Concurrent nucleophilic and general acid catalysis of the hydrolysis of a phosphate triester

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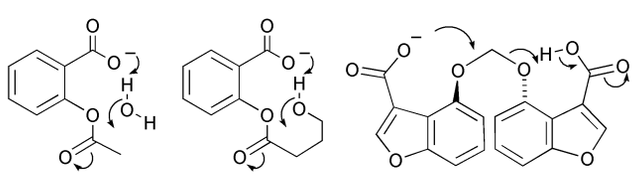
Received (in Cambridge, UK) 14th May 2002, Accepted 5th August 2002
First published as an Advance Article on the web 28th August 2002

The hydrolysis of diethyl 8-dimethylaminonaphthyl-1-phosphate is catalysed by the neighbouring dimethylammonium group, with a rate acceleration, compared with diethyl naphthyl-1-phosphate, of almost 10^6. The effective pK_a of the naphtholate leaving group is reduced from 9.4 to 3.4 by partial protonation in the transition state. The reaction is catalysed by oxyanion nucleophiles, and it is shown that a common nucleophilic mechanism, enhanced by general acid catalysis by the neighbouring dimethylammonium group, accounts for all the observed reactions. The efficiency of general acid catalysis depends on the extent of negative charge development on the leaving group oxygen in the transition state for P–O cleavage, and the strength of the intramolecular hydrogen bond in reactant and transition state.

We are interested in the mechanisms by which neighbouring groups, in systems ranging from small molecules to enzyme active sites, catalyse transformations of the main functional groups involved in metabolic processes. Many of the reactions concerned—like glycosyl transfer and phosphodiester cleavage—are intrinsically extraordinarily slow,[1] and to proceed at biologically useful rates require catalysis of correspondingly high efficiency. A logical way to break a very strong bond efficiently is to reinforce its natural polarity by the concerted application of complementary catalytic groups, to the point where cleavage becomes kinetically favourable.

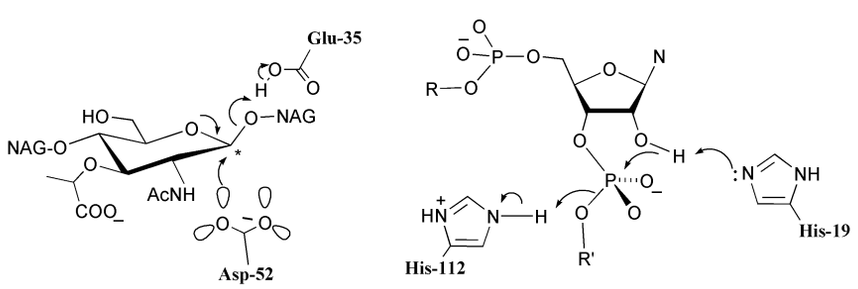
Such mechanisms have often been written, and more or less tacitly accepted, for many enzyme reactions. Examples are the reactions catalysed by lysozyme and ribonuclease (Scheme 1). However, it has proved suspiciously difficult to model such mechanisms in simple systems, and impossible in the few well-established cases to achieve very high efficiency. It may be that the reasons are simply practical: most of the relevant work has been done with relatively reactive systems, involving for example the displacement of convenient, good leaving groups; rather than the poor, non-chromophoric leaving groups typical of natural systems. And if two groups are both catalytic it often turns out that they act on different steps of the reaction.

We have been working to optimise the efficiency of intramolecular catalysis, most recently intramolecular general acid catalysis,[2] in a number of different systems, with a view to enhancing the most efficient monofunctional systems with catalysis by a second neighbouring group.[3] The most reliable designs start with an efficient intramolecular reaction in which a general acid or a general base catalyses the reaction of a target functional group with an external reactant. Thus the hydrolysis of aspirin involves intramolecular general base catalysis of the attack of water on the substrate ester group **1**.[4] The hydroxy group of a 4-hydroxy acid or ester is known to be a better nucleophile than water towards the neighbouring carboxy: combining the two systems give the hydroxyester acid **2**, which is hydrolysed by the mechanism shown, with the salicylate carboxylate acting as a general base to assist the addition of the 4-HO group on the common ester group.[5] In this system the effective molarities (EM) of the individual component parts of the mechanism are simply additive, as would be expected.



