tonated ethyl glycinate (11.8 kcal mol^{-1}),[25] ethyl acetate (11.4 kcal mol^{-1}),[28] and $Et_3N^+CH_2CO_2Et$ (12.7 kcal mol^{-1}).[29] The large increase in rate observed for the chelated ester results entirely from the large positive entropy of activation (~15 cal mol^{-1} deg^{-1}). The above hydrolysis reactions are all associated with negative $\Delta S^{\ddagger}$ values: glyOEt (−32 cal mol^{-1} deg^{-1}),[25] EtOAc (−27 cal mol^{-1} deg^{-1}),[28] $HglyOEt^+$ (−6 cal mol^{-1} deg^{-1}),[25] $^+NEt_3CH_2CO_2Et$ (−3 cal mol^{-1} deg^{-1}).[29] Also, the ~10^{11} rate increase for ^-OH compared to H_2O for the chelated ester may be attributed largely to a positive entropy charge of ~40 cal mol^{-1} deg^{-1}.

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Mechanisms of Thiamine-Catalyzed Reactions. A Kinetic Analysis of the Decarboxylation of Pyruvate by 3,4-Dimethylthiazolium Ion in Water and Ethanol[1]

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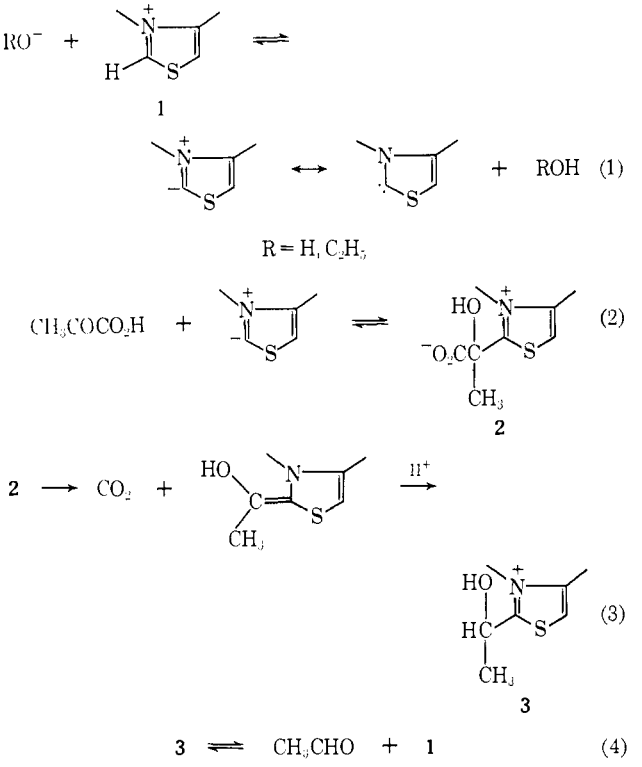
Abstract: This paper reports the kinetics of the hydrogen–deuterium exchange of 3,4-dimethylthiazolium ion (**1**) in ethanol and the kinetics of the breakdown of 2-(1-carbethoxy-1-hydroxyethyl)-3,4-dimethylthiazolium ion (**4**) and of 2-(1-hydroxyethyl)-3,4-dimethylthiazolium ion (**3**) to form **1** and ethyl pyruvate or acetaldehyde, in ethanol and in water. All three reactions are catalyzed by lyate ion. The rate constants for catalysis by ethoxide ion in ethanol are: for the hydrogen–deuterium exchange reaction of **1**-2-*d*, 4.6 × 10^9 M^{-1} min^{-1} at 25°; for the breakdown of **4**, 2.6 × 10^8 M^{-1} min^{-1} at 44.6°; for the breakdown of **3**, 1.1 × 10^5 M^{-1} min^{-1} at 45.6°. The lyate ion catalyzed hydrogen–deuterium exchange of **1**-2-*d* occurs 500 times more rapidly in ethanol than in water. The lyate ion catalyzed elimination reactions of **3** and **4** are 10^4–10^5 times faster in ethanol than in water. Also, the addition of **1** to the keto group of ethyl pyruvate to form **4**, the equilibrium constant for which is 20 M^{-1} in ethanol at 25.9°, probably is about 10^4 times faster in ethanol than in water when the rates are compared at the same concentration of lyate ion. In conjunction with earlier work, these results provide a kinetic analysis of the decarboxylation of pyruvate by **1** and provide further support for the hypothesis that catalysis in thiamine pyrophosphate dependent enzymatic reactions may be due in large part to binding of the thiazolium nucleus in a hydrophobic region of the enzymes.

The decarboxylation of α-keto acids is catalyzed by thiamine and other thiazolium salts. This catalysis is a model for the thiamine pyrophosphate dependent enzymatic decarboxylations of α-keto acids, which, however, are much more rapid. The major covalent changes which occur during catalysis have been clearly established, both for the model and the enzymatic reactions, largely through the efforts of Breslow, Krampitz, and Holzer.[2] These are given in eq 1–4 for the case of the decarboxylation of pyruvate catalyzed by 3,4-dimethylthiazolium ion (**1**).

The kinetics of ionization in aqueous solution of the hydrogen atom at C-2 of thiamine **1** and other thiazolium compounds have been previously determined.[3–6] The reaction is, as shown in eq 1, catalyzed by hydroxide ion. We have recently studied the kinetics



(1) This research was supported by grants (GB4848 and GB12848) from the National Science Foundation and a fellowship to J. C. from the William F. Milton Fund of Harvard University. Address inquiries to G. E. L.

(2) L. O. Krampitz (*Ann. Rev. Biochem.*, **38**, 213 (1969)) thoroughly reviews the enzymatic and model reactions.

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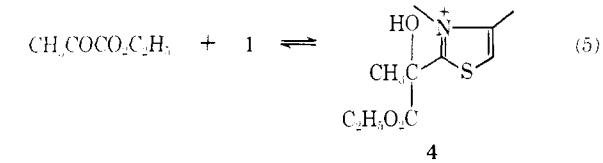
and mechanism of the decarboxylation of 2-(1-carboxy-1-hydroxyethyl)-3,4-dimethylthiazolium ion (**2**) in water and ethanol.[7] The decarboxylation, which has the mechanism given in eq 3, occurs 10^4 times more rapidly in ethanol than in water. Because of this large solvent effect and for other reasons, we proposed that a hydrophobic nature of the enzymatic active sites may be a major cause of catalysis in thiamine pyrophosphate dependent enzymatic reactions.[7] In order to complete the kinetic analysis of the model system with **1** and to examine the solvent effect upon the other steps in the model system, we have determined and report herein the kinetics of the ionization of the hydrogen atom at C-2 of **1** in ethanol, the kinetics of elimination of **1** from 2-(1-hydroxyethyl)-3,4-dimethylthiazolium ion (**3**) in water and ethanol (eq 4), and the kinetics of the addition of **1** to ethyl pyruvate to form 2-(1-carbethoxy-1-hydroxyethyl)-3,4-dimethylthiazolium ion (**4**) in water and ethanol (eq 5). This last reaction is a suitable analog of the addition of **1** to pyruvic acid (eq 1 and 2).



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Experimental Section

Proton magnetic resonance (pmr) spectra were taken with a Varian A-60 spectrometer operating at 60.00 MHz. Chemical shifts (δ in parts per million) are relative to the external standard of tetramethylsilane in chloroform. Ultraviolet spectra were measured with a Zeiss PMQ II or a Unicam SP 800 spectrophotometer. These instruments, equipped with thermostatted cell holders, were used to measure rates. pH values were measured with a Radiometer 25 SE pH meter.

Materials. Commercial ethyl pyruvate was purified by distillation under vacuum. Acidic impurities which remained in the distilled ethyl pyruvate were removed by shaking it with anhydrous sodium carbonate and filtering off the sodium carbonate. The preparations and ultraviolet and pmr spectra of **1** chloride, **4** boron tetrafluoride and chloride, **3** chloride, and **2** chloride have been described previously.[7]

1-2-*d* chloride was prepared by the exchange of the H-2 atom of **1** chloride for deuterium in deuterium oxide.[3,4] **1** chloride (3.0 g) was dissolved in 20 ml of deuterium oxide, and the pD[8] was adjusted from 6.7 to 7.2 by the addition of 1 μl of 1 *N* NaOH. After 0.75 hr at 22–23°, the reaction mixture was adjusted to pD 4.0 with 1 μl of 6 *N* HCl and divided into 4-mmol (4 ml) amounts in 50-ml flasks, from which the deuterium oxide was removed by flash evaporation and subsequent storage in an evacuated desiccator over P_2O_5.

