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Intramolecular Aminolysis of Esters and Transamidation

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Results obtained in aqueous solutions for the intramolecular aminolysis of a series of amino acid ethyl esters indicate that the reaction is general base catalyzed. At constant pH and ionic strength initial rates increase with buffer concentration at low buffer concentrations, but eventually level off at high buffer concentration. When allowance is made for the fraction of ester not in the free amino form, the plateau values of the observed first-order rate constant are inversely proportional to the hydrogen ion concentration. A mechanism involving rate-limiting general base catalyzed proton removal from an amine nucleophile and rate-limiting decomposition of a resultant tetrahedral addition intermediate is proposed. A detailed analysis of the results is presented in terms of this mechanism and deficiencies of previously proposed mechanisms to account for the results are outlined. Partitioning of a tetrahedral intermediate to yield products or reactants is almost independent of ring size for 5- and 6-membered rings. The more rapid reaction for the 6-membered ring compound is due mainly to the relatively greater rate of formation of a tetrahedral addition intermediate. The intramolecular transamidation of glutamine to pyrrolidonecarboxylic acid in basic solution is general acid catalyzed. Two virtually indistinguishable mechanisms are offered to account for the results.

Formation of pyrrolidonecarboxylic acid and derivatives from glutamine and related compounds has been of concern to biochemists in amino acid analysis for glutamine and in amino acid sequence studies. Furthermore, pyrrolidonecarboxylic acid formation appears to occur in living organisms both enzymatically and nonenzymatically. For these reasons we have undertaken a study of the mechanisms of the conversion of a series of amino acid esters and glutamine to the corresponding lactams in basic solutions. Glutamine conversion to pyrrolidonecarboxylic acid has been studied in a number of catalytic systems,[1] but the experiments were not designed for the elucidation of mechanism. Results and mechanisms advanced in this work are consistent with these earlier studies. Although not strictly valid because of differences of temperature, comparison of equilibrium measurements for pyrrolidonecarboxylic acid and glutamic acid[2] with those for glutamine and glutamic acid[3] reveals that under all pH conditions of this research pyrrolidonecarboxylic acid is favored over glutamine by several powers of ten.

The intramolecular aminolyses of the amino acid esters reported here are general base catalyzed. The effect of high concentrations of the boric acid–borate buffer system is discussed and the results indicate a mechanism different from other mechanisms suggested previously for ester aminolyses. The intramolecular transamidation of glutamine to pyrrolidonecarboxylic acid is general acid catalyzed and the data require a mechanism different from that for ester aminolysis for their interpretation.

Originally we had planned to study ring closure at acid pH values also. Slower rates, competitive ester hydrolysis, and less favorable or unfavorable equilibrium constants discouraged us from proceeding further.

Experimental

Both esters of glutamic acid and glutamine were commercial products. Other ethyl ester hydrochlorides were synthesized from commercial amino acids by passing dry HCl into an ethanolic solution of the amino acid and heating for about 18 hr. The recovered amino acid ester hydrochlorides were recrystallized from ethanol, then analyzed (found values followed by calculated values in parentheses): γ-aminobutyric acid ethyl ester·HCl, C, 43.0 (43.1); H, 8.3 (8.4); N, 8.4 (8.4); γ-aminovaleric acid ethyl ester·HCl, C, 46.7 (46.4); H, 9.1 (8.9); δ-aminovaleric acid ethyl ester·HCl, C, 47.2 (46.4); H, 8.9 (8.9); N, 7.9 (7.8); ε-aminocaproic acid ethyl ester·HCl, C, 49.1 (49.3); (9.2); N, 7.1 (7.2).

Initial rates of ring closure were followed by the appearance of absorption at 200 mμ in a Cary 11 spectrophotometer. The initial absorption was nearly zero except for glutamine where $\epsilon \simeq 1500$, varying slightly due to the variable ionization state. Rate constants were calculated from slopes of linear log $(A_\infty - A)$ *vs.* time plots; therefore it is necessary to know the extinction coefficients of products if accurate rate constants are to be obtained. Commercial pyrrolidonecarboxylic acid, γ-butyrolactam (pyrrolidone), δ-valerolactam, and ε-caprolactam show molar extinction coefficients of 5000, 4600, 6900, and 6600, respectively, at 200 mμ. Greater than 90% of the above readings were obtained from equilibrium readings with the corresponding ester starting materials and glutamine, except for γ-aminobutyric acid and ε-aminocaproic acid ethyl esters which gave final molar extinction coefficients of about 3300 and 400, respectively. Low final readings are probably due to parallel hydrolysis of ester which, if appreciable, renders the method for determination of the rate constants inaccurate. For this reason results for ε-aminocaproic ethyl ester are not reported and those for γ-aminobutyric acid ethyl ester are considered with reservation. Because hydrolysis is first order in hydroxide and hydroxide reacts more rapidly with amino acid esters in the protonated form, and since intramolecular aminolysis is first to second order in hydroxide throughout the pH range of this study, slightly higher equilibrium readings were observed at higher pH values. Independent experiments on the commercially available lactams revealed that their rates of hydrolysis are insignificant compared to their rates of formation. A product extinction coefficient of 4600 was assumed as the value for γ-valerolactam and a value of 5000 was assumed for pyrrolidone carboxylic acid ethyl ester. Any errors introduced by assumption of slightly incorrect extinction coefficients will affect only the relative values of rate constants and not any conclusions concerning mechanism.

All rate studies were performed in aqueous solutions at 25.0 ± 0.1° and 0.2 ionic strength controlled with $KClO_4$ or Na_2SO_4, which do not absorb appreciably at 200 mμ. Temperature equilibrated buffer solutions were adjusted on a pH meter to within ±0.02 pH unit of desired pH just before addition of a small volume of dilute substrate. More precise control was required at low pH values where the aminolysis rate is inversely proportional to almost the second power of the hydrogen ion concentration.

