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# Cobalt(III) Complex Catalyzed Hydrolysis of Phosphorus Esters

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A variety of cobalt(III) complexes were prepared and investigated with respect to their effects on hydrolysis of *p*-nitrophenyl methylphosphonate (PMP) and ethyl *p*-nitrophenyl methylphosphonate (EPMP). The complexes conformed to the formula $(N_4)Co(OH)(OH_2)$, where $N_4$ is a bis-bidentate or tetradentate ligand such as 1,3-diaminopropane (tn), 1,1,2,2-tetramethyl-1,2-diaminoethane (tme), 1,4,7,10-tetraazadecane (trien), or 2,2′-bipyridyl (bpy). The compounds accelerated the liberation of *p*-nitrophenolate (PNP) from PMP and from EPMP. The activities of the complexes as promoters for PMP and EPMP hydrolysis varied in the following order: $(bpy)_2Co(OH)(OH_2) << (trien)Co(OH)(OH_2) < (tn)_2Co(OH)(OH_2) < (tme)_2Co(OH)(OH_2)$. For $(tn)_2Co(OH)(OH_2)$ and $(tme)_2Co(OH)(OH_2)$ with PMP and EPMP initially present in excess, the complexes liberated greater than the stoichiometric amount of PNP; i.e. the reactions were catalytic with respect to complex. For 30 mM $(tme)_2Co(OH)(OH_2)$, in pH 7.6 aqueous solution at 25 °C, apparent second-order rate constants for PNP release from PMP and EPMP were $k_{app} = 15 \times 10^{-2}$ and $5.0 \times 10^{-2}$ $M^{-1}$ $s^{-1}$, respectively. These values compare with rate constants for alkaline hydrolysis ($k_{OH}$) of PMP and EPMP of $k_{OH} = 5 \times 10^{-5}$ and $3.9 \times 10^{-2}$ $M^{-1}$ $s^{-1}$, respectively.

## Introduction

Organophosphorus compounds are ubiquitous in nature and in modern society. To fully understand these compounds, it is important to establish mechanisms for their transformations in biological systems. Essentially all biological phosphorus chemistry is enzyme mediated, and either the relevant enzymes require metal ions for activity or the activities depend strongly on the metal ion content of the reaction media.[1]

To better characterize the role of metal ions in biological phosphorus chemistry, many investigators have examined metal ion complexes that model the activity of specific enzymes.[2] Early work[3-11] demonstrated that divalent metal complexes contribute to impressive rate enhancements in neutral phosphorus ester hydrolysis. However, the divalent metal ion complexes, as a class, are extremely labile toward solvolytic ligand displacement and the simultaneous presence in aqueous solution of monomeric, dimeric, and hydrated complexes seriously complicates reliable identification of the species actually involved in ester hydrolysis.

More recently, attention has focused on the effects of (tetraamine)cobalt(III) complexes on hydrolysis of anionic phosphates such as pyrophosphate,[12] adenosine di- and triphosphate,[13] *p*-nitrophenyl phosphate,[14] and triphosphate.[15] Compared with divalent metal ion complexes, tetraamine chelates of cobalt(III) offer two important advantages. First, the cobalt(III) complexes are simply prepared and are kinetically robust, thereby permitting characterization of all species present in solution. Second, the kinetics and mechanism of cobalt(III) complex substitution reactions are well-understood[16-23] and this knowledge should facilitate identifying mechanisms involved in complex-promoted phosphorus ester hydrolysis.

In view of the foregoing we have investigated reactions of various aquohydroxo(tetraamine)cobalt(III) complexes with *p*-nitrophenyl methylphosphonate (PMP) and its corresponding neutral ester, ethyl *p*-nitrophenyl methylphosphonate (EPMP). The chelates investigated conform to the general formula $(N_4)Co(OH)(OH_2)$, where $N_4$ is given by bis(1,2-diaminoethane) (en); bis(1,3-diaminopropane) (tn); bis(1,1,2,2-tetramethyl-1,2-diaminoethane) (tme); 1,4,7,10-tetraazadecane (trien), and bis(2,2′-bipyridyl) (bpy). Several of these complexes greatly accelerate *p*-nitrophenolate liberation from PMP and EPMP. Moreover, the reactions are truly catalytic with respect to cobalt chelate; i.e., PNP yields are greater than the stoichiometric amounts on the basis of complex as limiting reagent. These observations are relevant to previous work with cobalt(III) complexes and to the search for compounds that model phosphatase activity.

## Experimental Details

**Materials.** The following materials were used as obtained from the supplier: 3-(*N*-morpholino)propanesulfonic acid (MOPS) (Sigma Chemical Co.); 2,3-dinitro-2,3-dimethylbutane, 1,4,7,10-tetraazadecane (trien), 1,3-diaminopropane (tn), 2,2′-bipyridyl (bpy), pentanesulfonic acid (Aldrich Chemical Co.); $NaClO_4$, $CoCl_2 \cdot 6H_2O$ (Baker Chemical Co.); diethyl methylphosphonate, methylphosphonic acid (MPH) (Specialty Organics, Inc.).

PMP was obtained from Ash-Stevens, Inc., and purified by repeated crystallization until a sample gave analytical (>99% yield of *p*-nitrophenolate (PNP) on hydrolysis in 1 N NaOH. 1,1,2,2-Tetramethyl-1,2-diaminoethane, tme, was prepared by $SnCl_2$ reduction of

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the corresponding dinitro compound and purified by distillation at reduced pressure according to the procedure of Sayre.[24]

**Ethyl *p*-Nitrophenyl Methylphosphonate.** *Caution!* EPMP IS A VERY TOXIC NERVE POISON. The subcutaneous lethal dose (LD50)[25] in mice is 350 μg/kg, and the material should be handled with extreme care at all times. The reaction of diethyl methylphosphonate with *p*-nitrophenol as described by Fukuto and Metcalf[26] was used to prepare EPMP. Anal. Calcd for $C_7H_{12}NO_5P$: C, 44.1; H, 4.93; N, 5.71. Found: C, 43.9; H, 4.77; N, 4.59.

**β-*cis*-[(trien)Co(OH$_2$)$_2$](ClO$_4$)$_3$** was prepared from $CoCl_2 \cdot 6H_2O$ and trien·HCl by the method of Sargeson and Searle[27] and purified by precipitation from ethanol/ether. The β-cis configuration was confirmed by comparing UV–visible absorption bands (in 0.1 N $HClO_4$), $\lambda_{max}$ = 487 nm ($\epsilon$ = 123 $M^{-1}$ $cm^{-1}$) and $\lambda_{max}$ = 358 nm ($\epsilon$ = 80 $M^{-1}$ $cm^{-1}$) with literature values, $\lambda_{max}$ = 487 nm ($\epsilon$ = 122 $M^{-1}$ $cm^{-1}$) and $\lambda_{max}$ = 358 nm ($\epsilon$ = 85 $M^{-1}$ $cm^{-1}$).[27] Anal. Calcd for $C_6H_{22}N_4O_{14}Cl_3Co$: C, 13.38; H, 4.09; N, 10.41. Found: C, 14.07; H, 4.01; N, 11.03.

