SYNTHESES OF SELECTED MONO- AND DI-ESTERS OF PHOSPHORIC ACID AND STABILITY CONSTANTS OF THEIR COPPER(II) COMPLEXES

A thesis submitted to

THE UNIVERSITY OF CAPE TOWN

in fulfilment of the requirements of the degree of

MASTER OF SCIENCE

by

MOIRA M. ARMSTRONG

B.Sc. (HONS) (CAPE TOWN)

School of Chemical Sciences
University of Cape Town
Rondebosch
South Africa

September 1984

To my Parents

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to:

My supervisors - Associate Professor T.A. Modro, Professor P.W. Linder and Dr. R.G. Torrington - for their invaluable guidance, enthusiasm and support throughout the course of this project;

my friends and colleagues in the School of Chemical Sciences for their assistance and contributions towards this work;

A.E.C.I. Limited for a post-graduate research bursary.

CONFERENCE PROCEEDINGS

M.M. Armstrong, S. Cocks and T.A. Modro, "Structural Effects on the 'Energy-Rich' Phosphate Bond" - a poster presented at the Frank Warren Conference, University of Natal, Pietermaritzburg, 1983.

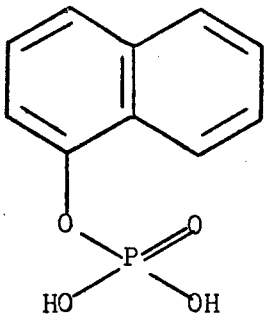
ABSTRACT

A number of different routes were attempted to the syntheses of organic phosphate models $(R_1O)(R_2O)PO_2^-$ where R_1 = 4-nitrophenyl group and R_2 = benzyl, 2-pyridylmethyl, 1-naphthyl or 8-quinolyl group. However, preparative difficulties and side reactions, due mainly to the susceptibility of the 4-nitrophenoxy moiety to nucleophilic displacement reactions, resulted in simplification of the target models to a pair of monoesters $(R_3O)PO_3H_2$ and a pair of diesters $(R_3O)(R_4O)P(O)O^-Na^+$ where R_3 = 1-naphthyl group (monoester (A) or diester (C)) or 8-quinolyl group (monoester (B) or diester (D)) and R_4 = methyl group. Such a set of substrates allowed comparison of the reactivities of phosphate monoesters and diesters and as the components of each pair differed only by the presence or absence of a tertiary amine function in one of the ester linkages, the effect on the reactivity of such a compound, due to the presence of a heterocyclic atom, could be gauged.

Complex formation between ligands (A), (B), (C) and (D) and copper(II) ions in aqueous solution at 25°C and I = 0,15 mol dm^{-3} $(Na)[Cl^-]$ was investigated by means of glass electrode potentiometry and computational analysis. Mononuclear and hydroxo complexes were found to occur with ligands (A), (B) and (D). No complexation was detected with ligand (C). The copper(II) ion was found to be only singly coordinated to ligands (A) and (D) through a terminal phosphate group and the quinolyl nitrogen respectively, while with ligand (B) a chelate structure was formed involving the quinolyl nitrogen and a terminal phosphate group to form a seven-membered ring. Formation constants are given for two protonation and four copper(II) complexes of ligand (B) and for one protonation and four copper(II) complexes, each, of ligands (A) and (D).

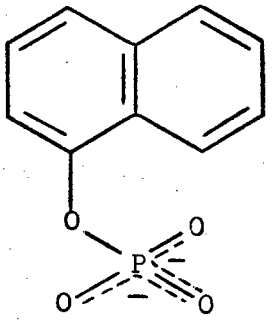
STRUCTURAL FORMULAE OF COMPOUNDS INVESTIGATED

(A)



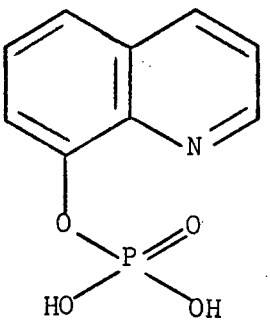
1-Naphthyl dihydrogen phosphate

Ligand (A)



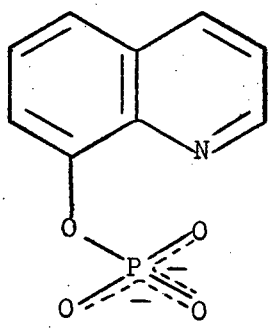
1-Naphthyl phosphate

(B)



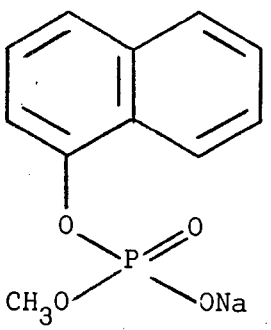
8-Quinolyl dihydrogen phosphate

Ligand (B)



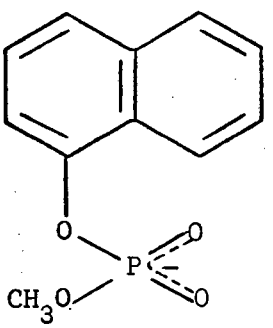
8-Quinolyl phosphate

(C)



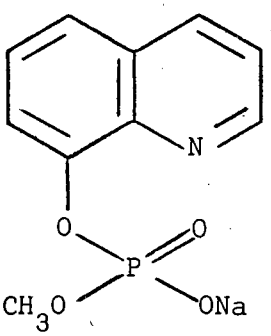
1-Naphthylmethyl phosphate
(mono-sodium salt)

Ligand (C)



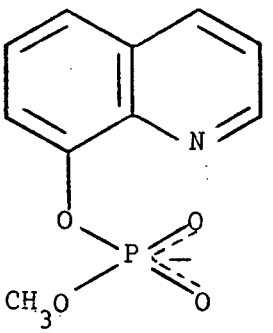
1-Naphthylmethyl phosphate

(D)



8-Quinolylmethyl phosphate
(mono-sodium salt)

Ligand (D)



8-Quinolylmethyl phosphate

GLOSSARY OF SYMBOLS

cyclen	1,4,7,10-tetraazacyclotetradodecane
PNP	4-nitrophenyl group
tn	trimethylenediamine
^{1}H NMR	proton nuclear magnetic resonance spectroscopy
Hz	Hertz
M^+	molecular ion
m/e	mass to charge ratio
tlc	thin layer chromatography
$^T\beta_{pqr}$	thermodynamic overall stability constant
β_{pqr}	stoicheiometric overall stability constant
$^TK_{pqr}$	thermodynamic stepwise stability constant
K_{pqr}	stoicheiometric stepwise stability constant
E	measured emf of the cell
E_o	collection of all constant potentials
I	ionic strength
Q_γ	activity coefficient quotient
R	crystallographic R-factor
S	Nernstein slope
T_H	total proton concentration
T_L	total ligand concentration
T_M	total metal concentration
$\bar{Z}$	average number of ligands bound to metal ion
$\bar{Z}_H$	average number of protons bound to ligand
{ }	denotes activity } Chapters 5 and 6
[ ]	denotes concentration } Chapters 5 and 6
pL	negative logarithm of ligand concentration

CONTENTS PAGE

CHAPTER 1

I N T R O D U C T I O N

1. INTRODUCTION

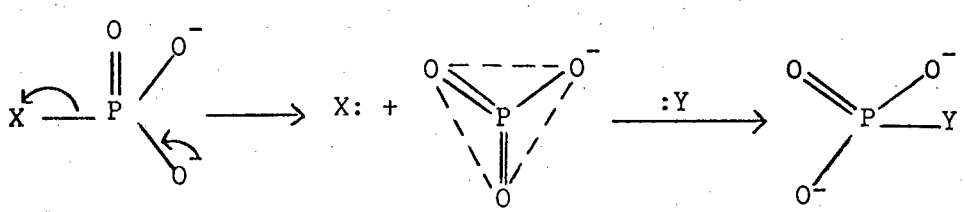
1.1 THE ROLE OF METAL IONS IN PHOSPHORYL TRANSFER - A REVIEW

Esters and diesters of phosphoric acid are known to be very stable towards hydrolysis and group transfer reactions[1-3] and yet these constitute some of the chief biological processes. In order to explain this phenomenon, the idea of intramolecular nucleophilic catalysis was conceived and a number of model systems demonstrating enhancement of hydrolysis rates due to suitable positioning of nucleophilic groups within the reacting molecule have since been described.[2,3]

Another striking feature of biological phosphate chemistry is that it all appears to be catalysed by metal ions, but although this topic has received much attention over the past three decades the exact role of the metal ion in promoting reaction is still speculative.

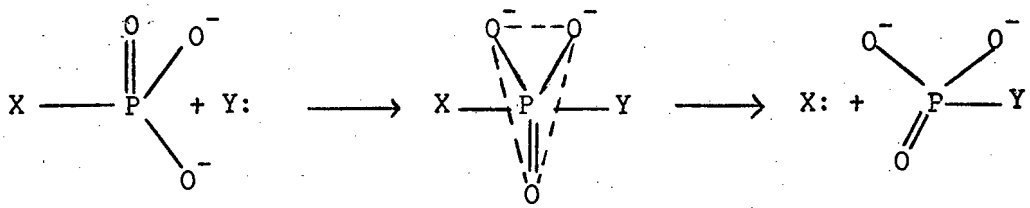
Phosphoryl and nucleotidyl transfer reactions are, in effect, nucleophilic displacements at phosphorus which may occur via two general mechanisms[4]:

i) dissociative (S_N1) mechanism which yields the planar, tricoordinate metaphosphate[5] anion as reactive intermediate on initial departure of the leaving nucleophile. Reaction with the entering ligand may then occur on either face of the metaphosphate plane in the second, usually fast, step:



Scheme 1

ii) associative (S_N2) reaction which results in formation of a pentacoordinate transition state or reactive intermediate species following initial binding of the entering nucleophile along the axis of the trigonal bipyramid. Unique to this mechanism is the ability of the intermediate phosphorus species to undergo pseudorotation.[6]



Scheme 2

In view of these two extreme mechanisms, the general absence of efficient metal ion assistance in the reactions of phosphate monoesters may be explained as a consequence of their undergoing dissociative, metaphosphate type phosphoryl transfers, in which much of the driving force for reaction derives from high electron density on the phosphoryl oxygens. Coordination of these ligands by a metal ion will effectively inhibit this pathway. Metal ion catalysis under these conditions will only occur in cases of specific coordination to the leaving group to increase its stability and decrease its pKa, or to a negatively charged nucleophile to decrease unfavourable electrostatic interactions. On the other hand, metal ion interaction with the transferable phosphoryl group itself will enhance the associative mechanism by charge neutralisation and possible electron withdrawal from phosphorus.

A number of discrete roles, then, may be envisaged for metal ion participation in phosphoryl transfer:[3,4,7]

a) charge neutralisation - it has been shown that anionic nucleophiles are prevented, by charge repulsion, from attacking dianionic phosphomonoesters and are slowed in attacking monoanionic diesters.[8a] A metal could overcome this charge barrier by charge shielding, a catalytic effect that

would be more significant for dianionic rather than monoanionic species;

b) template - a metal ion may serve as the bridging ligand in a ternary complex between a phosphate ester and a nucleophile, thus transforming a bimolecular into a unimolecular reaction;

c) coordination of the leaving group - the rates of phosphoryl transfer and nucleophilic attack on phosphate esters show a strong dependence on the pKa[8b] of the conjugate acid of the leaving group and therefore an increase in the acidity of the leaving group by metal ion coordination should be rate-enhancing;

d) complexation with a pentacovalent intermediate to stabilise the intermediate and enhance the rate, or control the stereochemical course, of the phosphoryl transfer reaction;

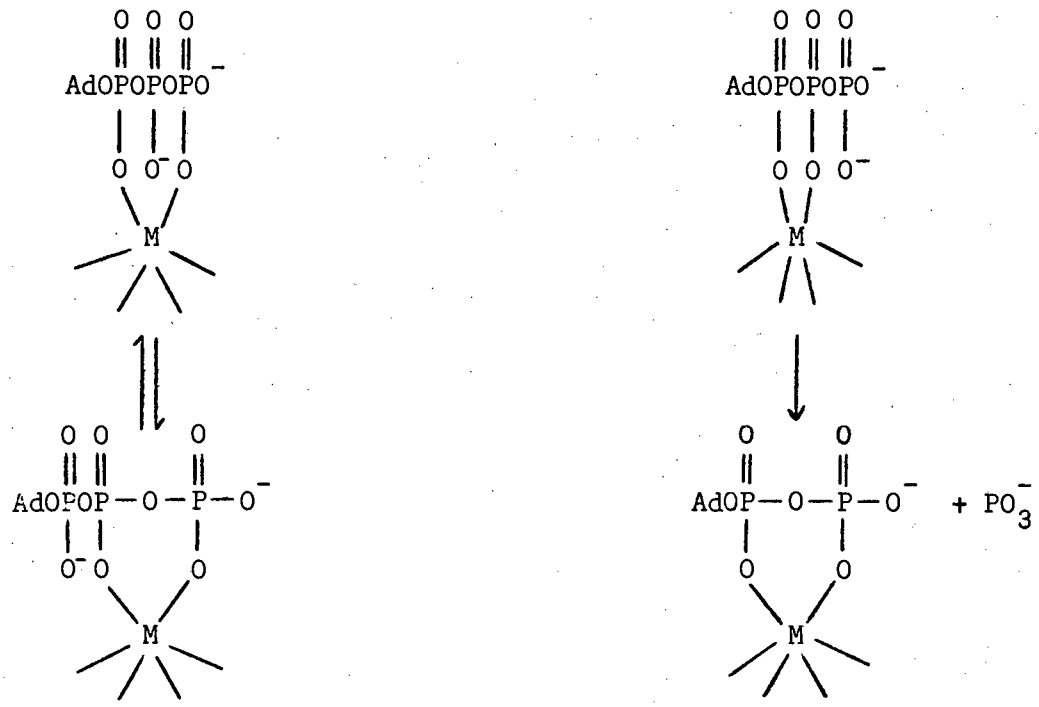
e) strain induction - bidentate-type binding of a metal ion to a single phosphoryl group could compress the O-P-O bond angle in the ground state and reduce the activation energy required for attainment of the transition state in phosphoryl transfer;

f) coordination of the entering ligand - metal ions may increase the nucleophilicity of a bound nucleophile by lowering its pKa eg. conversion of a bound neutral water molecule into a bound hydroxide.

Several more recent, representative model systems will be described to illustrate the above points.

Ramirez, Marecek and Szamosi[9] have shown that magnesium and calcium ions increase the rate of metaphosphate formation from the ATP tetraanion (S_N1 mechanism) at pH >3 in water by factors of approximately 10 and 50 respectively. If solutions of $MATP^{2-}$ contain three bidentate complexes of the cation and the polyphosphate chain in relatively rapid equilibrium with each other (Scheme 3) then formation of PO_3^- and $MADP^-$ from one of these complexes should be faster than formation of PO_3^- and ADP^- from

the tetraanion in the absence of the metal, due to negative charge neutralisation by the cation:



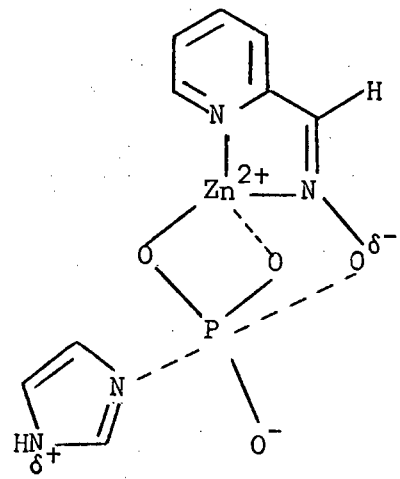
Ad = Adenosine

Scheme 3

The enhanced effect of Ca^{2+} vs Mg^{2+} on the hydrolysis of ATP^{4-} suggests that $CaADP^{-}$ is a better leaving group than $MgADP^{-}$ reflecting a higher binding of calcium than magnesium to the pyrophosphate oxyanions.

Lloyd and Cooperman[10] demonstrated the rapid transfer of a phosphoryl group from phosphorylimidazolium ion (PIm) to pyridine-2-carbaldoxime amine (PCA). Reaction via a ternary complex with Zn^{2+} serving as a bridging ligand was indicated by hyperbolic saturation kinetics and lack of reaction in the absence of the metal ion. The rates of phosphoryl transfer in the complex were from 1 to 4000 times faster than simple hydrolysis, indicating that if the charge barrier can be overcome, strong oxyanion

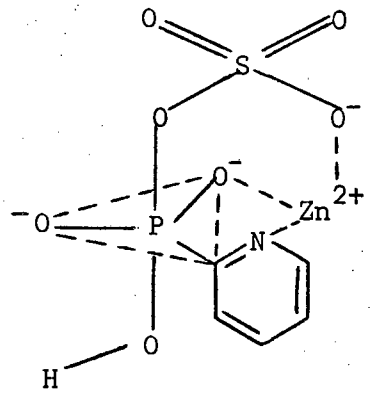
nucleophiles will attack phosphoryl dianions more rapidly than the weaker nucleophile, water. The results of this study are in accord with the mechanism (I) presented below:



(I)

Here the zinc ion has a double catalytic role. Firstly, it acts as a template to bring the phosphoryl group and oxime together with a favourable orientation for intramolecular reaction. Secondly, and more importantly, it shields the negative charge of the phosphoryl group, thus lowering the electrostatic barrier toward attack by the negatively charged oxime anion.

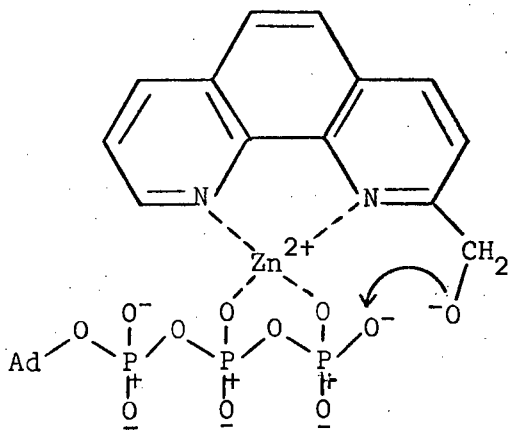
Eiki *et al*[11] studied the roles of hydrolysis of 2-pyridyl-(1) and 2-pyridylmethylphosphonosulfate (2) and compared them with those of their corresponding phenyl and benzylphosphonosulfate analogues, in the presence and absence of zinc. In the absence of the metal ion, intramolecular catalysis was observed for (2) but not for (1). The intramolecular catalysis was inhibited by the Zn^{2+} ion but was largely compensated by zinc ion catalysis. A more remarkable 20-fold rate enhancement for P-O bond cleavage of (1) in the presence of zinc was observed. The mechanism proposed for this catalysis is illustrated in (II):



(II)

The participation of the 2-pyridyl group provides an efficient metal binder for the favourable formation of the pentacovalent intermediate while the role of the metal ion is probably charge neutralisation at the phosphoryl oxyanion, facilitating the nucleophilic attack of hydroxide ion, coupled with a template effect in stabilising the intermediate.

The zinc ion has also been found to catalyse (26-fold) the phosphorylation of 1,10phenanthroline-2-carbinol by ATP, a reaction absolutely dependent on the metal ion.[12] The metal ion is thought to serve as a template for the reaction of two dissimilar ligands and the reactive ternary complex postulated to form is shown in (III):



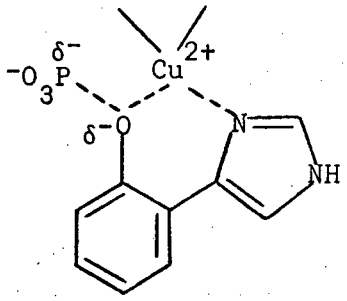
(III) Ad = Adenosine

Evidence for the formation of the ternary complex came from analysis of the phosphorylation reaction in terms of first-order reaction kinetics, together with a comparison of the reactivities of 1:1 and 1:2 Zn^{2+}-1,10-phenanthroline-2-carbinol complexes with p-nitrophenyl acetate and their reactivities with ATP. While the 1:2 complex reacted more rapidly than the 1:1 complex with the acetate, the former showed very little reaction with ATP. This was in agreement with the postulated phosphorylation mechanism - if the reaction of the 1:1 complex with ATP proceeded by complex formation rather than by way of a bimolecular process, no reaction of ATP with the 1:2 complex should be observed as the reactive ternary intermediate would not form because all the available coordinate positions around the zinc ion would be occupied.

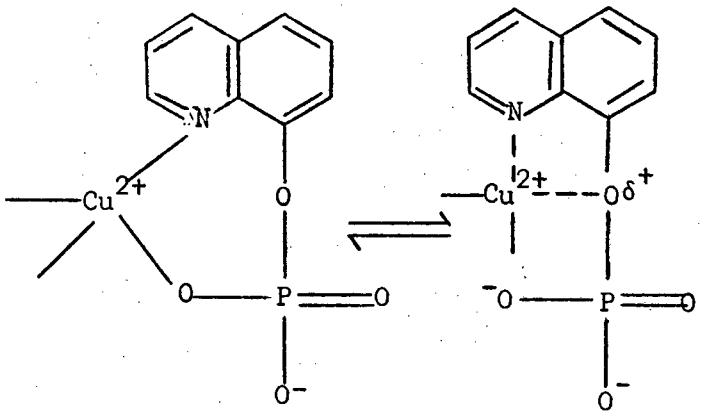
The zinc ion was also shown to reduce the pKa of the carbinol group (to 7,5), thus generating an effective nucleophile at neutral pH, which was suitably located for nucleophilic attack on the γ phosphate alone as the only derivative of 1,10-phenanthroline-2-carbinol detected was its monophosphate and very little AMP.

The third important feature of the metal ion is its coordination to the phosphates of ATP, which reduces the net negative charge on the γ phosphate and thus the electrostatic barrier to the approach of the alkoxide ion, and which also enables a stable chelated form of ADP to become the leaving group in the displacement reaction.

Examples of metal ion chelation of the leaving group are provided by (IV) and (V) which showed rate enhancements of 10^4 and 10^2 respectively, in the presence of cupric ions:



(IV)



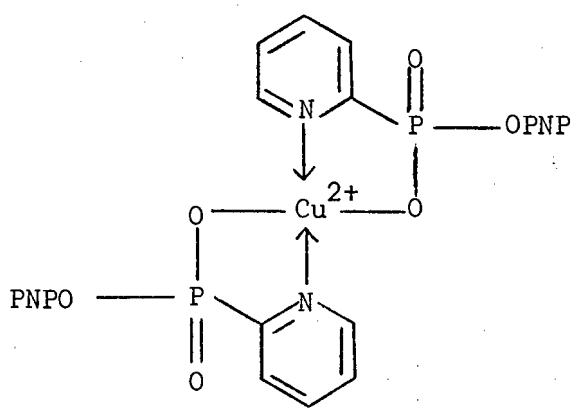
(a) (b)

(V)

For system IV, Benkovic and Dunikoski[13], found the rate acceleration to be directly proportional to the resultant decrease in leaving group pKa owing to the metal ion-oxyanion complex and to fit predictions based on the structure-reactivity correlation for the hydrolysis of phosphate monoester dianions. Murakami and Sunamoto[14a] postulate that the copper-assisted hydrolysis of 8-quinolyl phosphate involves initial formation of intermediate Va (due to a large stability constant of 5,24) followed by formation of a transition state Vb, at higher potential energy. The smaller rate acceleration for this system compared to that of IV may be due to a less favourable equilibrium between five- and seven-membered ring chelates. Takaku[14b] has in fact shown how the Cu^{2+}-8-quinolylphosphate system may be used as a phosphorylating agent for the synthesis of nucleoside 5' phosphates. If the role of the metal ion in the above cases was simply charge neutralisation, then the heteroatoms need not necessarily participate in coordination with the metal ion. These, and other examples[15-16] of specific chelation to the leaving group in order to enhance metaphosphate expulsion from a monoester dianion, all possess a common

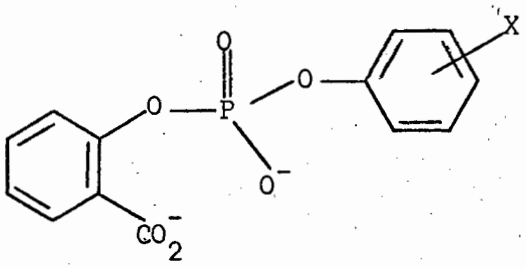
feature in that the organic phosphate demonstrates a chelate-forming tendency towards the metal ion in the initial stages by containing another functional group, besides the phosphoryl one, that displays a donating affinity for a metal ion.

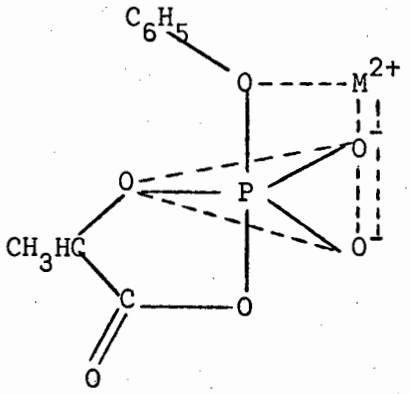
In the case of p-nitrophenyl-2-pyridylphosphonate however, Loran *et al*[17] argue that metal ion chelation serves rather to increase the electrophilicity of phosphorus through charge neutralisation and to provide steric acceleration due to ring strain on complexation, as the hydrolysis of substrate VI is thought to proceed via a pentacovalent phosphorus intermediate with the p-nitrophenoxide ion as leaving group.



(VI)

Benkovic *et al*[18a,b] have suggested that in certain cases divalent metal ions may be functioning mainly at the pentacoordinate level of the phosphoryl reaction. In the model systems studied (for example VIIa-d) hydrolysis was proposed to occur via a pentacovalent intermediate, such as that shown for another model, lactic acid-O-phenyl phosphate (VIII).





(VIII)

VIIa, X = H

b, X = M-NO_2

c, X = p-CN

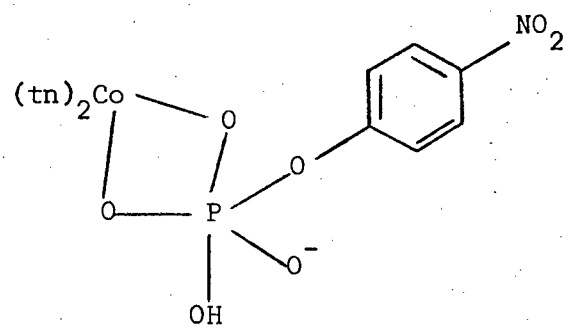
d, X = p-NO_2

The observation that exocyclic ligand expulsion accounted for >90% of the hydrolysis reactions, even if endocyclic bond cleavage was favoured on the basis of pKa values, was explained by invoking a pentacovalent intermediate that could not readily undergo pseudorotation as this would necessitate bringing a negatively charged oxygen into the apical position.[5] Such a molecule could only revert to starting materials or break-down by exocyclic ligand loss. It is noted that the stability of such a pentacovalent intermediate is enhanced relative to an acyclic species by inclusion of two of its ligands within a ring.[19] Presumably, the catalytic effect of metal ions (as much as 300-fold in the presence of Zn^{2+}) arises from further stabilisation of the intermediate (experimental evidence derived from spontaneous and zinc ion catalysed hydrolysis of VIIa-d indicates the locus of the metal ion in the reactive complex), together with facilitation of the breakdown of the intermediate, perhaps by complexation with the departing phenoxide

and/or carboxylate. A number of reasons are cited for the catalytic activity not being attributed to charge neutralisation. These model systems, then, show how both intramolecular and metal ion catalytic effects can be incorporated into a single system to confer great reactivity on a normally unreactive phosphate diester.

Recently, much interest has been generated in the catalytic effect of the cobalt(III) ion and in particular its effect on the hydrolysis of triphosphate species. This ion is especially useful in that it is substitution-inert.

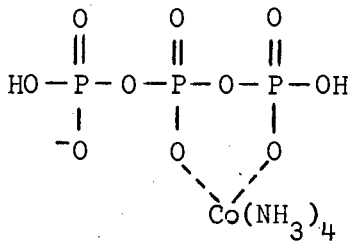
$[Co(tn)_2Cl_2]^+$ was found to chelate p-nitrophenylphosphate and to promote the hydrolysis of the ester 10^9-fold.[20] This rate enhancement was largely explained by the type of strain relief proposed by Westheimer[5] to account for accelerations observed with methylethylenephosphate relative to analogous esters lacking strained rings. Initial chelation of the phosphoryl oxygens will serve to decrease the electrostatic barrier to the approach of the hydroxide ion which then attacks to form a pentacovalent intermediate (IX)



(IX)

The strain in the parent chelate was revealed by X-ray crystallographic studies which showed O-Co-O and O-P-O bond angles of 76° and 98,7° respectively whereas, in order to maintain octahedral and tetrahedral geometries the preferred angles would be 90° and 109° respectively.

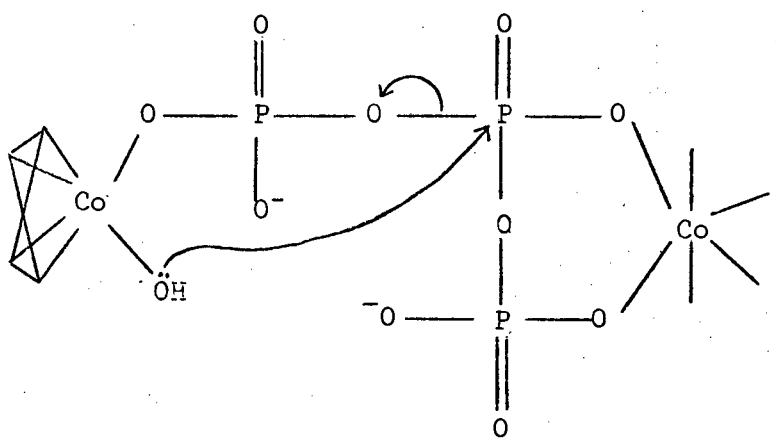
This analysis also indicated that the chelate ring was strained by the proximity of the Co and P atoms. An interesting feature of the hydrolysis of the system was that only exocyclic ligand loss was observed, whilst the hydrolysis of methylethylenephosphate occurs almost exclusively by ring opening. One would expect the four-membered ring to open more readily and thus C-O cleavage must occur in competition with that of P-O. Organic phosphate esters containing five-membered rings are more reactive than their acyclic counterparts and their six- and seven-membered ring homologues; therefore four-membered cyclic esters, to which the metal chelate approximates, will be very reactive. Indeed Cornelius[21] found that the rate of hydrolysis of bidentate triphosphate (X), which includes a six-membered ring, proceeded at two thirds the rate of that for the free ligand.



(X)

When the hydrolysis was conducted in frozen-solution (to retard the rate of dissociation of ligands from the coordination sphere of Co(III) while allowing the hydrolysis of the phosphate chain to proceed at a reasonable rate) the existence of $Co(NH_3)_4(HnPO_4)(HnP_2O_7)$ and $Co(NH_3)_4HnP_2O_7$ was revealed as intermediates in the reaction. Hydrolysis of the phosphate linkage in the six-membered chelate ring would account for the former species while the latter would arise from bond cleavage between the terminal, uncoordinated phosphate group and the chelate ring. Hydrolysis at the two sites was noted to proceed at essentially equal rates.

