nucleosides also hydrolyze with formation of a Schiff-base intermediate,[33-35] but this view has been criticized.[2,6,36,37] These reactions may proceed with C–N bond breaking and formation of an oxocarbonium ion intermediate.[32] Stabilization of a developing carbonium ion by nitrogen in nucleoside hydrolysis would be appreciably reduced in comparison with that of glycosylamine[2-4] and oxazolidine hydrolysis, and this could be a critical feature with respect to mechanism. The ease of protonation and leaving-group ability of nitrogen in combination with the ability of oxygen to stabilize a carbonium ion could then lead to rate-determining C–N bond breaking. However, it must be recognized that a reaction involving C–O bond breaking would be reversible as in the hydrolysis of I, and in view of the instability of a Schiff-base intermediate derived from a nucleoside, only low steady-state concentrations would be expected. Thus, the hydrolysis reaction might proceed via an undetectable Schiff base. Even in the case of the highly stabilized iminium ion derived from I, reversibility effects play a significant kinetic role at pH >8. It has recently been reported[7] that both anomerization and furanose → pyranose isomerization occur in the hydrolysis of thymidine and deoxyuridine in 2 M $HClO_4$, thereby removing an objection[2] to C–O bond breaking in the hydrolysis of pyrimidine nucleosides. There is, however, no evidence for anomerization in the hydrolysis of purine nucleosides. Regardless of whether C–O or C–N bond breaking is the predominant pathway in nucleoside hydrolysis, it is clear that an A-1 mechanism, in contrast with a mechanism involving general-acid catalysis in hydrolysis of the oxazolidine I, is due to the great difference in ease of stabilization of a developing carbonium ion with these compounds.

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Conclusions

In summary, the following conclusions may be drawn from the present work. (1) Ring opening of 2-substituted-1,3-oxazolidines proceeds by C–O bond breaking to give a cationic Schiff base at pH <10, even when there is a phenyl group substituent on nitrogen, and the substituent at the 2-position can significantly stabilize the developing carbonium ion. (2) General-acid catalysis occurs in a concerted process in oxazolidine ring opening (α = 0.5), which is due to the great ease of C–O bond breaking brought about by the highly stabilized carbonium ion in the transition state. (3) Ring opening of the oxazolidine neutral species at pH >5 involves pH-independent C–O bond breaking. (4) Rapid reclosure of the oxazolidine ring occurs at high pH by attack of alkoxide ion on the iminium ion, resulting in only a low steady-state concentration of Schiff base. (5) Apparent hydroxide ion catalyzed hydrolysis of cationic Schiff bases does not occur at high pH when there is an ionizable neighboring group whose attack will reclose the ring.

Acknowledgment. This work was supported by a research grant from the National Institutes of Health.

Registry No. I, 73178-24-2; *p*-(dimethylamino)cinnamaldehyde, 6203-18-5; *N*-phenylethanolamine, 122-98-5.

Hydrolysis of the Thiazolium Ion Ring of 1′-Methylthiaminium Ion. Rate and Equilibrium Constants

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Received December 11, 1979

The reversible hydrolytic cleavage of the thiazolium ion ring of 1′-methylthiaminium ion was studied by a pH-stat method in the forward (ring opening) and reverse (ring closing) directions. At 25.1 °C and 0.2 ionic strength, the observed pseudo-first-order rate constant, k_{obsd}, is given by $117[OH^-] + 1.55 \times 10^5[H^+]$ s^{-1}. The equilibrium is described by the expression $K_a^2 = [H^+]^2$[ring-opened compound]/[thiazolium ion], where $pK_a = 8.56$ at the same temperature and ionic strength used in the kinetic studies. It is estimated that the tetrahedral intermediate which reverts to hydroxide and thiazolium ions can have a half-life no greater than about 20 s.

