Effects of Hydrophobic Interaction on the Kinetics of the Reactions of Long Chain Alkylamines with Long Chain Carboxylic Esters of 4-Nitrophenol

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The kinetics of the reactions of some long chain alkylamines with long chain carboxylic esters of 4-nitrophenol have been studied. Hydrophobic interactions caused large increases (up to 10^7-fold) in the rate of the amine-catalysed (termolecular) reaction, compared with the corresponding reaction of ethylamine with 4-nitrophenyl acetate. In aqueous ethanol, the maximum effect from hydrophobic interaction occurred when the composition of the mixed solvent coincided with the maximum development of structure. In those cases where the hydrocarbon chain of the amine was at least as long as that of the ester, the decrease in the free energy of activation was that predicted from theoretical calculations of the free energy supplied to the transition state by the hydrophobic interaction.

HYDROPHOBIC interactions have been defined as 'interactions between molecules which cannot be accounted for by covalent, electrostatic, hydrogen bond, or charge transfer interactions'.[1] They are, essentially, the result of the free energy gained when hydrophobic molecules, in water, come into contact (approach to within their Van der Waals radii) and thereby reduce their contact with water.[2] Hydrophobic interactions are important in maintaining the tertiary structure of proteins[2,3] and nucleic acids[4] and in enzyme–substrate binding.[1] They provide the driving force for micelle formation[5] and for the formation of smaller aggregates such as hydrophobically enforced ion pairs[6] or 1 : 1 complexes of a carboxylic acid[7] or a carboxylic acid and its anion.[8]

Reactions between molecules with hydrophobic side chains are of interest as models for enzyme-catalysed reactions[8-10] and also as a means of investigating hydrophobic interactions. This paper reports some effects of hydrophobic side chains on the kinetics of aminolysis by long chain alkylamines of some long chain esters of 4-nitrophenol.

Ester aminolysis is particularly suitable for this investigation because much is already known about its mechanism.[11] In general, aminolysis of aryl acetates can proceed by both amine-catalysed and uncatalysed (water-catalysed) pathways so that the relationship between the reaction velocity ν and the amine concentration $[RNH_2]$ is given by equation (1) where

$$\nu = [\text{ester}]\,(k_0 + k_1[RNH_2] + k_2[RNH_2]^2) \quad (1)$$

k_1 and k_2 are, respectively, the second-order and third-order rate constants for the reaction with amine and k_0 accounts for hydrolysis. In the case of the reaction between a labile ester, such as 4-nitrophenyl acetate, and a strongly basic amine such as ethylamine ($pK = 10{\cdot}60$),[12] when the reactants do not carry hydrophobic side chains the catalysed pathway (k_2) does not significantly contribute to the observed rate.[13] (It can be detected in the reaction of phenyl acetate with ethylamine[14] but has not previously been detected in the reaction of 4-nitrophenyl acetate with ethylamine.) However, when the amine carries a hydrophobic side chain, as in the reactions of decylamine ($pK = 10{\cdot}64$)[15] the catalysed pathway is significant[16] and when both both amine and ester carry hydrophobic side chains, this work shows that the catalysed reaction becomes the dominant reaction pathway.

RESULTS

The Reactions of 4-*Nitrophenyl Acetate with Long Chain Alkylamines.*—The reactions were studied in borate buffer (0·10M) at a pH *ca.* 2 units below the p*K* of the amine. This procedure made it possible to keep the total amine concentration high enough for the reaction to follow first-order kinetics but with the concentration of the free (unprotonated) amine low enough for the reaction to proceed at a measurable rate. The amine hydrochloride was always below the critical micelle concentration (0·04M for decylamine hydrochloride in water at 25°)[17] and the borate buffer did not catalyse the reaction. (In an experiment in which the concentration of total decylamine was kept at 0·002M and the concentration of borate varied from 0·04 to 0·10M, rate constants agreed to within ±5%.) All these reactions were first order.

Reliable second- and third-order rate constants for the reaction of decylamine were difficult to obtain in pure water because the permissible concentration range was restricted by the low critical micelle concentration and the low solubility of free decylamine. Addition of ethanol

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[1] W. P. Jencks, 'Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, 1969.

[2] W. Kauzmann, *Adv. Protein Chem.*, 1959, **14**, 1.

[3] C. Tanford, *J. Amer. Chem. Soc.*, 1962, **84**, 4240.

[4] O. Sinanoglu and S. Abdulnur, *Fed. Proc.*, 1965, **24II**, S-12.

[5] P. H. Elworthy, A. T. Florence, and C. B. Macfarlane, 'Solubilization by Surface Active Agents,' Chapman and Hall, London, 1968, pp. 13—60.

[6] A. Packter and M. Donbrow, *Proc. Chem. Soc.*, 1962, 220.

[7] E. E. Schrier, M. Pottle, and H. A. Scheraga, *J. Amer. Chem. Soc.*, 1964, **86**, 3444.

