SYNTHETIC AND STRUCTURAL ASPECTS OF METAL COORDINATION TO PYRIMIDINES, PURINES AND NUCLEOTIDES

A Thesis submitted for the
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ABSTRACT

Complexes of pyrimidines, nucleosides and nucleotides have been prepared in order to investigate the coordination properties of these biologically important ligands.

The structures of the nucleotide complexes of cadmium, platinum and zinc were determined using X-ray crystallographic methods coupled with infrared spectroscopy to confirm modes of binding. The implications of these structures have been discussed with relevance to the interaction of metal ions with DNA and related molecules. A preliminary investigation on a new class of 'platinum blues' was also carried out with the pyrimidine bases, nucleosides and nucleotides.

The complexes of 2-pyrimidinethione and 2-pyrimidinone were studied with mainly first row transition metals and the stereochemistries were characterised using infrared and electronic reflectance spectroscopy and magnetic susceptibility measurements. X-ray crystallography was used to determine the structures of three of these complexes.

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To My Parents

To My Friends

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ABBREVIATIONS AND NOMENCLATURE

The abbreviations employed in this thesis are mostly those approved by the Commission on Biochemical Nomenclature (CBN) of the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB). In cases where units and trivial names remain largely unchanged in modern literature, these are used instead.

Bases

Ade	adenine
9MeAde	9-methyladenine (9-methyl-6-purinamine)
Cyt	cytosine
Gua	guanine
Hyp	hypoxanthine
9MeHyp	9-methylhypoxanthine (9-methyl-6-purinone)
9BenzPur6SH	9-benzyl-6-purinethione
Thy	thymine
Ura	uracil

Nucleosides

A	adenosine
C	cytidine
G	guanosine
I	inosine
T	thymidine
U	uridine

Complexes of bases and nucleosides containing deprotonated ligands are denoted by (') or ( )'

Nucleotides

5'-AMP	adenosine 5'-monophosphate
5'-ATP	adenosine 5'- triphosphate
5'-CMP	cytidine 5'-monophosphate
5'-GMP	guanosine 5'-monophosphate
5'-IMP	inosine 5'-monophosphate
5'-TMP	thymidine 5'-monophosphate
5'-UMP	uridine 5'-monophosphate

The free acid and sodium salt of these ligands are denoted by H_2 and Na_2 respectively. The above abbreviations are retained for complexes containing the dianion e.g. 5'-IMP^{2-}, but if a further proton is removed the symbol (') is used.

Polynucleotides

RNA	ribonucleic acid
mRNA	messenger RNA
tRNA	transfer RNA
DNA	deoxyribonucleic acid

Other Ligands

Pym2SH	2-pyrimidinethione
4,6Me_2Pym2SH	4,6-dimethyl-2-pyrimidinethione
Pym2OH	2-pyrimidinone
1MePym2OH	1-methyl-2-pyrimidinone
4,6Me_2Pym2OH	4,6-dimethyl-2-pyrimidinone
5MePym	5-methylpyrimidine
py	pyridine
Py2SH	2-pyridinethione
Py2OH	2-pyridinone
3,5Cl_2py	3,5-dichloropyridine
Imid	imidazole
2MeImid	2-methylimidazole

$1,2Me_2Imid$	1,2-dimethylimidazole
BenzImid	benzimidazole
$1,3Me_2Imid2SH$	1,3-dimethyl-2-imidazolethione
quin	quinoline
isoquin	isoquinoline
dipyam	di-2-pyridylamine
en	ethylenediamine
AcO	acetate
CPA	cyclopropylamine
HA	6-aminohexanoic acid
tet	N,N'-di-(3-aminopropyl)piperazine
trpn	tris-(3-aminopropyl)amine
Me_6 tren	tris-(2-dimethylaminoethyl)amine
Me_6 tpt	tris-(3-dimethylaminopropyl)amine

Abbreviations Used in Spectra

w	weak
m	medium
sh	shoulder
br	broad
v	very
s	strong

CHAPTER 1

INTRODUCTION

1.1. Metal Ions in Biological Systems

Although it has been known for many years that metals are necessary constituents for life to function normally, the ways in which these metals are employed in biological processes have been studied in detail only comparatively recently.[1]

The rapid development of this field has been largely due to the application of such modern physical techniques as n.m.r., e.p.r. and Raman spectroscopy in addition to the improved methods of X-ray diffraction.[2,3] By using these techniques, elucidation of metal - binding sites of many large molecules present in living matter has been achieved, for example in vitamin B_{12}.[1,4]

Of the metallic elements identified in our bodies, fewer than half have been shown to be indispensable. These elements may be separated into two groups; 1) the so-called 'bulk' elements, which account for 99% of all the metal ions in our bodies, i.e. K, Na, Ca and Mg; and 2) the 'trace' elements, which include Mn, Fe, Co, Cu, Zn, and Mo.

The roles of the four 'bulk' elements are varied, for they can function electrochemically, catalytically or structurally.[5] It should be recalled that water is the principal medium for the occurrence of metabolic processes, and with regard to electrochemical functions, it is essential for the transport of the ions K^+ and Na^+. These ions are mainly concerned with the osmotic regulation of body and tissue fluids,[6] but also serve as activators for special enzyme systems.[7,8] The more covalent metal ions, Mg^{2+} and Ca^{2+}, however, are involved with the catalytic and structural aspects of body processes. Magnesium is the

commonest enzyme activator and is important in the large class of phosphate transferases,[9] whereas calcium is primarily involved in the formation of bones.[10]

In recent years increasing interest has been centred on the 'trace' elements due to their role in enzymes.[11] Enzymes which contain metals can be divided into two classes; the 'metallo-enzymes' and the 'metal - activated' enzymes. The basic difference between these two types is that in the 'metallo-enzyme' system, the active metal is firmly bound to the protein and is described as a prosthetic group, whereas in the seoond case the metal is loosely bound and readily lost. This is termed a cofactor.

All the 'trace' elements are enzyme activators, but only transition metals, with variable oxidation states, are found in the 'metallo-enzyme' class. Compounds of this type and their associated proteins have been well studied and include ferredoxin (Fe),[12] glutamic isomerase (Co),[13] and tyrosinase (Cu).[14] It should be mentioned that zinc, although not strictly a transition element, does have similar properties as an activator and is an essential constituent of several important enzymes e.g. carboxypeptidase A (E.C. 3.4.12.2.) and alkaline phosphatase (E.C. 3.1.3.1.).[8]

It may be observed that some transition metals that are relatively abundant on the Earth's surface do not appear to have any function <u>in vivo</u> e.g. chromium and nickel. The suggestion has been made that these metals are so strongly held in minerals that they are unable to enter plants and then our bodies. However, recent evidence has shown that nickel may be needed in the enzyme Jack Bean Urease (E.C. 3.5.1.5.).[15]

Apart from the metal ions actually required for life, many undesirable elements are present in minute quantities.[1,5] Any metallic cation that enters the body (mainly from food) will spend a large part of its

time bound to various ligands e.g. amino acids, nucleic acids and proteins. This binding will often be at the expense of a vital 'trace' metal and hence the normal biological processes will be impaired. The most important elements of this kind are the toxic 'heavy' metals - Hg, Cd and Pb. These are extremely dangerous due to their accumulative properties, and examples of their poisoning effects are well known.[16,17]

Metal ions obviously play a very important role in biological systems, and hence it is often necessary to study in detail their behaviour with the many ligands in the body. This work therefore undertakes to investigate further the ways in which metals can coordinate to nucleic acids by examining the structures of the complexes containing related molecules.

1.2. The Nucleic Acids

The nucleic acids are complex compounds widespread in nature and present in all cells of animals, plants and microorganisms. They occur in combination with varying amounts of proteins, known as histones, and when attached are termed nucleoproteins.

Two distinct types of nucleic acids have been isolated from animal tissue, ribonucleic acid (RNA) which is found in the cytoplasm and nucleoli, and deoxyribonucleic acid (DNA) which is mainly confined to the nucleus.[18] These nucleic acids are large aggregates of purine and pyrimidine ribonucleotides or deoxyribonucleotides with molecular weights varying from several hundred thousand to several million.[19]

The nucleotides are similar for both DNA and RNA and differ only in the base constituents and type of sugar. In DNA the common bases are the pyrimidines thymine and cytosine and the purines adenine and guanine, whereas in RNA uracil replaces thymine (Fig.1.1.). Each base is then

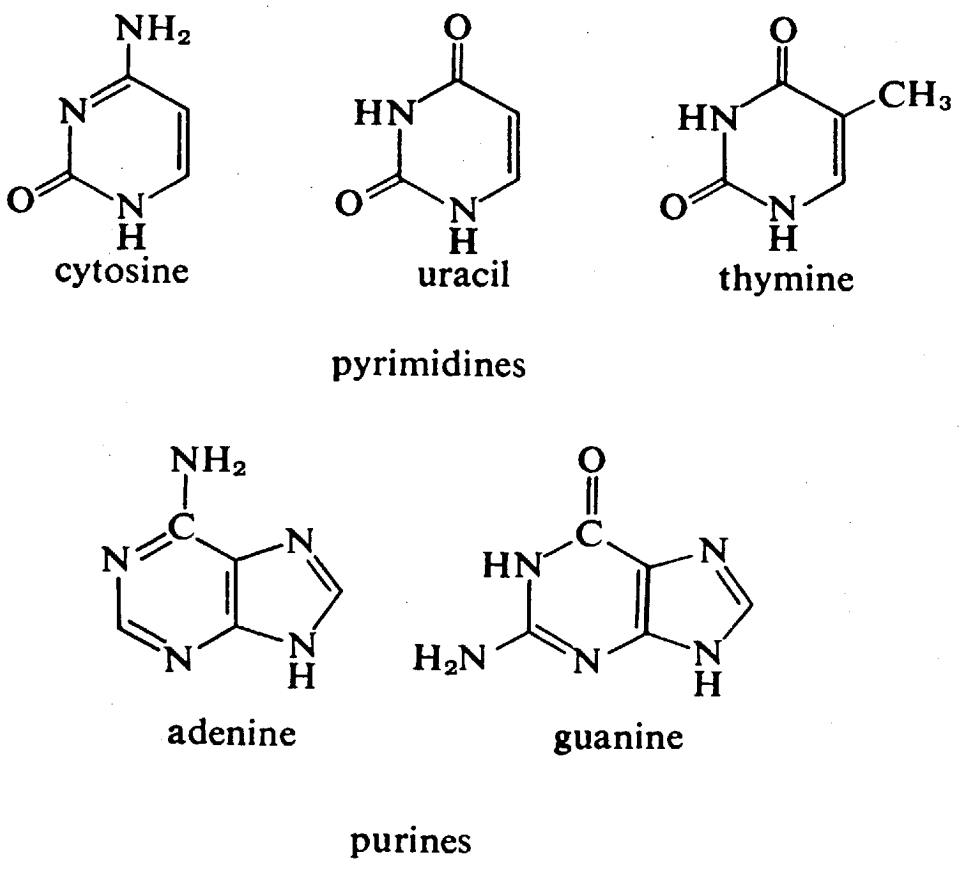


Fig.1.1. The major bases of the nucleic acids.

attached to a ribose (RNA) or 2'-deoxyribose (DNA) sugar (to form a nucleoside) which is in turn linked to a phosphate group via the 3' or 5' carbon atom.

Both DNA and RNA are constructed from these monomers by phosphodiester linkages of the 3'-hydroxyl group of one nucleotide to the 5'-hydroxyl group of the adjacent nucleotide. The primary structures of the acids are shown in Fig.1.2.

Investigations into the base content of DNA from many sources by Chargaff,[20] indicated that the pairs adenine and thymine and guanine and cytosine are present in equimolar amounts. Together with the X-ray diffraction studies by Wilkins[21] and Franklin[22], Watson and Crick[23] proposed the secondary structure of DNA. By arranging the purines and pyrimidines into 'base-pairs' (Fig.1.3.)[24] they put forward the view

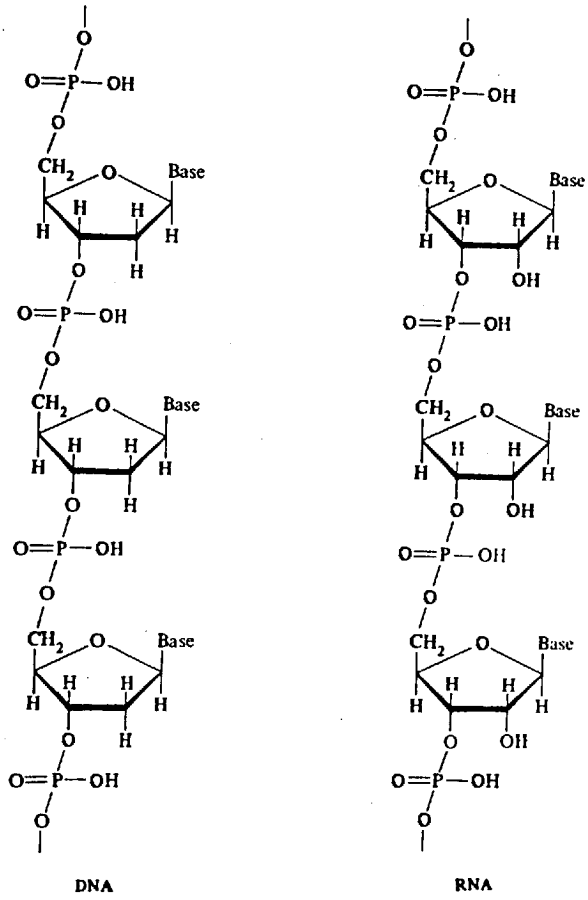


Fig.1.2. The primary structures of DNA and RNA.

that the DNA is double - stranded and in the form of a right-handed helix with the two polynucleotide chains wound round the same axis and held together by hydrogen bonds between their bases (Plate I). The less well defined tertiary structures of DNA and secondary structures of RNA are summarised by Davidson.[19]

The biological functions of the nucleic acids are now quite well understood. DNA is found in chromosomes (hereditary material in nuclei of cells), implying its role as a carrier of genetic information. The genetic message is stored in the sequence of the four bases arranged along the polynucleotide chain. With this knowledge, and that of the complementary nature of DNA, the replication process, whereby a parent molecule gives rise to two identical daughter molecules, was greatly clarified by the work of Kornberg.[25] He was the first to describe the

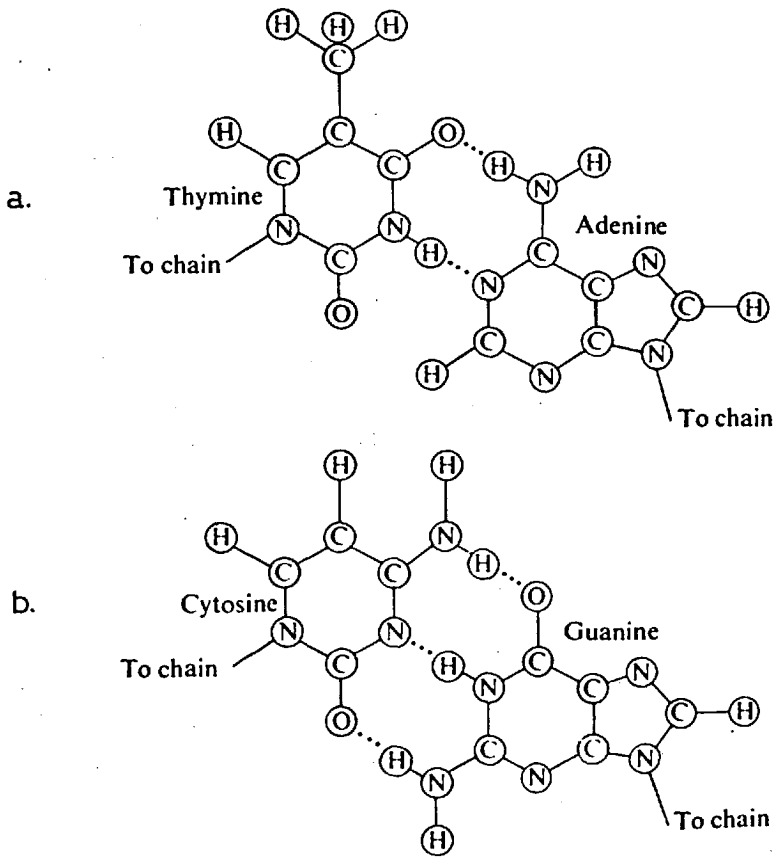


Fig.1.3. The Watson - Crick base pairing in DNA a) thymine and adenine; b) cytosine and guanine.

enzyme DNA polymerase (DNA nucleotidyltransferase - E.C. 2.7.7.7.) which uses as its substrates the deoxyribonucleoside triphosphates of the four chief bases.

The polymerase enzyme is unusual in that it requires a primer in the form of a single strand of DNA. A new chain is then built by the enzyme on this primer strand with the elimination of inorganic pyrophosphate from each triphosphate as it is attached in its turn. Since each primer strand is produced by the separation of the two component strands of the original DNA helix, the end result is the production of two new double helices, each identical with the original.

The DNA, in the nucleus of the cell, serves as a template for the formation of messenger RNA (mRNA), which then carries the genetic message

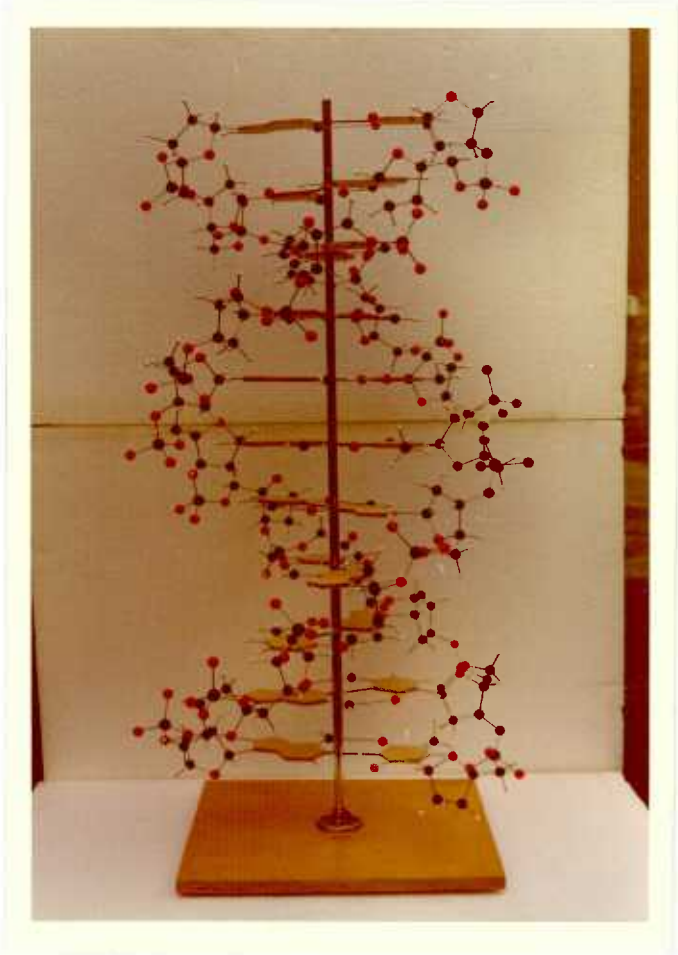


Plate I

A model of duplex DNA in the B-form.

into the cytoplasm. This is known as transcription. The genetic code in mRNA is contained in trinucleotide sequences, called codons, of which the arrangements are specific for each amino acid in the body. These codons are 'recognised' by anticodons, trinucleotide sequences in transfer RNA (tRNA), and comprises of the bases that are complementary to those in the codon. While one part of the tRNA contains the anticodon, another part binds the specific amino acid for which the particular tRNA possessing that anticodon is constructed. After the tRNA molecules (with the amino acids) have been attached to the mRNA in the right order, the amino acids are then joined together by peptide linkages to

form a protein molecule. The interpretation of the nucleotide code by the proper sequential placement of amino acids is called translation. A summary of the functions of nucleic acids is given in the concept of the 'Central Dogma' put forward by Crick in 1958[26] and later revised in 1970[27] (Fig.1.4.).

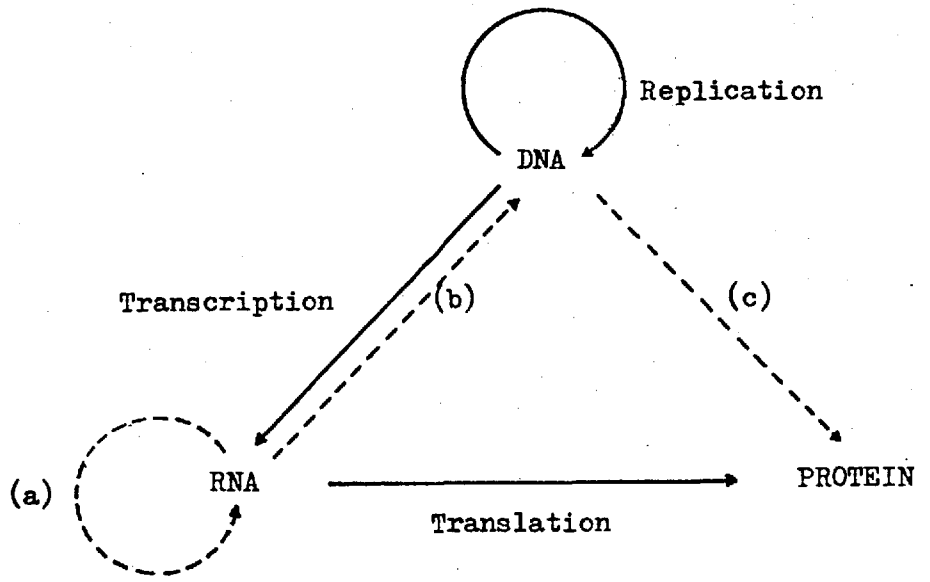


Fig.1.4. The Central Dogma. (a) Synthesis of viral RNA on a viral RNA template; (b) Action of the RNA - dependent DNA polymerase; (c) Reaction for which evidence is obscure.

Not only the replication of DNA, but virtually every step in the utilisation of the genetic code for the eventual production of the proteins specified by the code, is governed in some way by the presence of metal ions. The manner in which these ions influence the three fundamental processes, replication, transcription and translation is discussed in the following section.

1.3. Interactions of Metal Ions with Nucleic Acids.

The role of metal ions in the structure and function of nucleic acids has received increasing attention since the model for DNA was proposed.[23] The first clue to a possible implication of metal ions with the nucleic acids was the fact that Mg, Ca, Zn, Cu, Fe and Mn were found present in detectable amounts, tightly bound to RNA or DNA extracted from cells.[28] Although it has not been proved that these cations are directly linked to the nucleic acids *in vivo*,[29] their likely effect on the structure and function of RNA and DNA has been well documented.[30-32]

Firstly the possible roles or inhibitory effects of metal ions *in vivo* in the various processes of nucleic acids will be considered followed by a discussion of some *in vitro* studies.

1.3.1. Metal Ions in the Biological Function of Nucleic Acids

For the replication of DNA, the enzyme DNA polymerase requires divalent metal ions, generally magnesium, in addition to the DNA template and the four deoxyribonucleoside triphosphates. However Mn(II) can replace Mg under certain conditions causing non-selective incorporation of either ribo- or deoxyribonucleotides into the DNA.[33] This is probably due to the varied coordinating tendencies of Mn(II). The function of Mg in the polymerisation reaction appears to involve binding of the deoxyribonucleoside triphosphates to the enzyme.[34] Zinc is also found but its function is unknown.

Cobalt (II) is present in the enzyme terminal deoxyribonucleotidyltransferase (DNA nucleotidylexotransferase - E.C. 2.7.7.31.), as well as Mg and Mn(II), and is necessary for the selection of pyrimidine nucleotides for the incorporation on the polydeoxyribonucleotide chain.

Translation, leading to the formation of proteins, generally requires Mg^{34} and Mn(II).[38,39] However, it is important that the concentration of ions is not too high, as this may cause the incorporation of the wrong amino acids in the protein. Studies have shown that error in 'coding' may occur if there is mispairing of the bases in mRNA and tRNA leading to a different protein being formed.[40]

1.3.2. Metal Ion Interactions with Nucleic Acids in vitro

From an inspection of the nucleic acid molecules, it is evident that they contain several types of electron donors to which metal ions can attach themselves; phosphate groups, ribose hydroxyl groups (in the case of RNA) and the bases. Metal ions are diverse in their action and will bind to just about every available site under different conditions.

Both replication and transcription require the unwinding of double - stranded DNA and its subsequent rewinding. The way in which this happens is not known, but *in vitro* studies have shown that metal ions may be partly responsible.[32]

It is clear from the structure of DNA that metal ions binding to the surface of the molecule, i.e. to the phosphate groups, will exert quite a different effect than metals binding to the bases, because the latter mode means competition with hydrogen - bonding. In fact, metals binding to the phosphate groups stabilise the double helix, while base binding disrupts it.[41]

This stabilisation of DNA can be explained quite simply. The surface of the double - helix has the negatively charged phosphate groups arranged in close proximity, so that they tend to repel each other. When positive ions, that bind to the phosphate, are supplied, these negative charges are neutralised and the tendency to unwind is

overcome.

It has been shown that the metal ions Mg, Co(II) and Ni(II) increase Tm, (the melting temperature or the temperature at which DNA unwinds into single strands) whereas Mn(II), Zn(II), Cd(II), Cu(II) and Hg(II) decrease the value (usually after an initial increase).[42] From studies of this kind, the relative affinities of the binding of divalent metal ions to phosphate compared with base can be determined and placed in the order Mg > Co > Ni > Mn > Zn > Cd > Cu > Hg. Hence Mg will stabilise the structure while Hg will have the opposite effect.

RNA and DNA do not exhibit similar behaviour in all of their reactions with metal ions. The secondary structures of the various forms of RNA are less well-defined (except tRNA) and therefore the effects of metal ions on stabilisation are not clearly known. However, the destabilisation, or rather depolymerisation, of RNA has been well studied.[43-46]

The fact that degradation reactions occur with RNA, but not with DNA, has led to the assumption that the 2'-hydroxyl group of RNA must be involved. Eichhorn[46] explained this observation by a mechanism proceeding via a 2'-,3'- cyclic phosphate intermediate, instead of the formation of a metal chelate with phosphate and the 2'-hydroxyl group as he first proposed.

Berger[47] also postulated a theory of ribonucleoside recognition whereby an inorganic molecule, such as the copper (II) acetate dimer, could attach itself to the two hydroxyl groups of the ribose sugar and therefore be selective. However, an e.p.r. study concluded that differentiation between ribonucleosides and deoxynucleosides by copper (II) acetate does not result from bridging by the 2'- and 3'- hydroxy groups, but from the relative ease with which the dimeric structure is broken.[48]

To understand the <u>in vivo</u> processes of metal ions with nucleic

acids, it is of fundamental importance to first understand the nature of the metal complexes of their constituents. The next chapter deals with the structural aspects of nucleotide complexes of the biologically important metals cadmium, platinum and zinc. These elements have all been found to influence nucleic acid synthesis in some way. Zn ions are necessary for associated enzymes;[49,50] Cd ions for their inhibitory effect,[51,52] and, as shown in section 1.4., platinum complexes for their action as anti-cancer drugs. Later chapters look at the coordination properties of other 'trace' metals with related ligands.

1.4. Cancer and Chemotherapy

Of the 550,000 or so deaths recorded annually in the United Kingdom, about 110,000 are ascribed to the range of neoplastic diseases known collectively as cancer.[53] Roe[54] defined cancer as 'a disease of multicellular organisms which is characterised by the seemingly uncontrolled multiplication and spread within the organism of apparently abnormal forms of the organism's own cells.' This definition covers the key characteristics of cancer: autonomous growth and invasiveness - the latter property being the reason why cancer is so lethal.

It was previously stated that metals not usually associated with body functions were toxic and could affect normal metabolic processes in varying degrees. The extent of their influence is further shown by the fact that many of these same elements are known carcinogens,[55] with cadmium being one of the most potent.[56] It has been suggested that these metals penetrate living cells and either advance or retard the kinetics of anabolic or catabolic enzymes by instigating a competition between the invading and normal metals.[57]

There are four major modalities used in treating cancer: 1) surgery,

which cannot be applied when the disease is spread throughout the body; 2) radiation therapy, which damages normal as well as cancerous tissue; 3) chemotherapy, which often produces very unpleasant and sometimes dangerous side-effects, and 4) the new technique of immunotherapy - the manipulation of immune response. In all of these methods, it is essential to kill, or remove, virtually every neoplastic cell if recurrence is to be prevented.

Cancer chemotherapy attempts to do this selectively[58] by means of chemicals,[59] and exploits the few signifcant therapeutic differences between normal and cancer cells. Screening of potential drugs for anti-cancer activity is usually carried out against transplanted animal tumours.[60] Because animal cancers differ from those in humans, a variety of tumours must be used for the primary screen so that non-specific activity can be determined. However, recent work on this problem has shown that human tumours can be transplanted into mice and still keep their identity even after several weeks[61] inferring the possibility of better screening techniques being employed in the future.

Most drugs in current use inhibit cell division by interfering in one way or another with the synthesis or use of nucleic acids during mitosis.[62,63] These compounds are mostly highly poisonous substances and work on the principle that cancer cells, which divide rapidly, will be damaged more quickly than the relatively slowly growing cells of the body. Until recently, very few of these drugs were inorganic in nature but in 1969 the anti-tumour activities for a class of platinum coordination compounds were demonstrated.[64,65] Since their discovery, the properties of many platinum complexes have been extensively studied.[66-71]

It was observed by Rosenberg et al.[72] that when a low alternating current is passed through platinum electrodes in nutrient medium it can

inhibit cell division in Escherichia coli and cause the formation of long filaments. It was subsequently discovered that some of the platinum dissolves under these conditions to form the neutral species cis-$Pt(NH_3)_2Cl_4$. This compound was found to be a potent inhibitor of cell division while having only a small inhibitory effect on growth rate.

The property of inhibiting cell division but not cell growth indicated that platinum compounds may possess some anti-tumour activity, as other compounds (e.g. alkylating agents) also caused elongation and lysis in lysogenic bacteria. The testing of several platinum compounds against tumours in mice showed that cis compounds (especially cis-$Pt(NH_3)_2Cl_2$) were effective in inhibiting tumour growth, whereas the trans isomers gave no appreciable activity.

The distribution of platinum ions within E. coli was investigated using a tracer technique.[73] In the filamentous cells, platinum ions were associated not only with metabolic intermediates but also with cytoplasmic proteins and nucleic acids, whereas in the inhibited cell the platinum was combined only with the cytoplasmic proteins. This observation therefore provided the first indication that the filament forming ability of these compounds was due to nucleic acid interactions.

It was proposed that the primary mechanism of filamentation was the selective inhibition of DNA synthesis with no accompanying inhibition of RNA or protein synthesis, although binding to these molecules is known to occur. Conclusive evidence that the activity of platinum compounds was due to the interaction with nuclear DNA, came from studies on the incorporation of labelled precursors of DNA (thymidine - ^3H), RNA (uridine - ^3H) and protein (L - leucine - ^3H) in the presence of cis-$Pt(NH_3)_2Cl_2$.[74,75] These studies also showed that there was no inhibition of DNA polymerase, despite a proposal that its inactivation did occur,[76] and that the trans isomers, as expected, had no effect on

DNA synthesis. From this evidence and that of Drobnik et al.,[77] the possible mechanism of activity of the platinum compounds with DNA was investigated from the point of view of their chemical, physical and structural properties.

Many platinum complexes have been studied and tested to find a correlation between structure and activity.[69,78,79] It was shown that activity was generally confined to neutral compounds possessing cis leaving groups, and that charged or trans species were inactive. The most important types were found to be the square-planar cis-PtA_2X_2 complexes where A is an amine and X is the leaving group, usually chloride.

Although most investigations have been carried out on Pt(II) complexes, the anti-cancer activity of the Pt(IV) compounds has not been ignored. These were found to be moderately active and it was suggested[64] that Pt(IV) was reduced in vivo to form a Pt(II) complex. However, recent work with some substituted ethylenediamine compounds of Pt(II) and Pt(IV) show that this may not be the case.[80]

The fact that the trans isomers are fairly inactive and usually less toxic compared to the cis isomers is interesting and suggests that the difference lies in either their chemical properties or stereochemistries. The trans isomers are consistently more reactive than the cis analogues and thus are likely to react more quickly with the many available ligands in the body. Hence they will be less specific in their action. Also, the cis compounds are able to form chelates, which may imply that this type of interaction occurs in the mechanism of activity.

The ability of platinum compounds to attack DNA suggests a similarity with the drug action of the bifunctional alkylating agents. These are supposed to act by cross-linking opposite strands of DNA via the N7 positions of guanine.[81] However, the leaving groups in bifunctional

alkylating agents have a maximum separation of 0.8 nm while the chlorides in cis-$Pt(NH_3)_2Cl_2$ are only some 0.33 nm apart. This means that the mechanism of activity must be different. Nevertheless, some type of interstrand cross-linking of Pt(II) and Pt(IV) complexes has been demonstrated[82-87] although with other work,[88,89] it was concluded that this type of linkage bore little relationship to their cytotoxic properties. A study of platinum compounds with bacteriophages[90] confirmed the lack of importance of interstrand linking and, like Harder,[91] suggested that the main contribution to inactivation came from cross-linking of neighbouring bases on the same nucleic acid chain, a reaction which is also known to occur with the bifunctional alkylating agents. Other types of DNA binding have also been proposed. An intercalation reaction was suggested as a possible mode of interaction even though this has not been found in vivo.[92] Nevertheless, structural work has still been carried out on model compounds.[93,94]

A mechanism for the anti-tumour activity of platinum compounds has been put forward by Rosenberg[95] implying that the initial binding to DNA by interstrand or intrastrand linking or intercalation is only the 'primary step'. He postulated the 'enhanced antigenicity' hypothesis which states that the combination of platinum to tumour DNA is such that the antigenicity of the tumour is altered in favour of the host's ability to destroy it.

However, to help in the understanding of structure - activity relationships, it is of major importance to first determine the initial mode of interaction with DNA. The next chapter, therefore, examines the structures of cis-platinum complexes containing nucleic acid components to find out the prime coordination sites with a view to explaining the 'primary' mechanism.

CHAPTER 2

COMPLEXES OF NUCLEOTIDES

Many physical techniques have been employed to determine the nature of the electron donor atoms,[96-98] and some of these methods have been reviewed in detail.[99,100] This chapter describes results obtained in a continuation of the study of metal - nucleotide interactions and deals with the ribonucleotide complexes of cadmium, platinum and zinc.

2.1. Structural Studies

Ribonucleotides possess three major potential binding sites for metal ion coordination; the heterocyclic bases, the ribose hydroxyl groups and the phosphate groups. Of these, the last group has been generally considered to be the strongest donor for most transition metals, whereas the ribose sugar is the weakest.[99] Nevertheless, the HSAB (Hard and Soft Acids and Bases) approach suggests that the 'softer' metals, cadmium and platinum, would probably bind preferentially to nitrogen donors on the bases, rather than to oxygen atoms of the other sites.[101]

Investigations on the tautomeric structures of the bases shown in Fig. 1.1. (and hypoxanthine), have indicated that they exist predominantly in the amino and/or lactam forms.[102,103] This means that when the N1 (pyrimidine) and N9 (purine) positions are blocked by a ribose sugar, as in nucleosides and nucleotides, the most likely coordination would be to N3 of 5'-CMP, and N7 of the purine analogues.[100] For 5'-TMP and 5'-UMP, however, binding to base at N3 may only occur if the blocking proton is removed.

Recent n.m.r.[104-108] and e.p.r.[109] studies together with absolute structural determinations using X-ray diffraction methods (Table 2.6.), have shown that the metal ions do bind to mononucleotides at the base sites

proposed with little interaction from the oxygen and amino substituents. These investigations have also shown that phosphate binding occurs but not as readily as that of metal to base, and that the ribose groups only bind weakly, if at all.

Because metals are able to bind to all these various sites under different conditions, this investigation examines the reliability of another technique i.e. infrared spectroscopy, in determining modes of binding in Cd, Pt and Zn nucleotide complexes. First, however, the structures of several key compounds were determined by X-ray methods [110] to provide a firm basis for assessing the i.r. results.

The vibrational spectra of nucleic acids and their components have been well studied.[111-118] Although these spectra are extremely difficult to interpret because of the high complexity and low symmetry of the molecules, Ogawa and Sakaguchi [119-125] attempted to use this technique to investigate the interaction of metals with mononucleotides. They concluded that binding to the base and phosphate group could be demonstrated but not binding to the ribose sugar.

The determination of these sites was based simply on the comparison of the i.r. spectra of the Na, K, Ca, and Ba salts. The binding of metals to the base moieties was founded upon observing frequency shifts of the bands in the region 1800 - 1500 cm^{-1}. These are assigned to ring vibrations and are sensitive to interactions such as complex formation.[126] The proposal for identifying binding to the phosphate group, however, is assumed solely on whether the PO_3^{2-} symmetric stretch [115,127] is present or not.

The spectra of the Na, K, Ba, and Ca salts show this strong band at ca. 980 cm^{-1} but it is not found in the free acid. This is thought to be due to the position of the ions on the nucleotide. These Group I and II metals are known to be associated with the phosphate group [128] whereas the protons are situated near the base.[122] Ogawa and Sakaguchi then stated

that if on complexation the spectrum of the compound showed the PO_3^{2-} vibration, then the metal is bound to the phosphate group, but if this band is absent, binding to some other part of the molecule occurs.

Although the criterion for metal - binding to base has some theoretical justification, that of coordination to phosphate is clearly unsatisfactory. It should be noted, however, that despite the way this interaction is suggested, the final complexes prepared by Ogawa and Sakaguchi may indeed possess phosphate binding. This is due to the fact that some coordinated water molecules, in the original complexes, are removed by washing with ether and drying in vacuo over P_2O_5 therefore forcing the metal to accept other coordination sites i.e. phosphate. The question of using i.r. spectroscopy to determine interactions of this kind will now be re-examined.

The vibrational spectra of phosphate groups have been studied in some detail [129] including those occurring in natural nucleic acids, synthetic polynucleotides and mononucleotides.[115] The phosphate represented in mononucleotides is the phosphomonoester group, $ROPO_3^{2-}$. In general, this group exhibits three characteristic bands at ca. 1100 cm^{-1} (strong, broad), ca. 980 cm^{-1} (strong, sharp) and ca. 750 cm^{-1} (weak). These are assigned to the PO_3^{2-} asymmetric (ν as), symmetric (ν s), and P - O stretching modes respectively.[127,130]

The bands at 1100 and 750 cm^{-1} appear to be of little value in determining metal - coordination, for the P - O stretch is too weak and ν as is too broad. However, the PO_3^{2-} asymmetric stretch has been considered useful in suggesting metal - phosphate binding in various 5'-ATP complexes. It was proposed that a pronounced splitting of this band strongly indicated interaction of the terminal phosphate group.[131,132] As this group has similar properties to those found in mononucleotides, the PO_3^{2-} symmetric stretch vibration is also considered to be the band most sensitive to metal ion coordination.

From an i.r. study of some metal salts of $(RO)_2PO_2^-$ (the representative group of polynucleotide phosphates), it was noted that the more covalent metals caused the PO_2^- symmetric stretch mode to shift to higher frequencies.[133] This behaviour was also found with some $CH_3PO_3^{2-}$ salts where the PO_3^{2-} symmetric stretch moved from 973 to 990 cm^{-1} on going from sodium [134] to zinc.[135] The reason for these frequency shifts is not clear, but is thought to be due to a reduction in the symmetry of the phosphate group by its coordination to the metal ion.

From these observations the following study on the mononucleotide complexes will look closely at the PO_3^{2-} vibration (ν_s) to find if metal - phosphate interactions can be predicted with any certainty.

Attempts at preparing platinum complexes of 5'-AMP resulted in the formation of glasses, and therefore will not be considered further.

2.1.1. Cytidine 5'-Monophosphate

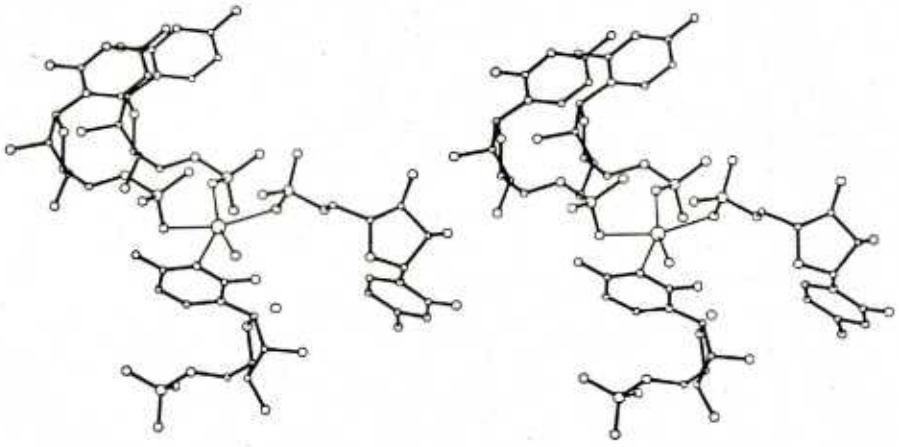
The platinum complexes are discussed in Chapter 3.

[Cd(5'-CMP)(H_2O)],H_2O [136]

This compound was obtained as colourless, fibrous crystals at pH 4.64. Preliminary oscillation and Weissenberg photographs showed the crystals to be orthorhombic and gave the space group as $P2_12_12_1$. Unit cell dimensions were found to be a = 0.5293, b = 1.6367 and c = 1.7063 nm, U = 1.4782 nm^3 and Z = 4.

The main part of the structure consists of a three - dimensional polymeric network in which the cadmium atom is pentacoordinate (Fig. 2.1., Plate II). It is bonded to the pyrimidine base at N3, to the phosphate groups of three neighbouring 5'-CMP molecules and to a water molecule. In turn, each phosphate group is bonded to three cadmium atoms. A distorted coordination geometry, intermediate between square - pyramidal and trig-

Fig. 2.1. Stereoscopic view of a fragment of the polymeric structure of $[\ Cd(5'\text{-CMP})(H_2O)\ ]_n$.



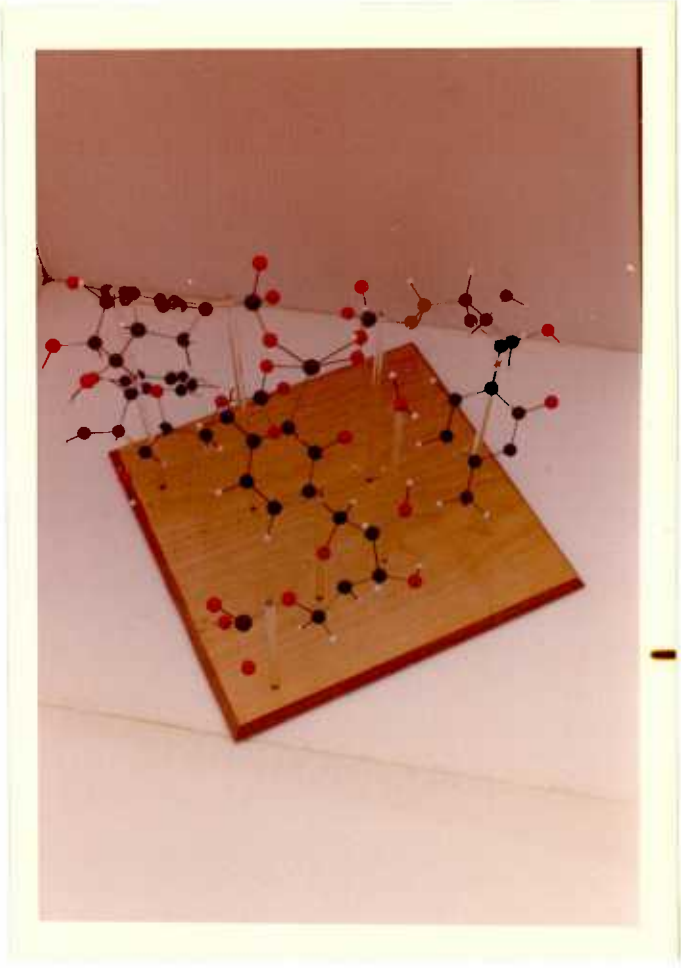


Plate II

A model of the structure of the complex $[\ Cd(5'\text{-CMP})(H_2O)\ ],H_2O$.

