action can effectively compete even with external solvent addition.

Experimental Section

Materials. 2-(4-Methylphenyl)-2,3-dimethyl-1,3-oxazolidine was prepared by refluxing for 2 weeks in toluene equivalent amounts of 4-methylacetophenone and 2-(methylamino)ethanol, water being continuously removed from the reaction by azeotropic distillation with the toluene. The toluene was removed on a rotary evaporator, and the residual liquid distilled to give III: bp 140 °C (30 mm); NMR ($CDCl_3$, $(CH_3)_4Si$) δ 7.25 (2 H, d), 6.93 (2 H, d), 3.78 (2 H, m), 2.85 (2 H, m), 2.32 (3 H, s), 2.27 (3 H, s), 1.53 (3 H, s).

Anal. Calcd for $C_{12}H_{17}NO$: C, 75.35; H, 8.96; N, 7.32. Found: C, 75.2; H, 9.12; N, 7.16.

The imine of 4-methylacetophenone and 2-methoxyethylamine was prepared as described for III, with a trace of *p*-toluenesulfonic acid being added to catalyze the addition. This imine had a boiling point of 90 °C (0.1 mm). The cationic Schiff base V, as a trifluoromethanesulfonate salt, was prepared by treatment of this imine dissolved in methylene chloride with an equivalent amount of methyl trifluoromethanesulfonate. The NMR spectrum of this solution showed that the methyl transfer was complete essentially on mixing. The presence of two isomers in about equal proportions was revealed by two NMe peaks (δ 3.96 and 3.76) and two OMe peaks (δ 3.50 and 3.38).

Kinetics. Stopped-flow experiments were carried out on a Durrum-Gibson stopped-flow spectrophotometer; slower kinetic experiments were monitored by using a Unicam sp 1800 spectrophotometer. The ring-opening reaction of III was studied by addition of a small amount of the oxazolidine in CH_3CN to an 0.002 M NaOH solution which had been thermostated in an external water bath. One syringe of the stopped-flow instrument was filled with this solution; while the other syringe was filled with the appropriate aqueous buffer. Six to eight successive mixings were carried out. The data were analyzed directly by using a Tektronix 4051 minicomputer linked to the stopped-flow instrument. First-order rate constants were evaluated by following the increase in absorbance at 290 nm. The ring-closing reaction of the Schiff base was studied in a similar manner. The experiment in this case involved the addition of oxazolidine to 0.002 M HCl, followed by mixing in the stopped-flow apparatus with an appropriate basic buffer. The decrease in UV absorbance at 290 nm was monitored. The formation of hydrolysis products was studied by using conventional spectroscopy, by addition of 1–3 μL of a solution of oxazolidine to a thermostated UV cell. Rate constants were obtained for the increase in absorbance at 257 nm due to the appearance of 4-methylacetophenone or, at pH <8, from the decrease in absorbance at 290 nm. The hydrolysis of the cationic Schiff base V was studied by adding 1 μL of the CH_2Cl_2 solution in which it had been prepared to a thermostated UV cell.

Acknowledgment. Financial support of the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

Registry No. III, 78456-50-5; (*E*)-V·$F_3CSO_3^-$, 78456-52-7; (*Z*)-V·$F_3CSO_3^-$, 78456-54-9; 4-methylacetophenone, 122-00-9; 2-(methylamino)ethanol, 109-83-1; *N*-(α,4-dimethylbenzylidene)-2-methoxyethylamine, 78456-55-0; 2-methoxyethylamine, 109-85-3.

Influence of Urea–Water Interactions on the Transition-State Structure for the Hydrolysis of 1-Acetylimidazolium Ion and 1-Acetyl-3-methylimidazolium Ion[1]

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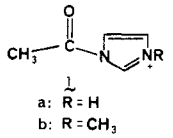
The hydrolysis of 1-acetylimidazolium ion and 1-acetyl-3-methylimidazolium ion has been investigated in the presence of 3 M urea. Urea significantly reduces the magnitude of the solvent deuterium isotope effect. The transition-state structure for hydrolysis is proposed to involve proton transfer from the nucleophilic water molecule of a water–urea complex. This proposed transition-state structure is consistent with the linear plot of the observed rate constant vs. the atom fraction of deuterium in the solvent. A pH–rate profile has also been determined.

