same basicity. The 6-cm^{-1} difference between them in the zinc–imidazole stretching frequency is attributed primarily to the steric effect of the 2-methyl group, which is expected to have short nonbonded contacts with the pyrrole rings.[22]

The reconstituted proteins do not show any band between 175 and 200 cm^{-1} (Figure 5). They both show a weak but well-resolved band at 148 cm^{-1}. This is assigned to the Zn–imidazole stretch in the proteins. It is much weaker and lower in frequency than the Zn–imidazole bands seen in the model complexes. The best reference complex is probably the 4-MeImH adduct, the 4-methyl group mimicking the methylene linkage of the imidazole side chains to the polypeptide backbone in the protein. If the Zn–imidazole stretch is modeled with a diatomic oscillator, with masses 65 (Zn) and 82 (4-MeImH), then the force constant is 0.76 mdyn/Å for the 4-methylimidazole adduct but only 0.48 mdyn/Å for ZnMb. Thus the Zn–imidazole bond is evidently much weaker in the protein than in the model complexes. This is not the case for native Mb, which shows an imidazole-stretching frequency,[23,24] 220 cm^{-1} (k = 1 mdyn/Å), that is in the range found for model complexes.[20,21] We conclude that the proximal imidazole is not optimally oriented for binding to Zn in the reconstituted protein.

One factor in differentiating Zn from Fe in the protein is that the out-of-plane displacement of the metal in five-coordinate complexes is 0.33 for Zn^{II} [5] but 0.55 for Fe^{II}.[18] Another factor is that the Zn–imidazole bond is intrinsically weaker than the Fe–imidazole bond. Thus in a non-H-bonding solvent the (2-MeImH)Fe^{II}PP frequency is 207 cm^{-1},[20] 30 cm^{-1} higher than for (2-MeImH)ZnPP. Consequently, a suboptimal position of the proximal imidazole can be accommodated by lengthening an already weak Zn–imidazole bond. Nevertheless, this phenomenon implies a relatively rigid positioning of the proximal histidine, presumably via the surrounding nonbonded contacts,[25] since an ~0.2-Å movement of a side chain would ordinarily be expected to require very little energy.

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Registry No. ZnPPDME, 15304-09-3; (ImH)ZnPPDME, 80481-01-2; (2-MeImH)ZnPPDME, 100228-83-9; (4-MeImH)ZnPPDME, 100243-48-9; L-His, 71-00-1.

(22) Collman, J. P.; Halbert, T. R.; Suslick, K. *Met. Ions Biol.* **1980**, *2*, 1–72.
(23) Kitagawa, T.; Nagai, K.; Tsubaki, M. *FEBS Lett.* **1979**, *104*, 376.
(24) Argade, P. V.; Sassaroli, M.; Rousseau, D. L.; Inubeushi, T.; Ikeda-Saito, M.; Lapidot, A. *J. Am. Chem. Soc.* **1984**, *106*, 6593–6596.
(25) Takano, T. *J. Mol. Biol.* **1977**, *110*, 537–568.
(26) The parameters K and A for ν_4 were omitted from ref 13, which gave the empirical core size plot. They are K = 133.3 cm^{-1}/Å and A = 12.32 Å. In support of the use of ZnTPP structure parameters, we note that bond lengths and core size are nearly independent of the porphyrin peripheral substituents for given central metal and axial ligands.[14]

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Intramolecular Phosphoryl Transfer: Chelated Phosphoramidate

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2,4-Dinitrophenyl phosphate (DNPP) coordinated to the pentaamminecobalt(III) moiety is rapidly lyzed in aqueous base to yield the previously unobserved N,O-chelated phosphoramidate and 2,4-dinitrophenolate (DNP) ions. The chelate ring then opens to monodentate N-bound phosphoramidate. The reaction is almost quantitative (98%) at 5 °C and obeys the rate law $v_{DNP} = k[CoDNPP][OH^-]$ with $k = (1.96 \pm 0.03) \times 10^{-2}$ L mol^{-1} s^{-1} at 25 °C and μ = 1.0 M. With increasing temperature Co–O bond rupture releases increasing amounts of unreacted phosphate ester. The mechanism is argued in terms of a deprotonated ammonia attacking the adjacent P center to give an aminophosphorane, which then decomposes to the chelate phosphoramidate. Subsequent ring opening and phosphoramidate loss are ascribed to the normal conjugate base chemistry of Co(III) amine complexes. The intramolecular substitution of NH_2^- for the phenolate ion at the P center is very rapid compared with analogous chemistry for the uncoordinated ion.

