Enhanced Hydrolysis of Neutral Phosphate Esters by the Zinc(II) Complex of 1,4,7,10-Tetra-azacyclododecane

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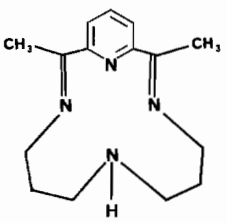
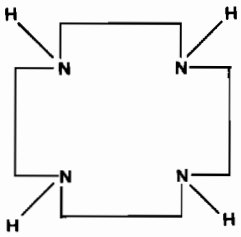
As a result of the important role that metal ions play in the biological chemistry of phosphorous and its compounds [1, 2], several investigations have been carried out into interactions of metal ions with phosphorous species. These studies have principally encompassed charged phosphate esters such as ATP [3], pyrophosphate [4], tripolyphosphate [5, 6], fluorophosphate [7] and *p*-nitrophenylphosphate [8] and in contrast, data available for neutral phosphorous esters is more scarce.

Studies have shown [9–11] that copper(II) species catalyse the hydrolysis of fluorophosphonates to yield fluoride ion and that cobalt(III) is demonstrably [12] active in the hydrolysis of neutral *p*-nitrophenyl esters of phosphorous. In addition, Breslow [13] has recently demonstrated the catalytic effect of the zinc(II) macrocyclic complex of CR (**I**) [14] on the hydrolysis of *p*-nitrophenyldiphenylphosphate in mixed solvent systems. In view of the potential of metal complexes to catalytically degrade these toxic materials, investigations into the mechanisms of such reactions have been initiated in this laboratory. The author wishes to report the synthesis of a zinc(II) complex of the macrocycle, 1,4,7,10-tetra-azacyclododecane (CYCLEN) (**II**) together with preliminary studies on its ability to enhance the rates of hydrolysis of ethyl(2,4-dinitrophenyl)-methylphosphonate (**III**, DNPEMP) and diethyl(2,4-dinitrophenyl) phosphate (**IV**, DNPDEP).



I



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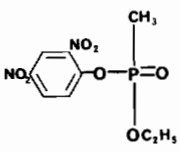
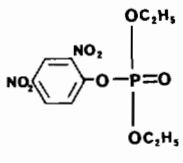


III



IV

Experimental*

Zinc Complex of CR (I)

This was synthesised as described by Wooley [15], using hydrated zinc perchlorate as the zinc salt. The product was precipitated at pH 10 using a minimum of saturated $NaClO_4$ solution (see Table I).

Zinc Complex of CYCLEN (II)

The ligand tetrahydrochloride was synthesised as described in the literature [16] by reacting the disodium salt of the tritosylate of diethylene triamine [17] with the tritosylate of diethanolamine. The zinc(II) complex was made thus: 954 mg of CYCLEN·4HCl (**II**) was dissolved in ~30 ml of 1 M NaOH and the organic fraction extracted with 4 × 25 ml portions of $CHCl_3$. The chloroform was removed under vacuum and the amine residue redissolved in ~40 ml of methanol. 800 mg of hydrated zinc(II) perchlorate was added and the whole refluxed for 3 h. After filtering, the solution was reduced in volume to about 20 ml whereupon 10 ml of distilled water was added together with 2 ml of saturated sodium perchlorate solution and sufficient 1 M NaOH to give the solution a pH of ~10. On cooling for several days at −5 °C, white microcrystals of the product were formed. These were filtered, washed with absolute ethanol and dried over silica-gel under vacuum; yield was 600 mg (see Table I). Phosphorous esters **III** and **IV** were synthesised by the method of Fukoto [18].

^1H NMR spectra were run on a Jeol FX100 spectrophotometer while ^{13}C measurements were obtained on a Jeol FX60 FT instrument. Samples were dissolved in the appropriate solvent ($CDCl_3$ for esters, D_2O, d_6-DMSO or d_3-nitromethane for zinc complexes) and chemical shifts measured *versus* TMS. IR spectra were run in KBr disc on a Perkin-Elmer 1750 FT-IR spectrophotometer.

Kinetic measurements were all made at 40 °C in solutions that were 10 mM in buffer and ionic strength made up to 1.0 ml l^{-1} with KNO_3. The series of 'Good' buffers, MES, PIPES, HEPES, TAPS, CHES and CAPS were used with 2,6-lutidine-3-sulphonate [19] being utilised at pH < 5.5. Reactions were carried out under pseudo-first order conditions by adding 50 μl of a 5 mM solution of the ester in CH_3CN to the spectrophotometer cell (total volume = 2.8 ml). Production of 2,4-dinitrophenoxide ion

*Note: the phosphorous esters described exhibit potent anticholinesterase activity. Great care should be exercised in their handling and use.

