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Hydrolysis of Pyrophosphate to Orthophosphate Promoted by Cobalt(III). Evidence for the Role of Polynuclear Species

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Complex formation between $N_4Co^{III}(aq)$ and pyrophosphate and its influence on the hydrolysis of pyrophosphate to orthophosphate have been studied by means of ^{31}P NMR over the pH range 0-12. Most experiments were undertaken with $N_4 = (tn)_2$; for comparison $(en)_2$ and $tren$ were also examined. There is evidence that the reactive species in regard to hydrolysis is a 3:1 N_4Co^{III} -pyrophosphate complex. At 25 °C and pH 7, $t_{1/2}$ is 1 h, compared with 10 years for corresponding solutions in the absence of cobalt complexes; the enhancement in rate is thus $\sim 10^5$.

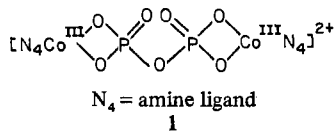
Introduction

Polyphosphates are essential to life processes, and understanding their hydrolysis and related phosphoryl transfer, involving cleavage within a P-O-P framework, is of recognized importance. In biological systems these reactions are catalyzed by enzymes, which quite generally contain metal cations and/or require them as added cofactors.¹ In the absence of enzymic catalysis hydrolysis reactions of simple polyphosphates such as pyrophosphate, linear triphosphate, ADP, and ATP normally proceed extremely slowly in all but highly acidic media.²

In model systems involving hydrolysis of polyphosphates it has not been possible to obtain other than very modest catalytic effects by addition of labile aquo cations such as Mg^{2+} , Ca^{2+} , Zn^{2+} , Cu^{2+} , Mn^{2+} , etc.³ Clearly such systems must have provided, at most, only the smallest fraction of polyphosphate in suitably reactive states.

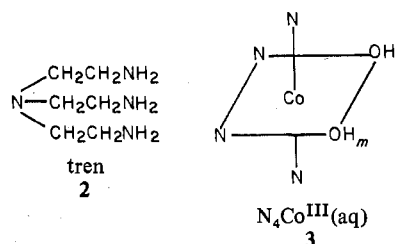
In an earlier study⁴ we were able to demonstrate that chelation of an orthophosphate ester to a cobalt(III) center can lead to extraordinarily large enhancements in rates for ester hydrolysis. The results raised the question as to whether it might be possible to obtain similar metal-promoted accelerations for hydrolysis of a P-O-P polyphosphate linkage. Recently Suzuki et al.⁵ have observed an increase in rate, by a factor of ~ 150 , for the hydrolysis of ATP to ADP and orthophosphate in the presence of $[(dien)CoCl_3]$.

In the present study we set out to determine if one can obtain effective catalysis for the hydrolysis of the simplest polyphosphate, viz., pyrophosphate. From the earlier work⁴ we knew that charge neutralization and introduction of ring strain at the phosphorus center are factors which should aid the process. This led to the idea that a binuclear complex of type 1 might be especially susceptible toward hydrolysis of the



diphosphate linkage, since here one would have electron-withdrawing cobalt centers associated with each phosphorus, as well as ring strain at both. Whether such a complex could be generated remained to be seen.

In an approach to this matter we have now examined aqueous solutions containing pyrophosphate and complexes of the type $N_4Co^{III}(aq)$ where $N_4 = (en)_2$, $(tn)_2$, or $tren$. Here $en = H_2N(CH_2)_2NH_2$ and $tn = H_2N(CH_2)_3NH_2$; we also use the following abbreviations:



In $N_4Co^{III}(aq)$, "aq" refers to $(OH_2)_2$, $(OH_2)(OH)$, or $(OH)_2$, depending on pH. For some situations some trans cobalt(III) species may also be present. In structural formulas the coordination number at the cobalt center is assumed to be 6, with solvent occupying any unspecified sites. The general formulas $H_nP_2O_7$ and H_nPO_4 are also used, where it is understood that n is a function of pH. Charges on ions are most often omitted for better readability.

Phosphorus-31 NMR was chosen as the principal experimental method for convenience, particularly since the approach seemed likely to be instructive concerning the nature of the various phosphorus species present.

Results and Discussion

Most experiments utilized $(tn)_2Co^{III}(aq)$, rather than the $(en)_2$ or $tren$ analogues, because of more favorable solubility characteristics⁶ and also because of more rapid rates of complex formation.⁷

As a prerequisite to detailed studies of pyrophosphate, the NMR spectra of solutions prepared from orthophosphate and $(tn)_2Co^{III}(aq)$ at different pHs were first obtained.⁸ The spectra were recorded on solutions which were kept at pH 3-4

- (1) Mildvan, A. S.; Grisham, C. M. *Struct. Bonding (Berlin)* 1974, 20, 1.
- (2) (a) Van Wazer, J. R.; Griffith, E. J.; McCullough, J. F. *J. Am. Chem. Soc.* 1955, 77, 287. (b) Greenfield, S.; Clift, M. "Analytical Chemistry of the Condensed Phosphates"; Pergamon: New York, 1975; p 22. (c) Holbrook, K. A.; Ouellet, L. *Can. J. Chem.* 1957, 35, 1496. (d) Bruce, T. C.; Benkovic, S. J. "Bioinorganic Mechanisms"; W. A. Benjamin: New York, 1966; Vol. II, Chapter 7. (e) Osterheld, R. K. *Top. Phosphorus Chem.* 1972, 7, 103.
- (3) (a) Cooperman, B. *Biochemistry* 1969, 8, 5005. (b) Schneider, P. W.; Brintzinger, H. *Helv. Chim. Acta* 1964, 47, 1717. (c) Tetas, M.; Lowenstein, J. M. *Biochemistry* 1963, 2, 350.
- (4) Anderson, B.; Milburn, R. M.; Harrowfield, J. M.; Robertson, G. B.; Sargeson, A. M. *J. Am. Chem. Soc.* 1977, 99, 2652.
- (5) Suzuki, S.; Higashiyama, T.; Nakahara, A. *Bioinorg. Chem.* 1978, 8, 277.

