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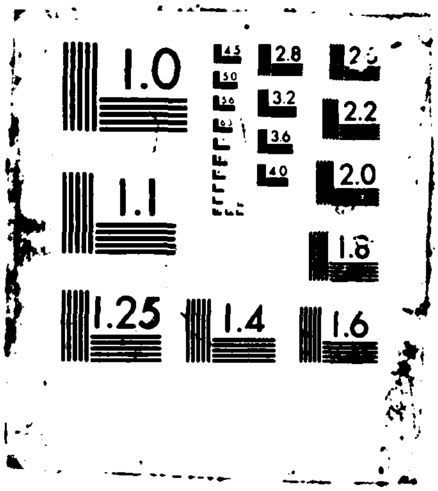
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The hydrolyses of tris-p-nitrophenylphosphate (5), tris-2-pyridylphosphate (6) and ethyl-p-nitrophenylphosphonate (7) catalyzed by two tris-imidazol-yl-phosphine:Co^{2+} complexes (Co^{2+}:3 and Co^{2+}:4) were studied as a function of pH. In the hydrolyses of both 6 and 7, Co^{2+}:3 (which contains no hydroxyethyl pendant) appears to act as its anionic form, generated by ionization of a Co^{2+} bound H_2O having a $pK_a \approx 8$ at 37°C in 80% ethanol-H_2O. On the other hand, Co^{2+}:4

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(which possesses a hydroxyethyl pendant) catalyzes the hydrolyses of **6** and **7** with an activity that increases linearly with pH up to at least pH 8.3. Above this pH, precipitation of Co^{2+} (OH^{-})x becomes a problem. In the latter case, the active form of Co^{2+}:**4** has a pK_a in excess of 8.3 that apparently obtains from ionization of the Co^{2+}-associated hydroxyethyl group. Evidence is presented that Co^{2+}:**4** functions as a general base catalyst in the hydrolysis of **6**.

(Keywords:

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Design, Synthesis and Study of Catalysts for Organophosphate Ester Hydrolysis

Annual Report

Robert S. Brown

July 1985

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Summary

This Annual Report details the work carried out during the contracting year (March 1984 - March 1985) under Contract DAMD17-83-C-3091. Section I describes very briefly the approach taken, namely, to design and synthesize catalysts for phosphate ester hydrolyses which are modelled after carbonic anhydrase (CA) and alkaline phosphatase (APase). Section II describes the kinetic studies undertaken since the last contracting year. We have investigated in considerable detail the hydrolysis of ethyl-*p*-nitrophenylmethylphosphonate (**7**), *tris*-*p*-nitrophenylphosphate (**5**), and *tris*-2-pyridylphosphate (**6**) catalysed by the Co^{2+}-complexes of two *tris*-imidazolylphosphines (**3** and **4**). In this study we found that both Co^{2+}-complexes facilitated the hydrolysis of **5** to **7** , but they do so by different mechanisms. While **3**:Co^{2+} reacts via a metal-coordinated OH_2 group having a pK_a of ~ 8.0 at 37°C in 80% ethanol-H_2O, **4**:Co^{2+} apparently reacts via the involvement of a metal-associated hydroxyethyl pendant.

Foreword

TABLE OF CONTENTS

LIST OF TABLES

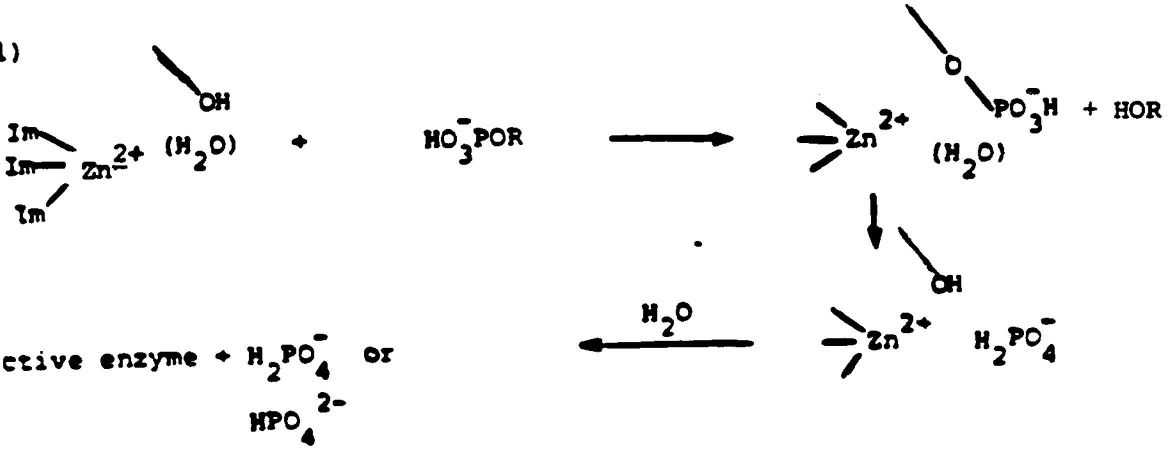
LIST OF FIGURES

I. Statement of Project

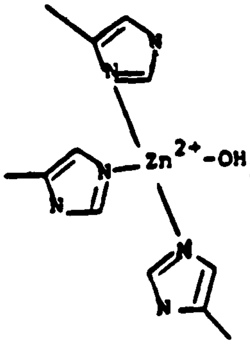
(a) Carbonic Anhydrase and Alkaline Phosphatase Models as Catalysts for Hydrolysis of Phosphate Esters.

