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Hydrolysis of Neutral Phosphate and Phosphonate Esters Catalysed by Co^{2+}-chelates of Tris-imidazolyl Phosphines

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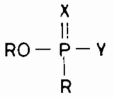
Abstract

The hydrolysis of two neutral phosphate esters, (tris-2-pyridylphosphate and tris-*p*-nitrophenylphosphate, **5** and **4** respectively) and a phosphonate ester (ethyl-*p*-nitrophenylmethylphosphonate (**6**)) were studied in the presence of Co^{2+}-complexes of two tris-imidazolylphosphines. In the hydrolyses of both **5** and **6**, the Co^{2+}-complex of bis-[4,5-diisopropylimidazol-2-yl]imidazole-2-yl phosphine (**2**) appears to act as the anionic form, generated by ionization of a Co^{2+}-bound H_2O having a pK_a of ~8 at 37 °C in 80% ethanol–H_2O. On the other hand, the Co^{2+}-complex of bis-[4,5-diisopropylimidazol-2-yl]-4(5)-hydroxyethylimidazol-2-yl phosphine (**3**) catalyses the hydrolyses of **5** and **6** with an activity which increases linearly with pH. In this case, the active form of Co^{2+}:**3** has a pK_a in excess of 8.3 which apparently obtains from ionization of the Co^{2+}-associated hydroxyethyl group. Evidence is presented that Co^{2+}:**3** functions as a general base catalyst in the hydrolysis of **5**.

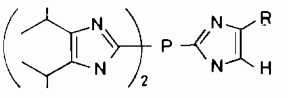
Introduction

Studies of the hydrolysis of neutral phosphorus esters (**1**) have received increasing attention not only because of the possible implications for biological phosphoryl transfers but also since certain of these materials inhibit acetyl-cholinesterase and disrupt normal neuro-transmission [1]. The latter substances include insecticides such as parathion (RO = R = OEt; X = S; Y = $OC_6H_5NO_2$) and paraoxon (RO = R = OEt; X = O; Y = $OC_6H_5NO_2$) and the widely stockpiled alkylphosphonofluoridic esters soman (RO = $(CH_3)_3CC(CH_3)HO$; R = CH_3; X = O; Y = F) and sarin (RO = $(CH_3)_2CHO$; R = CH_3; X = O; Y = F). In general, such neutral phosphorus esters undergo rate-limiting nucleophilic attack at P (sometimes involving general-base assistance) with the displacement of a suitable leaving group, Y [2].



$$RO-\overset{\overset{\displaystyle X}{\|}}{\underset{\underset{\displaystyle R}{|}}{P}}-Y$$

1



2 R = H **3** R = CH_2CH_2OH

The great bulk of the studies concerning methods of facilitating the cleavage of such esters has centered on the use of cationic micelles [3] and functionalized surfactants [4], the latter containing nucleophilic or basic pendants such as hydroxamic acids [4a], imidazole [3a, 4i], hydroximino [4b, g] and hydroxy-alkyl [4c, e, j]. The catalysis observed arises from aggregation effects that increase the concentration of reactive general base or nucleophile, and/or medium effects that reduce the pK_a of the nucleophilic precursor within the micellar interior and therefore allow larger [anion] at lower pH values.

In a limited number of cases, phosphate triester solvolysis has been shown to be accelerated by the presence of metal ions by routes that involve assisting departure of the leaving group [5a, b] or facilitating nucleophilic attack at P [5c, d]. In the hydrolysis of isopropylmethylphosphonofluoridate, Mg^{2+} ion as its hydroxo complex acts bimolecularly as a nucleophilic catalyst [6] with a possible Lewis acid role as well. Although the above studies refer to the metal ion effect with neutral phosphorus compounds, they may have a biological significance since enzymatic catalysis of phosphoryl transfer and phosphate mono ester hydrolysis generally requires metal ions as added cofactors [1, 7].