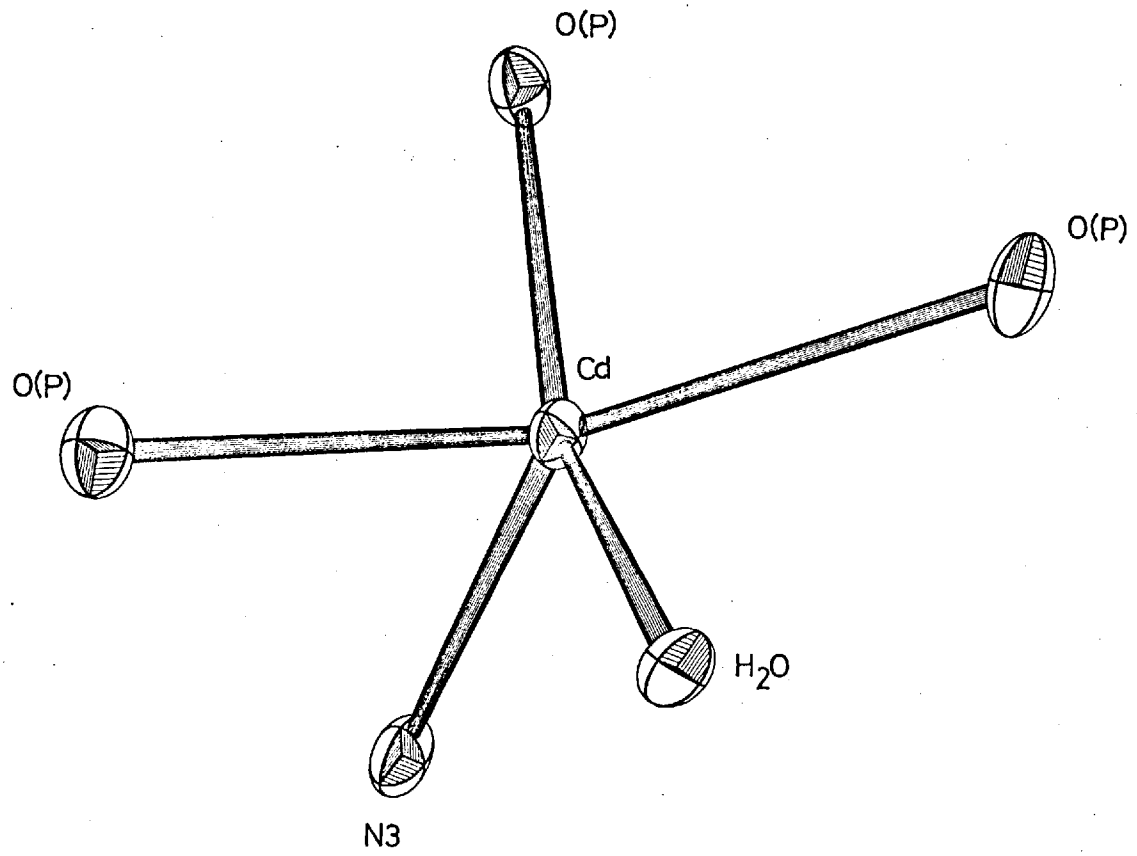


Fig. 2.2. The arrangement of donor atoms around the pentacoordinate cadmium ion. The view is directly related to Fig. 2.1.

onal - bipyramidal, is found for the metal atom, with metal - ligand distances of: Cd - N3 = 0.2327 nm, Cd - O (phosphate) = 0.2213 - 0.2280 nm and Cd - O (water) = 0.2387 nm (Fig. 2.2.). The second water molecule occurs as water of crystallisation in the interstices of the structure. The crystal structure is stabilised by a network of hydrogen bonds involving the coordinated water molecule, the water of crystallisation, phosphate oxygens, ribose oxygens, keto oxygen (O2) and the amino nitrogen (N4).

The i.r. spectral data, with assignments, of 5'-CMPNa_2 are given in Table 2.2. The stretching vibrations of the C = O, C = N, and C = C groups and the NH_2 scissoring mode are shown in the region 1700 - ca. 1500 cm^{-1} with the symmetric PO_3^{2-} stretch at 986 cm^{-1}.[137-139]

The spectrum of [Cd(5'-CMP)(H_2O)],H_2O displays some differences from

that of the sodium salt. The binding of cadmium to the base portion is indicated by a shift of bands in the 1700 - 1500 cm^{-1} region as proposed by Hartman.[126] Although extensive coupling occurs, it has been concluded [140] that binding to an unsaturated nitrogen site i.e. N3 in cytidine, can affect the π-electron distribution of the heterocyclic ring, thereby altering the C = N, C = C and associated stretching frequencies. The N3 coordination in the 5'-CMP complex therefore corresponds to the observed band shifts in the spectra.

A usual consequence of this type of binding is the shift to higher wavenumbers of the neighbouring carbonyl stretching band. However, the structure of this complex shows that O2 is quite close to the cadmium atom and hence may cause the band ascribed to mainly the ν(C = O2) vibration to shift to slightly lower frequencies. This behaviour has been noted with the cytosine complex, $Cu(Cyt)_2Cl_2$,[141,142] the structure of which shows the copper bound to N3 of the two pyrimidine bases with a weak bond to the O2 atoms. The infrared spectrum has demonstrated this interaction by displaying a shift downwards in the carbonyl stretch frequency as compared to cytosine itself.[143]

The PO_3^{2-} symmetric stretch is split with two bands at 988 and 980 cm^{-1}. This observation, therefore, does not agree with the postulation that metal - phosphate binding would shift the main band to higher frequencies. The reason may be due to the fact that the symmetry of the phosphate group is not lowered significantly because each oxygen is bound to the metal. Nevertheless, the more covalent cadmium ion (with respect to sodium) would still be expected to alter the band position more than is observed.

The i.r. spectrum of the above compound in the 1800 - 860 cm^{-1} region was found to be very similar to that reported by Ogawa and Sakaguchi,[125] who prepared a complex of the same stoichiometric formula even under severe drying conditions. Despite the uncertainty in predicting binding modes, they concluded, correctly, that cadmium is coordinated to both phosphate

and base.

During the course of this work, other structural determinations were carried out on the same compound and on [Co(5'-CMP)(H_2O)],[144] together with the seven - coordinate cadmium complex [Cd(5'-CMP)(H_2O)],$3H_2O$.[145]

Cd(5'-CMP),$5H_2O$ and Zn(5'-CMP),$4H_2O$

These compounds were prepared at pH 6.65 (Cd) and pH 5.70 (Zn). The relevant bands in their i.r. spectra are listed in Table 2.2.

In the region >1500 cm^{-1} the spectrum of Cd(5'-CMP),$5H_2O$ is essentially the same as that of [Cd(5'-CMP)(H_2O)],H_2O indicating N3 as the base binding site. The PO_3^{2-} vibration, νs, is slightly lowered suggesting that phosphate binding is not present due to the increase in water content.

Like the cadmium complex, Zn(5'-CMP),$4H_2O$ is thought to possess strong binding to N3 as denoted by the shift in the 1700 - 1500 cm^{-1} region. Nevertheless, the position of ν(C = O2) suggests that O2 is not as close to the metal as in [Cd(5'-CMP)(H_2O)],H_2O. The shift to higher frequency of the PO_3^{2-} vibration implies that coordination to the phosphate group may be present but is difficult to assess without further evidence.

2.1.2. Guanosine 5'-Monophosphate

[Cd(5'-GMP)(H_2O)$_5$],$3H_2O$

This compound was obtained as small feathery crystal clusters from a solution at pH 3.41. The crystals were not of very good quality, but were acceptable for preliminary Weissenberg and oscillation photographs. The data obtained showed that the cell dimensions are similar to those of the compound [Mn(5'-GMP)(H_2O)$_5$],$3H_2O$,[146] and so it is inferred that the complexes are isostructural (Table 2.1.).

The structure is therefore as shown in Fig. 2.3. The cadmium atom is bonded to the nucleotide only at N7 on the base, while the octahedral

Crystal Data	[Mn(5'-GMP)$(H_2O)_5$], $3H_2O$ [146]	[Cd(5'-GMP)$(H_2O)_5$], $3H_2O$
a	0.6757(1) nm	0.68 nm
b	1.1230(1) nm	1.14 nm
c	2.7809(3) nm	2.81 nm
Space Group	C2	C2
Z	4	4

Table 2.1. Comparison of the crystal data of hydrated cadmium derivative of 5'-GMP with that of its manganese analogue.

geometry is completed by five oxygen atoms from the coordinated water molecules.

Although the phosphate group is not bonded directly to the metal atom, an important factor in the stabilisation of the complex may be the presence of three intramolecular hydrogen bonds between the ligand water molecules and the O6 atom. There is also an extensive three dimensional network of intermolecular hydrogen bonds of the type O - H ..... O and N - H ..... O linking the complex molecules and the molecules of water of crystallisation.

In the same manner as the 5'-CMP complexes, the binding to base in the compound [Cd(5'-GMP)$(H_2O)_5$],$3H_2O$ is shown by a comparison of its spectrum with that of the sodium salt (Table 2.3.). Coordination to N7 only is signified by both the shift in the coupled ring vibrations and the movement to higher frequency of ν(C = O6). This is the converse of other infrared spectroscopic studies where a shift to lower frequencies is observed for Cu and Ag complexes of 5'-GMP and guanosine in solution [147] and in the solid state.[148,149] For these investigations it was suggested that the metal - oxygen (O6) interaction was quite strong. The PO_3^{2-} stretch is split into a band at 981 cm^{-1} and a shoulder at 966 cm^{-1}, indicating, as found, no binding to the phosphate group.

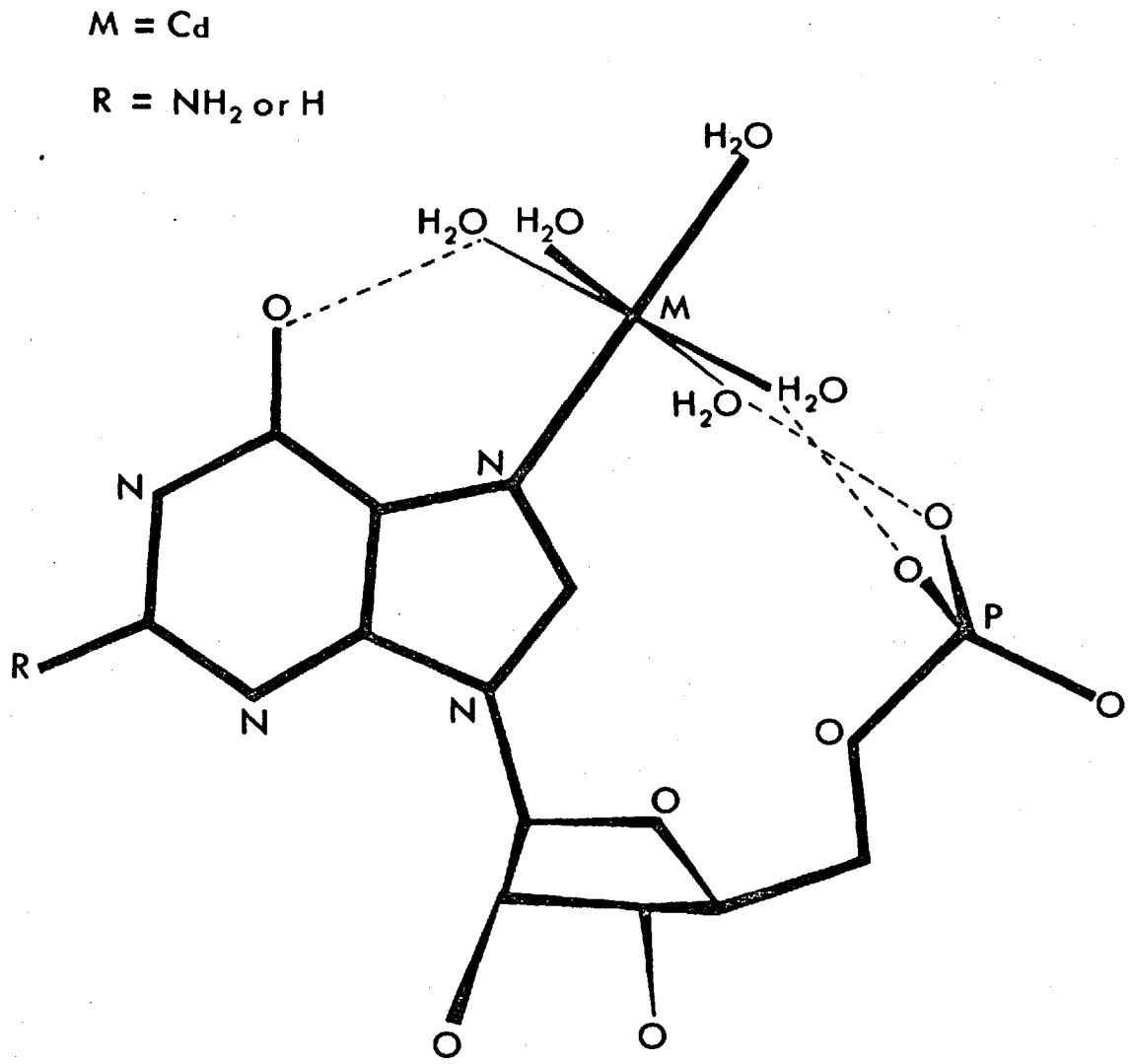


Fig. 2.3. A schematic view of the structures [Cd(5'-GMP)$(H_2O)_5$],$3H_2O$ and [Cd(5'-IMP)$(H_2O)_5$],nH_2O.

As this work was being completed an accurate determination of the structure of [Cd(5'-GMP)$(H_2O)_5$],$3H_2O$ was carried out confirming the proposed stereochemistry.[150] The crystals were prepared at pH 4.5 and gave cell dimensions very similar to those found from this investigation i.e. a = 0.6774(3), b = 1.1361(5) and c = 2.7849(7) nm.

Cd(5'-GMP),$6H_2O$

This compound was prepared as an amorphous solid at pH 5.62. The i.r.

spectral data (Table 2.3.) are very similar to those of [$Cd(5'\text{-GMP})(H_2O)_5$], $3H_2O$ and hence the same coordination and geometry about the cadmium atom is suggested even though νs is slightly different.

cis-[$Pt(NH_3)_2(5'\text{-GMP})_2H_2$],$5H_2O$

The complex was obtained as small colourless crystal clusters at pH 2.48. An oscillation photograph was taken and showed the compound to be rather unusual (Plate III). The strong 5th layer line suggested a polymeric structure with a repeating unit every 0.47 nm. The nature of this polymer

Plate III

An X-ray photograph of cis-[$Pt(NH_3)_2(5'\text{-GMP})_2H_2$],$5H_2O$ showing layer lines of a helical structure.

is not certain from the limited amount of data, but it is worth noting that 5'-GMP has been shown to form helices with a variable number of nucleotide residues per turn.[151] The possibility that the platinum complex assumes

this type of structure is supported by a recent X-ray structural determination of the compound [$PtenG_2$]$^{2+}$ possessing a helical geometry.[152] It is interesting that the manner of the base arrangement shows a distinct similarity to the complex cis-[$Pt(NH_3)_2(5'\text{-IMP})_2$]$^{2+}$ with platinum coordinating to the N7 positions (section 2.1.3.).

The shift of the bands in the 1700 - 1500 cm^{-1} region is similar to that found in the cadmium - 5'-GMP complexes, implying N7 binding only on the base (Table 2.3.). The weak PO_3^{2-} stretch vibration at 992 cm^{-1} suggests, from the proposed theory, that phosphate binding is present. However, this is extremely unlikely for platinum complexes with a choice of nitrogen donor atoms. Therefore the shift may arise because of extensive hydrogen - bonding.

$Zn(5'\text{-GMP}),nH_2O$ n = 4.5 or 5

The white solid was prepared at pH 5.08. The i.r. spectrum again showed shifts in band vibrations similar to the other 5'-GMP complexes inferring the same coordination to base (Table 2.3.). The position of the PO_3^{2-} stretch frequency, like $Zn(5'\text{-CMP}),4H_2O$, may suggest actual phosphate interaction.

2.1.3. Inosine 5'-Monophosphate

[$Cd(5'\text{-IMP})(H_2O)_5$],$2.5H_2O$

This was formed as crystal clusters from a solution at pH 5.85 and X-ray photographs showed that two forms existed, one of which was relatively unstable.

The stable form has the space group C2 with unit cell dimensions a = 0.69, b = 1.14, and c = 5.28 nm, $\beta = 93.2^{o}$, U = 4.15 nm^3 and Z = 4. Compared to the related complex [$Cd(5'\text{-GMP})(H_2O)_5$],$3H_2O$, a and b are very similar, with c approximately twice as large. This implies that [$Cd(5'\text{-IMP})(H_2O)_5$],

$2.5H_2O$ has the typical octahedral structure with binding to N7 of the base and five water molecules (Fig. 2.4.) but with a definite doubling of the long axis.

The unstable form was measured more accurately on a diffractometer and gave the dimensions a = 2.074, b = 1.145, and c = 2.641 nm. This means that there is a tripling of the short axis. It is observed that the crystals tend to change to the more stable 2:2 compound above very easily, suggesting that the three cadmium atoms are in similar environments. However, in the case of the stable manganese analogue (which shows distinct tripling) the stability comes from the metal having environments that are dissimilar.[153]

The i.r. spectrum of 5'-IMPNa_2 resembles that of the 5'-GMP salt and therefore the assignments are the same except for the absence of the NH_2 deformation (Table 2.4.). As for the cadmium - 5'-GMP complexes, the N7 coordination in [Cd(5'-IMP)$(H_2O)_5$],$2.5H_2O$, is shown by the band shifts in the double - bond region, especially the movement to higher frequencies of ν(C = O6). No shift in νs occurs as is consistent with there being no phosphate interaction.

[Cd(5'-IMP)$(H_2O)_5$],H_2O

This was prepared as small colourless prisms in a similar manner to the previous complex but at a lower pH i.e. pH 4.51. X-ray data showed that although the crystals were of a different habit, the structure is of the stable 2:2 compound. This conclusion is confirmed from the virtually identical i.r. spectrum (Table 2.4.). Hence the only difference is in the water of crystallisation.

[Cd_2(5'-IMP)$_3H_2(H_2O)_6$],$6H_2O$ [154]

These colourless crystal clusters were obtained from a solution at pH 3.88. The crystals are in the form of flat needles and oscillation and

Weissenberg photographs showed them to be monoclinic with the space group C2. Accurate unit cell dimensions were determined on a diffractometer to give a = 3.0377(4), b = 0.8760(1), and c = 2.0885(2) nm, β = 106.29(1)°, U = 5.3344 nm^3, and Z = 4.

The principal features of the structure of the compound are shown in Figs. 2.4. and 2.5. It consists of units of the type [Cd_2(5'-IMP^{2-})-(5'-IMP^{1-})$_2$(H_2O)$_6$] arranged in a polymeric array with about six non-coordinated water molecules of crystallisation distributed over nine positions.

There are two independent cadmium atoms, both having a distorted octahedral coordination, but attached to the nucleotides in differing ways. Cadmium 1 is bonded to a phosphate oxygen atom from 5'-IMP(3), to the N7 atoms of 5'-IMP(1) and 5'-IMP(2) with these atoms arranged in cis positions. The octahedron is completed by three coordinated water molecules. Cadmium 2, on the other hand, is bonded to N7 of 5'-IMP(3), three coordinated water molecules, and to the two exocyclic ribose oxygen atoms O2' and O3' of 5'-IMP(2).

Thus each 5'-IMP unit binds to cadmium in a different way. A common feature is that in all cases N7 is employed. However, whereas for 5'-IMP(1) this is the only bond formed to cadmium, with 5'-IMP(2) the two ribose exocyclic atoms are also utilised, and 5'-IMP(3) uses one phosphate oxygen atom. It may be noted that 5'-IMP(1) and (3) each carry a unit negative charge, whereas 5'-IMP(2) is doubly charged.

The differences in the environments of the cadmium atoms are reflected in the salient bond lengths (Fig. 2.6.). Thus in the case of cadmium 1 a rather short Cd - O (phosphate) bond of 0.223 nm is accompanied by two longer Cd - N7 bonds of 0.240 and 0.242 nm, while for cadmium 2 a short Cd - N7 bond of 0.227 nm is associated with two longer Cd - O (ribose) bonds of 0.242 nm (to O2') and 0.232 nm (to O3'). On the other hand, for both metal atoms all the Cd - O (water) bonds lie in a narrow range of 0.227 - 0.232 nm.

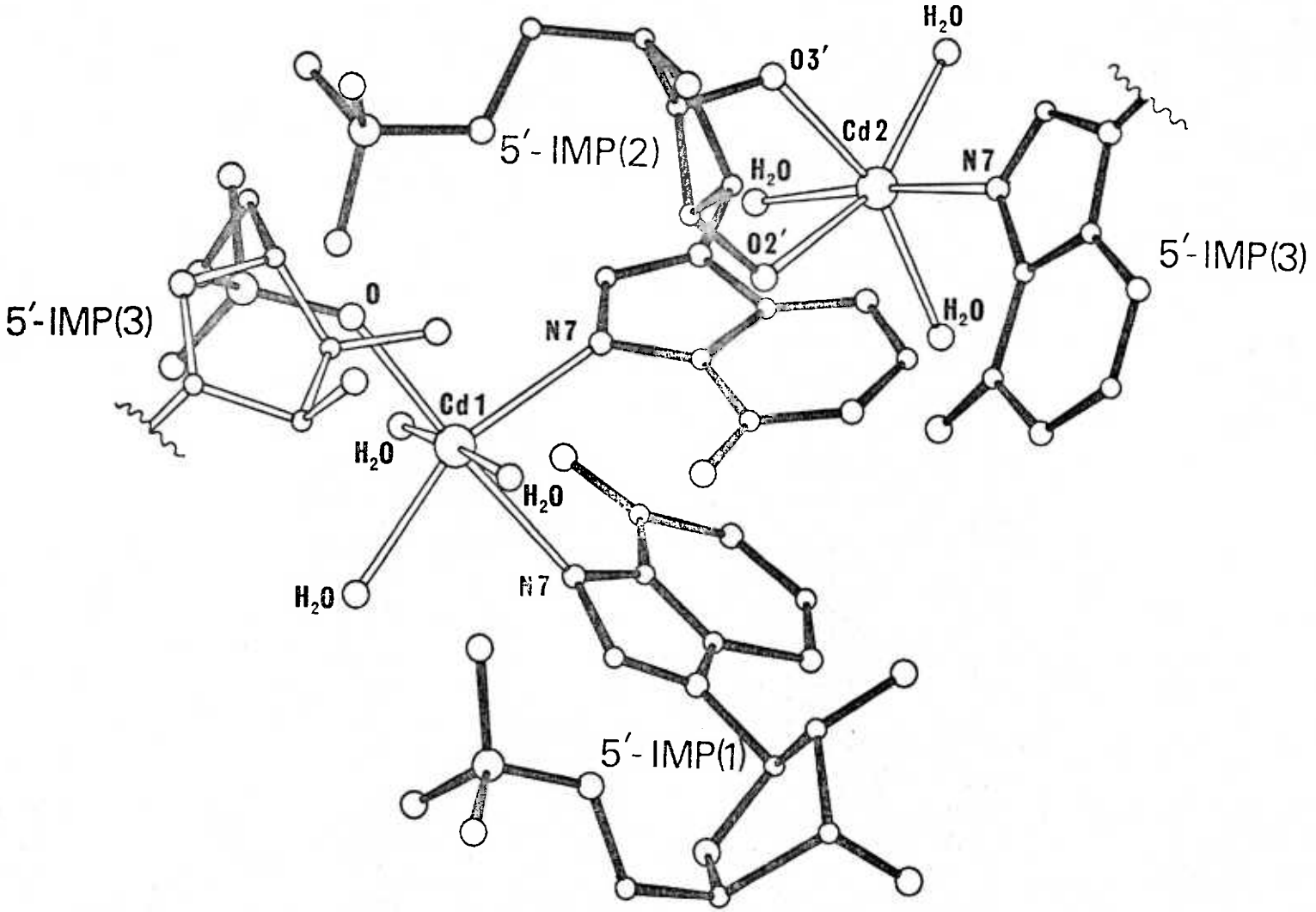


Fig. 2.4. A view showing the polymeric structure of [$Cd_2(5'\text{-IMP})_3H_2(H_2O)_6$].

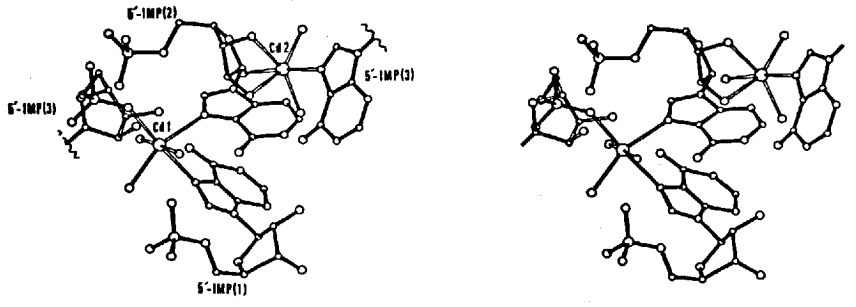


Fig. 2.5. A stereoscopic view of the structure of [$Cd_2(5'\text{-IMP})_3H_2(H_2O)_6$].

The overall structure is stabilised by numerous hydrogen bonds involving the coordinated water molecules, waters of crystallisation, phosphate oxygens, ribose oxygens, and the O6 and N1 atoms of the base etc.

A feature of particular interest concerns the geometry of the two hypoxanthine bases which are bonded in cis positions to Cd 1. The Cd - N7 bonds are very markedly out of the planes of the purine bases, such that Cd 1 is 0.092 nm out of the base plane of 5'-IMP(1) and 0.081 nm out of the plane of 5'-IMP(2). This results in a distortion towards pyramidal geometry at N7. The flexibility at N7 permits the planes of the two purine bases to move a considerable way towards achieving a parallel base stacking situation. Thus, although the N7 - Cd1 - N7 angle is 79.1°, the angle between the two base planes is only 31.4°. A similar effect in a platinum compound of 5'-IMP [155] is also observed, however, the angle between the two bases is somewhat larger (43°).

As cadmium binds to all the different available sites in the complex, the i.r. spectrum was studied closely to see if these types of coordination could be observed. The data (Table 2.4.) show a similarity to the other cadmium complexes of 5'-IMP in the 1650 - 1500 cm^{-1} region supporting the

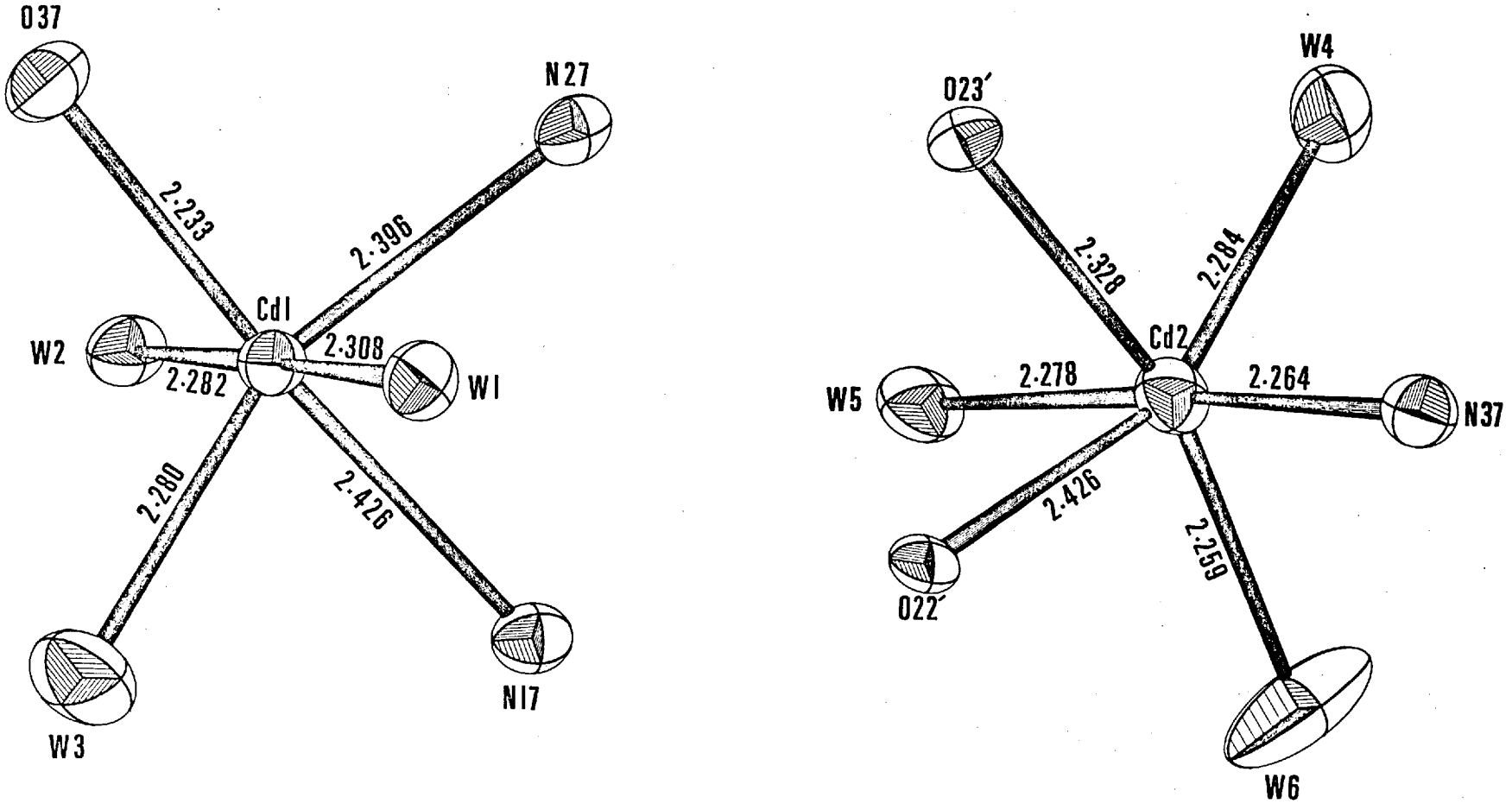


Fig. 2.6. The arrangement of donor atoms around the two cadmium ions in [$Cd_2(5'\text{-IMP})_3H_2(H_2O)_6$]. The bond lengths are given in $x10^{-1}$ nm.

observed N7 interaction. However, the ν(C =O6) band is not shifted to higher frequencies quite so much as compared with the sodium salt, probably indicating the extensive hydrogen - bonding.

It is noticeable that the PO_3^{2-} symmetric stretch hardly shifts at all even though phosphate binding has been shown in the complex. No information as to coordination to the hydroxyl groups of the ribose sugar could be obtained as these frequencies are obscured by bands due to water.

cis-[$Pt(NH_3)_2(5'\text{-IMP})_2$]$^{2-}$ [155]

This was formed as colourless prisms from a solution at pH 6.85. Preliminary single - crystal photographs showed the crystals to be orthorhombic with the space group $C222_1$. The unit cell dimensions are a = 0.8766, b = 2.2933, and c = 2.2436 nm, U = 4.5103 nm^3 and Z = 4.

The crystal structure is based on that of the sodium salts,[156,157] in which pairs of 5'-IMP moieties are connected to a common water molecule via hydrogen bonds to the N7 positions. In crystals of the platinum compound this water molecule is partially replaced by a $Pt(NH_3)_2$ group of a discrete anion of formula cis-[$Pt(NH_3)_2(5'\text{-IMP})_2$]$^{2-}$ shown in Fig. 2.7. The platinum atom lies on a crystallographic two-fold axis, and is bonded to the N7 atoms of two 5'-IMP moieties. The coordination is square - planar with Pt - N7 and Pt - NH_3 distances of 0.202 and 0.205 nm respectively. The metal atom is markedly out of the hypoxanthine base plane (approx. 0.059 nm) thus although the angle N7 - Pt - N7 is 89^o the angle between the two bases is only 43^o. (This bending at N7 may well be a crystal - packing effect).

The i.r. spectrum is very similar to that of 5'-IMPNa_2 except for the small shifts in the ring vibration band and ν(C = 06) indicating the N7 interaction (Table 2.4.). Also, as with the many other complexes showing no phosphate binding, there is no shift in the PO_3^{2-} symmetric stretch.

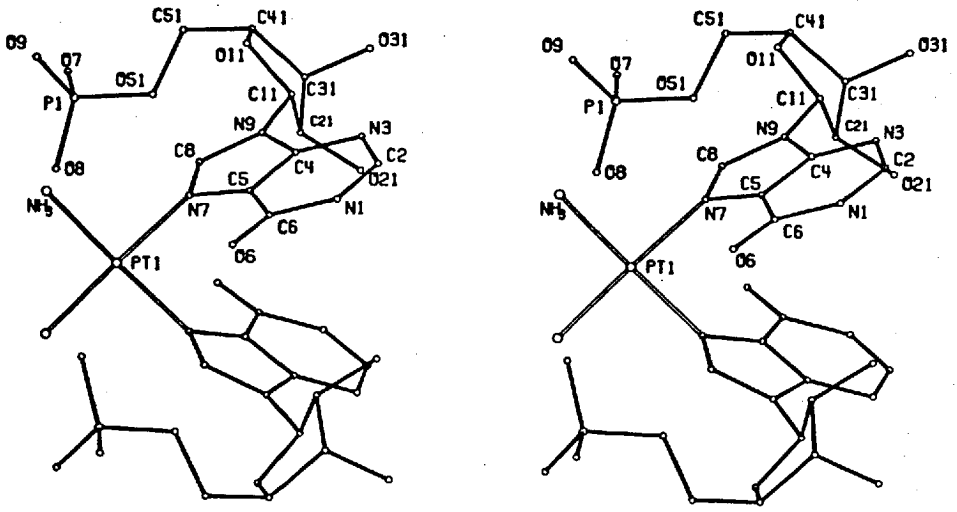


Fig. 2.7. Stereoscopic pair showing the structure of cis-[$Pt(NH_3)_2(5'\text{-IMP})_2$]$^{2-}$.

2.1.4. Uridine 5'-Monophosphate

The platinum complexes are discussed in Chapter 3.

$Cd(5'\text{-UMP}),nH_2O$ n = 6 or 6.5

This compound was prepared as large, fibrous crystals at pH 6.80. Good Weissenberg but poor oscillation photographs were obtained which showed the crystals as having a very short axis of about 0.50 nm. Two other compounds which have exhibited this behaviour both have metal - phosphate bonds, i.e. [$Zn(5'\text{-IMP})$],H_2O [158] with an axis of 0.5580 nm and [$Cd(5'\text{-CMP})(H_2O)$],H_2O [136] with one of 0.5293 nm. The limited X-ray data available for the compound therefore suggests that phosphate to metal bonding occurs. Furthermore, preliminary investigation of the cobalt complex of 5'-UMP has also shown it to be of a similar structure.[153]

The data from the i.r. spectra of this complex and $5'\text{-UMPNa}_2$ (Table 2.5.) do not give any real evidence for this proposed mode of binding. The PO_3^{2-}

stretch vibration in Cd(5'-UMP),nH_2O is split and the main peak is shifted only slightly to higher frequencies compared to 5'-$UMPNa_2$. However, only a small shift is observed in the pyrimidine nucleotide complex [Cd(5'-CMP)-(H_2O)],H_2O, which showed phosphate binding, hence no conclusions as to this mode of coordination can be made from i.r. measurements.

The bands in the 1800 - 1600 cm^{-1} region of the 5'-$UMPNa_2$ spectrum are very difficult to assign with any certainty due to the extensive coupling of the carbonyl and C = C stretch vibrations and possibly N - H deformation modes.[138] Nevertheless, a comparison with the spectrum of Cd(5'-UMP),nH_2O shows definite binding to the base. Because of the shift to high frequency of ν(C = O2), the site of coordination is probably N3, as was suggested for the Mn and Cu complexes.[104,105]

The structure of 5'-$UMPNa_2$ is known to be in the keto form with a proton attached to N3.[138] If this atom is the true donor, then the proton must be weakly bound and repositioned on another part of the molecule on complexation. Re-examination of the spectra show that the broad band ascribed to the PO_3^{2-} asymmetric stretch, at ca. 1100 cm^{-1} in 5'-$UMPNa_2$, is split in the cadmium complex like that found in uridylic acid. This infers that the proton is no longer bound to N3 but may still reside near the base portion.[122]

Support for the theory that this N3 - H bond is weak has come from several investigations. It is well known that for uracil itself, metal - binding to N3 usually occurs only if the proton is completely removed under alkaline conditions to form an inner complex.[159-161] Thymine behaves in this way but on substituting at N1, as in the case of 1-methylthymine, the N3 - H bond is considerably weakened and the proton is removed comparatively easily in neutral solution. This occurs in the 2:1 mercury complex.[162] Further evidence ofN3 interaction has come from n.m.r. studies of 5'-UMP complexes [104-106] and X-ray work on the uridine complex Cu(U)(ClO_4)$_2$(H_2O)$_2$.[163] However, recent

work on neutral complexes of uracil and related ligands has indicated that O4 may be the preferred site.[143]

In conclusion it would appear that the complex Cd(5'-UMP),nH_2O possibly contains both phosphate and base (N3 or O4) coordination. No information as to any ribose interactions could be obtained.

Zn(5'-UMP),$2H_2O$

This complex was prepared as small, white needles at pH 5.60. No X-ray work was carried out on this solid.

The same coordination as the cadmium complex is suggested from the i.r. spectrum (Table 2.5.). The shifts in bands are similar except for the ν_s mode of PO_3^{2-} which moves to a much higher frequency. Phosphate binding is almost certain to occur due to the low water content.

2.1.5. Conclusions from Structural Studies

Although infrared spectroscopy is an easy and quick technique, this investigation has underlined its limited value in determining metal - nucleotide binding sites.

The spectra have been shown to be complex, with the bands difficult to assign and this has precluded the positive identification of specific donor atoms. Nevertheless, in combination with X-ray work, coordination to the base moieties has been indicated from shifts in the carbonyl stretching vibrations and those pertaining to the ring. The donors appear to be N7 of the purine nucleotides, N3 of 5'-CMP and N3 or O4 of 5'-UMP. However, binding to phosphate is not denoted at all. No pattern was observed from shifts in the PO_3^{2-} symmetric stretch vibration of complexes with or without this type of coordination. Also no information as to ribose interaction could be achieved due to the overlapping ν(O - H) bands of the water molecules.

2.2. Implications of Metal - Nucleotide Structures

2.2.1. General Aspects

In the previous section, all the metal - nucleotide structures determined showed the proposed binding modes i.e. N7 of purines and N3 of pyrimidines, except in two cases. Additional phosphate binding was found for [$Cd(5'\text{-CMP})(H_2O)$],H_2O and phosphate and ribose in [$Cd_2(5'\text{-IMP})_3H_2(H_2O)_6$],$6H_2O$. This is not wholly unexpected as experimental work carried out with DNA showed that although cadmium bound to bases quite strongly, interaction with the phosphate residues also occurred.[100]

As a 2:2 complex of cadmium with 5'-IMP could also be made showing just N7 binding it was thought that for [$Cd_2(5'\text{-IMP})_3H_2(H_2O)_6$],$6H_2O$ the acidity must enhance the binding ability of the oxygen donors. Nevertheless, it would be expected that an increase in the concentration of a 'hard' donor, such as H^+, would tend to also 'harden' the oxygen atoms instead of the reverse.

A study of the list of known nucleotide complex structures given in Table 2.6. indicates that no rule can be made about the effect of low pH, as no two structures showing completely different binding modes have been found with the same ligand, metal and stoichiometry. The five and seven - coordinate cadmium - 5'-CMP structures appear to be the exception, but the arrangement around the metal is in fact very similar.

It would seem, therefore, that the reason for the preparation of [$Cd_2(5'\text{-IMP})_3H_2(H_2O)_6$],$6H_2O$ is inconclusive. This is borne out from several features of metal - nucleotide and nucleoside complexes. The inconsistency of ligand behaviour towards acidity is shown by 5'-GMP, for [$Cu_3(5'\text{-GMP})_3(H_2O)_8$],$4H_2O$, prepared at pH 3.50, exhibits phosphate binding,[164] whereas [$Cd(5'\text{-GMP})(H_2O)_5$],$3H_2O$ at pH 3.41 shows the 'normal' N7 interaction. In addition, the complexes of the pyrimidine nucleotide 5'-CMP (and possibly 5'-UMP) appear to bind to phosphate regardless of pH.

With respect to the ribose group, again there is no obvious reason for its interaction with cadmium, as work with copper(II) and the nucleosides adenosine and uridine [165] found that the hydroxyl groups bound to the metal in the pH region 9.5 - 12.

From the evidence given above, it would seem prudent to suggest that variations of pH in the acidic region does not affect the binding modes directly but probably influences the stabilisation of complexes and hence their structures. This then may explain the change of stoichiometry in going from the 2:2 Cd - 5'-IMP complex to the heavily hydrogen - bonded $[\ Cd_2(5'\text{-IMP})_3H_2(H_2O)_6\ ],6H_2O$.

The compound $[\ Cd(5'\text{-CMP})(H_2O)\ ],H_2O$ also exhibits an interesting structure (Fig. 2.1.). It shows a pentacoordinate cadmium atom in a distorted stereochemistry bound to the N3 of the 5'-CMP base, one water molecule and oxygen atoms from three phosphate groups. The O2 atom and a phosphate oxygen were found near to the metal but not actually bound.

A recent crystallographic study on the seven - coordinate complex $[\ Cd(5'\text{-CMP})(H_2O)\ ],3H_2O$ (Fig. 2.8.) has proved that a definite Cd - O2

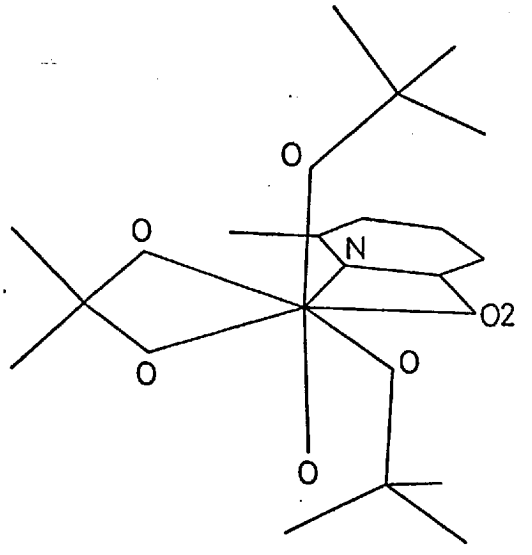


Fig. 2.8. Structure of the seven - coordinate complex $[\ Cd(5'\text{-CMP})(H_2O)\ ],3H_2O$

bond can, however, exist with the distance as 0.269 nm.[145] On close inspection of this structure there is a strong resemblance to the five - coordinate complex [Cd(5'-CMP)(H_2O)],H_2O which has a very similar arrangement of atoms but with O2 and the phosphate oxygen slightly farther away from the cadmium atom.

