Structure and Efficiency in Intramolecular and Enzymic Catalysis: Intramolecular General Base Catalysis. Hydrolysis of Monoaryl Malonates†

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The hydrolysis of monoaryl malonate anions is subject to intramolecular general base catalysis by the ionised carboxy-group. The mechanism does not change when the angle, and thus the distance also, between the catalytic and substrate groups is varied in a series of dialkylmalonic acid derivatives. This makes it possible to examine the dependence of reactivity on geometry, which includes such factors as orbital alignment, in a system uncomplicated by strain or steric effects. The sensitivity is small: over a range of structural variation sufficient to produce enormous effects on reactivity in intramolecular nucleophilic catalysis, the efficiency of intramolecular general base catalysis changes by a factor of less than ten. Catalytic efficiency is also low in absolute terms, reaching a limiting effective concentration of *ca.* 100M, to be compared with 10^8M for intramolecular nucleophilic catalysis uncomplicated by strain. The difference is ascribed to differences in transition state properties, particularly internal entropies, for the two mechanisms. The explanation has important implications for the origins of high efficiency in enzymic catalysis.

It has long been accepted that the extraordinarily high efficiency of enzymic catalysis can be accounted for in terms of normal reaction mechanisms, operating under the specially favourable conditions prevailing in the enzyme–substrate complex. Although the factors concerned are not fully understood in quantitative terms,[1] the mechanisms are thought to fall into a few well defined classes. Work on simple systems has identified three—general acid, general base, and nucleophilic catalysis—which appear to be of general importance.[2] The questions then arise: how efficient can these mechanisms be outside the special environment of the enzyme–substrate complex; and, are all three potentially highly efficient, or is one intrinsically more (or less) effective than the others?

Recent work in this [3] and other [4] laboratories has shown that the efficiency of intramolecular nucleophilic catalysis is extremely sensitive to structural variation, and can be very high indeed. The carboxy-group commonly has an effective concentration of 10^6—10^8M in cyclisation reactions, but systematic structural variation can raise this to 10^{10}M,[3] and in an extreme case a figure of over 10^{15}M has been reported.[5] General acid catalysis has not been systematically studied from this point of view,[6] but effective concentrations of the CO_2H group of at least 10^3M are observed in intramolecular reactions of acetals involving this mechanism,[7] and in the case of salicyl phosphate [8] figures higher still are observed.

General base catalysis appears to be far less efficient. The best documented reaction is intramolecular general base catalysis of ester hydrolysis, where the effective concentration of the ionised carboxy-group is never much greater than about 20M. This might be because the systems studied so far do not allow other than very inefficient catalysis; or because this type of catalysis is of its nature relatively inefficient, and thus cannot be a primary source of high catalytic efficiency in enzymic reactions. In this study we explore the limits of efficiency of intramolecular general base catalysis.

Our approach is in principle the same as that we have used successfully in recent similar work on intramolecular nucleophilic catalysis.[3,4] Taking an intramolecular reaction known to go by the mechanism concerned, we vary structure systematically in an attempt to maximise the efficiency of the catalysis. Page and Jencks [9] have reported a study of this general sort, where they varied the geometry of approach of the general base to the nucleophilic centre, using a series of diamines in the intramolecular general base catalysed aminolysis of acetylimidazole. They found little sensitivity to the structure of the diamine, and low effective concentrations (below 1M) of the general base, suggesting that the proton transfer part of the mechanism is not critically dependent on geometry. We planned to include the bond-formation part of the mechanism, specifically by observing how the efficiency of intramolecular general base catalysis of ester hydrolysis depends on the geometrical relationship between the general base and the carbonyl group under attack.

The choice of a suitable system for this investigation proved a major problem. When a basic group is brought close to an electrophilic centre it is easier, other things being equal, for it to react directly, as a nucleophile, rather than as general base in a reaction involving a third functional group. Intramolecular general base catalysis is observed in practice only when the nucleophilic reaction is specifically excluded or inhibited.

This may be for at least three reasons: (*a*) if the general base and the leaving group are identical the nucleophilic reaction simply regenerates starting material; then the

† No reprints available.

[1] W. P. Jencks, 'Catalysis in Chemistry and Enzymology,' McGraw–Hill, New York, 1969.

[2] T. C. Bruice and S. J. Benkovic, 'Bioorganic Mechanisms,' Benjamin, New York, vol. 1, 1966; M. L. Bender, 'Mechanisms of Homogeneous Catalysis, from Protons to Proteins,' Wiley, New York, 1971.

[3] A. J. Kirby and P. W. Lancaster, *J.C.S. Perkin II*, 1972, 1206.

[4] R. T. Borchardt and L. A. Cohen, *J. Amer. Chem. Soc.*, 1972, **94**, 9155, 9166; 1973, **95**, 8308, 8313, 8319.

[5] S. Milstein and L. A. Cohen, *J. Amer. Chem. Soc.*, 1972, **94**, 9158.

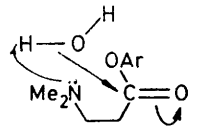
[6] Apart from work in progress in this laboratory, with R. Osborne.

[7] B. M. Dunn and T. C. Bruice, *Adv. Enzymol.*, 1973, **37**, 1.

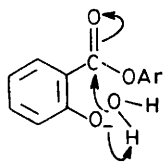
[8] R. H. Bromilow and A. J. Kirby, *J.C.S. Perkin II*, 1972, 149.

