and liquid in the reaction vessel were analyzed by GLPC for isobutane and isobutylene vs. authentic standards (OV-101 column at 25 °C); the total liquid- and gas-phase volumes were measured and the yields of isobutane and isobutylene were then calculated. (Most of the isobutane and isobutylene was found in the liquid phase.) The remaining products in the solution (2,2,3,3-tetramethylbutane, neopentylbenzene, and bibenzyl) were identified and quantitatively determined by GLPC analysis (using biphenyl as an internal standard). No *tert*-butylbenzene was detected. Table I shows the results with both 0.02 and 0.1 M AIB.

Neopentylbenzene and *tert*-butyltoluene can be distinguished by GLPC with temperature programming (50 to 100 °C, program started at 9 min after injection, 50 °C/min) using the OV-101 column. The presence of neopentylbenzene in the reaction mixture was indicated by comparison of its GLPC trace with authentic neopentylbenzene and *tert*-butyltoluene. The presence of neopentylbenzene and not *tert*-butyltoluene was confirmed by comparing the mass cracking patterns (HP 5985 GLPC/MS system with a 6 ft 2% SE-30 column) of the corresponding peak in the reaction mixture with those of authentic compounds.[38] The two have very different MS patterns. For example, the largest four peaks in the spectrum for *tert*-butyltoluene (with % peak height) are: 133.0 (100%), 105.1 (41%), 148.1 (23%), and 93.1 (11%); those for neopentylbenzene are 92.0 (100%), 91.1 (44%), 148.1 (30%), 57.1 (29%).

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Registry No. AIB, 927-83-3; benzene, 71-43-2; toluene, 108-88-3; *m,m*-dimethyltoluene, 108-67-8; *p*-methyltoluene, 106-42-3; *m*-methyltoluene, 108-38-3; *p*-chlorotoluene, 106-43-4; *m*-chlorotoluene, 108-41-8; *m,p*-dichlorotoluene, 95-75-0; *p*-cyanotoluene, 104-85-8; *p-tert*-butyltoluene, 98-51-1; *tert*-butyl radical, 1605-73-8; benzyl radical, 2154-56-5; *tert*-butyl phenylperacetate, 3377-89-7; neopentylbenzene, 1007-26-7; $PhCH_2C(CH_3)_2CH_2C(CH_3)_3$, 81195-34-8.

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Carboxylic Acid Participation in Amide Hydrolysis. Evidence That Separation of a Nonbonded Complex Can Be Rate Determining

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Abstract: Maleamic acids derived from aliphatic amines of a wide range of basicity (compounds **1–8**) undergo hydrolysis in solutions of acidity between 10^{-4} M and 1 M hydrogen ion concentration by a mechanism involving participation of the carboxyl group at the adjacent amide. Kinetic analysis of the reaction (50 °C) reveals that the identity of the rate-determining step (or steps) is a function of both the basicity of the leaving group and the acidity of the solution. At pH 4, the rate-determining step is O to N proton transfer to form a zwitterionic intermediate for compounds with weakly basic leaving groups. For compounds with more basic leaving groups, conversion of the zwitterionic intermediate to products is rate determining. In more acidic solutions, where proton transfer is facilitated, diffusion apart from the complex formed from C–N bond breakage of the zwitterionic intermediate is rate determining for compounds with the most basic leaving groups. C–N bond breakage is rate determining for compounds with the least basic leaving groups. This suggests that at pH 4 the breakdown of the zwitterion may also involve rate-determining diffusion. It is concluded that other facile elimination reactions may involve rate-determining diffusion and that changes in the rate-determining step can serve as an indicator of its occurrence and as a means of calibration of rate constants. It is also suggested that since pepsin may catalyze peptide hydrolysis by a carboxyl-participation mechanism, the occurrence of sequential covalent intermediates may exist to permit diffusion of the noncovalently held products.

The transfer of the acyl group of an amide to an oxygen nucleophile may proceed through a tetrahedral intermediate from which a carboxyl group is generated by elimination of the amine derived from the amide.[1] The detailed mechanism of the elimination process is important for arriving at an understanding of catalysis (enzymic and nonenzymic) of these acyl transfer reactions. If the reaction occurs in neutral or acidic water, expulsion of the amine from the intermediate occurs faster than expulsion of water or an alcohol.[1] Thus, the rate-determining step for the overall transfer process occurs during formation of the adduct, and a kinetic study does not provide information that is pertinent to elucidating the mechanism of elimination of amine from the adduct.

If a carboxyl group adds to an amide, that group should leave more readily than the amine from the adduct. However, if the reaction is carried out in water, the carboxyl is such a poor nucleophile that water adds preferentially. If a carboxyl group is adjacent to an amide within a molecule, the addition barrier is greatly reduced.[2] As a result, amine elimination is rate determining in formation of an internal anhydride from a carboxylic acid amide, and kinetic studies of such a reaction bear directly on the elimination reaction.[2] *N*-Alkylmaleamic acids react by such a mechanism,[3] but mechanistic analysis of the elimination process from kinetic data requires a relationship for amine basicity and observed rates. Such a relationship is observed for *N*-arylmaleamic acids but not for the limited number of *N*-alkylmaleamic acids that had been studied.[3,4]

In the case of 2,3-dimethylmaleamic acids, however, we recently showed that some mechanistic complexity results from the fact that the acidity of the medium affects the identity of the rate-

(1) Williams, A. *J. Am. Chem. Soc.* **1976**, *98*, 5645.

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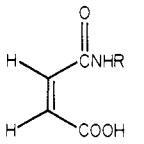
0002-7863/82/1504-2891$01.25/0

determining step.[5] We have extended the procedures of that study to the maleamic acid system. So that reaction patterns would be more readily discernible, we have utilized a series of primary alkyl amines as leaving groups whose basicities span a wide range (compounds **1-8**).



