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Reactions of Metal Ions with Vitamins.
III. Syntheses and Infrared Spectra of Metal Complexes with Pyridoxamine and Pyridoxine

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Syntheses and infrared spectra are reported for PM·2HCl, PM·HCl, PM·2H$_2$O, PN·HCl, PN, and 16 metal compounds containing PM and PN in various protonation states (PM and PN are pyridoxamine and pyridoxine, respectively). The pyridine ring breathing frequencies were identified by comparison of the spectra of the deuterated and nondeuterated compounds. They, together with the region for $-NH_3^+$ combination bands, are used to determine the tautomeric form of the vitamins and thus to identify the metal-binding sites in PM and PN. In all metal complexes of neutral PM and PN, the ligand exists as a zwitterion with the ring nitrogen protonated. The metal ions are chelated through the phenolate and adjacent hydroxymethyl or aminomethyl groups.

Introduction

Considerable research has been carried out on metal ion complexes of the B$_6$ vitamins and pyridoxylidene Schiff bases ever since Metzler, Ikawa, and Snell proposed that the metal-ion catalyzed reactions of pyridoxal with amino acids involves chelation of the pyridoxal to the metal ion and Schiff base formation with the amino acid [1]. The binding sites of the B$_6$ vitamins have been of interest, and most of the evidence, obtained in solution, has supported the idea of chelation through the phenolic and 4'-functional groups [2–8]. Bonding through other liganding sites has also been suggested, again on the basis of evidence obtained for solutions [9–14]. Our work has been concerned with the solid-state structures of vitamin B$_6$ complexes, and the present paper reports the synthesis and structural implications of the infrared spectra of complexes of pyridoxamine (PM) and pyridoxine (PN). X-ray crystal structures have been carried out on some of the compounds and are reported elsewhere [6, 8, 15, 16].

Experimental

Syntheses

Pyridoxamine dihydrochloride and pyridoxine hydrochloride were purchased from Sigma Chemicals. Deuterium oxide was obtained from Merck, Sharpe, and Dohme. Metal chlorides and nitrates were supplied by BDH and Fisher Scientific.

PM·2H$_2$O and PN were prepared by adding NaOH to an aqueous solution of PM·2HCl and PN·HCl until the pH was between 7 and 8. Small crystals of PM·2H$_2$O precipitated immediately; large plates of PN were obtained by partial evaporation of the solution. Block-shaped crystals of PM·HCl were grown from an equimolar solution of PM·2HCl and NaOH. Elemental analyses for PM·2HCl, PM·HCl, PM·2H$_2$O, PN·HCl, and PN agreed satisfactorily with the calculated values.

Most of the compounds reported in Table I were synthesized by partial evaporation of equimolar solutions of the metal salt and the appropriate form of the vitamin. The few exceptions are as follows. Brown needles of Cu(PM-H)$_2$·6H$_2$O precipitated upon cooling a hot solution of Cu(NO$_3$)$_2$·3H$_2$O and PM·2H$_2$O (1:2 mole ratio). Attempted recrystallization of the brown crystals gave chunky green crystals of Cu(PM-H)$_2$·2H$_2$O. The complexes CuCl$_2$-PM·H$_2$O and NiCl$_2$(PM)$_2$·2H$_2$O were obtained from equimolar solutions of the metal chloride and PM·HCl. Evaporation of a yellow solution containing CuCl$_2$·2H$_2$O and PM·2HCl (1:2 mole ratio) resulted in the deposition of pale blue crystals of (H$_2$PM)$_2$-CuCl$_6$·2H$_2$O.

Deuterations were carried out by recrystallizing the compounds from D$_2$O. Approximately 90% of the labile hydrogens were replaced in this way.

Measurements

Infrared spectra in the 4000–400 cm^{-1} range were recorded on Perkin-Elmer 237B and 225 grating infrared spectrometers. The spectra were calibrated with

TABLE I. Analytical Data.