For some time we have been interested in the development of simple biomimetic catalysts designed to approximate the active site on the metalloenzyme carbonic anhydrase (CA) [8] the active site of which consists of a requisite Zn^{2+}-ion coordinated in a nearly tetrahedral fashion to three histidine imidazoles [9]. 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 [10]. Given the above and the fact that a number of

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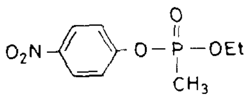
other less-well characterized enzymes have been shown to exhibit oxonase activities [11][*], it was of interest to determine whether the M^{2+}-complexes of ligands **2** and **3** [8c, d] were capable of catalysing the hydrolysis of neutral phosphate or phosphonate esters. Herein we report that the Co^{2+}-complexes of **2** and **3** do indeed catalyse the hydrolysis of phosphate triesters **4** and **5** as well as phosphonate **6**. It will be seen that while Co^{2+}:**2** appears to rely on a metal-bound hydroxide, the activity of Co^{2+}:**3** is in some way dependent on the intramolecularly associated hydroxyethyl pendant.

$(RO)_3P{=}O$

4 R = p-nitrophenyl

5 R = 2-pyridyl



6

Experimental

Tris-imidazol-2-yl phosphines **2** and **3** were prepared as previously described [8c, d] as were substrates **4** [12], **5** [13] and **6** [14]. Kinetics were monitored by observing the rate of appearance of *p*-nitrophenolate at 400 nm for **4** and **6** or 2-pyridinol at 305 nm for **5** in 80% ethanol–H_2O buffered solutions held at 37.1 ± 0.2 °C or 25.7 ± 0.2 °C as previously described [8d]. Buffers (0.047 M, μ = 0.047 $NaClO_4$) were CHES (cyclohexyalminoethanesulfonic 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 × 10^{-3} M THF stock solutions of **4**–**6**), ligand (0.025 M ethanol stock) and $CoCl_2$ (0.1314 M H_2O stock standardized by EDTA titration [15]) 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 ($A_t = A_{t\infty} + (A_{t0} - A_{t\infty})\, e^{-kt}$) 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 **6**, bis-2-pyridyl phosphate + 2-pyridinol in the case of **5**, and bis-*p*-nitrophenyl phosphate + *p*-nitrophenol in the case of **4**). 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 phosphate products are stable to further hydrolysis. **Caution.** Extreme care should be exercised when handling ethyl-*p*-nitrophenylmethylphosphonate **6** since it is a potential inhibitor of neurotransmission. Routine handling should only be performed while wearing protective clothing and gloves and all material transfers should be done in fume hoods.

Results

(a) Hydrolyses of 4 *and* 6

The hydroxide dependent rate constants for hydrolysis of **4** and **6** (H_2O, 25 °C) are reported to be 11.2 M^{-1} s^{-1} and 0.04 M^{-1} s^{-1} respectively [1b]. 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 **4** at pH 7.5 and 7.85 (MOPS) are 5.12 × 10^{-4} s^{-1} and 7.19 × 10^{-4} s^{-1} respectively (faster than that reported in 1:1 acetone/H_2O; 1 × 10^{-5} s^{-1} pH 7.5, 25 °C [1b]) while those of phosphonate **6** are too slow to be measured. Neither Co^{2+} nor ligands **2** or **3** alone at 5 × 10^{-4} M facilitate the decomposition of either substrate. However, equimolar Co^{2+} and **3** produce accelerations, the second order catalytic rate constants being given in Table I. Previous studies [8c, d] have shown that ligands **2** and **3** have high affinities for Co^{2+} such that at the concentrations employed, the complexes are >95% formed.[**] This is also verified by the fact that plots of k_{obs} *vs.* [Co^{2+}:**3**] are linear and show no upward curvature between 0.5–2.0 mM in added components. The linearity also argues against a pre-equilibrium formation of a phosphate or phosphonate: Co^{2+}:**3** ternary complex at these concentrations. The fact that the catalytic second order rate constants presented in Table I increase with pH indicates that the active form of Co^{2+}:**3** is in some way dependent on [OH^-] although from these data the exact nature of the active form is uncertain.

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[*] Diisopropylphosphorfluoridate (DFP) hydrolysing enzyme from squid head ganglia [11a]. DFPase [11b, c]. Phosphotriesterases from *Flavobacterium* species [11d]. Paraoxonse [11e]. Phosphorylphosphatase, paraoxonase [11f].

[**] The $pK_{Co^{2+}}$ values (defined in the direction of dissociation) for Co^{2+}:**2** and Co^{2+}:**3** in 80% ethanol H_2O are 7.7 and 5.7 respectively [8c, d]. The Eadie–Hofstee plot for the decomposition of **5** as a function of [Co^{2+}] yields an equivalent pK_{eq} for **5**:Co^{2+} of 2.2.

