The Mechanism of Hydrolysis of a Cobalt(III)-Bound Phosphate Ester: Transphosphorylation from Oxygen to Nitrogen

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Abstract: The (*p*-nitrophenylphosphato)pentaamminecobalt(III) cation (CoNPP) undergoes base hydrolysis to generate *p*-nitrophenolate ion (NP), *p*-nitrophenylphosphate ion (NPP), and hydroxo(phosphoramido)tetraamminecobalt(III) with a rate law of the form $-d(CoNPP)/dt = k[CoNPP][OH^-]$ (μ = 1.0 M, $NaClO_4$; 25 °C) where $k = 8.1 \times 10^{-4}$ M^{-1} s^{-1} (k is a composite rate constant for *p*-nitrophenol and *p*-nitrophenylphosphate production divided approximately equally between the two pathways) in the hydroxide range 0.05–1.0 M. The ester hydrolysis is accelerated at least 10^8-fold relative to uncoordinated *p*-nitrophenylphosphate in the presence of OH^- (1 M) and NH_3 (1 M). Product distribution, ^{18}O tracer, and ^{31}P NMR studies imply the participation of a five-coordinate aminophosphorane, generated by intramolecular attack of a deprotonated, coordinated ammonia at the phosphorus center. Both hydroxo(phosphoramido)tetraamminecobalt(III) and *p*-nitrophenolate are produced by this route. A competing conventional base-catalyzed S_N1cB mechanism for Co–O bond rupture accounts for the released *p*-nitrophenylphosphate. The intermediate hydroxo(phosphoramido)tetraammine complex is hydrolyzed slowly by base to liberate phosphoramidate anion by the same mechanism. The ester hydrolysis represents a rapid phosphoryl group transfer from oxygen to nitrogen, and it may model some aspects of amino transferase chemistry.

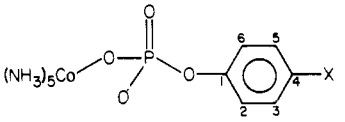
Introduction

Organic phosphate compounds are the major energy storage and transducing species in living organisms and most of the enzymes which are responsible for their metabolism require metal ions (Mg^{2+}, Mn^{2+}, Ca^{2+}, Zn^{2+}, etc.) for activity.[2] There has been much speculation as to the precise role of these ions in the mechanism of enzymic phosphate ester hydrolysis[3-6] since the rates of the enzyme-catalyzed reactions are many orders of magnitude faster than their nonenzymic counterparts. Proposals based on polarization of electron density, induction of steric strain, and the presence of metal-bound nucleophiles have been advanced to account for the rate increase. Implicit in these arguments is the assumption that the ester is coordinated to the metal ion in the active site of such metal ion-dependent phosphatase enzymes. An X-ray crystallographic study of the enzyme *E. coli* alkaline phosphatase (the most studied of the phosphate ester hydrolyzing enzymes) (7.7-Å resolution) places the Zn^{2+} ion in the vicinity of a cleft which would allow a substrate molecule to come within 3 Å of the Zn^{2+} ion.[7]

NMR relaxation measurements on the Mn(II) substituted enzyme have implied that phosphate is bound within the first or second coordination sphere of the metal ion. A tentative value of 8 Å was assigned to the metal ion–phosphorus distance in the Mn(II) enzyme–*p*-aminobenzylphosphonate adduct. Recently however, unequivocal NMR evidence for direct inner-sphere interaction of phosphate with a Cd(II) ion in the active site was obtained through the observation of ^{31}P–^{119}Cd(II) NMR coupling.[8,9]

Several inorganic model systems have been investigated previously in attempts to elucidate the dominant factors in metal ion catalysis of phosphate ester hydrolysis.[10] In these studies only modest rate enhancements were observed ($\sim 10^2$) and detailed interpretation of the mechanisms was hampered by uncertainties concerning the mode of binding of the metal ion to the phosphate ester and the strength of such binding. However, *p*-nitrophenylphosphate ion bound to the bis(propane-1,3-diamine)cobalt(III) moiety has shown enhanced reactivity[11] (10^9-fold) in the presence of OH^-. The enhancement was attributed to attack of OH^- at the P center and to strain in the four-membered chelate ring ester being relieved in the activated complex.

The efficacy of intramolecular nucleophiles in such reactions also needs to be evaluated, and in this paper we report the synthesis and mechanism of base hydrolysis of a well-defined and robust pentaamminecobalt(III) complex of *p*-nitrophenylphosphate (**1**, X = NO_2). Here there is a possibility for a coordinated amido



1, X = H, NO_2

ion to act as a intramolecular nucleophile at the P center. Co(III) ammine complexes of this type are slow to dissociate their ligands under the conditions used and such intramolecular reactions can be carried out uncomplicated by the ligand dissociation phenomena which make detailed interpretation of the mechanisms for more labile metal ion complexes rather difficult.

Experimental Section

Analytical grade reagents were used throughout except where otherwise specified. Sodium hydroxide solutions were freshly prepared from May and Baker "VOLUCON" concentrate by using CO_2-free water. Triflic acid (CF_3SO_3H) was obtained from the 3M Company and ^{18}O-enriched water (1.5 and 3 atom %) from BIORAD.

^1H NMR spectra were recorded by using a JEOL JNM-MH-100 (MINIMAR) spectrometer. All shifts are quoted as ppm downfield relative to NaTPS [sodium (3-(trimethylsilyl)propane)sulfonate] in aqueous solution or Me_4Si (tetramethylsilane) in nonaqueous media.

Routine ^{31}P NMR spectra were recorded by using a Brüker B-KR spectrometer operating at 24.281 MHz with external D_2O as a field frequency lock. For the kinetic studies JEOL JNM-FX-100 (^{31}P probe)

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or JEOL JNM-FX-90 (multi probe) 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 (±0.1 ppm) are reported relative to external 85% H_3PO_4. IUPAC nomenclature has been accepted for the signs of the chemical shifts: increasing values toward low field. $^{13}C\{H\}$ NMR spectra were recorded by using a JEOL FX-60Q spectrometer with chemical shift values in ppm quoted relative to 1,4-dioxane (internal reference).

Electronic spectra were recorded by using a Cary 118C spectrophotometer, and rate data were collected with a Cary 16K spectrophotometer. Extinction coefficients are quoted in units of M^{-1} cm^{-1}.

All evaporations were carried out by using a Büchi rotary evaporator at ~15mm saturated water vapor pressure so that the temperature of the solution did not exceed 25 °C.

$[Co(NH_3)_5O_3POC_6H_5]Cl$. To a vigorously stirred solution (pH adjusted to 4.0 with $HClO_4$) of disodium phenylphosphate[12] (27.6 g) in water (150 mL) at 80 °C was added $[Co(NH_3)_5OH_2](ClO_4)_3$ (30 g) in small portions. Stirring was continued for a further hour at 60 °C. The reaction mixture was filtered, diluted to a total volume of 6 L, and sorbed on a column (12 × 9 cm) of Dowex 50W-X2 (Na^+ form) cation-exchange resin. Elution with 0.8 M NaCl yielded 3 red bands. The first band, which contained the product, was collected and concentrated by evaporation until NaCl began to crystallize. Careful addition of ethanol in portions, followed by removal of the precipitated NaCl, yielded finally $[Co(NH_3)_5O_3POC_6H_5]Cl$ as red-orange crystals (10 g). These were recrystallized from a minimum volume of hot water (70 °C) to which was added six volumes of boiling absolute ethanol and one volume of a saturated ethanol solution of LiCl. After the solution was cooled for ~2 h in an ice bath, the product was collected, washed with methanol and ether, and finally dried in vacuo over P_2O_5 for 18 h. Anal. Calcd for $[Co(NH_3)_5O_3POC_6H_5]Cl$: C, 20.50; H, 5.73; N, 19.92; P, 8.81; Co, 16.76. Found: C, 20.7; H, 5.7; N, 20.2; P, 8.7; Co, 16.5. The ^1H NMR spectrum of a saturated solution of the complex in D_2O containing 1 drop of 11 M DCl gave the following chemical shifts (relative peak areas in parentheses): NH_3, δ 3.92 (12); NH_3, δ 2.72 (3); aromatic H, complex multiplet centered at δ 7.31 (5). The ^{13}C NMR assignments for the complex (**1**, X = H) are as follows C_1, 85.91 (d, $^2J_{POC}$ = 5.86 Hz); $C_{2,6}$, 53.21 (d, $^3J_{POCC}$ = 4.40 Hz); $C_{3,5}$, 63.11 (s); C_4, 56.72 ppm s. ^{31}P NMR data: 7.5 ppm (s, pH 5). Visible spectrum: ϵ_{518}^{max} = 77.3, ϵ_{356}^{max} = 61.1, H_2O.

The complex was treated with 1 M NaOH at 25 °C to yield cobalt oxide, $[(NH_3)_5CoOH]^{2+}$, and phenyl phosphate ion. There was no evidence for hydrolysis of the ester in the course of this reaction.

