Intramolecular Nucleophilic Catalysis in the Hydrolysis of 4-Nitrophenyl Quinolin-8-yl Phosphate

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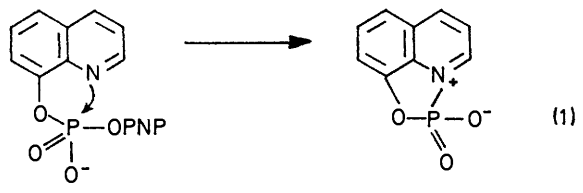
The hydrolysis of the title ester is shown to involve expulsion of 4-nitrophenol *via* intramolecular nucleophilic attack at phosphorus by the quinolinyl nitrogen. Strain in ring closure is proposed as the explanation for the less efficient action than carboxylate attack in the hydrolysis of 2-carboxyphenyl 4-nitrophenyl phosphate. The considerable mechanistic ambiguity due to the dibasic nature of the ester is resolved by comparison with appropriate models.

DIESTERS of phosphoric acid are notoriously stable to hydrolysis and transfer reactions [1] and yet these constitute some of the most important biological processes. The degradation of ribonucleic acid and deoxyribonucleic acid involves cleavage of a diester link as does cyclic 3',5'-adenosinemonophosphate. The latter reaction has a decisive role in regulating the concentration of the cyclic nucleotide, an important hormone.[2] Finally, reactivation of aged phosphorylated cholinesterases requires the hydrolysis of a phosphodiester [3] and a knowledge of the factors regulating phosphodiester hydrolysis has practical use in the design of therapeutic agents to ' phosphorus ' poisoning.

Intramolecular catalysis of phosphodiester hydrolysis has been demonstrated for neighbouring hydroxy-[4] and carboxy-groups.[5] The hydrolysis of nitrophenyl phosphodiesters is catalysed by pyridine type buffers *via* a nucleophilic pathway involving a phosphopyridine intermediate. Intramolecular catalysis by pyridine-like groups is therefore likely to involve nucleophilic attack unless this is constrained not to occur by steric or configurational reasons.

In this study we investigate the hydrolysis of 4-nitrophenyl quinolin-8-yl phosphate; we are interested in this ester because nucleophilic attack at phosphorus by the tertiary nitrogen produces a five-membered ring strained owing to the bridging of the 1- and 8-positions of the



(1) (1) PNP = 4-$NO_2C_6H_4$ (2)

quinoline nucleus. We observe that the strain is not sufficient to change the mechanism to, for example, general base catalysis but the efficiency of the mechanism is much reduced below that expected.

The decision as to whether catalysis involves nucleo-

[1] J. R. Cox and O. B. Ramsay, *Chem. Rev.*, 1964, **64**, 317.

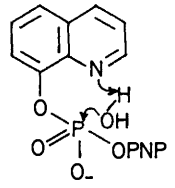
[2] G. I. Drummond and M. Yamamoto in ' The Enzymes,' ed. P. D. Boyer, Academic Press, New York, 1971, 3rd edn., vol. 4, p. 355.

[3] There are no reviews of a molecular nature on the problem of ' aging ' of phosphorylated cholinesterases but M. L. Bender and F. C. Wedler, *J. Amer. Chem. Soc.*, 1972, **94**, 2101, provide a useful introduction to the literature.

[4] (*a*) A. J. Kirby and S. G. Warren, ' The Organic Chemistry of Phosphorus,' Elsevier, Amsterdam, London, and New York, 1967, p. 339; (*b*) D. A. Usher, D. I. Richardson, and D. G. Oakenfull, *J. Amer. Chem. Soc.*, 1969, **92**, 469.

[5] S. A. Khan, A. J. Kirby, M. Wakselman, D. P. Horning, and J. M. Lawlor, *J. Chem. Soc.* (*B*), 1970, 1182.

philic attack [equation (1)] or general base catalysis (3) is not simple because the existence of ionising groups (POH and ≡NH⁺) allows for considerable ambiguity and a mechanism such as (4) gives the same kinetic rate law as (3) or (1).