There is general agreement, based on solvent isotope effects,[2,3] Brønsted plots,[2,3] and proton inventories,[4-6] that the hydrolysis of 1-acetylimidazolium ion (**1a**) and 1-acetyl-3-methylimidazolium ion (**1b**) occurs via a transition state containing a catalytic proton bridge between the reorganizing substrate and a water (base) molecule as shown in **2**. This transition-state structure has been suggested on the basis of the proton inventory studies done on **1**.[4-6] Isotope effect contributions from three protons (H_a and both H_b protons) are observed when water is the catalyzing base while a single proton (H_a) exhibits an isotope effect when a base such as imidazole catalyzes the reaction.[4-6]



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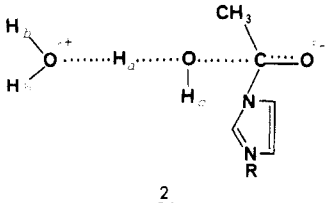

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Although water–urea systems have been the subject of much study, there is no general agreement as to whether

(1) This work was supported by the National Institutes of Health (Grant No. 1 R01 GM 25433) and, in part, by the Robert A. Welch Foundation.
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Table I. First-Order Rate Constants for the Hydrolysis of 1-Acetylimidazolium Ion in Mixtures of HCl-H_2O and DCl-D_2O in the Presence of 3 M Urea/Urea-d_4 at 25.00 ± 0.05 °C [a]

atom fraction of deuterium (n)	10^2k_n, s^{-1}	10^2k_n (calcd), [c] s^{-1}
0.000	4.74 ± 0.09 [b]	4.74
0.247	4.11 ± 0.06	4.10
0.495	3.40 ± 0.06	3.46
0.742	2.70 ± 0.04	2.82
0.989 [d]	2.13 ± 0.05	2.18

[a] Ionic strength was maintained at 0.2 M with KCl. Five runs were carried out in each case. [b] Error limits are standard deviations. [c] Calculated on the basis of model **6** by using eq 2 with $\phi^* = 0.455$. [d] Atom fraction of deuterium in "100"% 0.02 M DCl-D_2O as determined by Mr. Josef Nemeth.[30]

Table II. First-Order Rate Constants for the Hydrolysis of 1-Acetyl-3-methylimidazolium Ion in Mixtures of HCl-H_2O and DCl-D_2O in the Presence of 3 M Urea/Urea-d_4 at 25.00 ± 0.05 °C [a]

atom fraction of deuterium (n)	no. of runs	10^2k_n, s^{-1}	10^2k_n (calcd), [c] s^{-1}
0.000	11	5.03 ± 0.09 [b]	5.03
0.247	5	4.23 ± 0.17	4.32
0.495	7	3.52 ± 0.13	3.60
0.742	5	2.82 ± 0.10	2.89
0.989 [d]	6	2.12 ± 0.05	2.17

[a] Ionic strength was maintained at 0.2 M with KCl. [b] Error limits are standard deviations. [c] Calculated on the basis of model **6** by using eq 2 with $\phi = 0.426$. [d] Atom fraction of deuterium in "100"% 0.02 M DCl-D_2O as determined by Mr. Josef Nemeth.[30]

urea behaves as a nonelectrolyte, a base, or a zwitterion in aqueous solution.[7] However, the high solubility of urea in water, the ideal nature of the solutions it forms with water, and the similarity of the limiting heat of solution and heat of fusion of urea indicate that urea–water interactions in solution are energetically similar to those urea–urea interactions in the fused state and to water–water interactions in pure water. It is then of considerable interest to investigate the influence of added urea on the transition-state structure for the hydrolysis reactions of **1**.

