sodium azide, and heated to 130 °C for 18 h. After bicarbonate extraction, concentration,[37] and chromatography over silica gel (vide supra), azido ketal **23d** was crystallized from ethyl acetate–hexane mixtures to afford 980 mg of prisms (29%): mp 83.5–85 °C; IR (Nujol) 2110 (N_3), 1770, 1710 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 7.50–8.00 (m, 4 H, aromatic), 4.05 (s, 4 H, OCH_2CH_2O), 3.83 (t, 2 H, $CH_2Phthal$), 3.30 (s, 2 H, CH_2N_3), 2.17 (t, 2 H, CCH_2); mass spectrum, no molecular ion, but fragment ions at m/e 246 ($M^+ - CH_2N_3$) and 160 ($PhthalCH_2^+$).

Anal. Calcd for $C_{14}H_{14}N_4O_4$: C, 55.62; H, 4.67; N, 18.54. Found: C, 56.00; H, 4.90; N, 18.39.

On scaleup two unidentified more polar compounds were isolated in low yield from the chromatography. The less polar compound, crystallized as prisms from methanol–ethyl acetate mixtures, had the following: mp 154–160 °C; IR (Nujol) 1770, 1715 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 8.50 (s, 1 H), 7.60–7.90 (m, 4 H, aromatic), 4.80 (s, 2 H, CH_2), 4.10 (t, 2 H, $CH_2Phthal$), 3.80–4.10 (m, 4 H, OCH_2CH_2O), 2.20 (t, 2 H, CCH_2).

Anal. Found: C, 54.57; H, 4.68; N, 20.47.

The more polar compound, crystallized from chloroform–methanol mixtures, had the following: mp 225–227.5 °C; ^1H NMR ($CDCl_3$) δ 8.9 (s, 1 H), 7.70–7.90 (m, 4 H, aromatic), 4.60 (s, 2 H, CH_2), 3.85 (t, 2 H, $CH_2Phthal$), 3.60–4.00 (m, 4 H, OCH_2CH_2O), 2.05 (t, 2 H, CCH_2).

Anal. Found: C, 54.16; H, 4.85; N, 21.28.

Azido ketal **23d** was prepared in 64% isolated yield without chromatography by heating bromo ketal **23f** with 5 equiv of sodium azide in Me_2SO at 120 °C for only 18 h. Bromo ketal **23f** (mp 121–122 °C) was prepared in quantitative yield from the known bromo ketone **24f**[41] by refluxing for 5 h with 10 equiv of ethylene glycol in benzene containing several drops of concentrated H_2SO_4.

Amino Ketal Derivatives 2a,c-h. Azido ketal **23d** (14 g, 0.047 mol) in 250 mL of ethyl acetate was hydrogenated over 1.5 g of 10% Pd/C catalyst for 2 h in a Parr bomb. The resulting 12.7 g of oil was treated immediately with thione 1.

Phthalimido ketals **20a-d,f** (10–50 mmol) were dissolved in 100–250 mL of absolute EtOH and treated with 4 equiv of hydrazine hydrate at room temperature for 18 h. After the resulting white solid was filtered, the amines **2a,c,d,f** were obtained by concentration in vacuo and where appropriate distillation. Pertinent physical properties are reported in Table II.

Acknowledgment. We are indebted to Dr. E. C. Olson and his associates for physical and analytical data and to Mr. Paul A. Meulman, Mr. T. A. Scahill, and Dr. Lubomir Baczynskyj for invaluable discussions regarding infrared, ^1H NMR, and mass spectra, respectively. We thank Dr. R. P. Holysz and his associates for the preparation of several chemical intermediates and Mrs. Helen Branch and Ms. Suzanne Moyer for the preparation of this manuscript.

Intramolecular Aminolysis of Esters. *O*-Acetylserine and γ-Esters of Glutamic Acid

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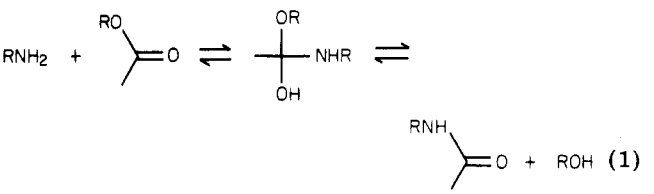
The kinetics of the concurrent hydrolysis and intramolecular aminolysis of γ-ethyl glutamate have been studied in aqueous solution (40 °C) in the range of pH 7.6–10.4. While hydrolysis contributes only 3% to the overall rate of reaction of γ-ethyl glutamate at pH 10.4, its importance relative to aminolysis increases with decreasing pH; at pH 8, the hydrolysis pathway accounts for 32% of the rate of disappearance of the ester. The pH–rate profile for the aminolysis pathway indicates the presence of a water and a hydroxide-catalyzed reaction and provides no evidence for intermediates. The conversion of diethyl glutamate to pyrrolidone-5-carboxylate may occur through either of two competing pathways: (a) rate-determining aminolysis to ethyl pyrrolidone-5-carboxylate, followed by rapid hydrolysis of the ester; (b) rate-determining hydrolysis to γ-ethyl glutamate, followed by rapid cyclization. The pH–rate profile for the intramolecular aminolysis of *O*-acetylserine, determined at zero buffer concentration (30 °C), has the complex appearance characteristic for acyl-transfer reactions involving neutral and anionic tetrahedral intermediates. Quantitative support for the interpretation of the pH–rate profile comes from the analysis of the nonlinear increases in the rate of aminolysis observed in the presence of increasing concentrations of phosphate buffers. The results of this and earlier studies suggest that there may not be major differences in the mechanisms of the intra- and intermolecular aminolysis of weakly acidic alcohols.

Considerable evidence exists that the aminolysis of esters[1-7] (and its reverse, the alcoholysis of amides)[8-12] in aqueous solution involves the participation of unstable tetrahedral addition intermediates (eq 1). Since these



(1)

intermediates do not usually accumulate, their presence has of necessity been demonstrated by indirect means,

(1) Hansen, B. *Acta Chem. Scand.* **1963**, *17*, 1307–1314.
(2) Martin, R. B.; Parcell, A.; Hedrick, R. I. *J. Am. Chem. Soc.* **1964**, *86*, 2406–2413.
(3) Blackburn, G. M.; Jencks, W. P. *J. Am. Chem. Soc.* **1968**, *90*, 2638–2645.
(4) Sawyer, C. B.; Kirsch, J. F. *J. Am. Chem. Soc.* **1973**, *95*, 7375–7381.
(5) Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7018–7031.
(6) O'Leary, M. H.; Marlier, J. F. *J. Am. Chem. Soc.* **1979**, *101*, 3300–3306.
(7) Kirby, A. J.; Mujahid, T. G.; Camilleri, P. *J. Chem. Soc., Perkin Trans. 2*, **1979**, 1610–1616.
(8) Cunningham, B. A.; Schmir, G. L. *J. Am. Chem. Soc.* **1967**, *89*, 917–922.
(9) Belke, C. J.; Su, S. C. K.; Shafer, J. A. *J. Am. Chem. Soc.* **1971**, *93*, 4552–4560.
(10) Okuyama, T.; Schmir, G. L. *J. Am. Chem. Soc.* **1972**, *94*, 8805–8811.
(11) Chiong, K. N. G.; Lewis, S. D.; Shafer, J. A. *J. Am. Chem. Soc.* **1975**, *97*, 418–423.
(12) Morris, J. J.; Page, M. I. *J. Chem. Soc., Perkin Trans. 2*, **1980**, 679–684; *Ibid.* **1980**, 685–691.

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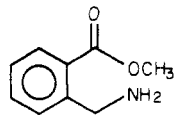
using mainly the following types of observations: (a) changes in rate-determining step with changing pH; (b) changes in rate-determining step with changes in the concentration or in the pK_a of general acid–base catalysts; (c) changes in isotope effects.

Not infrequently, however, such reactions fail to provide any evidence for intermediates. An example is the intramolecular aminolysis of 1, which shows no change in



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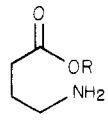

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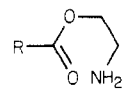

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rate-limiting step over the range of pH 6–13.[13] This is not to say that intermediates are not involved in this reaction; it may simply mean that the properties of the intermediates are such that the rate-determining step remains the same at all the pH values studied. In this case, it has been proposed[13] that intramolecular aminolysis may differ in mechanism from bimolecular aminolysis, for which a change in rate-determining step with varying pH is well documented.

