A STUDY OF SOME METAL COMPLEXES OCCURRING AS INTERMEDIATES IN THE METAL CATALYSIS OF ~~THE~~ TRANSAMINATION REACTION.

A thesis submitted to the University of London for the
degree of Master of Philosophy.

by

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ABSTRACT

The aim of this research was to isolate metal complexes which occurred as intermediates in the metal catalysis of the transamination reaction of pyridoxal phosphate and α-glutamic acid (pyridoxamine phosphate and α-keto glutaric acid). Metal ions used were gallium, copper, nickel, cobalt(II), iron(III) and aluminium.

The infra-red, ultra-violet and visible spectra of the complexes have been measured and assignments of bands made, with special reference to previous work in the field. Mass spectra were performed on several copper complexes, and fragmentation patterns have been evaluated,

It was found that mixed metal Schiff base species occurred in the reaction of pyridoxal phosphate with glutamic acid to form pyridoxamine phosphate and α-keto glutaric acid.

In all the metal species, there was a 1:1 ratio of metal ion to Schiff base species, and the remaining coordination positions of the metal ion were occupied by either an amino acid molecule, or anion radicals (such as OH^- or SO_4^{2-}). Bridging metal dimers tended to be more the rule than the exception.

ACKNOWLEDGEMENTS

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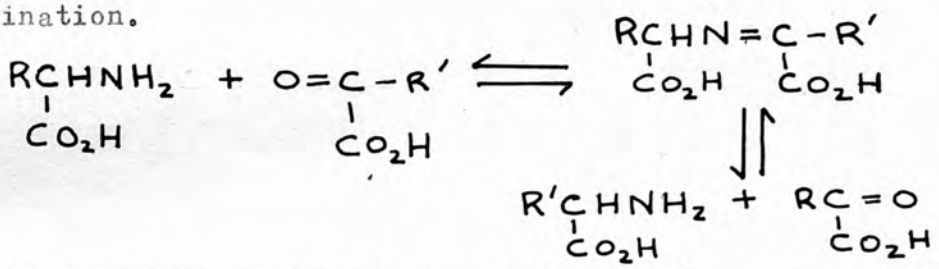
SECTION I

INTRODUCTION and THEORETICAL CONSIDERATIONS.

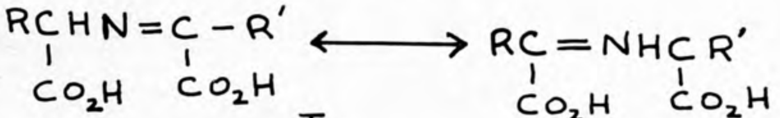
TRANSAMINATION REACTIONS.

Transamination was defined by Snell[1], as the intramolecular transfer of an amino group from an α-amino acid to an α-keto acid. This is also referred to as oxidative deamination.



$$\underset{CO_2H}{RCHNH_2} + O=\underset{CO_2H}{C}-R' \rightleftharpoons \underset{CO_2H}{RCHN}=\underset{CO_2H}{C}-R' \rightleftharpoons \underset{CO_2H}{R'CHNH_2} + \underset{CO_2H}{RC}=O$$

The intermediate in the transamination reaction is called a SCHIFF base which can exist as in tautomeric forms of aldimine and ketimine. (I and II)



$$\underset{CO_2H}{RCHN}=\underset{CO_2H}{C}-R' \longleftrightarrow \underset{CO_2H}{RC}=NH\underset{CO_2H}{C}R'$$

I II

SCHIFF BASE FORMATION.

Schiff bases are formed in the general reaction of an aldehyde with a primary amine. The intermediates formed are characterised by a prominent yellow colour which typifies the Schiff base, or imine class.

$$RCHO + R^1CH_2NH_2 \rightleftharpoons RCH = NHCH_2-R^1+H_2O$$

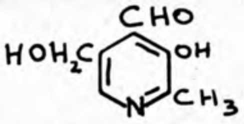
VITAMINS AND ENZYMES.

In 1945, Snell discovered that vitamin B_6 was comparable to enzymic transaminase*+ in its growth promoting properties. He postulated the existence of an aldehyde and amine form of the Vitamin which might be interconvertable in the course of transamination.

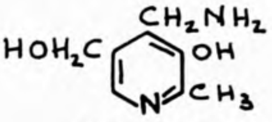
*An enzyme is defined as a protein with catalytic properties due to its power of specific activation.

+A coenzyme is an additional substance required, by some enzyme-substrate systems, in order that the reaction may proceed. The coenzyme is found to be unchanged at the end of the reaction These substances which are essential to the enzyme are called the cofactor of the enzyme or coenzyme

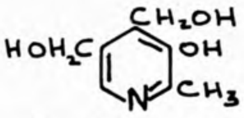
The two compounds which were synthesised were found to be 2-methyl-3-hydroxy-4-formyl-5-hydroxymethyl pyridine and 2-methyl-3-hydroxy-4 aminomethyl-5 hydroxymethyl pyridine and were subsequently named pyridoxal and pyridoxamine respectively. The chemical formulation, Vitamin B_6, was subsequently named pyridoxine.



III PYRIDOXAL



IV PYRIDOXAMINE



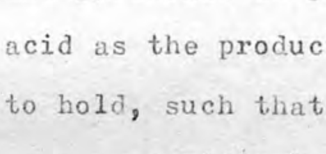
V PYRIDOXINE

REACTIONS of PYRIDOXAL and ANALOGS.

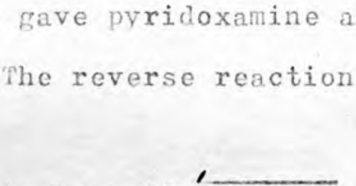
Snell[2] found that heating a solution of glutamic acid and pyridoxal at pH 6.8 gave pyridoxamine and α-ketoglutaric acid as the products. The reverse reaction was also found to hold, such that:

pyridoxal + glutamic acid $\rightleftharpoons$ pyridoxamine + ketoglutaric acid

This reaction was found to occur with other amino acids with pyruvic acid in non-enzymic reactions. The coenzyme or prosthetic group for full activity of the glutamic-aspartic transaminase and of glutamic-pyruvic transaminase, was found to be a phosphorylated derivative of pyridoxal and pyridoxamine. These were conclusively established to be pyridoxal-5'-phosphate (PLP) and pyridoxamine-5'-phosphate, (PMP),



VI
PYRIDOXAL PHOSPHATE



VII
PYRIDOXAMINE-5'-PHOSPHATE

PLP was proved to act as a catalyst in a variety of reactions with amino acids. However, the addition of a metal ion to pyridoxal seemed to simulate the enzymic reactions of pyridoxal-5'-phosphate. These reactions will be discussed in a later section, (p. 13).

Studies of the reaction of pyridoxal, analogous to enzymic reactions, led Braunstein & Snell to postulate the following mechanism: the interaction of pyridoxal with an α-amino acid gives a Schiff base with may exist in two tautomeric forms*, the Schiff base then yields pyridoxamine and α-ketoglutaric acid, on hydrolysis: (Fig. I)

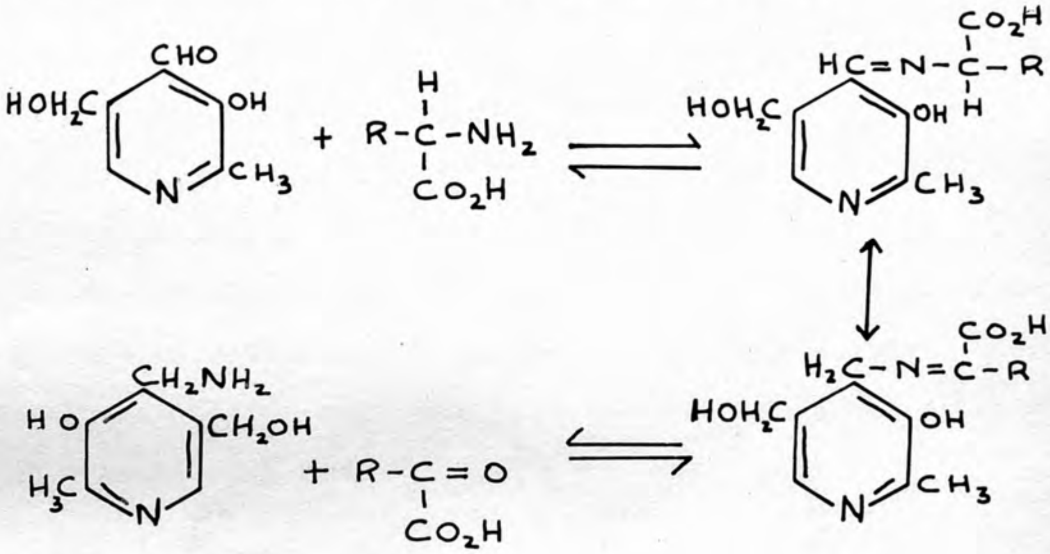


FIG. I.

The intermediate Schiff bases were characterised by their typical yellow colour and also their electronic spectra. This Braunstein-Metzler-Snell mechanism has been adopted by successive authors as the basis for transamination reactions for both the pyridoxal and pyridoxal phosphate catalysed reactions.

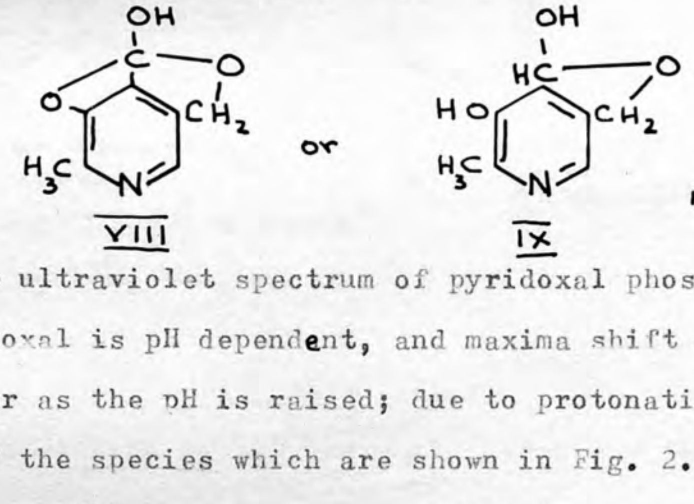
*A tautomer is a substance which can exist as two structural independent isomers; each isomer is capable of being changed rapidly into the second isomer when the equilibrium is disturbed. The internal energy of one of the structures overcomes the energy of transformation, which is generally small, to change spontaneously into the other isomer. A classical case is that of the keto-enol tautomers; in this research the tautomers are the aldimine-ketimine forms where the hydrogen is effectively transferred.

[105] Acid-base catalysis of the Schiff base in solution has been found to occur but the various mechanistic paths in particular those of the protropic shifts will not be considered in the present work.

PHYSICAL PROPERTIES of PYRIDOXAL and ANALOGS.

Spectra and ionisation constants of pyridoxal, pyridoxamine and their respective phosphates have been studied by various authors[103], and these are shown in Table 1.

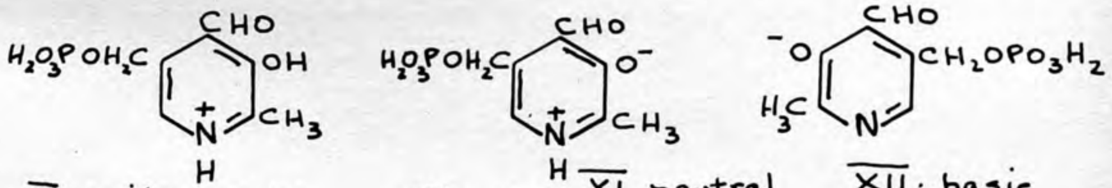
In addition to the change in spectra caused by proton uptake, the situation in the case of PL is complicated by hemi-acetal formation, and Snell suggested that the reason for the apparent effectiveness of pyridoxal phosphate compared to pyridoxal was due mainly to the fact that PLP would not exist in such a hemiacetal form.



VIII IX

HEMIACETAL FORMS of PYRIDOXAL

The ultraviolet spectrum of pyridoxal phosphate like that of pyridoxal is pH dependent, and maxima shift and bands often disappear as the pH is raised; due to protonation and deprotonation of the species which are shown in Fig. 2.



X: acid CATIONIC FIG. 2. XI neutral dipolar XII: basic ANIONIC

PLP exists in certain forms solvated. In methanol, the CH_3O^- bonds onto the formyl-carbon trans to the phenolic oxygen.

The following scheme has been seen suggested by Martell[6]. (Fig 3).

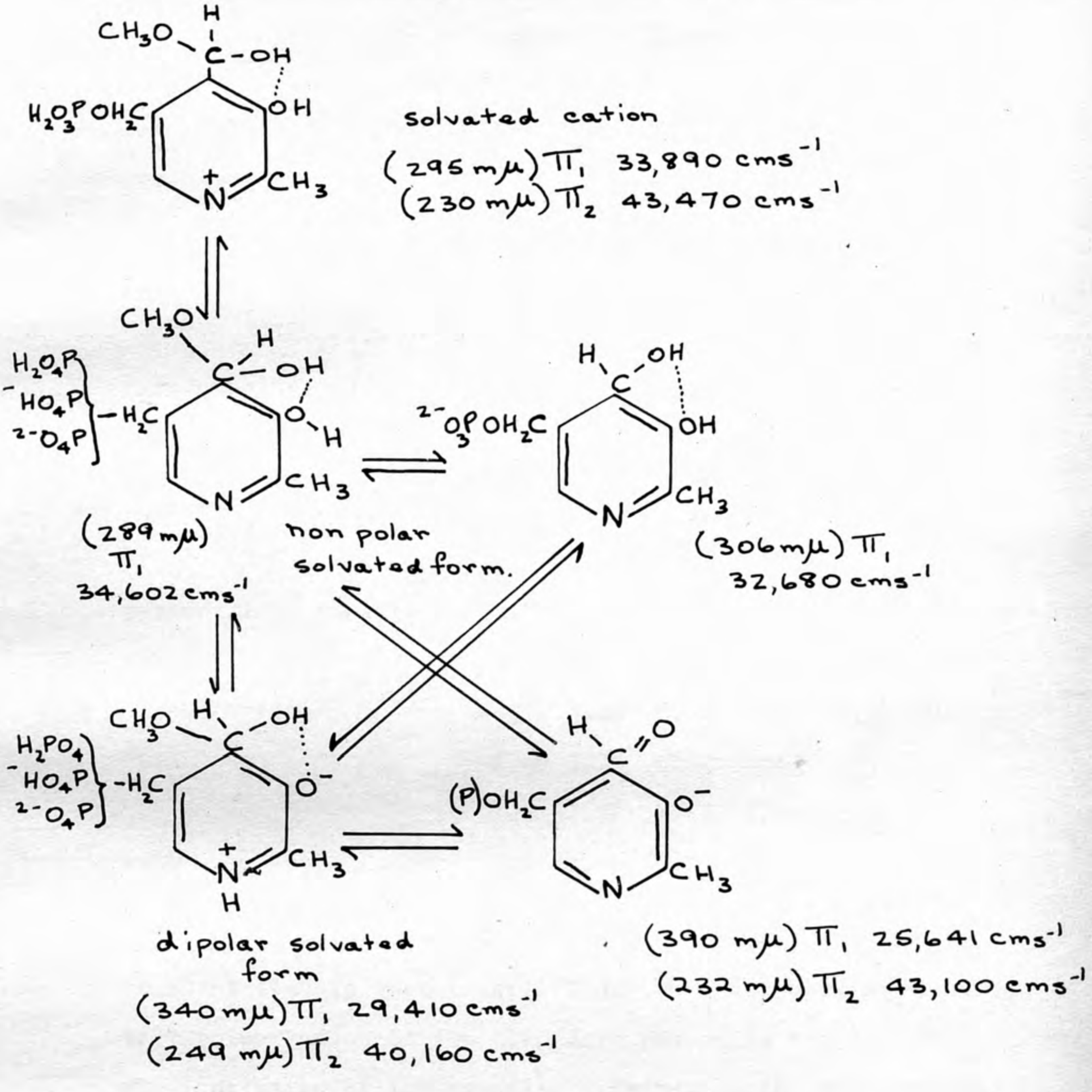


FIG. 3.

Dissociation of the phosphate hydrogens was not expected to have a large influence on the spectra.

TABLE I

Apparent acid dissociation constant of pyridoxal and various analogues

	pk_{a_1}	pk_{a_2}	pk_{a_3}	Reference.
pyridoxine	5.00	8.60		7
	5.00	8.96		8
pyridoxal	4.20	8.66		5
	4.23	8.70		
pyridoxamine	3.31	7.90	10.4	5
	3.54	8.21	10.6	7
		8.08	10.3	8

	pk_{a_1}	pk_{a_2}	pk'_{a_3}	pk_{a_4}	μ	Ref.
pyridoxal 5' phosphate	2.5	4.14	6.20	8.69	0.16	7
		4.00	6.40	8.40	0.20	9
	1.4	3.44	6.01	8.45	0.10	10
PLP	1.89	3.73	6.32	8.69		
	1.6	3.58	5.75	8.17	2.00	
PAMP	2.5	3.69	5.76	8.61	10.9	7

The ionic species of the above mentioned species have been detailed in recent work (11). pk'_{a_3} has been assigned to the ionisation of the secondary phosphate hydrogen,[5]. The ionisation of the phenolic hydrogen of the pyridine ring occurs in the course of excitation of the cationic species of the compounds investigated, thus decreasing the pk values in the excited state, [12].

PHYSICAL PROPERTIES OF SCHIFF BASES DERIVED FROM PYRIDOXAL.

The pk_a's for the various pyridoxal (and its phosphate) -amino acid Schiff bases vary according to the amino acid used. For the general Schiff base the pH is quoted as being 6 for the overall $-\underset{+}{N}H \longrightarrow -N= +H^+$ reaction. Table 2 sets out examples of the pk_a's of some Schiff bases.

TABLE 2

Some pk_a values of the Schiff base species.

	pk_{a_1}	pk_{a_2}		Ref.
3 hydroxy-4-aldehyde-alanine	4.05	6.77		
pyridoxal-phosphate valine	5.9		10.5 (9.0)	9
PLP-glutamate	pk_a 6.2 pk_e 5.9 pk_d 10.5			13
pyridoxal-glutamate		9.33 to 9.50		14
PLP-glutamate	6.4			110

If the pyridoxal phosphate -amino acid system is considered, there are three ways in which the amino acid could be bound [12].

PLP could be bound: (i) as a protonated Schiff base having absorption maxima in the 410-430mμ region.

(ii) as a non protonated Schiff base with absorption maxima in the 360mμ region.

(iii) as a substituted aldimine which absorbs in the 330mμ region.

Spectral properties and also prototropic shifts could therefore help to determine the nature of the Schiff base species.

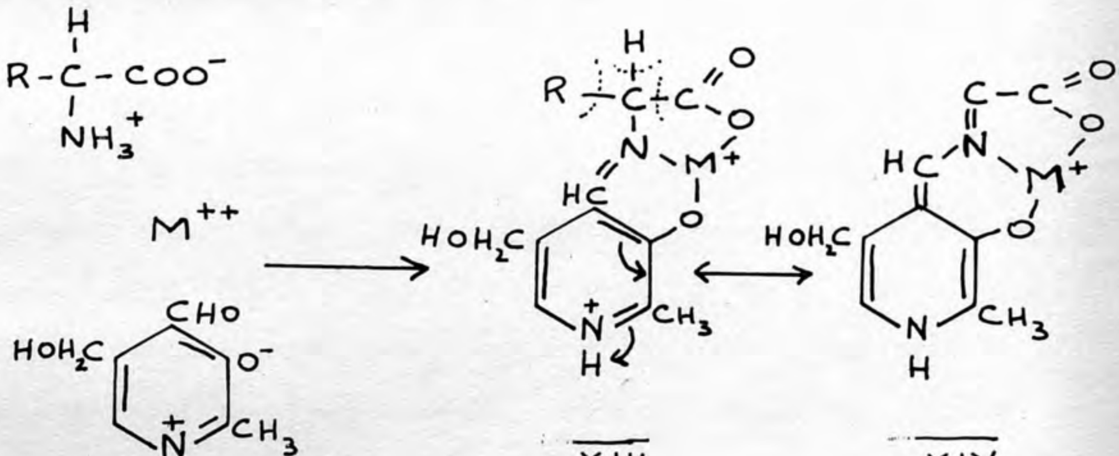
THE EFFECT of METAL IONS on the TRANSAMINATION REACTION.

In recent years, there has been speculation about the mechanism of the transamination reaction and the role of metal ions in this reaction. Further evidence has led to the suggestion that the reaction is 'catalysed' by the metal ions.

More recently however, it has been suggested that the metal ions form intermediates with the resulting Schiff base complexes. thereby facilitating the transfer by electrons from the α-carbon atom of the amino acid. This electron transfer has been related to the increase of the rate of the overall reaction.

The fact that the metal ions act as 'catalysts' in the reaction is liable to dispute, because by definition, the 'catalyst' does not actually take part in the reaction and only enables the reaction to proceed faster by lowering the energy barrier. Intermediates have been isolated in the metal ion catalyis of the transamination, so the fallacy of catalysis appears.

Metzler et al [15] have proposed a mechanism for the transamination reaction and have particularly referred to pyridoxal (and its phosphate). This mechanism shown in Fig.4, together with that proposed by Braunstein[3], has been considered to be the basis of the reaction as it is understood today.

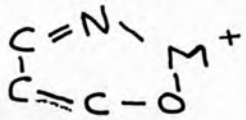


XIII

XIV

FIG. 4

These two tautomeric Schiff bases, XIII and XIV, decompose in three ways viz. racemisation of the amino acid, transamination or release of the R-group, and decarboxylation; all three of which are followed by hydrolysis of the C=N bond. Metzler [15] proposed that the phenolic and formyl group were vital in the chelation of the metal ions, while the <u>formyl</u> group is of primary importance in the transamination of pyridoxal to pyridoxamine[1,16]. The planarity of the system is maintained on chelation because the metal orbitals distort to attempt to stablise the planarity of the cyclic ring system formed by the formyl group-amino nitrogen-metal ion- phenolic oxygen and third carbon atom. (XV).



XV

Snell[5] suggested that the unoccupied co-ordination position of the metal chelate could be occupied by groups from the apoenzymes, thereby implying a linkage other than ionic. These facts combined with co-ordination and ligand field theory could be applied to the properties of the central metal atom.

Metzler et al [17] reported that the relative chelate strength of the bonds with the metal ions was in the following order,

$$Ga^{III} > Cu^{II} > Fe^{III} > Al^{III} > Ni^{II} > Co^{II} > Mn > Mg$$

in the pyridoxal-amino acid transamination reaction sequence. The method of continuous variation was used to determine the ratio of metal ion to Schiff base. 1:1, 1:2 and 1:3 ratios were found, depending on three factors viz: PL or PLP, the amino acid used, and the metal ion. According to both Matsuo [18] and Fasella [19], the ratio was found to be 1:1 with nearly all metal ions, in the case of PLP; while with the non-phosphorylated Schiff base, the ratio varied. Matsuo [18] suggested that "steric effects offered a problem in that the phosphate bridge is believed to have been bound to the enzyme". The stability of the compound is increased by ring formation and the stability was found to be even further increased if the complex could maintain maximum planarity. Hoard and coworkers[20] have studied the manganese (II) chelate of the pyridoxyledene-valinate Schiff base and have determined the structure of the complex crystallographically. They further found that the assumptions of Christenson[21,22], based or titration results, were doubtful. Christenson[21,22] had postulated that particularly weak complexing of the phenolic oxygen to the divalent metal ion occurred in all but the Cu(II) chelate. These conclusions were reached after an investigation of the acid dissociation constants of complexes, by titrimetric means.

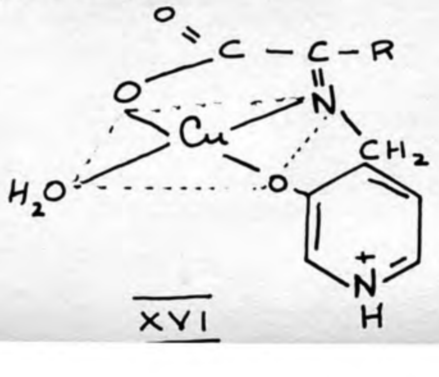
PROPOSED STRUCTURES OF METAL-SCHIFF BASE COMPLEXES.

Structures of complex compounds have been suggested by various authors:-

The Cu(II) pyridoxylidene-valinate chelate were discussed by Christensen[22], and the place of the co-ordinated water in structure XVI was occupied by an amino acid group which reacted with the complex, freeing the phenolic-oxygen from metal-oxygen bending. Metzler[17], although confirming the 1:1 nature of the pyridoxylidene metal chelate structures and their nature, stated that if the chelate was dissolved in a suitable concentration of the metal-amino acid, no appreciable dissociation occurs, The chelate strength was accounted for in the following order:

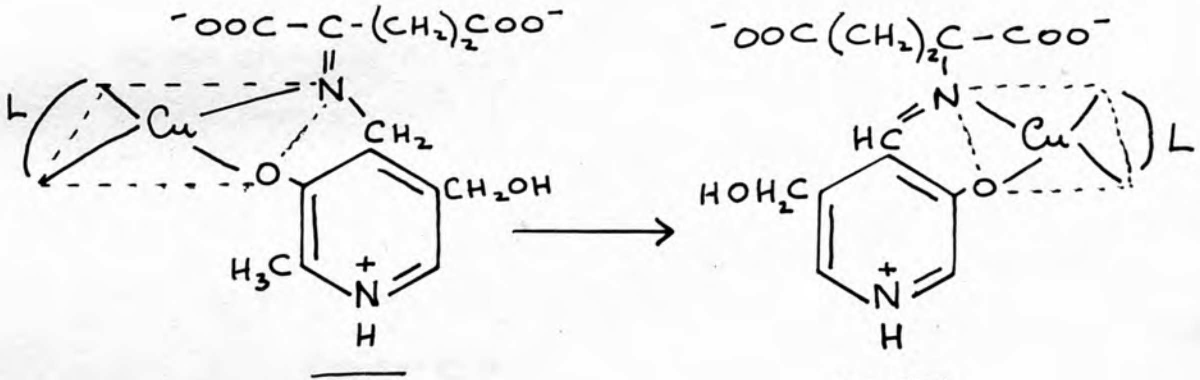
$$Cu > Ni > Zn > Mn > Mg$$

Recent studies of the Cu-pyridoxyledene-valinate complex by Hall[23], have confirmed the ratio 1:1 copper Schiff base and have elucidated the structure of the complex by X-ray methods. Hall[23] found the structure to be a 5-co-ordinated cupric complex (with a square pyrimidal base) such that overlap of molecular orbitals occurred and the intermediate had a polymeric structure. The overlap of molecular orbitals was said to stabilise the network, and maintain the essential planarity of the Schiff base system sterically.



XVI

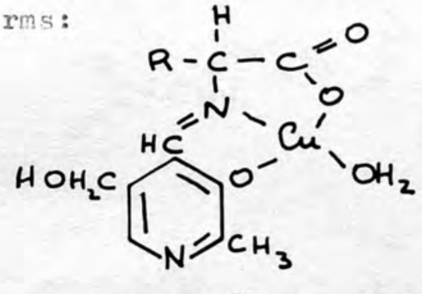
The structure in Fig. XVI was discussed by Longnecker[24] but was discounted because of its asymmetry. He suggested that structure XVII and XVIII were asymmetric but that the addition of an extra mole of amino acid attached itself to the molecule, allowing the attainment of partial symmetry.



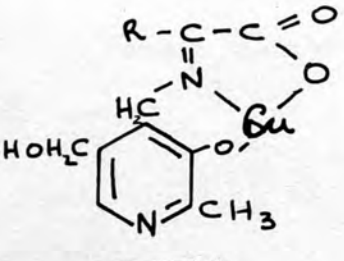
XVII   XVIII

However, the Cu-PL-Valinate chelate exhibited the extra stability accounted for by nitrogen co-ordination, but through the pyridine-nitrogen.

Structure XVI' has been quoted by Catannéo et al[25] and they suggested that the metal-Schiff base assumes the tautomeric forms:



XVI'



XVI^a

Metzler[26] suggested prior to this, that the 1:2 ratio of the aluminium to Schiff base existed in the case of the pyridoxyledene-glycine Schiff base in the "aldol-type condensation". Threonine and Allothreonine were reduced to α-ketobutyrate and acetaldehyde and glycine respectively, in the presence of pyridoxal, aluminium ions (or Cu^{++} or Fe^{3+}) and water, as shown in Fig. 5.

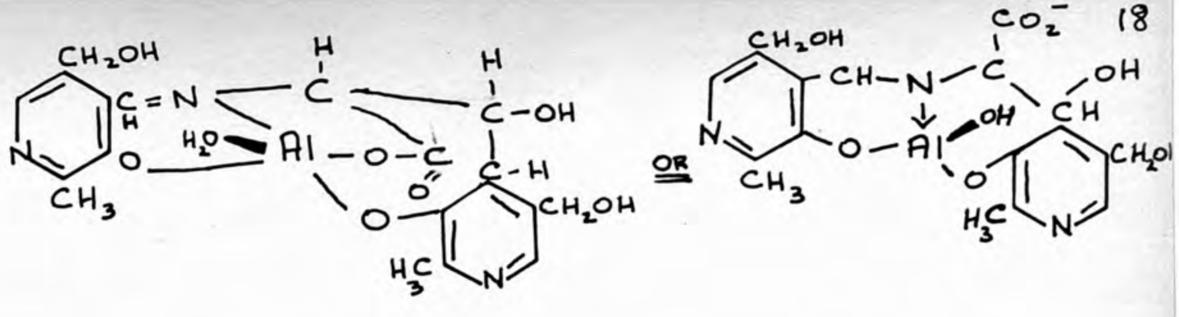
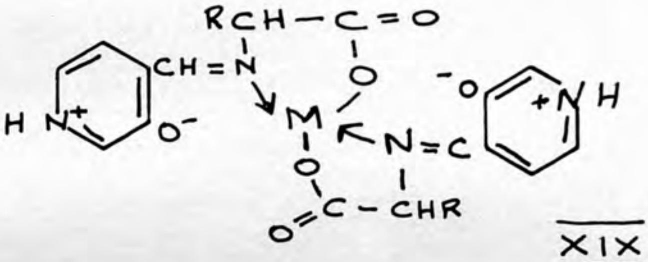


FIG. 5.

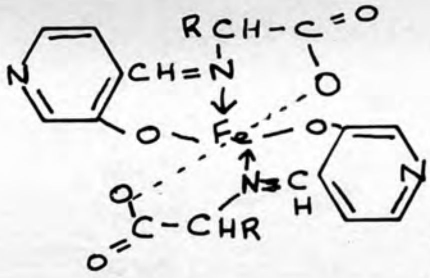
Fasella et al[27] studied the reaction with pyridoxal phosphate and aluminium. They suggested that the aluminium retained a single positive charge (Al^+) and also exhibits the same tautomeric form as XVI^1 and XVI^a. The ratio of the PLP Schiff base to aluminium was proved to be 1:1.

Christensen[21] proposed metal chelate structures with 1:1 and 2:1 ratios of S.B. to metal ions.



THE GENERAL CASE

XIX

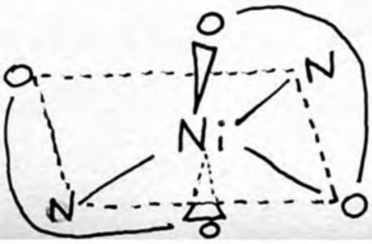


FERRIC CHELATE

XX

Various authors[28] have supported the claim that the formation of the Schiff base must occur before chelation with the metal ion, and the prior formation of the metal-amino acid chelate is said to inhibit the reaction almost completely.

Nickel-pyridoxylidene-alanine complexes were studied by Eichorn and Dawes[28] who postulated that the 2:1 Schiff base metal ion ratio existed and further that the structures were TRANS:-



XXI

The stability of TRANS- complexes relative to the CIS-type can be seen from models, to be greater in the case of the trans-isomer and therefore that form will predominate.

THE EFFECTS OF METAL IONS on the SCHIFF BASE SYSTEM.

Snell[29] has studied the effects of various activites of the metal ions and has quoted the following for the conversion of pyridoxal to pyridoxamine.

Ga(III) > Cu(II) > Al(III) > Fe(II) > Fe(III) > Zn(II) >
In(III) > Ni(II) > Co(II)

with only slight activity in the case of Mn(II) and Cd(II). However, Ga(III) was also found to be effective compared to Cu(II) Fe(II) and Fe(III) in the reaction of serine-3-phosphate with pyridoxal. This 'inefficiency' was also found in the transamination of pyridoxamine and α-ketoglutamate to form pyridoxal.

The general reaction for the series is considered to be

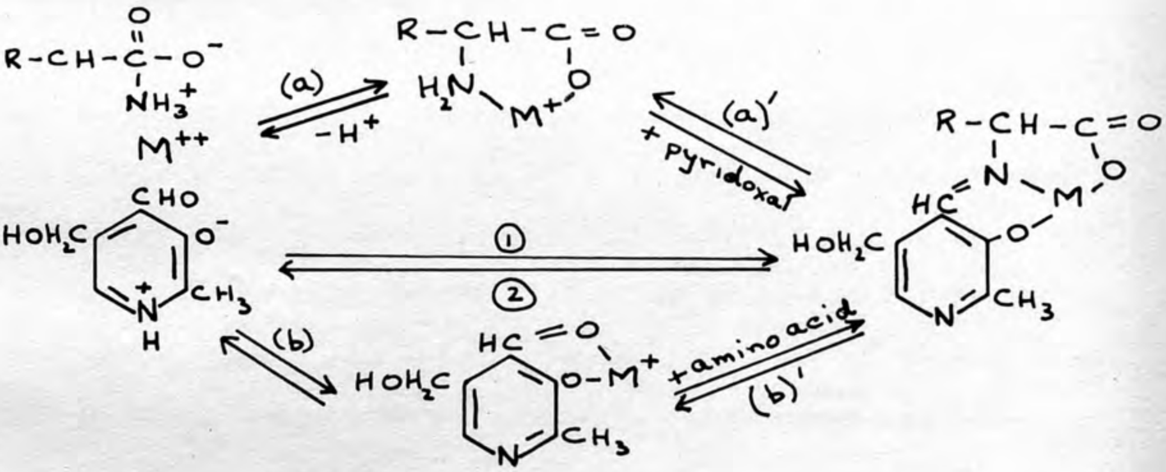
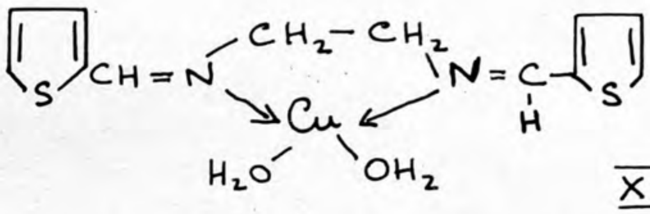


FIG. 6.

Note:-Reactions (a) and (b) proceed spontaneously but a' and b' require extra energy. The energy barrier of b' is much less than that of a' and therefore the reaction path b' seems more likely.

In 1954 Eichorn and Trachtenberg[30] found that the copper appearing in the bis(-2-thiophenol) ethylenediamine complex tended to weaken the C=N bond, thus rendering it more liable to hydrolysis. Consequently the assumption was that the stronger the complex, the more liable it is to be hydrolysed, seems rather strange. They further asserted that the stronger the metal ion bond, the lower the activation energy, ΔS^*, of the complex $\Delta S^*=R(\ln \frac{Kh}{Kt} + \frac{E}{RT} - 1)$. However, the stabilization of the C=N bond in the copper-salicylideneglycine chelates on metal co-ordination caused Eichorn[20] to study the thioethylenediamine system.



XXII

The relative electron distribution of the systems determined the stability and it was found that even after the double bond had been severed, the nitrogen atoms still remained co-ordinated to the metal ion.

Nunez and Eichorn[31] have also studied the salicylaldehyde-Glycine system and discovered that the salicylaldehyde is analogous to pyridoxal. The phenolic and o-aldehyde groups, necessary for chelation are both present, but the heteroatom in the pyridine ring tended further to stabilize the system electronically. This was due to the lone pair of electrons of the nitrogen which were in an orbital in the plane of ~~perpendicular to~~ the ring system.

Stability constants, formation constants and rate constants of these Schiff base metal complexes have seen evaluated. The pyridoxal-amino acid-metal ion system has been throughly studied with a variety of amino acids and metal ions. Absorption maxima[25] were found around 26,000cms^{-1}. and were suggested to be charge transfer bonds from ligand to the metal. Bender[32] suggested that the "electron sink" caused by the electrostatic effect of the metal ion facilitated the removal of the α-Hydrogen atom of the isomeric Schiff base. Electronic distribution seen in the electronic spectra, add to the knowledge of the system, and together with ligand field theory applied to the metal ion, help to solve the structure of the compounds. These will be dealt with in separate sections.

MASS SPECTROMETRY[51]

In 1898 Wien found that positive rays could be deflected in electric and magnetic fields, and later in 1912, Thomson demonstrated the existence of isotopes of neon (with differing mass to charge ratios). Accurate mass measurements were made by Aston and Dempster (1918-1919) on a photographic plate onto which the ions were then focused. Recently, mass spectrometry has become a fast and accurate way of analysis of complex mixtures, for both organic and inorganic chemists. Gases and volatile hydrocarbons are easily determined by this method.

Theoretically, an electron is accelerated in a magnetic field of strength H, with an ionisation potential eV. The magnetic deflection is then:-

$$eV = \tfrac{1}{2}Mv^2$$

where M = mass of molecule
v = velocity of accelerated molecule in field

The centripetal force is then $\frac{Mv^2}{r}$ balanced by the magnetic field.

$$Hev = \cancel{\frac{H^2r^2}{2V}}\ \frac{Mv^2}{r} \qquad r = \frac{Mv}{eH}$$

$$\therefore \quad \frac{M}{e} = \frac{H^2r^2}{2V}$$

The $^m/e$ ratio is one of comparis~~i~~on with fragmentations and the resolutions found by comparing $^{\Delta M}/M$. With a double beam mass spectrophotometer, constant H and V result in a spread of r. The fragmentation of a hypothetical molecule ABCD is considered to be

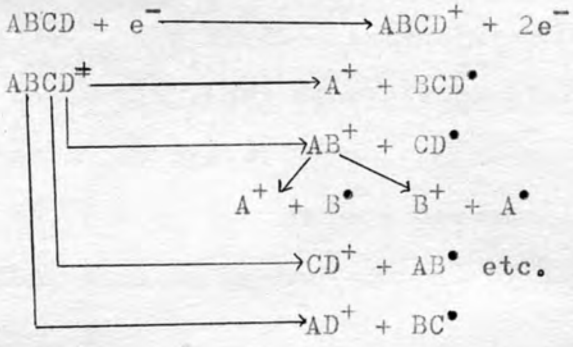


FIG. 7.

A further peak often occurs at M+1 where M is the molecular weight (or molecular ion). The reaction is as follows, where the molecular ion collides with a further molecule:

$$ABCD^{+} + ABCD \longrightarrow (ABCD.ABCD)^{+} \longrightarrow ABCDA^{+} + BCD.$$

Daughter ions will appear in the systems because of the life time 10^{-5} secs. of highest ~~wave~~ mass numbers and the base peak would be lowered.

The stability of the molecular ion (M^{+}) is said to be increased by the presence of π-electron systems and the loss of electrons is more easily accommodated by σ-bonds. Systems of various classes are considered according to their decreasing stability.

Aromatic compounds > alicyclic > unbranched hydrocarbons > ketones > amines > carboxylic acids > branched hydrocarbons > alcbhols.

π electron systems and stability towards fragmentation are said to be additive. Branching **favours** fragmentation and the intensity of the '<u>base</u>' peak is lowered.

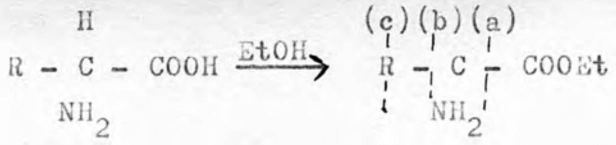
Biemann[51] has evolved a system of cleavage whereby different types of cleavage and rearrangements are grouped into classes. Substitution does effect fragmentation and further bond cleavages after the formation of a molecular ion.

Electron bombardment is the chief tool for the fragmentation of organic compounds, while for inorganic compounds, the technique involved is that of surface ionisation.

Amino acid mass spectra:

Amino acids have been studied extensively by Biemann[52, 53] There are found to be two types of cleavage i) of the carboxyl group, ii) of the amino group.

The amino acids studied by Biemann have previously been esterified and the degradation will be by the removal of the esterified carboxyl group.

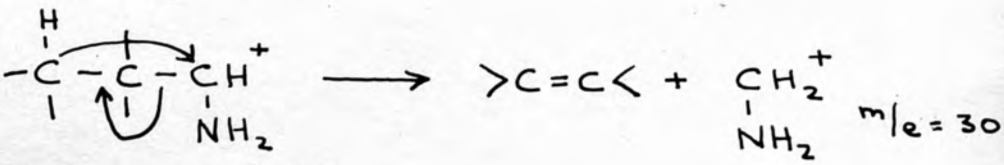


$$R-\underset{NH_2}{\overset{H}{C}}-COOH \xrightarrow{EtOH} \overset{(c)}{R}\,\vdots - \underset{NH_2}{\overset{(b)}{C}}\,\vdots - \overset{(a)}{\vdots}\,COOEt$$

(a) Cleavage will result in the formation of the $R-\underset{NH_2}{\underset{\|}{CH^+}}$ which will then rearrange and resonance will stabilise the ion as

$R-\underset{{}^{+}NH_2}{\underset{\|}{CH}}$

In α-amino acids, the amine fragment is the most prominent one with $^m/e$ ratio of (R+29)m̄.μ. Further fragmentation of the 'amine fragment' is the elimination of the olefin from the amine fragment.



Fragmentation of bond (b)

If $C_\alpha - C_\beta$ is broken, the positive ions is again stabilisation by attachment to the nitrogen

$^{+}CH_2 - CO_2C_2H_5$ $\quad ^m/e = 102$

$:NH_2$ $\quad$ or - Et = 86

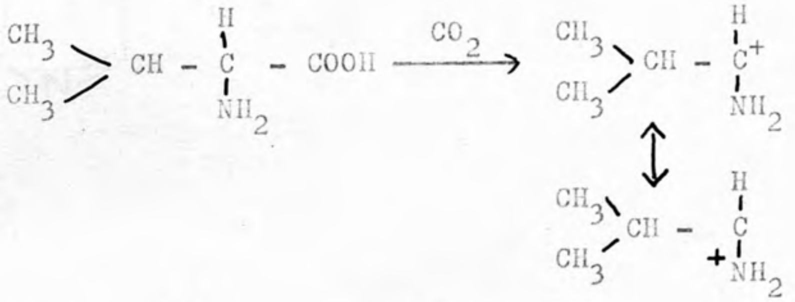
A high degree of branching (e.g. valine) facilitates the cleavage of the β-α bond.

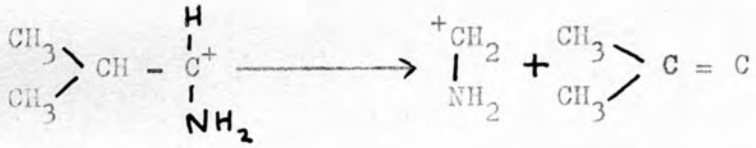
(c) Fragmentation of the molecular ion at the R-group: which is favoured by side chain fragmentation.

Amino acids used were glutamic acid and valine where the molecular ions of each are reported to be 84 and 72 $^m/e$ respectively.

Fragmentation of amino acids would follow the regular patterns, similar to those of the esterified amino acids.

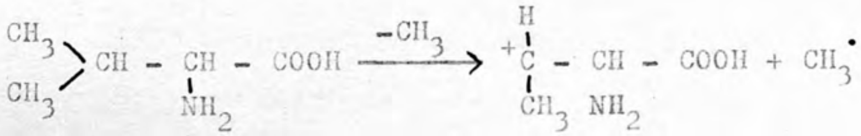
Valine


m/e=72


m/e=30

loss of 42 m/e.

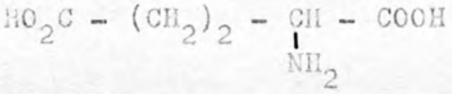
or loss of branching:


m/e=102

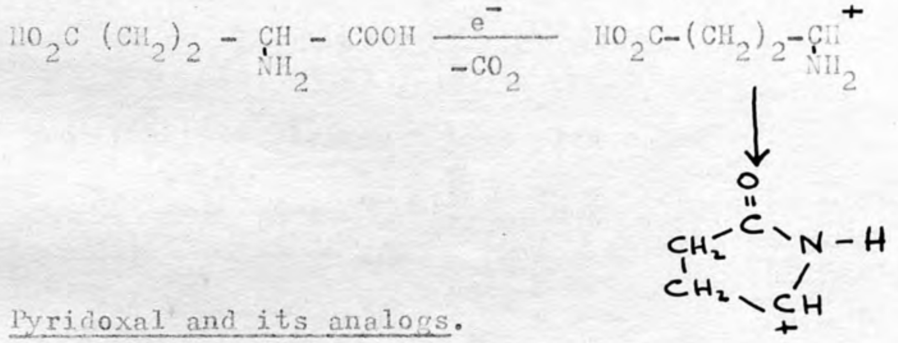
The molecular fragment m/e=102 would only be a relativily small peak since the former fragment is favoured.