**α-*cis*-[(trien)Co(OH$_2$)$_2$](ClO$_4$)$_3$** was prepared as described for the β isomer, except that the product was isolated by precipitation with acetone. Spectral data in 0.1 N $HClO_4$, $\lambda_{max}$ = 500 nm ($\epsilon$ = 85 $M^{-1}$ $cm^{-1}$) and $\lambda_{max}$ = 359 nm ($\epsilon$ = 60 $M^{-1}$ $cm^{-1}$), agreed with literature values,[27] $\lambda_{max}$ = 497 nm ($\epsilon$ = 90 $M^{-1}$ $cm^{-1}$) and $\lambda_{max}$ = 357 nm ($\epsilon$ = 58 $M^{-1}$ $cm^{-1}$). Anal. Calcd for $C_6H_{22}N_4O_{14}Cl_3Co$: C, 13.38; H, 4.09; N, 10.41. Found: C, 13.02; H, 4.22; N, 10.22.

**[(tn)$_2$Co(OH$_2$)$_2$](ClO$_4$)$_3$·2H$_2$O** was prepared by acid hydrolysis of $[(tn)_2CoCO_3]ClO_4$.[28] Thus $[(tn)_2CoCO_3]ClO_4$ (10 g, 24 mmol) was dissolved in 20 mL of 6 M $HClO_4$ and warmed to expel $CO_2$. The solution was cooled, and excess 70% $HClO_4$ was added. The solution was then placed in a vacuum desiccator for 3–7 days. Crystals formed slowly and were filtered and washed with ether; yield 4 g (31%). Anal. Calcd for $C_6H_{28}N_4O_{16}Cl_3Co$: C, 12.48; H, 4.88; N, 9.70. Found: C, 12.07; H, 4.25; N, 9.33.

**[(tme)$_2$CoCO$_3$]ClO$_4$·H$_2$O** was prepared from reaction of $NH_2C(CH_3)_2C(CH_3)_2NH_2$ (1.2 g, 10 mmol) and $Na_3Co(CO_3)_3 \cdot 3H_2O$ (1.8 g, 5.0 mmol) in 10 mL of $H_2O$; the reaction mixture was heated 4 h and filtered. Addition of $NaClO_4$ (1 g) to the filtrate precipitated the product, which was recovered by filtration. Concentrating the filtrate gave an additional 0.2 g; total yield 0.46 g (20%). Anal. Calcd for $C_{13}H_{34}N_4O_8ClCo$: C, 33.30; H, 7.31; N, 11.94. Found: C, 33.24; H, 7.01; N, 11.82.

**[(tme)$_2$Co(OH$_2$)$_2$](ClO$_4$)$_3$·H$_2$O** was prepared by dissolving $[(tme)_2CoCO_3]ClO_4 \cdot H_2O$ (0.66 g, 1.5 mmol) in 10 mL of 50% $HClO_4$ and filtering the precipitate that formed after 20 h; yield 0.4 g (40%). Spectral data in 0.1 N $HClO_4$: $\lambda_{max}$ = 510 nm, $\epsilon$ = 99.9 $M^{-1}$ $cm^{-1}$; $\lambda_{max}$ = 368 nm, $\epsilon$ = 89.7 $M^{-1}$ $cm^{-1}$. Anal. Calcd for $C_{12}H_{38}N_4O_{15}Cl_3Co$: C, 22.39; H, 5.95; N, 8.70. Found: C, 22.03; H, 5.84; N, 8.62.

**[(bpy)$_2$CoCO$_3$]ClO$_4$** was prepared by dissolving $Na_3Co(CO_3)_3 \cdot 3H_2O$ (1.8 g, 5.0 mmol) and 2,2′-bipyridyl (1.6 g, 10 mmol) in 20 mL of $H_2O$, allowing the solution to stand 8 h, and filtering hot. Addition of $NaClO_4$, 1 g in 10 mL of $H_2O$, precipitated a brown solid. The solid was filtered, washed with acetone to remove any $[(bpy)_3]Co(ClO_4)_3$, and dried; yield 2.2 g (88%). This compound was not further purified and was carried on to the desired diaquo product as described below.

***cis*-[(bpy)$_2$Co(OH$_2$)$_2$](ClO$_4$)$_3$·H$_2$O** was prepared from $[(bpy)_2CoCO_3]ClO_4$. The carbonato complex (2.2 g, 4.2 mmol) was dissolved in 10 mL of water and cooled in an ice bath. $HClO_4$ (70% in $H_2O$) was added dropwise until $CO_2$ evolution ceased. After 72 h, the resultant precipitate was filtered and washed with ether; yield 2.9 g (100%). Anal. Calcd for $C_{20}H_{22}N_4O_{15}Cl_3Co$: C, 33.19; H, 3.06; N, 7.74; Cl, 14.70; Co, 8.14. Found: C, 32.99; H, 2.99; N, 7.38; Cl, 14.63; Co, 8.00.

**Apparatus.** UV–visible spectra and PNP production kinetics ($\lambda_{max}$ = 392 nm, $\epsilon$ = 1.37 × 10$^4$ $M^{-1}$ $cm^{-1}$ at pH 7.6 in 0.5 M $NaClO_4$) were determined with a Perkin-Elmer Model 554 spectrophotometer. pH readings and pH-stat control were obtained with a potentiometric Metrohm Model E526 automatic titrator and E535 dosimat with 0.1 M NaOH. PNP production kinetic studies were performed in a jacketed, covered, glass reaction vessel under inert atmosphere. Temperature control was maintained to ±0.1 °C with a Haake Model FE circulating bath. A Milli-Q reverse osmosis/ion-exchange system was used to produce deionized water.

High-pressure liquid chromatography (HPLC) was performed with a Waters Associates system comprised of a Model U6K injector, two Model M-6000A solvent pumps, a Model 660 solvent programmer, and a Model 440 detector ($\lambda$ = 254 nm)9 A $C_{18}$-μBondapak reverse-phase column was used, and the samples were eluted with $H_2O$ (pH 4 acetate buffer plus pentanesulfonic acid), programmed to 20% $H_2O$ plus 80% MeOH. This system satisfactorily resolved PMP, PNP, *cis*-(trien)Co(OH)(OH$_2$) and two unidentified products of the reaction of *cis*-(trien)Co(OH)(OH$_2$) with MOPS buffer.

**Kinetic Procedures and Calculations.** All experiments were conducted at 25 °C in aqueous solution with 0.5 N $NaClO_4$ added. Unless otherwise stated, the reaction solutions were maintained at pH 7.6 by addition of 0.1 M MOPS buffer or by the pH-stat method. PNP production kinetics were monitored spectrophotometrically by removing aliquots from the reaction vessel at timed intervals.