Ionization constants were determined by extrapolation to zero time of pH meter readings made on solutions of substrate which were 25, 50, and 75% neutralized at 25° and 0.2 ionic strength, the same conditions of all rate runs. Values of pK_a recorded in Table I are probably reliable to within ±0.02 pH unit except for δ-aminovaleric acid ethyl ester, which reacts so rapidly that accurate extrapolation is difficult. Values of pH were read on Radiometer pH 4 or Beckman Model G pH meters calibrated with standard buffer solutions.

(1) J. B. Gilbert, V. E. Price, and J. P. Greenstein, *J. Biol. Chem.*, **180**, 209 (1949); P. B. Hamilton, *ibid.*, **158**, 375 (1945).
(2) H. Wilson and R. K. Cannan, *ibid.*, **119**, 309 (1937).
(3) F. H. Carpenter, *J. Am. Chem. Soc.*, **82**, 1111 (1960).

Results

Ester Aminolysis.—Rates of ring closure to the cyclic amides were followed by the increase in absorption at 200 mμ. Since neither esters nor acids absorb significantly at this wave length, appearance of absorption at 200 mμ characterizes amide bond formation independent of any concomitant ester hydrolysis. The extinction coefficients used for product and glutamine absorption are presented in the Experimental section. Checks throughout the study revealed that rates followed by increase in 200 mμ absorption were always first order in substrate at about 10^{-4} *M*.

A typical set of data is shown by the points in Fig. 1, where the observed first-order rate constants are plotted against total borate concentration for the conversion of glutamic acid γ-ethyl ester to pyrrolidonecarboxylic acid at pH 8.66. Plots of the observed first-order rate constants at constant pH and ionic strength *vs.* the total buffer concentration display an initial portion linear in buffer concentration at low buffer concentration. At greater buffer concentrations the plots exhibit curvature culminating in a horizontal straight line at the highest buffer concentrations. For our purposes such a plot can be characterized by the extrapolated ordinate intercept at zero buffer concentration, the value of the observed first-order rate constant in the plateau region, and the slope at low buffer concentrations of the initial straight line portion of the plot k_{obsd} *vs.* total buffer concentration. The results for the compounds studied are presented in Table I at each pH where a series of 8–20 runs was made from 0.01 to 0.4 *M* in total buffer concentration.

TABLE I

VALUES FROM PLOTS OF k_{obsd} *vs.* TOTAL BORATE CONCENTRATION AT CONSTANT pH, 0.2 IONIC STRENGTH, AND 25.0°

pH	*a* Intercept, 10^4 min.$^{-1}$	*b* Slope, 10^4 min.$^{-1}$ *M*$^{-1}$	*c* Plateau, 10^4 min.$^{-1}$
Glutamic acid γ-ethyl ester, pK_a = 9.06			
8.35	0.38	5.0	0.6
8.66	0.95	15	1.9
9.00	2.5	40	5.9
9.28	6.0	70	15
9.66	16	120	27
Glutamic acid diethyl ester, pK_a = 7.1			
8.75	0.8	25	2.1
9.15	1.2	40	3.7
9.80	4.0	70	13
γ-Aminobutyric acid ethyl ester, pK_a = 9.91			
8.50	0.13	0.7	0.18
9.00	0.8	4	1.5
γ-Aminovaleric acid ethyl ester, pK_a = 9.85			
8.54	0.15	3.0	0.26
9.00	1.2	20	2.4
9.47	9		
δ-Aminovaleric acid ethyl ester, pK_a = 10.11			
8.00	0.09	0.8	0.18
8.52	1.1	8	2.1
9.00	10	45	15
9.50	70		
Glutamine, pK_a = 9.10			
8.47	0.006	0.17	0.015
8.97	.009	.20	.024
9.60	.014	.16	.036

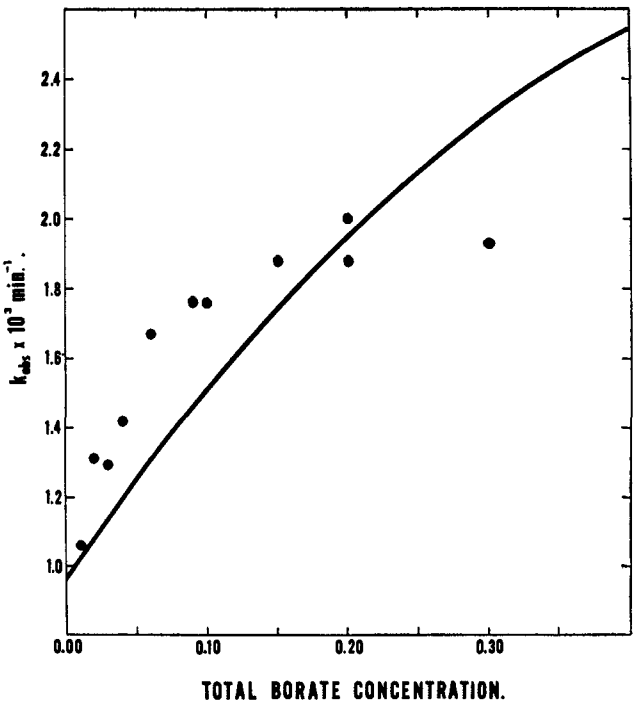


Fig. 1.—Points represent experimental values of observed first-order rate constant *vs.* total boron concentration for conversion of glutamic acid γ-ethyl ester to pyrrolidonecarboxylic acid at pH 8.66. The curved line represents borate ion concentration at the same pH *vs.* total boron concentration scaled to experimental points of rate constants at intercept and $0.2C_B$.

It is evident from the nature of the plots and the data of Table I that the aminolysis reaction is general base catalyzed at low buffer concentration. Catalysis was also observed with bicarbonate buffers from 9.2 < pH < 9.8, affirming that general base and not a specific borate ion catalysis is being studied. Unfortunately, only the borate buffer system is sufficiently nonabsorbing at 200 mμ to permit extended buffer concentration studies.