Glutamic acid

Fragmentation is similar to the ethyl ester, one the neutral EtOH has been eliminated[53]:



$$HO_2C - (CH_2)_2 - \underset{NH_2}{CH} - COOH \qquad {}^{m}/e = 147$$


m/e = 102

m/e = 84

Pyridoxal and its analogs.

Pyridoxine, pyridoxal and similar compounds have been studied[54] by means of mass spectrometry. The initial stage was found to be the abstraction of a water molecule followed by rearrangement of the molecular ion. This is typified by rearrangement of Biemann Type A_4 followed by cleavage of Type E.

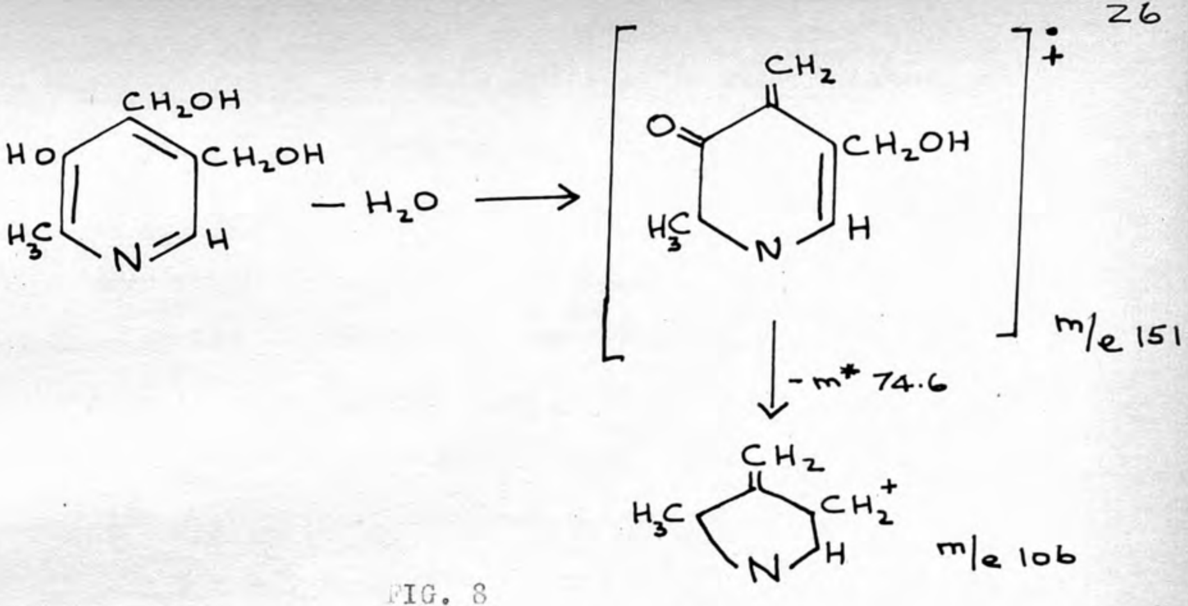


FIG. 8

There is an intermediate step of $^m/e = 122$ where the abstraction of either the phenolic-O or the CH_2 group is more probable.

All the vitamin B_6 analogs in this paper[54] showed similar cleavages and rearrangements and it was interesting to note whether this rearrangement could occur in the formation of the acetal of pyridoxal, whereas in the case of PLP this would be blocked by the phosphate group.

Schiff bases of amines.

Little work has been done on the Schiff base class. Fisher and Djarassi[55] discussed the aliphatic series and its fragmentation.

$$CH_3CH_2 \underset{(c)}{\wr} CH_2 \underset{(b)}{\wr} CH_2 \underset{(a)}{\wr} CH{=}NCH_3$$

Three possible cleavages have been noted:

(a) $\underline{-C_4H_9^{\circ}}$ $HC \equiv \overset{+}{N} - CH_3$ $^m/e = 42$

(b) ———— $\bullet CH_2 - CH = \overset{+}{N} - CH_3$ $^m/e = 56$

(c) ———— $^{\bullet}CH_2CH_2CH = \overset{+}{N} - CH_3$ $^m/e = 70$

Aromatic substitution would stabilize the double bond and a different system of cleavage would therefore follow.

Electronic spectra and their relation to configuration of the organic molecule.

GENERAL THEORY

Electronic transitions are produced by the excitation of an electron (or electrons) from one energy level to another. When this happens, energy is either transmitted or absorbed and the difference between the energy of initial level and final level is given by the relationship

$E = h\nu$ where E = energy difference

h = Plank's constant

$\nu = \frac{1}{\lambda}$ = (wavelength)$^{-1}$

In general, electrons can move about a system, via σ-(single) and π-(double) bonds, and energy (in the form of heat or light) can excite them to a higher level. The distribution of electrons and hence the electronic transitions directly affect the spectra of a system and the more complex it is, the more probable of ambiguity.

If the pyridine nucleus is considered separately, it can be seen that substitution on the pyridine ring will change the distribution of electronic charge, and hence the electronic transitions will change. The Schiff base, formed in the transamination reaction, demonstrates the shift of charge both in the pyridine nucleus and in the tautomeric forms of aldimine and ketimine*, shown in formulae XXIII - XXV on the proceeding page.

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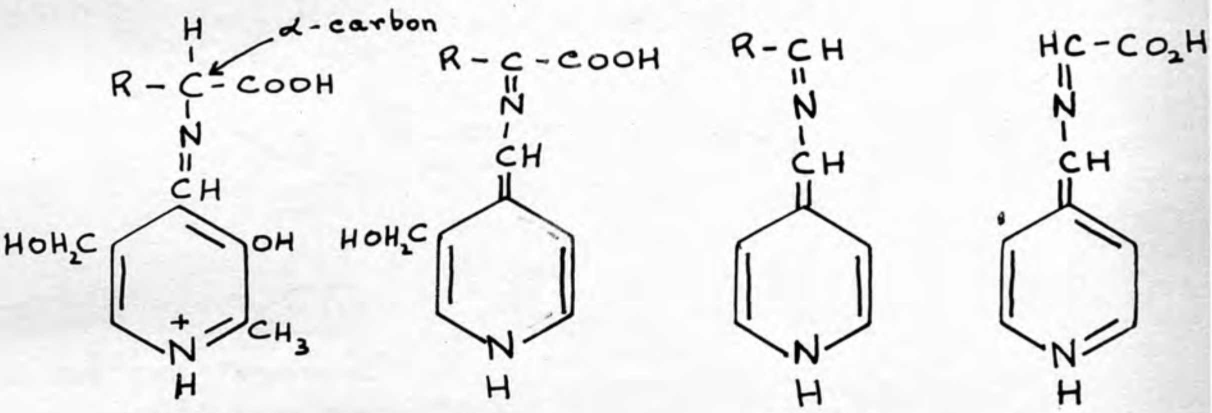
*See p. 9

MOLECULAR ORBITAL CALCULATIONS OF ELECTRON DISTRIBUTION IN THE SCHIFF BASE SYSTEMS,

The electronic distribution in the organic system of the Schiff base molecule has been considered in relation to the Braunstein-Snell theory of the transamination of Schiff bases. This was discussed in several papers by Pullman[56,57]. The three tautomeric forms XXIII, XXIV and XXV, of the amino acid-pyridoxylidene Schiff base are shown below:

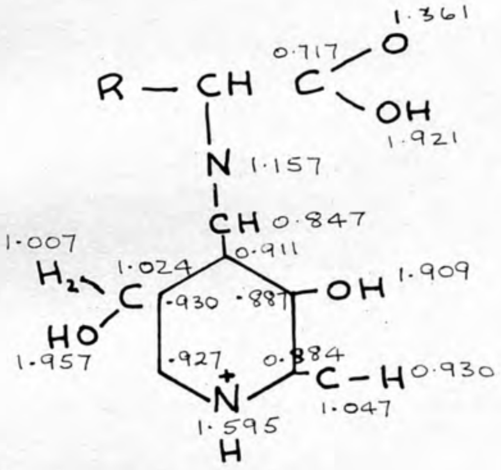


XXIII XXIV XXV XXVI

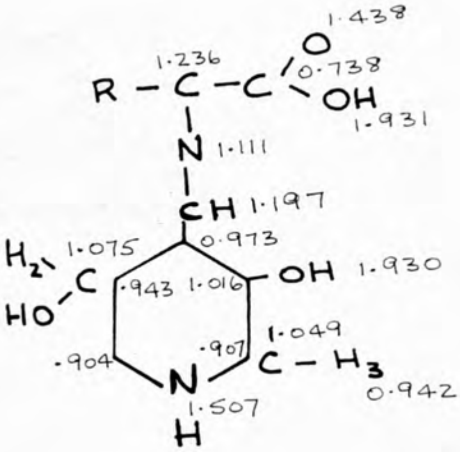
The Snell-Braustein theory proposed the intermediate XXIII, and assumed that the electron density about the α-carbon atom of the amino acid group was reduced, thus facilitating the electron displacement of the activation from αC to the N leading to either racemisation or transamination of the complex.

Pullman[57], using molecular orbital calculations, has found that formula I represents an increase in electron density at the α-carbon atom, whereas, Snell had calculated it to be a decrease. Calculation has shown that the nitrogen atom is sp^3 hybridized in the amino acid, whereas it has moved towards sp^2 trigonal hybridisation on complexing as a Schiff base. The nitrogen would therefore effectively increase in electronegativity thereby decreasing the charge on the α-carbon atom. Chelation with a metal cation would tend to cancel out the nett negative charge, then appearing on the nitrogen. However, for the present the effect of the metal ion is neglected.

The distribution of the π-electrons in the various Schiff bases as calculated by Pullman[56,57] are shown below and will be considered separately.



XXIII



XXIV

FOR THE FORMYL-C

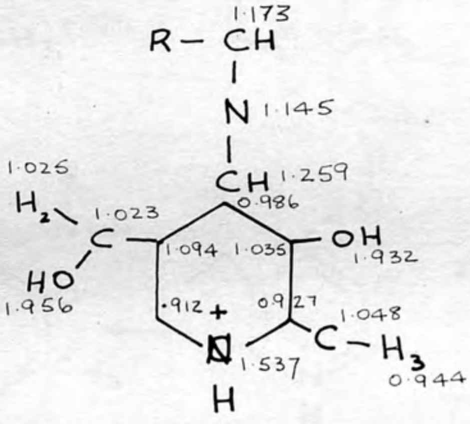
$\delta^+ = 1 - 0.847$

$= 0.153e.$

In formula XXIII, the two conjugated fragments are separated by the formyl carbon atom. Pullman[57] suggested that the decrease in electron density around the α-carbon atom could be due to "the appreciable gain in resonance energy which is associated with ionisation". The calculated resonance energy ΔE was found by molecular orbital calculations, to be of the order of 20 Kcals., This large resonance energy could be responsible for the transformation of the initial Schiff base form to another more "active" tautomeric form. If the $C_{formyl}-N-C$ chain is considered, in formula XXIV, the chain would be seen to bear a nett negative charge. C_α and C_{formyl} are more electronegative than the nitrogen and would therefore be centres for nucleophilic attack (racemisation and transamination respectively). In formula XXV (p.31) of the electron distribution diagram, both the formyl- and α-carbon atoms are more electronegative than the nitrogen (similar to the electron distribution in XXIV), but in this case, XXV, the formyl carbon is more electronegative than the α-carbon atom in XXIV. XXV differs from XXIV in that the carboxylate group is absent in XXV; the electron distribution in XXV would promote the hydrolysis of the N-C double bond, which would then be severed, and an "amine" would result from the electron rearrangement in the transamination intermediate.

Pullman[56] discounts the Snell-Braunstein postulation of formula XXIII because he considers the conjugated system does not extend as far as the α-carbon atom. In considering the molecule as a whole, it must be remembered that although the 'pyridine ring system' accounts for a large proportion of the electronic transitions of a conjugated nature; the molecule contains in addition the Schiff base-imine bond, which has a definite marked effect on electron distribution. Since $\pi \longrightarrow \pi^*$ transitions (of conjugated systems) are found to be much more intense than either $\sigma \longrightarrow \sigma^*$ or $n \longrightarrow \sigma^*$ or $n \longrightarrow \pi^*$ transitions, $\pi \longrightarrow \pi^*$ transitions will be considered of primary importance.

Recently, Dunathon[58] studied the conformation and reaction specificity of PLP enzymes. He found that "in a common step of all PLP enzyme reactions, the π-system of the Schiff base formed between PLP and an amino acid is extended by loss of a group from the amino acid α-carbon atom". Transamination and dehydroxymethylation are found to be increased when metals are chelated to the system, while decarboxylation is said to be inhibited. This inhibition could possibly be explained by hydrogen bonding and extension of charge-transfer through the metal atom from the phenolic oxygen to the carboxylate oxygen. This type of transfer has occurred in the Cu-PL-valinate chelate[23]. Bender[32] suggested that an "electron sink" took place due to the electrostatic effect of the metal ion. Clearly this will depend on the electronegativity of the metal ion, which will then effectively cancel out the negative charge on the imine nitrogen and stabilize the complex by co-ordination. Application of the knowledge of the distribution of electrons in this highly conjugated ring system certainly helps to understand the spectra of such complex molecules. However, the electronic distributions of the nitrogen lone-pair electrons has generally, been neglected by authors previously quoted. Brocklehurst[59] postulated that if either, the nitrogen lone-pair of electrons is localised by protonation or the molecule is constrained to a more planar form by ring formation, the intensity of the long wavelength absorption maxima is increased two or three times.



XXV

ULTRA-VIOLET SPECTRA of the SCHIFF-BASE SPECIES of PYRIDOXAL and its ANALOGS.

Formation of Schiff bases, with and without metal ions has been studies by many authors. Spectrophotometric evidence has been given for the various appearance and disappearance of species at specific wavelengths.

Christensen[9] proposed that the spectrophotometric observations of Schiff bases between pyridoxal and amino acids with bands at 26,547, 23,852 and 25,000 cms^{-1}, during the reactions:

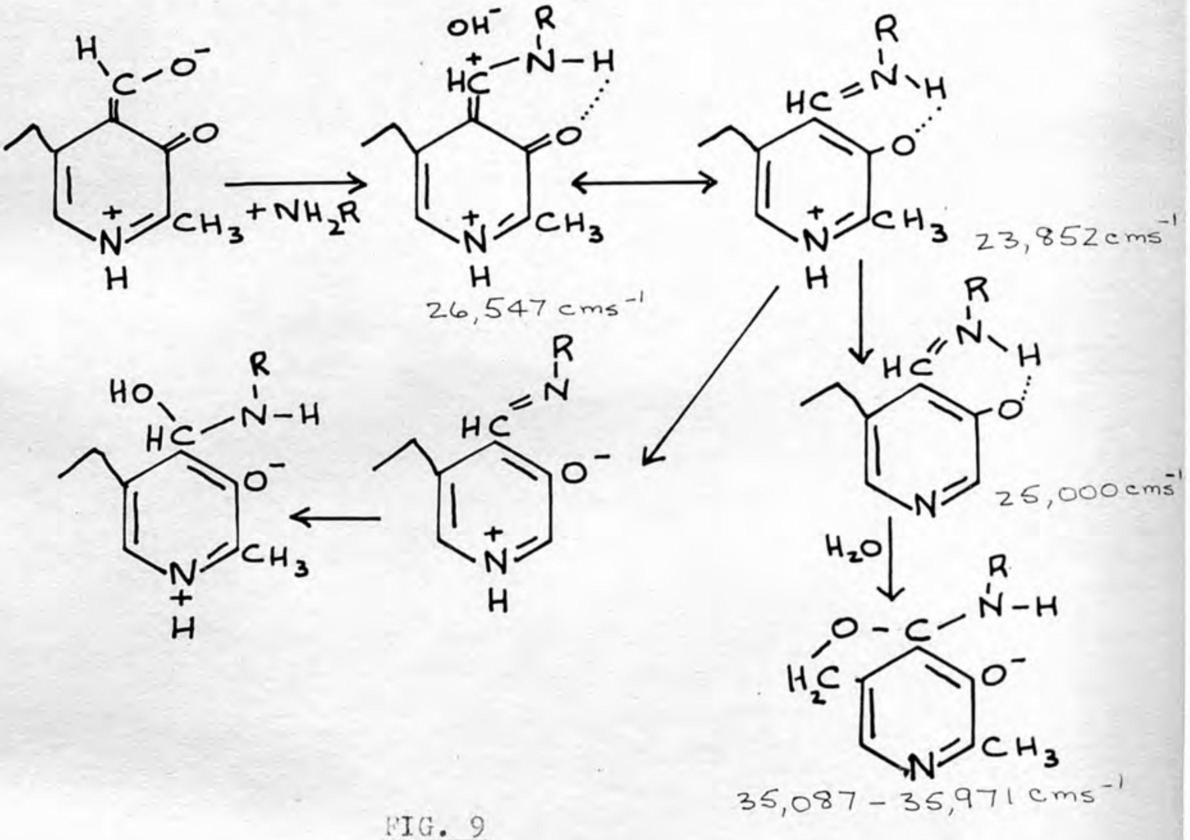
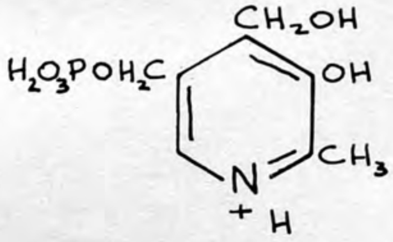


FIG. 9

The amino-acetal species of the cyclic hemiacetal (band 35,087 - 35,971cms^{-1}) cannot exist in the case of the pyridoxal phosphate because its formation is effectively blocked by the phosphate group[9]. There appears to be a difference of opinion between several authors as to the assignment of the hemi-acetal species of pyridoxal. Metzler[98] suggested that the band at 31,546cms^{-1} was due to the hemi-acetal form, while Christensen[9] believed that the species was present in the 35,087 - 35,971cms^{-1} region. The predominant species of Schiff base in neutral solution[98] was found to have an absorption maxima at 24,155cms^{-1}, while in acidic media the main protonated species absorbed at 27,397cms^{-1}. In the acid range however, the species change in the pH region 5-6; this idea is upheld by the pk$_a$'s of the Schiff bases[98].

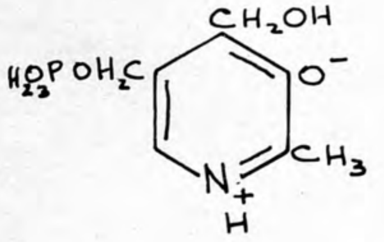
Snell[5] suggested that in aqueous media the various forms of pyridoxal phosphate are those shown below, together with their regions of absorption.



XXVII

acidic

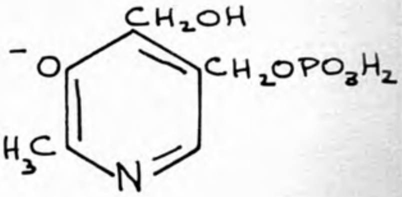
(33,900cms^{-1} 43,480cms^{-1})



XXVIII

neutral

(29,410 40,160cms^{-1})



XXIX

basic

(25,640 43,100 cms^{-1})

Matsushima and Martell[6] studied the molecular species of PLP in methanol and attributed the bands (in parenthesis) to the three species described by Snell[5]. They[6] further studied the pyridoxal valine Schiff base with the following band assignments with pH changes.

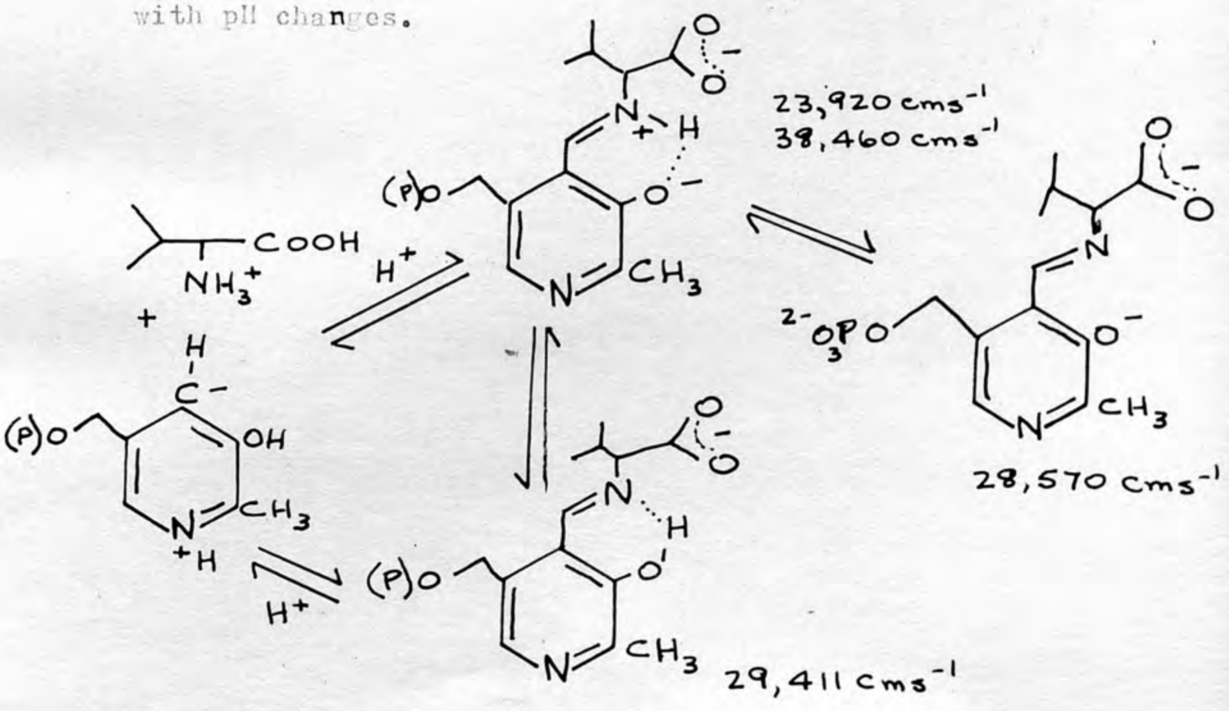


FIG 10.

Kinetic studies by Bruice and Topping[99, 100] showed that 25,316 and 40,650cms^{-1} bands were due to the disappearance of the aldehyde and appearance of the imine respectively. A further "transimination" could occur if the phosphate group is bound to an enzyme, (Fig. 11.)

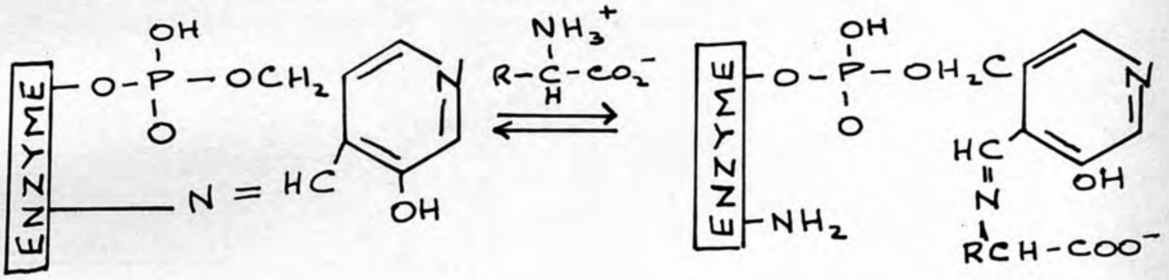


Fig. 11

It seemed rather strange that the 3-OH did not take part in the transamination reaction, especially since Thanassi et al[101] found that one of the functions of the phenolic group was to increase the concentration of aldimine species and promote the intramolecular acid catalysis. The presence of the 3-OH group was thought necessary to promote the shift from aldimine to ketimine species.

U.V. spectra of the imines and the effects of the addition of metal ions to the system, were studied by Matsuo[18]. Metal ions were found to shift the imine absorption maxima to shorter wavelengths. Conclusions were that the metal ion effect occurred after Schiff base formation in the salicyaldehyde-glycine system[31]. Shifts of specific bands species were found to obey the rule set out by Metzler and Snell[102]; that the red shift (to longer wavelengths) was due to the hydroxyl (phenolic) hydrogen while the pyridinium ion caused the blue shift.

Assignments of the various U.V. bands to species.

Nakamoto and Martell[103] have studied the vitamin B_6 analogs and assigned various transitions to the molecular species. Assumptions are made that the π — π^* transitions of the pyridine ring system and of conjugated $-C{=}N-$ imine will be the main contributors to the absorption. n — π^* and σ — σ^* are considered weak and generally < 0.01 absorption units ~~and~~ in 10^{-4}M solutions generally out of the spectral range considered.

Notation of the π-system bands is given according to the benzene notation and is set out for pyridine similarly.

TABLE 3.

Absorption maxima:

Benzene cms^{-1} (-mμ)	Pyridine cms^{-1} (-mμ)	Transition	π-transition
(180) 55,556		$A_{1g} \to E_{1u}$	forbidden & weak
(200) 50,000	(205-260) 48,780-48,750 cms^{-1}	$\pi_1 - \pi^*$ $A_{1g} \to B_{1u}$	Primary
(255) 39,216	(256) 39,060	$A_{1g} \to B_{2u}$	$\pi - \pi_2^*$. 2nd. band
	(281 sh) 35,590 cms^{-1}		$\pi - \pi_2^{**}$

It is important to point out that assignments of the π_1^*, of π_2^* transitions, considered analogous to the pyridine ring system, are bound to be those of the uncharged species of ring system. Table 4 will set out how the substitution of the ring systems shifts the maximal absorption, and further substitution causes even larger shifts in one direction or other. Bands shift obey the rule set out by Metzler and Snell[102], quoted previously.

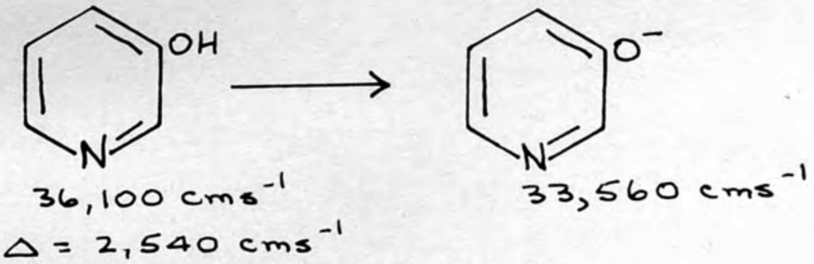
TABLE 4[102]

Compound.	Substituent.	Solvent.	max. cms^{-1}.
Pyridine	–	water	39,062
	–	alcohol	38,910
	3-OH	water	36,101
	3-OH	alcohol	35,971
	3-OH^-	water	33,557
	3-OH^-	alcohol	33,222

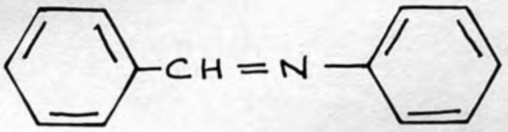
The π_1 and π_2 bands of the pyridine were calculated to be in the following order, of decreasing wavelength:

Zwitterion > anion > cation > neutral.

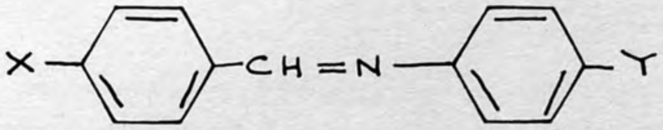
Shifts of up to $2,083\,\mathrm{cms}^{-1}$ were found in the ionisation of the hydroxyl group, and the stability is accounted for by resonance forms of the respective Schiff base.



The $\emptyset_1 - \pi^*$ peak at $38,168\,\mathrm{cms}^{-1}$[104] is said to be stabilised by an electron donating group in the p-position to the amine group, and would therefore absorb bathochromically relative to the isomeric Schiff base with the same p-position occupied by an aldehyde group.



XXX



XXXI

The steric requirement for maximum planarity was thought to account for increased absorption of the $38,168\,\mathrm{cms}^{-1}$ peak with a corresponding decrease in the $31,847\,\mathrm{cms}^{-1}$ region. Planarity of the Schiff base system[57] was achieved by the weakening of the σ-bonds around a specific carbon atom (the α-carbon atom of the amino acid in this case) which was adjacent to the cofactor π-system.

INFRA-RED TECHNIQUES.

Infra-red spectroscopy has been found a useful tool in analysis of compound structures, especially in the solid state. Much work has been sone in identifying IR absorption bands with compounds analogous to those in this research work and it is intended to use previous work to help in band assignments.

The analysis has been divided into several sections in order to observe what the varying of different bonding species would do to the I-R bonds typical of specific classes. It was thought that the different types of compounds prepared would fall into various catagories:-

i) pyridine bonds
ii) pyridoxal phosphate and pyridoxamine phosphate
iii) phenolic, aldehyde and amine bands
iv) -C=N-bands of Schiff bases
v) Shifts due to metal co-ordination

i) Pyridine-complexes.

Gill et al[33] have studied metal-pyridine complexes fairly thoroughly and have assigned bands mainly to the chief pyridine bands and have evaluated the shifts due to metal coordination. They found that the anion also influenced the exact positioning of the bonds. Characteristic bands in the 990-1217cms^{-1} region were found, on co-ordination to have shifted to higher frequencies. The appearance of a new band at 942cms^{-1} is reported when the metal is co-ordinated to the pyridine[40] and the assignment is to a -CH out of plane deformation.

Other studies by Greenwood and Wade[45] of the pyridine-metal complexes have shown the "the infra-red spectrum of the pyridine molecule is considerably modified when the non-bonding pair of electrons on the nitrogen atom is donated to the vacant orbital of an electron acceptor".

Metal-ligand bonds in the 200-400cms^{-1} region have been assigned by Clarke and Williams[34] to the series of the pyridine-metal complexes.

ii) Pyridoxal phosphate and pyridoxamine phosphate.

Previous work by Anderson and Martell[36] has linked up the infra-red bonds of pyridoxal and PLP and the pyridoxamine analogues. However, it must be pointed out that because PLP cannot exist as a hemi-acetal, bands assigned to the hemi-acetal will therefore not be present. Another factor which influences the I-R spectrum is the existance of the phosphate group, which according to Bellamy[47] absorbs in the 1,000-1,400cms^{-1} region and would therefore tend to blot out other bands due to the pyridoxal, especially the aldehyde bonds. Solution spectra of PLP were carried out by Parker[46]; these spectra demonstrated the strong absorption mode of the phosphate in both PLP and PAMP. However, better resolution was obtained in a phosphate buffer[46].

Martell[36] quotes the band due to carboxyl of the aldehyde to be at 1,645cms^{-1}, and this band would presumably be the one which would shift in the "simple" metal co-ordination. Other bands quoted are the 1,555 and 1,409cms^{-1} assigned to the pyridine ring and ($C-O^-$) vibrations respectively. Bands quoted for the PAMP are the 1,593 and 1,548cms^{-1} both assigned to the pyridine ring vibrations.

iii) Phenolic, aldehyde and amine bonds.

Two sets of bonds are quoted by Bellamy for the phenol viz - 1320 - 1420 and 1170 - 1230cms^{-1}. Precise positions vary according to the substitution on the basic phenol ring. Shifts of the benzene vibrations relative to the pyridine vibrations also occur, along with the extra vibration due to the lond pair of electrons on the nitrogen atom. -OH stretch vibrations occur in the 3200 - 2500 region but these could again be masked by such groups as phosphates. Martell[36] suggests that the pyridine-4-aldehyde has a strong band at 1645cms^{-1}. More generally aromatic -CHO according to Bellamy[47], are spread in the 3500-780cms^{-1} region, with four fairly strong bands. However, chelated aldehydes have been classified as having bonds in the 1645 - 1670cms^{-1} region. Also in this 1600cms^{-1} region is a band assigned by several workers, to be the amide I(C-O) stretching vibration.

Primary amine bonds (as in the case of PAMP) are reported to absorb in the 1590 - 1640cms^{-1} region and this would be affected by aromatic substition and spreading of the π-electron cloud from the conjugated system. N-H and OH stretching vibrations are found in the 3000 - 3300cms^{-1} region, and substitution of the pyridine nucleus could lead to shifting of the bands to lower frequencies.

Kovacic[43] reports the phenolic C-O stretch in the 1250 - 1330cms^{-1} region in salicylideneaniline chelates. This region is however, difficult to observe in the phosphoralated compounds.

iv) -C=N bonds of Schiff bases.

The general imine class R-CH=N-R^1 is discussed by Fabian[48] and bands assigned to the C=N amine group lie in the 1674 - 1665cms^{-1} region depending on substituents R and R^1. However, Gore <u>et al</u>[49] classify the C=N in a much wider region viz- 1640 - 1690cms^{-1}, with conjugated and cyclic imines in the 1650 - 1500cms^{-1} region.

Kovacic[43] reports the -C=N stretching frequency of the salicylidene anilines to occur in the 1620 - 1600cms^{-1} region, but account must be taken of the C=N stretching frequency of the pyridinium (and derivative) compounds. The C=N should increase the absorption in this area, and maybe even two bands could occur. Ueno[35] has described the C=N stretching modes in the bisbenzolyacetone-ethylene diamine chelates at 1500 - 1525cms^{-1}, but clearly the aryl ring is not involved in the chelation with the metal ion in forming the cyclic-quasiplanar structure and the aryl group only appears as a substituent on the chain.

Reports by Heinert[37] of Schiff base imine absorption occurring at 1510cms^{-1} are regarded as "anemolous" but possibly attributable to the C=C stretch in amide vinylogues. However, in a previous paper[42], C=C, C=O and C=N stretching modes of copper chelates of diamine Schiff bases are reported in the range 1650 - 1510cms^{-1}, with lowest absorption bands attributed to the C=N stretch.

Martell[42] described the unusual absorption at the 1525±2 cms^{-1} assigned to the C=N stretching vibrations as "displaced from the usual position at 1690 - 1640cms^{-1} as the result of strong hydrogen bonding". Bands in the 1525cms^{-1} region are presented as the C=N vibrations of substituted Schiff bonds of β-diketones and the copper chelates.

As discussed in the previous section, the 1690 - 1500cms^{-1} region appears to be full of controversy as a large number of bands which are important to the complexes in this research, appear in this region. There appears to be a slight controversy over the definite assignment of bands in the 1600 - 1630cms^{-1} region.

v) Shifts due to metal co-ordination

Metal co-ordination to any organic molecule will change the modes of vibration and the nature of the metal bond to the molecule will effect the intensity of position of the vibrations (if the M-N or M-O bonds are strong then the intensity will increase if the characteristic is enhanced). Shifts in the metal-pyridine stretching modes have been correlated by some writers[33,34]. However, no such correlation has been attempted for the metal-PLP and metal-PAMP complexes.

C=N and C-O bands shifts in metal Schiff base complexes have been studied[35,43,44,42], and effects due to the strength of the metal -O and M-N bonds quantitatively discussed. Manganese and zinc, and nickel are assumed to be weakly bonded through the phenolic ring, and therefore assumed a general overall polar nature (in the 1700 - 1900cms^{-1}) and display their ionic properties. However, the copper chelate has been said to be more strongly bonded through the phenolic oxygen and is therefore a "neutral molecule" displaying no absorption in the "ionic" zone.

Note:

Sulphate absorption[50] has a rather broad and engulfing mode. The free SO_4^{2-} ion is reported[106] to have two bands in the region under consideration in the present work. The frequencies are ν_3=1104cms^{-1} and ν_4= 613cms^{-1} both of which are very strong. The shifts of these modes will depend on the type of co-ordination of the sulphate and consequently its symmetry will change. This additive to the phosphate absorption region gives a rather large area where strong broad absorption would be found and the finer bonds of the organic molecule would be lost. The phosphate (PO_4^{3-}) modes are quoted as ν_3=1080cms^{-1} and ν_4=500cms^{-1}, with ν_2=970cms^{-1}.

LIGAND FIELD THEORY[60]

Historical.

In 1929, Bethe considered the perturbing effects of an electric field, produced by ligands, on the energy levels of a partly filled shell of the central ion. The energy levels, which were considered perturbed octahedra, were found to be split, in the case of partly filled d-shells, into two sub-shells. Van Vleck, however, pointed out that the molecular orbital theory, assuming partial covalent bonding, would also provide for the splitting of the d-shell into two sub-shells. The application of these two theories has led to the ligand field theory, and has provided a method whereby workers could represent the symmetry, bonding and energy level splitting of the transition metal chemistry.

Wave functions and symmetry.

Each overall wave function is defined by a set of quantum nos., n, l, m, and s. The wavefunction Ψ for a single electron (e.g. the hydrogen atom) is defined by four parameters which are dependent on these quantum nos.

$$\Psi = R(r)\cdot\Theta(\theta)\cdot\Psi_s\cdot\Phi(\phi)$$

r = radial distance

θ, ϕ are angles

Ψ_s spin function

The quantum number n is the principal quantum number and all the others depend on it. l can assume values of 0 to (n-l) and according to the number l, symbols are given to the states such that

l = 0 1 2 3 4 5 6 . . .

Symbol s p d f g h i . . .

m, the magnetic quantum no. contributes to both the angles θ and ϕ and can assume nos. -l to 0 and 0 to l

s, the spin quantum no., which governs Ψ_s, may only assume the values $\pm\frac{1}{2}$, The 4 quantum numbers are therefore sufficient to specify any electron in the system, and the most important one is <u>l</u>, which sums all the angular momenta of the various electrons.

Russell-Saunders coupling

The coupling of the quantum numbers L-S, which give the total angular momentum and the spin angular momentum, were used to characterise the configuration of various state. Table 5 shows the designations of the scheme for single electrons.

TABLE 5

L =	0	1	2	3	4	5	6	...
Letter symbols	S	P	D	F	G	H	I	....

Multiplicity was found to be another factor governing the electronic states and this was defined as (2S + 1), (where S is the spin quantum number) and appeated as superscript of L; the number also indicated the statė (singlet, triplet ...etc.)

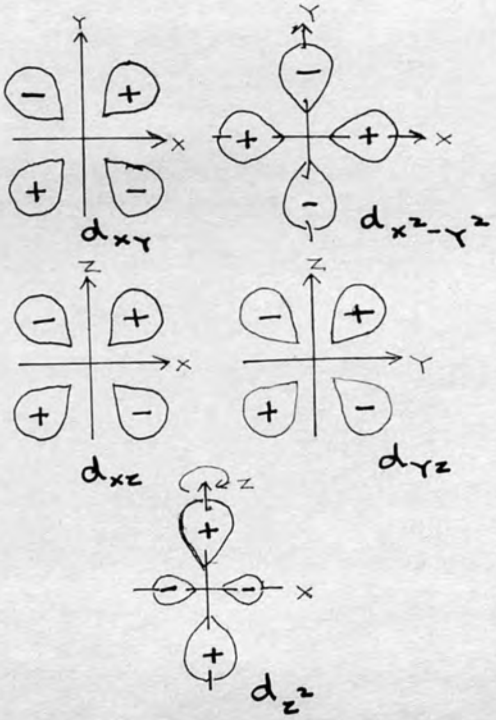
Table 6 indicates the L-S coupling for all d^{n} systems.

TABLE 6

d^{1}		$^{2}(D)$					
d^{2}	$^{1}(S,D,G)$		$^{3}(P,F)$				
d^{3}		$^{2}(D)$		$^{2}(P,D,F,G,H)$	$^{4}(P,F)$		
d^{4}	$^{1}(S.D.G)$		$^{3}(P,F)$	(S,D,F,G,I)	$^{3}(P,D,F,G,H)$	$^{5}(D)$	
d^{5}		$^{2}(D)$		$^{2}(P,D,F,G,H)$	$^{4}(P,F)$	$^{2}(S,D,F,G,I)$	$^{4}(D,G)$
d^{6}	same as d^{4}						
d^{7}	same as d^{3}						
d^{8}	same as d^{2}						
d^{9}	same as d^{1}						
d^{10}	$^{1}(S)$						

Symmetry of splitting of energy levels.

The five sets of d-wave functions are related to symmetry groups by their ligand environments and by the splitting and distortion of the atomic orbitals of the central metal ion. A diagram of the geometry of the five d-orbitals is shown below.



The d-orbitals.

FIG. 12

The spherical potential which exist around the free ions, such that the five d-orbitals have equivalent energies, and are therefore degenerate. The effect of the ligand is such that the degeneracy is broken, and the d-orbital energies are separated by an energy difference. This ligand field effect occurs most commonly in two ways, such that either octahedral or tetrahedral geometries occur. The full degenerate d-orbitals are split into two subshells, t_{2g} and e_g energy levels, and the symmetry of the resulting co-ordination complex, determines the position of the various orbitals.

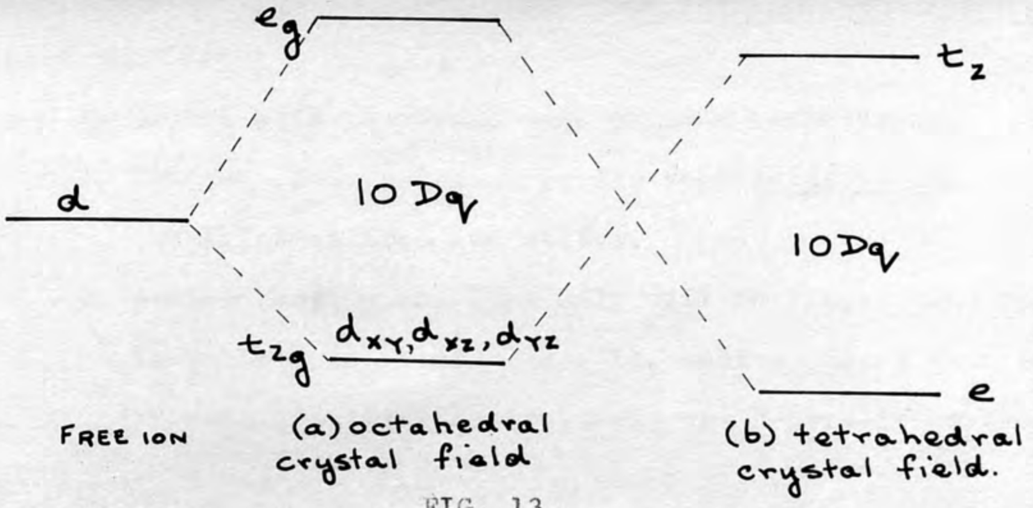


FIG. 13.

The separation between the two energy level sets, t_{2g} and e_g is exposed by an energy quantity called 10Dq, where q and D are cubic crystal field parameters. 10Dq varies with the symmetry of the molecule, and thus gives an indication of the stereochemistry of the molecule. This quantity 10Dq is an energy term and can be measured spectroscopically in the visible region of the spectrum. Electronic transitions between energy subshells depend directly on the symmetry of the molecule; Table 7 exemplifies the splitting of the Russell-Saunders states in the octahedral and tetrahedral electrostatic fields. In the case of the first row transition metals, the most important states of free ions will be those of S, P and D.

TABLE 7

State of free ion	States of crystal field.
S	A_1
P	T_1
D	$E + T_2$
F	$A_2 + T_1 + T_2$
G	$A_1 + E + T_1 + T_2$
H	$E + 2T_1 + T_2$

States represented bu the L - S energy diagrams obey three basic results:-

1. States with identical designations never cross.
2. Crystal field states have the same multiplicity as the original free ion states.
3. States having one type only will be linear with respect to the crystal field strength, whereas those with two or more identical designations are generally curved.

ELECTRON DISTRIBUTIONS IN CHEMICAL SYSTEMS.

Hund's general rule states that electronic states of a free atom with greater spin lie at lower energies than those with higher spin. It would therefore be expected that if there are two possible electronic states, singlet and triplet states, the triplet state will have a lower energy than the singlet state*. The Pauli exclusion principle requires the spins of the two electrons occupying the same molecular orbital to be opposed; but single electrons are allowed either spin orientation. However, the excitation of an electron from a full orbital to a half-filled orbital must be such that the electrons are of opposite spins, in order to satisfy the Pauli principle.

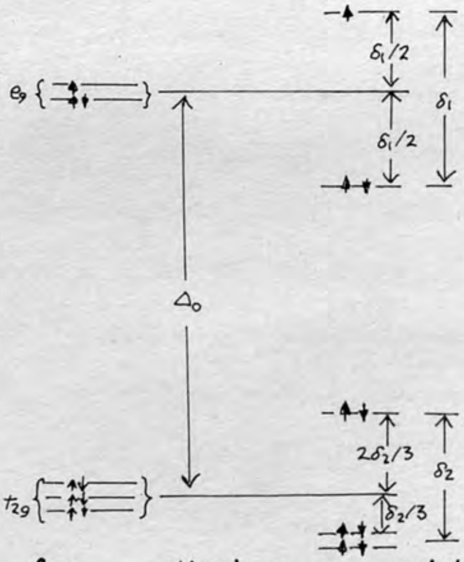
Electron distribution will play an important part in determining the symmetry, and consequent physical properties of the complex. Spin pairing causes the phenomenum of high spin and low spin complexes where there may be either three (or five) or one single electrons in similar number of d-

---

[*] Singlet and triplet electronic states of a molecule are related to the number of unpaired electrons in the molecule. These states are governed by multiplicity (see p.43).

molecular orbitals. The extent of high spin/low spin complexes is measurable in the magnetic properties of the complex, which will not be discussed in the present work.

