The Kinetic Consequences of Intermolecular Attraction. I. The Aminolysis of *p*-Nitrophenyl Decanoate and Acetate by *n*-Decylamine and Ethylamine

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Abstract: The rates of aminolysis in aqueous solution of the *p*-nitrophenyl esters of long and short chain fatty acids by long and short chain alkylamines have been determined. At reactant concentrations well below the critical micelle concentrations, the ratios of rate constants obtained demonstrate that, with the long chain reactants, the association arising from hydrophobic interactions (the proximity effect) kinetically outweighs the unfavorable steric effect. Product analyses confirm that the rate enhancement observed in the reaction between the long chain species arises almost entirely from the increased rate of the bimolecular aminolysis reaction.

The importance of approximation of substrate and catalytic groups in the binding step of an enzyme reaction has been discussed by Koshland,[2] Westheimer,[3] Jencks,[4] Bruice,[5] and others. In a simple bimolecular process the removal of independent translational degrees of freedom of the reactants, necessary to reach the activated complex, constitutes a formal contribution to the free energy of activation. If the two reacting species can be approximated, either at a surface or by various types of binding interaction, the overall free energy of activation of the reaction will be lowered. Examples of reactions where the approximating force is a covalent bond (neighboring group effects) are common enough,[5,6] but the kinetic effects of weak intermolecular attractions have not been so extensively studied.

Reactant molecules may attract one another by π complexation, electrostatic interaction, hydrogen bonds, or hydrophobic forces. Modest effects on reaction rates have been observed in systems where the reactants can interact by π complexation,[7] electrostatic attraction,[8] or London forces.[9] Larger changes in rate have been seen for multifunctional systems in which each reactant has more than one complementary binding function (*e.g.*, an alkyl hydrophobic chain carrying a charge,[10] or an aromatic nucleus bearing a charged substituent[11]). Large effects have been observed in systems containing a third species capable of interacting with one reactant, commonly by π complexing possibly involving charge transfer.[12] Reactants can also be approximated in micelles,[13] and in frozen solutions,[14] which show a variety of rate effects. Finally, synthetic polymeric reactants[15] and catalysts[16] have been studied, where reacting groups are brought together at polymer interfaces or surfaces.

In most of the systems examined so far, either the effects of approximation on the rate are less than an order of magnitude, or the interpretation of the origin of the rate effect is complicated by the existence of more than one type of reaction (*e.g.*, for reactions inside and outside micelles) or of more than one type of attractive interaction. Our aim here is to examine the kinetic consequences of approximation for a system where we can define a single attractive interaction. To our knowledge there is no report of a large rate enhancement in a simple bimolecular reaction between nonpolymeric and unambiguously monofunctional reactants.

Hydrophobic interactions have scarcely been investigated as a possible approximating force at reactant concentrations below the critical micelle concentration,[17] yet for reactions in aqueous solution this interaction is particularly powerful, and is, moreover, believed to dominate the binding free energy of many substrates to their enzymes.[18] We report here a large rate enhancement in the aminolysis of a long chain ester by a long chain amine, explicable as the result of the approximation of the two reactants in a simple bimolecular reaction.[19]

Experimental Section

1-Decanoic acid, bp 268–270° (760 mm), was obtained from British Drug Houses Ltd. Its purity, particularly freedom from near homologs, was checked by glc.

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N,N'-Dicyclohexylcarbodiimide was obtained from Koch-Light Laboratories Ltd., and distilled under reduced pressure before use, bp 114–116° (3 mm).

1,4-Dioxane for kinetic experiments was purified by the method of Beste and Hammett,[20] and stored under nitrogen. Immediately before use it was passed through a column of silica gel, the first and last fractions being rejected.

p-Nitrophenol was obtained from Koch-Light Laboratories Ltd. and recrystallized several times from deionized water, mp 114–114.5°.

