Electrophilic Participation in Phosphonodiester Hydrolysis

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The enhancement in rate constant for the alkaline hydrolysis of diethyl 2-acetamidophenylphosphonate over that of the 4-isomer increases significantly with increasing dioxan content of the medium consistent with intramolecular assistance by the amido NH in the former reaction.

THERE is considerable experimental evidence for the concept of electrophilic assistance in the reaction of nucleophiles with carboxylate esters.[1] For some time people have also been seeking evidence for a related catalysis of nucleophilic attack, namely general acid assistance, where proton transfer and attack of nucleophile are concerted.[2] The former type may involve a proton, for example *via* a hydrogen bond, but the distinction between it and general acid catalysis is that the proton is not transferred and the hydrogen bond has the same sense in the transition-state and product as in the ground-state (Schemes 1 and 2).

Recent structural work with proteases [3] has indicated that the notion of electrophilic catalysis could provide, in part, some of the enormous driving force in these reactions. This work is also supported by studies of the electronic requirements of protease-catalysed hydrolyses.[4] The picture is not so clear as yet for the

(I)

SCHEME 1 Electrophilic catalysis

(II).

SCHEME 2 General acid catalysis

phosphate-transferring enzymes although *X*-ray crystallographic studies with ribonuclease [5] and staphylococcal nuclease [6] indicate that electrophilic groups (lysine,

[1] See A. Williams and G. Salvadori, *J.C.S. Perkin II*, 1972, 883 for a bibliography of work prior to 1972.

[2] (*a*) J. P. Fox and W. P. Jencks, *J. Amer. Chem. Soc.*, 1974, **96**, 1436; (*b*) W. P. Jencks, *Chem. Rev.*, 1972, **72**, 705.

[3] (*a*) J. D. Robertus, J. Kraut, R. A. Alden, and J. J. Birktoft, *Biochemistry*, 1972, **11**, 4293; (*b*) R. Huber, 6th Harden Conference, Wye, Kent, September 1974; (*c*) J. Drenth, H. M. Swen, W. Hoogenstraaten, and L. A. AE. Sluyterman, *K. ned. Akademie Van Wetenschappen*, 1975, **78C**, 104; (*d*) R. M. Stroud, L. M. Kay, and R. E. Dickerson, *J. Mol. Biol.*, 1974, **83**, 185; (*e*) M. Krieger, L. M. Kay, and R. M. Stroud, *ibid.*, 1974, **83**, 209; (*f*) T. A. Steitz, R. Henderson, and D. M. Blow, *ibid.*, 1969, **46**, 337; (*g*) L. Polgár and B. Asbóth, *J. Theor. Biol.*, 1974, **46**, 543; (*h*) L. Polgár, *Acta Biochim. Biophys. Acad. Sci. Hung.*, 1972, **7**, 29, 319; (*i*) A. Williams and G. Salvadori, *J. Chem. Soc.* (*B*), 1971, 2401.

[4] (*a*) R. E. Williams and M. L. Bender, *Canad. J. Chem.* 1971, **49**, 210; (*b*) A. Williams, *Biochemistry*, 1970, **9**, 3383; (*c*) A. Williams and G. Woolford, *J.C.S. Perkin II*, 1972, 272; (*d*) A. Williams, E. C. Lucas, and A. R. Rimmer, *ibid.*, 1972, 621; (*e*) H. C. Hawkins and A. Williams, *ibid.*, 1976, 723.

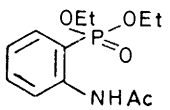
[5] F. M. Richards and H. W. Wyckoff, ' The Enzymes,' ed. P. D. Boyer, Academic Press, New York, 1971, 3rd edn., ch. 4, p. 647.

[6] F. A. Cotton and E. E. Hazen, ' The Enzymes,' ed. P. D. Boyer, Academic Press, New York, 1971, ch. 4, p. 153.

imidazolium, and arginine) lie close enough to the phosphate in the active site to interact *via* hydrogen bonding with the oxy-anion of the diester or its phosphoryl oxygen atom.