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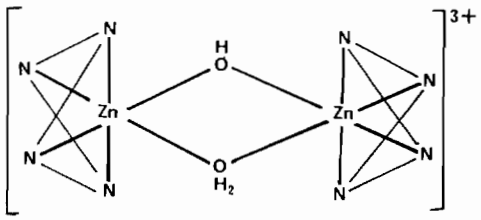
TABLE I. Spectroscopic and Analytical Data for Zinc(II) Complexes

	CYCLEN		CR	
^{1}H NMR	(d_6-DMSO) δ2.7 (16H); δ3.4 (4H)		(CD_3, NO_2) δ8.2–8.6 (3H); δ3.2–4.2 (12H); δ2.4 (6H)	
^{13}C NMR	(d_6-DMSO) δ43.4 (D_2O) δ45.75; δ46.48		(CD_3CN) δ13.05; δ29.15; δ51.24; δ125.05; δ144.82; δ146.89; δ162.51	
IR	γ(H_2O) 3400–3500 cm^{-1} γ(NH) 3175 cm^{-1} (ClO_4) 1100 cm^{-1}, 625 cm^{-1}		γ(H_2O) 3410–3500 cm^{-1} γ(NH) 3120 cm^{-1} γ(C=N) (imine) 1645 cm^{-1} (ClO_4) 1100 cm^{-1}, 625 cm^{-1}	
UV–Vis	198 (ε = 434)		296 (triplet) (ε = 18 × 10^3) 245 (sh) (ε = 5 × 10^3) 215 (ε = 8 × 10^3)	
	CYCLEN		CR	
	Calculated	Found	Calculated	Found
$[(Zn(L))_2OH\cdot H_2O](ClO_4)_3$				
C	23.76	21.24	36.72	36.49
H	5.35	4.65	4.82	4.64
N	13.85	12.03	11.42	11.33

(pK ~ 4.1) was monitored spectrophotometrically at 400 nm on either a Schimadzu UV-240 or Perkin-Elmer 402 instrument fitted with a data-logging device (Thurlby 1705a multimeter). In both cases temperature control was by means of a circulating water thermostat (40.0 ± 0.2 °C). Plots of $\ln(A_\infty - A_+)$ *versus* time were linear for at least 3 half-lives. Conductivity measurements were made at 25 °C on 1 mM solutions using a Bibby MSI conductivity meter fitted with a dip-cell of cell constant 1.05. Titrations were carried out in a cell thermostated at 40 °C under CO_2-free nitrogen gas. A Radiometer PHM64 meter was used fitted with a Corning micro combination electrode which was standardised with appropriate NBS buffers. Titrant was delivered by means of a micrometer driven syringe. Microanalyses were carried out at CDE on a Carlo-Erba CHN analyser.

Results and Discussion

Previous workers [15, 20] have assumed a five coordinate mono-aquo structure for similar zinc-(amine) complexes, but results of titrating 148 mg of the zinc(CYCLEN) complex (shown graphically in Fig. 1) clearly do not fit for a simple mono-aquo ion in solution. The isolated compound possesses both acid and base titratable groups in equimolar proportions and calculations show that the apparent molecular weight of the compound under these circumstances is 817 (±10). The simplest molecule to fit these criteria would be the aquo-hydroxy bridged dimer (**V**) shown below



V

The calculated molecular weight of **V** is 808, and the microanalytical data best fits this dimeric structure. The molar conductivity (Λ(M)) of **V**, based on MW = 808, is 381 ohm^{-1} cm^{-1}, within the expected range of a 1:3 electrolyte [21]. It should be noted that the Zn(CR) complex synthesised here exhibits

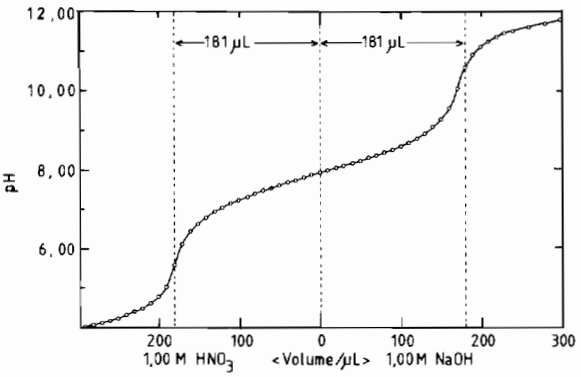


Fig. 1. Titration of 148 mg of zinc(CYCLEN) species at 40 °C and ionic strength 1.0 (KNO_3).

very similar titration and conductivity behaviour to the zinc(CYCLEN) species and its microanalytical data again best fits the dimeric type of structure described.