One way to look at the mechanism of these reactions is based on the view that it is the mode of partitioning of the intermediates which determines what is the rate-determining step under various conditions of reaction. Intermediates of this type break down to yield either an amine and an ester or an amide and an alcohol. Unfortunately, it is not yet possible to predict, or even merely to explain in any sort of quantitative manner, the partitioning of such tetrahedral intermediates between the two possible sets of products.[14]

To establish what kinds of differences may exist between the mechanisms of intramolecular as compared to bimolecular aminolysis, we have reinvestigated the intramolecular aminolysis of two types of amino esters, exemplified by 2 and 3.

With γ-esters of glutamic acid and related amino esters, the nonlinear increase in the rate of cyclization with increasing borate buffer concentration has been interpreted in terms of a change in the rate-determining step of the reaction.[2] The known tendency of borate to polymerize in aqueous solution complicates the interpretation of the nonlinear dependence of rate on catalyst concentration.[16] In the present research, we have sought to confirm the existence of a change in rate-determining step by means of a detailed study of the effect of pH variation on the rate of the intramolecular aminolysis of glutamic acid γ-ethyl ester and of diethyl glutamate. We have also studied the effects of variation in pH and buffer concentration on the rate of the intramolecular O to N acetyl transfer in *O*-acetyl serine.

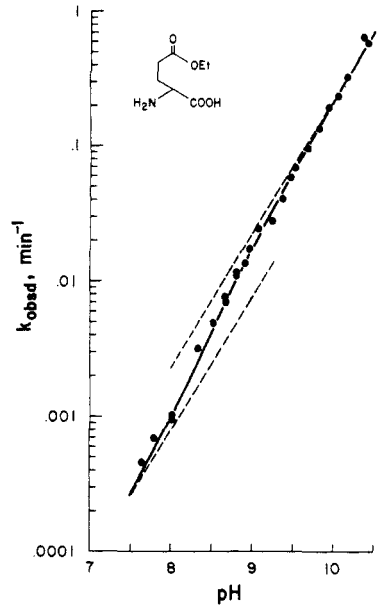


Figure 1. pH–rate profile for disappearance of γ-ethyl glutamate at 40 °C, μ = 0.5. Solid line is calculated from the sum of eq 4 and 7, using constants given in the text. Dashed lines have a slope of one.

Results

Glutamic Acid γ-Ethyl Ester. The rate of disappearance of γ-ethyl glutamate, in unbuffered aqueous solution (μ = 0.5, KCl) at 40 °C, was determined by measuring the rate of proton release, at constant pH, by means of a pH-stat. Observed first-order rate constants, obtained in the range of pH 7.6–10.4, increase with increasing pH (Figure 1) and the plot of log k_{obsd} vs. pH gives an acceptable straight line of slope 1.14 (r = 0.997). While it would have been desirable to carry out rate measurements over a larger pH range, especially at the lower end, the pH-stat technique is not suitable for the measurement either of very slow reactions (at pH 7.6, $t_{1/2}$ = 25 h) or of fast reactions (at pH 10.4, $t_{1/2}$ = 1 min). An additional complication is that, at pH >10, only a small fraction of the substrate is protonated (pK_a = 8.75 for the amino group), so that cyclization to pyrrolidone-5-carboxylate is accompanied by the release of relatively few protons. That the major reaction product is in fact the pyrrolidone was indicated in the earlier study[2] by measurements of UV absorbance at 200 nm. In the present study, exploratory experiments showed that the intramolecular aminolysis could also be readily followed by measurement of the increase in optical rotation at 225 nm, owing to the much more dextrorotatory character of the pyrrolidone as compared to its acyclic precursor.

The extent to which γ-ethyl glutamate undergoes hydrolysis concurrently with aminolysis was determined by ninhydrin assay for glutamic acid, after at least 8 half-lives of reaction. The fraction of substrate which is converted to glutamic acid increases with decreasing pH, from about 3% at pH 9.9–10.4 to 32% at pH 8.0. The pH–rate profile for hydrolysis (Figure 2B) was constructed from the observed rate constants for the overall disappearance of γ-ethyl glutamate and the mole fraction of glutamate produced at each pH value (eq 2). Although the rate of

$$k_H = k_{obsd}(\text{mole fraction of glutamate}) \quad (2)$$

(13) Fife, T. H.; DeMark, B. R. *J. Am. Chem. Soc.* **1976**, *98*, 6978–6982.

(14) Deslongchamps et al.[15] have proposed that the direction of breakdown of tetrahedral intermediates of the type considered here is under stereoelectronic control. Application of this theory has been generally limited to the distinction between two classes of intermediates, those which break down to yield exclusively amine and those which give rise to mixtures of amine and amide. In its present state, the theory does not yet lead to more quantitative conclusions.

(15) (a) Deslongchamps, P.; Lebreux, C.; Taillefer, R. *Can. J. Chem.* **1973**, *51*, 1665–1669. (b) Deslongchamps, P. *Pure Appl. Chem.* **1975**, *43*, 351–379. (c) Deslongchamps, P.; Dube, S.; Lebreux, C.; Patterson, D. R.; Taillefer, R. J. *Can. J. Chem.* **1975**, *53*, 2791–2807. (d) Deslongchamps, P.; Taillefer, R. J. *Ibid.* **1975**, *53*, 3029–3037. (e) Deslongchamps, P. *Tetrahedron* **1975**, *31*, 2463–2490; (f) *Heterocycles* **1977**, *7*, 1271–1317. (g) Deslongchamps, P.; Cheriyan, U. O.; Pradere, J.-P.; Soucy, P.; Taillefer, R. J. *Nouv. J. Chim.* **1979**, *3*, 343–350. (h) Deslongchamps, P.; Cheriyan, U. O.; Taillefer, R. J. *Can. J. Chem.* **1979**, *57*, 3262–3271.

(16) (a) Jencks, W. P. "Catalysis in Chemistry and Enzymology"; McGraw-Hill: New York, 1969; p 470. (b) Cotton, F. A.; Wilkinson, G. "Advanced Inorganic Chemistry" 3rd ed.; Interscience: New York, 1972; p 230–233.

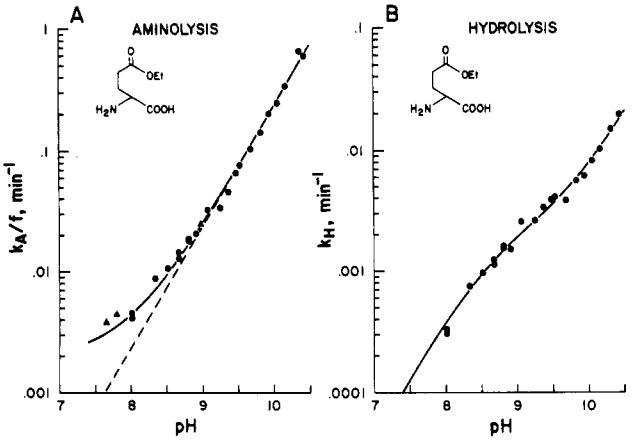


Figure 2. (A) pH–rate profile for the aminolysis of γ-ethyl glutamate. Solid line is calculated from eq 8, using constants given in the text. Triangles at pH 7.8–8 represent experiments in which the mole fraction of ester hydrolysis was not directly measured but was obtained by a short extrapolation of the data in Figure 2B. Dashed line has a slope of one. (B) pH–rate profile for the hydrolysis of γ-ethyl glutamate. Curve is calculated from eq 4, using constants given in the text.

hydrolysis increases with increasing pH, the plot of log k_H vs. pH is not linear. The rate expression of eq 3 and 4

$$\text{rate} = k^+[EH^+][OH^-] + k^\circ[E][OH^-] \quad (3)$$

$$k_H = \frac{k^+K_w + k^\circ K_a K_w/[H^+]}{[H^+] + K_a} \quad (4)$$

(where $[EH^+]$ and [E] represent ester with the amino group protonated and neutral, respectively) accounts satisfactorily for the data. The same rate equation has been found to describe the alkaline hydrolysis of several α- and γ-amino acid esters.[17] The curve in Figure 2B was calculated from eq 4, with $k^+ = 140\ M^{-1}\ min^{-1}$, $k^\circ = 20\ M^{-1}\ min^{-1}$, $pK_a = 8.75$, and $pK_w = 13.53$. The value for k° is less accurate than that for k^+, since it is based mainly on the small yields of glutamic acid found at pH 9.5–10.4. The absolute values of k^+ and k° for ethyl glutamate are not very different from those reported for the hydrolysis of methyl 4-aminobutyrate at 25 °C ($k^+ = 125\ M^{-1}\ min^{-1}$, k° = ca. 6 $M^{-1}\ min^{-1}$).[17d,18] Protonation of the amino group of amino acid esters is known to increase the rate of their alkaline hydrolysis[17] but the rate enhancement found with γ-ethyl glutamate is appreciably smaller than that of ca. 100-fold observed with α-amino acid esters.[17c] The rate-enhancing effect of protonating the amino group thus appears to be damped out when the distance between the amino group and the ester carbonyl is increased.

