through-bond contribution. Therefore, some of the difference in the benzyl alcohols and ethylbenzene could be due to a more effective transmittive ability of oxygen compared to carbon. In order to account for the differences between benzyl alcohols and ethylbenzenes by using only an enhanced σ-bond contribution through oxygen, it would be necessary for oxygen to be at least three times more effective than carbon in transmitting substituent information. There is no unambiguous data available to distinguish between the alternative explanations of transmission of substituent effects *via* dimethyl sulfoxide complexation or through σ-bond oxygen contributions. Both mechanisms could be operative.

Hydroxyl Group Participation in Amide Hydrolysis. The Influence of Catalysts on the Partitioning of a Tetrahedral Intermediate

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Abstract: The hydrolysis of 4-hydroxybutyranilide in weakly alkaline solution is catalyzed by phosphate and bicarbonate buffers, while imidazole buffers have little effect. The rate of hydrolysis varies linearly with catalyst at low buffer concentration but reaches a limiting value at high buffer concentration. These findings have been interpreted in terms of a change from rate-limiting breakdown to rate-limiting formation of an addition intermediate as catalyst concentration increases. It is suggested that intramolecular nucleophilic attack by the neighboring hydroxyl group is followed by bifunctional catalysis of the conversion of intermediate to products. This interpretation is supported by a quantitative comparison of buffer effects in 4-hydroxybutyranilide hydrolysis and in the hydrolysis of 2-phenyliminotetrahydrofuran, both reactions proceeding *via* identical intermediates.

The rapid hydrolysis of 4-hydroxybutyramide (relative to that of butyramide) in acidic, neutral, and basic solution has been explained on the basis of a pathway involving intramolecular nucleophilic displacement by the hydroxyl group on the amide function.[1,2] No direct evidence was obtained for the formation of intermediates in this process, although, in a number of recent investigations, it has been shown that nucleophilic attack at the carbonyl, acyl, or imino group affords tetrahedral addition intermediates.[3]

The present report describes our studies of the hydrolysis of 4-hydroxybutyranilide. Our findings provide kinetic evidence for the existence of an addition intermediate derived from intramolecular nucleophilic attack by the neighboring hydroxyl group. Furthermore, we demonstrate the susceptibility of this intermediate to bifunctional acid-base catalysis of its transformation to hydrolysis products, a process leading to major rate increases, in addition to those resulting from the initial intramolecular reaction.

Results and Discussion

The rate of hydrolysis of 4-hydroxybutyranilide in acid or alkaline solution (10% ethanol-water, 30°) depends linearly upon the concentration of hydronium or hydroxide ion (Table I). The value of the rate constant for the acid-catalyzed reaction is very similar to those reported for the hydrolysis of 4-hydroxybutyramide under comparable conditions.[1a,c]

Table I. Rates of Hydrolysis of 4-Hydroxybutyranilide[a]

pH	HCl, M	NaOH, M	$k_0 \times 10^3$,[b] hr^{-1}	$k_2 \times 10^2$,[c] M^{-1} hr^{-1}	
1.06	0.098		20.9	21.3	21.5 ± 0.3
1.36	0.049		10.8	22.0	
2.07	0.0098		2.08	21.2	
12.65		0.047	4.5	9.6	$9.5_5 \pm 0.1$
13.03		0.119	11.6	9.7	
13.27		0.237	22.8	9.6	
		0.474	44.0	9.3	

[a] At 30° in 10% ethanol-water, $\mu = 0.5$. [b] Observed first-order rate constants for anilide disappearance. [c] Second-order rate constants for acid- and base-catalyzed reactions.

For reasons which will become apparent in the sequel, rates of hydrolysis in weakly alkaline media (buffered solution, pH 9-10) were also determined at 30°, although reaction rates were very slow under these conditions. Nevertheless, acceptable first-order kinetics were followed during periods of measurement as long as 80 days (*e.g.*, Figure 1). The results of initial experiments possessed two features of interest: (a) hydrolysis at pH 9 was accelerated about 25-fold by 0.1 *M* phosphate or carbonate buffers, although an equal

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(1) (a) L. Zürn, *Ann. Chem.*, **631**, 56 (1960); (b) T. C. Bruice and F. H. Marquardt, *J. Am. Chem. Soc.*, **84**, 365 (1962); (c) R. B. Martin, R. Hedrick, and A. Parcell, *J. Org. Chem.*, **29**, 158 (1964).

(2) For a review of the mechanisms of neighboring hydroxyl group participation in ester and amide hydrolysis, see T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanism," Vol. 1, W. A. Benjamin, Inc., New York, N. Y., 1966, p 146.

(3) (a) W. P. Jencks, *Progr. Phys. Org. Chem.*, 2, 63 (1964). (b) For a compilation of references to recent work in this area, see A. J. Kirby and W. P. Jencks, *J. Am. Chem. Soc.*, **87**, 3217 (1965), footnote 3; (c) G. L. Schmir, *ibid.*, **87**, 2743 (1965); (d) G. E. Lienhard and W. P. Jencks, *ibid.*, **87**, 3855 (1965); (e) J. E. Reimann and W. P. Jencks, *ibid.*, **88**, 3973 (1966).

