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Imidazole Catalysis. IX.[1] The Bell-shaped *p*H-Dependence of the Rate of Imidazole Catalysis of δ-Thiovalerolactone Hydrolysis

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The pseudo-first-order constants of hydrolysis of δ-thiovalerolactone (L) in the presence of excess imidazole (IM_T) have been determined in water (T = 30 ± 0.1°, $\mu = 1.0$ *M* with KCl) between *p*H 5.9 and 8.4. The disappearance of L was found to follow the rate expression $-dL/dt = C_L[C_{IM_T}k_r[K_a/(K_a + a_H)][a_H/(\bar{K} + a_H)] + k_{OH^\theta}OH^\theta + k_{H_2O}]$. The mechanism relating to k_{H_2O} represents a general base-catalyzed hydrolysis by water ($k_{H_2O}/k_{D_2O} = 2.5$) and the mechanism relating to k_{OH^θ} represents a nucleophilic catalysis of hydrolysis by hydroxide ion ($k_{OH^\theta}/k_{OD^\theta} = 0.7$). The *p*H–$k_r$ profile is of a "bell-shape" and dependent on the fraction of IM_T in free base form [*i.e.*, $(K_a/(K_a + a_H))$] and the fraction of an intermediate species in the protonated form [*i.e.*, $a_H(\bar{K} + a_H)$]. The latter species must represent the intermediate N-(δ-thiolvaleroyl)-imidazole whose thiol group must, to prevent its return as a nucleophile, be protonated. The possible importance of this type of return mechanism in providing "bell-shaped" *p*H–rate profiles for enzymatic reaction of the double displacement type is discussed.

Introduction

This study represents an extension of previous investigations from this Laboratory on the reaction of nucleophiles with cyclic acyl compounds.[3,4] In nucleophilic displacement reactions at the carbonyl group of thiolactones the displaced thiol group, in its ionized form, represents a strong nucleophile. One would anticipate, therefore, that in a displacement reaction leading to a labile acyl intermediate (*i.e.*, an acyl derivative from which the attacking nucleophile is readily displaced) the internal return of the thiol anion would influence the over-all rate of thiolactone hydrolysis in such a way that a so-called "bell-shaped" *p*H–rate profile would be obtained. To test this expectation the kinetics of the reaction of imidazole with δ-thiovalerolactone has been investigated.

A decided interest in the relationship of *p*H-dependency curves to mechanism arises from the common employment of such curves to determine the dissociation constant of participating species in intramolecular organic reactions and particularly in enzymatic catalysis. We have previously pointed out the dangers in the assumption that kinetically apparent pK_a' values necessarily represent the true pK_a' values of the functional groups involved.[5] The present investigation reveals an additional example of a *pK* apparent value being decidedly different from that of the pK_a' of a participating group.

Experimental

Apparatus.—The spectrophotometer employed was a Zeiss PMQ II. The cell compartment of the spectrophotometer was modified to accommodate a hollow brass block cuvette holder through which was circulated water at a constant temperature of 30 ± 0.1° (Precision Scientific Co. circulating water-bath). All *p*H measurements were made with a Radiometer model 22 *p*H meter. The combined glass-calomel electrode (Radiometer GK 2021 C) and electrode cell compartment were thermostated at 30 ± 0.1° during the course of these investigations.

Compounds.—δ-Thiovalerolactone was prepared from thiolacetic acid (Eastman Kodak Co.) and 4-pentenoic acid (Aldrich Chemical Co.). by the procedures of Korte and Löhmer.[6] The thiolactone was obtained as a nearly colorless oil,[6] b.p. 85–88° (3.75–4.15 mm.), lit.[6] b.p. 70–72° (0.8 mm.); $\lambda_{max}^{CH_3OH}$ 237 mμ, log ε 3.59; lit. $\lambda_{max}^{CH_3OH}$ 237 mμ, log ε 3.61. The ultraviolet spectrum was obtained in water also: $\lambda_{max}^{H_2O}$ 239.5 mμ, log ε 3.94.

The imidazole used was Eastman Kodak Co. white label.

δ-Thiolvaleramide was prepared from the thiolactone by reaction with liquid ammonia in a sealed tube at room temperature for 3–4 hours. The amide was purified by recrystallization from ether under an atmosphere of oxygen-free nitrogen; m.p. 70–71.5°. The thiolamide was found to be readily oxidized by air while in ether solution, to yield the ether-insoluble disulfide m.p. 130°–135°. After several recrystallizations, the thiol was submitted for analysis.

