer. [i] All reaction mixtures for the study of thiols contained $10^{-4}$ $M$ ethylenediaminetetraacetic acid.

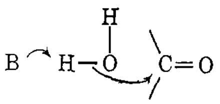
13 hr at 25°. Because of the slowness of the reaction, no further measurements were made at low pH. The deuterium-isotope effect upon the cleavage reaction was determined by measuring the rates in $D_2O$ with 0.05 $M$ potassium phosphate buffer, pD 7.13 and 7.62, at 25° and 0.5 $M$ ionic strength. The values of the observed rate constants were directly proportional to the deuteroxide ion activity, and from them a value of 25.2 $\times$ $10^6$ $M^{-1}$ min$^{-1}$ for $k_2$ in $D_2O$ was calculated. Thus, the deuterium isotope effect $k_2^D/k_2^H$ is 1.85.

**Reactions of Phosphate, Imidazole, and Thiols with the 2-Acetyl-3,4-dimethylthiazolium Ion.** In order to determine the extent of addition of phosphate anions, imidazole, and thiols to the carbonyl group of the 2-acetyl-3,4-dimethylthiazolium ion, the apparent extinction coefficients at 295 m$\mu$ of the ion in the presence of these compounds before the occurrence of any cleavage but after equilibration of the addition to the carbonyl group ($\epsilon'_{295}$, see Experimenatl Section), were obtained (Table III). The apparent extinction coefficients in the presence of 0.5 $M$ phosphate monoanion and 0.3 $M$ imidazole are only slightly less than that in their absence, a finding that shows that in water these compounds add very slightly, if at all. On the other hand, in the presence of phosphate dianion, methyl mercaptoacetate, potassium mercaptoacetate, and methanethiol, the apparent extinction coefficients are significantly lower, a fact which suggests that these compounds, like water, add to the carbonyl group. The equilibrium constants for these additions are given in Table III. The rates of these additions were not measured, but under the conditions given in Table III the reactions were complete in about 15 sec.

Two types of evidence indicated that acetyl transfer from the 2-acetyl-3,4-dimethylthiazolium ion to thiols, phosphate dianion, and imidazole does not occur to any significant extent in water. First, when the observed rate constants for the cleavage of the ion in the presence of these nucleophiles are compared with those expected at the same pH values solely on the basis of hydroxide ion catalyzed cleavage of the hydrate (Table III), it is seen that there were no accelerations with the thiols and only small accelerations (about 20%) with phosphate and imidazole. Second, attempts to detect the formation of a small amount of acetyl phosphate and thiol ester were unsuccessful. Thus, when 0.013 $M$ 2-acetyl-3,4-dimethylthiazolium iodide was allowed to undergo cleavage in the presence of 0.8 $M$ potassium phosphate buffer, pH 7.3, less than 5 $\times$ $10^{-4}$ $M$ acetyl phosphate could be detected with a hydroxylamine–ferric chloride test.[28,29] When the cleavage of a 0.011 $M$ solution was carried out in the presence of 0.1 $M$ ethanethiol at pH 7.2 and the reaction mixture extracted with $n$-hexane, examination of the ultraviolet spectrum of the hexane extract in the region of thiol ester absorption (220–250 m$\mu$), after removal of the ethanethiol by distillation, showed that less than 5 $\times$ $10^{-4}$ $M$ ethyl thioacetate had formed. Appropriate control experiments with authentic acetyl phosphate and ethyl thioacetate demonstrated that 5 $\times$ $10^{-4}$ $M$ acetyl derivatives would easily have been detected.

## Discussion

**Mechanisms of the Hydration and Carbon–Carbon Bond Cleavage Reactions.** A reasonable mechanism for the hydration of 2-acetyl-3,4-dimethylthiazolium ion is



(7)

where B is water, hydroxide ion, or the basic component of a buffer such as acetate ion or pyridine. This mechanism has been proposed previously to account for the general base catalyzed hydration of carbonyl compounds,[30] and the arguments in its favor have been considered in detail.[31] It is noteworthy that there is no significant catalysis of the hydration reaction by acids, although in general the hydration of ketones is strongly catalyzed by acids.[32–34] The explanation for the absence of acid catalysis must be that the positively charged thiazolium group lowers the basicity of the carbonyl oxygen to such an extent that the transition

---

(28) F. Lipmann and L. C. Tuttle, *J. Biol. Chem.*, **159**, 21 (1945).