[8] D. Eagland and F. Franks, *Trans. Faraday Soc.*, 1965, **61**, 2468.

[9] R. G. Shorenstein, C. S. Pratt, C. J. Hsu, and T. E. Wagner, *J. Amer. Chem. Soc.*, 1968, **90**, 6199.

[10] C. A. Blyth and J. R. Knowles, *J. Amer. Chem. Soc.*, 1971, **93**, 3021.

[11] T. C. Bruice and S. J. Benkovic, 'Bioorganic Mechanisms,' Benjamin, New York, 1966, pp. 66—89.

[12] H. T. S. Britton and W. G. Williams, *J. Chem. Soc.*, 1935, 796.

[13] T. C. Bruice and M. F. Mayahi, *J. Amer. Chem. Soc.*, 1960, **82**, 3067.

[14] W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, 1966, **88**, 104.

[15] C. W. Hoerr, M. R. McCorkle, and A. W. Ralston, *J. Amer. Chem. Soc.*, 1943, **65**, 328.

[16] D. G. Oakenfull, *Chem. Comm.*, 1970, 1655; *Proc. Austral. Biochem. Soc.*, 1971, **4**, 42.

[17] A. Veis and C. W. Hoerr, *J. Colloid Sci.*, 1960, **15**, 427.

made much higher concentrations possible, without exceeding the critical micelle concentration. The effect of increasing the concentration of decylamine on the first-order rate constant (ν/[ester]) in a solvent containing 0·10 mole fraction of ethanol is shown in Figure 1a. The reaction clearly shows a greater than first-order dependence on amine concentration and Figure 1b is the corresponding plot of apparent second-order rate constant, $(k_{\text{obs}} - k_0)/[\text{RNH}_2]$, which increases with increasing concentration of amine. (The slope of this line gives k_2 and the intercept, at zero amine concentration, gives k_1.)

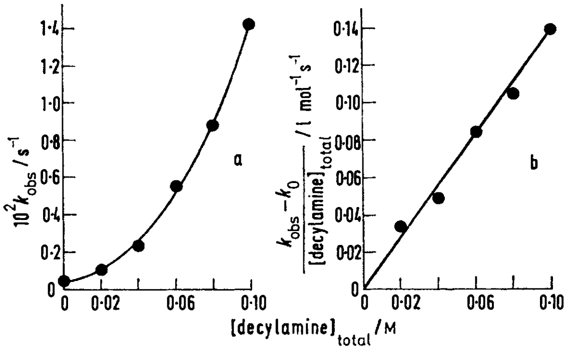

FIGURE 1 The effect of increasing amine concentration on observed first-order rate constants, k_{obs}, and apparent second-order rate constants, $(k_{\text{obs}} - k_0)/[\text{decylamine}]_{\text{total}}$, for the reaction of 4-nitrophenyl acetate with decylamine at 25° and ionic strength of 0·1M in aqueous ethanol containing 0·10 mole fraction of ethanol

TABLE 1

Rate constants * for the reaction of decylamine with 4-nitrophenyl acetate and decanoate in ethanol–water mixtures at 25° and ionic strength 0·1M

	Ethanol (mol fraction)	[Total amine]/M	Free amine (%)	No. of runs	k_1/m^3 mol^{-1} s^{-1}	k_2/m^6 mol^{-2} s^{-1}
4-Nitrophenyl acetate †	0	0—0·0040	1·2	10	0·0093	0·11
	0·05	0—0·010	1·2	6	<0·01 ‡	0·79
	0·10	0—0·010	1·3	6	<0·01 ‡	0·82
	0·15	0—0·010	1·3	6	0·082	0·31
	0·20	0—0·010	1·4	6	0·086	<0·06 ¶
	0·25	0—0·010	1·5	6	0·084	
4-Nitrophenyl decanoate §	0	0—0·0040	1·2	10	<0·03 ‡	30·3
	0·05	0—0·0046	1·2	8	<0·03 ‡	198
	0·10	0—0·0046	1·3	8	<0·03 ‡	215
	0·15	0—0·0046	1·3	9	<0·03 ‡	97
	0·20	0—0·0046	1·4	6	0·075	<0·1

* Calculated in terms of free amine concentration. † Initial concentration 3 × 10^{-5}M. ‡ Maximum intercept of $(k_{\text{obs}} - k_0)/[\text{amine}]$ *vs.* [amine] at zero amine concentration, which the data allow. § Initial concentration 5 × 10^{-6}M. ¶ Limit calculated by assuming that a 10% increase in k_{obs}, due to k_2, could have gone undetected.

