displacements at chlorine. The ρ value for this reaction is 3.87 indicating substantial negative charge buildup in the aromatic ring during the transition state. The acid-catalyzed reaction is more complex, presumably involving a protonation equilibrium for the *N*-chloroacetanilide prior to the rate-determining step. Consequently no clear correlation is discernable between the nature of the substituent and the rate of reaction.

Acknowledgment. Supported in part by Grants CA 25073 and CA28038 from the National Cancer Institute of the National Institutes of Health and by the Department of Energy contract DE-A C02-81 ER60015.

Registry No. *N*-Chloroacetanilide, 579-11-3; *N*-chloro-*p*-chloroacetanilide, 29551-85-7; *N*-chloro-*p*-nitroacetanilide, 79272-04-1; *N*-chloro-*p*-cyanoacetanilide, 14596-61-3; *N*-chloro-*p*-acetylacetanilide, 91238-44-7; *N*-chloro-*m*-chloroacetanilide, 29551-86-8; acetanilide, 103-84-4; *p*-chloroacetanilide, 539-03-7; *p*-nitroacetanilide, 104-04-1; *p*-cyanoacetanilide, 35704-19-9; *p*-acetylacetanilide, 2719-21-3; *m*-chloroacetanilide, 588-07-8; triethylamine, 121-44-8; phenoxide anion, 3229-70-7; phenol, 108-95-2.

Supplementary Material Available: A summary of specific reaction conditions and pseudo-first-order reaction rate constants (4 pages). Ordering information is given on any current masthead page.

Intramolecular Participation by a Neighboring Amide Group in the Hydrolysis of *N*-Acylimidazoles

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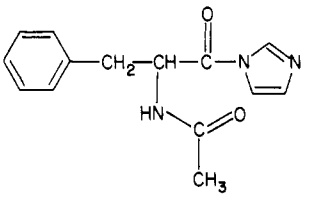
Rate constants have been determined for the disappearance of the *N*-acylimidazole derivatives of *N*-acetylphenylalanine and *N*-acetylvaline in H_2O at 30 °C. The pH–rate constant profiles are characterized by large pH-independent regions. These reactions are 2-fold slower in D_2O than in H_2O. The pH-independent reactions show rate enhancements of 100–250-fold in comparison with hydrolysis of the corresponding compounds *N*-(β-phenylpropionyl)imidazole and *N*-isovalerylimidazole, which lack an acetamido substituent. Thus, the neighboring acetamido groups are participating in the neutral species reactions. Nucleophilic attack by the acetamido oxygen occurs to form oxazolinone derivatives that were identified both spectrally and kinetically. Reversibility of this reaction was demonstrated in imidazole buffer. The reactions are general acid catalyzed by $H_2PO_4^-$. Therefore, proton transfer may take place in concert with nucleophile attack. The intramolecular nucleophilic reactions do not compete effectively with OH^--catalyzed hydrolysis, which illustrates the great facility of the latter reaction in the hydrolysis of *N*-acylimidazoles.

The hydrolysis reactions of *N*-acylimidazoles have been extensively studied.[1-8] General acid and general base catalysis by various buffers has been observed in these reactions.[2,4] However, there is little knowledge of intramolecular participation by neighboring groups other than carboxyl in the hydrolysis of such compounds.[9] Chemical intramolecular reactions bear a striking resemblance to the intracomplex reactions of enzymes.[10] Therefore, in view of the importance of determining the factors governing intramolecular reactions, we have studied the hydrolysis of the *N*-acylimidazole derivatives of *N*-acetylphenylalanine and *N*-acetylvaline (I and II), compounds having a neighboring acetamido group. Neighboring amide groups have been found to be powerful intramolecular nucleophiles in reactions of esters and amides.[1,11,12]



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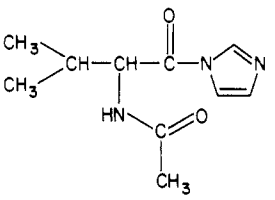

