Hydrolysis of Phosphate Esters Bound to Cobalt(III). Kinetics and Mechanism of Intramolecular Attack of Hydroxide on Coordinated 4-Nitrophenyl Phosphate

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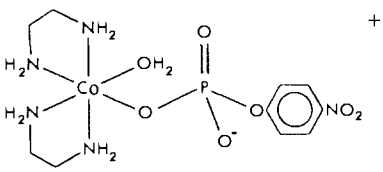
Abstract: The hydrolysis of coordinated 4-nitrophenyl phosphate ester in *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ has been studied over the pH range 7–14 by ^{31}P NMR spectroscopy and by monitoring nitrophenol release at 400 nm. Intramolecular attack by ^{18}O-labeled coordinated hydroxide yields initially a five-coordinate phosphorane, which decays to the tris chelate $[Co(en)_2PO_4]$ and nitrophenol ($k_{obsd} = 7.8 \times 10^{-4}$ s^{-1} at 25 °C in the pH range 9–11.8) with ^{18}O bonded between Co and P. The hydrolysis is 10^5-fold faster than that of the uncoordinated ester under the same conditions. At pH 10 there is evidence for ^{18}O exchange between solvent and the five-coordinate phosphorane, and therefore Co–OH addition and ester hydrolysis are not concerted processes. Above pH 11.8, paths first and second order in $[OH^-]$ dominate. They involve conjugate base (S_N1CB) paths for release of 4-nitrophenyl phosphate by Co–O bond rupture (>80%) and paths that give small yields of nitrophenol. The metal complex reaction is discussed in relation to the hydrolysis of β-hydroxyalkyl phosphate esters and possible implications for the mechanism of the enzyme *E. coli* alkaline phosphatase.

E. coli alkaline phosphatase is a Zn(II)-containing enzyme that catalyzes the nonspecific hydrolysis of phosphate monoesters at rates which are 10^{10}- to 10^{12}-fold faster than those in the absence of the enzyme.[2] One of the mechanisms that has been proposed for the enzyme hydrolysis implicates a coordinated hydroxide ion,[2] and such a proposal receives peripheral support from the observation that coordinated hydroxide is a very effective nucleophile for the intramolecular hydrolysis of nitriles,[3] olefins,[4] and amino acid esters.[5] However, no well-characterized models to test the efficacy of bound OH^- for the corresponding intramolecular hydrolysis of coordinated phosphate esters have yet been published.[6]

In this paper we report the synthesis and mechanism of base hydrolysis of a well-defined Co(III) complex ion, *cis*-$[Co(en)_2(OH_2)O_3POC_6H_4NO_2]^+$ (**1**), in which the coordinated water and



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monodentate 4-nitrophenyl phosphate occupy adjacent coordination sites. This work is parallel to a previous study in which the base hydrolysis of $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ was investigated and where a coordinated aminato ion was the intramolecular nucleophile.[7]

Experimental Section

Reagents and Instrumentation. Analytical-grade reagents were used throughout except where otherwise specified. Diethanolamine was dried and purified by distillation from NaOH pellets. Sodium hydroxide solutions were freshly prepared from May and Baker "Volucon" concentrate, using CO_2-free water. Triflic acid (CF_3SO_3H) was obtained from the 3M company and distilled before use. Disodium phenyl phosphate hexahydrate,[8] barium 2-hydroxypropyl 4-nitrophenyl phosphate,[9] $[Co(en)_2CO_3]ClO_4$,[10] *cis*-$[Co(en)_2Cl_2]ClO_4$,[10] $\{[Co(en)_2(\mu\text{-}O_3POC_6H_5]_2\}(CF_3SO_3)_2$,[11] and *cis*-$[Co(en)_2(H_2O)PO_4H]ClO_4$[12] were prepared by published methods.

^1H NMR spectra were recorded on a Jeol JNM-MH-100 (Minimar) spectrometer. Chemical shifts are quoted as downfield relative to NaTPS [sodium 3-(trimethylsilyl)propanesulfonate] in D_2O solution. For the ^{31}P NMR studies, Jeol JNM-FX-100 (^{31}P probe) or Jeol JNM-FX-90 (multiprobe) spectrometers operating at 40.32 and 36.20 MHz, respectively, with internal D_2O lock were used. All spectra were proton decoupled. ^{31}P chemical shifts are reported relative to external 85% H_3PO_4 with increasing values towards low field. The ^{17}O NMR spectra were recorded on a Bruker BK-R spectrometer operating at 8.139 MHz with external lock. Chemical shifts are reported relative to the solvent water ($H_2{}^{17}O$ natural abundance) peak. $^{13}C\{H\}$ NMR spectra were recorded at 15.04 MHz on a Jeol FX-60Q spectrometer with chemical shift values quoted relative to 1,4-dioxane (internal reference). Electronic spectra were recorded with a Beckman Acta IV spectrophotometer and rate data were collected on Zeiss PMQ III or Cary 118C spectrophotometers. Extinction coefficients are quoted in units of M^{-1} cm^{-1}.

A TPS digital pH meter equipped with an Ionode combination electrode was used for all pH measurements. Evaporations were carried out with a vacuum pump attached to a Buchi rotary evaporator such that the temperature of the solution did not exceed 15 °C.

***cis*-$[Co(en)_2(OH_2)O_3POC_6H_4NO_2]ClO_4 \cdot H_2O$.** $[Co(en)_2(\mu\text{-}O_3C_6H_5)]_2(CF_3SO_3)_2$[11] (2 g) was added in portions to vigorously stirred ice-cold triflic acid (30 mL). Stirring was continued until all of the solid had dissolved. Concentrated nitric acid (0.45 mL) was then added rapidly and the mixture stirred for 7 min. The reaction was quenched by slowly pouring the solution (**CAUTION**!!) into vigorously stirred anhydrous ether (1.4 L). Stirring was continued for a further 20 min. By this time the product had separated as a red-violet oil. The ether was decanted (retain) and a further 300 mL added to the crude product. The oil was triturated until it became a viscous gum. The ether was then discarded and the gum was dissolved in water (300 mL). This was combined with the aqueous extract of the ether retained from above and diluted to 800 mL. The pH of the solution was cautiously adjusted to 2.5 (pH meter) by the addition of 2 M NaOH. The resultant mixture was diluted to 5 L and the reaction products were adsorbed on Sephadex

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C-25 resin (Na^+ form, 12 × 8.5 cm) which had previously been washed with water (1 L) at pH 3. Elution with 0.2 M $NaClO_4$ revealed the presence of four bands. The first one (pale bluish violet) was discarded. The second band (red) contained the desired product and the eluate was therefore collected and concentrated to ~30 mL. The pH of the solution was adjusted to 4.5 by the cautious addition of dilute ammonia solution. Upon cooling of the solution in an ice bath for 2 h, reddish pink microcrystals of the complex were deposited. The crystals were collected and washed with ice-cold 0.4 M $NaClO_4$ (2 × 2 mL), ether/methanol (4:1 v/v, 10 mL), anhydrous ether (10 mL), and dried in vacuo over P_2O_5 for 18 h (yield, 1 g). Anal. Calcd for $[Co(C_4H_{16}N_4)(OH_2)O_3POC_6H_4NO_2]ClO_4 \cdot H_2O$: C, 23.29; H, 4.69; N, 13.58; P, 6.01; Co, 11.43. Found: C, 23.1; H, 4.7; N, 13.3; P, 5.6; Co, 11.7. The ^1H NMR spectrum of a saturated solution of the complex in 2 mL of D_2O containing 1 drop of concentrated $HClO_4$ gave the following chemical shifts (δ in ppm, relative peak areas and multiplicities in parentheses): ethylenediamine CH_2 2.64 (4, br), CH_2 3.03 (4, br), NH_2 ~4.5 (shoulder on HOD peak), NH_2 5.92 and 6.25 (total 4, overlapping br); aromatic H 7.34 (2, d), 8.24 (2, d). The ^{13}C NMR assignments for *cis*-$[Co(en)_2(OH_2)(O_3POC_6H_4NO_2)]^+$ are as follows: ethylenediamine CH_2 -20.61 (s), CH_2 -22.61 (s); Aromatic C_1 90.08 (d, $^2J_{POC}$ = 6.6 Hz), $C_{2,6}$ 54.12 (d, $^3J_{POCC}$ = 5.13 Hz), $C_{3,5}$ 59.31 (s); ^{31}P NMR 6.55 ppm (singlet) in D_2O soluiton at pH 10 with diethanolamine buffer; visible spectrum ϵ_{507}(max) = 103 in water, pH 4.

***cis*-$[Co(en)_2(^{18}OH_2)O_3POC_6H_4NO_2]C_7H_7SO_3$.** The product from above (0.6 g) was suspended in 5 atom % ^{18}O-enriched H_2O (10 mL). The pH of the suspension was adjusted to ~1.5 by the cautious addition of anhydrous triflic acid, and the residual solid was dissolved by warming to ~50 °C for a brief period. The solution was allowed to stand at 25 ± 2 °C for 4 days. At the end of this time a 0.5-mL aliquot was withdrawn for determination of the ^{18}O content of the solvent and the remainder filtered to remove suspended particles. The pH of the filtrate was adjusted to between 4 and 5 by the addition of concentrated ammonia solution. Excess solid ammonium toluenesulfonate (~2 g) was added and dissolved by vigorous agitation. The product began to crystallize ~5 min after this addition. Upon cooling of the solution at 0 °C for 2 h, the reddish pink product was collected, washed with ice-cold water (2 × 2 mL), and allowed to dry in air for 20 min. The anhydrous product was obtained by desication in vacuo over P_2O_5 for 18 h (yield, 90%). Anal. Calcd for $[Co(C_4H_{16}N_4)(^{18}OH_2)O_3POC_6H_4NO_2]C_7H_7SO_3$: C, 34.88; H, 4.99; N, 11.96; P, 5.29; Co, 10.07. Found: C, 34.7; H, 4.9; N, 11.7; P, 5.4; Co, 10.1. The solvent contained 5.13 atom % ^{18}O and the ^{18}O content of the complex was 0.694 atom %. This corresponds to 100% enrichment in one of the ten oxygen atoms in the toluenesulfonate salt. The natural abundance of ^{18}O was taken to be 0.202 atom % for this evaluation.