Assuming the dimeric nature of the zinc(II) species in solution, values for the practical p*K*s of the bridging groups can be determined as 7.3 and 8.5 (±0.1) at 40 °C and $I = 1.0$. IR data obtained by comparison of spectra of Zn(CYCLEN) recrystallised from H_2O and D_2O respectively, also lends support to the assignment of a dimeric structure for the Zn-(CYCLEN) species. Firstly, the bulk H_2O peak at 3400 cm^{-1} does not shift upon deuteration as might be expected if it were due to lattice water, but remains substantially intact, implying a more intimate interaction between the water molecules and the zinc complex than merely water of crystallisation. Secondly, there is a band shift upon deuteration from 960 to 750 cm^{-1}. Similar shifts have been noted for the bridging OH bending mode in copper(II)–bipyridyl dimers [22]. A new band also appears at 2360 cm^{-1} which may arise from deuterium oxide of crystallisation or deuteration of the bridging group protons. Although NMR data has been obtained (Table I), it is uninformative about the nature of the species in solution.

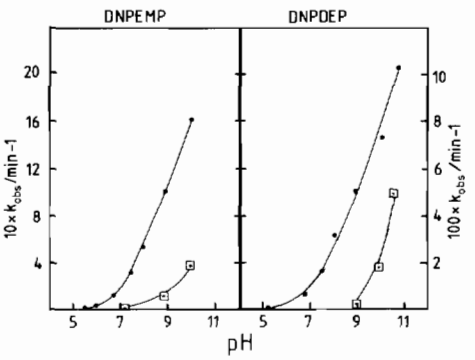


Fig. 2. pH profiles for hydrolysis of DNPDEP and DNPEMP by zinc(CYCLEN) species. [Zn(CYCLEN)] = 1.6×10^{-3} M, ●, catalysed rate; □, uncatalysed rate.

Preliminary kinetic data for the hydrolysis of the phosphate esters DNPDEP and DNPEMP is given in Fig. 2. Clearly the zinc(CYCLEN) species substantially enhances the rate of release of 2,4-dinitrophenoxide from both esters. Zinc(CYCLEN) behaves as does Zn(CR) [13] in that the catalytic effect increases markedly as the pH of the reaction approaches and passes the p*K* of coordinated water molecules in the complex. Thus, the complex would appear to function by providing a high concentration of the nucleophilic reagent (coordinated hydroxide) at comparatively low pH. This type of mechanism has been demonstrated [20] for other reactions of metal bound hydroxide ion, but some ambiguity

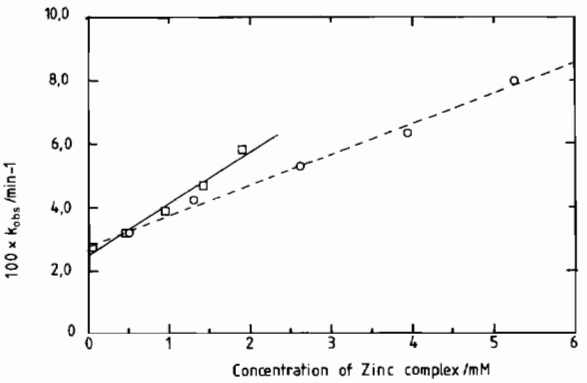


Fig. 3. Metal complex concentration dependence of hydrolysis of DNPDEP by Zn(CR) and Zn(CYCLEN) at pH 9.8. ○, Zn(CR); □, Zn(CYCLEN).

[14] as to whether direct nucleophilic or general base catalysis occurs still exists.

Under the conditions prevailing in these experiments (H^+ terms are very small and can be neglected), the rate law for the hydrolysis reaction can be expressed

$$k_{obs} = k_{aq} + k_{OH}[OH^-] + k_B[B^-] + k_m[M] \qquad (1)$$

where k_{obs} is the observed rate constant, k_{aq} is the first order aquation rate, k_{OH} the 2nd order rate constant for hydroxide catalysed hydrolysis, k_B the 2nd order rate constant for hydrolysis by buffer anion and k_m the 2nd order rate constant for hydrolysis by the metal complex.

Values for k_m, a measure of the catalytic effect of the metal complexes can be obtained by plotting [M] against k_{obs} keeping all other terms constant. When this is done (Fig. 3), values of k_m for different complexes and esters can be derived (Zn(CR) is included for comparison). The data gives k_m for Zn(CR) of 92.8 and 9.5 M^{-1} min^{-1} with DNPEMP and DNPDEP respectively while Zn(CYCLEN) gives 16.0 M^{-1} min^{-1} for its reaction with DNPDEP. The reaction of the Zn(CYCLEN) species with the ester DNPEMP was too rapid to be monitored at metal ion concentrations >1.6 mM given the experimental techniques used in this initial study.

Although the catalytic effect of the zinc-(CYCLEN) species is amply demonstrated, interpretation of results is hindered by a lack of knowledge of the exact nature of the zinc species in solution. In addition, complications arise from the use of buffer systems which must themselves contribute to the reactions under study. To remedy this, more detailed work using pH-stat techniques is in progress. Studies on other metal ions in similar hydrolytic systems should also yield pertinent information as to the nature of the interaction of metal hydroxide species with neutral phosphate esters.

Acknowledgement

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