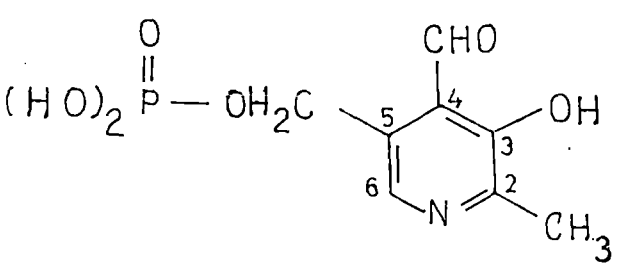
CHAPTER I

Introduction

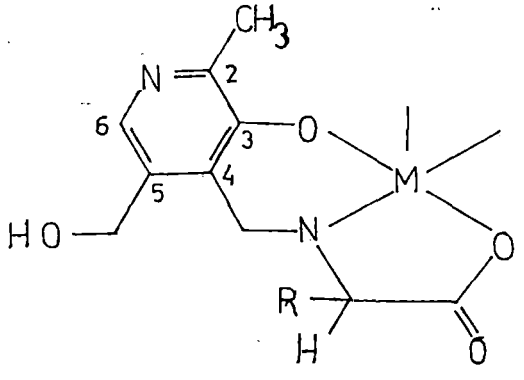
Aims and objectives of the work :

This work is concerned with synthetic, chiroptical and electrochemical studies on coordination compounds of aldimine derivatives of α -amino acids with well-defined oxocations like UO_2^{2+}, $[Mo_2^{V}O_3]^{4+}$, $[Mo_2^{VI}O_5]^{2+}$ [16,17,21,25,44,101,110,114,121,122,170,220] etc. The reasons behind this persuit are outlined below.

The biocatalytically active form of vitamin B_6 is pyridoxal phosphate (1); it functions in non-oxidative



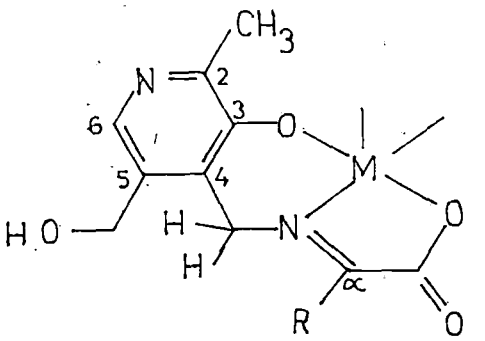
enzymatic transformations of amino acids and catalyzes a remarkably diverse series of reactions in amino acid metabolism including decarboxylation, racemization, transamination, β-elimination and γ elimination[4-7,34,203,221-22]. Despite the lack of proof that these enzymes function as metalloenzymes, a degree of interest continues to be maintained in systems which effect amino acid synthesis or transformations in the presence of pyridoxal or pyridoxamine, their phosphorylated derivatives, or their analogs and metal ions. The latter may simulate certain of the features of enzymatic active sites in the sense of facilitating Schiff base formation and of labilizing bonds formed by the α-carbon in the condensed amino acid portion of complexes of type (2). Obviously, the metal ion containing systems



Pyridoxylidene (aldimine) Schiff base complex

are commonly referred to as model systems for enzymatic reactions in spite of the lack of evidence for participation

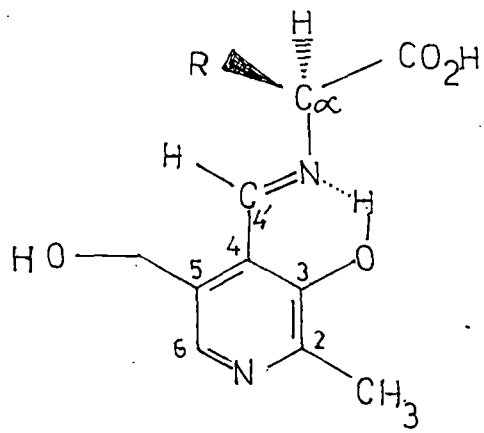
of metal ions in these reactions[6].



