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C(α)-Proton Transfer from Thiazolium Ions: Structure–Reactivity Correlations and the C(α)-H pK_a of 2-(1-Hydroxyethyl)thiamin[1]

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Rate constants for C(α)-proton transfer from racemic 2-(1-hydroxyethyl)thiamin (HET), 2-(1-hydroxyethyl)oxythiamin (oxy-HET), and several 2-(1-hydroxyethyl)-3-*R*-4-methylthiazolium ions catalyzed by H_2O, hydroxide ion, and various oxygen-containing and amine buffers in the pH range 2–9 were determined by iodination at 25°C and ionic strength 1.0 M in H_2O. Thermodynamically unfavorable C(α)-proton transfer from HET and oxy-HET shows general-base catalysis with a Brønsted β value of ≥0.9 and primary kinetic isotope effects of $(k_H/k_D)_{obsd} = 1$. These results are consistent with diffusion-controlled proton transfer to and from the C(α) position of HET in aqueous solution and a very late, enamine-like transition state for thermodynamically unfavorable C(α)-proton transfer. General base catalysis is detectable because there is a negative deviation from this correlation by hydroxide ion. Values of pK_a' = 18.4 and 19.8 for the C(α)-proton of N(1′)-protonated HET and free HET, respectively, were calculated from the rate constants for catalysis by H_2O and buffer bases. Values of $k_{-a} = 2 \times 10^{10}$ and 3×10^9 M^{-1} s^{-1} were assumed for diffusion-controlled protonation of the C(α)-enamine in the reverse direction by H_3O^+ and buffer acids, respectively. The Hammett ρ_1 value for C(α)-proton transfer catalyzed by hydroxide ion or phosphate dianion is 22 ± 3. The absence of a positive deviation of the rate constants for catalysis by phosphate dianion when *R* = Me or *n*-Pr from the correlation defined by bulky aryl substituents is inconsistent with significant steric inhibition of resonance in the enamine which affects the rate of C(α)-proton removal or the C(α)-H pK_a' value. The ≤15-fold positive deviations of the rate constants for catalysis by hydroxide ion from the correlation defined by bulky aryl substituents when R = Me > Et > *n*-Pr > *i*-Bu are attributed to a substituent-dependent "hydroxide ion anomaly." Mechanistic implications for thiamin diphosphate-dependent enzymes are discussed.

INTRODUCTION

Transfer of the C(α)-proton of 2-(1-hydroxyethyl)thiamin (HET)[3] (**1**, Scheme 1) gives a resonance-stabilized conjugate base (**2**) which has been implicated in

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[3] Abbreviations used: HET, 2-(1-hydroxyethyl)thiamin; HETDP, 2-(1-hydroxyethyl)thiamin diphosphate; oxy-HET, 2(1-hydroxyethyl)oxythiamin; TDP, thiamin diphosphate, Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; PDC, pyruvate decarboxylase; MET, 2-(1-methoxyethyl)-3,4-dimethylthiazolium ion; Me_2SO, dimethyl sulfoxide.

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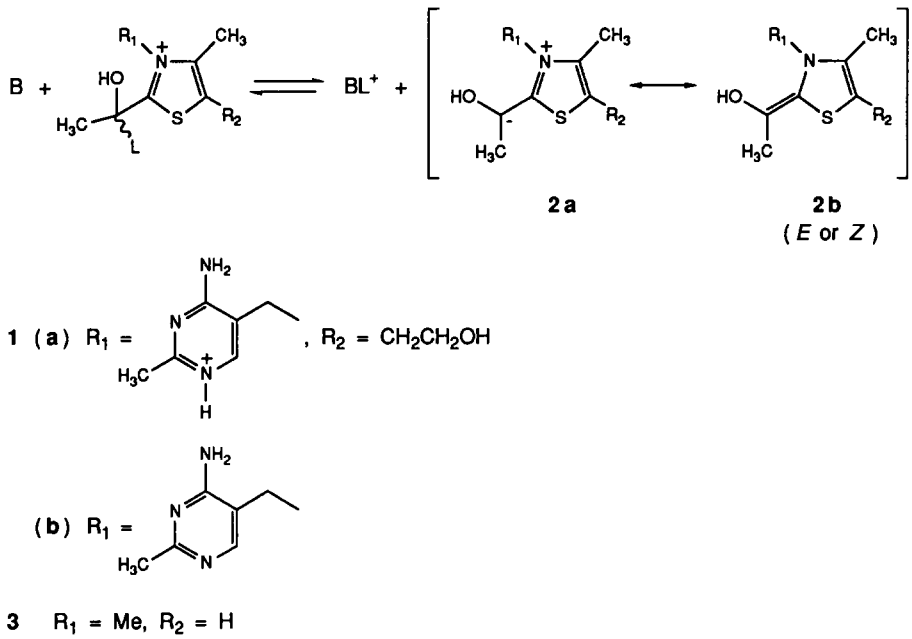


SCHEME 1

addition–elimination reactions catalyzed by several thiamin diphosphate (TDP)-dependent enzymes (*1*). For example, the C(α)-enamine (**2b**) derived from HETDP [$R_2 = (CH_2)_2OP_2O_6^{3-}$] is a proposed intermediate in the conversion of pyruvate to acetaldehyde by the TDP-dependent enzyme pyruvate decarboxylase (PDC) (2-oxo-acid carboxy-lyase; EC 4.1.1.1) (*2*). We are interested in understanding the factors which determine the rate of C(α)-proton transfer from HET, which is a reaction that is important for functioning of the coenzyme thiamin diphosphate on PDC and related enzymes.

An important mechanistic question is whether steric inhibition of resonance in **2b** derived from HET determines the rate of C(α)-proton removal. Stereoselective rates of proton abstraction α to carbonyl groups (*3*) and iminium ions (*4*) have been widely interpreted as reflecting the requirement for proper p–π orbital alignment between the acidic carbon atom and the activating group, although the evidence supporting this explanation is limited and not without controversy (*5*). Zoltewicz and co-workers proposed that steric interactions between the C(2)- and N(3)-substituents which limit formation of **2b** by preventing delocalization of the C(α)-carbanion (**2a**) in the transition state would decrease the rate of C(α)-proton removal (*6*). We previously described evidence that, in the absence of significant steric inhibition of rotation about the C(2)–C(α) bond, C(α)-proton abstraction occurs at the maximum possible rate for a given equilibrium constant (*7*).

We have examined the role of inductive effects on the rate of nonenzymatic C(α)-proton removal in aqueous solution in order to evaluate the importance of steric hindrance by bulky N(3)-substituents on the rate of proton removal. Our

results indicate that steric inhibition of resonance by the 3-aminopyrimidinyl group is not a significant factor in determining the rate of C(α)-proton removal from HET. The magnitudes of rate constants for catalysis by buffer bases and water of C(α)-proton removal from HET and related substrates and isotope effects for catalysis by buffer bases and hydroxide ion provide evidence for (i) normal acid behavior for ionization of C(α)-H in HET and (ii) C(α)-H pK_a' values of 18.4 for N(1')-protonated HET and 19.8 for free HET.