Alkaline phosphatases (APases) are Zn(II)- and Mg(II)-containing metalloenzymes found in virtually every living organism which catalyse the hydrolyses of phosphate monoesters ($ROPO_3H^-$) of wide structural variation.[1] It now seems reasonably certain that the active site catalytic groups consist of two Zn(II) ions ~ 5Å apart respectively coordinated by three histidine residues and a histidine and two aspartate residues.[1,2] The remaining ligand positions are occupied by H_2O. Also in the active site is an essential serine OH group which becomes transiently phosphorylated during the catalytic cycle. Although the exact mechanism of action is unknown, a minimum reaction scheme can be drawn as in equation 1.

(1)



Mechanistically, the role of the Zn^{2+} is, first, to activate the serine OH sufficiently to enable phosphoryl transfer to it, and second, to dephosphorylate that residue, probably by delivering a Zn^{2+}-bound H_2O or OH^- group. The parallel with the inhibition of acetylcholinesterase (AChE) by organophosphates is close, except that AChE has no internal mechanism by which it can hydrolyse the phosphoryl (phosphonyl) serine, since there is no Zn^{2+} at the active site.



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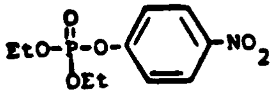

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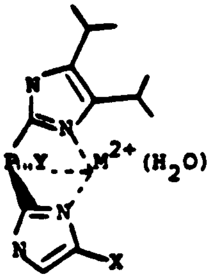
Carbonic anhydrase (CA) is a metalloenzyme with an active site consisting of a Zn^{2+}-ion coordinated to three histidine imidazole (Im) units as in **1**.[3] In addition to its normal physiological function of catalysing the interconversion of HCO_3^- and CO_2, CA has been shown to have oxonase activity in that it catalyses the hydrolysis of a dialkyl aryl phosphate triester analogue of paraoxon (**2**).[4] Given the above, and the fact that a number of less-well-characterized enzymes have been shown to exhibit oxonase activities,[5] it is of interest to know whether smaller, biomimetic models for the CA and APase active sites (**3** and **4**)[6] would act as catalysts in facilitating the hydrolysis of some selected phosphate triesters such as 5[7] and 6[8] and a phosphonate **7**.[9]



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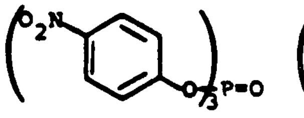

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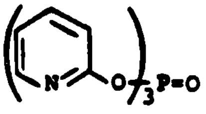

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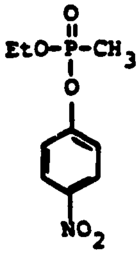

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3: X = H, Y = 4,5-diisopropylimidazol-2-yl

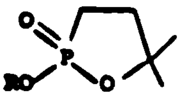
4: X = CH_2CH_2OH, Y = 4,5-diisopropylimidazol-2-yl

The latter compound can be taken as a model for the phosphonyl inhibited serine OH group in AChE.

(b) Overview of Catalysis of Phosphate (Phosphonate) Ester Hydrolyses

Considerable effort has been expended to define conditions under which the hydrolyses of phosphate and phosphonate esters are catalysed. Much of the work[10-13] centers on the use of micelles which contain functional groups such as Im[10], oximate[12], or alcohols.[13] Im-containing surfactants[10] have been found to hydrolyse phosphate triesters by nucleophilic and general base mechanisms. Oximate[12] and alcohol(ate) surfactants attack triesters and aryl alkyl phosphate diesters nucleo-philically, the main role of the tetraalkylammonium surfactant being to provide a positively charged medium in which the reaction can occur. Generally these studies used highly alkaline conditions.

Under non-micellar conditions, Macomber[14] has investigated the catalysis afforded by Im on the hydrolysis of oxa-phospholene esters (**8**). Although Im acts as a nucleophile toward tetra-benzylpyrophosphate (**9**)[15], its role with 8 is that of a general base.[14] When present in 2.4-fold excess over **8**, Im affords a 50-fold acceleration over the spontaneous hydrolysis rate.



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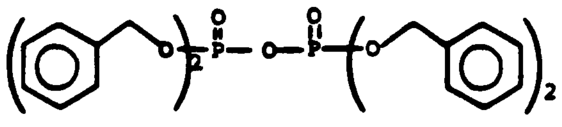

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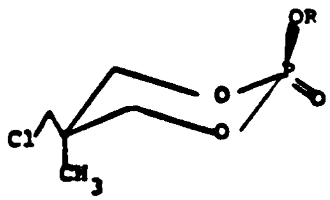

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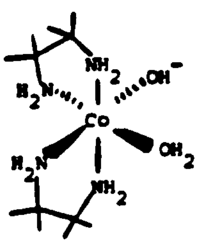
There have been relatively few reports of metal-ion catalysis of phosphate triester hydrolyses.[16,17] In the reported cases, the metal-ion can act either to assist in the departure of the leaving group, as in the methanolysis of **10**[16a,b], or in providing a metal-coordinated nucleophile.[16c,d] Earlier work demonstrated that divalent metal ions led to impressive rate accelerations in the hydrolysis of neutral phosphate esters, but the presence of several forms of the $M_x^{2+}(H_2O)y\ (OH^-)_z$ species seriously complicated the definition of which form was actually responsible for catalysis.

Most recently, Kenley et al.[17] have looked at the hydrolysis of 7 catalysed by several Co(III) chelates. The best of these chelates (**11**), at 25°C, pH 7.6, has an apparent second-order rate constant for catalysis of $5 \times 10^{-2}\ M^{-1}s^{-1}$[17], which compares favorably with that of OH^- ($3.9 \times 10^{-2}\ M^{-1}s^{-1}$)[18] and pralidoxime chloride (2-PAM) (**12**, $23.9 \times 10^{-2}\ M^{-1}s^{-1}$).[19]



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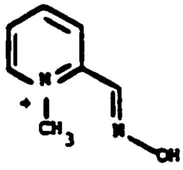

12

II. Progress Report

(a) Methodology

Over the contracting year we prepared ligands 3 and 4 and evaluated their Co^{2+}-complexes as catalysts for the hydrolysis of phosphates 5 and 6 and phosphonate 7. The syntheses of 3 and 4 are documented in last year's Annual Report[20], while the syntheses of 5-7 are reported in references 19-21, respectively.