$[Co(NH_3)_5O_2P(OH)OC_6H_4NO_2]X_2$ (X = Cl^-, Br^-, $CF_3SO_3^-$). $[Co(NH_3)_5O_3POC_6H_5]Cl$ (4 g) was dissolved, with rapid stirring, in CF_3SO_3H (30 mL) at 0 °C in a stoppered 50-mL conical flask. Fuming nitric acid (1 mL) was added and the reaction mixture allowed to stir for a further 6 min. The resulting orange-red solution was poured slowly (*Caution!!*) into stirred anhydrous ether (700 mL) to quench the reaction. The pink solid which precipitated almost immediately was collected and redissolved in a minimum volume of anhydrous acetone. The acetone-insoluble impurities (mainly NaCl) were removed, and slow addition of anhydrous ether to the filtrate yielded the product as a pale pink microcrystalline triflate salt (5.3 g, 95%). This material was recrystallized by dissolution in a minimum volume of hot water to which was added triflic acid (1 mL of 3 M) and solid sodium triflate until crystallization commenced. After the solution was cooled in an ice bath for 2 h, the red-orange crystalline product was filtered off and washed with ice-cold 3 M triflic acid and then anhydrous ether (50 mL). The crystals were dried for 18 h in vacuo over P_2O_5. Anal. Calcd for $[Co(NH_3)_5O_2P(OH)OC_6H_4NO_2](CF_3SO_3)_2$: C, 14.55; H, 3.05; N, 12.75; P, 4.69; F, 17.27; Co, 8.93. Found: C, 14.8; H, 3.1; N, 12.6; P, 4.9; F, 17.3; Co, 9.0. The crude product from the above procedure was also converted to the corresponding chloride and bromide salts. For all these salts ϵ_{517}^{max} = 78.5 (H_2O, pH 3.5). The ^1H NMR spectrum of a saturated solution of $[Co(NH_3)_5OPO(OH)OC_6H_4NO_2](CF_3SO_3)_2$ in D_2O containing 1 drop of DCl gave the following chemical shifts (relative peak areas in parentheses): NH_3, 4.11 (12); NH_3, 2.92 (3), aromatic H, 7.36 (2) doublet; aromatic H, 8.31 (2) doublet. ^{31}P NMR data: 4.3 ppm (s, pH 2); 6.7 ppm (s, pH >13). The ^{13}C NMR assignments for (**1**; X = NO_2) are as follows C_1, 92.10 (d, $^2J_{POC}$ = 5.86 Hz); $C_{2,6}$, 53.10 (d, $^3J_{POCC}$ = 5.13 Hz); $C_{3,5}$, 59.26 (s); C_4, 75.82 ppm (s).

$NaOP(O)(NH_2)OC_6H_4NO_2 \cdot 2H_2O$.[13] Sodium *p*-nitrophenylphosphate hexahydrate (1.1 g) was converted to the acid form by using Dowex 50W-X 2 (H^+ form) cation-exchange resin (4 × 2 cm). The effluent and washings were combined and evaporated under vacuum at ~15 °C to near dryness. Formamide (5 mL), *tert*-butyl alcohol (18 mL), dicyclohexylcarbodiimide (3 g), and aqueous ammonia (7.5 mL of 2 M) were added, and the two-phase system was transferred to a stoppered flask. The stirred reaction mixture was heated at 80 °C for 7 h during which time it became homogeneous and dicyclohexylurea crystallized out. This was removed by filtration and washed three times with 5-mL aliquots of water. The filtrate and washings were combined, and the *tert*-butyl alcohol was removed by evaporation. Residual dicyclohexylcarbodiimide was extracted with ether, and the aqueous formamide mixture containing the dicyclohexylguanidinium salt of the desired product was passed through Dowex 50W-X2 (Na^+ form) resin (4 × 2 cm) to convert the amidate to its sodium salt. The volume of this solution was reduced to ~4 mL, and slow addition of anhydrous acetone to the stirred mixture yielded a white, flocculent precipitate. This was filtered off, washed with acetone and ether, and finally dried in vacuo over P_2O_5 (yield 0.4 g). The crude product was recrystallized by suspending it in boiling acetone and adding hot water (50 °C) dropwise until all of the solid dissolved. When the mixture was cooled to room temperature, a microcrystalline product was obtained. After a further 2 h at 0 °C in the dark, the product was isolated as described above. It was dried for 18 h in vacuo over P_2O_5. (Immediately after isolation the crystals are white, but after a few days at room temperature, they develop a yellow discoloration. They are best stored at 0 °C over silica gel.) ^{31}P NMR spectroscopy and chromatography on DEAE Sephadex (Cl^- form) confirmed the purity of the product. Anal. Calcd for $NaOP(O)(NH_2)OC_6H_4NO_2 \cdot 2H_2O$: C, 26.10; H, 3.65; N, 10.14. Found: C, 25.9; H, 3.3; N, 10.1. ϵ_{294}^{max} = 9760; λ_{max_2} = 218 nm (H_2O). ^{31}P NMR: 5.66 ppm (s, pH ≥13 (H_2O solvent)). Base hydrolysis (1 M NaOH) of this compound yielded phosphoramidate ion (as the sole phosphorus-containing species) and *p*-nitrophenolate ion, confirming its constitution.[14]

$[Co(NH_3)_5PO_4] \cdot 3H_2O$. (Phosphato)pentaamminecobalt(III) was prepared as the trihydrate by a previously described method.[15] Anal. Calcd for $[Co(NH_3)_5PO_4] \cdot 3H_2O$: H, 7.22; N, 23.90; P, 10.57. Found: H, 7.2; N, 24.0; P, 10.8. ϵ_{524}^{max} = 90.7, H_2O at pH 8. (ϵ_{525}^{max} = 90.0.[15])

$[Co(NH_3)_4PO_4]$. (Phosphato)tetraamminecobalt(III) was synthesized by a published procedure.[16] Anal. Calcd for $[Co(NH_3)_4PO_4]$: H, 5.40; N, 25.20. Found: H, 5.3; N, 24.9.

$[Co(NH_3)_4(OH_2)PO_4H]ClO_4 \cdot 2H_2O$. Aquo(phosphato)tetraamminecobalt(III) perchlorate was prepared by a previously described method.[16] Anal. Calcd for $[Co(NH_3)_4(OH_2)PO_4H]ClO_4 \cdot 2H_2O$: H, 5.09; N, 14.88; P, 8.23; Co, 15.64. Found: H, 5.0; N, 14.9; P, 8.2; Co, 15.2.

Isolation of $[Co(NH_3)_4(OH_2)O_2P(OH)(NH_2)]Cl_2$. $[Co(NH_3)_5O_2P(OH)OC_6H_4NO_2]Cl_2$ (1 g) was dissolved in water (10 mL) at 25 °C. Aqueous sodium hydroxide (5 mL of 3 M) was added rapidly, with stirring, and the reaction was allowed to proceed for 28 min ($\sim 2t_{1/2}$), before quenching to pH 9 with 3 M HCl. The insoluble cobalt oxide which precipitated was removed by filtration, and the filtrate was diluted with water to 300 mL. Concentrated aqueous ammonia (0.2 mL) was added to keep the pH near 10 and the solution passed successively through columns of Sephadex SP C25 (Li^+ form, 9 × 6 cm) cation-exchange and Dowex Ag1-X 8 (Cl^- form, 200–400 mesh, 5 × 2 cm) anion-exchange resins. The final pale orange eluant was evaporated to ~3 mL, at which point a pink solid began to precipitate (solution pH 6). Methanol (6 mL) was added dropwise, with stirring, to precipitate the remainder of the material. The pink powder was filtered off, washed with ethanol (3 mL) and ether (10 mL), and finally dried in vacuo over P_2O_5 for 48 h. The complex was hygroscopic. Satisfactory elemental analyses for this compound could not be obtained. The element ratios were correct except for a persistently high P content which was attributed to contamination by a small amount of inorganic phosphate, presumably arising from slight decomposition of the complex during the evaporation procedure. The complex was characterized therefore by ^{31}P NMR spectroscopy: 6.9 ppm (s, (D_2O, 0.1 M NaOD)). Hydrolysis of the complex in 4 M $HClO_4$ yielded an orange-red solution of $[Co(NH_3)_5OH_2]^{3+}$ (identified by UV–vis spectroscopy). Hydrolysis in warm HBr gave violet crystals of slightly soluble $[Co(NH_3)_5Br]Br_2$. These chemical tests, together with the compound's neutrality at pH 10, and the ^{31}P NMR data provide strong evidence for the phosphoramidate being present as an N-bonded monodentate ligand. Anal. Calcd for $H_{17}N_5O_4PCoCl_2$: H, 5.49; N, 22.45; P, 9.93; Co, 18.89, Cl, 22.73. Found: H, 4.8; N, 19.9;

(12) Freeman, H. F.; Colver, C. W. *J. Am. Chem. Soc.* **1938**, *60*, 750.

(13) The procedure described is a modification of a previously published synthesis for dicyclohexylguanidinium phenylphosphoramidate. Chambers, R. W.; Moffat, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1957**, *79*, 4240–4241.

(14) Saponification of sodium diphenylphosphoramidate in concentrated alkali yields phosphoramidate as the sole phosphorus-containing product. Fluck, E. "Topics in Phosphorus Chemistry"; Grayson, M., Griffith, E. J., Eds.; Interscience: New York 1967; Vol. 4, pp 334. By analogy, sodium *p*-nitrophenylphosphoramidate would be expected to react in a similar fashion.

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(16) Von Siebert, H. *Z. Anorg. Allg. Chem.* **1958**, *296*, 280–286.

P, 9.9; Co, 16.8; Cl, 20.3. Ratio: H:N:P:Co:Cl = 17:5:1:1:2.

Kinetics. The release of *p*-nitrophenol from $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ was followed spectrophotometrically at 400 nm (λ_{max} nitrophenolate anion, $\epsilon = 18700\ M^{-1}\ cm^{-1}$)[11] in perchlorate media at constant ionic strength ($\mu = 1$ M) over the hydroxide concentration range 0.05–1.0 M at 25 ± 0.05 °C. Typically a stock solution of complex was prepared by dissolving $[Co(NH_3)_5O_2P(OH)OC_6H_4NO_2](CF_3SO_3)_2$ (17 mg) in 100 mL of CO_2–free water.

