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Reactions Involving Pyridoxal Phosphate or Pyridoxamine Phosphate in the Presence of Metal Ions. Part II. Complexes with Bivalent Metal Ions

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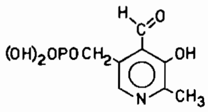
Complexes containing Pyridoxal Phosphate (PLP) or Pyridoxamine Phosphate (PMP) have been prepared and those with the following empirical formulae are reported: CuPLPO, CuPMPO, ZnPLPO, $ZnPMPOOH_2$, $CoPLPOOH_2$, $CoPMPOOH_2$. In no case is the pyridine ring involved in coordination. The complexes are insoluble, and appear to be polymeric, bridging involving the 5′ phosphate group.

Introduction

The aldehyde form of vitamin B_6, pyridoxal phosphate, PLP (I), is the co-factor for a number of enzymic reactions in the metabolism of amino acids[2]. The formyl group reacts with amino acids to form Schiff bases, which can then undergo a variety of reactions: transamination, racemization, deamination. Systems containing PLP or its analogues, amino acids, and metal ions have been used as enzymic models,[3,4] particularly in the case of the transamination reaction. This last reaction involves three steps: Schiff base formation, prototropic shift from aldimine to ketimine Schiff base, and finally hydrolysis of the latter to give pyridoxamine phosphate, PMP (II).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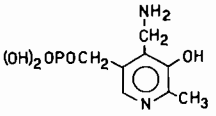

(II)

The mechanism of the metal catalysed reaction proposed by Snell and Metzler[2,3,5] implies the existence of both aldimine and ketimine Schiff base metal complexes. Metal complexes of PLP, of PMP, and of their derived Schiff bases have been shown to exist in solution and in the solid state. Work using PLP has elucidated equilibrium constants for some PLP–Zn(II)–amino acid systems,[6] and some kinetic data for these systems has been reported.[7] Recently equilibrium and kinetic data for the Zn(II)–pyruvate–PMP system have been published.[8]

In an attempt to characterise the species present in such reaction mixtures we have isolated and investigated the properties of bivalent metal complexes of PLP, PMP and their derived Schiff bases. In this note we present the results of studies of simple complexes of PLP and of PMP with bivalent metal ions. The compounds proved to be insoluble, intractable and probably polymeric, no crystallographic data could be obtained.

Experimental

Preparation of the Complexes

Complexes of PLP

5 mmol of the metal chloride (AnalaR) was added to a solution of PLP (5 mmol) (Fluka) in the minimum of water. The pH was adjusted to 7 by the addition of sodium bicarbonate and the mixture was cooled in ice. The precipitated complex was filtered off; washed with water, ethanol, and ether. After drying at the pump the complexes were stored over P_2O_5. The products were insoluble in water, methanol, ethanol, benzene, dioxan, toluene, chlorinated hydrocarbons, pyridine, dimethylformamide and all other common solvents.

Complexes of PMP

5 mmol each of PMP · HCl (Fluka) and metal chloride (AnalaR) were added to 20 cm^3 of 50% aqueous dioxan. The mixture was heated to 60° C and then cooled in ice. The resulting precipitates were treated as before.

Spectroscopic Measurements

Infrared spectra were measured variously on Beckman IR 9 or Perkin Elmer 457 instruments or by PCMU Harwell. In the region 560–200 cm^{-1} nujol mulls and silver chloride plates were used; above 400 cm^{-1} spectra of mulls in nujol or hexachlorobutadiene between potassium bromide plates were recorded. In some cases spectra of KBr discs were obtained, but these proved unsatisfactory since exchange appeared to take place.

Ultra violet and visible spectra of freshly ground samples were run on a Unicam SP700 spectrophotometer fitted with the SP 735 solid state attachment, against freshly prepared MgO as reference.

Chromatographic Studies

Ascending paper chromatographs were prepared using Whatman No. 1 paper and butanol/acetic/water (12:3:10 by volume, upper layer) as solvent. The dried papers were sprayed successively with ninhydrin and then dithizone (0.05% in chloroform). After coloured spots had been marked, the chromatographs were viewed by ultraviolet light.

