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MODEL REACTIONS FOR ENZYME ACTION : THE BIFUNCTIONAL REACTIVITY OF AMIDINES[1]

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Studies on simple model reactions for enzyme action are of basic interest as mechanistic features can be appreciated in much detail, though a satisfactory understanding of the mechanism of enzyme action cannot be expected other than from the examination of the reactions of enzymes themselves.[4] Such studies led to the theory of polyfunctional catalysis for enzyme action[5] which enjoys considerable popularity and is generally accepted for a number of model reactions.[6] However, considerable controversy has recently arisen in the important cases[6a,c] of aminolysis of esters.

Thus, the observation that p-nitrophenyl acetate in chlorobenzene acetylates benzamidine with second-order kinetics and some 15,000 times faster than an amine of comparable basicity, like n-butylamine,(where third-order overall kinetics and lack of catalysis by N-methylpiperidine were observed) was interpreted by Menger in terms of bifunctional reactivity for benzamidine and of n-butylamine participation in a cyclic transition state involving two molecules of amine.[7]

These conclusions have been refuted by Anderson[8] on the grounds that (a) an amidine like 1,4,5,6-tetrahydropyrimidine (for which they could not conceive a bifunctional adaptation in the transition state on the basis of examination of molecular models) is acetylated by p-nitrophenyl acetate in chlorobenzene with second-order kinetics and even faster than benzamidine, and (b) the corresponding n-butylaminolysis is catalysed by added triethylenediamine. Rather, they proposed that "high electron density on the imine nitrogen and stabilisation of the transition state by dispersal of the positive charge over three atoms" is responsible of the fast acetylation of amidines.[8]

We report here some results which throw much doubt on the latter conclusions[8] and give indirect support to Menger's mechanism.[7] These concern the reactions of 1-chloro-2,4-dinitrobenzene with n-butylamine or benzamidine in chlorobenzene where quantitative 2,4-dinitrophenylation occurs.[9,10] The choice of this substrate for comparison of amidinolysis with aminolysis was suggested by (i) the absence of catalytic phenomena of any sort,[11] and (ii) the considerable amount of bond formation between nucleophile and substrate at the transition state in the latter type of reactions[11,12] and by the most reasonable expectation that both these conditions should occur in the corresponding amidinolyses (even if no previous case of aromatic amidinolysis has been reported).

The above expectation is fulfilled as second-order kinetics are obtained and the outcome is that benzamidine is appreciably less reactive than n-butylamine, the lower reactivity being due

to enthalpy terms which are only partially compensated for by entropy terms. These results strongly argue against Anderson's mechanism[8] for amidinolysis which demands a fast amidinolysis for any substrate where, as in the aromatic one, a considerable amount of bond formation is involved at the transition state.[13] Our results are, however, consistent with Menger's mechanism[7] as amidinolysis is very fast with a substrate (ester) where catalysis is needed and slow when (as in aromatic substitution of a chlorine) no catalysis is necessary. Therefore, as far as no special factor, unrecognized by current theories, is involved in amidinolysis of esters, reactions of this type constitute a perfectly acceptable and interesting example of bifunctional reactivity. This eliminates the above discussed type of objections to the theory of polyfunctional catalysis for enzyme action.

Rate data for the reaction of CDNB with benzamidine* or n-buNH$_2$ in chlorobenzene

amine(10^3M)	temp(°C)	$10^3 k$ ($M^{-1} sec^{-1}$)	$\Delta H^{\neq}$ (kcal mol^{-1})	$-\Delta S^{\neq}$ (e.u.)
		benzamidine		
1.45	84.5	2.31		
2.90	84.5	2.37	15	28
2.90	59.8	0.486		
		n-butylamine		
10.0	84.5	15.5		
27.4	84.5	15.9	10	39
27.4	59.8	5.20		

*Menger's data[7] are nicely reproduced by our benzamidine.

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