METAL ION PROMOTED PHOSPHORYL TRANSFER REACTIONS

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by

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DECLARATION

The work presented in this thesis is

the original work of the candidate

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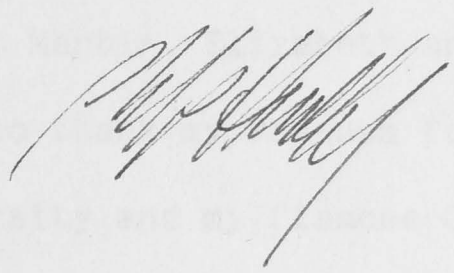

Philip Hendry

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ABSTRACT.

The aim of this work was to investigate the reactivity of various metal complex ions incorporating phosphate derivatives, with the intention of identifying the factors by which metal ions are able to enhance the rate of phosphoryl transfer reactions. Once identified it was the intention of this work to quantitate these factors and apply this information to the question of mechanism of action of several phosphoryl transfer enzymes.

Intermolecular attack of hydroxide ion on phosphate di- and triesters coordinated to a trivalent metal complex ion proceeds ~100-500 fold faster than the analogous reaction of uncoordinated esters. The enhancement of rates of reaction by this method could usefully augment other modes of activation by enzymes in the reactions of phosphate derivatives but, could not solely account for the reactivity encountered in such systems.

Intramolecular attack of coordinated nucleophiles on cis coordinated phosphate esters proceeds much more readily. The nucleophiles which in this study included coordinated amido and hydroxide ions attack at the phosphorus centre to yield initially a 4-membered chelate ring either as relatively stable products or transient intermediates. The reaction is quite general and is observed for a range of esters attached to Co(III) and Ir(III) complex ions. The lysis reaction can, in suitable systems, occur ~10^7 fold faster than that for the uncoordinated ligands.

During this study, it was observed that the reactions involving Co(III) as the central metal ion proceeded ~10^3 fold faster than the analogous Ir(III) reactions. This effect has been attributed to the energy required to form the chelate ring. Ir(III) is a larger ion than

Co(III), and it is argued that this results in greater strain in the chelate ring and a correspondingly reduced rate of cyclisation and lysis. This has important ramifications for biological phosphoryl transfer reactions; the ions typically involved in enzymic reactions of phosphate derivatives, Mg^{2+} and Zn^{2+}, have ionic radii even larger than Ir(III). The large size of these ions militates against metal ion promoted reactions of these derivatives occurring via formation of the 4-membered chelate ring. However, the studies do show the efficiency of the intramolecular pathway when compared to the intermolecular reactions. Enzymes of course could organize the substrates, metal ions and nucleophiles to react in this way without necessarily going via the intermediacy of the chelate ring. Many phosphoryl transfer enzymes require two or more metal ions for activity and it is conceivable that the efficiency of the enzyme arises from the combined effects of the metal ions as described above.

The 4-membered chelate esters formed by the intramolecular reactions did not hydrolyse with cleavage of the ester group despite the strained ring. The esters chelating the Ir(III) ion reacted rapidly with P-O cleavage to give monodentate esters, ie the ring opening of the chelate was the only reaction observed. In the Co(III) chemistry metal-ligand bond rupture prevailed. Intermediates of this type therefore do not seem relevant in the enzymic systems.

Co(III) complex ion mediated hydrolysis of triphosphate ion proceeds rapidly in the presence of two Co(III) complex ions. One of the Co(III) ions coordinates the triphosphate, neutralizing the charge and presumably making the phosphorus atoms more electrophilic. The second Co(III) ion coordinates to the preformed Co(III)-P_3O_{10} adduct and organizes a nucleophilic attack on a phosphorus atom by a coordinated hydroxide. At pH 8, the reaction proceeds 5×10^5 fold more rapidly

than the hydrolysis of triphosphate at that pH. It is a very efficient path considering that the reaction requires the breaking of a very stable 6-membered chelate ring.

Finally, the results of the model systems described above are applied to aspects of the mechanisms for several phosphoryl transfer enzymes which are relatively well characterized and have an absolute requirement for metal ion cofactors. In enzymic systems, the effect of several metal ions acting in concert, ie one to provide charge neutralization/electron density polarization and the other responsible for the positioning and activation of the nucleophile, together with the protonation of the leaving group by a suitable enzymic acid could accommodate the observed rate enhancements of up to 10^{11} fold in some enzymes.

TABLE OF CONTENTS.

ABBREVIATIONS

ADP	adenosine 5'-diphosphate
AMP	adenosine 5'-monophosphate
AP	alkaline phosphatase
ATP	adenosine 5'-triphosphate
BNPP	bis(4-nitrophenyl)phosphate
Caps	cyclohexylaminopropane sulfonic acid
Ches	2-(cyclohexylamino)ethane sulfonic acid
DMP	dimethylphosphate
DMSO	dimethylsulphoxide
DNP	2,4-dinitrophenolate
DNPP	2,4-dinitrophenylphosphate
DSS	sodium 2-dimethyl-2-silapentane-5-sulfonate
en	1,2-diaminoethane
ENPP	ethyl-4-nitrophenylphosphate
Hepes	N-2-hydroxyethylpiperazine-N'ethane sulfonic acid
Mes	2-(N-morpholino)ethane sulfonic acid
NMR	nuclear magnetic resonance
NP	4-nitrophenol(ate)
NPP	4-nitrophenylphosphate
NTP	Nucleoside 5'-triphosphate
Pipes	piperazine- N,N'-bis(2-ethane sulfonic acid)
Tacn	1,4,7-triazacyclononane
TMP	trimethylphosphate
tn	1,3-diaminopropane
Tris	Tris(hydroxymethyl)aminomethane
YIP	yeast inorganic pyrophosphatase

CHAPTER 1

INTRODUCTION

1.1 Rationale.

Phosphorus is an essential nutrient for all living organisms. It is taken up and utilized as phosphate and its derivatives, ie esters, amidates and anhydrides. These compounds are the major source of chemical energy for biological requirements including general synthesis and muscular movement. In addition, phosphate is required for nucleic acids and also plays an important role in the regulation of many enzymic activities.[1] The biological manipulation of phosphates is under the control of numerous phosphate utilizing enzymes, and many of these enzymes are activated by metal ions. The role that metal ions play in enzymatic phosphoryl transfer is in general unknown. This thesis will outline and quantitate effective roles for such metal ions in non-enzymic phosphoryl transfer. Further, these observations will be extrapolated to propose roles for the metal ions in several well characterised enzymes. An introduction to phosphoryl transfer both enzymic and non-enzymic, is provided as background to the work and will be described in this chapter.

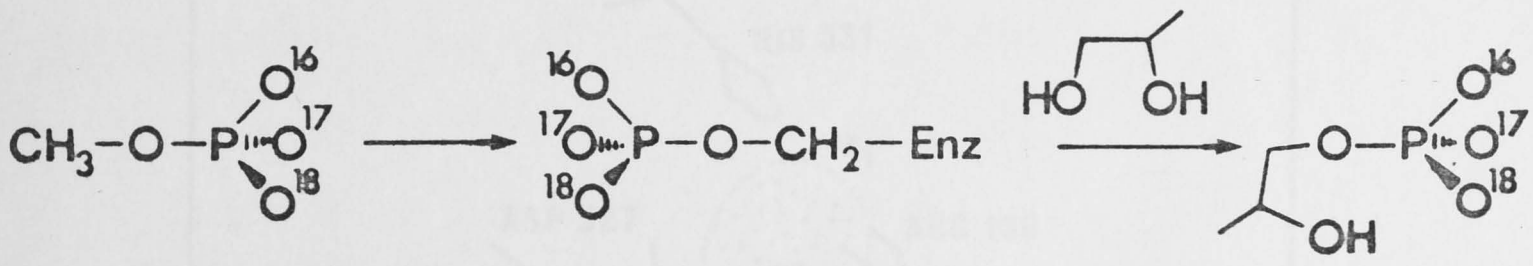
1.2 Alkaline Phosphatase.

Alkaline phosphatases (APs) are widespread in nature and catalyse the non-specific hydrolysis of phosphate monoesters with a rate maximum near pH 7-8. APs have been isolated from mammalian and bacterial sources and all characterised APs have been found to contain catalytically essential Zn^{2+} ions and Mg^{2+}.[2]

The best characterised AP is the one isolated from E. coli and this review will be confined to this particular enzyme. The function of the enzyme is not fully understood. However, the location of the enzyme in the periplasmic space and the recent studies that have localized the gene for AP in the region of the chromosome that codes for proteins of the phosphate transport system, suggest that the role of AP is to provide a source of inorganic phosphate to the cell. The non-specific nature of AP means that it is able to utilize a wide range of phosphate monoesters from the environment to provide a source of phosphate to the phosphate transport system. E. coli grown in limiting phosphate conditions show enhanced alkaline phosphatase activity, this activity is manifested in an increase in the amount of AP in the cell and is due to activation of the AP synthesizing gene by some effector molecule.[3]

E. coli AP is a dimer of molecular weight 94,000. As isolated it contains at least two and up to four Zn^{2+} ions and two Mg^{2+} ions per dimer. The metal ions appear to play two roles in the enzyme; two Zn^{2+} ions are required for activity and these are called "catalytic" and are bound very strongly to the enzyme. The two further Zn^{2+} ions and the two Mg^{2+} ions, termed "structural", in addition to enhancing the activity of the enzyme, stabilize its tertiary and quarternary structure. This terminology may be misleading since it is likely that two Zn^{2+} ions per subunit are involved in the catalytic process, (vide infra). At pH 6.5 addition of two Zn^{2+} ions to the apo-dimer results in an active enzyme. ^{113}Cd NMR of the corresponding $^{113}Cd^{2+}$ substituted enzyme shows the two metal ions to be in identical sites. However, when phosphate is added to the enzyme in this state both the metal ions appear to bind to a single subunit of the dimer. This is due to a cooperative effect between binding of the phosphate and binding the second or "structural" Cd^{2+} ion.

Hydrolysis of phosphate monoesters by AP occurs at a rate which is largely independent of the nature of the ester function. In the presence of alcohols AP also catalyses the transphosphorylation of monoesters.[2] The enzymic transphosphorylation (and presumably hydrolysis), proceeds with overall retention (probably via two inversions) of configuration at phosphorus as determined by the use of a stereospecifically $^{16}O^{17}O^{18}O$ labelled phosphate ester and enzymic transfer to 1,2-propanediol,[4] (Scheme 1.1).



Scheme 1.1

Transphosphorylation of (R)-Methyl$^{16}O^{17}O^{18}O$-phosphate catalysed by E. coli Alkaline Phosphatase.

The enzyme is phosphorylated at serine 102 in a rapid initial reaction preceding the rate determining step. The rate determining step depends on the pH of the reaction. In acid pH the dephosphorylation of the serine is rate limiting whilst at higher pH, dissociation of the enzyme·phosphate complex is rate limiting. This explains why the reaction rate is independent of the leaving group. Burst kinetics for release of alcohol have been observed with carefully dephosphorylated enzyme at low temperature.[4] Stopped flow kinetics at acid pH have shown that the rate of phosphorylation of serine 102 <u>is</u> dependent on the

nature of the leaving group.[2]

The native enzyme is extraordinarily stable to denaturation, indeed the isolation procedure involves heating the protein to 80°C for 15 minutes.[2] The metalloenzyme does not denature even in 8M urea, although it does dissociate into monomers. The halo-enzyme (natural) is fairly insensitive to pH changes in the region 4 - 9. Below pH 4 the enzyme dissociates into its subunits and unfolds; this change is fully reversible on raising the pH.

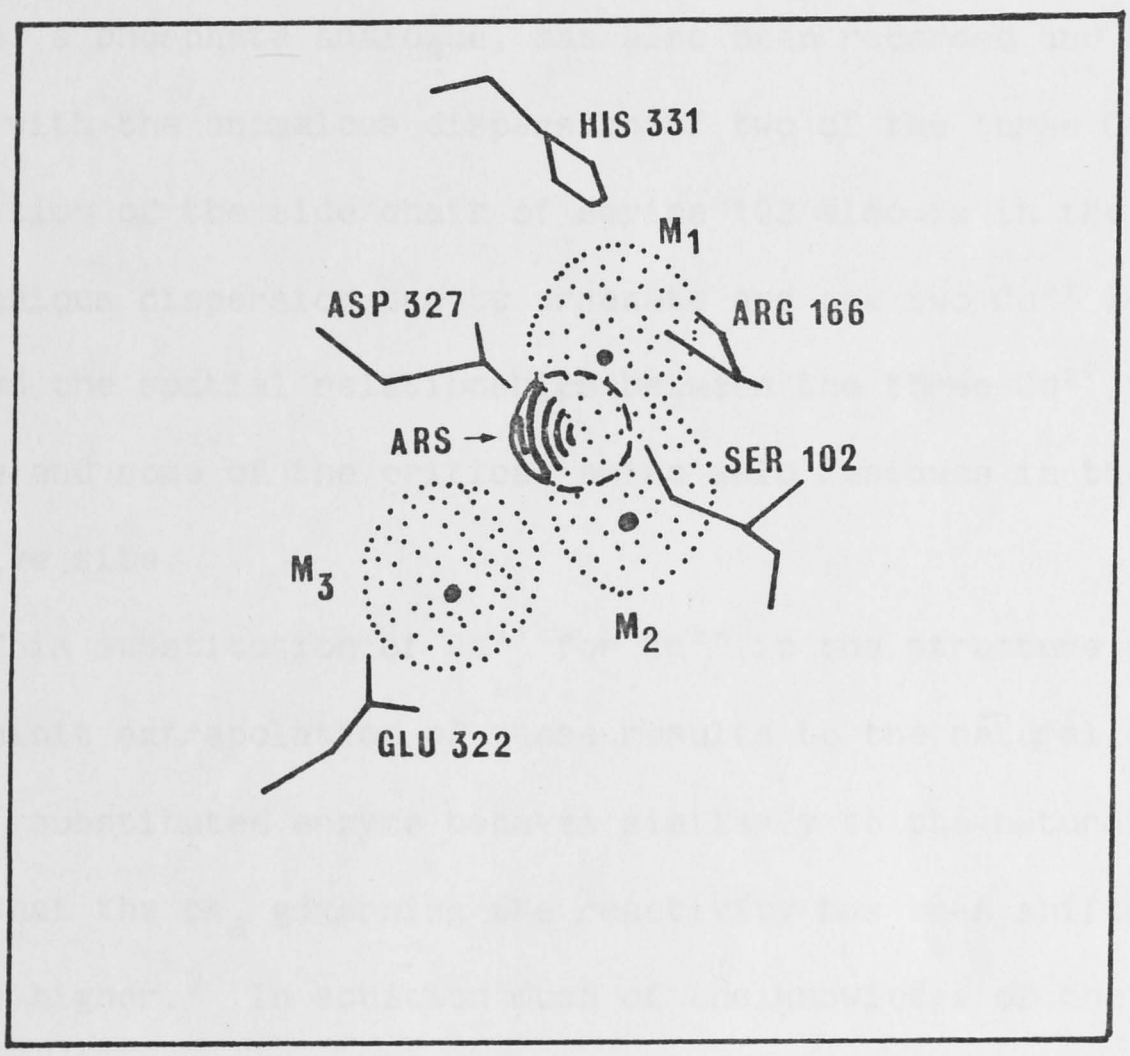


Figure 1.1

The active site of Alkaline Phosphatase showing the relative positions of the metal ions, arsenate and some of the important amino acid residues. (Modified from Reference 5)

An X-ray structural determination of E. coli alkaline phosphatase by Wyckoff et al[5] has recently been refined to a resolution of 3.1 A. This has been very helpful in answering many of the questions regarding the structure of the enzyme, especially with regard to the spatial relationships between the metal ions, the substrate and some key amino acid residues. The anomalous dispersion of the Cd^{2+} substituted enzyme has been recorded and the position of the Cd^{2+} ions corresponds to the active site of the native enzyme.

The anomalous dispersion map of the enzyme substituted with arsenate, a phosphate analogue, has also been recorded and found to overlap with the anomalous dispersion of two of the three Cd^{2+} ions. The position of the side chain of serine 102 also is in the region of the anomalous dispersion due to arsenate and the two Cd^{2+} ions. Figure 1.1 shows the spatial relationships between the three Cd^{2+} ions, arsenate and some of the critical amino acid residues in the region of the active site.

This substitution of Cd^{2+} for Zn^{2+} in the structure probably does not prohibit extrapolation of these results to the natural enzyme since the Cd^{2+} substituted enzyme behaves similarly to the natural enzyme except that the pK_a governing the reactivity has been shifted some 3.5 pH units higher.[2] In addition much of the knowledge of the metal binding sites has been gained from ^{113}Cd NMR of the Cd substituted enzyme.

The active site of AP has three metal ion sites, in the natural enzyme these sites are normally occupied by two Zn^{2+} ions and one Mg^{2+} ion. The three metal ion binding sites in each subunit have been termed A,B and C based on observations made by ^{113}Cd NMR. AP in the presence of excess phosphate at pH 8.2 when substituted with $^{113}Cd^{2+}$ shows three distinct ^{113}Cd NMR signals, 153 ppm, 70 ppm and 2 ppm. The signals are

due to Cd^{2+} in the metal sites A,B and C respectively. The protein ligands forming the three metal ion sites are probably, three histidines and an aspartate, two aspartates one histidine and possibly serine 102, and two aspartates a glutamate and a threonine for sites A,B and C respectively.[5]

Metal ion site A has the highest affinity for Cd^{2+} (and presumably Zn^{2+}), this was the site first filled when the apo-enzyme was titrated with Cd^{2+}. Since the enzyme was active in this state the metal in site A was called "catalytic". Metal site B was the next highest in affinity for Cd^{2+} and the affinity of this site was enhanced by addition of PO_4^{3-}. This site was termed "structural" even though addition of metal ion to this site enhances the reactivity of the enzyme. Metal site C bound Cd^{2+} most weakly of all three sites and is assumed to be the site that binds Mg^{2+} in the natural enzyme. Addition of Mg^{2+} to the enzyme containing four moles of Zn^{2+} or Cd^{2+} per dimer further enhances enzyme activity.

Although the natural enzyme binds four Zn^{2+} ions per dimer, the enzyme is active in the presence of only two ions per dimer albeit at about 50% activity of the fully metallated enzyme.[6] It seems probable that this activity is due to half-site reactivity where one of the sites is fully active and the other is inactive. This explanation seems to be preferable to the semi-active site argument in which both the sites are active but only at 50% of maximal activity. ^{113}Cd NMR experiments in which the spectrum of the enzyme in the presence of two moles of $^{113}Cd^{2+}$ per dimer changes dramatically upon addition of phosphate have been interpreted as showing a change from one Cd^{2+} per subunit in the absence of phosphate to two Cd^{2+} in one subunit and none in the other in the presence of phosphate.[2] This supports the half-site reactivity argument for the reactivity of the semi-metallated enzyme.

The chemical mechanism for AP is still a matter for conjecture. However there is now a considerable body of data on which a plausible mechanism may be based. The essential elements are as follows. Serine 102 is phosphorylated as an essential part of the mechanism. The enzymic hydrolysis proceeds with retention of configuration at phosphorus,[4] most likely via two inversions. (All enzymatic phosphoryl transfer reactions investigated stereochemically thus far have been shown to proceed with inversion except where double displacement reactions are known or suspected to occur.)[4,7] ^{31}P NMR of the $^{113}Cd^{2+}$ substituted enzyme has shown that the resonance attributed enzyme·phosphate complex is split into a doublet by coupling to the spin half $^{113}Cd^{2+}$ ions.[6] This implies that the phosphate is coordinated to the metal ion. The serylphosphate does not show splitting due to ^{113}Cd-^{31}P coupling. The X-ray structure has shown that the phosphate mimic, arsenate, displays anomalous dispersion that overlaps both the A and B metal sites. The same work shows that serine 102 is close enough to coordinate to metal ion A or B as well as being able to bond to arsenate, (and presumably phosphate). (Figure 1.1) Several proposals for the mechanism of hydrolysis have been advanced, the usual role of the metal ion is to either activate the substrate by coordination thereby increasing the electrophilicity at P or to activate the nucleophile. The nucleophile in question can be either the hydroxyl of serine 102 or water. The nucleophile, by coordinating to the metal ion, becomes more acidic and can be deprotonated easily and thereby becomes more nucleophilic.

The catalytically essential amino acid arginine 166 is located close to the phosphate binding site and is probably involved in charge neutralization of the bound phosphate.

Although much is now known about the structure of the active site

of Alkaline phosphatase, there is no definitive description of the chemical transformations that take place during a turnover of the enzyme. The role of the metal ions in the mechanism is still unclear and the functional group required to be deprotonated for the enzyme to be active remains to be conclusively identified.

1.3 Yeast Inorganic Pyrophosphatase.

Yeast inorganic pyrophosphatase (YIP) is an enzyme consisting of two identical subunits giving a molecular weight for the dimer of 64,000. YIP catalyses the reversible hydrolysis of pyrophosphate. In the presence of Mg^{2+}, YIP is specific for pyrophosphate, however, when other activating metal ions are employed, the enzyme becomes less specific and will hydrolyse a number of pyrophosphate esters.[8]

The native dimer binds divalent metal ions in the absence of phosphate, however in the presence of phosphate three divalent metal ions are bound with high affinity. These metal ions include Mg^{2+}, Mn^{2+}, Co^{2+}, Zn^{2+}, Ni^{2+}, Fe^{2+} and Cd^{2+} all of which activate the enzyme,[9] and Ba^{2+} and Ca^{2+} which inhibit YIP.[9] In vivo Mg^{2+} is the ion that is bound to the enzyme. ^{113}Cd NMR of YIP shows that in the presence of phosphate, three separate ^{113}Cd resonances are observed; all of which are different from free Cd^{2+} in solution.[10] The chemical shifts of all three Cd^{2+} signals appear to indicate that the ligands to the metal ions are almost all oxygen donors as might be expected for sites which normally bind Mg^{2+}.[10]

The enzyme has two binding sites for phosphate per subunit, one high affinity site and another site of lower affinity. The substrate for the enzyme in the hydrolytic direction is the Mg^{2+} pyrophosphate complex. There is still some discussion as to whether the substrate is

a P_1,P_2 chelate[9] or a monodentate species.[10] Cooperman et al are of the opinion that it is more likely that the natural substrate for the enzyme is the monodentate pyrophosphate.[10] They base this conclusion on the observation that for the $(Cd^{2+})_3$ enzyme in the presence of phosphate, apparently only one of the two bound phosphates interacts with the Cd^{2+} ion. This is argued from an increase in the ^{31}P NMR line width following a change from ^{112}Cd (I = 0) to ^{113}Cd (I = $^1/_2$). This experiment however is not conclusive. Evidence in favour of the chelate as the substrate has come from studies of the Mg^{2+}-$P_2O_7^{4-}$ equilibrium, the chelate is the most abundant species.[11] Evidence which is rather more compelling has come from the observation that both $[(H_2O)_4CrP_2O_7]^-$ and $[(NH_3)_4CoP_2O_7]^-$ are substrates for the enzyme in the presence of added divalent metal ions.[9] However, the monodentate complex $[(NH_3)_5CoOP_2O_6]^-$ is not a substrate for the enzyme.[11] Cr(III) and Co(III)-amine complexes unlike Mg(II) complexes are substitution inert, this allows the separation and purification and characterization of specific complexes. The $[(H_2O)_4CrP_2O_7]^-$ and $[(NH_3)_4CoP_2O_7]^-$ complex ions have been purified and unambigously characterized as the P^1,P^2 chelate including X-ray crystal structure analyses.[12,13]

These observations conclusively show that one metal ion is required to form the substrate and at least one other divalent metal ion is required for activity. More recent work employing paramagnetic probes has suggested that three metal ions are involved in the catalytic cycle of the enzyme.[14] When $[(NH_3)_4CoP_2O_7]^-$ was used as a substrate the initial product of the reaction was cis-$[(NH_3)_4Co(PO_4)_2]^{3-}$.[15]

There is an essential arginine located in the active site[16] which is required to be protonated for activity. Competitive inhibitors of the reaction such as phosphate, $[CaP_2O_7]^{2-}$ and $[MgO_3PCH(OH)PO_3]^{2-}$ also competitively inhibit the inactivation of the arginine 77 residue by

phenylglyoxal.[16] This suggests that the protonated arginine residue is involved in substrate binding.

Any consideration of the mechanism for the hydrolysis of pyrophosphate must account for the following observations: the substrate is a Mg^{2+}-pyrophosphate complex, more divalent metal ions are required for activity, and there appears to be an essential arginine residue in the active site.

The two most recently published proposals for the mechanism of YIP are quite similar,[9,16] both include metal ion binding to pyrophosphate to produce a substrate, both have proposed a metal bound hydroxo ligand as a nucleophile (the hydroxo ligand is produced by deprotonation of an aqua ligand by an adjacent basic amino acid residue), both involve stabilization of the substrate by interaction with the cationic arginine residue. The mechanisms differ in the spatial arrangement of the substrate, the various metal ions and essential amino acid residues.

1.4 Kinases.

As a major source of energy for chemical reactions in biology, nucleoside 5'-triphosphates (NTP's) are involved in many enzyme catalysed reactions which involve transfer of the terminal PO_3^- group to an acceptor molecule. The class of enzymes which catalyse these reactions are named NTP'ases when the acceptor molecule is water or kinases when the acceptor molecule is other than water. An outstanding feature of this group of enzymes is that with one possible exception all require at least one divalent metal ion (frequently Mg^{2+}), for activity.[17] Thus the overall catalysed reaction may be written as

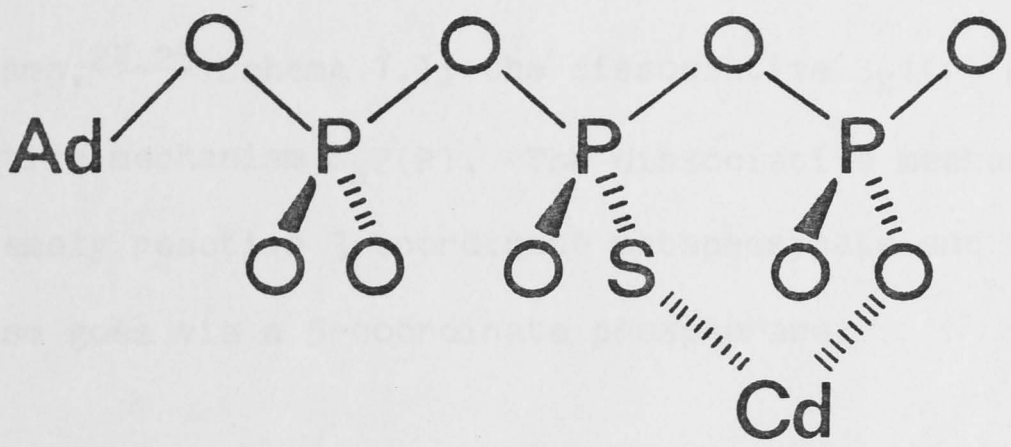
$$\text{Acceptor} + \text{Nucleoside triphosphate} \xrightarrow{M^{2+}} \text{Phosphorylated Acceptor} + \text{Nucleoside Diphosphate}$$

The metal ion appears to participate in the reaction as the NTP complex, in some cases additional metal ions may be required by the enzyme. The thesis that one substrate for some kinases is the β,γ-chelate Mg^{2+} - NTP complex has been supported by the fact that the substitution inert complexes β,γ-$[(NH_3)_4CoATP]^-$ and/or β,γ-$[(H_2O)_4CrATP]^-$ can also act as substrates for certain kinases. β,γ- bidentate coordination of NTP to a metal ion causes the β phosphorus atom to become chiral. The two diasteoromers thus produced (since the nucleoside is chiral) have been designated, somewhat misleadingly, Δ and Λ[18] to avoid the use of the R,S nomenclature which can change for the same screw sense isomer depending on the atomic number of the metal ion involved. All the enzymes investigated thus far utilize only one of the diastereomers as a substrate. Thus Yeast hexokinase will hydrolyse only the Λ isomer of β,γ-$[(NH_3)_4CoATP]^-$.[18] The β,γ-$[(NH_3)_4CoATP]^-$ complex is a substrate for the Ca^{2+} and Mg^{2+} ATP'ases.[19] β,γ-$[(H_2O)_4CrATP]^-$ is a substrate for, glycerokinase (Λ isomer), pyruvate kinase (Δ), phosphofructokinase (Δ), creatine kinase (Λ), myokinase (Δ) and arginine kinase (Λ).[20] These complexes in addition to their substrate activity, quite often act as competitive inhibitors of kinases utilizing their natural substrates. In cases where the inert complex is both a substrate and a competitive inhibitor it is often found that the k_m and k_i values are quite similar[19], thus reinforcing the notion that these molecules are reasonable substrate analogues.

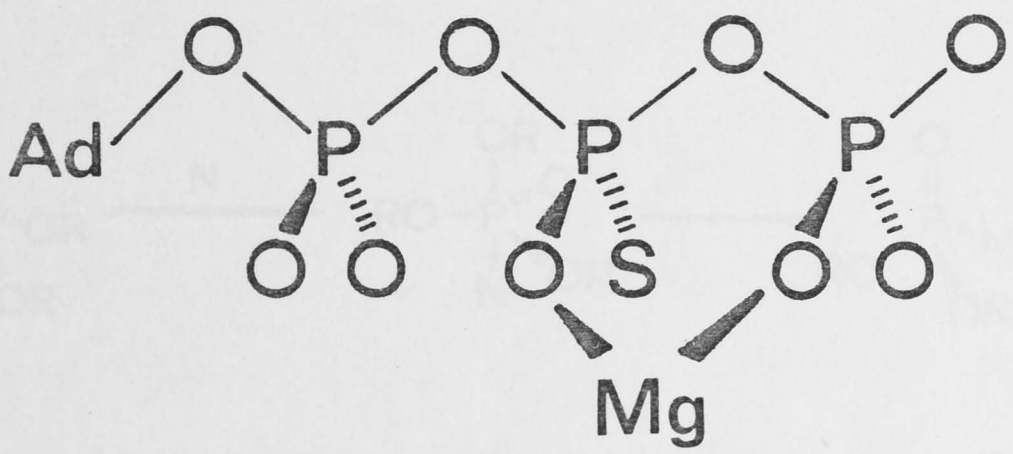
Sulphur substituted ATP analogues have been used as substrates for certain kinases.[21,22,23] When a sulphur atom is stereospecifically

substituted for an oxygen in a non-bridging position on the α or β phosphorus of ATP that phosphorus atom becomes chiral and the molecule becomes diastereomeric. These diastereomers have been separated and tested as substrates for some kinases including arginine kinase,[21] 3-phosphoglycerate kinase,[22] hexokinase,[23] and pyruvate kinase[23]. As shown previously, the substrate for most kinases is actually a metal ion NTP complex. When Mg^{2+} is the activating metal ion one of the diastereomers is preferred, ie one of the diastereomers has a lower K_m (stronger binding), and a higher V_{max} than the other, when Cd^{2+} is the activating metal ion the other diastereomer is preferred. The rationale for these observations is that Mg^{2+} prefers to bind oxygen whereas Cd^{2+} being a "softer" cation preferentially coordinates to sulphur. (Figure 1.2) The corollary of this is that Mg^{2+} and Cd^{2+} preferentially form opposite screw sense isomers with each of the diastereomers and therefore, because the enzyme prefers different diastereomers when activated by Mg^{2+} and Cd^{2+}, it is implied that the enzyme has a preference for one screw sense isomer over the other.

It appears that from the large number of kinases investigated by these two techniques that the enzymes will utilize only one conformation of the complex, this is typical of enzymes, which are usually stereospecific even when the reactant and product are achiral. It is also apparent from these studies that the kinases require the NTP substrate to be coordinated for activity.



The complex preferentially formed between ATP(S-βS) and Cd^{2+}



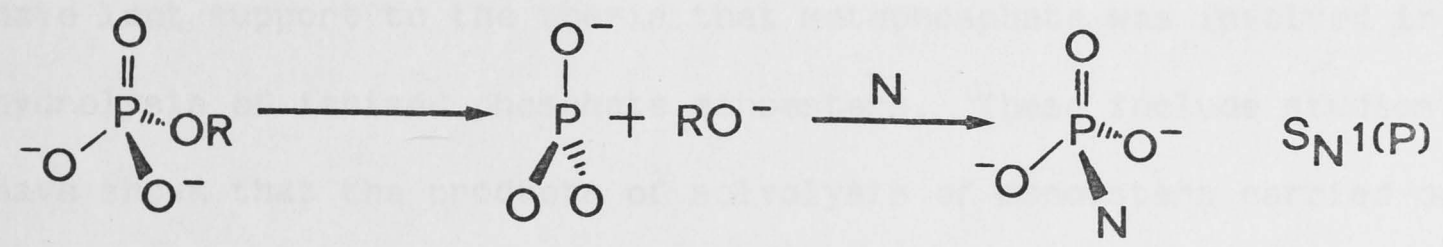
The complex preferentially formed between ATP(S-βS) and Mg^{2+}

Figure 1.2

A question that arises from this observation is, what is the effect that the metal ion has on the substrate to which it coordinates? Does it simply neutralize the negative charge of the NTP? Does it increase the electrophilicity, (susceptibility to nucleophilic attack), of the phosphorus centre by electron density withdrawal. Does it constrain the substrate into a conformation suitable to the enzyme? These questions may in part be answered by the use of suitable model systems.

1.5 Reactions of Organic Phosphates.

Nucleophilic substitution of phosphates can proceed via two basic mechanisms,[24,25] Scheme 1.1; the dissociative $S_N1(P)$ mechanism and the associative mechanism $S_N2(P)$. The dissociative mechanism proceeds via an extremely reactive 3-coordinate metaphosphate and the associative mechanism goes via a 5-coordinate phosphorane.



Scheme 1.1

Hydrolysis of Phosphate Esters.

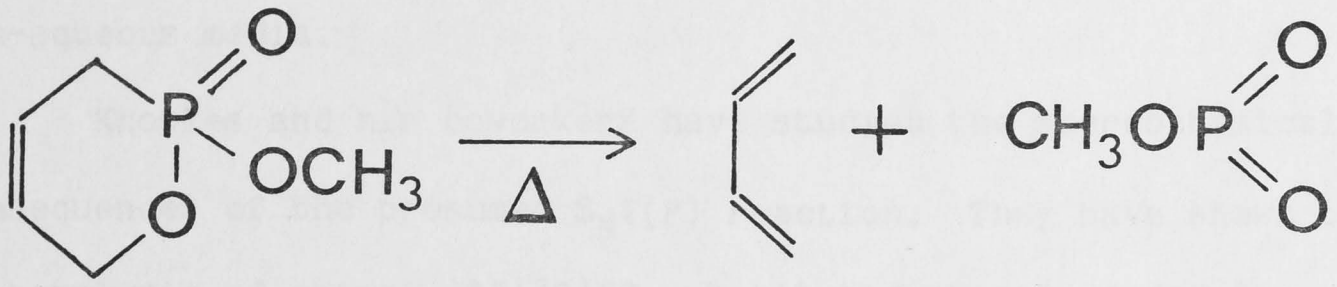
The Elimination-Addition Reaction, $S_N1(P)$.

The elimination addition reaction for phosphate ester was first proposed to account for the shape of the pH - rate profile of alkyl monoesters,[26] which are most reactive at the maximum concentration of the monoanion, ie about pH 4. The explanation for this observation was that the reaction proceeded via a metaphosphate "intermediate" by the expulsion of an alcohol group with the proton for the alcohol being transferred from the phosphate as the reaction proceeds. Further studies of the reactions of mono esters have implied that the above

mechanism operates for all alkyl and aryl monoesters where the pK_a of the leaving group exceeds about 5.5. In these cases, the alcohol or phenol needs to be protonated to be an effective leaving group. In contrast, phosphate monoesters with a phenol leaving group with a pKa less than 5.5 show rate maxima where the dianion predominates.[27,28] In this situation, the phenolate is an effective leaving group and therefore does not require protonation.

Since the mid 1950's many experiments have been performed which have lent support to the thesis that metaphosphate was involved in the hydrolysis of ionized phosphate monoesters. These include studies that have shown that the products of solvolysis of monoesters carried out in mixed alcoholic/aqueous media are alkylphosphate and phosphate in a ratio that is close to that of the molar ratio of the solvent.[29] This is interpreted to mean that a phosphorylating agent is produced which cannot distinguish between the two nucleophiles present. Also 2,4-dinitrophenylphosphate phosphorylates the sterically hindered tertiary butyl alcohol in acetonitrile at almost the same rate as it is hydrolysed in that solvent implying a dissociative type mechanism.[30] The oxygen kinetic isotope effect in the hydrolysis of 2,4-dinitrophenylphosphate, with the ^{18}O atom on the phenol group, the effect k_{16}/k_{18} is 1.02 ± .004 which is indicative of substantial P-O bond cleavage in the rate determining step.[31] The three phase test[32] in which the reactive phosphate is anchored to insoluble polymer beads and the receptor is attached to another type of bead, both in an inert solvent shows that the reactive phosphorylating agent has migrated through the solvent to the receptor molecule. A bimolecular reaction between the reactant and receptor is precluded by the fact that they are attached to separate insoluble polymers.

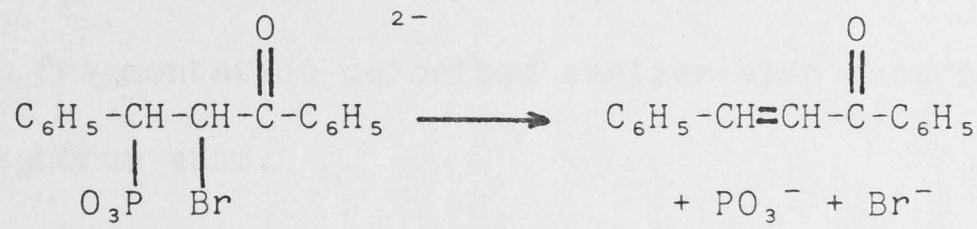
A number of experiments have been performed which appear to demonstrate the production of the metaphosphate fragment from other than phosphate. The pyrolysis of an appropriate precursor molecule for example, methyl-2-butenylphostonate, (Scheme 1.2), yields products attributable to the reaction products of methylmetaphosphate.[33]



Scheme 1.2

Pyrolysis of Methyl-2-butenylphostonate.

Westheimer has recently re-examined the Conant-Swan fragmentation of β-halophosphonates and has presented evidence for the fragmentation proceeding with production of metaphosphate,[34,35] (Scheme 1.3).



Scheme 1.3

The Conant-Swan Fragmentation (According to Westheimer).

The PO_3^- anion generated can be trapped by the addition of a suitable reagent.[34,35]

Recently, the monomeric metaphosphate anion has been directly

observed in the gas phase by mass spectrometry.[36] Ramirez and coworkers have utilized negative ion chemical ionization technique to study the mass spectroscopy of phosphotriesters. In many of these spectra a prominent peak occurs at m/e = 79, exact mass measurements of this peak confirm that it is PO_3^-. It is important to note that all the reactions where the metaphosphate anion has been trapped have been conducted in non-aqueous media.

Knowles and his coworkers have studied the stereochemical consequences of the presumed $S_N1(P)$ reaction. They have shown that the methanolysis of chiral $^{16}O^{17}O^{18}O$ substituted phenol- and 2,4-dinitrophenylphosphate and phosphocreatine, under conditions where they were expected to react by the metaphosphate pathway, all undergo complete inversion of configuration at phosphorus. This result implies that the putative metaphosphate is definitely not a free intermediate, in fact the metaphosphate is not even long-lived enough to equilibrate with the solvent cage in which it is formed. The authors of this report conclude that the reaction must be pre-associative at least in aqueous/alcoholic media. A very recent report[37] has shown that the Conant-Swan fragmentation described earlier also occurs with inversion at the phosphorus atom.

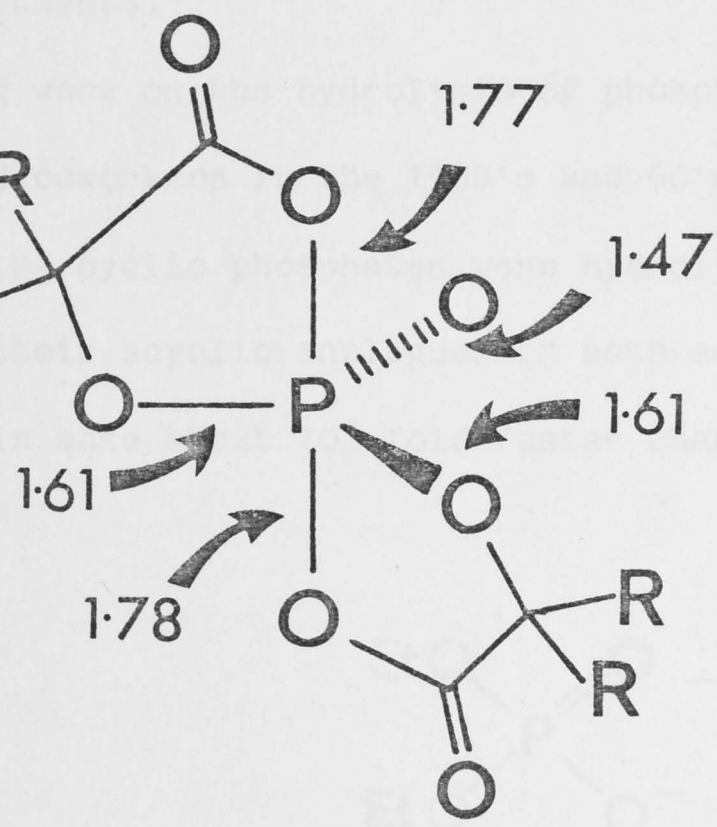
The Addition Elimination Reaction, $S_N2(P)$.

The addition elimination reaction proceeds via addition of a nucleophile at phosphorus to generate an intermediate or activated complex. The phosphorus atom is readily able to utilize its d-orbitals to expand its coordination number and it is argued that an attacking nucleophile such as OH^- adds to the 4-coordinate phosphorus to form a pentacoordinate phosphorane.[38] It appears that generally the pentacoordinate intermediate will have a trigonal bipyramidal structure, although spirocyclic phosphoranes appear to favour a square pyramidal

structure.[39]

Phosphate di- and tri- esters appear to react exclusively via this $S_N2(P)$ mechanism. Phosphate monoesters can react via either mechanism depending on their state of protonation;[25] the neutral diprotonated monoester appear to always react via the $S_N2(P)$ pathway.

X-ray structure analyses of available trigonal bipyramidal phosphoranes show that usually, apical bonds are longer (see for example **1**) and as a corollary, weaker than the equatorial bonds.[40,41]



1

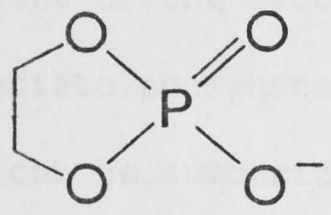
(Bond lengths in Å, R = C_6H_5[40])

X-ray structures determined for trigonal bipyramidal phosphoranes with mixed alkyl/alkoxy substituents invariably show that the alkoxy groups prefer apical coordination and alkyl groups prefer the equatorial sites.[42] An approximate scale of relative apicophilicities, a measure of a substituents preference for an apical position in the trigonal bipyramid, has been deduced for phosphoranes from NMR studies of these

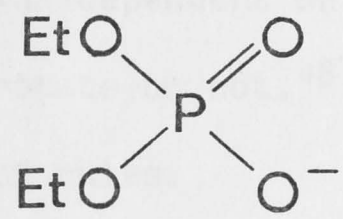
compounds.[43] The scale spans from F^- to alkyl groups in order of decreasing apicophilicity. Phosphoranes are stabilized by the presence of 4,5 and 6-membered rings incorporating the phosphorus atom as witnessed by the relative paucity of X-ray structures for non ring phosphoranes.[41]

Phosphorus however is not coordinatively saturated at five coordinate, there are many examples of hexacoordinate phosphorus compounds, for example PF_6^-, $P(OCH_3)_6^-$ and $P(OC_6H_5)_6^-$.[44] Like phosphoranes, hexacoordinate phosphorus compounds are stabilized by electronegative substituents.[44]

Much pioneering work on the hydrolysis of phosphate esters was done by Westheimer and coworkers in the 1950's and 60's. They observed that the 5 membered ring cyclic phosphates were hydrolysed many millions of times faster than their acyclic analogues in both acid and base.[38] Thus 2 is hydrolysed in base about 10^7 fold faster than 3.[45]



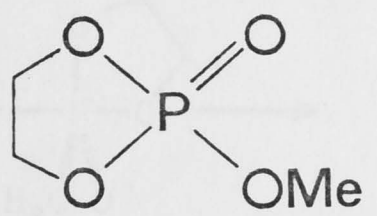
2



3

An important finding in this area of research is that in acid, exocyclic hydrolysis is accelerated by almost the same amount as is the ring cleavage reaction, thus in the acid hydroylsis of ethylene phosphate, 2, oxygen exchange in the reactant occurs at almost the same rate as hydrolysis. Similarly, the hydrolysis of methylethylenephosphate, 4, in acidic solution takes place with both

ring opening and loss of the methoxy group.[46] Prior to these observations the explanation for the rapid hydrolysis was simply the strain relief in the product,[47] however similar rates for exocyclic and endocyclic hydrolysis invalidated this argument.



4

During the 1960's Westheimer proposed a reaction mechanism which accounted for all of these observations and had predictive value as well.[38] He postulated that hydrolysis of these cyclic phosphates proceeded via a phosphorane which, because of the presence of the ring, was much lower in energy than the corresponding acyclic phosphorane. The extent of the exocyclic cleavage was dependent on whether the intermediate phosphorane could pseudorotate or not.[48] Westheimer's theory can be summarized by a number of rules:

1, the reaction proceeds via a trigonal bipyramidal phosphorane intermediate;

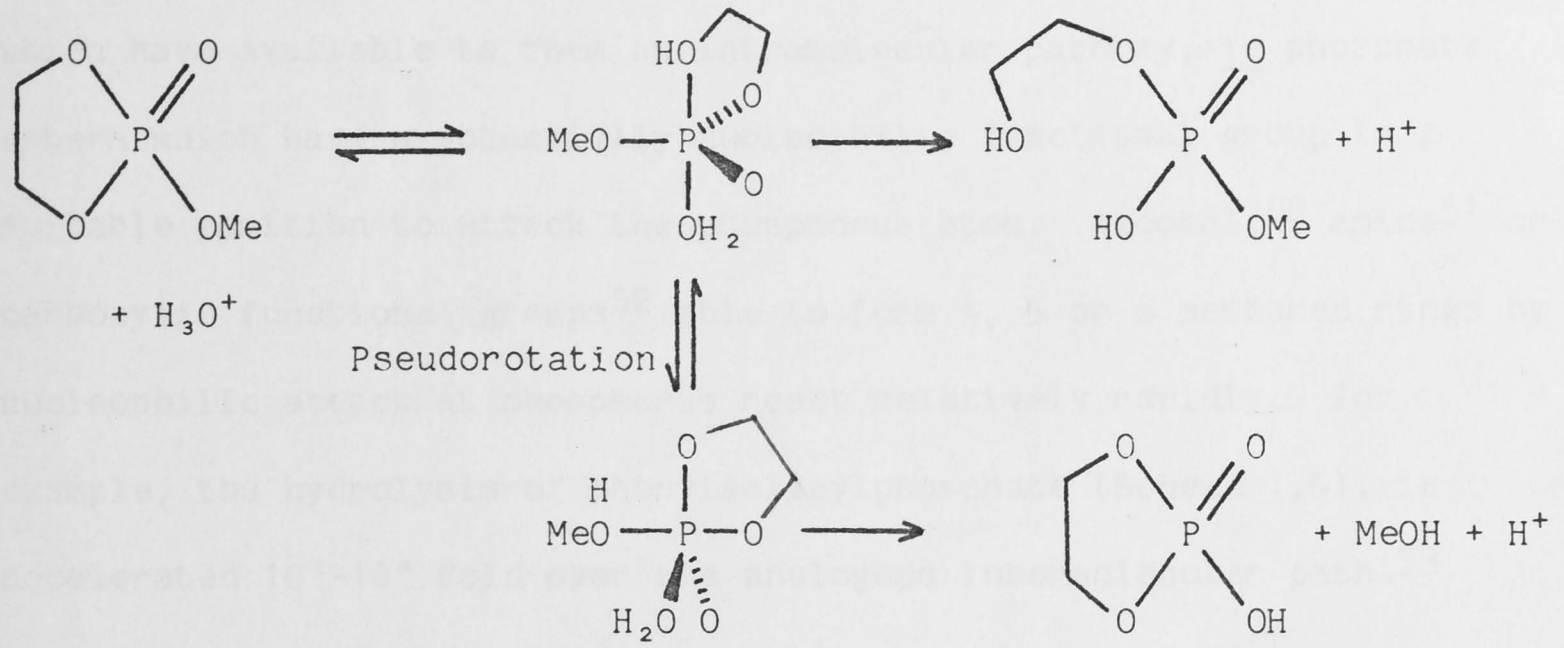
2, the nucleophile and leaving group enter and depart the phosphorane from the axial positions;

3, the trigonal bipyramidal intermediate may pseudorotate (with certain restrictions see rules 4 and 5);

4, 4 and 5-membered rings may only span axial-equatorial positions;

5, positioning of substituents in the intermediate is dependent on the relative apicophilicities of the substituents.

The theory is best illustrated by the acid hydrolysis of methylethylenephosphate, (Scheme 1.4)



Scheme 1.4

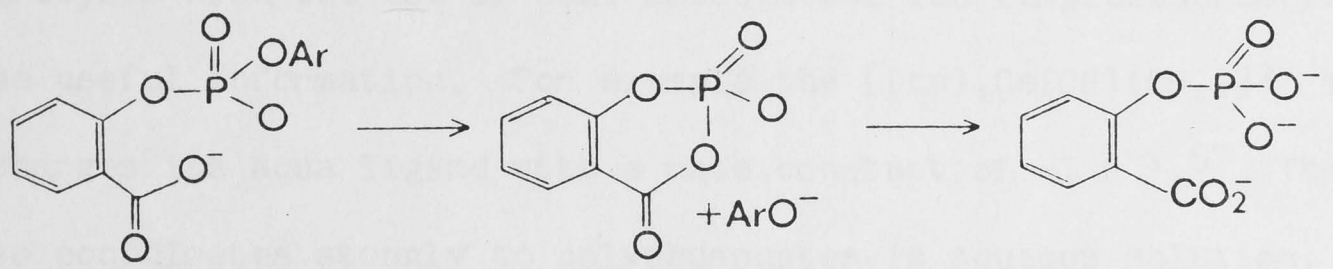
The Acid Hydrolysis of Methylethylenephosphate.[38]

Scheme 1.4 shows how pseudorotation of the phosphorane intermediate can accommodate exocyclic hydrolysis. The rate enhancement of the 5-membered cyclic phosphate arises from two effects of the ring. The cyclic phosphate is strained, the 5-membered ring must span the tetrahedral angle of ~109°, which raises the energy of the cyclic phosphate relative to the acyclic phosphate.[49] In going from the phosphate to the intermediate the angle that the ring is required to span is reduced to 90°, the presence of the ring therefore favours the formation of the intermediate. In addition the presence of the ring in the trigonal bipyramidal intermediate actually stabilizes the structure[49] which again favours formation of the intermediate from the cyclic phosphate. These effects are presumably also reflected in a lowering of the energy of the transition state for the reaction.

Intramolecular Hydrolysis

With two notable exceptions the di and tri-esters of phosphates are generally hydrolysed quite slowly. These exceptions include the 5-

membered ring phosphate esters described previously and phosphate esters which have available to them an intramolecular pathway, ie phosphate esters which have a potentially nucleophilic functional group in a suitable position to attack the phosphorus atom. Alcohol,[50] amine[51] or carboxylic functional groups[52] able to form 4, 5 or 6 membered rings by nucleophilic attack at phosphorus react relatively rapidly. For example, the hydrolysis of phenylsalicylphosphate (Scheme 1.5), is accelerated 10^7-10^8 fold over the analogous intermolecular path.[53]



Scheme 1.5

Hydrolysis of Phenylsalicylphosphate, Ar = C_6H_5.

1.6 Metal Ion Promoted Phosphoryl Transfer.

Metal ions promote polyphosphate and phosphate ester hydrolysis.[54] Studies on the hydrolysis of phosphoanhydride bonds in the presence of divalent metal ions have been carried out for many years. Several proposals for the mechanisms by which metal ions act have been advanced.[55] A major problem with experimental systems of this kind is that the divalent metal ions are substitutionally labile, and form numerous complexes, all of which are in rapid equilibrium. This situation makes it extremely difficult to unravel the complicated system and to say with certainty that any particular complex is wholly or partly responsible for the rate enhancement. It is an axiom however

that substitution lability is an essential requirement for efficient catalysis and presumably this is the reason enzymes choose mostly first row divalent metal ions as cofactors.

Lanthanide hydroxide gels have been known for about 50 years to catalyse the hydrolysis of polyphosphates and phosphate esters.[56] However the study of these systems is subject to many of the same difficulties as mentioned previously, ie the inability to be able to identify the complexes responsible for the activation.

More recently, attempts to study metal ion promoted polyphosphate hydrolysis with the use of semi-inert metal ion complexes has yielded some useful information. For example the $[(tn)_2Co(OH)(OH_2)]^{2+}$ ion exchanges its aqua ligand with a rate constant of ~1 s^{-1}.[57] The complex also coordinates stongly to polyphosphates in aqueous solution. Milburn has shown that in the presence of three mole equivalents of $[(tn)_2Co(OH)(OH_2)]^{2+}$, the hydrolysis of pyrophosphate is accelerated ~10^5 fold.[58] Apparently three moles of Co(III) reagent are required per mole of pyrophosphate to observe large rate enhancements. A similar result has been obtained using the $[(en)_2CoP_2O_7]^-$ as a substrate instead of $P_2O_7^{4-}$.[59] In this case the reaction proceeded rapidly in the presence of two moles of $[(tn)_2Co(OH)(OH_2)]^{2+}$, again displaying the requirement for three moles of Co(III) ion. More work with this and similar Co(III) and Pt(II) reagents[60] has shown that the rate of hydrolysis of ATP[61], ADP and triphosphate[62] can be enhanced by up to 10^6 fold by the use of these reagents. These large rate enhancements apparently arise from two separate effects of the metal complex. Firstly, the trivalent metal ion can effectively neutralize the negative charge and further polarize the P-O bond to which it coordinates making the phosphorus more electrophilic. Secondly, nucleophilic attack at a cis coordinated phosphate residue by coordinated OH^- proceeds quite

effectively. This subject will be developed in a later chapter.