1, R = $CH_2CH_2CH_3$
2, R = $CH_2CH_2CH_2OH$
3, R = $CH_2CH_2OCH_3$
4, R = CH_2CH_2F
5, R = CH_2CO_2Et
6, R = CH_2CHF_2
7, R = CH_2CF_3
8, R = CH_2CN

We find that at pH 4, the rate-determining step is a function of the basicity of the leaving group, varying between proton transfer and amine elimination processes. In 1 M acid, however, proton transfer is always faster than amine elimination from the intermediate, yet another change in rate-determining step with leaving group basicity variation occurs. This requires that the elimination step is actually at least two kinetically distinct processes, bond-breaking and diffusional separation. Thus, a case in which diffusion is rate limiting can be identified and analyzed. The information can be applied to elimination reactions in general, including related enzymic processes. If diffusional separation is the slow step in a nonenzymic reaction, a similar enzymic mechanism would normally lead to a *slower* reaction from an intermediate because of the affinity of an enzyme for its reaction product. We suggest that an enzyme, such as pepsin, could operate through a mechanism involving a number of reactive covalent intermediates in order to permit the enzyme to isomerize to a low affinity form so that diffusional separation of products can occur.

Experimental Section

Materials. *N*-Alkylmaleamic acids were prepared by the reaction of maleic anhydride and the corresponding amine in ether or tetrahydrofuran at room temperature.[3] All amines except 2,2-difluoroethylamine were commercially available and were purified prior to use. The preparation of 2,2-difluoroethylamine involved diborane reduction of difluoroacetamide produced from commercially available (Aldrich) difluoroacetic acid. Difluoroacetic acid (5 g) was converted to difluoroacetamide (4.6 g) via the ethyl ester.[6] Difluoroacetamide was reduced with diborane (from boron trifluoride etherate and sodium borohydride[7]) in the manner reported for production of 2-fluoroethylamine from fluoroacetamide,[8] yield 60%. Lithium aluminum hydride did not produce an isolable amount of product when attempts to use it in place of diborane were made. The corresponding maleamic acid, 2,2'-difluoroethylmaleamic acid, was analyzed for $CHN(C_6F_2H_7NO_3)$ by Galbraith Laboratories. The ^1H NMR spectrum of maleamic acids is characterized by the nonequivalence of the 2- and 3-position vinylic protons of the maleamic acid. The following are the chemical shifts (δ) of those protons and melting points of each compound: **1** (6.35, 6.10; 101 °C), **2**.(6.41, 6.13; 75 °C), **3** (6.47, 5.23; 77 °C), **4** (6.57, 6.23; 102 °C), **5** (6.53, 6.27; 86 °C), **6** (6.53, 6.23; 98 °C), **7** (6.53, 6.23; 105 °C), **8** (6.50, 6.19; 165 °C). All NMR spectra were taken on a Varian T-60 spectrometer. Deuterium oxide with internal DSS as a reference was used as solvent for **1**, **2**, and **4-7**. The spectrum of **3** was taken by using deuteriochloroform with internal Me_4Si. That of **8** was taken by using perdeuterioacetone with internal Me_4Si.

Kinetics. Conversion of the amides **1-8** to the corresponding amine and maleic acid was monitored with a Unicam SP1800A spectrophotometer set at 240 nm. The sample holder was maintained at 50.0 ± 0.1 °C. The decrease in absorbance followed first-order kinetics for all compounds under all reaction conditions (ionic strength of 1.0 was maintained with potassium chloride). Sulfuric acid was used to obtain data in the H_0 region (>1 M). Hydrochloric acid was used for acidities between 1 and 10^{-3} M. For lower acidities, pH was used as a measure of $[H^+]$ with chloroacetic acid–sodium chloroacetate buffers (0.1–0.4 M; no buffer catalysis was observed). Typically, 5 μL of an 0.1 M solution of substrate in acetonitrile was added to 3 mL of reaction solution contained in a preequilibrated quartz UV cell. All data were fitted to a least-squares line with a Texas Instruments calculator to a first-order rate equation by using a final value obtained after 10 half-times. Each line contained at least 20 data points and $r > 0.99$.

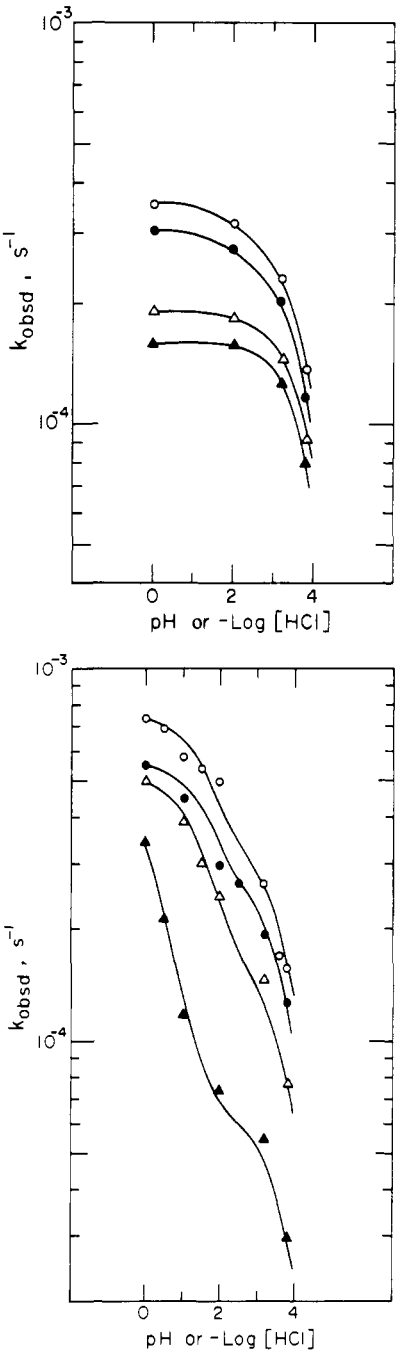


Figure 1. Dependence of first-order rate constants (observed) for hydrolysis of N-substituted maleamic acids on solution acidity at 50 °C. All curves were generated from eq 8 as discussed in the text. Diagram a: ▲, **1**; △, **2**; ●, **3**; ○, **4**. Diagram b: ○, **5**; ●, **6**; ▲, **7**, △, **8**. The rate of hydrolysis of **7** approaches a maximum at $H_0 = -1$ (data not shown) with $k_{obsd} = 4.3 \times 10^{-3}$ s^{-1}. For all other compounds, approach to maximum is within range of the data in the figure.

