Kinetic and Mechanistic Effects of Ease of C–N Bond Breaking in Amide Hydrolysis. The Mechanisms of Hydrolysis of *N*-Acylimidazoles and *N*-Acylbenzimidazoles

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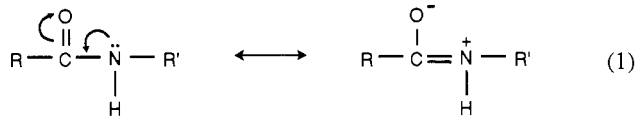
The hydrolysis reactions of *N*-acylimidazoles and *N*-acylbenzimidazoles have been actively studied since the early part of this century.[1-3] These compounds are amides but possess structural features that give rise to exceptional reactivity. For example, the second-order rate constant k_{OH} for alkaline hydrolysis of *N*-acetylimidazole is 316 M^{-1} s^{-1} at 25 °C,[4] whereas k_{OH} for hydrolysis of the reactive ester *p*-nitrophenyl acetate is only 15 M^{-1} s^{-1} at that temperature,[5] even though the pK_a of the imidazole leaving group is 14.5,[6] while that of *p*-nitrophenol is 7.0.

Resonance of the type shown in eq 1 markedly deactivates the carbonyl group of normal amides.



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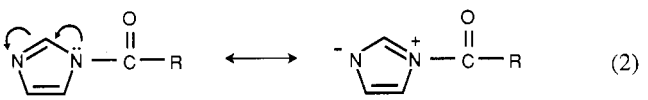
However, opposed resonance occurs with *N*-acylimidazoles in which partial negative charge is placed on N-3 (eq 2). Molecular orbital calculations on *N*-acetylimidazole[7] indicate that N-1 has a net charge of 0.475+, the carbonyl carbon has a net charge of 0.287+, and N-3 possesses a net negative charge of 0.23–. Thus, resonance involving N-1 and the carbonyl group is restricted, and the carbonyl group will be relatively reactive. Nucleophiles will attack the carbonyl carbon of *N*-acylimidazoles readily, and the C–N bond will break easily.



(2)

Twisting of an amide as it binds to the active site of a peptidase enzyme would also restrict the resonance of eq 1 and enhance the ease of C–N bond breaking. Such a twisting effect has often been suggested as an important feature of peptidase action (for an example, see ref. 8). *N*-Acylimidazoles, therefore, can be considered model amides which illustrate the results of restricted resonance with the carbonyl group in hydrolytic reactions. This is one of the features that has led to our interest in these reactive acyl derivatives, but their reactions also display other features that make them of general chemical interest. They display abnormal rate-accelerating steric effects in their hydrolytic reactions,[3,9-11] give extremely large rate enhancements in metal ion catalyzed reactions (the largest yet observed in amide hydrolysis),[12] and are excellent substrates for the proteolytic enzyme α-chymotrypsin,[13-15] thereby allowing discrimination between kinetically equivalent mechanistic possibilities for the enzyme reaction. *N*-Acylimidazoles are intermediates in bimolecular and intramolecular nucleophilic reactions of imidazole with esters[2b,16-18] and are possible intermediates in enzymatic acyl transfer reactions.[2] Mechanistic understanding of the reactions of *N*-acylimidazoles is, therefore, of both chemical and biochemical significance.

The observed rate constants for hydrolysis of *N*-acetylimidazole in H_2O at 25 °C follow eq 3, where K_a is the dissociation constant of the conjugate acid (pK_{app} = 3.6).[4] Thus, there is a reaction of the conjugate acid,

$$k_{obsd} = k_1\left[\frac{a_H}{(K_a + a_H)}\right] + [k_0 + k_{OH}a_{OH}]\left[\frac{K_a}{(K_a + a_H)}\right] \quad (3)$$

and there are also pH-independent and OH^--catalyzed reactions of the neutral species. Jencks and Carriuolo[4] found that k_1 is 2.5-fold less in D_2O than in H_2O, and the ΔS^* is –30.2 eu, which is in accord with attack of water on the protonated species and proton transfer in the transition state (1).[19]

Water Reactions. The reactions of *N*-acylimidazoles governed by k_0 also proceed more slowly in D_2O

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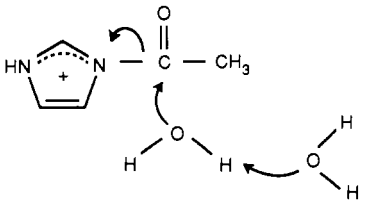

