Model Studies of Thiamin Catalysis. Comparison of the Effects of Heteroatoms at Annular Positions on Side-Chain Kinetic Acidity

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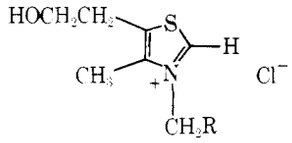
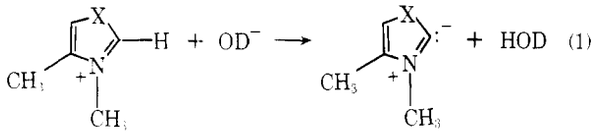
General-base-catalyzed deprotonation of the 2-methyl groups of 2,3-dimethylbenzothiazolium (II), 1,2,3-trimethylbenzimidazolium (III), and 1,2,3,-trimethylimidazolium (IV) ions was studied by means of hydrogen–deuterium isotope exchange. At 75 °C and 1.0 M ionic strength II, III, and IV show the following relative reactivity order toward deuterioxide ion: 3.0×10^5; 3.4×10^2; 1, respectively. Toward pyridine II is 9.5×10^3 times more reactive than III. The Brønsted β value for a series of bases, excluding water and lyate ion, reacting with II is 0.63. A more limited series of bases gives the value 0.8 for III. A comparison is made between the reactivities of sp^2- and sp^3-hybridized carbon centers in deprotonation reactions involving thiazolium ions.

Thiamin (I) or vitamin B-1 when catalyzing carbon–carbon bond forming reactions is converted to an ylide and subsequently to an enamine intermediate, both of which act as nucleophiles toward carbonyl electrophiles.[1-4] Many studies have been carried out to obtain an understanding of the various steps in the multistep catalyzed conversions. For example, the ability of several annular heteroatoms to facilitate ylide formation[5] in deprotonation reactions, eq 1, has been explored.



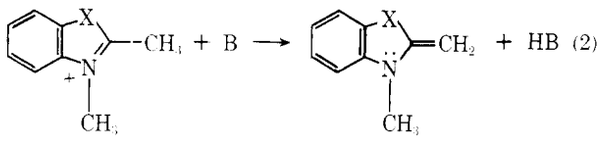
I, R = 2-methyl-4-amino-5-pyrimidinyl



(1)

Results show that reactivity increases in the order X=NCH_3, S, and O.[6] By contrast, little is known about the influence of similar heteroatoms on the kinetic acidity of an alkyl side chain which gives an enamine rather than an ylide on proton loss.

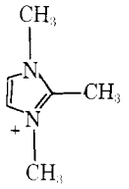
In this article we examine the effects of annular heteroatoms on the kinetic acidities of ions II–V containing an acidic methyl group. Equation 2 shows the enamine which forms



(2)

II, X = S
III, X = NCH_3
V, X = O

VI, X = S



IV

when fused-ring heteroaromatic ions II, III, and V undergo deprotonation. The accompanying paper explores the influence of methyl and hydroxy substituents bonded to the 2-methyl group of II on the kinetic acidity of this position.[7]

Results

No evidence exists to indicate that the 4-methyl and 5-β-hydroxyethyl substituents of the thiazolium ion ring of I provide other than expected, small, electronic effects on deprotonation reactions at position 2 under biological conditions. For this reason these substituents were not incorporated into our models. In order to simplify our models a methyl group was added to N-3 in place of the pyrimidinylmethyl substituent. Because of their ready availability and because they contain the essential structural elements, compounds II–V were selected as model substrates. The conjugate base of II, 3-methyl-2-methylenebenzothiazolene (VI), which is formed as an intermediate in our reactions has been isolated.[8]

All of the studies employ NMR to determine rates of carbon deprotonation. Reactions were carried out using D_2O as the reaction medium, leading to replacement of H by D in the side chains. Except where indicated, the reaction temperature is 75.0 °C.

2,3-Dimethylbenzothiazolium Ion (II). Deprotonation of the *C*-methyl group of this substrate was found to take place readily in 0.1 M DCl. Catalysis was also observed with the following buffers, listed in order of increasing basicity: 3-chloropyridine, phthalazine, formic acid, acetic acid, pyridine, and 2,6-lutidine (Table I). Buffer ratios and pD were varied in order to determine whether water, buffer base, and deuterioxide ion contributed to the general-base-catalyzed deprotonation reactions. Pseudo-first-order rate constants were analyzed using eq 3

$$k_\psi = k_{D_2O}[D_2O] + k_B[B]_{tot}\frac{K_a}{[D] + K_a} + k_{OD}\frac{K_w^D}{[D]} \quad (3)$$

where k_{D_2O}, k_B, and k_{OD} are second-order rate constants for water, buffer, and deuterioxide ion bases, respectively, and K_a and K_w^D are the dissociation constants of buffer and the ion product of D_2O, respectively.

Significant catalysis by deuterioxide ion was observed only with the most basic buffer, 2,6-lutidine. Thus, the value of k_{OD} was established using the data derived with this buffer. Application of the term $k_{OD}[OD^-]$ to the data obtained with other buffers indicates that deuterioxide ion measurably influences reactivity only in two other kinetic runs, those at pD 4.09 involving acetic acid and pyridine buffers. The kinetic contribution of this base represents about 13% of the total catalysis in both cases. With these two kinetic runs and also the lutidine studies as exceptions, deprotonation in the other buffered media was catalyzed only by water and by buffer base.