Magnetic Susceptibility Measurements

These were carried out on a Newport Variable Temperature Gouy Balance using $[Nien_3]S_2O_3$ as calibrant.[9] Diamagnetic corrections were estimated using Pascal's constants.[10] The susceptibilities are not corrected for TIP.

Results

Analytical Data

In work on the analysis of metal complexes of phosphorylated vitamin B-6 compounds we have found that the presence of the phosphate group interferes with carbon, hydrogen and nitrogen analyses. However, in the cases of the simple complexes both preparations and analyses are reproducible. Results for duplicate preparations are shown in Table I. Sodium and chloride were absent from all preparations.

The analytical results indicate that the complexes have a 1:1 metal to ligand ratio, and that one extra oxygen atom is required in the empirical formulae. The phenol group is ionized on coordination thus one further negative charge must be found amongst the ligands to give formal neutrality on coordination to a bivalent metal ion. Neutrality could be achieved either with an ionized phosphate group and the oxygen atom being present as neutral water; *or* by the presence of oxygen as hydroxide, and fully protonated phosphate.

Infrared spectra

Some indication of the structures of the compounds can be obtained from infrared data, although the molecules are too complicated for detailed analysis.

The infrared spectrum of pyridoxal (PL) has been discussed by Heinert and Martell,[11] who noted that in the solid state the C=O stretching band is absent due to the predominance of the hemi-acetal form. The suggested structured has been largely confirmed by investigation of the crystals.[12] The hydrochloride was shown to contain hemi-acetal and protonated ring nitrogen. Hemi-acetal formation is prevented by the presence of the 5'-phosphate group in PLP. In the dihydrate of PLP the ring nitrogen is reported[12] to be protonated by one hydrogen atom attached to the phosphate group, and the aldehyde group is hydrated and exists as a gem diol structure.

In PL there is no free OH stretching band,[11] but bands are assigned by Heinert and Martell at 3180–3100 cm^{-1} and at 2735 cm^{-1} to the chelated OH and intermolecular O–H · · · N bands respectively. PLP shows two sharp bands on the OH stretching region from the hydrated aldehyde at 3253 and 3420 cm^{-1}, which disappear on chelation to a metal. Bands at 1647 cm^{-1}

TABLE I. Analytical Data for Complexes of PLP and PMP.

Empirical Formula	Found %				Theoretical %			
	C	H	N	M	C	H	N	M[a]
CuPLPO	29.3	3.05	4.2	20.3	29.4	3.1	4.3	19.4
	29.4	3.0	4.2	19.7				
CuPMPO	29.2	3.9	8.5	19.1	29.3	4.0	8.55	19.4
	29.1	4.1	8.7	–				
ZnPLPO	29.8	3.5	4.3	19.6	29.2	3.1	4.3	19.9
	29.8	3.2	–	19.7				
$ZnPMPOH_2O$	26.7	4.4	7.2	199.2	27.6	4.35	8.1	18.8
	26.7	3.8	–	–				
$CoPMPOH_2O$	27.7	4.0	8.1	17.5	28.1	4.4	8.2	17.1
	26.4	5.0	8.5	–				
$CoPLPOH_2O$	29.7	3.5	4.3	18.2	28.8	3.6	4.2	17.7
	28.4	3.6	3.9	–				

[a] By atomic absorption spectrometry.

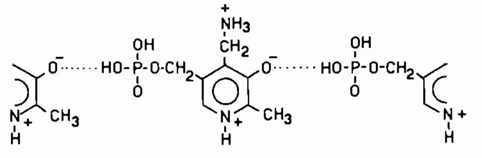
and 1560 cm^{-1} (probable C=C/C=N stretch) are changed in the complexes as follows: Cu, 1635 cm^{-1} and 1516 cm^{-1} (v. weak); Co, 1638 cm^{-1} and 1515 cm^{-1} (weak); Zn, 1661 cm^{-1} and 1536 cm^{-1} (v. weak).

