[CONTRIBUTION FROM COBB CHEMICAL LABORATORY, UNIVERSITY OF VIRGINIA, CHARLOTTESVILLE, VA.]

Hydrolysis of 2-Substituted Δ^2-Thiazolines

BY R. BRUCE MARTIN AND ALICE PARCELL

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Rate constants for hydrolysis and subsequent partition of the tetrahedral intermediates to yield the N- and S-acetyl derivatives are reported for several thiazoline compounds. Partitioning is independent of ring size, but is sensitive to substituents. All the thiazoline compounds as well as a 6-membered analog exhibit inhibition of hydrolysis in acidic solutions. Only in the case of bacitracin may the inhibition be ascribed to a decrease in the activity of water. For the thiazoline ring of bacitracin, $pK_a \simeq -1.8$. The six-membered analog of thiazoline is thermodynamically less stable but hydrates 17 times less rapidly than thiazoline. Formation of a 5-membered cyclic intermediate from the acetyl derivatives is 70 times more rapid than formation of the corresponding 6-membered ring. Results are also reported for the hydrolysis of 2-methylimidazoline.

The bell-shaped plot with a maximum at about pH 3 when the rate of hydrolysis is plotted against pH for 2-methyl-Δ^2-thiazoline has been described and a mechanism proposed to account for the results.[1] In addition, a relation of the proposed tetrahedral carbon intermediate to S–N transfer reactions has been formulated.[1] This latter subject is treated more extensively in a subsequent paper.[2] In this paper the hydrolysis of 2-methylthiazoline is given further study and the results of similar work on 2-ethylthiazoline, 2-(1-acetamino-2-methyl-propyl)-thiazoline (AMPT), the antibiotic bacitracin, 2-methylthiazoline-N-methylperchlorate (TMPC), 2-methyl-Δ^2-thiazine and 2-methyl-Δ^2-imidazoline are recorded.

Experimental

The 2-methylthiazoline was purchased from Aldrich Chemical Co., 2-ethylthiazoline synthesized according to Wenker[3]; 2-(1-acetamino-2-methyl-propyl)-thiazoline[4] was a gift from Lyman C. Craig, and bacitracin and zinc bacitracin were purchased from Nutritional Biochemicals Corp. 2-Methylthiazoline-N-methylperchlorate was made from the iodide[5] by dissolving 1 g. of the latter in methanol and adding 0.85 g. of $AgClO_4$ in a methanol solution. AgI was removed by filtration, the methanol permitted to evaporate and the product recrystallized from methanol as white crystals, m.p. 168°. 2-Methyl-Δ^2-thiazine[6] had a boiling point of 173–175°. 2-Methylimadazoline was prepared from N-monoacetylethylenediamine.[7]

The molar extinction coefficients and wave lengths of the maxima of the acidic and basic forms, respectively, in aqueous solution of the compounds not previously reported[1] are as follows: AMPT, ϵ_{267} 4600, ϵ_{249} 2400; bacitracin, ϵ_{278} 4150, ϵ_{250} 2950; TMPC, ϵ_{264} 6400, no basic form; thiazine, ϵ_{247} 4000, ϵ_{234} 2360; and imidazoline, ϵ_{218} 4800, ϵ_{219} 4950. The value for bacitracin in acid solution is considerably lower than the various values reported elsewhere. Titration with standard base indicates a purity of about 87%. The sample used here must be considered impure and the results only of qualitative significance.

All experiments were performed at 25° and 0.10 ionic strength unless otherwise noted. The spectrophotometric measurements were made on a Cary 11 spectrophotometer. Formate, acetate, phosphate, or borate buffers were used at about 10^{-2} M. Ionic strength was controlled with KCl.

