

Carboxylic Acid Participation in Amide Hydrolysis. External General Base Catalysis and General Acid Catalysis in Reactions of Norbornenylanilic Acids

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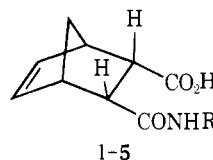
Abstract: Norbornenylanilic acids hydrolyze via assistance from the adjacent carboxylic acid with additional acceleration provided by buffer bases in certain cases. This permits an additional facet of the participation reaction to be analyzed. The rates of hydrolysis of norbornenylanilic acid and *p*-chloro-, *p*-methoxy-, *p*-nitro-, and *m*-nitronorbornenylanilic acid have been measured (50 °C, ionic strength 1.0 with KCl) over the range of acidity 1 M HCl to pH 7. Catalysis by hydronium ion, oxygen buffer acids, oxygen buffer bases, and water occurs for the reactions proceeding via intramolecular assistance from the carboxyl group. General base catalysis is observed for two compounds. The Brønsted β value for the *m*-nitro compound is 0.66 and for the *p*-nitro compound it is 0.62. The dependence of specific rate constants on the pK_a of the conjugate acid of the amine leaving group is linear with a slope (β_{LG}) of 0.16 for acid catalysis rate constants and 0.38 for water catalysis rate constants. The appearance of an external general base rate constant for carboxyl participation is assigned to a mechanism involving specific base-general acid catalysis since this best explains the β_{LG} value for this rate constant of 0.2. Furthermore, an analysis of the factors controlling the observed leaving group dependence suggests that the rate-determining step involves protonation of the amine nitrogen atom concerted with C-N bond breakage. A scheme to account for the observed patterns of reaction in carboxylic acid promoted amide hydrolysis is presented which involves intermediates of different protonation states, a variety of roles for acid and base catalysts, and concerted vs. stepwise buffer-catalyzed rate-determining steps. The irregularity of the kinetic patterns that has been observed for alkyl amine derivatives in related systems suggests that there are critical steric and solvation effects on the rate-determining step and the systematic basis provided by the use of aniline derivatives for our studies should permit these perturbations to be resolved. This will be especially important for enzymatic reactions. Finally, it is suggested that the results can explain the mechanism of action of the acid protease pepsin which contains adjacent carboxylic acid groups at its active site.

The participation of neighboring carboxylic acids in the hydrolysis of amides is a phenomenon pertinent to the understanding of enzyme-catalyzed amide hydrolysis.^{1,2} Although the major intermediates on the reaction pathway have been recognized for a long time, catalysis of reactions of these intermediates has only recently come under scrutiny.³⁻⁹ Since it is the conversion of intermediates to products that is most relevant in obtaining information that is applicable to enzymatic reactions, the modes of catalysis that can be observed in the nonenzymatic case are particularly important.

We have previously shown that external general acid catalysis is superimposed upon certain amide hydrolyses which proceed with assistance from a neighboring carboxylic acid group.^{5,6} However, generalization of a reaction mechanism requires that we obtain an understanding of the molecular characteristics which make a reaction susceptible to external buffer catalysis. No entirely satisfactory substrate had been studied previously. All compounds that were known to be subject to external catalysis contain conjugative unsaturation between the reacting functional groups. Highly complex, special reaction patterns appear in several of these systems.³ No systematic variation in catalytic pattern has been found, and applications to related systems have been hindered by a lack of knowledge of what precise catalytic components are necessary.

It appeared from other studies that compounds most likely to be subject to external catalysis are those which are inherently reactive owing to rigid geometric proximity of the interacting functional groups and those whose amine moiety is a derivative of aniline. In addition, since the reactions we are concerned with are those of the undissociated substrate, any factors which raise the acid's pK_a would be welcome in making the pH range for study sufficiently large.

We have found that anilic acid derivatives of *endo-cis*-5-norbornene meet the criteria we have sought. These substrates (1-5) are rigid, so that the entropy barrier to formation of an intermediate is minimized. They are not conjugated, and for



- 1, R = C₆H₅
- 2, R = C₆H₄-4'-OCH₃
- 3, R = C₆H₄-4'-Cl
- 4, R = C₆H₄-3'-NO₂
- 5, R = C₆H₄-4'-NO₂

that reason have a higher pK_a than α,β -unsaturated acids.

We find that both general acid catalysis and general base catalysis are readily observable over a wide pH range for certain of these compounds. The appearance of general base catalysis, under conditions which also lead to general acid catalysis and under which undissociated carboxylic acid is the kinetically dominant species, provides a new dimension for probing this reaction. Our results indicate the nature of intermediates and their breakdown patterns when a general acid or base catalyst is present. By having a system which provides a continuous spectrum of catalytic patterns, we are able to connect seemingly unrelated catalytic patterns observed by others and present a unified reactivity scheme which should be applicable to enzymatic cases.

Experimental Section

Materials. *endo-cis*-5-Norbornene-2,3-dicarboxylic acid monoanilide (1) and *endo-cis*-5-norbornene-2,3-dicarboxylic acid mono-*p*-anisilide (2) were prepared by procedures that have been published for these compounds.¹⁰ The reaction of purified amines with *endo-cis*-5-norbornene-2,3-dicarboxylic acid anhydride gave monoanilides (3-5). All new compounds (3-5) were analyzed satisfactorily for C, H, N by Galbraith Laboratories. Melting points for recrystallized compounds follow: 3, 169-170 °C; 4, 169-170 °C; 5, 171-172 °C (all uncorrected).

