

Enhanced Hydrolysis of a Phosphonate Ester by Mono-aquo Metal Cation Complexes

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(Received August 27, 1987)

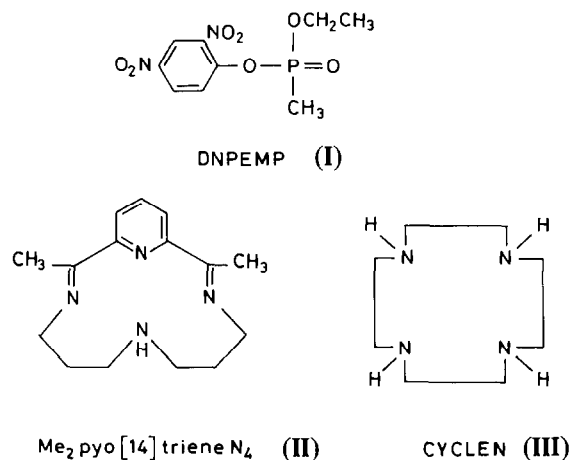
Abstract

The hydrolysis of the ester 2,4-dinitrophenylethyl methylphosphonate has been examined by both stop-flow spectrophotometric and pH-stat techniques. These reactions have been carried out in the presence of several nucleophiles including simple non-labile (w.r.t. substitution) mono-aquo metal ion complexes. Comparison of reaction rates of the metal complexes with sterically hindered organic nucleophiles has led to the conclusion that the metal ions function predominantly as general base catalysts in dilute aqueous solution. Reaction rates for the various nucleophiles studied are tabulated together with solvolysis constants for hydroxide ion and water at 35 °C and $I = 0.1 \text{ mol dm}^{-3}$ (KNO_3). These later two values are respectively $32.7 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ and $1.37 \times 10^{-4} \text{ s}^{-1}$. A Brønsted β value of 0.52 for the phosphonate ester studied has also been derived.

Introduction

The ability of metal ions or metal ion complexes to enhance rates of nucleophilic displacement reactions at neutral phosphorus(V) centres has been well known for a considerable time. Copper(II) chelates have been shown to be effective in assisting the displacement of fluoride ion from both diisopropylfluorophosphate (DFP) [1] and iso-propylmethylfluorophosphonate (Sarin) [2]. More general screening procedures [3, 4] have demonstrated that $\text{UO}_2(\text{VI})$, $\text{ZrO}(\text{IV})$, $\text{Th}(\text{IV})$, $\text{MoO}_2(\text{VI})$, $\text{Y}(\text{III})$ and $\text{Al}(\text{III})$ also enhance the degradation rates of similar phosphorus esters. More recently, both $\text{Co}(\text{III})$ [5] and $\text{Zn}(\text{II})$ [6, 7] species have been shown to be effective in assisting the hydrolysis of neutral phosphate and phosphonate esters.

However, despite the numerous publications that demonstrate the ability of metal complexes to catalyse hydrolysis reactions at neutral tetrahedral phosphorus(V) centres, there is little data on the precise mechanisms of these reactions. In view of



the potential that metal complexes possess to catalytically degrade such toxic materials, studies are being carried out in this laboratory to elucidate the interactions of metal ions and phosphorus esters. In order to probe these interactions, the hydrolysis reactions of the phosphonate ester 2,4-dinitrophenylethyl methylphosphonate (DNPEMP, I) with a variety of metallo and non-metallo reagents have been studied in aqueous solution. In this work, the relationship between the rate of degradation of the phosphate ester and the pK of the attacking agent is examined in order to ascertain whether general base catalysis or direct nucleophilic displacement is the mode of interaction of metal complexes with toxic phosphorus esters. Buffers have been excluded from the system [7], and metal complexes that are relatively non-labile (w.r.t. substitution) have been utilised in order to simplify interpretation of results. Such data is essential if the worth of metal complexes as catalysts in an aqueous based decontamination system for toxic phosphorus esters is to be assessed.

Experimental

Caution: The phosphonate ester used in this study, 2,4-dinitrophenylethyl methylphosphonate

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(DNPEMP, **I**) exhibits great anti-cholinesterase activity *in vitro*. Great care should be exercised in its handling and use.