EXPERIMENTAL PROCEDURES

Materials

Unless otherwise stated, chemicals were reagent grade. All deuterated compounds were ≥99 atom% D (Aldrich). Water was prepared on a four-bowl Milli-Q water system including an Organex-Q cartridge (Millipore). Catalysts were purified by redistillation or by recrystallization as the hydrochloride or potassium salt except for methanol and trifluoroethanol, which were used without further purification. Stock solutions of reagents in D_2O were prepared as described previously (*8*). The synthesis and purification of racemic HET (**1**) has been described (*9*); the synthesis of **1** with deuterium in the C(α) position was accomplished using acetaldehyde-1-*d* instead of acetaldehyde-1-*h*. The *R* and *S* enantiomers of **1** were prepared as described by Kluger *et al.* (*10*). The other racemic thiazolium salts were prepared by refluxing racemic 2-(1-hydroxyethyl)-4-methylthiazole (*11*) with an excess of the appropriate alkyl halide in 2-butanone and the salts were crystallized from methanol/diethyl ether unless otherwise stated. The diethyl ether was stored over activated alumina before use to dry the solvent and remove peroxides (*9b*).

2-(1-Hydroxyethyl)oxythiamin chloride hydrochloride (***4***). In a manner similar to that shown for the synthesis of oxythiamin (*12*), 2.7 g (90%) of racemic oxy-HET (**4**) was obtained from racemic HET (**1**) as a white solid: mp 197–199°C; ^{13}C NMR (D_2O) δ 14.6, 23.7, 25.2, 31.9, 50.7, 63.2, 118.5, 135.8, 146.7, 151.9, 165.3, 168.6, 181.6; ^1H NMR (D_2O) δ 1.71 (d, 3H), 2.4 (s, 3H), 2.6 (s, 3H), 3.1 (t, 2H), 3.9 (t, 2H), 5.5 (s, 2H), 5.7 (q, 1H), 7.9 (s, 1H). The product gave a negative thiochrome test and contained no detectable HET on the basis of visualization of silica gel TLC by fluorescence quenching after development in acetonitrile : water (40 : 10), which was adjusted to pH 6.5 with formic acid; HET, $R_f = 0$; oxy-HET, $R_f = 0.2$.

2-(1-Hydroxyethyl)-3-benzyl-4-methylthiazolium iodide (***5***). Melting point 163–165°C; ^{13}C NMR (Me_2SO-d_6, proton coupled) δ 13.4 (q), 23.4 (q) 52.6 (t), 64.5 (d), 119.8 (d), 124.9 (s), 127.0 (d), 127.9 (d), 130.0 (d), 132.8 (d), 146.7 (s), 183.1 (s); ^1H NMR (D_2O) δ 1.6 (d, 3H), 2.4 (s, 3H), 5.5 (q, 1H), 5.7 (s, 2H), 7.2 (m, 2H), 7.4 (M, 3H), 7.8 (s, 1H).

2-(1-Hydroxyethyl)-3-(4-nitrobenzyl)-4-methylthiazolium chloride (***6***). In a manner similar to that shown for the synthesis and purification of racemic HET (*9*), 0.7 g (30%) of racemic **6** was obtained from 3-(4-nitrobenzyl)-4-methylthiazolium

bromide (*8a*) as a hygroscopic white solid: ^{13}C NMR (D_2O) δ 15.9, 24.8, 55.2, 67.6, 122.7, 127.2, 128.8, 132.1, 142.5, 150.9, 181.2; ^{1}H NMR (D_2O) δ 1.6 (d, 3H), 2.4 (s, 3H), 5.4 (q, 1H), 5.9 (s, 2H), 7.3 (d, 2H), 7.9 (s, 1H), 8.2 (d, 2H).

2-(1-Hydroxyethyl)-3-pentafluorobenzyl-4-methylthiazolium bromide (*7*). Melting point 173.4–175.5°C (dec); ^{13}C NMR (D_2O, proton-coupled) δ 16.2 (m), 25.2 (q), 48.6 (t), 67.2 (d), 121.4 (d), 150.0 (s), 162.2 (s); ^{1}H NMR (D_2O) δ 1.5 (t, 3H) 1.7 (d, 3H), 2.6 (s, 3H), 4.4 (m, 2H), 5.5 (q, 1H), 7.7 (s, 1H).

2-(1-Hydroxyethyl)-3-propyl-4-methylthiazolium iodide (*8*). Melting point 137.2–138.2°C (dec); ^{13}C NMR (D_2O, proton coupled) δ 12.7 (q), 16.0 (q), 25.2 (t), 25.4 (q), 54.6 (t), 67.5 (d), 121.5 (d), 150.4 (s), 182.3 (s); ^{1}H NMR (D_2O) δ 1.0 (t, 3H), 1.7 (d, 3H), 1.9 (m, 2H), 2.6 (s, 3H), 4.3 (m, 2H), 5.5 (q. 1H), 7.7 (s, 1H).

2-(1-Hydroxyethyl)-3-(2-methylpropyl)-4-methylthiazolium Iodide (*9*). Melting point 156–157°C (dec); ^{1}H NMR (D_2O) δ 0.94 (m, 3H), 1.0 (m, 3H), 1.6 (d, 3H), 2.3 (m, 1H), 2.6 (s, 3H), 4.3 (m, 2H), 5.5 (q, 1H), 7.7 (s, 1H).

Methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM-300 NMR spectrometer using sodium 3-(trimethylsilyl)propanesulfonate as an internal standard for the ^{1}H NMR spectra and the instrument external standard for the ^{13}C NMR spectra. Melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. Measurements of pL (where L = H or D) were made at the reaction temperature on the buffered solutions of the thiazolium salts after proton transfer had occurred; the value of pD was obtained by adding 0.40 to the observed pH of solutions in D_2O (*8*).

Kinetics. Rate constants for C(α)-hydron abstraction were determined in the pH range 2–9 by following the irreversible iodination of **2** at 351 nm as described previously (*7*). All reactions were performed at 25.0 ± 0.1°C and the ionic strength was maintained at 1.0 M with KNO_3. Final substrate concentrations were in the range 1–40 mM. We previously established that the iodine reaction is following C(α)-proton transfer, that the reaction with iodine is not rate limiting, that the iodination reaction is not readily reversible, and that the stoichiometry of the reaction with iodine involves one molecule of iodine (*7*).[4]

Pseudo-first-order rate constants (k_{obsd}) for C(α)-proton transfer were calculated from the linear slopes of plots of absorbance at 351 nm against time (*7, 14*); where duplicate determinations of k_{obsd} were made, they agreed within ≤±5% of the average value. Because experiments with **1** were performed in the pH range 2–5, values of k_{obsd} for **1** were converted to corrected observed rate constants (k'_{obsd}) based on the fraction of the product in the N(3)-unprotonated and N(1′)-protonated form; this treatment normalizes the observed rate constants to one ionization state of the product. These fractions were calculated using the measured pL of the reaction mixture and apparent pK_a values for protonated N(3) and N(1′) as described elsewhere (*15*). A pK'_a value of 2.8 for N(3) at 25°C and ionic strength 1.0

[4] A complicating side reaction involving cleavage of the alcoholate anion of **1** or **4** [$pK_a^{ROH} \approx 12$ (*11*)] to acetaldehyde and thiamin or oxythiamin, respectively, is excluded because the rate of the cleavage reaction is ≤2% of the rate of C(α)-proton transfer at pH ≤ 10 (*7, 13*).

