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The spontaneous hydrolysis of 2-pyridyl phosphate is a good model for the special mechanism for the hydrolysis of phosphate monoester monoanions^{†‡}

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The microscopic pK_a of the pyridine N of the phosphate monoester 2-pyridyl phosphate (MPP) is 2.76, significantly higher than that expected for the leaving group (RO) oxygen of a typical monoester $ROPO_3^=$. This favours the prototropic equilibrium considered to be the key step in the rapid hydrolysis of a typical monoester, and the observed rate of hydrolysis of the monoanion MPP⁻ is shown to be close to that expected for the protomer MPP⁺⁼, with neutral 2-pyridone as the leaving group. Copyright © 2013 John Wiley & Sons, Ltd. Supporting information may be found in the online version of this paper.

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INTRODUCTION

Two defining points for the reactivity of phosphate monoesters, established many years ago, are the rate maximum in the region of pH=4 for the hydrolysis of the typical monoanion and the very high sensitivity of the rates of hydrolysis of the dianion to the pK_a of the leaving group.^[1–3] The high sensitivity observed for the reactions of phosphate ester dianions, combined with a sensitivity to the basicity of the nucleophile that is close to zero, can be understood in terms of a transition state for the S_N2(P) reactions of monoester dianions characterised by weak bonding to both incoming nucleophile and leaving group (Scheme 1).^[2,3]

Our recent work on the reactivity of phosphate triesters has focussed attention on the contributions of the non-leaving groups to the phosphate transfer process. In the case of triesters, this can be comparable with that of the leaving group itself,^[4] while the single non-leaving group present in the corresponding reactions of diesters has minimal effect on reactivity.^[5] This simple analysis excludes reactions of monoesters because there are no non-leaving groups present. None, that is, apart from the OH and O⁻ groups attached to P, which are always present and cannot be varied except by isotopic substitution. However, these groups also have major effects on reactivity and are indeed responsible for the defining points referred to earlier.

The high sensitivity to leaving group capability for the hydrolysis of monoester dianions, which makes esters of phenols with $pK_a < 5.45$ more reactive than the corresponding monoanions,^[2] is the result of the multiple $n-\sigma^*_{P-O}$ interactions available to a dianion, which lengthen and weaken the P—OAr bond in the ground state (Scheme 2).^[6]

The same mechanism is thought to account also for the much faster hydrolysis of alkyl monoester monoanions, which are believed to react via an initial proton transfer equilibrium to form the highly reactive $(ROH)^+PO_3^=$ form $(M^{+-}, Scheme 3)$. This

pre-equilibrium appears to account quantitatively for the observed rates of the reaction. $\ensuremath{^{[2]}}$

We have been interested recently in the mechanisms of hydrolysis of esters of 2-hydroxypyridine.^[4,7] Tri-2-pyridyl phosphate is hydrolysed to the di-pyridyl DPP without significant involvement of the pyridine nitrogens, because these are very weakly basic in the triester ($pK_a = -0.22$).^[4] But the more basic ($pK_a = 2.73$) pyridine nitrogens of DPP do appear to play a role, as general bases, in the hydrolysis of the diester. Which is hydrolysed at 25 °C some 10 000 times faster than expected for a diaryl phosphate with leaving groups of $pK_a = 9.09$.^[5] The immediate product of this reaction (Scheme 4) is the monoester 2-pyridyl phosphate (MPP), but this is hydrolysed faster than DPP over almost the complete pH region (Fig. 1), and so does not accumulate

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Scheme 1. The transition state for $S_N2(P)$ reactions of the dianions of phosphate monoesters is characterised by weak bonding to both leaving group and incoming nucleophile



Scheme 2. Multiple $n - \sigma^*_{P-O}$ interactions assist the cleavage of monoester dianions



Scheme 3. The prototropic pre-equilibrium mechanism that accounts for the pH-rate maximum for the hydrolysis of a typical phosphate monoester



Scheme 4. The hydrolysis of the DPP monoanion to inorganic phosphate

at pH's below 10.^[5] We needed to understand the kinetic properties of the monoester to calculate the accurate rate constants for the hydrolysis of DPP, and here report the details of this work, which offers a unique insight into the general mechanism of Scheme 3 for the hydrolysis of phosphate monoesters.

EXPERIMENTAL SECTION

General

Organic solvents were carefully dried, and synthetic reactions carried out in strictly anhydrous conditions under argon. Chemicals and inorganic salts were of the highest purity available and were used as purchased.

Monoester 2-pyridyl phosphate

The MPP was prepared by hydrolyzing the pure lithium salt of the diester $DPP^{[5]}$ at 80 °C in 1 M KOH and diluting to give a stock solution. The monoester dianion does not react at a significant rate with hydroxide anion and is stable under the conditions.