All reagents used were the purest available with non-commercial compounds being synthesised and purified as described below. Kinetic measurements were carried out at 35 °C unless otherwise stated with the final ionic strength of the reaction medium adjusted to 0.1 mol dm⁻³ with potassium nitrate. In general, reactions with nucleophiles with *pK* greater than 9 were monitored by stop-flow spectrophotometry while reactions of nucleophiles with *pK* less than 9 utilised a pH-stat technique. Production of 2,4-dinitrophenate anion was monitored by following the absorbance increase at 360 nm except in the case of stopped-flow measurements where for convenience, the visual absorption at 400 nm was used. In all experiments, a stock solution of DNPEMP (**I**) which was 2×10^{-2} mol dm⁻³ phosphonate ester in dry acetonitrile was used. pH electrodes were standardised with NBS buffers immediately prior to use.

Determination of Rate Constant by pH-stat Technique

Fifteen ml of the aqueous reaction medium containing a known concentration of the nucleophile and sufficient KNO₃ to maintain ionic strength at 0.1 mol dm⁻³ was added to the reaction vessel and maintained with constant stirring at the desired temperature by a circulating water thermostat. The pH of the solution was then adjusted and maintained throughout the reaction to a pH value sufficient to ensure complete dissociation of the nucleophile. The pH-stat consisted of a Radiometer PHM 64 pH meter attached to an Orion 'Ross' type 8103 semi-micro combination electrode containing 0.1 M KNO₃ in its salt bridge. The PHM 64 meter controlled a Radiometer ABU 13 autoburette (via a TTT60 controller) which delivered 0.1 M NaOH to the reaction vessel from a 0.25 ml capacity syringe. The mixture was then pumped by means of a Gilson 'Minipulse' peristaltic pump via capillary teflon tubing through a 1 cm flow cell contained in an LKB 4050 UV-Vis spectrophotometer and the effluent solution was passed back into the reaction vessel. Runs were initiated by injecting 100 μl of the stock DNPEMP solution to the reaction vessel. Rates were calculated from a linear regression analysis (unweighted) of plots of $\ln(A_{INF} - A_T)$ versus time. Plots were linear for at least 3 half-lives.

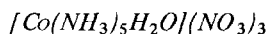
Determination of Rate Constants by Stop-flow Spectrophotometry

The system consists of a High-Tec Scientific Sample Handling Unit (SHU) fitted with a grating monochromator, tungsten-halogen light source and EMI

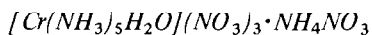
9798QB 11 stage end window photomultiplier to monitor absorbance changes. The output from the P.M. tube was fed into an Infotek AD 2000 Analog to Digital converter (high-speed) and thence via a Hewlett-Packard 98622 GPIO 16-BIT parallel interface into an HP-310 microcomputer where curve fitting routines based on the algorithm of Gampp [8] were used to calculate the desired rate constants. A total of 400 data points were collected during each run, irrespective of the time base used. Reaction was achieved by loading the sample syringe with (i) 0.020–0.10 mol dm⁻³ solutions of the nucleophile under investigation dissolved in *I* = 0.1(KNO₃), (ii) 150 μl of the stock, DNPEMP solution in 10 ml of 0.1 M KNO₃. The apparatus was thermostatted at the desired temperature (LKB 2219, Multitemp II circulator) and reactions initiated by pneumatically injecting 100 μl of each reactant into the mixing and observation chamber. Typically, a pressure of 60 psi on the activating ram yielded a dead time ≤ 2 milliseconds.

Synthesis of Metal Complexes

The zinc(II) complex of Me₂ pyo[14]triene N₄ (CR, **II**) was synthesised as described by Wooley *et al.* [9] using hydrated zinc(II) perchlorate as the zinc salt. The zinc(II) complex of 1,4,7,10-tetraazacyclododecane (CYCLEN, **III**) was synthesised by the literature method [7].



2.0 g of $[\text{Co}(\text{NH}_3)_5\text{CO}_3](\text{NO}_3)\frac{1}{2}\text{H}_2\text{O}$ [10] was dissolved in 30 ml of 1 M HNO₃ and warmed on a steam bath for 10 min. The solution was filtered and on cooling bright red crystals of the product were obtained. The crystals were washed with 5 ml of propan-2-ol and dried under vacuum. Spectral data (in 1 M HNO₃) was in agreement with literature values ($\lambda_{\text{max}} = 492$ nm, $\epsilon = 49$).