For most rate determinations, complex was added in at least 10-fold molar excess over PMP or EPMP, resulting in pseudo-first-order kinetics. Observed first-order rate constant ($k_{obsd}$) values were calculated according to eq 1, where $A_\infty$, $A_0$, and $A_t$ are experimentally determined absorbance values at long reaction time, at time zero, and at time $t$, respectively.

$$\ln ([PMP]_0 - [PNP]_t)/[PMP]_0 = \ln (A_\infty - A_t)/(A_\infty - A_0) = -k_{obsd}t \quad (1)$$

Assuming that the aquohydroxo complex is the only reactive species (see Discussion), $k_{obsd}$ values were used to calculate apparent bimolecular rate constants ($k_{app}$) according to eq 2. For experiments with

$$d[PNP]/dt = k_{app}[(N_4)Co(OH)(OH_2)][PMP \text{ or } EPMP] \quad (2)$$

PMP, $k_{app}$ values were directly calculated according to eq 3. However,

$$k_{app} = k_{obsd}[(N_4)Co(OH)(OH_2)]^{-1} \quad (3)$$

for experiments with EPMP, the rate constant, $k_0$, for spontaneous hydrolysis of EPMP was significant compared with $k_{obsd}$ and $k_{app}$ values were calculated according to eq 4.

$$k_{app} = (k_{obsd} - k_0)[(N_4)Co(OH)(OH_2)]^{-1} \quad (4)$$

In some kinetic runs with PMP, complex and PMP were added in comparable amounts. In these instances we assumed second-order kinetics as in eq 2 and calculated $k_{app}$ values according to eq 5, where $X$ is the extent of the reaction, (i.e., [PNP] determined spectrophotometrically) and $a$ and $b$, respectively, are initial concentrations of $(N_4)Co(OH)(OH_2)$ and PMP.

$$(a-b)^{-1} \ln \left[ \frac{b(a-X)}{a(b-X)} \right] = k_{app}t \quad (5)$$

## Results

**Chelate Structure and Stability.** Isomeric configurations and acid–base equilibria influence the reactivity of cobalt(III) complexes,[16-19] and we have characterized the chelates reported in this investigation with respect to these factors. The diaquo-, aquohydroxo-, and dihydroxo(tetraamine)cobalt(III) complexes are related by the acid–base equilibria shown in reactions 6 and 7.

$$(N_4)Co(OH_2)_2 \overset{K_1}{\rightleftharpoons} (N_4)Co(OH)(OH_2) + H^+ \quad (6)$$

$$(N_4)Co(OH)(OH_2) \overset{K_2}{\rightleftharpoons} (N_4)Co(OH)_2 + H^+ \quad (7)$$

We determined values for $pK_2$ and $pK_1$ by potentiometric titration under conditions where cis–trans isomerizations (see below) had already proceeded to equilibrium levels. For $N_4$ = trien, tn, tme, and bpy, respectively, the observed values ($pK_2$, $pK_1$) were as follows: 8.1, 5.9; 8.1, 5.6; 8.7, 4.8; 7.0, 4.6. Thus, at pH 7.6, the aquohydroxo species predominates for $N_4$ = trien, tn, and tme, while the dihydroxo species predominates for the bpy complexes.

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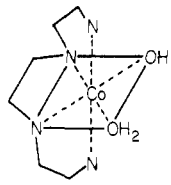
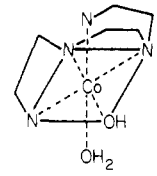
In the following, values reported for aquohydroxo(tetraamine)cobalt(III) concentrations were calculated from the concentration of initially added diaquo complex, $[N_4Co(OH_2)_2]_0$, the experimentally determined acid dissociation constants, and eq 8.

$$[N_4Co(OH)(OH_2)]/[N_4Co(OH_2)_2]_0 = [H^+]/([H^+] + K_2) \quad (8)$$

Regarding the complex structures, *cis*-(trien)Co(OH)($OH_2$) exists in $\alpha$ and $\beta$ configurations.



$\alpha$-*cis*-Co(trien)($OH_2$)(OH)



$\beta$-*cis*-Co(trien)($OH_2$)(OH)

Sargeson and Searle[27] report that the $\beta$ configuration is thermodynamically preferred and that $\alpha$-*cis*-(trien)Co($OH_2$)$_2$ spontaneously isomerizes to an 80:20 mixture of the $\beta$ and $\alpha$ forms. Thus, when the $\alpha$-cis isomer is specified as a reactant, it should be remembered that, for the reaction times typically employed, the active constituents in solution will be a mixture of isomers.

We have not assigned a configuration to the $[(tn)_2Co(OH_2)_2](ClO_4)_3$ isomer we isolated in crystalline form. In solution, however, *trans*-$[(tn)_2Co(OH)(OH_2)]$ isomerizes to the cis isomer rapidly ($t_{1/2}$ = 1 s at 25 °C)[28] and completely so that under our conditions the chemistry of this complex can be attributed exclusively to the cis complex. Similarly, we have not assigned a configuration to the crystalline $[(tme)_2Co(OH_2)_2](ClO_4)_3$ obtained as described in Experimental Details. However, in 0.1 M $HClO_4$ solution, $(tme)_2Co(OH_2)_2$ exhibited absorption maxima consistent with a cis configuration. As with $(tn)_2Co(OH)(OH_2)$, we attribute the observed chemistry of $(tme)_2Co(OH)(OH_2)$ to the cis isomer. For $(bpy)_2Co(OH)(OH_2)$ the cis configuration is preferred as a result of steric interactions between ligand atoms.[29]

***p*-Nitrophenyl Methylphosphonate (PMP) Hydrolysis.** We performed some preliminary experiments to elucidate the major effects of pH, buffer, and chelate structure on PNP liberation from PMP. These control experiments demonstrated that, under our experimental conditions, PMP was inert to reaction with $N_4Co(OH)_2$, $N_4Co(OH_2)_2$, $N_4Co(CO_3)$ or to reaction with $\alpha$-*cis*-(trien)Co(OH)($OH_2$) in pH 7.6 0.1 M phosphate buffer.

Preliminary experiments also showed rapid liberation of PNP from PMP in the presence of $N_4Co(OH)(OH_2)$, where $N_4$ = $(tn)_2$, $(tme)_2$, and $\alpha$- and $\beta$-*cis*-(trien). However, none of the $(bpy)_2$ cobalt complexes promoted PMP hydrolysis.

To probe these effects in more detail, we investigated the kinetics of production of PNP from PMP. We began by examining the effects of $\alpha$- and $\beta$-*cis*-(trien)Co(OH)($OH_2$) on the reaction. Figure 1 shows pseudo-first-order kinetic plots according to eq 1 for $\alpha$- and $\beta$-*cis*-(trien)Co(OH)($OH_2$). The figure shows that kinetics for the $\beta$ isomer adhered to eq 2. For three runs with 3.05 mM $\beta$-*cis*-(trien)Co(OH)($OH_2$) the observed first-order rate constant, $k_{obsd}$ (4.61 ± 0.007) × $10^{-5}$ $s^{-1}$, from which we calculate (see eq 3) the apparent bimolecular rate constant, $k_{app}$ = (1.51 ± 0.03) × $10^{-2}$ $M^{-1}$ $s^{-1}$. For

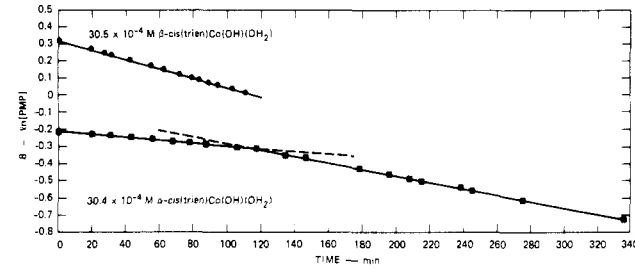



**Figure 1.** Natural logarithm of *p*-nitrophenyl methylphosphonate remaining at 25 °C and pH 7.6 in 0.10 M MOPS buffer with 0.5 M $NaClO_4$ for hydrolysis of PMP: (●) in the presence of 30.5 × $10^{-4}$ M $\beta$-*cis*-(trien)Co(OH)($OH_2$); (■) in the presence of 30.4 × $10^{-4}$ M $\alpha$-*cis*-(trien)Co(OH)($OH_2$).

the $\alpha$ isomer, Figure 1 shows two linear regions. Averaged over three runs, the initial apparent bimolecular rate constant was $(k_{app})_{in}$ = (0.486 ± 0.02) × $10^{-2}$ $M^{-1}$ $s^{-1}$ and the final rate constant was $(k_{app})_{fin}$ = (1.06 ± 0.17) × $10^{-2}$ $M^{-1}$ $s^{-1}$.

