Aminolysis of Esters. VII.[1] The Reaction of Lysine with Phenyl Acetate and Triacetin

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The report that lysine is a catalyst for the hydrolysis of tributyrin led to the study of the fate of phenyl acetate and triacetin in aqueous solutions of lysine. No catalysis of the hydrolysis of either ester was noted up to 1 *M* lysine when the reactions were studied by product analysis and kinetic methods. The reaction of lysine with phenyl acetate is an aminolysis reaction whose mechanistic pathway does not appear to differ from that anticipated from studies with monoamino acids and simple aliphatic mono- and diamines. Lysine does not appear to react with triacetin in water nor influence its rate of hydrolysis. Cursory experiments with the reaction of phenyl acetate and *trans*-1,2-diaminocyclohexane indicate that it also follows the anticipated mechanism from studies of simple aliphatic diamines.

The obvious involvement of the functional groups of protein-bound amino acids in the mechanism of hydrolytic enzyme action has led to an intensive investigation of the reaction of these groups with substrate models.[4] Consequently a rather large amount of data have been reported concerning the reaction of primary amines with esters. Although the best evidence would suggest that the role of the amine in this reaction is almost exclusively as a nucleophile and as a catalyst for self-addition,[1,4] it is not inconceivable that certain primary amines could serve as a catalyst for the hydrolysis of unactivated esters. The aniline-catalyzed hydrolysis of the acyl-activated ester, ethyl dichloroacetate, has been established by Jencks and Carriuolo.[5] Such a role has been attributed to lysine in the hydrolysis of tributyrin.[6] For this reaction the concerted involvement of both amino groups of lysine was proposed. It was felt that this reaction deserved a more detailed examination not only because of the exciting possibility of a bifunctional hydrolytic mechanism, but because of the equally exciting possibility of a bifunctional aminolytic mechanism. Simple α,ω-diaminoalkanes are not significantly more reactive toward phenyl acetate than simple primary and secondary amines[1e] but in reactions carried out at μ = 1.0 with KCl not all primary amines exhibit general base catalysis.[1g] On the other hand, α-amino acids and derivatives (*i.e.*, glycine, glycylglycine, glycine ethyl ester, etc.) exhibit kinetic terms which are second order in amine, attributed to self-assisted general base catalyzed aminolysis.[4] Thus, it seemed conceivable in the case of lysine that nucleophilic attack on an ester carbonyl by the α-amino group might be facilitated by intramolecular participation of the ϵ-amino group as a general base catalyst. It also seemed conceivable that the inability to observe intramolecular general base catalyzed aminolysis with the α,ω-diaminoalkanes could be due to the fact that they prefer an extended conformation in solution. A molecule such as *trans*-1,2-diaminocyclohexane, in which the amine functions are confined to a supposedly favorable conformation, might therefore show this desired catalytic mechanism. Herein we report our studies of the reaction of phenyl acetate and triacetin with lysine in aqueous solution at 30°[7] as well as cursory experiments with the reaction of phenyl acetate with *trans*-1,2-diaminocyclohexane.

Experimental Section

Materials.—Triacetin (Eastman) was redistilled *in vacuo* and a middle fraction was collected: bp 121° (3.4 mm), n^{20}D 1.4314 [lit.[8] bp 258–260° (760 mm), n^{20}D 1.4306]. L-Lysine hydrochloride (Calbiochem) was dried for 2 days over P_2O_5 *in vacuo*, and α-N-acetyl-L-lysine and ϵ-N-acetyl-L-lysine (Cyclo) were used as obtained. Phenyl acetate was from previous studies.[1d]