1 **2** **3**

Of potentially wider interest are systems where a general acid assists the departure of the leaving group in a concerted substitution reaction. The hydrolyses of methoxymethyl acetals and glycosides are both borderline enforced-concerted reactions, with water acting as the nucleophile:[6] and we have evolved systems showing very efficient intramolecular general acid catalysis of such reactions. It is only a matter of time (and synthesis) before we introduce an efficient intramolecular nucleophile to replace the water in such systems. Concerted

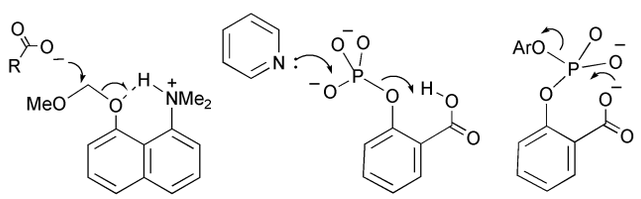


Scheme 1

 DOI: 10.1039/b204609a

catalysis might reasonably be expected—S_N2 at the acetal centre of methoxymethyl acetals is well-characterised[7]—and has recently been observed (**3**) in a simple test system with an intramolecular nucleophile.[3b] More interesting, and not easily predicted, is whether in the right system we will see synergy between the two mechanisms: specifically, a reaction in which the EM of each catalytic group is enhanced by the presence of the other. Very little relevant evidence is available.

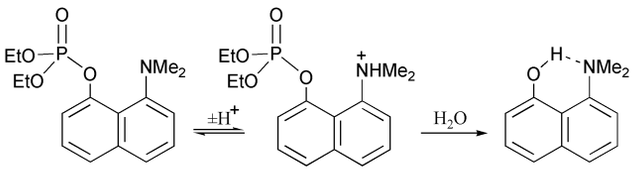
Perhaps surprisingly there is no significant catalysis by external nucleophiles of the hydrolysis of the methoxymethyl acetals derived from salicylic acid[8] or the 3-carboxybenzopyran nucleus of **3**:[9] although nucleophilic catalysis is well-established for methoxymethyl acetals with good leaving groups.[7] The only system we know which clearly shows general acid catalysis of the attack of (oxyanion) nucleophiles on an acetal centre is the 8-dimethylammonium-1-naphthol derivative **4** (suggesting that charge neutralisation might be a significant factor). On the other hand nucleophilic catalysis by pyridines is readily observed in the hydrolysis of the monoanion of salicyl phosphate, and best explained by the concerted mechanism shown for **5**.[10] The data suggest that the neighbouring carboxy group of **5** is a more efficient general acid than is the dimethylammonium group of **4**, but different centres are being attacked by different nucleophiles, so no firm conclusion can be drawn.



4 **5** **6**

The mechanism shown for **5** cannot be observed for phosphodi- or tri-esters of salicylic acid because the carboxylate anion is far more effective as a nucleophile (**6**) when an exocyclic leaving group is available.[11] When a second carboxy group is present, as in disalicyl phosphate (**6**, Ar = 2-carboxyphenyl), it does act as a general acid to enhance the highly efficient nucleophilic catalysis by the neighbouring carboxylate (overall rate acceleration for hydrolysis, compared with rate expected for the system with no carboxy groups, of the order of 10^{10}): but notably inefficiently (a contribution of less than one order of magnitude compared with the rate expected for a system **6** with a leaving group ArOH of a similar effective pK_a).[3a] The reaction of (**6**, Ar = 2-carboxyphenyl) is expected to be borderline concerted (see the Discussion Section below) so phosphate esters derived from 8-dimethylamino-1-napthol become of particular interest in the search for concerted nucleophilic and general acid catalysis.