We followed the experiments described above to low (~25%) conversions to facilitate observing the change in kinetics for $\alpha$-*cis*-(trien)Co(OH)($OH_2$). We performed additional experiments to examine the effects of long reaction times, chelate structure and concentration, and reaction medium on PNP production kinetics. In these experiments we used $\alpha$-*cis*-(trien)Co(OH)($OH_2$), $(tn)_2Co(OH)(OH_2)$, and $(tme)_2Co(OH)(OH_2)$. For $\alpha$-*cis*-(trien)Co(OH)($OH_2$), the rates were determined at reaction times ($6 \times 10^3 < t < 10^5$ s) after the change in kinetics (see Figure 1) had already occurred. For $(tn)_2Co(OH)(OH_2)$ and $(tme)_2Co(OH)(OH_2)$, we calculated kinetics on the basis of points from time zero to the time at the completion of the reaction.

For all reactions, we also calculated the yield of PNP relative to the concentration of initially added PMP. Because we were interested in elucidating the effect of the reaction medium on PNP production, we performed some experiments in 0.1 M MOPS buffer, while in others we used a pH-stat method and either deionized (DI) water or water distilled under $N_2$. In the latter case we maintained the reaction mixture under an inert atmosphere. Table I summarizes these experimental results.

The data in Table I demonstrate that the complex-promoted PMP hydrolyses did not quantitatively yield PNP in all cases. Generally, decreasing $(N_4)Co(OH)(OH_2)$ concentrations contributed to lower PNP yields and lower $k_{app}$ values. This trend held for all three complexes, although the overall complex activities (i.e. $k_{app}$ values) varied in the order $(tme)_2Co(OH)(OH_2)$ > $(tn)_2Co(OH)(OH_2)$ > $\alpha$-*cis*-(trien)Co(OH)($OH_2$). For nominally equivalent $(tn)_2Co(OH)(OH_2)$ concentrations, the change from buffered to nonbuffered solution resulted in higher values of $k_{app}$ and in higher yields of PNP at low chelate concentration. Similarly for $(tn)_2Co(OH)(OH_2)$, changing from deionized to distilled water increased $k_{app}$, though to a lesser degree.

To ensure that the low yields for PNP observed in some runs were not due to loss of PMP via side reactions with chelate, and also to probe for the reaction rate-determining step (vide infra), we spectrophotometrically monitored PNP production and simultaneously determined (by HPLC) PMP loss from the reaction with $\alpha$-*cis*-(trien)Co(OH)($OH_2$) in 0.1 M MOPS buffer.

For 0.169 mM PMP plus 2.85 mM $\alpha$-*cis*-(trien)Co(OH)($OH_2$) in 0.1 M MOPS buffer, we removed reaction mixture aliquots at timed intervals and calculated the ratio $([PMP]_t + [PNP]_t)/[PMP]_0$. From semilog plots of $[PNP]_t/[PMP]_0$ and $([PMP]_0 - [PMP]_t)/[PMP]_0$ vs. time we calculated $k_{app}$ values according to eq 1 and 3. Table II shows the results of these experiments.

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Table I. Rate Constants and Yields for the Production of PNP from the Reaction of PMP with Aquohydroxo(tetraamine)cobalt(III) Chelates at 25 °C in pH 7.6 Water plus 0.50 M $NaClO_4$

| run | chelate | $10^4 \times$ [chelate]$_0$, M | $10^4 \times$ [PMP]$_0$, M | buffer[a] | solvent[b] | $10^2 \times k_{app}$,[c] $M^{-1}$ $s^{-1}$ | [PNP]$_\infty$/[PMP]$_0$ |
|---|---|---|---|---|---|---|---|
| 1 | α-*cis*-(trien)Co(OH)($OH_2$) | 52.6 | 0.749 | MOPS | DI | 1.59 | 0.95 |
| 2 | | 38.4 | 0.749 | MOPS | DI | 1.61 | 0.90 |
| 3 | | 23.5 | 0.749 | MOPS | DI | 1.54 | 0.96 |
| 4 | | 24.2 | 0.749 | MOPS | DI | 1.33 | 0.98 |
| 5 | | 24.0 | 0.749 | MOPS | DI | 1.69 | 1.00 |
| 6 | | 9.72 | 0.636 | MOPS | DI | *d* | 0.49 |
| 7 | | 6.07 | 1.59 | MOPS | DI | *d* | 0.14 |
| 8 | | 4.98 | 0.749 | MOPS | DI | *d* | 0.06 |
| 9 | | 4.05 | 2.12 | MOPS | DI | *d* | <0.01 |
| 10 | | 5.36 | 30.0 | MOPS | DI | *d* | <0.01 |
| 11 | $(tn)_2$Co(OH)($OH_2$) | 19.7 | 0.783 | MOPS | DI | 2.46 | *f* |
| 12 | | 4.13 | 0.701 | MOPS | DI | *d* | 0.30 |
| 13 | | 1.91 | 0.842 | MOPS | DI | *d* | <0.01 |
| 14 | | 42.7 | 0.701 | none | DI | 3.03 | 1.00 |
| 15 | | 14.1 | 0.765 | none | DI | 2.74 | 1.01 |
| 16 | | 5.07 | 0.746 | none | DI | 2.09[e] | 0.95 |
| 17 | | 1.69 | 0.786 | none | DI | *d* | <0.01 |
| 18 | | 1.77 | 9.41 | none | DI | 1.15 | *f* |
| 19 | | 39.3 | 0.660 | none | dist | 3.40 | 0.88 |
| 20 | | 15.4 | 0.761 | none | dist | 3.26 | 0.97 |
| 21 | | 4.49 | 0.738 | none | dist | 3.02[e] | 1.14 |
| 22 | | 3.49 | 0.757 | none | dist[g] | 2.95[e] | 1.00 |
| 23 | | 1.52 | 0.750 | none | dist | 2.23[e] | 0.80 |
| 24 | $(tme)_2$Co(OH)($OH_2$) | 14.5 | 0.741 | none | dist | 14.7 | 0.87 |
| 25 | | 4.97 | 0.743 | none | dist | 11.0 | 1.14 |
| 26 | | 1.55 | 0.746 | none | dist | 4.80[e] | 0.84 |
| 27 | | 1.17 | 2.80 | none | dist | *d* | <0.01 |
| 28 | | 0.695 | 1.71 | none | dist | *d* | <0.01 |

[a] [MOPS] = 0.1 M; pH-stat control for experiments with no buffer. [b] DI = deionized water; dist = deionized water distilled from glass under $N_2$ and maintained under $N_2$. [c] $k_{app}$ calculated from eq 1-3 unless otherwise noted. [d] $k_{app}$ not calculated for experiments that did not go to completion. [e] $k_{app}$ calculated from eq 5. [f] Not determined. [g] [$NaClO_4$] = 1.0 M.

