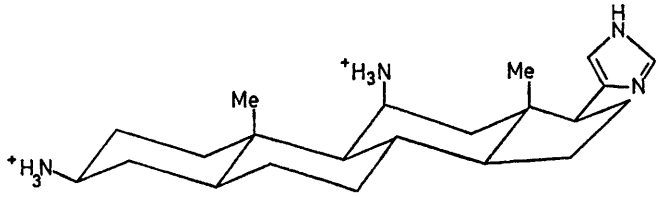
Electrostatic Catalysis and Inhibition in Aqueous Solution. Rate Effects on the Reactions of Charged Esters with a Cationic Steroid Bearing an Imidazolyl Substituent

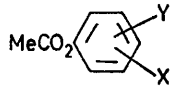
By J. PETER GUTHRIE and YASUTSUGU UEDA
(*Department of Chemistry, University of Western Ontario, London, Canada* N6A 3K7)

Summary The steroid (**1**) with a 17β-imidazoyl-and an 11β-ammonio-substituent catalyses the hydrolysis of aryl acetates; esters with an anionic substituent on the phenolic unit show rate enhancements, an ester with a cationic substituent shows a rate retardation, and the rate enhancement for a *meta*-anionic substituent is much larger than for *para*-anionic substituents.

ALTHOUGH electrostatic attractions between oppositely charged ions are believed to make important contributions to the free energy of enzyme substrate binding in many cases,[1,2] there are very few cases which permit the evaluation of the magnitude of ion binding for small well defined molecules. Most investigations of this phenomenon have involved ionic reactants with the charges on one or both species located at or very close to the reaction centre; such reactions sometimes show electrostatic effects on rate



(I)



(2)

a; X = H, Y = 4-NO_2
b; X = H, Y = 3-NO_2
c; X = 2-NO_2, Y = 4-NO_2
d; X = 3-CO_2^-, Y = 6-NO_2
e; X = 4-CO_2^-, Y = 6-NO_2
f; X = 4-SO_3^-, Y = 6-NO_2
g; X = 4-NMe_3^+, Y = 6-NO_2

constants of an order of magnitude, but sometimes show no such effects.[1,3,4] The most impressive model cases for electrostatic binding have involved polyions[1] for which it is difficult to specify the geometrical arrangement corresponding to a given free energy of interaction. We report a case of unambiguous electrostatic catalysis where the geometry of the interacting ions is well specified, and where there are marked geometrical requirements for the binding to occur.

The steroid (**1**) has been shown to act as a catalyst for the hydrolysis of active esters.[5–7] Initially our attention was focused on hydrophobic binding to the lower surface of the steroid, using esters of arylpropionic acids as substrates. It was noted that aryl ester substrates with ionic substituents in the phenolic unit showed rate enhancements for negative substrates and retardations for positive substrates.[7] These could be reasonably explained in terms of electrostatic interactions between the ionic substituent on the substrate and the 11-ammonio-group of the steroid. We now report a systematic study of this phenomenon, showing that the magnitude of the electrostatic effect is critically dependent on the positioning of the ionic substituent in the substrate, and depends on the ionic strength of the medium in a predictable way.

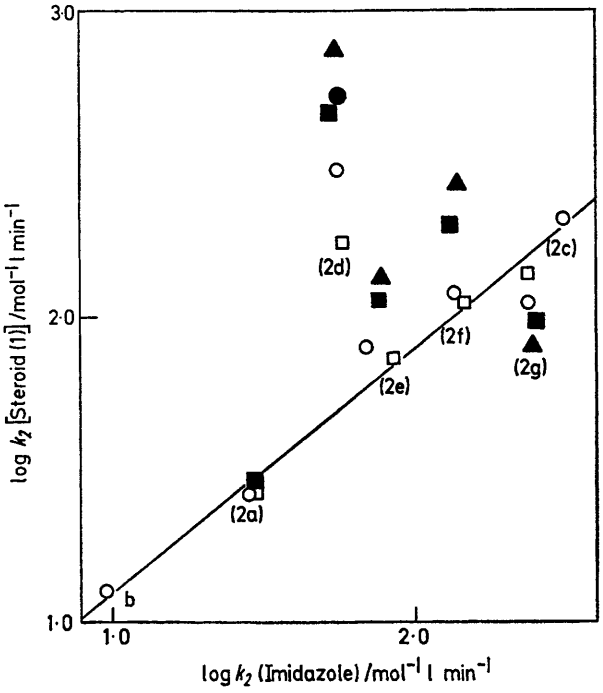
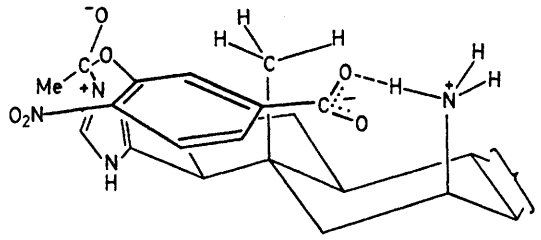