M (KNO_3) in H_2O was determined (*15*) from kinetic data for **1a** in the pH range 2–4 and $pK'_a = 3.3$ in D_2O was estimated from $\Delta pK_a = 0.5$ for the solvent isotope effect on the ionization of weak acids (*16*). A pK'_a value of 5.38 ± 0.03 for N(1′) in the 3-aminopyrimidinyl substituent of **1** was determined by potentiometric titration at 25°C and ionic strength 1.0 M (KNO_3) in H_2O and $pK'_a = 5.88$ in D_2O was estimated from $\Delta pK_a = 0.5$ for the solvent isotope effect on this ionization (*8a*). The values of k_{obsd} for the other substrates were obtained at pH ≥ 5 and required no correction because these reactions involved only the N(3)-unprotonated form of the product. Second-order rate constants for catalysis by buffer bases, water, and lyoxide ion were determined graphically as described previously (*7*) and are concentration-based; 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.

Values of pK'_a for the buffer catalysts were typically determined from the pL values of gravimetrically prepared buffer solutions containing the acid and base form at a 1 : 1 ratio. Values of $pK'_a = 7.62 \pm 0.03$ and 10.56 ± 0.05 for 1,2-diaminoethane dihydrochloride were determined by potentiometric titration at 25°C and ionic strength 1.0 M (KNO_3): data were analyzed by assuming overlapping ionization steps (*17*).

RESULTS

Second-order rate constants for catalysis of C(α)-hydron transfer from N(1′)-protonated **1a** and **3–9** (see Table 1) by buffer bases, water, and lyoxide ion in aqueous solution at 25°C and ionic strength 1.0 M, maintained with potassium nitrate, were determined in the pH range 2–9 by iodination under initial rate conditions and are reported in Tables 1 and 2. Identical values of k_{obsd} for C(α)-proton transfer from **3** were determined using the iodine assay and for C(α)-D → H exchange by ^1H NMR, which confirms that the iodine assay is following C(α)-proton transfer. Iodination of **4** that is first order in iodine was observed at pH ≤ 5.5: the usual zero-order kinetics in iodine were obtained at pH ≥ 5.5. Similar behavior was observed for **6** and **7** at pH ≤ 6.5. C(α)-proton transfer obeys the rate law described by Eq. [1],

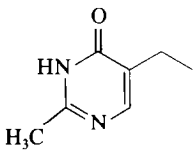
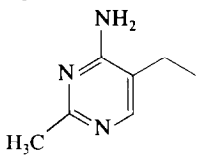
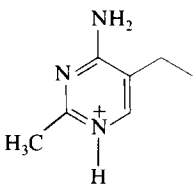
$$k_{obsd}\,(s^{-1}) = k'_{LOL} + k_{LO^-}[LO^-] + k_{B1}[Base_1] + k_{B2}[Base_2]. \qquad [1]$$

Typical data are shown in Fig. 1 for ionization of **1a** catalyzed by acetate, water, and hydroxide ion; no catalysis by the acid component of the buffer was observed. As observed previously for **3** (*7*), a second term for catalysis by buffer bases ($k_{B2}[Base_2]$) is required in the rate law when dibasic buffer catalysts are used. No significant consumption of iodine by 4-amino-5-(aminomethyl)-2-methyl pyrimidine hydrochloride that might suggest iodination of the 3-aminopyrimidinyl substituent was detected in the pH range 2–8. Table 1 also contains σ_I values for the 3-substituents (*8a, 19*).

C(α)-proton transfer from free HET (**1b**) could not be followed with the iodine assay at pH ≥ 4.7 because of a rapid side reaction involving the exocyclic 4′-amino

TABLE 1

Rate Constants for Hydroxide Ion and Phosphate Dianion-Catalyzed C(α)-Proton Transfer from 2-(1-Hydroxyethyl-3-*R*-4-methylthiazolium Ions[a]

3-R		σ_I[b]	k_{HO^-} (M^{-1} s^{-1})[c]	k_B (M^{-1} s^{-1})
Me	**3**	−0.01	1.5	5.3×10^{-7}
Et	**10**	−0.01	0.67	
n-Pr	**8**	−0.01	0.49	4.4×10^{-7}
i-Bu	**9**	−0.01	0.32	
Bzl	**5**	0.04	1.9	2.2×10^{-6}
	**4**	0.07[d]	2.9	2.2×10^{-5}
	**1b**	0.07[e]	2.9[f]	
4-NO_2-Bzl	**6**	0.09[e]	14	1.6×10^{-4}
$C_6F_5CH_2$	**7**	0.11[d]	45	
	**1a**	0.12[e]	73	5.6×10^{-4}[g]

[a] At 25°C and ionic strength 1.0 M (KNO_3) in H_2O. The rate constant k_B for general-base catalysis by phosphate dianion is defined in Eq. [1].

[b] Ref. (*19a*).

[c] Concentration-based, $\gamma_{HO^-} = 0.64$.

[d] Ref. (*19b*).

[e] Ref. (*8a*).

[f] This rate constant was calculated by dividing the corresponding rate constant for N(1')-protonated HET (**1a**) by 25 (see text).

[g] Extrapolated from Fig. 3.

group. No changes in the ultraviolet or ^1H NMR spectrum of **1b** that might suggest the nature of the side reaction were detected in the absence of iodine at pL $\geq$ 4.7. The side reaction cannot be attributed to the presence of contaminating thiamin in the HET sample (*9*). Intramolecular general-base catalysis of C(α)-proton transfer from **1b** by the exocyclic 4'-amino group is unlikely to be significant (*8a*). The side reaction was not observed for **4**, in which the exocyclic 4'-amino group is absent, and was not further characterized.

Rate increases from general-base catalysis were typically large ($\leq$1000%) at total buffer concentrations $\leq$0.4 M. Catalysis is detectable because the rate constant

TABLE 2

Rate Constants for General-Base Catalysis of C(α)-Proton Transfer from 2-(1-Hydroxyethyl)thiamin (**1**) and 2-(1-Hydroxyethyl)oxythiamin (**4**)[a]

Catalyst	pK_a'[b]	k_B (M^{-1} s^{-1})		
		1a	**1b**[c]	**4**
H_2O[d]	−1.74	1.3×10^{-10}	5.2×10^{-12}	
HO^-	15.74	73	2.9	2.9
$NCCH_2COO^-$	2.33	2.5×10^{-8}	1.0×10^{-9}	
$ClCH_2COO^-$	2.78	1.8×10^{-7}	7.2×10^{-9}	
$H_3COCH_2COO^-$	3.65	4.3×10^{-7}	1.7×10^{-8}	
H_3CCOO^-	4.58	8.6×10^{-7}	3.4×10^{-8}	
$F_3CCH_2NH_2$	5.66			2.6×10^{-6}
$(H_3C)_2AsO_2^-$	6.15			8.7×10^{-6}
HPO_4^{2-}	6.57			2.1×10^{-5}
$H_2NCH_2CH_2NH_3^+$	7.62			9.9×10^{-5}
$H_2NCH_2CH_2NH_2$	10.56			4.5×10^{-2}
$F_3CCH_2O^-$	12.3			0.14
H_3CO^-	15.6			≤6

[a] At 25°C and ionic strength 1.0 M (KNO_3) in H_2O. The rate constant k_B is defined in Eq. [1].