Table II. Production of PNP and Loss of PMP from Reaction of $1.69 \times 10^{-4}$ M PMP with $28.5 \times 10^{-4}$ M α-*cis*-(trien)Co(OH)($OH_2$) in 0.1 M MOPS plus 0.5 M $NaClO_4$ at 25 °C and pH 7.6

| $10^{-3}t$, s | $10^4 \times$ [PMP]$_t$,[a] M | $10^4 \times$ [PNP]$_t$,[b] M | ([PMP]$_t$ + [PNP]$_t$)/[PMP]$_0$ |
|---|---|---|---|
| 0 | 1.69 | 0 | (1) |
| 3.60 | 1.57 | 0.0877 | 0.98 |
| 7.20 | | 0.229 | |
| 10.8 | 1.24 | 0.395 | 0.97 |
| 14.4 | 1.07 | 0.552 | 0.96 |
| 18.0 | 0.945 | 0.691 | 0.97 |
| 21.6 | 0.774 | 0.822 | 0.95 |

[a] Determined by HPLC. [b] Determined spectrophotometrically at 392 nm.

From the table, it is evident that PMP loss equaled PNP production; the ratio ([PNP]$_t$ + [PMP]$_t$)/[PMP]$_0$ was 0.97 ± 0.02 averaged over all time points. Moreover, the $k_{app}$ values for PMP loss and PNP production were equivalent ($1.58 \times 10^{-2}$ and $1.46 \times 10^{-2}$ $M^{-1}$ $s^{-1}$, respectively) as calculated by using eq 1 and 3.

As a further probe for complicating side reactions we monitored the liberation of PNP from the reactions of 0.0752 mM PMP and 2.29 mM $(tn)_2$Co(OH)($OH_2$) in the presence of a potential reaction product, methylphosphonic acid (MPA). To guard against side reactions of the chelate with buffer, we used the pH-state technique. For the reaction with addition of 1.52 mM MPA the final PNP yield was 0.0725 mM, 96% of initially added PMP. For 1.52, 2.97, and 4.56 mM MPA added initially, semilog plots according to eq 1 were linear and gave $k_{app}$ values, respectively, of $1.21 \times 10^{-2}$, $1.14 \times 10^{-2}$, and $0.921 \times 10^{-2}$ $M^{-1}$ $s^{-1}$. These rate constants were significantly lower than values observed for the reaction of $(tn)_2$Co(OH)($OH_2$) with PMP under similar conditions in the absence of added MPA (see Table I, runs 19 and 20).

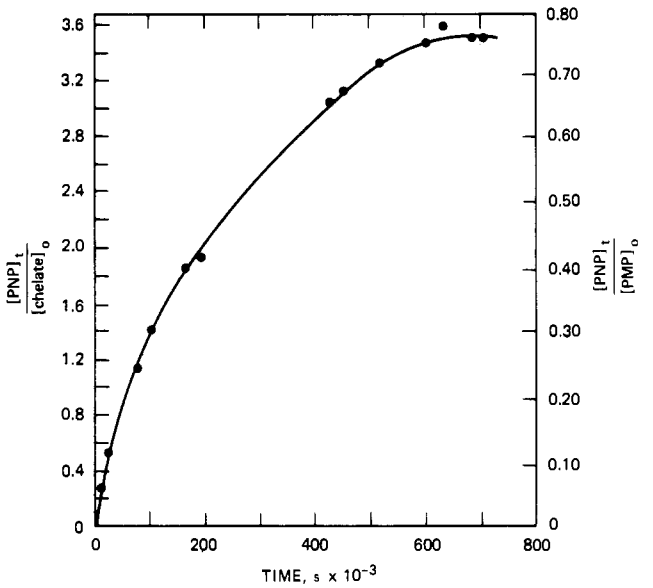



**Figure 2.** Plot of product formation relative to initial chelate, [PNP]$_t$/[chelate]$_0$, and product formation relative to initial PMP, [PNP]$_t$/[PMP]$_0$, vs. time for reaction of $7.46 \times 10^4$ M PMP and $1.62 \times 10^{-4}$ M $(tn)_2$Co(OH)($OH_2$) at 25 °C in pH 7.6 (pH stat) distilled water plus 0.50 N $NaClO_4$.

Finally, to test catalytic activity for the complexes, we determined rates and yields of PNP production from the reaction of PMP plus $(tn)_2$Co(OH)($OH_2$) or $(tme)_2$Co(OH)($OH_2$) with PMP initially present in approximately 3-fold molar excess. As in the previously described experiment, these determinations employed the pH-stat technique.

In both experiments PNP yields significantly exceeded the stoichiometric amounts on the basis of chelate as limiting reagent and approached 80–90% of the stoichiometric amount

Table III. Rate Constants and Yields for Production of PNP from Reaction of $0.725 \times 10^{-4}$ M EPMP with Chelates, Buffer, and Aqueous Base in 0.1 M MOPS Buffer plus 0.50 M $NaClO_4$ at 25 °C and pH 7.6

| chelate | $10^4 \times$ [chelate], M | $10^{-3}$(reacn time), s | $[PNP]_\infty/[EPMP]_0$[a] | $10^5 k_{obsd}$,[b] $s^{-1}$ | $10^2 k_{app}$,[c] $M^{-1}\ s^{-1}$ |
|---|---|---|---|---|---|
| $(tme)_2Co(OH)(OH_2)$ | 28.3 | 16.2 | 0.95 | 14.2 ± 0.97 | 5.00 |
| $(tn)_2Co(OH)(OH_2)$ | 22.9 | 99.9 | 0.65 | 0.985 ± 0.025 | 0.407 |
| $(tn)_2Co(OH)(OH_2)$ | 7.60 | 99.9 | 0.154 | 0.159 ± 0.0068 | 0.132 |
| $(tn)_2Co(OH)(OH_2)$ | 2.29 | 99.9 | 0.051 | 0.0450 ± 0.0053 | *d* |
| α-*cis*-(trien)Co(OH)$(OH_2)$ | 23.4 | 99.9 | 0.54 | 0.751 ± 0.096 | 0.298 |
| $(bpy)_2Co(OH)_2$ | 30.3 | 99.9 | 0.076 | 0.0974 ± 0.011 | 0.0144 |
| none[e] | | 99.9 | 0.054 | 0.0534 ± 0.00098 | *f* |
| none[g] | | 8.4 | 0.97 | 35.0 ± 0.51 | 11.6 |

[a] $[PNP]_\infty$ calculated at the indicated reaction time. [b] Calculated from eq 1; error limits are standard deviations from linear least-squares regression analysis. [c] Calculated from eq 4. [d] Not calculated since $k_{obsd} \leq k_0$; see eq 4. [e] No chelate added; run in 0.1 M MOPS plus 0.50 M $NaClO_4$ at pH 7.6 and 25 °C. [f] Not defined. [g] No chelate added; run in $30.3 \times 10^{-4}$ M NaOH plus 0.50 M $NaClO_4$ with no buffer. The value of $k_{app}$ was calculated from eq 4, with [NaOH] substituted for $[(N_4)Co(OH)(OH_2)]$.

based on PMP. These complex-catalyzed reactions did not exhibit simple pseudo-first-order kinetics. Figure 2, a plot of the PNP production data according to eq 1 for the reaction with $(tn)_2Co(OH)(OH_2)$, shows distinct downward curvature. The $(tme)_2Co(OH)(OH_2)$ reaction kinetics were similarly complicated by downward curvature. From initial slopes of the semilog plots we estimated $k_{app} = 3.43 \times 10^{-2}$ and $13.4 \times 10^{-2}\ M^{-1}\ s^{-1}$, respectively, for $(tn)_2Co(OH)(OH_2)$ and $(tme)_2Co(OH)(OH_2)$. These values were similar to $k_{app}$ values determined under conditions with complex initially present in excess over PMP (see Table I, runs 23, 25, 26).