In aqueous solution, a pH-dependent equilibrium may be established between glutamic and pyrrolidone-5-carboxylic acids. Despite the fact that in neutral or weakly alkaline solution the equilibrium favors the pyrrolidone, the cyclization of glutamate is expected to be extremely slow under the conditions of the present experiments and thus may be neglected.[19,20a]

First-order rate constants (k_A) for the intramolecular aminolysis of γ-ethyl glutamate are obtained from k_{obsd} for the disappearance of the substrate and the rate constant (k_H) for hydrolysis (eq 5). The aminolysis reaction obeys

$$k_A = k_{obsd} - k_H \quad (5)$$

the rate law of eq 6, and the pH-dependence of k_A is given by eq 7. Analysis of the pH–rate profile for aminolysis

$$\text{rate} = k_{H_2O}[E] + k_{OH}[E][OH^-] \quad (6)$$

$$k_A = \frac{k_{H_2O}K_a + k_{OH}K_wK_a/[H^+]}{[H^+] + K_a} \quad (7)$$

is simplified by expressing the rate constants in terms of ester containing an unprotonated amino group (eq 8). The

$$\frac{k_A}{f} = \frac{k_A}{K_a/([H^+] + K_a)} = k_{H_2O} + k_{OH}K_w/[H^+] \quad (8)$$

theoretical curve based on eq 8 was calculated by using $k_{OH} = 750\ M^{-1}\ min^{-1}$ and $k_{H_2O} = 2 \times 10^{-3}\ min^{-1}$, and the agreement between experiment and theory is acceptable (Figure 2A).

The calculated pH–rate profile for the overall disappearance of γ-ethyl glutamate (Figure 1) is obtained by the summation of eq 4 and 7. At both extremes of pH, the plot of log k_{obsd} vs. pH is expected to approach lines of unit slope, the line at low pH obeying eq 9, and the line at high pH being given by eq 10. The adherence of the observed

$$\text{(low pH)} \quad k_{obsd}\ (min^{-1}) = (k^+K_w + k_{H_2O}K_a)/[H^+] = (7.69 \times 10^{-12})/[H^+] \quad (9)$$

$$\text{(high pH)} \quad k_{obsd}\ (min^{-1}) = K_w(k^\circ + k_{OH})/[H^+] = (2.27 \times 10^{-11})/[H^+] \quad (10)$$

rate constants to a line of slope 1.14 when log k_{obsd} is plotted vs. pH is thus without precise mechanistic significance and is simply an empirical description of the transition of the log k_{obsd} values from one line of unit slope to another of the same slope displaced from the first one by 0.47 log units along the y-axis.

The rate equations for hydrolysis (eq 4) and for aminolysis (eq 7) have the same algebraic form, so that at the extremes of pH the ratio k_H/k_A will become independent of pH. As pH is decreased, the hydrolytic pathway will account for a maximum of 54% of substrate disappearance, while at pH >10, hydrolysis will reach a limiting value of 2.6% of the overall reaction. These conclusions will remain valid only as long as no new terms are added to the rate laws of eq 3 and 6 and as long as no change in mechanism occurs.

Efforts to determine whether the aminolysis reaction was susceptible to general acid–base catalysis by phosphate buffer (40 °C, $\mu = 0.9$) led to inconclusive results. Experiments at pH 9.1 or 9.5, with phosphate up to 0.3 M, showed small rate increases of no more than 20% above the rate at zero buffer concentration. Since small changes in rate constants were also found when the nature of the salt used to maintain constant ionic strength was varied from KCl to K_2SO_4 to KNO_3, the possibility of a specific salt effect of phosphate ions on the rate of aminolysis cannot be ruled out at present.

Diethyl Glutamate. Although kinetic data for the intermolecular aminolysis of diethyl glutamate[2] and the "hydrolysis" (see below) of dimethyl glutamate[17a] in weakly basic aqueous solution have been reported, it appears that the interpretation of these results is more complex than had been realized. The discussion that follows is simplified

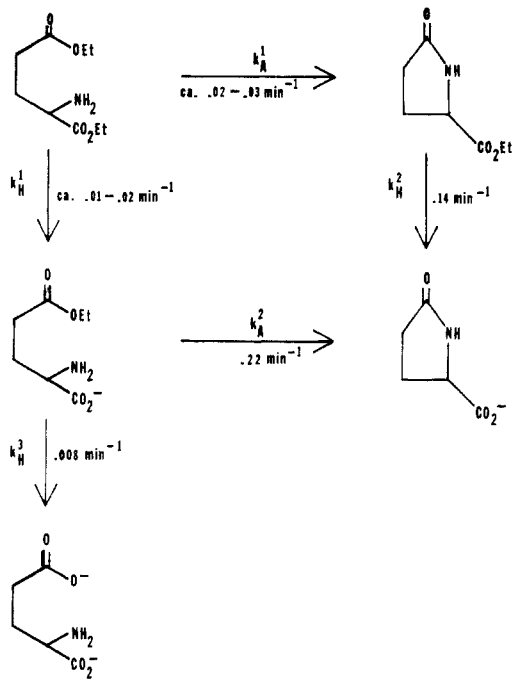
(17) (a) Hay, R. W.; Porter, L. J.; Morris, P. J. *Austr. J. Chem.* **1966**, *19*, 1197–1205. (b) Hay, R. W.; Porter, L. J. *J. Chem. Soc.* **1967**, 1261–1264. (c) Hay, R. W.; Morris, P. J. *Ibid.* **1970**, 1577–1582. (d) Hay, R. W.; Morris, P. J. *J. Chem. Soc., Perkin Trans. 2*, **1972**, 1021–1029.

(18) The similarity of k^+ for the hydrolysis of *γ-ethyl* glutamate (40 °C) and *methyl* 4-amino butyrate (25 °C) is the result of the near cancellation of two opposing effects. Increasing the reaction temperature from 25 to 40 °C is expected to increase the rate about twofold,[17b,c] but ethyl esters are hydrolyzed in alkali at about half the rate of methyl esters.[17a]

(19) Wilson, H.; Cannan, R. K. *J. Biol. Chem.* **1937**, *119*, 309–331.

(20) Greenstein, J. P.; Winitz, M. "Chemistry of the Amino Acids"; Wiley: New York, 1961; (a) Vol. 3, p 1935; (b) Vol. 2, p 927.

Scheme I



by reference to Scheme I, where the rate constants are for the reactions shown, at 40 °C and pH 10.0.

First-order rate constants for the hydrolysis of ethyl pyrrolidone-5-carboxylate in 10% acetonitrile–water (40 °C, μ = 0.5) were measured in the range of pH 9.7–10.5, by means of the pH-stat method. The plot of log k_{obsd} vs. pH is linear with unit slope, and the derived second-order rate constant is 460 M^{-1} min^{-1} (based on pK_w = 13.53). This value is 5–10 times greater than that expected on the basis of the known effects of N-acetylation[21] or N-benzoylation[22] on the rates of the alkaline hydrolysis of α-amino acid esters. With acyclic α-amino acid esters, the *N*-acyl derivative undergoes alkaline hydrolysis at a rate approximately equal to that for the neutral amino acid ester. The enhanced reactivity of ethyl pyrrolidonecarboxylate may be the result of diminished steric hindrance in the vicinity of the ester function of this cyclic compound, as compared to acyclic analogues.

