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Thiazolium Ions and Related Heteroaromatic Systems. III. Comparison of Rates of Hydrogen Exchange with ^{13}C–H Coupling Constants. Evidence for Stabilization of Heterocyclic Ylides by Sulfur[1-3]

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Abstract: The rates of exchange of 2-hydrogens in 3,4-dimethyloxazolium ion, 3,4-dimethylthiazolium ion, and 1,3,4-trimethylimidazolium ion have been measured at a variety of pH's and buffer concentrations. In each case the rate constant for exchange catalyzed by OD$^-$ has been evaluated. The relative second-order rate constants are respectively $10^{5.5}:10^{3.5}:1$. The ^{13}C–H coupling constants for ring hydrogens have also been measured. These data for rates are compared with knowledge of the ground states. The similarity of ^{13}C–H coupling constants in homologous imidazolium and thiazolium ions at the 2 position indicates that these C–H bonds are similar in the ground state. The exchange rate 3000 times greater in 3,4-dimethylthiazolium ion than in 1,3,4-trimethylimidazolium ion suggests the transition state for ylide formation must be considerably stabilized in the case of the sulfur heterocycle. This indicates a special role for sulfur and provides partial understanding of the importance of a thiazolium ion in the structure of thiamine.

Breslow has summarized[6] his research[7] on thiamine including his considerations of the possible role of sulfur in the mechanism of action of this vitamin. He has considered in some detail the structure of thiazolium ylides and has suggested overlap of a sulfur d_{xy} orbital with the filled sp_xp_y orbital at the 2-carbon.[6b]

We have attempted to extend this work to answer the question: Why a thiazolium ion as the active site of thiamine? We reported[8] some quantitative comparisons of oxazolium and thiazolium ions, especially the rates of proton exchange at the 2 position compared to ^{13}C–H coupling constants. These results gave some quantitative support to the suggestion[9] of d–σ overlap as a possible stabilizing factor in thiazolium ylides. Olofson and coworkers[9] later published a series of communications on rates of proton exchange in heterocycles. They found unusually large rates of exchange for protons α to sulfur.

We have also recently reported[3] the basicity of oxazoles, thiazoles, and imidazoles. The pK_a's of the conjugate acids, the respective azolium ions, give some quantitative information on the relative ease of proton loss at another position—the 3 position of the rings.

We report here our completed research[8] on the second-order rate constants for $^{-}$OH catalysis of proton exchange at the 2 position in azolium rings. The results have importance in understanding stabilization of thiazolium ylides and the suitability of thiazolium ions for their biological function.

Experimental Section

Nmr spectra were taken on a Varian Model A-60 spectrometer in CCl$_4$ with tetramethylsilane as internal standard or in D$_2$O with sodium 3-trimethylsilylpropanesulfonate as internal standard. Infrared spectra were taken in CCl$_4$ or in KBr windows on a Perkin-Elmer Model 421 spectrophotometer. Mass spectra were measured with an AEI MS-9 instrument using an ionizing voltage of 70 eV and a source temperature of 200°. All melting points are corrected, all boiling points are uncorrected.

^{13}C–H Coupling Constants. These measurements were made on concentrated solutions using either linear measurement (method A) or the side-band technique (method B). Since most of the ^{13}C satellite peaks were weak and broad, method A involved sweeping through the peaks several times, determining the centers at half-height, and measuring the average distance from center to center of the two satellite peaks. With the exception of 4-methylimidazole in trifluoroacetic acid and 3,4-dimethyloxazolium iodide, the coupling constants obtained by method B were determined by measuring the differences between the ^{13}C satellite peaks and 100.0, 110.0, or 125.0 cps side bands of the main peak. The side bands were imposed on the spectrum with a Hewlett-Packard Model 200 CD radiofrequency oscillator. The radiofrequency was measured with a Hewlett-Packard 5233L electronic counter.

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(3) Part II: P. Haake and L. P. Bausher, *J. Phys. Chem.*, **72**, 2213 (1968).

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In the case of 4-methylimidazole in trifluoroacetic acid, the upfield satellite of H-2 lay too close to H-5, and the downfield satellite of H-5 lay too close to H-2 to be observed under the high amplification required. Consequently, the coupling constants were estimated by measuring the separation of the satellites which could be seen and the respective side bands and applying an appropriate correction factor (1.6 cps for H-2 and 0.6 cps for H-5, obtained from the coupling constants of the 2- and 5-hydrogens of 1,3,4-trimethylimidazolium iodide) to correct for the difference between the chemical shifts of protons bound to ^{12}C and ^{13}C. The resultant ^{13}C center–^{13}C satellite frequency separation was doubled to obtain $J(^{13}C\text{–}H)$. For example, the downfield satellite of H-2 was located 9.3 cps downfield from the 100.0-cps side band of the ^{12}C peak. The separation between the ^{13}C satellite peak and the ^{12}C peak was, therefore, 109.3 cps. Using the 0.8-cps difference between the ^{12}C peak and the ^{13}C center, the separation between the ^{13}C center and the ^{13}C satelllte peak is $109.3 + 0.8 = 110.1$ cps. The ^{13}C–H coupling constant is, therefore, $2 \times 110.1 = 220.2$ cps.

Similarly, in the case of the 5-hydrogen of 3,4-dimethyloxazolium iodide, the ^{13}C–H coupling constants could be estimated by doubling the value obtained by measuring the separation between the ^{12}C resonance and the upfield satellite. This calculation assumes a negligible difference in chemical shift between protons bound to ^{12}C and ^{13}C. Since the chemical-shift difference for the 2-hydrogen is only 0.3 cps in this ion, this assumption is probably reasonable by analogy to the imidazolium system, in which the chemical shift difference for the 5-hydrogen is less than half as large as that for the 2-hydrogen.

All coupling constants were determined on a 250-cps sweep width except for the 2-hydrogen of 4-methylimidazole in deuterium oxide, which was determined on a 100-cps sweep width.