Kinetics were monitored by observing the rate of appearance of *p*-nitrophenolate at 400 nm for 5 and 7 or 2-pyridinol at 305 nm for **6** in 80% ethanol-H_2O buffered solutions held at 37.1±0.2°C or 25.7±0.2°C, as previously described.[20] Buffers (0.047 M, $\mu = 0.047$ $NaClO_4$) were CHES (cyclohexylaminoethanesulfonic acid), pH 7.5-8.3, or MOPS (morpholinoethanesulfonic acid), pH 5.4-7.2). pH values were measured with a radiometer GK 2322 C combination electrode immersed directly into the solution before and after each reaction and were not corrected for the organic solvent fraction. Reactions were initiated by injecting known quantities of substrate (5-10 x 10^{-3} M tetrahydrofuran [THF] stock solutions of **5-7**), ligand (0.025 M ethanol stock) and $CoCl_2$ (0.1314 M H_2O stock standardized by EDTA titration[21]) into 3.0 mL thermally equilibrated buffer. Pseudo-first-order rate constants were evaluated by fitting the absorbance vs. time data to a standard exponential model ($A_t = A_{t\infty} + (A_{to} - A_{t\infty})\ e^{-kt}$) by a non-linear least squares treatment. In all cases the reactions were followed in duplicate or triplicate to at least 80% completion and displayed good pseudo-first-order kinetics. Reported values are averages of two to three determinations and have a precision of better than 5%.

Product studies were conducted by comparing the uv-vis spectra obtained after reaction with a mixture comprised of the authentic catalytic components and expected hydrolytic products, (ethylmethylphosphonate + *p*-nitrophenol in the case of 7, *bis*-2-pyridylphosphate + 2-pyridinol in the case of **6**, and bis-*p*-nitrophenylphosphate + *p*-nitrophenol in the case of 5). In all cases the spectra obtained after reaction were identical with those of the anticipated product mixtures. Control experiments established that for the latter two cases only one leaving group is cleaved from the substrate and hence the initially formed phosphate products are stable to further hydrolysis.

(b) Results

i. Hydrolyses of 5 and 7.

The hydroxide-dependent rate constants for hydrolysis of 5 and **7** (H_2O, 25°C) are reported to be 11.2 $M^{-1}s^{-1}$ and 0.04 $M^{-1}s^{-1}$, respectively.[7a] In 80% ethanol-H_2O (this medium required for ligand solubility) at 25.7°C, the spontaneous pseudo-first-order rate constants for hydrolysis of 5 at pH 7.5 and pH 7.85 (MOPS) are 5.12 x $10^{-4}s^{-1}$ and 7.19 x $10^{-4}s^{-1}$, respectively (faster than that reported in 1:1 acetone/H_2O; 1 x $10^{-5}s^{-1}$, pH 7.5, 25°C[7a]), while those of phosphonate **7** are too slow to be measured. Neither Co^{2+} nor ligand **3** or **4** alone at 5 x 10^{-4} M facilitates the decomposition of either substrate. However, equimolar Co^{2+} and **4** produce accelerations, the second-order catalytic rate constants being given in Table 1. Previous studies[6] have shown that ligands **3** and **4** have high affinities for Co^{2+} such that at

Table 1. Hydrolysis of tris-p-nitrophenylphosphate (**5**) and ethyl-p-nitrophenylmethylphosphonate (**7**) catalysed by Co^{2+}:**4** at 25.7°C.[a,b]

pH	$k_5^{cat} \times 10^2 (M^{-1}s^{-1})^c$	$k_7^{cat} \times 10^2 (M^{-1}s^{-1})^c$
7.50	6.4	1.4
7.85	9.8	2.7
7.95	-	3.4
8.20	-	8.6
8.30	-	10.2

a. 80% ethanol-H_2O as in reference 8d; 0.047 M CHES buffer; μ = 0.047 $NaClO_4$.

b. 0.05 mM **5** and **7**; 0.5-2.0 mM Co^{2+}:**4**; followed at 400 nm.

c. Evaluated from the slopes of plots of k_{obs} vs $[Co^{2+}:\mathbf{4}]$; according to equation k_{obs} = intercept + $k_x^{cat}[Co^{2+}:\mathbf{4}]$; r > 0.99 for at least 5 concentrations.

the concentrations employed, the complexes are >95% formed.* This is also verified by the fact that plots of k_{obs} vs. $[Co^{2+}:\mathbf{4}]$ are linear and show no upward curvature between 0.5 and 2.0 mM in added components. The linearity also argues against a pre-equilibrium formation of a phosphate or phosphonate: Co^{2+}:**4** ternary complex at these concentrations. The fact that the catalytic second-order rate constants presented in Table 1 increase with pH indicates that the active form of Co^{2+}:**4** is in some way dependent on $[OH^-]$, although from these data the exact nature of the active form is uncertain.

Unfortunately, the relative activities of Co^{2+}:**4** and Co^{2+}:**3** toward substrates **5** and **7** cannot be compared at 25°C, since solutions of Co^{2+} and **3** become turbid during the course of the reactions. To make such a comparison, a temperature of 37.1°C was used for further hydrolysis studies of **7**. Given in Table 2 are the pseudo-first-order rate constants for the hydrolysis of 0.05 mM **7** observed at 37°C in the presence of 0.5 mM Co^{2+}:**3** or Co^{2+}:**4**. From these data it can be observed that Co^{2+}:**4** is about 3-7-fold more active than is Co^{2+}:**3**, the difference in activities being greater at higher pH. It is also evident from Fig. 1 that the activity of Co^{2+}:**3** levels off at about pH 8.0, indicating an ionization event, while the activity of Co^{2+}:**4** continues to increase with pH in a first-order fashion.