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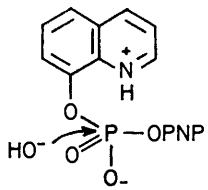


(4)

EXPERIMENTAL

Materials.—4-Nitrophenyl quinolin-8-yl phosphate was prepared by adding slowly, with stirring, a solution of 8-hydroxyquinoline (9 g, 63 mmol) in dichloromethane (50 ml) to a solution of 4-nitrophenyl phosphorodichloridate (16 g, 63 mmol) in dry dichloromethane (30 ml). A precipitate slowly appeared and after 1 h water was gently added to the mixture (cooled in ice). The precipitate dissolved and a further precipitate appeared. The *product* was a pale yellow crystalline solid which after recrystallisation from acetic acid gave a white crystalline powder, m.p. 198—200°. I.r., n.m.r., and mass spectra confirmed the identity of the material (Found: C, 51.6; H, 3.2; N, 8.0. $C_{15}H_{11}N_2O_6P$ requires C, 52.0; H, 3.2; N, 8.1%).

Phenyl 4-nitrophenyl phosphate was prepared by refluxing a mixture of 4-nitrophenyl phosphorodichloridate (28 g, 110 mmol), phenol (9.5 g, 100 mmol), and dry sodium chloride (0.5 g) as a catalyst using an oil-bath at a temperature which was slowly raised to 170°. After 6 h the reaction was complete as judged from the evolution of HCl fumes and volatile material was removed at 200° and 18 Torr. Phenyl 4-nitrophenyl phosphorochloridate was distilled at 203—209° and 1 Torr and crystallised on cooling and scratching, m.p. 78—80° (lit.,[6] 78—80°). The material (9.4 g, 30 mmol) was added in small portions to a solution of K_2CO_3 (4.25 g, 31 mmol) in water (15 ml) cooled in ice and stirred. The product was warmed to 75° and kept for *ca.* 30 min, then cooled, acidified with dilute sulphuric acid, and the precipitated oil extracted with diethyl ether. The ether extract was dried over Na_2SO_4 and evaporated. The resultant oil was induced to crystallise by cooling and scratching and the off-white solid recrystallised from benzene–light petroleum three times to yield needles, m.p. 98—101° (lit.,[6] 101—102°). The material was identified by i.r., n.m.r., and mass spectral analysis (Found: C, 48.7; H, 3.7; N, 4.7. Calc. for $C_{12}H_{10}NO_6P$: C, 48.8; H, 3.4; N, 4.7%).

Other reagents and buffer materials were of analytical reagent grade or were purified from bench grade reagents by distillation or recrystallisation. Deuterium oxide (99.8% D) was obtained from Prochem Ltd. and water, doubly distilled from glass, was used throughout the work.

Methods.—Measurements of pH and pD were carried out with a Pye Dynacap or Radiometer 25 pH meter both calibrated with E.I.L. buffer powders accurate to the second place of decimals. The pH meters were thermostatted. Measurement of pD utilised the meter reading and the equation: pD = meter reading + 0.4. M.p.s were determined using a Kofler Thermospan instrument and are corrected. Microanalyses were performed by Mr. G. M. Powell and Miss L. Tidy of this department using a Hewlett–Packard model 185 analyser. I.r. spectra were recorded on a Perkin-Elmer model 237 spectrophotometer using Nujol mulls or neat liquid samples. Mass spectra were measured by Dr. R. B. Turner using an A.E.I. MS902 high resolution mass spectrometer and n.m.r. spectra by Dr. D. O. Smith using a JEOL PS 100 MHz instrument or on a Perkin-Elmer R10 machine in both cases using tetramethylsilane as an internal or external standard.