The effect of O2 atoms (of pyrimidine bases) on metals has been examined in several recent crystallographic studies of copper(II) - cytosine [142,166,167] and copper(II) - cytidine [168] complexes. The structures clearly show two principal features common to all of the compounds; 1) binding of the pyrimidine or pyrimidine nucleoside through the N3 position of the ring, 2) an intramolecular Cu - O2 interaction from each base. The Cu - O2 distances range from 0.274 nm to 0.288 nm with all of the structures indicating a weak bond.

The formation of these semi-chelated compounds with cytosine and cytidine appears to have some theoretical justification. Molecular electrostatic potential calculations [169] for these ligands suggest that there is a wide attractive region for electrophilic agents with two deep minima, one in the direction of the lone pair on N3 and one at an angle of 55° to the C = O2 bond. The simultaneous binding of metal ions to N3 and O2 of cytosine and cytidine would seem, therefore, to be a natural consequence of the attractive potential inherent in the molecular framework.

Other related work with an N1 bonded Cu(II) - thymine complex [170] shows no meaningful Cu - O2 interaction. This is to be expected since the electrostatic potential at O2 of thymine is considerably less nucleophilic than O2 of cytosine.

The biological implications of the structures of [Cd(5'-CMP)(H_2O)],H_2O and [Cd_2(5'-IMP)$_3H_2$(H_2O)$_6$],$6H_2O$ are considered from their known metal - phosphate and ribose interactions. The significance of the complex cis-

$[\ Pt(NH_3)_2(5'\text{-IMP})_2\ ]^{2-}$ is discussed in the following section.

Although binding to the ribose moieties of nucleotides have been suggested for copper(II) [48,171] and cobalt(III) [172] on indirect evidence, $[\ Cd_2(5'\text{-IMP})_3H_2(H_2O)_6\ ],6H_2O$ provides the first direct demonstration that heavy metal - ribose binding can occur. There is therefore the possibility that such inter-actions may favour the known catalytic degradation of RNA by heavy metal ions.[173] However, it appears that the cadmium - ribose interaction is not an unduly strong one, as the Cd - O (ribose) bonds tend to be rather long and the O2' - Cd - O3' angle is only 68.3°. Therefore the effects of such interactions are likely to be secondary to metal - phosphate bonding in influencing RNA degradation (section 1.4.).

The similarity of the base stacking of two 5'-IMP ligands found in $[\ Cd_2(5'\text{-IMP})_3H_2(H_2O)_6\ ],6H_2O$ with that in cis-$[\ Pt(NH_3)_2(5'\text{-IMP})_2\ ]^{2-}$ is of some importance with the possible interaction of toxic metals with nucleic acids. If metals bind together adjacent purines in DNA, cell replication may be seriously affected, as proposed for the primary anti - tumour mechanism of platinum compounds. However, it should be noted that cadmium can bind in a less specific manner i.e. to base, phosphate and ribose sugar, and may therefore affect normal metabolic processes in this way.

It is well known that heavy metals replace certain essential elements in natural processes (section 1.1.) and therefore because of the chemical similarity of cadmium and zinc it is of interest to compare their nucleotide systems. The only zinc derivative for which X-ray information is available is $[\ Zn(5'\text{-IMP})\ ]_n,nH_2O$.[158] There is considerable similarity between the structures of this complex and $[\ Cd(5'\text{-CMP})(H_2O)\ ],H_2O$, for the zinc atom is bound to a base at N7 and to phosphate oxygen atoms from three other 5'-IMP groups. Although the stereochemistry around the metals are slightly different, the fact that similar binding modes are employed give further

evidence as to the possible ways cadmium may affect the functions of the body.[174,175]

2.2.2. cis-Platinum - Nucleotide Complex Structures and Anti - Cancer Mechanisms

It was stated in section 1.2. that the activity of the platinum compounds was due to inhibition of the DNA synthesis in cells and was thought to be caused by inter- or intrastrand linking of the double - helix. These two possible modes of binding are now discussed with regard to the crystal structure of cis-$[\ Pt(NH_3)_2(5'\text{-IMP})_2\ ]^{2-}$.

In high chloride medium, such as isotonic saline, replacement of the chloride groups in cis-$Pt(NH_3)_2Cl_2$ will be suppressed except for displacement by the strongest nucleophiles such as sulphur. Once inside the cell (where the chloride ion concentration is much lower) it is assumed that before interaction with DNA, the platinum complex converts to the diaquo species cis-$[\ Pt(NH_3)_2(H_2O)_2\ ]^{2+}$. It should be kept in mind that since the discovery of a stable platinum - pyrophosphate complex,[69] there is also the possibility that the chlorides may be replaced initially by a phosphate anion. However, the diaquo compound is considered as the major reactive intermediate.

Studies *in vitro*, using u.v. spectroscopy, showed that platinum compounds reacted with the bases of DNA rather than the sugar or phosphate residues,[176] and that the rate of reaction is enhanced as the G + C content is increased.[177,178] In all cases both cis- and trans-$Pt(NH_3)_2Cl_2$ interacted with the displacement of at least one chloride ion. Investigations into the binding of these Pt(II) compounds to DNA constituents using spectrophotometric [179] and kinetic [180] techniques, demonstrated that guanine was the preferred base followed by adenine and then cytidine. No immediate reaction was recorded with thymine although it is known to form a 'platinum - blue'

compound slowly (see Chapter 3). For the nucleosides, the major binding sites were shown to be the N7 position of the purine bases and N3 of the pyrimidines. However, the latter position will be blocked in helical DNA; this implies that the N7 atoms of guanine and adenine are the most likely sites for binding to platinum.

Although the previous studies showed some differences in the binding capabilities of cis- and trans-Pt(II) complexes, no evidence was given as to the specificity of action of the cis isomers. To account for this observation, interstrand cross-linking of double - stranded DNA was proposed,[82,83] and with studies using dinucleotides,[181] it was suggested that the amino groups on the adenine bases were important sites for cis bidentate binding. However, there is ample evidence that the hybridisation of these groups is essentially sp^2, with the formal lone electron pair participating in π-bonding to the purine ring. This greatly diminishes their coordinating ability as compared with the ring nitrogen atoms. This conclusion is supported by many structures of metal compounds with purine bases etc., in which the amine group is not directly bonded to the metal ion.[182-184] Instead N7 appears to be the most commonly involved atom.

It should be noted , however, that the results achieved by Roberts and Pascoe [83] using the method of alkaline caesium chloride density gradients, gave no certainty that platinum cross-links double - stranded DNA. The results do prove though that a) platinum binds to DNA and b) interstrand links are formed at some stage in the experimental procedure used, even if only during, or after, strand separation.

Because interstrand cross-linking of helical DNA can be discounted as the mode responsible for the activity shown by cis-platinum compounds, another proposal was put forward by Shooter [90] who suggested that intra-strand linkage occurred instead i.e. binding on the template strand only. This idea has since been widely supported.[185-189]

Morrison and Gale [190] studied the binding sites in DNA for possible intrastrand bifunctional interactions of the platinum compounds, and concluded that they may interact at electron - rich areas. It was suggested that intrastrand interaction occurred between either O6 or N7 of the guanine base and O2 of thymine (although this has been shown previously not to react easily).

Since the N7 position in DNA is the most open to attack, and as this nitrogen atom has been found bonded to platinum in the nucleotide complex cis-[$Pt(NH_3)_2(5'\text{-IMP})_2$]$^{2-}$, a mechanism indicating the anti-tumour action of the platinum compounds is now proposed. This accounts for the specificity of the cis isomers.

The complex ion cis-[$Pt(NH_3)_2(5'\text{-IMP})_2$]$^{2-}$, found both in the solid [155] and solution,[191] shows binding to the N7 position of two hypoxanthine bases. This implies that some sort of intrastrand linkage may be possible but it is doubtful that such an interaction occurs when the DNA is in the double - helical form. Thus the most likely attack on DNA by an active compound such as cis-[$Pt(NH_3)_2(H_2O)_2$]$^{2+}$ will be initially the formation of a bond to only one N7 position as indicated in Fig. 2.9.a. A hydrogen - bond may then be formed between a coordinated water molecule and the O6 position. This would entail the water molecule being markedly out of the plane of the guanine base, which could lead to steric problems in the helix. If these are severe, this water molecule could be lost, and a direct, weak bond formed to the keto oxygen (Fig. 2.9.b.). In the case of adenine both possibilities involving the amino group are less likely. Thus the most plausible situation in helical DNA would seem to be that platinum atoms are attached mainly to the guanine bases, firmly at N7 and weakly via O6. Each platinum atom then has one relatively labile bond.

A consequence of this would be that as soon as the two strands separate, and the restrictions of base stacking are largely removed, the metal is

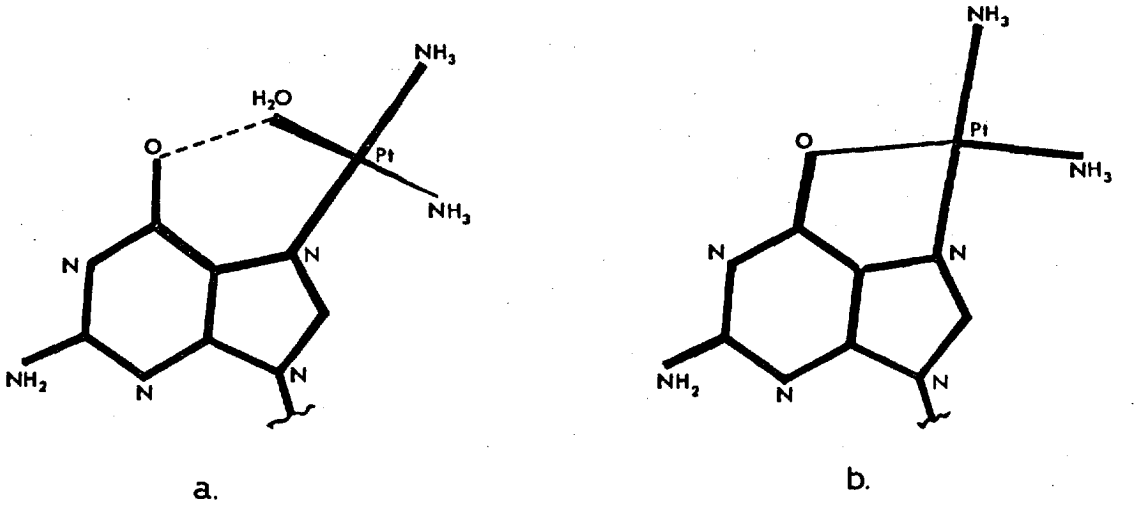


Fig. 2.9. Postulated modes of initial binding of platinum to a guanine base in double - stranded DNA.

free to replace the labile bond by a stronger Pt - N bond to a neighbouring base. This is more likely to be a base within the same strand. Thus guanine could be linked by platinum to the N7 positions on adenine or another guanine, or to N3 on cytidine. In this way most of the bound platinum should be able to 'capture' a second base, since only a TGT sequence would be unaffected. Such a process would be very effective in preventing replication.

Although most of the DNA will be double - stranded, the active platinum species may encounter single - stranded segments. These less restricted systems offer wider opportunities for attack by platinum. For instance, the N1 position on a hypoxanthine base,[192] N7 and N1 on adenine [193-197] and N3 on cytosine [142] would now also be open to initial attack.[198] The availability of a number of potential binding sites, and the flexibility of the system increase the chance of interstrand linking. However, in these circumstances one would expect the non-active trans complexes to be at least as effective in forming interstrand links as their cis counterparts.[199]

The above considerations apply equally well to all forms of RNA, but the consequences on cell replication of such platinum attack will depend on how readily that particular form of RNA can be replaced.

Evidence for the first step of this mechanism has come from potentiometric [200] and X-ray photoelectron spectroscopy [201] techniques which concluded that cis-$Pt(NH_3)_2Cl_2$ formed a specific chelate with N7 and O6 of guanine. However, the second step, leading to inhibition of DNA replication, was thought to be slightly different to that proposed here. The cis-platinum compounds are thought to first attack and saturate all the N7 sites of guanine in DNA, then these square - planar complexes form a strong bond to O6 which breaks the hydrogen - bond between O6 (Gua) and NH_2 (Cyt). As a result O6 (Gua) is not available to form hydrogen - bonds in the replication process. This reduction of hydrogen - bonds prevents the recognition of guanine by cytosine because the Gua - Cyt approach is very specific (three hydrogen - bonds). The inability of guanine to form three hydrogen bonds with cytosine can stop the DNA synthesis, but may not affect the growth. This is also the process by which cadmium is thought to destabilise DNA.[202]

The objection to this mechanism is that it would be expected that the Pt - O6 bond is much weaker than a Pt - N bond. Hence, if a nitrogen atom is available (as after strand separation) the initial bond would break forming the more stable PtN_4^{2+} moiety. Furthermore, it is interesting to note that although the work has shown the cis-platinum isomer binding to N7 and oxygen, ESCA is not able to differentiate easily between O6 or any other oxygen atom in DNA. This means that while the presence of a Pt - O6 bond is not discounted, a Pt - H_2O bond, also proposed for the first stage of the previous mechanism, should cause a similar shift in the spectrum.

Absorption studies and related techniques were also used to confirm the initial attack of cis-platinum compounds on guanine.[203] This work supported the proposed second step of the mechanism whereupon binding to the second

purine molecule led to a loss of base stacking. This was observed for DNA bound with platinum.

To conclude this section about the implications of cadmium and platinum in nucleic acid chemistry, it is interesting to note ' Haddow's Paradox' [204] which states that 'agents which cause cancer also cure it'. This is emphasised by the fact that both anti-tumour and carcinogenic mechanisms [205,206] show that electrophiles, such as cations, undergo substitution at selected nucleophilic sites, mainly N7 and O6 of the guanine residues in nuclear DNA of cells. This therefore endorses the importance of these sites with regard to the different effects metals may have on metabolic processes and underlines how specific drugs must be.

5'-CMPNa_2 [a]	[Cd(5'-CMP)$(H_2O)_{H_2O}$][a,b],	Cd(5'-CMP),5H_2O	Zn(5'-CMP),4H_2O	Assignment [115,137-139]
1668 sh	-	-	-	δNH_2
-	1667 s	1658 s	-	δNH_2 + ν(C = 02) [c]
1648 s	1640 s	1640 s	1652 s,br	Mainly ν(C = 02) [c]
1614 sh	1601 s	1603 sh	1619 sh	Coupled ν(C = C) + [ν(C = N)]
1583 sh	-	-	-	Overtone
1525 m	1527 s	1527 m	1536 sh	Coupled ring
1490 s	1502 s	1505 s	1511 s	vibrations
986 s	988 s	980 s	995 s	Symmetric PO_3^{2-}
978 sh	980 s			stretch

a. Crystalline solids.

b. X-ray shows metal binding to phosphate and base (N3).

c. Also contribution from δNH_2.

Table 2.2. Selected i.r. data for 5'-CMP complexes. Measurements were carried out using Nujol mulls between NaCl plates and KBr discs. Values are in cm^{-1}.

5'-GMP$Na_2\frac{1}{2}H_2O$ [a]	$[Cd(5'\text{-GMP})(H_2O)_5]$, $3H_2O$ [a,b]	$Cd(5'\text{-GMP}),6H_2O$	cis-$[Pt(NH_3)_2H_2(5'\text{-GMP})_2],5H_2O$ [a]	$Zn(5'\text{-GMP}),nH_2O$ n = 4.5 or 5	Assignment [115,138]
1692 s	1697 s	1698 s	1695 s	1695 s	$\nu(C = O6)$ [c]
1660 sh	1638 s	1632 s	1640 s	1640 s	Coupled $\nu(C = O6)$ [c] + $\nu(C = C)$
1601 s	1609 s	1601 sh	1590 s	1602 s	Coupled $\nu(C = N)$ [c] + $\nu(C = C)$
1585 sh	1571 m	1577 sh			
1545 s	1533	1535	1540	1538	
1480 m	1478	1483	1500	1489	Ring vibration
975 s	981 s 966 sh	986 s	992 w	993	Symmetric PO_3^{2-} stretch

a. Crystalline solids.

b. X-ray shows metal binding to base only (N7).

c. Also contribution from the NH or NH_2 deformations.

Table 2.3. Selected i.r. data for 5'-GMP complexes. Measurements were carried out using Nujol mulls between NaCl plates and KBr discs. Values are in cm^{-1}.

5'-IMPNa$_2$ [a]	[Cd(5'-IMP)(H$_2$O)$_5$], 2.5H$_2$O [a,b]	[Cd(5'-IMP)(H$_2$O)$_5$], H$_2$O [a,b]	[Cd$_2$(5'-IMP)$_3$H$_2$-(H$_2$O)$_6$],6H$_2$O [a,c]	cis-[Pt(NH$_3$)$_2$-(5'-IMP)$_2$]$^{2-}$ [a,b]	Assignment [115]
1678 s	1693 s 1675 sh	1693 s 1676 sh	1685 s	1688 s	ν(C = 06)
1645 sh	1625 sh	1625 sh	1625 m	1642 sh	Coupled ν(C = 06) [d] + ν(C = N)
1591 m	1586 m	1587 m	1589 m	1593 m	Coupled ν(C = N) [d] + ν(C = C)
1551 m	1554 m	1555 m	1550 m	1561 m	
1521	1510	1512	1512	1525	
1480	-	-	1497	1494	Ring vibration
977	976 s 958 sh	976 s 958 sh	978 s	976 s	Symmetric PO_3^{2-} stretch

a. Crystalline solids.

b. X-ray shows metal binding to base only (N7).

c. X-ray shows metal binding to phosphate, ribose and base (N7).

d. Also contribution from the NH deformation.

Table 2.4. Selected i.r. data for 5'-IMP complexes. Measurements were carried out using Nujol mulls between NaCl plates and KBr discs. Values are in cm^{-1}.

5'-UMPNa_2 [a]	Cd(5'-UMP),nH_2O n = 6 or 6.5 [a]	Zn(5'-UMP),2H_2O [a]	Assignment [115,137,138,207,208]
1775 vw	1785 vw	1785 vw	Overtone or combination
1678 s	1717 m ?	1695 s	Mainly ν(C = O2) [b]
1655 sh	1656 s	1668 s	Coupled ν(C = O4) + [ν(C = C)] [b]
1635 sh	-	1610	Coupled ν(C = C) + [ν(C = O4)] [b]
981 s	985 s 971 sh	992 986 sh	Symmetric PO_3^{2-} stretch

a. Crystalline solids.

b. Also contribution from the NH deformation.

Table 2.5. Selected i.r. data for 5'-UMP complexes. Measurements were carried out using Nujol mulls between NaCl plates and KBr discs. Values are in cm^{-1}.

Table 2.6. Details of the binding modes from the known structures of transition metal - nucleotide complexes.

	Nucleotide Complex	pH of Prep.	Binding Modes	Reference
1.	[Ni(5'-AMP)$(H_2O)_5$],H_2O	5.0	N7 of base + five water molecules	184
2.	[Co(5'-CMP)(H_2O)]	5.4	N3 of base + one water molecule + two phosphate oxygens	144
3.	[Cd(5'-CMP)(H_2O)],H_2O	a) 4.64	N3 of base + one water molecule + three phosphate oxygens	This Work (136)
		b) 6.5	As for 3	144
4.	[Cd(5'-CMP)(H_2O)],$3H_2O$	6.5	N3 and O2 of base + one water molecule + four phosphate oxygens	145
5.	[Mn(5'-GMP)$(H_2O)_5$],$3H_2O$	4.19	N7 of base + five water molecules	146
6.	[Co(5'-GMP)$(H_2O)_5$],$3H_2O$	6.5	As for 5	209
7.	[Ni(5'-GMP)$(H_2O)_5$],$3H_2O$	-	As for 5	210
8.	[Cu_3(5'-GMP)$_3(H_2O)_8$],$4H_2O$	3.5	N7 of base + three water molecules + one phosphate oxygen	164

9.	[$Cd(5'\text{-}GMP)(H_2O)_5$],$3H_2O$	a)	3.41	As for 5	This Work
		b)	4.5	As for 5	150
10.	[$Mn(5'\text{-}IMP)(H_2O)_5$],$2H_2O$		5.5	A trimer with binding to N7 of base + five water molecules	153
11.	[$Co(5'\text{-}IMP)(H_2O)_5$],$2H_2O$	a)	4.5	N7 of base + five water molecules	211
		b)	6.5	As for 11a)	209
12.	[$Ni(5'\text{-}IMP)(H_2O)_5$],$2H_2O$		4.5	As for 11	211,212
13.	[$Zn(5'\text{-}IMP)$]$_n$,nH_2O		4.0	N7 of base + three phosphate oxygens	158
14.	[$Cd(5'\text{-}IMP)(H_2O)_5$],H_2O		4.51	A dimer with binding to N7 of base + five water molecules	This Work
15.	[$Cd(5'\text{-}IMP)(H_2O)_5$],$2.5H_2O$		5.85	Both dimer and trimer forms were obtained with binding to N7 of base + five water molecules	This Work

16.	$[Cd_2(5'\text{-IMP})_3H_2(H_2O)_6],6H_2O$	3.88	Cadmium 1 is bound to the N7 atom of two bases + three water molecules + one phosphate oxygen Cadmium 2 is bound to the N7 atom of one base + three water molecules + two ribose oxygens	This Work (154)
17.	cis-$[\ Pt(NH_3)_2(5'\text{-IMP})_2\ ]^{2-}$	6.85	N7 of two bases + two NH_3 groups	This Work (155)

CHAPTER 3

COMPLEXES OF PURINES AND NUCLEOSIDES, AND THE PLATINUM BLUES

The importance of chelation in biochemical processes has long been known and the use of metal chelates in all areas of medicine[1] and their relationship with carcinogens and anti-cancer agents have been well studied.[213] The significance of this type of binding is now discussed with regard to the first step in the proposed mechanism of the anti-tumour action of platinum drugs, and also concerning the possible structures of the platinum-blue compounds.

3.1. Guanosine and 9-methylhypoxanthine

In Chapter 2, it was proposed that the initial binding of cis-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ to DNA was to the guanine base at N7 and to O6, either directly with a weak bond, or via a water molecule. The type of binding in the latter case has been widely demonstrated in many nucleotide complexes (Table 2.6), but direct oxygen binding has not been conclusively shown. To investigate whether the guanine base can chelate in this way, a study was carried out using platinum with the compounds guanosine and the nucleoside analogue 9-methylhypoxanthine.

Examination of the complexes of guanosine and related compounds has shown that N7 is the normal binding site for most metals,[100,149,214] including platinum[215-218] (see also purine nucleotide structures). This is confirmed by X-ray crystallographic studies of the copper - 9-methylhypoxanthine[219] and 9-methylguanine[220] complexes. These structures show the O6 atom hydrogen - bonded to a water molecule which itself is coordinated to the metal. The absence of chelate ring formation was explained on geometric grounds.[221]

From these studies it is obvious that if chelation is to take place, the metal - oxygen bond would be expected to be weak, as indicated in section 2.2.2. This means that to force bidentate binding onto the ligand, the deprotonated form should be used. This was the approach employed in this work.

The first method followed that of Heitner and Lippard,[222] who prepared a platinum complex with 9-benzyl-6-purinethione. This crystalline compound, of formula $Pt(9\text{-}BenzPur6S)_2$, DMA, has a square - planar structure with two deprotonated ligands chelating to platinum via the N7 and S6 atoms (Fig.3.1.). This structure has since been reported for the analogous thionucleoside complex with platinum.[223]

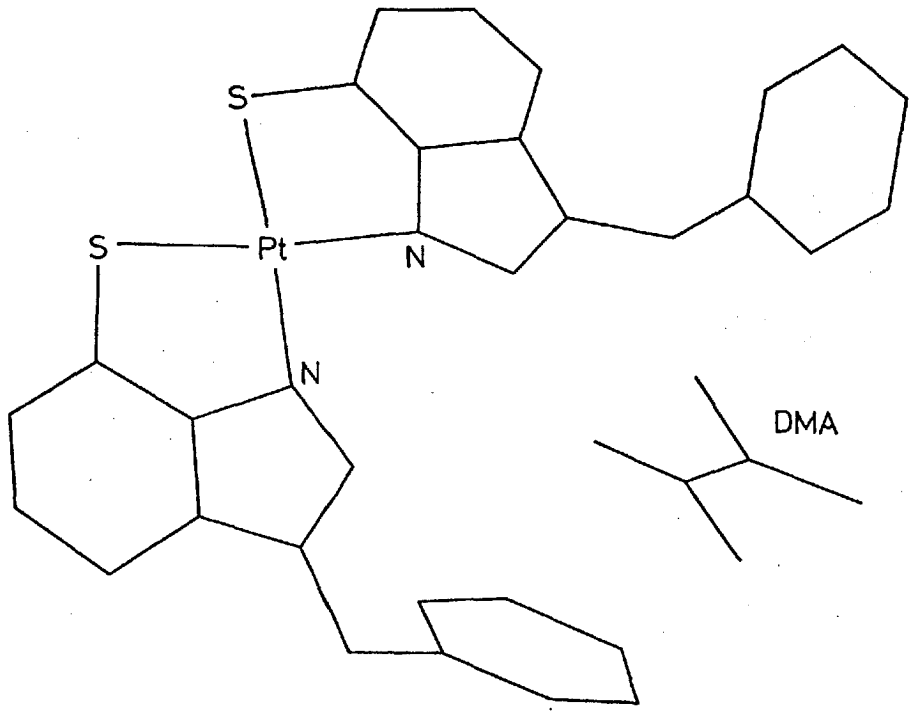


Fig. 3.1. Structure of Bis(9-benzyl-6-purinethione)platinum(II) - dimethylacetamide.

The observed cis-configuration (Fig.3.1.) permits maximum back-bonding to sulphur and the orbitals of the two sulphur atoms can overlap different metal orbitals (d_{xz} and d_{yz}).

An attempt to obtain a similar chelated platinum - guanosine complex led to a brown solid with a poor analysis. However, 9-methylhypoxanthine, gave better results and the complex $Pt(9MeHyp)'_2$, 0.5 DMA was prepared in the same manner. Unlike the structure shown in Fig.3.1., the compound probably has a trans structure for if a platinum - oxygen bond is formed, this will certainly be weak (Fig.3.2.a.).

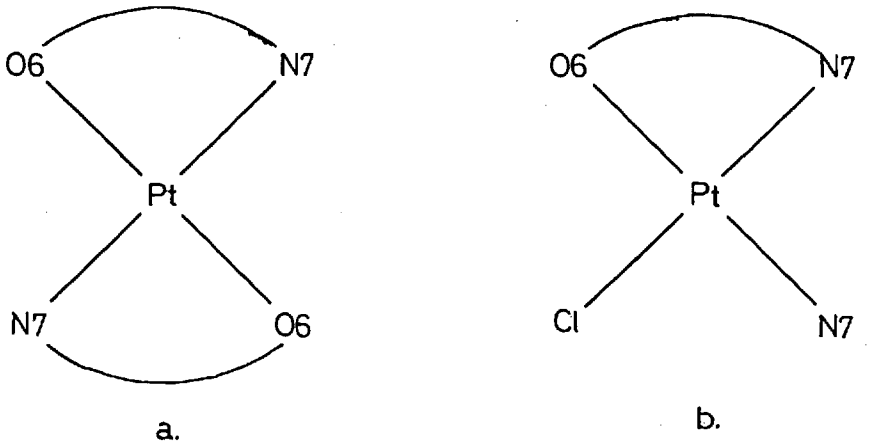


Fig.3.2. Proposed structures of a) $Pt(9MeHyp)'_2$, 0.5 DMA and b) $Pt(9MeHyp)(9MeHyp)'Cl$.

The preparation of the inner complex of 9-methylhypoxanthine was also attempted via the N7 bound cis-$Pt(9MeHyp)_2Cl_2$ compound. This solid was heated under reflux with 2,6-dimethylpyridine whereupon one molecule of HCl was removed to form the compound $Pt(9MeHyp)(9MeHyp)'Cl$. The structure is most likely to be in the cis form shown in Fig.3.2.b. This has one 9-methylhypoxanthine molecule chelated, with the remaining positions taken up by N7 of the other neutral ligand and a chlorine atom. Due to the presence of different carbonyl groups in both of these

complexes, i.e. from a neutral hypoxanthine molecule and DMA, coordination to O6 of the deprotonated hypoxanthine could not be detected using i.r. spectroscopy.

Recently, similar inner complexes with guanosine and inosine have been obtained from aqueous solution at pH > 9.[224] Binding to O6 (as well as to nitrogen) was confirmed using i.r. spectroscopy, and they were considered to have the same trans structure as shown in Fig.3.2.a. However, it is known that with Cu(II), inosine tends to bind to the N1 and O6 atoms as the pH increases.[225,226] Therefore, it was suggested that these inner complexes may possibly have a polymeric structure with the metal binding to O6, N1 and N7 (Fig.3.3.), or a trans chelated structure interacting via N1 and O6. Nevertheless the most probable structure is the one first proposed.

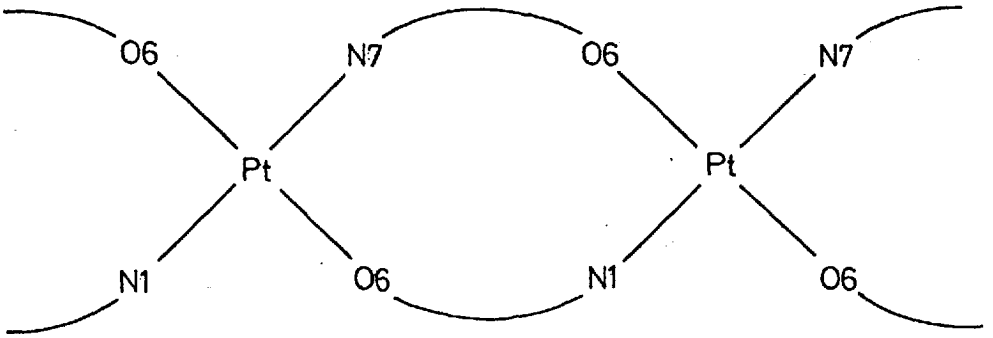


Fig.3.3. Proposed polymeric structure of PtG'_2 and PtI'_2.

Finally in this investigation, it was also attempted to prepare a crystalline sample of a metal - 9-methylhypoxanthine chelate (using copper perchlorate) for an X-ray determination. Instead of forming, as hoped, a complex with three chelated neutral ligands, the compound $Cu(9MeHyp)_4(ClO_4)_2$ was isolated as blue triclinic crystals. The

electronic reflectance spectrum showed a single medium intensity band at 16130 cm^{-1} indicative of a square - planar CuN_4^{2+} moiety but with axial interaction to form a tetragonally distorted octahedral ligand field.[227] This interaction is most likely from the O6 atoms arranged in a similar manner to $Cu(Pym2OH)_4(ClO_4)_2$,EtOH (Fig.4.17.).

Although the inner complexes PtG'_2, PtI'_2, $Pt(9MeHyp)'_2$, 0.5 DMA and $Pt(9MeHyp)(9MeHyp)'Cl$ have been formed, chelation by these guanine - like ligands has not been fully shown. However, binding of platinum to O6 is definitely indicated and therefore it can be concluded that the first stage of the anti-tumour mechanism proposed in section 2.2 is certainly feasible.

3.2. The Platinum Blues

Since the discovery of cis-$Pt(NH_3)_2Cl_2$ as an anti-tumour agent there has been much work carried out to find similar compounds with greater activity but less toxicity. It was suggested[68] that this could be achieved through combination of the platinum complexes with DNA and its components to enhance selectivity. The method of uptake by cells of large complex molecules requires pinocytosis after which lysozyme digestion releases the active portion. Because tumour cells often have pinocytic, lysozomal and mitotic activity higher than normal cells, these combined drugs may be more effective. This type of mechanism is thought to explain the activity of the newly discovered platinum-pyrimidine blues.

These compounds have been shown to be more active than cis-$Pt(NH_3)_2Cl_2$ against a wide variety of animal tumour systems and significantly less toxic, especially to kidneys.[228] They are advantageous in drug use due to their high solubility in water. The formation of the blue colour

has also been found to have useful applications in electron microscopy in staining nucleic acids of tumourigenic cells.[229,230]

One of the first platinum blue complexes to be discovered, was that made by Hofmann and Bugge[231] who prepared a compound they called 'Platinblau' by the reaction of Ag_2SO_4 on $Pt(CH_3CN)_2Cl_2$. Gillard and Wilkinson[232] re-examined this compound, $Pt(CH_3CONH)_2,H_2O$, and postulated that its structure consisted of polymeric chains with bridging acetamide groups, and divalent platinum.

The compound has since been shown to be monomeric by ebulliometry;[68] this was considered an important result, for some highly coloured platinum compounds are known which owe their colour entirely to a Pt-Pt interaction arising either in a polymer or in the solid state. It was considered, therefore, that the acetamide acts as a bidentate chelate as shown in Fig.3.4.a.

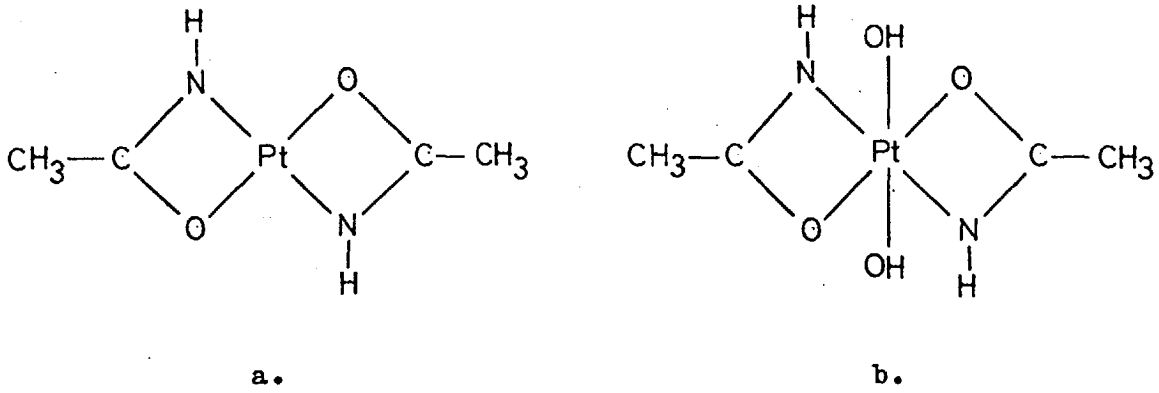


Fig.3.4. Proposed structures of 'Platinblau', a) $Pt(II)(CH_3CONH)_2,H_2O$ and b) $Pt(IV)(CH_3CONH)_2(OH)_2$.

However, a group of workers, using ^1H n.m.r. spectroscopy, have suggested that Pt(IV) is present as $Pt(IV)(CH_3CONH)_2(OH)_2$.[233] An octahedral structure was proposed with the OH groups residing in the fifth and sixth positions (Fig.3.4.b.). Nevertheless, recent

photoelectron spectroscopy (ESCA) measurements on 'Platinblau' have shown that it contains Pt(II).[228]

A blue complex, not possessing these potential chelating molecules, was also prepared.[232] An X-ray crystal structure determination on a compound formed from cis-$Pt(NH_3)_2Cl_2$ and conc. H_2SO_4 showed that there was a Pt-Pt distance of only 0.306 nm implying strong interaction. It was suggested that the complex contained layers of cis-$Pt(NH_3)_2Cl_2$ held together by Pt-Pt bonds, with the sulphate ion hydrogen - bonded to the coordinated ammonia groups.

From these studies the blues have been thought to be both polymeric and monomeric, have platinum in varying oxidation states and that Pt-Pt interaction occurs. It appears then that there may be no common structure for these blues and that the colour may arise differently depending on the ligands and method of preparation employed.

Because the structure of acetamide is similar to those of the naturally occurring pyrimidine bases (Fig.3.5.), it was suggested that these compounds may also produce blues with cis-PtL_2X_2 isomers. This was indeed found to be the case, for cis-$Pt(CH_3CN)_2Cl_2$ formed blue solutions with both 1-methylthymine and cytosine. However, no coloration was observed with the purines, guanine and adenine.[68]

Since this discovery, several classes of platinum - pyrimidine blues have been isolated incorporating the ligands uracil, thymine, cytosine and their substituted analogues.[228] These compounds were classified according to the method of preparation and the platinum complex from which they were made. The Class I blues were studied in more detail and synthesised from the reaction of cis-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ and the pyrimidine in aqueous solution at pH 7-8 and 37°C. The analyses showed that the complexes were 1:1, containing two ammonia ligands, one

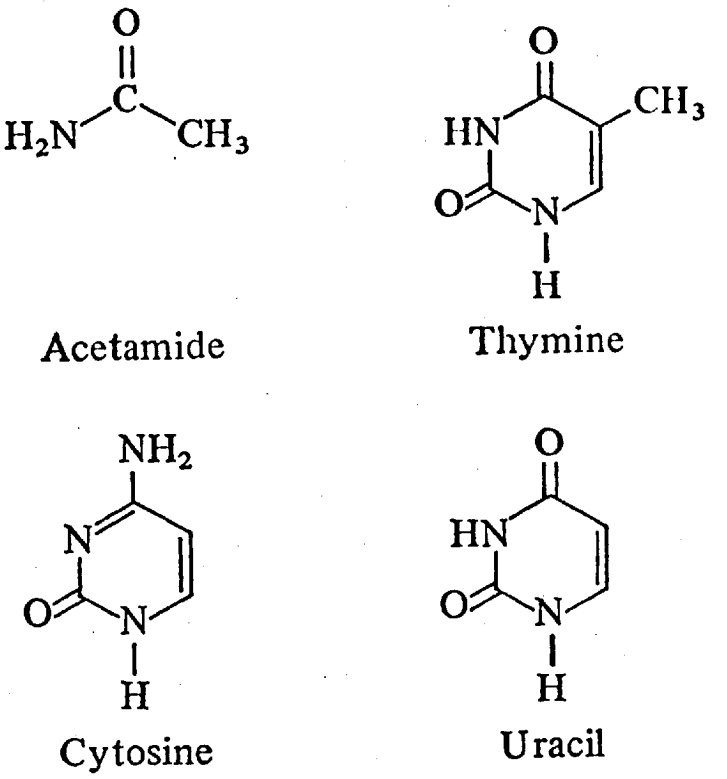


Fig. 3.5. The structures of the pyrimidine bases and acetamide.

pyrimidine anion, and one hydroxide ion per platinum but with two additional oxygen atoms at an unspecifiable location. Physical measurements gave little information as to the possible structures.

During the study of the reaction of cis-[$Pt(NH_3)_2(H_2O)_2$]$(NO_3)_2$ with nucleotides (Chapter 2), it was found that 5'-CMP and 5'-UMP, at low pH, gave blue glasses. This was unlike the behaviour with 5'-GMP and 5'-IMP which formed colourless crystalline solids under the same conditions. It was seen that the colour deepened as the pH decreased but that colourless solutions were produced at pH 7 and above. These observations contrast with those reported previously[228] in which the pyrimidine bases gave blues at pH 7-8. Therefore a preliminary investigation was carried out using the above method with various pyrimidine nucleotides, nucleosides and bases and related ligands.

Each ligand was added to a solution of cis-[$Pt(NH_3)_2(H_2O)_2$]$(NO_3)_2$ in a 1:1 stoichiometric ratio at two different pH's i.e. pH 4.00 and pH 8.50. The colours of the resulting solutions were noted, and if blue, the solids were isolated, analysed for carbon and hydrogen and tested for the presence of nitrate (Table 5.1.).

The naturally occurring pyrimidines, e.g. the uracil, cytosine and thymine nucleotides, nucleosides and bases, all gave dark blue or green solutions at pH 4.00, but at pH 8.50 they were yellow or brown (Table 3.1.). However, of the other ligands employed e.g. 2- pyrimidinethione, 2- pyrimidinone and their methyl derivatives and acetamide (Table 3.2.), a blue was formed only with the amide.

For comparison, trans-[$Pt(NH_3)_2(H_2O)_2$]$(NO_3)_2$ and [$Pten(H_2O)_2$]$(NO_3)_2$ were also used as starting solutions, but these did not produce any coloration with 5'-CMP or 5'-UMP. However, the platinum - ethylenediamine solution did form a blue with acetamide.

From the analyses of the isolated blues (Tables 3.3. and 5.1.), the nucleotides formed inner complexes of 1:1 and 2:3 stoichiometry with 5'-CMP and 5'-UMP respectively; the latter ligand behaving as a trivalent anion. The complexes of the nucleosides and bases all had 1:1 stoichiometry but were of variable nitrate content depending on whether the pyrimidines were deprotonated. Although these blue compounds could not be obtained in a pure state, certain important features were noted.

Blue solutions could only be formed using cis-[$Pt(NH_3)_2(H_2O)_2$]$(NO_3)_2$ with acetamide and the 2,4-disubstituted pyrimidines. The isolated blue solids were hygroscopic and had variable water content, and most compounds had 1:1 stoichiometries with the retention of the NH_3 groups bound to platinum.

The acetamide blues have been well studied and reported to have

structures with the ligand either bridging[232] or chelating.[233] The similarity in its structure with the naturally occurring pyrimidines suggest that these binding modes may also occur in the platinum - pyrimidine blues assuming that the coordination is to the base portion only for nucleotides and nucleosides.

The possibility of chelation was examined using the compounds 2-pyrimidinethione and 2-pyrimidinone, for these are known to bind in this manner with other metal ions (Chapter 4). However, with cis-$[\ Pt(NH_3)_2(H_2O)_2\ ](NO_3)_2$ at either pH 4.00 or 8.50, no blue coloration was observed. It may therefore be supposed that the acetamide blues have bridged structures and the pyrimidine blues involve the use of a donor group or atom in the C4 position as well as O2 and N3.

It has been reported that like acetamide, acetic acid is also able to form a dark blue solution at pH 4.00 with the cis-platinum isomer.[234] These compounds are structurally very similar and so it may be significant that bridging acetate ions have been found in the structures of two platinum complexes. The black compound, $Pt_4(AcO)_6(NO)_2,2AcOH$ [A][235] has a structure in which the platinum atoms are a distance of 0.2944 nm apart and are close enough for some type of interaction. However, the complex $[\ Pt(AcO)_2\ ]_4$ [B],[236] is brown and possesses distinct metal - metal bonds of distances 0.2492 - 0.2498 nm.

It would appear that from these observations, the criterion for producing a dark colour is to have two platinum atoms close together, but not too close so that an actual Pt - Pt bond is formed i.e. not less than ca. 0.275 nm.

The second possibility of binding in the platinum - pyrimidine blues is to have the natural pyrimidines bridging. When N1 is substituted, as in the nucleotides and nucleosides, the donors available on the base portion include O2, O4 or NH_2, and when deprotonated, N3. To determine whether

this mode is present in the structure of the blues the starting solution, [Pten$(H_2O)_2$]$(NO_3)_2$ was reacted at pH 4.00 with acetamide, 5'-CMP, 5'-UMP and recently uracil and cytosine.[234] It was observed that only acetamide was able to form a blue solution. This means that if one assumes that the ethylenediamine is still bound to the platinum, the structure of the acetamide blue cannot be a simple bis-bidentate chelate as proposed previously.[68,233] However, this cis arrangement would allow the acetamide ion to bridge two platinum atoms quite easily as shown in Fig. 3.6.