**Ethyl *p*-Nitrophenyl Methylphosphonate Hydrolysis.** We examined the effects of $(tn)_2Co(OH)(OH_2)$, $(tme)_2Co(OH)(OH_2)$, α-*cis*-(trien)Co(OH)$(OH_2)$, and $(bpy)_2Co(OH)_2$ on the rates and yields of PNP liberated from EPMP. The experiments were performed in 0.1 M MOPS buffer. For comparison, we monitored PNP production in buffer without added complex and in 3.03 mM NaOH without complex and without buffer. For all runs we calculated observed pseudo-first-order rate constants according to eq 1. We also calculated apparent bimolecular rate constants with a correction for spontaneous hydrolysis according to eq 4. Table III summarizes these experimental results.

The results show that $(tme)_2Co(OH)(OH_2)$, $(tn)_2Co(OH)(OH_2)$, and α-*cis*-(trien)Co(OH)$(OH_2)$ significantly accelerated EPMP hydrolysis compared with the spontaneous hydrolysis rate in MOPS buffer. For $(tme)_2Co(OH)(OH_2)$, the PNP yield was essentially quantitative, and the plot according to eq 1 was linear to 95% conversion. The reaction with $(bpy)_2Co(OH)_2$ was approximately 2 times more rapid than the spontaneous hydrolysis in buffer and considerably slower than the reactions in the presence of the other complexes added in comparable amounts.

We also checked for catalytic activity of $(tme)_2Co(OH)(OH_2)$ toward EPMP. To do so, we determined PNP production from the reaction of 0.740 mM EPMP with 0.412 mM $(tme)_2Co(OH)(OH_2)$ with pH-stat control. For this reaction, the PNP concentration increased with time and reached maximum values of $[PNP]_t/[complex]_0 = 1.36$ and $[PNP]_t/[EPMP]_0 = 0.76$. The kinetic behavior was similar to that seen in Figure 2 for PMP reaction with $(tn)_2Co(OH)(OH_2)$.

## Discussion

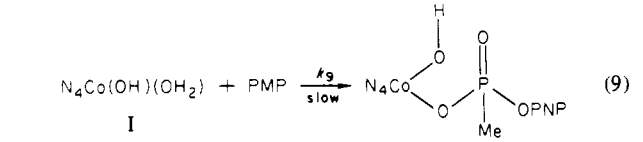
***p*-Nitrophenyl Methylphosphonate Hydrolysis.** The foregoing demonstrates that (1) certain aquohydroxo(tetraamine)cobalt(III) complexes greatly accelerate hydrolysis of *both* PMP and EPMP and (2) the complexes function as true catalysts for the reaction. To illustrate the degree to which the chelates enhance PMP hydrolysis, compare (Table IV) our apparent bimolecular rate constants for PMP/complex reactions with literature values for rate constants of PMP reactions with various nucleophiles. Table IV shows that $(tme)_2Co(OH)(OH_2)$, for example, is 60 times more reactive toward PMP than hydroperoxide ion, the most powerful known nucleophile toward organophosphorus esters. To put the chelate-catalyzed reactions in another perspective, the PMP half-life in the presence of 1.5 mM $(tme)_2Co(OH)(OH_2)$ at 25 °C and pH 7.6 is about 1 h, whereas the alkaline hydrolysis rate proceeds under the same conditions with an estimated half-life of 11 years.

Table IV. Bimolecular Rate Constants for Reaction of PMP with Cobalt(III) Chelates and with Selected Nucleophiles

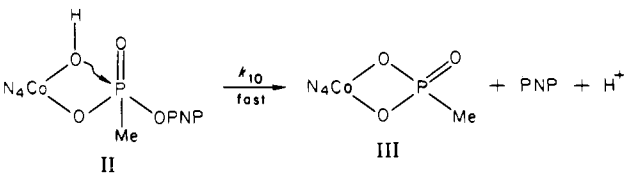
| reactant | temp, °C | rate constant, $M^{-1}\ s^{-1}$ | relative rate | ref |
|---|---|---|---|---|
| $(tme)_2Co(OH)(OH_2)$ | 25 | $1.47 \times 10^{-1}$ | 9.3 | this work |
| $(tn)_2Co(OH)(OH_2)$ | 25 | $3.40 \times 10^{-2}$ | 2.1 | this work |
| (trien)Co(OH)$(OH_2)$ | 25 | $1.59 \times 10^{-2}$ | 1 | this work |
| $HOO^-$ | 30 | $2.6 \times 10^{-3}$ | $1.6 \times 10^{-1}$ | 30 |
| $HO^-$ | 30 | $5.0 \times 10^{-5}$ | $3.1 \times 10^{-3}$ | 31 |
| $C_6H_5O^-$ | 30 | $2.0 \times 10^{-7}$ | $1.3 \times 10^{-5}$ | 30 |
| pyridine | 30 | $1.1 \times 10^{-7}$ | $6.9 \times 10^{-6}$ | 32 |
| $H_2O$ | 30 | $3.6 \times 10^{-11}$ | $2.3 \times 10^{-9}$ | 31 |

That the complexes are true catalysts for PMP hydrolysis is also important. Figure 2 clearly shows that, with PMP initially present in excess over $(tn)_2Co(OH)(OH_2)$, the PNP yield was greater than the stoichiometric amount of chelate and that PMP conversion to PNP was nearly quantitative. Similar results were obtained with PMP in excess over $(tme)_2Co(OH)(OH_2)$.

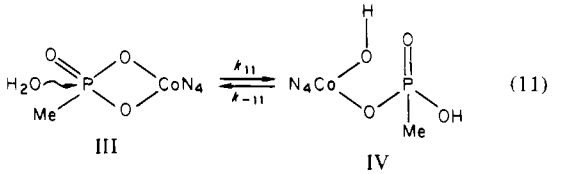
We believe our data are consistent with the mechanism given by reactions 9–12. In this mechanism, and throughout the




I II (9)




II III (10)




III IV (11)

$$IV \xrightarrow{k_{12}} I + MPA \quad (12)$$

$$I + MPA \xrightarrow{k_{-12}} IV \quad (-12)$$

following discussion, we note that I–IV participate in acid–base equilibria and that at pH 7.6 the predominant species may not all be protonated to the degree shown above. We cannot completely identify these equilibria at present, but this is not strictly required to understand the major aspects of the mechanism given in eq 9–12.

According to this mechanism, the reaction proceeds via rate-limiting substitution of PMP for water in the aquohydroxo complex, I. PNP liberation from the hydroxo(*p*-nitrophenyl methylphosphonato)(tetraamine)cobalt(III) complex II is fast compared with reaction 12 and yields III, the bidentate (methylphosphonato)(tetraamine)cobalt(III) complex. Attack by solvent water at the phosphorus of III (reaction 11) yields the monodentate hydroxo(methylphosphonato)(tetraamine)-cobalt(III) complex IV. IV in turn hydrolyzes to reversibly generate the aquohydroxo(tetraamine)cobalt(III) complex and MPA.