Kinetic Measurements. The rates of exchange of the deuterium atom of **1-2-*d*** chloride with the hydroxyl hydrogen atom of ethanol were followed by a pmr method. For each run, a dry 4-mmol sample of **1-2-*d*** chloride in a 50-ml flask was dissolved in 3.0 ml of 10^{-3} *N* ethanolic HCl. The solution was incubated in a water bath at 25.0° for 15 min, a 0.37-ml sample was removed and added to 10 μl of 1 *N* HCl, and a minute later reaction was initiated by the addition of 0.87 ml of ethanolic lithium acetate buffer, which had been temperature equilibrated at 25.0°. At various times thereafter, 0.50-ml samples were removed and added to 10 μl of 1 *N* HCl, which was sufficient to give 0.013–0.016 *N* ethoxonium ion after protonation of the acetate ion in the buffer. Pmr spectra of the samples were taken within 5 hr; the fraction of hydrogen atom at C-2 was given by the ratio of the integrated intensity of its signal at 10.0 to that of the signal at 7.80, which is the signal of the stable hydrogen atom at C-4 and so serves as an internal standard of one proton. The sample taken before initiation of the reaction with buffer showed less than 5% hydrogen atom at C-2. The observed first-order rate constants for the hydrogen–deuterium exchange reaction were calculated from semilogarithmic plots of the difference between the fraction of hydrogen atom at C-2 after completion of the reaction and the fraction at various times during the first two half-times against time, by use of the equation, $k_{obsd} = 0.69/t_{1/2}$, in which $t_{1/2}$ is the half-time for the reaction. These plots were linear. The fraction of hydrogen atom at C-2 after completion of the reaction varied from 0.90 to 0.92. If there were no equilibrium isotope effects and no error in the integrations of the pmr signals, this value would be 0.944, since the deuterated reactant was 1 *M* and ethanol is 17 *M* in exchangeable hydrogen atoms.

The rates of breakdown of **4** and of **3** to ethyl pyruvate or acetaldehyde and **1** in ethanolic buffers were measured by the decrease in absorbance at 270 and 260 nm, respectively. Reaction was initiated by the addition of substrate, either as an accurately weighed amount of the solid compound or as a small aliquot from a concentrated stock solution in ethanol, to the buffer in an amount which was sufficient to give a 1–4 × 10^{-4} *M* solution. In the case of **4**, the buffer was equilibrated beforehand at the reaction temperature, and a portion of the reaction mixture was quickly transferred to Teflon-stoppered, 3-ml, 1-cm cuvettes which were placed in the thermostatted compartment of the spectrophotometer. In the case of **3**, ten 5-ml aliquots of the reaction mixture were dispensed into test tubes with Teflon-lined, screw-on caps. These were placed in a constant-temperature bath. After a period of 15 min of temperature equilibration, tubes were withdrawn at appropriate time intervals and cooled in ice; the optical densities of these samples were then measured.

The simultaneous hydrolysis of **4** to **2** and breakdown of **4** to ethyl pyruvate and **1** in aqueous buffers at 44.9° were followed by the decrease in absorbance at 254 nm. The procedure for these kinetic studies was the same as that for **4** in ethanol, with the exception that the buffer was not temperature equilibrated beforehand. After an initial period of 6 min, which was found to be the time required for a solution in the thermostatted cell compartment to reach the constant temperature, recording of absorbance as a function of time against a blank of the buffer was commenced.

The total changes in absorbance during these reactions were in the range of 0.20–1.0 absorbance units. The reactions showed endpoint absorbances that were stable for at least three half-times, except for the reaction of **4** in aqueous buffers. In this latter case there was a very slow decrease in absorbance which followed the initial reactions and which is probably due to the destruction of the thiazolium ring (see Results); it was still possible to estimate accurately the values of the endpoint absorbance. The observed first-order rate constants were calculated from semilogarithmic plots of the difference between the absorbance of each reaction mixture at intervals during the first two or three half-times and the endpoint absorbance against time, by use of the equation, $k_{obsd} = 0.69/t_{1/2}$. These plots were linear. Where duplicate determinations of k_{obsd} were made, they agreed within ±5% of the average value.

Product Compositions. The composition of the products from the reaction of **4** in aqueous solution was determined spectrophotometrically. The absorbance at 254 nm after completion of the reaction (A_∞) is equal to the sum of the absorbance of **1** and that of **2** plus that of any **3** which has formed by decarboxylation of the acid. Because 254 nm is the isobestic wavelength for **3** and the dipolar ion species of **2**, the fraction of **1** in the product, x, is given by the equation

$$A_\infty = x[\mathbf{4}]\epsilon_1 + (1 - x)[\mathbf{4}]\epsilon_{2,3}$$

where [**4**] is the initial concentration of reactant, and ϵ_1 and $\epsilon_{2,3}$ are the extinction coefficients at 254 nm of **1** and of **3** or of the dipolar ion species of **2**, respectively, under the conditions of the kinetic run.

Product Isolations. **4** chloride (194 mg) was dissolved in 950 ml of 1.3 × 10^{-5} *M* ethanolic sodium acetate. The progress of the elimination reaction was followed spectrophotometrically at 265 nm; it was complete in 2.5 hr at 21–23°, but the solution was left a further 14 hr prior to the work-up. The solution was evaporated under reduced pressure at 25–30° in a rotary evaporator, and 600 ml was collected at −10° in the receiving flask. 2,4-Dinitrophenylhydrazine (440 mg) was added to this ethanolic solution, and then dry HCl gas was bubbled through the solution for 30 min. After 16 hr at 21–23°, the ethanol and HCl were removed with the flash evaporator and the residue was extracted with ether. The ethereal

(7) J. Crosby, R. Stone, and G. E. Lienhard, *J. Amer. Chem. Soc.*, **92**, 2891 (1970).

(8) pD = reading of pH meter + 0.4; P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).

solution was taken to dryness and the resulting material was dissolved in a little hot ethanol. After cooling, 90 mg (42%) of needles of ethyl pyruvate 2,4-dinitrophenylhydrazone appeared: mp 148–151°, mmp 149–152° with authentic hydrazone of mp 154–156°.[9] The residue from the evaporation of the reaction mixture was recrystallized from ethanol–ether to give 91 mg (83%) of **1** chloride: mp 179–181°, mmp 180–184° with authentic compound of mp 183–185°; nmr spectrum in D_2O identical with that of authentic compound.[7]

A solution of 193 mg of **3** chloride in 50 ml of 7×10^{-5} *M* ethanolic lithium acetate was refluxed under argon for 16 hr. The course of the reaction was followed spectrophotometrically, and the spectrum of the product in ethanol (λ_{max} 250 nm, ϵ 4200 M^{-1} cm^{-1}) was nearly identical with that of authentic **1** chloride (λ_{max} 249 nm, ϵ 3700 M^{-1} cm^{-1}). The ethanolic solution was neutralized with HCl and 141 mg (95%) of crude **1** chloride was obtained from it by flash evaporation of the solvent and desiccation of the residue *in vacuo* over P_2O_5. The pmr spectrum of the crude product in D_2O was that of **1**; recrystallization of the crude product yielded a compound with mp 176–180°.

Equilibrium Constants. The equilibrium constant for the addition of ethanol to the keto group of ethyl pyruvate to form the hemiketal was determined spectrophotometrically by measurement of the absorbance at 330 nm, which is due to the keto group.[10] Over a period of 9 hr the absorbance of 8.6×10^{-2} *M* ethyl pyruvate in absolute ethanol at 25.9° fell from an initial value of 1.58 to a final value of 0.53, which did not change in a subsequent period of 15 hr. Consequently, the equilibrium constant, expressed as $[CH_3C(OH)(OC_2H_5)CO_2C_2H_5]/[CH_3COCO_2C_2H_5]$, is 1.58–0.53/0.53 = 1.98. The equilibrium constants for the hydration of methyl pyruvate and pyruvic acid in water at 25° have values of 3.1[11] and 2.3,[10] respectively.