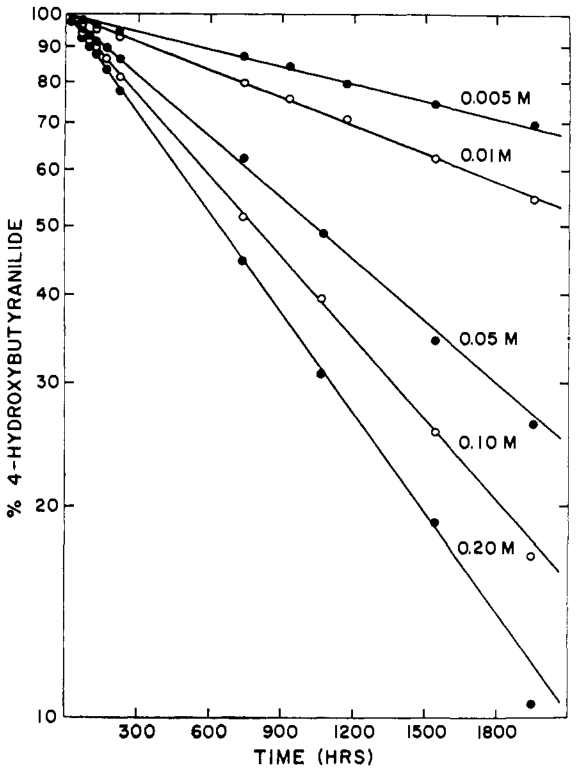

Figure 1. First-order plots for disappearance of 4-hydroxybutyranilide at pH 9.02 ± 0.03, 30°. All reaction mixtures contain 0.03 M Tris buffer and varying amounts of bicarbonate buffer. Numbers indicate total concentration of bicarbonate buffer.

concentration of imidazole buffer had nearly no effect; (b) there was little dependence of the rate of hydrolysis upon hydroxide ion concentration in the range of pH 9–10 (Table II).

Table II. Rates of Hydrolysis of 4-Hydroxybutyranilide in Buffered Solutions[a]

	Buffer		
pH	Tris, M	Other, M	$k_0 \times 10^4$,[b] hr^{-1}
9.07	0.03	. . .	0.32
9.07	0.03	Imidazole, 0.10	0.44
9.03	0.03	Phosphate, 0.10	7.63
9.00	0.03	Carbonate, 0.10	8.74
9.12	0.10	. . .	0.52
9.86	0.10	. . .	0.60

[a] At 30° in 10% ethanol-water, $\mu = 0.5$. [b] Observed first-order rate constants for aniline formation.

Comparison of the markedly differing abilities of imidazole, phosphate, and bicarbonate–carbonate buffers to catalyze the hydrolysis of the anilide is made in Figures 2 and 3. A significant aspect of the phosphate and carbonate catalyses is the nonlinear dependence of rate of hydrolysis upon total buffer concentration at fixed pH. The effect of pH variation upon the carbonate catalysis is shown in Figure 4. At high total buffer concentration, hydrolysis proceeds about three times more rapidly at pH 9.9 than at pH 9.0. Low (<0.01 M) catalyst concentrations, however, produce nearly equal rate increases at each pH value examined. For clarity, the rate constants determined at low carbonate buffer concentration are also presented in Table III. It will be noted (Figure 4) that the tendency of the reaction rate to reach a limiting value at high buffer concentration is seen at all three pH values, although it is most pronounced at lowest pH.

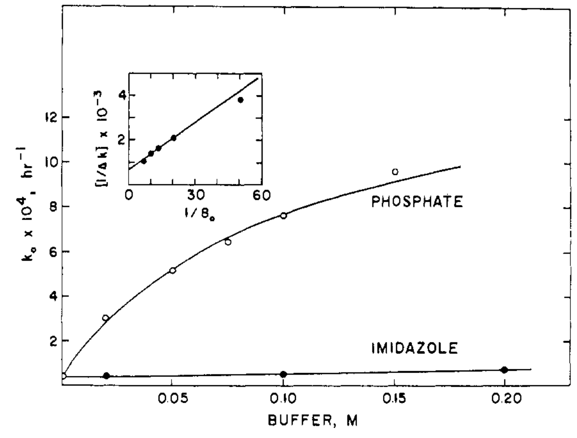

Figure 2. Effect of phosphate and imidazole buffers on rate of hydrolysis of 4-hydroxybutyranilide at pH 9.0, 30°. All reaction mixtures contain 0.03 M Tris. Insert: double reciprocal plot of the increase in rate of anilide hydrolysis as a function of phosphate buffer concentration.

Table III. Effect of pH on Rates of Hydrolysis of 4-Hydroxybutyranilide in Bicarbonate Buffers[a]

	$k_0 \times 10^4$, hr^{-1} [b]		
	Bicarbonate buffer		
pH	0.0 M	0.005 M	0.01 M
9.07[c]	0.32	1.78	2.94
9.38[c]	0.29	1.71	2.84
9.85[d]	0.65	1.72	2.94

[a] At 30° in 10% ethanol-water, $\mu = 0.5$. [b] Observed first-order rate constants for aniline formation. [c] All reaction mixtures contain 0.03 M Tris buffer. [d] All reaction mixtures contain 0.10 M Tris buffer.

