Intramolecular Participation of the Amide Group in Acid- and Base-catalysed Phosphonate Monoester Hydrolysis

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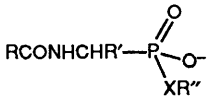
Rates of hydrolysis of monoaryl α-amidophosphonates in both acid and alkaline solutions are much greater than those of comparable compounds lacking the amido substituent; intramolecular nucleophilic catalysis seems likely. This phenomenon may be important in the employment of α-amidophosphonate derivatives as enzyme inhibitors.

Phosphonate monoester and phosphonamidate monoanions, in the form of depsipeptide and peptide analogues respectively (**1**), have been much employed as inhibitors of hydrolytic



1a X = O
1b X = NH

enzymes in recent years.[1–3] In most cases,[1,2] no cleavage of the phosphonyl derivative has been observed, and the inhibitors bind non-covalently as substrate or transition state analogues. In at least one case however, that of the β-lactamases,[3] inhibition arises through phosphonylation of the enzyme; slow hydrolytic turnover is also observed. The resistance of **1** to hydrolytic enzymes is in accord with the well-known low susceptibility of phosphonate monoanions to nucleophilic attack at phosphorus.[4] Phosphonates of structure **1** however do present the potential for intramolecular catalysis by the amido group. We provide evidence here for this phenomenon; compounds **1a** are much more labile in both acidic and basic media than would be predicted on the basis of the reactivity of phosphonates lacking the amide group.

Experimental

Synthesis.—N-(*Phenylacetyl*)*aminomethylphosphonic acid.* A mixture of aminomethylphosphonic acid (2 g, 18 mmol), water (10 cm^3), sodium hydrogencarbonate (5 g, 60 mmol), potassium carbonate (11 g, 5 mmol) and phenylacetyl chloride (3.86 g, 25 mmol) was stirred at room temperature for 4 h. Water was then added to *ca.* 100 cm^3 and the pH of the solution lowered to *ca.* 2 by addition of concentrated HCl. A further 10 cm^3 of concentrated HCl was then added and the mixture evaporated to dryness by rotary evaporator and then oil pump. The solid residue was extracted with 100 cm^3 of methanol and the mixture filtered. The solid obtained on evaporation of the methanol was extracted with 100 cm^3 of boiling 1:1 methanol–acetonitrile and the hot solution filtered. Evaporation of the filtrate yielded *ca.* 3 g of a white solid of m.p. 155–160 °C. The ^1H NMR spectrum (below) showed it to contain the required product as the only organic component. This material was adequate for the syntheses described below. Recrystallization from acetonitrile yielded colourless needles, m.p. 165–166 °C; $\delta_H(D_2O)$ 3.57 (d, *J* 12 Hz, 2 H, CH_2P), 3.65 (s, 2 H, CH_2Ph), 7.4 (m, 5 H, Ph).

Sodium aryl N-(*phenylacetyl*)*aminomethylphosphonates.* The preparation of the aryl phosphonates was based on the method described by Wasielewski *et al.*[5] In a typical synthesis, a mixture of *N*-(phenylacetyl)aminomethylphosphonic acid (0.2 g, 0.8 mmol), the phenol (0.8 mmol), trichloroacetonitrile (0.88 cm^3) and pyridine (distilled from BaO, 3 cm^3) was heated in an oil bath at 100 °C for *ca.* 6 h. The reaction mixture was then dried by rotary evaporation and the residue partitioned between saturated aqueous sodium hydrogencarbonate and ethyl acetate (3–5 cm^3 each). The layers were separated and the aqueous layer extracted several more times with ethyl acetate before being freeze-dried. The dry residue was extracted with ethanol at room temperature and the ethanol extracts evaporated to dryness. The residue, which contained the desired product, was then purified by Biogel P-2 chromatography (elution by water). Column fractions containing the required product were pooled and freeze-dried, yielding hygroscopic sodium salts. These were unsuitable for combustion microanalysis and consequently were characterized by NMR and mass spectroscopy. ^1H NMR spectra of the dried sodium salts showed that the desired compounds were the only organic species present, except for traces of the parent phenol in the cases of the more reactive *ortho*-nitrophenyl and *para*-nitrophenyl derivatives. FAB mass spectra showed the expected molecular ions. ^1H NMR spectra (400 MHz) are reported below; sodium 3-(trimethylsilyl)-1-propane sulphonate was used as an internal standard and coupling constants are in Hz. The carboxyphenyl esters were prepared similarly, as described elsewhere (ref. 3 and J. Rahil and R. F. Pratt, manuscript in preparation).

