The True Magnitude of the Hydrophobic Rate Enhancements obtainable from Hydrocarbon Alkyl Chain Contacts in Aqueous Solutions

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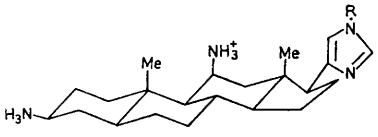
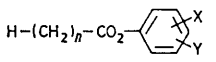
Summary Rate enhancements attributable to hydrophobic interaction of linear alkyl groups with a steroidal enzyme model have been measured and are much smaller than the values reported previously for less well defined enzyme model systems, but are in satisfactory agreement with independent evaluations of these interactions.

HYDROPHOBIC interactions are believed to be important in the free energy of enzyme-substrate binding in many cases.[1] There have been several attempts to use hydrophobic interactions to provide rate enhancements in enzyme model systems.[2-8] Most of these have used linear alkyl groups as hydrophobic groups,[2-6] and large rate effects, attributed to 1:1 interactions of these groups have been reported. Reports suggest that for interaction of two alkyl chains of 9 or 10 carbons (or more) rate enhancements of 100—1000 (or more) times are to be expected.[3,4] We have previously reported[9] that neutral *p*-nitrophenyl alkanoates are aggre-

gated at very low concentrations and that in some cases these compounds have been used in enzyme model esperiments at concentrations in excess of their aqueous solubility limit. We have developed an enzyme model system based upon a steroid nucleus as a hydrophobic binding region.[10] We now report evaluations of the rate enhancements attributable to linear alkyl group–steroid interactions with 1:1 stoicheiometry; these rate enhancements are very small, although they increase in a regular way with increasing chain length.



(1a); R=H
(1b) R=CHMe$_2$



(2); X=2-NO$_2$, Y=5-CO$_2^-$; a; n=1
b; n=7
c; n=9

(3); X=H, Y=4-NO$_2$; a; n=1
b; n=5
c; n=7
d; n=9

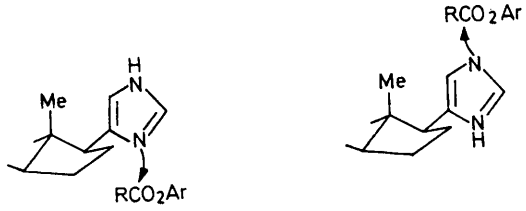
(4); X=2-NO$_2$, Y=4-NMe$_3^+$; a; n=1
b; n=7
c; n=9

Three sets of substrates were used with the steroid (**1a**);[10] these were negative, neutral and positive aryl esters of fatty acids, [(**2a**)–(**2c**)], [**3a**)–(**3c**)] and [(**4a**)–(**4c**)].† With negative aryl acetates, (**1a**) is more reactive than imidazole; with neutral aryl acetates it is very similar and with positive aryl acetates, it is less reactive. To compensate for this electrostatic effect, and also for any systematic errors in the concentration of (**1a**) solutions we calculate relative rates with the acetate ester as standard in each series, using a rate constant for the acetate measured with the same stock solution of (**1a**). The ratio of the relative rates for (**1a**) and for imidazole-catalysed reactions, R_2, measures the effect of steroid-alkyl chain interactions on the rate of reaction, all other effects (inherent reactivities of substrate and catalyst, and electrostatic interactions) having been cancelled (Table).

The values obtained for log R_2 were plotted as a function of n, the number of alkyl chain carbons in H-(CH$_2$)$_n$-C$_2$OAr (Figure). All the points could be fitted by a line of slope 0·027. For the decanoate esters, the observed rate enhancement is *ca.* 1·6 fold, in marked contrast to values of 600 fold[3] previously reported.

It could be suggested that (**1a**) has the option of reacting at either imidazole nitrogen, and that reaction at the more hindered nitrogen is more appropriate for hydrophobic interactions. Examination of space-filling models however suggested that reaction at N-1 would lead to almost as favourable a set of hydrophobic interactions for alkyl chain bearing substrates as reaction at N-3. We have examined the reaction of (**2a**)—(**2c**) with the modified steroid (**1b**) which is constrained to react at N-3. The values of R_2 obtained are virtually identical to those obtained for (**1a**).

The experiments reported demonstrate that 1:1 steroid-alkyl chain interactions are very weak in aqueous solution. We must now consider why these interactions are so much weaker than might be expected on the basis of equilibrium constants for binding of alkyl chains to micelles and also why the effects which we observe are so much smaller than those observed in other systems.



Okenfull and Fenwick have recently reported[11] results of a systematic study of the binding of carboxylate ions to micelles of alkyltrimethylammonium ions. To attempt to compensate for the electrostatic contribution to their binding constants, we take the ratio of the binding constant for an alkanoate to that of acetate. The ratios calculated are *ca.* 11 for hexanoate, 60 for octanoate and 140 for decanoate. These values are markedly larger than the rate enhancements which we report, however, they

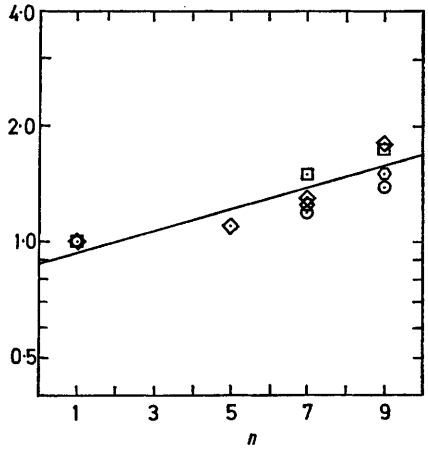


FIGURE. Log R_2, the ratio of k_{rel} for (**1**) catalysed reaction of an alkanoate ester, with the acetate as standard, to k_{rel} for imidazole catalysed reaction, as a function of n, the number of carbons in the alkyl chain of the alkanoate. (**1a**) as catalyst: (○) [**2a**—(**2c**)] (◇) [(**3a**)—(**3d**)]; (□) [(**4a**)—(**4c**)] (**1b**) as catalyst; (⬡) [**2a**)—(**2c**)].