[b] Apparent pK_a of the conjugate acid at 25°C and ionic strength 1.0 M (KNO_3) in H_2O.

[c] Rate constants for free HET (**1b**) were calculated by dividing the corresponding rate constant for N(1′)-protonated HET (**1a**) by 25 (see text).

[d] The second-order rate constant was calculated using a standard state of 55.4 M for pure H_2O at 25°C.

for hydroxide ion falls below the Brønsted line (see below). Because of the large β values in these reactions, catalysis by hydroxide ion would overwhelm general-base catalysis if the hydroxide ion also obeyed the Brønsted correlation. Changing the concentration of the acid component of the buffer or substituting potassium trifluoroacetate for potassium nitrate had no effect on k_B, which indicates that medium and specific salt effects are small.

The value of the observed pseudo-first-order rate constant ($k'_{HOH} = 7.2 \times 10^{-9}$ s^{-1}) for the pH-independent, buffer-independent C(α)-proton transfer reaction of **1a** with H_2O was obtained from the ordinate intercept of a plot of k'_{obsd} against [HO^-] (Fig. 2). The value of $k'_{HOH} = 2.9 \times 10^{-10}$ s^{-1} for **1b** was calculated by dividing the value of k'_{HOH} for **1a** by 25, to correct for the smaller electronic effect of the N(1′)-unprotonated 3-aminopyrimidinyl substituent in **1b**. The value of 25 was calculated from the ratio of the second-order rate constants for catalysis of C(α)-proton transfer by hydroxide ion from **1a** and **4** (Table 2); this value of 25 is not significantly different from the value of 22 ± 3 which was estimated using a value of $k_{HO^-} = 3.3 \pm 0.5$ M^{-1} s^{-1} for **1b**.[5] The second-order rate constants for general-base catalysis of C(α)-proton transfer from **1a** were divided by 25 for

[5] This value of k_{HO^-} was calculated by dividing $k_{DO^-} = 4.6$ M^{-1} s^{-1} by 1.4 to correct for a secondary isotope effect of $k_{DO^-}/k_{HO^-} = 1.4$. The value of $k_{DO^-} = 4.6$ M^{-1} s^{-1} at 25°C was estimated by dividing $k_{DO^-} = 16$ M^{-1} s^{-1} (50°C) (*18*) by 3.5 = (25)(0.14) to correct for the 25°C difference in reaction temperature and the temperature dependence for **1a** of $\Delta k_{DO^-}/°C = 0.14$ (this work).

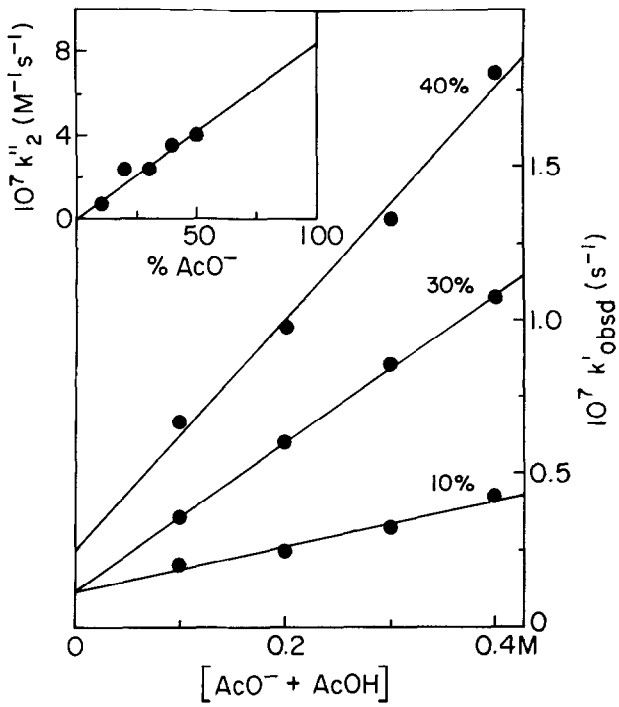


FIG. 1. Dependence of the observed rate constant for C(α)-proton transfer from N(1')-protonated 2-(1-hydroxyethyl)thiamin (**1a**) on the total concentration of acetate buffer containing 10, 30, and 40% acetate ion at 25°C, I = 1.0 M (KNO_3). Values of k'_{obsd} are corrected for the fraction of the N(3)-unprotonated enamine (see text). The slope of the plot against buffer concentration gives the apparent rate constant of the acetate buffer-catalyzed reaction, k''_2 ($= k'_{obsd} - k'_0[\text{buffer}]_{tot}$). The inset shows the dependence of the apparent catalytic constant for buffer catalysis, k''_2, on the composition of the acetate buffer.

comparison of the rate constants for free HET (**1b**) (or **4**) and N(1')-protonated HET (**1a**) on a single Brønsted correlation (see below) and are reported in Table 2.

Values of $(k_H/k_D)_{obsd}$ = 1.3 ± 0.5 and 1.1 ± 0.1 for the observed primary deuterium kinetic isotope effect on C(α)-L transfer from **1a** catalyzed by acetate ion and hydroxide ion, respectively, were determined by iodination in H_2O at 25°C, I = 1.0 M (KNO_3). A value of the secondary solvent deuterium isotope effect of k_{DO^-}/k_{HO^-} = 1.4 ± 0.1 for C(α)-H transfer from **1a** was determined in L_2O at 25°C, I = 1.0 M (KNO_3). Similar values of k_{DO^-}/k_{HO^-} = 1.4 ± 0.1 and 1.3 ± 0.1 were determined under these reaction conditions for C(α)-H transfer **4** and **5**, respectively.

Values of k_{obsd} = (5.0 ± 0.2) × 10^{-8} s^{-1} for C(α)-proton transfer from racemic **1a** and the R and S enantiomers of **1a** were determined by iodination in 0.20 M aqueous potassium acetate buffer (pH 4.00) at 25°C, I = 1.0 M (KNO_3).