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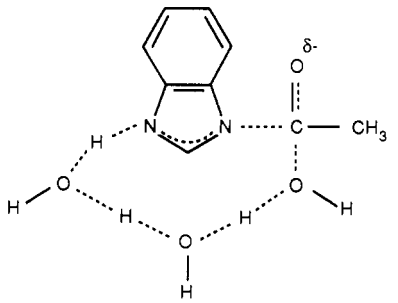
than in H_2O ($k_0^{H_2O}/k_0^{D_2O}$ = 2.7–3.5).[4,20] The k_0 values for pH-independent hydrolysis of *N*-acylbenzimidazoles are 5–10-fold less than those of the corresponding *N*-acylimidazoles.[20] A concerted process involving attack of a water molecule and simultaneous transfer of a proton to the leaving group **2** is consistent with the kinetic data. The *N*-acylbenzimidazole conjugate acid



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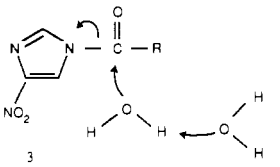
and leaving group pK_a values are ~2 pK_a units less than those of *N*-acylimidazoles. The facilitation of the nucleophilic reaction provided by the lower pK_a of the benzimidazole leaving group (12.8) will be offset by the greater difficulty of proton transfer to N-3. A similar mechanism was suggested in the hydrolysis of *N*-acetylimidazole,[19] acetyl-1,2,4-triazole,[21] and *N*-acetylbenzotriazole.[22] Partial proton transfer from water to the leaving group probably also occurs in other reactions of the neutral species. The D_2O solvent isotope effects in the OH^- reactions of substituted *N*-benzoylimidazoles (k_{OH}/k_{OD} ~ 1.0)[23] could be explained in that manner (the ratio is generally much less than 1.0).[2]

The pH-independent hydrolysis reactions of *N*-acyl-4(5)-nitroimidazole derivatives are rapid and extend from pH 6–7 to at least pH 1.0.[20] There is no indication of a conjugate acid reaction at pH 1 because of the low pK_a of the conjugate acid and the very favorable k_0. The k_0 for hydrolysis of *N*-(β-phenylpropionyl)-4(5)-nitroimidazole (10^{-2} s^{-1} at 30 °C) is 33-fold larger than in the hydrolysis of *N*-(β-phenylpropionyl)imidazole. Strong electron withdrawal in the leaving group will facilitate both nucleophilic attack by water and C–N bond breaking but will retard protonation. Therefore, partial proton transfer from water to the leaving group is not as important as in the reactions of unsubstituted *N*-acylimidazoles. Nevertheless, the D_2O solvent isotope effect ($k_0^{H_2O}/k_0^{D_2O}$) is 3.5. Nucleophilic attack of water is very likely assisted by general base catalysis involving a second water molecule **3**.

Marked catalysis by the base species of imidazole was also found in the hydrolysis of *N*-acetylimidazole,[4] and the solvent isotope effect ($k_{Im}^{H_2O}/k_{Im}^{D_2O}$) is 3.6. A



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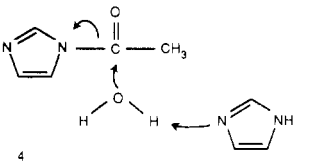

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general base mechanism **4** was suggested. General acid catalysis by imidazolium ion or a kinetic equivalent also occurs.[9] Kinetic general acid catalysis by carboxylic acids such as acetic acid was observed,[4] but in the hydrolysis of the 4(5)-nitro derivatives there is strict general base catalysis by acetate,[20] which again indicates the lessened importance of proton transfer to the leaving group when its pK_a is low [4(5)-nitroimidazole has a pK_a of 9.1].[2b,24] Imidazole acts as a nucleophile toward the 4(5)-nitro derivatives.[20]

In the general base catalyzed reactions the Bronsted coefficient, β (slope of a plot of the logarithms of the second-order rate constants vs the pK_a of the catalyst conjugate acid), is 0.55 with *N*-acetylimidazole,[25] 0.34 with *N*-acetylimidazolium ion,[25] and 0.50 with *N*-(3,3-dimethylbutyryl)-4(5)-nitroimidazole.[20] Thus, with the pK_a of the leaving group varying from 14.5 to 7, the β value changes but slightly. The most likely rate-determining step involves nucleophilic attack by a water molecule as in **4**. Choi and Thornton[23] have argued that the proton is not transferred in this transition state but is hydrogen bonded to the base. The transition state was considered to resemble a tetrahedral intermediate. A transition state with nearly complete C–O bond formation is not, however, in accord with the steric rate acceleration produced by branching in the acyl group.[9,10]