Measurements of Rates of Hydrogen Exchange. Buffers were prepared in D_2O using phosphate (from NaH_2PO_4, Na_2HPO_4, and Na_3PO_4), acetate (from glacial acetic acid and sodium acetate), or ethylenediamine (from ethylenediamine dihydrochloride and NaOD solution). All pH values were measured on a Beckman Model G pH meter. The values were measured on the buffered solutions of the salts after the exchange had occurred. The pD was then calculated by adding 0.40 to the observed "pH."[10]

Solutions of the salts studied were prepared by dissolving about 0.5 mmol of salt in 0.5 ml of buffered D_2O. For fast runs, the solutions were transferred to thin-walled nmr tubes and placed in the probe of the spectrometer, and integrated areas measured for the 2-hydrogen signal compared to the 4-methyl signal (as nonexchanging standard) as a function of time. The probe temperature was measured before and after each kinetic run with a copper–constantan thermocouple. For slow runs, the nmr tube was placed in a constant-temperature bath and the tube removed for about 15 min every 6 hr in order to measure the integrated area of the 2-hydrogen signal and the 4-methyl signal. The pseudo-first-order rate constants were obtained by plotting and by least-squares analysis using the equation: $\ln [4\text{-}CH_3/3(2\text{-}H \text{ signal})] = kt + C$ where $4\text{-}CH_3$ is the integrated area of the 4-methyl signal and 3(2-H signal) is three times the integrated area of the 2-proton signal. Table II contains typical per cents of reaction over which the exchanges were followed.

Preparation of Salts. The synthesis of 4-methyloxazole and 4-methylthiazole have been described.[3]

4-Methylimidazole was prepared from both acetoxyacetone and chloroacetone.[11] The final product was distilled at 125° (0.1 mm): nmr (8% in D_2O) τ 2.33 (singlet, 1 H), 3.17 (singlet, 1 H), and 7.77 (doublet, 3 H).

1,3,4-Trimethylimidazolium Iodide. A solution was prepared by dissolving 8.8 g of 4-methylimidazole in 20 ml of methanol. Methyl iodide (145.6 g) was added to the solution, which was stirred magnetically and heated at slow reflux for 22 hr and 30 min. The reaction mixture was allowed to cool and was stirred into 125 ml of ether, whereupon a yellow oil separated from the solution. Water (30 ml) was added to the mixture, the aqueous phase was separated, and the ether phase was extracted with another 20 ml of water. The two aqueous phases were combined and washed twice with 20-ml portions of ether. The water was stripped from the resulting solution, leaving 22.9 g of a thick oil, which solidified upon standing overnight. The salt was recrystallized as a fine white powder which was filtered from the solution and stored over phosphorus pentoxide. The hygroscopic crystals gradually discolored on standing; yield 4.5 g, mp 160–165° (lit.[12] mp 160–163°);. nmr (54% in D_2O) τ 1.20 (singlet, 1 H), 2.62 (singlet, 1 H), 6.05 and 6.12 (singlets, total of 6 H), and 7.60 (doublet, 3 H); λ_{max}^{KBr} 3.18, 3.27, 3.33, 6.23, 6.39, 6.92, 6.98, 8.45, 8.55, 8.66, 9.30, 9.65, 11.88, 12.79, and 16.18 μ.

Anal. Calcd for $C_6H_{11}IN_2$: C, 30.27; H, 4.68. Found: C, 30.53; H, 4.83.

3,4-Dimethyloxazolium Iodide. Methyl iodide (1.50 g) and 4-methyloxazole[3] (0.45 g) were mixed in a dry, thick-walled glass tube which had been sealed at one end. The mixture was frozen in Dry Ice–acetone and the other end of the tube was sealed. The tube was wrapped in aluminum foil and heated in a steam bath. During heating it was shaken at 2- to 5-min intervals. After 1 hr of heating and shaking, the contents of the tube consisted of a dark red upper layer (80% of the liquid volume) and a yellow lower layer (20% of the liquid volume). Heating was discontinued, whereupon the tube was broken and its contents rinsed into a 10-ml flask with absolute ethanol. The iodide salt was then crystallized twice from absolute ethanol–ether. The yellow crystals were rapidly filtered from the mother liquor, washed with a small amount of absolute ethanol, and dried at 80° over phosphorus pentoxide. Upon drying, the salt turned white; yield 0.74 g, mp 106–109°; nmr (29% in D_2O) τ 0.17 (singlet, 1 H), 1.74 (multiplet, 1 H), 6.05 (doublet, 3 H), and 7.60 (doublet, 3 H).

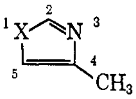
Anal. Calcd for C_5H_8INO: C, 26.68; H, 3.58. Found: C, 26.37; H, 3.91.

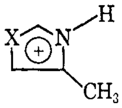
3-Benzyl-4-methylthiazolium chloride was prepared[13] by reaction of freshly distilled benzyl chloride with 4-methylthiazole,[3] mp 191–192° (lit.[13] 188°); nmr (D_2O) τ 0.10–0.16 (doublet, 1 H), 1.99 (broad singlet, 1 H), 2.30–2.59 (multiplet, 5 H), 4.22 (singlet, 2 H), 5.20 (H_2O), and 7.39 (doublet, 3 H).

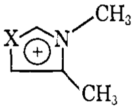
3,4-Dimethylthiazolium iodide was prepared[14] by reaction of 4-methylthiazole[3] with excess methyl iodide. After several days at room temperature, the crystals were collected, washed with cold methanol, and recrystallized from ethanol–ether, mp 118.5–119.5° (lit.[14] 119–120°); nmr (D_2O) τ 0.12 (1 H), 2.06 (1 H), 5.72 (3 H), and 7.25 (3 H).