The main feature of a series of runs performed at constant pH and ionic strength with increasing buffer concentration is the eventual leveling off of the observed first-order rate constant, k_{obsd}, at high buffer concentrations. Since boric acid–borate buffer systems form trimers and higher polymers as the buffer concentration is increased, it is pertinent to consider to what extent plateaus such as that exhibited by the points in Fig. 1 might be due to these buffer effects.

Boric acid reacts with water to yield a proton according to

$$B(OH)_3 + H_2O \rightleftharpoons B(OH)_4^- + H^+$$
$$K_B = (H^+)[B(OH)_4^-]/[B(OH)_3]$$

The value of the equilibrium constant[4] $K_B = 10^{-9.0}$ at 25° in 0.1 *M* $NaClO_4$, the conditions for which the constant has been determined nearest to those of this study. The trimerization reaction may be written

$$3B(OH_3) + H_2O \rightleftharpoons B_3(OH)_9(OH^-) + H^+$$

for which

$$K_T \times (H^+)[B_3(OH)_9(OH^-)]/[B(OH)_3]^3$$

Also at 25° in 0.1 *M* $NaClO_4$ the value[4] of $K_T = 10^{-7.3}$. The total concentration of boron in all its forms is

$$C_B = [B(OH)_3] + [B(OH)_4^-] + 3[B_3(OH)_9(OH^-)]$$

(4) N. Ingri, *Acta Chem. Scand.*, **16**, 439 (1962).

Higher polymers are not important at the highest (C_B = 0.4 M) concentration of this study.

From the above equations it is possible to calculate the concentration of either basic or acidic monomeric forms of boron for various total concentrations of boron in all three forms. The solid curve in Fig. 1 is a plot of $B(OH)_4^-$ concentration *vs.* C_B, scaled to an approximate ordinate intecept and k_{obsd} at 0.2 M C_B for pH 8.66. It is evident that the formation of trimer in the borate buffer system does not account for the leveling off of the aminolysis reaction as indicated by the experimental points in Fig. 1. The experimental points level off much more rapidly with increasing C_B than the borate *vs.* C_B solid curve. If the trimer species were also catalytic, an even steeper curve would be obtained. Similar results were obtained with the other esters for which plateau values are listed in Table I.

In a study to be reported,[5a] acyl transfer in the conversion of O-acetylethanolamine to N-acetylethanolamine is also an intramolecular general base catalyzed ester aminolysis, but in this case the curvature of experimental points in a plot similar to Fig. 1 may be wholly accounted for in terms of reduction of catalytic borate at high buffer concentrations. Such a system provides a kinetic method for the elucidation of complex equilibria in borate systems. It is concluded therefore that in the ester aminolyses reported here a genuine plateau due to a change in rate-limiting step is observed. However, the possibility that the plateaus may be accounted for by interactions between boric acid–borate buffers and reactants or tetrahedral intermediates more profound than general acid–base catalysis cannot be excluded. Evidence for a change in the rate-limiting step has recently been presented for transacetylation of O-acetylethanolamine.[5b]

Rate experiments by two independent methods provide a check on ordinate intercepts obtained from spectrophotometric data. In the first, the initial rate of decrease of pH with time of reactant esters 25, 50, and 75% neutralized was followed on a pH meter. Since the ring product contains no basic amino group, the initial rate of change of pH with time is a measure of the concentration of unprotonated ester.[6] Unlike analogous studies on other compounds,[6,7] the initial rate of change of pH with time for esters in this study is dependent upon the percentage neutralization. Therefore, we plotted the observed rate constant, obtained from the slope of pH *vs.* time plot, *vs.* hydroxide ion concentration and recorded the intercepts and slopes from the best straight lines through the points. For glutamic acid γ-ethyl ester, γ-aminobutyric acid ethyl ester, and γ-aminovaleric acid ethyl ester the intercepts are 2, 7, and 8 (all $\times 10^{-3}$ min.$^{-1}$), respectively, while the slopes are 430, 550, and 580 min.$^{-1}$ M^{-1}, respectively. Glutamic acid diethyl ester and glutamine react too slowly in the pH region of their pK_a values for the method to be useful. Parallel hydrolysis is so extensive in the case of ε-aminocaproic acid ethyl ester that interpretation of such data is not considered. Since δ-aminovaleric acid ethyl ester reacts so rapidly in the same pH region, only an average value for the second-order rate constant for ring closure with hydroxide ion of 12×10^3 min.$^{-1}$ M^{-1} obtained at 25% neutralization of the hydrochloride salt is considered.

The rate of ring closure of 5–20 $\times 10^{-3}$ M δ-aminovaleric acid ethyl ester was also followed on a pH-Stat at pH 8.51 and 8.99. In this pH region the rate is nearly second order in hydroxide ion concentration. The observed first-order rate constant for liberated protons was divided by $1/(1 - f)$ to allow for the fraction of unprotonated amine, f, yielding a first-order rate constant for ester reacting. This last rate constant divided by f was plotted against hydroxide ion concentration yielding a slope of 13×10^3 min.$^{-1}$ M^{-1} and an ordinate intercept of about 14×10^{-3} min.$^{-1}$.

Interpretation.—In the preceding section it was remarked that the data of Table I are in accord with general base catalysis of ester aminolysis. The results reported in the last two paragraphs for the pH meter and pH-Stat studies may be interpreted immediately on this basis. The first-order rate constants correspond to the $k'(H_2O)$ term of eq. 2 below while the second-order rate constants are represented by k''.

The results summarized in the a and b columns of Table I may also be described by general base catalysis as described in succeeding paragraphs. The interpretation and analysis presented here were originally performed on the first ester in Table I, glutamic acid γ-ethyl ester. After the requirements for any mechanism were clearly established, work on the other esters was examined in order to ascertain if they fit the mold established by the first ester. Glutamine presents a special situation and is considered separately.