One of the first attempts to synthesize and study the hydrolysis of a discrete, inert metal ion phosphate complex was undertaken by Spiro and coworkers.[63] They attempted to avoid the problems associated with labile metal ions by working with a Co(III) phosphate ester complex. They were investigating the effect of chelation on the rate of hydrolylsis of methylphosphate. Unfortunately, these workers had no evidence for the structure of the putative chelate complex and therefore the origin of the observed rate enhancement (~10^2) could not be specified. It seems possible that the actual structure may have been a dimer possessing an eight membered ring of the type described recently.[64] Since this original publication of Spiro _et al_,[63] two more publications claiming synthesis of the chelate ester have appeared.[65,66] One of the compounds has since been shown to be a dimer,[64] whilst the other is most likely a simple 4-nitrophenylphosphate salt of some $(en)_2Co(III)$ species.[67]

Recent work on well characterized substitution inert Co(III) phosphate complexes have advanced the knowledge of mechanisms of metal ion promoted phosphate chemistry. Sargeson _et al_ have synthesized several phosphate ester complexes incorporating features of interest for rapid phosphate ester hydrolysis, in one such model, a cis aqua ligand was incorporated into a phosphate ester complex[68], upon deprotonation the hydroxo ligand attacked the phosphate ester resulting in the loss of the ester group with a rate enhancement of ~10^5. In another, nitrophenylphosphatopentaamminecobalt(III) was hydrolysed in base by attack of a _cis_ coordinated deprotonated ammonia also resulting in hydrolysis of the ester group.[69]

1.7 This Work.

The work to be described in the following chapters was carried out in an attempt to define and quantitate the effects that a metal ion can exert on the reactions of phosphate esters and anhydrides. To this end a number of phosphate ester complexes of various metal ions have been synthesized. These compounds were designed to test the efficacy of various possible modes by which a metal ion might conceivably influence the reactivity of phosphate derivatives. The manner in which the metal ion may influence and specifically increase the rate of reaction of phosphates was thought to include;

1. charge neutralization and electron density polarization,
2. provision of a locus for the reaction, ie by coordination of the nucleophile and the phosphate derivative and making the reaction unimolecular,
3. chelating the phosphate, and inducing strain into the molecule and enhancing the rate of reaction in a manner similar to that described by Westheimer[38] for 5-membered ring organic phosphates.

This thesis will be divided into chapters with each chapter relating to a particular type of phosphate complex and will conclude by summarizing the observed reactivity and relating the results to some of the questions of mechanism in enzymic phosphoryl transfer.

1.8 ^{31}P NMR Chemical Shifts.

In this work much of the kinetics and product analysis has been conducted by ^{31}P NMR spectroscopy. Identification of products from their ^{31}P NMR spectra was aided by the application of a set of "additivity rules". These rules have been empirically derived from observations made during the course of this investigation and by

others.[64,68,69,70,71,72] ^{31}P NMR spectra of phosphate derivatives has shown that many substituents have a reproducible and consistent effect ($\Delta\delta$) on the chemical shift of the phosphorus nucleus. The substituents include the metal ions, Co(III), Rh(III) and Ir(III), esterifying groups, the phosphoryl (PO_3^-) group and protons. As an example coordination of a phosphate or derivative to the Co(III) centre through a basic oxygen, (ie not through the phosphoryl oxygen) shifts the phosphorus resonance downfield by + 6-8 ppm. Table 1.1 contains a list of substituents and their effect on the chemical shift of phosphate derivatives to which they add via an oxygen.

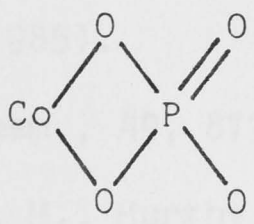
Table 1.1

Substituent Effects for Addition to a Basic Oxygen of Phosphate Derivatives.

Substituent	$\Delta\delta$ (ppm)
Co(III)	+6 to +8
Rh(III)	+6 to +8
Ir(III)	+6 to +8
H^+	-2 to -4
$-CH_3$	0 to -2
$-C_2H_5$	0 to -2
$-C_6H_4NO_2$	-5 to -6
$-PO_3^-$	-10 to -13

There are several points that need elaboration. Coordination to one of the trivalent metal ions via the phosphoryl oxygen yields a $\Delta\delta$ value of +3 to +4 ppm as evidenced by the chemical shift of coordinated trimethylphosphate. Chelation of a phosphate has a greater effect than

that which would be predicted simply by adding the Co(III) effect twice to the chemical shift of free phosphate. This result seems quite understandable, it has been shown that the chemical shift of various phosphate derivatives is strongly dependent on the O-P-O bond angles.[73] An empirical correction of +5 ppm must be added to the chemical shift of phosphate derivatives incorporating a 4-membered chelate ring including the Co(III) ion. It seems probable that this $\Delta\delta$ effect for the strain/bond angle in the ring will be metal ion dependent, given that the differing sizes of metal ions will alter the strain and bond angles at phosphorus.

<u>eg</u>. PO_4^{3-} + Co(III) ⟶ 

+5.5 +7 +7 +5 = 24.5

The chemical shifts of phosphoramidate derivatives behave differently to phosphate derivatives. Coordination of phosphoramidate derivatives to Co(III) and Ir(III) via the nitrogen shifts the chemical shift of the phosphorus atom involved <u>upfield</u> by 1 - 2 ppm. Deprotonation of the metal bound NH_2 group shifts the phosphorus resonance downfield by 16 - 18 ppm. The effect of N-deprotonation on the uncoordinated phosphoramidate derivatives cannot be measured because the protons are too basic, <u>ie</u> the pKa for the NH_2 group of phosphoramidates is much higher than 14.

References, Chapter 1.

1. Chock, P.B., Rhee, S.G., Stadtman, E.R., Ann. Rev. Biochem., **49**, 813-843, (1980).

2. Coleman, J.E., Chlebowski, J.F., in "Adv. in Inorg. Biochem." Vol **1**, Eichorn, G.L., Marzilli, L.,(Eds), Elsevier, New York, 1979, pp 1-662. Coleman, J.E., Gettins, P., in "Adv. in Enzymology" Vol **55**, Meister, A.,(Ed), Interscience, New York, 1983, pp 381-452.

3. Surin, B.P., Rosenberg, H. and Cox, G.B., in "Proceedings of the Australian Biochemical Society" abstracts of the 1985 A.B.S. meeting, Canberra. **17**, S50, (1985).

4. Knowles, J.R., Ann. Rev. Biochem., **49**, 877-919, (1980).

5. Wyckoff, H.W., Handschumacher, M., Murthy, H.M.K., Sowadski, J.M., in "Adv. in Enzymology" Vol **55**, Meister, A., (Ed), Interscience, New York, 1983, pp 453-480.

6. Vallee, B.L., Galdes, A., in "Advances in Enzymology" Vol **56**, Meister, A., (Ed), Interscience, New York, 1984, pp 283-430.

7. Frey, P.A., Tetrahedron, **38**, 1514-1567, (1982).

8. Butler, L.G., in "The Enzymes", 3rd. Edition, Boyer, P.D., (Ed), Academic Press, New York, Vol. **4**, pp 529-541

9. Knight, W.B., Fitts, S.W. and Dunaway-Mariano, D., Biochem, **20**, 4079-4086, (1981).

10. Welsh, K.M., Armitage, I.M. and Cooperman, B.S., Biochem, **22**, 1046-1054, (1983).

11. Knight, W.B., Ting, S-J., Chuang, S., Dunaway-Mariano, D., Haromy, T. and Sundaralingam, M., Arch. Biochem. Biophys., **227**, 320-309, (1983).

12. Merritt, T.P., Sundaralingam, M., Acta Cryst., **B36**, 2576-2584, (1980).

13. Merrit, E.A., Sundaralingam, M. and Dunaway-Mariano, D., J. Am. Chem. Soc., 1981, **103**, 3565-3567.

14. Knight, W.B., Dunaway-Mariano, D., Ranson, S.C. and Villafranca, J.J., J. Biol. Chem., **259**, 2886-2895, (1984).

15. Haromy, T.P., Knight, W.B., Dunaway-Mariano, D. and Sundaralingam, M., Biochemistry, **21**, 6950-6956, (1982).

16. Cooperman, B.S., in "Methods in Enzymology", Purich, D.L., (Ed.), Academic Press, New York, 1982, Vol **87**, 526-548.

17. Morrison, J.F. and Heyde, E., Ann. Rev. Biochem. **41**, 29-54, (1972).

18. Cornelius, R.D. and Cleland, W.W., Biochem. **17**, 3279-3286, (1978).

19. Gantzer, M.L., Klevickis, C. and Grisham, C.M., Biochem., 21, 4083-4088, (1982).

20. Cleland, W.W. and Mildvan, A.S., in "Adv.in Inorg. Biochem." Vol. **1**, Eichorn, G.L., Marzilli, L.G., (Eds), Elsevier, New York, 1979, pp 163-191.

21. Cohn, M., Shih, N. and Nick, J., J. Biol. Chem., **257**, 7646-7649, (1982).

22. Jaffe, E.K., Nick, J. and Cohn, M., J. Biol. Chem., **257**, 7650-7656, (1982).

23. Jaffe, E.K. and Cohn, M., J. Biol. Chem., **253**, 4823-4825, (1978).

24. Westheimer, F.H., Pure App. Chem., **49**, 1059-1067, (1977).

25. Ramirez, F. and Marecek, J.F., Pure App. Chem., **52**, 1021-1045, (1980).

26. Cox, J.R. and Ramsay, O.B., Chem. Rev., **64**, 317-352, (1964).

27. Kirby, A.J. and Varvoglis, A.G., J. Am. Chem. Soc., **89**, 415-423, (1966).

28. Bunton, C.A., Fendler, E.J. and Fendler, J.H., J. Am. Chem. Soc., **89**, 1221-1230, (1966).

29. Jencks, W.P. and Gilchrist, M., J. Am. Chem. Soc., **86**, 1410-1417, (1964).

30. Ramirez, F. and Marecek, J.F., J. Am. Chem. Soc., **101**, 1460-1465, (1975).

31. Gorenstein, D.G., Lee, Y-G, and Kar, D., J. Am. Chem. Soc., **99**, 2264-2267, (1977).

32. Rebek, J., Gavina, F. and Navarro, C.J., J. Am. Chem. Soc., **100**, 8113-8117, (1978).

33. Westheimer, F.H., Chem. Rev., **81**, 313-326, (1981).

34. Calvo, K.C. and Westheimer, F.H., J. Am. Chem. Soc., **105**, 2827-2831, (1983).

35. Calvo, K.C. and Westheimer, F.H., J. Am. Chem. Soc., **106**, 4205-4210, (1984).

36. Meyerson, S., Harvan, D.J., Hass, J.R., Ramirez, F. and Marecek, J.F., J. Am. Chem. Soc., **106**, 6977-6983, (1984).

37. Calvo, K., J. Am. Chem. Soc., **107**, 3690-3694, (1985).

38. Westheimer, F.H., Acc. Chem. Res., **1**, 70-78, (1968).

39. Holmes, R.R., J. Am. Chem. Soc., **96**, 4143-4149, (1974).

40. Dubourg, A., Roques, R., Germain, G., Declercq, J-P., Garrigues, B., Boyer, D., Munoz, A., Klaebe, A. and Comtat, M., J. Chem. Res. (S), **1982**, 180-181.

41. Ramirez, F., Ugi, I., in "Adv. Phys. Org. Chem." Gold, V., (Ed.) Academic Press, London, 1971, pp 25-126.

42. Ul-Haque, M., Caughlan, C.N., Ramirez, F., Pilot, J.F., Smith, C.P., J. Am. Chem. Soc., **93**, 5229-5235, (1971). Swank, D.D., Caughlan, C.N., Ramirez, F., Pilot, J.F., J. Am. Chem. Soc., **93**, 5236-5241, (1971).

43. Trippett, S., Pure Appl. Chem., **40**, 595-605, (1974).

44. Denney, D.B., Denney, D.Z., Hammond, P.J., Wang, Y-P., J. Am. Chem. Soc., **103**, 1785-1789, (1981).

45. Kumamoto, J., Cox, J.R. and Westheimer, F.H., J. Am. Chem. Soc., **78**, 4858-4860, (1956).

46. Covitz, F. and Westheimer, F.H., J. Am. Chem. Soc., **85**, 1773-77, (1963).

47. Cox, J.R., Wall, R.E., Westheimer, F.H., Chem. Ind., **1959**, 929.

48. Pseudorotation is a ligand reorganizational isomerization in which two of the equatorial ligands replace the axial ligands which become equatorial. See for example ref. 38

49. Gerlt, J.A., Westheimer, F.H. and Sturtevant, J.M., J. Biol. Chem., **250**, 5059-5067, (1975).

50. Brown, D.M. and Usher, D.A., J. Chem. Soc., **1965**, 6558-6564.

51. Lazarus, R.A., Benkovic, P.A. and Benkovic, S.J., J.C.S. Perkin Trans. II, **1980**, 373-379.

52. Abel, K.W.Y. and Kirby, A.J., J.C.S. Perkin Trans. II, **1980**, 1171-74.

53. Khan, S.A., Kirby, A.J., Wakselman, M., Horning, D.P. and Lawlor, J.M., J. Chem. Soc. B, **1970**, 1182-1187.

54. Tetas, M. and Lowenstein, J.M., Biochem., **2**, 351-357, (1963).

55. Spiro, T.G., Kjellstrom, W.A., Zeydel, M. and Butow, R.A., Biochem., **7**, 859-865, (1968). Sigel, H., Hofstetter, F., Martin, R.B., Milburn, R.M., Scheller-V. and Scheller, K.H., J. Am. Chem. Soc., **106**, 7935-7946, (1984). Sigel, H. and Amsler, P.E., J. Am. Chem. Soc., **98**, 7390-7400, (1976).

56. Butcher, W.W. and Westheimer, F.H., J. Am. Chem. Soc., **77**, 2420-2424, (1955).

57. Jonasson, I.R., Lincoln, S.F. and Stranks, D.R., Aust. J. Chem., **23**, 2269-2278, (1970).

58. Hubner, P.W.A. and Milburn, R.M., Inorg. Chem., **19**, 1267-1272, (1980).

59. Creaser. I.I., Haight, G.P., Peachey, R., Robinson, W.T. and Sargeson, A.M., J. Chem. Soc. Chem. Commun., **1984**, 1568-1571.

60. Bose, R.N., Cornelius, R.D. and Viola, R.E., Inorg. Chem., **23**, 1182-1183, (1984).

61. Hediger, M. and Milburn, R.M., J. Inorg. Biochem., **16**, 165-182, (1982).

62. Cornelius, R.D. and Norman, P.R., Inorg. Chim. Acta., **65**, L193-195, (1982). Norman, P.R. and Cornelius, R.D., J. Am. Chem. Soc., **104**, 2356-2361, (1982).

63. Spiro, T.G., Kjellstrom, W.A. and Farrell, F.J., Science, **164**, 320-321, (1969).

64. Jones, D.R., Lindoy, L.F. and Sargeson, A.M., J. Am. Chem. Soc., **106**, 7807-7819, (1984).

65. Anderson, B., Milburn, R.M., Harrowfield, J.MacB., Robertson, G.B. and Sargeson, A.M., J. Am. Chem. Soc., **99**, 2652-2661, (1977).

66. Hay, R.W. and Bembi, R., Inorg. Chim. Acta., **78**, 143-149, (1983).

67. Hendry, P. unpublished observations.

68. Jones, D.R., Lindoy, L.F. and Sargeson, A.M., J. Am. Chem. Soc., **105**, 7327-7336, (1984).

69. Harrowfield, J. MacB., Jones, D.R., Lindoy, L.F. and Sargeson, A.M., J. Am. Chem. Soc., **102**, 7733-7741, (1980).

70. Seel, V.F. and Bohnstedt, G., Z. Anorg. Alleg. Chem., **435**, 257-267, (1977).

71. Cornelius, R.D., Hart, P.A. and Cleland, W.W., Inorg. Chem., 16, 2799-2805, (1977).

72. Lin, I., Knight, W.B., Ting, S-J. and Dunaway-Mariano, D., Inorg. Chem., 23, 988-991, (1984).

73. Gorenstein, D.G., J. Am. Chem. Soc., 97, 898-900, (1975).

Chapter 2

INTERMOLECULAR ATTACK OF HYDROXIDE

2.1 General Introduction

It has been shown that a phosphate ester with a good leaving group is rapidly hydrolysed when coordinated cis to a good nucleophile, e.g. M-OH^- or M-NH_2^-. However the hydrolysis of a coordinated phosphate ester by intermolecular nucleophilic attack at phosphorus has never been observed. This mode of reaction is of interest because it will allow quantitation of the effect of charge neutralization/electron density polarization on the reactivity of coordinated phosphate esters.

As early as 1963 the $[(NH_3)_5CoOP(OMe)_3]^{3+}$ ion had been synthesized and its base hydrolysis studied.[1] The products were found to be the trimethylphosphate (TMP) and the $[(NH_3)_5CoOH]^{2+}$ ion, the rate of loss of the TMP ligand being much faster than the rate of attack of OH^- on the phosphorus or carbon centre. Later, tracer studies on this reaction showed that the reaction proceeded with almost 100% Co-O bond cleavage,[2] presumably by the usual $S_N1(CB)$ mechanism of cobalt amine complexes.[3] This indicated that the rate of Co-O cleavage was at least 100 fold slower than that of attack of OH^- on the TMP moiety.

A recent publication has also shown that "soft" nucleophiles less basic than OH^- will demethylate $[(NH_3)_5CoTMP]^{3+}$.[4] In this case the less basic conditions allow nucleophilic attack at coordinated TMP to be competitive with the $S_N1(CB)$ path for loss of TMP from the complex. However, all the nucleophiles employed in this study, SCN^-, I^- and $S_2O_3^-$, attack only the methyl carbon to produce the methylated derivatives of the nucleophiles and do not attack at the phosphorus centre.

It was thought that if TMP was coordinated to a more

substitutionally inert complex moiety than $[(NH_3)_5Co]^{3+}$ the intermolecular attack of hydroxide at TMP may become competitive with metal-O bond rupture. In this way, some idea of the effect that coordination to a metal centre has on the rate of attack of OH^- at phosphorus may be gained.

Base hydrolytic studies of the uncoordinated TMP have been carried out by previous workers[5] and it was shown to be first order in hydroxide and TMP. Moreover, the reaction proceeded with 100% P-O bond cleavage via an associative type mechanism, $S_N2(P)$.

2.2 $[(NH_3)_5IrOP(OCH_3)_3]^{3+}$

2.2.1 Introduction

The substitution reactions of Ir(III) complexes generally occur much more slowly than the corresponding Co(III) derivatives, for example the rate of aquation of the complexes $[(NH_3)_5MCl]^{2+}$ in water at 25°C is, for Co, 1.7×10^{-6} s^{-1} and for Ir 1×10^{-10} s^{-1}.[6] This substitutional inertness prompted work on the base hydrolysis of $[(NH_3)_5IrTMP]^{3+}$ which should enhance the prospect of observing some reaction at the phosphorus centre, unobservable with the corresponding Co(III) complex because of rapid dissociation of the TMP ligand.

2.2.2 Experimental

^1H NMR spectra were recorded with a JEOL FX-200 spectrometer at 200 MHz. ^{31}P NMR spectra were recorded with either a Bruker CXP-200 at 80.98 MHz or a JEOL FX-60 instrument at 24.21 MHz. Electronic spectra were recorded with a Hewlett Packard 8450A diode array spectrophotometer.

$\underline{[(NH_3)_5IrTMP](ClO_4)_3}$ (1)

$[(NH_3)_5IrOSO_2CF_3](CF_3SO_3)_2$ (1.05g) was dissolved in TMP (20 mL) and heated to 70°C for 16 hours. The solution was extracted with ether (3x) to yield a white powder which was washed with ether and dried in vacuo. Yield 0.95g. Analysis Calculated for $C_6H_{24}N_5F_9IrO_{13}PS_3$: C, 8.32; H, 2.79; N, 8.09; P, 3.58; S, 11.12. Found; C, 8.0; H, 2.7; N, 7.9; P, 3.5; S, 11.1. ^1H NMR; 3.95 ppm (d), J = 11.2 Hz, D_2O. $^{31}P\{H\}$ NMR; +6.2 ppm (s) H_2O/D_2O. Electronic spectrum, ε^{max}_{272} = 96 $M^{-1}cm^{-1}$, ε^{max}_{231} = 98 M^{-1} cm^{-1} (shoulder).

$[(NH_3)_5IrDMP](ClO_4)_2$.

Dimethylphosphoric acid (DMP) was prepared by passing a sample of sodium dimethylphosphate through a Dowex 50W X2 (H^+) column and evaporating the eluant to dryness. The resulting oil was desiccated for several days over P_2O_5. $[(NH_3)_5IrOSO_2CF_3](CF_3SO_3)_2$ (0.5 g) was dissolved in dimethylphosphoric acid (8 ml), and 2,4,6-collidine, (200 µl), and heated to 60°C for 6 hours. Excess DMP was extracted with ether and the yellowish oil remaining was dissolved in methanol (50 ml) and $LiClO_4$ (3 g) added to the solution. Ethanol (10 ml per day) was added to the solution over 3 days, the resulting white solid was washed with ethanol then ether and dried in vacuo. Yield 100 mg. The sample analysed reasonably with the exception of nitrogen which was somewhat lower than expected. However, the septet observed in the ^{31}P NMR spectrum of the complex clearly established the identity of the product. Analysis Calculated for $C_2H_{21}N_5Cl_2IrO_{12}P$; C, 3.99; H, 3.52; N, 11.65, Cl, 11.79. Found; C, 3.7; H, 3.3, N, 10.7; Cl, 11.3. ^1H NMR; 3.65 ppm (d) J_{P-H} 10 Hz, D_2O/NaOD. ^{31}P NMR; {H}, +9.8 ppm (s), ^1H coupled +9.8 ppm (septet) J_{P-H} 11 Hz.

Kinetics.

All kinetics were run under pseudo first order conditions. The ionic strength of the reacting solutions was maintained at 1.0 M with

NaCl or $NaClO_4$. NaCl was generally used because the complexes were more soluble in those solutions.

The hydrolysis of $[(NH_3)_5IrTMP]^{3+}$ was followed by two methods, ^1H NMR spectroscopy and U.V. spectroscopy at 250 nm. The hydrolysis was slow enough to be conducted under pseudo-first order conditions in NaOH solutions. For kinetics followed by ^1H NMR spectroscopy, the reaction was conducted in D_2O and the hydroxide concentration was at least 10 times the complex concentration. The reaction was conducted at 27°C, the probe temperature of the NMR spectrometer. (Acquisition parameters; acquisition frequency 199.5 MHz, acquisition time 2.048 s, pulse delay 1.0 s, 8K points per spectrum, 8 scans per spectrum). Rate constants for the reaction were determined graphically by plotting $\ln(I_t - I_{infinty})$ versus time, where I_t was the normalized integral for a particular signal. The integrals for each signal were normalized by reference to the integral of the standard, sodium 2-dimethyl-2-silapentane-1-sulfonate (DSS). Errors in the rate constants were estimated to be ± 15%.

The rate of hydrolysis of the iridium complex was also determined by following the change in absorbance at 250 nm. The absorbance change was largest at this wavelength but still extremely small. The kinetics were followed with either a Cary 16, Cary 118C or a Hewlett Packard 8450A spectrophotometer. The reaction was initiated by mixing equal volumes of thermostatted solutions containing the required amounts of complex, hydroxide and salt. Temperature control was achieved by means of a recirculating water bath for the Cary instruments or by the Hewlett Packard 89100A temperature controlled cell holder. The temperature dependence for the reaction in 0.1 M OH^- (μ = 1.0 M NaCl) was also determined.

The rate of hydrolysis of TMP was determined by ^1H NMR

spectroscopy under conditions identical to those employed for the hydrolysis of $[(NH_3)_5IrTMP]^{3+}$. The pseudo first order rate constant was determined at two hydroxide concentrations.

Tracer Study

Approximately 80 mg of $[(NH_3)_5IrTMP](CF_3SO_3)_3$ was dissolved in 1.8 mL of 23% $H_2{}^{18}O$ and 0.2 mL of 2.7 M NaOD was added. The final $H_2{}^{18}O$ concentration was 21%. The reaction was allowed to proceed at ~25°C. After 10 $t_{1/2}$ a ^{31}P NMR spectrum of the product was accumulated. The experiment was duplicated using a $H_2{}^{18}O$ concentration of 9.5%.

A sample of $[(NH_3)_5IrDMP](ClO_4)_2$ was dissolved in a solution containing 0.15 M NaOH, 9.5% $H_2{}^{18}O$ and 10% D_2O. The solution was kept at 25°C for 90 minutes and the ^{31}P NMR spectrum was recorded.

A sample of ~80mg of $[(NH_3)_5IrTMP](CF_3SO_3)_3$ was hydrolysed in 0.2 M NaOH, without added $H_2{}^{18}O$ and a ^{31}P NMR spectrum of the product recorded. (Acquisition parameters for all tracer experiments; acquisition frequency 80.98 MHz, sweep width 500 Hz, pulse angle 90°, pulse repetition time 16.4 s).

2.2.3 Results and Discussion

The synthesis of **1** was achieved by heating $[(NH_3)_5IrOSO_2CF_3](CF_3SO_3)_2$ in TMP. Good yields of analytically pure complex were obtained by precipitation of the complex with addition of ether.

Hydrolysis of the $[(NH_3)_5IrTMP]^{3+}$ ion in hydroxide solutions obey the rate law,

$$v = k_1[IrTMP][OH^-]$$

The rate constant obtained by ^1H NMR spectroscopy in D_2O at ~27°C was $(5.8 \pm 1.0) \times 10^{-2}$ $lmol^{-1}$ s^{-1}, (See Figure 2.1 and Table 2.1). The products of the reaction were identified as $[(NH_3)_5IrDMP]^{2+}$ and methanol by their chemical shifts in ^1H and ^{31}P NMR spectra. Their authenticity

was verified by the addition of genuine samples of these compounds to the reaction mixture and observing an increase in the appropriate signal

Table 2.1

Rate constants for the Hydrolysis of $[(NH_3)_5IrTMP]^{3+}$ in D_2O, at 27°C, μ = 1.0 M (NaCl).[a]

[NaOD] M	k_{obs} s^{-1} (x 10^3)
0.05	3.1 ± 0.5
0.10	5.8 ± 0.9
0.20	11.7 ± 1.5

[a] Determined by ^1H NMR spectroscopy.

Table 2.2

Rate Constants for the Hydrolysis of $[(NH_3)_5IrTMP]^{3+}$, μ = 1.0 M ($NaClO_4$).[a]

Temperature	$[OH^-]$	k_{obs} s^{-1} (x 10^3)
5	0.1	0.76 ± .02
15	0.1	2.17 ± .06
25	0.1	5.56 ± .05
35	0.1	12.4 ± .6
25	0.05	2.6 ± .2
25	0.25	17.3 ± .4

[a] Determined Spectrophotometrically at 250 nm.

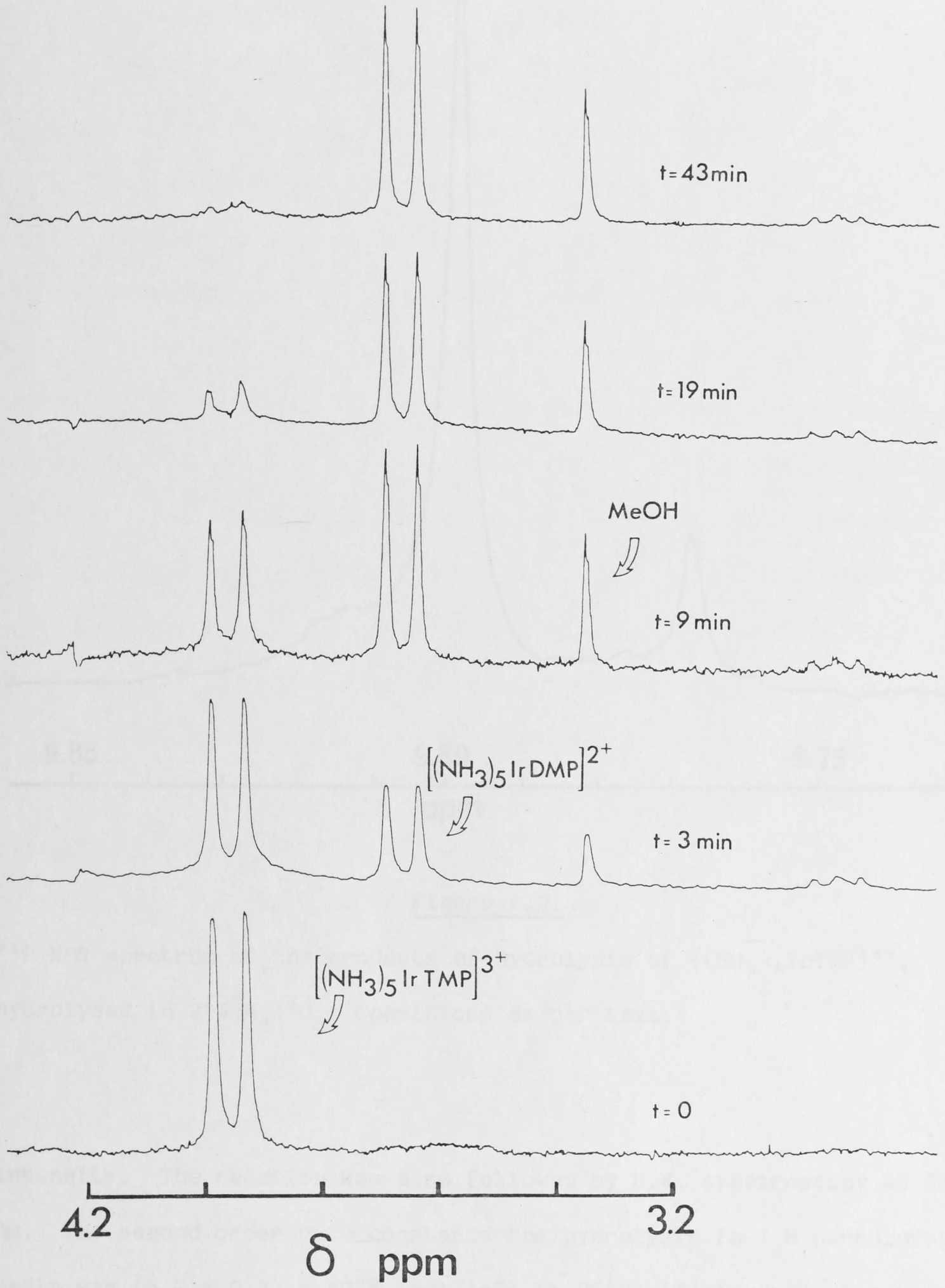


Figure 2.1.

Hydrolysis of $[(NH_3)_5IrTMP]^{3+}$ followed by ^1H NMR spectroscopy.

Conditions; [NaOD] = 0.05 M, $[[(NH_3)_5IrTMP]^{3+}]$ = 0.005 M

μ = 1.0 M (NaCl), Temp. = 27°C, Acquisition parameters as per text.

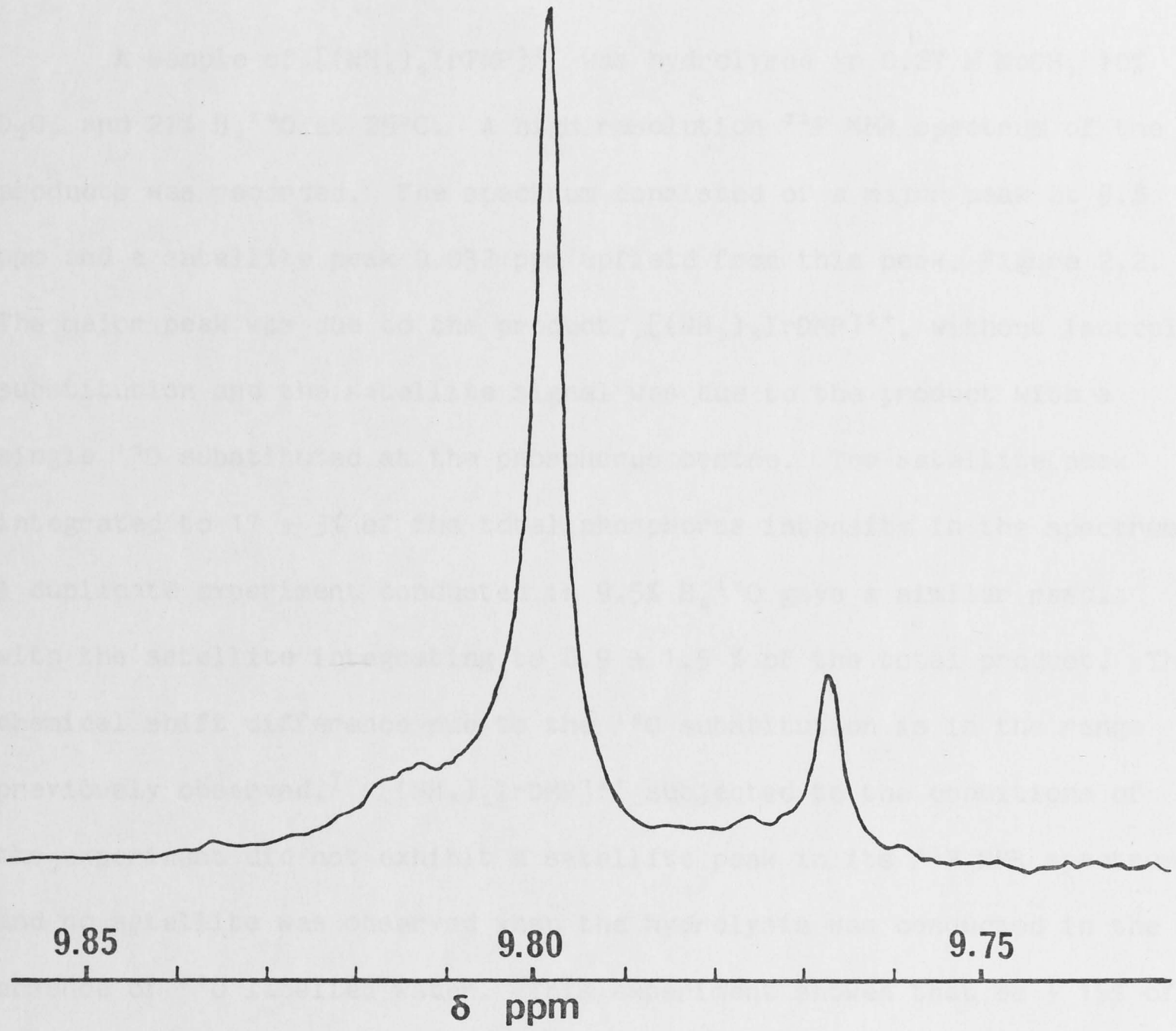
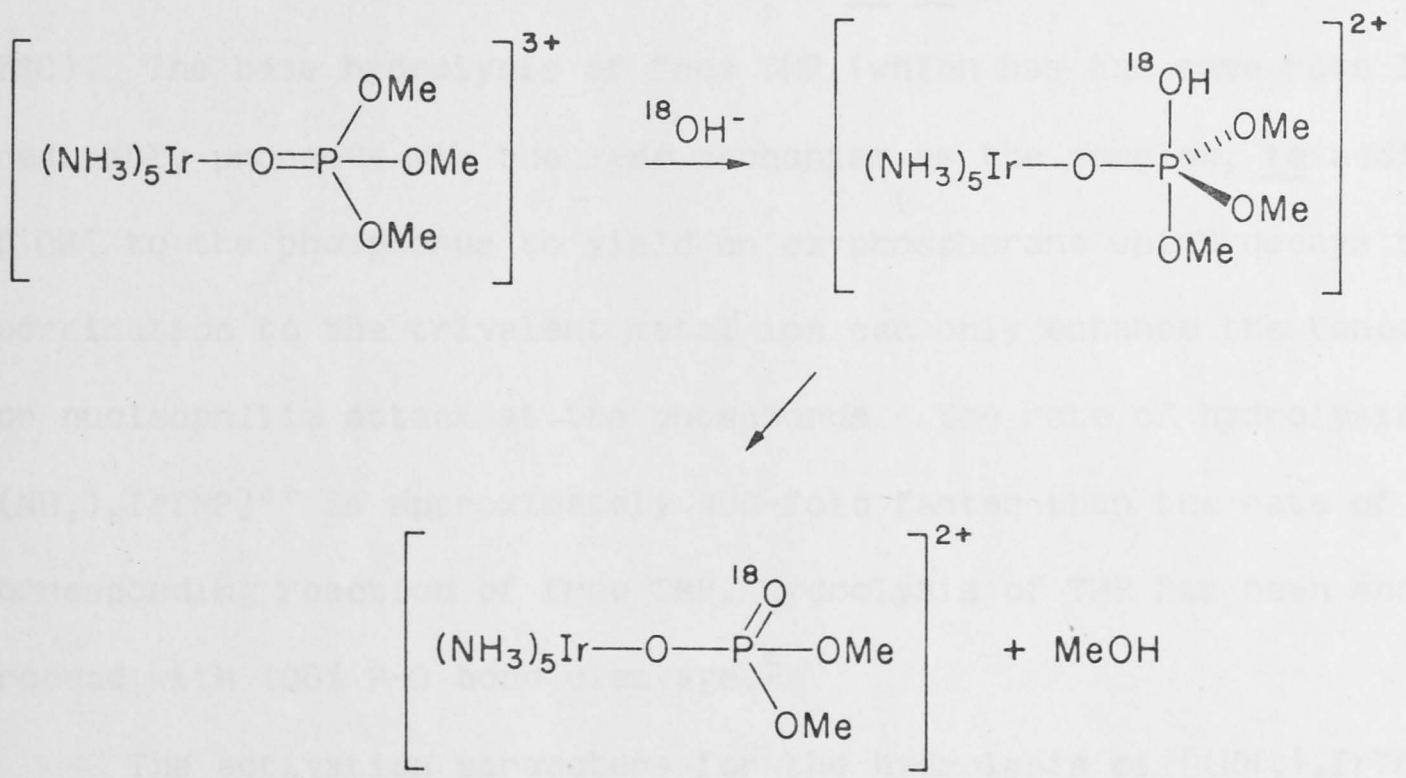


Figure 2.2.

^{31}P NMR spectrum of the products of hydrolysis of $[(NH_3)_5IrTMP]^{3+}$, hydrolysed in 21% $H_2{}^{18}O$. Conditions as per text.

intensity. The reaction was also followed by U.V. spectroscopy at 250 nm. The second order rate constants for hydrolysis in 1 M perchlorate media was $(6.7 \pm 0.4) \times 10^{-2}$ $lmol^{-1}s^{-1}$ at 25°C. (Table 2.2) The temperature dependence of the rate of hydrolysis was investigated spectrophotometrically in 0.1 M hydroxide and an ionic strength of 1.0 M ($NaClO_4$), at four temperatures between 5° and 35°C. A plot of 1/T versus ln k_{obs} was linear and yielded the activation parameters $\Delta H^{\neq}$ 64 ± 5 $KJmol^{-1}$ and $\Delta S^{\neq}$ -74 ± 15 JK^{-1} mol^{-1}.

A sample of $[(NH_3)_5IrTMP]^{3+}$ was hydrolysed in 0.27 M NaOH, 10% D_2O, and 21% $H_2{}^{18}O$ at 25°C. A high resolution ^{31}P NMR spectrum of the products was recorded. The spectrum consisted of a major peak at 9.8 ppm and a satellite peak 0.032 ppm upfield from this peak, Figure 2.2. The major peak was due to the product, $[(NH_3)_5IrDMP]^{2+}$, without isotopic substitution and the satellite signal was due to the product with a single ^{18}O substituted at the phosphorus centre. The satellite peak integrated to 17 ± 3% of the total phosphorus intensity in the spectrum. A duplicate experiment conducted in 9.5% $H_2{}^{18}O$ gave a similar result with the satellite integrating to 8.9 ± 1.5 % of the total product. The chemical shift difference due to the ^{18}O substitution is in the range previously observed.[7] $[(NH_3)_5IrDMP]^{2+}$ subjected to the conditions of the experiment did not exhibit a satellite peak in its ^{31}P NMR spectrum and no satellite was observed when the hydrolysis was conducted in the absence of ^{18}O labelled water. This experiment showed that 88 ± 15% of the label was incorporated into the DMP product presumably by intermolecular attack of $^{18}OH^-$ at the phosphorus.



SCHEME 2.1

Proposed Reaction Mechanism for the Base Hydrolysis of $[(NH_3)_5IrTMP]^{3+}$.

The base hydrolysis of the $[(NH_3)_5IrTMP]^{3+}$ ion using OH^- yields $[(NH_3)_5IrDMP]^{2+}$ and methanol quantitatively. The oxygen tracer experiment shows conclusively that the reaction proceeds largely with P-O cleavage with incorporation of the labelled solvent oxygen in the $[(NH_3)_5IrDMP]^{2+}$ product as shown in Scheme 2.1.
The uncertainty in the tracer experiment (88 ± 15% incorporation of label at P) means that some attack of hydroxide at carbon cannot be eliminated as a possible minor reaction path. The reaction presumably goes via the usual $S_N2(P)$ mechanism in which attack of OH^- at the P centre yields the five-coordinate oxyphosphorane complex which decays rapidly to the dimethylphosphate complex product.

The hydrolysis of TMP was followed by ^1H NMR spectroscopy under pseudo first order conditions at 27°C and μ = 1.0 M (NaCl). The pseudo first order rate constant was linear with hydroxide concentration to yield a second order rate constant of $(1.4 \pm 0.2) \times 10^{-4}$ $lmol^{-1}s^{-1}$. The reaction yielded only DMP and methanol as shown by ^1H and ^{31}P NMR spectroscopy. The rate constant was very similar to the value obtained by extrapolation of the data of Barnard *et al*[5], (1.6×10^{-4} $lmol^{-1}s^{-1}$ at 27°C). The base hydrolysis of free TMP (which has the same rate law) presumably proceeds via the same mechanism as the complex, *ie* addition of OH^- to the phosphorus to yield an oxyphosphorane which decays to DMP. Coordination to the trivalent metal ion can only enhance the tendency for nucleophilic attack at the phosphorus. The rate of hydrolysis of $[(NH_3)_5IrTMP]^{3+}$ is approximately 400-fold faster than the rate of the corresponding reaction of free TMP. Hydrolysis of TMP has been shown to proceed with 100% P-O bond cleavage.[5]

The activation parameters for the hydrolysis of $[(NH_3)_5IrTMP]^{3+}$ in 0.1 M NaOH show that the rate enhancement is due solely to the more favourable $\Delta S^{\neq}$ term, i.e. the $\Delta H^{\neq}$ terms for the reactions of the free

and coordinated TMP appear to be identical; for the complex, $\Delta H^{\neq}$ 64 $KJmol^{-1}$, $\Delta S^{\neq}$ -74 $JK^{-1}mol^{-1}$; for the uncoordinated TMP, $\Delta H^{\neq}$ 64 $KJmol^{-1}$ $\Delta S^{\neq}$ -103 $JK^{-1}mol^{-1}$.[5] This result conflicts with what one might predict by a preliminary inspection; if the rate determining step was a simple nucleophilic addition and the rate enhancement were due to the metal ion withdrawing electron density from the phosphorus centre making it more susceptible to nucleophilic attack, the effect should be observed in the $\Delta H^{\neq}$ term. Similarly, if the rate enhancement was due to the the anionic nucleophile attacking a neutral species on one hand and a tripositively charged ion on the other, the difference in the work required to bring the two reactancts together should also be reflected in the $\Delta H^{\neq}$ term. This, however is not the case, the rate enhancement resides in the larger $\Delta S^{\neq}$ term. An explanation for this may reside in the considerable difference in solvation of the two activated complexes. The free TMP in going from ground state to transition state, a neutral molecule to an anion, must experience a more profound change in solvation than the corresponding change for the complexed TMP, a +3 ground state to a +2 transition state. In this argument, it is tacitly assumed that the activated complex resembles the pentacoordinate intermediate more than the reactants. An alternative explanation may be that attack of OH^{-} on the phosphorus atom is not the rate determining step; rather, a pre-equilibrium is established between the reactant and the phosphorane and decomposition of the pentacoordinate phosphorane intermediate is rate determining. For example, the methoxy group may require protonation before leaving, in which case the change in $\Delta S^{\neq}$ may be due to effects associated with transfer of a proton from the hydroxy group of the intermediate to the methoxy group and such effects could give rise to the entropy changes observed.

Similar results have been observed in the base hydrolysis of

coordinated acetonitrile and dimethylformamide, ie the difference in the activation parameters between the coordinated and free molecule is solely in the more positive $\Delta S^{\neq}$ term for the coordinated molecule.[8] Clearly this area requires a more detailed study of the microscopic events and energetics.

2.3 $[(NH_3)_5RhOP(OCH_3)_3]^{3+}$

2.3.1 Introduction

The substitution reactions of Rh(III) ions are usually intermediate in rate between the corresponding Co(III) and Ir(III) complexes.[6] Hydrolytic studies on the $[(NH_3)_5IrTMP]^{3+}$ complex have shown that in hydroxide solution the reaction yields $[(NH_3)_5IrDMP]^{2+}$ and MeOH exclusively, whereas $[(NH_3)_5CoTMP]^{3+}$ yields only $[(NH_3)_5CoOH]^{2+}$ and free TMP. It was therefore of interest to observe the products and the rate of the base hydrolysis of $[(NH_3)_5RhTMP]^{3+}$.

2.3.2 Experimental

$[(NH_3)_5)RhTMP](ClO_4)_3$ (2)

$[(NH_3)_5RhOSO_2CF_3](CF_3SO_3)_2$ (2.0g) was dissolved in TMP (35 mL) and heated to 70°C for 2 hours. The solution was extracted with ether (3x) and the resulting powder dissolved in methanol (35 mL). A solution of $LiClO_4$ in methanol (4.0 g in 40 mL) was added dropwise until no more precipitation occurred (~1.0 mL). The yellowish precipitate was washed with methanol and dried in vacuo. Yield 1.5g. Analysis calculated for $C_3H_{24}N_5Cl_3O_{16}PRh$; C, 5.75; H, 3.86; N, 11.18; P, 4.94. Found; C, 5.8; H, 3.8; N, 10.8; P, 4.6. ^1H NMR; 3.93 ppm (d) J = 11.2 Hz, D_2O, 3.68 (d) J = 11.2 Hz 9H, 4.29 (br) 3H, 3.94 (br) 12H d_6-DMSO/TMS. $^{31}P\{H\}$ NMR; 7.0 ppm (d) J_{Rh-P} = 2.3 Hz, H_2O/D_2O. Electronic spectrum,

$\varepsilon^{max}_{264} = 88\ M^{-1}\ cm^{-1}$, $\varepsilon^{max}_{328} = 108\ M^{-1}\ cm^{-1}$.

The base hydrolysis of $[(NH_3)_5RhTMP]^{3+}$ was followed at 290 nm in a thermostatted cell holder. The kinetics were run in buffer solutions in the pH region 10.20 to 11.70, containing 1-butylamine and HCl. Seven pH values were chosen and four buffer concentrations were used at each pH. The rate of acid hydrolysis of 2 in 0.1 M HCl, μ = 1.0 M (NaCl) was followed spectrophotometrically at 340 nm. Each rate constant was the average of at least 2 separate determinations. The reaction was initiated by rapidly dissolving a small amount of the complex in the required buffer. This was achieved by means of a thermostatted syringe designed for the rapid dissolution of solid samples and their subsequent injection into sampling devices, eg spectrophotometric cells.[9] The data sets obtained followed single exponential decays and were analysed by curve fitting using the LSTSQR program. Measurement of the pH of the buffer solutions was conducted using deaerated buffer solutions at 25°C with a Radiometer PHM 26 pH meter equipped with a G202C glass and K4122 calomel electrodes and calibrated using standard buffers.

The yield of products of the hydrolytic reactions were determined by ^1H and ^{31}P NMR spectroscopy. For ^1H NMR spectroscopy, the reactions were conducted in D_2O. ^{31}P NMR spectroscopic analysis of the reaction products was carried out by addition of D_2O (10% by volume), to the reaction mixture immediately prior to the accumulation of the spectrum.

2.3.3 Results and Discussion.

The complex 2 was synthesized by heating $[(NH_3)_5RhOSO_2CF_3](CF_3SO_3)_2$ in TMP and recrystallized from methanol as the perchlorate salt. ^1H and ^{31}P NMR spectra are in agreement with the proposed structure. The rhodium complex shows a small $^{103}Rh,^{31}P$ coupling confirming the inner sphere nature of the complex.

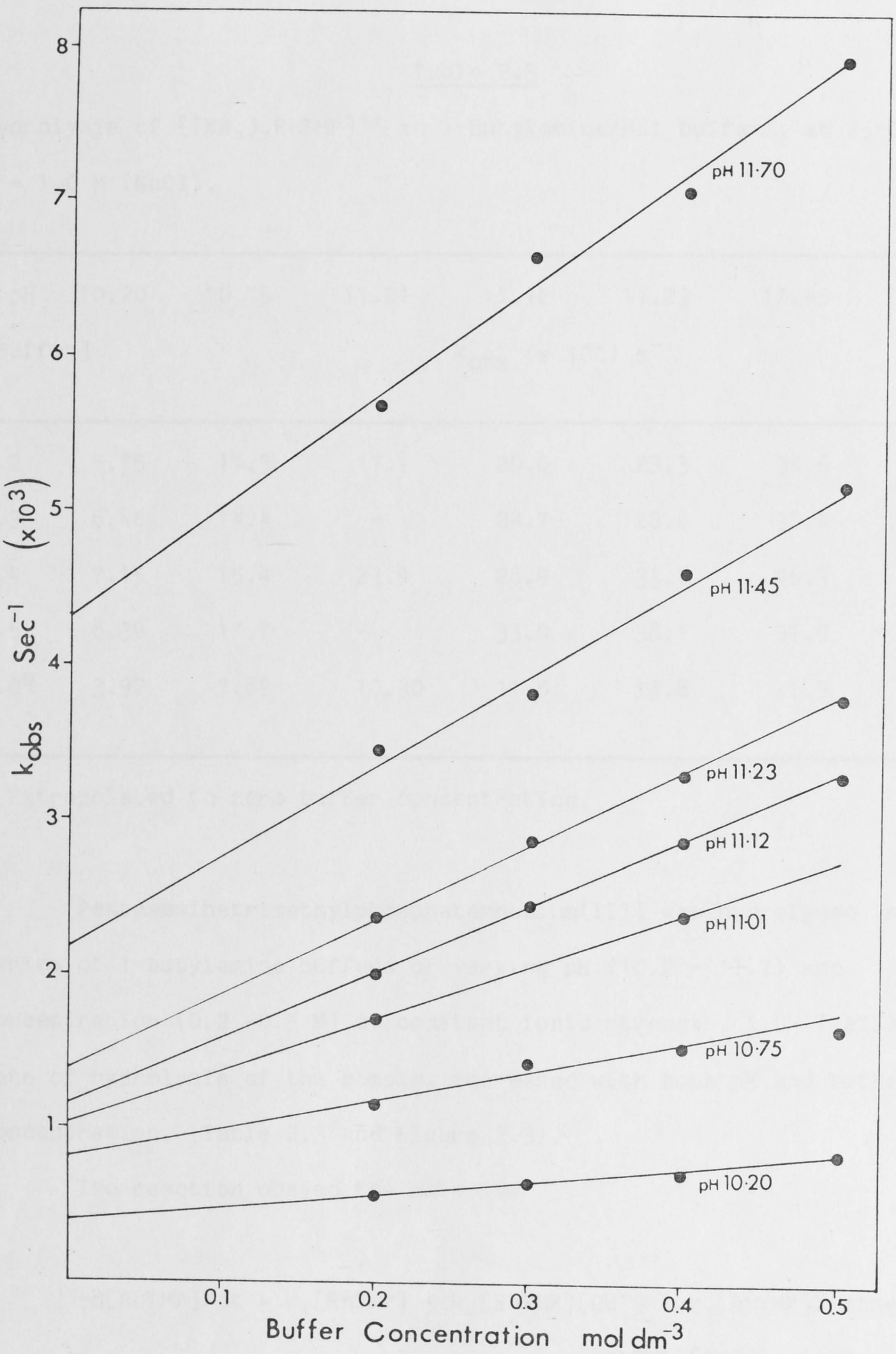


Figure 2.3.

Dependence of the rate of hydrolysis of $[(NH_3)_5RhTMP]^{3+}$ on pH and buffer concentration. Conditions; 1-butylamine buffers, μ = 1.0 M (NaCl), Temp. = 25°C.

Table 2.3

Hydrolysis of $[(NH_3)_5RhTMP]^{3+}$ in 1-Butylamine/HCl buffers, at 25°C, μ = 1.0 M (NaCl).

pH	10.20	10.75	11.01	11.12	11.23	11.45	11.70
[Buffer]				k_{obs} (x 10^4) s^{-1}			
0.2	5.75	11.5	17.1	20.0	23.3	34.6	56.9
0.3	6.46	14.4	-	24.7	28.9	38.4	66.6
0.4	7.15	15.4	23.9	28.9	33.1	46.3	70.8
0.5	8.39	17.7	-	33.0	38.1	51.9	79.5
0.0[a]	3.92	7.89	10.30	11.5	13.8	21.9	43.2

[a] Extrapolated to zero buffer concentration.

Pentaamminetrimethylphosphaterhodium(III) was hydrolysed in a series of 1-butylamine buffers of varying pH (10.2 - 11.7) and concentration (0.2 -0.5 M) at constant ionic strength, 1.0M (NaCl). The rate of hydrolysis of the complex increased with both pH and buffer concentration, (Table 2.3 and Figure 2.3).

The reaction obeyed the rate law:

$$-d[RhTMP]/dt = k_1[RhTMP] + k_2[RhTMP][OH^-] + k_3[RhTMP][amine] + k_4[RhTMP][OH^-][amine] \quad (2)$$

The rate constants k_1 to k_4 were determined graphically using a linear least squares analysis of the data; k_1 and k_2 were determined from the intercept and slope, respectively, of a plot of $[OH^-]$ versus $k_{obs'}$, where $k_{obs'}$ is the rate of hydrolysis extrapolated to zero buffer

concentration, (Figure 2.3). The -log K_W under these conditions was taken as 13.75. From this plot, $k_1 = (1.8 \pm 1.0) \times 10^{-4}$ s^{-1} and $k_2 = (4.9 \pm 0.3) \times 10^{-1}$ $lmol^{-1}$ s^{-1}.

From equation (1) it is apparent that

$$k_{obs} = k_1 + k_2[OH^-] + k_3[amine] + k_4[OH^-][amine] \quad (3)$$

which can be rearranged to

$$(k_{obs}-(k_1 + k_2[OH^-]))/[amine] = k_3 + k_4[OH^-] \quad (4)$$

A linear plot of $(k_{obs}-(k_1 + k_2[OH^-]))/[amine]$ versus $[OH^-]$ was obtained with an intercept, $k_3 = (4.8 \pm .1) \times 10^{-3}$ $lmol^{-1}$ s^{-1} and a slope, $k_4 = (4.5 \pm .4) \times 10^{-1}$ $lmol^{-2}$ s^{-1}.