Results

The hydrolysis of **1a** and **1b** has been investigated at pH 1.5 and 2.0 or the equivalent pD, respectively, at 25 °C in the presence of 3 M urea (urea-d_4) in H_2O, D_2O, and H_2O–D_2O mixtures. The ionic strength was maintained at 0.2 M with potassium chloride. The rate constants for hydrolysis are collected in Tables I and II. Also included in the tables are rate constants calculated on the basis of a transition-state structure discussed below.

Figures 1 and 2 are plots of the hydrolysis rate constant (k_n) vs. the atom fraction of deuterium (n) in the solvent for **1a** and **1b**, respectively. The solvent isotope effects, k_{H_2O}/k_{D_2O}, of 2.20 and 2.38 are lower than the literature values of 2.58 and 2.56, respectively, for **1a** and **1b**, in the absence of urea.[4,6] The solid lines drawn through the data points in these figures are based on the chemical transition-state model discussed below and represent the calculated rate constants in Tables I and II.

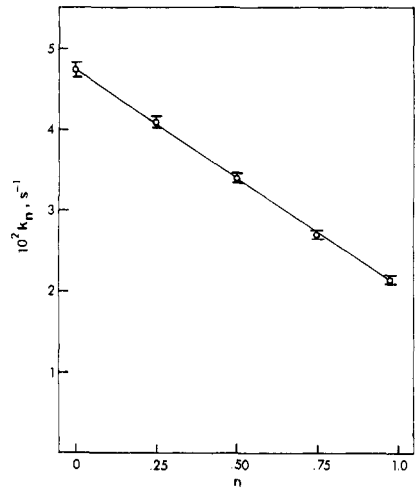

Figure 1. Proton inventory for the hydrolysis of 1-acetylimidazolium ion in the presence of 3 M urea/urea-d_4. The data are from Table I. The solid line is drawn through the calculated rate constants based on eq 2 with $\phi^* = 0.455$. Error bars represent standard deviations.

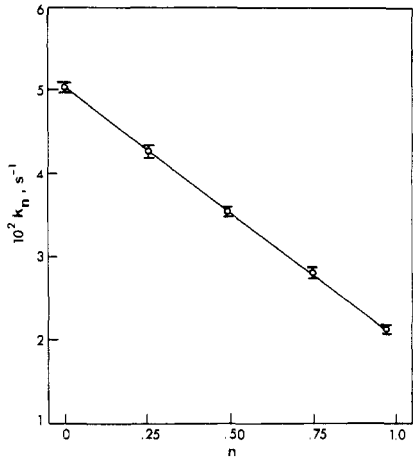

Figure 2. Proton inventory for the hydrolysis of 1-acetyl-3-methylimidazolium ion in the presence of 3 M urea/urea-d_4. The data are from Table II. The solid line is drawn through the calculated rate constants based on eq 2 with $\phi^* = 0.426$. Error bars represent standard deviations.

Table III. First-Order Rate Constants for the Hydrolysis of 1-Acetylimidazolium Ion at Different Ionic Strengths in HCl-H_2O Containing 3 M Urea at 25.00 ± 0.05 °C

ionic strength [a]	10^2k, s^{-1}	ionic strength [a]	10^2k, s^{-1}
0.2	4.74 ± 0.09	1.2	3.35 ± 0.19
0.4	4.32 ± 0.12	2.0	2.61 ± 0.11
0.8	3.87 ± 0.08		

[a] Maintained with potassium chloride.

The influence of ionic strength upon the hydrolysis rate constants for 1-acetylimidazolium ion (**1a**) is reported in Table III. The decrease in rate constant with increasing ionic strength is similar to the effect observed in the absence of urea.[2] The rates of hydrolysis of **1a** and **1b** as a function of pH in the presence of urea are shown in Figure 3. These pH–rate profiles are very similar to those observed in the absence of urea.[2,3,10]

Discussion

The utilization of solvent isotope effects to identify the role of proton transfers is well documented.[11] Recently,