- (6) For example, if one attempts to prepare solutions which are ~ 0.1 M in both $(en)_2Co^{III}(aq)$ and pyrophosphate, thick precipitation occurs as soon as the pH is brought above 3-4. Heating such mixtures provides clear solutions again, but with unwanted side effects. Thus some paramagnetic Co^{2+} is produced, which causes the NMR lines to broaden or even disappear. Further, especially at lower pHs, heating by itself results in noticeable hydrolysis of free pyrophosphate to orthophosphate (see ref 2b, Chapter 1).
- (7) (a) Jonasson, I. R.; Lincoln, S. F.; Stranks, D. R. *Aust. J. Chem.* 1970, 23, 2267. (b) Jonasson, I. R.; Murray, R. S.; Stranks, D. R.; Yandell, J. K. *Proc. Int. Conf. Coord. Chem., 12th, 1969* 1969, 32.
- (8) It should be noted that high-quality NMR spectra, especially for the middle pH region, require meticulous attention to purification of the N_4Co complexes. See the Experimental Section.

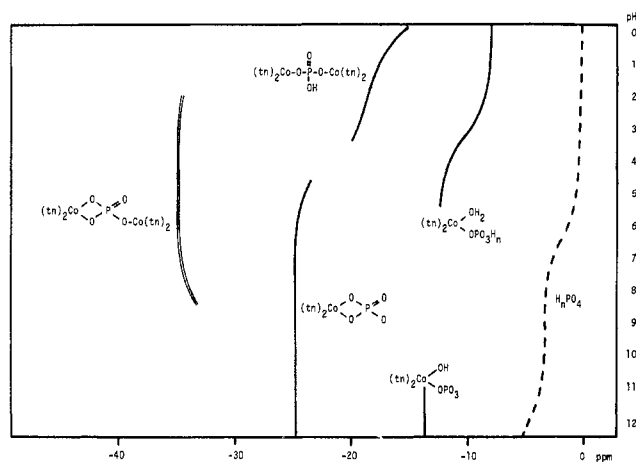


Figure 1. Interpretation of P NMR signals of solutions containing $(tn)_2Co^{III}(aq)$ and H_nPO_4 .

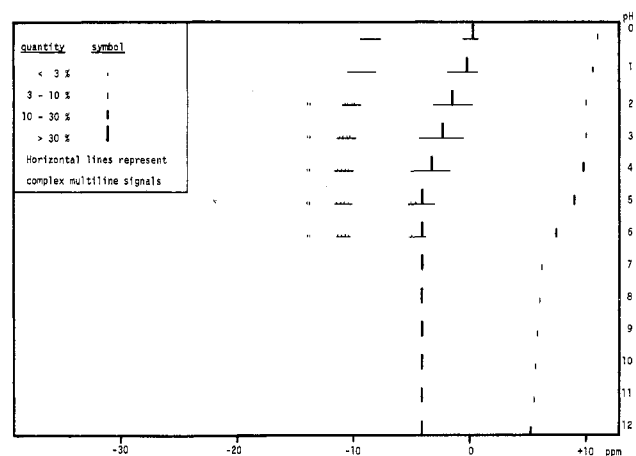


Figure 2. P NMR signals of solutions containing 0.1 M $(tn)_2Co^{III}(aq)$ and 0.1 M $H_nP_2O_7$.

for 1 h and then brought to the respective pH by means of a pH stat and equilibrated for at least 30 min.^{9,10} Detailed results are available.¹¹ Comparison of our results with literature data^{12,13} leads to a straightforward assignment for all major signals, except for the pair of equally intense signals at -35 ppm. We assign the latter signals on the basis that influences of different modes of complexing are incremental and noninterfering.^{12a} Confirmation of this assignment to a binuclear species is provided by the fact that the signal intensities are markedly enhanced (integrals more than doubled) for the 2:1 compared to the 1:1 cobalt to phosphate mixtures. Such a closely spaced pair of equally intense signals is to be expected for a binuclear species containing two *cis*- $(tn)_2Co^{III}$ moieties; thus the configurations may be the same ($\Delta\Delta$ or $\Lambda\Lambda$) or, equally probably, opposite ($\Delta\Lambda$ or $\Lambda\Delta$). Figure 1 summarizes our results and assignments.

The NMR spectra for solutions prepared from pyrophosphate and $(tn)_2Co^{III}(aq)$ were then examined. For solu-

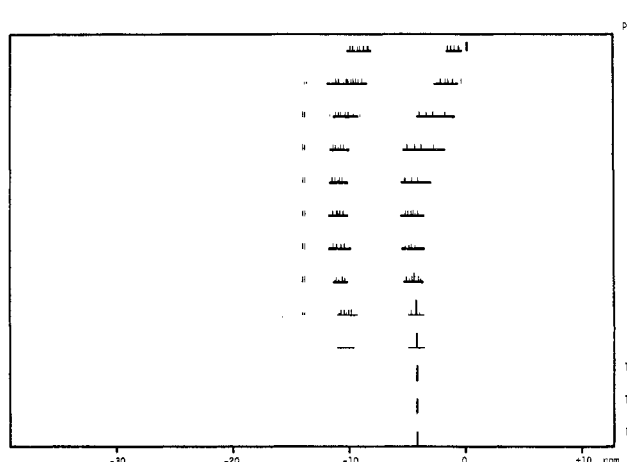


Figure 3. P NMR signals of solutions containing 0.2 M $(tn)_2Co^{III}(aq)$ and 0.1 M $H_nP_2O_7$.

