Intramolecular General Base Catalysis of Intramolecular Nucleophilic Catalysis of Ester Hydrolysis

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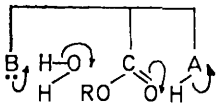
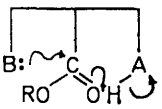
The hydrolysis of the anion of 2-carboxyphenyl 4-hydroxybutyrate at 39° is 85 times faster than that of aspirin anion, and some 500 times faster than the spontaneous hydrolysis of phenyl 4-hydroxybutyrate. The expected mechanism is concerted intramolecular general base–intramolecular nucleophilic catalysis, by the carboxylate and hydroxy-groups, respectively, and the expected products are salicylate and γ-butyrolactone. All the evidence is consistent with this mechanism, which constitutes the first clear-cut example of bifunctional catalysis of ester hydrolysis.

ALTHOUGH we do not yet understand in detail the catalytic mechanism of any enzyme, suggested mechanisms appear regularly in the literature. Frequently such mechanisms include steps which involve bifunctional catalysis; that is to say the concerted operation of two of the catalytic groups known or thought to be present at the active site of the enzyme. Now it may be that such suggestions are well founded; though in many cases the reactions concerted are complex, and involve several distinct steps which might be expected to require separate catalytic assistance. But we have no evidence on which to base an assessment, because such catalysis has never been identified even in a simple system. Maugh and Bruice [1] have reviewed the small number of cases in which the efficiency of intramolecular catalysis of ester hydrolysis has been shown to be enhanced by the introduction of a second functional group, and conclude that there is no evidence for concerted general acid–general base (1) or general acid–nucleophilic catalysis (2) of ester hydrolysis in water.



(1)



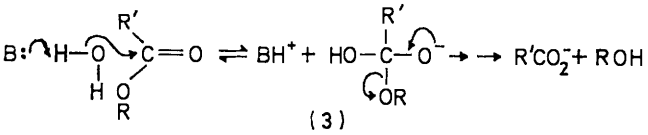
(2)

One reason for this is undoubtedly that general acid catalysis is not an important mechanism for ester hydrolysis, especially under conditions where the addition of the nucleophile is rate determining. Ester hydrolysis under mild conditions of temperature and pH normally involves a general base catalysis mechanism,[2] in which nucleophilic attack of water on the carbonyl group is assisted by a second molecule of solvent or other general base (3).

[1] T. Maugh and T. C. Bruice, *J. Amer. Chem. Soc.*, 1971, **93**, 3237.

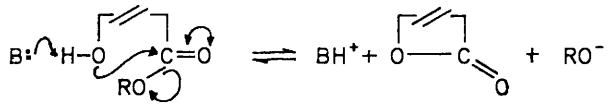
[2] A. J. Kirby in 'Comprehensive Chemical Kinetics,' eds. C. H. Bamford and C. P. H. Tipper, Elsevier, Amsterdam, 1972, vol. 10, p. 150.

The corresponding intramolecular reaction (4) has recently been reported by two groups. Capon *et al.*[3]



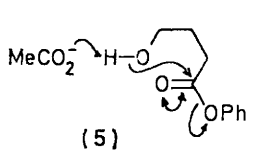
(3)

have studied aryl esters derived from several hydroxy-acids and find that lactone formation is rapid at high pH, where significant amounts of the alkoxide form are present, but occurs also near neutrality, where the



(4)

reaction is buffer catalysed. The likely mechanism is general base catalysis: for example, catalysis by acetate of the formation of γ-butyrolactone from phenyl 4-hydroxybutyrate would involve the mechanism shown in (5). A similar reaction studied by Fife and Benjamin[4] is the cyclisation to phthalide of ethyl 2-



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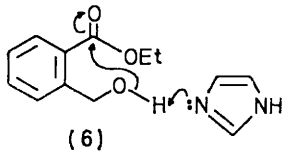
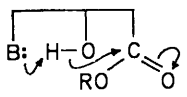


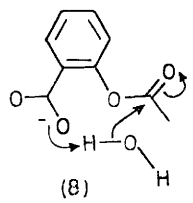
(6)

hydroxymethylbenzoate, which is catalysed by imidazole. These authors also suggest a general base catalysis mechanism (6).

