Thiazolium C(2)-Proton Exchange: General-Base Catalysis, Direct Proton Transfer, and Acid Inhibition[1]

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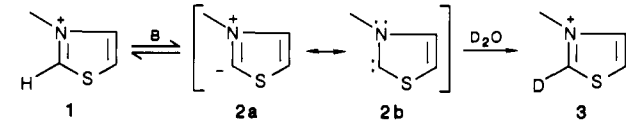
Abstract: Rate constants for C(2)-L → D exchange from 3,4-dimethylthiazolium ion, N(1')-protonated thiamin, and 3-(cyanomethyl)-4-methylthiazolium ion were determined by ^{1}H NMR and detritiation in carboxylate and amine buffers at 30 °C and ionic strength 2.0 M in D_2O. Thermodynamically unfavorable hydron transfer from thiazolium C(2)-L (pK_a = 16.9-18.9) to oxygen-containing bases ($RCOO^-$ and RO^-) and amines shows general-base catalysis and follows almost completely normal "Eigen curves" with Brønsted β values of ≥0.95; the thermodynamically favorable reverse protonation reaction has a Brønsted α value of ≤0.05. This is consistent with diffusion-controlled protonation of the C(2) ylide by most acids. General-base catalysis is detectable because there is a small negative deviation from this correlation for deuterioxide ion. Values of k_H/k_T in the range 1.2 ± 0.2 for C(2)-L → D exchange catalyzed by buffer bases are in the range expected for rate-limiting diffusional separation of buffer acids from the C(2) ylide in the rate-limiting step, as expected for "normal" acids. The absence of strong inhibition by D_3O^+ of C(2)-H → L exchange from 3-(cyanomethyl)-4-methylthiazolium ion is inconsistent with proton transfer through water according to the Swain-Grunwald mechanism. The secondary solvent deuterium isotope effect of k_{H_2O}/k_{D_2O} = 2.8 for transfer of C(2)-H to water indicates that the initial product is HOL_2^+, and not $HOL{\cdot}L_3O^+$, that undergoes diffusion-controlled separation. It is concluded that hydrogen bonding of solvent to the carbanion is weak and that strong hydrogen bonding in the transition state is not necessary for rapid proton transfer. The observed rate constants for C(2)-H → L exchange from 3-(cyanomethyl)-4-methylthiazolium ion were used to define an H_0 acidity function for the ionization of this thiazolium ion in aqueous HCl solutions.

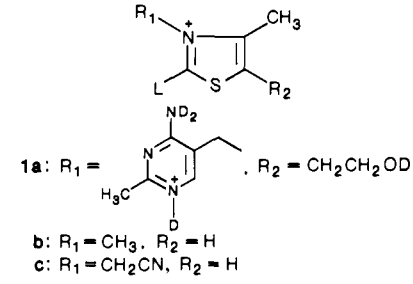
In spite of the importance of proton-transfer reactions in chemistry and biochemistry and the large amount of research that has been performed in this area, there are still several aspects of these reactions that are incompletely understood. A particularly important mechanistic question is the problem of what determines whether proton transfer is fast or slow.[2]

Proton transfer from carbon and the electronegative atoms of "normal" acids differs in that proton transfer from carbon is usually much slower and is generally believed to occur directly, rather than through a hydroxylic solvent molecule.[3-5] The main reason for slow proton transfer from carbon is the low acidity of most carbon acids because of the low electronegativity of carbon.[6] Some functional groups that decrease the pK_a of carbon acids also tend to make the transition state more complex and less stable; this increase in transition state complexity is accompanied by a decreased reaction rate.[2,3b] The behavior of substituted acetylenes, HCN, and other carbon acids that give highly localized carbanions supports the conclusion that delocalization and the accompanying structural and solvation changes are responsible for the slow rates and large intrinsic barriers for proton transfer from most carbon acids.[3c,7,8] Hydrogen bonding in the transition state may stabilize the transition state, but this stabilization appears to be small for carbon compared with more electronegative atoms.[9]

Scheme I



The problem for biochemistry is illustrated by the coenzyme thiamin (**1a**). Several thiamin-dependent enzymes catalyze aldol-type addition reactions between thiamin pyrophosphate (R_2 = $CH_2CH_2OP_2O_6H_3$) and carbonyl compounds;[10] the base-catalyzed abstraction of the C(2) proton from the thiazolium ring of thiamin forms a reactive thiazolium ylide (**2**; Scheme I), which is both a potent carbon nucleophile and a reasonably stable leaving group.[11] Thiazolium ions are especially simple carbon acids that presumably require relatively little of the electron delocalization



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by resonance and the resulting changes in geometry and solvation of delocalized charge that are believed to be responsible for the slow ionization of most carbon acids;[3c,7,8] changes in bond lengths and angles are restricted by the five-membered ring. We are interested in the factors that contribute to the kinetic barriers for C(2)-proton transfer from thiamin, which is required for functioning of the coenzyme.

In this and the subsequent paper in this issue we describe an examination of hydron[12] transfers involving thiamin (**1a**) and related thiazolium ions (**1b,c**) in aqueous solution. The results indicate that the behavior of thiazolium ions is similar, but not identical, to that of normal acids; thiazolium ions undergo proton loss with a minimal intrinsic barrier and C(2)-proton removal by most bases occurs at the maximum possible rate for a given equilibrium constant.

In this paper we show that the rate constants for proton transfer from thiazolium ions follow an "Eigen curve" similar to that for normal acids and have a similar barrier for proton transfer near $\Delta pK = 0$. Kemp and O'Brien have reported catalysis by water, methoxyacetate, acetate, and hydroxide ion of C(2)–T → H exchange from 3-benzylbenzothiazolium ion that is consistent with a Brønsted β value of ≥0.9;[13] our results confirm and extend the conclusion that general-base catalysis is detectable for thiazolium C(2)–L exchange. The absence of strong inhibition of proton exchange by acid and a solvent isotope effect of $k_{H_2O}/k_{D_2O} = 2.8$ show that proton transfer to water does not follow the Swain–Grunwald mechanism[4] and does not proceed through a water molecule. The relatively small inhibition is used to define an acidity function that reflects stabilization of the C(2) ylide in concentrated acid solution. We conclude that thiazolium ions undergo proton loss with a small intrinsic barrier and are even more normal than HCN,[8] but they still have properties characteristic of other carbon acids.

Experimental Section

Materials. All organic chemicals were reagent-grade and were purified by recrystallization or distillation; pentafluorophenol (Aldrich) was sublimed. Reagent-grade inorganic chemicals were used as received. Water was glass distilled. Acetic-d_3 acid-d, acetonitrile-d_3, deuterium chloride, deuterium oxide, dimethyl-d_6 sulfoxide, phosphoric acid-d_3, sodium deuterioxide, and thiamin hydrochloride were purchased from Aldrich; all deuteriated compounds were ≥99 atom % D. [^{3}H]H_2O (1 Ci/mL) and Aquasol-2 were purchased from New England Nuclear. Thiamin hydrochloride was recrystallized from methanol/ethanol: mp = 242–243 °C dec. The synthesis of 3,4-dimethylthiazolium chloride[14] and 3-(cyanomethyl)-4-methylthiazolium chloride[15] has been described. C(2)-[^{3}H]Thiazolium ions were prepared from unlabeled thiazolium salts by exchange with [^{3}H]H_2O in H_2O at 25 °C for 24 h and pH 4.8 [for N(1′)-protonated thiamin; the pH was adjusted with NaOH] or pH 5–6 (for other thiazolium salts; the pH was maintained by ambient dissolved CO_2). The solvent was removed by evaporation under a water aspirator vacuum in a Savant Speed-Vac centrifugal concentrator; all operations were performed in a hood. The C(2)-[^{3}H]thiazolium ions, which were typically 0.3–0.5 mCi/mol, were stored in vacuo over P_2O_5 at 25 °C in a hood. Parallel experiments in deuterium oxide and ^{1}H NMR examination of the exchanged thiazolium ions showed that isotopic labeling under these conditions occurs exclusively by exchange at C(2)–H. Stock solutions of reagents in D_2O were prepared by first exchanging exchangeable protium from the reagents with D_2O to give <2 atom % exchangeable protium in the stock solution except for methoxyethanol and propargyl alcohol, which were used without exchanging protium and at concentrations that gave <4 atom % exchangeable protium in the stock solution.

Methods. Solution pH was measured at 30 °C with an Orion Model 701A pH meter and a Radiometer GK2321C combination electrode standardized at pH 6.99 and 4.01 or 9.96. The electrode was free of the anomalous ionic strength effects reported by Illingworth.[16] ^{1}H NMR spectra in D_2O were recorded on a Varian XL-300 NMR spectrometer. Proton spin–spin coupling of C(2)–H ($\delta \simeq 10$ ppm) to C(5)–H ($\delta \simeq 8$ ppm) through sulfur was observed for all thiazolium salts studied. This coupling results in splitting of the C(2)–H peak to a doublet ($J = 3.0 \pm 0.5$ Hz) and an increased peak width for C(5)–H.[15] Radioactivity was measured with a Beckman LS 6800 liquid scintillation counter.

Kinetics. Rate constants for thiazolium C(2)–H → D exchange in D_2O were determined by ^{1}H NMR spectroscopy at 30 ± 1 °C as described previously.[15] The ionic strength was maintained at 2.0 M with NaCl. Solutions of the amine catalysts were prepared by partial neutralization of the amine deuteriochlorides with NaOD. The carboxylic acid and alcohol catalysts were prepared by partial neutralization of the sodium salts with DCl, except for aqueous solutions of methoxyethanol and propargyl alcohol, which were prepared from the alcohol. Aqueous solutions of 0.01–2.7 M DCl in D_2O were used for exchange reactions at pD ≤ 2. The exchange reaction was initiated by dissolving 0.0625 mmol of thiazolium salt in 0.5 mL of buffer, which was previously equilibrated at 30 °C, giving a final concentration of 0.125 M thiazolium salt, unless stated otherwise.