$[Co(en)_2PO_4] \cdot 2H_2O$ has been reported previously,[12] but an improved synthesis is described here. $[Co(en)_2CO_3]Cl$ (10 g) was added in small portions to 4 M $HClO_4$ (14.8 mL) in order to generate $[Co(en)_2(OH_2)_2]^{3+}$ in situ. Nitrogen was bubbled through the resultant deep red solution for 20 min to expel dissolved CO_2 and H_2O (80 mL) was then added. The solution was heated to 80 °C over a steam bath and $Na_3PO_4 \cdot 10H_2O$ (11 g), dissolved in a minimum volume of hot (80 °C) water, was added with stirring. An orange precipitate formed initially but this soon dissolved. The mixture was heated for a further 30 min during which time the color changed from deep red to intense red–violet. The solution was then cooled to 50 °C and the pH was adjusted to 10 by the addition of concentration aqueous ammonia. Boiling methanol was added, with stirring, until the development of the first trace of permanent turbidity. Cooling at 0 °C for 1 day resulted in the formation of a pale red–violet microcrystalline product. This was collected and washed with methanol/water mixture (10 mL, 2:1 *V/V*), methanol, and finally ether (yield, 7 g).

The crude product was dissolved in a minimum volume of 0.2 M acetate buffer at pH 4 and the solution filtered. Concentrated aqueous ammonia was used to adjust the pH to 10. Almost immediately red violet microcrystals were deposited. After the solution was cooled overnight at 0 °C, the product was collected, washed with methanol and diethyl ether, and finally dried in vacuo over P_2O_5. Anal. Calcd for $[Co(C_4H_{16}N_4)PO_4] \cdot 2H_2O$: C, 15.49; H, 6.50; N, 18.07. Found: C, 15.7; H, 6.7; N, 17.9.

Determination of pK_a. The pK_a of the coordinated water molecule in the complex *cis*-$[Co(en)_2(OH_2)O_3POC_6H_4NO_2]^+$ was determined spectrophotometrically.[13] Equal volumes (1 mL) of 10^{-2} M complex solution and the appropriate buffer (CH_3COOH/CH_3COO^-, Tris/$HClO_4$, diethanolamine/$HClO_4$; μ = 2 M, $NaClO_4$) were placed in the compartments of a split cell. After equilibration at 25 ± 0.1 °C for 8 min the two solutions were mixed rapidly and the absorbance at 550 nm recorded immediately. The aqua complex is rather reactive above pH 7 and the development of an intense absorption band at 400 nm, due to free nitrophenolate ion, interferes with the measurement at 550 nm if it is not made within 30 s of mixing. This procedure was repeated three times for each of the ten buffers used.

Product Distribution. Three series of experiments were carried out:

(a) The yields of nitrophenol were determined under the same conditions as used for the kinetic runs by mixing equal volumes (4 mL) of the complex and buffer or NaOH solutions. The absorption due to the nitrophenolate anion (λ_{max} 400 nm (ϵ = 18 700)) was recorded at $10t_{1/2}$. The total nitrophenol (nitrophenol plus nitrophenyl phosphate) was determined from a basified aliquot of the reaction mixture, which had been hydrolyzed in 1 M HCl at 100 °C for 4 h.

(b) *cis*-$[Co(en)_2(OH_2)O_3POC_6H_4NO_2]ClO_4 \cdot H_2O$ (0.24 g) was suspended in water (10 mL) at 25 °C. Diethanolamine buffer (10 mL, pH 10; total amine concentration = 0.4 M; μ = 2 M, $NaClO_4$) or NaOH solution [10 mL, 0.8 M (μ = 2 M, $NaClO_4$) or 4 M] at 25 °C was added with vigorous stirring and the suspended complex dissolved immediately. The reaction was quenched after $8t_{1/2}$ by the rapid addition of 2 M $HClO_4$ until the pH was 4 (the pH should not be allowed to go below 3.5). The resultant solution was then diluted to 1400 mL with ice-cold water and sorbed rapidly on Sephadex C-25 resin (Na^+ form; 12 × 3 cm), which had been washed immediately beforehand with ice-cold water (300 mL, at pH 3.5 with 0.005 M acetate buffer). The initial effluent containing the free nitrophenol and nitrophenyl phosphate was collected, and the column was then washed with cold water until the effluent no longer developed a yellow color at pH >12. The initial effluent and washings were combined and analyzed for nitrophenol and total nitrophenol as described in part a. The difference between the two values is equal to the amount of nitrophenyl phosphate that was produced. Elution of the cationic species with 0.1 M $NaClO_4$ (pH 4) revealed the presence of three bands. The first band (pale blue-violet) was easily removed and corresponded to *trans*-$[Co(en)_2(OH_2)O_3POC_6H_4NO_2]^+$. The second and third bands, which were not easily eluted by 0.1 M $NaClO_4$, were colored intense red and orange-red, respectively.

After the first band had eluted, the column was washed with ice-cold water (400 mL). Dilute aqueous ammonia solution (400 mL of 0.02 M) was then passed through the column. The second band was eluted by this procedure since it corresponded to zero-charged $[Co(en)_2PO_4]$ for the pH 10 hydrolysis or anionic $[Co(en)_2(OH)PO_4]^-$ for the 0.4 or 2.0 M OH^- hydrolyses. At pH 4 these complexes are protonated and cationic whereas at pH 10 they are fully deprotonated and no longer bind to the cation exchange resin. The red band that remained on the column was $[Co(en)_2(OH)_2]^+$, and this was eluted with 0.3 M $NaClO_4$ solution at pH 9.5 (adjusted with ammonia). All fractions were analyzed for cobalt by atomic absorption spectrometry and for nitrophenol by the procedure described above.

(c) The product distribution of nitrophenol was also determined at μ = 10 for NaOH concentrations between 0.1 and 10 M. The hydroxide solutions were made to constant ionic strength with $NaClO_4$, and the reaction mixture was obtained by mixing the precooled base solution with the complex solution (9.37 mg ClO_4 complex/100 mL) so that the final temperature was close to 25 °C. These experiments show the following results (% nitrophenol given in parentheses): $[OH^-]$ = 0.1 (83); 0.5 (65); 1.0 (51,54); 2.0 (39); 5.0 (22,20); 7.0 (23,20); 10.0 (22).

^{31}P NMR Studies. A saturated solution (~5 × 10^{-2} M) of *cis*-$[Co(en)_2(OH_2)O_3POC_6H_4NO_2]^+$ was prepared by gently warming (~40 °C) and stirring a suspension of the perchlorate salt and Dowex AG1-X8 (Cl^- form) resin in H_2O (2.0 mL) containing trimethyl phosphate (0.21 mL of TMP in 100 mL of H_2O) as an internal reference. The resin and undissolved complex were removed by filtration and 1.5 mL of the filtrate was transferred to a 10-mL NMR tube. D_2O (0.3 mL) was added and a spectrum of the starting material obtained. The reaction was then initiated by the rapid addition of 4 M diethanolamine buffer (0.2 mL, pH 10) or NaOH solution (0.2 mL, 2 or 4 M). Spectra were accumulated until the reaction had proceeded for $8t_{1/2}$. Spectral parameters: band width, 2100 Hz; pulse repetition, 1.0 s; pulse angle, 60°; 4K data points; 100 transients per spectrum.

^{17}O NMR Studies. A series of ^{17}O-enriched compounds were prepared with 20% $H_2{}^{17}O$. A sample of $[Co(NH_3)_5OH_2](ClO_4)_3$ was labeled by a procedure that was known to result in 100% exchange of the aqua ligand.[14] A solution of $(Na^{17}O)_2P(O)OC_6H_4NO_2$, Na_2NPP, was prepared by decomposing 4-nitrophenyl phosphorodichloridate in the 20% $H_2{}^{17}O$. The $H_2{}^{17}O$ was recovered on a vacuum line and the product dissolved in H_2O (2 mL). The pH of the solution was then adjusted to 11 with sodium hydroxide. *cis*-$[Co(en)_2(^{17}OH_2)O_3POC_6H_4NO_2]^+$ and *cis*-$[Co(en)_2(^{17}OH_2)O_3POH]^+$ were produced in solution by analogous

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procedures to those used in the ^{18}O studies. The former was obtained as its toluenesulfonate salt, but the latter was not isolated from solution. Spectral parameters: frequency, 8.14 MHz; band width, 10000 Hz; pulse repetition, 40 ms; pulse angle, 90°; 4K data points; 20000–50000 transients per spectrum.

Kinetics (by Spectrophotometry). The release of 4-nitrophenol from $[Co(en)_2(OH)O_3POC_6H_4NO_2]$ was followed spectrophotometrically at 400 nm in perchlorate media at constant ionic strength (μ = 1 M) over the pH range 6.8–14 at 25 ± 0.1 °C. Typically, a stock solution of complex was prepared by dissolving the perchlorate salt (8 mg) in 100 mL of CO_2-free water. Buffers were prepared by titrating the appropriate amine with standardized perchloric acid and adjusting the ionic strength (2 M) with sodium perchlorate. The reaction was initiated by mixing equal volumes of complex and buffer (or sodium hydroxide) solutions in a split cell. First-order rate constants were obtained by linear least-squares fitting of the log ($A_\infty - A_t$) vs. time data. The buffer dependence of the hydrolysis reaction was investigated for diethanolamine by varying the total amine concentration from 0.05 to 0.4 M for a constant ratio of the buffer species (pH 10).

Barium 2-hydroxypropyl 4-nitrophenyl phosphate was converted to the Na^+ form by passing a solution of the salt through Dowex 50W-X2 (Na^+ form) resin. The rate of 4-nitrophenolate production was measured spectrophotometrically (400 nm) at pH 9.1 and 10.65 as well as over a range of hydroxide ion concentration (0.05–1.0 M; μ = 1 M, $NaClO_4$).