II

Experimental Section

Materials. The *N*-acylimidazoles I and II were prepared from *N*-acetyl-L-phenylalanine or *N*-acetyl-L-valine by reaction in dichloromethane with equimolar amounts of imidazole and dicyclohexylcarbodiimide. After 2 h at room temperature the mixture was filtered, and the dichloromethane was evaporated under reduced pressure. The residue was dissolved in chloroform and filtered. Ether was then added to the filtrate. The product crystallized from this mixture upon standing in the cold and was recrystallized from the same solvent. *N*-[α-(acetylamino)-β-phenylpropionyl]imidazole (I) had mp 99–101 °C. Anal. Calcd for $C_{14}H_{15}N_3O_2$: C, 65.35; H, 5.88; N, 16.33. Found: C, 65.14; H, 6.09; N, 16.09. *N*-[α-(Acetylamino)isovaleryl] imidazole (II) had mp 95–97 °C. Anal. Calcd for $C_{10}H_{15}N_3O_2$: C, 57.38; H, 7.22;

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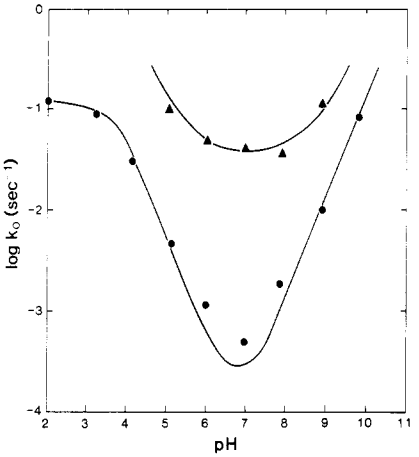

Figure 1. Plot of log k_{obsd} vs. pH for the disappearance of *N*-[α-(acetylamino)-β-phenylpropionyl]imidazole (▲) and *N*-(β-phenylpropionyl)imidazole (●) in H_2O at 30 °C and μ = 0.1 M.

Table I. Rate Constants for Disappearance of *N*-Acylimidazoles at 30 °C in H_2O (μ = 0.1 M)

compd	k_H,[a] M^{-1} s^{-1}	k_{OH}, M^{-1} s^{-1}	10^2k_2, s^{-1}
I	10000	5400	4.0
II	790	1070	1.5
N-(β-phenylpropionyl)imidazole	500	860	0.03
N-isovalerylimidazole	50	260	0.006

[a] $k_H = k_1/K_a$.

N, 20.08. Found: C, 57.46; H, 7.12; N, 20.07. *N*-(β-phenylpropionyl)imidazole and *N*-isovalerylimidazole were the same as previously reported.[5,7]

2-Oxazolin-5-ones were prepared from *N*-acetyl-L-valine or *N*-acetyl-L-phenylalanine by reaction in dichloromethane with an equimolar amount of dicyclohexylcarbodiimide. After 2 h at room temperature the mixture was filtered, and the dichloromethane was evaporated. The residue was then distilled under reduced pressure. 2-Methyl-4-benzyl-2-oxazolin-5-one had bp 83–85 °C (0.2 mm) (lit.[13] bp 118 °C (0.8 mm)). 2-Methyl-4-isopropyl-2-oxazolin-5-one had bp 52–53 °C (5.0 mm) (lit.[14] bp 45–48 °C (1.0 mm)).

Kinetic Methods. The rates of reaction of the *N*-acylimidazoles and the corresponding oxazolinones were measured by following the decrease in absorbance at 245 nm with a Pye Unicam SP8-100 or Beckman-25 recording spectrophotometer at 30 °C and μ = 0.1 M with KCl. In the kinetic experiments 25 μL of a stock solution of the *N*-acylimidazole in acetonitrile was added to 3 mL of aqueous buffer in the cuvette with stirring. Two consecutive pseudo-first-order reactions with widely separated rate constants, corresponding with formation and slower hydrolysis of an intermediate, were observed in the study of I and II in buffers other than imidazole. Pseudo-first-order rate constants were calculated by employing an IBM-370 computer. Reaction pH values were determined with a Radiometer Model 22 pH meter.