A useful tool in predicting geometres is that of the Jahn-Teller theorem. This theorem states that: If a molecule is seen to give rise to an orbitally degenerate ground term, it will be found to have distorted itself so as to remove that degeneracy. One can therefore predict that if there is either, a vacant orbital, or a half filled orbitals (for example, the Cu^{2+} ion), the molecule will distort until the degeneracy is removed, and the symmetry will also have changed. The splitting of the d-orbitals is such that the centres of gravity of the two subshells, t_{2g} and e_g, are unchanged by the presence of the lower symmetry component.



Schematic diagram of the splittings caused by elongation of an octahedron along one axis. δ_1 and δ_2 are much smaller relative to Δ_o, than indicated.

The two e_g orbital separate so that one goes up and the other goes down as much, while the t_{2g} orbitals separate so that the doubly degenerate pair goes down only half as far as the single orbital goes up.

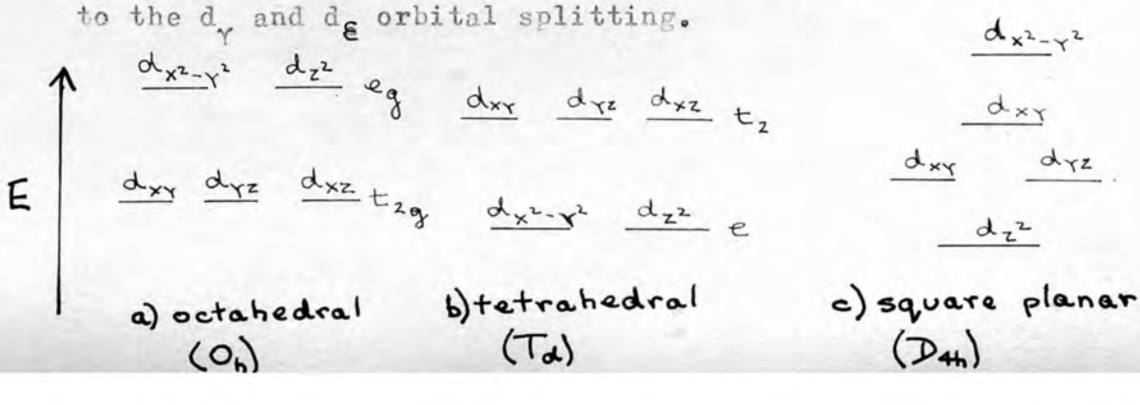
In the case of octahedral complexes, the Jahn-Teller distortion occurs if unsymmetrical distribution of electrons in e_g orbitals is present, viz. e_g^1 or e_g^3.

It is therefore expected to occur for $t_{2g}^3 e_g^1 (d^4)$, $t_{2g}^6 e_g^1 (d^7) Co^{II}$, and $t_{2g}^6 e_g^3 (d^9) Cu^{II}$ electron distributions.

PREVIOUS REVIEWS OF LIGAND FIELD THEORY and STEROCHEMISTRY

Gillespie and Nyholm[61] have made a fairly extensive review of inorganic sterochemistry. They review the d-orbitals with respect to splitting of the orbitals and rearrangement of the electronic charge. The five d-orbitals are considered equivalent and degenerate until they are arranged specifically. The d_{z^2}, and $d_{x^2-y^2}$ are directed along the x, y and z axes and in the case of octahedral complexes, are considered of highest symmetry and lowest energy and therefore, are filled first. They are called t_{2g} or e_g orbitals. A further feature which is found to occur, together with electronic transitions within the d-orbitals, is the charge transfer from ligand to metal ion or visa versa. This happens if dπ - dπ or dπ - pπ bonding is also present[62]. The t_{2g} and e_g ratio depend on the shapes of the complex molecule. The stereochemical shape of the molecule depends directly on the number of non-bonding d-electrons on the metal ion. t_{2g} and e_g orbital arrangement of electrons determines the shape, and in the case of the number of non-bonding electrons exceeding 4, the magnetic properties of the molecules as well.

Griffith and Orgel[63] have studied the ligand theory with special reference to the orbital energies derived from electrostatic theory. The following is the table of electron arrangements in various electrostatic fields, with reference to the d_γ and d_ε orbital splitting.



If any of the regular symmetries becomes distorted than the energy level (the lowest, and therefore most stable) will be found to split.

Valence Bond Theory[64]

The valence bond theory regards a molecule as being composed of atoms which retain their individual character on bonding. This theory was formulated by Heitler-London who described the bonding in terms of wave functions. These wave functions are explicit in the definition if the position of the electronic charge. It is therefore difficult to apply this theory to a system where there is a large amount of delocalised charge.

In the valence bond theory the σ, π character of separate bonds is preserved but the <u>bond order</u> is simply the number of pairs of electrons whose spins are coupled together. Repulsion will only occur where there are unpaired sets of electrons. In the hetronuclear bond however, as in the unfilled d-system, the complexity of the definition of the system led workers to revert to the more simple molecular orbital theory.

However, it has been found useful to represent the various sets of energy levels in the 3d, 4s and 4p by means of the valence bond "boxes". This together with the knowledge of the various numbers of electrons entering the system, have been used to attempt to describe the probably shapes of the molecules, with respect to the metal ions. Symmetry considerations and molecular orbital calculations are then used to determine the physical properties of the complex molecule.

STEREOCHEMISTRY and CONFIGURATION of the METAL IONS.

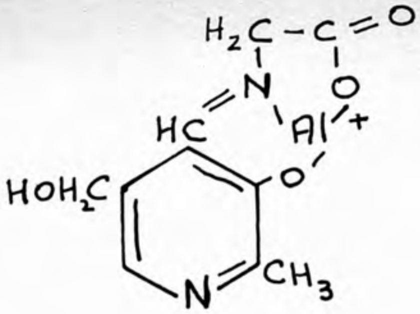
Aluminium stereochemistry.

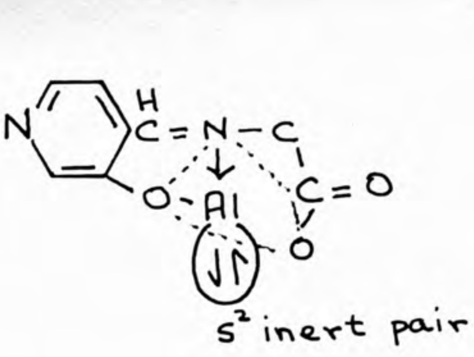
The nature of the more common aluminium complexes is essentially ionic which is party due to the lack of d-electrons, and the Al(III) state which is similar to the inert neon-electronic structure. However, covalent features have been found in several complexes. The stability of the Al(I) oxidation state has been attributed to the $3s^2$ "inert pair effect" which is said to characterise and stabilize group III elements.

The ability for the metal to accept neutral donor molecules decreases on descending the group, so that the ability to co-ordinate is of the order Al > Ga > In., and dimeric species of both Al and Ga have been found. Polymerisation has been more commonly found in the Al(III) oxidation state.

Stereochemically, aluminium (III) occurs in both tetrahedral and octahedral configurations. The shape of the molecule of the Schiff base complex, will depend on the ratio of aluminium to the Schiff base molecule, and whether it acts as a bi- or tri-dentate ligand. In compounds with a 2:1 Schiff base to metal ratio, the complex would most likely be trans-octahedral, whereas in the case of a 1:1 ratio, the structure would be a distorted tetrahedron.

However, in complexes of the pyridoxal phosphate Schiff bases, Fasella et al[66] have found the ratio of Al to Schiff base to be 1:1. They postulated the existence of the Al(I) oxidation species which would imply tetrahedral or trigonal configuration. Account must be taken of the $3s^2$ inert pair and this would seem to signify that the aluminium has attained the co-ordination number of 4 with a tridentate ligand. The configuration of the Al(I) species would then appear to be a trigonal pyramid with the "inert $3s^2$" pair at the apex of the pyramid.





If the aluminium has a octahedral configuration, then either the two Schiff bases act as tridendate ligands, and no further substitution is possible, or, they act as bidentate ligands (with either the phenolic or carboxylate oxygens non bonding) which would allow either water or hydroxyl molecules to substitute for the carboxylate donor group.

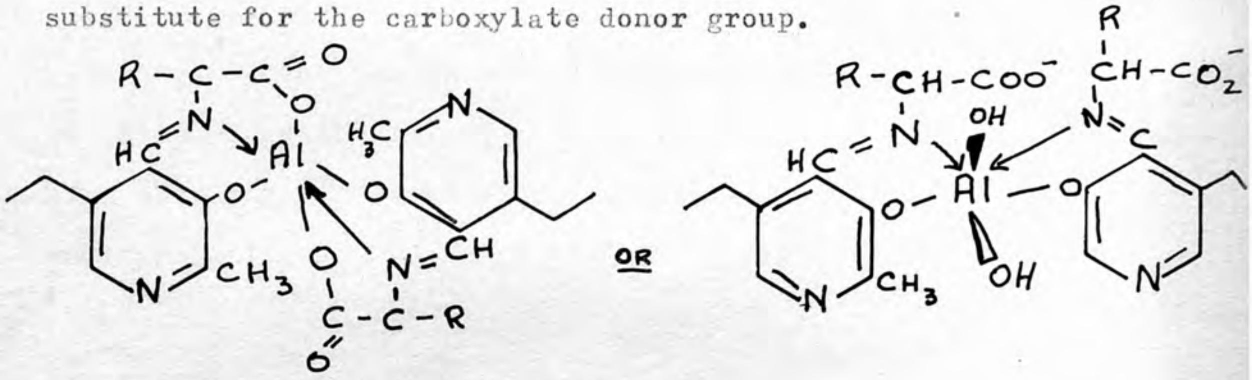


FIG. 14

Gallium[67]

The chemistry of gallium is similar to that of aluminium except that gallium has a completely filled d-shell which shields the nucleus more effectively than the aluminium nucleus. Because of the d^{10} configuration there is no ligand field stabilisation and therefore no d-d visible transitions.

The Ga(III) oxidation state allows the following configurations; the sp^3 hybridisation predicts a tetrahedral configuration. The sp^3d^2 octahedral configuration predicts the 2:1 ratio of Schiff base to gallium ion in the +3 oxidation state.

The gallium^{+1} oxidation state has an inert pair, the $4s^2$ electron pair, which accounts for the extra stability of the gallium ion as a catalyst. Stereochemically, the configurations possible for the Ga(I) state are the trigonal pyramid, tetrahedron, and square pyramid, depending on the Schiff base to metal ion ratio. The lone s^2 pair influences the shape of the molecule and forms an integral part of it.

The structure of unipositive gallium with donor ligand is assumed to involve the use of the sp^3d orbitals, one of which is occupied by an lone pair of the gallium atom. This hybridisation corresponds to a trigonal bipyramid configuration[95]. Calculations of the stability constant indicated[96] that bonds with a co-ordination number of 4 are ~~found~~ formed by the p^3d orbitals of gallium and the arrangement of four atoms at the basal angles of a tetragonal pyramid with a gallium atom, at its vertex most probable.

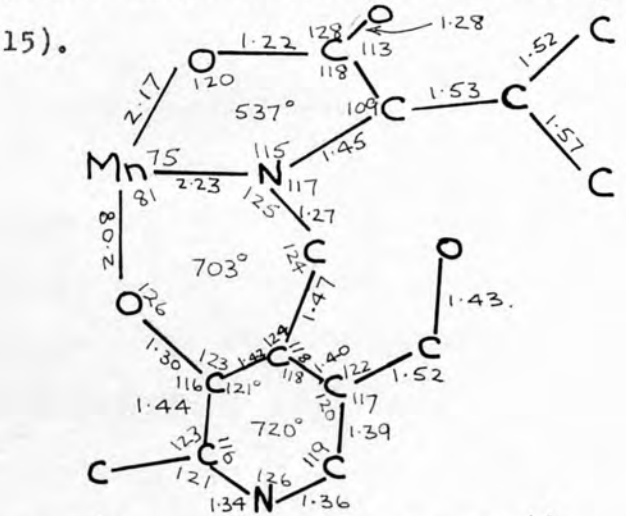
Manganese.

The manganese (II) complexes cannot really be compared to the divalent states of the suceeding transition row elements. This is due to the fact that the d^5 configuration has little or no ligand field stabilization energy, except in the case of low spin complexes. High spin complexes of Mn(II) have the $t_{2g}{}^3e_g{}^2$ electron distribution. The energies of the orbitals will therefore be equivalent and degenerate, and the Jahn-Teller effect will not operate.

Ligands such as ethylenediamine, EDTA, and tridentate amines are able to form penta- and 7- co-ordinate species with Mn(II). These will low spin complexes of Mn(II) because the donor atoms of the ligands have sufficient energy to force the d-electrons of the free Mn^{2+} ion to spin-pair.

Mn(II) tetrahedral complexes have been examined but they are not very stable and require strong donors such as phosphines and arsines to maintain the necessary stability.

Hoard *et al*[20] have determined the structure of the Mn(II) pyridoxylidene valinate chelate by X-ray analysis. The configuration is that of a distorted octahedron, which thus allows the maintaince of planarity of the pyridine ring system. The quasi planar shape of this part of the system is shown below (Fig. 15).

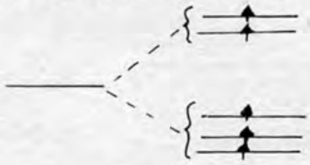


ONE $(PL-val)^-$ chelated to Mn^{++}

FIG 15.

The ratio of Schiff base to Mn(II) ion is 2:1 completing the octahedron. The two-fold axis bisects the opposite pairs of octahedral edges and renders the two pyridoxyledene-valinate molecules structurally equivalent. The symmetry of the molecule is therefore dihedral and the point group is D_{2h}.

The Russell-Saunders coupling scheme of the d^5 electronic configuration is shown in Table 5.



The ground state of the d^5 ion, with parallel spins gives rise to the $t_{2g}^3 e_g^2$ configuration, with sextuplet, quartet and doublet states. Hence transitions from the ^6S ground state to any excited state will involve spin pairing, and then these will be weak, since they are all spin forbidden. Fig 16[65], shows the Orgel diagram of the possible transition of the Mn(II) ion, showing only the sextuplet ground state and quartet excited states.

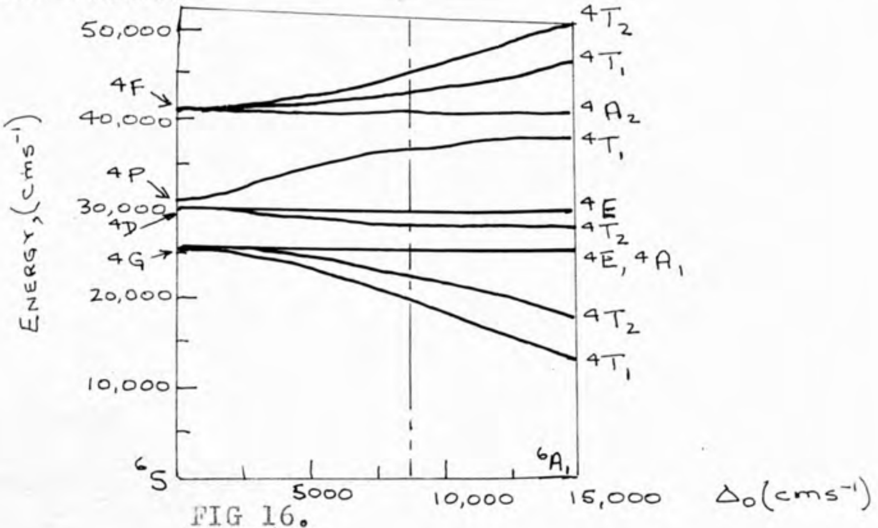


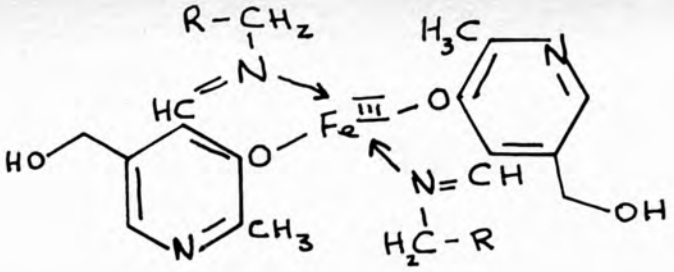
FIG 16.

Tetrahedral Mn(II) complexes are typified by their yellow green colour and sparsity of the visible ligand field bands. However, the bands in tetrahedral symmetries do appear to be more intense than the Fe(III).

Iron (ferric)

The ferric state of ion is considered fairly analogous to the manganese (II) case, because it also has a half filled 3d subshell containing five electrons. High spin complexes $(t_{2g}^{3}e_{g}^{2})$ will have no ligand field stabilisation energy and therefore no bands in the visible region. Tetrahedral and octahedral (tending to tetragonal ~~configurations~~ lengthening of the d_{z^2} axis) configurations are the expected shapes of ferric complexes. The main difference between the Mn2+ and ferric 3+ configuration will be dependent on the size of the ion, and the increase in ionic charge in the case of the ferric ion. High spin states of iron (III) $((t_{2g}^{3}e_{g}^{2}))$ will have a stable sextuplet state, while spin states will contain quartet and doublet states.

2:1 Schiff base to metal ion ratio have been suggested [67,39] with the following octahedral - tetragonal configurations.



XXXII

A *trans* octahedral structure gave the most stable ionic configuration bands of octahedral complexes, and this is partially accounted for by the overlap of the p- and d- orbitals. This combination of molecular orbitals by the overlap of the metal d-orbitals with the ligand orbitals of the same symmetry.

Low spin Mn(II) complexes (and therefore quartet states) have been reported where π-bonding of the ligand was assumed to be strong enough to cause spin pairing.

Cobalt (II)

The most common geometries for the cobaltous ion are four co-ordinate tetrahedral, or six co-ordinate octahedral[107]. However, because of the d^7 electronic configuration there will be doublet states of the ground state (A_2), and the Jahn-Teller effect will minimise the degeneracy by distorting the ideal geometry to a pseudo-shape.

Splitting of the degenerate d-orbitals will later place in the following manner.

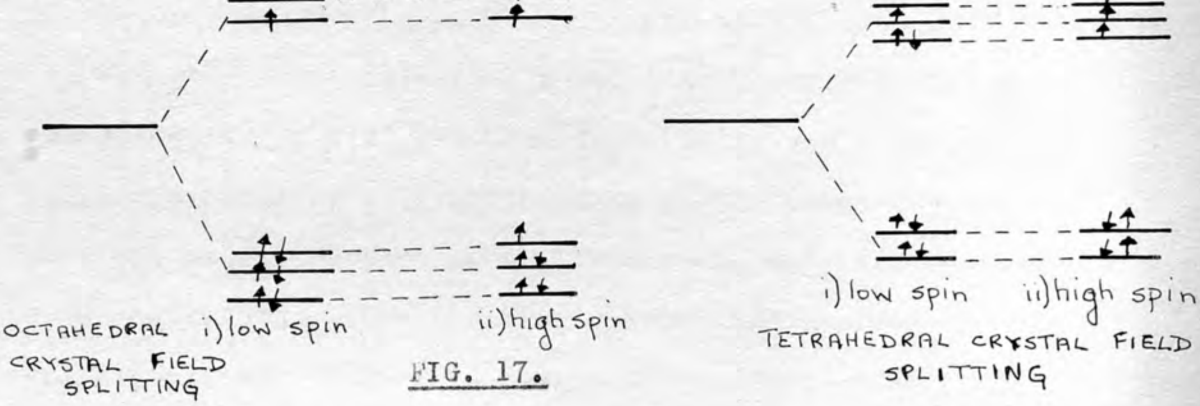


FIG. 17.

The differences in the electronic spectra of the tetrahedral and octahedral species of cobalt(II) lies in their molar extinction co-efficients; $\mathcal{E}_M$ of the tetrahedral configuration is approximately 10^2 greater than the octahedral extinction co-efficient. Absorption bands of the two geometries lie in similar regions, but there can be more reason for assuming that a band in the 15,000cms^{-1} region belongs to the tetrahedral species, as calculations by Leihr[108] have shown.

An unusual geometry for four-co-ordinate Co(II) if that of square planar[107]. This could be either spin-free or spin-paired but generally these have been found to be spin-paired in all the compounds investigated in the bis(salicylald) ethylene-dimine cobalt (II) and phthalocyanine series[109]. The order of energy levels was deduced[109] from a simple point charge to be in the following order, if the ligands were on the x and y axes:

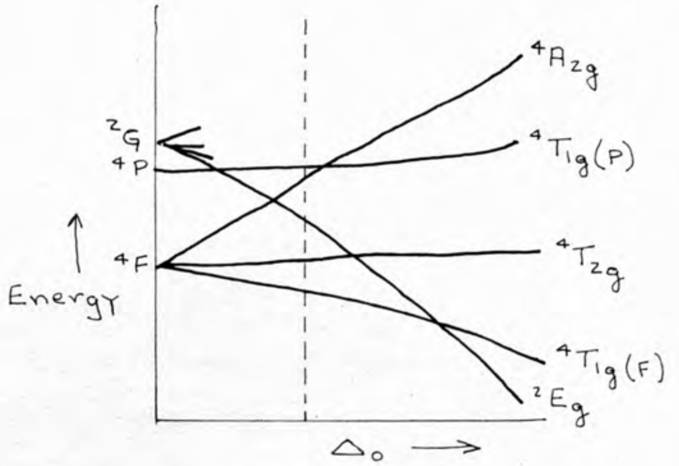
$$d_{z^2} < d_{xz}, d_{yz} < d_{xy} < d_{x^2-y^2}$$

with the energy separation $d_{xy} - d_{x^2-y^2}$ of the order of $t_{2g}-e_g$ separation in the octahedral case.

Five co-ordinate Co(II) is becoming increasing more prominent, with both square pyramidal and trigonal bipyramid geometries being reported, in particular with Schiff base types of ligands.

Co(II) bis(N-methylsalicylaldiminato)[69] was found to be a dimer with cobalt five co-ordinate. Magnetic moment studies have shown the trigonal bipyramidal nature of the complex and the combination of the orbitals. High spin or low spin states are expected with quartet or doublet transitional states respectively. Co(II) has been found to assume a trigonal bipyramid geometry with poly-amines of the (tren Me) series[70]. From the quartet states of the free ions, and from equivalent dipole strengths, four electronic transitions occur in the visible region: viz. (fig. 18).

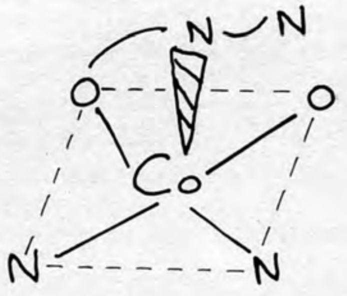
A partial energy level diagram for d^7 ions in an octahedral field.



$^2E_g \rightarrow {}^4T_{1g}(F)$
$\rightarrow {}^4T_{2g}$
$\rightarrow {}^4T_{1g}(P)$
$\rightarrow {}^4A_{2g}$.

FIG. 18.

Square pyramidal configuration of Co(II) have been ascertained for Co(II) bis(salicylaldimine)^{71} by X-ray analysis. Three bands were found at 6,700, 11,400 and 16,800 cms^{-1} and was said to exemplify a distorted square pyramidal configuration.



XXXIII

These substituted Schiff base-metal complexes of Co(II) are analogous to the pyridoxylidene (phosphate) amino acid chelates as they contain the same donor atoms (Nitrogen and Oxygen) and therefore the essential stereochemical factors could be considered similar. However, various substitution studies have shown that substitution on the nitrogen donor <u>does</u> have an effect on the shape of molecule.

Nickel (II) complexes.

In considering the stereochemistry of Ni(II), it is important to take account of the two common co-ordinations numbers of the +2 oxidation state. These are 6 and 4 which give rise to the basic octahedral and tetrahedral $(e_g{}^4t_{2g}{}^4)$ configurations, respectively. Electronic distributions of $t_{2g}{}^6e_g{}^2$ exemplify a regular octahedral configuration and possible paramagnetic species could occur. Ligand field stabilisation energy was found to be greater in the case of the octahedral complex[77] when compared to the tetrahedral configuration[77], and the tetrahedral species was therefore felt to be a less stable shape for the molecule.

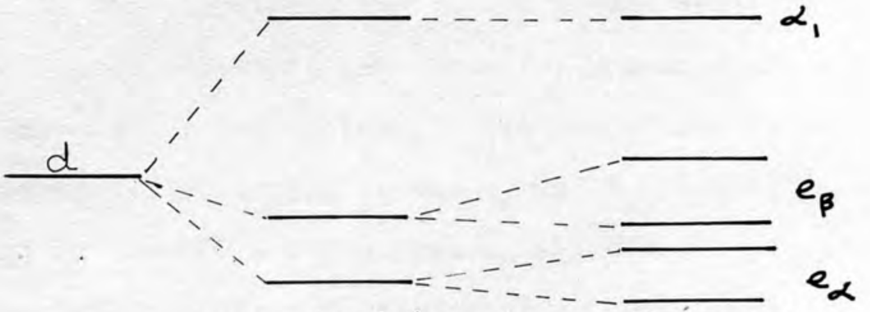
The co-ordination number of 4 is exemplified by several configurations: the regular tetrahedron , and the square planar configuration. Tetrahedral complexes, as reported by Sacconi[72], are paramagnetic with characteric intense electronic spectra, have $e_g{}^4t_{2g}{}^4$ electronic configuration, with two unpaired electrons. When compared to the tetrahedral configuration, the square planar nickel molecule can be either diamagnetic (singlet) or paramagnetic (triplet). Visible bands of medium intensity are expected in the 450 - 600cms^{-1} region. The differences in the band around 400mμ are said[73] to differentiate between the tetrahedral and square planar configurations. The square planar complexes studied exhibited a medium intensity band in this region, which appeared to be absent in the tetrahedral configurations.

An anomolous paramagnetic complex of bis(N-methyl salicylaldime)Ni(II) was found to[74,75] be tetragonal. This was explained by Nyholm[76] (in valence bond terms) to be four-covalent $(4s4p^3$ hybridised) in a basic tetrahedral arrangment, and was then increased to a tetragonal configuration $(4s4p^34d^2)$ by polymerisation with two orthogonal molecular orbitals. One band only was found in the visible region at 16,000cms^{-1}. [75].

The intermediate co-ordination number of 5 has also been found to occur in nickel complexes[78,79], as either the square pyrimidal or trigonal bipyramidal configurations. If one considered the symmetry properties of these two configurations it would be found that the square pyramid belongs to the D_{4h} class while the trigonal bipyramid belongs to the lower C_{3v} class. However, the symmetry of the ligand would govern the shape of the molecule, because of the necessity to overlap orbitals of similar symmetry.

Sacconi[80] has studied the five co-ordinated species of Ni(II) of the general formula $-NR_2-N(C_2H_5)_2$. He found that $\mu_{eff} = 3.25 - 3.30$BM and reflectance spectra absorption maxima to contain three bands viz: 7,700, 9,800 and 16,200cms^{-1}. Calculations explained these properties to be attributable to a distorted square planar configuration.

The Orgel diagram for the trigonal bipyramidal configuration was found to exhibit a large number of possible electronic transitions. However, Dyer[78], found that only two of these were spin allowed transitions, and explained this in a field of C_{3v} symmetry by the following ligand field splitting (Fig.19).



(a) regular trigonal Bipyramid b) distorted

FIG. 19

The $e_\alpha \rightarrow \alpha_1$ and $e_\beta \rightarrow \alpha_1$ electronic transitions are said to belong to the $A_1 \rightarrow E_1(D)$ class. Einstein's tables[81] help to elucidate the shape of the molecule since the d_{z^2} and p_z orbitals of the metal ion have a_1 symmetry, with two filled subshells $(e_\alpha)^4(e_\beta)^4$. Anomolous magnetic moments of Ni(II) complexes were due to 1)square planar-tetrahedral equilibrium.

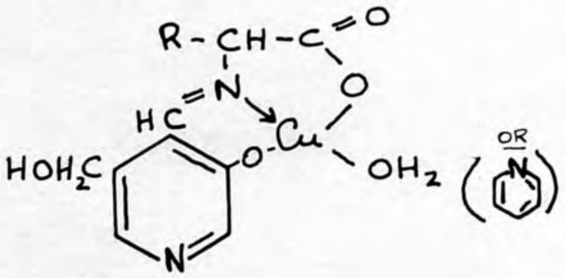
2)in octahedral equilibrium by ligand

3)octahedral equilibrium by solvent

4)polymerisation

Copper

The Cu(II) posses the d^9 electronic configuration which is subject to large distortions, bourne out by the Jahn-Teller theorem. It is felt that in the case of the Cu(II) pyridoxylidene amino acid chelates, the Schiff base acts as a tridentate-donor, with two oxygen and one nitrogen co-ordinating to the metal. The fourth position of the tetrahedron (or square planar complex) could be occupied by a water molecule or a hydroxyl group or by pyridine donor. If the structure is to be trigonal bipyramid then the hybridisation would be $4s4p^34d$, with a magnetic moment of μeff = 1.73B.M.



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It is therefore felt logical to assume that the Cu(II) assumes either a tetrahedral or square planar form, with the usual Jahn-Teller distortions. Electronic spectra predicts that tetragonal splitting of the e_g and t_{2g} levels would produce one band in the 600 - 900mμ region, which could possibly be resolved into 3 bands. Charge transfer bonds in the blue end of the visible spectrum could also occur, as the donation of electrons from ligand to metal does occur in the case of the nitrogen.

The following transitions could occur:-

$d_{x^2-y^2} \longrightarrow d_{xy}$))These two occur close together
$d_{x^2-y^2} \longrightarrow d_{z^2}$))usually as an envelope.
$d_{x^2-y^2} \longrightarrow (d_{zx}, d_{yz})$) -often escape detection.

However, Cu(II) bis(salicylaldimato) and bisacetylacetenato Cu(II) complexes are known to react with pyridine to form pentaco-ord. species of Cu(II) with trigonal bipyrimidal and square pyramidal configurations[82-4].

$\underline{Cu(H_2O)_6^{++}}$ tetragon. pyramid[85]
trig. bipyrid

max. 7,00mμ' (14,300cms^{-1})

$Cu(NH_3)_4^{++}$ [86]

600mμ narrower (16,500cms^{-1})

d-d bonds around 750mμ are broad. (13,400cms^{-1})

Sacconi[87] quoted the case of Cu++ both tetra- and penta-co-ordinate with substituted salicyladehyde-ethylediamine Schiff bases. Here for 5 co-ordination, one ligand acts as a bidentate and the other as a tridentate ligand. These 5 co-ordinated compounds are characterised by a single band around 15,000cms^{-1} similar to trans-planar tetrahedral complexes[88].

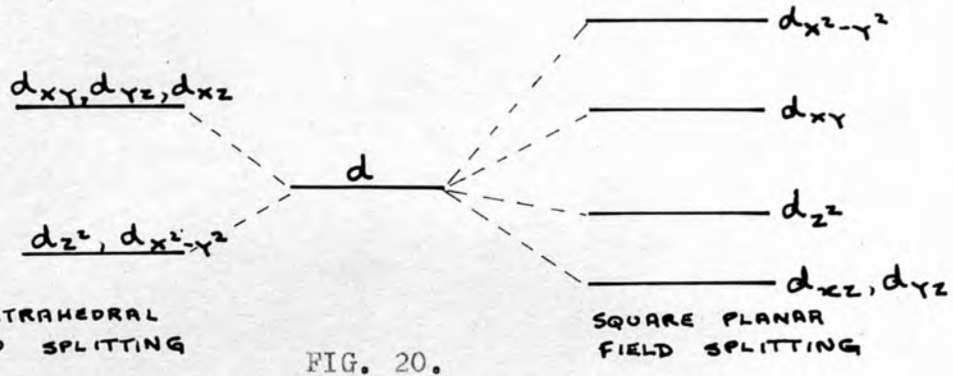
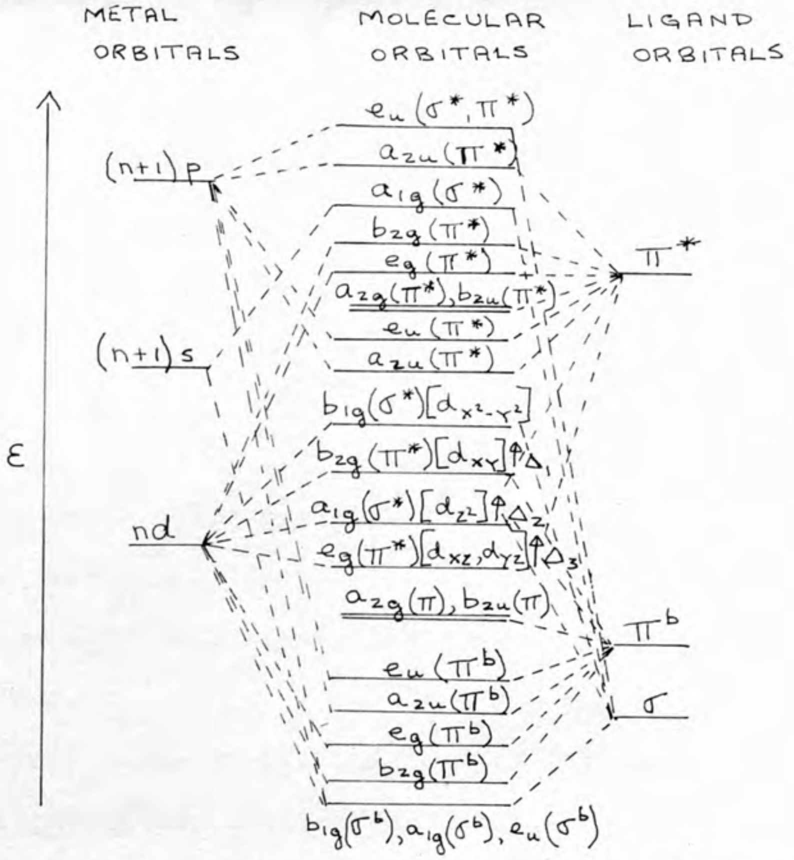


FIG. 20.

Assuming the solvent to form the penta-co-ordinated position along the z-axis, the compound could be called a flattened tetragonal pyramid - flattened pyramidal structure[89].

The metal orbitals involved in π-bonding in square planar complexes[90] are the ~~ndz~~^{2}, $\underline{ndx^2-y^2}$ ~~(n+1)s~~, (n+1)px, (n+1)py. The most important π-orbital is the a_{2u} from the (n+1)pz molecular orbital + four π_r- ligand orbitals, according to molecular orbital theory[90].



MOLECULAR ORBITAL ENERGY LEVEL SCHEME for SQUARE PLANAR METAL COMPLEXES in which the LIGANDS THEMSELVES HAVE A π-ORBITAL SYSTEM.

FIG.21.

The addition of the fifth group above the square planar ring system greatly reduces the ring π-bonding by tying up the $(n+1)p_z$ in π bonding.

Ligand to metal charge transfers are usually separated by 10,000 - 13,000 cms^{-1}.

Jorgensen[91] states that the shortening of the Cu-N bond increases the strength of the $d_{x^2-y^2}$ and d_{z^2} and therefore produces a blue shift,

Hall and coworkers[23] have analysed the Cu(II) (pyridoxyledene valinate) chelate by X-ray analysis. They reported the existence of a five co-ordinate copper atom, with a square pyramidal configuration.

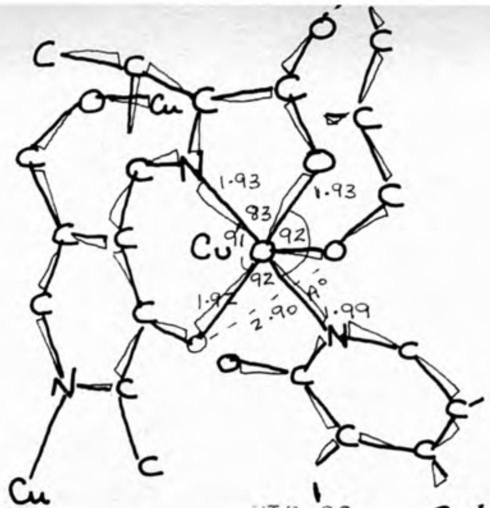


FIG. 22 Cu(II) pyridoxylidene(±)-valine.

The complex has a polymeric structure, and in the solid state it has the essential planarity postulated by Metzler and thought necessary in facilitating the reaction of the cofactor (PLP ⟶ PMP).

A further X-ray analysis of interest in this research is that of the Cu(II) bis(N-methylsalicylaldenato)[92]. Each copper atom was found to be five co-ordinate, with a $Cu\langle^{O}_{O}\rangle Cu$ unit giving a square distorted planar bridge.

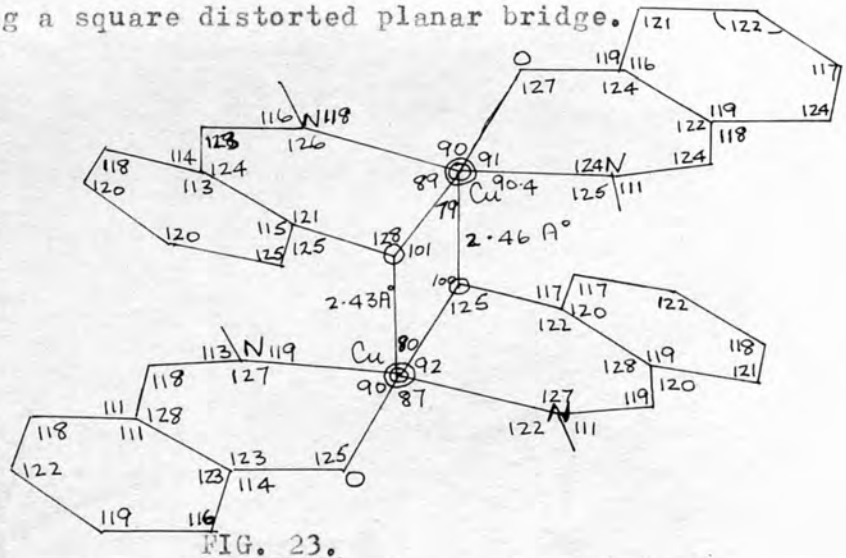
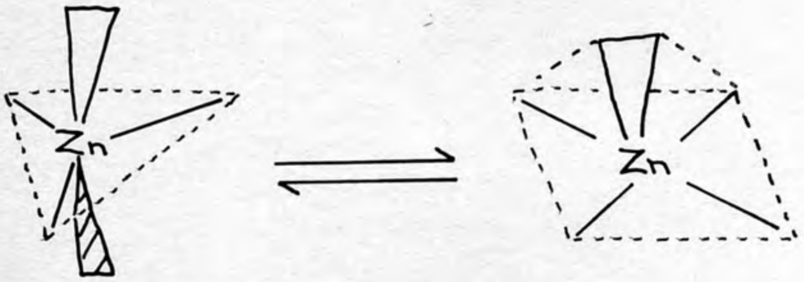


FIG. 23.

Corbridge[98] discussed the 5-co-ordinate nature of copper and suggested the dsp^3 and d^2sp^2. hybridisation would result in a trigonal bipyramid configuration, whereas the $3d4s4p^3$ hybridisation would result in tetrahedral pyramidal configuration. Distortion of any configuration would most likely result in a splitting of vibrational levels and splitting of the visible region bands.

Zinc.

The electronic state of the free Zn(II) ion is characteristic ~~by~~ of the full 3d subshell, and therefore either extremely stable or very labile complexes are expected. The completed 3d subshell rules out the possibility of ligand field stabilization and no bands are found in the visible region of the spectrum. The most common co-ordination number of zinc is 4, with sp^3 hybridisation. Stereochemically the configuration of the molecule would probably be tetrahedral. However, the bis(glycinyl)zinc has been reported to be a square planar configuration. 5-co-ordinate zinc complexes have been reported by Sacconni and coworkers[94]. Ligands are reported to contain nitrogen and oxygen donor atoms which Sacconni[80] concluded gave rise to this unusual co-ordination number of 5. Polyamine complexes have been reported[70] to be a trigonal bipyramid configuration. Substitution of the N,O donors would obviously alter the ligand field and the 5-co-ordinate configuration has been reported to alternate between a trigonal bipyramid and square structure. Hybridisation of the sp^3d orbital would occur[94,87].



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SECTION II

RESULTS and DISCUSSION.

EXPERIMENTAL

Preparations:

A. Metal-pyridoxal phosphate-L sodium glutamate complex.

5 mM of each of pyridoxal phosphate and sodium glutamate were separately dissolved in a minimum volume of water. Solid sodium bicarbonate was used to adjust the pH to approximately 7. These were then mixed and the mixture was added to an equivalent weight of metal salt*, which has been dissolved in a minimum volume of water. The solution was cooled in ice. A precipitate was filtered off, and further yields were obtained by adding methanol or methylated spirits. In the case of the zinc complex, dioxane was found to be a more suitable precipitating agent.

The precipitates were washed with water, methyl alcohol or methylated spirits then ether, and dried in vacuo. Recrystallisation of the products from water was impossible because of the possibility of hydrolysis of the complex. Recrystallisation from organic solvents was also impossible, due to the low solubility of the complexes in the solvents attempted, which included methanol, methylated spirits, ethanol, benzene, dioxan, toluene, chloroform, carbon tetrachloride, pyridine and dimethyl formamide.

B. Metal-pyridoxamine phosphate-α-keto glutaric acid complexes.

5 mM of each of pyridoxamine phosphate and αKG were dissolved in a minimum of warm water and the respective solutions were neutralised by adding solid sodium bicarbonate. The mixture of these solutions was added to 5mM of the metal salt, previously dissolved in a minimum of warm water. The final mixture was warmed to 60° for a few minutes, and then left in ice for three hours (depending on the metal-complex). Methanol or methylated spirits was then added, to assist precipitation. The precipitate was then filtered off and washed with methanol, ether and then dried in vacuo. However, precipitation occurred in several cases

(cobalt, zinc, aluminium and iron) without the addition of the polar solvent, and soon after removing from the heat.

Recrystallisation was similarly found impossible in any aqueous or organic solvents.

3. Metal-pyridoxamine phosphate complexes.

Equimolar quantities of pyridoxamine phosphate and metal chloride were dissolved in 20mls. of 50% aqueous dioxane. The resulting mixture was heated to 60° for several minutes and then cooled in ice. The precipitates were washed with methylated spirits and dried in vacuo.

4. Metal salt-pyridine complexes.

A concentrated methanolic solution of the metal salt (chlorides in this case) was added to about 5mls. of pyridine. Coloured precipitates formed immediately and these were filtered off, washed with methylated spirits, and dried in vacuo. The products themselves were fairly crystalline and pure and did not require recrystallisation.

* Metal salts used were research grade $CuCl_2$, $Ni(SO_4)_2$, $Ga_2(SO_4)_3$ $CoCl_2$, $MnCl_2$, $(NH_4)Al(SO_4)_2$, $(NH_4)Fe(SO_4)_2$, $Zn(SO_4)_2$.

TABLE 8.

PHYSICAL PROPERTIES

Colour and Texture

Compound	Metal ion. Ga^{3+}	Cu^{2+}	Ni^{2+}	Co^{2+}	Fe^{3+}	Mn^{2+}	Al^{3+}	Zn^{2+}
Schiff base series A	light yellow powder	green powder	yellow brown powder	yellowy brown powder	tan fine powder	light brown powder	light yellow powder	canary yellow
Schiff base series B	"	olive green powder	"	^{1}light blue ^{2}grey sandy brown pdr.	tan yellow pwder	——	"	light yellow powder
Pyridoxal phosphate (all powders).	cream	lime green	light green	yellow brown	light brown		light yellow	yellow
Pyridoxamine phosphate (all powders)	yellowy	bright green	yellow green	yellow	light brown		cream	cream
Pyridine salt (chlorides)		sky blue	dirty yellow	a)pink mauve b)bright blue		brown		white

TABLE 9

ANALYSIS of METAL COMPLEXES.

METAL-PYRIDOXAL PHOSPHATE CHELATES

COMPOUND	ANALYSIS %				THEORETICAL %			
	C	H	N	M^{n+}	C	H	N	M^+
$Cu^{II}(PLP)H_2O$	29.37	3.63	4.18	19.66	29.31	3.38	4.27	19.22
$Ni^{II}(PLP)$~~2~~H_2O.OH	29.17	3.62	5.20	17.05	28.10	3.83	4.11	17.22
$Co^{II}(PLP)2H_2O$	29.76	3.53	4.26	15.20	28.19	3.84	4.11	17.22
$Zn^{II}(PLP)H_2O$	29.81	3.53	4.26	19.67	29.16	3.36	4.25	19.83
$Al^{III}(PLP)(SO_4)_2$	19.91	3.46	2.75	6.95	19.88	2.29	2.29	5.58
$Fe^{III}(PLP)(SO_4)(H_2O)$	22.93	2.68	3.90	12.35	22.13	3.02	3.23	12.87
Ga^{III}	32.42	4.11	4.17	13.82				

METAL-PYRIDOXAMINE PHOSPHATE CHELATES.