Ethylamine and 1-decylamine were obtained as anhydrous liquids described as specially pure, from Koch-Light Laboratories Ltd. These materials were purified by recrystallization of the alkylammonium chlorides. Their purity (particularly freedom from homologs) was checked by glc of the *N*-acetyl derivatives.

Buffer solutions were made up from AnalaR sodium carbonate and sodium bicarbonate from British Drug Houses Ltd., with freshly deionized water. All other materials were standard grade laboratory chemicals. Microanalyses were carried out by Drs. Weiler and Strauss in this laboratory.

***p*-Nitrophenyl esters** were prepared by the method of Bodansky and du Vigneaud.[21] The carboxylic acid (0.05 mol) and *p*-nitrophenol (0.05 mol) were dissolved in dry ethyl acetate (100 ml). The mixture was cooled to 0° and dicyclohexylcarbodiimide (0.08 mol) was added. The mixture was kept at 0° for 1 hr and then at room temperature overnight. Dicyclohexylurea was removed by filtration, and the solvent evaporated under reduced pressure. The resulting yellow solid was recrystallized twice from dry ethanol. The yield of *p*-nitrophenyl decanoate was 45%, mp 35–35.7°. *Anal.* Calcd for $C_{16}H_{23}NO_4$: C, 65.50; H, 7.85; N, 4.78. Found: C, 65.66; H, 7.85; N, 4.79. The yield of *p*-nitrophenyl acetate was 62%, mp 78.5–80°. *Anal.* Calcd for $C_8H_7NO_4$: C, 53.00; H, 3.86; N, 7.74. Found: C, 52.86; H, 3.78; N, 7.75. The purity of the esters was checked spectrophotometrically by measuring the absorbance at 400 nm due to *p*-nitrophenoxide ion, after complete hydrolysis in base.

***N*-Decyl-1-decanoylamide.** Anhydrous 1-decylamine (2.6 g) and *p*-nitrophenyl 1-decanoate (4.75 g) were dissolved in dimethylformamide (50 ml) and heated under reflux for 10 min. The solvent was evaporated off and a yellow solid remained which consisted of equimolar amounts of the amide and *p*-nitrophenol. This mixture was dissolved in chloroform (50 ml) and washed with three 10-ml portions of sodium carbonate solution, then dilute hydrochloric acid (10 ml). The chloroform layer was dried over magnesium sulfate and the solvent removed under reduced pressure. The residue was recrystallized twice from dry ethanol, yield 67%, mp 67.5–68°. *Anal.* Calcd for $C_{20}H_{41}NO$: C, 77.21; H, 13.20; N, 4.50. Found: C, 77.51; H, 13.20; N, 4.50. This material gave a single symmetrical peak in glc.

***N*-Ethyl-1-decanoylamide.** The above procedure was adopted, using 4.75 g of *p*-nitrophenyl decanoate and 0.75 g of anhydrous ethylamine. The amide was recrystallized from aqueous methanol, yield 45%, mp 38–39°. *Anal.* Calcd for $C_{12}H_{25}NO$: C, 72.40; H, 12.55; N, 7.04. Found: C, 72.24; H, 12.57; N, 6.95.

Methods. Kinetic Experiments. Reaction rates were obtained from the change in absorbance at 400 nm due to release of *p*-nitrophenoxide ion. A Unicam SP800 recording spectrophotometer was fitted with an SP870 scale expansion unit, an SP820 constant-wavelength scan unit, and an SP850 constant-temperature cell block maintained at 35 ± 0.1° by circulating water from an external thermostat bath. This instrument was coupled to a slave recorder, Sunvic Type 10.S. Matched stoppered 10-mm silica cells containing 2.5 ml of buffer solution (and in aminolysis experiments 25 μl of alkylammonium chloride solution) were brought to 35° in the cell block and 25 μl of ester solution was added to the sample cell. Initial reaction rates were obtained in terms of change in absorbance per second and used to calculate bimolecular hydrolysis and aminolysis rate constants. The latter were calculated on the basis of the *free* base concentration, obtained from the pK_a of the amine and the pH of the run. Rates were measured at a series of pH values between pH 8.8 and 10.7 in the following buffer solutions, 0.15, 0.10, 0.05, and 0.01 *M* sodium carbonate–sodium bicarbonate, and 50% (v/v) 1,4-dioxane–0.01 *M* sodium carbonate–sodium bicarbonate. Apparent rate constants were plotted against total buffer concentration, and the rate constant at zero buffer concentration was obtained by extrapolation.