Steric Effects. The reactions of *N*-acylimidazoles provide highly unusual examples of steric acceleration of bimolecular reactions. Staab[3] had found that *N*-(trimethylacetyl)imidazole hydrolyzes faster than *N*-acetylimidazole in conductivity water (pH not specified). We found that the abnormal steric effect also extends to the OH^--catalyzed, conjugate acid,[10] water,[20] and imidazole-catalyzed reactions.[9] In these reactions the rate constants are approximately 2–8-fold larger for hydrolysis of the trimethylacetyl derivative than the acetyl. In contrast, a normal steric effect in a bimolecular reaction produced by a trimethylacetyl acyl group should give a rate retardation of 50–100-fold, as exemplified by the imidazole and OH^- nucleophilic reactions of *p*-nitrophenyl esters.[9] The steric accelerating effect is due primarily to branching at the α-carbon of the acyl group. Branching at the β-carbon retards the rate; *N*-(3,3-dimethylbutyryl)imidazole hydrolyzes approximately 6-fold more slowly than *N*-acetylimidazole. However, the effect is 150 in the reaction of imidazole with the corresponding *p*-nitrophenyl esters.[9] Thus, the accelerating effect caused by acyl group branching is superimposed on the rate-retarding effect of steric restriction of the approach of a nucleophile to the carbonyl group. Little C–O bond making with the nucleophile in the transition state would explain the absence of a major rate-retarding effect of acyl group branching, but could not explain the observed rate-enhancing effect.

Steric inhibition of resonance between N-1 and the carbonyl group (see eq 1) is clearly not responsible for

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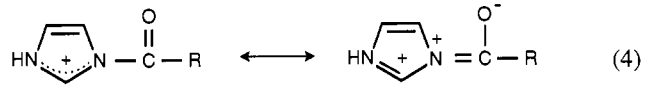
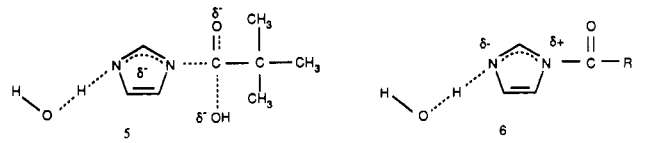
the accelerating effect of acyl group branching. Such a resonance effect would be greatly diminished by protonation of N-3 (eq 4), yet the order of reactivity for a large series of *N*-acylimidazole conjugate acids is the same as in the OH^- reaction, and differences in the rate constants are nearly the same in these reactions.[10]



(4)

N-Acetyl-*N'*-methylimidazole is a good model for reactions of protonated *N*-acetylimidazole (rate constants are nearly the same),[19a] thereby specifying that the proton is on N-3 in the conjugate acid.

A possible explanation for the observed steric effects is that while nucleophilic attack is retarded by the branched acyl groups, the ease of C–N bond breaking is enhanced by the relief of steric strain.[9] Breakdown of a tetrahedral intermediate is probably not rate limiting in either the OH^- or conjugate acid reactions of *N*-acylimidazoles, as shown by the slight ^{18}O incorporation into the carbonyl group when the hydrolysis reactions are carried out in water enriched with ^{18}O.[26,27] The β_{lg} value of –0.28 in the OH^--catalyzed reaction for substitution in the imidazole leaving group[20] (slope of a plot of log k_{OH} vs the pK_a of the substituted imidazole) is consistent with rate-determining nucleophilic attack by OH^-.[2] Therefore, if the enhancement of C–N bond breaking is a factor in producing the steric effects, then the hydrolysis reactions must be concerted **5**, i.e., a stable tetrahedral intermediate does not exist. A concerted reaction should be greatly accelerated by the presence of a water molecule functioning as a general acid. Likewise, either the reactions of *N*-acetylimidazole and *N*-acetylimidazolium ion with trifluoroethoxide ion are concerted or, if a tetrahedral intermediate is formed, it cannot be sufficiently stable to be at equilibrium with respect to proton transfer.[25]



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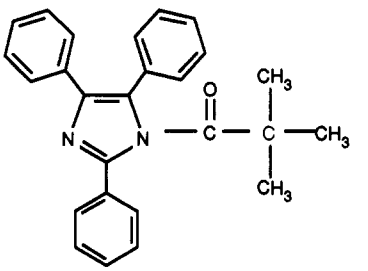
Restriction of water in the ground state of the *N*-acylimidazoles could also explain in part the abnormal steric effects. The translational entropy of some or all of the water molecules required for solvation of the transition state would not then be lost. The partial negative charge of N-3 would promote hydrogen bonding of a water molecule **6**,[28] and the ordering of solvent about the molecule could be further enhanced by a large hydrocarbon residue in the acyl group. The normal steric effects for aminolysis reactions of *N*-acylimidazoles in tetrahydrofuran[3] suggest that the solvent plays an important role in determining the relative reactivity.