Anal. Calcd. for $C_5H_{11}NOS$: C, 45.08; H, 8.32; N, 10.52; S, 24.07. Found: C, 45.37; H, 8.32; N, 10.29; S, 24.05.

Kinetics.—The hydrolysis of thiolactone in water at 30 ± 0.1° was followed by observing the decrease in thiolactone absorption at 239.5 mμ. A stock solution of 0.24 g. of δ-thiovalerolactone in 5 ml. of ether was prepared. Two drops of this solution from a calibrated dropper when diluted to 10 ml. with oxygen-free water gave a solution approximately 10^{-4} *M* in thiolactone. The absorption due to imidazole was compensated for by using the appropriate concentration of imidazole in the reference cell. The imidazole-catalyzed hydrolysis of δ-thiovalerolactone was carried out in ₮ cuvettes, employing oxygen-free solutions of imidazole. Reactions were routinely carried out at several imidazole concentrations at various *p*H values between *p*H 5.9 and 8.4. In all instances the concentration of added imidazole was far in excess of thiolactone so that pseudo-first-order kinetics were obtained. In addition, all reactions were carried out at a calculated $\mu = 1.0$ *M* with KCl and at 30 ± 0.1°. The pseudo-first order rate constants were calculated from the slope of plots of log $O.D._0/O.D._t$ *vs.* *t*. Reactions were routinely followed to at least two half-lives and were found to be invariably first order.

D_2O Solvent Kinetic Isotope Effect.—The rates of imidazole catalysis of δ-thiovalerolactone hydrolysis were determined in D_2O by the same procedure followed in H_2O, with the added care taken that all solutions remained anhydrous. The pseudo-first-order rate constants for thiolactone disappearance were determined for imidazole concentrations of 1.0, 0.7, 0.5 and 0.2 *M* at *p*D 8.37, 8.02, 7.34 and 6.97 ($T = 30 \pm 0.1°$, $\mu = 1.0$ *M* with KCl). In the determination of *p*D a Metrohm type X glass electrode was employed and the *p*H meter reading was corrected to read *p*D *via* the equation of Fife and Bruice.[7] The autoprotolysis constant of D_2O employed in the calculations was 2.24×10^{-15}.[4] The pK_a' of imidazole in D_2O at an ionic strength of 1.0 *M* was determined by half neutralization in D_2O with a standard DCl solution. The value obtained, pK_a' (D_2O) 7.54,

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[1] For previous papers in this series see: (a) T. C. Bruice and G. L. Schmir, *J. Am. Chem. Soc.*, **79**, 1663 (1957); (b) **80**, 148 (1958); (c) G. L. Schmir and T. C. Bruice, *ibid.*, **80**, 1173 (1958); (d) T. C. Bruice and R. Lapinski, *ibid.*, **80**, 2265 (1958); (e) T. C. Bruice and J. M. Sturtevant, *ibid.*, **81**, 2860 (1959); (f) T. C. Bruice, *ibid.*, **81**, 5444 (1959); (g) U. K. Pandit and T. C. Bruice, *ibid.*, **82**, 3386 (1960); (h) T. C. Bruice and T. H. Fife, *ibid.*, **83**, 1124 (1961).

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[3] T. C. Bruice and U. K. Pandit, *J. Am. Chem. Soc.*, **82**, 5858 (1960).

[4] T. C. Bruice and J. J. Bruno, *ibid.*, **83**, 3494 (1961).

[5] T. C. Bruice and G. L. Schmir, *ibid.*, **81**, 4552 (1959).

[6] F. Korte and K. H. Löhmer, *Ber.*, **91**, 1397 (1958).

[7] T. H. Fife and T. C. Bruice, *J. Phys. Chem.*, **65**, 1079 (1961).