Kinetics.—Hydrolysis of the esters was carried out in buffer (2.5 ml) in a silica cell in the thermostatted cell compartment of a Unicam SP 800 instrument. A portion of the ester (25—50 λ) on the flattened tip of a thin glass rod was introduced into the buffer (equilibrated for 15 min) and ' pumped ' three times to effect homogeneity. Repeat scans enabled selection of the best wavelength for fixed wavelength kinetic measurements and allowed us to detect possible intermediate build-up. First-order rate constants were obtained from plots of $A_t - A_\infty$ *versus* time on semi-logarithmic graph paper for the quinolinyl ester but the method of initial rates was required for the phenyl ester up to 0.1M-NaOH since the rate constants were so low even at 60°: *ca.* 4 mg of phenyl ester were accurately weighed (to 0.01 mg) into a volumetric flask (1 ml) and buffer added to the mark. Complete dissolution of the ester was ensured before placing the flask in a thermostatted water-bath set at 60°. Portions (50 λ) were pipetted into small sample tubes and frozen at −20°. After *ca.* 10 portions had been removed all samples were allowed to reach room temperature and borate buffer (2.5 ml) at pH 10.00 added. The optical density (A) at 400 nm, 25°, was measured using a Unicam SP 600 u.v. spectrophotometer. The infinity reading was measured by diluting the sample in 1M-NaOH at 60° and allowing complete reaction to occur. Suitable volume corrections were then made to enable the reading to correspond to those for the timed samples.

Measurement of the pK_a of the quinolinyl ester was carried out spectrophotometrically at 290 nm using a series of buffers in the pH range 2.7—5.8. The pK_a was estimated by a curve-fitting technique using a modification of an Algol program described by us.[7] ' Basic Language ' was employed using the University of Kent ' On-line ' system. Titration was used to try to determine the POH acidity but owing to the low pK_a of such groups the method was unsuccessful.[8] The ' kinetic ' pK_a was estimated *via* a similar manner to that from the spectrophotometric method.

RESULTS

Spectral scanning experiments indicated that both esters were hydrolysed with 1 : 1 stoicheiometry since good isosbestic wavelengths were observed. Also, the amount of 4-nitrophenol released was equivalent to the amount of ester used as substrate. The step measured in the kinetic experiments is therefore presumably the cleavage of the

[6] C. Stuebe, W. M. LeSuer, and G. R. Norman, *J. Amer. Chem. Soc.*, 1955, **77**, 3526.

[7] A. Williams, ' Introduction to the Chemistry of Enzyme Action,' McGraw-Hill, London, 1969, p. 123.

[8] (*a*) W. P. Jencks and J. Regenstein, ' Handbook of Biochemistry,' ed. H. A. Sober, Chemical Rubber Co., Cleveland, Ohio, 1970, 2nd edn., pp. J150—189; (*b*) A. Albert and E. P. Serjeant, ' The Determination of Ionisation Constants,' Chapman and Hall, London, 1971.

4-nitrophenyl ester linkage, the quinolinyl or phenyl cleavage providing a negligible contribution. The reactions followed good first-order kinetics up to *ca.* 90% of the total reaction when it was fast enough to follow to completion.

TABLE 1

Hydrolysis of 4-nitrophenyl quinolin-8-yl phosphate [a]

Buffer	pH	$10^4 k_0/s^{-1}$ [c]
1M-NaOH	13.02	5.0
0.1M-NaOH	12.02	4.8
0.1M-NaOH	12.02	4.0
Glycine	9.5	2.0
Tris [b]	7.92	3.5
Tris [b]	7.20	4.3
Tris [b]	7.00	2.8
Tris [b]	6.60	3.4
Tris [b]	5.98	2.7
Acetate	5.63	4.1
Acetate	4.88	3.7
Acetate	3.90	1.9
Formate	3.47	0.96
Formate	2.93	0.34
Glycine	2.43	0.084
Triethylamine (D_2O)	12.23	3.1
Triethylamine (H_2O)	11.53	3.3

[a] 60°, 0.1M ionic strength except for pH 13.02. [b] Tris = trishydroxymethylaminomethane. [c] Values are the average of duplicate runs.