^{18}O Tracer Experiments. Two series of experiments were carried out at pH 10 and 0.2 M OH^-. The first (at pH 10) was designed to study solely the intramolecular cyclization reaction to produce chelate phosphate. Two sets of conditions were employed: (1) ^{18}O-labeled complex in unenriched buffer and (2) unlabeled complex in $H_2{}^{18}O$ buffer solutions. Each experiment was carried out in duplicate. For (1), the toluenesulfonate salt of $[Co(en)_2(^{18}OH_2)O_3POC_6H_4NO_2]^+$ (0.8 g) was suspended in unlabeled water at 25 °C. A solution of diethanolamine (0.4 mL) in water (5 mL) was then added rapidly with vigorous agitation. The suspended complex dissolved almost immediately. The reaction was allowed to proceed for 3 h ($10t_{1/2}$) before the solvent (0.5 mL) was sampled for determination of its ^{18}O content. The intense red-orange solution was diluted to 1.4 L with ice-cold water and the pH adjusted to 3.8 by the cautious addition of 2 M $HClO_4$. (Note: It is imperative that the pH does not fall below 3; otherwise acid-catalyzed ring opening of the $[Co(en)_2PO_4]$ occurs.) Acetic acid/acetate buffer (20 mL of 0.05 M at pH 3.8) was then added and the solution chromatographed by a procedure identical with that used for the product distribution experiment.

The fraction containing the $[Co(en)_2PO_4]$ was reduced in volume until the product began to crystallize (ca. 4 mL). One volume of absolute ethanol was then added slowly, with stirring. The solution was cooled overnight at 0 °C, and then the pale red-violet microcrystals were collected and washed with methanol and ether and dried in vacuo over P_2O_5 for 18 h. The product analyzed as the dihydrate but was converted to the monohydrate for the purposes of the ^{18}O experiment by heating it at 95 °C in an oven for 12 h. Anal. Calcd for $[Co(C_4H_{16}N_4)PO_4]\cdot H_2O$: C, 16.46; H, 6.22; N, 19.19. Found: C, 16.4; H, 6.1; N, 19.0. The solution containing the nitrophenolate anion was reduced in volume to 30 mL, acidified with HCl to pH 0, and then extracted six times with 40-mL aliquots of chloroform. Evaporation of the combined chloroform extracts to dryness yielded the crude nitrophenol as pale yellow needles. Final purification was achieved by vacuum sublimation (85 °C at 0.05 mmHg). Anal. Calcd for $C_6H_5NO_3$: C, 51.80; H, 3.62; N, 10.07. Found: C, 52.2; H, 3.7; N, 10.8. The ^{18}O contents of the solvent and solid samples obtained from the above experiment were determined by using published methods.[7]

A similar procedure was followed for the reaction of unlabeled complex in labeled solvent. The experiment was performed in duplicate, but different solvent enrichments (1.5 and 3 atom %) were used for each run. The diethanolamine was added to 5 mL of the appropriate enriched solvent, and this was then rapidly mixed with the complex which was suspended in 5 mL of the same solvent.

Duplicate experiments using unlabeled complex dissolved in ~3 atom % $H_2{}^{18}O$, which was 0.2 M in hydroxide ion (final concentration), were also carried out. The nitrophenol and nitrophenyl phosphate products were obtained in solution together by the cation-exchange procedure discussed above and separated by chromatography on DEAE-Sephadex. The cationic products were not investigated in these experiments. The full details of the anion-exchange chromatography and subsequent isolation procedures are described elsewhere.[7] The averaged results for the experiments are as follows: solvent, 2.60 atom % excess; nitrophenyl phosphate, 0.00 atom % excess; nitrophenol, 0.00 atom % excess (±0.01 atom %).

In order to provide a control for the $[Co(en)_2PO_4]$ produced in the experiments at pH 10, the exchange in an authentic sample of $[Co(en)_2PO_4]$ was investigated under identical conditions. $[Co(en)_2PO_4]\cdot 2H_2O$ (0.25 g) was dissolved in ~3 atom % $H_2{}^{18}O$ (10 mL) by gentle warming (40 °C). Diethanolamine buffer (0.3 mL of 4 M; HA/A = 1:2) was then added to produce a pH of 10 and the solution was allowed to stand at 25 °C for 5 h. The solvent was then sampled (0.5 mL for solvent ^{18}O determination) and the solution diluted to 1 L with ice-cold water. The pH was adjusted to 3.8 and the solution chromatographed on Sephadex C-25 resin (as described above) in order to reisolate the material. Evaporation of the eluate containing the complex to ~3 mL yielded a microcrystalline product, which was collected by vacuum filtration and washed with a methanol/water mixture (4 mL; 3:1 v/v), methanol, and finally ether. Overnight drying at 100 °C yielded $[Co(en)_2PO_4]\cdot H_2O$. The reaction solvent and metal complex were processed by the methods used previously. The results were as follows: solvent, 2.93 atom % excess; $[Co(en)_2PO_4]\cdot H_2O$, 0.00 atom % excess.

The phosphate chelate ring in $[Co(en)_2PO_4]$ is cleaved in strong base to yield *cis*-$[Co(en)_2(OH)PO_4]^-$.[12] Since the latter complex is generated from the former during the base hydrolysis of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ in 0.4 M hydroxide, the ^{18}O tracer chemistry of the chelate ring-opening reaction was investigated. A 0.8 M solution of hydroxide ion in ~5 atom % $H_2{}^{18}O$ was prepared by dissolving sodium hydroxide pellets (0.32 g) in 10 mL of the enriched water. This was divided into two 5-mL aliquots and $[Co(en)_2PO_4]\cdot 2H_2O$ (0.5 g) was suspended in each. When all of the solid had dissolved (indicating completion of the reaction since $[Co(en)_2PO_4]$ is sparingly soluble under basic conditions), the intense red-purple solution was allowed to stand for a further 10 min. The pH of each solution was then adjusted to 6 with glacial acetic acid, and excess zinc dust was added to reduce the cobalt(III) to cobalt(II). The color of the solution faded rapidly to pale pink, which is characteristic of a high concentration of cobalt(II); qualitative tests for the presence of this species were strongly positive. The pH was maintained at 6 throughout the period required for complete reduction by the addition of more acetic acid. The solutions were mixed intermittently for 15 min, and then Dowex 50W-X2 resin (Na^+ form, 2-g wet weight) was added with stirring. After 3 min, the suspensions were diluted with water (20 mL) and glacial acetic acid (0.5 mL). Finally, the resin and unreacted zinc dust were removed by filtration. The filtrate was diluted to 100 mL and the cationic reaction products (Zn^{2+} and Co^{2+}) were adsorbed on a column of Dowex 50W-X2 resin (Na^+ form, 11 × 1 cm). The effluent plus washings (ca. 150 mL) were reduced in volume (40 mL), and the pH was adjusted to 12 with sodium hydroxide solution. Saturated barium hydroxide solution (6 mL) was then added to precipitate the phosphate as insoluble $Ba_3(PO_4)_2$. The white amorphous product was collected and washed with water, methanol, and finally ether.

The $Ba_3(PO_4)_2$ was converted to KH_2PO_4 for ^{18}O analysis by a method similar to that of Haake and Westheimer.[15] The average results from the duplicate ^{18}O determinations were as follows: solvent, 4.77 atom % excess; KH_2PO_4, 0.00 atom % excess.

Results

Spectrophotometric Studies. The release of 4-nitrophenolate anion from *cis*-$[Co(en)_2(OH_2)O_3POC_6H_4NO_2]^+$ was followed spectrophotometrically under pseudo-first-order conditions over the pH range 7–14 (μ = 1 M, $NaClO_4$; T = 25 ± 0.05 °C). The log ($A_\infty - A_t$) vs. time data were linear to at least $5t_{1/2}$. Rate constants were obtained by linear least-squares analysis of these data (Table I).

The pH–rate profile for the hydrolysis reaction is illustrated in Figure 1. It is obvious that the observed pseudo-first-order rate constants do not show a simple dependence on pH. From pH 6.5 to 9 there is a sigmoidal increase in rate to a pH-independent maximum. The rate is constant up to pH 12, but above this value it again becomes pH dependent. A least-squares fitting of the data to a rate law of the following type

$$k_{obsd} = \frac{[OH^-]}{a + [OH^-]}\{k_1 + k_2[OH^-] + k_3[OH^-]^2\} \quad (1)$$

gives k_1 = 7.6 (±0.1) × 10^{-4} s^{-1}, k_2 = 4.2 (±0.1) × 10^{-3} M^{-1} s^{-1}, k_3 = 1.5 (±0.1) × 10^{-3} M^{-2} s^{-1}, and a = 6.3 × 10^{-7}. Over the pH range 9–12 the buffer type (successively Tris/$HClO_4$, di-

(15) Haake, P. C.; Westheimer, F. H. *J. Am. Chem. Soc.* **1961**, *83*, 1102–1109.
(16) Fischer, R.; Bye, J. *Bull. Soc. Chim. Fr.* **1964**, 2920–2929.
(17) Kirsch, J. F.; Jencks, W. P. *J. Am. Chem. Soc.* **1964**, *86*, 837–846.

Table I. Kinetic and Product Distribution Data for the Base Hydrolysis of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$[a]

pH[b]	[NaOH], M	10^4k, s^{-1} [c]	nitrophenol,[d] %	calculated nitrophenol,[h] %
8.20[e]		6.0	86	
8.97[e]		7.5	86	
10.66[f]		7.6	86	
11.83[g]	0.01	7.8	83	
	0.025		77	
	0.05	9.9	67	
	0.1	12.0	56	56
	0.25	19.0	40	39
	0.4	27.0	31	
	0.5	32.0	27	26
	0.75	48.0	20	20
	1.0	65.0	17	18
	1.1	71.0		
	2.0		14	14

[a] μ = 1 M, $NaClO_4$; T = 25 °C. [b] pH = $-\log p a_{OH}$, where pK_w = 13.80[16] and γ_{OH^-} = 0.67[17] for 25 °C and μ = 1. [c] Observed spectrophotometric rate constant for nitrophenol release. Each entry is the average of three values (maximum deviation 3%). [d] Percentage yield of nitrophenol after $10t_{1/2}$. Each entry is the average of three values which differ by not more than 5%. [e-g] Tris/$HClO_4$, diethanolamine/$HClO_4$, and triethylamine/$HClO_4$ buffers, respectively. Total (buffer) = 0.2 M in each case. [h] Calculated by assuming the bifurcation of paths in Scheme II and that the rate law in eq 2 holds up to μ = 2 M.