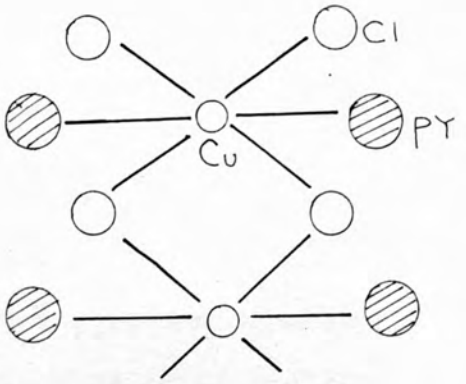
COMPOUND	ANALYSIS %				THEORETICAL %			
	C	H	N	M^{n+}	C	H	N	M^{n+}
$Cu^{II}(PAMP)H_2O$	29.11	4.09	8.72	19.11	29.31	3.99	8.55	19.27
$Ni^{II}(PAMP)$	26.86	5.04	6.94	15.76				
$Co^{II}(PAMP)2H_2O$	27.68	3.97	8.09	13.42	28.19	3.84	8.22	17.22
$Zn^{II}(PAMP)2H_2O$	26.67	3.83	6.72	20.83	27.64	3.77	8.06	18.11
$Al^{III}(PAMP)(SO_4)_2H_2O$	15.38	4.00	4.26	4.47	19.92	2.51	5.81	5.59
Fe^{III}	32.19	4.63	5.93	13.23				
$Ga^{III}(PAMP)(SO_4)$	23.60	4.28	7.63	16.14	23,33	3.18	7.80	16.93

METAL-CHLORIDE-PYRIDINO COMPLEXES.

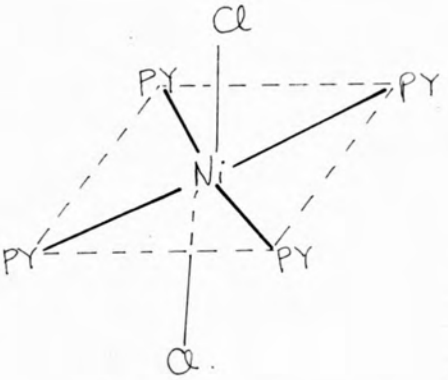
COMPOUND	ANALYSIS %				THEORETICAL %			
	C	H	N	M	C	H	N	M
$CuCl_2.2py$	40.81	3.06	8.64	21.58	41.60	3.49	9.71	22.00
$NiCl_2.4py$	52.53	4.88	12.88	14.73	56.40	4.93	13.15	13.70
$CoCl_2.2py.2H_2O$	42.90	4.08	9.18	18.66	42.28	3.55	9.86	20.74
$CoCl_2.2py.10H_2O$	46.64	3.39	9.94	18.60	42.28	3.55	9.86	20.74
$ZnCl_2.2py.$	40.41	3.55	9.34	21.19	41.33	3.47	9.64	22.50

TABLE 10

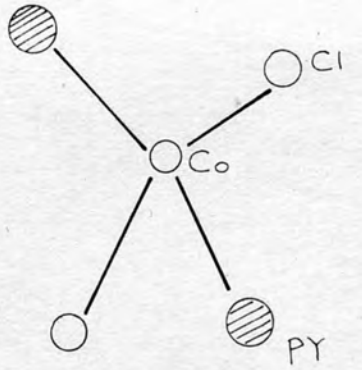
METAL - PYRIDINO complex structures.



$Cu(py)_2.Cl_2$
OCTAHEDRAL, polymeric

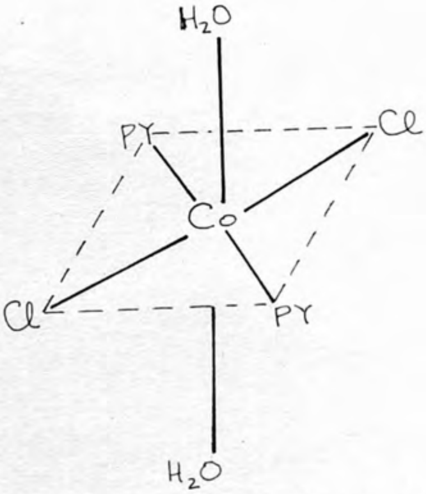


$Ni(py)_4.Cl_2$
trans - OCTAHEDRAL



$CoCl_2.2py$ (blue)
TETRAHEDRAL

($ZnCl_2.2py$ is similar)



$CoCl_2.2py.2H_2O$ (violet)
trans-OCTAHEDRAL

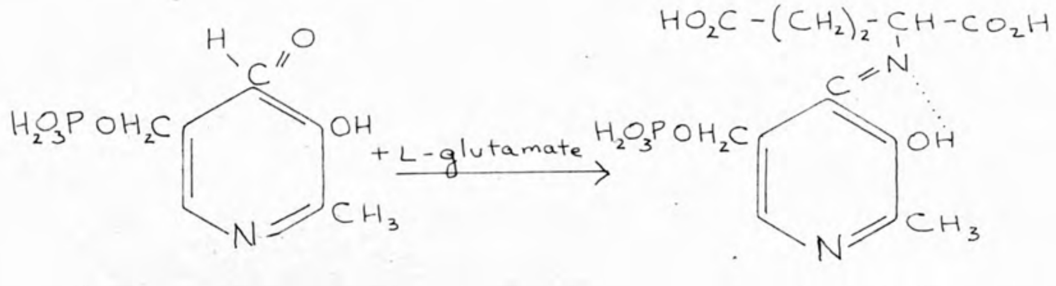
ANALYSIS:-

Analysis of carbon, nitrogen and hydrogen were measured by Bernhardt[119]. Copper and nickel were determined by electro-gravermetric means[120]; cobalt by colourmetric method[121]; aluminium and zinc gravimetrically with oxime[122]; ferric determination and gallium[122] with EDTA. EDTA was back titrated using bismuth nitrate (and pyrocatechol violet indicator) in the case of the gallium determination.

It was generally found that the phosphate group tended to interfere with the analysis of the C, H and N percentages, and to lower them to a small degree.

ANALYSIS of the PYRIDOXAL-PHOSPHATE-L GLUTAMATE SCHIFF BASE

(a) Proposed reaction:



M.W. 376.

(b) Analysis %				Theoretical %				
C	H	N	Resid. H_2PO_3	C	H	N	Resid. H_2PO_3.	Resid. O
36.48	4.03	5.91	20.91	41.49	4.55	7.45	21.52	25.51

The difference between the actual analysis (69.83%) is probably due to the oxygen percentage, which in this case is then 32.67%, in excess of the theoretical if one does not consider the 3 oxygen molecules removed with the H_2PO_3 ash.

(It was found that the phosphate group tended to interfere with all the analysis of C, H and N percentages. In the Schiff base, however, it is highly probable that the compound partially hydrolysed on the way to Bernhardts, and that there is a small amount of PLP present, plus water.)

TABLE 11

From analysis of Schiff bases: ratio of metal ions to C, H, N

Series I

Compound cation.	Ratio/metal ion C	H	N	Inference.
Cu^{2+}	9.96	12.01	1.68	$Cu_2(C_{20}H_{24}N_3)$
Cu^{2+}	9.24	10.99	1.57	$Cu_2(C_{18}H_{22}N_3)$
Cu^{2+}	7.78	12.00	1.19	$Cu(C_8H_{12}N)$
Cu^{2+}	9.32	15.49	1.65	$Cu_2(C_{18}H_{31}N_3)$
Ni^{2+}	20.56	28.10	3.03	$Ni(C_{20}H_{28}N_3)$
Ni^{2+}	19.19	33.14	2.32	$Ni(C_{19}H_{33}N_2)$
Co^{2+}	16.71	24.20	2.65	$Co_2(C_{16}H_{24}N_{2\frac{1}{2}})_2$
Zn^{2+}	20.81	22.35	2.74	$Zn(C_{20}H_{22}N_3)$
Al^{3+}	14.29	23.99	2.40	$Al_2(C_{28}H_{48}N_5)$
Fe^{3+}	9.36	13.72	1.60	$Fe_2(C_{19}H_{27}N_3)$
Ga^{3+}	18.63	28.90	2.94	$Ga(C_{18}H_{28}N_3)$

TABLE 12

RATIO of C, H, N to metal ions:

Schiff base series 2

Compound cation	C	H	N	Inference.
Cu^{2+}	11.83	14.40	1.51	$Cu_2(C_{24}H_{28}N_3)$
Ni^{2+}	8.23	12.88	1.53	$Ni_2(C_{16}H_{24}N_3)$
Co^{2+}	22.00	36.80	4.95	$Co(C_{22}H_{36}N_5)$
Zn^{2+}	9.29	17.78	1.55	$Zn_2(C_{18}H_{35}N_3)$
Ga^{3+}	11.86	19.64	2.54	$Ga_2(C_{24}H_{40}N_5)$
Ga^{3+}	22.80	32.79	3.49	$Ga_2(C_{45}H_{64}N_7)$
Al^{3+}	12.36	24.51	2.05	$Al(C_{12}H_{24}N_2)$
Fe^{3+}	10.05	15.96	2.02	$Fe(C_{10}H_{16}N_2)$

TABLE 13

ANALYSES of SCHIFF BASE METAL COMPLEXES

Series 1.

Analysis:

Metal ion.	% Found C	H	N	Metal ion	Na^+
Ga^{III}	16.53	2.15	3.03	5.131Ga^{3+}	21.41

Possible theoretical:

$Na_2(Ga^{III}(PLP\text{-}glu)(gly))^{2+}(SO_4)$

22.84 2.34 4.44 7.37

Series 2.

Analysis:

Metal ion	C	H	% Found N	Mn^+	Na^+	Cl
Cu^{2+}	28.56	2.92	5.90	12.76Cu^{2+}	7.35	-
Co^{2+}	33.25	4.67	8.73	7.415Co^{2+}	-	14.71
Ga^{3+}	28.55	3.90	7.02	13.74Ga^{3+}	4.0	-
Ga^{3+}	33.12	4.00	5.92	8.46Ga^{3+}	-	-
Al^{3+}	20.79	3.46	4.02	3.78Al^{3+}	-	-

Possible theoretical:

$(Cu^{2+}(PAMP\text{-}\alpha KG)(glu)(\alpha KG)(H_2O)_2)$

33.75 3.76 5.05 15.28

$Co^{2+}((PAMP\text{-}\alpha KG)(glu)_2)Cl_3$

33.59 4.16 5.60 7.85

$Na_2(Ga^{III}(SB)(gly))(SO_4)_3$

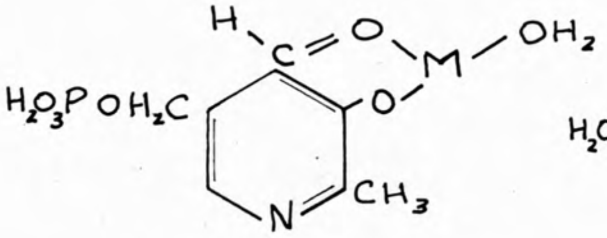
22.84 2.34 4.44 7.37

$Al^{III}(PAMP\text{-}\alpha KG)(SO_4)_2(H_2O)_2$

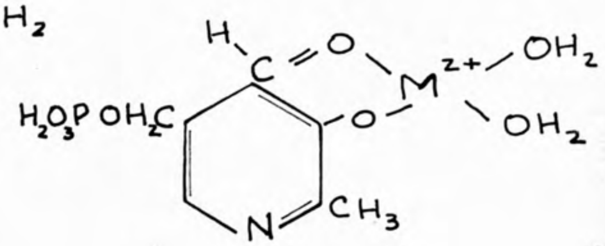
25.26 3.53 4.91 4.73

PROPOSED STRUCTURES

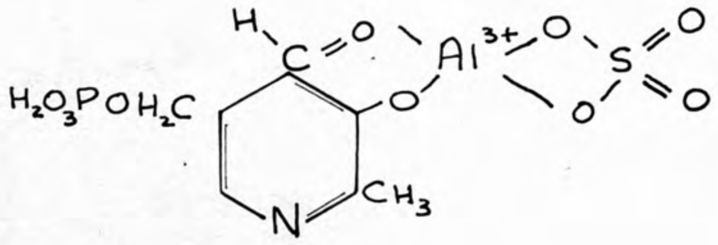
Metal-pyridoxal phosphate complexes



For Cu^{2+}, Zn^{2+} and Fe^{3+}

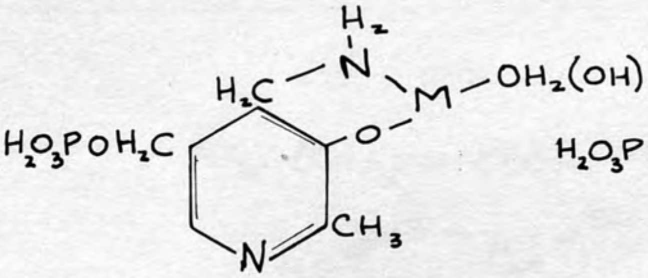


for Co^{2+} and Ni^{2+}

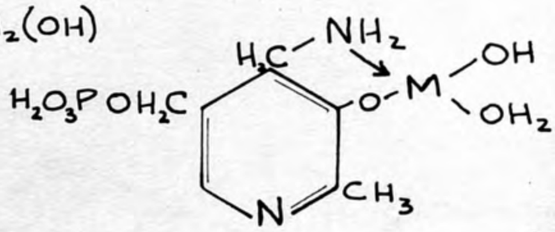


Aluminium

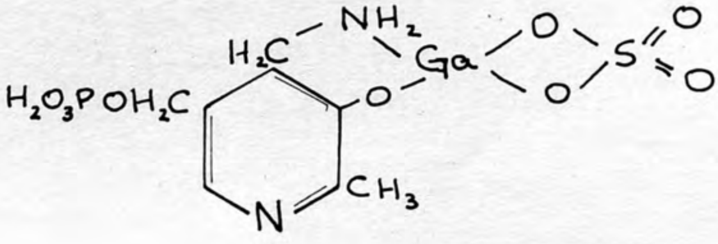
Metal-pyridoxamine phosphate complexes



for Cu^{2+} and $Al^{3+}(SO_4)_2$

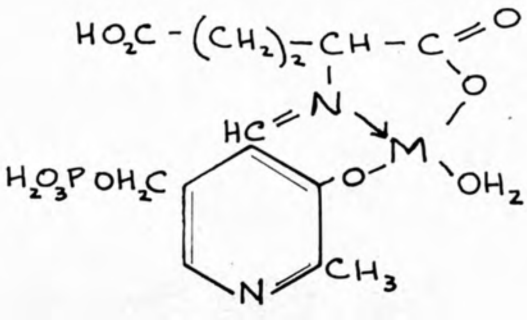


for Co^{2+} and Zn^{2+}

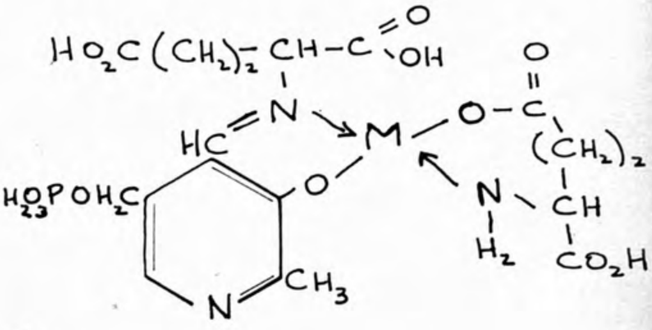


PROPOSED STRUCTURES for the METAL-SCHIFF BASE COMPLEXES

General Structures



A



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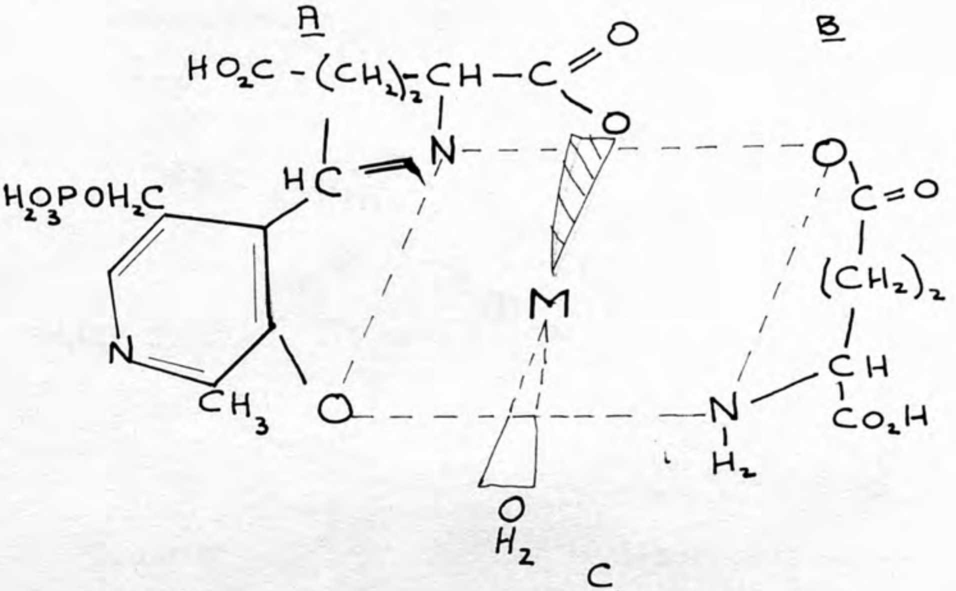
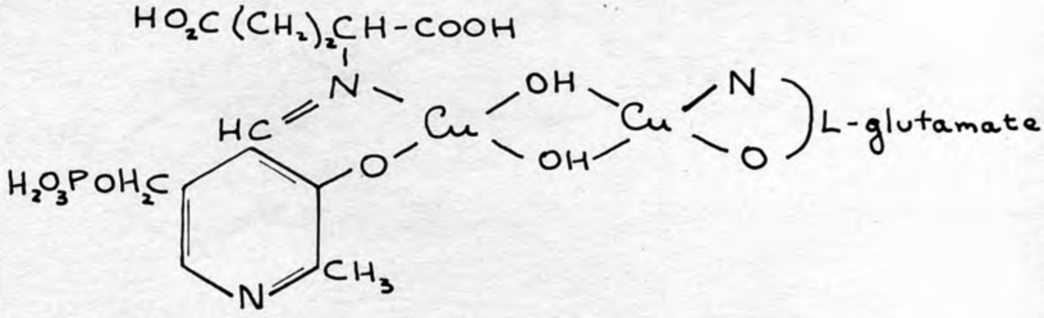


C

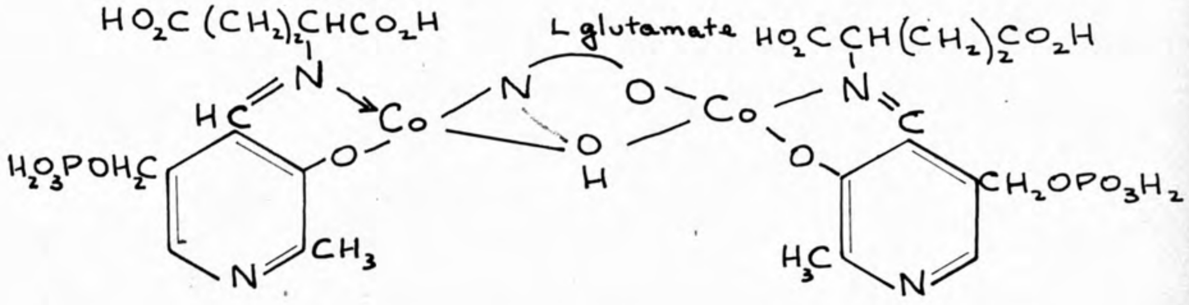
Structures of Metal-Schiff base complexes, series 1



Theoretical: C: 31.62% H: 3.98% N: 6.15% Cu: 18.59%

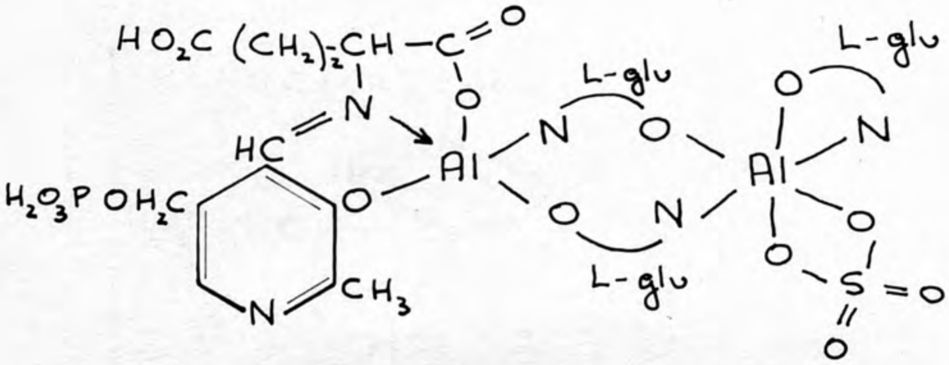
Analysis: C: 30.12% H: 4.20% N: 6.20% Cu: 17.10%

D



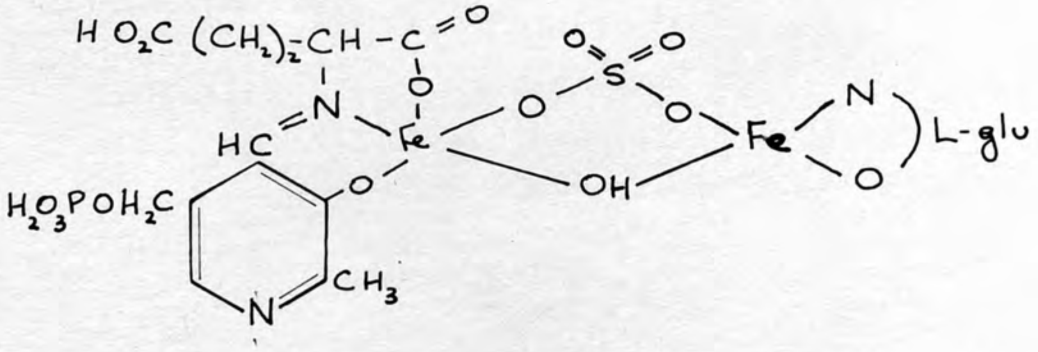
Theoretical: C: 37.21% H: 4.13% N: 7.00% Co: 11.78%
Analysis: C: 34.73% H: 4.22% N: 6.52% Co: 11.80%

E



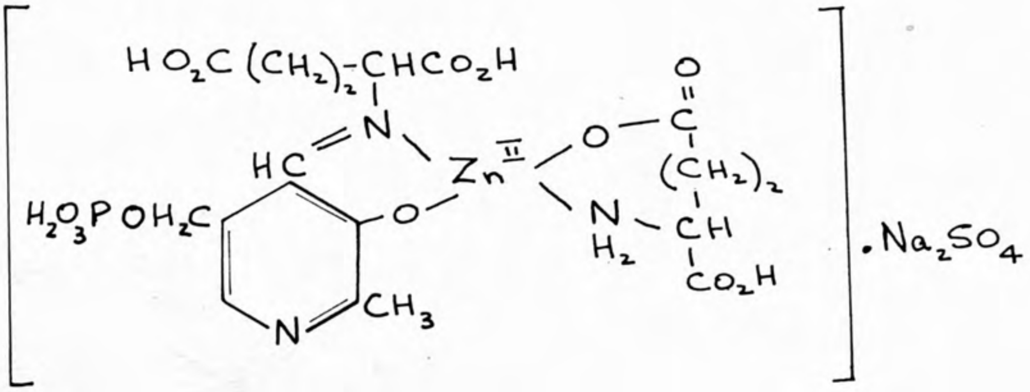
Theoretical: C: 31.85% H: 3.53% N: 6.63% Al: 5.11%
Analysis: C; 30.42% H: 4.28% N: 5.96% Al: 4.77%

F



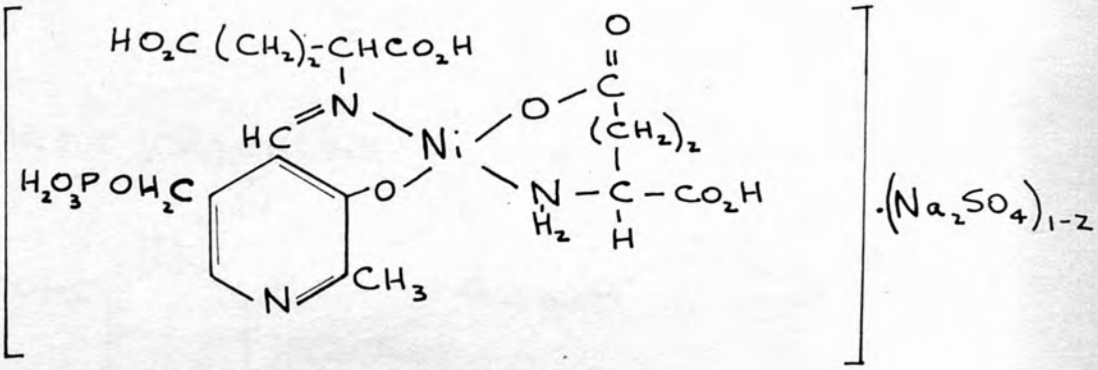
Theoretical: C: 28.93% H: 3.24% N: 5.62% Fe: 14.95%
Analysis: C: 28.37% H: 3.49% N: 5.67% Fe: 14.10%

G



Theoretical: C: 29.74% H: 3.32% N: 5.78% Zn: 8.99%
Analysis: C: 28.62% H: 3.07% N: 6.43% Zn: 8.90%

H

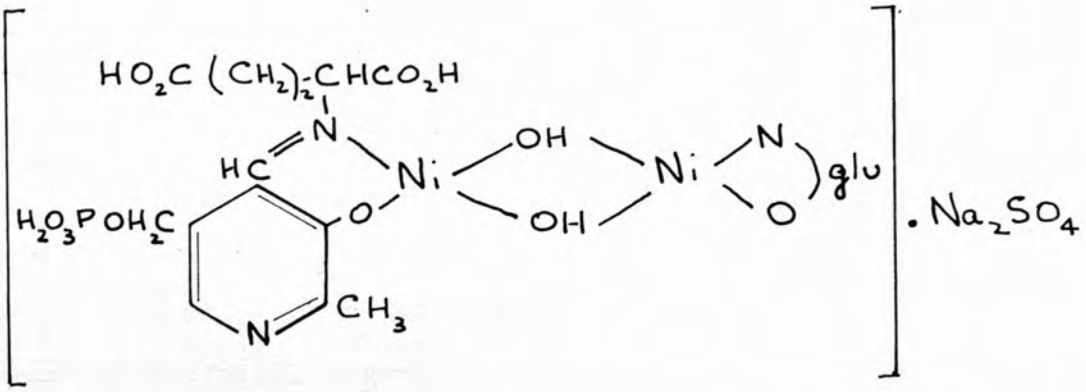


Theoretical:	%C	%H	%N	Ni%
(S.B.)Na_2SO_4	30.02	3.22	5.88	8.15
(S.B.)$(Na_2SO_4)_2$	26.49	2.84	5.15	7.19

Analysis:	%C	%H	%N	Ni%
	30.38	3.49	5.22	7.20
	26.77	3.88	3.79	6.82

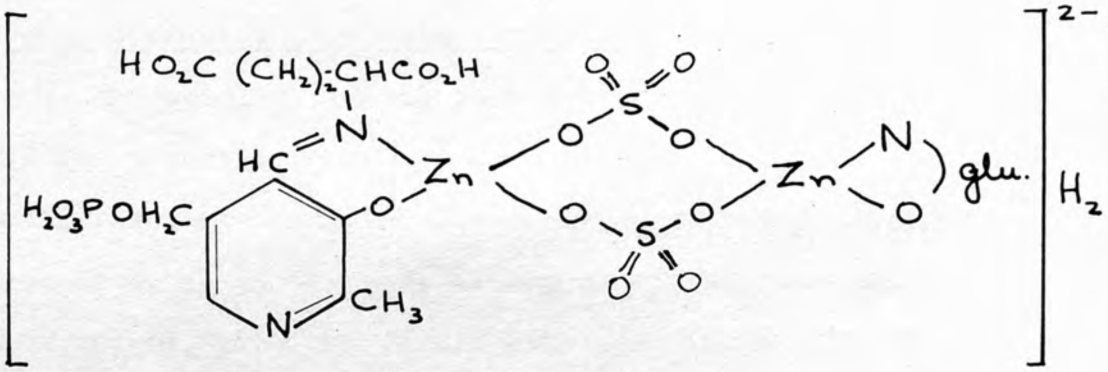
I

SERIES 2. METAL SCHIFF BASE COMPLEXES



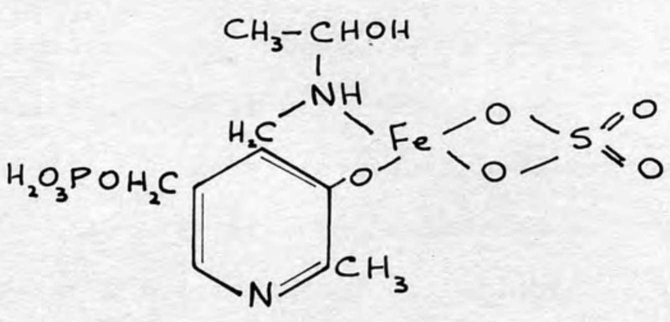
Theoretical: C: 27.74% H: 5.06%% N: 5.71% Ni: 15.95%
Analysis: C: 26.88% H: 3.33% N: 5.86% Ni: 15.03%

J



Theoretical: C: 26.45% H:4.25% N: 5.16% Zn: 15.48%
Analysis: C: 25.63% H: 2.74% N: 4.98% Zn: 15.50%

K



Theoretical: C: 26.98% . H: 3.62% N: 6.29% Fe: 12.54%
Analysis: C: 27.18% H: 3.62% N: 6.36% Fe: 12.59%

L

Discussion of structures, assigned from analysis results

Metal-pyridine complexes.

The metal chloride-pyridino complexes (general formula $M^{2+}Cl_2.2py$) were studied by Gill, Nyholm and co-workers[111], and their assignments of structures are shown in Table 10 . The general form is octahedral or distorted octahedral, generally achieved either by coordination of water (as in the violet form of the cobalt isomer), or by polymerisation (as in the polymeric single chain of the copper-pyridino complex). However, the structural assignment of the zinc isomer and also the blue isomer of the Co^{II} complex is that of tetrahedral arrangement of ligand molecules.

Metal-pyridoxal phosphate structures.

The metal-pyridoxal phosphate complexes all have a 1:1 ratio of PLP to metal ion. The remaining co-ordination positions of the metal ions are occupied either by coordinated water, or by sulphate, as in the case of the aluminium complex. Analysis of the copper, zinc and ferric complexes would indicate that the fourth (or possibly fifth) coordination position would either be occupied by the pyridinium-nitrogen from another complex molecule, or be bound through the phosphate group of a third molecule. This process would indicate a small degree of polymerisation. However, in the case of the ferric complex the remaining position is occupied by a sulphate radical. The cobalt and nickel complexes have a 1:1 PLP to metal ion ratio, with two molecules of coordinated water. This fact would imply either a square planar or tetrahedral structure, but if there was any polymerisation, then the resulting five- or six-coordinate metal ion structure should be differentiated in the electronic visible absorption spectra. The aluminium chelate has a 1:1 PLP to metal ion ratio, in agreement with that proposed by Fasella[119], but there are two anion radicals (Sulphates) attached to the aluminium, to achieve the required coordination number.

The gallium chelate does not appear to fit in with any theoretical analysis results of C, H and N, although the ratio of nitrogen and carbon to the gallium would imply a momomeric complex with one PLP molecule per gallium.

Metal-pyridoxamine phosphate complexes

The PAMP chelates all have a 1:1 PAMP ratio to the metal ion. The copper chelate is similar to the PLP chelate in that the third coordination position is occupied by a water molecule, with the fourth (and possibly fifth) positions occupied by donor nitrogens from other molecules, thereby forming a polymerised complex. The aluminium PAMP complex, like the copper one, contains one molecule of coordinated water, with the remaining electronic charge satisfied by two sulphate radicals. The gallium chelate is similar to the aluminium, except that there is no coordinated water, and only one sulphate radical. This is probably bridged, which accounts for the four-coordinate, tetrahedral gallium. If the sulphate is not bridged, then the fourth position of the gallium may be occupied by the "inert pair" of electrons, which would indicate the gallium to be in the Ga^{I} oxidation state.

Both the cobalt and zinc PAMP complexes have two molecules of water coordinated and therefore are four-coordinate. The zinc complex will be four-coordinate. In the cobalt complex, however, there may be polymerisation to achieve a five- or six-coordinate structure, alternatively the complex may be either square planar or tetrahedral like the zinc chelate.

The Schiff base-metal complexes structures

Series I: Schiff bases of pyridoxal phosphate and L-glutamate

Table II shows the ratio of C, H and N atoms per metal ion. In all the structures considered, there is one Schiff base unit per metal ion, plus an amino acid molecule and other molecules or radicals to constitute the correct coordination number of the atom. In all complexes, it is assumed that the Schiff base is bonded through the phenolic-oxygen and the imine-nitrogen; bonding through the carboxylate can take place, but this would imply that the ligand-Schiff base was tridentate. This basic unit of the Schiff base in terms of C, H and N is ($C_{13}N_2H_{14-15}$). In all complexes, except the nickel and zinc ones, the complex appear to be dimeric, with either hydroxy-, glutamate or sulphate radicals bridging the two metal atoms.

General structures for the metal-monomers are proposed in figs. A-C. However, distinctive analysis for each metal ion are set out in figs. D- I . The theoretical results are in good agreement with analysis results and it is therefore maintained that these are the most likely structures for the complexes. The only exception to the 1:1 ratio of Schiff base to metal ion is that of the cobalt dimer, where analysis points to two Schiff base molecules per complete molecule (one per cobalt atom) with the twocobalt atoms bridged by one hydroxyl radical and one glutamate molecule.

Ratios of C, H and N to the gallium metal ion would seem to indicate that there is one Schiff base molecule and one amino acid molecule per gallium atom; up to three sulphates could possibly occur, but theoretical analysis does not agree with the actual percentages of elements found.

The appearance of anion radicals, other than water or hydroxide, would seem to contradict the assumptions of several authors. Predictions that the structures of pyridoxal phosphate Schiff bases would simulate the structures of pyridoxylidene-amino acid metal complexes[15,18,23] have not been found to be true in the present work.

Schiff base-metal complexes: series 2

Schiff bases of pyridoxamine phosphate and α-keto glutaric acid

The Schiff bases of pyridoxamine phosphate and αKG of nickel, zinc and iron(III) are shown in figs. J- L . The nickel complex is dimeric, bridged by hydroxy- radicals; the zinc complex is similarly dimeric, this time bridged by sulphate groups. It is interesting to note that an amino acid molecule is coordinated to the second metal ion; this would indicate that the transfer of the amino-group from pyridoxamine phosphate to the α-keto glutaric acid had taken place to some extent. Whether the original complex had two Schiff base molecules is still to be proved; if this had occurred then the complexes isolated had transaminated to a small degree. The ferric Schiff base complex is monomeric but is already slightly decomposed. The dicarboxylic acid of the amino acid has been doubly decarboxylated, and the ketimine bond has been hydrated.

According to analysis ratios in Table 12, the copper and gallium complexes are both dimeric with an odd number of nitrogen atoms per metal ion; this would indicate that there are either one or three molecules of amino acid also coordinated to the metals. However, theoretical analysis of various forms do not seem to fit in with experimental analyses. The cobalt and aluminium complexes are monomeric, the cobalt with an odd number of nitrogen atoms per metal ion and the aluminium with an even number of nitrogen atoms per aluminium ion.

Conclusions

The above results and discussion confirm the author's suggestion that the aldimine-ketimine species of Schiff base govern the nature of the bonding with the 'catalytic' metal ions.

The dimeric species of metal ion coordinates only with one tautomeric form of the Schiff base, while the other tautomeric Schiff base assumes a monomeric species with the metal ion. The exceptions to this idea are the copper complexes, both of which appear to be dimeric; these structural forms may account for the abnormal stability of the copper complexes.

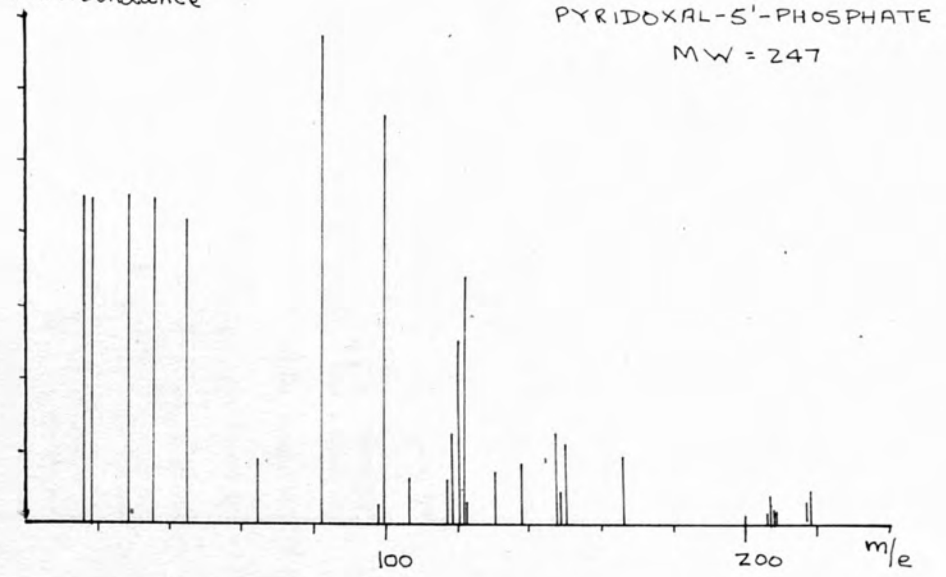
MASS SPECTROMETRY STUDIES

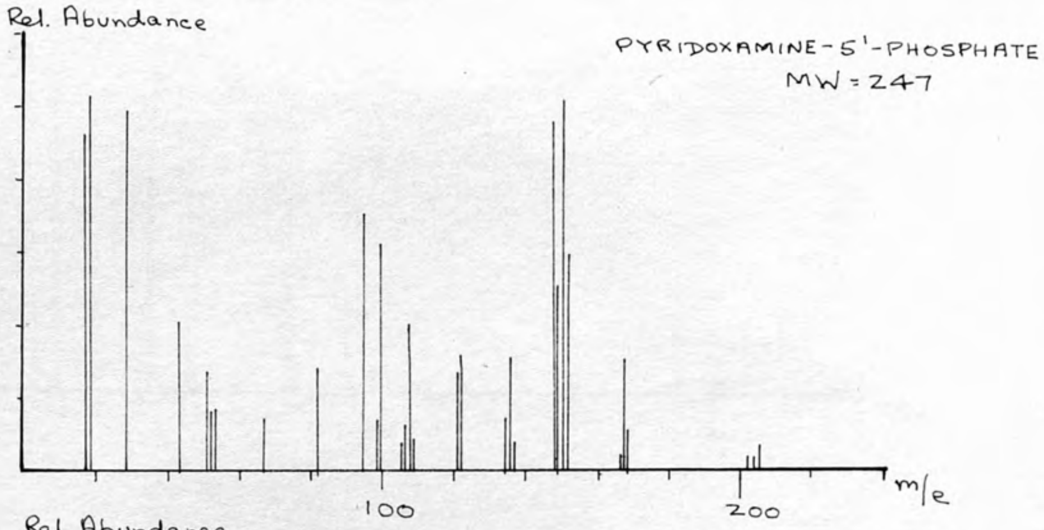
Experimental

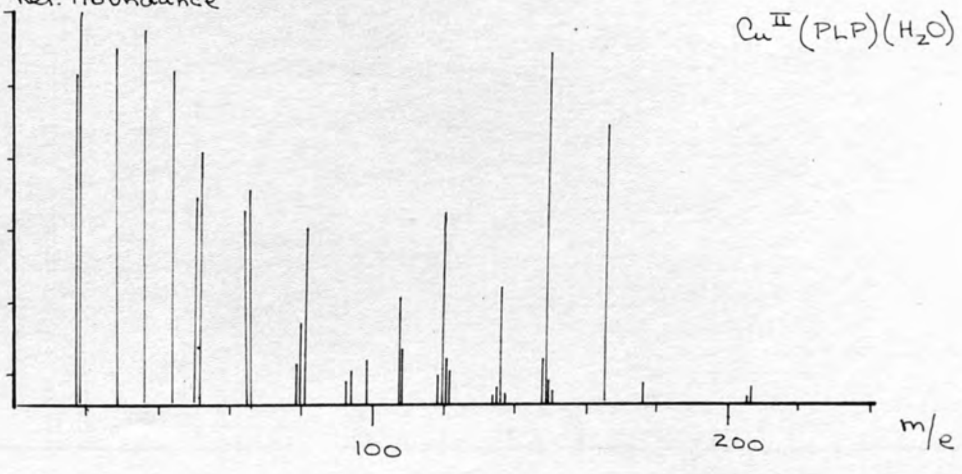
The mass spectra were measured on the Mass Spectrometer MS 902 at the School of Pharmacy, University of London, Brunswick Square, by Dr. B. J. Millard and Mr. D. Carter.

The mode of insertion was that of direct insertion at a temperature of 240 - 245° with a magnetic field of 5B.M. The ionisation potential was kept at 70 eV throughout. Preliminary spectra were run in the same conditions, except that the temperature was 105°, but little ionisation and fragmentation took place at this temperature.