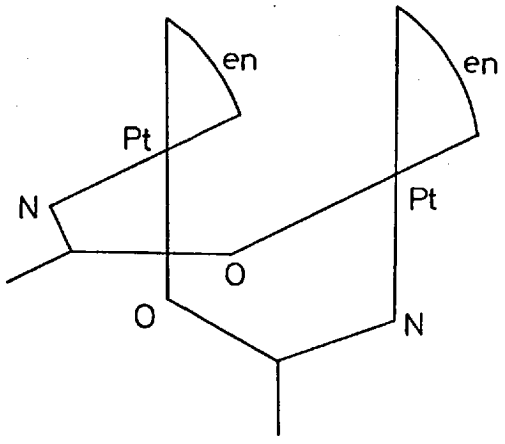


Fig. 3.6. The proposed bridge structure of an acetamide blue.

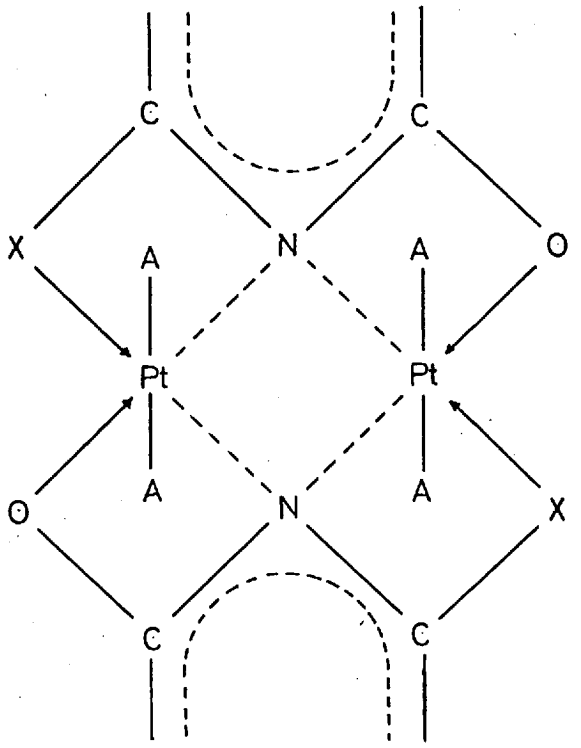
In this case the Pt - Pt distance would be at least 0.25 nm. However, it can be seen from this structure that if bridging did occur in the pyrimidine - blues, using any two of the three available sites, there would be little steric hindrance. It is considered, therefore, that because no blue is formed with these pyrimidines, bridging is not the mode of binding in the blue products containing ammonia groups. It is also important to notice that as ethylenediamine is a bidentate ligand, there is a suggestion that the above blues do not have platinum ions in a cis configuration.

As the results show that the pyrimidine blues are not simply bridging or chelating, it is not easy to see how the proposed criterion of bringing two platinum atoms close together, can be fulfilled. However, it has been reported that the i.r. spectrum of the purple complex formed from the reaction of cis-[$Pt(CPA)_2Cl_2$] with 1-methylthymine suggests that the amines, pyrimidine and chlorine atoms are all coordinated to the platinum atoms.[237] This implies that the platinums in the blue compounds may not be restricted to being four - coordinate. Also, cytosine and uracil have been shown to have chelation tendencies[142,238] and the majority of analyses of these blues indicate the presence of deprotonated ligands with N3 available for binding. Two structures are now proposed which satisfy many of the observations, and are given in Fig. 3.7. These show that the platinum atoms are either in a pseudo-octahedral or pseudo-trigonal - bipyramidal environment, the amine groups are trans, and the Pt - Pt distance is ca. 0.3 nm.

The two structures are alike but differ in the involvement of N3. In Fig. 3.7.a., the lone pair of electrons is used to bridge the platinum atoms to form an octahedral - type structure. As octahedral Pt(II) compounds are not common, the other possibility is the trigonal - bipyramidal structure shown in Fig. 3.7.b. However, from the work on the acetamide and acetate complexes, it appears that it may be necessary for the platinum atoms to interact via a delocalised system. It seems likely, then, that a possible structure of the pyrimidine blues should be one that lies between the two proposed here.

As the structures have trans amine groups it is difficult to see why the reaction is specific for cis-platinum compounds. A possible answer may be due to the relative stabilities of the pyrimidine complexes and the inclination of cis- and trans-platinum square - planar compounds to go to other stereochemistries. Taking the reaction with cytosine as an

a.



A = NH_3

X = O or NH_2

b.

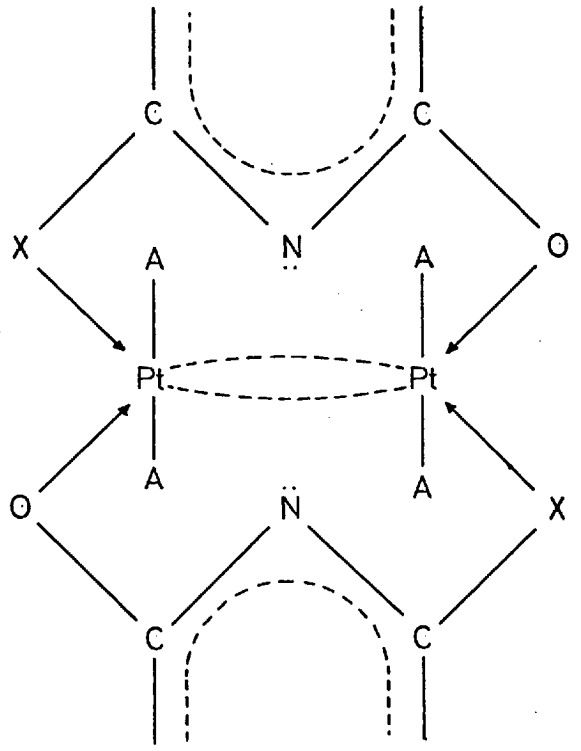


Fig. 3.7. Proposed structures for the platinum - pyrimidine blues.

example, the affinity of cis- and trans-platinum compounds to bind to various donors can be seen. As demonstrated from a crystal structure determination, the trans-Pt(II) isomer is able to form a stable complex with the ligand exhibiting coordination to N3 only.[239] However, from a ^{1}H and ^{13}C n.m.r. study,[359] a cis-platinum compound was shown to bind to N3, but that the involvement of N3 and NH_2 or N3 and O2 chelates was also a possibility. As two bonds from platinum are therefore available for coordination to the base, it is not obvious why two ligand molecules are not bound to N3. In the case of cis-$[\ Pt(NH_3)_2(H_2O)_2\ ](NO_3)_2$ with cytosine, a stable PtN_4^{2+} chromophore would be expected. The reason for the lack of formation is not due to steric factors as a square - planar palladium complex of formula Pden(Cyt)$_2$ has been shown to be present in solution.[240] Compounds of 2:1 stoichiometry with thymine and uracil were also indicated. With these ligands, it is interesting to note that the palladium atom is able to substitute N3 well below the normal pH for deprotonation.

It would appear, therefore, that cis-platinum compounds do not coordinate to the pyrimidine molecules in a straight forward manner and that the exocyclic groups may be involved. This may account for both the difference in rates of reaction between cis- and trans-Pt(II) and the behaviour of cis-$Pt(NH_3)_2^{2+}$ with pyrimidines. The fact that platinum blues are not formed with platinum - ethylenediamine solutions indicate, however, that stability may possibly be achieved only through a slow rearrangement of the monoamine ligands with the subsequent increase in coordination number of the platinum ion.

Although the proposed structures explain some of the various observations, the fact that pH has such an effect on the colour cannot be answered from this limited investigation. Further work obviously needs to be done employing two approaches. Firstly, the purification of a platinum - pyrimidine blue followed by the determination of various

physical measurements. Secondly, it will be necessary to continue the use of model compounds to find certain patterns in both ligand and amine structure and to vary the conditions of all preparations.

However, from this preliminary study, it can be concluded that the pyrimidine bases, nucleosides and nucleotides most likely react with cis-[$Pt(NH_3)_2(H_2O)_2$]$(NO_3)_2$ in the same manner and that the colour in the platinum - pyrimidine blues probably arises from the interaction between two platinum atoms via a delocalised system.

Pyrimidine	Acid (pH 4.00)	Alkaline (pH 8.50)
A. cis-[$Pt(NH_3)_2(H_2O)_2$]$^{2+}$		
5'-CMP	blue - black	dark brown
5'-UMP	blue - black	deep yellow
Cytidine	blue - purple	dark brown
Thymidine	blue - green	deep yellow
Uridine	blue - black	yellow
Cytosine	purple	yellow - brown
Thymine	dark green	dark brown
Uracil	dark green	deep yellow [a]
B. trans-[$Pt(NH_3)_2(H_2O)_2$]$^{2+}$		
5'-CMP	colourless	-
5'-UMP	colourless	-
C. [$Pten(H_2O)_2$]$^{2+}$		
5'-CMP	colourless	-
5'-UMP	colourless	-

a. pH 9.00

Table 3.1. Solution colours from the reaction of [$Pt(NH_3)_2(H_2O)_2$]$(NO_3)_2$ and [$Pten(H_2O)_2$]$(NO_3)_2$ with the pyrimidine nucleotides, nucleosides and bases in acid and alkaline media.

Compound	Acid (pH 4.00)	Alkaline (pH 8.50)
A. cis-[$Pt(NH_3)_2(H_2O)_2$]$^{2+}$		
Pym2OH	colourless	colourless
1MePym2O	faint yellow	yellow - brown
4,6Me_2Pym2OH	colourless	yellow
1,4,6Me_3Pym2O	faint yellow	orange
Pym2SH	-	red - brown
4,6Me_2Pym2SH	orange - yellow [a]	orange - yellow [a]
Acetamide	dark - blue	-
B. [$Pten(H_2O)_2$]$^{2+}$		
Acetamide	dark - blue	-

a. Yellow precipitate also formed

Table 3.2. Solution colours from the reaction of cis-[$Pt(NH_3)_2(H_2O)_2$]$(NO_3)_2$ and [$Pten(H_2O)_2$]$(NO_3)_2$ with acetamide, 2-pyrimidinethione, 2-pyrimidinone and their methyl derivatives in acid and alkaline media.

Pyrimidine	Acid (pH 4.00)	Alkaline (pH 8.50)
5'-CMP	blue	fawn
5'-UMP	blue	fawn
Cytidine	blue - grey	fawn
Thymidine	dark blue	yellow - brown
Uridine	blue	white
Cytosine	purple	fawn
Thymine	dark green	fawn
Uracil	blue	cream [a]

a. pH 9.00

Table 3.3. Colours of the isolated solids from the reaction of cis-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ and the pyrimidine nucleotides, nucleosides and bases.

CHAPTER 4

COMPLEXES OF PYRIMIDINES

4.1. Introduction

4.1.1. Biological Aspects

Sulphur containing groups are of major importance in biological processes and much research has been carried out, especially on the properties of thiol compounds.[241] Naturally occurring thiols are found as enzymes, proteins and hormones, and include coenzyme A and glutathione. However, relatively little work has been done on those compounds associated with the nucleic acids, namely the pyrimidinethiols (or thiones).

The thio-bases, 2- and 4-thiouracil and 2-thiocytosine have been found as normal constituents of some tRNA species,[242-244] but their function in the molecule is as yet unknown. Although these bases appear to be essential in small quantities, experiments have shown that the compounds can, under certain conditions, inhibit RNA synthesis leading to anti-tumour and anti-thyroid activity.[245-248] A similar inhibiting behaviour has also been shown by recent work with the minor base, 2-pyrimidinethione.[249,250]

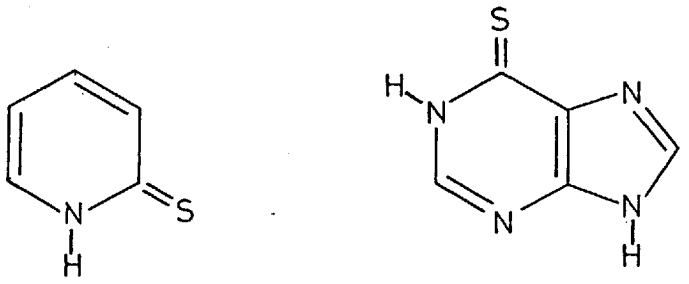
Unless masked in some proteins, thiols are chemically the most active groups found in cells and they often react quantitatively with various thiol-combining agents such as metal ions. Mercury, silver, arsenic and copper tend to form simple stable complexes (e.g. with glutathione [251]) whereas those of the naturally occurring metals, iron, molybdenum, cobalt and zinc often only find stability through chelation i.e. by binding to thiol and amino or carboxyl groups.[241] Manganese and cadmium usually bind only weakly to thiol groups, however metallothioneine, a protein

isolated from the kidneys, contains cadmium bound quite strongly to SH.[252]

In pursuing the theme of the interactions of metal ions with nucleic acids and their constituents, this chapter deals with the binding and possible chelation of transition metals (mainly first row) to the minor base 2-pyrimidinethione (Pym2SH) and its oxygen analogue, 2-pyrimidinone (Pym2OH).

4.1.2. Metal Ions and Ambidentate Ligands

Interest has recently been shown in the complex formation of the ambidentate sulphur containing ligands 2-pyridinethione [253,254] and the anti-cancer agent 6-purinethione.[255,256] The structures are shown in Fig. 4.1. Study has found that, as an anion, 2-pyridinethione can chelate



a. b.

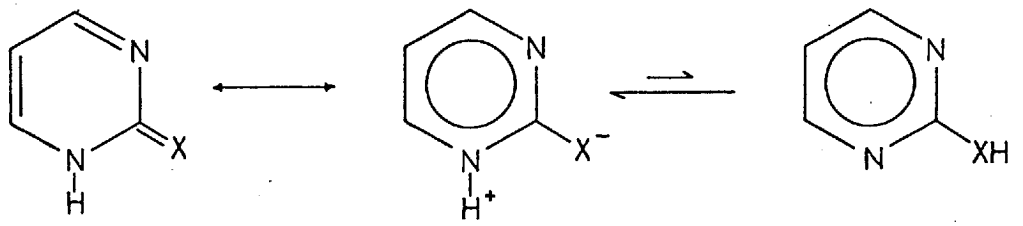
Fig. 4.1. The structures of a) 2-pyridinethione and b) 6-purinethione.

to metals via nitrogen and sulphur, as in the ruthenium complex $Ru(Py2S)_2(PPh_3)_2$.[257] Similar compounds are formed with iridium [258] and rhodium.[259] Likewise, complexes of some 9-substituted 6-purinethiones show chelation by binding via the N7 atom and sulphur. Both platinum and palladium form square - planar compounds by coordinating to two deprotonated ligands,[222] but it has been shown that copper(II) is able to bind

to the neutral substituted 6-purinethione molecule in the same manner.[260]

Other work on related ligands has included investigations of the complexes of 2-thiouracil [261-263] which as a dianion, has the possibility of coordinating via N and S or N and O. At high pH's, both of these modes of binding in fact occur.

To understand the way in which metal ions coordinate with ambidentate ligands of this type, it is first necessary to look at certain aspects of their structures. Oxo and thio derivatives of N-heteroaromatic compounds are capable of lactim - lactam or thiol - thione tautomerism respectively.[103,264] There is evidence that in the solid state and in solution, pyrimidines (and pyridines) substituted in the 2 (or 4) position by potentially tautomeric groups -XH (X being oxygen or sulphur) exist predominantly in the lactam or thione forms (Fig. 4.2.a and b) rather than in the lactim or thiol forms (Fig. 4.2.c.).[265-267] The existence



a. b. c.

Fig. 4.2. The major tautomeric structures of 2-pyrimidinethione and 2-pyrimidinone.

of the stable, aromatic, zwitterionic form (Fig. 4.2.b.) in these molecules has been well established by infrared [268-270] and electronic spectroscopy.[271]

Another characteristic feature of these compounds is their ability to form strong intermolecular hydrogen bonds of the type N - H .... X where X = O or S. In the case of 2-pyridinethione [272] the molecular structure consists of dimers whereas for 2-pyridinone [273] and 2-pyrimidinone [274] a helical arrangement occurs.

Strong hydrogen - bonding of this type can be detected using vibrational spectroscopy by examination of the N - H and N - D stretching frequencies. The band assigned to the free N - H stretch is usually sharp and found in the 3300 - 3500 cm^{-1} region but hydrogen - bonding can broaden this band and lower its frequency substantially i.e. ν(N - H) of 2-pyridinethione - 2885 cm^{-1} and 2-pyridinone - 2825 cm^{-1}.[275,276] This therefore, shows that the N - H .... O hydrogen - bonds, as expected, are stronger than those of the sulphur analogues.[277]

Of the ligands used in this study of metal ion interactions, only the structures of 2-pyrimidinone [274] and the dihydrate of its 4,6-dimethyl derivative [278] have been determined. However, because the latter compound contains water of crystallisation, only the hydrogen - bonding effects in 2-pyrimidinone is of any significance. To understand the hydrogen - bonding properties in the other pyrimidines, an infrared spectral study was carried out in the region 4000 - 1800 cm^{-1} (Table 4.4.).

All the compounds showed a lowering and broadening of the N - H stretching frequency band confirming the existence of the lactam or thione forms and that hydrogen - bonding was present. Deuteration gave ν(N - D) bands higher than expected (normal NH:ND ratio is 1.35)[279] and it was proposed by Bellamy [275] that association is weaker in the deuterated derivative giving correspondingly greater N ... S or N ... O distances.

On comparing the results in Table 4.4. it can be seen that the molecular structure of 4,6-dimethyl-2-pyrimidinone resembles its parent compound whereas 4,6-dimethyl-2-pyrimidinethione appears similar to

2-pyridinethione. 2-Pyrimidinethione, however, is rather unusual in that the ν(N - H) band is considerably lower than for the others (even lower than the strongly hydrogen - bonded 2-pyrimidinone). This behaviour is consistent with the view that the tautomeric structure is almost entirely in the aromatic stabilised zwitterionic form (Fig. 4.2.b.).[275] If this is so, then it would explain the appearance of the three ring overtone bands at about 2000 cm^{-1} similar to those found in the aromatic pyridine molecule.[280] It would appear that 2-pyrimidinethione, therefore, is most likely to have a dimer structure resembling that of 2-pyridinethione but with stronger hydrogen - bonds.

Apart from ν(N - H) bands, infrared spectroscopy is also used to observe C = O and C = S stretching frequencies. It is well known that bands pertaining to these vibrations often show shifts in their frequencies upon coordination to metal ions. These shifts can give information about the bond forces between the ligand and the metal ion [281] or sometimes about the donor site of the ligand.[282]

Nevertheless, as in the case of determining metal - sulphur coordination [283] in heterocyclic compounds, caution must be taken in assigning the proper bands as extensive coupling within the ring system gives rise to mixed vibrations. This problem is shown in ligands containing the - N - C = S fragment (e.g. Py2SH and Pym2SH) for vibrations known as thioamide bands occur in the region 1500 - 850 cm^{-1} arising from the coupling of ν(C = S), ν(C = N), δNH and δCH.[283-285] Despite this, the strong band at 1200 - 1100 cm^{-1} is considered to possess the greatest amount of ν(C = S) character and has been shown to be the most sensitive to metal - sulphur binding.[283] This band is thus termed 'ν(C = S)' and is found at 1137 cm^{-1} (Py2SH) and 1186 cm^{-1} (Pym2SH).

The use of this and other techniques in the investigation of transition metal complexes of Pym2SH, Pym2OH and their methyl derivatives are now discussed.

4.2. Transition Metal Complexes

To study the modes of binding of the potentially ambidentate 2-substituted ligands, a variety of transition metal complexes were prepared and investigated using i.r., far i.r., electronic reflectance spectroscopy and magnetic susceptibility measurements together with X-ray powder pattern data. For both 2-pyrimidinethione and 2-pyrimidinone, structural determinations were carried out on some cobalt and copper complexes.

In the case of the thione ligands, i.r. spectroscopy was employed to determine its usefulness as a guide to metal - sulphur coordination, but a similar study with the 2-pyrimidinone complexes was not possible as the $\nu(C = O)$ bands were difficult to assign.

4.2.1. Complexes of 2-Pyrimidinethione

The majority of 2-pyrimidinethione complexes were of 2:1 stoichiometry despite variations in the ligand to metal ratio and had the formulae $M(Pym2SH)_2X_2$ (M = Mn, Fe, Co, Ni, X = Cl, Br; M = Zn, X = Cl; M = Cd, X = Cl, Br, I), $M(Pym2S)_2$ (M = Co, Ni, Zn), and $Ni_5(Pym2S)_7I_2OH,6H_2O$. The formation of complexes containing non-coordinating anions were attempted but none were made with acceptable or reproducible analyses. Also copper(II) compounds could not be prepared due to the reducing ability of the ligand.

The data obtained from conventional physical measurements are collected in Tables 4.5. to 4.8.

Iron(II)

The dark brown crystalline complexes FeL_2X_2 (X = Cl, Br) had room - temperature magnetic moments of 5.25 B.M. (X = Cl) and 5.42 B.M. (X = Br). The electronic spectra of the complexes were similar, with the bromide

Fig. 4.3.

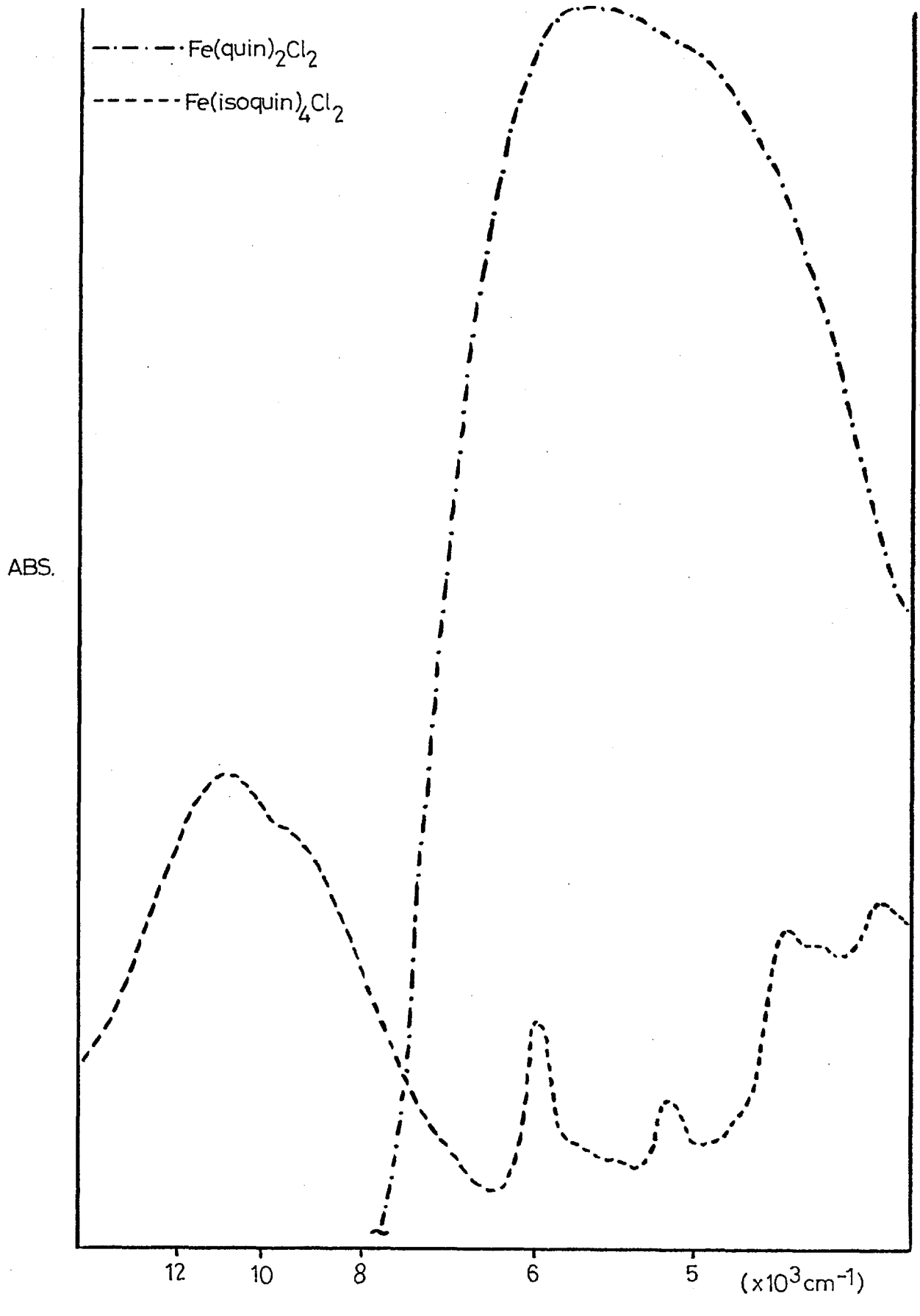
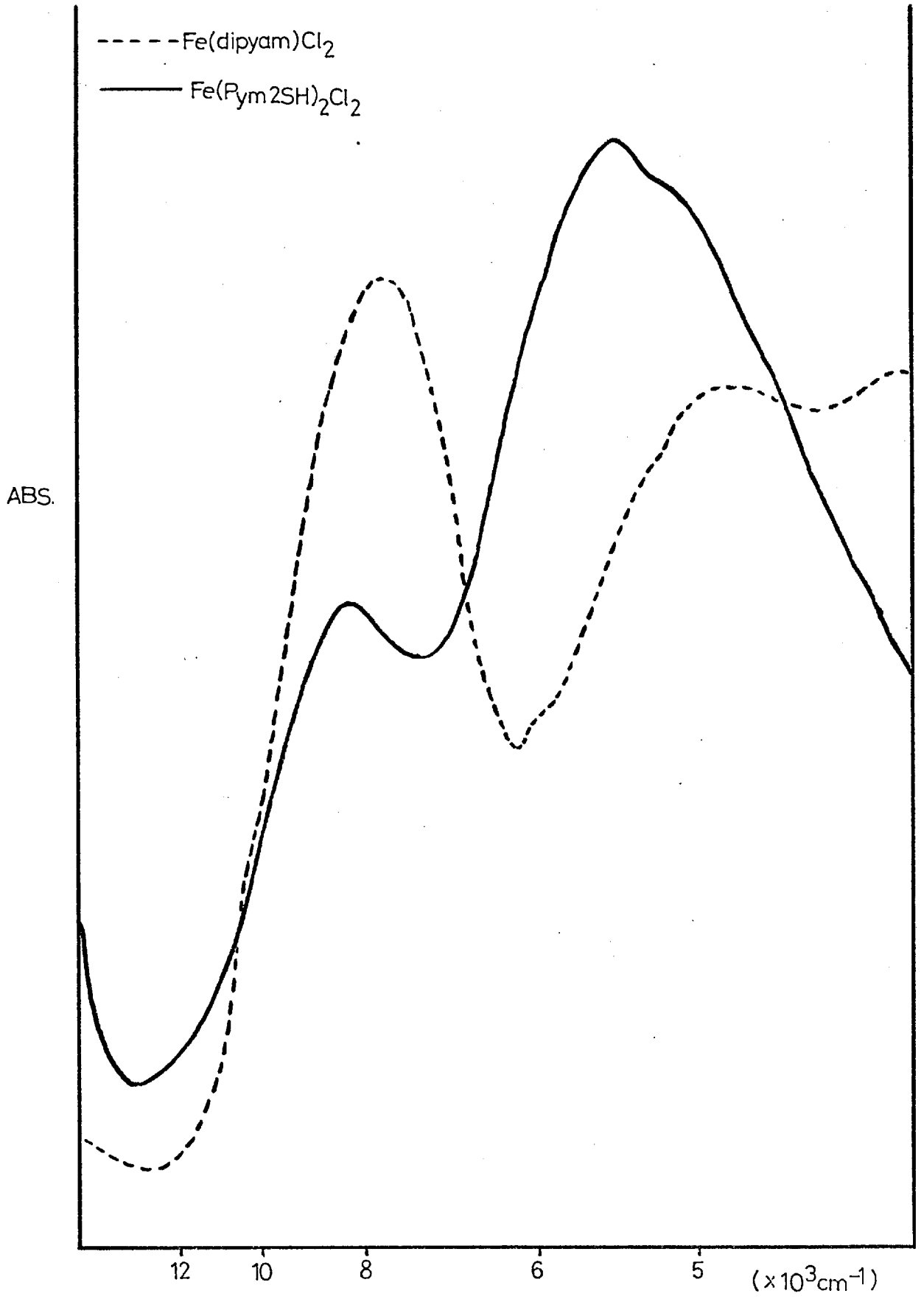


Fig. 4.4.



showing a more pronounced splitting of the two bands in the 9000 - 5000 cm^{-1} region. The spectra of other iron(II) compounds with known geometries are shown in Fig. 4.3. i.e. $Fe(isoquin)_4Cl_2$ [286] (D_4h symmetry) and $Fe(quin)_2Cl_2$ [287] (C_2v symmetry). These indicate that FeL_2Cl_2 has a greater similarity to a tetrahedral geometry. This is confirmed to some extent by comparing with the spectrum of the highly distorted tetrahedral complex $Fe(dipyam)Cl_2$ [288] (Fig. 4.4.).

Cobalt(II)

The magnetic moments of the green cobalt complexes CoL_2X_2 (X = Cl, Br) are 4.62 B.M. (X = Cl) and 4.75 B.M. (X = Br) which are in the range for typical tetrahedral cobalt(II).[289] Indeed these values are close to those reported [253] for the related tetrahedral $Co(Py2SH)_2X_2$ complexes in which the ligand, 2-pyridinethione, is thought to bind via sulphur only.

The reflectance spectra of both complexes are similar with a larger splitting of the two bands in the 11500 - 6000 cm^{-1} region for the bromide. Comparison of the spectra of CoL_2Cl_2 with those in Fig. 4.5. i.e. the compounds $Co(isoquin)_4Cl_2$ [290] (D_4h symmetry) and $Co(quin)_2Cl_2$ [291] (C_2v symmetry), show that the 2-pyrimidinethione complex has a coordination geometry closer to a tetrahedron than an octahedron. However, spectra resembling CoL_2Cl_2 have been reported [292] for some cobalt nitrate complexes (e.g. $Co[\ (C_6H_5)_3PO\ ]_2(NO_3)_2$, Fig. 4.6.). From the crystal structure determination of $Co[\ (CH_3)_3PO\ ]_2(NO_3)_2$,[293] it was shown that the metal atoms are surrounded by an irregular array of oxygen atoms with each nitrate group serving as a bidentate ligand. It is interesting to note that although this complex is highly distorted octahedral, the magnetic moments, like the complexes reported here,are more indicative of a tetrahedral geometry.

The spectrum of the inner complex $Co(Pym2S)_2$ is not well resolved

Fig. 4.5.

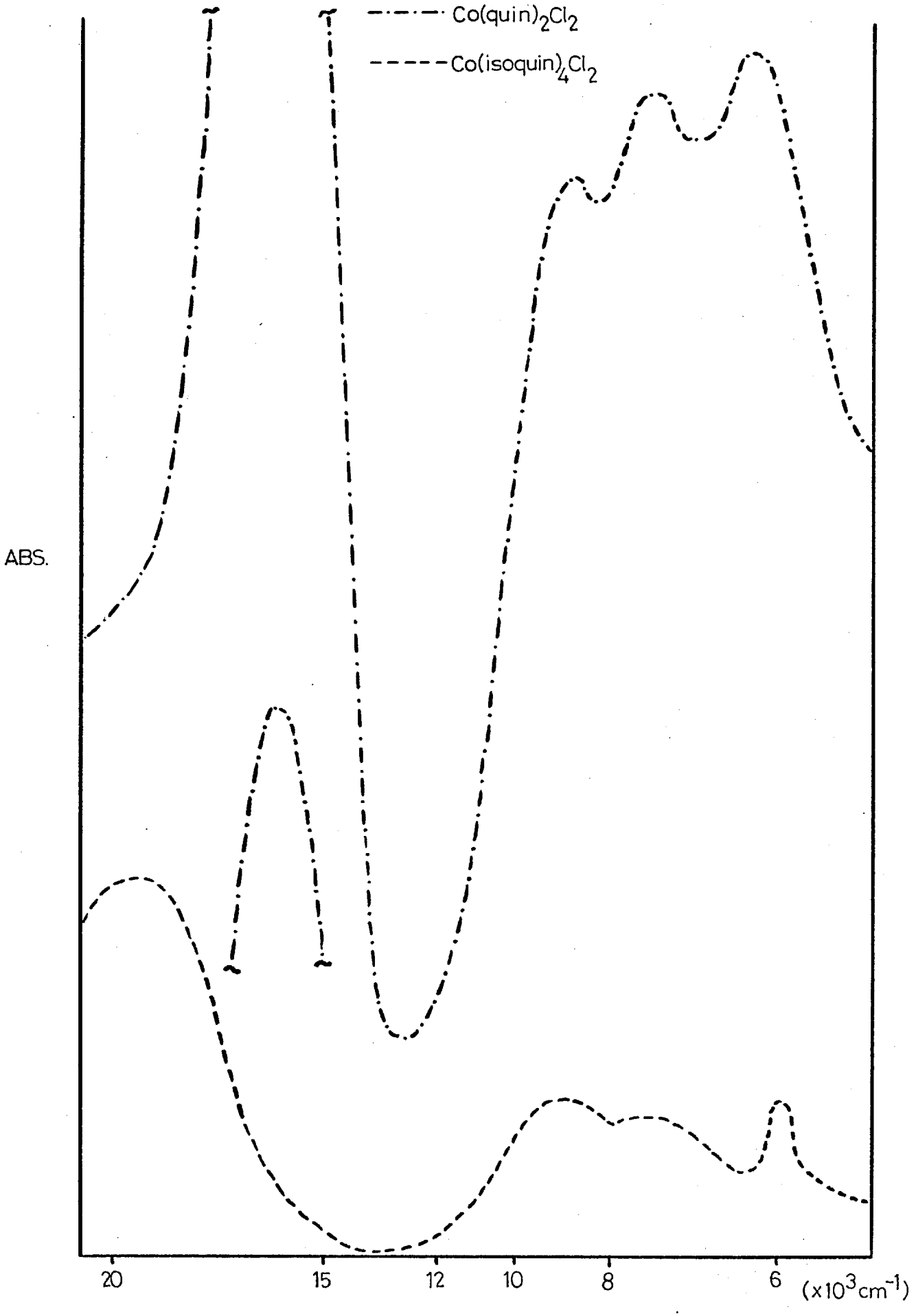
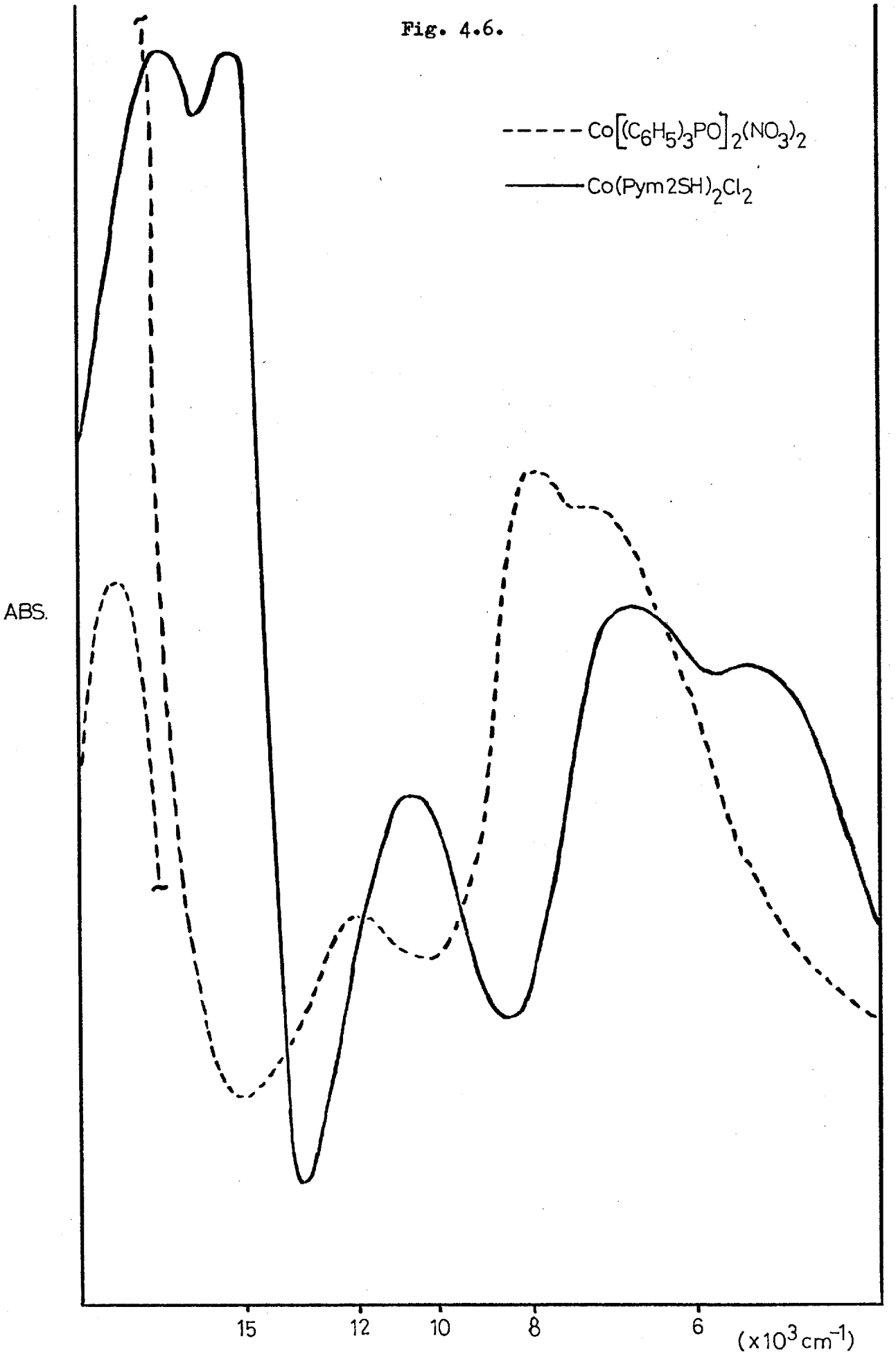


Fig. 4.6.



but shows bands in positions similar to those found in the spectrum of the compound bisbenzimidazolatocobalt(II).[294] It is reasonable to assume then that $Co(Pym2S)_2$ has a structure of an infinite polymer with the 2-pyrimidinethione anions functioning as bidentate ligands forming bridges between cobalt ions. The cobalt, therefore, may be present in a tetrahedral environment bound by nitrogen and sulphur donor atoms, but a structure involving sulphur bridges must also be considered.[295,296]

Nickel(II)

The magnetic moments of the nickel halide complexes NiL_2X_2 (X = Cl, Br) are consistent with octahedral coordination. These were found to be 3.21 B.M. (Cl) and 3.20 B.M. (Br).

As shown in Fig. 4.7., the electronic spectrum of NiL_2Cl_2 (Fig. 4.8.) has little in common with those of typical octahedral (e.g. $Ni(isoquin)_4Cl_2$, D_4h [297]) or tetrahedral (e.g. $Ni(quin)_2Cl_2$, C_2v [291]) nickel complexes. There is, however, a close resemblance to the spectrum of $Ni(2MeImid)_2Cl_2$ [298] (Fig. 4.8.), and hence the structures of the NiL_2X_2 compounds are probably polymeric octahedral with bridging chloride atoms. It is also significant that the magnetic moment of the 2-methylimidazole complex is virtually the same as NiL_2Cl_2 being 3.20 B.M.[298]

The spectrum of the iodide complex $Ni_5(Pym2S)_7I_2OH,6H_2O$ is complicated. Nevertheless, the major bands indicate an essentially octahedral environment around the nickel ion. Vibrational bands due to water are found at 7040 cm^{-1} (shoulder) and 5130 cm^{-1}. The spectrum of $Ni(Pym2S)_2$ is also difficult to interpret but again an octahedral geometry is thought to be the most likely structure.

On the whole, the data from the electronic spectra and magnetic measurements of the iron, cobalt and nickel complexes have shown that the

Fig. 4.7.

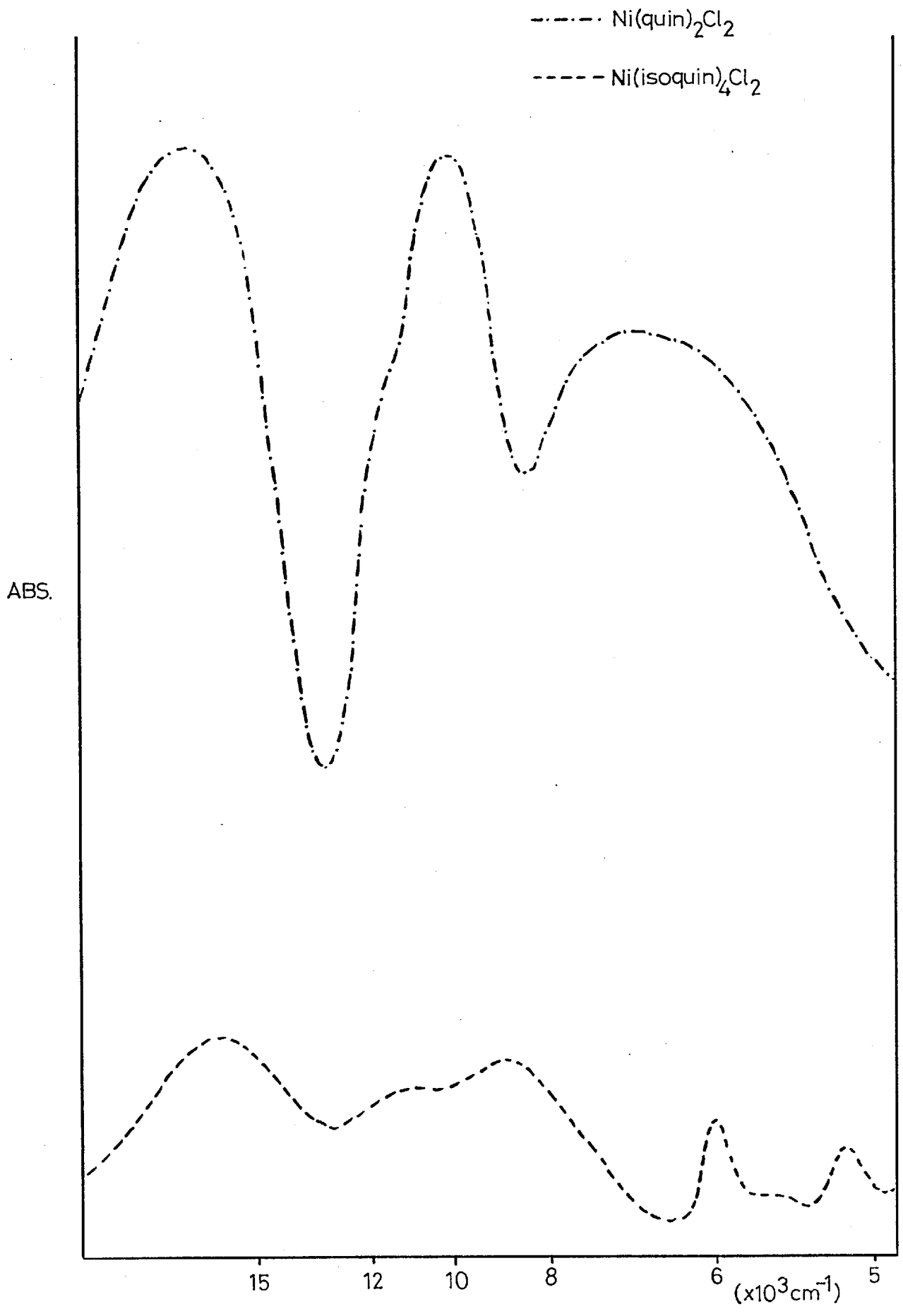
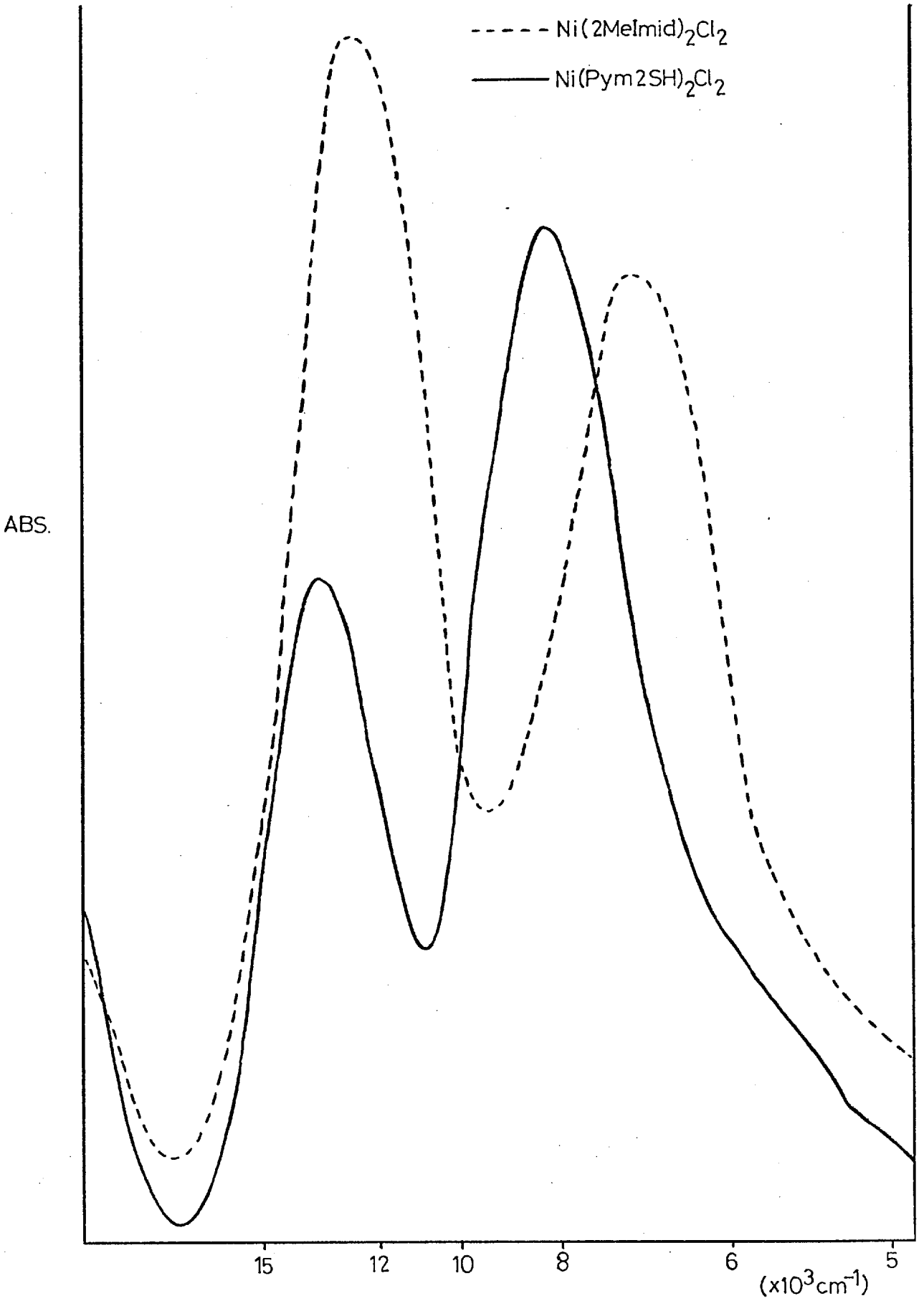


Fig. 4.8.



structures are highly distorted irrespective of the main coordination geometry from which they are derived.