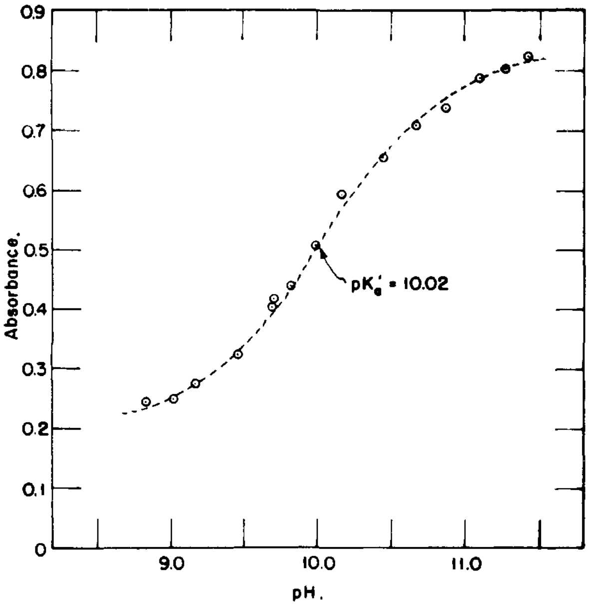

Fig. 1.—Spectrophotometrically determined ionization curve for δ-thiolvaleramide.

compares reasonably well with that calculated (7.68) from the empirical equation of Li.[8] The experimentally determined constants are

pD	k_2', l. mole^{-1} min.$^{-1}$ $\times 10^4$	k_h', min.$^{-1}$ $\times 10^4$
6.97	1.8	0.4
7.34	3.8	0.9
8.02	5.6	2.4
8.37	4.7	5.9

Determination of the pK_a' of the thiol group of δ-thiolvaleramide was carried out spectrophotometrically employing the trailing absorption of the thiol anion at 212 mμ. A sample of δ-thiolvaleramide (0.016 g.) was weighed into a 50-ml. volumetric flask. To the thiolvaleramide was added 10 ml. of acetone followed by dilution to volume with dearated water. A like solution without the valeramide was also prepared. A series of borate and phosphate buffers varying in pH between 8.8 and 11.5 were prepared. One ml. of the solution of δ-thiolvaleramide was pipetted into a 10-ml. volumetric flask and diluted to volume with deaerated buffer which had been pre-equilibrated at 30°. The absorbance at 212 mμ was taken immediately. The absorption due to buffers and acetone was compensated for by employing a solution of 1.0 ml. of the acetone-water solution, diluted to 10 ml. with the appropriate buffer, in the reference cell. The optical densities at the various pH values were plotted against pH and the pK_a' was determined by fitting the points to a theoretical titration curve (Fig. 1). In this manner the pK_a' of the thiol group of δ-thiolvaleramide was found to be 10.0.

Results[9]

In Fig. 2 are presented plots of the observed pseudo-first-order rate constants (k_{obs}) for the reaction of imidazole with δ-thiovalerolactone at the various pH values employed. The slopes of the lines provide the pseudo-second-order rate constants (k_2') for the imidazole catalysis of δ-thiolactone hydrolysis and the intercepts provide the pseudo-first-order hydrolytic constants for lyate species catalysis (k_h').

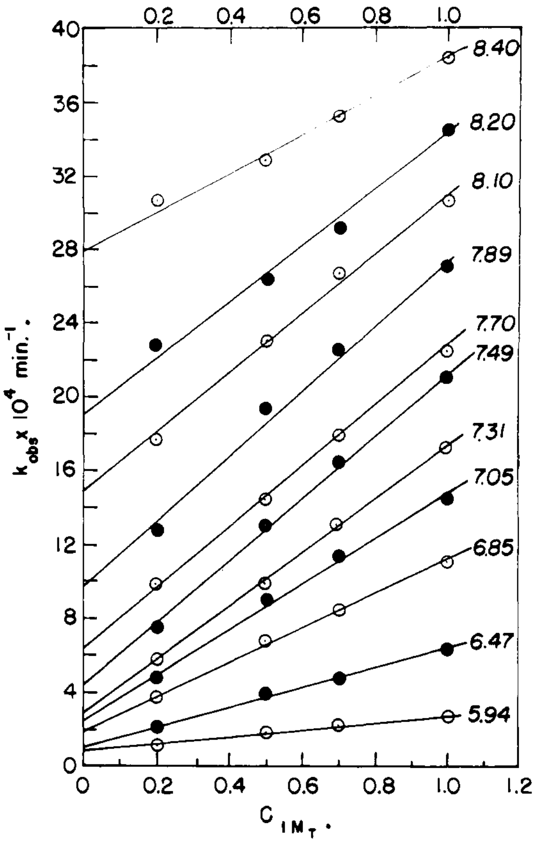

Fig. 2.—Plots of total imidazole concentration *vs.* the observed pseudo-first-order rate constants for thiolactone disappearance at various pH values.