Results

pH–k_{obsd} Profiles. The dependence of the observed first-order rate constants for hydrolysis of **1-8** on solution acidity (pH) is presented in Figure 1 (parts a and b). For compounds **1-4**, the

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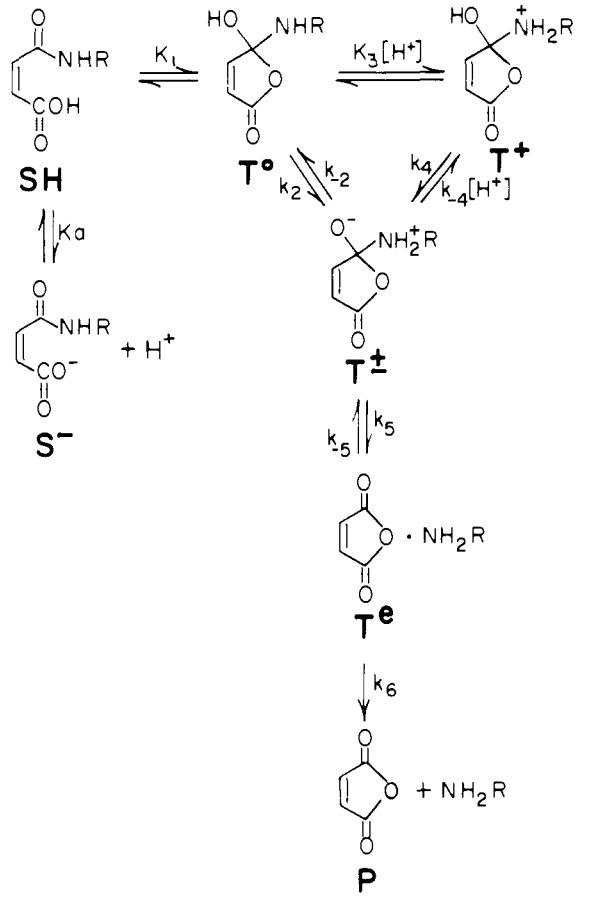
(6) Park, J. D.; Gerjovich, H. J.; Lycan, W. R.; Lacher, J. R. *J. Am. Chem. Soc.* **1952**, *74*, 2189.
(7) (a) Zweifel, G.; Brown, H. C. *Org. React.* **1963**, *13*, 1. (b) Brown, H. C.; Murray, K. J.; Murray, L. J.; Snover, J. A.; Zweifel, G. *J. Am. Chem. Soc.* **1960**, *82*, 4233.
(8) Papanastassiou, Z. B.; Bruni, R. J. *J. Org. Chem.* **1964**, *29*, 2870.

Scheme I



results plotted in Figure 1a follow a titration curve of an acid of $pK_a = 4$. That is, the observed rate constants for those substrates (those with the most basic leaving groups) indicate simply that ionization of a substrate causes it to become reduced in reactivity. No other change in reactivity with acidity is seen. It is well-known that dissociation of the carboxyl group of substituted maleamic acids produces an inactive conjugate base.[2-5] Thus, the pH–rate profiles simply reflect the common pK_a of the carboxyl group in each of **1–4** (that is, SH going to S^- in Scheme I).

The order of the magnitude of the rate constants at any pH in Figure 1a has the compound with the most basic amine associated with the smallest observed rate constant. This is in agreement with the established mechanism,[2-5] which is presented in Scheme I. If formation of $T^{\pm}$ from SH is rapid compared to conversion of $T^{\pm}$ to amine and anhydride, then the relative strength of the C–N bond appears to control the order of reactivity since this parallels the basicity of the amine. This suggests that bond breaking is kinetically significant;[5] that is, it occurs prior to or during the rate-determining step.

We also know from analysis of reactions of N-substituted 2,3-dimethylmaleamic acids[5] that for substrates with less basic amines as leaving groups, C–N bond breaking can compete with proton transfer as a rate-determining process. Thus, for the compounds whose hydrolysis rate constants are plotted in Figure 1b (those with the weakest bases as leaving groups), the dependence of observed rate on pH is more complex due to variation in identity of the rate-determining step with changing pH. If, as in the case of N-substituted 2,3-dimethylmaleamic acids, proton transfer (k_2 in Scheme I) is rate determining at pH 4, the order of reactivity as a function of amine basicity is expected to be opposite to that observed in Figure 1a. This is the case. The compound with the most basic amine as its leaving group reacts most *rapidly*. Therefore C–N bond strength is apparently not a factor in determining relative rates (prior equilibria do not favor the less basic amine substituent), and the rate-determining step occurs prior to breaking of the C–N bond.

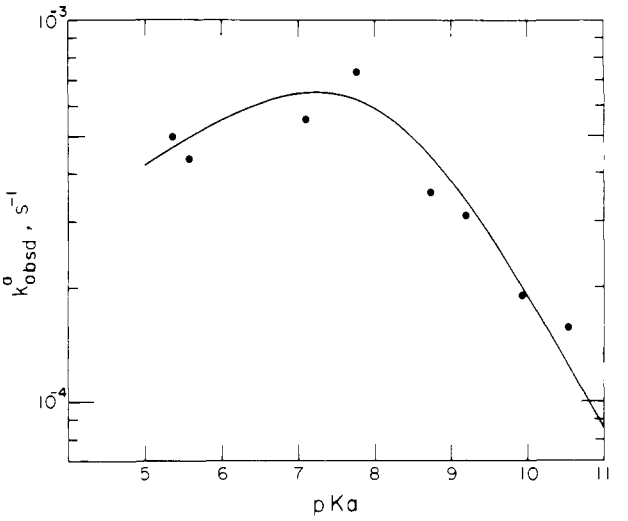


Figure 2. Observed first-order rate constants for hydrolysis of **1–8** at 50 °C at the maximum, in acid. The curve is a plot of eq 12 in the text.