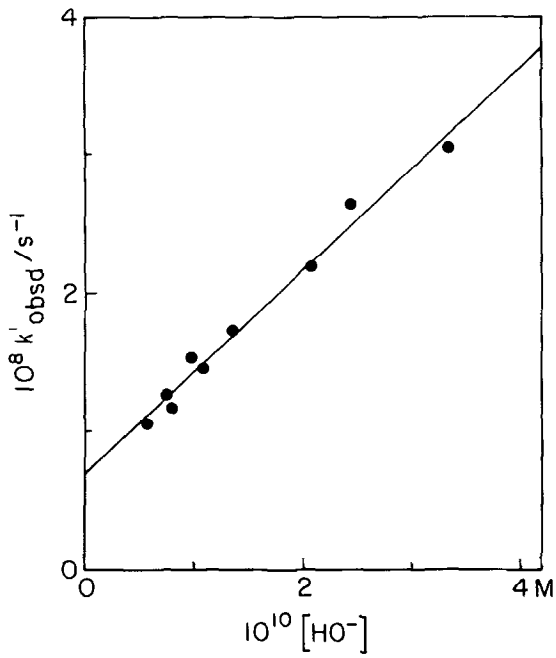


FIG. 2. Catalysis by water and hydroxide ion of C(α)-proton transfer from N(1′)-protonated 2-(1-hydroxyethyl)thiamin (**1a**) in aqueous solution at 25°C, I = 1.0 M (KNO_3). The experiments were carried out in HCl at pH ≤ 4.1.

DISCUSSION

General-Base Catalysis and the Nature of the Transition State

The second-order rate constants for C(α)-proton transfer catalyzed by general bases from free HET (**1b**) and oxy-HET (**4**, see Table 1) give a combined Brønsted plot of slope $\beta \geq 0.9$, as shown in Fig. 3. General-base catalysis is detectable because there is a negative deviation of 2700-fold of the rate constant for catalysis of C(α)-proton transfer by hydroxide ion from the Brønsted plot. There are also 45- and ≥2700-fold negative deviations in Fig. 3 of the rate constants for catalysis by the anions of trifluoroethanol and methanol, respectively. Statistical correction of the catalyst pK'_a values and the rate constants for base catalysis (*21*) does not improve the fit of the data to the Brønsted plot or change β significantly.

A combined Brønsted plot is required because C(α)-proton transfer from free HET (**1b**) could not be followed with the iodine assay at pH ≥ 4.7 due to a rapid side reaction involving the exocyclic 4′-amino group. To obtain a Brønsted plot with catalysts that cover as large a range of reactivity as possible, C(α)-proton transfer from oxy-HET (**4**) was used as a model of **1b** for catalysts with pK'_a values ≥6. The structurally similar N(1′)-unprotonated 3-aminopyrimidinyl and 3-oxopyrimidinyl substituents (see Table 1) in **1b** and **4**, respectively, have identical steric

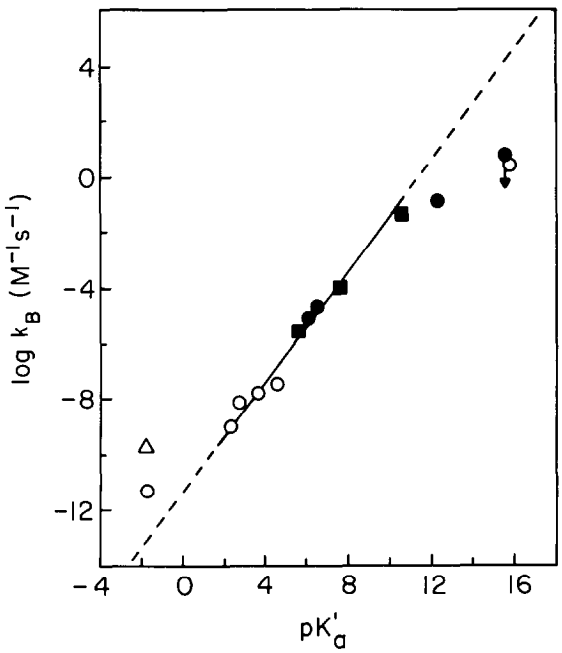


FIG. 3. Brønsted plot for general-base catalysis of C(α)-proton transfer from N(1')-unprotonated 2-1-(hydroxyethyl)thiamin (**1b**) (open symbols) and 2-(1-hydroxyethyl)oxythiamin (see text) (**4**) (solid symbols) in aqueous solution at 25°C, I = 1.0 M (KNO_3). The solid line of slope β = 0.9 is based on a least-squares fit (r = 0.99) to the rate constants for catalysis by oxygen-containing buffers (circles) and primary amines (squares) (see text). The upper limit for catalysis by methoxide is indicated.

and electronic effects on rates of C(α)-proton transfer from **1b** and **4**: (i) the 3-substituents of **1b** and **4** have identical values of the inductive substituent constant σ_I (*19b*); (ii) **1b** and **4** have identical second-order rate constants for catalysis by deuteroxide ion of C(α)-proton transfer in D_2O at 50°C (*18*); and (iii) the rate constants for catalysis by hydroxide ion of C(α)-proton transfer from **1a, 1b,** and **4** follow the same Hammett correlation with the inductive substituent constant σ_I for the 3-substituent (see below). The ≤two-fold deviations of the second-order rate constants for general-base catalysis about the combined solid Brønsted line (Fig. 3) are not unusual, are randomly scattered about the Brønsted line, and provide no evidence that a unique Brønsted correlation is required for **1b** and **4**.

Large Brønsted β values have been reported for general-base catalysis of thermodynamically unfavorable proton transfer from other carbon acids, and this behavior is similar to that expected for "normal" acids with electronegative atoms when the proton transfer step is strongly favorable in one direction (*22*). By analogy, the Brønsted β value of ≥0.9 suggests (i) that C(α)-proton transfer from **1b** and **4** is thermodynamically unfavorable in aqueous solution, (ii) that the rate-limiting step for C(α)-proton transfer from **1b** and **4** involves diffusion-controlled separation of the products (Eq. [2]),

$$\geq C{-}H + B \rightleftharpoons \geq C^- \cdot HB^+ \xrightarrow{\text{slow}} \geq C^- + HB^+, \qquad [2]$$

and (iii) that the reverse, thermodynamically favorable protonation of **2** is diffusion-controlled with almost all acids and has a Brønsted α value of ≤ 0.1. Values of $(k_H/k_D)_{obsd} = 1$ for C(α)-L transfer catalyzed by acetate ion and hydroxide ion also provide evidence for a very late, product-like transition state that does not involve rate-limiting proton transfer. Nearly identical results were obtained previously for C(α)-proton transfer from **3** ($pK_a' = 21.8$) in aqueous solution (*7*).

The curvature of the Brønsted plot in Fig. 3 might suggest a change in the rate-limiting step from diffusional separation of the products to proton transfer itself or diffusion-controlled encounter of the reactants, as described by Eigen for normal acids (*23*). Alternatively, a curved line through the data points (including hydroxide ion) might imply a rapid change in transition state structure with changing catalyst basicity (*24*). However, these interpretations are excluded by the following:

(i) The primary kinetic isotope effect of $(k_H/k_D)_{obsd} = 1$ for hydron exchange catalyzed by hydroxide ion is inconsistent with rate-limiting hydron transfer.

(ii) The second-order rate constant of $k_{HO^-} = 10^{0.5}$ M^{-1} s^{-1} for the reaction of hydroxide ion is at least five orders of magnitude smaller than expected for a diffusion-controlled reaction in aqueous solution (*25*).

(iii) In general, sharply curved Brønsted plots result from perturbations due to solvation of the catalytic bases and not changes in transition-state structure (*24*). Changes in transition-state structure give rise to a relatively gradual change in slope, are observable only over a very large change in ΔpK_a, and cannot be perceived with the single substrate and homologous set of catalysts typically found in a Brønsted plot (*24*). The identical Hammett ρ_I values for catalysis by phosphate dianion and hydroxide ion are consistent with this explanation (see below).