These reactions are of particular interest because they are simple models for a mechanism generally believed to be involved in the action of the serine proteinases. In the hydrolysis of amides and esters catalysed by α-chymotrypsin, for example, the imidazole group of histidine-57 is thought to act as a general base to assist the attack of the OH group of serine-195 on the carbonyl group of the substrate.[5] We now report a further step in the elaboration of the model system, which involves the incorporation of the second catalytic group [the general base in (4)] into the substrate molecule, to give a system of the type shown in (7). If the mechanism



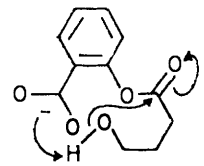
(7)



(8)

shown in (5) is correct, and a system can be devised in which the three functional groups can interact freely, we would expect to observe concerted intramolecular general base–nucleophilic catalysis of the acyl transfer reaction.

The hydrolysis of the aspirin anion[6] is a reaction in which the carboxylate group acts as a general base to assist the attack of a water molecule on an ester carbonyl group (8). In the lactonisation of phenyl 4-hydroxybutyrate, and the other esters studied by Capon[3] and by Fife,[4] the effective concentration of the substrate hydroxy-group is much greater than that of water, so that a combination of the structural features of (5) and (8) should provide a system with the required properties. The simplest molecules of this type are 2-carboxyphenyl 4- or 5-hydroxyalkanoates: the mechanism of hydrolysis of 2-carboxyphenyl 4-hydroxybutyrate, for example would be expected to be as shown in (9). We have prepared three such compounds [including (10; $n = 3$ or 4)], and find that their behaviour is exactly as predicted.



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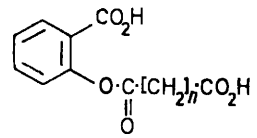


(10)

EXPERIMENTAL

Materials.—Inorganic salts were of analytical grade, and were used without further purification. Distilled water was distilled twice more from all-glass apparatus. The esters used were synthesised by conventional methods, using the benzyl group to protect the hydroxy- and carboxy-functions.

4-Benzyloxybutyric acid was prepared by the method of Eyre *et al.*[7] as modified by Raftery,[8] and had b.p. 130—135° at 0·1 mmHg (lit.,[8] 140—144° at 1 mmHg). 5-Benzyloxyvaleric acid was prepared according to Eyre,[7] and had b.p. 158—163° at 0·5 mmHg (lit.,[3] 171—175° at 1·5 mmHg). *4-Benzyloxyvaleric acid*, prepared by the same method, had b.p. 158—160° at 0·25 mmHg; δ ($CDCl_3$) 1·2 (3H, d), 1·85 (2H, m), 2·05 (2H, m), 3·55 (1H, m), 4·5 (2H, q), 7·28 (5H, m), and 11·55 (1H, s) (Found: C, 69·1; H, 7·45. $C_{12}H_{16}O_3$ requires C, 69·2; H, 7·1%).

2-Benzyloxycarbonylphenyl 4-Benzyloxybutyrate.—Benzyl salicylate and 4-benzyloxybutyric acid (0·03 mol each) were dissolved in AnalaR pyridine (10 ml) and the solution cooled to 0°. Thionyl chloride (1 equiv.) was added over 1 h with stirring, and the mixture was kept for a further 12 h at 0°. The products were poured into water (50 ml), and an oil separated: the aqueous layer was acidified with 10M-HCl and shaken well, and the oil was extracted into ether. Drying and evaporation gave a colourless oil. To remove unchanged benzyl salicylate the oil was chromatographed in ether–petroleum (b.p. 60—80°) (1 : 7) mixture

[3] B. Capon, S. T. McDowell, and W. V. Raftery, *J.C.S. Perkin II*, 1973, 1118.
[4] T. H. Fife and B. M. Benjamin, *J. Amer. Chem. Soc.*, 1973, **95**, 2060.
[5] K. Kirsch in ' The Enzymes,' ed. P. Boyer, Academic Press, New York, 1971, p. 43; H. C. Froede and I. B. Wilson, *ibid.*, p. 87.
[6] A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, 1967, **89**, 4853, 4857.
[7] D. H. Eyre, J. W. Harris, and B. Lythgoe, *J. Chem. Soc.* (*C*), 1967, 452.
[8] W. A. Raftery, Ph.D. Thesis, Glasgow University, 1971.