Apparatus.—Autotitrimetric kinetic measurements were made with a Radiometer TTT 1b autotitrator equipped with a PHA 630Ta scale expander and a water-jacketed 100-ml cell equipped with ⅋ openings to accomodate a salt bridge leading to a calomel electrode, an EA 115 Metrohm glass electrode, an inlet capillary for titrant base addition, inlet and outlet tubes for nitrogen, and a thermometer. A port in the side of the cell sealed with a serum cap allowed withdrawal of aliquots by means of a hypodermic syringe and needle. The titrimetric cell employed to determine pK_{a_1}' and pK_{a_2}' of L-lysine was that previously described by Bruice and Bradbury.[9] Values obtained (30° ± 0.1) were pK_{a_1}' = 9.05 and pK_{a_2}' = 10.25 (μ = 1 with KCl) in 0.00926 *M* lysine. The method of pK_a' calculation is that of Britton.[10]

Spectrophotometric measurements were performed with a Zeiss PMQ II spectrophotometer equipped with a thermostated brass cuvette holder through which water was circulated at 30 ± 0.1°. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter. The polarimeter cell was water jacketed and thermostated by circulating water at 30 ± 0.1°.

The pH values of the buffer solutions were determined prior to and at the completion of the reactions. pH measurements were made with a Radiometer GK 2021B combined glass calomel electrode and a Radiometer Model 22 pH meter with a Radiometer Model PHA 630Pa scale expander. The electrode was kept at the temperature of the reaction being investigated and reaction solutions were prepared to a tolerance of 0.03 pH unit.

Product Analyses. The Lysinolysis of Phenyl Acetate.—Aminolysis of phenyl acetate in 0.3 *M* L-lysine was allowed to proceed to completion as in the kinetic runs at pH values 8.80 and 10.54. The N-acetyllysines present were converted to the acetohydroxamic acid–ferric ion complex according to the method of Bruice and Bruno[1b] and their optical densities were determined at 540 mμ. The optimum heating period for the conversion of the acetyllysines to acetohydroxamic acid was determined in

(1) For the previous papers in this series, see (a) T. C. Bruice and M. F. Mayahi, *J. Am. Chem. Soc.*, **82**, 3067 (1960); (b) T. C. Bruice and J. J. Bruno, *ibid.*, **83**, 3494 (1961); (c) T. C. Bruice and S. J. Benkovic, *ibid.*, **85**, 1 (1963); (d) *ibid.*, **86**, 418 (1964); (e) T. C. Bruice and R. G. Willis, *ibid.*, **87**, 531 (1965); (f) L. R. Fedor, T. C. Bruice, K. L. Kirk, and J. Meinwald, *ibid.*, **88**, 108 (1966); (g) T. C. Bruice, A. Donzel, R. W. Huffman, and A. R. Butler, *ibid.*, **89**, 2106 (1967).

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(4) T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin Inc., New York, N. Y., 1966, Chapter 1.

(5) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **83**, 1743 (1961).

(6) A. Gero and C. L. Withrow, *Nature*, **180**, 1354 (1957); W. R. Chesbro and L. R. Hedrick, *ibid.*, **183**, 994 (1959); A. Gero, *ibid.*, **183**, 995 (1959).

(7) Studies of the hydrolysis of tributyrin in the presence of lysine (as carried out by Gero[6]) in water are precluded owing to the limited solubility of the former.

(8) "The Merck Index," Merck and Co., Inc., Rahway, N. J., 1960.

(9) T. C. Bruice and W. C. Bradbury, *J. Org. Chem.*, **28**, 3403 (1963).

(10) H. T. S. Britton, "Hydrogen Ions," Chapman and Hall, London, 1955.

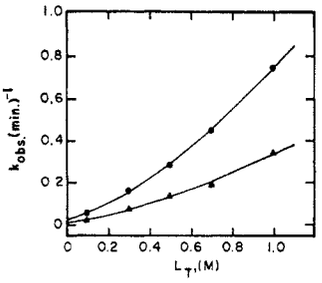

Figure 1.—Plots showing the variation of the observed rate constants with the concentration of L-lysine for the reaction of amino acid with phenyl acetate (O, pH 9.64; Δ, pH 9.22; 30 ± 0.1° at μ = 1.0 with KCl).