Since the k_{OD} term was obtained with only one buffer, another method was employed as a check. This involved the use of a pH-stat in place of a buffer to adjust the pD of the reaction medium. From runs at pD 5.25 and 5.45 this approach gave

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Table I. Kinetic Results for Hydrogen–Deuterium Exchange at the 2-Methyl Group of 2,3-Dimethylbenzthiazolium Ion (II) in Buffered D_2O at 75.0 °C and 1.0 M Ionic Strength

Buffer	pK_a	pD[a]	Total buffer, M	Obsd $10^5 k_\psi$, s^{-1}	Calcd[b] $10^5 k_\psi$, s^{-1}	k_B, $M^{-1} s^{-1}$
DCl	- -	- -	0.1	2.65 ± 0.23[c]		$(4.90 \times 10^{-7}$ [d])
3-Chloropyridine	3.07	2.49	1.20×10^{-1}	13.15	12.3	3.84×10^{-3}
		2.65	4.80×10^{-2}	8.00	7.80	
		2.73	2.40×10^{-2}	5.23	5.61	
Phthalazine[e]	3.79	3.20	1.01×10^{-1}	34.9	34.9	1.56×10^{-2}
		3.37	2.12×10^{-2}	11.45	11.7	
Formic acid	4.03	3.22	4.40×10^{-2}	12.1	12.2	1.62×10^{-2}
		3.33	3.33×10^{-2}	11.5	11.6	
		3.33	1.10×10^{-2}	5.17	5.61	
		3.35	2.20×10^{-2}	8.48	8.82	
Acetic acid	5.12	3.75	4.20×10^{-2}	23.7	20.4	1.00×10^{-1}
		4.09	8.33×10^{-3}	9.60	11.0	
Pyridine	5.27	3.98	4.20×10^{-2}	22.1	21.8	8.90×10^{-2}
		4.09	8.44×10^{-3}	8.33	8.42	
2,6-Lutidine	6.44	5.10	1.20×10^{-1}	99.3	96.5	
		5.20	6.05×10^{-2}	60.8	68.8	1.57×10^{-1} (B)
		5.43	6.05×10^{-3}	38.7	35.8	3.08×10^{4} (OD^-)
		5.51	2.00×10^{-3}	40.6	35.7	
DCl[f,g]	- -	- -	0.1	0.010	- -	$(1.81 \times 10^{-9}$ [d])
Acetic acid[f,h]	5.25	4.27	4.54×10^{-2}	0.270	0.260[i]	5.81×10^{-4} [i]
		4.27	8.48×10^{-3}	0.0544	0.0567[i]	

[a] Measured at 75.0 °C. [b] Using equation 3. [c] Average of four determinations. [d] $k_\psi/[D_2O]$. [e] 2,3-Diazanaphthalene. [f] At 25.0 °C and 0.5 M ionic strength. [g] $\Delta H^\ddagger = 22.4$ kcal/mol; $\Delta S^\ddagger = -4.12$ eu. [h] $\Delta H^\ddagger = 20.7$ kcal/mol; $\Delta S^\ddagger = -4.12$ eu. [i] Neglects any contribution from OD^-.

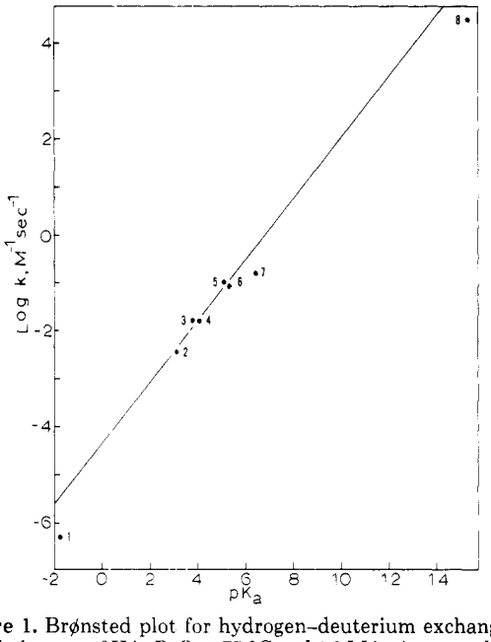


Figure 1. Brønsted plot for hydrogen–deuterium exchange at the 2-methyl group of II in D_2O at 75 °C and 1.0 M ionic strength. Buffers include: 1, water; 2, 3-chloropyridine; 3, phthalazine; 4, formic acid; 5, acetic acid; 6, pyridine; 7, 2,6-lutidine; and 8, deuterioxide ion. No statistical corrections have been applied. The least-squares line does not include points 1, 7, and 8.

a value of $3.80 \pm 0.16 \times 10^4$ $M^{-1} s^{-1}$ which is only 23% larger than that derived from the lutidine studies.

The quality of our results may be assessed by comparing the observed pseudo-first-order rate constants with those calculated with the aid of eq 3 and the second-order constants listed in Table I. In carrying out the computations the buffer-derived value of k_{OD} was employed. The largest differences are found in the results obtained with acetic acid and lutidine buffers where the results with the poorest agreement differ by about 14%. Differences between observed and calculated values are substantially less in all other cases.

The spread in second-order rate constants between the least (D_2O) and most (OD^-) reactive bases is a factor of 6.3×10^{10}. The variation in reactivity for buffer bases unrelated to solvent is a factor of 41.