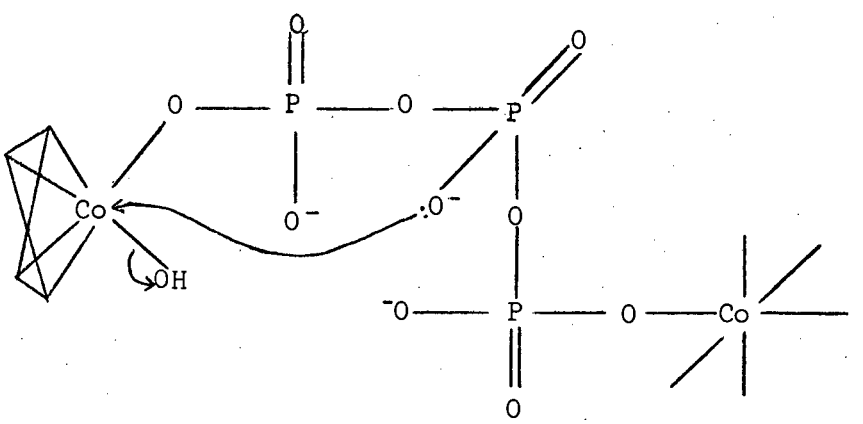
Norman and Cornelius[22] have reported that the β-γ-$[Co(NH_3)_4HnP_3O_{10}]$ complex, in which the triphosphate ion is coordinated as a bidentate ligand, undergoes a 5×10^5 hydrolytic rate acceleration, over the rate for the free triphosphate ion, in the presence of the macrocyclic tetraamine ligand, cyclen. The proposed mechanism involved coordination of the free end of the phosphate chain in the $Co(NH_3)_4H_2P_3O_{10}$ by the cyclen complex to form a dinuclear complex, followed by internal attack on the phosphate chain by a coordinated hydroxide ion (see XI):



(XI)

The active species in the reaction was shown to have a pKa of 7.9 as a rapid increase in the rate was observed on changing the pH from 7-9. The only ionisable proton to which such a value could be assigned was the coordinated water molecule in the proposed intermediate, XI. It has been suggested[23] that the function of the cobalt(III) ions in such a system is two-fold: i) to provide a good, coordinated nucleophile (hydroxide ion) and ii) to block the nucleophilic characteristics of the phosphates themselves. Two conditions must be met in order for such metal ion catalysis to occur:

i) reduction of the phosphate chain nucleophilicity through suitable chemical modification - this is exemplified by the observation that neither β-$[Co(NH_3)\ HP_2O_7]$ nor γ-$[Co(NH_3)_5HnP_3O_{10}]$ when substituted for βγ-$[Co(NH_3)_4HnP_3O_{10}]$ show any tendency towards hydrolysis over the pH range 6-9.[23] This lack of rate enhancement is thought to be due to a competing reaction, namely chelation of the polyphosphate chain onto the macrocyclic cobalt(III) complex which causes release of the co-ordinated hydroxide ion into solution and subsequent loss of activity (see XII).



(XII)

ii) simultaneous coordination of the cobalt(III) ion of the substrate polyphosphate and the attacking nucleophile 'cis' to one another[24] - the introduction of $[Co(NH_3)_4(H_2O)_2]^{3+}$ into a solution containing $[Co(NH_3)_4HnP_3O_{10}]$ causes a much larger increase in reaction rate than does $[Co(NH_3)_5(H_2O)]^{3+}$, an observation that cannot be explained by charge neutralisation.

It is proposed that the tetraamine cobalt species is the more effective catalyst as it can lose one water molecule on binding to the phosphate and still have one coordinated water molecule for nucleophilic attack

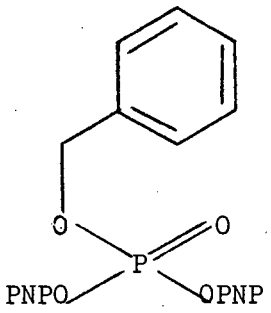
on phosphorus, whilst the pentaamine would have to lose its only water molecule on binding to the free phosphate group.

Cationic micelles have also been shown to catalyse phosphoryl transfer, although the heterogeneity of such systems makes mechanistic determinations difficult. Bunton *et al*[25] have reported that cetyltrimethylammonium bromide catalyses the hydrolysis of dianions of 2,4- and 2,6-dinitrophenyl phosphate but does not affect the hydrolysis of monoanionic forms nor the rate of attack of the dianions by hydroxide ion. They suggest that the dianion splits into two monoanions, a phenoxide ion and a metaphosphate ion, such that the electrostatic energy of the two monoanions, when incorporated into a cationic micelle, is greater than that of the parent dianion. The fact that the micelle does not assist the reaction between hydroxide ion and the dianion is thought to be due to competition between the anions for identical sites on the micelle. The incorporation of one anion greatly inhibits approach of the other. Another research group[2b] has, however, found the reaction of hydroxide ion with bis-2,4-dinitrophenyl phosphate to be catalysed some 30-fold in the presence of cationic micelles of cetyltrimethylammonium bromide. Here the phosphate and micelle form submicellar aggregates allowing the dinegatively charged transition state resulting from hydroxide attack to be formed in the micelle rather than in the water so as to lower its free energy of activation.

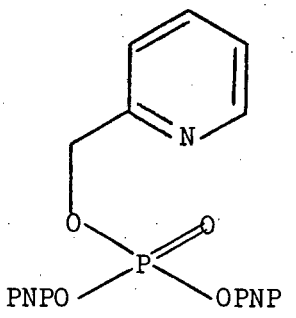
It is well known that most enzymes that catalyse phosphoryl transfer require divalent metal ions[27] and therefore the determination of how these metal ions facilitate simple, non-enzymic phosphoryl-transfer reactions is an important primary approach to understanding their roles in enzymic systems. The model studies discussed present clear evidence for the versitility of metal ions in catalysing nucleophilic attack on phosphoryl compounds and suggest intermediates which may simulate those formed in enzyme-substrate complexes.

1.2 OBJECTIVES OF THE RESEARCH

In view of the scarcity of literature pertaining to the reactivity of organic phosphate esters containing heteroatomic moieties such as pyridyl nuclei, the following compounds (XII) and (XIII) have been synthesised in this laboratory.[28]



(XII)



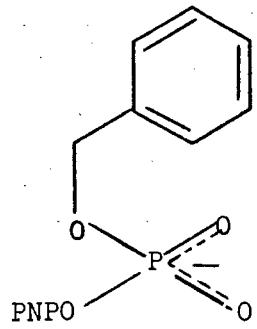
(XIII)

PNP = 4-nitrophenyl group

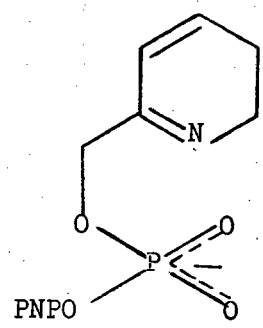
System (XIII) may be described as a simple model for the ATP molecule as it contains an "energy-rich" phosphorus oxygen bond (P~OPNP), a good leaving group ($PNPO^-$) and a tertiary nitrogen atom in one of the ester groups which may display anchimeric assistance in the hydrolysis of the triester. In fact, preliminary studies of the rates of hydrolysis by aqueous NaOH of substrates (XII) and (XIII) revealed the pyridyl phosphate to be 14 times more reactive than its benzyl analogue. Intramolecular nucleophilic catalysis by the pyridyl nitrogen may be responsible for the rate acceleration as such a form of catalysis would not be possible in the benzyl compound. However the low order of magnitude of the rate enhancement may indicate that the differences in hydrolytic rates are rather due to the inductive effect of the pyridyl ring.

Due to the difficulty experienced with systems (XII) and (XIII)

in determining when the second 4-nitrophenyl ester linkage started to be cleaved together with the first, attention was turned to a pair of diesters (XIV) and (XV) which, as a chemical group, are biologically more important than triesters.

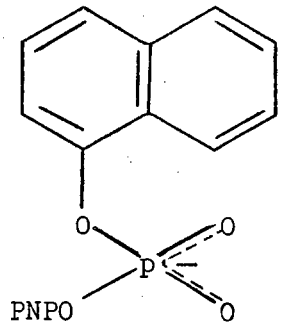


(XIV)

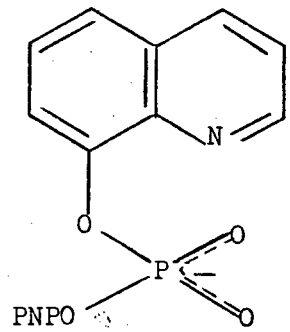


(XV)

With the idea that chelate formation between the phosphate ester and a metal ion could result in greatly enhanced rates of hydrolysis, an investigation of the stability constants of some metal chelates of (XIV) and (XV) was proposed. In both systems, catalysis may involve interactions of the metal ion with the phosphoryl group and/or the ester oxygen, but in system (XV), a third binding site, namely the pyridyl nitrogen is available. However, synthetic difficulties (see Chapter 2) led to the formulation, rather, of another pair of phosphate diesters (XVI) and (XVII), that are very similar to (XIV) and (XV) respectively, but that, having more rigid skeletons, it was hoped would be more stable and easier to prepare.



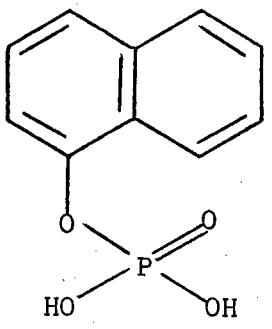
(XVI)



(XVII)

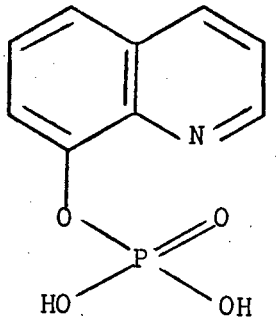
Unfortunately, synthetic routes to these substrates were also found not to be without major complications.

Once again a new set of simpler target models (A-D) were selected for investigation and successfully prepared and characterised:



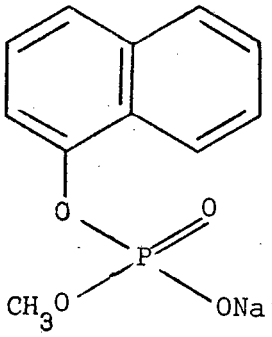
1-naphthyl dihydrogen phosphate

(A)



8-quinolyl dihydrogen phosphate

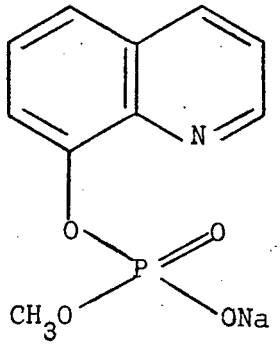
(B)



1-naphthylmethyl phosphate

(mono-sodium salt)

(C)



8-quinolylmethyl phosphate

(mono-sodium salt)

(D)

The 4-nitrophenyl moiety proved to be too good a leaving group and its replacement with a methyl group, which is easily identified, more stable towards nucleophilic displacements and very stable towards other types of reaction, allowed relatively trouble-free preparation of the above substrates (see Chapter 3). A study of the stability constants of metal ion (copperII) complexes formed with each of the esters (A-D) was of interest in order to determine the importance of a heterocyclic atom in

one of the ester groups, and also to compare the reactivities of phosphate monoesters with those of their diester analogues. As has been mentioned before, monoesters and diesters are the most important forms of biological phosphate species.

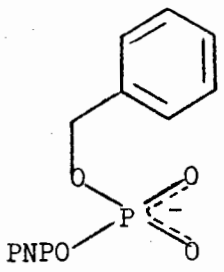
CHAPTER 2

ATTEMPTED SYNTHESES OF BIOLOGICALLY REPRESENTATIVE PHOSPHATE DIESTERS

2. ATTEMPTED SYNTHESES OF BIOLOGICALLY REPRESENTATIVE PHOSPHATE DIESTERS

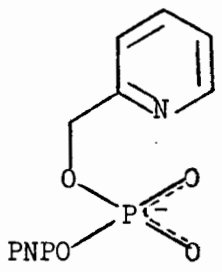
2.1 BENZYL (4-NITROPHENYL)PHOSPHATE and (2-PYRIDYLMETHYL)(4-NITROPHENYL)PHOSPHATE

As discussed in Chapter 1.1, the initial set of model phosphate diesters formulated for synthesis and subsequent solution chemistry studies, comprised benzyl(4-nitrophenyl)phosphate - (XIV) and (2-pyridylmethyl)(4-nitrophenyl)phosphate (XV); two analogous substrates differing only in the chemical group at position two in the unsubstituted aryl ring:



(XIV)

benzyl(4-nitrophenyl)phosphate

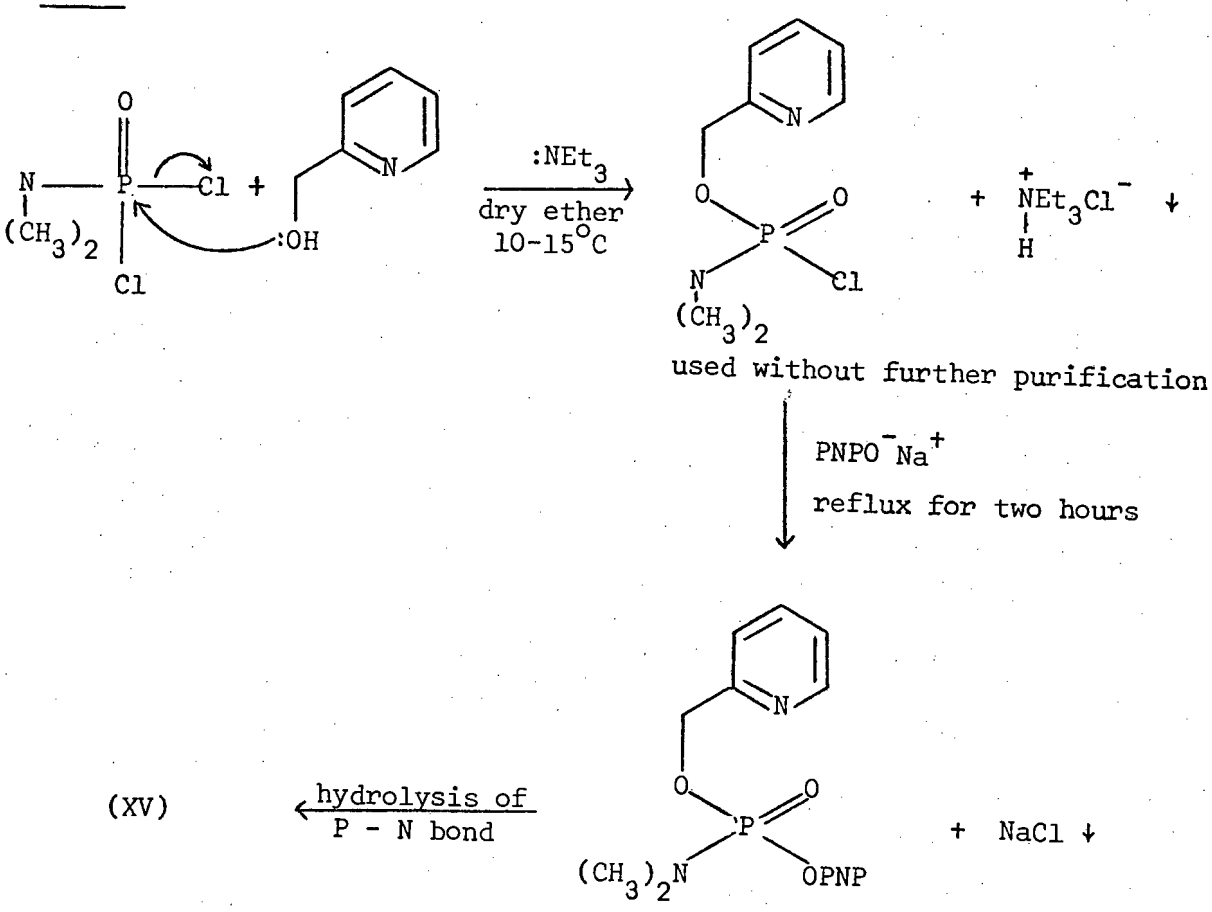


(XV)

(2-pyridylmethyl)(4-nitrophenyl)phosphate

A number of routes to the syntheses of compounds (XIV) and (XV) were attempted, with varying degrees of success. As the pyridyl phosphate was known to be more reactive towards the hydroxide ion,[28] it was envisaged that it might well be the more difficult to prepare and therefore, in most cases, its synthesis was attempted first; the idea being to use similar preparative methods for both substrates.

2.1.2



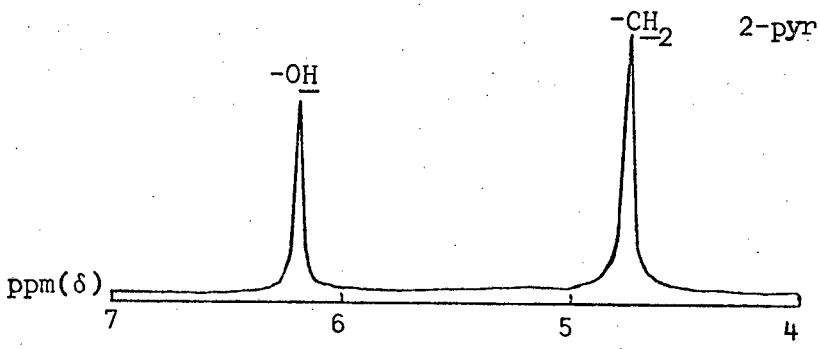
Scheme 4

Scheme 4 depicts the phosphorylation of 2-pyridylmethanol by N,N-dimethylphosphoroamidodichloridate followed by substitution of the second chloro-group for a 4-nitrophenoxy moiety. Effective hydrolysis of the P-N bond would then result in production of the desired substrate (XV).

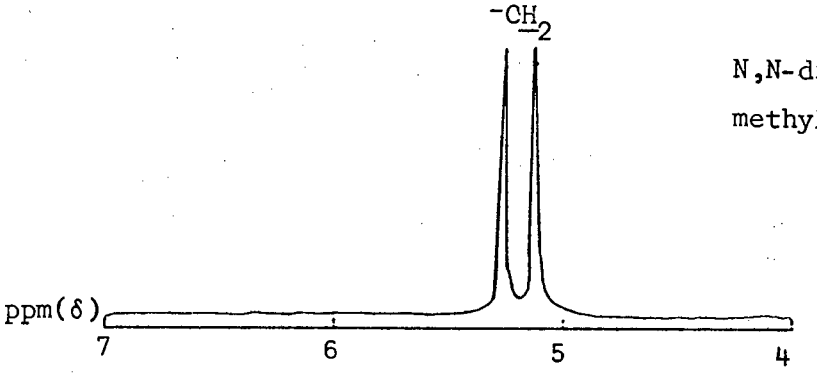
To a solution of N,N dimethylphosphorodichloridate was added dropwise, with stirring and cooling in ice (10-15°C), a mixture of equimolar volumes of both 2-pyridylmethanol and triethylamine. A fine white precipitate of triethylamine hydrochloride formed immediately. Once addition was completed the reaction mixture was stirred for a further two hours and then left to stand for sixteen hours to ensure complete reaction. The successful coupling between the phosphate and the alcohol was confirmed by thin layer chromatography (tlc)(acetone:chloroform; 7:3)

which indicated no traces of alcohol and by ^{1}H NMR($CDCl_3$) which showed the appearance of a symmetrical doublet at δ5,20 ($^{3}J_{H,P}$ = 8 Hz). This splitting of the $-CH_2$ signal (due to coupling with the ^{31}P nucleus) and its downfield shift (owing to the electron-withdrawing effect of the phosphoryl group) relative to the singlet (δ4,77) in the spectrum of the pure alcohol, is a useful probe for the formation of the $P-O-CH_2$ bond.

Compare:



2-pyridylmethanol



N,N-dimethyl(2-pyridyl-methyl)phosphorochloridate

The sodium salt of 4-nitrophenol was prepared in almost quantitative yield by dissolving 4-nitrophenol in a minimum volume of ethanol and adding, with stirring, a stoicheiometric amount of sodium hydroxide dissolved in a minimum volume of water. An exothermic reaction produced a solid yellow precipitate of the hydrated salt which was dried to constant weight at 115°C to yield a red-orange powder.

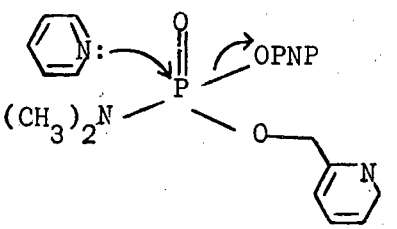
The N,N-dimethyl(2-pyridylmethyl)phosphoroamidochloridate prepared previously was used without further purification or removal of the triethylamine salt, so as to reduce the chance of hydrolysis of the P-Cl bond. 100% yield of the phosphate was assumed and to the crude ethereal solution was added slowly (due to the heat effect), with stirring, an equimolar portion of the sodium salt of 4-nitrophenol. When the addition was completed, the mixture was stirred for 30 minutes and then refluxed for 2 hours. After cooling, the precipitates of sodium chloride, triethylamine hydrochloride and any excess of sodium 4-nitrophenoxide were removed by gravity filtration; the filtrate was washed with water to remove all traces of ionic products and dried over sodium carbonate. Removal of the ether *in vacuo* left a pale brown solid which tlc and ^{1}H NMR revealed to be a mixture of the desired phosphate (76%) and 4-nitrophenol (24%).

Various methods were tried in attempts to remove the unwanted 4-nitrophenol including:

i) treatment with alumina and acid alumina - the alumina turned yellow but tlc still showed signs of 4-nitrophenol indicating perhaps some further hydrolysis of the product;

ii) column chromatography - a benzene:pyridine; 95:5 solvent system was found to give a good separation of the phosphate and 4-nitrophenol, although the latter moved faster (r.f. 4-nitrophenol - 0,52; r.f. N,Ndimethyl-(4-nitrophenyl)(2-pyridylmethyl)phosphate - 0,23). Efforts to find a solvent system that retarded the movement of the phenol relative to that of the desired product were unsuccessful. Eventually the crude phosphate was purified on a short silica gel column using a benzene:pyridine; 95:5 solvent to elute the 4-nitrophenol followed by a benzene:acetone; 80:20 system to elute the phosphate. The purity of the phosphate was confirmed by tlc, ^{1}H NMR, sharp melting point and microanalysis. However

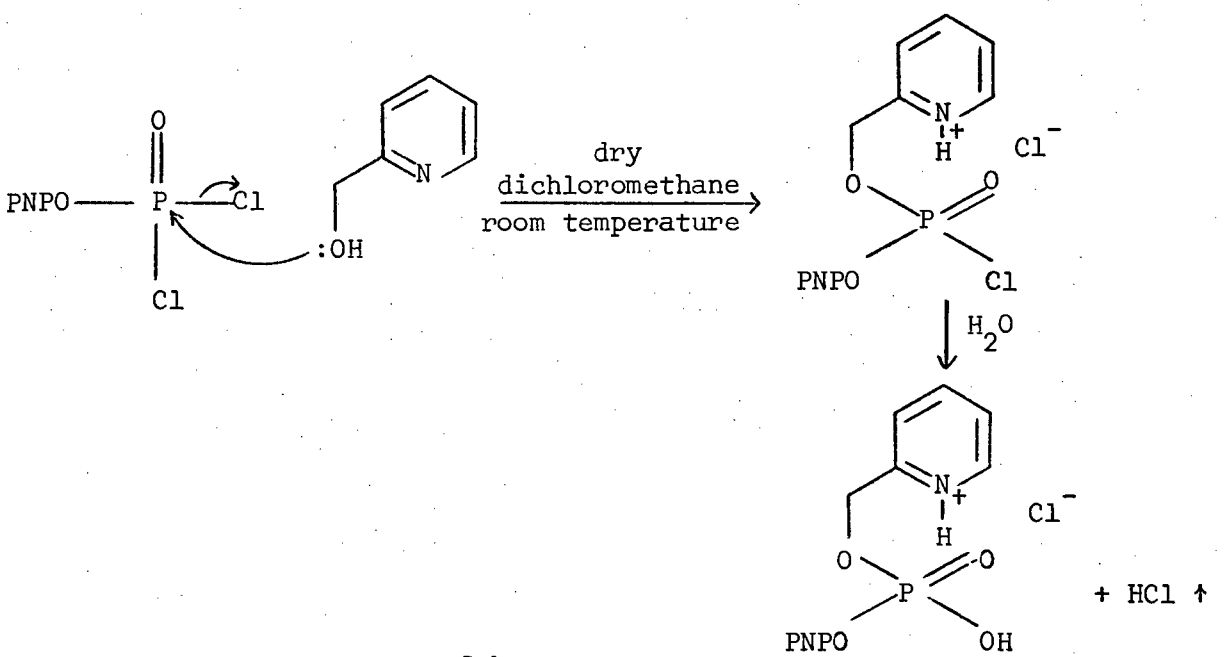
the yield was only 3%. It has been shown that the hydrolysis of 4-nitrophenyl phosphate dianion is catalysed by pryidine type buffers via a nucleophilic pathway involving a phosphopyridine intermediate.[29] Possibly the phosphate was in the presence of pyridine long enough for the substantial hydrolysis of the P-OPNP bond to occur through interactions of the type:



Due to this low yield and the fact that hydrolysis of the amine function had still to be effected in order to obtain the target compound, (2-pyridylmethyl)(4-nitrophenyl)phosphate, this route was abandoned in favour of the method described in 2.1.2 below.

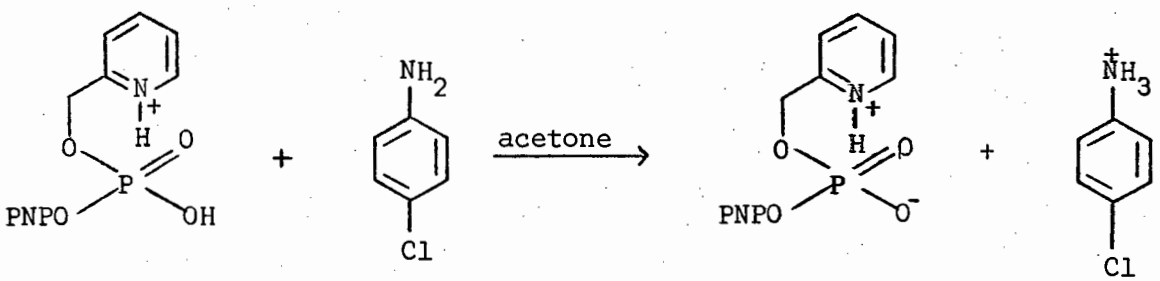
2.1.2

Using 4-nitrophenylphosphorodichloridate as a phosphorylating agent, Scheme 5 was then proposed as a means for synthesising (XV) after a report by Loran and Williams[30] for the phosphorylation of 8-quinolinol:



Scheme 5

To a suspension of 4-nitrophenylphosphorodichloridate in dry dichloromethane was added dropwise, with stirring, an equivalent of 2-pyridylmethanol dissolved in dry dichloromethane. The phosphate suspension dissolved as the reaction proceeded to leave a clear solution. Once addition was complete stirring was continued for one hour and the mixture was then left to stand for 18 hours. Two layers were seen to have separated out - a smaller lower layer [probably a solution of dichloromethane in the (4-nitrophenyl)(2-pyridylmethyl)phosphorochloridate product] and a larger upper layer [probably a solution of phosphate product in dichloromethane]. A stoicheiometric amount of water was added slowly with vigorous stirring, which was continued for a further sixty minutes after addition was finished. Initial removal of solvent gave rise to a highly viscous oil from which complete removal of dichloromethane was not possible. Also unsuccessful were efforts to isolate the desired phosphate product from the oil; for example by recrystallisation or by treatment with 4-chloroaniline until the isoelectric pH of the phosphate was reached - theoretically:



It may well have been preferable to have included in the reaction an external base, stronger than that of the nitrogen centre in 2-pyridylmethanol, to trap the HCl generated on esterification and hydrolysis, rather than relying on the internal, pyridyl base.

2.1.3

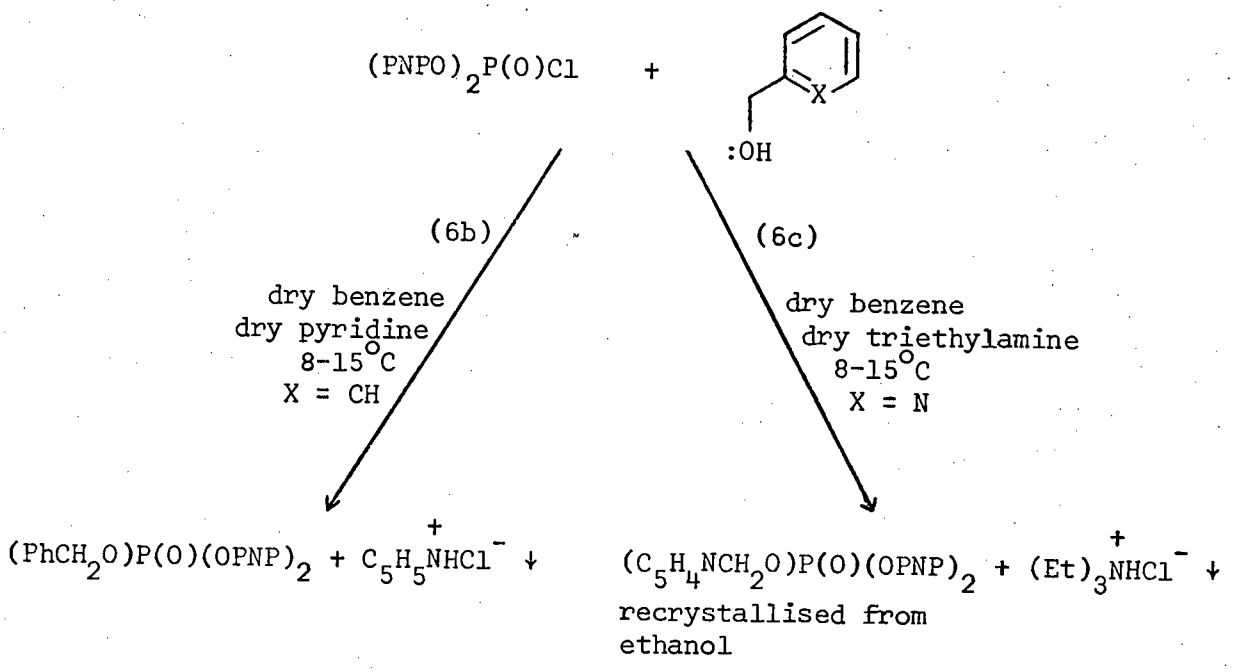
Two analogous triesters, benzyldi(4-nitrophenyl)phosphate and (2-pyridyl)di(4-nitrophenyl)phosphate have previously been prepared in

in this laboratary and the rates of the hydrolysis of one P-OPNP bond in the presence of base have been studied.[28] It was proposed, then, to use these esters to generate the corresponding diesters of interest, (XIV) and (XV), by the careful base-catalysed hydrolysis of a single P-OPNP bond.

The phosphate triesters were synthesised as outlined in Schemes 6a, b and c.