Results

Repeating the previous formulation[1] for the hydrolysis of 2-methylthiazoline we have

$$TH^+ \rightleftharpoons T + H^+ \qquad K_1 = (T)(H^+)/(TH^+)$$

$$TH^+ + H_2O \underset{k_2}{\overset{k_1}{\rightleftharpoons}} H^+ + D$$

$$N \underset{k_4}{\overset{k_3}{\rightleftharpoons}} D \underset{k_6}{\overset{k_5}{\rightleftharpoons}} S$$

$$SH^+ \rightleftharpoons S + H^+ \qquad K_2 = (S)(H^+)/(SH^+)$$

where T represents thiazoline, D a hydroxythiazolidine intermediate, and N and S the N and S-acetyl-β-mercaptoethylamines, respectively. Assuming a steady state for D, the initial rate of disappearance of thiazoline is given by

$$-\frac{dC_T}{dt} = \frac{k_1 C_T (H^+)[(k_3 + k_5)/k_2]}{[K_1 + (H^+)][(H^+) + (k_3 + k_5)/k_2]} = k_1' C_T \quad (1)$$

where $C_T = (T) + (TH^+)$. This reaction is conveniently followed by the decrease in absorption in the 250–270 mμ range and k_1' determined by eq. 4 of ref. 1. The midpoints of curves like the dashed one of Fig. 1 are determined by K_1 on the right and $(k_3 + k_5)/k_2$ on the left-hand descent. From the change in absorption at 230 mμ the rate of appearance of the S-acetyl derivative is simultaneously determined by an equation similar to 5 of ref. 1. The apparent rate constant, k_5', for S-acetyl formation when divided by k_1' gives $k_5'/(k_3 + k_5)$, and hence k_3/k_2 and k_5/k_2 may be determined. Results for the compounds studied are given in Table I.

Equilibrium constants may be defined and related to the rate constants in this way

$$K_{ST} = (SH^+)/(TH^+) = k_1 k_5 / k_2 k_6 K_2 \quad (2)$$

$$K_{NS} = (N)(H^-)/(SH^+) = k_3 k_6 K_2 / k_4 k_5 \quad (3)$$

$$K_{NT} = (N)/(T) = k_1 k_3 / K_1 k_2 k_4 \quad (4)$$

The three equilibrium constants are not independent: evaluation of any two determines the third.

Equilibrium analyses were performed by following the absorption of solutions at several pH values until equilibrium was obtained. Absolutely constant readings are not observed due to the slow hydrolysis of the thiol ester in most solutions. Since there are three species present at equilibrium, the parent thiazoline and the N- and S-acetyl derivatives, absorption measurements at three wave lengths are required for characterization of the system. Fortunately thiazolines absorb maximally about 260 mμ, S-acetyl compounds about 230

(1) R. B. Martin, S. Lowey, E. L. Elson and J. T. Edsall, *J. Am. Chem. Soc.*, **81**, 5089 (1959).
(2) R. B. Martin and R. Hedrick, *ibid.*, in press.
(3) H. Wenker, *ibid.*, **57**, 1079 (1935).
(4) W. Stoffel and L. C. Craig, *ibid.*, **83**, 145 (1961).
(5) L. G. S. Brooker, *ibid.*, **58**, 662 (1936).
(6) F. M. Hamer and R. J. Rathbone, *J. Chem. Soc.*, 243 (1943).
(7) A. J. Hill, *J. Am. Chem. Soc.*, **61**, 822 (1939).

TABLE I

RATE AND EQUILIBRIUM CONSTANTS FOR HYDROLYSIS OF THIAZOLINE DERIVATIVES AT 25° AND 0.10 IONIC STRENGTH

	Thiazoline	AMPT	TMPC	Thiazine
pK_1	5.22	3.64	[b]	7.6
k_1, $\times 10^4$ min.$^{-1}$	10.5	5.0	1.7	0.6
$(k_3 + k_5)/k_2$, M	0.11	1.2	0.3	0.2
k_5/k_2, M	0.06	1.1	0.3	0.1
k_3/k_2, M	0.05	0.1	(1×10^{-3})	0.1
k_5/k_3	1.2	11	(250)	1.0
K_{ST}	11	35	0.8	90
K_{NS}, M	4.5×10^{-2}	7×10^{-2}	0.25×10^{-2}	(4.5×10^{-2})
K_{NT}	8.5×10^4	1.1×10^4	[b]	(2×10^8)
$k_6K_2 \times 10^6$ min.$^{-1}M^{-1}$	4.5	16	64	0.07
pK_2	(9.1)	[a]	[a]	[a]
k_6	(6×10^3) min.$^{-1}$	[a]	[a]	[a]
k_4, min.$^{-1}$	1.0×10^{-4}	0.2×10^{-4}	(1×10^{-4})	1.5×10^{-6}
k_7, min.$^{-1}$ M^{-1}	[a]	[a]	13×10^8	0.76×10^3

[a] Not measured. [b] Not defined. Values in parentheses are assumed or derived from an assumed value.

mμ and N-acetyl derivatives far in the ultraviolet. For practical reasons, readings were taken at 202 mμ, which is on the slope of the N-acetyl absorption curve rising to a peak further in the ultraviolet.