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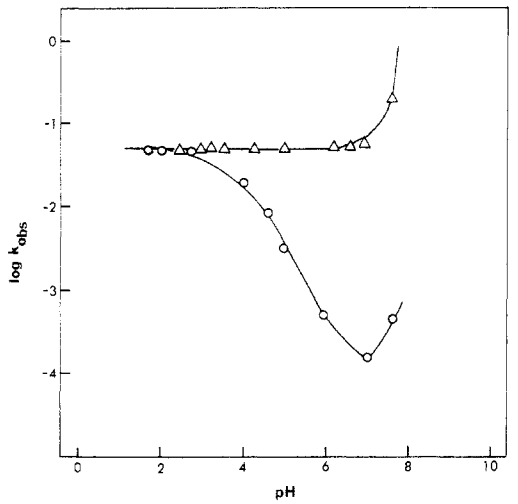


Figure 3. Hydrolysis of 1-acetylimidazolium ion (○) and 1-acetyl-3-methylimidazolium ion (△) as a function of pH at 25.00 ± 0.05 °C. The pH was adjusted by adding different concentrations of HCl to water containing 8 M urea.

the proton inventory technique[12] has been treated in several reviews,[11-13] so our discussion of the theory will be limited to those aspects directly related to the present situation. Discussions of the technique as applied in detail to the hydrolysis of **1a** and **1b** in the absence of urea may be found in previous studies.[4-6]

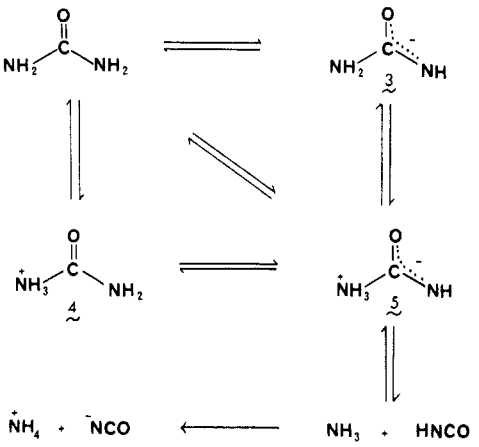
It can be shown that curvature in proton inventory plots results from multiple proton contributions to the observed solvent deuterium isotope effect. Linear proton inventories will result when only a single proton contributes the entire solvent isotope effect except under highly fortuitous conditions.[5,11f,14]

The linear dependence of k_n on n for the hydrolysis and **1a** and **1b** in the presence of 3 M urea, apparent in Figures 1 and 2, indicates that the hydrolysis of both compounds involves a transition state in which a single proton contributes the entire observed solvent isotope effect. This is in obvious contrast to the previous studies in the absence of urea where the proton inventories exhibited significant downward curvature resulting from the contributions of three protons.[4,6]

The observed pH–rate profile (Figure 3) in the presence of 3 M urea and its similarity with the earlier results indicate that the hydrolysis is obeying the same rate law as previously found.[2,3,10] This result, coupled with the similarity of the ionic strength effect in the absence or presence of urea, indicates that the kinetics of the hydrolysis of **1a** and **1b** remain essentially the same with regard to the nature of the substrate, the nature of the interactions, and the nature of charge distributions. The problem is how to reconcile these results with the highly dissimilar proton inventories observed in the absence and presence of urea.

A similar linear proton inventory has been observed for the intermolecular, imidazole-catalyzed hydrolysis of 1-acetylimidazole and ethyl trifluorothioacetate.[5] Here imidazole simply acts as a general-base catalyst to abstract a proton from the attacking water molecule. In the present case it is unlikely that urea serves as a base in the classic sense for three reasons: (i) it is not certain whether urea behaves as a base in aqueous solution,[7] (ii) a linear proton inventory is obtained only at urea concentrations ≥3 M, and (iii) no substantial increase in the rate of hydrolysis is observed with an increase in urea concentration at a constant pH.