TABLE I. Hydrolysis of tris-*p*-nitrophenylphosphate (4) and Ethyl-*p*-nitrophenylmethylphosphonate (6) Catalysed by Co^{2+}:3 at 25.7 °C.[a, b]

pH	$k_4^{cat} \times 10^2$ ($M^{-1} s^{-1}$)[c]	$k_6^{cat} \times 10^2$ ($M^{-1} s^{-1}$)[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; $\mu = 0.047$ $NaClO_4$. [b] 0.05 mM 4 and 6; 0.5–2.0 mM Co^{2+}:3; followed at 400 nm. [c] Evaluated from the slopes of plots of k_{obs} *vs.* [Co^{2+}:3]; according to equation k_{obs} = intercept + k_x^{cat}[Co^{2+}:3]; $r \geqslant 0.99$ for at least 5 concentrations.

TABLE II. Pseudo-first-order Rate Constants for the Hydrolysis of 6 in the Presence of Co^{2+}:2 and Co^{2+}:3 at $T = 37.1$ °C.[a, b]

pH	k_{obs} (Co^{2+}:2) $\times 10^5$ s^{-1}[c]	k_{obs} (CO^{2+}:3) $\times 10^5$ 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; $\mu = 0.047$ $NaClO_4$. [b] Followed at 400 nm. [c] 0.5 mM Co^{2+}:2 or Co^{2+}:3; 0.05 mM 6; values averages of at least duplicate measurements; uncertainties ±5%.

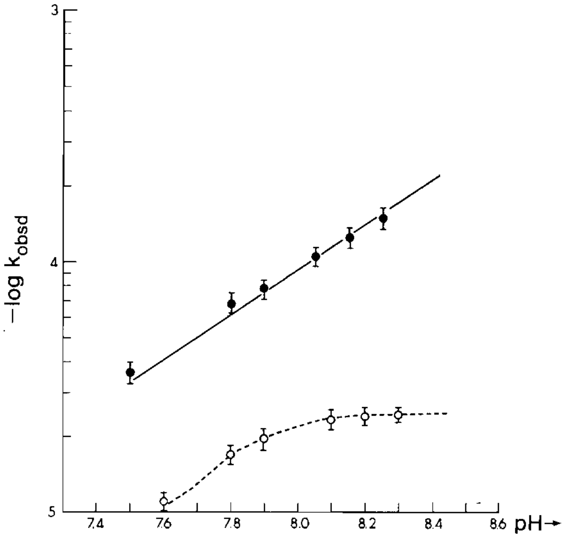


Fig. 1. Plot of $-\log k_{obs}$ (s^{-1}) *vs.* pH for the hydrolysis of 5×10^{-5} M phosphonate 6 in the presence of 5×10^{-4} M Co^{2+}:2 (○) and Co^{2+}:3 (●). $T = 37.1$ °C, 0.047 μ, CHES buffers, $\mu = 0.047$ $NaClO_4$, 80% ethanol–H_2O. Straight line drawn through Co^{2+}:3 data is first order in [OH].

Unfortunately the relative activities of Co^{2+}:3 and Co^{2+}:2 toward substrates 4 and 6 cannot be compared at 25 °C since solutions of Co^{2+} and 2 become turbid during the course of the reactions. To make such a comparison, temperatures of 37.1 °C were used for further hydrolysis studies of 6. Given in Table II are the pseudo-first-order rate constants for the hydrolysis of 0.05 mM 6 observed at 37 °C in the presence of 0.5 mM Co^{2+}:2 or Co^{2+}:3. From these data it can be observed that Co^{2+}:3 is about 3–7 times more active than is Co^{2+}:2, the difference in activities being greater at higher pH. It is also evident from Fig. 1 that the activity of Co^{2+}:2 levels off at about pH 8.0, indicating an ionization event, while the activity of Co^{2+}:3 continues to increase with pH in a first order fashion.