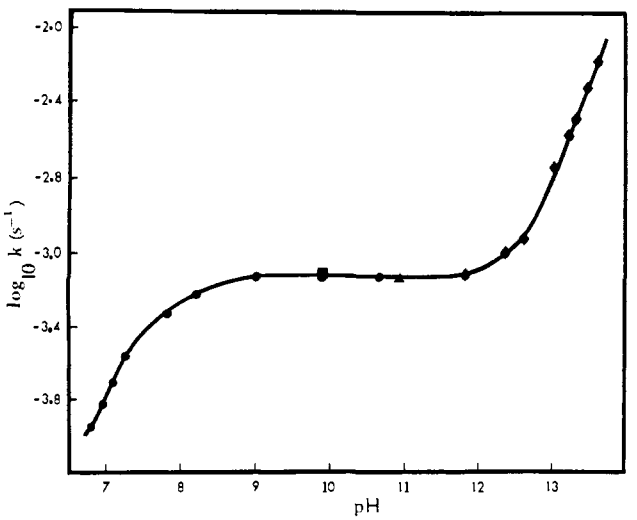


Figure 1. Plot of log k_{obsd} vs. pH for the base hydrolysis of *cis*-$[Co(en)_2(OH_2)O_3POC_6H_4NO_2]$ where k is the observed pseudo-first-order rate constant for nitrophenolate production (T = 25 °C; μ = 1 M, $NaClO_4$). Buffers: Tris/$HClO_4$ (●); diethanolamine/$HClO_4$ (■); triethylamine/$HClO_4$ (▲); NaOH solutions cis in Table I (♦).

ethanolamine/$HClO_4$, and triethylamine/$HClO_4$) and concentration (for a constant pH) had no effect on the value of the rate constant. Therefore, general acid or general base catalysis can be excluded.

Rate data obtained at pH 10 for the temperature range 25–45 °C resulted in a linear (Eyring) plot of log (k/T) vs. $1/T$ with $\Delta H^{\ddagger}$ = 71 ± 4 kJ mol^{-1} and $\Delta S^{\ddagger}$ = −65 ± 5 J mol^{-1} K^{-1}. The percentage yield of nitrophenol remained constant over the temperature range studied. Rate data for the hydrolysis in D_2O at pD 10 yielded a value of 6.8 (±0.1) × 10^{-4} s^{-1} for k_{obsd} and therefore a k_H/k_D ratio of 1.2. Since this rate was determined in the pH-independent region of the rate profile, the different values of pH and pD (pD = pH + 0.4)[18] will have no effect on the k_H rate selected for comparison (for pH 9–11.8, k_H = 7.8 × 10^{-4} s^{-1}). The measured kinetic isotope effect of 1.2 is consistent with proton transfer not being rate limiting and the lack of general acid and base catalysis.

Spectrophotometric titration of the complex with Tris buffers (μ = 1 $NaClO_4$; T = 25 °C) yielded a value of 7.61 ± 0.05 for the pK_a of the aqua ligand.

Product Identification and Distribution. The reaction products in the pH-independent and high-base regions were investigated in detail by both ^{31}P NMR and quantitative product distribution

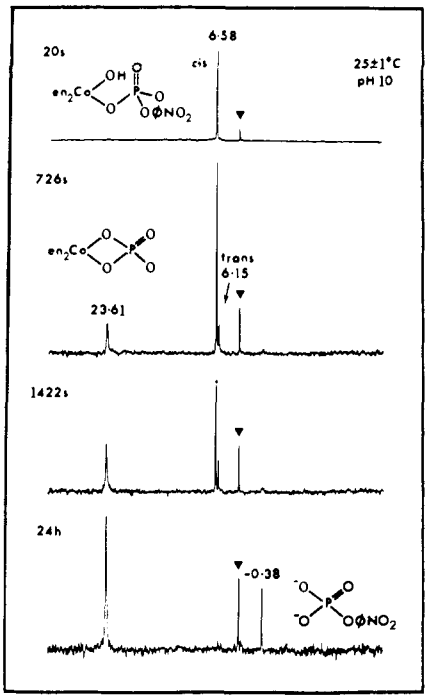


Figure 2. ^{31}P NMR spectra of the products of base hydrolysis of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ at pH 10. Trimethyl phosphate (▼) was used as an internal reference. Spectral parameters: frequency, 40.3 MHz; bandwidth, 2100 Hz; pulse rate, 1.0 s; pulse angle, 60°; 100 pulses per spectrum. Chemical shifts in ppm.

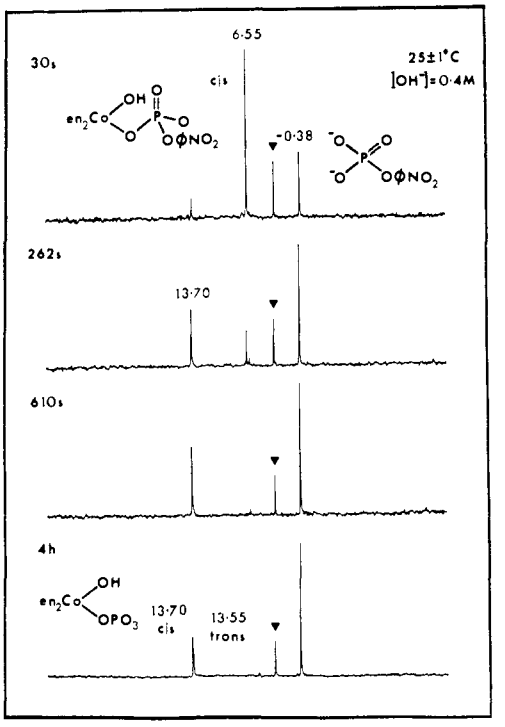


Figure 3. ^{31}P NMR spectra for the base hydrolysis of ($[OH^-]$ = 0.4 M) of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$. Spectral parameters are the same as for Figure 2.

(18) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188–190.

Table II. Product Distributions for the Base Hydrolysis of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ after $8t_{1/2}$[a]

product	conditions: pH 10	$[OH^-]$ = 0.4 M	$[OH^-]$ = 2.0 M
4-nitrophenol	87[b]	30[b]	16[b]
4-nitrophenyl phosphate	8	67	84
trans-$[Co(en)_2(OH_2)O_3POC_6H_4NO_2]^+$	6	3	
$[Co(en)_2PO_4]$ or $[Co(en)_2(OH_2)PO_4H]^+$ [c]	87	30	16
$[Co(en)_2(OH_2)_2]^{3+}$	8	68	84

[a] $T = 25$ °C. [b] Expressed as a percentage of the total material recovered. Recovery was 100 ± 1% in all cases. The values quote quoted are an average of duplicate determinations (accuracy ±1%). [c] $[Co(en)_2PO_4]$ for pH 10, $[Co(en)_2(OH_2)PO_4H]^+$ for 0.4 and 2.0 M OH^-.

studies. The ^{31}P NMR spectra clearly show that the major product of the reaction at pH 10 (Figure 2) is the chelate phosphate complex $[Co(en)_2PO_4]$ (δ 23.6). This species presumably arises from intramolecular hydrolysis of the phosphate ester by the coordinated hydroxide ion. The other product of ester hydrolysis is nitrophenol. A small amount of *trans*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ (δ 6.15) was also generated, and this reacted much more slowly to liberate 4-nitrophenyl phosphate (δ −0.38) plus $[Co(en)_2(OH_2)_2]^+$. Thus ester hydrolysis is the dominant pathway at pH 10. A similar experiment was carried out for $[OH^-] = 0.4$ M (Figure 3), and the yields of the products were found to be different from those obtained above. A small amount of *trans*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ was still produced, but the major products of the reaction were *cis*-$[Co(en)_2(OH)PO_4]^-$ (δ 13.70) and 4-nitrophenyl phosphate. It is clear that, under these conditions, release of unhydrolyzed ester is the dominant pathway. This is in contrast to the situation at pH 10.

The origin of the *cis*-$[Co(en)_2(OH)PO_4]^-$ complex in strong base needs elaboration. The hydrolysis of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ was also investigated at $[OH^-] = 0.2$ M and in this case the chelate phosphate complex $[Co(en)_2PO_4]$ was observed as a transient peak at δ 23.9. This established that the *cis*-$[Co(en)_2(OH)PO_4]^-$ ion is produced by rapid opening of the phosphate chelate ring. The result is consistent with previous studies[19] on the base hydrolysis of $[Co(en)_2PO_4]$. At pH 10 the chelate is stable toward ring opening, but in 0.2 M hydroxide solution the $t_{1/2}$ is only 3 s at 25 °C. The reaction is first order in hydroxide ion concentration ($k = 1.8$ M^{-1} s^{-1}),[19] and therefore $[Co(en)_2PO_4]$ is observed for $[OH^-] = 0.2$ M but not for $[OH^-] = 0.4$ M. The rate of disappearance of the chelate becomes too fast relative to its rate of production in the latter case to enable its detection on the NMR time and sensitivity scales.

The results of quantitative product distribution studies at pH 10 and $[OH^-] = 0.4$ and 2.0 M, are presented in Table II. These are in agreement with the ^{31}P NMR studies and the data for nitrophenol release as a function of pH, which are tabulated in Table I. From pH 8 to 11.8 the yield of nitrophenol is a constant 86 ± 2%, but above pH 11.8 it drops rapidly until for $[OH^-] = 1.0$ M it is only 17 ± 2%. The ^{31}P NMR spectra for the hydrolysis reaction at pH 10 show that the nitrophenyl phosphate arises solely from the decomposition of *trans*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$. Since the yield of the phosphate ester is constant (14 ± 2%) from pH 8 to 11.8, this implies that the yield of the trans isomer is also constant over this range. Thus the rate of cis to trans isomerization is independent of pH in this range.