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*The $pK_{Co^{2+}}$ values (defined in the direction of dissociation) for Co^{2+}:**3** and Co^{2+}:**4** in 80% ethanol H_2O are 7.7 and 5.7, respectively.[6] The Eadie-Hofstee plot for the decomposition of **6** as a function of $[Co^{2+}]$ yields an equivalent pK_{eq} for **6**:Co^{2+} of 2.2.

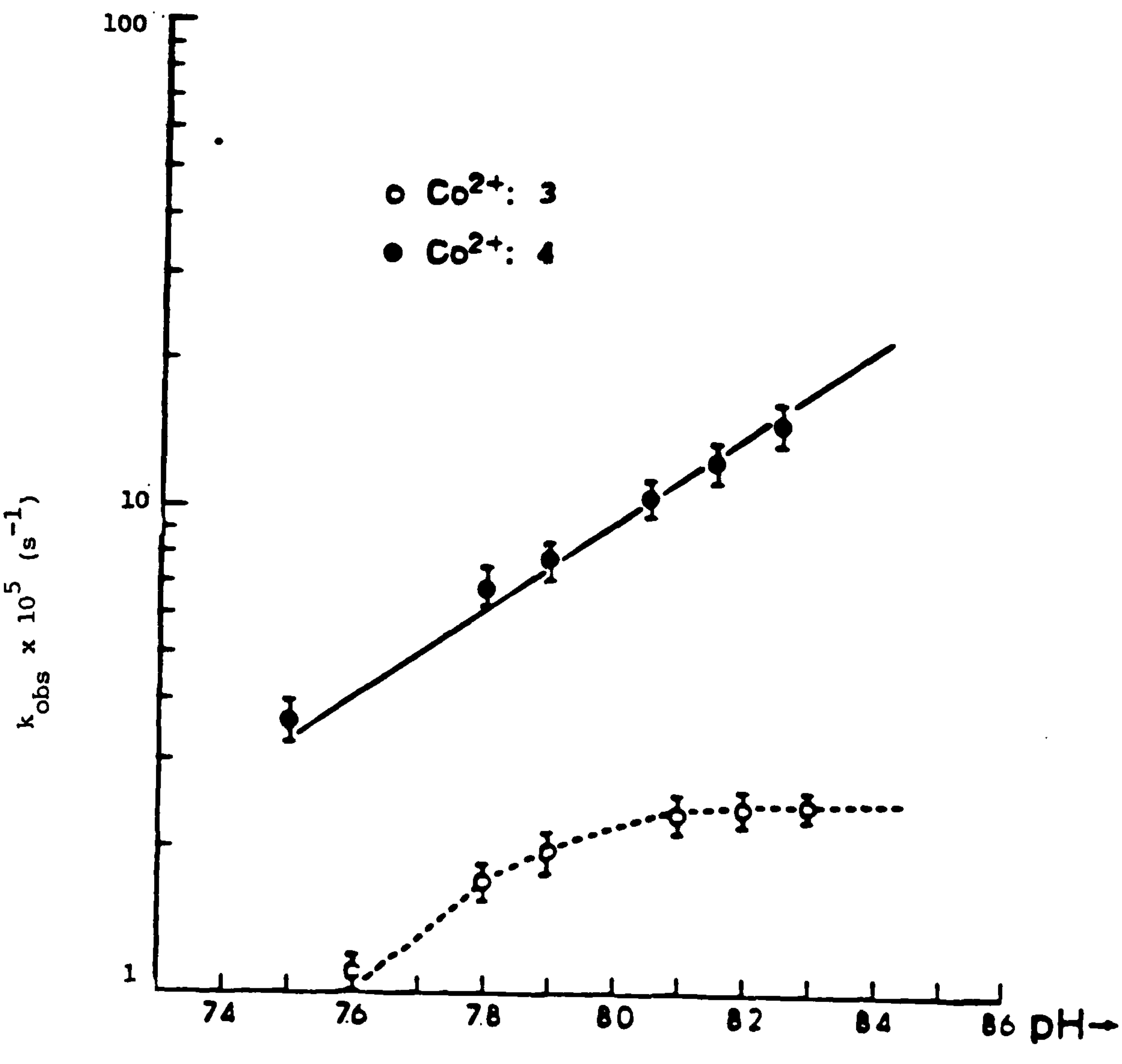

Figure 1 Plot of $k_{obs} \times 10^5$ for the hydrolysis of $5 \times 10^{-5}M$ phosphonate 7 in the presence of $5 \times 10^{-4}M$ Co^{2+}:3 (O) and Co^{2+}:4 (●). T = 37.1°C, 0.047μ, CHES buffers, $\mu = 0.047$. $NaClO_4$, 80% ethanol-H_2O. Straight line drawn through Co^{2+}:4 data is first order in $[OH^-]$. Data from Table 2 with error limits being $\leq \pm 5\%$ of the value as determined by averages of duplicate experiments.

Table 2. Pseudo-first-order rate constants (k_{obs}) for the hydrolysis of 7 in the presence of Co^{2+}:3 and Co^{2+}:4 at 37.1°C.[a,b]

pH	$k_{obs}(Co^{2+}:3) \times 10^5 s^{-1}$ [c]	$k_{obs}(Co^{2+}:4) \times 10^5 s^{-1}$ [c]
7.50	-	3.64
7.80	1.70	6.75
7.90	1.95	7.80
8.10	2.35	10.6
8.20	2.41	13.5
8.30	2.45	15.1

a. 80% ethanol-H_2O; 0.047 M CHES; μ = 0.047 $NaClO_4$.
b. Followed at 400 nm.
c. 0.5 mM Co^{2+}:3 or Co^{2+}:4; 0.05 mM 7; values are averages of at least duplicate measurements.

ii. Hydrolysis of *tris*-2-pyridylphosphate (6).