Pyridoxamine 5′ phosphate hydrochloride (PMP HCl) shows only a shoulder at 3200 cm^{-1}, and no bands in the free O–H stretching region. A strong band is shown at 26,600 cm^{-1} whereas in PLP there is only a broad absorption in this region. The band at 26,600 cm^{-1} together with the weak absorption can be assigned to the N^+–H stretch of the protonated hetero-atom. Three bands are present in PMP HCl in the1500–1600 cm^{-1} region: at 1636, 1549 and 1509 cm^{-1}. The 1549 cm^{-1} band is likely to be the C=C/ C=N stretching which shifts on coordination to: Cu, 1531 cm^{-1}; Co, 1525 cm^{-1}; and Zn, 1530 cm^{-1}. The other two bands are assigned to the deformations of the R–NH_3^+ group. This coordinates as the NH_2 group and the metal complexes show bands at: Cu, 1600 cm^{-1}, Co, 1625 cm^{-1}, 1558 cm^{-1}; Zn, 1619 cm^{-1}. Thus in the 1500–1600 cm^{-1} region the bands may be assigned as in Table II. In the region 900–1200 cm^{-1} the spectra of the phosphorylated vitamin B-6 compounds and their derivatives are dominated by the bands from the phosphate group. The free phosphate ion, PO_4^{3-}, like sulphate, SO_4^{2-}, belongs to the symmetry group *Td*. The infrared spectra of the latter and its coordination complexes have been discussed from the point of view of the changes in symmetry on coordination.[13]

On bonding one of the oxygen atoms of the phosphate group its symmetry is lowered to C_{3v} and this would apply to the fully ionised 5′ phosphate group of the phosphorylated vitamin B-6 compounds. Addition of one proton to or coordination of such a phosphate group would lower the symmetry to *quasi* C_{2v}. Addition of a further proton in either case would "raise" the symmetry to *quasi* C_{3v}. In the free phosphate ion both stretching (ν_3) and bending (ν_4) are infrared active and triply degenerate. In symmetry C_{3v} each of these is split into two bands (A_1+E) and the $\nu_1(A_1)$ and $\nu_2(E)$ vibrations become i.r. active, thus giving six bands. When the symmetry is lowered to C_{2v} then the degenerate bands are split completely and the three components of ν_3 and $\nu_4(A_1+B_1+B_2)$ and the A_1 component of ν_2 become i.r. active, thus giving eight bands. In sulphate[13] and phosphate[14] ν_1 appears around 950–1000 cm^{-1}, ν_2 around 450 cm^{-1}, ν_3 in the 1050–1150 cm^{-1} region, and ν_4 in the region of 600–650 cm^{-1}. Interpretations of the spectra along these lines must be treated with caution since site symmetry is not taken into account.

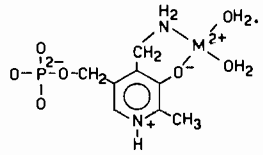
The infrared spectra in the P–O region of PLP, PMP HCl and their metal complexes are shown in Figures 1 and 2, and the bands assigned to the phosphate group are in Table III.

The band pattern of PLP is similar to that of sulphate in $[Co(NH_3)_5SO_4]^+$,[13] suggesting full protonation of the phosphate group. The band pattern in PMPHCl in this region is complicated. However, the presence bands due to $^+$N–H and NH_3^+ together with the absence of free stretching bands would lead to the structure III.



(III)

The i.r. spectra of the metal complexes $ZnPMPOOH_2$, $CoPMPOOH_2$ and $CoPLPOOH_2$ are nearly identical in the phosphate region, with the band pattern suggesting *quasi* C_{3v} phosphate group as in IV or V.



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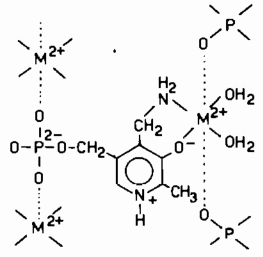


(V)

TABLE II. Bands (cm^{-1}) in the 1500–1600 cm^{-1} Region in Metal Complexes[a] of PLP and PMP.