Pyridoxylimino (ketimine) Schiff base complex

The formation of aldimine or ketimine Schiff base is an essential feature of all currently accepted mechanisms of vitamin B_6 catalysis, many of which require lability of the α-hydrogen in those reactions proceeding to products through an intermediate aldimine[203,222].

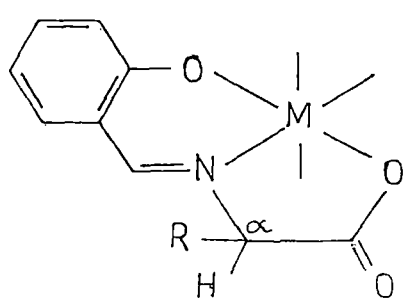
The above-mentioned reactions of Schiff bases involve a number of electron and hydrogen shifts and most likely are controlled by subtle conformational effects. It has been suggested that the bond perpendicular to the π system of the Schiff base is most easily broken and that the enzymes govern reaction specificity by controlling the conformation of the N-C_α bond. Another important structural feature is



the conformation of the C_4-C_4' bond, which is predominantly cis[2].

The fundamental factors are variations in resonance energy and electronic distribution among the possible states[6].

Our present-day understanding of enzyme transamination is based to a great extent on the work with chemical model



reactions ("biomimetic") rather than an experiment with enzyme-catalyzed reactions. In such model studies the salicyladimine complexes (5) are also considered whose structural and electronic properties are similar in several respects to those of pyridoxylidene (2) and pyridoxylimino (3) Schiff base complexes. However, salicylaldehyde does not possess the catalylic properties of pyridoxal or the simpler hydroxy pyridine aldehyde in amino acid transformation[6].

In such model studies the first transition metal ions have been employed extensively. Apart from physico-chemical and kinetic studies on such complexes, several of them have been isolated in the solid state and their structures determined through X-ray crystallography[6,31-33]. The usual M^{n+}:aldehyde:amino acid ratio is either 1:1:1 or 1:2:2. The metal containing model systems regarding transformations of amino acids, still continue to attract considerable attention for a couple of reasons.

(1) There is no other group of metal complexes which manifest as great a diversity of reactions of coordinated ligands, whether of biological significance or not, as do pyridoxylidene and pyridoxylimino chelates. Elucidation of reactions of coordinated ligands is one of the significant research activities in coordination chemistry[6,7].

(2) There remains much to be understood about the basic structural and electronic features of the complexes themselves[6]. For an understanding of reactivities it is essential to possess knowledge regarding the configurational/conformational aspects of such complexes in solution in the light of chiroptical methods like CD spectra supported by other evidences especially ^{1}H NMR data. Smith and coworkers have developed a salicylideneamino (SA) chirality rule (using the coupled oscillator model for the generation of Cotton effects) which correlates unambiguously the sign of the relevant CD bands (especially the one around 315-320 nm) with the absolute configurations/conformations of chiral Schiff base derivatives of a wide ranging chiral amines including amino acids and amino sugars[1]. In recent years chiroptical methods have been developed considerably for determining absolute configurations of in situ transition metal complexes of ligating natural products (e.g., carboxylic acids, glycols, amines, amino-alcohols, diamines, peptides and nucleoside derivatives) from CD spectra[28].

Chang and workers have carried out conformational analyses of a series of pyridoxal-amino acid Schiff bases using ^{13}C and ^{1}H NMR spectral techniques[2]. In spite of all these developments, comprehensive chiroptical studies are

still needed on the coordination compounds of aldimine derivatives of amino acids, for bringing the inferences drawn regarding their absolute configurations/conformations from CD and NMR spectra, within a single frame work.