We report results for the hydrolysis of the simple triester **7**, which brings together for the first time an oxyanion leaving group from phosphorus and the neighbouring dimethylammonium general acid. In the parent naphthol **8** the phenolic OH forms a strong intramolecular hydrogen bond to the neighbouring amino-group nitrogen, and this is expected[12] to assist the departure of an alcohol or phenol leaving group from phosphorus, insofar as this H–bond has developed in the transition state for P–O cleavage. Substitution at the triester phosphorus undoubtedly involves the S_N2(P) mechanism, possibly by way of a pentacovalent addition intermediate: so the scene is set for general acid catalysis of nucleophilic catalysis. (The *peri*-dimethylamino group should be a less effective nucleophile towards P than carboxylate,[13] and we considered in-line displacement of the ethoxy groups unlikely to compete with the reaction of interest.) The question is how efficient this reaction will be. It turns out to be highly efficient.



7 **7H$^+$** **8**

Results

The pH–rate profile for the hydrolysis of the triester **7** (to the naphthol **8**, as predicted) shows a pH-independent region ($k_0 = 8.68 \pm 0.89 \times 10^{-5}$ s^{-1}) below pH 4, as the only feature in the pH range 0–7 (see Fig. 1). This is readily assigned to the

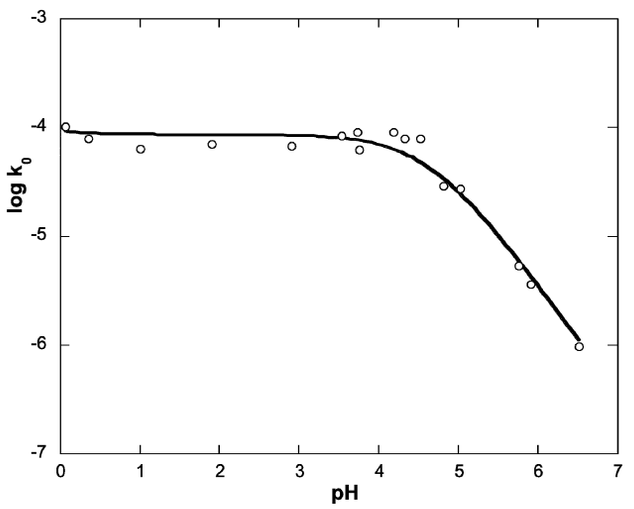


Fig. 1 pH–rate profile for the hydrolysis of triester **7**, at 60 °C and ionic strength 1.0 M. The points for pH > 1 represent extrapolations to zero buffer from data measured at three or four different buffer concentrations. Data from Table 1.

reaction of the conjugate acid, **7H$^+$**, the rate falling off at higher pH as expected if the neutral triester **7** is unreactive, according to an apparent pK_a of 4.63. This pK_a is in the region expected for a naphthylamine, but differs significantly from the pK_a of the 8-methoxymethyl acetal **4** (7.40 at 65 °C);[12] presumably because there is significant hydrogen bonding between the N^+H and the more basic acetal oxygen, but not—or much less so—to the less basic P–OAr group of the phosphate triester.

The absence of a significant acid-catalysed reaction at low pH is explained by electrostatic inhibition by the adjacent dimethylammonium group,[14] combined with the high rate of the spontaneous reaction.

Hydrolysis above pH 2 shows strong catalysis by the buffers used to maintain the pH. For example, hydrolysis is 11.5 times faster in the presence of 0.5 M formate: so the data points used to construct the pH–rate profile represent extrapolations to zero buffer concentration. Analysis of the buffer catalysis shows that it involves exclusively the basic component in each case, and thus represents either general base or nucleophilic catalysis of the hydrolysis of **7H$^+$**. (The kinetically equivalent mechanism involving the buffer conjugate acid and the neighbouring NMe_2 group as a nucleophile can be ruled out because of (i) the strong dependence on the basicity of the buffer anion (see below), (ii) the product is the naphthol **8** (which would require a pseudorotation, putting the Me_2N^+ group in an equatorial position) and because (iii) no reaction is observed at pH > 7, where the NMe_2 predominates; until alkaline hydrolysis sets in at pH > 10.)