Results

^{13}C–H Coupling Constants. Coupling constants for the neutral azoles **1**, **2**, and **3**, the N-protonated azolium ions (**1**-H$^+$, **2**-H$^+$, and **3**-H$^+$), and the N-methylated azolium ions (**1**$^+$, **2**$^+$, and **3**$^+$) are given in Table I.


1, X = O
2, X = S
3, X = NH


1-H$^+$, X = O
2-H$^+$, X = S
3-H$^+$, X = NH


1$^+$, X = O
2$^+$, X = S
3$^+$, X = NCH_3

N-Protonated ions were formed by dissolving the neutral heterocycles in CF_3CO_2H. Comparison of ^{13}C–H couplings for these solutions and for the N-methylated ions indicates that the heterocycles were completely protonated in CF_3CO_2H. The pK_a's of 4-methylimidazole,[15] 4-methyloxazole,[3] and 4-methylthiazole[3] have been determined; 4-methyloxazole is the weakest base, pK_a = 1.1. Substitution of the H_0 value for trifluoroacetic acid ($H_0 = -3.03$[16]) and the pK_a of 4-methyloxazole into the Hammett equation

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Table I. ^{13}C–H Coupling Constants

Azole or azolium ion	Solvent	Method[a]	$J(^{13}C$–2H)	$J(^{13}C$–5H)
4-Methylimidazole (**3**)	D_2O	B	206	187
4-Methylthiazole (**2**)	Neat	A	209	187
4-Methyloxazole (**1**)	Neat	A	231	209
4-Methylimidazolium (**3-H**$^+$)	CF_3CO_2H	B	220[b]	201[b]
1,3,4-Trimethylimidazolium (**3**$^+$)[d]	D_2O	B	220	201
4-Methylthiazolium (**2-H**$^+$)[c]	CF_3CO_2H	A	218	200
3,4-Dimethylthiazolium (**2**$^+$)[d]	D_2O	A	216	202
4-Methyloxazolium (**1-H**$^+$)[c]	CF_3CO_2H	A	247	224
3,4-Dimethyloxazolium (**1**$^+$)[d]	H_2O	B	246	224[b]

[a] Method A is linear measurement; method B involves use of side bands; see Experimental Section. [b] Only one ^{13}C satellite seen; see Experimental Section for method. [c] Prepared by dissolving **1**, **2**, and **3** in CF_3CO_2H; therefore $CF_3CO_2^-$ is the counterion. [d] Iodide salt.

Table II. Rate Constants for Exchange of 2-Hydrogen of 1,3,4-Trimethylimidazolium Iodide in Phosphate Buffers[a]

pD	10^8 [OD^-],[d] *M*	[$D_2PO_4^-$], *M*	[DPO_4^{2-}], *M*	10^6 [PO_4^{3-}], *M*	% R[b]	$10^5 k_{obsd}$, sec^{-1} [c]
6.62	1.20	0.0050	0.0027	0.011	35	0.192 ± 0.013
7.01	2.95	0.0042	0.0057	0.056	75	0.358 ± 0.012
7.22	4.68	0.054	0.119	1.9	91	1.10 ± 0.03
7.58	11.0	0.022	0.108	3.9	93	2.18 ± 0.06
7.72	15.1	0.0091	0.063	3.2	90	2.36 ± 0.08
7.75	16.2	0.012	0.089	4.9	96	2.16 ± 0.13
7.78	17.4	0.011	0.088	5.1	98	2.63 ± 0.08
8.11	37	0.006	0.094	12	80	5.65 ± 0.00[e]
7.46	8.3	0.021	0.080	2.2	80	0.528 ± 0.023[f]

[a] At 34.4°. [b] Per cent reaction over which rates were measured. [c] Listed deviations are standard deviations. [d] Using p*K*(D_2O) = 14.54. [e] Two-point rate. [f] Sodium chloride (1.67 *m*) added.

Table III. Rate Constants for Exchange of the 2-Hydrogen of 1,3,4-Trimethylimidazolium (**3**$^+$) Iodide in Ethylenediamine Buffers[a]

pD	10^7 [OD^-],[b] *M*	10^2 [E^+],[c] *M*	$10^5 k_{obsd}$, sec^{-1} [d]	$10^{-2}(k_{obsd}/[OD^-])$
8.08	3.47	9.63	4.14 ± 0.11	1.19
8.03	3.09	10.66	3.19 ± 0.15	1.03
7.82	1.91	12.53	1.87 ± 0.11	0.98
8.03	3.09	4.80	3.52 ± 0.07	1.25
7.92	2.40	5.28	2.56 ± 0.02	1.07
7.82	1.91	6.27	1.77 ± 0.09	0.93

[a] At 33.2°. [b] Using $pK_c(D_2O)$ = 14.54.[20] [c] E^+ = monoprotonated ethylenediamine. [d] Listed deviations are standard deviations.

Table IV. Rate Constants for Exchange of the 2-Hydrogen of 3,4-Dimethyloxazolium (**1**$^+$) Iodide in Acetate Buffers[a]

pD	10^{11} [OD^-],[b] *M*	10^3 [OAc^-],[c] *M*	% R[d]	$10^3 k_{obsd}$, sec^{-1} [e]	10^{-7} ($k_{obsd}/[OD^-]$)
3.80	1.82	5.6	91	0.70 ± 0.01	3.85
4.10	3.63	10.7	88	1.40 ± 0.04	4.23
4.39	7.08	18.9	92	2.34 ± 0.04	3.31
3.72	1.51	2.4	90	0.66 ± 0.01	4.70
4.04	3.16	4.7	92	1.29 ± 0.03	4.08
4.32	6.03	8.3	91	2.44 ± 0.07	4.05

[a] At 33.5 ± 0.20. [b] Using $pK_c(D_2O)$ = 14.54.[20] [c] Using pK_{DOAc} = 5.03. [d] Per cent reaction over which rate was observed. [e] The listed deviations are standard deviations.