Uncatalysed, the pseudo-first-order rate constant for hydrolysis of 6 between pH 6.0 and 8.2 varies by only a factor of 3 from 5.27 to 18.7 x $10^{-6} s^{-1}$, suggestive of a dominant water term and the onset of a small, OH^--dependent term at the high pH end of this region. Addition of a 10-fold excess of Co^{2+} (0.5 mM) accelerates the reaction by nearly 100-fold at all pH's, these data being given in Table 3 along with data for the

Table 3. Pseudo-first-order rate constants (k_{obs}) for the hydrolysis of 0.05 mM *tris*-2-pyridylphosphate (6) in the presence of Co^{2+}, Co^{2+}:3, and Co^{2+}:4.

	$k_{obs} \times 10^4 s^{-1}$		
pH	Co^{2+} [b]	Co^{2+}:3 [c]	Co^{2+}:4 [c]
5.40	4.52	-	-
5.80	4.19	-	-
6.00	4.55	3.82	2.66
6.40	4.37	4.55	3.17
6.80	5.02	5.93	3.44
7.10	6.31	8.28	5.61
7.45	13.1	19.0	13.7
7.85	20.0	30.4	27.0
8.10	-	38.4	-
8.15	-	41.1	68.4

a. T = 37.1°; 0.047 M MOPS (pH 5.4-7.1) or 0.047 M CHES (pH 7.1); μ = 0.047 $NaClO_4$; 80% ethanol H_2O; followed at 305 nm; uncertainties ± 5%.
b. 0.493 mM $CoCl_2$.
c. 0.484 mM complex.

observed in the presence of Co^{2+}:**3** and Co^{2+}:**4**. The latter data are displayed in Fig. 2 and show that the catalysis exhibited by Co^{2+}:**3** levels off above pH 8 while Co^{2+}:**4** catalysis increases linearly from pH 7 to 8.2. Unfortunately, precipitation is observed above pH 8.2, which prevents complete definition of the pH profile. Ligand alone does not accelerate the reaction.

Plots of k_{obs} against $[Co^{2+}]$ alone show definite signs of curvature between 0.5 and 5.5 mM Co^{2+} suggesting a saturation phenomenon as in equation 2. At a given pH, an Eadie-Hofstee plot of k_{obs} against $k_{obs}/[Co^{2+}]$ yields a straight line with intercept reaction k_{cat} and a slope $-K_{eq}$, the latter value being defined as the dissociation constant of **6**:Co^{2+}. At pH 6.4, the derived constants are k_{cat} = (4.67±.17) x $10^{-3}s^{-1}$ and K_{eq} = (4.68±.41) x 10^{-3} M (r = 0.98, 8 data), while at pH 7.15, the values are (7.32±.22) x $10^{-3}s^{-1}$ and (5.13±.37) x 10^{-3} M (r = 0.98, 9 data). These data indicate that (within experimental error) the dissociation constant for **6** and Co^{2+} is equivalent at the two pH's, while the catalysed rate constant increases with pH, indicating a dependence on $[OH^-]$.

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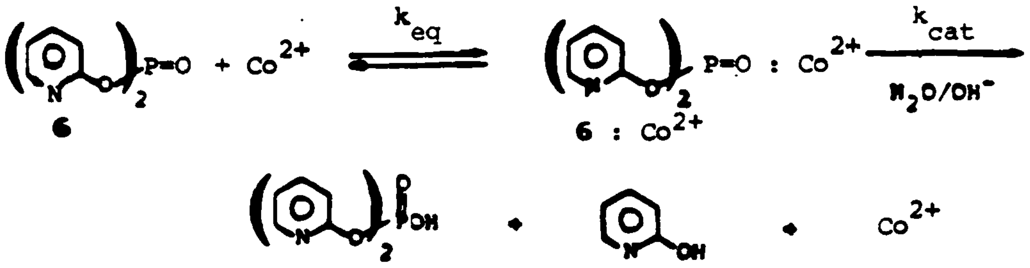

Hydrolysis of **6** is also promoted by the Co^{2+} complexes of 3 and **4**, although from the data given in Table 3, the two complexes are approximately as reactive as Co^{2+} alone. Since 3 and 4 bind Co^{2+} at least 10^3 more tightly than does **6***, the great bulk of Co^{2+} will be coordinated to those ligands, leaving little free metal in solution. Hence, whatever acceleration occurs in hydrolysis must result from the 3:Co^{2+} or 4:Co^{2+} complexes. Plots of k_{obs} vs. [**4**:Co^{2+}] between 0.4 and 5.0 mM at pH 6.4 and 7.15 yield straight lines with slopes of 0.58±.07 $M^{-1}s^{-1}$ and 2.0±.15 $M^{-1}s^{-1}$, indicating that the second-order catalytic rate constants increase with pH in (within experimental error) a first-order fashion. No evidence of saturation behavior is evident.