TABLE 2

Rate constants and conditions for the reaction of 4-nitrophenyl acetate * with alkylamines in water at 25° and ionic strength 0·1M

Amine	Free base (%)	Concn./M	No. of runs	k_1/m^3 mol^{-1} s^{-1}	k_2/m^6 mol^{-2} s^{-1}
Decylamine	1·2	0—0·004	10	0·0093	0·11
Nonylamine	†	†	†	0·010	0·0105
Octylamine	1·71	0—0·01	9	0·0069	0·015
Heptylamine	3·44	0—0·01	11	0·0082	*ca.* 8 × 10^{-3}
Hexylamine	3·28	0—0·01	9	0·0083	<6 × 10^{-3} ‡
Ethylamine	30	0—0·1	17	0·0112 ± 0·0006 §	6 ± 2 × 10^{-5} §

* Initial concentration 5 × 10^{-6}M. † Refer to Figures 2—4 for full details. ‡ Upper limit (see text). § Estimated statistically, mean square errors are given.

The concentration of free amine was calculated from the pH of the solution and the p*K* of the amine, both measured with conventional electrodes standardised against aqueous buffers. The response of the glass electrode is unimpaired in solvents containing <90% (w/w) ethanol (mole fraction 0·78) and the liquid junction potential, although large, is constant (independent of acidity) for a particular solvent composition.[18] The p*K* was measured for each solvent composition by potentiometric titration.[19] The low solubility of decylamine made it necessary to correct for hydroxide ion (and ethoxide ion) concentration.[19] This was done by choosing (by the use of a computer) the value of the effective ionic product of the mixed solvent, $K_{\text{W,E}}$,* which gave the most consistent set of values of p*K* for each solvent composition.

Results obtained in a series of ethanol–water mixtures are summarised in Table 1, which also includes rate constants measured without added ethanol. Here the maximum concentration of decylamine was 0·004M but catalysis was detectable as curvature in the plot of k_{obs} *vs.* [RNH$_2$] although this was not as obvious as in Figure 1. (These results are presented more fully in ref. 16.)

Nonylamine is more soluble in water than decylamine, so it was possible to make a detailed study of the kinetics of its reaction with 4-nitrophenyl acetate, without adding organic solvent. Figure 2 shows plots of second-order rate constant (in terms of total nonylamine concentration) against total nonylamine concentration, for three values of pH. The intercepts represent the uncatalysed reaction, k_1', and the slopes, the catalysed reaction, k_2' (with the rate

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* $K_{\text{W,E}} = (\text{H}^+)_{\text{opp}}\{[\text{OH}^-] + [\text{OEt}^-]\}$ where $(\text{H}^+)_{\text{opp}}$ is the 'operational' hydrogen ion activity, calculated from the pH meter reading.

[18] R. G. Bates, M. Paabo, and R. A. Robinson, *J. Phys. Chem.*, 1963, **67**, 1833.

[19] A. Albert and E. P. Serjeant, 'Ionization Constants of Acids and Bases,' Methuen, London, 1962.

constants defined in terms of total concentration of amine). The relative contributions from free and from unprotonated nonylamine to k_1' and k_2' are obtained by plotting k_1' and k_2'/(fraction of free nonylamine) against the fraction of free nonylamine (calculated from the pK and the measured pH). Both of these plots (Figure 3) have zero intercepts when the fraction of free amine is zero, so only the free amine takes part in the reaction.

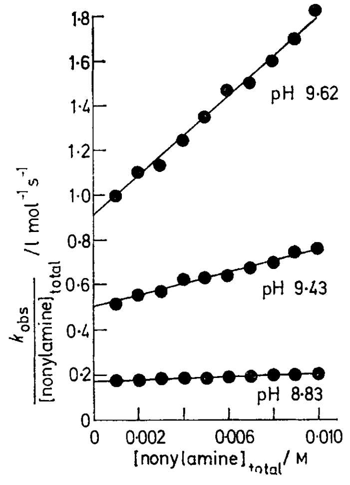

FIGURE 2 Plots of apparent second-order rate constants against amine concentration for the reaction of 4-nitrophenyl acetate with nonylamine in water at 25° and ionic strength 0·1M

Rate constants for other long chain amines are given in Table 2. In the case of hexylamine, no curvature could be detected and an upper limit for k_2 was calculated by assuming that a 10% increase in the rate, due to the catalysed reaction, could have gone undetected.

The Reactions of 4-Nitrophenyl Decanoate and Dodecanoate with Long Chain Alkylamines.—The rapid rates of these reactions made it again necessary to use borate buffer, or, in the case of the reaction of the dodecanoate with dodecylamine, imidazole buffer. There was no detectable catalysis by either of these buffers. The reactions were not necessarily first order. Figure 4 shows a typical ' first-order plot ' for the reaction of 4-nitrophenyl decanoate (initial concentration 3·3 × 10^{-5}M) with decylamine (0·004M). At high ester concentration the reaction is inhibited, presumably by aggregation of the ester by hydrophobic interaction. A similar effect has been observed by Menger and Portnoy in the hydrolysis of 4-nitrophenyl dodecanoate.[20] Strictly first-order kinetics were observed, however, when the initial concentration of 4-nitrophenyl decanoate was below 3 × 10^{-6}M. The rate constants shown in Figure 5 and Table 3 were obtained with ester concentrations low enough for the reaction to be first order. The catalysed pathway dominates these reactions. The plot of first-order rate constant against amine concentration for the reaction of 4-nitrophenyl decanoate with decylamine (Figure 5a) is very obviously curved. The corresponding plot of apparent second-order rate constant against amine concentration (Figure 5b) shows that the intercept (k_1') is so small that the catalysed pathway must account for at least 98% of the overall reaction. Plots corresponding to those in Figure 3 show that, again, only the free amine takes part in the reaction. Rate constants for this series of experiments are summarised in Table 3.