(6a)

$$(PhO)_2P(O)Cl \xrightarrow[HNO_3/H_2SO_4,\ 5\text{-}10^{\circ}C]{\text{nitration}} (PNPO)_2P(O)Cl$$



Scheme 6

The nitration of diphenylphosphorochloridate was performed as outlined by Murayama *et al*.[31]

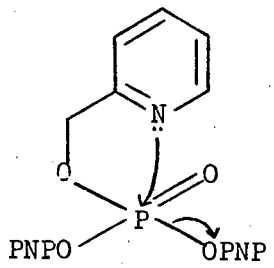
As with the other phosphorylations described earlier, the esterification

of di(4-nitrophenyl)phorphorochloridate with benzyl alcohol or 2-pyridylmethanol resulted from nucleophilic attack at the phosphorous centre by the alcohol. Benzyldi(4-nitrophenyl)phosphate was prepared by dissolving equimolar amounts of benzyl alcohol and pyridine in dry benzene and adding dropwise to this stirred solution, with cooling in ice, an equimolar portion of di(4-nitrophenyl)phosphorochloridate dissolved in dry benzene. Removal of HCl formed during the reaction was effected by the pyridine and a dense white precipitate of pyridinium hydrochloride was seen to form in the reaction solution. Tlc was used to monitor the reaction (r.f. benzyl alcohol 0,32; r.f. benzyldi(4-nitrophenyl)phosphate 0,43 in a solvent system of acetone:chloroform; 7:3). Once the reaction was considered complete the precipitate was removed by suction filtration and the solvent removed from the filtrate *in vacuo* to yield the desired product.

Initially the preparation of (2-pyridylmethyl)di(4-nitrophenyl)-phosphate was attempted in an analogous fashion to Scheme 6b, but using an excess of pyridine, as a solvent, in order to remove the HCl formed during the esterification and also to prevent protonation of the pyridyl nitrogen of the substrate alcohol. However, cleavage of the P-OPNP bond was shown to occur by the yellowing of the reaction solution, due to the release of the 4-nitrophenolate ion, and tlc monitoring of the reaction [r.f. 4-nitrophenol 0,60; r.f. (2-pyridyl)di(4-nitrophenyl)phosphate 0,42; r.f. 2-pyridylmethanol 0,22 in a solvent system of acetone:chloroform, 7:3]. Again, this was thought to be due to the nucleophilic attack at the phosphorus centre by pyridine. It was then decided to revert to using triethylamine as a base in 1:1 stoicheiometric ratio with both the alcohol and di(4-nitrophenyl)phosphorochloridate and employ benzene as a solvent.

In both cases the identities of the phosphate products were confirmed by the presence of a symmetrical doublet (δ5,33; $^{3}J_{H,P}$ = 11 Hz for

the benzyl phosphate and δ5,43; $^{3}J_{H,P}$ = 10 Hz for the pyridylmethyl phosphate) for the $-CH_2$ group in the ^{1}H NMR spectra and by microanalysis. The phosphorylation of benzyl alcohol always gave yields >70% after filtration to remove the pyridinium hydrochloride salt followed by removal of the solvent from the filtrate. A little of the desired product was always found to be adsorbed to the salt and was recovered by washing the salt with water. With the pyridyl analogue, however, the best yield obtained after filtration and removal of the solvent was 48%. The salt of triethylamine hydrochloride was always found to be greatly contaminated with the pyridylmethyl phosphate - washing with water was not effective. The (2-pyridylmethyl)di(4-nitrophenyl)phosphate always required purification - mainly to remove 4-nitrophenol - when isolated from the benzene medium. Recrystallisation from ethanol was found to be the best method as decomposition was found to occur when wet column chromatography was attempted, possibly due to intramolecular interactions of the type:



The difficulties experienced in the synthesis of the pyridylmethylphosphate relative to that of its benzyl analogue were an early indication of the greater instability or reactivity of the former.

With reference to a paper by Moffatt and Khorana;[32] mild alkaline hydrolysis was used to remove one of the 4-nitrophenyl groups. Sodium hydroxide was the hydrolysing agent of choice as the sodium salt of the phosphate diester would result - the sodium ion is commonly found in

biological systems. The procedure used is outlined below for the benzyl phosphate (Scheme 7).

$(PNPO)_2P(O)(OCH_2Ph)$

| 2NaOH

| i) acetonitrile (solvent)

| 30 mins

$(PNPO)P(O)(OCH_2Ph)O^-Na^+$ + $PNPO^-Na^+$ + H_2O

| ii) partial evaporation to remove acetonitrile

| iii) dropwise addition of acetic acid to adjust the pH of the solution to 5 and ensure complete protonation of the 4-nitrophenolate ion (pKa 4-nitrophenol - 7,15[33a]):

$PNPO^-Na^+ \xrightarrow[pH5]{H^+} PNPOH + Na^+$

| iv) extraction with ether, until the ether layer gave no yellow colour with alkali, to remove 4-nitrophenol:

$PNPOH \xrightarrow{-H^+} PNPO^-$

colourless yellow

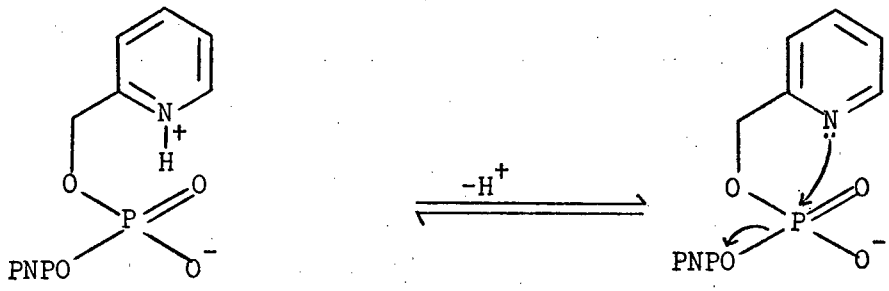
| v) removal of the solvent *in vacuo*

↓

$(PNPO)P(O)(OCH_2Ph)O^-Na^+ + CH_3COO^-Na^+$

Scheme 7

Before trying to purify this benzyl phosphate by ion exchange chromatography or recrystallisation, an attempt was first made to convert its pyridyl analogue in the same way. This was unsuccessful as both nitrophenyl groups were removed. On learning that the pKa for the nitrogen centre in 2-pyridylmethanol is 4,89[33b] it was surmised that loss of the second nitrophenyl function was again probably due to intramolecular effects:



pH < 5 pH > 5

2.1.4.

A publication by Niewiarowski, Stec and Zielinski[35] for the preparation of nucleoside-O-(4-nitrophenyl)phosphoroanilidates by the action of O-(4-nitrophenyl)N-phosphoroamidochloridate on alcohols and their subsequent conversion to the corresponding 4-nitrophenyl phosphate esters, encouraged the performance of the following experiments in the attempts to synthesise (XIV) and (XV).

$$(PNPO)P(O)Cl_2 + 2NH_2Ph \xrightarrow[\text{room temperature}]{\text{benzene}} \underset{68\%}{(PNPO)P(O)(NHPh)Cl} + \overset{+}{N}H_3PhCl^- \downarrow$$

Scheme 8

This reaction was achieved by dissolving (4-nitrophenyl)phosphorodichloridate in benzene and adding a two molar equivalent of aniline dissolved in benzene, with stirring, over a period of sixty minutes. A white precipitate of anilinium hydrochloride separated out immediately. Once all the aniline had been added, the solution was stirred for four hours. It was then heated to almost boiling and the precipitate filtered off while hot in an effort to reduce loss of product due to adsorption to the precipitate. The phosphate was then left overnight to recrystallise from the benzene solution. Once the first crop of crystals had been obtained, the mother liquor was reduced to a third of its volume and a second crop of crystals was formed on leaving this solution to stand

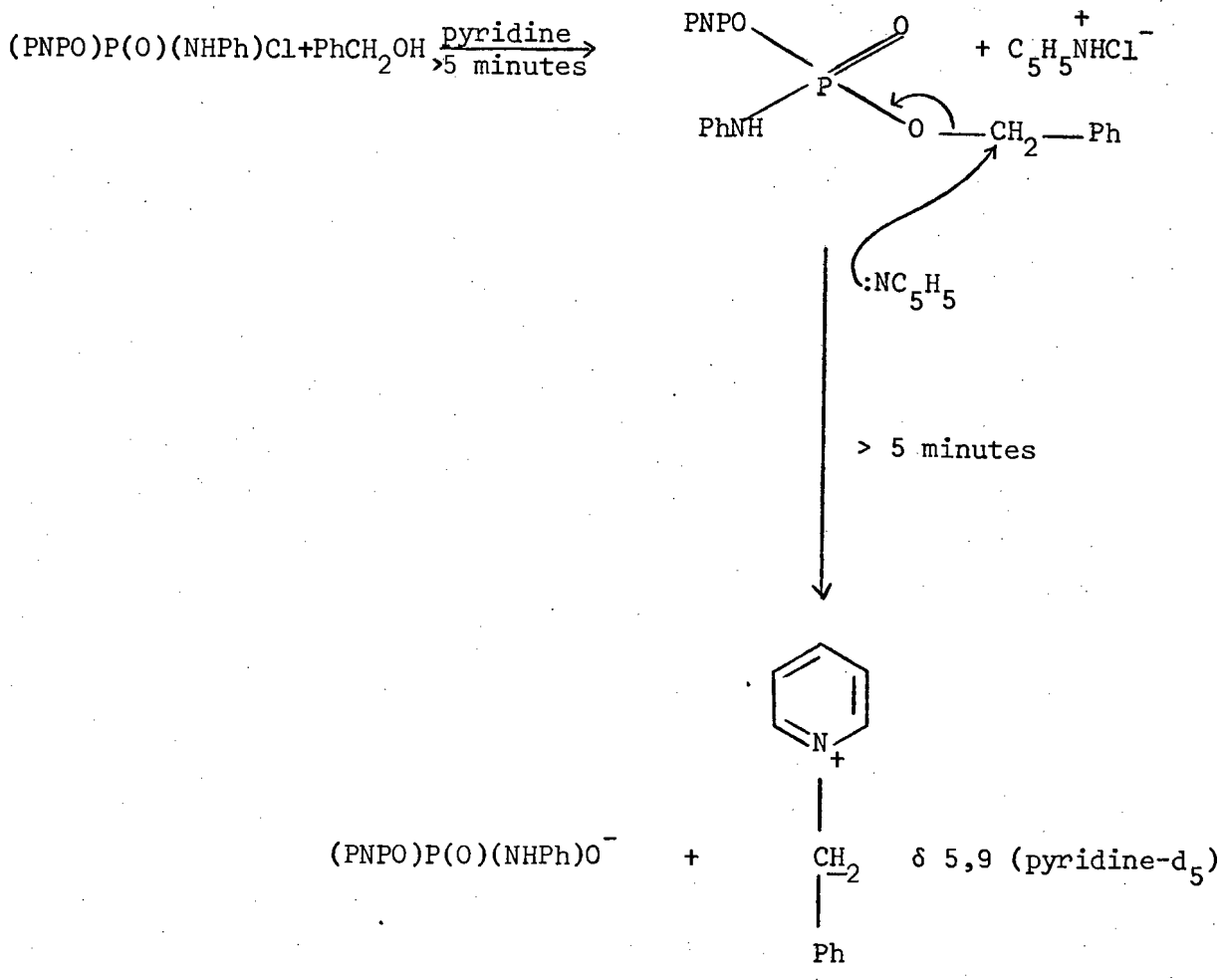
overnight. The phosphate was characterised by ^{1}H NMR, microanalysis and melting point determination.

The second step concerned the phosphorylation of benzyl alcohol or 2-pyridyl methanol:

$$(PNPO)P(O)(NHPh)Cl + PhCH_2OH \xrightarrow[5\ mins]{pyridine} (PNPO)P(O)(NHPh)(OCH_2Ph) + C_5H_5\overset{+}{N}HCl^- \quad 74\%$$

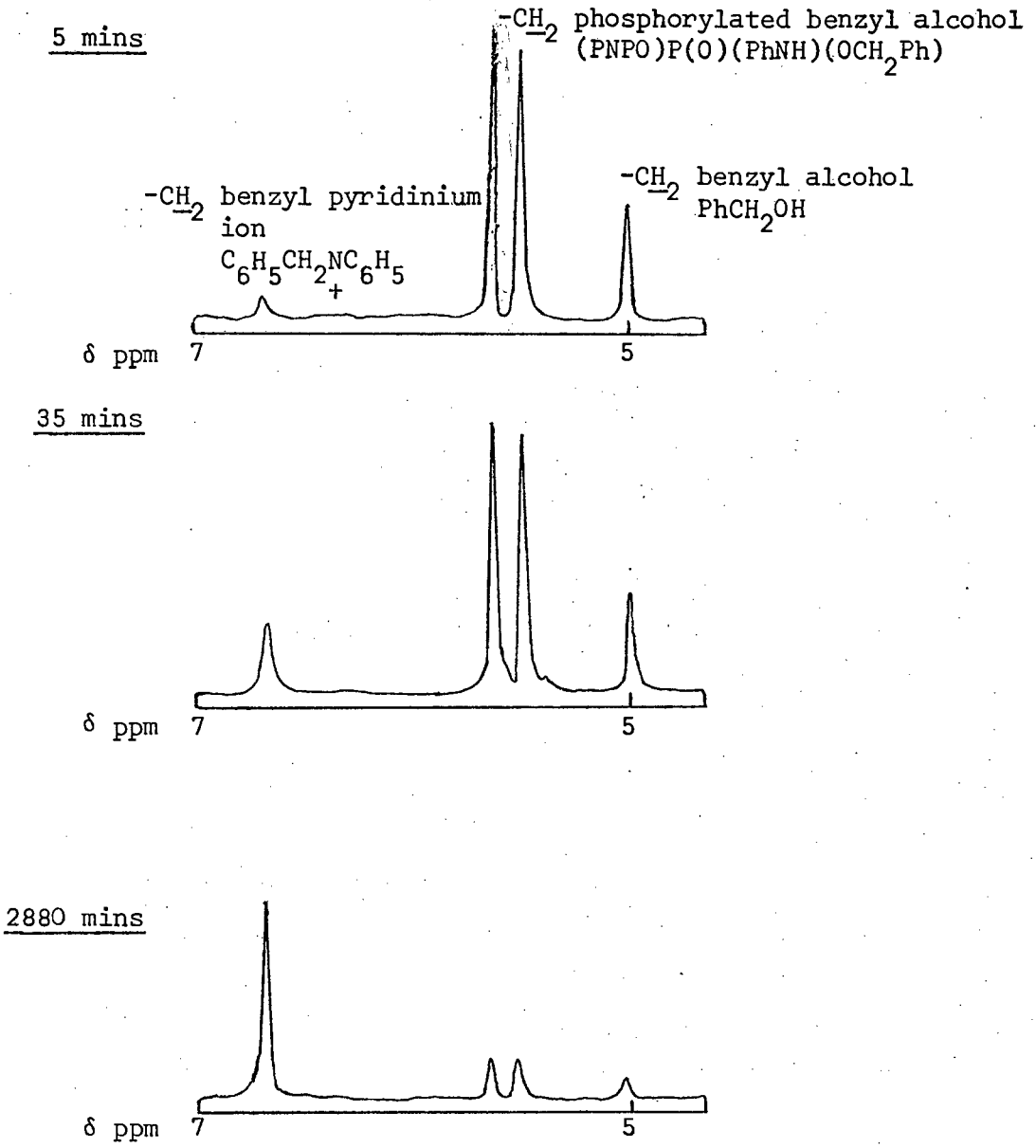
Scheme 9

^{1}H NMR experiments using pyridine-d_5 showed the phosphorylation of benzyl alcohol to be very time-dependent due to the effective N-benzylation of pyridine which started after five minutes:



Scheme 10

The production of the N-benzyl pyridinium ion was confirmed by a control experiment in which benzyl chloride was added to a two-fold excess of pyridine and allowed to stand for 12 hours. A new singlet appeared in the ^{1}H NMR spectrum at δ6,4($CDCl_3$) due to substitution of the chloride by pyridine [-CH_2 benzyl chloride-δ4,6($CDCl_3$);-CH_2 N-benzyl pyridinium ion-δ5,9 but measured in pyridine which would account for the upfield shift of this experiment relative to that in the control experiment].

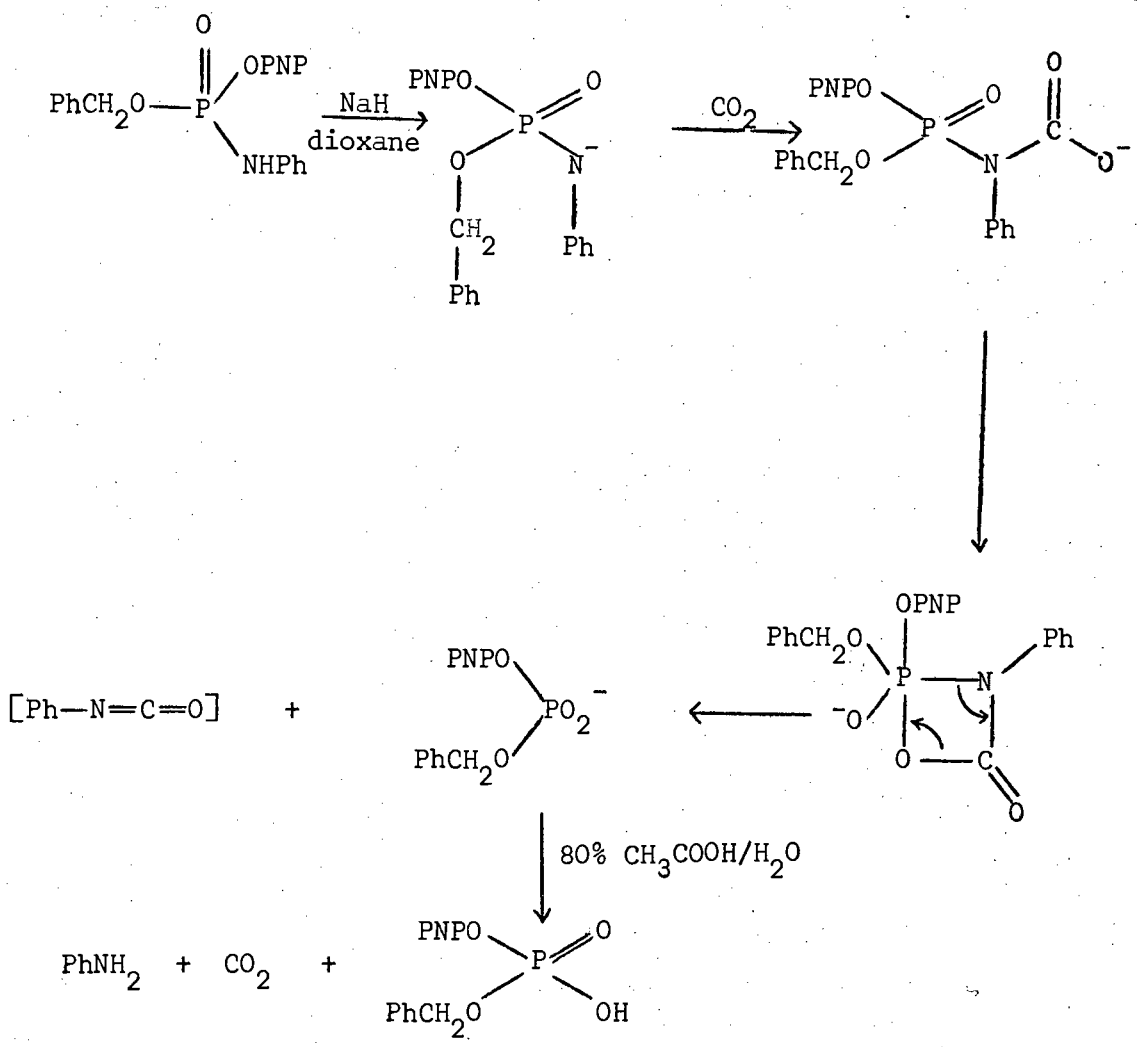


Sequence of ^{1}H NMR(pyridine-d^5) Spectra Providing Evidence for the N-Benzylation of Pyridine Leading to the Decomposition of Benzyl(4-nitrophenyl)phosphoranilidate

In order to ensure the maximum yield, then, of O-benzyl(O-4-nitrophenyl)-phosphoranilidate the reaction between the chlorophosphate and the alcohol was quenched after five minutes by pouring the reaction solution into cold water. The phosphorylated alcohol precipitated out and was recovered by suction filtration. No further purification was required (74% yield; characterised by ^{1}H NMR, microanalysis, melting point).

The phosphorylation product formed with 2-pyridyl methanol was found to be more stable in pyridine than that formed with the benzyl alcohol and did not decompose. After a thirty minute reaction period the reaction solution was poured into cold water and the pyridyl phosphate separated out as a red oil. It was purified by column chromatography using a solvent containing chloroform:acetone; 10:3 and characterised by ^{1}H NMR, microanalysis and sharp melting point. (Yield 40%.)

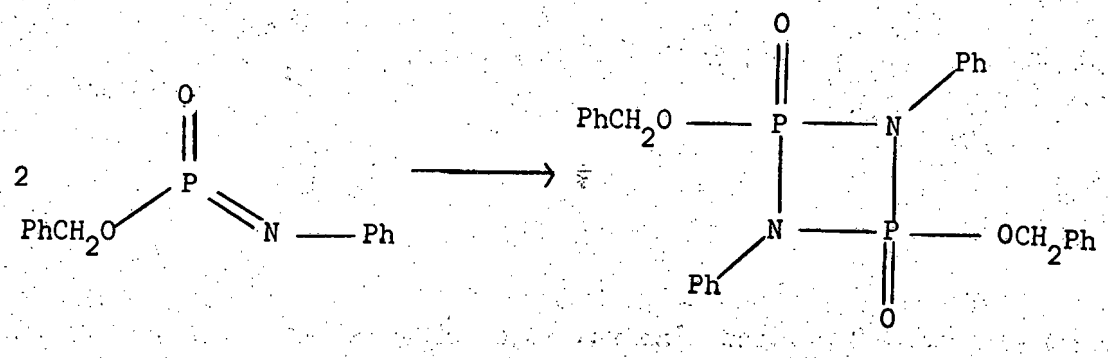
The next step was to hydrolyse the P-N bond. Following a method reported by Stec *et al*[34,35] for the cleavage of such bonds in sugar phosphates, the ideal reaction scheme was formulated as shown in Scheme 11:



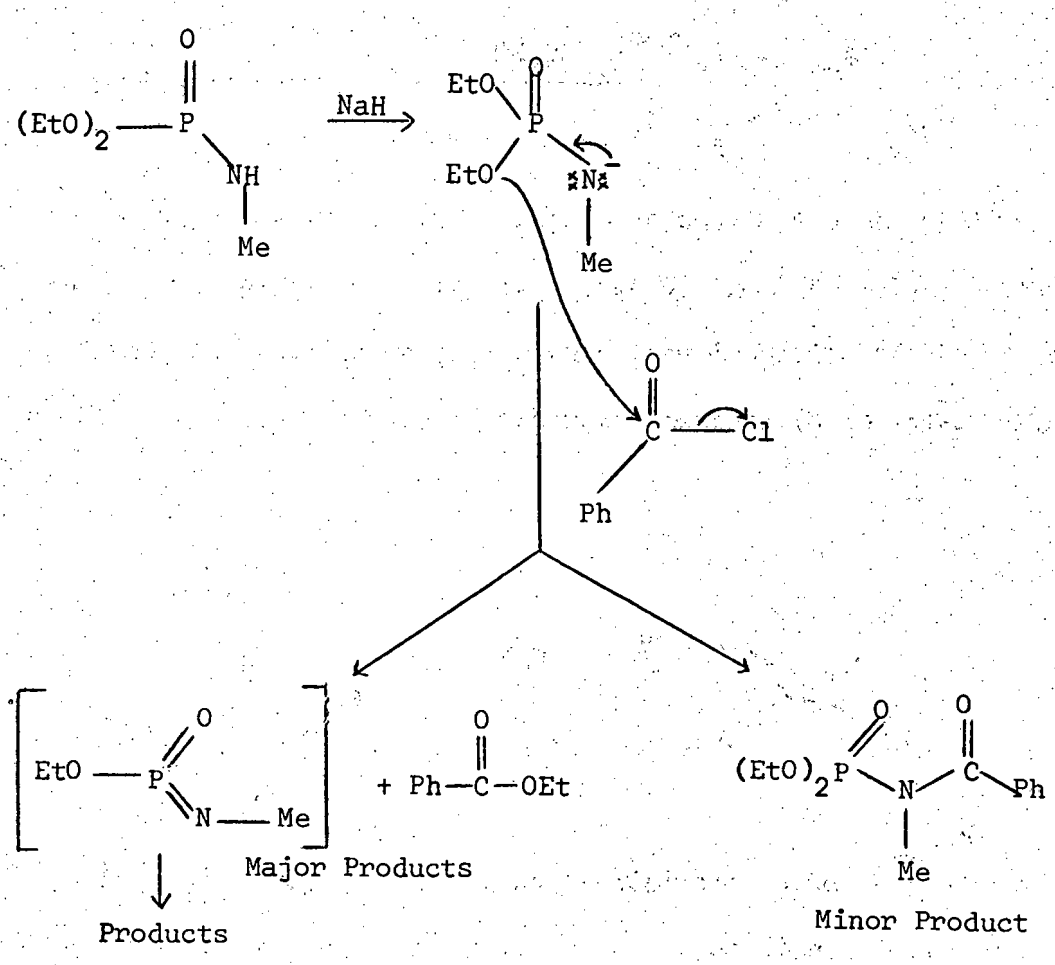
Scheme 11

All reagents were rigorously dried before use. Dioxane was dried by refluxing for three hours over sodium wire and distilling over fresh sodium wire onto 4Å molecular sieves. The O-benzyl-(O-4-nitrophenyl)-phosphoranilidate was dissolved in dioxane and added dropwise, with stirring, to a 100% molar excess of sodium hydride suspended in dioxane. The reaction mixture turned from green to yellow to a light orange. Once addition of the phosphate was complete, stirring was continued for a further twenty minutes and then dry carbon dioxide was bubbled through the mixture for three hours. Tlc showed little evidence of starting material but the pH of the mixture remained very alkaline (pH 8-9) whereas the pH of a sodium salt of an organic ester should be about 7. The solution was filtered in a glove box and the precipitate was identified as sodium 4-nitrophenoxide (36%) by ^{1}H NMR, high melting point and orange

colour. The filtrate was protected from moisture to prevent any dimerisation of the suspected species:



As the following reactions have been observed in this laboratory:[36]



Scheme 12

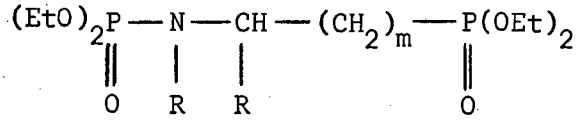
it was thought that a similar reaction may be occurring in the case of O-benzyl-(O-4-nitrophenyl)phosphoranilidate:

only a molar equivalent of hydride this time) was effected as quickly as possible and the carbon dioxide treatment was commenced as soon as the addition was complete so as to try to minimise the intramolecular effect giving rise to P-OPNP bond cleavage. Once tlc showed no signs of starting material, (a further spatula head of sodium hydride had to be added to the reaction mixture in order to react all the phosphoranilidate), the reaction mixture was left to stand for 17 hours and then poured into a large excess of benzene. A light brown precipitate settled out of solution. After 24 hours the mother liquor was decanted off and the solid dried under vacuum and over H_2SO_4 to remove any traces of pyridine. The ^1H NMR spectrum proved the solid to be very impure and indicated only a small amount of the desired phosphate ester relative to aromatic impurities of possibly sodium 4-nitrophenoxide and aniline (small integration for the $-CH_2$ doublet relative to that for the aromatic peaks).

In an effort to purify the crude product, it was dissolved in a minimum volume of methanol and poured into a large excess of diethyl ether. A fine yellow solid precipitated out of solution and was collected by centrifugation. The ^1H NMR spectrum still showed it to be impure and the integration for the $-CH_2$ doublet was very low. This precipitate was then dissolved in a minimum volume of water and acidified to pH 1 in water to derive the corresponding phosphoric acid. The acid was extracted into ether, washed and dried, and treated with cyclohexylamine with the idea of producing the large and bulky cyclohexylamine salt of benzyl(4-nitrophenyl)phosphate which should precipitate out of an ethereal solution leaving behind, in solution, all impurities. Once again, though, there was seemingly no reaction and definitely no precipitation.

With the (2-pyridylmethyl)(4-nitrophenyl)phosphoranilidate, hydrolysis of the P-N bond was attempted in an analogous way to a method

outlined by Savignac[37] for the hydrolysis of the P-N bond in:



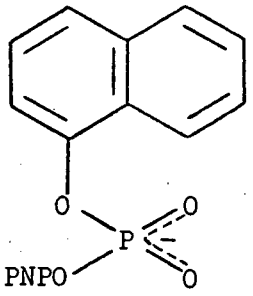
R = simple alkyl group

Effectively the procedure involved passive acid hydrolysis (8MHCl) of the P-N bond over a period of 24 hours, followed by purification of the (2-pyridylmethyl)(4-nitrophenyl)phosphate by ion exchange chromatography. The ^{1}H NMR spectrum indicated a very wet sample and prolonged drying did not appear to improve the recording and sharpen the peaks. Covering the phosphate with methanol and allowing it to evaporate off slowly did not help to drive off the water either.

At this stage it was decided rather to formulate another set of phosphate esters which would incorporate the same features of study as found in (XIV) and (XV) but which might be easier to prepare.

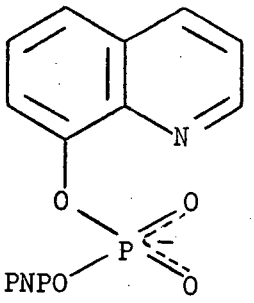
2.2 (1-NAPHTHYL)(4-NITROPHENYL)PHOSPHATE AND (8-QUINOLYL)(4-NITROPHENYL)PHOSPHATE

The pair of phosphate diesters (1-naphthyl)(4-nitrophenyl)-phosphate (XVI) and (8-quinolyl)(4-nitrophenyl)phosphate (XVII) are very similar to the pair (XIV) and (XV) discussed above in that both pairs i) comprise diesters of phosphoric acid, ii) contain one 4-nitrophenyl ester linkage in each phosphate, iii) contain one component carrying a heteroatom in the second ester group, the effect of which on the reactivity of the phosphate as a whole can be compared with that of the >CH centre in the ester group of the other component in each pair.