(c) Discussion

Neutral phosphate and phosphonate esters at pH values above 7 generally decompose by pathways dependent on the concentration of anionic nucleophiles.[7,22] Hydroxide is a reasonably good nucleophile, but at pH values approaching neutrality, its concentration is so low that the pseudo-first-order rate constants for hydrolysis of esters such as 5 or 7 are 10^{-6}-10^{-8} s^{-1} in H_2O.[22a]

In a variety of systems, it has been shown that the pK_a of an M^{2+}-coordinated H_2O is drastically reduced but it retains sufficient nucleophilicity to attack electrophilic centers.[3,23] Several studies have shown that M^{x+}-coordinated hydroxyalkyl or oxime groups also have reduced pK_a values and retain sufficient nucleophilicity as their anions to attack esters, CO_2 and phosphate esters.[24] Coordination of metal ions to normally hydrolysis-resistant anionic phosphates and polyphosphates has been reported to lead to large accelerations in hydrolysis by mechanisms which could involve a combination of Lewis acid charge neutralization, intramolecular delivery of M^{x+}-OH^-, and/or electrostatic stabilization of the leaving group.[23a,25]

Ligand **3**, when coordinated to Zn^{2+} or Co^{2+}, appears to be a reasonable model for the active site of carbonic anhydrase, while M^{2+}-coordinated **4** is a model for a portion of the active site of APase.‡ Earlier studies have shown that Co^{2+}:**3** and Co^{2+}:**4** both undergo ionization of a Co^{2+}-OH_2 with a kinetic pK_a of 7.6-7.8 at 25.0°C in 80% ethanol-H_2O to produce a Co^{2+}-OH^- unit that acts as a bimolecular catalyst in promoting the hydrolysis of p-nitrophenyl picolinate (pNPP) (equation 3).[6b] No evidence of the formation of a ternary pNPP:Co^{2+}:**3** or pNPP:Co^{2+}:**4** complex is evident. This study[6b] also indicated that the hydroxyethyl group in Co^{2+}:**4** is of no catalytic benefit in pNPP hydrolysis, even though in related examples[24] a hydroxyalkyl pendant is reported to act as a nucleophile. The major catalytic advantage of Co^{2+}:**3** and Co^{2+}:**4** over $Co^{2+}(H_2O)_x$ is two-fold: first, to reduce the pK_a of a coordinated H_2O and second, to bind Co^{2+} tightly enough to

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‡The active site of CA has been determined by X-ray crystallography to consist of an essential Zn^{2+}-ion coordinated in a tetrahedral manner to three protein-based histidine Im units.[3] The active site of AP, while not yet completely elucidated by X-ray crystallographic analysis, appears to consist in part of an essential Zn^{2+}-ion coordinated to at least three histidine Im units.[1] Also in the active site is an essential serine OH group, which during the hydrolytic event becomes transiently phosphorylated. The latest structural determinations indicate that the active site is dinuclear in Zn^{2+}, the second Zn^{2+} being bound by 1 His Im and two oxygen-containing ligands.