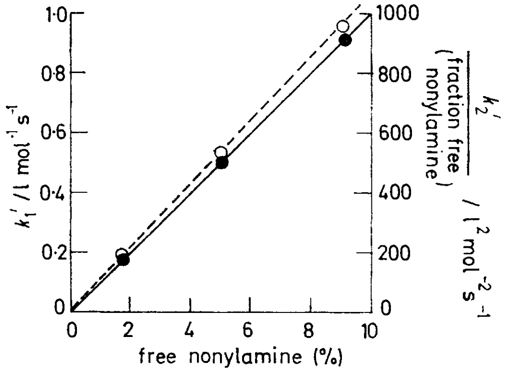

FIGURE 3 Dependence of first-order rate constants (k_1') and second-order rate constants (k_2') for the reaction of 4-nitrophenyl acetate with nonylamine on the fraction of nonylamine as the free base

TABLE 3

Rate constants and conditions for the reactions of 4-nitrophenyl decanoate and dodecanoate with alkylamines in water at 25° and ionic strength 0·1M

	Amine	Free base (%)	Concn./M	No. of runs	k_1/m^3 mol^{-1} s^{-1}	k_2/m^6 mol^{-2} s^{-1}
4-Nitrophenyl decanoate *	Dodecylamine †	0·025	0—0·001	6		193
	Decylamine	1·20	0—0·004	10	<0·03 ‡	30·3
	Nonylamine	1·43	0—0·010	12	<0·01 ‡	1·93
	Octylamine	3·58	0—0·010	10	<0·01 ‡	0·272
	Heptylamine	5·10	0—0·020	5	*ca.* 0·007	0·00967
	Hexylamine §	30	0—0·02	9	0·0063	0·00400
4-Nitrophenyl dodecanoate ¶	Dodecylamine †	0·025	0—0·001	6		833
	Decylamine	2·34	0—0·004	8	<0·03 ‡	10·2

* Initial concentration 3 × 10^{-6}M. † In 0·1M-imidazole buffer (half-neutralised). ‡ Calculated from the maximum intercept of $(k_{obs} - k_0)$/[amine] *vs.* [amine] at zero concentration which the data allow. § Hexylamine was used as its own buffer. ¶ Initial concentration 1·8 × 10^{-6}M.

The effect of exceeding the critical micelle concentration is shown in Figure 6 in which first-order rate constants for the reaction of 4-nitrophenyl decanoate with dodecylamine are plotted against the concentration of total amine. Once micelles have formed (the critical micelle concentration of dodecylamine hydrochloride is 0·0013M),[17] k_{obs} remains independent of the concentration of amine (within the concentration range of the experiment). The hydrocarbon chains of the free amine and the ester

[20] F. M. Menger and C. E. Portnoy, *J. Amer. Chem. Soc.*, 1968, **90**, 1875.

are likely to be absorbed,[5,21,22] so the reaction most probably takes place on the surface of the micelle.[21-23]

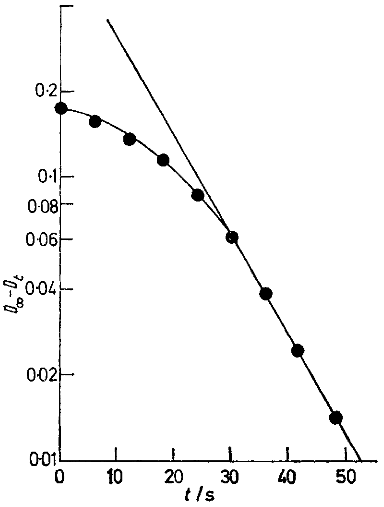

FIGURE 4 A typical 'first-order plot' for the reaction of 4-nitrophenyl decanoate (initial concentraton 3·3 × 10^{-5}M) with decylamine (0·004M) in water at 25° and ionic strength 0·1M (D is the absorbence at 400 nm)

The Reaction of 4-*Nitrophenyl Acetate with Ethylamine*.—A knowledge of the value of k_2 for this reaction is needed to get a quantitative estimate of the kinetic effect of hydrophobic side chains. A very approximate value can be calculated from the rate constant for water-catalysed aminolysis ($k_1/[H_2O]$) by using the Brønsted catalysis law with β having the same value as in the reaction of ethylamine with phenyl acetate.[14] This value (*ca.* 2 × 10^{-5} m^6 mol^{-2} s^{-1}) indicated that k_2 might be directly measurable if the amine concentration were large enough and prompted reinvestigation of this reaction.