ZnPLPO	CuPLPO	CoPLP	ZnPMP	CuPMP	CoPMP	Assignment
1661	1635	1638	1622	1600	1625	ν(C–C)
1536	1516	1515				ν(C–O)
			1533	1531	1538	$\delta(NH_2)$

[a] Formula as in Table I.

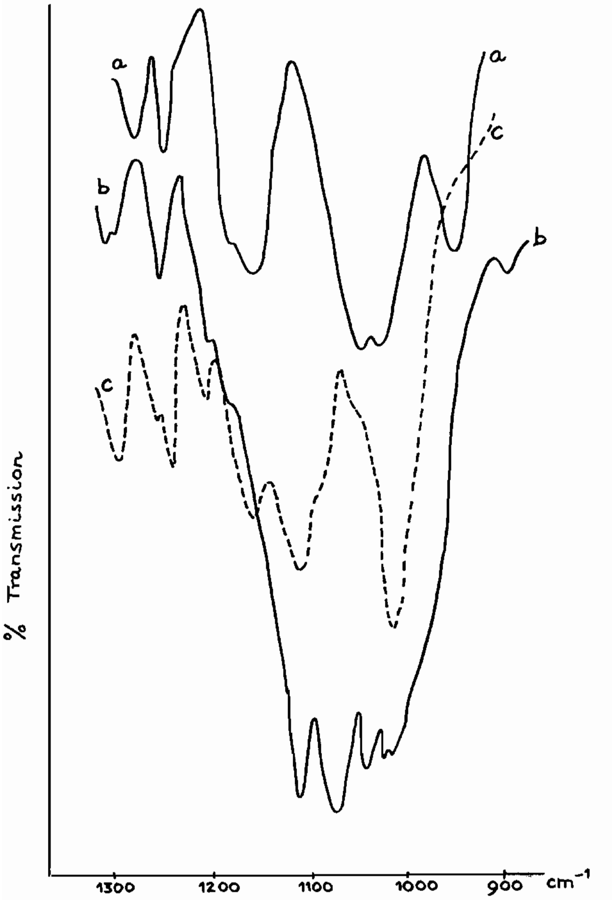


Figure 1. Bands in the i.r. spectrum of (a) PLP; (b) CuPLPO; (c) ZnPLPO.

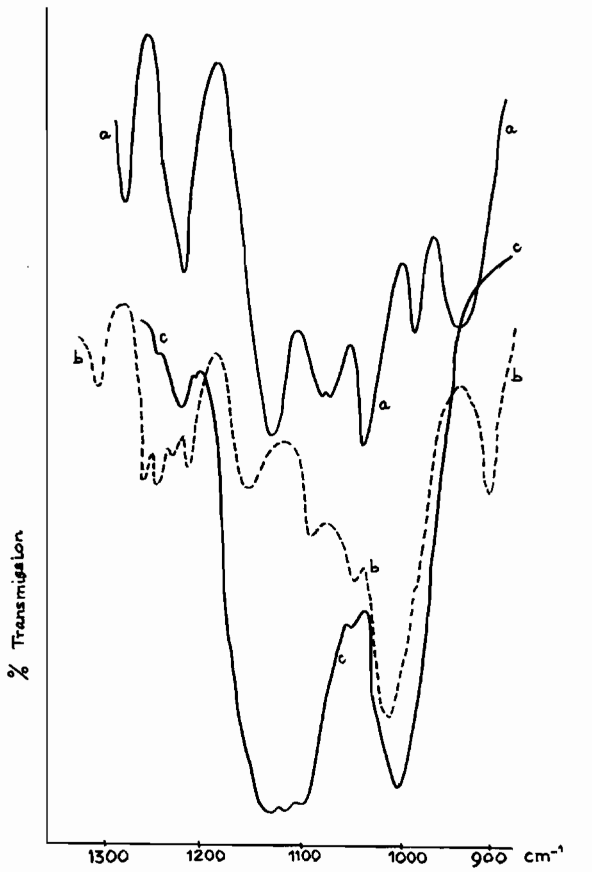


Figure 2. Bands in the i.r. spectrum of (a) PMPHCl; (b) CuPMPO; (c) ZnPMP.

