Intramolecular catalysis. Part 9.[1] The hydrolysis of *p*-nitrophenyl acetate catalysed by imidazoles having proximate carboxylate groups

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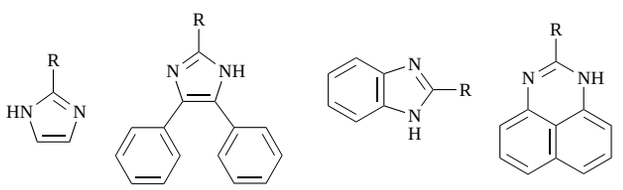
The rate coefficients for the hydrolysis of *p*-nitrophenyl acetate catalysed by a series of imidazoles, 4,5-diphenylimidazoles, benzimidazoles and perimidines in 30 mol% aqueous dimethyl sulfoxide at 30.0 °C have been measured, as have the pK_a values of the catalysts in the same medium. The effect of a 2-(2-carboxyphenyl) substituent in each system has been studied. The pK_a values of the imidazolium and related ions show that the 2-carboxylate group increases their acidity by 1.03 to 1.32 pK_a units. The same effect on the nucleophilicity of the corresponding imidazole and related compounds gives rise to rate increases of a factor of 1.4 to 3.2. These effects are related to the structure of the imidazoles, indicating the importance of the environment of carboxylate groups.

It has been considered that the active-site serine-195 residue in chymotrypsin has its nucleophilicity increased by the imidazole base of histidine-57 acting as a general-base catalyst.[2] Likewise, the carboxylate group of aspartic-102 was considered to be involved as a base activating the imidazole by removing the proton. This catalytic triad has become known as the 'charge-relay' system. Furthermore, imidazole is well-known as a nucleophilic catalyst in organic, model enzymic and enzymic systems. For esters with good leaving groups, such as *p*-nitrophenyl esters, nucleophilic catalysis by imidazoles is well established.[3] However, the study[4] of the cooperativity in the 2-benzimidazoleacetate anion catalysed hydrolysis of ethyl chloroacetate involved a general-base hydrolytic process, in which a twelvefold acceleration was found arising from the increased nucleophilicity of water towards the substrate, catalysed by imidazole enhanced by proton removal by carboxylate anion from the second nitrogen. Similar studies[5] have investigated the effect of proximate carboxylate groups on imidazole and benzimidazole systems acting as general-base in hydrolysis which indicate very small or no assistance from the carboxylate group. A recent study[6] has suggested that the role of aspartic-102 is to fix the tautomeric form of imidazole and to anchor histidine-57 in the correct orientation in chymotrypsin. It was proposed that a *syn*- and an *anti*-orientated carboxylate may give very different results and investigations of a number of models indicated that the *syn*-carboxylate raises the pK_a value of a proximate imidazolium ion by *ca.* 0.4 to 0.6 pK_a unit over that of an *anti*-carboxylate system.

Bender *et al.*[7] prepared some elegant model systems for chymotrypsin. Thus, the *endo*,*endo*-5-[4(5)-imidazolyl]bicyclo-[2.2.1]heptan-2-yl *trans*-cinnamate, unlike the *exo*,*endo*-isomer, showed participation by imidazole in the deacylation hydrolysis by general-base catalysis, with the rate tremendously increased by the presence of benzoate anions. The latter catalysis was effected intramolecularly by the synthesis of the *endo*, *endo*-system having a 2-(2-carboxyphenyl)imidazole substituent and this model had a rate of hydrolysis 1.54×10^5-fold faster than the previous model. This research group then developed a full model for the enzyme using a binding/nucleophilic hydroxy subsite, cyclodextrin, bonded to a catalytic subsite, 2-imidazoylbenzoate anion. Using 4-*tert*-butylphenyl acetate as substrate, they demonstrated the occurrence of hydrolysis by a nucleophilic hydroxy/general-base mechanism, rather than nucleophilic attack by imidazole. Kinetic studies indicated that the model enzyme is as efficient as the enzyme itself. Brown and his co-workers[8] have addressed the issue of the aspartic carboxylate group acting either as a general base or to enhance electrostatically the basicity of imidazole. Their study of a model system indicated that the latter occurs.