(29) S. Hestrin, *ibid.*, **180**, 249 (1949).

(30) R. P. Bell, M. H. Rand, and K. M. A. Wynne-Jones, *Trans. Faraday Soc.*, **52**, 1093 (1956).

(31) W. P. Jencks, *Progr. Phys. Org. Chem.*, **2**, 63 (1964).

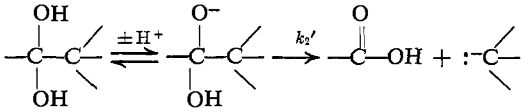
(32) M. Cohn and H. C. Urey, *J. Am. Chem. Soc.*, **60**, 679 (1938).

(33) R. P. Bell, and M. B. Jensen, *Proc. Roy. Soc.* (London), **A261**, 38 (1961).

(34) M. Byrn and M. Calvin, *J. Am. Chem. Soc.*, **88**, 1916 (1966).

states for acid catalysis, which would involve partial or complete protonation of this oxygen, are of too high energy for acid catalysis to contribute significantly to the rate.

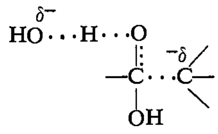
The cleavage of the carbon–carbon bond in the hydrate probably occurs by a mechanism in which the rate-determining step is the unimolecular decomposition of the alcoholate anion which is derived from and in equilibrium with the hydrate (eq 8). On the basis of this mechanism the second-order rate constant for hydroxide ion catalyzed cleavage of the carbon–carbon bond, $k_2$, is equal to $k_2'K_a/K_w$, where $K_a$ is the acid dissociation constant of the hydrate and $K_w$ is the ion product of water. The value of $K_a$ can be estimated as about $10^{-12}$ from a plot of p$K_a$ for a number of alcohols[35,36] against the sum of the polar substituents constants $(\Sigma\sigma^*)$[23] for the substituents bonded to the alcoholic carbon; in this treatment the value of $\sigma^*$ for the 3,4-dimethylthiazolium group was taken as that for the $(CH_3)_3N^+CH_2$ group. Thus at 25° $k_2'$ is about $13.6 \times 10^4$ $M^{-1}$ min$^{-1}$. The deuterium isotope effect for this mechanism is described by



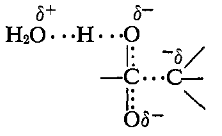
(8)

$$k_2^D/k_2^H = k'^D_2K_a^DK_w^H/k'^H_2K_a^HK_w^D \qquad (9)$$

Application of the method of Bunton and Shiner[37] for the estimation of secondary solvent isotope effects yields a value of approximately 1.5 for the ratio $k'^D_2/k'^H_2$. The ratio $K_a^D/K_a^H$ can be taken as approximately 0.22, which is the value for 2,2,2-trifluoroethanol[35] (p$K$ = 12.4), and $K_w^H/K_w^D$ is 6.5. Consequently, the predicted deuterium isotope effect is 2.1. The observed value of 1.85 is close to this predicted value and is thus consistent with the proposed mechanism (eq 8). Two alternative transition states for cleavage are not excluded by the kinetic data; in one hydroxide ion removes the proton from the hydroxyl group simultaneously with carbon–carbon bond cleavage (VI) and in the other water acts as a general base to assist in the expulsion of the thiazolium zwitterion from the alcoholate anion (VII).



VI

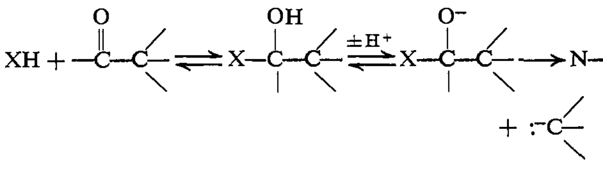


VII

These mechanisms seem less likely because reactions that occur in $H_2O$ with proton transfer in the rate-determining step have generally been found to be faster than the corresponding reactions in $D_2O$ with deuteron transfer[38,39] and because there is no significant general base catalysis of the carbon–carbon bond cleavage by phosphate dianion or imidazole (Table III).