The hydrolysis of *N*-acylimidazolium ions is characterized by large compensating changes in ΔH^* and ΔS^* as HCl concentration is increased from 0.1 to 6 M.[11] There is no effect on k_{obsd} of increasing HCl in that concentration range in the hydrolysis of *N*-(3,3-dimethylbutyryl)imidazolium ion (in contrast with the negative effect found when the acyl group is small), but ΔS^* (at 30 °C) becomes more favorable by +13.9 eu as ΔH^* becomes less favorable by 4.2 kcal/mol. Increasing the concentration of NaCl at 0.1 M HCl produces increases in ΔH^*. When the activity of water is held nearly constant in LiCl–HCl solutions of constant Cl^-, k_{obsd} increases (nonlinearly) as the acid molarity is increased, even though the substrate is completely in the conjugate acid form. The increasing concentration of protons specifically produces the rate increase and the more positive ΔS^*. The proton is a strong water structure former[29] and may facilitate a favorable orientation of water about the branched *N*-acylimidazolium ion.[11]

Steric bulk in the imidazole leaving group has a large accelerating effect on the pH-independent water reaction.[30] *N*-Acetyl-2,4,5-triphenylimidazole has at 25 °C a k_{OH} that is 2-fold less than that of *N*-acetylimidazole, but the water reaction is enhanced 39-fold by the triphenyl substitution. This results in a hydrolysis reaction that is pH independent in the range pH 4–9. The pK_a of the triphenylimidazole leaving group is less than that of imidazole,[31] but steric bulk in the leaving group is large. *N*-(Trimethylacetyl)-2,4,5-triphenylimidazole (**7**) hydrolyzes 20-fold faster than the corresponding acetyl derivative in the OH^- reaction and 3-fold faster in the water reaction, even though steric hindrance to approach of the carbonyl is extreme. Thus,



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steric accelerating effects in the acyl group and the leaving group are additive, which again suggests an effect on the ease of C–N bond breaking. *N*-(Trimethylacetyl)-4,5-diphenylimidazole hydrolyzes about 3-fold faster than the 2,4,5-triphenyl derivative, thereby making it one of the most reactive *N*-acylimidazoles that has been studied.

Intramolecular Reactions. The participation of an intramolecular carboxyl group[32] and a neighboring acetamido group[33] has been studied in reactions of *N*-acylimidazoles. Intramolecular acetamido attack on esters[34,35] proceeds via attack of the anionic species; plots of log k_{obsd} vs pH have slopes of 1.0. An oxazolinone

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(31) The pK_a values for 2,4-diphenylimidazole and 4,5-diphenylimidazole are 12.53 and 12.80, respectively. Schofield, K.; Grimmet, M. R.; Keene, B. R. T. *Heteroaromatic Nitrogen Compounds, The Azoles*; Cambridge University Press: Cambridge, 1976; p 283.
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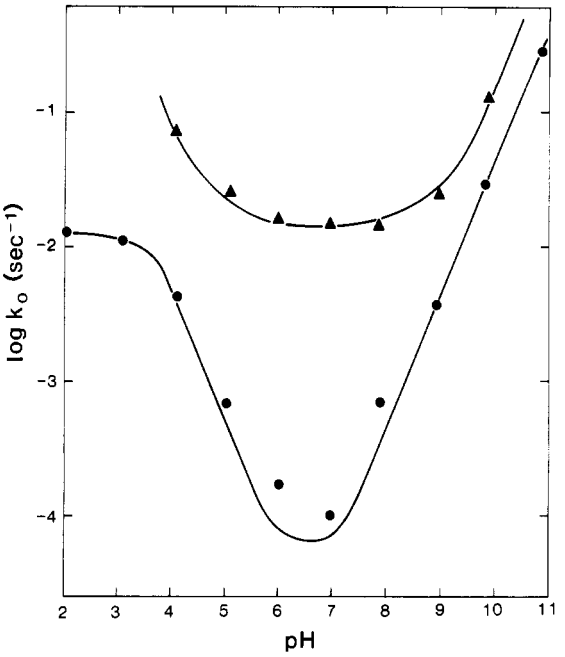
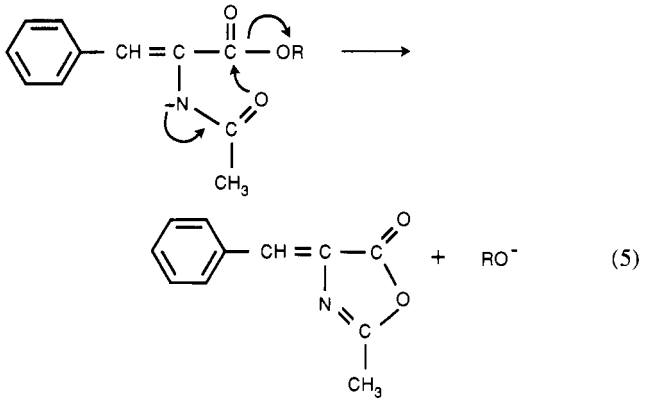