The rate constant for the disappearance of diethyl glutamate at pH 10.0 (40 °C, μ = 0.5) is 0.037 min^{-1}, as determined eiter from the rate of proton release (pH-stat) or from the rate of increase of the optical rotation at 228 nm. The rate of the *hydrolysis* of the α-ester function of diethyl glutamate at pH 10 is estimated to be in the range of 0.01–0.02 min^{-1}, based on the detailed studies of Hay et al. on the alkaline hydrolysis of other amino acid esters.[17b,c] The only reaction considered here is that between hydroxide ion and the neutral ester, no significant reaction with the protonated ester (pK_a 6.85) being expected at pH 10. It follows that the rate constant for the intramolecular *aminolysis* of diethyl glutamate (Scheme I, k_A^1) is approximately 0.02–0.03 min^{-1} (pH 10).

The measured and estimated values of the rate constants in Scheme I indicate that there are two possible pathways for the conversion of diethyl glutamate to pyrrolidonecarboxylate ion. The rate-determining step in the formation of the pyrrolidone is either the aminolysis step k_A^1 or the hydrolysis step k_H^1 (or a combination of both these reactions) since each of these primary reactions is followed by a relatively fast subsequent reaction. The kinetics of the overall reaction will probably show little or no deviation from first-order behavior since no more than 10% of the total material will accumulate as either of the two intermediates, γ-ethyl glutamate or ethyl pyrrolidonecarboxylate.[23] The observed first-order rate constant will be equal to $k_A^1 + k_H^1$. Thus, the increase in UV absorbance, which results from the formation of the cyclic products,[2] will obey first-order kinetics even if the major reaction pathway consists of rate-limiting hydrolysis (k_H^1) followed by rapid cyclization to the pyrrolidone carboxylate ion via step k_A^2. For the same reason, proton release will be approximately first-order, even if the main pathway is the (neutral) intramolecular aminolysis step k_A^1, followed by a rapid hydrolysis step which generates the proton monitored by the pH-stat.

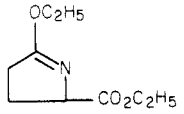
These considerations explain why earlier workers, having observed the expected release of 1 equiv of protons per mole of dimethyl glutamate, concluded that the derived rate constant was solely that for hydrolysis of the α-ester group.[17a] Since the rates of aminolysis and hydrolysis of diethyl glutamate are not very different, it is not surprising that the rate constant for the "hydrolysis" of dimethyl glutamate fell on or near a line which correlated the rate constants for the alkaline hydrolysis of several α-amino acid esters with Taft's polar substituent constants.[17b,24]

The plot of log k_{obsd} for the reaction of diethyl glutamate (by all pathways) vs. pH is a line of unit slope in the range of pH 9.5–10.7 and follows eq 11, with k_{OH} = 130 M^{-1} min^{-1}.

$$k_{obsd}\ (min^{-1}) = k_{OH}[OH^-] = (3.7 \times 10^{-12})/[H^+] \qquad (11)$$

The ratio of the reactivities of γ-ethyl glutamate and diethyl glutamate at pH ≥10 is 5.9, which may be compared to the value of 7.5 reported for these reactions at 25 °C.[2] In view of the difficulty in determining the contribution of the aminolysis reaction (Scheme I, step k_A^1) to the overall rate of the disappearance of diethyl glutamate, no further attempt was made to construct the pH–rate profile for the intramolecular aminolysis of this compound.

An unsuccessful effort was made to predict the pH value at which a change in rate-limiting step would be expected to occur in the aminolysis of diethyl glutamate by studying the effect of pH variation on the *initial* products of the hydrolysis of the imidate ester **4**.[3,8,25] Preliminary ex-



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periments, carried out at room temperature, showed that

(21) Purdie, J. E.; Benoiton, N. L. *Can. J. Chem.* **1971**, *49*, 3468–3476.
(22) (a) Kirsch, J. F.; Katchalski, E. *Biochemistry* **1965**, *4*, 884–890. (b) Hay, R. W.; Morris, P. J. *Chem. Commun.* **1967**, 663–664. (c) de Jersey, J.; Willadsen, P.; Zerner, B. *Biochemistry* **1969**, *8*, 1959–1967.

(23) Calculated from the equations for consecutive first-order reactions: Frost, A. A.; Pearson, R. G. "Kinetics and Mechanism"; Wiley: New York, 1953; p 153.
(24) (a) Although in a later publication,[17d] Hay and Morris discuss the reactions of two γ-amino acid esters in terms of competing aminolysis and hydrolysis, no further mention is made of dimethyl glutamate. (b) The claim[17a] that γ-methyl glutamate is unreactive at pH 11 is evidently erroneous. That no proton release was found in the pH-stat is understandable, since only about 1% of the amino group (pK_a ca. 9.0 at 25 °C) is protonated, and, in addition, the aminolysis reaction has an estimated half-life of 20 s under these conditions. The statement[17a] that "the unreactive nature of the γ-methoxycarbonyl group" is shown by the (alleged) preparation of γ-methyl glutamate by hydrolysis of dimethyl glutamate with triethylamine rests on a probable misinterpretation of the reference cited.[20b] Triethylamine was used most likely to neutralize excess HCl and to ionize the carboxyl group of the γ-ester prepared by selective γ-esterification of glutamic acid.
(25) Schmir, G. L. *J. Am. Chem. Soc.* **1968**, *90*, 3478–3486.

Table I. Rate Constants for Phosphate Buffer Catalysis of the Aminolysis of *O*-Acetylserine[a]

pH	Δk_{max}, min^{-1} [b,d]	K_{app}, M [b,d]	k_{max}, min^{-1} [c]	k_{max}/K_{app}, M^{-1} min^{-1}	k_{PO_4}, [e] M^{-1} min^{-1}
8.03	0.12 ± 0.007	0.008 ± 0.002	0.14	18	71
8.31	0.44 ± 0.05	0.021 ± 0.006	0.50	24	56
8.61	1.09 ± 0.10	0.033 ± 0.009	1.19	36	59
8.91	2.09 ± 0.17	0.042 ± 0.009	2.25	54	70
					64 ± 6

[a] 30 °C, μ = 0.5. [b] See eq 13. [c] $k_{max} = \Delta k_{max} + k_0$, where k_0 = rate at zero buffer. [d] Standard deviation is given. [e] Second-order rate constant for catalysis by phosphate, calculated as described in the text.

the rate of hydrolysis decreased with increasing pH (pH 2–4, $t_{1/2}$ = ca. 10 min; pH 7.5, $t_{1/2}$ = 22 h), as expected[26] from the behavior of other imidate esters of relatively low pK_a (the pK_a for **4** estimated from the pH–rate profile for its hydrolysis is ca. 5.1). Control experiments indicated that, at pH values near neutrality, diethyl glutamate underwent considerable cyclization during the extended period of time required for complete hydrolysis of the imidate ester. In these circumstances, it would be quite difficult to determine accurately how much pyrrolidone was formed directly from the imidate ester, rather than by subsequent cyclization of initially formed diethyl glutamate.