Sodium phenyl N-(*phenylacetyl*)*aminomethylphosphonate* (**2a**). $\delta_H(D_2O)$ 3.58 (d, *J* 12, 2 H, CH_2P), 3.60 (s, 1 H, CH_2Ph), 7.0–7.4 (m, 10 H, 2 Ph).

Sodium m-*nitrophenyl* N-(*phenylacetyl*)*aminomethylphosphonate* (**2d**). $\delta_H(D_2O)$ 3.58 (s, 2 H, CH_2Ph), 3.63 (d, *J* 12, 2 H, CH_2P), 7.2–8.0 (m, 9 H, 2 ArH).

Sodium o-*nitrophenyl* N-(*phenylacetyl*)*aminomethylphosphonate* (**2e**). $\delta_H(D_2O)$ 3.60 (d, *J* 12, 2 H, CH_2P), 3.63 (s, 2 H, CH_2Ph), 7.3–8.0 (m, 9 H, 2 ArH).

Sodium p-*nitrophenyl* N-(*phenylacetyl*)*aminomethylphosphonate* (**2f**). $\delta_H(D_2O)$ 3.57 (s, 2 H, CH_2Ph), 3.65 (d, *J* 12, 2 H, CH_2P), 7.11 (d, *J* 9, 2 H, $ArNO_2$), 7.3–7.4 (m, 5 H, Ph), 8.12 (d, *J* 9, 2 H, $ArNO_2$).

Triethylammonium p-*nitrophenyl* N-(*benzyloxycarbonyl*)-*aminomethylphosphonate* (**2g**). *N*-(Benzylcarbonyl) aminomethylphosphonic acid was prepared from reaction of benzyloxycarbonyl chloride with aminomethylphosphonic acid, as described above for the *N*-phenylacetyl analogue. In the present case, however, the required product precipitated directly on acidification of the reaction mixture with hydrochloric acid. This material, m.p. 178–180 °C after drying *in vacuo*, was directly employed in the next step of the synthesis. The condensation of *N*-(benzyloxycarbonyl)aminomethylphosphonic acid with *p*-nitrophenol was carried out in the presence of trichloroacetonitrile, as also described above. The crude product was purified by anion exchange chromatography (elution by a 0–1

mol dm^{-3} triethylammonium hydrogencarbonate gradient from a Sephadex QAE-25-120 column). The purified, but also hygroscopic, triethylammonium salt yielded the expected FAB mass and ^1H NMR spectra. $\delta_H(D_2O)$ 3.53 (d, *J* 12, 2 H, CH_2P), 5.03 (s, 2 H, CH_2O), 7.18 (d, *J* 9, 2 H, $ArNO_2$), 7.3–7.5 (m, 5 H, Ph), 8.11 (d, *J* 9, 2 H, $ArNO_2$).

Triethylammonium p-*nitrophenyl methylphosphonate* (**2i**). A solution of *p*-nitrophenol (2.78 g, 20.0 mmol) and freshly distilled triethylamine (3.1 cm^3, 22 mmol) in dry diethyl ether (50 cm^3) was added dropwise with stirring to a solution of methylphosphonic dichloride in dry diethyl ether (50 cm^3) at 0 °C. After 1 h, triethylammonium chloride was removed by filtration and 1 mol dm^{-3} aqueous sodium hydroxide (44 cm^3) was added to the ether layer with stirring. The diethyl ether was removed by rotary evaporation, the pH of the solution was adjusted to pH 7–8 with saturated aqueous sodium hydrogencarbonate solution, and the neutral solution freeze-dried. The product was purified by anion exchange chromatography as described above. The purified product had the expected FAB mass and ^1H NMR spectra. $\delta_H(D_2O)$ 1.46 (d, *J* 17, 3 H, CH_3P), 7.27 (d, *J* 9, 2 H, $ArNO_2$), 8.21 (d, *J* 9, 2 H, $ArNO_2$).