In interpreting the results of Table I, all the observed first-order rate constants are first corrected for the fraction, f, of the substrate in the amino free base form. Since the amino rather than the charged form is presumably the reactive one, the rate constants are divided by $f = K_a/[(H^+) + K_a]$. For most compounds studied the range of pH values is less than or passes through the pK_a value of the substrate, so that this correction is important.

In the first column of Table II the intercept value of Table I (a) is divided by the fraction of substrate in the amino form (f), a constant subtracted, and the difference multiplied by the hydrogen ion concentration (H^+) to obtain a relatively constant set of values for each compound. A constant set of values is not always obtained in the first column of Table II unless a small constant is subtracted as indicated. The constant so subtracted is recorded after the name of the ester in Table II.

In the second column of Table II the initial slopes recorded in column b of Table I are divided by the fraction of substrate in amino form (f) and by the fraction of the borate buffer system in the basic form, $K_B/[(H^+) + K_B]$ where K_B, the acid ionization constant for boric acid, is taken as 1.0×10^{-9} M for 25° and 0.2 ionic strength.[4] The constant sets of values so obtained in the second column of Table II for a range of pH values passing through the pK_B value of the buffer system affirms apparent general base catalysis in ester aminolysis.

The third column of Table II lists values of plateau (c) recorded in Table I divided by f and multiplied by the hydrogen ion concentration. Since it is necessary

(5) (a) R. B. Martin, R. I. Hedrick, and A. Parcell, submitted for publication; (b) B. Hansen, *Acta Chem. Scand.*, **17**, 1307 (1963).

(6) G. R. Porter, H. N. Rydon, and J. A. Schofield, *J. Chem. Soc.*, 2686 (1960).

(7) R. B. Martin and A. Parcell, *J. Am. Chem. Soc.*, **83**, 4835 (1961).

TABLE II

OPERATIONS ON COLUMNS *a*, *b*, AND *c* OF TABLE I IN ORDER TO OBTAIN CONSTANT VALUES FOR EACH AMINO ACID ETHYL ESTER

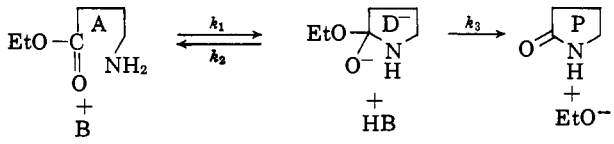
$(a/f - \text{const.})(H^+)$, 10^{12} min.$^{-1}$ M	$b](H^+) + K_B]/fK_B$, min.$^{-1}$ M^{-1}	$c(H^+)/f$, 10^{12} min.$^{-1}$ M
Glutamic acid γ-ethyl ester, const. = 1.5×10^{-3}		
4.1	0.17	17
4.2	.17	15
3.9	.17	13
4.3	.17	13
4.1	.18	8
Glutamic acid diethyl ester, const. = 0.5×10^{-3}		
0.54	0.07	3.8
.50	.07	2.6
.56	.08	2.1
γ-Aminobutyric acid ethyl ester, const. = 2×10^{-3}		
5.1	0.08	16
5.5	0.08	14
γ-Aminovaleric acid ethyl ester		
9.5	0.25	17
9.8	0.32	20
δ-Aminovaleric acid ethyl ester		
120	1.1	230
150	1.3	250
140	1.2	210
110		

to multiply by the hydrogen ion concentration in order to obtain constant values for each ester at any pH, plateau values corrected for the fraction of substrate in amino form are pH dependent. This result is important because it eliminates a whole set of possible mechanisms, as discussed in the next section.

Any mechanism for the aminolysis of the esters studied here must account for the relatively constant values of Table II obtained for each ester at any pH.

Discussion

It is perhaps simplest to begin with discussion of a mechanism that can satisfactorily account for the results of ester aminolysis and then mention why some other postulated mechanisms are unsatisfactory for the systems studied here. The mechanism proposed for the aminolysis of esters as a result of this work as written for γ-aminobutyric acid ethyl ester is



Applying a steady state approximation to the tetrahedral intermediate D^- we obtain

$$\frac{1}{(A)}\frac{d(P)}{dt} = \frac{k_{obsd}}{f} = \frac{k_3[k_1'(H_2O) + k_1''(OH^-) + k_1'''(B)]}{k_3 + [k_2'(H_3O^+) + k_2''(H_2O) + k_2'''(HB)]} \quad (1)$$

where (A) is the molar concentration of free amine and (B) and (HB) are related by $K_B = (H^+)(B)/(HB)$ for acid and base systems present in the reaction mixture. In eq. 1 catalytic rate constants for general bases, k_1, are written out for H_2O, OH^-, and B, the last representing only borate anion in this study. Similarly, catalytic rate constants, k_2, for the acids H_3O^+, H_2O, and boric acid are specified by 1, 2, and 3 primes, respectively. The first two terms in brackets in the denominator of eq. 1 are always less than k_3 under the conditions studied here.

Since the observed first-order rate constant k_{obsd} were calculated from the total ester concentration, they must be divided by the fraction of substrate in amino form, $f = (A)/[(A) + (HA^+)] = K_a/[K_a + (H^+)]$ where $K_a = (H^+)(A)/(HA^+)$. At low buffer concentrations eq. 1 predicts rate-limiting general base catalyzed amine attack

$$k_{obsd}/f = k_1'(H_2O) + k_1''(OH^-) + k_2'''(B) \quad (2)$$

Owing to its low concentration of about 10^{-4} M, general base catalysis by amino acid ester is not important, consistent with the observed first-order dependence of rate on ester concentration.