$$\frac{-\mathrm{d}(C_L)}{\mathrm{d}t} = k_2' C_{IM_T}\, C_L + k_h' C_L \qquad (1)$$

$$k_{obs} = k_2' C_{IM_T} + k_h'$$

In Fig. 3 the values of k_h' have been plotted *vs.* the hydroxyl ion activity as determined by the value of K_W/a_H, where K_W is the autoprotolysis constant of water (1.47×10^{-14}) and a_H the hydrogen ion activity as determined by the glass electrode at 30 ± 0.1° ($\mu = 1.0$ with 1 M KCl). The slope of the linear relationship of Fig. 3 was found to equal 740 l. mole^{-1} min.$^{-1}$ and represents the second-order rate constant for the reaction of thiolactone with hydroxide ion (k_{OH^θ}). The intercept of Fig. 3 at $K_W/a_H = 0$, is not negligible but equal to 1×10^{-4} min.$^{-1}$ and represents the pseudo-first-order rate constant for spontaneous hydrolysis of the thiolactone by water (k_{H_2O}')

$$k_h' = k_{OH^\theta}(OH^\theta) + k_{H_2O}' \qquad (2)$$

The inability to observe an acid-catalyzed term in the pH range studied is in agreement with expectations based on the findings for the hydrolysis of alkyl thiol acetates.[10-12] Spontaneous hydrolysis has not previously been noted for a thiol ester.

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(8) N. C. Li, Abstract of paper presented to the A.C.S. Meeting in St. Louis, Mo., March 21–30, 1961, Division of Phys. Chem., Paper 14.

(9) Abbreviations employed are: δ-thiovalerolactone concentration, C_L; total imidazole concentration, C_{IM_T}; concentration of imidazole neutral base, C_{IM}; concentration of imidazole anion, C_{IM^θ}; N-(δ-thiolatevaleroyl)-imidazolium ion concentration, $C_{IH'}$; N-(δ-thiolvaleroyl)-imidazole concentration, C_{IH^2}; N-(δ-thiolvaleroyl)-imidazole anion concentration, C_I.

(10) P. M. Rylander and D. S. Tarbell, *J. Am. Chem. Soc.*, **72**, 3021 (1950).

(11) B. K. Morse and D. S. Tarbell, *ibid.*, **74**, 416 (1952).

(12) T. C. Bruice, in "Organic Sulfur Compounds," N. Kharasch, ed., Pergamon Press, New York, N. Y., 1961, Chapt. 35.

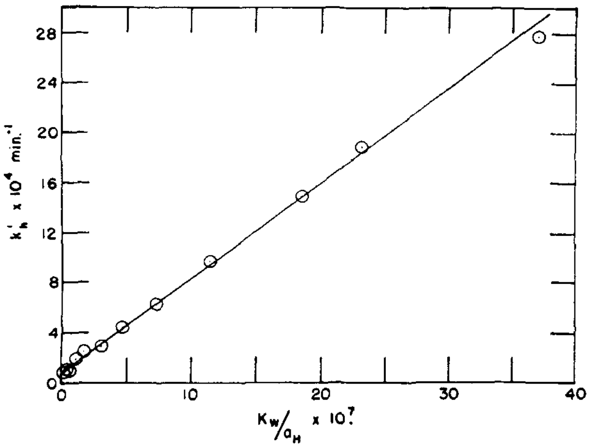


Fig. 3.—Plot of k_h' (the pseudo-first-order rate constant for lyate species catalysis) *vs.* hydroxide ion concentration (K_w/a_H).

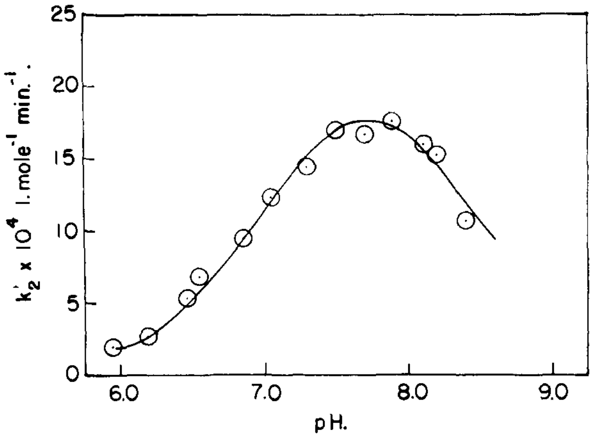
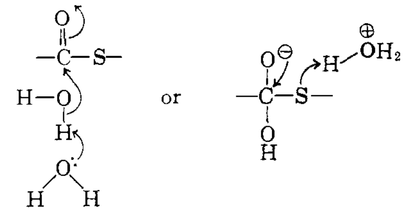


Fig. 4.—*p*H-rate profile for the reaction of imidazole with δ-thiovalerolactone. The values of k_2' are pseudo-second-order rate constants calculated on the basis of total imidazole added.