Hydrolytic cleavage of the thiazolium ion ring of thiamin (vitamin B_1) is part of the characterisitic chemistry of thiamin and was known to the earliest investigators in the field.[2] The ring-opened hydrolysis product has intrinsic interest because it passes through biological membranes more easily than thiamin itself.[3]

In view of the prominent position hydrolytic ring cleavage occupies in the chemistry and biochemistry of thiamin, it is understandable that several groups have studied this process not only for thiamin but also for structurally simpler thiazolium ions.[4-13] All these studies

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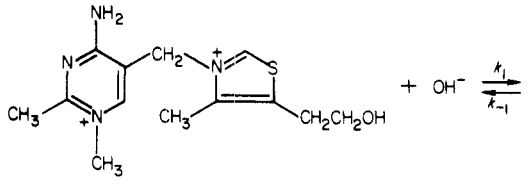
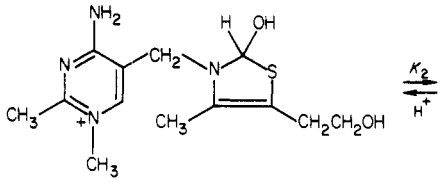
are limited. No investigation has yet produced values for all the significant rate and equilibrium constants involved in the hydrolysis reaction.

We report results for the hydrolytic cleavage of a thiamin derivative, 1'-methylthiaminium ion[14] ($NMeB_1$ or 1), eq 1. Our study is the most complete to date on a thiazolium ion. It provides several important experimentally determined rate and equilibirum constants along with the best estimates of values for constants not easily accessible to experimental verification.



1



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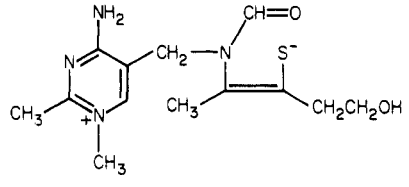
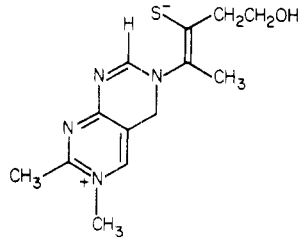


3 (1)

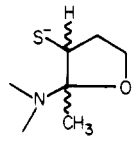
Results

Product Studies. Thiamin reacts with alkali to give several different products.[4,11,12] One study which reassigns some of these product structures is controversial.[15] The following is based on the assumption that $NMeB_1$ will give products similar to those formed from thiamin.

Reaction of $NMeB_1$ with 2 equiv of alkali may produce not only formamide product 3, resulting from the addition of hydroxide ion to the thiazolium ion ring, but also amidine product 4 due to intramolecular addition of the amino group to the thiazolium ion. The precursor of 3 is tetrahedral intermediate 2. The tricyclic precursor of 4[12] produced in a reaction with 1 equiv of base is not shown.



4



5

In the case of both 3 and 4, an isomer containing a tetrahydrofuran ring (5) may form by the intramolecular addition of the β-hydroxyethyl side chain to the double bond of the enamine. Of course, *E* and *Z* isomers reflecting restricted rotation about the amide and ene groups of 3 and 4 are possible along with cis–trans isomers associated with the tetrahydrofuran ring of 5. Structure 3 with the *Z* configuration about the double bond is expected to be formed directly by a hydrolytic ring-cleavage reaction.

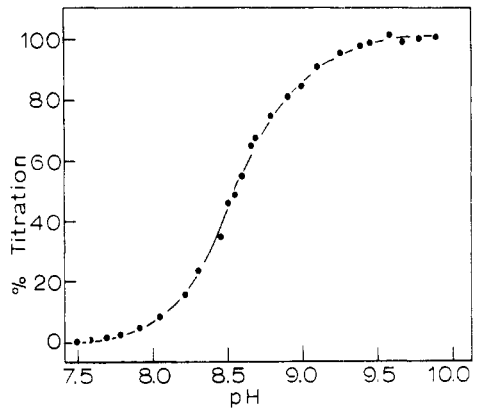


Figure 1. Titration curve for $NMeB_1$ at 25.1 °C and 0.2 ionic strength. Points are experimental while the line is a calculated one using $pK_a = 8.56$ and eq 2.