Introduction

The enzymic hydrolysis of phosphates and phosphate esters is remarkable in that many of the known enzymes require at least one divalent metal ion for activity.[1] This observation has stimulated research into the role that metal ions play in both the enzymic and nonenzymic hydrolysis of phosphates.[1,2] Work in this laboratory has concentrated on the synthesis and hydrolysis of well-defined, substitution-inert metal ion phosphate complexes designed to test the efficacy of certain types of reaction paths for their possible role in enzymic phosphate chemistry.[3-5] In this context, the reactivity of phosphate derivatives in the pentaamminecobalt(III) complexes[6,7] $[(NH_3)_5CoOPO_3C_6H_4NO_2]^+$ and $[(NH_3)_5CoOPO_2F]^+$ has been studied. In both these cases, it was postulated that reaction proceeded via attack of a cis-coordinated deprotonated ammonia on the phosphorus center to yield a five-coordinate aminophosphorane activated complex, which decayed to a presumed N,O chelate phosphoramidate (Scheme I). The chelate, however, was never observed and was presumed to undergo ring opening rapidly under the conditions in which it was produced.[6,7] The aim of the present study was to observe the chelate formation and decay. This aim might be achieved by making the precursor complex more reactive, i.e. by improving the leaving-group ability of the ester function in comparison to that used in the previous studies. To this end, the leaving group chosen was 2,4-dinitrophenolate ion since its phosphate ester is well characterized[8] and is known to be more reactive than 4-

(1) Morrison, J. F.; Heyde, E. *Annu. Rev. Biochem.* **1972**, *41*, 29.
(2) Cooperman, B. S. *Met. Ions Biol. Syst.* **1976**, *2*, 79–125.
(3) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1983**, *105*, 7327.
(4) Anderson, B.; Milburn, R. M.; Harrowfield, J. M.; Robertson, G. B.; Sargeson, A. M. *J. Am. Chem. Soc.* **1977**, *99*, 2652.
(5) Hendry, P.; Sargeson, A. M. *J. Chem. Soc., Chem. Commun.* **1984**, 164.
(6) Harrowfield, J. M.; Jones, D. R.; Lindoy, L. F.; Saregeson, A. M. *J. Am. Chem. Soc.* **1980**, *102*, 7733.
(7) Creaser, I. I.; Dubs, R. V.; Sargeson, A. M. *Aust. J. Chem.* **1984**, *37*, 1999.
(8) Ramirez, F.; Marecek, J. F. *Synthesis* **1978**, 601.

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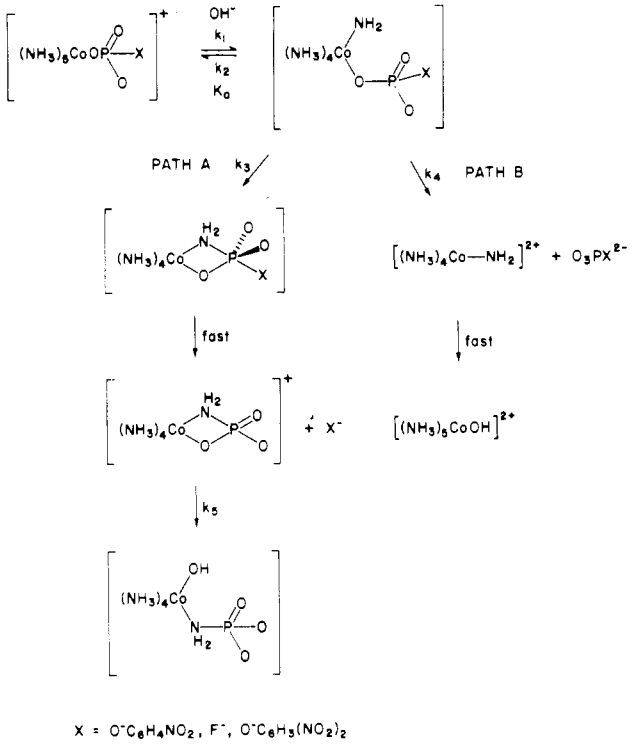
Scheme I



nitrophenyl phosphate and fluorophosphate.[6,7,9] The complex ion $[(NH_3)_5CoO_3POC_6H_3(NO_2)_2]^+$ has now been synthesized, and this paper examines its reactivity in basic conditions.

Experimental Section

Analytical grade reagents were used throughout. ^{31}P NMR spectra were recorded with either a JEOL JNM-60 or a Bruker CXP-200 instrument at 24.21 and 80.98 MHz, respectively. Chemical shifts (ppm) are quoted relative to 85% H_3PO_4 as an external standard. ^1H NMR spectra were recorded with a JEOL FX-200 spectrometer and DSS as an internal standard. All evaporations were carried out in a Buchi rotatory evaporator at ~20 torr such that the solution did not exceed 25 °C. Electronic spectra and kinetic traces were recorded with a Hewlett-Packard HP8450A diode array spectrophotometer equipped with a thermostated cell holder.