The kinetic data show clearly that catalysis is nucleophilic. (i) The solvent kinetic isotope effect k_{H_2O}/k_{D_2O} for the formate catalysed reaction is <1.39 (Table 1: the observed ratio includes

Table 1 First-order rate constants for the hydrolysis of triester **7** at 60 °C and ionic strength 1.0 M

Buffer/% free base	pH	k_0/s^{-1}
1.0 M HCl	0.06	$1.03 \pm 0.06 \times 10^{-4}$
0.5 M HCl	0.35	$7.93 \pm 0.56 \times 10^{-5}$
0.1 M HCl	1.01	$6.48 \pm 0.56 \times 10^{-5}$
H_3PO_4/50%	1.9	$7.06 \pm 0.21 \times 10^{-5}$
HCOOH/20%	2.9	$6.89 \pm 1.06 \times 10^{-5}$
HCOOH/50%	3.53	$8.49 \pm 0.62 \times 10^{-5}$
HCOOH/60%	3.73	$9.12 \pm 1.35 \times 10^{-5}$
HCOOH/80%	4.19	$9.21 \pm 1.37 \times 10^{-5}$
HCOOH/80%/30 °C[a]		$1.04 \pm 0.04 \times 10^{-4}$
HCOOH/80%/40 °C[a]		$2.55 \pm 0.18 \times 10^{-4}$
HCOOH/80%/50 °C[a]		$6.11 \pm 0.12 \times 10^{-4}$
HCOOH/80%/60 °C[a]		$1.27 \pm 0.07 \times 10^{-3}$
MeCOOH/20%	3.75	$6.30 \pm 0.47 \times 10^{-5}$
MeCOOH/40%	4.32	$7.97 \pm 1.29 \times 10^{-5}$
MeCOOH/50%	4.52	$8.01 \pm 0.99 \times 10^{-5}$
MeCOOH/60%	4.81	$2.92 \pm 0.60 \times 10^{-5}$
MeCOOH/75%	5.02	$2.79 \pm 0.64 \times 10^{-5}$
$H_2PO_4^-$/20%	5.76	$5.42 \pm 1.98 \times 10^{-6}$
$H_2PO_4^-$/25%	5.91	$3.64 \pm 1.01 \times 10^{-6}$
$H_2PO_4^-$/50%	6.51	$9.83 \pm 2.89 \times 10^{-7}$
In D_2O		
1.0 M DCl	[−0.13]	5.37×10^{-5}
0.5 M DCl	[0.23]	3.33×10^{-5}
HCOOD/50%[b]	[3.85]	6.10×10^{-5}

[a] In 1 M formate: $\Delta H^{\ddagger}$ 67.6 ± 1.0 kJ mol^{-1}; $\Delta S^{\ddagger}_{333}$ −96.2 ± 3.3 J K^{-1} mol^{-1} [b] Mean of 2–4 measurements in 1 M formate buffer at the temperature indicated. The reaction is 95% buffer catalysed in 80% free base buffer at 60 °C, 91% buffer catalysed at 50% free base.

a small contribution from the spontaneous hydrolysis reaction, which shows a higher isotope effect). This is fully accounted for by the typically low isotope effect associated with intramolecular general acid catalysis through a developing, strong hydrogen bond,[12] without the significant contribution from reaction expected for catalytic formate acting as a general base.[13] The kinetic solvent deuterium isotope effect for the spontaneous reaction, in which solvent water would be expected to be involved as both nucleophile and general base, is higher, at >1.61 (Table 1), but mechanistically opaque. (ii) The entropy of activation for the formate-catalysed reaction (< 96 J K^{-1} mol^{-1}, Table 1) lies squarely in the region characteristic of bimolecular processes. (iii) Catalysis by oxyanions is expected to be exclusively nucleophilic for a leaving group with an effective pK_a corresponding to that of **7H**$^+$.[13]

The second-order rate constants for the reaction with water and the (more basic) oxyanions $H_2PO_4^-$, formate, acetate and HPO_4^{2-} fall on the same Brönsted line (Fig. 2), consistent with water acting by the same mechanism as the oxyanions.