50 g of potassium chromic sulphate dodecahydrate was finely powdered and mixed with 120 ml of 0.88 ammonia solution. 125 g of ammonium nitrate was added, the flask was then warmed to 55 °C for 1 h with occasional shaking. The mixture was cooled to 20 °C and a fast stream of air passed through it for 30 min whereupon the mixture was poured onto 50 g of crushed ice and 125 ml of conc. nitric acid and kept at 0 °C for 1 h. The resulting orange crystals were filtered, redissolved in 100 ml of water and re-filtered. To the filtrate was added 100 g of NH₄NO₃ in 100 ml of water. On cooling overnight, orange crystals of the product were obtained, washed with ethanol saturated with ammonium nitrate and finally with a mixture of 1:1 ethanol/diethylether and dried under vacuum. The yield was 5 g of orange

crystals with a visible spectrum in 0.1 M NH_4NO_3 of λ_{max} 480 ($\epsilon = 35$), 358 ($\epsilon = 31$).

$[\text{Cu}(\text{dipyridyl})(\text{H}_2\text{O})_2](\text{NO}_3)_2$

25 ml of a 50 mM solution of $\text{Cu}(\text{NO}_3)_2$ (standardised by titration of protons liberated from a cation exchange column) was added to 25 ml of a 50 mM solution of 2,2'-dipyridyl in methanol. The solution was reduced by dryness on the rotary evaporator and redissolved in 50 ml of water to yield a 25 mM solution of $[\text{Cu}(\text{dipy})(\text{H}_2\text{O})_2](\text{NO}_3)_2$ (by stoichiometric proportion).

Synthesis of 2,4-Dinitrophenylethyl methylphosphonate, DNPEMP (I)

Ethyl methylphosphonochloridate (28.8 g, 0.2 mol) was added dropwise to a stirred suspension of sodium 2,4-dinitrophenate (41.2 g, 0.2 mol) in dry toluene (200 ml). On completion of the addition, the mixture was refluxed for four hours, cooled, filtered and concentrated *in vacuo* to give crude DNPEMP in 70% yield. Purification immediately before use was carried out thus. 1.0 g of the crude ester was dissolved in 20 ml of ice cold chloroform and then shaken with 25 ml of ice cold 5% NaHCO_3 , 25 ml of ice cold water, 25 ml of ice cold 0.1 M HCl and 2×25 ml aliquots of ice cold water. The organic layer was kept over Na_2SO_4 for several hours and finally the solvent was removed at 40 °C under vacuum. The phosphonate ester was then used as required. ^{13}C NMR (rel TMS) 7.2 (CH_3); 16.2 (CH_3); 63.0 (CH_2); 121.5 (CH); 124.5 (CH); 128.1 (CH); 141.3 (C-X); 143.6 (C-X); 148.7 (C-X). IR 1540 (NO_2); 1250 (P=O); 1040 (P-O-alkyl); 1250 (P-O-aryl) cm^{-1} . Purity was checked by HPLC. Measurements were performed on a Hypersil ODS column (Michrom) at 30 °C. Products were monitored spectrophotometrically at 270 nm or over the wavelength range 190–370 nm using an LKB 2140 Rapid Spectral Detector. The mobile phase was 50 mM $\text{H}_3\text{PO}_4/\text{CH}_3\text{CN}$ (3:2 *v/v*). HPLC gave a purity >96% for the phosphonate ester. ^{13}C spectra were run on a Jeol FX60 FT instrument in CDCl_3 , solvent using TMS as internal standard. IR spectra were run as a mull on KBr plates (for ester) or as KBr discs (for metal complexes) on a Perkin–Elmer 1750 FT–IR spectrophotometer. UV–Vis spectra were run on a Shimadzu UV-240 instrument.