$[(NH_3)_5CoOPO_3HC_6H_3(NO_2)_2]Cl_2 \cdot {}^1/_2H_2O$. 2,6-Lutidinium 2,4-dinitrophenyl hydrogen phosphate was synthesized (analytically pure) by the method of Rawji and Milburn.[10] $[(NH_3)_5Co(CF_3SO_3)](CF_3SO_3)_2$ (2 g) and 2,6-lutidinium 2,4-dinitrophenyl hydrogen phosphate (1 g) were dissolved in dry sulfolane (30 mL) and heated to 40 °C for 5 h. The sulfolane solution was extracted with ether (1 L), the residue dissolved in water (800 mL), and the solution acidified to ~pH 2 with HCl and applied to a Sephadex SP-C25 (Na^+) column (18 × 5 cm). The column was eluted with 0.2 M NaCl acidified to pH 2 with HCl. The first eluted band was evaporated (to 35 mL) and cooled at 4 °C for 16 h. A red microcrystalline solid was collected, washed twice with cold 2 M NaCl (pH 2), twice with ethanol, and thrice with ether, and dried in vacuo for 8 h (yield 230 mg, 19%). Anal. Calcd for $C_6H_{19}N_7Cl_2CoO_8P \cdot {}^1/_2H_2O$: C, 14.79; H, 4.14; N, 20.13; Co, 12.10; P, 6.36. Found: C, 15.0; H, 4.1; N, 20.0; Co, 12.1; P, 6.2. $^{31}P\{^1H\}$ NMR (H_2O, 10% D_2O): +6.5 (s) 0.1 M NaOH) + 5.0 (s) (0.025 M HCl). ^1H NMR (D_2O, 0.01 M DCl): 8.94 (d, J = 2.7 Hz, 1 H), 8.56 (d of d, J = 9.3, 2.7 Hz, 1 H), 7.72 (d, J = 9.3 Hz, 1 H), 4.13 (br, 12 H), 2.92 (br, 3 H). ϵ^{max}_{518} = 75 M^{-1} cm^{-1} (0.02 M HCl).

Fresh solutions of $[(NH_3)_5CoOPO_3HC_6H_3(NO_2)_2]Cl_2 \cdot {}^1/_2H_2O$ (~10^{-2} M, 10 μL) were syringed into 2 mL of hydroxide solution in a thermostated cell in the spectrophotometer. The solution was rapidly mixed, and the change in absorbance at 360 nm was recorded with time. The molar absorptivity of the dinitrophenolate ion was taken to be 14 700 at 360 nm.[11] The data were processed by a VAX-11/750 computer using a nonlinear least-squares package, LSTSQR. All data fitted well to a single-exponential function. All quoted rate constants are the average of at least two separate determinations; the errors are standard deviations.

Table I. Hydrolysis of $[(NH_3)_5CoOPO_3C_6H_3(NO_2)_2]^+$ by OH^- (μ = 1.0 M)

temp, °C	[NaOH], M	10^3k_{obsd}, s^{-1}	yield of DNP, %
5	0.5	0.808 ± 0.003	98.3
15	0.5	2.86 ± 0.04	95.9
25	0.1	1.79 ± 0.01	93.6
25	0.2	3.62 ± 0.01	92.8
25	0.5	9.33 ± 0.05	93.0
25	1.0	19.9 ± 0.4	94.0
35	0.5	26.6 ± 0.1	91.4

The data at different temperatures were plotted as $1/T$ vs. ln k for each reaction (vide infra) and gave a straight line for both reactions; the slopes and standard deviations were determined by computer fitting of these points. The activation parameters for the reactions were determined with use of the slopes of these lines; the quoted errors originate from the standard deviation of the slope.

A known weight of $[(NH_3)_5CoOPO_3C_6H_3(NO_2)_2]Cl_2 \cdot {}^1/_2H_2O$ (20–50 mg) was dissolved in H_2O (1.35 mL containing ~0.03 M Na_3PO_4 as a standard) and D_2O (0.4 mL) Enough NaCl was added to the solution to make the final ionic strength 1.0 M. A ^{31}P NMR spectrum of this solution was recorded at 5 °C, then ice-cold NaOH (0.25 mL) of the required concentration was added, and the solution was replaced in the thermostated NMR probe. The final NaOH concentration was always at least 10 times the complex concentration. ^1H-coupled spectra (^1H decoupling irradiation heats the sample) were recorded at intervals and stored on disk. Acquisition parameters: Acquisition frequency 80.98 MHz; spectral width 9 kHz, pulse angle 90°, pulse repetition time 0.5 s. The integrated spectra showed only three signals at 6.5, 22–31 (dependent on base concentration), and 7.1 ppm. The intensities of the signals were plotted relative to standard. The variation in the intensity of the signal at 6.5 ppm vs. time was fitted by the least-squares program to a single-exponential function to yield the rate constant for the disappearance of the starting material. The rate constant for the hydrolysis of the intermediate was estimated by using the relationship easily derived for the reaction scheme