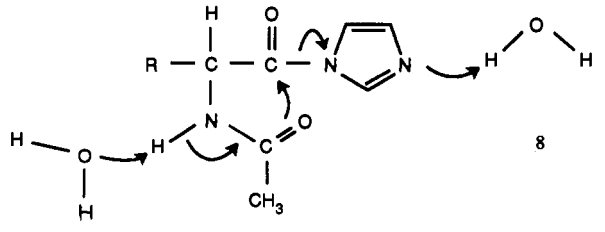
Figure 1. Plot of log k_{obsd} vs pH for the disappearance of *N*-[α-(acetylamino)isovaleryl]imidazole (▲) and *N*-isovalerylimidazole (●) in H_2O at 30 °C and μ = 0.1 M.

can be identified as the product (eq 5). Rate enhance-



(5)

ments for expulsion of the leaving group are large when its pK_a is low (10^6-fold with the *p*-nitrophenyl ester in comparison with OH^- attack on the corresponding ester without a neighboring group). In contrast, there is little enhancement of the OH^- reactions of *N*-acylimidazoles by a neighboring acetamido group.[33] However, the pH-independent reactions are accelerated by factors of 100–250-fold, as seen in Figure 1. The cyclization reactions proceed 2.0–2.2-fold more slowly in D_2O than in H_2O and are subject to kinetic general acid catalysis, which indicates that proton transfer occurs in the transition state. Therefore, the pH-independent water reactions very likely occur via the neutral species 8 rather than



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the kinetically equivalent attack of the acetamido anion

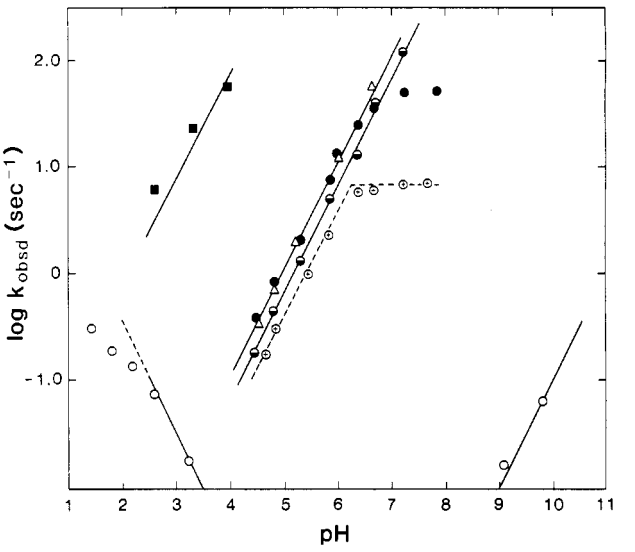
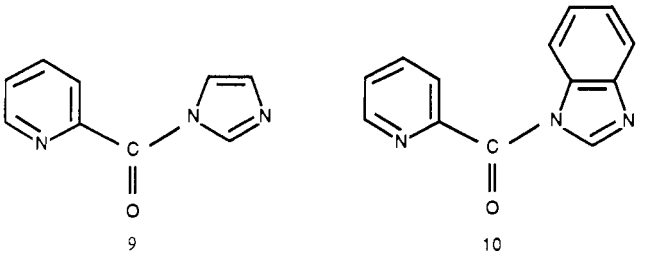


Figure 2. Plot of log k_{obsd} vs pH for the hydrolysis of *N*-(6-carboxypicolinoyl)benzimidazole (11) in H_2O at 30 °C with μ = 0.1 M (with KCl) in the presence of 0.001 M Cu^{2+} (■), 0.01 M Ni^{2+} (●), Co^{2+} (⊖), and Zn^{2+} (△), and 0.001 M Ni^{2+} (⊕) and in the absence of metal ions (○).

on the protonated species; C–N bond breaking must be sufficiently easy that the high concentration of the neutral species is more important than the greater nucleophilicity of the anion. The small rate enhancements at pH > 8 must be due primarily to competition from the facile OH^- hydrolytic reaction.

Metal Ion Catalysis. There are pronounced rate enhancements by the divalent metal ions Cu^{2+}, Ni^{2+}, Co^{2+}, and Zn^{2+} in the OH^--catalyzed hydrolysis of **9**–**11**.[12]



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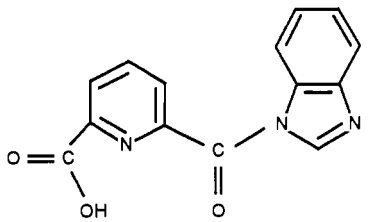

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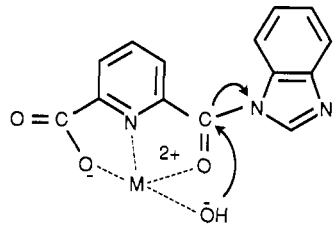
Significant metal ion effects were not observed in the hydrolysis of the *N*-isonicotinoylbenzimidazole. Therefore, the coordinating ligand must be adjacent to the carbonyl group. A chelation effect involving the pyridine nitrogen is very likely important.