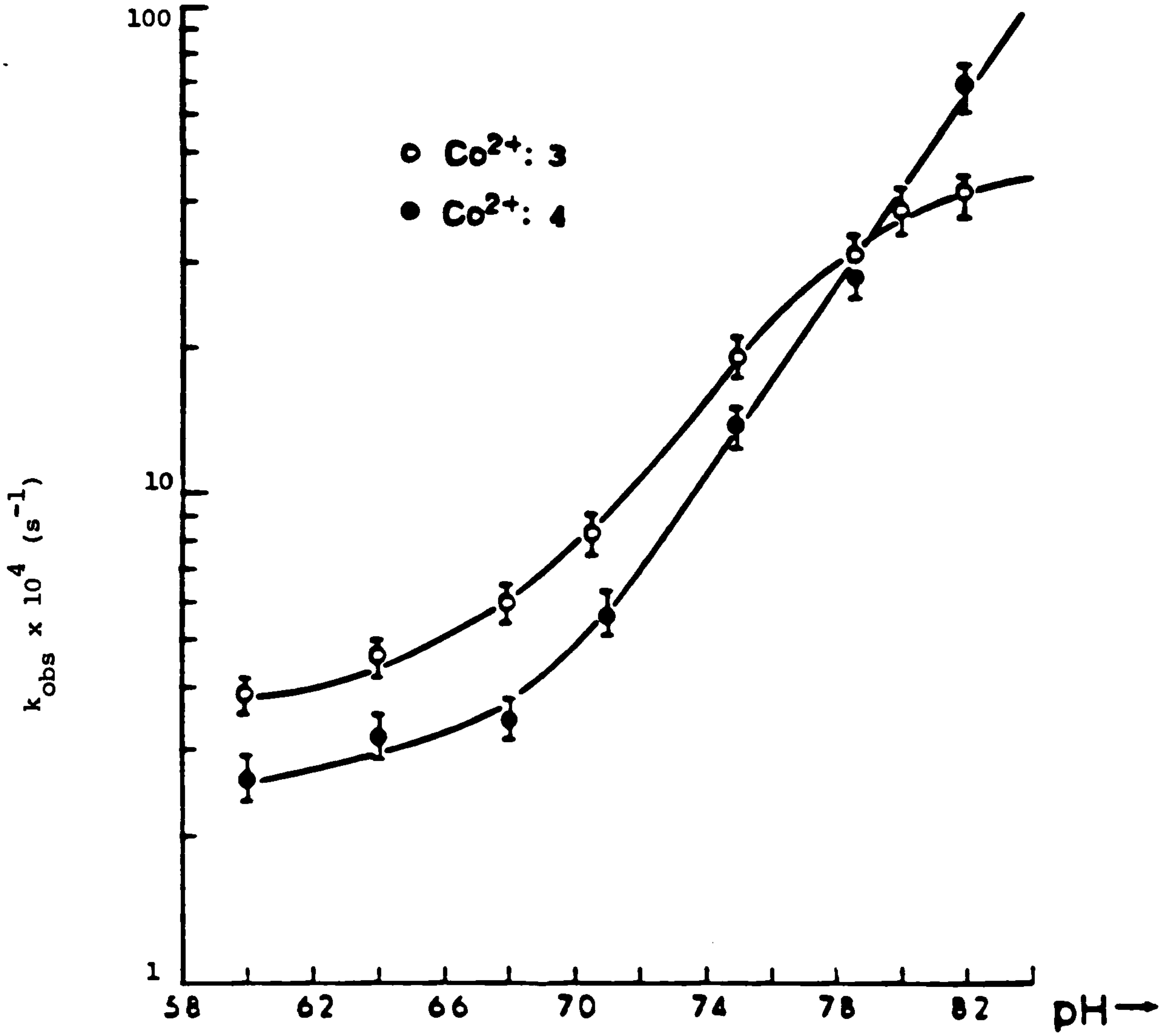

Figure 2 Plot of $k_{obs} \times 10^4$ for the hydrolysis of 5×10^{-5}M tris-2-pyridylphosphate (6) in the presence of 4.8 $\times 10^{-4}$M Co^{2+}:3 (O) and Co^{2+}:4(●). T = 37.1°C, 0.047 M MOPS or CHES buffers, μ = 0.047 $NaClO_4$, 80% ethanol-H_2O. Error limits < ±5% of value determined by average of duplicate experiments.

prevent precipitation of $Co^{2+}(OH^-)_x$ during the course of the reaction.

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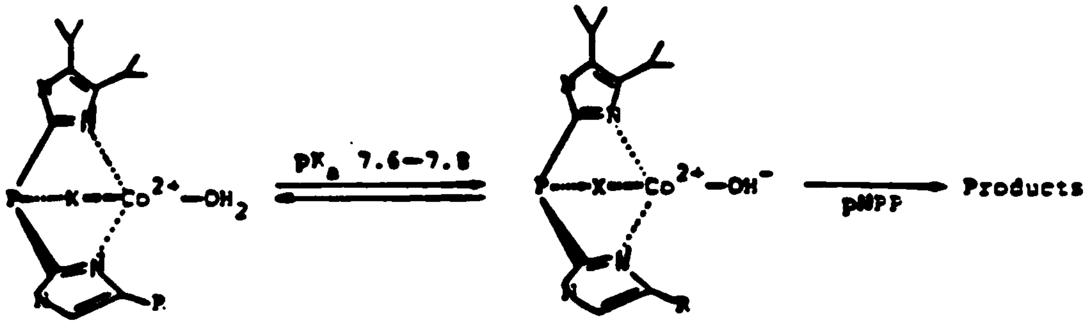


Co^{2+}:**3**: R = H; X = diisopropylimidazole

Co^{2+}:**4**: R = CH_2CH_2OH; X = diisopropylimidazole

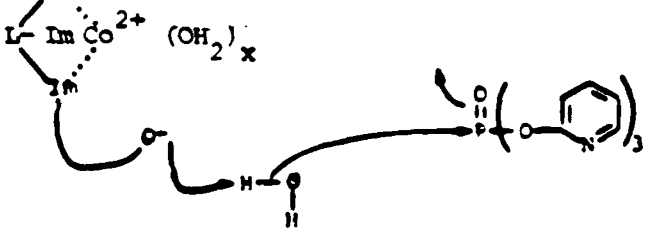
Toward phosphonate **7** at 37.1°C, both Co^{2+}:**3** and Co^{2+}:**4** exhibit activity, but the plots given in Fig. 1 indicate that they behave differently. Co^{2+}:**3** shows an increasing activity with pH below pH 7.8 and levels off above 8, indicating the participation of a deprotonated form of the complex. We tentatively ascribe this to an ionizing Co^{2+}:**3**-bound H_2O with a pK_a ~8.0. This value is somewhat higher than the 7.6-7.8 noted for the same ionization in the catalysed hydrolysis of pNPP[6b] and may be attributable to the slightly different conditions and 12°C higher temperature used for the present study. Admittedly, the pH range covered here is not wide enough to make a definite conclusion concerning the pK_a. This is because the reactions are quite slow at pH values lower than 7.4, where they are complicated by ligand instability while at pH values higher than 8.3, precipitation becomes a problem.

Apparently Co^{2+}:**4** behaves differently from Co^{2+}:**3**, since the former's activity toward phosphonate **7** continues to increase with pH with no evidence of curvature. This implies that a basic form of Co^{2+}:**4** is active but that the ionizing group pK_a is higher than that of the Co^{2+}-bound H_2O, which was shown in a previous study to be 7.6-7.8 at 25°C.[6b] By analogy to related systems bearing a pendant hydroxyalkyl group,[24] we ascribe the ionization to the Co^{2+}-associated hydroxyethyl group which should have a higher pK_a than 8. Whereas the activity of Co^{2+}:**4** toward pNPP involves the metal-associated H_2O, reaction toward these phosphorus centers apparently involves the hydroxyethyl pendant. Plots of k_{obs} vs. [Co^{2+}:**4**] at each pH are linear and do not indicate formation of a ternary **7**:Co^{2+}:**4** complex.

Hydrolysis of phosphate **6** is markedly catalysed by Co^{2+} and saturation behavior is observed in plots of k_{obs} vs. [Co^{2+}]. This indicates pre-equilibrium formation of a **6**:Co^{2+} complex, which suffers attack by H_2O and or OH^- as in equation 2. From the Eadie-Hofstee plot, the k_{cat} values for the decomposition of $6:Co^{2+}$ at pH 6.4 and 7.15 are $4.67 \times 10^{-3} s^{-1}$ and $7.32 \times 10^{-3} s^{-1}$ and show a slight but not first-order dependence on $[OH^-]$. The coordinated Co^{2+} likely acts both as a Lewis acid to facilitate attack on the central P and to stabilize the departing alkoxypyridine, both processes having precedent.[24,25]

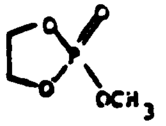
Co^{2+}:**3** and Co^{2+}:**4** also catalyse the solvolysis of **6** in a way which increases with pH (Fig. 2). However, the plot for Co^{2+}:**3** levels off, indicative of an active species having a pK_a ~8, while that for Co^{2+}:**4** continues to increase linearly with pH. The implication is that the activity for the former is associated with $3:Co^{2+}\text{-}OH^-$, while the different behavior of Co^{2+}:**4** is tied to the presence of the hydroxyethyl group, which in its basic form is promoting the solvolysis of **6**. Plots of k_{obs} vs. $[Co^{2+}:4]$ show a strict linearity, the slopes of which increase with pH and give no evidence of saturation behavior.