Kinetic Methods. Procedures described in our earlier papers^{5,6,11} were used in these studies. Ionic strength of the reaction medium was maintained at 1.0 through the addition of potassium chloride. Reaction temperature was maintained at 50.0 ± 0.1 °C. Spectrophotometric recording of the absorption at the following wavelengths was used to monitor hydrolysis of the amides: 1, 242 nm; 2, 260 nm; 3, 250 nm; 4, 330 nm; 5, 325 nm. Amides were added in 5 μ L of methanol to 3

Table I. Buffer Catalysis and Hydronium Catalysis of Hydrolysis of Norbornenylanilic Acids^a

Compd	pK _a ^b	10 ² k _{H+} , M ⁻¹ s ⁻¹	10 ³ k _w , s ⁻¹	Buffer component ^c	10 ⁴ k _B , M ⁻¹ s ⁻¹ ^d	Buffer pK _a ^e
1	4.8	3.11	8.20	Phosphoric acid	86.7	2.12 ^f
				Dihydrogen phosphate	13.1	2.12, ^g 7.21 ^f
2	5.0	3.84	14.1	Phosphoric acid	87.1	2.12 ^f
				Dihydrogen phosphate	26.7	2.12, ^g 7.21 ^f
3	4.7	2.54	3.96	Phosphoric acid	48.9	2.12 ^f
				Dihydrogen phosphate	11.4	2.12, ^g 7.21 ^f
				Acetate	96.4	4.76 ^g
4	5.0	1.28	1.01	Phosphoric acid	19.5	2.12 ^f
				Dihydrogen phosphate	6.05	2.12, ^g 7.21 ^f
				Cyanoacetate	1.28	2.43 ^g
				Chloroacetate	2.45	2.86 ^g
				Methoxyacetate	11.9	3.53 ^g
				Acetate	68.7	4.76 ^g
				Hydrogen phosphate	1790.	7.21, ^g 12.32 ^f
5	4.8	0.905	0.39	Phosphoric acid	9.40	2.12 ^f
				Dihydrogen phosphate	6.80	2.12, ^g 7.21 ^f
				Cyanoacetate	0.80	2.43 ^g
				Chloroacetate	1.07	2.86 ^g
				Methoxyacetate	3.92	3.53 ^g
				Acetate	24.0	4.76 ^g
				Hydrogen phosphate	660.	7.21, ^g 12.32 ^f

^a Ionic strength maintained at 1.0 with KCl; temperature, 50 °C. ^b Determined kinetically (from pH-rate profiles). ^c Determined from plot of buffer rate constant vs. buffer fraction. ^d Observed buffer rate constant corrected by extrapolation of buffer fraction plot. ^e W. P. Jencks and J. Regenstein in "Handbook of Biochemistry", H. A. Sober, Ed., Chemical Rubber Co., Cleveland, Ohio, 1968, pp J153-J154. ^f Acid component of the buffer system. ^g Base component of the buffer system.

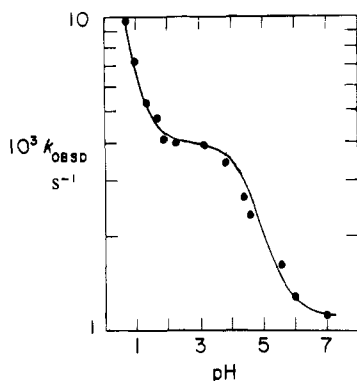


Figure 1. The observed first-order rate constant for the hydrolysis of 4'-chloro-endo-cls-5-norbornene-2,3-dicarboxylic acid monoanilide at 50 °C.

mL of reaction solution. In all cases first-order plots were linear for at least four half-lives.

Since all reactions were carried out at pH < 7, no formation of imides was expected to occur.¹⁰ This was confirmed by spectroscopic observations, using repetitive scanning. Therefore, we do not have complications due to side reactions to consider.

Results

Rate constants observed for hydrolysis of compounds 1–5 are summarized in Table I.

A typical pH-log k_{obsd} profile for the disappearance of the amide absorbance of compounds 1–5 is given in Figure 1. An adequate fit to the data is given by the lines plotted according to eq 1 for acidities below pH 7. Parameters defined follow:

$$k_{\text{obsd}} = [k_{\text{H}_2\text{O}} + k_{\text{H}^+}(\text{H}^+)] / [1 + K_a/(\text{H}^+)] \quad (1)$$

$k_{\text{H}_2\text{O}}$, the apparent spontaneous rate constant for hydrolysis of the undissociated substrate (water rate); k_{H^+} , the rate constant (second order) for the specific acid-catalyzed hydrolysis of the undissociated substrate; and K_a , the dissociation constant of the substrate carboxylic acid. Values for these parameters for each substrate are given in Table I.

For all compounds we examined in this series, catalysis by buffers was observable. Buffer catalysis rate constants (Table I) were obtained from the slopes of plots of k_{obsd} as a function of buffer concentration. We determined which component of the buffer is catalytically active by plotting observed buffer rate constants as a function of mole fraction of specific buffer components. Buffer concentration was varied from 0.1 to 1.0 M (phosphate to 0.5 M due to ionic strength) and buffer fraction from 0.20 to 0.80. With phosphoric acid–dihydrogen phosphate buffer, we observed contributions from both components of the buffer. A secondary analysis, discussed later, was used to sort out the contributions of dihydrogen phosphate into general acid and general base components. When we used substituted acetic acids as buffers, we observed catalysis for the hydrolysis of 4 and 5 by the basic component of the buffer.