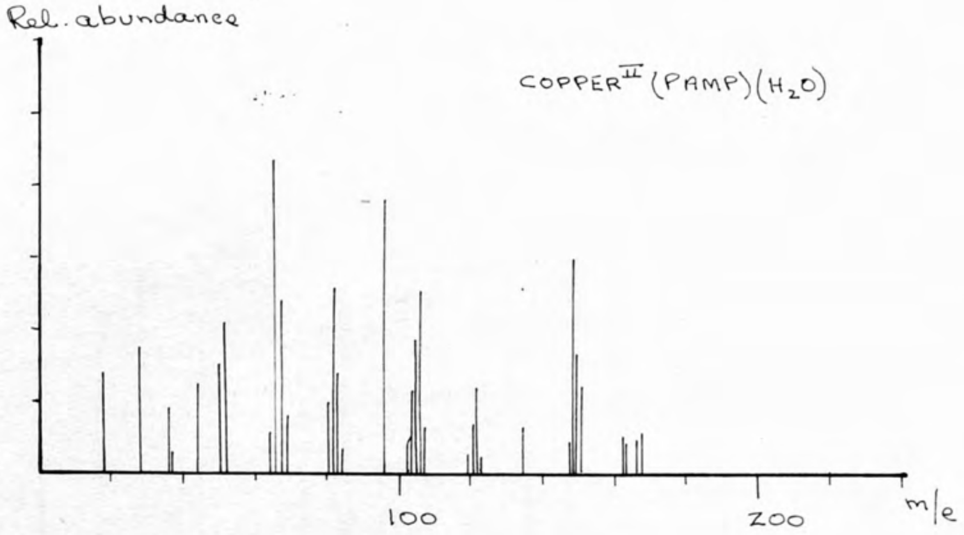
RESULTS OF MASS SPECTRA

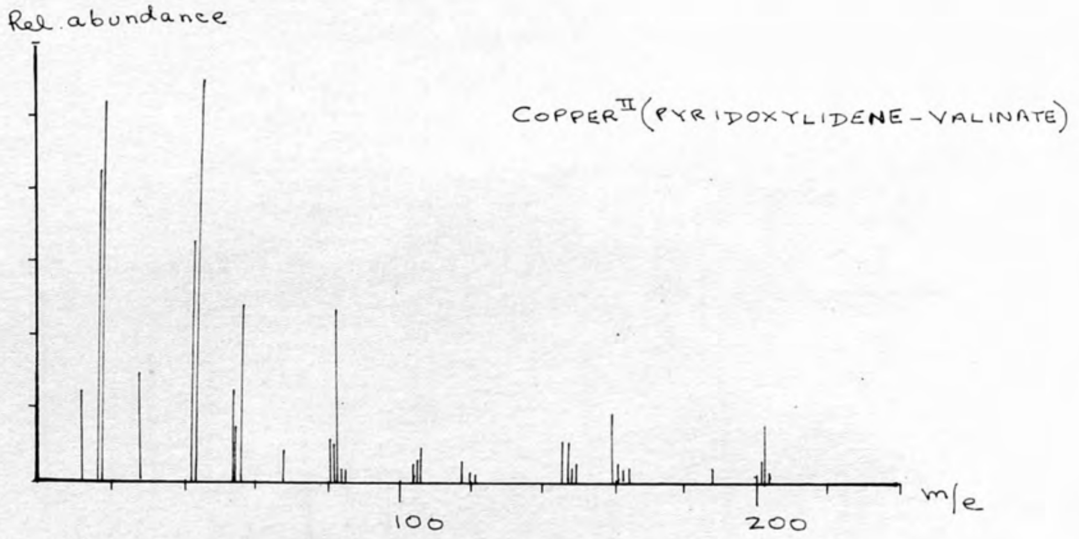


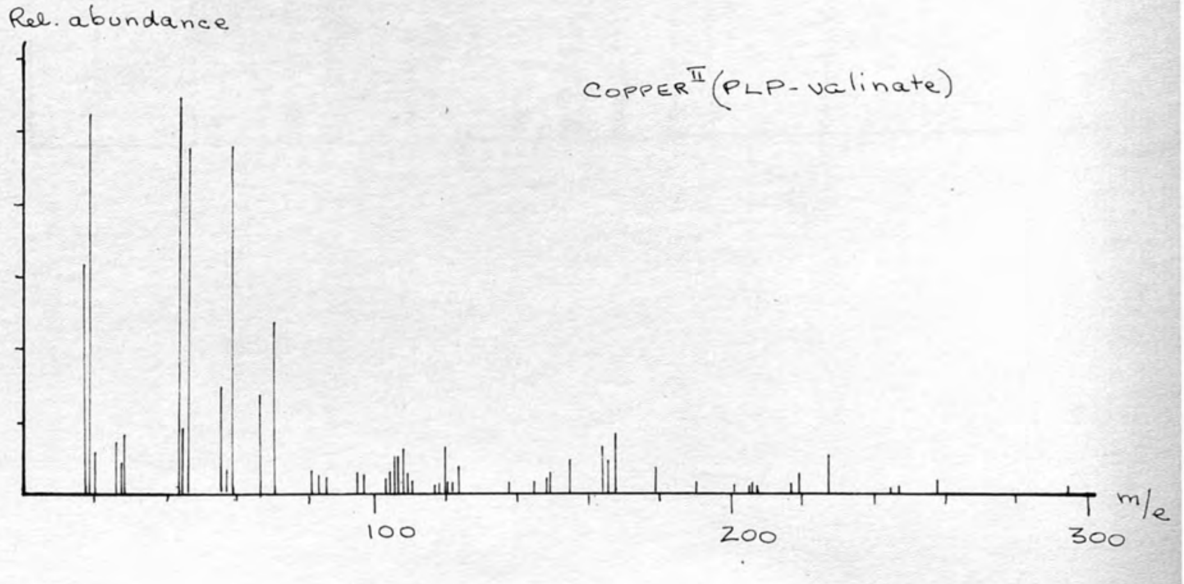




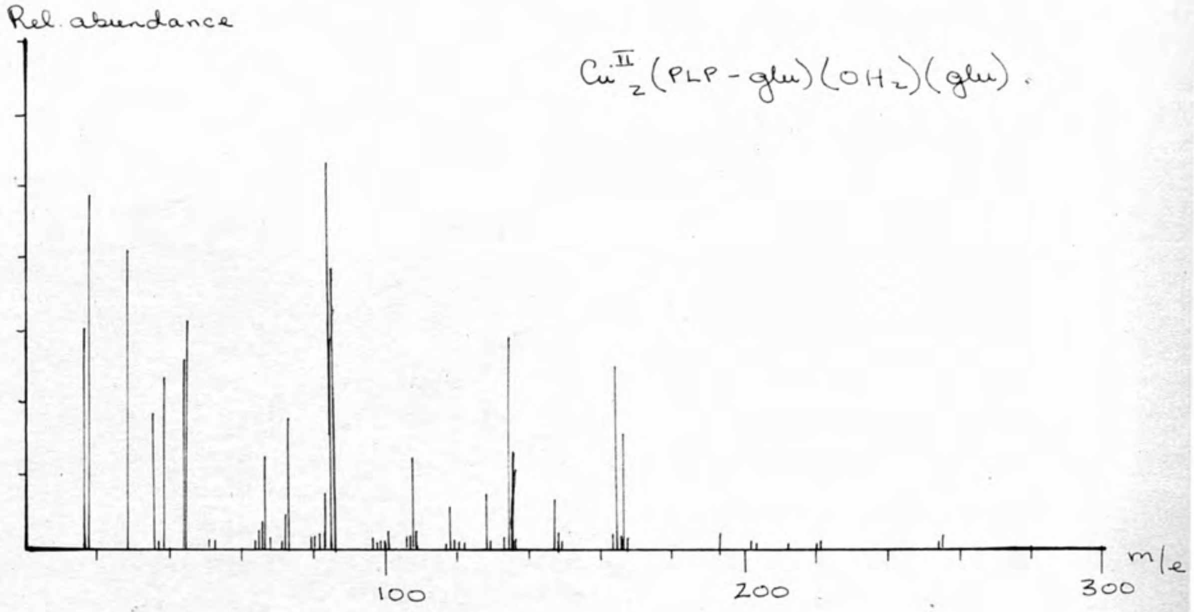
RESULTS OF MASS SPECTRA

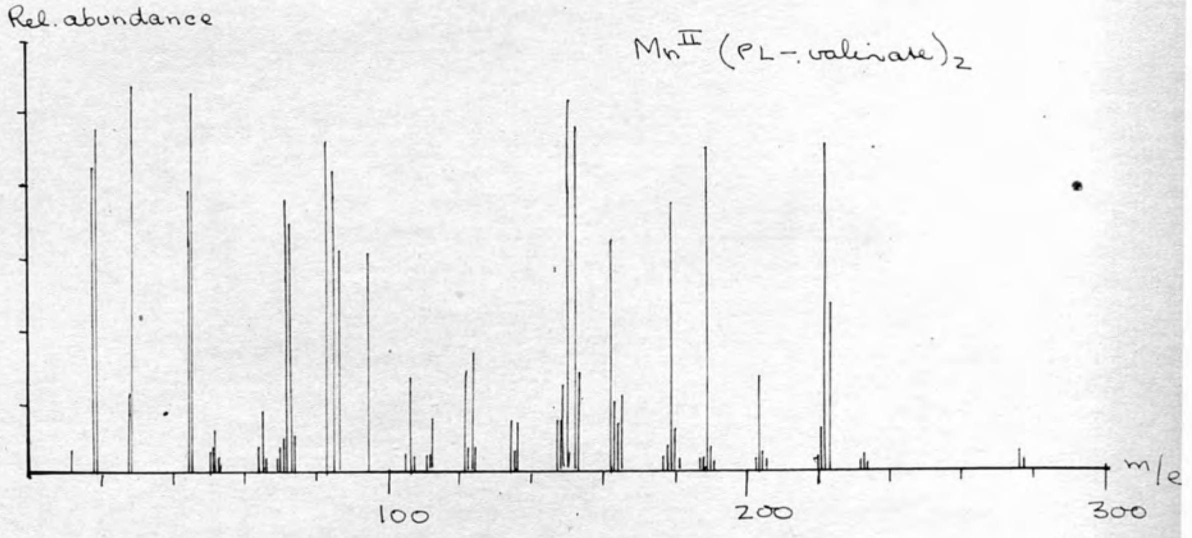






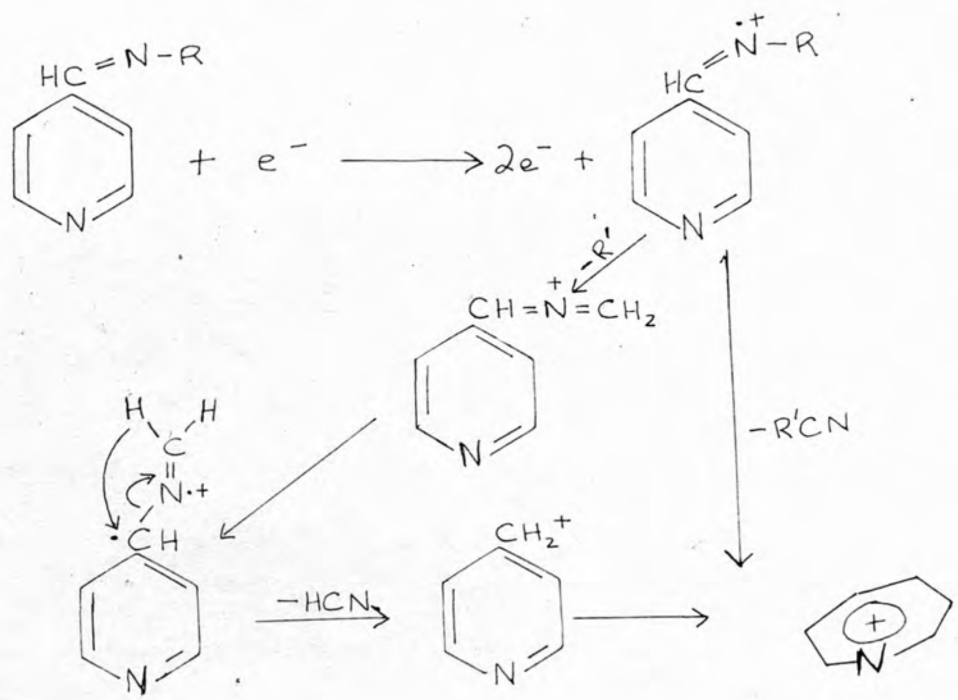
MASS SPECTRA RESULTS



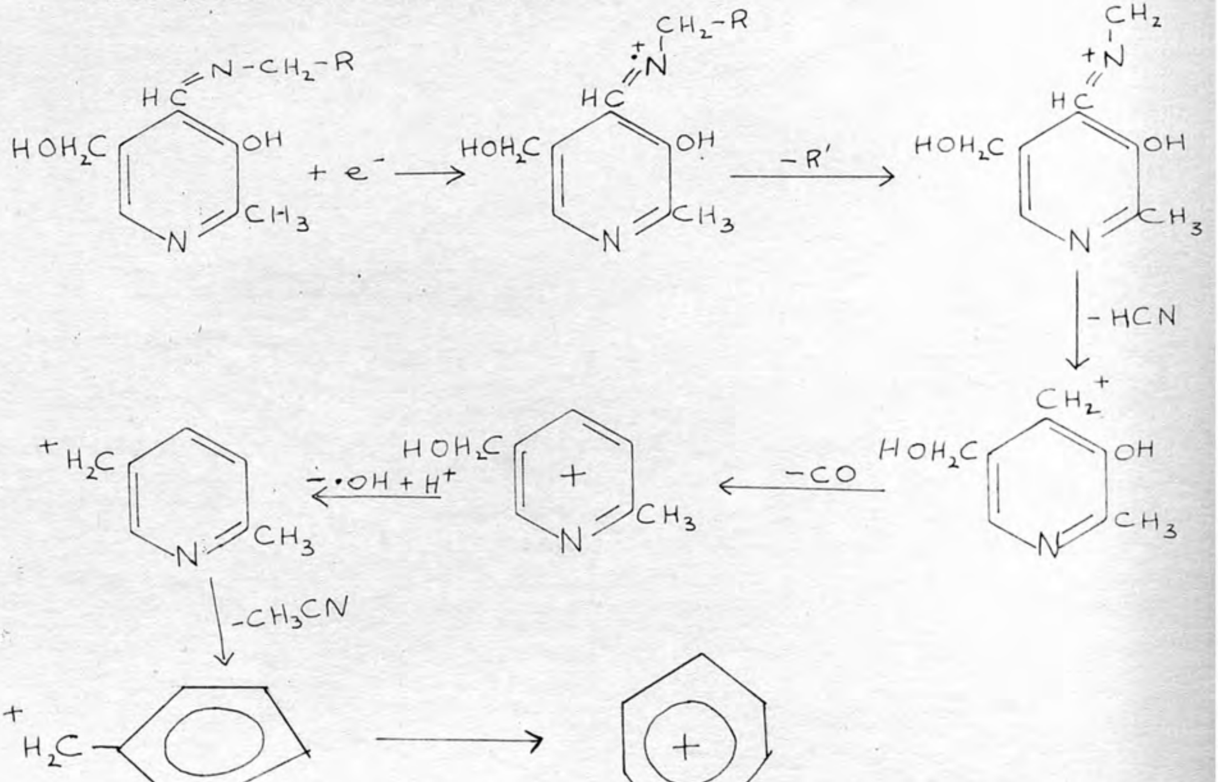


SCHEMES FOR DISSOCIATION and FRAGMENTATION of SCHIFF BASES and their METAL CHELATES by mass spectrometry.

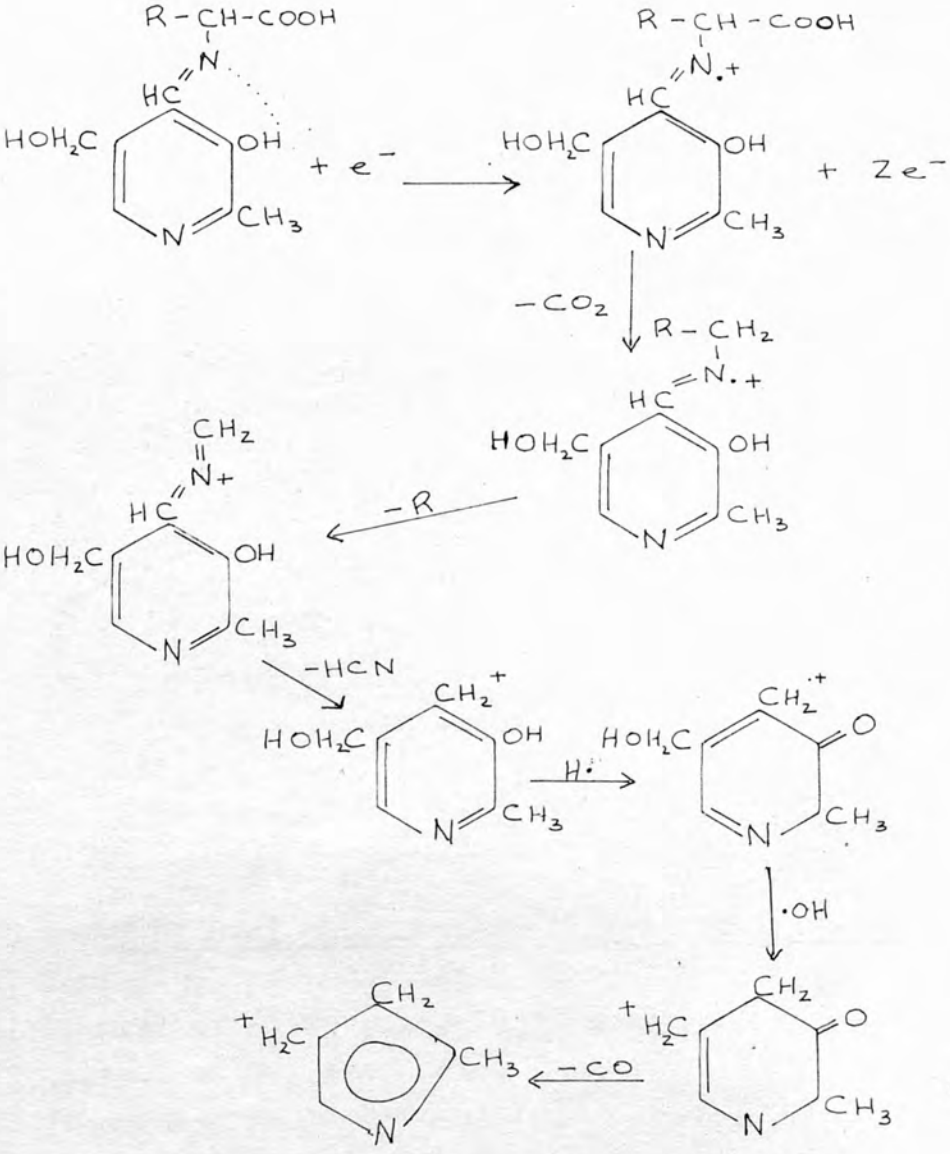
a) SCHIFF BASE of PYRIDINE-4-ALDEHYDE



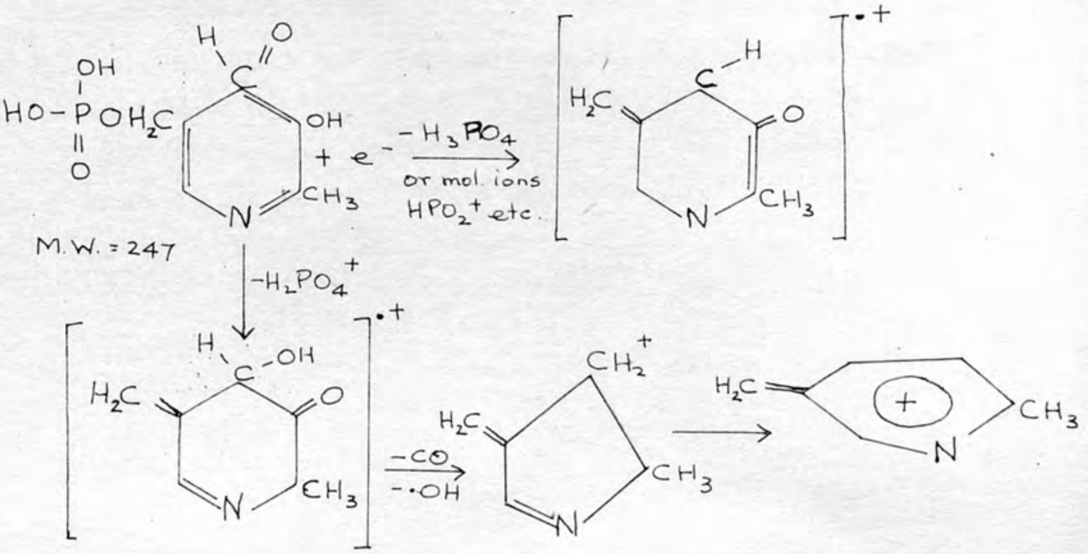
b) SCHIFF BASE of PYRIDOXAL



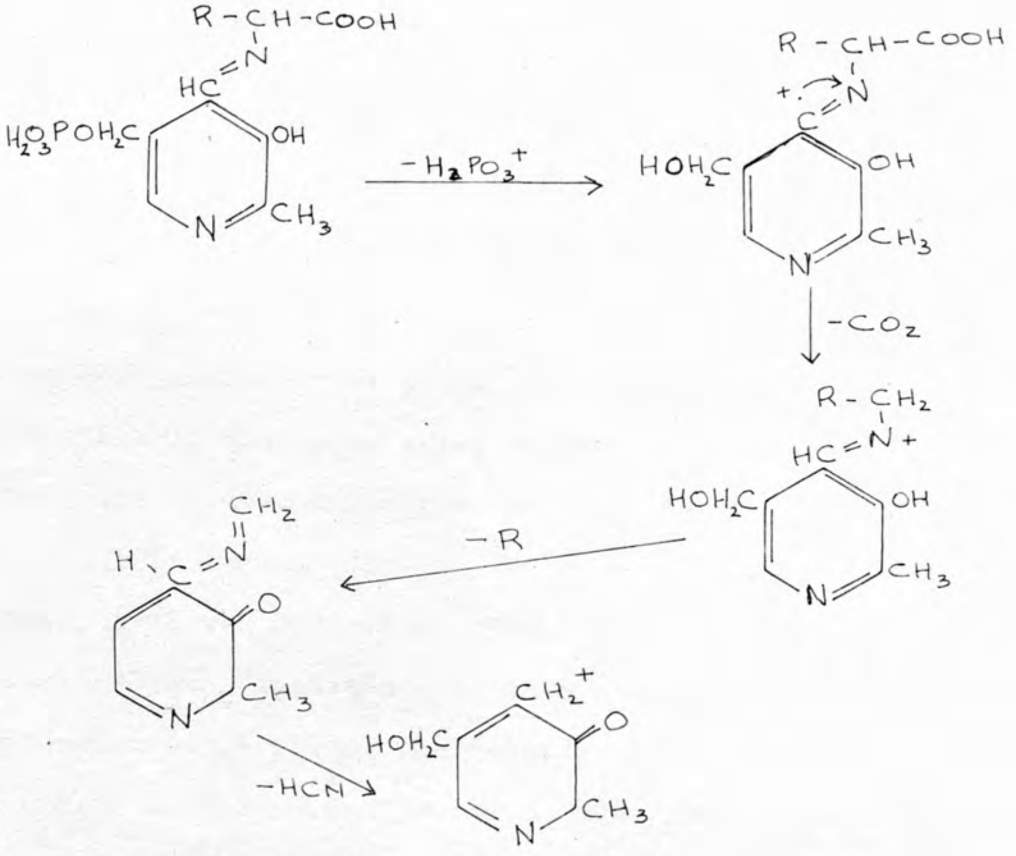
c) SCHIFF BASE of PYRIDOXAL and L-AMINO ACID



d) PYRIDOXAL-5'-PHOSPHATE



e) PYRIDOXAL PHOSPHATE- AMINO ACID SCHIFF BASES



f) Metal-pyridoxal phosphate-amino acid Schiff bases

i) Ionisation of metal ion.
ii) Removal of H_3PO_4 molecule or substituent ions.
iii) Removal of CO_2 from acid.
iv) Fragmentation of R-radical.
v) Rearrangement and abstraction of HCN.
vi) molecular ion now rearranges as pyridoxine.

Removal of R-radical

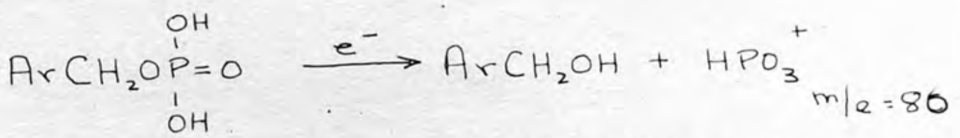
In the case of glutamic acid, the CO_2 removes, and then the CH_2=CH_2 molecule, because it is a straight chain.

For valine, fragmentation is more difficult due to the branched chain. Probably the $CH_3^{\bullet}$ radical comes off first, followed by the rest of the alkyl radical.

Discussion.

Schiff bases in general and those of pyridine aldehydes have been discussed and fragmentation processes have been suggested[113]. If it is assumed that similar reasoning can be applied to the pyridoxal-Schiff bases, then the imine fragmentation will take place, followed by the pyridoxal rearrangement, and subsequent fragmentation. However, with the amino acid present in the species, carbon dioxide would probably be released, prior to the removal of the alkyl fragment of the amino acid, followed by cleavages similar to those discussed for the pyridine Schiff bases.

It is thought that in the case of the 5'-methoxy phosphorylated species of pyridoxal and their analogous Schiff bases, that the phosphate group is ionised off immediately as either the neutral H_3PO_4 or that of fragments similar to those which occur in the fragmentation of alkyl phosphates[114], in the ionisation chamber. Similarly in the case of the metal chelates, the metal is ionised before it enters the chamber and hence the highest m/e peak is that leass the m/e of the metal ion and the phosphate in the case of the metal chelates.



$$ArCH_2O\overset{OH}{\underset{OH}{P}}=O \xrightarrow{e^-} ArCH_2OH + HPO_3^+ \quad m/e = 80$$

Other fragments

	m/e
H_3PO_4	98
$H_2PO_3^+$	81
HPO_2^+	64

Pyridoxal and related compounds[54] were found to lose a molecule of water, first, to form a molecular ion-radical. Rearrangement and further radical abstraction followed the pattern with m/e ratio peaks at 151, 122, 106 and 94 as shown in Fig. 24 .

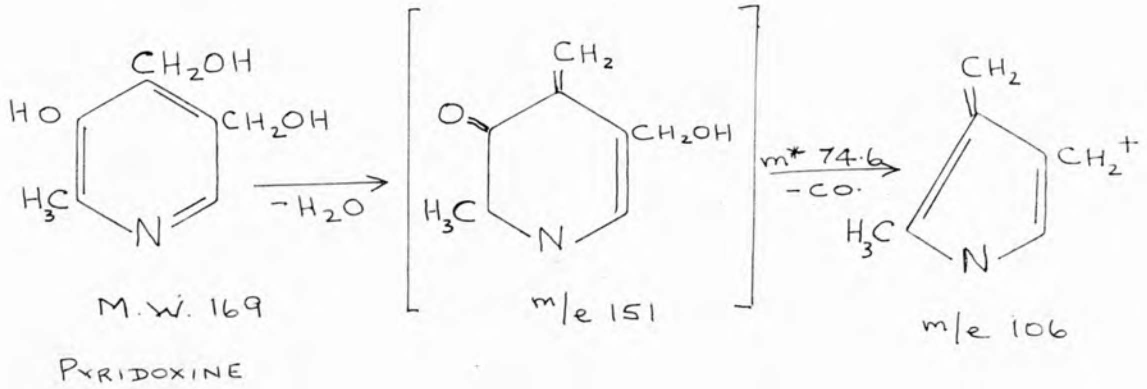


FIG.24

The rearrangement of the pyridoxal-5'-phosphate is expected to follow a similar pattern to that of the pyridoxal. A peak occurs at m/e=64, which can be attributed to the HPO_2^+ molecular ion, which would therefore leave the 5-methyoxy group as an entity to rearrange as in Fig.24 . A weak peak, however, does appear at m/e=165 which could be accounted for by the metastable transition states of the following molecular ion. (Fig.25)*

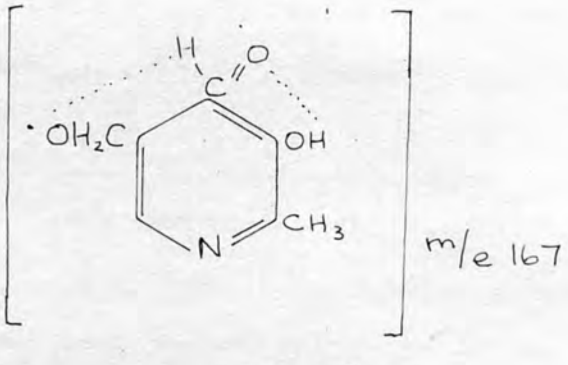


FIG.25

Internal rearrangement of the molecular ion in Fig.25 would than allow the molecule to lose a molecule of water to form the molecular ion with m/e=105, as found in the mass spectrum. Other peaks of consequence are those at m/e ratios of 122, 106 and 99, together with a strong peak at 81-82. The cupric-pyridoxylidene phosphate chelate has similar peaks to PLP and therefore fragmentation and bond cleavage, followed by rearrangement, is essentially the same. The peak at m/e=151 is much stronger than that given by the unbound PLP and so it is assumed that the breaking of the metal-ligand bonds facilitates a greater abundance of the molecular ion fragment at that m/e ratio.

Investigation of the fragmentation of the pyridoxamine phosphate and metal chelate seem to follow the same type of pattern. The neutral molecule eliminated in the first instance is that of ammonia (NH_3) after the ionisation of the phosphate, as shown in Fig. 26.

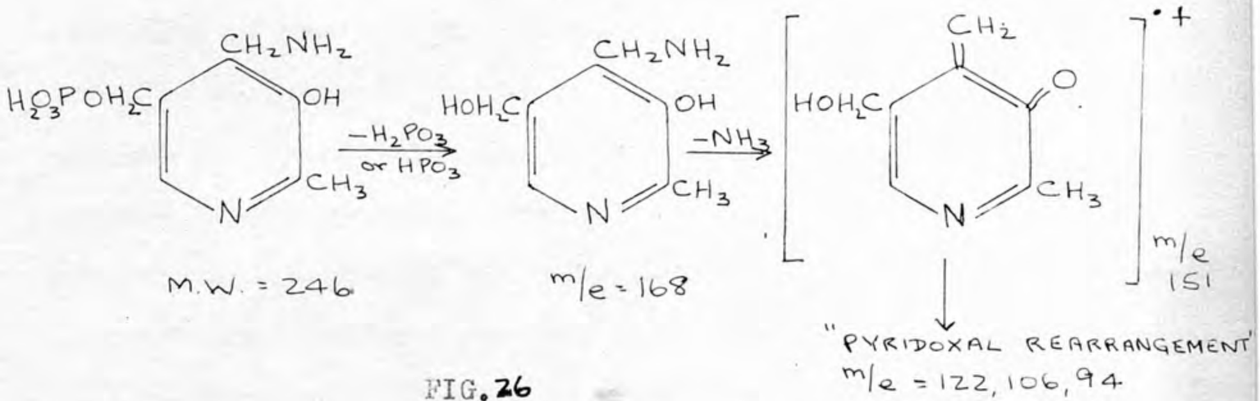
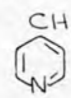


FIG. 26

This fragmentation and rearrangement is upheld by the results of the mass spectra for both the pyridoxal phosphate and pyridoxamine phosphate, and their respective copper chelates.

The Schiff base and their metal chelates.

Complications arise in the analysis of the mass spectra of these Schiff bases because of certain assumptions. If in fact, the Schiff base rearranges as in Scheme (a), the other (viz. the phenolic and 5-methoxy) are neglected and if considered are likely to complicate the issue. The  molecular ion is not vastly different from the

molecular ion suggested for the "pyridoxal rearrangement", except for the other substituent groups (phenolic and 5-methoxy groups.) It is thus easy to assume that the usual rearrangement will take place as in Scheme (b). Kortytnyk[54] pointed out that if the 5-methoxy group was substituted and oxidised to an aldehyde, then the expulsion of the CH_3CN group from the nucleus of the pyridine ring would take place prior to the expulsion of the CO. Either way, rearrangement and fragmentation would eventually lead to an m/e ratio of 65-66, which is present in all the spectra of the compounds investigated. The only rearrangement foreign to that of the amine-aldehyde of the pyridoxal-pyridoxamine is the migration of the phenolic hydrogen, not to be extracted as a neutral molecule but to promote the cleavage of the formyl- C=N bond and supply the necessary charge for the formation of the CH_2^+ molecular ion.

The elimination of the carbon dioxide from the amino acid is set out in Scheme (c), The carboxyl hydrogen is effectively donated to the α-carbon atom of the Schiff base which is then effectively negatively charged. The fragmentation of the Schiff base is then similar to Scheme (b). However, it must be noted that the rupture of the $R'-\underset{N}{C}H_2$ bond does depend on the nature of R'; if R' is a simple straight chain, then the cleavage will take place in one step, whereas if there is a branched chain, then the fragmentation of the R' fragment will follow a different pattern.

In this research, it is clear that the fragmentation of the valinate and glutamate fragments of the amino acid uphold this theory. Beimann and coworkers[52,53] studied the fragmentation of ethyl esters of valine and glutamate and came to the same conclusions. Added to this is the fact that the glutamate is a dicarboxylic acid, thus evolving two volumes of CO_2 per Schiff base molecule. However, this does not complicate the mass spectrum as the acid is essentially a straight chain.

Results of the manganese (II) and copper (II) complexes of pyridoxylidene-valinate Schiff base shows that the m/e peaks are far more intense in the case of the manganese(II) chelate and this fact is scarcely surprising, seeing that the ratio of Schiff base to metal ion is 2:1 in the Mn(II) case and 1:1 in the copper chelate. Peaks are almost identical for the fragmentation process, so one can assume that despite the stereochemical differences, the break-down of the complexes follows the same pattern.

Cu(II) (pyridoxylidene phosphate-valinate) showed an unusually intense peak at m/e=266 and it is assumed that the phosphate is therefore completely ionised off, but the CO_2 has not been extracted. A stronger peak occurred at m/e=221 which could represent the molecular ion after loss of CO_2. The abstraction of the $\begin{matrix}CH_3\\CH_3\end{matrix}\!\!> CH^{\bullet}$ radical would then give the m/e=178 molecular ion, which is in good agreement with results obtained Removal of HCN (m/e now 151) and then rearrangement to m/e 122, 106 and 94 follow the typical "pyridoxal cleavage pattern".

In the copper complex of pyridoxal phosphate and glutamate, the cleavage and rearrangement is similar to that above. The phosphate ionises off to give the molecular ion of m/e=278. This however, is not observed and the first molecular ion which occurs is that at m/e=254 which would mean that a molecule of CO_2 had already been abstracted. Peaks at m/e=204 indicate the further removal of CO_2. Fig. 27 indicates the proposed fragmentation pattern.

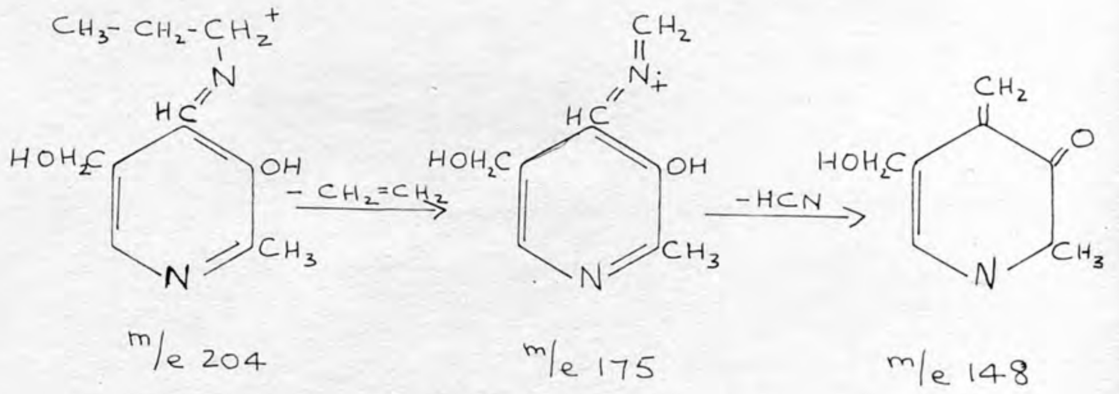


FIG 27

Peaks at m/e=204, 175, 148, 118, 107 and 94 mass unit were also observed.

Conclusions:

Fragmentations and rearrangements in these pyridoxal-Schiff bases follow a similar pattern, and although the purpose of the research in this field was to attempt to determine the molecular ion and hence the molecular weight, this was found impossible due to the elimination of both the metal ion and the various phosphate ions, before the compound had time to reach the ionisation chamber.

The results of the fragmentation of the Schiff bases do, however, agree with previous work done on fragmentation processes.

EXPERIMENTAL

INFRA-RED MEASUREMENTS.

The infra-red absorption spectra were recorded on a Unicam SP 200 spectrophotometer, and on a Grubb Parsons GS 2A spectrophotometers. The spectra were taken as a dispersion in Nujol in the 5-15μ region. Mulls were difficult to make as the compounds tended to hydrolyse and become rather sticky on mulling.. Sulphate and phosphate groups interferred in the 12 - 14μ region and tended to envelop the resolution and other bands in the region. Sodium chloride plates were used for routine spectra on a Unicam SP 200, while potassium bromide plates were used on the Grubb Parsons GS 2A.

Both spectrophotometers were calibrated using a polystyrene film.

TABLE 14

INFRA-RED SPECTRA OF PYRIDINO-METAL COMPLEXES (cms^{-1}).

PYRIDINE	ASSIGNMENT for 	$CuCl_2$ 2py	$NiCl_2$ 2py	$CoCl_2$-2py $2H_2O$	$CoCl_2$-2py $10H_2O$	$ZnCl_2$ 2py
1980						
1924						
1868						1661^{1+6b}
		1604^{8a}	1594	1604	1604	1614^{8a}
1681_{6a+12}						
1629^{1+6b}						
1594^{1+6a}	anti-					
1579^{8a}	symmetric					
1502^{8b}	C=C C=N					1525^{19a}
1437^{19b}	stretch symmetric stretch	1492^{19a}		1483	1483	1492^{19b}
1375^{14}						
1356^{6a+10b}						
1308						
		1246				1241
1216^{9a}	C-H in		1210^{9a}	1205	1214	1220^{9a}
1144	plane	1151^{15}	1156	1148	1151	1163^{15}
1067	deformation	1086^{18a}	1071	1066	1071	1036
1030^{12}	2nd. breathing ν	1055^{12}	1032 1006	1043 1014	1034 1015	1008^{1}
990^{1}	1st. breathing ν		980		996	
		947^{5}				
882		872				891^{5}
741^{4}	out-of-plane ring	760^{4}	755	756	761	763
	deformation	719^{11}	717	719	719	752
700^{11}	out-of-plane C-H def.	687	692	694	696	692^{11}
653^{6b}		667^{6b}			669	
602^{6a}		644^{6a}	625	642	642	642^{6a}
402^{16b}		440^{16b}			623	

Note: superscripts denote pyridine assignments.[33,112]

TABLE 15

INFRA-RED SPECTRA OF PYRIDOXAL PHOSPHATE AND ITS METAL CHELATES (cms^{-1})

Assignment	PLP	Cu(PLP)	Ni(PLP)	Co(PLP) violet	Co(PLP)	Zn(PLP)
					1647	1655
C=0	1648^{6a+12}	1629	1633	1629		
C=C/C=N ring	1555^{8b}					
	1471^{19a}				1511	
	1465^{1}					
	1456^{19b}					
$(C=0^{-})$	1404					
	1383^{14}					
	1334^{6a+10b}					
P=0free stretch	1325				1304	1301
	(1291	1290		1294		
P=0 stretch	(1288					
(H-bonded)	(1258	1255		1258		1250
	$(1238^{9a}$	1242		1243		
		1203		1204	1202	1200
	1183^{15}					1154
	1170					
		1114		1116		1103
	1041^{18a}	1076		1081		
		1042		1043	1040	
	1024^{12}	1025				1014
	982^{5}					
	947^{5}					
	881					
	876					
	862					
	850	826		821		
	795					
	789^{4}					774
	726^{11}	722	718	720	724	
	718					
	669^{6b}		667	669	666	668
	629^{6a}					

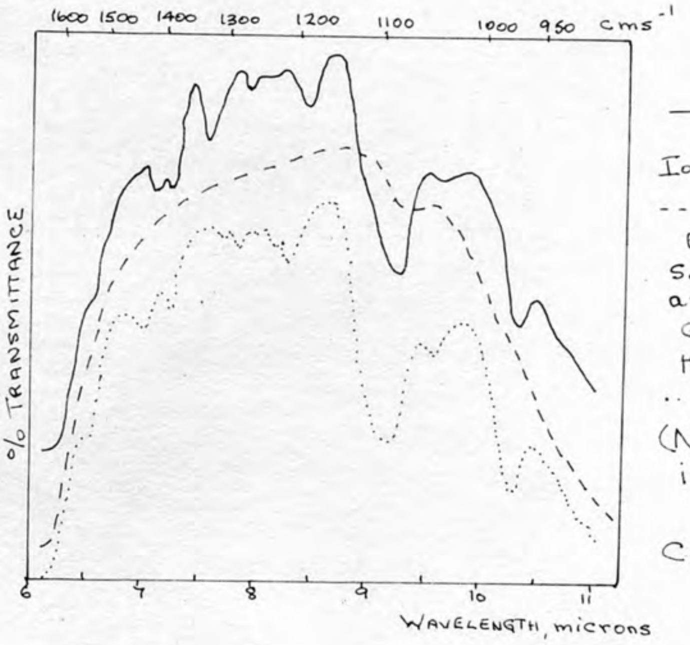
Note: superscripts denote py assignments[33,112]

TABLE 16

INFRA-RED SPECTRA OF PYRIDOXAL-5'-PHOSPHATE METAL COMPLEXES
(cms^{-1})

Al(PLP)	Fe(PLP)	Ga^{III}(PLP)	Assignment
1640	1637	1650	6a+12) 1+6b) +C=0 anti-symmetric stretch.
	1527	1555	8b C=C/C=N ring
1470			19a
1304	1298	1301	P=0 free stretch
		1238	P=0 stretch (H-bonded)
		1219	
	1124	1143	SO_4, PO_4 broad bands
1030–1016		1026	
		781	4
	724	724	11 py modes.
699			
	671	671	6b
649			

Ref: [46]PARKER, F.S.



——— pH 7.3 PHOSPHATE BUFFER Ionic Strength 0.05

- - - - - SCHIFF BASE FORMED BETWEEN 0.03g. SODIUM PYRIDOXAL PHOSPHAT and 0.0084g. of GLYCINE in 5ml. of the BUFFER.

······ PYRIDOXAL PHOSPHAT (Na salt) 0.03g dissolv in 5.0ml. of the buffe

Cells: 0.32mm BaF_2

TABLE 17

INFRA-RED SPECTRA OF PYRIDOXAMINE PHOSPHATE AND ITS METAL CHELATES.

(Cms^{-1})

PAMP	Assignment	Cu(PAMP)	Ni(PAMP)	Co(PAMP)	Zn(PAMP)
1976					
1629	6a+12) 1+6b)+amine	1611	1624	1629	1621
1536	pyridine ring vibrations	1532	1529	1519	1555
1505					
1342	6a+10b				
1328		1324		1309	1304
1223	9a		1228	1229	1223
1128	15	1173			
1072	18a	1067		1076	
995	1	996			996
			972	979	
		912			
864		857			
		811			
780	4	776			
721	11	756			
		720	721	721	720
669	6b	652		668	667
618	6a				

Note: superscripts denote py assignments[33,112].

TABLE 18

INFRA-RED SPECTRA OF PYRIDOXAMINE-5'-PHOSPHATE METAL CHELATES

(cms^{-1})

Al(PAMP)	Fe^{III}(PAMP)	Ga^{III}(PAMP)	Assignment.
1632	1627	1629	6a+12) 1+6b)+ primary amine vibration.
1546	1529	1532	pyridine ring vibration
1301	1311	1301	P=O stretch
1176- 1053	1111- 1026	1143- 1081 1000	SO$_4^=$, PO$_4^=$ absorption
	781		4 PY. mode
724	724	721	11 PY mode
671	671	670	6b PY mode

TABLE 19

INFRA-RED SPECTRA of METAL-SCHIFF BASES of PYRIDOXAL PHOSPHATE and L-GLUTAMIC ACID (SERIES I).

ABSORPTION MAXIMA(cms^{-1})

Metal ion			
Cu^{++}	Ni^{++}	Co^{++}	Assignment of band.
1619		1624	C=N imine
	1550	1522	C=C/C=N ring
1308	1304	1308	phenolic C-O^{-}
	1263		P=0 and H bonded
	1230		
		1211	
1151		1167	^{15}mode of py
1079		1034	1170 PLP band
971	971	935	^{5}py mode on co-ordination
	826		new co-ordination band
719	719	722	^{11}py mode out-of-plane C-H def.

TABLE 20

INFRA RED SPECTRA of METAL-SCHIFF BASES of PYRIDOXAL PHOSPHATE and L-GLUTAMIC ACID (series I)

ABSORPTION MAKIMA (cms^{-1}).

Metal ion

Zn^{++}	Fe^{+++}	Al^{+++}	Assignment
1624	1635	1639	C=N imine + C=C/C=N ring antisymm str.
1510	1532	1555	C=C/C=N stretch
1402			19b py mode
1315	1311	1304	phenolic $C-O^-$
1264			(PO_4^{2-}, SO_4^{2-} region.
1110			
	1045	1036	12_{py} 2nd breathing frequency
980	877		1_{py} 1st breathing frequency
833			weak py band increased on co-ordination
	781		$11/_4$ out-of-plane def. (PY)
719	723	719	out-of-plane def. (PY)

TABLE 21

INFRA-RED SPECTRA of METAL-SCHIFF BASES of PYRIDOXAMINE PHOSPHATE and α-KETO GLUTARIC ACID (series 2)

ABSORPTION MAXIMA (cms^{-1})

Metal ion.

Cu^{++}	Ni^{++}	Co^{++}	Assignment
1635		1629	C=N imine
1580	1584		C=C/C=N ring vib.
		1541	
		1500	
1311	1311	1311	phenolic $C-O^-$
		1226	$PO_4^{=}$, P=O stretch frequencies
1195			
		1126	
		1072	
		1034	2^{nd} py breathing ν
971	985		1^{st} py breathing ν
892	862		
833	826	830	864 band of PMP
769		~780	^{4} py mode
		753	
722	719	721	^{11} py mode

TABLE ZZ

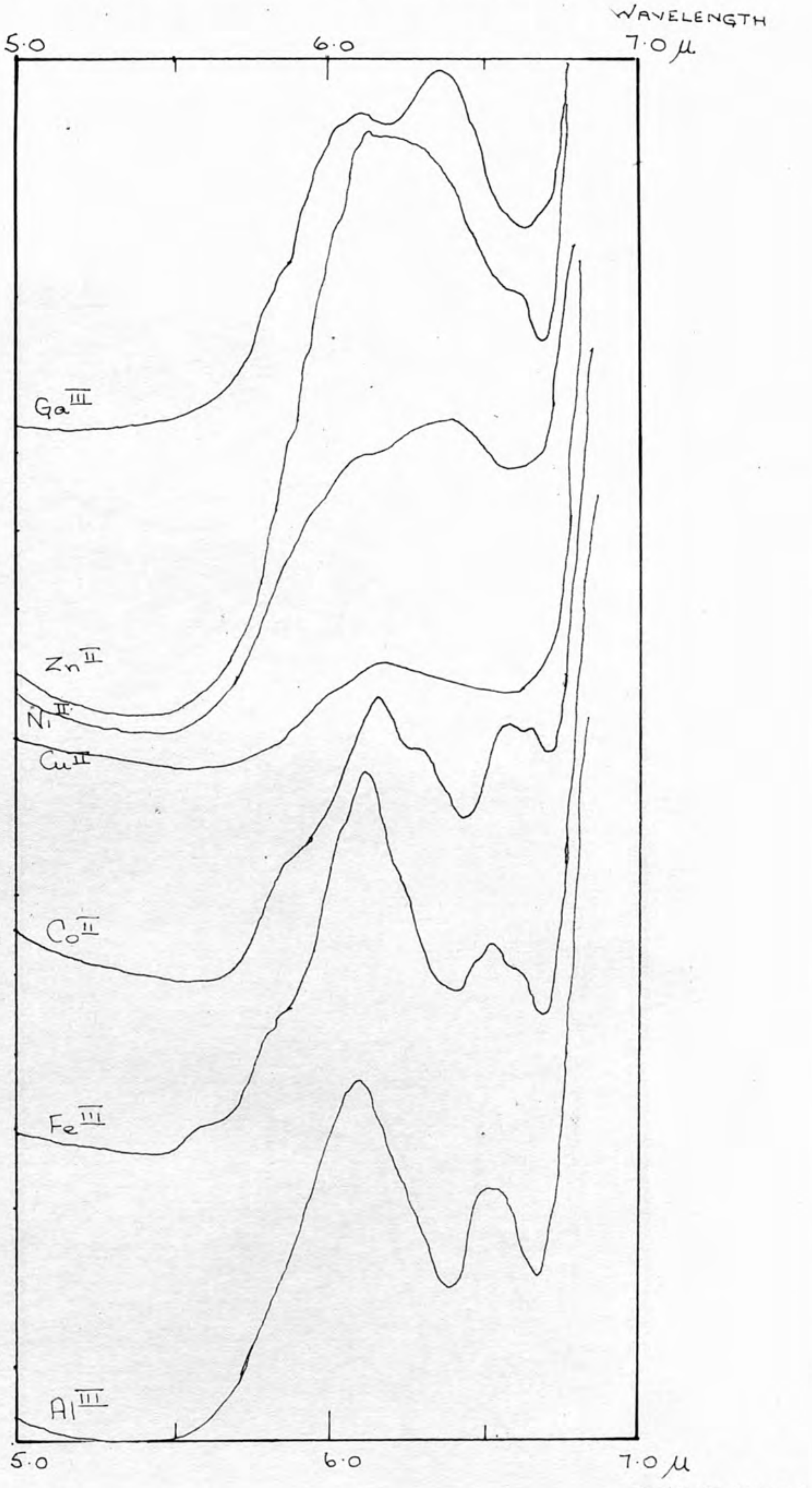
INFRA-RED SPECTRA of METAL-SCHIFF BASES OF PYRIDOXAMINE PHOSPHATE AND α-KETO GLUTARIC ACID (series 2)

ABSORPTION MAXIMA (cms^{-1}).