A problem associated with the spectrophotometric study of the base hydrolysis of this complex was that insoluble Co(III) oxide was one of the ultimate products of the reaction. In this work appreciable interference from oxide precipitation occurred after $3t_{1/2}$ and the absorbance data were unreliable after this time. The Guggenheim procedure was therefore employed to calculate the pseudo-first-order rate constants; all correlation coefficients for the computer fits of the data were greater than 0.998. The validity of this method of data analysis was demonstrated by allowing the reaction at a single hydroxide concentration to proceed to $\sim 20t_{1/2}$, filtering out the cobalt oxide, and measuring the absorbance of the filtrate. This provided the infinity value for nitrophenol production. The plot of log $(A_\infty - A_t)$ against time was linear until $3t_{1/2}$ (at which point cobalt oxide interference became appreciable) and yielded a rate constant which was identical with that calculated by the Guggenheim procedure.

Anion-Exchange Paper Chromatography. Descending chromatography on Amberlite SB-2 paper was carried out on the $20t_{1/2}$ hydrolysis products ($[OH^-] = 1$ M) of $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ by using sodium orthophosphate, sodium phosphoramidate, and sodium *p*-nitrophenylphosphate as reference standards. The standards and reaction mixture were applied to a 9 × 30 cm strip of the ion-exchange paper. Aqueous sodium sulfate (0.025 M) solution, adjusted to pH 10 with ammonia solution, was used as the eluant. The three anions were well separated on allowing the solvent front to migrate to the end of the strip. The chromatogram was dried in a current of warm air and developed by the Hanes and Isherwood procedure.[17]

Quantitative Product Distribution. $[Co(NH_3)_5O_2P(OH)OC_6H_4NO_2]Cl_2$ (0.4 g) was dissolved in 5 mL of water, the pH of the solution was adjusted to ~6 with 3 M NaOH, and then the solution was diluted to 10 mL. After the solution was equilibrated for 10 min at 25 °C, the hydrolysis reaction was initiated by rapid addition of 3 M NaOH (5 mL). When the desired number of half-lives had elpased, the reaction mixture was quenched to ~pH 9 by the addition of 1 M HCl. It was then quickly filtered to remove the precipitated cobalt oxide and diluted to 1.1 L with ice-cold water. This solution was loaded under pressure on to Na^+ SP Sephadex C 25 resin (9.5 × 3.8 cm uncompressed length) to give a flow rate of ~35 mL/min. After absorption of the complex, the column was washed with water (100 mL) at pH 9 and the initial effluent, rinse effluent, and water wash were combined. The column was washed once more with water (300 mL) at pH 3.5. Elution with 0.2 M and then 0.4 M $NaClO_4$ was carried out to yield a red-orange species followed by an orange species—$[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ and $[Co(NH_3)_5OH_2]^{3+}$, respectively. Both fractions were collected for analysis. The initial effluent plus water wash from above was divided into two portions. Half was retained and the other was sorbed on Dowex AG1-X8 resin (Cl^- form, 4 × 3 cm) at pH 9 to remove the free nitrophenolate and phosphate-containing anions. The resin was washed with water (50 mL), and the washings and initial effluent were combined.

All eluates and the cobalt oxide[18] (dissolved in concentrated HCl) were analyzed for nitrophenylphosphate by heating aliquots with 1 M HCl for 4 h at 100 °C, adding excess NaOH, and then recording the absorbance due to nitrophenolate. The initial effluent (plus water wash) from the Sephadex C-25 cation-exchange chromatography was analyzed for nitrophenylphosphate and nitrophenol by measuring the nitrophenol absorbance on a basified aliquot before and after acid hydrolysis. *p*-Nitroaniline (generated by attack of a deprotonated ammonia at the aromatic carbon) was a possible product. In basic conditions the UV–vis and ^1H NMR spectra of an authentic sample of the aniline are very similar to those for the nitrophenolate anion. However, at pH 4, nitrophenol is protonated and colorless while the much more acidic nitroaniline is still yellow ($\lambda_{max} \approx 400$ nm). Aliquots of the nitrophenol-containing solution were therefore also assayed spectrophotometrically at pH 4 (400 nm). No nitroaniline was detected in the product distribution experiments. All fractions were also analyzed for cobalt by atomic absorption spectroscopy and phosphate by the King modification of the Fiske and Subbarrow procedure.[19]

Measurements of the $10t_{1/2}$ ratio of nitrophenylphosphate to nitrophenol were made under the same conditions of ionic strength and complex concentration as the rate constant determinations. Equal volumes of complex and base solution ($\mu = 2$ M, $NaClO_4$) were mixed, and the reaction was allowed to proceed to $10t_{1/2}$. The absorbance due to nitrophenol was measured after removal of the precipitated cobalt oxide by centrifugation. Acid hydrolysis (as above) of a known volume of this mixture provided the value for total nitrophenol (nitrophenol plus nitrophenylphosphate).

Anion-Exchange Column Chromatography. $[Co(NH_3)_5O_2P(OH)OC_6H_4NO_2]Cl_2$ (0.03 g) was dissolved in 1 M NaOH (2 mL) and the hydrolysis allowed to proceed for 17 min at 25 °C. The reaction mixture was then quenched to pH 9 with HCl and diluted to 200 mL with NH_3/NH_4Cl buffer (prepared by diluting 5 mL of 1 M HCl and 5 mL of 2 M NH_3 solution to 200 mL). The diluted reaction mixture was passed through Sephadex SP C-25 (Na^+ form, 4 × 3 cm) to sorb the cationic species, and 60 mL of the effluent was loaded on to DEAE Sephadex (Cl^- form, 12.5 × 2.5 cm) resin at pH 9. The absorbance of the effluent from this column was monitored spectrophotometrically at 310 nm by using a flow-through cell. Elution with 0.05 M NaCl (200 mL at pH 9) followed by 0.1 M NaCl yielded only two bands: the first corresponding to *p*-nitrophenylphosphate and the second to *p*-nitrophenol. A previous experiment with authentic samples of *p*-nitrophenolate, *p*-nitrophenylphosphate, and *p*-nitrophenylphosphoramidate had demonstrated that the three anions could be separated by this procedure. The order of elution was *p*-nitrophenylphosphoramidate, *p*-nitrophenylphosphate, and *p*-nitrophenolate. Similar experiments to that described above were performed for samples of complex allowed to react for $5t_{1/2}$ and $10t_{1/2}$, respectively.

^{18}O Tracer Experiment. $[Co(NH_3)_5O_2P(OH)OC_6H_4NO_2](CF_3SO_3)_2$ (1.0 g) was dissolved in H_2O (10 mL) containing 3 atom % $H_2{}^{18}O$ at 25 °C. The hydrolysis was initiated by rapid addition of ~3 M NaOH (5 mL), prepared by dissolving NaOH pellets in ^{18}O-enriched water. After 1, 2 or $10t_{1/2}$ had elapsed, the reaction mixture was sampled (0.5 mL) and then rapidly quenched to ~pH 9 with 1 M HNO_3. The solution was filtered to remove precipitated cobalt oxide, and the filtrate was diluted to 2.5 L with H_2O at 0 °C. The pH was readjusted to ~9 with dilute aqueous ammonia and the solution was sorbed on Sephadex SP C-25 resin (Na^+ form, 9 × 6 cm). The column was washed with 500 mL of H_2O, and the effluent and washings were combined (fraction 1). Elution with 0.2 and 0.4 M NaBr enabled the hydroxopentaammine and unreacted starting material to be separated. The latter was isolated by reducing the volume of the solution to ~10 mL and adding 6 drops of 48% HBr. Red crystals formed almost immediately. These were collected, washed with ice-cold 3 M HBr (5 mL) and methanol (10 mL), and dried in vacuo over P_2O_5 for 18 h. Anal. Calcd for $[Co(NH_3)_5O_2P(OH)OC_6H_4NO_2]Br_2$: C, 13.81; H, 3.86; N, 16.10; P, 5.93; Co, 11.29; Br, 30.30. Found: C, 13.8; H, 4.1; N, 16.1; P, 5.6; Co, 11.3; Br, 30.7. The solution containing the hydroxopentaamminecobalt(III) complex was reduced in volume to ~10 mL and chilled to 0 °C in an ice bath. Addition of 6 drops of ice-cold 48% HBr resulted in the immediate formation of red-orange crystals of $[Co(NH_3)_5OH_2]Br_3$. These were collected, washed with ice-cold 3 M HBr (3 mL) and methanol (10 mL), and dried in vacuo over P_2O_5 for 18 h. Anal. Calcd for $[Co(NH_3)_5OH_2]Br_3$: H, 4.26; N, 17.43; Co, 14.67; Br, 59.66. Found: H, 4.0; N, 17.6; Co, 14.3; Br, 59.4.

Fraction 1 was sorbed on DEAE Sephadex resin (NO_3^- form, 14.0 × 6.5 cm) and eluted initially with 0.04 M $NaNO_3$ at pH 9. Under these conditions phosphoramidate anion, *p*-nitrophenylphosphate, and *p*-nitrophenolate anion are easily separated. The phosphoramidate was detected by analyzing 1-mL aliquots of sequentially collected 100-mL samples of the effluent for phosphate.[20] The nitrophenol-containing chromophores were monitored by the flow-through cell technique mentioned above. The phosphoramidate-containing fraction was eluted first. When this fraction had been collected, the eluant was changed to 0.1 M $NaNO_3$. Nitrophenylphosphate was next eluted, followed by nitrophenolate.

(17) Hanes, C. S.; Isherwood, F. A. *Nature (London)* **1949**, 1107–1112.