The spontaneous rate, as we shall see, most likely represents a general base-catalyzed hydrolysis in which a water molecule acts as the nucleophile to attack the thiol ester bond and a second water molecule plays the role of general base, *viz.*



(3)

The values of the pseudo-second-order rate constants for the reaction of imidazole with δ-thiovalerolactone are plotted *vs.* *p*H in Fig. 4. It may be noted that the plot of Fig. 4 describes a so-called bell-shaped *p*H-rate profile. The points of Fig. 4 represent experimentally determined constants while the curve is a theoretical one calculated from eq. 4

$$k_2' = k_r(F_{\text{IM}})(F\text{s})$$

$$k_2' = k_r\left[\frac{K_1'}{K_1' + a_{\text{H}}}\right]\left[\frac{a_{\text{H}}}{\bar{K} + a_{\text{H}}}\right] \qquad (4)$$

In 4, k_r represents the true second-order constant for imidazole catalysis, K_1' the first dissociation constant for imidazole (7.25×10^{-8} mole l.$^{-1}$)[4] and $\bar{K}$ an apparent dissociation constant. The value of $\bar{K}$ and k_r were determined graphically as those values which provide the best fit of 4 to the experimental points. The value of F_{IM} represents the fraction of total imidazole in the neutral basic form. The value of F_s represents the apparent fraction of some ionizable group in the acid form. This group can only represent the thiol function produced *via* the nucleophilic displacement of thiol from the thiolactone by imidazole. The requirement by the kinetic expression of 4 for this group to be in the acid form strongly suggests return of the thiolate ion to displace imidazole[1d] from the N-(δ-thiolvaleroyl)-imidazole which most certainly is an intermediate in the reaction.[1d] If one includes all possible equilibria and rate steps the mechanism of imidazole catalysis of δ-thiolactone hydrolysis is given in Chart I. Assuming a steady state in $C_{\text{IH}'}$, C_{IH^2} and C_{I}, then

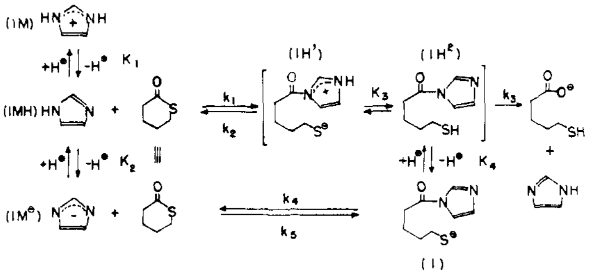


CHART I

$$\frac{-\text{d}(C_{\text{L}})}{\text{d}t} = \left[\frac{k_3(k_1a_{\text{H}} + k_5K_2)}{a_{\text{H}}\left(k_3 + \dfrac{k_2}{K_3}\right) + k_4K_4}\right] C_{\text{L}} \times C_{\text{IM}} \qquad (5)$$

In the *p*H range employed C_{IM^-} would be vanishingly small so that k_5K_2 may be ignored[1b] and $C_{\text{IM}_\text{T}} = C_{\text{IM}} + C_{\text{IMH}^\oplus}$ so that

$$k_2' = \frac{k_3k_1}{k_3 + \dfrac{k_2}{K_3}}\left[\frac{a_{\text{H}}}{a_{\text{H}} + \dfrac{k_4K_4K_3}{k_3K_3 + k_2}}\right]\left[\frac{K_1}{K_1 + a_{\text{H}}}\right] \qquad (6)$$

The values of k_2 and K_3 must be very large. With this in mind two simplifying assumptions may be made (7 and 8).

$$k_3 > k_2/K_3$$

$$k_2' = k_1\left[\frac{a_{\text{H}}}{a_{\text{H}} + \dfrac{k_4K_4}{k_3}}\right]\left[\frac{K_1}{K_1 + a_{\text{H}}}\right] \qquad (7)$$

$$k_2/K_3 > k_3$$

$$k_2' = \frac{k_3k_1K_3}{k_2}\left[\frac{a_{\text{H}}}{a_{\text{H}} + \dfrac{K_3K_4k_4}{k_2}}\right]\left[\frac{K_1}{K_1 + a_{\text{H}}}\right] \qquad (8)$$