Compound	Color	Found, %					Calcd., %				
		M	C	H	N	Cl	M	C	H	N	Cl
$(H_2PM)_2CuCl_6 \cdot 2H_2O$	Blue	9.35	29.58	4.89	8.54	31.91	9.74	29.44	4.95	8.58	32.58
$(H_2PM)ZnCl_4 \cdot H_2O$	White	16.45	24.11	4.16	–	35.30	16.53	24.30	4.09	–	35.86
$(HPM)_2CdCl_4 \cdot 2H_2O$	White	17.88	30.30	4.79	–	22.35	17.89	30.57	4.82	–	22.56
$(HPM)ZnCl_3$	White	19.13	28.33	3.87	–	30.99	19.17	28.18	3.85	–	31.19
$Cu(NO_3)_2(PM)_2 \cdot H_2O$	Green	11.65	35.58	4.86	15.11	–	11.72	35.45	4.84	15.51	–
$Ni(NO_3)_2(PM)_2 \cdot 2H_2O$	Green	10.42	34.92	5.13	14.78	–	10.57	34.61	5.09	15.14	–
$CdNO_3(PM\text{-}H)PM \cdot 3H_2O$	White	20.06	34.22	5.32	12.10	–	19.93	34.08	5.19	12.42	–
$Zn(NO_3)_2(PM)_2 \cdot 2H_2O$	White	11.84	33.81	5.01	14.76	–	11.64	34.19	5.03	14.96	–
$CoCl_2(PM\text{-}H)(PM)_2 \cdot 3H_2O$	Black	8.60	41.96	6.14	12.22	11.13	8.57	41.94	6.02	12.23	10.29
$CuCl_2PM \cdot H_2O$	Green	18.81	30.10	4.37	–	21.94	19.83	29.95	4.40	–	22.11
$NiCl_2(PM)_2 \cdot 2H_2O$	Green	11.33	38.30	5.75	11.13	14.27	11.69	38.27	5.63	11.16	14.12
$Cu(PM\text{-}H)_2 \cdot 6H_2O$	Brown	12.53	38.88	6.62	11.36	–	12.56	37.97	6.79	11.07	–
$Cu(PM\text{-}H)_2 \cdot 2H_2O$	Green	14.30	43.99	5.98	12.59	–	14.64	44.28	6.05	12.91	–
$CdCl_2PN$	White	31.65	27.14	3.13	3.99	20.08	31.89	27.26	3.15	3.97	20.11
$MnCl_2(PN)_3 \cdot 2H_2O$	Yellow	8.20	43.09	5.59	6.28	9.66	8.21	43.05	5.58	6.28	10.59
$Zn(NO_3)_2(PN)_3 \cdot 2H_2O$	White	9.01	36.56	5.07	9.37	–	8.92	39.32	5.10	9.56	–