When 2 equiv of alkali is added quickly to an aqueous solution of 1 at room temperature and the NMR spectrum is recorded, the proton spectrum is that of a single major product (see Experimental Section for chemical shifts). Acidification regenerates the spectrum of $NMeB_1$. Hence, the hydrolysis reaction is clean, rapid, and reversible.

Addition of dimethyl sulfate to an alkaline solution prepared by the addition of 2 equiv of OH^- rapidly traps ring-opened material and leads to the formation of a precipitate of a SMe derivative.[16] This precipitate is quite stable. A solution of it in Me_2SO–H_2O may be heated at 100 °C for 1 h and no change in its NMR spectrum is observed.

Elemental analysis and ultraviolet absorption and NMR spectra quite conclusively show that the SMe material is a derivative of 3 or its *E* isomer and not 4 or 5. The UV absorption spectrum of the SMe product lacks the long wavelength absorption expected for the amidine form of thiamin.[4] The SMe product in Me_2SO-d_6 shows NH_2 and OH signals, clearly eliminating 4 and 5.

Finally, because the UV and NMR spectra of the SMe compound and its precursor ion are so similar, the two must have the same basic structure. The hydrolysis product of $NMeB_1$ produced by a reaction with 2 equiv of alkali must be the *E* or *Z* form of 3.

Equilibria. Figure 1 shows the percentage of ring-cleaved product 3 which is formed from 1 as a function of pH at 25.1 °C and 0.2 ionic strength. In calculations of this percentage, the presence of intermediate 2 was neglected. A similar assumption has been made for the titration data associated with thiamin[4,5] and other organic substrates reacting with 2 equiv of titrant when the second step is more favorable to products than the first.[17]

A titration curve was fitted to the experimental values by using the equilibrium expression given by eq 2, where

$$K_a^2 = \frac{[H^+]^2[3]}{[1]} \text{ or } K^2 = \frac{[3]}{[OH^-]^2[1]} \qquad (2)$$

$K_a^2 = K^2K_w^2$ and K_w is the ion product of water. Note the

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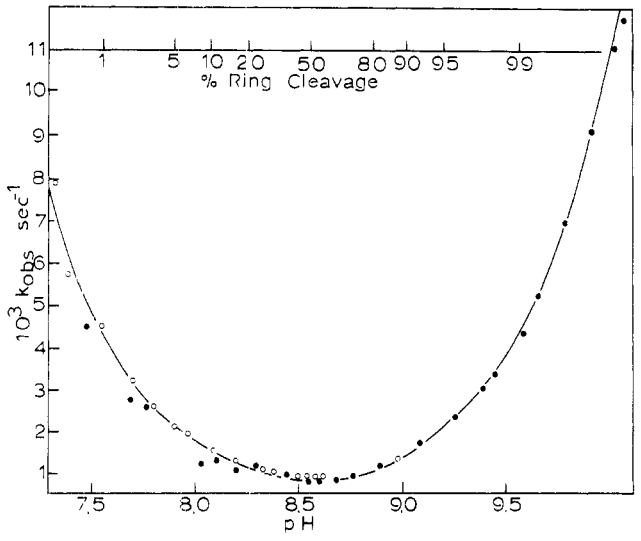

Figure 2. pH-rate constant profile for the hydrolysis of the thiazolium ion ring of $NMeB_1$ at 25.1 °C and 0.2 ionic strength. Filled circles result from studies of the ring-opening reaction using KOH titrant with a pH-stat while open circles reflect the reverse, ring-closure reaction using an acidic titrant. The line is calculated by using eq 4 and rate constants $k_1 = 117\ M^{-1}\ s^{-1}$ and $k_{-1}/K_2 = 1.55 \times 10^{-9}\ M\ s^{-1}$. The horizontal line gives the percentage of ring-opened product **3** at various pH values.

traditional use of the square of the equilibrium constant.[17] The calculated solid line in Figure 1 shows the excellent fit obtained when $K_a = 10^{-8.56}$ or 2.75×10^{-9} M.