Let the fraction of the initial thiazoline that is TH^+ at equilibrium be α, that is T be β, that is SH^+ be γ and the fraction that is N be δ. However, α and β are related by $\beta = K_1\alpha/(H^+)$. The observed extinction in the 260 mμ region is given by

$$\epsilon^6 = \alpha[\epsilon_{TH^+}{}^6 + K_1\epsilon_T{}^6/(H^+)] + \gamma\epsilon_S{}^6 + \delta\epsilon_N{}^6$$

Similar equations may be written for the 230 and 200 mμ regions where the superscript 6 is replaced by 3 and 0, respectively. The three simultaneous equations may then be solved for α, γ and δ, but more directly, their numerator determinants may be divided to yield, $K_{ST} = \gamma/\alpha$ and $K_{NS} = \delta(H^+)/\gamma$. The third equilibrium constant is related to the two just determined by $K_{NT}K_1 = K_{ST}K_{NS}$. The equilibrium constants obtained by this interpretation of the equilibrium absorptions are given in Table I. The extinction coefficients used are listed under the heading for each compound.

2-Methylthiazoline.—The previous results[1] shown by the dashed line of Fig. 1 have been confirmed. A detailed study was made of the k_5'/k_1' ratio as a function of *p*H, especially at *p*H <2, where the back reaction of the thiol ester is insignificant. The ratio reaches a constant value at higher acidities and does not tend to unity. This result means that the N- and S-acetyl derivatives must come from intermediates of the same over-all charge; otherwise the k_5'/k_1' ratio would become unity (or zero) at high acidities. At the higher acidities, thiol ester hydrolysis becomes a complicating factor. However, if an acid dependency did exist, the k_5'/k_1' ratio should vary from near unity to almost zero over a range of only two *p*H units. This behavior is not observed because the ratio is 0.5 at *p*H 0 and still greater than zero at *p*H 4.

In order to evaluate the equilibrium constants, the following extinction coefficients were substituted into the determinant expressions previously described.

mμ	TH^+	SH^+	N
260	5200	80	0
230	830	4250	100
202	0	1000	5400

All the values were determined on preparations of the pure compounds except for the N-acetyl values, which were measured on solutions of thiazoline or S-acetyl which stood for some time at *p*H 4.7. No extinction coefficients for the free base T form of thiazoline need be considered because all equilibrium results were obtained on solutions of *p*H <3. At greater *p*H values the N-acetyl form predominates to such an extent that reliable equilibrium constants cannot be obtained. The

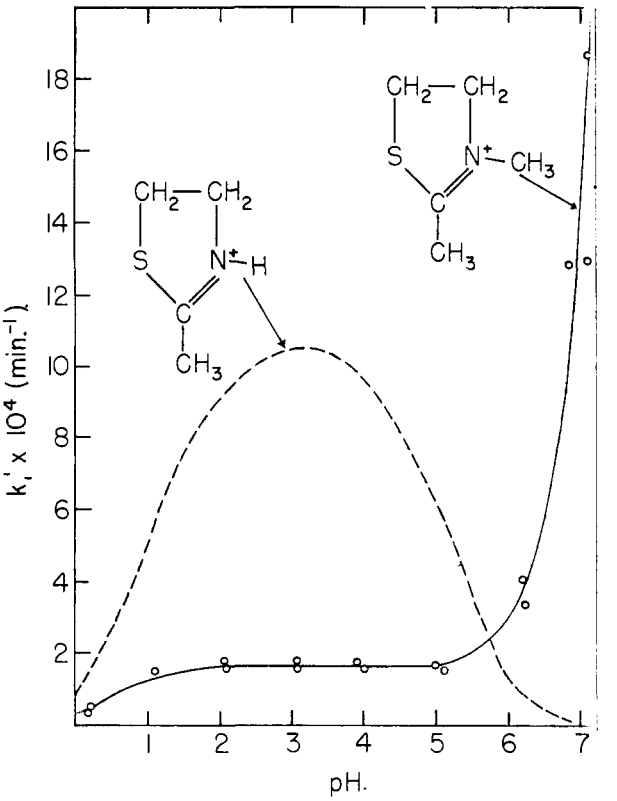

Fig. 1.—Initial rates of hydrolysis at 25° and 0.10 ionic strength. Points are experimental; curves are drawn with equations of text and constants of Table 1. Two additional points off the scale of the graph for thiazoline-N-methylperchlorate are $k_1' = 119$ and 131×10^{-4} min.$^{-1}$ at *p*H 7.95 and 8.00, respectively.

