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Retrograde Aldol-Type Reactions Involving Thiamin in Aqueous Solution: Evidence for Changes in Transition-State Structure[1]

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Cleavage of racemic 2-(1-hydroxyethyl)- and 2-(1-hydroxyaryl)-3-R-4-methylthiazolium ions to the corresponding aldehyde and thiazolium ion in aqueous solution is catalyzed by oxygen-containing and amine buffer bases at 40°C and ionic strength 1.0 M (KCl). The buffer base-catalyzed reactions are formulated as general acid catalysis of the departure of thiazolium ion from the alcoholate anion of the substrate (general base catalysis of thiazolium ion attack on the aldehyde in the reverse direction). Brønsted α values decrease from 0.38 to 0.21 for general acid catalysis of the cleavage of (2-1-hydroxyethyl)-R-4-methylthiazolium ions (R = $C_6F_5CH_2$, 4-aminopyrimidinyl, Bzl). The decrease in α with decreasing pK_a of C(2)-H in the leaving thiazolium ion is described by a positive interaction coefficient $p_{xy'} = \partial\alpha/\partial pK_{lg} = 0.3 \pm 0.1$. Brønsted α values increase from 0.39 (R′ = Ph) to 0.48 (R′ = 4-CF_3-Ph) for the corresponding reactions with 2-(HOCH(R′))thiamin. The increase in α as the carbon electrophile becomes less stable is described by a positive interaction coefficient $p_{xy} = \partial\alpha/\partial\sigma_{para} = 0.2 \pm 0.1$. These positive interaction coefficients support a nonenforced concerted reaction mechanism with an important component of proton transfer in the transition state. Mechanistic implications for thiamin diphosphate-dependent enzymes are discussed.

INTRODUCTION

We previously described evidence that is inconsistent with a stepwise mechanism (Scheme 1, upper path) involving the free C(2)-ylide as a discrete intermediate for retrograde aldol-type reactions involving thiamin in aqueous solution and suggests that a nonenforced concerted mechanism (lower path) which avoids the relatively unstable C(2)-ylide is available for aldol-type addition reactions catalyzed by thiamin diphosphate (TDP)-dependent[3] enzymes (*1*). Most of this evi-

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[3] Abbreviations used: TDP, thiamin diphosphate; MBTH, 3-methyl-2-benzothiazolinone hydrazone hydrochloride; Me_2SO, dimethyl sulfoxide; HBT, 2-(1-hydroxybenzyl)thiamin; HET, 2-(1-hydroxyethyl)thiamin; 4-AP, 4-aminopyrimidinyl.

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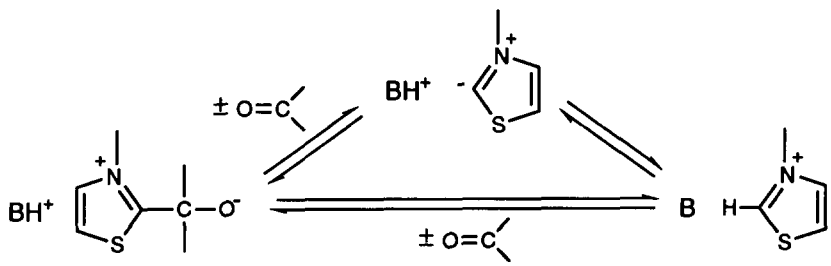


SCHEME 1

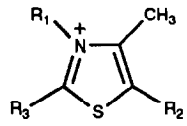
dence is based on the observation of buffer catalysis of the elimination reaction, but strong support for a concerted mechanism can also be obtained from the demonstration of an interaction between the effects of substituents in different parts of the substrate on the energy of the transition state. These interactions can be conveniently described by shifts in the position of the transition state on an energy contour diagram that is defined by the observed substituent effects (*2*). Interaction coefficients are useful for distinguishing between concerted and stepwise mechanisms (*3*), even when the structure of the transition state closely resembles the carbanion intermediate of the stepwise mechanism (*4*).

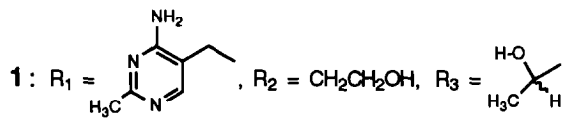
In this paper, we describe retrograde aldol-type reactions of the substrates **1–4**. We have measured the effects of catalyst acidity, leaving group (thiazolium ion) basicity, and increasing the energy of the electrophile (aldehyde) on the rate and have estimated the interactions between these substituent effects. The signs of the interaction coefficients that describe the changes in the substituent effects provide evidence for coupling between proton donation and leaving group expulsion; they confirm the concerted mechanism for these model elimination reactions. The results support the idea that stepwise and concerted mechanisms can coexist for substitution at carbon involving a relatively unstable nucleophile derived from a "normal" carbon acid (*1, 4*).

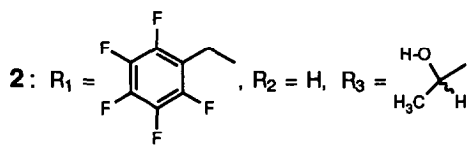
EXPERIMENTAL PROCEDURES

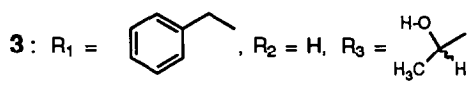
Materials. All chemicals were analytical or reagent grade and used as received unless stated otherwise. All water was prepared on a four-bowl Milli-Q water system including an Organex-Q cartridge (Millipore). 3-Methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) and semicarbazide hydrochloride were purchased from Aldrich. Thiamin chloride hydrochloride (Aldrich) was dried *in vacuo* at 80°C against P_2O_5 before use: mp 242–243°C dec. The synthesis of racemic 2-(1-hydroxyethyl)thiamin chloride hydrochloride (**1**) (*5*), 2-(1-hydroxyethyl)-3-pentafluorobenzyl-4-methylthiazolium bromide (**2**) (*6*), and 2-(1-hydroxyethyl)-3-benzyl-4-methylthiazolium iodide (**3**) (*6*) has been described.