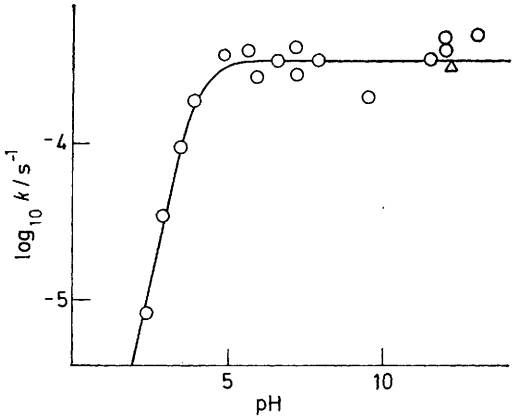


FIGURE 1 Hydrolysis of 4-nitrophenyl quinolin-8-yl phosphate at 60° over a pH range; ionic strength maintained at 0.1 except for pH 13.02 (1M-NaOH). Line is theoretical for an apparent pK_a 4.2 and $k_{plateau} = k_0 = 3.5 \times 10^{-4} s^{-1}$; ○ water solvent; △ deuterium oxide solvent

TABLE 2

Hydrolysis of 4-nitrophenyl phenyl phosphate [a]

Buffer	pH	$10^6 k_0/s^{-1}$ [c]
1M-NaOH	13.02	3 500
0.1M-NaOH	11.21	125
0.01M-NaOH	10.58	11
Carbonate	9.68	3.3 [d]
Tris [b]	7.97	3.9 [d]
Tris [b]	6.60	1.6 [d]

[a] 60°, ionic strength made up to 0.1M with NaCl except in the case of 1M-NaOH. [b] Tris = trishydroxymethylaminomethane. [c] Duplicate measurements; estimated errors are shown in Figure 2. [d] These are values extrapolated to zero buffer concentration.

The quinolinyl ester exhibited a sigmoid pH dependence (Table 1 and Figure 1) with a kinetically determined pK_a (4.20) which is close to the thermodynamic pK_a (4.34) measured spectrophotometrically. The phenyl ester pH dependence exhibited a plateau region and hydroxide ion-catalysed hydrolysis at high pH (Table 2 and Figure 2). No buffer catalysis was observed for the quinolinyl ester but perusal of Table 2 indicates such participation for the phenyl ester; the data for Figure 2 are from results extrapolated to zero buffer concentration.

Table 1 includes results for the effect of deuterium oxide solvent on the hydrolysis of the quinolinyl ester.

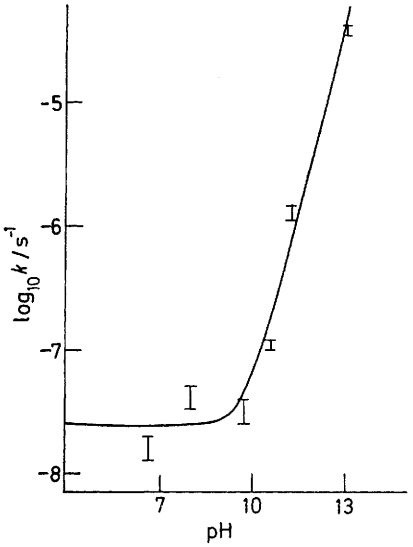
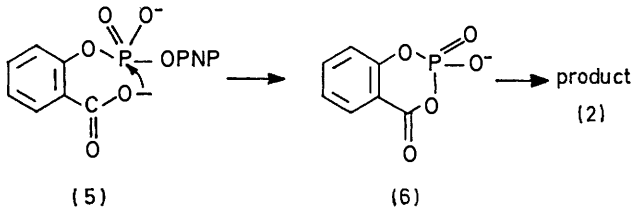


FIGURE 2 Dependence on pH of the hydrolysis of 4-nitrophenyl phenyl phosphate at 60°, ionic strength maintained at 0.1 except for pH 13.02 (1M-NaOH). Line is theoretical for $k_{plateau} = k_0 = 2 \times 10^{-8}$ s^{-1} and $k_{OH} = 5 \times 10^{-5}$ l mol^{-1} s^{-1}. Rate constants are those extrapolated to zero buffer concentration (see Table 2).