Kinetics.—Concentrated stock solutions of the phosphonates were prepared in water. Since the phosphonate salts were hygroscopic, solutions of accurately known concentrations could not readily be prepared by direct weighing. Concentrations of stock solutions were therefore determined from the absorption of the phenoxides after complete hydrolysis of the phosphonates in alkali. Extinction coefficients of the relevant phenoxides were determined using solutions prepared from purified phenols.

The hydrolyses of **2a**–**2g** were followed spectrophotometrically at wavelengths appropriate to the phenol(phenoxide) being formed and the pH. In a typical kinetic run, a small aliquot (5–10 mm^3) of a phosphonate stock solution (yielding a final concentration of *ca.* 0.1 mmol dm^{-3}) was added to a thermostatted (25.0 °C) cuvette containing 1.0 cm^3 of acid or alkali in the cell compartment of a Perkin-Elmer Lambda 4B spectrophotometer. After initiation of the reaction in this way, the absorbance at the appropriate wavelength was followed for at least five half-lives of the reaction. Hydrolyses were carried out in hydrochloric acid and potassium hydroxide solutions of concentrations between 0.01 and 1 mol dm^{-3}. The ionic strength of these solutions was maintained at 1.0 by addition of potassium chloride. Pseudo-first-order rate constants were derived from the recordings of absorbance *vs.* time by means of a non-linear least squares procedure[6] which treated the final absorbance and the rate constant as adjustable parameters. Rate constants for **2h** and **2i** were obtained from initial rate measurements on solutions prepared as described above.

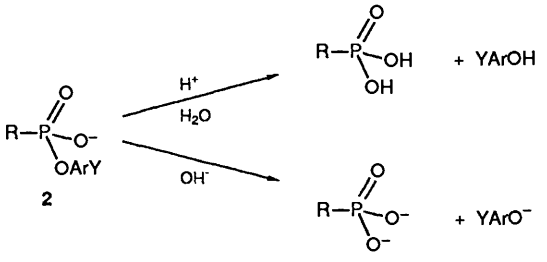
Results and Discussion

A series of monoaryl phosphonates **2** was prepared as described above and their hydrolyses studied under acidic and basic conditions. ^1H NMR studies showed that the sole products of hydrolysis under both of these regimes were the phosphonic acid and the phenol (Scheme 1). Kinetic measurements showed that the hydrolysis reactions were first order in hydrogen ion and hydroxide ion, respectively. The pK_as of these amides are most likely greater than 14,[7] and thus no deviation from first order behaviour would be expected in the hydroxide solutions employed. Second-order rate constants for the two types of hydrolysis are reported in Table 1.

It is clear, first, from the data for compounds **2a**–**2f**, that both the acid and base-catalysed reactions are accelerated by electron-withdrawing substituents in the leaving group, the former to a lesser extent than the latter. This difference in sensitivity would be expected if protonation of the substrate were required for the former. ρ_{lg} values were calculated, taking the data for compounds **2a**–**2d** and **2f**, to be 2.2 ± 0.4 for the base-catalysed hydrolysis and 0.4 ± 0.1 for the acid. In both instances, a better (least squares) correlation was obtained, giving the quoted ρ_{lg} values, on using σ^- parameters for the leaving group, rather than σ, in the Hammett plots. This indicates the importance of bond-breaking to the leaving group in the transition states of both mechanisms. This point is also made for the base-catalysed mechanism by a β_{lg} value (determined from a plot of log k_{OH^-} *vs.* pK_a of the phenol corresponding to the leaving group) of 1.0 ± 0.1. This also indicates significant P–OAr bond fission in the transition state, probably to an extent greater than the half-way point.[8]