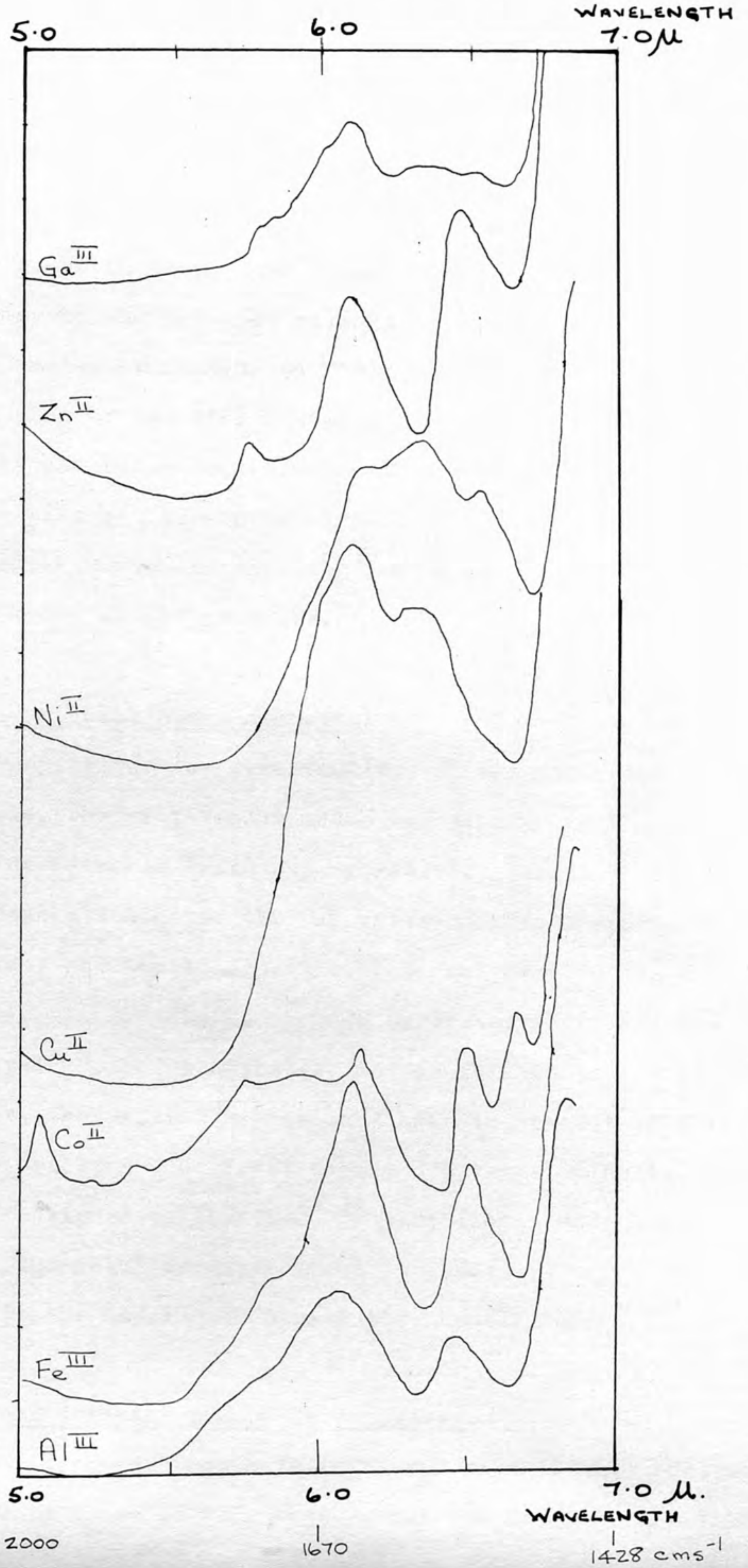
Metal ion.

Zn^{++}	Fe^{+++}	Al^{+++}	Assignment
1734			
1640		1643	C=N imine stretch
1546	1536	1541	C=C/C=N ring stretch.
1294	1308	1301	phenolic $C-O^-$
775	781		^{4}py mode 780 band of PAMP.
721	720	720	^{11}py mode C-H out-of-plane deformatio

INFRARED SPECTRA OF METAL-SCHIFF BASE COMPLEXES OF PYRIDOXAL PHOSPHATE and L-GLUTAMIC ACID



INFRA-RED SPECTRA OF METAL-SCHIFF BASE COMPLEXES OF PYRIDOXAMINE PHOSPHATE and α-KETO GLUTARIC ACID



Discussion on Infra-Red Results.

Metal-pyridine complexes.

The metal salt-pyridino complexes were discussed by Gill[33] They assigned the bands due to the pyridine nucleus according to the notation of Kline and Turkevich[112]. The only new absorption band in the metal-pyridino series is a mode at 942 cms^{-1} which is assigned to the C-H out of plane deformation. An increase in the 882cms^{-1} mode has also been observed. The symmetry of the pyridine nucleus is not effectively upset, since the co-ordination to the metal ion occurs through the hetero-atom of the ring system. The symmetry about the metal atom is generally regular; and the greatest distortion occurs in the case of the cupric complex where the polymeric distorted octahedral structure has very little effect on the IR vibrational modes of the molecule.

Metal-pyridoxal phosphate complexes.

Despite the low symmetry (C_1) of the pyridoxal phosphate molecule, the vibrational modes are similar to those of the pyridine molecule (with C_{2v} symmetry). Martell[38] has assigned the 1648cms^{-1} mode to the C=O antisymmetric stretch of the aldehyde, and the 1555cms^{-1} vibrational mode to the C=C/C=N ring vibration. The "C=O" mode is preserved in all the metal-pyridoxal phosphate chelates, and is shifted to a small degree. However, the C=C/C=N vibrational mode is present in only the ferric, gallium and violet cobalt complexes. The 1404cms^{-1} of PLP assigned to the $(C-O^-)$[37] vibrational mode has disappeared in the metal complexes, and therefore it may be suggested that all the metal bond through the phenolic group.

Metal-pyridoxamine phosphate complexes.

Pyridoxamine phosphate infra red vibrational modes are similar to those of PLP, except that the C=C/C=N ring vibration has shifted to a shorter wavenumber at 1536cms^{-1} [38]. The 1629 cms^{-1} vibrational mode is assigned to the amine band, and also the (6a+12) pyridine[112] .mode.

The 1629cms^{-1} amine vibrational mode is shifted to shorter wavenumber on co-ordination to the metal ion, in all the metal complexes, except the aluminium one. The C=C/C=N ring vibration is also shifted to shorter wavelengths, except in the aluminium and zinc chelates. The essential ionic nature of the aluminium complex could account for its deviation from the patterns of the transition metal complexes.

The 1404cms^{-1} mode of PLP assigned to the (C-O$^-$)[37] vibrational mode is absent in PAMP and in all its metal chelates. It is therefore impossible to assign the loss of the band to metal-coordination, as in the PLP case.

The metal-Schiff base chelates,

Imine bands.

The most important band shift of the metal-Schiff base series is that in the 1620-1630cms^{-1}region. This is assigned to the -C=N imine stretch frequency[39] and shifts are expected to vary according to the metal ion and the strength of the nitrogen and phenolic oxygen bands with the metaliion. If one considers the reference of the -C=N band to be that of the free PAMP at 1629cms^{-1}, then shifts can be correlated on metal coordination together with that of the free Schiff base.

The maximum shift in the first metal-Schiff base series was noticed in the copper complex, and the strong band is noted at 1619cms^{-1}. No band was found in this region in the nickel complex, but bands similar, but not as strong as that of the copper complex, were found at 1624cms^{-1}, in the cobalt and zinc complexes. This would seem to confirm opinions that metal coordination tended to shift the imine bands to longer wavelengths. However, in the aluminium and ferric chelates, -C=N imine bands occured at 1639 and 1635 cms^{-1} respectively. This could possibly be attributed to the C=O band occuring at 1648cms^{-1} of pyridoxal-5'-phosphate, shifted by +9 and +13cms^{-1} respectively. These shifts were obviously larger than any other shifts, and so there is some doubt as to the correct assignment.

The (C-O$^-$) frequency was quoted by Martell[37] to occur at 1410cms^{-1} in the PLP molecule. This band also overlaps on the 19b mode of the pyridine which is quoted to be that of the C=C, C=N antisymmetrical stretch. No such bands occur in any of the Schiff base metal chelates, except for that of the zinc-Schiff base complex of the first series. In this, absorption occurs at 1402cms^{-1} and could quite reasonably assigned to the (C-O$^-$) frequency and the Zn-O/phenolic bond would therefore, be presumably fairly weak, if it existed at all.

Kovacic[43] has assigned the C-O phenolic band to the 1130-1310cms^{-1} region in bonded metal chelates. The phenolic C-O frequency occurs at 1324 and 1328 cms^{-1} in PLP and PAMP respectively. These are taken for the reference of the first and second Schiff base series respectively. The maximum shift in the first series occurs in the nickel and aluminium chelates with the band occuring at 1304cms^{-1} (a shift of -20cms^{-1}). The copper and cobalt bands occur at 1308cms^{-1}, with bands at 1311 and 1315cms^{-1} for the ferric and zinc chelates respectively. The following series for the shift of the phenolic band is suggested:

$$Al = Ni > Cu = Co > Fe > Zn$$

This series would not appeat to agree with any other series which appears, associated with transamination catalysis of metal ions.

In the second Schiff base series, the maximum shift is that of the zinc chelates where the (C-O$^-$) frequency occurred at 1294cms^{-1}. The next in the series is that of the aluminium chelate at 1301cms^{-1}, and the 1308cms^{-1} mode found in the ferric chelate. The copper, nickel and cobalt chelates all absorb at 1311cms^{-1}. The shift from the 1328cms^{-1} PAMP band ranges from +34 to +17 cms^{-1} depending on the metal ion. The series of (C-O$^-$) band shifts is not in agreement with the first Schiff base series; viz.

$$Zn > Al > Fe > Co = Ni = Cu$$

However it is possible that the P=O stretch frequencies occurring in this region, mask the (C-O$^-$) frequency and distort the metal coordination shift of bands.

C-H deformation

It is interesting to note in all the complexes, the existence of the weak band due to the pyridine ring as reported by Popov^{40}. An increase in the absorption is expected in the 882cms^{-1}mode. A new band at 924cms^{-1} appears in the metal-pyridino complexes and is assigned to a C-H out-of-plane deformation. The bands occurring in the PLP molecule are the 881cms^{-1} mode and the 947cms^{-1} mode attributed to the C-H deformation. However, in the PAMP case these modes appear to be absent as the nearest bands are 864 and 995cms^{-1} respectively. The copper and Zinc pyridino complexes are the only ones in the series that have bands in the 890cms^{-1}region, at 872 and 891 respectively. The copper pyridine complex is the only one which has the 942cms^{-1} band, at 947cms^{-1}. In the "simple" metal complexes with PLP and PAMP both the 882 and 942cms^{-1} bands are absent, except in the case of the Cu (PAMP) where the pyridine band occurs at 947cms^{-1}.

The first series of Schiff bases exhibit an absorption band in a range 980-971cms^{-1} region, Cu = Ni > Zn. The 882 cms^{-1} coordination C-H deformation is absent from all this series except for the ferric complex of Schiff base series 1, which occurs at 877cms^{-1}.

In the second Schiff base series the "942cms^{-1}" band region extends from 985-971cms^{-1}. However, the only complexes which have this band are those of the copper and nickel (at 971 and 985cms^{-1}). In all the others the coordination band is absent. Similarly the 882cms^{-1} mode is present only in the copper and nickel complexes (at 892 and 862cms^{-1} respectively). The 864cms^{-1} band of PAMP is shifted on coordination and chelation to longer wavelengths in the case of $Ni > Co > Cu$.

A mode common to all the complexes, is the C-H out-of-plane deformation[112], of the pyridine ring at 719cms^{-1}. The maximum shift in the metal-pyridine series is $\pm 2cms^{-1}$, $\pm 5cms^{-1}$ in the PLP series, and $\pm 2cms^{-1}$ in the PAMP series. In both Schiff base series the C-H out-of-plane deformation is shifted by 4cms^{-1}, and it is therefore thought that this mode must be a basic one which is attributable only to the ring system. If this is the case, then it would not be affected by the consequent chelation of the ring system to the metal ion.

The -OH and N-H region.

All the compounds prepared were examined in the 2000-5000cms^{-1} region. The few peaks occuring there were very indistinct, and such groups as -OH and bridging hydroxyl groups could not be detected.

Ultra violet solid state spectra: EXPERIMENTAL

The ultra violet and near-ultra violet spectra of freshly ground samples were run on the Unicam SP700 recording spectrophotometer using the SP735 solid state attachment. Freshly prepared magnesium oxide was used in the reference beam.

TABLE 23

ULTRAVIOLET SPECTRA of the METAL-PYRIDINE COMPLEXES.

Solid state spectra: Unicam SP 700 spectrophotometer.

Complex assignment	Absorption maxima:cms^{-1} (Rel.absorb.) $CuCl_2.2py$	$NiCl_2.2py$	$CoCl_2.2py$	$CoCl_2.2py$ $10H_2O$	$ZnCl_2.2py$
π-π$_1$*			43,000*′ (0.853)		
π-π$_2$*	38,333 (0.956)	38,333 (1.006)	38,267 (0.977)	38,467 (1.01)	38,000 (1.45)
π-π$_2$*	37,333 (0.973)	37,267 (1.00)	37,200 (1.00)	37,667 (1.00)	
n-π*	35,933 (1.000)				
ligand field	30,200 (0.962)				
		25,600 (0.246)			

*′ denotes shoulder.

TABLE 24

ULTRAVIOLET SOLID STATE SPECTRA of the METAL-PYRIDOXAL-5'-PHOSPHATE COMPLEXES.

Metal assignment.	Absorption maxima:cms^{-1} (Rel. absorbs). Cu^{++}	Ni^{++}	Co^{++}	Zn^{++}	Ga^{+++}
π-π$_1$*	46,000 (1.058)	45,733 (1.124)	46,467 (0.937)	45,800 (1.068)	46,467 (1.182)
π-π$_2$*	38,500 (1.00)	39,133 (1.00)	37,333 (1.00)	38,933 (1.00)	39,400 (1.00)
3-OH→O$^-$		33,333 (0.977)		33,400 (0.956)	33,000 (1.032)
ligand field.	24,667 (0.910)	31,333*′ (0.956)	28,600 (1.104)	27,667 (1.115)	
		24,333*′ (1.045)			

TABLE 25

ULTRAVIOLET SOLID STATE SPECTRA of the METAL-PYRIDOXAMINE PHOSPHATE COMPLEXES.

Metal Assignment	Absorption maxima: cms^{-1} (Rel. absorb.) Cu^{++}	Ni^{++}	Co^{++}	Zn^{++}	Ga^{+++}
$\pi-\pi_1{}^*$	43,400 (0.878)	46,333 (0.851)	46,267 (0.986) 45,600 (0.983)	46,533 (1.236)	46,400 (1.252)
$\pi-\pi_2{}^*$	38,267 (0.991) 37,000 (1.00)	37,333 (1.00)	38,133 (1.00)	40,000 (1.00)	39,467 (1.00)
neutral or anionic form of molecule.	33,467 (1.002)	32,333 (1.072)	31,467 (1.00)	32,267 (1.292)	32,267 (1.282)

TABLE 26

ULTRAVIOLET SOLID STATE SPECTRA of the FERRIC and ALUMINIUM COMPLEXES of PYRIDOXAL PHOSPHATE (PLP) and PYRIDOXAMINE PHOSPHATE (PAMP)

Complex Assignment	Absorption maxima: cms^{-1} (Rel. abs.) Fe^{3+}(PLP)	Fe^{3+}(PAMP)	Al^{3+}(PLP)	Al^{3+}(PAMP)
$\pi-\pi_1{}^*$	47,300 (0.940)	47,300 (0.900)	46,400 (1.213)	47,400 (0.850)
$\pi-\pi_2{}^*$			39,667* (1.00)	
neutral depolar form	34,267 (1.00)	34,667 (1.00)	32,133 (1.140)	34,333 (1.00)
$\pi-\pi_1{}^*$ligand	27,733 (0.663)	27,733 (0.874)	25,000 (1.028)	

*denotes shoulder

TABLE 27

SOLID STATE of the METAL–SCHIFF BASE COMPLEXES.

SERIES I: PYRIDOXAL PHOSPHATE– L-GLUTAMIC ACID.

Absorption maxima:cms^{-1}(Rel. absorb.)

	Cu^{++}	Cu^{++}	Cu^{++}	Cu^{++}
Assignment				
$\pi-\pi 2^*$	45,500 (0.91)	45,333 (0.979)	45,333 (0.92)	44,800 (1.012)
$\pi-\pi_1^*$ (py)	36,500 (1.00)	36,000 (1.00)	35,400 (1.00)	36,500 (1.00) 34,000* (0.89)
$\pi-\pi_1^*$ (imine)	26,800 (0.976)	25,133 (0.95)	26,267 (1.012)	25,333 (0.78)

Absorption maxima:cms^{-1}(Rel. abs.)

Metal	Ni^{2+}	Ni^{2+}	Co^{2+}	Zn^{2+}
Assignment				
$\pi-\pi_2^*$	45,333 (1.07)	44,800 (0.87)	45,600 (1.06)	45,333 (1.03)
$\pi-\pi_1^*$ (pyridine)	38,467 (1.00)	36,467 (1.00)	38,000 (1.00)	36,500 (1.00)
neutral dipolar forms	30,533 (0.95)	27,667* (1.03)	33,800 (1.02)	27,133 (1.03)
$\pi-\pi_1^*$ (imine)	25,667 (0.98)	25.600 (1.06)	25,867 (0.88)	

Absorption maxima:cms^{-1}(Rel. abs.)

Metal	Ga^{3+}	Al^{3+}	Fe^{3+}
Assignment			
$\pi-\pi_2^*$	45,400 (0.90)	46,200 (1.05)	44,800 (0.87) 36,533 (1.00)
$\pi-\pi_1^*$ (py)	34,800 (1.00)	33,200 (1.00)	35,000 (1.005) 28,067* (0.73)
$\pi-\pi_1^*$ (imine)	26,400 (0.90)	26,784 (0.86)	24,333* (0.62)

TABLE 28

ULTRAVIOLET SOLID STATE SPECTRA of the METAL-SCHIFF BASE COMPLEXES.

SERIES II: PYRIDOXAMINE PHOSPHATE - αKETO GLUTARIC ACID.

Absorption maxima: cms^{-1} (Rel. abs.)

Metal Assignment	Cu^{2+}	Ni^{2+}	Co^{2+}(12)	Co^{2+}(36)
$\pi-\pi_2^*$	45,600 (0.99)	45,333 (1.07)	46,555 (0.88)	46,000 (1.04)
$\pi-\pi_1^*$ (py)	36,400 (1.00)	38,467 (1.00)	34,667 (1.00)	38,133 (1.00)
neutral dipolar		30,533 (0.95)		33,200* (0.94)
$\pi-\pi_1^*$ (imine)	25,400 (0.98)	25,667 (0.98)	27,000* (0.36)	

Absorption maxima: cms^{-1} (Rel. abs.)

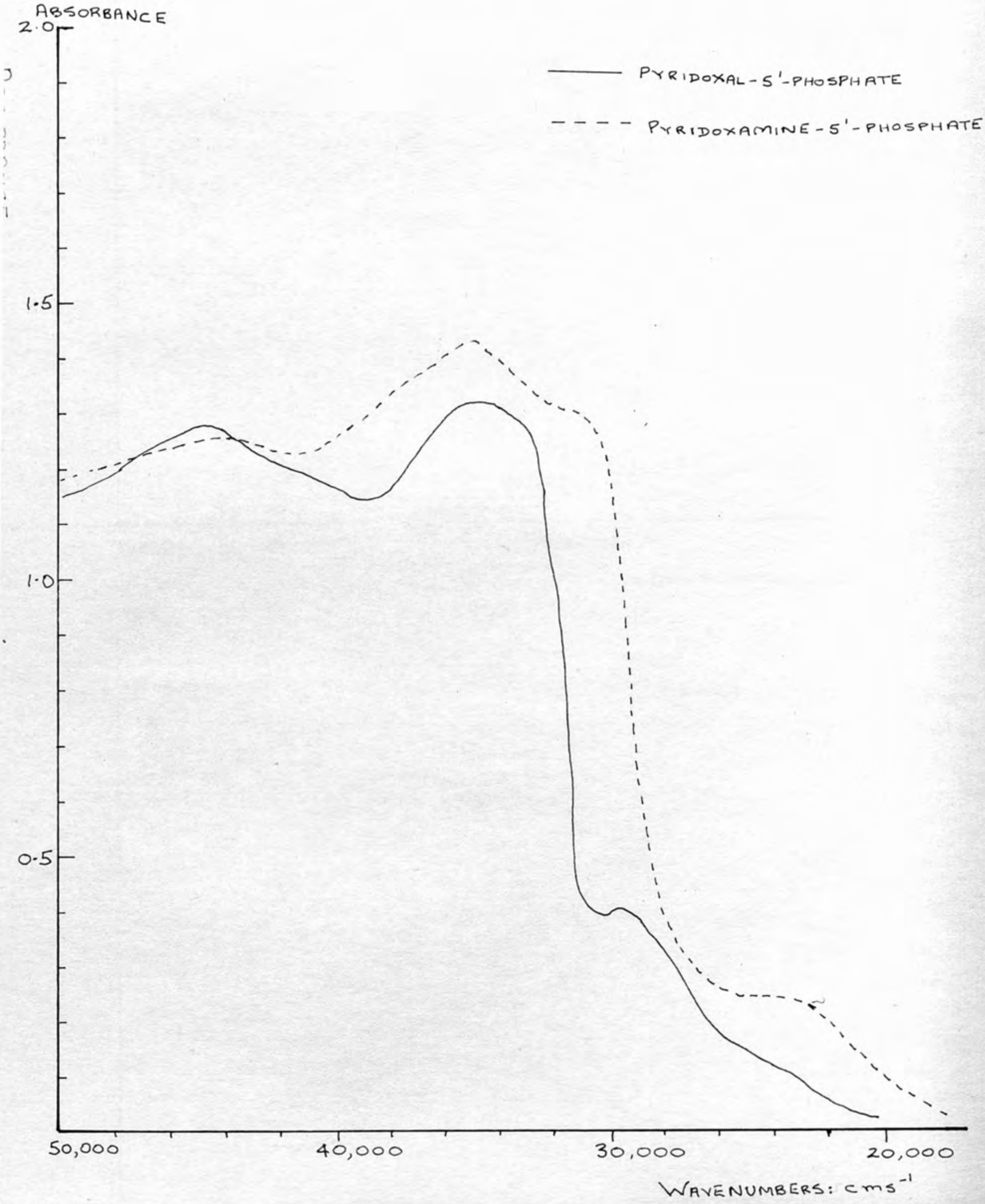
Metal Assignment	Zn^{2+}(22)	Zn^{2+}(49)	Al^{3+}	Fe^{3+}
$\pi-\pi_2^*$	44,333 (0.78)	46,800 (1.11)	46,500 (1.10)	44,333 (0.84)
$\pi-\pi_1^*$ (py)	35,400 (1.00)	39,467 (1.00)	30,600 (1.00)	34,000 (1.00)
		32,400 (1.17)		27,900*
$\pi-\pi_1^*$ (imine)	25,000 (0.55)	25,400 (1.72)		24,000*

Absorption maxima: cms^{-1} (Rel. abs.)

Assignment	Ga^{3+}(26)	Ga^{3+}(42)	Ga^{3+}
$\pi-\pi_2^*$	44,867 (1.05)	46,200 (1.20)	46,000 (1.20)
$\pi-\pi_1^*$ (py)		39,733 (1.00)	39,733 (1.00)
neutral dipolar	35,467 (1.00)		
	33,533 (0.99)	32,400 (1.21)	33,200 (1.19)
$\pi-\pi_1^*$ (imine)	25,733 (0.93)	25,733 (0.80)	26,200 (0.95)

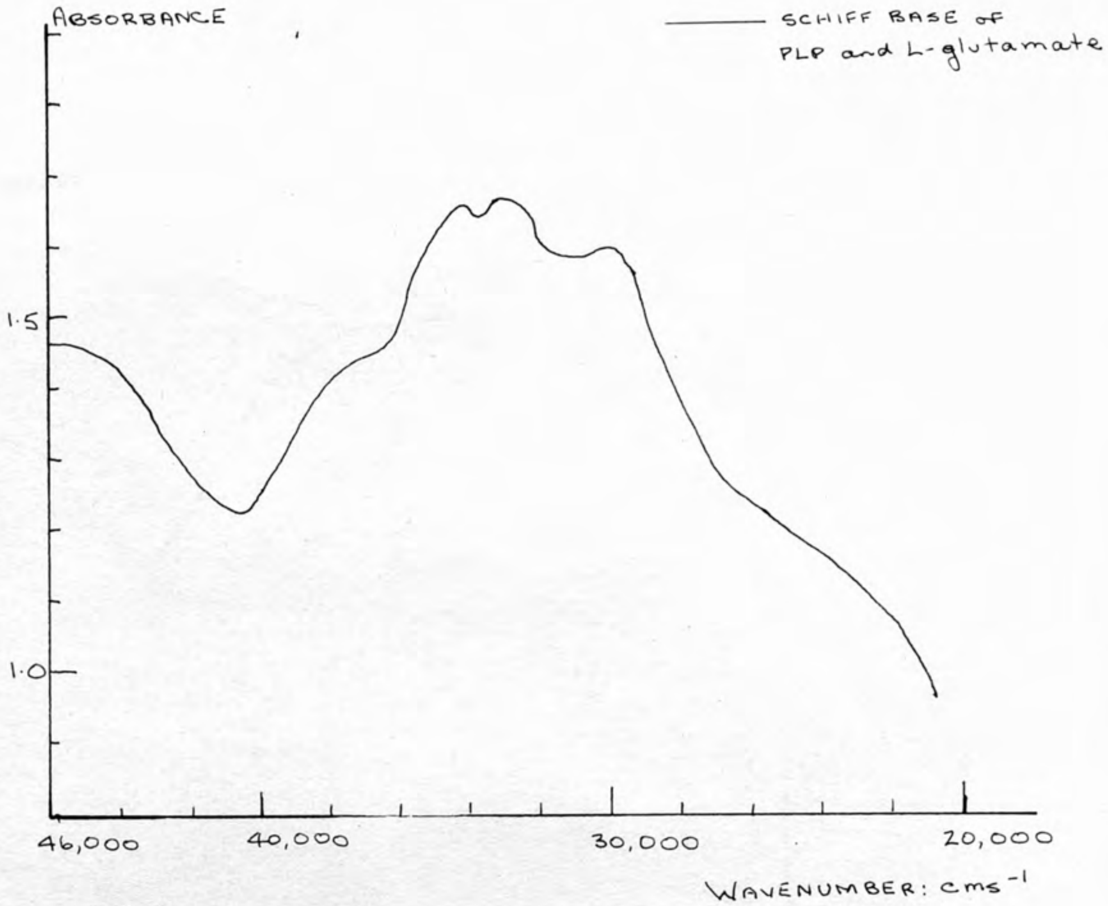
SOLID STATE SPECTRA

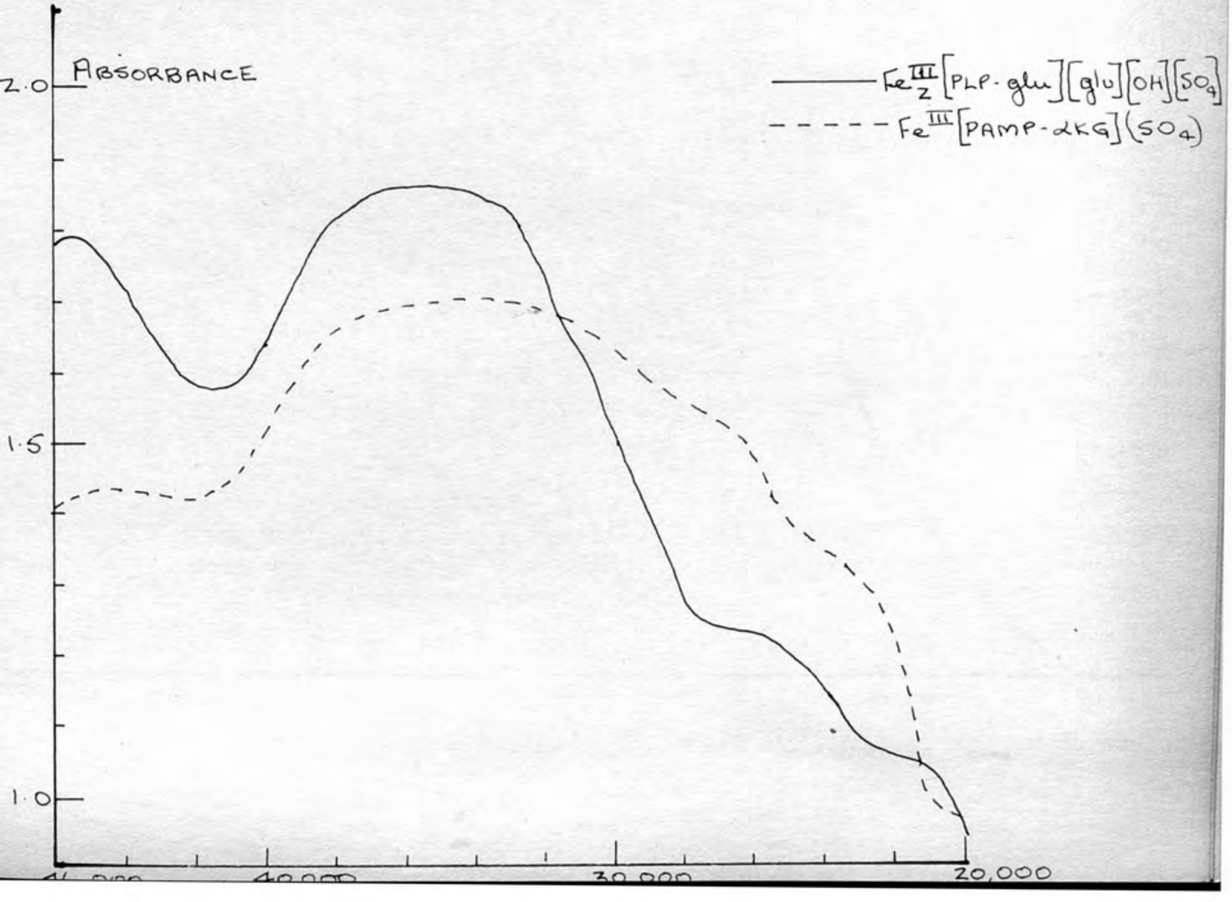
UNICAM SP 700 + SP 735
Reference: MgO



SOLID STATE SPECTRA

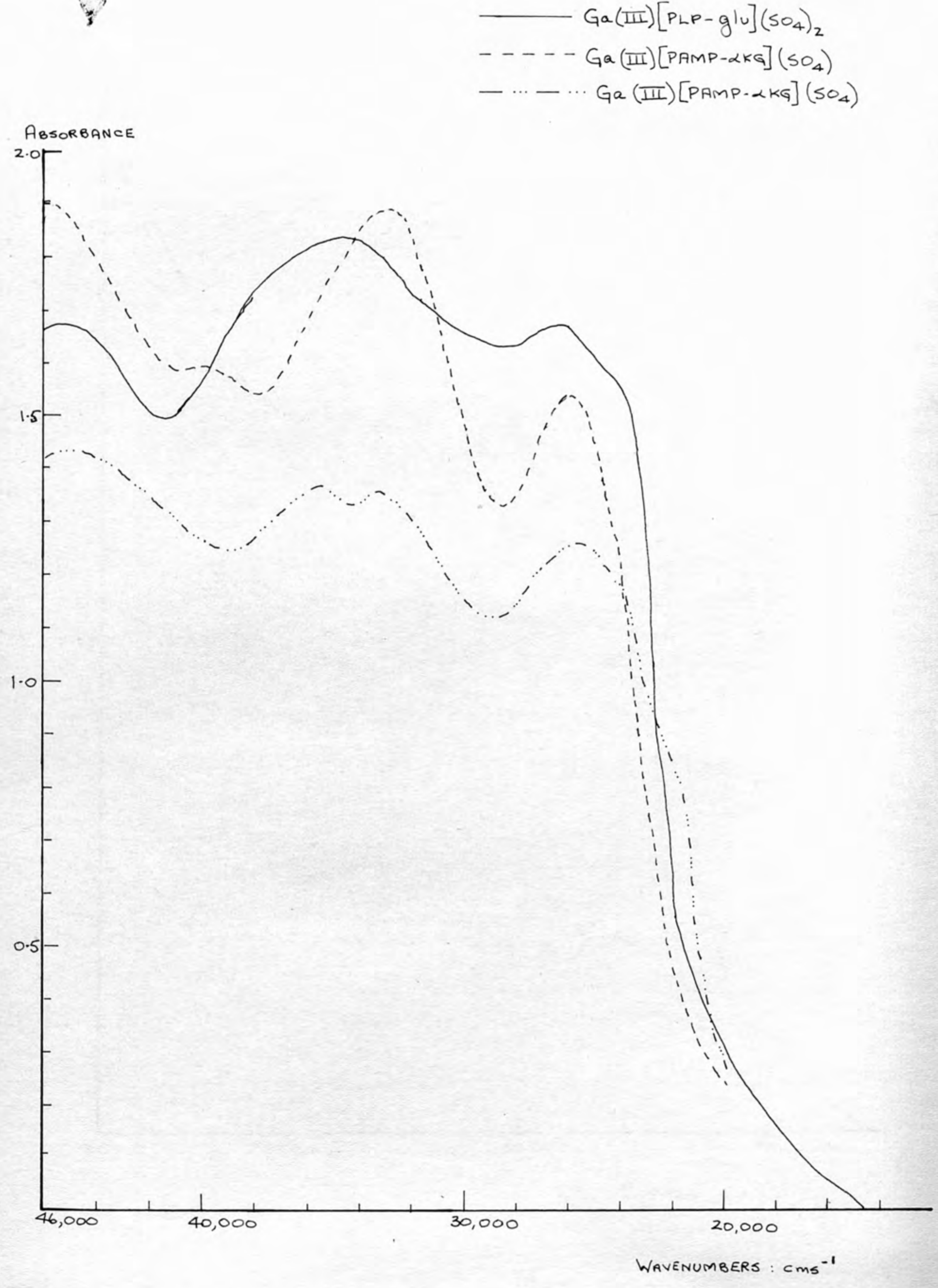
UNICAM SP 700 + SP735
Reference: MgO



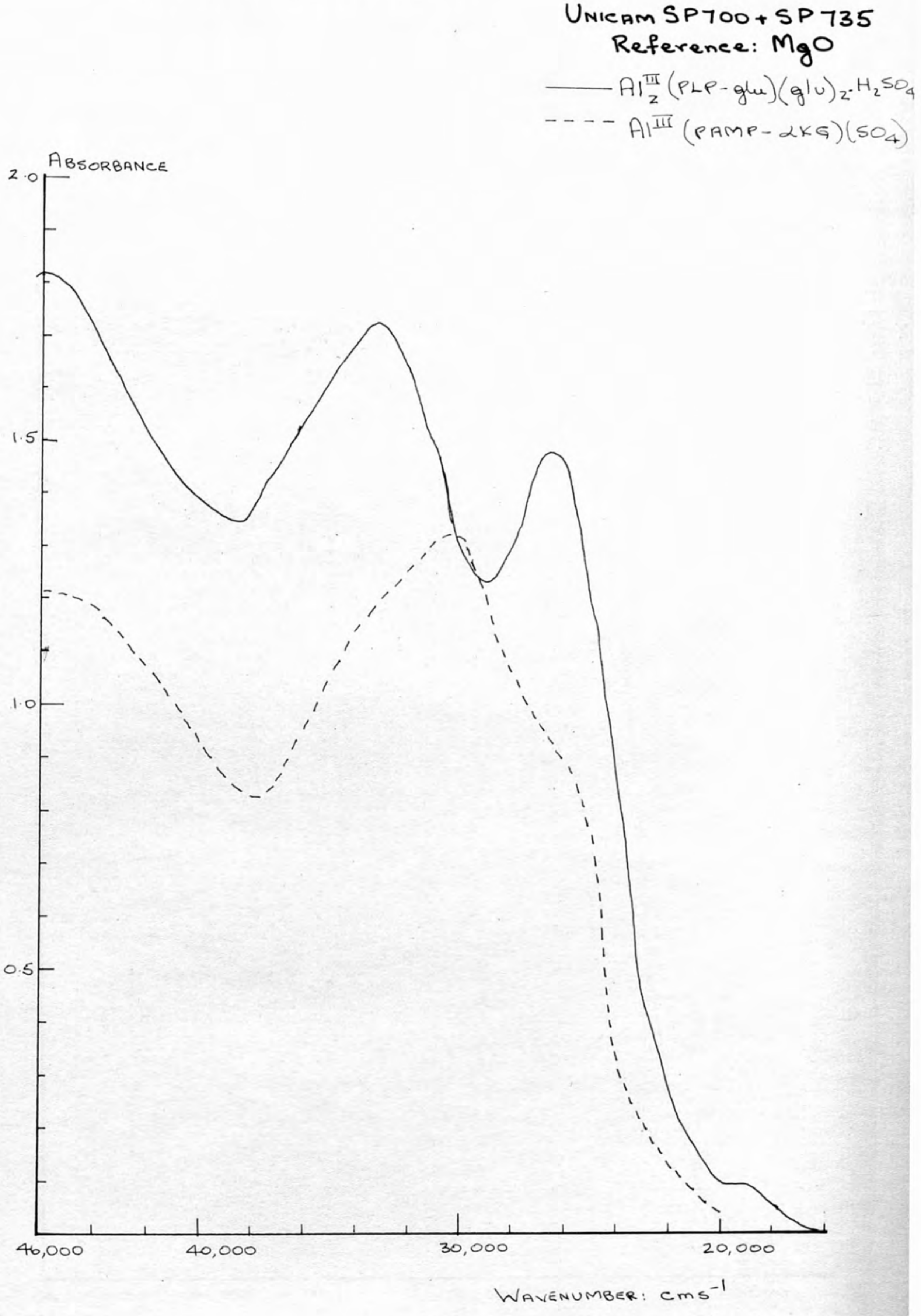


SOLID STATE SPECTRA

UNICAM SP 700 + SP735
Reference: MgO



SOLID STATE SPECTRA



SOLID STATE SPECTRA

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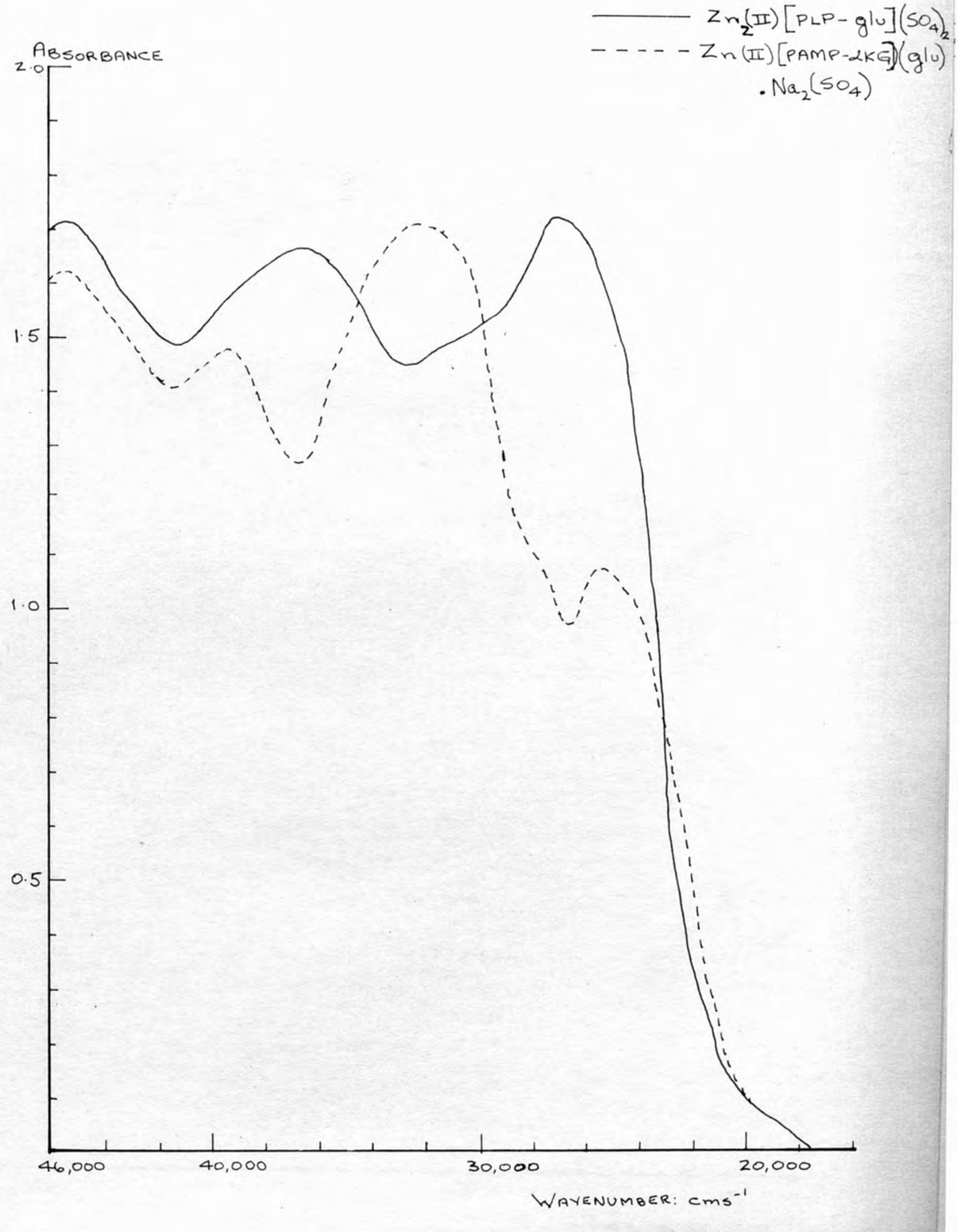
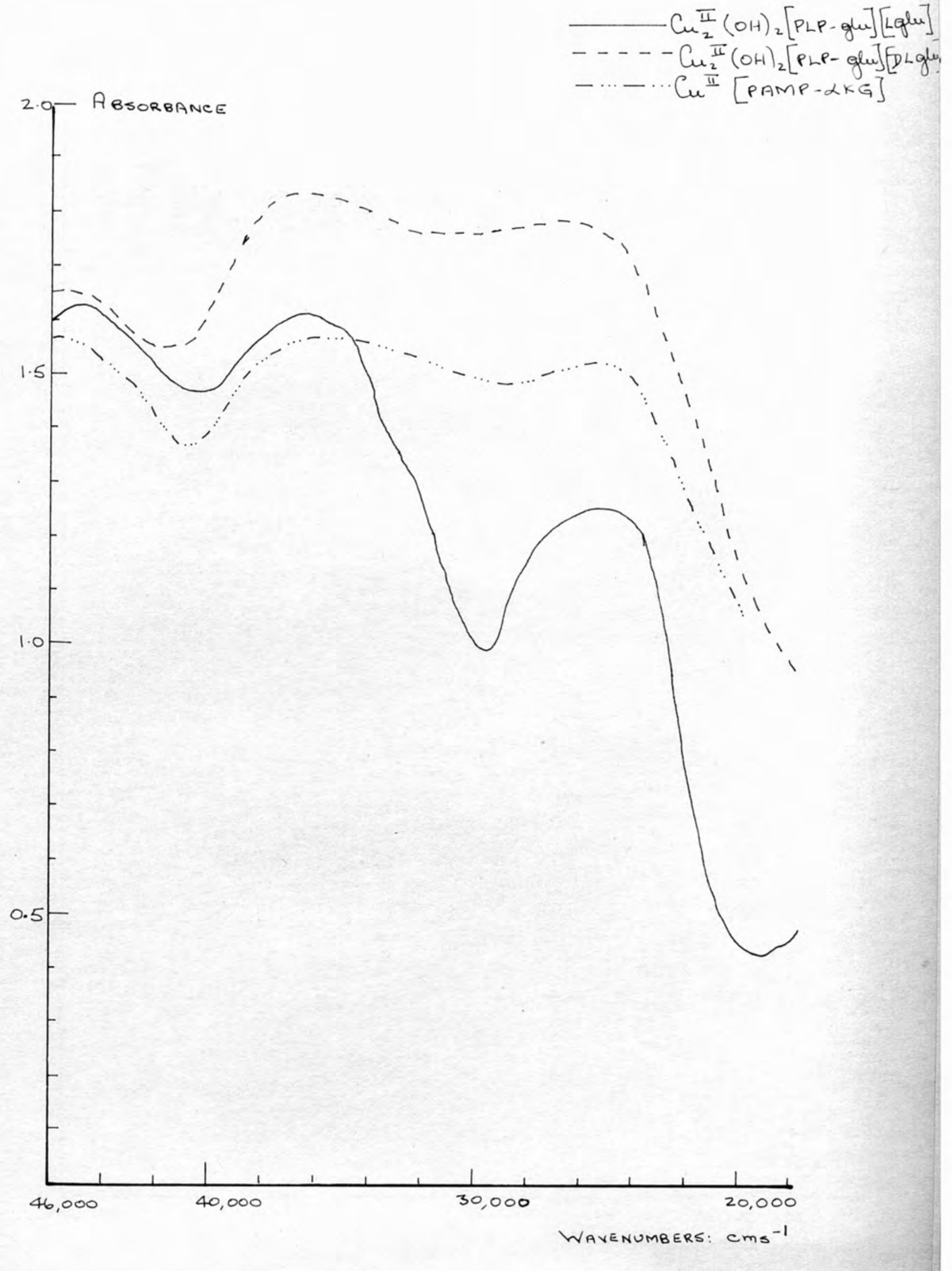