The determinations of the equilibrium constant for the formation of **4** from ethyl pyruvate and **1** were carried out by dissolving the reactants in 25 ml of ethanol containing 0.005 *M* acetic acid and 0.005 *M* lithium acetate. The reaction mixture was held in a water bath at 25.9°. The progress of the reaction was followed by measuring the increase in absorbance at 290 nm (see Figure 1). When equilibrium had been established, the reaction was stopped by the addition of 25 ml of ethanolic 2 *N* HCl and the volume was reduced to about 3 ml by evaporation under reduced pressure at less than 35°. The thiazolium salts were precipitated by the addition of anhydrous ether, collected, thoroughly washed with further portions of ether, and dried over P_2O_5. The pmr spectrum of the precipitate dissolved in D_2O was in each case that expected for a mixture of **1** and **4**, and the percentage of the adduct was obtained from the integrated intensities of the signal at 2.01 from the adduct side-chain methyl and the signal at 2.50 from the 4-methyl of both thiazolium compounds. In a control experiment, a mixture of **1** and **4** chlorides in the molar ratio of 74/26 was prepared in ethanolic 1 *N* HCl–0.01 *M* acetic acid and analyzed according to the above procedure; the analysis gave a molar ratio of 76/24.

Under the conditions that were used, the establishment of the equilibrium between the thiazolium compounds requires 2–3 hr (see Figure 1). By use of the spectroscopic method described above, it was found that under these same conditions the equilibrium between ethyl pyruvate and its hemiketal is established within 5 min of solution of the ethyl pyruvate. The values of the equilibrium constants for the formation of **4** from **1** and ethyl pyruvate have been expressed in terms of the concentration of ethyl pyruvate itself, which was calculated from the concentration of ethyl pyruvate and its hemiketal by use of the equilibrium constant for hemiketal formation.

Results

Rates of Deuterium–Hydrogen Exchange of 1-2-*d*. The observed first-order rate constants for the exchange of the deuterium atom of **1**-2-*d* with the hydroxyl hydrogen atoms in ethanolic acetate buffers are given in Table I. The fact that the value of k_{obsd} increased only slightly or did not change when the concentration of the buffer was almost doubled shows that there is little or no catalysis of the reaction by acetate ion at low concentrations. The value of k_{obsd} obtained for the buffers with a ratio of [acetate ion] to [acetic acid] of 0.10 is twice as large as the value obtained for the buffers with a ratio of 0.05. Since ethoxide ion is the only species the concentration of which varies directly with this buffer ratio, the exchange reaction under these conditions is catalyzed almost entirely by ethoxide ion.

Table I. Rate Constants for Deuterium–Hydrogen Exchange of **1**-2-*d* Chloride in Ethanol at 25.0° [a]

Buffer	k_{obsd}, min^{-1}
0.0034 *M* Li acetate–0.034 *M* acetic acid	0.69
0.0064 *M* Li acetate–0.064 *M* acetic acid	0.80
0.0035 *M* Li acetate–0.070 *M* acetic acid	0.38
0.0067 *M* Li acetate–0.133 *M* acetic acid	0.38

[a] The ionic strength was 1 *M* because of the presence of 1 *M* reactant.

Calculation of the second-order rate constant for catalysis by ethoxide ion requires values for the concentration of ethoxide ion. This concentration is related to the buffer ratio, *r*, by eq 6 where K_{HOAc}

$$[C_2H_5O^-] = r\frac{K_{1P}f_{OAc^-}a_{C_2H_5OH}}{K_{HOAc}f_{C_2H_5O^-}f_{HOAc}} \quad (6)$$

is the thermodynamic acid dissociation constant of acetic acid in ethanol at 25°, K_{1P} is the thermodynamic ion product of ethanol at 25°, $a_{C_2H_5OH}$ is the activity of ethanol in 1 *M* ethanolic **1** chloride relative to an activity of 1 for pure ethanol, and f_{OAc^-}, $f_{C_2H_5O^-}$, and f_{HOAc} are the activity coefficients of acetate ion, ethoxide ion, and acetic acid, respectively, in 1 *M* ethanolic **1** chloride. The values of K_{HOAc} and K_{1P} have been found to be 6×10^{-11} [12] and 10^{-19}.[13] There are no values in the literature for $a_{C_2H_5OH}$, f_{HOAc}, f_{OAc^-}, and $f_{C_2H_5O^-}$. However, since ethoxide ion and acetate ion are both negative ions and since ethanol and acetic acid are both neutral molecules, the assumption that the ratios, $f_{OAc^-}/f_{C_2H_5O^-}$ and $a_{C_2H_5OH}/f_{HOAc}$, have values close to 1 seems permissible. With the use of this assumption, we calculate a second-order rate constant of 4.6×10^9 M^{-1} min^{-1}.

Breslow and McNelis have reported that the observed first-order rate constant for deuterium–hydrogen exchange of 1 *M* **1**-2-*d* bromide in aqueous 0.02 *M* phthalate buffer, pH 5.37, at 25° is 0.030 min^{-1}.[3] Also, in agreement with our results, Haake, *et al.*, have shown that the only significant term in the rate law for the hydrogen–deuterium exchange reaction of **1** iodide in acetate buffers in D_2O at 33° is catalysis by deuteroxide ion.[4] Consequently, the single rate constant of Breslow and McNelis may be used to calculate the second-order rate constant for the hydroxide ion catalyzed exchange of **1**-2-*d*. The activity coefficient of hydroxide ion in 1 *M* **1** bromide at 25° probably has approximately the same value as it does in 1 *M* KBr, which value is 0.62,[14] so that the concentration of hydroxide ion at pH 5.37 is $10^{-14}/[(0.62)(4.25 \times 10^{-6})]$ and the value of the second-order rate constant is

(9) H. H. Strain, *J. Amer. Chem. Soc.*, **57**, 760 (1935).
(10) M. Becker and H. Strehlow, *Ber. Bunsenges. Phys. Chem.*, **64**, 813 (1960).
(11) P. Greenzaid, Z. Luz, and D. Samuel, *J. Amer. Chem. Soc.*, **89**, 749 (1967).
(12) (a) H. O. Spivey and T. Shedlovsky, *J. Phys. Chem.*, **71**, 2171 (1967); (b) E. Grunwald and B. J. Berkowitz, *J. Amer. Chem. Soc.*, **73**, 4939 (1951).
(13) G. Briere, B. Crochon, and N. Felici, *C. R. Acad. Sci.*, **254**, 4458 (1962).
(14) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolyte Solutions," 3rd ed, Reinhold, New York, N. Y., 1958, pp 593–594.

7.9×10^6 M^{-1} min^{-1}. Thus, under these conditions, the lyate ion catalyzed deuterium–hydrogen exchange reaction of **1**-2-*d* occurs about 500 times faster in ethanol than in water.

Elimination of 1 from 4 and Its Reversal in Ethanol. **4** at low concentrations in ethanolic acetate buffers forms **1** and ethyl pyruvate. These products have been directly identified by isolation (see Experimental Section). Moreover, ultraviolet spectra of the reaction mixtures used in the kinetic runs which were taken after completion of the reaction were identical with that of **1** in ethanol and showed that the yields of this product were quantitative. Spectra which were obtained at intervals during the reaction in ethanolic 0.015 *M* Li acetate–0.09 *M* acetic acid at 25.9° exhibited sharp isobestic points at 229.5 and 245.5 nm. Consequently, no ultraviolet-absorbing intermediate accumulates in detectable amounts during the course of the reaction.

The observed first-order rate constants for this elimination reaction are summarized in Table II.

Table II. Kinetic Data for the Reaction of **4** BF_4 in Ethanolic Lithium Acetate Buffers at 25.9°

[B]/[HB][a]	Li acetate, m*M*	LiCl, m*M*	Ionic strength, m*M*	10^2k_{obsd}, min^{-1}	$10^2k_{obsd} \times$ ([BH]/[B])
0.50	2	17	19	0.62	
	4	17	21	0.61	
	6	17	23	0.57	
	8	17	25	0.54	
2.00	4	8	12	3.82	
	9	8	17	3.35	
	13	8	21	2.99	
	17	8	25	2.82	
1.78	16		16	3.82	
	16	10	26	2.21	
	16	22	38	1.48	
	16	33	49	1.12	
2.00	17	33	50	1.28	
	33	17	50	1.65	
	50		50	2.15	
2.00	17	283	300	0.182	
	33	267	300	0.198	
	50	250	300	0.204	
0.50	10	10	20	0.77	1.54
1.00	10	10	20	1.57	1.57
2.00	10	10	20	3.00	1.50
4.00	10	10	20	5.90	1.48
0.30[b]	12		12	20.3	67.8
0.60[b]	12		12	43.3	72.1

[a] [B] and [HB] are the concentrations of the basic and acidic species of the buffer, respectively. [b] At 44.6°.