It is interesting to compare these findings about the kinetics and mechanism of the hydrolysis of the 2-acetyl-3,4-dimethylthiazolium ion to those for a similar reaction, the hydrolysis of diethyl acetylmalonate, $CH_3C(=O)CH(COOC_2H_5)_2$, to acetic acid and diethyl malonate. Lienhard and Jencks[40] have shown that hydration of this compound and the cleavage of the carbon–carbon bond of its hydrate occur by the same mechanisms of hydroxide ion catalysis as presented here for the 2-acetyl-3,4-dimethylthiazolium ion. However, the ratio of the rate constant for hydroxide ion-catalyzed dehydration of the hydrate to that for the cleavage of the carbon–carbon bond is $1.4 \times 10^{-8}$ for the diethyl acetylmalonate hydrate whereas it is about 10 for the hydrated 2-acetyl-3,4-dimethylthiazolium ion. These ratios are a measure of the rate of expulsion of hydroxide ion from the hydrate anion relative to the rate of expulsion of the carbanion from the hydrate anion. The difference between the two values can be accounted for by the fact that diethyl malonate anion is a weaker base (p$K$ of diethyl malonate, 15.2)[41] and thus a better leaving group than hydroxide ion (p$K$ of water, 15.7), whereas the thiazolium zwitterion is a stronger base (upper limit for the p$K$ for dissociation of the C-2 hydrogen of 3,4-dimethylthiazolium ion, 20.5)[15] and thus a poorer leaving group than hydroxide ion.

Originally one goal of this work was to study the kinetics, mechanisms, and equilibria in water of the transfer of the acetyl group of the 2-acetyl-3,4-dimethylthiazolium ion to thiols, phosphates, and imidazole. These reactions would be of more relevance than hydrolysis as models for the enzymatic reactions in which 2-acylthiamine pyrophosphate may participate. The results of this study demonstrate that in water the rates of acetyl transfer to thiols, phosphate, and imidazole are not significant compared to the rate of hydrolysis, although thiols and phosphate dianion do appear to add to some extent to the carbonyl group of 2-acetyl-3,4-dimethylthiazolium ion. The explanation for the lack of reactivity of these nucleophiles involves a consideration of the equilibrium position for formation of the adduct, the equilibrium position for ionization of the adduct, and the rate of expulsion of the thiazolium zwitterion from the ionized adduct relative to those for the water reaction (eq 10). The concentrations of the thiol (mercaptoacetate), phosphate dianion, and imidazole adducts relative to that of the hydrate can be calculated from the equilibrium constants in Tables I and III and were 1.0, 1.7, and <0.18, respectively, at the highest concentrations of these nucleophiles at which acetyl transfer was examined. Thus, at least with the thiols and phosphate dianion, the lack of acetyl transfer must be the result of a higher ioni-



(10)

XH ≡ $H_2O$, RSH, $HPO_4^{2-}$, imidazole

---

[35] P. Ballinger and F. A. Long, *J. Am. Chem. Soc.*, **82**, 795 (1960).

[36] R. P. Bell and P. T. McTigue, *J. Chem. Soc.*, 2983 (1960).

[37] C. A. Bunton and V. J. Shiner, Jr., *J. Am. Chem. Soc.*, **83**, 3207 (1961).

[38] M. L. Bender, E. J. Pollock, and M. C. Neveu, *ibid.*, **84**, 595 (1962).

[39] Several exceptions to this generalization have been found, however; see G. E. Lienhard and W. P. Jencks, *ibid.*, **88**, 3982 (1966), and references therein.

[40] G. E. Lienhard and W. P. Jencks, *ibid.*, **87**, 3855 (1965).

[41] C. Vermesse-Jacquinot, R. Schaal, and P. Rumpf, *Bull. Soc. Chim. France*, 2030 (1960).

zation constant for the hydroxyl group of the adduct and/or a lower rate of expulsion of the thiazolium zwitterion from the ionized adduct. Since the inductive effect of the HO group and the RS group are the same[42] and since the thiazolium group, which is common to all the adducts, probably has a major effect in determining their acidities, the p$K$ values of hemithioketal and the hydrate may be estimated as about the same. Consequently, the expulsion of the thiazolium zwitterion from the ionized hemithioketal is probably slower than that from the ionized hydrate. In fact, on the basis of these considerations and of the observation of Diago and Reed[18] that the reaction of 2-acetyl-3,4-dimethylthiazolium iodide with 0.1 $M$ butanethiol and 1.1 $M$ $H_2O$ in dimethoxyethane under basic conditions yields 35% thiol ester, the ratio of the rate of cleavage of the ionized hemithioketal to that of the ionized hydrate can be estimated as 0.045. This result is expected because the less basic RS group (p$K$ of RSH, 8–10) should be less able to aid in the cleavage by electron donation than the HO group (p$K$ of $H_2O$, 15.7).