separate experiments to be *ca.* 2 hr at 90°. Beer's law plots were determined at each pH value with mixtures of α-N-acetyl-L-lysine and ε-N-acetyl-L-lysine in solutions identical with those employed in the kinetic runs. The ratio of acetyllysines employed at each pH was determined as follows. At pH 8.8 terms $k_3[LH]^2$ and $k_1[LH]$ account for about 80% of the reaction product while $k_2[L^-]$ accounts for about 16% (see eq 2).[11] Since α-acetyl-L-lysine should be the product from the first two terms and ε-acetyl-L-lysine should be the product of the latter term, the Beer's law plot at pH 8.8 was made by dilution of a sample containing 80% α- and 20% ε-acetyl-L-lysines. The kinetic terms $k_4[L^-]^2$ and $k_2[L^-]$ account for 90% of the reaction product at pH 10.54. These two terms were assumed to produce ε-acetyl-L-lysine, the remainder being α-acetyl-L-lysine. The composition of the sample for the pH 10.54 plot was thus 90% ε-acetyl-L-lysine and 10% α-acetyl-L-lysine. The slope of the Beer's plot at pH 8.87 was 170 and the slope at pH 10.54 was 100.

Lysinolysis of Triacetin.—The optical rotations of aqueous solutions of α-N-acetyl-L-lysine and ε-N-acetyl-L-lysine were measured at 589 mμ (30°). The pH of these solutions was adjusted with aqueous potassium hydroxide. Kinetic runs of the triacetin reaction were conducted as described below at pH 8.5 and 9.9. The optical rotation of these solutions was measured at the beginning and end of the reaction at 589 mμ. The data are shown in Table I.

TABLE I

SPECIFIC ROTATIONS OF L-LYSINE AND DERIVATIVES AT 589 mμ (30°)

Compd	Concn, mole/l.	pH	$[\alpha]^{30}_{589}$	[Triacetin], *M*
ε-N-Acetyl-L-lysine	0.144	9.92	6.39	0
	0.144	8.25	3.45	0
α-Acetyl-L-lysine	0.288	9.98	4.36	0
	0.288	8.09	4.26	0
Kinetic runs	Concn, mole/l.	pH	$[\alpha]^{30}_{589}$	[Triacetin], *M*
L-Lysine				
Beginning	0.303	8.50	12.50	0.3
End		8.50	12.00	0
L-Lysine				
Beginning	0.303	9.90	11.80	0.3
End		9.90	11.50	0

Kinetics. Phenyl Acetate.—The L-lysine concentration was maintained in large excess over that of the substrate ester in order to obtain pseudo-first-order kinetics and provide buffering capacity. The lysine–lysine hydrochloride buffers were prepared shortly before use by the addition of calculated amounts of standardized aqueous potassium hydroxide to aqueous solutions of known concentration of the amine hydrochloride. Ionic strength was maintained at 1.0 by the addition of potassium chloride. The water employed was either doubly distilled from glass or saturated with nitrogen immediately before use. Five buffer dilutions were prepared at each pH over a tenfold concentration range. A dilute solution of phenyl acetate was prepared in dioxane and stored at −18°. The reactions were initiated by addition of about 0.01 ml of phenyl acetate solution to a cuvette containing 2 ml of lysine buffer solution. The lysine buffer had been previously thermostated in the Zeiss instrument and after momentary shaking was returned to that position where the appearance of absorption at 274 mμ (due to phenol) was followed spectrophotometrically.