Saturation effects occur in the metal ion enhanced hydrolysis of *N*-(6-carboxypicolinoyl)benzimidazole (11); plots of k_{obsd} vs metal ion concentration are hyperbolic at constant pH.[12] At saturating metal ion concentrations only OH^- reactions occur. As seen in Figure 2, the plot of log k_{obsd} vs pH in the Cu(II)-promoted reaction of 11 is linear with a slope of 1.0 at pH values as low as 3. The enhancements in the second-

order rate constants k_{OH} for the reactions of the 1:1 metal ion complexes range from 10^5–10^6 with Zn(II), Ni(II), and Co(II) to 10^9 with Cu(II). These are the largest effects by metal ions that have been found in amide hydrolysis. The k_{OH} for the Cu(II)-promoted reaction is 5.4×10^{11} M^{-1} s^{-1} at 30 °C, a value which exceeds that of a rate constant for a diffusion-controlled reaction (10^{10} M^{-1} s^{-1}). Therefore, the reaction cannot involve attack of external OH^- on the metal ion complex but must be an example of intramolecular attack of metal ion bound OH^- as in **12**. This is also very likely



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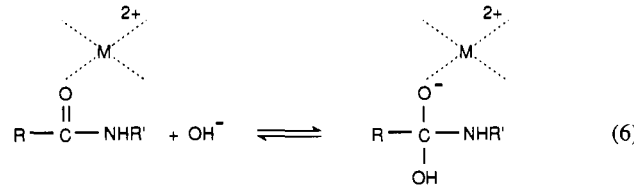
the case with the other metal ions, although the rate constants k_{OH} at 30 °C are less than the diffusion limit, ranging from 4.6×10^8 M^{-1} s^{-1} with Co(II) to 7.6×10^8 M^{-1} s^{-1} with Ni(II) and Zn(II). Increased strength of metal ion binding to the reactant, as with **11** in comparison with **9** or **10**, generally facilitates metal ion promoted OH^- reactions.[36]

At pH > 6 the Ni(II)-promoted hydrolysis of **11** becomes pH independent. The downward bend in the plot of log k_{obsd} vs pH cannot be due to an ionization [the pK_a of the aquo complex of Ni(II) is 10.6][37] but must reflect a change in rate-determining step with increasing pH. Replacement of a liganded water molecule in aquo complexes of Ni(II) is characterized by a rate constant of 3×10^4 s^{-1}, which is much less than the corresponding rate constants with Cu(II), Zn(II), and Co(II).[38] This step is probably rate determining in the formation of Ni(II) complexes. Formation of the Ni(II) complex of **11** must become rate limiting in the hydrolysis reaction when the OH^--catalyzed step becomes sufficiently rapid. Thus, at pH > 6.7 the hydrolysis reaction of **11** is so facile at a saturating concentration of Ni^{2+} (0.01 M; k_{obsd} = 50 s^{-1} at 30 °C) that formation of the metal ion complex is the rate-determining step.

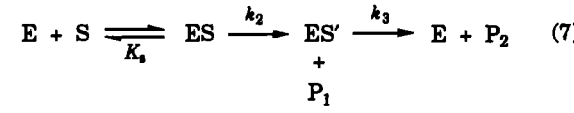
The large rate enhancements in the metal ion promoted hydrolysis reactions of *N*-acylimidazoles can be compared with the small effects that have often been found in amide hydrolysis.[2b,39–42] The poor leaving group of amides results in very unfavorable OH^--catalyzed reactions that proceed with rate-determining breakdown of a tetrahedral intermediate (C–N bond breaking).[2] Metal ion complexation of the carbonyl oxygen will then enhance the nucleophilic attack step but will retard C–N bond breaking by preventing electron release from oxygen (eq 6), thereby reducing the efficiency of metal ion catalysis. In contrast, the



(6)

imidazole and benzimidazole leaving groups of **9**–**11** permit nucleophilic attack by OH^- to be rate determining (nucleophilic attack will be rate determining if the reaction is concerted or if the leaving group departs from a tetrahedral intermediate faster than the intermediate reverts back to reactants). Polarization of the carbonyl group by a metal ion should be effective in producing large catalytic effects when nucleophilic attack is the rate-determining step. Thus, the key factor leading to the effective metal ion catalysis in the hydrolysis of *N*-acylimidazoles must be the easily broken C–N bond.