A Brønsted plot may be constructed using the results in Table I. Without making any statistical corrections for the number of basic sites in a buffer a single plot (Figure 1) is obtained, excluding points for water, deuterioxide ion, and 2,6-lutidine. The slope, β, is 0.63 and the intercept is −4.305. The correlation coefficient is satisfactory, being 0.991. On the basis of this plot, 2,6-lutidine is 3.7 times less reactive than expected from a consideration of its pK_a value, no doubt reflecting a modest steric hindrance to general base catalysis.[9] Water and deuterioxide ion deviate; observed reactivity in both cases is about 8 times less than that calculated. Negative deviations for these are not uncommon. The reduced reactivities of the two bases with carbon acids is said to be a consequence of a lack of hydrogen bonding between reactants in the ground state.[10,11]

When rate and equilibrium constants are statistically corrected for the two basic centers found in phthalazine and in the carboxylate anions, two correlation lines now are generated, one for carboxylic acids and one for pyridines. The line for carboxylate ion bases is displaced above that for the pyridines. Phthalazine now deviates from the pyridine correlation line in the sense that its reactivity is about 4 times greater than that predicted by its basicity. Phthalazine is known to show an enhanced reactivity (α effect) toward esters,[12] but it is likely that the present deviation is not significant. Thorough studies of α-effect nucleophiles in carbon deprotonation reactions have failed to uncover enhanced reactivities.[13,14] Although it is customary to make statistical corrections in constructing Brønsted plots,[15] good correlations using uncorrected data are known for carbon deprotonation.[13]

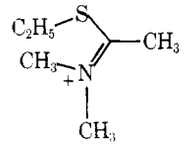
Some kinetic information was also obtained at 25.0 °C so that the reactivity of II could be compared with that of the *C*-methyl group of its acyclic relative VII, *N,N*-dimethyl-

Table II. Kinetic Results for Hydrogen–Deuterium Exchange at the 2-Methyl Groups of 1,2,3-trimethylbenzimidazolium Ion (III) and of 1,2,3-Trimethylimidazolium Ion (IV) in Buffered D_2O at 75.0 °C and 1.0 M Ionic Strength

Compd	Buffer	pK_a	pD[a]	Buffer, M	Obsd k_ψ, s^{-1}	Calcd[b] k_ψ, s^{-1}	k_B[c] M^{-1} s^{-1}
III	DCl	- -	- -	0.1	$<0.5 \times 10^{-7}$	- -	- -
	Pyridine	5.27	5.60	0.303	2.30×10^{-6}	2.33×10^{-6}	9.36×10^{-6} (B)
			5.76	0.0303	7.46×10^{-7}	7.94×10^{-7}	31.1 (OD^-)
	Phosphate	7.10	6.61	0.140	2.25×10^{-5}	2.26×10^{-5}	5.40×10^{-4} (B)
			6.98	0.0200	1.64×10^{-5}	1.43×10^{-5}	41.4 (OD^-)
			7.12	0.280	9.46×10^{-5}	9.05×10^{-5}	
			7.50	0.300	1.59×10^{-4}	1.47×10^{-4}	
	Glycine	9.17	7.83	0.0550	1.07×10^{-4}	1.17×10^{-4}	2.02×10^{-2} (B)
			7.87	0.110	1.72×10^{-4}	1.81×10^{-4}	29.2 (OD^-)
			8.17	0.220	5.32×10^{-4}	5.53×10^{-4}	
IV	Carbonate	9.93	10.00	0.120	2.88×10^{-5}	3.01×10^{-5}	
			10.25	0.350	5.35×10^{-5}	5.38×10^{-5}	1.01×10^{-1} (OD^-)
			10.86	0.275	2.26×10^{-4}	2.15×10^{-4}	

[a] At 75.0 °C. [b] Using eq 3, the k_B values listed for each base and the average value for k_{OD} of 33.9 m^{-1} s^{-1}. [c] For buffer base and/or deuterioxide ion.

thioacetimidate, under similar conditions.[16] Rate constants were obtained for water and acetate ion bases (Table I). Our second-order rate constant for acetate ion may be somewhat too large if deuterioxide ion makes a contribution to catalysis. No attempt was made to obtain a k_{OD} term at the lower temperature in order to estimate the magnitude of any contribution by lyate ion. However, a fivefold dilution of buffer failed to change the apparent second-order rate constant for acetate ion catalysis, suggesting that lyate ion catalysis is small.



VII

The enthalpies of activation, $\Delta H^{\ddagger}$, for water and acetate ion reacting with II are 22.4 and 20.7 kcal/mol, respectively, while the entropies, $\Delta S^{\ddagger}$, are −23.6 and −4.1 eu, respectively. Very similar entropy values have been reported for quinolinium ion–cyanine dye deprotonation reactions.[17] The less negative value associated with the ionic base probably reflects the release of solvent molecules in the transition state as the charges on the two reactants are being neutralized. By comparision, when water acts as a base, charge is maintained.