At high buffer concentrations when any term in the brackets in the denominator of eq. 1 exceeds k_3, decomposition of the tetrahedral addition intermediate is rate limiting. We then obtain

$$k_{obsd}/f = k_3k_1'''(B)/k_2'''(HB) = k_3K_s/(H^+) \quad (3)$$

where the equilibrium constant $K_s = (D^-)(H^+)/(A)$. Equation 3 is consistent with the pH dependence of the plateau for each ester.

Intercept values of the spectrophotometric experiments and the pH meter and pH-Stat experiments performed in the absence of borate may be interpreted by the first three terms of eq. 2. The $k_1'(H_2O)$ term of eq. 2 is identified with the constant term after each ester in Table II and K_wk_1'' corresponds to the values in the first column of Table II. The ion product constant for water is taken as $K_w = 10^{-14}$. A summary of average values of $k_1'(H_2O)$ and k_1'' so obtained from spectrophotometric measurements for each ester is given in Table III.

TABLE III

SUMMARY OF RATE CONSTANTS FOR ESTER AMINOLYSIS DERIVED FROM EQ. 2 AND 3 AND TABLE II

Ethyl ester of	$k_1'(H_2O)$, 10^3 min.$^{-1}$	k_1'', min.$^{-1}$ M^{-1}	k_1''', min.$^{-1}$ M^{-1}	k_3K_s, 10^{12} min.$^{-1}$ M	$k_s/k_2''(H_2O)$
γ-Glutamic acid	1.5	410	0.17	14	3.4
Glutamic acid γ-ethyl ester	0.5	55	.07	3	5
γ-Aminobutyric acid	2	530	.08	15	2.8
γ-Aminovaleric acid		980	.28	19	1.9
δ-Aminovaleric acid		13,000	1.2	230	1.8

These values may be compared directly with the intercepts and slopes obtained from the initial rates of change of pH with time on the pH meter described near the end of the Results section. For glutamic acid γ-ethyl ester, agreement between $k_1'(H_2O)$ and k'' of Table III and the intercept and slope of the pH meter study is as good as can be expected. The intercept of the pH meter study is high compared to $k_1'(H_2O)$ for γ-aminobutyric acid ethyl ester. This discrepancy may be due to parallel ester hydrolysis which is also indicated by the low equilibrium extinction coefficient of the γ-aminobutyric acid ester system as mentioned in the Experimental section. The slope of the pH meter data agrees with k'' for γ-aminobutyric acid ethyl ester.

On the other hand, k'' from Table III for γ-aminovaleric acid ethyl ester is greater than the slope obtained from pH meter data. With all the uncertainties involved in interpreting both kinds of results the agreement obtained, though less than optimum, is sufficient to lend support to the interpretations of the two different experimental methods of rate determination. In addition, the slope from the pH-Stat experiment described in the last paragraph of the Results section represents the rate of attack of hydroxide ion on unprotonated δ-aminovaleric acid and is identical with the value obtained spectrophotometrically and recorded in Table III. The intercept value of 14×10^{-3} min.$^{-1}$ obtained from the pH-Stat experiment is greater than all other $k_1'(H_2O)$ values for slower reacting esters.

At low buffer concentrations all terms in eq. 2 must be considered. The constant k_1''' may be determined from the slope of the k_{obsd} *vs.* buffer concentration plots at each pH. This slope, when divided by the fraction of substrate in amino form and the fraction of buffer in basic form, gives k_1'''. This is precisely the calculation used to obtain the nearly constant values in the second column of Table II. The average k_1''' values so obtained for each ester are summarized in Table III. Comparison of the three catalytic coefficients k_1 for the first three esters of Table III with the ionization constants for the corresponding catalysts yields a Brønsted exponent of about 0.4.

At sufficiently high boric acid concentrations an equilibrium exists between reactant and tetrahedral addition intermediate so that decomposition of the latter is rate limiting. According to eq. 3, plateau values of k_{obsd} *vs.* total borate concentration plots when divided by the fraction of substrate in amino form and multiplied by (H^+) should yield a constant value corresponding to k_3K_s. Values for each ester at several pH values are collected in the third column of Table II and average values are listed in Table III. Since the concentration of free boric acid increases with increasing total borate concentration regardless of occurrence of the trimerization reaction, once a denominator term in eq. 1 exceeds k_3, a plateau is observed and the reduced eq. 3 applies.

The partitioning ratio k_3/k_2''' may be evaluated by rearranging terms in eq. 3 to obtain $k_3/k_2''' = k_3K_s/k_1'''K_B$. All the terms on the right hand side are known and values of k_3/k_2''' are 0.08, 0.04, 0.2, 0.07, and 0.2 M, respectively, for the 5 esters of Table III. Similarly, the unitless partitioning ratio $k_3/k_2''(H_2O)$ is given by $k_3K_s/10^{-14}k''$. These values for partitioning of the tetrahedral intermediate to products or reactants in the hydroxide ion–water catalytic system are presented in the last column of Table III. These ratios indicate that partitioning of a tetrahedral addition intermediate to a trigonal ring structure or to a trigonal open chain structure is little affected by ring size, the ratio for a 6-membered ring formed from δ-aminovaleric acid ethyl ester being similar to the ratios for 5-membered rings. Thus the more rapid rate of aminolysis of δ-aminovaleric acid ethyl ester is due mainly to a more rapid initial ring closure step which yields the tetrahedral addition intermediate. Unfortunately, concomitant hydrolysis of ϵ-aminocaproic acid ester makes it impossible to extend this analysis to 7-membered ring formation.