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

When $d[B]/dt = 0$, $k_1[A] = k_2[B]$. From the plot of the data as in Figure 2 it was possible to determine the relative concentrations of A and B (starting material and intermediate) at the maximum concentration of B (where $d[B]/dt = 0$). Knowing k_1 and the ratio of [A] to [B], it was possible to deduce k_2.

The hydrolysis of DNPP was studied in 1 M aqueous NH_3 at 25 °C under pseudo-first-order conditions, spectrophotometrically at 360 nm. The relative yields of the two products, phosphate and phosphoramidate, were determined by integration of ^{31}P NMR spectra recorded at intervals over several half-lifes. The identities of the signals were verified by adding authentic specimens of the presumed products to the sample and observing the increase in the intensity of the appropriate signal. Acquisition parameters: acquisition frequency 24.21 MHz; spectral width 5 kHz; pulse width 8 μs; pulse repetition time 1 s. The relative integration of the two signals did not change significantly upon increasing the pulse repetition time to 2 s. As expected, the yield of phosphoramidate increased with increasing NH_3 concentration.

The complex $[(NH_3)_5CoDNPP]^+$ was hydrolyzed in 25% aqueous NH_3 to yield predominantly (>95%) a complex with a ^{31}P NMR chemical shift of 18.7 ppm. The complex behaves as a monocation on a Sephadex SPC-25 cation-exchange column upon elution with $NaClO_4$. Hydrolysis of the phosphoramidate complex in 1 M HCl yields $[(NH_3)_5CoOPO_3]$, identified by its ^{31}P NMR chemical shift under acidic (1.0 M HCl, 7.6 ppm) and basic (0.1 M NaOH, 13.6 ppm) conditions.

Results

$[(NH_3)_5CoOPO_3HC_6H_3(NO_2)_2]Cl_2 \cdot {}^1/_2H_2O$ was synthesized from the $[(NH_3)_5CoOSO_2CF_3]^{2+}$ ion and dinitrophenyl phosphate ion and purified by ion-exchange chromatography.

Hydrolysis of the complex was followed by ^{31}P NMR (Figures 1 and 2) and visible spectroscopy at 360 nm. At 5 °C, the complex reacts almost quantitatively to produce dinitrophenolate (DNP) and an intermediate species ~98% (Table I). At elevated temperatures, a competing path (B) results in the loss of some phosphate ester ligand (Table I). The intermediate decays to the monodentate N-bound phosphoramidate complex (δ 7.1), which in a slower subsequent reaction yields free phosphoramidate (δ 8.6) and dihydroxotetraamminecobalt(III) ions along with some

(9) Bunton, C. A.; Fendler, E. J.; Fendler, J. H. *J. Am. Chem. Soc.* **1967**, *89*, 1221.
(10) Rawji, G.; Milburn, R. M. *J. Org. Chem.* **1981**, *46*, 1205.
(11) Younas, M.; Bokhari, S. S. *Pak. J. Sci. Ind. Res.* **1978**, *21*, 111.