(b) Hydrolysis of tris-2-pyridyl Phosphate (5)

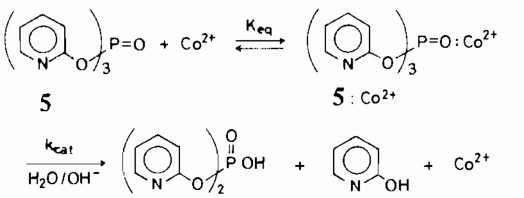
Uncatalysed, the pseudo-first-order rate constant for hydrolysis of 5 between pH 6.0 and 8.2 varies by only a factor of 3 from 5.27 to 18.7×10^{-6} s^{-1} suggestive of a dominant water term and the onset of a small OH^--dependent term at the high pH end of this region. Addition of a 10-fold excess of Co^{2+} (0.5 mM) accelerates the reaction by nearly 100 times at all pH values, these data being given in Table III along with data for the reaction observed in the presence of Co^{2+}:2 and Co^{2+}:3. The latter data are graphically displayed in Fig. 2 and show that the catalysis exhibited by Co^{2+}:2 levels off above pH 8 while Co^{2+}:3 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 eqn. (1).

TABLE III. Hydrolysis of 0.5 mM tris-2-pyridylphosphate (5) in the Presence of Co^{2+}, Co^{2+}:2, and Co^{2+}:3.[a]

pH	$k_{obs} \times 10^4$ s^{-1}		
	Co^{2+} [b]	Co^{2+}:2[c]	Co^{2+}:3[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] $T = 37.1$ °C; 0.047 M MOPS (pH 5.4–7.1) or 0.047 M CHES (pH 7.1); $\mu = 0.047$ $NaClO_4$; 80% ethanol H_2O; followed at 305 nm; uncertainties ±5%. [b] 0.493 mM $CoCl_2$. [c] 0.484 mM complex.



(1)

At a given pH, an Eadie–Hofstee plot of k_{obs} against $k_{obs}/[Co^{2+}]$ yields a straight line with intercept k_{cat} and a slope $-K_{eq}$, the latter value being defined as the dissociation constant of **5**:Co^{2+}. At pH 6.4 the derived constants are $k_{cat} = (4.67 \pm 17) \times 10^{-3}$ s^{-1} and $K_{eq} = (4.68 \pm 0.41) \times 10^{-3}$ M (r = 0.98, 8 data) while at pH 7.15 the values are $(7.32 \pm 0.22) \times 10^{-3}$ s^{-1} and $(5.13 \pm 0.37) \times 10^{-3}$ M (r = 0.98, 9 data) respectively. These data indicate that (within experimental error) the dissociation constant for **5** and Co^{2+} is equivalent at the two pH values, while the catalysed rate constant increases with pH indicatint a dependence on $[OH^-]$.

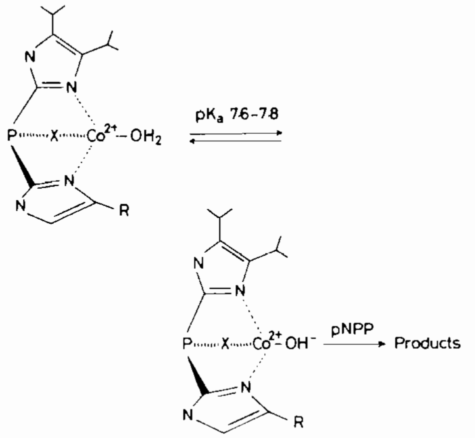
Hydrolysis of **5** is also promoted by the Co^{2+} complexes of **2** and **3**, although from the data given in Table III, the two complexes are approximately as reactive as Co^{2+} alone. Since **2** and **3** bind Co^{2+} at least 10^3 more tightly than does **5***, the great bulk of Co^{2+} will be coordinated to those ligands leaving little free metal in solution. Hence whatever acceleration in hydrolysis must result from the **2**:Co^{2+} or **3**:Co^{2+} complexes. Plots of k_{obs} *vs.* [**3**:Co^{2+}] between 0.4 and 5.0 mM at pH 6.4 and 7.15 yield straight liens with slopes of 0.58 ± 0.07 M^{-1} s^{-1} and 2.0 ± 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.

Discussion

Neutral phosphate and phosphonate esters at pH values above 7, generally decompose by pathways dependent on [anionic nucleophiles] [1, 2, 3f, 16]. 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 **4** or **6** are 10^{-6}–10^{-8} s^{-1} in H_2O [2a].

In a variety of systems it has been shown that the pK_a of a M^{2+}–coordinated H_2O is drastically reduced but it still retains sufficient nucleophilicity to attack electrophilic centers [7d, 8c, d, 17, 18]. 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 [19]. Coordination of metal ions to normally hydrolysis-resistant anionic phosphate 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 [7d, 20].