Scheme I



One should also consider the influence of possible hydrolysis products of urea upon the system. The hydrolysis of urea in water to cyanate and ammonia is thought to involve a stepwise mechanism with the formation of an intermediate zwitterion (**5**) as shown in Scheme I.[15] The zwitterion is calculated to break down to cyanic acid and ammonia with a rate constant of 8×10^4 s^{-1}.[15] Therefore, its existence in a concentration high enough to effectively compete with water for a proton is unlikely.[15]

The possibility of general-base catalysis by species such as **3** and **4** appears to be ruled out for the following reasons: (i) the pH employed in the present study is unfavorable for the formation of **3**, (ii) the observed increase in pH on dissolving urea in water previously adjusted to a known pH is inconsistent with the formation of **3**, and (iii) a decrease in pH which should favor the formation of **4** did not increase the rate.

A qualitative test, suggested by Werner,[16] indicated the presence of cyanate ions in 3 M aqueous urea solution under the experimental conditions. However, two experimental observations rule out a general-base role for cyanate ion. First, no enhancement of rate is observed upon addition of potassium cyanate to the system at constant pH. Second, proton inventory curves for the hydrolysis of **1a** and **1b** in the presence of initially added 0.02 M cyanate exhibited significant downward curvature in contrast to the linear inventories observed without added cyanate. It thus appears that urea or one of its hydrolysis products does not function as a classic general base to produce the observed linear proton inventory.

There are two diverse schools of thought regarding the influence of urea on water structure. Urea has been both suggested as a disruptor of water structure[17,18] and an agent which strengthens and extends the hydrogen bonding of water.[19] The former view originates from observations of

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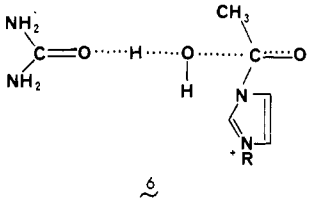
the effect of urea on phenomena such as hydrocarbon solubility,[17] micelle formation or association of colloids[18] whereas the latter rests on the observed increase in the dielectric constant of water produced by urea.[19]

Frank and Franks concluded, on the basis of thermodynamic properties of urea solutions, that urea displaces the equilibrium between two species of water, a bulky form involving long-range order and a dense form involving only short-range structure, in favor of the dense form.[20] This is due to the planar nature of urea which prevents it from taking part in extended tetrahedrally hydrogen-bonded clusters, although it most resembles the water molecule in its capacity to form hydrogen bonds.

Finer, Franks, and Tait used NMR relaxation and chemical shift measurements to conclude that in a concentrated urea solution the long range order characteristic of water is destroyed without being replaced by extended structures involving urea molecules.[21] On the other hand, there are extensive short-range, short-lived interactions between water and urea involving hydrogen bonds to the urea NH_2 groups and possibly to the carbonyl group. Thus, urea not only shifts the rapid equilibrium between polymeric and monomeric water completely in favor of the latter, but it also establishes a new equilibrium between monomeric water and the species resulting from the water–urea short-lived interactions. Thus, it should be possible to interpret the action of urea on the hydrolysis of **1a** and **1b** in terms of its influence on the water structure which in turn alters the nature of substrate–water interactions.

The short-range, short-lived, urea–water association through hydrogen bonds should induce a partial negative charge on the water–oxygen atom relative to the monomeric water. This association will lead to an enhanced kinetic nucleophilicity of water. These bimolecular associative clusters can be recognized as urea–water complexes and distinguished from the monomeric solvent waters which have recently been shown to bear no significant charge on the oxygen atom.[22] Thus, it is likely that it is the water of the water–urea complex which acts as the nucleophile to attack the carbonyl carbon of the substrate to give a tetrahedral intermediate as shown in **6**. We



6

propose the following mechanism to explain the influence of urea. The nucleophilic water–urea complex attacks to form the tetrahedral intermediate with partial transfer of the proton to the urea carbonyl in the transition state. Here the urea is an essential part of the tetrahedral complex, and so it acts as an internal base to remove a proton and generate a primary solvent isotope effect. In this context it is pertinent to point out that activated complexes may generate momentary sites of extreme acid–base character, which might produce extremely strong hydrogen-bonded complexes.[23] As a result, the slow step becomes independent of the urea concentration. It has been suggested that water is the dominant hydrogen bond donor in aqueous solution, leaving little or no chance for other hydrogen bond donors to compete with water for available acceptor sites on solute molecules.[24] In the present case, urea overcomes the competition from water by destroying the hydrogen-bonded network. This converts the network into the monomers which are converted into the water–urea complexes.