(eq 1) and calculation of the ratio of the protonated to the unprotonated form of the amine reveals that $[BH^+]/$

$$H_0 = pK_a + \log([B]/[BH^+]) \qquad (1)$$

[B] should be greater than 10^4. This calculation assumes that the protonation behavior of 4-methyloxazole will approximate that of a Hammett base. Consideration must also be given to the fact that the solutions of the heterocycles are quite concentrated (molar ratios of trifluoroacetic acid:azole were about 2:1). Nevertheless, the ratio $[BH^+]/[B]$ should be sufficiently large so that the ^{13}C–H coupling constants are correct for the protonated heterocyclic cation. Also, it has been found[17] that N-methylacetamide, a weaker base[18] than 4-methyloxazole, is completely protonated in CF_3CO_2H. The *J*(C–H) values for the neutral heterocycles are consistent with results on other heterocycles.[19]

Rates of Hydrogen Exchange in Azolium Ions. Rates of exchange of the 2-hydrogen of 1,3,4-trimethylimidazolium ion (**3**$^+$) were measured in two buffers by observing the change in area of the signal due to the 2-hydrogen in the nmr spectrum. Table II gives the results of the studies in phosphate buffers. The oxide concentration was calculated from pOD = 14.54 − pD which uses the value of pK_c, the p*K* value of D_2O on a molar basis, determined recently at 35°.[20] This p*K* value of D_2O was not corrected for salt effects. The expected correction factor is ~0.2 log unit[21]—absolute values of rate constants due to OD^- may be in error due to this approximation, but relative values are not. The phosphate monoanion ($D_2PO_4^-$) and dianion (DPO_4^{2-}) concentrations were calculated using an ionization constant (K_2) corrected for (1) deuterium isotope effect (eq 2)[22] and (2) salt effect—the solutions studied contain about 1.0 *m* **3**$^+$ I^- (eq 3).[21] This assumes that the salt effect of 1.0 *m* **3**$^+$ I^- will be approximately equal to the salt effect of 1.0 *M* NaCl. The phosphate trianion concentrations were calculated from $K_{DPO_4^{2-}} = 9.7 \times 10^{-13}$ *M* which was obtained using the same corrections as were used for $K_{D_2PO_4^-}$. This is certainly not valid, but actual measurements of the deuterium isotope effect and salt effect on $K_{HPO_4^{2-}}$ are apparently not available. The PO_4^{3-} concentrations in Table I may be in error, but the relative magnitudes from run to run are in the right order. It

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Table V. Rate Constants for Exchange of the 2-Hydrogen of Thiazolium (**2**+) Ions in Phosphate Buffers[a]

pD	10^{10}[OD$^-$],[b] *M*	[D$_2$PO$_4^-$],[c] *M*	10^3[DPO$_4^{2-}$],[c] *M*	10^{11}[PO$_4^{3-}$],[d] *M*	$10^4 k_{obsd}$, sec^{-1}	$10^{-6}(k_{obsd}$/[OD$^-$])
			3-Benzyl-4-methylthiazolium Chloride			
4.58	1.07	0.27	1.34	4.9	2.7	25.2
4.62	1.23	0.068	0.37	1.37	1.6	13.0
4.69	1.41	0.132	0.86	4.1	2.4	16.7
4.73	1.55	0.134	0.95	4.9	3.2	20.6
4.85	2.04	0.099	0.92	6.3	2.4	11.9
6.49	89.1	0.0082	3.18	950	38	4.3
			3,4-Dimethylthiazolium Iodide			
4.69	1.41	0.132	0.86	4.1	0.69	4.9
4.73	1.55	0.134	0.95	4.9	0.78	5.0

[a] At 38° except for the data at pD = 4.85 where T = 32.4°. [b] Using pK(D$_2$O) = 14.54.[20] [c] Using pK(D$_2$PO$_4^-$) = 6.88. [d] Using pK(DPO$_4^{2-}$) = 12.01.

Table VI. Rate Constants for Exchange of the 2-Hydrogens of 3,4-Dimethylthiazolium (**2**+) Iodide in Acetate Buffers[a]

pD	10^9 [OD$^-$],[b] *M*	[OAc$^-$], *M*	$10^3 k_{obsd}$, sec^{-1} [c]	10^{-5} (k_{OD^-}/[OD$^-$])
6.21	4.68	0.26	1.61 ± 0.10	3.44
5.96	2.63	0.27	1.14 ± 0.07	4.33
5.63	1.23	0.29	0.50 ± 0.075	4.07
6.23	4.90	0.13	1.93 ± 0.095	3.65
5.94	2.51	0.14	1.18 ± 0.025	4.70
5.59	1.12	0.14	0.56 ± 0.017	5.001

[a] At 33.3°. [b] pK(D$_2$O) = 14.54.[20] [c] Deviations are standard deviations.

should therefore be possible to tell if PO$_4^{3-}$ is an important base in proton removal to give imidazolium ylide.

$$pK_{D_2PO_4^-} = 7.19 + 0.56 = 7.75 \qquad (2)$$

or

$$K_{D_2PO_4^-} = 1.78 \times 10^{-8}\ M$$

$$K'_{D_2PO_4^-}(\text{in } 1.0\ m\ 3^+\ I^-) = K_{D_2PO_4^-} \times \left[\frac{K_{H_2PO_4^-} \text{ in } 1.0\ M \text{ NaCl}}{K_{H_2PO_4^-}}\right] = 1.31 \times 10^{-7}\ M \qquad (3)$$

However, because of this potential uncertainty in the interpretation of rates of deuterium exchange of **3**+ in phosphate buffers, an alternative buffer system was sought. It was found that the ethylenediamine monocation–dication buffer system was suitable. Table III contains the results of measurements of exchange rates using this buffer system prepared from (H$_3$NCH$_2$–)$_2^{2+}$ 2Cl$^-$ and NaOD. The pK_1 of (H$_3$NCH$_2$–)$_2^{2+}$ is of primary concern. It was corrected for the deuterium isotope effect by addition of 0.5 (eq 4) to the pK,[22–24] but no correction was made for the salt effect. Obviously, the salt effect will be large on this pK, but the

$$pK_1(\text{H}_3\text{NCH}_2\text{–})_2^{2+} = 6.97 + 0.5 = 7.47 \qquad (4)$$

relative values for the concentration of ethylenediamine monoanion will be valid and this will be sufficient to exclude this as an important base in the exchange reaction.