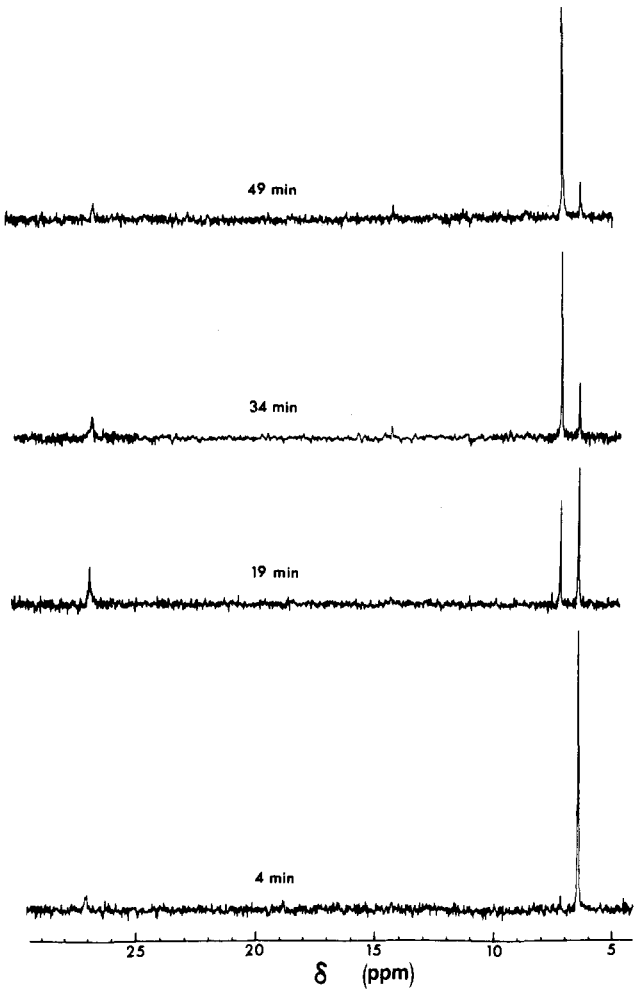


Figure 1. Sequential ^{31}P NMR spectra of hydrolysis of $[(NH_3)_5CoO_3POC_6H_3(NO_2)_2]^+$ ion. Conditions: $[OH^-] = 0.35$ M, $\mu = 1.0$ M, temperature 5 °C. Acquisition parameters: pulse repetition time 0.5 s, spectral width 9 kHz, acquisition frequency 80.98 MHz, pulse angle 90°.

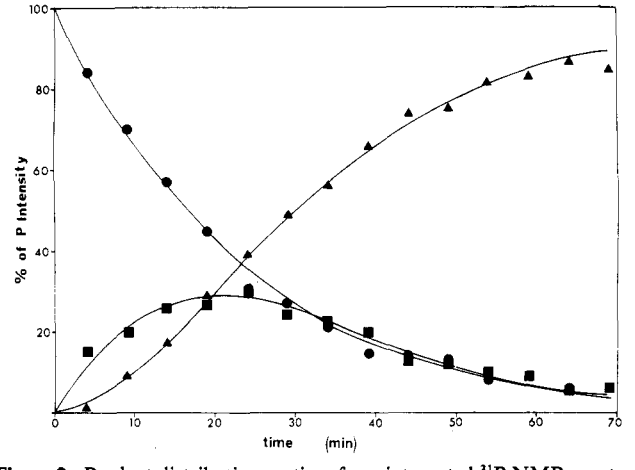


Figure 2. Product distribution vs. time from integrated ^{31}P NMR spectra (same conditions as for Figure 1): (●) reactant; (■) N,O chelate phosphoramidate; (▲) monodentate N-bound phoshoramidate.

decomposition of the latter. These chemical shifts (δ 7.1 and 8.6) are identical with those observed for N-bound and free phosphoramidate respectively established in related studies.[6,7] The observed rate of decay of N-bound to free phosphoramidate is also close to that observed in the previous study.[6]

The initial reaction, releasing both DNP and DNPP, followed at 360 nm, was found to be first order in both reactants, complex and hydroxide ion, up to 1.0 M OH^- (Table I). It had a second-order rate constant at 25 °C ($\mu = 1.0$ M ($NaClO_4$)) of (1.96 ± 0.03) × 10^{-2} L mol^{-1} s^{-1}. The temperature dependence of the reaction was also investigated (Table I). The observed rate at each temperature was partitioned into rate constants for each pathway by determining the yield of DNP from the infinity absorbance value at 360 nm. ^{31}P NMR studies of the reaction showed that the partition of the products was constant throughout the reaction. Plots of $1/T$ vs. ln k were linear for both reactions. The activation parameters for each process were evaluated to be as follows for production of DNP: path A, $\Delta H^\ddagger = 79 \pm 2$ kJ mol^{-1}, $\Delta S^\ddagger = -19 \pm 4$ J K^{-1} mol^{-1}; path B, $\Delta H^\ddagger = 119 \pm 7$ kJ mol^{-1}, $\Delta S^\ddagger = 93 \pm 20$ J K^{-1} mol^{-1}.

Hydrolysis of the complex was also followed by ^{31}P NMR spectroscopy at a probe temperature of 5 ± 1 °C ($\mu = 1.0$ M NaCl, complex concentration ~0.025 M). The second-order rate constant for loss of the reactant was (2.5 ± 0.3) × 10^{-3} L mol^{-1} s^{-1}, which is larger than that determined spectrophotometrically, 1.62 × 10^{-3} L mol^{-1} s^{-1}, but is in reasonable agreement given that the reaction was conducted in 20% D_2O and that there were difficulties involved in the ^{31}P NMR method, i.e. temperature control, inaccuracies in the integrations, and errors in time and temperature involved in initiating and mixing the solution and then transferring the sample into the probe. The starting material decayed to yield an intermediate with a ^{31}P NMR chemical shift of between 22 and 31 ppm; the plot of pH vs. chemical shift describes a titration curve for a pK_a of 13.1 ± 0.1 (Figure 3). The decay of the intermediate to yield N-bound phosphoramidate (δ 7.1) was also followed at the same temperature. The determination of the rate constants for this subsequent reaction was complicated by the fact that the rate of production of the intermediate was not greatly different from its rate of decay over the limited range of OH^- concentration used. The rate of decay of the intermediate was approximately independent of hydroxide concentration from 0.15 to 0.5 M ($k = (1.0 \pm 0.1) \times 10^{-3}$ s^{-1} at 25 °C). At higher base concentrations, the rate increases, up to (2.0 ± 0.2) × 10^{-3} s^{-1} at 0.975 M.