Product Analysis. Qualitative product analysis for amides was achieved by extracting the solution from a scaled-up kinetic run, with chloroform, and comparing the infrared spectra obtained with those of genuine samples of the amides.

Quantitative product analysis was carried out on scaled-up kinetic experiments. After the reaction was complete, the solutions were acidified with 1 *N* hydrochloric acid, and the amide products were extracted into ether. The amides and acid present were shown to be efficiently extracted in test extractions of solutions of known composition. The ethereal extracts were then analyzed by glc and compared with standard mixtures of the products. The instrument used was a Perkin-Elmer F-11 flame ionization gas chromatograph. Resolution of the product mixtures was achieved on a stainless steel column (2 m × 1 mm), packed with fluorosilicone oil FS1265 on HMDS Chromosorb W (80–100 mesh), the ratio of stationary phase to support being 1.5:98.5. Carrier gas was nitrogen at 24 psi and the temperature programming unit was set for 16 min at 160° followed by temperature rise at 6°/min to 200°. Under these conditions, decanoic acid and the ethyl- and *n*-decylamides of decanoic acid were completely resolved, with retention times of 1.1, 2.9, and 10.8 min, respectively. Peak areas were measured by triangulation.

pH measurements were carried out on a Radiometer pH meter TTTlc, fitted with scale expander pHA630Ta, and a thermostated sample cell. The meter was standardized against standard buffer solutions from British Drug Houses Ltd.

Results

Kinetic Experiments. Table I shows second-order rate constants (in M^{-1} sec^{-1}) for the hydrolysis and aminolysis by ethylamine and 1-decylamine of *p*-nitrophenyl acetate and decanoate. Aminolysis rate constants were calculated on the basis that the free base is the reacting species (see below). All reactions were carried out in aqueous carbonate buffer solutions of pH 8.8–10.7 and molarity 0.01–0.15, 0.99% (v/v) in acetone, and at 35°. The pH was measured before and after each experiment and was constant to 0.05 unit. Different reactions were found to be subject to different buffer effects (see Table II), and all rate constants in Table I were obtained by extrapolating the experimental data at a given pH to zero buffer concentration. The procedure adopted is illustrated for the aminolysis of *p*-nitrophenyl decanoate by *n*-decylamine. Values of the rates at particular free amine concentrations (or hydroxyl ion concentrations in the case of hydrolysis) were obtained by interpolation of plots of the experimental rates as a function of amine concentration (at given buffer concentrations). The rates at a given amine concentration were then plotted against buffer

Table I. Second-Order Rate Constants for the Hydrolysis and Aminolysis of *p*-Nitrophenyl Esters[a]

Ester	k_{OH^-}	$k_{ethylamine}$	$k_{decylamine}$	$\frac{k_{decylamine}}{k_{ethylamine}}$
p-Nitrophenyl acetate	9.45[b]	5.74[c]	39.1[d]	6.8
p-Nitrophenyl decanoate	1.19[e]	0.42[f]	133[g]	317
$k_{decanoate}/k_{acetate}$	0.13	0.073	3.4	46.6

[a] All rate constants in M^{-1} sec^{-1}, extrapolated to zero buffer concentration. Reactions in 0.99% aqueous acetone, 35°, in carbonate–bicarbonate buffers. Duplicate runs gave rate constants agreeing to better than 1%. [b] Ester, 51.3 μ*M*. [c] Ester, 3.14–24.3 μ*M*; total amine, 0.181–1.43 m*M*. [d] Ester, 3.4–25.3 μ*M*; total amine, 0.045–0.36 m*M*. [e] Ester, 4.53 μ*M*. [f] Ester, 6.0–48 μ*M*; total amine, 0.0371–0.297 m*M*. This rate constant was erroneously quoted as 0.19 in ref 19, and consequent errors in the rate ratios appear therein. [g] Ester, 4.53 μ*M*; total amine, 0.0408 m*M*.