Metal ions provide another type of electrophilic catalyst and the three-dimensional structure of carboxypeptidase would appear to support activation of the substrate's amide carbonyl by interaction of the oxygen with zinc(II) at the active-site.[7] Although metal-ion activation has been postulated for a number of other hydrolases and transfer enzymes [8] there is little evidence save that the metal is required for enzyme action. The small electronic requirement in phosphate hydrolysis catalysed by bacterial alkaline phosphatase would seem to argue for electrophilic catalysis possibly by the essential zinc(II).[9a]

Here we examine a simple model of electrophilic assistance in phosphate hydrolysis; we compare the hydrolysis in alkali of 2-acetamidophenylphosphonate (III) and its 4-isomer in increasing dioxan concentrations in order to test the existence of intramolecular



(III)

electrophilic assistance in the former case. We choose this ester as a model because an acid stronger than the amide would be susceptible to ionisation and hence introduce ambiguities of interpretation. We use the neutral phosphonodiester rather than the anionic monoester because of the absence of similar ambiguities and because the higher rates of hydrolysis allow us to use less forcing conditions than are needed for the anionic substrates; under the latter conditions there is the possibility of C–O cleavage.

EXPERIMENTAL

Materials.—Reagents and buffers were of analytical reagent grade or were redistilled or recrystallised from bench grade products. 4-Nitrophenyl hippurate was from a previous study.[9b] Dioxan was freed from peroxides by passage through a column of neutral activated alumina; potassium iodide solution was employed to test for the absence of peroxides; water was twice distilled from glass.

Diethyl 2-nitrophenylphosphonate. 1,2-Dinitrobenzene (29.2 g) was recrystallised from ethanol and refluxed with triethyl phosphite (50 ml) and acetonitrile (100 ml) for 8.5 h in an atmosphere of nitrogen. The solvent was removed *in vacuo* and the remaining oil distilled from an oil-bath under reduced pressure to give an orange oil (b.p. 158 °C at 0.5 Torr) which solidified on cooling. The solid was recrystallised from light petroleum (b.p. 60—80 °C) to give colourless needles, m.p. 55 °C (lit.,[10] b.p. 128 °C, 0.02 Torr, m.p. 55—56 °C) in 71% yield.

Diethyl 2-aminophenylphosphonate. The usual methods for reducing aromatic nitro-groups to amino-functions failed for diethyl 2-nitrophenylphosphonate. Catalytic hydrogenation over 5% Pd/C at atmospheric pressure, sodium borohydride reduction in the presence of 5% Pd/C and Sn/HCl reduction all gave brown oils containing some amine derivative; distillation resulted in decomposition. A successful preparation involved ferrous sulphate/ammonia reduction as described by Smith and Opie for 2-nitrobenzaldehyde.[11] Diethyl 2-nitrophenylphosphonate (10.4 g, 40 mmol) was added to a solution of ferrous sulphate heptahydrate (105 g, 380 mmol) in water (175 ml) containing concentrated sulphuric acid (0.5 ml). The mixture was stirred and warmed to 90 °C in a water-bath and saturated ammonia solution (25 ml) was added, followed at two-minute intervals by three 10-ml portions of ammonia. The mixture was steam-distilled until 500 ml of distillate had been collected; the latter was extracted with ethyl acetate (3 × 200 ml) and the ethyl acetate layer dried (Na_2SO_4) and evaporated to give a yellow oil (100 mg). The residue from the steam-distillation was also extracted with ethyl acetate (5 × 200 ml) and 7.2 g of a brown oil was obtained which gave 5 g of a pale yellow oil (57%) on vacuum distillation (b.p. 124—128 °C at 0.01 Torr). A further preparation gave a 75% yield. The structure was confirmed by mass-spectral analysis and n.m.r. and i.r. spectroscopy.