Because the conversion of **1** to **3** includes two steps, an attempt was made to estimate the values of the two equilibrium constants. A linear equation has been derived for reactions involving two stages of ionization.[18] It has only two variables, the measured hydrogen ion concentration and the fractional amount of substrate titrated. Equilibrium constants for both reactions may be obtained from the slope and intercept values for this equation. Naturally, the sensitivity of the equation to this dissection into equilibrium constants decreases as the difference between the two values increases, i.e., as the relative amount of intermediate decreases. Unfortunately, this mathematical approach did not work for our data on $NMeB_1$. It would appear that the second equilibrium constant is so much larger than the first that the concentration of intermediate **2** must be small relative to **1** and **3** under our conditions. We estimate from this examination of our titration data and simulated data based on pairs of equilibrium constants having progressively larger differences that the second step in the reaction of **1** must have an equilibrium constant at least 100 times larger than the first. Unfortunately, no such estimate has yet been published for thiamin to provide a comparison.

Kinetics. The reaction of $NMeB_1$ to give **3** (eq 1) is reversible. The rate of formation of **3** from **1** at 25.1 °C and 0.2 ionic strength was observed by using a pH-stat. Under the same conditions, the conversion of **3** back to **1** was followed by the same technique. The forward reaction requires the consumption of hydroxide ion in order to maintain constant pH while the reverse needs protons. Under these conditions, the kinetics are those of a reversible reaction pseudo-first-order in both directions. The observed rate constant, k_{obsd}, has a minimum at about pH 8.6 and then increases as the pH is lowered or raised (eq 3). Experimental values are given in Figure 2.

$$k_{obsd} = k_f[OH^-] + k_r[H^+] \quad (3)$$

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An initial estimate of k_f, the apparent rate constant for ring cleavage, may be obtained by considering the data at the highest pH values where ring opening is essentially complete and k_r, the rate constant for ring closure, is insignificant. It is convenient to rearrange eq 3 as follows to obtain k_f and k_r. A plot of $k_{obsd} - k_f[OH^-]$ vs. $[H^+]$ provides k_r as the slope while a plot of $k_{obsd} - k_r[H^+]$ vs. $[OH^-]$ gives k_f. The quantities subtracted from k_{obsd} are small when data are taken from one side of the rate minimum where ring opening or closure is the major reaction. Neither plot has a significant intercept, suggesting that a "water" reaction is unimportant.

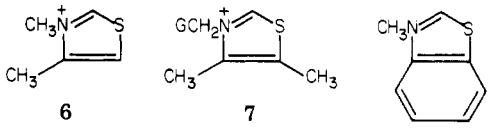
The solid line in the rate constant–pH profile given in Figure 2 is a calculated one, using eq 3 and $k_f = 117\ M^{-1}\ s^{-1}$ and $k_r = 1.55 \times 10^5\ M^{-1}\ s^{-1}$. Except for a few points involving the forward reaction, where 1–15% ring opening was measured in the presence of a slow side reaction (see Experimental Section), the fit of the curve to the data is excellent.

The rate constants may be converted to equilibrium constant K_a, eq 2. Thus $(k_f K_w/k_r)^{0.5} = K_a$ or 2.75×10^{-9} M which is exactly the same value obtained by titration. This good agreement provides strong support for our data and the way in which it was obtained.

Discussion

Equilibrium Constant. Our equilibrium constant for the hydrolytic cleavage of the thiazolium ion ring of $NMeB_1$ may be compared with other values. For thiamin at 25 °C, pK_a's of 9.0,[2] 9.23,[4] and 9.33[10] have been reported, all for solutions of very low ionic strength.