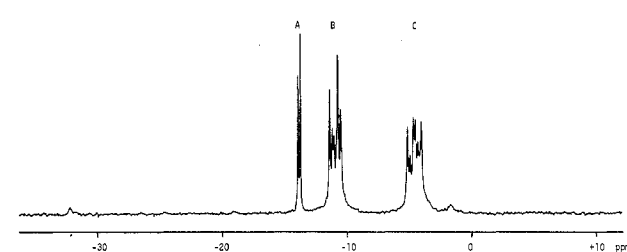
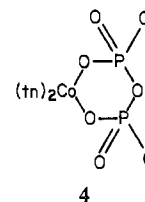


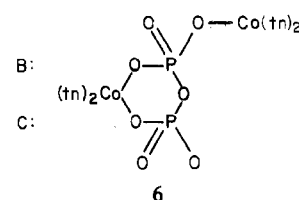
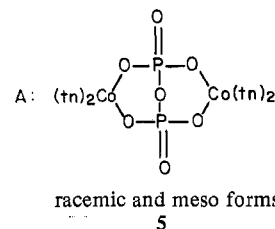
Figure 4. P NMR spectrum of a solution containing 0.2 M $(tn)_2Co^{III}(aq)$ and 0.1 M $H_nP_2O_7$; pH 5.

tions which were 1:1 in $(tn)_2Co^{III}(aq)$ and $H_nP_2O_7$ (0.1 M in each), over the entire pH range there is only one dominant pyrophosphate species, giving rise to the signal at 0 to -5 ppm (see Figure 2). Comparison with literature data^{12,13} leads to the conclusion that this is the bidentate six-membered chelate 4. As shown in Figure 2, besides this dominant complex, there



are weak signals arising from free pyrophosphate (+5 to +10 ppm) and from higher complexes (see below).

For solutions of the 2:1 type, free pyrophosphate is no longer apparent, and the weak signals seen for the 1:1 mixtures are enhanced. Figure 3 gives a summary and Figure 4 a typical spectrum. We offer the following assignments:



- (9) Equilibrations were at ambient temperature, except where otherwise indicated.
- (10) Experiments in which solutions were equilibrated at temperatures higher than ambient gave generally similar spectra, although there were increases in the integrals for some of the smaller peaks which we believe were due to different extents of *cis*-*trans* equilibration for the N_4Co^{III} unit.
- (11) Supplementary material.
- (12) (a) Seel, F.; Bohnstedt, G. *Z. Anorg. Allg. Chem.* **1977**, *435*, 257. (b) Seel, F.; Bohnstedt, G. *Ibid.* **1978**, *441*, 237. (c) Bohnstedt, G. Ph.D. Thesis, Universität des Saarlandes, Saarbrücken, BRD, 1974.
- (13) Cornelius, R. D.; Hart, P. A.; Cleland, W. W. *Inorg. Chem.* **1977**, *16*, 2799.

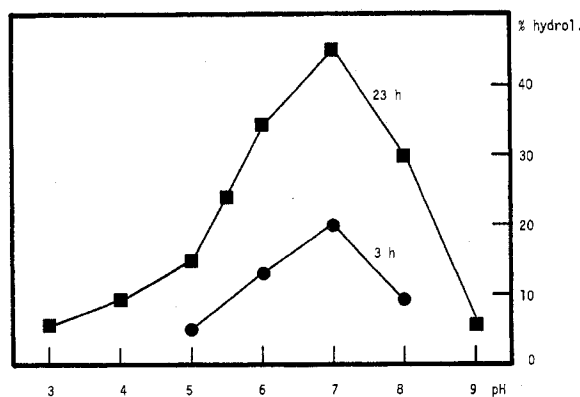
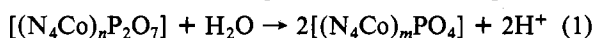


Figure 5. Percent hydrolysis after 3 and 23 h for 0.2 M $(tn)_2Co^{III}(aq)$ and 0.1 M $H_nP_2O_7$.

The above explanation fits the incremental approach and the equal integral value for B and C, as well as the fact that there is apparent coupling between pairs of signals in B and C. B and C would appear to arise from the existence of at least three very similar isomers (probably different configurations of the N_4CoO_2 unit and different ring conformations). If one goes to lower pHs, the B and C patterns shift upfield and display even more signals. The chemical shifts for the two signals labeled A are pH independent, consistent with there being no protons associated with the P_2O_7 unit.¹⁴

For the 2:1 series with $(tn)_2Co^{III}(aq)$ and pyrophosphate, if one records an NMR spectrum of a sample some time after preparation, the spectrum contains signals which are not observed earlier. The intensities of these signals increase with the time elapsed after preparation; further, the same signals are observed in the orthophosphate experiments. In addition, it was found that the pH of such samples drifts noticeably toward lower values. These facts are a clear indication that pyrophosphate complexes hydrolyze to orthophosphate complexes. Schematically, the process may be represented by eq 1. For investigation of this process, a series of experiments



was set up in which carefully prepared 2:1 solutions were held at constant pH by means of a pH stat. During the 24 h following preparation, aliquots were quenched several times with aqueous sodium hydroxide. In the resulting strongly basic medium, hydrolysis of pyrophosphate stops and the complexes are decomposed to release pyrophosphate and orthophosphate as free ligands. Their ratio can then easily be determined by means of the integrals for their NMR signals.