Rate constants for thiazolium C(2)–T → D exchange were determined by measuring nonvolatile, unexchanged tritium remaining in the C(2)-[^{3}H]thiazolium salt at 30 °C. The ionic strength was maintained at 2.0 M with NaCl. All operations were performed in a hood. The exchange reaction was initiated as described above in a 1.5-mL Eppendorf centrifuge tube. The reaction solution was incubated in a constant-temperature bath (30 ± 0.2 °C) and was removed for about 10 s every 2–30 min in order to obtain a 10-μL aliquot that was immediately mixed with 10 μL of 2 M HCl in a 400-μL flat-bottomed well of a rigid polystyrene microtiter plate (Becton-Dickinson Falcon 3075 96-Well MicroTest III tissue culture plate) to quench the exchange reaction. A 0.5 × 2.0 cm strip of Whatman 3mmChr paper was placed in the well to absorb the quenched reaction solution. The solvent, which contained exchanged tritium as [^{3}H]L_2O and aqueous HCl, was allowed to evaporate leaving any unexchanged C(2)-[^{3}H]thiazolium salt on the paper strip; the paper strips were handled only with tweezers at this point. The paper strips were pinned to an aluminum foil covered board and dried under a heat lamp to ensure that the strips were completely dry. Each paper strip was transferred to a liquid scintillation counting vial, suspended in 1.0 mL of water, and mixed with 15 mL of Aquasol-2; the vials were shaken intermittently over several hours until no schlieren were observed around the paper strips. The samples were counted for at least 10^5 counts. A 10-μL aliquot of the reaction solution typically gave ~5000 cpm for the initial time point. The end point was obtained after $>10t_{1/2}$ and was typically 50–100 cpm. The pseudo-first-order rate constants were obtained from semilogarithmic plots of $(A_t - A_\infty)$ against time (A = cpm) and the relationship $k_{obsd} = 0.693/t_{1/2}$. These plots were linear for $>4t_{1/2}$ with 10–15 time points. When duplicate determinations of k_{obsd} were made, they agreed within ±5% of the average value.

Rate constants for C(2)–H → T exchange of 3-(cyanomethyl)-4-methylthiazolium ion catalyzed by H_2O or D_2O in 1.75–10.4 M LCl containing 0.43 mCi/mol [^{3}H]L_2O were determined from initial rate measurements, in which the reaction had proceeded ≤10% to completion, at 30 ± 0.2 °C by using the assay described above. The pseudo-first-order rate constant, k_{obsd}, was obtained by dividing the initial rate of appearance of nonvolatile thiazolium ion C(2)–T by 0.23 M, which was the initial concentration of thiazolium ion C(2)–H.

Measurements of pH were made at 30 ± 0.2 °C on the buffered solutions of the thiazolium salts after exchange had occurred. The value of pD was obtained by adding 0.40 to the observed pH of solutions in D_2O.[17] Based on measurements of pH at known concentrations of hydroxide ion at 30 °C and 2.0 M ionic strength, maintained with KCl, eq 1 was used to calculate the concentration of deuterioxide ion at any

$$[OD^-] = 1.18 \times 10^{(pD-14.70)} \quad (1)$$

pD. This equation includes the ion product of deuterium oxide at 30 °C.[18]

Second-order rate constants for general-base-catalyzed exchange were obtained from the slopes of plots of ≥3 values of k'_{obsd} against [B], where B is the catalytic general base. Values of k'_{obsd} were calculated from k_{obsd} by subtracting the rate from catalysis by OD^-, D_2O, and any additional buffer base present to maintain pH, according to $k'_{obsd} = k_B[B] = k_{obsd} - k_{OD}[OD^-] - k'_{D_2O}$. This treatment corrects for small changes in $[OD^-]$ with increasing buffer concentration that had the effect of increasing or decreasing the dependence of k_{obsd} on buffer concentration.[19] The cat-

(12) The term "hydron" refers to the hydrogen cation (L^+) without regard to nuclear mass. The specific names "proton" (^{1}H), "deuteron" (^{2}H), and "triton" (^{3}H) refer to the specific isotopes (Commission on Physical Organic Chemistry, IUPAC *Pure Appl. Chem.*, in press) and are abbreviated here as: $^1H^+$, H; $^2H^+$, D; $^3H^+$, T.

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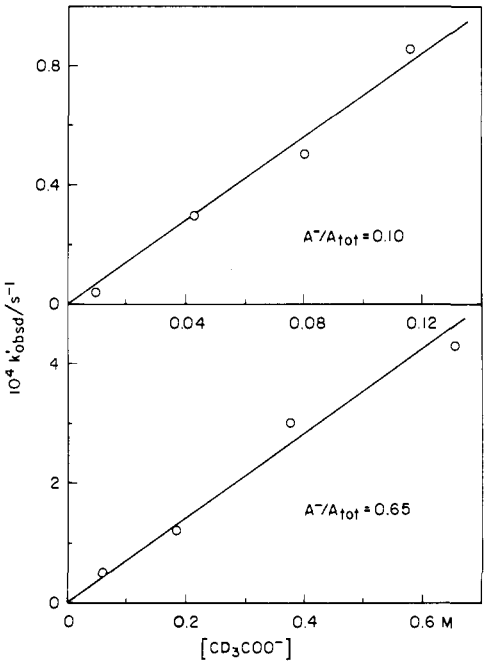


Figure 1. Dependence of the rate constant for 3-(cyanomethyl)-4-methylthiazolium ion C(2)–T → D exchange on the concentration of acetate-d_3 ion at 30 °C and I = 2.0 M (NaCl) in D_2O. Values of k'_{obsd} ($=k_B[CD_3COO^-]$) were calculated from k_{obsd} after correcting for catalysis by deuterioxide ion and D_2O (see text). The slope of both lines (k_B = 6.7 × 10^{-4} M^{-1} s^{-1}) is the rate constant for general-base catalysis by acetate-d_3 ion and is independent of the fraction of buffer base. This general-base catalysis represents a 36% rate increase at 0.13 M acetate-d_3 for A^-/A_{total} = 0.10 and a 16% rate increase at 0.66 M acetate-d_3 for A^-/A_{total} = 0.65.

alyst concentration, [B], at the pD of the reaction was calculated from its apparent pK_a (see below). We estimate error limits of ±15% and ≤±40% in the second-order rate constants for general-base catalysis from **1c** and **1b**, respectively, based on the maximum and minimum slopes that could be drawn in plots of k'_{obsd} against [B] assuming an error of ±12% in k'_{obsd}.[20] Second-order rate constants for deuterioxide ion catalyzed exchange were obtained as described previously.[15]

Determination of Catalyst pK_a' Values. Amine hydrochlorides and the sodium salts of carboxylic acids and pentafluorophenol were dried in vacuo over P_2O_5 to constant weight before use. A 0.01 M solution of the catalyst at ionic strength 2.0 M (NaCl) in freshly boiled water was titrated potentiometrically under an argon atmosphere with either 0.100 M KOH or 0.100 M HCl at 30.0 ± 0.2 °C according to the method of Albert and Serjeant.[21] For catalysts with pK_a > 9 the ionic strength was maintained with KCl to avoid electrode sodium ion errors. Data were analyzed by using K_w = $10^{-13.837}$ at 30 °C[18] and a correction for H^+ and OH^- derived from hydrolysis of the salt being formed at pH ≤ 4 and pH ≥ 10.[21] Errors in the pK_a' values in H_2O were ≤±0.04. The catalyst pK_a' values in D_2O were calculated by adding ΔpK_a = 0.5 ($RCOO^-$) or 0.6 (amines, ArO^-) for the solvent deuterium isotope effect on the ionization of weak acids[22] to the values for pK_a' in H_2O.

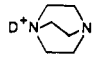
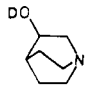
Results

The kinetics of general-base-catalyzed C(2)–L → D exchange from N(1′)-protonated thiamin (**1a**), 3,4-dimethylthiazolium ion (**1b**), and 3-(cyanomethyl)-4-methylthiazolium ion (**1c**) at 30 °C and ionic strength 2.0 M, maintained with sodium chloride, in D_2O were followed by ^1H NMR or detritiation under pseudo-first-order conditions. The kinetics of L_2O-catalyzed C(2)–H → L exchange from 3-(cyanomethyl)-4-methylthiazolium ion (**1c**) at 30 °C were followed by a combination of ^1H NMR and tritium-incorporation methods under pseudo-first-order and initial rate conditions, respectively. The exchange reactions obey the rate law described by eq 2. The rate constants for base catalysis

$$k_{obsd} = k_{OD}[OD^-] + k_B[B] + k'_{D_2O} \quad (2)$$

of C(2)–L → D exchange were determined as described in the Experimental Section. The rate constants for C(2)–H → D exchange catalyzed by deuterioxide ion and D_2O under these reaction conditions were reported previously.[15] Typical data are shown in Figure 1 for detritiation of **1c** catalyzed by acetate-d_3. Values of k_{obsd} and $k_B[B]$, calculated from eq 2, are summarized in supplementary Tables S1–3. The second-order rate constants, k_B, for general-base catalysis of C(2)–L → D exchange are summarized in Table I. The rate constant for deuterioxide ion catalyzed H → D exchange of the two protons from the exocyclic 3-methylene position in **1c** under these reaction conditions was also determined by ^1H NMR and was found to be 8.4 × 10^5 M^{-1} s^{-1}, which is 55-fold slower than H → D exchange of the single C(2)–H in this compound. This shows that the activating effects for proton transfer of the thiazolium S atom, sp^2 hybridization