Simple thiazolium ions show similar values. Thus, dimethylthiazolium ion **6** has values of 9.4[7] (30 °C?) and 9.5[6]



6 **7** **8**

G = H, C_6H_5

(25 °C, 0.5 ionic strength), while trisubstituted ions **7** have larger values of 10.3 (20 °C).[4] Our value of 8.56 for $NMeB_1$ which is lower than any of these presumably largely reflects the electron-withdrawing effect of the positively charged pyrimidine ring. The electron-withdrawing group preferentially destabilizes the aromatic ring with its positive charge. As expected,[19] a benzothiazolium ion has a still lower pK_a value, 6.35 being reported for 3-methylbenzothiazolium ion **8**.[20]

Mechanism. Several groups[6,7] have stated that the rate-limiting step in the hydrolysis of thiazolium ions is the addition of hydroxide ion to the ring to give a tetrahedral intermediate such as **2**. In the reverse direction, the rate-limiting step is expulsion of hydroxide ion to reform the aromatic ring.

We agree with this conclusion and in support of it point out that there is at least one low-energy pathway to **2** from **3**. This implies that the formation of **2** in the direction of ring closure is not rate limiting and that another step, such as hydroxide ion expulsion, must be rate limiting.

A pathway which could rapidly give **2** from **3** involves the intramolecular addition of the thiolate ion to the amide carbonyl group. The resultant cyclic oxide ion then reacts with hydronium ion to give **2**. The proton-transfer step is in the thermodynamically favored direction and is likely

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to have a rate constant approaching that for a diffusion-controlled process, 10^{10} M^{-1} s^{-1}. The magnitude of the equilibrium constant involving the intramolecular-addition step is of critical importance if this pathway is to be a low-energy one. Thus, the oxide ion form of **2** would have to be disfavored over **3** by a factor of about 10^{-5}, or $k_r/10^{10}$, in order for ring closing to be rate limiting in our case. The NMR spectra show that amide indeed is favored over oxide ion, as evidenced by the similar proton and carbon chemical shifts for the formamide group in thiolate ion and its SMe derivative where ring opening must be complete. But a comparison with a literature value makes it seem unlikely that the oxide ion of **2** is disfavored to such a large extent. Thus, the intermolecular addition of $HOCH_2CH_2S^-$ to the amide *N*-acetylimidazole has an estimated equilibrium constant of 10^{-4} M^{-1}.[21] Our reaction involving **3** and the conjugate base of **2** is likely to have a more favorable value because the reaction is intramolecular.[22,23]

It is possible to associate observed rate constants with the mechanism and to rewrite the kinetic expression (eq 4). The first and rate-limiting step has rate constants k_1

$$k_{obsd} = k_1[OH^-] + \frac{k_{-1}}{1 + K_2[OH^-]} = k_1[OH^-] + \frac{k_{-1}[H^+]}{[H^+] + K_2K_w} \quad (4)$$

and k_{-1} for the forward and reverse reactions, respectively. Because **2** and **3** are in rapid equilibrium and because **2** and not **3** is present in the transition state for the rate-limiting step, the rate expression for the reverse reaction must include a term to indicate the fractional amount of product in reactive form **2**. This fraction is given by $1/(1 + K_2[OH^-])$ or its equivalent $[H^+]/([H^+] + K_2K_w)$ where $K_2 = [3]/[2][OH^-]$.

Attempts were made to obtain estimates of K_2, k_{-1}, and $k_1/k_{-1} = K_1$ as follows. Equation 4 may be rearranged and then written in its inverse form, eq 5, which now is linear.

$$\frac{1}{k_{obsd} - k_1[OH^-]} = \frac{K_2[OH^-]}{k_{-1}} + \frac{1}{k_{-1}} \quad (5)$$