All of the above points to the fact that the Co^{2+}:**4** behaves differently from Co^{2+}:**3** in terms of its relative propensity to attack the neutral phosphates and phosphonate studied here. The basic ligand structure in Co^{2+}:**3** and Co^{2+}:**4** is the same with respect to numbers, orientation, and types of Im's. Earlier studies[6b] have revealed that the pK_a value of the Co^{2+}-coordinated H_2O units is the same for both complexes. Hence, it seems reasonable that the different behavior of the two toward phosphate ester hydrolysis is ascribable to the anionic form of the hydroxyethyl pendant, which apparently has a pK_a in excess of 8.3 in the medium used. The pendant could act either as a nucleophile or general base, although for several reasons we favor the latter role. First, although the bulk of the studies reported here employed an excess of complex relative to phosphate **6** or phosphonate 7, when equimolar concentrations (5×10^{-4} M) of Co^{2+}:**4** and **6** or 7 were employed, the production of p-nitrophenoxide exhibited clean first order kinetics. Second, product studies revealed that hydrolysis of an equivalent of **6** catalysed by Co^{2+}:**4** yielded only one equivalent of 2-pyridinol. Dipyridylphosphate is not hydrolysed under the conditions employed here nor is its hydrolysis catalysed visibly by Co^{2+} or either complex. These two observations suggest strongly that the role of the anionic hydroxyethyl pendant in Co^{2+}:**4** is that of a general base as in 13.



13 L • 3

General base roles are well documented, for example, in the hydrolyses of phosphate **14**[14] or phosphonate **15**.[15] Were Co^{2+}:**4** to



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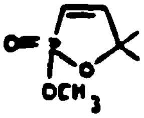

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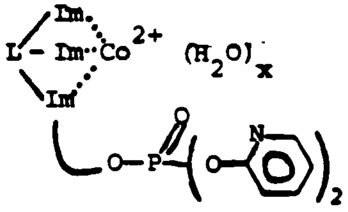
behave as a nucleophile toward **6**, intermediate **16** would be formed. To satisfy the kinetics and the observation that hydrolysis of **6** produces only one molecule of 2-pyridinol



16 L = **3**

would require that **16** hydrolyse at least 10 times faster than it is formed (to maintain a constant [Co^{2+}:**4**]) by a pathway that involves exclusive cleavage of the L:Co^{2+}O-P bond. However, it is anticipated that cleavage of a 2-pyridinol unit from **16** would be a much more rapid process, since the heterocycle is a far better leaving group than is the hydroxyethyl group of Co^{2+}:**4**. Unfortunately, synthesis of the putative intermediate **16** proved impossible in our hands, yielding only complex mixtures of what appeared to be a cyclic phosphate having both the N and O of the hydroxyethyl Im unit covalently attached to phosphorus.

The above account provides evidence that complexes such as Co^{2+}:**4** and Co^{2+}:**3** can be reasonably effective catalysts in promoting the hydrolysis of selected neutral phosphonate and phosphate substrates. In 80% ethanol-H_2O at 25°C, the apparent second order rate constant of Co^{2+}:**4** toward phosphonate **7** increases linearly with pH up to at least pH 8.3, where its value of $k_7^{cat} = 10.2 \times 10^{-2} M^{-1}s^{-1}$ exceeds that of OH^- ($k_{OH^-} = 3.9 \times 10^{-2} M^{-1}s^{-1}$), even though the active form of Co^{2+}:**4** is not completely generated. This value compares favorably with the apparent second-order rate constants for some Co^{3+} chelates[17] and aldoximates[19] toward **7**, recently published by Kenley et al. For example, in H_2O at 25°, pH 7.6, the apparent k_{cat} terms for complex **11** and 2-PAM (**12**) are $5 \times 10^{-2}\ M^{-1}s^{-1}$ and $24 \times 10^{-2}\ M^{-1}s^{-1}$‡, respectively. Of course, the medium required for

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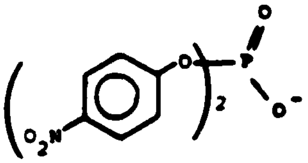
‡Calculated from the reported second-order rate constant of 5.70 $M^{-1}min^{-1}$ and pK_a of 7.99 for 2-PAM.[19]

solubility is such that a strict comparison cannot be made and future complexes will be designed to overcome the solubility and lability[26] problems of these phosphine-based ligands. Nevertheless, it appears that related complexes with ionizable pendants could provide attractive catalysts for facilitating the hydrolysis of phosphate and phosphonate esters.

Since January 1985, we have been investigating another area of catalysis of phosphate ester hydrolysis, namely, the ability of 3:Co^{2+} and 4:Co^{2+} to catalyse the hydrolysis of phosphate mono- and diesters. Over the course of a 3 month period, we looked at a variety of esters such as 17-19 and attempted to demonstrate catalysis of their hydrolysis under a variety of conditions of pH, concentration of catalyst and temperature. The invariable result was that under no circumstances could either metal complex be demonstrated to be active toward these anionic phosphates. Prolonged reaction times were complicated by ligand instability, as was seen in the above study.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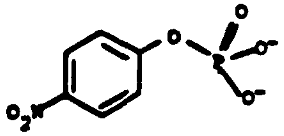
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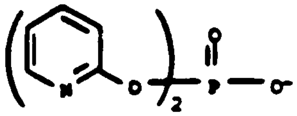
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