The rate constant k_1 was determined more precisely in 0.1 M HCl, μ = 1.0 M (NaCl) to yield a value of $(2.9 \pm 0.06) \times 10^{-4}$ s^{-1} at 25°C in agreement with the value extracted from the hydrolysis in basic conditions. ^{31}P NMR analysis of the products of acid hydrolysis showed that ~11% coordinated DMP was produced with the complement being free TMP. When the reaction was conducted in the absence of butylamine the reaction products were free TMP and the pentaamminerhodiumDMP^{2+} ion. In sodium hydroxide the yields of coordinated DMP and free TMP were found to be ~14% and ~86% respectively. In 1-butylamine buffered solutions the yield of coordinated DMP increases with concomitant production of N-methylbutylamine.

The rate constant for attack of OH^- on the Rh complex, 2, was ~8 times greater than for the corresponding Ir complex, 1. The reactions also gave different yields of products; for the rhodium complex both free TMP, (86%) and coordinated DMP, (14%) were produced, whereas the iridium complex yielded coordinated DMP as the sole phosphorus containing product. The bimolecular rate constant for attack of hydroxide on the rhodium complex, 2, k_2, 4.9×10^{-1} $lmol^{-1}s^{-1}$, may therefore be partitioned into two contributing rate constants. Thus,

the rate constant for the reaction between the rhodium complex and hydroxide to produce $[(NH_3)_5RhDMP]^{3+}$ and methanol is 6.9×10^{-2} $lmol^{-1}s^{-1}$ and that to produce free TMP is 4.2×10^{-1} s^{-1}.

Hydrolysis of the complex in hydroxide solution to yield coordinated DMP most likely proceeds by the same mechanism as that for the Ir complex, given that the rate constants are almost identical. The loss of the TMP ligand intact probably occurs by the $S_N1(CB)$ mechanism.[3] The acid hydrolysis of $[(NH_3)_5RhTMP]^{3+}$ yields ~ 11% coordinated DMP and 89% free TMP. The reaction is independent of acid concentration as observed for the corresponding Co(III) complex and probably goes by water attack at the phosphorus atom to produce coordinated DMP, and a simple first order dissociation of the complex to produce TMP and $[(NH_3)_5RhOH_2]^{3+}$.

In butylamine buffers the rate of hydrolysis of $[(NH_3)_5RhTMP]^{3+}$ is enhanced. This is due to attack of the amine on the methyl carbon to produce coordinated DMP and N-methylated butylamine. Moreover, there was no evidence for attack of amine at the phosphorus atom. The product of such a pathway would have been a phosphoramidate but it was not observed by ^{31}P NMR and phosphoramidates generally are very stable in basic conditions.[10] The butylamine dependent reaction apparently consists of 2 paths, one of which is first order in complex and butylamine, k_3, and another which is first order in complex, butylamine and hydroxide, ie overall third order, k_4. The products of the k_4 path are assumed to be the same as for k_3 but its contribution to the overall reaction rate is insignificant at all but the highest pH where it accounts for only ~10% of the reaction.

2.4 General Discussion.

The rate of attack of OH^- on the phosphorus atom of TMP coordinated to both $(NH_3)_5Ir$- and $(NH_3)_5Rh$- is essentially the same, Ir ~6 x 10^{-2}, Rh ~7 x 10^{-2} $lmol^{-1}s^{-1}$. This assumes that the reaction to produce $[(NH_3)_5RhDMP]^{2+}$ and methanol occurs by attack of hydroxide on the phosphorus and not at the methyl carbon. This is not an unreasonable assumption given that the hydrolysis of both the iridium complex and free TMP proceed by attack at phosphorus. The similarity of the rate of attack at the phosphorus is not unexpected; such effects have been observed previously. The base hydrolysis of coordinated benzonitrile, acetonitrile and dimethylformamide show only a slight dependence on the metal ion in the series $(NH_3)_5Co$, $(NH_3)_5Rh$ and $(NH_3)_5Ir$.[8,11,12,13] Indeed, it seems to be a general observation that when metal to ligand bond making or fission is not directly involved in the reaction of a coordinated ligand, there is little variation in the rate constants along the series Co(III), Rh(III) and Ir(III).[14]

These results imply a rate constant for the rate of attack of OH^- on the phosphorus atom of $[(NH_3)_5CoTMP]^{3+}$ of ~8 x 10^{-2} $lmol^{-1}s^{-1}$ which is consistent with the fact that no methanol or coordinated DMP is observed in the reaction[2] since the rate constant for the $S_N1(CB)$ reaction (Co-O cleavage) for this complex is ~10^3 fold larger, 79 $lmol^{-1}s^{-1}$, at 25°C. The predicted yield of coordinated DMP and methanol is therefore ~0.1% which is less than the detection limit for the conditions of the experiment.

The observations made here show the effect that coordination to a metal ion can have on the rate of intermolecular nucleophilic attack on a phosphate ester. In this case, coordination of TMP to a trivalent metal centre increases the rate of attack of hydroxide at the phosphorus atom by ~400 fold in aqueous solution. This result is interesting

because it gives, for the first time, some idea of the ability of metal ions to activate phosphates for intermolecular nucleophilic attack. This information should be relevant for mechanistic enzymologists who, confronted with the fact that metal ions are often required for enzymic phosphoryl transfer, need to know roughly the efficacy of the various possible ways that a metal ion might promote such phosphoryl transfers.

Coordination to a tripositive metal centre clearly has a marked effect on the rate of intermolecular attack of hydroxide on TMP, (and presumably other phosphate esters), in aqueous solution. However, compared with the magnitude of the rate enhancement achievable by enzymes, up to ~10^{11}, this effect (~400) is quite minor. Nevertheless, it could be responsible for part of the rate enhancement observed in enzymic hydrolysis in relevant circumstances, eg E. coli alkaline phosphatase.[15] There is no doubt however, that monodentate coordination of a phosphate ester alone is inadequate to explain the enormous rate enhancements observed with enzymic systems.

References, Chapter 2.

1. Schmidt, W. and Taube, H., Inorg. Chem., **2**, 698-705, (1963).

2. Dixon, N.E., Jackson, W.G., Marty, W. and Sargeson, A.M., Inorg. Chem., **21**, 688-697, (1982).

3. Tobe, M.L., in "Adv. Inorg. Bioinorg. Mech." Sykes, A.G.,(Ed.) Academic Press, London, 1983, Vol 2, pp 1-94.

4. Jackson, W.G. and McGregor, B.C., Inorg. Chim. Acta, **83**, 115-124, (1984).

5. Barnard, P.W.C., Bunton, C.A., Llewellyn, D.R., Vernon, C.A. and Welch, V.A., J. Chem. Soc., **1961**, 2670-2676.

6. Tobe, M.L., in "Inorg. Reaction Mechanisms", Nelson, London, 1972, pp 87.

7. Webb, M.R., Trentham, D.R., J. Biol. Chem., **255**, 1775, (1980). Gorenstein, D.G. and Taira, K., J. Am. Chem. Soc., **104**, 6130, (1982). Sammons, R.D., Frey, P.A., Bruzik, K. and Tsai, M-D., J. Am. Chem. Soc., **105**, 5455-5461, (1983).

8. Curtis, N.J. and Sargeson, A.M., J. Am. Chem. Soc., **106**, 625-630, (1984).

9. Lawrance, G.A., J. Chem. Ed., **60**, 663, (1983).

10. Preobrazhenskaya, N.N., Russ. Chem. Rev. (Eng. Trans.), **41**, 54-65, (1972).

11. Zanella, A.W. and Ford, P.C., Inorg. Chem., **14**, 700-701, (1975).

12. Buckingham, D.A., Keene, F.R. and Sargeson, A.M., J. Am. Chem. Soc., **95**, 5649, (1973).

13. Buckingham, D.A., Harrowfield, J. MacB. and Sargeson, A.M., J. Am. Chem. Soc., **96**, 1726, (1974).

14. Palmer, D.A. and Harris, G.M., Inorg. Chem., **13**, 965-969, (1974).

15. Coleman, J.E. and Gettins, P., in "Advances in Enzymology", Meister, A., (Ed.), Interscience, New York, 1983, Vol **55**, pp 453-480.

CHAPTER 3

INTRAMOLECULAR ATTACK OF AMIDO ION - THE CHELATE PHOSPHORAMIDATE.

3.1 General Introduction.

The [4-nitrophenylphosphatopentaammminecobalt]$^{+1}$ and [fluorophosphatopentaammminecobalt]$^{+2}$ ions react in hydroxide to yield significant amounts of 4-nitrophenolate and F^- respectively by attack of deprotonated ammonia at the phosphorus centre. This observation raised several interesting possibilities with regard to metal ion activated phosphoryl transfer.

The reaction presumably goes via a chelate phosphoramidate, incorporating the phosphorus into a strained 4- membered ring. This ring was not observed in the reaction and the question of the stability of the ring therefore remained unanswered. The possibility that the chelate might be observable by improving the leaving group was envisaged; to this end the [2,4-dinitrophenylphosphato-pentaammminecobalt]$^+$ ion was synthesized and its hydrolysis in basic conditions studied.

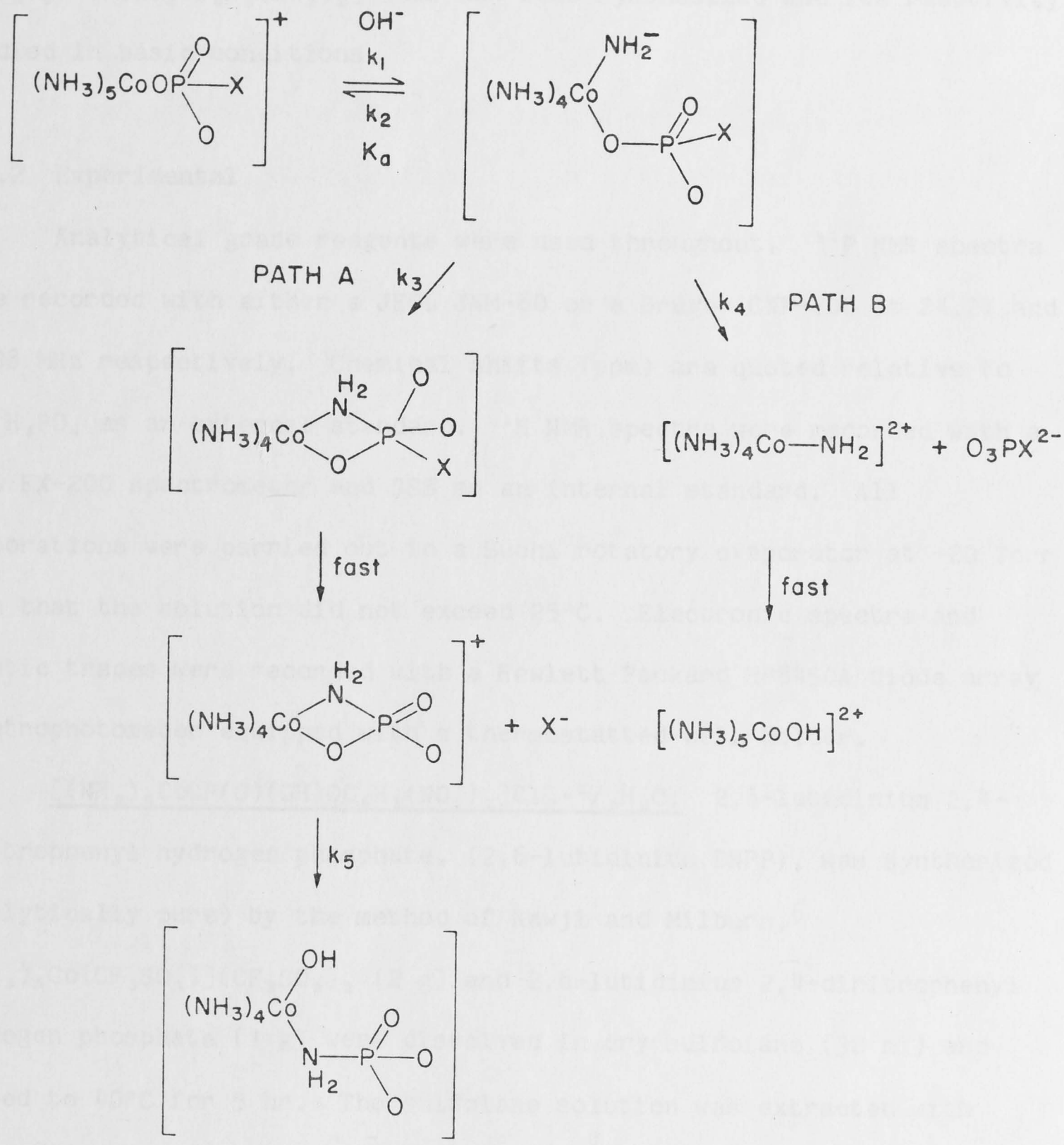
The demonstration that a cis-coordinated amido ion was an effective nucleophile towards phosphorus raised the prospect of being able to synthesize a chelated phosphoramidate ester. Such an ester should be formed at least as an intermediate in the hydrolysis of a coordinated phosphodiester. The interest in this type of molecule arose from the work of Westheimer on the extraordinary reactivity of 5-membered ring organic phosphate esters. The prospect of observing substantial reactivity with phosphate esters involved in 4-membered rings prompted the synthesis of and hydrolytic studies on the [ethyl,4-nitrophenylphosphatopentaammminecobalt]$^+$ ion.

Lastly the uncoordinated basic oxygen of the coordinated 4-nitrophenylphosphate provided the opportunity for investigation of the effect of coordination of another metal on the reaction. Many phosphoryl transfer enzymes require two or more metal ions for activity[3], and this system provides an ideal opportunity to study the effects that two coordinated metal ions can have on the reactivity of a phosphate ester. The complex ion [μ-(4-nitrophenylphosphato)-decaamminedicobalt]$^{4+}$ was synthesized and its hydrolysis studied and compared with the corresponding mononuclear complex.

3.2 $[(NH_3)_5CoOP(O)_2(OC_6H_3(NO_2)_2)]^+$.

3.2.1 Introduction.

The hydrolysis of the pentaammine cobalt(III) complexes $[(NH_3)_5CoOP(O)_2OC_6H_4NO_2]^{+1}$ and $[(NH_3)_5CoOPO_2F]^{+2}$ have been studied in this laboratory by other workers. In both these cases, it was postulated that reaction proceeded via attack of a cis- coordinated deprotonated ammonia on the phosphorus centre to yield a 5-coordinate amino-phosphorane activated complex which decayed to a presumed N,O chelate phosphoramidate, (Scheme 3.1). The chelate, however, was never observed and was presumed to undergo ring opening rapidly under the conditions in which it was produced.[1,2] The aim of the present study was to observe the chelate formation and decay, which might be achieved by making the precursor complex more reactive, ie 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[4] and is known to be more reactive than 4-nitrophenylphosphate and



Scheme 3.1

Proposed Reaction Scheme for Phosphatopentaamminecobalt Derivatives.[6,7]

fluorophosphate.[1,2,5] The complex ion $[(NH_3)_5CoOP(O)_2OC_6H_3(NO_2)_2]^+$ has now been synthesized and its reactivity studied in basic conditions.

3.2.2 Experimental

Analytical grade reagents were used throughout. ^{31}P NMR spectra were recorded with either a JEOL JNM-60 or a Bruker CXP-200 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 thermostatted cell holder.

$\underline{[(NH_3)_5CoOP(O)(OH)OC_6H_3(NO_2)_2]Cl_2 \cdot {}^1/_2H_2O.}$ 2,6-lutidinium 2,4-dinitrophenyl hydrogen phosphate, (2,6-lutidinium DNPP), was synthesized (analytically pure) by the method of Rawji and Milburn.[6] $[(NH_3)_5Co(CF_3SO_3)](CF_3SO_3)_2$ (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 hr. The sulfolane solution was extracted with ether (1 l) and the residue dissolved in water (800 ml), acidified to ~ pH 2 with HCl and absorbed on a Sephadex SP-C25 (Na^+) column (18 x 5 cm). The column was eluted with 0.2 M NaCl acidifed to pH 2 with HCl. The first eluted band was evaporated (to 35 ml) and cooled at 4°C for 16 hr. A red microcrystalline solid was collected, washed twice with cold 2 M NaCl (pH 2) twice with ethanol, thrice with ether and dried <u>in vacuo</u> for 8 hr. (Yield, 230 mg, 19%). Analysis calculated 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. ^1H NMR (D_2O, 0.01M

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. $^{31}P\{^{1}H\}$ NMR (H_2O/D_2O); (0.1 M NaOH); +6.5, (0.025 M HCl); +5.0. ε^{max}_{518} 75 $M^{-1}cm^{-1}$ (0.02 M HCl).

Fresh solutions of known concentrations of $[(NH_3)_5CoDNPP]^{2+}$, (~ 10^{-2} M, 10 µl) were syringed into 2 ml of hydroxide solution in a thermostatted cell in the spectrophotometer. The solution was rapidly mixed and the change in absorbance at 360 nm was recorded with time. The molar absorbtivity of the dinitrophenolate, (DNP), ion was taken to be 14,700 at 360 nm.[7] The data were processed by a VAX-11/750 computer using a non-linear least-squares package, LSTSQR. The sets of data fitted well to single exponential functions. All quoted rate constants are the average of at least two separate determinations, the errors are standard deviations.

A known weight of $[(NH_3)_5CoDNPP]^{2+}$, (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). 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 replaced in the thermostatted NMR probe. The final NaOH concentration was at least 10 times the complex concentration. ^{1}H coupled spectra (^{1}H decoupling irradiation heats the sample) were recorded at intervals at 5°C and stored on disc. (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 3 signals at, 6.5 ppm, 22 to 31 ppm (dependent on base concentration) and 7.1 ppm. The intensities of the signals were plotted relative to the standard. The variation in the intensity of the signal at 6.5 ppm versus 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 determined 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 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 spectrophotometrically at 360 nm in 1 M aqueous NH_3 at 25°C under pseudo first order conditions. 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 lives. The identity of the signals were verified by addition of authentic specimens of the presumed products to the sample and by 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 two seconds. As expected the yield of phosphoramidate increased with increasing NH_3 concentration.

The complex $[(NH_3)_5CoDNPP]^+$ was hydrolysed 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 mono-cation on a Sephadex SP C-25 cation exchange column upon elution with $NaClO_4$ and is presumed to be the O-bonded phosphoramidate complex, $[(NH_3)_5CoOP(O)_2NH_2]^+$. Hydrolysis of the phosphoramidate complex in 1 M HCl yields $[(NH_3)_5CoOPO_3]$,

identified by its ^{31}P NMR chemical shift in acidic (1.0 M HCl, 7.6 ppm) and basic (0.1 M NaOH, 13.6 ppm) conditions.

3.2.3 Results and Discussion

$[(NH_3)_5CoDNPP]Cl_2 \cdot {}^1/_2H_2O$, was synthesized from the $[(NH_3)_5CoOSO_2CF_3]^{2+}$ ion and dinitrophenylphosphate ion,(DNPP), and purified by ion exchange chromatography.

Hydrolysis of the complex was followed by ^{31}P NMR spectroscopy (Figures 3.1 and 3.2) and spectrophotometrically at 360 nm. At 5°C, hydrolysis of the complex results in almost quantitative production of dinitrophenolate (DNP), ~ 98% (Table 3.1), by attack of a cis-coordinated amido ion at the phosphorus atom to produce the N,O chelate phosphoramidate (Path A, Scheme 3.1). The chelate phosphoramidate then undergoes a ring opening reaction to yield monodentate N-bound phosphoramidate which in a slower subsequent reaction yields free phosphoramidate and bis(hydroxo)tetraamminecobalt(III) ions along with some decomposition of the latter. At higher temperatures, a competing reaction (Path B, Scheme 3.1), which results in the loss of the DNPP ligand becomes more significant.

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 3.1). It had a second order rate constant at 25°C (μ = 1.0 M, $NaClO_4$) of (1.96 ± .03) x 10^{-2} $lmol^{-1}s^{-1}$. The temperature dependence of the reaction was also investigated, (Table 3.1), to yield the activation parameters for both reaction pathways. 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. Plots of 1/T versus ln k were linear for both reactions. The activation parameters for each process

were found to be; for production of DNP, Path A, $\Delta H^{\neq}$ 79 ± 2 $KJmol^{-1}$, $\Delta S^{\neq}$ -19 ± 4 $JK^{-1}mol^{-1}$, and for Path B, $\Delta H^{\neq}$ 119 ± 7 $KJmol^{-1}$, $\Delta S^{\neq}$ 93 ± 20 $JK^{-1}mol^{-1}$.

Table 3.1.

Hydrolysis of $[(NH_3)_5CoDNPP]^{2+}$ by OH^-, μ = 1.0 M

Temp.	[NaOH]	k_{obs} x 10^3 s^{-1}	Yield DNP %
5	0.5	.808 ± .003	98.3
15	0.5	2.86 ± .04	95.9
25	0.1	1.79 ± .01	93.6
25	0.2	3.62 ± .01	92.8
25	0.5	9.33 ± .05	93.0
25	1.0	19.9 ± .4	94.0
35	0.5	26.6 ± .1	91.4

Hydrolysis of the complex was also followed by ^{31}P NMR at 5 ± 1°C, (μ = 1.0 M NaCl, complex concentration ~0.025 M). (See for example Figures 3.1 and 3.2.) The second order rate constant for loss of the reactant was (2.5 ± .3) x 10^{-3} $lmol^{-1}s^{-1}$, larger than that determined spectrophotometrically, 1.62 x 10^{-3} $lmol^{-1}s^{-1}$, but in reasonable agreement given that the reaction was conducted in 20% D_2O and the difficulties involved in the ^{31}P NMR method, ie temperature control, inaccuracies in the integrations and errors in time and temperature involved in initiating and mixing the solution then transferring the sample into the probe.

The starting material decayed to yield an intermediate with a

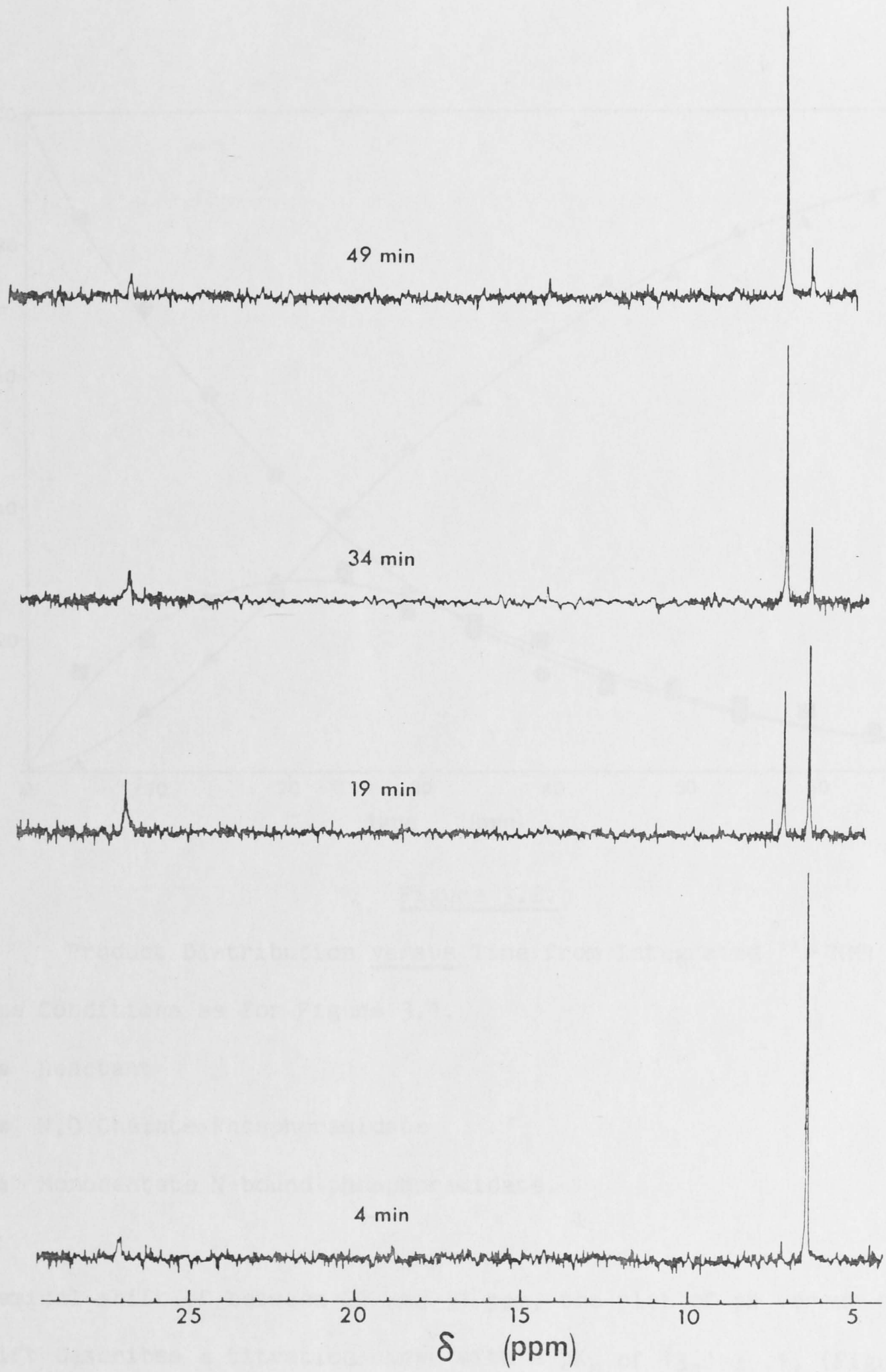


Figure 3.1.

Sequential ^{31}P NMR Spectra of Hydrolysis of $[(NH_3)_5CoDNPP]^{2+}$, ion. Conditions: $[OH^-]$ = 0.35 M, u = 1.0 M, Temp = 5°C. Acquisition parameters: Pulse repetition time 0.5 s, Spectral width 9KHz, Acquisition frequency 80.98 MHz, Pulse angle 90° .

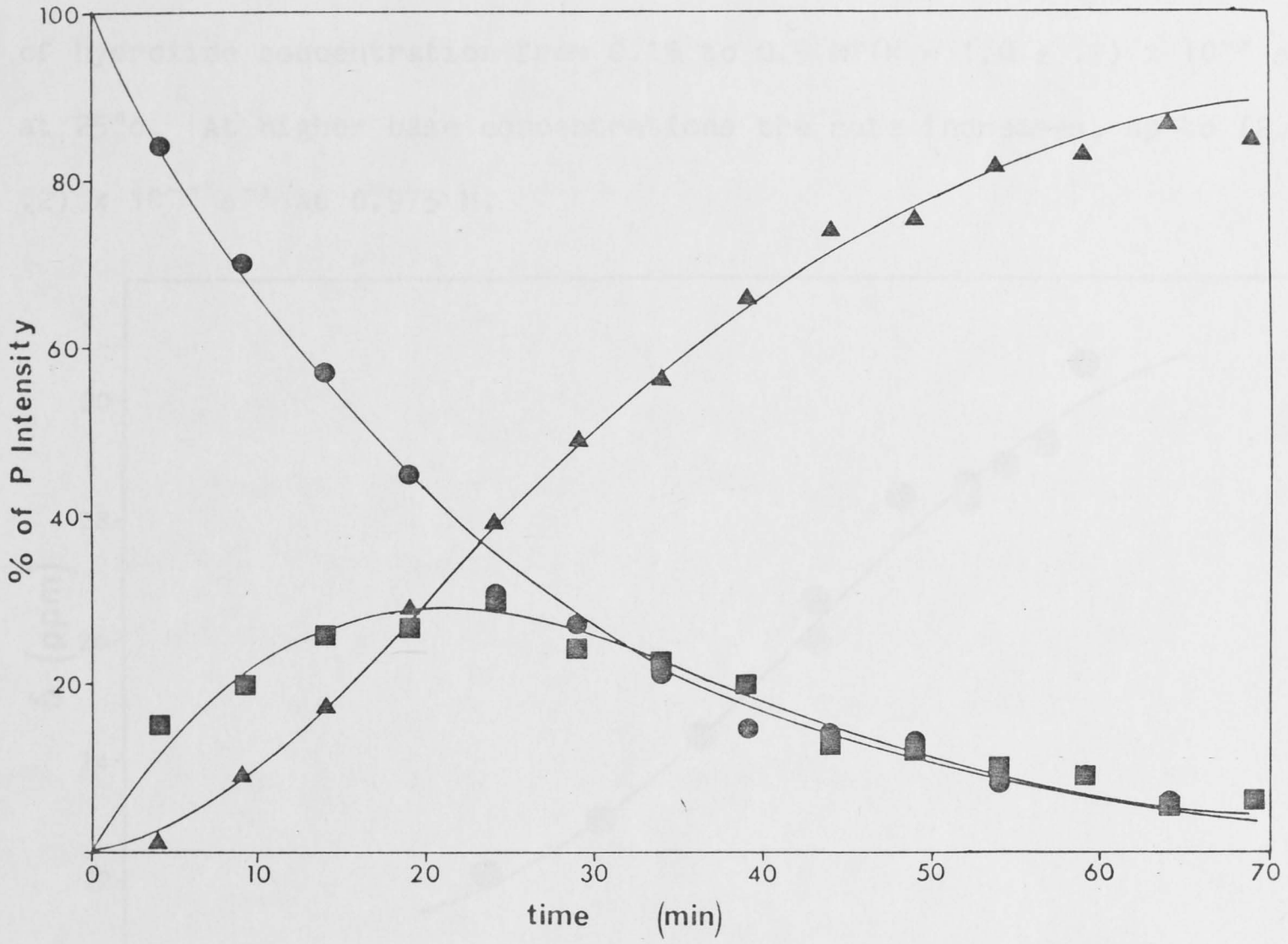


Figure 3.2.

Product Distribution versus Time from Integrated ^{31}P NMR Spectra, same Conditions as for Figure 3.1.

- ● Reactant
- ■ N,O Chelate Phosphoramidate
- ▲ Monodentate N-bound phosphoramidate.

chemical shift of between 22 and 31 ppm, the plot of pH versus chemical shift describes a titration curve with a pK_a of 13.1 ± .1, (Figure 3.3). The reaction of the intermediate to yield a compound with a chemical shift of 7.1 ppm was also followed by ^{31}P NMR. The determination of the rate constants for the reaction was complicated by the fact that the rate of production of the intermediate was not greatly different from its rate of decay and an equilibrium between two possible forms of the intermediate in the hydroxide concentration range of the experiment.

The rate of hydrolysis of the intermediate was approximately independent of hydroxide concentration from 0.15 to 0.5 M ($k = 1.0 \pm .1$) x 10^{-3} s^{-1} at 25°c. At higher base concentrations the rate increases, up to ($2.0 \pm .2$) x 10^{-3} s^{-1} at 0.975 M.

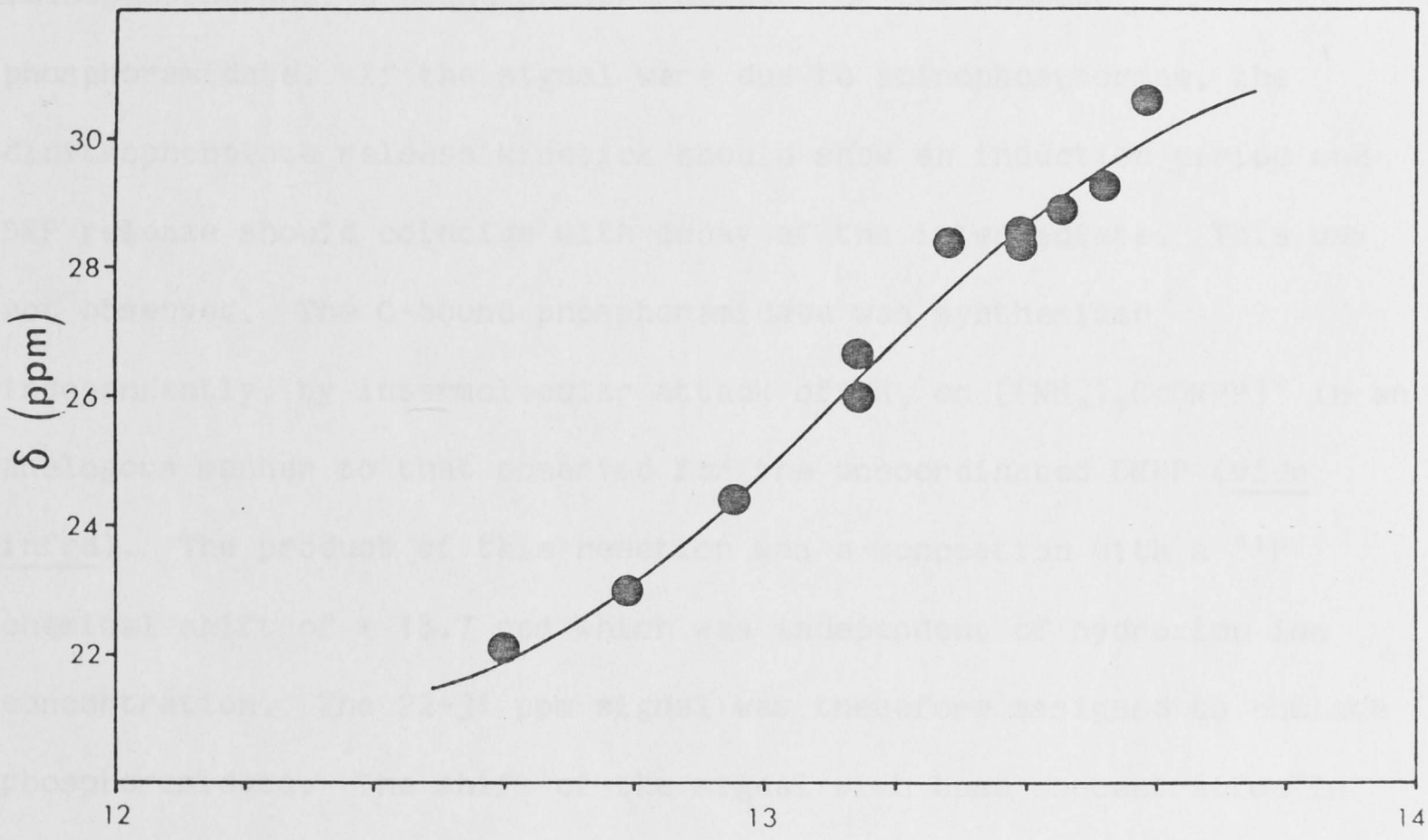


Figure 3.3.

^{31}P NMR Chemical Shift versus 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

The decay of the 7.1 ppm signal was followed by ^{31}P NMR spectroscopy in 0.5 M NaOH, μ = 1.0 M, at approximately 27°C. This was coincident with the growth of a signal at 8.6 ppm with a rate constant of approximately 3 x $10^{-4}s^{-1}$ in these conditions. These chemical shifts are identical to those observed for N-bound and free phosphoramidate respectively in related studies.[1,2] The observed rate of decay is also similar to that observed in a previous study.[1]

The unusual feature of the ^{31}P NMR spectra, however, was the

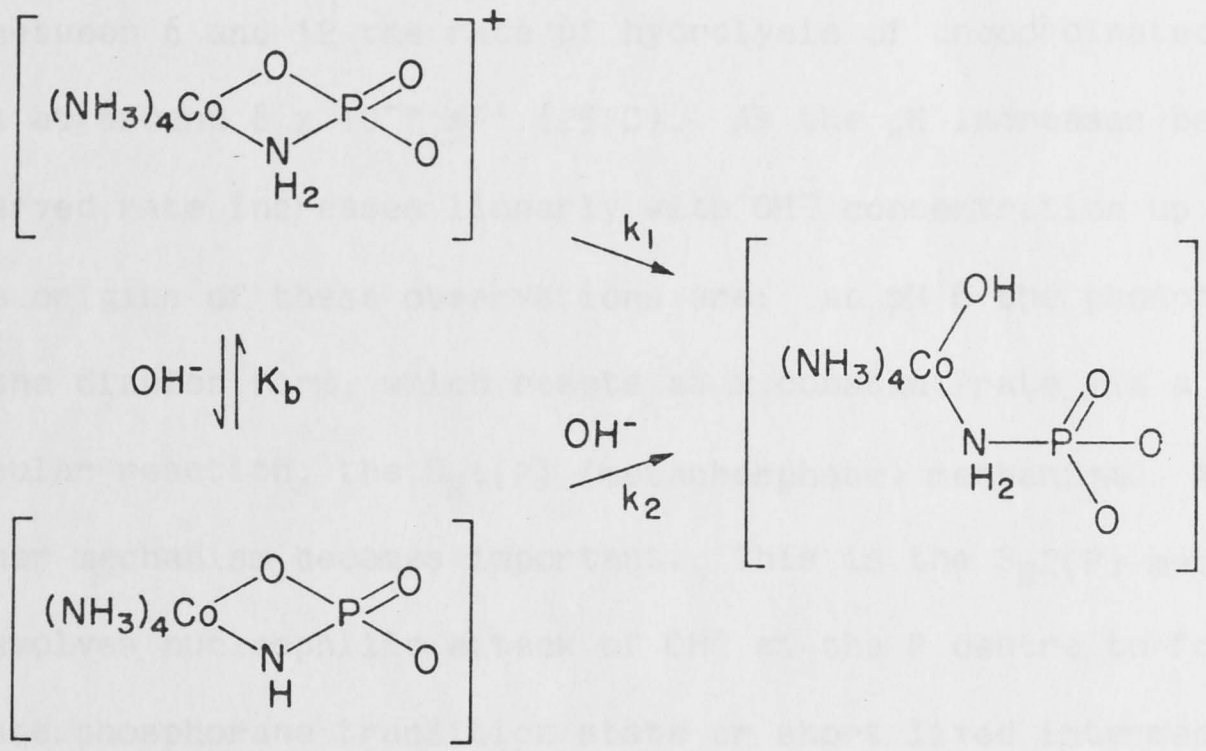
broad signal at 22 to 31 ppm, depending on hydroxide ion concentration. At 0.15 M OH^- the signal was at 22.1 ppm, whilst in 0.975 M OH^- the chemical shift was 30.6 ppm. Since the signals at 8.6 and 7.1 ppm have been definitely assigned, the low field signal could be attributed to aminophosphorane, O-bound phosphoramidate or the chelate phosphoramidate. If the signal were due to aminophosphorane, the dinitrophenolate release kinetics should show an induction period and DNP release should coincide 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 an analogous manner to that observed for the uncoordinated DNPP (vide infra). The product of this reaction was a monocation with a ^{31}P 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 shift of the signal with base concentration is ascribed to deprotonation at the bridging nitrogen. The chemical shift observed on deprotonation is much larger than that observed with 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 that instance, the effect of protonation was to shift the signal ~ 5 ppm upfield,[8] 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 centre since there is no other source of ammonia in the reaction. This path would yield initially a 5-coordinate aminophosphorane either as a transition state or short lived intermediate. It is possible that the phosphorane is an intermediate since some oxyphosphoranes and aminophosphoranes are stable especially

when ring systems are involved.[9] However when an ^{18}O tracer experiment was carried out with an analogous system,[1] vis the $[(NH_3)_5CoOP(O)_2OC_6H_4NO_2]^+$ ion in labelled 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.[10] 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 hydrolyses 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.[1] The liberation of dinitrophenylphosphate via Path B (Scheme 3.1) also presumably occurs via the $S_N1(CB)$ mechanism[11] by analogy with the 4-nitrophenylphosphate complex.[1]

The unusual hydroxide dependence of the ring opening reaction requires some explanation. A possible mechanism for the reaction is shown in Scheme 3.2.



SCHEME 3.2

Possible Reaction Scheme for the Ring-Opening of the N,O-Chelate Phosphoramidate.

The rate law derived for this reaction scheme is,

$$k_{obs} = \frac{k_1 + k_2 K_b [OH^-]^2}{1 + K_b [OH^-]}$$

Using the value of K_b obtained from the pH dependence of the chemical shift of the chelate, (Figure 3.3) and values for k_1 and k_2 of 1.4×10^{-3} s^{-1} and 2.2×10^{-3} $lmol^{-1}s^{-1}$ respectively the derived rate law adequately describes the dependence of k_{obs} on $[OH^-]$. The proposed hydroxide independent ring opening of the N-diprotonated phosphoramidate chelate, (k_1 path) might proceed via the equivalent structure where one of the protons has migrated from the phosphorus nitrogen to the bridging oxygen, although this oxygen is much more acidic than the nitrogen the complex thus formed would be expected to be extremely reactive. The k_2 path probably goes via the usual $S_N1(CB)$ mechanism of cobalt ammine chemistry[11] where a proton has been lost from one of the NH_3 groups.

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 around 8 x 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.[5] The origins of these observations are: 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 another mechanism becomes important. This is the $S_N2(P)$ mechanism which involves nucleophilic attack of OH^- at the P centre to form a 5-coordinate phosphorane transition state or short lived intermediate. Another possibility which was not addressed in the original publication is that OH^- attacks the carbon atom of the phenol. Alkaline methanolysis for example yielded significant amounts of 2,4-dinitroanisole from attack of MeO^- on the phenol carbon. 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.[12,13] 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 rate constant for attack of OH^- on DNPP is 2.5 x 10^{-5} $lmol^{-1}s^{-1}$. The observed rate of hydrolysis in 1 M NaOH is therefore ~3.3 x 10^{-5} s^{-1}. Hydrolysis of free DNPP in 1 M aqueous ammonia occurs with an observed rate constant of ~ 2.8 x 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 P centre is ~1.5 x 10^{-5} s^{-1}. (The phosphate arises from two reactions, intermolecular attack of OH^- on P 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 enhancement for attack of the nitrogen nucleophile

on the P centre is therefore ~1300 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 3.1), is established before the reaction is underway the rate of loss of reactant is

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

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 $lmol^{-1}s^{-1}$ for monopositive ions at 25°C.[14] Equation 1 yields

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

Since $K_a[OH^-] << K_w$,[15] equation 2 reduces to

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

which is the form of the observed rate law with

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

The pK_a of cobalt(III) coordinated ammonia for mono-cationic complexes is ~ 17,[16] which means that the rate of aminolysis of the cis-amido complex, k_3, is about 10^3k or 20 s^{-1} (because $k_4 << k_3$). This is a rate increase on coordination of ~ 10^6 compared with the attack of NH_3 on DNPP in 1 M NH_3.

The work described here has confirmed predictions[1,2] about the intermediacy of the N,O chelate phosphoramidate in the hydrolysis of the phosphate monoesters coordinated to the pentaamminecobalt(III) moiety.

3.3 $[(NH_3)_5CoOP(O)(OC_2H_5)(OC_6H_4NO_2)]^{2+}$

3.3.1 Introduction

As shown in the previous section, phosphomonoesters coordinated to the $(NH_3)_5Co^{3+}$ moiety react via a cis-amido ion to yield as an intermediate the chelate phosphoramidate. It seems likely that a similarly coordinated phosphodiester would react by a similar path. The initial product of such a reaction would be expected to be a chelate phosphoramidate ester. The reactivity of this class of compounds is interesting because of the presence of the 4-membered ring incorporating the phosphorus. This ring strain might cause an increase in the reactivity of the phosphorus atom by lowering the energy of the transition state for the $S_N2(P)$ reaction[17,18,19] as observed for the reactions of 5-membered ring organic phosphate esters[20], (vide supra).

3.3.2 Experimental

All chemicals used were analytical grade unless otherwise stated, 4-nitrophenol was recrystallized from ethanol before use.

Electronic spectra were recorded with either a Cary 118C or a Hewlett Packard 8450A spectrophotometer.

^1H NMR spectra were recorded with a JEOL FX-200 instrument operating at 200 MHz and referenced with respect to DSS (in D_2O) or TMS (in other solvents). ^{31}P NMR were recorded with JEOL FX-60 and Bruker CXP-200 instruments at 24.21 and 80.98 respectively and referenced with respect to external 85% H_3PO_4 (Downfield shift is positive).

Synthesis of $HOP(O)(OC_2H_5)(OC_6H_4NO_2)$ (ENPP).

To 4-nitrophenylphosphorodichloridate (5.12 g) in dry ether (30 ml) was added dry pyridine (1.58 g) in ether (10 ml). Nitrogen was passed over the surface of the resulting suspension and ethanol (0.92 g) in ether (15 ml) was added dropwise over 15 minutes. When the addition

was complete, pyridine (1.58 g), in water (30 ml) was added and the mixture stirred for a further 10 minutes. An excess of 5 M HCl solution was added to the biphasic mixture which was then extracted 3 times with ether. The ethereal solution was dried with sodium sulphate and evaporated to yield the product as a white solid. Yield 3.8 g. The crude product was recrystallized from hot ether/pentane. mp. 102-103°C

Analysis calculated for $C_8H_{10}NO_6P$; C, 38.88; H, 4.08; N, 5.67; P, 12.54. Found; C, 38.9; H, 4.1; N, 5.7; P, 12.4.

^1H NMR, (D_2O ~ pH 7); 1.33 (tr) J = 7.2 Hz (3H), 4.12 (d of quart) J_{H-H} ~ J_{P-H} ~ 7.2 Hz (2H), 7.33 (d) J = 8.1 Hz (2H), 8.20 (d) J = 8.1 Hz (2H). $^{13}C\{H\}$ NMR, (D_2O ~ pH 7); -51.1 (d) J = 7.5 Hz, -2.95 (d) J = 6.0 Hz, 53.89 (d) J = 4.5 Hz, 59.15 (s), 76.78 (s), 90.69 (d) J = 5.9 Hz. $^{31}P\{H\}$ NMR, (H_2O/D_2O pH 7); -5.0 (s).

Synthesis of $[(NH_3)_5CoOP(O)(OC_2H_5)(OC_6H_4NO_2)](ClO_4)_2 \cdot H_2O$ ($[(NH_3)_5CoENPP](ClO_4)_2H_2O$.

To $[(NH_3)_5CoOSO_2CF_3](CF_3SO_3)_2$ (2.1 g) in dry sulfolane was added ethyl4-nitrophenylphosphoric acid and several drops of tri(n-butyl)amine. The solution was stirred at 37°C for 3 hr, the sulfolane and excess phosphate ester were extracted twice with ether. The solid thus obtained was dissolved in water (1600 ml) and absorbed on a Sephadex SP C25 (Na^+ form) column. The column was eluted with a 0.1 M $NaClO_4$ solution, two minor bands which eluted first were discarded, elution then with 0.2 M $NaClO_4$ yielded the major band containing the desired product. The solution containing the product was evaporated to ~ 40 ml and cooled at 4°C for 16 hr. The complex crystallized as fine red needles which were washed with ethanol and ether and dried in vacuo. Yield 1.52 g.

Analysis calculated for $C_8H_{26}N_6Cl_2CoO_{15}P$; C, 15.81; H, 4.32; N, 13.84; Cl, 11.68; Co, 9.71; P, 5.10. Found, C, 15.8; H, 4.2; N, 13.7; Cl,

11.6; Co, 9.5; P, 5.1.

^{1}H NMR, (D_2O); 1.05 (d of tr) J = 1.0 Hz, 7.3 Hz (3H), 3.87 (d of quart) $J_{H-H} \sim J_{P-H} \sim$ 7.3 Hz (2H), 7.15 (d) J = 9.3 Hz (2H), 8.09 (d) J = 9.3 Hz (2H). $^{31}P\{H\}$ NMR,(H_2O/D_2O); +1.3 (s).

ε^{max}_{517} = 58.9 M^{-1} cm^{-1}, ε^{max}_{282} = 9.27 x 10^3 $M^{-1}cm^{-1}$.

Kinetics.

The kinetics of hydrolysis of the complex was followed spectrophotometrically in aqueous solution, by monitoring the release of 4-nitrophenolate at 400 nm. The reaction was followed under pseudo first order conditions at a constant ionic strength, 1.0 M ($NaClO_4$). Two methods for initiation of the reaction were used. The first involved use of a rapid hand mixing device which was required by the rapid rate observed at the higher hydroxide concentrations. A solution of known concentration of the complex (~10^{-4} M) in CO_2 free water was placed in one thermostatted compartment, in the other, a $NaOH/NaClO_4$ solution of double the required final concentration. The device mixes equal volumes of these solutions and injects the mixed solution into a flow-through cell in the light path of the spectrophotometer. The second method involved injection of a solution of known concentration the complex (10 µl, ~10^{-2} M) into a $NaOH/NaClO_4$ solution (2.00 ml) at the required temperature. The solution was rapidly mixed and placed in the thermostatted cell holder of the spectrophotometer. Both methods gave identical results. The data were processed by computer (VAX-11/750) using a non-linear least squares package, (LSTSQR). The sets of data fitted well to single exponential functions. The yield of 4-nitrophenolate was calculated from the final absorbance of the reaction mixture using a molar absorbtivity of 18,700 $M^{-1}cm^{-1}$ at 400 nm.

The hydrolysis of $[(NH_3)_5CoENPP]^{2+}$, was also followed by ^{31}P NMR spectroscopy. $[(NH_3)_5CoENPP]^{2+}$, (60 mg) and NaCl (82 mg) were dissolved

in H_2O (1.35 ml) containing Na_3PO_4 (0.02 M) and D_2O (0.4 ml). The solution was cooled in an ice bath and NaOH (0.25 ml, 2 M) added, the solution was placed in the probe of the spectrometer at 5°C and consecutive spectra accumulated. (Acquisition parameters; acquisition frequency 80.98 MHz, spectral width 9 KHz, acquire 8 K data points, zero fill to 16 K points, pulse repetition time 0.5 s, 120 scans per spectrum.).

The second step of the reaction was followed by ^{31}P NMR spectroscopy at 25°C. $[(NH_3)_5CoENPP]^{2+}$, (30 mg) was dissolved in H_2O (1.35 ml), D_2O (0.4 ml) containing Na_3PO_4 (~0.05 M) and NaCl was added to make the final ionic strength 1.0 M. The solution was cooled in an ice bath then a solution of NaOH (0.25 ml, 1,2,4,6 or 8 M) added. The reaction was allowed to proceed for 5-10 minutes, then the solution was warmed to 25°C and placed in the probe of the CXP-200 spectrometer thermostatted at 25 ± 1°C. ^{31}P NMR spectra were accumulated every 5 minutes for 1 hour. (Acquisition parameters; acquisition frequency 80.98 MHz, spectral width 9 KHz, accumulate 8192 data points, transform to 16K data points, pulse angle 90°, pulse repetition time 0.5 s, number of scans per spectrum 600). The spectra were integrated and the integral of the signals was normalized with respect to the integral of the standard, (PO_4^{3-}). The rate of decay of the signal of interest was determined by plotting the log of the normalized integral of the signal versus time.

$[(NH_3)_5CoENPP]^{2+}$, (50 mg) was dissolved in $H_2{}^{18}O$ (0.9 ml, 9.8% ^{18}O) and cooled to ~0°C in an ice bath, NaOH (0.1 ml, 5M) was added and the reaction allowed to proceed in the ice bath for 10 minutes. The solution was then warmed to ~ 27°C and allowed to react for a further 30 minutes. The solution was passed through a Dowex 50W-x2 (Na^+) column (0.4 cm x 3 cm), the column was then washed with D_2O (1 ml) and the

effluent and washings combined and placed in a 10 mm NMR tube and a ^{31}P NMR spectrum recorded. (Acquisition parameters; acquisition frequency 80.98 MHz, spectral width 2 KHz, accumulate 16 K data points, pulse angle 90°, pulse repetition time 4.1s, 300 scans, resolution enhancement by trapezoidal multiplication).

The rate of hydrolysis of the uncoordinated ligand was determined at 25°C, μ = 1.0 M ($NaClO_4$) by the initial rate method. The reaction was monitored at 400 nm. The initial concentrations of both $(O)_2P(OC_2H_5)(OC_6H_4NO_2)^-$, (ENPP) and NaOH were varied from run to run. Only data from ~ the first 1% of the reaction were used in the calculation of the initial rate.

$[(NH_3)_5CoENPP]^{2+}$, (40 mg) was dissolved in H_2O (1.0 ml) at ~0°C in an ice bath, and ice cold NaOH (1.0 ml, 1.0 M) was added and the reaction allowed to proceed at ~0°C for 10 minutes. Concentrated HCl (0.5 ml) was then added, after two minutes the solution was diluted to 200 ml and the cationic products absorded on a Sephadex SP-C-25 (Na^+ form) column (0.8 x 8.0 cm). Elution with 0.1 M NaCl yielded apparently only two bands; a fast moving purplish band (~80% of the product), and a slow moving red coloured product, (~20%). The major purplish band was collected, reduced in volume and ^{31}P NMR spectra recorded of the product(s) at various pH's. In another experiment the major band was collected and after the pH was adjusted to ~10 by addition of NH_3, the Co(III) was reduced by the addition of $Co(ClO_4)_2 \cdot 6H_2O$ (~1 mg) and KCN (0.2 g) and a ^{31}P NMR spectrum of the reduced products recorded. The reduction procedure was checked for its effect on phosphoramidate esters by reduction of the complex initially formed by hydrolysis at 0°C before addition of HCl where the identity of the phosphorus containing ligand is known.

3.3.3 Results and Discussion.

The complex was synthesized by heating $[(NH_3)_5CoOSO_2CF_3](CF_3SO_3)_2$ and ENPP in an inert solvent, ion exchange chromatography yielded the pure complex $[(NH_3)_5CoENPP]^{2+}$. The complex displayed the expected ^{31}P NMR chemical shift, +1.3 ppm, ~ 6 ppm downfield from the ionized free ester, the magnitude expected for coordination of phosphate derivatives to Co(III).

The hydrolysis of the complex was followed spectrophotometrically at 400 nm over the hydroxide concentration range, 0.01 to 0.5 M (μ = 1.0 M $NaClO_4$). The reaction obeyed the rate law,

$$v = k_1[(NH_3)_5CoENPP^{2+}][OH^-],$$

the second order rate constant k_1 was $(5.48 \pm 0.03) \times 10^{-1}$ $lmol^{-1}s^{-1}$ at 25°C.

Table 3.2

Hydrolysis of $[(NH_3)_5CoENPP]^{2+}$, μ = 1.0 M ($NaClO_4$).