Of more immediate significance however is the considerable difference in rate, both in acid and in base, between compounds **2b** and **2h**, both with *meta*-carboxyphenoxide leaving groups, and between **2f** and **2i**, both with *para*-nitrophenoxide leaving groups. It is likely that little of this difference can be due to the greater electron withdrawing power of the alkyl substituent in **2b** and **2f** because compounds **2f** and **2g**, with the same leaving group and very similar alkyl substituents, also hydrolyse at quite disparate rates, again both in acid and in base. A rate increase of 5–10 fold would probably be produced in the alkaline hydrolysis of **2h** or **2i** on addition of the acylamido substituents present in **2a**–**2g**. This estimate was made on the basis of inductive substituent effects on the dissociation of phosphonate monoanions,[9] of carboxylic acids,[9] and on the rates of alkaline hydrolysis of ethyl acetates.[10] The influence of this change on the acid-catalysed reaction would presumably be less, as seen above in the leaving group substituent effects, because the substituent would have opposing effects on the protonation and nucleophilic attack steps.

We therefore interpret the data of Table 1 to imply that the acylamido group of **2a**–**2f**, absent in **2h** and **2i**, promotes phosphonate ester hydrolysis by some mechanism other than a simple inductive effect. Since **2g** is so much less reactive than **2f**, nucleophilic participation is suggested: it would be expected on the basis of analogy with other systems[11,12] that such participation in **2g** would be significantly less effective than in **2f**. Such intramolecular catalysis would presumably first involve amide anion attack at phosphorus in the hydroxide ion mediated hydrolysis, probably aided by general-acid participation by water (**3**), and neutral amide attack on the protonated phosphonate, under the acidic regime (**4**). In each case, after



Scheme 1

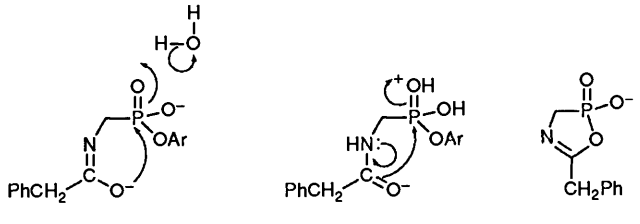


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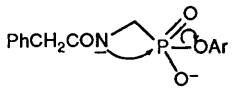
Table 1 Second-order rate constants for hydrolyses of aryl phosphonates catalysed by hydrogen and hydroxide ions

Compound	R	Y	k_{OH^-}/dm^3 mol^{-1} min^{-1}	k_{H^+}/10^{-2} dm^3 mol^{-1} min^{-1}
2a	$PhCH_2CONHCH_2$	H	0.083	0.86
2b	$PhCH_2CONHCH_2$	*m*-CO_2^-	0.24	1.48
2c	$PhCH_2CONHCH_2$	*p*-CO_2^-	0.94	1.47
2d	$PhCH_2CONHCH_2$	*m*-NO_2	8.32	1.83
2e	$PhCH_2CONHCH_2$	*o*-NO_2	175	11.8
2f	$PhCH_2CONHCH_2$	*p*-NO_2	120	2.94
2g	$PhCH_2OCONHCH_2$	*p*-NO_2	0.88	$<2 \times 10^{-3}$
2h	CH_3	*m*-CO_2^-	1.4×10^{-5}	$<2 \times 10^{-3}$
2i	CH_3	*p*-NO_2	1.7×10^{-3}	$<2 \times 10^{-3}$

departure of the leaving group from the pentacoordinated intermediate (or transition state), which, from the substituent effects, is likely to be, at least partly, rate determining, a cyclic phosphonate intermediate **5** would remain. This presumably would react rapidly with water to give *N*-(phenylacetyl)-aminomethylphosphonate, the observed product. Under alkaline conditions at least, **5** could not be detected by ^{1}H NMR spectroscopy during hydrolysis of **2f**. Although there seem to be no exact analogues of **5** in the literature, five-membered cyclic phosphates and phosphonates in general are known to hydrolyse rapidly.[13]