When the reaction was carried out at one buffer ratio with varying concentrations of lithium acetate and with a constant concentration of LiCl, the values of k_{obsd} decreased as the concentration of lithium acetate was increased (first and second series of Table II). Similarly, when the reaction was carried out at one buffer ratio with varying concentrations of LiCl and with a constant concentration of lithium acetate, the values of k_{obsd} decreased as the concentration of LiCl was increased (third series, Table II). These results show that the reaction is slower at higher ionic strengths. However, the LiCl appeared to slow the reaction more markedly than lithium acetate, and when the reaction was carried out at a constant buffer ratio and a constant ionic strength, adjusted with LiCl, the value of k_{obsd} increased with increasing concentration of buffer (fourth and fifth series, Table II). Because of this effect, the dependence of the rate upon the buffer ratio was determined in the presence of constant concentrations of both lithium acetate and LiCl (sixth and seventh series, Table II). Under these conditions, the value of k_{obsd} was found to vary directly with the buffer ratio over an eightfold range. Consequently, the reaction is catalyzed by ethoxide ion. On the basis of the data in Table II, it is not possible to decide definitely whether the reaction is also subject to general base catalysis by acetate ion. The apparent catalysis by 0.017–0.05 *M* acetate ion which was observed when the ionic strength was maintained at 0.05 *M* with LiCl (fourth series, Table II) may be explained by the alternative hypothesis that the activity coefficients of the reactants and/or transition state in the presence of chloride ion differ from those in the presence of acetate ion. This latter interpretation is supported by the finding that catalysis by acetate ion at constant ionic strength was much less marked when the ionic atmosphere was determined predominantly by chloride ion throughout the 0.017–0.05 *M* range of acetate ion (fifth series, Table II).

The second-order rate constant for the ethoxide ion catalyzed breakdown of **4** in ethanol at 44.6° can be calculated from the last series in Table II. Equation 6 gives the concentration of ethoxide ion in the lithium acetate buffers. At 45°, the value of K_{IP} is 1.6×10^{-19}.[15] The value of K_{HOAc} in ethanol at 45° has not been determined, but it is known that pK_{HOAc} in 80 wt% ethanol–20 wt% water increases by only 0.11 unit with an increase in the temperature from 0 to 35°.[12a] Consequently, it seems unlikely that our calculation will be in serious error if we assume that the value of K_{HOAc} in ethanol at 25°, which is 6×10^{-11},[12] is also the value at 45°. This treatment yields a second-order rate constant of 2.6×10^8 M^{-1} min^{-1}. In a similar way, we calculate from the sixth series in Table II a second-order rate constant of 9.1×10^6 M^{-1} min^{-1} for the reaction at 25.9°.

The reaction of ethyl pyruvate with **1** in ethanol was followed by the increase in absorbance at 290 nm, where the extinction coefficients of ethyl pyruvate (itself plus hemiketal), **1**, and **4** are 2.1, 2, and 94 M^{-1} cm^{-1}, respectively. The results are illustrated by the example presented in Figure 1. After the reaction had appeared to come to equilibrium, the absorbance continued to increase linearly with time. This further increase in absorbance may be due to some side reaction of ethyl pyruvate, since under the same conditions the absorbance of ethyl pyruvate alone also increases with time (Figure 1), whereas that of **1** is constant. Some hours after the linear portions of the absorbance *vs.* time plot had been reached, the reaction mixtures were analyzed for **4** and **1** by a pmr method (see the Experimental Section). Table III summarizes the values of the equilibrium constant for the addition reaction (K_{add}) calculated on the basis of these analyses. The assumption that the equilibrium had been established

(15) G. Briere, N. Felici, and E. Piot, *C. R. Acad. Sci.*, **255**, 107 (1962).

Table III. Equilibrium Constants for the Formation of **4** from Ethyl Pyruvate and **1**[a]

Ethyl pyruvate,[b] mM	**1** Cl,[b] mM	**4** Cl,[c] mM	Reaction time, hr	K_{add},[d] M^{-1}
22.4	25.4	3.1	7	21.6
52.0	28.4	6.6	9.5	19.1
101.3	25.6	8.6	24	16.5
22.4	101.2	9.4	24	23.7

[a] In ethanol at 25.9°. [b] Initial concentrations. The concentration of ethyl pyruvate given here includes the concentration of hemiketal. [c] Concentration at equilibrium. [d] K_{add} = [adduct]/(initial [ethyl pyruvate] − [adduct])(initial [**1**] − [adduct]). In these calculations, the concentrations of ethyl pyruvate itself were used.

is supported by the fact that these values are approximately constant over a range of initial concentrations of the reactants. The third-order rate constant for ethoxide ion catalyzed formation of **4** is equal to the product of the equilibrium constant K_{add} and the second-order rate constant for the reverse reaction. Consequently, its value at 25.9° is $1.8 \times 10^8\ M^{-2}\ min^{-1}$.

In addition to the above treatment, it is possible to obtain approximate values of first-order rate constants for approach to equilibrium directly from the spectrophotometric data, of which Figure 1 is an example. Since less than 15% of the initial concentration of either ethyl pyruvate or of **1** was consumed in the establishment of equilibrium under the conditions described in Table II, the kinetics of the reaction should be approximately first order, with the observed first-order rate constant for approach to equilibrium (k_{obsd}) equal to the sum of the first-order rate constant for the forward reaction (k_f[X], where [X] is the concentration of the reactant in excess) and the first-order rate constant for the reverse reaction (k_r).[16] In fact, in each case, when the logarithm of the difference between the absorbance given by extrapolation of the linear portion of the plot of absorbance against time (solid line, Figure 1) and the measured absorbance was plotted against time, an approximately straight line was described. The values of k_{obsd} obtained in this way were found to be in fair agreement with the values which are expected on the basis of the equilibrium constant and the value for k_r determined at the same buffer ratio. For example, the data in Figure 1 yield a value of 0.029 min^{-1} for k_{obsd}; at the same buffer ratio, but with 0.01 M LiCl–0.005 M Li acetate, the value of k_r is 0.0157 min^{-1} (Table II), so that the expected value for k_{obsd} (k_f[X] + k_r = $K_{add}k_r$[X] + k_r) is about 0.026 min^{-1}.

The assumption that extrapolation of the linear portions of the plots of absorbance against time is a valid way to correct for side reactions also allows calculation of the equilibrium constant for adduct formation from the spectrophotometric data. According to this assumption, the ordinate intercepts of the extrapolations are the endpoint absorbances in the absence of side reactions (A_∞). The concentration of the adduct at equilibrium, [**4**], is given by A_∞, according to the equation

$$A_\infty = [\mathbf{4}]\epsilon_{\mathbf{4}} + ([\mathbf{1}] - [\mathbf{4}])\epsilon_{\mathbf{1}} + ([EP] - [\mathbf{4}])\epsilon_{EP}$$

where [**1**] and [EP] are the known initial concentrations of **1** and ethyl pyruvate, respectively, and the ϵ's are the extinction coefficients of the species at 290 nm. The values of the equilibrium constant obtained with these values for [**4**] were found to agree approximately with the value from the pmr method. For example, the value of K_{add} which is calculated from A_∞ in Figure 1 is 10.8 M^{-1}.

(16) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, Wiley, New York, N. Y., 1961, pp 186–187.

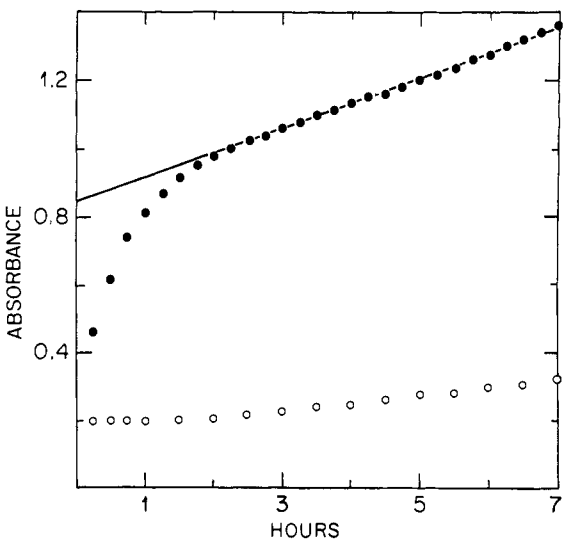


Figure 1. Change in absorbance during the reaction of 0.0256 M **1** chloride with 0.1013 M ethyl pyruvate in ethanolic 0.005 M Li acetate–0.005 M acetic acid, at 25.9°. The open circles (O) show the change in absorbance of 0.102 M ethyl pyruvate under the same conditions. Absorbances were measured with 1-cm cuvettes against a blank of the buffer alone.