Kinetic methods

Reactions were initiated by adding 10 or 30 μ L of the stock solution of the substrate MPP (5 × 10⁻³ M) to 1 or 3 mL of buffered aqueous solution at the appropriate pH. Buffers used were HCl (pH < 2), chloroacetate (pH = 2–3.5), acetate (pH = 4–5.5), BIS-TRIS (pH = 6–7), TRIS (pH = 8–9), CHES (pH = 8.7–10.4), carbonate (pH \approx 10) and KOH (pH > 10). Reactions



Figure 1. pH-rate profiles for the spontaneous hydrolysis reactions of monoester 2-pyridyl phosphate (MPP) and DPP, at $25 \,^{\circ}$ C and ionic strength 1.0 M (KCI). The points are experimental, and the curves are calculated using the data of Tables 1 and S.1 (Supporting Information)

were followed, at constant temperature and ionic strength 1.0 M (KCI), by monitoring the appearance of pyridone at 294 nm for 4 days on a Varian Cary 50 spectrophotometer (Mulgrave, Victoria, Australia) equipped with a thermostatted cell holder. Rate constants were obtained by fitting to the first order equation, or, where appropriate, by the initial rate method.

RESULTS AND DISCUSSION

The hydrolysis of monoester 2-pyridyl phosphate

The pH-rate profile (Fig. 1) for the hydrolysis of MPP shows an acid-catalysed reaction at low pH, minimal reactivity at high pH and the rate maximum near pH = 4 expected for the hydrolysis of a phosphate monoester.

The pH-rate profile reflects both the mole fractions of the various ionic forms present (Scheme 5) and their reactivity as functions of pH. We consider the five distinct ionic species shown in Scheme 5, which includes the pK_{as} obtained by analysing the kinetic results shown in Table 1.

The reactions at low pH, involving the cation MPP⁺ and the zwitterion MPP[±], proceed at rates similar to the hydrolysis of the diester DPP, as might be expected. The predominant ionic form between pH = 2.62 and 5.62, the region of the rate maximum of Fig. 1 near pH = 4, is the monoanion MPP⁻. As discussed earlier, the rate maximum near pH = 4 is typical of a phosphate monoester, but the rate observed for the hydrolysis of MPP⁻ (Table 1) is a great deal faster than that expected for an ester with a comparable leaving group. (300 000 times faster, at 25 °C, than that observed for the hydrolysis of the monoanion of monophenyl phosphate at 39 °C.^[2]) What is special about MPP⁻ is the basic nitrogen of the 2-pyridyl leaving group, which makes the equilibrium $K_e = [MPP^{+=}]/[MPP^-]$ much more favourable.



Scheme 5. The ionic forms of monoester 2-pyridyl phosphate (MPP) significant in the pH region. The apparent pK_as shown are derived from the kinetics (Fig. 1)

Table 1. Kinetic data (rate constants and pK_a s) derived from the pH-rate profile of Fig. 1 for the hydrolysis of monoester 2-pyridyl phosphate (MPP), at 25 °C and ionic strength 1.0 M (KCl)

lonic form	pK _a (observed)	$k_{\rm hyd}/{ m s}^{-1}$
$\begin{array}{l} MPP^+ \\ MPP^\pm \\ MPP^- \rightleftharpoons MPP^{+=} \end{array}$	-1.02 ± 0.3 2.62 ± 0.3 5.62 ± 0.2	2.87 ± 1.04e-2 1.02 ± 0.70e-3 1.32 ± 0.38e-2

Full kinetic data appear in Table S.1 of the Supporting Information. The dianion MPP^{2-} is stable to hydrolysis under the conditions used, while MPP^+ and MPP^{\pm} carry the reaction in strong acid and near pH = 0, respectively.



Scheme 6. Microscopic dissociation constants for the ionic forms of monoester 2-pyridyl phosphate (MPP) significant in the pH region



Figure 2. The hydrolysis of monoester 2-pyridyl phosphate (MPP) in the region of the pH-rate maximum shows an inverse solvent deuterium isotope effect



Scheme 7. Kinetic (blue) and microscopic (red) pK_as derived for the various ionic forms of monoester 2-pyridyl phosphate (MPP) present in the pH region. See the text



Scheme 8. $MPP^{+=}$, with a partial positive charge on the leaving group oxygen, is kinetically equivalent to the O-protonated form $M^{+=}$ of a phosphate monoester (Scheme 3)

The curve fit for the pH-rate profile for the hydrolysis of the monoester MPP (Fig. 1) gives a reasonable set of rate constants and three apparent pK_{as} (Table 1), of which the two in the pH region, pK_2 and pK_3 , are composed of pairs of microscopic constants. Of the four ionic forms present in water near pH=7 (Scheme 6), MPP⁺⁼ is of particular interest as it can be cleaved by a uniquely readily accessible version of the mechanism (Scheme 3) generally accepted for the hydrolysis of the monoanions of phosphate monoesters.^[2]

