# A New Enzyme Model System Showing Marked Substrate Specificity

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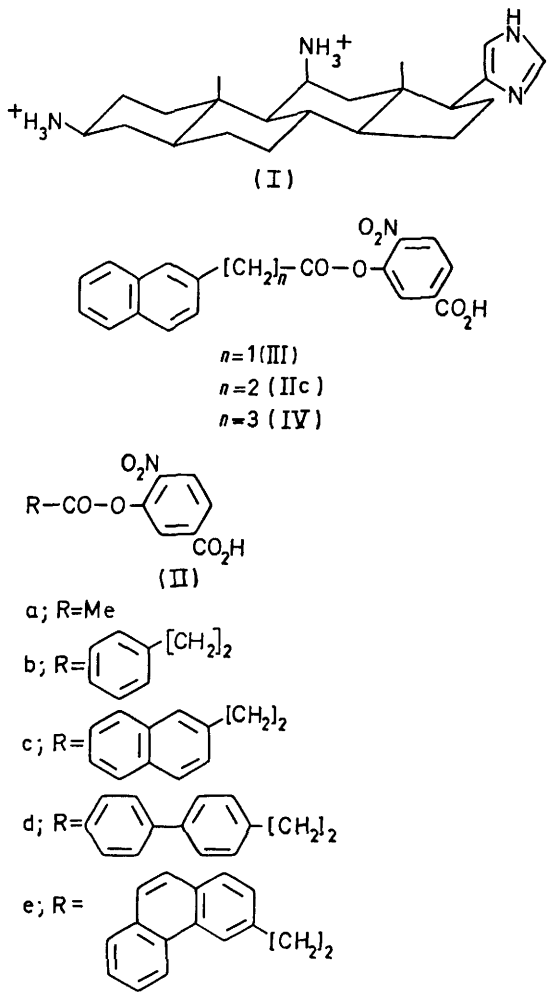
*Summary* The water-soluble steroid (I) acts as a catalyst for the hydrolysis of active esters, with a definite specificity for esters of 3-arylpropionic acids, the ester of 3-(3-phenanthryl)propionic acid being the most rapidly hydrolysed substrate found to date; it is concluded that imidazole-catalysed hydrolysis of the esters is facilitated by hydrophobic interactions between substrate and catalyst.

SEVERAL examples of enzyme model systems, in which hydrophobic interactions between substrate and catalyst lead to stabilisation of the transition state for the catalysed reaction, have been reported in recent years.[1–5] For such a system to give readily interpreted results, it is essential that the stoicheiometry be clearly specified and that there be little ambiguity about the orientation of the interacting molecules in the complex. We describe the behaviour of a new enzyme model, the water-soluble steroid (I), which was designed with these considerations in mind. The synthesis of this steroid has been reported.[6] Substrates (IIa—e)† were prepared to explore the specificity of (I) as a catalyst for the hydrolysis of active esters. We have found that, as the size of the hydrophobic group in the ester increased, the rate of the (I)-catalysed hydrolysis increased (Figure 1); these esters show essentially identical reactivity toward imidazole as nucleophile (Figure 1). The increase in reactivity of the esters toward (I) with increasing size of the

† All new compounds gave satisfactory elemental analyses.

hydrophobic group of the ester almost certainly reflects hydrophobic interactions with the steroid in the transition state. Figure 1 also shows that the rate constants for the




reaction of (I) with (IIb, c, and e) increase linearly with the Hansch $\pi$ value[7] for the acyl substituent; the small deviation observed for (IId) is explicable in terms of the non-planarity of the biphenyl system. It seems reasonable that (IIb, c, and e) which differ only in the size of the hydrophobic substituent should react with (I) at rates related simply to $\pi$; (IIa), which can react by a less cluttered transition state, might be expected to deviate.

It proved necessary to work at extremely low concentrations of substrate to evaluate true second-order rate constants for (I)-catalysed hydrolysis. As Figure 2 shows, the apparent second-order rate constants are concentration-independent at very low concentrations of substrate, but rise rapidly once a critical value is exceeded. This may represent the onset of mixed micelle formation; it does not represent simple micellisation of the substrate, since the apparent second-order rate constants for the imidazole-catalysed hydrolyses are concentration-independent over the concentration range employed. Alternatively there may be smaller aggregates forming, with other than 1:1 stoicheiometry.