(XVI)

(1-naphthyl)(4-nitrophenyl)-phosphate



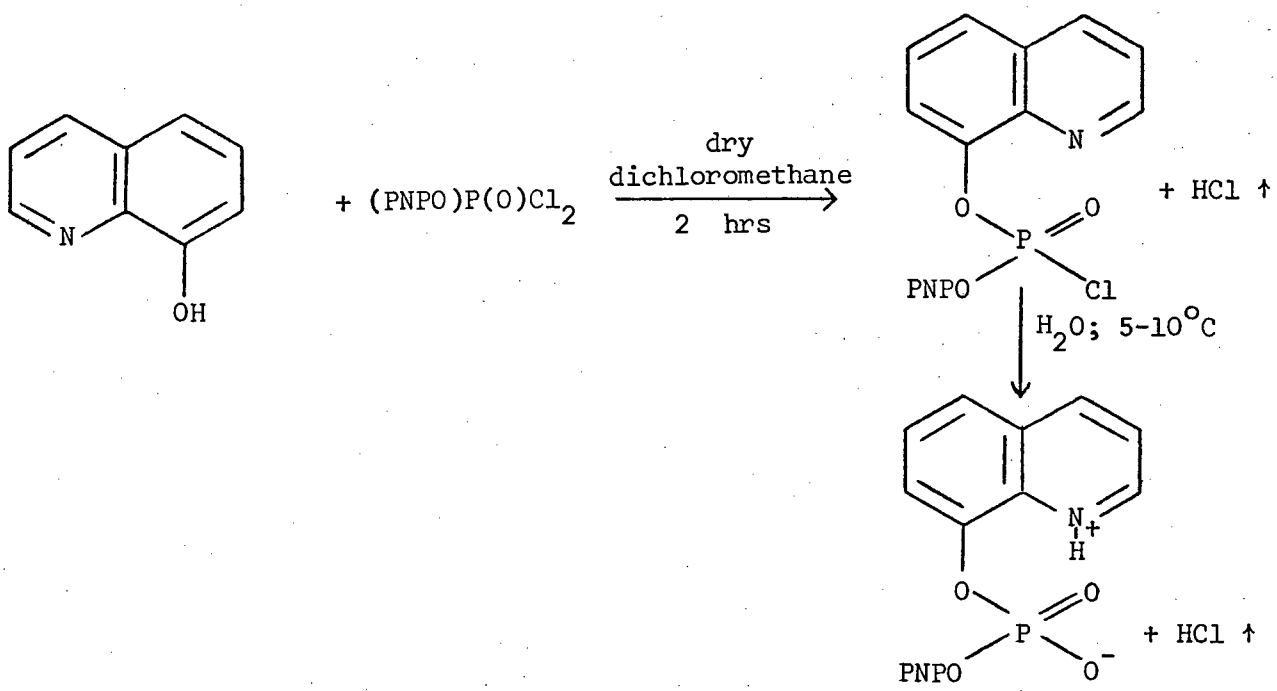
(XVII)

(8-quinolyl)(4-nitrophenyl)-phosphate

It was hoped that the more rigid skeleton of the fused aryl rings would add stability to the phosphate structures and allow them to be more easily prepared than the single-(aryl)-ringed ester systems in (XIV) and (XV).

2.2.1

The synthesis of (XVII) was achieved by following a method reported by Loran and Williams[30]:



Scheme 13

recrystallised from 1-butanol

8-hydroxyquinoline was recrystallised from a mixture of ethanol: water; 6:3.

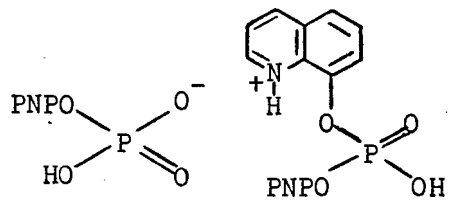
4-nitrophenylphosphorodichloridate was dissolved in dichloromethane and to this solution an equimolar portion of 8-hydroxyquinoline, dissolved in dichloromethane, was added dropwise with stirring, at room temperature. A yellow precipitate formed in the mixture and after a further two hours of stirring an excess of water was slowly added, with cooling in ice. The initial precipitate disappeared and another paler yellow one formed. After stirring for 18 hours the precipitate was recovered by suction filtration and found to be a sample of the desired product, contaminated with a little of both starting materials.

Efforts to purify the product included:

i) recrystallisation from acetic acid, as suggested by Loran and Williams;[30] this proved to be unsuccessful;

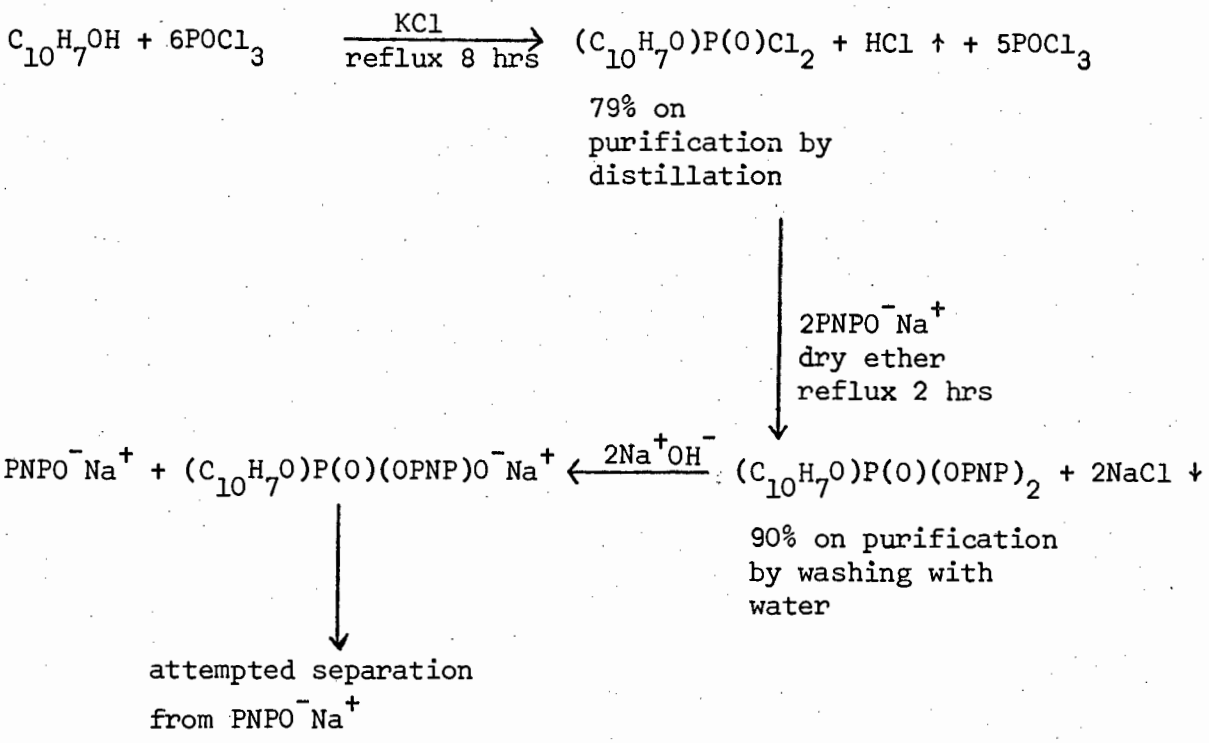
ii) column chromatography using the solvent system chloroform:methanol:water; 65:25:4 (the only suitable solvent found). However, the sample was not easily soluble in this solvent and no clean separation of any fraction was obtained;

iii) washing the solid with ethanol removed the 8-hydroxyquinoline impurity. The microanalysis of the resulting powder gave a good result for the following salt which resulted probably from the hydrolysis of some unreacted 4-nitrophenylphosphorodichloridate



Eventually the (8-quinolyl)(4-nitrophenyl)phosphate was purified by recrystallisation from 1-butanol.

The preparation of (1-naphthyl)(4-nitrophenyl)phosphate is summarised in Scheme 14:



Scheme 14

The first problem to overcome in this synthesis was the purification of 1-naphthol (obviously a rather old sample). Of the procedures tried, including steam distillation, sublimation, vacuum distillation and recrystallisation, the last technique (using distilled water and activated charcoal) became the one of choice for the sake of convenience and yield of pure naphthol (~ 50%).

The condensation of 1-naphthol with phosphoryl chloride in the presence of a catalytic amount of potassium chloride is described in the literature[38]. However the $3\frac{1}{2}$ hour reaction time advocated by Katyshkina and Kraft[38] was found to be insufficient for the complete coupling of 1-naphthol with phosphoryl chloride - some 8 hours of refluxing was required. The 1-naphthylphosphorodichloridate so formed was isolated by distillation. Confirmation of the phosphorylation was obtained by the

downfield shift of the signals for the naphthyl group in the ^{1}H NMR spectrum, relative to their positions and greater spacing in the spectrum of the free alcohol.

1-naphthylphosphorodichloridate was dissolved in dry ether and a molar excess of sodium 4-nitrophenoxide was added in portions, with stirring, so as to maintain a reaction temperature below 20°C. Separation of the triester so produced, from the precipitate of sodium chloride and other impurities, was achieved by pouring the reaction mixture into an excess of water. The phosphate triester was then collected by suction filtration. Mild alkaline hydrolysis (as described in 2.1.3) was employed to remove one of the 4-nitrophenyl groups and generate the sodium salt of (1-naphthyl)(4-nitrophenyl)phosphate. In an attempt to separate the diester from the sodium acetate also produced during the hydrolysis of the P-OPNP triester linkage, the salt mixture was dissolved in water and treated with IR 120(H^{+}) resin to convert both salts to their acid forms. However, the diester of phosphoric acid precipitated out of solution and no suitable solvent could be found to separate it from the beads of resin.

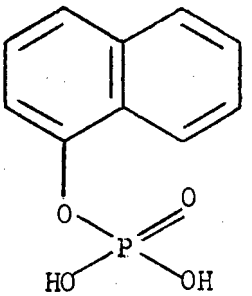
Once again, due to the time factor, it was decided to select a new set of more simple target models for investigation and, in particular, to dispense with the 4-nitrophenyl ester function as it was so reactive in the sense of susceptibility to nucleophilic displacement. This nitro-group was originally chosen so as to facilitate possible kinetic studies of hydryolyis rates but would not especially convenience studies of complexation of the phosphate with metal ions, which was, after all, to be the essence of this particular research project.

C H A P T E R 3

SYNTHESES OF SELECTED MONO- AND DI-ESTERS OF PHOSPHORIC ACID

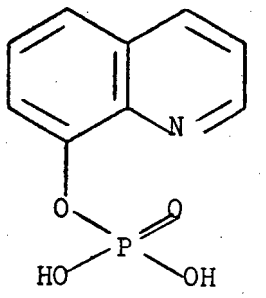
3. SYNTHESES OF SELECTED MONO- AND DIESTERS OF PHOSPHORIC ACID

As explained in Chapter 1.1, the set of phosphate esters eventually successfully prepared, characterised and used in solution chemistry studies, comprised 1-naphthyl dihydrogen phosphate (A), 8-quinolyl dihydrogen phosphate (B), 1-naphthylmethyl phosphate (mono-sodium salt) (C) and 8-quinolylmethyl phosphate (mono-sodium salt) (D):



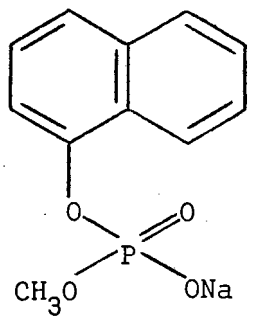
1-naphthyl dihydrogen phosphate

(A)



8-quinolyl dihydrogen phosphate

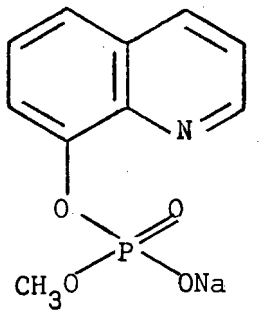
(B)



1-naphthylmethyl phosphate

(mono-sodium salt)

(C)



8-quinolylmethyl phosphate

(mono-sodium salt)

(D)

It was thought that such a set of model phosphate esters could yield some interesting information on i) the relative stabilities of metal ion (copperII) complexes of monoesters and diesters of phosphoric acid and ii) the effect of a heteroatom such as nitrogen (which is present in many nucleotide bases) on these stability constants.

3.1 1-NAPHTHYL DIHYDROGEN PHOSPHATE (A)

The preparation of 1-naphthyl dihydrogen phosphate is outlined below:

$$C_{10}H_7OH + 6POCl_3 \xrightarrow[\text{reflux 8 hrs}]{KCl} (C_{10}H_7O)P(O)\,Cl_2 + 5POCl_3 + HCl\uparrow$$

$(C_{10}H_7O)P(O)\,Cl_2$ → purified by vacuum distillation → KOH(aq), partial vacuum, 3 days → (A)

Scheme 15

The synthesis of 1-naphthylphosphorodichloridate is reported in the literature[38] and has been described in Chapter 2.2. Another procedure for the preparation of this phosphate has been reported by O.M. Friedman and A.M. Seligman[39] but was found to be unsatisfactory as coupling of the 1-naphthol with the phosphoryl chloride did not go to completion under the conditions specified, and the presence of unreacted naphthol in the crude product solution made distillation (purification) difficult. A method involving spontaneous hydrolysis by water molecules[40] was employed in the conversion of 1-naphthylphosphorodichloridate to its corresponding di-acid form (A). In order to facilitate the process, it was conducted under partial vacuum and in the presence of aqueous potassium hydroxide so as to maintain an atmosphere of water and to allow the hydrogen chloride generated in the reaction to be trapped by the base. The white solid so produced was analysed, initially, by ^1H NMR(D_2O) which showed a doublet of doublets at δ 8,61 integrating for one proton and a multiplet at δ 7,38 - 7,92 integrating for six protons. If one considers the phosphoryl group to be electron withdrawing, this spectrum showed, at least, the successful coupling between 1-naphthol and the phosphate (the spectrum of the free

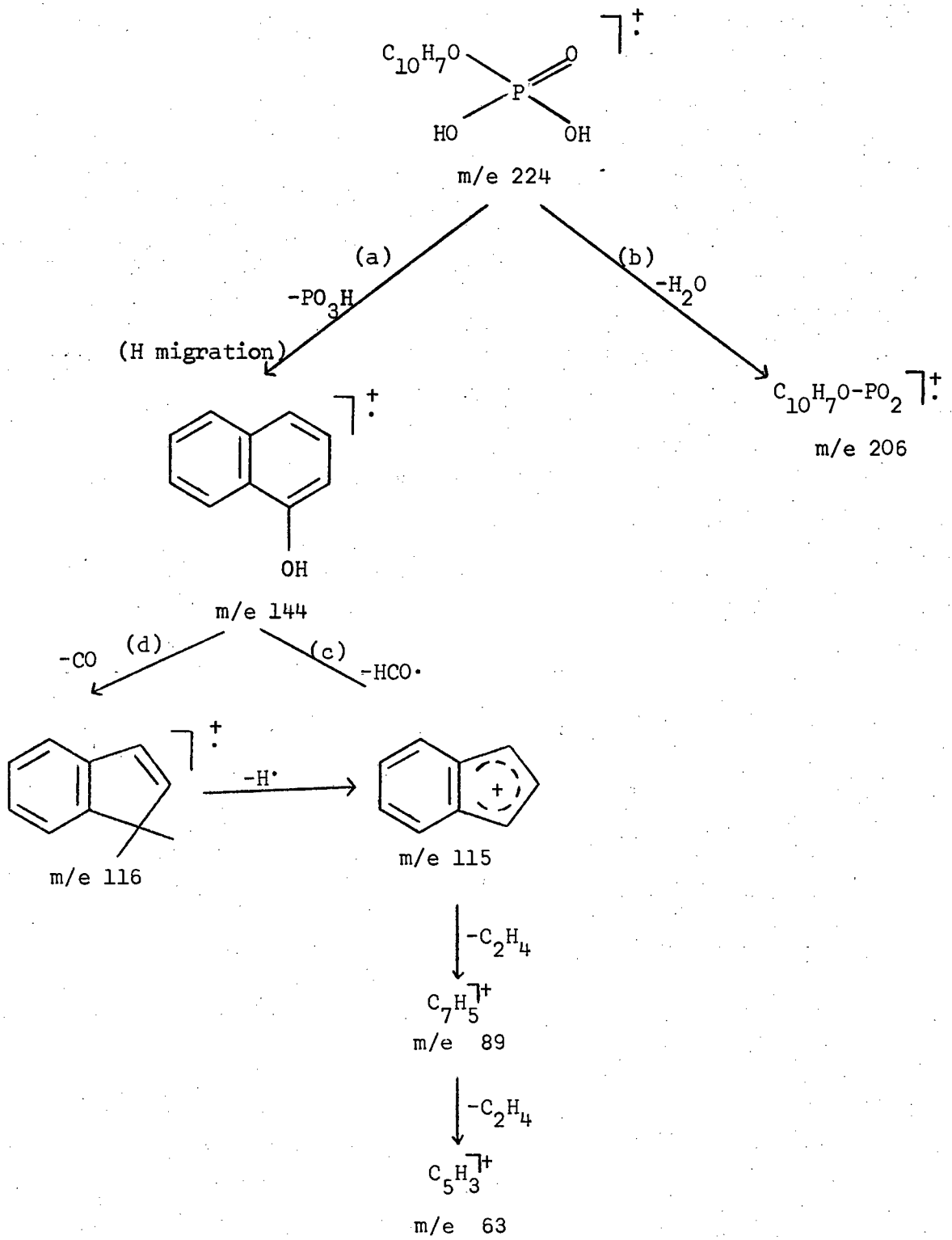
naphthol shows just a broad multiplet at higher field) with the more low field signals being assigned to the highly deshielded proton at position two in the naphthyl ring. As the ^{1}H NMR spectrum was obtained in deuterated water, the two P-O<u>H</u> protons could not be distinguished due to their exchange with the deuterium in solvent. The identity of the product as 1-naphthyl dihydrogen phosphate was therefore confirmed by microanalysis and a melting point determination.

The phosphate mono- and di-esters of interest in this project were submitted for mass spectral analysis in order to determine what effect, if any, the nitrogen heteroatom might have on their electron induced fragmentation patterns. Also, a survey of the literature revealed a great lack of information on the mass spectrometry of mono- and di-esters of phosphorus acids.

The mass spectrum of 1-naphthyl dihydrogen phosphate is very simple and concerns mainly the fragmentation behaviour of 1-naphthol. The main ions observed are listed in Table 1 and the proposed fragmentation patterns are presented in Scheme 16.

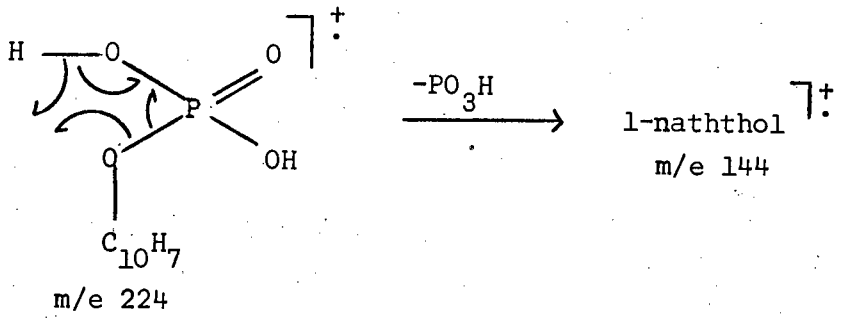
TABLE 1. Mass spectrum of 1-naphthyl dihydrogen phosphate (at 70eV)

m/e	Relative Intensity %
224 (molecular ion)	20
206	4
144	100
116	26
115	50
89	5
63	5

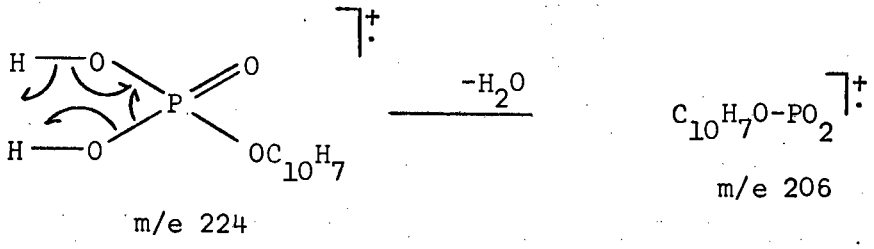


Scheme 16: Proposed fragmentation patterns for 1-naphthyl dihydrogen phosphate (at 70eV)

The base peak (m/e 144) is due to the 1-naphthol molecular ion resulting from expulsion of metaphosphoric acid (HPO_3) from the molecular ion [pathway (a) in Scheme 16]:



Minor, but interesting is the fragmentation consisting of the dehydration of the molecular ion to give the molecular ion of 1-naphthyl-metaphosphate [pathway (b) in Scheme 16]:



The most important fragmentations of the 1-naphthol molecular ion are the losses of the formyl radical and neutral carbon monoxide (pathways c and d respectively in Scheme 16). The elimination of CO is characteristic for phenols.[41] It has been shown[42] that the loss of 29 mass units from 1-naphthol is a two step process occurring either as (H·+CO) or (CO+H·). Deuterium labelling studies[42] also indicate much scrambling of the hydroxyl group hydrogen under electron impact but that three quarters of this hydrogen remains in the benzocyclopentadienyl-carbonium ion (m/e 115).

3.2 8-QUINOLYL DIHYDROGEN PHOSPHATE (B)

A number of methods[14b,43] have been published for the synthesis of

the above phosphate; that reported by H. Takaku[14b] was found to be the most efficient and is illustrated in Scheme 17

$$C_9H_6NOH + 3POCl_3 \xrightarrow[\text{dioxane}]{\text{pyridine/}} C_9H_6NO\text{-}P(O)Cl_2 + C_5H_5\overset{+}{N}HCl^- \downarrow + 2POCl_3$$

isolated by filtration and removal of $POCl_3$ *in vacuo*

$\downarrow$ water/pyridine

$$C_9H_6NO\text{-}PO_3H_2 + 2C_5H_5\overset{+}{N}HCl^- \downarrow$$

(B)

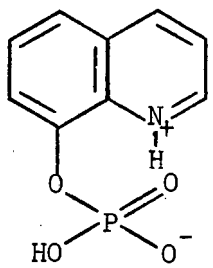
purified by recrystallisation

Scheme 17

The phosphorylation of 8-hydroxyquinoline was effected by phosphoryl chloride in the presence of pyridine (to remove the hydrogen chloride formed during the reaction and prevent protonation of the quinolyl nitrogen). After a 5 hour reaction period the pyridinium hydrochloride by-product was collected by filtration and the excess phosphoryl chloride was removed *in vacuo* to leave the crude 8-quinolylphosphorodichloridate. This residual phosphate was added portionwise to a solution of water and pyridine in order to hydrolyse the P-Cl bonds. Again the pyridinium hydrochloride was filtered off, the solvents were removed *in vacuo* and white crystals of 8-quinolyl dihydrogen phosphate were obtained by repeated recrystallisations from acetonitrile/water solutions.

^{1}H NMR(D_2O) was employed in the preliminary characterisation of (B). Phosphorylation of 8-hydroxyquinoline was indicated by the shift to lower field of the 8-hydroxyquinoline signals due to electron withdrawal by the phosphoryl group. The multiplet at δ 9,20-9,37 integrating

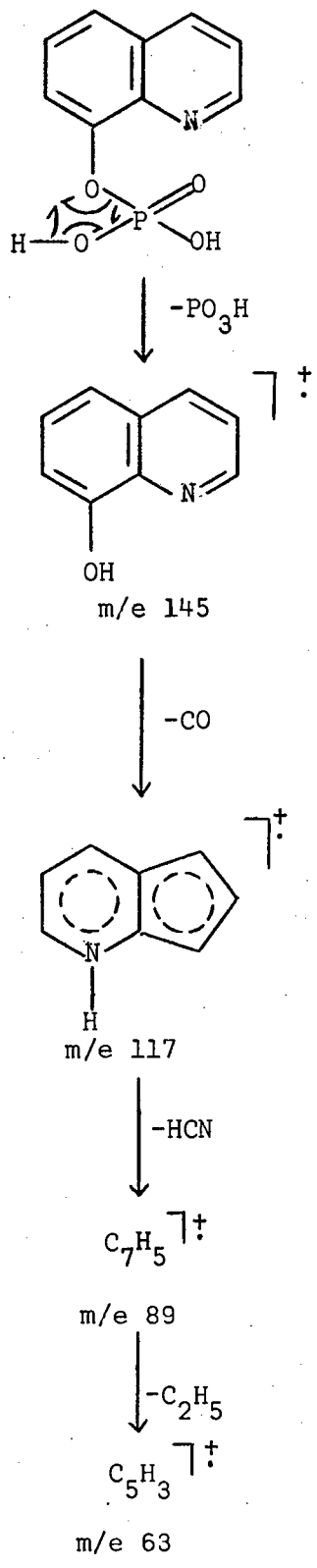
for two protons was assigned to the protons at the two and seven positions in the quinolyl ring as these would be most subject to the inductive withdrawal of electrons by the nitrogen and phosphoryl centres respectively. The four remaining ring protons were seen to resonate as a multiplet at higher field - δ 8,08-8,33. As the P-OH protons could not be identified due to the use of deuterated water as a solvent, the 8-quinolyl dihydrogen phosphate was fully characterised by microanalysis and a melting point determination. The discrepancy between the melting point of these crystals (170-174°C) and that reported in the literature (218-220°C) is probably due to the fact that in the solid state the phosphate is most likely to exist as a zwitterion (this compound was also found to be relatively insoluble in water a fact that may also be attributed to the separation of charge within the molecule):



The mass spectrum of 8-quinolyl dihydrogen phosphate is essentially that of 8-hydroxyquinoline. Table 2 lists the main ions formed and Scheme 18 outlines the proposed fragmentation patterns.

TABLE 2: Mass spectrum of 8-quinolyl dihydrogen phosphate (at 70 eV)

m/e	Relative Intensity %
225 (molecular ion)	-
145	100
117	29
89	15
63	7



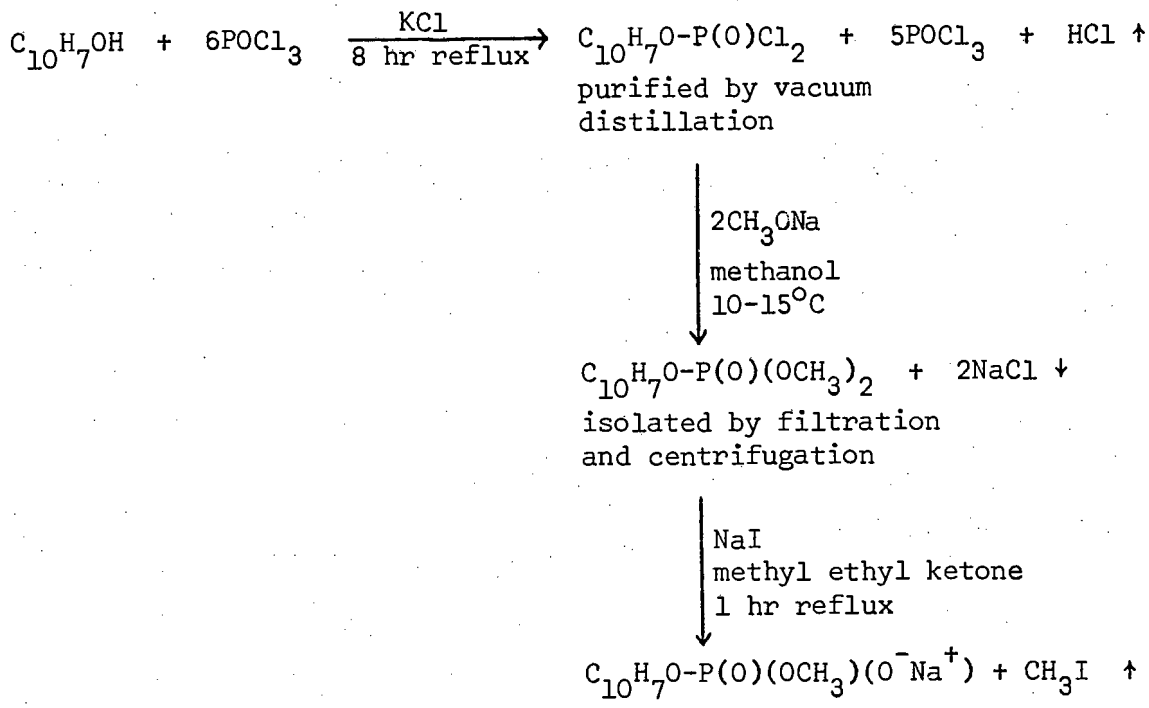
Scheme 18 : Proposed fragmentation patterns for 8-quinolyl dihydrogen phosphate (at 70 eV)

The most striking feature of this spectrum, compared with that of its naphthyl analogue, is the absence of a molecular ion. As the base peak is due to the 8-hydroxyquinoline molecular ion, a more facile expulsion (in the gas phase) of the metaphosphate group from 8-quinolyl phosphate than from 1-naphthyl phosphate is indicated and is obviously due to the introduction of the heteroatom into the ester ring system.

The main fragmentations of the 8-hydroxyquinoline ion are loss of CO, followed by loss of HCN and further decomposition of the resulting carbonium ion. The preferential fragmentation of the phenolic ring is presumably aided by the close proximity of the nitrogen centre which accepts the hydrogen of the 8-hydroxy function, thereby allowing for the facile loss of CO.[41] Fragmentation of the pyridine ring then occurs through expulsion of hydrogen cyanide. Burton and Davis[44] have lent support for the initial fragmentation of the phenolic ring, followed by decomposition of the pyridine ring, through their LCAO calculations of electron densities which indicate that carbonium ion formation is favoured in the phenolic ring and thus primary decomposition should involve the loss of CO from the phenoxy cation.

3.3 1-NAPHTHYLMETHYL PHOSPHATE (MONO-SODIUM SALT)(C)

The synthesis of the mono-sodium salt of 1-naphthylmethyl phosphate is presented schematically below:



Scheme 19

It is well known[45] that under mild conditions anionic cleavage, through the use of salts such as sodium or barium iodide, of a single primary alkyl-oxygen (or benzyl-oxygen) bond in the triester of phosphoric acid may be effected to yield the salt of the diester and the alkyl (or benzyl) iodide. Thus, it was decided to use this "phosphotriester approach" in the syntheses of the phosphate diesters (C) and (D).

1-naphthyldimethyl phosphate was chosen as the triester precursor to 1-naphthylmethyl phosphate and was prepared by condensation of 1-naphthylphosphorodichloridate (see Chapter 2.2 for a description of the synthesis of 1-naphthylphosphorodichloridate) with sodium methoxide at 10-15°C.

The reaction was monitored by tlc and when complete the precipitate of NaCl was collected by filtration and centrifugation. Removal of the solvent *in vacuo* yielded a brown oil which, on analysis by ^{1}H NMR(D_2O), was found to contain the desired phosphate triester (0.8 mole fraction) together with a small amount of the target phosphate diester. Successful methylation of the 1-naphthylphosphorodichloridate was indicated by the splitting of the P-OCH_3 signal at δ 3,23 due to coupling of the 6 methyl protons with the ^{31}P nucleus of the triester. The appearance of another doublet, δ 3,87, was attributed to the 3 methyl protons in the diester as deshielding of these protons would occur on removal of an electron donating group and the production of a negative charge on a phosphoryl oxygen. The premature demethylation of the triester may have been due i) to the introduction of water with one of the reagents used in its synthesis, ii) to contact with Cl^- during the course of the reaction, or iii) to the incomplete removal of Cl^- (by filtration and centrifugation) combined with heating on removal of the solvent from the triester. The preparation of the triester was repeated with strict attention being paid to the exclusion of water during the course of the reaction and to the efficient removal of sodium chloride from the product solution, which included washing quickly with a little water, but the same mixture of esters was obtained in similar proportions. However, as the diester was the target compound anyway, its presence at this stage in the synthetic procedure was not of too much concern.