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Table II. Effects of Buffer Concentration on the Hydrolysis and Aminolysis Rates[a]

Ester	Nucleophile	Apparent rate constants, M^{-1} sec^{-1} — Buffer concn, M 0.01	0.05	0.10	0.15
Acetate	Hydroxide ion	9.70	10.8	13.3	13.5
Acetate	Ethylamine	5.80	6.00	6.16	6.25
Acetate	Decylamine	35.6	17.8	11.9	7.50
Decanoate	Hydroxide ion	1.17	1.15	1.13	1.08
Decanoate	Ethylamine	0.388	0.352	0.269	0.217
Decanoate	Decylamine	128	104	72.5	46.2

[a] Reactions in 0.99% aqueous acetone, 35°, in carbonate–bicarbonate buffers.

molarity (Figure 1) and the intercepts, representing rates at particular amine concentrations and zero buffer concentration were plotted against amine concentration (Figure 2). The slope of this line gives the rate constant at zero buffer concentration, in Table I.

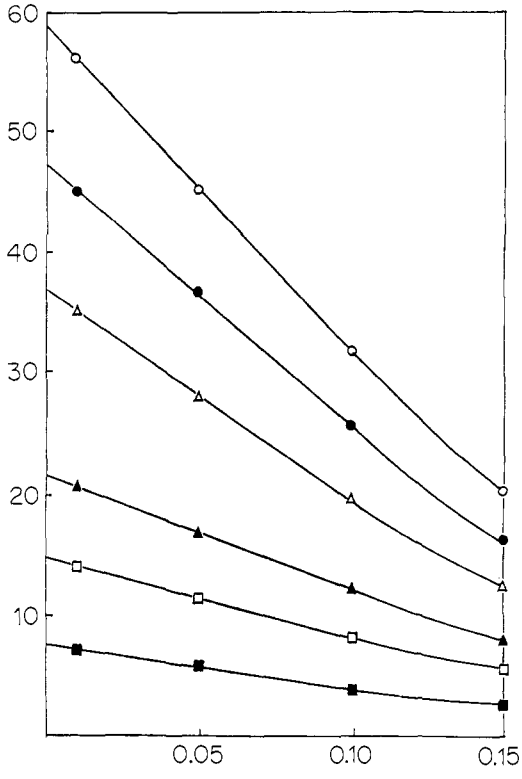

Figure 1. Aminolysis rates of *p*-nitrophenyl decanoate by given concentrations of free *n*-decylamine as a function of buffer molarity. Values of aminolysis rate obtained from the least-squares lines of aminolysis rate *vs.* amine concentration by interpolation. Lines relate to amine concentrations as: ○, 16 μM; ●, 13 μM; △, 10 μM; ▲, 6 μM; □, 4 μM; ■, 2 μM; ordinate, 10^5(aminolysis rate) (M min^{-1}); abscissa, buffer molarity (M).

Free amine concentrations were calculated on the basis of pK_a values determined at 25° of 10.67 and 10.64 for ethylamine and decylamine, respectively.[22] The pK_a values for both amines are not available at 35° (the temperature of our kinetic experiments), but since only rate *ratios* are of interest in this work, and since the heats of ionization of straight-chain amines do not differ markedly,[22] the above values have been used. The validity of this approach is confirmed by the results of product analysis from competitive aminolysis reactions described below.

In the experiments performed in exactly analogous conditions in solutions containing 50% (v/v) 1,4-dioxane, the rate constants could not be estimated with accuracy, since the aminolysis rates were very small compared with the hydrolysis rates in the reactions of both amines with the decanoate ester. At the same concentrations of ethylamine and decylamine, however, it was possible to estimate that the decylamine rate was marginally the smaller. This is in contrast to the rates in 99% aqueous solution, where decylamine attacks the decanoate ester more than 300 times as fast as ethylamine (Table I).