A Brønsted plot (Figure 2) for the general base rate constants observed for the hydrolysis of 4 has a slope, β , of 0.66 (correlation coefficient, 0.999) and a plot for 5 has a slope of 0.62. Points used to construct the plot were taken from data of four substituted acetates and phosphate dianion catalysts. The appearance of dihydrogen phosphate above the extended plot (this point was not used to construct the plot), is presumably due to a contribution by this buffer as a general acid catalyst.

Figure 3 plots the effect of variation of pK_a of the conjugate acid of the departing amine on the various observed rate constants. All observed rate constants increase monotonically with pK_a except the rate constant for catalysis due to dihydrogen phosphate ion which is independent of pK_a for the lowest pK_a values. We assume that the anomalous values for dihydrogen phosphate ion are due to its functioning both as a Brønsted acid and as a Brønsted base. Extrapolation of the plot in Figure 2 through the pK_a of dihydrogen phosphate for compounds 4 and 5 gives an expected value for the rate constant owing to general base catalysis. The remainder of the observed buffer rate constant has been assigned to general acid catalysis.

Discussion

The conversions of 1–5 to the corresponding amines and

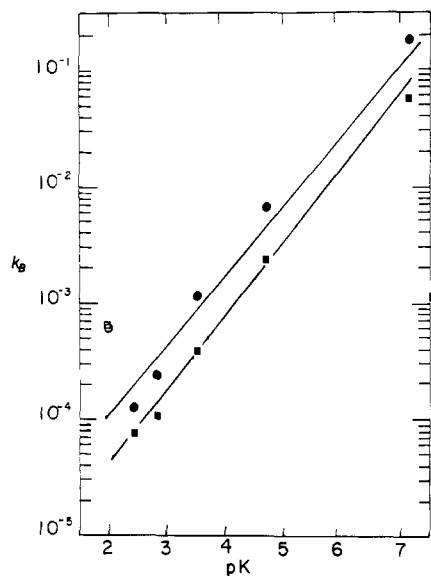


Figure 2. Observed buffer rate constants ($M^{-1} s^{-1}$) as a function of pK_a of acid component of buffer for 3'-nitro-(●) and 4'-nitro-*endo-cis*-5-norbornene-2,3-dicarboxylic acid monoanilides (■) at 50 °C. Open symbols are for dihydrogen phosphate ion/phosphoric acid buffer.

endo-cis-5-norbornene-3,4-dicarboxylic anhydride (which subsequently is hydrolyzed to the dicarboxylic acid) are subject to hydronium ion catalysis and general acid catalysis, as are those of other vicinal anilic acids.^{5,6} The presence of an observable kinetic term in the rate law for the hydrolysis of vicinal amic acids gives an indication that general acid catalysis may also be observable, although this aspect has been overlooked in many cases. Empirically, for anilic acids the rate constant for hydronium ion (using the assumption that $pK_a = -\log 55$ and $p/q = 1$)¹² falls on or near the extended Brønsted plot for general acid catalysis. This suggests a common acid catalysis mechanism and tests of this involving studies in concentrated acid bear this out.⁶

The presence of a large spontaneous (water) rate for the undissociated substrate has often made it difficult to observe buffer catalysis of the hydrolysis of amic acids. In addition, ionization of the substrate reduces the *rate* of reaction in pH regions where significant buffer concentrations and low hydronium ion concentrations, along with a Brønsted coefficient less than unity, would make catalysis observable. However, for compounds **1–5**, the involvement of a buffer term in the hydrolytic rate law is quite explicit. The separation of buffer catalysis into general acid and general base catalysis is easily seen. The observations of various modes of catalysis in a closely related series of compounds permits a comprehensive mechanistic picture to be constructed which may be applied to less obvious cases.

The hydrolyses of **4** and **5** are significant since they are clearly subject to both general acid and general base catalysis. The values of the Brønsted slopes are clearly not unity and not zero, indicating a conventional mechanistic analysis may be applied.

Intermediates. The mechanism of formation of intermediate T (eq 2) has been discussed elsewhere^{4,5,10} and our discussion will begin with the knowledge that the rate-determining step in the reaction occurs after the intermediate has formed. Furthermore, since we are observing disappearance of the amide absorbance, and the intermediate is present only in very low concentrations, the conversion of anhydride to diacid is not observable and the buildup of intermediate is not a complication.¹⁰ We have previously shown that the related N-protonated intermediate ($T \cdot H^+$) undergoes general base catalyzed

