feature of strain which makes the Phillips mechanism for lysozyme plausible. Perhaps the most pleasing mechanism[4a] now stands as general acid catalyzed, by Glu-35, formation of an oxocarbonium ion–alkoxide ion stereoretained ion pair followed by oxocarbonium ion capture by Asp-52 to form an acylal which can spontaneously regenerate[24] oxocarbonium ion.

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Direct Determination of C-Protonation and Hydrolysis Rates in Enamines. Application to Ethyl β-Cyanomethylaminocrotonate[1]

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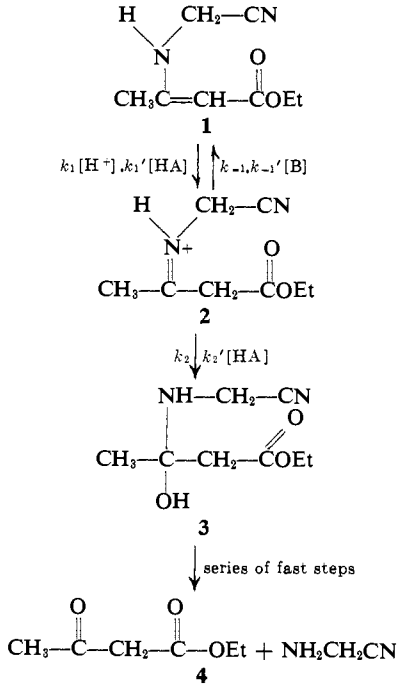
Abstract: Nmr studies were performed on the rates of hydrolysis of ethyl β-cyanomethylaminocrotonate (**1**), the enamine of ethyl acetoacetate, and the primary amine, cyanomethylamine. Simultaneous observation of the rates of C-protonation and overall hydrolysis enables unequivocal assignment of the steps in the mechanism involving a change in rate-limiting step with changing buffer concentration. As previously suggested, in the limit of zero buffer concentration C-protonation of the enamine is found to be rate limiting.

Recently, the detailed mechanism of the hydrolysis of ethyl β-cyanomethylaminocrotonate (**1**) was presented[3] and the intermediacy of the closely related enamine of acetoacetic acid in the amine catalyzed decarboxylation of acetoacetic acid was substantiated.[4] For the hydrolysis of **1** the mechanism in Scheme I was proposed.

Scheme I. Mechanism of Enamine Hydrolysis



At pH 4, 5, and 6 the rate of hydrolysis of the enamine was found to be subject to both specific acid and general acid–base catalysis. The perceptably curved k_{obsd} *vs.* buffer_{total} relationship found at these pH values was interpreted to mean a change in rate-limiting step with changes in buffer concentration as discussed by Jencks for such behavior.[5] Based on precedent, isotope effects, etc. the buffer behavior was interpreted as a change from rate-limiting C-protonation at low buffer concentration to rate-limiting hydration of Schiff base (or Schiff base salt) at high buffer concentration in the pH range studied.[3] C-Protonation at or near zero buffer concentration was suggested because of the sensitivity of the rate to buffer catalyst concentration and the observed isotope effect ($k^0_{H^+}/k^0_{D^+} = 2.3$) found in this buffer range.

Based on extensive indirect evidence such rate-determining C-protonation had also been proposed for two related enamines (nonconjugated), the hydrolysis of 1-*N*-morpholino-1-isobutene[6] above pH 4 (measured isotope effect $k_{H_3O^+}/k_{D_3O^+} = 2.5 \pm 0.7$, rate subject to general acid catalysis) and for the hydrolysis of the morpholine enamine of propiophenone[7] above pH 5 (this reaction also sensitive to general acid catalysis).

The present results support the mechanism proposed for the hydrolysis of the enamine **1**, prove that C-protonation can in fact be rate limiting under certain conditions (in this study at low buffer concentrations) and suggest a general method for identifying rate-limiting C-protonation steps in analogous mechanisms.