The demethylation of 1-naphthyldimethyl phosphate was performed by refluxing the triester with sodium iodide in methyl ethyl ketone. The sodium halide was the demethylating agent of choice as it would yield the sodium salt of the phosphate diester; the sodium ion is commonly found in biological systems. Once again though, ^{1}H NMR analysis of the reaction

product indicated that the reaction had, to a small extent, gone one step further than anticipated. A doublet at δ 3,72, that is at slightly higher field to the P-O-CH_3 doublet of the diester at δ 3,80, but having the same coupling constant, was tentatively attributed to the anionic cleavage of the P-Onaphthyl bond in the diester to yield the di-sodium salt of methyl phosphate (~7%). Perhaps this second reaction was due to a slight excess of sodium iodide as it was difficult to calculate from the ^1H NMR spectrum the exact molar equivalent of halide to be used in the reaction with the triester which was already partially demethylated.
Separation of the two sodium salts in order to obtain pure 1-naphthyl-methyl phosphate (mono-sodium salt) was achieved by fractional recrystallisation from butanol/water and then from acetone/water.

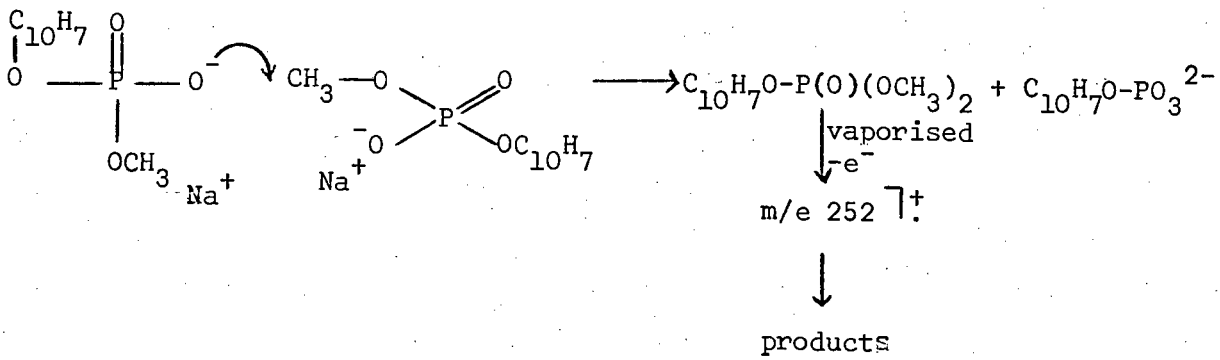
The ^1H NMR spectrum (D_2O) of the naphthyl protons in (C) shows a shift to slightly higher field (δ 8,33-8,50) of the multiplet for the proton at position two in the ring that is possibly due to the electron donating effect of the -OCH_3 group and the presence of the negative charge on the phosphate group (cf. δ 8,61 for the same proton in 1-naphthyl dihydrogen phosphate). Firm evidence for the demethylation, however, is found in the integration of the methyl doublet for only three protons. (C) was further characterised by microanalysis and a melting point determination (246-248°C) which was in the range typical for such sodium salts of phosphate esters.

The main ions formed in the mass spectrum of the mono-sodium salt of methyl phosphate are listed in Table 3.

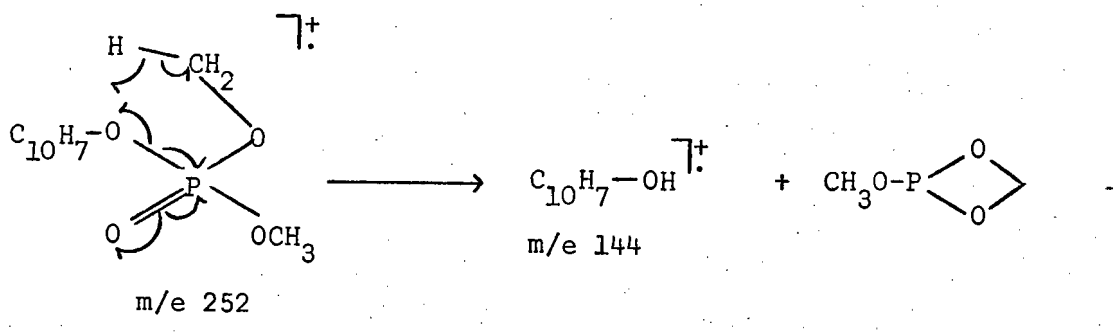
TABLE 3: Mass spectrum of the mono-sodium salt of 1-naphthylmethyl phosphate (at 70eV)

m/e	Relative Abundance %
260 (molecular ion)	-
252	48
158	54
144	62
140	41
115	100
89	9
63	12

Conspicuous by its absence in the mass spectrum of (C) is the molecular ion. However, a fairly strong peak at m/e 252 suggests that the mono-sodium salt of 1-naphthylmethyl phosphate is not volatile at the ion source and is indeed methylated by another molecule of (C) to yield 1-naphthyldimethyl phosphate (m/e 252) and 1-naphthyl phosphate. It is then this 1-naphthyldimethyl phosphate that is volatilised and the mass spectrum of which is recorded.

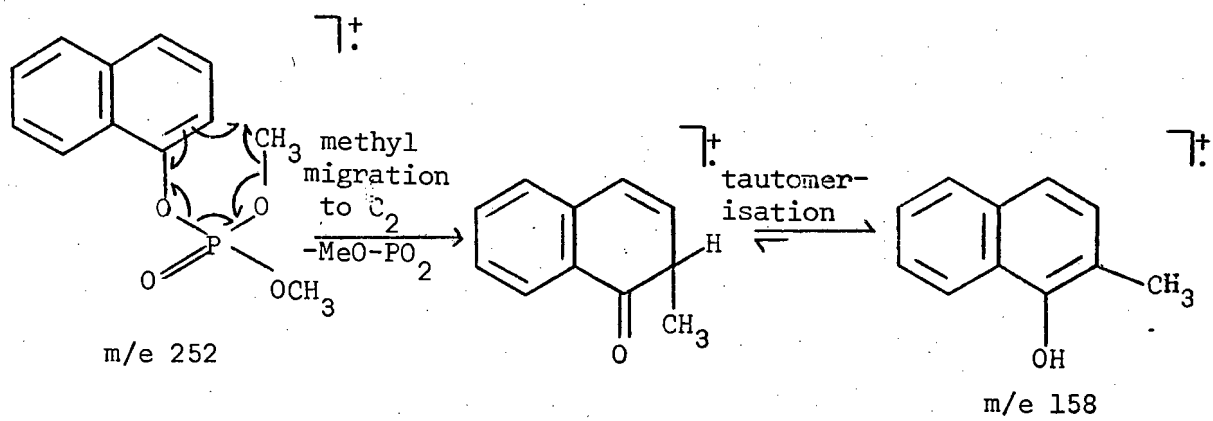


The intense peak (relative intensity 62%) at m/e 144 involves hydrogen migration and loss of methyl methylene phosphite[47] from 1-naphthyldimethyl molecular ion to give the molecular ion of 1-naphthol at m/e 144:

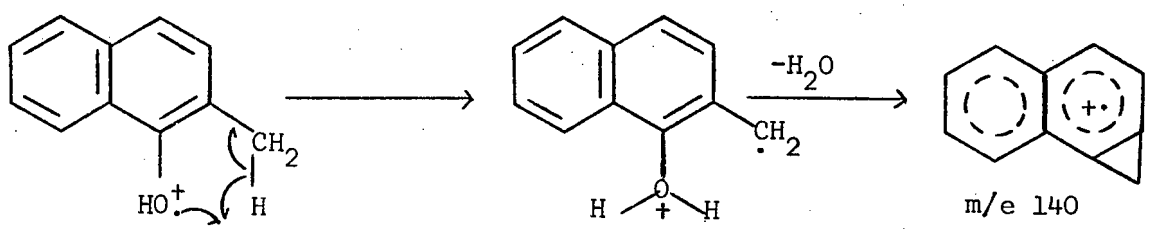


The base peak at m/e 115 and the peaks at m/e 89 and 63 are due to the fragmentations of the 1-naphthol molecular ion which have been discussed previously.

Methyl migration to C_2 in the naphthyl ring of 1-naphthyldimethyl phosphate followed by expulsion of methyl metaphosphate group yields the 2-methyl-1-naphthol ion at m/e 158:



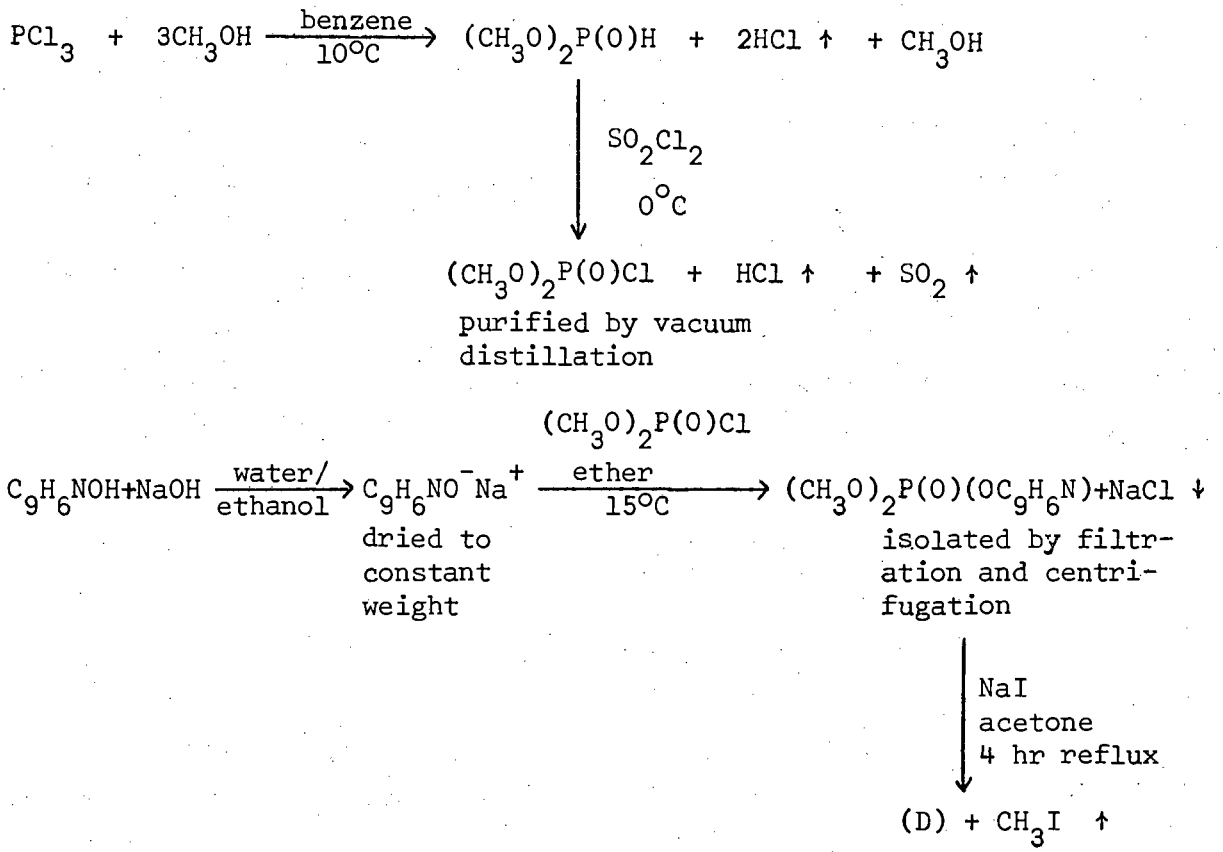
Dehydration of the 2-methyl-1-naphthol ion via a fragmentation typical for O-methylphenols[48] yields the peak at m/e 140:



3.4 8-QUINOLYLMETHYL PHOSPHATE (MONO-SODIUM SALT)(D)

The preparation of this salt by the anionic dealkylation of a single

(O-methyl) bond in its triester precursor (see Chapter 3.3) is outlined below:



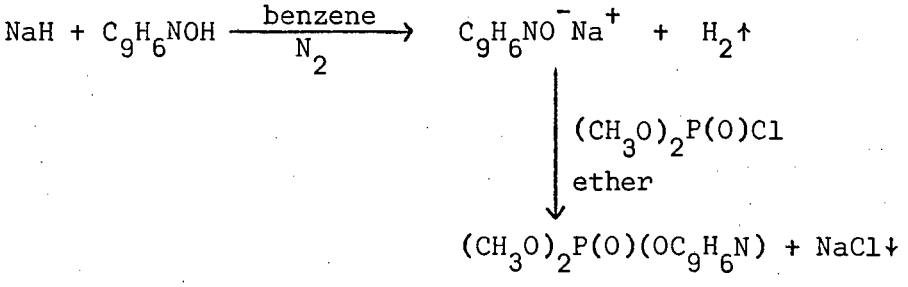
Scheme 20

Dimethylphosphorochloridate was prepared by following closely the method reported by Fiszer and Michalski.[49] The further esterification of this phosphate by the sodium salt of 8-hydroxyquinoline (8-hydroxyquinoline was activated for nucleophilic attack at the phosphorus centre by conversion to its sodium salt) was carried out in ether at 15°C and monitored by tlc. Once the reaction was complete the precipitate of sodium chloride was recovered by suction filtration and centrifugation.

^{1}H NMR($CDCl_3$) confirmed the successful phosphorylation of 8-hydroxyquinoline. The proton ortho to the nitrogen centre was still very deshielded (δ 9,1) but the resonance of the proton at position 7 in the quinolyl ring (which was the same as that for the aforementioned proton in 8-quinolyl

dihydrogen phosphate) was shifted upfield to δ 8,23 due to the presence of the electron donating methyl groups. The presence of a doublet at δ 4,05, integrating for six protons, confirmed the presence of the two P-O-CH_3 linkages and the coupling of these protons with the ^{31}P nucleus. The purity of the phosphate was further checked by microanalysis and a melting point determination.

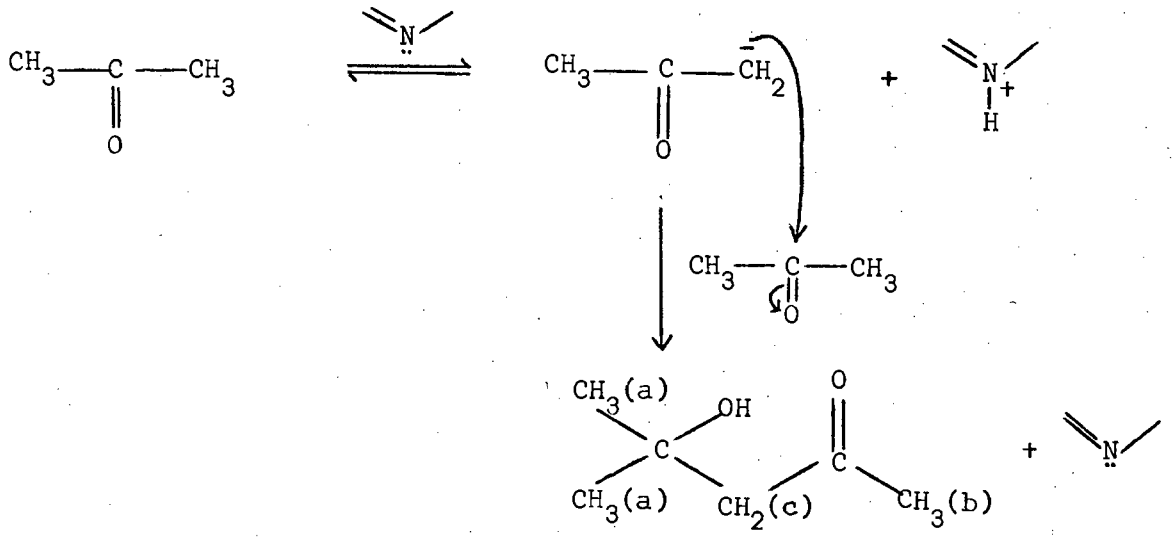
The 8-quinolyldimethyl phosphate has also been prepared by following a method detailed by Lacey and Loew[50] for the synthesis of alkyl(2-dimethylamino)ethyl methyl phosphates. Scheme 21 formulates the reactions involved in this alternative approach to the esterification of dimethylphosphorodichloridate.



Scheme 21

If one compares Scheme 21 with the second part of Scheme 20 it can be seen that they only differ really in the mode of activation of 8-hydroxyquinoline for nucleophilic displacement of chloride in dimethylphosphorochloridate. The use of sodium hydride in the formation of the sodium salt of 8-hydroxyquinoline is more tedious as it is far more reactive than sodium hydroxide and requires an atmosphere of nitrogen for successful reaction. The sodium salt so formed had then to be siphoned, under positive nitrogen pressure, from the excess sodium hydride used in its preparation into an ethereal solution of dimethylphosphorochloridate. As this procedure was more troublesome and less efficient, in terms of yield of phosphate triester, than that already described it was not the method of choice for the synthesis of 8-quinolinyldimethyl phosphate.

The demethylation of the phosphorotriester with sodium iodide, in order to obtain the sodium salt of the phosphorodiester, proved to be more difficult than that for the analogous 1-naphthyldimethyl phosphate due to the presence of the reactive quinolyl nitrogen centre. Initially methyl ethyl ketone was the solvent of choice as the demethylation reaction was reported[45b] to proceed faster in this solvent than in solvents such as acetone or methyl cyanide. However some N-methylation of the quinolyl nitrogen was found to occur and difficulty was experienced in separating the two sodium salts. The reaction was then attempted on a small scale, in acetone. This solvent has a lower boiling point than methyl ethyl ketone but a higher boiling point than methyl iodide, thus allowing the use of an air condenser, just, during reflux so as to ensure the rapid escape of the methyl iodide generated in the course of the demethylation reaction. The reaction in acetone, however, required four hours of hard refluxing for completion. The light yellow precipitate formed in the reaction solution was found to a mixture of (D) and a dimer of acetone formed, presumably, in the presence of the basic quinolyl nitrogen centre:



Scheme 22

Evidence for this dimer came from the ^{1}H NMR(D_2O) spectrum of the precipitate isolated from the reaction mixture by centrifugation. A new series of singlets appeared at δ 1,40, 2,37 and 2,83 integrating for 6, 3 and 2 proton(s) respectively and were assigned to the $CH_{3(a)}$, $CH_{3(b)}$ and $CH_{2(c)}$ groups respectively in Scheme 22. The removal of one of the methyl groups from the phosphate centre was also evident from the ^{1}H NMR spectrum by the integration of the methyl doublet for only three protons, and the shift to slightly lower field (δ 8,08) of the doublet for the proton at position one in the quinolyl ring due to the decrease in electron donating effect from the phosphoryl group (when compared with the spectrum for 8-quinolyldimethyl phosphate).

Separation of the sodium salt of 8-quinolylmethyl phosphate from the dimer of acetone could not be achieved by efforts to volatilise the acetone compound in a vacuum. It appeared rather that the dimer was in some way bonded to the phosphate. Isolation of the sodium salt in a pure form was eventually effected by dissolving the mixture in a minimum volume of acetonitrile and pouring it into a large volume of ether. The monosodium salt of 8-quinolylmethyl phosphate precipitated out of solution and was collected by centrifugation as it was found to be very hygroscopic. Microanalysis further confirmed the identity of the phosphate.

However, when the above procedures were performed on a large scale, N-methylation of the quinolyl nitrogen was again found to have occurred; obviously a larger reaction volume to phosphate reactant ratio was required. Fortunately, though, enough phosphate diester had been produced in the small scale reaction for the purpose of solution chemistry studies and therefore no further attempts were made, at this stage, to perfect the reaction conditions for the efficient synthesis of (D).

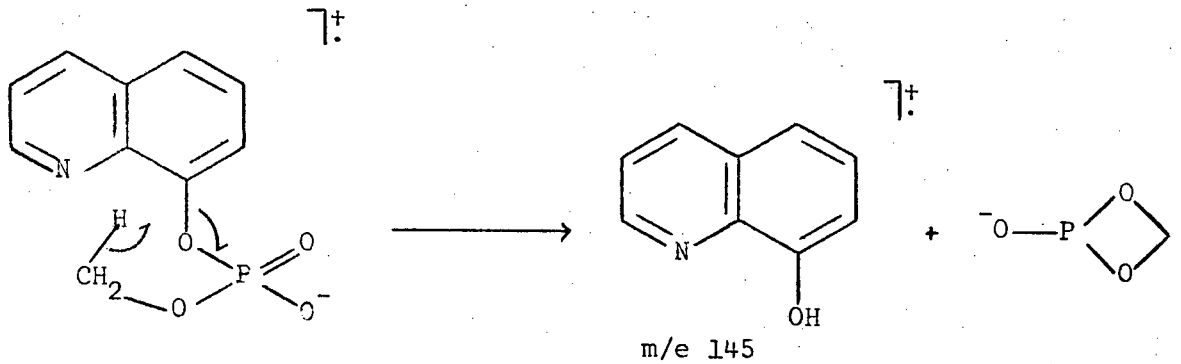
Table 4, below, lists the main ions formed in the electron-induced

fragmentation of the mono-sodium salt of 8-quinolylmethyl phosphate.

TABLE 4: Mass spectrum of the mono-sodium salt of 8-quinolylmethyl phosphate (at 70 e/V)

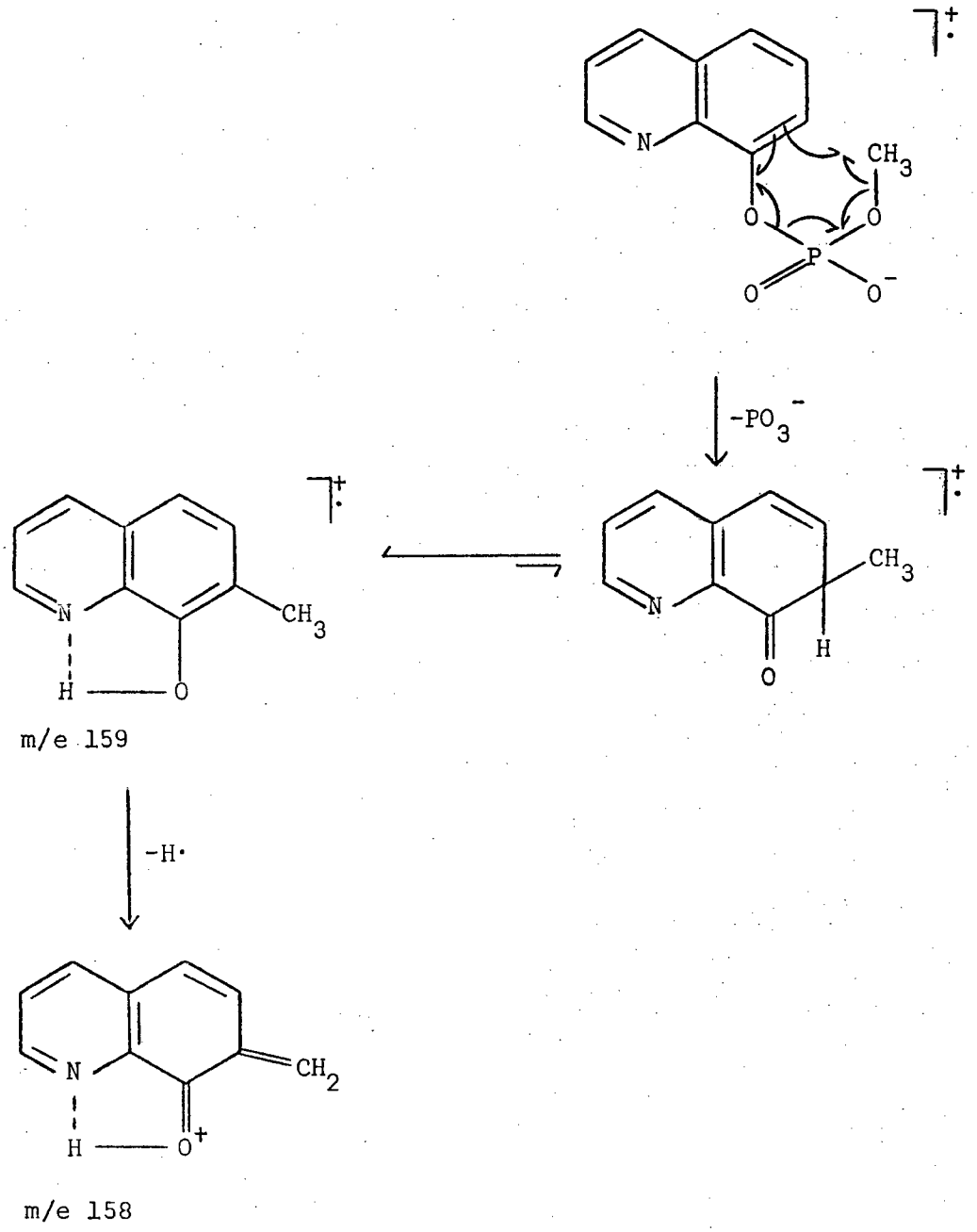
m/e	Relative Abundance %
261 (Molecular ion)	-
159	43
158	39
145	100
130	31
129	36
117	55
89	27
63	21

Once again there is no molecular ion present nor is there any indication of disproportion of (D) to 8-quinolyl phosphate and 8-quinolyldimethyl phosphate as observed in the mass spectrum of (C). The base peak at m/e 145 is due to the 8-hydroxyquinoline molecular ion which, as discussed for 8-quinolyl dihydrogen phosphate, gives rise to fragments at m/e 117, m/e 89 and m/e 63:



m/e 145

The peak at m/e 159 is assigned to the 2-methyl-8-hydroxyquinoline molecular ion arising from methyl migration in, and expulsion of $-PO_3^-$ from, D. Further loss of H· generates the fragment at m/e 158:



No dehydration, as observed for the 2-methyl-1-naphthol molecular ion, is seen here for the corresponding 2-methyl-8-hydroxyquinoline ion, perhaps because of the stabilising effect of hydrogen bonding (N--H-O).

CHAPTER 4

EXPERIMENTAL PROCEDURES FOR THE SYNTHESES OF SELECTED MONO- AND DI-ESTERS OF PHOSPHORIC ACID

4. EXPERIMENTAL PROCEDURES FOR THE SYNTHESES OF SELECTED MONO- AND DI-ESTERS OF PHOSPHORIC ACID

4.1 General

^{1}H NMR were recorded in deuterated water (with the sodium salt of 3[trimethylsilyl]-propanesulfonic acid as internal reference) or in deuterated chloroform (with tetramethylsilane as internal reference) on a Varian EM-360A spectrometer. Mass spectra were measured on a VG Analytical Micromass 16F spectrometer operating at 70eV with an ion source temperature of 200°C.

Melting points (uncorrected) were determined on a Fisher-Johns m.p. apparatus.

Aluminium-backed silica gel plates (Merck, Kieselgel 60 F_{254} Art. 5554) were used for thin layer chromatography.

Analyses for C, H, N were performed on a Heraeus Universal combustion analyser by Mr. W.R.T. Hemsted of the Organic Chemistry Department, University of Cape Town.

4.2 Reagents

All reagents used were of analytical grade and all water was glass distilled.

The following reagents were used as supplied: charcoal (BDH), d^{2}-water (Goss), d^{1}-chloroform, diphosphorus pentoxide (Merck), potassium hydroxide, sodium hydroxide (Laboratory and Scientific Equipment), sodium metal.

The following reagents were purified by drying and/or distillation,

according to standard procedures: acetone, acetonitrile, potassium chloride, sodium iodide (Laboratory and Scientific Equipment), dioxane, phosphoryl chloride, phosphorus trichloride, sulfuryl chloride (Merck), benzene, ether, methyl ethyl ketone (N. and T. Laboratory Supplies), methanol (UnivAR).

8-hydroxyquinoline (Merck) was purified by recrystallisation from ethanol:water (6:3).
1-naphthol (Hopkin and Williams) was purified by recrystallisation from distilled water, in the presence of activated charcoal.

4.3 Substrates

The following syntheses were carried out with the strict exclusion of moisture.

1-Naphthyl Dihydrogen Phosphate

1-naphthyl phosphorodichloridate[38] was prepared by refluxing 1-naphthol (0.1072 moles) and phosphoryl chloride (0.6432 moles) for 8 hours in the presence of a catalytic amount of potassium chloride (0.0001 moles). The solution was cooled and then distilled, the fraction boiling at 164°C (0.1mmHg) being collected as the pure, desired product (79%).

The conversion of 1-naphthyl phosphorochloridate to 1-naphthyl dihydrogen phosphate[40] was effected by placing the former as a 2-3mm thick layer in a flat dish over aqueous potassium hydroxide, in a partially evacuated desiccator for 3 days. The white solid so formed was dried over silica gel and diphosphorus pentoxide (to remove traces of water) and potassium hydroxide pellets (to remove traces of HCl).

Yield: 96% m.p.: 159-161°C (lit.[40] m.p. 155-157°C)

^{1}H NMR(D_2O): δ 8,61 (1H, dd, $J_{H,H}$ [ortho] 8Hz, $J_{H,H}$ [meta] 2Hz) proton ortho to naphthyl oxygen

δ 7,38 7,92 (6H,m) remaining aromatic protons

MS: m/e 224(M^+); m/e 144 (base, M-HPO_3)

Anal.calc. for $C_{10}H_7O_4P$: C 53,56; H 4,05% Found: C 53,60; H 4,05%.

8-Quinolyl Dihydrogen Phosphate[14b]

8-hydroxyquinoline (0.0499 moles) was dissolved in pyridine (40ml) and added dropwise, over a period of 2 hours, to a solution of phosphoryl chloride (0.2490 moles) and dioxane (40ml), with stirring and cooling in ice. Immediate formation of a white precipitate of pyridinium hydrochloride was observed. Once the addition was completed stirring was continued for 30 mins and then the resulting mixture was allowed to stand overnight at room temperature. The pyridinium salt was collected by gravity filtration and the excess phosphoryl chloride, pyridine and dioxane were removed from the filtrate, *in vacuo*, at 50°C, to leave a thick yellow liquid. To this was added pyridine (100ml), dioxane (100ml) and then, with good stirring and dropwise addition, water (2,5ml). The reaction mixture was stirred for a further 30 minutes before being left to stand overnight. The solvents were removed *in vacuo* and the desired 8-quinolyl dihydrogen phosphate was separated, as white crystals, from the pyridinium hydrochloride by repeated recrystallisations from water:acetonitrile (1:1).

Yield: 40% m.p. 170-174°C (lit.[14b] m.p. 218-220°C)

^{1}H NMR(D_2O): δ 9,20-9,37 (2H,m) protons at positions 2 and 7 in the 8-hydroxyquinoline system

δ 8,08-8,33 (4H,m) remaining aromatic protons

MS: no M^+, m/e 145 (base, 8-hydroxyquinoline)

Anal. calc. for $C_9H_8O_4NP$: C 48,01 H 3,59 N 6,22% Found: C 47,95; H 3,65; N 6,25%.

1-Naphthylmethyl Phosphate (Mono-Sodium Salt)

1-naphthyl phosphorodichloridate was prepared as described previously.[38]

Sodium methoxide was obtained by the portionwise addition, with stirring and cooling in ice, of sodium metal (0.1350 moles) to "super-dry" methanol[46] (2.500 moles). Once the sodium was completely dissolved, 1-naphthyl phosphorochloridate (0.0676 moles) dissolved in benzene (20ml) was added dropwise, with stirring and cooling, to the reaction flask. A cream precipitate of sodium chloride formed in the reaction mixture. When the addition was complete, stirring, at room temperature, was continued overnight. The sodium chloride was collected by gravity filtration and centrifugation. The solvents were removed *in vacuo* to leave a brown oil, which was determined, by ^{1}H NMR to be a crude mixture of 1-naphthyl-dimethyl phosphate (0.8 mole fraction) and 1-naphthylmethyl phosphate (mono-sodium salt) (0.2 mole fraction) (see discussion of experimental procedures).