The rates of exchange of the 2-hydrogen of 3,4-dimethyloxazolium (**1**+) iodide were measured in acetate buffers. The pK of acetic acid was corrected for deuterium isotope effect (eq 5)[22] and salt effect (eq 6).[25]

$$pK_{DOAc} = 4.74 + 0.52 = 5.26 \qquad (5)$$

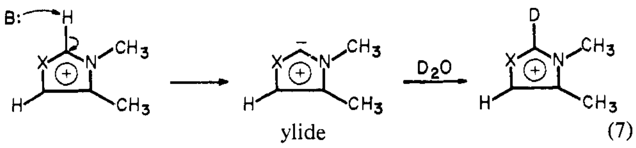
$$pK_{DAOc}(\text{in } 1.0\ M \text{ NaCl}) = 5.26 - \log(1.7) = 5.03 \qquad (6)$$

Using this corrected pK = 5.03, the concentrations of $^-$OAc in the exchanging solutions were calculated. Table IV gives rate constants and concentrations of bases.

The rates of exchange of the 2-hydrogens of thiazolium (**2**+) salts were measured in phosphate buffers (Table V) and acetate buffers (Table VI). The concentrations of anions in the buffer systems were calculated using the pK's given above (eq 3 and 6).

Discussion

Hydrogen Exchange Rates. Studies of exchange of hydrogens bonded to the 2 position of azolium ions (eq 7) have played a central role in understanding thiamine. Our work is characterized by two straightforward methods which are necessary for a clear understanding of experimental data on the rates of ylide formation (eq 7). (1) We have studied azolium ions which resemble one another closely. (2) We have varied buffer concentrations and pH, so that, for each azolium ion, it is possible to tell which basic species is abstracting the 2-proton in the rate-determining step for proton exchange (eq 7).



(7)

The rate constants for exchange of imidazolium ions in phosphate buffers (Table II) are plotted against OD$^-$ concentration in Figure 1. It is clear that the predominant exchange pathway for imidazolium ions, under the conditions used here, involves OD$^-$ as base, *i.e.*, D$_2$O and buffer anions do not significantly contribute to the k_{obsd} values of Table II and these terms in eq 8

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

(24) Ammonia and glycine have pK_D about 0.5 unit greater than pK_H.[22]

(25) M. Kilpatrick and R. D. Eanes, *J. Am. Chem. Soc.*, 75, 586 (1953).

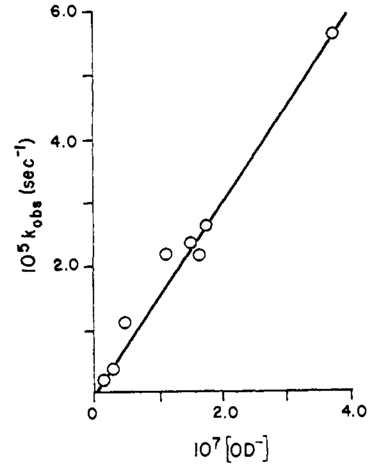


Figure 1. Observed rate constants (Table II) for exchange of 2-hydrogen of 1,3,4-trimethylimidazolium ion plotted against deuterioxide concentration in phosphate buffers.

$$k_{\text{obsd}} = k_{D_2O} + k_{OD^-}[OD^-] + k_{B^-}[B^-] \qquad (8)$$

may be neglected. Therefore, the slope of Figure 1 gives $k_{OD^-} = 1.5 \times 10^2\ M^{-1}\ \text{sec}^{-1}$. The data in Table III substantiate this conclusion; $k_{\text{obsd}}/[OD^-]$ is quite constant, average $= 1.1 \times 10^2\ M^{-1}\ \text{sec}^{-1} = k_{OD^-}$ in this system. These rate constants for exchange are not very accurate because they have been done with crudely controlled temperature, by integration of nmr signals, and in concentrated solutions. Nevertheless, better agreement of the k_{OD^-} values from Table II and III may be expected and there may be some systematic error in one of these sets of data. However, the most important fact is that most or all of the exchange rates are due to OD^-; the average value of k_{OD^-} (eq 9) will be sufficiently accurate to establish the main conclusions in this paper.

k_{OD^-}(imidazolium) =

$$k_{OD^-}{}^{3^+} = 1.3 \times 10^2\ M^{-1}\ \text{sec}^{-1} \qquad (9)$$

The rate constants of oxazolium ion (Table IV) are also linearly dependent on OD^- concentration (Figure 2). In this case, there appears to be a small intercept indicating some contribution from k_{D_2O} or k_{B^-} or both. The slope of Figure 2 yields

k_{OD^-}(oxazolium) =

$$k_{OD^-}{}^{1^+} = 3.8 \times 10^7\ M^{-1}\ \text{sec}^{-1} \qquad (10)$$

The same conclusions result from consideration of $(k_{OD^-}/[OD^-])$ values.

The situation for exchange of the 2-hydrogen of thiazolium ions is somewhat more complex. The data for the 3-benzyl ion (Table V) result in $k_{OD^-}/[OD^-]$ values which vary widely. Therefore, there must be catalysis by bases other than OD^-. The rate at pD = 6.49 can be assumed to involve only OD^- and from this $k_{OD^-}{}^{2^+} \sim 4.3 \times 10^5\ M^{-1}\ \text{sec}^{-1}$. A fair fit can then be obtained to the other data with $k_{DPO_4{}^{2-}}{}^{2^+} = 0.24\ M^{-1}\ \text{sec}^{-1}$.