Results

In Figures 1 and 2 plots of log k_0 vs. pH are shown for the disappearance of *N*-(β-phenylpropionyl)imidazole and *N*-isovalerylimidazole at 30 °C in H_2O (μ = 0.1 M). Hydronium ion, hydroxide ion, and water-catalyzed reactions can be observed. The equation for k_0 is given in eq 1,

$$k_0 = k_1\left[\frac{a_H}{K_a + a_H}\right] + [k_{OH}(OH^-) + k_2]\left[\frac{K_a}{K_a + a_H}\right] \quad (1)$$

where K_a is the dissociation constant of the *N*-acylimidazole conjugate acid, k_1 is the rate constant for reaction of the protonated species, k_2 is the rate constant for the water-catalyzed reaction, and k_{OH} is the second-order rate constant for OH^- catalysis. Values of the constants are given in Table I. Measurements of k_{obsd} were made in HCl solutions or in 0.01 M buffer solutions. Buffer catalysis takes place, but it was shown that such low buffer concentrations do not have an experimentally significant effect on the observed rate constants. Also shown in Figures 1 and 2 are the plots of log k_0 vs. pH for the disappearance of I and II (the first step measured at 245 nm). Large plateau regions are observed in the profiles. The values of k_2 in Table I are over 100–250-fold larger than those for the compounds without a neighboring acetamido group. Thus, the neighboring group is participating in the neutral species reaction. However, it can be seen in Figures 1 and 2 and Table I that rate constants for the apparent OH^--catalyzed reactions differ only by 4–5-fold for the corresponding compounds. The pH-independent reaction is considerably slower in D_2O than in H_2O. Values of k_{obsd} measured at pD 6.25 and 7.05 gave $k_2^{H_2O}/k_2^{D_2O}$ = 2.2 for I and 2.0 with II.

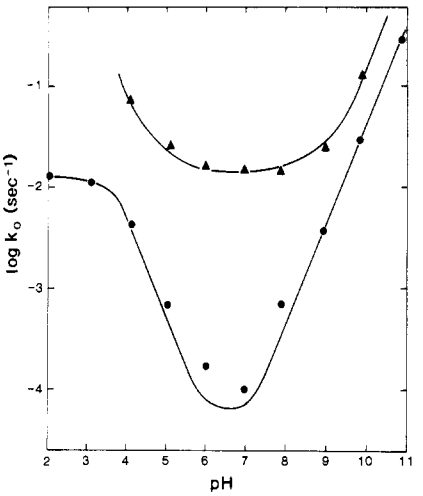

Figure 2. Plot of log k_{obsd} vs. pH for the disappearance of *N*-[α-(acetylamino)isovaleryl]imidazole (▲) and *N*-isovalerylimidazole (●) in H_2O at 30 °C and μ = 0.1 M.

The pH-independent reactions of I and II are strongly catalyzed by buffer. A plot (not shown) of k_{obsd} for the disappearance of I vs. the total concentration of phosphate buffer (5 points, 0.01 to 0.2 M) at the constant pH value of 6.45 was linear and showed 240% catalysis at the total phosphate concentration of 0.2 M. A similar buffer dilution at pH 7.02 also showed significant catalysis, but the slope of the linear plot of k_{obsd} vs. total buffer concentration was less than at lower pH. Therefore, it is clear that the acid species of the buffer or a kinetic equivalent is catalytically active. Second-order rate constants for phosphate catalysis k_{HA} are 0.77 M^{-1} s^{-1} for I and 0.18 M^{-1} s^{-1} with II. The values of k_{obsd} in phosphate buffers are large and extrapolate to the k_0 values on the log k_0 – pH profiles for the disappearance of I and II.