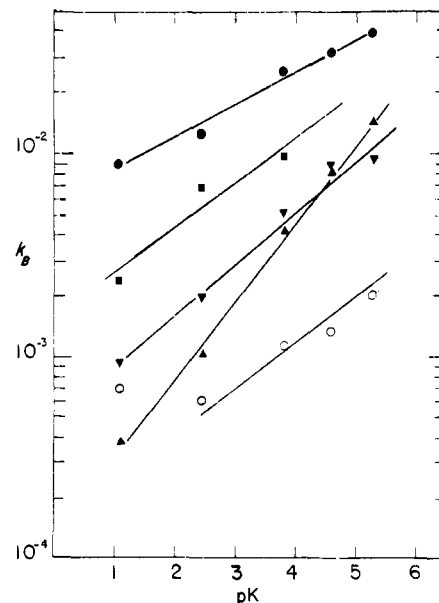
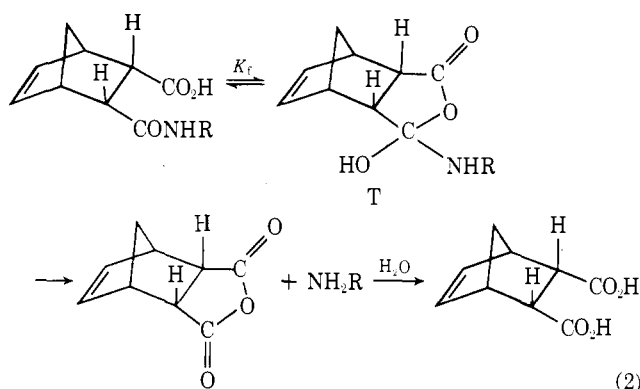
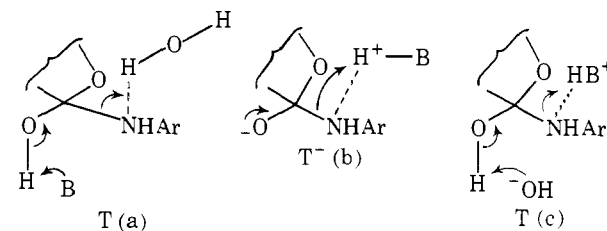


Figure 3. Observed buffer rate constants ($M^{-1} s^{-1}$; ■, acetate; ▼, phosphoric acid; ○, dihydrogen phosphate ion), acid rate constants ($M^{-1} s^{-1}$; ●, and water rate constants (s^{-1} ; ▲) for hydrolysis of substituted *endo-cis*-5-norbornene-2,3-dicarboxylic acid monoanilides vs. pK_a of the conjugate acid of the departing amine. The line for dihydrogen phosphate has not been drawn through points which result from a combination of rate constants (see text).



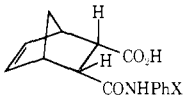
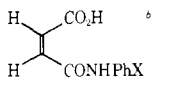
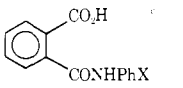
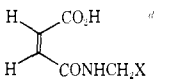
decomposition in reactions in which general acid catalysis is observed.

For the general acid catalyzed reaction of **1–5**, the similarity in leaving group dependence of observed rate constants to those for maleanilic acids^{5,6} (Table II) and the appearance of general acid and hydronium ion terms in the rate law support a similar mechanism. However, the appearance of observable general base catalysis in the reactions of **3**, **4**, and **5** cannot be due to that mechanism. The simplest mechanistic possibilities for the rate-determining step that would yield the observed kinetic results involve: (a) decomposition of a neutral intermediate catalyzed by a Brønsted base, (b) decomposition of the con-



jugate base of T (T^-) catalyzed by a Brønsted acid, or (c) hydroxide-catalyzed removal of the proton from T concerted with general acid catalysis of departure of the amine.

Table II. Dependence of Observed Rate Constants on pK_a of Amine Moiety

Compd	$\beta_{LG}^{H^+}$	$\beta_{LG}^{H_2O}$	$\beta_{LG}^{H_3PO_4}$	Temp, °C
	0.16	0.38	0.25	50
	0.23	0.38	0.19	50
		0.34		65.8
		Irregular		39

^aThis work. ^bData from ref 5. ^cIn 20% v/v dioxane; $\beta_{LG}^{H_2O}$ was obtained by plotting the data from ref. 9. ^dData of Table 4, ref. 3.

Mechanism a explicitly involves general base catalysis in the rate-determining step and therefore relates directly to the observed kinetic pattern. Mechanism b is slightly more complex but yields the same observable equation. Where K_f is the equilibrium constant for formation of T and K_A is the acid dissociation constant for T to T^- , k is the rate constant for conversion of T^- to products in the rate-determining step, and K_{BH^+} is the acid dissociation constant of the buffer, the observed rate constant for mechanism b is given by eq 3.

$$k_{\text{obsd}} = kK_fK_A(BH^+)/(H^+) = kK_fK_A(B)/K_{BH^+} = k_B(B) \quad (3)$$

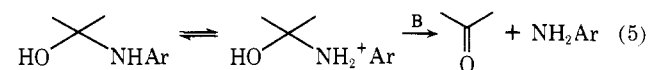
For mechanism c, with K_W the ion product of water and k' the rate constant for the rate-determining step, eq 4 yields the same observable rate constant.

$$k_{\text{obsd}} = k'K_f(BH^+)(OH^-) = k'K_f(K_W/K_{BH^+})(B) = k_B(B) \quad (4)$$

We can use eq 4 and the data from Table I to estimate the reasonableness of mechanism c. For acetate, $k_B = 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$. Applying eq 4, we obtain $k'K_f = 10^6 \text{ M}^{-2} \text{ s}^{-1}$. Since k' involves assembly of three species and carbon-nitrogen bond breakage, a reasonable upper limit for k' is $10^8 \text{ M}^{-2} \text{ s}^{-1}$. This would require K_f to be equal to 10^{-2} , which is probably unreasonably large for formation of a tetrahedral intermediate,¹⁰ even an intramolecular one.