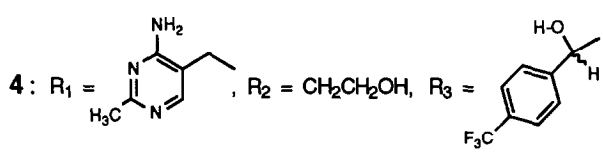
2-[1-Hydroxy-(4-(trifluoromethyl)benzyl)]thiamin chloride hydrochloride (**4**). In a manner similar to that shown for the synthesis of **1** (*5*), 250 mg (5%) of racemic

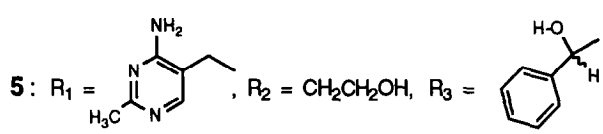












4 was obtained as a white solid: mp 183.7–185.4°C; ^{1}H NMR (Me_2SO) δ 2.31 (s, 3H), 2.39 (s, 3H), 3.04 (t, 2H), 3.69 (t, 2H), 5.52 (d, 1H), 5.94 (d, 1H), 6.65 (s, 1H), 7.34 (s, 1H), 7.57 (d, 2H), 7.80 (d, 2H), 8.25 (s, 1H), 9.10 (s, 2H). The product is unhydrated and free of NaCl on the basis of the calculated molecular weight obtained from a mercurimetric titration of chloride using diphenylcarbazone (*7*).

Methods. ^{1}H NMR spectra were recorded on a Bruker WM-300 NMR spectrometer using sodium 3-(trimethylsilyl)propanesulfonate as an internal standard. Melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. Measurements of pH were made at the reaction temperature on the buffered solutions of the thiazolium salts after the reaction had occurred as described previously (*1*).

Kinetics. Rate constants for cleavage of **1–4** in aqueous solution were determined from initial rate measurements, in which the reaction had proceeded ≤2%, at 40 ± 0.2°C with the ionic strength maintained at 1.0 M (KCl) throughout. The reaction of **4** was followed spectrophotometrically by trapping the 4-(trifuoromethyl)benzaldehyde formed with semicarbazide (0.02–0.04 M, pH 2.5–6.1) and following the increase in absorbance at 295 nm (*1*). The extinction coefficient of 4-(trifluoromethyl)-benzaldehyde semicarbazone is 1.72×10^4 M^{-1} cm^{-1} under the

reaction conditions in dilute aqueous solution and acetic acid/acetate buffers. The concentration of semicarbazide was such that there was no induction period in the initial rate of appearance of semicarbazone and was low enough for catalysis to be negligible; control experiments with double the concentration of trapping reagent were shown to give the same rate constants. Stock solutions of the trapping reagents were adjusted to the pH of the buffer before use.

Rate constants for the cleavage of **1–3** were determined by measuring acetaldehyde with a discontinuous colorimetric assay and MBTH. A kinetic run was initiated in a 4-ml screw-capped HPLC sample vial fitted with a Teflon septum by injecting 0.2–2.0 ml of a 0.2–40 mM substrate solution in H_2O into 2.0–3.8 ml of reaction mixture to give a volume of 4.0 ml and a final substrate concentration of 12–27 mM. The reaction solution was incubated in a constant-temperature bath (40 ± 0.2°C) and was removed for about 10 s every 0.5–12 h in order to obtain a 250-μl aliquot that was placed in a screw-capped 2.0-ml Eppendorf centrifuge tube, immediately frozen in liquid nitrogen to quench the reaction, and stored at −80°C. The quenched samples (5–10 time points) were thawed at 0°C in an ice-water bath and acetaldehyde at each time point was determined colorimetrically with MBTH at 670 nm using the procedure of Paz *et al.* (*8*) with freshly distilled acetaldehyde as the standard. Losses of acetaldehyde due to evaporation under the reaction, quenching, and storage conditions and increases in acetaldehyde concentration upon thawing of the quenched samples were shown to be insignificant.

The observed pseudo-first-order rate constant, k_{obsd}, was obtained by dividing the initial rate of appearance of aldehyde by the initial concentration of substrate. When duplicate determinations of k_{obsd} were made they agreed within ±5% of the average value. Second-order rate constants for general base-catalyzed cleavage were determined graphically (*9*); in the case of **1** and **4**, the values of k_{obsd} were corrected for the fraction of free and protonated N(1′). We estimate that the second-order rate constants are accurate to within ±10% based on the maximum and minimum slopes that could be drawn in these plots.

RESULTS

The kinetics of the cleavage of 2-(1-hydroxyethyl)- and 2-(1-hydroxyaryl)-3-R-4-methylthiazolium ions (**1–4**) to 3-R-4-methylthiazolium ions and aldehyde in aqueous solution were followed by trapping the aldehyde with semicarbazide at 295 nm or MBTH at 670 nm under initial rate conditions. Identical initial rates for the release of aldehyde were measured using ^{1}H NMR as described by Gallo and Sable (*10*).