Enzymatic Reactions. α-Chymotrypsin follows the scheme shown in eq 7, in which ES′ is an acyl derivative of Ser-195.[2,43] This scheme is followed with both specific



(7)

and nonspecific ester and amide substrates. *N*-Acylimidazoles are excellent substrates for α-chymotrypsin.[13–15,44] For example, *N*-(β-phenylpropionyl)imidazole has a k_2/K_m (pH 7.5 and 30 °C) of 1.2×10^6 M^{-1} s^{-1},[13] which makes it the best amide substrate known for the enzyme. In contrast, the specific amide substrate *N*-acetylphenylalaninamide has k_2/K_m = 1.5 M^{-1} s^{-1} at pH 7.9 and 25 °C.[45] The constant k_2/K_m is the second-order rate constant for reaction of substrate with the free enzyme to give the acyl enzyme intermediate. It is the constant of choice for relative rate studies because it is not affected by nonproductive binding. The formation of the same acyl derivative of Ser-195 in the reactions of the *N*-acylimidazoles and the corresponding *p*-nitrophenyl esters was demonstrated.[13]

The rate constants for acylation of α-chymotrypsin by the *N*-acylimidazoles are highly dependent on the hydrophobic nature of the acyl group. The plot of $\log(k_2/K_m)$ at pH 8 vs the Hansch hydrophobicity constants π[46] is linear[15] with a slope of 1.71 (Figure 3). Employing both the π constants and the Taft steric constants E_s,[47] in the four-parameter equation 8,

$$\log(k_2/K_m) = \delta E_s + \gamma\pi + C \qquad (8)$$

γ = 1.88 and δ = 1.0 were obtained. Thus, there is a

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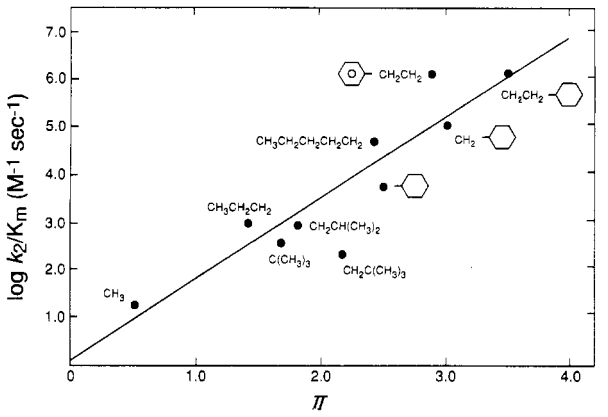
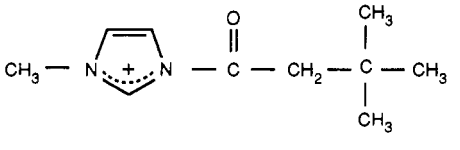


Figure 3. Plot of $\log(k_2/K_m)$ for acylation of α-chymotrypsin by *N*-acylimidazoles at 30 °C and pH 8.0 vs the Hansch hydrophobicity constant π.

more normal influence of steric bulk in the acyl group than in the nonenzymatic hydrolysis reactions,[9,47] but these steric effects are outweighed by the much greater dependence on π. In contrast, steric effects are the major influence in acylation by the corresponding *p*-nitrophenyl esters ($\delta = 0.95$, $\gamma = 0.63$).[48] There is a hydrophobic region adjacent to the enzyme active site,[2,49] and the acyl group of the substrate is very likely bound in that region. The differences in hydrophobic effects for the two types of substrates must be due in part to differences in transition-state structure. A hydrophobic region in the active site would be most favorable in a reaction having a relatively nonpolar transition state. A transition state in which there is only moderate bond making with the nucleophile (Ser-195) is indicated by the small Hammett ρ in acylation by substituted *N*-benzoylimidazoles[14] (0.9 at pH 7.5).

The values of k_2/K_m for acylation of the enzyme by the *N*-acylimidazoles are nearly pH independent from pH 5 to 9.[13-15] This is because both the conjugate acid ($pK_a < 4$) and neutral species are substrates with rate constants that do not differ greatly. The K_m values for nonionizing amide substrates are not affected by pH in the range pH 5–9.[45] Acylation reactions of α-chymotrypsin are normally influenced by an apparent pK_a near 6.5, which is presumably due to the dissociation of the conjugate acid of His-57. Thus, if k_2/K_m values for the *N*-acylimidazole conjugate acids are 10^2–10^3 greater than those for the neutral species, then the observed reactions will be nearly pH independent. That this is the case is shown by sigmoidal plots of k_2/K_m vs pH when the reaction of the neutral species is abolished with *N*-(3,3-dimethylbutyryl)-*N'*-methylimidazolium ion (**13**) ($pK_{app} = 6.6$),[13] or the reaction of the protonated species is abolished with *N*-(3,3-dimethylbutyryl)-4(5)-nitroimidazole ($pK_{app} = 6.5$).[30] The maximum value of



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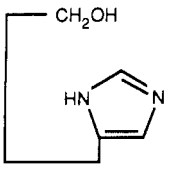
k_2/K_m for **13** at pH > 7.5 is only 160-fold larger than that of the neutral species *N*-(3,3-dimethylbutyryl)-imidazole, even though the pK_a of the *N*-methylimidazole leaving group (**7**) is over 7 pK_a units less than that of neutral imidazole. This corresponds to a β_{lg} of –0.3, comparable to that in the OH^--catalyzed hydrolysis of *N*-(3,3-dimethylbutyryl)-4(5)-substituted-imidazoles (–0.28).[20] The β_{lg} for acylation of the enzyme (k_2/K_m) by these 4(5)-substituted derivatives is also –0.3.[30] Thus, the transition states for the enzymatic and nonenzymatic reactions must be similar in regard to the amount of C–N bond breaking.