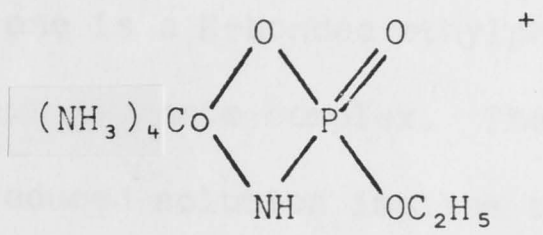
Temperature °C	[NaOH] M	k_{obs} (x 10^3) s^{-1}	Yield NP %	k_{NP} (x 10^3) s^{-1}	k_{CB} (x 10^3) s^{-1}
5	0.05	2.12	76.2	1.62	0.50
15	0.05	7.83	66.2	5.18	2.65
25	0.05	28.5	54.3	15.5	13.0
35	0.05	91.3	42.6	38.9	52.4
25	0.01	5.13	53	2.72	2.41
25	0.025	12.93	53	6.85	6.08
25	0.10	53.1	51	27.1	26.0
25	0.25	135	53	72	63
25	0.50	276	49	135	141

The temperature dependence of the reaction was investigated over the range 5° to 35°C. The yield of 4-nitrophenolate (NP) increased with decreasing temperature. The yield of NP at each temperature was calculated and the observed rate constant partitioned into rate constants for two competing reactions, one producing NP, the other not. (Table 3.2) The activation parameters for the two processes were determined by plotting ln k versus 1/T. The plot was linear for both reactions and the resulting activation parameters were: for the NP producing reaction, $\Delta H^{\neq}$ 75 ± 2 $KJmol^{-1}$, $\Delta S^{\neq}$ -27 ± 6 $JK^{-1}mol^{-1}$, for the conjugate base pathway, $\Delta H^{\neq}$ 108 ± 2 $KJmol^{-1}$ and $\Delta S^{\neq}$ +82 ± 5 $JK^{-1}mol^{-1}$.

^{31}P NMR studies on the reaction showed that at ~5°C the starting material gave two products, one with a chemical shift of 25.6 ppm (80 ± 3 %) and the free diester, ENPP (-4.5 ppm).

The identity of the -4.5 ppm ^{31}P NMR signal was verified by the addition of authentic ENPP to the sample and observing the increase in the signal intensity. Not only were the chemical shifts of the product and authentic ENPP identical, but the P-O-C-H coupling constants were also identical. This reaction presumably occurs by the well known $S_N1(CB)$ mechanism.[11]

The product with the chemical shift of ~25 ppm is assumed to be associated with the NP producing reaction, since the yield of the product by ^{31}P NMR and the yield of NP were essentially identical. The initial product of the NP producing reaction is a species which has a chemical shift of + 25.6 ppm. The identity of this product was, by analogy to the $[(NH_3)_5CoDNPP]^+$ system assigned as the chelate (1).



(1)

The complex is drawn as a mono-cation (deprotonated on the bridging N), since the complex would be expected to be more acidic than the chelate phosphoramidate, and its ^{31}P NMR chemical shift is independent of hydroxide concentration in the range 0.25 - 1.0 M unlike the chelate phosphoramidate. The assignment is supported by the hydrolytic behaviour of the compound. In hydroxide solution, the complex decomposes to yield ethylphosphoramidate, + 9.5 ppm, and cobalt oxide. The presence of the P-N bond in the decomposition product of the intermediate seems to indicate that this bond also occurs in the precursor complex.

When the precursor complex was hydrolysed in 2.5 M HCl, chromatographed on Sephadex SP-C25 and ^{31}P NMR spectra accumulated on the initially eluted purple band two phosphorus containing products were observed. At pH 2-3 the ^{31}P NMR shows the two products to be triplets (J = 6 Hz), resonating at + 8.5 and + 7.0 ppm. When the products of acid hydrolysis were chromatograpically separated and the fast moving purple product(s) reduced by the $Co(II)/CN^-$ method, two products were observed in the ^{31}P NMR spectrum of the reduced solution. The signals of approximately equal intensity resonated at + 9.5 and + 3.9 ppm and correspond to ethylphosphoramidate and ethylphosphate respectively. The reaction procedure does not effect the integrity of phosphoramidates; reduction of the complex initially formed by hydrolysis at 0°C yields only ethylphosphoramidate. These experiments indicate that the fast

moving purple band eluted from the cation exchange column actually contains two products, one is a N-bonded ethylphosphoramidate complex and the other is an ethylphosphate complex. The presence of ethylphosphate in the reduced solution implies that the acid hydrolysis of the original precursor complex yielded an ethylphosphate complex, this in turn implies that the original precursor complex possessed the Co-O-P linkage. The presence of ethylphosphoramidate in the reduced solution shows that its immediate precursor complex was N-bonded since free phosphoramidates are hydrolysed extremely rapidly in acidic conditions.[21] Coordination of phosphoramidates through the nitrogen obviously protects the P-N bond from hydrolysis in acidic conditions. The rationale for this is that the protonation site on the nitrogen is blocked by the metal ion. These arguments have indicated that both the Co-O-P and the Co-N-P links exist in the intermediate and support the structure depicted in complex **1**.

The previous studies of this type all involved intramolecular aminolysis of coordinated monoesters. In those studies the chelate phosphoramidate decayed to the N-bound phosphoramidate which decayed slowly to the free phosphoramidate.[1,2] In this case, the chelate phosphoramidate ester apparently decays to a product which itself decays rapidly to free ethylphosphoramidate. No signal attributable to N-bound or O-bound ethylphosphoramidate was observed.

The rate of decomposition of the chelate (**1**) in hydroxide solution was followed by ^{31}P NMR at 25°C. The initial reaction to yield (**1**) was conducted at low temperature (~0°C) to maximize the yield of the chelate. The rate of loss of the chelate was determined at 4 hydroxide concentrations from 0.25 to 1.0 M and at constant ionic strength of 1.0 M. The rate of decomposition, ~10^{-3} s^{-1}, was independent of hydroxide concentration in the range studied and was accompanied by the production

of the insoluble Co(II) oxide. The base independent loss of the chelate ethylphosphoramidate poses an awkward question about the mechanism of the reaction. As discussed previously, the chelate complex is probably deprotonated at the bridging nitrogen in the experimental conditions used here, ie pH>12. Such a deprotonated nitrogen is akin to that required for the $S_N1(CB)$ reaction of Co(III) amine complexes.[11] It is possible therefore that this chelate is the reactive deprotonated coordinated amine complex required for the $S_N1(CB)$ process. Another possibility is that the chelate complex, **1**, is readily reduced to Co(II), releasing the ethylphosphoramidate into solution.

The question of whether the reaction proceeds with Co-O or P-O fission is a vital one. To answer this question a tracer experiment was conducted. The reaction of $[(NH_3)_5CoENPP]^{2+}$ in 0.5 M NaOH was conducted in 8.8% ^{18}O labelled water, the ultimate phosphorus containing products, ethylphosphoramidate and ENPP were separated from the reaction by ion exchange chromatography, and high resolution $^{31}P\{H\}$ NMR spectra of the two products recorded simultaneously. (Figure 3.4) This tracer experiment relies on the fact that ^{18}O coordinated to phosphate and its derivatives has a measurable effect on the ^{31}P NMR chemical shift, typically between 0.02 and 0.04 ppm upfield shift relative to the unsubstituted phosphate.[22] The spectrum of ENPP consisted of a singlet of 0.6 Hz width at half peak height. As expected for a $S_N1(CB)$ reaction there was no incorporation of ^{18}O label into the product. The spectrum of ethylphosphoramidate also consisted of a single peak, however the half height width of the peak is 2.4 Hz. This makes the observation of the satellite peak more difficult since it is quite possible that the upfield peak was obscured by the broadness of the major peak. However close inspection of the peak reveals that the peak is extremely symmetrical and it appears unlikely that a satellite peak of 8.8%

intensity could be obscured under the wing of this peak. The reason for the broadness of the ethylphosphoramidate resonance is unknown although it may be due to that fact that the quadrapolar nitrogen atom (^{14}N, I = 1) is bonded to the P atom. The question of whether the products exchange oxygen or not was not determined experimentally, however it is known generally that phosphate esters and amidates exchange oxygen very slowly under the conditions of this experiment[23].

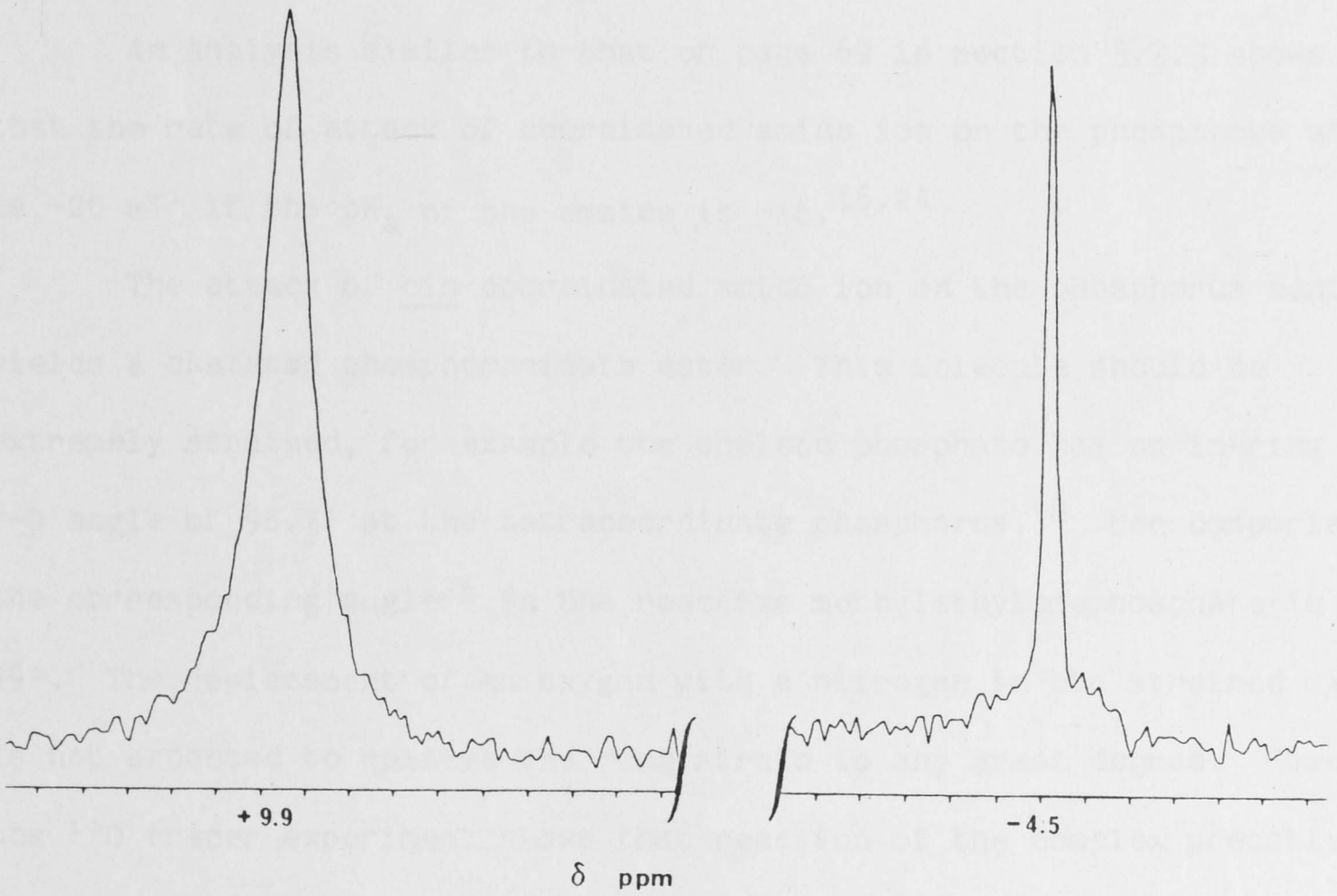


Figure 3.4

^{31} P NMR Spectrum of the Anionic Products of The Hydrolysis of $[(NH_3)_5CoENPP]^{2+}$ in 8.8% $H_2{}^{18}O$. Conditions as per Experimental Section. Scale: 2 Hz per division.

The hydrolysis of free ENPP was followed at 400 nm by the initial rate method. The reaction in the presence of hydroxide ion obeyed the rate law,

$$v = k_1[ENPP][OH^-]$$

with k_1 = (3.3 ± 0.3) x 10^{-7} $lmol^{-1}s^{-1}$ at 25°C, μ = 1.0 M. The reaction includes both attack at phosphorus and at carbon, the extent of attack at phosphorus has not been determined in this case, although hydroxide ion attack on the methyl-2,4-dinitrophenylphosphate ion occurs with 55% P-O cleavage.[13]

The rate of release of nitrophenolate from ENPP in hydroxide ion solution is enhanced more than 10^6 fold upon coordination to the $(NH_3)_5Co$ moiety.

An analysis similar to that on page 69 in section 3.2.3 shows that the rate of attack of coordinated amido ion on the phosphorus atom is ~20 s^{-1} if the pK_a of the ammine is ~16.[16,24]

The attack of cis-coordinated amido ion on the phosphorus centre yields a chelated phosphoramidate ester. This molecule should be extremely strained, for example the chelate phosphate has an in-ring O-P-O angle of 98.7° at the tetracoordinate phosphorus.[18] For comparison the corresponding angle[25] in the reactive methylethylenephosphate is 99°. The replacement of an oxygen with a nitrogen in the strained ring is not expected to relieve the ring strain to any great degree. However the ^{18}O tracer experiment shows that reaction of the complex probably proceeds with Co-ligand bond rupture, ie there was no reaction at the presumably strained phosphorus centre.

This result may call into question the assumption,[17,18,19] which until now has gone unchallenged, that the chelate phosphate ester will be extremely reactive.

It should be pointed out however that the hydrolysis of ethylenephosphate in hydroxide solution proceeds with a rate constant of 4.7 x 10^{-4} s^{-1} at 25°C.[26] The in-ring O-P-O angle in the ethlyene phosphate is presumably similar to the angle in methylethylenephosphate, 99°.[25] This is about the same as would be predicted for the N,O

ethylphosphoramidate chelate in this case. For the complex, the Co-ligand bond rupture to release the ethylphosphoramidate proceeds with a rate of ~10^{-3} s^{-1} at 25°C in strongly basic solutions. It is possible therefore that the reaction at the strained phosphorus centre would be markedly enhanced, if it were not obscured by the faster Co(III) bond rupture.

3.4 $[(NH_3)_5CoOP(O)(OC_6H_4NO_2)OCo(NH_3)_5]^{4+}$.

3.4.1 Introduction.

The coordination of another Co(III) ion to the basic oxygen of the $[(NH_3)_5CoOP(O)_2(OC_6H_4NO_2)]^+$ ion provides an ideal opportunity to study the effect that an additional metal ion has on the rate of reaction. This is of interest because of the number of phosphoryl transfer reactions in enzymes that appear to require two or more metal ions to proceed rapidly.[27]

3.4.2 Experimental.

Synthesis of $[(NH_3)_5Co\text{-}O\text{-}P(O)(OC_6H_4NO_2)O\text{-}Co(NH_3)_5](ClO_4)_4\cdot 1^1/_2NaClO_4\cdot H_2O$, $([(NH_3)_5CoNPPCo(NH_3)_5]^{4+}$.

To $[(NH_3)_5CoOSO_2CF_3](CF_3SO_3)_2$ (4.0 g) in sulfolane (25 ml) was added $(HO)_2P(O)(OC_6H_4NO_2)$ (0.5 g) and 2,4,6-collidine (0.75 ml). The solution was heated to 60°C for 2 hr. The sulpholane was extracted 3 times with ether and the residue dissolved in water (500 ml) and absorbed on a Sephadex SP C-25 (Na^+) column. The column was eluted with aqueous $NaClO_4$ (0.3 M) and bands corresponding to $[(NH_3)_5CoOP(O)_2(OC_6H_4NO_2)]^+$ and $[(NH_3)_5CoOH_2]^{3+}$ were observed. The desired product eluted slowly in 0.5 M $NaClO_4$, This band was collected, evaporated to ~80 ml and then stored at 4°C for several days. The red

needles of product that formed were collected and washed 5 times with methanol, three times with ether, and the dried in vacuo. Yield 0.25 g. Analysis calculated for $C_6H_{36}N_{11}Cl_{5.5}Co_2Na_{1.5}O_{29}P$; C, 6.52; H, 3.29; N, 13.95; Cl, 17.65; Co, 10.65; P, 2.80. Found; C, 6.4; H, 3.4; N, 14.3; Cl, 17.6; Co, 10.7; P, 2.7.

^{1}H NMR, (d_6-DMSO); 8.18 (d) J = 9 Hz (2H), 7.29 (d) J = 9 Hz (2H), 3.89, 3.73 (br) (30H).

^{31}P{H} NMR, (H_2O/D_2O); + 12.6 (s).

Kinetics.

A solution of known concentration of the complex, $[(NH_3)_5CoNPPCo(NH_3)_5]^{4+}$, (10 ul, ~2 x 10^{-2} M) was added to a $NaOH/NaClO_4$ solution (2.00 ml) in a spectrophotometric cell at the required temperature, rapidly mixed and placed in the spectrophotometer. The change in absorbance at 400 nm was monitored with time. The data sets were processed as usual by the LSTSQR program, all data sets fitted to single exponential functions. The quoted rates and errors are the mean and standard deviation of at least 3 determinations. The yield of 4-nitrophenolate (NP) was determined from the infinity absorbance value, using a molar absorbtivity of 18,700 $M^{-1}cm^{-1}$.

The reaction of $[(NH_3)_5CoNPPCo(NH_3)_5]^{4+}$, in hydroxide solution was also followed by ^{31}P NMR spectroscopy. $[(NH_3)_5CoNPPCo(NH_3)_5]^{4+}$, (30-50 mg) was dissolved in H_2O (1.35 ml) and D_2O (0.4 ml) containing Na_3PO_4 (0.015 M) and NaCl to make the final ionic strength 1.0 M. A spectrum was recorded, then NaOH (0.25 ml) of the required concentration was added and consecutive spectra recorded at the required temperature, 25° or 15°C. The spectra were recorded on disc then displayed and integrated. The integrals of the signals were normalized with respect to the standard (PO_4^{3-}). (Acquisition parameters; acquisition frequency 80.98 MHz, sweep width 9 kHz, 8 K points per spectrum, zero fill to 16 K

points, pulse repetition time 0.5 s, 600 transients per spectrum).

3.4.3 Results and Discussion.

The complex, $[(NH_3)_5CoNPPCo(NH_3)_5]^{4+}$, was synthesized by heating 4-nitrophenylphosphoric acid, (NPP), and an excess of $[(NH_3)_5CoOSO_2CF_3](CF_3SO_3)_2$ in an inert solvent and purifying the resulting mixture by cation exchange chromatography. The desired product was easily separated by virtue of its high charge.

The hydrolysis of the complex was followed spectrophotometrically at 400 nm over a range of hydroxide concentration. The rate of hydrolysis of $[(NH_3)_5CoNPPCo(NH_3)_5]^{4+}$ was first order in hydroxide up to 0.5 M, (Table 3.3), yielding a second order rate constant of (6.23 ± .07) x 10^{-2} $lmol^{-1}s^{-1}$ at 25°C, μ = 1.0 M ($NaClO_4$).

The temperature dependence of the reaction was determined, the yield of nitrophenolate, (NP), decreasing with increasing temperature. The observed rate constant for each hydroxide concentration was partitioned into rate constants for the two competing reactions by determining the amount of NP produced. A plot of ln k_{obs} versus 1/T was linear for both reactions, (Figure 3.5). The activation parameters for the two processes were; NP producing reaction, $\Delta H^{\neq}$ 87 ± 2 KJ mol^{-1}, $\Delta S^{\neq}$ -1 ± 4 $JK^{-1}mol^{-1}$, the other reaction, $\Delta H^{\neq}$ 108 ± 2 KJ mol^{-1} and $\Delta S^{\neq}$ 67 ± 6 $JK^{-1}mol^{-1}$.

The reaction was also followed by ^{31}P NMR spectroscopy. The product of the non NP releasing pathway had a chemical shift identical to that of the $[(NH_3)_5CoOP(O)_2(OC_6H_4NO_2)]^+$ ion,[1] as would be expected from the previous results, ie $S_N1(CB)$ loss of the ligand being competitive with phenolate release, in this case the ligand is actually another complex metal ion. The product of the NP yielding pathway has a ^{31}P NMR chemical shift of 17.9 ppm. The expected product (by analogy

with the previous reactions) from attack of cis amido ion on P with loss of NP would initially be a N,O chelate phosphoramidate with an attached pentaamminecobalt moiety, (Scheme 3.4) this species might be expected to ring open to an N,O bridging phosphoramidate. The chemical shift of this species was predicted to be ~16 to 18 ppm, (see Section 1.8). So the signal at 17.9 was tentatively assigned as the N,O-bridging phosphoramidate.

The bridging phosphoramidate dinuclear species reacts to yield a compound with a chemical shift of 18.6 ppm. The 18.6 ppm signal is coincident with the signal of added $[(NH_3)_5CoO_3PNH_2]^+$ synthesized by intermolecular attack of NH_3 on $[(NH_3)_5CoDNPP]^+$ as described in section in 3.2.2. The identification of the 18.6 ppm signal as the O-bonded phosphoramidate supports the assignment of the 17.9 ppm precursor species as the N,O-bridging phosphoramidate species. The proposed reaction sequence is shown in Scheme 3.4.

Table 3.3

Hydrolysis of $[(NH_3)_5CoNPPCo(NH_3)_5]^{4+}$, (Ionic strength = 1.0 M $NaClO_4$)

[NaOH]	Temperature	kobs (x 10^3) s^{-1}	Yield of NP %
0.1	5	0.346 ± 0.001	70.3
0.1	15	1.43 ± 0.01	66.4
0.1	25	5.83 ± 0.1	59.0
0.1	35	20.7 ± 0.6	49.5
0.01	25	0.53 ± 0.01	-
0.05	25	2.87 ± 0.01	-
0.2	25	11.9 ± 0.1	-
0.5	25	31.5 ± 0.4	-

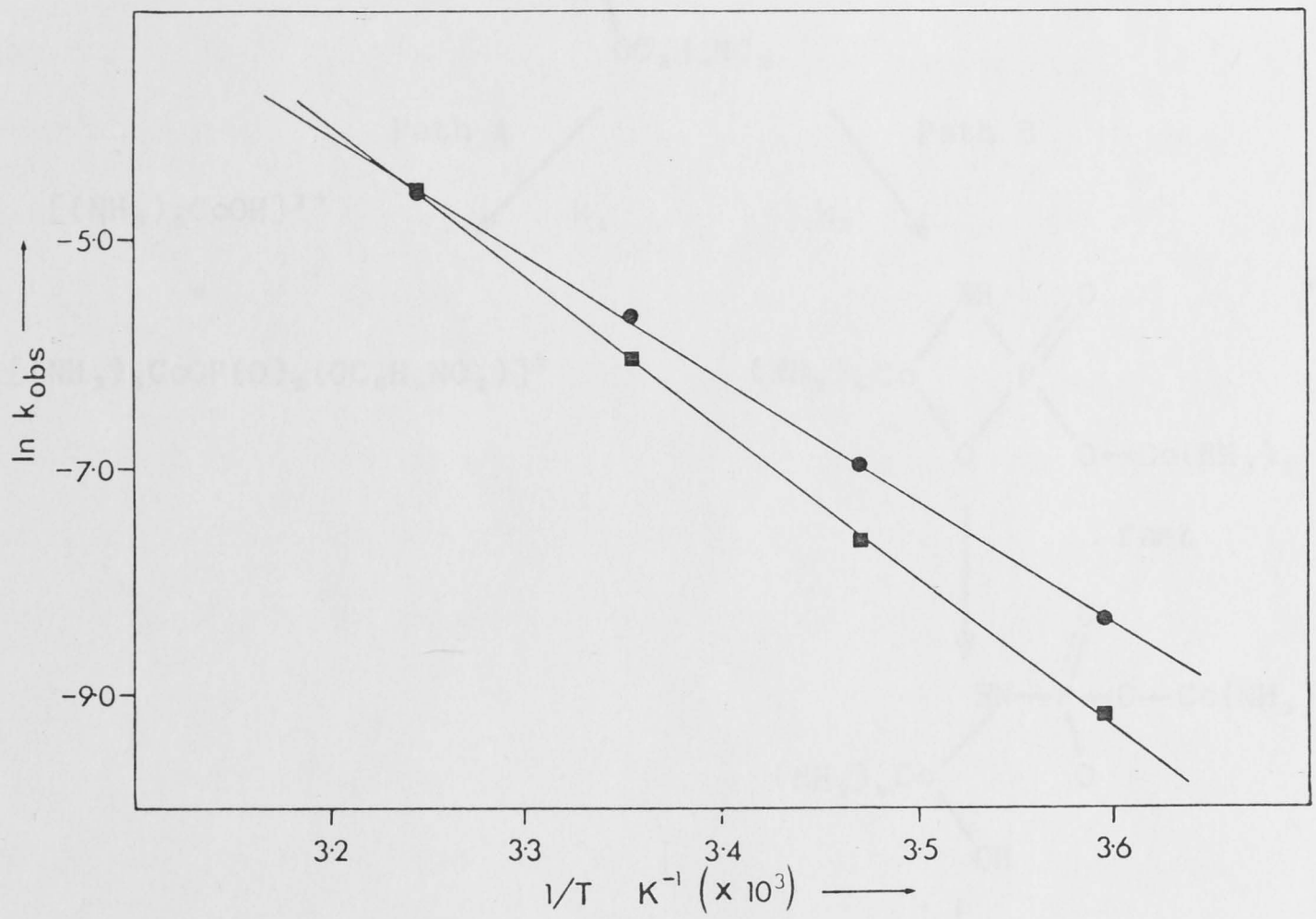
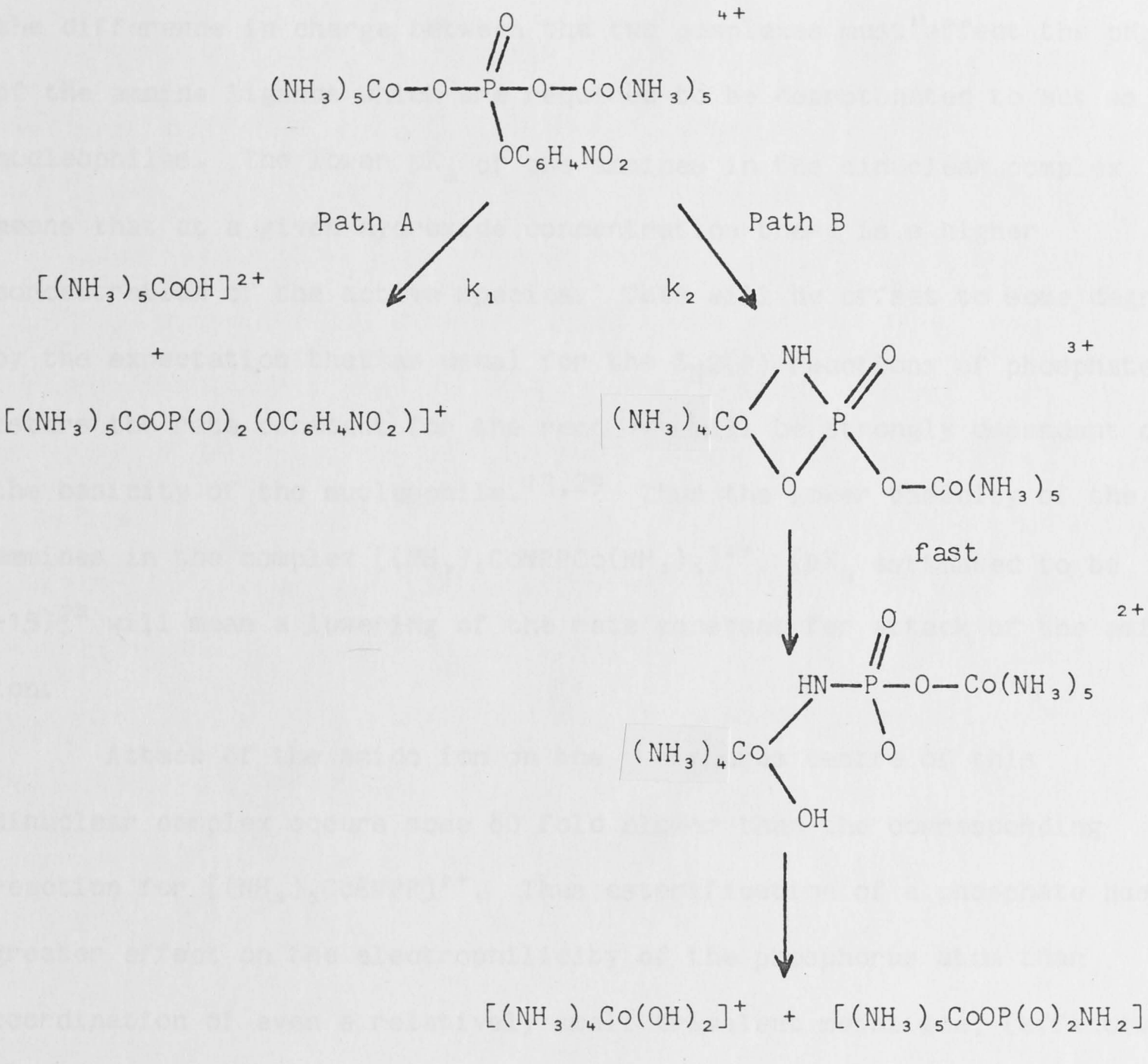


Figure 3.5

Eyring plot, ln k_{obs} versus 1/T for both reactions of $[(NH_3)_5CoOP(O)(OC_6H_4NO_2)OCo(NH_3)_5]^{4+}$ in NaOH = 0.1 M, ● NP producing reaction, ■ $S_N1(CB)$ reaction.

The rate constant for the intramolecular aminolysis reaction of this dinuclear bridging phosphate ester species is 3.44×10^{-2} $lmol^{-1}s^{-1}$ at 25°C, μ = 1.0 M. The rate constant for the attack of amido ion on the phosphorus centre of $[(NH_3)_5CoNPPCo(NH_3)_5]^{4+}$ is estimated to be ~0.3 s^{-1}, using the arguments set out in section 3.2.3, and estimated pK_a for the ammine ligands in the 4+ ion of ~15.[24]

The rate constant for the corresponding reaction of the mononuclear complex is 3.6×10^{-4} $lmol^{-1}s^{-1}$ under the same conditions.[1] This 100 fold increase in rate is due solely to the effect of the



Scheme 3.4

Proposed Reaction Sequence for $[(NH_3)_5CoNPPCo(NH_3)_5]^{4+}$, in Hydroxide Solution.

additional metal ion. The origin of this effect is probably due to several factors, the additional metal ion coordinated to the phosphate ester must increase the electrophilicity of the phosphorus atom[28]. There is also a statistical effect which is simply that there is twice the number of possible nucleophiles in the dinuclear complex, in the absence of other effects this would double the rate of reaction of the mononuclear complex. In addition to the two effects already mentioned,

the difference in charge between the two complexes must effect the pK_a of the ammine ligands which are required to be deprotonated to act as nucleophiles. The lower pK_a of the ammines in the dinuclear complex means that at a given hydroxide concentration there is a higher concentration of the active species. This will be offset to some degree by the expectation that as usual for the $S_N2(P)$ reactions of phosphate esters the rate constant for the reaction will be strongly dependant on the basicity of the nucleophile.[13,29] Thus the lower basicity of the ammines in the complex $[(NH_3)_5CoNPPCo(NH_3)_5]^{4+}$, ($pK_a$ estimated to be ~15)[24] will mean a lowering of the rate constant for attack of the amido ion.

Attack of the amido ion on the phosphorus centre of this dinuclear complex occurs some 80 fold slower than the corresponding reaction for $[(NH_3)_5CoENPP]^{2+}$. Thus esterification of a phosphate has a greater effect on the electrophilicity of the phosphorus atom than coordination of even a relatively small trivalent metal ion, (effective ionic radius for Co(III) 0.55 Å).[30]

3.5 General Discussion.

This work has shown that a number of phosphate esters are rapidly lysed when coordinated to the $[(NH_3)_5Co-]^{3+}$ moiety, the reaction occurs in basic conditions by attack of coordinated amido ion at the phosphorus centre to yield initially the N,O phosphoramidate chelate. Coordination of the diester ENPP to the $[(NH_3)_5Co-]^{2+}$ moiety results in the production of the N,O-chelate ethylphosphoramidate. This chelated ester does not hydrolyse with exocyclic cleavage of the ester group. The reaction proceeds with Co-ligand bond cleavage. The large rate enhancement observed upon coordination appears to come mainly from the

intramolecular nature of the reaction, as distinct from the activating effect of the metal ion *per se*. This is shown by the fact that the dinuclear complex, $[(NH_3)_5CoOP(O)(OC_6H_4NO_2)Co(NH_3)_5]_4^+$ releases NP only ~10^2 times faster than the corresponding mononuclear complex. In contrast, release of NP from the mononuclear complex is enhanced by 8 x 10^4 over free nitrophenylphosphate in hydroxide solution. This observation is in agreement with the conclusions of chapter 2, *ie* coordination of phosphate esters by a trivalent metal centre activates the phosphorus centre to nucleophilic attack by a relatively small amount, ~ several hundred fold. Esterification of the basic oxygen has a greater effect on the rate of amido attack than coordination by a trivalent metal centre.

References, Chapter 3.

1. Harrowfield, J.MacB., Jones, D.R., Lindoy, L.F. and Sargeson, A.M., J. Am. Chem. Soc., **102**, 7733-7741, (1980).

2. Creaser, I.I., Dubs, R.V. and Sargeson, A.M., Aust. J. Chem., **37**, 1999-2003, (1984).

3. Morrison, J.F. and Heyde, E., Ann. Rev. Biochem., **41**, 29-54, (1972).

4. Ramirez, F. and Marecek, J.F., Synthesis, **1978**, 601-603.

5. Bunton, C.A., Fendler, E.J. and Fendler, J.H., J. Am. Chem. Soc., **89**, 1221-1230, (1967).

6. Rawji, G. and Milburn, R.M., J. Org. Chem., **46**, 1205-1206, (1981).

7. Younas, M. and Bokhari, S.S., Pak. J. Sci. Ind. Res., **21**, 111-114, (1978).

8. Febray, J., Casabianca, F. and Ries, J.G., J. Am. Chem. Soc., **106**, 7985-7986, (1984).

9. Ramirez, F., Acc. Chem. Res., **1**, 168-174, (1968).

10. Jones, D.R., Lindoy, L.F. and Sargeson, A.M., J. Am. Chem. Soc., **105**, 7327-7336, (1983).

11. Tobe, M.L. in "Adv. Inorg. Bioinorg. Mech."; Sykes, A.G., (Ed.), Academic Press: London, 1983; Vol 2, pp 1-94.

12. Kirby, A.J. and Vargolis, A.G., J. Chem. Soc. B., **1968**, 135-141.

13. Kirby, A.J. and Younas, M.J., Chem. Soc. B, **1970**, 1165-1172.

14. Bramley, R., Creaser, I.I., Mackey, D.J. and Sargeson, A.M., Inorg. Chem., **17**, 244-248, (1978).

15. This holds if $[OH^-]$ is low, i.e. less than ~ 1 M since $K_a \sim 10^{-17}$ (Ref.16) and $K_w \sim 10^{-14}$.

16. Basolo, F. and Pearson, R.G. "Mechanisms of Inorganic Reactions", 2nd Edition, Wiley, New York, 1967, pp 183-184.

17. Cooperman, B.S. in "Metal Ions in Biological Systems", Sigel, H., (Ed.), Marcel Dekker, New York, 1976, Vol 2, pp 79-125.

18. Anderson, B., Milburn, R.M., Harrowfield, J.MacB., Robertson, G.B. and Sargeson, A.M. J. Am. Chem. Soc., 99, 2652-2661, (1977).

19. Farrell, F.J., Kjellstrom, W.A. and Spiro, T.G., Science, 164, 320-321, (1969).

20. Westheimer, F.H., Acc. Chem. Res., 1, 70-78, (1968).

21. Preobrazhenskaya, N.N., Russ. Chem. Rev. (Engl. Trans.), 41, 54-65, (1972).

22. Webb, M.R., Trentham, D.R., J. Biol. Chem., 255, 1775-1778, (1980). Gorenstein, D.G. and Taira, K., J. Am. Chem. Soc., 104, 6130- (1982). Sammons, R.D., Frey, P.A., Bruzik. K., Tsai, M-D., J. Am. Chem. Soc., 105, 5455-5461, (1983).

23. Frey, P.A., Tetrahedron, 38, 1541-1567, (1982).

24. The pK_a of Co(III) ammines should decrease with increasing charge on the complex, Pt(IV) ammine complexes decrease in pK_a about 1 unit per charge on the complex. Basolo, F. and Pearson, R.G., in "Mechanisms in Inorganic Reactions" 1st edition, Wiley, New York, 1958, pp 388-389.

25. Steitz, T.A. and Lipscomb, W.N., J. Am. Chem. Soc., 87, 2488-2489, (1965).

26. Kumamoto, J., Cox. J.R. and Westheimer, F.H., J. Am. Chem. Soc., 78, 4858-4860, (1956).

27. Coleman, J.E. and Gettins, P., in "Advances in Enzymology", Meister, A., (Ed.), Interscience, New York, 1983, Vol 55, pp 381-452.

28. Hanzlic, R.P., in "Inorganic Aspects of Biological and Organic Chemistry" Academic Press, New York, 1976, pp 229-242.

29. Khan, S.A. and Kirby, A.J., J. Chem. Soc. (B), 1970, 1172-1182.

30. Shannon, R.D., Acta Cryst., A32, 751-767, (1976).

Chapter 4

PHOSPHATOPENTAAMMINEIRIDIUM(III) DERIVATIVE COMPLEXES

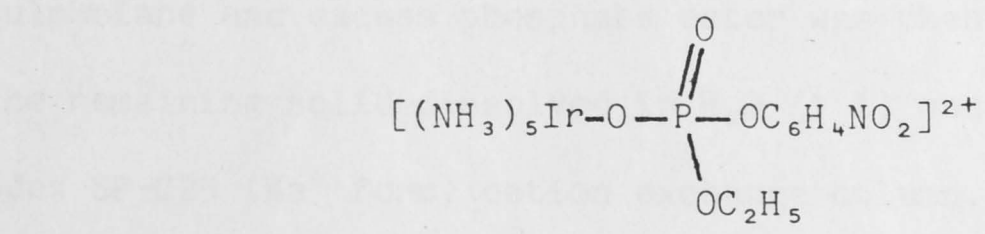
4.1 General Introduction.

The previous chapter demonstrated the efficacy of intra-molecular cis-amido ion attack at coordinated phosphate esters. The chelated phosphoramidate and its derivatives thereby produced reacted with metal-ligand bond cleavage without reaction at the phosphorus centre. The replacement of Co(III) with a more substitution inert metal ion in these systems may allow the apparently slow phosphorus chemistry in the chelate phosphoramidate moiety to compete with metal-ligand bond rupture.

4.2 $[(NH_3)_5IrOP(O)(OC_2H_5)(OC_6H_4NO_2)]^{2+}$.

4.2.1 Introduction.

In the preceding chapter it was shown that it is possible to produce, as an intermediate, the chelate ethylphosphoramidate. Under these conditions the chelate ethylphosphoramidatotetraamminecobalt(III) was slowly cleaved to yield free ethylphosphoramidate. This section is concerned with the reactions of the analogous Ir(III) ion, (1), where it is expected that Ir-O bond rupture will be substantially



(1)

reduced relative to Co-O rupture. It has been shown in general

chemistry that the rate of Ir(III)-ligand bond rupture is much slower than the analogous Co(III) reaction, for example the rate of aquation of Cl^- ion for $[(NH_3)_5IrCl]^{2+}$ is $>10^4$ fold slower than that of the analogous Co(III) complex.[1] The reduced rate of metal-ligand bond rupture means that if the chelate phosphoramidate ester is produced, reaction at the strained phosphorus centre may become competitive with the metal centered ring opening reactions.

4.2.2 Experimental.

Analytical grade reagents were used throughout except where otherwise stated. ^{31}P NMR spectra were recorded with either a JEOL JNM-60 or a Bruker CXP-200 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 thermostatted cell holder, or with a Cary 118C spectrophotometer thermostatted with recirculating water.

$\underline{[(NH_3)_5IrOP(O)(OC_2H_5)(OC_6H_4NO_2)](ClO_4)\cdot NaClO_4\cdot 2H_2O}$ **(1)**

$[(NH_3)_5IrOSO_2CF_3](CF_3SO_3)_2$ (1.0g), ethyl,4-nitrophenylphosphoric acid, (ENPP),(2.7g, not recrystallized) and 2,4,6-collidine (0.1 ml) were dissolved in sulpholane (30 ml) and heated to 45°C for 24 hours. The sulpholane and excess phosphate ester was then extracted with ether and the remaining solid dissolved in H_2O (1 l) and absorbed on a sephadex SP-C25 (Na^+ form) cation exchange column. The column was eluted with 0.1 M $NaClO_4$, the effluent was monitored at 285 nm, two minor bands were the first to elute, these were collected separately and retained. A third band eluted with 0.2 M $NaClO_4$ and containing the

desired product, it was collected and evaporated to ~70 ml. The solution was cooled at 4°C for 16 hours. The white solid thus formed was collected, washed twice with ethanol and thrice with ether and dried in vacuo. Yield 0.32 g. Analysis calculated for $C_8H_{28}N_6O_{20}PCl_3IrNa$; C, 10.90; H, 3.20; N, 9.54; Cl, 12.08; Na, 2.61. Found; C, 10.8; H, 3.2; N, 9.4; Cl, 11.9; Na, 3.0.

^1H NMR; (D_2O), 1.28 ppm (tr) J_{H-H} 7 Hz (3H), 4.12 ppm (d of quart) $J_{P-H-H-H}$ ~7 Hz (2H), 7.39 ppm (d) J_{H-H} 9 Hz (2H), 8.29 ppm (d) J_{H-H} 9 Hz.

$^{31}P\{H\}$ NMR; (H_2O/D_2O), + 1.3 ppm (s).

$\varepsilon^{max}_{282} = 8.7 \times 10^3\ M^{-1}cm^{-1}$, $\varepsilon^{max}_{216} = 6.5 \times 10^3\ M^{-1}cm^{-1}$.

4-Nitrophenyl(l)-menthylphosphate

4-nitrophenylphosphodichloridate (5.0 g) was dissolved in dry ether (20 ml) and pyridine (1.54 g) added. The suspension was stirred whilst (l)-menthol (3.05 g) in ether (10 ml) was added. The stirring was continued for 80 minutes then water (20 ml) and pyridine (2 g) were added and the aqueous solution extracted with ether (3 x 100 ml). The extract was dried (Na_2SO_4), and evaporated to dryness. The crude product was redissolved in ether (20 ml) and poured into water (1.5 l). On prolonged standing (3 weeks), white needles of the desired product separated which were collected and dried in vacuo. Yield 1.05 g. Analysis calculated for $C_{16}H_{24}NO_6P$; C, 53.78; H, 6.77; N, 3.92. Found; C, 55.6; H, 7.0, N, 4.12.

^1H NMR; ($CDCl_3$) 8.20 (d) J = 9 Hz (2H), 7.34 (d) J = 9 Hz (2H), 4.25 (m) (1H), 2.3 to 1.0 complex series of multiplets (8H), 0.89 (tr) J = 6 Hz (7H), 0.73 (d) J = 7 Hz (3H).

^{31}P NMR; (H_2O/D_2O) -5.0 (d) J = 7 Hz.

$[(NH_3)_5IrOP(O)(OC_{10}H_{19})(OC_6H_4NO_2)]Cl_2$

4-nitrophenyl(l)-menthylhydrogenphosphate (0.5 g), $[(NH_3)_5IrOSO_2CF_3](CF_3SO_3)_2$ (0.5 g) and 2,4,6-collidine (0.1 g) were

dissolved in dry sulpholane and stirred at 65°C for 8 hr. The solution was then extracted with ether (3 x 250 ml); the residue from the extraction was a white powder insoluble in water. The powder (0.25 g) was dissolved in ethanol (10 ml) and a saturated solution of LiCl in ethanol added (0.5 ml). The resulting precipitate was extremely fine and was collected by repeated centrifugation and decantation of the supernatant. The off white solid was dried in vacuo. Yield 105 mg. ^{1}H NMR; (D_2O,DSS) 8.30 (d) J = 8 Hz (2H), 7.40 (d) J = 8 Hz (2H), 2.0 to 0.5 complex multiplets (~15 H)

^{31}P NMR; (H_2O/D_2O), + 0.70 (d) J = 7 Hz (1P), + 0.56 (d) J = 7 Hz (1P).

The kinetics of hydrolysis of $[(NH_3)_5IrENPP]^{2+}$, 1 were followed by observing the rate of release of nitrophenolate at 400 nm. Equal volumes of solutions of **1** (~5 x 10^{-5} M) and $NaOH/NaClO_4$ (total concentration 2.00 M) were mixed in a cuvette at 25°C and the increase in absorbance at 400 nm recorded with time. The data sets were processed using the LSTSQR program, and all fitted to single exponential functions. Each rate constant is the average of at least three determinations and the quoted error is the standard deviation.

The complex, **1**, (25 mg) and NaCl (58 mg) were dissolved in H_2O (1.35 ml, containing PO_4^{3-} 0.02 M) and D_2O (0.40 ml) and a ^{31}P NMR spectrum recorded. To this solution was added a solution of NaOH (0.25 ml, 4.0 M) then further spectra were recorded over a period of time. The spectra were plotted and the integrated signals were normalized with respect to that of the standard, PO_4^{3-}. (Acquisition parameters; acquisition frequency 80.98 MHz, spectral width 9 KHz, record 8 K data points, zero fill to 16 K data points, pulse repetition time 0.5 s, 2400 scans per spectrum.) When the reaction was complete, NH_4Cl (112 mg) was added to the solution and a $^{31}P\{H\}$ NMR spectrum was recorded within 3 minutes of the addition. Then a ^{1}H coupled ^{31}P NMR spectrum of the

products was recorded.

The complex, **1**, (2 x 20 mg) was dissolved in NaOH solutions (1.5 ml, 1.0 M and 0.1 M, μ = 1.0 M, $NaClO_4$) and the reaction allowed to proceed at 25°C for several half lives. D_2O (0.25 ml) was added to the solutions then integrated ^{31}P NMR spectra of the two solutions were recorded. The solutions were stored at 25°C in a thermostatted water bath over a period of 49 days and ^{31}P NMR spectra recorded periodically. (Acquisition parameters; acquisition frequency 24.21 MHz, spectral width 5 KHz, pulse repetition time 1.0 s, pulse angle 90°.)

The complex, $[(NH_3)_5IrENPP]^{2+}$, **1**, (8 mg) was dissolved in a D_2O solution of NaOD (0.5 ml, 1.0 M) with a trace of DSS as standard. The solution was filtered into a 5 mm diameter NMR tube and consecutive ^1H NMR spectra recorded at approximately 15 minute intervals. When the reaction was complete ethanol (~1 μl) was added and a further spectrum accumulated. (Acquisition parameters; acquisition frequency 199.5 MHz, spectral width 2 KHz, acquisition time 2.0 s, pulse delay 2.0 s, temp 27°C)

The complex, **1**, (5-10 mg) was hydrolysed in NaOH solution, ~25% D_2O, acid was added to the solution to produce a buffered solution. Trimethylphosphate (2 μl) was added as standard. Acids used were Mes, Hepes, Caps, guanidinium, tetramethylguanidinium and n-butylammonium. The pH of the solutions was estimated from the acid/base ratio. ^{31}P NMR spectra of the solutions were recorded.

The complex, **1**, (14 mg) was dissolved in NaOH solution (250 μl, 1.0 M) and allowed to react at 25°C for 2 hr. The solution was then diluted to 500 μl with water and absorbed onto a Dowex 50W-X2 (Na^+) column (0.5 x 2 cm) pre-equilibrated with 0.5 M NaOH, 75% D_2O. The column was washed with 0.5 M NaOH (75% D_2O) until most of the nitrophenolate was eluted (1 ml). The washings were collected and a ^{31}P

NMR spectrum recorded. (Acquisition parameters; acquisition frequency 80.98 MHz, spectral width 9 KHz, record 8 K data points, zero fill to 16 K data points, pulse repetition time 0.5 s.

4.2.3 Results and Discussion.

The complex $[(NH_3)_5IrENPP]^{2+}$, **1**, was synthesized by heating $[(NH_3)_5IrOSO_2CF_3](CF_3SO_3)_2$ and ENPP in sulpholane and purifying the resulting mixture by cation exchange chromatography. The complex crystallized as the $NaClO_4$ adduct, and it analysed correctly for the determined elements including sodium. The ^1H and ^{31}P NMR spectra were in agreement with the proposed structure; the ^{31}P NMR chemical shift of the phosphate ester showed the expected downfield shift for coordination to the trivalent metal centre as discussed in Section 1.8.

The reaction of **1** in NaOH solution was studied by following the release of nitrophenolate ion from the complex spectrophotometrically at 400 nm. The reaction was conducted at 25°C and an ionic strength of 1 M ($NaClO_4$). The release of nitrophenolate from **1** obeyed the rate law,

$$v = k_1[1][OH^-] + k_2[1][OH^-]^2$$

The rate constants k_1 and k_2 were determined by fitting the data in Table 4.1 to an equation of the above form using the LSTSQR program. The rate constants k_1 and k_2 have the values $(2.4 \pm 0.2) \times 10^{-4}$ $lmol^{-1}s^{-1}$ and $(2.9 \pm 0.2) \times 10^{-4}$ $l^2mol^{-2}s^{-1}$ respectively. ^1H NMR spectra of the reaction solution on completion of the reaction showed that the only nitrophenol containing product is the nitrophenolate ion.

The reaction was followed also by ^{31}P NMR spectroscopy, with $[OH^-]$ = 0.5 M, 25°C and μ = 1.0 M ($NaClO_4$), the rate constant for disappearance of $[(NH_3)_5IrENPP]^{2+}$, **1** was $(1.7 \pm 0.4) \times 10^{-4}$ s^{-1}, almost identical to the rate of appearance of NP. The reaction yielded two products, one with a chemical shift of ~25 ppm, A, and another with a

Table 4.1

Observed rates of production of NP from $[(NH_3)_5IrENPP]^{2+}$, 1 at 25°C, μ = 1.0 M ($NaClO_4$).

[NaOH]	k_{obs} (x 10^4) s^{-1}
0.10	0.319 ± .002
0.25	0.85 ± .01
0.50	1.90 ± .02
0.60	2.46 ± .01
0.75	3.31 ± .07
0.90	4.44 ± .02
1.00	5.3 ± .2

chemical shift of 12.5 ppm, B. The chemical shift of A was dependent on OH^- concentration. The chemical shift of B was independent of OH^- concentration. A and B were produced in constant relative yields irrespective of hydroxide concentration, 81 ± 2%, 19 ± 2% respectively. The compounds were stable in hydroxide solution; no change in the ^{31}P NMR spectrum of the products was observed over a period of 49 days in either 1.0 or 0.1 M NaOH solution at 25°C.

Identification of the products of this reaction was made from their ^{31}P NMR chemical shifts and coupling patterns, the charge on the compounds and their reactivity. Numerous attempts were made to crystallize the products of the reaction, without success.

The product B is probably the monodentate ethylphosphate complex produced by intermolecular attack of hydroxide on the coordinated ENPP. The observed ^{31}P NMR chemical shift is in the region expected for this

complex and the ^{1}H coupled ^{31}P NMR spectrum of this complex displays a triplet with a P-H coupling constant of ~6 Hz. The stability of this complex in both acid and base supports this assignment.

The product A displays a triplet in its ^{31}P NMR spectrum in the absence of proton decoupling, which implies that the phosphate still has the ethyl group bound. When the reaction was conducted in D_2O the ^{1}H NMR spectrum of the products showed only a phosphate bound ethyl group and no free ethanol was produced. By analogy with the corresponding Co(III) complex a substantial proportion of the reaction was expected to proceed via attack of deprotonated ammonia on the phosphorus atom. The first stable molecule expected from this reaction path was the chelate ethylphosphoramidate ester. The similarity of the chemical shift of A to the chemical shift of the Co(III) chelate ethylphosphoramidate suggested that the signal was due to the corresponding Ir(III) complex. However, the effect of pH on the chemical shift of the signal made this assignment doubtful. The chemical shift of the species in question was dependant on a protonation with a pK_a of ~12.5 (Figure 4.1) The titration was fully reversible and the chemical shifts of the limiting species were 9.0 ppm, fully protonated, and ~27 ppm fully deprotonated. The magnitude of the chemical shift variation upon deprotonation and the observed pK_a of the group suggested that the deprotonation was occurring at the bridging nitrogen of an N-bound phosphoramidate, since protonation on the oxygen atom of phosphates has a much smaller effect on the ^{31}P NMR chemical shift.

A chemical shift of 9.0 ppm was thought to be incompatible with a 4-membered ring chelate phosphoramidate but was that predicted for the N-protonated N-bound ethylphosphoramidate. This is in accord with the previous observation that coordination of phosphoramidate to the Co(III) metal centre results in an upfield shift of 1.5 ppm[2] in the ^{31}P NMR

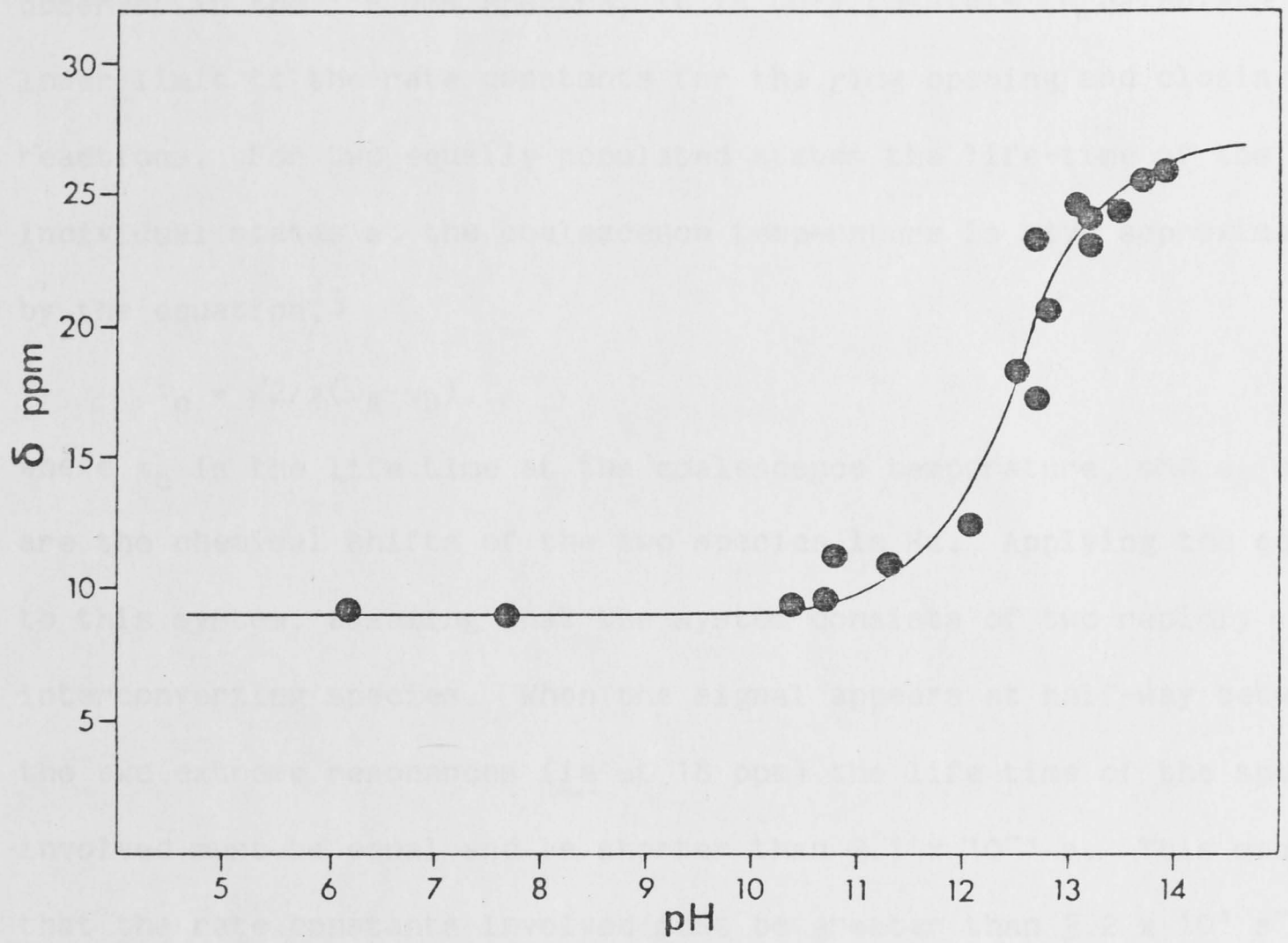


Figure 4.1

^{31}P NMR Chemical Shift Vs pH for product A, Solid line was calculated for pK_a = 12.5, fully protonated species = 9.0 ppm, fully deprotonated species = 27.0 ppm.