The pH–rate profiles in Figure 1b differ from those in Figure 1a in their general shape. In Figure 1b, the observed rate constant increases with acidity beyond the pK_a of the substrate. As in the case of N-substituted 2,3-dimethylmaleamic acids,[5] we propose that since "proton switch" from O to N is rate determining at pH 4, the ascent in rate with acidity represents the involvement of a kinetically significant acid-catalyzed proton-switch process (via $T^{\pm}$). The limit of the acid catalysis process occurs at the point where a process other than proton transfer from O to N becomes rate determining. Since conversion of SH to T^0 is always rapid,[2,9] the rate-determining step at higher acidity must involve a step *after* the formation of $T^{\pm}$.

The first assumption that we might make is that the conversion of $T^{\pm}$ to products (k_5) has become rate determining, as is the case for the compounds with the most basic leaving groups. However, the maxima in Figure 1b have an opposite order of dependence on leaving-group basicity to those in Figure 1a. Therefore, the two groups of compounds cannot react by a mechanism which involves the same rate-determining step. The resolution of this paradoxical situation can be accomplished by considering that conversion of $T^{\pm}$ to products involves two steps in series. Each has a different dependence of rate on leaving-group basicity. The relative reactivities of the compounds in both parts of Figure 1 reflect the variation of the identity of the rate-determining step. It is clear that if conversion of $T^{\pm}$ to products involves two steps, the actual breakage of the C–N bond can occur in only one of these steps. The other step must be associated with separation of the two reaction products after the C–N bond has broken. Fitting of the kinetic data to free energy relationships establishes with considerable confidence the identity of the rate-determining step in each case.

Relationship of Data to Equations. The relationship between basicity of an amine leaving group and observed rate constants for hydrolysis of **1–8** a pH 4 is the nonlinear plot in Figure 2. This curved relationship is consistent with different steps being rate determining for different compounds since the curve is concave downward.[10] The curvature under these conditions, as discussed in the previous section, is due to a change from the proton switch step, k_{-2}, being rate determining for the compounds whose data are plotted in Figure 1b to bond breaking or subsequent events being rate determining for the compounds of Figure 1a. (See Scheme I).

The data obtained at pH 4 permit an extrapolation to the expected rates of hydrolysis of all compounds at their maxima in acid. Under more acidic conditions, the proton-switch step cannot be rate determining since the presence of an acid-catalysis

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(10) Chin, J. *J. Chem. Soc., Chem. Commun.* **1980**, 1269.

route assures that the rate will increase until another step is rate determining ($k_{-2} < k_{-4}[H^+]$). (The rate will increase until the unimolecular decomposition process of a steady-state intermediate becomes rate limiting.) Thus, the relative rates at the plateau that is achieved for the observed rate in acid would be expected to follow an extrapolation from the linear free energy relation established for compounds **1–4** (the ascending limb with decreasing pK_a of Figure 2) if no other change in rate-determining step occurs with increasing acidity. Our analysis of the pH–rate profiles above suggests that this is not the case since the relative order of reactivity of **5–8** is not consistent with such an interpretation. Figure 3 plots the rates at the stronger acid plateaus as a function of leaving group conjugate acid pK_a. The concave downward curvature[10] confirms that all the substrates do not react by a mechanism involving a common rate-determining step.

As we stated before, this requires that the bond-breaking process of $T^{\pm}$ be followed by a kinetically significant process which involves separation of the resulting amine and anhydride.

The mechanism in Scheme I summarizes the minimal number of species and kinetic parameters necessary to describe the behavior of any of **1–8** in the range of our study. Equation 1 relates the

$$k_{obsd} = \left(\frac{[H^+]}{[H^+] + K_a}\right)\frac{K_1(K_3k_4[H^+] + k_2)(k_5k_6/(k_{-5} + k_6))}{k_{-4}[H^+] + k_{-2} + k_5k_6/(k_{-5} + k_6)} \quad (1)$$

observed rate constant for the first-order disappearance of amide to the mechanism in Scheme I, assuming the "T" species are steady-state intermediates. The species T^e is the "encounter complex" formed after breaking of the C–N bond of $T^{\pm}$ has occurred;[11] this exists prior to diffusional separation of the amine and anhydride or their recombination. The mean lifetime of such a complex is expected to be 10^{-11} s or less.[12]

Relationship of Observed Data to Rate Law. Although eq 1 is complex, it can be seen to be a steady-state equation based on any of the "T" species. At the extremes of the range of acidity of the medium in our study and at the extremes of reactivity of the substrates, the equation reduces to forms which relate directly to the observed data in a simplified form.

At high acidity, the favored pathway between T^0 and $T^{\pm}$ is via T^+ since that pathway is acid catalyzed. Also bond breaking and diffusion are always slower than reprotonation so that

$$k_{-4}[H^+] > k_{-2},\ k_5k_6/(k_{-5} + k_{-6}) \quad (2)$$

The microscopic reverse also holds

$$K_3k_4[H^+] > k_2 \quad (3)$$

Since at high acidity the substrate is present as the undissociated carboxylic acid, $[H^+] > K_a$. We can consider that $k_2/k_{-2} = K_2$, $k_4/k_{-4} = K_4$. Therefore, from Scheme I, $K_2 = K_3K_4$ in a three-point equilibrium. Hence, eq 1 becomes

$$k_{obsd}^{a} = K_1K_2k_5k_6/(k_{-5} + k_6) \quad (4)$$

at high acidity.