The present work has been undertaken with this goal in mind using the dioxouranium (VI) entity as a diamagnetic NMR probe; the 2D NMR technique has proved to be a valuable tool in this endeavour. The usually pentagonal planar coordination geometry of the UO_2^{2+} entity, coupled with its high atomic weight, allows strong ligand coordination. The three principal donor atoms (CH=N, phenoxide oxygen and carboxylate oxygen) of the aldimine ligand are restricted to the equatorial coordination plane and in most cases essentially one set of signal is observed for each of the ligand protons in the immediate vicinity of the coordination zone (e.g., CH=N, H_α etc.), leading to a simplified NMR spectrum. For a corresponding octahedral complex, which normally involves diastereoisomer formation through λ-/δ - puckering of chelate rings, additional signals are observed[36]. Besides this, the spatial relationship between the H_α and CH=N protons, is to be probed by a more direct method, e.g., 2D NMR technique, for rationalizing its implications regarding the nature (multiplicity) of CH=N signal as well as its relation to the sign of the decisive CD maximum (ca.315-320 nm) of the aldimine derivatives. Occasional

splitting of the original singlet CH=N signal in spin decoupling experiment throws light on the subtle spin interaction between the H_{α} and CH=N protons.

The dioxouranium (VI) entity has a well-developed coordination chemistry[16,17,21,25,40,44,53-57,60]. The electronic structure of the UO_2^{2+} entity has been the subject of considerable attention for the interpretation of electronic spectra of the UO_2^{2+} entity and its analogs[58,59,65]. The recent surge of publications on the different aspects of coordination compounds of dioxouranium (VI) is a sure indication of the current interest that centres around this dioxometal entity as a versatile coordinating agent[12,45-49,68-71,106,147-149,224-228].

Another part of this work is addressed to the CD property of "oxo-type" molybdoenzymes[178,194,196]. These molybdoenzymes possess a common cofactor (Mo-Co), which is a complex of a novel 6-alkyl pterin (called molybdopterin) with the molybdenum atom through the dithiolene function[104,146,195]. In addition to the pterin component, the Mo-Co is associated with a peptide[175,189]; both of them are vital for the oxo-transfer activity of these enzymes. The pterin group is most probably involved in electron transfer with the molybdenum centre during turn over[205], while the peptide chain is likely to create a sterically demanding ligand environment around the molybdenum atom,

thereby preventing the irreversible oxo-dimerization reaction during the oxo-transfer cycle[206-208].

The analyses of amino acid composition of Mo-Co from typical 'oxo-type' molybdoenzymes like sulfite oxidase and xanthine oxidase, indicated the presence of several amino acids including histidine and arginine[175,189].

At this stage a brief pause may be appropriate for having a bird's-eye view of the facinating aspects of chemistry and biochemistry of molybdenum[99,101,110,114,119,121,122,137,164,169-171,173-175,182,183,189,194-196,206,208-210,214,219,220].

Long ago Nature recognized the chemical uniqueness of molybdenum that scientists have only recently learned to appreciate. This second-transition-row metal is an essential micronutrient for most if not all life forms. It has unusual chemical capabilities as an oxo-transfer agent, a redox -partner and as an easily transportable oxyanion. Well -developed molybdenum chemistry is known for oxidation states 0 through +6. Although the other Group VI metals, chromium and tungsten, display a similar range of oxidation states in their compounds, it is the overlap in the redox potentials available to biological systems and to molybdenum that makes it the preferred metal. Organisms in most cases find chromium too difficult to oxidize from the trivalent state

and tungsten too hard to reduce from the hexavalent state. Molybdenum is not as "oxophilic" as the early transition metals of group IV and V. Its strong tendency to bind oxo groups (=O) is balanced by a capacity to lose a single oxygen atom easily. The biochemical transformations involving oxo-type molybdoenzymes are oxidation-reduction reactions and may involve at least formal oxygen atom transfer processes. The one-and two-electron transfer capability associated with molybdenum and its ability to couple ion (H^+ or O^{2-}) transfer with electron transfer may be crucial to the chemical role played by molybdenum in enzymes[169,220].

The lack of knowledge of details of molybdenum biochemistry gives us some liberty to speculate in the hope that some suggestions may be of use as working hypotheses to be tested in enzyme or models. The coordination bioinorganic chemist can play a crucial role in devising and evaluating such proposals in many ways, e.g., by preparing, isolating and thoroughly characterizing molybdenum compounds which mimic the spectral and/or chemical behaviour of oxo-type molybdoenzymes[101,174].