Diethyl 2-acetamidophenylphosphonate. Diethyl 2-aminophenylphosphonate (11.5 g, 50 mmol) and triethylamine (5.1 g, 50 mmol) were dissolved in dry dichloromethane (28 ml). Redistilled acetyl chloride (3.9 g) in dry dichloromethane (200 ml) was then added dropwise to the stirred solution. After being stirred for 2 h the suspension was washed with 2M-HCl (2 × 50 ml), water (2 × 50 ml), and $NaHCO_3$ solution (2 × 50 ml). The solution was dried (Na_2SO_4) and evaporated to give a brown oil (11.3 g, 87%) which was vacuum distilled to give a pale yellow oil (b.p. 134—136 °C, 0.01 Torr) (Found: C, 53.2; H, 6.6; N, 5.4. $C_{12}H_{18}NO_4P$ requires C, 53.1; H, 6.6; N, 5.2%); i.r. and n.m.r. spectra are consistent with the proposed structure.

Diethyl 4-aminophenylphosphonate. A photolytic method previously described by Obrycki and Griffin [12] for the dimethyl derivative was employed. 4-Iodoaniline (22 g, prepared by the method of Vogel) [13] was dissolved in triethyl phosphite (100 ml). The solution was placed in a Pyrex vessel fitted with a Hanovia 450 W medium-pressure mercury arc housed in a quartz, water-cooled, jacket. Oxygen-free nitrogen was bubbled through the solution overnight to remove dissolved oxygen. Cooling water was passed through the jacket and the lamp ignited; after about 20 h of photolysis the lamp jacket became coated with crystals. These were scraped off and the presence of some sticky brown material suggested some decomposition; after a total of 40 h of photolysis only 3 g of crystalline product was obtained. The solution was vacuum distilled to remove triethyl phosphite and the product oil appeared

[7] W. N. Lipscomb, *Chem. Soc. Rev.*, 1972, **1**, 319.
[8] (*a*) J. E. Coleman, *M.T.P. Internat. Rev. Sci. Biochem.*, Series 1, 1974, **1**, 185; (*b*) I. M. Klotz and W. C. L. Wang, *J. Amer. Chem. Soc.*, 1954, **76**, 805.
[9] (*a*) A. Williams and R. A. Naylor, *J. Chem. Soc.* (*B*), 1971, 1973; (*b*) A. Williams, *J.C.S. Perkin II*, 1975, 947.
[10] J. I. G. Cadogan, D. J. Sears, and D. M. Smith, *J. Chem. Soc.* (*C*), 1969, 1314.
[11] L. I. Smith and J. W. Opie, *Org. Synth.*, Coll. Vol. 1955, **3**, 56.
[12] R. Obrycki and C. E. Griffin, *J. Org. Chem.*, 1968, **33**, 632.
[13] A. I. Vogel, 'Practical Organic Chemistry,' Longmans Green, London, 1956, 3rd edn., p. 647.

to be largely starting material. The solid material was recrystallised from carbon tetrachloride and had m.p. 120—122 °C (lit.,[14] 115—119 °C).

Diethyl 4-acetamidophenylphosphonate. The diethyl 4-aminophenylphosphonate was acetylated by the method used for the 2-amino-derivative. The product, obtained in 82% yield, was recrystallised from benzene–light petroleum (b.p. 60—80 °C) and had m.p. 138—140 °C (Found: C, 53.1; H, 6.5; N, 5.3. $C_{12}H_{18}NO_4P$ requires C, 53.1; H, 6.6; N, 5.2%); i.r. and n.m.r. spectra are consistent with the proposed structure.

Methods.—Microanalyses were carried out by Mr. G. M. S. Powell and Miss F. Duckworth using a Hewlett-Packard Model 185 CHN analyser; under ideal conditions the error is ±0.3% absolute. I.r. spectra were recorded on a Perkin-Elmer 257 spectrophotometer using Nujol mulls except where stated. ^{1}H N.m.r. spectra were measured by Mr. P. Horton and Mr. P. Simmonds on a Perkin-Elmer R10 instrument and mass spectra by Dr. R. B. Turner on an A.E.I. MS902 high resolution machine. M.p.s were determined with a Kofler Thermospan instrument.