Ethylamine was used as its own buffer and a direct measurement was made (titrimetrically; see Experimental section) of the rate at zero buffer concentration (k_0). First-order rate constants are plotted against ethylamine concentration in Figure 7a. Curvature is just detectable and Figure 7b shows that the apparent second-order rate constant increases with increasing concentration of ethylamine. These data were analysed statistically. The absolute value of the error in the rate constant (as opposed to percentage error) increased with the concentration of ethylamine so the regression of rate constant on concentration, as a quadratic, was calculated using weights. The quadratic coefficient was significant at $P = 1\%$. The rate constants, with their standard errors, for ethylamine in Table 2 were obtained statistically.

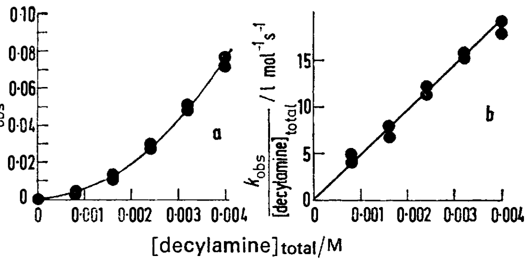

FIGURE 5 The effect of increasing amine concentration on the observed first-order rate constants, k_{obs}, and the apparent second-order rate constants, k_{obs}/[amine], for the reaction of 4-nitrophenyl decanoate with decylamine in water at 25° and ionic strength 0·1M

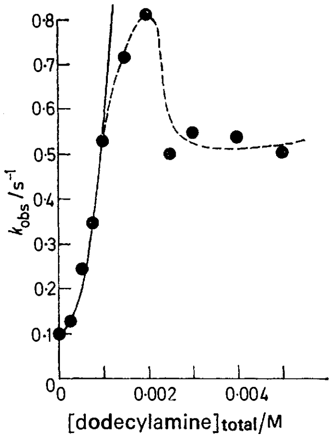

FIGURE 6 The effect of exceeding the critical micelle concentration (0·0013M) [17] on the observed first-order constant, k_{obs}, for the reaction of 4-nitrophenyl decanoate with dodecylamine in water at 25° and ionic strength 0·1M [the solid curve was calculated from equation (1) and the rate constants given in Table 3.]

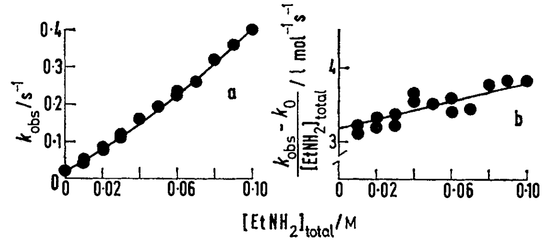

FIGURE 7 The effect of increasing amine concentration on the observed first-order rate constants, k_{obs}, and the apparent second-order rate constants, $(k_{obs} - k_0)/[RNH_2]$, for the reaction of 4-nitrophenyl acetate with ethylamine at 25° and ionic strength 0·1M. The curves were calculated from the rate constants in Table 2, with $k_0 = 0{\cdot}012\ s^{-1}$

DISCUSSION

The Origin of the Rate Increase.—The rate constants reported in Tables 2 and 3 show that when the reactants carry hydrophobic side chains there can be a large increase in the rate of amine-catalysed aminolysis (k_2) compared with the corresponding reaction of ethylamine with 4-nitrophenyl acetate. (For the reaction of dodecylamine with 4-nitrophenyl dodecanoate this ratio of rate constants is 1·38 × 10^7.) This effect seems best explained by hydrophobic interactions.

The basicities of the amines are not sufficiently different to provide an explanation. (Their pK values differ by, at most, 0·06 units.[15]) Steric effects can be discounted because amine catalysis of aminolysis of phenyl

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[21] L. R. Romsted and E. H. Cordes, *J. Amer. Chem. Soc.*, 1968, **90**, 4404.

[22] T. C. Bruice, J. Katzhendler, and L. R. Fedor, *J. Amer. Chem. Soc.*, 1968, **90**, 1333.

[23] E. J. Fendler and J. H. Fendler, *Adv. Phys. Org. Chem.*, 1970, **8**, 271.

acetate is more difficult to detect for n-butylamine than for methylamine.[14] Micellar effects [23] can also be discounted because the reactants were always below the critical micelle concentration. An explanation in terms of hydrophobic interactions is supported by both theoretical calculations and the effects observed when ethanol was added to the system.

Very different rate constants (Tables 2 and 3) were obtained for the catalysed reaction of a single amine (for example, decylamine) with the three esters of different chain length. This must mean that hydrophobic interaction between two amine molecules, alone, is not sufficient to explain the observed rate increase. Hydrophobic interaction of the ester is also important.