Table I. Rate Constants for General-Base Catalysis of 3-R-4-Methylthiazolium Ion C(2)–L → D Exchange[a]

			log k_B, M^{-1} s^{-1}		
catalyst	pK_a[b]	C(2)–L	R = Me	thiamin	R = CH_2CN
D_2O[c]	−1.74	H	≤−10.5	−9.57	−8.77
OD^-[c]	16.44[d]	H	5.63	6.92	7.67
		T	5.16	6.13	6.50
$NCCH_2COO^-$	2.49	T			−5.30
$CH_3OCH_2COO^-$	3.60	H	−6.25	−5.03	−4.19
		T			−4.31
$HCOO^-$	3.81	H			−3.82
D_3CCOO^-	4.97	H	≤−4.89	−3.25	
		T			−3.18
$CH_3CH_2COO^-$	5.03	H			−2.20
DPO_4^{2-}	6.62	H	−2.72		
		T	−2.64		
$C_6F_5O^-$	5.82	H	−4.64		
$CF_3CH_2O^-$	13.0[e]	T			4.90
$HCCH_2O^-$	14.2[e]	H	3.78		
		T			5.90
$CH_3OCH_2CH_2O^-$	15.4[e]	H			7.36
		T			6.77
CH_3O^-	16.3[e]	T			≤7.28
$CH_3CH_2O^-$	16.6[e]	T			≤7.51
$CF_3CH_2ND_2$	6.38	H	−3.78		
		T	−3.82		−1.17
$NCCH_2CH_2ND_2$	8.58	H	−1.37		
		T			0.59
$DOCH_2CH_2ND_2$	10.25[f]	H		1.53	
		T			3.08
	4.06	H	−5.39		−3.57
		T			−3.64
	10.75[f]	T			2.89

[a] At 30 °C and ionic strength 2.0 M (NaCl) in D_2O. The rate constant k_B is defined in eq 2. [b] Apparent pK_a of the conjugate acid at 30 °C and ionic strength 2.0 M (NaCl) in D_2O (see text). [c] Reference 15. Rate constants for catalysis by OD^- are concentration based, γ_{OD} = 0.85. [d] Calculated from the ion product of D_2O[18] based on a standard state of 55.1 M for pure D_2O at 30 °C,[53] with $K_a^{D_2O} = K_w/[D_2O]$ = $10^{-14.70}/55.1$. [e] Estimated from pK_a values at low ionic strength in H_2O and ΔpK_a = 0.7 for the solvent deuterium isotope effect on the ionization of alcohols.[22] [f] Apparent pK_a of the conjugate acid at 30 °C and ionic strength 2.0 M (KCl) in D_2O (see text).

(19) Methods for handling "buffer failure" have been discussed: see ref 7a and Keeffe, J. R.; Kresge, A. J. In *Investigations of Rates and Mechanisms of Reactions*; Bernasconi, C. F., Ed.; Wiley: New York, 1986; Vol. 6, part 1, pp 747–790.

(20) The propagated standard error(s) of ±12% in k'_{obsd} was calculated from $k'_{obsd} \equiv k_B[B] = k_{obsd} - k_{OD}[OD^-]$ and $s = (s^2_{k_{obsd}} + s^2_{k_{OD}[OD^-]})^{1/2}$: Skoog, D. A.; West, D. M. *Fundamentals Of Analytical Chemistry*, 4th ed.; CBS College Publishing: New York, 1982; pp 75–76. Catalysis by D_2O does not significantly contribute to k_{obsd} under these reaction conditions and was neglected. Errors of $s_{k_{obsd}}$ = ±5% in k_{obsd} and $s_{k_{OD}[OD^-]}$ = ±10% in $k_{OD}[OD^-]$ were assumed.

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(22) Laughton, P. M.; Robertson, R. E. In *Solute–Solvent Interactions*; Coetzee, J. F., Ritchie, C. D., Eds.; Marcel Dekker: New York, 1969; Vol. 1, pp 399–538.

of C(2)–H and, possibly, the aromatic ring, are significantly larger than that of a CN group.

The amount of buffer catalysis observed was larger for C(2)–T → D than for C(2)–H → D exchange and for the more electron-withdrawing substituents at N(3) on the thiazolium ring, but was typically small. Experiments to measure general-base catalysis were generally performed at total buffer concentrations ≤2 M and buffer ratios of $B/BH^+ \leq 0.35$ in order to obtain maximal rate increases from general-base catalysis.[19] Catalysis by lyoxide ion is large in these reactions because of the large β values; it would overwhelm general-base catalysis if the lyoxide ion also obeyed the Brønsted correlation.[23] Catalysis is detectable because the rate constant for lyoxide ion falls below the Brønsted line (see below) and the reactions were followed at low buffer ratios (B/BH^+) in order to minimize the contribution of catalysis by lyoxide ion. For C(2)–H → D exchange from N(1′)-protonated thiamin (**1a**) and 3,4-dimethylthiazolium ion (**1b**), general-base catalysis gives a rate increase of only ~9% for buffer ratios of $B/BH^+ \leq 0.35$ and total buffer concentrations up to 1.0 M; however, a larger rate increase of 29% was observed for catalysis of C(2)–T → D exchange by 0.13 M DPO_4^{2-} from **1b** at a buffer ratio of $B/BH^+ = 0.35$. Larger rate increases were observed for C(2)–H → D and C(2)–T → D exchange from 3-(cyanomethyl)-4-methylthiazolium ion (**1c**), where general-base catalysis gave rate increases of ~15% and ~36–93%, respectively, for buffer ratios of $B/BH^+ \leq 0.40$ and total buffer concentrations up to 2.0 M (Figure 1).

When working with high concentrations of buffer components, it is important to assess the effects of varying $[M^+B^-]$ and [BH] even when ionic strength and the buffer ratio are kept constant.[24] The following points suggest that medium effects are small: (1) No detectable curvature at high buffer concentration was observed in plots of k'_{obsd} against [buffer] (see Figure 1). (2) The rate constants for catalysis of thiazolium C(2)–H → D exchange by OD^- are not sensitive to variations in the nature or concentration of salts used to maintain the ionic strength at 2.0 M (except for an anion-specific effect of ClO_4^- and SO_4^{2-}); there is no significant change in k_{OD} (≤±5%) upon substituting sodium trifluoroacetate, sodium methoxyacetate, sodium iodide, or tetramethylammonium chloride for sodium chloride.[25] (3) There is no significant change in k_B (≤±5%) for catalysis of C(2)–H → D exchange from **1b** by DPO_4^{2-} ($B/BH^+ = 0.23$) upon substituting 0.11–1.71 M sodium methoxyacetate for sodium chloride to maintain the ionic strength at 2.0 M. (4) The pD-independent rate constant for C(2)–H → D exchange catalyzed by D_2O from **1c** is not affected (≤±3%) by substitution of 0.125 or 2.0 M tetramethylammonium chloride for sodium chloride in 1.75 M DCl at a total ionic strength of either 2.0 or 3.9 M. (5) Values of k_B for catalysis of C(2)–H → D exchange from **1b** by DPO_4^{2-} (A^-/A_{total} = 0.36–0.59) and from **1c** by D_3CCOO^- (A^-/A_{total} = 0.10–0.65; see Figure 1) do not change significantly (≤±5%) with the fraction of buffer base, which indicates that there is no significant effect of the acid component of the buffer on the rate constants for general-base-catalyzed thiazolium C(2)–H → D exchange. (6) We examined the effect of adding small organic molecules to the reaction medium with 5.5–11 vol % acetonitrile, 7–14 vol % dimethyl sulfoxide, and 6–12 vol % ethanol, which correspond to 1.0–2.0 M organic solvent in the reaction. There is no significant change (≤±5%) in k_{OD} with increasing organic solvent in the reaction medium.

Even though the ionic strength was kept constant at 2 M with sodium chloride there was usually a small increase in the observed pD of ≤0.1 unit for oxygen-containing catalysts or a small decrease of ≤0.1 unit for amines between the lowest and highest buffer concentrations examined. This variation in pD with [buffer] had the effect of increasing or decreasing the dependence of k_{obsd} on [buffer]; in some reactions it resulted in decreases in k_{obsd} with increasing [buffer] for several amine buffers, as shown in supplementary Tables S1–3. Consequently, a correction of k_{obsd} was made for the rate resulting from the variation in $[OD^-]$ as described in the Experimental Section. There is no significant effect of errors in pD and catalyst pK_a on k_B because, for the buffer ratios of $B/BH^+ \leq 0.4$ that were used to determine k_B, the fractional rate increase resulting from general-base catalysis is constant; $[OH^-]$ changes in parallel with $[M^+B^-]$.[19]

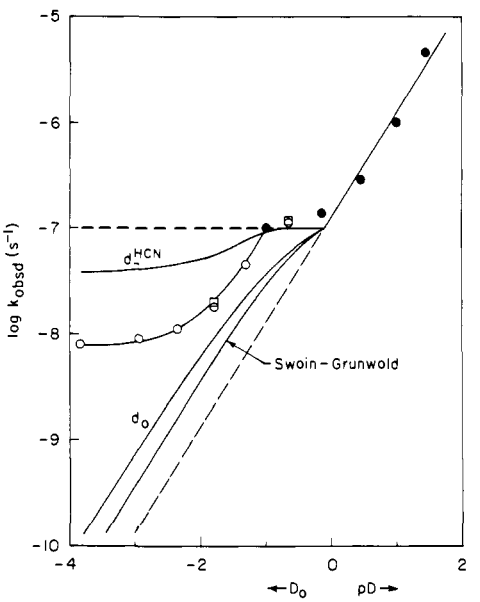


Figure 2. Dependence of the observed first-order rate constants for 3-(cyanomethyl)-4-methylthiazolium ion C(2)–H → L exchange in aqueous DCl solutions on the Hammett D_0 acidity function[26] at 30 °C in D_2O. Above $D_0 = -0.7$ the ionic strength was maintained at 2.0 M (NaCl). The reaction was followed by incorporation of T (○) or D (●) into the C(2)–H substrate. The square symbols represent duplicate determinations of k_{obsd}. The solid lines represent the inhibition in DCl expected for ionization of an acid of $pK_a = 16.9$ by the Swain–Grunwald mechanism and by acidity function effects (see text).