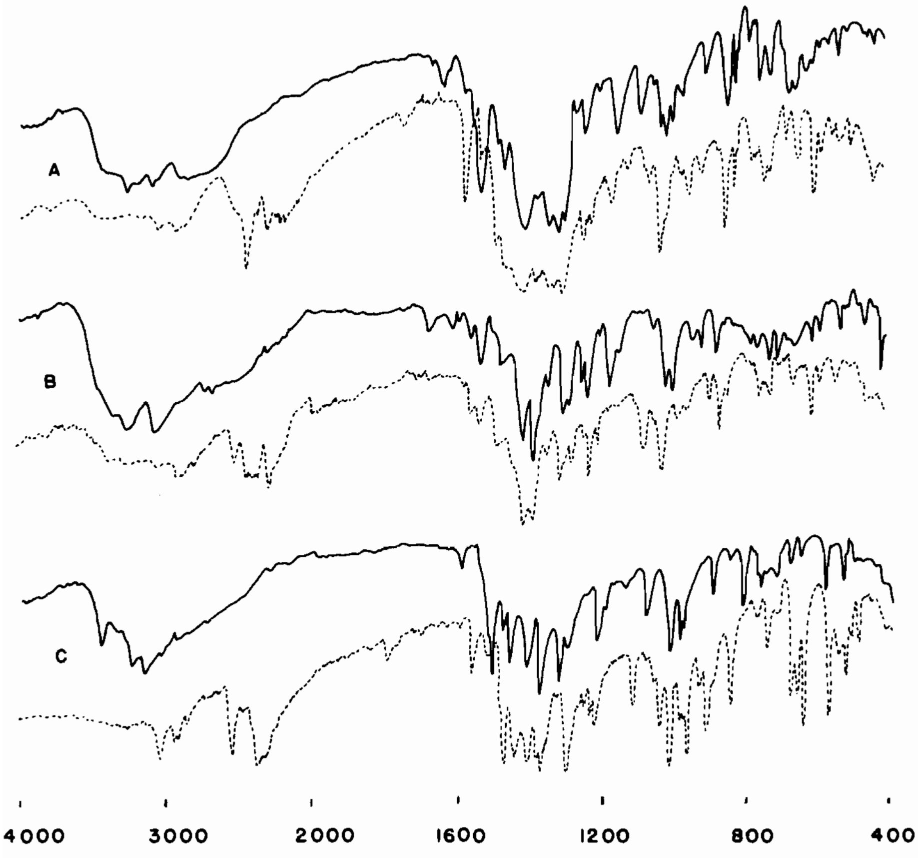

Fig. 1. Ir spectra from 400 to 4000 cm^{-1} of (A) $Cu(NO_3)_2(PM)_2 \cdot H_2O$; (B) $Cu(PM\text{-}H)_2 \cdot 6H_2O$; (C) $CdCl_2PN$. Dashed lines show the spectra of the deuterated compounds.

the polystyrene bands at 3027.9, 1601.8, and 906.9 cm^{-1}. The samples were run as mulls in mineral oil or Fluorlube. Mulls for the deuterated compounds were prepared in glove bags under a dry nitrogen atmosphere.

X-ray powder patterns were taken on all compounds using a 57.3 mm diameter powder camera and CuK_α radiation.

The magnetic susceptibility was determined with a Gouy balance adapted from a Mettler type 415 balance. The standard used was $Hg[Co(SCN)_4]$.

Analyses were performed by F. Pascher, Mikroanalytisches Laboratorium, Bonn, Germany.

Results

Syntheses and Compositions of the Compounds

The syntheses were relatively straightforward, due allowance being made for the fact that the protonation states of PM and PN depend both on pH and the presence of metal ions. For example, solutions containing PM·HCl and $ZnCl_2$ gave crystals of $HPMZnCl_3$, whereas when $CuCl_2$ was present, HPM^+ was deprotonated and a copper complex was obtained ($CuCl_2PM·H_2O$). Similarly, a 1:1 ratio of PM and $Cu(NO_3)_2$ yielded a complex with neutral PM, $Cu(NO_3)_2(PM)_2·H_2O$, whereas a 2:1 ratio resulted in a complex containing anionic pyridoxamine, $Cu(PM\text{-}H)_2·6H_2O$.

The compositions of the complexes were established by elemental analyses (Table I). X-ray powder patterns confirmed that new compounds had been formed, since their powder patterns were different from those of the starting materials.

Magnetic measurements on the cobalt complex showed that the compound is diamagnetic, and it was formulated as a low-spin complex of Co(III). Only two chloride ions are present in the compound, and one of the three PM ligands is presumed to be anionic in order to balance the charge: $[Co(PM)_2(PM\text{-}H)]Cl_2·3H_2O$.

Infrared Spectra

Infrared spectra were obtained for all of the compounds listed in Table I as well as for PM·2HCl, PM·HCl, $PM·2H_2O$, PN·HCl, and PN, and for their deuterated analogs (labile hydrogens only were exchanged). The spectra of members of a given class of compounds, *e.g.*, metal complexes with neutral PM, are similar to each other. Representative spectra are shown in Fig. 1 for the metal complexes, and spectra of the ligands are tabulated in Table II.

The compounds exhibit several peaks in the 1450–1650 cm^{-1} range, which is the region for HOH bending and NH_2 deformation modes as well as the ring breathing frequencies of pyridine [17]. The H_2O and NH_2 peaks shift to ~1200 cm^{-1} upon deuteration, thus allowing the pyridine ring modes to be isolated. Also deuteration results in characteristic changes of the ring modes when a pyridinium ion is present but has no effect on the ring frequencies if the pyridine nitrogen is not protonated. Thus, Cook [18] and others [19, 20] have observed four ring breathing modes of pyridine and substituted pyridines between ~1400 and 1610 cm^{-1}. These shift to higher frequencies in pyridinium salts [18–20], and two of them are sensitive to deuteration. The band at 1610 cm^{-1} in $C_5H_5NH^+$ shifts to 1585 cm^{-1} in $C_5H_5ND^+$, and the one at 1535 cm^{-1} moves to 1475 cm^{-1} [18, 20], Similar changes are observed for substituted pyridinium salts [19].