The *N*-acylimidazole substrates have allowed kinetically equivalent mechanistic possibilities to be distinguished in reactions catalyzed by α-chymotrypsin for the first time. A reaction via a neutral active site **14** or a zwitterionic active site **15** would give equivalent kinetic results.[50] From the dissociation constant of the



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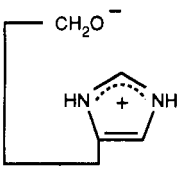

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serine hydroxyl group ($pK_a = 13.6$)[51] and the histidine conjugate acid ($pK_a = 6.6$), it follows that the concentration ratio of neutral to zwitterionic active sites is 10^7 at any pH. Consequently, if the acylation reaction of *N*-(β-phenylpropionyl)imidazole involved the zwitterionic active site **15**, then the true value of k_2/K_m would be 1.2×10^{13} M^{-1} s^{-1}. This value exceeds that of a diffusion-controlled reaction (10^8–10^9 M^{-1} s^{-1} in an enzyme reaction) and is therefore impossible. Attack of the serine anion on the *N*-acylimidazole conjugate acid can be ruled out for the same reason and also because serine anion attack does not take place in the reactions of the N-methylated derivatives. Thus, the reaction must involve the neutral active site (**14**). The near identity of the rate constants at low pH for the N-methylated and unmethylated derivatives indicates that the reaction involves the N-protonated species of the acylimidazole, not general acid catalysis by the histidine-57 conjugate acid.

The D_2O solvent isotope effect, $[k_2/K_m(H_2O)]/[k_2/K_m(D_2O)]$, is 3.1 (pD 7.5) in the acylation of α-chymotrypsin by **13**[13] and 2.1 in acylation by *N*-[*p*-(dimethylamino)benzoyl]-*N'*-methylimidazole.[14] Since $K_m(D_2O)$ would be expected to be less than $K_m(H_2O)$,[45,52] this indicates proton transfer in the critical transition state. Proton transfer to the leaving group of these substrates cannot occur; therefore, the only reasonable mechanism must involve proton transfer from serine-195 (**16**). The D_2O solvent isotope effect can distinguish between general base and nucleophilic mechanisms in simple chemical reactions but is ambiguous when applied to the reactions of most enzymes[53] because of unknown effects of D_2O on the enzyme conformation. However, Bender and co-workers[52] argued that the solvent isotope effect observed in α-chymotrypsin-catalyzed reactions is a genuine indication of mechanism. This argument is strongly supported by a small

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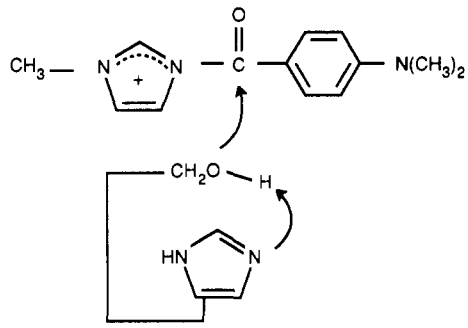

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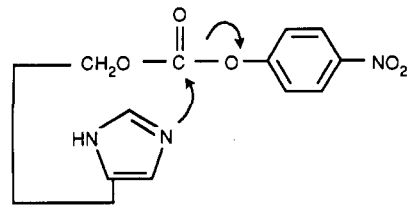
solvent isotope effect ($k_{H_2O}/k_{D_2O} \sim 1.5$) in the release of *p*-nitrophenol from *p*-nitrophenoxy carbonyl α-chymotrypsin **17**, a reaction that proceeds with nucleophilic attack by histidine.[54] Mechanism **16** is similar to that previously postulated for specific amide substrates, but without a histidinyl conjugate acid component in the breakdown of a tetrahedral intermediate and without the large uncertainty of kinetically equivalent possibilities.

Thus, *N*-acylimidazoles have been employed in both enzymatic and nonenzymatic reactions to answer questions that cannot be approached with conventional types of amides. It appears that the key structural features that give rise to the novel properties that these compounds display are the easily broken C–N bond and a partial negative charge on N-3, which will facilitate hydrogen bonding of the reactant with a water molecule or a general acid (an important factor when the leaving group has a $pK_a > 9.1$). The exceptional ease of C–N bond breaking then leads to hydrolytic reactions that have early transition states and that may proceed without stable tetrahedral intermediates. Qualitatively similar effects could occur in enzymatic reactions of peptides because of restriction of the nitrogen–carbonyl group resonance and a strained C–N bond.[55]



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