Discussion

The efficient general acid catalysis that made the 8-dimethylamino-1-naphthol system an exceptionally good leaving group from the acetal **4** is observed also for the phosphate triester **7**. Linear free energy relationships for the reactions of nucleophiles with a series of phosphotriesters **9** allow good estimates of rate constants at 39 °C for a triester with a phenol leaving group with pK_a = 9.40. (We use the value for 1-naphthol for the pK_a of the leaving group oxygen in the absence of the *peri*-substituent.[12] The measured value of 14.9[15] for **8** itself (in 80% DMSO) is raised by the strong hydrogen bonding possible in the free base form, and not directly relevant to the reactant **7**.) The cyclic system **9** is more reactive than the corresponding diethyl esters, by a factor of 1.66 for the attack of hydroxide on the *p*-nitrophenyl phosphates.[16] Correcting by this factor the rate constants that can be calculated for the spontaneous,

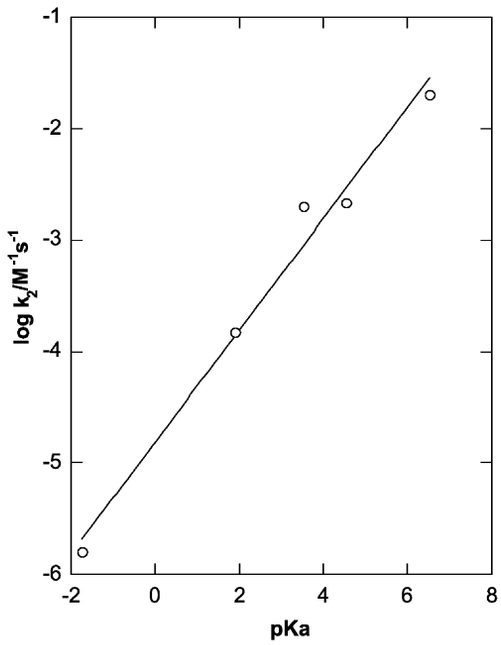
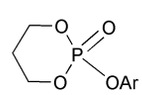


Fig. 2 Brönsted plot for catalysis by water and by oxyanions (phosphate mono and dianion, formate and acetate) for the hydrolysis of triester **7H**$^+$. Conditions as for Fig. 1. The slope of the (least-squares) line drawn is 0.50 ± 0.04.

acetate and phosphate (dianion)-catalysed hydrolysis of **9** at 39 °C are slower than the corresponding figures for the reaction of **7H**$^+$ at 60 °C by factors of 2.5×10^6, 4.9×10^5 and 8.2×10^4: the range reflecting the lower sensitivity of the reactions of **9** with more basic nucleophiles to the pK_a of the leaving group.[13] (Only the figure for acetate can be corrected with confidence for the temperature difference: if we assume that $\Delta H^{\ddagger}$ is the same as that for catalysis by formate (Table 1) the ratio is reduced by a factor of 4.8 to 5.3×10^5 at 39 °C.)



9

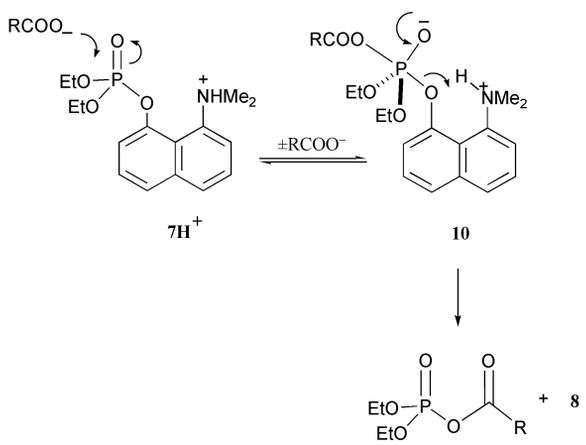
General acid catalysis by the dimethylammonium group of the hydrolysis of **7** is thus substantially more efficient than the corresponding reaction of the methoxymethyl acetal **4**, for which an acceleration of 1.9×10^3 has been estimated at 39 °C.[12] Accelerations for the attack of nucleophiles at the acetal centre of **4** are not easily estimated, but are smaller than for the attack of water.