As reported previously for 2-(1-hydroxybenzyl)thiamin (HBT) (**5**) (*1*), the cleavage reactions of **1–4** are catalyzed by oxygen-containing and amine buffers and follow the rate law described by Eq. [1]. The rate constants for catalysis of the cleavage reaction by the basic

$$v/[\mathrm{ROH}] = k_{obsd} = k_{HO^-}[HO^-] + k_B[B] + k_{HOH} \qquad [1]$$

species of buffers were determined as described under Experimental Procedures;

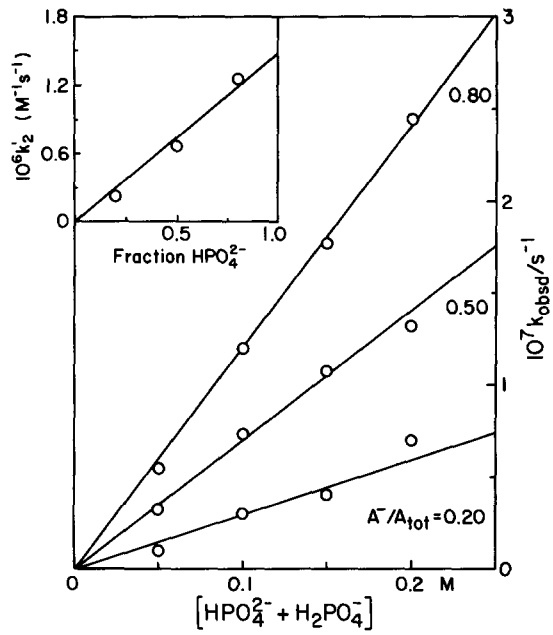


FIG. 1. Dependence of the observed rate constant for cleavage of N(1')-unprotonated 2-(1-hydroxyethyl)thiamin (**1**) on the total concentration of phosphate buffer containing 20, 50, and 80% phosphate dianion in aqueous solution at 40°C, I = 1.0 M (KCl). The slope of the plot against buffer concentration gives the apparent rate constant of the phosphate dianion-catalyzed reaction, k_2' ($= k_{obsd} - k_0[\text{buffer}]_{tot}$). Inset: dependence of the apparent catalytic constant for buffer catalysis, k_2', on the composition of the phosphate buffer.

no general acid catalysis was detected. Representative data are shown in Fig. 1 for catalysis of the cleavage of N(1')-unprotonated 2-(1-hydroxyethyl)thiamin (HET) (**1**) by phosphate dianion. Rate constants for cleavage of **1–4** catalyzed by a series of different buffer bases are summarized in Table 1.

Experiments to measure general base catalysis were generally performed at relatively high total buffer concentrations of ≤0.4 M in order to obtain maximal rate increases (≈100%) from general base catalysis. Even though the ionic strength was kept constant at 1.0 M with potassium chloride, it is important to assess the effects of varying $[M^+B^-]$ and [BH] when working with high concentrations of buffer components. The following points suggest that medium effects, if present, are small: (i) The rate constant for catalysis by acetate ion or HPO_4^{2-} is not sensitive to variations in the nature or concentration of salts used to maintain the ionic strength at 1.0 M; there is no significant change in k_B (≤5%) upon substituting potassium trifluoroacetate for potassium chloride to maintain the ionic strength. (ii) We examined the effect of adding small organic molecules to the reaction medium with 0–3 vol% acetonitrile, which corresponds to 0–1.0 M organic solvent in the reaction. There is no significant change (≤5%) in the value of k_B for catalysis by acetate ion with increasing organic solvent in the reaction medium. This suggests that there is no significant effect of the acid component of the buffer on the

TABLE 1

Rate Constants for General Base Catalysis of the Cleavage of 2(1-Hyroxyethyl)- and 2-(1-Hydroxyaryl)-3-R-4-Methylthiazolium Ions[a].

Catalyst	pK_a'[b]	log k_B(M^{-1} s^{-1})				
		1	**2**	**3**	**4**	**5**[c]
H_2O	−1.74				−9.04	−9.75
$NCCH_2COO^-$	2.35				−7.07	−7.40
$ClCH_2COO^-$	2.74				−7.00	
$CH_3OCH_2COO^-$	3.50				−6.60	−6.96
$HCOO^-$	3.65				−6.26	−6.41
CH_3COO^-	4.65	−7.36	−7.08	−8.15	−5.85	−6.00
$CF_3CH_2NH_2$	5.46[d]	−6.60	−6.29	−7.54		
$(CH_3)_2AsO_2^-$	6.23					−5.04
HPO_4^{2-}	6.47	−5.85	−5.33	−6.70		−4.70
$H_2NCH_2CH_2NH_3^+$	7.18	−5.37	−4.82	−6.42		
$H_2NCH_2CH_2NH_2$	9.64	−3.60		−4.92		

[a] At 40°C and ionic strength 1.0 M (KCl) in H_2O. The rate constant k_B is defined in Eq. [1].

[b] Apparent pK_a of the conjugate acid at 40°C and ionic strength 1.0 M (KCl) in H_2O.

[c] Ref. (*1*).

[d] Calculated by subtracting (0.016)(15) = 0.24 from pK_a = 5.70 at 25°C to correct for the pK_a decrease of a monoacidic nitrogenous base when the temperature is raised by 15°C (*11*).

rate constants for general base catalyzed cleavage of **1–4**. (iii) No significant curvature at high buffer concentration was observed in plots of k_{obsd} against [buffer] (see Fig. 1). (iv) The amounts of buffer catalysis observed are different for **1–4**. A medium effect would be expected to cause similar or identical changes in the rate constants for the different substrates.