The demethylation[45] of 1-naphthyldimethyl phosphate to give the mono-sodium salt of 1-naphthylmethyl phosphate was performed by dissolving the former (0.0434 moles) together with sodium iodide (0.0347 moles) in methyl ethyl ketone (100ml) and heating, under reflux, for 1 hour. The white precipitate which formed during the reaction was collected by gravity filtration and recrystallised fractionally from butanol/water and then acetone/water to yield the pure mono-sodium salt of 1-naphthyl methyl phosphate.

Yield: 65% m.p. 246-248°C

^{1}H NMR(D_2O): δ 8,33-8,50 (1H,m) proton in 2 position in naphthyl ring

δ 7,53-8,17 (6H,m) remaining aromatic protons

δ 3,80 (3H,d,$^3J_{H,P}$ 12Hz) methyl protons

MS: no M^+; m/e 115 (base, benzocyclopentadienylcarbonium ion)

Anal. cal. for $C_{11}H_{10}O_4PNa$: C 50,78 H 3,85% Found: C 50,40 H 3,95%.

8-Quinolylmethyl Phosphate (Mono-Sodium Salt)

The sodium salt of 8-hydroxyquinoline was prepared by dissolving 8-hydroxyquinoline in a minimum volume of ethanol and adding, with stirring, an equimolar amount of sodium hydroxide dissolved in a minimum volume of water. A thick yellow precipitate of the desired salt formed immediately; it was collected by suction filtration and dried at 120°C to an olive green powder of constant mass and almost quantitative yield.

Dimethyl phosphorochloridate[47] was prepared by the dropwise addition, over a period of 1 hour, of a solution of phosphorus trichloride (0.5040 moles) in benzene (44cm^3), to a stirred solution of methanol (1.5041 moles) in benzene (150ml), while maintaining a reaction temperature of between 5 and 10°C. Once this addition was completed the temperature was lowered further to 0°C as sulphuryl chloride (0.5001 moles) was slowly added, with stirring. This mixture was then left to stand overnight at room temperature to allow the gaseous products to escape. Benzene was removed *in vacuo* and the crude dimethyl phosphorochloridate was purified by distillation at the water pump (yield: 80%).

8-quinolyldimethyl phosphate was synthesised by dissolving dimethyl phosphorochloridate (0.0845 moles) in ether (200ml) and adding, portionwise, the sodium salt of 8-hydroxyquinoline (0.0845 moles) with

stirring and cooling in ice such that the temperature of the reacting solution remained below 15°C. As the reaction proceeded, a light brown precipitate of sodium chloride was observed to form. After addition of the salt was completed, the mixture was allowed to stand overnight in order to ensure complete reaction. The precipitate of sodium chloride was removed by gravity filtration and centrifugation. The ether was evaporated from the filtrate *in vacuo* to yield pale yellow crystals of 8-quinolyldimethyl phosphate.

Yield: 74% m.p. 64-68°C

^{1}H NMR($CDCl_3$): δ 9,1 (1H, dd, $J_{H,H}$ [ortho]4Hz; $J_{H,H}$ [meta] 1Hz) aromatic proton ortho to quinolyl nitrogen

δ 8.23 (1H, dd, $J_{H,H}$ [ortho]9Hz; $J_{H,H}$ [meta] 1Hz) aromatic proton ortho to hydroxyl function of 8-hydroxyquinoline system

δ 7.38 - δ 7.90 (4H,m) remaining aromatic protons

δ 4.05 (6H, dd, $^3J_{H,P}$12Hz) methyl protons

MS: m/e 153 (M^+); m/e 158 (base, M^+ - $MeOPO_2H$)

Anal. calc. for $C_{11}H_{12}NO_4P$: C 52,17; H 4,79; N 5,53%

Found: C 52,20; H 4,80; N 5,45%.

8-quinolylmethyl phosphate (sodium salt) was obtained by the demethylation[45] of 8-quinolyldimethyl phosphate. 8-quinolyldimethyl phosphate (0.0040 moles) and sodium iodide (0.0040 moles) were dissolved in acetone (100ml) and heated under reflux, using an air condenser, for four hours. After cooling, the acetone was removed *in vacuo* to leave a yellow powdery solid which was found, by ^{1}H NMR, to be a mixture of the desired sodium salt and an acetone dimer. 8-quinolylmethyl phosphate was isolated by dissolving the crude product in acetonitrile (40ml) and pouring this solution into ether (200ml). A fine

yellow precipitate fell out of solution; it was collected by centrifugation and identified as pure 8-quinolylmethyl phosphate (mono-sodium salt).

Yield: 85% m.p. undetermined as the salt is very hygroscopic

^{1}H NMR(D_2O): δ 8,70 (1H, dd, $J_{H,H}$ [ortho] 4Hz, $J_{H,H}$[meta] 1Hz) proton ortho to quinolyl nitrogen

δ 8,08 (1H, dd, $J_{H,H}$ [ortho]10Hz, $J_{H,H}$[meta] 1Hz) proton ortho to the hydroxyl function in 8-hydroxyquinoline system

δ 7,27 - 7,70 (4H,m) remaining aromatic protons

δ 3,77 (3H,d, $^3J_{H,P}$ 11Hz) methyl protons

MS: no M^+; m/e 145 (base, 8-hydroxyquinoline)

Anal. calc. for $C_{10}H_9NO_4PNa.H_2O$: C 43,02; H 3,98; N 5,02%;

Found: C 42,55; H 3,85; N 4,8%.

CHAPTER 5

THE USE OF POTENTIOMETRY AND COMPUTATIONAL ANALYSIS IN THE DETERMINATION OF STABILITY CONSTANTS

5. THE USE OF POTENTIOMETRY AND COMPUTATIONAL ANALYSIS IN THE DETERMINATION OF STABILITY CONSTANTS

5.1 INTRODUCTION

As discussed in Chapter 1, the stimulus for this research project derived from the fact that esters of phosphoric acid in biological systems have in common their relative lack of reactivity in hydrolytic and nucleophilic processes, and their association with metal ions (particularly divalent metal ions) in the great majority of their biochemical transformations. In view of the above statement it was decided to synthesise a series of mono- and di-esters of phosphoric acid (substrates A - D) and determine their ionisation constants and the stability constants of the complexes they formed with a metal ion commonly found in biological media. A knowledge of such constants was deemed important in order that the effects of pH and metal ion concentration on the equilibria and kinetics of the reactions of organic phosphate compounds be more fully understood.

As a result of their work on the hydrolysis of pyridylmethyl phosphates in the presence of bivalent metal ions, Murakami and Sunamoto[14a,15] have proposed that two successive interactions between a metal ion and an organic phosphate are required to yield catalytic efficiency:

i) preliminary chelate formation - a significant chelate-forming affinity for an organic phosphate must be demonstrated by a metal ion. Both the neighbouring functional group in the leaving alcohol group (e.g. the quinolyl nitrogen in A and D) and the phosphate group, in which one of the oxygen atoms may act as a donor atom, may participate

in such coordination interaction;

ii) transitional chelate formation - an appropriate affinity for the ester oxygen must be demonstrated by a metal ion in the transitional state so that the chelate ring involving both the neighbouring functional group and the ester oxygen can be formed in the activated complex. This affinity can be judged from the chelating tendency of a metal ion with the alcohol, a hydrolysis product.

We chose then, to investigate the complex formations between the organic phosphate ligands, A - D, and the copper(II) ion which is known to have a strong chelation tendency and which, being a borderline metal ion (Lewis acid) in the HSAB[51] classification of metals and ligands, will interact to a certain extent with both hard and soft donor groups (Lewis bases).

5.2 EQUILIBRIA INVOLVED IN COMPLEX FORMATION

In any aqueous solution containing a metal ion, M, a ligand, L, and a strong acid, H, a number of complex formations may occur. The reaction between a metal ion and n ligands, for instance, may be expressed in terms of two related, but different equilibrium constants;[52,53] namely stepwise[54] stability constants, K's and overall stability constants, β's:

$$\begin{aligned}
M + L &\longrightarrow ML & {}^{T}K_1^M &= {\{ML\}}/{\{M\}\{L\}} \\
ML + L &\longrightarrow ML_2 & {}^{T}K_2^M &= {\{ML_2\}}/{\{ML\}\{L\}} \\
&\vdots & &\vdots \\
ML_{(n-1)} + L &\longrightarrow ML_n & {}^{T}K_n^M &= {\{ML_n\}}/{\{ML_{(n-1)}\}\{L\}}
\end{aligned}$$

$$\begin{aligned}
M + L &\longrightarrow ML & {}^{T}\beta_1^M &= {\{ML\}}/{\{M\}\{L\}} = {}^{T}K_1^M \\
M + 2L &\longrightarrow ML_2 & {}^{T}\beta_2^M &= {\{ML_2\}}/{\{M\}\{L\}^2} = {}^{T}K_1^M\,{}^{T}K_2^M \\
&\vdots & &\vdots \\
M + nL &\longrightarrow ML_n & {}^{T}\beta_n^M &= {\{ML_n\}}/{\{M\}\{L\}^n} = {}^{T}K_1^M\,{}^{T}K_2^M \cdots {}^{T}K_n^M
\end{aligned}$$

In the same way stepwise and overall stability (protonation) constants may be defined to describe the competition for the ligand, L, by n protons, H:

$$L + nH \longrightarrow LH_n \qquad {}^{T}\beta_n^H = {\{LH_n\}}/{\{L\}\{H\}^n} = {}^{T}K_1^H \, {}^{T}K_2^H \ldots {}^{T}K_n^H$$

For such a three component system L, M and H, the following generalised equilibrium may be established:

$$pL + qM + rH \longrightarrow L_pM_qH_r$$

and the overall thermodynamic stability constant (${}^{T}\beta_{pqr}$) defined in terms of activities thus:

$${}^{T}\beta_{pqr} = {\{L_pM_qH_r\}}/{\{L\}^p\{M\}^q\{H\}^r}$$

(The subscripts of β may account for the possibility of the formation of protonated, hydroxo or oligonuclear complexes. When r = -1 this refers to a proton removed to a water molecule or the addition of an hydroxide ligand).

In practice it is often difficult to obtain the activities of all the reacting species but one can usually determine their concentrations and define the overall stoicheiometric stability constant (β_{pqr}) in terms of concentrations:

$$\beta_{pqr} = {[L_pM_qH_r]}/{[L]^p[M]^q[H]^r}$$

The above expression will only remain constant, though, if the associated activity coefficient (γ) quotient remains constant:

$$Q_\gamma = {\gamma_{LMH}}/{\gamma_L^p \; \gamma_M^q \; \gamma_H^r}$$

In order to accomplish this all investigations must be carried out in the presence of a large excess of an inert background electrolyte which does not react with any of the metal, ligand or proton species present. If one assumes that activity coefficients are functions of ionic strength then the

activity coefficient quotient will remain constant. It is important to remember that stability constants determined in this way are only applicable to the concentration and nature of the background electrolyte in which they were measured as the activity coefficients depend on the electrolyte.

In the above formulations the reactions have effectively been represented as taking place in the gas phase. In an aqueous solution, the metal ion and proton will be solvated and reaction of these two species with the ligand will involve displacement of coordinated water molecule(s) by the ligand and therefore the activity of the water molecule should also appear in the definition of the thermodynamic stability constant. This term is, however, normally neglected for very dilute solutions when the water is effectively in its standard state[55] and its activity thus negligibly different from unity.

5.3 CHOICE OF EXPERIMENTAL METHODS AND CONDITIONS AND COMPUTATIONAL ANALYSIS OF POTENTIOMETRIC TITRATION DATA

The reliability of a stability constant is determined by the following factors:

i) the adequacy of the experimental method

ii) the exactness of the experimental work

iii) the consideration of all relevant equilibria

iv) the calculation method

v) the reliability of the auxiliary data used.

The methods used for the determination of stability constants are based on the measurement of a physical quantity, X, which is then used for the calculation of the concentration of all species present in the system.

The potentiometric, pH, method is the most popular method due to its high accuracy and precision and because it permits the determination of both protonation and stability constants. With the pH method, X is a function of one species only, namely proton concentration, and a glass electrode reversible to hydrogen ions is used in electrical contact with a calomel reference electrode.

The measured emf of the cell may be expressed as:

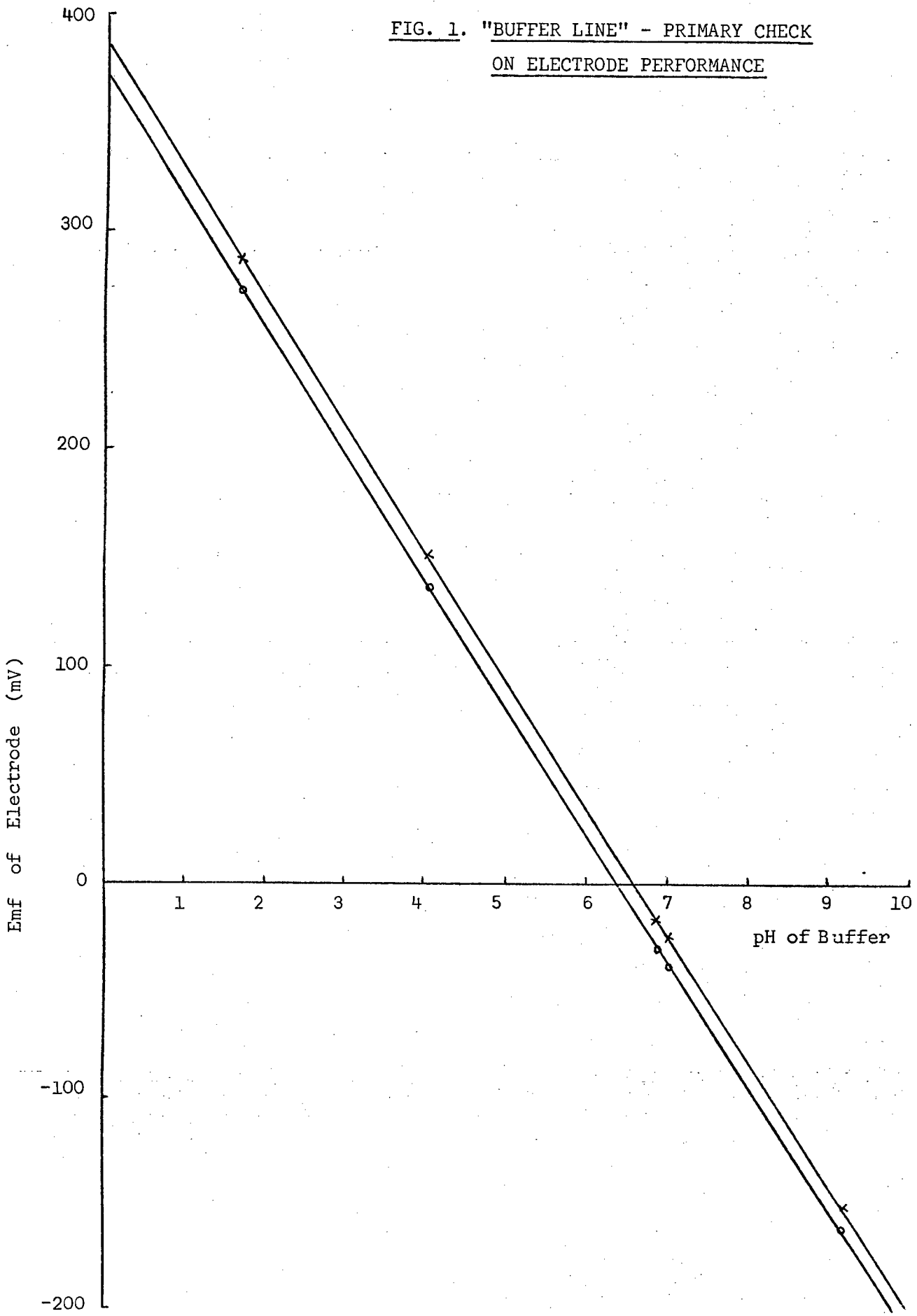
$$E = E_0 + S \log h \quad h = [H^+] \qquad \text{EQN 1}$$

where E is the measured emf of the cell, E_0 is a measure of all the constant potentials including the hydrogen ion activity coefficient and factors arising from it, and S is the Nernstian slope, $^{RT}/_{F}$.

The importance of controlling the experimental temperature is evident from the above equation. The temperature chosen for this research was 25°C. A temperature of 37°C (that of biological blood plasma) would have been more ideal were it not for the fact that at this higher temperature thermostatting of the complete system of vessel and electrode would be needed to prevent condensation in the cooler parts and the tubing linking the burette to the titration vessel would also need to be maintained at 37°C to minimise temperature fluctuations to the vessel. To overcome these problems careful thermostatting, perhaps of the whole room, would have been required.

The background electrolyte of choice was 0,15 mol dm^{-3} NaCl as this approximates to biological blood plasma conditions. The chloride ion does not have a great tendency for complexation to copper(II) (log β_{110} 0,40 at 25°C, ionic strength 0; log β_{110} 0,09 at 25°C, ionic strength 2[56]) but as any such complexation constants will be incorporated into the copper(II)-phosphate ligand stability constants the latter constants may still be compared directly with any obtained in a blood medium.

FIG. 1. "BUFFER LINE" - PRIMARY CHECK ON ELECTRODE PERFORMANCE



O Electrode 1 (E1) slope: - 58,67mV; intercept (E_0); 371,3mV

X Electrode 2 (E2) slope: - 58,96mV; intercept (E_0); 385,0mV

Actual calibration of the electrodes and testing of the potentiometric technique was effected by:

i) the CALIBT determination of E_0 and pKw from potentiometric titration data generated by titrating HCl (0,01 mol dm^{-3} H^+; 0,15 mol dm^{-3} Cl^-) against NaOH (0,02 mol dm^{-3} OH^-; 0,15 mol dm^{-3} Cl^-).

The input parameters for the CALIBT subroutine included:

pKw : scan between 13,68 and 13,78

electrode intercept (E_0): 350,0 mV

CALIBT optimisation produced the following values:

pKw : 13,74

$E_0(E_1)$: 367,7 mV

$E_0(E_2)$: 385,0 mV

ii) the determination of the protonation constants of glycine using the MAGEC-MINIQUAD cycling routine and the E_0 and pKw values obtained in i). Four sets of titration data (two sets per electrode) were generated by titrating 0,008 mol dm^{-3} ligand solutions in 0,01 mol dm^{-3} HCl (0,15 mol dm^{-3} Cl^-) with 0,02 mol dm^{-3} NaOH (0,15 mol dm^{-3} Cl^-) in the absence of metal ions up to a pH of 10. The refined values of a) the formation constants (MINIQUAD) and b) E_0's (MAGEC) appear in Tables 5 and 6 respectively:

TABLE 5

LOGARITHMS OF PROTONATION CONSTANTS FOR GLYCINE
(0,15 mol dm^{-3} NaCl; 25°C) R = 0,0017

COMPLEX	Log β_{por}	Log K_{por}	Std. deviation in log β_{por}	log β(lit)[59]
LH	9,57	9,57	0,002	9,62
LH_2	11,97	2,40	0,001	12,06

In a mixture containing several species of various types (mononuclear, protonated, hydroxo, oligonuclear) the total concentrations of the components may be expressed in terms of their free concentrations and the various stability constants of the N complexes. These expressions are known as mass balance equations:

$$[L]_T = [L] + \sum_{r=-R}^{R} \sum_{q=0}^{Q} \sum_{p=1}^{P} p\beta_{pqr} [L]^p [M]^q [H]^r \qquad \text{EQN. 2}$$

$$[M]_T = [M] + \sum_{r=-R}^{R} \sum_{q=1}^{Q} \sum_{p=1}^{P} q\beta_{pqr} [L]^p [M]^q [H]^r \qquad \text{EQN. 3}$$

$$[H]_T + [H] + \sum_{r=-R}^{R} \sum_{q=1}^{Q} \sum_{p=1}^{P} r\beta_{pqr} [L]^p [M]^q [H]^r \qquad \text{EQN. 4}$$

The MAGEC program makes use of equations 1, 2 and 4 and the Nelder and Mead[60] simplex routine in minimising the sum of squares of the individual residuals.

MINIQUAD uses equations 2, 3 and 4 with the consideration that if there are n_p titration points and $n_{M.B.E.}$ mass balance equations at each point, there will be a total of $n_p \times n_{M.B.E.}$ free concentration. As the free hydrogen ion concentration is known at each titration point there will, in fact, be $n_c = n_p(n_{M.B.E.} - 1)$ unknown free concentration. If there are n unknown β's there will be a total of $n_c + n$ parameters to be determined. Thus, both unknown stability constants and unknown free concentrations are refined by means of a squared residuals minimisation using a generalised Gauss-Newton algorithm.

Another useful program is ZPLOT[61] which introduces the concept of $\bar{Z}$, the average number of ligands bound to the metal ion:

$$\bar{Z} = [\text{L bound to M}]/[M] = [L]_T - [L]/[M] = \Sigma i\beta_i[L]^i / 1 + \Sigma\beta_i[L]^i$$

and the analogous $\bar{Z}_H$, the average number of protons bound to the ligand.

This allows one to plot a formation curve of $\bar{Z}$ versus pL ($-\log[L]$) or $\bar{Z}$ versus pH ($-\log[H^+]$), to give a pictorial representation of the equilibria. If only mononuclear binary complexes are formed in solution, $\bar{Z}$ is a function of hydrogen ion concentration and protonation constants only and is independent of the total component concentrations.[55] This means that all the formation curves for different titrations concerning the same ligand and metal ion should overlap. If the major species present in solution are simple binary complexes an estimation of the formation constants can be obtained from the pL values corresponding to half integral $\bar{Z}$ values[52] i.e. $^1/_2$, $^3/_2$, $^5/_2$. If, however, other complexes such as hydroxo or protonated complexes are present then the formation curves tend to 'fan' out (i.e. they are definitely not superimposable).

As a test of the proposed model the refined β's together with the relevant titration data and experimental conditions are used to construct a theoretical $\bar{Z}$ plot. This can easily be done using the program PSEUDOPLOT[62] and if the model is valid the experimental and theoretical formation curves should overlap.

The statistical output from MINIQUAD allows the determination of the "goodness of fit" between experimental and calculated data, in terms of the crystallographic R factor:[63]

$$R = \sqrt{\frac{\sum_{1}^{3N} (f_i^{calc} - f_i^{obs})^2}{\sum_{1}^{3N} (f_i^{obs})^2}}$$

where N is the number of titration points and f_i^{calc}'s are the total component concentrations as defined by equation 2. Another statistical test of the reliability of the proposed model is in the value of χ^2 (chi-squared) which is also part of the MINIQUAD output. This is a

measure of how "normal" or Gaussian the distribution of residuals is and from a scan of the residuals it is possible to see which of the component concentrations is in error. However, the usefulness of χ^2 is questionable due to the inevitable occurrence of systematic errors in the titration data.

Finally, the program MINEQL[64] was employed in the determination of the concentrations of the different ligand-copper(II) species at various pH. MINEQL uses the equilibrium constant approach to the problem of finding the equilibrium composition/speciation of an aqueous system. This begins with an initial guess for a set of components from which the minimum Gibbs free energy composition is readily calculated from equilibrium constants; then the mass balance equations are solved by iteration using a Newton Raphson algorithm. Thus, a chemical equilibrium problem is solved at every step towards the final solution (that is, the computed composition represents the minimum Gibbs free energy for a set of mass balance constraints), the task, then, being to iterate the chemical problem with the desired set of mass balance constraints.

C H A P T E R 6

STUDIES OF PHOSPHORIC ACID ESTER-PROTON AND COPPER(II) EQUILIBRIA : RESULTS AND DISCUSSION

6. STUDIES OF PHOSPHORIC ACID ESTER-PROTON AND COPPER(II) EQUILIBRIA : RESULTS AND DISCUSSION

6.1 PROTONATION CONSTANTS

Four titrations of each ligand (A-D), initially in the presence of hydrochloric acid (0,01 mol dm^{-3} H^+; 0,15 mol dm^{-3} Cl^-), were performed against sodium hydroxide (0,02 mol dm^{-3} OH^-; 0,15 mol dm^{-3} Cl^-) up to a pH of ca. 7,5 using two different glass electrodes. The total ligand concentration was fixed at 0,005 mol dm^{-3}.

No attempt was made to determine:

i) the second protonation constant of the monoanion of 8-quinolylmethyl phosphate (D), (hereafter referred to as ligand (D)) or the dianion of 1-naphthyl phosphate (A) (hereafter referred to as ligand (A));

ii) the third protonation constant of the dianion of 8-quinolyl phosphate (B) (hereafter referred to as ligand (B));

as these pK values are known to lie below 2[65] where the ionic strength cannot be maintained sensibly constant at 0,15 mol dm^{-3} and where the glass electrodes become "non-linear".

The overall protonation constants (β_{por} referring to the general complex $L_pM_qH_r$ where L = ligand, M = metal ion, H = proton, q = 0 and r = OH) for ligands A-B, as determined by MINIQUAD, are listed in Table 7.

The monoanion of (C) (hereafter referred to as ligand (C)) has only one protonation site, namely the non-esterified PO_2^- group, the pKa of which could not be determined within the range of experimental pH used. This was to be expected, however, following the discussion in paragraph two above.

TABLE 7

LOGARITHMS OF THE OVERALL PROTONATION CONSTANTS OF LIGANDS A-D
(0,15 mol dm^{-3} NaCl; 25°C)

LIGAND	COMPLEX	PROTONATION SITE	LOG β_{por}	STD. DEVIATION in LOG β	NUMBER OF EXPERIMENTAL OBSERVATIONS	R FACTOR
A	LH	$P\text{-}O^-$	5,722	0,003	173	0,0060
B	LH	$P\text{-}O^-$	6,396	0,002	276	0,0016
	LH_2	$=N-$	10,560	0,002		
C	-	-	-	-	131	-
D	LH	$=N-$	4,750	0,002	131	0,0048

FIG. 2. EXPERIMENTAL PROTONATION CURVES FOR LIGAND (A)
(4 titrations)

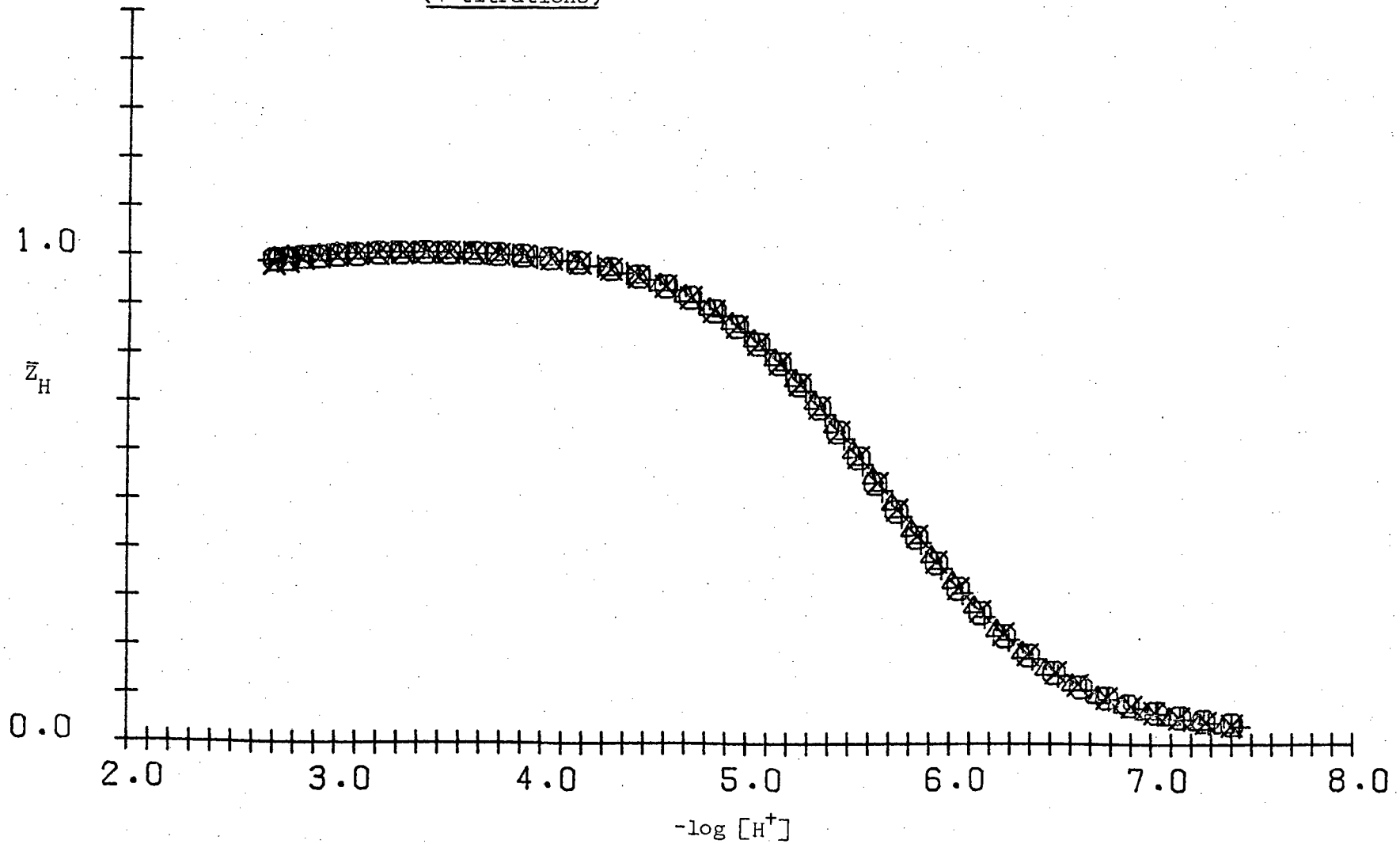


FIG. 3. EXPERIMENTAL PROTONATION CURVES FOR LIGAND (B)
(4 titrations)