These values of k_{OD^-} and $k_{DPO_4{}^{2-}}$ lead to a Brønsted value of 0.7 (eq 11), using $K_a(D_2O) = 2.9 \times 10^{-15}/55.0 = 5.3 \times 10^{-17}$ and $K_a(D_2PO_4^-)$ from eq 3. This β value could be in error because of the approximate nature of these data and because of the high concentrations of substrate which were used.

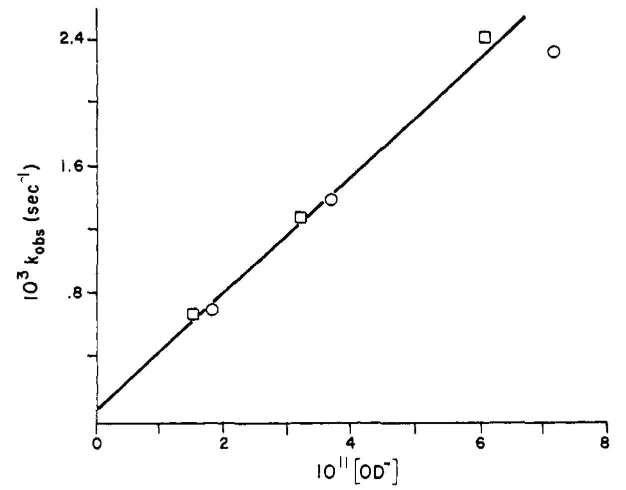


Figure 2. Observed rate constants (Table IV) for exchange of 2-hydrogen of 3,4-dimethyloxazolium ion plotted against deuterioxide concentration in acetate buffers: ○, low [$^-$OAc]; □, high [$^-$OAc].

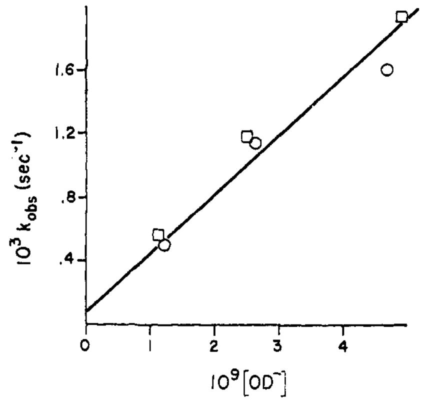


Figure 3. The rate constants (Table VI) for exchange of the 2-hydrogen of 3,4-dimethylthiazolium ion plotted against OD^- concentration in acetate buffers: ○, low [$^-$OAc]; □, high [$^-$OAc].

$$\beta = \frac{\Delta \log k}{\Delta \log K_a} = \frac{6.3}{9.4} = 0.7 \qquad (11)$$

Because of the complication from $DPO_4{}^{2-}$ catalysis of exchange, additional rate constants were evaluated on exchange of 3,4-dimethylthiazolium ion in acetate buffers. The data of Table VI are plotted against $[OD^-]$ in Figure 3; these data appear to involve mainly exchange of OD^- as base. The slope of Figure 3 or analysis of $(k_{OD^-}/[OD^-])$ values yields

k_{OD^-}(thiazolium) =

$$k_{OD^-}{}^{2^+} = 3.7 \times 10^5\ M^{-1}\ \text{sec}^{-1} \qquad (12)$$

The agreement with the data on the benzylthiazolium ions (both $K_{OD^-} \sim 4 \times 10^5\ M^{-1}\ \text{sec}^{-1}$) substantiates the above analysis of that data. However, the value of k_{OD^-} in eq 12 will be used because it comes from study of the N-methyl ion and is therefore the best value to compare to k_{OD^-} values (eq 9 and 10) for the imidazolium and oxazolium ions which are N-methylated.

Comparison of the k_{OD^-} values for exchange in the three azolium ions yields the following relative rates of ylide formation with OD^- abstracting a proton from the

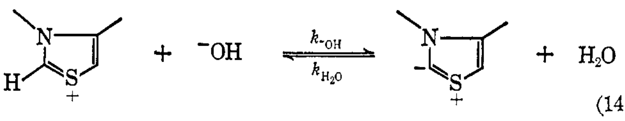
2 position. This result contrasts with previous work[26]

$$\begin{aligned} k_{\text{OD}^-}{}^{3^+} &= 1.3 \times 10^2 = 1 \\ k_{\text{OD}^-}{}^{2^+} &= 3.7 \times 10^5 = 10^{3.5} \\ k_{\text{OD}^-}{}^{1^+} &= 3.8 \times 10^7 = 10^{5.5} \end{aligned} \tag{13}$$

where a diphenylimidazolinium ion was found to exchange at the same rate as a thiazolium ion. Because the earlier data[26] were obtained with azolium ions differing considerably in their structure and because insufficient rates were measured to evaluate the main pathway for exchange, we believe that the true comparison is given in eq 13: *deuterioxide abstracts a proton from thiazolium ions to give the thiazolium ylide ~3000 times faster than the same process in structurally similar imidazolium ions.* This result also affects previous conclusions[26, 27] about the reasons for a *thiazolium* ring as the active site of thiamine.

Relative Stability of Ylides. It would be good to be able to use these data to decide whether the sulfur atom, a third-period element, stabilizes the thiazolium ylide by some special effect not available with the second-period elements, oxygen and nitrogen. The above relative rates only indicate the relative differences in free energy between ground states and transition states. Therefore, in order to approach the question of stability of ylides, we must attack two questions. (1) How closely do the transition states approximate the ylides? (2) How do the ground states differ in stability?