The mechanism suggested at the beginning of this section can account for the results of other ester aminolysis studies which exhibit base catalysis. However, the mechanisms proposed in several other studies cannot accommodate our results. The mechanism proposed by Jencks and Carriuolo[8] for phenyl acetate aminolysis is a special case of our proposal when the k_1 step is rate limiting. As a consequence their formulation is adequate only at low buffer concentrations and cannot account for the leveling off of the rate at constant pH and high buffer concentrations reported in this study. As originally put forth, the mechanism of Bunnett and Davis[9] also cannot account for the leveling off of the rate at high buffer concentrations. If modified so that formation of a tetrahedral addition intermediate is rate limiting under some conditions, their mechanism then predicts a plateau as observed, but also indicates that rates at a plateau should be independent of pH when due account is taken of the amount of substrate in protonated form. However, from the constancy of the values for each ester in the third column of Table II, plateau rates are pH dependent; a constant value is obtained only on multiplication by (H^+). Since the mechanism of Hawkins and Tarbell[10] is kinetically indistinguishable from that of Bunnett and Davis, the same objections also apply to it. To make it apply to our conditions and account for our results, the concerted type mechanism suggested by Bruice and Mayahi[11] would seemingly have to be altered beyond recognition.

Though the mechanisms suggested by these four sets of investigators cannot accommodate all of our results, the mechanism of ester aminolysis proposed in this paper seems consistent with experimental results obtained in other studies. Therefore, we offer the mechanism at the beginning of this section as a general one for base-catalyzed ester aminolysis applicable also to cases where ester and amine are not part of the same molecule. This mechanism predicts that the reverse reaction, the alcoholysis of amides, will be specific or general base catalyzed depending upon conditions.

Other authors[9,10] have suggested the formation of a tetrahedral addition intermediate in ester aminolysis, but their results are not nearly as compelling evidence for this intermediate as those reported here where a leveling off of the rate is observed at high buffer concentrations. The argument of Bunnett and Davis[9] that their mechanism with a catalyst operating upon a preformed tetrahedral intermediate in a rate-limiting step accounts for slow ester aminolysis in liquid ammonia may not be relevant. At temperatures so far from room temperature, an entirely separate order of relative reaction rates exists, so that results at one temperature cannot be applied to another so different without a detailed knowledge of activation energies. Simple mechanisms with catalysts in the second or both steps cannot accommodate our results.

In order to account for the general base catalyzed aminolysis of *p*-nitrothiolbenzoate and the absence of aminolysis of *p*-nitrobenzoate by butylamine, Connors

(8) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **82**, 675 (1960).
(9) J. F. Bunnett and G. T. Davis, *ibid.*, **82**, 665 (1960).
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and Bender[12] suggested that partitioning of the tetrahedral addition intermediate to yield products was unfavorable in the case of the oxygen ester. Since the partitioning ratio k_3/k_2 is a function of substrates and both the catalytic system and its concentration, this suggestion is consistent with the ratios reported in this work. Connors and Bender[12] also propose that aminolysis proceeds by general base catalyzed proton abstraction from the tetrahedral addition intermediate in the case of the thiol ester. This proposal is not in accord with their suggested k_2/k_3 ratio of 0.1 for thiol ester aminolysis. Both their results and those for the general base catalyzed aminolysis of ethyl thiolacetate by glycine[13] may be analyzed to yield a Brønsted exponent of about 0.3 for the three general bases water, amine, and hydroxide ion. When assigned to the k_3 step, such a value for the Brønsted exponent cannot be realized if the k_1 reaction is to be the predominant rate-limiting one as proposed by Connors and Bender.[12] Accordance could be achieved by increasing the suggested k_2/k_3 ratio by at least a factor of ten. To do so, however, would tend to defeat other features of the argument ascribing the difference between oxygen and thiol ester aminolysis to partitioning of the tetrahedral addition intermediates. The simplest way to account for the observed general base catalysis while at the same time preserving other features of the argument of Connors and Bender is to postulate general base catalysis in the k_1 step.

Not all reactions of amines with esters proceed by the mechanism put forth in this section. In the reaction of imido esters with amines to yield amidines, Hand and Jencks[14] observed maxima in the pH–rate profiles and showed that condensation of ester and amine to yield an addition intermediate is rate limiting on the alkaline side of the maxima while decomposition of the intermediate is rate limiting on the acidic side of the pH–rate profile. They formulated a mechanism and pictured transition states where the main mode of decomposition was *via* an uncharged addition intermediate, but their interpretation of the results also required that some decomposition occur through a protonated intermediate.

Their results may be described more compactly by the mechanism

$$EH^+ + M \rightleftharpoons DH^+$$

$$DH^+ + B \longrightarrow \text{products}$$

where EH^+ represents protonated imido ester, M basic form of amine, DH^+ protonated addition intermediate, and B general base catalyst. Rather than write a transition state for the main mode of decomposition through an uncharged intermediate species D, it is simpler to consider the kinetically equivalent formulation $DH^+ + OH^-$. Hydroxide ion is a much more effective general base than water so that reaction of $DH^+ + H_2O$ would be much slower. Hand and Jencks observed general base catalysis only for those amines where the acid side of the maxima in the pH–rate profile occurs at pH <7. For the more strongly basic amines, where the ascent on the acidic side of the maxima was studied at 7 < pH < 10, no general base catalysis was observed because, according to the mechanism presented here, of the dominant effect of hydroxide ions in this pH region. This formulation and conclusion are consistent with Brønsted type plots for several general bases studied.