In Figure 5 we summarize the pH dependence of the hydrolysis. There is a clear maximum at pH 7. Analysis of rate data for pH 7 shows the process is not a simple first-order one; rather there is a tailing off after the reaction has proceeded to about 65%. Therefore the influence of the stoichiometric ratio of $(tn)_2Co^{III}(aq)$ to pyrophosphate was checked. Figure 6 shows remarkable differences for the 1:1, 2:1, and 3:1 ratios at pH 7. Thus, for the 3:1 ratio half of the pyrophosphate hydrolyzes within about 2 h; in contrast the 1:1 ratio shows no detectable pyrophosphate hydrolysis even after 22 days!

A series of 3:1 samples was then prepared for examination of the NMR. The spectra are generally similar to those for the 2:1 series (although it is now almost impossible to prepare samples which contain no orthophosphate species). Figure 7 shows a spectrum for pH 5. There is, however, an interesting

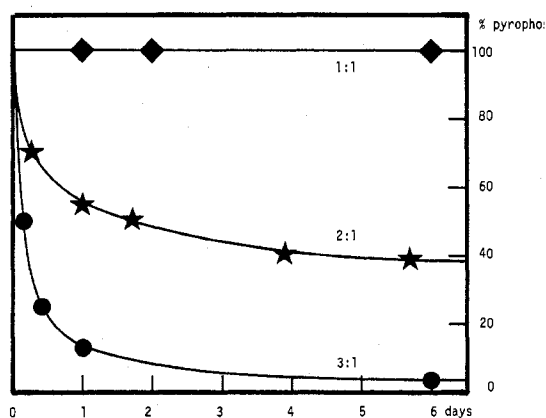


Figure 6. Hydrolysis vs. time for 0.1/0.2/0.3 M $(tn)_2Co^{III}(aq)$ and 0.1 M $H_nP_2O_7$; 25 °C.

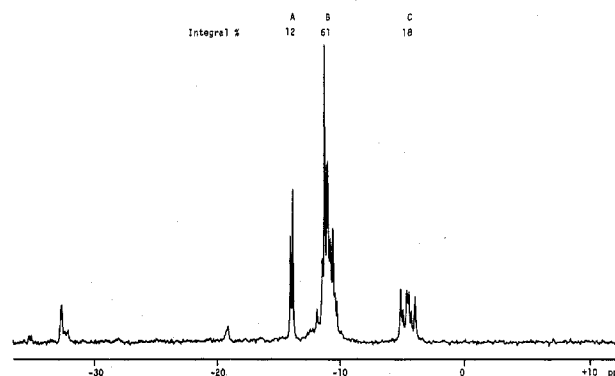
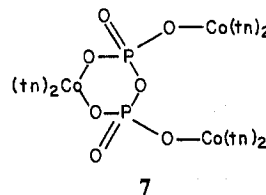


Figure 7. P NMR spectrum of a solution containing 0.3 M $(tn)_2Co^{III}(aq)$ and 0.1 M $H_nP_2O_7$; pH 5.

detail in this spectrum: In contrast to the 2:1 situation (where the integrals for the regions B and C are equal) we now observe a much larger integral at B than at C. This result points to the presence of an additional species in which *both* phosphorus atoms have chemical shifts similar to that of P(B) in formula 6. The following structure is consistent with this idea:



Attempts to prepare solutions of the 4:1 type failed because of thick red precipitations.¹⁵

NMR spectra and solubility data indicate that 1:1, 2:1, and 3:1 complexes are formed in solution. Hydrolysis experiments (Figure 6) make it clear that the 1:1 complex is not reactive at all and that the 3:1 complex is much more reactive than the 2:1 complex. It is not yet clear whether the 2:1 complex is reactive itself or whether it is only part of the preequilibrium process. That the kinetics do not exhibit simple first-order behavior is easily explained by the fact that an essential partner to the preequilibrium, $(tn)_2Co^{III}(aq)$, is not present in large excess and that the $(tn)_2Co^{III}$ becomes more and more tied up in the products as the reaction proceeds. Therefore, we have

(14) Alternative assignments for the signals, based on four-membered chelates, were discounted on the basis of comparison with the orthophosphate results. In addition, for the en series, Seel and Bohnstedt^{12b} report a remarkable stability for the six-membered chelate $(en)_2CoP_2O_7^-$ and its protonated forms.

(15) The solid was centrifuged, washed, and dissolved in aqueous sodium hydroxide. The NMR showed that it contained neither phosphate nor pyrophosphate. The solubility of $[(tn)_2Co^{III}(aq)](ClO_4)_3$ in neutral solutions was therefore checked and found to be ~ 0.05 M. Stranks et al.⁷ report the preparation of $[(tn)_2Co(OH)OH_2](ClO_4)_2(s)$ under essentially the same conditions as for the above experiment. This substance has the same appearance as the precipitate in our experiment.

used kinetic data only for the very early stages of reaction to compare reaction rates. With this approach, and considering the fact that in the 3:1 series only about half of the pyrophosphate is initially present as a 3:1 complex (integral in Figure 7), we estimate $t_{1/2}$ to be of the order of 1 h. Compared to hydrolysis of free pyrophosphate,¹⁶ which has a half-life of the order of 10 years for comparable pH and temperature, the hydrolysis of the $[(\text{tn})_2\text{Co}]_3\text{P}_2\text{O}_7$ is about 10^5 times faster.