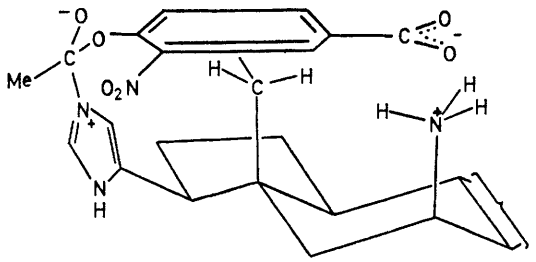
FIGURE. Plot of log k_2 for the reaction of (**1**) with (**2a-g**) *vs.* log k_2 for the reaction of imidazole with these esters. All reactions at 25·0 ± 0·1 °C, pH 7·9; apparent second-order rate constants calculated from the observed pseudo-first-order rate constant (corrected for uncatalysed hydrolysis) by dividing by the total nucleophile concentration. The solid line was drawn to fit the points for (**2a—c**): □, μ 0·94M; ○ μ 0·10M; ■, μ 0·014M; ●, μ 0·0054M; ▲, μ 0 (extrapolated); ionic strength adjusted with KCl; all solutions contained 0·01M Tris buffer; [(**1**)] = 0·00097M; [imidazole] = 0·0097M.

The substrates used were the acetate esters (**2a—g**). In order to separate the electrostatic effect from other effects of changing leaving group, the reaction of (**2a–g**) with imidazole was used as a measure of the rate of reaction in the absence of electrostatic interactions. The rate constant for imidazole-catalysed hydrolysis is almost independent of the ionic strength; for neutral substrates there is a good linear free energy relationship between the rate constants for imidazole- and (**1**)-catalysed hydrolysis, as is shown in the Figure. Substrate (**2d**) with a negative substituent in the *meta*-position shows a positive deviation from the correlation line, while substrates with negative

substituents in the *para*-position show little deviation. Substrate (**2g**) with a positive substituent shows a small negative deviation. Experiments were performed at



(I) + (2d)



(I) + (2e)

0·0054, 0·014, 0·1 and 0·94 M ionic strength; linear plots of log k_2 *vs.* log $\gamma_\pm$ (calculated using the Davies equation[8]) were obtained, permitting evaluation of the rate constants at zero ionic strength, which are included in the Figure. At zero ionic strength, substrate (**2d**) reacts 15 times faster with the steroid than would be expected from its reactivity towards imidazole, whereas substrate (**2e**), with the carboxylate displaced from the *meta*- to the *para*-position shows only a 2-fold rate enhancement; these rate enhancements correspond to free energies of interaction of 1·6 and 0·3 kcal/mol respectively.

These results constitute the first clear demonstration of catalysis resulting from the electrostatic interactions of charged groups remote from the reaction centre interacting with reasonably well specified geometry. Construction of space-filling models, subject to the constraints of: (partial) bonding between the imidazole nitrogen and the ester carbonyl; reaction at N-1 of the steroidal imidazole;[10] and closest possible contact between carboxylate and ammonium ions, leads for (**2d**) to a conformation with no obvious strain and contact between the ionic groups, presumably associated with a hydrogen bond, although this would be non-linear. A similar exercise with (**2e**) or (**2f**) leads to the conclusion that close electrostatic contact is unavoidably associated with uncompensated desolvation of one of the N^+H hydrogens, which is shielded from water by the phenolic ring without being in contact with an O^- of the anionic substituent. Displacing a water molecule solvating an ammonio-group is believed to cost about 15 kcal/mol[11], so that it is to be expected that this desolvation will not occur and that for substrates (**2e**, **f**) the ionic groups will remain separated by at least one layer of solvent molecules. We conclude that the geometrical requirements for efficient electrostatic catalysis are quite severe.

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

(*Received*, 12*th November* 1973; *Com.* 1557.)

[1] W. P. Jencks, 'Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, 1969, pp. 351—392 and references cited therein.
[2] H. C. Froede and I. B. Wilson, 'The Enzymes,' ed. P. D. Boyer, 3rd edn., vol. 5, Academic Press, New York, 1971, p. 87.
[3] S. L. Johnson, *Adv. Phys. Org. Chem.*, 1967, **5**, 237, and references cited therein.
[4] B. Holmquist and T. C. Bruice, *J. Amer. Chem. Soc.*, 1969, **91**, 2985.
[5] J. P. Guthrie, *Canad. J. Chem.*, 1972, **50**, 3993.
[6] J. P. Guthrie and Y. Ueda, *J.C.S. Chem. Comm.*, 1973, 898.
[7] J. P. Guthrie and Y. Ueda, *Canad. J. Chem.*, in the press.
[8] R. A. Robinson and R. H. Stokes, 'Electrolyte Solutions,' Academic Press, New York, 1955, p. 231.
[9] K. B. Wiberg, 'Physical Organic Chemistry,' Wiley, New York, 1964, p. 395.
[10] J. P. Guthrie and Y. Ueda, *J. Amer. Chem. Soc.*, submitted for publication.
[11] H. A. Scheraga, *Adv. Phys. Org. Chem.*, 1968, **6**, 140.