Mechanism a requires water to function as a Brønsted acid in the rate-determining step or that a strongly basic aniline anion be generated in that step with water acting only as a solvent. However, these possibilities do not provide for any development of positive charge on the nitrogen atom during the course of the rate-determining step. This appears to be a requirement in order to explain the observed dependence of the rate constant on the basicity of the aniline derivative. From the data in Table I, the values of k_B are $3 > 4 > 5$. Since the effects of substituents on formation of T are in the reverse order,⁶ the specific rate constant for the rate-determining step must reflect strongly the order which makes the least basic amine the slowest to depart. Since the C-N bond strength order is $5 > 4 > 3$, but the basicity order is $3 > 4 > 5$, proton transfer to make the nitrogen atom have positive character in the transition state is necessary. Mechanism a does not provide for such a transition state since water is a very weak acid. For these reasons, we will not consider mechanism a any further although its gross kinetic properties are in accord with the observed rate law.

Mechanism b involves catalysis by a Brønsted acid of departure of the amine from T^- . This mechanism is a logical extension of the specific acid general base catalysis pattern that we observed for maleanilic acids, where more acidic buffers were involved in observable buffer-catalyzed reactions (5). We

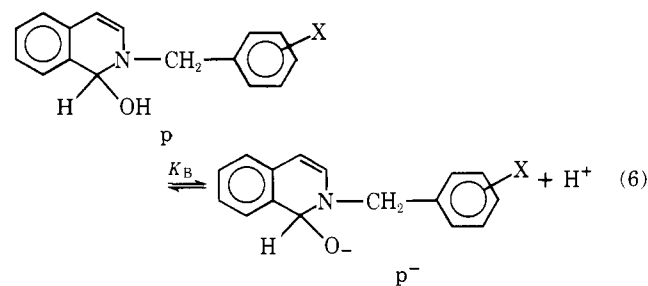


observe general base catalysis for **3**, **4**, and **5** with the most weakly acidic buffers. These substrates contain strongly electron-withdrawing substituents. This encourages ionization of T (a quantitative estimate follows) and makes preequilibrium protonation of the nitrogen atom more difficult. However, a positive nitrogen atom is necessary in the transition state. This should tip the balance in favor of the specific base-general acid mechanism. The transition state for mechanism b involves avoidance of formation of the nitroaniline anion by the Brønsted acid, giving a nearly neutral transition state. For these reasons we prefer mechanism b. Our analysis will be in terms of this mechanism but since mechanisms a and c are kinetically indistinguishable from b on direct analysis, we cannot rule them out rigorously.

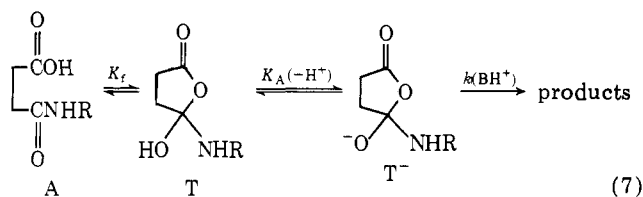
From the data in Figure 2 we obtain Brønsted β values of 0.66 for the general base catalyzed hydrolysis of **4** and 0.62 for that of **5**. Since mechanism b involves specific base-general acid catalysis, these observed values correspond to α values for general acid catalysis in the rate-determining step of $1 - \beta$ or 0.34 and 0.38, respectively. These values probably reflect partial conversion from the ionic pair of T^- and BH^+ to the neutral products in the transition state and an "early" transition state. For further information on mechanistic detail, we can examine the dependence of rate constants on the amine leaving group.

We observe that the logarithm of the rate constant, k_B , for buffer catalysis varies with leaving group pK_a with a slope of ca. (+) 0.2 (Figure 3). The slope of the dependence of $\log K_f$ (eq 4) on pK_a of the leaving group should be approximately -0.2.^{6,13} The dependence of $\log K_A$ on the amine substituent should be relatively small and identical in sign with that of $\log K_f$, since a weaker base will stabilize the anionic form. Whatever the absolute magnitude of the slopes of $\log K_f$ and $\log K_A$ vs. amine pK_a , both will be in the same direction and opposite to what the observed dependence is.

We can arrive at an approximate value for the slope relating $\log K_A$ to substituent amine pK_a if we develop a linear free-energy relationship for alcohol pK_a s as a function of geminally substituted amine pK_a s (carbinolamines). If the amine is protonated, then the substituent will have little differential effect. However, when we have a neutral intermediate, a more significant dependence may develop. A quantitative estimate is available from the data of Bunting and Lee-Young¹⁴ for the ionization of pseudobase ($p \rightarrow p^- + H^+$) (eq 6). Their data



indicate the ρ value for equilibrium K_B is 0.10. Since the ρ value for anilinium ions is 2.8 times that for benzylammonium ions,¹⁵ the ρ value for ionization of T (K_A , eq 7) is proportional: $2.8 \times 0.10 = 0.28$. The ρ for ionization of anilinium ions (compared with the ionization of benzoic acids) is 2.9.¹⁵ The



Brønsted slope for the value of $\log K_A$ as a function of anilinium ion pK_A is approximately $(0.28/2.9) = 0.1$ if the proportionality assumption is used.