A least-squares treatment of 10 data points according to this equation yields slope $K_2/k_{-1} = 5.83 \times 10^8$ M^{-1} s, intercept $1/k_{-1} = 16.4$ s ($k_{-1} = 6.1 \times 10^{-2}$ s^{-1}), and slope to intercept ratio of 3.6×10^7 for K_2 (correlation coefficient 0.9989). Because we are especially interested in obtaining an estimate of k_{-1}, only those data points at the lowest pH were used, corresponding to not less than 80% ring closure in the reverse reaction. However, our value for k_{-1} must only be an estimate because if we use the Student t-test at the 95% confidence level $1/k_{-1}$ is found to have the value of 16 ± 16 s. This wide range indicates that k_{-1} is not well-defined. But it can be said with 95% confidence that the half-life for the conversion of **2** to **1** cannot be greater than about 20 s. This is the first estimate for such a reaction.

The values of K_1 ($k_1/k_{-1} = 117/0.061 = 1.9 \times 10^3$) and K_2 derived with the aid of k_{-1} also are not well-defined. However, these estimates are consistent as evidenced by the good agreement between the observed value of $K_a = 2.75 \times 10^{-9}$ M produced by titration and the value of $K_a = 2.6 \times 10^{-9}$ M calculated from $(K_1K_wK_2K_w)^{0.5}$. These values suggest that the equilibrium constants for the second step is some 20000 times larger than the first.

One other study dealing with the hydrolysis of $NMeB_1$ has appeared. From a spectrophotometric measurement at a single pH value (pH 9.6, 30 °C, and 0.1 ionic strength) where ring cleavage is complete, a second-order rate constant of 38 M^{-1} s^{-1} was obtained.[9,24] Comparison with our equivalent term, $k_1 = 117$ M^{-1} s^{-1}, shows poor agreement. We have no explanation why the reported value at a higher reaction temperature is so much smaller than our own. But it should be noted too that the same group reports a much lower second-order rate constant than that for the hydrolysis of thiamin.[4]

In a pioneering study on thiamin (19.2 °C and 0.2 ionic strength) only k_1 was derived. The value 12 M^{-1} s^{-1} [4] is considerably smaller than that for $NMeB_1$. Another study involving thiamin, where both the forward and reverse reactions were examined, did not employ a kinetic analysis such as that given by eq 4, making comparisons of data difficult.[8]

An extensive study of the ring-opening reaction of **6**[7] (30 °C?) provides $k_1 = 23$ M^{-1} s^{-1} and $k_1/K_2 = 1.7 \times 10^{-8}$ M s^{-1}. The former is 5.1 times smaller than that for $NMeB_1$ while the latter is 10 times larger than the corresponding value for $NMeB_1$. The individual values for k_1, k_{-1}, K_1, and K_2 given for ion **6** must be regarded as more uncertain than our own, because they are based on results obtained over a more limited range.

Another study of ion **6**[6] (25 °C, 0.5 ionic strength) gives k_1 as 3.0 M^{-1} s^{-1}. Thus, $NMeB_1$ is attacked by hydroxide ion faster than thiamin and other thiazolium ions, reflecting the electron-withdrawing inductive effect of the positively charged pyrimidine ring.

The majority of past investigations have concentrated on the ring-opening hydrolysis reaction of thiazolium ions. The future, however, lies in studying the reverse, ring-closing pathway. By use of rapid reaction techniques, it seems likely that it will be possible to determine directly the remaining rate and equilibrium constants we have been able only to estimate. Only then will a reasonably complete understanding of this old problem emerge.

Experimental Section

Apparatus. Titrations were carried out with a Radiometer titration assembly consisting of a PHM 64 digital pH meter, a TTT60 titrator, REA 160 titrigraph module, and an ABU autoburette with a 2.5-mL assembly. Through the jacketed reaction vessel was circulated thermostated water, the temperature (25.1 ± 0.1 °C) being adjusted with a certified NBS thermometer. A Radiometer GK 2321C combined electrode was employed.

NMR spectra were recorded on either a Varian A-60A or a JEOL FX-100 spectrometer, using either $Me_3Si(CH_2)_3SO_3Na$ (DSS) or Me_4Si as internal standard. Atlantic Microlab performed the analyses.