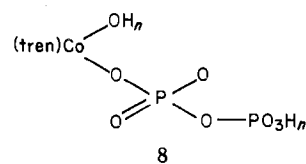
The complex formation between $(\text{tn})_2\text{Co}^{\text{III}}(\text{aq})$ and $\text{H}_n\text{P}_2\text{O}_7$ can easily be monitored by means of base consumption in the pH stat. At pH 4 it is largely accomplished within 1 h; at lower pHs it takes considerably longer. Above pH 4, complex formation is even faster, but now liberation of protons due to the hydrolysis of pyrophosphate to orthophosphate becomes noticeable as another source of base consumption. The increase of the complex formation rate, as one goes to less acidic media, can be explained by the increasing fraction of $(\text{tn})_2\text{Co}^{\text{III}}(\text{aq})$ present as $[(\text{tn})_2\text{Co}(\text{OH}_2)(\text{OH})]^{2+}$, the latter having very much greater lability than the diaquo species.^{7b}

The relative rates of complex formation and subsequent hydrolysis of the P–O–P bond system are especially favorable in the case of the ligand tn. Here one can build up the 2:1 complexes at pH 4 within 1 h without any detectable hydrolysis and then adjust the pH and study the hydrolysis (see Figure 5).

As mentioned above, with en attempts to prepare solutions lead to thick precipitations as one brings the pH above 3–4. The solution becomes clear again under basic conditions. We therefore prepared a solution $[(\text{en})_2\text{Co}^{\text{III}}(\text{aq})$, 0.2 M; $\text{H}_n\text{P}_2\text{O}_7$, 0.1 M; pH ~ 10] and lowered its pH slowly. Between pH 8 and 7 precipitation again occurred. The experiment was carefully repeated, keeping the pH at 8, where precipitation was not observed. After 23 h, quenching and NMR measurements showed that 10% of the pyrophosphate had been converted into orthophosphate. Although this is only one-third of the amount found in a comparable experiment with $(\text{tn})_2\text{Co}^{\text{III}}$ (cf. Figure 5), we conclude that the hydrolysis behavior is generally similar. The difference in the amount of hydrolysis may be attributed to the changed experimental approach and the fact that the $(\text{en})_2\text{Co}^{\text{III}}(\text{aq})$ system is considerably less labile^{7b} than the tn analogue. For the en system it is likely that hydrolysis is no longer the rate-limiting step.

For the ligand tren geometric reasons require that the fifth and sixth coordination positions for $(\text{tren})\text{Co}^{\text{III}}$ complexes be cis, a fact which eliminates ambiguities concerning cis/trans isomerism. In addition, for $(\text{tren})\text{Co}^{\text{III}}$ these two remaining coordination positions are not equivalent (one is trans and the other cis to the tertiary amine N). It turned out that the solubility problems in experiments with $(\text{tren})\text{Co}^{\text{III}}(\text{aq})$ are similar to those with $(\text{en})_2\text{Co}^{\text{III}}(\text{aq})$. On the other hand, the tendency to decompose to Co^{2+} appears to be reduced (probably because of the extraordinary stability of the tetradentate chelation). We therefore attempted to get around the solubility problems by applying higher temperatures. These experiments provided evidence for complex formation as well as for production of orthophosphate species. Complex formation for $(\text{tren})\text{Co}^{\text{III}}(\text{aq})$, however, is even slower than with $(\text{en})_2\text{Co}^{\text{III}}(\text{aq})$. Another consequence of the relative inertness of the tren complexes is that the quenching procedure with aqueous sodium hydroxide (well established with tn complexes) does not fully decompose the complexes within 1 week, which makes the quantitative measurements difficult.

The same inertness allows the observation of species which could not be detected for the tn series such as **8**, which gives an AB pattern in the P NMR spectrum (Figure 14).¹¹ In the same spectrum the signals for the six-membered chelate

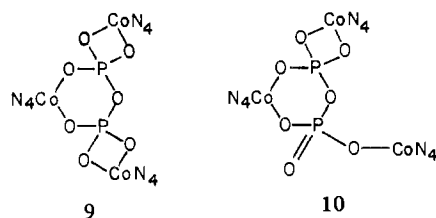


$(\text{tren})\text{CoP}_2\text{O}_7$ are also of the AB type, which is a nice illustration of the inequality of the two coordination sites of $(\text{tren})\text{Co}^{\text{III}}$. The spectra of the higher complexes become rather complex (Figure 15).¹¹

Conclusions

For the 1:1 mixtures of $\text{N}_4\text{Co}^{\text{III}}(\text{aq})$ and $\text{H}_n\text{P}_2\text{O}_7$, production of the six-membered chelate (**4**) is clearly dominant, and this species exhibits no detectable reactivity toward hydrolysis of the P–O–P linkage. On the other hand, with increased metal to pyrophosphate ratios, we observe evidence for dinuclear and trinuclear complexes paralleled by marked reactivity toward pyrophosphate hydrolysis. It therefore seems clear that efficient hydrolysis requires the formation of a complex containing more than a single metal center. For the $(\text{tn})_2$ system, the significantly greater hydrolysis rates observed for 3:1 compared to 2:1 mixtures points to the 3:1 complex being much more reactive, and indeed we can quantitatively explain the rate behavior on the basis that reaction proceeds entirely through a 3:1 species. Geometrical considerations render it unlikely that 4:1 or higher complexes are of any importance.