DISCUSSION

Evidence for a class n mechanism. Figures 2 and 3 show Brønsted plots for general base catalysis of the cleavage reactions of **1–5** by oxygen-containing and amine buffers. The slopes in Fig. 2A for compounds **1–3** are in the range β = 0.62–0.79 (Table 2) and increase with increasing acidity and increasing leaving ability of C(2)-H in the N(3)-substituted thiazolium ion. The slopes in Fig. 3A for compounds **4** and **5** are in the range β = 0.61–0.52 (Table 2) and decrease as the 4-substituted benzaldehyde electrophile becomes less stable. The ≤two-fold deviations of the second-order rate constants for general base catalysis about a single Brønsted line defined by the heterogeneous set of buffers in Fig. 2A and 3A are not unusual, are randomly scattered about the Brønsted line, and provide no evidence that a unique Brønsted correlation is required for each buffer class.

The change in β with decreasing pK_a of the leaving group and decreasing stability of the carbon electrophile provides the strongest direct experimental evidence for

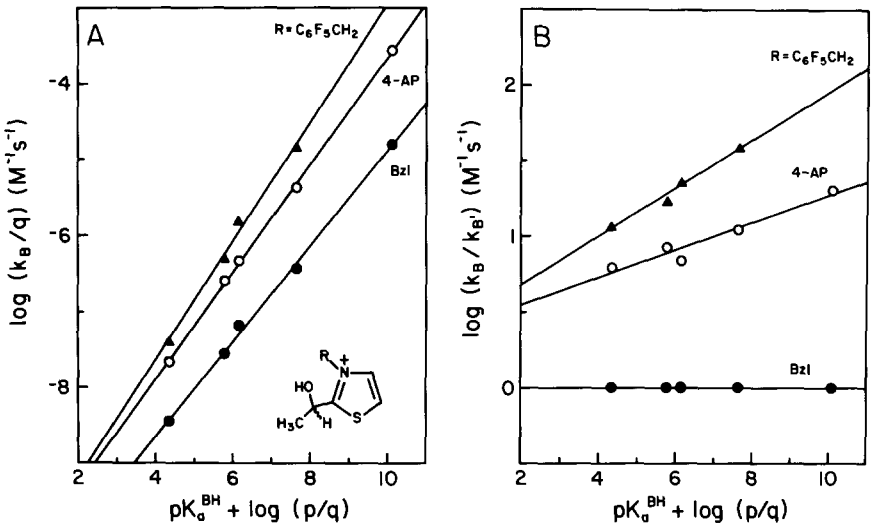


FIG. 2. The effect of stabilizing the carbon acid (leaving group). (A) Brønsted plots for general base catalysis of the cleavage of 2-(1-hydroxyethyl)-3-pentafluorobenzyl-4-methylthiazolium ion (**2**) (pK_{lg} = 17.6) (▲), N(1′)-unprotonated 2-(1-hydroxyethyl)thiamin (**1**) (pK_{lg} = 18.0) (○), and 2-(1-hydroxyethyl)-3-benzyl-4-methylthiazolium ion (**3**) (pK_{lg} = 18.2) (●). Statistical corrections were made according to Bell and Evans (*12*). (B) The change in the dependence of the rate constants, k_B, for decomposition of **1** (○) and **2** (▲) in aqueous solution catalyzed by oxygen-containing and amine buffers, relative to the rate constant k'_B, for decomposition of **3** (●), on the pK_a of the buffer catalyst. 4-AP, 4-aminopyrimidinyl substituent of thiamin (see text). The lines are least-squares fits to the data.

a coupled, concerted class *n* reaction mechanism (Scheme 2).[4] The change in β with a change in the substituent at N(3) or C(α) in the thiazolium ion is most clearly shown by the correlation of the ratios of rate constants for different substituents with the pK_a of the base catalyst, as shown in Figs. 2B and 3B. If there were no change in β with the different leaving groups and different electrophiles the lines in these figures would be parallel, with slope zero. The changes in β with decreasing pK_a of the leaving group and decreasing stability of the carbon electrophile are in the direction expected for a class *n* mechanism involving the kinetically equivalent *general acid catalysis* of the breakdown of the *alcoholate anion* of **1–5** (*1, 2, 13*). The changes in β are in the opposite direction from that expected for general base catalysis between the catalyst and the oxygen atom in a class *e* mechanism (*2*).

In a class *n* mechanism there is a rapid equilibrium dissociation of the thiazolium carbinol hydroxyl proton ($pK_a \geq 10$) (*1*), followed by concerted leaving group expulsion coupled to partial proton donation to this group in the transition state.

[4] The problem of assigning the developing lone pair of electrons formed at C(2) upon carbon–carbon bond cleavage in the proposed transition state of Scheme 2 has been discussed (*1*): proton transfer to the sp^2-hybridized orbital that is involved in the C–C bond that undergoes cleavage is unlikely because of unfavorable steric requirements. Formation of a termolecular complex in solution does not imply a termolecular collision.