Ligand **2** 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 **3** is a model for a portion of the active site of alkaline phosphatase.* Earlier studies have shown that Co^{2+}:**2** and Co^{2+}:**3** 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) (eqn. (2)) [8d]. No evidence of the formation of a ternary pNPP:Co^{2+}:**2** or pNPP:Co^{2+}:**3** complex is evident. This study [8d] also indicated that the hydroxyethyl group in Co^{2+}:**3** is of no catalytic benefit in pNPP hydrolysis even though in related examples [19b–d] a hydroxyalkyl pendant is reported to act as a nucleophile. The major catalytic advantage of Co^{2+}:**2** and Co^{2+}:**3** over $Co^{2+}(H_2O)_x$ is two-fold: firstly to reduce the pK_a of a coordinated H_2O and secondly to bind Co^{2+} tightly enough to prevent precipitation of $Co^{2+}(OH^-)_x$ during the course of the reaction.



(2)

Co^{2+}:**2**; R = H; X = diisopropylimidazole
Co^{2+}:**3**; R = CH_2CH_2OH; X = diisopropylimidazole

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*See footnote** page 202.

*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 imidazole units [9]. 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 imidazole units [22]. Also in the active site is an essential serine OH group which during the hydrolytic event becomes transiently phosphorylated [7c, 22d]. The latest structural determinations [21, 22e] indicate that the active site is dinuclear in Zn^{2+}, the second Zn^{2+} being bound by 1 His imidazole and two oxygen-containing ligands.

Toward phosphonate **6** at 37.1 °C, both Co^{2+}:**2** and Co^{2+}:**3** exhibit activity, but the plots given in Fig. 1 indicate that they behave differently. Co^{2+}:**2** 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+}:**2**-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 [8d] and may be attributable to the slightly different conditions and 12 °C higher temperature used for the present study. Admittedly, a wide enough pH range is not converted here 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+}:**3** behaves differently from Co^{2+}:**2** since the former's activity toward phosphonate **6** continues to increase with pH with no evidence of curvature. This would imply that a basic form of Co^{2+}:**3** 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 [8d]. By analogy to related systems bearing a pendant hydroxyalkyl groups [19], we ascribe the ionization to the Co^{2+} associated hydroxyethyl group which should have a higher pK_a than 8. It is worthwhile to reiterate that whereas the activity of Co^{2+}:**3** 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+}:**3**] at each pH are linear and do not indicate formation of a ternary **6**:Co^{2+}:**3** complex.

Hydrolysis of phosphate **5** 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 **5**:Co^{2+} complex which suffers attack by H_2O and/or OH^- as in eqn. (1). From the Eadie–Hofstee plot, the k_{cat} values for the decomposition of **5**:Co^{2+} at pH 6.4 and 7.15 are 4.67×10^{-3} s^{-1} and 7.32×10^{-3} s^{-1} and show a slight but not first order dependence on [OH^-]. The coordinated Co^{2+} likely acts as both a Lewis acid to facilitate attack on the central P and to stabilize the departing alkoxypyridine, both processes having precedent [19, 20].

Co^{2+}:**2** and Co^{2+}:**3** also catalyse the solvolysis of **5** in a way which increases with pH (Fig. 2). However, the plot for Co^{2+}:**2** levels off, indicative of an active species having a pK_a ~ 8, while that for Co^{2+}:**3** continues to increase linearly with pH. The implication is that the activity for the former is associated with **2**:Co^{2+}–OH^-, while the different behavior of Co^{2+}:**3** is tied to the presence of the hydroxyethyl group which in its basic form is promoting the solvolysis of **5**. Plots of k_{obs} *vs.* [Co^{2+}:**3**] show a strict linearity, the slopes of which increase with pH and give no evidence of saturation behavior.

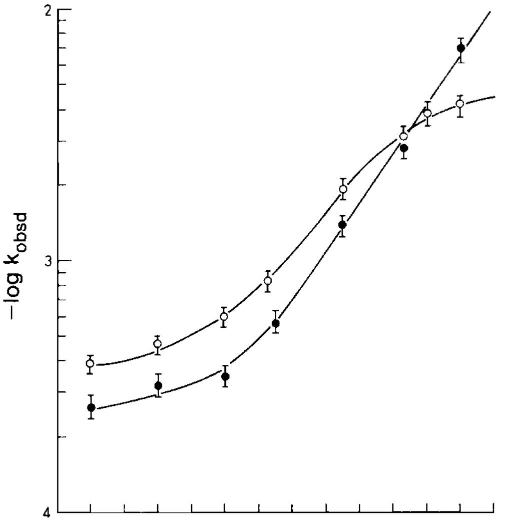
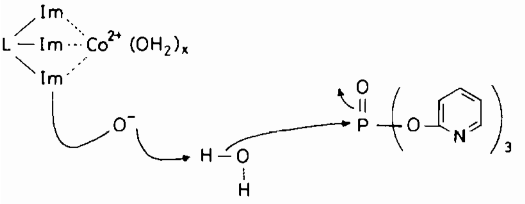
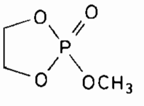