hydrochloride salt of β-mercaptoethylamine, a possible further hydrolysis product, only absorbs appreciably at 202 mμ with ε 950. The equilibrium constants listed in Table I are probably accurate to about ±10%. Identical values were obtained from initial solutions of the S-acetyl derivative, indicating that an equilibrated system is being measured. In addition, an independent method[8] involving the measurement of the final *p*H of partially neutralized solutions gives $K_{NT} + 1 = 7 \times 10^3 (K_{ST} + 1)$, a result consistent with those of Table I. The initial rate of change of *p*H with time[8] also leads to the same k_1 value.

The combination of constants k_6K_2 for S-acetyl disappearance in Table I have been determined directly[2] by use of eq. 5

$$k_6' = \frac{k_6K_2[(H^+) + k_3/k_2]}{(H^+)[(H^+) + (k_3 + k_5)/k_2]} \qquad (5)$$

with the refined rate constants $k_3/(k_3 + k_5)$ of this work. Substitution of the k_6K_2 value with the other rate constants in the equilibrium expression (2) yields $K_{ST} = 14$, a value somewhat different from the purely equilibrium value of 11 in Table I. Nonetheless, the values are sufficiently close to lend confidence to both the equilibrium and kinetic approaches for the evaluation of the equilibrium constants.

It is not possible to measure directly the pK_2 value for the S-acetyl derivative because of the rapid transfer reaction. The value of $pK_2 = 9.1$ in Table I is chosen on the basis of similar measured values for monoacetylethylenediamine (below) and O-acetylethanolamine.[8] Unlike the previous study[1] we have not synthesized the N-acetyl-β-mercaptoethylamine and prefer to infer the k_4-value of Table I from the equilibrium constants. The kinetic method for the evaluation of k_4 suffers from the necessity of working in strong acids with concomitant amide hydrolysis occurring at a comparable rate. The values determined by the two methods, however, are in good agreement.

On the acid side of the maximum of the hydrolysis curve no catalysis is evident; on the basic side some general catalysis is noticed.[2] Experiments performed at 35° and 0.10 ionic strength give $k_1 = 2.5 \times 10^{-1}$, and the same value of $k_5'/k_1' = 0.55$ obtained at 25°. Thus the partitioning of the intermediate is not markedly affected by temperature. Comparison of the 35° and 25° k_1-values yields an activation energy $E_1 = 16$ kcal./mole and a preexponential term $A_1 = 10^{6.9}$ sec.$^{-1}$. At the higher temperature the midpoint of the acid side of the curve yields $(k_3 + k_5)/k_2 = 0.15$.

2-Ethylthiazoline.—The hydrolysis curve as a function of *p*H is so similar to that of 2-methylthiazoline that the values obtained are not separately recorded in Table I. The value of $k_1 = 12.5 \times 10^{-4}$ min.$^{-1}$, and pK_1 and k_5'/k_1' are nearly the same as for 2-methylthiazoline. No equilibrium experiments were performed.

2-(1-Acetamino-2-methylpropyl)-thiazoline (AMPT).—The hydrolysis curve of this compound is of the same shape as that of the dashed curve of Fig. 1, but is half as high and has a maximum at *p*H 1.8. Thus inhibition of hydrolysis in acid solutions is also observed for this thiazoline derivative. The rate and equilibrium constants were determined by the general methods already outlined. The pK_1 value determined from direct titration in a *p*H meter with standard base agreed with the value obtained from spectrophotometric measurements. This K_1-value fits the right-hand descent of the hydrolysis curve according to eq. 1. The values of k_4 and k_6K_2 have not been measured directly, but are calculated by substitution of the already known rate constants into the expressions for the known equilibrium constants. To evaluate the equilibrium constants the following molar extinction coefficients were used

mμ	TH$^+$	T	SH$^+$	N
266	4600	930	100	0
230	1150	2300	4250	400
202	2100	5500	500	9600

The SH$^+$ values were assumed by analogy with other S-acetyl compounds, and the N values were measured after complete decomposition of the parent thiazoline at *p*H 4.7, where the N-acetyl compound is the sole product.