The mechanism of Scheme 3 involves a pre-equilibrium protonation of the weakly basic oxygen of the leaving group of the monoester dianion, to generate very small amounts of the kinetically equivalent, highly reactive species $M^{+=}$, with the very good leaving group ROH attached to the PO₃²⁻ phosphoruscentre. In the MPP system, the corresponding equilibrium $MPP^{-} \rightleftharpoons MPP^{+=}$ (K_e, Scheme 6) is substantially more favourable, because the pK_{as} of the phosphate OH and the pyridinium N of the leaving group of MPP^{\pm} (K_{22} and K_{21} , Scheme 6) are much closer. We can derive reliable values for these microscopic pK_{as} from estimates of the pK_as of the phosphoric acid groups of the ArOPO₂OH⁻ systems of MPP[±] and MPP⁻, as follows. Measured pK_a values for a series of monoaryl phosphates ArOPO₂OH⁻ show a good linear free energy relationship with the $pK_{a}s$ of the parent phenols ArOH.^[8] This correlation provides estimates for pK_{22} and pK_{31} (Scheme 6) of 3.17 and 5.46, respectively, based on the literature values of 9.09 and 0.75 for the $pK_{a}s$ of 2-hydroxypyridine and its conjugate acid.^[9] Full details are presented in the Supporting Information.

The complementary values of pK_{21} and pK_{32} are readily obtained from the expressions $K_2 = K_{21} + K_{22}$ and $1/K_3 = (1/K_{31} + 1/K_{32})$ as 2.76 and 5.11, respectively. This allows the calculation of the equilibrium constant K_e for MPP⁻ \rightleftharpoons MPP⁺⁼ as 0.39 ± 0.08 (Scheme 7), indicating that 28% of the monoanion is present in what we can assume to be the reactive form MPP⁺⁼.

The rate constant obtained for the hydrolysis of the monoanion of the monoester MPP⁻ (Table 1) is $1.32 \pm 0.38 \text{ e-}2 \text{ s}^{-1}$: so if the reaction is carried out – as we would expect – exclusively by the 28% of MPP⁺⁼ present, this must be hydrolysed with a rate constant of $4.7 \pm 0.6 \text{ e-}2 \text{ s}^{-1}$. The linear free energy relationship for the dependence of the rate constant for hydrolysis of the dianions of phosphate monoesters on the pK_a of the conjugate acid of the leaving group^[2] (log $k_{hyd} = 0.70 \pm 0.44 - 1.19 \pm 0.087 pK_{LG} \text{ s}^{-1}$ at 39°C; see Supporting Information) predicts a rate constant for the hydrolysis of MPP⁺⁼ of 0.64 s^{-1} at 39°C, corresponding at 25°C to a figure of 0.13 (between 0.04 and 0.41) s⁻¹. This is in reasonable agreement with the observed value of approximately 0.05 s^{-1} . (For a detailed discussion of the short extrapolation leading to this conclusion, see the Supporting Information).

The original calculation supporting the mechanism of Scheme 3 was for the hydrolysis of the methyl phosphate monoanion, a

system with a much less basic leaving group than MPP⁺⁼, and an unfavourable equilibrium constant for the prototropic pre-equilibrium of the order of 10^{-11} . The estimated rate constant was one order of magnitude larger than necessary to account for the observed rate.^[2] The corresponding preequilibrium for the MPP system is unfavourable by only some $3 pK_a$ units (Scheme 5), so that the equivalent calculation can be made with greater confidence. The convincing conclusion is the same in both cases that M⁺⁼ is the species that carries the reaction.

For an independent test of this mechanistic conclusion, we measured the solvent deuterium isotope effect for the hydrolysis of MPP in the region of the pH-rate maximum. The pH-rate profile (Fig. 2) indicates clearly that the kinetic isotope effect is inverse: curve fitting gives a ratio $k_{H2O}/k_{D2O} = 0.73 \pm 0.22$, consistent with the value close to unity expected for the proposed prototropic pre-equilibrium of Scheme 3 but inconsistent with a mechanism involving the spontaneous attack of solvent water on MPP⁻.

CONCLUSIONS

The positive charge produced by protonation of the pyridine N of MPP²⁻ is partially delocalised onto the leaving group oxygen (Scheme 8), making the system a simple model for the kinetically equivalent but far less accessible O-protonated intermediate $M^{+=}$ of Scheme 3, considered to account for the rate maximum in the region of pH=4 observed for the hydrolysis of phosphate monoesters.

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