A series of hydrolyses were conducted in very high ionic strength solutions, $\mu = 10$ M $NaClO_4$ with $[OH^-]$ varying between 0.1 and 10 M (see Experimental Section). The ionic strengths of these solutions are too high to enable the rates to be compared to the $\mu = 1.0$ M kinetics described above. However, the product distribution does indicate a limit for nitrophenol production in the high base region.

(19) Lincoln, S. F.; Stranks, D. R. *Aust. J. Chem.* **1968**, *21*, 57–65.

Table III. ^{17}O NMR Chemical Shifts for ^{17}O-Enriched Compounds

compound	chemical shift,[a] ppm	pH
$[Co(NH_3)_5{}^{17}OH_2]^{3+}$	−127 s	4
$[Co(NH_3)_5{}^{17}OH]^{2+}$	−214 br	10
$[Co(en)_2(^{17}OH_2)PO_4H]^+$	−122 s	3
$[Co(en)_2(^{17}OH_2)O_3POC_6H_4NO_2]^+$	−125 s	4
$(Na^{17}O)_2P(O)OC_6H_4NO_2$	106 d (J_{PO} = 68 Hz)[b]	12
$(Na^{17}O)_3PO$	113 d (J_{PO} = 52 Hz)[b]	12

[a] The solvent water peak was used as the reference; downfield values are positive (accuracy ±3 ppm). s = singlet, d = doublet, br = broad, poorly resolved multiplet. [b] Quoted coupling constants are approximate values only (accuracy ±10 Hz).

Table IV. ^{18}O Tracer Results for the Hydrolysis of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ at pH 10[a]

solvent	starting material		products[b]: $[Co(en)_2PO_4]\cdot H_2O$		nitrophenol	
	atom %[c] excess	E %[d]	atom %[c] excess	E %[d]	atom %[c] excess	E %[d]
0.00	0.45		0.99		0.00	
0.00	0.50		1.08		0.00	
1.32	0.00	0	0.033	13	0.00	0
2.91	0.00	0	0.109	17	0.00	0

[a] $T = 25$ °C. [b] The products of the reaction were isolated after $10t_{1/2}$ for nitrophenol release. [c] Atom % excess is equal to the ^{18}O content of the sample (expressed in atom %) minus the natural abundance of ^{18}O in CO_2 (0.202 atom %); accuracy ±0.01 atom %. [d] The percentage enrichment (E) of a compound is given by E = [(atom % excess for compound)/(atom % excess for solvent)] × n × 100% where n is equal to the number of oxygen atoms per mole of the pure compound.

^{18}O Tracer Studies. In order to obtain further mechanistic details about the intramolecular pathway it was necessary to prepare ^{18}O-labeled *cis*-$[Co(en)_2(^{18}OH_2)O_3POC_6H_4NO_2]^+$. This was achieved by equilibration of unlabeled complex with $H_2{}^{18}O$ in dilute acid solution. However, the position of the label needed to be unambiguously established since there was a low probability that one of the phosphate oxygens may have undergone significant exchange in the acidic medium. ^{17}O-labeled complex was also prepared by the same procedure. The ^{17}O NMR chemical shifts for this complex and a number of other ^{17}O-enriched compounds are listed in Table III. Clearly the label resides on the cobalt-bound water and not on the phosphate since the ^{17}O resonance has a chemical shift that is characteristic of a coordinated water molecule and does not exhibit splitting due to ^{17}O–^{31}P coupling.

The first series of ^{18}O-tracer experiments were designed to study the mechanism of hydrolysis of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ at pH 10. The reactions were carried out in duplicate under two sets of conditions: (a) ^{18}O-labeled complex was dissolved in isotopically normal water and (b) unlabeled complex was dissolved in ^{18}O-enriched water. The pH of the solutions was maintained at 10 with diethanolamine buffer. The results of these experiments are summarized in Table IV. It is clear that there is complete retention of label in the $[Co(en)_2PO_4]\cdot H_2O$ product when ^{18}O-labeled starting material is used, since the atom % excess of ^{18}O in the product is twice that of the starting material (starting material and product contain 10 and 5 oxygen atoms per molecule, respectively). No incorporation of ^{18}O was observed in the nitrophenol that was isolated in this experiment.

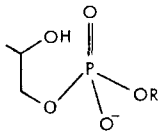
The corresponding experiment with unlabeled complex in labeled solvent resulted in some incorporation of ^{18}O into the chelate phosphate product (13% and 19% enrichment in duplicate experiments). A control experiment was carried out in order to investigate the origin of this enrichment. $[Co(en)_2PO_4]$ was dissolved in 5 atom % $H_2{}^{18}O$, and the solution pH adjusted to 10 by the addition of diethanolamine buffer. After 5 h at 25 °C the chelate phosphate complex was reisolated. Isotopic analysis revealed that no incorporation of ^{18}O into the complex had occurred

over this period. Therefore the exchange described above must have occurred prior to or during the production of $[Co(en)_2PO_4]$ by the intramolecular hydrolysis of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$.

The mechanism of base hydrolysis of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ in 0.2 M hydroxide solution was also investigated by ^{18}O tracer experiments. The nitrophenol and nitrophenyl phosphate products of the reaction were isolated and their ^{18}O contents were determined. No ^{18}O incorporation into either of the products was observed.

cis-$[Co(en)_2(OH)PO_4]^-$ was also one of the hydrolysis products in strong base. The base-catalyzed chelate ring opening of $[Co(en)_2PO_4]$ has previously (but erroneously; see below) been reported to occur with 70% Co–O and 30% P–O bond rupture.[12] The ^{18}O tracer chemistry of this process was reinvestigated during the present study. The hydrolysis of $[Co(en)_2PO_4]$ was carried out in $H_2{}^{18}O$ solution that contained 0.4 M hydroxide ion. The phosphate was removed from the *cis*-$[Co(en)_2(OH)PO_4]^-$ product by initially reducing the cobalt(III) to cobalt(II) with zinc dust (and then precipitating phosphate from solution as $Ba_3(PO_4)_2$, which was subsequently converted to KH_2PO_4 for isotopic analysis[15]). No ^{18}O incorporation was detected. Since the reduction was carried out in the enriched solvent, loss of label could not have occurred via P–O cleavage in the zinc reduction step. If any P–O cleavage had occurred, label incorporation would have resulted. The hydrolysis reaction was also carried out in 20% $H_2{}^{17}O$ and the ^{17}O NMR spectrum of the product obtained. No ^{17}O was detected in the bound phosphate, and the only resonance observed corresponded to a $Co-{}^{17}OH_2$ moiety. From the ^{17}O NMR experiments (Table III) it is quite clear that 30% label incorporation at phosphorus would have given rise to a readily detectable resonance exhibiting $^{17}O-{}^{31}P$ coupling. The ^{18}O and ^{17}O results therefore both establish that the original observation of P–O cleavage was in error.

Analogous Organic Hydrolysis. The hydrolysis in base of 2-hydroxypropyl 4-nitrophenyl phosphate anion (**2**, R = $PhNO_2$)[9]



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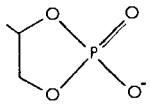
was proposed to occur via intramolecular attack of the deprotonated hydroxyl moiety at the phosphorus center. This conclusion was confirmed in the present work by a ^{31}P NMR experiment, which showed that the cyclic phosphate diester (**3**) was the sole



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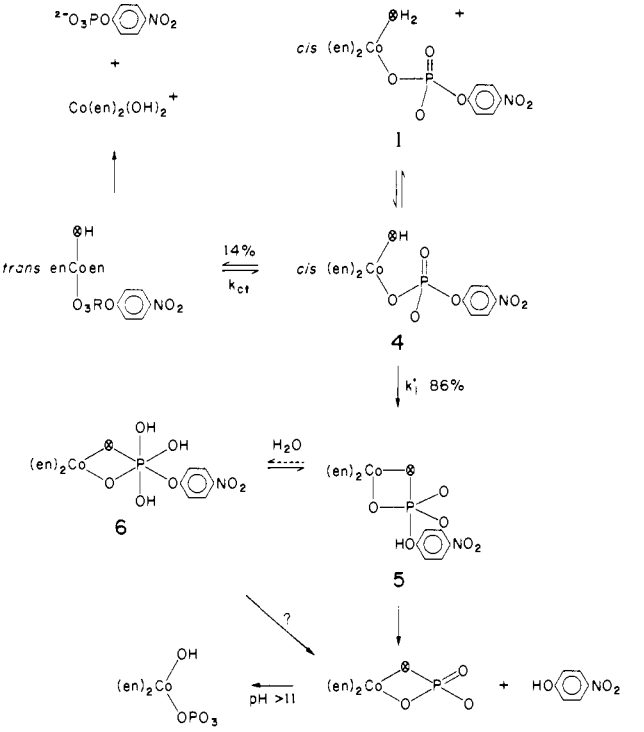
initial product of the hydrolysis reaction in 0.2 M hydroxide [δ (starting material) −5.1, δ (product) 17.8].

Unfortunately the rate of the reaction was measured only at pH 11.37 and 11.82 in the original work, while the complete pH–rate profile was required for comparison in the present study. The liberation of 4-nitrophenol from a sample of **2** was therefore followed spectrophotometrically at 400 nm under pseudo-first-order conditions over the pH range 9–14 (Table V). The reaction is first order in $[OH^-]$ over this range with $k = 2.1 \times 10^{-1}$ M^{-1} s^{-1} (μ = 1 M, $NaClO_4$; T = 25 °C).

Discussion

The mechanism for the base hydrolysis of *cis*-$[Co(en)_2(OH_2)O_3POC_6H_4NO_2]^+$, Schemes I and II, accounts for the following observations: (i) the sigmoidal dependence of the reaction rate constant on pH (6–14), (ii) the change in product distribution at high pH and also the apparently limiting production of nitrophenol, (iii) the tracer results, and (iv) the isomerization to *trans*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ and subsequent loss of nitrophenyl phosphate.