TABLE III. Bands (cm^{-1}) Assigned to the Phosphate Group in PLP, PMP and Their Metal Complexes.

	ν_1	ν_3	ν_2	ν_4	*quasi*-symmetry of PO_4 Group
$PLPOH_2$	950	1050–1025 1181–1156	nm	600 628 572	C3v
PMPHCl	944	1044 1141 994	nm	604 625 571	C_{2v}
CuPMPO	908	1066	445	644	Td
CuPLPO	900	1028 1051 1129	–	647 600 538	C_{2v}
$ZnPMPOOH_2$	998	1048–1142	440	610 660	C_{3v}
ZnPLPO	1013	1108 1167	–	640 615 600	C_{3v}

TABLE IV. Band Maxima (cm^{-1}) in the Solid State Reflectance Spectra of PLP, and PMP and Their Complexes.

	ν_1	ν_{2a}	ν_{2b}	ν_3
$PLPOH_2$	43,000	35,000	33,300	28,000
CuPLPO	46,000	38,500	30,000	25,000
ZnPLPO	45,700	38,700	33,000	27,600
$CoPLPOOH_2$	45,000	38,700	–	25,900
PMPHCl	44,000	34,000	31,000	24,000
CuPMPO	46,700	38,500	30,000	25,000
$CoPMPOOH_2$	45,600	37,000	31,600	~25,000
$ZnPMPOOH_2$	46,500	40,000	32,700	–

The spectra of the anhydrous compounds are all quite different. The phosphate in CuPMPO appears to have almost *Td* symmetry.

Ultraviolet and Visible Spectra

The ultraviolet and visible spectra of PLP and its complexes are shown in Figure 3 and those of PMP in Figure 4, band positions are shown in Table IV.

The PLP complexes show four bands in the uv region (Table III). ν_1 and ν_2 can be assigned to the $\pi_L \rightarrow \pi_L^*$ bands.

$\nu_1(\pi_L \rightarrow \pi_L^*)$ shifts to higher energy on coordination, the magnitude of the splitting of ν_2 increases on coordination. The band near 27000 cm^{-1} is of high intensity like the $\pi_L \rightarrow \pi_L^*$ band and could be assigned $3d \rightarrow \pi_L^*$ or $\sigma_L \rightarrow \pi_L$. A similar band appears in diketoenolate complexes of copper and nickel and its origins have been discussed. Cotton and Wise[15] have assigned this band to the $3d \rightarrow \pi_L^*$ transition, and it seems likely that in PLP complexes with a similar chelate ring, the band has a similar origin. Support for this assignment comes from the fact that the PLP complex of Ga(III)[16] which has no occupied $3d$ orbitals does not show a band in this region. The zinc complex with $3d^{10}$ configuration shows a band at a slightly higher energy than the other complexes. The PMP complexes show bands with a much lower intensity in this region, the Co(III) complex having only a shoulder which is not resolved from the $\pi_L \rightarrow \pi_L^*$ band.

The visible region bands are much less intense and can be assigned to $d \rightarrow d$ transitions, these are absent from the spectra of the Zn(II) complexes. The PLP complex of copper shows a band at 12,500 cm^{-1} while that of the PMP complexes is at 14000 cm^{-1}. This is good evidence for oxygen coordination in the PLP complex (since $Cu(OH_2)_6^{2+}$ has a band at 12,600 cm^{-1}) and for mixed nitrogen–oxygen coordination in the PMP complex ($Nigly_2$ has a band at 15,800 cm^{-1}).[17]

Magnetic Measurements

The susceptibility of CuPLPO, CuPamPO, and Co PLPO are shown in Table V.