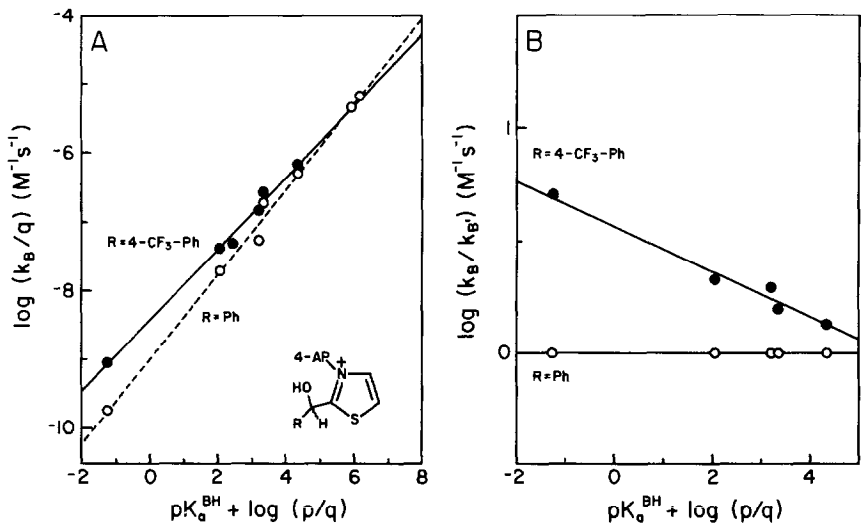


FIG. 3. The effect of destabilizing the aldehyde (electrophile). (A) Brønsted plots for general base catalysis of the cleavage of N(1')-protonated 2-[1-hydroxy-(4-(trifluoromethyl)benzyl)]thiamin (**4**) ($\sigma_{para} = 0.53$) (●, solid line) and N(1')-protonated 2-(1-hydroxybenzyl)thiamin (**5**) ($\sigma_{para} = 0$) (○, dashed line) (*1*). Statistical corrections were made according to Bell and Evans (*12*). (B) The change in the dependence of the rate constants, k_B, for decomposition of **4** (●) in aqueous solution catalyzed by oxygen-containing and amine buffers, relative to the rate constant, k'_B, for decomposition of **5** (○), on the pK_a of the buffer catalyst. 4-AP, 4-aminopyrimidinyl substituent of thiamin (see text). The lines are least-squares fits to the data.

TABLE 2

Brønsted β Values for the General Base-Catalyzed Cleavage of 2-(1-Hydroxyethyl)- and 2-(1-Hydroxyaryl)-3-R-4-Methylthiazolium Ions

Substrate	pK_{lg}[a]	σ_I[b]	σ_{para}[c]	β[d]	$\alpha(= 1 - \beta)$	ρ_I[e]	p_{xy}[f]	p_{xy}[g]
2	17.6	0.11		0.79	0.21			
1	18.0	0.07		0.71	0.29	16	0.3	
3	18.2	0.04		0.62	0.38			
5			0	0.61[h]	0.39			0.2
4			0.53	0.52	0.48			

[a] Apparent pK_a of the C(2) proton in the 3-R-4-methylthiazolium ion (leaving group) at 30°C and ionic strength 2.0 M (NaCl) in H_2O (*15*).

[b] σ_I for the N(3)-substituent in the thiazolium ion (*16*).

[c] σ_{para} for the 4-substituent in 4-R-benzaldehyde (electrophile) (*17*).

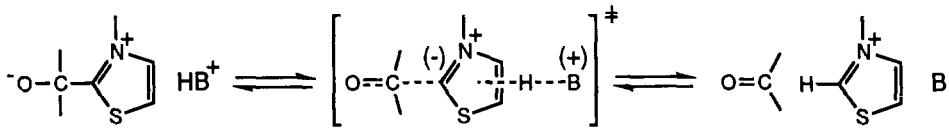
[d] Slopes of lines in Figs. 2A and 3A. We estimate that these values are accurate to within ±0.04.

[e] Calculated using $\partial \log k_B/\partial\sigma_I$ with values of log k_B listed in Table 1 for catalysis by CH_3COO^-, $CF_3CH_2NH_2$, HPO_4^{-2}, and $H_2NCH_2CH_2NH_3^+$ We estimate that this value is accurate to within ±3.

[f] Defined as $\partial\alpha/\partial pK_{lg}$ [See text and Ref. (2)].

[g] Defined as $\partial\alpha/\partial\sigma_{para}$ [see text and Ref. (2)].

[h] Ref (*1*).



SCHEME 2

A concerted mechanism for cleavage of **1–5** is supported by the observed Brønsted slopes in the range $\beta = 0.52–0.79$, which are equivalent to values of $\alpha = 1 - \beta$ in the range 0.48–0.21 (Table 2) for catalysis by BH^+; a stepwise mechanism with a rate-limiting thermodynamically unfavorable proton transfer from thiazolium C(2)-H to form the C(2)-ylide would give an α value approaching zero for rate-limiting diffusion-controlled protonation of the C(2)-ylide in the cleavage direction. A class *n* mechanism was proposed previously for the buffer base-catalyzed reactions of **5** (*1*) because (i) addition–elimination reactions of the carbonyl group and strongly basic nucleophiles, like the C(2)-ylide, are not subject to general acid–base catalysis at the carbonyl oxygen atom (*3*), and (ii) a thermodynamically unfavorable rate-limiting proton transfer to form the alcoholate anion should give a Brønsted slope of $\beta = 1.0$ for general base catalysis rather than the observed slope of $\beta = 0.61$, which shows that the alcoholate anion is at equilibrium and the formation of the alcoholate anion cannot be rate determining.

The observation of general base catalysis means that the transition state for cleavage of **1–5** is stabilized by proton transfer driving the reaction or by simple hydrogen bonding, or "solvation" (*18*), or both. The observed values of β in the range 0.52–0.79 are much larger than the calculated maximum value of $\beta \approx 0.23$ for hydrogen bonding between electronegative atoms (*1*, *19*) and are outside of the range of values for α and β (in the range 0.06–0.26) for stabilization of the transition state by hydrogen bonding to a general-acid or -base catalyst (*20*). Because the observed catalysis is much larger than can be attributed to hydrogen bonding for a reaction involving carbon as a hydrogen bond donor–acceptor (*1*), only a small fraction of the catalysis can be explained by stabilization of the developing negative charge on the C(2)-ylide by hydrogen bonding in aqueous solution. We conclude that concerted proton donation to the leaving C(2)-ylide withdraws electrons from carbon and provides the driving force for cleavage of the C–C bond. The reverse, aldol-type condensation reaction involves the removal of the C(2)-proton from the attacking thiazolium C(2)-position by the conjugate base of the catalyst, as it attacks the carbonyl group.