Scheme I. k_1 Path



Scheme II

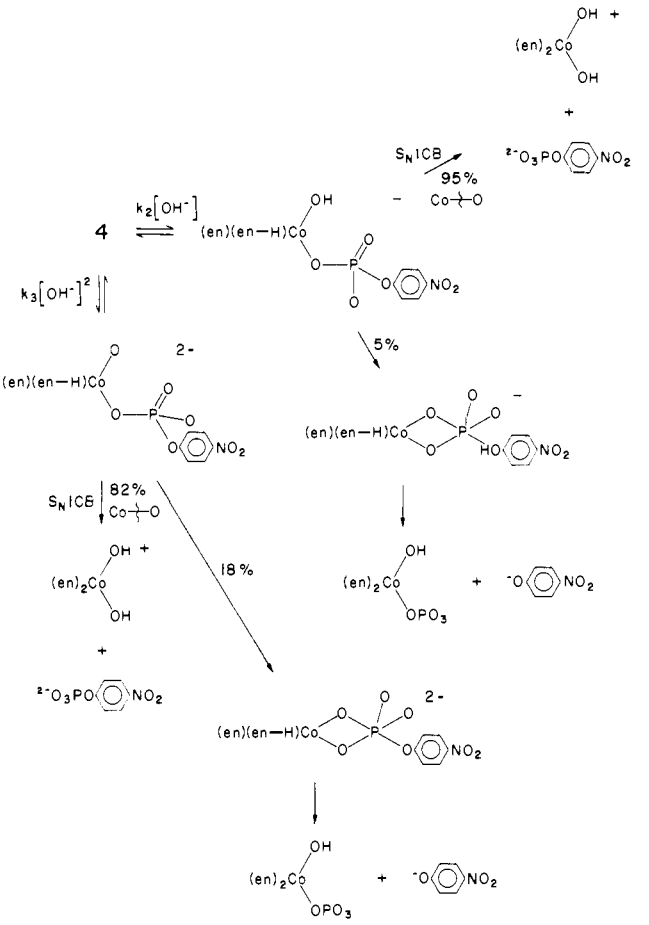


Table V. Kinetic Data for the Base Hydrolysis of 2-Hydroxypropyl 4-Nitrophenyl Phosphate[a,b]

[NaOH], M	α_{OH^-}[c]	pH	k, s^{-1} [d]
		9.10[e,f]	3.8×10^{-6} [i]
		10.65[e,g]	1.45×10^{-4}
0.01	0.0067	11.63[h]	9.2×10^{-4} [j]
0.025	0.017	12.03[h]	2.56×10^{-3}
0.1	0.067	12.63[h]	9.5×10^{-3}
0.5	0.335	13.3[h]	5.2×10^{-2}
1.0	0.67	13.7[h]	1.04×10^{-1}

[a] [Ester] = 5×10^{-4} M. [b] μ = 1 M, $NaClO_4$; T = 25 °C. [c] Activity coefficient γ_{OH^-} = 0.67 for μ = 1 M, $NaClO_4$. [d] The rate constants quoted are the average of duplicate values, which differ by not more than 3%. [e] Measured pH for buffer solutions. [f] 0.2 M tris/$HClO_4$ buffer. [g] 0.2 M $CH_3NH_2/HClO_4$ buffer. [h] $pH = pK_w - p\alpha_{OH^-}$; pK_w = 13.80 for μ = 1 M, $NaClO_4$. [i] This rate constant was determined by the method of initial velocities. [j] From the original study (ref 14) $k = 9.9 \times 10^{-4}$ s^{-1} for pH 11.82 with piperidine/HCl buffer (μ = 1 M, KCl).

The rate expression for the production of nitrophenol in these schemes follows:

$$k_{obsd} = \frac{K_a[OH^-]}{K_w + K_a[OH^-]} (k_{ct} + k'_1 + k_2[OH^-] + k_3[OH]^2) \quad (2)$$

K_a is the dissociation constant for the coordinated water molecule, K_w is the dissociation constant for water ($-\log K_w$ = 13.80 for μ = 1[16]), k_{ct} is the pH-independent rate constant for cis → trans isomerization of $[Co(en)_2(OH)O_3POC_6H_4NO_2]$, k'_1 is the pH-independent rate constant for intramolecular phosphate ester hydrolysis (Scheme I), k_2 is a second-order rate constant for the path leading largely to loss of bound phosphate ester, and k_3 is a third-order rate constant for a path also leading largely to loss of bound phosphate ester (Scheme II).

Equation 2 is derived assuming the hydroxo form of the reactant (**4**) gives rise to cis ⇄ trans isomerization. It follows that in eq 1 $k_1 = k_{ct} + k'_1$ and $a = K_w/K_{a'}$ and in the low pH region, the limiting form of (1) is

$$k_{obsd} = \frac{k_1K_a[OH^-]}{K_w + K_a[OH^-]} = \frac{k_1K_a}{K_a + [H^+]} \quad (3)$$

Least-squares fitting of the rate constant data for the pH range 6.8–9 to eq 3 using a value of 7.6×10^{-4} s^{-1} for k_1 yielded a value of 2.66×10^{-8} for K_a (pK_a = 7.58 ± 0.05), in agreement with the directly measured $pK_{a'}$ 7.61.

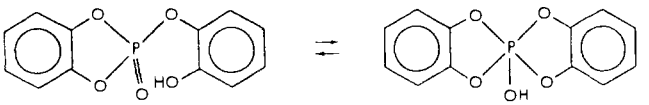
The percentage yields of nitrophenol and *trans*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ are the same at pH 8.2 (k_{obsd} ~ 76% of pH-independent rate constant maximum) and pH 10 (Table I), and it follows that both must be produced from the hydroxo complex (**4**). A precedent for this type of cis to trans isomerization is provided by the $[Co(en)_2(NH_3)(OH)]^{2+}$ system[20] where the cis and trans ions isomerize in aqueous solution at a rate ~400-fold faster than that of the aqua complexes. The equilibrium is also pH independent, and the ^{18}O-tracer results imply the isomerization of the hydroxo amine complexes occurs via an intramolecular path. It may involve a unimolecular dissociation of one end of an 1,2-ethanediamine chelate ring under the labilizing influence of the hydroxo group[20] or it may rearrange by a twisting mechanism along a trigonal or a rhombic axis.

trans-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ is one of the minor products of base hydrolysis of the cis isomer over the entire pH range studied. In the current study the trans isomer was not isolated, but it was characterized in solution by ^{31}P NMR spectroscopy, UV/vis spectrophotometry, and the determination of its cobalt:phosphate:nitrophenol ratio (1:1:1). The close proximity of the ^{31}P NMR resonance for this complex to that observed for *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ ($\Delta\delta$ 0.4) at pH 10 (Figure 2) is also consistent with its assignment as the trans isomer. At pH 10 the trans isomer reacts ~10 times more slowly than the cis isomer to liberate nitrophenol (10%) and nitrophenyl phosphate (90%).

The values of the rate constants for cis–trans isomerization and the intramolecular hydrolysis of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ were obtained by partitioning the pH-independent rate constant k_1 by the ratio of nitrophenyl phosphate (14 ± 2%) to nitrophenol (86 ± 2%) production whence k'_1 = 6.5×10^{-4} s^{-1} and $k_{ct} = 1.1 \times 10^{-4}$ s^{-1}. The increase in rate up to ~pH 9 is accommodated by the conversion of the aqua complex to the hydroxo form. Thereafter the rate is constant until pH 12 whereupon it commences to increase again. This latter aspect will be considered later as it involves a change in mechanism and is not especially germane to the main thrust of this paper.

For the pH-independent region, combined ^{31}P NMR (Figure 2) and ^{18}O-tracer studies (Table IV) establish that ester hydrolysis (86%) arises from intramolecular attack of coordinated OH^- at the phosphorus center (k'_1, Scheme I) to give 4-nitrophenol and $[Co(en)_2PO_4]$.

The nature of the species involved in the conversion of **4** to **6** in Scheme I is of major interest. Unless the reaction is concerted, a five-coordinate phosphorane intermediate (**5**) must arise as a result of the intramolecular attack by the coordinated OH^- ion. It is generally accepted that phosphoranes are involved in many of the hydrolysis reactions of phosphate di- and triesters.[21] In addition, a dynamic equilibrium in solution between the hydroxyphenyl phosphate **7** and the hydroxyphosphorane **8** has been



7 **8**

detected by ^{31}P NMR spectroscopy.[22] Apart from the ring sizes involved, this system is an organic analogue of the metal complex reaction reported here.

The majority of stable pentaoxyphosphoranes containing one five-membered ring that have been structurally characterized exhibit a trigonal-bipyramidal geometry with the five-membered ring spanning apical–equatorial positions.[23,24] The general rules that have evolved from the study of the solid-state structures and the solution NMR spectra of these compounds place electron-withdrawing groups preferentially in apical sites and electron-donating groups in equatorial sites.[24,25] The phosphorane **5** is therefore depicted as a trigonal bipyramid with its chelate ring subtending apical–equatorial positions. The negatively charged oxygens occupy the remaining equatorial positions, and the nitrophenol is in an apical position, ready to leave. By analogy with organic phosphate ester systems, whose hydrolyses in base are attributed to formation and decay of five-coordinated phosphorane intermediates, the decomposition of **5** would be expected to be fast.[24,26] The absence of a peak that could be assigned to **5** in the ^{31}P NMR spectra is consistent with this expectation.