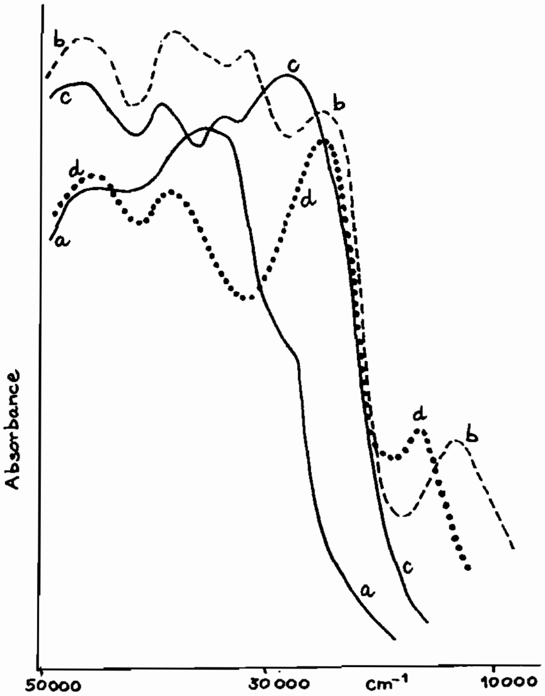


Figure 3. Solid state reflectance spectra of (a) PLP; (b) CuPLPO; (c) ZnPLPO; (d) $CoPLPOOH_2$.

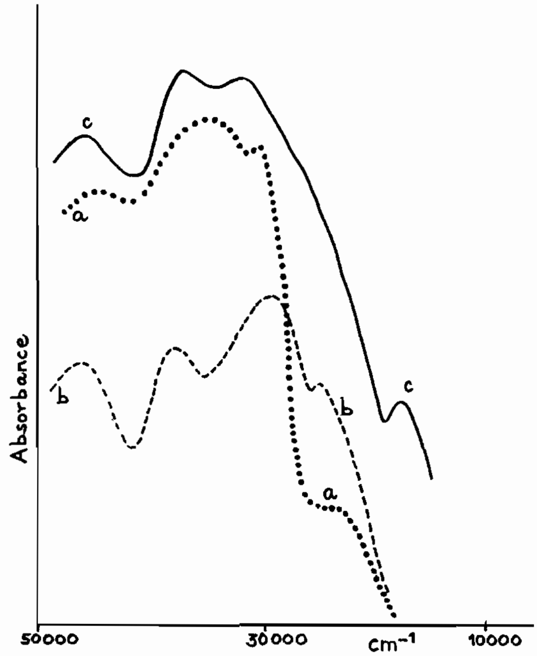


Figure 4. Solid state reflectance spectra of (a) PMPHCl; (b) CuPMPO; (c) $CoPMPOOH_2$.

TABLE V. Magnetic Susceptibility Data ($10^6 \chi_m'$ (c.g.s.u.)) and Effective Magnetic Moments (BM) with Temperature in K.

CuPLPO								
T	297	273	253	213	193	153		
$10^6\chi_m'$	1633	1721	1855	2072	2211	2611		
μ_{eff}	1.98	1.95	1.95	1.89	1.86	1.79		
CuPMPO								
T	303	295	273	253	213	193	153	
$10^6\chi_m'$	1197	1285	1437	1462	1623	1697	2141	
μ_{eff}	1.71	1.75	1.79	1.73	1.67	1.62	1.62	
T	133	113	105					
$10^6\chi_m'$	2348	2737	2826					
μ_{eff}	1.61	1.62	1.55					
$CoPLPOOH_2$								
T	301	273	253	213	193	153	133	105
$10^6\chi_m'$	11,688	12,836	13,898	16,176	17,757	21,963	24,870	30,787
μ_{eff}	5.33	5.31	5.32	5.27	5.26	5.21	5.16	5.10

Plots of $1/\chi_m'$ for CuPLPO and CuPMPO give $\Theta \sim 45$ and 25° K respectively.

Chromatography

Chromatographic studies were initiated as an aid in the interpretation of transamination reactions. By use of only two sprays (ninhydrin and dithizone) and ultraviolet light components from the ligands, Zn^{2+} and Cu^{2+} were identified. The R_f values are shown in Table VI.