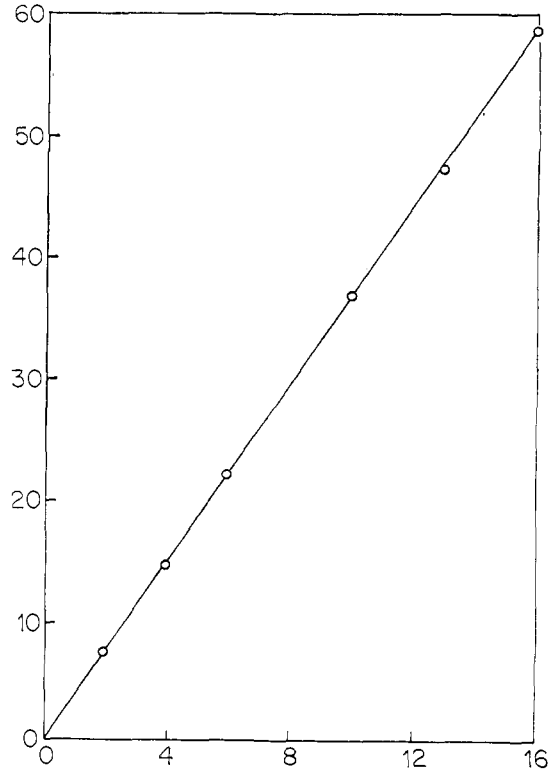

Figure 2. Aminolysis rates of *p*-nitrophenyl decanoate by *n*-decylamine at zero buffer concentration (by extrapolation of Figure 1): ordinate, 10^5(aminolysis rate) (M min^{-1}); abscissa, 10^6(free amine concentration) (M).

Product Analysis. Since changes in the rate of *p*-nitrophenoxide ion formation can arise either from changes in hydrolysis rates or in aminolysis rates or both, it is necessary to demonstrate that these two reactions occur independently in all systems. This is required for the interpretation of the rate constants,

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since we must be certain that an apparent change in aminolysis rate constant is not in reality due to a more rapid hydrolysis in the presence of amine. Accordingly, product analyses of three reaction mixtures were performed, in which the nucleophiles hydroxyl ion, ethylamine, and decylamine were allowed to compete for *p*-nitrophenyl decanoate.

For ethylaminolysis the relative amounts of products found by glc agreed within 2% with the ratio predicted on the basis of the experimental rates. However, the very high decylaminolysis rate compared with the hydrolysis rate for *p*-nitrophenyl decanoate made product analysis for this system less accurate (±7%). The two amines were therefore allowed to compete for the ester under exactly the conditions of the kinetic experiments, except that the concentration of ethylamine was increased in an inverse ratio of the relative aminolysis rate constants

Analysis of the ethylamide–decylamide ratio from this competitive reaction mixture showed that 94 ± 3% of the expected amount of *N*-1-decyl-1-decanoylamide had been formed (relative to the amount of *N*-ethyl-1-decanoylamide). The expected amount was based on the relative rate constants of Table II. These analyses validate the assumption that the hydrolysis and aminolysis reactions are occurring independently in these systems, and mean that the rate constants of Table I relate only to the aminolytic process.