The rate of the tautomerization of the enamine **1** to Schiff base (or Schiff base salt) **2** can be followed by nmr

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(3) J. P. Guthrie and F. Jordan, *J. Amer. Chem. Soc.*, **94**, 9132 (1972).
(4) J. P. Guthrie and F. Jordan, *J. Amer. Chem. Soc.*, **94**, 9136 (1972).
(5) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969, pp 477–480; E. H. Cordes and W. P. Jencks, *J. Amer. Chem. Soc.*, **84**, 4319 (1962).
(6) (a) E. J. Stamhuis and W. Maas, *J. Org. Chem.*, **30**, 2156 (1965); (b) W. Maas, M. J. Janssen, E. J. Stamhuis, and H. Wynberg, *ibid.*, **32**, 1111 (1967).
(7) P. Y. Sollenberger and R. B. Martin, *J. Amer. Chem. Soc.*, **92**, 4261 (1970).

by monitoring the rate of disappearance of the vinyl proton resonance. The overall hydrolysis rate can be followed in the same experiment by monitoring the disappearance of the γ-methyl protons of the enamine or the appearance of the γ-methyl protons of product.

A comparison of the rates of C-protonation and overall hydrolysis under a variety of buffer and pH conditions can be employed in elucidating the rate-limiting steps under these conditions.

Experimental Section

Materials and Methods. The enamine, **1**, was prepared and purified as in ref 3. Aqueous buffers were prepared from commercial grade chemicals and used without further purification. Distilled, deionized water was used in the preparation of aqueous buffers. D_2O was obtained from Bio-Rad (99.88% D). 99.5% minimum isotopic purity acetic acid-d_4, 20% DCl in D_2O, and CD_3CN were all obtained from Diaprep. NaOD in D_2O was prepared by dissolving Na metal in D_2O.

Buffers in D_2O were prepared by weighing accurate amounts of DCl or acetic acid-d_4, titrating with known normality NaOD, and diluting to required volume. Serial dilutions were accomplished by mixing the buffer with appropriate volumes of KCl in D_2O to assure constant ionic strength.

Fisher Spectrograde CH_3CN (stored over molecular sieves) was used in the kinetics.

Nmr Kinetics. The kinetics were performed on Varian A-60 or HA-100 spectrometers, both equipped with variable-temperature attachments. The solid enamine, **1**, was first dissolved in CD_3CN and an appropriate volume of deuterated buffer was added to it immediately prior to insertion into the probe. Zero time was taken as the time of buffer addition. Temperature of the probe was monitored by measurement of the temperature dependent chemical shift differences of the methanol resonances. All volumes were measured with 100-μl calibrated Hamilton syringes. A TMS capillary (10% in CCl_4) served as an internal lock. The HA-100 was used for the low % CD_3CN (low enamine concentration) kinetics.

Kinetic data were taken from the integrated peak intensities. The disappearance of the vinyl proton peak of the enamine (s, δ 4.6 ppm) and the appearance of the γ-methyl group of the product, ethyl acetoacetate (s, δ 2.2), were monitored as a function of time. The signals from the ester methyl groups were also integrated and this served as an internal standard. The disappearance of the signal from the γ-methyl group of the enamine could also be followed (s, δ 2.0) but turned out to be somewhat inconvenient since this peak is located in the middle of the CD_2HCN multiplet. The rate of disappearance of the latter peak was shown to equal the rate of appearance of product.