[9] M. I. Page and W. P. Jencks, *J. Amer. Chem. Soc.*, 1972, **94**, 8818.

general base catalysed reaction may be observed even if it is much slower, as in Kilpatrick's original observation of catalysis by acetate of the hydrolysis of acetic anhydride;[10] (*b*) the faster nucleophilic reaction is reversible, and thermodynamically unfavourable, as in the case of aspirin hydrolysis;[11] (*c*) the nucleophilic reaction is inhibited or impossible for structural reasons, as in the case of aryl 3-dimethylaminopropionates (1)[12] and salicylates (2).[13]

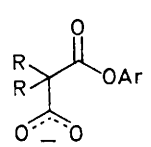


(1)

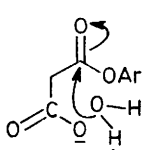


(2)

Of the systems known to exhibit intramolecular general base catalysis when this work was started, none allowed the type of structural variation we required, in which the distance and geometry of approach of catalytic and substrate groups are varied step by step over a wide range. So we set out to develop new systems for the purpose. The aryl 3-dimethylaminopropionates (1) looked likely candidates, allowing considerable possibilities for varying geometry by substitution at the two methylene groups. In fact these compounds did prove to be hydrolysed by the general base catalysis mechanism shown (1). But for reasons described in the previous paper[12] it proved unexpectedly difficult to distinguish general base from nucleophilic catalysis in these compounds. We therefore went on to examine the monoaryl malonate system (3), patterned on (2), which we expected to show intramolecular general base catalysis (4) for the same reason [(c) above] as in (1) and (2). In comparison with the dialkylaminopropionate system, the possibilities for structural variation are reduced to varying the geometry of the central carbon atom; but since the catalytic group is the more weakly basic carboxylate the reaction (4) should be characterised by a more substantial solvent deuterium isotope effect, which may be used as a convenient criterion of mechanism.



(3)



(4)

In this paper we show that the hydrolysis of substituted-phenyl malonate anions (3) does involve intramolecular general base catalysis[14] (4), and describe the effects on the efficiency of catalysis of varying the substituents R, and thus the angle between the catalytic and substrate groups.

EXPERIMENTAL

Inorganic chemicals, and simple alcohols used as buffer and solvent constituents were of analytical grade, and were used without further purification. Fison's AR dioxan was purified by refluxing then distilling over sodium, and was stored over sodium wire.

Aryl Hydrogen Malonates.—These (except the 4-nitrophenyl ester) were prepared from the phenol and the half acid chloride of malonic acid[15] [m.p. 61—62° (decomp.) (lit., 65°)]. In each case the acid chloride (2.5 g) and the phenol (1 mol. equiv.) were refluxed together in dry benzene (50 ml) for 4—6 h. Then the benzene was evaporated off to leave an oily semisolid. This was decolourised if necessary (charcoal in ether) then recrystallised from dry benzene–light petroleum (b.p. 60—80 °C) (dry benzene for the 4-methoxyphenyl ester). The properties of these compounds are given in Table 1.

TABLE 1

Properties of aryl hydrogen malonates (3)

Compound				Found (%)			Required (%)		
	R_2	Ar	m.p. (°C)	C	H	N	C	H	N
(3a)	H_2	Ph	65—67	60.2	4.7		60.0	4.5	
(3b)	H_2	4-MeO·C_6H_4	89—91	57.3	4.65		57.1	4.75	
(3c)	H_2	4-MeC_6H_4	69.5—71.5	62.1	5.2		61.8	5.15	
(3d)	H_2	3-ClC_6H_4	73—74	50.2	3.45		50.2	3.25	
(3e)	H_2	3-$NO_2C_6H_4$	90—92	47.7	3.15	6.25	47.6	3.1	6.15
(3f)	H_2	4-$NO_2C_6H_4$	92.5—93.5	47.7	3.25	6.3	47.6	3.1	6.15
(3g)	Me_2	Ph	95.5—97.5	63.2	5.95		63.4	5.8	
(3h)	Me_2	4-$NO_2C_6H_4$	107.5—110	52.3	4.45	5.6	52.2	4.35	5.53
(3j)	Et_2	4-$NO_2C_6H_4$		55.6	5.25	5.25	55.5	5.35	5.0
(3m)	CH_2·CH_2	Ph	66.5—68.5	64.0	4.85		64.1	4.85	
(3n)	Me_2C=	4-$NO_2C_6H_4$	134—136	54.2	4.35	5.1	54.3	4.15	5.3

4-Nitrophenyl hydrogen malonate.—Finely powdered sodium 4-nitrophenolate (5.7 g) was added slowly to a stirred solution of malonyl chloride (b.p. 43—45° at 10 mmHg; 5 g) in dry ether (40 ml). After this the solution was filtered and evaporated to leave a buff-coloured solid. This solid (3 g) was stirred with water (75 ml). 1M-Sodium hydrogen carbonate solution was added to bring the pH up to 7, and the undissolved solids were filtered off. The filtrate was extracted with ether, then acidified (2N-HCl). The acidified solution was extracted with ether, and the extracts were

[10] M. Kilpatrick, *J. Amer. Chem. Soc.*, 1928, **50**, 2891.
[11] A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, 1967, **89**, 4857.
[12] A. J. Kirby and G. J. Lloyd, preceding paper.
[13] M. L. Bender, F. J. Kezdy, and B. Zerner, *J. Amer. Chem. Soc.*, 1963, **85**, 3017.
[14] Preliminary communication, A. J. Kirby and G. J. Lloyd, *Chem. Comm.*, 1971, 1538.
[15] H. Staudinger and E. Ott, *Chem. Ber.*, 1921, **41**, 2208.

dried and evaporated to give 4-nitrophenyl hydrogen malonate; this formed orange plates (see Table 1), m.p. 92.5—93.5° (from dry benzene) (lit.,[16] 92.5—94°).

Phenyl Hydrogen Dimethylmalonate.—Dimethylmalonyl chloride (b.p. 46.5—49° at 10 mmHg) was prepared in the same way as malonyl chloride. To a stirred solution of the chloride (4 g) in dry benzene (40 ml) was added sodium phenolate (2.74 g) in portions. After 12 h stirring at room temperature the benzene was evaporated off. The resulting white solid was dissolved in ether (30 ml) and shaken vigorously for 30 min with an equal volume of water, then with aqueous $NaHCO_3$ (25 ml). The resulting aqueous layer (pH 7) was acidified (HCl) and extracted with ether to give phenyl hydrogen dimethylmalonate (Table 1).

4-Nitrophenyl Hydrogen Dimethylmalonate.—A solution of dimethylmalonyl chloride (6.3 g), 4-nitrophenol (5.18 g), and dry pyridine (2.95 g) in dry benzene (50 ml) was refluxed for 6 h, cooled, filtered, and evaporated to leave an orange solid. This was taken up in ether (25 ml); the solution was filtered, and then treated as already described for the isolation of the phenyl ester. The 4-nitrophenyl hydrogen dimethylmalonate produced was recrystallised from benzene–light petroleum (Table 1).