An alternative way to compare the efficiency of intramolecular catalysis in these systems is to estimate the effective pK_a of the leaving group oxygen (*i.e.* the pK_a of ROH for the cleavage of a P–OR bond). Extensive data are available for the hydrolysis of a series of triesters **9** and for the reactions with nucleophiles of the 2,4-dinitrophenyl derivative.[13] The Brönsted (leaving group) equations for the reactions of **9** then allow us to calculate the effective pK_as of the leaving group, for the spontaneous and acetate-catalysed reactions of **7H**$^+$, as 3.4 and 3.6, respectively. For the spontaneous and acetate-catalysed reactions of **4** we estimate values of 6.1 and 6.3.

The evidence thus shows that catalytic efficiency depends not only on the general acid involved and the geometry of the system,[17] but also the reaction being catalysed. A reasonable assumption is that efficiency depends on the extent of involvement of the catalytic general acid—*i.e.* the extent of proton transfer—in the transition state, and this in turn will depend on the amount of negative charge that has developed on the leaving group oxygen. In the case of a nucleophilic substitution

process this will depend also on the extent of bond formation to the nucleophile. We have a measure of this factor in the shape of the Brönsted coefficient, β_{nuc}, which is 0.50 ± 0.04 for the attack of oxyanions and water on **7H**$^+$. This figure can be compared with β_{nuc} for the attack of anions on triesters **9**; which varies significantly with the leaving group: ranging from 0.30 for Ar = 2,4-dinitrophenyl to 0.48 for Ar = 4-nitrophenyl.

It is immediately clear that the transition state for the intramolecular general acid catalysed reaction of **7H**$^+$ is significantly different from that expected for either a triester hydrolysing at a similar rate (for which β_{nuc} is expected to be <0.30) or one with a comparable leaving group (for pK_{LG} of 9.40 the expected β_{nuc} would be in the region of 0.7). Evidently more bond formation to the nucleophile is necessary (later TS) to generate the negative charge on the leaving group needed to support efficient proton transfer catalysis. This would explain the similar difference observed for the reactions of pyridine with 2,4-dinitrophenyl phosphate and salicyl phosphate **4** (the second-order rate constants differ only by a factor of 2), for which β_{nuc} = zero[18] and 0.21,[10] respectively. These latter reactions, at the phosphorus centre of phosphate monoester dianions, certainly involve synchronous, concerted $S_N2(P)$ mechanisms. Displacements at the phosphorus centre of the phosphate triester **7**, on the other hand, are more likely to involve pentacovalent addition intermediates. The differences in the apparent degree of bond formation in the transition state are consistent with the rate-determining formation of the pentacovalent addition intermediate for the reactions of reactive triesters **9**, but breakdown being rate determining for the reaction of **7H**$^+$, with an initially poorer leaving group (Scheme 2). In this latter case it appears from the observed pK_a

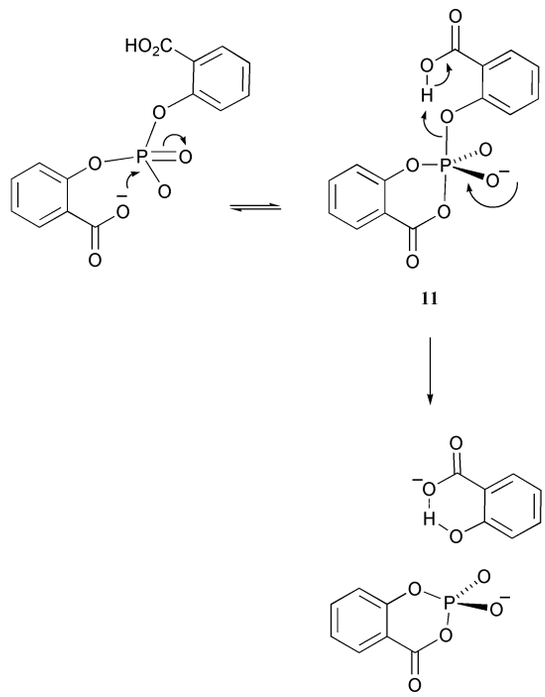


Scheme 2

that there is little hydrogen bonding from the dimethylammonium group to the leaving group oxygen of **7H**$^+$; so that proton transfer becomes significant only during the (rate determining) breakdown of the pentacovalent intermediate **10**.