The present studies were designed to assess the effect of a proximate carboxylate group on the nucleophilic reactivity of imidazoles and the effect of the environment of the catalytic sites. We have prepared a series of 2-substituted imidazoles, **1**, 4,5-diphenylimidazoles, **2**, benzimidazoles, **3** and perimidines, **4**,



1 **2** **3** **4**

a R = H
b R = Ph
c R = 2-Carboxyphenyl

and measured the imidazole-catalysed hydrolysis of *p*-nitrophenyl acetate, together with the pK_a of the catalysts. The effects of substitution, proximate carboxylate groups and their interrelations are discussed.

Results and discussion

pK_a values

The pK_a values of the imidazoles, benzimidazoles and perimidines in 30 mol% aqueous dimethyl sulfoxide (DMSO) at 30 °C are shown in Table 1. The pK_a values of imidazole, 2-phenyl- and 4,5-diphenyl-imidazole, benzimidazole and 2-phenylbenzimidazole have been measured in water at 25 °C by Walba and Isensee[9] previously. The first four compounds have pK_a values which are *ca.* 0.1 to 0.2 pK_a units greater in 30 mol% aqueous DMSO. However, the present results show a consistent decrease in pK_a of *ca.* −0.6 to −0.7 pK_a unit for 2-phenyl substitution in the four parent systems, *i.e.* imidazole, 4,5-diphenylimidazole, benzimidazole and perimidine. The major effect causing this increase in acidity appears to be the relative stabilisation of the protonated imidazoles by resonance delocalisation interactions involving the π-electron system of the substituted cationic imidazolium. A similar effect causes the acid-strengthening effect of the 4,5-diphenyl (−1.40 pK_a units) and benzo (−1.61 pK_a units) substitution, relative to imidazole. The pK_a of perimidine

Table 1 Rate coefficients (k_2 and k_2^{max}/dm^3 mol^{-1} s^{-1}) for the hydrolysis of *p*-NPA catalysed by imidazoles in 30 mol% aqueous DMSO at 30.0 °C, together with the pK_a values of the imidazoles[a,b]

Imidazole catalyst	k_2/10^{-2} dm^3 mol^{-1} s^{-1}	k_2^{max}/10^{-2} dm^3 mol^{-1} s^{-1}	pK_a
Unsubstituted	41.3	46.5	7.10
2-Phenyl	9.60	9.78	6.27
2-(2-Carboxyphenyl)	15.6	18.7	[4.13],[c] 7.30
4,5-Diphenyl	11.1	11.2	5.70
2,4,5-Triphenyl	9.70	9.70	4.96[d]
2-(2-Carboxyphenyl)-4,5-diphenyl	14.3	14.5	[4.19],[c] 6.10[d]
Benz	13.9	13.9	5.49
2-Phenylbenz	8.05	8.06	4.85
2-(2-Carboxyphenyl)benz	11.1	11.2	6.01
Perimidine	14.3	14.4	6.02
2-Phenylperimidine	2.20	2.20	5.28[d]
2-(2-Carboxyphenyl)perimidine	6.75	7.01	6.60[d]

[a] Rate coefficients were reproducible to ±3% and pK_a values to ±0.03 pK_a unit. [b] $k_1^{H_2O}$ equal to 1.19 × 10^{-4} s^{-1}. [c] pK_a[1]. [d] pK_a values to ±0.05 pK_a unit.