(18) It is known that metal oxides adsorb phosphate. Vissers, D. R. *J. Phys. Chem.* **1968**, *72*, 3236–324. Previous reference has been made to problems which this caused in the study of the base hydrolysis of $[Co(NH_3)_5PO_4]$. Lincoln, S. F.; Jayne, J.; Hunt, J. P. *Inorg. Chem.* **1969**, *8*, 2267–2270. When the precipitated cobalt oxide in the present study was dissolved in concentrated hydrochloric acid and analyzed for phosphate, significant incorporation of this species was found. Thus phosphoramidate also binds to cobalt oxide since no phosphate is produced in the initial base hydrolysis reaction. (The phosphoramidate would have been rapidly hydrolyzed to phosphate when the oxide precipitate was dissolved in concentrated acid. See ref 20.)

(19) Lindberg, O.; Ernster, L. "Methods of Biochemical Analysis"; Glick, D., Ed.; Wiley-Interscience: New York, 1956; Vol. 3, pp 1–6.

(20) Phosphoramidate is rapidly hydrolyzed to phosphate by concentrated acid. The colorimetric phosphate analysis procedure in ref 19 can therefore be used for its estimation. Preobrazhenskaya, N. N. *Russ. Chem. Rev.* (*Engl. Transl.*) **1972**, *41*, 54.

The phosphoramidate fraction was reduced in volume to 10 mL. After the solution was cooled to 0 °C, its pH was adjusted to 6. The phosphoramidate was then precipitated as its silver salt by the addition of excess silver nitrate. The white solid was collected, washed with 5 mL of ice-cold water, and dried in vacuo (0.07mmHg) over P_2O_5 in the dark for 48 h. An identical procedure was followed to isolate the *p*-nitrophenylphosphate as its disilver salt. Anal. Calcd for $Ag_2O_3POC_6H_4NO_2$: C, 16.65; H, 0.93; N, 3.24; P, 7.16. Found: C, 16.7; H, 1.0; N, 3.2; P, 6.9. The *p*-nitrophenolate anion was the final species to elute. After the volume of the fraction was reduced to ~20 mL, it was acidified to pH 1 with HNO_3 and extracted eight times with 40-mL aliquots of chloroform.[21] The chloroform extracts were combined, dried with anhydrous sodium sulfate, and evaporated to dryness. The resulting yellow microcrystalline product was dried in vacuo over P_2O_5 for 48 h.

Samples (~50 mg) of the silver phosphoramidate, silver p-nitrophenylphosphate, and p-nitrophenol were heated with a $HgCl_2/Hg(CN)_2$[22] mixture in sealed tubes for 12 h at 400 °C. The condensable gases were then passed through a gas chromatograph, and the CO_2 was trapped in a Urey tube by cooling with liquid N_2. The solvent distilled from the reaction mixture was equilibrated with CO_2 (~2 mL) of normal enrichment at 80 °C, and the enriched CO_2 was collected as above. The $[Co(NH_3)_5OH_2]Br_3$ was processed by a previously described method.[23] A MICROMASS 602D mass spectrometer was used to measure the 46:44 mass ratio in the CO_2 samples. Control experiments with authentic specimens of ^{18}O-enriched $[Co(NH_3)_5OH_2]Br_3$ and $Na_2O_3POC_6H_4NO_2$ confirmed that negligible loss of label occurred from these compounds during the chromatographic isolation procedures.

All of the tracer experiments were performed at least twice. For the silver nitrophenylphosphate, nitrophenol, and silver phosphoramidate samples the ^{18}O contents were 0.202, 0.200, and 0.205 atom %, respectively; the natural ^{18}O abundance of CO_2 is 0.202 atom %. The reaction solution contained 3.23 atom % ^{18}O. The ^{18}O content of the $[Co(NH_3)_5OH_2]Br_3$ complex was 1.32 atom % and solvent, 1.33 atom % (errors ±0.01%).

$[Co(NH_3)_5{}^{18}OH_2]Br_3$ was prepared and analyzed mass spectrometrically as described previously.[23] A sample (0.2 g) was subjected to the same isolation procedure as $[Co(NH_3)_5OH]^{2+}$ produced in the base hydrolysis of the nitrophenylphosphate complex. The enrichment was retained to the level of 99%.

$Ag^{18}O_2P(O)OC_6H_4NO_2$. *p*-Nitrophenylphosphorodichloridate ($PO_2Cl_2C_6H_4NO_2$) was prepared as described elsewhere[24] (bp 128 °C (0.02mmHg)). The dichloridate (0.5 g) was added with stirring to 1.5 atom % $H_2{}^{18}O$ (6 mL). Stirring was continued until the solution became clear (signifying that decomposition of the dichloridate to *p*-nitrophenylphosphoric acid and hydrogen chloride was complete). It is important to ensure that the temperature of the solution does not exceed 50 °C since nitrophenylphosphate hydrolyzes under acidic conditions at elevated temperatures. Excess $BaCl_2$ was added to precipitate the product as its barium salt. The off-white solid was collected by filtration and washed with 2 × 3 mL aliquots of ice-cold H_2O, 2 mL of an ice-cold 1:1 (v/v) ethanol/ether mixture, and finally 5 mL of ether. The solid (0.75 g) was dried in vacuo over P_2O_5 for 45 h. Anal. Calcd for $BaO_2P(O)OC_6H_4NO_2{\cdot}2H_2O$: C, 18.46; H, 2.07; N, 3.59. Found: C, 18.7; H, 2.6; N, 3.4.

The barium salt (0.1 g) was suspended in 5 mL of H_2O and shaken with an excess of Dowex 50W-X2 (Na^+ form) resin until all of the solid dissolved. The resin was removed by filtration and washed with 100 mL of H_2O. The filtrate and washings were combined and diluted to 2000 mL. A chromatography procedure identical with that employed for the ^{18}O tracer studies discussed above was followed, and ultimately the nitrophenylphosphate was isolated as its disilver salt. The isotope enrichment was 30%. A sample of the silver salt was also prepared by precipitating the nitrophenylphosphate after the resin treatment to remove the Ba^{2+} ions. The ^{18}O contents observed were as follows: solvent $^{18}OH_2$, 1.59 atom %; Ag_2NPP before column elution, 0.63 atom %; Ag_2NPP after column elution, 0.61 atom % (errors ±0.01%).

Azide Competition. $[Co(NH_3)_5O_2P(OH)OC_6H_4NO_2]Cl_2$ (0.1 g) was dissolved in 4 M NaN_3 (4 mL) at 25 °C. Sodium hydroxide (4 mL of 2 M) was added with stirring and the reaction allowed to proceed for ~$1t_{1/2}$ (14 min). After being quenched to pH 5 with 1 M $HClO_4$, the solution was diluted with ice-cold water to 2000 mL. The reaction products were loaded under pressure (to produce a flow rate of 50 mL/min) onto a column (12 × 3.5 cm) of Sephadex C-25 (Na^+ form) resin. When loading was complete, the column was washed with 200 mL of cold H_2O. The 1+ charged species (residual starting material and phosphoramidate complex) were removed from the column by eluting successively with 200 mL of 0.1 M $NaClO_4$ (pH 4.5) and 200 mL of 0.2 M $NaClO_4$. The azidopentaammine (2+) and aquapentaammine (3+) complexes were eluted with 0.4 M $NaClO_4$. The two bands were collected and assayed spectrophotometrically ($\epsilon_{518}{}^{max}[Co(NH_3)_5N_3]^{2+}$ = 272, $\epsilon_{492}{}^{max}[Co(NH_3)_5OH_2]^{3+}$ = 47.7).[25] The competition ratio (*R*) is defined as $-[(NH_3)_5CoN_3{}^{2+}]/[(NH_3)_5CoOH_2{}^{3+}][N_3^-]$.[25]

Table I. Base Hydrolysis of $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ (μ = 1.0 M $NaClO_4$, T = 25 °C)

[NaOH], M	[base], M	NP,[a] %	NPP,[b] %	NPP/NP	10^4k,[c] s^{-1}
0.05		45	55	1.2	0.39 ± 0.01
0.1		45	55	1.2	0.83 ± 0.03
0.25		45	55	1.2	2.0 ± 0.1
0.5		45	55	1.2	4.1 ± 0.1
0.75		46	54	1.2	6.05 ± 0.05
0.9		46	54	1.2	7.3 ± 0.3
1.0		47	53	1.2	8.16 ± 0.05
	$[CH_3NH_2]$[d]				
0.1	0.5	45		1.2	1.02 ± 0.04
0.1	1.0	48		1.1	1.19 ± 0.03
0.1	2.0	52		1.0	1.48 ± 0.03
0.5	0.5	46		1.2	4.8 ± 0.1
0.5	1.5	48		1.1	5.9 ± 0.1
0.5	2.0	50		1.0	6.4 ± 0.1
	$[NH_3]$				
0.5	1.0	47		1.1	4.9 ± 0.1
0.5	2.0	48		1.1	5.6 ± 0.1
	$[N(CH_3)_3]$				
0.5	0.5	48		1.1	4.6 ± 0.1
0.5	1.2	51		0.9	6.0 ± 0.1
	[imidazole]				
0.1	1.0	45		1.2	0.77 ± 0.03
	[dioxane]				
0.5	1.0	18		4.5	11.2 ± 0.4
0.5	2.0	15		5.8	18.1 ± 0.7

[a] Percent *p*-nitrophenol after $10t_{1/2}$. Each entry is the average of three values which differ by not more than 3%. [b] Percent nitrophenylphosphate at $10t_{1/2}$; 100% − NP (%) (recovery 100%). [c] Each entry is the average of three values which differ by not more than 5%. [d] μ = 1.0 M, KCl.