A pK_a value for II may be estimated using the rate constants for water acting as a base and an assumed rate constant for reverse reaction in which conjugate base VI reacts with D_3O^+. If it is assumed that the reverse reaction proceeds with a diffusion-controlled rate constant, about 10^{10} M^{-1} s^{-1}, the pK_a of II is 15 and 17 at 75 and 25 °C, respectively. These are likely to be upper limits because the reverse reaction, as suggested by the Brønsted β value of less than 1, is likely to be slower than estimated.[18]

1,2,3-Trimethylbenzimidazolium Ion (III) and 1,2,3-Trimethylimidazolium Ion (IV). Similar but more limited studies involving base-catalyzed H–D exchange at the *C*-methyl groups of III and IV were carried out. Both of these ions undergo deprotonation much more slowly than II, IV being less reactive than III. Fused ring substrate III undergoes deprotonation by the action of water extremely slowly; our value in Table II is only an estimate of an upper limit based on <5% hydrogen isotope exchange observed over 3 weeks. Convenient rates of deprotonation of III were obtained using pyridine, phosphate ion, and glycine buffers. Under these conditions both buffer base and deuterioxide ion catalyze deprotonation. Rate constants k_B and k_{OD} were obtained for each buffer. The three values obtained for k_{OD} using these buffers are in satisfactory agreement (Table II). The variation in k_B is a factor of 2.2×10^3.

An estimation of the experimental scatter in our results may be obtained by comparing observed and calculated pseudo-first-order rate constants. Calculations were performed using eq 3 and the average value of k_{OD} obtained from the three buffers and the appropriate k_B value (Table II). Calculated values for pyridine base are high by an average of 3.2%; for phosphate buffer they fall within ±6.1% of the observed value and for glycine they are systematically high by 6.1%. The systematic, small deviations found for pyridine and glycine result because the values of k_{OD} determined using these buffers separately are smaller than the average calculated from the data for the three buffers.

A Brønsted plot constructed from these data, excluding k_{OD}, without making any statistical corrections has $\beta = 0.85$, intercept −9.454, and a correlation coefficient of 0.998. The observed value of k_{OD} is 108 times smaller than that calculated using this correlation. Making statistical corrections for the number of acidic and basic sites in a buffer improves the correlation, β now being 0.76, the intercept −9.130, and the correlation coefficient 0.9997. Deuterioxide ion now is found to be only 11 times less reactive than predicted. Both treatments produce remarkably good correlations. Superior correlations generally, but not always,[13] are observed when data for structurally similar bases having the same charge are correlated.[11] Caution should be exercised when attempting to interpret our results in view of the limited number of buffers employed.

The reactivity of compound IV was examined using only a carbonate ion buffer. Apparently, deuterioxide ion and not carbonate ion catalyzes deprotonation of this substrate as evidenced by the good agreement between observed pseudo-first-order rate constants and those calculated solely with the term $k_{OD}[OD^-]$ (Table II). In this study the carbonate ion concentration was varied by a factor of 3.8, deuterioxide ion by 7.1. Note that this conclusion is to be expected in view of the Brønsted plots observed for II and III.

2,3-Dimethyloxazolium Ion (V). No information about the rate of deprotonation of the *C*-methyl group of this ion could be obtained. After just 15 min of heating in 0.1 M DCl at 75 °C there was clear evidence in the NMR spectrum of substrate degradation. This decomposition is to be expected on the basis of studies which show that cleavage of the het-

erocyclic ring is a facile process, having a half-life of 3.1 h at pH 1 and 25 °C.[19]

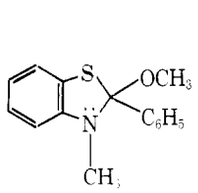
Discussion

Reactivities. Comparison of the reactivities of cations II–IV when undergoing deprotonation at a 2-methyl group by the action of deuterioxide ion shows that II is 9.1×10^2 times more reactive than III and 3.0×10^5 times more reactive than IV.[20] Toward pyridine, II is 9.5×10^3 times more reactive than III. These large values confer a distinct advantage in kinetic carbon acidity on the thiazolium derivative over both diazolium substrates. In addition, comparison of the reactivities of III and IV toward deuterioxide ion reveals that fusing a benzene ring onto the imidazolium ion leads to a 340-fold increase in kinetic acidity.

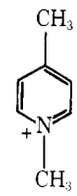
An interesting comparison involves II and its acyclic relative VII. Both cations undergo deprotonation by water and acetate ion bases. At 25 °C II is just 2.2 times more reactive than VII when acetate ion is the base and approximately 14 times less reactive when water is the catalyst.

An understanding of the origin of the coincidentally similar kinetic acidities of II and VII can be found in a consideration of the resonance energy of cyclic acid II and the extent of the change in this stabilization in the transition state for deprotonation.[22] The resonance energy of II is estimated to be about 52 kcal/mol and that for a thiazolium ion not having a fused benzene ring as about 21 kcal/mol.[23] These large values clearly show that a thiazolium ion ring has a substantial delocalization energy. Significantly, the delocalization energy of VIII, a model of VI in which the aromatic thiazolium ion ring no longer is present, is expected to be about the same as that for III. This resonance energy which is well above the value of 36 kcal/mol associated with benzene itself is due to interactions of the two heteroatoms with the aromatic ring.[23]

In view of the expected similar resonance energies of II, VI, and VIII, we suggest that the energy of activation associated with the conversion of II to VI will be largely free of a contribution reflecting a change in resonance energy. This is true in spite of the fact that II with its two aromatic rings is being converted to VI with essentially a single aromatic ring. Substrates II and VII are similar in that the reactivities of both are affected in only a minor way by changes in resonance energies associated with proton loss. Clearly, if the fused-benzene ring were not present in II to provide extensive activation, the resultant single-ring thiazolium ion would be very much less reactive than VII.