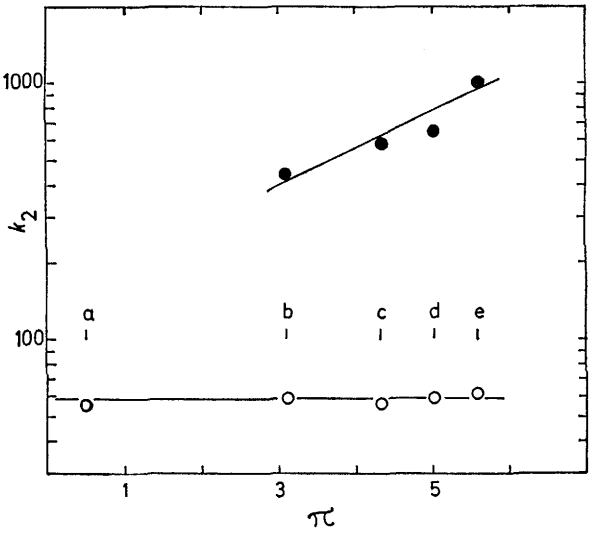



FIGURE 1. Dependence of the logarithm of the second-order rate constants ($M^{-1}$ $min^{-1}$) for imidazole and (I)-catalysed hydrolysis of esters $RCO_2Ar$ upon the Hansch $\pi$ value for R. All rate constants measured at concentrations of ester low enough to give simple kinetic behaviour; 0·78M-MeCN, pH 7·9, $\mu$ = 0·097, 25·0 ± 0·1°; ○, imidazole-catalysed; ●, (I)-catalysed.

Examination of space-filling models suggested that two methylene groups were required between the aromatic ring system and the ester group in order to permit good hydrophobic contact of the aromatic rings with the α-surface of the steroid while nucleophilic attack by the imidazole was occurring. To test this idea, substrates (III) and (IV) were prepared. The rate constants for (I)-catalysed hydrolysis (relative to imidazole) were; (III), 7·25; (IIc), 10·1; (IV),

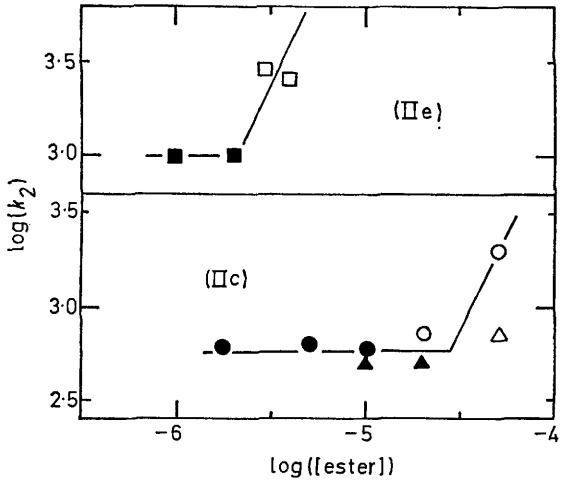
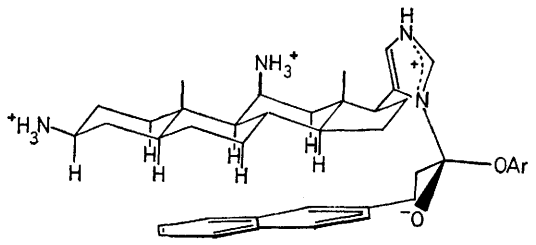



FIGURE 2. Dependence of the logarithm of the second-order rate constant ($M^{-1}$ $min^{-1}$) for (I)-catalysed hydrolysis of (IIg), (IIb), and (IIe), on the logarithm of the ester concentration. Filled symbols: rate constants obtained from linear pseudo-first-order plots; open symbols, rate constants obtained from the initial slopes of curved pseudo-first-order plots; (■, □) 2·00 × $10^{-5}$M-(I); (●, ○) 3·33 × $10^{-5}$M-(I); (▲, △), 9·68 × $10^{-4}$M-(I).

10·3; thus, as expected, with fewer than two methylene groups reaction with (I) becomes less favourable.



The system described above has the advantage of having very little flexibility in either substrate or catalyst, so that there can be little uncertainty concerning the nature or extent of hydrophobic contact between reacting molecules. Also we believe that we have measured true second-order rate constants corresponding to 1:1 stoicheiometry of reaction, since the apparent second-order rate constants are independent of substrate and catalyst concentration, provided the substrate concentration is low enough. There is a disturbing contrast between the modest rate enhancements which we find in the system described above and the large rate enhancements which have been reported for enzyme models with linear alkyl groups as hydrophobic binding sites.‡ Nontheless the rate enhancements which we have observed are unambiguous and can only be ascribed to hydrophobic interactions.§ In view of the propensity for compounds with long alkyl chains to aggregate at very low concentrations[8] and of the concentration dependence observed for the (I)-catalysed hydrolyses, it seems possible that in at least some cases, the large rate enhancements reported previously may reflect aggregation of reagents. There appears to be a need for a critical re-examination of the question of how much rate enhancement can be obtained by hydrophobic binding of modest sized (12—16 carbon atom) molecules.

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‡ Rate enhancement (group acting as binding site): 27 (decanoyl);[1] 550 (decanoyl);[2] 2250 (dodecyl);[3] 7600 (dodecanoyl).[4]

§ The hydrophobic effect will be somewhat underestimated for (I), since the two nitrogens of the imidazole group are not equivalent; the effect is not expected to be large. We hope to be able to measure rates of reaction at N-3 alone by alkylating N-1.

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