The secondary solvent deuterium isotope effect on C(2)–T → L exchange from **1c** catalyzed by acetate-d_3 was also determined at 30 °C and ionic strength 2.0 (NaCl). In parallel experiments with $A^-/A_{total} = 0.19$ and 0.25, values of $k_B^{H_2O} = (7.4 \pm 1.0) \times 10^{-4}$ M^{-1} s^{-1} and $k_B^{D_2O} = (7.0 \pm 0.8) \times 10^{-4}$ M^{-1} s^{-1} were obtained. The solvent isotope effect, $k_B^{H_2O}/k_B^{D_2O}$, is 1.1 ± 0.2 and is independent of the concentration of the acid component of the buffer.

In summary, the following provide evidence that this treatment of k_{obsd} is valid and provides a reliable measure of general-base catalysis:

(1) The amount of buffer catalysis observed is larger for C(2)–T → D than for C(2)–H → D exchange. A medium effect that changes the concentration of OD^- would be expected to have the same effect on the exchange of T and H.

(2) The observed catalysis is independent of buffer base charge type, while the variation in pD depends on the buffer base charge type.

(3) No medium effects were observed upon addition of several different organic solvents or change in the nature of salts at constant ionic strength.

(4) Changing the concentration of the acid component of the buffer has no effect on k_B (Figure 1), although it changes pD.

(5) The relative effectiveness of different buffer bases is the same for the three thiazolium ions examined, although the amounts of catalysis are different. A medium effect would be expected to cause similar or identical changes in the rate constants for the three compounds.

(6) Although trifluoroethanol, propargyl alcohol, and methoxyethanol give catalysis and values of k_B that are consistent with a Brønsted correlation, ethanol has no effect on k'_{obsd} for C(2)–L → D exchange from **1c**. Ethanol is not expected to show significant catalysis because of the curvature in the Brønsted plot for pK_a values >14 (see below).

(23) Bell, R. P. *Acid–Base Catalysis*; Oxford University Press: London, 1941; p 94.
(24) Hand, E. S.; Jencks, W. P. *J. Am. Chem. Soc.* **1975**, *97*, 6221–6230.
(25) Washabaugh, M. W.; Jencks, W. P. *J. Am. Chem. Soc.*, following paper in this issue.

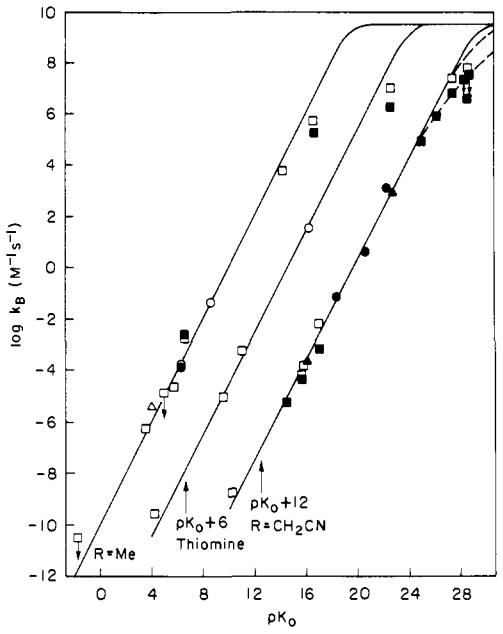


Figure 3. Brønsted plots for general-base catalysis of C(2)–H (open symbols) or C(2)–T (solid symbols) → D exchange from 3-R-4-methylthiazolium ions and N(1′)-protonated thiamin at 30 °C and I = 2.0 M (NaCl) in D_2O; the plots for N(1′)-protonated thiamin and for R = CH_2CN are displaced by 6 and 12 pK_a units, respectively, for clarity of presentation. The squares are for oxygen-containing catalysts (pK 2–5 are RCOOD, and pK 7–16 are ROD), circles are for primary amines, and triangles are for tertiary amines. Upper limits for catalysis by D_2O and acetate-d_3 (R = Me), as well as methoxide and ethoxide (R = CH_2CN), are indicated. The solid lines are theoretical Eigen curves (see text) for $k_d = 3 \times 10^9$ M^{-1} s^{-1}, $k_d/k_{-d} = 0.1$ M^{-1}, and $k_p^0 = 10^{12}$ s^{-1}; the broken lines are theoretical Eigen curves for $k_p^0 = 10^{10.05}$ and $10^{8.85}$ s^{-1}, respectively.

Figure 2 shows the dependence on pD and the Hammett acidity function D_0[26] of the observed pseudo-first-order rate constants for C(2)–H → L exchange of 3-(cyanomethyl)-4-methylthiazolium ion (**1c**). The results are summarized in supplementary Table S4.

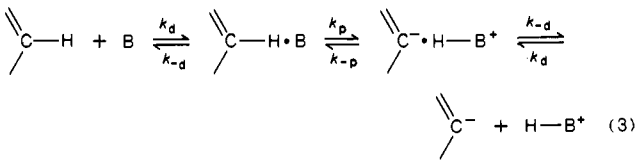
The secondary solvent deuterium isotope effect on C(2)–H → T exchange for 3-(cyanomethyl)-4-methylthiazolium ion (**1c**) with the solvent (L_2O) was determined by tritium incorporation at 30 °C under pseudo-first-order conditions in the D_0-independent region at $D_0 = -0.65$ with [LCl] = 1.75 M and ionic strength 2.0 M (NaCl) or in the D_0-dependent region at $D_0 = -1.80$ with [LCl] = 4.90 M. In separate experiments, three determinations of k_{obsd} were made at each [LCl]. The observed rate constants are $k_{H_2O} = (7.5 \pm 0.3) \times 10^{-7}$ s^{-1} and $k_{D_2O} = (2.6 \pm 0.1) \times 10^{-7}$ s^{-1} for [LCl] = 1.75 M, while $k_{H_2O} = (1.2 \pm 0.1) \times 10^{-7}$ s^{-1} and $k_{D_2O} = (4.7 \pm 0.3) \times 10^{-8}$ s^{-1} for [LCl] = 4.90 M. The solvent isotope effect, k_{H_2O}/k_{D_2O}, is 2.8, with an uncertainty of 0.2 based on the errors in the observed rate constants, and is independent of [LCl] within experimental error. No changes in the ultraviolet or ^1H NMR spectrum of **1c** that might suggest hydrolysis of the nitrile were detected in the presence of 1–10 M LCl.

Discussion

General-Base Catalysis and the Nature of the Transition State. The second-order rate constants for thermodynamically unfavorable C(2)–L → D exchange catalyzed by general bases from N(1′)-protonated thiamin (**1a**) (pK_a = 17.7), 3,4-dimethylthiazolium ion (**1b**) (pK_a = 18.9), and 3-(cyanomethyl)-4-methylthiazolium ion (**1c**) (pK_a = 16.9)[15] can be described by almost completely normal "Eigen curves", with Brønsted β values of ≥0.95, as shown in Figure 3; the curves for **1a** and **1c** are offset by 6 and 12 pK_a units, respectively, for clarity of presentation. Though general-base catalysis of thiazolium ion C(2)–L → D exchange is unmistakable, it is also weak. General-base catalysis is detectable because there is a negative deviation of ≥6-fold of the rate constant for catalysis of C(2)–L → D exchange by deuterioxide ion from the Brønsted plot for all three thiazolium ions examined; there is a constant 8-fold negative deviation of the rate constant for C(2)–H → D exchange. Statistical correction of the catalyst pK_a values and the rate constants for base catalysis[27] does not improve the fit of the data to the Brønsted plots or change β significantly.

The Brønsted β value of ≥0.95 suggests (1) that diffusional separation of the conjugate acid from the C(2) ylide is rate limiting for thermodynamically unfavorable C(2)–L → D exchange, as expected for a normal acid, and (2) that the reverse, thermodynamically favorable protonation of the thiazolium C(2) ylide, with a Brønsted α value of ≤0.05, is diffusion-controlled with almost all acids. These conclusions are supported by values of k_H/k_T in the range 1.2 ± 0.2 for thermodynamically unfavorable C(2)–L → D exchange catalyzed by general bases (Table I).