Fig. 2. Plot of −log k_{obs} (s^{-1}) for the hydrolysis of 5×10^{-5} M tris-2-pyridylphosphate (**5**) in the presence of 4.8×10^{-4} M Co^{2+}:**2** (○) and Co^{2+}:**3** (●). $T = 37.1$ °C, 0.047 M MOPS or CHES buffers, $\mu = 0.047$ $NaClO_4$, 80% ethanol–H_2O.

All of the above points to the fact that the Co^{2+};**3** behaves differently from Co^{2+}:**2** in terms of its relative propensity to attack the neutral phosphates and phosphante studied here. The basic ligand structure in Co^{2+}:**2** and Co^{2+}:**3** is the same with respect to numbers, orientation, and types of imidazoles. Earlier studies [8d] 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. Firstly, although the bulk of the studies reported here employed an excess of complex relative to phosphate **5** or phosphonate **6**, when equimolar concentrations (5×10^{-4} M) of Co^{2+}:**3** and **5** or **6** were employed, the production of *p*-nitrophenoxide exhibited clean first order kinetics. Secondly product studies revealed that hydrolysis of an equivalent of **5** catalysed by Co^{2+}:**3** yielded only one equivalent of 2-pyridinol. Dipyridyl phosphate is not hydrolysed under the conditions employed here nor is its hydrolysis catalysed visibly by Co^{2+} or either complex. These two observations strongly suggest that the role of the anionic hydroxyethyl pendant in Co^{2+}:**3** is that of a general base as in **7**. General base roles are well documented [3b, 23] for example in the hydrolyses of phosphate **8** [23a] or phosphonate **9** [23b].



7 L = 3



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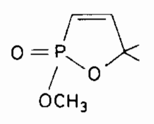

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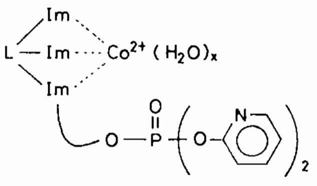
Were Co^{2+}:**3** to behave as a nucleophile toward **5**, intermediate **10** would be formed. To satisfy the kinetics and the observation that hydrolysis of **5** produces only one molecule of 2-pyridinol would require that **10** hydrolyse at least 10 times faster that it is formed (to maintain a constant [Co^{2+}:**3**]) 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 **10** would be a much more rapid process since the heterocycle is a far better leaving group than is the hydroxyethyl group of Co^{2+}:**3**. Unfortunately, synthesis of the putative intermediate **10** 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 imidazole unit covalently attached to phosphorus.



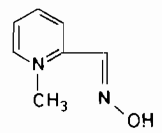
10 L = 3

The above account provides evidence that complexes such as Co^{2+}:**3** and Co^{2+}:**2** 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+}:**3** toward phosphonate **6** increases linearly with pH up to at least pH 8.3 where its value of $k_6{}^{cat}$ 10.2×10^{-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+}:**3** is not completely generated. This value compares favorably with the apparent second-order rate constants for some Co^{3+} chelates [24a], and aldoximates [24b] toward **6**, 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 5×10^{-2} M^{-1} s^{-1} and 24×10^{-2} M^{-1} s^{-1}*, respectively [24]. Of course the medium required for solubility is such that a strict comparison cannot be made and future complexes will be designed to overcome the solubility and lability [25]** 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.

$(tme)_2Co^{3+}(OH^-)(OH_2)$

11

tme = 1,1,2,2-tetramethyl-1,2-diaminoethane



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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 given in [24b].

This study indicates that ligand **2 in the presence of Zn^{2+} or Co^{2+} undergoes slow oxidation and hydrolysis to produce bis[4,5-diisopropylimidazol-2-yl]phosphinic acid. The catalysed hydrolysis of pNPP is a much faster reaction and ligand instability is not a problem.

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