The special stability of the six-membered ring (as in **4**) is likely to work against the development of significant concentrations of dinuclear complexes with structures of type **1**, at least for our reaction conditions. Indeed, the NMR results support the view that the principal 2:1 species has the general features of **6**. The NMR spectrum is likewise consistent with the idea that the principal 3:1 species has the features of **7**. It is noteworthy, though, that structure **7** relates very closely to **1**. In fact, if all three cobalts become chelated to the pyrophosphate, we obtain **9**, which has the structural features of **1** together with the six-membered ring system. One would suppose that **9**, with three electron-withdrawing metal centers and two four-membered and presumably strained rings, would be every bit as reactive toward pyrophosphate hydrolysis as our earlier proposed structure **1**. The related 3:1 structure **10**,



in which two cobalts are chelated, would also be expected to be highly reactive. It should be emphasized that in the NMR data we see no direct evidence for **9** or **10**, nor would one expect to observe such species directly if they are as reactive toward hydrolysis as we suppose.

In Figure 8, we formulate a general scheme which summarizes some of the important features of this system. The distribution of pyrophosphate between 1:1, 2:1, and 3:1 complexes will depend on such factors as the metal to pyrophosphate ratio, time after mixing, identity of N_4 , and pH. With $\text{N}_4 = (\text{tn})_2$, for a 2:1 ratio and at appropriate pH, we have been able to build up the 2:1 species until it represents close to 100% of the total phosphate (i.e., before significant hydrolysis to orthophosphate has occurred). At a 3:1 ratio, we have obtained up to about 50% of the total phosphate as the 3:1 species, but limiting factors are the high reactivity toward hydrolysis to orthophosphate as well as the stability of the 3:1 complex. However, the $(\text{tn})_2$ system comes fairly close to a

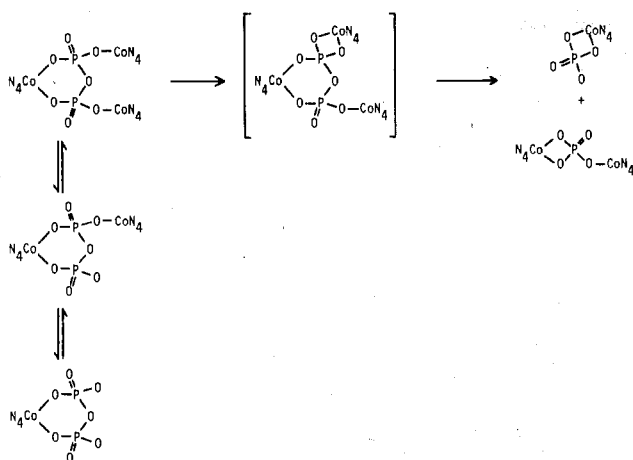


Figure 8. General reaction scheme.

situation where one has preequilibrium formation of the pyrophosphate complexes followed by rate-determining hydrolysis to orthophosphate, and here we can certainly obtain a buildup of high concentrations of polynuclear pyrophosphate complexes. For the (en)₂ and tren systems, formation of the pyrophosphate complexes is much slower and now appears to be rate limiting; the relatively faster hydrolysis leading to orthophosphate now precludes significant buildup of the 3:1 pyrophosphate complexes.

The formation of the polynuclear species is certainly pH dependent, and we believe this aspect is the main contributor to the pH dependency of the overall hydrolysis process.

The requirement of polynuclear metal complexation for efficient hydrolysis of polyphosphates may turn out to be quite general. Some evidence for this phenomenon has been seen in previous studies, such as those by Suzuki et al.⁵ A related observation is that polyphosphate hydrolysis can be markedly accelerated by solid metal hydroxides;¹⁷ for such systems the polyphosphate will almost certainly be part of a polynuclear cluster. The difficulties of obtaining stoichiometric and mechanistic information for systems of the latter type are well recognized.^{3a}

The present study demonstrates a dramatic enhancement in the rate of pyrophosphate hydrolysis for the biologically important neutral pH region. This is, to our knowledge, the first case where a catalytic effect of this magnitude ($\sim 10^5$) has been seen for hydrolysis of the P–O–P linkage in a homogeneous model system.

Experimental Section

en, tn, tren, Na₄P₂O₇·10H₂O, and Na₂HPO₄, as well as all other reagents, were commercially obtained (purest available) and used without further purification.

[(en)₂Co(CO₃)](ClO₄). The chloride salt, synthesized following the procedure of Schäffer and Springborg,¹⁸ was dissolved in water and the perchlorate precipitated by addition of a saturated solution of NaClO₄. The product was recrystallized twice from hot water (70 °C).

[(tn)₂Co(CO₃)](ClO₄). The chloride was obtained and converted to the perchlorate salt according to the preparation of Schäffer and Springborg.¹⁹ The product was recrystallized twice from hot water (70 °C).

[(tren)Co(CO₃)](ClO₄). The preparation published by Scheidegger²⁰ was slightly modified. A 2.93-g sample of tren (20 mmol) was dissolved in 75 mL of water and neutralized with 60 mmol of concentrated HClO₄. Then 8.2 g of NaHCO₃ and 9.55 g of PbO₂ were

added. This inhomogeneous slurry was cooled to 0 °C and a solution of 7.32 g of Co(ClO₄)₂·6H₂O in 20 mL of water slowly added over a period of 1 h with stirring while the mixture was kept in an ice bath. Stirring was continued for 3 h at 60 °C and 30 min at 70 °C. After being cooled, the solution was filtered (Celite) and a solution of 1.56 g of AgClO₄ slowly added. After another filtration (Celite), the filtrate was reduced to ~ 20 mL. At ~ 70 °C, 60 mL of ethanol was added. When the solution was cooled, the complex crystallized as red crystals. The product was recrystallized (by dissolving in 20 mL of water at 70 °C and then adding 70 mL of hot ethanol and cooling overnight). The overall yield was 4.32 g (55%).