The observed base catalysis can be interpreted in terms of the simple three-step model for proton transfer described by Eigen (eq 3).[28] For thermodynamically favorable proton transfer the



(3)

rate-limiting step is diffusion-controlled encounter of the reactants (k_d); for unfavorable transfer the rate-limiting step is diffusion-controlled separation of the products (k_{-d}) and near $\Delta pK = 0$, diffusion together (k_d), proton transfer within the encounter complex (k_p), and diffusion apart (k_{-d}) are all partially rate limiting. Theoretical curves were calculated[8,29] from eq 4, which

$$k_B = k_d k_p k_{-d}/(k_p k_{-d} + (k_{-d})^2 + k_{-d}k_{-p}) \quad (4)$$

$$k_p = k_p^0 10^{0.5(\Delta pK)} \quad (5)$$

is the steady-state solution to eq 3; the value of k_p is given by eq 5, assuming that $\beta = 0.5$ near $\Delta pK = 0$, in which k_p^0 is the value of k_p at $\Delta pK = 0$.

The solid lines in Figure 3 are theoretical Eigen curves for proton transfer from an acid with essentially no intrinsic barrier and were calculated from values of $k_p^0 = 10^{12}$ s^{-1}, $k_d = 3 \times 10^9$ M^{-1} s^{-1},[30] and $k_d/k_{-d} = 0.1$ M^{-1}.[8] The broken lines in Figure 3 for catalysis of C(2)–H → D and C(2)–T → D exchange from **1c** by buffer bases were calculated similarly from $k_p^0 = 10^{10.05}$ s^{-1} and $k_p^0 = 10^{8.85}$ s^{-1}, respectively; these values for k_p^0 correspond to second-order rate constants of $k_B = 10^{8.81}$ M^{-1} s^{-1} for C(2)–H → D exchange and $k_B = 10^{7.83}$ M^{-1} s^{-1} for C(2)–T → D exchange at $\Delta pK = 0$. The observed rate constants for buffer bases agree with these calculated lines within the experimental error of the measurements (±15%) and give values for k_p^0 of $10^{8.80}$–$10^{8.90}$ s^{-1} for C(2)–T → D exchange and $10^{9.75}$–$10^{10.35}$ s^{-1} for C(2)–H → D exchange; the rate constants for OD^- fall below the lines.

Transfer of the C(2) proton from N(1′)-protonated thiamin (**1a**) and related N(3)-substituted thiazolium cations (**1b,c**) is very similar to proton transfer from normal acids with electronegative atoms. Proton transfers between normal acids and bases are almost completely diffusion-controlled; only in a small region near $\Delta pK = 0$ is the proton-transfer step even partly rate determining, and for thermodynamically favorable ($\Delta pK > 3$) or unfavorable

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(28) Eigen, M. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 1–19.

(29) Fischer, H.; DeCandis, F. X.; Ogden, S. D.; Jencks, W. P. *J. Am. Chem. Soc.* **1980**, *102*, 1340–1347. No correction was made for effects of charge, which are not expected to be large at ionic strength 2.0 M.

(30) Keeffe, J. R.; Kresge, A. J.; Toullec, J. *Can. J. Chem.* **1986**, *64*, 1224–1227. These workers measured the rate constant directly for the diffusion-controlled reaction of acetophenone enol with bromine in aqueous hydrobromic acid at 25 °C and I = 0.10 M (NaBr).

($\Delta pK < -3$) proton transfers, diffusion-controlled encounter of the reactants or separation of the products becomes fully rate determining.[28,29,31] The Brønsted plots for such reactions follow Eigen curves with slopes of 0 and ±1.0 in the favorable and unfavorable directions, respectively, and have a small transition region near $\Delta pK = 0$ of slope ~0.5 where the proton-transfer step gives rise to a deuterium isotope effect[31] when it becomes partially rate limiting. Most carbon acids are not normal in this respect; the proton-transfer step is partly or completely rate determining over a large range of ΔpK.

These results show that the rate constants for C(2)–proton transfer from thiazolium ions are nearly identical with those observed for normal acids with electronegative atoms;[28] thiazolium ions undergo proton loss with a small intrinsic barrier. This is relevant to the physiological role of thiamin because it means that C(2)–proton removal occurs at nearly the maximum possible rate for a given equilibrium constant. The value of $k_p = 10^{10.05}$ s^{-1} at $\Delta pK = 0$ for thiazolium C(2)–H exchange is ~14-fold larger than the values of $10^{8.6}$–$10^{9.1}$ s^{-1} ($k_B = 10^{7.6}$–$10^{8.0}$ M^{-1} s^{-1} at $\Delta pK = 0$) obtained by an analogous treatment for HCN,[8] so that thiazolium ions are even more normal than HCN; even for errors of ±30% in the second-order rate constants for general-base catalysis, the value of $k_p^0 = 10^{9.55}$–$10^{11.5}$ s^{-1} for C(2)–H → D exchange is significantly greater than the values for HCN. The curvature in the Brønsted plot for C(2)–T → D exchange from **1c**, which does not involve significant internal return,[25] can be fit to the Marcus equation[32] with values of 1.3 ± 0.3 and 3.7 kcal mol^{-1} for the intrinsic barrier and the constant work term, respectively. These are similar to the values of 2 and 3 kcal mol^{-1} for the intrinsic barrier and the work term for proton transfer from normal acids.[2] They correspond to a second-order rate constant of $k_B = 10^{7.83}$ M^{-1} s^{-1}, or a total barrier of 5.0 kcal mol^{-1}, at $\Delta pK = 0$. It appears that the work term must represent, in part, the difference in zero-point energy estimated from $RT \ln (k_H/k_T)_{obsd}$ = 1.6 kcal mol^{-1} for C(2)–H → D and C(2)–T → D exchange.

Large Brønsted β values have been reported for several other carbon acids that require little electron delocalization and desolvation upon ionization, such as acetylenic, cyano, halogen, and sulfonyl-activated acids[7,33,34] when the proton-transfer step is strongly favorable in one direction. However, the rate constants of ~10^8 M^{-1} s^{-1} for reaction with buffers in the thermodynamically favorable direction for malononitriles and disulfones and of ~10^7 M^{-1} s^{-1} at $\Delta pK = 0$ for bromomalononitrile are significantly smaller than those for the corresponding reactions of thiazolium ions;[33] the rate constants for proton transfer to and from thiazolium ions are close to those observed for normal acids and bases with electronegative atoms.[28] On the basis of the observation that bromination of the dicyanomethyl carbanion is twice as fast as reprotonation by H_3O^+, Kresge and co-workers concluded that reprotonation is not a diffusion-controlled process and that malononitrile is not a normal acid.[35]

Thiazolium C(2)–L → D exchange catalyzed by deuterioxide ion is near the region of $\Delta pK = 0$ and shows significant primary kinetic isotope effects. The values of k_H/k_T for this exchange (Table I) are 2.9 for 3,4-dimethylthiazolium ion (**1b**) ($\Delta pK = -2.5$), 6.2 for N(1′)-protonated thiamin (**1a**) ($\Delta pK = -1.3$), and 14 for 3-(cyanomethyl)-4-methylthiazolium ion (**1c**) ($\Delta pK = -0.5$). The following paper[25] demonstrates on the basis of the breakdown of the Swain–Schaad equation[36,37] that this increase in k_H/k_T over the range 2.9–14.7 as electron-withdrawing N(3) substituents are added to the thiazolium ring is consistent with internal return and diffusion-controlled separation of water from the C(2) ylide, which decreases the observed isotope effect and is more important for the more basic carbanions. Relatively small primary tritium kinetic isotope effects for deuterioxide ion catalyzed thiazolium C(2)-proton exchange were observed previously; $k_H/k_T = 5.2 \pm 1.0$ for *N*-methylthiazolium ion (26.5–28 °C),[38] 2.7 for 3-benzyl-4,5-dimethylthiazolium ion, and 4.8 for 3-benzylbenzothiazolium ion (30 °C, I = 1.0 M).[13]

The negative deviations from the Brønsted plot of the rate constants for catalysis by OD^- of ~10-fold are in the lower range of the negative deviations of between 10- and 1000-fold for catalysis by lyoxide ion that are usually observed for thermodynamically unfavorable hydron transfers from carbon; the fact that no such negative deviation is found for proton transfer[39] between H_2O and H_3O^+ or H_2O and OH^- at $\Delta pK = 0$ may reflect proton transfer through solvent molecules, which does not ordinarily occur with carbon acids.[5,34,40] The rate constants for catalysis of C(2)–L → D exchange by deuterioxide ion are smaller than those for catalysis by the less basic anion of methoxyethanol, which shows that the "lyoxide ion anomaly" does not arise simply from the high pK of lyoxide ion. The lyoxide ion anomaly will be examined further in the following paper.[25]

Gilbert and Bernasconi and their co-workers have shown that 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.[41] Therefore, it is virtually certain that there is also a very small intrinsic barrier for the addition–elimination reactions involving the C(2) ylide that occur during the turnover of thiamin-dependent enzymes.

Thiazolium ions require little or no delocalization by resonance or change in the lengths and angles of bonds to heavy atoms upon ionization; ionization gives an electron lone pair in an sp^2-hybridized orbital that cannot be stabilized by p–π delocalization and the five-membered ring restricts changes in bond lengths and angles. Although overlap with antibonding σ^* orbitals is possible, more conventional resonance delocalization is unlikely to be important for this electron pair.[42] The ρ_I value of 8.4 for the ionization of both thiazolium ions and normal acids provides evidence that there is little, if any, resonance that decreases the positive charge on N(3) in the C(2) ylide; N(3) is closer to the substituent and a decrease in its charge would give a larger value

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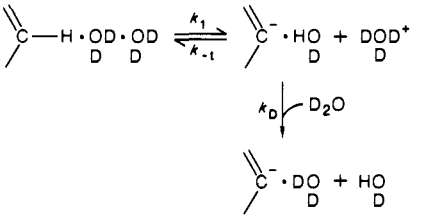
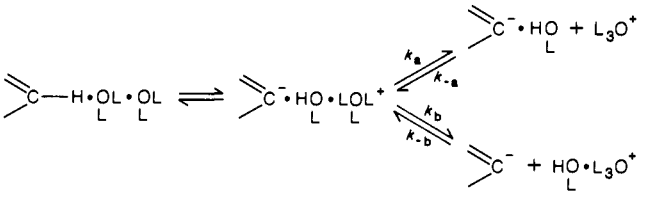
Scheme II



Scheme III



of ρ_I.[43] Solvation of delocalized charge on electronegative atoms in the carbanions of nitro- and carbonyl-activated carbon atoms has been suggested as a reason for the large intrinsic barrier to ionization of these compounds,[44] but there is no such delocalization or solvation in thiazolium ylides; the carbanion is stabilized internally by the adjacent sulfur and cationic nitrogen atoms.