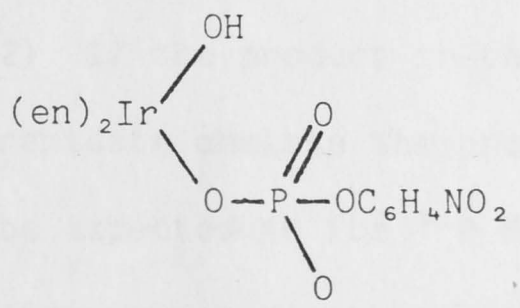
spectrum of the phosphoramidate. The chemical shift of free ethyl phosphoramidate is 9.9 ppm. The large downfield shift on deprotonation at the nitrogen is in agreement with the result obtained in section 3.2.3 where deprotonation at the nitrogen of the N,O chelate ethylphosphoramidate resulted in a downfield shift of ~16 ppm.

There may be another explanation for the chemical shift variation with pH, that is, that there is a pH dependent equilibrium present between two molecules, possibly the N,O-chelate ethylphosphoramidate and the N-bonded ethylphosphoramidate. The equilibrium would involve rapid ring opening and closing reactions. Since only one signal was always

observed in the ^{31}P NMR spectra, it is only possible to calculate a lower limit to the rate constants for the ring opening and closing reactions. For two equally populated states the life-time of the individual states at the coalescence temperature is give approximately by the equation,[3]

$$\tau_c = \sqrt{2}/\pi(\nu_A - \nu_B),$$

where τ_c is the life time at the coalescence temperature, and ν_A and ν_B are the chemical shifts of the two species in Hz. Applying the equation to this system, assuming that the system consists of two rapidly interconverting species. When the signal appears at half-way between the two extreme resonances (ie at 18 ppm) the life time of the species involved must be equal and be shorter than 3.1×10^{-4} s. This means that the rate constants involved must be greater than 3.2×10^{3} s^{-1}. In comparison with this figure the estimated rate of intramolecular attack of hydroxide ion on the phosphorus centre of cis coordinated 4-nitrophenylphosphate bound to the Ir(III) centre in the complex (6), is estimated to be ~10^{-6} s^{-1} at 25°C, (vide infra section 5.3.3). The magnitude of the rate constant required to produce coalescence in the ^{31}P NMR spectra seems to preclude the possibility that there is two rapidly interconverting species in solution.



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Thus, it seemed that product A was the N-bonded ethylphosphoramidate. The possibility remained however that the the product was actually the N,O chelate. Several experiments were performed in an attempt to distinguish between these possibilities. A 0.5 M NaOH solution containing the two products of the reaction, was applied to a Dowex 50W-X2 column pre-equilibrated with 0.5 M NaOH, elution with 0.5 M NaOH (1.5 ml) yielded the complex having the 25 ppm signal but not that showing the 12.5 ppm signal. Under the conditions of this experiment the N,O chelate ethylphosphoramidate would be a monocation whereas the N-bonded ethylphosphoramidate should be zero charged if the bridging nitrogen was fully deprotonated. This experiment indicated that the species responsible for the 25 ppm signal was less positively charged than the 12.5 ppm signal which was a monocation.

In addition, an analogous complex possessing a chiral ester group was synthesized, viz the 4-nitrophenyl(l)-menthylphosphatopentaammineiridium(III) ion. This ion displays a pair of doublets in its ^{31}P NMR spectrum as a result of the diastereomers in the molecule; both the phosphorus atom and the menthyl group were chiral. The hydrolysis of this molecule proceeded at about the same rate as the corresponding ethyl,4-nitrophenylphosphato complex and yielded similar products. The products displayed only singlets in their ^{31}P NMR spectra. (Figure 4.2) If the product in the region of 25 ppm were the N,O-menthylphosphoramidate chelate the phosphorus atom would be chiral and a doublet might be expected in its ^{31}P NMR. Admittedly this experiment cannot be conclusive unless diasteromers are observed, however the negative observation does lend some support to the argument that the phosphorus atom is not chiral.

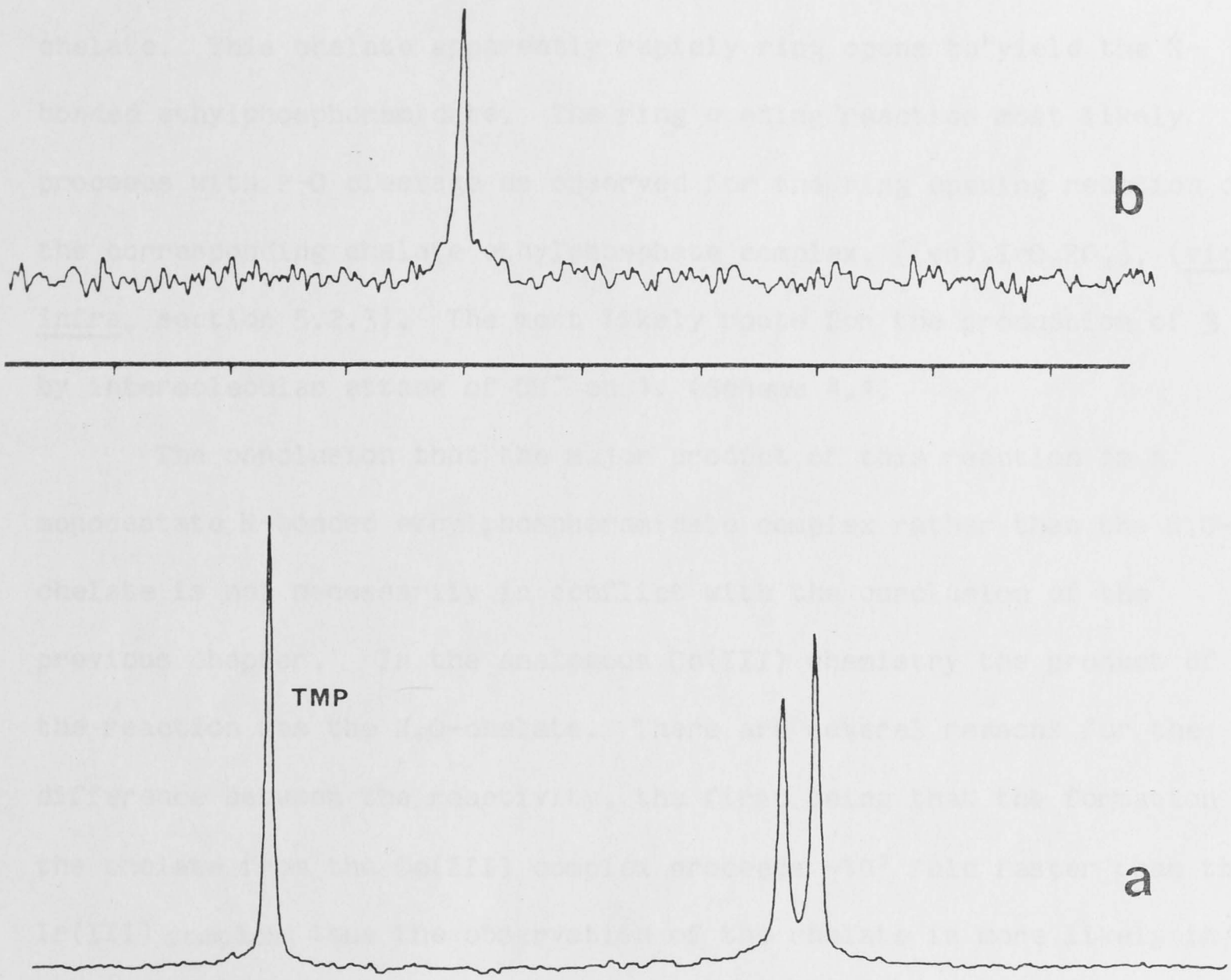


Figure 4.2

^{31}P NMR Spectra of 4-nitrophenyl(l)-menthylphosphate complex.

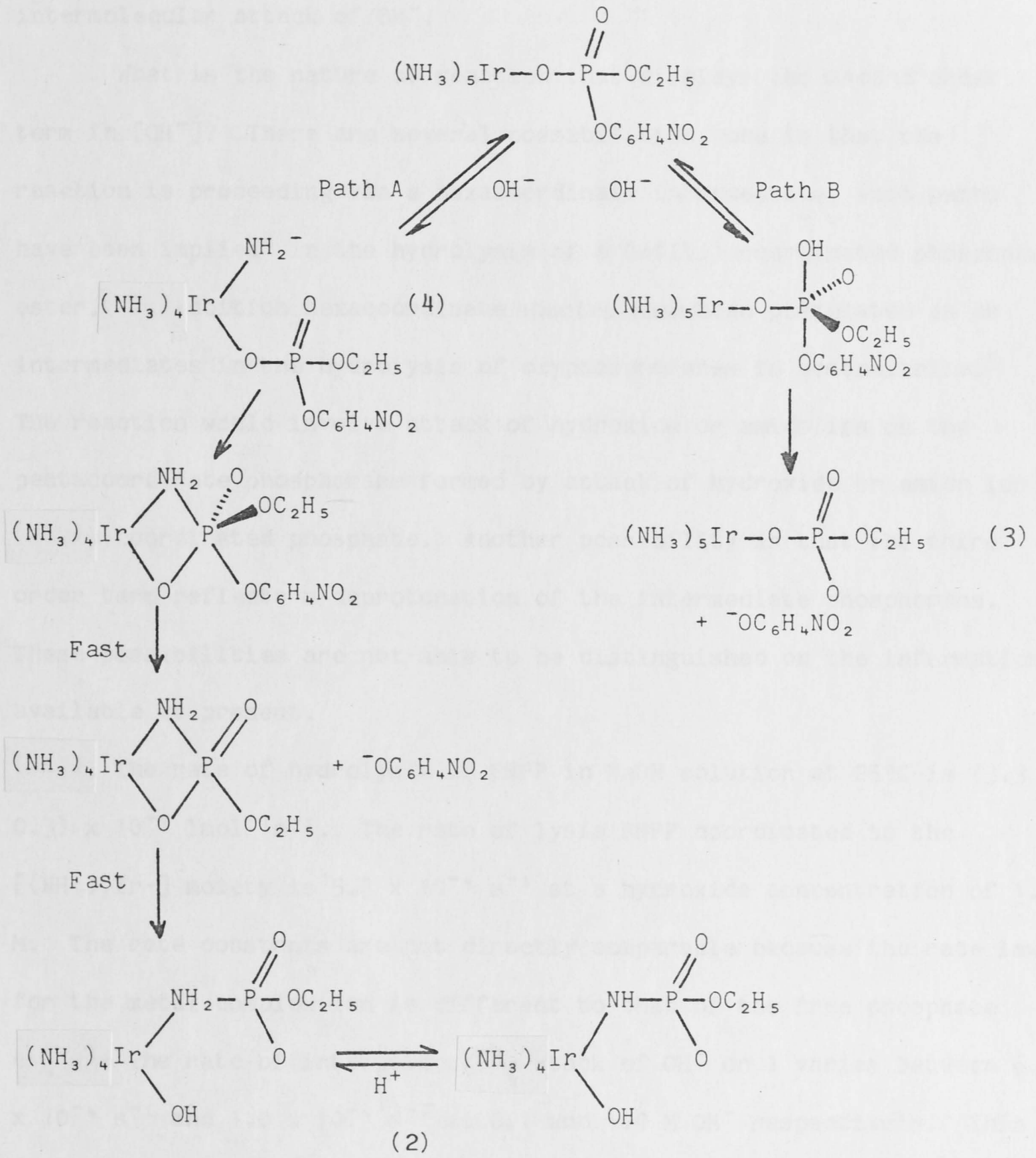
a) $[(NH_3)_5IrOP(O)(OC_{10}H_{19})(OC_6H_4NO_2)]^{2+}$ and standard (TMP). b) Major hydrolytic product, 24.2 ppm, Hydrolysed in 1.0 M OH^- at 30°C. Scale for both spectra 0.5 ppm/division.

The products of the hydrolysis of 1 are therefore most likely to be the N-bound ethylphosphoramidate (2) and the monodentate ethylphosphate (3). The question of how these products arise appears to be relatively simple. By analogy with the reactions described in the previous chapter, intramolecular attack of amido ion should produce an aminophosphorane which decays to yield the N,O ethylphosphoramidate

chelate. This chelate apparently rapidly ring opens to yield the N-bonded ethylphosphoramidate. The ring opening reaction most likely proceeds with P-O cleavage as observed for the ring opening reaction of the corresponding chelate ethylphosphate complex, $[(en)_2IrO_2PO_2]$, (vide infra, section 5.2.3). The most likely route for the production of 3 is by intermolecular attack of OH^- on 1. (Scheme 4.1)

The conclusion that the major product of this reaction is a monodentate N-bonded ethylphosphoramidate complex rather than the N,O-chelate is not necessarily in conflict with the conclusion of the previous chapter. In the analogous Co(III) chemistry the product of the reaction was the N,O-chelate. There are several reasons for the difference between the reactivity, the first being that the formation of the chelate from the Co(III) complex proceeds ~10^3 fold faster than the Ir(III) complex, thus the observation of the chelate is more likely in the case of the Co(III) complex. In addition the increased amount of strain, (vide infra, section 5.4), in the ring incorporating the larger Ir(III) ion is likely to make the ring opening reaction, (with P-O bond cleavage) much more facile as compared with the Co(III) complex.

The kinetics of hydrolysis of $[(NH_3)_5IrENPP]^{2+}$, 1, shows a two term rate law, one first order in 1 and OH^- and another first order in 1 and second order in OH^-, ie overall third order. The products and their relative yields however, do not vary over the hydroxide concentration range 0.1 to 1.0 M despite the considerable difference in the relative contributions of the two paths to the observed rate. This implies that either there is an accidental coincidence and the two paths produce the same products in the same ratio or that there is some common intermediate after the RDS from which both products are formed. This second explanation would seem to be hard to justify given that 2 is



SCHEME 4.1

Proposed Reaction Path for $[(NH_3)_5IrENPP]^{2+}$, 1, in Hydroxide Solution.

produced by intramolecular attack of amido ion and 3 is produced by intermolecular attack of OH^-.

What is the nature of reaction that displays the second order term in $[OH^-]$? There are several possibilities, one is that the reaction is proceeding via a hexacoordinate intermediate, such paths have been implied[4] in the hydrolysis of a Co(III) coordinated phosphate ester. In addition hexacoordinate species have been postulated as an intermediates in the hydrolysis of oxyphosphoranes in acetonitrile.[5] The reaction would involve attack of hydroxide or amido ion on the pentacoordinate phosphorane formed by attack of hydroxide or amido ion on the coordinated phosphate. Another possibility is that the third order term reflects a deprotonation of the intermediate phosphorane. These possibilities are not able to be distinguished on the information available at present.

The rate of hydrolysis of ENPP in NaOH solution at 25°C is (3.3 ± 0.3) x 10^{-7} $lmol^{-1}s^{-1}$. The rate of lysis ENPP coordinated to the $[(NH_3)_5Ir-]$ moiety is 5.3 x 10^{-4} s^{-1} at a hydroxide concentration of 1.0 M. The rate constants are not directly comparable because the rate law for the metal complex ion is different to that of the free phosphate ester. The rate of intermolecular attack of OH^- on 1 varies between 6.1 x 10^{-6} s^{-1} and 1.0 x 10^{-4} s^{-1} at 0.1 and 1.0 M OH^- respectively. This is a rate enhancement upon coordination of 180 to 300 fold which is only slightly lower than the rate enhancement for trimethylphosphate upon coordination to a trivalent metal centre as described in chapter 2.

The intramolecular reaction, attack of coordinated amido ion on the phosphorus centre dominates the reactivity of 1 in hydroxide solution accounting for 81% of the products. An analysis similar to that in chapter 3, ie calculation of the rate of attack of amido ion on the phosphorus in complex 4 (Scheme 4.1) yields a rate constant for the

process of ~0.4 s^{-1} if the pK_a of the cis ammine ligands is 17. The pK_a of the Ir(III) bound ammines is estimated to be ~17 because generally the pK_a of Ir(III) bound species is ~1 pK unit less than the corresponding Co(III) complex,[6] and the pK_a of for dicationic Co(III) ammine complexes has been estimated at ~16, (vide supra, section 3.3.3). The rate calculated for the analogous Co(III) reaction was 20 s^{-1}. In 1 molar OH^- the rate of intramolecular attack of Ir(III) bound amido ion at the phosphorus centre proceeds ~10^6 fold faster than intermolecular attack of hydroxide on the free ligand. This rate enhancement is due in large part to the intramolecularity of the reaction, and in small part to the effect of the metal ion on the electrophilicity of the phosphorus atom.

4.3 $[(NH_3)_5IrOP(O)(OC_6H_4NO_2)]^{2+}$.

4.3.1 Introduction.

The previous section, 4.2, demonstrated that the N,O-chelate ethylphosphoramidate produced by intramolecular attack of coordinated amido ion on ENPP, rapidly ring opens without loss of the ethyl group. This section will describe the synthesis and reactivity of the bis(4-nitrophenyl)phosphate, (BNPP), complex, $[(NH_3)_5IrBNPP]^{2+}$. This complex was investigated with the expectation that incorporation of a good leaving group, (ie NP) in the strained 4-membered ring might make the exocyclic cleavage reaction competitive with the ring opening reaction.

4.3.2 Experimental.

$[(NH_3)_5IrOP(O)(OC_6H_4NO_2)]Cl_2 \cdot 2H_2O$ (5).

$[(NH_3)_5IrOSO_2CF_3](CF_3SO_3)_2$ (1.35g), $HOP(O)(OC_6H_4NO_2)_2$ (BNPP)

(3.0g) and 2,4,6-collidine (0.5ml) were dissolved in sulpholane and heated to 50°C for 20 hours. The complex was precipitated as an oil by the addition of H_2O (150 ml) and cooling in an ice bath. The water was decanted and the oil dissolved in H_2O (2 l) by stirring the oil with a suspension of Dowex AG-1X8 (Cl^- form) anion exchange resin for 4 hours. The resin was removed and the dissolved complex absorbed onto a Sephadex SP-C25 (Na^+ form) cation exchange column. The column was eluted with NaCl (0.1-0.3 M) and the effluent monitored at 285 nm. Two minor bands eluted before the major band which contained the desired product. The solution containing this band was evaporated to ~150 ml and cooled in ice. The white precipitate which formed was collected, washed with ice cold H_2O (2 ml) and dried in vacuo. Yield 0.25g. Analysis calculated for $C_{12}H_{27}N_7Cl_2IrO_{10}P$. Calc; C, 19.92; H, 3.76; N, 13.55; Cl, 9.80. Found; C, 19.9; H, 3.5; N, 13.3; Cl, 10.0.

^1H NMR; (D_2O), 8.26 ppm (d) J_{H-H} 9 Hz, 7.38 ppm (d) J_{H-H} 9 Hz,. (d_6-DMSO) 8.24 ppm (d) J_{H-H} 9 Hz (4H), 7.50 ppm (d) J_{H-H} 9Hz (4H), 5.12 ppm (br) (3H), 4.85 ppm (br) (12H).

$^{31}P\{H\}$ NMR; (H_2O/D_2O, pH 7, 1.0 M NaOH), -5.2 ppm (s).

$\varepsilon^{max}_{297} = 2.0 \times 10^4\ M^{-1}cm^{-1}$, $\varepsilon^{max}_{214} = 1.6 \times 10^4\ M^{-1}cm^{-1}$.

[$(NH_3)_5IrOP(O)_2H(OC_6H_4NO_2)$]$(ClO_4)_2$ (6).

The second of the two bands from the preparation of **1** (Section 4.2.2, page 93) to be eluted with 0.1 M $NaClO_4$ was evaporated to ~15 ml and cooled to 4°C for 16 hr. The microcrystalline white solid which had precipitated was collected, washed with ethanol (2 x 2 ml), ether (2 x 2 ml) and dried in vacuo for 8 hr. Yield 95 mg. Analysis calculated for $C_6H_{20}N_6Cl_2IrO_{14}P$. Calc; C, 10.38; H, 2.90; N, 12.10; Cl, 10.21. Found; C, 10.8; H, 3.0; N, 11.9; Cl, 10.2.

^1H NMR; (D_2O, 0.1 M DCl), 8.30 (d) J = 9 Hz (2H), 7.39 (d) J = 9 Hz (2H), 4.64 (br) (12 H), cis NH_3 probably obscured by HOD peak (4.9 ppm)

$^{31}P\{H\}$ NMR; (H_2O/D_2O, 0.1 M HCl), +2.9 (s), (H_2O/D_2O, 1.0 M NaOH), +7.1 (s).

$\varepsilon^{max}_{220} = 6.04 \times 10^3\ M^{-1}cm^{-1}$, $\varepsilon^{max}_{300} = 8.88 \times 10^3\ M^{-1}cm^{-1}$.

A known weight of $[(NH_3)_5IrBNPP]^{2+}$, **5**, (~8 mg) was dissolved in H_2O (1 ml). A solution of NaOH (2.00 ml, μ = 1.0 M $NaClO_4$) of the required concentration was pipetted into a cuvette and equilibrated at 25.0°C. 5 µl of the solution of **5** was syringed into the cuvette and the absorbance at 400 nm recorded. The data showed biphasic kinetics, ie two nitrophenolate releasing reactions. The rates of the two reactions in the $[OH^-]$ range studied, 0.1 to 1.0 M, were different enough to be able to treat the two reactions independently.

A known weight of **5** (3-5 mg) was dissolved in a solution of NaOH (500 µl, 1.00M) and allowed to react at 25°C for 10 minutes, then stored as a frozen solution at -10°C. This solution was used within 24 hours of its preparation. A small volume of this stock solution (5.0 µl) was added to a buffer solution (2.00 ml) at 25°C and the required pH (μ = 1.0 M, $NaClO_4$). The increase in absorbance at 400 nm was recorded. The data from this reaction followed a single exponential decay and its pseudo first order rate constant was evaluated using the LSTSQR program.

A known weight of $[(NH_3)_5IrBNPP]^{2+}$, **5**, (3-5 mg) was dissolved in a solution of NaOH (500 µl, 1.00 M) and allowed to react at 25°C for 10 minutes. To this solution was added glacial acetic acid (100 µl), and the solution stored at -10°C. This solution was used within 12 hours of its preparation. A small volume of this stock solution (5.0 µl) was added to a buffer solution (2.00 ml) at 25°C and the required pH or hydroxide concentration (μ = 1.0 M, $NaClO_4$). The data from this reaction followed a single exponential decay and the rate constants were determined using the LSTSQR curve fitting program.

The complex, **5** (20 mg) was dissolved in H_2O (1.35 ml) and D_2O

(0.4 ml) then NaOH (0.25 ml, 8.0 M) was added and consecutive ^{31}P NMR spectra accumulated at 25° ± 1°C and stored on disc. The spectra were displayed and integrated and the decrease in intensity of the 21.9 ppm signal plotted on a log scale versus time. The spectra contained no standard, however the intensity of the 21.9 ppm signal was normalized with respect to the signal at 6.9 ppm which is constant throughout the reaction.

The complex, **5** (8 mg) was dissolved in NaOH (1.5 ml, 0.2 M NaOH, 20% D_2O) and after ~50 minutes a ^{31}P NMR spectrum was recorded. Glacial acetic acid (34 µl) was added to this solution and another ^{31}P NMR spectrum accumulated, to this solution was added NaOH (100 µl, 8 M) and another spectrum recorded.

The complex, **5** (15 mg) was dissolved in NaOH (1.5 ml, 0.2 M NaOH, 20% D_2O) and the reaction was maintained at ~25°C for ~30 minutes. A ^{31}P NMR spectrum was then recorded, and to this solution was added two moles of acid to produce a buffer of the required pH. Acids used were Mes (pH 6.1), Caps (pH 10.4) and NH_4Cl (pH 9.5). After addition of the acid, integrated spectra were recorded at intervals. (Acquisition parameters for all ^{31}P NMR experiments; acquisition frequency 80.98 MHz, spectral width 9 KHz, accumulate 8 K data points, zero fill to 16 K data points, pulse repetition time 0.5 s).

The hydrolysis of the free BNPP was determined spectrophotometrically by release of NP, the initial rate method was used and the concentration of hydroxide was varied between runs. A stock solution of BNPP was prepared by dissolving the free acid (93.29 mg) in H_2O (25.00 ml). Stock solution (1.00 ml) and hydroxide solution (1.0 ml, 1.0, 2.0 M, μ = 2.00 $NaClO_4$) were pipetted into a cuvette and placed in a thermostatted cell holder (25°C), after temperature equilibration the increase in absorbance at 400 nm was recorded. Only

data from the first 0.5% of the reaction was used.

4.3.3 Results and Discussion.

The complex $[(NH_3)_5IrBNPP]^{2+}$, **5**, was synthesized by heating $[(NH_3)_5IrOSO_2CF_3](CF_3SO_3)_2$ and BNPP in sulpholane solution and purifying the resulting mixture by ion exchange chromatography. The product was characterized by elemental analysis, NMR and electronic spectroscopy. The ^{31}P NMR spectrum of **5**, shows the expected shift for coordination to a tripositive metal ion as discussed in section 1.8.

The reaction of $[(NH_3)_5IrBNPP]^{2+}$, **5**, in basic solution was followed spectrophotometrically at 400 nm. At hydroxide concentrations above ~0.05 M the reaction proceeds in two distinct nitrophenolate releasing steps. The first step of the reaction was followed at $[OH^-]$ between 0.1 and 1.0, at these concentrations the second step is slow enough to be separated by a simple extrapolation procedure. This procedure involved extrapolation of the straight line observed for the following reaction back to zero time and the difference between this line and the absorbance at any time, t, recorded. The pseudo-first order rate constants were determined by fitting that data to curves using the program LSTSQR, all sets of data fitted well to single exponential functions. The observed pseudo-first order rate constants are shown in Table 4.2. The yield of NP from the first step of the reaction was 98 ± 2% per mole of **5**. The observed rate of hydrolysis of **5** in OH^- solution obeyed the rate law,

$$v = k_1[(NH_3)_5IrBNPP^{2+}][OH^-] + k_2[(NH_3)_5IrBNPP^{2+}][OH^-]^2 \qquad \{1\}$$

The rate constants k_1 and k_2 were determined by fitting the data in table 4.2 to an equation of the form of {1} by the curve fitting program LSTSQR. The values for k_1 and k_2 were $(4.8 \pm 0.7) \times 10^{-3} l mol^{-1} s^{-1}$ and $(1.2 \pm 0.09) \times 10^{-2}\ l^2 mol^{-2} s^{-1}$ respectively.

Table 4.2

Pseudo-first order rate constants for the first step in the hydrolysis of **5**, followed at 25.0°C spectrophotometrically at 400 nm.

[NaOH]	k_{obs} (x 10^2) s^{-1}	Yield NP (%)
0.10	0.085 ± 0.005	-
0.25	0.24 ± 0.01	-
0.50	0.530 ± 0.01	97
0.60	0.71 ± 0.02	-
0.75	1.01 ± 0.02	-
1.00	1.7 ± 0.05	99

At and below $[OH^-]$ = 0.01 M, the reaction was monophasic, ie only one nitrophenolate releasing step was observed. However, under these conditions more than one mole of nitrophenolate was released per mole of reactant. Therefore at any pH below this point, it was necessary first to produce the intermediate at a high $[OH^-]$ concentration then reduce the pH and follow the second step of the reaction. The second step of the reaction was followed between pH ~6 and $[OH^-]$ = 1.0 M.

The rate of hydrolysis of the intermediate displayed a bell-like dependence on pH, with a maximum in the rate occurring at pH ~8.0. (Figure 4.3 and Table 4.3) The second step of the reaction released about 65 ± 4% of the amount of NP obtained from the initial step.

The reaction of $[(NH_3)_5IrBNPP]^{2+}$, **5**, in hydroxide was also followed by ^{31}P NMR spectroscopy. In 1.0 M NaOH solution the disappearance of **5** was too fast to follow, the initial products of the reaction however were compounds with chemical shifts of 21.9 ppm and

6.9 ppm, (Figure 4.4). By analogy with the reactivity of the compound 1 described in the previous section in this chapter these resonances are assigned to compounds containing, N-bound 4-nitrophenylphosphoramidate (7) and the monodentate 4-nitrophenylphosphate (6) respectively. The signal at 6.9 ppm is coincident with the signal due to added $[(NH_3)_5IrNPP]^+$ (6). 6 and 7 were produced in the ratio ~30:70%. The product 7 decayed further in 1.0 M OH^- to yield two products, C and D.

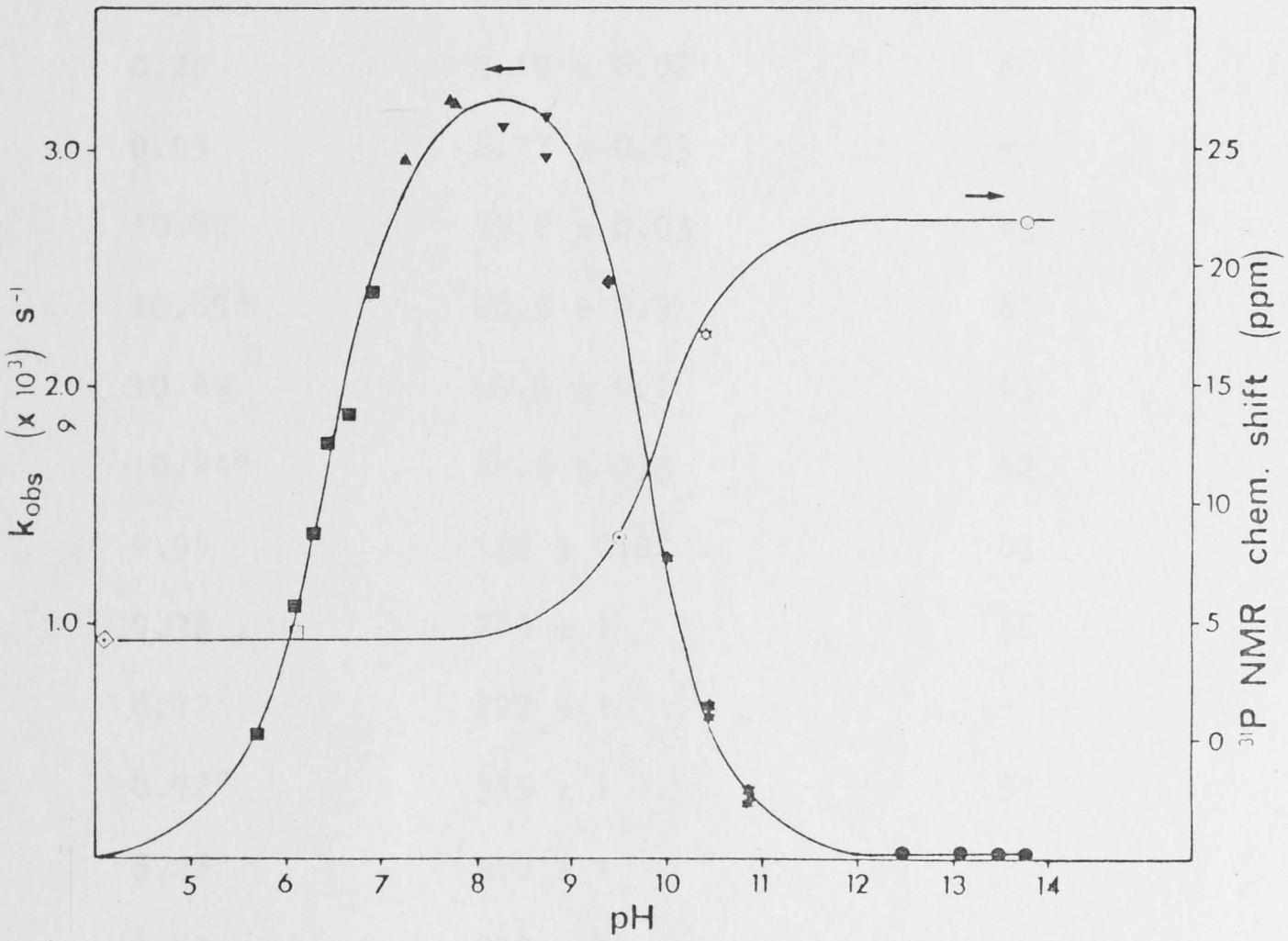


Figure 4.3

pH versus Rate of Second Step in the Reaction of $[(NH_3)_5IrBNPP]^{2+}$, filled symbols. Chemical Shift of the intermediate, open symbols. Conditions; μ = 1.0 M ($NaClO_4$), T = 25°C, Buffers, ■ Mes, ▲ Hepes, ▼ Tris, ◆ Ches, ★ Caps, ● OH^-, ⊙ NH_4^+, ◈ Acetate.

Table 4.3

Observed rate constants for the second step in the hydrolysis of **5**, same conditions as for Table 4.2.

[NaOH]/pH	k_{obs} (x 10^5) s^{-1}	Yield NP (%)
1.00	2.03 ± 0.002	69
1.00[a]	2.13 ± 0.01	-
0.50	1.99 ± 0.01	69
0.20	2.19 ± 0.02	66
0.05	2.77 ± 0.03	-
10.85	23.2 ± 0.03	63
10.85[a]	28.5 ± 0.3	61
10.44	60.0 ± 0.1	63
10.44[a]	64.5 ± 0.3	62
9.99	128 ± 0.2	63
9.38	244 ± 1	65
8.72	297 ± 1	-
8.72[a]	315 ± 1	57
8.26	310 ± 1	-
7.73	320 ± 1	-
7.72	322 ± 2	57
7.24	295 ± 1	63
6.90	240 ± 1	57
6.66	188 ± 1	-
6.45	176 ± 3	-
6.29	138 ± 1	-
6.10	108 ± 3	-
5.69	53 ± 3	56

[a] These runs were initiated with the complex at ~ pH 3 (see text)

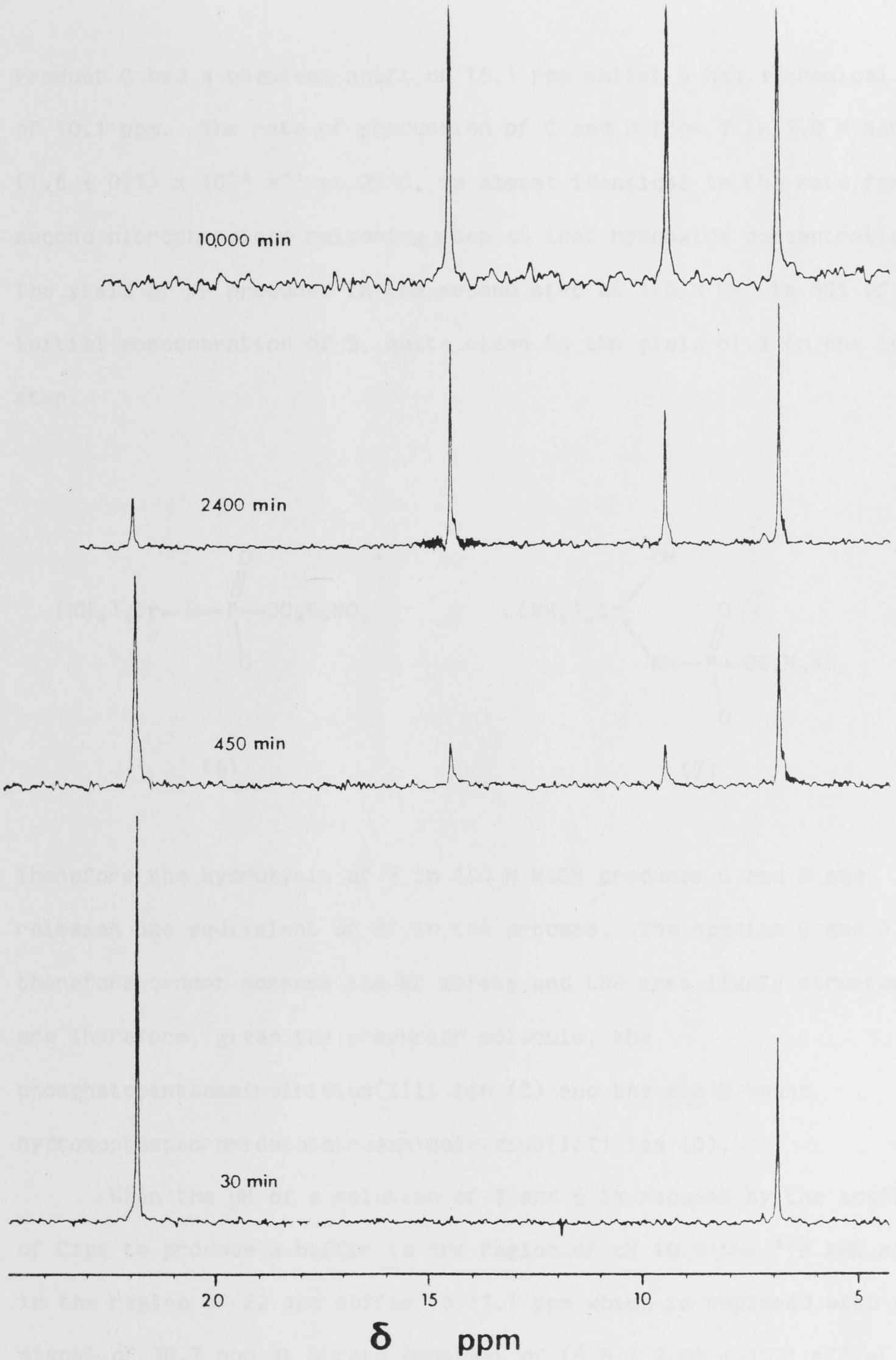
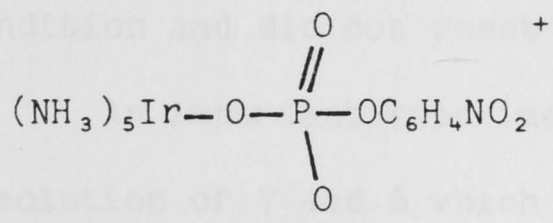


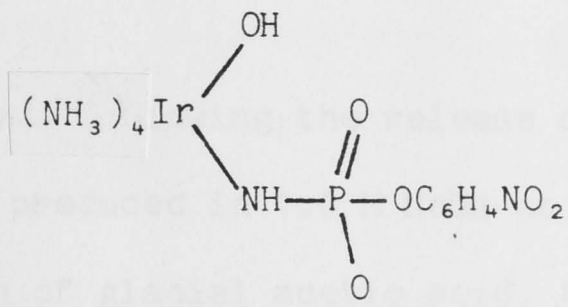
Figure 4.4

^{31}P NMR spectra showing the second step in the reaction of $[(NH_3)_5IrBNPP]^{2+}$, **5**, Conditions; NaOH = 1.0 M, T = 25°C, Spectrometer parameters as per text.

Product C had a chemical shift of 15.1 ppm whilst D has a chemical shift of 10.1 ppm. The rate of production of C and D from **7** in 1.0 M NaOH, $(1.6 \pm 0.1) \times 10^{-5}\ s^{-1}$ at 25°C, is almost identical to the rate for the second nitrophenolate releasing step at that hydroxide concentration. The yield of NP produced in the second step at 1.0 M OH^- is 69% of the initial concentration of **5**, quite close to the yield of **7** in the initial step.



(6)



(7)

Therefore the hydrolysis of **7** in 1.0 M NaOH produces C and D and releases one equivalent of NP in the process. The species C and D therefore cannot possess the NP moiety and the most likely structures are therefore, given the precursor molecule, the phosphatopentaammineiridium(III) ion (C) and the <u>cis</u> N bound hydroxophosphoramidatotetraammineiridium(III) ion (D).

When the pH of a solution of **7** and **6** is reduced by the addition of Caps to produce a buffer in the region of pH 10.4 the ^{31}P NMR signal in the region of 22 ppm shifts to 17.1 ppm which is replaced with a signal of 30.7 ppm at a rate constant of $(8.5 \pm 2.0) \times 10^{-4}\ s^{-1}$ at 25°C. Whilst the peak at 30.7 ppm is the predominant product, a signal at 9.3 ppm also appears in the spectrum.

When the pH of a solution of **7** and **6** is reduced by the addition of Mes to produce a buffer in the region of 6.1 the ^{31}P NMR signal at

~22 ppm is replaced by a signal at 4.6 ppm which decays to form a signal at 30.7 ppm with a rate constant of $(7.2 \pm 0.5) \times 10^{-4}$ s^{-1} at 25°C. Slowly the molecule responsible for the signal at 30.7 ppm yields a product with a chemical shift of 11.4 ppm.

When the pH of a solution of **7** and **6** is reduced by the addition of NH_4Cl to produce a buffer in the region of 9.5 the ^{31}P NMR signal of the intermediate is seen only fleetingly at ~8.5 ppm. In a similar experiment in acetate buffer pH ~4 the chemical shift of the intermediate was 4.3 ppm, however the intermediate was stable in this condition and did not react further.

An important experiment involved following the release of NP from a solution of **7** and **6** which had been produced in 1.0 M NaOH at 25°C then reduced to about pH 4 by the addition of glacial acetic acid, the rate of release of NP from **7** treated in this way was identical to the rate of release of NP from **7** reduced from 1.0 M OH^- to the required pH. This showed that it was simply two reversible protonations that were determining the shape of the pH-rate profile. This observation eliminated the possibility that the ring opening reaction of the N,O-chelate was rate determining in the pH 9-11 region. The supposition that it is the <u>cis</u> hydroxo, N-bound phosphoramidate ester that is the active species with respect to loss of the NP group is supported by the ^{31}P NMR chemical shifts of the species undergoing reaction.

The bell shaped pH-rate profile for the second NP releasing step in the reaction of $[(NH_3)_5IrBNPP]^{2+}$, can be explained by two protonations. In the pH region 5-8 the rate is controlled by a deprotonation with a pK_a of 6.39 ± 0.02, assigned the <u>cis</u>-aqua ligand. In the region pH 8-10 the rate is controlled by a deprotonation with a pK_a of 9.76 ± 0.05, which is most likely the deprotonation of the phosphoramidate nitrogen. The chemical shift of the intermediate, **7** was

dependent on pH, the pH dependence followed a titration curve with a pK_a ~9.9, (Figure 4.3).

The rate diminution upon deprotonation at the phosphoramidate nitrogen is expected since the deprotonation must reduce the electrophilicity of the phosphorus atom.

Attack of the cis-hydroxo ion on the N-bound phosphoramidate ester should yield initially the N,O chelate phosphoramidate and NP. ^{31}P NMR observations show that at both pH 10.4 and 6.1 the initial product of the reaction had a chemical shift of 30.7 ppm, and this may be due to the N,O chelate phosphoramidate. In addition to the product at 30.7 ppm, a signal at ~10 ppm appears in the spectra, this is possibly the N-bound ring opened species.

The hydrolysis of the free ligand in hydroxide solution was determined by the initial rate method and the reaction was first order in hydroxide. The second order rate constant for the reaction was (1.3 ± 0.1) x 10^{-5} $lmol^{-1}s^{-1}$ at 25°C and μ = 1.0 M ($NaClO_4$).

With this system as with the previous one, the rate law for the reaction includes a second and a third order pathway. This make the comparison of of the rates of hydrolysis of the free ligand and the complex more difficult. In 1.0 M OH^- the complex is lysed ~1300 fold faster than the free ligand.

In 1.0 M OH^- the intermolecular attack of hydroxide on the phosphorus centre of **5** is 5.1 x 10^{-3} s^{-1}, in 0.1 M OH^- the rate is 2.6 x 10^{-4} s^{-1}. These figures represent a rate enhancement of 200 to 390 fold in the rate of attack of OH^- on the phosphorus atom upon coordination to the Ir(III) centre.

The intramolecular reaction proceeds with a rate of ~10 s^{-1} in 1.0 M NaOH if the pK_a of the cis ammines is assumed to be ~17, (vide supra). This value was estimated by an analysis similar to that in

chapter 3, page 69. Therefore the rate of attack of cis-coordinated amido ion on the phosphorus centre occurs ~10^6 fold faster than the rate of attack of OH^- in 1.0 M NaOH solution.

This work has shown that the N,O-chelate 4-nitrophenylphosphoramidate ester hydrolyses readily without loss of the nitrophenol group even though the ester is a good leaving group. The only reaction of the chelate is the ring opening reaction. However the chelate ring opens to yield the cis hydroxo N-bound 4-nitrophenylphosphoramidate complex which subsequently hydrolyses by attack of the cis hydroxo ligand at the phosphorus centre.

4.4 General Discussion.

The reactions described above demonstrate the efficacy of the intramolecular attack of cis-coordinated amido ion on coordinated phosphate esters when the metal ion involved is Ir(III). This has not been observed before. In comparison with the analogous Co(III) chemistry the reactions are about 10^3 fold slower. The reduction in rate could be due to several factors. The pK_a of the ammine ligands are certainly higher for Ir(III) as compared to Co(III) complexes[6], and deprotonation of the ammine ligands is critical for the reaction to occur. However there are several arguments against the possibility that this is the main cause of the difference in rate between the two metal ions. Firstly the difference in the pK_a's between the two complexes for example, $[(NH_3)_5MOP(O)(OC_2H_5)(OC_6H_4NO_2)]^{2+}$, M = Co, Ir, is not likely to be large enough to account for the almost 10^3 fold difference between the rates of attack of amido ion on the phosphorus centre in the two ions. The difference in the pK_a's of the ammines between analogous Co(III) and Ir(III) is generally of the order of 1 unit[6]. Secondly

phosphoryl transfer reactions which proceed via the $S_N2(P)$ mechanism usually show a marked dependence on the basicity of the attacking nucleophile. For example, the Bronsted β coefficient for the attack of a series of nucleophiles on the phosphorus atom of methyl2,4-dinitrophenylphosphate anion is 0.31, the nucleophiles include substituted pyridines, primary amines and oxy anions,[7] (each class of nucleophile has a separate line but all have the same slope). The reaction of a series of substituted pyridines and primary amines with a phosphotriester displayed a β value of 0.61,[8](separate lines for each class), indicating that the rate of the reaction has a strong dependence on the pK_a of the nucleophile. So even though less of the Ir(III) complex will be in the ionized form, the greater basicity of the Ir(III)-amido ligand should compensate for this to some degree.

The reason for the reduced rate of aminolysis in the Ir(III) complexes is more likely to be due to the size of the Ir(III) ion compared to the Co(III) ion. The larger Ir(III) ion forces the attacking nucleophile and the phosphorus centre further apart, thus reducing the rate of the reaction. This idea will be developed further in the following chapter.

Interestingly a comparison of the rates of reaction of ENPP and BNPP for all three reactions, attack of OH^- on the free and coordinated ligand and intramolecular attack of amido ion yields very similar rate ratios. For attack of hydroxide ion on the uncoordinated ligand the ratio is 40, for the coordinated ligand the ratio is 46 and for intramolecular attack of amido ion the ratio is 30. This is an interesting result because it shows that the influence of the metal ion is constant and reproducible, ie it increases the rate of a particular reaction to a similar extent for the two different phosphodiesters.

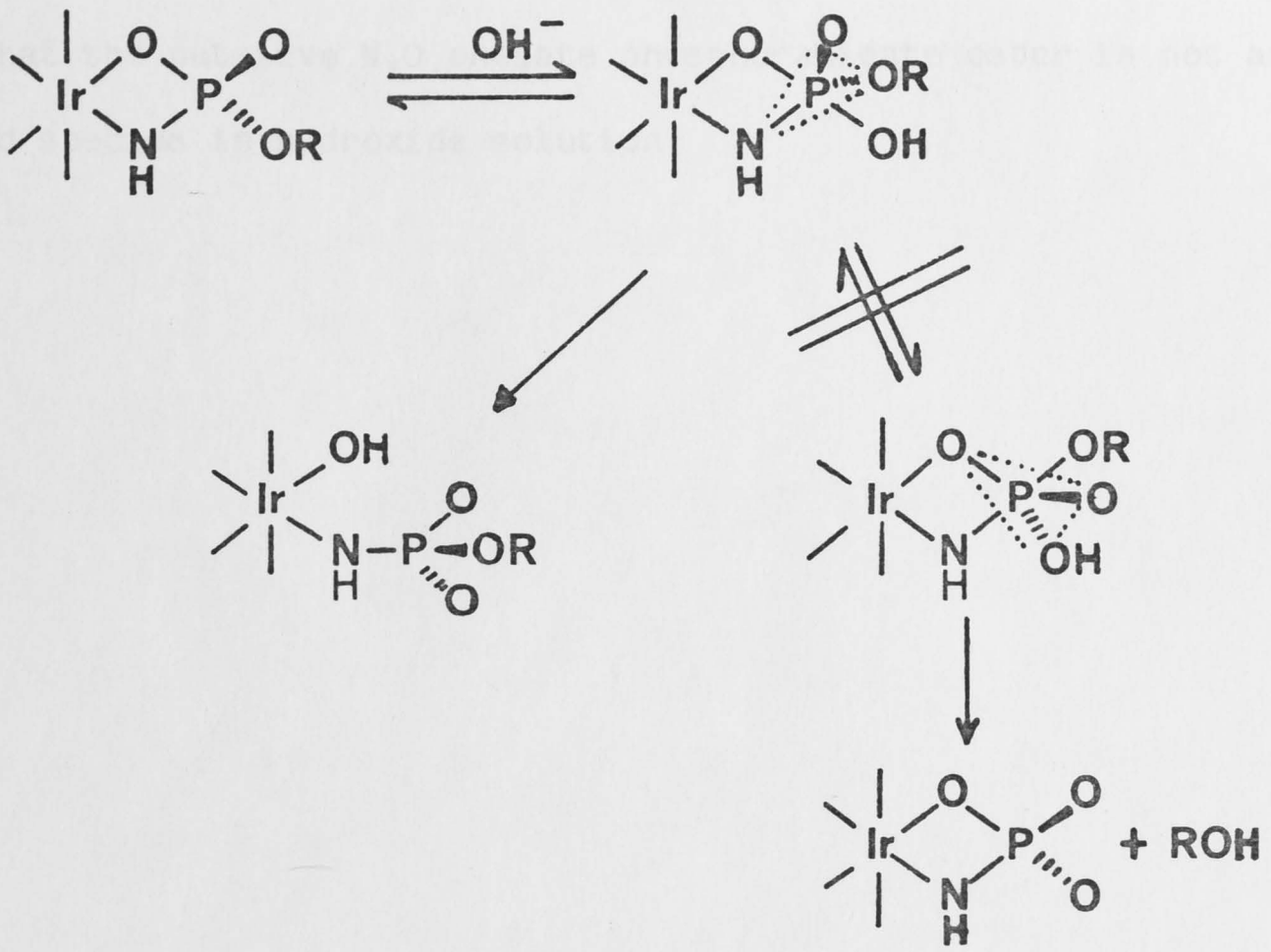
This work has shown that the chelate phosphoramidate ester which

is believed to be an intermediate in the reaction of 1 and 5 ring opens without loss of the esterifying group. The ring opening reaction presumably goes with substitution at phosphorus although this has not been shown. However, the ring opening reaction of the chelate ester $[(en)_2IrO_2PO_2Et]$ goes with P-O cleavage, (vide infra, Chapter 5). The rate at which the reaction occurs, especially in the case of $[(NH_3)_5IrBNPP]^{2+}$, seems to preclude the reaction involving metal-ligand bond cleavage given the very slow rates normally associated with Ir(III)-O ligand cleavage.[1]

It is generally accepted that a nucleophile attacking a four-coordinate phosphorus atom will attack at such a position so as to occupy an axial position in the resulting phosphorane[9,10]. In addition to this requirement, the four-membered ring must span axial-equatorial positions since the energy required for the ring to span equatorial-equatorial positions (120°) is prohibitive.[9,11] These requirements mean that the esterifying group must occupy an equatorial position in the aminophosphorane as it is initially formed, (Scheme 4.2)

The principle of microscopic reversibility[11] means that the leaving group, if it is of a similar nature to the nucleophile[12], is required to leave from an axial position in the phosphorane. In this case therefore, since the esterifying group is located equatorially in the initially formed aminophosphorane, some form of ligand reorganization, eg pseudorotation[13] or the turnstile mechanism[14], is required to place the ester group in an axial position. The fact that the sole product of the reaction is an N-bound phosphoramidate ester means that the required pseudorotation does not readily occur.

In the case of the 4-nitrophenol complex, 5, the phenol should be a superior leaving group to the metal-oxo substituent however it is only the oxo group that leaves. The reason for the inability of the



Scheme 4.2

Proposed Reaction Scheme for the Ring-Opening of the Ir(III) Chelated Phosphoramidate Esters.

aminophosphorane to pseudorotate is unknown, there are examples of 4-membered ring phosphoranes which are able to readily pseudorotate[15]. It may be that the aminophosphorane in this case is not long-lived enough to undergo such isomerization.