Triacetin.—The disappearance of triacetin in aqueous and aqueous lysine solutions was followed by the hydroxamic acid method.[12] About 10^{-3} *M* aqueous solutions of triacetin were prepared and maintained at a constant pH in the pH-Stat cell over the period necessary for the kinetic run. Two milliliters of a 7% aqueous hydroxylamine solution (0.1 *M* in acetate buffer) was added to 1 ml of suitably diluted aliquots taken periodically from this kinetic solution. The mixture was left in a thermostated bath at 30° for 1 hr at which time 2 ml of 3 *N* hydrochloric acid and 1 ml of a solution of 5% ferric chloride in 3 *N* hydrochloric acid were added. The optical density of this solution was determined immediately at 540 mμ and the concentration of acetohydroxamic acid–ferric ion complex present was determined from a standard Beer's law plot obtained from solutions of triacetin by the same hydroxamate method as that employed for the kinetic solutions. Five aqueous solutions, which were from 1.0 to 0.1 *N* in L-lysine, were prepared at each of two pH values (9.07 and 9.70) as described for the reaction with phenyl acetate. Three drops of triacetin was added to 50 ml of each solution to provide a lysine buffered solution *ca.* 10^{-4} *M* in triacetin. The solutions were maintained at 30 ± 1° in a thermostated aluminum block during the course of the time required for triacetin disappearance. The concentration of triacetin in 2-ml aliquots of these solutions was determined at various time intervals by treatment as described in the above hydroxamic acid procedure. The k_{obsd} values were determined from the slopes of log $(OD_0 - OD_\infty)/(OD_t - OD_\infty)$ *vs.* time. Good first-order kinetics were obtained to at least 3 half-lives. Under the conditions used for the analysis of triacetin the acetyl-L-lysines do not yield detectable concentrations of the acetohydroxamic acid–ferric ion complex.

Results[11]

Lysinolysis of Phenyl Acetate.—The appearance of phenol in the reaction of L-lysine with phenyl acetate was followed under pseudo-first-order conditions. The rate law of eq 1 was found to correlate the experimental data (for definition of abbreviations see footnote 11).

$$\frac{-d[PA]}{dt} = (k_1[LH] + k_2[L^-] + k_3[LH]^2 + k_4[L^-]^2 + k_5[L^-][LH] + k_{hyd}[OH^-])[PA]$$

$$k_{obsd} - k_{hyd}[OH^-] = k_1[LH] + k_2[L^-] + k_3[LH]^2 + k_4[L^-]^2 + k_5[L^-][LH]$$

$$\frac{k_{obsd} - k_{hyd}[OH^-]}{L_T} = \frac{k_1 a_H K_1 + k_2 K_1 K_2}{a_H^2 + a_H K_1 + K_1 K_2} + \left[\frac{k_3(a_H K_1)^2 + k_4(K_1 K_2)^2 + k_5 K_1^2 K_2 a_H}{(a_H^2 + a_H K_1 + K_1 K_2)^2}\right] L_T \quad (1)$$

Very good pseudo-first-order kinetics were obtained up to *ca.* 4 half-lives. The value of k_{hyd} was that of a previous study.[1a] Figure 1 shows plots of k_{obsd} *vs.* L_T. The definite upward curvature of the lines at all pH values examined establishes a second-order dependence of the reaction on amino acid. Plots of the left side of eq 1 *vs.* L_T at each pH afforded straight lines whose slopes (slopes II) were pH dependent. Ex-

(11) Abbreviations employed are

$$H_3\overset{+}{N}(CH_2)_4CH(\underset{(LH_2^+)}{\overset{+}{N}H_3})COO^- \xrightleftharpoons{K_1} H^+ + H_3\overset{+}{N}(CH_2)_4CH(\underset{(LH)}{NH_2})COO^- \xrightleftharpoons{K_2} H^+ + H_2N(CH_2)_4CH(\underset{(L^-)}{NH_2})COO^-$$

$$l_T = [LH_2^+] + [LH] + [L^-]$$

PA = phenyl acetate, a_H = activity of hydrogen ion as measured by the glass electrode, and k_{hyd} = rate of [$^-$OH]-catalyzed ester hydrolysis. The assumption is made that the macroscopic ionization constants represent the microscopic ionization constants; see J. T. Edsall and J. Wyman, "Biophysical Chemistry," Vol. I, Academic Press Inc., New York, N. Y., 1958, pp 465, 487–504.