Warning! The potential hazards of compounds containing both perchlorate and organic ligands always require precautions, including limitations in the amounts handled!

[N₄Co^{III}(OH₂)₂]³⁺. The conversion of the carbonato to the diaquo species is usually achieved by adding excess acid and expelling the CO₂ by heating.^{7a} It turned out, especially for the tn series, that heating results in production of notable amounts of paramagnetic Co^{II} species. Better results were obtained by addition of 2.5 mmol of HClO₄/mmol of finely ground [N₄Co(CO₃)](ClO₄) (caution!) and stirring under an aspirator vacuum for 20 min in the dark.

Preparation of N₄Co–Pyrophosphate Complex Solutions (Example). Ten milliliters of 0.4 M [(tn)₂Co^{III}(aq)]³⁺ (freshly prepared as perchlorate; see above) and 10 mL of 0.2 M Na₄P₂O₇ were mixed in the thermostated titration jar of a pH stat apparatus. After 30 min, the pH was raised to 4 and maintained there for 1 h (the building up of complexes being monitored by the base consumption). Then the final pH was adjusted and the NMR spectrum recorded as soon as the solution was in equilibrium.

Monitoring the Hydrolysis. A solution prepared according to the above procedure was maintained at a given pH with a pH stat. Samples (0.6 mL) were taken at intervals and quenched by the addition of aqueous 1 M NaOH (0.6 mL). Three days later, all phosphate and pyrophosphate were present as free ligands and the integral of their P NMR signals revealed the amount of hydrolysis at the time of the quenching. The quenching process itself causes no significant hydrolysis, as seen from the fact that solutions quenched immediately after preparation show no detectable amounts of orthophosphate.

Attempted Separation. Attempts were made to separate into its components a 2:1 mixture prepared from (tn)₂Co^{III}(aq) and H₇P₂O₇ (which was first equilibrated for 1 h at pH 4). Thin-layer electrophoresis showed one large spot moving toward the cathode, but there was a marked tailing, reaching almost back to the starting point. Another experiment with Sephadex ion-exchange resin SP C25 120 in an acetic acid buffer at pH 4.7 gave three bands, but again there was a lot of tailing. After the collection and evaporation to small volumes, we obtained NMR spectra of only poor quality. All fractions gave spectra indicating that there are signals in the areas A, B, and C (cf. Figure 4), but no details or quantitative information could be gained. We believe these results are compatible with our ideas about the species in solution: for these 2:1 mixtures almost all pyrophosphate is present as 2:1 complexes as shown in 5 and 6, charge and size being similar. These complexes are in equilibrium with each other and their components, with rates of formation and decomposition fast compared with the time scale of the above-mentioned separation experiments.

³¹P NMR. Measurements were made with a JEOL JNM-FX60Q Fourier transform NMR spectrometer at a field of 14 kG, using a 10-mm P-probe (24.15 MHz). All spectra were taken at ambient temperature (22 °C). Chemical shifts are relative to 1 M aqueous H₃PO₄, positive values being upfield. Since broad-band [H] decoupling did not improve spectra quality, it was not applied, in order not to affect the quantitative information for the integral. Typical parameters used were as follows: spectrum width, 1208 Hz (=50 ppm); offset frequency, 56.74 kHz; pulse width, 7 μs; pulse repetition time, 3.5 s; number of accumulations, 200. Varying the pulse repetition time demonstrates that 3.5 s is long enough to avoid partial saturation. In a calibration series it was shown that peak areas are proportional to concentrations. The internal lock was accomplished by using 25% D₂O in the solvent or a concentric inner cell containing D₂O. The initial stoichiometric concentrations of pyrophosphate (or phosphate) were always 0.1 M. Higher concentrations caused solubility problems, and lower concentrations would require many more accumulations.

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Registry No. [(en)₂Co^{III}(OH₂)₂]³⁺, 21247-59-6; [(tn)₂Co^{III}(OH₂)₂]³⁺, 51286-38-5; [(tren)Co^{III}(OH₂)₂]³⁺, 41884-78-0; [(en)₂Co(CO₃)](ClO₄), 15155-12-1; [(tn)₂Co(CO₃)](ClO₄), 31188-

12-2; [(tren)Co(CO₃)](ClO₄), 41593-06-0; H₄P₂O₇, 2466-09-3.

Supplementary Material Available: Figures 9-15, providing sample spectra, detailed results for pH 0-12 at both 1:1 and 2:1 molar ratios of (tn)₂Co^{III}(aq) to orthophosphate, and a summary of related literature data^{12,13} (7 pages). Ordering information is given on any current masthead page.