The decay of the N-bound phosphoramidate (δ 7.1) was followed by ^{31}P NMR spectroscopy in 0.5 M NaOH, $\mu = 1.0$ M, at approximately 27 °C. This was coincident with the growth of the signal due to free phosphoramidate (δ 8.6) and had a rate constant of approximately 3 × 10^{-4} s^{-1} under these conditions.

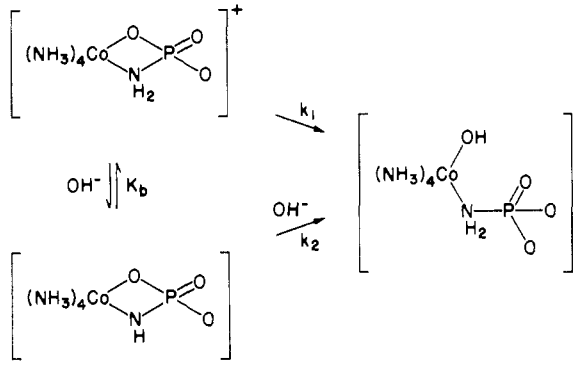
Discussion

Like the previously studied reactions of phosphato derivative–pentaamminecobalt(III) complexes[6,7] with OH^-, $[(NH_3)_5CoDNPP]^+$ reacted to yield two phosphorus-containing products: the free phosphate ester and a major product (<90%) resulting from the loss of the ester group from the complex. The yield of this product corresponded closely to the yield of DNP determined by UV/vis spectroscopy (Table I). The initial product of this major pathway displayed a ^{31}P NMR signal that was broad, and its position was dependent on OH^- concentration. At 0.15 M OH^- the signal was observed at 22.1 ppm, while in 0.975 M OH^- the chemical shift was 30.6 ppm. This intermediate reacted further to yield N-bound phosphoramidate (δ 7.1), which subsequently yielded free phosphoramidate (δ 8.6).

Since the signals at 8.6 and 7.1 ppm have been definitely assigned, the low-field signal could be attributed to aminophosphorane, O-bound phosphoramidate, or the chelate phosphoramidate. If the signal were due to aminophosphorane, the dinitrophenolate release kinetics should have shown an induction period and DNP release should have coincided with decay of the intermediate. This was not observed. The O-bound phosphoramidate was synthesized independently, by intermolecular attack of NH_3 on $[(NH_3)_5CoDNPP]^+$ in a manner analogous to that observed for the uncoordinated DNPP (vide infra). The product of this reaction was a monocation with a ^{31}P NMR chemical shift of +18.7 ppm, which was independent of hydroxide ion concentration. The 22–31 ppm signal was therefore assigned to chelate phosphoramidate. The observed chemical shift was in the region of that observed for chelate phosphate, 24 ppm,[12] which is con-

(12) Seel, F. V.; Bohnstedt, G. *Z. Anorg. Allg. Chem.* **1977**, *435*, 257.

Scheme II



sistent with the assignment of the signal as the chelate phosphoramidate. The shift of the signal with base concentration is ascribed to deprotonation at the bridging nitrogen. However, the chemical shift observed on deprotonation is much larger than that observed for deprotonation at P oxygen. To our knowledge there is only one report of the consequence of N protonation of a P–N bond on the ^{31}P NMR chemical shift. In this case, the effect of protonation was to shift the signal ~5 ppm upfield,[13] which is also greater than the effect of protonation on oxygen, typically ~1–2 ppm upfield shift.