At low acidity, $k_{-4}[H^+] < k_{-2}$ and, in reverse, $K_3k_4[H^+] < k_2$, so that eq 1 becomes

$$k_{obsd}^{b'} = \left(\frac{[H^+]}{[H^+] + K_a}\right)\left(\frac{K_1k_2(k_6k_5/(k_{-5} + k_6))}{k_{-2} + k_6k_5/(k_{-5} + k_6)}\right) \quad (5)$$

Since K_a is known independently $k_{obsd}^{b'}$ can be converted to k_{obsd}^{b} (with $pK_a = 3.8$ for all compounds; see ref 3)

$$k_{obsd}^{b} = \frac{K_1k_2(k_5k_6/(k_{-5} + k_6))}{k_{-2} + (k_6k_5/(k_{-5} + k_6))} \quad (6)$$

Thus k_{obsd}^{b} is the same as k_{obsd}^{a} if $(k_6k_5/(k_{-5} + k_6)) \ll k_{-2}$. If not, then k_{obsd}^{b} is smaller than k_{obsd}^{a}.

(11) Leffler, J. E.; Grunwald, E. "Rates and Equilibria of Organic Reactions"; Wiley: New York, 1963; pp 106–109.
(12) Amdur, I.; Hammes, G. G. "Chemical Kinetics"; McGraw Hill: New York, 1966; Chapters 2, 6.

Table I. Rate Constants Derived from Data in Figure 1

compd	$10^5K_1K_2K_5k_6$	$10^4K_1K_2k_5$, s^{-1}	$10^4K_1K_2K_4k_6$, s^{-1}
1	3.54	26.0	1.64
2	2.16	21.6	2.06
3	1.12	16.9	3.84
4	78.7	14.8	4.93
5	32.1	12.3	17.6
6	18.1	7.25	22.6
7	3.73	4.65	99.0
8	8.58	5.18	123

Variation in the nature of the leaving group of the substrate also changes k_{obsd}. If the conjugate acid of the leaving group has a $pK_a < 7$, conversion of $T^{\pm}$ to products is more rapid than conversion of $T^{\pm}$ to the original maleamic acid, SH. That is, $k_{-2} < k_6k_5/(k_{-5} + k_6)$. For these compounds $k_{obsd}^{b} < k_{obsd}^{a}$. If the leaving group is more basic, then proton switch is faster than conversion to products from $T^{\pm}$. Therefore $k_{-2} > k_6k_5/(k_{-5} + k_6)$ and $k_{obsd}^{a} = k_{obsd}^{b}$. (This gives a log k vs. pH plot which curves only at the pK_a of the carboxylic acid group of the substrate.) The rate-determining step for the compounds with less basic leaving groups changes from conversion of $T^{\pm}$ to SH to conversion of $T^{\pm}$ to P with changes in acidity of the medium. Hence the pH–log k plot for the less basic amines shows additional complexity.

The general expression for k_{obsd} can be related to k_{obsd}^{a} and k_{obsd}^{b}:

$$k_{obsd} = \{[H^+]/([H^+] + K_a)\} \times \{K_1K_3K_4k_5k_6/(k_{-5} + k_6) + K_1k_2k_5k_6/(k_{-4}[H^+](k_{-5} + k_6))\}/\{1 + (k_{-2}/k_{-4}[H^+]) + k_5k_6/(k_{-4}[H^+](k_{-5} + k_6))\} \quad (7)$$

This is

$$k_{obsd} = \left(\frac{[H^+]}{[H^+] + K_a}\right)\left(\frac{k_{obsd}^{a}(1 + (k_{-2}/k_{-4}[H^+]))}{1 + \frac{k_{obsd}^{a}}{k_{obsd}^{b}}\left(\frac{k_{-2}}{k_{-4}[H^+]}\right)}\right) \quad (8)$$

The only unknown parameter affecting the value of k_{obsd} then is k_{-2}/k_{-4}. This is the ratio of the rate constants for solvent-catalyzed proton switch from $T^{\pm}$ to T^0 and the rate constant for protonation of $T^{\pm}$. The ratio is generally independent of the leaving group. The data in the pH–rate profiles were fit with the reasonable value of k_{-2}/k_{-4} of 0.01 M, except for the trifluoroethylamine compound, **1**, where a value of 0.05 M gives a curve that fits the data. (This suggests that the proton switch is facilitated in the trifluoroethylamine case or that protonation of the alkoxide is slowed.)

Relationship of Rate Constants in Scheme I to Observed Rate Constants. Figure 2 is a plot of log k_{obsd}^{a} vs. the pK_a of the conjugate acid of the leaving amine. The points are related by a curve that is concave downward, suggesting that there is a different rate-determining step for the least basic and most basic leaving groups. The equation for k_{obsd}^{a} allows for such a variation since the denominator is $(k_{-5} + k_6)$. Thus for the more basic amines, in the nonbonded complex, T^e, addition of the amines to the carboxyl groups should be faster than with less basic amines. Thus, for high pK_a, $k_{-5} > k_6$, and for low pK_a, $k_6 > k_{-5}$. Then k_{obsd}^{a} (strongly basic amines) $= K_1K_2K_5k_6$ and k_{obsd}^{a} (weakly basic amines) $= K_1K_2k_5$. It is reasonable to assume that the quantitites $K_1K_2k_5$ and $K_1K_2K_5k_6$ obey linear free energy relationships with respect to amine pK_a. These were evaluated to fit the data.

$$\log K_1K_2K_5k_6 = -0.38\ pK_a + 0.12 \quad (9)$$

$$\log K_1K_2k_5 = 0.14\ pK_a - 4.1 \quad (10)$$

The value of k^a can be generated from these equations by rearranging eq 4 so that it can be fit to the data in Figure 2. This gives the values generated in Table I and the resultant curve using eq 11 (from eq 4) which is plotted in Figure 2. That is,

$$k^a = \frac{1}{(K_1K_2K_5k_6)^{-1} + (K_1K_2k_5)^{-1}} \quad (11)$$

$$k^a = (10^{0.38pK_a-0.12} + 10^{-0.14pK_a+4.1})^{-1} \quad (12)$$

The data for log $k_{obsd}{}^b$ vs. pK_a also show a downward curvature. For the most basic amines, conversion of $T^{\pm}$ to products is slower than reversion to T^0. Therefore, k^b is close to k^a. For weakly basic amines, $(k_5k_6/(k_{-5} + k_6)) > k_{-2}$ and k^b approaches K_1k_2. Thus, for all substrates, the data were fit to eq 13. Similarly to the

$$\log K_1k_2 = -0.14\ pK_a - 6.4 \quad (13)$$

case for k^a

$$k^b = ((K_1k_2)^{-1} + (k^a)^{-1})^{-1} \quad (14)$$

The curve in Figure 3 was obtained from the relationships above and eq 13. The values of K_1k_2, $K_1K_2k_5$, and $K_1K_2K_5k_6$ for all compounds are given in Table I.