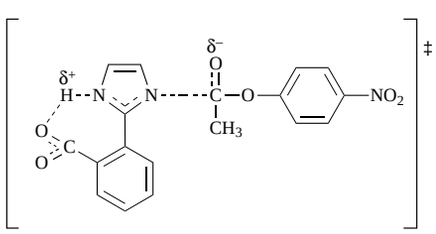
is even lower than that of benzimidazole. Perimidine has the 1,3-diaza system in a six-membered ring and is not strictly an imidazole. However, the acid-strengthening effect (−1.08 pK_a units), relative to imidazole, apparently arises from the same resonance delocalisation.

The pK_a[1] values of the 2-carboxyphenyl systems are very similar, *i.e.* 4.1 to 4.2, and are *ca.* 2.9 pK_a units stronger than that of imidazole. The pK_a values of a series of *ortho*-substituted benzoic acids have been measured in aqueous DMSO.[10] The first ionisation observed here is that of the benzoic acid group. The carboxylate anion would be expected to be intramolecularly hydrogen-bonded, which would be acid-strengthening;[11] as well as having the electrostatic influence of the *ortho*-cationic imidazolium substituent and a specific *ortho* effect.[10] On the other hand, the imidazolium cations would be acid-weakened by the electrostatic effect of the cationic and anionic centres and by an intramolecular hydrogen bond. The latter would occur as a seven-membered ring which is larger than ideal. The effect of the 2-carboxylate group is as expected from related studies[4,6] and gives here ΔpK_a values equal to between +1.03 and +1.32 pK_a units, increasing in the order: 2-phenylimidazole ≤ 2,4,6-triphenylimidazole < 2-phenylbenzimidazole < 2-phenylperimidine. Thus, it would appear that 'flanking' steric bulk increases the acid-weakening effect.

Catalysed hydrolysis

Imidazoles have been used extensively as catalysts to study the hydrolysis of *p*-nitrophenyl acetate (*p*-NPA).[12,13] In Table 1 are shown the apparent second-order rate coefficients, k_2, and the maximal second-order rate coefficients, k_2^{max}, for the hydrolysis of *p*-NPA catalysed by the substituted imidazoles, benzimidazoles and perimidines in 30 mol% aqueous DMSO at pH 8.0 and 30 °C.

Bruice and Schmir[12] investigated the reaction of a series of 4(5)-substituted imidazoles with *p*-NPA in 28.5% (v/v) aqueous ethanol at 25 °C. The relationship between basicity and nucleophilicity given allowed the construction of a Brønsted relation with β equal to 0.66. More recently, Zimmerman *et al.*[6] studied the reactivity of a series of substituted imidazoles with relatively similar steric environments of the reaction site in water at 25 °C and found a Brønsted β value of 0.49. For the present results in 30 mol% aqueous DMSO at 30 °C, a Brønsted relation can be constructed for the four catalysts without a 2-phenyl substituent giving β equal to 0.37 (±0.09) with a correlation coefficient equal to 0.951. Considering the differences in the series, solvent and temperature, the agreement is reasonable. The present series is effectively a 4,5-disubstituted series and a modest steric effect can be expected to reduce the β value observed. Substitution by a 2-phenyl group results in the decreased reactivity expected arising, in the main, from decreased basicity, with a contribution from steric bulk retardation. The latter is most severe for the 2-phenylperimidine. Bruice and Schmir[12] found that 4(5)-substituents had a much smaller steric effect than 2-substituents. The 4,5-diphenyl substitution apparently results in a small steric effect which is probably minimised by the mutual buttressing of phenyl groups. This causes the phenyl groups not to be coplanar with each other or imidazole in 4,5-diphenylimidazole, similar to the situation found in *cis*-1,2-diphenylethylene.[14] The 2-carboxylate group increases the nucleophilic reactivity of the 2-phenyl systems. However, for the imidazole, 4,5-diphenylimidazole and benzimidazole systems, the reactivity increases by a factor of *ca.* 1.6, whereas, for the perimidine, by a factor of 3.2. The 2-phenylperimidine appears to have the most steric shielded or crowded reaction site. It appears likely that solvating water is excluded from the twin nitrogens of 2-phenylperimidine. The carboxylate group can effectively replace this stabilisation. While the carboxylate group activates all the systems, it becomes more critical when water is excluded. There is good evidence[15] that water is partially excluded from the active site of enzymes such as chymotrypsin. Thus, the role of catalysis by the carboxylate anion at that site may well be even more important. In terms of the source of the general effect of the 2-carboxylate groups here, an electrostatic effect appears less likely than that of a general-base, *cf.* ref. 8. A structure for the transition state involving the 2-carboxylate substituted catalyst is shown in **5** below.