4-Nitrophenyl Hydrogen Diethylmalonate.—Diethylmalonic acid was converted into the bis-acid chloride (b.p. 85° at 25 mmHg) by the method of Speck.[17] The ester was prepared by adding 4-nitrophenol (706 mg) in dry pyridine (5 ml) to the acid chloride (1 g). The mixture was stirred for 2 h at room temperature, then water (91.3 μl, 1 equiv.) was added from a microsyringe. Stirring was continued for a further 8 h, by which time the i.r. band of the acid chloride near 1 790 cm^{-1} had disappeared. The mixture was then acidified with 30 ml of 3M-HCl (an oil separated) and extracted with ether. The ether layer was shaken with aqueous $NaHCO_3$, and the resulting aqueous layer acidified (HCl) and itself extracted with ether. After drying and evaporation this gave 4-nitrophenyl hydrogen diethylmalonate, which was recrystallised from benzene–light petroleum (Table 1).

Phenyl Hydrogen Cyclopropane-1,1-dicarboxylate.—Cyclopropane-1,1-dicarboxylic acid (1 g) was stirred with trifluoracetic anhydride (1.62 g) for 1 h at room temperature in a tube protected from atmospheric moisture. Reagent grade phenol (0.724 g) was added to the resulting clear solution, and stirring continued for a further 24 h. The resulting liquid was evaporated (oil-pump) to leave a solid. This was taken up in aqueous sodium hydrogen carbonate, and purified as described for the other half esters (above). The solid phenyl half ester was recrystallised from benzene–light petroleum (see Table 1).

4-Nitrophenyl Hydrogen Isopropylidenemalonate.—The bis-acid chloride was prepared from isopropylidenemalonic acid (6 g) and thionyl chloride (5.0 g), refluxed for 2 h in dry ether (20 ml) with dimethyl formamide (3 drops). The solution was evaporated and 4-nitrophenol (5.58 g) in dry benzene (25 ml) was added to the residual oil. The mixture was refluxed for 2½ h and cooled, whereupon crystals separated. These were filtered off, dissolved in aqueous sodium hydrogen carbonate, and purified as for the other half esters. The orange product which crystallised on acidification of the final solution was washed with water, dried, and found to be analytically pure.

All the aryl hydrogen malonates prepared had strong i.r. bands between 1 780 and 1 750 and between 1 725 and 1 680 cm^{-1}, due to the different C=O stretching vibrations, and the expected n.m.r. spectra.

4-Nitrophenyl Hydrogen Cyclopent-1-ene-1,2-dicarboxylate, (5).—This was prepared from the anhydride;[3] the orange solid *product* had m.p. 125—127.5° (from benzene–light petroleum) (Found: C, 56.2; H, 4.2; N, 4.95. $C_{13}H_{11}NO_6$ requires C, 56.3; H, 3.95; N, 5.05%).

TABLE 2

Hydrolysis data for aryl hydrogen malonates (3) at 39.6 °C and ionic strength 1.0

Compd.	Followed at (λ/nm)	Method	pK_{app}	k_H/l mol^{-1} min^{-1}	k_0/min^{-1}	k_n/min^{-1}	k_{OH}/ l mol^{-1} min^{-1}
(3a)	270, 288 [a]	*b*	3.15	3.99×10^{-3}	2.85×10^{-4}	8.6×10^{-4}	145
(3b)	280	*b*	3.15	4.37×10^{-3}	2.35×10^{-4}	6.7×10^{-4}	80.5
(3c)	291	*b*	3.01	4.3×10^{-3}	2.35×10^{-4}	6.6×10^{-4}	97.4
(3d)	281, 293 [a]	*b*		4.08×10^{-3}	7.0×10^{-4}	1.95×10^{-3}	1 265
(3e)	340, 395 [a]	*c*	3.18	5.31×10^{-3}	1.39×10^{-3}	5.6×10^{-3}	2 520
(3f)	325, 400 [a]	*c*	3.15	4.65×10^{-3}	2.3×10^{-3}	1.4×10^{-2}	3 810
(3g)	270, 288 [a]	*b*	3.11	1.97×10^{-4}	1.54×10^{-4}	4.0×10^{-4}	0.71
(3h)	325, 400 [a]	*b*	3.01		8.5×10^{-4}	5.7×10^{-3}	9.6
(3j)	325, 400 [a]	*b*	3.2			1.74×10^{-4}	0.19
(3m)	270, 288 [a]	*b*	3.2	8.6×10^{-4}	3.8×10^{-4}	2.09×10^{-3}	4.0
(3n)	325, 400 [a]	*b*				1.6×10^{-5}	3.8

[a] At pH values below and above the pK_a of the phenol, respectively. [b] Followed for at least three half-lives. [c] Followed by initial rates.

Kinetic Methods.—Rate constants for hydrolysis were measured under first-order conditions by following the release of the phenol, or phenolate ion, at or near a maximum in its u.v. spectrum (see Table 2), in the thermostatted cell compartment of a Zeiss PMQII spectrophotometer. Temperature was maintained, usually at 39.6°, by circulating water, and the ionic strength of all solutions was adjusted to 1.0M (KCl). Reactions were initiated by injecting a solution of the ester in dioxan (20—40 μl) into the buffer solution at the temperature of the run. The measured rate constants were independent of the size of the sample injected. Reactions were followed for at least three half-lives, and end points taken after at least ten, for the more reactive esters (see Table 2). Slower reactions were followed by the initial rate method, and end points measured by using aliquot portions of the same stock solution, hydrolysed in M-NaOH, neutralised, and diluted with the buffer solution used in the relevant run. Several of the slower reactions were measured by both methods: the rate constants obtained were independent of the method. The pH of the solution was measured at the end of every run, with an E.I.L. Vibron electrometer

[16] T. Nakata, *Nippon Kagaku Zasshi*, 1957, **78**, 1780 (*Chem. Abs.*, 1960, **54**, 1506b).
[17] S. B. Speck, *J. Amer. Chem. Soc.*, 1952, **74**, 2876.

with C33B pH-measuring attachment and Pye-Ingold EO2, 40NS glass electrode, at the temperature of the reaction. Hydroxide ion concentrations below 0.01M were calculated from the measured pH by using a value of 13.32 for pK_w for water at 40 °C and ionic strength 1.0,[18] and an activity coefficient of 0.68.