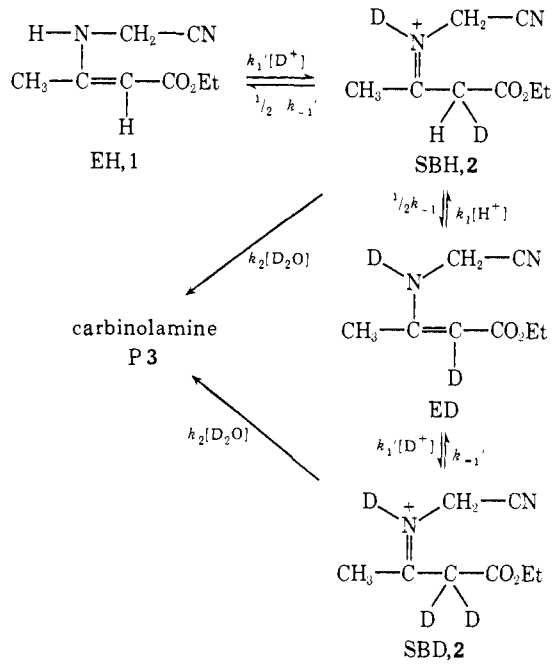
The data were plotted as fraction vinyl proton remaining and fraction unhydrolyzed enamine (equal to 1 − fraction of product formed) *vs.* time on semilog paper for the exchange and overall hydrolysis rates, respectively. The hydrolysis rates were followed for nearly 2 half-lives and were found to obey pseudo-first-order kinetics in this range. An average of five integrations were used to determine each point on the kinetic plot with average time values used as abscissas. The data are subject to considerable error, especially those concerning the exchange rates which process is rather fast under most conditions employed in this study. Estimated errors for the disappearance of vinyl proton signal and overall hydrolysis rates are 20–30% and 10–15%, respectively. The absolute rates cannot easily be compared for any two sets of data since very small deviations in solvent composition can lead to measurable rate differences in both processes.

Uv Kinetics. The rate of disappearance of the enamine can be followed very conveniently at 275 nm by uv under a variety of conditions.[3] Such determinations were done on a Cary 15 uv–vis spectrophotometer equipped with a thermostated cell block.

pH Determinations. The pH of all solutions was measured with a Radiometer pH M 4b meter employing a combination glass electrode. The pH_{app} values quoted in Table I refer to measurement of pH in D_2O–CD_3CN mixtures using such an electrode. Since deuteron activity in such solutions is not well defined an attempt was made to establish an H_0 scale in such mixtures using the indicator method.[8] A comparison of the apparent pH values with H_0 indicates that such glass electrode values are probably about 1 pH unit lower than the H_0 values.[8]

It is not anticipated that such a shift in solution pH to higher values would alter the mechanism, especially the rate-limiting step in this region of acidity.

Scheme II. Vinyl Proton Exchange in D_2O and D_2O Hydrolysis[a]



[a] All rate constants shown are composites of buffer catalytic terms as well as water terms; prime indicates breaking of a C–D bond.

Results and Discussion

The Effect of CH_3CN on Rate Constants. The overall enamine hydrolysis rate can be studied conveniently and with relatively good precision by uv spectroscopy. Extensive studies indicated[3] that addition of CH_3CN decreases the rate of hydrolysis well beyond that expected on the basis of the effect on hydronium ion activity alone. For the same buffer concentration at a given apparent pH (measured by glass electrode or H_0) the solution having the larger acetonitrile concentration leads to much slower rates.

Results of Nmr Kinetic Studies. Representative kinetic results are listed in Table I. Due to the relatively large errors in the exchange kinetics as well as to the complex specific and general acid catalytic behavior of each step in the mechanism[3] no exact analysis of the rate data is feasible in terms of second-order specific rate constants incorporating the hydronium ion concentration. The trends, however, are clear cut and easily interpretable.

First of all, both the protonation (exchange) and overall hydrolysis rate constant are suppressed by the addition of CH_3CN.

Secondly, in all mixtures studied the ratio of exchange to hydrolysis rate constants decreases with decreasing buffer concentration at all pH's employed. The exact behavior of the ratio of rate constants (*i.e.*, as to whether k_{obsd} *vs.* $buffer_{total}$ is linear or curved) cannot be determined but is not essential to the present analysis.