Reactions of 4 in Water. Preliminary spectrophotometric measurements suggested that **4** undergoes simultaneous hydrolysis to **2** and elimination to ethyl pyruvate and **1** in aqueous buffers at 44.9°. Since **2** was known to decarboxylate to **3**,[7] measurements of the rates of the simultaneous reactions were carried out at the isobestic wavelength for **3** and the dipolar ion species of **2**, 254 nm, in order to avoid interference from the decarboxylation. The lower spectrum in Figure 2 is the pmr spectrum in D_2O of the thiazolium products from the reaction of **4** after 6 half-times. This spectrum is that of a mixture of 38% **1**, 54% **2**, and 8% **3**.[7] The presence of **2** in this mixture was confirmed by the pmr spectrum of the mixture after the thiazolium salts had been treated with neutral ethanol (Figure 2, upper spectrum), in which solvent **2** is known to decarboxylate rapidly.[7] This spectrum shows that the peaks due to **2** have disappeared and those due to **3** have intensified.

The product composition and rate constants for the reactions of **4** in aqueous solution are summarized in Table IV. Neither the elimination nor the hydrolysis reaction appears to be catalyzed significantly by phosphate buffer (first series, Table IV). Within the pH range in which the rates of the reactions were determined, both reactions are catalyzed by hydroxide ion (second series, Table IV). The rates of reaction were not measured at pH values higher than 7.7, since at higher pH values thiazolium salts undergo a ring-opening reaction (eq 7).[17–19] For example, at 25°

(17) G. D. Maier and D. E. Metzler, *J. Amer. Chem. Soc.*, **79**, 4386 (1957).
(18) E. Yatco-Manzo, F. Roddy, R. G. Yount, and D. E. Metzler, *J. Biol. Chem.*, **234**, 733 (1959).
(19) R. G. Yount and D. E. Metzler, *ibid.*, **234**, 738 (1959).

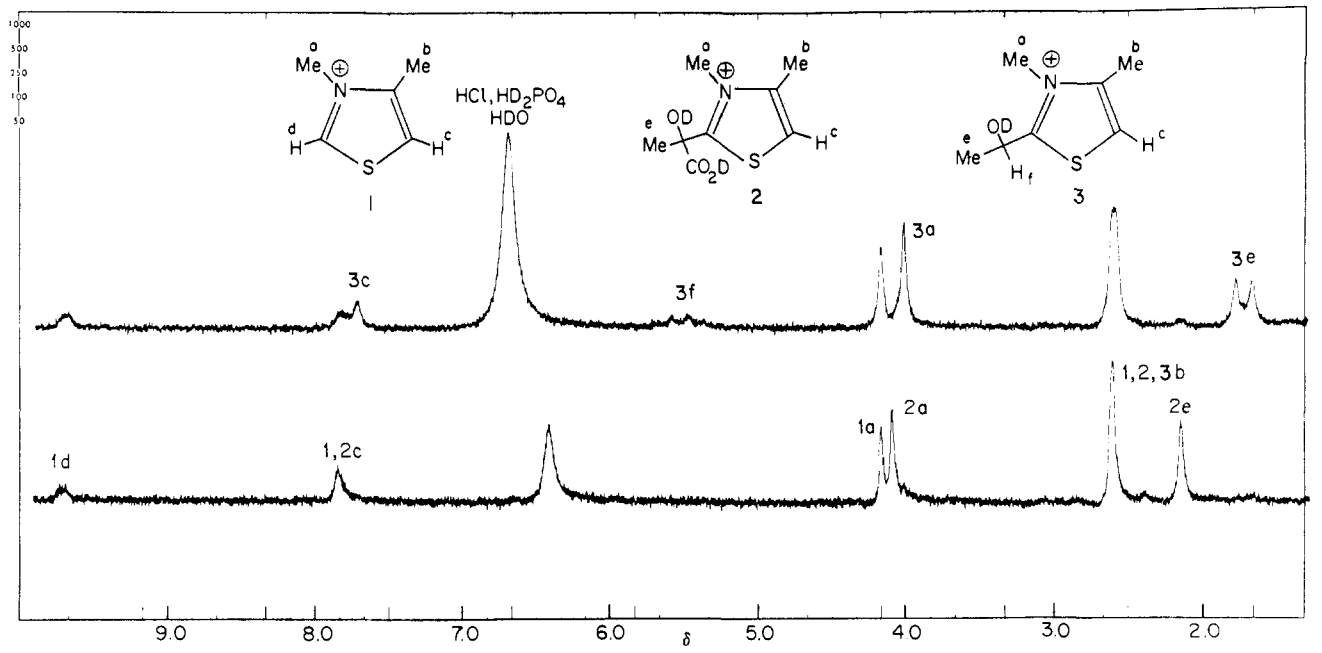


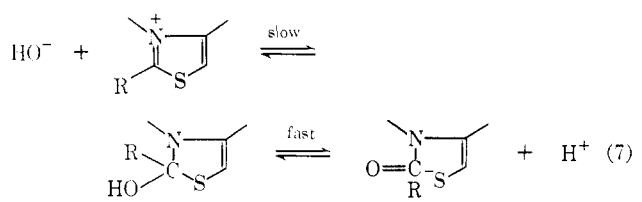
Figure 2. Pmr spectra of the thiazolium salts from the reaction of **4** chloride in aqueous solution. **4** chloride (150 mg) in 25 ml of 0.025 *M* K phosphate buffer, $[HPO_4^{2-}]/[H_2PO_4^-]$ = 11, was maintained at 45.6° for 2 hr. The reaction mixture was then cooled, acidified with 12.5 ml of 1 *N* HCl, and taken to dryness at 30–35° on the flash evaporator. The residue was extracted five times with 10 ml of 1.1 *N* ethanolic HCl. The ethanolic HCl was evaporated on the flash evaporator at 30–35°. The pmr spectrum of the oily residue was taken in 0.8 ml of D_2O (lower spectrum). The D_2O was removed with the flash evaporator at 30–35°, and the oily residue was dissolved in 25 ml of neutral ethanol. After 2.75 hr at room temperature, the ethanol was taken off on the flash evaporator, and the pmr spectrum of the residue in D_2O was again taken (upper spectrum). There were no signals in the region between δ 0 and 1.4, which is not shown. In a control experiment, 60 mg of **2** Cl was treated in the same way as the 150 mg of **4** Cl; the pmr spectrum of the thiazolium salts in the acidic ethanol extract showed no **1**, 88% **2**, and 12% **3**. In another control experiment, a mixture of **1** Cl and **2** Cl in the molar ratio of 36/64 was treated in the same way as the **4** Cl; the pmr spectrum of the recovered thiazolium salts showed 38% **1**, 54% **2**, and 8% **3**.

and 0.5 *M* ionic strength, the second-order rate constant for attack of hydroxide ion upon **1** to initiate the ring opening is 180 M^{-1} min^{-1}, and the equilibrium equal amounts of **1** and the ring-opened form and only a very small amount of the intermediate adduct of hydroxide to **1**.[20]

Table IV. Rate Constants for the Reactions of **4** in Aqueous Potassium Phosphate Buffers at 44.9°

Buffer, *M*	pH[a]	$10^7[OH^-]$,[b] *M*	10^2k_{obsd}, min^{-1}	**1**,[c] %	10^3k_{elim},[d] min^{-1}	10^3k_{hyd},[d] min^{-1}	$10^{-3}k_{2elim}$,[e] M^{-1} min^{-1}	$10^{-3}k_{2hyd}$,[e] M^{-1} min^{-1}
0.05[f]	7.03	6.45	0.53	32	1.70	3.60	2.64	5.57
0.10[f]	7.09	7.40	0.63	30	1.89	4.41	2.56	5.96
0.15[f]	7.12	7.98	0.75	34	2.54	4.96	3.18	6.20
0.20[f]	7.18	9.15	0.82	33	2.70	5.50	2.95	6.00
0.025[g]	6.68	2.15	0.34	44	1.50	1.70	6.98	7.90
0.025[g]	7.17	6.65	0.82	45	3.68	4.52	5.52	6.80
0.025[g]	7.47	13.3	1.87	43	8.05	10.65	6.05	8.02
0.025[g]	7.71	23.2	3.38	42	14.2	19.6	6.08	8.45