The Effect of Ethanol.—Rate constants for the reactions of decylamine with 4-nitrophenyl acetate and 4-nitrophenyl decanoate are given in Table 1. Ethanol has little effect on the value of k_1. Most of its effect is on k_2 which increases until the mole fraction of ethanol reaches 0·1, and then decreases. Large concentrations of ethanol can be expected greatly to reduce, or destroy, hydrophobic interactions.[24-26] The fact that k_2 cannot be detected when the mole fraction of ethanol is 0·2, or greater, is strong evidence that it is hydrophobic interaction which makes catalysed aminolysis a significant reaction pathway when the solvent is pure water.

The maximum observed value of k_2 occurs when the mole fraction of ethanol is 0·1. This concentration of ethanol corresponds to maximum development of structure in the mixed solvent.[27,28] On the basis of a statistical mechanical study of hydrophobic interaction, Ben-Naim [26] has suggested that the free energy required to make a cavity in the solvent, to accommodate the solute, is a practical index for comparing hydrophobic interactions in various media. He calculated the free energy from an expression derived from the Scaled Particle Theory. This is not feasible for a mixed solvent but an alternative approach is to exploit the relationship* $(\partial G/\partial V)_T = V(\partial P/\partial V)_T = -1/\beta$, where β is the isothermal compressibility, from which equation (2) is obtained where V_m is the molar volume of the solute.

$$\Delta G_{\text{cavity}} = -V_m/\beta \tag{2}$$

For a series of single component solvents, this treatment gives values of ΔG_{cavity} roughly agreeing (±20%) with those calculated by Ben-Naim (Table 4), so it seems reasonable to use it to 'predict' the strength of hydrophobic interactions in ethanol–water mixtures.

Values of ΔG_{cavity} for a series of ethanol–water mixtures are given in Table 5. ΔG_{cavity} Passes through a maximum value when the mole fraction of ethanol is 0·1, following the experimentally observed trend in the strength of hydrophobic interaction.

TABLE 4

Values of the free energy of cavity formation for a spherical solute of diameter 0·34 nm

Solvent	$10^{11}\,\beta$ */m^2N^{-1}	ΔG_{cavity}/kJ mol^{-1} From equation (2)	From Ben-Naim
Ether	186·5 †	6·64	7·65
Chloroform	97·4 †	12·7	11·2
Carbon disulphide	93·1	13·3	12·6
Methanol	121·8 †	10·2	8·73
Chlorobenzene	75·1	16·5	13·6
Acetone	123·9	10·0	9·68
Water	45·7	27·1	23·7

* At 1 atm and 25° (unless otherwise stated). Obtained from 'Handbook of Chemistry and Physics,' The Chemical Rubber Co., Cleveland, Ohio, 1967. † Measured at 20°.

TABLE 5

Values of the free energy of cavity formation for a spherical solute of diameter 0·34 nm in ethanol–water mixtures

Ethanol (mol fraction)	$10^{11}\,\beta$ */m^2N^{-1}	ΔG_{cavity}/kJ mol^{-1}
0	41·9	29·6
0·05	39·1	31·7
0·10	38·1	32·5
0·15	41·2	30·1
0·20	46·7	26·5
0·25	53·4	23·2
0·30	61·2	20·2

* Obtained from Figure 1 of ref. 30.

Comparison with Theoretical Calculations of the Free Energy of Hydrophobic Interaction.—The effect on the rate constant of hydrophobic side chains can be expressed as the corresponding change in the free energy of activation [equation (3) where k^{H}/k is the ratio of

$$\Delta\Delta G^{\ddagger} = -RT\ln(k^{\text{H}}/k) \tag{3}$$

rate constants for corresponding reactions, with and without, hydrophobic side chains]. Table 6 compares $\Delta\Delta G^{\ddagger}$, for amine-catalysed aminolysis, with the free energy supplied to the transition state by the hydrophobic interaction between the two amine molecules and the ester, as calculated by using the theoretical treatment of Némethy and Scheraga (ΔG_{HI}).[30]

Némethy and Scheraga's [30] equations (1)—(5) give the free energy very simply. All that is required is values

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* The derivation of this relationship is as follows:

$$G = U + PV - TS \tag{i}$$

$$\therefore \quad \left(\frac{\partial G}{\partial V}\right)_T = \left(\frac{\partial U}{\partial V}\right)_T + P + V\left(\frac{\partial P}{\partial V}\right)_T - T\left(\frac{\partial S}{\partial V}\right)_T \tag{ii}$$

We know that (iii), the thermodynamic equation of state, and

$$\left(\frac{\partial U}{\partial V}\right)_T + P = T\left(\frac{\partial P}{\partial T}\right)_V \tag{iii}$$

(iv) apply. Consequently equation (v) is obtained.

$$\left(\frac{\partial S}{\partial V}\right)_T = \left(\frac{\partial P}{\partial T}\right)_V \tag{iv}$$

$$\left(\frac{\partial G}{\partial V}\right)_T = V\left(\frac{\partial P}{\partial V}\right)_T \tag{v}$$

[24] A. Ben-Naim, *J. Chem. Phys.*, 1971, **54**, 3696.