on a Silicar CC7 column (50 × 3 cm) (benzyl salicylate runs much faster than the product on t.l.c.). After elution of the fast-running impurity the desired product was eluted with a more polar solvent mixture containing 60% of ether. Fractions containing the product were combined and evaporated to give an oil, which was pumped for many hours to remove the last traces of solvent. T.l.c. showed a homogeneous *product*, $\nu_{max.}$ 1760 and 1720 cm^{-1}, δ ($CDCl_3$) 2·0 (2H, m), 2·5 (2H, m), 3·5 (2H, t), 4·5 (2H, s), 5·35 (2H, s), 7·0 (1H, q), 7·3 (12H, m), and 8·05 (1H, q) (Found: C, 74·4; H, 6·1. $C_{25}H_{26}O_5$ requires C, 74·2; H, 5·95%).

2-*Benzyloxycarbonylphenyl* 5-*benzyloxyvalerate* was prepared by the same method from 5-benzyloxyvaleric acid; $\nu_{max.}$ 1760 and 1780 cm^{-1}, δ ($CDCl_3$) consistent with the structure (Found: C, 74·7; H, 6·35. $C_{26}H_{28}O_5$ requires C, 74·6; H, 6·2%).

2-*Benzyloxycarbonylphenyl* 4-*benzyloxyvalerate* was prepared in the same way, from 4-benzyloxyvaleric acid; $\nu_{max.}$ 1760 and 1720 cm^{-1}, n.m.r. data consistent with the structure (Found: C, 74·6; H, 5·95. $C_{26}H_{28}O_5$ requires C, 74·6; H, 6·2%).

Removal of Protecting Groups.—The benzyl groups from the three diesters were removed by catalytic hydrogenation in dioxan. The hydroxy-ester acids produced are reactive and analytical samples were not obtained. Instead the dioxan solutions produced by hydrogenation of the analytically pure dibenzyl derivatives were used directly as stock solutions for the kinetic experiments, as described by Capon *et al.*[3] The products were characterised by their spectroscopic properties, and their structures follow from the method of preparation.

2-*Carboxyphenyl* 4-*Hydroxybutyrate.*—2-Benzyloxycarbonylphenyl 4-benzyloxybutyrate (400 mg) and 5% palladium–charcoal (400 mg) in dry dioxan (10 ml) were hydrogenated at room temperature (19°) under atmospheric pressure. After 2 h uptake (2·26 equiv.) was complete. The catalyst was filtered off and the bulk of the dioxan solution was stored at −20 °C, to be used for the kinetic experiments. T.l.c. of this solution showed a single spot, with R_F different from that of the starting material. A small portion of the solution was evaporated to dryness, and gave a colourless oil which did not crystallise. This had the spectroscopic properties expected for 2-carboxyphenyl 4-hydroxybutyrate. Two broad, strong i.r. bands had appeared at 3400 and 3100—2300, in the region expected for the OH stretching absorptions of OH and CO_2H groups, and the bands at 1760 (phenolic ester) and 1720 cm^{-1} (now CO_2H) remained. When a sample of the dioxan solution was dissolved in acetate buffer the u.v. spectrum showed that no salicylic acid was present initially but salicylate was rapidly produced under these conditions; δ ($CDCl_3$) 2·05 (2H, m), 2·75 (2H, t), and 3·90 (2H, t) (β-, α-, and γ-CH_2 groups, respectively), and a group of aromatic H peaks [δ 7·0—7·6 (m) and 8·05 (q)] superimposed on a broad absorption, δ 6·5—8·2, ascribed to the CO_2H and OH protons (total 6H).

2-*Carboxyphenyl* 5-*Hydroxyvalerate.*—2-Benzyloxycarbonylphenyl 5-benzyloxyvalerate was hydrogenated under the same conditions. Uptake was complete at 2·10 equiv. The solution of product was treated as before. The oil produced from a small portion did not crystallise, but had the expected strong i.r. absorptions at 3400, 3100—2300, 1760, and 1710 cm^{-1}; δ 1·8 (4H, m), 2·6 (2H, m), 3·7 (dioxan), 7·0—7·6 (5H, m), and 8·05 (1H, q). The u.v. properties were the same as those of the butyrate. T.l.c. of the dioxan solution after hydrogenation showed a single spot, with R_F different from that of the starting material.