Thirdly, there is a strong suggestion in the data that the lower the acetonitrile concentration, the closer to

(8) The H_0 determinations in H_2O–CH_3CN mixtures and their correlation with pH_{app} (measured with a glass electrode) are reported elsewhere: F. Jordan, *J. Phys. Chem.*, 77, 2681 (1973).

Table I. Nmr Kinetics of Enamine C-Protonation and Hydrolysis[a]

Run no.	Temp, °C	[Enamine][b]	% CD_3CN[c]	Buffer[d] concn	pH_{aq}[e]	pH_{app}[f]	k_{exch},[g] sec^{-1}	k_{hydr},[g] sec^{-1}	k_{exch}/k_{hydr}
76,77	14.5	0.15	50	0.278	4.0	5.04	1.05E-2	1.00E-3	10.
78,79	14.5	0.15	50	0.0556	4.0	5.04	3.10E-3	5.40E-4	5.8
80,81	14.5	0.15	50	0.0140	4.0	5.04	1.30E-3	3.20E-4	4.0
99	30	0.140	40.4	0.0420	4.97	5.80	3.70E-3 (20)	8.40E-4 (10)	4.4
98	30	0.140	40.4	0.1070	4.97	5.80	2.40E-3 (30)	4.30E-4 (20)	5.6
97	30	0.140	40.4	0.00568	4.97	5.80	5.80E-4 (30)	1.65E-4 (10)	3.5
96	30	0.143	40.4	0.00284	4.97	5.80	9.24E-5 (20)	5.66E-5 (10)	1.63
106	30	0.138	40.4	0.0334	4.11	4.80	8.00E-3 (20)	2.26E-3	3.50
107	30	0.138	40.4	0.0334	4.11	4.80	5.63E-3 (20)	1.58E-3	3.50
104	30	0.138	40.4	0.0167	4.11	4.80	2.89E-3	8.50E-4 (20)	3.40
105	30	0.138	40.4	0.0167	4.11	4.80	1.83E-3 (25)	5.80E-4 (10)	3.17
102	30	0.138	40.4	0.00668	4.11	4.80	5.90E-4 (15)	2.26E-4 (10)	2.61
103	30	0.138	40.4	0.00668	4.11	4.80	7.45E-4 (15)	3.72E-4	2.00
100	30	0.138	40.4	0.00334	4.11	4.80	2.09E-4 (10)	9.79E-5 (10)	2.12
101	30	0.138	40.4	0.00334	4.11	4.80	3.56E-4 (15)	2.06E-4 (5)	1.72
112[h]	30	0.071	29.1	0.00788	4.11	4.62	1.44E-3	5.80E-4	2.4
113[h]	30	0.071	29.1	0.00788	4.11	4.62	1.72E-3 (40)	4.44E-4 (25)	3.8
110[h]	30	0.071	29.1	0.00394	4.11	4.62	n.a.	n.a.	1.50
111[h]	30	0.071	29.1	0.00394	4.11	4.62	4.13E-4 (15)	2.14E-4 (10)	1.93
108[h]	30	0.071	29.1	0.00332	4.11	4.62	1.70E-4 (25)	1.39E-4 (20)	1.22
109[h]	30	0.071	29.1	0.00332	4.11	4.62	1.93E-4 (30)	1.07E-4 (10)	1.80
119[h]	30	0.074	29.1	0.020	4.97	5.57			>3
120[h]	30	0.074	29.1	0.020	4.97	5.57			>3
121[h]	30	0.074	29.1	0.020	4.97	5.57			8.
117[h]	30	0.074	29.1	0.00994	4.97	5.57	1.26E-3 (30)	3.79E-4 (20)	3.3
118[h]	30	0.074	29.1	0.00994	4.97	5.57	1.58E-3 (40)	4.36E-4 (30)	3.6
114[h]	30	0.074	29.1	0.00664	4.97	5.57	6.80E-4 (30)	3.85E-4 (15)	1.77
116[h]	30	0.074	29.1	0.00664	4.97	5.57	6.08E-4 (30)	5.10E-4 (10)	1.20