***O*-Acetylserine.** The pH–rate profile for the conversion of *O*-acetyl- to *N*-acetylserine by intramolecular aminolysis was determined by the pH-stat method at 30 °C, μ = 0.5, in the range of pH 6.9–10.2 (Figure 3A). Small amounts of serine (4% at pH 9.9; 8% at pH 7.2), arising presumably from the hydrolysis of the ester, were detected by ninhydrin assay at the end of the reaction. No correction was applied to the observed rate constants for this minor side reaction; the quantitative conclusions deduced from the pH–rate profile would be affected very little if the correction had been made. Fragmentary data reported in the literature yield rate constants consistent with those in Figure 3A.[27,28]

Correction of the pH–rate profile for the extent of protonation of the amino group of *O*-acetylserine (pK_a = 8.30) gives the profile of Figure 3B for the pH dependence of the aminolysis expressed in terms of amine free base. The existence of two regions of slope approaching unity, separated by a region of lesser slope, is characteristic for acyl-transfer reactions involving anionic and netural tetrahedral intermediates and a change in rate-determining step with changing pH.[3,5,8,25] Though the reaction mechanism is almost certainly more complex (see Discussion), it is sufficient for the moment to consider the formulation of Scheme II, in which rate constants may include proton-transfer steps. The corrected rate constants (k_{obsd}/f) obey eq 12, in which P^- describes the partitioning of the

$$\frac{k_{obsd}}{f} = \frac{k_{obsd}}{K_a/([H^+] + K_a)} = \frac{k_1'(1 - P^-)K''(1 + K''P^-/[H^+])}{[H^+] + K''} \quad (12)$$

anionic intermediate and pK'' gives the pH value where the change in rate-determining step occurs ($P^\circ = k_2'/(k_2' + k_3')$; $P^- = k_2''/(k_2'' + k_3'')$; $K'' = K_2(k_2'' + k_3'')/(k_2' + k_3')$). For the derivation of eq 12 from Scheme II, the steady-state approximation has been applied to T° and T^-, and it has been assumeed that the partitioning ratio P° for

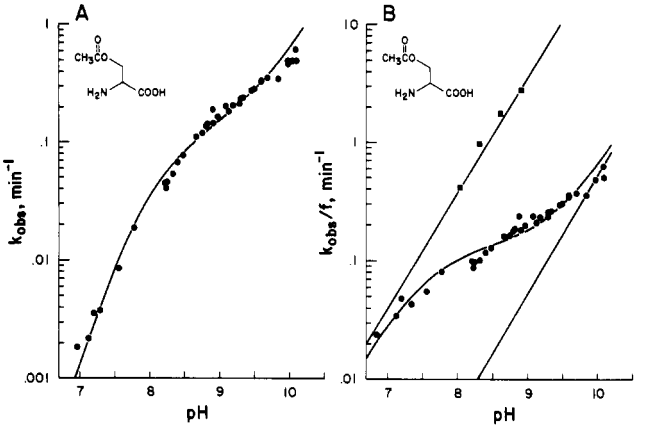
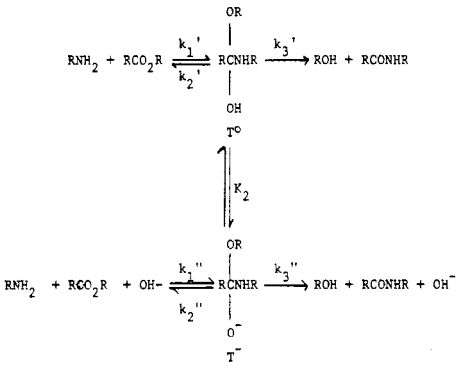


Figure 3. (A) pH–rate profile for the aminolysis of *O*-acetylserine at 30 °C, μ = 0.5. Curve is calculated from eq 12 (using constants given in the text) and then multiplying the resulting rate constants by the mole fraction of amine in the free base form. (B) pH–rate profile for the aminolysis of *O*-acetylserine, expressed in terms of amine free base. Curve is calculated from eq 12, using constants given in the text. Straight lines have a slope of one and represent the limiting values of the rate constants at low and high pH. Squares represent the maximum rate constants approached at high phosphate buffer concentration (see Figure 4).

Scheme II



the neutral intermediate is ≃1.0 (i.e., $k_2' \gg k_3'$). The constants used to compute the theoretical curve of Figure 3B are k_1' = 0.14 min^{-1}, pK'' = 7.6, and P^- = 0.015.[51]

The aminolysis of *O*-acetylserine is subject to catalysis by buffer species. Increasing concentrations of phosphate buffer cause a nonlinear increase in rate (Figure 4). At high buffer concentration, the rate reaches a constant value, which is pH dependent. The curves in Figure 4 fit a rectangular hyperbola (eq 13, where Δk and Δk_{max} are,

$$k_{obsd} - k_0 = \Delta k = \frac{k_{max}\,[\text{phosphate}]}{[\text{phosphate}] + K_{app}} \quad (13)$$

respectively, the increase in rate caused by a given buffer concentration and the maximum possible increase in rate, K_{app} is the buffer concentration required to give half the maximum possible rate increase, and k_0 is the rate at zero buffer concentration). Use was made of a nonlinear

(26) Chaturvedi, R. K.; Schmir, G. L. *J. Am. Chem. Soc.* **1968**, *90*, 4413–4420.
(27) Josefsson, L.; Edman, P. *Biochim. Biophys. Acta* **1957**, *25*, 614–623.
(28) Rabinowitz, J. L. *C. R. Trav. Lab. Carlsberg* **1960**, *31*, 483–506.

Table II. Partitioning of Tetrahedral Intermediates Formed in the Aminolysis of Esters and the Alcoholysis of Amides

reactant(s)	pK'' [a]	k^{RNH_2}/k^{ROH} [b]	k^{RO^-}/k^{RNH^-} [c]	ref [d]
O-acetylethanolamine	7.76		33	1
O-acetylserine	7.6		67	this study
methyl formate + glycine amide	6.34		234	3
methyl formate + morpholine	7.36		780	3
methyl formate + hydrazine	8.74	220		3
ethyl acetate + hydrazine	8.96		146-352	5
4-hydroxybutyranilide	7.17		1500	8
13	8.43	59	43	9
14	7.68	40	11	11

[a] pH where transition in rate-limiting step occurs. [b] Partitioning of T^o, given by $1/1 - P^o$. [c] Partitioning of T^-, given by $1/P^-$. For definitions of K'', P^o, and P^-, see Scheme II and text. [d] In the case of ref 3 and 5, the constants presented in this table are calculated from other constants cited in those papers. For the reactions from ref 1, 9, and 11, the constants were calculated by nonlinear least-squares curve fitting of the original rate data to eq 12 (for *O*-acetylethanolamine) and to the complete equation derived from Scheme II (i.e., without the assumption that $P^o = 1$; see ref 30 and 31) for compounds 13 and 14.

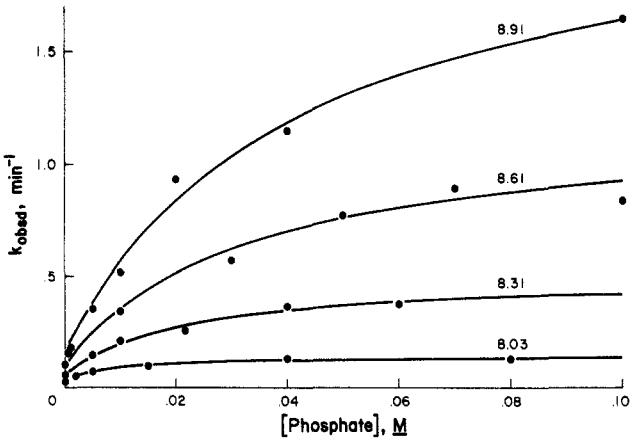
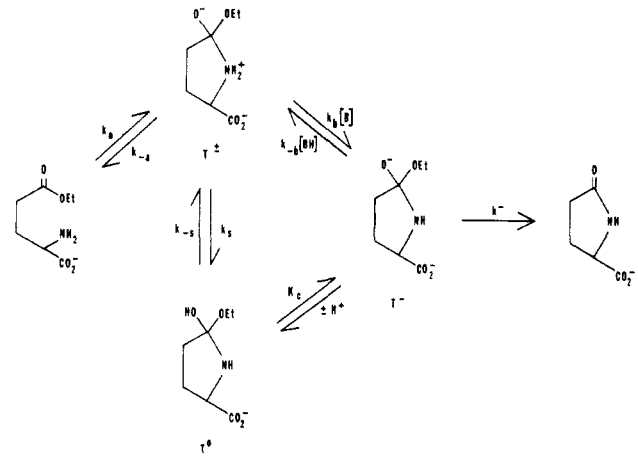


Figure 4. Dependence of the rate of aminolysis of *O*-acetylserine on phosphate buffer concentration. Numbers give pH values. Curves are calculated from eq 13, using constants given in Table I.

Scheme III



least-squares computer-fitting procedure[29] to obtain Δk_{max} and K_{app} (Table I). The squares in Figure 3B represent the values of k_{max} (where $k_{max} = \Delta k_{max} + k_0$) after correction for the extent of protonation of the amino group of *O*-acetylserine.