Microscopic Rate Constants. The individual rate constants in Scheme I can be obtained from the data we have presented along with assumptions concerning the rates of diffusional processes and estimates of the acidity of the intermediates. The k_{-4} step is a diffusion-controlled proton transfer in the energetically favorable direction for which a rate constant of 1×10^{10} s^{-1} M^{-1} is reasonable.[12] The k_6 step is diffusional separation for which a rate constant of 1×10^{11} s^{-1} is an upper limit.[12] The value of K_2 can be estimated by eq 15 which is derived as we have discussed earlier[5]

$$\log K_2 = 0.9\ pK_a - 12.33 \quad (15)$$

as can the value of K_3 by eq 16. The value of k_4 (eq 17) is

$$\log K_3 = -pK_a - 6.28 \quad (16)$$

$$k_4 = K_2k_{-4}/K_3 \quad (17)$$

obtained from these quantities. The values are in Table II. The value of k_{-2} is available from k_{-2}/k_{-4} and k_{-4}, k_2 from K_2, K_1 from K_1k_2, k_5 from $K_1K_2k_5$, and k_{-5} from $K_1K_2K_5k_6/K_1K_2k_5$. The results of these calculations are summarized in Table III.

Discussion

The relationship of the data and the mechanism is obtained from the equations and analysis we have presented. The curvature of log k_{obsd} vs. pK_a at different acidities is a result of variation of rate-determining step with leaving-group basicity. This is a consequence of differences in the terms in the denominator of eq 1. Most important is our finding that for the most basic leaving groups, diffusion apart (k_6) can be the rate-determining process.

Table III lists the values for the bond-breaking and bond-forming steps between $T^{\pm}$ and T^e. The equilibrium constant $k_5/k_{-5} = K_5$ favors $T^{\pm}$ for **1**–**6** and for **7** and **8** is close to unity. Under the latter conditions, rate constants measure the intrinsic barrier to bond cleavage which is very low. For the most basic compounds, bond formation ($T^e \rightarrow T^{\pm}$) is favored over diffusion ($T^e \rightarrow P$) so that the rate-determining process is diffusion. This point is a general one. More thermodynamically favorable bond formation processes will compete most effectively with diffusion.

Elimination in More Reactive Maleamic Acids. Maleamic acids with alkyl substituents in the 2- and 3-positions are hydrolyzed more rapidly than their unsubstituted counterparts.[5,13] In principle, formation of T^0 (K_1) or conversion of $T^{\pm}$ to T^e (or both) could be responsible for the difference in reactivity.

With data we have now obtained (Table III), the contributions of individual steps can be compared. The value of K_1 (formation of T^0) is larger for 2,3-dimethylmaleamic acids[5] than the values in Table III by a factor of about 100. The conversion of $T^{\pm}$ to T^e is more favorable in the 2,3-dimethylmaleamic acid system by a factor of about 3.5. Since the data for the present study were obtained at a higher temperature, the inherent difference in k_{obsd} in the two systems amounts to a factor of about 10 000 in favor of the 2,3-dimethylmaleamic acids over the unsubstituted compounds.

(13) Aldersley, M. F.; Kirby, A. J.; Lancaster, P. W.; McDonald, R. S.; Smith, C. R. *J. Chem. Soc., Perkin Trans. 2* **1974**, 1487.

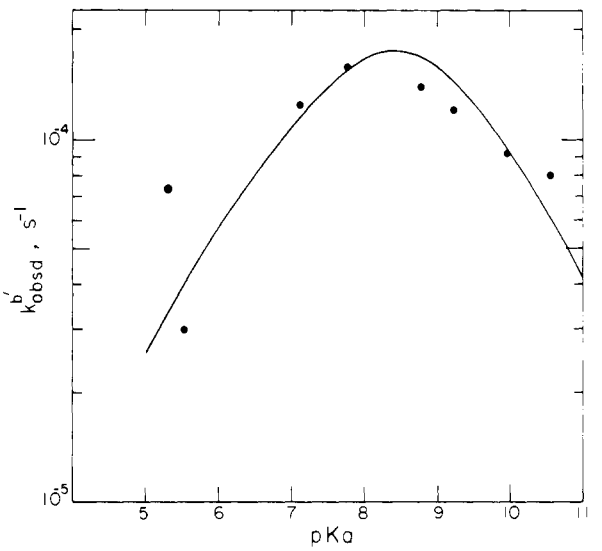


Figure 3. Observed first-order rate constants for hydrolysis of **1**–**8** at 50 °C, pH 4. The value of $k^{b\prime}$ is a plot of eq 14 for k^b reduced by the effect of dissociation of the carboxylic acid group of the substrate, assuming the pK_a' of each substrate is 3.8.