A number of tracer experiments in the current study have provided evidence which suggests that although the pathway involving a five-coordinated intermediate state is the dominant one, it does not completely account for all of the observations. When *cis*-$[Co(en)_2(^{18}OH_2)O_3POC_6H_4NO_2]^+$ reacted at pH 10 in unlabeled solvent, complete retention of the label was observed

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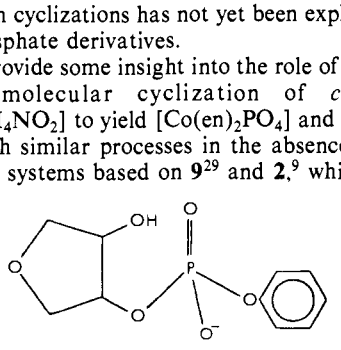
in the chelate–phosphate product. Therefore there is no loss of ^{18}O from the starting material or from the $[Co(en)_2PO_4]$ once it is formed. This means that the cobalt-bound hydroxide and the oxygens bridging the cobalt and phosphorus centers are effectively inert toward exchange. However, a small amount (~ 17%) of ^{18}O incorporation into the chelate–phosphate product was observed when unlabeled complex was reacted in labeled solvent (at pH 10). The label must reside on one or both of the exocyclic oxygens of the $[Co(en)_2PO_4]$ product since the results from the first experiment show that the oxygen atoms in the chelate ring are inert to exchange. Two control experiments showed that $[Co(en)_2PO_4]$ did not undergo exchange when it was dissolved in $H_2{}^{18}O$-enriched water at pH 10. Therefore, the exchange described above must have occurred prior to $[Co(en)_2PO_4]$ production during the course of ester hydrolysis since reactant **4** does not undergo exchange either. These conclusions imply that solvent adds to the phosphorane center (**5**) via a six-coordinate phosphorus intermediate or activated complex (**6**). This exchange path competes partly with the decay of the five-coordinated phosphorane and establishes the latter as a genuine intermediate along the reaction path. Clearly, such a path leads to isotopic enrichment in the exo-oxygen atoms of the chelated phosphate product.

It is not clear if the short-lived phosphorane also decomposes by the six-coordinate path, but the proposed involvement of the six-coordinate species in the reaction is in accord with their involvement in some organic phosphate hydrolyses that contain ring systems.[26,27] The increased coordination numbers are also consistent with known five- and six-coordination states of phosphorus(V) containing electronegative ligands, e.g., PF_5 and PF_6^-. We presume also that the five- and six-coordinate phosphorus species would lead to pseudorotation and that the stereochemical course of the addition could be obscured by this process. This aspect, of course, needs further investigation.

The addition of the coordinated nucleophile to the P center leads to a strained four-membered ring. However, the strain is considerably less in the five-coordinate phosphorane than in the four-coordinate chelated phosphate product, provided the 4-chelate spans an axial–equatorial position. In the former instance, the preferred angle at P is only 90° while in the latter it is 108–109°. The acceleratory effect observed therefore presumably comes largely from the intramolecular reaction and the consequent gain in translational entropy compared to an intermolecular reaction.[28] There would also be little loss from vibrational and rotational sources in this instance since the P atom is placed so that it can hardly avoid the adjacent nucleophile. The strain developed in the chelation step may reduce the rate enhancement somewhat over that expected for a strain-free cyclization, but the effect of ring size on such cyclizations has not yet been explored with the complexed phosphate derivatives.

In order to provide some insight into the role of the metal ion in the intramolecular cyclization of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ to yield $[Co(en)_2PO_4]$ and nitrophenol, a comparison with similar processes in the absence of metals is desirable. Two systems based on **9**[29] and **2**,[9] which contain β-



9

hydroxy functions relative to the phosphate group, have been synthesized by other workers. The published log k vs. pH profile for **9** as well as the data for **2** (R = $-C_6H_4NO_2$) and *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ (for comparison) are illustrated in Figure 4.

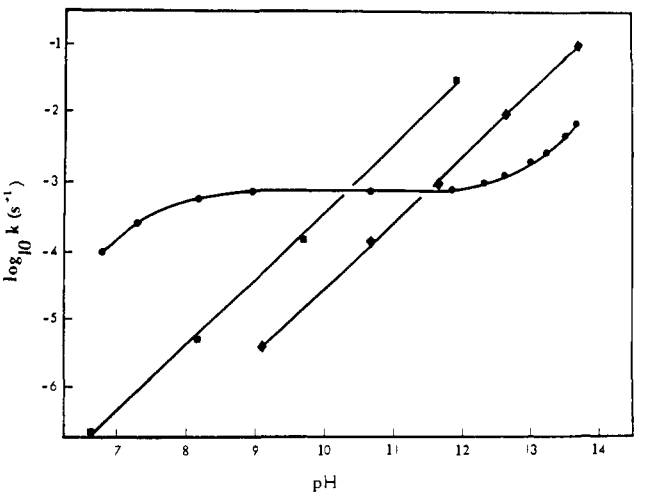


Figure 4. Plots of log k_{obsd} vs. pH for the base hydrolysis of *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ (●), (**2**) (■), and (**10**) (♦). T = 25 °C.

Clearly there are a number of differences between 2 and 9 and the metal complex. Both of the organic phosphate diesters react by the intramolecular attack of alkoxide anion at the phosphorus center. The resultant five-coordinate phosphoranes decompose exclusively by exocyclic cleavage to yield the corresponding phenol and cyclic phosphate diester. The pK_a of the alcohol function in **9** was calculated to be 13.8 from kinetic results,[29] and the pK_a of the 2-hydroxypropyl moiety in **2** would be expected to be somewhat higher because of the negative inductive effect that is exerted by the tetrahydropyran moiety in **9**. The importance of this effect is illustrated by the observation that *cis*-1-phenyl-cyclopentan-2-ol phosphate reacts 70-times slower in base than does **9**.[29]

In contrast to **2** and **9**, the pK_a of the coordinated water in the metal complex is only 7.6 and the hydrolysis rates for 2 and 9 in the high base region are much faster than those for the analogous region of the pH–rate profile for the metal complex (pH 7–9, corresponding to dissociation of a proton from the coordinated water). This is presumably due to the much greater nucleophilic character of an alkoxide anion compared with coordinated hydroxide (assuming a direct relationship between nucleophilic strength and pK_a for these reactions). Sufficient data are available for the systems under discussion to enable a quantitative test of this proposal. The values to be compared are the logarithm of the ratio of the plateau rate constants for the nitrophenol analogue of **9** and the metal complex and the difference between their respective pK_a values. Unfortunately the leaving group in **9** is phenol whereas for the metal complex it is nitrophenol. However, the second-order rate constants for the base hydrolysis of **9** and the analogous methyl ester are known. If the log k values are plotted against their corresponding pK_a values, a line of slope −0.59 and intercept 6.61 results. There is good evidence for presuming that a linear relationship applies over the whole range of pK_a values for potential alcohol leaving groups, even through it is defined in this case by only two points. For **2** there is a linear correlation (slope = −0.56) for six different alcohols,[9] and the similarity to the mechanism of hydrolysis of **9** suggests that the latter should display analogous behavior. In fact, both systems should exhibit a similar sensitivity to the nature of the leaving group—the close agreement between the two slopes provides support for this assertion.

The approximate value for the rate of hydrolysis of the nitrophenyl analogue of **9** can thus be calculated from the relation log $k = -0.59\ pK_a + 6.61$, yielding a $k_{nitrophenol}/k_{phenol}$ ratio of 42. The plateau rate constant for nitrophenol is equal to the product of the plateau rate constant for **9** with R = C_6H_5 (5.5 s^{-1})[32] and $k_{nitrophenol}/k_{phenol}$. Therefore the rate of reaction of the nitrophenol analogue of **9** is predicted to be 210 s^{-1} if it is assumed that

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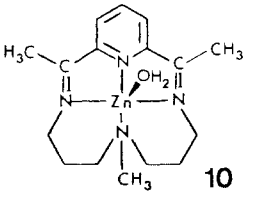
replacement of phenol by nitrophenol in **9** has little effect on the pK_a of the hydroxyl group. The results of the above analysis are that the logarithm of the ratio of the rate constant for **9** to that for the metal complex is 5.5 and the difference between the corresponding acid dissociation constants is 6.2. This implies that the difference in basicity of the nucleophiles, Co–OH and C–O^-, accounts almost entirely for the plateau rate differences between the intramolecular pathways for the two systems.

It is clear, however, from the pH–rate profiles for *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ and both **2** and **9** (see Figure 4) that coordinated hydroxide is a much more effective intramolecular nucleophile than is an alcohol; at pH 9 the metal complex reacts much faster than either of the phosphate diesters. In both the organic and metal complex systems, the close proximity of the appropriate reactive groups facilitate efficient intramolecular hydrolysis of the phosphate esters despite the fact that the metal complex involves forming a somewhat strained four-membered ring. However, the unique attribute of the metal complex is its ability to generate a high concentration of the reactive nucleophile at a much lower pH than would otherwise be possible. The pH for which the rate of the intrmolecular process reaches a maximum is thereby shifted into the physiological range—a result of potential significance to the mechanism of action of the enzyme *E. coli* alkaline phosphatase.

Alkaline phosphatase is a Zn(II) metalloenzyme that catalyzes the nonspecific hydrolysis of phosphate monoesters.[30] The enzyme activity is dependent on pH, with maximum rate in the pH range 8.2–11.0.[31] The native enzyme is dimeric and consists of two identical monomer subunits; there are normally four Zn(II) ions and one or two Mg(II) ions associated with each dimer.[32] Two of the Zn(II) ions are essential for catalytic activity,[32] and the X-ray structure of the enzyme places them in the vicinity of clefts that would allow a substrate molecule to come within 3 Å of the metal ion.[33] Recent ^{113}Cd NMR experiments with the ^{113}Cd-substituted enzyme have shown unequivocally that phosphate does bind to the metal ion in the active site.[34]

The catalysis of phosphate ester hydrolysis by the enzyme occurs in two steps:[2,30] (i) a phosphorylated enzyme intermediate is generated by attack of the hydroxyl moiety of serine-99 at the phosphorus of the bound substrate (the ester is concomitantly hydrolyzed) and (ii) the phosphorylated enzyme is hydrolyzed and free enzyme is regenerated. Both substrate binding and dephosphorylation of the enzyme intermediate have a requirement for Zn(II). The pH–activity profile of alkaline phosphatase reflects the pH dependence of the dephosphorylation reaction[2,30,32] and the sigmoidal increase in rate between pH 7 and 9 has been attributed to the dissociation of an acidic group in the enzyme with $pK_a = 7.4$.[31] It has been suggested previously that the acidic group is a water molecule that is coordinated to Zn(II) ($Zn–OH_2 \rightleftarrows Zn–OH + H^+$) in the active site.[2] At least two questions must be considered before this proposal can be accepted as chemically feasible: (i) can the pK_a of a water molecule that is coordinated to Zn(II) be as low as 7.4, and (ii) is coordinated hydroxide a good nucleophile for phosphate ester hydrolysis?