Therefore, the net dependence of the value of $\log k$ on the amine's pK_a can be obtained since we can estimate the dependence of all other steps on the leaving group's pK_a . Referring to eq 7, the slope of $\log K_f$ for formation of T is -0.2 ; the slope for dissociation of T to T^- is -0.1 . The observed dependence of $\log k_B$, the rate constant for general base catalysis (eq 8), on the pK_a of the

$$k_B = K_f K_A k / K_{BH^+} \quad (8)$$

conjugate acid of the amine is given by eq 9 and 10.

$$\frac{\Delta \log k_B}{\Delta pK_a} = 0.2 = \frac{\Delta \log K_f}{\Delta pK_a} + \frac{\Delta \log K_A}{\Delta pK_a} + \frac{\Delta \log k}{\Delta pK_a} \quad (9)$$

$$0.2 = -0.2 - 0.1 + \Delta \log k / \Delta pK_a$$

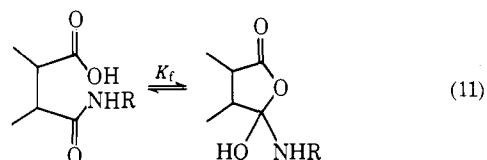
$$(\Delta \log k / \Delta pK_a) = +0.5 \quad (10)$$

The rate-determining step in the water-catalyzed reaction also proceeds from a neutral intermediate. Since the cyclized intermediate is the same and the product is the same as in the general base catalyzed reaction, the dependence of $\log k$ for the "water" reaction upon the leaving group's pK_a (which gives a $\log k_{\text{obsd}}$ dependence of $+0.3$) is therefore $+0.6$.

These values suggest that, in all cases, the rate constant for the rate-determining step involves a factor dependent on the basicity of the amine. If proton transfer alone occurred in that step, the dependence would have a slope of unity under our reaction conditions. The derived values of 0.5 and 0.6 for general base and spontaneous reactions suggest that an effect opposite in direction to that favoring proton transfer is affecting the rate. The obvious alternate effect is kinetic C-N bond strength, suggesting that the transition state involves proton transfer to nitrogen (from a Brønsted acid or water) concerted with breakage of the C-N bond. That value of the slope of the Brønsted catalysis equation suggests that proton transfer is about midway in the transition state if no new interactions are introduced.¹⁶

General Considerations

Molecules containing a carboxylic acid in a geometrical relationship to an amide that permits covalent interaction appear to proceed readily to an intramolecular adduct. The factors influencing the formation (K_f) of this adduct (eq 11)

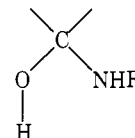


have been examined in detail by Kirby et al.^{3,4} and in our earlier work.^{5,6} The subsequent reaction involving expulsion of the amine from the adduct is the portion of the reaction which is susceptible to catalysis by buffers. The spontaneous decomposition to products can occur without additional catalysts in all cases so far examined, since solvent water molecules appear to promote the decomposition efficiently. The

nature of the substrate may present special situations which increase susceptibility to catalysis.

We have divided the decomposition into several rate-controlling factors and we shall discuss the reaction in terms of these factors: (1) basicity of the amine nitrogen; (2) kinetic stability (and by linear free-energy approximation, thermodynamic stability) of $>C-N$ bond; (3) acidity of hydroxyl group; (4) geometric relationships of hydroxyl and amino groups.

The decomposition formally requires removal of the proton of the hydroxyl group, breaking of the C-N bond, and pro-



tonation of the amine nitrogen atom. The portions of this process that are potentially subject to acid-base catalysis are the two facets involving proton transfer.

In terms of the factors which we have outlined, a sufficiently basic nitrogen atom of the intermediate will be protonated under our reaction conditions (if the rate of proton transfer to nitrogen is rapid compared with the rate of reversion of the intermediate to the starting compound). If the intermediate is not sufficiently basic, then a form of acid catalysis will be necessary to avoid formation of an amine anion in the elimination step.

To avoid generation of a protonated anhydride, the proton must be removed from the hydroxyl group. Since we have accumulated evidence that the pK_a of this hydroxyl is relatively insensitive to the basicity of the adjacent amine, we can regard the acidity of this group as being constant ($pK_a \sim 9$). For the case in which the nitrogen atom of the intermediate is protonated, removal of the hydroxylic proton by a base may be kinetically significant (if the zwitterion that is generated decomposes rapidly or does not exist as a metastable intermediate). The controlling factor will be the strength of the C-N bond. A strong C-N bond will promote formation of a stable zwitterion; a weak C-N bond will lead directly to products.

The facts that a proton is removed from the hydroxyl group and a proton is added to the amino group in this reaction lead to the possibility of a direct (solvent mediated) transfer between these groups—a "proton switch". The spontaneous (water) reaction is best accommodated by such a mechanism.

We have observed general acid catalysis, which is in fact the result of specific acid-general base catalysis, for maleilic acids with the full range of departing aniline derivatives. This suggests that significant protonation on nitrogen occurs so that proton removal from the hydroxyl group becomes rate limiting. For aliphatic amine leaving groups, Kirby^{3,4} has shown that in most cases C-N bond strength is sufficient to make decomposition of the zwitterion rather than proton removal rate limiting. For aniline-derived leaving groups, C-N bond breaking occurs sufficiently readily that proton removal is kinetically significant. The basis for the selectivity of buffer catalysis observed by Kirby^{3,4} for other maleamic acids appears more complex.

Norbornenylanilic acid derivatives, have structural features which permit observation of cases in which protonation of the amino nitrogen atom becomes part of the rate-determining step. We have shown that our results are best accommodated by a mechanism in which equilibrium removal of the hydroxylic proton precedes protonation of the amine nitrogen when the nitrogen is very weakly basic (and is part of what becomes a very good leaving group).