The normal behavior of thiazolium ions provides additional support for the conclusion that the slow proton transfers of other carbon acids involve a larger intrinsic barrier that arises from electron delocalization, rehybridization, changes in bond lengths and angles of heavy atoms, and solvation changes in the transition state.[3c,7,8] It appears that the properties of the C–H bond itself are not directly responsible for the slow proton-transfer reactions that are observed with most carbon acids; carbon acids can be as normal as any other acid with respect to rates of proton transfer if these mechanisms for stabilization of the carbanion are not required.

The conclusion that proton transfer from carbon is not intrinsically slower than that of more electronegative atoms is surprising, because hydrogen bonding certainly stabilizes transition states for proton transfer and carbon acids are not believed to form strong hydrogen bonds in either ground or transition states.[9] Measurements of hydrogen bonding between cyanide ion and Brønsted acids in the gas phase have shown that cyanide is similar to chloride ion in its hydrogen-bonding properties.[45] There is spectral evidence for a weak intramolecular $C^-\cdot HOR$ hydrogen bond and specific solvation of fluorenide and indenide anions by hydrogen bonding.[46] Stabilization by hydrogen bonding is expected to be most favorable when the negative charge is largely localized at carbon, as for the acetylide anion,[7b] cyanide, or the C(2) ylide,[15] and will be maximal for late transition states with a large amount of charge development; however, there appears to be no strong hydrogen bond between water and the C(2) ylide (see below). The conclusion that transition-state stabilization by hydrogen bonding is not significant in thiazolium C(2)-proton exchange implies that poor hydrogen bonding is not likely to contribute significantly to the slow rate of formation of delocalized carbanions.

Direct Proton Transfer and Weak Hydrogen Bonding to the Carbanion. For carbon acids and bases it is generally believed that proton transfer occurs directly, rather than through an intervening water molecule.[5,34] This conclusion is based mainly on the failure to detect the primary and secondary solvent deuterium isotope effects,[40] the inhibition by acid,[8,35] and saturation transfer of the proton NMR signal[5] that are expected if proton transfer occurred through water. Figure 2 shows that there is a pD-independent "water" reaction of **1c** in 0.8–2.7 M DCl, in the region of $D_0 = 0$ to -1, followed by a small additional decrease in rate as the acid concentration is increased to 10.4 M ($D_0 = -3.8$). The solid lines show the inhibition by DCl that is expected for ionization of this carbon acid according to the Swain–Grunwald mechanism[4] (Scheme II) and as a consequence of acidity function effects.

Proton transfer from several protonated amines to water occurs through a hydrogen-bonded water molecule according to the Swain–Grunwald mechanism, as shown in Scheme II. Proton exchange is inhibited in the presence of acid because the H^+ that has been transferred to an adjacent water molecule is returned to the base (k_{-1}) faster than the H_2O molecule can diffuse away from the base (k_D). No such inhibition is observed for carbon acids such as HCN[8] or *tert*-butylmalononitrile.[35]

It is evident from the data in Figure 2 that there is no inhibition of C(2)-proton exchange from **1c** in the range of acid concentration that causes inhibition of reactions that occur through the Swain–Grunwald mechanism; in fact, there is only modest inhibition in more concentrated acid solutions. The rate equation that describes Scheme II for thiazolium ions is given in eq 6, in

$$\frac{\text{rate}}{[\text{C–H}]} = \frac{K_a k_{-1}(\eta_0/\eta)([D^+]/d_0)k_D(\eta_0/\eta)}{k_D(\eta_0/\eta) + k_{-1}(\eta_0/\eta)} \quad (6)$$

which K_a is the equilibrium constant for ionization of **1c** ($pK_a = 16.9$),[15] η_0/η is the viscosity relative to the viscosity in dilute aqueous solution,[47] k_{-1} is the reverse rate constant in dilute aqueous solution, and d_0 is an acidity function for the ionization of a cationic acid. The acid inhibition that is expected for proton transfer through water by this mechanism was calculated from eq 6 and the Hammett D_0 acidity function with $k_D = 1 \times 10^{11}$ s^{-1}, which corresponds to the rate constant for dielectric relaxation of water,[48] and $k_{-1}^0 = 2 \times 10^{10}$ M^{-1} s^{-1}. Values for k_{-1}^0 in the range $(1–4) \times 10^{10}$ M^{-1} s^{-1} have been reported[28] for protonation of amines by H_3O^+ and a rate constant of 4×10^{10} M^{-1} s^{-1} has been measured for the diffusion-controlled protonation of CN^- by H_3O^+, which is a localized carbanion similar to the thiazolium C(2) ylide.[8] The D_0 acidity function in DCl/D_2O mixtures (10^{-4}–1.5 M) is identical with the H_0 acidity function in aqueous HCl for substituted aniline indicators[26,49] and is temperature independent.[50]

For the Swain–Grunwald mechanism, the rate-limiting step for proton transfer is the diffusion together or apart of $C^-\cdot HOL$ and L_3O^+ (k_a or k_{-a}, Scheme III). The rate-limiting step of an alternative mechanism that involves proton transfer through water bridges is diffusion together or apart of C^- and $HOL\cdot L_3O^+$ (k_b or k_{-b}).[8,51] Consequently, the absence of inhibition through the Swain–Grunwald mechanism means that either (1) there is no proton transfer through water or (2) transfer through water occurs

(43) Reference 15. The identical ρ_I values for normal acids and thiazolium C(2) ylides provide no evidence that stabilization of the C(2) ylide by a resonance contribution from a carbene-like structure (**2b**, Scheme I) is important. The formation of a rearranged dimer from thiamin, 3-benzylthiazolium, and 3-benzylbenzothiazolium salts was suggested to occur through a mechanism involving carbene dimerization followed by a 1,3 sigmatropic rearrangement of the benzyl (or aminopyrimidinyl) group: Doughty, M. B.; Risinger, G. E. *Bioorg. Chem.* **1987**, *15*, 1–14. However, an ionic mechanism involving C(2)-ylide addition to the thiazolium ring at C(2) followed by deprotonation and rearrangement was not ruled out.

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(45) Larson, J. W.; McMahon, T. B. *J. Am. Chem. Soc.* **1987**, *109*, 6230–6236.

(46) Ahlberg, P.; Johnsson, B.; McEwen, I.; Rönnqvist, M. *J. Chem. Soc., Chem. Commun.* **1986**, 1500–1501.

(47) The relative viscosity of aqueous HCl solutions at 30 °C was calculated by using $\log(\eta_0/\eta) = 9.66 \times 10^{-6} - 0.0269[\text{HCl}]$, which is based on $\eta_0 = 0.7975$ cp for pure H_2O at 30 °C (Weast, R. C., Ed. *CRC Handbook of Chemistry and Physics*, 66th ed.; CRC Press: Boca Raton, FL, 1985; p F-37) and the viscosity of aqueous HCl solutions at 30 °C (Nishikata, E.; Ishii, T.; Ohta, T. *J. Chem. Eng. Data* **1981**, *26*, 254–256); we assume that the relative viscosities of aqueous HCl and DCl solutions are equal.

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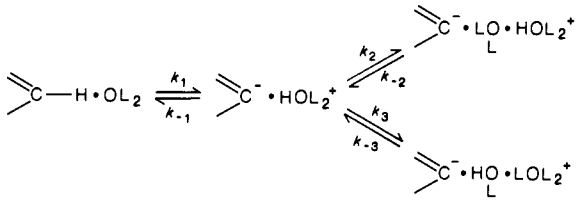
Scheme IV



to give the hydrogen-bonded species $HOL{\cdot}L_3O^+$, which diffuses away from the carbanion as a unit faster than it separates. Microscopic reversibility requires that the same pathway must be followed in both directions. Therefore, we are left in either case with the surprising conclusion that in the protonation direction there is no significant protonation of the thiazolium C(2) ylide by the solvated proton through the approach of L_3O^+ to the solvated C(2) ylide, followed by proton transfer through the solvating water molecule (upper pathway, Scheme III), in spite of the difference of $\sim$19 pK units between the acid and the base.