Results

The release of *p*-nitrophenolate anion from $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ was followed spectrophotometrically under pseudo-first-order conditions at 400 nm over the hydroxide concentration range 0.05–1.0 M (μ = 1.0 M, $NaClO_4$; 25 ± 0.05 °C). The hydrolysis rate was first order in ester complex and first order in OH^- with a rate law of the form $-d[CoNPP]/dt = k_{OH}[CoNPP][OH^-]$ with k = 8.1 (±0.1) × 10^{-4} M^{-1} s^{-1} (Table I).

An experiment for comparison was carried out in order to determine the rate of hydrolysis of uncoordinated nitrophenylphosphate in 1 M NaOH solution which was also 1 M in ammonia. Under these conditions less than 1% hydrolysis occurred in 2 days.

Proton NMR spectroscopy showed that free *p*-nitrophenol and *p*-nitrophenylphosphate were the major nitrophenol-containing products of the reaction (Figure 1). Quantitative determination of the yields of these anions at $10t_{1/2}$ revealed that their ratio was constant over the range of OH^- concentrations studied (Table I). The ratio of nitrophenylphosphate to nitrophenol at $1t_{1/2}$ and $5t_{1/2}$ (Table III) was also close to that observed at $10t_{1/2}$ (Table III). Clearly the nitrophenol and nitrophenylphosphate arise from competing parallel paths, each first order in $[OH^-]$. No 4-nitroaniline was detected in the reaction.

In the presence of other solutes and solvents such as dioxane, ammonia, methylamine, and trimethylamine (0–2 M) the rates changed appreciably but the product ratios did not alter comparably (Table I) for the added base. The decomposition to cobalt

(21) Bunton, C. A.; Hellyer, J. M. *J. Org. Chem.* **1969**, *34*, 2798–2799.
(22) Anbar, M.; Guttmann, S. *Int. J. Appl. Radiat. Isot.* **1959**, *5*, 233–235.
(23) Sargeson, A. M.; Taube, H. *Inorg. Chem.* **1966**, *5*, 1094–1100 and references therein.
(24) Methoden *Org. Chem.* (*Houben-Weyl*), *4th Ed.* **1964**, *12*(2), p 216.
(25) Buckingham, D. A.; Olsen, I. I.; Sargeson, A. M. *J. Am. Chem. Soc.* **1966**, *88*, 5443.

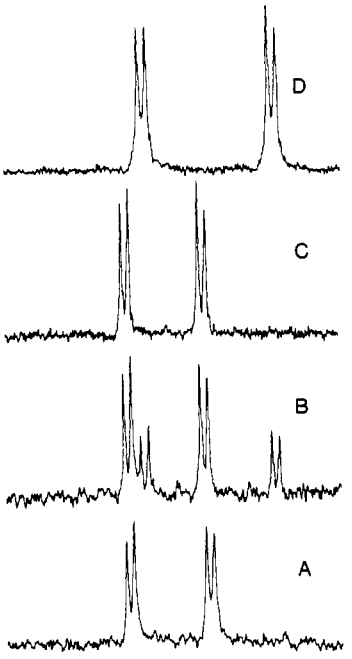


Figure 1. ^1H NMR spectra of the base hydrolysis of $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ (5×10^{-2} M) in 1 M NaOD (NMR parameters, sweep width = 1080 Hz, sweep time = 250 s): A, $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ starting material; B, products at $10t_{1/2}$; C, authentic *p*-nitrophenylphosphate in 1 M NaOD; D, authentic *p*-nitrophenol in 1 M NaOD.

oxide was depressed however in the presence of CH_3NH_2 and NH_3 but not in the presence of $(CH_3)_3N$ or dioxane. The variation in rate without corresponding variation in products is ascribed to solvent changes induced by the additives rather than to any alteration in mechanism. General-base paths have been observed for phosphate ester hydrolysis.[26] However, the same effects have not been seen for the conjugate base path for Co–ligand cleavage in Co(III) amine complexes except in those instances where the coordinated amine is more acidic than the coordinated ammonia used here, and proton removal is rate determining.[27,28]

^{31}P NMR spectroscopy proved to be an excellent technique for monitoring the phosphate-containing species produced. Initially, analytically pure samples of a range of phosphate-containing tetraammine and pentaammine complexes were used to compile known chemical shifts to aid assignment of the products from the base hydrolysis reaction (Table II). A solution of $[Co(NH_3)_5O_2P(OH)OC_6H_4NO_2]Cl_2$ at 25 °C was made 1 M in NaOH, and spectra were recorded at 1, 2, 10, and $20t_{1/2}$ (Figure 2). From this series it was apparent that the major phosphorus-containing products at $10t_{1/2}$ were phosphoramidate ($PO_3NH_2^{2-}$), nitrophenylphosphate, and a species which hydrolyzed to release phosphoramidate. At $20t_{1/2}$ phosphoramidate (45%) and nitrophenylphosphate (55%) were the sole products. Within the relative limits of detection, no (phosphato)pentaammine or free phosphate were produced. This allows the hydrolysis rate constant to be separated into two competing paths with rate constants of 3.6×10^{-4} and 4.5×10^{-4} M^{-1} s^{-1}, respectively.

An inherently more sensitive procedure was used to check this result. The reaction mixture was analyzed by descending anion-exchange paper chromatography after $20t_{1/2}$; individual standards of phosphate, phosphoramidate, and nitrophenylphosphate as well as mixed standards were run. The only phosphorus-containing species detected corresponded to phosphoramidate and nitrophenylphosphate.

The ^{31}P NMR studies indicated that the phosphoramidate-containing complex decomposed over several days at pH 9 to

Table II. ^{31}P NMR Chemical Shifts of Phosphate Compounds[a]

compd	chem shift, ppm	conditns
$[Co(NH_3)_5O_3POC_6H_5]Cl$	7.5	1.0 M NaOH
$[Co(NH_3)_5O_3POC_6H_4NO_2]Cl$[b]	6.7	1.0 M NaOH
$[Co(NH_3)_5O_2P(OH)OC_6H_4NO_2]Cl_2$[b]	4.3	pH 3
$[Co(NH_3)_4(H_2O)(HPO_4)]ClO_4$	13.7	pH 5.5
$[Co(NH_3)_5PO_4H]ClO_4$	12.0	pH 5.5
$[Co(NH_3)_5PO_4]$	13.7	0.2 M NaOH
$Na_2O_3POC_6H_4NO_2$[b]	−0.1	1.0 M NaOH
$NaOP(O)(NH_2)OC_6H_4NO_2$[b,c]	5.7	1.0 M NaOH
$Na_2O_2P(O)NH_2$[d]	8.6	1.0 M NaOH
$NaOP(O)(OH)NH_2$[d]	3.0	pH 7
Na_3PO_4[e]	5.5	1.0 M NaOH
$Na_2O_3POC_6H_5$[f]	0.0	1.0 M NaOH

[a] Downfield from 85% H_3PO_4 (external reference). Precision is ±0.1 ppm. All resonances are singlets. [b] The $-NO_2$ substituent is para in all instances. 4-Nitrophenylphosphoric acid, pK_1 = 0.21 and pK_2 = 5.18 (see: Desjobert, A. *Bull. Chim. Soc. Fr.* 1963, 683). [c] Sodium-4-nitrophenylphosphoramidate. [d] Disodium phosphoramidate and sodium hydrogen phosphoramidate literature values for chemical shifts are 8.9 and 3 ppm, respectively (see: Nielsen, M.; Pustinger, J. V.; Strobel, J. *J. Chem. Eng. Data* 1964, *9*, 167–170). Phosphoramidic acid, pK_1 = 2.83 and pK_2 = 8.03 (see: Jencks, W. P.; Gilchrist, M. *J. Am. Chem. Soc.* 1965, *87*, 3199–3208). [e] Trisodium phosphate (lit. 5.5–6.0 ppm; see: Grayson, M., Griffith, E., Eds. "Topics in Phosphorus Chemistry"; Vol 5, 1967; p 319). The exact value of the chemical shift is concentration dependent. [f] Phenylphosphoric acid, pK_1 = 1.45 and pK_2 = 6.12 (see Desjobert, footnote *b*).