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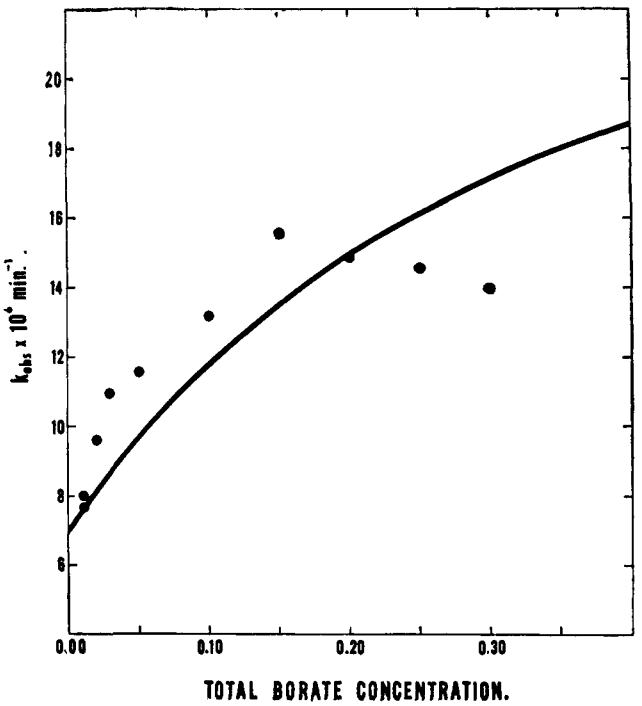


Fig. 2.—Points represent experimental values of observed first-order rate constant *vs.* total boron concentration for conversion of glutamine to pyrrolidonecarboxylic acid at pH 8.47. Curved line represents boric acid concentration at the same pH *vs.* total boron concentration scaled to experimental points of rate constants near intercept and $0.2C_B$.

A kinetically equivalent mechanism for the reaction of imido esters with amines is

$$EH^+ + M \rightleftharpoons DH^+$$

$$D + HB \longrightarrow \text{products}$$

where concentrations of charged DH^+ and uncharged D forms of an addition intermediate are in equilibrium. Instead of general base catalyzed decomposition of protonated intermediate as in the previous paragraph, this last mechanism involves the kinetically equivalent general acid catalyzed decomposition of uncharged intermediate. No matter which kinetically equivalent mechanism is more accurate, it seems necessary to postulate general catalysis in the second step of the reaction of imido esters with amines in contrast to its appearance in the first step of the reaction in aminolysis of the esters discussed above.

Transamidation.—The points in Fig. 2 show the data obtained at pH 8.47 for conversion of glutamine to pyrrolidonecarboxylic acid. At high buffer concentrations in the plot of observed first-order rate constant *vs.* total boron concentration, a leveling off of the rate constant occurs. The curved line in Fig. 2 represents the boric acid concentration at pH 8.47 *vs.* total boron concentration scaled to approximate ordinate intercept and k_{obsd} value at $0.2C_B$. As discussed above in connection with ester aminolysis, polymerization reactions in the buffer system cannot account for the plateau which we conclude is due to a change in rate-limiting step in the transamidation as the catalyst concentration is increased.

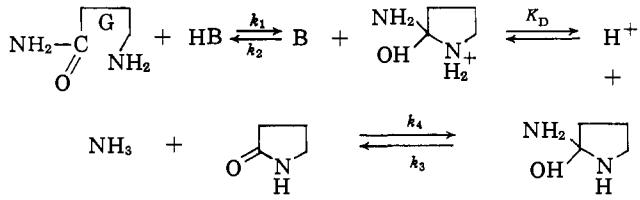
Values from plots of initial rates *vs.* buffer concentration at constant pH for transamidation of glutamine to pyrrolidonecarboxylic acid are presented in Table I. It is immediately evident that values in columns *a*, *b*, and *c* for transamidation are much less pH dependent than those for ester aminolysis. The manipulations performed on Table I data for glutamine in columns *a*, *b*, and *c* to obtain constant values of Table IV are not the same as those used in Table II for ester aminolysis. The manipulations of Table II do not give constant values for glutamine transamidation. The first and third columns of Table IV are not multiplied by (H^+), and the second column was obtained by dividing by the fraction of buffer in the acid form. Transamidation of glutamine to pyrrolidonecarboxylic acid is apparently general *acid* catalyzed. The two separate pH dependences observed in transamidation and ester aminolysis lend confidence that we are studying these reactions and not some properties of a boric acid–borate buffer system. Furthermore, more profound interaction than general acid–base catalysis of the buffer system with reactants and intermediates is rendered less likely by the occurrence of similar plateaus at high buffer concentration with both ester and amide reactants.

TABLE IV

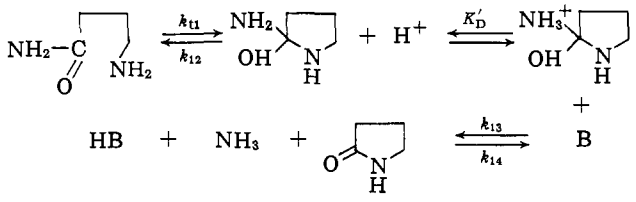
OPERATIONS ON COLUMNS *a*, *b*, AND *c* OF TABLE I TO OBTAIN CONSTANT VALUES FOR GLUTAMINE

a/f, 10^6 min.$^{-1}$	$b[(H^+) + K_B]/f(H^+)$, 10^3 min.$^{-1}$ M^{-1}	c/f, 10^6 min.$^{-1}$
32	1.2	79
21	0.9	56
19	1.1	48

Either of two formulations of the same basic mechanism can account for the results of glutamine transamidation. The second formulation is essentially the reverse of the first, and the choice between the two would seem to be mainly a matter of personal preference. As written for γ-aminobutyramide, these mechanisms are



and



A mechanism with catalytic components in both rate-limiting steps cannot account for the leveling off of rate at high buffer concentrations unless quite different Brønsted exponents are invoked for general catalysts in each step. Our data do not test such a detailed mechanism and the two simpler ones proposed here may be considered as limiting cases of a more general common mechanism.

To be brief and specific we consider only the rate law in terms of the first mechanism, derived on the assumption that the steady state approximation may be applied to tetrahedral addition intermediates.

$$\frac{1}{(G)}\frac{d(P)}{dt} = \frac{k_{obsd}}{f} = \frac{k_3[k_1'(H_2O) + k_1''(HB)]}{k_3 + [k_2'K_w + k_2''(HB)K_B]/K_D} \quad (4)$$

Symbols used in eq. 4 are those of the above diagram or the same as those defined in the previous sections. In the absence of buffers eq. 4 reduces to

$$k_{obsd}/f = k_1'(H_2O) = 24 \times 10^{-6}\ \text{min.}^{-1}$$

where the numerical value is the average of values in the first column of Table IV. From the average value of the second column of Table IV the catalytic coefficient for acid catalysis by boric acid, $k_1'' = 1.1 \times 10^{-3}$ min.$^{-1}$ M^{-1}. Comparison with the above catalytic constant for water yields a Brønsted exponent of about one-half.