Table II. Rate Constants Estimated by Procedures Described in Text

compd	pK_a	K_2	K_3	k_4, s^{-1}
1	10.53[a]	1.4×10^{-3}	1.8×10^4	7.8×10^2
2	9.96[a]	4.3×10^{-4}	4.9×10^3	8.8×10^2
3	9.20[a]	8.9×10^{-5}	8.3×10^2	1.1×10^3
4	8.79[b]	3.8×10^{-5}	3.2×10^2	1.2×10^3
5	7.75[a]	4.4×10^{-6}	3.0×10	1.5×10^3
6	7.09[b]	1.1×10^{-6}	6.5×10	1.7×10^3
7	5.59[b]	5.0×10^{-8}	2.0×10^{-1}	2.5×10^3
8	5.34[a]	3.0×10^{-8}	1.2×10^{-1}	2.6×10^3

[a] From: Jencks, W. P.; Regenstein, J., "Handbook of Biochemistry"; Sober, H. A., Ed.; Chemical Rubber Publishing Co.: Cleveland, OH, 1968; pp J202-J205. [b] Love, P.; Cohen, R. B.; Taft, R. W. *J. Am. Chem. Soc.* 1958, *90*, 2455.

Table III. Rate and Equilibrium Constants Derived from Experimental Data

compd	10^8k_1	$10^{-8}k_5$, s^{-1}	$10^{-10}k_{-5}$, s^{-1}	k_2, s^{-1}
1	2.5	0.73	160	1.4×10^5
2	5.0	1.3	110	4.3×10^4
3	13	1.8	44	8.9×10^3
4	21	2.2	30	3.8×10^3
5	73	4.4	7	4.4×10^2
6	170	4.5	3.2	1.1×10^2
7	150	71	0.47	2.5×10
8	290	70	0.42	3.0

For the 2,3-dimethylmaleamic acid case, a smaller range of leaving group pK_a was studied (10.5–7.8), leading to no observation of curved plots and thus no change in rate-determining step.[5] Thus, for the 2,3-dimethylmaleamic acids it was assumed that bond breaking is rate determining. However, a plot of the kinetic data for the 2,3-dimethylmaleamic acid case corresponding to $k_{obsd}{}^a$ in this study has the same pK_a dependence as do the points for $k_{obsd}{}^a$ for the more basic leaving groups in this study.[5] Therefore, formation of products occurs with rate-determining diffusion, and bond breaking is a preequilibrium. If bond breaking were rate determining, then a slope corresponding to that for the less basic compounds in this study should have been observed in the previous case. A comparison of rate constants reveals that the substitution of alkyl groups on the 2- and 3-positions of the maleamic acids has its greatest effect on k_5 (bond breaking) rather than on k_{-5}. If k_{-5} decreased with respect to k_6, then we would have observed a change in rate-determining step to C–N bond breaking from diffusion.

Comparison with Concerted Eliminations. Maleanilic acids[4] have substituted anilines as leaving groups. Due to enhanced reactivity of the C–N bond in that case, reactions proceed via concerted, base-catalyzed eliminations from T^+ to P.[14] These should also involve formation of a T^e species prior to diffusion of form P. The observed hydronium ion catalyzed process involves water-catalyzed conversion of T^+ to P, via T^e. Since diffusion ($T^e \rightarrow P$) should actually be the rate-determining process in these reactive systems, we can consider the observed dependence of log k_{H^+} on acidity for maleanilic acid. For a preequilibrium followed by rate-determining diffusion, the results of the current study (eq 9) suggest that the rate constant will level at 2.4×10^{-2} s^{-1}, corresponding to an acidity of $H_0 \sim 1.3$ if k_{H^+} is 1.3×10^{-3} M^{-1} s^{-1}. This closely corresponds to the (temperature-corrected) maximum observed in the maleanilic acid study of 2.6×10^{-2} s^{-1}.[4] A similar agreement for aniline-substituted compounds holds.

Decreases in observed rates from the plateau values are a likely result of protonation of the amide substrate by the strongly acidic medium.[4] Maleanilic acid has a conjugate acid whose pK_a should resemble that of the conjugate acid of acetanilide ($pK_a = -1.4$).[15] The maximal rate of reaction of maleanilic acid occurs at an acidity that is somewhat less than that necessary to half-protonate the substrate. For 4-nitromaleanilic acid we expect a maximum rate constant of 5.5×10^{-1} s^{-1} at $H_0 = -2.5$. In this case, however, the expected pK_a of −2.2 indicates that a rate decrease must be observed at an acidity below that which would give the maximal rate.

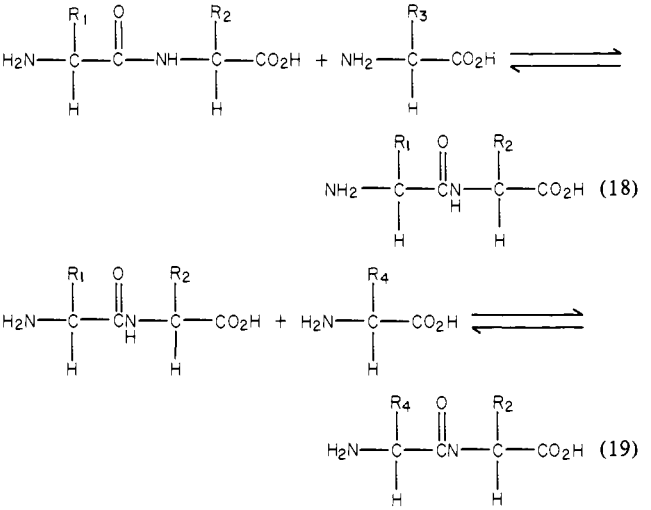
Addition Reactions. The microscopic reverse of the elimination of the amine from the zwitterionic intermediate $T^{\pm}$ to form maleic anhydride is the nucleophilic addition of the amine to a carbonyl group of the anhydride. The interpretation we have applied to our results obtained for the elimination reaction must be applicable to the addition reaction as well. Thus, the rate-determining step for the addition of basic primary amines to maleic anhydride is formation of the encounter complex T^e. For less basic amines, conversion of T^e to $T^{\pm}$, in which a C–N bond is formed, is rate determining. This suggests, in other words, that the processes of separation of the nonbonded T^e complex and formation of a covalent bond within the complex are comparable in activation energy.