(12) T. C. Bruice and F. H. Marquardt, *J. Am. Chem. Soc.*, **84**, 365 (1962).

trapolation of the lines to $L_T = 0$ at each pH afforded intercept values (intercepts II) which were also pH dependent. From eq 1 it is apparent that

$$\text{slope II} = \frac{k_3(a_H K_1)^2 + k_4(K_1K_2)^2 + k_5 K_1^2 K_2 a_H}{(a_H^2 + a_H K_1 + K_1K_2)^2} \quad (2)$$

and

$$\text{intercept II} = \frac{k_1 a_H K_1 + k_2 K_1 K_2}{a_H^2 + a_H K_1 + K_1K_2} \quad (3)$$

The best values of k_1, k_2, k_3, k_4, and k_5 to provide calculated slope and intercept II values nearest to those observed were obtained by the use of an IBM 1620 computer. They are $k_1 = 0.18$ l. mole^{-1} min^{-1}, $k_2 = 4.1$ l. mole^{-1} min^{-1}, $k_3 = 0.23$ l.^{2} mole^{-2} min^{-1}, $k_4 = 7.1$ l.^{2} mole^{-2} min^{-1}, and $k_5 = 2.1$ l.^{2} mole^{-2} min^{-1}. The calculated values for slope and intercept II points are shown along with the experimental data at each pH in Table II. The results show deviations between calculated and observed slopes at pH 11.40. The derived rate law appears to be incorrect at high pH values at least in terms second order in amino acid. A hydroxide-catalyzed lysinolysis term was not included in eq 1 because an equation which contains a hydroxide term which satisfies the observed intercept II values from pH 8.36 to 11.15 has *ca.* twice the observed value at pH 11.4.

TABLE II

COMPARISON OF VALUES OF SLOPE II AND INTERCEPT II OBTAINED BY EXPERIMENT WITH THOSE OBTAINED BY CALCULATION USING EQUATIONS 2 AND 3 AND EMPLOYING THE CONSTANTS TABULATED FOR THE LYSINOLYSIS OF PHENYL ACETATE

pH	Slope II (calcd)	Slope II (obsd)	Intercept II (calcd)	Intercept II (obsd)
8.36	0.00547	0.0067	0.0323	0.033
8.74	0.0234	0.027	0.0763	0.073
9.0	0.0555	0.054	0.136	0.127
9.22	0.1069	0.0925	0.22	0.200
9.64	0.3214	0.328	0.532	0.398
10.11	1.014	0.83	1.253	1.127
10.46	2.138	2.05	2.03	1.89
11.15	5.079	5.10	3.40	3.40
11.40	5.83	11.10	3.68	3.60

That the reaction followed was indeed aminolysis and not a lysine-catalyzed hydrolysis, as reported by Gero and Withrow[6] for tributyrin, was shown by product analysis. At pH 8.80, 97% of the ester was shown to be converted to acetyllysines while at pH 10.54, 84% conversion was confirmed by the hydroxamate test.[1b]

Lysinolysis of Triacetin.—The rate constants for disappearance of triacetin in aqueous solutions at 30° with and without added lysine are shown in Table III. Constant pH during the kinetic runs in which no lysine was present was maintained by a pH-Stat. When L-lysine was added it was in large excess over the concentration of triacetin so that constant pH was maintained by its buffer capacity. Aliquots were withdrawn periodically from the reaction vessels and the unreacted triacetin was converted to the acetohydroxamic acid–ferric ion complex the concentration of which was determined spectrophotometrically at 540 mμ. From Table III it can be seen that changing the lysine concentration has little effect on the rate of disappearance of triacetin. A plot of the rate constants of Table III *vs.* pH (shown in Figure 2) indicates that the disappearance of triacetin is due to reaction with the hydroxide ion.