To help explain the reason for such distortions, an X-ray structural determination was carried out on the complex $Co(Pym2SH)_2Cl_2$.

Structure of $Co(Pym2SH)_2Cl_2$ [299]

X-ray photographs of the green crystals gave the space group as C2/c with unit cell dimensions a = 1.2371(1), b = 0.8301(4), and c = 1.4851(1) nm, $\beta = 117.18(6)^o$, U = 1.3567 nm^3 and Z = 4.

The molecular structure of the complex is shown in Fig. 4.9. viewed down the diad axis. The coordination about the cobalt atom is distorted octahedral with the 2-pyrimidinethione ligands chelating strongly through nitrogen and weakly via sulphur. The nitrogen atoms are trans, with the pairs of chlorine and sulphur atoms cis. The important bond angles and bond lengths are given in Table 4.1.

The Co - S bond, however, is very long and the sulphur atom may be considered to be non-bonded. The structures in Fig. 4.10. show that this is not the case and a weak Co - S interaction does in fact exist. The S - C - N angle in 2-pyridinethione [272] is 119^o. If it is assumed that 2-pyrimidinethione is similar, then complexation decreases the angle to 116.2^o and indicates some sort of involvement of sulphur with the metal. The structure of $Ru(Py2S)_2(PPh_3)_2$ [257] shows the angles which can be obtained if a strong metal - sulphur bond is formed.

The molecular packing diagram (Fig. 4.11.) shows that the crowding is considerable and a hydrogen - bond is formed between a chlorine atom of one molecule and N3 of another. It is interesting to note that a proton is found 0.15 nm away from the nitrogen atom and almost midway along the bond. This, therefore, gives the reason why no $\nu(N - D)$ band is observed in the i.r. spectrum of the deuterated complex (Table 4.4.).

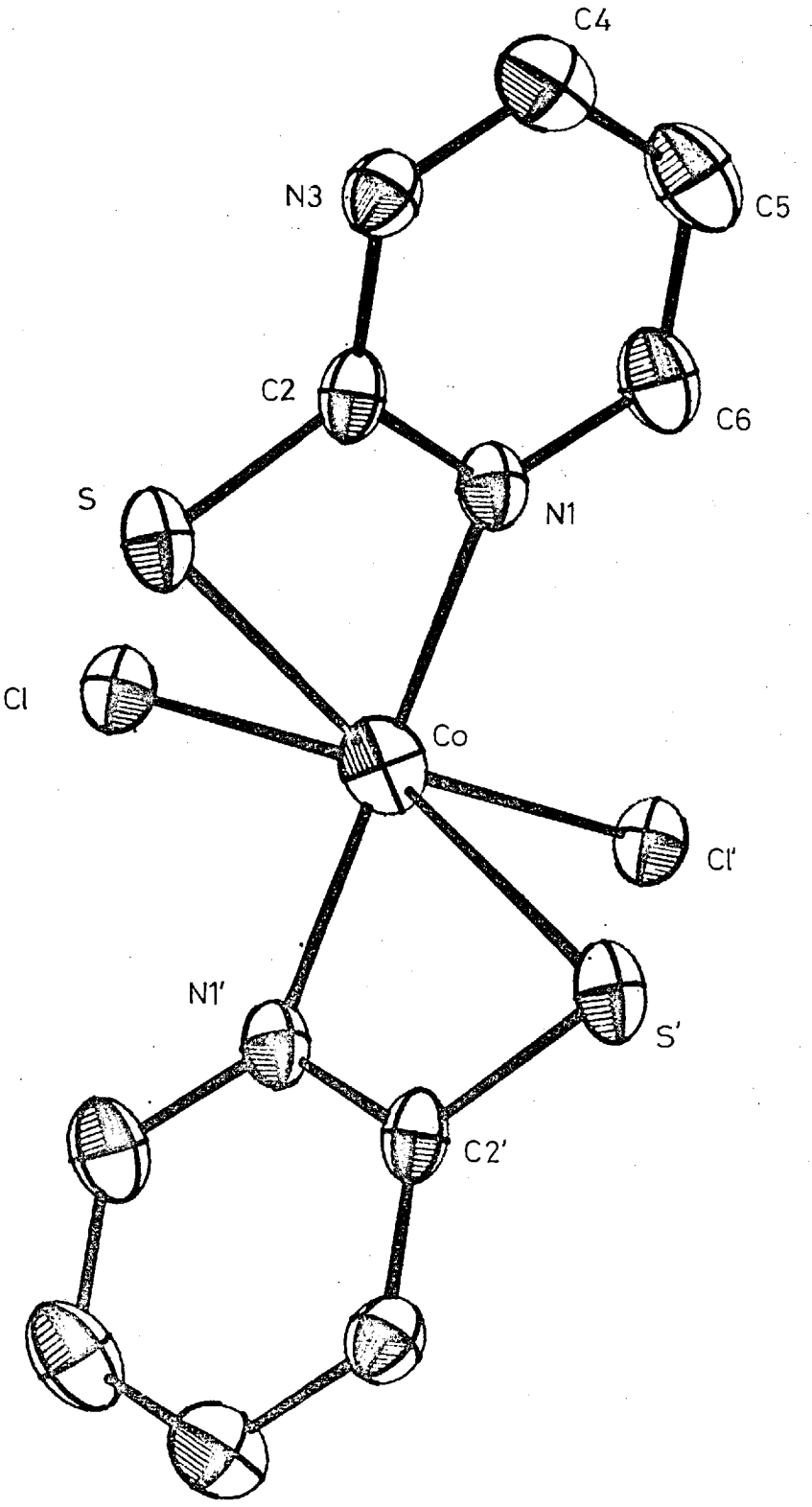


Fig. 4.9. The molecular structure of $Co(Pym2SH)_2Cl_2$.

Bond Angles

Cl - Co - Cl'	98.58(7)°
Cl - Co - S	89.36(5)°
Cl - Co - N1	105.26(14)°
Cl - Co - N1'	99.10(13)°
Cl - Co - S'	158.18(3)°
S - Co - S'	90.65(6)°
S - Co - N1'	93.39(14)°
S - Co - N1	59.12(13)°
N1 - Co - N1'	142.32(17)°
S - C2 - N1	116.2(3)°
Co - N1 - C2	111.8(3)°
C2 - S - Co	71.9(2)°

Bond Lengths

Co - Cl	0.2369(2) nm
Co - S	0.2960(2) nm
Co - N1	0.2098(4) nm
N1 - C2	0.1388(8) nm

Table 4.1. Selected bond angles and bond lengths of the $Co(Pym2SH)_2Cl_2$ structure with estimated standard deviations in parentheses.

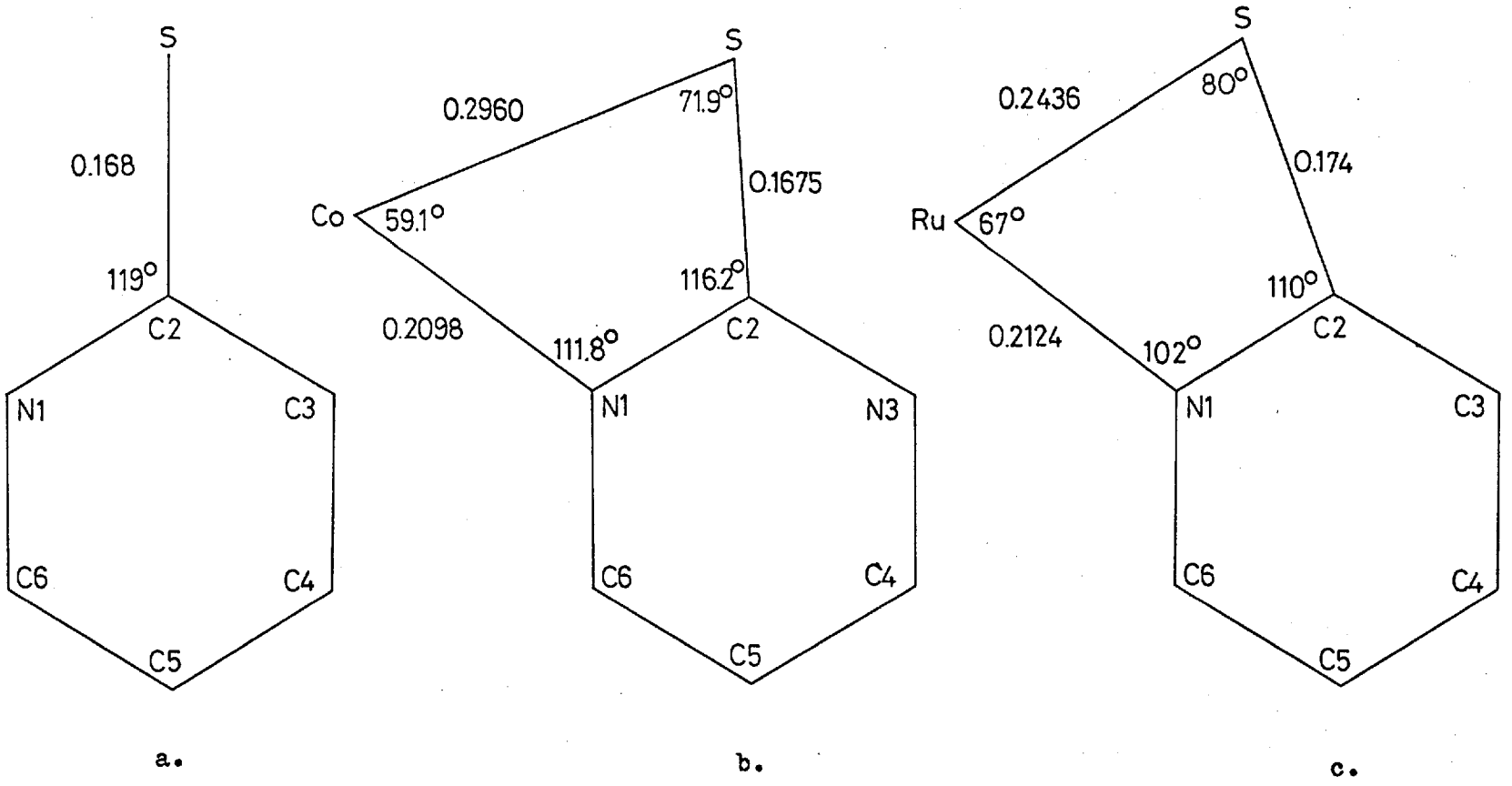


Fig. 4.10. Comparison of some important bond angles and bond lengths in a) 2-pyridinethione, b) $Co(Pym2SH)_2Cl_2$ and c) $Ru(Py2S)_2(PPh_3)_2$.

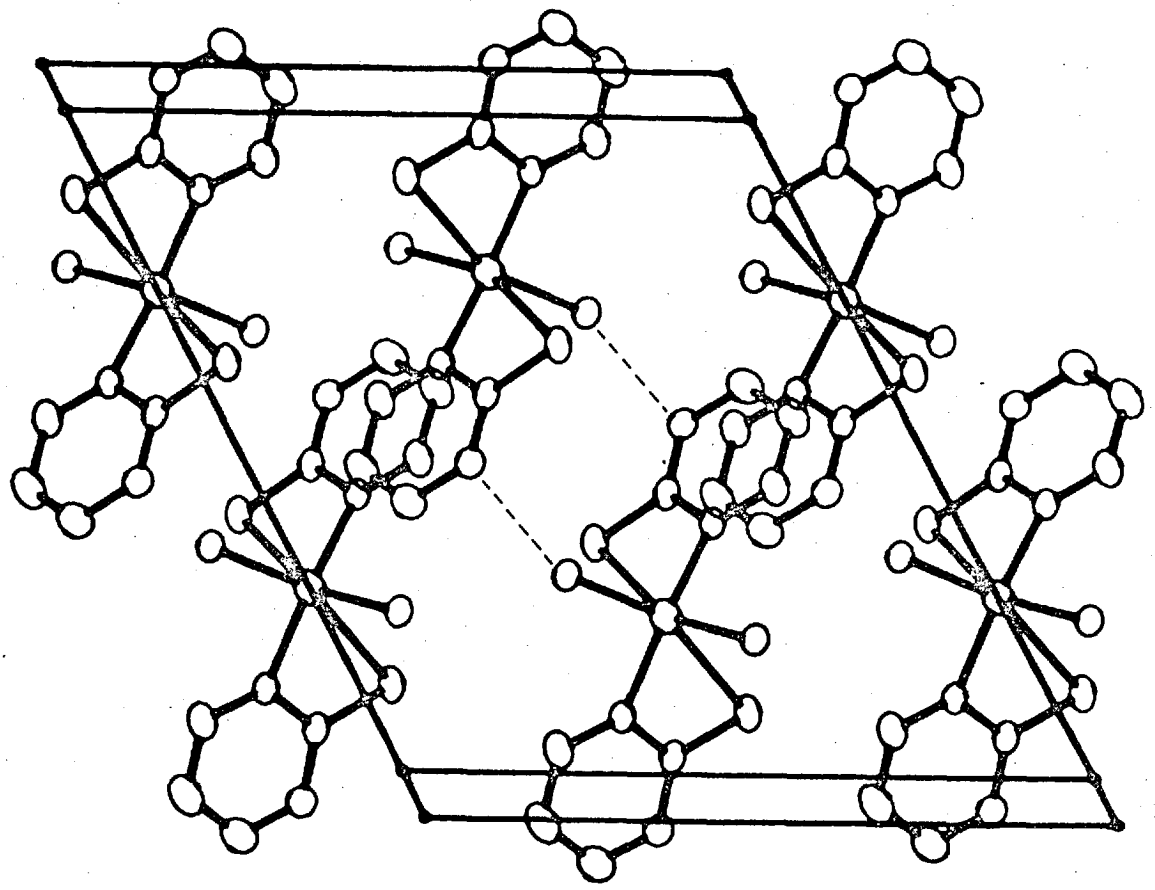


Fig. 4.11. A molecular packing diagram of $Co(Pym2SH)_2Cl_2$ showing hydrogen - bonding between N3 and Cl of different complex molecules.

It was previously shown that the electronic spectrum of $Co(Pym2SH)_2Cl_2$ was remarkably similar to $Co[\ (C_6H_5)_3PO\ ]_2(NO_3)_2$.[292] A comparison of the structures of the thione complex and $Co[\ (CH_3)_3PO\ ]_2(NO_3)_2$ [293] shows how closely related the geometries are. In $Co(Pym2SH)_2Cl_2$ the angles N - Co - S (59.1°) and Cl - Co - Cl' (98.6°) correspond quite well to those of the bidentate nitrate and cobalt (57.2°) and the angle made by the phosphine oxides (106.1°) in $Co[\ (CH_3)_3PO\ ]_2(NO_3)_2$. This may imply that this type of distorted stereochemistry may not be so uncommon in cobalt complexes containing halides (or pseudohalides) and chelating ligands.

The results of other physical techniques will now be discussed in the light of the structural details found for $Co(Pym2SH)_2Cl_2$.

Infrared Spectra (4000 - 650 cm^{-1})(Table 4.7.)

The determination of metal - sulphur coordination using i.r. spectroscopy has often been attempted by observing the shift in the frequency of the band assigned to the 'ν(C = S)' vibration. If the complex shows a shift to lower frequency, sulphur bonding is indicated; if a shift to higher frequency is observed, then coordination to some other part of the molecule must be present.[300-302] The danger in using this technique for definitive information is now exemplified from the results of the $Co(Pym2SH)_2Cl_2$ spectrum.

Comparison with the spectrum of Pym2OH shows that the 'ν(C = S)' band of Pym2SH occurs at 1186 cm^{-1}. On forming the complex CoL_2Cl_2, this vibration shifts to the higher frequency of 1199 cm^{-1} even though the molecule contains sulphur coordination. This behaviour is attributed to the electronic structure of the ligand.

In theory, a shift to higher frequency indicates that the bond order (B.O.) of C = S has apparently increased. Consequently, this implies that in the ligand itself, the B.O. is less than 2. If the actual structure is a zwitterion, as proposed (Fig. 4.2.b.), then complexing of cobalt via the nitrogen and sulphur atoms would give a less aromatic ring which will increase the bond order of C = S and the frequency of its stretching vibration.

Similar shifts in the 'ν(C = S)' band were observed for all of the other complexes except CdL_2I_2 and those of nickel. These compounds gave very small shifts and suggest that either their structures or coordination modes are very different from CoL_2Cl_2. Also, NiL_2X_2 (X = Cl, Br) and CdL_2I_2 were the only compounds to exhibit the N - H stretching vibration (Table 4.7.). Deuteration studies confirmed its presence from the observation of ν(N - D) in $Ni(Pym2SD)_2Cl_2$. This work also showed that an N - H bond did not exist in the complex CoL_2Cl_2 (Table 4.4.).

The conclusions from this infrared study are twofold. Firstly, it emphasises that this is not a good technique for determining metal - ligand coordination, with this type of ligand, as many of the vibrations are not pure. Secondly, the nickel compounds and CdL_2I_2 appear to be different from the other halide complexes.

Far Infrared Spectra (Table 4.8.)

It is well known that because the coupling of vibrations occur in a molecule, few, if any, bands in an infrared spectrum can be regarded as 'pure'.[303-305] The title 'metal - halogen' (M - X) or 'metal - ligand' (M - O, M - N, or M - S) stretch, as used here, should be taken to infer that a particular absorption band arises primarily, but not necessarily totally, from such a normal coordination.

As expected for a complex with C_{2v} symmetry,[304] two infrared active ν(M - Cl) vibrations are found at 243 and 232 cm^{-1} for CoL_2Cl_2. The corresponding bromide exhibits only one ν(M - Br) band at ca. 188 cm^{-1}. These values are consistent with the distorted octahedral structure shown in Fig. 4.9. The medium intensity band found at 258 cm^{-1} in the chloride was fairly insensitive to a change in halogen and was present at 252 cm^{-1} in the bromide. This can be assigned to a metal - ligand band of which the donor atom is either nitrogen or sulphur.

The structure of CoL_2Cl_2 shows the Co - N distance to be normal but Co - S long i.e. 0.2960 nm. Typical Co - S bond lengths are exhibited by the distorted tetrahedral complex $Co(1,3Me_2Imid2S)_2Br_2$ at 0.2319 and 0.2349 nm.[306] Although C_{2v} cobalt complexes show a metal - sulphur stretching vibration in the 258 cm^{-1} region,[307] clearly the weakness of the Co - S bond in CoL_2Cl_2 must lower the frequency of ν(Co - S) considerably. An unambiguous assignment of ν(Co - S) was, therefore, not possible because of the complexity of the spectra below ca. 180 cm^{-1} with

the likely presence of bending and lattice modes in this region.

The positions of the ν(M - X) and ν(M - L) bands in the other complexes infer that MnL_2X_2, FeL_2X_2 (X = Cl, Br) and ZnL_2Cl_2 have stereochemistries rather similar to those of CoL_2X_2. However, the spectra of the compounds NiL_2X_2 (X = Cl, Br) are slightly different as the ν(Ni - X) bands are lower than expected. As indicated by electronic spectra, this implies that the nickel complexes may have an octahedral structure with bridging halogen atoms. The cadmium and inner complexes gave spectra that were complicated and difficult to interpret reliably.

The ligand vibrations were also studied. Ligand band I did not alter its intensity a great deal and tended to move to lower frequencies on complexation. The ligand II band, however, behaved differently. In uncomplexed 2-pyrimidinethione, the band was at 353 cm^{-1} and found to be very weak in the i.r. but strong in the Raman. When the ligand is complexed this vibration is i.r. activated by all except CdL_2I_2 and the cobalt and inner complexes. As the intensity of the band decreases on going from chloride to bromide, it appears that this vibration is related to the mode of coordination. If so, then the cobalt halide complexes must be different from the rest, despite previous infrared evidence.

X-Ray Powder Data

Comparison of the X-ray powder photographs suggested that none of the complexes were isostructural although certain similarities were found. The cobalt chloride complex appeared to have some resemblance to ZnL_2Cl_2 but no other metal, whereas the halide complexes of manganese, iron and nickel were alike. The only other information obtained was that the chloride and bromide cadmium compounds had the same type of structure.

Summary and Conclusions

The structure of $Co(Pym2SH)_2Cl_2$ was determined and found to have a distorted octahedral geometry with the ligand chelating strongly via nitrogen but weakly via sulphur. The physical measurements of this complex were recorded and then compared with those obtained for the other halogen compounds. Although the normal infrared data were similar for all of the chloride and bromide complexes except nickel, the far infrared spectra and X-ray powder patterns indicate that the structure is only typical of the cobalt halides and ZnL_2Cl_2.

However, the implications drawn from the physical data of the remaining compounds are also contradictory. Therefore, it would seem that no definite conclusions can be made as to their structures. Nevertheless, it is likely that the chloride and bromide complexes of manganese, iron, zinc and cadmium have geometries more closely related to those of cobalt than nickel, which is suggested to be octahedral with bridging halogen atoms. All of the inner complexes are probably polymeric with nickel being octahedral and cobalt and zinc tetrahedral.

4.2.2. Complexes of 2-Pyrimidinone

Metal complexes of 1:1, 2:1, 4:1 and 5:1 stoichiometry were obtained with this ligand. These include $M(Pym2OH)X_2$ (M = Fe, Ni, Cd, X = Cl; M = Cu, X = Br), $M(Pym2OH)_2X_2$ (M = Mn, X = Cl; M = Co, Cu, Zn, X = Cl, Br; M = Cd, X = Br, I), $M(Pym2OH)_2Br_2,2H_2O$ (M = Mn, Ni), $M(Pym2O)_2$ (M = Co, Ni), $M(Pym2OH)_4X_2$ (M = Co, X = Cl, Br; M = Ni, X = I; M = Zn, X = NO_3), $Cu(Pym2OH)_4(ClO_4)_2,S$ (S = EtOH, 1-PrOH) and $Co(Pym2OH)_5X_2$ (X = I, ClO_4).

The discussion of these complexes are in the order of ascending atomic number except for manganese(II) which is placed after copper(II). The results of physical measurements are given in Tables 4.9. and 4.10.

Iron(II)

The only complex formed with satisfactory analysis was the yellow $FeLCl_2$. Its near infrared spectrum shows two clearly resolved bands at 9520 and ca. 6560 cm^{-1} which resemble those obtained for the compounds $Fepy_2Cl_2$ and $Fe(3,5Cl_2py)_2Cl_2$.[286] These 2:1 complexes are known to have a polymeric octahedral structure with bridging chlorine atoms and pyridine molecules in the trans positions. Because the presence of an OH group is indicated in the normal i.r. region, a similar arrangement to the above is possible for the 2-pyrimidinone complex. The only difference is that the ligand molecules, now with two available nitrogen donors, can bridge as well as the chlorine atoms. This behaviour is also found in the 1:1 compound $Fe(5MePym)Cl_2$.[308]

Cobalt(II)

Using various ratios of ligand to metal halides, the complexes CoL_2X_2, CoL_4X_2 (X = Cl, Br) and CoL_6I_2 were prepared. The 2:1 compounds had two forms i.e. blue [A] and purple [B], however, the compound CoL_2Br_2 [B] could not be made easily as it readily converts to the blue form in the reaction solution. Because of this, only the chloride was studied. The compound $CoL_5(ClO_4)_2$ and the inner complex $Co(Pym2O)_2$ were also made.

The electronic spectra of the blue CoL_2X_2 complexes were typical of tetrahedral C_{2v} stereochemistry[291] except that the bromide exhibited slight band splitting, indicating that the oxygen at C2 may be closer to the metal than in the chloride. However, there is no interaction of O2 like the sulphur atom in the analagous $Co(Pym2SH)_2X_2$ complexes. In the far i.r. spectra, both Co - X stretch vibrations expected for a tetrahedral molecule were observed[304] i.e. 310 and 288 cm^{-1} (X = Cl) and 254 and 238 cm^{-1} (X = Br). The band at 241 cm^{-1} for CoL_2Cl_2 [A] is regarded as the metal - nitrogen stretch vibration[309] although this mode is obs-

cured in the bromide.

Because no definite interaction was indicated in these complexes, it was hoped that a crystal structure determination could be carried out for comparison with $Co(Pym2SH)_2Cl_2$. However, only small crystals could be obtained initially as slower reactions resulted in the formation of the purple compound [B]. The blue bromide complex did not appear to be crystalline at all.

The reflectance spectra of the other halides and $CoL_5(ClO_4)_2$ showed a remarkable similarity, despite the differences in stoichiometry. All of the compounds possess two major bands at ca. 18500 and 9000 cm^{-1} except CoL_2Cl_2 [B] which shows two extra bands attributed to $CoCl_4^{2-}$ (Fig. 4.12.). This compound can be formulated as $[CoL_4][CoCl_4]$. These two components are shown from the combination of the spectra of $CoL_5(ClO_4)_2$ and $(Bu_4N)_2CoCl_4$ (Fig. 4.13.).

The far i.r. spectra of these compounds are also rather similar. No metal - halogen stretch vibrations were found for any complex except CoL_2Cl_2 [B]. This exhibited a single strong band at 310 cm^{-1} due to $CoCl_4^{2-}$.[310] It can be considered that the halogen atoms in the other complexes are either binding very weakly or not at all. The metal - ligand vibrations of all the cobalt complexes were fairly consistent, having similar bands in the 275 - 255 cm^{-1} region. These bands are assigned to ν(Co - N) and correspond to the complexes having low symmetry.

Because the electronic and far i.r. spectra are similar for the 4:1, 5:1 and CoL_2Cl_2 [B] compounds, the arrangement of ligands around the cobalt ion must also be similar. If it is assumed that all of the 2-pyrimidinone molecules coordinate to the metal, the general structure must be five - coordinate with one long cobalt - ligand bond. This ligand would be 2-pyrimidinone in the 5:1 complexes and halogen in the 4:1 compounds and CoL_2Cl_2 [B]. On the other hand, if the halogen atoms are

Fig. 4.12.

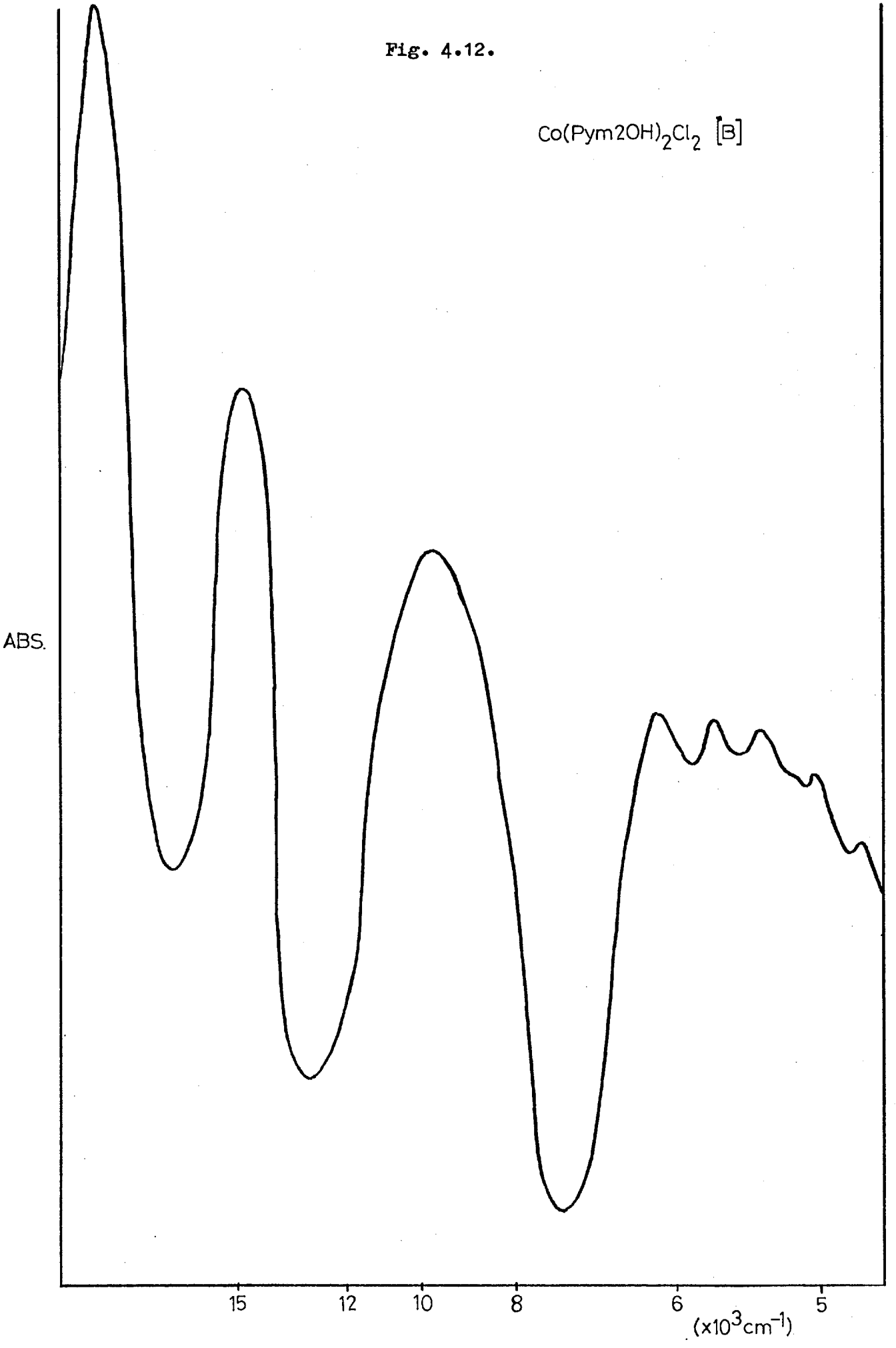
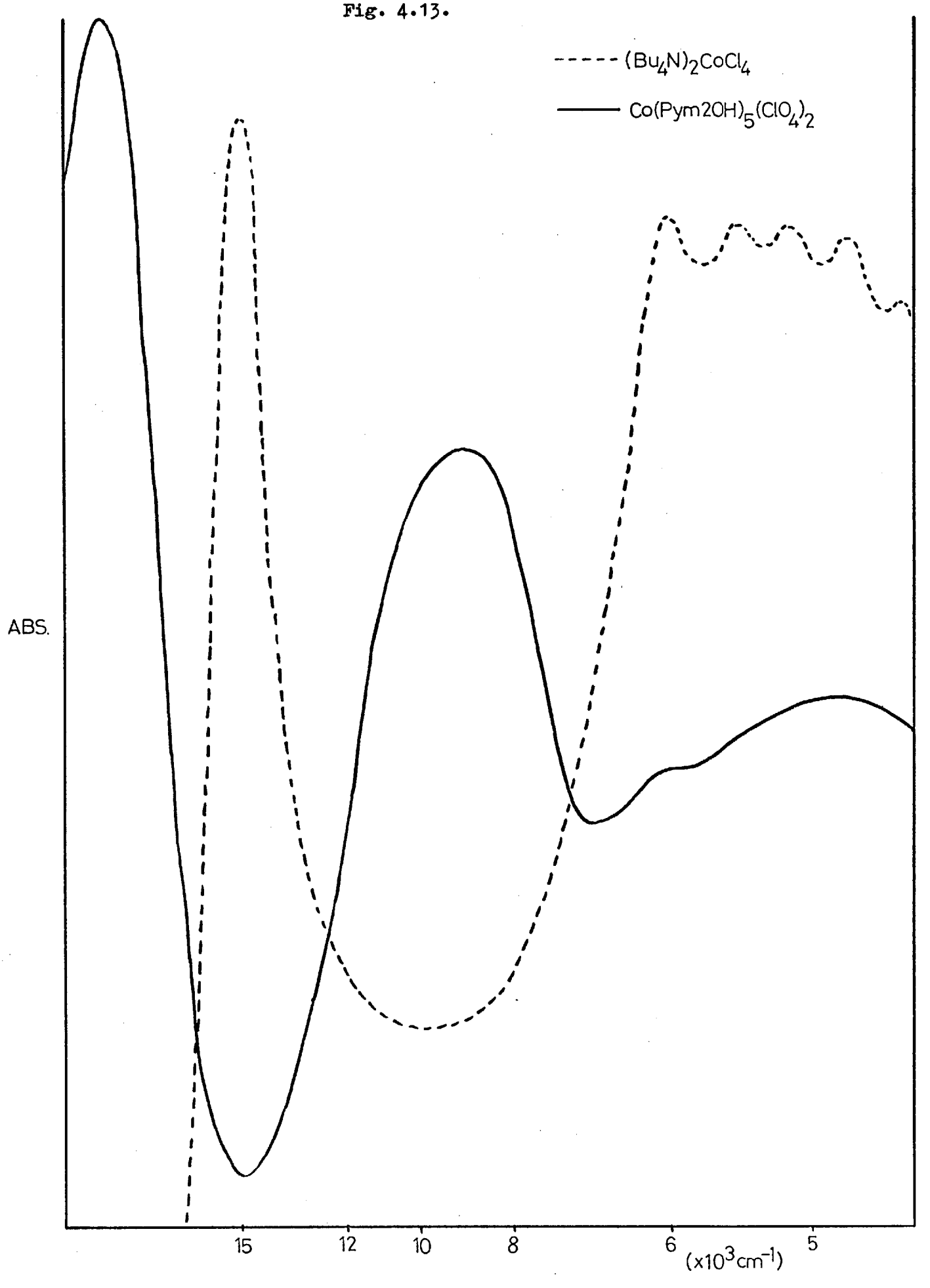


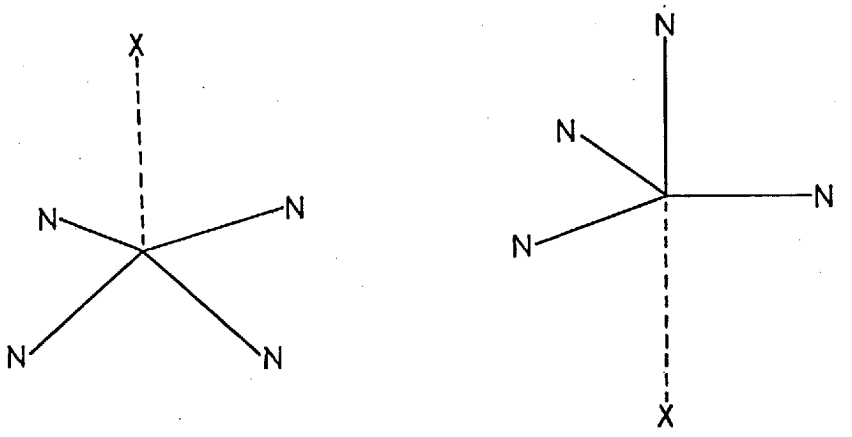
Fig. 4.13.



completely ionic, a four - coordinate structure results. There would then be a non-coordinating ligand in the 5:1 compounds similar to that found in some iron(II) - pyridine complexes.[311] The relative merits of these geometries are now examined. The five - coordinate structural possibilities are discussed first.

The chemistry of pentacoordinate cobalt complexes has been well reviewed in recent years.[312-314] Five equivalent donor atoms can be symmetrically arranged around a metal ion to form either a square based pyramidal (C_4v) or a trigonal - bipyramidal (D_3h) structure. It should be noted that these can be interconverted by means of simple angular distortions. For compounds of the type $[CoL_4X]^{n+}$, where X is a non-equivalent ligand, the Co - X bond distance is significantly longer than those of Co - L and the difference has been shown to be as much as 30% longer . The donor atom in an elongated metal - ligand bond can be considered as partially coordinated to the metal so that the effective coordination number is somewhere between four and five. This structure would certainly explain the far i.r. results of the 2-pyrimidinone complexes as it can be expected that weak Co - X bonds would give no obvious stretching vibrations in the 450 - 200 cm^{-1} region.

Compounds which exhibit this type of behaviour include the square - pyramidal $[Co(tet)Br]Br$[315,316] (Fig. 4.14.a.) and trigonal - bipyramidal $[Co(Me_6tren)Br]Br$[317,318] and $[Co(trpn)Br]Br$[319,320] (Fig. 4.14.b.). Both of these structures have long axial Co - Br bonds. The electronic spectra of the complexes showing the two geometries were recorded and compared with that of CoL_4Br_2 (Fig. 4.15.). No similarity could be found. As the fifth cobalt - ligand bond gets longer, due to steric hindrance, the structures progressively approach four - coordination. The square - pyramidal structure becomes square - planar whereas trigonal - bipyramidal changes to tetrahedral. Of these, the latter is the most common stereo-



a. b.

Fig. 4.14. The basic structures of $[CoN_4X]^{n+}$ complexes, a) square-pyramidal and b) trigonal - bipyramidal.

chemistry for cobalt complexes.

An example of a complex containing a 'tripod' ligand with no competing fifth atom is given by $[Co(Me_6tpt)](BPh_4)_2$.[317] This is related to the trigonal - bipyramidal structure in that the original equatorial nitrogen atoms move to the coordination positions of a tetrahedron. Although the stereochemistry is not perfect, the electronic spectrum (Fig. 4.16.) does begin to show some resemblance to the cobalt - 2-pyrimidinone complexes. A more regular tetrahedron has been reported for a complex incorporating 2-methylimidazole, which appears sterically related to 2-substituted pyrimidines (section 4.2.). The compound $Co(2MeImid)_4I_2$ [298] gives a spectrum remarkably similar to that of $CoL_5(ClO_4)_2$ (Fig. 4.16.). However, the same kind of spectrum is also shown by the complex $Co(2MeImid)_4(NO_3)_2$ [B] [298] (Fig. 4.16.).

The structure of this nitrate is described as a very distorted octahedron with a bidentate nitrate group and four donor nitrogen atoms from the 2-methylimidazole ligands.[321] It has been suggested by various

Fig. 4.15.