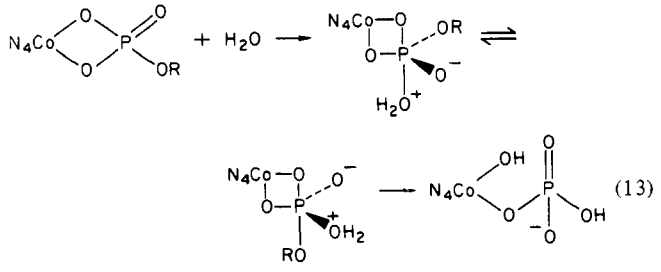
To demonstrate that reactions 9–12 best describe PMP reactions with $N_4Co(OH)(OH_2)$, we cite the following experimental results and literature comparisons. First, the overall reactivity order (tme > tn > trien > bpy) of the aquohydroxo complexes toward PMP parallels the reactivity order of analogous substitution reactions for cobalt complexes with these ligands.[18,28,33-35] These literature substitutions proceed via a mechanism that is essentially characterized by rate-limiting, $S_N1$, reaction of the cobalt complex.

Second, Table II shows that for the *cis*-(trien)Co(OH)-$(OH_2)$-catalyzed reaction the quantities and rates of PMP loss and PNP production were precisely equivalent. This excludes the possibility that the reaction proceeds via rapid prior coordination of PMP to cobalt, followed by rate-determining liberation of PNP. If rapid prior coordination were predominant, PNP production would lag behind PMP loss.

Third, we show reaction 10 as proceeding via intramolecular attack of coordinated hydroxide on phosphorus. Entropic effects favor this mechanism over the alternative intermolecular attack of solvent water. Additionally, intramolecular attack by coordinated hydroxide has been reported[34,36-39] for various analogous cobalt(III) complexes. The requirement[15] for cis-coordinated hydroxide in establishing reactivity of macrocyclic cobalt(III) complexes toward triphosphate anion hydrolysis is particularly compelling evidence for a similar mechanism in the reactions reported here.

Finally, by analogy to the reaction of $(tn)_2Co(OH)(OH_2)$ with *p*-nitrophenyl phosphate,[14] reaction 11 probably proceeds via attack by water at phosphorus to yield a pentacoordinate intermediate that converts to products via pseudorotation[40] and cobalt–oxygen bond cleavage as shown in reaction 13.

Apart from the overall mechanism, several observations in our kinetic studies deserve comment. These include relative reactivities of the $\alpha$- and $\beta$-*cis*-(trien)Co(OH)$(OH_2)$ isomers (Figure 1), nonlinear kinetics for PNP production with PMP initially present in excess over complex (Figure 2), and medium (i.e. buffer and solvent) effects on PNP yields (see Table I).



(13)

For the *cis*-(trien)Co(OH)$(OH_2)$ isomers, different rates for ligand (water) substitution plus the fact that the $\alpha$ isomer spontaneously reverts to the $\beta$ configuration[27] readily explain the rate differences for PMP hydrolysis as shown in Figure 1. To estimate the relative amounts of $\alpha$- and $\beta$-aquohydroxo isomers present in the equilibrium mixture, we compare $k_{app}$ values for PMP hydrolysis. For the $\beta$ isomer we set $(k_{app})_\beta = 1.51 \times 10^{-2}$ $M^{-1}$ $s^{-1}$ (see Results for PMP hydrolysis), and for the $\alpha$ isomer we set $(k_{app})_\alpha = 0.486 \times 10^{-2}$ $M^{-1}$ $s^{-1}$, where the latter value is given by the initial slope in Figure 1. For the equilibrium mixture we set $(k_{app})_{eq} = 1.06 \times 10^{-2}$ $M^{-1}$ $s^{-1}$ (i.e. the final slope in Figure 1). From the available rate constants and eq 14 (where $[\alpha] + [\beta] = 1$ and $[\alpha]$ and $[\beta]$

$$(k_{app})_{eq} = (k_{app})_\alpha[\alpha] + (k_{app})_\beta[\beta] \quad (14)$$

are the fractions of each isomer in the equilibrium mixture) we calculate $[\alpha] = 0.44$ and $[\beta] = 0.56$. By comparison, Sargeson and Searle[27] report $[\alpha] = 0.23$ and $[\beta] = 0.77$ for the isomeric diaquo(1,4,7,10-tetraazadecane)cobalt(III) chelates in aqueous acid. Thus the preference for the $\beta$ isomer is more pronounced in the diaquo chelates.

For the reaction with PMP added in excess over $(tn)_2Co(OH)(OH_2)$, Figure 2 shows an apparent decrease in the PNP production rate at long reaction times. We attribute this behavior to inhibition of the reaction by the hydrolysis product, methylphosphonic acid (MPA). We expect MPA to compete (reaction −12) with PMP for the coordinatively unsaturated intermediate I. To the extent that reaction of I with MPA predominates over reaction 9, the observed PNP production rate will decrease. Consistent with this, for the reaction of $(tn)_2Co(OH)(OH_2)$ with PMP in the presence of added MPA we found (see Results) an inverse dependence of $k_{app}$ on [MPA].

The final point concerns the apparent (Table I) decreases in $k_{app}$ values and PNP yields with decreasing initial chelate concentration. A likely explanation is reaction of the chelates with impurities in parallel with PMP catalysis. Thus, HPLC analysis of $\alpha$-*cis*-(trien)Co(OH)$(OH_2)$ in 0.1 M MOPS buffer revealed the appearance of two unidentified products. Furthermore, we performed parallel determinations of the $(tn)_2Co(OH)(OH_2)$-catalyzed hydrolysis of PMP in buffered and nonbuffered solutions. Comparing runs 11–13 with runs 14–18 in Table I reveals a substantial increase in PNP yields at low chelate concentrations in nonbuffered solution.

Because aquohydroxo(tetraamine)cobalt(III) chelates react rapidly with dissolved $CO_2$ and because $\alpha$-*cis*-(trien)$CoCO_3$ does not accelerate PMP hydrolysis, we took precautions to exclude $CO_2$ from the reaction mixture by using buffer-free water distilled and maintained under an inert atmosphere. For runs 19–23 in Table I, excluding $CO_2$ from the reaction mixture elevated $k_{app}$ values and PNP yields, relative to those for the reactions (runs 14–18) for which no precautions were taken. Even under these carefully controlled conditions the trend toward lower values for $k_{app}$ at lower chelate concentration remained for $(tn)_2Co(OH)(OH_2)$ and $(tme)_2Co(OH)(OH_2)$. However, the phenomenon was not highly reproducible (compare runs 26 and 27). Thus reactions with unidentified adventitious impurities may have been involved. In any event, in carefully purified water, with complex con-

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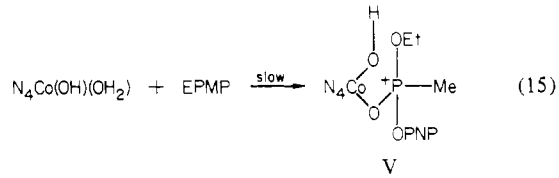
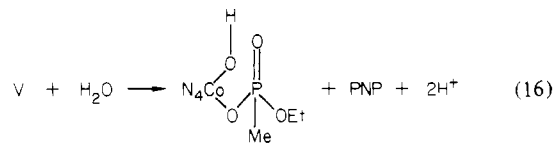
centrations greater than approximately $10^{-3}$ M, the PMP hydrolysis reactions appear to be well-behaved, and $k_{app}$ values can be confidently regarded to be good measures of complex reactivity.