The nonlinear variation of rate with increasing catalyst concentration suggests the existence of an addition intermediate, the formation of which is rate determining at some catalyst concentrations, while, at others, the rate-limiting step is the conversion of intermediate to products. According to the mechanism discussed below, it is proposed that low catalyst concentrations accelerate the breakdown of the intermediate to products, until, at high buffer concentrations, the rate-limiting step becomes the formation of the intermediate, a process insensitive to buffer catalysis in this system. Similar interpretations of nonlinear rate–catalyst relationships have been given for several instances of nucleophilic reactions at the acyl or imino function.[3d,4]

The facility with which phosphate buffer catalyzes the hydrolysis of 4-hydroxybutyranilide is in striking contrast to the ineffectiveness of the equally basic imidazole buffer. In nucleophilic displacement at the acyl carbon, imidazole has been found to be 100–4000 times as reactive as phosphate dianion;[5] imidazole

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(4) (a) E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, **84**, 4319 (1962); (b) R. B. Martin, A. Parcell, and R. I. Hedrick, *ibid.*, **86**, 2406 (1964); (c) W. P. Jencks and M. Gilchrist, *ibid.*, **86**, 5616 (1964).

(5) (a) T. C. Bruice and R. Lapinski, *ibid.*, **80**, 2265 (1958); (b) W. P. Jencks and J. Carriuolo, *ibid.*, **82**, 1778 (1960); (c) G. Di Sabato and W. P. Jencks, *ibid.*, **83**, 4393 (1961); (d) K. Koehler, R. Skora, and E. H. Cordes, *ibid.*, **88**, 3577 (1966).

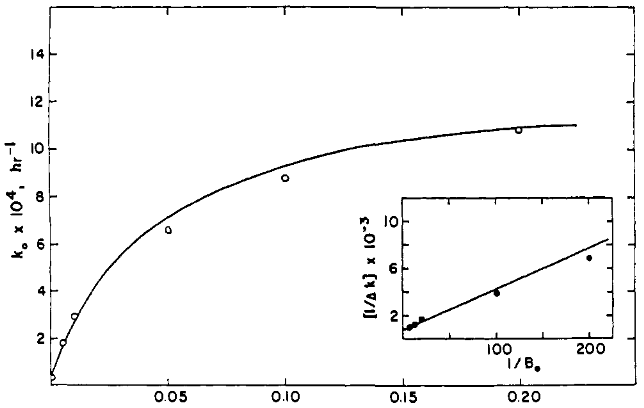

Figure 3. Effect of bicarbonate buffer on rate of hydrolysis of 4-hydroxybutyranilide at pH 9.0, 30°. All reaction mixtures contain 0.03 *M* Tris. Insert: double reciprocal plot of the increase in rate of anilide hydrolysis as a function of bicarbonate buffer concentration.

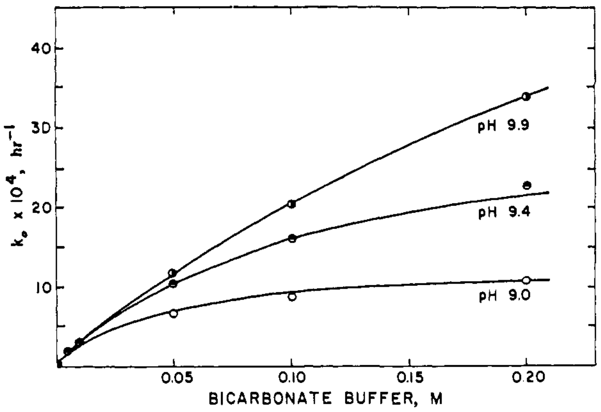

Figure 4. Effect of bicarbonate buffer on rate of hydrolysis of 4-hydroxybutyranilide at three pH values. All reaction mixtures at pH 9.0 and 9.4 contain 0.03 *M* Tris. Reactions at pH 9.9 contain 0.10 *M* Tris. Points at the two lowest concentrations of bicarbonate buffer are common to all three curves.

and phosphate dianion or imidazolium ion and phosphate monoanion exhibit nearly equal effect in the general base or general acid catalysis of nucleophilic reactions at acyl or imino functions.[3d,4a,6-8] These unusual aspects of the hydrolysis of an amide are elaborated in what follows.

General Considerations. In a previous study,[6c,9] evidence was adduced that the hydrolysis of the iminolactone 2-phenyliminotetrahydrofuran in neutral or alkaline solution proceeded, in general outline, accord-to Scheme I.[10] It was proposed that rate-determining

Scheme I

$$\begin{tikzcd}
 & I + H_2O \arrow[d] & \\
A \arrow[r, shift left, "k_1"] & X \arrow[l, shift left, "k_2"] \arrow[r, "k_3"] & B
\end{tikzcd}$$

hydration of the iminolactone led to an addition intermediate capable of yielding either aniline (and butyrolactone) or 4-hydroxybutyranilide (*via* steps k_3 and k_2, respectively). The fate of the intermediate depended on pH, alkaline media favoring the formation of the anilide (A). In addition, at fixed pH, the presence of certain buffers caused increased yields of aniline (B), as a result of a (proposed) bifunctional acid-base catalysis of step k_3. Although product distribution changed, these catalysts did not increase the rate of iminolactone disappearance since intermediate formation (and not breakdown) was rate determining. Under the selected experimental conditions, product interconversion *via* step k_1 occurred at a negligible rate.