We conclude that the relatively sharp curvature in the Brønsted plot shown in Fig. 3 arises from some type of solvent reorganization in the rate-limiting transition state.

The 2700-fold negative deviation from the Brønsted plot of the rate constants for catalysis by hydroxide ion is in the upper range of the negative deviations between ~10- and ~1000-fold for catalysis by hydroxide ion that are usually observed for thermodynamically unfavorable proton transfers from carbon. It is possible to explain the negative deviations for the reactions in which hydron transfer is largely rate limiting by a requirement for partial desolvation of the lyoxide ion before reaction; however, we do not have a satisfactory explanation for deviations when diffusional separation of the products is rate limiting (*8c*). The fact that no hydroxide ion anomaly has been found for proton transfers between electronegative atoms might reflect the fact that these reactions occur through intervening solvent molecules whereas those involving carbon do not (*8b*, *26*).

Values of k_{DO^-}/k_{HO^-} have been interpreted as an index of transition-state structure, based on the assumption that values derived from Eq. [3],

$$k_{DO^-}/k_{HO^-} = (2.4)^{\beta}, \qquad [3]$$

describe the fractional extent of hydron transfer to lyoxide ion in the rate-limiting transition state for proton transfer (*8c*). A systematic dependence of k_{DO^-}/k_{HO^-} on

transition-state structure is reasonable, but the quantitative significance of the value of β obtained from k_{DO^-}/k_{HO^-} and Eq. [3] has been questioned (*27*). The discrepancy between the value of $\beta = 0.4$ derived from Eq. [3] for C(α)-proton abstraction from **1a** by lyoxide ion and the observed Brønsted slope of $\beta \geq 0.9$ for C(α)-proton abstraction by buffer bases provides additional evidence that values of β calculated from Eq. [3] should be interpreted cautiously.[6]

We conclude that, regardless of whether the N(3)-substituent is small or bulky, these thiazolium ion-activated carbon acids exhibit "normal" acid behavior (*23*), but they still have properties characteristic of other carbon acids. Their thermodynamically uphill C(α)-proton transfer reactions are processes in which the proton-transfer step is rapid and reversible, and separation of the proton-transfer products is rate-limiting and involves solvent reorganization (Eq. [2]). Rate-limiting diffusion-controlled protonation and deprotonation in this system mean that C(α)-proton transfer occurs with internal return and provides additional proof that **2** has a significant lifetime in aqueous solution (*7*). Kluger *et al.* also concluded that **2** has a significant lifetime in aqueous solution because racemic **1b** is formed upon decarboxylation of optically active 2-(lact-2-yl)thiamin (*30*). This is important because the lifetime of intermediates determines whether elimination and other reactions of carbanions can proceed through a stepwise mechanism; if the intermediate is too unstable to exist, the reaction must be concerted (*8a*).

A reliable estimate of the lifetime of **2** from the kinetics of the iodination of **2** under conditions in which halogenation is the rate-determining step is not possible at this time. Zero-order kinetics in iodine are expected when reprotonation of an "intermediate" is slow compared to the rate of reaction with iodine. A change to first-order kinetics in iodine is expected when reprotonation of the "intermediate" by buffer acids or H^+ is faster than iodination (*20*). Such a change from zero- to first-order kinetics in iodine was observed for **4, 6,** and **7** (see above). However, a reliable estimate of the lifetime of **2** is not possible because of uncertainty in the rate constant for iodination. This rate constant depends on whether the halogenation reaction occurs with a product complex or after diffusional separation of the products (Eq. [2]) (*20c*). Minimally, this change from zero- to first-order kinetics in iodine provides evidence that the C(α)-enamine "intermediate" partitions between reprotonation and iodination, which supports the conclusion that the C(α)-enamine has a significant lifetime in aqueous solution.

Inductive Effects

The second-order rate constants for reactions of hydroxide ion and phosphate dianion with 2-(1-hydroxyethyl)-3-*R*-4-methylthiazolium ions containing bulky N(3)-substituents, including HET, follow Hammett correlations with the inductive substituent constant σ_I for R_1 (see Scheme 1) with a slope of $\rho_I = 22 \pm 3$ for both catalysts, as shown in Fig. 4. Hammett correlations with σ_I for R_1 of slope $\rho_I =$

[6] There is also a discrepancy between the value of $\beta = 0.31$ derived from Eq. [3] for the thermodynamically unfavorable reaction of lyoxide ion with 9-[(dimethylamino)methyl[fluorene and the observed Brønsted β value of ≥ 0.65 for general-base catalysis of this reaction (*28*). The apparent discrepancy for proton abstraction from 2-nitropropane (*29*) has been discussed (*8c*).

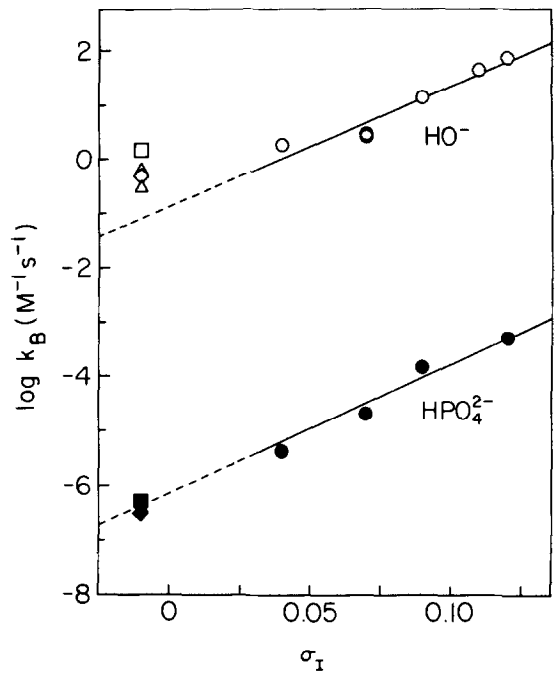


FIG. 4. Hammett correlation with σ_I for C(α)-proton transfer from 2-(1-hydroxyethyl)-3-*R*-4-methylthiazolium ions catalyzed by hydroxide ion (open symbols) and phosphate dianion (solid symbols) at 25°C and I = 1.0 M (KNO_3) in H_2O. The squares are for R = Me, the triangle is for R = Et, the diamonds are for R = *n*-Pr, and the inverted triangle is for R = *i*-Bu. The solid lines of slope ρ_I = 22 ± 3 are based on a least-squares fit to the rate constants for catalysis by phosphate dianion (r = 0.99) and for catalysis by hydroxide ion of C(α)-proton transfer from substrates with bulky aryl N(3)-substituents (r = 0.96).

22 ± 3 were also obtained for catalysis by acetate ion, cacodylate, and trifluoroethylamine (data not shown). We conclude that there is no significant change in the value of ρ_I for C(α)-proton transfer from substrates containing bulky N(3)-substituents with a change in catalyst basicity or structure. A dependence of the C(α)-proton transfer rate on an electron-withdrawing inductive effect by substituents on the nitrogen atom of the thiazolium ring has been noted by others (*6b*, *13*).