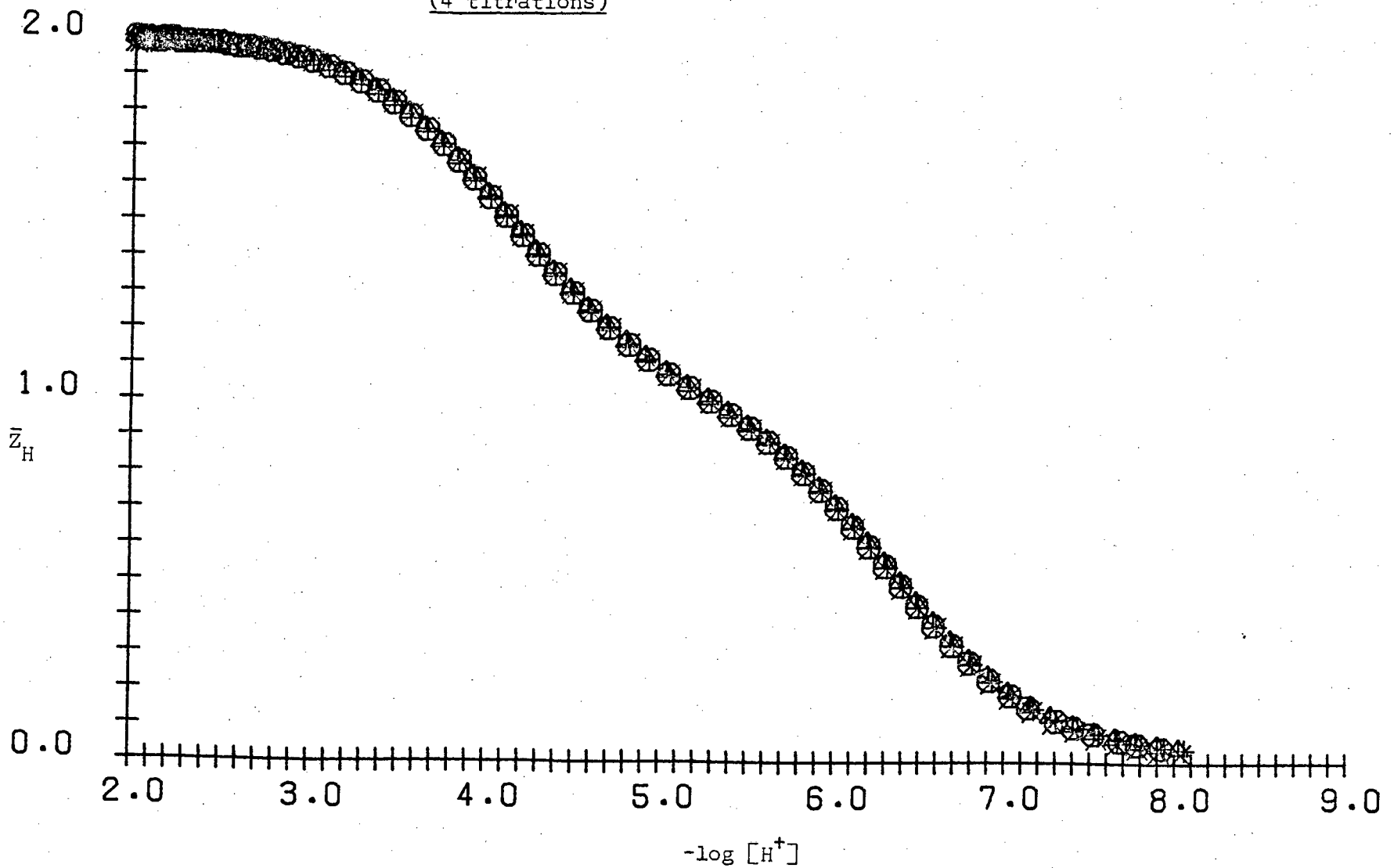
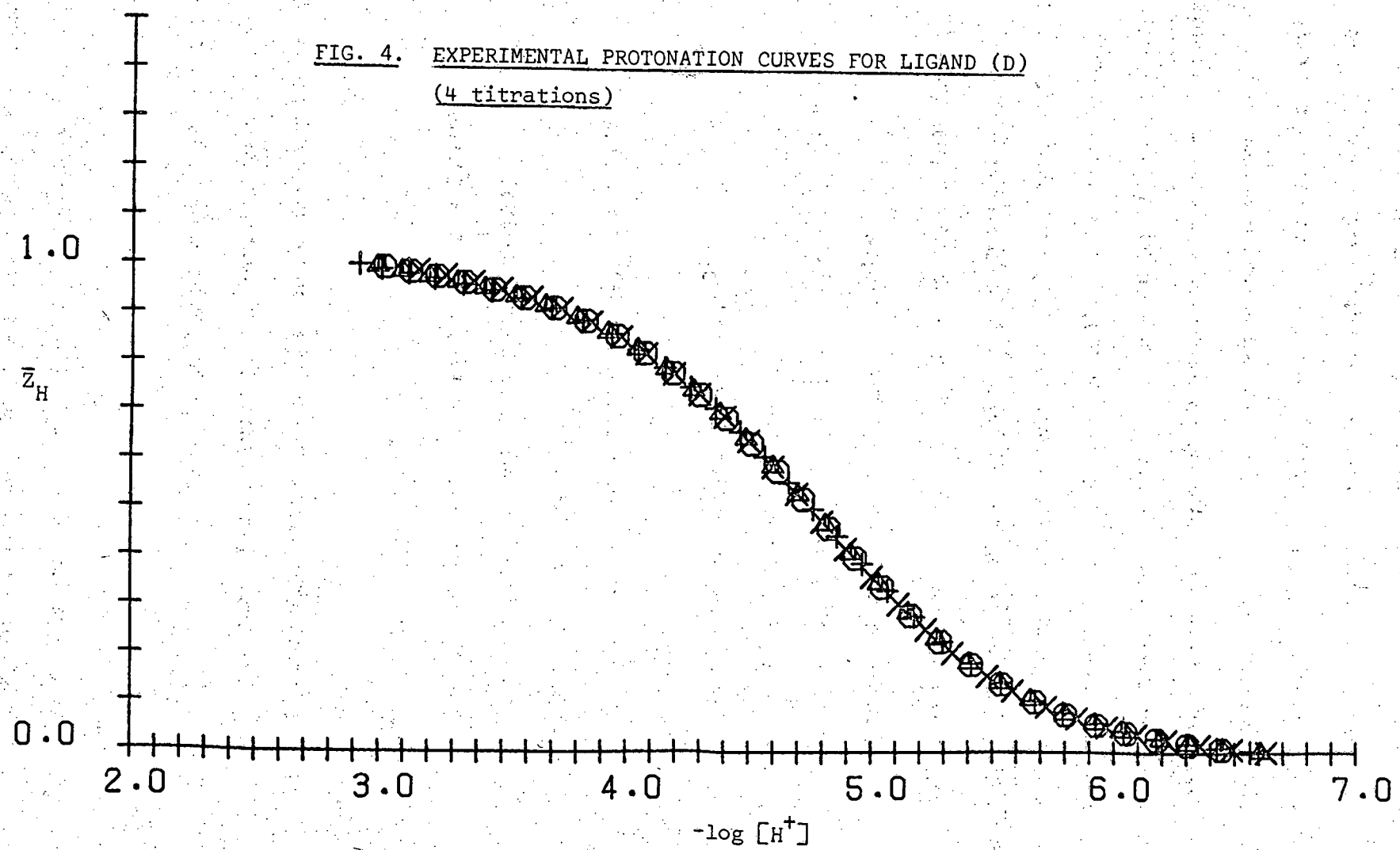


FIG. 4. EXPERIMENTAL PROTONATION CURVES FOR LIGAND (D)
(4 titrations)



All the sets of titration data yielded superimposable curves of $(\bar{Z}_H)_{obs}$ against pH and indicated the presence solely of simple LH (ligands (A) and (D)), and LH and LH_2 (ligand (B)) protonated species (see Figs. 2, 3 and 4).

For ligand (B), the first protonation constant was assigned to a phosphoryl oxygen and the second to the quinolyl nitrogen by:

i) comparison with literature values for related ligands at 25°C and I = 0,1:

1-naphthyl dihydrogen phosphate -

pKa of the more basic phosphate oxygen = 5,74[65]

(c.f. pKa for the same atom in ligand (B))

phenyl dihydrogen phosphate -

pKa of the more basic phosphate oxygen = 5,76[65]

8-hydroxyquinoline -

pKa of the quinolyl nitrogen = 4,99[33a]

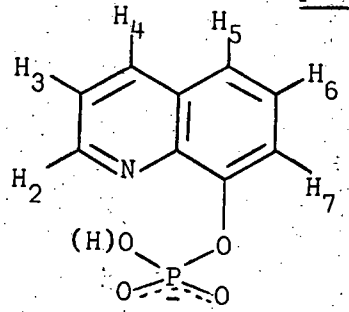
8-quinolyl dihydrogen phosphate -

pKa of the more basic phosphate oxygen = 6,42[14a]

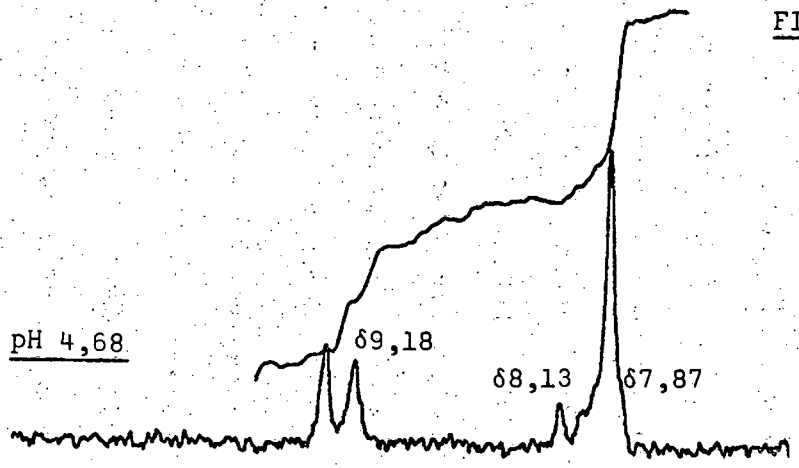
pKa of the quinolyl nitrogen = 4,17[14a].

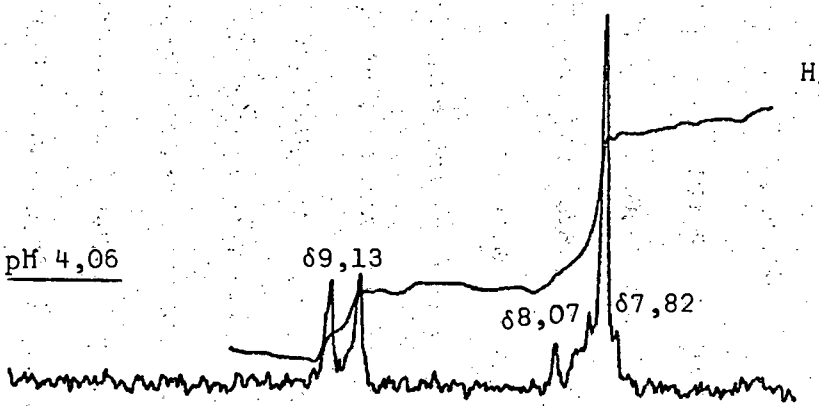
ii) recording the ^1H NMR(D_2O) spectrum of ligand (B) in a pH range of 2-7 (see Fig. 5). At pH 2,68 the protons in positions 2 and 7 in the quinolyl ring resonate as a multiplet (δ 9.23). By pH 4,06, this multiplet has separated to a broad doublet(δ9,13)(due to deprotonation of the nitrogen atom) which, as the pH rises to 6 and 7, further refines to the distinct doublet of doublets (δ8,90 proton ortho to the quinolyl nitrogen; δ8,67 proton ortho to the hydroxyl function)characteristic of the spectrum of 8 -hydroxyquinoline (in $CDCl_3$). The fact that the proton ortho to the quinolyl nitrogen only becomes distinctly deshielded relative to the proton orthoto the hydroxyl function at a pH above 6,3 (the pKa of one

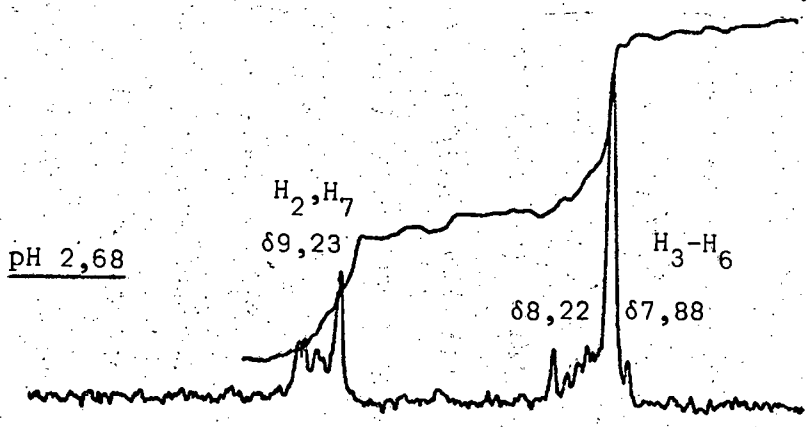
FIG. 5. ^1H NMR SPECTRA (D_2O) OF QUINOLYL PROTONS IN LIGAND (B) AT pH 2-7

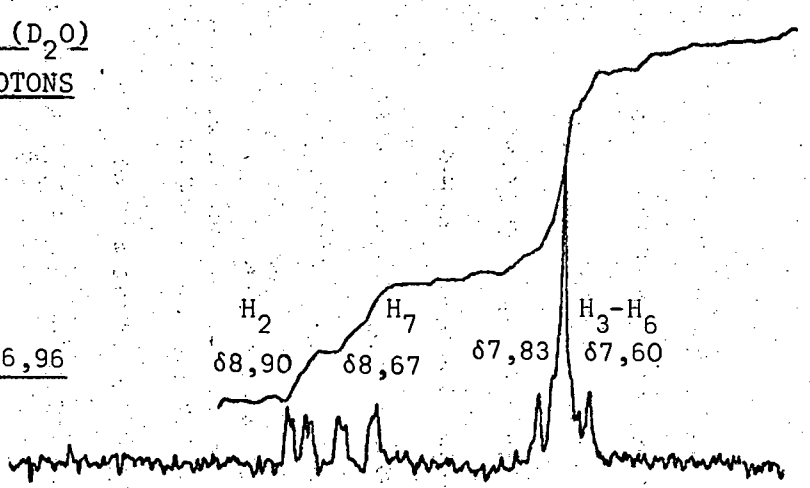


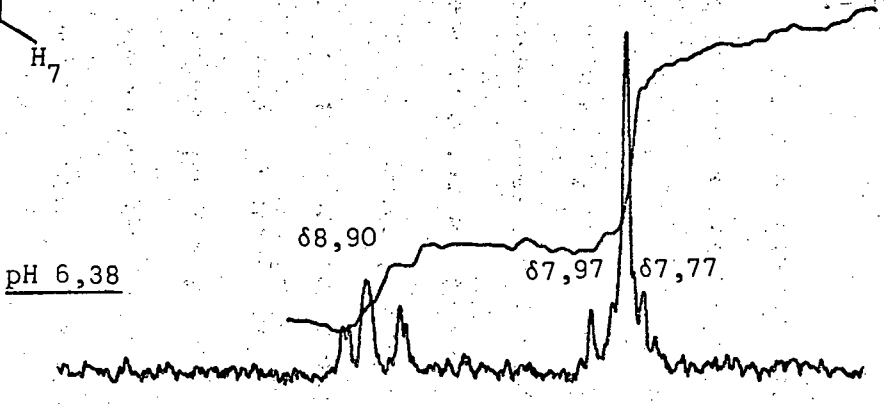
Ligand (B)

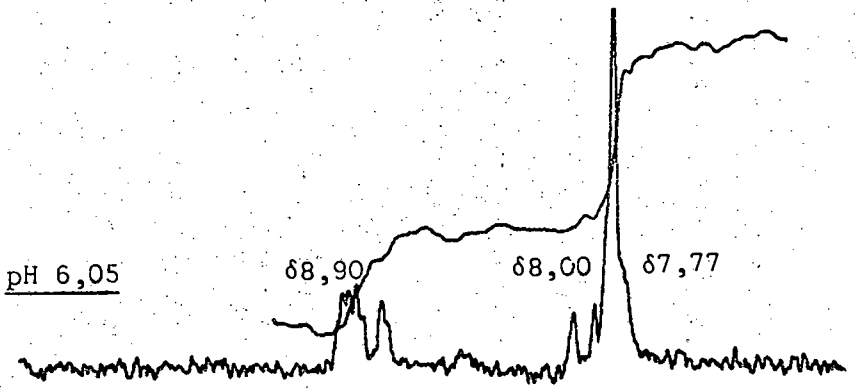












of the phosphate groups being 6,396) may suggest some hydrogen bonding by the quinolyl nitrogen of the proton on the phosphoryl oxygen. This idea of hydrogen bonding may also account for the slightly higher basicity of the phosphoryl oxygen in ligand (B) (pKa 6,396) compared to that for the same group in ligand (A) (pKa 5,722). The increased acidity of the quinolyl nitrogen in (B) (pKa 4,164), compared to that of the analogous function in (D) (pKa 4,750), may also be due to the fact that in the conjugate base of ligand (B) the neutral nitrogen atom can form a hydrogen bond with the free phosphate group, while in ligand (D) this is not possible because of the second (methyl) ester group. One might also speculate that the higher pKa of the nitrogen atom in ligand (D) may be due to the electron-donating effects of the methyl ester group present in ligand (D) but absent in ligand (B). However, such electronic effects would have to be transmitted through six bonds and would probably not be felt to any great extent.

6.2 COPPER(II) COMPLEXATIONS

A series of replicated titrations of each phosphate ester ligand in the presence of copper(II) ions and hydrochloric acid (0,01 mol dm^{-3}; 0,15 mol dm^{-3} Cl^-), was carried out against sodium hydroxide (0,02 mol dm^{-3}; 0,15 mol dm^{13} Cl^-) up to respective pH values limited by the appearance of precipitates. Total concentrations of the ligands varied from 0,003 to 0,004 mol dm^{-3}. Ligand : metal ratios of 1:1 and 2:1 were employed in order to facilitate the search for not only mononuclear binary complexes but also protonated, hydroxo- and maybe oligonuclear species. Indeed, Figs. 6, 7 and 8 show the computed $\bar{Z}_{obs}$ and pL points for the phosphate ester-copper(II) systems to fall on sets of non-overlapping curves with "fanning back" patterns. If only mononuclear binary complexes had formed (with q = 1 and r = 0 throughout), then the individual formation curves

FIG. 6a. EXPERIMENTAL FORMATION CURVES FOR LIGAND (A) IN THE PRESENCE OF COPPER(II)

FIG. 6b. THEORETICAL FORMATION CURVES FOR LIGAND (A) IN THE PRESENCE OF COPPER(II)

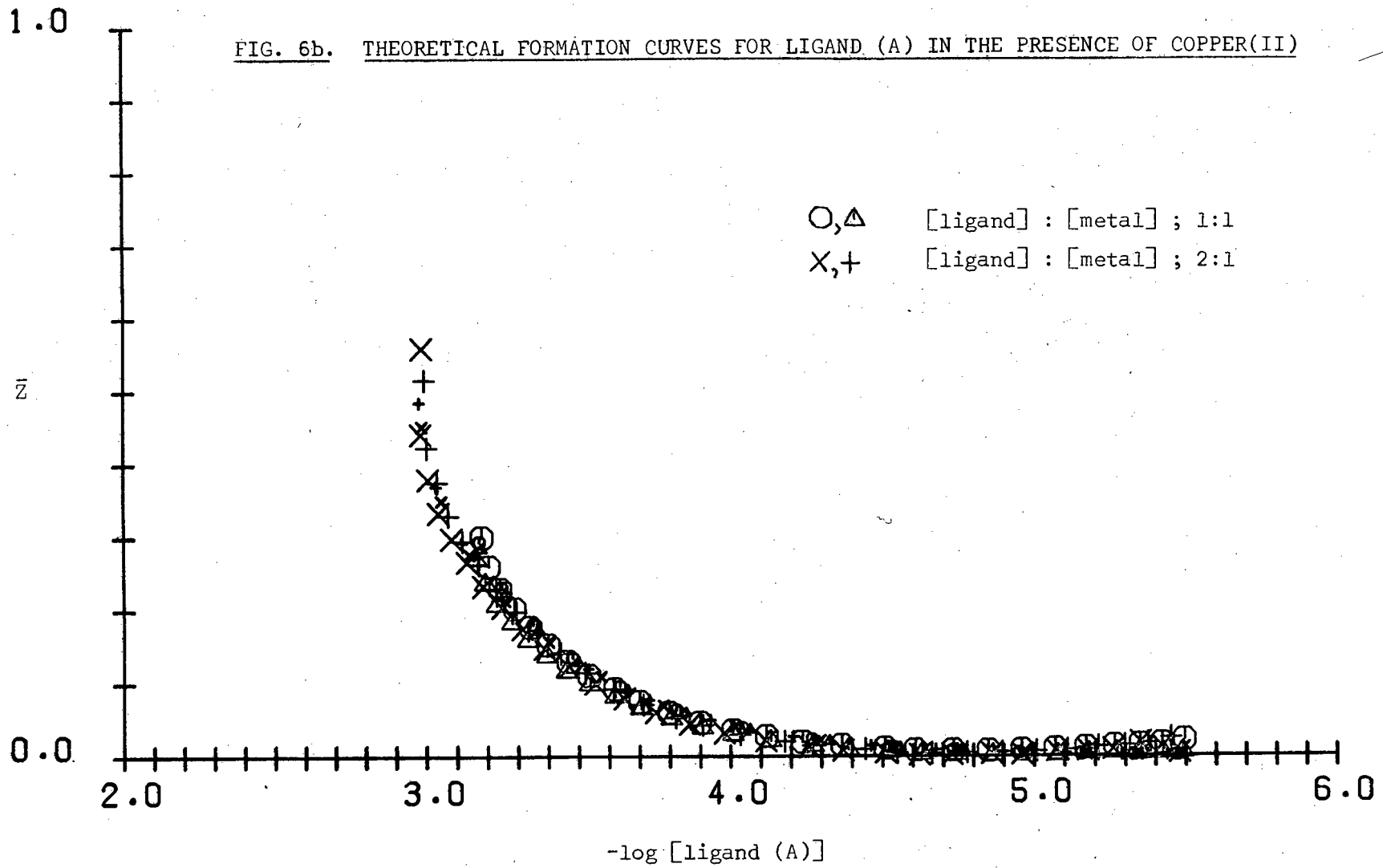


FIG. 7a. EXPERIMENTAL FORMATION CURVES FOR LIGAND (B) IN THE PRESENCE OF COPPER(II)

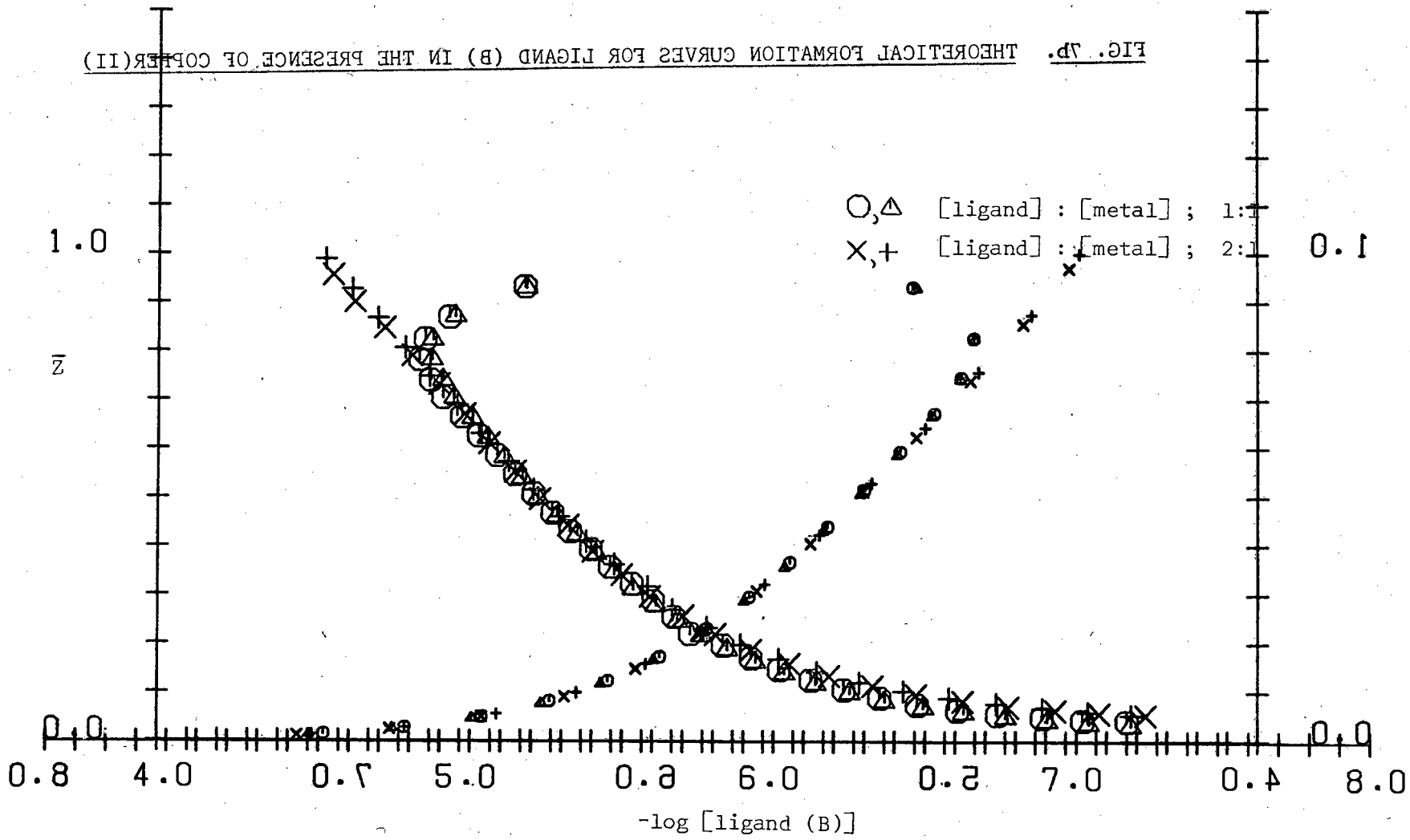
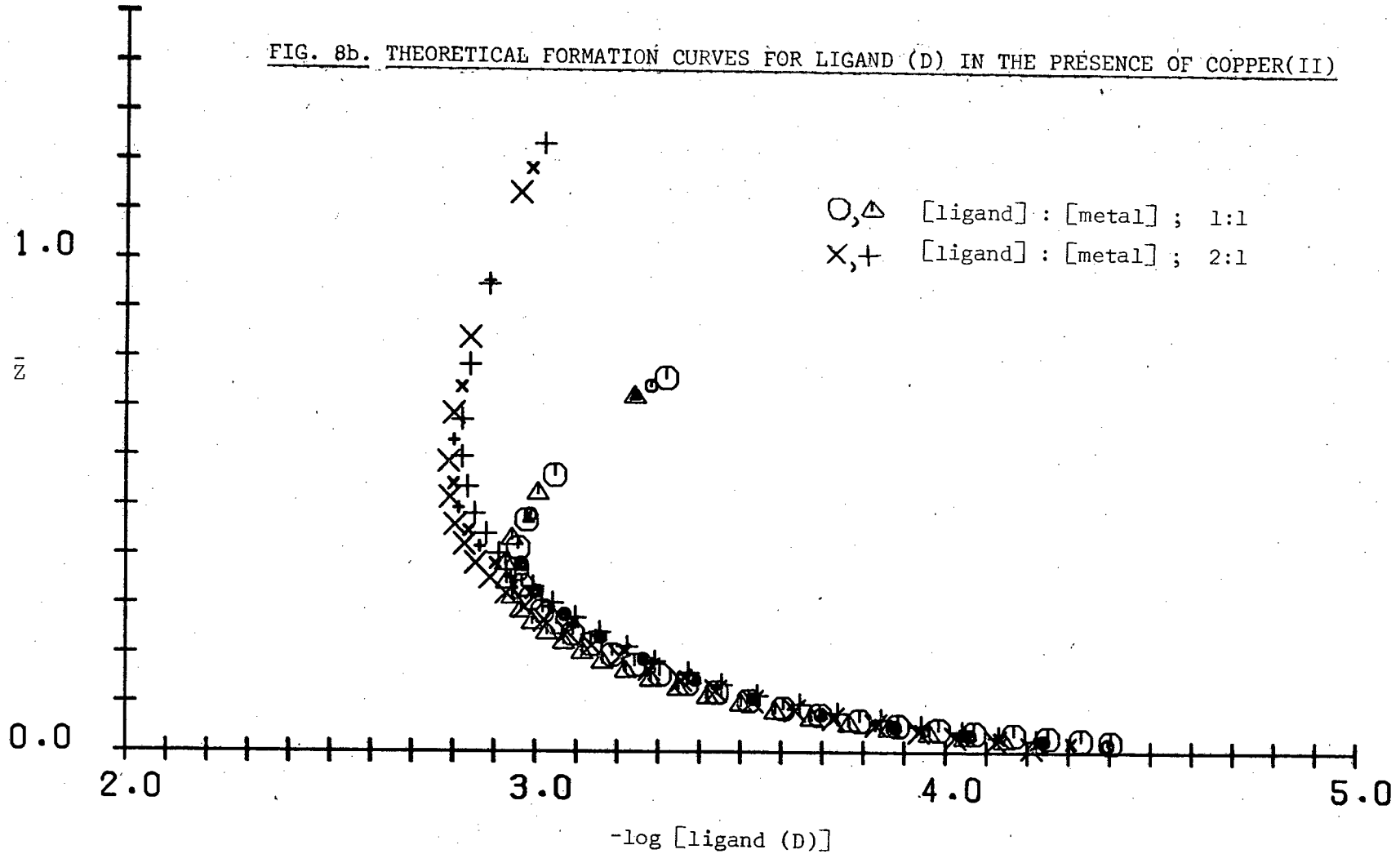


FIG. 8a. EXPERIMENTAL FORMATION CURVES FOR LIGAND (D) IN THE PRESENCE OF COPPER(II)

FIG. 8b. THEORETICAL FORMATION CURVES FOR LIGAND (D) IN THE PRESENCE OF COPPER(II)



corresponding to different ligand : metal concentrations would have overlapped exactly.[62] The shapes of the curves in Figs. 6 - 8 indicate the presence of other species (i.e. with $r \neq 0$ and/or $q > 1$) as $\bar{Z}_{obs}$ was computed assuming $q = 1$ and $r = 0$ for all complexes present (Williams[62] has named the resulting $\bar{Z}$, "pseudo $\bar{Z}$").

MINIQUAD computations were applied to the two sets of titration data obtained for each ligand (A, B and D) and in all refinements the protonation formation constants of the ligand were fixed and the hydrolysis reactions[66] (EQN'S 5 and 6) were incorporated into every trial.

$$Cu^{2+} + H_2O \rightleftharpoons [Cu(OH)]^{+} + H^{+} \quad \log K = -8,0 \qquad \text{EQN 5}$$

$$2Cu^{2+} + 2H_2O \rightleftharpoons [Cu(OH)]^{2+} + 2H^{+} \quad \log \beta = -10,6 \qquad \text{EQN 6}$$

Numerous combinations of β_{pqr} were tried and the most consistent set of complexes found, for each phosphate ester-copper(II) system, together with their respective stability constants, is shown in Table 8 (β_{pqr} referring to the general complex $L_p M_q H_r$ where L = ligand, M = metal ion, H = proton and -r = OH).

The validity of the model is illustrated by the high degree of matching of the experimental $\bar{Z}_{obs}$ and pL points in Figs. 6, 7 and 8 to corresponding theoretical formation curves computed by PSEUDOPLOT, using the formation constants from Table 8. Figs. 9, 10 and 11 show the distribution, as calculated by MINEQL, of the postulated complexes in the 2:1 ligand : metal ion titrations.