The most direct experimental consequence of this interpretation is the dependence of the rate of formation of an amide from an amine and maleic anhydride in acidic solutions. The observed rate constant should be independent of amine basicity for those cases in which formation of T^e is rate determining, that is, in the cases of amines whose conjugate acid pK_a is greater than about 7. For less basic amines, where C–N bond formation is rate determining, the dependence of rate on amine basicity should be considerable. The absolute value of the rate constant observed at the highly basic amine limit will depend on the rate of diffusion of the amine and the anhydride reduced by unfavorable solvation changes, which must result from the amine-to-water hydrogen bond being disrupted as well as other solvation phenomena. The results observed by Pitman and co-workers[16] for the addition of amines to phthalic and succinic anhydrides are at least in rough agreement with the proposal we are making for the maleic anhydride case. Gresser and Jencks[17] and Fersht and Jencks[18] have interpreted pK_a(amine) vs. log k(addition to reactive acyl compounds) plots which show a change in slope toward zero for basic nucleophiles as a "Hammond postulate" phenomenon due to changing structure of a common transition state[18] or change from formation to breakdown of a covalent intermediate being rate determining with curvature at an anomalous pK_a due to the presence of an anomalously poor leaving group.[17] We find those explanations difficult to apply in our case since it has been established that for hydrolysis reactions of compounds like maleamic acids, breakdown of the intermediate, rather than its formation, must be rate determining.[9,13]

Our results also suggest that other elimination reactions and addition reactions may be subject to rate-determining processes involving the formation and decomposition of nonbonded encounter complexes. Compounds with more basic leaving groups may be subject to a rate-determining step involving diffusion whereas those with "better" leaving groups must be subject to rate-determining bond breaking. This interpretation is a direct consequence of the fact that diffusion apart will not depend on basicity significantly but addition in the encounter complex will be faster for better nucleophiles.

Implications for Enzyme Mechanisms. The active sites of enzymes classified as acid proteases contain adjacent carboxylic acid functional groups.[19] Pepsin is the most thoroughly studied member of this family, and the high-resolution X-ray crystal structure of penicillinopepsin has been reported.[20] The results of our study may be extended to explain the mechanism of action of pepsin.

Pepsin may catalyze exchange of either the amino portion and acyl portion of a substrate peptide or both[21,22] (without proceeding to hydrolysis). Equation 18 describes "amino" exchange and



(18)

(19)

implies the existence of a covalent acyl-enzyme intermediate or its equivalent. Equation 19 describes "acyl" exchange and implies the existence of a covalent amino enzyme or its equivalent. The degree to which exchange occurs, and the type of exchange, depends on the type of peptide substrate.[21,22]

The existence of several possible covalent intermediates has caused mechanistic conjecture. Why should the enzyme be capable of these exchanges and how do they relate to the overall mechanism?[19]

The work we report in this paper indicates that in amide hydrolysis promoted by an adjacent carboxylic acid group, the rate-limiting process can be diffusion of the products. If we assume that an enzyme can be as efficient as a model system, the enzyme using a related mechanism would still encounter the problem of diffusion. However, in the enzymic case the diffusional problem is complicated by the existence of attractive forces between it and the substrate. We propose that pepsin could operate through sequential formation of both acyl and amino enzyme intermediates that permit escape of products that otherwise would be tightly held. The mechanism we propose is a modification and combination of those proposed by other workers.[23,24] If the R_2 fragment

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(19) Fruton, J. S. *Adv. Enzymol.* **1976**, *44*, 1.
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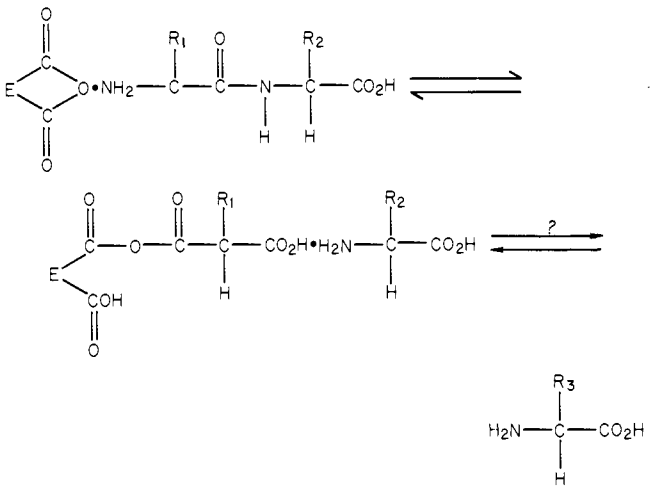
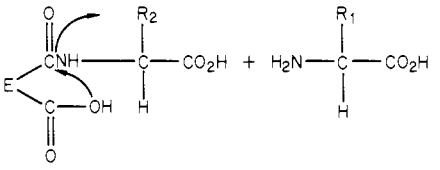


is not held tightly, it can depart and be replaced by $R_3CHNH_2CO_2H$ in an exchange reaction. If R_2 is held tightly, it remains to the next stage.



Since R_2 has not diffused away, it recombines to form a derivative that permits the enzyme to isomerize so that the R_1 fragment is no longer held and can escape. At this point, reversal will generate acyl exchange via the amino enzyme. Finally, the adjacent carboxyl permits the amino enzyme to split readily while the anhydride re-forms. Isomerization of the enzyme allows the R_2 fragment to diffuse. After the anhydride forms between the carboxyl groups, as a result of the amide being cleaved, water from the medium reacts to produce the two carboxyl groups if direct reaction of the substrate carbonyl group with water has not occurred in an earlier step.

Conclusion

Our result indicate the importance of diffusional separation as a possible rate-determining step in elimination reactions leading to formation of carboxyl groups. Consideration of the behavior of a nonbonded encounter complex can explain apparently anomalous behavior in a number of reaction systems.[14] The existence of complexes and the importance of diffusion vs. recombination of species have been widely recognized in the chemistry of compounds producing reactive pairs.[11] The ability to extrapolate to a diffusional limit in the case of less reactive species is potentially useful in the study of many reactions which should be amenable to similar kinetic analysis.

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