Evidence for changing transition-state structure. The decrease in the calculated values of α (Table 2) with increasing acidity of the leaving groups, in the cleavage of **1** and **2** compared with **3**, is described by $p_{xy'} = \partial\alpha/\partial pK_{lg} = 0.3 \pm 0.1$ (*2*). The increase in the calculated values of α as the carbon electrophile (4-R-benzaldehyde) becomes less stable, in the cleavage of **4** (R = CF_3) compared with **5** (R = H), is described by $p_{xy} = \partial\alpha/\partial\sigma_{para} = 0.2 \pm 0.1$ (*2*). The $p_{xy'}$ and p_{xy} values for this small series of substrates are not of high precision but the *positive values* of $p_{xy'}$ and p_{xy} reflect the role of C(2) in the leaving thiazolium ion as the central group in the

transition state, with a net charge that is determined by the relative amounts of proton transfer from the general acid and bond formation to carbon. This reaction is analogous to the addition and loss of ROH at a carbonyl group and involves electrophilic displacement on the central atom, C(2) in this case, by the proton in one direction or by the carbonyl group in the other. We conclude that the cleavage reactions of this series of substrates exhibit coupling between proton donation and leaving group explusion in the transition state that is described by the positive interaction coefficients $p_{xy'}$ and p_{xy}.

A change in α with a change in substituents on the leaving group or electrophile can result from an electrostatic interaction between the catalyst and the reactant, without changes in the amount of bond formation and cleavage in the transition state, but this change is generally small and often opposite in direction from observed $p_{xy'}$ and p_{xy} coefficients (*2*). A negative p_{xy} coefficient of -0.10 was attributed to an electrostatic interaction between an electron-donating substituent on the alcohol and an electron-withdrawing substituent on the phenyl ring in the addition of alcohols to substituted 1-phenylethyl carbocations (*21*). If such an electrostatic interaction is significant in the reactions described here the observed p_{xy} coefficient is an underestimate. Although an electrostatic interaction between a polar substituent on one reactant with a charge or dipole on another that stabilizes the transition state relative to the separated reactants ("Hine effect") undoubtedly contributes to the observed $p_{xy'}$ coefficient (*22*), it is unlikely that the contribution of $p_{xy'}$ (electrostatic) is much larger than the value of 0.024 originally suggested by Hine (*22c*), so that another explanation is required for the greater part of the observed $p_{xy'}$ coefficient of ≈ 0.3. Although **1**, **4**, and **5** have a different C(5)-substituent ($R_2 = CH_2CH_2OH$) than **2** and **3** ($R_2 = H$), it is unlikely that differences in electronic and steric effects of these C(5)-substituents significantly affect the cleavage rate. Changing the C(5)-substituent in a thiazolium ion from R = CH_2CH_2OH to R = H has no affect on the rate of C(2)-proton transfer (*15*), C(α)-proton transfer (*6*), or nucleophilic attack at C(2) by water or hydroxide ion (*16b*).

The nonenforced ("free choice")[5] concerted cleavage reactions of **1–5** may be described by the reaction coordinate-energy diagram of Fig. 4, in which the horizontal axis represents proton transfer (α) and the vertical axis represents C–C bond formation and cleavage (ρ) (*2, 13*). The x coordinate of the transition state at the saddle point is defined by the calculated values of α (Table 2), but the y coordinate is not as well established. The Hammett ρ_I value of 16 ± 3 for cleavage of a small series of 2-(1-hydroxyethyl)-3-R-4-methylthiazolium ions (**1–3**) (Table 2) is not of high precision but does indicate that there is a significant amount of negative charge development on the leaving C(2) carbon atom in the transition state. The contour lines are not shown in Fig. 4.

The positive $p_{xy'}$ coefficient is consistent with a diagonal reaction coordinate on the diagram and requires a reaction coordinate with an important vertical component (*2*). Stabilization of the C(2)-ylide will lower the energy of the C(2)-ylide in the lower right corner of the diagram, as shown by the heavy arrows in Fig.

[5] The C(2)-ylide has a short, but significant, lifetime in aqueous solution in the presence of buffer acids and the concerted mechanism is not enforced ("determined") by the presence of buffer acids (*1*).