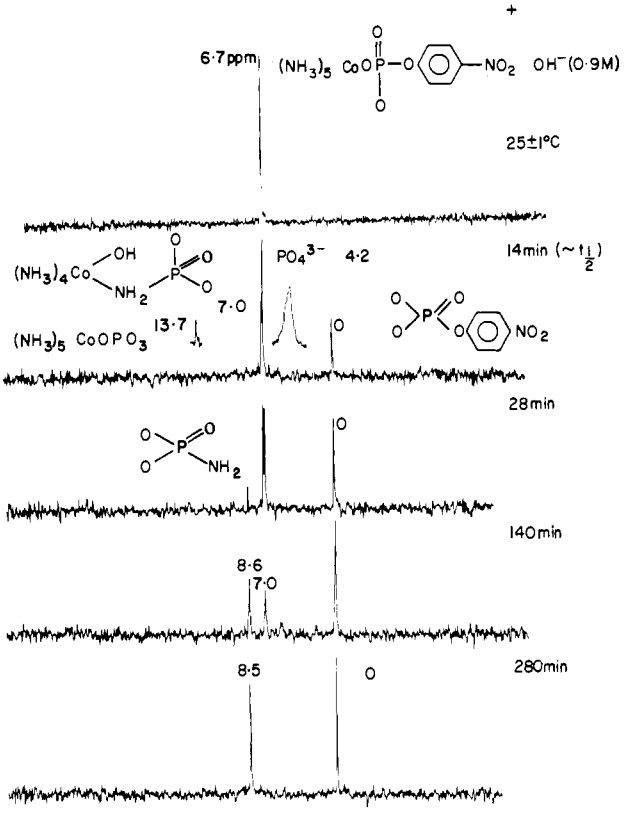
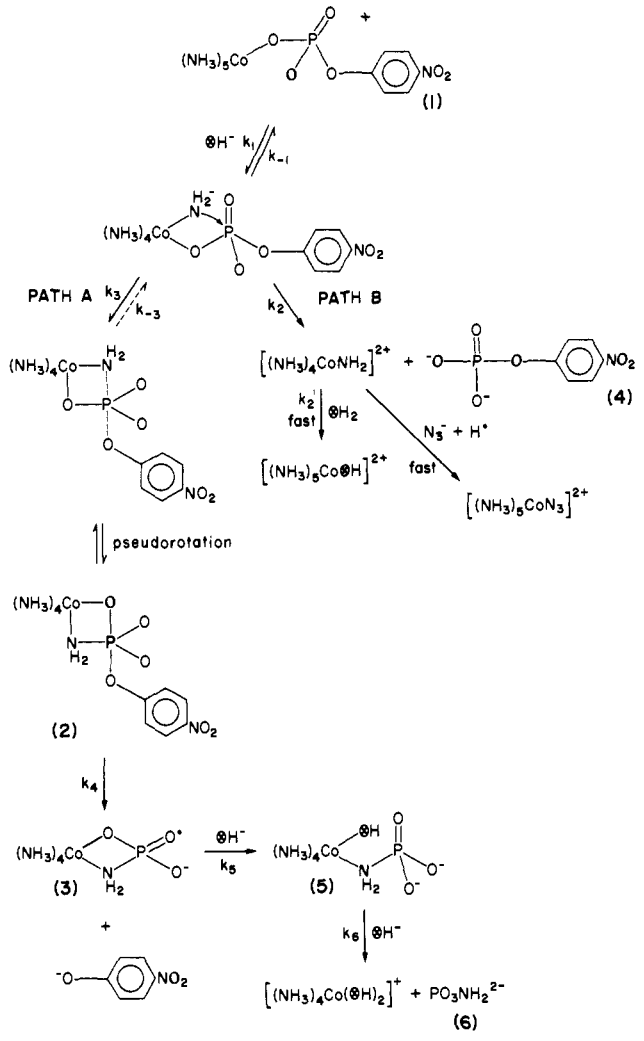


Figure 2. ^{31}P NMR spectra of the base hydrolysis of $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ (5×10^{-2} M) in 1.0 M NaOH (T = 25 ± 1 °C). Total sample volume was 2.2 mL containing 20% D_2O for internal lock. NMR parameters: band width, 2100 Hz; pulse repetition rate, 1.0 s; pulse angle, 60°; 4K data points; 100 transients per spectrum. The sample solution was rapidly filtered immediately prior to the accumulation of each spectrum in order to remove precipitated cobalt oxide (Jeol JNM-FX-100, 40.3 MHz).

phosphoramidate anion and cobalt oxide. This relatively long lifetime enabled a procedure to be devised for its isolation following the base hydrolysis of $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$. Since it is a zero-charged species at pH 10, it can be isolated free of the

(26) Benkovic, S. J.; Sampson, E. J. *J. Am. Chem. Soc.* **1971**, *93*, 4009 and references therein.
(27) Tobe, M. L. *Acc. Chem. Res.* **1970**, *3*, 377–385 and references therein.
(28) Sargeson, A. M. *Pure Appl. Chem.* **1973**, *33*, 527.

Scheme I



cationic ($[Co(NH_3)_5OH]^{2+}$ and starting complex) and anionic (phosphoramidate, *p*-nitrophenolate, and p-nitrophenylphosphate) species involved in the reaction by passing the solution through cation- and anion-exchange resins consecutively. The ^{31}P NMR chemical shift (7.0 ppm in 1 M NaOH) of the orange-red complex obtained in this way was identical with that of the phosphoramidate-producing species detected in the ^{31}P NMR kinetics experiments (Figure 2). Both complexes hydrolyzed at the same rate to free phosphoramidate ion. The compound's neutrality at pH 9 implied that the phosphoramidate was present as an N-bonded monodentate ligand (**5** in Scheme I). Strong evidence for this proposal was also provided by the products of acid hydrolysis of this complex. Organic phosphoramidate compounds characteristically undergo P–N cleavage in acidic media.[20] Thus **5** (Scheme I) would be expected to generate $[Co(NH_3)_5OH_2]^{3+}$ in a strongly acidic medium. In perchloric acid red-orange crystals were produced with an absorption spectrum ($\epsilon_{492}^{max} = 49$, H_2O) which was identical to that of an authentic specimen of $[Co(NH_3)_5OH_2](ClO_4)_3$. In hydrobromic acid red-violet crystals of the slightly soluble $[Co(NH_3)_5Br]Br_2$ complex were deposited on standing over several days or more rapidly on heating. If the phosphoramidate anion had been O bonded, $[Co(NH_3)_4(OH_2)_2]^{3+}$ would have been generated in acidic solutions.

The results of quantitative product distribution studies for the hydrolysis of $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ (in 1.0 M NaOH) are presented in Table III. In separate experiments, reaction solutions were quenched with HCl after 1- and $5t_{1/2}$ had elapsed. Total recovery of complexes was 94% for $1t_{1/2}$, but cobalt oxide became a much more significant component over longer reaction times. The mechanism outlined in Scheme I requires that the amount of nitrophenol produced be equal to the sum of phosphoramidate

Table III. Stoichiometry for $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ Hydrolysis in 1 M NaOH (25 °C)

compd	quenching time, % $1t_{1/2}$	$5t_{1/2}$
$[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ [a]	51.8[b]	4.6[b]
$[Co(NH_3)_5OH_2]^{3+}$ [c]	25.7	48.3
p-nitrophenylphosphate	25.6	51.1
p-nitrophenol	22.6	44.7
phosphoramidate complex	18.5	23.9
phosphoramidate	5.6	19.9
cobalt oxide	5.6	19.2

[a] Unreacted starting material. [b] Expressed as a percentage of the total material recovered (94% was recovered for both $1t_{1/2}$ and $5t_{1/2}$). [c] $[Co(NH_3)_5OH_2]^{3+}$ plus a small amount of $[Co(NH_3)_4(OH_2)_2]^{3+}$.

Table IV. Hydrolysis of $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ in ^{18}O Enriched Water[a]

product isolated	enrichment,[b] % $1t_{1/2}$	$2t_{1/2}$	$10t_{1/2}$
$[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ [c]	0	...	...
$[Co(NH_3)_5OH_2]^{3+}$ [c]	100	100	...
p-nitrophenol	0	...	0
p-nitrophenylphosphate[d]	0	...	0
phosphoramidate[d]	...	...	0

[a] 1.0 M NaOH. [b] Enrichment percent = (atom % sample − 0.202)/(atom % solvent − 0.202), where 0.202 atom % is the natural abundance of ^{18}O in CO_2; the values quoted were duplicated in two separate studies; error ±1%. [c] Isolated as bromide salt. [d] Isolated as silver salt.

complex and free phosphoramidate. The requirement is satisfied by both of the experiments. The amounts of $[Co(NH_3)_5OH_2]^+$ and PNPP should also be equal. However, it was not possible to establish this assertion due to the decomposition of the hydroxopentaammine cation to cobalt oxide which is also one of the ultimate products of the base hydrolysis of the phosphoramidate complex. Another complication was that attempts at chromatographic separation of $[Co(NH_3)_5OH_2]^{3+}$ and $[Co(NH_3)_4(OH_2)_2]^{3+}$ over a range of eluant pH values (3–8) were unsuccessful.

Tracer studies, involving hydrolysis of $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ in ^{18}O-enriched water, were also carried out (Table IV). From the tracer data it can be concluded that (i) there is no incorporation of label into the starting material, (ii) the nitrophenol is produced by 100% P–O cleavage, (iii) the nitrophenylphosphate arises by 100% Co–O cleavage, and (iv) the phosphoramidate anion is unlabeled.

These conclusions are justified provided the products do not exchange under the reaction or isolation conditions. Authentic specimens of ^{18}O-enriched $[Co(NH_3)_5OH_2]Br_3$ and $Na_2O_3POC_6H_4NO_2$ were treated and isolated in the same manner as the reaction products and negligible loss of label occurred. The phenol product exchanges slowly in acidic solution at high temperatures and apparently not at all in basic conditions.[29] Phosphate exchanges ^{18}O in acidic solution slowly and very slowly, if at all, above pH 8.[30]

The ^{18}O-exchange experiments do not seem to have been carried out with the phosphoramidate ion, but it can be presumed to be very slow in keeping with HPO_4^{2-} and related oxyanions.[31]

Discussion

The mechanism for the base hydrolysis of $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ needs to accommodate the production of a phosphoramidate complex and the release of unreacted nitrophenylphosphate from competitive rate-determining paths which

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(30) Bunton, C. A.; Llewellyn, D. R.; Vernon, C. A.; Welch, V. A. *J. Chem. Soc.* **1961**, 1636.
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are both first order in $[OH^-]$ and approximately equal.