Results

As the pK of 2,4-dinitrophenol is ~ 4.1 at 35 °C, it is impossible to assay reactions by direct spectrophotometric observation below this pH. Thus, experiments to determine the second order rate constant for H^+ catalysed hydrolysis of the ester, by analysing aliquots of reactions carried out in 0.1

to 0.01 M nitric acid, were carried out by HPLC techniques (as described earlier). The value for k_{H} at 35 °C by this technique was found to be $\sim 2 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$. Thus hydrogen ion terms are negligible at the pH values used for these experiments and the kinetic course of the reactions can be described by the following equation.

$$k_{\text{OBS}} = k_{\text{aq}} + k_{\text{Nu}}[\text{Nu}] + k_{\text{OH}}[\text{OH}^-] \quad (1)$$

where k_{OBS} = observed rate constant, k_{aq} = spontaneous first order reaction rate with water, k_{Nu} = second order rate constant for the reaction with nucleophile, k_{OH} = second order rate constant for the reaction with hydroxide ion.

When species other than H_2O and OH^- are excluded from the reaction medium (with the exception of KNO_3 which must be included to maintain ionic strength although it does not itself contribute to the reaction) eqn. (1) reduces to

$$k_{\text{OBS}} = k_{\text{aq}} + k_{\text{OH}}[\text{OH}^-] \quad (2)$$

Thus, a plot of $[\text{OH}^-]$ versus k_{OBS} in this situation will yield a straight line of slope k_{OH} and intercept k_{aq} . An example of such a plot is given in Fig. 1 for the hydrolysis of DNPEMP at 30 °C and $I = 0.1 \text{ mol dm}^{-3}$ (KNO_3). The slope of this graph yields values of $k_{\text{OH}} = 32.7 \text{ mol}^{-1} \text{ s}^{-1}$ and $k_{\text{aq}} = 1.37 \times 10^{-4} \text{ s}^{-1}$. The data obtained from the pH stat experiments are compiled in Table I. Hydroxide ion concentrations were derived from the pH readings, by application of Debye–Hückel theory and the Davies equation [11]. Also included in Table I is data derived from stop-flow experiments using much stronger (~ 0.01 – 0.05 mol dm^{-3}) hydroxide ion concentrations where as a result of the high value of $[\text{OH}^-]$, no pH-statting was required since only $10^{-4} \text{ mol dm}^{-3}$ acid was produced by the reaction and the change in $[\text{OH}^-]$ was insignificant. The stop-flow technique is unsuitable for assessment of k_{aq} , as the very small intercepts found on the large ordinate value are inaccurate.

TABLE I. Hydrolysis and Aquation Rates for DNPEMP at $I = 0.1 \text{ M}$ (KNO_3)

Temperature (°C)	pH-stat		Stop-flow k_{OH} ($\text{mol}^{-1} \text{ s}^{-1}$)
	$10^4 \times k_{\text{aq}}$ (s^{-1})	k_{OH} ($\text{mol}^{-1} \text{ s}^{-1}$)	
10.0			10.0
15.0			13.3
20.0			18.0
25.0	0.99	26.4	24.2
30.0	1.38	32.7	31.6
35.0	2.30	41.9	43.0
40.0	2.90	53.7	55.6

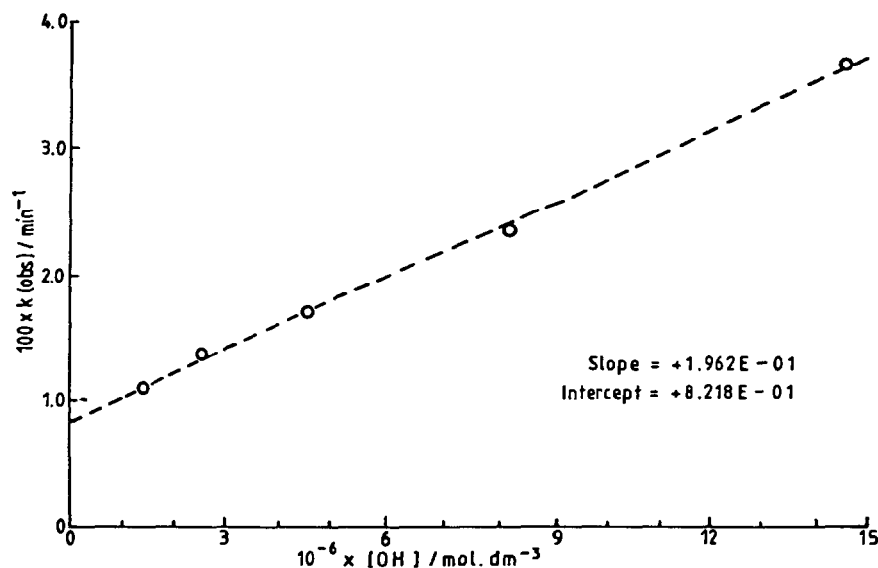


Fig. 1. Hydrolysis of DNPEMP at 30.0 °C and $I = 0.1$ M (KNO_3) monitored by pH-stat.