DISCUSSION

This study shows that the hydrolysis of 4-nitrophenyl quinolin-8-yl phosphate possesses a sigmoid pH dependence with a plateau well into the alkaline region consistent with intramolecular nucleophilic participation by the tertiary nitrogen to produce a reactive cyclic intermediate [equation (1)]. The mechanism is reasonable because it is known that both phosphodiesters and phosphomonoesters are susceptible to intermolecular nucleophilic attack by pyridines [9] to yield phosphopyridines which rapidly decompose to products.



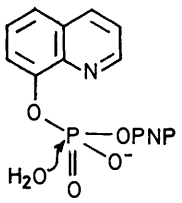
Catalysis by neighbouring carboxy-participation in the hydrolysis of 2-carboxyphenyl 4-nitrophenyl phosphate [equation (2)] is some 100-fold *more* efficient than

[9] (*a*) A. J. Kirby and M. Younas, *J. Chem. Soc.* (*B*), 1970, 1165; (*b*) A. J. Kirby and W. P. Jencks, *J. Amer. Chem. Soc.*, 1965, **87**, 3209; (*c*) G. DiSabato and W. P. Jencks, *ibid.*, 1961, **83**, 4393; (*d*) W. P. Jencks and M. Gilchrist, *ibid.*, 1965, **87**, 3199; (*e*) E. J. Behrman, M. J. Biallas, H. J. Brass, J. O. Edwards, and M. Isaks, *J. Org. Chem.*, 1970, **35**, 3069; (*f*) H. J. Brass, J. O. Edwards, and M. J. Biallas, *J. Amer. Chem. Soc.*, 1970, **92**, 4675.

that provided by the quinolinyl group in our system [equation (1)]. The different temperatures at which these compounds were investigated (39 and 60° respectively) makes this efficiency difference even greater. Normally the carboxylate is less nucleophilic than the pyridine-type nitrogen.[10] In the case of phosphoramidate hydrolysis Jencks and Gilchrist [9d] were unable to obtain detectable reaction with acetate and Kirby and Younas [9a] found acetate some 10^3-fold *less* effective than pyridine in the catalysed hydrolysis of methyl 2,4-dinitrophenyl phosphate. This difference presumably resides, in part, in the electrostatic repulsion felt by anionic substrate and the acetate. We propose that the reverse order of efficiency in the intramolecular attack is due to the difference in strain in forming the six- and five-membered ring transition states in carboxylate and quinolinyl participation respectively. Perusal of Dreiding models of the two cyclic transition states indicates this to be so with an angle strain of *ca.* 30° in the quinolinyl case and complete freedom in the carboxylate.

The pyridine-catalysed hydrolysis of methyl 2-nitrophenyl phosphate at 39° (4.38×10^{-8} l mol^{-1} s^{-1}) [9a] allows us to estimate an upper limit for the effective molarity of the intramolecular reaction compared with the intermolecular case (*ca.* 7 000). This is an upper limit because the intermolecular hydrolysis rate constant is for 39° and the model is a methyl rather than a phenyl ester; also the 2-nitrophenyl group probably makes the ester slightly less reactive than the 4-nitrophenyl ester. This upper limit is rather small compared with other effective molarities for nucleophilic attack [11] and part of this is presumably due to angle strain in forming the five-membered ring transition state. The low effective molarity would also be consistent with a 'loose' transition state; reactions of pyridines with phosphodiesters, phosphonomonoesters, and phosphomonoesters have low β_{nuc} values [9] consistent with only partial P–N bond formation in the transition state.