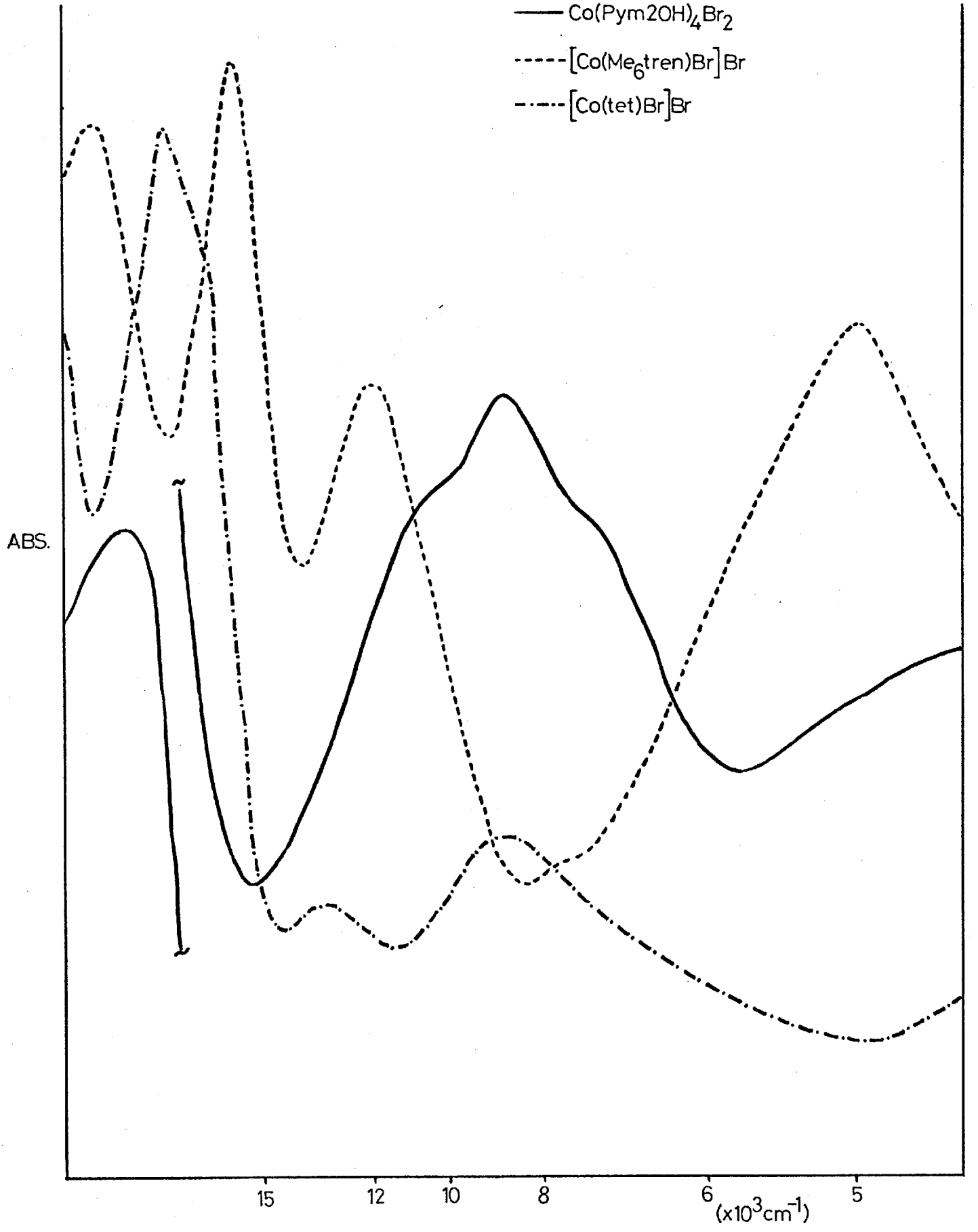
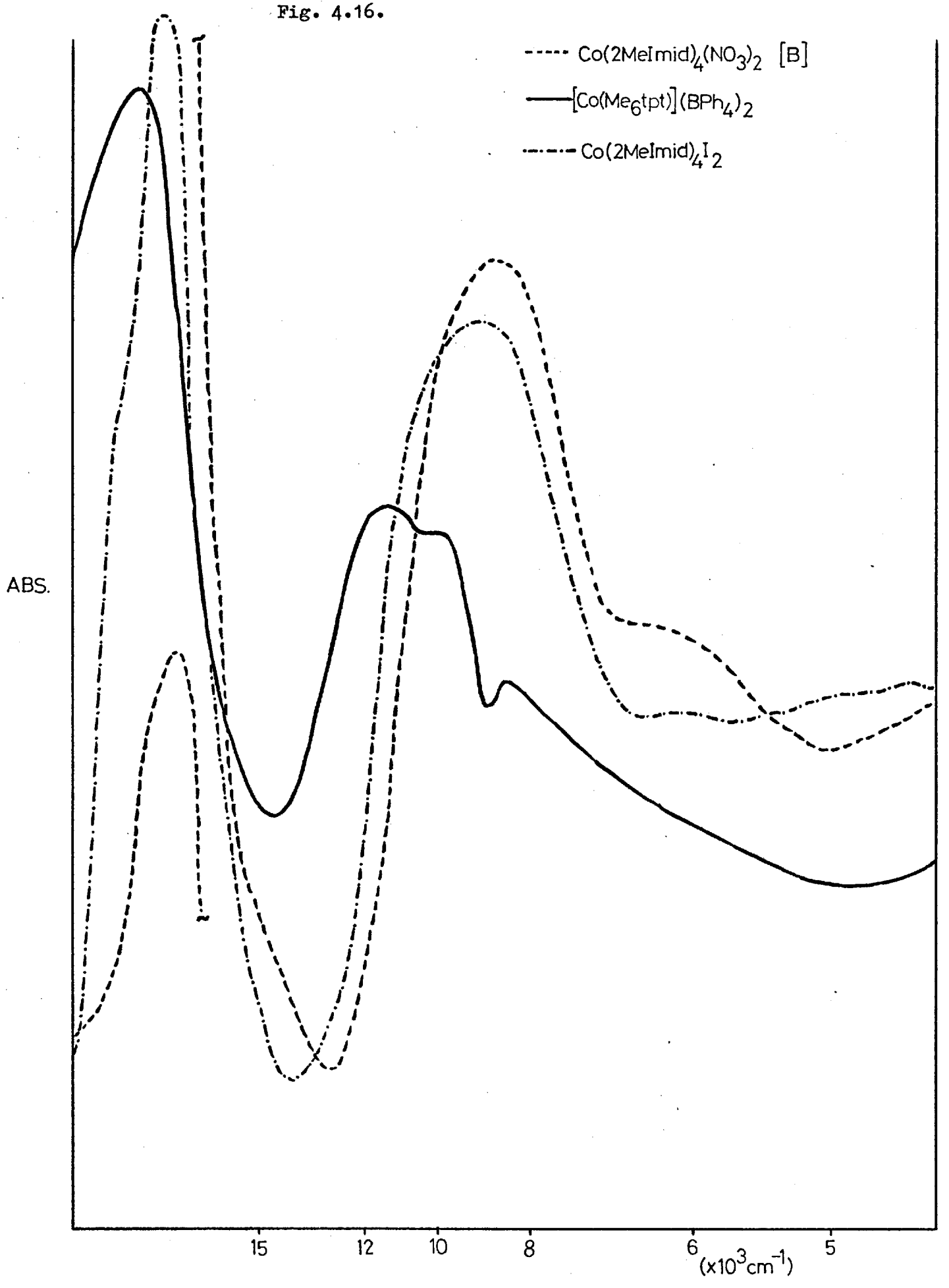

Co(Pym2OH)4Br2
[Co(Me6tren)Br]Br
[Co(tet)Br]Br
ABS.
15
12
10
8
6
5
(x10³cm⁻¹)

Fig. 4.16.


Co(2MeImid)4(NO3)2 [B]
[Co(Me6tpt)](BPh4)2
Co(2MeImid)4I2
ABS.
15
12
10
8
6
5
(x10³cm⁻¹)

workers[292,322-325] that in some cases a bidentate group may be considered as occupying one coordination site about a metal ion. Such a concept has its limitations, but the assumption of a pseudo-trigonal - bipyramidal geometry for $[Co(2MeImid)_4(NO_3)]^+$ (with NO_3 in the equatorial position) correlates well with the observed spectrum.

It can be concluded that the complexes under investigation may be either four or five - coordinate. In the former case, little change in the electronic spectra of the various compounds would be observed as the 2:1 and 4:1 complexes would possess ionic halide atoms (and $CoCl_4^{2-}$), and 5:1 compounds, a non-coordinating ligand.

If the complexes are five - coordinate one may expect the chromophore $[CoL_4X]^{n+}$ to exist with X = Cl, Br, $CoCl_4^{2-}$ or a non-equivalent ligand molecule. As the Co - X bond is usually significantly longer than the others, again the ligand field would not be altered to any great extent. Like the nitrate group in $Co(2MeImid)_4(NO_3)_2$ [B], the Co - X bond would probably be equatorial.

Because of the difficulty in interpreting the results of the physical measurements absolutely, a crystal structure determination is now being carried out on the complex CoL_4Br_2.

The electronic and far i.r. spectra of the inner complex $Co(Pym2O)_2$ showed certain similarities to the 5:1 iodide and perchlorate compounds. However, this probably has a polymeric tetrahedral structure with N and O coordination.

Nickel(II)

The complexes of the nickel halides $NiLCl_2$, $NiL_2Br_2,2H_2O$ and NiL_4I_2 and the inner complex $Ni(Pym2O)_2$ were prepared.

The electronic spectrum of the chloride was found to resemble that

of $NipyCl_2$ which has a distorted octahedral structure with the metal ions surrounded by five chlorines (all bridging) and one nitrogen.[326] This is the most likely structure for $NiLCl_2$. The spectrum of $NiL_2Br_2,2H_2O$ again shows an octahedral - type structure indicative of four strong and two weak bonds.[297] It is probable that the bromide atoms are situated in the trans position with elongated bonds. The band associated with the presence of water is found at 4940 cm^{-1}. The far i.r. spectrum of this compound (450 - 200 cm^{-1}) does not show the ν(Ni - Br) band but confirms the fact that water is coordinated to the metal with a band at 360 cm^{-1} assigned to ν(Ni - H_2O). The metal - nitrogen stretch vibration is found at 263 cm^{-1}.

The reflectance spectra of NiL_4I_2 and $Ni(Pym2O)_2$ also show octahedral geometry. The iodide exhibits a ligand field stronger than $Ni(H_2O)_6^{2+}$ but weaker than $Ni(NH_3)_6^{2+}$ and may be indicative of a pseudo-octahedral structure similar to that found in $CuL_4(ClO_4)_2,EtOH$ (vide infra). The iodine atoms are therefore non-coordinated. The inner complex, however, has a weak field and probably has an octahedral polymer structure with both nitrogen and oxygen atoms coordinating.

Copper(II)

The reaction of 2-pyrimidinone with $CuCl_2,6H_2O$ gave two complexes of 2:1 stoichiometry. The electronic spectra showed intense bands at 16390 cm^{-1} with a shoulder at 10100 cm^{-1} for the blue compound [A] and 16670 and 10000 cm^{-1} for the turquoise isomer [B]. These spectra suggest a tetragonally distorted octahedral structure.[308] In the far i.r. region, bands at ca. 300 cm^{-1} and 282 - 254 cm^{-1} were assigned to ν(Cu - Cl) and ν(Cu - N) respectively. These values are consistent with the proposed geometry containing four short and two long bonds,[304,327] but with non-bridging halogens.

The compound $Cu(Cyt)_2Cl_2$ has been shown to have similar physical properties to the 2-pyrimidinone complexes.[143] This confirms that the structures of the 2:1 complexes are distorted octahedral with weak Cu - O2 bonds.[142] The reason for the formation of two isomers is not clear but is probably due to the variation in the amount of metal - oxygen interaction. This type of coordination is discussed later.

With copper bromide, both 1:1 and 2:1 complexes were formed. The electronic spectra showed intense bands at ca. 16950 cm^{-1} for CuL_2Br_2 and 14810 cm^{-1} for $CuLBr_2$ but no positive information as to their structures could be obtained. The far i.r. spectrum of $CuLBr_2$ gave no metal - bromine stretch vibration >200 cm^{-1}, only a band at 282 cm^{-1} corresponding to ν(Cu - N). The 2:1 complex exhibited a band at 233 cm^{-1} due to ν(Cu - Br) which is in the same region as a distorted octahedral structure.[327] The metal - nitrogen stretch vibrations are found at 280 and 254 cm^{-1}.

The normal i.r. spectra indicate the presence of OH in both complexes implying the likelihood of the compounds being polymeric with bridging ligands. For the 1:1 complex, the structure is probably similar to that of $Fe(Pym2OH)Cl_2$ with both the 2-pyrimidinone molecule and halogen atoms acting as bridges. The compound CuL_2Br_2, however, is not quite so simple. The e.p.r. spectrum was recorded[234] and showed that the structure is not a polymer in the accepted sense, but the metal atoms do appear to be in different coordination sites.

The formation of a perchlorate complex from ethanol and 1-propanol gave compounds of the formula $CuL_4(ClO_4)_2,S$ where S = EtOH or 1-PrOH. The reflectance spectra of these two lilac compounds were virtually identical, with a single medium intensity band at 18690 cm^{-1}. This is indicative of the compounds having four nitrogen atoms in a square - coplanar arrangement similar to $Cu(NH_3)_4^{2+}$.[227] The far i.r. spectra exhibited strong bands at ca. 280 cm^{-1}, typical of the metal - nitrogen

stretching vibrations found in other $CuL_4(ClO_4)_2$ complexes.[328]

Although a square - planar arrangement is suggested, recent work on copper complexes of the related cytosine ligand has shown that weak metal - oxygen (O2) binding is present in the structures.[142,168] To find out if this type of interaction occurs in these 2-pyrimidinone complexes, a crystal structure determination was carried out on $Cu(Pym2OH)_4(ClO_4)_2$,EtOH.

Structure of $Cu(Pym2OH)_4(ClO_4)_2$,EtOH [329]

Preliminary oscillation and Weissenberg photographs showed the crystals to be orthorhombic and gave the space group as Aba2 and approximately 42m symmetry. Unit cell dimensions were found to be a = 2.0022(2), b = 1.2263(1) and c = 1.1073(1) nm, U = 2.7188 nm^3 and Z = 4.

The structure (Fig. 4.17.) is best described as being square - planar with the copper ion bound to the tertiary nitrogen atoms of four pyrimidine rings. The planes of these rings are arranged normal to the copper - nitrogen plane causing the keto oxygen atoms, which are very nearly in the plane of the rings, to reside above and below the CuN_4 plane effectively blocking the axial positions of the copper. The ethanol molecule, however, is not coordinated to the metal atom but is kept in the lattice through hydrogen - bonding. The important bond angles and bond lengths are given in Table 4.2.

It has been stated that copper compounds of this type rarely attain pure square - coplanar geometry due to weak interactions with groups in the axial positions. These binding effects have given rise to the introduction of the term 'semi-coordination' which suggests that in tetragonal octahedral copper(II) complexes, the axial fifth and sixth ligands are weakly bonded at definite distances. It is therefore of interest to examine the possibility of Cu - O2 interaction in the complex $CuL_4(ClO_4)_2$, EtOH. This will now be discussed.

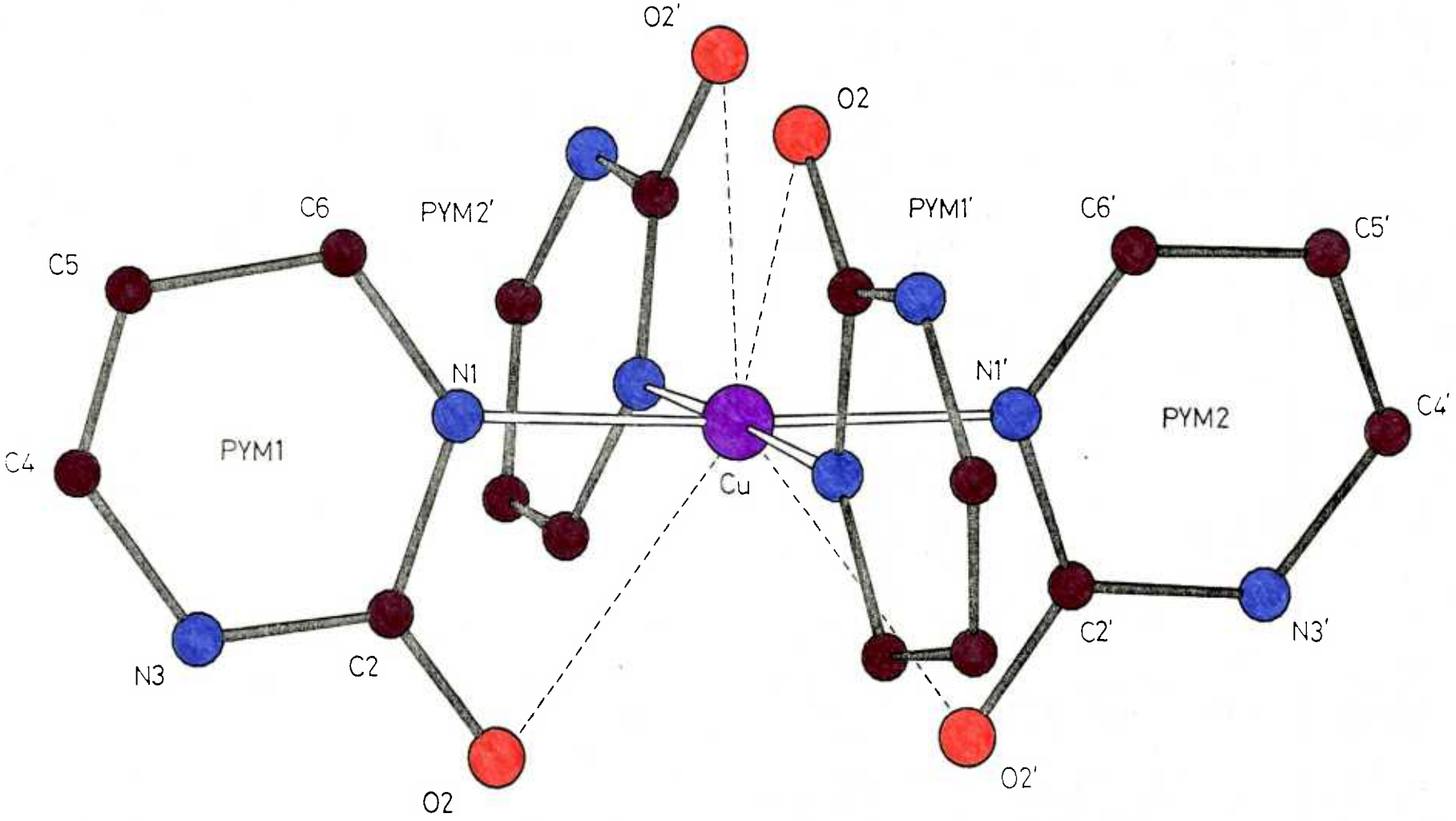
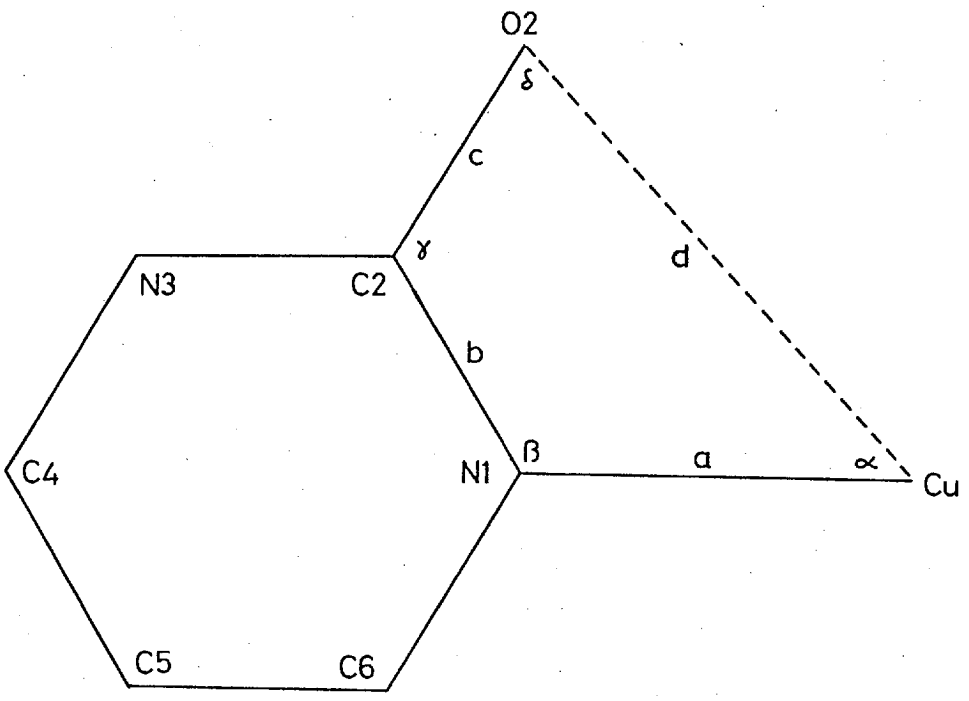


Fig. 4.17. The structure of $Cu(Pym2OH)_4(ClO_4)_2$,EtOH



PYM1 and 1'

a = 0.2004(6) nm	α = 53.6(2)°
b = 0.1374(10) nm	β = 109.7(5)°
c = 0.1247(9) nm	γ = 119.3(7)°
d = 0.2776(6) nm	δ = 77.4(4)°

PYM2 and 2'

a = 0.1992(6) nm	α = 50.6(2)°
b = 0.1368(10) nm	β = 112.7(5)°
c = 0.1190(9) nm	γ = 122.7(7)°
d = 0.2901(7) nm	δ = 74.1(5)°

N1(PYM1) - Cu - N1(PYM1') = 90.0°
N1'(PYM2) - Cu - N1'(PYM2') = 90.6°
N1(PYM1) - Cu - N1'(PYM2') = 89.8°
N1(PYM1') - Cu - N1'(PYM2) = 89.8°

O2 - O2' = 0.367(1) nm

Table 4.2. Selected bond angles and bond lengths of the structure of $Cu(Pym2OH)_4(ClO_4)_2$,EtOH with estimated standard deviations in parentheses.

The possibility of some oxygen interaction is shown from a comparison of the structures of 2-pyrimidinone,[274] uracil,[331] and the Cu - PYM1 fragment of $CuL_4(ClO_4)_2$,EtOH (Fig. 4.18.). It can be seen that the relevant angles O - C - N and C - N - X (X = H or Cu) both decrease on complexation. This indicates that a very weak Cu - O2 bond may exist for the PYM1 ring. However, the analagous Cu - PYM2 fragment shows the above angles to be 122.7° and 112.7° respectively and implies that for this ring a very small amount of interaction, if any, is present.

An example of the effect of weak binding is noted in the electronic spectra of a series of copper complexes viz. $Cu(Imid)_4X_2$ (X = Cl, Br and I). The crystal structure of the iodide has been determined[332] and shown to have an essentially square - planar arrangement like that found in $CuL_4(ClO_4)_2$,EtOH. The iodine atoms, however, were found to be 0.3423 and 0.3866 nm from the metal and were assumed to be non-bonded or only very weakly at the best. The electronic reflectance spectrum gave the normal single medium intensity band at 18200 cm^{-1} indicative of a CuN_4^{2+} chromophore.[227] As the iodines are replaced by bromine and then by chlorine atoms, the energy of this electronic band decreases i.e. 17400 cm^{-1} (Br) and 16900 cm^{-1} (Cl).[333] This demonstrates the greater tendency of smaller halogen atoms to coordinate and hence the formation of a more octahedral structure.

This type of behaviour can also be applied to other systems i.e. $Cu(1,2Me_2Imid)_4(ClO_4)_2$. This structure is closely related to both $CuL_4(ClO_4)_2$,EtOH and $Cu(Imid)_4I_2$ except that no coordinating atoms occupy the positions above or below the CuN_4 plane due to the 'blocking' effect of the 2-methyl groups. The electronic spectrum exhibited a band at 18800 cm^{-1} which is some 110 cm^{-1} higher in energy than $CuL_4(ClO_4)_2$,EtOH. The implication of this value, considering the differences in the ligands, is that the Pym2OH complex is <u>less</u> square - planar and therefore some weak

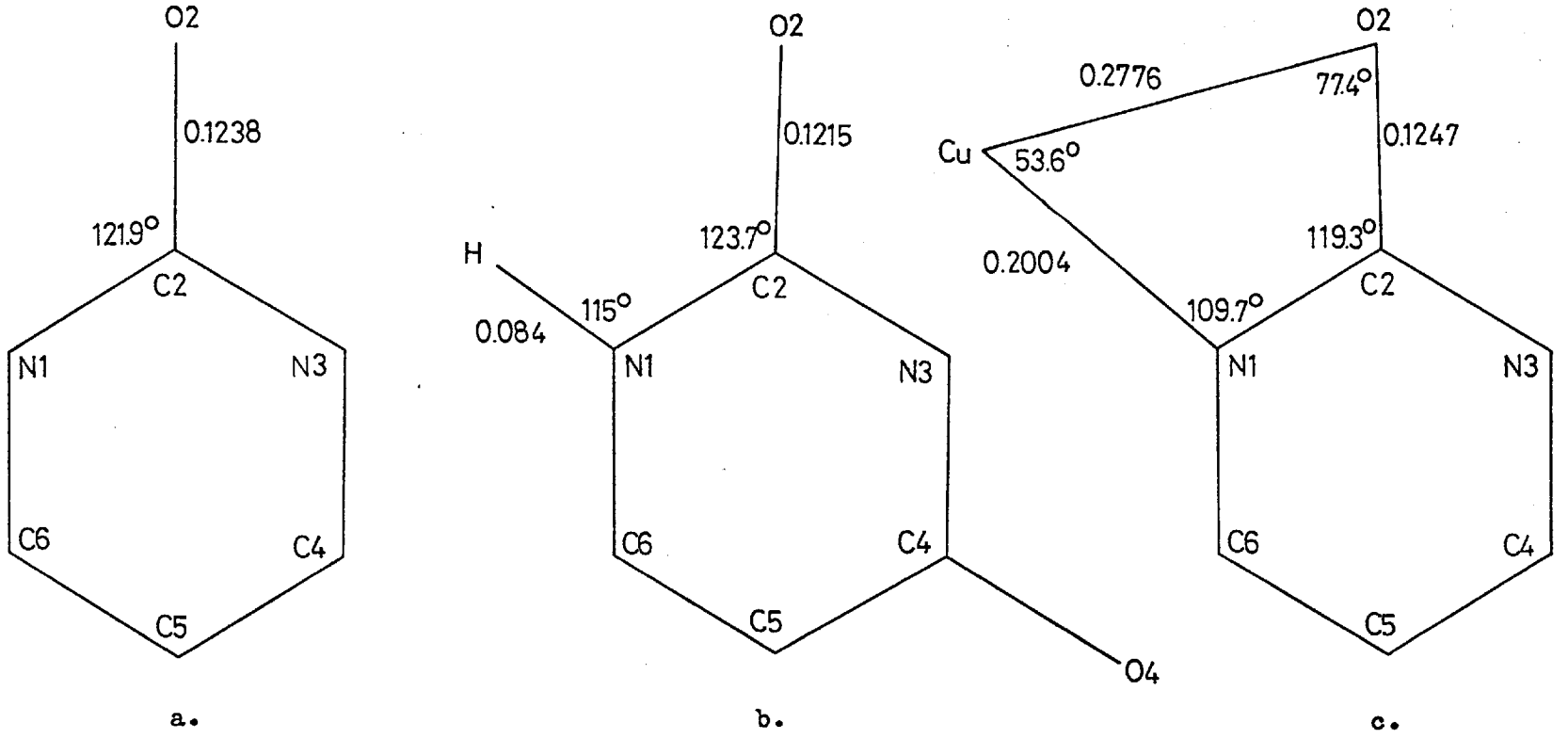


Fig. 4.18. Comparison of some important bond angles and bond lengths in a) 2-pyrimidinone, b) uracil and c) the Cu - PYM1 fragment of $Cu(Pym2OH)_4(ClO_4)_2$,EtOH.

axial interaction must be occurring, as suggested, between copper and the oxygen atom of the pyrimidine ring. The e.p.r. spectra of these compounds confirm this proposal.[234]

Because this weak interaction is undoubtedly all from the oxygen atoms, a comparison can now be carried out with the structures of the copper - cytosine and cytidine complexes which are also thought to possess metal - oxygen binding. The relative bond lengths and angles are given in Table 4.3. These values are similar to those found in the Cu - PYM1 fragment and therefore confirm that a weak Cu - O2 (PYM1) bond occurs. However, although the Cu - O2 (PYM2) distance is long, it is not impossible for this oxygen to still be involved in the overall interaction above and below the CuN_4 plane. This means that two structures are possible either with two or four oxygens interacting. These proposals are considered with respect to the structures of some copper acetate complexes.

If all the oxygen atoms in $CuL_4(ClO_4)_2$,EtOH interact with the metal, a pseudo-dodecahedral structure would result. This type of stereochemistry (Fig. 4.19.) has been found in the complexes $CaCu(AcO)_4,6H_2O$ [334] (Cu - O′ = 0.2790 nm) and $Cu(HA)_4(ClO_4)_2$ [335] (Cu - O′= 0.288 nm). In both cases

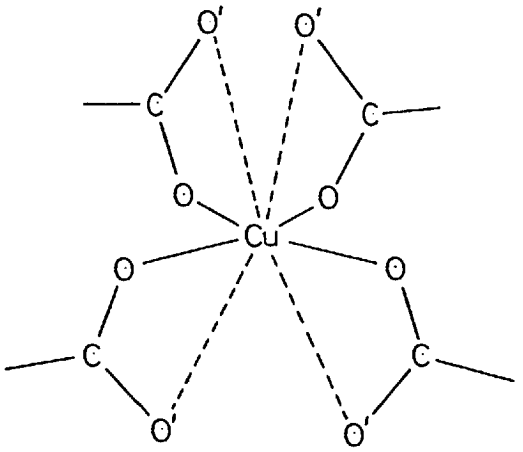
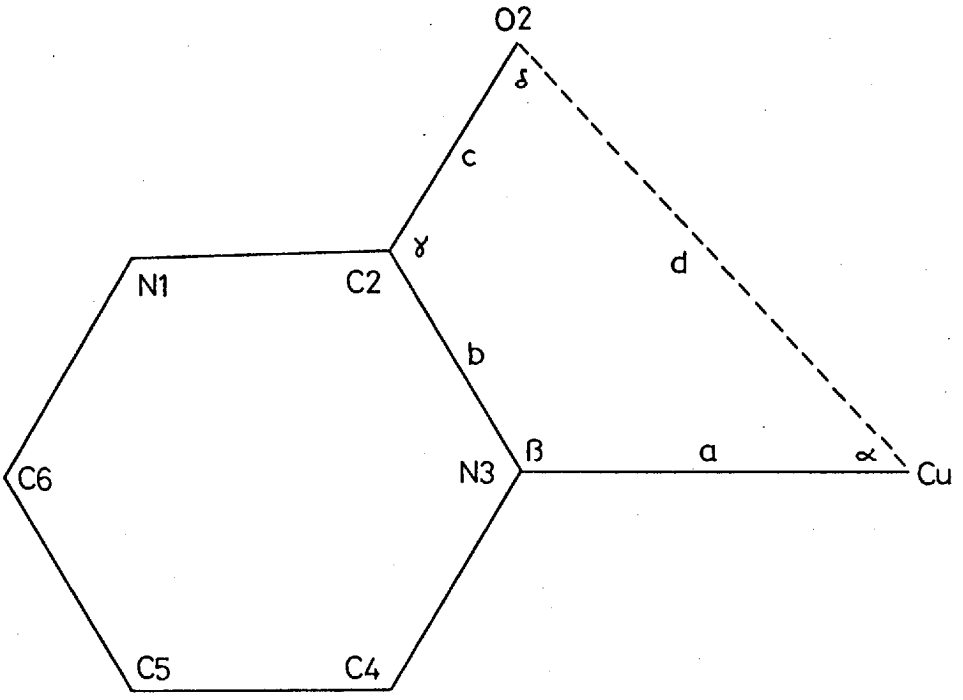


Fig. 4.19. The molecular stereochemistry of $CaCu(AcO)_4,6H_2O$ and $Cu(HA)_4(ClO_4)_2$.



	A	B		C	D	E[a]
a =	0.2004	0.197	0.195	0.1979	0.2008	0.201
b =	0.1374	0.134	0.140	0.1377	0.1368	0.134
c =	0.1247	0.122	0.123	0.1234	0.1237	0.126
d =	0.2776	0.274	0.288	0.2819	0.2772	0.274
α =	53.6	53.8	52.0	52.9[b]	53.8[b]	
β =	109.7	109.2[b]	113[b]	110.3[b]	108.4	
γ =	119.3	121	120	121.3	121.1	
δ =	77.4	76	75	75.5	76.7	77

A. The Cu - PYM1 fragment of $Cu(Pym2OH)_4(ClO_4)_2$,EtOH

B. $Cu(Cyt)_2Cl_2$ [142]

C. [(glycylglycinato)(Cyt)Cu(II)],$2H_2O$ [167]

D. [(N-salicylidene-N'-methylethylenediamine)(Cyt)Cu(II)]NO_3,H_2O [166]

E. [(glycylglycinato)(C)Cu(II)],$2H_2O$ [168]

a. Averaged values

b. Calculated values assuming planarity of Cu-N3-C2-O2 ring

Table 4.3. A comparison of some bond lengths (nm) and bond angles (^{o}) of various copper - cytosine and cytidine complex structures with the Cu - PYM1 fragment of $Cu(Pym2OH)_4(ClO_4)_2$,EtOH.

it has been reported[336] that the four off-axis oxygen atoms of the CuO_4O_4' chromophore are considered to be weakly bonded. However, in the compound $Cu(HA)_4(ClO_4)_2$ the amount of interaction is quite small, therefore as the Cu - 02 (PYM2) bond is even longer in the 2-pyrimidinone complex, i.e. 0.2901 nm, it would be expected that all of the interaction must come from Cu - 02 (PYM1).

Examples of just two off-axis oxygen atoms are given by the complexes $Cu(HA)_2,2H_2O$ [335,337] (Cu - O′= 0.2768 nm, $\alpha = 51.8^0$) and $Cu(AcO)_2(NH_3)_2$ (Cu - O′= 0.277 nm, $\alpha = 50.0^0$).[338,339] These have semi-elongated rhombic structures (Fig. 4.20.).

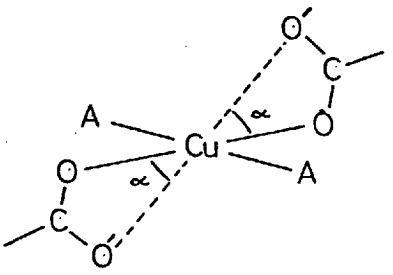


Fig. 4.20. The molecular stereochemistry of $Cu(HA)_2,2H_2O$ and $Cu(AcO)_2(NH_3)_2$.

On comparing both the Cu - O distances and the angles made by the acetate group with the corresponding values in $Cu(Pym2OH)_4(ClO_4)_2,EtOH$, it can be seen that the structures are extremely similar. It is concluded, therefore, that although the crystal structure of $Cu(Pym2OH)_4(ClO_4)_2,EtOH$ shows an essentially square - coplanar arrangement, a comparison study with related complexes has indicated that weak interaction probably occurs from the PYM1 and 1′ oxygen atoms 36.4^0 away from the z-axis.

Manganese(II)

Two halide complexes were prepared of formulae MnL_2Cl_2 and $MnL_2Br_2,2H_2O$. The far i.r. spectrum of the chloride gave little information, as only a broad band at 231 cm^{-1} was observed and may be due to ν(Mn - Cl) and ν(Mn - N). If the chlorine atoms are assumed to be coordinated then the structure is most likely octahedral with bridging halogen atoms. The presence of OH in the normal i.r. does, however, suggest the possibility of bridging ligand molecules.

The bromide complex also appears to be octahedral in nature. The far i.r. spectrum shows a metal - water stretch vibration at 370 cm^{-1} and ν(Mn - N) at 249 cm^{-1}. The e.p.r. spectra of both of these complexes[234] are consistent with their having polymeric structures.

Zinc(II) and Cadmium(II)

The zinc halide complexes of 2-pyrimidinone were of the type ZnL_2X_2 (X = Cl, Br). The far i.r. spectrum of the chloride showed two Zn - Cl stretching vibrations at 296 and 280 cm^{-1} and the ν(Zn - N) band at 241 cm^{-1}. These are in the region for tetrahedral species.[304] The bromide also exhibited a Zn - N stretch at 241 cm^{-1}, but a Zn - Br stretch vibration at 215 cm^{-1} is much lower than expected for tetrahedral coordination. However, this type of behaviour has been noted for other tetrahedral complexes including $Zn(Me_3NO)_2X_2$ [340] and $Zn(NH_3)_2X_2$ [341] (X = Cl, Br).

The only other zinc complex to be prepared was the compound $ZnL_4(NO_3)_2$. The normal infrared spectrum showed the presence of an ionic nitrate group and therefore the existence of a tetrahedral ZnL_4^{2+} cation.[342] Also the far i.r. spectra exhibited a broad band at ca. 240 cm^{-1}, indicative of several metal - nitrogen bonds.

The cadmium complexes that were prepared were all halides and of the

formulae $CdLCl_2$ and CdL_2X_2 (X = Br, I). The 1:1 chloride showed the presence of OH in the normal i.r. but no Cd - Cl stretching vibrations above 200 cm^{-1} in the far i.r. spectra. This implies that the structure is octahedral and possibly polymeric like the other 1:1 2-pyrimidinone complexes, incorporating both bridging ligands and chlorides. The far i.r. spectra of the 2:1 compounds also showed that the ν(Cd - X) and ν(Cd - N) bands must be below 200 cm^{-1}. This means that the structures are probably similar to $Cdpy_2X_2$ (X = Br, I).[343]

4.2.3. Complexes of the Methyl Derivatives

For comparison with $Co(Pym2SH)_2X_2$ and $Co(Pym2OH)_2X_2$ (X = Cl, Br), the cobalt complexes of the methyl derivatives of these ligands were prepared. The formulae of these compounds are CoL_2X_2 (L = 4,6Me$_2$Pym2SH, 4,6Me$_2$Pym2OH, 1MePym2O; X = Cl, Br) and $Co(4,6Me_2Pym2SH)_2X_2,2H_2O$ (X = Cl, Br). The physical measurements are given in Tables 4.11 and 4.12.

4,6-Dimethyl-2-Pyrimidinethione

With $CoX_2,6H_2O$ (X = Cl, Br) as the starting materials, two anhydrous compounds of stoichiometry CoL_2X_2 could be prepared in each case, one blue and the other green. In addition, each halide gave a pink dihydrate of composition $CoL_2X_2,2H_2O$. The initial solid product was the blue complex CoL_2X_2 [A] (X = Cl, Br) but, as shown in Fig. 4.21., this changes after ca. ten minutes to the dihydrate when left in contact with the reaction solution. After isolation, the pink complexes could be converted to the green compounds [B] by heating to 80°C or by using 2,2-dimethoxypropane. However, no interconversion of the blue and green forms could be achieved.

The chloride and bromide complexes of the blue form [A] were found to have electronic spectra very similar to each other. This showed that

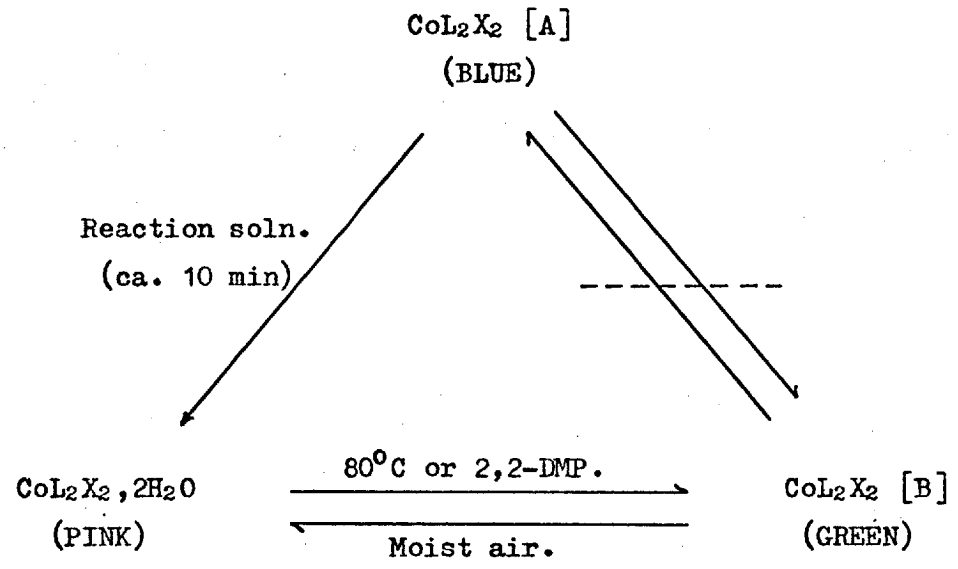


Fig. 4.21. A scheme showing the methods employed for the preparation of the pink and green 2:1 complexes from the initially formed blue compound.

the bands were due to a common structure with non- or weakly coordinating halogen atoms. The far i.r. spectra confirmed this proposal as no metal - halogen stretching bands were observed. A conductivity measurement was taken of the chloride according to recommended procedures[344] and the molar conductance was found to be 2.8 mS m^2 mol^{-1}; 10^{-3}M in nitroethane. Although this figure is much smaller than expected for a 2:1 electrolyte i.e. 16 - 20 mS m^2 mol^{-1}, later conductivity measurements showed this value was decreasing. This implies that the blue compound is in fact ionic but is unstable in solution giving a fully coordinated complex. The structure of form [A] is therefore likely to be tetrahedral with both N and S donor atoms chelating.

The electronic spectra of the pink dihydrates are typical of octahedral complexes. The far i.r. spectra gave no halogen dependent bands but showed ν(M - H_2O) at 395 cm^{-1} (Cl) and 384 cm^{-1} (Br). The metal - ligand stretch vibrations (either Co - N or Co - S) were found at 229 and 228 cm^{-1}

for chloride and bromide respectively. From both of these techniques, the structures of both hydrates appear to be octahedral with no coordinated halogens. The coordination positions are then made up of two nitrogen and two sulphur atoms with two water molecules arranged in trans positions.

The green complexes (form B) have reflectance spectra closely resembling those of the parent compounds $Co(Pym2SH)_2X_2$ (X = Cl, Br). Hence their structures can be assumed to be very similar to the distorted octahedron shown in Fig. 4.9. However, as the far i.r. spectrum of $Co(4,6Me_2Pym2SH)_2Cl_2$ gave a low ν(M - Cl) vibration at 222 cm^{-1}, the binding of the halogen atoms are probably weaker due to steric hindrance caused by the methyl groups.

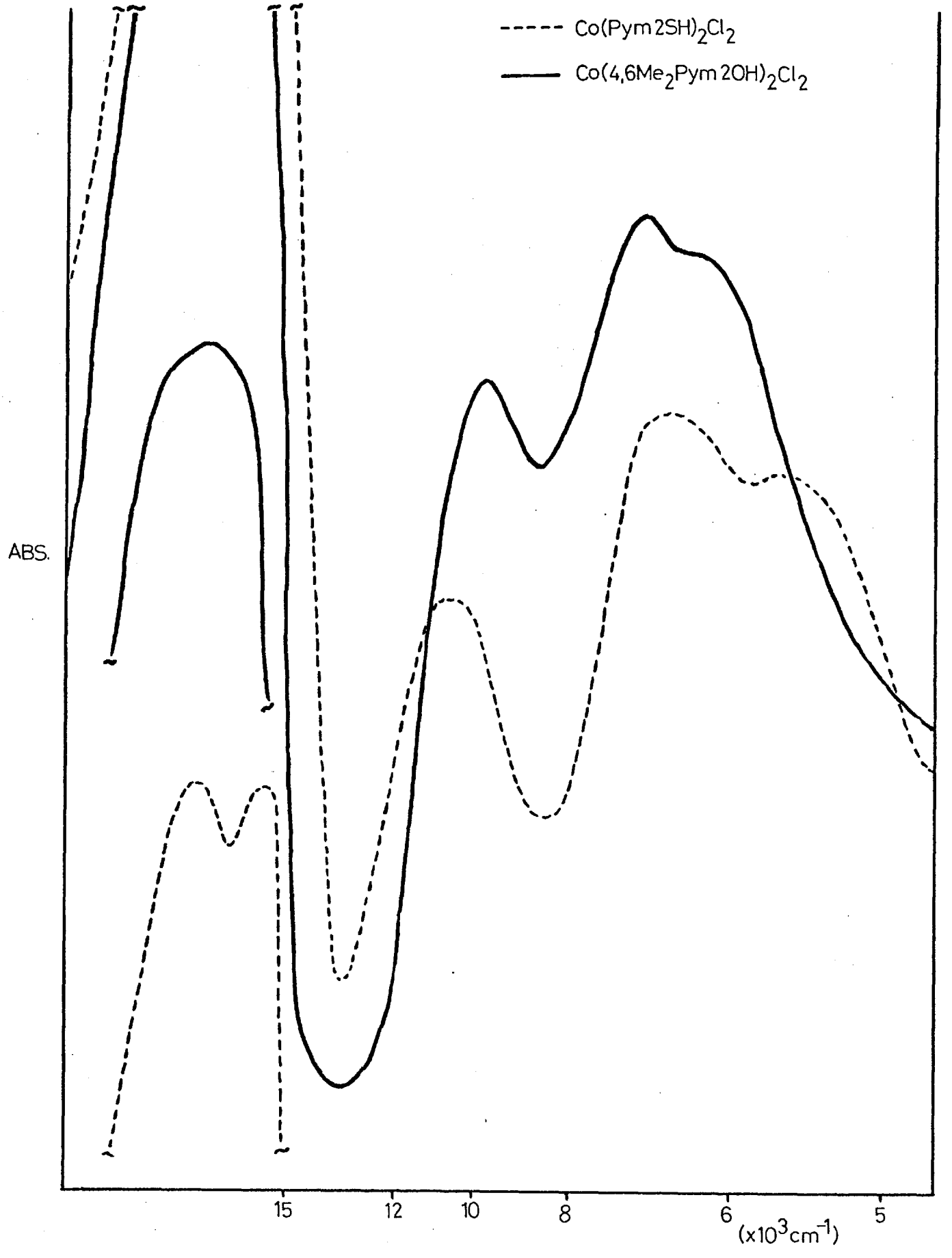
4,6-Dimethyl-2-Pyrimidinone

Two complexes of this ligand were formed with the formula CoL_2X_2 (X = Cl, Br). The electronic spectra (the chloride is shown in Fig. 4.22.) rather unexpectedly exhibited a certain similarity with the compounds $Co(Pym2SH)_2X_2$. The splitting of the three bands in the 10000 - 6000 cm^{-1} region is rather less than in the 2-pyrimidinethione complexes, but nevertheless, a distorted structure is indicated probably being caused by a small amount of Co - O2 interaction.