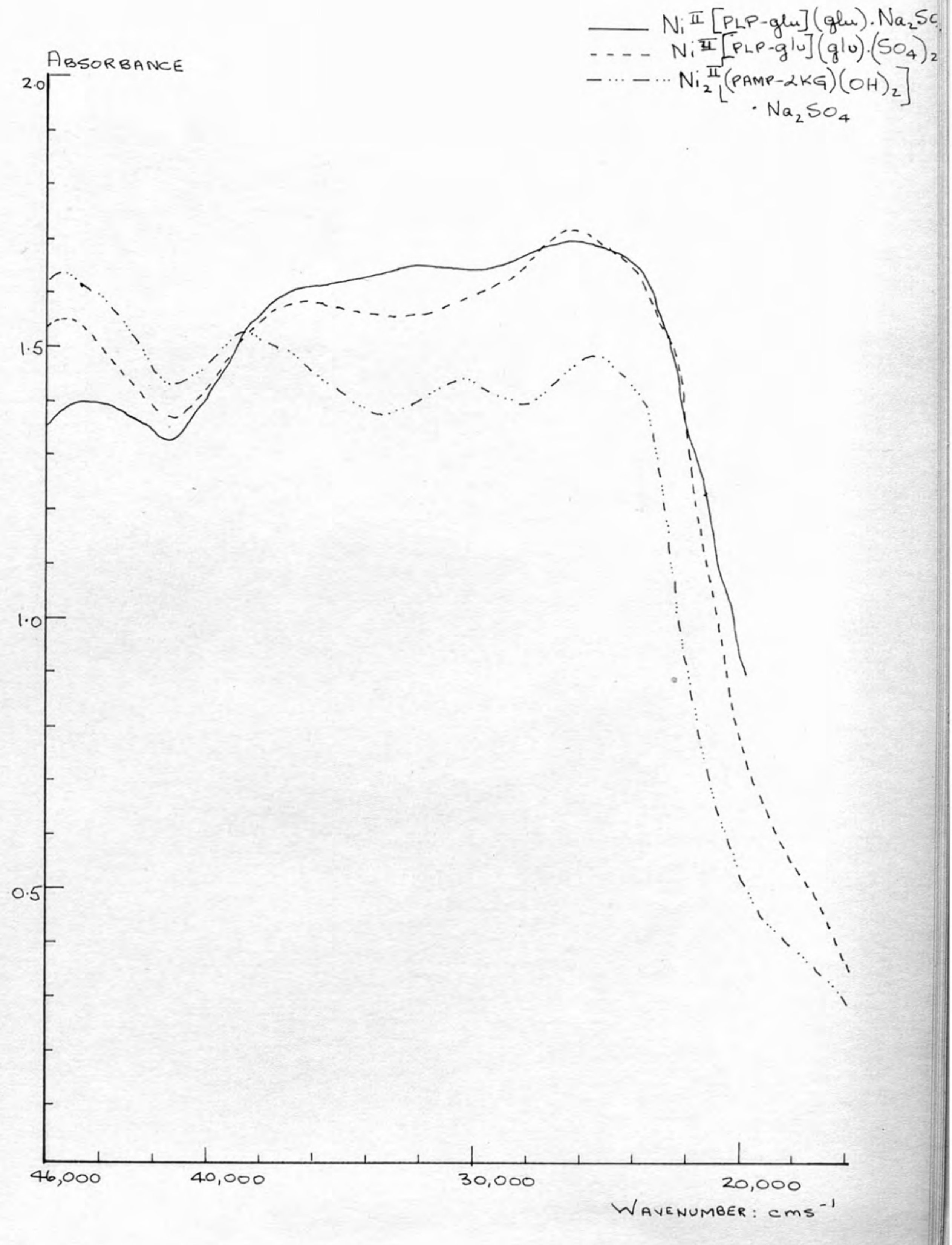
SOLID STATE SPECTRA

UNICAM SP 700 + SP 735
Reference: MgO



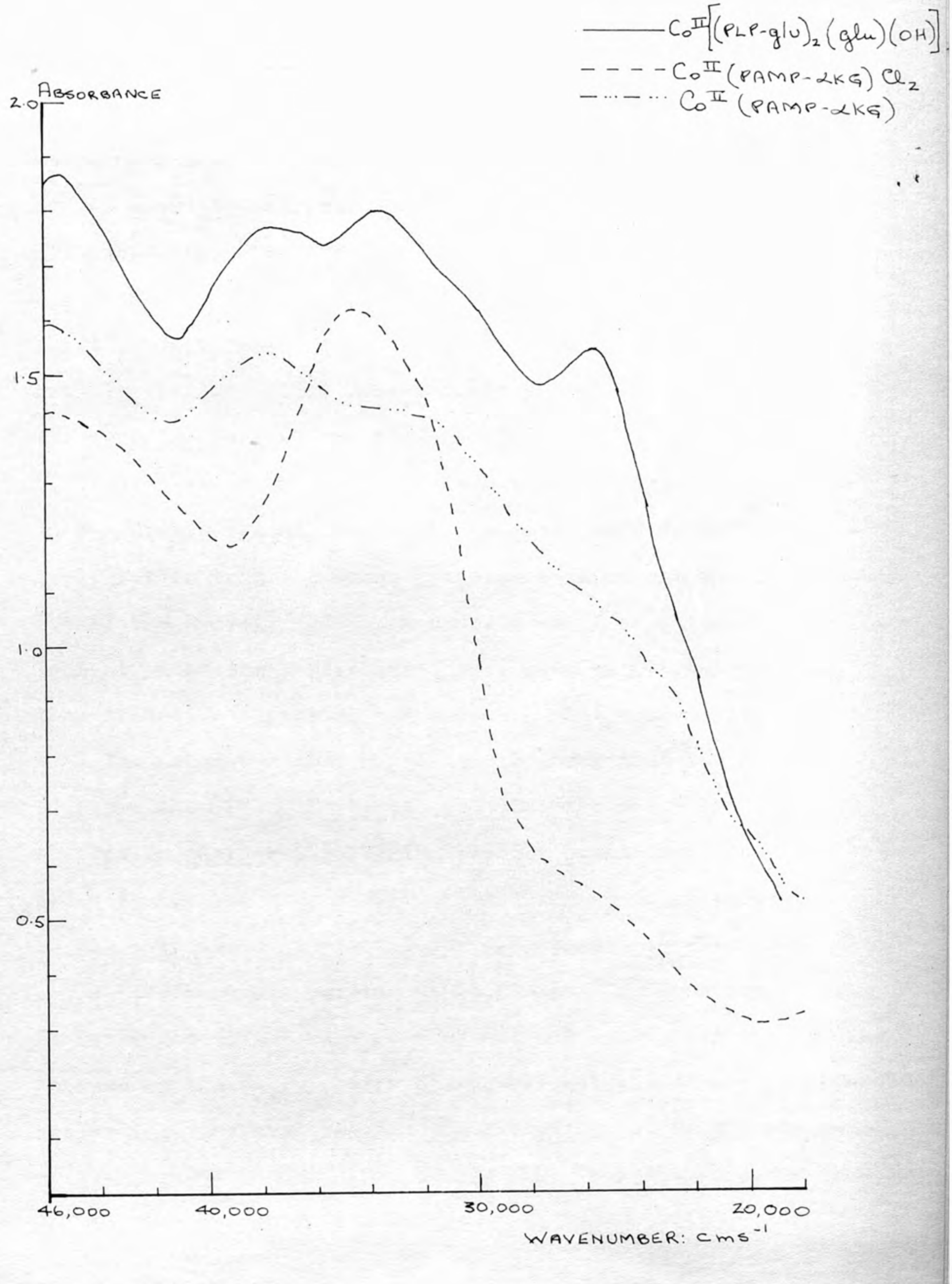
SOLID STATE SPECTRA

UNICAM SP700+ SP735
Reference: MgO



SOLID STATE SPECTRA

UNICAM SP700 + SP735
Reference: MgO



Discussion:

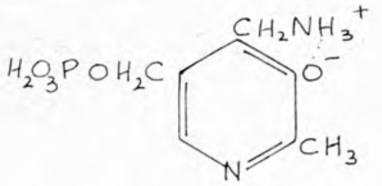
In assigning electronic transitions to the spectroscopic states and bands of the metal complexes, it is necessary, firstly, to consider the electronic distributions and possible transition in the basic pyridine ring system, and in its substituted derivatives.

The pyridine nucleus has three main bands quoted on p. 35 which have been assigned to the π-π_1*, and π-π_2*[38] transitions of the aromatic nucleus. n-π* transition have been neglected although it has been reported[115] that a singlet n-π* transition has been observed at 34,769cms^{-1}, which can subsequently shift to 36,000cms^{-1} if the nitrogen lone pair forms an N-H band (as in alcohol). However, although the protonation of the pyridine nucleus can cause shifts of $\pm$5mμ, the greater shift has been found to be in the case of hydroxy-pyridines. In 3-hydroxypyridine, the uncharged species differs comparitively little from the basic pyridine species but the ionisation of the hydroxy group can cause a shift of up to 2,500cms^{-1}. Ionisation of the species can cause such significant shifts, that transitions overlap and become indistinguishable.

The metal-pyridino complexes indicate that the nature of the metal-ligand band, is such that the π-π_2* band is shifted to <u>shorter</u> wavelengths (longer wavenumber) on coordination. The copper-pyridine chloride complex seems to be the only one of the metal-pyridino complexes which exhibits the π-π_1*, band of pyridine at 35,933 cms^{-1}. This transition is forbidden in the case of benzene, but is allowed in pyridine because of the C_{2v} symmetry of the molecule. One can therefore assume that the copper-N bond is the strongest of the three-metal-nitrogen bonds. (Co, Cu and Ni). π-π_2* band shift to shorter wavelength in the order:

$$Co^{++} > Cu^{++} = Ni^{++} > Zn^{++}$$

There have been no previous assigments of electronic transitions of either PLP or PAMP, in the solid state. Both the π_1^* and π_2^* bands have been shifted to longer wavelength than in both cases and π-π_1^* species occur around 30,000 cms^{-1} for the basic O^- species. However, PAMP does contain an extra transition at 24,000 cms^{-1} (as a shoulder) which could be attributed to the neutral dipolar species of PAMP.



XXXVI

Shift of the π-π^* bands on coordination

Shifts due to metal co-ordination of PLP and PAMP would appear (in the solid state) to shift the π-π_1^* band to shorter wavelengths, whereas the π-π_2^* band is shifted to longer wavelengths (i.e. relative to the transition states of PLP and PAMP).

PLP $\pi \rightarrow \pi_2^*$ 34,333 cms^{-1}

PAMP $\pi \rightarrow \pi_2^*$ 35,000 cms^{-1}

π-π_2^* band shift of the metal pyridine complexes to shorter wavelength is in the order:

$$Co^{++} > Cu^{++} = Ni^{++} > Zn^{++}$$

For PLP complexes: (see Table 24 and 26)

π-π_1^* band: shift to shorter wavelength:

44,000 cms^{-1}

$$Fe^{3+} > Co^{2+} = Ga^{3+} > Al^{3+} > Cu^{2+} > Zn^{2+} > Ni^{2+}$$

π-π_2^*. shift to longer wavelengths:

35,000 cms^{-1}

$$Al^{3+} > Ga^{3+} > Ni^{2+} > Zn^{2+} > Cu^{2+}$$

For PAMP complexes: (see Tables 25 and 26)

π-π_1* band: shift to shorter wavelength:

$Al^{3+} > Fe^{3+} > Zn^{2+} > Ga^{3+} > Ni^{2+} > Co^{2+}$

Cu^{2+} shift to longer wavelengths.

π-π_2* band: shift to longer wavelength:

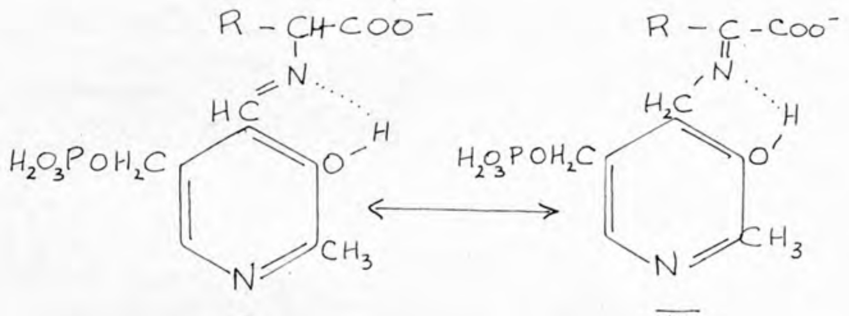
$Zn^{2+} > Al^{3+} > Ga^{3+} > Cu^{2+} > Co^{2+} > Ni^{2+}$

There appears to be no order for the amount of effective shift of the bands, and it was hoped that spectra of the 25,000-35,000cms^{-1} would provide further information. It was generally found in both the PLP and PAMP metal complexes that the shift of the existing bands was to shorter wavelengths; added to this fact that there were new bands as well. These "new" bands in the 25,000-26,000cms^{-1} region were most likely to be 'ligand field bands'. According to Metzlers' rule[16]: possible shifts to longer wavelengths would be due to the ionisations of the hydroxyl hydrogen. In the solid states, however, this does not appear to be the case, and therefore, the phenolic oxygen-metal band is assumed to be strong enough to prevent ionisation. Shifts to shorter wavelengths (due to the pyridinium ion) do occur, as might be expected if the metal ion is not attached through the pyridium-nitrogen.

The Schiff base of pyridoxal-5'-phosphate and L-glutamic acid(page 122), was found to have bands at 45,375, 34,200 and 33,300cms^{-1}, with shoulders at 37,200, 30,000, 26,000 and 24,375cms^{-1}. Free pyridoxal phosphate can be detected in the 24,000, 30,333, 34,867 and 44,200cms^{-1}. The remaining absorption peaks are the peaks at 33,300cms^{-1} and shoulders at 26,000 and 37,200cms^{-1}. The 33,300cms^{-1} will be attributed to the π-π_1* band, and the 37,200cms^{-1} to the π-π_2* band of the Schiff base. The band at 26,000cms^{-1} must therefore be π-π_1* band of the aldimine ~~vibrational~~ mode of the Schiff base.

The metal-Schiff base series of complexes.

The species expected among these complexes are the tautomeric aldimine-ketimine Schiff bases. Each tautomer has a distinctive spectrum. It had been pointed out by Heinert[37] that a tautomeric mixture occurred in the solid state, with bands around 26,000cms^{-1} attributable to the two forms of Schiff bases I and II.



I II

In the case of metal complexes, the metal ion would effectively replace the phenolic hydrogen and stronger bonding would result in two ways; viz. the donation of a lone pair of electrons from the nitrogen to the vacant d- (or metal) orbitals of the metal ion and the particully covalent nature of the phenolic oxygen-metal bond. This donation of electrons from the nitrogen to the metal ion would effectively cancel out the nett negative charge on the nitrogen as calculated by Pullman[56,57]. A rearrangemnt of electronic charge would therefore ensure with a probable "electron sink"[32] and a shift to shorter wavelength would be expected because of the protonation of the pyridine-nitrogen. Brocklehurst[59] predicted an increase in intensity of two to three times in the 38,100 cms^{-1} band.

If one compares each of the various Schiff base chelates with the metal-amine or aldehydes complexes which could have been their derivatives, it will be seem that the "imine" band around 25,000cms^{-1} and the band around 44,000cms^{-1} both shift to <u>shorter</u> wavelengths compared to the simpler metal complexes.

This factor could be taken as indicative that there is a greater degree of chelation and stability in the Schiff base metal complexes. However, it is found that in the 40,000-29,000cms^{-1} region, the maxima do appear to vary with metal ion, and it is this region which one hopes will indicate the ionic species present.

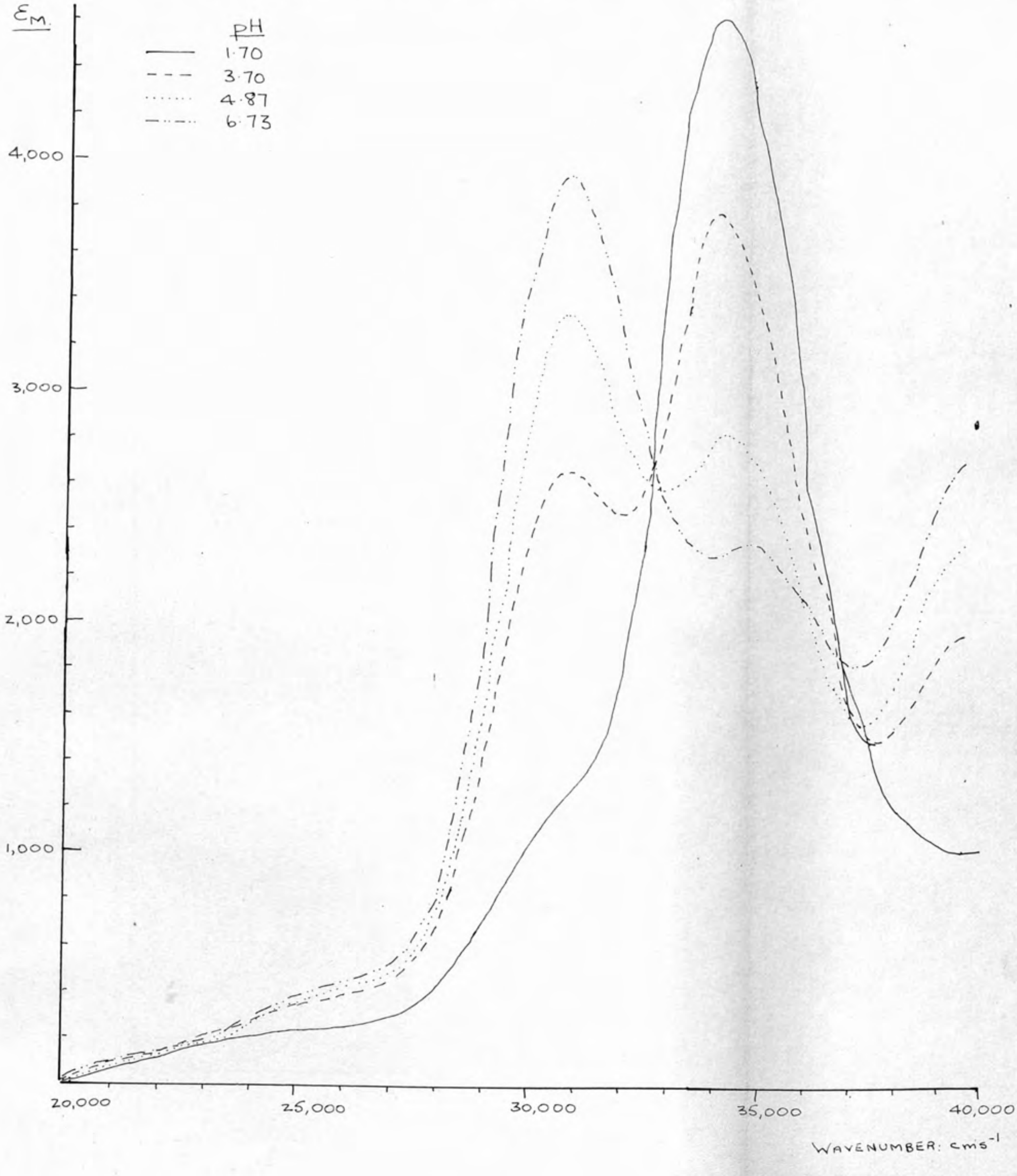
An advantage of solid state spectra, is that one can calculate the number of ionic species present, and also if there is any free pyridoxal or pyridoxamine phosphate present.

ULTRA VIOLET SPECTRA in SOLUTION.

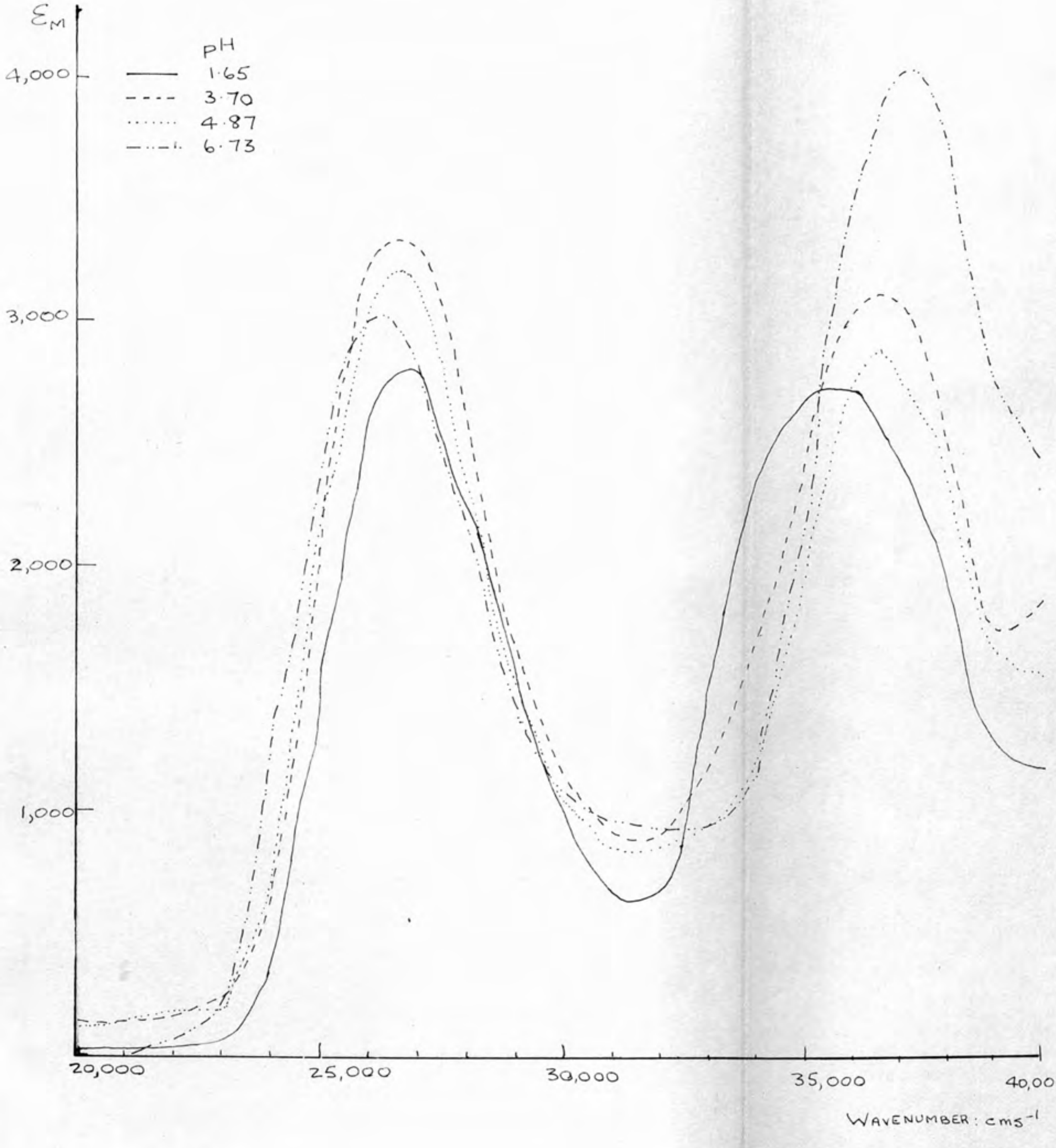
Experimental

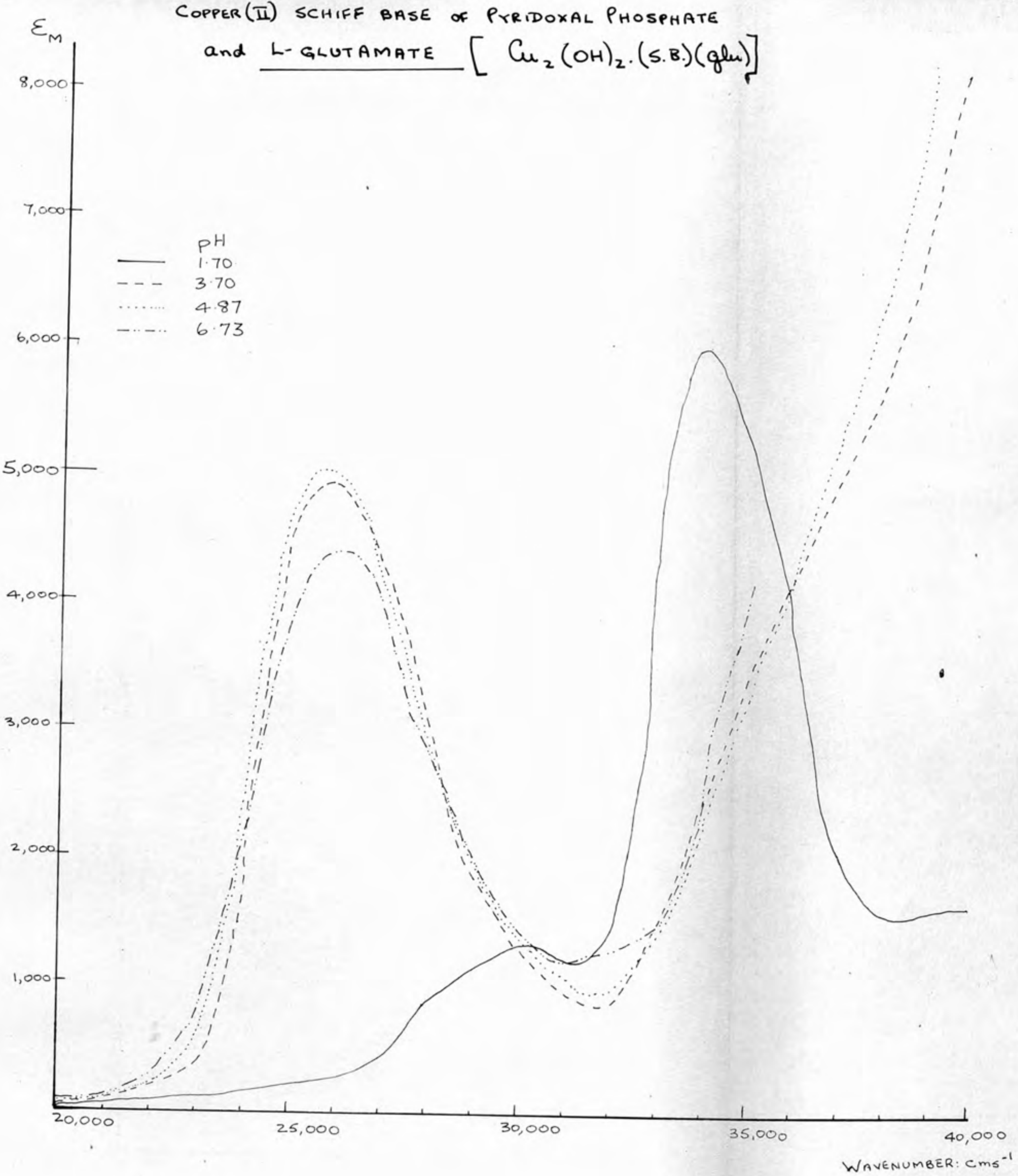
The ultra violet and near ultra violet spectra were measured on the Unicam SP 800 recording spectrophotometer. Cells used were 10mm. silica cells, with the various acetate buffers in the reference cell. The frequency range studied was 20,000-40,000cms^{-1} and the pH range was from 1.6 to 6.7. The buffers used were acetate buffers, adjusted with hydrochloric acid, (see Appendex I). The concentration of the complexes were generally kept at 1-2 x 10^{-4}M. Molecular weights were those assumed in the analytical section.

SCHIFF BASE OF PYRIDOXAL PHOSPHATE
and L-GLUTAMIC ACID

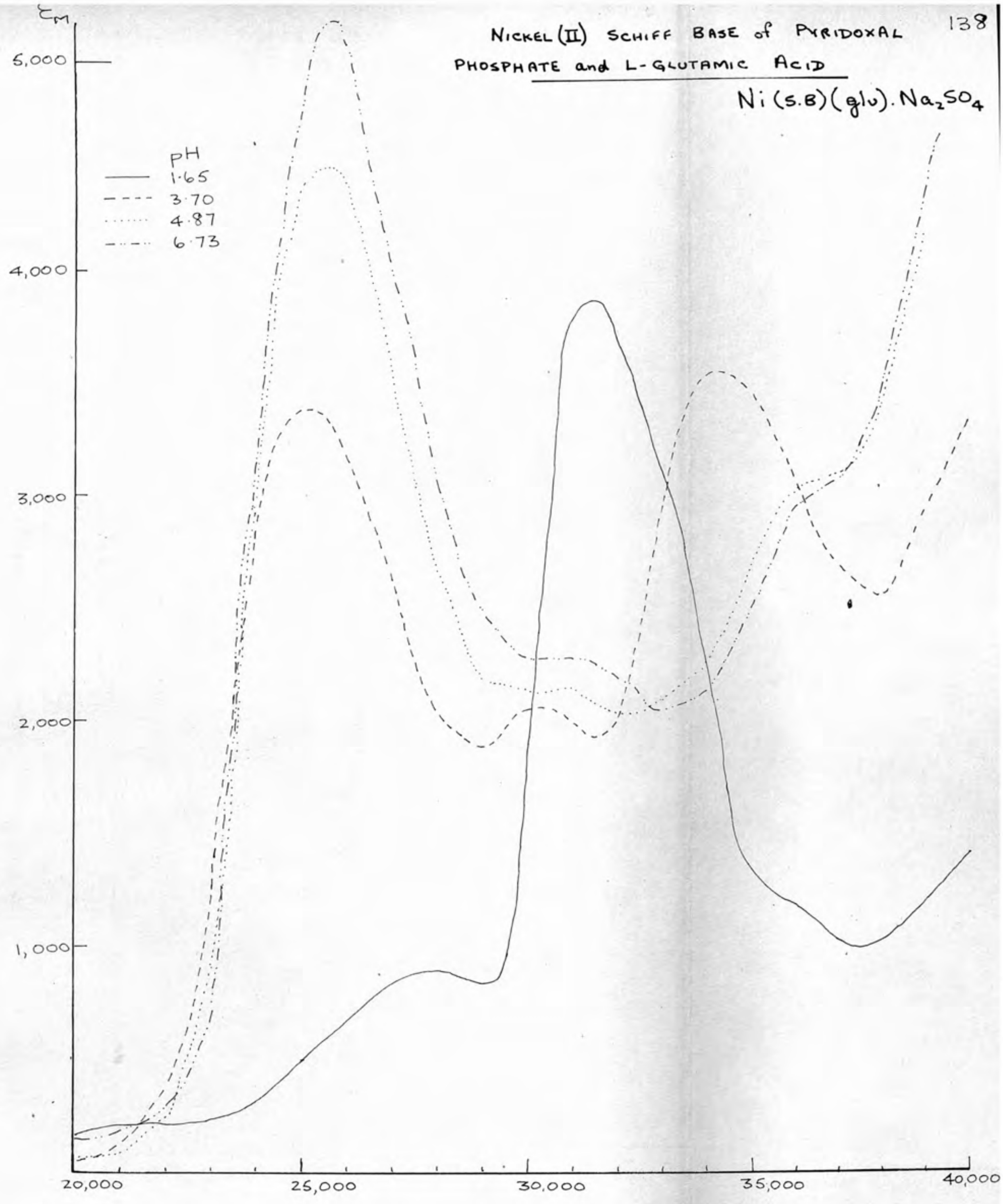


GALLIUM (III) SCHIFF BASE of PYRIDOXAL PHOSPHATE and L-GLUTAMIC ACID





COPPER(II) SCHIFF BASE OF PYRIDOXAL PHOSPHATE and L-GLUTAMATE [$Cu_2(OH)_2.(S.B.)(glu)$]

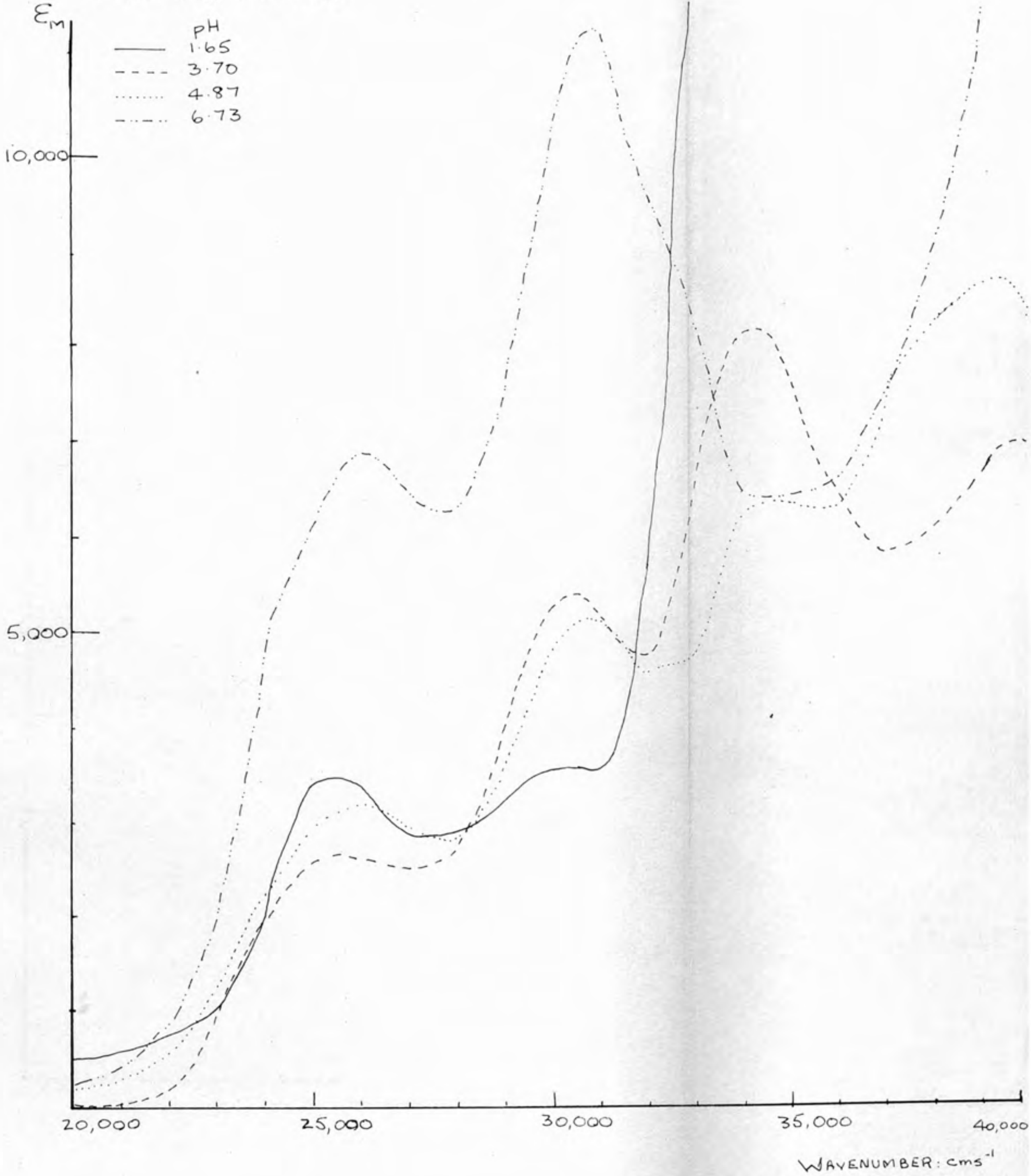


NICKEL (II) SCHIFF BASE of PYRIDOXAL PHOSPHATE and L-GLUTAMIC ACID

$Ni(S.B)(glu).Na_2SO_4$

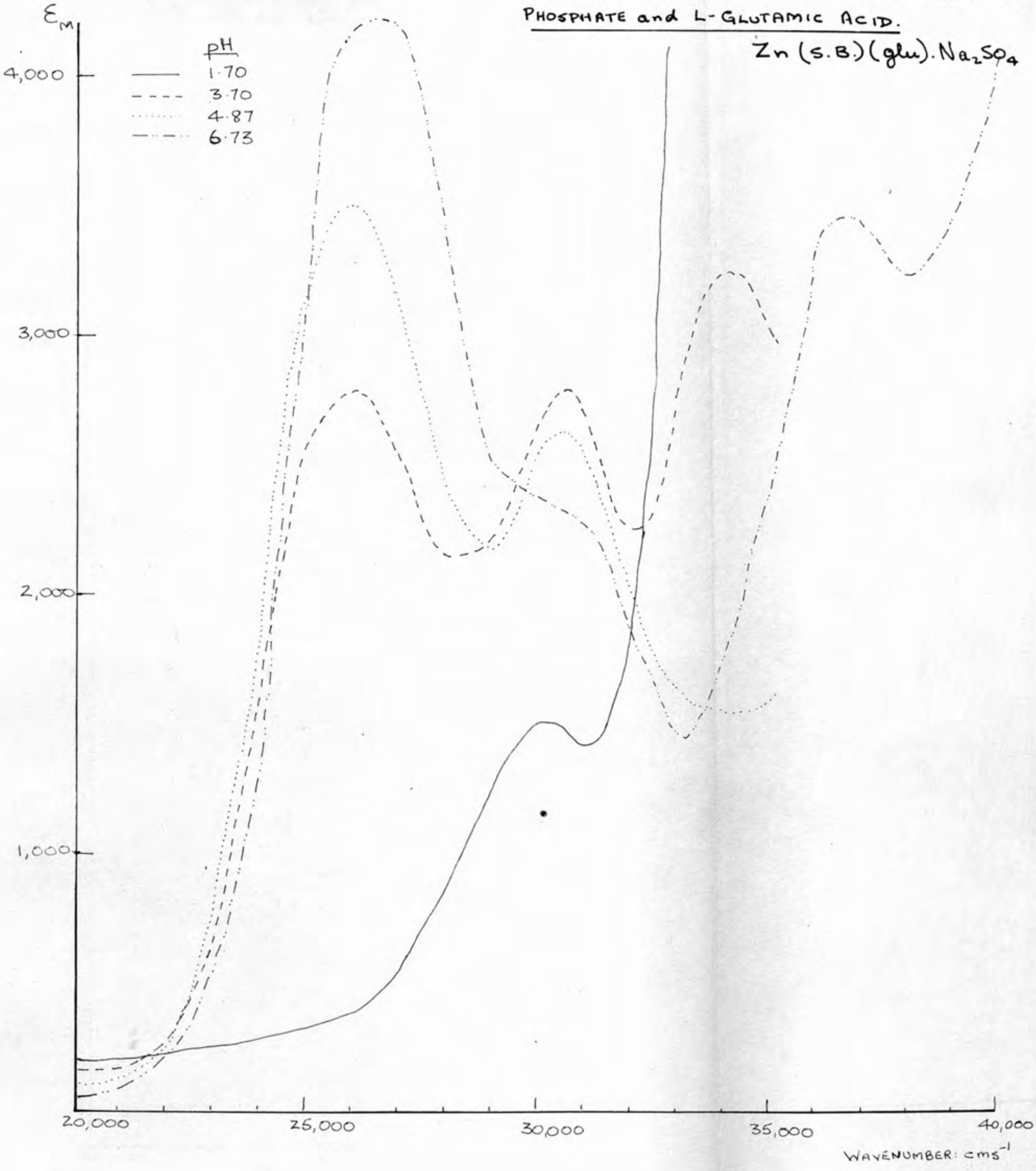
COBALT(II) SCHIFF BASE of PYRIDOXAL PHOSPHATE and L-GLUTAMIC ACID

$Co_2(OH)(glu)(S.B.)_2$

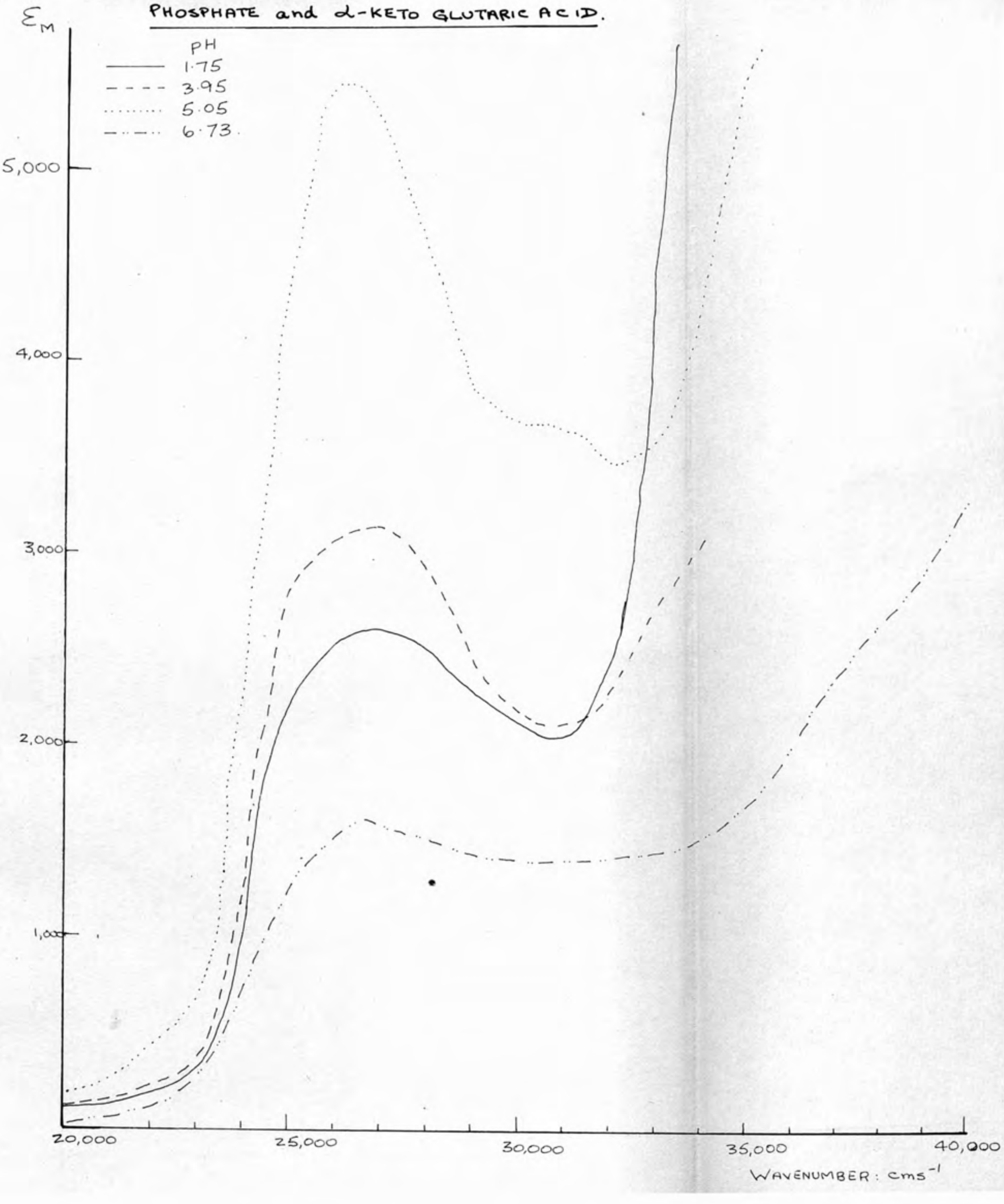


ZINC(II) SCHIFF BASE of PYRIDOXAL PHOSPHATE and L-GLUTAMIC ACID.