The spectra of all of the metal complexes of neutral PM and PN, as well as those of PM·2HCl, PM·HCl, PN·HCl, $(H_2PM)_2CuCl_6·2H_2O$, $H_2PMZnCl_4·H_2O$, $(HPM)_2CDCl_4·2H_2O$, and $HPMZnCl_3$, have features which are characteristic for a pyridinium ion. For example, $CdCl_2PN$ has a weak peak at 1600 cm^{-1} and a strong one at 1515 cm^{-1} which shift to 1565 and 1480 cm^{-1}, respectively, upon deuteration (Fig. 1). The peak positions and magnitude of the deuteration shift establish these peaks as ν_{8b} and ν_{19b} for a pyridinium ring [18, 20]. There should also be peaks near 1630 and 1450 cm^{-1} (ν_{8a} and ν_{19a}). The latter peak occurs in the same region as the CH_2 deformations [17], and so cannot be distinguished from them. The former peak was not observed in any of the compounds, but it is known that the ring modes are of variable intensity and depend on the substitutents [17].

The spectrum of $Cu(NO_3)_2(PM)_2·H_2O$ resembles that of $CdCl_2PN$, particularly in the shift of the strong peak at 1525 cm^{-1} upon deuteration (Fig. 1). The peak at 1570 cm^{-1} is much more intense in the deuterated compound that the corresponding peak (1610 cm^{-1}) in the nondeuterated substance. This phenomenon has been observed occasionally in other pyridinium salts and has been attributed to Fermi resonance [18].

The spectra of PN, $PM·2H_2O$, $Cu(PM\text{-}H)_2·2H_2O$, and $Cu(PM\text{-}H)_2·6H_2O$ are quite different from those of the compounds discussed above. They show no strong peaks above 1500 cm^{-1}, and the weak peaks in the 1500–1600 cm^{-1} region are essentially unchanged in spectra of the deuterated materials (Table II; Fig. 1). The spectrum of $Cu(PM\text{-}H)_2·6H_2O$ is typical (Fig. 1). The peaks at 1665 and 1599 cm^{-1} disappear upon deuteration and are assignable as the H_2O and NH_2 bending frequencies. The peaks at 1524 and 1554 cm^{-1} occur at 1522 and 1548 cm^{-1} in the deuterated compound. The absence of a significant deuteration shift indicates that the pyridine ring is not protonated in these four compounds.

One other region of the spectrum is important for establishing the tautomeric structure of the ligand, and this is the region for the $-NH_3^+$ combination bands [17]. PM·HCl and PM·2HCl each have several

TABLE II. Infrared Spectra of PM and PN and Their Hydrochlorides (cm^{-1}).[a]

PM·2HCl		PM·HCl		PM·2H_2O	
H	D	H	D	H	D
3430 s	2540 m	3325 s	3040 m	3280 s	2510 m,sh
3360 s	2425 s	3217 w	2465 s	3150 s	2440 s
3300 s	2295 s	3040 m	2310 m	2450 m,br	2250 m,br
3120 s	2130 s	2800 s	2240 m	2080 w	1900 w,br
2750 s,br	1980 m,br	2500 m,br	2160 s	1620 w	1655 w
2350 w	1825 w	2140 w	1850 m,br	1570 w	1575 w
2030 w	1615 w	2075 m	1765 w	1548 w	1553 m
1940 w	1587 m	1940 m	1584 w	1535 w	1534 m
1840 w	1523 m	1890 w	1570 m	1480 m	1477 m
1755 w	1503 s	1820 w	1530 w	1405 s	1465 m
1630 m	1480 m	1709 w	1490 m	1362 s	1430 m
1610 m	1455 s	1645 w	1473 s	1289 m	1403 s
1588 m	1430 s	1528 s	1443 s	1246 m	1375 m
1541 s	1396 sh	1486 w	1432 s	1227 m	1360 s
1475 m	1385 m	1473 s	1400 w	1210 sh	1350 w,sh
1430 w	1375 m	1455 s	1385 s	1140 sh	1302 m
1390 s	1362 sh	1429 s	1360 w	1130 m	1290 w
1365 s	1320 m	1385 s	1332 w	1075 m	1264 m
1345 sh	1251 m	1370 s	1322 m	1015 s	1240 w
1298 s	1228 w	1328 m	1272 w	917 m	1222 s
1279 s	1172 w	1302 m	1240 m	876 w	1187 w
1220 w	1161 m	1248 w	1225 w	785 w	1125 m
1125 m	1138 m	1216 m	1190 w	768 w	1102 m
1059 w	1088 m	1166 m	1155 m	720 br	1069 m
1032 s	1047 s	1127 m	1145 m	675 w	1013 s
1020 m	1033 sh	1064 m	1130 m	650 w	942 w
973 s	970 m	1034 w	1060 w	585 w	910 w
914 m	936 m	1018 s	1035 m	546 w	874 m
871 s	920 m	1011 s	1020 w	513 w	819 m
840 w	853 w	985 w	1001 w	417 w	777 m
787 w	828 w	975 w	983 w		746 m
763 w	718 m	919 w	969 w		668 w
730 m	646 w	852 s	935 w		646 m
668 w	630 w	786 s	920 w		576 m
638 m	552 w	744 w	885 m		575 m,br
628 m	510 w	715 w	855 w		500 m
570 w,br	478 w	678 m	845 w		410 w
500 m	453 w	640 m	785 m		
415 w		622 m	765 w		
		580 m	750 m		
		552 m	725 m		
		531 w	720 m		
		494 m	667 m		
		425 m	637 m		
		400 w	559 m		
			551 m		
			519 m		
			495 m		
			455 w		
			427 w		