If, in the present study, conversion of 4-hydroxybutyranilide to aniline occurs *via* the addition intermediate X, then the observed first-order rate constant (k_0) for aniline production will be given[11a] by $k_0 = k_1k_3/(k_2 + k_3)$. When $k_3 \gg k_2$, intermediate formation is the rate-limiting step ($k_0 \simeq k_1$). If $k_2 \gg k_3$, the intermediate exists at a steady-state concentration in equilibrium with reactant A, and the over-all reaction rate is a function of the rate-determining step k_3 and the preequilibrium level of the intermediate ($k_0 = k_1k_3/k_2$). Furthermore, environmental factors capable of increasing step k_3 will increase the over-all rate constant k_0, at least until step k_3 is no longer rate determining. Since, at alkaline pH, iminolactone hydrolysis yielded hydroxyanilide rather than aniline, then, necessarily, k_2 was greater than k_3 under these conditions. It was therefore to be expected that if the hydrolysis of 4-hydroxybutyranilide were studied under the same conditions, those catalysts effective in increasing the *yield* of aniline from the iminolactone (by increasing the rate of step k_3) would increase the *rate* of hydrolysis of the anilide.

The Reaction Mechanism. The favored reaction mechanism (Figure 5) for the hydrolysis[11b] of 4-hydroxybutyranilide states that cyclization of the hydroxyanilide anion yields an addition intermediate whose anionic form may either cleave to products or revert to reactant, and whose neutral (or zwitterionic) form gives invariably aniline and butyrolactone.

At pH >11, aniline production occurs *via* the anionic carbinolamine only, and the rate constant for the over-all reaction is first order in hydroxide ion concentration. At pH 8–10, the availability of a path for aniline formation *via* the neutral carbinolamine results in a constant rate of hydrolysis, the increase in neutral intermediate offsetting exactly the unfavorable decrease in hydroxyanilide anion. At pH 5.5–7, aniline arises solely through the neutral carbinolamine, present in

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(6) (a) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **83**, 1743 (1961); (b) L. R. Fedor and T. C. Bruice, *ibid.*, **87**, 4138 (1965); (c) G. L. Schmir and B. A. Cunningham, *ibid.*, **87**, 5692 (1965).

(7) T. C. Bruice, J. J. Bruno, and W. S. Chou, *ibid.*, **85**, 1659 (1963). It has not been established whether, in the hydrolysis of δ-thiolvalerolactone, the buffer effects represent nucleophilic or general base catalysis.

(8) In the general acid catalyzed formation of *p*-chlorobenzaldehyde oxime,[3e] phosphate monoanion is 12 times as reactive as imidazolium ion, owing both to the large (0.77) value of the Brønsted coefficient and to a small positive deviation of the point for phosphate monoanion from the linear Brønsted plot.

(9) B. A. Cunningham and G. L. Schmir, *J. Am. Chem. Soc.*, **88**, 551 (1966).

(10) I = iminolactone; A = 4-hydroxybutyranilide; B = aniline + butyrolactone; X = tetrahedral addition intermediate. For the purposes of the present discussion, no attention is being given to the important effects of pH variation on the individual steps in Scheme I.

(11) (a) In this and the more detailed treatments that follow, the steady-state approximation is applied to all carbinolamine intermediates. (b) Strictly speaking, the present study deals with the intramolecular alcoholysis of the anilide. Since the alkaline hydrolysis of γ-butyrolactone is rapid relative to the appearance of aniline (k_{OH} for γ-butyrolactone at 25° is 0.81 M^{-1} sec^{-1}: H. K. Hall, M. K. Brandt, and R. M. Mason, *J. Am. Chem. Soc.*, **80**, 6420 (1958); see also H. C. Brown, J. H. Brewster, and H. Schechter, *ibid.*, **76**, 467 (1954)), the over-all reaction consists of the hydrolysis of 4-hydroxybutyranilide.

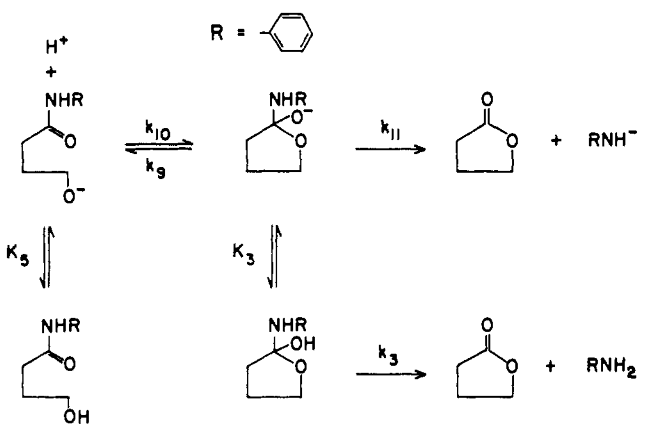


Figure 5. Mechanism of hydrolysis of 4-hydroxybutyranilide in neutral and alkaline solution.

maximal amounts, and decreasing pH causes only a further unfavorable decrease in hydroxyanilide anion. Eventually, acid catalysis leads to an increase in the rate of hydrolysis at lower pH.