In principle, the solvent deuterium isotope effect can determine if any water bridges are involved in the rate-limiting step of the proton-transfer reaction, regardless of the kinetic unit to which the water belongs.[51] The observed solvent deuterium isotope effect of $k_{H_2O}/k_{D_2O} = 2.8$ for C(2)–H $\rightarrow$ T exchange indicates that the initial product is HOL_2^+, not $HOL{\cdot}L_3O^+$, that undergoes rate-limiting diffusion-controlled separation. Therefore, proton transfer does not occur through a water molecule followed by rate-limiting diffusional separation, k_b, according to the lower pathway in Scheme III. This solvent isotope effect is consistent with the calculated isotope effect of $k_{H_2O}/k_{D_2O} = 2.6$ from a fractionation factor of $1/l^2 = 1/(0.69)^2 = 2.1$ for HOD_2^+,[52] a solvent isotope effect of $\eta_{D_2O}/\eta_{H_2O} = 1.22$ for diffusional separation of HOD_2^+ from the C(2) ylide,[53] and a fractionation factor for the thiazolium C(2)–H of $\phi_{CH} = 1.0$.[54] We assume a fractionation factor of $\phi_{C^-} = 1.0$ for the thiazolium C(2) ylide in this range of [LCl]. A solvent isotope effect of $k_{H_2O}/k_{D_2O} = 3.7$ is calculated for proton transfer through solvent (k_b) from the same parameters and a fractionation factor of $1/l^3 = 1/(0.69)^3 = 3.0$ for D_3O^+.

Exchange of C(2)–H $\rightarrow$ T involves the diffusion-controlled reaction of either TOH_2^+ or TOD_2^+ with the thiazolium C(2) ylide. This is demonstrated by the observation that the solvent deuterium isotope effect of $k_{H_2O}/k_{D_2O} = 2.8$ is independent of [LCl] in the range 1.75–4.9 M. Our mechanistic interpretation of this solvent isotope effect requires that there is little, if any, solvent isotope effect for diffusion of the triton in L_2O. If there were a solvent isotope effect of $k_{TOL{\cdot}L_3O^+}/k_{LOL{\cdot}L_3O^+} = 1.3$ for reprotonation of the C(2) ylide (k_{-b}), the observed solvent deuterium isotope effect would be consistent with the through-water pathway (k_b) because $(2.8 \pm 0.2) \times 1.3 \simeq 3.7$. However, the rate-limiting step for reprotonation of the C(2) ylide by the through-water pathway is diffusion-controlled encounter of the C(2) ylide and either $TOL{\cdot}L_3O^+$ or $LOL{\cdot}L_3O^+$ (k_{-b}, Scheme III). The solvent isotope effect on diffusion of HTO in H_2O and DTO in D_2O is small ($\leq$3%)[55] and would not be expected to be significantly larger if LTO is hydrogen bonded to L_3O^+. This means that $k_{TOL{\cdot}L_3O^+}/k_{LOL{\cdot}L_3O^+} \simeq 1.0$.

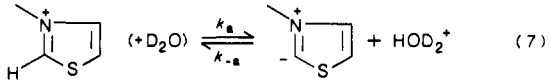
The solvent isotope effect of $k_{H_2O}/k_{D_2O} = 2.8$ shows that the rate-limiting step for thiazolium C(2)–H $\rightarrow$ T exchange is diffusional separation of HOL_2^+ from the C(2) ylide to form a solvent-separated ion pair with the rate constant k_2 (Scheme IV); the reaction does not occur through a mechanism involving a proton jump to an adjacent water molecule (k_3). This means that breaking of the hydrogen bond between the strongly acidic L_3O^+ and the strongly basic C(2) ylide (k_2) is extremely fast, faster than a proton jump to an adjacent water molecule (k_3).

It is likely that $C^-{\cdot}LOL{\cdot}OL_3^+$ is formed initially upon encounter of L_3O^+ and the C(2) ylide. However, microscopic reversibility requires that protonation occur through the k_2–k_{-2} pathway, so that when L_3O^+ approaches the C(2) ylide in the diffusion-controlled reverse reaction any water molecule that is hydrogen bonded to the C(2) ylide is expelled from the solvent-separated ion pair (k_{-2}) faster than a proton jumps to this water molecule to give protonation of the C(2) ylide (k_{-3}). Consequently, if there is a water molecule hydrogen bonded to this highly localized carbanion, it dissociates faster than a proton can jump to it from L_3O^+. We conclude that the dissociation of water from the C(2) ylide must be extremely fast, on the order of 10^{11} s^{-1}, which corresponds to the rate constant for dielectric relaxation of water,[48] and that there is no strong hydrogen bond between water and the C(2) ylide. The separation of a hydrogen-bonded water molecule from a more delocalized carbanion is expected to be at least as fast.

The absence of any detectable proton transfer through water to such strongly basic and relatively normal carbanions as thiazolium C(2) ylides ($\Delta pK \simeq 19$) supports the conclusion that bimolecular proton transfer is direct with other less normal carbon acids and their carbanions that have more delocalization of negative charge and larger intrinsic barriers for proton transfer than thiazolium ions. Proton transfer between HCN, which is slightly less normal than thiazolium ions, and nitrogen and oxygen bases has been shown to be >99% direct for reactions with $\Delta pK \leq 5$ units.[5] An upper limit of $\leq$30% for the fraction of thiazolium C(2)–H $\rightarrow$ L exchange that occurs by the through-water pathway (k_b, Scheme III) can be calculated from an upper limit for $k_{H_2O}/k_{D_2O} = 3.0$ (from 2.8 + 0.2). Although the reaction of H_3O^+ and CN^- ($\Delta pK > 10$) does not occur through water by the Swain–Grunwald mechanism (k_a, Scheme III), the possibility has not been excluded that it occurs by the through-water pathway (k_b).[8]

It has been suggested that proton transfer to or from carbon might occur through a solvent molecule for protonation of carbanions with intramolecular assistance by groups that are close enough to permit direct proton transfer; the same mechanism must hold for the reverse deprotonation reaction in which the carbanion is formed.[5,56] Such intramolecular assistance of proton transfer to carbon through an intervening water molecule has been demonstrated for facilitation of the protonation of carbanions by neighboring amines.[57] The solvent deuterium kinetic isotope effects of $k_{H_2O}/k_{D_2O} \simeq 3.6$ for cyanocarbon acids,[40c] which are almost normal acids, have been explained as being consistent with the through-water pathway.[35]

Exchange of Thiazolium C(2) Protons in Concentrated Acid. Inhibition of the ionization rate of 3-(cyanomethyl)-4-methylthiazolium ion (**1c**) is expected in strong acid media because of acidity function effects; the large increase in the activity of HOD_2^+ in strong acid solutions would be expected to shift the equilibrium in eq 7 to the left.[58] The inhibition that is expected from acidity



(7)

$$k_a = k_{-a}K_a[D_3O^+]/d_x \quad (8)$$

function effects was calculated from eq 7 and 8, taking d_x as either

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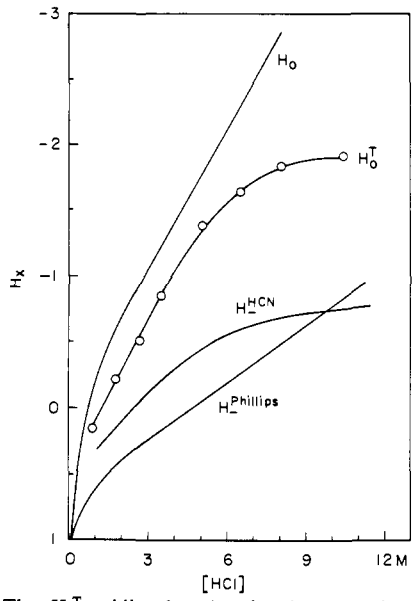


Figure 4. The H_0^T acidity function for deprotonation of 3-(cyanomethyl)-4-methylthiazolium ion in HCl. The values of H_0^T were calculated from the observed rate constant for C(2)–H → L exchange, after correction for a secondary solvent deuterium isotope effect of k_{H_2O}/k_{D_2O} = 2.8, by using eq 7, 9, and 10 with pK_a = 16.9; $k_{-a}^0 = 2 \times 10^{10}$ M^{-1} s^{-1}; $k_a = k_{obsd}$; relative viscosity values at 30 °C.[47] The Hammett H_0,[26] Phillips H_-,[60] and H_-^{HCN} acidity functions[8] are shown for comparison.

the Hammett D_0[26] or the D_-^{DCN} acidity function[59] and k_{-a} = 2 × 10^{10} M^{-1} s^{-1}.

Since k_{-a} (eq 7) is diffusion limited, the rate constant for the ionization of C(2)–H (k_a) is expected to decrease in direct proportion to the ratio $[HOD_2^+]/d_x$, as shown in eq 8, in which $d_x \equiv a_{HOD_2^+}(f_{C^-}/f_{CH})$. Figure 2 shows that the amount of inhibition by acid of D_2O-catalyzed C(2)–H → D exchange from **1c** in DCl/D_2O mixtures is much less than that predicted by the Hammett D_0 acidity function, but is larger than the inhibition predicted by eq 8 and the D_-^{DCN} acidity function.

The observed decrease in the rate constant for C(2)–H → D exchange from **1c** ($k_a = k_{obsd}$, eq 7) was used to calculate an H_0 acidity function for 3-(cyanomethyl)-4-methylthiazolium ion, after correction for the solvent deuterium isotope effect of k_{H_2O}/k_{D_2O} = 2.8, by using eq 9 and 10. The values of this H_0 acidity function

$$H_x \equiv pK_a + \log I \quad (9)$$

$$I \equiv k_a/(k_{-a}[L_3O^+]) = k_a/(k_{-a}^0(\eta_0/\eta)[L_3O^+]) \quad (10)$$

for **1c** in HCl (designated H_0^T) are shown graphically in Figure 4 and in tabular form in supplementary Table S4. The values of the H_-^{HCN},[8] Phillips H_-,[60] and Hammett H_0 acidity functions are shown for comparison.