Alternative Mechanisms.—The simple rate law involving monoanionic decomposition of the quinolinyl



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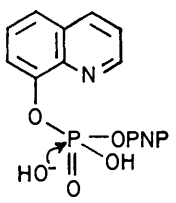
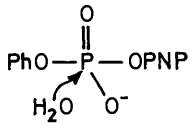


(8)

ester could result from a number of equivalent mechanisms including nucleophilic catalysis [equation (1)]. These involve water attack on the monoanion (7), water attack on the monoanion catalysed by tertiary nitrogen (3), hydroxide attack on the zwitterion (4), or on the neutral species (8). The deuterium oxide solvent isotope effect (Table 1) seems to rule out the intramolecular general base mechanism (3) and the very high rate constant for the plateau (3.5×10^{-4} s^{-1}) compared with that for phenyl 4-nitrophenyl phosphate (2×10^{-8} s^{-1}) would remove the uncatalysed possibility (7). We are sure that the hydrolysis of phenyl 4-nitrophenyl phosphate is attack of water on the anion (9) rather than hydroxide attack on the neutral species (10) because the bimolecular rate constant for the latter is related to the observed plateau rate constant (k_0, see Table 2 and Figure 2) by the equation: $k = k_0 K_a^{POH}/K_w$ * and has the value 2×10^4 l mol^{-1} s^{-1} which is four orders of magnitude larger than for the reaction of hydroxide with methyl 4-nitrophenyl phenyl phosphonate (0.15 l mol^{-1} s^{-1} at 60°) [12] which, with a P–C link, should be *more* reactive than the true model with a P–O link.



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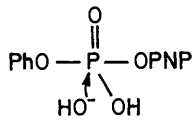

(10)

Hydroxide attack on the zwitterionic species (4) has the rate constant $k = k_0 K_a^{QH}/K_w$ and has the value 3.5×10^5 l mol^{-1} s^{-1} (for k_0 see Table 1) which is some ten orders of magnitude in excess of that for hydroxide attack on phenyl 4-nitrophenyl phosphate and it is inconceivable that $\equiv NH^+$ is such an exceptionally good electrophilic catalyst. Only some 20-fold enhancements have been found for example with amides acting as electrophiles in phosphonodiester hydrolysis [13] and in carboxylic ester hydrolysis [14] and we therefore rule out this mechanism. Similarly, the rate constant for attack of hydroxide on the neutral species (8) may be estimated from the equation: $k = k_0 K_a^{POH}/K_w = 3.5 \times 10^8$ l mol^{-1} s^{-1} which is some seven orders of magnitude larger than for reaction of hydroxide with methyl 4-nitrophenyl phenyl phosphonate (0.15 l mol^{-1} s^{-1} at 60°) [12] which is more reactive than the true model with a P–O link.

Absence of Hydroxide Ion Catalysis in the Quinolinyl Ester.—No hydroxide term is noticed in the hydrolysis of the quinolinyl ester and this may be explained on the grounds that it is too small to compete with the intramolecular catalysis even at the high pH values employed. 4-Nitrophenyl phenyl phosphate has a rate constant of 5×10^{-5} s^{-1} at 1M-NaOH representing hydroxide reaction with the monoanion and is a good model of the reaction with the quinolinyl monoanion. Thus the hydroxide reaction with the latter will contribute only

* We assume in these calculations that the pK_a of the POH group is unity [8] because it proved impossible to measure this value (see Experimental section); we use $pK_w = 13.02$ for 60°. K_a^{POH} and K_a^{QH} represent ionisation constants for phosphate and quinolinyl conjugate acids respectively.

[10] W. P. Jencks and J. Carriuolo, *J. Amer. Chem. Soc.*, 1960, **82**, 1778.
[11] M. I. Page. *Chem. Soc. Rev.*, 1973, **2**, 295.
[12] (*a*) J. S. Loran, Ph.D. Thesis, University of Kent, 1975; (*b*) see also ref. 1 for similar values.
[13] R. A. Naylor and A. Williams, *J.C.S. Perkin II*, 1976, in the press.
[14] A. Williams and G. Salvadori, *J.C.S. Perkin II*, 1972, 883.

ca. 10% of the total reaction flux and hardly be noticeable. A similar explanation holds for the absence of a hydroxide term in the hydrolysis of 2-carboxyphenyl 4-nitrophenyl phosphate.[5]

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