Comparing 7 and 8 to the equation employed to correlate the experimental data (*i.e.*, 4) we see that $K_1' = K_1$ and $\bar{K} = k_4K_4/k_3$ (in 7) or $\bar{K} = K_3K_4k_4/k_2$ (in 8). In both instances the $\bar{K}$ term, an apparent dissociation constant, contains the true dissociation constant of the returning thiol group, K_4, only as a factor in a product. The second-order rate constant k_r in 4 is equal to k_1 in 7 and $k_3k_1K_3/k_2$ in 8. To remove impossible complications the k_3 step is assumed to be a spontaneous hydrolysis. This assumption is probably valid since the hydrolysis of N-acylimidazoles are essentially *p*H independent from *p*H 6–8.[1e,13,14] The plateau rate of N-acetylimidazole hydrolysis at *p*H 7.0 was shown by Jencks and Carriuolo[14] to be associated with a deuterium oxide kinetic isotope effect (k^H/k^D) of 2.7–2.5 (general base catalysis by water). This fact leads to a means of distinguishing between the limiting cases of 7 and 8. In 8, k_3 forms a portion of the experimentally determined values of k_r while in 7 it does not. The values of k_2' were determined at *p*D values of 8.37, 8.02, 7.34 and 6.97 as described previously for the studies in water. From the known pK_1 values of imidazole in H_2O and D_2O the values of k_r^H/k_r^D were calculated to be 3.6, 3.1, 2.8 and 3.2, respectively (see Experimental). Values of k^H/k^D of this magnitude strongly suggest the involvement of a general base mechanism.[14–16] Case 8 rather than 7 is favored and subsequent discussion assumes the mechanism is as described in case 8.

In the course of determining the ratio of k^H/k^D for the imidazole catalysis we obtained the value of $k_{OD^\ominus}$ and k_{D_2O} (as previously described for the experiments in water). From these values the k^H/k^D ratio for hydrolysis by specific base ($OD^\ominus$ and $OH^\ominus$) was estimated to be 0.7 in reasonable agreement with previously determined values for specific base catalysis.[16] The kinetic ratio k^H/k^D for the water rate was determined to be 2.5 which supports a general base mechanism for the spontaneous hydrolysis of thiolactone in water (*i.e.*, mechanism 3).

The value of K_4 in Chart I may be assumed to be essentially identical to the dissociation constant of the thiol group in δ-thiolvaleramide which has been determined (see Experimental) to be $10^{-10.0}$. The kinetically determined dissociation constant $\bar{K}$ is equal to 4.78×10^{-9}. The value of $p\bar{K}$ is then 1.68 pK units less than pK_1'. From the expression $\bar{K} = K_3K_4k_4/k_2$ and with the assumption that K_4 is as determined for δ-thiolvaleramide, we can calculate the value of K_3k_4/k_2 to be 47.8. The constant k_2 would be expected to be many orders of magnitude greater than k_4 as shown in comparisons of the rate constants for the reaction of bases with protonated and neutral N-acylimidazoles.[17] Therefore, K_3 must be a very large number as expected from the pK_a values of acylimidazoles (pK 3–4)[17] and the mercapto group ($pK_a \sim 10$).

The various rate constants determined in this study are presented in Table I.

TABLE I

A TABULATION OF KINETIC AND EQUILIBRIUM CONSTANTS DETERMINED IN THIS STUDY

$$-\frac{dL}{dt} = C_LC_{IMT}\left[\left(\frac{K_a'}{K_a' + a_H}\right)\left(\frac{a_H}{\bar{K} + a_H}\right) k_r + k_{OH}(OH^-) + k_{H_2O}\right]$$

$K_a' = 7.25 \times 10^{-8}$ mole/liter

$\bar{K} = K_3K_4k_4/k_2 = 4.78 \times 10^{-9}$

$k_r = k_3k_1K_3/k_2 = 2.80 \times 10^{-3}$ l. mole^{-1} min.$^{-1}$

$k_r^{H_2O}/k_r^{D_2O}$ = 3.6(*p*D 8.37), 3.1(*p*D 8.02), 2.8(*p*D 7.34), 3.2(*p*D 6.97)

$k_{OH^\ominus} = 740$ l. mole^{-1} min.$^{-1}$; $k_{OD^\ominus} = 1000$ l. mole^{-1} min.$^{-1}$