VIII



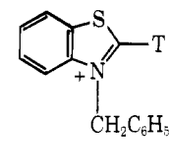
IX

Deprotonation of II then represents a most interesting and unusual example of an aromatic carbon acid which undergoes a small change in resonance energy on deprotonation at an exocyclic α position in spite of extensive proton transfer in the transition state. This conclusion contrasts with that for carbon acid IX, for example, which has a large energy barrier opposing conversion to its nonaromatic conjugate base by removal of a proton from the 4-methyl group.[24] Part of this barrier reflects the fact that the conjugate base of IX has about 19 kcal/mol less resonance energy.[25]

A number of widely varying estimates have been made concerning the resonance energies of III and IV[25] and little is known about the magnitudes of the changes in their delocalization energies when the acids are converted to their conjugate bases. However, it seems likely that the 340-fold greater reactivity of III over IV reflects in large measure both a smaller energy of activation due to a smaller loss of resonance energy on proton transfer and the possibility of more extensive electron delocalization. The aromaticity of the heterocyclic ring in the fused-ring compound is likely to be less than that of the single-ring compound.

Ylide and Enamine Formation. Our results show that the reactivity of II is 908 times greater than that of III toward lyate ion. In other terms, the annular sulfur atom increases kinetic, carbon acidity much more than an annular nitrogen atom. Here proton loss produces an enamine. Similarly, ylide formation, eq 3, is promoted by a factor of 3200 when the annular atom is sulfur rather than nitrogen.[6] These two comparisons show that at both the ylide and also the enamine stages of deprotonation sulfur confers greater reactivity than nitrogen and by similar amounts for the substrates considered. This conclusion reinforces an earlier suggestion based only on data pertaining to ylide formation:[26] nature selected a heterocyclic ring for the enzyme cofactor which contains a sulfur atom rather than a nitrogen or oxygen atom because the thiazolium ion ring has the proper combination of reactivity and stability toward hydrolysis.[27]

Using our results, a comparison can now be made of the rate constants for ylide and enamine formation. Consider, for example, benzothiazolium ion X forming an ylide and II forming an enamine. The second-order rate constant for X reacting



X

with lyate ion[28] is about 10^4 times greater than that for II under similar conditions.[29] However, deprotonation of II is subject to marked general base catalysis while that for X is not.[28] Therefore, the reactivity difference under other conditions need not be as great as that just indicated. As an illustration, consider a buffer base of pK_a 8 at pH 8 present in 0.1 M concentration; assume its rate constant is that predicted by the Brønsted equation observed for II. Under these conditions deprotonation of II is only about 460 times slower than that of X, not the factor of 10^4 reflecting lyate ion reactivities. Although this comparison involves benzothiazolium ion reactants, similar conclusions are expected to apply to thiamin-like molecules containing thiazolium ion rings.[32]

Now that kinetic data have become available for deprotonation reactions leading to ylide and enamine intermediates, future kinetic studies should focus on reactions involving capture of these intermediates by carbonyl electrophiles. A detailed understanding of the way in which thiamin functions as a catalyst will begin to emerge.

Experimental Section

Reagents. 3-Chloropyridine hydrochloride was prepared by bubbling hydrogen chloride into an etheral solution of 3-chloropyridine (Aldrich); the white precipitate was sublimed and then titrated with standard alkali. Alkylation of 2-methylbenzimidazole (Aldrich) in methanol gave 1,2,3-trimethylbenzimidazolium iodide, mp 265–266 °C (lit.[33] mp 258–259 °C) while 2-methylbenzothiazole (Aldrich) gave 2,3-dimethylbenzothiazolium iodide, mp 221–222 °C (lit.[34] mp 221–222 °C), or perchlorate,[35] mp 122–123 °C, and 2-methylbenzoxazole (Aldrich) gave 2,3-dimethylbenzoxazolium iodide, mp 194–196 °C (lit.[19] mp 196 °C). Pyridine (Mallinckrodt) and 2,6-dimethylpyridine (Eastman) were dried over sodium and distilled from zinc powder. Sodium acetate (Mallinckrodt), sodium formate (Fisher), phthalazine (Aldrich), and reagent grade inorganic chemicals were used as received. Deuterium oxide (99.8%) was obtained from Columbia Organic Chemicals. Buffer solutions were prepared by

mixing DCl (D_2O and concentrated HCl) or KOD (D_2O and KOH) with appropriate materials.

1,2,3-Trimethylimidazolium perchlorate, mp 250–251 °C, was prepared by treating its methosulfate with perchloric acid dissolved in a mixture of ethanol–ethyl acetate. The methosulfate was prepared by the method used for benzimidazoles.[36]

Anal. Calcd for $C_6H_{11}ClN_2O_4$: C, 34.20; H, 5.27; N, 13.30. Found: C, 34.38; H, 5.40; N, 13.16.

Instrumentation. Nuclear magnetic resonance spectra were recorded on a Varian Associates A-60A instrument while pD was determined on a Beckman Model 1019 Research pH meter equipped with either a Corning (476050) or a Sargent (S-300-70-10) semimicro combination electrode. Measurements of pD and kinetic runs were carried out at 75.0 °C using a Lauda/Brinkman Model K-2 constant-temperature bath. Temperature was measured using a National Bureau of Standards certified thermometer. A Radiometer TTTl-c titrator with jacketed reaction vessel was used in pH-stat work. Least-squares calculations were performed on a Texas Instruments SR-51 calculator.