2-*Carboxyphenyl* 4-*hydroxyvalerate* was prepared from 2-benzyloxycarbonylphenyl 4-benzyloxyvalerate under the same conditions. Total uptake was 2·3 equiv. The oil slowly deposited an oily solid, which had the expected spectroscopic properties and moved as a single spot on t.l.c., with R_F different from that of the starting material. The solid appeared to be volatile when analysis was attempted, and presumably decomposed by loss of the γ-lactone. Correct analytical figures could not be obtained, but the solid had the expected i.r. bands at 3450, 3300—2300, 1760, and 1690 cm^{-1}, and the same u.v. properties as the other compounds. The n.m.r. spectrum ($CDCl_3$) showed the expected changes from that of the dibenzyl compound: the signal for 10 aromatic protons at δ 7·3 and the peaks due to the benzylic methylene groups at δ 4·5 and 5·25 had disappeared, and in their place had appeared absorption due to the OH produced, in the same region as the aromatic protons of the salicylate residue (6H multiplet at δ 6·9—8·2). The other absorptions appeared in the expected positions, together with a small peak at δ 3·7 (traces of dioxan).

Kinetic Methods and Results.—The stock solutions used for the kinetic experiments were obtained from the dioxan solutions of the hydroxy-ester acids produced by hydrogenolysis, by diluting 10 times with dry dioxan (giving final concentrations of substrate of about 10^{-2}M) for all but the initial rate measurements (see below). Reactions were started by adding 40 μl of stock solution to 2 ml of buffer solution incubated at the temperature of the experiment in the thermostatted six-compartment cell-holder of an automatic Zeiss PMQ II spectrophotometer. In each case the reaction was followed by monitoring the increase in absorbance at 298·5 nm, the isosbestic point for salicylic acid and its anion. Reactions were followed at 39° and ionic strength 1·0, normally for 3 half-lives, except that the slow hydrolysis of the 5-hydroxyvalerate below pH 2·5 was measured by the initial rate method; and the pH was measured at the end of each run. Repeated scans of the reaction mixture showed in each case a rapid appearance of salicylate ($\lambda_{max.}$ 296 nm), with tight isosbestic points at 246 and 268 nm. Thus the reaction produced salicylic acid as the only u.v.-absorbing product, with no detectable build-up of any intermediate.

Hydrolysis was also followed by n.m.r. in the case of 2-carboxyphenyl 4-hydroxybutyrate (D_2O; pyridine buffer, pD 5·41, at about 30°, on a Varian HA-100 spectrometer). The spectrum was scanned repeatedly from 0 to 3·5 p.p.m. downfield from t-butyl alcohol. The spectrum of γ-butyrolactone was recorded under the same conditions. In the course of 1 h the triplet at δ 3·64 (from Me_4Si) in the spectrum of the starting material disappeared, and was replaced by a new triplet centred at δ 4·35, the position of the corresponding peak in the spectrum of γ-butyrolactone. No peaks other than those of γ-butyrolactone appeared during the reaction. The lactone must be the initial product of hydrolysis, because its formation from 4-hydroxybutyric acid is much slower under these conditions.[9] So all the evidence is consistent with a direct conversion of starting material into salicylate and lactone.

A full set of data for the hydrolysis of 2-carboxyphenyl

[9] D. R. Storm and D. R. Koshland, *Proc. Nat. Acad. Sci. U.S.A.*, 1970, **66**, 445.

4-hydroxybutyrate is given in Table 1, and data for hydrolysis in the pH-independent region between pH 4 and 7 are given for the 4- and 5-hydroxyvalerates in Tables 2 and 3. Full pH–rate profiles for all three compounds are shown in the Figure. Catalysis by buffer constituents was negligible in each case.