$k_{OH^\ominus}/k_{OD^\ominus} = 0.7$; $k_{H_2O} = 1 \times 10^{-4}$ min.$^{-1}$

$k_{D_2O} = 0.4 \times 10^{-4}$ min.$^{-1}$; $k_{H_2O}/k_{D_2O} = 2.5$

Discussion

The bell-shaped *p*H–rate profile obtained for the reaction of imidazole with δ-thiovalerolactone has been established to be due to the return of thiolate ion (Chart I). The mechanism provides an additional example of a kinetic scheme leading to a bell-shaped *p*H–rate profile. The general expression for the new scheme can be abbreviated as in 9.

$$\mathrm{BH}^{\oplus} \overset{K_1}{\rightleftarrows} \mathrm{B} + \mathrm{H}^{\oplus}$$

$$\begin{tikzcd}
\mathrm{B} + \mathrm{A} \arrow[r, "k^1"] & \mathrm{CH} \arrow[r, "k_2"] \arrow[d, shift right, "-\mathrm{H}^+"'] & \text{products} \\
 & \mathrm{C} \arrow[u, shift right, "+\mathrm{H}^+ \quad K_2"'] \arrow[ul, bend left, "k_3"] &
\end{tikzcd} \qquad (9)$$

where

$$\frac{-dC_A}{dt} = k_1\left[\frac{K_1}{K_1 + a_H}\right]\left[\frac{a_H}{\frac{k_3K_2}{k_2} + a_H}\right]C_{BT}C_A \qquad (10)$$

Bell-shaped *p*H dependency curves are common phenomena in enzymatic reactions and in the acid-base catalytic processes of organic chemistry. The most commonly encountered kinetic scheme providing a bell-shaped profile is that of 11[28]

$$\begin{tikzcd}
\mathrm{ES}^- \arrow[d, shift left, "+\mathrm{H}^{\oplus}"] & \\
\mathrm{ESH}^{\ominus} \arrow[u, shift left, "K_2 \quad -\mathrm{H}^{\oplus}"] \arrow[r, "k_r"] \arrow[d, shift left, "+\mathrm{H}^{\oplus}"] & \text{products} \\
\mathrm{ESH}_2 \arrow[u, shift left, "K_1 \quad -\mathrm{H}^{\oplus}"] &
\end{tikzcd} \qquad (11)$$

where K_1 and K_2 are acid dissociation constants. For 11, the observed pseudo-first-order rate constant (k_{obs}) determined at any constant acidity is related to the value of a_H as in 12 and the *p*H at the maximum rate will be related to K_1 and K_2

$$k_{obs} = \frac{k_rK_1a_H}{a_H^2 + K_1a_H + K_1K_2} = \frac{V_M}{E_T} \qquad (12)$$

by 13, while the maximum observed rate will be

$$pH_{max} = 1/2\,(pK_1 + pK_2) \qquad (13)$$

given by the expression

$$k_{obs(max)} = \frac{k_r}{1 + 2\sqrt{K_2/K_1}} \qquad (14)$$

Examples of enzymatic reactions whose kinetic constants exhibit bell-shaped curves and whose

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[13] E. R. Stadtman, "The Mechanism of Enzyme Action," W. D. McElroy and B. Glass, ed., The Johns Hopkins Press, 1954, Baltimore, Md., p. 581.

[14] W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1272 (1959).

[15] W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **83**, 1743 (1961).

[16] F. A. Long, *Ann. N. Y. Acad. of Sci.*, **84**, 596 (1960).

[17] W. P. Jencks and J. Carriuolo, *J. Biol. Chem.*, **234**, 1280 (1959).

mechanism have been interpreted on the basis of 11–14 include the hydration of fumaric acid to L-malic acid by fumarase[18]; and the hydrolysis of esters by acetylcholinesterase[19,20]; *pseudo*-cholinesterase,[20] dog-liver esterase[20] and papain.[21,22]

Organic reactions whose over-all rates follow 12 and that have been interpreted on the basis of the involvement of an acidic and a basic functional group include the hydration of fumaric acid at 175° in water,[23] the catalyzed hydrolysis of chloramphenicol by dibasic acids,[24] intramolecular general acid assistance to nucleophilic catalysis of the hydrolysis of certain ω-aminoacyl methyl salicylates[25,26] and intramolecular nucleophilic general acid catalysis of the hydrolysis of the monosuccinate ester of salicyclic acid.[27] Bender and Zerner[28] have recently pointed out two additional possibilities for obtaining bell-shaped pH-rate profiles (15 and 16).