***N*-[(1,2-Dimethyl-4-amino-5-pyrimidinium)methyl]-*N*-[4-hydroxy-1-methyl-2-(methylthio)-1-butenyl]formamide Perchlorate.** To a suspension of 4.79 g (0.010 mol) of 1'-methylthiaminium diperchlorate[14] in 5 mL of nitrogen-saturated water was quickly added with stirring 10.5 mL of 2 M NaOH. Shortly after the disappearance of the deep yellow color, 1.00 mL (1.06 equiv) of dimethyl sulfate was added. The pale yellow color disappeared quickly and after about 10 min a colorless precipitate began to form. The filtered material was washed with cold water and gave 3.2 g (78%) of product, mp 161–162 °C. Examination of the mother liquor showed that more product was present, but no attempt was made at additional recovery. Recrystallization from water afforded the analytical sample, mp 164–165 °C. Anal. Calcd for $C_{14}H_{23}N_4ClO_4S$: C, 40.93; H, 5.64; N, 13.64. Found: C, 40.87; H, 5.64; N, 13.63. The ultraviolet spectrum of this SMe

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(24) This value was calculated from the first-order constant by using $pK_w = 14.0$.

compound in H_2O has an absorption maximum at 251 nm (log ϵ 4.17): ^{13}C NMR (Me_2SO-d_6, Me_4Si) δ 14.4, 17.9 (SCH_3, CCH_3), 21.4 (2'-CH_3), 33.5, 37.7 (CCH_2, NCH_2), 41.6 (NCH_3), 59.1 (OCH_2), 111.2 (5'-C), 131.6, 133.4 (alkene), 147.2 (6'-C), 162.0, 162.4 (2'-C, 4'-C), 164.2 (CHO); ^1H NMR (Me_2SO-d_6, Me_4Si) δ 1.99, 2.03 (SCH_3, CCH_3), 2.52 (CCH_2, J = 6.7 Hz), 2.58 (2'-CH_3), 3.51 (CH_2O, J = 6.7, 5.7 Hz), 3.77 (NCH_3), 4.40 (NCH_2), 4.68 (OH, J = 5.7 Hz), 7.98 (6'-H), 8.18 (CHO and NH), 9.17 (NH). With the exception of 6'-H the CH chemical shifts are nearly the same as those for the S-methyl derivative of thiamin in water having a cleaved thiazolium ring.[25] Very small signals are also found at δ 1.75 and 8.24; they were not assigned. An isomeric iodide which has a dimethyl sulfonium ion structure has been reported.[26]

NMR Analysis of Reaction Mixtures. To 100 mg (0.284 mmol) of 1'-methylthiaminium dichloride[14] in 0.5 mL of nitrogen-saturated water was quickly added NaOH to raise the pH to about 10. The proton NMR spectrum indicated that starting material had been changed to a single major product: δ (DSS) 1.88 (CH_3), 2.63 (2'-CH_3), 2.65 (CCH_2), 3.78 (CH_2O), 3.82 (NCH_3), 4.58 (NCH_2), 8.06, 8.18 (CHO and 6'-H). Raising the pH to about 12 leads to small additional changes especially of the two signals at lowest field which now coalesce. The spectrum of aromatic starting material was regenerated when the solution was acidified to pH 3 with HCl. These observations show that the thiazolium ion ring is rapidly and reversibly cleaved to a single major product. Thiamin in alkaline solution shows similar chemical shifts.[27]

However, when a solution of $NMeB_1$ slowly is made alkaline, small new signals appear along with those described above. The signals at δ 2.37 and 8.79 lead us to believe that some free thiazole is being produced. Consequently, in kinetic studies in which ring-opened material was generated and then closed by the addition of acid, care was always taken to add alkali in a few seconds to minimize this complication.