Figure 5 shows a logarithmic plot of $k_{obsd}(\eta/\eta_0)/[H_3O^+]$ against several acidity functions. The H_0^T acidity function, by definition, falls on a straight line with slope 1.0. The H_0^T acidity function for thiazolium C(2)–H is more sensitive to acid concentration than both the Phillips H_- acidity function for the first ionization of a series of phosphorus-containing acid indicators and the H_-^{HCN} acidity function. It parallels the Hammett H_0 acidity function for substituted aniline indicators at [HCl] < 6 M, but deviates negatively from H_0 for [HCl] > 6 M. The weaker acidity

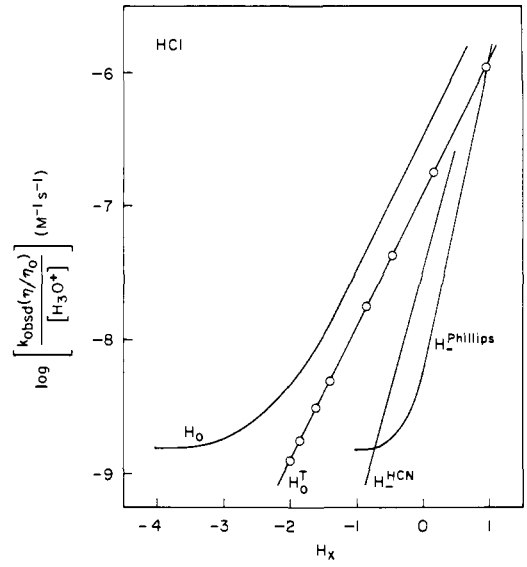
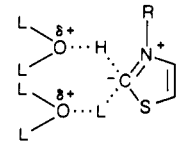


Figure 5. Logarithmic plot of $k_{obsd}(\eta/\eta_0)/[H_3O^+]$ against several acidity functions.

Chart I



functions observed for HCN and for phosphates and phosphonates compared to thiazolium C(2)–H indicate that there is less decrease in f_{C^-}/f_{CH} for thiazolium ionization than for these acids in moderately strong acid solutions to offset the large increase in $f_{H_3O^+}$; however, f_{C^-}/f_{CH} does decrease at [HCl] > 6 M.

The modest amount of acid inhibition of thiazolium C(2)–H ionization indicates that some component of proton donation must destabilize CH (increase f_{CH}) or stabilize C^- (decrease f_{C^-}) in order to offset the inhibition that is expected from the increase in $a_{HOD_2^+}$. The decrease in f_{C^-}/f_{CH} at [HCl] > 6 M in Figure 4 could result from stabilization of the C(2) ylide by formation of a $C^-{\cdot}H_3O^+$ ion pair or formation of a hydrogen bond between H_3O^+ and the π electrons of the thiazolium ring, an "Hπ bond".[61] The stabilization of sulfate ion in aqueous sulfuric acid solutions may result from the formation of an ion pair, $SO_4^{2-}{\cdot}H_3O^+$, and provides precedent for the stabilization of anions in strong acid media;[62] however, the C(2) ylide (see above) and other carbanions form relatively weak hydrogen bonds. The strong stabilization of CN^- in strong acid media[8] may represent ion pair formation by attachment of H_3O^+ to the nitrogen rather than the carbon atom of CN^- because the CN^- ion is believed to have a dipole moment with negative charge on the more electronegative nitrogen atom.[63] There is precedent for a π complex between an aromatic ring and a hydrogen bond donor; an NH–π interaction was recently demonstrated by ^1H NMR between the amide groups of Gly-37 and Asn-44 and the aromatic ring of Tyr-35 in basic pancreatic trypsin inhibitor.[64]

(59) The D_-^{DCN} acidity function was calculated from the observed rate constants for HCN proton exchange in H_2O at 20 °C, after correction for a secondary solvent deuterium isotope effect of k_{H_2O}/k_{D_2O} = 4.5 (ref 8), using eq 7, 9, and 10 with $k_{-a}^0 = 4 \times 10^{10}$ M^{-1} s^{-1} and pK_a = 9.4 for DCN in D_2O; k_{-a}^0 is the value of k_{-a} in dilute acid solution. The pK_a for DCN was calculated from pK_a = 9.0 in H_2O at ionic strength 1.0 M (KCl) at 25 °C (Reenstra, W. W.; Jencks, W. P. *J. Am. Chem. Soc.* **1979**, *101*, 5780–5791) and ΔpK_a = 0.4 for the solvent deuterium isotope effect on the ionization of HCN (Reenstra, W. W.; Abeles, R. H.; Jencks, W. P. *J. Am. Chem. Soc.* **1982**, *104*, 1016–1024).

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An alternate explanation for the modest inhibition of thiazolium C(2)-proton exchange in strong acid should be considered in which acid has a direct role in the chemistry of C(2)-proton exchange, rather than the indirect role of stabilization of the C(2) ylide. Specifically, an increase in the C(2)-proton exchange rate due to acid-catalyzed exchange might not be observed because of incomplete cancellation of activity coefficient effects, rather than from a large decrease of f_{C^-} in strong acid.[8] However, acid-catalyzed exchange involving electrophilic displacement at carbon with a transition state in which the leaving and entering protons interact weakly with the C(2) ylide, as shown in Chart I, is unlikely because (1) there is no increase in the rate of exchange with increasing acidity from $D_0 = -2$ to -4 (Figure 2), (2) there is no clear precedent for such electrophilic assistance to proton exchange, and (3) the strong inhibition by acid of proton exchange of protonated amines[4] is inconsistent with this mechanism.

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Supplementary Material Available: Tables showing rate constants for general-base catalysis of 3-R-4-methylthiazolium ion and N(1')-protonated thiamin C(2)-L → D exchange, and for exchange of 3-cyanomethyl-4-methylthiazolium ion C(2)-H in aqueous LCl solutions (6 pages). Ordering information is given on any current masthead page.

Thiazolium C(2)-Proton Exchange: Isotope Effects, Internal Return, and a Small Intrinsic Barrier[1]

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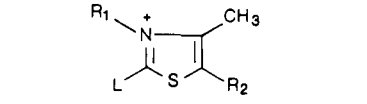
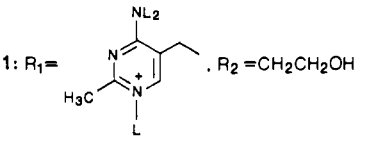
Abstract: Rate constants are reported for C(2)-hydron exchange catalyzed by lyoxide ion from thiazolium ions of pK_a 16.9–18.9 at 30 °C and ionic strength 2.0 M in aqueous solution. The reactions with deuterioxide ion, which are close to $\Delta pK = 0$, show primary kinetic isotope effects that increase over the range $(k_H/k_T)_{obsd}$ = 2.9–14.7 with increasing acidity of the thiazolium ion. Deviations of $(k_D/k_T)_{obsd}$ and $(k_H/k_T)_{obsd}$ from the Swain–Schaad equation are consistent with internal return of the transferred proton to the C(2) ylide from water. This corresponds to an Eigen mechanism for proton transfer, in which both proton transfer and diffusional separation of the C(2)-ylide·water complex are partially rate limiting, and a small intrinsic barrier for C(2)-hydron exchange. A disappearance of the temperature dependence of $(k_H/k_T)_{obsd}$ with decreasing thiazolium ion acidity is also consistent with internal return. Correction of $(k_H/k_T)_{obsd}$ for internal return gives the primary isotope effect on the proton-transfer step, which increases over the range of ~9.6–18.7 as the acidity of the carbon acid increases. This is consistent with a changing structure of the transition state for proton transfer. A large decrease in the secondary solvent isotope effect for C(2)–T → L exchange from k_{OD}/k_{OH} = 2.4 to 1.3 provides evidence for a decrease in the amount of triton transfer to lyoxide ion in the transition state as the acidity of the carbon acid increases. The values of k_{OD}/k_{OH} and the negative deviation of deuterioxide ion from the Brønsted plot for general-base catalysis are consistent with a requirement for the removal of a solvating water molecule from lyoxide ion before abstraction of a C(2) hydron, with K_{desolv} = 0.02 and pK_a = 18.1 for the partially desolvated deuterioxide ion at 30 °C. The rate constants for C(2)–H → D exchange catalyzed by deuterioxide ion increase with decreasing ionic strength.

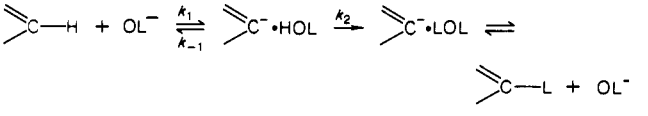
The preceding paper[2] describes evidence that thermodynamically unfavorable C(2)–proton transfer from N(1')-protonated thiamin (pK_a = 17.6)[3] (**1**), 3,4-dimethylthiazolium ion (pK_a = 18.9) (**2**), and 3-(cyanomethyl)-4-methylthiazolium ion (pK_a = 16.9) (**3**) in aqueous solution is similar to proton transfer from "normal" acids. Proton transfer between the electronegative atoms of normal acids and bases involves diffusion-controlled separation of the products in the unfavorable direction and there is a small region near $\Delta pK = 0$ in which the proton-transfer step itself is partially rate limiting.[4-6] The Brønsted plots for such reactions follow "Eigen curves" with slopes of 0 and ±1.0 in the favorable and unfavorable directions, respectively, and have a small transition region near $\Delta pK = 0$ of slope ~0.5 where the proton-transfer step gives rise to a deuterium isotope effect.[5] Abstraction of the C(2) proton from these thiazolium ions follows almost completely





2: $R_1 = CH_3$, $R_2 = H$
3: $R_1 = CH_2CN$, $R_2 = H$
4: R_1 = Bzl, $R_2 = H$

Scheme I



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