The ring opened species however, is not subject to the same constraints, this species can ring close to form an aminophosphorane which conforms to the requirements described above, ie the nucleophile entering the phosphorane axially and the ring substituents spanning axial equatorial positions, and still allow the phenol group to be axial. In this manner the esterifying group is readily cleaved from the complex.

Unfortunately this system has not allowed any estimation of the effect of chelation on the rate of exocyclic cleavage. It has shown

however that the putative N,O chelate phosphoramidate ester is not a long lived species in hydroxide solution.

References, Chapter 4.

1. Tobe, M.L. in "Inorg. Reaction Mechanisms", Nelson, London, 1972, pp 87.

2. Harrowfield, J. MacB., Jones, D.R., Lindoy, L.F. and Sargeson, A.M., J. Am. Chem. Soc., **102**, 7733-7741, 1980.

3. Martin, M.L., Martin, G.J. and Delpuech, J.J., in "Practical NMR Spectroscopy", Heyden, London, 1980, pp 301-302.

4. Jones, D.R., Lindoy, L.F. and Sargeson, A.M., J. Am. Chem. Soc., **105**, 7327-7336, (1983).

5. Lerman, C.L. and Westheimer, F.H., J. Am. Chem. Soc., **98**, 179-184, (1976).

6. Palmer, J.W. and Basolo, F., J. Inorg. Nucl. Chem., **15**, 279-286, (1960). Zanella, A.W. and Ford, P.C., Inorg. Chem., **14**, 700-701, (1975).

7. Kirby, A.J. and Younas, M., J. Chem. Soc. (B), **1970**, 1165-1172.

8. Khan, S.A., Kirby, A.J., J. Chem. Soc. (B), **1970**, 1172-1182.

9. Trippett, S., Pure Appl. Chem., **40**, 595-605, (1974).

11. Westheimer, F.H. Acc. Chem. Res. **1**, 70-78, (1968).

10. Gillespie, P., Ramirez, F., Ugi, I. and Marquarding, D., Angew. Chem. (Eng. Ed.), **12**, 91-172, (1973).

12. It is generally accepted that hydroxide and alkoxide ions are sufficienty alike to require the principle of microscopic reversibility to apply, see for example reference 11.

13. Mislow, K., Acc. Chem. Res., **3**, 321-331, (1970).

14. Holmes, R.R., J. Am. Chem. Soc., 96, 4143-4149, (1974).

15. Denney, D.Z., White, D.W. and Denney, D.B., J. Am. Chem. Soc., **93**, 2066-2067, (1971).

Chapter 5

INTRAMOLECULAR ATTACK OF HYDROXIDE ION - THE CHELATED PHOSPHATE ESTER

5.1 General introduction

The work of Westheimer[1] and others[2] in the 1950's and '60's demonstrated that organic phosphates possessing a five-membered ring hydrolyse up to 10^7 fold faster than their acyclic analogues.[1] The reason for this reactivity is that the ring structure destabilizes the phosphate ester and stabilizes the phosphorane intermediate relative to the non-cyclic ester. These effects are presumably reflected in a lowering of the activation energy for the reaction of the cyclic ester. This subject is discussed at greater length in section 1.5. Since the work of Westheimer, several groups of researchers[3,4,5] have suggested that the origin of the rate enhancement in the enzymic hydrolysis of phosphate monoesters may in part, be due to chelation of the substrate by a metal ion. The presence of this strained ring may induce rapid exocyclic hydrolysis of the ester group by a mechanism similar to that described by Westheimer, ie the presence of the ring favours the formation of the intermediate phosphorane which may result in the cleavage of the ester group.

Many Co(III) amine complexes of orthophosphate are quite stable, and the chelate phosphate is the thermodynamically preferred isomer in the pH range ~4 to 9 for some complexes.[6] Despite this stability several attempts[3,5,7] to synthesize a chelate phosphate ester have proven fruitless, although interesting dimeric species have arisen from such attempts.[8] The difficulties encountered in the attempted syntheses of the chelate ester indicated that this molecule might be particularly reactive and therefore worth pursuing further.

The strategy used in the current attempt to synthesize the chelated phosphate ester was indicated by the results obtained in the two previous chapters, ie intramolecular attack of a nucleophile on a coordinated diester. The precursor complex should possess a phosphodiester coordinated cis to a water molecule. After deprotonation, the coordinated hydroxide ion should attack the phosphorus centre to yield a phosphorane which should decay to the chelated phosphate monoester.

Ir(III) was the metal ion chosen for this study in order to avoid complications arising from metal-ligand bond rupture and cis-trans isomerization.

5.2 cis-$[(en)_2Ir(OH_2)(OP(O)(OC_2H_5)(OC_6H_4NO_2))]^{2+}$

5.2.1 Introduction

This section of work describes the reactions of a system, where a hydroxide ion and the ethyl,4-nitrophenylphosphate ligand are coordinated cis to each other about an iridium(III) complex ion.

5.2.2 Experimental.

All chemicals used were of analytical grade unless otherwise stated. Electronic spectra were recorded using a Hewlett Packard 8450A spectrophotometer. Kinetic traces were recorded on either the Hewlett Packard instrument above equipped with a 89100A temperature controlled cell holder, or a Cary 118C instrument thermostatted with a recirculating water bath. ^1H NMR spectra were recorded with a JEOL FX-200 spectrometer. ^{31}P NMR spectra were recorded with either a JEOL FX-60 or a Bruker CXP-200 spectrometer operating at 24.21 and 80.98 MHz respectively.

Buffers were made up from standardized $HClO_4$ or NaOH (Volucon) in CO_2-free glass distilled water. The buffer components were used as

supplied by the manufacturers; Tris, Caps - Sigma chemical Co., Hepes, Mes - B.D.H. chemicals. The ionic strength of the buffers was maintained at 1.0 M ($NaClO_4$). The pH of the deaerated buffers was measured using a Radiometer PMH 26 pH meter fitted with G202C glass and K4122 calomel electrodes calibrated using standard buffers.

<u>cis-$[(en)_2Ir(OH_2)(OP(O)(OC_2H_5)(OC_6H_4NO_2))]S_2O_6 \cdot {}^1/_2H_2O$, (1).</u>

To <u>cis</u>-$[(en)_2Ir(OSO_2CF_3)_2]CF_3SO_3$ (4.0 g) dissolved in dry sulfolane (40 ml) was added $HOP(O)(OC_2H_5)(OC_6H_4NO_2)$, (ENPP), (1.35 g) and the solution heated to 50°C for 22 hr. To this solution was added water (10 ml) acidified with concentrated $HClO_4$ (0.5 ml), and the solution stirred for a further 3 hr. The solution was extracted twice with ether, and the residue dissolved in water, (1 l), acidified to ~pH 1 with $HClO_4$. The solution was absorbed on a Dowex 50W-X2 column (H^+) column, the column was washed with water and eluted with 2 M HCl. The eluate was monitored at 285 nm and the major band, containing the desired product, was collected after several minor bands had eluted. The solution was evaporated to dryness and the resulting oil dissolved in warm methanol (20 ml), a warm solution of $Li_2S_2O_6$ (1.0 g in 10 ml methanol) was added dropwise until no more precipitation occurred, (excess dithionate redissolves the precipitate). The solution was cooled, and the solid collected, washed twice with methanol, twice with ether and dried <u>in vacuo</u> for 18 hr. Yield 0.53 g. Analysis calculated for, $C_{12}H_{29}N_5IrO_{14}PS_2$; C, 19.10; H, 3.87; N, 9.28; P, 4.10; Found; C, 19.2; H, 3.7; N, 9.2; P, 4.0. ^1H NMR; (D_2O, 0.05 M $DClO_4$), 1.28 (tr) J_{H-H} = 7.1 Hz (3H), 2.59 (br) 2.75 (br) (8H total), 4.14 (d of quart) $J_{P-H,H-H}$ ~ 7 Hz (2H), 5.70 (br) 6.00 (br) 6.27 (br) (8H total), 7.41 (d) J = 9.3 Hz (2H), 8.30 (d) J = 9.3 Hz (2H). $^{31}P\{H\}$ NMR; (H_2O/D_2O, pH 2), 1.600 ppm (1P), 1.624 (1P).

A solution of the complex, <u>cis</u>-$[(en)_2Ir(OH_2)ENPP]^{2+}$, 1, (10 µl,

~10^{-2} M) was syringed into a thermostatted cell containing the required hydroxide or buffer solution (2.0 ml). The solution was rapidly mixed and the increase in absorbance at 400 nm was recorded with time. The data sets were processed by the LSTSQR curve fitting program, and fitted well to single exponentials. At pH 6.29 the rate constant was determined by the initial rate method. The molar absorbtivity of NP at this pH was determined to be 2.61 x 10^3 mol l^{-1} cm^{-1}. Only the first 1-2% of the reaction was used to determine the rate of NP production.

The hydrolysis of the complex was, in some buffers, subject to buffer catalysis. Where this was apparent, the reaction was followed in buffers of constant pH but of varying buffer concentration. The rate of hydrolysis in the absence of buffer was determined by extrapolation to zero buffer concentration. The activation parameters for the reaction were determined in 0.01 M NaOH at five temperatures varying between 15 and 35°C.

The reaction was also followed by ^{31}P NMR spectroscopy. The complex, **1**, (30 mg) was dissolved in a solution of Tris/$HClO_4$ buffer pH 8.6, μ = 1.0 M ($NaClO_4$), D_2O, 20% was added and consecutive ^{31}P NMR spectra recorded at 25°C.

The complex, **1**, (20 mg) and NaCl (105 mg), were dissolved in H_2O (1.5 ml) containing Na_3PO_4 (0.01 M), NaOH (0.1 ml, 2 M) was added and consecutive spectra recorded at 25°C. (Acquisition parameters; acquisition frequency 80.98 MHz, sweep frequency 9 KHz, acquire 8 K data points, zero fill to 16 K points, pulse repetition time 0.5 s, number of scans per spectra 7200-14400).

The complex, **1**, (40 mg), Tris (109 mg) and NaCl (70 mg) were dissolved in $H_2{}^{18}O$ (1.8 ml, 9.84% ^{18}O) and HCl (50 µl, 9.0 M) to make a buffered solution of pH ~8.2, 9.57% $H_2{}^{18}O$, μ = 1.0 M (NaCl). The solution was maintained at ~20°C for 16 hrs, then ^{31}P NMR spectra were

recorded on the solution after the addition of D_2O (250 µl) and trimethylphosphate (4 µl). (Acquisition parameters; acquisition frequency 80.98 MHz, sweep frequency 2000 Hz, pulse angle 90°, pulse repetition time 4.5 sec).

5.2.3 Results and Discussion

The complex, cis-$[(en)_2Ir(OH_2)ENPP]^{2+}$, **1**, was synthesized by heating equal amounts of cis-$[(en)_2Ir(OSO_2CF_3)_2](CF_3SO_3)$ and ENPP in sulfolane then quenching with water. The complex was purified by cation exchange chromatography, isolated as the dithionate salt and characterized by elemental analysis, ^1H and ^{31}P NMR spectroscopy and electronic spectroscopy. The ^{31}P NMR spectrum of cis-$[(en)_2Ir(OH_2)ENPP]^{2+}$ consisted of two signals of equal intensity separated by 0.024 ppm. This indicated the presence of the two diastereomers expected since both the Ir and P centres have chiral configurations. The chemical shift of the complex was shifted ~7 ppm downfield from the ionized free ligand as expected, (vide supra, section 1.8).

The hydrolysis of cis-$[(en)_2Ir(OH_2)ENPP]^{2+}$, was followed spectrophotometrically at 400 nm in a series of buffers from pH 5.94 to 10.43, and in hydroxide solutions from 0.01 to 1.0 M. (Table 5.1 and Figure 5.1) In the pH range 6 to 12 the reaction can be described by the rate law:

$$k_{obs} = k_m K_a/(K_a+[H^+])$$

where k_m is assigned as the rate constant for the reaction of the deprotonated complex and K_a is the dissociation constant for the coordinated H_2O ligand. Non linear least squares fitting by computer of the data in the range pH 6 to 10.5 to equation (1) resulted in values for these two constants of, $k_m = (4.6 \pm .1) \times 10^{-5} s^{-1}$ and $K_a = (1.02 \pm .08) \times 10^{-7}$ ($pK_a = 6.99 \pm .04$). This pH dependence was as expected for

Table 5.1

Rate Constants for the Hydrolysis of cis-$[(en)_2Ir(OH_2)(ENPP)]^{2+}$, **1**, μ = 1.0 M ($NaClO_4$), followed spectrophotometrically at 400 nm.

pH/[NaOH]	Buffer	Temperature (°C)	k_{obs} (x 10^5) s^{-1}
5.94	Mes	25	0.292 ± 0.007
6.29	Mes	25	0.63 ± 0.05
6.45	Mes	25	0.96 ± 0.01
6.90	Mes	25	1.99 ± 0.01
7.24	Hepes	25	3.06 ± 0.02
7.72	Hepes	25	3.95 ± 0.05[a]
8.22	Hepes	25	4.25 ± 0.03[a]
8.57	Tris	25	4.50 ± 0.1
8.94	Tris	25	4.62 ± 0.01
10.43	Caps	25	4.32 ± 0.02
0.01	NaOH	25	4.62 ± 0.05
0.05	NaOH	25	6.53 ± 0.05
0.10	NaOH	25	8.90 ± 0.1
0.20	NaOH	25	14.4 ± 0.1
0.50	NaOH	25	34.7 ± 0.3
0.80	NaOH	25	65.4 ± 1.0
1.00	NaOH	25	98.8 ± 0.5
0.01	NaOH	15	1.71 ± 0.01
0.01	NaOH	20	2.84 ± 0.05
0.01	NaOH	30	7.50 ± 0.02
0.01	NaOH	35	11.7 ± 0.1

[a] extrapolated to zero buffer concentration.

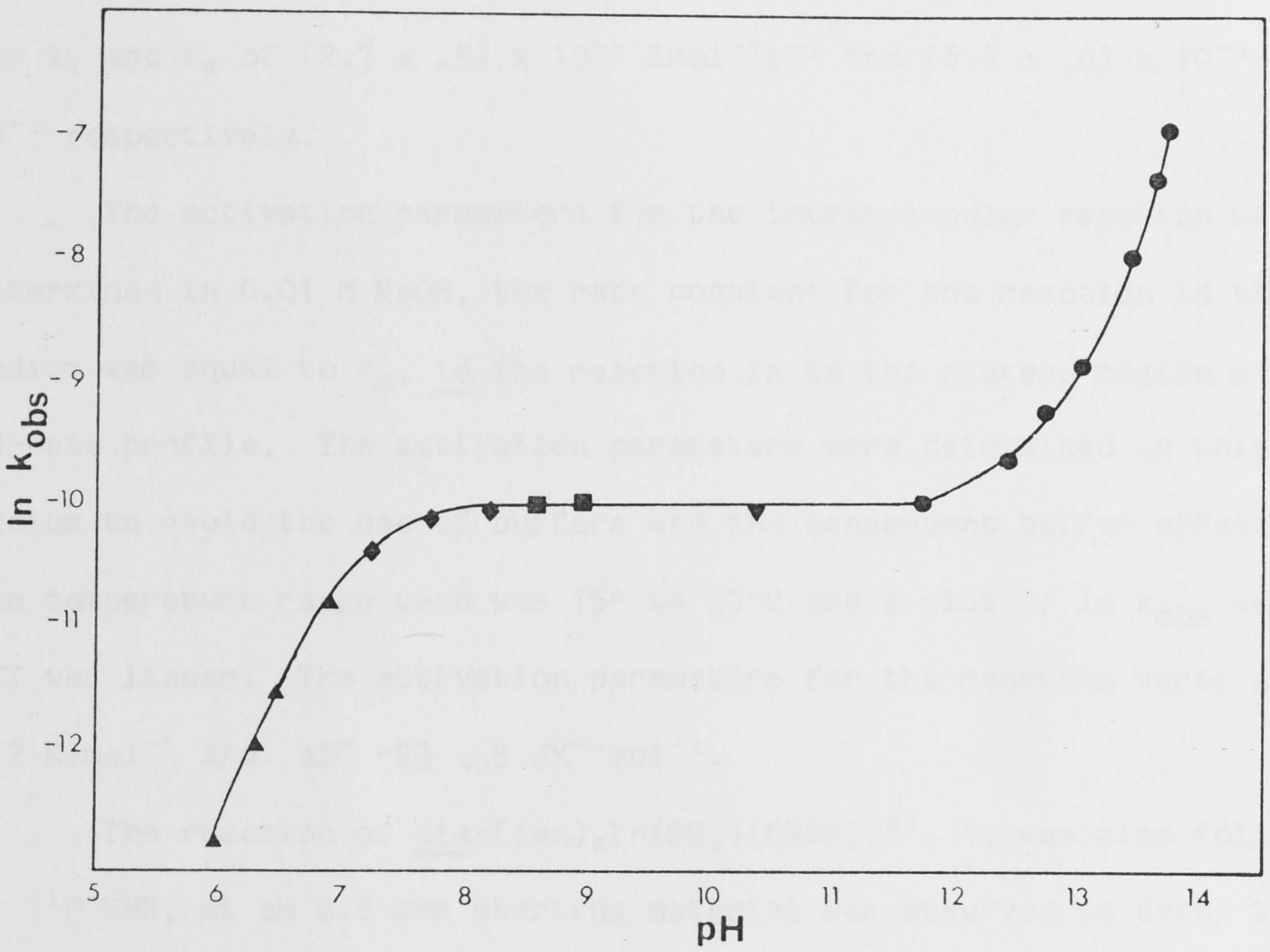


Figure 5.1

pH Versus Rate Profile for the Hydrolysis of cis-$[(en)_2Ir(OH_2)(ENPP)]^{2+}$, **1**, μ = 1.0 M ($NaClO_4$), 25°C. The solid line was calculated using the rate constants given in the text.

the reaction involving attack of the cis-hydroxo ion on the phosphorus centre. The pK_a of the coordinated H_2O is in the expected area for a dicationic Ir(III) species.[9]

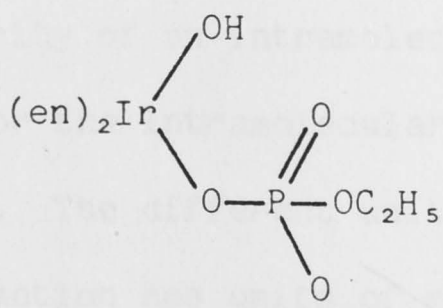
At higher pH values the rate of hydrolysis increased. The reaction above pH ~12 was dependent on $[OH^-]$ with a two term rate law as observed for the corresponding pentaammine complex, $[(NH_3)_5IrENPP]^{2+}$. The rate of hydrolysis of **1** in the pH range 6 to 14 is described by the rate law:

$$k_{obs} = k_m K_a/(K_a + [H^+]) + k_1[OH^-] + k_2[OH^-]^2$$

Non linear least squares fitting of the data in table 5.1 gave values for k_1 and k_2 of (2.7 ± .5) x 10^{-4} $lmol^{-1}s^{-1}$ and (6.6 ± .6) x 10^{-4} $lmol^{-2}s^{-1}$ respectively.

The activation parameters for the intramolecular reaction were determined in 0.01 M NaOH, the rate constant for the reaction in this medium was equal to k_m, ie the reaction is in the plateau region of the pH-rate profile. The activation parameters were determined in this medium to avoid the use of buffers and the consequent buffer effects. The temperature range used was 15° to 35°C and a plot of ln k_{obs} versus 1/T was linear. The activation parameters for the reaction were; $\Delta H^{\neq}$ 69 ± 2 $KJmol^{-1}$ and $\Delta S^{\neq}$ -93 ± 5 $JK^{-1}mol^{-1}$.

The reaction of cis-$[(en)_2Ir(OH_2)(ENPP)]^{2+}$, **1**, was also followed by ^{31}P NMR, at pH 8.6 the starting material was observed to decay to a single product displaying a triplet, J = 6 Hz, at 13.8 ppm in its ^{31}P NMR spectrum. The rate constant for loss of **1** and concomitant production of the 13.8 ppm signal was (4.7 ± 0.5) x 10^{-5} s^{-1} at 25°C, μ = 1.0 M (NaCl), ie identical to the rate constant for nitrophenolate, (NP), production. The reaction product was a net zero charged compound at pH 9; the product in dilute NH_4^+/NH_3 buffer passed through both Dowex 50W-X2 cation exchange, and Dowex AG-1X8 anion exchange, columns unchanged. Its ^{31}P NMR chemical shift, ^1H coupling and lack of charge at pH 9 strongly implies that the species is **2**.



(2)

The reaction of **1** in 1 M OH^- solution also yielded a single product, which displayed a triplet at 12.5 ppm in its ^{31}P NMR spectrum. The product was stable to further hydrolysis. When the product of the reaction at 1 M OH^- was added to the product of the hydrolysis at pH 8.6 the signals were identical with respect to both their ^{31}P NMR chemical shift and proton coupling. Therefore in both 1.0 M NaOH and at pH 8.6 the reaction of **1** yields only one product, apparently **2**. The mechanism by which these reactions occur would appear to be intramolecular attack of coordinated hydroxide at pH 8.6, followed by a rapid ring opening reaction (Path B, Scheme 5.1). There is another possibility which would account for the pH versus rate profile and the formation of the ring opened species as the sole product. That is, that the cis hydroxo group acts as a base and deprotonates a water molecule as it attacks the phosphorus centre. The activated complex for such a reaction would involve a cyclic system where a proton from the water nucleophile is being transferred to the coordinated hydroxide moiety as the oxygen of the water molecule approaches the phosphorus atom. The product of such a reaction would also be the monodentate ethylphosphate complex.

On the basis of a very large number of observations Kirby has proposed a method for distinguishing between general base catalysis and nucleophilic catalysis of intramolecular reactions.[10] The system is based on the difference in the "effective molarities", (EM), between the two possibilities, general base catalysed reactions have EM values less than 80 and nucleophilic catalysed reactions have EM values greater than 80. The effective molarity of an intramolecular reaction is the ratio of the rate constants for the intramolecular reaction and the comparable intermolecular reaction. The different units for the two rate constants means that the EM of reaction has units of mol l^{-1}.

The effective molarity for the reaction of cis-

$[(en)_2Ir(OH_2)ENPP]^{2+}$, could be calculated if the rate constant for the corresponding intermolecular reaction were known. The reaction in question should be the intermolecular attack of a Ir(III) bound hydroxide nucleophile on ENPP coordinated to another Ir(III) ion. Such a reaction has not been observed, however an estimate of the rate constant can be made. The rate of attack of free hydroxide ion on the ENPP moiety coordinated to Ir(III) was measured in the previous chapter and the rate constant for attack in 1.0 M hydroxide was $\sim 10^{-4}$ s^{-1}. The difference in the basicities of the two nucleophiles in question is quite large, the pK_a's of their conjugate acids are, 16[11] for H_2O and 7 for $Ir\text{-}OH_2$. The dependence of the rate for this reaction on the basicities of the nucleophiles is not known. However, the reaction rates of di-[12] and triesters[13] of phosphoric acid generally show a marked dependence on the basicity of the attacking nucleophile, Bronsted β's of 0.31[12] and 0.61[13] respectively. Therefore the rate of attack of the $Ir(III)\text{-}OH^-$ nucleophile would be expected to be much slower than the rate of attack of free OH^-. Assuming an intermediate Bronsted β value of 0.5; the rate constant for intermolecular attack of $Ir(III)\text{-}OH^-$ would be $\sim 3 \times 10^{-9}$ $lmol^{-1}s^{-1}$. This would yield a EM of 2×10^4 mol l^{-1} for the intramolecular reaction of cis-$[(en)_2Ir(OH_2)ENPP]^{2+}$.

Using Kirby's criteria, the large EM implies that the reaction proceeds via intramolecular nucleophilic attack of coordinated OH^- attack on the phosphorus centre, despite the formation of the strained ring in the process.[10]

In neither the kinetics by ^{31}P NMR spectroscopy or visible spectroscopy was there observed any evidence that the two diastereomers reacted at different rates.

The chelate phosphate ester formed therefore must rapidly ring open under the conditions that it is formed. The question is then, does

the ring opening reaction occur with Ir-O or P-O bond cleavage? To answer this question an ^{18}O tracer study was conducted. The hydrolysis was conducted in tris buffer at pH 8.2 in a solution containing 9.6% $H_2{}^{18}O$, the result of the experiment as shown in Figure 5.2 suggests that ~100% incorporation of the label occurs during the reaction. The quantitation of the result was result was made difficult by the broadness of the product peak at 13.7 ppm. The half-height width of the standard trimethylphosphate was in the region of 0.5 to 0.9 Hz depending on the resolution enhancement technique employed. The product peak had a half height width of ~3 Hz, this was of the order of the shift expected for the ^{18}O substituted product. The shoulder on the high field side of the product peak was reproducible using a number of resolution enhancement techniques including trapezoidal multiplication, gaussian multiplication and convolution difference, in addition the shoulder was clearly evident in standard spectra; exponential multiplication of the free induction decay. Spectra recorded under identical conditions in the absence of $H_2{}^{18}O$ do not show the satellite peak at the high field side of the product, (Figure 5.2). The measured isotopic shift of 0.048 ± 0.01 ppm was in the region expected for ^{18}O isotope shifts on phosphate esters.[14] The large error associated with the shift is due to the broadness of the peaks and the noise in the spectrum concealing the true peak position. The spectra were not able to be usefully integrated, however inspection of Figure 5.2 shows that the intensity of the shoulder on the product peak is in the region expected for 100% incorporation of label. Oxygen exchange in the product and the reactant are assumed to be very slow at pH 8.2 in keeping with observations on both free phosphate,[15] phosphate esters[16] and Co(III) coordinated phosphate.[17] Therefore it was concluded that, the ring opening reaction most likely proceeded with P-O cleavage.

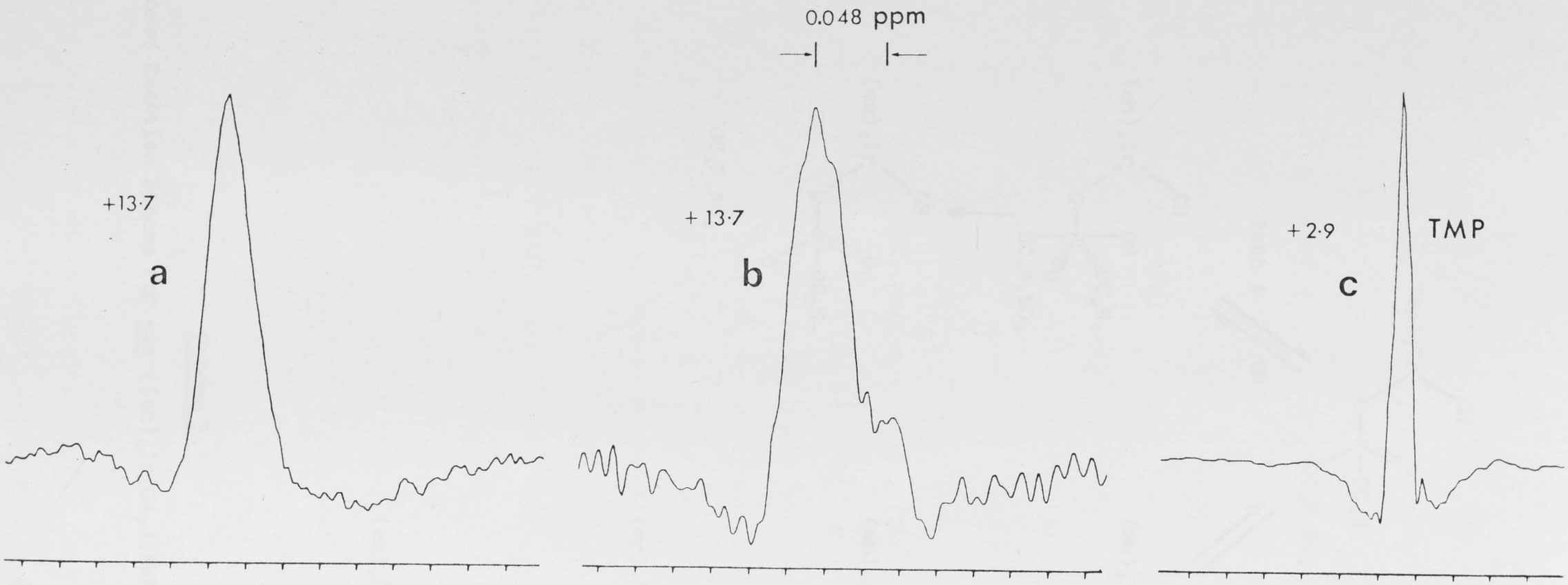
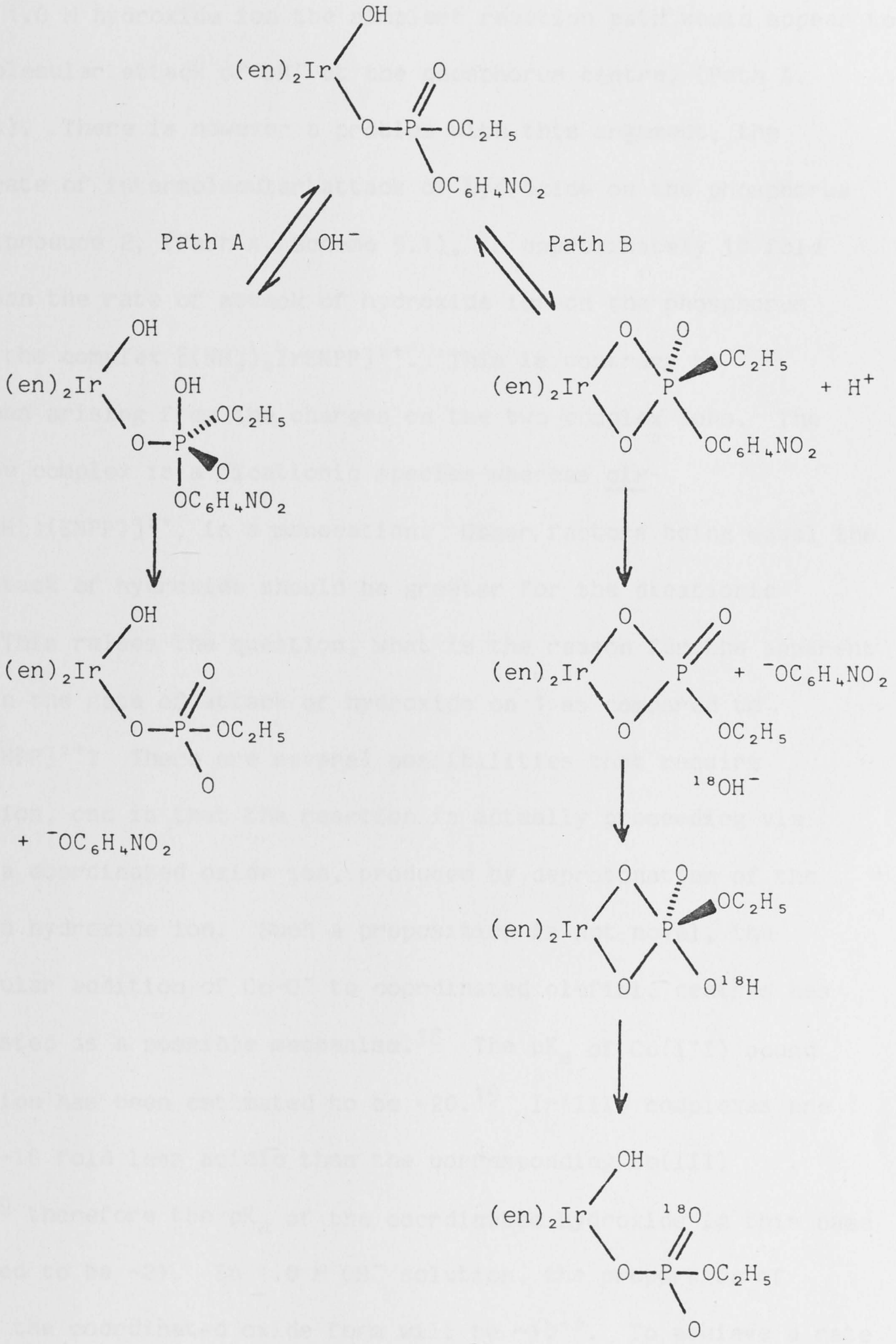


Figure 5.2

$^{31}P\{H\}$ NMR Spectrum of the Products of Hydrolysis of cis-$[(en)_2Ir(OH_2)(ENPP)]^{2+}$, 1, at pH 8.2, Tris buffer, μ = 1.0 M (NaCl). a). product of hydrolysis in the absence of $H_2{}^{18}O$, b). product of hydrolysis in 9.6% $H_2{}^{18}O$, c). standard, trimethylphosphate same spectrum as b. The Vertical scale for signal b is 4 times that of a. The Horizontal scale is 2 Hz per division. Acquisition parameters as per text, Resolution enhancement by trapezoidal multiplication; TM1 = 500, TM2 = 3000.



Scheme 5.1

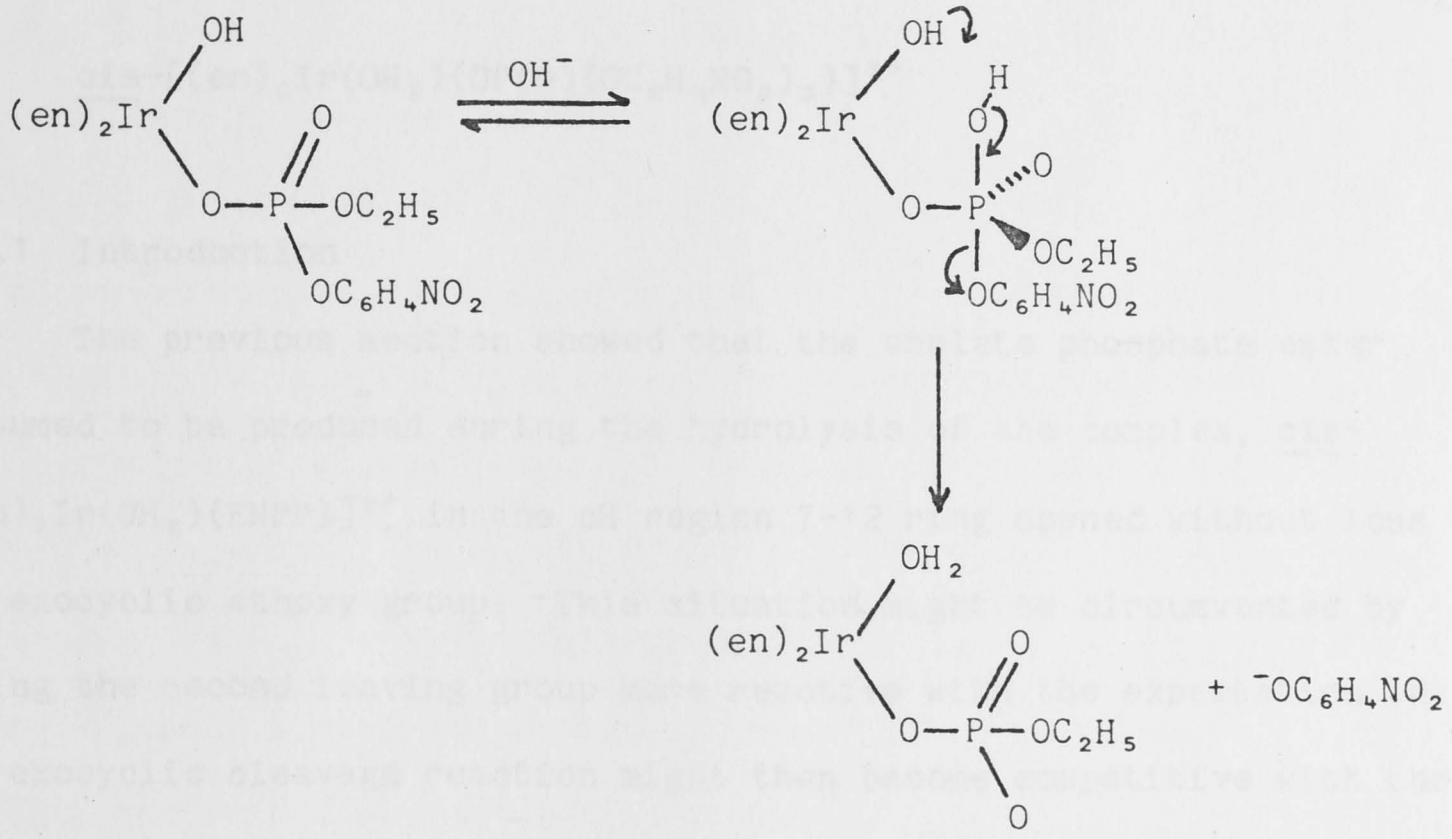
Proposed Reaction Scheme for cis-$[(en)_2Ir(OH_2)(ENPP)]^{2+}$, **1**, in Basic pH.

In 1.0 M hydroxide ion the simplest reaction path would appear to be intermolecular attack of OH^- at the phosphorus centre, (Path A, Scheme 5.1). There is however a problem with this argument, the apparent rate of intermolecular attack of hydroxide on the phosphorus centre to produce **2**, (Path A, Scheme 5.1), is approximately 10 fold greater than the rate of attack of hydroxide ion on the phosphorus centre in the complex $[(NH_3)_5IrENPP]^{2+}$. This is contrary to expectations arising from the charges on the two complex ions. The pentaammine complex is a dicationic species whereas cis-$[(en)_2Ir(OH_2)(ENPP)]^{2+}$, is a monocation. Other factors being equal the rate of attack of hydroxide should be greater for the dicationic species. This raises the question, what is the reason for the apparent increase in the rate of attack of hydroxide on **1** as compared to $[(NH_3)_5IrENPP]^{2+}$? There are several possibilities that require investigation, one is that the reaction is actually proceeding via attack of a coordinated oxide ion, produced by deprotonation of the coordinated hydroxide ion. Such a proposition is not novel, the intramolecular addition of $Co\text{-}O^-$ to coordinated olefinic centres has been suggested as a possible mechanism.[18] The pK_a of Co(III) bound hydroxide ion has been estimated to be ~20.[19] Ir(III) complexes are generally ~10 fold less acidic than the corresponding Co(III) complexes,[9] therefore the pK_a of the coordinated hydroxide in this case is estimated to be ~21. In 1.0 M OH^- solution, the proportion of complex in the coordinated oxide form will be $\sim10^{-7}$. To achieve a rate constant of $\sim10^{-3}$ s^{-1} the rate of attack of the coordinated oxide on the phosphorus centre needs to be $\sim10^{4}$ s^{-1}. This is quite a substantial rate constant considering that the reaction involves formation of a highly strained four membered ring. The rate constant is 10^4 fold greater than that estimated for the rate of attack of amido ion on

Ir(III) coordinated ethyl,4-nitrophenylphosphate, (vide supra, section 4.2.3), and makes this route difficult to support as a possible mechanism.

An alternative path for this reaction is through an intermolecular attack of hydroxide ion to give a phosphorane and deprotonation of this species by the coordinated hydroxide ion. The intermediate phosphorane is in equilibrium with the starting material and the decomposition of the phosphorane is rate determining. The adjacent coordinated hydroxide ion acts as a base, deprotonating the intermediate phosphorane thereby accelerating its rate of decomposition into products, (Scheme 5.2).

However, these mechanistic proposals cannot be distinguished on the basis of the rate law or any evidence to hand at the moment.



Scheme 5.2

Intramolecular Base Catalysis of the Hydrolysis of cis-$[(en)_2Ir(OH_2)(ENPP)]^{2+}$, at High pH.

In general, the phosphodiesters are the least reactive of the phosphate esters in mild aqueous conditions ie pH 2-10.[20] There has

been no study on the hydrolysis of ENPP or similar compound, eg methyl4-nitrophenylphosphate, under these conditions. The reason for this is readily apparent, the reaction would be prohibitively slow even at elevated temperatures. It would be safe to assume however, that the rate of hydrolysis of ethyl,4-nitrophenylphosphate, (ENPP), at pH 8 will be at least an order of magnitude slower than that of bis(4-nitrophenyl)phosphate, (BNPP), at that pH. The hydrolysis of BNPP at pH 8, 25°C, has been estimated to be ~10^{-9} s^{-1}, (vide infra, section 5.3.3). Therefore at pH 8 the hydrolysis of ENPP should not proceed with a rate constant greater than ~10^{-10} s^{-1}. At pH 8 cis-$[(en)_2Ir(OH)(ENPP)]^+$ is hydrolysed at a rate of ~5 x 10^{-5} s^{-1}, ~10^5 fold faster than the free ligand at this pH.

5.3 cis-$[(en)_2Ir(OH_2)(OP(O)(OC_6H_4NO_2)_2)]^{2+}$

5.3.1 Introduction

The previous section showed that the chelate phosphate ester presumed to be produced during the hydrolysis of the complex, cis-$[(en)_2Ir(OH_2)(ENPP)]^{2+}$ in the pH region 7-12 ring opened without loss of the exocyclic ethoxy group. This situation might be circumvented by making the second leaving group more reactive with the expectation that the exocyclic cleavage reaction might then become competitive with the ring opening reaction. To this end the analogous bis(4-nitrophenyl)phosphate complex, cis-$[(en)_2Ir(OH_2)(BNPP)]^{2+}$, was synthesized.

5.3.2 Experimental

cis-$[(en)_2Ir(OH_2)(OP(O)(OC_6H_4NO_2)_2](ClO_4)_2$, (3).

cis-$[(en)_2Ir(OSO_2CF_3)_2](CF_3SO_3)$ (1.0 g) and $HOP(O)(OC_6H_4NO_2)_2$

(BNPP) (0.45 g) were dissolved in sulfolane (20 ml) with the addition of 2,4,6-collidine (0.2 g), the solution was heated to 40°C for 19 hr. After this time dilute triflic acid (CF_3SO_3H) (1 ml, 0.2 M) was added and the solution stirred for a further 3 hr. The solution was then extracted with ether (2 x 300 ml), and the residue dissolved in H_2O (2000 ml). After the pH was adjusted to ~2 with HCl the mixture was absorbed on a Sephadex SP-C25 column (Na^+ form). The column was washed with water, (pH ~2), then eluted with NaCl (0.1 to 0.2 M, pH ~2). The eluate was monitored at 285 nm. Several minor bands eluted before the major band containing the desired product came off the column with 0.2 M NaCl. This solution was evaporated to 150 ml and $LiClO_4$, (10 g in 20 ml H_2O), was added. The solution was kept at 4°C for 16 hr. The fine precipitate which formed was collected, washed with cold water (2 ml) and dried in vacuo. Yield 0.25 g.

Analysis calculated for $C_{16}H_{26}N_6Cl_2IrO_{16}P$; C, 22.13; H, 3.02; N, 9.68. Found; C, 22.52; H, 3.09; N, 9.40.

The perchlorate salt of 3 was very insoluble in water and was not useful for experiments requiring a reasonable concentration of complex, eg NMR experiments. The chloride salt was used for these experiments and it was obtained by evaporation of the solution from the ion-exchange column to ~50 ml and cooling it at 4°C for 16 hours. The chloride salt of the complex precipitated and was collected and dried in vacuo for 18 hours. Elemental analysis indicated that 3 moles of NaCl co-precipitated with the monohydrated complex.

Calculated for $C_{16}H_{28}N_6Cl_5IrNa_3O_{10}P$; C, 20.58; H, 3.02; N, 9.00; Cl, 18.98. Found; C, 20.6; H, 3.0; N, 9.0; Cl, 18.5. ^{1}NMR; (D_2O, 0.01 M DCl), 2.59 (br) (4H), 2.77 (br) (4H), 5.7, 5.9, 6.2, 6.45, 6.63 (all br) (8H), 7.40 (d) J = 9 Hz (4H), 8.28 (d) J = 9 Hz (4H). ^{31}P NMR; (H_2O/D_2O, pH 10), - 6.0 (s).

The kinetics of hydrolysis of cis-$[(en)_2Ir(OH_2)BNPP]^{2+}$, 3, were followed by observing the rate of release of NP from the complex spectrophotometrically at 400 nm. A stock solution of 3 (~1 mg in 20 ml H_2O) was prepared and equal volumes of this solution and buffer or hydroxide solution at twice the required final concentration and ionic strength were mixed at 25°C and the increase in absorbance at 400 nm followed with time. The data sets obtained normally encompassed consecutive reactions and the rate constants for the two reactions were extracted from the data by established procedures. When the initial reaction was completed, the following reaction fitted well to single exponential decays and rate constants were obtained by curve fitting using the LSTSQR program. The rate constant for the initial reaction was extracted from the data in a variety of ways depending on the ratio of the two rate constants. When the difference between the rate constants was more than 100 fold the following reaction was ignored and the data sets processed by the LSTSQR program and fitted well to single exponential functions. When the difference was between 10 and 100 fold, the plot of log $(A_\infty - A_t)$ versus time for the following reaction was linear and was extrapolated to zero time. The difference between this line and the points in the initial portion of the plot were replotted on a log scale versus time to determine the rate constants for the initial reaction.

The reaction was also followed by ^{31}P NMR, a known weight of cis-$[(en)_2Ir(OH_2)BNPP]^{2+}$, 3, and NaCl (to make final ionic strength 1.0 M) were dissolved in a mixture of D_2O and H_2O (final D_2O concentration 25%) and concentrated NaOH or buffer solutions were added to make the solution up to the required volume and NaOH concentration or pH. Trimethylphosphate (2 µl), was added to the solution as an internal standard. (^{31}P NMR acquisition parameters; acquisition frequency 80.98

MHz, sweep frequency 9 KHz, acquire 8 K data points, zero fill to 16 K points, pulse repetition time 0.5 s, number of scans per spectra 600-14400).

5.3.3 Results and Discussion

The complex cis-$[(en)_2Ir(OH_2)(BNPP)]^{2+}$, 3, was prepared by a method similar to that employed in the synthesis of 1, ie heating cis-$[(en)_2Ir(OSO_2CF_3)_2]CF_3SO_3$ with one equivalent of bis(4-nitrophenyl)phosphate, (BNPP), then quenching the reaction with water. The crude product was purified by ion exchange chromatography, isolated and characterized by elemental analysis, ^1H and ^{31}P NMR spectroscopy. The ^{31}P NMR chemical shift variation of the BNPP moiety upon coordination was slightly less than usual, only 5 ppm downfield.

The hydrolysis of 3 was followed by observing the release of NP from the complex. The release of NP showed biphasic kinetics, ie there were at least two consecutive reactions occurring. The rate constants for the two reactions were extracted from the data by established procedures described in the experimental section. In all cases the rate constant for the first step refers to reaction of 3 to yield products, and the second step refers to the subsequent reaction of the product(s) of the initial reaction. The assignment of the rate constants was established by two procedures; firstly ^{31}P NMR spectroscopy of the reaction mixture showed that when the initial reaction was over the starting material had disappeared. Also the yield of nitrophenolate from the two steps of the reaction was equal which implies that the initial reaction was the reaction of the starting material. Table 5.2 and Figure 5.3 show the variation of the rate of the initial reaction with pH.

The overall rate of reaction of cis-$[(en)_2Ir(OH_2)BNPP]^{2+}$, 3, in

the range pH 5 to 14 was described by the rate law;

$$k_{obs} = k_m K_a/(K_a + [H^+]) + k_1[OH^-] + k_2[OH^-]^2.$$

As expected, the rate of the initial reaction of **3** was dependent on pH, with a group required to be deprotonated for reaction to occur. The group had a pK_a of 7.06 ± 0.02 and was assigned as the cis-aqua ligand.

Table 5.2

Rate Constants for the Initial Step in the Hydrolysis of **3**, 25°C, μ = 1.0 M $NaClO_4$, determined spectrophotometrically at 400 nm.

pH/$[OH^-]$	Buffer	k_{obs} ($\times 10^4$ s^{-1})
5.83	Mes	0.34 ± 0.01
6.35	Mes	0.764 ± 0.002
6.72	Hepes	1.21 ± 0.01
7.12	Tris	2.09 ± 0.02
7.29	Tris	2.51 ± 0.01
7.68	Tris	3.19 ± 0.04
8.18	Tris	3.60 ± 0.03
8.67	Tris	3.84 ± 0.05
10.98	Caps	3.95 ± 0.1
0.01	NaOH	6.14 ± 0.05
0.03	NaOH	12.9 ± 0.1
0.05	NaOH	20.5 ± 0.1
0.10	NaOH	39 ± 1
0.25	NaOH	105 ± 2
0.50	NaOH	241 ± 3
1.00	NaOH	809 ± 3

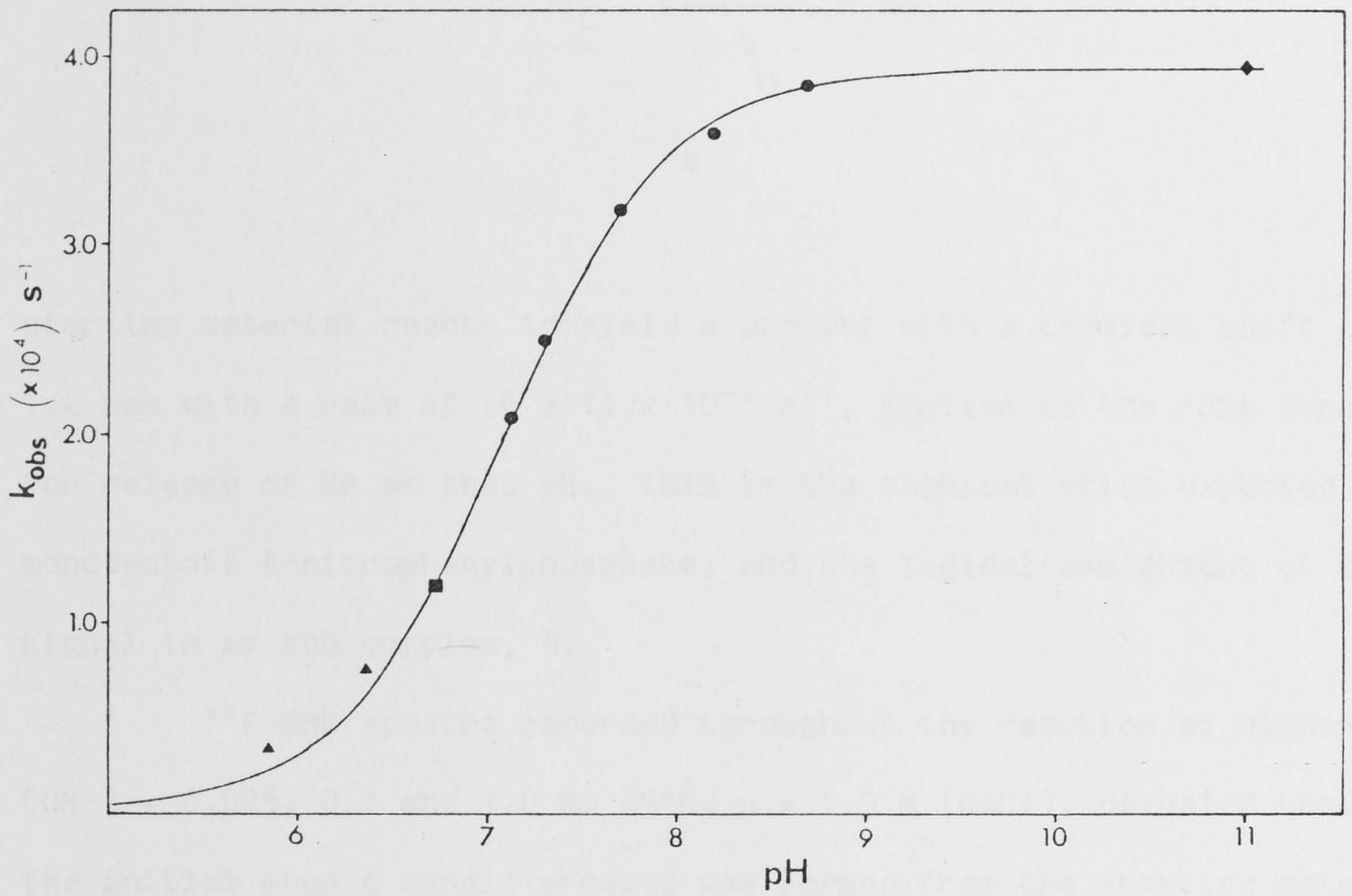
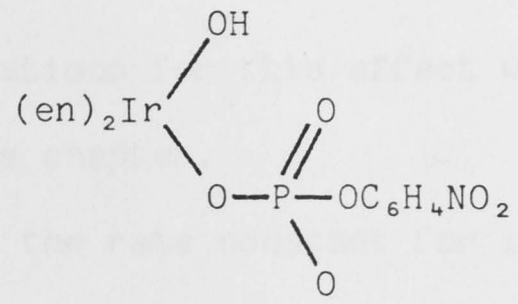


Figure 5.3

pH Versus Rate Profile for the Initial Step of the Reaction of cis-$[(en)_2Ir(OH_2)(BNPP)]^{2+}$ at 25°C, μ = 1.0 M ($NaClO_4$), the solid line was calculated using a pK_a of 7.06 and a k_m of 3.95×10^{-4} s^{-1}.

The rate of reaction above pH 9.5 becomes independent of pH with a rate constant, $k_m = (3.95 \pm 0.05) \times 10^{-4}$ s^{-1} at 25°C. The pK_a of the aqua ligand was virtually identical to the pK_a of the aqua ligand in the complex, cis-$[(en)_2Ir(OH_2)(ENPP)]^{2+}$, **1**. The rate constant k_m refers to the attack of the hydroxo ligand on the phosphorus centre. At higher pH the rate increases with both first and second order dependence on $[OH^-]$. The rate constants k_1 and k_2 were calculated to be $(2.0 \pm 0.3) \times 10^{-2}$ $lmol^{-1}s^{-1}$ and $(6.0 \pm 0.3) \times 10^{-2}$ $l^2mol^{-2}s^{-1}$ respectively.

The reaction of cis-$[(en)_2Ir(OH_2)(BNPP)]^{2+}$, **3**, was also followed by ^{31}P NMR spectroscopy. At pH 9.5 in Ches/OH^- buffer, the



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starting material reacts to yield a product with a chemical shift of + 7.2 ppm with a rate of (6 ± 1) x 10^{-4} s^{-1}, similar to the rate constants for release of NP at that pH. This is the chemical shift expected for a monodentate 4-nitrophenylphosphate, and the logical assignment of this signal is as the complex, 4.

^{31}P NMR spectra recorded throughout the reaction at higher pH, [OH-] = 0.025, 0.1 and 1.0 M, 25°C, μ = 1.0 M (NaCl), revealed that in the initial step a single product was formed from the starting material at -6.0 ppm. The product had a chemical shift of + 7.1 ppm and was coincident with the signal of the product of the reaction at pH 9.5.

The intramolecular reaction again apparently yields only monodentate phosphate ester, like the previous reaction the ring opening reaction occurs very rapidly as the chelate ester is formed. The reaction almost certainly proceeds by P-O bond cleavage analogously to the reaction of the ethylphosphate chelate derived from cis-$[(en)_2Ir(OH_2)ENPP]^{2+}$.