The expected effect of pH on the rate of hydroxyanilide hydrolysis is summarized in Figure 6 (curve ABC). In the absence of a reaction pathway *via* the neutral intermediate, anilide hydrolysis would have been expected to follow line CD. We do not find it necessary to invoke a mechanism involving nucleophilic attack by the undissociated hydroxyl group to explain the existence of a pH-independent region, as was done[1b] in the case of 4-hydroxybutyramide. If such a pathway occurred, the trough (at point B) would be either lessened or abolished. The data of the present study give no information on this point.

The favored mechanism leads to eq 1 for the dependence of the observed first-order rate constant (k_0) for anilide hydrolysis on pH. It may be shown[12]

$$k_0 = \left(\frac{k_{10}K_5}{K_5 + [\mathrm{H}^+]}\right)\left(\frac{k_3 + k_{11}K_3/[\mathrm{H}^+]}{k_3 + (k_9 + k_{11})K_3/[\mathrm{H}^+]}\right) \quad (1)$$

that, at pH >11, k_0 is directly proportional to hydroxide ion concentration and includes terms for the mole fraction of hydroxyanilide anion and the partitioning ratio (k_{11}/k_9) of the anionic tetrahedral intermediate (eq 2). In the pH-independent region, eq 3

$$k_0 = \frac{k_{10}k_{11}K_5}{k_9[\mathrm{H}^+]} \quad (2)$$

$$k_0 = k_{10}k_3K_5/k_9K_3 = k_{10}K_5/K' \quad (3)$$

obtains, with $K' = k_9K_3/k_3$, while at pH <7, k_0 is again proportional to hydroxide ion concentration, but the partitioning term for the intermediate is absent (eq 4).

$$k_0 = k_{10}K_5/[\mathrm{H}^+] \quad (4)$$

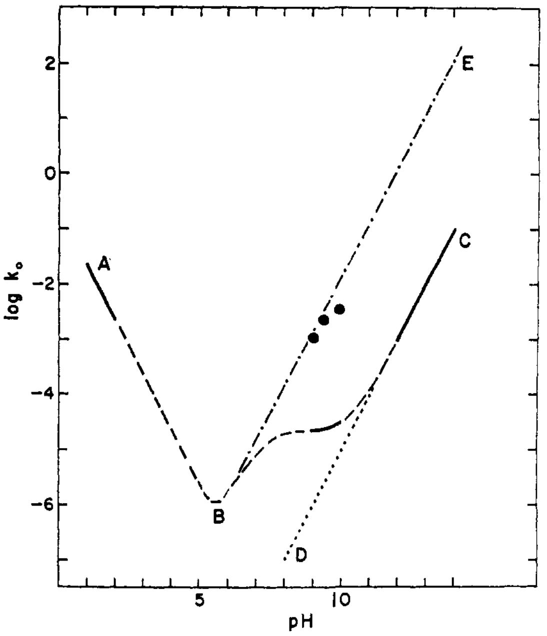
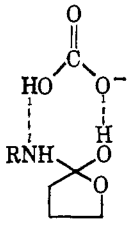
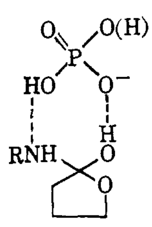


Figure 6. Summary of observed and predicted rates of hydrolysis of 4-hydroxybutyranilide as a function of pH. Rate constants are expressed in units of hr^{-1}. (1) Curve ABC: effect of hydronium and hydroxide ion catalysis (the latter involving both anionic and neutral intermediates); solid portions (pH 1-2, 9-10, 12-14) represent regions of actual rate measurements; dashed portions are predicted for mechanism discussed in the text. (2) Curve CD: rate calculated for hydroxide ion catalysis involving anionic intermediate only. (3) Curve BE: maximum predicted rate if intermediate invariably breaks down to products; ●, highest rates actually observed in presence of bicarbonate buffers.