Plots of k_{obsd} for the disappearance of the acylimidazoles I and II vs. the concentration of imidazole buffer show a large linear effect of increasing buffer concentration. In Figure 3 the plot of log k_{obsd} vs. total imidazole concentration is shown for hydrolysis of II at pH 6.98. Values of the second-order rate constant k_{Im} for imidazole catalysis are 0.10 M^{-1} s^{-1} with I and 0.012 M^{-1} s^{-1} with II. However, the plots extrapolate to zero buffer concentration at points considerably below the pH-independent values of k_0 in Figures 1 and 2. The value of k_0 for II at zero imidazole

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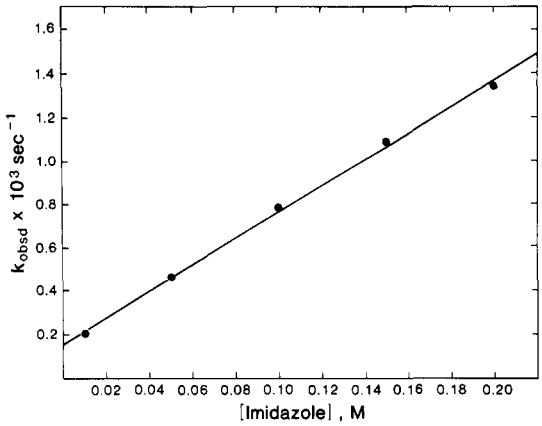


Figure 3. Plot of k_{obsd} vs. total imidazole buffer concentration for hydrolysis of II at pH 6.98 at 30 °C, μ = 0.1 M.

Table II. Values of the Rate Constants (k_{obsd}, s^{-1}) for Hydrolysis of 2-Methyl-4-benzyl-2-oxazolin-5-one and 2-Methyl-4-isopropyl-2-oxazolin-5-one in H_2O at 30 °C, μ = 0.1 M

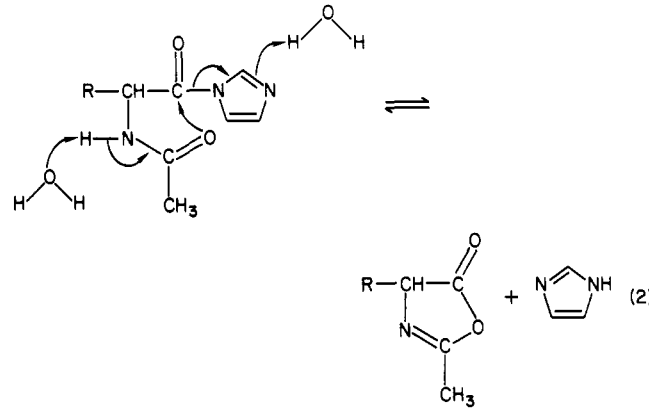
pH	buffer (0.01 M)	4-benzyl	4-isopropyl
5.09	acetate	7.45×10^{-5}	5.11×10^{-4}
6.00	cacodylate	3.44×10^{-4}	8.31×10^{-4}
6.98	phosphate	8.71×10^{-4}	8.46×10^{-4}
7.87	tris	1.92×10^{-3}	1.23×10^{-3}
8.97	borate	1.67×10^{-2}	5.31×10^{-3}

concentration in Figure 3 is only 1.6×10^{-4} s^{-1} at pH 6.98, near 100-fold less than the k_0 value from Figure 2. This indicates that an intermediate is being produced in the process which reacts with imidazole to regenerate the reactant, i.e., imidazole reverses the reaction. This behavior would be expected if oxazolinone intermediates are formed from I and II. Conclusive evidence that oxazolinone intermediates are formed in the cyclization reactions of I and II in 0.01 M buffers other than imidazole was provided by the UV spectra at the conclusion of N-acylimidazole disappearance; the spectra were those of authentic samples of the oxazolinones. The absorbance at 245 nm then declined slowly, and rate constants were the same as obtained in the hydrolysis of synthetically prepared samples of the appropriate oxazolinones.