[a] Ionic strength in aqueous portion always 0.1; acetate buffers used throughout. [b] Enamine molar concentration in total solution. [c] % by volume CD_3CN. [d] Total molar buffer concentration in total solution. [e] pH of buffer measured on pH meter; to get pD just add 0.40 unit. [f] pH_{app} estimated for initial mixture. [g] In parentheses the estimated per cent error; E notation used for exponentials, *i.e.*, E-3 implies $\times 10^{-3}$. [h] Runs on Varian HA-100; all others on Varian A-60.

unity tends the ratio of exchange to hydrolysis rate constant with decreasing buffer concentration.

One should note also that the range of buffer concentrations employed in the nmr study is very much smaller than the one in the uv study, since the former method is very limited in the range of reaction rates it can measure (*i.e.*, need a rather low buffer concentration for the nmr studies to slow down the rates).

The tendency to similar exchange and hydrolysis rate constants with very low buffer concentration can be interpreted in terms of the mechanism in Scheme II as solved for limiting conditions in the Appendix. Independently of the assumption made in the derivation, the experimental findings demand that the C-protonation be at least partially rate determining at low buffer concentrations and very fast at high buffer concentrations. Qualitatively this can be rationalized easily since one may expect the C-protonation to exhibit much more pronounced general acid catalysis than would the Schiff base hydration step. That deamination of the carbinolamine to ketone is fast under the conditions here reported has been suggested by several groups before: for example, in the hydrolysis of *p*-chlorobenzylideneaniline,[9] hydrolysis of 1-*N*-morpholino-1-isobutene,[6a] and in the hydrolysis of the morpholinoenamine of propiophenone.[7]

Some of the studies on simple enamines indicated a change in rate-determining step from the hydration of the Schiff base to C-protonation in the pH range here employed.[6a,7] According to the present results at pD 5 and above (estimated pD for the D_2O–CD_3CN mix-

(9) E. H. Cordes and W. P. Jencks, *J. Amer. Chem. Soc.*, **84**, 832 (1962).

tures) there is still a change in rate-limiting step from tautomerization to Schiff base hydration with increased buffer concentration. Reference 3 presents arguments for a further change in rate-limiting step to expulsion of the amine from the carbinolamine as the pH is lowered to below 4.

The method here proposed can clearly distinguish between a rate-limiting protonation step or some other slow step in the mechanism and should find applications in analogous systems.

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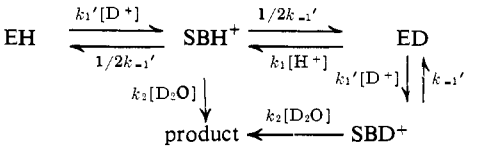
Appendix

Rewriting Scheme II in abbreviated form



The rate of the disappearance of the vinyl proton is

$$-\frac{d[EH]}{dt} = k_1'[D^+][EH] - 1/2k_{-1}'[SBH^+] \quad (1)$$

The rate of product formation (experimentally shown to equal rate of total enamine disappearance) is

$$-\frac{d[EH]}{dt} - \frac{d[ED]}{dt} = \frac{d[P]}{dt} = k_2[D_2O]([SBH^+] + [SBD^+]) \quad (2)$$

Since the Schiff base intermediate is present in small quantities and under the experimental conditions the reaction is essentially irreversible one can assume steady-state conditions for SBD^+ and SBH^+.