[a] At 25°. [b] At 45°. The concentration of hydroxide ion was calculated from the pH value at 25° in the following way. The pH value at 45° was obtained by subtracting 0.03 unit from the pH value at 25°, since the apparent p*K* of 0.05 *M* phosphate buffer decreases by this amount (V. Gold, "pH Measurements," Methuen, London, 1956, p 118). The ion product of water at 45° (4×10^{-14}) gave the activity of hydroxide ion. Its concentration was calculated from the activity by assuming that the activity coefficients for hydroxide ion in aqueous KCl at 25° and at 0.2 and 1.0 *M* ionic strength, which values are 0.83 and 0.62, respectively,[14] also hold at 45°. [c] Calculated from the endpoint absorbance; see Experimental Section. [d] $k_{obsd} = k_{elim} + k_{hyd}$, where k_{elim} and k_{hyd} are the first-order rate constants for breakdown to ethyl pyruvate and **1** and for hydrolysis to **2**, respectively, and $k_{obsd} \times$ % elimination = k_{elim}. See ref 16. [e] $k_{2elim} = k_{elim}/[OH^-]$; $k_{2hyd} = k_{hyd}/[OH^-]$. [f] This series was done at an ionic strength of 1.0 *M*, adjusted with KCl. [g] This series was done at an ionic strength of 0.2 *M*, adjusted with KCl.



constants for the steps are such that at pH 9.5 there are

Comparison of the second-order rate constant for hydroxide ion catalyzed breakdown of **4** to ethyl pyruvate and **1** with the second-order rate constant for catalysis by ethoxide ion in ethanol shows that at the same concentration of base the reaction occurs 4×10^4 times more rapidly in ethanol. The large second-order rate constant for hydrolysis of the ester function of **4**

(20) G. E. Lienhard and D. Packard, unpublished results.

is presumably due to the strongly electron-withdrawing effect of the substitutents in the acyl portion, which effect is also shown by the very low pK value of 1.3 for the carboxyl group of **2**.[7] The rate constant for hydroxide ion catalyzed hydrolysis of $(CH_3)_3N^+CH_2CO_2CH_3$, a somewhat similar compound, is 8500 M^{-1} min^{-1} at 42°.[21]

Elimination of 1 from 3. A product isolation experiment showed that in ethanolic acetate buffers **3** breaks down to **1**. The other product, which was not isolated, is presumably acetaldehyde. Complete ultraviolet spectra of reaction mixtures which were taken at intervals during the kinetic runs exhibited a change from the spectrum of **3** to one which resembled that of **1**, although after long periods the extinction coefficient of the maximum at 249 nm was less than that of **1** and a new band with λ_{max} at 318 nm appeared. These changes are due to a slow decomposition of the products, since an equimolar mixture of **1** and acetaldehyde showed the same behavior. The rate constants for the elimination reaction are summarized in Table V.

Table V. Rate Constants for the Elimination of 1 from 3 Chloride[a]

10^4[3], M	[B]/[HB][b]	$10^3 k_{obsd}$, min^{-1}
1	1	4.6
2	1	4.6
4	1	4.9
2	0.5	2.2
2	1	4.6
2	2	11.9
2	2	0.60[c]

[a] In ethanol at 71.7° and 0.025 M ionic strength, adjusted with LiCl. [b] The buffer used was Li acetate (B)–acetic acid (HB) at a total concentration of 0.025 M. [c] At 45.6°.

The value of k_{obsd} is independent of the concentration of **3** in the 1–4 × 10^{-4} M range; consequently, there is no significant reverse reaction at these concentrations. The values of k_{obsd} are directly proportional to the buffer ratio, and therefore, as is the case with the elimination reaction of **4**, the reaction is catalyzed by ethoxide ion. The second-order rate constant for this catalysis, which has been calculated in the same way as the constant for **4** (see above), is 1.1 × 10^5 M^{-1} min^{-1} at 45.6°.

An attempt was made to investigate the elimination of **1** from **3** in aqueous solution. The spectrum of 1.7 × 10^{-4} M **3** Cl in 0.025 M sodium phosphate buffer–0.95 M KCl, pH 7.28 at 25°, was recorded after the solution had been maintained at 67° for various periods. The absorbance decreased with time, but λ_{max} remained at 255 nm; thus, little or no **1**, which has λ_{max} at 249 nm, was formed. After the reaction(s) was complete, the spectrum was transparent above 240 nm. The disappearance of **3** followed first-order kinetics; the value of k_{obsd} was 3.3 × 10^{-4} min^{-1}. Further study of this reaction was not made, but it seems likely that **3** decomposes *via* the reversible ring opening reaction (eq 7) followed by hydrolytic reactions of the ring-opened form. If it is assumed that the elimination reaction occurs no more than one-third as rapidly as the observed reaction, we can estimate (see Table IV) that the second-order rate constant for hydroxide ion catalyzed elimination is 30 M^{-1} min^{-1} or less at 67° and, with the assumption of a twofold increase in rate per 10° rise in temperature, 7 M^{-1} min or less at 46°. Thus, the acceleration of this reaction upon change in the solvent from water to ethanol is 10^4 or more, a magnitude which is similar to that found for the elimination reaction of **4**.

(21) M. R. Wright, *J. Chem. Soc. B*, 548 (1968).

Discussion

Ionization at C-2 of Thiazolium Ions. No pK_a value[8] for the dissociation of the hydrogen atom at C-2 of thiazolium compounds to form the ylide and hydronium ion have been determined. However, it is possible to estimate upper and lower limits for the pK_a of **1** in water at 25°. The upper limit is estimated by assuming that the hydroxide ion catalyzed deuterium–hydrogen exchange reaction is a diffusion-controlled reaction[22] (eq 8, $k_t' > k_{-d}'$ and $k_t' > k_t$). In this case, the second-

$$HO^- + \underset{(H)}{D\text{–}\overset{+}{C}} \underset{k_{-d}}{\overset{k_d}{\rightleftharpoons}} HO^- \cdots \underset{(H)}{D\text{–}\overset{+}{C}} \underset{k_t'}{\overset{k_t}{\rightleftharpoons}}$$

$$\underset{(H)}{HO\text{–}D} \cdots \overset{\pm}{C} \underset{k_d'}{\overset{k'_{-d}}{\rightleftharpoons}} \underset{(H)}{HO\text{–}D} + \overset{\pm}{C} \quad (8)$$

order rate constant for catalysis by hydroxide ion, k_{OH^-}, is equal to $k_d k_t k_{-d}'/k_{-d}k_t'$ and is related to the pK_a of **1**-2-*d* by eq 9, where pK_{aHOD} is the pK_a for dis-

$$\log k_{OH^-} = \log k_d' + pK_{aHOD} - pK_{a1\text{-}2\text{-}d} \quad (9)$$

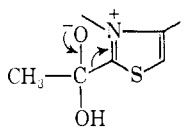
sociation of the deuterium of HOD and k_d' is the second-order rate constant for the diffusion encounter of HOD and the ylide of **1** in water at 25°. Since the deuterium isotope effects upon the equilibrium constant for the ionization of H_2O and **1** are probably about the same,[23] eq 9 becomes

$$\log k_{OH^-} = \log k_d' + pK_{H_2O} - pK_{a1} \quad (10)$$

The value of k_{OH^-} is 7.9 × 10^6 M^{-1} min^{-1}, and the value of k_d' should be about 6 × 10^{11} M^{-1} min^{-1}.[24] Thus, the upper limit for pK_{a1} in water at 25° is 20.6. It is interesting to note that Haake, *et al.*,[4] were unable to detect catalysis of the hydrogen–deuterium exchange of **1** by acetate ion and that the slope of the plot of the logarithms of the second-order rate constants for proton transfer from **1** to acetate and hydroxide ions against the pK_a's of acetic acid and water must therefore have a value of greater than 0.8. If the proton transfer is, in fact, a diffusion-controlled reaction, the slope of the Brønsted plot should be 1.0.[24]

The lower limit for the pK_a of **1** can be estimated from the finding that the ratio of the rate of expulsion of hydroxide ion to the rate of expulsion of **1** ylide from the hydrate dipolar ion of 2-acetyl-3,4-dimethylthiazolium ion (**5**) is 10,[25] whereas the ratio of the rate of expulsion of hydroxide ion to that of diethyl malonate anion from the hydrate anion of diethyl acetylmalonate (**6**) is 1.4 × 10^{-3}.[26] Thus, the ylide is a poorer leaving group and therefore probably a stronger base than diethyl malonate anion (pK_a of diethyl malonate, 15.2) and hydroxide ion (pK_a of water, 15.7). If the dif-

(22) E. J. NcNelis, Ph.D. Thesis, Columbia University, New York, N. Y., 1960.
(23) R. P. Bell and A. T. Kuhn, *Trans. Faraday Soc.*, **59**, 1789 (1963).
(24) M. Eigen, *Angew. Chem., Int. Ed. Engl.*, **3**, 1 (1964).
(25) G. E. Lienhard, *J. Amer. Chem. Soc.*, **88**, 5642 (1966).
(26) G. E. Lienhard and W. P. Jencks, *ibid.*, **87**, 3863 (1965).