The formation of the phosphoramidate species requires a deprotonated ammonia cis to the coordinated ester to attack the phosphorus center (path A, Scheme I). The resultant aminophosphoranes (**2**) can then eliminate nitrophenol to give presumably a chelated phosphoramidate anion (**3**) initially. A logical route for the production of nitrophenylphosphate is via the conjugate base dissociative (S_N1cB) mechanism which is frequently found in cobalt(III) ammine chemistry (path B, Scheme I).[27,28] It is characterized by the production of a presumed five-coordinate cobalt intermediate which is able to compete for a variety of nucleophiles in aqueous solution.[28] In the presence of N_3^- for example, both H_2O and N_3^- are captured to give the hydroxo- and azidopentaammine complexes, simultaneously. Such behavior was observed in the chemistry recorded here. When the hydrolysis of $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ was carried out in 1 M NaOH which was also 2 M in NaN_3, a competition ratio of 0.07 was measured after 1 half-life had elapsed.

The proposed conjugate base mechanism is also supported by the ^{18}O-tracer experiments (Table IV) which show no enrichment in the free nitrophenylphosphate ion and by the fact that phenylphosphate is cleaved entirely from the $[Co(NH_3)_5O_3POC_6H_5]^+$ ion without any hydrolysis of the ester.

The tracer results eliminate a process where $^{18}OH^-$ adds to the P center and decomposition of the resulting phosphorane leads to $[(NH_3)_5CoOH]^{2+}$ and nitrophenylphosphate ions. Such a reaction is not unreasonable since the leaving group would be a cation, but it requires incorporation of ^{18}O in the cleaved phosphate ester and this was not observed. Presumably the Co–O bond is ruptured directly during the rate-determining dissociation k_2 almost at the same rate as the formation and decomposition of the aminophosphorane by path A. This condition is imposed by the essentially constant ratio of nitrophenylphosphate ion to nitrophenol over the range of OH^- concentrations studied.

Nitrogen proton-exchange rates in complexes of this type are specifically catalyzed by OH^- and not by general bases. Moreover the exchange rates are much faster than the hydrolysis rate observed here (usually $>10^3$ M^{-1} s^{-1} at 25 °C). The preequilibrium therefore can be assumed to be established well before hydrolysis is advanced.[32,33] Moreover, the pK_a for the bound ammonia probably is of the order of 17 for the single positively charged reactant.[28,33,34] With use of this background the rate equation for ester hydrolysis and Co–O cleavage in Scheme I can be formulated as

$$-d[CoNPP^+]/dt = k_3[CoNPP^+\text{–}H^+] + k_2[CoNPP^+\text{–}H^+]$$

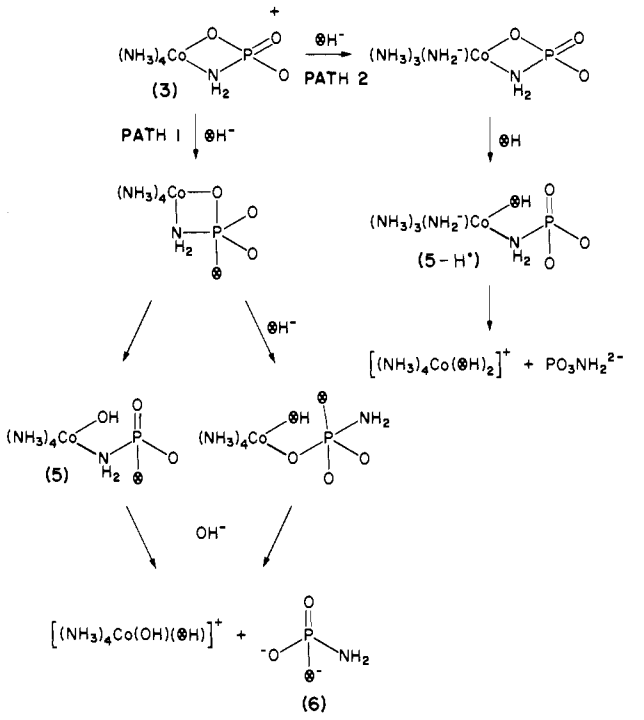
whence

$$k_{obsd} = \frac{(k_2 + k_3)K_a[OH^-]}{K_a[OH^-] + K_w}$$

provided $k_2' \gg k_2$ and $k_4 \gg k_3$, and since $K_a[OH^-] \ll K_w$, this reduces to $k_{obsd} = (k_2 + k_3)(K_a/K_w)[OH^-]$ so that $k = (k_2 + k_3)(K_a/K_w)$. Certainly the inequality $k_2' \gg k_2$ holds since there is every indication that intermediates of reduced coordination number of this type survive few encounters with nucleophiles in solution.[28]

By analogy with organic phosphate ester systems whose hydrolyses in base are attributed to formation and decay of five-coordinate phosphorane intermediates,[35-39] the decomposition of

Scheme II



2 to release nitrophenol would be expected to be fast, i.e., $k_4 > k_3$. The absence of a peak which could be assigned to **2** in the ^{31}P NMR spectra supports this argument. The proposed mechanism, Scheme I, therefore is consistent with known and expected chemistry and accommodates the observed rate law. On this basis, if K_w/K_a is $\sim 10^3$, the rate of ester hydrolysis for the amido complex is ~ 0.4 s^{-1}. This is to be compared with the hydrolysis of the uncoordinated nitrophenylphosphate under similar conditions[40] ($k = 2 \times 10^{-9}$ s^{-1} for 0.5 M OH^- at 25 °C). The intramolecular nucleophile therefore gives rise to a very substantial rate enhancement ($\sim 10^8$) for cleavage of nitrophenol. Obviously if the concentration of the actual amido ion is not taken into account then the rate enhancement is less substantial ($\sim 10^5$).

Decomposition of the chelated phosphoramidate could conceivably occur via several routes. Addition of $^{18}OH^-$ to the phosphorus and decomposition of the resulting aminophosphorane via path 1 in Scheme II would ultimately lead to the labeled phosphoramidate which was not observed. The obvious route which does not lead to incorporation of label in the final product is a conjugate base-dissociative path involving only cobalt–donor atom bond rupture (path 2). In this context it is most likely that Co–O rupture occurs first to give the hydroxo, N-bonded monodentate phosphoramidate complex. Cleavage of the Co–N phosphoramide bond would follow more slowly and the expected tetraammine dihydroxo complex would decompose rapidly in the basic medium to cobalt oxide.[41] A good precedent for fast Co–O rupture accompanying ring opening is provided by the rapid base hydrolysis of the $[Co(en)_2PO_4]$ phosphate chelate complex.[42] In 1 M OH^- at 25 °C the half-life for this process is ~ 0.5 s. The phosphoramidate complex observed in the ^{31}P NMR spectra and isolated after ion-exchange chromatography is therefore presumably the ring-opened form **5** $[(NH_3)_4Co(OH)(NH_2PO_3)]$, containing the phosphoramidate bound to the cobalt through the N atom. The evidence for this method of binding has been presented in the Experimental Section and Results.

In Scheme I the aminophosphorane generated by intramolecular attack of the deprotonated ammonia ligand is depicted as five

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(33) Buckingham, D. A.; Marzilli, L. G.; Sargeson, A. M. *J. Am. Chem. Soc.* **1967**, *89*, 825 and references therein.
(34) K_a is estimated to be $\sim 10^{-17}$ for ammine complexes. Basolo, F.; Pearson, R. G. "Mechanisms of Inorganic Reactions", 2nd ed.; Wiley: New York, 1967; pp 183–184. Therefore if $[OH^-] \leq 1.0$ M, $K_a[OH^-] \ll K_w$.
(35) Cox, J. R.; Ramsay, O. B. *Chem. Rev.* **1964**, *64*, 317–352.
(36) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70–78.
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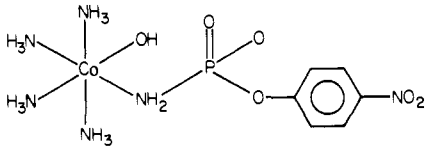
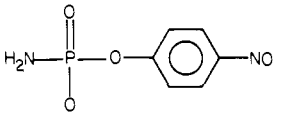
(38) Mislow, K. *Acc. Chem. Res.* **1970**, *3*, 321–332.
(39) Gillespie, P.; Ramirez, F.; Ugi, I.; Marquarding, D. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 91–119.
(40) Extrapolated from the data of: Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209–3216.
(41) Werner, A. *Chem. Ber.* **1907**, *40*, 4113.
(42) Lincoln, S. F.; Stranks, D. R. *Aust. J. Chem.* **1968**, *21*, 57–65.

coordinate with a trigonal-bipyramidal geometry. Alternatively square-pyramidal[43,44] or octahedral[39,45,46] phosphorus intermediates could arise from this step—the latter by intervention of a solvent molecule. Any of these proposed intermediates could decay to the chelated phosphoramidate complex with release of nitrophenol. If a six-coordinate phosphorus intermediate is involved, then significant return to the deprotonated reactant (k_{-3} in Scheme I) is not allowed by the ^{18}O-tracer experiment (no incorporation of ^{18}O in the reactant was observed). More importantly, decay of such an intermediate should lead to labeled phosphoramidate, and this was not observed either. We presume therefore that only trigonal-bipyramidal or square-pyramidal intermediates or activated complexes can be involved.