The observation of phosphoramidate formation requires that the mechanism of ester cleavage involve attack by coordinated amido ion at the phosphorus center since there is no other source of ammonia in the reaction. This path would yield initially a five-coordinate aminophosphorane as either an activated complex or short-lived intermediate. It is possible that the phosphorane is an intermediate since some oxyphosphoranes and aminophosphoranes can be stabilized especially when ring systems are involved.[14] However, when an ^{18}O tracer experiment was carried out with an analogous system,[4] viz. the $[(NH_3)_5CoO_3POC_6H_4NO_2]^+$ ion in ^{18}O-labeled water, it was found that ^{18}O was not incorporated into the phosphoramidate product. This result implies that if the aminophosphorane is an intermediate it does not have the opportunity to exchange oxygen with the solvent, unlike its oxyphosphorane equivalent.[3] This experiment is relevant to the current question since the systems are identical except for the leaving group (DNP in this case). It is even less likely then that oxygen exchange will be observed in the current system since the proposed aminophosphorane should be shorter lived, as 2,4-dinitrophenolate is a better leaving group than 4-nitrophenolate. We cannot say therefore if the reaction is a concerted addition–elimination process of if the aminophosphorane has a lifetime, albeit short.

The expulsion of dinitrophenolate from the aminophosphorane yields the N,O chelate phosphoramidate. This is the first time that this chelate has been observed. It hydrolyzes readily to yield the N-bound monodentate phosphoramidate, and this and the subsequent reaction to yield free phosphoramidate have been shown to go without ^{18}O incorporation at phosphorus.[6] Presumably both reactions occur via the usual conjugate base ligand–metal ion cleavage characteristic of Co(III) amine chemistry.[15] The liberation of dinitrophenyl phosphate via path B (Scheme I) also presumably occurs via the $S_N1(cb)$ mechanism by analogy with the case for the 4-nitrophenyl phosphate complex.[6]

The unusual hydroxide dependence of the ring-opening reaction requires some discussion. A possible mechanism for the reaction is shown in Scheme II. The rate law derived for this reaction scheme is

$$k_{obsd} = \frac{k_1 + k_2K_b[OH^-]^2}{1 + K_b[OH^-]}$$

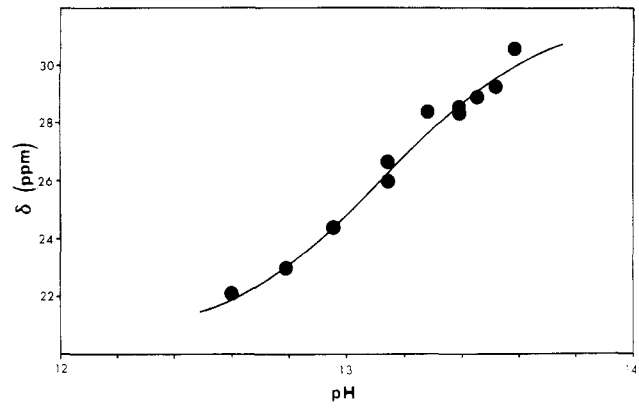


Figure 3. ^{31}P NMR chemical shift vs. pH for the intermediate. The line is calculated for a pK_a of 13.1 and a chemical shift for the protonated species of 18 ppm and for the deprotonated species of 34 ppm.

With use of the value of K_b obtained from the pH dependence of the chemical shift of the chelate (Figure 3) and values for k_1 and k_2 of 1.4×10^{-3} s^{-1} and 2.2×10^{-3} L mol^{-1} s^{-1}, respectively, the derived rate law adequately describes the dependence of k_{obsd} on [OH$^-$]. The proposed hydroxide-independent ring opening of the N-diprotonated phosphoramidate chelate (k_1 path) may proceed via the equivalent structure where one of the protons has migrated from the phosphorus nitrogen to the bridging oxygen, i.e. an intramolecular conjugate base path. Although this oxygen site is more acidic than the nitrogen site, the complex thus formed would be expected to be very sensitive to ring opening. Not only does the protonated oxygen assist in the Co–O rupture but the deprotonated amide could also labilize the bond. The k_2 path probably also occurs via the conjugate base mechanism[15] except that a deprotonated NH_3 group now labilizes the Co–O bond.