Kinetics of Hydrogen–Deuterium Exchange. Substrate and KCl to maintain constant ionic strength were weighted into a 3-mL volumetric flask. Buffer was added by weight or by syringe when dealing with a stock solution. After diluting to the mark with D_2O, substrate concentration was 0.15–0.20 M and the ionic strength 1.0 M. A sample in a sealed NMR tube was heated in a bath at 75.0 °C and then was removed periodically and quenched in an ice bath. The area of the *C*-methyl peak was integrated several times using the *N*-methyl area as a reference. Reactions using 2,6-lutidine buffer were so fast that 0.6-mL aliquots were removed from a sample and quenched with 1 M DCl prior to analysis. Reactions generally were followed for 2–3 half-lives, except the slowest runs. Rate constants were obtained in the usual way from plots based on the *C*-methyl to *N*-methyl area ratio.[37,38] Plots were nicely linear. Rate constants reflect the removal of a single proton. The NMR signals employed have the following shifts (N–CH_3 listed before C–CH_3): II, τ 5.76 and 6.80; III, 6.01 and 7.09; IV, 6.22 and 7.42.

Following a kinetic run, pD measurements were made at 75.0 °C on the heated mixture as well as the unheated stock solution according to the method of Bates.[39] The electrode was allowed to equilibrate at 75 °C in 4 M KCl for 20 min prior to use. Reproducibility of the measurements was about 0.03 while the difference in pD between heated and unheated samples was about 0.04 or less. When differences exceeded these values, runs were discarded. The pD of a buffer solution was obtained by adding the factor 0.35[38] to the meter reading.[40] The concentration of deuterioxide ion was calculated from the expression $pOD = pK_w^D - pD$ where K_w^D is the dissociation constant for D_2O. The value of pK_w^D, 13.526 at 75.0 °C, was calculated from reported data[41] and is uncorrected for salt effects which are expected to be small.[42]

A pH-stat also was employed to obtain kinetic data on II. A 0.11 M solution of the perchlorate salt of II brought to 1 M ionic strength with NaCl was acidified with DCl and raised to 75.0 °C in a jacketed titration vessel. The pD of the mixture then was increased to the desired value by adding KOD from the titrator. Aliquots of this mixture were removed from the sample and analyzed by NMR. The following results were obtained: at pD 2.5 where only D_2O catalyzes the reaction $k_\psi = 2.54 \times 10^{-5}\ s^{-1}$. This value is 4.1% lower than the average value derived from the 0.1 M DCl runs. At pD 5.25 $k_\psi = 2.19 \times 10^{-4}\ s^{-1}$ and at pD 5.45, $3.59 \times 10^{-4}\ s^{-1}$. After correcting for water catalysis these last two runs give $k_{OD} = 3.80 \pm 0.16 \times 10^4\ M^{-1}\ s^{-1}$. No base was consumed during these runs. The run at pD 5.45 was followed for 3 half-lives, the rate plot being linear.

The pK_a values for buffers were calculated from the known composition of the buffer mixtures employed in the kinetic studies and the measured pD of these mixtures at 1.0 M ionic strength. Hydrolysis corrections of concentrations were made as required.[43] The pK_a of 2,6-lutidine was determined using samples which did not contain substrate. Uncertainties in values reflect the scatter in the pD measurements.

Control Runs to Determine the Stability of Substrates. In order to learn whether substrate might have degraded during kinetic studies and escaped detection by NMR because of conversion to deuterated materials, controls were run using H_2O as the medium. This supplements attempts to detect degradation by comparing the pD of heated and unheated samples. Generally, in performing proteo control runs, conditions were selected which matched the most severe, i.e., the most alkaline solution with the highest concentration of buffer used in kinetic studies.

The stability of 2,3-dimethylbenzthiazolium iodide and perchlorate toward hydrolysis was ascertained using a pH-stat. The method of sample preparation and analysis was the same as that employed in kinetic studies. After 30 min at 75 °C and pD 6.4, NMR spectra failed to provide evidence of hydrolysis; no alkaline titrant was consumed even after 6 h. But at pD 7.5 new signals were evident in NMR spectra after 20 min, indicating decomposition. Since pD values in kinetic runs were much lower than those in the control studies, substrate degradation is likely to be unimportant. Ring cleavage studies support this conclusion.[35]

1,2,3-Trimethylbenzimidazolium iodide was heated at 75 °C in a mixture of 0.105 M glycine–0.005 M glycinate ion in H_2O containing KCl for 20 h, corresponding to a period of >10 half-lives for H–D exchange. The NMR spectrum of this mixture showed no sign of substrate degradation; no pH change was observed.

1,2,3-Trimethylimidazolium perchlorate was heated at 75 °C in 0.25 M sodium carbonate–0.025 M sodium bicarbonate for 8.5 h, a period corresponding to 10 half-lives for isotope exchange. No evidence for decomposition was detected by NMR.

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Registry No.—I, 59-43-8; II iodide, 2785-06-0; II perchlorate, 706-67-2; III iodide, 3805-38-7; IV perchlorate, 65086-11-5; IV methosulfate, 65086-12-6; 2-methylbenzimidazole, 615-15-6; 2-methylbenzothiazole, 120-75-2.