The rate constants quoted represent extrapolations to zero buffer concentration, where appropriate. Buffer catalysis is a simple second-order reaction for most of the esters used, but the mononitrophenyl hydrogen malonates showed complex buffer catalysis, which is the subject of the following paper.[19]

TABLE 3

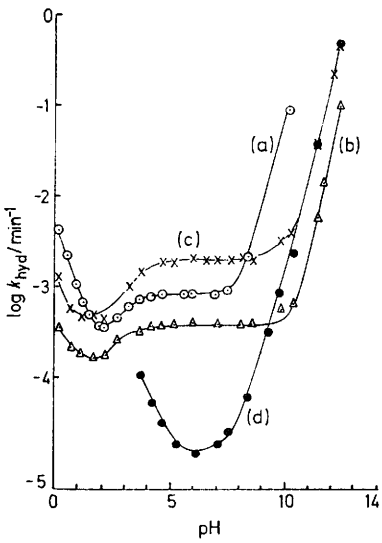
Comparison of data for the hydrolysis of the anions of four ester acids, at ionic strength 1.0

	Aspirin [a]	(3a)	(3f)	(5)
k_n (39.6 °C)	6.65×10^{-4}	8.6×10^{-4}	1.4×10^{-2}	
k_n (T/°C)		1.95×10^{-4} (25.1)	5.92×10^{-3} (31.2)	5.74×10^{-2} (20.6)
		4.29×10^{-4} (32.3)	3.15×10^{-2} (47.6)	1.47×10^{-1} (27.3)
		1.58×10^{-3} (45.9)	7.22×10^{-2} (55.5)	3.23×10^{-1} (33.8)
$\Delta H^{\ddagger}$/kcal mol^{-1}	18.4	17.9	20.4	22.7
$\Delta S^{\ddagger}$/cal mol^{-1} K^{-1}	22.5	−23.7	−10.1	5.1
k_H/k_D (39.6 °C)	2.2	2.28	1.54	1.09
ρ	0.96 ± 0.04 [b]	0.97 ± 0.05		
k_n (rel) [c] MeOH	10.1	5.05		
k_n (rel) [c] EtOH	3.5	1.63		
k_n (rel) [c] Pr^{i}OH	2.3	0.71		
k_n (rel) [c] Bu^{t}OH	0.84	0.59		

[a] Data at 39.0 °C, from ref. 11, for the aspirin anion. [b] For the leaving group. [c] k_n at 39.6 °C in 0.05M-phosphate buffer (pH 6.73 in water), ionic strength 0.1, in 50% v/v aqueous alcohol, relative to k_n in water under the same conditions.

RESULTS

The pH-rate profile for the hydrolysis of phenyl hydrogen malonate appears in our preliminary communication.[14]



pH–Rate profiles for hydrolysis of (a) phenyl hydrogen malonate (3a), (b) phenyl hydrogen dimethylmalonate (3g), (c) phenyl hydrogen cyclopropane-1,1-dicarboxylate (3m), and (d) 4-nitrophenyl hydrogen isopropylidene-malonate (3n), all at 39.6 °C and ionic strength 1.0

Profiles for the 4-nitrophenyl esters used in this work are shown in the Figure. In the typical case it is possible to identify four separate reactions: the acid-catalysed hydrolysis of the acid form and the alkaline hydrolysis of the anion, at low and high pH, respectively; and pH-independent reactions of the two forms at intermediate pH values. The pH–rate profiles appear to follow the ionisation curves of the carboxy-groups, since the kinetic pK_a values lie between 3.1 and 3.2, as expected for substituted malonic acids. The four rate constants and the apparent pK_a required to describe the rate of hydrolysis as a function of pH for each ester are given in Table 2; k_o is the first-order rate constant for the hydrolysis of the aryl hydrogen malonate, and k_n the corresponding constant for hydrolysis of the ester anion. In all cases where it could be measured k_n was greater than k_o, although simple electronic effects must act to make the anion less electrophilic than the acid ester. This is the first evidence that the ionised carboxy-group catalyses the hydrolysis of the ester. Apart from k_n, the other rate constants have values close to those expected for unassisted hydrolysis: k_H and k_o for 4-nitrophenyl hydrogen malonate, for example, are almost identical with the corresponding rate constants measured by Pratt and Bruice [20] for the hydrolysis of ethyl 4-nitrophenyl malonate. The reaction of interest for this work is that represented by k_n, and more data were collected for three esters in the pH-independent region above pH 4 (Table 3). 4-Nitrophenyl hydrogen diethylmalonate and isopropylidenemalonate were both unreactive enough to require the initial rate method, and too insoluble for this to be practicable, so measurements could not be made on these esters below pH 3—4.

The rate constants k_n for the pH-independent hydrolysis reactions of the six substituted-phenyl hydrogen malonates are correlated by the Hammett equation (correlation coefficient 0.993), using σ^- for the *p*-nitro group, with Hammett's $\rho = 0.92 \pm 0.06$. This rate constant also shows a significant solvent deuterium isotope effect for the malonate esters, and a large negative entropy of activation.

The products of hydrolysis of 4-nitrophenyl malonate catalysed by *N*-methylmorpholine were isolated in one experiment, and shown by proton n.m.r. in [$^2H_6$]acetone to consist of an equimolar mixture of malonic acid and 4-nitrophenol.

DISCUSSION

Mechanism.—The pH–rate profiles for hydrolysis of all the substituted-phenyl hydrogen malonates we have

[18] Calculated from data of H. S. Harned and B. B. Owen, 'The Physical Chemistry of Electrolytic Solutions,' Reinhold, New York, 1973, pp. 485, 578.

[19] A. J. Kirby and G. J. Lloyd, following paper.