TABLE 1

Data for the hydrolysis of 2-carboxyphenyl 4-hydroxybutyrate, at 39° and ionic strength 1·0

pH	Buffer	k_{obs}/min^{-1}
	1M-HCl	$5{\cdot}50 \times 10^{-2}$
	0·5M-HCl	$1{\cdot}59 \times 10^{-2}$
	0·1M-HCl	$7{\cdot}21 \times 10^{-3}$
	0·05M-HCl	$3{\cdot}59 \times 10^{-3}$
	0·01M-HCl	$3{\cdot}79 \times 10^{-3}$
2·77	Formate	$1{\cdot}10 \times 10^{-2}$
3·22	Formate	$1{\cdot}98 \times 10^{-2}$
3·71	Formate	$3{\cdot}43 \times 10^{-2}$
4·17	Acetate	$4{\cdot}60 \times 10^{-2}$
5·16	Acetate	$5{\cdot}52 \times 10^{-2}$
[a]	Acetate in D_2O	$2{\cdot}46 \times 10^{-2}$
5·68	Acetate	$5{\cdot}48 \times 10^{-2}$
6·02	Phosphate	$5{\cdot}82 \times 10^{-2}$
6·60	Phosphate	$6{\cdot}13 \times 10^{-2}$
7·06	Phosphate	$8{\cdot}27 \times 10^{-2}$
8·04	TRIS	0·225
8·57	TRIS	0·542
9·05	Carbonate	1·50
9·48	Carbonate	4·32
5·13	Acetate, at 31·9°	$2{\cdot}95 \times 10^{-2}$
5·13	Acetate, at 47·0°	0·101

[a] pD 5·66.

TABLE 2

Data for the hydrolysis of 2-carboxyphenyl 5-hydroxyvalerate, at 39° and ionic strength 1·0

pH	Buffer	k_{obs}/min^{-1}
4·58	Acetate	$1{\cdot}30 \times 10^{-2}$
5·07	Acetate	$1{\cdot}40 \times 10^{-2}$
5·59 (pD)	Acetate in D_2O	$5{\cdot}58 \times 10^{-3}$
5·97	Phosphate	$1{\cdot}39 \times 10^{-2}$
6·47	Phosphate	$1{\cdot}45 \times 10^{-2}$
6·93	Phosphate	$1{\cdot}49 \times 10^{-2}$
5·07	Acetate, at 20·7°	$2{\cdot}53 \times 10^{-3}$
5·07	Acetate, at 26·7°	$4{\cdot}50 \times 10^{-3}$
5·07	Acetate, at 32·8°	$7{\cdot}54 \times 10^{-3}$

TABLE 3

Data for the hydrolysis of 2-carboxyphenyl 4-hydroxyvalerate, at 39° and ionic strength 1·0

pH	Buffer	k_{obs}/min^{-1}
4·18	Acetate	$7{\cdot}88 \times 10^{-2}$
5·15	Acetate	$9{\cdot}43 \times 10^{-2}$
5·59	Acetate	$9{\cdot}56 \times 10^{-2}$
5·59 (pD)	Acetate in D_2O	$3{\cdot}87 \times 10^{-2}$
6·02	Phosphate	$1{\cdot}01 \times 10^{-1}$
6·48	Phosphate	$1{\cdot}02 \times 10^{-1}$
6·96	Phosphate	$1{\cdot}06 \times 10^{-1}$

The data of Tables 1—3 and the pH–rate profiles of Figure 1 can be interpreted in terms of the acid-catalysed hydrolysis of the hydroxy-ester acid, and the spontaneous (k_0) and hydroxide-catalysed hydrolyses of the anion. The spontaneous hydrolysis rate of the acid is not large enough to contribute significantly to the observed rate at any pH, and has been neglected. Analysed on this basis, the data for the three esters give the rate constants listed in Table 4, which also includes the deuterium solvent isotope effects and activation parameters for the hydrolysis of the anions.