Bacitracin.—This antibiotic of known structure contains a thiazoline ring which does not accept a proton, on the basis of titration evidence,[9] down to *p*H 1.4. We have determined an approximate ionization constant for the thiazoline ring in bacitracin by measuring the change in absorption at 278 mμ in solutions of HCl and H_2SO_4 and relating the concentration of acid to the acidity function.[10] On this basis $pK_a \simeq -1.8$ and $K_a \simeq 60$. Despite probable concomitant protonation elsewhere on the molecule, as at amide bonds, the spectrophotometric method permits separate evaluation of the thiazoline ring ionization constant.

The absorption due to the thiazoline ring in bacitracin disappears at a maximum rate in about 4 *M* acid with an over-all curve not unlike the dashed curve of Fig. 1, but displaced on the abscissa to negative *p*H values. The maximum in the curve occurs at $k_1 \simeq 0.035$ min.$^{-1}$. A sample of zinc bacitracin gave similar results. Rather than accounting for the inhibition of hydrolysis by the proposed mechanism we use an equation of the form

$$k_1' = k_1 a_{H_2O}{}^p \left(\frac{h_0}{h_0 + K}\right) \qquad (6)$$

which fits the data tolerably well. The term in parentheses amounts to the fraction of the thiazoline ring in the protonated form if h_0 is the acidity function and K is the ionization constant of the ring. A satisfactory fit is obtained with $K = 35$ with the power p of the activity of water a_{H_2O} set at 3.0 in H_2SO_4 solutions. Fewer points were obtained in HCl solutions where the most satisfactory $p = 3.5$. Unfortunately, the K-value is not in good agreement with the K_a-value independently determined as described above, but is at least in the same range. If our sample is impure (see Experimental), our results could be in error, and we have not pursued the matter further. The

(8) R. B. Martin and A. Parcell, *J. Am. Chem. Soc.*, **83**, 4835 (1961).

(9) G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **53**, 604 (1953).

(10) M. A. Paul and F. A. Long, *Chem. Revs.*, **57**, 1 (1957).

power of p = 3.0–3.5 is what is expected of water acting as a nucleophile[11] as in the proposed mechanism. In H_2SO_4 solutions k_1 of eq. 6 is about 0.15 min.$^{-1}$.

2-Methylthiazoline-N-methylperchlorate (TMPC).—The initial rates of hydrolysis *versus* pH are shown in Fig. 1 with the curve for 2-methylthiazoline for comparison. Unlike the latter compound, the former cannot exist in the free base form and the rapidly ascending curve in basic solutions, first order in OH^-, is ascribed to

$$TH^+ + OH^- \underset{k_8}{\overset{k_7}{\rightleftharpoons}} D \qquad (7)$$

where for TMPC TH^+ is TCH_3^+. This step is easily incorporated into the mechanism already given to yield

$$\frac{-d(TH^+)}{dt} = \frac{[k_1 + k_7(OH^-)](TH^+)[(k_3 + k_5)/k_2]}{(H^+) + (k_3 + k_5 + k_8)/k_2} \qquad (8)$$

The rate constants in Table I are derived from the data drawn in Fig. 1 assuming $k_8 << k_3 + k_5$.

The equilibrium constants of Table 1 are evaluated with the aid of the following extinction coefficients derived by methods already illustrated.

mμ	TH^+	SH^+	N
264	6400	80	0
232	1100	4250	45
202	0	0	9100

Since only the S-acetyl derivative is obtained at pH <1, k_3/k_2 cannot be determined without some additional information. Therefore, the k_4-value has been assumed from analogy with thiazoline and the k_5/k_3 and k_3/k_2 values computed from the other rate and equilibrium constants.

The broad constant region of the TMPC curve of Fig. 1 confirms that H_2O is attacking the cationic thiazoline molecule. An experiment in this pH-independent region in 80% D_2O gave only half the hydrolysis rate obtained in pure H_2O. In this same region general base catalysis is indicated by increased hydrolysis rates in strongly buffered solutions at constant ionic strength and controlled pH.