It seems likely that the primary reason for the higher efficiency of intramolecular general acid catalysis of phosphate ester compared with acetal hydrolysis, by both COOH and Me_2NH^+ groups, is the naturally more extensive development of negative charge on the leaving group oxygen in the rate-determining transition state for P–O cleavage. This is consistent with the low efficiency of general acid catalysis in the hydrolysis of the diester **6** (Ar = 2-carboxyphenyl); which was explained[3a] in terms of the necessarily very early transition state, and thus minimal development of negative charge on the leaving group oxygen, for the breakdown of the high energy intermediate **11** (Scheme 3).

Brönsted (leaving group) coefficients β_{LG} are −1.23[18] and −0.96[13] at 39 °C for the hydrolyses of phosphate monoester dianions and triesters, respectively, compared with −0.82 for



Scheme 3

the hydrolysis of a series of comparable methoxymethyl acetals under similar conditions (35 °C).[19] The effective charge on the leaving group oxygen of a simple aryl ester has been estimated as + 0.35 for a monoester dianion, compared with + 0.87 for a diethyl triester.[20] This information is not available for the acetal system, though the similar absolute reactivities suggest that effective charges could be similar for the leaving group oxygens of phosphate monoester dianions and acetals $MeOCH_2OAr$. However, these effective charges will be increased, perhaps substantially, by strong intramolecular hydrogen-bonding to the leaving group oxygen in the systems of interest for this work. We can estimate the order of magnitude of this effect from the increase in the pK_a of the catalytic dimethylammonium group. At 4.63 this is normal (for a naphthylammonium cation) for **7H**$^+$, but raised to 7.40 for the corresponding methoxymethoxy acetals. So we have no sound basis for assessing the effective charge that has developed on the leaving group oxygen in the cleavage reactions of these systems, and still less basis for comparing them. It could be that the main factor responsible for the less efficient catalysis in the acetal system is stabilisation of the reactant by the intramolecular hydrogen bond. General acid catalysis is only possible if the hydrogen bond stabilises the transition state more effectively.

Our results with **7H**$^+$ show that the more stable pentacovalent species (intermediate or transition state) formed from a phosphate triester (or, presumably, from the congugate acid form of a diester[21]) can support efficient intramolecular general acid catalysis if the geometry is right: as can the concerted reactions of phosphate monoester dianions (*e.g.* **5**,[10] above). Thus the reactivity at pH < 7 of a phosphodiester derived from **7**, for which $S_N2(P)$ processes are expected to be close to concerted, now becomes of special interest.

Experimental

General procedures, methods and materials

^{1}H and ^{31}P NMR spectra were measured on a BRUKER Avance DPX 400 spectrometer at 300 K with tetramethylsilane as internal and 85% H_3PO_4 as external standard, respectively. Chemical shifts are reported in δ (ppm). All solvents used were dried with great care, and reactions carried out in an anhydrous argon atmosphere.

Table 2 Second-order rate constants for buffer catalysis of the hydrolysis of triester conjugate acid **7H**$^+$, at 60 °C and ionic strength 1.0 M

Buffer base	pK_a	k_2/dm^3 mol^{-1} s^{-1}
H_2O	−1.74	$1.58 \pm 0.16 \times 10^{-6}$
$H_2PO_4^-$	1.90	$1.50 \pm 0.13 \times 10^{-4}$
$HCOO^-$	3.53	$2.02 \pm 0.03 \times 10^{-3}$
$MeCOO^-$	4.52	$2.39 \pm 0.39 \times 10^{-3}$
HPO_4^{2-}	6.51	$2.01 \pm 0.14 \times 10^{-2}$