The value of ρ_I = 22 for C(α)-proton transfer is much larger than ρ_I = 8.4 for hydroxide ion-catalyzed C(2)-proton exchange from thiazolium ions in aqueous solution (*8a*). The ρ_I value of 8.4 for both C(2)-proton exchange from thiazolium ions and normal acids provides evidence that there is little resonance that decreases the positive charge on N(3) in the C(2)-ylide; N(3) is closer than C(2) to R_1 (Scheme 1) and a decrease in its charge would be expected to give a value of $\rho_I > 8.4$ (*8a*). Accordingly, the ρ_I value of 22 supports the conclusion that there is development of a large amount of resonance that decreases the positive charge on N(3) in the product-like transition state for C(α)-proton transfer. We conclude that rates of C(α)-proton transfer from thiazolium carbinols with bulky N(3)-substituents are

controlled by inductive effects which are amplified by electron delocalization by resonance into the thiazolium ring in the rate-limiting transition state.[7,8]

Steric Inhibition of Resonance

There is no significant positive deviation in Fig. 4 of the rate constants for catalysis by phosphate dianion for R_1 = Me (**3**) or *n*-Pr (**8**). These substituents are not expected to sterically hinder electron delocalization into the enamine resonance form (**2b**) on the basis of models. There were also no significant positive deviations for **3** and **8** from the Hammett correlations of slope $\rho_I = 22 \pm 3$ for catalysis by acetate ion, cacodylate, and trifluoroethylamine (see above). The absence of a significant upward deviation for **3** and **8** shows that changing the N(3)-substituent has no effect, other than electronic, on the rate of C(α)-proton transfer for catalysis by buffer bases. This does not mean that steric interactions between the bulky N(3)- and 2-(1-hydroxyethyl)-substituents are absent, it just means that steric interactions that are present do not hinder formation of the C(α)-enamine (**2b**) in a manner that affects the rate of C(α)-proton transfer or the stability of the product. Their are sufficient steric interactions between the 3-aminopyrimidinyl and 2-(1-hydroxyethyl) substituents of **1a** in the ground state to show AB-type spin–spin couplings for the N(3)-methylene bridge protons (Fig. 5B).[9] Different values of k_{obsd} for C(α)-proton transfer from racemic **1a** and the *R* and *S* enantiomers of **1a** might have been expected if steric hindrance gave rise to different degrees of orbital overlap in the enantiomers (*5b*), but no differences were observed. We conclude that the extent of electron delocalization in the very late, product-like transition state for C(α)-proton transfer from these thiazolium ions is independent of the size of the N(3)-substituent, at least for the substituents examined here.

We attribute the $\leq$15-fold positive deviations in Fig. 4 of the rate constants for catalysis by hydroxide ion for R_1 = Me (**3**) > Et (**10**) > *n*-Pr (**8**) > *i*-Bu (**9**) to a substituent-dependent "hydroxide ion anomaly" (*8c*); the relatively sharp upward curvature in Fig. 4 for catalysis by hydroxide ion is inconsistent with a change in transition-state structure (see above). Evidence for a N(3)-substituent-dependent change in the magnitude of the hydroxide ion anomaly includes: (i) the increase in the negative deviation from the corresponding Brønsted plot of the rate constant for catalysis by hydroxide ion from 85-fold for R_1 = Me (**3**) (*7*) to 2700-fold for **1b** and **4**; and (ii) the large discrepancy between the observed and calculated (Eq. [3])

[7] The covarience of σ_I and σ_R (r = 0.69) or σ_{R^-} (r = 0.87) for the N(3)-substituents in this work precludes more detailed analysis of the contributions of inductive and resonance effects. Solvation and steric effects involving the substituent on N(3) can conceivably be involved.

[8] The absence of a positive deviation in Fig. 4 of the rate constant for catalysis by either hydroxide ion or phosphate dianion for **1** also shows (i) that the 3-aminopyrimidinyl and 5-(2-hydroxyethyl) groups do not provide significant intramolecular base catalysis of C(α)-proton removal and (ii) that electronic and steric effects of the 5-(2-hydroxyethyl) group do not significantly affect the rate of C(α)-proton removal.

[9] The absence of line broadening for the methyl resonance in the 2-(1-hydroxyethyl) substituent of **1a** (data not shown), compared to the line widths for the isolated 4-methyl and 2′-methyl resonances, provides no evidence that the steric interactions between the C(2)- and N(3)-substituents hinder rotation about the C(2)–C(α) bond.

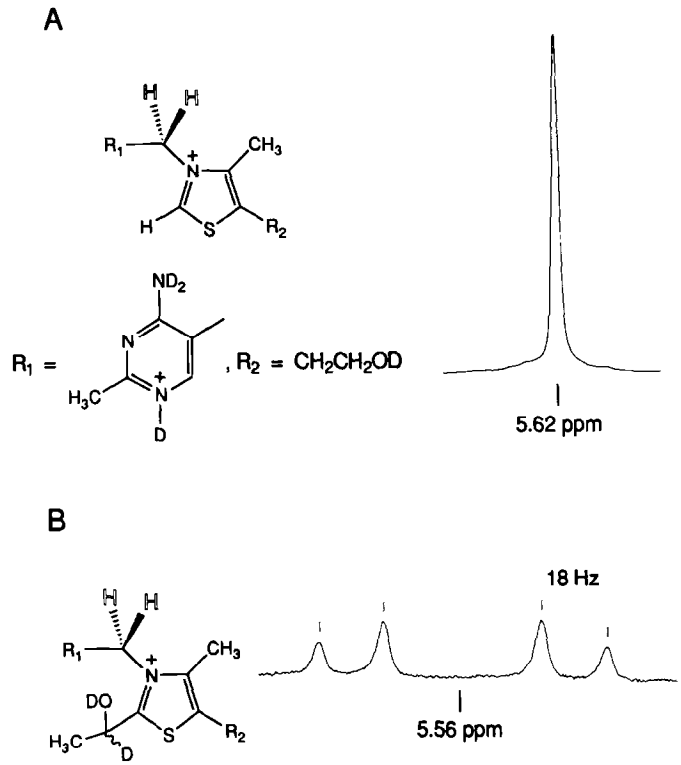


FIG. 5. ^{1}H NMR spectra (300 MHz; D_2O) corresponding to (A) methylene bridge protons of thiamin chloride deuterochloride and (B) methylene bridge protons of racemic HET C(α)-*d*-chloride deuterochloride.

Brønsted β values for **1b** and **4**, but not for R_1 = Me (**3**) (*7*). The absence of positive deviations in the Hammett plots for catalysis of C(α)-proton transfer by several different buffer bases (see above) also provides evidence for anomalous behavior of hydroxide ion. There is precedence for varying steric hindrance causing a dramatic change in the magnitude of the hydroxide ion anomaly for nucleophilic reactions that may be relevant (*24b*).