Exchange Rates.

$$-\frac{d[EH]}{dt} = \frac{k_1'(k_2[D_2O] + 1/2k_{-1})[EH][D^+] - 1/2k_1k_{-1}'[H^+][ED]}{k_2[D_2O] + 1/2k_{-1}' + 1/2k_{-1}} \quad (3)$$

Now assuming that in D_2O $[H^+] \ll [D^+]$ or rather $[H^+]\cdot[ED] \ll [D^+][EH]$ a condition certainly true in the first portion of the reaction in which most exchange data were taken, for a given $[D^+]$ and $[EH]$

$$k_{obsd}^E = \frac{k_1'(k_2[D_2O] + 1/2k_{-1})}{k_2[D_2O] + 1/2k_{-1}' + 1/2k_{-1}} \quad (4)$$

Overall Hydrolysis Rates.

$$\frac{d[P]}{dt} = k_2[D_2O]\left(\frac{k_1[H^+][ED] + k_1'[D^+][EH]}{k_2[D_2O] + 1/2k_{-1} + 1/2k_{-1}'} + \frac{k_1'[D^+][ED]}{k_2[D_2O] + k_{-1}'}\right) \quad (5)$$

There are three limiting conditions to contend with: (a) $[ED] \ll [EH]$ and $k_1[H^+][ED] < k_1'[D^+][EH]$ since $[H^+] \ll [D^+]$

$$\frac{d[P]}{dt} = \frac{k_1'k_2[D_2O][D^+][EH]}{k_2[D_2O] + 1/2k_{-1} + 1/2k_{-1}'} \quad (6)$$

$$k_{obsd}^H = \frac{k_1'k_2[D_2O]}{k_2[D_2O] + 1/2k_{-1} + 1/2k_{-1}'} \quad (7)$$

(b) $[ED] \cong [EH]$ and to simplify $k_{-1}' \cong 1/2k_{-1} + 1/2k_{-1}'$

$$\frac{d[P]}{dt} = \frac{2k_1'k_2[D^+][EH][D_2O]}{k_2[D_2O] + 1/2k_{-1} + 1/2k_{-1}'} \quad (8)$$

$$k_{obsd}^H = \frac{2k_1'k_2[D_2O]}{k_2[D_2O] + 1/2k_{-1} + 1/2k_{-1}'} \quad (9)$$

(c) $[ED] \gg [EH]$ and $k_1'[D^+] \gg k_1[H^+]$

$$\frac{d[P]}{dt} = \frac{k_1'k_2[D_2O][D^+][ED]}{k_2[D_2O] + k_{-1}'} \quad (10)$$

$$k_{obsd}^H = \frac{k_1'k_2[D_2O]}{k_2[D_2O] + k_{-1}'} \quad (11)$$

Ratio of Exchange to Hydrolysis Rate Constants. Under identical conditions of initial enamine concentration and pH as is the case in the nmr measurements of the two constants: (a) $[ED] \ll [EH]$

$$\frac{k_{obsd}^E}{k_{obsd}^H} = \frac{k_2[D_2O] + 1/2k_{-1}}{k_2[D_2O]} \quad (12)$$

The ratio is near unity for $k_2[D_2O] \gtrsim 1/2k_{-1}$ and much greater than unity for $1/2k_{-1} \gg k_2[D_2O]$; (b) $[ED] \simeq [EH]$

$$\frac{k_{obsd}^E}{k_{obsd}^H} = \frac{k_2[D_2O] + 1/2k_{-1}}{2k_2[D_2O]} \quad (13)$$

with the same results in the limits as in case a; (c) $[ED] \gg [EH]$

$$\frac{k_{obsd}^E}{k_{obsd}^H} = \frac{(k_2[D_2O] + 1/2k_{-1})(k_2[D_2O] + k_{-1}')}{(k_2[D_2O])(k_2[D_2O] + 1/2k_{-1}' + 1/2k_{-1})} \quad (14)$$

Two further limits obtain: (i) when $k_{-1} \cong 5k_{-1}' \gg k_2[D_2O]$ (assuming an isotope effect of 5), the ratio is much larger than unity; (ii) when $k_2[D_2O] > 1/2k_{-1}$, the ratio is clearly unity.