As can be seen from Fig. 1, the data observed in Table I is self-consistent and yields a good line over a 14 fold change in $[\text{OH}^-]$ concentration. For this reason, the value of the intercept was taken as being a reasonably accurate value of the aquation rate (k_{aq}), which is difficult to determine independently. Similarly consistent data was obtained using the pH-stat technique at other temperatures, and this coupled with an experimentally acceptable overlap of pH-stat and stop-flow results, as shown in Table I, lends confidence to the assigned values. Analysis of the data in Table I can be carried out to yield activation parameters for the H_2O and OH^- reaction with DNPEMP. These values are respectively for H_2O and OH^- ΔH^\ddagger (KJ mol^{-1}) 55.4, 39.9; ΔS^\ddagger_{98} ($\text{J mol}^{-1} \text{K}^{-1}$) -92, -84.

Having characterised the water and hydroxide reactions, closer detail can be paid to the metal based reactions. Experiments were carried out with the non-labile metal complexes. $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]^{3+}$;

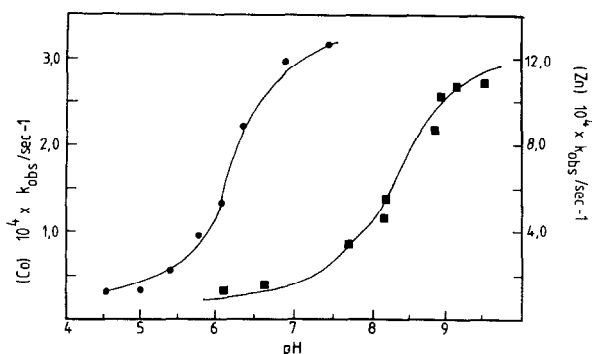
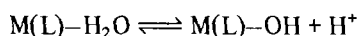


Fig. 2. Hydrolysis of DNPEMP by $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]^{3+}$ (●) and $[\text{Zn}(\text{CR})(\text{H}_2\text{O})]^{2+}$ (■) at 35 °C and $I = 0.1$ M (KNO_3). The solid line signifies calculated rates based on pK values in Table II.

$[\text{Cr}(\text{NH}_3)_5(\text{H}_2\text{O})]^{3+}$ and $[\text{Zu}(\text{CR})(\text{H}_2\text{O})]^{2+}$ in order to ascertain the effect of pH on the reactions. Figure 2 summarises the results of two experiments carried out at 35 °C using $[\text{Co}(\text{NH}_3)_5(\text{H}_2\text{O})]^{3+}$ and $[\text{Zn}(\text{CR})(\text{H}_2\text{O})]^{2+}$. Clearly, (Fig. 2) the rate enhancements due to these metal complexes coincide with deprotonation of the coordinated water molecule (see pK values in Table II below). Thus the process



is an important first step in the aquo metal ion enhanced hydrolysis of neutral phosphate esters by simple mono-aquo metal ions. Further experiments also showed that the same criterion was true for all species studied and that the reaction required complete deprotonation of the conjugate acid to achieve maximum rate enhancements. In the light of this observation, further experiments were carried out to determine the values of k_{Nu} for a series of different metallo and non-metallo (for comparison) bases (Fig. 3).