Zoltewicz *et al.* proposed that steric inhibition of resonance caused the 35- to 100-fold rate decrease for C(α)-proton abstraction upon substitution of 1-hydroxyethyl for methyl at C(2) of 3-methylthiazolium ions (*6*). However, the rate of C(α)-proton removal from the C(2)-methyl group must be divided by three to remove the statistical advantage of the three equivalent methyl protons over the single C(α)-proton in the 2-(1-hydroxyethyl) group; this adjustment gives a 12- to 33-fold rate decrease that could be attributed to steric effects. Furthermore, an unfavorable steric effect on diffusional encounter of the catalytic base and substrate upon substitution of 1-hydroxyethyl for methyl at C(2) was not strictly excluded. Tenfold rate decreases for catalysis of C(α)-proton transfer from **3** by bulky buffer bases were attributed to such a steric effect (*7*). In the absence of evidence to the

contrary, this suggests that only a ≤3-fold rate decrease could be attributed to steric inhibition of resonance in the rate-limiting transition state for C(α)-proton transfer from these 3-methylthiazolium ions.

Determination of pK'_a

Values of pK'_a for C(α)-H in N(1′)-protonated (**1a**) and free HET (**1b**) were found to be 18.4 and 19.8 in H_2O, respectively. These equilibrium constants in H_2O were obtained according to Eq. [4] with $k'_{HOH} = 7.2 \times 10^{-9}$ s^{-1} for **1a** and $k'_{HOH} = 2.9 \times 10^{-10}$ s^{-1} for **1b**.

$$\geqslant C{-}H\,(+\,H_2O) \underset{k_{-a}}{\overset{k'_{HOH}}{\rightleftharpoons}} \geqslant C^- + H_3O^+ \qquad [4]$$

A value of $k_{-a} = 2 \times 10^{10}$ M^{-1} s^{-1} was assumed for the diffusion-controlled reaction in the reverse direction. Values for k_{-a} in the range (1–4) $\times 10^{10}$ M^{-1} s^{-1} have been reported for protonation of amines by H_3O^+ (*23*), and a rate constant of 4×10^{10} M^{-1} s^{-1} has been measured for the diffusion-controlled reaction of CN^- by H_3O^+ (*31*). This uncertainty in k_{-a} results in an uncertainty of ±0.3 in the calculated pK'_a values. The assumption of diffusion-controlled reprotonation of **2** by H_3O^+ is supported by the Brønsted β value of ≥0.9 and the primary kinetic isotope effect of $(k_H/k_D)_{obsd} = 1$ for catalysis of C(α)-proton removal from **1b** and **4** by buffer bases, which indicates that the reverse protonation reaction is diffusion-controlled with strong acids.

The C(α)-H pK'_a values of **1a** and **1b** obtained from exchange catalyzed by H_2O are confirmed by values calculated from the second-order rate constants for catalysis by oxygen-containing buffer bases (Table 2) and the equation $pK_a'^{CH} = pK_a'^{BH} - \log (k_B/k_{-a})$ according to Eq. [5] (*7*).

$$\geqslant C{-}H + B^- \underset{k_{-a}}{\overset{k_B}{\rightleftharpoons}} \geqslant C^- + BH \qquad [5]$$

If a value of $k_{-a} = 3 \times 10^9$ M^{-1} s^{-1} is assumed for the diffusion-controlled protonation of **2** by conjugate buffer acids (*7, 8a*), these rate constants give pK'_a values of 19.3 and 20.6 for C(α)-H of **1a** and **1b**, respectively. These pK'_a values (±0.5) are not significantly different than the pK'_a values obtained from exchange catalyzed by H_2O.

The C(α)-H pK'_a values of 18.4 and 19.8 for the C(α)-proton of N(1′)-protonated HET and free HET in H_2O are in the same range as previous estimates of 17–23 and are similar to the C(2)-H pK'_a values for thiamin of 17.6–18.0 (*8a*). The C(α)–H pK'_a value of 17 was estimated (*32*) from the rate of C(α)-H → D exchange from **1b** (*18*) using a linear free energy relationship that relates C(α)-H pK'_a values in ketones to the rate of C(α)-proton abstraction in ketones (*33*). A C(α)-H pK'_a value of 23 = 18 + 5 can be estimated from Kluger's suggestion (*1a*) that the C(α)-H pK'_a value for HET would be five pK'_a units greater than the C(2)-H pK'_a value of 18 for thiamin (*8a*). The value of $\Delta pK'_a = 5$, which was based on values of k_{HO^-} for these two carbon acids (*1a*), is an overestimate of $\Delta pK'_a$ because corrections for the anomalous behavior of hydroxide ion were not utilized (*8a*). Second-

order rate constants for catalysis by buffer bases were used to calculate a C(α)-H pK_a' value of 21.8 for **3** in H_2O (*7*).

These C(α)-H pK_a' values are much higher than a recent estimate of 14 ± 1 for 2-(1-methoxy*ethyl*)-3,4-dimethylthiazolium ion (MET) in H_2O. This C(α)-H pK_a' value in H_2O is based on (i) a C(α)-H pK_a' value of 14 for MET in Me_2SO and (ii) the conclusion that C(α)-H pK_a' values for 2-(1-methoxy*benzyl*)thiazolium ions are similar (±1) in Me_2SO and H_2O (*34*). We do not have a satisfactory explanation at the present time for the large difference between the results reported here based on experiments performed entirely in aqueous solution and those based, in part, on experiments in Me_2SO. We are currently examining the role of inductive effects on the rate of nonenzymatic C(α)-proton removal from 2-(1-hydroxybenzyl)-3-*R*-4-methylthiazolium ions and the stability of **2** derived from these substrates in order to evaluate effects of resonance delocalization of the C(α)-carbanion into the 2-(1-hydroxybenzyl)-substituent.

Implications for Enzyme-Catalyzed Reactions

The normal acid behavior for C(α)-H in HET, the absence of stereoelectronic control of the rate of C(α)-proton abstraction, and the pK_a' values that are reported here for C(α)-H show the following:

(i) Like C(2)-proton exchange (*8*), enzymatic and nonenzymatic C(α)-proton abstraction from HETDP occurs at the maximum possible rate for a given equilibrium constant, even in the presence of steric interactions between the N(3)- and C(2)-substituents.

(ii) The conclusion that steric inhibition of resonance does not determine the rate of nonenzymatic C(α)-proton removal or the C(α)-H pK_a' of HET means that any steric control of the rate of enzyme-catalyzed C(α)-proton removal from HETDP or the stability of the enzyme-bound C(α)-carbanion/enamine would be a result of steric interactions imposed by the enzyme.

(iii) The C(α)-enamine, like the C(2)-ylide (*8*), is quite unstable, but has a significant lifetime in aqueous solution. This means that enzymatic and nonenzymatic aldol-type addition reactions of **2b** must follow either a stepwise pathway or a nonenforced ("free choice") concerted pathway (*35*). The stepwise pathway must exist, but it might be bypassed by a nonenforced concerted pathway because of the instability of the carbanion. Cleavage of 2-(1-hydroxybenzyl) thiamin, a retrograde aldol-type reaction, proceeds by a nonenforced concerted mechanism in aqueous solution that is determined by the short lifetime of the C(2) – ylide (*36*).

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