$Zn(S.B.)(glu).Na_2SO_4$



COPPER(II) SCHIFF BASE of PYRIDOXAMINE PHOSPHATE and α-KETO GLUTARIC ACID.



NICKEL (II) SCHIFF BASE of PYRIDOXAMINE PHOSPHATE and α-KETO GLUTARIC ACID

$Ni_2(OH)_2(S.B.)(glu) \cdot Na_2SO_4$

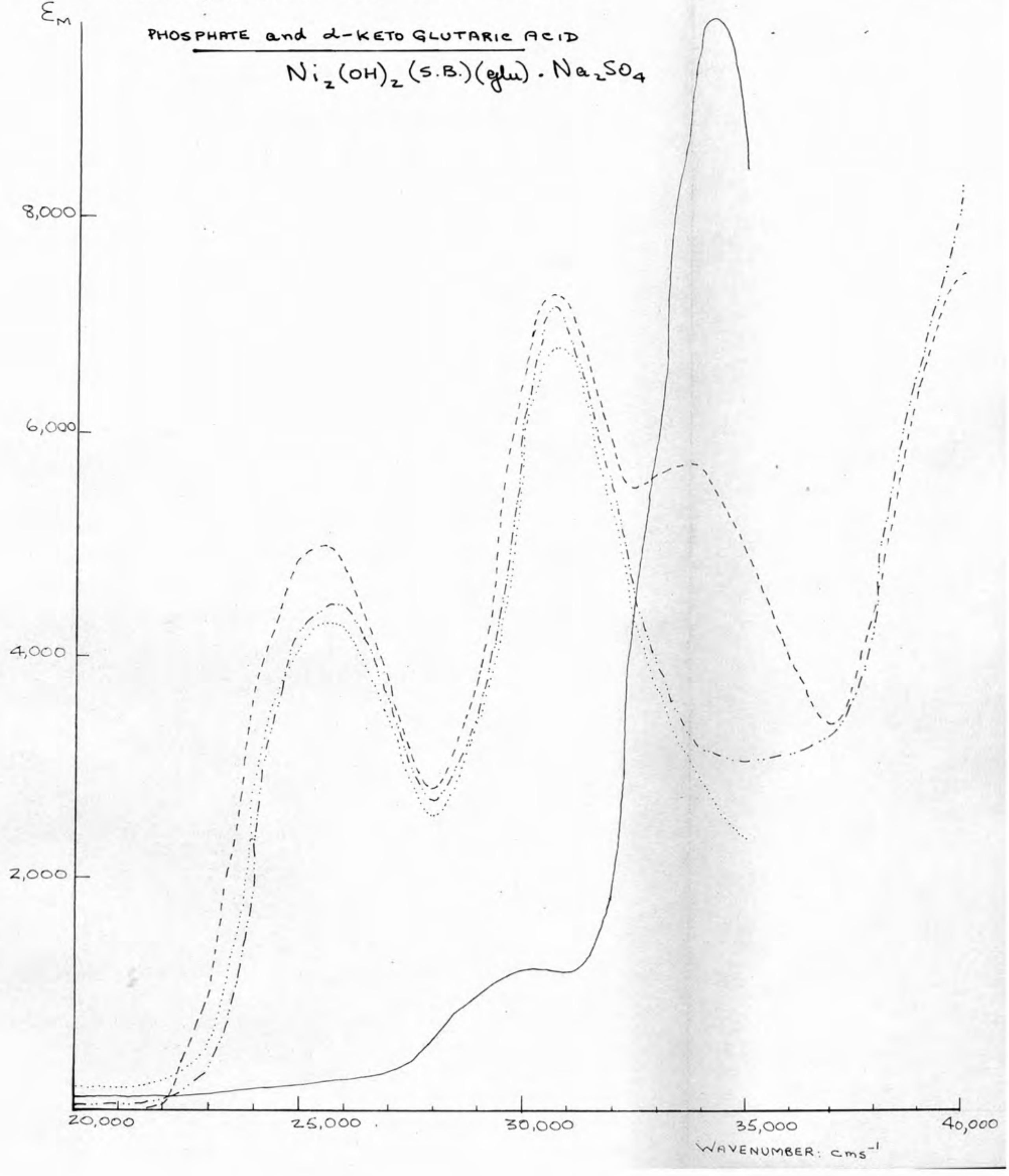


TABLE 29

Variation of ε_M with pH

Acetate buffer

Pyridoxal-5'-phosphate

pH.	maxima cms^{-1}	ε_M
1.94	29,600	1207
	34,000	6088
3.96	25,850	3507
	30,000sh.	1998
	33,800	2813
5.02	25,800	4842
	30,500sh.	2262
5.99	25,800	4955
	30,500sh.	2390

Pyridoxamine-5'-phosphate

pH.	maxima cms^{-1}	ε_M
1.88	34,100	10,807
4.02	30,700	8251
	34,000sh.	4300
	39,500	4532
5.11	30,700	9995
	39,500	5462
5.99	30,700	10,343
	39,500	5462

TABLE 3a

ABSORPTION MAXIMA OF THE SCHIFF BASES AT VARIOUS pH's.

Solution spectra of the metal chelates of the pyridoxal phosphate-glutamate schiff base.

Schiff base complex.	pH of buffer	Absorption maxima (cms^{-1}) $\pi-\pi_1^*$ aldimine	$\pi-\pi_1^*$ ketimine	$\pi-\pi_2^*$
PLP-glutamate	1.65			34,100
	3.70		30,800	34,100
	4.87		30,800	34,100
	6.73		30,800	35,000
Gallium^{3+}	1.65	26,600		34,100
	3.70	26,500		36,400
	4.87	26,500		34,200
	6.73	26,300		34,500
Copper^{2+}	2.01	26,500		34,300
	3.95	26,000		
	5.05	26,600	31,000	
	6.73	27,500		
Copper^{2+}	2.01	26,000		34,000
	3.70	25,800		
	4.87	25,800		34,000
	6.73	26,200		35,700
Nickel^{2+}	1.87		30,000	34,000
	3.95	25,500		34,500
	5,05	25,800		34,700
	6.73	25,800		
Nickel^{2+}	1.87		31,500	
	3.70	25,200		34,100
	4.87	25,700	30,500	
	6.73	25,800	30,500	
Zinc^{2+}	1.87		30,200	34,100
	3.95	25,900	30,500	34,000
	4.87	25,900	30,500	
	6.73	25,800	30,800	36,500

Schiff base complex.	pH of buffer.	Absorption maxima (cms^{-1}) $\pi-\pi_1^*$ aldimine	$\pi-\pi_1^*$ ketimine	$\pi-\pi_2^*$
Co^{2+}	1.87	25,500		34,300
	3.95	25,700	30,400	34,000
	5.05	25,800	30,800	34,500
	6.73	26,000	30,800	
Al^{3+}	1.88	27,000	33,700	
	3,90	27,100	33,500	
	4.96	27,000*		
	6.95	25,200	31,000	
Fe^{3+}	1.88		33,900	
	3.90		33,900	
	4.96))virtually insoluble. Sol. < ·1mg/ml. 6.95)			

TABLE 31

Solution spectra absorption maxima of metal chelates of the pyridoxamine phosphate - α-ketoglutaric acid Schiff base.

Schiff base complex.	pH of buffer.	Absorption maxima:cms^{-1}		
		$\pi-\pi_1^*$ aldimine	$\pi-\pi_1^*$ ketimine	$\pi-\pi_2^*$
Cu^{2+}	1.75	26,000		34,200
	3.95	26,400		34,100
	4.99	25,900	31,000	
	6.73	26,800		
Ni^{2+}	1.87		30,200	34,100
	3.95	25,500	30,800	33,600
	5.05	25,700	30,700	
	6.73	25,800	30,700	
Co^{2+}12	1.87	25,500		34,200
	3.95		30,000	34,100
	5.05	26,200	30,700	35,200
	6.73		30,700	

Schiff base complex.	pH of buffer.	Absorption maxima (cms^{-1})		
		$\pi-\pi_1^*$ aldimine	$\pi-\pi_1^*$ ketimine.	$\pi-\pi_2^*$.
Co^{2+}36B	1.87			34,500
	3.95	25,700	30,800	34,200
	4.87	26,500	30,800	
	6.73	26,800	30,700	
Al^{3+}	1.88			34,000
	3.90		30,600	(33,400 (39,500
	4.96		30,600	39,400
	6.95		30,700	39,300
Fe^{3+}	1.88			34,000
	3.90		30,500	33,800*
	4.96		30,500	
	6.95		30,600	39,000
Ga^{3+}26	1.88	26,700		34,200
	3.90	26,200*	30,600	39,200
	4.96	26,400	30,700	36,500*
	6.95	26,000	30,700	
42	1.88	26,300		33,700
	3.90	25,900	30,500	(33,400 (39,300
	4.96	25,800	30,500	39,400
	6.95	26,000	30,700	39,500
Zn^{2+}22	1.88		30,200*	34,200
	3.90		30,600	33,500* 39,300
	4.96		30,600	39,400
	6.95		30,700	39,600
49	1.88		30,200*	34,000
	3.90	25,200*	30,600	33,500* 39,200
	4.96	25,500*	30,500	39,300
	6.95	25,000*	30,700	39,500

Discussion on solution spectra.

Bands in the pH range 1.8-6 have been measured for the pyridoxal-5'-phosphate and pyridoxamine-5'-phosphate. Both molecular extinction co-efficients and absorption maxima are in good agreement with literature values[117].

The pyridoxal phosphate-glutamic acid Schiff base has been studied in the same pH range, and the various ionic species present-cationic and neutral- have been noted. It is interesting to note that the band at 34,000cms^{-1}, assigned by Martell[37] to the neutral non-polar Schiff base, is present been in acidic media. The intensity of the peak appears to 'decrease with increasing pH. This fact would tend to contradict Martell's assumption[37] unless it is that this band is due in part to free pyridoxamine phosphate. However, a shoulder does appear at 31,000cms^{-1} which would indicate that the complex is not completely dissociated. The Schiff base could break down in two possible ways: i)into the constituent pyridoxal phosphate and racemized amino acid.

ii)transamination could occur, in which case the product would be pyridoxamine phosphate.

In the case of the metal-Schiff base complexes of pyridoxal phosphate, the band around 25,000-26,000cms^{-1} which has been assigned to the imine π-π^*_1 band of the Schiff base[37], is considerably enhanced when compared to the band in the Schiff base. In all but the gallium complex, the band appears to be rather weak, and almost absent in the low pH range around 1.6-1.8. In the pH range of 3.7-4.87 the molar extinction co-efficient increases with increasing pH and around 6-7, ε_M decreases again to a small extent. This would seem to imply that around pH5-6, there is a change of ionic species as would have been expected, since the pk$_a$ of the Schiff base is expected to be around 6.

Absorption maxima occurring in the 30,000cms^{-1} region are thought to be attributable to the $\pi - \pi^*_1$ transition of the ketimine form of the Schiff base. Only the gallium complex is anomolous in that there is an absence of this band in this pH region (1.65-6.73).

Rate constants for the acid hydrolysis of the imine band appear below. They appear to be first order with respect to "imine" concentration, at ν =26,300cms^{-1}, (the wavelength at which Matsuo[18] determined stability constants of the Schiff base metal complexes).

Rate constants k (sec^{-1}) at 26,300cms^{-1}. temp = 25°C

pH = 1·70

Schiff base	Cu^{++}	Ni^{++}	Zn^{++}	Fe^{3+}	Al^{3+}	Ga^{3+}
1.8 $\times 10^{-4}$	14.6 $\times 10^{-4}$	0.249 $\times 10^{-4}$	0.160 $\times 10^{-4}$	5.14 $\times 10^{-4}$	0.461 $\times 10^{-4}$	2.59 $\times 10^{-6}$

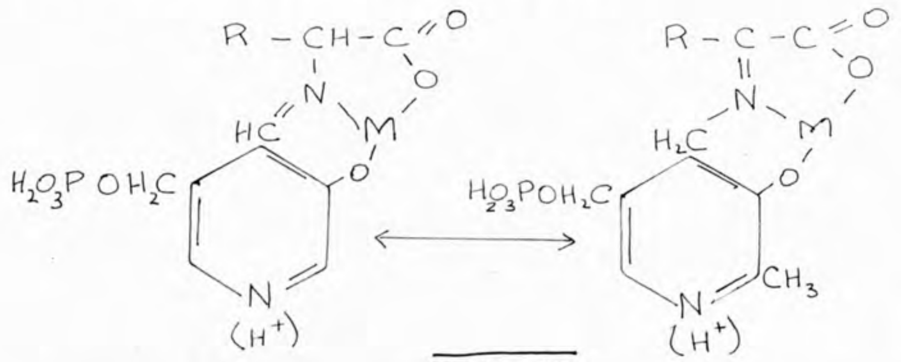
where $k = \frac{dc}{dt}$ (c = conc. measured in optical density units)
(t = time in secs.)

The rates of hydrolysis of Schiff base-metal complexes in acid solution are in the order:

$$Cu^{2+} > Fe^{3+} > Ga^{3+} > \text{Schiff base} > Al^{3+} > Ni^{2+} > Zn^{2+}$$

Shifts in the absorption maxima can. therefore, be correlated with increasing pH and change in ionic species. Christensen[9] proposed the intermediate species of aldimine-ketimine tautomers shown in table 9 (p.32) at 26,550 and 23,850cms^{-1} with subsequent hydrolysis products at 35,100-36,000cms^{-1} which would agree with results obtained for the acid catalysis of the metal-Schiff bases.

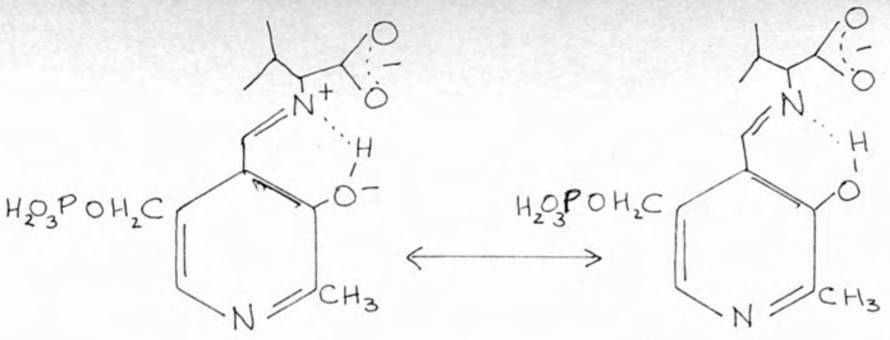
The metal Schiff bases derived from the pyridoxamine-α-ketoglutaric acid Schiff bases have similar absorption maxima to the first-series considered. However, the 26,500cms^{-1} band, together with the band around 34,000 cms^{-1}, appears in the pH region 1.6-1.8, compared with bands in the 30,000cms^{-1} and 34,000cms^{-1} in the first Schiff base series. The facts represent a shift from the ketimine to the aldimine Schiff base, which is apparently promoted by acid conditions. The overall reaction would then appear to be:-



XXXVII

Clearly, the protonation of the pyridine-nitrogen will therefore shift the bonds to longer wavelengths in the ketimine Schiff base, but to shorter wavelengths in the aldimine. Therefore, an additional shift to shorter wavelength, caused by chelation of the Schiff base to the metal ion, will sufficiently stabilise the system so that the aldimine/ketimine forms are interconvertable. Large shifts to shorter wavelengths also indicate the predominance of the aldimine form of the Schiff base. The nickel Schiff base of pyridoxamine phosphate and α-keto glutaric acid does however, seem to simulate its anolog of the pyridoxal phosphate-glutamic acid Schiff base, in that at pH 1.9 there is no band at 25-26,000cms^{-1} but a band at 30,000 (assigned to the ketimine $\pi-\pi_1{}^*$ band). As previously suggested, nickel does not appear to trap the aldimine as effectively as the copper or gallium.

The anionic form of the Schiff base (where the phenolic group has been ionised) was quoted by Martell to occur at 28,570cms^{-1} with the single band only, for the pyridoxal phosphate-valine Schiff base. No band was encountered in this region in any of the metal chelates or the Schiff base, in the pH range investigated. Therefore, the only possible species occurring are those as seen in the species XXXVIII



23,920 / 38,460 cms^{-1}

29,410 cms^{-1}

XXXVIII

Protonation will shift maxima to shorter wavelengths (i.e. to larger wavenumbers).

ELECTRONIC SPECTRA in the VISIBLE REGION.

Experimental

Both solid state and solution spectra were measured on the Unicam SP700 recording spectrophotometer. Those in the solid state were carried out using the SP735 solid state attachment, with freshly prepared magnesium oxide in the reference beam. The solution spectra were recorded using 3 cm. silica cells, against buffer solutions. The pH was adjusted to approximately 7 with acetate buffer and excess sodium glutamate.

TABLE 32

VISIBLE BANDS of the METAL-PYRIDINE COMPLEXES general formula: $MCL_2.2py$

Absorption maxima:cms^{-1} (Rel. abs.)

Metal state	Copper	Nickel	Cobalt	
			pink	blue
solid state	22,600 (0.390)	25,600 (0.246)		22,600
	17,100 (0.32)	17,400 (0.183)	19,733 (0.294)	17,100
			18,800 (0.291)	
		8,667 (0.154)	8,733 (0.115)	7,200

TABLE 33

VISIBLE BANDS of the METAL-PYRIDOXAL-5'-PHOSPHATE COMPLEXES general formula: $M^{2+}(PLP)_n$ where n=1,

Absorption maxima:cms^{-1} (Rel. abs.)

Metal	Copper	Nickel	Cobalt
solid state spectra	24,667 (1.410)	24,333*′ (1.60)	
	12,533 (0.313)	13,000	18,933 (0.235)
		8,150 7,933	8,250*

TABLE 34

VISIBLE BANDS of the METAL-PYRIDOXAMINE-5'-PHOSPHATE COMPLEXES general formula:$M^{2+}(PAMP)_n$ n=1,

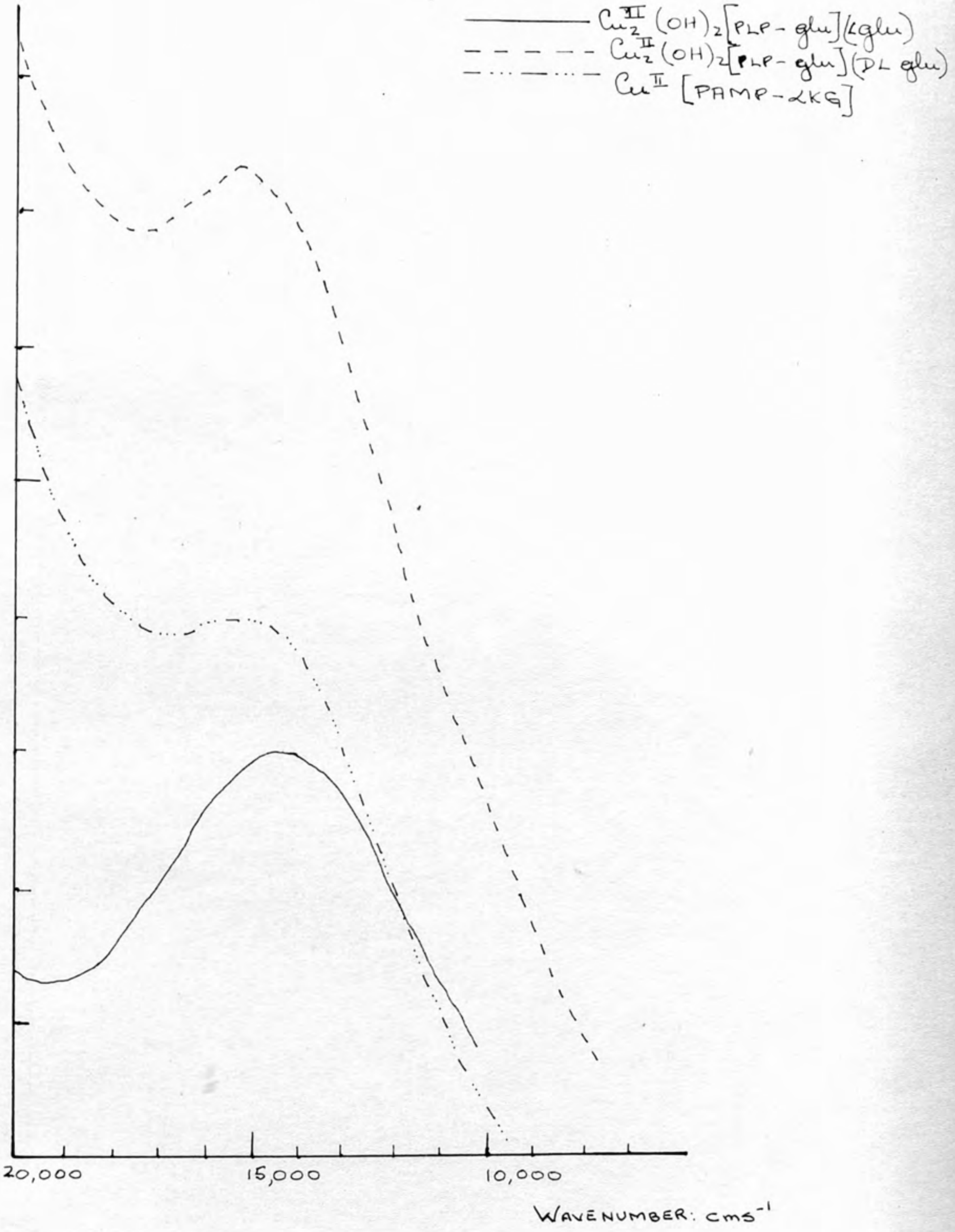
Absorption maxima: cms^{-1} (Rel. abs.)

Metal	Copper	Nickel	Cobalt
Solid state spectra		25,200*′ (0.597)	
	14,210 (0.503)	15,050 (0.174)	18,333 (0.271)
			17,133 (0.505)
		9,260 (0.177)	5,800 (0.165)

*′ denotes shoulder

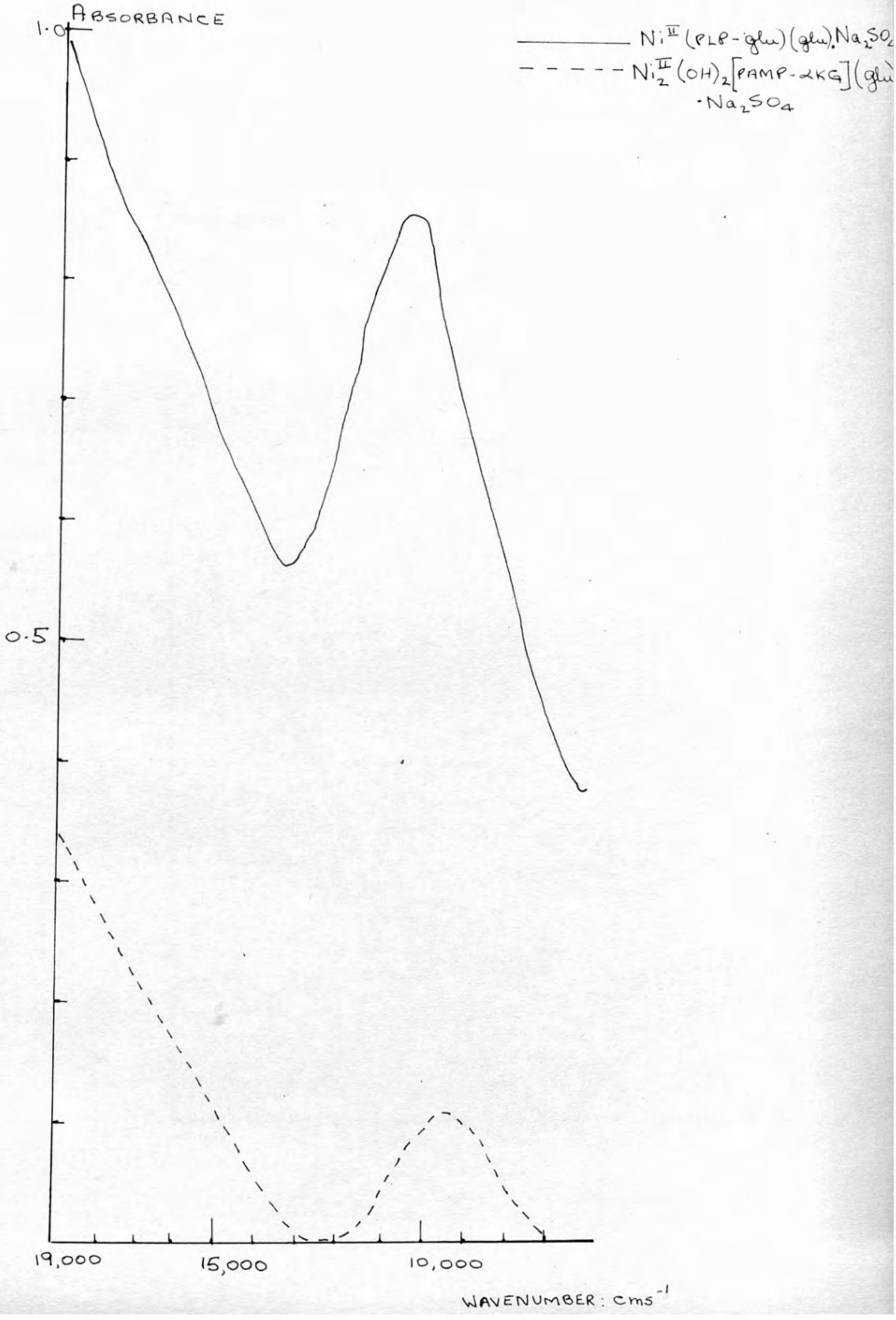
SOLID STATE SPECTRA

UNICAM SP 700 + SP 735
Reference: MgO



SOLID STATE SPECTRA

UNICAM SP 700 + SP 735
Reference: MgO



SOLID STATE SPECTRA

UNICAM SP 700 + SP 735
Reference: MgO

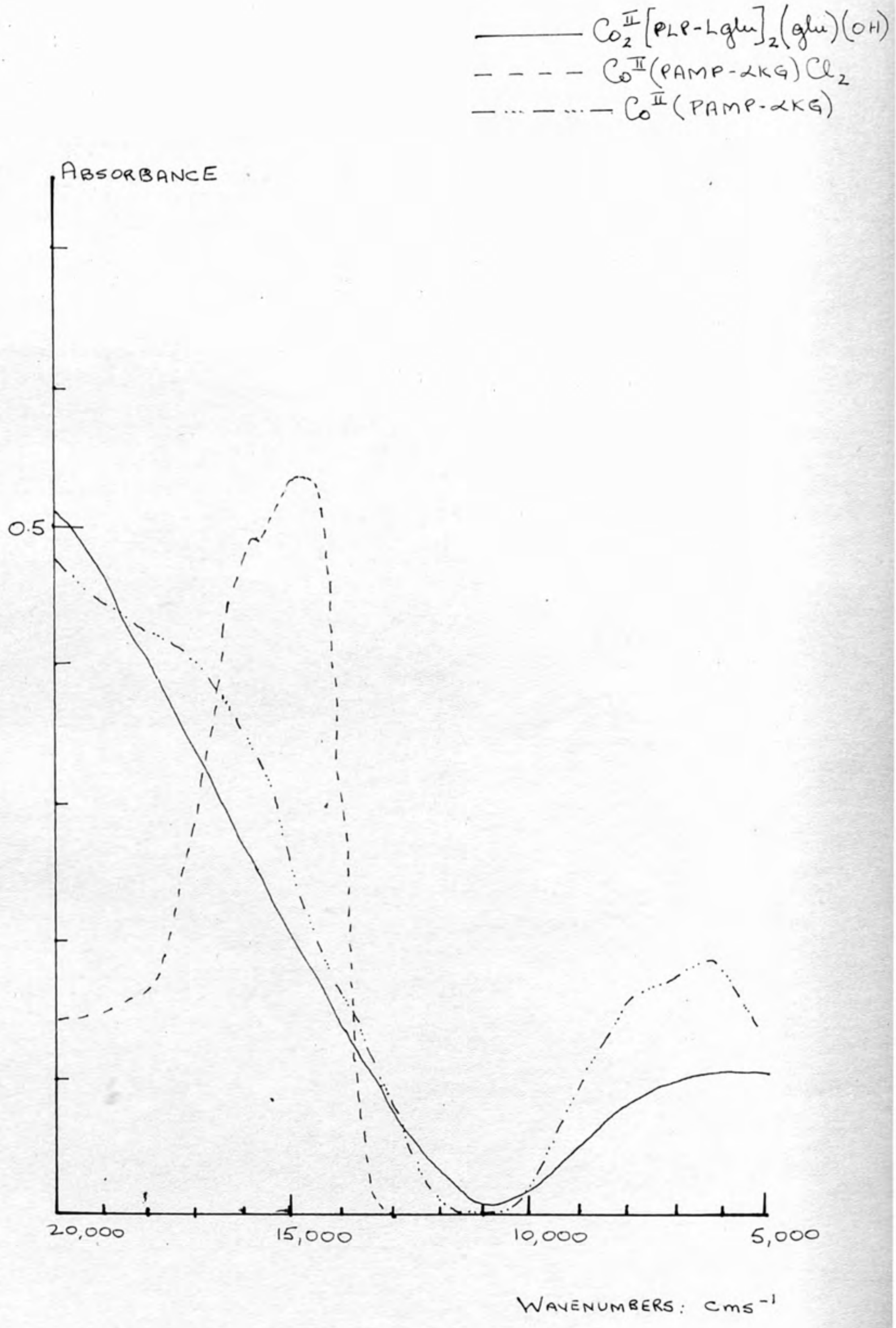


TABLE 35

Electronic visible spectra of Schiff base molecules

Copper compounds	solid state cms^{-1}	neutral buffered. (ε_M)
SCHIFF BASE SERIES 1	14,800	14,300 (39)
	14,714	
	15,133	14,800 (39)
SCHIFF BASE SERIES 2	15,400	14,800 (148)
SCHIFF BASE SERIES 1	17,000	
Nickel compounds		
SCHIFF BASE SERIES 1	26,714 10,500	16,600sh. 12,900sh. 10,300
SCHIFF BASE SERIES 2	25,667 9,267	16,600sh. 13,400 10,400
SCHIFF BASE SERIES 1	25,667 10,333	17,000sh. 13,300 10,400
Cobalt compounds		
6SB$_1$	only shoulders	—
12SB$_2$	14,800	19,600 19,400
36 SB.2.	1900sh 18,400 17,000cms^{-1}	14,500

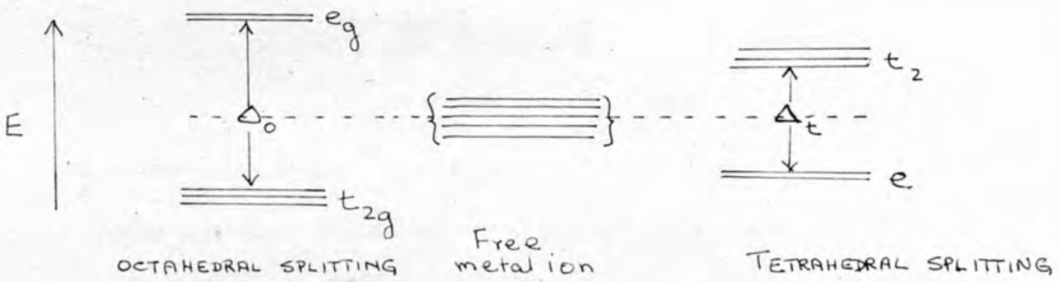
Visible electronic spectra.

Discussion:

General:

Electronic transitions for transition metal ions are expected in the visible region of the spectrum when d-d electron transfers take place. The nature of the ligands, the stereochemical configuration of the molecule and the multiplicity all effect the energy of the transition and their position of the absorption bands,

The most common stereochemistries for the metal ions are octahedral or tetrahedral. Splitting of the d-electrons shell into [redacted] subshells has been shown as following energy level diagrams[65]:



Any distortion from these two regular symmetry configurations will result in a probably splitting of the subshells and the rearrangement of the specific d-orbitals ($d_{x^2-y^2}$; d_{XY} etc.) Distortions of the octahedral configuration include that of the tetragonal (lengthening of the d_z2 axis) and square configurations (where d_z2 ligands are removed to infinity). The John-Teller theorem[65] predicts distortions for unstable electronic states, which will lower the symmetry of the molecule and split the degenerate state. The best example of this is the Cu^{2+} ion.

Results:

(a) Copper (Cu^{2+})

The most common structures for copper are square and distorted octahedral. However, in the case of the copper

(II) (pyridoxylidene-valinate)[23], the molecule has been found to be five co-ordinate with respect to copper, with a square pyrimidal configuration. One band only was expected for this copper complex case and it is found at 14,060cms^{-1}. According to Belford[118] this would seem to imply a $d_{xy} \rightarrow d_{x^2-y^2}$ transition, and the base part of the square pyridimid would therefore seem to be extremely planar. The splitting of the energy levels would be as follows:

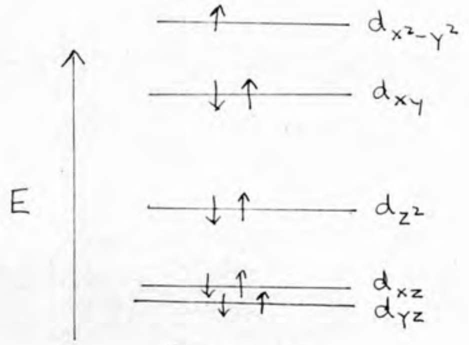


FIG. 28

The electron transition can then be assigned to $^2T_{2g} \rightarrow {}^2E_g$.

Bands of the other copper complexes appear in the same region, but shifted slightly. This is expected because of:

1) the different substituent chain on the amino acid (imine).
2) the fact that the pyridine nitrogen might not be bonded to the metal (resulting in a more square planar molecule).
3) the phosphate group has not been expected to bond in the pyridoxal phosphate-aminc acid chelates, This co-ordination position could be occupied by either a water molecule or a second amino acid (keto acid) molecule.

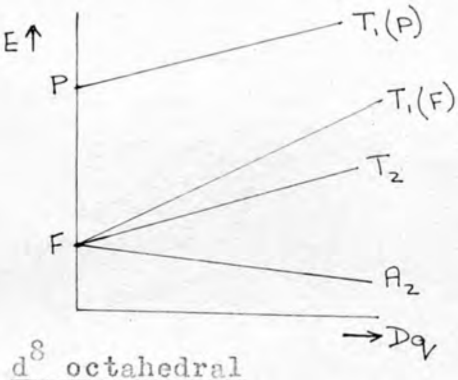
In view of these factors, the transition would still appear to be a $d_{xy} \rightarrow d_{x^2-y^2}$ and $^2T_{2g} \rightarrow {}^2E_g$.

The copper chloride-pyridine conplex is know to have a octahedral polymeric (single chain) structure[111] and the two bands have been found at 22,700 and 17,100cms^{-1} in the solid spectrum. These copper-pyridino complex absorption bands differ from both the Schiff base complexes and the pyridoxamine phosphate complexes spectra. The pyridoxal phosphate copper complex (with two donor oxygen atoms) has an absorption maximum in the red at 12,533 cms^{-1} whereas the cupric pyridoxamine phosphate complex has a band at 14,210cms^{-1}. This band at 14,210cms^{-1} is similar to the Schiff base transitions, because it has the same co-ordinating atoms (nitrogen and oxygen).

Solution spectra of the complexes exhibit a shift to the red in all cases, which may represent partial aquation.

Nickel:

Octahedral nickel has been found to occur most frequently in biological systems.



d^8 octahedral **FIG. 29**

Expected transitions[70,71] are the

$$^3A_{2g} \rightarrow {}^3T_{2g} \quad (9{,}000 - 11{,}000\text{cms}^{-1})$$
$$^3A_{2g} \rightarrow {}^3T_{1g(F)} \quad (14{,}000 - 19{,}000\text{cms}^{-1})$$
$$^3A_{2g} \rightarrow {}^3T_{1g(P)} \quad (25{,}000 - 30{,}000\text{cms}^{-1}).$$

In the solid state of the nickel Schiff base complexes, two bands have been found, the $^3A_{2g} \rightarrow {}^3T_{2g}$ and $^3A_{2g} \rightarrow {}^3T_{1g}(P)$ regions. The band due to the $^3A_{2g} \rightarrow T_{1g(F)}$ transition appears to be masked by the other two more intense bands. However, in solution, a split band appears in this region in all the Schiff base complexes. This would appear to substantiate the claim made by Balhausen[90] that it is an octahedral complex in the singlet state, in solution. He calculated the following estimated energies for the various transitions:

	cms^{-1}
$^3A_{2g} \rightarrow {}^3T_{2g}$	(10,000)
$\rightarrow {}^1E_{1g}$	(12,000)
$\rightarrow {}^3T_{1g(F)}$	(17,000)
$\rightarrow {}^3T_{1g(P)}$	(25,000)

Magnetic moment studies would therefore help to correlate whether the complexes do in fact, change the spin multiplicity on going into solution.

Cobalt (II)

The d^7 electronic configuration of the Co^{2+} ion, in its high spin form, gives rise to a quartet state[65], with a Jahn-Teller distortion due to the $t_{2g}{}^5$ configuration. Sacconi has studied the various configuration of Co^{II} salicylaldehyde Schiff bases and bands due to the various configurations are shown below:

Absorption maxima: cms^{-1}

cis octahedral	tetrahedral	distorted sq. pyrimidal.
15,600	18,000	16,800
11,500	11,200	11,400
9,300	7,700	6,700

Clearly then the position of the bands in the 15,500 - 18,000 cms^{-1} region will indicate the nature of the configuration of the molecule. The cobalt Schiff base of pyridoxal phosphate and L-glutamic acid shows no definite bands in this region although the strong absorption of the bands at band in the 24-25,000cms^{-1} could overshadow it and envelop it as a shoulder - around the 17-18,000cms^{-1} region. There is however, <u>no</u> band in the 11,000cms^{-1} region. The two Schiff bases of pyridoxamine phosphate and α-ketoglutaric acid appear to be rather different in the solid state. Both have fairly strong bands in the 16,000-17,000cms^{-1} region, but the No 36 has a definite specific split band typical of the tetrahedral configuration, whereas the No 12 complex seems to be cis-octahedral with two chlorine atoms. Suspected transitions for the cobaltous complex are :

	cms^{-1}
$^4T_{1g(F)} \rightarrow {}^4T_{2g}$	12,000
$\rightarrow {}^4A_{2g}$	18,000
$\rightarrow {}^4T_{1g}$	19,400

If one compares these bands with those of the pyridino-, pyridoxal phosphate- and pyridoxamine phosphate cobaltous complex, one finds that the pink cobalt-pyridino and cobalt pyridoxamine phosphate and cobalt - (PLP) have similar spectra which have been found to be tetrahedral whereas the blue cobaltous-pyridino complex has been confirmed to be octahedral tetragonal configuration.

SECTION III

GENERAL DISCUSSION and CONCLUSIONS.

General Discussion:

Analysis results of the metal complexes confirm the 1:1 ratio of metal ion to the Schiff base species. Further investigation of analysis showed that in the case of the first Schiff base series (pyridoxal phosphate and L-glutaric acid) that there was at least one amino acid per metal ion, as well as the Schiff base molecule. With the exceptions of the nickel and zinc complexes, this Schiff base-metal complex series favoured a dimeric-bridged structure. The second Schiff base series (pyridoxamine phosphate and α-keto glutaric acid Schiff base) would appear to favour a monomeric structure with the metal ion whereas the PLP-glutamate Schiff base favoured the dimeric structure, These isolated metal complexes differed from previous structures quoted[20,23,67], and those assumed to occur in solution. Metal ions, such as nickel and zinc, cobalt, and aluminium (to small extent), which are reported to have a reduced catalytic power, compared to copper, appear to precipitate as sodium salts, or acid salts. It is doubted that the Na_2SO_4 or H_2SO_4 is an impurity, but the nature of bonding is unknown.

Infra-red analysis of the complexes confirm the presence of the C=N imine vibrational band, and in several complexes, the phenolic C-O$^-$ stretch frequency. Bridging groups such as hydroxyl and sulphate radicals could not be detected, owing to the high absorption of phosphate group, in the same region as the sulphate and hydroxyl absorptions would have been expected.

Ultra violet spectroscopy provided the most powerful tool in this research; the phosphate group absorption in this region did not interfere with bands due to the pyridine ring system, the imine double bond absorption and the visible d-d electronic transitions of the metal ion. The solid state spectra was of invaluable assistance in determining the exact ketimine-aldimine characteristic of the complex, and also the stable electronic stereochemistry of the metal ions. Solution spectra indicated that the trapping by the metal ion promoted the concentration of the Schiff base species (predominantly the aldimine tautomer, as indicated by the absorption band at 25,000 - 26,000cms^{-1}). The dissociation of the Schiff base complexes was confirmed to be acid catalysed especially in the region of 25,000 - 26,000cms^{-1} where the aldimine species predominated. The shift of the absorption band to the 30,000 cms^{-1} region could indicate that the initial change was from aldimine to ketimine, followed by hydrolysis of the ketimine band. This mechanism agrees with that proposed by Metzler[15].

The visible electronic spectra of the copper, nickel and cobalt complexes exemplified the probable stereochemical shapes of the metal ions. The copper complexes all exhibited an absorption band in the 14,000 - 14,500cms^{-1} region, and this according to Belford[118] is due to the $d_{xy} - d_{x^2-y^2}$ transitions and would substantiate the square-planar form of the copper complex, specifically underlined by the dimeric copper square-planar bridging. Proposed structures for the nickel complexes are four-coordinate (either square planar or tetrahedral). The square planar structure would maintain essentially planar structure of the ring system attaching itself to the metal ion, and this structure is therefore thought to be more probable. Possible bonding of the amino acid carboxylate group to the metal ion is thought unlikely as this would involve a five-co-ordinate metal ion and consequent splitting of the d-orbitals; This has not been observed. The dimeric, four-co-ordinate structure of the cobalt complexes indicate an overall square planar configuration. However, the Schiff base of pyridoxamine phosphate and α-keto glutaric acid appears to have a tetrahedral structure, from its visible electronic spectrum.

Conclusions:

The phosphate group of the PLP and PAMP would appear to block any type of bonding which accurred between the 5-methoxy group and the metal ion, as in the case of the Cu(II)-pyridoxylidene valinate[23]. However, if there are vacant co-ordination positions on the metal ion, these could be filled by the bonding of the pyridinium nitrogen to the metal ion. This factor of the phosphate group would seem to be the only factor which influences the different bonding and symmetry of the stereochemistry of the metal ions. Further studies are needed in the fields of; a)molecular weight determinations (in fields other than mass spectrometry.)

b)theoretical considerations; where symmetries of the molecules should be considered (after structure determination) and possible vibrational modes computed.

c)X-ray powder photographs, to determine the absolute structure of the metal complexes.

The study of metal complex formation in biological systems poses many interesting problems, both chemical and physiological. The choice of the pyridoxal phosphate-pyridoxamine phosphate system was chosen for the present work because the PLP-PAMP system appears to similate the body-enzyme function with the peptide-amino acid.

APPENDIX I

BUFFERS.

pH range 1.6 to 6.73.

50ml. N-sodium acetate + Xml. H-HCl made up to 250ml.

pH.	X(ml.) HCl	pH	X ml. HCl
1.65)) 2.01)	52.5	4.87)) 5.10)	15.0
3.70)) 3.97)	40.0	6.73	0.25

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APPENDIX II

ABBREVIATIONS.

PLP	pyridoxal-5'-phosphate
PAMP, PMP	pyridoxamine-5'-phosphate
PL	pyridoxal
S.B.	Schiff base
I.R.	Infra-red
tren	tris(2-aminoethyl)amine
py	pyridine
def.	deformation
glu	glutamic acid
αKG	α-Keto glutaric acid.

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