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Experimental

Materials

Imidazole, benzimidazole and perimidine, as well as their derivatives, were prepared by standard literature methods.[16,17] After recrystallisation from suitable solvents and drying under reduced pressure (P_2O_5), their mps were in good agreement with literature values,[18] with the exception of 2-(2-carboxyphenyl)perimidine which did not melt below 400 °C, as reported.[17] *p*-Nitrophenyl acetate and *p*-nitrophenol were available pure commerically. Solvents were purified by standard methods,[19] while other analytical grade reagents were used without further purification.

Kinetic procedure

Rate coefficients were determined spectrophotometrically by use of a Perkin-Elmer lambda 5 or 16 UV–VIS spectrometer. The reactions were followed at 408 nm. The procedure used was similar to that described previously.[12,13,20] Solutions in DMSO–water (30 mol%) were prepared by adding the aqueous buffer to the DMSO in a volumetric flask. The ionic strength (μ) was 0.1 mol dm^{-3} using KCl. The substrate ester was 0.5×10^{-4} mol dm^{-3}. The first-order rate coefficients, k_1, were determined in the presence and absence of catalyst, being reproducible to ±3%. The rate coefficient in the absence of catalyst is given in Table 1. The apparent second-order rate coefficients, k_2, were obtained at least in duplicate and at three concentrations of the catalyst, *i.e.* normally 1×10^{-4}, 2×10^{-4} and 3×10^{-4} mol dm^{-3}. The maximal second-order rate coefficient[21] was calculated from the apparent rate coefficient using eqn. (1) shown below,

$$k_2^{max} = k_2 ([H^+] + K_a)/K_a \quad (1)$$

which corrects for concentration of the cationic and non-catalytic species.

pH′ and pK_a measurements

The pH′ values of the reactant solutions were measured at 30.0 °C using a combined glass and reference electrode and a Phillips PW9409/10 direct reading pH meter. The pK_a measurements were made according to either the potentiometric method of Bruice and Schmir[12,13] or the spectrophotometric method of Bowden *et al.*[20] The solutions were prepared as described above. The compounds were studied at 2×10^{-2} mol dm^{-3} when using the potentiometric method. The solubility of the 2,4,5-triphenylimidazoles and 2-phenylperimidines required the use of dilute solutions at 2×10^{-4} mol dm^{-3} and the spectrophotometric method. The uncertainty of the pK_a values are shown in Table 1. Measurements were made at least in duplicate.

Acknowledgements

One of us (A. B.) thanks the UK SERC for a research studentship.