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Structural Studies on Polynuclear Osmium Carbonyl Hydrides. 15.¹ Preparation and X-ray Crystal Structure of (μ-H)Os₃(CO)₁₀(μ-NHSO₂C₆H₄Me), a Complex with an Os(μ-H)(μ-N)Os Bridge

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The complex (μ-H)Os₃(CO)₁₀(μ-NHSO₂C₆H₄Me) has been synthesized from (μ-H)₂Os₃(CO)₁₀ and MeC₆H₄SO₂N₃. This species crystallizes in the triclinic space group *P* $\bar{1}$ with *a* = 8.246 (1) Å, *b* = 8.668 (1) Å, *c* = 18.572 (3) Å, α = 89.92 (1)°, β = 98.61 (1)°, γ = 113.05 (1)°, *V* = 1205.4 (2) Å³, ρ_{calcd} = 2.82 g cm⁻³ for mol wt 1021.95, and *Z* = 2. Diffraction data (4.5° < 2θ < 50°; Mo Kα) were collected with a Syntex P2₁ diffractometer, and the structure was solved by standard Patterson, Fourier, and least-squares refinement techniques. The resulting discrepancy indices were *R*_F = 4.0% and *R*_{wF} = 2.9% for all 4276 reflections (none rejected) and *R*_F = 3.2% and *R*_{wF} = 2.8% for those 3786 data with |*F*_o| > 3σ(|*F*_o|). The molecule contains a triangular arrangement of osmium atoms; Os(1) and Os(2) are each linked to three terminal carbonyl groups, while Os(3) is linked to four. Atoms Os(1) and Os(2) are additionally bridged by a hydride ligand (which was located and refined) and by a >NHSO₂C₆H₄Me ligand. Osmium-osmium bond lengths are Os(1)-Os(3) = 2.858 (1) Å and Os(2)-Os(3) = 2.847 (1) Å (unbridged) and Os(1)-Os(2) = 2.814 (1) Å (μ-H, μ-N bridged). Within the Os(1)-H(br)-Os(2) system, interatomic parameters are Os(1)-H(br) = 1.91 (8) Å, Os(2)-H(br) = 1.96 (8) Å, and Os(1)-H(br)-Os(2) = 93.5 (34)°. Within the Os(1)-N-Os(2) system, parameters are Os(1)-N = 2.162 (7) Å, Os(2)-N = 2.145 (7) Å, and Os(1)-N-Os(2) = 81.6 (2)°.

Introduction

The unsaturated cluster compound (μ-H)₂Os₃(CO)₁₀ has been shown to react with certain diazo compounds RHC⁻-N⁺≡N (R = H⁴, CO₂Et⁵) with loss of dinitrogen to give the insertion products (μ-H)Os₃(CO)₁₀(μ-CH₂R). These results prompted us to examine the interaction of (μ-H)₂Os₃(CO)₁₀ with an analogous dipolar compound, an azide RN⁻-N⁺≡N. This article describes the characterization of the insertion product (μ-H)Os₃(CO)₁₀(μ-NHSO₂C₆H₄Me) that results from reaction with *p*-toluenesulfonyl azide.

Experimental Section

Os₃(CO)₁₂ was prepared by the method of Nyholm⁶ from osmium tetraoxide. The synthesis of H₂Os₃(CO)₁₀ is described by Kaesz et al.⁷ Tosyl azide was prepared by a literature procedure.⁸

Infrared spectra were obtained on a Perkin-Elmer 467 spectrophotometer. Proton NMR spectra were recorded on a Varian HA-100 spectrometer. Electron-impact mass spectra were obtained by Mr. Joe Wrona with a Varian MAT CH-5 instrument at 70 eV. Elemental analyses were performed at the University of Illinois by the micro-analytical laboratory of the School of Chemical Sciences.

Table I. Experimental Data for the Diffraction Study of (μ-H)Os₃(CO)₁₀(μ-NHSO₂C₆H₄Me)

(A) Crystal Parameters at 25 °C ^a	
<i>a</i> = 8.2462 (14) Å	<i>V</i> = 1205.4 (2) Å ³
<i>b</i> = 8.6681 (14) Å	<i>Z</i> = 2
<i>c</i> = 18.5723 (28) Å	mol wt 1021.95
α = 89.923 (13)°	ρ(calcd) = 2.82 g cm ⁻³
β = 98.614 (13)°	μ(calcd) = 168.6 cm ⁻¹
γ = 113.046 (12)°	space group <i>P</i> $\bar{1}$
(B) Measurement of Intensity Data	
radiation: Mo Kα (λ = 0.710 730 Å)	
monochromator: highly oriented graphite, 2θ _{mono} = 12.2°, equatorial mode	
rflctns measd: + <i>h</i> , ± <i>k</i> , ± <i>l</i>	
2θ range: 4.5-50.0°	
scan type: coupled θ(cryst)-2θ(detector)	
scan speed: 3.0°/min in 2θ	
scan width: [2θ(Mo Kα ₁) - 0.85]° → [2θ(Mo Kα ₂) + 0.85]°	
bkgd measmt: stationary crystal-stationary counter at beginning and end of each scan, each for one-third of the total scan time	
std rflctns: 0, 0, 13, 263, and 403; these were measured after every 97 rflctns. No decay in intensity was observed	
rflctns collected: 4267 unique data	

^a Unit cell parameters were derived from a least-squares fit to the setting angles at the unresolved Mo Kα components of 12 Friedel pairs of reflections well scattered in reciprocal space with 2θ values between 25 and 30°.

Synthesis of (μ-H)Os₃(CO)₁₀(μ-NHSO₂C₆H₄Me). To a stirred solution of (μ-H)₂Os₃(CO)₁₀ (30 mg) in chloroform (5 mL) was added an excess (4 drops, ca. 60 mg) of *p*-toluenesulfonyl azide. After 24 h the bright yellow solution was evaporated to dryness, and the residue was extracted with a small amount of diethyl ether. This solution was cooled to -25 °C for several hours, and the yellow precipitate was collected. Recrystallization from dichloromethane and drying

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