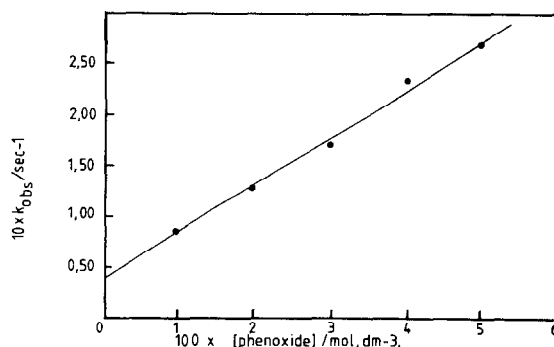


Fig. 3. Hydrolysis of DNPEMP by sodium phenate at 35 °C, pH 10.7 and $I = 0.1$ M (KNO_3), monitored by stop-flow spectrometry.



general base catalysis (GBC)

nucleophilic displacement

When general base catalysis (GBC) takes place, water plays an important role in the mechanism of the reaction whereas the solvent is not necessarily involved in any reaction involving direct nucleophilic displacement. There is much data in the literature [14, 15] concerning the kinetics of degradation of phosphorus esters which offers conflicting evidence as to whether GBC or nucleophilic attack occurs. For instance, heterocyclic amines such as pyridine and imidazole as well as ionic nucleophiles such as acetate, bicarbonate and phosphate had been demonstrated to react with phosphonyl phosphorus [16, 17]; these reactions have been regarded as general base catalysis since they failed to demonstrate any steric hindrance effects. Thus, 2-methyl pyridine has the same reactivity as pyridine [18]. From these and other studies [19] lack of steric effect has come to be regarded as diagnostic of general base catalysis. There is, however, also good experimental evidence for direct nucleophilic attack on phosphorus. Ashbolt and Rydon [20] have demonstrated that if tyrosine is reacted with di-isopropyl-fluorophosphate at pH 7.8 in aqueous solution di-isopropylphosphonylation of the phenolic hydroxyl group occurs. There are also reports that phosphonylated phenols can be obtained from the reactions of Sarin with phenols.

In considering the possibility that GBC is the preferred mechanistic pathway of the reactions examined in the present study, it is beneficial to relate the structure of a catalyst to its catalytic activity by means of its pK_a . The general form of

this relationship which is known as the Brønsted Catalysis Law is given below (eqn. (3))

$$k_B = G_B(1/K_a)^\beta \quad (3)$$

where k_B is a rate constant, G_B is a constant for a particular reaction, β is the Brønsted slope and K_a is the dissociation constant of the base. This can be rearranged to give

$$\log(k_B) = \beta pK_a + \text{constant} \quad (4)$$

Hence, a plot of $\log(k_B)$ versus pK_a will yield a straight line for a series of nucleophiles operating by a similar GBC mechanism.

The evidence available in this research clearly shows that the majority of the nucleophiles investigated lie on a fairly good GBC Brønsted plot. Care should be taken in immediately assigning a GBC mechanism solely on the criterion of a Brønsted plot. A group of similarly related reactions occurring by way of direct nucleophilic attack will also yield a straight Brønsted line. These can be distinguished by examining the data obtained for the bases studied, which because of steric hindrance, are known to be non-nucleophilic in character. It has already been stated that, if such bases behave in a manner predicted by the given Brønsted plot, this is good evidence for GBC rather than direct nucleophilic attack. Several such non-coordinating bases were included in the compounds examined, triethylamine-(10), DBU(11) and DBN(12). The fact that all these hindered bases lie on the same Brønsted plot as all the others investigated, including the metal complexes, is reasonable evidence that all the reactions are proceeding via a general base catalysis.

Accordingly, values for k_{Nu} for a variety of compounds were determined by use of eqn. (1). When the pH is maintained at a constant value, a plot of k_{OBS} versus $[Nu^-]$ should yield a straight line of slope k_{Nu} and intercept ($k_{aq} + k_{OH}[OH^-]$). This was well supported in practice as can be seen from

TABLE II. Values of pK and k_{Nu} for Bases Considered in This Work

No.	Compound	Reference	$\sim pK$	k_{Nu} (mol s ⁻¹)	$\log_{10}(k_{Nu})$
1	2-aminopropane	12	10.4	6.82	0.8337
2	azide ion	13	4.7	0.015	-1.8239
3	[Cu(dipy)(H ₂ O) ₂] ²⁺	1	6.5	1.33	0.1249
4	[Cr(NH ₃) ₅ (H ₂ O)] ³⁺	this work	5.2	0.065	-1.1871
5	Phenol	12	10.0	4.70	0.6721
6	[Zn(Me ₂ [14]pyoN ₄)(H ₂ O)] ²⁺	this work	8.1	1.2	0.0792
7	[Zn(cyclen)(H ₂ O)] ²⁺	7	7.9	2.1	0.3046
8	[Co(NH ₃) ₅ (H ₂ O)] ³⁺	this work	6.3	0.143	-0.8436
9	Water	13	-1.7	4.16 × 10 ⁻⁶	-5.3802
10	Triethylamine	13	10.7	11.43	1.0582
11	DBU	this work	10.1	26.88	1.4295
12	DBN	this work	9.6	12.73	1.1048