The far i.r. spectra showed metal - halogen stretch bands at 281 and 259 cm^{-1} (chloride) and 234 cm^{-1} (bromide). These values are considered to be lower than those found for normal CoL_2X_2 tetrahedral complexes,[304] but higher than for octahedral. This, therefore, implies that there is some involvement of the pyrimidine oxygens with the metal.

Because of the evidence of possible 'semi-chelation', it was attempted to prepare a crystalline sample of the complex $Co(4,6Me_2Pym2OH)_2Cl_2$ for a structural determination. However, although the solid appeared to be in the form of nice cubic crystals, no diffraction pattern could be obtained.

Fig. 4.22.



1-Methyl-2-Pyrimidinone

As with the previous ligand, only two cobalt halide complexes were prepared for comparative purposes. The blue $Co(1MePym2O)_2X_2$ (X = Cl, Br) compounds gave reflectance spectra typical for tetrahedral stereochemistry. No splitting of the ν_2 band was observed for either compound indicating that there is no interaction of the O2 oxygen atoms with cobalt. The M - X stretching vibrations in the far i.r. spectra confirmed that the structures are tetrahedral.

Compound	ν(N - H)[I]	ν(N - D)[II]	I/II
Py2SH	ca. 2885	2210	1.31
Pym2SH	ca. 2550	ca. 1980[a]	1.29
Pym2OH	ca. 2770	2180	1.27
4,6Me$_2$Pym2SH	ca. 2920	2240	1.30
4,6Me$_2$Pym2OH	ca. 2800	2165	1.29
Co(Py2SH)$_2$Cl$_2$	ca. 3020	2295	1.32
Co(Pym2SH)$_2$Cl$_2$	b	b	-
Ni(Pym2SH)$_2$Cl$_2$	ca. 2700	2115	1.28

a. Obscured by ring overtone bands

b. Not observed

Table 4.4. Selected i.r. data of deuterated ligands and their complexes. Nujol and hexachlorobutadiene mulls were used with NaCl plates Values are in cm^{-1}.

Complex	X_g ($m^3kg^{-1}x10^{-8}$)	X_M' [a] ($m^3mol^{-1}x10^{-11}$)	u_{eff} (B.M.)	T (K)
FeL_2Cl_2	41.17	14683	5.25	294.5
FeL_2Br_2	34.95	15636	5.42	295.0
CoL_2Cl_2	31.33	11325	4.62	296.0
CoL_2Br_2	26.64	12062	4.75	294.5
NiL_2Cl_2	14.80	5470	3.21	295.5
NiL_2Br_2	11.79	5221	3.20	295.0

a. Pascal's constants from ref. 358

Table 4.5. Magnetic susceptibility data of selected 2-pyrimidinethione complexes.

Compound	Bands
FeL_2Cl_2	5480, 8200
FeL_2Br_2	5120, 8770
CoL_2Cl_2	5530, 6640, 10530, 15630, 17240
CoL_2Br_2	5240, 6150, 11490, 14810, 17180
$Co(Pym2S)_2$	5200 br,w, 10100 br, 18350
NiL_2Cl_2	8260, 13610, 23530 br [a]
NiL_2Br_2	8260, 13510, 24630 br [a]
$Ni_5(Pym2S)_7I_2OH,6H_2O$	9660, 14490, 25000 br [a]
$Ni(Pym2S)_2$	8970, 15270 sh, 19840 sh, 22520 sh

a. Tentative assignment only due to interference of ligand band

Table 4.6. Electronic reflectance spectra showing d - d transitions of the 2-pyrimidinethione complexes. Values are in cm^{-1}.

Compound	'ν(C = S)'	ν(N - H) [b]
Pym2SH	1186	ca. 2550
MnL_2Cl_2	1199	a
MnL_2Br_2	1194	a
FeL_2Cl_2	1198	a
FeL_2Br_2	1193	a
CoL_2Cl_2	1199	a
CoL_2Br_2	1194	a
$Co(Pym2S)_2$	1198	a
NiL_2Cl_2	1188	ca. 2700
NiL_2Br_2	1188	ca. 2710
$Ni_5(Pym2S)_7I_2OH,6H_2O$	a	a
$Ni(Pym2S)_2$	1182	a
ZnL_2Cl_2	1199	a
$Zn(Pym2S)_2$	1200	a
CdL_2Cl_2	1197	a
CdL_2Br_2	1193	a
CdL_2I_2	1161	ca. 2575

a. Not observed

b. Refer to Table 4.4.

Table 4.7. Selected i.r. data for 2-pyrimidinethione complexes. Nujol mulls were used with NaCl plates. Values are in cm^{-1}.

Compound	Ligand Bands		ν(M - L)	ν(M - X)	Unass.
	I	II			
Pym2SH	405	353 vw [a]			
MnL_2Cl_2	398	353 mw	b	230, 209	160
MnL_2Br_2	397	352 w	231	181 sh	166
FeL_2Cl_2	398	354 m	250 sh	236 sh, 217	166
FeL_2Br_2	398	356 w	246	177 sh [c]	168
CoL_2Cl_2	403	d	258	243 sh, 232	168
CoL_2Br_2	401	d	252	188 sh	170
$Co(Pym2S)_2$	392	d	259		230, 202
NiL_2Cl_2	401	365 w	263	215, 204 sh	
NiL_2Br_2	400	365 w	264	179	
$Ni_5(Pym2S)_7I_2OH,6H_2O$	393	d			287, 271, 237 sh, 230
$Ni(Pym2S)_2$	402	360 w	256		213
ZnL_2Cl_2	403	361 w	260 sh	252, [c] 238 [c]	202, 163
$Zn(Pym2S)_2$	391	d			216
CdL_2Cl_2 [e]	397	355 m		211	
CdL_2Br_2 [e]	395	354 mw			
CdL_2I_2 [e]	405	d	311	240 [c]	

a. Raman active

b. Band obscured

c. Tentative assignment

d. Not observed

e. Only 450 - 200 cm^{-1} range

Table 4.8. Far i.r. data for 2-pyrimidinethione complexes. Vaseline mulls were used with polythene plates. Values are in cm^{-1}.

Compound	Bands
$FeLCl_2$	ca. 6560, 9520
CoL_2Cl_2 [A]	4080, 6900, 16450
CoL_2Cl_2 [B]	5520, 9900, 14930, 18690
CoL_4Cl_2	5260, 9520, 18690
CoL_2Br_2 [A]	6250, 7250, 9090, 16130
CoL_4Br_2	ca. 4380, 7350, 8930, 10700, 18350
CoL_5I_2	5710, 9660, 18520
$CoL_5(ClO_4)_2$	ca. 4820, 8770, 18250
$Co(Pym2O)_2$	ca. 4670, 9302, 18520
$NiLCl_2$	8000, 12990, 20410 sh, 22990
$NiL_2Br_2,2H_2O$	7630, 14810, ca. 24300 sh
NiL_4I_2	9050, 15380, ca. 22830 sh
$Ni(Pym2O)_2$	8550, 14930, 25250
CuL_2Cl_2 [A]	ca. 10100 sh, 16390
CuL_2Cl_2 [B]	ca. 10000 sh, 16670
$CuLBr_2$	ca. 16950 br
CuL_2Br_2	14810
$CuL_4(ClO_4)_2$,EtOH	18690
$CuL_4(ClO_4)_2$,1-PrOH	18690
$Cu(1,2Me_2Imid)_4(ClO_4)_2$	18800

Table 4.9. Electronic reflectance spectra showing d - d transitions of the 2-pyrimidinone complexes. Values are in cm^{-1}.

Compound	Ligand	ν(M - L)	ν(M - X)	Unass.
Pym2OH	403			
MnL_2Cl_2	404			231 br
$MnL_2Br_2,2H_2O$	406 w	370,[a] 249		
$FeLCl_2$	419			250, 204
CoL_2Cl_2 [A]	417	241	310, 288	
CoL_2Cl_2 [B]	410		309	
CoL_4Cl_2	423 w	274 sh, 260		220
CoL_2Br_2 [A]	416		254, 238	
CoL_4Br_2	414	273, 260		237
CoL_5I_2	409	274 sh, 258		309, 220
$CoL_5(ClO_4)_2$	409	260		220
$Co(Pym2O)_2$	b	274 sh, 261		219
$NiLCl_2$	426			258, 234 211
$NiL_2Br_2,2H_2O$	423 w	360,[a] 263		210
NiL_4I_2	411 w			300, 240 sh
$Ni(Pym2O)_2$	b			ca. 274 br
CuL_2Cl_2 [A]	424 w	273, 261	306	
CuL_2Cl_2 [B]	425	254	299	413, 313, 213
$CuLBr_2$	416	282		
CuL_2Br_2	417	280, 254	233	
$CuL_4(ClO_4)_2,S$	419	281		236
$CuL_4(ClO_4)_2,S'$	419	279		236
ZnL_2Cl_2	412	241	296, 280 sh	318, 306 sh 218
$ZnL_4(NO_3)_2$	414	240 br		

(Continued overleaf)

Compound	Ligand	ν(M - L)	ν(M - X)	Unass.
$CdLCl_2$	402 w			210 sh
CdL_2Br_2	407			211 sh
CdL_2I_2	406			

a. $\nu(M - H_2O)$

b. Not observed

S = EtOH, S' = 1PrOH

Table 4.10. Far i.r. data for 2-pyrimidinone complexes. Vaseline mulls were used with polythene plates. Values are in cm^{-1}.

Compound	Bands
$Co(4,6Me_2Pym2SH)_2Cl_2$ [A]	5000, 9760, 15380 sh, 16670
$Co(4,6Me_2Pym2SH)_2Cl_2$ [B]	5040, 9300, 15380, 16390
$Co(4,6Me_2Pym2SH)_2Cl_2,2H_2O$	5970, 7870, 20620
$Co(4,6Me_2Pym2SH)_2Br_2$ [A]	5050, 9710, 16130
$Co(4,6Me_2Pym2SH)_2Br_2$ [B]	6250, 9520, 16670 br
$Co(4,6Me_2Pym2SH)_2Br_2,2H_2O$	5880, 7970, 20620
$Co(4,6Me_2Pym2OH)_2Cl_2$	6230 sh, 6900, 9620, 17180
$Co(4,6Me_2Pym2OH)_2Br_2$	4930 sh, 6250 sh, 6920, 9090 sh, 16670
$Co(1MePym2O)_2Cl_2$	4850 sh, 6450, 16670
$Co(1MePym2O)_2Br_2$	4870 sh, 6990, 16130

Table 4.11. Electronic reflectance spectra showing d - d transitions of the methyl derivative complexes of 2-pyrimidinethione and 2-pyrimidinone. Values are in cm^{-1}.

Compound	Ligand		ν(M - L)	ν(M - X)	Unass.
	I	II			
4,6Me$_2$Pym2SH	285	268			
CoL_2Cl_2 [A]	294	270	314, 246		211
CoL_2Cl_2 [B]	290	271	304 sh	222	
$CoL_2Cl_2,2H_2O$	296	276	395,[a] 229		
CoL_2Br_2 [A]	291	271	316, 251		212
CoL_2Br_2 [B]	287	271	303, 225		
$CoL_2Br_2,2H_2O$	294	277	384,[a] 228		
4,6Me$_2$Pym2OH	364	283			
CoL_2Cl_2	353	300		281, 259	
CoL_2Br_2	352	300	267	234	
1MePym2O	356	262			
CoL_2Cl_2	352	264	247	330, 300	
CoL_2Br_2	351	b	b	264, 243	

a. ν(M - H_2O)

b. Obscured

Table 4.12. Far i.r. data for complexes of the methyl derivatives of 2-pyrimidinethione and 2-pyrimidinone. Vaseline mulls were used with polythene plates. Values are in cm^{-1}.

CHAPTER 5

EXPERIMENTAL

5.1. General

Analyses were carried out by the Microanalytical Laboratory, Imperial College, except where platinum or confirmatory determinations were needed. The Pregl method was used for carbon and hydrogen analyses for compounds discussed in Chapters 2 and 3 because of the better combustion as compared with other methods. Normal titrimetric and gravimetric techniques were employed for other elements.

I.r. spectra were recorded on the following spectrophotometers, a Perkin-Elmer 257 (4000-625 cm^{-1}), a Perkin-Elmer 457 (4000-250 cm^{-1}), a Perkin-Elmer 325 (4000-200 cm^{-1}) and a Beckman FS 720 Fourier spectrophotometer (400-40 cm^{-1}).

Diffuse reflectance spectra were measured in the region 26000-4000 cm^{-1} on a Beckman DK2 spectrometer and a Cary 14 spectrometer using a Cary Model 1411 diffuse reflectance accessory.

Magnetic moments were measured on solid samples at room temperature using the Evans modification [345,346] of the Gouy-Rankine balance. The balance was calibrated with cobalt mercury thiocyanate.

Measurements of pH were taken using a Pye Unicam Model 292 pH meter with a Russell CT 72 electrode, and X-ray powder photographs were obtained on a Guinier Nonius Mark II camera.

5.2. Ligands

The nucleotides 5'-AMP (BDH), 5'-CMP and 5'-UMP (Koch-Light), 5'-GMP (Aldrich) and 5'-IMP (Sigma) were purchased as their sodium salt; and with cytidine (Sigma), guanosine (BDH), thymidine (Cambrian), uridine

(Sigma), cytosine (Sigma), thymine (BDH) and uracil (BDH), were used without further purification. 2-Pyridinethione and 2-pyrimidinethione (Aldrich) were also purchased, and the latter was purified by recrystallisation from a 1:1 ethanol-water mixture (1g 55 cm^{-3}).

Most of the other ligands were prepared according to known methods, i.e. 9-methyladenine,[347] 9-methylhypoxanthine,[348] 2-pyrimidinone,[349,350] 1-methyl-2-pyrimidinone[351] and 1,4,6-trimethyl-2-pyrimidinone,[352] but 4,6-dimethyl-2-pyrimidinethione and its oxygen analogue were synthesised as follows:-

4,6-Dimethyl-2-Pyrimidinethione

Concentrated hydrochloric acid (25 cm^3) was added to a warm suspension of finely powdered thiourea (7.60g, 0.1 mol) in 2,4-pentanedione (12.0g, 0.12 mol) and ethanol (250 cm^3), and the mixture boiled under reflux for 2 hours. After cooling, the yellow needles (14.0g, 80%) of 4,6-dimethyl-2-pyrimidinethione hydrochloride were collected. To the mother - liquor were added more powdered thiourea (7.60g), 2,4-pentanedione (11.00g), ethanol (10 cm^3), and concentrated hydrochloric acid (15 cm^3), and the mixture was boiled as before. Cooling and filtration then gave the hydrochloride (16.00g, 90%). After two such preparations, making use of the original mother - liquor, the final solution was discarded.

The combined yield of hydrochloride (46.10g, total - 87%) was dissolved in water (200 cm^3), neutralised with NaOH solution and evaporated to dryness on a water bath. The residue was then powdered and the 4,6-dimethyl-2-pyrimidinethione extracted with ethanol, from which it was later recrystallised, to give the pure compound (26.10g, 62%; M.P. - 208-210°C, Lit.M.P. - 209-210°C[353]).

4,6-Dimethyl-2-Pyrimidinone

The hydrochloride of this compound was prepared in a similar manner to 4,6-dimethyl-2-pyrimidinethione using urea and 2,4-pentanedione.

The combined yield of hydrochloride (39.47g, 82%) was dissolved in the minimum of cold water, neutralised with NaOH solution and evaporated to one third of its volume on a water bath. The solution was cooled overnight to give colourless crystals of the 4,6-dimethyl-2-pyrimidinone dihydrate. This was recrystallised from water and the anhydrous ligand then prepared by heating in vacuo at 80°C. (17.80g, 48%; M.P.201-202°C., Lit.M.P.201-202°C.[354]).

5.3. Complexes of Chapter 2

For the syntheses of these compounds, the pH values refer to those preparations that gave either crystals for X-ray examination or precipitates for comparison analyses. The pH ranges in which experiments were conducted are also given. The compounds with platinum, involving the pyrimidine nucleotides 5'-CMP and 5'-UMP (the platinum blues), are reported in the experimental section of Chapter 3.

In all cases the solids were filtered under vacuum, washed with a little ice-cold water and air-dried.

5.3.1. Cytidine 5'-Monophosphate

A. Cadmium

pH Range 3.91 - 6.65

[Cd(5'-CMP)(H_2O)],H_2O

A solution of 5'-CMPNa_2 (0.183g, 0.5 mmol) in water (12.5 cm^3) was added, with stirring, to one of $Cd(NO_3)_2.4H_2O$ (0.154g, 0.5 mmol) in water

($7.5\ cm^3$). The white gelatinous precipitate that immediately formed was redissolved with a few drops of nitric acid (2M) and the resultant clear solution (pH 4.71) heated at 60°C for 15 minutes. When cool, this solution was filtered (pH 4.64), and gave colourless crystals after one week at room temperature.

[Cd(5'-CMP)(H_2O)],H_2O	requires	C, 23.01%	H, 3.43%
	found	C, 23.32%	H, 3.52%

This is the normal procedure used to obtain crystals and so for future preparations is referred to as Method I.

Cd(5'-CMP),$5H_2O$

This compound was prepared as for [Cd(5'-CMP)(H_2O)],H_2O except that the white precipitate (formed by mixing the solutions - resultant solution pH 6.65) was filtered, washed with cold water and air dried.

Cd(5'-CMP),$5H_2O$	requires	C, 20.64%	H, 4.24%
	found	C, 20.54%	H, 4.25%

The synthesis involving the formation of solids in this way is termed Method II.

B. Zinc

pH Range 3.50 - 5.70

Zn(5'-CMP),$4H_2O$

As for Method II using 5'-$CMPNa_2$ (0.183g, 0.5 mmol) and $Zn(NO_3)_2$, $4H_2O$ (0.130g, 0.5 mmol). The white precipitate that formed at pH 5.70 was isolated as before.

$Zn(5'\text{-CMP}),4H_2O$	requires	C, 23.57% H, 4.40%
	found	C, 23.46% H, 4.44%

5.3.2. Guanosine 5'-Monophosphate

A. Cadmium

pH Range 3.41 - 5.62

$[\ Cd(5'\text{-GMP})(H_2O)_5\ ],3H_2O$

As for Method I using $5'\text{-GMPNa}_2,0.5H_2O$ (0.208g, 0.5 mmol) and $Cd(NO_3)_2,4H_2O$ (0.154g, 0.5 mmol) giving a final solution of pH 3.41. After 2 days, small feathery crystal clusters had formed.

$[\ Cd(5'\text{-GMP})(H_2O)_5\ ],3H_2O$	requires	C, 19.44% H, 4.57%
	found	C, 19.45% H, 4.60%

$Cd(5'\text{-GMP}),6H_2O$

This was prepared according to Method II using $5'\text{-GMPNa}_2,0.5H_2O$ (0.208g, 0.5 mmol) and $Cd(NO_3)_2,4H_2O$ (0.154g, 0.5 mmol). The white precipitate that formed at pH 5.62 was isolated as before.

$Cd(5'\text{-GMP}),6H_2O$	requires	C, 20.65% H, 4.16%
	found	C, 20.72% H, 3.99%

B. Platinum(II)

pH Range 2.48 - 6.95

To synthesise complexes involving platinum(II) it was first necessary to prepare a stock solution of the compound cis-$[\ Pt(NH_3)_2(H_2O)_2\ ](NO_3)_2$[355]:-

A solution of K_2PtCl_4 (4.151g, 10 mmol) in water (30 cm^3) was added

to a solution of KI (6.40g, 40 mmol) in water (20 cm^3) and stirred at room temperature for 3 minutes. A slight excess of ammonia solution (5M) was added dropwise, with stirring, to produce an immediate deep yellow precipitate. After 10 minutes the cis-$Pt(NH_3)_2I_2$ was filtered, washed copiously with water and dried in vacuo over P_2O_5. (Yield 4.590g, 95%).

A mixture of cis-$Pt(NH_3)_2I_2$ (2.415g, 5 mmol) and $AgNO_3$ (1.699g, 10 mmol) in water (40 cm^3) were stirred overnight and the silver iodide precipitate was filtered off and the filtrate collected. A very small amount of this liquid was tested for silver ions with dilute hydrochloric acid (0.1 M). If the test was negative, the volume of the filtrate was made up to 100 cm^3 producing a solution of cis-[$Pt(NH_3)_2(H_2O)_2$]$(NO_3)_2$ (0.05 M). For each experiment, 5 cm^3 aliquots of this were used (ca. 0.097g, 0.25 mmol).

cis-[$Pt(NH_3)_2(5'\text{-GMP})_2H_2$],$5H_2O$

A solution of cis-[$Pt(NH_3)_2(H_2O)_2$]$(NO_3)_2$ (5 cm^3 - 0.097g, 0.25 mmol) was added, with stirring, to one of 5'-$GMPNa_2.0.5H_2O$ (0.208g, 0.5 mmol) in water (10 cm^3). To the resulting colourless solution (pH 6.50) was added dropwise dilute HNO_3 (2M) to produce a solution of pH 2.48. After one hour small colourless clusters of crystals had formed.

cis-[$Pt(NH_3)_2(5'\text{-GMP})_2H_2$],$5H_2O$	requires	C, 23.02%	H, 4.06%
	found	C, 23.07%	H, 4.07%

C. Zinc

pH Range 3.04 - 5.08

$Zn(5'\text{-GMP})nH_2O$

As for Method II using 5'-$GMPNa_2,0.5H_2O$ (0.208g, 0.5 mmol) and $Zn(NO_3)_2,4H_2O$ (0.131g, 0.5 mmol). A white gelatinous precipitate was formed at pH 5.08.

$Zn(5'\text{-GMP}),nH_2O$	n = 5	requires	C, 23.25% H, 4.29%
	n = 4.5	requires	C, 23.66% H, 4.17%
		found	C, 23.57% H, 4.58%

5.3.3. Inosine 5'-Monophosphate

A. Cadmium

pH Range 2.20 - 6.80

$[\ Cd(5'\text{-IMP})(H_2O)_5\ ],2.5H_2O$

A solution of 5'-$IMPNa_2$ (0.205g, 0.5 mmol) in water (12.5 cm^3) was added, with stirring, to one of $Cd(NO_3)_2,4H_2O$ (0.154g, 0.5 mmol) in water (7.5 cm^3) to produce a white gelatinous precipitate at pH 5.90. The mixture was then stirred and heated at 60°C for 15 minutes and cooled (pH 5.85). On standing at room temperature overnight, large white crystal clusters had formed from the precipitate.

$[\ Cd(5'\text{-IMP})(H_2O)_5\ ],2.5H_2O$	requires	C, 20.23% H, 4.41%
	found	C, 20.28% H, 4.41%

$[\ Cd(5'\text{-IMP})(H_2O)_5\ ],H_2O$

This was prepared in the same manner as the previous compound except that the initial white precipitate was redissolved with a few drops of HNO_3 (2M) before heating, to produce a solution at pH 4.52. The cooled solution (pH 4.51) then gave on leaving for some hours, small colourless prisms.

$[\ Cd(5'\text{-IMP})(H_2O)_5\ ],H_2O$	requires	C, 21.20% H,4.09%
	found	C, 21.17% H, 4.10%

[$Cd_2(5'\text{-IMP})_3H_2(H_2O)_6$],$6H_2O$

Further addition of HNO_3 (2M) to the final solution of the above preparations gave a solution of pH 3.88. On leaving for 3 weeks at room temperature this solution yielded colourless crystal clusters.

[$Cd_2(5'\text{-IMP})_3H_2(H_2O)_6$],$6H_2O$	requires	C, 24.30%	H, 4.01%
	found	C, 24.34%	H, 3.97%

B. Platinum(II)

pH Range 5.10 - 6.90

cis-[$Pt(NH_3)_2(5'\text{-IMP})_2$]$^{2-}$

A solution of cis-[$Pt(NH_3)_2(H_2O)_2$]$(NO_3)_2$ (5 cm^3 - 0.097g, 0.25 mmol) was added, with stirring, to one of 5'-IMPNa$_2$ (0.205g 0.5 mmol) in water (10 cm^3) to form a colourless solution at pH 6.85. After ten days, colourless prisms were formed.

Microanalytical results are consistent with the compound being non-stoichiometric and of formula Na_{4-2x}[$\{Pt(NH_3)_2\}_x$ $(5'\text{-IMP})_2$],nH_2O where $x \approx 0.56$ and $n \approx 16$.

required	C, 20.48%	H, 4.93%	N, 10.89%	Na, 5.45%	Pt, 9.31%
found	C, 20.20%	H, 4.57%	N, 11.24%	Na, 5.75%	Pt, 9.25%

5.3.4. Uridine 5'-Monophosphate

A. Cadmium

pH Range 3.90 - 6.80

Cd(5'-UMP)nH_2O

This compound was prepared as for Method I using 5'-UMPNa$_2$

(0.184g, 0.5 mmol) and $Cd(NO_3)_2,4H_2O$ (0.154g, 0.5 mmol) except that no precipitate came down on mixing the solutions. The final solution (pH 6.80) was left for 2 weeks at room temperature whereupon large fibrous crystals had formed.

$Cd(5'\text{-UMP})nH_2O$	n = 6	requires	C, 19.92% H, 4.27%
	n = 6.5	requires	C, 19.60% H, 4.39%
		found	C, 19.68% H, 4.29%

B. Zinc

pH Range 2.28 - 6.60

$Zn(5'\text{-UMP})2H_2O$

A solution of $5'\text{-UMPNa}_2$ (0.184g, 0.5 mmol) in water (12.5 cm^3) was added, with stirring, to one of $Zn(NO_3)_2,4H_2O$ (0.131g, 0.5 mmol) in water (7.5 cm^3). To the resulting colourless solution (pH 6.40) was added a few drops of HNO_3 (2M) to produce a solution of pH 5.69. This solution was heated at 60°C for 20 minutes and when cool, the white flocculent precipitate that had come down was filtered off. The filtrate (pH 5.60) was allowed to evaporate at room temperature and after 1 week small white needles had formed.

$Zn(5'\text{-UMP})2H_2O$	requires	C, 25.52%	H, 3.57%
	found	C, 25.55%	H, 3.53%

Preparations using 5'-AMP and platinum(II) were also carried out but no crystals could be made. At pH's 2.20 - 6.80, glasses were formed on evaporation of the reaction mixture.

5.4. Complexes of Chapter 3

5.4.1. 9-Methylhypoxanthine

$Pt(9MeHyp)_2'$,0.5DMA

K_2PtCl_4 (0.133g, 0.33 mmol) and 9-methylhypoxanthine (0.100g, 0.66 mmol) were heated under reflux for 30 min. in N,N-dimethylacetamide (4 cm^3). The yellow solution was filtered into a 10 cm^3 beaker which was placed in a desiccator containing 1-propanol. After 4 days a cream-yellow solid was filtered off, washed with 1-propanol and ether and dried in vacuo over P_2O_5.

$Pt(9MeHyp)_2'$,0.5DMA	requires	C, 31.32%	H, 2.72%	N, 22.17%
	found	C, 31.61%	H, 2.88%	N, 22.31%

cis-$Pt(9MeHyp)_2Cl_2$

A solution of K_2PtCl_4 (0.083g, 0.2 mmol) and 9-methylhypoxanthine (0.060g, 0.4 mmol) in water (10 cm^3) was left for 24 hours after which time a yellow solid had come down. The precipitate was filtered, washed with a small amount of water and dried in vacuo over P_2O_5.

$Pt(9MeHyP)_2Cl_2$	requires	C, 25.45%	H, 2.14%	N, 19.79%
	found	C, 25.23%	H, 2.37%	N, 19.63%

$Pt(9MeHyp)(9MeHyp)'Cl$

This compound was prepared by heating under reflux $Pt(9MeHyp)_2Cl_2$ (0.050g) in 2,6-dimethylpyridine for 1 hour. The resulting yellow solid was then filtered, washed with ether and dried as above.

$Pt(9MeHyp)(9MeHyp)'Cl$	requires	C, 27.20%	H, 2.09%	N, 21.15%
	found	C, 26.93%	H, 2.32%	N, 20.91%

$Cu(9MeHyp)_4(ClO_4)_2$

A solution of 9-methylhypoxanthine (0.150g, 1 mmol) in water (3 cm^3) was added to a warm solution of $Cu(ClO_4)_2,6H_2O$ (0.124g, 0.33 mmol) in water (4 cm^3). The resulting blue solution was allowed to evaporate at room temperature and after 5 days dark blue cubic crystals were formed. These were filtered, washed with a small amount of ethanol and air dried.

$Cu(9MeHyp)_4(ClO_4)_2$	requires	C, 34.03%	H, 2.86%	N, 26.46%
	found	C, 33.90%	H, 3.10%	N, 26.33%

5.4.2. The Platinum Blues

For cis-platinum(II) compounds, the preparation of the initial solutions, containing the various pyrimidines, was essentially the same. Where possible, two experiments were carried out on each compound: i.e. one in acid (pH 4.00) and the other in alkaline media (pH 8.50).

General Procedure

To a solution of pyrimidine (0.5 mmol) in water (5 cm^3) was added, with stirring, a solution of cis-$[\ Pt(NH_3)_2(H_2O)_2\ ](NO_3)_2$ (10 cm^3, 0.5 mmol) (see Section 5.3.2.) and the pH of the resulting solution was adjusted as required, filtered, and the filtrate left to evaporate at room temperature. When the volume had become ca. 3 cm^3 the colour was noted (Table 3.1 and 3.2) and those complexes of the naturally occurring bases were precipitated by the addition of ethanol (10 cm^3), methanol in the case of the nucleotides, with stirring. These very hygroscopic compounds were then separated by centrifuging. They were washed twice with ethanol (or methanol), transferred in a slurry to a petri dish, and finally dried in vacuo over P_2O_5. The colours of the solids are given in Table 3.3.

The Pregl method was used for the analysis of C and H and the brown-ring and diphenylamine tests were employed for the detection of the nitrate ion.

As a comparison, experiments using trans-[$Pt(NH_3)_2(H_2O)_2$]$(NO_3)_2$ with 5'-$CMPNa_2$ and 5'-$UMPNa_2$ and [$Pten(H_2O)_2$]$(NO_3)_2$ with acetamide, 5'-$CMPNa_2$ and 5'-$UMPNa_2$ were carried out. These platinum(II) starting solutions were prepared in the same manner as the cis compound from the complexes trans-$Pt(NH_3)_2Cl_2$ and $PtenI_2$.

Analytical Results

The analyses, (Table 5.1) were variable. This may be due to the products being mixtures. However, from the C and H analyses and nitrate ion tests, it was possible to deduce some sort of stoichiometric formulae for each compound.

Pyrimidine	Nitrate	Complex (ex. pH4.00)
5'-CMP	-	[$Pt(NH_3)_2$(5'-CMP)],4H_2O C, 17.37%(17.34%) H, 4.21%(3.94%)
5'-UMP	-	[$Pt_3(NH_3)_6$(5'-UMP')$_2$] C, 16.62%(16.66%) H, 2.95%(3.03%)
Cytidine	+	[$Pt(NH_3)_2$(C')]NO_3,H_2O C, 19.97%(20.32%) H, 3.72%(3.75%)
Thymidine	+	[$Pt(NH_3)_2$(T)]$(NO_3)_2$ C, 20.21%(20.16%) H, 3.22%(3.46%)
Uridine	+	[$Pt(NH_3)_2$(U')]NO_3 C, 20.23%(20.29%) H, 3.21%(3.29%)
Cytosine	+	[$Pt(NH_3)_2$(Cyt')]NO_3,H_2O C, 11.46%(11.38%) H, 2.89%(2.75%)
Thymine	+	a
Uracil	+	[$Pt(NH_3)_2$(Ura')]NO_3 C, 11.94%(12.00%) H, 2.26%(2.41%)

a. Too hygroscopic for analysis

(') Deprotonated ligand (e.g. (5'-UMP') = 5'-UMP^{3-})

Analysis figures in brackets represent those found

Table 5.1. Analytical data for the isolated platinum - pyrimidine blue complexes from solutions at pH 4.00.

5.5. Complexes of Chapter 4

In all preparations, dried and de-oxygenated solvents were used and the complexes were filtered under nitrogen. Because of the slight solubility of the ligand, only solution/suspensions could be used in the syntheses of the 2-pyrimidinethione complexes.

5.5.1. 2-Pyrimidinethione

$Mn(Pym2SH)_2X_2$ X = Cl, Br.

A warm solution of $MnX_2,4H_2O$ (2 mmol) in ethanol (10 cm^3) was added, with stirring, to a hot solution/suspension of ligand (0.393g, 3.2 mmol) in ethanol (10 cm^3). After heating and stirring for 15 mins., the deep-yellow crystalline solids precipitated. These were filtered from the hot solution, washed with ethanol and diethyl ether and dried in vacuo over P_2O_5.

$Mn(Pym2SH)_2Cl_2$	requires	C, 27.44%	H, 2.30%	N, 16.00%
	found	C, 27.63%	H, 2.37%	N, 16.14%
$Mn(Pym2SH)_2Br_2$	requires	C, 21.88%	H, 1.84%	N, 12.76%
	found	C, 21.93%	H, 1.96%	N, 12.88%

$Fe(Pym2SH)_2Cl_2$

$FeCl_2,4H_2O$ (0.398g, 2 mmol) was dissolved under nitrogen in a mixture of ethanol (25 cm^3) and 2,2-dimethoxypropane (8 cm^3) which had been degassed with nitrogen. Iron wire (ca. 1.5g) was added, with conc. HCl (0.2 cm^3), to provide a reducing system. The flask was sealed and shaken periodically until the solution was colourless. This was then filtered under nitrogen directly into a warm solution/suspension of 2-pyrimidinethione (0.393g, 3.5 mmol) in ethanol (15 cm^3). The mixture was heated and stirred for 10 mins. whereupon a dark brown, crystalline

precipitate formed. This was filtered off, washed with ethanol and ether and dried in vacuo over P_2O_5.

$Fe(Pym2SH)_2Cl_2$	requires	C,	27.35%	H,	2.30%	N, 15.96%
	found	C,	27.39%	H,	2.55%	N, 15.82%

$Fe(Pym2SH)_2Br_2$

This dark brown, crystalline solid was prepared as above using 40% aqueous hydrobromic acid.

$Fe(Pym2SH)_2Br_2$	requires	C,	21.84%	H,	1.83%	N, 12.73%
	found	C,	21.97%	H,	1.81%	N, 12.78%

$Co(Pym2SH)_2X_2$ X = Cl, Br

These dark green crystalline solids were prepared in a similar manner to the manganese complexes.

$Co(Pym2SH)_2Cl_2$	requires	C,	27.11%	H,	2.28%	N, 15.83%
	found	C,	27.11%	H,	2.31%	N, 15.81%
$Co(Pym2SH)_2Br_2$	requires	C,	21.69%	H,	1.82%	N, 12.65%
	found	C,	21.96%	H,	1.72%	N, 12.78%

$Co(Pym2S)_2$

The inner complex was made by adding a solution of $Co(CH_3COO)_2,4H_2O$ (0.249g, 1 mmol) or $Co(NO_3)_2,6H_2O$ (0.291g, 1 mmol) in ethanol (10 cm^3) to a hot solution/suspension of 2-pyrimidinethione (0.168g, 1.5 mmol) in ethanol (10 cm^3) and heating, with stirring, for 30 mins. The red powder which precipitated was filtered, washed copiously with ethanol and ether and dried in vacuo over P_2O_5.

$Co(Pym2S)_2$	requires	C, 34.17%	H, 2.15%	N, 19.92%
	found	C, 34.01%	H, 2.27%	N, 19.65%

$Ni(Pym2SH)_2X_2$ X = Cl, Br

The yellow-green chloride and the deep yellow bromide complexes were prepared as for the manganese compounds. These solids were not crystalline.

$Ni(Pym2SH)_2Cl_2$	requires	C, 27.15%	H, 2.28%	N, 15.83%
	found	C, 27.46%	H, 2.65%	N, 15.75%
$Ni(Pym2SH)_2Br_2$	requires	C, 21.70%	H, 1.82%	N, 12.65%
	found	C, 22.12%	H, 2.24%	N, 12.89%

$Ni(Pym2S)_2$

This yellow-green compound was formed from $Ni(CH_3COO)_2,4H_2O$ as for the cobalt inner complex.

$Ni(Pym2S)_2$	requires	C, 34.20%	H, 2.15%	N, 19.94%
	found	C, 34.35%	H, 2.10%	N, 20.17%

'$Ni_5(Pym2S)_7I_2(OH),6H_2O$'

A nickel iodide solution, made metathetically from $NiCl_2,6H_2O$ (0.47g, 2 mmol) in ethanol ($10cm^3$) and NaI (0.600g, 4 mmol) in ethanol ($10\ cm^3$), was added to a solution/suspension of 2-pyrimidinethione (0.449g, 4 mmol) in ethanol ($15\ cm^3$). The mixture was heated under reflux, with stirring, for 2 hours during which a rust-coloured precipitate formed. The solid was filtered from the hot solution, washed with ethanol and ether, and dried in vacuo over P_2O_5.

This preparation was carried out 3 times and reproducible analytical results were obtained, e.g.:-

$Ni_5(Pym2S)_7I_2(OH),6H_2O$ requires C, 23.19% H, 2.36% N, 13.52%
Ni, 20.24% I, 17.50%

found C, 23.12% H, 1.82% N, 13.31%
Ni, 20.34% I, 17.48%

$Zn(Pym2SH)_2Cl_2$

This was made by the same method as $Mn(Pym2SH)_2Cl_2$, except that 1 drop of conc. HCl was added to prevent partial hydrolysis. A lemon-yellow crystalline precipitate was formed.

$Zn(Pym2SH)_2Cl_2$ requires C, 26.45% H, 2.24% N, 15.54%
found C, 26.67% H, 2.18% N, 15.87%

$Zn(Pym2S)_2$

This lemon-yellow powder was made in a similar way to that of $Co(Pym2S)_2$.

$Zn(Pym2S)_2$ requires C, 33.40% H, 2.10% N, 19.48%
found C, 33.68% H, 2.01% N, 19.71%

$Cd(Pym2SH)_2X_2$ X = Cl, Br, I

These compounds were prepared in the same manner as for $Zn(Pym2SH)_2Cl_2$ producing lemon-yellow micro-crystalline solids for the chloride and bromide, and a cream crystalline solid for the iodide.

$Cd(Pym2SH)_2Cl_2$ requires C, 23.57% H, 1.98% N, 13.75%
found C, 23.69% H, 2.03% N, 13.83%

$Cd(Pym2SH)_2Br_2$ requires C, 19.35% H, 1.62% N, 11.28%
found C, 19.42% H, 1.61% N, 11.26%

$Cd(Pym2SH)_2I_2$	requires	C, 16.27%	H, 1.37%	N, 9.49%
	found	C, 16.22%	H, 1.36%	N, 9.55%

5.5.2. 2-Pyrimidinone

$Mn(Pym2OH)_2Cl_2$

A warm solution of $MnCl_2,4H_2O$ (0.396g, 2 mmol) in ethanol (10 cm^3) was added dropwise, with stirring, to a warm solution of 2-pyrimidinone (0.384g, 4 mmol) in ethanol (10 cm^3). After heating and stirring for 10 mins. the white solid that had deposited was filtered from the hot solution, washed with ethanol and ether, and dried in vacuo over P_2O_5.

$Mn(Pym2OH)_2Cl_2$	requires	C, 30.21%	H, 2.54%	N, 17.62%
	found	C, 30.13%	H, 3.10%	N, 18.21%

$Mn(Pym2OH)_2Br_2,2H_2O$

This was prepared from 1-propanol in the same way as $Mn(Pym2OH)_2Cl_2$ except that the solid came down as pink crystals after 3 days at 0°C. The complex was washed only with 1-propanol and dried for a short time in vacuo over silica gel.

$Mn(Pym2OH)_2Br_2,2H_2O$	requires	C, 21.69%	H, 2.73%	N, 12.65%
	found	C, 21.52%	H, 2.39%	N, 12.83%

$Fe(Pym2OH)Cl_2$

A solution of ferrous chloride in 1-propanol (see $Fe(Pym2SH)_2Cl_2$) was filtered under nitrogen directly into a warm solution of 2-pyrimidinone (0.192g, 2 mmol) in 1-propanol (10 cm^3). On heating and stirring for 5 minutes a deep orange solid was produced which was filtered and washed as before.

$Fe(Pym2OH)Cl_2$	requires	C, 21.56%	H, 1.81%	N, 12.57%
	found	C, 21.66%	H, 2.08%	N, 12.62%

$Co(Pym2OH)_2X_2$ X = Cl, Br [A]

A solution of 2-pyrimidinone (0.384g, 4 mmol) in 1-propanol (10 cm^3) was added quickly, with stirring, to a solution of anhydrous cobalt halide (2 mmol) in 1-propanol (10 cm^3). The royal-blue solid that formed almost immediately, was filtered and washed as before.

$Co(Pym2OH)_2Cl_2$	requires	C, 29.84%	H, 2.50%	N, 17.40%
	found	C, 30.04%	H, 2.73%	N, 17.52%
$Co(Pym2OH)_2Br_2$	requires	C, 23.38%	H, 1.96%	N, 13.63%
	found	C, 23.59%	H, 2.24%	N, 13.71%

$Co(Pym2OH)_2Cl_2$ [B]

A solution of 2-pyrimidinone (0.384g, 4 mmol) in ethanol (10 cm^3) was slowly added dropwise, with stirring, to a warm solution of $CoCl_2,6H_2O$ (0.476g, 2 mmol) in ethanol (15 cm^3). After heating and stirring for 15 mins. the solution was allowed to stand for 2 hours at room temperature. The purple solid that precipitated, was filtered, washed with ethanol and dried in vacuo over P_2O_5.

$Co(Pym2OH)_2Cl_2$	requires	C, 29.84%	H, 2.50%	N, 17.40%
	found	C, 30.03%	H, 2.55%	N, 17.64%

$Co(Pym2OH)_4Cl_2$

A solution of $CoCl_2,6H_2O$ (0.238g, 1 mmol) in ethanol (10 cm^3) was added dropwise, with stirring, to a warm solution of 2-pyrimidinone (0.384g, 4 mmol) in ethanol (15 cm^3). The solution mixture was heated under reflux for 30 mins. during which a red solid formed. This was filtered from the hot solution and washed as before.