[25] C. Tanford, *J. Amer. Chem. Soc.*, 1962, **84**, 4240.

[26] A. Ben-Naim, *J. Chem. Phys.*, 1971, **54**, 1387.

[27] E. M. Arnett, W. G. Bentrude, J. J. Burke, and P. McC. Duggleby, *J. Amer. Chem. Soc.*, 1965, **87**, 1541.

[28] F. Franks and D. J. G. Ives, *Quart. Rev.*, 1966, **20**, 1.

[29] K. H. Jung and J. B. Hyne, *Canad. J. Chem.*, 1970, **48**, 2423.

[30] G. Némethy and H. A. Scheraga, *J. Phys. Chem.*, 1962, **66**, 1773.

for the number of water molecules ' squeezed out ' (ΔY_S) and the number of hydrocarbon pair ($CH_3 \cdots CH_3$, $CH_2 \cdots CH_3$, $CH_2 \cdots CH_2$) interactions (Z_R) when the hydrocarbon chains come into contact. ΔY_S Was estimated (following ref. 30) by using space-filling molecular models (Courtauld). Z_R Was obtained in the following way. In the transition state, the carboxylate group and the two amino-groups must be in contact. Given this restriction, the models show that the hydrocarbon chains minimise their contact with water by adopting the fully extended configuration, with contact along their entire length. Consequently Z_R was assigned the value of 3 for each set of methylene groups in contact between three hydrocarbon chains and the value of one for each set of methylene groups where only two hydrocarbon chains were in contact. The small (and uncertain) correction for the loss of rotational free energy[30] has been neglected.

TABLE 6

Comparison of observed changes in the free energy of activation for catalysed aminolysis in water at 25° with the free energy supplied to the transition state by hydrophobic interaction

Ester	Amine	k_2/m^6 mol^{-2} s^{-1}	k_2^H/k_2 *	$-\Delta\Delta G^‡$/kJ mol^{-1} *	ΔY_S †	Z_R ‡	$-\Delta G_{HI}$/kJ mol^{-1} §
4-Nitrophenyl acetate	Decylamine	0·11	1830	18·6	30	14	18·1
	Nonylamine	$1·05 \times 10^{-2}$	175	12·8	28	13	16·6
	Octylamine	$1·5 \times 10^{-2}$	250	13·7	26	12	15·3
	Heptylamine	*ca.* 8×10^{-3}	*ca.* 130	*ca.* 12	24	11	13·9
	Hexylamine	$<6 \times 10^{-3}$	<100	<11	22	10	12·5
	Ethylamine	6×10^{-5}	1	0			
4-Nitrophenyl decanoate	Dodecylamine	193	$3·22 \times 10^{6}$	35·4	42	30	35·0
	Decylamine	30·3	$5·05 \times 10^{5}$	32·5	38	28	35·2
	Nonylamine	1·93	$3·22 \times 10^{4}$	25·7	36	27	30·8
	Octylamine	0·272	$4·53 \times 10^{3}$	20·9	32	24	27·4
	Heptylamine	$9·67 \times 10^{-3}$	161	12·6	28	21	23·9
	Hexylamine	$4·0 \times 10^{-3}$	66·7	10·4	24	18	20·5
4-Nitrophenyl dodecanoate	Dodecylamine	833	$1·38 \times 10^{7}$	40·7	46	34	39·0
	Decylamine	10	$1·70 \times 10^{5}$	29·8	40	30	34·2

* Defined by equation (3). † The number of water molecules ' squeezed out ' when the hydrocarbon chains come into contact (estimated by using molecular models). ‡ The number of hydrocarbon pair interactions (see text). § The free energy of hydrophobic interaction, calculated according to Némethy and Scheraga.[30] The calculations are for interaction between two amine molecules and the ester.

Models show that with 4-nitrophenyl acetate, the aromatic ring can interact with the long chain amine and this interaction has been included in the calculations. With the long chain esters, it is not possible for *both* the aromatic ring and the alkyl chain to interact with the alkyl chain of the amine and only the stronger of these interactions, that of the alkyl chain, has been included. Table 6 includes the values of ΔY_S and Z_R.

Némethy and Scheraga's treatment is based on a model for the structure of water which is possibly unsound.[31] Experimental tests, however, have shown that it gives reliable results[7,8,32,33] (possibly because there are sufficient adjustable parameters for the results to be independent of the details of the model). Within the context of this paper, it only matters that the calculations are reliable enough to permit meaningful speculation as to why the kinetic results (expressed as $\Delta\Delta G^‡$) do not always agree with ΔG_{HI}.

Table 6 shows that there is excellent agreement between $\Delta\Delta G^‡$ and ΔG_{HI} when the hydrocarbon chain of the amine is at least as long as that of the ester. However, with the shorter amines, $\Delta\Delta G^‡$ decreases with decreasing chain length by much more than ΔG_{HI}. In these cases the interaction between the amine molecules and the ester may be ' nonproductive ' in that the reacting groups are not properly aligned. (Storm and Koshland[34] have shown that the rates of some simple lactonisations are sensitive to the orientation of the reacting groups.)