The resemblance of the transition states to the ylides depends on the barrier for reprotonation of the ylides, *e.g.*, the barrier to the back reaction of eq 14. In the case of vinyl carbanions, it has been assumed that there is essentially no barrier to protonation by CH_3OH.[28] This approximation was part of an estimation of the pK_a of *cis*-1,2-dibromoethylene. It was also used to estimate pK_a ~ 20 for 3,4-dimethylthiazolium ion.[6b, 29] Although second-order rate constants for the protonation of carbanions by H^+ often approximate diffusion control, this is not necessarily true where the proton donor is a neutral solvent molecule.[30] The transition state for protonation then involves breaking the H–O bond of neutral solvent with generation of hydroxide or alkoxide ion. However, there are some data[31] which can be used to give a more exact estimate of the barrier to protonation and provide a better estimate of the pK_a's of the azolium ions studied here. The data for acetone[31] indicate that the rate constant for protonation of the anion by water is about 3 × 10^5 sec^{-1}.



(14)

$$CH_3C(O)CH_3 + HO^- \underset{k_{H_2O}}{\overset{k_{HO^-}}{\rightleftarrows}} CH_3C(O)CH_2^- + H_2O$$

$$K_a = \frac{[A^-][H^+]}{[HA]} \therefore \frac{K_a}{K_w} = \frac{k_{HO^-}}{k_{H_2O}}$$

$$k_{H_2O} = \frac{K_w k_{HO^-}}{K_a}$$

$$k_{H_2O} = \frac{10^{-14} \times 2.7 \times 10^{-1}}{10^{-20}} \sim 3 \times 10^5 \text{ sec}^{-1}$$

There may be problems in applying these data, because removal of a proton from acetone yields an enolate anion quite unlike a neutral azolium ylide. However, applying the same equations to data for HCN (pK_a = 9.1)[31] leads to k_{H_2O} ~ 5 × 10^4 sec^{-1}. Therefore, for purposes of estimation, we may take 10^5 sec^{-1} as an approximate value for k_{H_2O} for protonation of azolium ylides (eq 14). This suggests that there is a barrier to protonation approximately 7–9 kcal/mol greater than expected of a diffusion-controlled reaction. It also suggests the following approximate K_a's: **1**$^+$, K_a ~ 10^{-12}; **2**$^+$, K_a ~ 10^{-14}; **3**$^+$, K_a ~ 10^{-17}. Since k_{H_2O} is derived from known data on protonation of anions and the ylides are neutral, k_{H_2O} may be too high. Also, the values of k_{H_2O} may vary considerably. Therefore, these K_a's are only approximate estimations.

The intermediate ylides are therefore considerably more stable than the transition states for proton exchange. However, the same factors that stabilize the ylides probably stabilize the transition states. In the transition states for removal of a proton by HO^-, the C–H bond is probably fairly well broken. The same factors that stabilize an unshared pair at the 2 position in the ylides should therefore be present in the transition state although to a smaller extent.

In order to understand the rates of proton exchange in terms of heterocyclic structure, we have to look at both sides—ground state and transition state—of the pseudoequilibrium. Many effects can be involved: solvation, strengths of σ bonds, π bond energies, etc. This is, therefore, a very complex question, but we have one possible approach, grounded in experiment, which appears worth consideration. This involves ^{13}C–H coupling constants.

The original correlation[32] of C–H couplings with per cent s character in the carbon orbital of the C–H bond has been criticized[33] and an alternative correlation with effective nuclear charge has been proposed.[34] However, either correlation would lead one to expect, in the absence of perturbations, that *J*(C–H) values would provide an indication of acidity.[35] Support for this view also is provided by the increase in *J*(C–H) with increase in electronegativity of heteroatoms bonded to the same carbon.[36, 37] The known experimental correlation of *J*(C–H) with C–H acidity therefore appears

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[26] W. Hafferl, R. Lundin, and L. L. Ingraham, *Biochemistry*, **2**, 1298 (1963); **3**, 1072 (1964).

[27] (a) R. L. Collin and B. Pullman, *Arch. Biochem. Biophys.*, **108**, 535 (1964); (b) B. Pullman and C. Spanjaard, *Biochim. Biophys. Acta*, **46**, 576 (1961).

[28] S. I. Miller and W. G. Lee, *J. Am. Chem. Soc.*, **81**, 6313 (1959).

[29] E. McNelis, Ph.D. Thesis, Columbia University, New York, N. Y., 1960.

[30] As in this case and in ref 28 and 29.

[31] M. Eigen *Angew. Chem. Intern. Ed. Engl.*, **3**, 1 (1964).

[32] N. Muller and D. E. Pritchard, *J. Chem. Phys.*, **31**, 768 (1959); **31**, 1471 (1959).

[33] G. J. Karabatsos and C. E. Orzech., Jr., *J. Am. Chem. Soc.*, **86**, 3574 (1964); **87**, 560 (1965).

[34] D. M. Grant and W. M. Lichtman, *ibid.*, **87**, 3994 (1965).

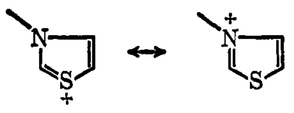
[35] E. M. Kosower, "An Introduction to Physical Organic Chemistry," John Wiley & Sons, Inc., New York, N. Y., 1968, Chapters 1–2.

to be quite reasonable. However, J(C–H) values will only indicate the expected acidity in the absence of such perturbations as might be expected in the transition state for the proton exchange studied here (eq 7). That is, the J(C–H) values in Table I are only useful in indicating the potential acidity of the ground states (the J(C–H) clearly are due only to the nature of the C–H bond in the ground state).[38]

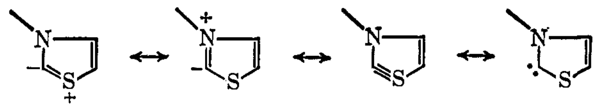
We are mainly concerned with the C–H bond at the 2 position in the three azolium ions whose rates of exchange have been studied. The $J(^{13}$C–2H) value for $\mathbf{1}^+$, 246 cps, is very high—nearly the same as for an acetylenic C–H bond.[35] Consequently oxazolium ions should exchange rapidly as observed.[35] The $J(^{13}$C–2H) values for $\mathbf{3}^+$ and $\mathbf{2}^+$ are similar and considerably lower than for $\mathbf{1}^+$. This indicates that *in the ground state, the 2-hydrogens of structurally similar imidazolium and thiazolium ions have similar potential acidity.* In fact, on this basis, one would predict a slightly greater exchange rate for the imidazolium ion.