Ohishi and Fukui[43] have reported that ultraviolet irradiation of a dilute solution of 2-(1-hydroxyethyl)-thiamine and oxidized lipoic acid in 50% aqueous ethanol yields, upon paper chromatography and electrophoresis at pH 5, a compound which appeared to be the hemithioketal of reduced lipoic acid and 2-acetylthiamine. Because of the small equilibrium constants for hemithioketal formation with 2-acetyl-3,4-dimethylthiazolium ion reported here and the rapidity of hemithioacetal formation and breakdown,[44] it seems unlikely that the hemithioketal of reduced lipoic acid and 2-acetylthiamine could be isolated under these conditions.

**Enzymatic Reactions.** Previous studies of 2-acetylthiazolium salts have emphasized their rapid solvolysis[3,12,13] and thus have created the impression that the direct isolation of 2-acylthiamine pyrophosphate from enzymatic reactions would not be possible. This study has shown that 2-acylthiazolium salts are stable in acid. Thus, the isolation of 2-acylthiamine pyrophosphate from those enzymatic reactions in which it or a carbonyl adduct of it has been implicated as an enzyme-bound intermediate (see introductory section) may be possible by acidifidation of substrate amounts of the enzymes. In addition, the marked ultraviolet absorption of the 2-acetyl-3,4-dimethylthiazolium ion in the 300–310-m$\mu$ region ($\epsilon$ 6600–3900) suggests that in favorable cases spectrophotometric identification of an enzyme-bound 2-acylthiamine intermediate may be possible. For instance, in the phosphoketolase reaction neither the substrates nor products absorb in this region, and estimates of the absorbance of the protein in the 300–310 m$\mu$ range[45] indicate that if an appreciable fraction of the enzyme-bound thiamine pyrophosphate were in the 2-acyl form, its detection would not be obscured by protein absorption.

**Acknowledgment.** The author thanks W. P. Jencks for a valuable discussion.

---

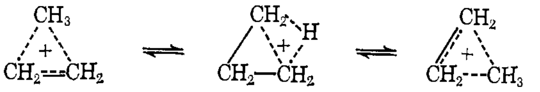
(42) R. W. Taft, Jr., and I. C. Lewis, *J. Am. Chem. Soc.*, **80**, 2436 (1958).
(43) N. Ohishi and S. Fukui, *Biochem. Biophys. Res. Commun.*, **20**, 21 (1965).
(44) See Lienhard and Jencks, ref 39.
(45) D. B. Wetlaufer, *Advan. Protein Chem.*, **17**, 310 (1962).

# Communications to the Editor

## Carbonium Ions. VIII. The Question of Protonated Cyclopropanes in the Nitrous Acid Deamination of Isobutylamine

*Sir:*

We have shown[1] that in the aqueous nitrous acid deamination of *n*-propylamine methyl-bridged ions (I) and edge-protonated cyclopropanes (II) are intermediates leading to isotope-position-rearranged *n*-propyl alcohol. In contrast, the intermediacy of III and IV could not be detected in the corresponding deamination of neopentylamine.[2] Although IV was rigorously excluded, III might have been an intermediate leading solely to the *t*-amyl cation.


I II I′


III IV

To assess the factors associated with the relative stability of simple bridged ions and edge-protonated cyclopropanes with respect to their classical ions, we have studied the deamination of suitably labeled isobutylamines. The isobutyl system is structurally intermediate between the *n*-propyl and the neopentyl. Under deamination conditions *n*-propylamine gives cyclopropane, isobutylamine gives methylcyclopropane to a lesser extent, and neopentylamine gives no dimethylcyclopropane.[3]

---

(1) G. J. Karabatsos, C. E. Orzech, Jr., and S. Meyerson, *J. Am. Chem. Soc.*, **87**, 4394 (1965). See also C. C. Lee, J. E. Kruger, and E. W. C. Wong, *ibid.*, **87**, 3985 (1965); C. C. Lee and J. E. Kruger, *ibid.*, **87**, 3986 (1965).
(2) G. J. Karabatsos, C. E. Orzech, Jr., and S. Meyerson, *ibid.*, **86**, 1994 (1964).
(3) P. S. Skell and I. Starer, *ibid.*, **82**, 2971 (1960); M. S. Silver, *ibid.*, **82**, 2971 (1960); J. H. Bayless, F. D. Mendicino, and L. Friedman, *ibid.*, **87**, 5790 (1965).