Kinetic Measurements. All reactions were carried out under nitrogen at 25.1 ± 0.1 °C in glass-distilled water at a constant ionic strength of 0.2 with KCl as the added electrolyte. Acidic (0.0750 M HCl) and basic (0.0603 M KOH) titrant were at the same ionic strength. Several different stock solutions of substrate were used to give reaction solutions ranging from 0.90 to 40 × 10^{-3} M; 20-mL aliquots were titrated. The pH meter was adjusted with phosphate and borate buffers according to Bates.[28] The ion product of water (pK_w) is 14.000 at 25 °C, 14.169 at 20 °C, and 13.837 at 30 °C.[29] No correction was applied to these values to reflect the influence of ionic strength. But the correction at 25 °C and 0.2 ionic strength is small, being given by log $[OH^-]$ = pH + log K_w − log f, where f = 0.72.[30]

When the ring-opening reaction was studied, the pH of the reaction mixture was quickly increased to the desired value by using alkaline titrant. The volume required for this purpose was recorded and the kinetic run commenced. Titrations were run to completion, i.e., at least 10 half-lives. Plots were constructed by using ln (V_∞ − V) vs. t, where V_∞ is the volume of titrant at "infinite" time and V the volume at any other time. These pseudo-first-order plots generally were linear over 5 half-lives. The highest concentrations of substrate were used at the lowest pH values in order to increase the amount of alkali consumed.

Difficulties were encountered while studying ring opening at pH values below about 8.5 where cleavage proceeded to a small extent. A constant infinity value was not observed. Instead, titrant was consumed at what appeard to be a constant (zero order) rate. This side reaction was followed for about the same length of time as that for the main reaction; this linear portion was then back extrapolated to the start of the reaction. Volume changes were calculated relative to the extrapolated "infinity" line. In other words, it is assumed that the side reaction takes place at the same rate throughout the course of the ring-opening process. First-order plots constructed in this way were linear over 3 half-lives. However, the magnitude of the rate constant obtained from such a plot was very sensitive to the slope of the "infinity" line, varying in some cases by as much as 20% when the slope of this line was reestimated.

The extent of the side reaction may be estimated as follows. After an additional period corresponding to 7 half-lives for the main reaction to reach equilibrium, the side reaction required the consumption of about 50% more titrant at pH 7.5 where there is only 1% ring opening. From the initial rate and an assumed 1 to 1 stoichiometry, the half-life of the competing reaction is estimated to be about 24 h while that for the main reaction is 2.5 min. The relative importance of the unidentified process steadily decreases as the pH is raised, requiring essentially no additonal titrant at pH 8.7.

When ring closure was studied, 2 mL of a 4.00 × 10^{-2} M stock solution of substrate was added quickly to 20 mL of aqueous KCl solution containing 2 equiv of KOH. The pH now was about 10.5; about 20 min was allowed to elapse to enable the ring-opening reaction to reach completion. The pH now was about 9.4. Acid titrant then was added to maintain the desired pH. Again pseudo-first-order plots were linear for at least 5 half-lives. No complications were observed.

Equilibria. The percentage of 1'-methylthiaminium diperchlorate converted to ring-opened product in titrations with alkali was calculated as follows: the V_∞ value first was corrected for the amount of alkali required to raise the pH of the substrate stock solution to the desired pH without any ring opening taking place. The volume to be applied as a correction was estimated from an aqueous blank. This blank consisted of a 20-mL aliquot of 0.2 M KCl free of substrate which was titrated with the standardized KOH solution to the desired pH. The correction from the blank (V_{cor}) never exceeded 5% of the total volume of alkali consumed in a ring-opening kinetic run. The percentage was then calculated with the aid of eq 6, using the factor 0.5 because 2 equiv of base are needed to open the thiazolium ion ring.

$$\% \text{ ring opening} = \frac{(V_\infty - V_{cor})M_{KOH} \times 0.5 \times 100}{\text{mmol of } NMeB_1} \quad (6)$$

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