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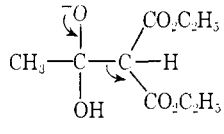

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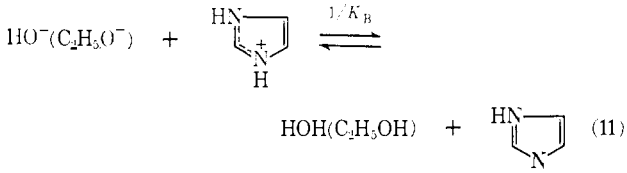
ference between the rates of expulsion of the two carbon acids relative to the rate of expulsion of hydroxide ion is an approximately quantitative measure of the difference between their basicities, then the pK_a of **1** is about 19.2, which is close to the upper estimate of 20.6.

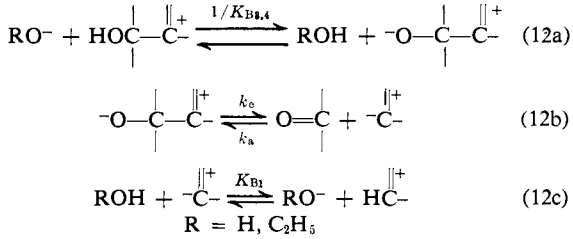
Our finding that in the presence of 1 *M* salt proton transfer to lyate ion occurs 500 times more rapidly in ethanol than in water is in agreement with the expectation for a reaction in which two charged species form two uncharged ones. The solvent effect would probably be even larger if the comparison were made at a lower ionic strength. In terms of the scheme given in eq 8, if the reaction is diffusion controlled, then the solvent effect is predominantly an effect upon the equilibrium constant, $k_d k_t/k_{-d}k_t'$, since k_{-d}' should have about the same value in ethanol and water; if it is not diffusion controlled, then the solvent effect is the effect upon the rate constant $k_d k_t/k_{-d}$. An estimate of the expected magnitude of the solvent effect upon the equilibrium constant for the overall reaction (eq 1) is given by data for the corresponding reaction of imidazolium ion (eq 11). The ratio of the



(11)

equilibrium constant for this reaction, expressed with the activity of the solvent as one, in ethanol to that in water is 2×10^4.[27]

The Elimination of 1 from 3 and 4. The most likely mechanism for hydroxide and ethoxide ion catalyzed elimination of **1** from **3** and **4** is shown in eq 12a–c.



(12a)

(12b)

(12c)

The fact that the observed first-order rate constants for the elimination reaction of **4** in water are proportional to the concentration of hydroxide ion at concentrations below 10^{-6} *M* and that the first-order rate constants for the elimination reaction of **3** and **4** in ethanol are proportional to the concentration of ethoxide ion at values less than 10^{-8} *M* show that only small fractions of **3** and **4** are ionized at these concentrations of lyate ion. Consequently, the K_B value of **4** in water, expressed with the activity of the solvent equal to one, must be 10^{-5} *M* or greater, and the values of K_{B3} and K_{B4} in ethanol, similarly expressed, must be 10^{-7} *M* or greater. The K_B value of **3** in water has been found to be 2.5×10^{-3} *M* at 25° and 0.5 *M* ionic strength.[20] The pK_B values of alcohols are known to be approximately proportional to the sum of Taft's polar substituent constants, σ^*, for substituents bonded to the carbinol carbon atom.[28-30] Using this linear free energy relationship and the pK_{B3} value, we estimate that σ^* for the 3,4-dimethyl-2-thiazolium group is 3.65 and that the K_B value of **4** is 10^{-5} *M* in water at 25°. In turn, these values of K_B allow estimates of the values of k_e, the rate constant for the unimolecular elimination of the **1** ylide, since the second-order rate constants for lyate ion catalysis are equal to k_e/K_B. The value of k_e for **3** in water at 45° is less than 2×10^{-2} min^{-1} and that for **4** is about 6×10^{-2} min^{-1}.

The fact that the second-order rate constants for the ethoxide ion catalyzed elimination reactions of **3** and **4** are $>10^4$ and 4×10^4 times greater, respectively, than the rate constants for the hydroxide ion catalyzed reactions is probably due to solvent effects upon both the equilibrium for ionization of the hydroxyl group (eq 12a) and upon the rate of the elimination step itself (eq 12b). Since the ionization reaction is one in which two ions yield a neutral species and a dipolar ion, it does not seem likely that the ratio of the value of $1/K_B$ in ethanol to that in water would be as large as 10^4–10^5, which is the range found for reactions in which two ions form two neutral species,[31] such as the neutralization of imidazolium ion described above. This supposition is in agreement with the above discussion which indicates that the value of $1/K_B$ for **4** is no more than about 10^2 times greater in ethanol than in water. In this regard, it has been determined that the ratio of $1/K_A$ for the reaction, $CH_3CO_2^- + H_3^+O \rightleftharpoons CH_3CO_2H + H_2O$, in 70% dioxane–30% water (dielectric constant = 18) to that for the reaction in water is 3×10^3, whereas the corresponding ratio of $1/K_A$ for the reaction, $H_2NCH_2COO^- + OH_3^+ \rightleftharpoons H_3N^+CH_2CO_2^- + H_2O$, is 3×10^1.[32] If it is assumed that the values of $1/K_B$ for **3** and **4** increase by a factor of 10^2 in ethanol, then the values of k_e (eq 12b) for **3** and **4** in ethanol would be >100 and 400 times larger, respectively, than the values in water. Such an acceleration is expected, since there should be less charge separation in the transition state for elimination of the ylide than in the dipolar ion (see below).

The second-order rate constant for ethoxide ion catalyzed elimination from **4** exceeds that for elimination from **3** at 45° by a factor of 2.4×10^3. This difference appears to be due largely to the difference between the $1/K_B$ values for these two alcohols, which was estimated above to be a factor of 2.5×10^2 in water. It is interesting that under conditions where the elimination reaction of **4** goes to completion, **2** is stable to elimination (Figure 2). Consequently, there is no facile intramolecular general base catalyzed pathway for elimination (eq 13). The value of K_B for ionization of the hydroxyl group of the dipolar ion of **2** can be estimated from the $K_B - \Sigma\sigma^*$ relationship for alcohols and is 0.4 *M*. This large value explains

(27) H. Goldschmidt and E. Mathiesen, *Z. Phys. Chem. Stoechiom. Verwandschaftslehre*, **119**, 439 (1926).

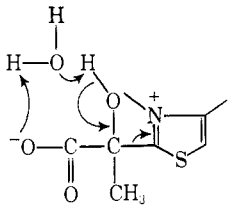
(28) R. P. Bell, *Advan. Phys. Org. Chem.*, **4**, 15 (1966).

(29) P. Ballinger and F. A. Long, *J. Amer. Chem. Soc.*, **82**, 795 (1960).

(30) M. Charton, *J. Org. Chem.*, **29**, 1222 (1964).

(31) R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, Chapter IV.

(32) See ref 14, p 756.



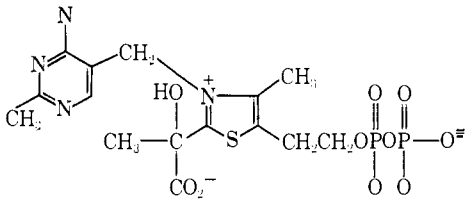
(13)

the lack of reactivity of **2** relative to **4**. The alcoholate anion itself from **2** would be expected to undergo the elimination reaction more rapidly than the dipolar ion of **4**, since the CO_2^- group is less electron withdrawing than CO_2Et.