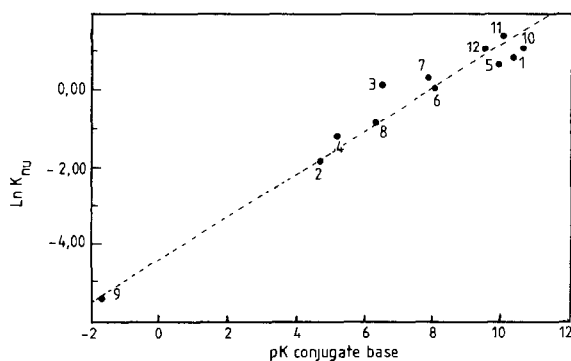


Fig. 4. Brønsted plot for the hydrolysis of DNPEMP by various nucleophiles.

an example of the results shown in Fig. 3 where the slope of the graph, k_{Nu} for phenate ion at 35 °C, $I=0.1$, is $4.7 \text{ mol}^{-1} \text{ s}^{-1}$. The intercept of 0.04197 s^{-1} is in good agreement with the calculated value of 0.044 s^{-1} , based on a pH value of 10.7 (the pH of the phenate reaction). A complete list of bases and nucleophiles investigated at 35 °C and $I=0.1 \text{ mol dm}^{-3}$ (KNO_3) is given in Table II and shown graphically in Fig. 4. Where possible, pK values were recovered from the literature and as such may be slightly in error where temperature effects had to be interpolated; the magnitude of these errors is not great and is insignificant in the context in which they are applied. The sources of the pK data are referenced in Table II.

Discussion

From the data in Table II and Fig. 4, it is amply demonstrated that metal complexes and other nucleophiles enhance the rate of hydrolysis of DNPEMP. That formation of a conjugate base is an initial step in the process is apparent from data in Fig. 2, thus two likely mechanisms exist for the displacement of the leaving group, these being outlined below.

Additional evidence for a GBC mechanism is yielded by a study of the deuterium isotope effect on the rate of reaction. Nucleophilic reactions do not involve proton transfer in the rate determining step and as such, a value of k_H/k_D of unity or very close to unity would be expected, as for example is obtained for the imidazole catalysed hydrolysis of *p*-nitrophenylacetate which is recognised to take place via a nucleophilic displacement [21]. The criterion of a positive deuterium isotope effect also should only be used in conjunction with other evidence since other effects such as solvation can contribute to the result.

In these experiments, the reaction of triethylamine with DNPEMP was carried out in both H_2O and D_2O at 35.0 °C in order to determine a value of

k_H/k_D . Values obtained for H_2O and D_2O reaction were 11.43 and 8.17 s^{-1} respectively giving a k_H/k_D of 1.40; this again is evidence for a GBC mechanism being operative for triethylamine which in turn leads weight to the metal complexes operating by a GBC mechanism. Further experiments on deuterium isotope effects in the metal ion based reactions are underway to clarify this point.

Further consideration of eqn. (4) will also demonstrate that the slope of the graph in Fig. 4 will be β , the Brønsted coefficient for hydrolysis of DNPEMP. The value obtained in this case is 0.52, which is typical of other esters. For example DFP has a β value of 0.42 [18] at 25 °C while that of Sarin [21] is 0.49 at 25 °C. The value of β gives some measure of the degree of GBC in the reaction, a value of 0 implies no general base catalysis. Thus, the evidence so far collected would seem to suggest that for simple mono-aquo metal ions (which are comparatively inert with respect to substitution), the reaction path with DNPEMP in dilute aqueous solution is one in which the aquo metal ion takes on the role of a general base in a manner similar to that which occurs in metal ion enhanced hydrolysis of carbocyclic acid esters and anhydrides [22]. This observation is important in considering the applicability of such metal complexes in catalytic decontamination systems since the nature of the solvent enters into the design of the catalyst.

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