An alternative mechanism in base would involve intramolecular nucleophilic attack by the nitrogen atom of the amide anion (**6**), leading to an azaphosphiridine oxide intermediate (**7**). Derivatives of the latter, either unsubstituted



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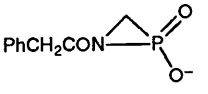

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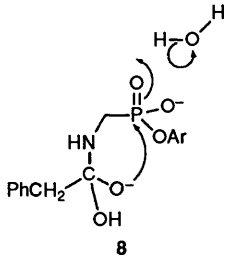
at nitrogen, *N*-alkylated or *N*-arylated, have been proposed as intermediates in rearrangements of α-chlorophosphonamidates.[14] *N*-Acyl derivatives, such as **7**, would however be much less stable. The mechanism represented by **6** would therefore seem unlikely in the present case, despite the fact that the nucleophilic centre of amide anions is usually the nitrogen atom.[15] It might be noted that the cyclization analogous to **6** does not occur in acyl systems,[12] probably for the analogous reason, the instability of the intermediate *N*-acylaziridinone. Even in intramolecular alkylation of amide anions, oxazolines, from oxygen participation, are often obtained rather than aziridines.[11,16] At any event, the rate difference between **2f** and **2g** is less suggestive of amide anion nitrogen participation than of oxygen participation.[11] Another alternative possibility in base, that of cyclization through a tetrahedral intermediate generated by hydroxide attack on the amide carbonyl group (**8**), seems unlikely because of the instability and short lifetime



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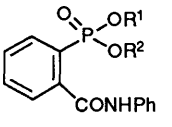
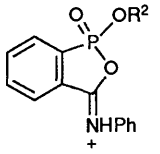
of such intermediates;[17] such a mechanism would be more likely with an adjacent aldehyde or ketone carbonyl group where the tetrahedral adducts are stable.[18]

Rates of hydrolysis of **2** (R = $PhCH_2CONHCH_2$) in neutral buffered solution were also much greater than those of **2** (R = CH_3), suggesting that spontaneous water and/or buffer-catalysed hydrolysis of **1** also proceeds with intramolecular amide catalysis. Bartlett and co-workers[2] have previously suggested intramolecular amide participation in phosphonate hydrolysis on the basis of the instability in neutral solutions of certain phosphonopeptides **1b**.

There are other precedents for intramolecular nucleophilic amide catalysis of phosphonate ester hydrolysis. Kluger and Chan[19] demonstrated amide participation in the acid catalysed hydrolysis of phosphonates **9**. Here the intermediate is an exocyclic imidate, **10**, rather than endocyclic as in **5**. Interestingly, in these cases, no catalysis of alkaline hydrolysis by the *o*-amido group was observed. This may simply reflect a strong leaving group dependence, since Steinberg *et al.*[20] reported that **11**, which would also yield an exocyclic imidate



9 R^1 = alkyl
R^2 = H or alkyl



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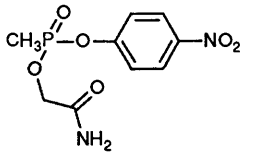

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intermediate, hydrolysed in neutral buffer more than 100-fold more rapidly than alkyl *p*-nitrophenyl methylphosphonates. Kluger and co-workers have also demonstrated amide participation in the acid-catalysed hydrolysis of phosphoenolpyruvamides[21] and urea oxygen participation in the hydrolysis of ureidophosphonates.[22]

Thus, intramolecular amide group participation is likely to be a general phenomenon in the reactions of amidophosphonates where a five-membered cyclic intermediate can be generated; rate accelerations of at least 10^4 are apparently possible. The intervention of this possibility should be borne in mind in all reactions of **1**, including those where XR″ represents the functional group of a modified enzyme.

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