Kinetic measurements were carried out by adding an aliquot of a stock solution of the substrate in dioxan (10—50 μl) to sodium hydroxide solution (2.5 ml) in a 1 cm path-length silica cell in the thermostatted cell compartment of a Unicam SP 800 spectrophotometer. Repetitive scans using 0.1M-NaOH at 25 °C indicated the best wavelength to follow the hydrolyses and first-order rate constants were estimated from plots of $\log_{10}(A_t - A_\infty)$ *versus* time at constant wavelength; values of optical density at infinite time were measured after at least 9 half-lives had elapsed.

RESULTS

Repeat scanning experiments on the 2- (and 4-)acetamido esters in 0.1M-NaOH indicated excellent isosbestic wavelengths consistent with 1 : 1 stoicheiometry for the reactions (Table 1). Hydrolysis followed at constant wavelength

TABLE 1

Spectral data for the hydrolysis of diethyl 2-(and 4-) acetamidophenylphosphonates [a]

Ester	$\lambda_{isosbestic}$/nm	$\lambda_{kinetic}$/nm	Δε [b]
2-Acetamido	225	250	5 410
	275		
	290.5		
4-Acetamido	250	275	4 210

[a] 0.1M-NaOH at 25 °C. [b] Measured at $\lambda_{kinetic}$.

was first order in ester up to *ca.* 90% of the total reaction and the first-order rate constants are proportional to hydroxide ion concentration up to 0.1M (0.1M ionic strength, 50 °C, see Figure 1). The second-order rate constants for hydroxide ion hydrolysis agree with those in the literature (3.75×10^{-2} l mol^{-1} s^{-1} at 100 °C) [15a] for similar reactions of ethyl phosphonates. Reaction of the *ortho-* and *para-*isomers with equivalent amounts of sodium hydroxide (in 0.1M-solution) gave material which when worked up yielded the sodium salts of ethyl 2-(and 4-) acetamidophenylphosphonates as characterised by their ^{1}H n.m.r. spectra (yield was *ca.* 90—100%). We therefore assign the kinetics to simple P–OEt cleavage. The hydroxide-ion rate constant was measured for both isomers in solutions of increasing dioxan content and the results are reported in Table 2; increasing dioxan reduced the rate constants in both cases.

The hydrolysis of 4-nitrophenyl *N*-benzoylglycinate in increasing dioxan concentrations at constant pH (tris-hydroxymethylaminomethane buffer) and ionic strength (0.1M) had k_{OH} = 7 200, 2 500, and 700 l mol^{-1} s^{-1} at 25 °C for 0, 20, and 40% dioxan respectively.

The n.m.r. spectrum of the two acetamido-esters in $CDCl_3$ was measured at different concentrations using tetramethylsilane as internal standard. The 4-isomer has a strongly concentration-dependent NH singlet (Table 3)

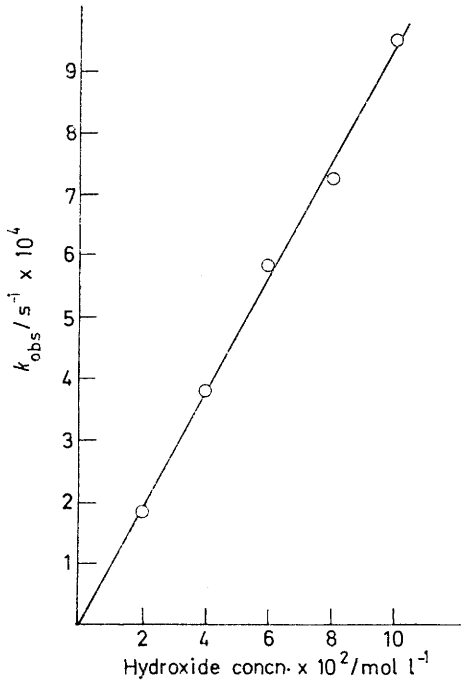


FIGURE 1 Alkaline hydrolysis of diethyl 2-acetamidophenylphosphonate at 50 °C, ionic strength made up to 0.1M with NaCl, substrate concentration 5.3 × 10^{-4}M