Most simple complexes of Zn(II) that contain a coordinated water molecule have pK_a values in the range 9–10.[35] However, five-coordinate complexes are known that appear to have substantially lower values. For example, the distorted square-pyramidal complex **10** is reported to have a pK_a of 8.12 in aqueous

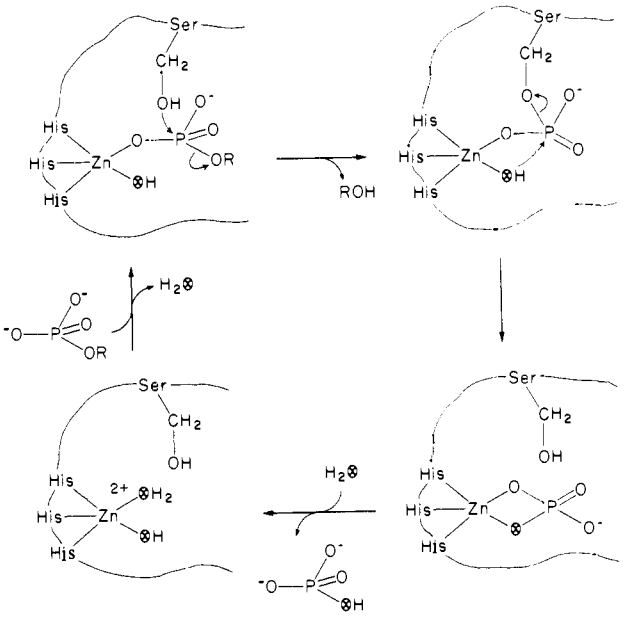


10

solution.[36] This value is only slightly higher than the kinetic pK_a of 7.4 observed for the dephosphorylation step of phosphorylated Zn(II)–alkaline phosphatase so it is feasible that imidazole residues bound to Zn^{2+} could achieve the appropriate pK_a for coordinated OH_2. Thus, a distorted five-coordinate environment for Zn(II) in the enzyme could account for the observed pK_a.[32,37]

The coordinated hydroxide ion in the deprotonated form of *cis*-$[Co(en)_2(OH_2)O_3POC_6H_4NO_2]^+$ is a very effective intramolecular nucleophile for phosphate ester hydrolysis even though coordination to the metal ion lowers the pK_a of the water molecule from 14 to 7.6. Thus hydroxide ion coordinated to Zn(II) could also be an efficient intramolecular nucleophile for the hydrolysis of a phosphate ester which is bound in an adjacent (cis) coordination site. The Zn–OH mechanism shown in Scheme III could therefore account for the observed features of the dephosphorylation step of *E. coli* alkaline phosphatase.[38] Mechanisms in which a Zn-bound hydroxide nucleophile plays an effective role have been advanced previously,[2] but until now their feasibility has not been tested in a well-defined model system. The proposal would also be consistent with the overall retention observed by Knowles et al.,[39] presumably arising from consecutive inversion paths.

Scheme III



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(36) Woolley, P. *Nature (London)* **1975**, *258*, 677–682.
(37) EPR and spectrophotometric data for the catalytically active Co(II)-substituted enzyme provide evidence that the metal ion is in a distorted tetrahedral of five-coordinate environment: Anderson, R. A.; Bosron, W. F.; Kennedy, F. S.; Vallee, B. L. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 2989–2993. Applebury, M. L.; Coleman, J. E. *J. Biol. Chem.* **1969**, *244*, 709–718.
(38) One referee pointed out that, while the results of the ^{113}Cd NMR studies in ref 34 provide unequivocal evidence for phosphate binding to the metal in the noncovalent phosphoenzyme, they do not provide any evidence for coordination of the phosphoserine intermediate. Thus, the possibility must also be entertained that the proximity and entropic advantages conveyed by cis coordination of the nucleophile and ester in *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ could be achieved without such dual coordination in the enzyme, by the metal-bound nucleophile and the phosphorserine being held in close proximity.
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The rate constants for each of the individual steps for alkaline phosphatase have been determined by a combination of stopped flow, ^{18}O-tracer, and NMR line-broadening experiments.[2] The precision of the NMR measurements is such that the rate-limiting step of the enzyme cannot be unambiguously assigned to either the hydrolysis of the phosphoserine intermediate or to the dissociation of the resultant enzyme–product adduct. Accordingly, a lower limit of $\sim 100\ s^{-1}$ has been selected for the overall rate constant of the enzymic hydrolysis, and this will be used for comparison with the nonenzymic systems. The rate of hydrolysis of 4-nitrophenyl phosphate at pH 9 ($k = 2 \times 10^{-9}\ s^{-1}$ at 25 °C)[40] leads to a calculated rate enhancement of 10^{11} for the enzyme. This can be compared with the rate enhancement of 10^5 observed for ester hydrolysis in *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$ ($k = 6.5 \times 10^{-4}\ s^{-1}$ at pH 9, 25 °C), assuming that the assignment of an intramolecular pathway to the dephosphorylation step in the enzyme is correct. Thus, the intramolecular (proximity) component could contribute 10^5 toward the 10^{11}-fold enhancement observed for the enzyme. Additional contributions might arise from strain induced in the substrate by the enzyme[41] or an intramolecular acid that protonates the series alkoxide group, thereby assisting it to leave. Factors of up to 10^6 in enhancement of the rate would not be unreasonable from the latter source.[28]

The kinetic paths effective in the higher pH regions (k_2 and k_3) for the hydrolysis of *cis*-$[Co(en)_2(OH)O_3POC_6H_5NO_2]$ lead to a sharp change in the product distribution (Table I). Cleavage of nitrophenyl phosphate from the metal center becomes paramount, and in 1 M OH^- the amounts of nitrophenol and ester are almost reversed in magnitude relative to their yields for pH 7–11. However, no ^{18}O appears in either the nitrophenol or the ester, and the amount of *trans*-$[Co(en)_2(OH)(O_3POC_6H_5NO_2)]$ remains constant.

The implication in all these results is that conjugate base reactions characteristic of cobalt(III)–amine complexes[42] are now dominating the kinetics, leading to metal–oxygen cleavage and substantial loss of intact nitrophenyl phosphate. Both the first and second order paths in $[OH^-]$ are required to give large amounts of Co–O (ester) cleavage, 95% and 82%, respectively. At the same time, these paths are required to produce small amounts of nitrophenol (5% and 18%, respectively). The product distribution and observed rate constants appear to require this bifurcation in the pathways. The calculated and observed nitrophenol production assuming such routes is given in Table I.

The high base mechanisms are formulated in Scheme II. For the path first order in $[OH^-]$, 95% is proposed to occur by the S_N1CB mechanism involving deprotonation of an ethylenediamine amine site leading to loss of the phosphate ester by a unimolecular dissociation. A small pathway (5%) is required to produce some nitrophenol, and this is argued to occur from the same deprotonated intermediate via a five-coordinate phosphorane.

The path second order in hydroxide, $k_2[OH^-]^2$, can be argued to occur via deprotonation of an ethylenediamine amine group and the hydroxo group. Both deprotonations would assist the labilization of the phosphate ester, and the deprotonation of the hydroxo group would probably make attack of the O^- on the phosphorus atom concerted with removal of the nitrophenoxide ion. Given the overall negative charge on the complex and on the phosphorus moiety at this point, nitrophenoxide ion should be able to leave the P center readily.

Other proposals can be advanced. The fraction of the $k_1[OH^-]$ path for production of nitrophenoxide, for example, could arise from deprotonation of the phosphorane derived directly from **4**. Also, it could come from deprotonation of the hydroxo group on **4**. Similarly, the nitrophenoxide path associated with the term $k_2[OH^-]^2$ could come from deprotonation of the phosphorane arising from cyclization in *cis*-$[(en)(en\text{-}H)Co(OH)O_3POC_6H_5NO_2]^-$. At present we have no way to distinguish between these proposals.

Acknowledgment. We are indebted to Dr. K. Barrow and G. Grossman of the Biochemistry Department, University of New South Wales, as well as to Dr. D. Fenn and M. Whittaker of the Australian National University for assistance in obtaining the ^{31}P NMR spectra. We acknowledge Dr. J. MacB. Harrowfield for some preliminary observations on this system and P. Hendry for assitance with calculations.

Registry No. *cis*-$[Co(en)_2(OH_2)O_3POC_6H_4NO_2]ClO_4$, 87183-71-9; *cis*-$[Co(en)_2(^{18}OH_2)O_3POC_6H_4NO_2]C_7H_7SO_3$, 87183-73-1; $Co(en)_2PO_4$, 19169-67-6; *cis*-$[Co(en)_2(OH)O_3POC_6H_4NO_2]$, 87183-66-2; $[Co(NH_3)_5{}^{17}OH_2]^{3+}$, 75522-70-2; $[Co(NH_3)_5{}^{17}OH]^{2+}$, 87183-67-3; $[Co(en)_2(^{17}OH_2)PO_4H]^+$, 87183-68-4; $[Co(en)_2(^{17}OH_2)O_3POC_6H_4NO_2]^+$, 87183-69-5; $(Na^{17}O)_2P(O)OC_6H_4NO_2$, 87174-81-0; $(Na^{17}O)_6PO$, 87174-82-1; 2-hydroxypropyl 4-nitrophenyl phosphate, 87174-80-9.

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(41) Distortion of the substrate could lead to an analogous enhancement in the rate of hydrolysis to that observed for the hydrolysis of phosphate triesters containing a strained five-membered ring. ^{31}P NMR evidence suggests that the geometry of the phosphate ester substrate is distorted when it binds to the enzyme. Chlebowski, J. F.; Armitage, I. M.; Tusa, P. P.; Coleman, J. E. *J. Biol. Chem.* **1976**, *251*, 1207–1216.

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