The reaction rate is not significantly affected by the presence or absence of urea for either **1a** or **1b**. This implies that proton transfer occurs with about equal ease irrespective of the presence of urea. This does not mean that partial proton transfer to urea is not occurring but only that such removal does not lead to a large rate acceleration because both water and urea are part of the hydrogen-bonded system of the tetrahedral complex.

Because of the stronger hydrogen bonding between urea and water and the enhanced nucleophilicity of the water of the urea–water complex, the transition state may be reached at an earlier stage relative to imidazole catalysis. This could account for the reduced solvent deuterium isotope effect compared to the classical general-base catalysis by imidazole. An earlier transition state has been postulated to account for the linear proton inventory observed for the water-catalyzed hydrolysis of bis(4-nitrophenyl) carbonate.[25]

It has been assumed that the hydrogen bonding in the urea–water complexes occurs through the carbonyl oxygen for the following reasons: (i) the pK_a of the protonated urea (carbonyl oxygen) is 0.2 while the pK_a for protonation on nitrogen is significantly less;[26] (ii) ND_3 is more electron donating than NH_3.[27]

We can analyze the proton inventory data using the "γ method" of Albery.[28] This method consists of the calculation of a quantity γ, defined by eq 1, by using the ex-

$$\gamma = 8 \ln [(k_{0.5}/k_0)/(k_1/k_0)^{1/2}]/[\ln (k_1/k_0)]^2 \quad (1)$$

perimental rate constants in a solvent of atom fraction deuterium of 0, 0.5, and 1.0. Although it is not obvious, $\gamma = 1$ when the proton inventory is linear and approaches zero as the number of hydrogenic sites contributing to the isotope effect approaches infinity. The γ values calculated in the present study are 0.84 ± 0.13 and 0.83 ± 0.14 for the hydrolysis of compounds **1a** and **1b**, respectively. These values and the experimental rate constants can be used to calculate the fractionation factor for the "in-flight" proton as 0.455 and 0.426 for **1a** and **1b**, respectively. These values and values for k_0 and n can be substituted into the appropriate form of the Gross–Butler equation for a single transition-state proton generating the entire solvent isotope effect (eq 2) to generate the calculated proton inventory (Tables I and II) represented by the solid lines in Figures 1 and 2.

$$k_n = k_0(1 - n + n\phi^*) \quad (2)$$

Conclusion

The influence of urea on the structure of water has been shown to have a very dramatic effect on the proton inventory for the hydrolysis of simple amides. This repre-

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(30) Mr. Josef Nemeth, Urbana, IL.

sents yet another case in which the proton inventory technique has been used to probe hydrolytic reactions in a perturbed aqueous system.[29] It will be interesting to see if this urea effect extends to other hydrolytic reactions.

Experimental Section

Materials. 1-Acetylimidazole, 1-acetyl-3-methylimidazole, and acetonitrile were prepared and purified as previously reported.[4,6] Deuterium oxide (99.75 mol % deuterium, Bio-Rad), deuterium chloride (20% solution in D_2O, Aldrich), urea (Fisher), and urea-d_4 (98+ atom % deuterium, Aldrich) were used as obtained.

Solutions. Stock solutions of urea were prepared by dissolving the appropriate amount of urea in water containing the necessary concentration of HCl to maintain the pH and enough potassium chloride to maintain the ionic strength at 0.2 M. Urea-d_4 was used in the same way with DCl in deuterium oxide to maintain the proper pD. The DCl-D_2O solutions were analyzed for deuterium content. Solvent mixtures were prepared by mixing appropriate volumes of the two stock solutions.

Kinetics. Hydrolysis of **1a** and **1b** was followed at 245 nm as reported earlier.[4-6] Spectral data were collected by using a Micromation computer interfaced to a Cary 118C spectrophotometer. Data points were taken at 1-s intervals for greater than 3 half-lives. The data were then analyzed by using a nonlinear least-squares computer program.