To explain the ability of certain buffers to alter the product distribution in iminolactone hydrolysis, interaction between the buffer and the neutral carbinolamine was postulated.[9] For the hydroxyanilide system, we propose the same interaction between neutral intermediate (X) and the acidic form (BH) of a buffer (acid dissociation constant K_2) to form a complex (X′)





whose decomposition yields aniline (eq 5 and 6).

$$\mathrm{X} + \mathrm{BH} \overset{K_4}{\rightleftharpoons} \mathrm{X}' \quad (5)$$

$$\mathrm{X}' \xrightarrow{k_{13}} \text{aniline} \quad (6)$$

The model under consideration leads to the following predictions. (a) At fixed pH, the plot of $1/\Delta k$ *vs.* $1/[\mathrm{B}_0]$ is linear (Δk is the difference between the rate obtained at a given total buffer concentration B_0 and the rate in the absence of catalyst). K_{app} is the ratio of the slope to the intercept of this plot and is numerically equal to the concentration of catalyst required to obtain half the maximum possible rate increase ($\Delta k_{\max}$ = 1/intercept). (b) The dependence of K_{app} on pH is given by eq 7, where $K' = K_3k_9/k_3$. Since K_2 is

$$K_{\mathrm{app}} = \frac{(K_4k_3/k_{13})([\mathrm{H}^+] + K')([\mathrm{H}^+] + K_2)}{[\mathrm{H}^+]^2} \quad (7)$$

known and K' was obtained in the iminolactone study,[9] relative values of K_{app} are easily computed. (c) For a given catalytic buffer at fixed pH, the value of

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[12] The derivations of all equations mentioned in the text are given in the Appendix.

K_{app} describing its effect on the rate of anilide hydrolysis should be identical with the value of K_{app} describing its effect on the aniline yield from the iminolactone. (d) While different buffers (*e.g.*, phosphate and bicarbonate) are characterized by different values of K_{app} at a given pH, Δk_{max} at that pH should be independent of the nature of the catalyst. At this point, tetrahedral intermediate formation has become rate determining and k_{max} (the sum of Δk_{max} and of the rate constant in absence of catalyst) is given by eq 8 (see also Figure 6, curve BE).

$$k_{max} = k_{10}K_5/[H^+] \tag{8}$$

Comparison of Predictions with Observations. The linearity of the double reciprocal plots ($1/\Delta k$ *vs.* $1/[B_0]$) is seen in Figures 2 and 3. The derived values of K_{app} at pH 9.0 for phosphate and bicarbonate buffers are 0.10 and 0.045 *M*, respectively. When iminolactone hydrolysis was studied under comparable conditions (except that 10% acetonitrile-water was used instead of 10% ethanol-water), the corresponding values of K_{app} (for aniline formation) were 0.06 and 0.02 *M*. While the general agreement is satisfying, we are unable to account for the differences between the two sets of results. The asymptotically reached rate constants for anilide hydrolysis were nearly identical for phosphate and bicarbonate catalysis, being 15.3 and 13.3 × 10^{-4} hr^{-1}, respectively. The curves drawn in Figures 2 and 3 are calculated from the expressions $\Delta k/\Delta k_{max} = [B_0]/([B_0] + K_{app})$ and $k_0 = \Delta k + k_0'$, with k_0' being the rate constant for hydrolysis in the absence of catalyst.

The curves shown in Figure 4 are computed similarly using the values of K_{app} and Δk_{max} listed in Table IV (columns 2 and 3). Table IV (columns 4 and 5) also includes a comparison of relative values of K_{app} (chosen to give satisfactory fits to the data of Figure 4) to relative values expected on the basis of eq 7,[13] and a comparison of relative experimental and calculated Δk_{max} values. The predictable effects of pH on the catalytic efficiency of the buffer (as denoted by K_{app}) and on the maximally achievable rates of hydrolysis are in accord with the proposed mechanism. It is noteworthy that, at low buffer concentration, the opposing effects of decreasing catalytic efficiency (owing both to loss of the reactive neutral carbinolamine and to some decrease in the mole fraction of bicarbonate ion as pH increases) and of increasing k_{max} values nearly cancel. Consequently, the catalytic effect of bicarbonate buffer seems nearly independent of pH (Table III). At high catalyst concentration, relative rates (as pH increases) reflect mainly the increasing k_{max} values, masking the fact that bicarbonate ion (and not carbonate ion) is the reactive species of the catalyst.

Table IV. Summary of Values of K_{app} and Δk_{max} for Hydrolysis of 4-Hydroxybutyranilide in Bicarbonate Buffers

pH	K_{app}, *M*	Δk_{max} $\times 10^4$, hr^{-1}	Relative values			
			K_{app}		Δk_{max}	
			Found	Calcd	Found	Calcd
9.02	0.045	13	1.0	...	1.0	...
9.38	0.11	33	2.45	2.48	2.54	2.28
9.88	0.43	105	9.6	10.3	8.1	7.2

The constant K' is a measure of the partitioning of the intermediates (*via* steps k_3 and k_9) in the pH-independent region (where step k_{11} is negligible). This constant can be evaluated from the pH-independent rate constant $k_{10}K_5/K'$ (eq 3) and eq 8, using data at pH 9 (the pH-independent rate constant being 0.22 × 10^{-4} hr^{-1} after extrapolation to zero Tris concentration, and k_{max} = 15 × 10^{-4} hr^{-1}). The resulting K' value of 6.8 × 10^{-8} *M* is in good agreement with that of 8.5 × 10^{-8} *M* obtained[9] from the inflection point of the plot of aniline yield *vs.* pH in iminolactone hydrolysis.