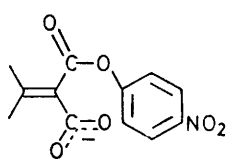
[20] R. F. Pratt and T. C. Bruice, *J. Amer. Chem. Soc.*, 1970, **92**, 5956.

studied, with the single exception of the isopropylidene derivative (3n), show four distinct reactions (Figure): the acid-catalysed and spontaneous hydrolysis (h_H and k_o) of the ester acids, and the alkaline and spontaneous hydrolysis of the ester anions (k_{OH}, k_n). Three of these reactions are normal for aryl esters of this sort (see above). The spontaneous hydrolysis of the anions, however, is substantially faster than expected. The ester anions must be significantly less electrophilic than the ester acids (k_{OH} for the hydrolysis of diethyl malonate is 87 times greater [21] than for the monoethyl ester anion at 35 °C), yet in every case where we could measure it k_n is several times greater than k_o. In comparison with phenyl acetate the rate of spontaneous hydrolysis of phenyl malonate anion is enhanced by a factor of over 150.

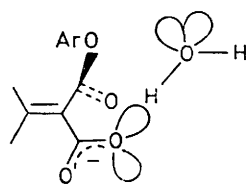
The absolute rate, and thus the rate enhancement also, is similar to that observed [11] for the hydrolysis of the aspirin anion. Nor do the similarities end here. In Table 3 all the kinetic parameters we have measured for the hydrolysis of the anion of phenyl malonate (3a) are compared with the corresponding values for the hydrolysis of aspirin anion. Not only the rate enhancement, but also the thermodynamic parameters, and the solvent deuterium isotope effect, are almost identical for the two compounds. And Hammett's ρ for the pH-independent reaction (based on k_n values, and the same σ values as used for aspirin [22]) also has the same value as ρ_{phenol} for aspirin hydrolysis. Intramolecular *nucleophilic* catalysis by the carboxylate group is characterised by a much higher sensitivity to the leaving group. For the hydrolysis of substituted-phenyl succinates and glutarates,[23] $\rho = 2.6$, a value which suggests that the breakdown of the tetrahedral intermediate is rate-determining. The low ρ value found for aryl malonate anions is consistent with a mechanism involving rate-determining formation of a tetrahedral intermediate. For reasons discussed in the preceding paper [12] we can rule out the possibility that the rate-determining step in intramolecular nucleophilic catalysis might change to formation of the tetrahedral intermediate because of the strain involved in the formation of a four-membered ring. Finally, solvolysis is faster in 50% aqueous methanol and ethanol, reflecting the higher nucleophilicity of the lower alcohols in comparison with water, as previously observed in the solvolysis of aspirin.[11]

The mechanism of hydrolysis of the aspirin anion is firmly established as intramolecular general base catalysis, largely on the basis of the data quoted in Table 3, which are considered typical for this mechanism.[11] The evidence is therefore strong that the very similar reaction of phenyl malonate anion proceeds by this mechanism (4) also. The good linear free energy relationship is evidence that the mechanism does not change over the series of substituted-phenyl hydrogen malonates (3a—f), even though k_H/k_D falls to 1.54 for the 4-nitrophenyl compound. We also measured the solvent deuterium isotope effect for the anion of 4-nitrophenyl diethylmalonate (3j), the compound we considered most likely to show the nucleophilic mechanism. The value observed (k_H/k_D = 1.7) is consistent with intramolecular general base catalysis in this case also. We believe that this mechanism accounts for the hydrolysis of the anions of all the simple malonic acid derivatives we have measured.

Just two compounds behave differently. The isopropylidene derivative (3n) does not have a detectable pH-independent reaction of the anion (k_n) significantly greater than k_o, and thus intramolecular general base catalysis is probably not operating in this case. This is simply explained on stereoelectronic grounds. In the highly substituted structure (3n) at least one and possibly both carboxy-groups will be rotated out of plane to reduce non-bonded interactions. This will be less likely for the ester group because it would interrupt significant delocalisation, but seems certain for the ionised carboxy-group. Since the lone-pair electrons of the oxygen atoms, through which it acts as a general base, are in the plane of the carboxylate group, the optimum geometry for intramolecular general base catalysis will be close to the conformation where these are in the plane of the ethylene system, and the *ester* carboxy-group is rotated out of plane, as in (6). If the system cannot attain the required geometry without undue strain, it is reasonable that the mechanism should be excluded.

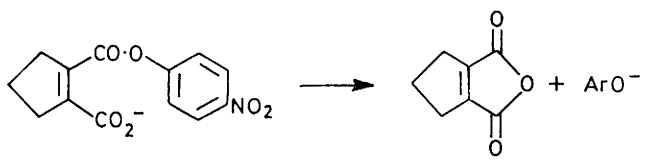


(3n)



(6)

The second case is the cyclopent-1-ene-1,2-dicarboxylate (5). This system forms the strained anhydride (7) only with difficulty, and we thought that this might be sufficient to tip the balance towards general base catalysis. The kinetic parameters observed for this compound (Table 3), however, are quite different from those found for malonate monoesters, and are entirely consistent with intramolecular nucleophilic catalysis. The greater efficiency of catalysis in this system therefore reflects



(5) (7)

no more than the change of mechanism. Strain in the intermediate has reduced the efficiency of the nucleophilic mechanism [the effective concentration of the

[21] P. S. Rhadakrishnamurti and P. C. Patro, *Tetrahedron*, 1970, **24**, 5503.

[22] A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, 1967, **89**, 4853.

[23] E. Gaetjens and H. Morawetz, *J. Amer. Chem. Soc.*, 1960, **82**, 5328.

carboxylate group in the hydrolysis of (5) is about 1 100M, much less than observed for the flexible succinate system; see discussion below], but it is still evidently at least ten times more efficient than general base catalysis.