In the presence of high concentrations of boric acid eq. 4 becomes

$$k_{obsd}/f = k_3k_1''K_D/k_2''K_B = k_3K_sK_D = 6 \times 10^{-5}\ \text{min.}^{-1} \quad (5)$$

where $K_s = (DH^+)/(G)(H^+)$, the ionization constant for the tetrahedral addition intermediate $K_D = (D)\cdot(H^+)/(DH^+)$, and the numerical value is the average of those in the third column of Table IV. Equation 5 is consistent with the relative pH independence of the plateau for reaction of glutamine.

Partitioning of the tetrahedral intermediate to products or reactants may be indicated by rearranging eq. 5 to obtain

$$k_3K_D/k_2'' = k_3K_sK_DK_B/k_1'' = 5 \times 10^{-11}\ M^2$$

where the numerical value is obtained by substitution of values already discussed into the second term. Since the ionization constant K_D should be greater than the numerical value, k_3/k_2'' is evidently less than unity, as is k_3/k_2'.

In spite of the difference in results, interpretation, and mechanism for ester aminolysis and transamidation, a study[15] of transamidations in a series of glutamines to the corresponding cyclic amides shows over-all rate correlations with structure similar to those observed in this study for aminolysis in a series of amino acid esters.

Results obtained in this work may have significance for other observations. Increased formation rates of lactams or hydroxamates from amides observed in presence of simple phosphate or arsenate ions may be only general catalytic and not specific anion effects, but more study is required before a definitive answer is available.

In the enzymatic formation of glutamine from glutamic acid and ammonia, an enzyme linked γ-carboxyl activated glutamate occurs as an intermediate.[16] In the absence of ammonia this intermediate forms pyrrolidonecarboxylic acid more rapidly than free glu-

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tamine at a rate which suggests that activated glutamic acid may be bound to the enzyme in a γ-ester link.

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Concerning the Mechanism of the Hydrolysis and Aminolysis of Schiff Bases[1]

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The pH–rate profiles and the effect of structure on reactivity for the hydrolysis of Schiff bases derived from benzophenone and aliphatic amines are similar to those previously obtained for the hydrolysis of benzylidene-1,1-dimethylethylamines. The second-order rate constant for the attack of hydroxide ion on the cationic Schiff base, benzhydrylidenedimethylammonium ion, is similar to those calculated from the first-order rate constants for the pH-independent reaction above pH 9 for the attack of hydroxide ion on protonated benzhydrylideneamines. This finding, together with the observed lack of dependence of the first-order rate constants for the pH-independent reaction on the nature of polar substituents, strongly suggests that this reaction does involve the attack of hydroxide ion on the protonated substrates rather than the attack of water on the free base of the substrate. The attack of water on benzhydrylidenedimethylammonium ion is subject to general base catalysis by carboxylate ions. The Brønsted β-value for this reaction is 0.27. Under conditions in which the Schiff bases exist as the conjugate acids, the reactivity of Schiff bases derived from benzophenone and ammonia, methylamine, and dimethylamine, toward hydroxylamine, methoxyamine, semicarbazide, and water decreases with increasing methyl substitution on nitrogen. Values of ρ^+ for the reaction of water, hydroxylamine, and methoxyamine with the conjugate acids of a series of substituted benzhydrylidenemethylamines are 1.1, 0.92, and 0.96, respectively. The similarity of the effect of structural alteration in either the amine or carbonyl component of the Schiff base on reaction rates for the reaction with water, for which attack of the nucleophilic reagent is rate determining, and for the reactions with the amines suggests that the attack of the amines is also rate determining. This suggestion is also supported by the finding that the aminolysis reactions, like the hydrolysis reaction, are subject to general base catalysis. The reaction of a series of substituted benzaldehyde semicarbazones with hydroxylamine and the reaction of a series of substituted benzaldehyde oximes with semicarbazide are dependent upon acid catalysis. Both sets of data are correlated by Hammett ρ-values of -1.7. The pK_a values for the conjugate acids of benzaldehyde oximes have been determined.

Introduction

Numerous studies have established that imines, particularly when present in cationic form $>C{=}N^+<$, readily undergo reaction with a variety of nucleophilic reagents.[3-8] By their apparent involvement in numerous biochemical processes, attention has been focused on two classes of such reactions, those involving water and amines, as nucleophilic reagents. Enzymatic aldolization,[9-12] enzymatic decarboxylation,[13-15] and, perhaps, the visual process[16,17] all appear to involve Schiff base formation and hydrolysis. In addition, pyridoxal phosphate-dependent enzymatic reactions very probably involve both the hydrolytic and aminolytic cleavage of Schiff bases.[18-20]

The formation and hydrolysis of Schiff bases, particularly those involving benzaldehydes as carbonyl components, have received considerable study and, in consequence, the basic features of the mechanism and catalysis of these reactions are reasonably well understood.[21-27] On the other hand, the mechanism and catalysis of transimination reactions, the reactions of Schiff bases with amines, are considerably less well understood although such reactions have received some study.[3,4,7,28]

Results reported herein are concerned principally with the mechanism of the reactions of Schiff bases derived from benzophenone with water and with weakly basic amines. The reaction of these Schiff bases with water was studied for two reasons: first, to extend previous investigations of the mechanism of hydrolysis of Schiff bases to substrates involving benzophenone as the carbonyl component; second, to establish relationships between structure and reactivity for a reaction with these substrates which is reasonably well understood. This reaction, then, will serve as a standard of com-

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