Second-order rate constants for catalysis by phosphate buffer are obtained from the ratio k_{max}/K_{app} (Table I, column 5), whose values are then divided by the terms $K_a/([H^+] + K_a)$ and $K''/([H^+] + K'')$ to correct, respectively, for the extent of amino group protonation and for the partial change in rate-limiting step as pH is varied. Though there is some scatter, no trend is apparent in these corrected values (Table I, last column), indicating that, in this pH range, catalysis is entirely due to phosphate dianion.

Experiments at pH 8.3 and 8.6 showed that imidazole is ca. 8–10 times less effective than phosphate in catalyzing the aminolysis reaction.

Discussion

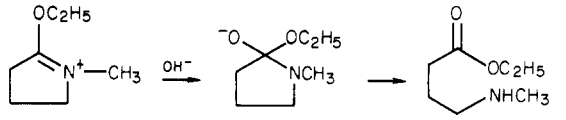
The results of the present study will be considered within the framework of the mechanism of the aminolysis of esters of aliphatic alcohols, proposed by Satterthwait and Jencks[5] and illustrated for γ-ethyl glutamate in Scheme III. The pH–rate profile for the intramolecular aminolysis of the ester (Figure 2A) provides no evidence for a change in rate-limiting step with changing pH or, for that matter, for the existence of any intermediates at all, despite the fact that this reaction was studied over a range of pH which was twice as large as that previously[2] reported. However, since tetrahedral intermediates have been shown to occur both in intramolecular[1,7] as well as in intermolecular[3-6] aminolysis of esters, it will be assumed that they are formed here also.

The rate law of eq 8 could refer either to rate-determining proton-transfer steps (k_s and k_b) or to rate-determining breakdown of the anionic intermediate T^-.[5] Assignment of these terms to the proton-transfer steps seems more reasonable than the alternative. In most of the cases studied to date (Table II), a transition in rate-limiting step takes place in the range of pH 7–9, from rate-limiting breakdown of T^- at low pH to rate-limiting proton transfer at higher pH. If there is to be such a change in rate-determining step in this reaction, it is more likely to occur at pH <7 than at pH >10.5, so that the data of these experiments probably refer to the proton-transfer steps. In terms of Scheme III, constants k_{H_2O} and k_{OH} of eq 8 become $k_a k_s/k_{-a}$ and $k_a k_b^{OH}/k_{-a}$,[36] respectively. The water term represents a rate-determining proton switch which converts $T^\pm$ to T^o, with $T^\pm$ having been formed from the ester in a rapid equilibrium step. The hydroxide term refers to the diffusion-controlled encounter of hydroxide ion with $T^\pm$, which serves to trap the highly reactive zwitterionic intermediate and prevents its reversal to the reactant.[5]

There is some reason to believe that perhaps no change in rate-determining step should be expected to occur at all in the intramolecular aminolysis of glutamate esters and of related esters derived from the γ-aminobutyric acid system. As we have discussed in previous publications,[25,30,31] an acyl-transfer reaction involving two inter-

(29) Hanson, K. R.; Ling, R.; Havir, E. *Biochem. Biophys. Res. Commun.* **1967**, *29*, 194–197.

mediates of different net charge (or some kinetic equivalent) should exhibit a change in rate-limiting step as pH is varied, if the two intermediates partition differently between reactants and products. The determination of the products of hydrolysis of an appropriate imidate ester often[3,8,32] (though not always)[33] provides a useful way of assigning the nature of the rate-limiting step of an acyl-transfer reaction thought to involve the same intermediates as in the hydrolysis of the imidate. In alkaline solution, the cationic imidate **5** is hydrolyzed to yield the γ-amino



5 **6** **7**

ester **7** in 65% yield, presumably via the anionic intermediate **6**, which resembles closely the anionic intermediate T^- of Scheme III.[15c] The pH–rate profile of an acyl-transfer reaction proceeding via a neutral intermediate, which breaks down always (or almost always) to amine and ester, and via an anionic intermediate which yields 65% amine and 35% amide will show at the most a slight deviation from the rate law of eq 8;[30] given the not insignificant experimental error in the points of Figure 2A, such a deviation might well be missed.[34] Put another way, an aminolysis reaction which involves two intermediates which each give mostly amine proceeds to amide largely via rate-determining breakdown of the intermediates at all pH values and thus is not expected to show a major change in the nature of the rate-determining step. We will consider below the question of whether anionic intermediate **6** is an adequate model for T^- in Scheme III.

The pH dependence of the intramolecular aminolysis of *O*-acetylserine (Figure 3) is quite similar to that of *O*-acetylethanolamine;[1] the latter has previously been interpreted in terms of Scheme II[25] which includes neutral and anionic intermediates in acid–base equilibrium. Applying now the more detailed Satterthwait–Jencks formulation[5] (Scheme III), the pH–rate profile for acetyl transfer in *O*-acetylserine (Figure 3B) may be described as follows. At pH >7.6, the rate-limiting steps consist of a pH-independent proton switch (k_s) and of the reaction of hydroxide ion with $T^\pm$ (k_b, where B = OH^-). As the pH is lowered below 7.6, the rate of breakdown of T^- to amide via k^- becomes smaller than the rate of reversal of T^- to *O*-acetylserine. The rate-determining step in this pH region is the breakdown of T^- to *N*-acetylserine. This transition in rate-limiting step is responsible for the observed decrease in rate of aminolysis at pH below 8.

The complete steady-state rate law (not shown) for Scheme III may be cast in the form of eq 12, from which the following definitions of the parameters of eq 12 are deduced (eq 14–16).[36] The values of k_{obsd}/f approach

$$k_1' = k_a k_s / k_{-a} \tag{14}$$

$$K'' = \frac{(k_{-b}^{H_2O} + k^-)K_c}{k_{-s}} \tag{15}$$

$$P^- = \frac{k_{-b}^{H_2O}}{k_{-b}^{H_2O} + k^-} \tag{16}$$

limiting lines of unit slope both at low and at high pH (eq 17 and 18). Comparison of the parameters obtained for

$$\text{(low pH)} \quad \frac{k_{obsd}}{f}\,(\text{min}^{-1}) = k_1'(1 - P^-)K''/[H^+] = \frac{k_a k_s k^- K_c}{k_{-a}k_{-s}[H^+]} = (3.4 \times 10^{-9})/[H^+] \tag{17}$$

$$\text{(high pH)} \quad \frac{k_{obsd}}{f}\,(\text{min}^{-1}) = k_1'(1 - P^-)K''P^-/[H^+] = \frac{k_a k_b^{OH}[OH^-]k^-}{k_{-a}(k_{-b}^{H_2O} + k^-)} = (5.2 \times 10^{-11})/[H^+] \tag{18}$$

O-acetylethanolamine[37] and *O*-acetylserine (Table II) shows that the breakdown of T^- in the ethanolamine reaction produces twice as much amine as in the serine system, though both intermediates give predominantly the corresponding amide. It is worth reiterating that very small numerical values such as those for P^- may be known with high precision, since the distance along the pH axis between the two lines of unit slope (Figure 3B) is proportional to $1/P^-$.[25,31] It should be noted that the numerical values of P^- do not necessarily give the ratio of the rates of the actual bond-breaking processes, i.e., the ratio of the rate of cleavage of the C–N bond to that of the C–O bond. What P^- indicates is the ratio of the extent of overall amine expulsion to that to overall alcohol explusion from T^-. In terms of elementary processes, the detailed interpretation of P^- depends on the mechanism assumed. For example, if the mechanism of Scheme II is employed, P^- may be considered to provide the ratio of the rates of C–N and C–O bond breakage. In terms of Scheme III, P^- gives the ratio of the rate of protonation of T^- (to $T^\pm$) to the rate of breakdown of T^- via C–O bond cleavage (eq 16).