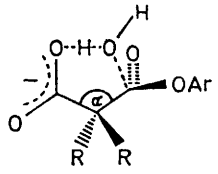
TABLE 4

Dependence on structure of efficiency of intramolecular general base catalysis in the hydrolysis of aryl malonates

Compd.	k_n(rel)	k_{OH}(rel)
(3a)	1.0	1.0
(3g)	0.46	4.9×10^{-3}
(3h)	0.41	2.5×10^{-3}
(3j)	1.25×10^{-2}	5.0×10^{-5}
(3m)	2.4	2.8×10^{-2}
(3n)	1.1×10^{-3}	1.0×10^{-3}

Structure and Efficiency in Intramolecular General Base Catalysis.—In Table 4 we compare reactivity in intramolecular general base catalysis for the five systems we have studied, with relative rates of alkaline hydrolysis for the same compounds. Both reactions involve the rate-determining addition of hydroxide ion to the carbonyl group, and have closely similar electronic requirements. It is apparent that the two reactions depend quite differently on structure. The hydroxide reaction shows normal sensitivity to steric effects,[24] with chain branching at the α-carbon atom sharply decreasing the rate. Intramolecular general base catalysis, on the other hand, shows little if any sensitivity to this factor: only in the case of the isopropylidene derivative (3n) are the relative values of k_n and k_{OH} similar, as expected if both reactions involve external attack for this compound (see above). For the other esters the effects are quite different, and in the case of the cyclopropane derivative (3m) actually in the opposite direction. The only way that alkyl substituents are likely to affect the transition state (8) for the intramolecular reaction, other than by affecting the angle α, is by interfering with the free rotation of the carboxy-groups. Models show that this is only likely to be significant with the diethyl compound (3j), and even here large effects on reactivity are not expected.

The results in Table 4 refer in some cases to phenyl esters, and in some cases to 4-nitrophenyl esters. 4-Nitrophenyl malonate shows anomalous reactions with strong neutral bases, but not with hydroxide ion;[19] and the similarity of the relative values for both k_n and k_{OH} found for phenyl and 4-nitrophenyl dimethylmalonate (3g and h) confirms that the figures given in Table 4 are characteristic of the structure of the malonic acid, and depend little on the leaving group. We therefore consider that in the four systems (9)—(12) (Table 5), the major factor affecting reactivity is the geometry of the malonic acid, and in particular the angle α in the ground state leading to the transition state (8). (The values of α quoted are those obtained for the parent malonic acids by *X*-ray structure determinations; the differences for the esters in solution should not be significant for the purposes of this work.) The most remarkable feature of these results is the very small dependence of reactivity on

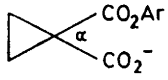
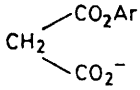
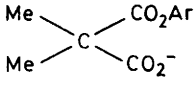
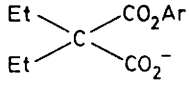
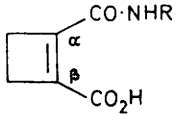
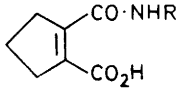
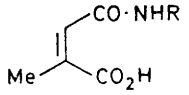
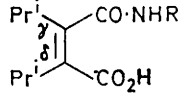


(8)

geometry. For comparison we include similar data for a reaction involving intramolecular nucleophilic catalysis,

TABLE 5

Structure and reactivity in intramolecular catalysis by the carboxy-group

	System	Angle * (α, β)	Effective concentration
(9)		118.4°	60M
(10)		110°	25M
(11)		106.2°	11M
(12)			0.3M
(13)		(132.1, 133.4°) †	>10M ‡
(14)		127.7,[e] 131.5°	$>10^2$M ‡
(15)		126.8, 131.7°	$>10^7$M ‡
(16)		121.0[g] 121.7°	$>10^{10}$M ‡

* Angle between the carboxy-groups, from *X*-ray structures of malonic acid,[a] dimethylmalonic acid,[b] and cyclopropane-1,1-dicarboxylic acid.[c] † Angles found for the diacid.[d] ‡ Minimum values, based on our estimated maximum rate constant for the intermolecular reaction, which is not detectable.

[a] J. A. Goedkoop and C. H. MacGillavry, *Acta Cryst.*, 1957, **10**, 125. [b] D. J. Haas and S. A. Brenner, *Acta Cryst.*, 1966, **20**, 709. [c] M. A. M. Mester, H. Scheuk, and C. H. MacGillavry, *Acta Cryst.*, 1971, **27**, 630. [d] D. Bellus, H.-C. Mez, and G. Rihs, *J.C.S. Perkin II*, 1974, 844. [e] Ref. 27. [f] F. H. Allen and O. Kennard, *Cryst. Struct. Comm.*, 1973, 145. [g] Ref. 31.

[24] A. J. Kirby in 'Comprehensive Chemical Kinetics,' eds. C. H. Bamford and C. F. H. Tipper, Elsevier, Amsterdam, 1972, vol. 10, p. 57.

taken from our recent work,[3] with *X*-ray structural parameters measured for the actual compounds used. The changes in geometry cover a larger range for the maleamic acids, twice 11—12° of angle, compared with a change of just over 12° for the malonates, but are of the same order of magnitude. A trend is clear in each case: for intramolecular general base catalysis efficiency increases as the angle α increases, but by a factor of less than 10 over the full range [α may be a little smaller still for the diethylmalonate system (12), but the efficiency of catalysis is probably also diminished by a steric effect, as discussed above]. For the nucleophilic reaction catalytic efficiency decreases as the angles α and β increase, by a factor of over 10^9 over the range of structural variation possible.

Clearly quite different factors operate to control catalytic efficiency in the two systems. Since the geometrical changes are of the same order of magnitude in each case, it is evident that the consequent changes in orbital alignment [25] cannot be responsible for the very large range of catalytic efficiency observed in the nucleophilic reaction. One crucial difference * between the two systems is that the transition state for the general base catalysis mechanism is essentially strainless, because the cycle (8) is made up from two separate molecules, while in the cyclisation reactions the transition state (which involves the breakdown of the tetrahedral intermediate, and thus has the ring fully formed [3]) is only reached after large deformations of bond angle, associated with the build-up or release of substantial amounts of strain. For example, the very high reactivity of the di-isopropylmaleamic acid system (16) is associated with an important decrease in non-bonded interactions among the four groups on the crowded tetrasubstituted ethylene: the two reacting groups move closer together to form a five-membered ring, allowing the two alkyl groups on the other side of the double bond more space, as the relevant bond angles (γ and δ) open up from about 123° [26] to the value of about 130° found for groups on a cyclopentene double bond.[27] In the cyclopentenyl and cyclobutenyl derivatives (13) and (14) all these adjustments are prevented, or severely inhibited, and reactivity falls dramatically.