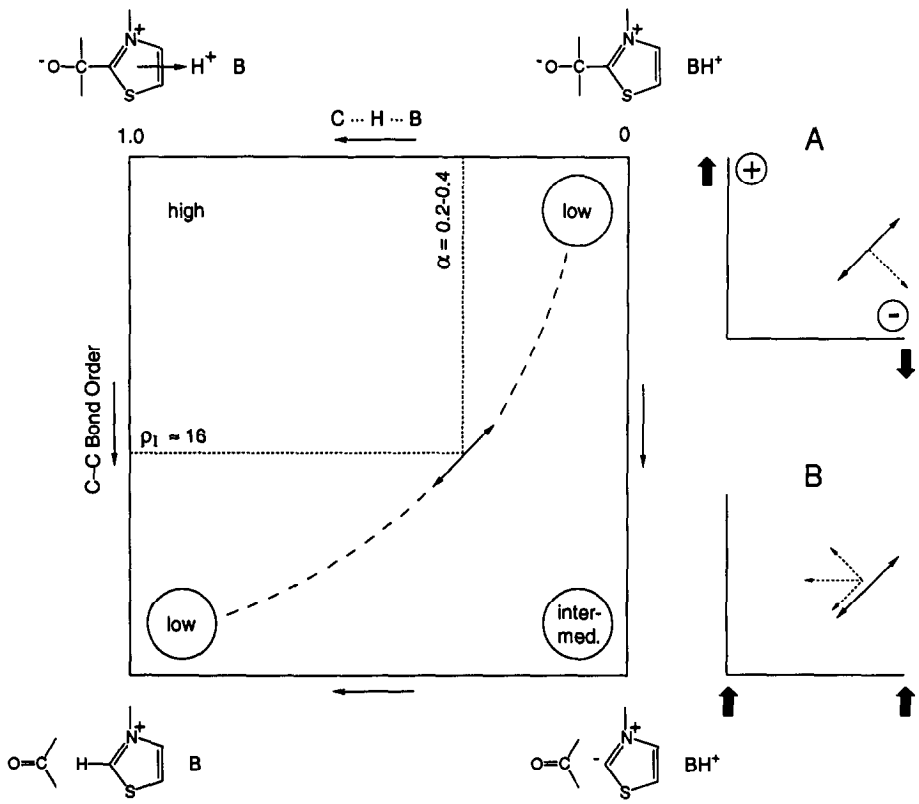


FIG. 4. Reaction coordinate-energy contour diagram for general acid catalysis of the decomposition of N(3)-substituted thiazolium carbinols to the corresponding thiazolium ion and aldehyde in aqueous solution (general base catalysis of thiazolium ion attack on the aldehyde in the reverse direction) (class *n* mechanism). The *x* and *y* axes represent proton transfer and C–C bond formation/breaking as measured by α and ρ_I (Table 2), respectively. The approximate position of the transition state at the saddle point is indicated by the double-headed arrow. Transfer of electron density from the aromatic system to the proton is represented by the cationic complex in the upper left corner; this complex may involve interactions of the proton with antibonding σ^* orbitals or the sulfur atom of the thiazolium ion, or formation of an "Hπ bond" between the proton and the π electrons of the thiazolium ring (*1*).[4] The reactants, products, and C(2)-ylide are in potential wells: the cationic complex in the upper left corner may be too unstable to exist in a potential well (*13*). The effect of increased acidity of C(2) in the thiazolium ion is shown in A and the effect of destabilization of the aldehyde in B.

4A, so that the transition state will tend to slide downhill toward the C(2)-ylide, perpendicular to the reaction coordinate in an "anti-Hammond" direction. The movement of the transition state toward the right corresponds to a decrease in the amount of proton transfer and α, as observed. The same behavior has been observed previously for the general acid-catalyzed explusion of water and alcohols from carbonyl and related addition compounds (*3*) and general acid-catalyzed cleavage of cyclopropanol anions (*13*).

As the carbon electrophile becomes less stable there is a tendency for the

transition state to move toward the left with an increase in the Brønsted α value, as described by the positive p_{xy} coefficient (*2*). Increasing the energy of the electrophile at the bottom of the diagram will tend to shift the transition state toward the lower left corner, parallel to the reaction coordinate, and toward the upper left corner, perpendicular to the reaction coordinate, to give the observed net movement toward the left as shown in Fig. 4B. This net movement corresponds to an increase in the amount of proton transfer and α for acid catalysis of the expulsion of C(2)-H.

The absence of significant curvature in the Brønsted plots shown in Fig. 2A and 3A, which corresponds to $p_x = \partial\alpha/\partial \mathrm{p}K_a^{\mathrm{BH}} \approx 0$ in the class *n* mechanism of Scheme 2, is also consistent with a concerted or coupled reaction mechanism, in which C–C bond cleavage and proton transfer are both occurring in the transition state (*2*).

The large value of the $p_{xy'}$ interaction coefficient is expected if there is a small intrinsic barrier for group transfer (either H or carbonyl) from the C(2)-position of thiazolium ions and small curvature of the reaction coordinate, so that there is a large change in the position of the transition state when the stability of a reactant is varied by a small amount (*23*). Although the precision of $p_{xy'}$ is limited by the range of $\mathrm{p}K_{\mathrm{lg}}$ values that is experimentally accessible for these substrates in aqueous solution, the value of $p_{xy'} \approx 0.3$ can be compared to the relatively large values of $p_{xy'} \approx 0.1$ for cleavage of acetaldehyde hemiacetals (*3*) and formaldehyde hemiacetals (*24*). This value of $p_{xy'}$ is consistent with a large "anti-Hammond effect," or substantial movement of the transition state perpendicular to the reaction coordinate as the leaving ability of the thiazolium ion is varied (*2*). A small intrinsic barrier and a small curvature of the reaction coordinate for group transfer reactions involving the C(2) position of thiazolium ions is supported by the value of $p_{xy} = 0.31 \pm 0.09$ for C(2)-T $\rightarrow$ H exchange from thiazolium ions (*23*). This value of $p_{xy} = 0.31$ is much larger than the value of 0.053 for proton transfer from substituted phenylacetonitriles, which are carbon acids with comparatively large intrinsic barriers (*25*).[6,7]

Implications for enzyme-catalyzed reactions. These results confirm and extend the previous conclusion that one of the several mechanisms that are utilized by TDP-dependent enzymes to catalyze aldol-type reactions may involve assistance to proton removal by interaction with an electrophile in the transition state, and assistance to carbon–carbon bond cleavage and formation by a significant amount of proton transfer in the transition state (*1*). This mechanism would avoid the formation of the relatively unstable C(2)-ylide as a discrete intermediate and the transition state leading to its formation (*1*, *15*). Enzymatic stabilization of the C(2)-

[6] There are large intrinsic barriers for reactions involving nitro- and carbonyl-activated carbanions that do not involve proton removal; these barriers are much smaller for nitriles, which also have smaller intrinsic barriers for proton transfer (*26*).