In our analysis of possible mechanisms of catalyzed reactions in the carboxylic acid-amide system, we considered that for-

mation of a zwitterion could be a rate-determining process. In addition we have suggested that the zwitterion may not be stable and direct expulsion from the intermediate may occur. A quantitative distinction between the possibilities can be made if we know the dependence of the rate-determining step on the pK_a of the conjugate acid of the amine substituent (β_{LG}).

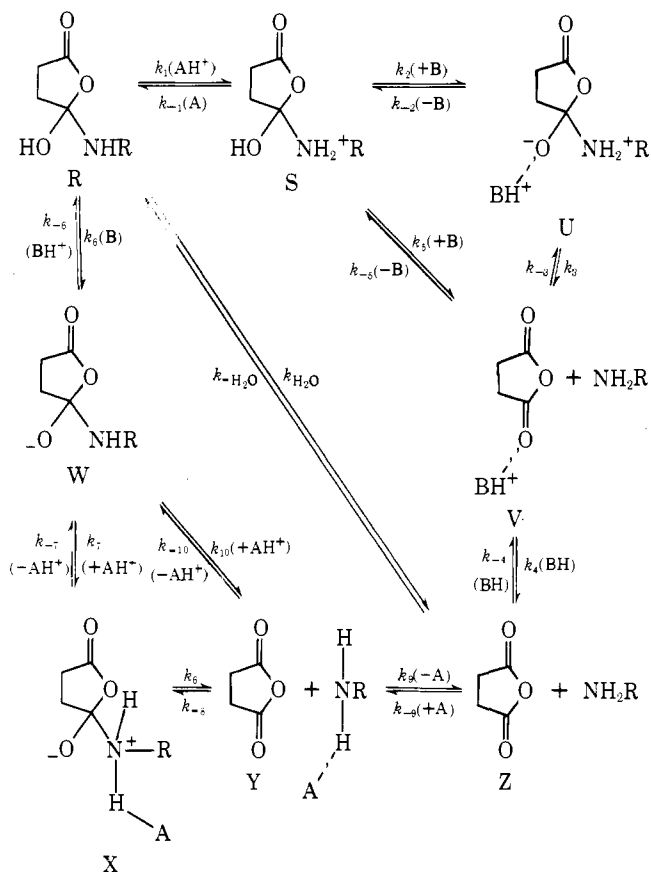
We can measure the dependence of observable rate constants but we cannot measure the dependence of the rate constant for the rate-determining step. To estimate that dependence we have analyzed the factors controlling formation of the intermediate, that is the β_{LG} for K_f . The approximate value for β_{LG} is -0.2 .^{6,13} Any steps following formation of T can be related to the difference between β_{LG} observed and -0.2 .

The value of β_{LG} for the rate-determining step in each mechanism can be arrived at by adjustment of the observed β_{LG} value, using the β_{LG} value of K_f and any further nitrogen protonic equilibria (which have values of $\beta_{LG} = 1.0$). In Table II, we have summarized the β_{LG} for observed rate constants. These can be related by equilibrium assumptions to the extent of positive charge on nitrogen in the transition state. Depending on the structure of the precursor, a measure of transition state structure can be obtained. For cases in which a charge of a value other than zero or unity results, it would appear that C-N bond breaking must be part of the rate-determining step. This can be related to the degree of proton transfer in the transition state according to conventional analysis of β or α values. It appears that, although proton transfer alone may be rate limiting in some reactions in this sense, in the cases which we have examined it appears that C-N bond breakage is likely to be involved as well.

Accessible Mechanisms

The reactions in Scheme I provide the necessary pathways to explain the variety of catalytic patterns seen by us and by

Scheme I



others in the decomposition of amide-carboxylic acid adducts. The relative magnitude of rate constants and catalyst concentrations control the flux through any species. The magnitude of each rate constant is controlled by factors including (1) the nature of leaving groups, (2) relative stability of tetrahedral (orthoamide) centers compared with trigonal (carbonyl) centers, and (3) acidity-basicity of intermediates. For clarity, amine leaving groups are depicted as primary amines and the carbocycle is represented as that derived from succinic acid.

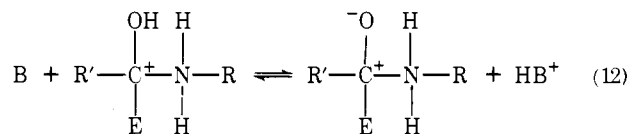
For the cases studied by Kirby,⁴ involving aliphatic amine leaving groups, the pathway $R \rightarrow S \rightarrow U \rightarrow V \rightarrow Z$ is applicable. Normally k_3 is a rate determining and buffer need not be involved. However, when interaction of the carbocyclic ring and the tetrahedral center increase k_3 so that it is large compared with $k_{-2}(BH^+)$, the situation involving proton transfer alone being rate determining ($k_2(B)$) applies. This is characterized by Brønsted slopes of zero and unity and saturation effects in buffer concentration dependence. The conversion of U to V requires that the buffer be present for "enforced catalysis" since conversion is fast compared with diffusion.¹⁷ The dependence of observed rate constants on leaving groups (Table II) reflects prior equilibria.

When leaving groups are aniline derivatives, the direct conversion of S to V predominates for maleic acid derivatives, presumably because U is too unstable to exist for any time.^{5,6} In these cases, the buffer is involved in a step also involving C-N bond breaking, so that partial proton transfer occurs in the transition state of the rate-determining step, leading to Brønsted coefficients between zero and unity.