Diethyl-(8-dimethylamino)-1-naphthyl phosphate 7

To a stirred solution of 8-dimethylamino-1-naphthol **8**[22] (2 mmol) in 5 ml of dry dichloromethane were added 1.3 equiv. of pentane-washed sodium hydride in 2 ml of dichloromethane. After 15 min 1.2 equiv. of diethyl phosphorochloridate in 2 ml of dichloromethane were added dropwise to the resulting suspension and the mixture stirred for 15–20 min, when reaction was complete (RP-HPLC, TLC). Then the reaction mixture was poured into 50 ml of sodium bicarbonate solution, the organic layer washed with water, evaporated and chromatographed (silica; EtOAc–hexane, 7 : 3) to yield the triester **7**, 60–70%. ^{31}P NMR ($CDCl_3$) − 6.31, quintet, $J_{31P\text{-}1H}$ 8.08, 14.9 Hz. ^{1}H NMR ($CDCl_3$) 7.645 (1H, dd, 8 and 1.5 Hz), 7.42 (4H, m), 7.045 (1H, dd, 7.5 and 1Hz), 4.2 (4H, m, CH_3CH_2), 2.83 (6H, s, $(CH_3)_2N$), 1.28 (6H, dt, 1 and 7.1 Hz, CH_3CH_2).

Kinetic procedures

All buffer reagents were of AnalaR grade. Deionised water was further doubly distilled in all glass apparatus and degassed with argon. KOH and HCl stock solutions (2 mol dm^{-3}) were made by dilution of BDH Convol concentrates. Buffer solutions were made by appropriate dilutions in grade A volumetric flasks. KCl was added to adjust the ionic strength of the reaction mixtures to 1.0 mol dm^{-3}. Dioxane was freshly distilled from $NaBH_4$ prior to use. Cosolvent content is quoted as a (v/v) percentage. The pH of each buffer solution was recorded at the temperature used in the kinetic investigation using a Radiometer PHM82 pH meter fitted with a Russel CTWL electrode calibrated to standard buffer solutions.

UV–visible spectroscopic data were recorded using a Varian Cary 3 spectrometer fitted with a thermostated cell holder maintained at 30, 40, 50 or 60 °C. Stock solutions (~5×10^{-3} mol dm^{-3}) of kinetic substrates were prepared in acetonitrile. Kinetic runs were started by adding stock solution (10 μl) to preheated buffer solution (2 cm^3) in a quartz cuvette (1.0 cm path length). Repetitive wavelength scans were carried out in a series of buffer solutions to determine whether the reaction exhibited one or more isosbestic points and to allow the selection of an appropriate wavelength at which to record absorbance data *vs*. time. Experimental data were measured under pseudo-first order conditions and fitted to the appropriate equation using Kaleidagraph v.3.08 (Synergy Software). Correlation coefficients were better than 0.999 for all data used: accuracies are quoted as ± one standard deviation.

Data are summarised in Tables 1 and 2. Rate constants k_0 for the spontaneous hydrolysis of triester **7** were obtained over a range of pHs by extrapolation to zero buffer concentration of rate constants measured in buffered solutions of different concentrations. Some of these (k_0) data are not of high accuracy, because buffer catalysis is strong and intercepts relatively small at higher pH. But we have enough data points to define the simple pH–rate profile (Fig. 1) accurately. The measured pH varied significantly with concentration for buffers of low pK_a at low pH; and the minor variations at high pH were also significant because the concentration of the reactive, conjugate acid form of the substrate changes rapidly with pH above its pK_a. So for the calculation of second-order rate constants for buffer catalysis individual first-order rate constants were corrected by subtracting the rate constant for the spontaneous reaction. Second-order rate constants obtained in this way are collected in Table 2. Thermodynamic parameters were measured for the formate catalysed reaction, by measuring the rate of hydrolysis of **7** at a range of temperatures in 1 M buffer, 80% free base. The reaction is almost exclusively buffer catalysed under these conditions.

Acknowledgements

N. A. is grateful to the Engineering and Physical Sciences Research Council of Great Britain for the award of a Studentship.

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