[7] The values of $p_{xy} = 0.31$ for C(2)-hydron exchange and $p_{xy} \approx 0.2$ for cleavage of **4** and **5** are not directly comparable without being converted ("normalized") to a common energy scale using $\partial\sigma_{\mathrm{norm}} = \partial\sigma\rho_{\mathrm{eq}} = \partial \log K$ (*3*). The values of p_{xy} have not been normalized because accurate experimental values of the equilibrium constant, K, for the formation of thiazolium carbinols derived from 4-substituted benzaldehydes are not currently available.

ylide (or destabilization of TDP) in a concerted mechanism could serve to facilitate proton transfer rather than simply increase the lifetime of the C(2)-ylide for a stepwise mechanism.

The results also suggest that addition–elimination reactions involving the C(2)-ylide that occur during the turnover of TDP-dependent enzymes have a very small intrinsic barrier. This is important because a small barrier has a small curvature that allows the position of the transition state to slide easily over the energy surface as the energy of the reactants, products, or intermediates is changed, which gives a change in transition-state structure (*23*). A change in transition-state structure which leads to a progressive increase in the importance of general acid catalysis would allow a larger potential advantage from general acid catalysis in an enzymatic reaction (*27*). For example, the decrease in α for general acid catalysis of the expulsion of ROH as the electrophile becomes more stable, as occurs for the formation of oxocarbonium ions in the acid-catalyzed cleavage of acetals (*3*), leads to a progressive increase in the importance of general acid catalysis.

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REFERENCES

1. CRANE, E. J. III, AND WASHABAUGH, M. W. (1991) *Bioorg. Chem.* **19,** 351–368.
2. JENCKS, W. P. (1985) *Chem. Rev.* **85,** 511–527 and references cited therein.
3. SØRENSEN, P. E., AND JENCKS, W. P. (1987) *J. Am. Chem. Soc.* **109,** 4675–4690 and references cited therein.
4. See, for example, BANAIT, N. S., AND JENCKS, W. P. (1990) *J. Am. Chem. Soc.* **112,** 6950–6958.
5. STIVERS, J. T., AND WASHABAUGH, M. W. (1990) *Bioorg. Chem.* **18,** 425–434.
6. STIVERS, J. T., AND WASHABAUGH, M. W. (1992) *Bioorg. Chem.* **20,** 155–172.
7. SCHALES, O., AND SCHALES, S. S. (1941) *J. Biol. Chem.* **140,** 879–884.
8. PAZ, M. A., BLUMENFELD, O. O., ROJKIND, M., HENSON, E., FURFINE, C., AND GALLOP, P. M. (1965) *Arch. Biochem. Biophys.* **109,** 548–559.
9. JENCKS, W. P. (1987) Catalysis in Chemistry and Enzymology, pp. 577–585, Dover, New York.
10. GALLO, A., AND SABLE, H. Z. (1976) *J. Biol. Chem.* **251,** 2564–2570.
11. ALBERT, A., AND SERJEANT, E. P. (1984) The Determination of Ionization Constants, 3rd ed., pp. 11–12, Chapman and Hall, London.
12. BELL, R. P., AND EVANS, P. G. (1966) *Proc. R. Soc. London, A* **291,** 297–323.
13. THIBBLIN, A., AND JENCKS, W. P. (1979) *J. Am. Chem. Soc.* **101,** 4963–4973.
14. (a) DO AMARAL, L., SANDSTROM, W. A., AND CORDES, E. H. (1966) *J. Am. Chem. Soc.* **88,** 2225–2233; (b) Ref. (9), p. 198.
15. WASHABAUGH, M. W., AND JENCKS, W. P. (1988) *Biochemistry* **27,** 5044–5053.
16. (a) CHARTON, M. (1981) *Prog. Phys. Org. Chem.* **13,** 119–251; (b) WASHABAUGH, M. W., YANG, C. C., HOLLENBACH, A. D., AND CHEN, P., submitted for publication.
17. PERRIN, D. D., DEMPSEY, B., AND SERJEANT, E. P. (1981) pK_a Prediction for Organic Acids and Bases, pp. 109–126, Chapman and Hall, London.
18. SWAIN, C. G., KUHN, D. A., AND SCHOWEN, R. L. (1965) *J. Am. Chem. Soc.* **87,** 1553–1561.

19. STAHL, N. S., AND JENCKS, W. P. (1986) *J. Am. Chem. Soc.* **108,** 4196–4205.
20. JENCKS, W. P. (1980) *Acc. Chem. Res.* **13,** 161–169.
21. ROTHENBERG, M. E., RICHARD, J. P., AND JENCKS, W. P. (1985) *J. Am. Chem. Soc.* **107,** 1340–1346.
22. (a) HINE, J. (1959) *J. Am. Chem. Soc.* **81,** 1126–1129; (b) HINE, J. (1972) *J. Am. Chem. Soc.* **94,** 5766–5771; (c) See Ref. (*26*) in FUNDERBURK, L. H., AND JENCKS, W. P. (1978) *J. Am. Chem. Soc.* **100,** 6708–6714.
23. WASHABAUGH, M. W., AND JENCKS, W. P. (1989) *J. Am. Chem. Soc.* **111,** 683–692.
24. PALMER, J. L., AND JENCKS, W. P. (1980) *J. Am. Chem. Soc.* **102,** 6472–6481.
25. BERNASCONI, C. F., AND HIBDON, S. A. (1983) *J. Am. Chem. Soc.* **105,** 4343–4348.
26. (a) GILBERT, H. F. (1980) *J. Am. Chem. Soc.* **102,** 7059–7065; (b) BERNASCONI, C. F., AND KANAVARIOTI, A. (1986) *J. Am. Chem. Soc.* **108,** 7744–7751.
27. HERSCHLAG, D., AND JENCKS, W. P. (1987) *J. Am. Chem. Soc.* **109,** 4665–4674.