It remains to demonstrate that intramolecular general base catalysis in the hydrolysis of aryl malonate anions is a typical reaction involving this mechanism. In Table 6 we compare the effective concentrations of intramolecular general base found in the present work with values taken or calculated from the literature for other reactions thought to involve this mechanism. The data show clearly that the range of catalytic efficiency obtained by varying the geometry of the monoaryl malonate system is essentially the same as that available from varying the system. The limit of efficiency of intramolecular general

TABLE 6

Efficiency of intramolecular general base catalysis

Reaction	Effective concentration of catalytic group
Hydrolysis	
of phenyl cyclopropane-1,1-dicarboxylate (9) [a]	60M
of phenyl malonate (10) [a]	25M
of *p*-nitrophenyl diethylmalonate (12) [a]	0.3M
of aspirin	13M
of *p*-nitrophenyl 5-nitrosalicylate [b]	6M
of phenyl 3-dimethylaminopropionate [c]	20M
of *p*-nitrophenyl *o*-dimethylaminobenzoate [c]	0.25M
of 2-(imidazol-4-yl)phenyl acetate [d]	15M
of phenyl quinoline-8-carboxylate [e]	>100M †
Aminolysis	
of acetylimidazole, by ethylenediamine [f]	0.55M
of acetylimidazole, by 1,3-diaminopropane [f]	0.94M
of methyl formate, by 1,3-diaminopropane [f]	0.6M
of methyl formate, by ethylenediamine [f]	0.5M
Enolisation	
of *o*-carboxyacetophenone [g]	5M
of *o*-carboxybutyrophenone [h]	56M
of 4-diethylaminobutan-2-one [i]	0.45M
of 5-diethylaminopentan-2-one [i]	0.10M

[a] This work. [b] Ref. 12. [c] Ref. 11. [d] S. M. Felton and T. C. Bruice, *J. Amer. Chem. Soc.*, 1969, **91**, 6721. [e] P. Y. Bruice and T. C. Bruice, *J. Amer. Chem. Soc.*, 1974, **96**, 5523. [f] Ref. 9. [g] R. P. Bell, B. G. Cox, and J. B. Henshall, *J.C.S. Perkin II*, 1972, 1232. [h] E. T. Harper and M. L. Bender, *J. Amer. Chem. Soc.*, 1965, **87**, 5625. [i] R. P. Bell and B. A. Timimi, *J.C.S. Perkin II*, 1973, 1519.

† Based on our very large extrapolation of a two-point Bronsted plot to the measured pK_α of the quinoline N, which appears to be particularly efficient also in the hydrolysis of 8-acetoxyquinoline.

* We are concerned here specifically with factors affecting the *range* of catalytic efficiency observed in intramolecular general base *vis à vis* nucleophilic catalysis. We discuss below a second crucial difference between the two mechanisms, which gives rise to the much greater *absolute* efficiencies observed for nucleophilic catalysis even in unstrained systems.

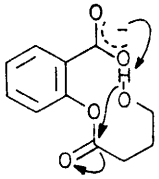
[25] D. R. Storm and D. E. Koshland, *Proc. Nat. Acad. Sci. U.S.A.*, 1970, **66**, 445; A. Dafforn and D. E. Koshland, *ibid.*, 1971, **68**, 2463.

[26] P. J. Roberts and O. Kennard, *Cryst. Struct. Comm.*, 1973, 153.

[27] F. H. Allen and O. Kennard, *Cryst. Struct. Comm.*, 1973, 149.

base catalysis appears to be about 100M, and there is no evidence that this depends significantly on the type of reaction.

We conclude that general base catalysis, though probably the commonest part-mechanism in enzymic reactions, cannot be a primary source of the enormous rate accelerations observed. While there is no doubt that proton transfers involving active site general bases are an essential part of the catalysis process, the evidence is that their contribution to high catalytic efficiency is only a supporting one. This contribution takes the form of a modest enhancement of the rate of the main reaction, whatever its intrinsic reactivity. For example, we have shown recently [28] that the effective molarity of the carboxylate group in the lactonisation reaction (17) is the same as in aspirin hydrolysis (18), though the reaction is



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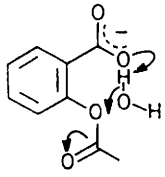


(18)

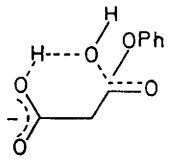
faster by some two orders of magnitude. The high reactivity of the system (17) is determined by the ease of the cyclisation process, leading to γ-butyrolactone. It is well known that this process can be made very fast indeed by appropriate structural modification: [25] but the evidence suggests that there is no prospect of increasing the contribution to catalytic efficiency of the general base significantly above the level observed for (17).

General Conclusions.—There is now a good deal of evidence that very high rate accelerations in intramolecular, and presumably therefore in enzymic reactions also, are associated specifically with the process of bond formation or cleavage between heavy atom centres (C, N, O, P, S, *etc.*). The most efficient intramolecular reactions involve the formation of five- or six-membered rings.[3-5,25] Reactivity in these systems can be controlled by systematic structural variation, and the highest rate accelerations, associated with decreases in activation enthalpy,[3,4] are found with molecules having strained ground states,[29] as revealed by deformations of bond length [30] and angle.[26,30] But very substantial rate accelerations are possible even in unstrained systems, where reactivity depends largely on conformational flexibility, and is therefore controlled by the entropy of activation.[31] Effective concentrations of the carboxy-group of the order of 10^6M are found in intramolecular reactions of open-chain monoaryl succinates and glutarates,[32,33] where ground-state strain cannot be a factor. From these and similar experimental results, and to some extent on theoretical grounds also,[34] it appears that the limit of efficiency of intramolecular nucleophilic catalysis, in the absence of complications due to strain is in the region 10^6—10^8M.

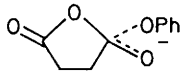
We have shown that the corresponding limit for intramolecular general base catalysis is about 100M, and it is of great interest to identify the factors responsible for the disparity—perhaps as large as 10^6-fold—between the efficiencies of the two mechanisms. The effective concentrations quoted are the ratios of the first-order rate constants for the intramolecular reaction and the second-order rate constants for the corresponding intermolecular process. In the case of general base catalysis of hydrolysis the rate constants represent termolecular and bimolecular processes, but the effective concentration as calculated has the same significance as for nucleophilic catalysis, where the order and molecularity are the same. This factor, then, is not the source of an artificial difference between the two types of mechanism.