correspond to what is probably inclusion of the alkyl chain in the micelle with almost complete removal from water, while our values correspond to 1:1 interaction with only partial removal of the alkyl chain from water. Taking the fraction of total surface removed from contact with solvent as approximately one quarter (since a space-filling model of an all staggered alkyl chain is roughly square in cross section) the equilibrium constants predicted for 1:1 contact are 1·8 for the hexanoate, 2·8 for the octanoate and 3·4 for the decanoate. These are only slightly larger than we observed; the discrepancy may be attributed to the loss in conformational freedom entailed in coming in contact with the steroid since a large fraction of the available conformations are inaccessible or would have the alkyl chain hanging off in solution. There is likely to be little loss in conforma-

† All new compounds gave satisfactory elemental analyses.

tional freedom on binding to a micelle, since the interior of the micelle is considered to resemble liquid hydrocarbon.[12]

TABLE. Relative rate constants for steroid and imidazole catalysed hydrolysis of aryl alkanoates.[a]

Substrate	Imidazole	k_{rel}[b] Catalyst (**1a**)	(**1b**)
(**2b**)	0·769[c]	0·911[d]	0·948[e]
(**2c**)	0·663[c]	1·02[d]	0·995[e]
(**3b**)	0·718[f]	0·800[g]	
(**3c**)	0·671[f]	0·860[g]	
(**3d**)	0·398[f]	0·726[g]	
(**4b**)	0·745[h]	1·14[i]	
(**4c**)	0·741[h]	1·33[i]	

[a] In aqueous solution, 25·0 ± 0·1°, pH 7·9 (0·01 M Tris buffer), μ = 0·097 (KCl), 0·62 M acetonitrile. All kinetics performed at concentrations low enough that constant second order rate constants were obtained. (**1a**) $< 1 \times 10^{-4}$M; (**2b**) and (**2c**) $< 2 \times 10^{-6}$M; (**3b**) and (**3c**) 1×10^{-6}M; (**3d**) 7×10^{-7}M; (**4b**) and (**4c**) 1×10^{-4}M; all substrate concentrations were less than the aggregation or critical micelle concentrations. For (**2c**) and (**4c**), the apparent second order rate constants were independent of both substrate and catalyst concentrations; for [(**3a**)—(**3d**)] the second order rate constants were independent of catalyst concentrations. All pseudo first order rate plots were linear within experimental error for 3 or 4 half lives. [b] Relative apparent second order rate constants, with the reaction of the appropriate acetate ester as standard. [c] For the acetate, k_2 = 0·866 M^{-1} s^{-1}. [d] For the acetate, k_2 = 4·66 M^{-1} s^{-1}. [e] For the acetate, k_2 = 0·158 M^{-1} s^{-1}. [f] For the acetate, k_2 = 0·426 M^{-1} s^{-1} [g] For the acetate, k_2 = 0·401 M^{-1} s^{-1}. [h] For the acetate, k_2 = 4·12 M^{-1} s^{-1}. [i] For the acetate, k_2 = 1·65 M^{-1} s^{-1}.

Thus the rate enhancements which we report are in satisfactory agreement with micelle binding and extraction experiments, and the large rate enhancements attributed to 1:1 complex formations are out of line. It seems highly probable in view of the relatively high concentrations used in these experiments (high at least relative to the solubilities to be expected[13] for these very hydrophobic materials) that the stoicheiometry has not been 1:1 but rather that complex aggregates have been the actual reacting species.‡

Oakenful has recently re-investigated the reaction of alkylamines and *p*-nitrophenyl alkanoates and reported that the second-order terms either showed little rate enhancement with increasing chain length or were undetectably small.[6] However Oakenful reported[6] very large rate enhancements for the "termolecular" reaction between two molecules of alkylamine and one of *p*-nitrophenyl alkanoates when three long alkyl chains were present. Unfortunately, as Oakenful points out, there is an inconsistency here, since large termolecular rate enhancements imply marked bimolecular rate enhancements, which were not observed.§ Furthermore the concentrations of *p*-nitrophenyl decanoate and dodecanoate used were above the critical concentration where aggregation sets in. We doubt that these large rate enhancements really reflect true 2:1 stoicheiometry; it seems more probable that aggregation of less well-defined stoicheiometry is involved.

We thank the National Research Council of Canada for financial support of this study.

(*Received*, 13*th September* 1974; *Com.* 1161.)

‡ The enzyme model of Ref. 5 is a macrocycle which may be able to bind alkyl chains with more complete removal from contact with water than (**1a**) leading to larger rate enhancements. Nonetheless it seems probable[9] that some of their substrates were used at concentrations above the aggregation limit.

§ The rate enhancements reported in ref. 6 are for formally termolecular reactions, and amount to 10^7 fold for three chains of 9 or 10 carbons. Examination of models suggests that about three times as much surface area is removed from contact with water when three alkyl groups interact as when two are in contact; our results would predict a rate enhancement of 3·4 fold.

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