Molybdoenzymes are important for catalyses within the metabolic cycles of carbon, nitrogen, sulfur and chlorine[169,170]. The structural and functional aspects (more than 12) of molybdenum enzymes have been probed intensively over the past two and half decades.

The current absence of x-ray crystallographic data for any of the molybdoenzymes has meant that spectroscopic techniques have been the main source of information concerning the nature of these molybdenum centres. Spectroscopic methods including EXAFS, Mössbauer, EPR, ENDOR, CD and MCD spectroscopy have provided detailed information about the molybdenum site, while its biological activity has been increasingly well defined by kinetic, biological and genetic investigations.

EPR spectroscopy has been extensively applied to oxo-type molybdoenzymes[164,169,173,182,183]. These studies have established that all of these enzymes have similar molybdenum centres, at which the substrate undergoes the catalytic conversion. Strong evidence has been obtained to indicate that the redox states of molybdenum involved in the normal cycle of these enzymes, are Mo(VI), Mo(V) and Mo(IV). Furthermore, for xanthine oxidase, EPR spectroscopy has provided direct evidence for the hypothesis developed by Stiefel that the mechanisms of all the molybdoenzymes involve coupled proton-electron transfer at the molybdenum centre. The EPR spectra obtained by Gutteridge and Bray using ^{17}O-enriched H_2O have demonstrated that oxygen ligands are bound to the Mo(V) centres of the enzymes, the exchangeability of these ligands with bulk solvent molecules and the strength of the hyperfine coupling varying from one

reduced form of an enzyme to another. These and other EPR studies have documented important features of the catalytic mechanisms of the oxo-type molybdoenzymes and have shown that there are significant differences in the nature and reactions of the active centres of these enzymes.

EXAFS studies on the oxo-type molybdoenzymes are complementary to the EPR studies, since recording EXAFS data for the oxidized [Mo(VI)] or fully reduced [Mo(IV)] state is much more readily accomplished than for the partially reduced [Mo(V)] state. The nature, number and distance of the ligand donor atoms attached to molybdenum in the oxo-type molybdoenzymes, so far studied by EXAFS are well documented[101,169,170]. In view of the recent EPR results demonstrating that the molybdenum centres of the oxo-type molybdoenzymes are accessible to chloride it may be necessary to revise these data, since it is difficult a priori for the distinction between Cl and S donor atoms to be established from the molybdenum K-edge EXAFS. The detailed EPR and EXAFS studies presently constitute the most definitive view of the immediate environment of molybdenum in the oxo-type molybdoenzymes[170].

The detailed nature of the Mo-Co of several oxo-type molybdoenzymes has been probed extensively using biochemical methods by Rajagopalan, Johnson, Coughlan and other workers[88,104,146,175,195,230,231].

EPR titrations and CD potentiometry have been employed nicely for elucidating the interactions among the various redox centres of nitrate reductase[171,178].

Structurally characterized oxomolybdenum centres including the polynuclear species have been summarized in a couple of reviews[101,110,170,220]. They invariably incorporate accounts of their coordination chemistry.

Model reactions of molybdenum complexes have been discussed vis-a-vis the mechanisms of reactions of molybdenum enzymes by Spence, Wentworth, Stiefel and others[122,169,170,210,219,234].

Several authors have attempted to explain the electron transfer processes in the oxo-type molybdoenzymes[122,178,219,232,233].