Calculation predicts that the bimolecular reaction (k_1) should also be increased by hydrophobic interaction. [For example, in the reaction of 4-nitrophenyl decanoate with decylamine, $\Delta G_{HI} = -12·5$ kJ mol^{-1} which corresponds to a rate ratio (k_1^H/k_1) of 155.] Experimentally, however, k_1 is almost independent of chain length. This cannot be satisfactorily explained but could, again, be because the interactions are nonproductive as a result of incorrect alignment.

The Work of Knowles and his Co-workers.—The reactions of 4-nitrophenyl acetate and 4-nitrophenyl decanoate with decylamine have also been investigated by Knowles and Parsons[35] and Blyth and Knowles.[36] They found the reactions to be strictly second order and their reaction rates were slower than those reported here, even though they used a higher temperature (35°). In the case of the acetate, amine catalysis is easily missed when the reaction is carried out in pure water because the range of amine concentrations is restricted by the low critical micelle concentration. The rate constants reported here were only obtained after the reaction had been carried out in aqueous ethanol which permitted use of higher concentrations

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of amine and made the catalysis more obvious. In the case of the decanoate, the discrepancy is probably explained by the fact that Knowles and his co-workers obtained their rate constant for the decylamine reaction from the determination of the initial rate but used only one ester concentration. This concentration ($4{\cdot}53 \times 10^{-6}$M) would have been high enough for the reactions to be inhibited by aggregation (see Figure 4 and its explanation in the text), if they had been carried out at 25°. Hydrophobic interaction can be expected to increase with increasing temperature * [2,24] and be stronger at 35 than at 25° so it seems likely that Knowles and his co-workers' reactions were inhibited by aggregation.

A Comment on Enzyme Models.—Reactions such as those described here, in which the reactants are held together by weak, reversible binding forces have been discussed as models for enzymic catalysis,[9,10,37,38] particularly as a means of assessing the importance of the 'proximity effect'.[1,39] It seems worth pointing out, in this connexion, that special effects, such as that of proximity, have to be 'paid for' in free energy. In an enzyme-catalysed reaction this free energy is supplied by the various mutual interactions between the enzyme, the substrate, and the solvent. Much the same is true of these model reactions, free energy being supplied by interactions between the catalyst, the substrate, and the solvent. In these model reactions, however, the possibility should be considered that the rate increase is limited by the availability of free energy and not by an intrinsic limitation of the proximity effect.

The rate increases in the reactions described here are entirely accounted for by the free energy supplied by hydrophobic interaction, which suggests that, here at least, availability of free energy is the limiting factor.

EXPERIMENTAL

Materials.—4-Nitrophenyl acetate was recrystallised from ether; 4-nitrophenyl decanoate and dodecanoate were recrystallised from light petroleum. The long chain alkylamines (C_6—C_{12}) were redistilled (under nitrogen and under reduced pressure); ethylamine was recrystallised as the hydrochloride. G.l.c. showed that the purity of the amines was >99% (10 ft column; 10% SF 96 on Chromosorb-G). Water was glass-distilled, and for the experiments with ethylamine it was also passed through a mixed-bed ion-exchange column [Bio-Rad AG 501-X8(D)]. The conductivity of the effluent was *ca.* 6×10^{-7} $\text{ohm}^{-1}\,\text{cm}^{-1}$.

Kinetic Methods.—In one experiment (see Results section) the hydrolysis of 4-nitrophenyl acetate (k_0) was followed titrimetrically using a pH-stat (Radiometer SBR2C/TTT11b/ABU1b/PHM26c). In all other cases reactions were followed spectrophotometrically at 400 nm (Zeiss PMQII) directly in the thermostatted (25 ± 0·1°) cell compartment. When the half-life was <*ca.* 45 s the spectrophotometer was fitted with a pen recorder (Beckman), otherwise data were recorded manually. Reactions were started by adding a dioxan solution (3 μl) of the ester to the amine solution (3 ml) in a thermostatted, Teflon-stoppered cuvette. Reactions were always under first-order conditions (at least 100-fold excess of amine over ester) and were followed for at least four half-lives. First-order rate constants were calculated from half-lives obtained from semilogarithmic plots of the difference between the absorbance, and the absorbance at the end point, against time. The ionic strength was made up to 0·1M by adding potassium chloride. The pH was measured routinely after each run using a Radiometer PHM26c pH meter, standardised against two buffers, and fitted with Radiometer electrodes (G2222C and K4112). (This same equipment was used to measure the pK of decylamine in ethanol–water mixtures.)

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* Hydrophobic interaction is believed to be accompanied by a gain of entropy [2] which, because $(\partial G/\partial T)_P = -S$, implies that the temperature coefficient of ΔG is negative.

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