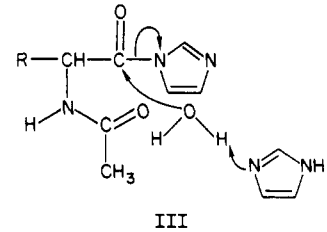
2-Methyl-4-benzyl-2-oxazolin-5-one and 2-methyl-4-isopropyl-2-oxazolin-5-one, the respective intermediates formed in reactions of I and II hydrolyze relatively slowly in the same buffers employed for monitoring acylimidazole disappearance. Values of k_{obsd} are given in Table II. There is a pH-independent water reaction at pH 5–7, and at higher pH values, hydroxide ion catalysis occurs. Thus, Figures 1 and 2 and the rate constants in Table I refer strictly to cyclization of the N-acylimidazoles to the oxazolinones. The slow hydrolysis of the intermediate does not interfere with measurement of those rate constants. Addition of oxazolinone to imidazole buffer resulted in a rapid large increase in absorbance at 245 nm followed by a slow decrease. Values of k_{obsd} determined for the absorbance decrease at 245 nm were the same in imidazole buffers for N-acylimidazole disappearance in reactions of I and II and oxazolinone hydrolysis, indicating that the equilibrium in those buffers lies very far on the side of N-acylimidazole.

Discussion

The log k_0–pH profiles for the disappearance of the N-acylimidazoles I and II indicate that both hydroxide ion and hydronium ion catalysis are occurring, and at pH 5–8 a large plateau is observed. The values of k_{OH} and k_H are similar to those of the appropriate reference compounds, N-(β-phenylpropionyl)imidazole and N-isovaleryl-imidazole, but the pH-independent reactions proceed approximately 100–250-fold more rapidly with the compounds having a neighboring acetamido group. Such large effects should not arise from the small inductive effect of an acetamido group (σ_I = 0.28),[15] and it will be noted that such effects in the OH^--catalyzed reaction must be small. Thus, it is clear that the neighboring group is participating but only in the neutral pH range. The neighboring acetamido group is clearly acting as an intramolecular nucleophile in view of the fact that an oxazolinone intermediate in the reaction was identified both spectrally and kinetically. The large D_2O solvent isotope effect in the pH-independent reaction and the observed general acid catalysis suggests that a proton transfer is taking place in the critical transition state rather than the kinetically equivalent intramolecular nucleophilic reaction of a zwitterionic species resulting from preequilibrium proton transfer. Therefore, at pH 5–8 the reaction of eq 2 is possibly occurring.



The oxazolinone intermediates hydrolyze relatively slowly to the N-acetyl amino acids. Therefore, the observed kinetics for acylimidazole disappearance are not affected by hydrolysis of the intermediate. This was confirmed by independently measuring the rate constants for hydrolysis of synthetically prepared oxazolinones. The reversibility of the reaction of eq 2 was demonstrated by measuring rate constants in imidazole buffers. These constants are the same for the disappearance of the N-acylimidazole or the oxazolinone determined separately. The rate constants extrapolate to a value at zero imidazole concentration far below the plateau in the log k_0–pH profiles for cyclization of the N-acylimidazole. Consequently, the equilibrium of eq 2 lies far to the left in the presence of high concentrations of imidazole, and the k_{obsd} values in imidazole buffer refer to general base catalyzed hydrolysis of the N-acylimidazole (III).



III

The equation for k_{obsd} in imidazole buffers derived from the scheme assuming reversible oxazolinone formation and rate-determining hydrolysis of the acylimidazole is given

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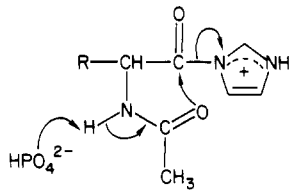
in eq 3. From the linear plot of Figure 3, which shows a

$$k_{obsd} = \frac{k_r(\mathrm{Im})^2}{K_{eq} + (\mathrm{Im})} \quad (3)$$

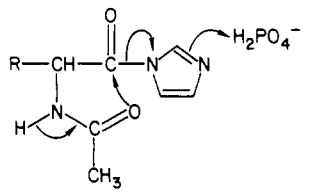
first-order dependence on imidazole concentration, it is clear that K_{eq}, the equilibrium constant for the reaction of eq 2, cannot be greater than 10^{-2} M (a second-order dependence on imidazole concentration would result if $K_{eq} > (\mathrm{Im})$).