The most interesting result from the chromatographic studies is the appearance of a third spot from the PLP complexes. Apart from the R_f value the characteristics of the spot are those of the non phosphorylated pyridoxal. If this were the case then metal promoted dephosphorylation would be suggested. Further studies of this component are being undertaken.

Discussion

The spectral properties of $ZnPMPOOH_2$, $CoPMPOOH_2$ and $CaPLPOOH_2$ are not incompatible with either four coordinate square planar or six coordinate tetragonal coordination as in IV or V.

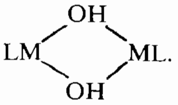
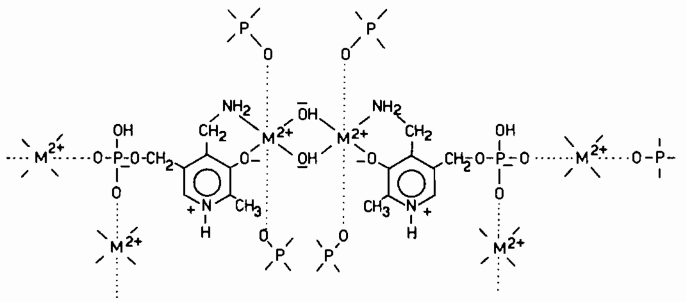
The anhydrous compounds are unlikely to be three coordinate, therefore we suggest that these compounds are bridged hydroxide complexes of the type . Recent work has demonstrated the

TABLE VI. R_f Values for PL, PLP, PM and PMP and Metal Complexes.

Compound		Approximate R_f Values and Conditions	
PL and PLHCl		60	blue under uv light; (v. pale yellow with ninhydrin after few hours)
PLP		20	bright blue under uv light; no colour with ninhydrin
PM		43	bright orange with ninhydrin; black under uv light
PMP, PMPHCl		16	bright orange with ninhydrin; black under uv light
CuPMPO	PMP	15	orange with ninhydrin; purple under uv light
	Cu	26	yellow with dithizone
$ZnPMPOOH_2$	PMP	15	orange with ninhydrin; purple under uv light
	Zn	20	pink with dithizone
CuPLPO	PLP	17	blue under uv light
	Cu	26	yellow with dithizone
	?	45	yellow with ninhydrin, fades; blue under uv light
ZnPLPO	PLP	17	blue under uv light
	Zn	20	pink with dithizone
	?	44	yellow with ninhydrin, fades blue under uv light



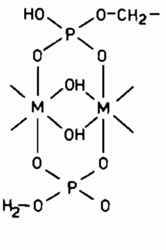
(VI)

existence of the dimeric structural unit where M is Cu^{2+} [18–21].

It has been suggested[19] that Cu(II) hydroxo complexes with N substituted 2-(2-(aminoethyl)pyridines should contain dimers of this type, and that within this group of compounds structural differences between compounds containing mono and di substituted amine groups produce different magnetic properties. The dimethyl and diethyl substituted complexes obey the Curie–Weiss law above 80° K whereas the mono methyl and isopropyl ligands show large antiferromagnetic interactions. These suggestions have been confirmed recently.[20,21] The complex containing the monoethyl substituted ligand $[Cu(EAEP)OH]_2\ (ClO_4)_2$ has been shown to contain the hydroxo bridged unit, and tetragonal pyramidal copper.[20] $\alpha[Cu(DMAEP)OH]_2(ClO_4)_2$ containing the dimethyl derivative contains the dihydroxo bridges, the copper having distorted octahedral coordination, with two intramolecular perchlorate bridges.[21]

It seems possible that the hydroxo bridged unit exists in the anhydrous compounds prepared in this work. The phosphate group in CuPMPO appears to have almost *Td* symmetry, and tentative suggestions can be made for a formula such as VI, which gives a three-dimensional polymer and six coordination about the metal.

It is also possible that the phosphate groups in this compound form intermolecular bridges giving the unit, as in VII.



(VII)

VII gives a structure similar to that of $\alpha[Cu(DMAEP)OH]_2(ClO_4)_2$ which is also reported to obey the Curie–Weiss law above 80° K.

Acknowledgements

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