References

1 Part 8. K. Bowden, A. Butt and M. Streater, *J. Chem. Soc., Perkin Trans. 2*, 1992, 567.
2 D. M. Blow, J. J. Birktoft and B. S. Hartley, *Nature*, 1969, **221**, 337; D. M. Blow, *Acc. Chem. Res.*, 1976, **9**, 145.
3 W. P. Jencks, *Catalysis in Chemistry and Enzymology*, McGraw-Hill, New York, 1969; G. A. Rogers and T. C. Bruice, *J. Am. Chem. Soc.*, 1974, **96**, 2463; M. J. Gresser and W. P. Jencks, *J. Am. Chem. Soc.*, 1977, **99**, 6970.
4 M. Komiyama and M. L. Bender, *Bioorg. Chem.*, 1977, **6**, 13.
5 J. B. Jones and K. E. Taylor, *Can. J. Chem.*, 1977, **55**, 1653; G. Wallenberg, J. Broger and P. Haake, *J. Am. Chem. Soc.*, 1971, **93**, 4938; F. Menger and A. C. Vitale, *J. Am. Chem. Soc.*, 1973, **95**, 4931; F. Menger, S. Wrenn and H. S. Rhee, *Bioorg. Chem.*, 1975, **4**, 194; F. D'Andrea and U. Tonellato, *J. Chem. Soc., Chem. Commun.*, 1975, 659.
6 S. C. Zimmerman, J. S. Korthals and K. D. Kramer, *Tetrahedron*, 1991, **47**, 2649.
7 I. M. Mallick, V. T. D'Souza, M. Yamaguchi, J. Lee, P. Chalabi, R. C. Gadwood and M. L. Bender, *J. Am. Chem. Soc.*, 1984, **106**, 7252; V. T. D'Souza, K. Hanabuse, T. O'Leary, R. C. Gadwood and M. L. Bender, *Biochem. Biophys. Res. Commun.*, 1985, **129**, 727; M. Komiyama, T. R. Roesel and M. L. Bender, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 23; M. Komiyama, M. L. Bender, M. Utaka and A. Takedo, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 2634.
8 K. I. Skorey, V. Somayaji and R. S. Brown, *J. Am. Chem. Soc.*, 1989, **111**, 1445.
9 H. Walba and R. W. Isensee, *J. Am. Chem. Soc.*, 1955, **77**, 5488; H. Walba and R. W. Isensee, *J. Org. Chem.*, 1961, **26**, 2789.
10 M. Hojo, M. Utaka and Z. Yoshida, *Tetrahedron*, 1971, **27**, 2713.
11 D. D. Perrin, B. Dempsey and E. P. Serjeant, pK_a *Prediction for Organic Acids and Bases*, Chapman and Hall, London, 1981.
12 T. C. Bruice and G. L. Schmir, *J. Am. Chem. Soc.*, 1957, **79**, 1663.
13 M. L. Bender and B. W. Turnquest, *J. Am. Chem. Soc.*, 1957, **79**, 1656; T. C. Bruice and G. L. Schmir, *J. Am. Chem. Soc.*, 1958, **80**, 148.
14 E. L. Eliel and S. H. Wilen, *Stereochemistry of Organic Compounds*, Wiley, New York, 1994.
15 A. Fersht, *Enzyme Structure and Mechanism*, Freeman, Reading, 1977.
16 E. C. Horning, *Org. Synth.*, 1943, Coll. Vol. III, p. 471; P. Keller, F. G. Eggart, G. Wehrli, H. Schaffner and O. Jeger, *Helv. Chim. Acta*, 1967, **50**, 2259; C. Giordao, *Synth.*, 1975, 167; J. B. Hendrickson and S. Hussoin, *J. Org. Chem.*, 1989, **54**, 1144; R. A. Duncan and M. G. Sturrock, *J. Org. Chem.*, 1968, **33**, 2149.
17 F. Sachs, *Annalen*, 1909, **365**, 53.
18 T. H. Fife, R. J. Bambery and B. R. DeMark, *J. Am. Chem. Soc.*, 1978, **100**, 5500; G. E. Philbrook, M. A. Maxwell, R. C. Taylor and J. R. Totter, *Photochem. Photobiol.*, 1965, **4**, 1175; R. Meyer and J. Maier, *Annalen*, 1903, **327**, 41.
19 D. D. Perrin and W. L. F. Armarego, *Purification of Laboratory Chemicals*, 3rd edn., Pergamon Press, Oxford, 1988.
20 K. Bowden, D. Law and R. J. Ranson, *J. Chem. Soc., Perkin Trans. 2*, 1977, 1799.
21 T. C. Bruice and S. J. Benkovic, *J. Am. Chem. Soc.*, 1964, **86**, 418.

Paper 6/05649K
Received 13*th August* 1996
Accepted 2*nd October* 1996