The hydroxide dependent reaction at high pH, produced only monodentate 4-nitrophenylphosphate. As observed with cis-$[(en)_2Ir(OH_2)(ENPP)]^{2+}$, **1**, the hydroxide dependent reaction had rate constants of the order of 10-20 fold greater than those found for the rate of intermolecular attack of hydroxide ion on the phosphorus centre of the analogous pentaammine complex, $[(NH_3)_5IrBNPP]^{2+}$ in this case.

Several possible explanations for this effect were proposed in the previous section of this chapter.

An estimate of the rate constant for intermolecular attack of the Ir(III)-OH^- nucleophile on coordinated BNPP using arguments presented in the previous section of this chapter is 5×10^{-7} $lmol^{-1}s^{-1}$. This yields an EM for the reaction of $\sim 10^3$ mol l^{-1}, supporting the argument that the reaction proceeds via intramolecular attack of coordinated hydroxide.[10]

The second step of the reaction was followed spectrophotometrically at 400 nm, ie by observing the release of nitrophenolate, (NP), from the complex. The reaction was very slow at

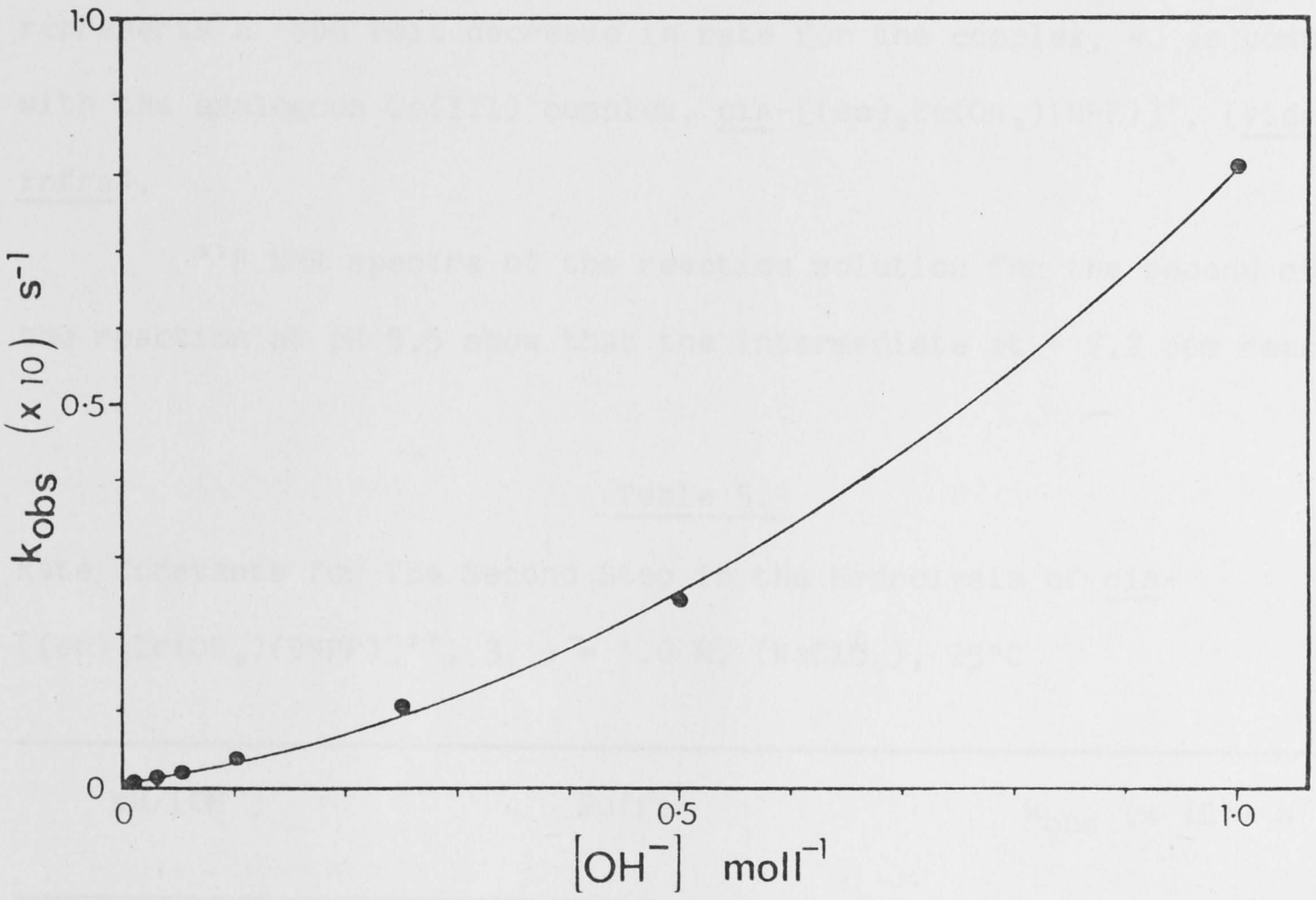


Figure 5.4

k_{obs} versus $[OH^-]$ for the Initial Step in the Reaction of cis-$[(en)_2Ir(OH_2)(BNPP)]^{2+}$, at 25°C, μ = 1.0 M ($NaClO_4$), solid line is calculated for the equation;

$k_{obs} = 4.95 \times 10^{-4} + 2.0 \times 10^{-2}[OH-] + 6.0 \times 10^{-2}[OH-]^2$.

25°C and therefore was conducted at 40°C. The rate of release of NP from the intermediate complex was independent of pH from pH 8.7 up to an hydroxide concentration of 0.1 M. The yield of NP in this second step was identical to the yield of NP from the first step. These observations are what would be expected for the reaction of **4**. The reaction again presumably goes via coordinated OH^- attack at the phosphorus centre to yield the chelated phosphorane which rapidly decays to the chelate phosphate. The intramolecular attack of the coordinated OH^- on the phosphorus centre proceeds with a rate constant of ~8 x 10^{-6} s^{-1} at 40°C. If the activation parameters for the intramolecular reaction are similar to those for cis-$[(en)_2Ir(OH_2)(ENPP)]^{2+}$, **1**, the rate constant for the reaction at 25°C would be ~2 x 10^{-6} s^{-1}. This represents a ~500 fold decrease in rate for the complex, **4**, as compared with the analogous Co(III) complex, cis-$[(en)_2Co(OH_2)(NPP)]^+$, (*vide infra*).

^{31}P NMR spectra of the reaction solution for the second step of the reaction at pH 9.5 show that the intermediate at + 7.2 ppm reacts to

Table 5.3

Rate Constants for The Second Step in the Hydrolysis of cis-$[(en)_2Ir(OH_2)(BNPP)]^{2+}$, **3**, μ = 1.0 M, ($NaClO_4$), 25°C

pH/$[OH^-]$	Buffer	k_{obs} (x 10^6) s^{-1}
8.67	Tris	7.4 ± 0.1
0.01	NaOH	8.3 ± 0.1
0.10	NaOH	7.5 ± 0.04

yield a single product with a chemical shift of ~15 ppm. This product has the chemical shift expected for the coordinated monodentate phosphate. Thus even at pH 9.5 the $[(en)_2IrO_2PO_2]$ complex is not stable and ring opens, unlike the corresponding Co(III) complex which at this pH is predominantly in the form of the chelate.[6]

A study of the hydrolysis of the free ligand in the pH region 1-10 has been carried out by Kirby and Younas.[20] The reaction rate goes through a minimum at ~pH 4, at pH 8 the rate constant for loss of NP was ~8 x 10^{-7} s^{-1}, 100°C, μ = 1.0 M (KCl). Unfortunately the activation parameters for the reaction have not been measured, however if they are similar to those for the reaction of bis(2,4-dinitrophenol)phosphate,[20] the rate constant at 25°C would be ~10^{-9} s^{-1}. Thus at pH 8, NP release from BNPP is enhanced ~10^6 fold by coordination to Ir(III) in the complex, cis-$[(en)_2Ir(OH_2)(BNPP)]^{2+}$.

5.4 General Discussion

Coordination of ENPP and BNPP to the Ir(III) centre cis to a hydroxo ligand results in an enhanced rate of cleavage of these esters at moderate pH. At pH 8 the rate of release of nitrophenolate from these esters is enhanced ~10^6 fold upon coordination in the complexes, cis-$[(en)_2Ir(OH_2)ENPP]^{2+}$ and cis-$[(en)_2Ir(OH_2)BNPP]^{2+}$. The reaction proceeds by attack of the cis-coordinated hydroxide ion and the large rate enhancements are attributed to the intramolecular nature of the reaction, (vide supra).

The anticipated product of the reaction, the chelated phosphate monoester was not observed, instead, the product was the cis-hydroxo monodentate phosphate ester complex. The reaction to yield the monodentate ester complex from the intermediate chelate ester complex

proceeded with P-O cleavage. The phosphorane intermediate for this ring opening reaction was apparently incapable of any ligand reorganizational process which would result in the placing of the ester group in an axial position in the phosphorane, (vide infra, section 7.1.4). This conclusion was drawn from the observation that ester cleavage does not occur concurrently with the ring opening of the chelate.

It is of interest to note that the intramolecular reactions described in this chapter appear to proceed much more slowly than would have been predicted by comparison with the rate of hydrolysis of cis-$[(en)_2Co(OH)(NPP)]^+$.[21] In the pH region 9-12 the hydrolysis of $[(en)_2Co(OH)(NPP)]^+$ is independent of pH and proceeds by attack of the cis coordinated hydroxide at a rate of ~8 x 10^{-4} s^{-1} at 25°C.[21] ENPP is much more susceptible to nucleophilic attack than NPP;[17] attack of OH^- on the free ligand proceeds about 10^2 fold faster, and the intramolecular attack of amido ion proceeds about 10^3 fold faster when the phosphate esters are coordinated to the $[(NH_3)_5Co-]^{3+}$ moiety.[17] Attack of cis coordinated hydroxide on coordinated ENPP was therefore expected to proceed with a rate constant at least 10^2 fold greater than 10^{-3} s^{-1}. In contrast to this simplistic expectation the rate observed was only 4.6 x 10^{-5} s^{-1} at 25°C, a **decrease** of ~20 fold over the rate observed for the Co(III) NPP complex. In addition, the complex cis-$[(en)_2Ir(OH)(NPP)]$, produced as an intermediate in the hydrolysis of cis-$[(en)_2Ir(OH_2)(BNPP)]^{2+}$, reacted via the intramolecular pathway. At 25°C the rate constant for the reaction was estimated to be ~2 x 10^{-6} s^{-1}. This is a decrease of ~500 fold upon going from Co(III) to Ir(III).

Thus the reactions proceed at ~10^3 fold slower than expected. The reason for this decrease cannot lie in a difference in the degree of activation of the phosphate ester by the different metal ions since this

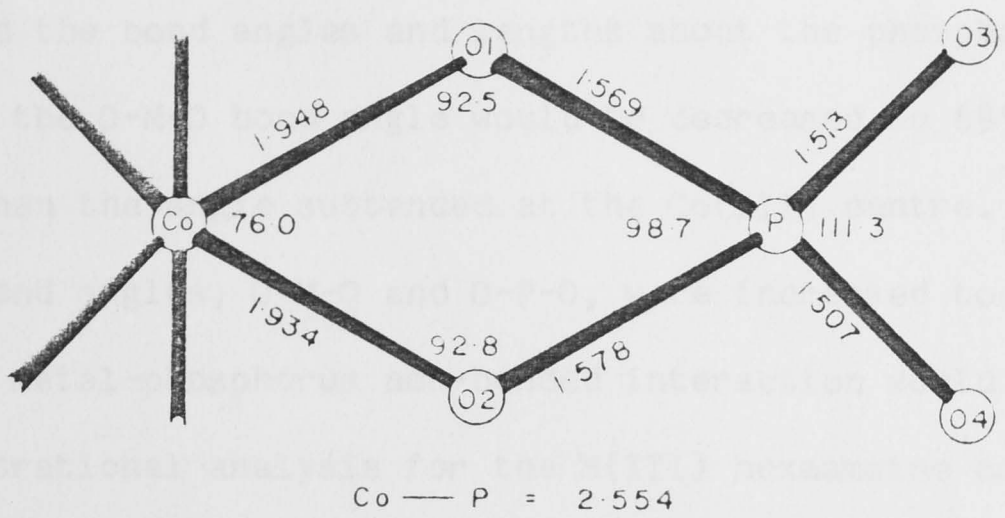


Figure 5.5

The Bond Lengths and Angles in the Chelate Phosphate Ring in the Complex $[(en)_2CoO_2PO_2]^3$ (bond lengths in A)

effect was shown to be small in Chapter 2. The reason for this phenomenon most likely lies in the fact that the two metal ions are quite different in size. The effective ionic radii of the two ions are 0.545 A and 0.68 A for Co(III) and Ir(III) respectively.[22] This large difference in size must have an effect on the energy required to form the necessary four membered ring. X-ray crystal structural analyses of the complexes $[(en)_2CoO_2PO_2]^3$ and $[(NH_3)_4CoO_2PO_2]^{23}$ have been performed. The in-ring bond angles and lengths were almost identical for both complexes. In both cases, the four membered ring containing the Co(III) and the phosphorus atoms was extremely strained. This was ascribed to bond angle strain and the proximity of the cobalt and the phosphorus atoms,[3] (Figure 5.5). In the case of the bis ethylenediamine complex, the non-bonded interatomic distance between the Co and P is only 2.554 A, which is close to the sum of the covalent radii of the two atoms involved. The larger Ir(III) ion must cause further strain in this ring. Simple geometric considerations dictate that if the metal-O bond lengths were increased to 2.1 A, a reasonable estimate for the Ir-O

bond,[24] and the bond angles and lengths about the phosphorus centre were invariant, the O-M-O bond angle would be decreased to 69°, much more strained than the angle subtended at the Co(III) centre. If these critical bond angles, O-M-O and O-P-O, were increased to relieve the strain the metal-phosphorus non-bonded interaction would increase.

Vibrational analysis for the M(III) hexaammine complexes, M = Co and Ir, suggest that the intrinsic resistance to compression of the N-M-N bond angle, ie the portion of the resistance that is due to orbital overlap and electron exchange factors, should be greater for Ir than Co.[25] This result probably also applies to similar systems having the O-M-O bond angle. From an inspection of molecular geometries it has been anticipated that the O-Ir-O bond angle may need to compress to nearly 70° to achieve a reasonable transition state geometry for the reaction involving the intramolecular attack of hydroxide ion on a coordinated phosphate derivative, (vide supra). In contrast to this expectation a suitable transition state geometry for the corresponding Co(III) complex would be closer to 78-80°, based on the geometry in the product ground state.[3,23] Large energy increases per unit angle compression are expected when the O-M-O bond is required to to achieve such a configuration. The more compression required in the case of the Ir(III) complex is therefore expected to markedly increase the energy required to reach the transition state in this reaction.[25]

References, Chapter 5.

1. Westheimer, F.H., Acc. Chem. Res., **1**, 70-78, (1967), and references therin.

2. Cox, J.R. and Ramsay, O.B., Chem. Rev., **64**, 317-352, (1964), and references therein.

3. Anderson, B., Milburn, R.M., Harrowfield, J. MacB., Robertson, G.B. and Sargeson, A.M., J. Am. Chem. Soc., **99**, 2652-2661, (1977)

4. Cooperman, B.S., in "Metal ions in Biological Systems", Sigel, H., (Ed.), Marcel Dekker, New York, 1976, vol **5**, pp 80-125.

5. Farrell, F.J., Kjellstrom, W.A. and Spiro, T.G., Science, **164**, 320-321, (1969).

6. Lincoln, S.F. and Stranks, D.R., Aust. J. Chem., **21**, 37-56 and 56-65, (1968). Woodside, A., Unpublished observations.

7. Hay, R.W. and Bembi, R., Inorg. Chim. Acta, **78**, 143-149, (1983).

8. Jones, D.R., Lindoy, L.F. and Sargeson, A.M., J. Am. Chem. Soc., **106**, 7807-7819, (1984).

9. The pK_a of $[(NH_3)_5IrOH_2]^{3+}$ is 6.1 and the reduction in charge for the complex cis-$[(en)_2Ir(OH_2)ENPP]^{2+}$, should increase the pK_a. Palmer, J.W. and Basolo, F., J. Inorg. Nucl. Chem., **15**, 279-286, (1960).

10. Kirby, A.J., in " Adv. in Phys. Org. Chem." **17**, 183-278, (1980).

11. Bell, R.P., "The Proton in Chemistry", 2nd Edition, Cornell University Press, New York, 1972, pp 92.

12. Kirby, A.J. and Younas, M., J. Chem. Soc. (B), **1970**, 1166-1172.

13. Khan, S.A. and Kirby, A.J., J. Chem. Soc. (B), **1970**, 1172-1182.

14. Sammons, R.D., Frey, P.A., Bruzik, K. and Tsai, M-D., J. Am. Chem. Soc., **105**, 5455-5461, (1983).

15. Gamsjager, H. and Murman, R.K., in "Adv. Inorg. Bioinorg. Mech.", Sykes, A.G., (Ed.), Academic press, New York, 1984, pp. 338-339.

16. Frey, P.A., Tetrahedron, **38**, 1541-1567, (1982).

17. Harrowfield, J.MacB., Jones, D.R., Lindoy, L.F. and Sargeson, A.M., J. Am. Chem. Soc., **102**, 7733-7741, (1980).

18. Sargeson, A.M., Pure Appl. Chem., **50**, 905-913, (1978).

19. Boreham, C., Buckingham, D.A. and Keene, F.R., J. Am. Chem. Soc., **101**, 1409-1421, (1979).

20. Kirby, A.J. and Younas, M., J. Chem. Soc. (B), **1970**, 510-513.

21. Jones, D.R., Lindoy, L.F. and Sargeson, A.M., J. Am. Chem. Soc., **105**, 7327-7336, (1983).

22. Shannon, R.D., Acta Cryst., A32, 751-767, (1976).

23. Haromy, T.P., Knight, W.B., Dunaway-Mariano, D. and Sundaralingam, M., Biochem., **21**, 6950-6956, (1982).

24. Ir-N bond lengths have been estimated to be ~2.1 Å, Borch, G., Klaeboe, P. and Nielsen, P.H., Acta Chem. Scand., **A33**, 19-29, (1979). The Co-O and Co-N bond lengths in the complex, $[(en)_2CoO_2PO_2]$ are very similar, therefore the bond length for the Ir(III)-O bond was estimated to be 2.1 Å.

25. Geue, R.J., Personal communication.

CHAPTER 6

POLYPHOSPHATE HYDROLYSIS

6.1 Introduction

The hydrolysis of phosphoanhydride bonds is a major source of chemical energy in biological systems and is mediated by a large number of enzymes, most of which require metal ions as essential cofactors.[1] The rapid enzymic hydrolysis of phosphoanhydride bonds contrasts with the extremely slow hydrolysis of these bonds under aqueous laboratory conditions, rate differences of the order of 10^{10} are observed typically. For example, the hydrolysis of pyrophosphate by Yeast Inorganic Pyrophosphatase, (YIP), occurs about 10^{11} fold faster than hydrolysis in the absence of the enzyme.[2] One role of the metal ion in the enzymic hydrolysis of polyphosphates seems to be to coordinate to the polyphosphate to form the substrate. (vide supra, section 1.3 and 1.4) In some enzymes there is a proven requirement for multiple metal ions,[3,4,5] the roles of which are not well understood.

The non-enzymic hydrolysis of polyphosphates is catalysed by metal ions, with di- and tri-valent metal ions being the most effective reagents[6,7] although monovalent ions also affect the reaction rate.[8] The mechanism by which such metal ions effect the rate is difficult to ascertain because the ions are generally substitutionally labile; there are numerous complex species in solution and it is difficult to ascribe the observed rate enhancement to any particular complex, ie to identify the most effective intermediate and or separate the equilibria and or rate constants.

However, by the use of specific types of metal complex ions it is

possible to dramatically enhance the rate of polyphosphate cleavage.[9,10,11,12,13] The semi-inert nature of the complex ions utilized has allowed assertions to be made about the mechanism of hydrolysis. These metal complex ions are generally cobalt (III) ions which have a poly-amine poly-aqua donor set, the amines being chosen for their ability to labilize the aqua ligands. By the use of such reagents it has been possible to enhance the rate of hydrolysis of pyrophosphate,[9,10] triphosphate,[11] ATP[12,14] and ADP[13] by up to 10^6 fold. Experiments to date indicate that more than one, and up to three moles of these reagents, per mole of anhydride bond to be cleaved, are required for efficient hydrolysis. The mode by which these reagents hydrolyse the phosphoanhydride bonds is generally accepted to be a combination of effects including charge neutralization, electron density polarization and the provision of an appropriately placed nucleophile, usually a metal bound hydroxide. Also, a report of the hydrolysis of pyro- and tri-phosphate by the complex ion $[Pt(NH_3)_2(OH_2)_2]^{2+}$ was published.[15] This reagent increases the rate of hydrolysis of condensed phosphates by ~100 fold at pH 4. The proposed mechanism for this reaction was similar to that of the Co(III) complex ion mediated reactions mentioned previously.

This chapter describes the efficient hydrolysis of a substitutionally inert tridentate triphosphate complex, $[tacnCoP_3O_{10}]^{2-}$, (tacn = 1,4,7-triazacyclononane), mediated by the Co(III) reagent, $[(tn)_2Co(OH)(OH_2)]^{2+}$, (tn = 1,3-propanediamine), and outlines aspects of the mechanism of the process.

An account of some of this work has appeared[15a], the X-ray crystal structure analysis described herein was performed by T.W. Hambley.

6.2 Hydrolysis of Triphosphate.

6.2.1 Experimental.

Electronic spectra were recorded with a Hewlett Packard 8450A spectrophotometer. Solutions were buffered with a 0.4 M Pipes/0.2 M NaOH buffer pH 7.3. Pipes was used as supplied by Calbiochem.

[tacnCo(OSO_2CF_3)$_3$]

[tacnCoCl_3] (1.6 g) was dissolved in anhydrous CF_3SO_3H and heated to 100°C for 2 hr. The solution was cautiously added to stirring ether (250 ml), filtered, and the purple solid resuspended in warm chloroform. The suspension was filtered and the solid washed with ether and dried *in vacuo*. Yield 3.5 g.

[tacnCo$P_3O_{10}H_2$], (1).

[tacnCo(OSO_2CF_3)$_3$] (1.0 g) was dissolved in dilute NaOH (25 ml of 0.1 M) then neutralized with concentrated $HClO_4$. $Na_5P_3O_{10}$ (2.0 g) was added and the pH adjusted to ~4 with concentrated $HClO_4$ and the solution heated to ~80°C for 15 minutes. The pH was then adjusted to ~1 with $HClO_4$, the volume reduced to ~10 ml and the solution cooled to 4°C for 16 hr. A purple crystalline solid was collected, washed twice with cold dilute $HClO_4$ and dried in vacuo. Yield 0.40 g. Analysis calculated for $C_6H_{17}N_3CoO_{10}P_3$; C, 16.27; H, 3.87; N, 9.48; Co, 13.30; P, 20.97. Found, C, 16.4; H, 3.9; N, 9.4; Co, 13.1; P, 20.9. ^{1}H NMR. (D_2O, 0.1 M $DClO_4$); 2.73 (br) (6H), 3.25 (br) (6H), 6.77 (br) (2H), 7.34 (br) (1H). ^{31}P NMR. (H_2O/D_2O, pH 7); +3.8 (d) J = 16.5 Hz (2P), -6.3 (tr) J = 16.5 Hz (1P). ε^{max}_{381} = 50 $lmol^{-1}cm^{-1}$, ε^{max}_{542} = 135 $lmol^{-1}cm^{-1}$.

[(tn)$_2$Co(OH)(OH_2)](ClO_4)$_2$ (2) was synthesized analytically pure by a published procedure.[16] (tn = 1,3-diaminopropane)

The hydrolytic reaction involving (1) and (2) was followed at pH 7.3 by several methods. The first was simply to conduct the reaction in

the ^{31}P NMR probe. Integrated spectra were recorded and the change in the relative intensities of the peaks with time noted. To obtain rate constants from this method it was necessary to assign all the peaks observed in the spectra to their respective class, ie due to triphosphate, pyrophosphate or monophosphate containing species. Assignment of the spectra was aided by the observation that ^{31}P NMR chemical shifts for phosphate derivatives seem to obey simple additivity rules, (See section 1.8). The intensity due to each class of phosphate was plotted against time.

The second method for following the reaction was to quench an aliquot from the reaction at various time intervals and determine the triphosphate, pyrophosphate and monophosphate in the quenched sample. The reaction was quenched by the addition of an excess of CN^- and a trace of Co(II). For a typical quenching experiment where the initial concentration of (1) was 0.025 M and (2) was 0.05 M, KCN (~1g) and $Co(ClO_4)_2 \cdot 6H_2O$ (~1 mg) were added to a 1 ml aliquot of the reaction mixture at the required time. The mixture was shaken for about 30 s and filtered into a 10 mm NMR tube. The solids were washed with D_2O (~ 1 ml), the washings added to the NMR tube and a ^{31}P NMR spectrum accumulated. (Acquisition parameters; acquisition frequency, 24.21 MHz, sweep width 5 KHz, acquisition time 0.4 s, pulse rate 1.0 s^{-1}). The spectra show phosphate (+ 4 ppm) and the β phosphorus of triphosphate (-19 ppm) well separated, however the pyrophosphate and the α and γ phosphorus resonance of triphosphate (~ -6 ppm) were not resolved. This problem was resolved by integrating the 3 regions of the spectrum and subtracting twice the intensity of the -19 ppm region from the -6 ppm region, the residual intensity was therefore due to pyrophosphate. Changing the pulse delay did not appreciably alter the relative intensities of the three phosphate types and therefore it was assumed

that the phosphorus nuclei of all the phosphate types were fully relaxed in the pulse repetition time, or that the various types relaxed with a similar rate. In later experiments a standard, triethylphosphate which was unaffected by the quenching procedure, was added to the reaction mixture. The ratio of each phosphate type to the standard was then calculated. The rate constants for the reaction were calculated by plotting the log of the ratio of triphosphate to standard versus time. The plots were linear with the slope equal to the rate constant. This procedure eliminated any errors due to differences in the relaxation rates of the various phosphates and this method gave the same result as the method without added standard. The quenching procedure did not result in the hydrolysis of phosphoanhydride bonds, as shown by the appearance of only triphosphate, and pyrophosphate, respectively when fresh samples of (1) and $[(NH_3)_4CoP_2O_7]^-$ were reduced by this method. The precipitate that arose during the quenching procedure was redissolved in a 0.5 M NaOH solution and a ^{31}P NMR spectrum of that solution was collected, no phosphorus resonance was observed after 5 times the usual accumulation time.

Kinetics by visible absorption spectroscopy were performed using a 1 mm path length cell and a Hewlett Packard HP 8450A spectrophotometer with a thermostatted cell holder. The concentration of **1** used in these experiments was 0.025 M.

6.2.2 Results and Discussion

The complex, $[tacnCoP_3O_{10}]^{2-}$, **1**, was synthesized by heating an aqueous solution of $[tacnCo(OH_2)_3]$ and $P_3O_{10}H_2^{3-}$ and crystallized by reducing the volume and acidifying the solution to protonate and precipitate the complex.

The solid state structure of **1** has been determined by X-ray crystallography. The small size of the macrocyclic ring obligates the

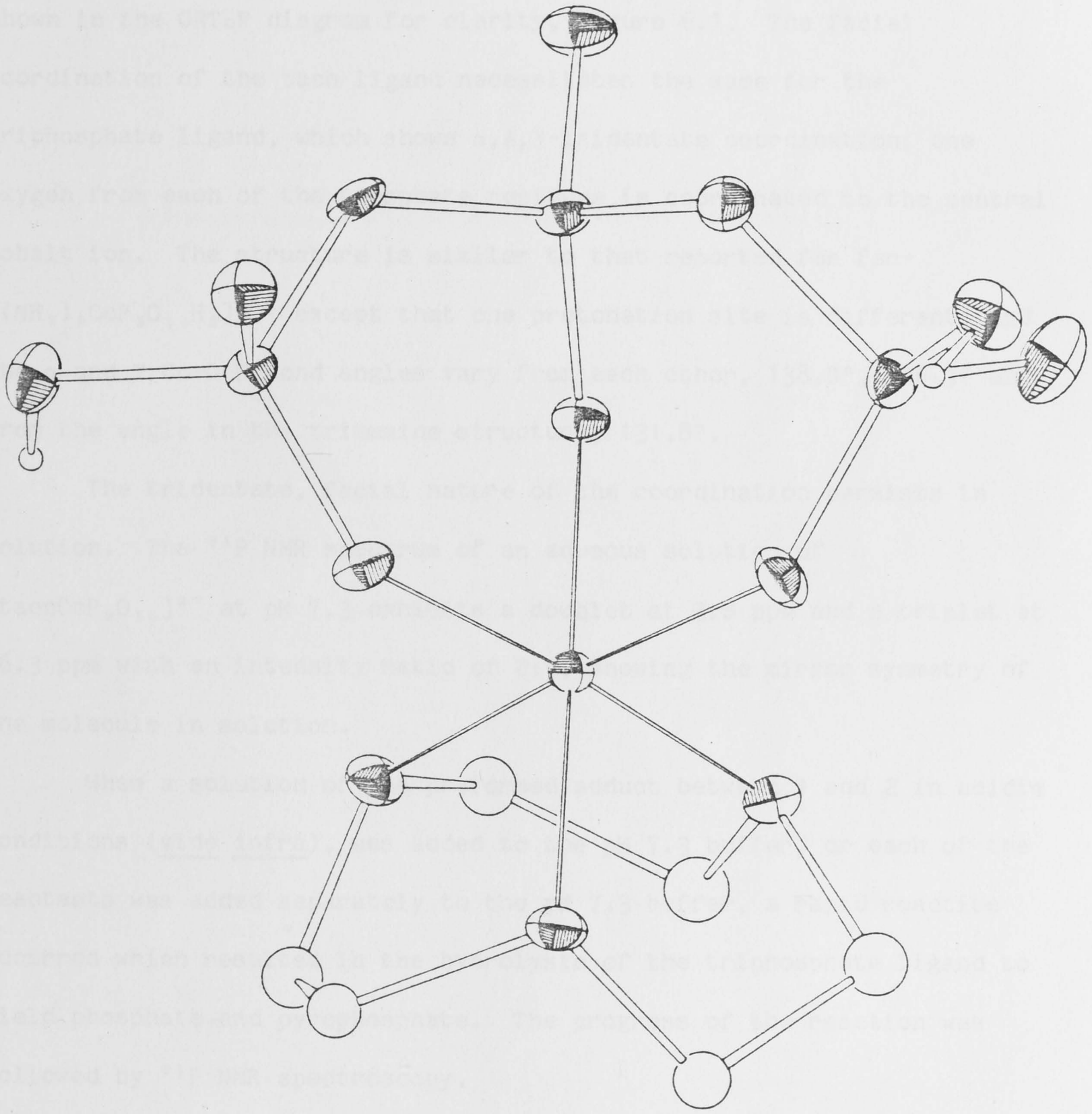


Figure 6.1

Molecular Structure of $[tacnCoP_3O_{10}H_2]\cdot 2H_2O$, **1**.

ring to coordinate facially. The carbon atoms of the tacn ligand were disordered 52:48% ± 2 over two sites, only one of the conformations is shown in the ORTEP diagram for clarity, Figure 6.1. The facial coordination of the tacn ligand necessitates the same for the triphosphate ligand, which shows α,β,γ-tridentate coordination; one oxygen from each of the phosphate residues is coordinated to the central cobalt ion. The structure is similar to that reported for fac-$[(NH_3)_3CoP_3O_{10}H_2]$,[17] except that one protonation site is different, and the α and γ Co-O-P bond angles vary from each other, 138.0°, 126.3° and from the angle in the triammine structure, 131.8°.

The tridentate, facial nature of the coordination persists in solution. The ^{31}P NMR spectrum of an aqueous solution of $[tacnCoP_3O_{10}]^{2-}$ at pH 7.3 exhibits a doublet at 3.8 ppm and a triplet at -6.3 ppm with an intensity ratio of 2:1, showing the mirror symmetry of the molecule in solution.

When a solution of the preformed adduct between **1** and **2** in acidic conditions (vide infra), was added to the pH 7.3 buffer, or each of the reactants was added separately to the pH 7.3 buffer, a rapid reaction occurred which resulted in the hydrolysis of the triphosphate ligand to yield phosphate and pyrophosphate. The progress of the reaction was followed by ^{31}P NMR spectroscopy.

The interpretation of the complex ^{31}P NMR spectra obtained when $[tacnCoP_3O_{10}]^{2-}$, **1**, and $[(tn)_2Co(OH)(OH_2)]^{2+}$, **2**, are mixed at pH 7.3, (as in Figure 6.2), was aided by applying additivity rules which have been empirically derived by this group[10,18] and others.[11,19] These rules consist of a series of approximate Δδ values to be added to the known chemical shift of a particular phosphate moiety following addition of a metal ion, proton or PO_3^- residue to obtain the chemical shift of the derivative. Some of these Δδ values are: Co^{3+} + (6-8), H^+ - (1-3);

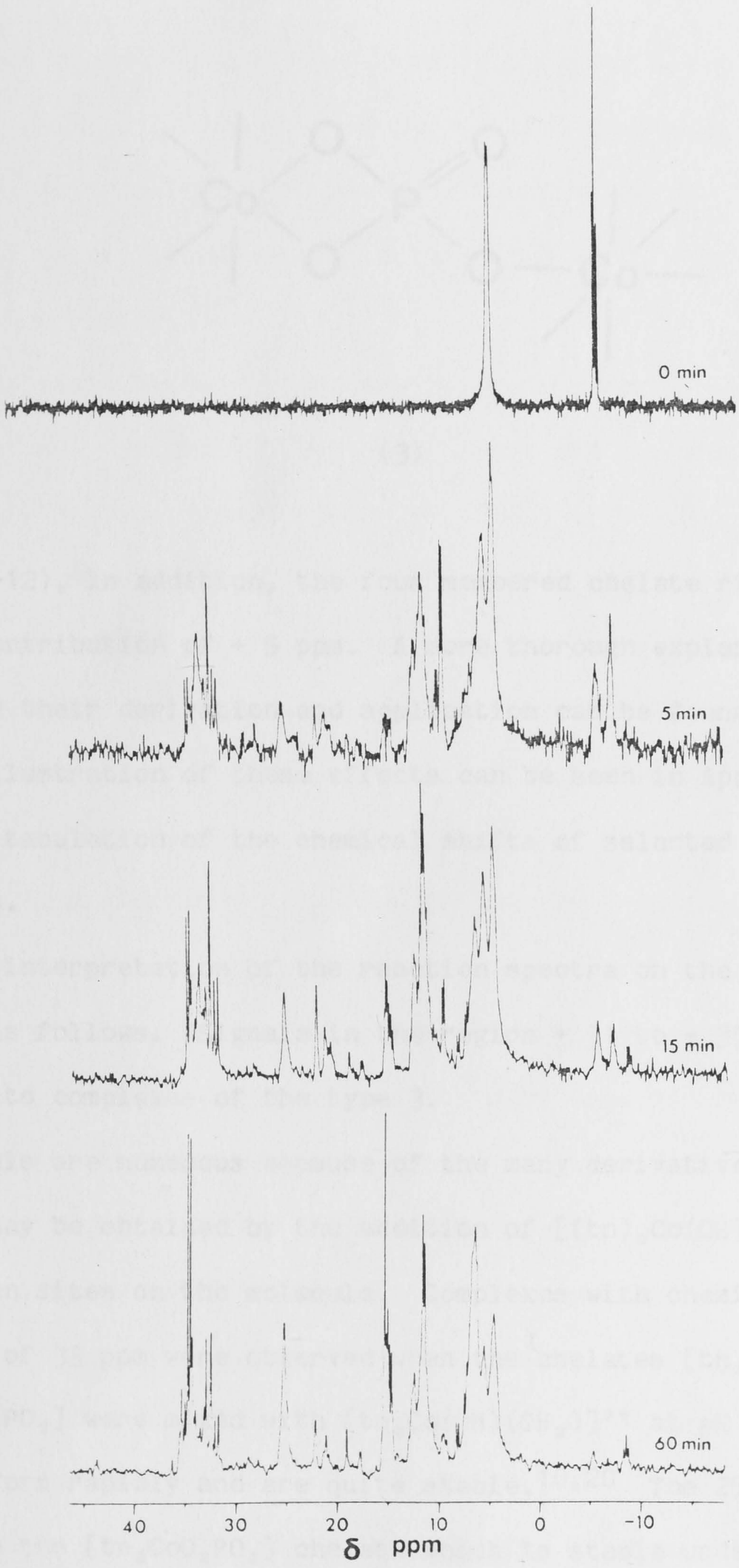
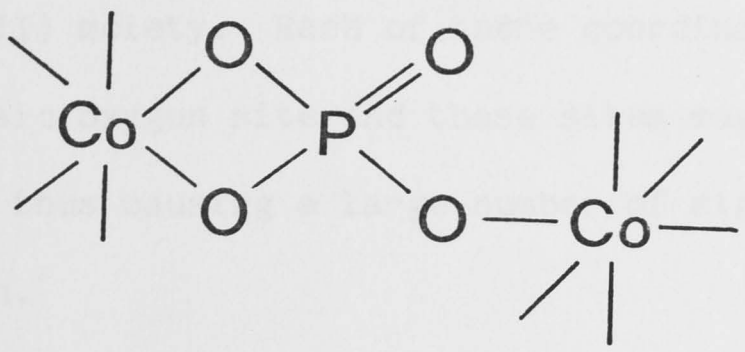


Figure 6.2.

Successive 80.98 MHz ^{31}P NMR spectra of $[tacnCoP_3O_{10}]^{2-}$, 1, (0.05M) reacting with $[(tn)_2Co(OH)(OH_2)]^{2+}$, 2, (0.10 M) at pH 7.3, 20°C.



(3)

PO_3^- - (11-12), in addition, the four membered chelate ring requires an addition contribution of + 5 ppm. A more thorough explanation of these effects and their derivation and application can be found in section 1.8. An illustration of these effects can be seen in Appendix 1 which contains a tabulation of the chemical shifts of selected phosphate derivatives.

The interpretation of the reaction spectra on the basis of these rules was as follows. Signals in the region + 31 to + 35 ppm are attributed to complexes of the type 3.

These signals are numerous because of the many derivatives of this basic unit that may be obtained by the addition of $[(tn)_2Co(OH)(OH_2)]^{2+}$ to any basic oxygen sites on the molecule. Complexes with chemical shifts in the region of 33 ppm were observed when the chelates $[tn_2CoO_2PO_2]$ or $[(NH_3)_4CoO_2PO_2]$ were mixed with $[tn_2Co(OH)(OH_2)]^{2+}$ at pH 7.3. These complexes form rapidly and are quite stable.[10,20] The 25 ppm signal is ascribed to the $[tn_2CoO_2PO_2]$ chelate which is stable under these conditions.[16,21] This fragment has obviously been completely cleaved from the $[tacnCo]^{3+}$ moiety. Independent synthesis of this complex confirms the assignment. Signals in the region + 4 to + 15 ppm are numerous and are not easily assigned individually. However, it is possible to say with some certainty that the signals are due to

pyrophosphate and the α and γ residues of triphosphate coordinated at least once to a Co(III) moiety. Each of these coordinated residues possesses another basic oxygen site and these sites may be coordinated to a Co(III) moiety, thus causing a large number of signals in the chemical shift region.

The signals below - 5 ppm are due to the coordinated β phosphorus of triphosphate. The only other possibility for the assignment of these signals would be free pyrophosphate or free α or γ residues of triphosphate. The possibility that the signal was due to uncoordinated pyro- or triphosphate was considered to be remote given the stability of the six membered rings formed by α,β chelation of these ligands by $[tn_2Co(OH)(OH_2)]^{2+}$. The simplest method of determining the rate constants from these complex spectra was simply by plotting the loss of the -5 to -8 ppm signals versus time in the usual way, log % triphosphate remaining versus time.

An alternative method for determining the extent of the reaction was also developed. The substitionally inert Co(III) complexes were destroyed by the addition of an excess of CN^- and a trace of Co(II). The catalytic amount of $[Co(CN)_6]^{4-}$ thus formed is a potent reductant for Co(III) complexes. The reduction of the inert Co(III) complex ions to the much more labile Co(II) species releases the various phosphate species into solution and their relative concentrations were determined by ^{31}P NMR. The net concentration of Co(III) in the solution does not alter significantly and is trapped as $[Co(CN)_6]^{3-}$. Both methods gave comparable results.

The tridentate coordinated triphosphate complex, **1**, was hydrolysed to coordinated phosphate and pyrophosphate in the presence of $[(tn)_2Co(OH)(OH_2)]^{2+}$ with an apparent first order rate constant of ~4 x 10^{-3} at 25°C and pH 7.3. The rate constant does not have a strong

dependance on the concentration of $[(tn)_2Co(OH)(OH_2)]^{2+}$, (Figure 6.3). The rate of hydrolysis of uncoordinated triphosphate at this pH is estimated to be about 1 x 10^{-8} s^{-1} at 25°.[8] Thus the action of the two metal ions increases the rate of hydrolysis of triphosphate by about 5 x 10^5 fold.

Whilst the rate constant for the reaction does not depend on the concentration of added $[tn_2Co(OH)(OH_2)]^{2+}$, the extent of the reaction does depend on the concentration of $[(tn)_2Co(OH)(OH_2)]^{2+}$, **2**, (Figure 6.3). In the absence of **2**, the reaction does not proceed; no hydrolysis of $[tacnCoP_3O_{10}]^{2-}$, **1**, was observed even after one week in solution at pH 7.3 and 25°C. In the presence of ~3 moles of **2**, the hydrolysis of

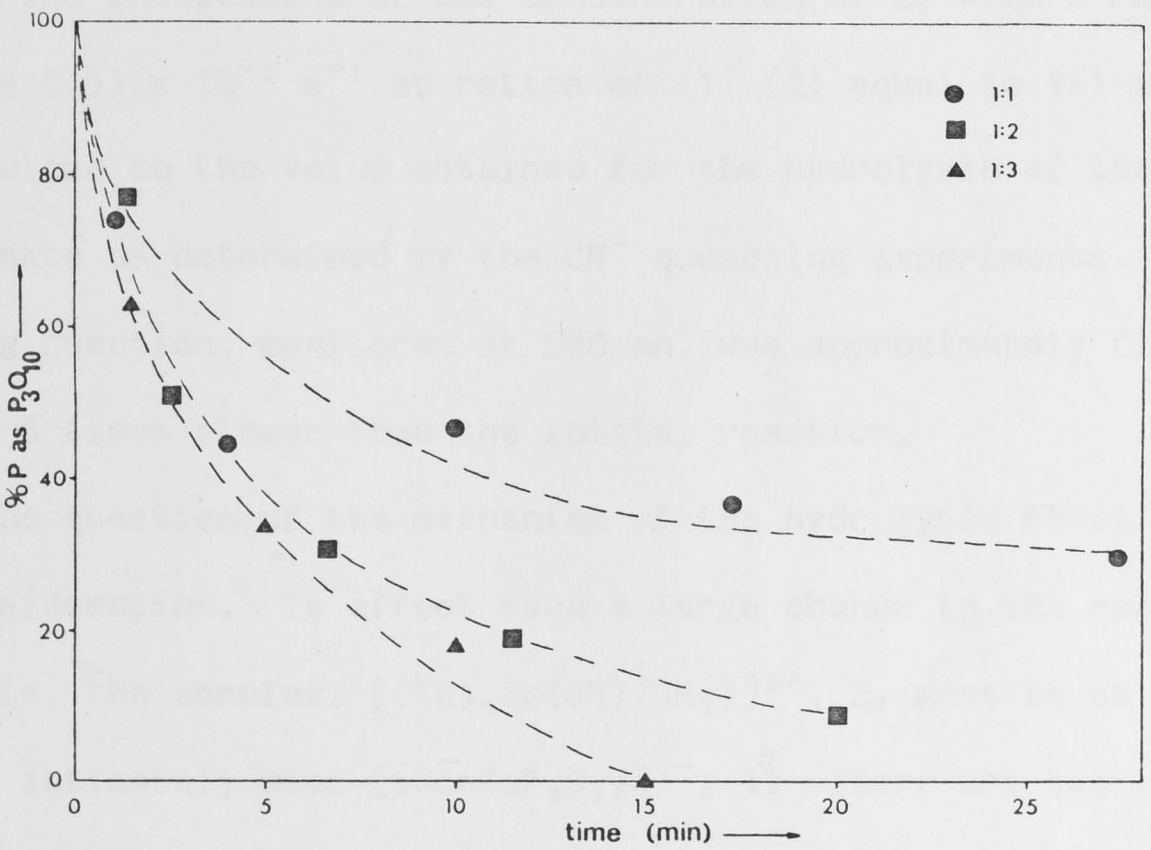


Figure 6.3.

Decrease in $P_3O_{10}^{5-}$ concentration with time, with varying ratios of 1:2. Relative $P_3O_{10}^{5-}$ concentrations were determined by ^{31}P NMR spectroscopy of cyanide quenched samples of the reaction mixture. Reaction conditions: $[tacnCoP_3O_{10}]^{2-}$ = 0.025 M, $[(tn)_2Co(OH)(OH_2)]^{2+}$ = 0.025, 0.050, 0.075 M, 25°C, pH = 7.3 (Pipes buffer 0.4 M)

the triphosphate goes to completion. However, with less than this ratio of 1:2, the reaction does not go to completion. The reason for this appears to be that hydrolysis of the triphosphate ligand results in the production of a number of basic oxygen sites which are able to compete effectively with the α and γ sites of the unreacted triphosphate complex for coordination of 2.

The reaction between 1 and 2 when followed by visible absorption spectroscopy proceeded in two consecutive steps. The slower of the two reactions has an isobestic point at 552 nm. When the reaction was monitored at pH 7.3, 25°C at this wavelength, the observed absorbance increase fitted well to a single exponential growth. The rate of this reaction was independent of the concentration of 2, with a rate constant of $(4.5 \pm 0.5) \times 10^{-3}$ s^{-1} at ratios of (1):(2) equal to 1:1 and 1:2. This is close to the value obtained for the hydrolysis of the triphosphate as determined by the CN^- quenching experiments. The following reaction, monitored at 580 nm, was approximately first order and was ~5 times slower than the initial reaction.

The question of the mechanism of the hydrolytic reaction needs some consideration. To effect such a large change in the rate of hydrolysis, the complex, $[(tn)_2Co(OH)(OH_2)]^{2+}$, 2, must be able to interact intimately with $[tacnCoP_3O_{10}]^{2-}$, 1. There are two identical basic oxygen sites on the coordinated triphosphate ion and it is a reasonable assumption that 2 will coordinate to these sites.

More information about the binding of 2 to 1 can be gleaned from the ^{31}P NMR spectra of the reaction in acidic conditions. At pH 4.6, 2 binds to the triphosphate complex but the subsequent hydrolytic reaction is very slow. Therefore at this pH the binding of the tn_2Co moiety to the triphosphate complex can be observed without the complication of the following reaction. When $[tacnCoP_3O_{10}]^{2-}$, 1, and $[tn_2Co(OH)(OH_2)]^{2+}$, 2,

were mixed at pH 4.6 a fairly slow change in the ^{31}P NMR was observed. A new set of signals was seen to grow into the spectrum at around 8-9 ppm at the expense of the signal at 3.8 ppm. Concomitant with this, was the loss of identity of the original doublet and triplet. These, which were originally sharp multiplets, change into broader more complex signals although the positions of the peaks does not alter much. This change in the NMR spectrum is interpreted as showing the coordination of the tn_2Co moiety to the α position of the coordinated triphosphate. The signals around 10 ppm are sharp doublets and they are numerous because the configuration about the α-P atom is now chiral as is the $[tn_2Co]$ moiety added. This results in diastereomers. These diastereomers are easily observed at the α phosphorus, ie the signals are distinct and sharp but the effect on the β and γ phosphorus atoms is to produce overlapping multiplets which appear as a broad single resonances.

It is possible, from the above observations to postulate a plausible mechanism for the hydrolytic reaction between $[tacnCoP_3O_{10}]^{2-}$ and $[tn_2Co(OH)(OH_2)]^{2+}$. The reaction between $[tacnCoP_3O_{10}]^{2-}$, 1, and $[tn_2Co(OH)(OH_2)]^{2+}$, 2, under acidic conditions showed that 2 appeared to bind to a uncoordinated basic oxygen on the α phosphorus of 1, under these conditions the hydrolytic reaction did not proceed. The interaction of 1 and 2 at pH 7.3 showed very complex changes in the ^{31}P NMR, (see Figure 6.2). The most easily followed change in the ^{31}P NMR spectra was the loss of the signals in the region of -5 to -8 ppm with time. These signals are due to the coordinated β phosphate residue, and their loss is a convenient way of following the loss of triphosphate.

The first spectrum after addition of 2, (time = 5 minutes, Figure 6.2), shows that most of the phosphorus resonances occur in two regions, + 4 to + 15 and +31 to +35. The signals in the first region include the adduct proposed previously; the triphosphate complex 1, with

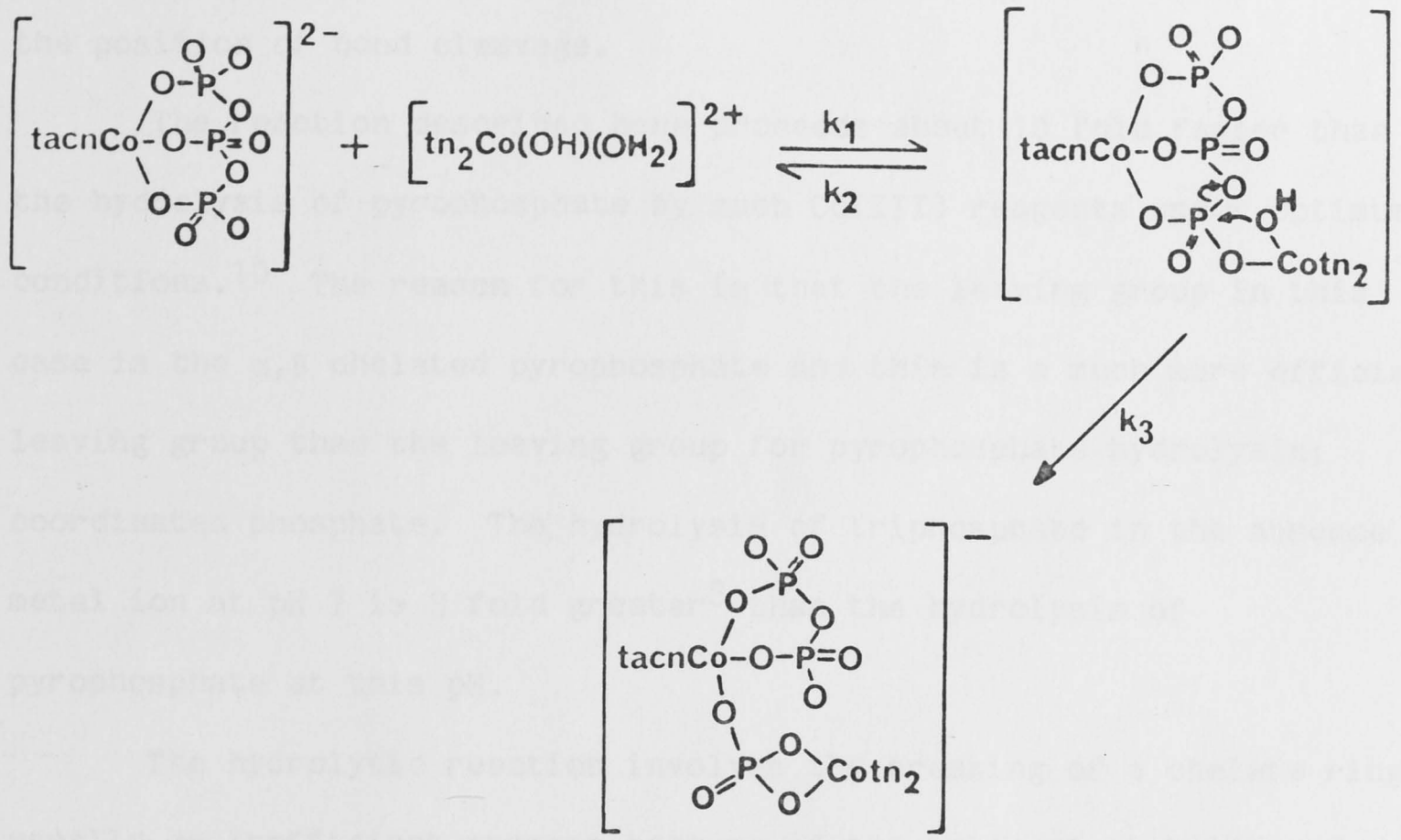
$[tn_2Co(OH)(OH_2)]^{2+}$, 2, coordinated to the α phosphate residue. This is most likely the reactive complex, poised to attack the α phosphorus atom with the coordinated hydroxo ligand. The ^{31}P NMR spectrum of such a reactive complex would consist of a pair of doublets in the area of +4 and +10 to +12 ppm, this is consistent with what was observed. This first region also displays signals due to the pyrophosphate containing products of the reaction. The signals in the second region, +31 to +35 ppm are ascribed to complexes of the type 3, (vide supra). The initially formed product of the reactive complex describe above, would be complexes containing a nucleus of the form of 3.

Over time two signals appear which seem to be secondary products, ie formed from the initial product. These signals appear at +15 and +25 ppm and are attributed to monodentate phosphate and chelate phosphate complexes respectively.

The visible absorption studies support the ^{31}P NMR kinetic studies, ie that the initial triphosphate hydrolysing reaction is first order with a rate independent of the concentration of $[tn_2Co(OH)(OH_2)]^{2+}$. This implies that the reaction between 1 and 2, (Scheme 6.1), occurs on a very rapid time scale compared with the hydrolytic reaction. This is not unreasonable given that the rate of water exchange in the complex, $[tn_2Co(OH)(OH_2)]^{2+}$ is of the order of 1.0 s^{-1} at 25°C.[16]

Scheme 6.1 shows the simplest plausible mechanism for the hydrolysis of $[tacnCoP_3O_{10}]^{2-}$ by $[tn_2Co(OH)(OH_2)]^{2+}$.

The observations made in this study have confirmed and extended many of the previously held notions regarding the hydrolysis of phosphoanhydride bonds by this type of Co(III) reagent. These notions are that, at least two and often three moles of metal complex ion per mole of polyphosphate are required for hydrolysis and a cis hydroxo group on one of the complex ions is essential for rapid hydrolysis.