while that of the 2-isomer is relatively invariant. I.r. spectra ($CHCl_3$) also show a concentration-invariant peak for the 2-isomer (3 260 cm^{-1}) and a concentration-dependent peak for the 4-ester (3 250 to 3 300 cm^{-1}, 100 to 10 mg ml^{-1}). Unfortunately the P–O bands in chloroform were obscured by solvent cut-off but in CCl_4 the phosphoryl oxygen band is 17 cm^{-1} lower for the *ortho*-isomer than for the *para-* case (1 215 and 1 232 cm^{-1}) whereas the P–OEt bands are hardly affected.

DISCUSSION

The ratio of k_{OH} for 2-(and 4-) acetamido esters increases from 5 at zero dioxan content to 20 at 50% (Figure 2 and Table 2). Both isomers show a decrease in the alkaline hydrolysis rate as the polarity of the

[14] L. D. Freedman and H. H. Laffee, *J. Amer. Chem. Soc.*, 1955, **77**, 920.

[15] (*a*) R. F. Hudson and L. Keay, *J. Chem. Soc.*, 1956, 2463; (*b*) B. Capon and M. I. Page, *J. Chem. Soc.* (*B*), 1971, 741; (*c*) T. C. Bruice and T. H. Fife, *J. Amer. Chem. Soc.*, 1962, **84**, 1973.

solvent decreases (Figure 3) and the 4-isomer is more sensitive to the changing solvent composition than the 2-isomer.

The increase in the ratio of k_{OH} for 2- *versus* 4-acetamido-esters may be explained by the availability of internal stabilisation of the transition-state of the

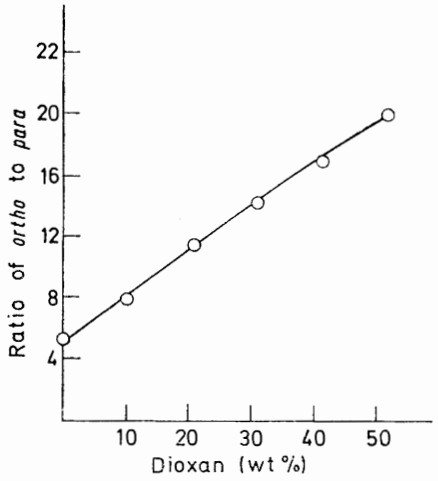


FIGURE 2 Variation of *ortho*/*para* ratio (see Table 2) with the dioxan content of the medium for the alkaline hydrolysis of diethyl 2-(and 4-) acetamidophenylphosphonates. For conditions see Table 2

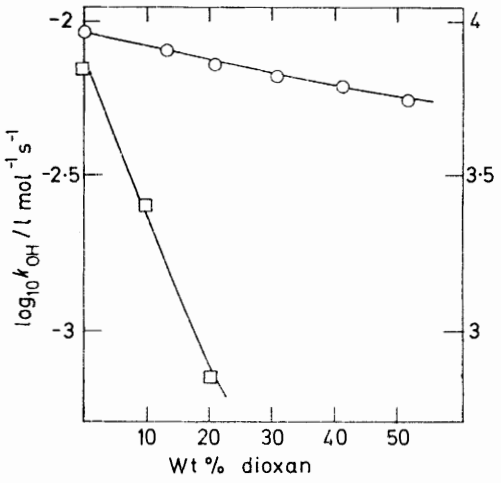


FIGURE 3 Dependence of hydroxide rate constants on dioxan content of the medium for the hydrolysis of diethyl 2-acetamidophenylphosphonate at 50 °C (○) and for 4-nitrophenyl *N*-benzoylglycinate (□) at 25 °C. Right-hand ordinate refers to the latter reaction and the left to the former; data are from Table 2 and the text