Page and Jencks [34] reckon that losses of translational and rotational entropy of up to 40—50 cal K^{-1} mol^{-1} are to be expected for the formation of the transition state for many bimolecular reactions. The size of this factor for a given reaction depends on the residual entropy of the transition state, associated with a variable number of low frequency vibrations. The greater this residual entropy—that is, the looser the transition state—the greater (more favourable) is the entropy of activation for the bimolecular process, and thus the smaller is the advantage of the corresponding intramolecular reaction.

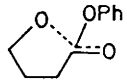
In formulae (19) and (20) we compare the transition state for one of the reactions described in this paper, involving classical general base catalysis of hydrolysis, with the transition state for nucleophilic catalysis of hydrolysis, involving the same functional groups, in a system (phenyl succinate) which shows a typically high effective concentration of the catalytic group. In the transition state (20) for nucleophilic catalysis only one



(19)



(20)



(21)

σ-band is being broken.* It is clear that the transition state (19) for the general base catalysed reaction is substantially looser, with no less than three covalent bonds being made or broken simultaneously. The proton transfer part of the transition state is expected to involve particularly low frequency vibrations, as discussed by

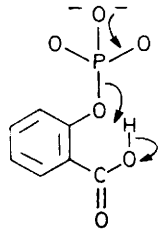
* Jencks has recently made this point in an extensive review [35] of these matters.

[28] A. J. Kirby and G. J. Lloyd, *J.C.S. Perkin II*, 1974, 637.
[29] T. C. Bruice, *Ann. Rev. Biochem.*, 1976, **45**, 331.
[30] J. M. Karle and I. L. Karle, *J. Amer. Chem. Soc.*, 1972, **94**, 9182.
[31] T. C. Bruice and S. J. Benkovic, ref. 2, p. 178.
[32] T. C. Bruice and U. K. Pandit, *Proc. Nat. Acad. Sci. U.S.A.*, 1960, **46**, 402.
[33] T. C. Bruice and W. C. Bradbury, *J. Amer. Chem. Soc.*, 1965, **87**, 4846; 1971, **90**, 3808.
[34] M. I. Page and W. P. Jencks, *Proc. Nat. Acad. Sci. U.S.A.* 1971, **68**, 1678; M. I. Page, *Chem. Soc. Rev.*, 1973, **2**, 295.
[35] W. P. Jencks, *Adv. Enzymol.*, 1975, 219, especially p. 278.

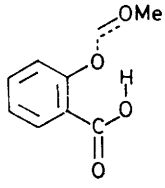
Bell and his co-workers;[36] and intramolecular general base catalysed proton transfer processes, such as those studied by Bell,[36] and by Page and Jencks,[9] do show characteristically low effective molarities of general base. This is probably the dominant factor; but there is also a little evidence that the effective molarity of an intramolecular nucleophile may be lower than the 10^5—10^6M observed for unstrained systems when the *formation* of the tetrahedral intermediate is securely rate-determining: as it is in general base catalysed hydrolysis. Thus the effective molarity of the alkoxide group in the specific base catalysed lactonisation of phenyl 4-hydroxybutyrate appears to be only a few thousand,[37] consistent with a transition state (21) significantly looser than (20).

The larger number of low frequency vibrations associated with transition states [such as (19)] for general base catalysis must give rise to increased residual entropies of such transition states, and thus increased (more favourable) entropies of activation for bimolecular reactions involving this mechanism. This effect should reduce the advantage of the corresponding intramolecular reactions substantially, and could be large enough to account for the difference, amounting to 20—25 cal K^{-1} mol^{-1}, between the limits of efficiency of intramolecular nucleophilic and general base catalysis.

If this simple picture is accepted, some interesting conclusions follow. General acid catalysis is mechanistically simply the microscopic reverse of general base catalysis. Since the same transition states are involved, the limits of efficiency of intramolecular general acid catalysis should be similar to those for the general base catalysis mechanism. And low effective molarities are indeed observed for some reactions involving intramolecular general acid catalysis. In a number of much studied reactions, however, involving derivatives of salicylic acid, effective molarities of the carboxy-group as high as 10^3—10^4M are observed.[38,39] We have already suggested,[38] on quite different grounds, that these reactions do not involve fully concerted general acid catalysis, with three bonds being made or broken simultaneously. In our picture of the transition states for the hydrolysis of salicyl phosphate (22)[8] and 2-methoxymethoxybenzoic acid (23)[38] the CO_2H group is immobilised, but the entropy-rich proton-transfer process is not yet substantially under way, and the reaction is dominated by the breaking of the bond to the leaving group. Under these circumstances the internal entropy associated with very early (or presumably, very late[39]) transition states for proton transfer, with the proton tightly bonded to one basic centre, could be significantly smaller than for more symmetrical transition states, with looser bonds to two. If this is true, then since this type of transition state is peculiar to the salicylate



(22)



(23)

system, so too may be the high effective molarities of general acid observed.

The entropy problem arises for any mechanism which involves an extended sequence of bond making and breaking, particularly if it involves proton transfers, and may account for the originally rather surprising difficulty in observing bifunctional general acid–base catalysis.[40] The conclusion for a mechanism such as Blow's original suggestion[41] for a charge-relay system in the chymotrypsin reaction, is that the overall efficiency of catalysis must be determined largely by factors controlling the approach of the active site nucleophile (serine oxygen) to the substrate carbonyl group. The assistance of the general base (histidine-57) probably contributes a modest further rate enhancement, but elaboration of the general base is not likely to make catalysis significantly more efficient. (Bruice[29] has come to similar conclusions, on slightly different grounds.)

Finally, it is instructive to consider the extreme case, of a mechanism with a transition state so ' loose ' that the reactants lose no translational or rotational degrees of freedom—a case which occurs, as Page[34] has pointed out, for diffusion-controlled reactions. Here the effective concentration of a catalytic group is indeed determined by the statistical factors considered in the early model of Koshland.[42]

We thank the Salters' Company for a Scholarship (to G. J. L.).

[6/592 *Received*, 29*th March*, 1976]

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