The mechanism of Scheme III leads to the expectation that there may occur a change in rate-determining step at constant pH and increasing buffer concentration. In the pH region where the rate-limiting step is proton transfer (k_s or k_b), increasing the rate of step k_b will be accompanied by a parallel increase in step k_{-b}. It may be possible to reach a concentration of buffer high enough that $k_{-b}[BH] \gg k^-$. At this point, the rate of aminolysis becomes independent of buffer concentration, and the rate-determining step has changed from k_b to k^-. This is precisely what is observed with phosphate buffer (Figure 4). At high buffer concentration, the rate approaches a constant value, and these maximum rates, obtained by extrapolation of the data in Figure 4, fall on or near the line of unit slope defined by the data obtained at zero buffer concentration for the region of rate-limiting breakdown of T^- (Figure 3B and eq 17). The fact that the rate data obtained at high buffer concentration can be merged with the quite independent data obtained in the absence of buffer to provide

(30) Chaturvedi, R. K.; Schmir, G. L. *J. Am. Chem. Soc.* **1969**, *91*, 737–746.
(31) Hershfield, R., Schmir, G. L. *J. Am. Chem. Soc.* **1973**, *95*, 3994–4002.
(32) (a) Schmir, G. L.; Cunningham, B. A. *J. Am. Chem. Soc.* **1965**, *87*, 5692–5701. (b) Cunningham, B. A.; Schmir, G. L., *Ibid.* **1966**, *88*, 551–558.
(33) Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7031–7044.
(34) The hydrolysis of methyl thiolformate[35] provides an example of the pH–rate profile of an acyl-transfer reaction involving two intermediates of different charge and whose partitioning ratios differ, but not greatly.
(35) Hershfield, R.; Schmir, G. L. *J. Am. Chem. Soc.* **1972**, *94*, 1263–1270.

(36) Superscripts in k_b or k_{-b} terms (see eq 15 and 16) identify the general base or general acid being considered. In eq 15, for example, the k_{-b} term refers to water acting as a general acid in the conversion of T^- to $T^\pm$.
(37) The values of the parameters reported here for *O*-acetylethanolamine are slightly different from those previously calculated,[25] though the same data[1] were used in both calculations. In the earlier work, the parameters were estimated by trial and error, while the present set of values was calculated by using nonlinear least-squares curve fitting.

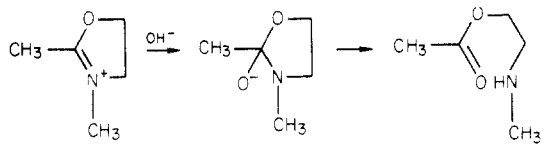
a unified explanation offers strong support for the validity of Scheme III.

In terms of Scheme III, the ratio k_{max}/K_{app}, which was used to evaluate the catalytic constant for phosphate, is given by eq 19, so that k_{PO_4} (Table I) is equivalent to

$$\frac{k_{max}}{K_{app}} = \frac{k_a k_b^{PO_4}}{k_{-a}} \left(\frac{K''}{K'' + [H^+]} \right) (1 - P^-) \qquad (19)$$

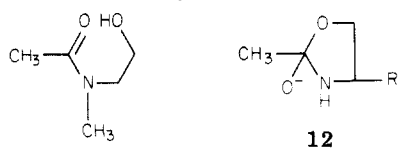
$k_a k_b^{PO_4}/k_{-a}$. Assuming that the rate constant k_b^{OH} for the diffusion-controlled abstraction of a proton from $T^{\pm}$ by hydroxide ion has the value[38] of 10^{10} M^{-1} s^{-1} leads to an estimate of 6×10^{-9} for the equilibrium constant (k_a/k_{-a}) for the formation of $T^{\pm}$ from *O*-acetylserine (see eq 18) and of ca. 10^{-9} for γ-ethyl glutamate (but, in the case of the glutamate, only if k_{OH} represents rate-limiting conversion of $T^{\pm}$ to T^-). These values are considerably smaller than that of 7×10^{-7} estimated[13] for **1**; the differences may reflect the greater flexibility of the serine and glutamate esters when compared to the benzoate derivative **1**. For *O*-acetylserine, estimates of $k_s = 4 \times 10^5$ s^{-1} and $k_b^{PO_4} = 2 \times 10^8$ M^{-1} s^{-1} are obtained, using eq 14 and 19, respectively. The observation that proton transfer from $T^{\pm}$ to phosphate dianion is 50-fold slower than proton removal by hydroxide ion is in accord with expectation when going from a thermodynamically favored proton transfer (with OH^-) to a proton transfer (with phosphate) which is thermodynamically unfavorable.[38,39] The enhanced reactivity of phosphate as compared to the equally basic imidazole suggests that phosphate dianion may be acting as a bifunctional acid–base catalyst,[3,8,26,32b] though considerably more data on general acid–base catalysis in this system would be needed to establish this point.

The hydrolysis of the *N*,2-dimethyloxazolinium cation **8** in the presence of sodium carbonate has been reported



8 **9** **10**

to yield mainly the amino ester **10** in a rapid reaction under kinetic control, which is followed by a slower rearrangement of **10** to the thermodynamically favored amide **11**.[15c]



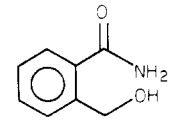
11

12

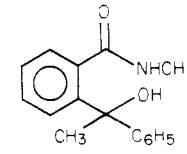
a, R = H; **b**, R = COO^-

Under these alkaline conditions, the hydrolysis of **8** probably proceeds via the anionic intermediate **9**, which is closely related to the T^- species **12a** and **12b** formed in the aminolyses of *O*-acetylethanolamine and *O*-acetylserine. It is therefore puzzling that the kinetics of the acetyl-transfer reaction of these two amino esters indicate that the breakdown of **12** yields only 1.5–3% amine, while that of **9** appears to give almost exclusively amine. This observation raises the more general question of how close in structure must two tetrahedral intermediates be before one can be considered an adequate model for the other. The startling difference in behavior of intermediates **9** and **12a** suggests that caution must be taken in drawing conclusions about one tetrahedral intermediate, using as a basis the behavior of a second, even only slightly dissimilar, intermediate.

A summary is presented in Table II of those aminolysis of alcoholysis reactions involving intermediates for which partitioning ratios are known. No clear trends are apparent in the values of the partitioning ratios of T° and T^- and there seems to be no obvious distinction between intra- and intermolecular reactions. Where data are available, the breakdown of T° strongly favors amine expulsion, while the reversal is true for T^-. For reactions in which cyclic tetrahedral intermediates are formed, three classes of intermediates should be distinguished: (a) intermediates with both O and N endocyclic (e.g., from *O*-acetylserine) (this is the only class in which both the aminolysis reaction and the reverse alcoholysis are intramolecular); (b) O endocyclic and N exocyclic (e.g., from hydroxy amides **13** and **14**) (the alcoholysis reaction is



13



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intramolecular, but the reverse is the bimolecular aminolysis of a lactone); (c) O exocyclic and N endocyclic (the aminolysis reaction is intramolecular, but the alcoholysis is bimolecular). It may be significant that this third class is the only one not represented in Table II. In the three examples of this class of which we are aware, the aminolysis of **1** proceeds without a change in rate-determining step over a wide range of pH,[13] while in the aminolysis of γ-ethyl glutamate, the existence of a change in rate-limiting step is still in doubt. With esters of 4-aminobutyric acid[2,17d] insufficient information is available to permit conclusions concerning the properties of tetrahedral intermediates in the aminolysis reaction.

Experimental Section

Ethyl L-pyrrolidone-5-carboxylate[42] was prepared from L-glutamic acid. Distillation of the crude product gave a colorless liquid, bp 129–131 °C (0.5 mm) [lit.[42] bp 152–153 °C (3 mm)], which crystallized slowly on standing: mp 47–51 °C (lit.[42] mp 51–53 °C); IR (neat) 5.75 (ester C=O), 5.89 μm (amide C=O).

L-2-Ethoxy-5-carbethoxy-1-pyrroline (4). A solution of 9.4 g (60 mmol) of ethyl L-pyrrolidone-5-carboxylate in 10 mL of $CHCl_3$ was added dropwise to a stirred solution of 12.5 g (66 mmol) of triethyloxonium tetrafluoroborate[43] in 10 mL of $CHCl_3$ kept at 5 °C. After 4 h, the reaction mixture was shaken with 14 mL of a 50% aqueous solution of potassium carbonate. The aqueous layer was extracted again with $CHCl_3$, and the combined $CHCl_3$ extracts were dried over Na_2SO_4. Distillation yielded the imidate ester as a colorless liquid: bp 70–72 °C (0.45 mm) [lit.[50] bp 102–103 °C (8 mm)]; 69% yield; IR (neat) 5.75 (ester C=O), 6.10 μm (C=N) (lit.[50] 5.75, 6.10 μm). Anal. Calcd for $C_9H_{15}NO_3$ (mol wt 185.22): C, 58.36; H, 8.16; N, 7.56. Fd: C, 58.31; H, 8.14; N, 7.52.