Scheme 6.1

Possible Mechanism for the Hydrolysis of $[tacnCoP_3O_{10}]^{2-}$, 1, mediated by $[tn_2Co(OH)(OH_2)]^{2+}$, 2.

The reaction mechanism that has been proposed here (Scheme 6.1) is similar to that proposed for the hydrolysis of other polyphosphates by similar Co(III) reagents.[9,10,11,12] The novel aspects of this particular reaction was that only one mole of semi-labile Co(III) ion per mole of triphosphate was required for hydrolysis, and the addition of excess of this reagent did not appreciably enhance the rate of hydrolysis. The rationale for these observations, which are obviously related, is that tridentate coordination of the triphosphate highly activates the ligand and leaves only two identical possible coordination sites on the triphosphate. One of these sites will be quite distant from the position of the hydrolysis. Therefore coordination of one Co(III) reagent is sufficient for hydrolysis, and the addition of an extra mole of Co(III) reagent does not significantly alter the rate of hydrolysis because it can only coordinate at a site quite distant from

the position of bond cleavage.

The reaction described here proceeds about 10 fold faster than the hydrolysis of pyrophosphate by such Co(III) reagents under optimum conditions.[10] The reason for this is that the leaving group in this case is the α,β chelated pyrophosphate and this is a much more efficient leaving group than the leaving group for pyrophosphate hydrolysis; coordinated phosphate. The hydrolysis of triphosphate in the absence of metal ion at pH 7 is 3 fold greater[8] than the hydrolysis of pyrophosphate at this pH.

The hydrolytic reaction involves the breaking of a chelate ring, usually an inefficient process because of the inherent stability of chelate rings. However, in this case the unfavourable energetics are offset by the formation of another chelate ring in the product, the chelate phosphate.

One of the best characterized polyphosphate hydrolysing enzymes in terms of its requirements for metal ions and substrates is the yeast enzyme, inorganic pyrophosphatase (YIP), (see section 1.3). This enzyme requires two or three moles of divalent metal ions per active site for activity.[2,3] The proposed mechanism for this enzyme,[3] (Figure 6.4) is very similar to that proposed here for the hydrolysis of triphosphate and to the mechanism proposed for the cobalt (III) complex mediated hydrolysis of pyrophosphate.[9,10]

There are a number of similarities between the enzymic and metal complex ion mediated hydrolysis of polyphosphates, the most striking of these is their requirement for metal ions. As mentioned previously, (see Chapter 1) almost all enzymes involved in polyphosphate bond rupture require at least one and often more divalent metal ions for activity.[1] This apparent contrast, the fact that some enzymes are able

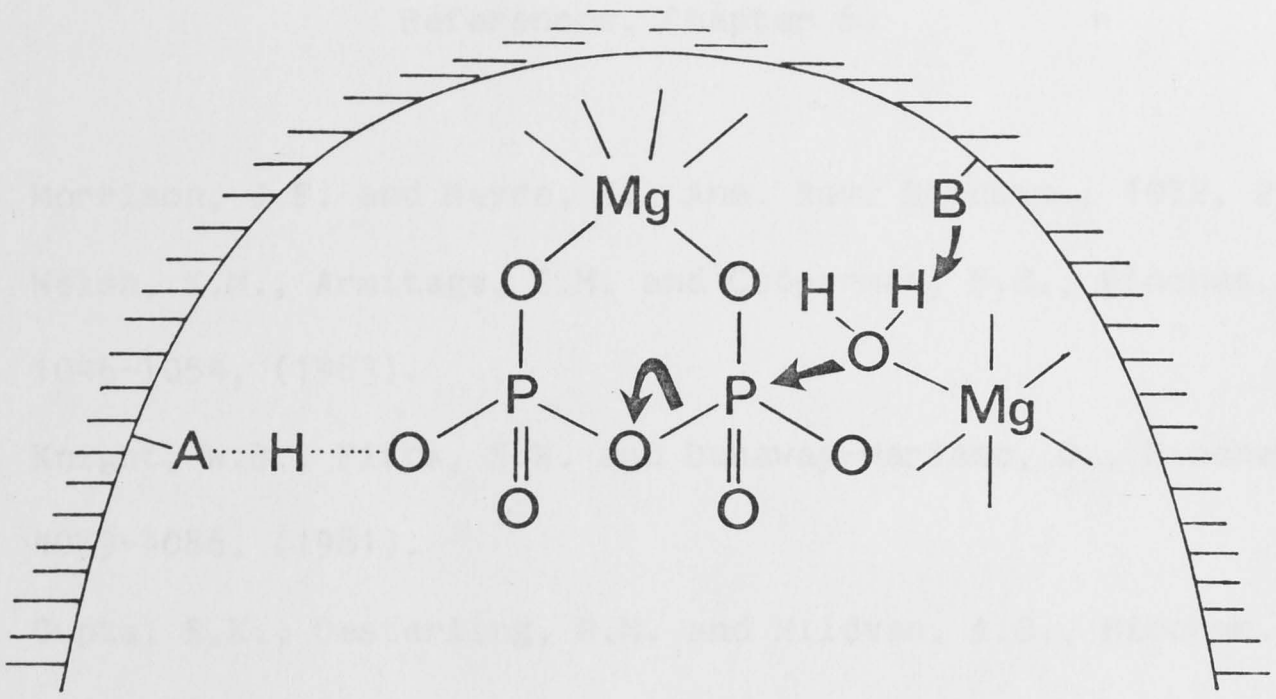


Figure 6.4.

Proposed Mechanism of Action of Yeast Inorganic Pyrophosphatase, B = Base, AH = Acid.

(Redrawn from reference 3.)

to function with only one metal ion, may be explained by the fact that the role of at least some of the metal ions in the non-enzymic hydrolysis appears to be charge neutralization. Enzymes, on the other hand, have evolved purpose-built cavities for the binding and hydrolysis of polyphosphates. These cavities are often rich in positively charged amino acid residues[1,22] which are able to reduce the negative charge on the polyphosphate.

References, Chapter 6.

1. Morrison, J.F. and Heyde, E., Ann. Rev. Biochem., 1972, 29-54.

2. Welsh, K.M., Armitage, I.M. and Cooperman, B.S., Biochem., 22, 1046-1054, (1983).

3. Knight, W.B., Fitts, S.W. and Dunaway-Mariano, D., Biochem., 4079-4086, (1981).

4. Gupta, R.K., Oesterling, R.M. and Mildvan, A.S., Biochem., 15, 2881-2887, (1976).

5. Li, T.M., Mildvan, A.S. and Switzer, R.L., J. Biol. Chem., 253, 3918-3923, (1978).

6. Sigel, H. and Hofstetter, F., Eur. J. Biochem., 132, 569-577, (1983).

7. Imamura, T., Hinton, D.M., Belford, R.L., Gumport, R.I. and Haight, G.P., J. Inorg. Biochem., 11, 241-259, (1979) and references therein.

8. Van Wazer, J.R., Griffith, E.J. and Mccullough, J.F., J. Am. Chem. Soc., 77, 287-291, (1955).

9. Hubner, P.W.A., and Milburn, R.M., Inorg. Chem., 19, 1267-1272, (1980), and references therein.

10. Creaser, I.I., Haight, G.P., Peachey, R., Robinson, W.T. and Sargeson, A.M., J. Chem. Soc. Chem. Commun., 1984, 1568-1571.

11. Norman, P.R. and Cornelius, R.D., J. Am. Chem. Soc., 104, 2356-2361, (1982).

12. Hediger, M. and Milburn, R.M., J. Inorg. Biochem., 16, 165-182, (1982).

13. Hediger, M. and Milburn, R.M., in "Phosphorus Chemistry", ACS Symposium Series no. 171., Quin, L.D. and Verkade, J., (Eds.), Am. Chem. Soc., 1981, pp 211-216.

14. Tafesse, F., Massoud, S.S. and Milburn, R.M., Inorg. Chem., 24, 2591-2593, (1985).

15. Bose, R.N., Cornelius, R.D. and Viola, R.E., Inorg. Chem., 23, 1182-1183, (1984).

15a Haight, G.P. Jr., Hambley, T.W., Hendry, P., Lawrance, G.A. and Sargeson, A.M., J. Chem. Soc. Chem. Commun., 1985, 488-491.

16. Jonasson, I.R., Lincoln, S.F. and Stranks, D.R., Aust. J. Chem., 23, 2266-2278, (1970).

17. Merrit, E.A. and Sundaralingam, M., Acta Cryst., B37, 1505-1509, (1981).

18. Jones, D.R., Ph.D. Thesis, James Cook University of North Queensland, (1980).

19. Seel, F.V. and Bohnstedt, G., Z. Anorg. Alleg. Chem., 435, 257-267, (1977).

20. Hendry, P., Unpublished observations.

21. Woodside, A., Unpublished observations.

22. Cooperman, B.S. and Chui, N.Y., Biochem., 12, 1676-1682, (1973).

CHAPTER 7

CONCLUSIONS AND ENZYMIC PHOSPHORYL TRANSFER

7.1 The Sources of Rate Enhancement.

The aim of this work has been to identify the roles that metal ions play in enhancing phosphoryl transfer reactions and to gauge the efficacy of the different paths. It remains to be seen how such paths could influence the enzymic chemistry as far as it is known at the present time.

7.1.1 Charge Neutralization and Electron Density Polarization.

Coordination of a metal ion to a phosphate ester effects the nature of the phosphate ester in several ways. The positive charge on the metal ion reduces the negative charge on the phosphate, and the electron withdrawing ability of the metal ion should reduce the electron density at the phosphorus centre. Both these effects should increase the rate of attack of a charged or polarized nucleophile on the phosphorus centre. The effects are not easily separated and are treated together in the following discussion.

The intermolecular attack of hydroxide ion on coordinated trimethylphosphate, (TMP), as described in chapter 2 proceeds analogously to the reaction of uncoordinated TMP,[1] ie by attack of OH^- on the phosphorus centre. The reactions have the same rate law,

$$v = k_1[OH^-][TMP],$$

and the rate of loss of methanol was enhanced 400 fold when TMP was coordinated to a trivalent metal centre. The rate of the reaction was essentially independent of the trivalent metal ion for the complexes, $[(NH_3)_5IrTMP]^{3+}$ and $[(NH_3)_5RhTMP]^{3+}$, investigated.

The reactivity of [μ-4-nitrophenylphosphatodecaamminedicobalt]$^{4+}$ also allows an estimate of the effect of a trivalent metal ion on the reactivity of the phosphorus centre. This arises because the first metal ion gives the reaction its intramolecularity and the second metal ion acts to further withdraw electron density from the phosphorus centre. The rate enhancement observed upon addition of the second metal ion is 80 fold, quite a modest amount. Half of this enhancement is probably attributable to the fact that the dinuclear complex has twice the number of potentially nucleophilic ammine ligands available to attack the phosphorus centre. The decreased pK_a of the ammines in the 4+ complex increases the proportion of the nucleophile present, however, this is offset to some degree by the expectation that that the rate of attack of the amido ion will be reduced because of its decreased basicity, (*vide supra*). The effect that the second metal ion has on the reactivity of the phosphorus atom is much less than the effect of the addition of the first metal ion, which increases the rate of hydrolysis by some 10^5 fold[2] for the intramolecular reaction path.

The intermolecular reactions described in chapter 4, OH^- attack on the phosphorus centre of two phosphodiesters coordinated to the $[(NH_3)_5Ir-]^{3+}$ moiety, proceed at rates of the order of 200-400 fold faster than the attack of hydroxide on the free ligand. The rate law for the reaction of the complex includes both first and second order terms in $[OH^-]$. However, the magnitude of the effect due to the metal ions is easily discernible. In these instances, the intermolecular hydrolytic reactions are competitive with intramolecular aminolytic reactions; the intermolecular reactions accounting for 20-30% of the reaction products. The rate enhancement of the intermolecular attack is consistent for both pentaammineiridium(III) diester complexes and for the trimethylphosphate complexes.

Coordination of phosphate esters to a trivalent metal centre increases the rate of intermolecular attack of hydroxide on the phosphorus centre by ~10^2-10^3 fold. This is quite a modest amount compared with the rate enhancements observed in enzymic phosphate hydrolysis reactions. However, the effect may contribute in a more substantial way to the reactivity observed in some enzymes where the substrate is coordinated to more than one metal ion.

7.1.2 Intramolecular attack of Amido ion.

Intramolecular attack of coordinated amido ion on cis-coordinated phosphate esters proceeds comparatively rapidly. Chapter 3 described the attack of coordinated amido ion on several phosphate esters coordinated to the pentaamminecobalt(III) moiety. The reactions of the three complexes are all first order in hydroxide, and the reaction products dictate that coordinated amido ion is the nucleophile.

Table 7.1 contains the rate constants for the reactions of some phosphate esters coordinated to the $[(NH_3)_5Co-]^{3+}$ moiety. The table includes results of work performed previously.[2,3]

It can be seen from Table 7.1 that coordination of the phosphate esters to the pentaamminecobalt(III) moiety results in enhanced rates of cleavage of the ester groups. The effect can be quite substantial, as much as ~10^7 fold. The values quoted in the rate enhancement column are simply comparisons of the observed rate of phosphate ester cleavage in OH^- solution for the free ligands and the complex. A better estimate of the effect of the metal complex ion might be to compare the rate of attack of NH_3 on the phosphorus centre with the rate of $Co\text{-}NH_2^-$ attack on the phosphorus atom. Unfortunately, the comparison is not able to be made for all the complexes, due to lack of data. Nevertheless, the one

Table 7.1

Rate Constants for the Hydrolysis of Some Phosphatopentaamminecobalt(III) Complex Derivatives, 25°C, μ = 1.0 M ($NaClO_4$).

$[(NH_3)_5CoX]$ X[a]	k_{OH^-} (X) $lmol^{-1}s^{-1}$	k_{NH_3} (X) $lmol^{-1}s^{-1}$	k_{OH^-} (Co-X) $lmol^{-1}s^{-1}$	k_{Co-NH_2} (Co-X) s^{-1}	Enhancement $k_{OH^-}(Co-X)/k_{OH^-}(X)$
NPP[1]	4×10^{-9}	$<6 \times 10^{-8}$	3.9×10^{-4}	0.4	10^5
O_3PF[2]	$\sim 10^{-10}$	-	1.7×10^{-3}	2	2×10^7
DNPP	2.5×10^{-5}	1.5×10^{-5}	1.83×10^{-2}	20	7×10^2
NPP-$Co(NH_3)_5$	4×10^{-9}	-	3.68×10^{-2}	0.3	10^7
ENPP	3.3×10^{-7}	-	2.85×10^{-1}	20	9×10^5

[a] NPP = 4-nitrophenylphosphate, O_3PF = fluorophosphate, DNPP = 2,4-dinitrophenylphosphate, ENPP = ethyl,4-nitrophenylphosphate.

comparison of this type that can be made results in a value of $k_{CoNH_2^-}/k_{NH_3}$ ~10^6 mol l^{-1} for the DNPP system. Inspection of the other data would lead one to conclude that this figure will be much higher for the other complexes. As high as 10^{10} mol l^{-1} if the rate of attack of NH_3 is similar to the rate of attack of OH^- as is the case for DNPP. Two major factors contribute to this value; the intramolecularity of the reaction and the higher nucleophilicity of $Co\text{-}NH_2^-$ as compared with NH_3. An extraction of the effect due to the increased nucleophilicity of the reaction will yield the enhancement due to the intramolecularity of the reaction. As argued previously the $S_N2(P)$ reactions of phosphates have a marked dependence of basicity of the attacking nucleophile.[4,5] Arguments have been advanced that an intermediate value of the Bronsted β coefficient ~0.5 might be appropriate for this type of reaction, (vide supra, section 4.4). The pK_a's for the nucleophiles in question are 9.5[6] and between 15 and 17 for NH_3 and Co(III) bound amido ions respectively. Therefore a reduction in the $k_{Co\text{-}NH_2^-}/k_{NH_3}$ value of between ~10^4 and 10^3 should account for effects due to differences in the basicities of the nucleophiles. Taking these effects into account, the $k_{Co\text{-}NH_2^-}/k_{NH_3}$ value becomes about an order of magnitude less than the value of the enhancement quoted in Table 7.1. Therefore, is possible to say that the enhancement of the hydrolytic reaction upon coordination to the pentaamminecobalt(III) moiety arises mainly from the intramolecular nature of the reaction. The activating effect of the metal ion by the charge neutralization/electron density polarization (as discussed in the previous section of this chapter), might be responsible for the remainder of the observed rate enhancement.

This conclusion is supported by the fact that intermolecular attack of hydroxide is not competitive with the intramolecular attack of

amido ion. Even though the relative concentrations of the two species differ greatly; thus the concentration of the hydroxide is 1.0 M whereas the concentration of the deprotonated ammonia ligand is ~10^{-2}-10^{-3} (depending of the pK_a of the coordinated ammine), of the total concentration of the Co(III) complex. The two species are comparable in basicity, (pK_a values for their conjugate acids; 16 for H_2O[6] and ~15-17 for Co(III) coordinated ammonia),[7] and would be expected to attack the phosphorus centre at comparable rates, (vide infra). The argument implies again that the intramolecularity of the reaction is responsible for the major part of the rate enhancement.

The table also reveals that for fluorophosphate and 2,4-dinitrophenylphosphate the rate of attack of hydroxide ion at the phosphorus centre differs by more than 10^5 fold but the difference in the rate of intramolecular attack of amido ion is only 10 fold. The significance of this difference may not be fully understood at this time but, it does seem to indicate a reduced dependence of the leaving group in determining the rate of the reaction. Greater understanding of this observation will require further experimentation, perhaps with a series of substituted phenolic phosphate monoester complexes.

When the phosphate esters, ENPP and BNPP, are coordinated to the pentaammineiridium(III) moiety, similar reactions occur, ie the reaction products dictate that the reactions proceed largely via intramolecular attack of coordinated amido ion. However, the reactions proceed much more slowly than for the corresponding Co(III) complexes; the intramolecular aminolysis reaction for the ENPP complexes occurs ~10^3 fold faster for the Co(III) complex than for the Ir(III) analogue. This phenomenon cannot be due to the differences in the basicities of the ammines of the two complexes because the difference in the pK_a's of the complexes is only of the order of 1 pK unit.[8,9] This difference means

that at a given pH the Co(III) complex would have a larger proportion of the complex in the form of the amido complex. This effect however, would be offset by the expectation that the more basic Ir(III) amido ion would be more nucleophilic than the Co(III) complex.

The reason for the rate decrease is most likely the varying sizes of the two metal ions involved. In addition to reducing the charge density on the metal, the larger size of the Ir(III) ion must increase the energy required to form the obligatory and strained four-membered ring. This subject was discussed in some detail in section 5.4.

The reduced rate of attack of the amido ion in the Ir(III) complexes means that intermolecular attack of hydroxide becomes competitive with the intramolecular reaction. Despite the fact that the concentration difference between the coordinated amido ion and the hydroxide ion is substantial ($[OH^-]$ = 1.0 M, $[Ir\text{-}NH_2^-]$ = $\sim 10^{-3}$ of total [Ir] given a pK_a for the coordinated NH_3 of ~17[8]), the reaction still proceeds with ~70 - 80% attack by the amido ion. This attests to the efficacy of the intramolecular reaction which is still quite efficient despite the highly unfavourable geometry required to from the the 4-membered ring.

Intramolecular attack of coordinated amido ion on <u>cis</u> coordinated phosphate esters proceeds relatively rapidly and rate enhancements over the free ester of the order of up to 10^7 fold are observed for Co(III) complexes. The rate enhancement has been shown to arise mainly from the intramolecularity of the reaction. The efficiency of this route contrasts with the rate enhancements observed in intermolecular attack of nucleophiles. The work summarized here has described a facile route for O to N transphosphorylation. This is of interest in enzymes such as creatine kinase where such reactions readily occur and multiple metal ion cofactors are often required.[10]

7.1.3 Intramolecular attack of Hydroxide ion.

Intramolecular attack of coordinated hydroxide ion on phosphate esters proceeds readily about the Ir(III) ion. The two complexes investigated in this study are hydrolysed at pH 8, ~5 x 10^5 fold faster than the free ligand at that pH.

An interesting comparison can be made between the rate of intermolecular attack of hydroxide ion and the rate of intramolecular attack of the Ir-OH^- nucleophile. Table 7.2 shows the rate constants for intermolecular attack of OH^- on the phosphorus atom of two pentaammineiridium(III) phosphodiester complexes and the rate constants for intramolecular attack of coordinated hydroxide ion on the same esters coordinated to the bis(ethylenediamine)iridium(III) moiety. At a concentration of 1.0 M, the hydroxide ion attacks the phosphorus centre of the coordinated phosphate esters with a rate constant only slightly larger than that for the intramolecular attack of coordinated hydroxide. Based on their relative basicities, OH^- should be a much better nucleophile than Ir-OH^-. This comparison is based on the observations of Kirby et al.[4,5] A conservative estimate of the difference in nucleophilicity between the two species would be ~10^5 fold. The difference in the rate of intermolecular attack of hydroxide and intramolecular attack of coordinated hydroxide is ~10 or less. Thus, the intramolecular attack of hydroxide proceeds at a rate ~10^4 fold faster than that predicted for intermolecular attack of coordinated hydroxide at a concentration of 1 M. This figure gives a quantitation of the efficacy of the intramolecular reaction. Moreover it is shown to be quite efficient despite the obligatory formation of an extremely strained 4-membered ring in the course of the reaction.

Intramolecular attack of coordinated hydroxide ion on phosphate esters coordinated to a Co(III) complex ion proceed ~10^3 fold faster

Table 7.2

Comparison of Rate Constants for Attack of Hydroxide and Coordinated Hydroxide on ENPP and BNPP Coordinated to Ir(III) complex ions.

Phosphate Ester X	Rate constant for attack of OH^- on $[(NH_3)_5IrX]^{2+}$ in 1.0 M OH^-	Rate Constant for Attack of Ir-OH^- in <u>cis</u>-$[(en)_2Ir(OH)(X)]^+$
ENPP	1×10^{-4} s^{-1}	4.6×10^{-5} s^{-1}
BNPP	4.9×10^{-3} s^{-1}	3.95×10^{-4} s^{-1}

than the analogous reaction involving the Ir(III) complex. The difference has been attributed to the difference in the sizes of the two metal ions especially as it relates to the ease of formation of the 4-membered ring required in the product. It has been argued that the larger Ir(III) ion makes the 4-membered chelate phosphate ring more difficult to form. The effect also applies to the formation of 4-membered rings by intramolecular attack of amido ion.

The metal ions most commonly associated with enzymic phosphoryl transfer, Mg^{2+} and Zn^{2+} have effective ionic radii even larger than that of Ir(III). For a six-coordinate environment, the effective ionic radii are; Co(III) 0.545 Å, Ir(III) 0.68 Å, Mg(II) 0.72 Å and Zn(II) 0.74 Å.[11] The large size of these biologically active metal ions must raise doubts as to the efficacy of reactions involving the formation of 4-membered chelate rings about these metal ions.

The intramolecular attack of hydroxide on the phosphorus in the complex, <u>cis</u>-$[(NH_3)_4Ir(OH)NH_2P(O)_2(OC_6H_4NO_2)]^+$ is of interest because it allows the quantitation of the effect of protonation on the reactivity

of the phosphorus atom. The NH_2 moiety bridging the Ir and P atoms has a pK_a of the order of 9.8 and the rate of intramolecular attack of hydroxide on the phosphorus has been determined in both the fully protonated and fully deprotonated conditions. The difference in rate is ~2 x 10^2 fold with the protonated species being hydrolysed faster as expected since the protonation should increase the electrophilicity of the phosphorus.

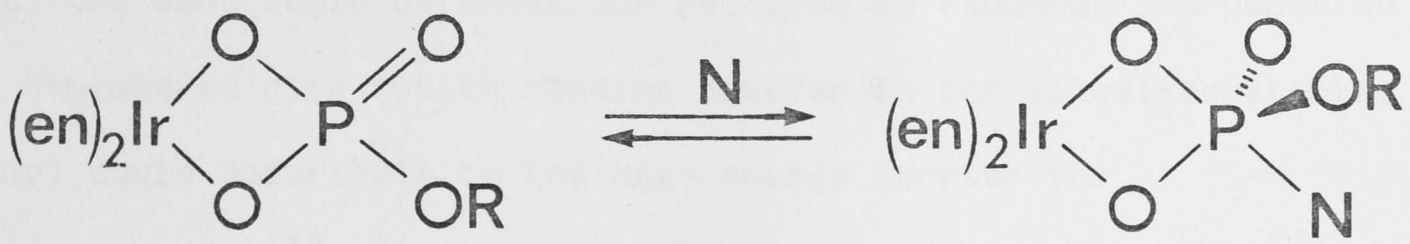
7.1.4 Chelation of the Phosphate.

Chelation of phosphate esters and phosphoramidate esters did not result in the cleavage of the ester group. In only one instance was the chelate ester actually deduced; this was the N,O-ethylphosphoramidate chelated to the tetraamminecobalt(III) moiety. This complex did not hydrolyse rapidly and was easily observable by ^{31}P NMR spectroscopy; the complex hydrolysed with Co-ligand bond rupture. However, both chelate phosphate ester and chelate phosphoramidate ester species were deduced as intermediates along the reaction path of various precursor complexes.

Unlike the Co(III) complex above, the hydrolysis of the pentaammineiridium(III) phosphodiester complexes probably did not yield the chelate, rather the initial product observed in the ^{31}P NMR was the ring opened N-bound phosphoramidate ester. The product dictates that the chelate must be an intermediate in the reaction. The chelate must rapidly ring open as it is formed, most likely with P-O bond rupture as observed for the phosphate ester chelate. The shift between metal-O and P-O bond rupture is probably related to the increased ring strain in the Ir(III) chelate complex and the relative inertness of the Ir(III)-O bond compared to the Co(III)-O bond.

Several complexes were prepared in which an adjacent coordinated hydroxide ion attacked a coordinated phosphodiester. The aim of this

work was to gain some idea of the efficacy of the chelated phosphate ester as an intermediate in the hydrolysis of phosphate esters. The reaction proceeds initially as expected with the cis hydroxide ion attacking the phosphorus centre and cleaving one of the ester groups. However, the chelate ester presumably formed, rapidly ring opens to yield the monodentate phosphate ester complex. The ring opening reaction proceeds with P-O cleavage rather than Ir-O cleavage. This ring opening process presumably occurs via the $S_N2(P)$ mechanism, ie, via the phosphorane intermediate. Arguments similar to those used by Westheimer[12] show that the phosphorane formed by attack of water or hydroxide ion on the chelate phosphate ester must have one Ir-O ligand axial and the other equatorial so that the 4-membered ring spans axial-equatorial positions in the phosphorane. The other axial position is occupied by the entering nucleophile, thus leaving two equatorial positions which must be occupied by the ester function and the oxo ligand, Scheme 7.1. The phosphorane that is formed apparently does not



Scheme 7.1

Formation of the Phosphorane Complex by Attack of a Nucleophile on the Chelate Phosphate Ester complex.

have a life-time long enough to pseudorotate or is in some manner inhibited from psuedorotating since the only leaving group is the Ir-O^{2-} substituent which is axial in the initially formed phosphorane. The degree of inhibition of pseudorotation must be quite severe because in the case of the phosphorane containing the 4-nitrophenolate leaving group, the phenol is a much more efficient leaving group than Ir-O^{2-} and yet the phenol is not cleaved in this process. The question of why the phosphorane does not pseudorotate does not seem to be a simple one. In most organic phosphoranes the most common reason for inhibition of pseudorotation is that the process would place an apicophobic substituent in an apical position, eg $(CH_3)_3PF_2$ in which the fluorines are apical and the methyls are equatorial, does not readily pseudorotate.[12] There are no such demanding requirements in this case; all the substituents are similar in electronegativity and apicophilicity. The presence of the strained four-membered ring may inhibit the pseudorotation. The inhibition of pseudorotation on a phosphorane containing a 4-membered ring (with oxygen and carbon substituents), has been attributed largely to the relative apicophilicity of the substituents.[13] However, these workers suggested that "the bond angle deformations required to exchange the position of the 4-membered ring (which remains however in the apical-equatorial plane) could contribute to the high energy barrier for pseudorotation".[13] There are, however, examples of phosphoranes containing a 4-membered ring, with two carbon atoms bonding to the phosphorus, which can readily pseudorotate.[14]

There does not seem to be any compelling argument for inhibition of pseudorotation of the intermediate phosphorane on steric or electronic grounds in the ring opening reaction of the chelate phosphate ester. Therefore it seems likely that the lack of exocyclic hydrolysis

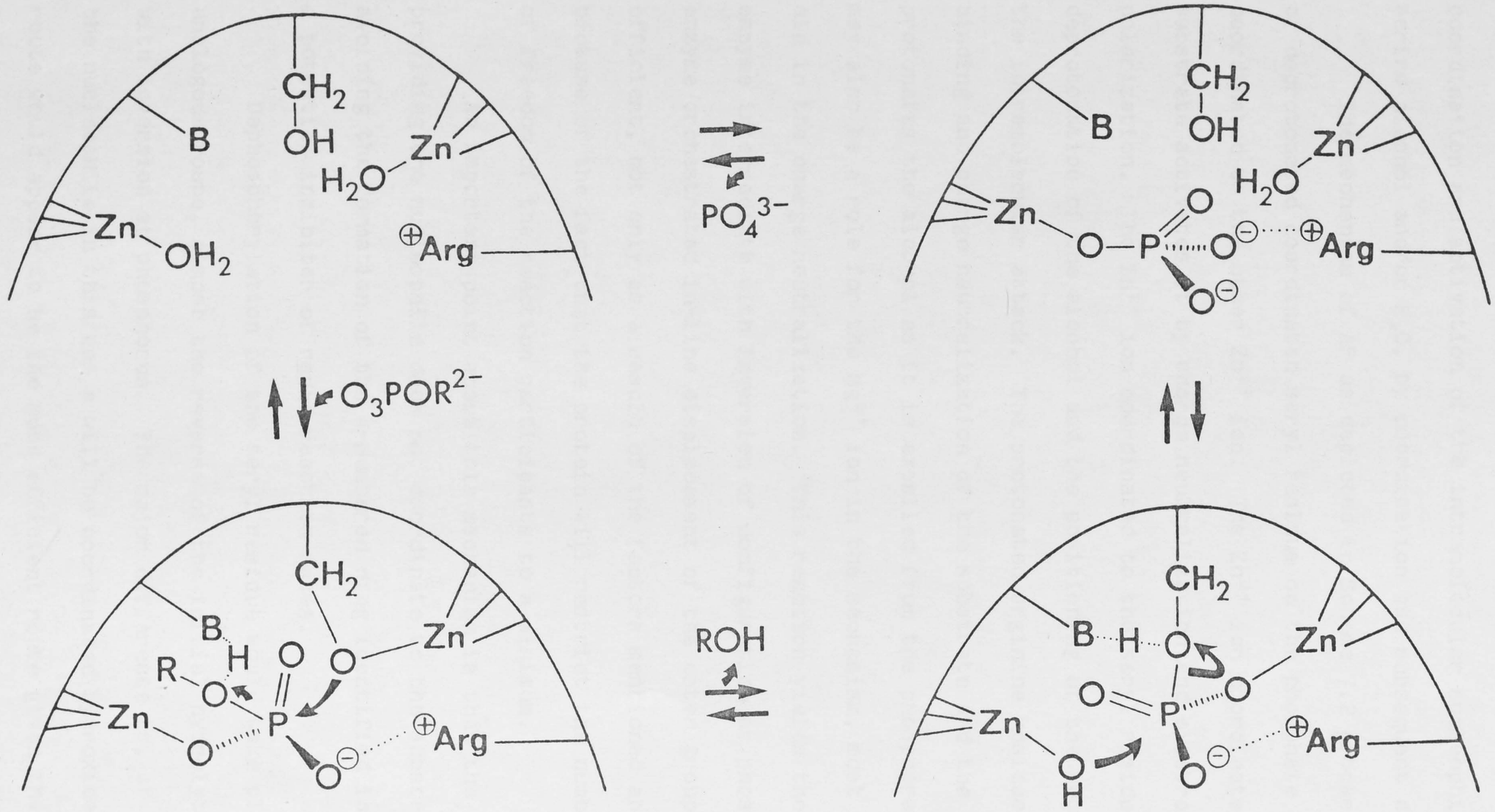
of the ester group is due to the lifetime of the phosphorane being too short to allow any ligand reorganization.

7.2 Enzymic Phosphoryl Transfer.

7.2.1 Alkaline Phosphatase.

Biochemical and structural studies on the E. coli enzyme, alkaline phosphatase (AP) have revealed important features about the enzyme and the mechanism by which it catalyses the hydrolysis of phosphate monoesters. Several of these features are; the enzyme binds two Zn^{2+} ions and one Mg^{2+} ion per active site, the reaction proceeds via the intermediacy of a phosphorylated serine residue with overall retention of configuration at phosphorus, most probably via two inversions. The two Zn^{2+} ions appear to be essential for activity but the Mg^{2+} ion is not. A more detailed description of the structure and function of the enzyme is given in the Introduction. The mechanistic scheme to be proposed here is based on the current knowledge about the enzyme, including structural and kinetic information, along with information gained from model studies including those described in the previous chapters of this thesis.

The proximity of the two metal ions to the site of binding of the substrate and serine residue is compelling evidence for the proposal



Scheme 7.2

Proposed Mechanism of Action of Alkaline Phosphatase.

that the enzyme uses the metal ions in the hydrolytic reaction. The most likely roles for the metal ions are substrate activation by coordination and activation of the intramolecular nucleophiles, the serine alcohol and/or H_2O, by coordination and subsequent deprotonation.

The mechanism of AP as depicted in Scheme 7.2 proceeds by attack of deprotonated coordinated seryl residue on the phosphate ester coordinated to the other Zn^{2+} ion. The Zn^{2+} ion coordinated to the substrate activates it by charge neutralization and electron density polarization. The Zn^{2+} ion coordinated to the seryl residue aids in the deprotonation of the alcohol and the positioning of the nucleophile for the intramolecular attack. The protonated arginine residue aids in the binding and charge neutralization of the substrate and the acid (BH) protonates the alcohol as it is expelled from the phosphorane. There may also be a role for the Mg^{2+} ion in the mechanism, most probably, to aid in the charge neutralization. This reaction yields the phosphoseryl enzyme intermediate with inversion of configuration at phosphorus. The enzyme orchestrated in-line displacement of the ester group will be very efficient, not only as a result of the factors mentioned above, but also because of the fact that the protein will restrict the number of degrees of freedom of the reaction participants to a minimum.

An important point about this mechanism is that the metal ion providing the nucleophile does not coordinate to the substrate thereby avoiding the formation of the 4-membered ring identified in this work as a potential inhibitor of rapid reaction rates.

Dephosphorylation of the seryl residue would take place by an analogous route, almost the reverse of the initial hydrolytic reaction, with inversion at phosphorus. The major difference is, of course, that the nucleophile in this cas e will be coordinated hydroxide ion. This route would appear to be the most efficient route given the results of

the model studies described herein. One point of contention with this proposal is the coordination of the phosphorylated serine to the Zn^{2+} ion. $^{31}P\{^{113}Cd\}$ NMR studies[15] which failed to show coupling between the spin half nuclei ^{31}P and ^{113}Cd, have been interpreted as indicating that the serylphosphate does not coordinate to the metal ions in the active site. It must be born in mind however, that the failure to observe coupling does not exclude direct coordination of the metal ion to the serylphosphate. The model studies described herein would argue for the coordination of the Zn^{2+} ion to the serylphosphate, as an aid to its hydrolysis.

The dephosphorylation of the serine residue yields the Zn^{2+} bound phosphate ion stabilized in the active site by interaction with the protonated arginine residue as required by phosphate binding studies, ie a protonated residue, probably arginine, is required for phosphate binding.[16]

The dissociation of the phosphate returns the enzyme to the resting state, ie ready to bind another molecule of phosphate ester.

7.2.2 Yeast Inorganic Pyrophosphatase.

The hydrolysis of coordinated triphosphate proceeded rapidly in the presence of the $[(tn)_2Co(OH)(OH_2)]^{2+}$. As observed with the hydrolysis of other condensed phosphates and their esters, the reaction required more than one metal ion to proceed efficiently. The reaction described herein required two trivalent metal complex ions for efficient hydrolysis of triphosphate. The metal ions play two distinct roles in the hydrolytic reaction. The $[tacnCo-]^{3+}$ moiety coordinates the triphosphate, partially neutralizing the negative charges and making the phosphorus atoms more susceptible to nucleophilic attack. This complex however is itself not rapidly hydrolysed and might be expected to

actually be stabilized to hydrolysis because of the formation of the chelate rings in the complex. The second metal complex ion, the $[(tn)_2Co-]^{2+}$ moiety, provides an intramolecular nucleophile to cleave the polyphosphate chain.

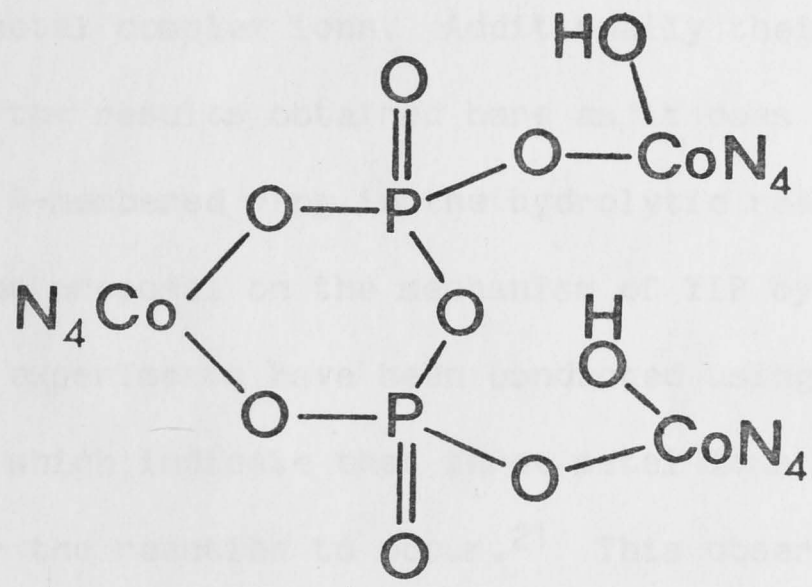


Figure 7.1

Postulated Intermediate in the Hydrolysis of Pyrophosphate by the Co(III) Metal Complex Ions.

Pyrophosphate hydrolysis mediated by these types of complex metal ions proceeds by a similar mechanism except that the reactive complex in this case seems to be one where three complex metal ions are bound to the pyrophosphate.[17,18] Figure 7.1 shows the most commonly postulated intermediate in the hydrolysis of pyrophosphate by these metal complex ions.

The similarity between this structure and the arrangement of substrate and metal ions in the active site of yeast inorganic pyrophosphatase (YIP), as proposed by Dunaway-Mariano *et al*[19] (Figure 6.4) is striking.

The substrate for the reaction is the P_1,P_2-Mg^{2+} chelate, and the nucleophile for the reaction is provided by a Mg^{2+} bound hydroxide ion.

The leaving group is aided by the donation of a proton from an adjacent general acid moiety to the basic P_2 phosphate moiety as it is expelled.

The mechanism proposed by Dunaway-Mariano et al[19] is therefore in agreement with the mechanism that is proposed here and by others[17,18,20] on the basis of the experiments on the hydrolysis of polyphosphates using Co(III) metal complex ions. Additionally their mechanism is in agreement with the results obtained here as it does not require the formation of a 4-membered ring in the hydrolytic reaction.

Since the proposal on the mechanism of YIP by Dunaway-Mariano et al,[19] further experiments have been conducted using paramagnetic metal ions as probes which indicate that three metal ions are required in the active site for the reaction to occur.[21] This observation is readily accommodated by the results of the hydrolysis of pyrophosphate by the Co(III) reagents. The third metal ion may replace the acid moiety and coordinate to the phosphate leaving group to improve its leaving group ability. The interatomic distances estimated for the metal ions in the active site[21] are also consistent with such a proposal.

7.2.3 Kinases.

Kinases catalyse the reversible phosphorylation of acceptor molecules by nucleosidetriphosphates, (NTP's). In general, kinases require the NTP to be in the form of a M^{2+} (usually Mg^{2+}) complex before it accepts the NTP as a substrate, vide supra section 1.4. The work described in this thesis and elsewhere by other workers, assists in the understanding of why the polyphosphate substrate is required to be coordinated. The enzymes seem to have evolved to accept only the M^{2+}-NTP complex and not the uncomplexed NTP as a substrate. There must have been an advantage to the organisms involved for such an evolution to occur. The question is, why do most kinases require the phosphate donor

to be complexed? A possible explanation seems to have been gained from studies on the reactivity of coordinated polyphosphates; without exception more than one coordinated metal ion is required for rapid hydrolysis.[17,18,20,22,23] The first metal ion appears to neutralize the negative charge on polyphosphate. This is the role of the $[tacnCo-]^{3+}$ moiety described in chapter 6. The second metal ion's role is to provide an intramolecular nucleophile for the hydrolytic cleavage. Both metal ions appear to be vital for the reaction to proceed efficiently. In the case of the triphosphate hydrolysis described in chapter 6, the $[tacnCoP_3O_{10}]^{2-}$ complex does not hydrolyse in the absence of the complex responsible for the provision of the nucleophile, $[(tn)_2Co(OH)(OH_2)]^{2+}$. The reaction between free triphosphate and $[(tn)_2Co(OH)(OH_2)]^{2+}$ is not expected to result in greatly enhanced hydrolysis of triphosphate since the corresponding reaction between the more reactive ATP and $[(tn)_2Co(OH)(OH_2)]^{2+}$ does not result in the hydrolysis of ATP.[20]

These observations indicate a possible rationale for the strict requirement of kinases for the Mg^{2+}-NTP complexes as substrates. The coordination of the NTP by the metal ion activates the NTP by electron density polarization and charge neutralization so that the complex is "primed" for intramolecular attack by enzyme organized nucleophiles. The question of activation of the nucleophile is another matter which will have to be answered individually for each enzyme nonetheless activation of the nucleophile by the coordination/deprotonation route as described for the serine residue of alkaline phosphatase would seem to be one obvious possibility. The combination of charge neutralization and the intramolecularity of the reaction lead to efficiency in the phosphoryl transfer.

References, Chapter 7.

1. Barnard, P.W.C., Bunton, C.A., Llewwllyn, D.R., Vernon, C.A. and Welch V.A., J. Chem. Soc., **1961**, 2670-2676.

2. Harrowfield, J.MacB., Jones, D.R., Lindoy, L.F. and Sargeson, A.M., J. Am. Chem. Soc., **102**, 7733-7741, (1980).

3. Creaser, I.I., Dubs, R.V. and Sargeson, A.M., Aust. J. Chem., **37**, 1999-2003, (1984).

4. Kirby, A.J. and Younas, M., J. Chem. Soc. (B), **1970**, 1165-1172.

5. Khan, S.A. and Kirby, A.J., J. Chem. Soc. (B), **1970**, 1172-1182.

6. Bell, R.P., "The Proton in Chemistry" 2nd edition, Cornell University Press, New York, 1973, pp 96.

7. Tobe, M.L., Acc. Chem. Res., **3**, 377-385, (1970).

8. The pK_a of Ir(III)-NH_3 in 2+ complexes is estimated to be ~17, this is based on the estimated pK_a of Co(III)-NH_3 for 2+ complexes of 16^7 and the observation that Co(III) complexes are between $10^{0.5}$ and $10^{1}{}^{15}$ more acidic than the corresponding Ir(III) complex.

9. Palmer, J. W. and Basolo, F., J. Inorg. Nucl. Chem., **15**, 279-286, (1960).

10. Watts, D.C., in "The Enzymes", 3rd Edition, Boyer, P.D. (Ed.), Academic Press, New York, 1973, Vol 8A, pp 384-431.

11. Shannon, R.D., Acta Cryst. **A32**, 751-767, (1976).

12. Westheimer, F.H., Acc. Chem. Res., **1**, 70-78, (1968).

13. Ul-Haque, M., Caughlan, C.N., Ramirez, F., Pilot, J.F. and Smith, C.P., J. Am. Chem. Soc., **93**, 5229-5235, (1971).

14. Denney, D.Z., White, D.W. and Denney, D.B., J. Am. Chem. Soc., **93**, 2066-2076, (1971).

15. Cohn, M. and Reed, G.H., Ann. Rev. Biochem., **51**, 365-394, (1982).

16. Coleman, J.E. and Gettins, P., Adv. in Enzymology, **55**, 381-452, (1983).

17. Creaser, I.I., Haight, G.P., Peachey, R., Robinson, W.T. and Sargeson, A.M., J. Chem. Soc. Chem. Commun., **1984**, 1568-1571.

18. Hubner, P.W.A. and Milburn, R.M., Inorg. Chem., **19**, 1267-1272, (1980).

19. Knight, W.B., Fitts, S.W. and Dunaway-Mariano, D., Biochem., **20**, 4079-4086, (1981).

20. Hediger, M. and Milburn, R.M., J. Inorg. Biochem., **16**, 165-182, (1982).

21. Knight, W.B., Dunaway-Mariano, D., Ransom, S.C. and Villafranca, J.J., J. Biol. Chem., **259**, 2886-2895, (1984).

22. Milburn, R.M., Gautam-Basak, M., Tribolet, R. and Sigel, H., J. Am. Chem. Soc., **107**, 3315-3321, (1985).

23. Norman, P.R. and Cornelius, R.D., J. Am. Chem. Soc., **104**, 2356-2361, (1982).

APPENDICES

Table of ^{31}P NMR Chemical Shifts of Selected Phosphates and Derivatives.[a]

Compound	Conditions	Chemical Shift (multiplicity)	Reference
H_3PO_4	pH 0	0.00 (s)	-
PO_4^{3-}	1 M NaOH	+ 5.5 (s)	3
$NO_2C_6H_4OPO_3^{2-}$	pH 10, 0.4 M NaOH	- 0.3 (s)	2
$(NO_2)_2C_6H_3OPO_3^{2-}$	pH 12	- 4.1 (s)	3
$(EtO)(NO_2C_6H_4O)PO_2^-$	pH 7	- 5.0 (s)	3
$(MeO)_3PO$	pH 0 to 14	+ 2.9 (s)	3
$(EtO)_3PO$	pH 0 to 14	- 0.6 (s)	3
$[(NH_3)_5CoOPO_3]$	0.2 M NaOH	+ 13.7 (s)	2
$[(tn)_2Co(OH)(OPO_3)]^-$	1.0 M NaOH	+ 13.1 (s)	8
$[(NH_3)_5CoOPO_3H]^+$	pH 6	+ 11.0 (s)	1
$[(NH_3)_5CoOPO_3H_2]^{2+}$	pH 1	+ 8.0 (s)	1
$[(NH_3)_4CoO_2PO_2]$	0.01 M NaOH	+ 24.3 (s)	8
$[(en)_2CoO_2PO_2]$	pH 10	+ 23.6 (s)	2

$[(tn)_2CoO_2PO_2]$	0.1 M NaOH	+ 24.3 (s)	8
$[(NH_3)_5CoO_3POC_6H_4NO_2]^+$	1 M NaOH	+ 6.7 (s)	2
$[(NH_3)_5CoO_3POC_6H_3(NO_2)]^+$	pH 10, 0.5 M NaOH	+ 6.4 (s)	3
$[(NH_3)_5CoO_2P(OEt)(OC_6H_4NO_2)]^{2+}$	pH 7, 0.5 M NaOH	+ 1.3 (s)	3
$[(NH_3)_5CoOP(O)(OC_6H_4NO_2)OCo(NH_3)_5]^{4+}$	0.4 M OH^-	+ 12.6 (s)	3
$H_4P_2O_7$	pH 0	- 12 (s)	3
$P_2O_7^{4-}$	1 M NaOH	- 5.6 (s)	3
$P_3O_{10}^{5-}$	1 M OH^-	- 4.7 (d) - 19 (t)	3
$[(NH_3)_4CoP_2O_7]^-$	pH 7	+ 4.0	7
$[(en)_2CoP_2O_7]^-$	pH 7	+ 4.3 (s)	7
$[(tn)_2CoP_2O_7]^-$	pH 7	+ 3.9 (s)	8
$[(tn)_2CoP_2O_7H]$	pH 2	+ 0.1 (s)	8
$[(NH_3)_5CoP_2O_7]^-$	pH 8	+ 5.6 (d), - 1.6 (d)	4
α,β,γ-$[(Tacn)CoP_3O_{10}]^{2-}$	pH 7	+ 3.8 (d), - 6.3 (t)	3
α,β,γ-$[(NH_3)_3CoP_3O_{10}]^{2-}$	pH 8	+ 4.2 (d), - 5.6 (t)	4
α,β-$[(NH_3)_4CoP_3O_{10}]^{2-}$	pH 8	+ 4.2 (d), - 5.1 (d), - 9.1 (dd)	4
α,γ-$[(NH_3)_4CoP_3O_{10}]^{2-}$	pH 6.5	+ 1.0 (d), - 18.0 (t)	5

α-$[(NH_3)_5CoP_3O_{10}]^{2-}$	pH 8	+ 3.5 (d), - 3.2 (d), - 18.0 (dd)	6
β-$[(NH_3)_5CoP_3O_{10}]^{2-}$	pH 8	- 2.4 (d), - 9.9 (t)	6
$[(H_2O)_4RhP_2O_7]^-$	pH 2	+ 5.6 (s)	9
$[(H_2O)_4Rh(PO_4)_2]^{3-}$	pH 2	+ 15 (s)	9
α,β,γ-$[(H_2O)_3RhP_3O_{10}]^{2-}$	pH 3	+ 6.3 (d), - 5.4 (t)	9
α,β-$[(H_2O)_4RhADP]$	pH 3	+ 3 (d), + 8 (d)	9
β,γ-$[(H_2O)_4RhATP]^-$	pH 3.9	+ 7.6 (d), - 8.6 (t), - 11.5 (t)	9
α,β,γ-$[(H_2O)_3RhATP]^-$	pH 3.9	+ 8.0 (m), + 0.6 (m), - 7.5 (m)	9
$\{[(en)_2Co(\mu\text{-}O_3POC_6H_4NO_2)]_2\}^{2+}$	pH 7	+13.3 (s)	2
$(NO_2C_6H_4O)_2PO_2^-$	0.1 M NaOH	- 10.9 (s)	3
$[(NH_3)_5CoO_2P(OC_6H_4NO_2)_2]^{2+}$	pH 7	- 5.8 (s)	3
$[(NH_3)_5IrO_2P(OC_6H_4NO_2)_2]^{2+}$	pH 7	- 5.2 (s)	3
$[(NH_3)_5IrOP(OMe)_3]^{3+}$	pH 7	+ 6.2 (s)	3
$[(NH_3)_5RhOP(OMe)_3]^{3+}$	pH 7	+ 7.0 (d)	3
$[(NH_3)_5IrO_2P(OMe)_2]^{2+}$	pH 7	+ 9.8 (s)	3
$[(NH_3)_5IrO_2P(OEt)(OC_6H_4NO_2)]^{2+}$	pH 7	+ 1.2 (s)	3
$[(NH_3)_5IrO_2P(O)(OC_6H_4NO_2)]^+$	pH 7, 1.0 M NaOH	+ 7.1 (s)	3

cis-$[en_2Ir(OH_2)OP(O)(OEt)(OC_6H_4NO_2)]^{2+}$	pH 1	+ 1.6 ("d" diasteromers)	3
cis-$[en_2Ir(OH_2)O_2P(OC_6H_4NO_2)_2]^{2+}$	pH 2	- 5.6 (s)	3
$NH_2PO_3^{2-}$	1 M NaOH	+ 8.6 (s)	2
$NH_2PO_2OEt^-$	1 M NaOH	+ 9.5 (s)	3
$NH_2PO_2OC_6H_4NO_2^-$	1 M NaOH	+ 5.7	2
$[(NH_3)_4Co(OH)(NH_2PO_3)]$	0.05 M NaOH	+ 7.0 (s)	2
$[(NH_3)_5CoOP(O)NH_2]^+$	0.1 M NaOH	+ 18.7 (s)	3
cis-$[en_2Co(OH_2)(OPO_3C_6H_4NO_2)]^+$	pH 10, 0.4 M NaOH	+ 6.6	2

a ^1H decoupled in aqueous solution.

References, Appendix 1.

1. Seel, V.F. and Bohnstadt, G., Z. Anorg. Allg. Chem., 435, 257-267, (1977).

2. Jones, D.R., Ph.D. Thesis, James Cook University of North Queensland, 1981.

3. This Work.

4. Cornelius, R.D., Hart, P.A. and Cleland, W.W., Inorg. Chem., 16, 2799-2805, (1977).

5. Reibenspies, J. and Cornelius, R.D., Inorg. Chem., 23, 1563-1565, (1984).

6. Haromy, T.P., Gilletti, P.F., Cornelius, R.D. and Sundariligam,M., J. Am. Chem. Soc., 106, 2812-2818, (1984).

7. Creaser, I.I., Haight, G.P., Peachey, R., Robinson, W.T. and Sargeson, A.M., J. Chem. Soc. Chem. Commun., 1984, 1568-1571.

8. Woodside, A., Unpublished observations.

9. Lin, I., Knight, W.B., Ting, S-J., Dunaway-Mariano, D., Inorg. Chem., 1984, 23, 988-991.

APPENDIX 2

LEAST SQUARES FITTING PROGRAM.

A fitting program for the analysis of curves was utilized extensively in this thesis. The original program, using a generalized weighted least squares fitting procedure was written for an IBM computer and has been modified to run on the school Vax 11/750.

The program allows the user to describe the function to be fit in terms of a number of constants and a number of parameters. The user specifies the required equation, the number and value of the constants and the number parameters and estimates of the value of these parameters. The data is entered in the form of a table of X,Y values where X is the independent variable and Y is the dependent variable.

The program uses an iterative procedure minimizing the value of "Q" which is a weighted least squares measure of the goodness of fit.

The output from the program includes the "Q" values after each successive iteration, the calculated values of the parameters with associated errors and the residuals for each point. A plot of the data with the calculated curve is also available.

APPENDIX 3

PUBLICATIONS

1. Hendry, P. and Sargeson, A.M., "Base Hydrolysis of Pentaamminetrimethylphosphate Iridium (III)." J. Chem. Soc. Chem. Commun., 1984, 164-165.
2. Haight, G.P., Hambley, T.W., Hendry, P., Lawrance, G.A. and Sargeson, A.M., "Rapid Cleavage of Tridentate Cobalt(III)-Coordinated Triphosphate." J. Chem. Soc. Chem. Commun., 1985, 488-491.
3. Hendry, P. and Sargeson, A.M., "Base Hydrolysis of Coordinated Trimethylphosphate." Aust. J. Chem., Accepted for publication.
4. Hendry, P. and Sargeson, A.M., "Intramolecular Phosphoryl Transfer: Chelated Phosphoramidate." Inorg. Chem. Submitted.