References and Notes

(1) L. O. Krampitz, *Annu. Rev. Biochem.*, **38**, 213 (1969).
(2) R. Breslow, *J. Am. Chem. Soc.*, **80**, 3719 (1958).
(3) R. Breslow, *Ann. N.Y. Acad. Sci.*, **98**, 445 (1962).
(4) For examples of carbon–carbon bond formation catalyzed by thiazolium ions in purely chemical systems, see H. Stetter and H. Kuhlmann, *Synthesis*, 379 (1975).
(5) For a review of ylide formation, see J. A. Elvidge, J. R. Jones, C. O'Brien, E. A. Evans, and H. C. Sheppard, *Adv. Heterocycl. Chem.*, **16**, 1 (1974).
(6) P. Haake, L. P. Bausher, and W. B. Miller, *J. Am. Chem. Soc.*, **91**, 1113 (1969).
(7) J. A. Zoltewicz and L. S. Helmick, *J. Org. Chem.*, following article in this issue.
(8) J. R. Owen, *Tetrahedron Lett.*, 2709 (1969); J. Metzger, H. Larive, E. J. Vincent, and R. Dennilauler, *J. Chim. Phys.*, **60**, 944 (1963).
(9) F. Covitz and F. H. Westheimer, *J. Am. Chem. Soc.*, **85**, 1773 (1963).
(10) A. J. Kresge and A. C. Lin, *Chem. Commun.*, 761 (1973).
(11) A. J. Kresge, *Chem. Soc. Rev.*, **2**, 475 (1973).
(12) J. A. Zoltewicz and L. W. Deady, *J. Am. Chem. Soc.*, **94**, 2765 (1972); J. A. Zoltewicz and H. L. Jacobson, *Tetrahedron Lett.*, 189 (1972).
(13) R. F. Pratt and T. C. Bruice, *J. Org. Chem.*, **37**, 3563 (1972).
(14) M. J. Gregory and T. C. Bruice, *J. Am. Chem. Soc.*, **89**, 2327 (1967).
(15) R. P. Bell and P. G .Evans, *Proc. R. Soc. London, Ser. A.*, **291**, 297 (1966).
(16) G. E. Lienhard and T.-C. Wang, *J. Am. Chem. Soc.*, **90**, 3781 (1968).
(17) P. J. Dynes, G. S. Chapman, E. Kebede, and F. W. Schneider, *J. Am. Chem. Soc.*, **94**, 6356 (1972).
(18) D. J. Cram, "Fundamentals of Carbanion Chemistry", Academic Press, New York, N.Y., 1965, p 10.
(19) P. F. Jackson, K. J. Morgan, and A. M. Turner, *J. Chem. Soc., Perkin Trans. 2*, 1582 (1972).
(20) A linear free energy relationship exists between the kinetic acidities of II–IV in water and the kinetic acidities of their unquaternized precursors in ethanol.[21] The scale of relative values is larger for the less reactive, uncharged acids.[21]
(21) N. N. Zatsepina and I. F. Tupitsyn, *Khim. Geterotsikl. Soedin.*, **12**, 1587 (1974).
(22) For considerations of linear free energy relationships involving proton transfer reactions of cyanine dyes and π-electron delocalization energies, see ref 17 and references cited therein.
(23) M. J. Cook, A. R. Katritzky, A. D. Page, R. D. Tack, and H. Witek, *Tetrahedron*, **32**, 1773 (1976).
(24) J. A. Zoltewicz and H. L. Jacobson, *J. Org. Chem.*, **43**, 19 (1978).
(25) M. J. Cook, A. R. Katritzky and P. Linda, *Adv. Heterocycl. Chem.*, **17**, 256 (1974).
(26) J. M. Duclos and P. Haake, *Biochemistry*, **13**, 5358 (1974).
(27) It is assumed that the relative equilibrium acidities of the two classes of carbon acids being considered are reflected in their relative kinetic acidities.
(28) D. S. Kemp and J. T. O'Brien, *J. Am. Chem. Soc.*, **92**, 2554 (1970).
(29) The comparison makes corrections to reflect the approximately threefold rate retarding effect of an *N*-methyl relative to an *N*-benzyl group,[30] the roughly twofold greater reactivity of OD^-/D_2O relative to OH^-/H_2O[31] and an estimated 150-fold reduction in reactivity of II due to lowering the temperature by 45 °C.
(30) R. Breslow and E. McNelis, *J. Am. Chem. Soc.*, **81**, 3080 (1959).
(31) R. L. Schowen, *Prog. Phys. Org. Chem.*, **9**, 275 (1972).
(32) Removal of the fused benzene ring will decrease reactivity at the sp^2 carbon by about 10-fold[28] and by about 10^2-fold at sp^3 carbon (III vs. IV). Replacement of a 2-methyl by a 2-hydroxyethyl substituent diminishes re-