Discussion

The Kinetics of the Reaction. As shown by Tommila and Hinshelwood,[23] acid-catalyzed hydrolysis of *p*-nitrophenyl acetate is negligible compared with the hydroxide ion promoted rate. Assuming that this is true for *p*-nitrophenyl decanoate also, we can neglect terms involving H_3O^+ and ammonium ion. This is confirmed by the experimental observation that the second-order aminolysis rate constants (calculated on the assumption that only free amine reacts, using the pK_a values and heats of ionization in the literature[22]) are pH independent from pH 8.8 to 10.2. This also excludes hydroxide ion catalysis of aminolysis. All aminolysis rates are first order in free amine concentration, which rules out amine catalysis of aminolysis. Since plots of $v_{hydrolysis}$ against $[OH^-]$ are linear with intercepts close to zero, the water rate is negligible. The correspondence between the amounts of acid and amide product observed from glc analysis of aminolysis reactions, and the amounts predicted on the basis of the second-order hydrolysis and aminolysis rate constants, demonstrates that the hydrolytic reaction is not significantly enhanced by the presence of amine. The overall rate equation therefore reduces to $-d(\text{ester})/dt = k_{OH}[\text{ester}][OH^-] + k_{amine}[\text{ester}][\text{amine}]$.

Buffer Effects. The second-order rate constants for both hydrolysis and aminolysis reactions depend in strikingly different ways on the concentration of the carbonate–bicarbonate buffer (see Table II and Figure 1). Primary and secondary salt effects are smaller than many of the variations observed here, and it is likely that factors in addition to these are responsible. It is notable that increasing buffer concentration decreases the observed rate constant for all reactions involving a long chain reactant. The largest inhibitory effects are seen in the aminolysis reactions of decylamine, and for these reactions a considerable increase in the rate constant may arise from complexation due to hydrophobic interactions prior to the lysis itself (see below). The buffer effects are explicable, therefore, if any or all of the ions, Na^+, HCO_3^-, or CO_3^{2-}, organize the structure of liquid water, thereby weakening the hydrophobic interaction between the reactants. For the reactions of the acetate ester with both hydroxide ion and with ethylamine, increasing buffer concentration increases the observed rate constant. These increases may arise from catalysis by carbamate (arising from buffer species with amine), or from the stabilization of the transition state leading to the formation of the tetrahedral intermediate which is likely to be rate determining in these systems.[4] Several instances of oxyanion catalysis in similar systems have been reported.[24] In summary, the buffer effects observed here probably arise from a combination of primary and secondary salt effects, disruption of reactant approximation by hydrophobic forces, general catalysis, and possible specific stabilization of nearly tetrahedral transition states by buffer species.

Rate Constants and Rate Ratios. From Table I, the following points are apparent. (1) The ratio $k_{decanoate}/k_{acetate}$ for the nucleophiles hydroxide ion (0.13) and ethylamine (0.073) are less than one, as expected if attack on the decanoate ester is subject to greater steric hindrance than is attack on the acetate. Consistent with this is the fact that the above ratio is smaller for the larger nucleophile, though this difference can also be explained on a selectivity–reactivity basis. (2) Despite the greater steric hindrance expected in reactions of decylamine compared with ethylamine, the ratio $k_{decylamine}/k_{ethylamine}$ for *p*-nitrophenyl acetate is 6.8. This ratio is unlikely to arise from differences in intrinsic nucleophilicity, since the pK_a values of ethylamine and decylamine are 10.67 and 10.64, respectively (at 25°). (The latter value is slightly concentration dependent even at concentrations below the critical micelle concentration, very probably because of hydrophobic interactions of the type at issue here.[25]) In the light of what follows, the fact that the $k_{decylamine}/k_{ethylamine}$ ratio is greater than one is best explained on the basis that the aromatic group in *p*-nitrophenyl acetate encourages some hydrophobic approximation of this ester with decylamine. (3) The ratio $k_{decylamine}/k_{ethylamine}$ for *p*-nitrophenyl decanoate is 317. That is, decylamine attacks the long chain ester nearly 50 times faster than expected on the basis of its reactivity toward *p*-nitrophenyl acetate. The possibilities that this large ratio arises from (i) an increased rate of *hydrolysis* by hydroxide ion attack on the ester in a partially cationic micelle of 1-decylammonium ions, or (ii) general catalysis of hydroxide ion attack by neighboring 1-decylammonium ions or 1-decylamine, have been ruled out by product analysis. Both the above mechanisms would lead to decanoic acid, and product analysis of a competitive aminolysis by decylamine and ethylamine showed that 94 ± 3% of the expected amount of *N*-1-decyl-1-decanoylamide is formed relative to the *N*-ethyl-

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amide. Thus nearly all of the rate enhancement arises from a bimolecular aminolysis reaction, and only about 6% from the above-mentioned mechanisms for enhanced hydrolysis.