TABLE 4

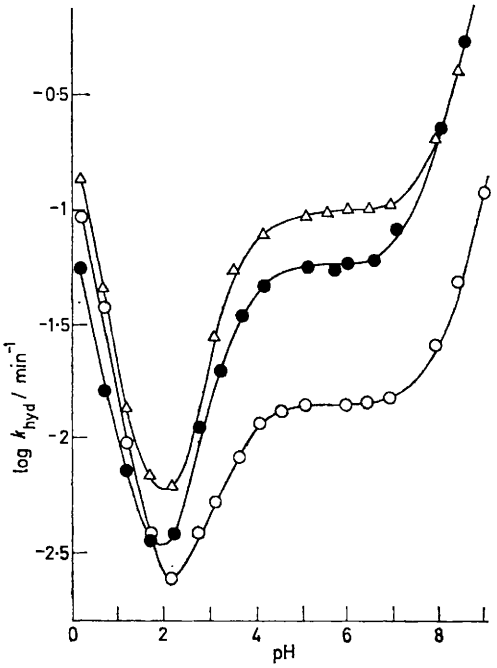
Summary of kinetic data derived from Tables 1—3, at 39° and ionic strength 1·0

	2-Carboxyphenyl esters		
	4-HO-butyrate	4-HO-valerate	5-HO-valerate
pK_{app}	3·45	3·45	3·40
k_H/l mol^{-1} min^{-1}	$5{\cdot}3 \times 10^{-2}$	$1{\cdot}38 \times 10^{-1}$	$9{\cdot}4 \times 10^{-2}$
k_{OH}/l mol^{-1} min^{-1}	$3{\cdot}0 \times 10^{4}$	$2{\cdot}53 \times 10^{3}$	$2{\cdot}31 \times 10^{4}$
k_0/min^{-1}	$5{\cdot}6 \times 10^{-2}$	$9{\cdot}6 \times 10^{-3}$	$1{\cdot}4 \times 10^{-2}$
k_H/k_D [a]	2·28	2·48	2·52
$\Delta H^{\ddagger}$ [b] /kcal mol^{-1}	15·95		16·2
$\Delta S^{\ddagger}$ [b] /e.u.	−21·8		−23·4

[a] In the pH-independent region, pD 5·6. [b] In the pH-independent region, pH 5·10.

DISCUSSION

The pH–rate profile for hydrolysis of each of the three hydroxy-ester acids (Figure) can be accounted for quantitatively in terms of three reactions. Hydrolysis



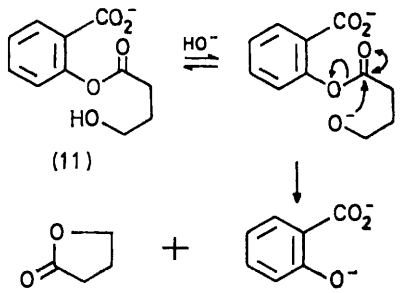
pH–Rate profiles for 2-carboxyphenyl 5-hydroxyvalerate (○), 4-hydroxybutyrate (●), and 4-hydroxyvalerate (△), at 39° and ionic strength 1·0; data from Tables 1—3

is specific acid catalysed at low pH, where the carboxy-group is protonated. The rate constants for this catalysis (Table 4) are substantially higher than that observed for the hydrolysis of aspirin ($4{\cdot}72 \times 10^{-3}$ l mol^{-1} min^{-1} under the same conditions [10]), which itself shows a modest rate enhancement attributed [10] to intramolecular catalysis by the CO_2H group. On the other hand, k_H for the hydrolysis of 2-carboxyphenyl 4-hydroxybutyrate is a few times slower than for the hydrolysis of phenyl 4-hydroxybutyrate ($4{\cdot}05 \times 10^{-1}$ l mol^{-1} min^{-1} at 50°).[8] This suggests that acid catalysed hydrolysis involves participation by the 4-hydroxy-group, but that the carboxy-group does not assist the

[10] A. R. Fersht and A. J. Kirby, *J. Amer. Chem. Soc.*, 1968, **90**, 5826.

reaction (it probably slows it slightly by steric hindrance). The initial product of hydrolysis would be γ-butyrolactone, as suggested also by Capon [3] for the corresponding reaction of phenyl 4-hydroxybutyrate.