In the present study, the region of Scheme I corresponding to $R \rightarrow W \rightarrow Y \rightarrow Z$ predominates. When the leaving group is a weakly basic aniline derivative and there are no conjugative interactions between the anhydride carbonyl and carbocyclic ring, buffer catalysis, again with nonzero, nonunit slope, is observed. However, general base catalysis, or its kinetic equivalent, can be the major buffer-catalyzed path.

Reaction proceeding stepwise via $W \rightarrow X \rightarrow Y$ has not been specifically observed as yet. This will require a poor leaving group that is a weak base in order to stabilize X. The difficulty of combining these apparently mutually exclusive properties into one group makes this route seem unlikely ever to be significant.

Application to Enzyme Reactions. Reactions of peptides with serine proteases proceed via initial addition of a group of the enzyme to the amide, forming a neutral addition product. This proceeds to form an acyl enzyme by expulsion of the amine portion of the adduct. Since amino groups of peptides are relatively strong bases, it is likely that a cationic (N protonated) intermediate will be present (eq 12). In these cases general base



catalysis to remove the hydroxyl proton will be necessary to form a zwitterion or ester. Since aliphatic amines are relatively poor leaving groups and there is little that can be done to improve their leaving ability, it is likely that formation of the zwitterionic tautomer will occur.

The possibility that the acid protease, pepsin, may form a covalent amino enzyme derivative between its active site aspartic acid group and the substrate peptide² suggests that a reactive amide may form. Since a second carboxylic acid is available at the active site,⁸ a mechanism analogous to those that we have seen in this study may be relevant. Although alkyl amine derivatives bear a better structural resemblance to the substrates, steric and solvation irregularities in those systems have prevented a systematic analysis. The regular variation

provided by aniline derivatives makes possible systematic observations which we find do bear on the mechanism of pepsin.

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Ozonolysis of *trans*-Di-*tert*-butylethylene. Mechanistic Considerations¹

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Abstract: In general, ozonolysis of *trans*-di-*tert*-butylethylene (**1**) in nonpolar solvents leads to ozonide formation. Ozonolysis of **1** in polar solvents, however, does not give ozonide. Decomposition of the initial ozonide of **1** in a variety of solvents leads to similar results. Thus, the solvent effect is not due to a change in the structure of the first intermediate in the reaction. Decomposition of the initial ozonide of **1** in a variety of environments leads to no radical formation as indicated by ESR measurements. Treatment of the initial ozonide of **1** with increasing amounts of propionaldehyde leads to reduced and eventually no ozonide formation. The significance of these results to the ozonolysis mechanism problem is discussed, including a suggestion that ozonide formation may be nonconcerted under some circumstances.

Introduction

Studies on the ozonolysis of *trans*-di-*tert*-butylethylene (**1**) have proven particularly useful for gaining information on the mechanism of ozonolysis. In large part, this is due to the relatively high stability of the initial olefin-ozone adduct in this case. It is this stability, for example, which permitted Criegee and Schröder³ to show that the initial adduct in this case is a crystalline compound which can be reduced to racemic di-*tert*-butylethylene glycol, thus demonstrating that the initial adduct is formed stereospecifically and that one carbon-carbon bond is still intact in this intermediate. The stability of the initial adduct also permitted Bailey et al.⁴ to measure its NMR spectrum and to conclude that its structure is that of a 1,2,3-trioxolane.

While these studies on the ozonolysis of **1** have proven extremely useful regarding the general problem of the mechanism of ozonolysis, several reports using the same substrate have produced results which are somewhat perplexing. In the same report in which they observed formation of crystalline initial adduct upon ozonolysis of **1** in pentane at -75°C Criegee and Schröder³ also reported that similar ozonolyses carried out in ethyl ether, methylene chloride, and ethyl acetate gave *no ozonide*. Indeed in the cases of methylene chloride and ethyl acetate they also reported that no pivalaldehyde was formed. In the diethyl ether case, the aldehyde was found to be present, and an addition of methanol at the low temperature and subsequent warming led to formation of the methoxyhydroperoxide expected from addition of methanol to the carbonyl oxide produced from ozone cleavage of **1**.

Likewise, Bailey et al.⁴ found that warming of the low-temperature solutions of the trioxolane of **1** in acetone-*d*₆ or Freon 11 led to pivalaldehyde, *but no ozonide formation*. In all of these cases, the carbonyl oxide is presumed to give polymeric product. Again in these studies⁴ addition of methanol at the low temperature to a Freon 11 solution of the presumed trioxolane, formed from addition of ozone to **1**, followed by warming, led to methoxyhydroperoxide formation. Thus, in several of these studies the carbonyl oxide appears to be able to react with methanol or to polymerize, but to be unable to react with pivalaldehyde to produce the ozonide of **1**. Only in the nonpolar solvent pentane is ozonide formation consistently observed to follow decomposition of the trioxolane.

A related study is that of Story et al.⁵ in which they report that addition of propionaldehyde to a low-temperature pentane solution of the trioxolane of **1**, followed by warming, gave no cross ozonide incorporating the propionaldehyde, and also led to a lower yield of the normal ozonide of **1** and propionic acid formation.

Most generally accepted schemes for the ozonolysis process call for initial adduct formation, usually a trioxolane, decomposition to give aldehyde and carbonyl oxide, and subsequent recombination of these latter fragments to give ozonide.⁶ While stereochemical aspects of the process have led to further suggestions⁷⁻¹¹ for the mechanism, all have retained these basic elements.

The observations³⁻⁵ on the ozonolysis of **1** referred to above suggest that aspects of the mechanism other than stereochemical require further examination. In particular these