The absence of groups which might stabilize the transition state of the reaction between the long chain species by electronic effects, the likely similarity between ethylamine and decylamine in terms of intrinsic nucleophilicity, and the fact that the reactant concentrations (ester, 4.53 μM; total amine, 40.8 μM) are well below the expected critical micelle concentrations (for decylamine, this is 0.04 M[25]) limit the possible explanations of this rate enhancement. The factors which affect true rate constants are electronic, steric, and medium effects. Electronic and medium changes will be minimal in going from a reaction involving ethylamine to one involving decylamine, and the effect observed is in the opposite direction from any prediction of steric phenomena. The rise in the observed rate constant is probably best explained by the association of the reactants in solution prior to reaction, so that the system is more appropriately described in Michaelis–Menten terms.

At concentrations of material well below the critical micelle concentration, it is known that molecules containing long alkyl chains associate in aqueous solution. Thus the observed pH of solutions of sodium salts of long chain fatty acids at concentrations as low as 10^{-6} M is best accounted for on the basis of the formation of 1:1 complexes between the carboxylic acid and its anion.[26] In the present system, we suggest that such complexes form, and that we are observing the kinetic consequences of this approximation. From our results it is not possible to say whether favorably aligned 1:1 complexes dominate the distribution of complex species at the concentrations used, but we can say that the complexes contain only one kinetically important amine molecule, since the reaction between long chain ester and long chain amine is cleanly first order in amine. The fact that kinetic saturation could not be observed at higher amine concentrations is not surprising (the apparent dissociation constant could easily be as high as 10^{-2} or 10^{-2} M), but this failure precludes any measure of the association constant between reactants, and effectively rules out a direct test of the above description by the determination of the true rate constant for the breakdown of the proposed complex.

When the reactions are carried out in 50% (v/v) aqueous dioxane, the rate enhancement, though it can be estimated only very approximately, disappears. It is known that hydrophobic interactions are seriously disrupted in this medium.[27] In accordance with the interpretation offered for the data of Table I, the ratios $k_{\text{decylamine}}/k_{\text{ethylamine}}$ for the reactions in 50% aqueous dioxane have fallen to values near unity for both esters, compared with values of 6.8 and 317 in more completely aqueous solution (1% (v/v) acetone). This dramatic fall in rate ratio supports the proposal that the ratios in water for both esters are larger than one, due to approximation by hydrophobic forces.

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The Kinetic Consequences of Intermolecular Attraction. II. The Hydrolysis of a Series of Fatty Acid *p*-Nitrophenyl Esters Catalyzed by a Series of *N*-*n*-Alkylimidazoles. A Very Simple Esterase Model[1a]

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Abstract: Second-order rate constants have been determined for the *N*-*n*-alkylimidazole catalysis of the hydrolysis of straight chain *p*-nitrophenyl carboxylates, for alkyl chain lengths 2, 4, 6, 8, and 10, at 25°. At concentrations of reactants well below the critical micelle concentrations, larger rate constants are seen for the reactions between long chain reactants despite the expected steric hindrance. Allowing for the steric effect, the rate enhancement for the decanoate ester with *N*-decylimidazole as catalyst is some 550-fold, and is explained on the basis of a hydrophobic interaction between the reactants. This is supported by the smaller size of the enhancement in a "denaturing" medium, 4 *M* aqueous urea. Thermodynamic parameters for typical "short" and "long" pairs indicate that the approximated reaction is favored mainly in the entropic contribution to the activation free energy.

The catalytic importance of enzyme–substrate binding has long been realized,[2–5] but assessment of the contribution of such binding to the reaction rate is bedevilled by lack of unambiguous model systems. In

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