The equilibrium constant for the addition of **1** to the keto function of ethyl pyruvate in water was not determined because of the competing hydrolysis of the ester function of **4**. However, since the equilibrium constant for the addition of water to the carbonyl group of methyl pyruvate in water (0.056 M^{-1}) is only slightly smaller than the equilibrium constant for the addition of ethanol to the carbonyl group of ethyl pyruvate in ethanol (0.12 M^{-1}), it seems likely that the equilibrium constant for the reaction of **1** with ethyl pyruvate in water is about the same as the value of 20 M^{-1} which was found for the reaction in ethanol. Consequently, the third-order rate constant for lyate ion catalyzed reaction of **1** with ethyl pyruvate is also approximately 4×10^4 times greater in ethanol than in water. This constant is equal to k_a/K_{B1} (eq 12), and the above discussion has shown that the solvent effect upon $1/K_{B1}$ is probably about 10^4. Thus, the rate constant for attack of the ylide upon the keto function, k_a, probably has about the same value in ethanol and water. This conclusion suggests that the transition state for the addition of the ylide to the keto group is not significantly more polar than the reactants and so probably very closely resembles the reactants. Such a structure for the transition state is consistent with the Hammond postulate:[33] the equilibrium constant for this reaction, k_a/k_e (eq 12b), is equal to $K_{add}K_{B1}/K_{B4}$, and on the basis of the above estimates for K_{B1} and K_{B4}, its value is roughly 10^{12} M^{-1} in water and 10^{10} M^{-1} in ethanol. If the value of pK_{a1} in ethanol is taken to be 20, the value of k_a at 25.9° is 1.8×10^9 M^{-1} min^{-1}. In the past 2-hydroxyalkylthiazolium compounds have been prepared by condensation of the carbonyl compound and thiazolium salt in slightly basic aqueous solution.[34] This analysis suggests that ethanol would be a better solvent in which to carry out the condensation.

The mechanism given for the elimination–addition reactions of **1** (eq 12) is exactly analogous to that which has been established for the addition of hydrogen cyanide to aldehydes to form cyanohydrins[35] and for base-catalyzed aldol condensations.[36,37] In some aldol condensations, when the concentration of aldehyde is high enough, the rate of reaction of the carbanion with the aldehyde carbonyl group (eq 12b) is greater than the rate at which it is protonated by the solvent (eq 12c).[36,37] Then the formation of the carbanion is the rate-determining step in the addition reaction and the protonation of it is the rate-determining step in the reverse direction. The rates of the elimination reactions of **3** and **4** were measured with low concentrations of **3** and **4** in the absence of added acetaldehyde or ethyl pyruvate. If protonation of the ylide had been rate determining under these conditions, the velocity of the reaction would have decreased more rapidly than does that of a pseudo-first-order reaction because the increasing concentration of the carbonyl compound would have depressed the concentration of the **1** ylide. Since the kinetics were first order, we have been correct in taking the breakdown of the dipolar alcoholate ion as the rate-determining step. When this step is rate determining, the ratio of the rate of protonation of the ylide by solvent to the rate of reaction of the ylide with 1 M carbonyl compound is equal to the ratio of the second-order rate constant for proton transfer from **1** to lyate ion to the third-order rate constant for lyate ion catalyzed reaction of **1** with the carbonyl compound.[36] Our data show that the value of this ratio for ethyl pyruvate in ethanol at 25–26° is roughly 25.

Relationship between the Model Reactions and Enzymatic Reactions. In our recent paper on the kinetics of decarboxylation of **2** we showed that the rate constant for the decarboxylation of 2-(1-carboxy-1-hydroxyethyl)thiamine pyrophosphate (**7**) in water at 30° is 10^5–10^6 times smaller than the turnover number of purified yeast pyruvate decarboxylase,[7] the value of which is 2340 mol of pyruvate/min/mol of enzyme-bound thiamine pyrophosphate at 30° and pH 6.[38]



7

Because there is evidence that the thiazolium group of thiamine pyrophosphate is bound in a hydrophobic region of pyruvate decarboxylase and because the dipolar ion species of **2** was found to decarboxylate 10^4–10^5 times more rapidly in ethanol than in water, we suggested that the catalysis can largely be explained as the result of the hydrophobicity of the active site. On the basis of the following considerations, the solvent effects which have been described here may be taken as a model for the effect which a hydrophobic active site would have upon the other steps in the enzymatic decarboxylation of pyruvate.

Since the basicity of ethoxide ion in ethanol is very nearly the same as that of hydroxide ion in ethanol,[39a] the rate of proton transfer from C-2 of **1** to ethoxide ion in ethanol is a correct model for the reaction of hydroxide ion with thiamine pyrophosphate in the hydrophobic active site. Also, it is evident

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that a comparison between rates in ethanol and water is a logical model in the case of the breakdown of the dipolar alcoholate anion and its reversal (eq 12b), which is a reaction that does not involve lyate ion. Finally, use of the ethanolic equilibrium constants for the formation of the dipolar alcoholate ion and the ylide from ethoxide ion and the corresponding conjugate acids (eq 12a and c) shows in a qualitative way the effect that a nonpolar active site would have upon the concentration of these reactive species. It should be recognized, however, that the analogy is not exact: the hydroxide ion that participates in the equilibrium between the enzyme-bound species ($E-CH^+$, $E-C^{\pm}$ in eq 14) is solvated by water, whereas the ethoxide ion that participates in the model equilibria (eq 12a and c) is solvated by ethanol, and it appears that the transfer of anions from water to ethanol is much more unfavorable than the transfer of cations.[39b]

$$HO^- + E{-}CH^+ \rightleftharpoons E{-}C^{\pm} + H_2O \qquad (14)$$

One of the pathways that can be envisioned for the formation of enzyme-bound **7** in the pyruvate decarboxylase reaction is the binding of pyruvate anion adjacent to the thiazolium ring, proton transfer from C-2 to the carboxylate anion of pyruvate, and addition of the ylide to the keto carbonyl group of the pyruvic acid.[7] The electrostatic interaction between the carboxylate and thiazolium groups would be a significant binding force in a hydrophobic environment. Attack of the ylide upon pyruvic acid should occur more readily than upon pyruvate anion. It is interesting to consider that if the pK_a values of pyruvic acid and C-2 of thiamine pyrophosphate were the same on the enzyme as they are in water (pK_a of pyruvic acid, 2.5; pK_a of thiamine is estimated on the above basis to be about 18), then the fraction of the holoenzyme–pyruvate complex which would be in the ylide–pyruvic acid form at pH 6 would be 3×10^{-16}. Thus, even if the rate constant for the addition of the ylide to the keto group were that of a molecular vibration (10^{15} min^{-1}), the observed first-order rate constant for the formation of enzyme-bound **7** would only be 0.3 min^{-1}, much less than the turnover number of 2340 min^{-1}. This calculation and similar ones for other hypothetical mechanisms for the formation of enzyme-bound **7** strongly suggest that the pK_a value for dissociation of C-2 of enzyme-bound thiamine pyrophosphate is markedly less than the value of about 18 which is expected in water.

The final step in the enzymatic reaction is the breakdown of enzyme-bound 2-(1-hydroxyethyl)thiamine pyrophosphate to form acetaldehyde.[2] 2-(1-Hydroxyethyl)thiamine pyrophosphate has been found to be stable for 2 hr in deuterium oxide at pD 8.6 and 60°;[40,41] our data indicate that the rate constant for elimination of **1** from the model compound **3** in water at pH 6 and 30° is less than 10^{-7} min^{-1}. Thus, the enzyme must accelerate the final step enormously. Even the rate constant of 3.3 min^{-1} for the breakdown of the dipolar alcoholate anion of **3** in ethanol at 46° (k_e, eq 12b), which can be crudely estimated from our results on the basis of the above discussion, is less than the turnover number of the enzyme by a factor of 10^3. This comparison suggests that the active site is probably less polar then ethanol and that the pK_a of the alcohol group of enzyme-bound 2-(1-hydroxyethyl)thiamine pyrophosphate may be as much as 5 units lower than the value of about 11 which is expected in water. Although this change in pK_a and that change which we suspect occurs in the pK_a of C-2 hydrogen of thiamine pyrophosphate upon binding to the enzyme are large, it is known that the pK_a values of imidazole and phenol groups buried in the hydrophobic interior of proteins may differ by more than 3 units from their values in water.[42,43]

Since all enzymatic reactions in which thiamine pyrophosphate is a coenzyme appear to be initiated by the addition of the ylide to a carbonyl group of the substrate and to end by the elimination of the ylide,[2] the accelerations in ethanol of the model reactions described herein are of general significance. It may be that catalysis in many thiamine pyrophosphate dependent enzymatic reactions is due in large part to the binding of the thiazolium portion of the coenzyme in a hydrophobic region of the proteins.

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