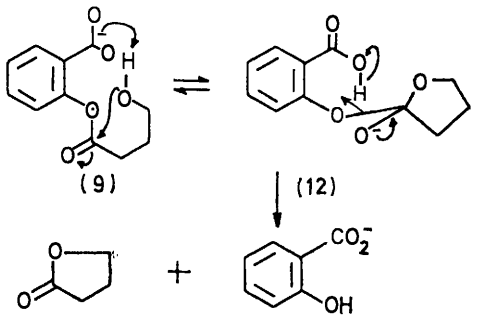
Alkaline hydrolysis is also slower for 2-carboxyphenyl 4-hydroxybutyrate than for the phenyl ester. The rate constant (k_{OH}) observed by Capon [3] for the latter compound, $3{\cdot}25 \times 10^5$ l mol^{-1} min^{-1} at 30°, corresponds to a greater than 10-fold difference at 39°. This effect is larger than the steric effect of the CO_2H group on the acid catalysed reaction, noted above, and may include a contribution from electrostatic repulsion between the attacking alkoxide anion and the neighbouring carboxylate group. An effect of similar magnitude is observed on k_{OH} for the hydrolysis of aspirin,[6] which is 8—10 times smaller than for the hydrolysis of phenyl acetate.[3] On the other hand, the values of k_{OH} observed by us for 2-carboxyphenyl 4-hydroxybutyrate, and by Capon *et al.* for the phenyl ester, are both several orders of magnitude greater than the corresponding rate constants for the hydrolysis of aspirin or phenyl acetate, and it seems safe to assume that the reaction concerned in each case is intramolecular nucleophilic displacement of aryloxide by the ionised hydroxy-group [see (11)].



We conclude that both acid and alkaline hydrolysis give the lactone as the initial product, and thus involve intramolecular nucleophilic catalysis by the hydroxy-group: but that the neighbouring carboxy-group plays no part in either reaction.

Between pH 2 and 7 the only significant reaction is the spontaneous hydrolysis of the hydroxy-ester anions. The pH–rate profiles follow the expected dissociation curves of the CO_2H groups, with apparent pK_a values of 3·40—3·45. (The pK_a of aspirin, measured [6] under the conditions of these experiments, is $3{\cdot}36 \pm 0{\cdot}02$.) There is no significant contribution from the spontaneous hydrolysis of the hydroxy-ester acids, which must be hydrolysed over 100 times more slowly than the anions. In fact the undissociated form of aspirin,[10] like phenyl 4-hydroxybutyrate,[3] is hydrolysed several orders of magnitude more slowly than the anion of 2-carboxyphenyl 4-hydroxybutyrate, so that the reaction of the acid form would be detectable only if it showed substantial bifunctional catalysis. Our evidence is that it does not: the carboxy-group of aspirin acid is thought [10] to assist the hydrolysis of the ester group by a nucleophilic mechanism: intramolecular general acid catalysis of hydrolysis must be less efficient than this already not-very-efficient process, and so no substantial catalysis of lactonisation by this mechanism is to be expected for undissociated 2-carboxyphenyl 4-hydroxybutyrate.

The anion (11) of 2-carboxyphenyl 4-hydroxybutyrate is hydrolysed 85 times as fast as the aspirin anion [6] at 39°, and some 500 times faster * than phenyl 4-hydroxybutyrate.[3] The initial product of hydrolysis is γ-butyrolactone, as shown by our spectroscopic evidence,



and the pH–rate profile follows the ionisation curve of the CO_2H group. This is convincing evidence that both OH and CO_2^- groups are involved in the reaction, and since the formation, rather than the breakdown, of the tetrahedral intermediate (12) is expected to be rate determining (aryloxide is the better leaving group) the carboxylate group must be involved in this step.

The simplest mechanism consistent with all the facts is intramolecular general base catalysis by carboxylate of the addition of the 4-hydroxy-group to the carbonyl carbon atom, shown in (9). It is likely that the CO_2H group of (12) will act as a general acid to assist the departure of the leaving group—this is a common feature of reactions in which salicylate is lost.[11] Because the breakdown of (12) is not rate determining this will not affect the observed rate of hydrolysis of (9); but it is of interest for the further development of the enzyme model, since it is easy to introduce structural features that will accelerate the formation of the tetrahedral intermediate.

A number of alternative mechanisms can be written for participation by the carboxylate group in this reaction. These have been discussed previously [6] and will not be reproduced here. We are satisfied that the general base catalysis mechanism accounts for the observed properties of the hydrolysis of 2-carboxyphenyl 4-hydroxybutyrate just as well as it does for the hydrolysis of aspirin.[6] The most direct evidence is the similarity in kinetic properties between the two reactions. The solvent deuterium isotope effect (k_H/k_D = 2·2 for aspirin anion hydrolysis) is almost identical in the two cases, and the efficiency of catalysis by the CO_2^- group is also similar: compared with external catalysis by acetate [3] the carboxylate group of 2-carboxyphenyl 4-hydroxybutyrate has an effective molarity of 14M.