activity by about 50.[7] These changes result in an additional decrease in the reactivity of the sp^3 relative to the sp^2 carbon by an approximate factor of $10^{2.7}$.
(33) N. A. Zakharava, B. A. Poral-Koshits, and L. S. Efros, *Zh. Obshch. Khim.*, **23**, 1225 (1953); *Chem. Abstr.*, **47**, 12367*f* (1953).
(34) A. I. Kiprianov and Z. N. Pazenko, *Zh. Obsch. Khim.*, **19**, 1515 (1949); *Chem. Abstr.*, **44**, 1097d (1950).
(35) H. Vorsanger, *Bull. Soc. Chim. Fr.*, 551 (1967).
(36) H. Quast and E. Schmitt, *Chem. Ber.*, **101**, 4012 (1968).
(37) J. A. Zoltewicz and G. M. Kaufmann, *J. Org. Chem.*, **34**, 1405 (1969).
(38) J. A. Zoltewicz and R. E. Cross, *J. Chem. Soc., Perkin Trans. 2*, 1363 (1974).
(39) R. Bates, "Determination of pH. Theory and Practice", Wiley, New York, N.Y., 1964.
(40) A. K. Covington, M. Paabo, R. A. Robinson, and R. G .Bates, *Anal. Chem.*, **40**, 700 (1968).
(41) A. K. Covinton, R. A. Robinson, and R. G. Bates, *J. Phys. Chem.*, **70**, 3820 (1966); G. S. Kell, *J. Chem. Eng. Data*, **12**, 66 (1967).
(42) H. S. Harned and B. B. Owen, "The Physical Chemistry of Electrolytic Solutions", 3rd ed, Reinhold, New York, N.Y., 1958, pp 639–643.
(43) A. Albert and E. P. Serjeant, "The Determination of Ionization Constants", Chapman and Hall, London, 1971.

Model Studies of Thiamin Catalysis. Steric Inhibition of Deprotonation of a Hydroxyethyl Side Chain

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Rate constants have been obtained for deprotonation of the 2 position of a series of 2-substituted 3-methylbenzothiazolium ions involving formate ion and water as bases in D_2O. Relative rate constants for the two bases are very similar in spite of the reactivity of formate ion being about 3.4×10^4 times greater than that for water. Second-order rate constants for formate ion at 75.0 °C and 1 M ionic strength are: 2-ethyl, 2.28×10^{-2}; 2-hydroxymethyl, 1.10×10^{-2}; 2-isopropyl, 2.28×10^{-4}; and 2-(1-hydroxyethyl), 4.50×10^{-4} M^{-1} s^{-1}. The major influence on reactivity is found in the latter two substances where steric inhibition of resonance is dominant. It is suggested that a similar steric effect will be present in the conjugate base of 2-(1-hydroxyethyl)thiamin and will influence rates and equilibria in nonenzymatic and possibly in enzymatic reactions as well.

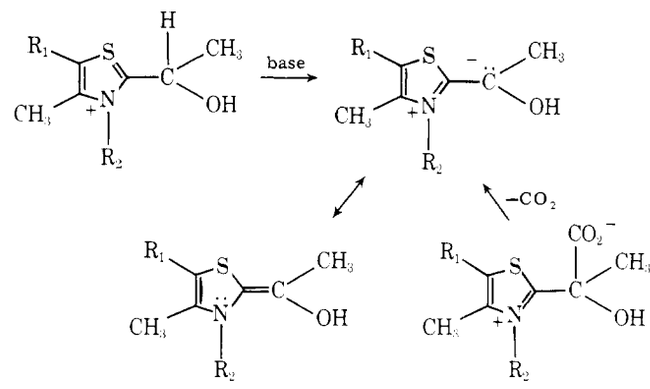
The enzyme cofactor thiamin pyrophosphate participates in a number of significant biological transformations. One very important derivative, an "enamine", is shown in Scheme I where it is formed by deprotonation of the corresponding 2-(1-hydroxyethyl) modification of the cofactor as well as by decarboxylation.[1] Prior to this study information has not been reported above how the methyl and hydroxy groups bonded to the exocyclic carbon atom of the enamine affect its rate of formation.

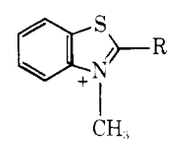
Generally, the effects of methyl and hydroxy or alkoxy substituents on the rates of deprotonation of a carbon atom to which they are bonded are variable and complex. The net effect is likely to be dependent on the hybridization of carbon in the transition state leading to deprotonation. Effects produced when the reactive site is pyrimidal can be different from those when the site is planar. Factors affecting reactivity include inductive and resonance effects, bond strengths as influenced by hybridization,[2] and electron pair repulsion associated both with electrostatic and Pauli exclusion principle effects.[3,4] Examples are known where an alkoxy group hinders[2] as well as facilitates[5] deprotonation of an adjacent sp^3 hybridization carbon.

In addition, the methyl and hydroxy substituents of the thiamin derivative in Scheme I may influence the rate of deprotonation by a steric effect. Interaction of the side chain and R_2 group may prevent the substituents and the ring from adopting a planar configuration, thereby hindering electron delocalization leading to effective charge neutralization. That is, there may be steric inhibition of resonance in the transition state and in the conjugate base.

We have studied a series of 2-substituted 3-methylbenzothiazolium ion model compounds I–V in order to obtain insight into the effects operating in the deprotonation reaction given in Scheme I. Starting with a 2-methyl substituent, the hydrogen atoms have been systematically replaced, first by a methyl and then by a hydroxy group, to yield 2-ethyl (II) and 2-hydroxymethyl (III) substrates. Next, two such substitutions give rise to 2-isopropyl (IV) and 2-(1-hydroxyethyl) (V) model compounds. We believe the effects of these replacements on rates of deprotonation provide considerable insight into the factors affecting reactivity of the enzyme cofactor not only in deprotonation but also in decarboxylation reactions as well.

Scheme I





I, R = CH_3
II, R = CH_2CH_3
III, R = CH_2OH
IV, R = $CH(CH_3)_2$
V, R = $CHOHCH_3$

Results

Hydrogen–deuterium exchange reactions were carried out at 75.0 °C in D_2O solution, generally using a formate buffer.

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