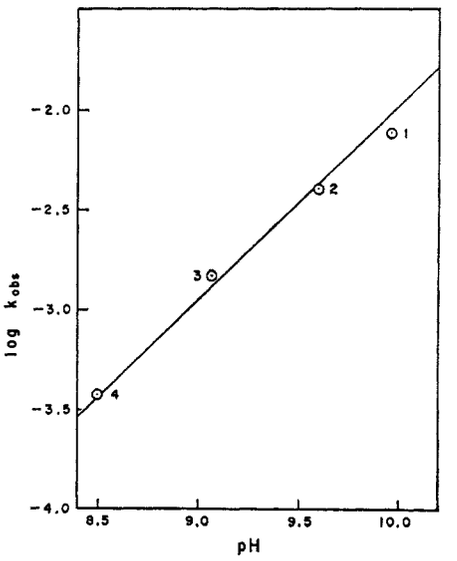

Figure 2.—Plot of the log of the pseudo-first-order rate constant for the hydrolysis of triacetin *vs.* pH [1 and 4, pH-Stat; 2 and 3, L-lysine buffer (average of five values of the rate constant at buffer concentrations of 0.1 to 1.0 *M*); 30 ± 0.1°, μ = 1.0 with KCl].

TABLE III

RATE CONSTANTS FOR DISAPPEARANCE OF TRIACETIN IN THE PRESENCE AND ABSENCE OF L-LYSINE (H_2O, μ = 1.0, 30°)

[L-Lysine], M	pH	$k_{obsd} \times 10^4$ min^{-1}
1.0	9.07	1.55
0.7	9.07	1.44
0.5	9.07	1.44
0.3	9.07	1.47
0.1	0.07	1.39
		Av 1.48 ± 0.09
1.0	9.70	5.20
0.7	9.70	3.97
0.5	9.70	3.74
0.3	9.70	3.82
0.1	9.70	3.82
		Av 4.11 ± 1.09
0	8.50	0.373
0	9.96	7.78

It can be seen from Table I that the specific rotation of the triacetin reaction solutions remains constant during the course of the reaction. No acetyl-L-lysines were thus formed because their formation would be accompanied by a significant lowering of the specific rotation of the reaction solutions (see specific rotations of acetyllysine solutions Table I). It is apparent that L-lysine does not participate in the disappearance of triacetin under these conditions.

Discussion

A priori the lysinolysis of phenyl acetate would be anticipated to proceed in a manner very similar to the aminolysis of phenyl acetate by a mixture of an amino acid such as glycine and a primary amine such as *n*-propylamine.[1e] The expected pathways would be self-assisted general base catalyzed aminolysis and un-

assisted aminolysis. No general acid catalytic pathway would be expected on this basis and indeed LH_2, the strongest general acid present, does not enter into the derived rate equation (1).

Each of the experimentally observed square terms (*i.e.*, $k_3[LH]^2$, $k_4[L^-]^2$, and $k_5[L^-][LH]$) have more than one kinetically equivalent mechanism. Only general base catalysis involving the α-amino function of two molecules of LH is possible for k_3. The value of 0.225 for k_3 lies on a Brønsted plot composed of the log k_{gb} terms for many amines in their reaction with phenyl acetate if the pK_{a_1}' of lysine is employed.[1g] The k_4 and k_5 terms may represent more than one possible but kinetically equivalent general base mechanism. Thus, for k_4 either the α-amino or ϵ-amino group may act as either a nucleophile or a general base, providing four kinetically equivalent paths to acylated lysines. For k_5 there are between the two species L^- and LH two unprotonated α-amino groups and one unprotonated ϵ-amino group leading to three possible kinetically equivalent mechanisms. Table IV shows a tabulation of the possible pathways involved in each of the square terms along with associated numbers. These numbers were assigned as follows. The path [α-NH_2][α-NH_2] (meaning that the α-NH_2 groups of two lysines are acting as nucleophile and general base, respectively) is simply k_3. Subtraction of k_3 from k_5, therefore, affords the number 1.87 associated with [α-NH_2][ϵ-NH_2]. The remaining pathway is [ϵ-NH_2][ϵ-NH_2]. The number 4.94 associated with this path was obtained by subtracting k_5 from k_4. The log of this number falls on the previously mentioned Brønsted plot[1g] when plotted against the pK_{a_2}' for lysine. Thus, the kinetic terms involving the products of two amino acid species are rationalized in terms of a general base mechanism involving all possible combinations of the two amino groups of two lysine molecules.