ortho-isomer which is not available for the *para*. Inspection of a Dreiding model of the diethyl 2-acetamidobenzoate indicates that the amido NH group is within hydrogen-bonding distance (*ca.* 0.15 nm) of either the P=O or P–OEt oxygens in the ground-state and a similar distance in the pentacovalent addition intermediate (presumably of similar structure to the transition-state). Since the ground- or transition-state will not have to rely totally on intermolecular solvation with the solvent molecules the change in solvent composition is not going to alter the relative energies of ground- and transition-states for the *ortho*-isomers as much as for the *para* where no such internal solvation may occur. This explanation has been advanced previously for hydrolyses of hydroxy-esters where the substrate has been supposed to carry its own solvation and hence be largely

TABLE 2

Rate constants for hydroxide ion-catalysed hydrolysis of diethyl 2-(and 4-) acetamidophenylphosphonates in dioxan–water mixtures [a–c]

Dioxan/wt%	0	10.33	20.73	31.01	41.34	51.68
2-ester						
k_{OH}/l mol^{-1} s^{-1} $\times 10^3$	9.4	8.2	7.4	6.6	6.2	5.5
4-ester						
k_{OH}/l mol^{-1} s^{-1} $\times 10^4$	18	10	6.5	4.6	3.7	2.8
k_{ortho}/k_{para}	5.2	8.2	11	14	17	20

[a] 50 °C 0.1M ionic concentration, except for the zero dioxan content the rate constants are from duplicate runs using 0.1M-NaOH. [b] The second-order rate constants lie within 5% of the quoted figures. There are considerably better confidence limits for the rate constants at zero dioxan. [c] Substrate concentrations of the order 10^{-4}M.

independent of solvent structure.[15b,c] Internal solvation has some support from n.m.r. (Table 3) and i.r. observations on the 2(and 4-) acetamido esters where intramolecular hydrogen bonding is seen to exist in the *ortho*-form but not in the *para*-.

There has been considerable discussion as to the site of electrophilic assistance by hydroxy and ammonium moieties in ester hydrolysis [1] and a similar choice is obtained here. Hydrogen bonding in the pentacovalent intermediate will be most stable between the NH and the oxy-anion since the latter is the most basic oxygen

TABLE 3

N.m.r. data for solutions of diethyl 2-(and 4-) acetamidophenylphosphonates [a]

Conc.[b]	NH	$POCH_2CH_3$	$POCH_2CH_3$	Amide CH_3
Diethyl 4-acetamidophenylphosphonate				
170	0.15	8.7	5.9	7.81
85	0.48	8.7	5.9	7.81
42.5	0.84	8.7	5.9	7.81
21.3	1.26	8.7	5.9	7.81
Diethyl 2-acetamidophenylphosphonate				
200	−0.67	8.69	5.88	7.82
100	−0.66	8.69	5.88	7.82
50	−0.66	8.69	5.88	7.82

[a] The measured chemical shifts are quoted in τ values; the solvent was $CDCl_3$ and tetramethylsilane was used as an internal standard. [b] Concentration is quoted in mg ml^{-1}.

(V). I.r. studies show that (IV) (where hydrogen bonding is between NH and the phosphoryl oxygen) is the most stable form. We may therefore picture a free-energy/reaction co-ordinate diagram involving these four species (Figure 4). If we assume that Hammond's

postulate [16] holds and that each transition-state is close to the least-stable state in a reaction pair then the mechanistic path directly from (IV) to (V) carries most of the reaction flux. This argument can be transferred to other cases of electrophilic assistance for example by amide NH, by ammonium, and by hydroxy on ester hydrolysis.[1] It might be argued that the