2-Methyl-Δ^2-thiazine.—Figure 2 shows the initial rates of hydrolysis *versus* pH for the six-membered analog of 2-methyl-Δ^2-thiazoline. Because of the slower rates the results for thiazine are not as precise as those for thiazoline. Once again acid inhibition of hydrolysis is observed at low pH values. The curve as a whole is more reminiscent of that obtained for TMPC rather than that for thiazoline (solid rather than dashed curve of Fig. 1). However, the curve for thiazine levels off at about pH 10; whereas no leveling was observed for TMPC. This result is consistent with pK_1 = 7.6 for thiazine as determined spectrophotometrically. At pH regions 10 and higher the product $(TH^+)(OH^-)$ is a constant for thiazine. The curve of Fig. 2 may be accounted for by an extension of eq. 1 with the incorporation of reaction 7 to yield

$$\frac{-dC_T}{dt} = \frac{[k_1 + k_7(OH^-)]\ C_T(H^+)[(k_3 + k_5)/k_2]}{[K_1 + (H^+)][(H^+) + (k_3 + k_5 + k_8)/k_2]} \qquad (9)$$

(11) J. F. Bunnett, *J. Am. Chem. Soc.*, **82**, 499 (1960).

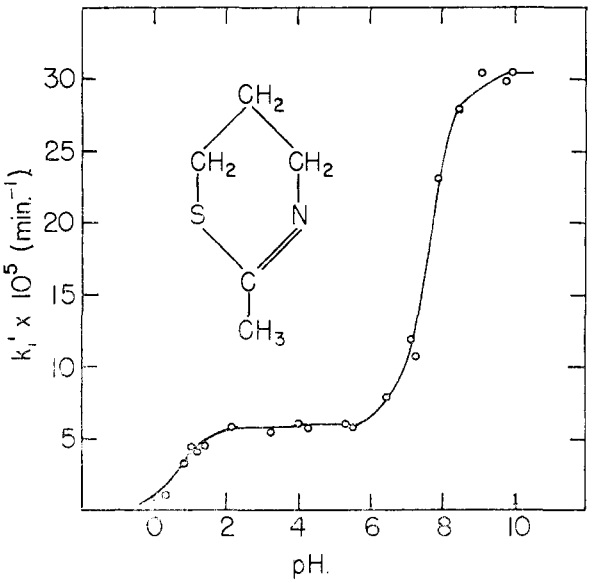

Fig. 2.—Initial rates of hydrolysis *versus* pH for 2-methyl-Δ^2-thiazine. Points are experimental and solid curve corresponds to the constants of Table I substituted into eq. 9.

This equation reduces to eq. 8 in the case where no loss of the positive charge from TH^+ can occur. The constants required to fit eq. 9 are given in Table I. The K_1-value determined independently by spectrophotometry when used in eq. 9 provides a good fit to the curve of Fig. 2 and lends support to the interpretation. Reaction 7 is not significant in the case of thiazoline because pK_1 is 2.4 log units less than for thiazine, and the $(TH^+)(OH^-)$ product is never appreciable in the thiazoline case. For thiazine, $k_5'/k_1' = 0.5$ for $0.3 < p\text{H} < 3$, a larger range than for thiazoline because the back reaction of the S-acetyl product is slower.

No equilibrium measurements were attempted on thiazine because of the sluggishness of the system. Therefore, the constants of Table I not deduced from eq. 9 are derived by less empirical procedures. The rate of acetyl transfer of S-acetyl-γ-mercaptopropylamine is $^1/_{60}$ as fast as that of S-acetyl-β-mercaptoethylamine.[12] Since the constants in eq. 5 are known for thiazoline, substitution of the $^1/_{60}$ factor and the known constants for thiazine permits evaluation of k_6K_2 for the latter compound because (H^+) is much less than all the additive constants in eq. 5 under the conditions of the comparison. The k_6K_2 value so obtained for thiazine is listed in Table I. Sufficient rate constants are now known to evaluate K_{ST} by eq. 2.

The remaining constants of Table I cannot be deduced without more data unless an assumption is made. We assume K_{NS} is the same for thiazoline and thiazine since the equilibrium position for N- and S-acetyl derivatives to transfer should not depend greatly on the length of the chain separating the functional groups. With this assumption K_{NT} and k_4 are quickly evaluated from the constants already known. The ratio of k_4 for the thiazoline and thiazine systems is about the same as the k_6K_2 ratio for the two systems. Since pK_2 is probably similar for both S-acetyl deriva-

(12) T. Wieland and H. Hornig, *Ann.*, **600**, 12 (1956).

tives, the 70-fold greater rate of formation of the 5- over the 6-membered cyclic intermediate from either the N- or S-acetyl direction implies that the assumed K_{NS} value for thiazine is a reasonable one.