* The Arrhenius plot of the data for k_0 (Table 1) gives a value of 0·135 min^{-1} at 50°, 485 times larger than Capon's value for the spontaneous hydrolysis of the phenyl ester at this temperature.

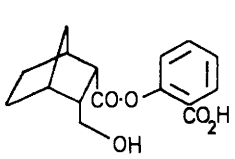
[11] G. A. Craze and A. J. Kirby, *J.C.S. Perkin II*, 1974, 61.

The effective molarity of the CO_2^- group of aspirin is 9M, and rises to 13M if the difference in basicity of the CO_2^- and acetate groups is taken into account.[6] More surprisingly, the entropies of activation for hydrolysis are also identical within experimental error. The observed value of −22 e.u. is presumably accounted for in the case of aspirin by the involvement of the second (water) molecule in the transition state. If mechanism (9) is correct a major contribution to the observed negative entropy of activation for the hydrolysis of 2-carboxyphenyl 4-hydroxybutyrate must come from the loss of rotational degrees of freedom in the butyrate chain: in which case other cyclisation reactions in which γ-butyrolactone is formed should also show moderately large negative values of $\Delta S^{\ddagger}$. This is in fact the case: $\Delta S^{\ddagger}$ for the acid-catalysed lactonisation of 4-hydroxybutyric acid is −31·5 e.u.,[12] and for the acid-catalysed lactonisation of phenyl 4-hydroxybutyrate is −18·7 e.u.[3] This latter value is only slightly greater than the value ($\Delta S^{\ddagger}$ −22·4 e.u.) found[3] for the *A*-2 hydrolysis of phenyl butyrate, confirming that intramolecular attack by the oxygen atom of the 4-hydroxybutyryl group is not significantly more favourable entropically than external attack by water.

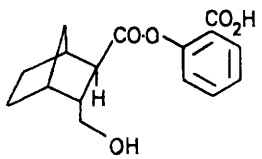
We have discussed in detail only the reactions of 2-carboxyphenyl 4-hydroxybutyrate, but our conclusions apply equally to the corresponding reactions of the 4- and 5-hydroxyvalerates. The relative rates of the acid and base-catalysed processes are similar to those expected on the basis of previous work using the hydroxybutyrate and valerate systems,[3,13] and the relative rates of the carboxylate-catalysed reactions (4 : 1 : 7 for 4-hydroxybutyrate, 5-hydroxyvalerate, and 4-hydroxyvalerate, respectively), probably also reflect changes in the structure of the hydroxyalkyl group of the starting material.

[12] D. R. Storm and D. E. Koshland, *J. Amer. Chem. Soc.*, 1972, **94**, 5815.

Because of the small spread in reactivities observed for this reaction we have not been able to show whether the efficiency of catalysis by the carboxylate group in this type of bifunctional catalysis is sensitive to the rate of lactonisation. This is an important question if we are to apply our conclusions to discussions of similar processes occurring in enzymic reactions, where the overall process is faster by many orders of magnitude. What evidence we have suggests that general base catalysis is intrinsically a relatively inefficient form of catalysis: the ionised carboxy-group attains only modest effective concentrations, in the range 10—60M, in reactions involving intramolecular general base catalysis.[14] Any variation outside this range would be of considerable interest. Before this work was interrupted we attempted to prepare the 2-carboxyphenyl ester (13) of a system known[12,13] to lactonise much faster than the compounds used in this work. The ester (13) was approached by the same general methods used for the



(13)



(14)

preparation of the compounds used in this work; but the hydroxy-ester acid obtained turned out to be (14), resulting presumably from base-catalysed epimerisation during the synthesis of the benzyl ester. Compound (14) is not hydrolysed faster than aspirin near pH 7.

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[13] D. R. Storm and D. E. Koshland, *J. Amer. Chem. Soc.*, 1972, **94**, 5805.

[14] A. J. Kirby and G. J. Lloyd, in preparation.