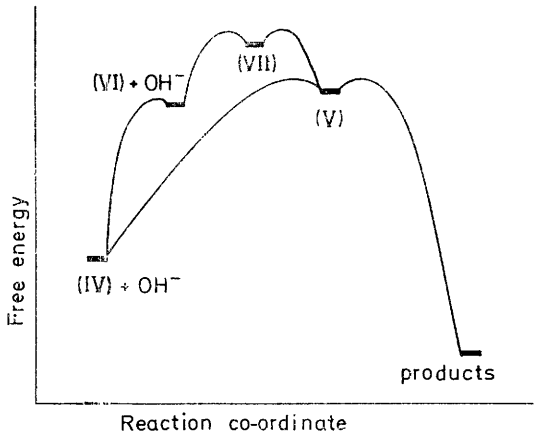
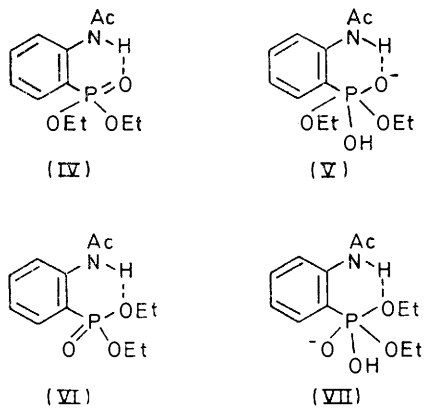


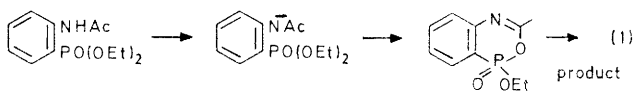
FIGURE 4 Reaction co-ordinate-free energy diagram for the attack of hydroxide on diethyl 2-acetamidophenylphosphonate; energy levels are identified in the text



hydrogen bond to the ether (VI) might raise the reactivity of the ester with hydroxide so much as to counteract any effect due to its presence in small concentration. However, the intermediate (VII) (hydrogen-bonded to ether oxygen) in this reaction has a higher energy than that derived from direct attack on the phosphoryl oxygen (V) and provided Hammond's postulate holds the transition-state for (IV) to (V) will always be lower than (VI) to (VII).

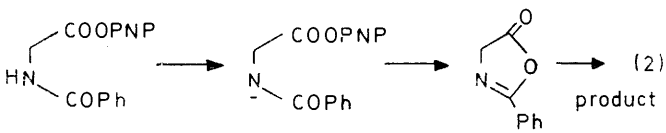
Simple electrostatic theory [17] predicts that the rate constant for an ion-dipole interaction should increase as the dielectric constant decreases. More complicated theories [18] discuss the reaction in terms of displacement of a solvent molecule by an ion from the solvated substrate to form the transition-state. Bender and Glasson [19a] proposed that the decrease in rate constant for alkaline hydrolysis of esters was due to the transition-state being more polar than the ground-state; if the solvation of the hydroxide ion was unchanged as the dioxan content increased any solvation differences would reside in the ground- and transition-states of the ester moiety of which the latter is the more polar.

It is possible that the 2-acetamido-ester hydrolyses *via* a different route from the 4-isomer and involves nucleophilic participation by the amide [equation (1)].



Undoubtedly this mechanism is a strong possibility for the phosphate esters with better leaving groups than ethanol. The cyclic intermediate [equation (1)] is likely to hydrolyse much faster than the free diethyl ester because the leaving isoamide oxy-anion group corresponds to that of a phenolate and presumably has a similar leaving capacity; in accord with this the 3,1-benzoxazin-4-one (VIII) [19b] hydrolyses some one thousand-fold faster than the corresponding ethyl 2-acetamidobenzoate (IX).[1] We therefore expect that the hydrolysis of the intermediate is fast enough to sustain the mechanism of equation (1).

