Aggregation of *p*-Nitrophenyl Alkanoates in Aqueous Solution: A Caution Concerning their Use in Enzyme Model Studies

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Summary. *p*-Nitrophenyl octanoate and decanoate are aggregated in aqueous solution at the usual spectrophotometric concentrations; conclusions drawn from earlier work using these compounds as substrates for enzyme models must be re-examined.

SEVERAL recent studies[1-4] of enzyme models have used *p*-nitrophenyl alkanoates as substrates, with the aim of achieving non-covalent binding of the alkyl group of the ester to the enzyme model by virtue of hydrophobic interactions. These substrates appeared attractive because of

the ease with which hydrolysis can be followed, their simplicity of structure, and their commercial availability. I wish to point out that in aqueous solution the octanoate and decanoate esters are aggregated at much lower concentrations than has been previously assumed, and that indeed the rates of reaction of the monomeric esters have not previously been measured. Since in previous studies[1-4] using these esters as substrates, it was assumed that the esters were not aggregated, when in fact they were, the conclusions drawn from these experiments must be re-examined.

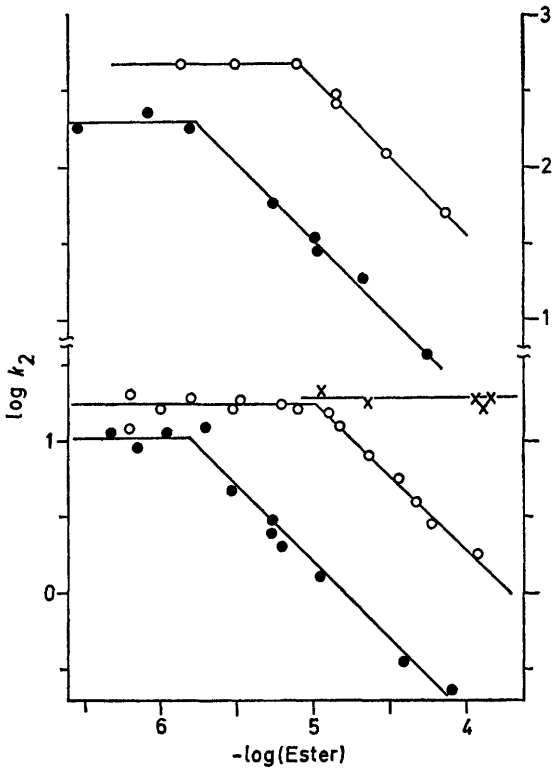

FIGURE 1 *Reactions of* p-*nitrophenyl hexanoate* (×), *octanoate* (O) *and decanoate* (●) *with imidazole and hydroxide ion. Dependence of the logarithm of the second-order rate constant on the logarithm of the ester concentration. Left-hand ordinate: imidazole-catalysed hydrolysis. Right-hand ordinate: hydroxide-ion catalysed hydrolysis.*

Reactions of *p*-nitrophenyl hexanoate, octanoate, and decanoate were studied over a wide concentration range.† The results, Figure 1, clearly show that for *p*-nitrophenyl hexanoate, the rate constant for imidazole-catalysed hydrolysis is independent of concentration. For *p*-nitrophenyl octanoate, the rate of hydroxide ion or imidazole-catalysed hydrolysis is independent of ester concentration below $1{\cdot}0 \times 10^{-5}$ M, but falls dramatically at higher concentrations. For *p*-nitrophenyl decanoate, the critical concentration below which aggregation is unimportant is $1{\cdot}6 \times 10^{-6}$M. Menger has shown[5] that for *p*-nitrophenyl dodecanoate, the critical concentration is less than 10^{-6}M.

It has been suggested[4] that aggregation can be prevented by using 10% methanol–90% water as solvent. However,

† 10 cm cells were used at the lower concentrations.

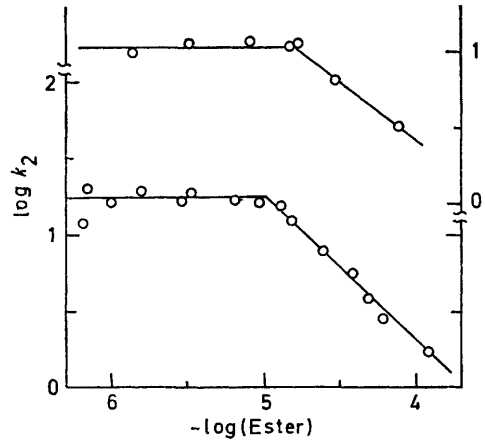

FIGURE 2. *Effect of added methanol on the imidazole-catalysed hydrolysis of* p-*nitrophenyl octanoate. Left-hand ordinate: reaction in* 0·64M-*acetonitrile,* $\mu = 0{\cdot}097$M (KCl). *Right-hand ordinate: reaction in* 10% (v/v) *methanol* 0·64M-*acetonitrile,* $\mu = 0{\cdot}087$ M (KCl).