TABLE IV

Term	Position of nucleophilic amino group	Position of catalytic amino group	k_r, l.$^{-2}$ mole^2 min^{-1}
$k_3[LH]^2$	α	α	$k_3 = 0.225$
$k_4[L^-]^2$	α	α	0.23
	α / ϵ	ϵ / α	1.87
	ϵ	ϵ	4.94
			$k_4 = 7.04$
$k_5[L^-][LH]$	α	α	0.23
	α / ϵ	ϵ / α	1.87
			$k_5 = 2.10$

The second anticipated mechanism, nucleophilic attack by both amino groups, is also a reality as is indicated by terms $k_1[LH]$ and $k_2[L^-]$ in eq 1. These have the numerical values 0.183 for k_1 and 4.12 for k_2, respectively. These values lie on the Brønsted plot for second-order constants in the aminolysis of phenyl acetate[1g] tending to eliminate the possibility of intramolecular catalysis. Thus, these data parallel the earlier findings by Bruice and Willis[1e] who found no compelling evidence for intramolecular general acid or general base catalyzed aminolysis of PA with a series of aliphatic diamines. The fact that no term due to hydroxide-catalyzed lysinolysis is observed, is probably due to masking of this term by the multitude of terms in the derived rate law.

Lysine and the α,ω-diamines previously investigated[1g] may prefer an extended conformation in aqueous solutions. If this is true, intramolecular general base or general acid catalysis of the aminolysis of phenyl acetate would be prohibited owing to the distance between the amine functions in a single molecule. An extended conformation of this type has been established for the glutamic acids in aqueous solution.[13] To probe this possibility, the reaction of *trans*-1,2-diaminocyclohexane with phenyl acetate was investigated. From nmr evidence the two amine functions of this molecule have been assigned a diequatorial conformation in aqueous solution,[14] thus allowing a frozen spatial configuration in which proton transfer in the aminolysis transition state(s) between the amine functions could be very facile. The pK_a values for this molecule have been determined to be 6.92 and 10.15 at 26–27°,[14] nearly identical with ethylenediamine.[1e] The rate law shown in eq 4, which predicts the rate of

$$v = 0.022[\text{diamine monocation}][\text{ester}] + 1.68[\text{diamine}][\text{ester}] + 1000[\text{diamine}][\text{ester}][\text{hydroxide}] \quad (4)$$

reaction of ethylenediamine[1e] with phenyl acetate, gives pseudo-first-order rate constants which are near to or greater than the observed constants in the reaction of phenyl acetate with *trans*-1,2-diaminocyclohexane. Five kinetic runs ranging over a tenfold dilution of amine concentration at each of seven pH values in the vicinity of the higher pK_a' were conducted. Although difficulties were experienced in obtaining and using absolutely pure *trans*-1,2-diaminocyclohexane dihydrochloride and no rate law for this reaction was derived, enough data were obtained to indicate no enhancement of the rate of reaction of this diamine compared to ethylenediamine. The monoprotonated diamines [$NH_2(CH_2)_nNH_3^+$; n = 2 to 6)] are about ten times as reactive as simple primary and secondary amines toward phenyl acetate.[1e] This small enhancement may represent intramolecular general acid catalysis. On the other hand, the diamines themselves exhibit no rate enhancement establishing the fact that intramolecular general base catalysis does not seem to occur.

Registry No.—L-Lysine, 56-87-1; phenyl acetate, 122-79-2; triacetin, 102-76-1; ϵ-*N*-acetyl-L-lysine, 692-04-6; α-acetyl-L-lysine, 1946-82-3.

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(13) T. C. Bruice and W. C. Bradbury, *J. Am. Chem. Soc.*, **87**, 4846, 4851 (1965).

(14) J. L. Sudmeier and C. N. Reilley, *J. Anal. Chem.*, **36**, 1707 (1964).