***O*-Acetyl-L-serine hydrochloride**[44] was recrystallized from ethanol–ether and had mp 159–160 °C (lit. mp 160 °C,[45] 167 °C[44]). *N*-Acetyl-L-serine was prepared by a method previously described[48] for *N*-acetyl-DL-serine and had mp 118 °C (lit. mp 114.5–115 °C,[28] 123–124 °C[49]).

(38) Eigen, M. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 1–19.
(39) The pK_a value for the conversion of $T^{\pm}$ to T^- is estimated to be in the range of 7.5–9.5, based on the argument that the pK_a of $T^{\pm}$ is generally not very different from that of the corresponding amine (in this case, *O*-acetylserine, pK_a 8.3).[5,40,41]
(40) Jencks, W. P. *Chem. Rev.* **1972**, *72*, 705–718.
(41) Fox, J. P.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 1436–1449.
(42) Adkins, H.; Billica, H. R. *J. Am. Chem. Soc.* **1948**, *70*, 3121–3125.
(43) Meerwein, H. *Org. Synth.* **1966**, *46*, 113–115.
(44) Wilchek, M.; Patchornik, A. *J. Org. Chem.* **1964**, *29*, 1629–1630.
(45) Sheehan, J. C.; Goodman, M.; Hess, G. P. *J. Am. Chem. Soc.* **1956**, *78*, 1367–1369.

γ-Ethyl L-glutamate (Sigma) was recrystallized twice from water–ethanol. Methyl Cellosolve was purified by treatment with alumina and then Na_2CO_3 and was finally distilled from calcium hydride. **Diethyl L-glutamate hydrochloride** (Mann) and buffer salts were commercial products which were used without further purification.

Kinetics. The hydrolysis and/or aminolysis of γ-ethyl glutamate, diethyl glutamate, *O*-acetylserine, and ethyl pyrrolidone-5-carboxylate were studied by measuring the rate of proton release with a Radiometer TTTlc pH-stat, equipped with a PHA 630 scale expander, a SBR 2c titrigraph, and a Metrohm AC 9100 combination electrode. Reactions were initiated by the addition of 10–200 μL of 1 N NaOH to 10 mL of reaction solution which had been previously equilibrated at the desired temperature. The concentration of reactant was ca. 5×10^{-3} M. Carbon dioxide was excluded from the reaction vessel by maintaining a gentle flow of argon or nitrogen (previously bubbled through saturated aqueous NaOH) over the surface of the reaction mixture. Reactions were generally followed to completion (>6 half-lives) except for very slow reactions, which were followed for 2–3 half-lives. Less than 0.4 mL of titrant was used in each case.

First-order rate constants were generally calculated from semilogarithmic plots of (volume of base added at infinite time − volume of base added at time, *t*) vs. time. For very slow reactions, the infinity value was obtained by a modified Guggenheim procedure.[46]

Rate measurements with γ-ethyl glutamate and diethyl glutamate were carried out at 40 °C, in aqueous solution, ionic strength 0.5, maintained with added KCl. In the experiments designed to determine the effects of phosphate buffer on the aminolysis of γ-ethyl glutamate, the ionic strength was kept constant at 0.9, so that phosphate buffers at concentrations up to 0.3 M could be used. The aminolysis of *O*-acetyl serine was studied at 30 °C, $\mu = 0.5$ (KCl). The rates of hydrolysis of ethyl pyrrolidone-5-carboxylate were determined at 40 °C, in 10% acetonitrile–water (v/v), $\mu = 0.5$ (KCl).

The formation of glutamic acid or serine as a result of the hydrolysis of the amino esters was determined after completion of the reaction by assay with ninhydrin.[47] The almost quantitative conversion of *O*-acetyl-L-serine to *N*-acetyl-L-serine was verified by comparison of the optical rotatory dispersion curve of the reaction product ($[\alpha]_{230}$ −1570° ± 100, in the pH range of 8–10) to that of authentic *N*-acetyl-L-serine ($[\alpha]_{230}$ −1590° at pH 8.3).

pK_a values for the amino groups of γ-ethyl glutamate, diethyl glutamate, and *O*-acetylserine were determined under the conditions of the kinetic studies by partial neutralization of the protonated amino group and rapid measurement of the pH (extrapolating the pH readings to zero time, if necessary).

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Registry No. 4, 76529-79-8; ethyl L-pyrrolidone-5-carboxylate, 7149-65-7; L-glutamic acid, 56-86-0; *O*-acetyl-L-serine hydrochloride, 66638-22-0; *N*-acetyl-L-serine, 16354-58-8; γ-ethyl-L-glutamic acid, 1119-33-1; *O*-acetyl-L-serine, 5147-00-2.

(46) Swinbourne, E. S. *J. Chem. Soc.* **1960**, 2371–2372.

(47) Rosen, H. *Arch. Biochem. Biophys.* **1957**, *67*, 10–15.

(48) Narita, K. *Biochim. Biophys. Acta* **1958**, *30*, 352–359.

(49) Akabori, S.; Otani, T. T.; Marshall, R.; Winitz, M.; Greenstein, J. P. *Arch. Biochem. Biophys.* **1959**, *83*, 1–9.

(50) Glushkov, R. G.; Granik, V. G.; Volskova, V. A.; Chernov V. A.; Minakova, S. M. *Pharm. Chem. J. (Engl. Transl.)* **1974**, *7*, 688–690.

(51) We have pointed out previously[52] that rate expressions derived for mechanisms such as that of *Scheme* II give rise to identical calculated curves through the use either of a set of constants (P^0, P^-) or of the set ($1 - P^0$, $1 - P^-$). In the present instance, this means that the data of Figure 3B can equally well fit eq 12 with the assumption that $P^0 \simeq 0$ and $P^- = 0.985$. This assumption, however, is equivalent to saying that the neutral intermediate T^0 breaks down exclusively to amide ($k_3' \gg k_2'$) and T^- gives mainly amine, which is contrary to earlier results with closely related systems.[3,10,32]

(52) Hershfield, R.; Schmir, G. L. *J. Am. Chem. Soc.* **1973**, *95*, 7359–7369.

Metal–Ammonia Reduction: Effect of Methyl Substituents and a Question Concerning Protonation Sites in Dianions

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The effect of substituents on Birch reductions and other metal–ammonia processes has received considerable attention. Since the intermediate(s) is negatively charged, activation and orientation effects have been dealt with in terms of electron donation or withdrawal of substituents. The methyl group displays somewhat irregular behavior in naphthalenes and biphenyls, and an explanation is offered in terms of methyl location on a charge-bearing or non-charage-bearing carbon in the intermediates. The observation is also made that protonation of dianions (produced by electron addition) always seems to lead to the most stable monoanion. Reasons for this are discussed.

The addition of alkali metals to aromatic compounds in liquid anhydrous ammonia, a reaction known generally as the Birch reduction,[1] provides an important method for the reduction of aromatic rings. Initial electron addition produces a radical anion, which in the case of many benzene derivatives requires the presence of a proton source (e.g., alcohol) to shift the equilibrium to the right (Scheme I, lower path). In the case of polynuclear compounds (and some highly activated benzenes), a second electron addition may take place to produce a dianion which is highly basic,[2] and except for cases of unusual stability (e.g., aromatic dianions, etc.), protonation by ammonia then takes place to produce a monoanion[3] (Scheme I, upper path). If this monoanion is sufficiently delocalized (usually two pathways are necessary such as

(1) (a) Birch, A. J. *Q. Rev., Chem. Soc.* **1950**, *4*, 69. (b) House, H. O. "Modern Synthetic Reactions", 2nd ed.; W. A. Benjamin: Los Angeles, CA, 1972. (c) Harvey, R. G. *Synthesis* **1970**, *4*, 161.

(2) Szwarc, M., Ed. "Ions and Ion Pairs in Organic Reactions"; Wiley-Interscience: New York, 1972.

0022-3263/81/1946-1593$01.25/0