Finally, the partitioning ratio k_9/k_{11} for the anionic carbinolamine, evaluated from eq 2 and 8 using rate data at pH 13 and 9, is found to be approximately 1500.[14]

Conclusions

The introduction of a hydroxyl group in the γ position of butyranilide increases the rate of alkaline hydrolysis of the anilide function by a factor of about 11.[15,16] The advantage of spatial proximity of the reacting groups is largely offset by an unfavorable partitioning ratio for the anionic carbinolamine. Considerable rate acceleration results, however, from the availability of a mechanism involving breakdown of a neutral (or zwitterionic) intermediate (Figure 6, compare curves BC and CD). In addition, bifunctional catalysis of the conversion of the neutral addition intermediate to products leads to further rate enhancement (Figure 6, circles). If it is assumed[1b] that a neutral (pH-independent) pathway is not available to butyranilide, the three factors cited above cause over-all rate increases of 12,600-fold at pH 9 and 4500-fold at pH 10 (the theoretically maximum rate increase being a factor of 1500 × 11 = 16,500).

We doubt that the catalytic phenomena described here are unique to the 4-hydroxybutyranilide system. A bifunctional role has been ascribed to phosphoric acid in the cyclization of glutamic acid esters[17] and in the N,N′-diacylhydrazine rearrangement.[18] The conversion of glutamine to pyrrolidonecarboxylic acid[19] and the reaction of urea with formaldehyde[20] are specifically accelerated by phosphate and bicarbonate

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(13) pK_2 for bicarbonate = 10.13; K' = 8.5 × 10^{-8} *M*.[9]

(14) The corresponding partitioning ratio for the alkaline hydrolysis of acetanilide is about 10: M. L. Bender and R. J. Thomas, *J. Am. Chem. Soc.*, **83**, 4183 (1961). The possibility of forming the dipolar species $CH_3C(O^-)_2NH_2^+C_6H_5$ may be responsible for the relatively facile expulsion of amine from the anionic carbinolamine.

(15) The second-order rate constant for alkaline hydrolysis of butyranilide is estimated to be about 0.009 M^{-1} hr^{-1} at 30°, by extrapolation of the data of J. M. A. Hoeflake and F. E. C. Sheffer, *Rec. Trav. Chim.*, **51**, 673 (1932), for acetanilide and from the ratio (3.45) of the rate constants for the alkaline hydrolysis of acetanilide and butyranilide at 100°: O. C. M. Davis, *J. Chem. Soc.*, **95**, 1397 (1909); see also ref 14.

(16) 4-Hydroxybutyramide has been found[1b] to be 22 times as reactive as butyramide with respect to alkaline hydrolysis.

(17) A. J. Hubert, R. Buyle, and B. Hargitay, *Helv. Chim. Acta*, **46**, 1429 (1963).

(18) M. Brenner and W. Hofer, *ibid.*, **44**, 1794 (1961); M. Brenner in "Peptides. Proceedings of the Fifth European Symposium, Oxford, September, 1962," G. T. Young, Ed., The Macmillan Co., New York, N. Y., 1963, p 126; W. Hofer and M. Brenner, *Helv. Chim. Acta*, 47, 1625 (1964); M. Brenner, *Acta Chim. Acad. Sci. Hung.*, 44, 81 (1965).

(19) P. B. Hamilton, *J. Biol. Chem.*, **158**, 375 (1945); J. B. Gilbert, V. E. Price, and J. P. Greenstein, *ibid.*, **180**, 209 (1949); A. Meister, *ibid.*, **210**, 17 (1954).

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ions. Clearly, the combination of intramolecular nucleophilic addition with effective catalysis of the breakdown of the intermediate constitutes a powerful catalytic mechanism, the scope of which merits further investigation.[21]

Experimental Section

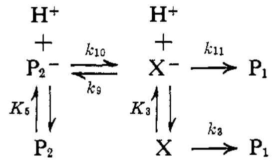
Imidazole and 4-hydroxybutyranilide were materials used in an earlier study.[6c] Buffers and inorganic salts were of reagent grade and were used without further purification. All reactions at alkaline pH were carried out with freshly boiled, glass-distilled water.

The medium used for the kinetic studies was 10% ethanol-water (v/v), at ionic strength 0.50, maintained with added KCl. The rate of hydrolysis of the anilide (1-2 × 10^{-4} *M*) in dilute NaOH or HCl solution was determined spectrophotometrically by the decrease in absorbance at 260 mμ (in base) or at 240 mμ (in acid), using the apparatus described.[6c] Hydrolysis of the anilide (5 × 10^{-4} *M*) at pH 9-10 was measured by following the appearance of aniline, by means of a modified Bratton-Marshall diazotization assay.[6c,9] All reactions were carried out in sealed ampoules and were allowed to proceed to 25-90% of completion with the exception of the slowest reactions (in Tris buffer alone) which reached only 3-5% of completion. Rate constants were generally calculated by means of the integrated first-order rate expression (usually employing calculated infinity values). For reactions which did not exceed 5% of completion, rate constants were evaluated from the slope of linear plots of per cent reaction *vs.* time.