It is expected that the combined ionisation (K_a) and cyclisation (k_2) steps of equation (1) will involve a greater solvent dependence than bimolecular hydroxide attack. We use as a model the hydroxide ion-catalysed hydrolysis of 4-nitrophenyl *N*-benzoylglycinate a reaction known to proceed *via* intramolecular nucleophilic attack on an ester by amide oxygen [equation (2)] to give oxazolinone.[9b] This is a good model because the

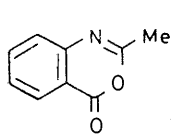


ionisation step is an exact analogue of that in equation (1); the intramolecular attack step should also have a similar solvent effect because hydroxide-catalysed hydrolysis of diethyl 4-acetamidophenylphosphonate and ethyl 4-acetamidobenzoate have almost identical solvent effects in dioxan–water mixtures (see Table 2 of this work and Table 2 of ref. 1).

The effect of increasing dioxan concentration on the alkaline hydrolysis of 4-nitrophenyl *N*-benzoylglycinate is compared with that for the hydrolysis of diethyl 2-acetamidophenylphosphonate in Figure 3. The rate constant derived from the mechanism of equation

[16] G. S. Hammond, *J. Amer. Chem. Soc.*, 1955, **77**, 334.
[17] A. A. Frost and R. G. Pearson, ' Kinetics and Mechanism,' J. Wiley, New York, 1961, 2nd edn., 147.
[18] (*a*) K. J. Laidler and H. Eyring, *Ann. New York Acad. Sci.*, 1940, **39**, 303; (*b*) E. S. Amis and G. Jaffee, *J. Chem. Phys.*, 1942, **10**, 588.
[19] (*a*) M. L. Bender and W. A. Glasson, *J. Amer. Chem. Soc.*, 1959, **81**, 1590; (*b*) A. Williams and G. Salvadori, *J. Chem. Soc.* (*B*), 1971, 1105; (*c*) L. G. Hepler, E. M. Woolley, and D. G. Hurkot, *J. Phys. Chem.*, 1970, **74**, 3908.

(2) ($K_a \cdot k_2/K_w$) owes its high solvent dependence to a large decrease in the K_a term as dioxan concentration is increased because the K_w term decreases [19c] thus tending to *increase* the composite observed rate constant.



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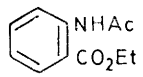
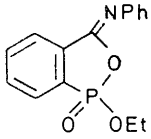


(IX)



(X)

Nucleophilic amide participation in phosphate ester hydrolysis has been noted by Kluger and Chan [20] where the leaving group is good (ethanol) in the acid-catalysed hydrolysis of diethyl 2-*N*-phenylcarboxamidophenylphosphonate. There is no evidence for the cyclic intermediate (X) in the form of a rate enhancement and this reaction ought to be more favourable than in our case; this provides further evidence against the mechanism of equation (1).

The hydrolysis of ribonucleic acid catalysed by ribonuclease consists of base removal of the proton of the 2′-hydroxy concerted with expulsion of the primary alcohol moiety to give the cyclic 2′,3′-nucleotide.*,[21] The driving force for this reaction may include electrophilic catalysis (as opposed to catalysis by proton donation by imidazolium—which undoubtedly occurs) by the ε-ammonium group of lysine-41 which is known to be close to the phosphate group at the active site in phosphate-inhibited enzyme.[5] Electrophilic catalysis could also involve one of the two imidazole groups (as imidazolium) which are also close to the active-site. This possibility has been cited previously [23] and recent work by Walter and Wold [24] has provided evidence for the participation of lysines (numbers 7, 41, 112 in the peptide backbone sequence) in the catalytic steps of ribonuclease action. A similar observation of arginine moieties (that is the guanidino-group) close to the phosphate in staphylococcal nuclease [6] suggests some sort of electrophilic assistance in this enzyme also. The advantage of such interactions is to reduce the charge on the phosphodiester nucleus to render it more susceptible to nucleophilic attack and the model work of this report is consistent with this activation.

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* This intermediate contributes to the major reaction flux but some 4% of the total flux proceeds *via* a route not involving this species.[22]

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