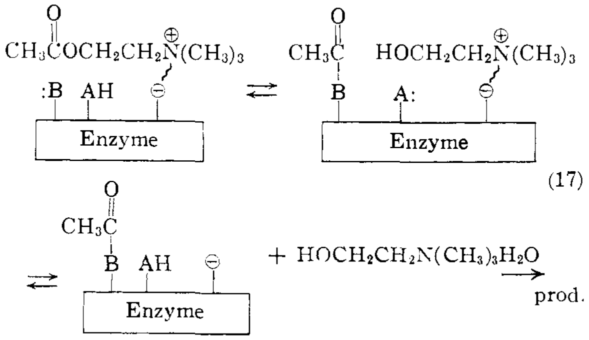
$$\begin{gathered} AH^{\oplus} \overset{K_1}{\rightleftarrows} A + H^{\oplus} \\ A \underset{k_2}{\overset{k_1}{\rightleftarrows}} B \\ B + H^{\oplus} \overset{K_2}{\rightleftarrows} BH^{\oplus} \xrightarrow{k_3} \text{prod.} \end{gathered} \tag{15}$$

$$\begin{gathered} AH^{\oplus} \overset{K_1}{\rightleftarrows} A + H^{\oplus} \\ AH^{\oplus} \underset{k_2}{\overset{k_1}{\rightleftarrows}} BH^{\oplus} \\ BH^{\oplus} \overset{K_2}{\rightleftarrows} B \xrightarrow{k_3} \text{prod.} \end{gathered} \tag{16}$$

Either 15 or 16 have been suggested to represent the kinetics for the hydrolysis of o-carboxyphthalimide.[28]

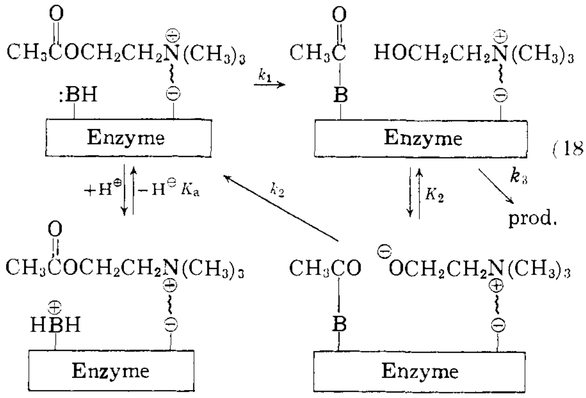
In the kinetic scheme of 9 and 10, the fact that the value of k_3K_2/k_2 is not that of the dissociation constant of the returning group (K_2) serves as an additional example of the danger in employing pH-rate profiles to determine the pK_{app} values as values of participating groups.[5]

The kinetic scheme of 9 could be of particular pertinence in enzymatic reactions where the pH-dependence of the catalysis is employed as one of the chief tools to identify the functional group at the active site. The main difference between the kinetic schemes of 9 and 11 is the involvement of only one basic species as catalyst *vs.* the involvement of one acidic and one basic species as catalyst. Though the arguments presented are intended to be general, a consideration of the possible mechanisms for acetylcholinesterase serves particularly well to demonstrate the concept. Present suggestions for the mechanism of action of acetylcholinesterase include an anionic binding site (⊖) and an active center composed of a basic (B:) + acidic (AH) group (17).[29] Reaction 17 is kinetically of type 11



(17)

and would follow eq. 12. However, mechanism 17 cannot be differentiated kinetically from 18 whose constants are given correctly by 10.



(18)

On the basis of kinetic evidence alone 18 could serve equally well as the mechanism for acetylcholinesterase.[30] The value of the kinetically apparent dissociation constant of the choline hydroxyl group in 18 might (as in the case of the kinetically apparent dissociation constant of the thiol group produced in the imidazole-catalyzed hydrolysis of δ-thiovalerolactone) be several orders of magnitude less than the true dissociation constant. For this reason the pK_{app}' value of 10.0 determined for the acidic group in cholinesterase[20] may not be the pK_a' of a phenolic hydroxyl group but a function of the pK_a' of the enzyme-bound returning choline molecule (18).

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(30) This is not to propose that eq. 18 actually does depict the mechanism for acetylcholinesterase. This enzyme has been employed only as an illustrative example.