The trigonal-bipyramidal complex shown in Scheme I could readily eliminate nitrophenoxide ion or nitrophenol via exocyclic P–O cleavage to produce the phosphoramidate chelate (**3**) with a minimum of rearrangement. The ^{18}O-tracer experiments preclude any exchange between the phosphate oxygens and solvent in the lifetime of the intermediate since no enrichment was detected in the final phosphoramidate product. The possibility that the intermediate could decompose by endocyclic P–O or P–N bond rupture also arises. The latter would regenerate starting material but the former would yield **7**. Base-catalyzed Co–N cleavage in **7** would produce **8**, and this species would be expected to undergo



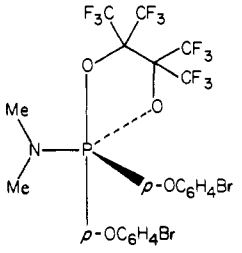
7



8

hydrolysis in base to liberate nitrophenol and phosphoramidate.[14] In order to check this possibility, we synthesized an authentic sample of sodium *p*-nitrophenylphosphoramidate. The compound had a ^{31}P chemical shift of 5.9 ppm in 1 M NaOH and, under these conditions, hydrolysis to nitrophenol and phosphoramidate did occur. However the rate (4.3×10^{-5} s^{-1}) was 40 times slower than the production of *p*-nitrophenol from $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ and at least 20 times slower than phosphoramidate release. Thus it would have been detected by ^{31}P NMR spectroscopy if it had been present as a major product. To check whether it was present as a minor product, we passed the reaction mixture first through cation-exchange resin to remove the cationic species and then through an anion-exchange column to absorb the anionic species. Under these conditions authentic samples of nitrophenol, nitrophenylphosphate, and nitrophenylphosphoramidate were easily separated by elution with 0.04 M NaCl. The absorbance of the effluent was monitored at 305 nm. Since the species of interest had very large extinction coefficients, this method was much more sensitive than ^{31}P NMR. (Detection limit, approximately 1% of total nitrophenol species present.) No nitrophenylphosphoramidate was detected at 1-, 5-, or $10t_{1/2}$ for the hydrolysis reaction.

The four-membered chelate ring in the cyclic aminophosphorane complex (**2** in Scheme I) should span axial–equatorial (a–e) positions in the trigonal bipyramid.[47] Chelated phosphate ion in an analogous octahedral complex[11] subtends angles of 76 and 98.7° at the Co and P atoms, respectively. An a–e chelate in a trigonal bipyramid would not be expected to be very different from this. Spanning the a–e positions (90°) is clearly more favorable than spanning the edge of a trigonal plane (120°). The question is whether O or N occupies the apical position? The general rules which have evolved from studies of organic phosphoranes containing five-membered heterocyclic rings place electron-withdrawing groups preferentially in apical sites and electron-donating groups preferentially in equatorial sites.[39,48] This would favor oxygen apical in an N–O ring system. X-ray structures of the aminophosphoranes **9** and **10**[49] demonstrate the preference for



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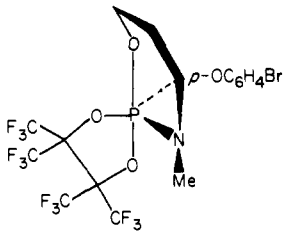
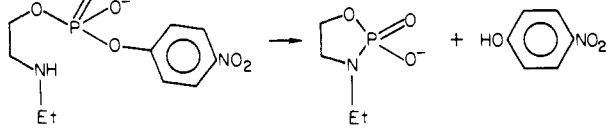


10

nitrogen in the equatorial plane of trigonal-bipyramidal phosphorus molecules.

In the above discussion it is tacitly assumed that the aminophosphorane is an intermediate with a lifetime long enough to enable it to pseudorotate at least once before decomposing. This assumption is in keeping with the general proposals for nucleophilic attack at such P centers.[36-39] The most likely structure for the aminophosphorane after pseudorotation and consistent with apical expulsion of the nitrophenoxide anion is that with the N atom equatorial (**2** in Scheme I). The structure is also consistent with observations in phosphorus compounds of ligancy five that the most electronegative groups or atoms tend to occupy apical positions.

An alternative explanation for the results however could be a concerted S_N2 reaction where apical entry of the coordinated amido ion at the P atom leads directly to apical loss of the 4-nitrophenolate ion. The reaction is very dependent on the leaving group, and this is consistent with analogous intramolecular amine addition to phosphorylethanolammine diesters (**11**).[50] Intramolecular amine attack in **11** is responsible for the product **12**.



11 12

In this example a limiting rate is reached when the pH exceeds the pK_a for the amine moiety ($k = 2.7 \times 10^{-3}$ s^{-1}, 35 °C, μ = 1.0 M (KCl)). The process is much slower than that evaluated for the deprotonated ammine on the cobalt complex ($k \approx 0.4$ s^{-1}, 25 °C, μ = 1.0 M ($NaClO_4$)). However, the basicity of the ethanolamine moiety (pK_a = 9.4) is also much less than that of the coordinated amido ion ($pK_a \approx 16$–17). The different reactivities may be reflecting in part this pK_a difference. Reductions in rate were also observed for the less basic phosphoryl ethanolamines and for those systems with poor leaving groups. Structure-reactivity correlations derived by changing the pK_a of the amine and leaving group and the absence of general-base catalysis were used to support a concerted mechanism. In the complex chemistry

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the failure to observe the aminophosphorane using ^{31}P spectroscopy, the lack of incorporation of ^{18}O label from the solvent, and the absence of general-base catalysis are also consistent with such an explanation, but they are not compelling reasons for choosing between a concerted and nonconcerted process. However, an interesting possibility arises from this discussion since the concerted mechanism clearly requires inversion of the configuration about phosphorus and the other allows pseudorotation. While a distinction cannot be made with the present system, a suitable phosphate diester coordinated to the cobalt moiety would allow it provided pseudorotation does occur.

The acceleratory effect in the base hydrolysis of $[Co(NH_3)_5O_3POC_6H_4NO_2]^+$ relative to uncoordinated nitrophenylphosphate presumably arises largely from the proximity of the coordinated nucleophile NH_2^- to the phosphorus center and the rapid decay of the aminophosphorane which is generated. There are four ammonia molecules in the complex which are cis to the bound ester. During rotation of the ester about the Co–O bond the P center never escapes the nucleophile. Presumably the rate gain from the loss of translational entropy by carrying out the reaction in an intramolecular manner is maximized ($\sim 10^8$) since there are minimal losses from vibrational and rotational degrees of freedom.[51] Note also that no detectable intermolecular hydrolysis occurred. It is surprising perhaps that the rate enhancement is so substantial when strain is introduced by ring formation in the aminophosphorane. The contribution of strain to a decrease in hydrolysis rate would be expected to be even more substantial in the decomposition of the five-coordinate molecule where a chelated four-membered ring is generated at the tetrahedral P center. The other factor which could contribute substantially to the rate enhancement compared to the uncoordinated molecule is the neutralization of charge on the phosphorus residue by coordinating it to the metal ion. While it is clear that the metal ions are not as efficient in this manner as H^+, they do make it much easier to add an electron-rich reagent to what is basically an anionic residue. Even so, addition of a simple trivalent metal ion to neutralize the charge on the phosphorus residue is insufficient to completely account for the rate enhancement observed here.[10]

The chemistry described indicates how a substantial increase in rate can be obtained for attack of an amine nucleophile at a phosphorus center by using a restricted intramolecular pathway despite the ring strain involved in making the chelate. It is conceivable that the metal ion in the enzymic phosphoryl amino transferases functions partly in this way by grouping the reagents so that efficient intramolecular transfer from an oxygen to a nitrogen base can occur.

Acknowledgment. We are indebted to Dr. K. Barrow of the Biochemistry Department, University of New South Wales, Dr. D. Fenn and Mr. M. Whittaker of ANU for the ^{31}P spectra, and the Microanalytical Unit of ANU for C, H, N, Co, and P analyses.

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Steric Effects in Conformationally Mobile Systems. The Iodomethylation of 1-Methyl-2-arylpyrrolidines Related to Nicotine[1]

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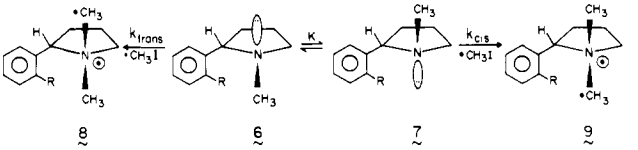
Abstract: The ground-state equilibrium distribution, the total observed iodomethylation rate constant, and the corresponding iodomethylation stereoselectivity were determined for 1-methyl-2-phenylpyrrolidine and the related 1-methyl-2-(2-alkylphenyl)pyrrolidines where the alkyl substituents include methyl, ethyl, isopropyl, and *tert*-butyl. From these parameters, the iodomethylation rate constants for attack cis and trans to the aromatic ring (k_{trans} and k_{cis}) were calculated by using the Curtin–Hammett and Winstein–Holness equations. The dependence of k_{cis} and k_{trans} on K was evaluated. The results are examined in light of the three important conformational features present in these systems: nitrogen inversion, rotation about the aromatic ring–pyrrolidine ring C–C single bond, and rotation about the bond linking the ortho substituent to the aromatic ring. Implications of the combined usage of the Curtin–Hammett/Winstein–Holness equations are analyzed.

An understanding of the role of steric interactions in organic chemistry has been advanced by various statistical treatments and enhancements of the Taft equation.[2] The concept that substituents have specific spatial requirements[3] has engendered attempts to quantitate steric phenomena independent of localized (field and/or inductive) and delocalized (resonance) electronic effects.[4] One approach has focused on the evaluation of steric parameters as a function of branching of alkyl substituents.[4e] Alkyl groups have relatively minor electronic effects which are usually unrelated to

Scheme I



(1) (a) Presented in part at the Second Chemical Congress of the North American Continent, 180th National Meeting of the American Chemical Society, Las Vegas, NV, August 24-29, 1980; American Chemical Society: Washington, D.C., 1980; Abstract No. ORGN-335. (b) For the previous paper in this series, see Seeman, J. I.; Sanders, E. B.; Farone, W. A. *Tetrahedron* **1980**, *36*, 1173-1177.

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