The linear plots of k_{obsd} vs. phosphate buffer concentration in the reactions of I and II show that the intramolecular nucleophilic reactions involve kinetic general acid catalysis. Thus, the mechanism is very likely general base abstraction of a proton from the acetamido nitrogen



IV

in concert with nucleophilic attack by oxygen on the protonated species (IV) or the kinetically equivalent reaction of the neutral species in which a general acid donates a proton to the leaving group (V). Nucleophilic attack would be favored in IV in comparison with V be-



V

cause of the greater ease of negative charge development on the nucleophile, and the leaving group of IV would be improved by the complete protonation. On the other hand, the concentration of the reactive species would be higher in the case of V at pH values near neutrality (the pK_a of an *N*-acylimidazole is approximately 4).[2]

Intramolecular nucleophilic attack by a neighboring amide group occurs with great facility in reactions of esters and amides;[1,10-12] the minimum rate enhancement is 6×10^4 in the intramolecular reaction of *O*-acetylsalicylamide in comparison with bimolecular attack of 1 M acetamide on *p*-nitrophenyl acetate.[11] These reactions take place predominantly through the anionic species, i.e., apparent hydroxide ion catalysis is observed. It appears that nucleophilic reactions via the anionic species occur most readily with attack by nitrogen, whereas neutral species reactions take place with attack by oxygen.[1] In the reactions of the *N*-acylimidazoles I and II intramolecular attack by nitrogen is not favorable because of the steric situation; such attack would require a three-membered ring transition state. However, oxygen attack can occur via a kinetically favorable five-membered ring transition state, and consequently, rate enhancements are observed in the neutral species reactions at pH values where the concentration of OH^- is small.

Oxazolinone intermediates have previously been detected in the hydrolysis of *N*-acyl amino acid nitrophenyl esters,[16,17] but cyclization does not occur when the leaving group is poor, e.g., with the methyl or benzyl esters.[17] Only apparent OH^- catalysis was observed in the reaction of *p*-nitrophenyl hippurate even at pH values as low as 6, in contrast with the pH-independent reactions of the *N*-acylimidazoles I and II. The value of k_{OH} at 30 °C in the ester cyclization is 1.05×10^4 M^{-1} s^{-1},[16] which is only 2-fold larger than the k_{OH} value in reactions of I. Thus, the relatively small rate enhancements in the neutral species and OH^--catalyzed reactions of the acetamido-substituted *N*-acylimidazoles are not entirely a reflection of the fact that oxygen must act as the nucleophile but must be due primarily to the facile hydrolytic reactions with which the intramolecular nucleophilic reaction is in competition.

The lack of significant rate enhancements due to intramolecular participation by the neighboring acetamido group at high pH illustrates the great facility of the OH^--catalyzed hydrolysis reactions of *N*-acylimidazoles.[2,4] The rate constants for these reactions are remarkable when it is considered that the leaving group has a pK_a of 14.5,[18] only slightly less than that of methanol (15.5)[19] and over 7 pK_a units greater than that of *p*-nitrophenol. Nevertheless, k_{OH} at 25 °C for hydrolysis of *N*-acetylimidazole[2] is 316.6 M^{-1} s^{-1}, whereas k_{OH} for hydrolysis of *p*-nitrophenyl acetate[20] is only 14.8 M^{-1} s^{-1}. Hydrogen bonding of the solvent water to the leaving group of an *N*-acylimidazole may be improving the ease of bond breaking beyond that expected for an ester or an amide with a relatively poor leaving group. It should be noted that the OH^--catalyzed reactions of *N*-benzoylimidazoles have abnormal D_2O solvent isotope effects[21] which can be explained by proton transfer from H_2O in the transition state. On the other hand, the normal inverse D_2O solvent isotope effect (k_{OD}/k_{OH} = 1.2–1.4) is found in hydrolysis of N′-methylated derivatives[21] with which hydrogen bonding of water to the leaving group cannot occur. It is very likely the facility of the spontaneous hydrolysis reactions of I and II that is responsible for the small rate enhancements in the intramolecular reactions in comparison with those that have been observed in reactions of other types of esters and amides.[1,10,22]

Acknowledgment. This work was supported by research grants from the National Institutes of Health.

Registry No. I, 50676-36-3; II, 92220-20-7; D_2, 7782-39-0.

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