**Ethyl *p*-Nitrophenyl Methylphosphonate Hydrolysis.** The reaction of EPMP with $(N_4)Co(OH)(OH_2)$ is analogous to the catalyzed PMP reactions. Thus, Table III shows not only that the reactivities of the various complexes toward EPMP parallel the reactivities toward PMP but also that the $k_{app}$ values are nearly identical for both reactions. This strongly suggests rate-limiting dissociation of water from the complex, followed by coordination of the EPMP phosphonyl oxygen to cobalt and subsequent rapid hydrolysis as shown in reactions 15 and 16.




(15)

V




(16)

The rate accelerations provided by the complexes in EPMP hydrolysis are substantial. The value ($k_{app} = 5.0 \times 10^{-2}$ $M^{-1}$ $s^{-1}$) for $(tme)_2Co(OH)(OH_2)$-promoted hydrolysis compares with hydroxide-promoted hydrolysis ($k_{OH} = 3.9 \times 10^{-2}$ $M^{-1}$ $s^{-1}$) at the same temperature.[41] At 25 °C and pH 7.6, for the reaction with hydroxide ion, EPMP thus has a half-life of 22 years, while in the presenceof 3 mM $(tme)_2Co(OH)(OH_2)$ the half-life is 80 min.

**Relevance to Other Metal Ion and Enzyme Catalyzed OP Ester Hydrolyses.** It is noteworthy that the aquohydroxo(tetraamine)cobalt(III) complexes *catalytically* hydrolyze organophosphorus compounds. Previous work with divalent metals[3-11] and cobalt(III) complexes[12-15] did not directly demonstrate greater than stoichiometric hydrolysis product yields.

In the proposed mechanism for $(N_4)Co(OH)(OH_2)$-catalyzed hydrolysis of PMP, the metal ion plays a dual role as a Lewis acid and also as template for the reaction. The catalytic nature of the reaction is derived form the relatively rapid hydrolysis of intermediate phosphato or phosphonato cobalt(III) complexes to an aquohydroxo complex. For the case of a bidentate phosphonato ligand such as III, a "seesaw" effect prevails, wherein chelate ligand geometry and basicity control metal ion reactivity, ligand substitution, and the initial hydrolytic step, while relief of ring strain on the phosphorus atom provides the driving force for water attack and regeneration of the catalytic species, $(N_4)Co(OH)(OH_2)$.

With respect to related cobalt(III)-catalyzed reactions, mechanistic similarities are certain. Anderson et al.,[14] for example, found that 50 mM $(tn)_2Co(OH)(OH_2)$ at 25 °C in pH 7.6 buffer hydrolyses PNPP with an observed pseudo-first-order rate constant $k_{obsd} = 1.2 \times 10^{-4}$ $s^{-1}$. From this we calculated an apparent bimolecular rate constant ($k_{app} = 2.4 \times 10^{-2}$ $M^{-1}$ $s^{-1}$) that essentially equals the $k_{app}$ values reported here for PMP and EPMP hydrolyses catalyzed by the same complex. Anderson et al. also observed that low (0.10 mM) $(tn)_2Co(OH)(OH_2)$ concentrations failed to hydrolyze *p*-nitrophenyl phosphate (PNPP). Complicating side reactions with buffer constituents or dissolved $CO_2$ probably account for the poor reactivity of <1 mM $(tn)_2Co(OH)(OH_2)$ in the cited investigation as well as in our own work.

Regarding divalent metal ion complex catalyzed organophosphorus ester hydrolysis, we noted above that the extreme lability of these complexes with respect to ligand substitution and a tendency to form dimeric species in solutions complicate the interpretation of kinetic data. Extrapolating our results to the case of divalent metal ion complexes suggests specifically that coordination of organophosphorus ester to the metal ion precedes the hydrolytic step and that acid catalysis by protons on coordinated water,[3,4] "charge-effects",[9] or leaving-group polarization[8] need not be invoked.

Finally, concerning metalloenzyme-catalyzed organophosphorus ester reactions, current understanding of the role played by active-site metal ions remains imprecise. Although Lewis-acid and also nucleophilic effects due to coordinated hydroxide can obviously be important, we believe a mechanism involving prior coordination followed by hydrolysis and subsequent liberation of bound organophosphorus ester to be entirely consistent with data pertaining, for example, to alkaline phosphatase activity.[1b] We do not expect our proposed mechanism to apply universally to metalloenzyme reactions: nevertheless, reinterpreting existing information in view of our findings might add new insight to a number of important systems.

## Conclusions

Our observations concerning aquohydroxo(tetraamine)cobalt(III) chelate catalyzed hydrolysis of organophosphorus esters have important practical and fundamental ramifications.

In a practical context we can identify some experimental techniques that should facilitate future investigations. Our HPLC procedure for separating and detecting oppositely charged species is convenient and should have general application to metal ion complex promoted reactions of organic compounds. We find that buffer constituents and dissolved $CO_2$ interfere with aquohydroxo(tetraamine)cobalt(III) reactions and recommend that care be taken to avoid these complicating factors in future work. We have demonstrated that the chelates can provide enormous accelerations of neutral organophosphorus ester hydrolyses. This suggests that cobalt(III) chelates have potential application for rapidly degrading hazardous organophosphorus substances.

Fundamentally, our findings contribute to understanding the mechanism of metal ion catalyzed organophosphorus ester hydrolysis reactions. This understanding permits us to predict with increasing confidence the effect of structural modification on inherent reactivity and to design novel materials with enhanced reactivity. The demonstrated catalytic hydrolysis of PMP reveals that the aquohydroxo(tetraamine)cobalt(III) complexes possess the principle property (i.e. catalytic activity) common to all enzymes. Cobalt(III) complexes in solution are well-characterized species and are, in this regard, considerably superior to similar complexes based on divalent metal ions. Since the factors controlling the activity of cobalt(III) chelates are well established, this class of complexes could serve as an extremely powerful probe for the complex mechanisms involved in metalloenzyme reactions.

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**Registry No.** PMP, 1832-64-0; EPMP, 3735-98-6; PNP, 100-02-7; α-*cis*-$[(trien)Co(OH_2)_2](ClO_4)_3$, 89890-77-7; β-*cis*-$[(trien)Co(OH_2)_2](ClO_4)_3$, 75363-51-8; $[(tn)_2Co(OH_2)_2](ClO_4)_3$, 89921-38-0; $[(tme)_2Co(OH_2)_2](ClO_4)_3$, 89890-81-3; *cis*-$[(bpy)_2Co(OH_2)_2](ClO_4)_3$, 10170-75-9; α-*cis*-$[(trien)Co(OH)(OH_2)]^{2+}$, 46135-48-2; β-*cis*-$[(trien)Co(OH)(OH_2)]^{2+}$, 50859-73-9; $[(tn)_2Co(OH)(OH_2)]^{2+}$, 61687-74-9; $[(tme)_2Co(OH)(OH_2)]^{2+}$, 89890-82-4; $[(tn)_2CoCO_3]ClO_4$, 31188-12-2; $[(tme)_2ClCO_3]ClO_4$, 89890-79-9; $[(bpy)_2CoCO_3]ClO_4$, 10170-74-8; $Na_3Co(CO_3)_3$, 23311-39-9.

(41) Hudson, R. F.; Keay, L. *J. Chem. Soc.* **1956**, 2463.