an investigation of the imidazole-catalysed reaction of *p*-nitrophenyl octanoate, Figure 2, clearly showed that even with 10% methanol added, aggregation is still a serious problem at the usual spectrophotometric concentrations. The added organic solvent has increased the critical concentration from 1×10^{-5} M to $1{\cdot}6 \times 10^{-5}$ M and decreased the rate constant for reaction of monomeric ester from 17 to 11 l mol^{-1} min^{-1}.

The relative rate constants for hydrolysis of the monomeric esters, shown in the Table, decrease with increasing

TABLE

Relative rate constants for hydroxide ion and imidazole-catalysed hydrolysis of p-*nitrophenyl esters in aqueous solution at* 25°

Ester	This work		Earlier work		
	k_{OH^-}[a,b]	k_{Im}[a,c]	k_{OH^-}[a,d]	k_{EtIm}[e]	k_{II}[f]
Acetate	1·00	1·00	1·00	1·00	1·00
Hexanoate ..	0·53	0·68	0·53	0·81	0·51
Octanoate ..	0·55	0·61	0·27	0·11	0·28
Decanoate ..	0·23	0·39	0·04	0·03	—
Dodecanoate ..					0·03

[a] 0·64 M-Acetonitrile, $\mu = 0{\cdot}097$ M (KCl).

[b] Carbonate buffer, 0·026 M, pH 9·7 or 10·1; k_2 for the acetate was 852 l mol^{-1} min^{-1} (observed pseudo-first-order rate constant divided by [OH$^-$]).

[c] Tris buffer, 0·01 M, pH 7·9, with 0·01 M-imidazole; k_2 for the acetate was 28 l mol^{-1} min^{-1}.

[d] Ref. 3, 0·99% (v/v) acetone; k_2 for the acetate was 724 l mol^{-1} min^{-1}.

[e] Ref. 3, 0·99% (v/v) acetone; relative rate constants for reaction with *N*-ethylimidazole, k_2 for the acetate was 24 l mol^{-1} min^{-1}.

[f] Ref. 4, pH 6·80 phosphate buffer, $\mu = 0{\cdot}088$, with 1·75% acetonitrile 10·55% methanol. Rate constants for reaction with $(Me_2CHCH_2)_2NCH_2CON(Me)OH$, relative to that for the acetate, 0·693 l mol^{-1} s^{-1}.

length of the alkyl chain. This decrease most probably represents the effect of the alkyl chain coiling back upon itself and shielding the ester carbonyl,[3] but it should be noted that the effect is much smaller than has been pre-

viously believed. In each case the values for *p*-nitrophenyl hexanoate are in reasonable agreement with earlier work but the values for the octanoate and decanoate esters reported here are larger. This implies that in the earlier work the conditions were such that these esters were aggregated.

Thus it appears that the large rate enhancements which have been reported[1-4] for *p*-nitrophenyl octanoate, decanoate, and dodecanoate represent not the consequences of 1:1 complex formation, but rather of incorporation of reactants into a common, micelle-like aggregate. Furthermore there is to date no unambiguous case of a large rate enhancement for the hydrolysis of *p*-nitrophenyl alkanoates not based on micellar catalysis[6,7] or related phenomena.

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[1] J. R. Knowles and C. A. Parsons, *Chem. Comm.*, 1967, 755.
[2] C. A. Blyth and J. R. Knowles, *J. Amer. Chem. Soc.*, 1971, **93**, 3017.
[3] C. A. Blyth and J. R. Knowles, *J. Amer. Chem. Soc.*, 1971, **93**, 3021.
[4] R. Hershfield and M. L. Bender, *J. Amer. Chem. Soc.*, 1972, **94**, 1376.
[5] F. M. Menger and C. E. Portnoy, *J. Amer. Chem. Soc.*, 1968, **90**, 1875.
[6] E. H. Cordes and R. B. Dunlap, *Accounts Chem. Res.*, 1969, **2**, 329.
[7] E. J. Fendler and J. H. Fendler, *Adv. Phys. Org. Chem.*, 1970, **8**, 721.