The base hydrolysis of uncoordinated DNPP consists of several competing pathways, all of which produce DNP and phosphate. At pH values between 6 and 12 the rate of hydrolysis of uncoordinated DNPP is constant at 8.2×10^{-6} s^{-1} (25 °C). As the pH increases beyond 12, the observed rate increases linearly with OH$^-$ concentration up to 1.0 M.[9] The origins of these observations are as follows: at pH 6 the phosphate is all in the dianion form, which reacts at a constant rate via a supposed unimolecular reaction, the $S_N1(P)$ (metaphosphate) mechanism. Above pH 12 the $S_N2(P)$ mechanism may become important. This involves nucleophilic attack of OH$^-$ at the phosphorus center to form a five-coordinate phosphorane-activated complex or short-lived intermediate. Another possibility, not addressed in the original publication,[9] is that OH$^-$ attacks the carbon atom of the phenol. Alkaline methanolysis however yielded significant amounts of 2,4-dinitroanisole from attack of MeO$^-$ on the phenol carbon.[9] This pathway (attack at C) has been shown to be very significant in other cases of hydrolysis of aryl phosphates by hydroxide and other nucleophiles.[11,16,17] The hydrolysis reaction was first order in OH$^-$ and DNPP up to 1.0 M with a large non-zero intercept attributable to the $S_N1(P)$ reaction, which is zero order in OH$^-$. The derived second-order rate constant for attack of OH$^-$ on DNPP is 2.5×10^{-5} L mol^{-1} s^{-1}. The observed rate constant for hydrolysis in 1 M NaOH is therefore 3.3×10^{-5} s^{-1} (the sum of the first- and second-order paths). Hydrolysis of free DNPP in 1 M aqueous ammonia occurs with an observed rate constant of 2.8×10^{-5} s^{-1} at 25 °C. The products were phosphoramidate (54%) and phosphate (46%). Assuming that all the phosphoramidate is produced by an intermolecular attack of NH_3 on DNPP, the rate constant for attack of NH_3 on the phosphorus center is 1.5×10^{-5} s^{-1}. (The phosphate arises from two reactions, intermolecular attack of OH$^-$ on phosphorus and the metaphosphate process.) Proton NMR spectra of the reaction after ~1 $t_{1/2}$ showed that only 2,4-DNP was produced; no peaks attributable to 2,4-dinitroaniline were observed. The rate en-

(13) Febray, J.; Casabianca, F.; Ries, J. G. *J. Am. Chem. Soc.* **1984**, *106*, 7985.
(14) Ramirez, F. *Acc. Chem. Res.* **1968**, *1*, 168.
(15) Tobe, M. L. *Adv. Inorg. Bioinorg. Mech.* **1983**, *2*, 1–94.
(16) Kirby, A. J.; Vargolis, A. G. *J. Chem. Soc. B* **1968**, 135.
(17) Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 1165.

hancement for attack of the nitrogen nucleophile on the phosphorus center is therefore $\sim 10^3$, comparing the rate of NH_3 attack in 1 M NH_3 to the rate of Co–NH_2^- attack in 1 M OH^-. This apparent rate enhancement is greatly increased when it is appreciated that the amount of nucleophile, i.e. cis-coordinated amido ion, present is very low. If it is assumed that the equilibrium, K_a (Scheme I), is established before the reaction is under way, the rate of loss of reactant is

$$-d[\text{CoDNPP}]/dt = k_3[\text{CoDNPP-H}] + k_4[\text{CoDNPP-H}] \quad (1)$$

This assumption is well-grounded since it is known that exchange of ammine protons on cobalt(III) complexes is rapid in hydroxide solution, usually of the order of 10^3 M^{-1} s^{-1} for monopositive ions at 25 °C.[18] Equation 1 then yields

$$k_{obsd} = \frac{(k_3 + k_4)K_a[OH^-]}{K_a[OH^-] + K_w} \quad (2)$$

Since $K_a[OH^-] << K_w$,[19] eq 2 reduces to

$$k_{obsd} = (k_3 + k_4)\frac{K_a}{K_w}[OH^-] \quad (3)$$

which is the form of the observed rate law with

$$k = (k_3 + k_4)\frac{K_a}{K_w} \quad (4)$$

The pK_a of cobalt(III)-coordinated ammonia for a 1+ ion is ~ 17,[20] which means that the rate of aminolysis by the cis-amido complex, k_3, is about 10^3k or 20 s^{-1} (because $k_4 << k_3$). This is a rate enhancement on coordination of $\sim 10^6$ compared with the attack of NH_3 (1 M) on DNPP.

The work described here has confirmed predictions[6,7] about the intermediacy of the N,O chelate phosphoramidate in the hydrolysis of the phosphate monoesters coordinated to the pentaammine-cobalt(III) moiety. It is also consistent with the substantial rate enhancement arising from attack by the intramolecular nucleophile on the coordinated substrate, in keeping with previous examples of this phenomenon.

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Registry No. $[(NH_3)_5CoOPO_3HC_6H_3(NO_2)_2]Cl_2$, 100113-26-6; $[(NH_3)_5CoOPO_3C_6H_3(NO_2)_2]^+$, 100113-27-7; OH^-, 14280-30-9.

(18) Bramley, R.; Creaser, I. I.; Mackey, D. J.; Sargeson, A. M. *Inorg. Chem.* **1978**, *17*, 244.

(19) This holds if $[OH^-]$ is low, i.e. less than ~ 1 M, since $K_a \approx 10^{-17}$[15] and $K_w \approx 10^{-14}$.

(20) Basolo, F.; Pearson, R. G. "Mechanisms of Inorganic Reactions", 2nd ed.; Wiley: New York, 1967; pp 183–184.