Early attempts to model the reactivity of oxo-type molybdoenzymes were hindered by the formation of μ-oxo Mo(V) dimers through reaction of freshly made MoO^{2+} with unreacted MoO_2^{2+} according to reaction $2(MoO_2^{2+} + MoO^{2+} \longrightarrow Mo_2O_3^{4+})$.

$$MoO_2L_n + X \rightleftharpoons MoOL_n + XO \quad \ldots (1)$$

$$MoO_2L_n + MoOL_n \rightleftharpoons Mo_2O_3L_{2n} \quad \ldots (2)$$

Recently, catalysis of oxygen atom transfer has been accomplished by using an oxo-bridged Mo(V) complex as an

active participant in the catalytic cycle. Hence the role of oxo-bridged Mo(V) complexes in oxygen atom transfer reactions of systems designed to model enzyme behavior cannot necessarily be ignored[103,177]. Years earlier while explaining mechanisms for the reactions of oxo-type molybdoenzymes, Wentworth postulated the participation of an oxo-bridged dimer at one stage of the catalytic cycle[219]. Spence suggested the possible occurrence of an EPR active (triplet) paramagnetic Mo(V) dimer in this process through involvement of a dimer-monomer equilibrium (strongly in favour of the dimer) with Mo(V)[122].

Due cognizance should be taken about the role of tetranuclear species in oxomolybdenum chemistry, since the enzyme nitrate reductase exists as a homotetramer, with each subunit (MW 96,000) containing FAD, a b-type cytochrome and a Mo-Co possessing a complex of molybdenum-molybdopterin in a ratio of 1:1:1[229]. Parallel examples exist in synthetic systems, e.g., in recent years several tetranuclear molybdenum-containing complexes have been structurally characterized[107-110].

Actually there are other important chemical results whose biochemical relevance are yet to be perceived.

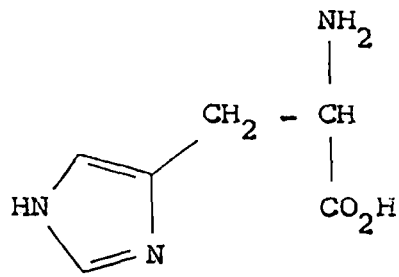
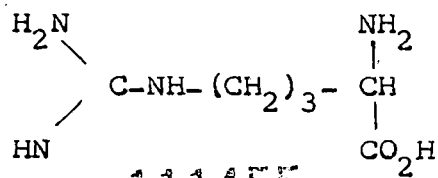
These works give a vivid impression of the richness of molybdenum chemistry as it has evolved in biology. The field now stands on a solid foundation and is poised for

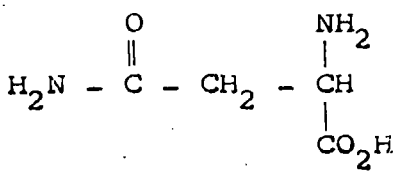
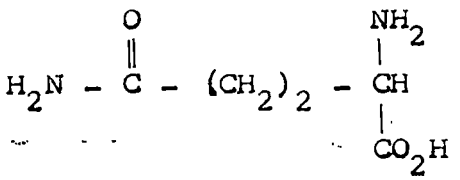
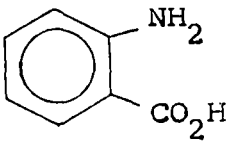
major advances in the coming years.

Finally electrochemical studies integrate the two above-mentioned parts of this work. Their main purpose is to demonstrate experimentally the dependence of electron transfer reactions on conformational factors[75,76,138,203].

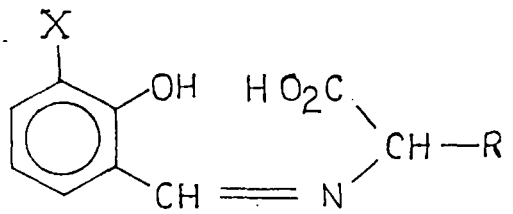
The present study :

This thesis presents physico-chemical studies on new coordination compounds of dioxouranium(VI) and bi-/tetranuclear molybdenum (V,VI) species with aldimine ligands derived from α-amino acids (especially with imidazole, amine and amide R groups). A pair of ligands containing anthranilic acid has been included in this study, to follow the difference in their coordinating ability towards UO_2^{2+} entity.