2-Methyl-Δ^2-imidazoline.—In a subsequent paper[8] we report the results of the oxygen analog (oxazoline) of thiazoline; here the results obtained on the nitrogen analog are described. For the parent compound $pK_1 = 11.1$, and for the sole product, monoacetylethylenediamine, $pK_2 = 9.05$, as determined by titration of each compound with standard base on the pH meter. Since no hydrolysis of imidazoline is observed until high pH values are obtained, only reaction 7 is significant, and the k_1 step is very slow. Three hydrolysis experiments on imidazoline performed through the ionization region at pH 9.16, 11.85 and 12.74 yield a constant $k_7 = 2.6 \pm 0.1$ min.$^{-1}$ M.$^{-1}$ when eq. 9 is applied with $(k_3 + k_5)/k_2 >> (H^+)$ in this pH range.

Discussion

An extraordinary range of pK$_a$ values is exhibited by thiazoline derivatives. From 5.2 for 2-methylthiazoline to -1.8 for bacitracin is a range of 7 log units. Interestingly, AMPT, prepared as an approach to the bacitracin structure,[4] has an intermediate value of pK_a 3.6. It would be of interest to check pK_a values and hydrolysis rate constants on thiazoline compounds substituted in the 4-position.

All the ring compounds discussed in this paper exhibit inhibition of hydrolysis in acid solution. Only in the case of bacitracin may this inhibition be accounted for by the decrease in activity of water in strongly acid solutions. In all other cases the proposed mechanism can account for the data by suitable selection of constants. Bell-shaped curves are often obtained in kinetic and enzymatic studies and it is standard practice to ascribe their ascending and descending limbs to two different proton transfers on the molecule. An ionization constant is used in this work to account only for the descending limb in the least acidic solutions. The ascending limb is accounted for by a collection of rate constants $(k_3 + k_5)/k_2$ in the mechanism arising from the acid-inhibited k_2 step. The explanation of one or even both limbs of bell-shaped curves in terms of a collection of rate constants may be of more general utility than heretofore realized. Some kind of intermediate seems to be required by the results. The proposed hydroxythiazolidine intermediate can account for the observations.

The initial reaction of the protonated form of the thiazoline compounds with water seems to be required by the plateaus observed for TMPC and thiazine and is consistent with the catalysis[2] and D_2O experiments, the pre-exponential A_1 term for thiazoline, and the bacitracin results in strongly acid solutions. That the independently determined pK_a value for thiazine when substituted into eq. 9 provides so satisfactory a fit to the experimental points of Fig. 2 is strong evidence for the proposed mechanism. In addition, the leveling off of the thiazine results at pH >10 supports the formulation of reaction 7. Evidently OH^- is reacting with TH^+ and not functioning as a basic catalyst in some intermediate step.

Many comparisons, some of which have already been mentioned, may be made by reference to Table I. When the 5- and 6-membered ring analogs, thiazoline and thiazine are compared, the equilibrium constants indicate the greater thermodynamic stability of the 5-membered ring even though it hydrates 17 times faster than its 6-membered homolog. However, the k_5/k_2 and k_3/k_2 ratios and hence the partitioning of the intermediate to the S- and N-acetyl derivatives are unaffected by ring size.

For both TMPC and thiazine, $k_7 \simeq 10^7\ k_1$: reactions of the charged rings with OH^- and H_2O occur in a constant ratio. If this result may be applied to imidazoline with $k_7 = 2.6$ min.$^{-1}$ M.$^{-1}$, then $k_1 \simeq 2.6 \times 10^{-7}$ min.$^{-1}$ (half-life of 5 years). The low value of k_1 accounts for the stability of imidazoline in acid solutions even though our formulation requires the cationic form to be less stable than the free base. The statements in the literature to the effect that the cationic form of imidazoline is more stable than the free base are kinetically incorrect. Due to the low k_1-value the main mode of decomposition is by reaction 7 which becomes appreciable only at high pH values.

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