Appendix

Scheme II summarizes the proposed mechanism (Figure 5).[22]

Scheme II



A. The Effect of pH on the Rate Constant for Anilide Hydrolysis. Assuming that the two forms of the tetrahedral intermediate, X and X^-, maintain steady states with respect to reactants and using the expressions $[X^-][H^+]/[X] = K_3$, $[P_2^-][H^+]/[P_2] = K_5$, and $[P_t] = [P_2^-] + [P_2]$, it follows that the observed first-order rate constant (k_0) for anilide hydrolysis is given by

$$k_0 = \left(\frac{k_{10}K_5}{K_5 + [H^+]}\right)\left(\frac{k_3 + (k_{11}K_3/[H^+])}{k_3 + (k_9 + k_{11})K_3/[H^+]}\right) \quad (1)$$

a. The Hydroxide-Dependent Region. At pH >11, aniline formation occurs mainly *via* step k_{11}. Assuming that $k_{11}K_3/[H^+] \gg k_3$, $[H^+] \gg K_5$, and $k_9 \gg k_{11}$

$$k_0 = \frac{k_{10}k_{11}K_5}{k_9[H^+]} \quad (2)$$

b. The pH-Independent Region. At pH 8-10, aniline formation occurs mainly *via* the neutral intermediate X (*i.e.*, $k_3 \gg k_{11}K_3/[H^+]$). Since the partitioning of X yields largely reactant rather than product (*i.e.*, $k_9K_3/[H^+] \gg k_3$)

$$k_0 = k_{10}k_3K_5/k_9K_3 = k_{10}K_5/K' \quad (3)$$

c. At pH <7. Aniline formation proceeds *via* X and the partitioning of X yields largely product (*i.e.*, $k_3 \gg k_9K_3/[H^+]$)

$$k_0 = k_{10}K_5/[H^+] \quad (4)$$

B. Dependence of Rate of Hydrolysis on Buffer Concentration. Variation of K_{app} with pH. In Tris (or other unreactive buffer), anilide hydrolysis follows eq 1. Consider an additional reaction path involving interaction of X with a reactive buffer B_0 (reactive ionic species unspecified)

$$X + B_0 \overset{K_4}{\rightleftharpoons} X' \quad (5a)$$

$$X' \xrightarrow{k_{13}} P_1 \quad (6a)$$

Letting $k_3' = k_3 + k_{13}[B_0]/K_4$ and proceeding as for the derivation of eq 1, it follows that the rate constant (k_0') for anilide hydrolysis in the presence of a catalytic buffer is given by

$$k_0' = \left(\frac{k_{10}K_5}{K_5 + [H^+]}\right)\left(\frac{k_3' + k_{11}K_3/[H^+]}{k_3' + (k_9 + k_{11})K_3/[H^+]}\right) \quad (9)$$

Let $\Delta k = k_0' - k_0$. From eq 1 and 9, the expression $K' = K_3k_9/k_3$, and the assumption that $k_9 \gg k_{11}$, eq 10 is derived.

$$\frac{1}{\Delta k} = \left(\frac{K_5 + [H^+]}{k_{10}K_5}\right)\left(\frac{(K_4k_3/k_{13})([H^+] + K')^2}{[B_0][H^+]K'} + \frac{[H^+] + K'}{K'}\right) \quad (10)$$

From the linear plot of $1/\Delta k$ *vs.* $1/[B_0]$

$$\text{slope/intercept} = K_{app} = \frac{(K_4k_3/k_{13})([H^+] + K')}{[H^+]} \quad (11)$$

$$1/\text{intercept} = \Delta k_{max} = \left(\frac{k_{10}K_5}{[H^+]}\right)\left(\frac{K'}{[H^+] + K'}\right) \quad (12)$$

For the range of pH 9-10, eq 1 is approximately equivalent to

$$k_0 = \left(\frac{k_{10}K_5}{[H^+]}\right)\left(\frac{[H^+]}{[H^+] + K'}\right) \quad (13)$$

Combination of eq 12 and 13 yields

$$k_{max} = \Delta k_{max} + k_0 = k_{10}K_5/[H^+] \quad (8)$$

By the method employed earlier,[9] it is readily shown that for interaction of X with the buffer conjugate acid only (acid dissociation constant K_2), eq 11 becomes eq 7.

$$K_{app} = \frac{(K_4k_3/k_{13})([H^+] + K')([H^+] + K_2)}{[H^+]^2} \quad (7)$$

Acknowledgment. We are grateful to the National Institutes of Health, U. S. Public Health Service, for financial support (Grant No. AM-04288).

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[21] The mechanism of the alkaline hydrolysis of trifluoro-N-methylacetanilide appears to involve an addition intermediate whose breakdown is subject to general base catalysis: R. L. Schowen and G. W. Zuorick, *J. Am. Chem. Soc.*, **88**, 1223 (1966); R. L. Schowen, H. Jayaraman, and L. Kershner, *ibid.*, **88**, 3373 (1966).

[22] P_2 = 4-hydroxybutyranilide; P_2^- = 4-hydroxybutyranilide anion; P_1 = aniline + butyrolactone; X = neutral carbinolamine; X^- = anionic carbinolamine.