$Co(Pym2OH)_4Cl_2$	requires	C, 37.37%	H, 3.14%	N, 21.79%		
	found	C, 37.26%	H, 3.28%	N, 21.63%		

$Co(Pym2OH)_4Br_2$

A hot solution of $CoBr_2,6H_2O$ (0.327g, 1 mmol) in ethanol was added, with stirring, to a hot solution of 2-pyrimidinone (0.384g, 4 mmol) in ethanol (35 cm^3). After 10 mins. the solution was cooled quickly to room temperature and the purple solid which precipitated (probably the B-form of $Co(Pym2OH)_2Br_2$) was filtered off. After leaving the filtrate to evaporate slowly overnight, nice deep red crystals were formed. These were washed with ethanol and ether and dried in vacuo over P_2O_5.

$Co(Pym2OH)_4Br_2$	requires	C, 31.86%	H, 2.67%	N, 18.58%		
	found	C, 32.00%	H, 2.66%	N, 18.78%		

$Co(Pym2OH)_5I_2$

A cobalt iodide solution, made metathetically from $CoCl_2,6H_2O$ (0.238g, 1 mmol) in ethanol (5 cm^3) and NaI (0.300g, 2 mmol) in ethanol (5 cm^3), was added, with stirring, to a hot solution of 2-pyrimidinone (0.480g, 5 mmol) in ethanol (10 cm^3). The dark red complex that immediately precipitated was isolated as before.

$Co(Pym2OH)_5I_2$	requires	C, 30.29%	H, 2.54%	N, 17.66%	I,	32.00%
	found	C, 29.71%	H, 2.26%	N, 17.47%	I,	31.82%

$Co(Pym2OH)_5(ClO_4)_2$

A solution of $Co(ClO_4)_2,6H_2O$ (0.366g, 1 mmol) in ethanol (15 cm^3) was added to a warm solution of 2-pyrimidinone (0.480g, 5 mmol) in ethanol (10 cm^3) to form an immediate pink precipitate.

$Co(Pym2OH)_5(ClO_4)_2$	requires	C, 32.54%	H, 2.73%	N, 18.97%		
	found	C, 32.60%	H, 3.12%	N, 19.08%		

$Co(Pym2O)_2$

This pink inner complex was prepared from $Co(CH_3COO)_2,4H_2O$ and 2-pyrimidinone in the same way as $Co(Pym2S)_2$.

$Co(Pym2O)_2$	requires	C, 38.58%	H, 2.43%	N, 22.49%
	found	C, 38.63%	H, 2.65%	N, 22.67%

$Ni(Pym2OH)Cl_2$

This light yellow complex was made in an analagous manner to that of $Mn(Pym2OH)_2Cl_2$ except that a 1:1 metal to ligand ratio was used.

$Ni(Pym2OH)Cl_2$	requires	C, 21.29%	H, 1.79%	N, 12.41%
	found	C, 21.34%	H, 1.86%	N, 12.53%

$Ni(Pym2OH)_2Br_2,2H_2O$

This emerald green compound was prepared from nickel bromide (0.436g, 2 mmol) and 2-pyrimidinone (0.384g, 4 mmol) in the same way as for $Mn(Pym2OH)_2Br_2,2H_2O$, but in 1-butanol.

$Ni(Pym2OH)_2Br_2,2H_2O$	requires	C, 21.51%	H, 2.71%	N, 12.54%
	found	C, 21.72%	H, 2.82%	N, 12.63%

$Ni(Pym2OH)_4I_2$

A nickel iodide solution, made metathetically from $NiCl_2,6H_2O$ (0.238g, 1 mmol) in 1-propanol (5 cm^3) and NaI (0.300g, 2 mmol) in 1-propanol (5 cm^3), was added to 2-pyrimidinone (0.384g, 4 mmol) in 1-propanol (10 cm^3). The solution was heated and stirred for 5 mins. and then evaporated to half volume on a water bath. After leaving for 24 hours at 0^oC, a yellow-green solid was formed.

$Ni(Pym2OH)_4I_2$	requires	C, 27.58%	H, 2.31%	N, 16.08%
	found	C, 27.35%	H, 2.54%	N, 15.79%

$Ni(Pym2O)_2$

The preparation of this pale green inner complex was similar to that of $Co(Pym2O)_2$ using $Ni(CH_3COO)_2,4H_2O$ (0.498g, 2 mmol) and 2-pyrimidinone (0.384g, 4 mmol).

$Ni(Pym2O)_2$	requires	C, 38.61%	H, 2.43%	N, 22.51%
	found	C, 38.72%	H, 2.68%	N, 22.64%

$Cu(Pym2OH)_2Cl_2$ [A]

A solution of 2-pyrimidinone (0.192g, 2 mmol) in ethanol (10 cm^3) was added, with stirring, to a hot solution of $CuCl_2,2H_2O$ (0.241g, 2 mmol) in ethanol (10 cm^3) to form an immediate green solid. After heating and stirring for 10 mins., the green solid redissolved and in its place a blue precipitate formed. On further heating no change occurred and therefore this solid was isolated in the usual way.

$Cu(Pym2OH)_2Cl_2$	requires	C, 29.42%	H, 2.47%	N, 17.15%
	found	C, 29.60%	H, 2.56%	N, 17.29%

$Cu(Pym2OH)_2Cl_2$ [B]

A solution of $CuCl_2,2H_2O$ (0.341g, 2 mmol) in ethanol (10 cm^3) was added in the usual manner to a warm solution of 2-pyrimidinone (0.384g, 4 mmol) in ethanol (10 cm^3) to produce an immediate light green precipitate. This precipitate redissolved on further heating and stirring to form a deep turquoise solid.

$Cu(Pym2OH)_2Cl_2$	requires	C, 29.42%	H, 2.47%	N, 17.15%
	found	C, 29.65%	H, 2.39%	N, 17.30%

$Cu(Pym2OH)Br_2$

This dark grey complex was prepared as for the blue form [A] of $Cu(Pym2OH)_2Cl_2$ using a 1:1.5 ligand to $CuBr_2$ mole ratio.

$Cu(Pym2OH)Br_2$	requires	C, 15.04%	H, 1.26%	N, 8.77%
	found	C, 14.87%	H, 1.43%	N, 8.65%

$Cu(Pym2OH)_2Br_2$

Preparation of this dark green complex was carried out in an analagous manner to that of the turquoise form [B] of $Cu(Pym2OH)_2Cl_2$.

$Cu(Pym2OH)_2Br_2$	requires	C, 23.12%	H, 1.94%	N, 13.48%
	found	C, 23.01%	H, 1.94%	N, 13.36%

$Cu(Pym2OH)_4(ClO_4)_2,S$ S = EtOH, 1-PrOH

These purple complexes were prepared by the slow addition of a solution of $Cu(ClO_4)_2,6H_2O$ (0.371g, 1 mmol) in ethanol or 1-propanol (10 cm^3) to a solution of 2-pyrimidinone (0.384g, 4 mmol) in ethanol or 1-propanol (10 cm^3) and heating with stirring for 10 mins.

$Cu(Pym2OH)_4(ClO_4)_2,EtOH$	requires	C, 31.20%	H, 3.20%	N, 16.17%
	found	C, 31.26%	H, 3.35%	N, 16.20%
$Cu(Pym2OH)_4(ClO_4)_2,1\text{-}PrOH$	requires	C, 32.28%	H, 3.42%	N, 15.85%
	found	C, 32.02%	H, 3.20%	N, 15.82%

$Zn(Pym2OH)_2X_2$ X = Cl, Br

To the solution of 2-pyrimidinone (0.384g, 4 mmol) in ethanol (10 cm^3) was added, with stirring, a solution of zinc halide (2 mmol) in ethanol (10 cm^3) plus 1 drop of the corresponding concentrated acid to prevent partial hydrolysis. After heating and stirring for 10 mins., the white microcrystalline solids were filtered off and washed as before.

$Zn(Pym2OH)_2Cl_2$	requires	C, 29.25%	H, 2.46%	N, 17.06%
	found	C, 29.55%	H, 2.62%	N, 17.27%
$Zn(Pym2OH)_2Br_2$	requires	C, 23.02%	H, 1.93%	N, 13.42%
	found	C, 22.79%	H, 1.54%	N, 13.56%

$Zn(Pym2OH)_4(NO_3)_2$

This white compound was formed immediately on the addition of $Zn(NO_3)_2,4H_2O$ (0.262g, 1 mmol) in ethanol (5 cm^3) to a warm solution of 2-pyrimidinone (0.384g, 4 mmol) in ethanol (10 cm^3), with stirring.

$Zn(Pym2OH)_4(NO_3)_2$	requires	C, 33.50%	H, 2.81%	N, 24.41%
	found	C, 33.82%	H, 2.93%	N, 24.60%

$Cd(Pym2OH)Cl_2$

A solution of 2-pyrimidinone (0.192g, 2 mmol) in ethanol (10 cm^3) was added slowly, with stirring, to a hot solution of $CdCl_2,2.5H_2O$ (0.456g, 2 mmol) in ethanol (5 cm^3) with conc. HCl (ca. 0.2 cm^3). The white precipitate that immediately formed, was filtered off after heating and stirring for 5 mins.

$Cd(Pym2OH)Cl_2$	requires	C, 17.20%	H, 1.44%	N, 10.03%
	found	C, 17.26%	H, 1.48%	N, 10.12%

$Cd(Pym2OH)_2X_2$ X = Br, I

The preparation of these white complexes was analagous to that of $Zn(Pym2OH)_2X_2$.

$Cd(Pym2OH)_2Br_2$	requires	C, 20.69%	H, 1.74%	N, 12.06%
	found	C, 20.54%	H, 1.68%	N, 12.17%
$Cd(Pym2OH)_2I_2$	requires	C, 17.21%	H, 1.44%	N, 10.03%
	found	C, 17.12%	H, 1.46%	N, 9.97%

5.5.3. The Methyl Derivatives

$Co(4,6Me_2Pym2SH)_2X_2,2H_2O$ X = Cl, Br

A solution of $CoX_2,6H_2O$ (2 mmol) in acetone (10 cm^3) was added to a warm solution of 4,6-dimethyl-2-pyrimidinethione (0.560g, 4 mmol) in acetone (15 cm^3). After heating and stirring for 15 mins., the pink solids were filtered, washed with acetone and dried in vacuo over silica gel.

$Co(4,6Me_2Pym2SH)_2Cl_2,2H_2O$	requires	C, 32.30%	H, 4.52%	N, 12.55%
	found	C, 32.43%	H, 4.33%	N, 12.49%
$Co(4,6Me_2Pym2SH)_2Br_2,2H_2O$	requires	C, 26.93%	H, 3.77%	N, 10.47%
	found	C, 27.09%	H, 3.63%	N, 10.38%

$Co(4,6Me_2Pym2SH)_2X_2$ X = Cl, Br [A]

These microcrystalline blue complexes were prepared in the same way as the above, except that the anhydrous halides were used.

$Co(4,6Me_2Pym2SH)_2Cl_2$	requires	C, 35.13%	H, 3.93%	N, 13.66%
	found	C, 35.34%	H, 4.04%	N, 13.52%
$Co(4,6Me_2Pym2SH)_2Br_2$	requires	C, 28.87%	H, 3.23%	N, 11.22%
	found	C, 28.98%	H, 3.26%	N, 11.14%

$Co(4,6Me_2Pym2SH)_2X_2$ X = Cl, Br [B]

The pink $Co(4,6Me_2Pym2SH)_2X_2,2H_2O$ complexes were heated in vacuo at 80°C for 4 hours to produce green anhydrous compounds. The chloride was darker than the bromide.

$Co(4,6Me_2Pym2SH)_2Cl_2$	requires	C, 35.13%	H, 3.93%	N, 13.66%
	found	C, 34.97%	H, 4.06%	N, 13.49%
$Co(4,6Me_2Pym2SH)_2Br_2$	requires	C, 28.87%	H, 3.23%	N, 11.22%
	found	C, 28.81%	H, 3.37%	N, 11.18%

$Co(4,6Me_2Pym2OH)_2Cl_2$

A solution of $CoCl_2,6H_2O$ (0.476g, 2 mmol) in 1-propanol (10 cm^3) was added dropwise to a hot solution of 4,6-dimethyl-2-pyrimidinone (0.496g, 4 mmol) in the minimum volume of 1-propanol, to produce a dark blue microcrystalline precipitate. This was filtered, washed with 1-propanol and ether and dried in vacuo over P_2O_5.

$Co(4,6Me_2Pym2OH)_2Cl_2$	requires	C,	38.12%	H,	4.27%	N, 14.82%
	found	C,	37.86%	H,	4.34%	N, 15.01%

$Co(4,6Me_2Pym2OH)_2Br_2$

The preparation of this complex was similar to the one above using $CoBr_2,6H_2O$ (0.654g, 2 mmol), except that 2,2-dimethoxypropane (10 cm^3) was added to the stirred solution to precipitate a royal blue solid.

$Co(4,6Me_2Pym2OH)_2Br_2$	requires	C,	30.86%	H,	3.45%	N, 12.00%
	found	C,	31.02%	H,	3.54%	N, 12.13%

$Co(1MePym2O)_2X_2$ X = Cl, Br

These royal blue complexes were made by the addition of $CoX_2,6H_2O$ (2 mmol) in ethanol (10 cm^3) to a hot solution of 1-methyl-2-pyrimidinone (0.440g, 4 mmol) in ethanol (15 cm^3). The solids were isolated by the usual procedure.

$Co(1MePym2O)_2Cl_2$	requires	C,	34.31%	H,	3.46%	N, 16.00%
	found	C,	34.36%	H,	3.63%	N, 16.12%
$Co(1MePym2O)_2Br_2$	requires	C,	27.36%	H,	2.76%	N, 12.76%
	found	C,	27.51%	H,	3.02%	N, 12.79%

5.5.4. Miscellaneous Syntheses and Deuteration Studies

Ligands

Py2SD, 4,6Me_2Pym2SD, Pym2OD and 4,6Me_2Pym2OD were prepared by dissolving the protonated ligand (0.2g) in D_2O (0.5 cm^3), heating for 5 mins. and then removing the excess liquid under vacuum over P_2O_5. This process was repeated twice. Further heating under vacuum at 80°C was needed for 4,6Me_2Pym2OD.

Due to the poor solubility of Pym2SH in D_2O, a slightly different method of deuteration was employed. A hot solution/suspension of Pym2SH (0.1g) in dry acetone (20 cm^3) was filtered into D_2O (0.75 cm^3) and the resulting liquid removed as before.

Complexes of these ligands were prepared but analyses were not performed. However, all were very similar in appearance to the analagous compounds with protonated ligands.

$Co(Py2SD)_2Cl_2$

A solution of anhydrous $CoCl_2$ (0.020g) in the minimum of dry acetone was added, with stirring, to a warm solution of Py2SD (0.034g) in dry acetone. The green precipitate that immediately came down was filtered, washed with acetone and dried in vacuo over P_2O_5.

$Co(Pym2SD)_2Cl_2$

A hot solution of anhydrous $CoCl_2$ (0.005g) in dry acetone (5 cm^3) was added to a solution/suspension of Pym2SD (0.009g) in dry acetone (10 cm^3). The resulting mixture was stirred and heated until the volume decreased to about 5 cm^3 at which point a dark green solid was produced. This was isolated as before.

$Ni(Pym2SD)_2Cl_2$

This yellow complex was prepared in an analagous way to the above except that the anhydrous nickel chloride was first dissolved in a very small amount of D_2O.

$Cu(1,2Me_2Imid)_4(ClO_4)_2$

A warm solution of $Cu(ClO_4)_2,6H_2O$ (0.185g, 0.5 mmol) in ethanol (5 cm^3) was added, with stirring, to a warm solution of 1,2-dimethylimidazole (0.192g, 2 mmol) in ethanol (5 cm^3). A purple solid was immediately precipitated and this was filtered, washed with ethanol, and dried in a desiccator over silica gel.

$Cu(1,2Me_2Imid)_4(ClO_4)_2$	requires	C,	37.13%	H,	4.99%	N, 17.32%
	found	C,	37.00%	H,	4.94%	N, 17.55%

CHAPTER 6

CONCLUSION

The interaction of metals with the nucleic acids (especially DNA) has been of considerable interest in relation to the biological activity displayed by some of these ions. It has been thought that the variations in behaviour of metals is caused by differences in the modes of binding and thus it is important to understand the exact nature of these sites.

The preparation and subsequent structural determination of several metal - nucleotide complexes has provided some evidence for the significance of various binding modes on both toxic and anti-tumour properties of heavy metals. cis-[$Pt(NH_3)_2(H_2O)_2$]$(NO_3)_2$ was found to bind only to the heterocyclic nitrogen atoms of two inosine bases (i.e. N7), to give a structure with the ligands in a base - stacked conformation, and it was shown that this may have some bearing on the anti-cancer activity of cis-platinum complexes. The result suggested that intrastrand linking of two purine bases may occur in DNA (of tumour and normal cells), during replication, thus preventing the reformation of the duplex structure. Tumour cells, however, are affected to a greater degree than normal cells because the DNA molecules replicate at a faster rate. This is coupled with the fact that there is also less time for repair mechanisms to work before the process is completely stopped.

Cadmium, on the other hand, has been shown to bind to phosphate oxygens as well as the heterocyclic nitrogen atoms in the complex [Cd(5'-CMP)(H_2O)],H_2O. This is considered to be important, for the binding modes resemble those in the only zinc - nucleotide structure to be determined i.e. [Zn(5'-IMP) $]_n$,nH_2O.[158] It is proposed, therefore, that because of the similarity in coordination tendencies, it endorses the fact that cadmium is able to easily substitute zinc in many biological

processes. Furthermore, the toxicity of cadmium may also be related to its non-specific coordination behaviour at various pH's. It was observed that two types of complexes were isolated with 5'-IMP, one with just nitrogen binding (i.e. [$Cd(5'\text{-IMP})(H_2O)_5$],H_2O) and the other, prepared at low pH, possessing coordination to nitrogen of the base, phosphate oxygens and the ribose sugar (i.e. [$Cd_2(5'\text{-IMP})_3H_2(H_2O)_6$],$6H_2O$).

The exocyclic groups of the bases i.e. O, NH_2 (and S in the minor bases) have also been considered important in the effect metals may have on the steric and electrostatic properties of nucleic acids.[360] Their involvement in the anti-cancer mechanism of cis-Pt(II) complexes and the platinum blues has been suggested as well as the influence of these groups on the structures of 2-substituted pyrimidine - metal complexes.

The reaction of cis-platinum complexes with purine nucleosides, and the nucleotides, nucleosides and bases of pyrimidine have indicated that O6 of guanine (and inosine) and O2, O4 and NH_2 of the pyrimidines may be involved in binding. The significance of the purine interaction comes from the fact that chelate formation between N7 and O6 was proposed as the initial step in the anti-cancer mechanism of cis-Pt(II) complexes. The groups of the pyrimidines, however, are thought to be important in the structures of the platinum - pyrimidine blues.

The effect of exocyclic atoms on complex structures was also investigated using 2-pyrimidinethione, 2-pyrimidinone and their methyl derivatives with mainly first row transition metals. In all cases, the metal bound to the heterocyclic nitrogen atom but only in the 2-pyrimidinethione complexes was coordination to the exocyclic group (i.e. S) taking place for certain. However, some involvement of oxygen in the complexes of 2-pyrimidinone and related ligands was indicated from physical measurements of two compounds i.e. $Co(4,6Me_2Pym2OH)_2Cl_2$ and $Cu(Pym2OH)_4(ClO_4)_2$, EtOH, and possibly other copper complexes.

It can be concluded, therefore, that transition metal ions are able to coordinate to all the available donors in nucleic acid components under certain conditions, and that the nature of these binding sites do have some biological significance.

APPENDIX I

THE STRUCTURE OF TETRAKIS(2-PYRIMIDINONE)COBALT(II) DIBROMIDE [153]

Preliminary oscillation and Weissenberg photographs showed the crystals to be orthorhombic with space group Pbcn. Unit cell dimensions were found to be a = 1.7350, b = 1.1399, and c = 1.1203 nm, U = 2.2156 nm^3 and Z = 4. R is now 0.067.

The structure shows that the complex has the formula $[Co(Pym2OH)_4]Br_2$; the geometry of the cation (Fig. A.1.) is a slightly distorted tetrahedron with the cobalt atom bound to four ring nitrogens and lying on a two-fold axis. The bromine atoms are not coordinated but are situated 0.3219 and 0.3269 nm from the N3 atoms to which they hydrogen - bond. The important bond angles and bond lengths are given in Table A.1.

The O2 atoms are virtually in the plane of the pyrimidine rings and 0.2819 and 0.2844 nm away from the cobalt. The O - C - N and Co - N - C angles of Co - PYM1 are 120.84° and 108.97° respectively. Although these are similar to the corresponding Cu - PYM1 values in the complex $Cu(Pym2OH)_4(ClO_4)_2$,EtOH (Table 4.2.), the Co - O2 distance is longer and therefore these oxygen atoms are unlikely to be interacting with the metal. This is confirmed to some extent by the electronic spectrum. Similar bands, including the shoulders at about 8900 cm^{-1}, have been observed in the spectrum of the tetrahedral complex $Co(BenzImid)_4(ClO_4)_2$ [294] which has no other available ligand donor atoms near to the metal.

Consequently, it is probable that the complexes $[Co(Pym2OH)_4][CoCl_4]$, $Co(Pym2OH)_4Cl_2$, $Co(Pym2OH)_5I_2$ and $Co(Pym2OH)_5(ClO_4)_2$, which have similar electronic reflectance and i.r. spectra, also have four - coordinate structures with a CoN_4 chromophore. This means, therefore, that the 5:1 compounds possess a non-coordinating ligand molecule. However, the

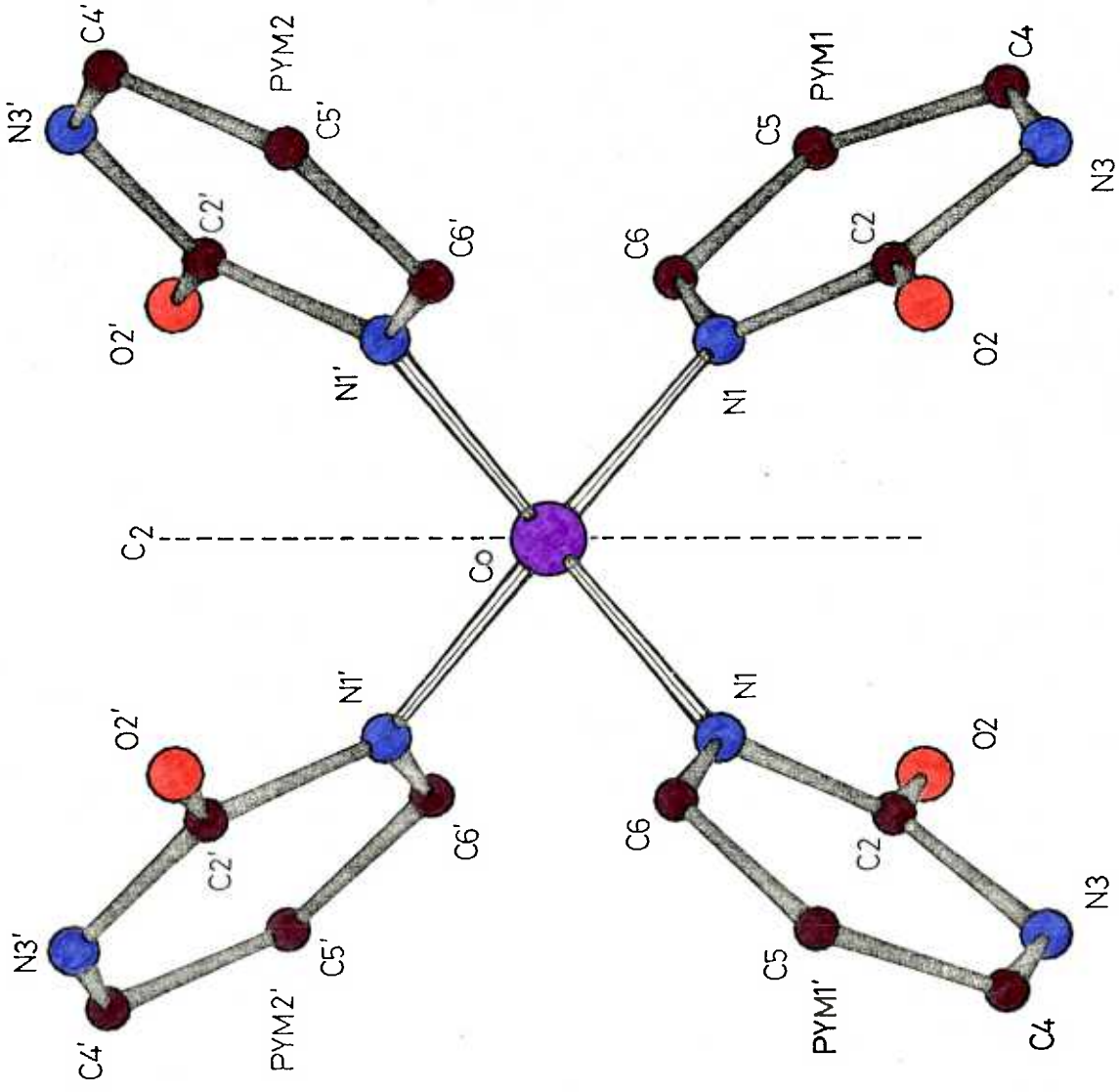
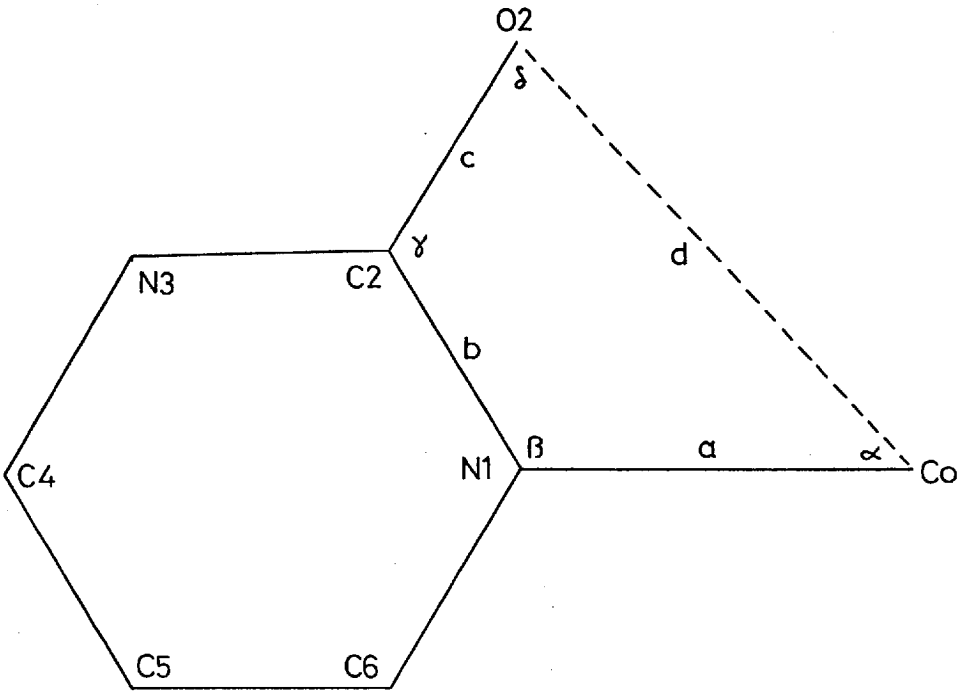


Fig. A.1. The structure of the complex cation $[Co(Pym2OH)_4]^{2+}$.



PYM1 and 1'

a = 0.2034 nm	α = 52.6° [a]
b = 0.1387 nm	β = 109.0°
c = 0.1215 nm	γ = 120.8°
d = 0.2819 nm	δ = 77.6° [a]

PYM2 and 2'

a = 0.2017 nm	α = 52.0° [a]
b = 0.1373 nm	β = 110.2°
c = 0.1210 nm	γ = 121.9°
d = 0.2844 nm	δ = 75.9° [a]

N1(PYM1) - Co - N1'(PYM2) = 101.93°
N1(PYM1) - Co - N1(PYM1') = 119.20°
N1(PYM1) - Co - N1'(PYM2') = 108.45°
N1'(PYM2) - Co - N1'(PYM2') = 117.74°

a. Calculated values assuming planarity of Co-N1-C2-O2 ring

Table A.1. Selected bond angles and bond lengths of the structure of $Co(Pym2OH)_4Br_2$.

re-examination of both normal and far i.r. spectra of the iodide and perchlorate complexes gave no indication as to the presence of this unbound ligand. Perhaps this is not unexpected as the nature of 2-pyrimidinone is such that it would probably be strongly hydrogen - bound in the complex, hence altering the band frequencies and therefore precluding positive identification.

APPENDIX II

A NEW CONSTRUCTION METHOD FOR 'BALL AND SPOKE' MODELS

In the course of the investigations on metal complexes of nucleotides, nucleosides and pyrimidines, it was found useful to construct models to show both the structural and biological aspects of the work. Commercially available kits were initially employed, but were found to be rather expensive and, more importantly, not very versatile. Because of this, it was decided to develop a model system, which would fulfill the necessary requirements, incorporating easily obtainable parts with the use of normal workshop facilities. By using the 'ball and spoke' approach,[356] several models were constructed very cheaply (approx. 10% of the cost of normal models) yet these were accurate, robust and not restricted in their stereochemistries. These features are exhibited in two large models.

Part of a DNA molecule in the B-form (1.1 turns) was built (Plate I), to help to understand the manner in which platinum compounds act as anti-cancer agents for the determination of potential binding sites. A model of the complex [Cd(5'-CMP)(H_2O)],H_2O was also made to show the distortion around the cadmium atom (Plate II). The general method of construction is now discussed.

The atoms of the structures were represented by various coloured polythene spheres of different sizes and stainless-steel rod was used for the bonds. The scale of the models was 0.025 m ≡ 0.1 nm.

The spheres were of three sizes, 1) 0.006 m diameter for hydrogen, 2) 0.013 m diameter for elements of the second period and 3) 0.016 m diameter for the heavier atoms. Also, the colours corresponded to the widely accepted code of black for carbon, blue for nitrogen and red for oxygen etc. Connection of the spheres was achieved by using bonds made

from 0.0016 m diameter rod. A guillotine[357] was employed to cut this rod to prescribed lengths accurately (and cleanly), and the resulting bonds were inserted into holes made to a specific depth using a No. 52 drill. By this method, a tight fit was accomplished which accounted for the robust nature of the models. The exact depths of the holes depended on the size of the spheres and were 0.0035 m, 0.0055 m and 0.007 m for the 0.006 m, 0.013 m and 0.016 m diameter spheres respectively.

To obtain the correct stereochemistry around each atom (sphere), drilling was carried out using two jigs. These were made especially for use with normal workshop bench drilling machines. A hole is first made in a sphere (i.e. termed 'initial hole') using Jig I [357] which is essentially a cup with a locating hole (Fig. A.2.). After positioning Part B of the jig correctly on the drill table by means of the wooden base, a sphere is placed into the cup. For safety reasons (and to prevent movement of the sphere during the operation), a cover (Part A) is placed over the lower portion of the jig and held with the fingers. The hole is then drilled to the required depth (which is often achieved after several attempts) and the travel distance of the drill carriage locked. This is now set for the production of 'initial holes' in other spheres of the same diameter.

The sphere is then transferred to Jig II, which is shown in Plates IV and V, and fixed onto the spindle axis A using the 'initial hole'. This axis, which controls the angle 'around' the initial hole (θ^{o}), is connected to a perspex disc marked 0^{o}, 90^{o}, 120^{o}, 180^{o}, 240^{o} and 270^{o}. Axis T however, controls the angle 'to' the initial hole (ϕ^{o}) and the corresponding disc is marked 90^{o}, 109.5^{o} and 120^{o}. The sphere can now be moved into the positions for all the common stereochemistries (Table A.2.).

The drilling procedure can now be explained by taking as an example a tetrahedron. After setting up the jig, a sphere with an initial hole is

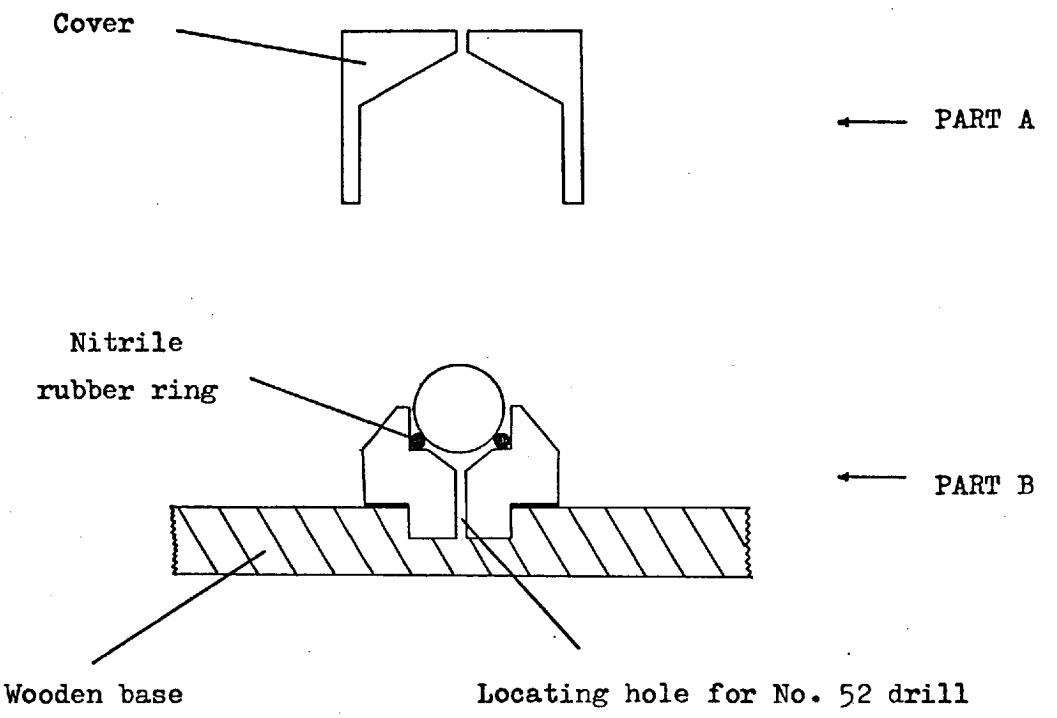


Fig. A.2. Cross-sectional diagram of Jig I.

pressed onto the spindle axis A up to the shoulder which exactly centralises the sphere. The disc T is first adjusted to 109.5° and the position locked. Disc A is then moved to 0° (which is held in place by a locating spring B) and the support raised to 'cup' the sphere and take the downward pressure when the second hole is drilled. The support is lowered, so that disc A can be turned to 120°, returned to its raised position and the operation repeated. The fourth and last hole is drilled at $\theta = 240^{\circ}$.

Distorted stereochemistries can also be produced if ϕ and θ are calculated for each angle relative to the initial hole. The discs A and T are then adjusted and the holes drilled in the same manner as before.

Although the resulting models justify the use of this method in building all types of structures, the system has one major disadvantage.

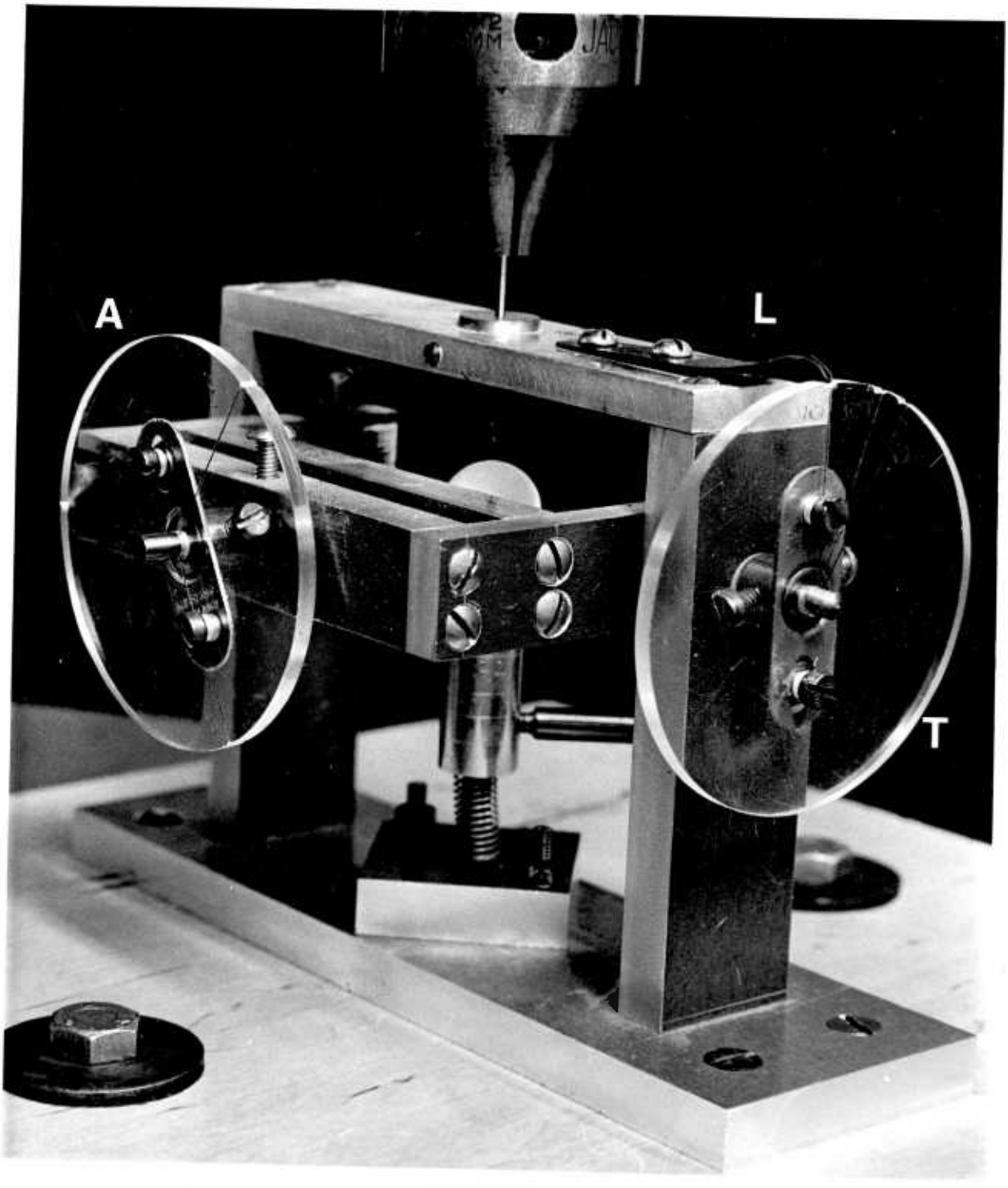


Plate IV

View of Jig II from 'closed' side

A = Axis '<u>around</u>' initial hole (θ^{o})

T = Axis '<u>to</u>' initial hole (ϕ^{o})

L = Locating spring

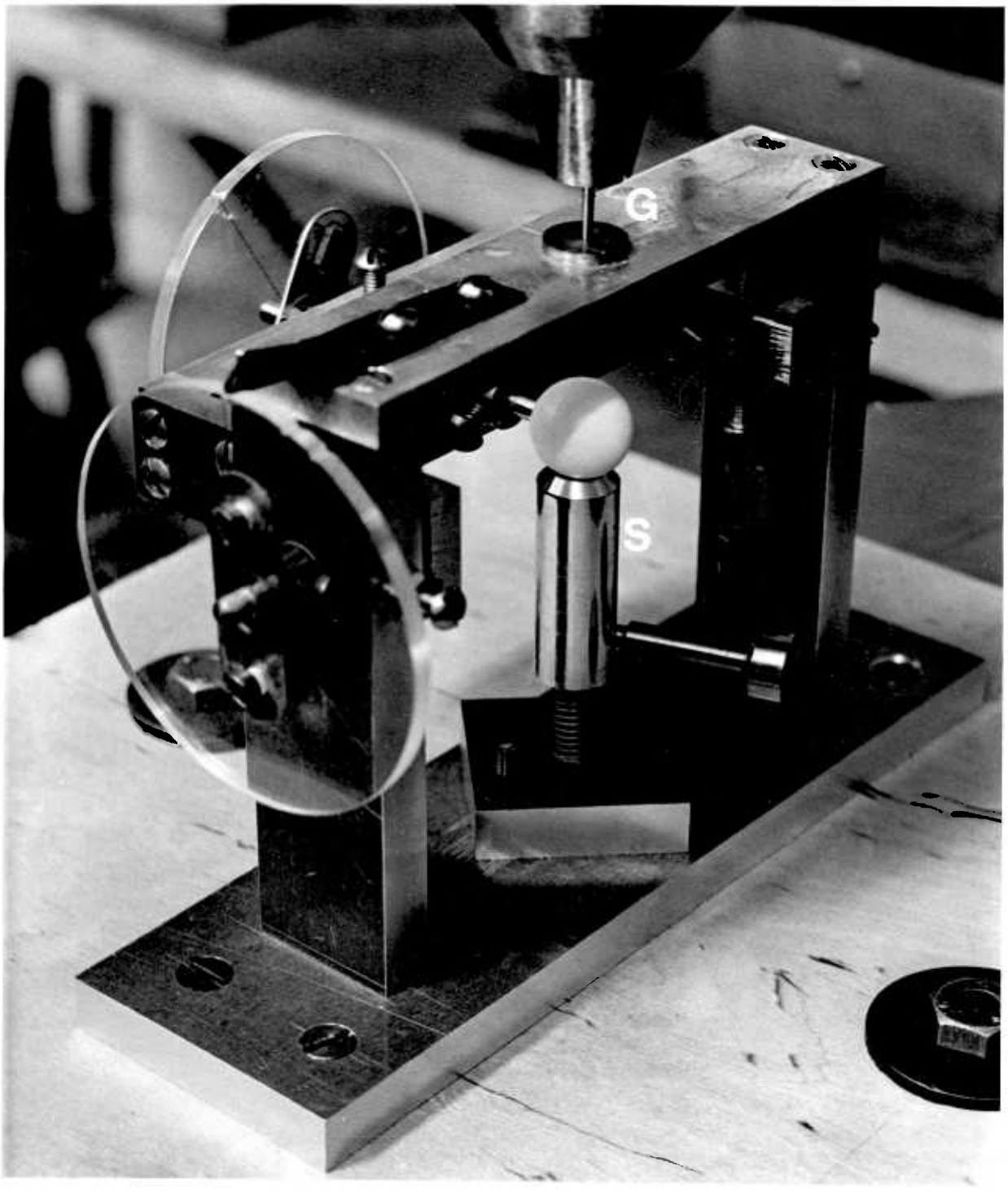


Plate V

View of Jig II from 'open' side

G = Drill guide

S = Sphere support

	Stereochemistry	Jig I	Jig II	
			ø	θ
1.	Linear	Initial hole + 180° hole	-	-
2.	Trigonal planar	Initial hole	120°	0° 180°
3.	Tetrahedral	Initial hole	109.5°	0° 120° 240°
4.	Trigonal bipyramidal	Initial hole + 180° hole	90°	0° 120° 240°
5.	Square planar	Initial hole + 180° hole	90°	0° 180°
6.	Octahedral	Initial hole + 180° hole	90°	0° 90° 180° 270°

Table A.2. The method of producing drilled spheres with regular stereochemistries.

This concerns the production of only permanent models, for any rearrangement of the structures (e.g. boat - chair conformations) would tend to make the bonds fit less tightly in the polyethylene spheres. The problem is overcome to some extent by the low cost of the components and hence several models can be produced to show the difference in geometries. Nevertheless, because of the easy use of the system, its future application undoubtedly lies in 1) schools and colleges which have limited resources but with the necessary equipment, and 2) the construction of models of compounds with unusual stereochemistries.

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