This conclusion is reinforced by the other J(C–H) values in Table I. There is similarity in the C–H bonds of all similar thiazoles and imidazoles, but the C–H bonds of oxazoles appear considerably more acidic. Although electronegativities are only approximate, they are consistent with the prediction, based on ground-state properties, that thiazolium ions should be no more acidic, perhaps less acidic, than imidazolium ions.

In fact, $\mathbf{2}^+$ exchanges 3000 times faster than $\mathbf{3}^+$. Therefore, the thiazolium ylide probably has considerably greater stability than the imidazolium ylide. It is also significant that $\mathbf{2}^+$ only exchanges 10^2 times slower than $\mathbf{1}^+$ despite the much larger $J(^{13}$C–2H) in $\mathbf{1}^+$. An explanation of the greater stability of thiazolium ylides is d–σ overlap—stabilization through interaction of a d orbital (or a mixed orbital with considerable d character) at sulfur with the σ orbital directed away from the ring at the 2-carbon. Since **4** is probably as important, perhaps more important, than **5** in the structure of thiazolium ions, **6** might be expected to contribute significantly to the structure of the thiazolium ylide—the partial positive charge at S would cause orbital contraction so that d orbitals might be suitable for bonding with the neighboring carbon.[8,39]



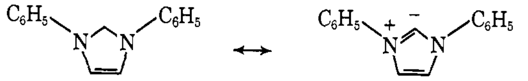
4 **5**



6 **7** **8** **9**

Although this is one effect that might explain the role of sulfur, there are certainly others. A very simple alternative is that the filled π orbitals of imidazolium ylide are of higher energy relative to the thiazolium ylide because sulfur can better adjust to changes in structure of the atoms in the π system.[40] The partial negative charge at the 2-carbon in the imidazolium ylide might lead to poor overlap with p orbitals of the neighboring, partially positively charged nitrogens. In this regard, it may be significant that our recent study[3] of the basicity of azoles revealed the following pK's for the conjugate acids of the neutral azoles: **3**-H$^+$, pK_{BH^+} = 7.5; **2**-H$^+$, pK_{BH^+} = 3.1; **1**-H$^+$, pK_{BH^+} = 1.1. The acidities of **3**-H$^+$, **2**-H$^+$, and **1**-H$^+$ are therefore in the ratio $1:10^{4.4}:10^{6.4}$. This order is not terribly different from that found for the relative rates of proton exchange at the 2 position. In both cases $AH^+ \rightleftarrows A + H^+$ has been studied. The similarity of the results may indicate that $\Delta E_\pi(AH^+ - A)$ may be considerably smaller in the thiazolium case than in the imidazolium case.

It was been suggested[6,26] that the carbene resonance form **8** is important in the structure of azolium ylides. From the viewpoint of valence-bond theory, this is at least questionable, for **8** has an electron-deficient carbon and therefore might be of considerably higher energy than the other contributors (**6**, **7**, and **9**. The reactions of **10** which have been explained by the carbene structure[41] can be explained as well by the ylide structure. From the viewpoint of molecular orbital theory, the 2-carbon is part of an aromatic π system. Although the density of π electrons will be low at C-2 due to the presence of a free pair of σ electrons and the adjacent heteroatoms, there will certainly be a net partial negative charge at C-2.



10

These data make it clear that ylide formation, essential for thiamine action, proceeds much faster in thiazolium ions that in imidazolium ions. Biological selection at the biochemical level has apparently been exercised at this point.[42] Oxazolium ions seem to be unsuited for the role which thiamine plays because of their instability.

Acknowledgment. We thank Jean P. McNeal for carrying out the rate measurements on the thiazolium ion in acetate buffers.

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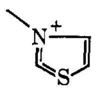
(36) P. Haake, D. A. Tyssee, and W. B. Miller, *J. Am. Chem. Soc.*, **86**, 3577 (1964), and unpublished data. Also, it does appear, despite misgivings,[33,34] that J(C–H) values are a function of per cent s character —see especially G. A. Olah and M B. Comisarow, *ibid.*, 88, 1818 (1966), and R. A. Alden, J. Kraut, T. G. Traylor, *ibid.*, 90, 74 (1968).

(37) A. W. Douglas, *J. Chem. Phys.*, **45**, 3465 (1966); D. A. Tyssee, Ph.D. Thesis, University of California at Los Angeles, Los Angeles, Calif., 1967.

(38) We emphasize that J(C–H) values only provide information about the nature of C–H bonds in the ground state and that it is not necessary to correlate them with per cent s character although this correlation does appear valid. Olofson (ref 9b, footnote 8) provides additional data supporting our view; 1,2,3-thiadiazole is reported to exchange at the 5 position but not at the 4 position although the J-(C–H) values are almost equal at the two positions. That is, the 4-H (α to sulfur) appears to exchange faster due to stabilization of the transition state—not due to any difference in C–H bonds in the ground state.

(39) D. P. Craig, A. Maccoll, R. S. Nyholm, L. E. Orgel, and L. E. Sutton, *J. Chem. Soc.*, 332 (1954). For this reason,



seems unlikely to be a significant contributor.

(40) The stability of aromatic systems in which S contributes an electron pair certainly supports the strength of S–C π bonding in aromatic rings.

(41) H. W. Wanzlick and E. Schikora, *Angew. Chem.*, **72**, 494 (1960).

(42) The presence of imidazole rings in other biologically important materials may indicate a simpler evolutionary development of thiamine with an imidazolium ring.