The pH of the solutions used to determine the pH-rate profile was adjusted in two ways with the kinetic results being identical. Different amounts of urea introduced into water previously adjusted to a known pH and the addition of HCl to solutions containing fixed urea concentrations were the methods used. All pH measurements were made by using a Corning 130 pH meter equipped with a combination electrode.

Acknowledgment. We are thankful to Mr. William P. Huskey for technical assistance in the early stages of this work.

Registry No. 1a, 31346-45-9; **1b**, 31399-05-0; urea, 57-13-6; water, 7732-18-5.

Ionization and Intramolecular Reactions of *N,N*-Bis[(2-pyridyl)ethyl]- and *N,N*-Bis[(2-pyridyl)methyl]maleamic Acids. An Enzyme Model

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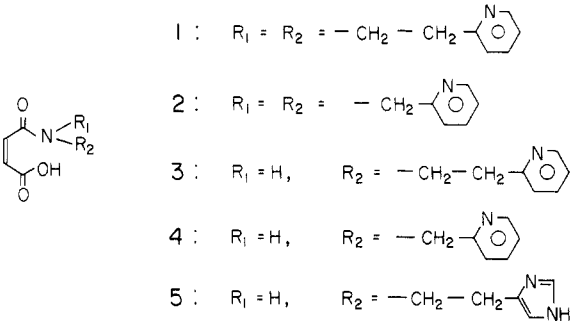
N,N-Bis[(2-pyridyl)ethyl]maleamic acid (1) and *N,N*-bis[(2-pyridyl)methyl]maleamic acid (2) underwent exclusive amide hydrolysis and intramolecular Michael-type addition, respectively. The pH profile of the pseudo-first-order rate constant for the reaction of 1 was a simple descending sigmoid inflecting at the pK_a of the carboxyl group. The pH profile of 2 was a composite of two bell-shaped curves which disclosed the abnormally low pK_a's of the carboxyl group and one of the two pyridinium groups. The change in the reaction path and the abnormal pK_a's observed with the structural variation in maleamic acid derivatives suggest that the change in enzyme specificity and the perturbed pK_a's of the active site functional groups can be achieved with a relatively loose geometry of the enzyme-substrate complex. The failure to observe the metal ion catalysis of the amide hydrolysis of 1 and 2 indicates that the metal complexation of the compounds is inefficient.

The conformation of an enzyme-substrate (ES) complex is extremely important for enzyme catalysis as it determines the relative positions of the functional groups of the enzyme and the substrate.[1,2] Thus, a slight modification of active-site residues or substrate structures often causes remarkable rate changes. However, a completely different reaction can occur upon the alteration of the conformation because both the enzyme active site and the substrate contain more than one reaction site.

One of the most important tools for the elucidation of enzyme mechanisms is the kinetic method.[3,4] Among the information provided by enzyme kinetics, the pH dependence of an enzymatic reaction discloses pK_a values which are utilized in identifying catalytically or conformationally essential functional groups. However, the ionization of an active site functional group can be perturbed by its environment so drastically that the assignment of the pK_a values is often very difficult.

Maleamic acid derivatives **1-5** can be viewed as a model for an ES complex with multiple functional groups. For example, the nucleophilic groups in **1**, the two pyridyl and



the carboxylate, correspond to the functional groups on the active site, and the electrophilic groups, the carbonyl and the olefinic, to those on the substrate. Thus, we have been interested in the effect of the structural variation in the maleamic acid derivatives on the relative reactivity of the two reaction paths indicated by eq 1 and 2, in relation to the conformational requirements for the changes in enzyme specificity. With respect to the ionization of active-site functional groups, the maleamic acid derivatives can serve as a model since the electrostatic interactions of the charged atoms may alter the ionization behavior of the functional groups. In addition, the amide hydrolysis of **1-5** in the presence of transition-metal ions can be regarded as a model for carboxypeptidase A,[5,6] a carboxyl-

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