α-Amino acid	Formula	Number of ligands derived from the amino acid
Imidazole		
Histidine (His)		2
Amine R		
Arginine (Arg)		2

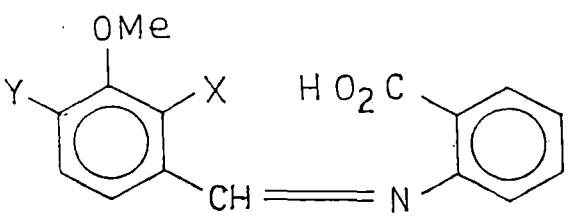
Amide R		
Asparagine (Asn)		2
Glutamine (Gln)		1
Other amino acid		
Anthranilic acid		2

The pertinent aldimine ligands conform to the following types.



X = H or OCH_3;

N-(salicyledene/orthovanillidene) amino acid.



X = H, Y = OH;

N-(Vanillidene) anthranilic acid.

X = OH, Y = H;

N-(orthovanillidene) anthranilic acid.

Different stages of this work represent persuits for achieving the following research goals.

(1) Use of the UO_2^{2+} entity as a diamagnetic NMR probe for the simplification/interpretation of ^1H NMR signals of chelated aldimine ligands.

(2) Study of conformational control of metal-/ligand-centred electron transfer processes in coordination compounds of aldimine ligands.

(3) To develop a synthetic CD model of oxo-type molybdo-enzymes even at a rudimentary level, for understanding the CD properties of such enzymes.

The new coordination compounds synthesized in course of this investigation, have been thoroughly characterized by elemental analysis and different physico-chemical studies including IR, UV-visible, 2D NMR (H-H COSY) and CD spectroscopy. Some of these compounds have been subjected to the scrutiny of FAB mass spectrometry. The crystal and molecular structures of $[UO_2(OV\text{-}L\text{-}His)(bipy)]\cdot CH_3OH \cdot H_2O$, have been determined by x-ray crystallography; this complex has been used as a model for understanding the chiroptical properties of this and related systems, using a coupled oscillator model and taking the assistance of NMR data. Such studies on quasi-enantiomeric dioxouranium (VI) complexes are helpful for ascertaining the relative contributions of configurational,

conformational and vicinal factors, to the observed optical activity. Their electrochemical properties have been studied using cyclic voltammetry and coulometry; for the μ-oxo -bridged Mo(V) complexes, differential pulse voltammetry has served as an additional valuable tool. One of these Mo(V) dimers is paramagnetic as well as chiral; its EPR and CD spectra have been recorded and compared vis-a-vis with those of corresponding data of oxo-type molybdoenzymes.

For the UO_2^{2+} - aldimine acid systems it has been possible, to block dimerization reactions through base adduct formation (with 2,2'-bipyridyl and 1,10-phenanthroline, i.e., formation of mononuclear complexes with a UO_2^{2+} : aldimine acid : bipy or phen ratio 1:1:1). This a vital step for chiroptical studies. Experimental data point towards extensive M$\rightarrow$L π bonding in such cases.

The thesis is composed of five chapters. In chapter I brief reviews on different aspects of aldimine ligands and bioinorganic chemistry of molybdenum, have been presented along with the objectives of the present work. Salient aspects of the chemistry/coordination chemistry of the relevant oxo metal species, have also been touched upon.

Chapter II delineates the coordination chemistry of dioxouranium(VI) with aldimine ligands derived from L-/D-histidine (type 6). Histidine is an α-amino acid with a side chain containing 'soft' donor centres, i.e., the aromatic nitrogen donors.

Chapter III presents similar studies with aldimine ligands which contain L-/D-arginine as well as anthranilic acid (type 6 & 7).

Chapter IV reports the studies on coordination compounds of the same dioxocation with aldimine ligands (type 6), conaining asparagine and glutamine. Here a couple of 2,2'-bipyridyl/1,10-phenanthroline bridged dimers have been isolated in the solid state, which may be regarded as structural models of the active centre of the metalloenzyme, copper-zinc superoxide dismutase.

Chapter V is concerned with the studies on the different facets of the bi-/tetranuclear molybdenum (V,VI) complexes with aldimine ligands derived from L-/D-histidine and arginine. Interpretation of some of the experimental results, e.g., electrochemical data, EPR and CD spectra, stirs up considerable intellectual interest.