TABLE 8

LOGARITHMS OF THE OVERALL STABILITY CONSTANTS OF THE COPPER(II) COMPLEXES OF LIGANDS A, B AND D ($0{,}15$ mol dm^{-3} NaCl; $25^{\circ}C$)

LIGAND	COMPLEX	LOG β_{pqr}	STD. DEVIATION IN LOG β_{pqr}	NO. OF EXPERIMENTAL OBSERVATIONS	R FACTOR
A	LM	2,645	0,011	58	0,0037
	L_2M	4,416	0,104		
	L_2MH_{-1}	-1,645	0,115		
	L_2MH_{-2}	-8,057	0,283		
B	LM	5,293	0,010	58	0,0090
	L_2M	8,905	0,088		
	L_2MH_{-1}	3,625	0,093		
	L_2MH_{-2}	-2,068	0,237		
ℰ D	LM	2,600	0,014	55	0,0059
	L_2M	4,257	0,158		
	L_2MH_{-1}	-1,213	0,040		
	L_2MH_{-2}	-8,042	0,317		

Species distribution in the phosphate ligand-copper(II) systems as computed by MINEQL

Total concentration of ligand = 0,004 mol dm^{-3}

Total concentration of copper(II) = 0,002 mol dm^{-3}

KEY:

× LM

o L_2M

▲ L_2MH_{-1}

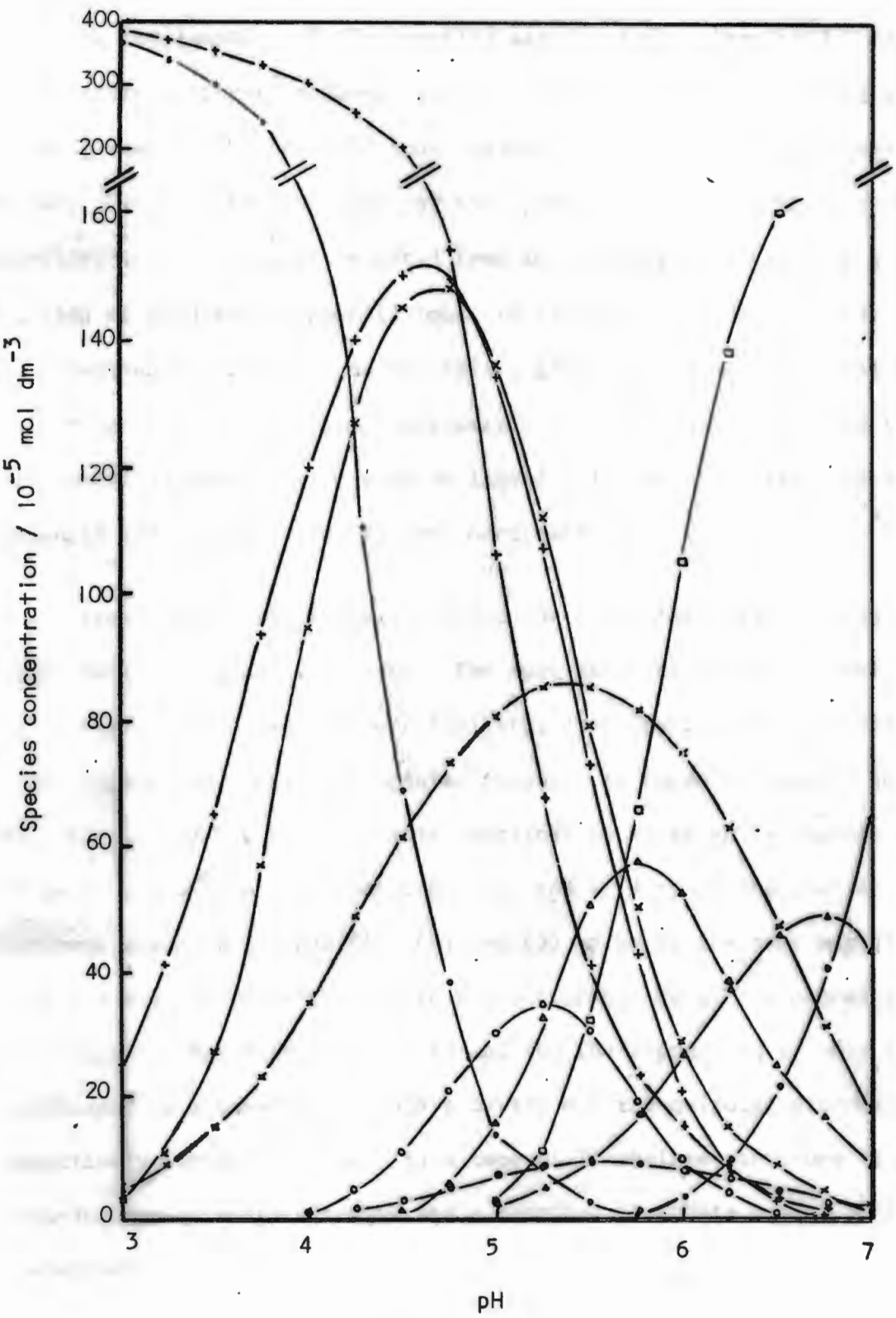
□ L_2MH_{-2}

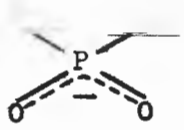
\+ LH

• LH_2

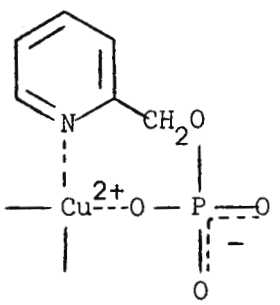
FIG. 11. Ligand (B)

FIG. 10. Ligand (C)





The fact that ligand (B) is acting as a bidentate ligand can also be illustrated using a principle introduced by Sigel[66] According to this, the difference in the logarithms of the stepwise formation constants for the mono and bis complexes formed from a bidentate ligand should have a value between 1 and 2. As K_{110} and K_{210} may be defined as $[LM]/[L][M]$ and $[L_2M]/[L][ML]$, respectively, $\log K_{110} - \log K_{210} = \sim 1{,}68$ for the copper(II) complexes of ligand (B). On the other hand, $\log K_{110} - \log K_{210}$ for ligands (A) and (D) have values less than one. It is not unreasonable for 8-quinolyl phosphate to yield a seven-membered chelate ring when examined by means of a Dreiding structural model, and chelate rings involving more than six members have been proposed for other phosphate-containing ligands eg. 2-pyridylmethyl phosphate;[68] O-phosphorylated peptides[69] and salicyl phosphate?[70] The greater stability of the 1:1 ligand (B) : copper(II) complex relative to that of the analogous 1:1 2-pyridylmethyl phosphate : copper(II) complex[68] [see (XVIII) below] :

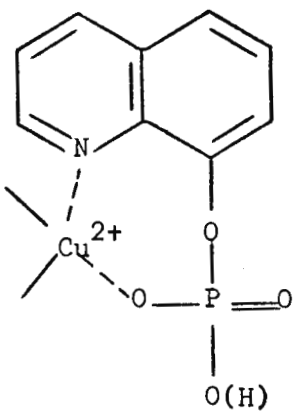


(XVIII)

may be attributed to the greater structural rigidity of ligand (B) compared to that of 2-pyridylmethyl phosphate, and the restricted freedom of rotation about the bonds between the phosphate and the quinolyl ring. A 2 : 1 pyridylmethyl phosphate : copper(II) complex was not detected and the low stability of such a species ascribed to the low basicity of the pyridyl nitrogen (pKa 4,42) and to the steric effect of the pyridyl ring which, it was thought, might generate a strong repulsive force against a second ligand. However, the strength of the 2 : 1 8-quinolyl phosphate : copper(II) complex found here, together with the lower

basicity of the 8-quinolyl nitrogen (pKa 4,16) and the lack of steric hindrance in the Dreiding model, suggests that the instability of the higher complex of 2-pyridylmethyl phosphate is due purely to structural factors.

Murakami and Sunamoto;[14b] in their studies on 8-quinolyl phosphate, report also the formation of the protonated complex $[M(HL)^{+}]$ and propose that the copper assisted hydrolysis of 8-quinolyl phosphate involves the intermediate (XIX):



(XIX)

Protonated complexes were, indeed, found (using MINIQUAD) for ligands (A), (B) and (C) but were deemed to be statistical artefacts, possibly due to activity quotient fluctuations, as they could not be rationalised, MINEQL found them to be present in very small concentrations and only at low pH, and their removal from the model did not affect the theoretical curves produced by PSEUDOPLOT, nor to any considerable extent the MINIQUAD R-factors. Structure (XIX) is inconsistent with the fact that the stability constants for copper complexation with ligand (D) indicate only unidenticity of ligand (D) (through the quinolyl nitrogen) as the second possible coordination site, the terminal phosphate group is presumably too acidic for metal coordination. (The lack of complexation with ligand (C) was also attributed to the great acidity of the only potential

coordination site, the terminal phosphate group).

A number of reports[71-73] on the stability constants of bivalent metal ions with various phosphates (adenosine mono-, di- and tri-phosphate; 2-pyridylmethyl phosphate) indicate that the donor group playing the major role in the metal complex formation is the phosphate group, with the heterocyclic nitrogen atom(s) playing a merely minor or nearly a negligible coordination role. From Table 8 it can be seen that the stabilities of the copper complexes formed with ligand (A) through metal-oxygen coordination are, in fact, slightly stronger than those formed with ligand (D) involving metal-nitrogen coordination. Perhaps this effect is due to the relatively high electronegativity of the sp^2 hybridised quinolyl nitrogen atom and to the presence of the negative charge at the phosphate oxygen.

The formation of hydroxo-complexes only occurred with the 2:1 phosphate : copper(II) species and at higher pH's, which suggests that coordinate bond formation is fairly strong and hydrolysis unfavourable. Hence, the dimerisation reported [72b] for 1:1 chelates of adenosine di- and tri-phosphates was not found to occur with copper(II) complexes of ligands (A), (B) and (D).

In conclusion then, the availability and suitability of coordination sites on a phosphate ester ligand is an important factor in determining the stability of its complexes with a metal ion, such as copper. In this respect chelate-formation, which enhances stability, is only possible for monoesters [eg. ligand (B)] which possess at least one more functional group, besides the phosphate moiety, which shows a satisfactory donating affinity for a metal ion. Monoesters such as ligand (A) which do not fulfil this requirement show only unidentate coordination to copper(II), with a corresponding decreased stability, as do diesters

such as ligand (D). In a diester like ligand (C) where the only metal ion coordination site is rather too acidic to provide a suitable donating affinity for a metal ion such as copper(II) (which is itself a Lewis acid), no complexation occurs.

CHAPTER 7

EXPERIMENTAL PROCEDURES USED IN THE DETERMINATION OF STABILITY CONSTANTS

7. EXPERIMENTAL PROCEDURES USED IN THE DETERMINATION OF STABILITY CONSTANTS

7.1 GENERAL

The experimental procedures used in this study were similar to those described by Williams[60b] and other workers in this field. In general they involved the potentiometric titration of a solution of the phosphate ligand of initial concentration 0,005 mol dm^{-3} and a solution of copper(II) ions of varying initial concentration 0,005 - 0,0005 mol dm^{-3}. The protonation constants were obtained from solutions containing no metal ions. Each titration was carried out under an atmosphere of nitrogen and checked by duplicate runs. The data obtained (ml, mV) were analysed using the computational methods described in Chapter 5.3.

7.2 APPARATUS

The titrations were carried out in a Metrohm titration vessel (EA876-20) equipped with a thermometer, magnetic stirrer, nitrogen inlet and outlet tubes, a Metrohm automatic burette (DOSIMAT E 635) delivery tube, a Metrohm glass electrode (1047) and a Metrohm calomel reference electrode (1028). The titrations were controlled by a Metrohm automatic titration controller (TITROPROCESSOR E636) which also recorded the experimental readings of volume added and emf measured. (See Figs. 12, 13).

The titration vessel was thermostatted at 25°C ± 0,1°C using a Lauda thermostat. The high purity nitrogen, (Afrox), which was passed through the titration vessel in order to exclude carbon dioxide and oxygen from the experimental solution, was (before being admitted to the titration vessel) further purified by bubbling through a series of gas wash bottles:

i) 50% KOH to remove CO_2

ii) Fiesers[74] solution to remove O_2

iii) distilled water (to wash)

iv) an empty bottle (a trap)

v) a solution of the support electrolyte (0,15 mol dm^{-3} NaCl) thermostatted at 25°C.

Back diffusion of the gas after passage through the titration vessel was prevented by its release to the atmosphere via a 0,15 mol dm^{-3} NaCl solution trap.

7.3 REAGENTS

All reagents used were of analytical grade. 'A' grade volumetric flasks and calibrated pipettes were employed in solution preparations. Boiled out glass distilled water (CO_2-free) was used throughout and all solutions were made up to 0,15 mol dm^{-3} with respect to $[Cl^-]$ using sodium chloride ampoules (Merck).

Sodium hydroxide (0,02 mol dm^{-3} OH^-; 0,15 mol dm^{-3} Cl^-) (Merck ampoules) was freshly prepared (at least once a week) under nitrogen and protected from atmospheric CO_2 by soda lime. Standardisation of the solution was effected with AnalaR potassium hydrogen phthalate (Merck) which had been dried according to the procedure in Vogel[75].

Hydrochloric acid (0,01 mol dm^{-3} H^+; 0,15 mol dm^{-3} Cl^-) (Merck ampoules) was standardised against sodium hydroxide.

A solution of copper(II) ions (0,01M Cu^{2+}; 0,15M Cl^-) was prepared from crystals of $CuCl_2.2H_2O$ (Merck) and standardised by EDTA titration[75].

A 0,005 mol dm^{-3} solution of each phosphate ester was prepared in the mineral acid such that each 20ml aliquot of the solution would deliver 0,1 mmoles of ligand to each titration. Each ligand solution was used within twelve hours (the ligands were found to be stable to acid hydrolysis for at least twelve hours). The titrations in this study involved solutions containing ligand:metal ion ratios of 1:0 (determination of protonation constants); 1:1 and 2:1 (determination of formation constants) and were checked by duplicate runs.

FIG. 12. Apparatus used in the determination of formation constants including (from left) thermostat, nitrogen purification train, electrode assembly, titration vessel, magnetic stirrer, automatic burette and automatic titration controller.

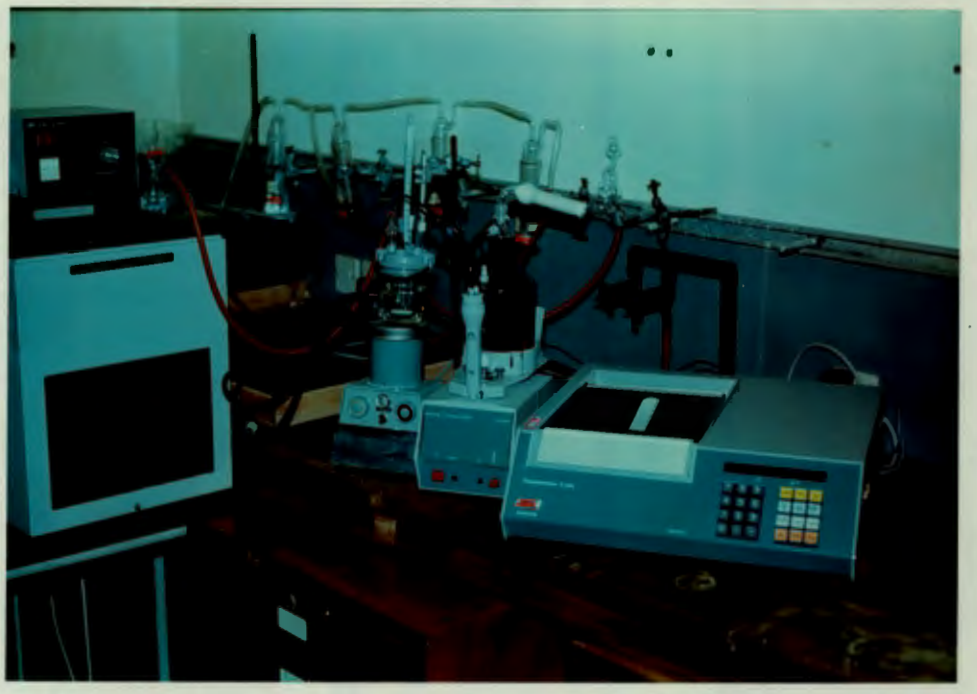
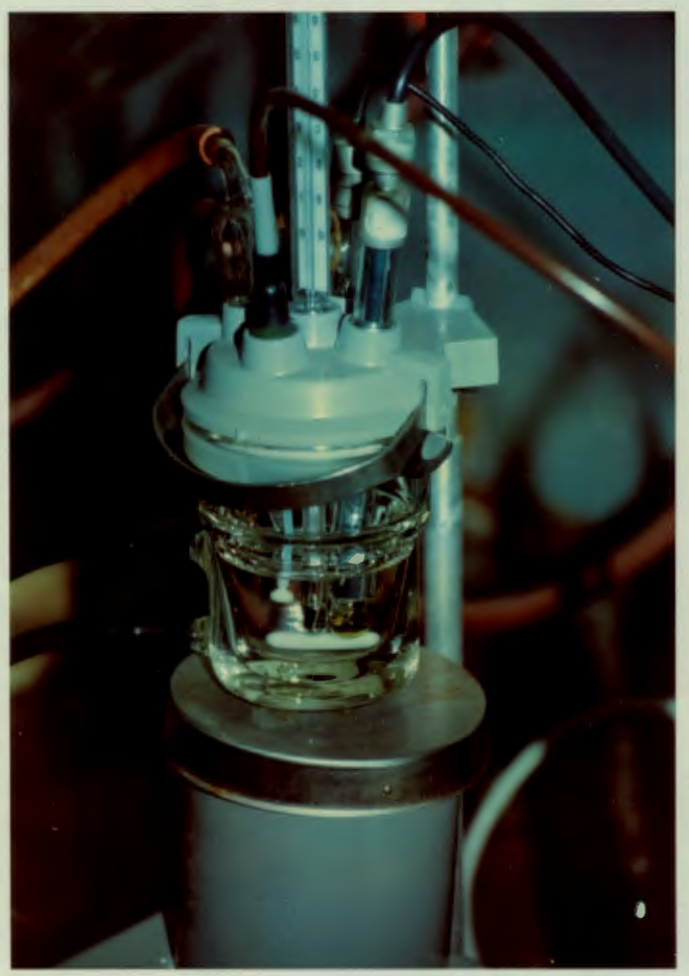


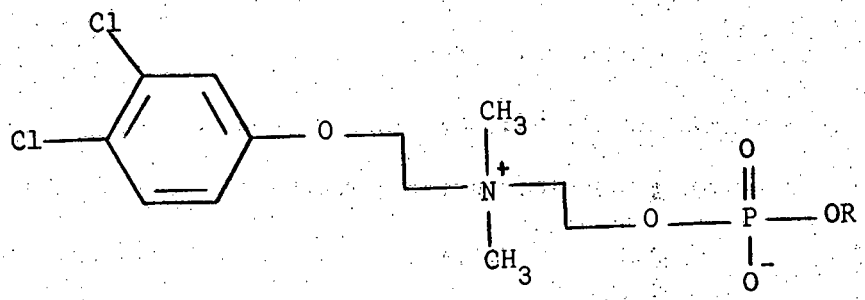
FIG. 13. Electrode assembly, thermostatted titration vessel and magnetic stirrer used in the determination of formation constants.



APPENDIX

APPENDIX

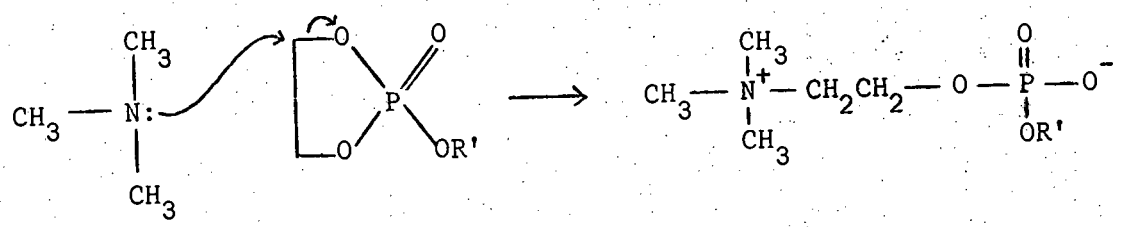
Colleagues in this (Organic Chemistry) laboratory had experienced some difficulty with the synthesis of the substituted lecithin (L):



R = cetyl or diacyl glycerol

(L)

via the route reported by Chandrakumer and Hadju[76] for the formation of a phosphatide link by reaction of triethylamine with a 1,3,2-dioxaphospholane:



R' = disubstituted glycerol.

Whereas ring opening by trimethylamine is facile and complete after 24 hrs at 60°C, the analogous reaction using 2-(3,4-dichlorophenoxy)ethyldimethylamine was extremely slow even at 100°C and led rather to degradation of the starting materials.

Initially it was thought that the lack of reactivity of the nitrogen moiety in 2-(3,4-dichlorophenoxy)ethyldimethylamine might be due to steric factors. However, a study of space-filling models did not fully support this idea, but led to the proposal that the electron-withdrawing properties of the 3,4-dichlorophenoxy group might play an important role

REFERENCES

REFERENCES

1a. C.A. Bunton, *Acc. Chem. Res.*, 3, 257, (1970).

b. C.A. Bunton and S.J. Farber, *J. Org. Chem.*, 34, 767, (1969).

c. A.J. Kirby and A.G. Varvoglis, *J. Amer. Chem. Soc.*, 89, 415,(1967).

2. J.R. Cox Jr. and O.B. Ramsay, *Acc. Chem. Res.*, 64, 317,(1964) and refs. therein.

3. S.J. Benkovic and K.J. Schray, In: "The Enzymes" (ed. P.D. Boyer), 8,201, (1973) and refs. therein.

4. A.S. Mildvan and C.M. Grisham, *Structure and Bonding*, 20, 1,(1974).

5. J. Emsley and D. Hall, "The Chemistry of Phosphorus", Harper and Row, London, 1976.

6. F.W. Westheimer, *Acc. Chem. Res.*, 1, 70,(1968).

7. B. Cooperman, In: "Metal ions in biol. systems" (ed. H. Sigel), 5, 79, (1976).

8a. A.J. Kirby and M. Younas, *J. Chem. Soc. (B)*, 1165, (1970).

b. A.J. Kirby and M. Younas, *ibid*, 510, (1970).

9. F. Ramirez, J.F. Marecek and J. Szamosi, *J. Org. Chem.*, 45, 4748, (1980).

10. G.J. Lloyd and B.S. Cooperman, *J. Am. Chem. Soc.*, 93, 4883, (1971).

11. T. Eiki, T. Horiguchi, M. Ono, S. Kawada, W. Tagaki, *J. Am. Chem. Soc.*, 104, 1986, (1982).

12. D.S. Sigman, G.M. Wahl and D.J. Creighton, *Biochem.*, 11, 2236, (1972).

13. S.J. Benkovic and L.K. Dunikoski Jr., *J. Am. Chem. Soc.*, 93, 1526, (1971).

14a. Y. Murakami and J. Sunamoto, *Bull. Chem. Soc. Jap.*, 44, 1827, (1971).

b. H. Takaku, *Chem. Pharm. Bull.*, 25, 2121, (1977).

15. Y. Murakami and M. Takagi, *J. Am. Chem. Soc.*, 91, 5130, (1969).

16. S.J. Benkovic and E.M. Miller, *Bioinorganic Chem.*, 1, 107, (1972).

17. J.S. Loran, A. Naylor and A. Williams, *J.C.S. Perkin II*, 418, (1977).

18a. J.J. Steffens, I.J. Siewars, S.J. Benkovic, *Biochem.*, 14, 2431, (1975).

b. E.J. Sampson, J. Fedor, P.A. Benkovic, S.J. Benkovic, *J. Org. Chem.*, 38, 1301, (1973).

19. R.H. Bromilow, S.A. Kahn, A.J. Kirby, *J.C.S. Perkin II*, 911, (1972).

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

21. R.D. Cornelius, *Inorg. Chem.*, 19, 1286, (1980).

22. P.R. Norman and R.D. Cornelius, *J. Am. Chem. Soc.*, 104, 2356. (1982).

23. P.R.Norman, P.F.Gilletti, E.D.Cornelius, *Inorg.Chim. Acta*, 82, L5, (1984).

24. R.D.Cornelius, *Inorg.Chim. Acta*, 46, L109, (1980).

25. C.A. Bunton, E.J. Fendler, L. Sepulveda, K.U. Yang, *J. Amer. Chem. Soc.*, 90, 5512, (1968).

26. G.J. Buist, C.A. Bunton, L. Robinson, L. Sepulveda, M. Stam, *J. Amer. Chem. Soc.*, 92, 4072, (1970).

27. A.S. Mildvan, In: "The Enzymes", (ed. P.D. Boyer), 2, 446, (1970).

28. M.M. Armstrong, T.A. Modro, unpublished results.

29a. A.J. Kirby and W.P. Jencks, *J. Amer. Chem. Soc.*, 87, 3209, (1965).

b. A.J. Kirby and W.P. Jencks, *ibid.*, 3217, (1965).

30. J.S. Loran and A. Williams, *J.C.S. Penkin II*, 64, (1977).

31. A. Murayama, B. Jastorff,F. Cramer and H. Hettler, *J. Org. Chem. Soc.*, 36, 3029, (1971).

32. J. Moffatt and H.G. Khorana, *J. Amer. Chem. Soc.*, 79, 3741, (1957).

33a. A.E. Martell and R.M. Smith, "Critical Stability Constants", Vol.3 - Other Organic Ligands, Plenum Press, 1977, New York.

b. D.G Perrin "Stability Constants of Metal Ions", Part B - Organic Ligands, IUPAC, Pergamon Press (1979, New York.

34. W. Niewiarowski, W.J. Stec and W.S. Zielinski, *J.C.S. Chem. Comm.*, 524, (1980).

35. W.J. Stec, A. Okruszek, K. Lesiak, B. Uznanski and J. Michalski, *J. Org. Chem.*, 41, 227, (1976).

36. V. Mizrahi, T.F. Hendrikse and T.A. Modro, *Phosphorus and Sulphur*, in press.

37. D. Bricot, N. Collignon and P. Savignac, *Tetrahedron*, 35, 1345, (1979).

38. V.V. Katyshkina and M.Ya Kraft, *Chem. Abs.*, 51, 8028f, (1957).

39. O.M. Friedman and A.M. Seligman, *J. Amer. Chem. Soc.*, 72, 624, (1950).

40. O.M. Friedman and A.M. Seligman, *J. Amer. Chem. Soc.*, 72, 625, (1950).

41. H. Budzikiewicz, C. Djerassi and D. Williams, "Mass Spectrometry of Organic Compounds", Holden-Day Inc., San Francisco, 1967.

42. L. Klasinc and H. Gusten, *Z. Naturforsch*, 27, 1681, (1972).

43a. Y. Murakami, J. Sunamoto, H. Sadamori, H. Kondo and M. Takagi, *Bull. Chem. Soc., Japan*, 43, 2518, (1970).

b. H. Takaku, M. Kato and T. Hafa, *J. Chem. Soc. Chem. Comm.*, 6, 190, (1977).

44. R.E. Burton and W.J. Davis, *J. Chem. Soc.*, 344, 1766, (1964).

45a. L. Zervas, I. Dilaris, *J. Am. Chem. Soc.*, 77, 5354, (1955).

b. R.J.W. Cremlyn, G.W. Kenner, J. Mather and A. Todd, *J. Chem. Soc.*, 528, (1958).

46. Vogel's Textbook of Practical Organic Chemistry, 4th Ed., Longman, Suffolk; p.269, 1980.

47. B. Davidowitz and T.A. Modro, *Org. Mass. Spec.*, 19, 128, (1984).

48. F.W. McLafferty, Interpretation of Mass Spectra, 3rd Ed. Univ. Science Books, Mill Valley, U.S.A., p. 194, (1980).

49. B. Fiszer and J. Michalski, *Roczn.Chem.*, 26, 688, (1952).

50. C.J. Lacey and L.M. Loew, *Tet. Let.*, 21, 2017, (1980).

51. R.G. Pearson, *J. Chem. Ed.*, 45, 581 and 643, (1968).

52. F.J.C. Rossotti and H. Rossotti, "The Determination of Stability Constants", McGraw-Hill, New York, 1961.

53. F.R. Hartley, C. Burgess, R. Alcock, "Solution Equilibria", Longman, New York, 1978.

54. Niels Bjerrum first suggested in 1915 and later proved that the formation of complexes occurs in a stepwise manner with ML being formed first and then ML_2, such that it is not possible to form ML_n without first forming ML_{n-1}.

55. H. Rossotti, "The Study of Ionic Equilibria", Longman, New York, 1978.

56. R.M. Smith and A.E. Martell "Critical Stability Constants", Vol. 4. *Inorganic Complexes*, Plenum Press, 1976, New York.

57a. M. May, D.R. Williams, P.W. Linder and R.G. Torrington, *Talanta*, 29, 249, (1982).

b. P.W. Linder, R.G. Torrington and D.R. Williams, "Analysis using Glass Electrodes", Open University Press, Milton Keynes, 1984.

58a. A. Sabatini, A. Vacca and P. Gans, *Talanta*, 21, 53, (1974).

b. P. Gans, A. Sabatini and A. Vacca, *Inorg. Chim. Acta*, 18, 237, (1976).

59. R.P. Martin and R.A. Paris, *Societe Chim. de France Bull.*, 110, 570, (1968).

60. J.A. Nelder and R. Mead, *Comput. J.*, 7, 308, (1965).

61a. D.R. Williams, *J. Chem. Ed.*, 48, 480, (1971).

b. D.R. Williams, *J.C.S.*, Dalton, 1064, (1973).

62. A.M. Corrie, G.K.R. Makar, M.L.D. Touche and D.R. Williams, *J.C.S.*, Dalton, 105, (1975).

63. A. Vacca, A. Sabatini and M. Gristina, *Coord. Chem. Rev.*, 8, 45, (1972).

64. J. Westell, J. Zachary and F. Morel, "MINEQL", Technical Note 18, Water Quality Lab., M.I.T. (1975).

65. R.M. Smith, and A.E. Martell, "Critical Stability Constants", Vol. 2, *Amines*, Plenum Press, 1975, New York.

66. C. Berecki-Biedermann, *Arkiv. Kemi.*, 9, 175, (1956).

67. H. Sigel in "Metal ions in biological systems", (ed. H. Sigel) 2, 65, (1973).

68. Y. Murakami and M. Takagi, *J. Phys. Chem.*, 72, 116 (1968).

69. R. Österberg, *Acta Chem. Scand.*, 16, 2434, (1962).

70. G.E. Mont and A.E. Martell, *J. Am. Chem. Soc.*, 88, 1387, (1966).

71. A.E. Martell and G. Schwarzenbach, *Helva. Chim. Acta*, 39, 653, (1956).

72a. M. Taqui Khan and A.E. Martell, ?

b. M. Taqui Khan and A.E. Martell, *J. Am. Chem. Soc.*, 84, 3037, (1962).

73. R.M. Smith and R.A. Alberty, *J. Am. Chem. Soc.*, 78, 2376 (1956).

74. A. Albert and D. Serjeant, "Ionisation Constants of Acids and Bases" Methuen, p. 20, 1962.

75. A. Vogel, "Textbook of Quantitative Inorganic Analysis", 4th Ed. Longman, New York, 1978.

76. N.S. Chandrakumer and J. Hadku, *J. Org. Chem.*, 47, 2144, (1982).

77. D. Perrin, B. Dempsey and E.P. Serjeant, "pKa Prediction for Organic Acids and Bases", Chapman and Hall, New York, p.22, 1981.