PN·HCl		PN	
H	D	H	D
3320 s	3909 w	3260 s	2435 s

(continued on facing page)

TABLE II. *(continued)*

PN•HCl		PN	
H	D	H	D
3230 s	2460 s	3140 s	2320 s
2810 s	2405 s	2850 s	2190 s
1617 w	2305 s	2660 s	2025 s
1537 m	2170 s	2600 sh	1980 sh
1517 m	1790 w	1616 w	1602 w
1478 m	1580 m	1555 w	1561 w
1455 m	1535 sh	1524 w	1523 w
1412 m	1510 m	1430 s	1427 s
1380 s	1470 s	1416 s	1410 s
1278 m	1427 s	1380 s	1380 s
1215 m	1395 s	1355 s,br	1288 m
1150 w,br	1380 s	1283 m	1256 w
1086 m	1292 s	1276 m	1232 m
1016 m	1240 w	1250 w	1143 w
988 w	1240 m	1235 w	1085 m
970 w	1225 w	1221 s	1055 w
960 w	1140 w	1084 s	1033 w
923 w	1125 w	1052 w	1010 s
871 m	1088 m	1018 s	984 w
795 m	1063 m	984 w	955 m
746 m	1038 m	956 m	922 m
720 m	980 m	884 w	881 m
684 m	947 m	850 w,br	854 w
618 m	904 m	756 m	755 m
591 m	855 w	738 m	723 w
574 m	731 s	723 m	687 w
514 w	725 s	692 w	646 w
472 w	714 s	645 m	573 m
	675 m	574 m	548 m,sh
	586 s	518 w	513 w
	520 m	485 w	478 w
	501 w	413 w	451 w
	465 m		
	455 m		
	430 m		

[a] Abbreviations: s, strong; m, medium; w, weak; br, broad; sh, shoulder.

weak peaks between 1700 and 2200 cm^{-1} which show that the amino group is protonated in these two compounds. PM•$2H_2O$ also absorbs in this region, having a peak at 2080 cm^{-1}. Neutral PM is thus in a dipolar form having a protonated amino group.

The remaining regions of the spectra are less important in determining ligand structure. Several N–H and O–H stretching frequencies occur in each compound. When the crystal structure is known, the individual O–H and N–H frequencies can probably be assigned with the aid of curves showing ν_{X-H} as a function of the X•••Y distance in an X–H•••Y hydrogen bond [21]. However, if the structure is not known in detail this region has little diagnostic value in distinguishing tautomeric forms of the ligands.

Metal–nitrogen and metal–oxygen frequencies should appear in the region below 600 cm^{-1} [22]. However, the ligands themselves have several absorptions between 400 and 600 cm^{-1} (Table II) so that no peaks can be assigned definitely to metal–ligand vibrations.

The peaks associated with C–N–H and C–O–H deformations should shift upon deuteration [18–20]. Although there are changes, with several peaks disappearing and new ones appearing in the spectra of the deuterated compounds (Fig. I and Table II), there seems to be no easily interpretable pattern. The dependence of the peak positions on hydrogen bonding complicates the assignment of these bands, and no attempt was made to interpret the spectra in the region below 1400 cm^{-1}.

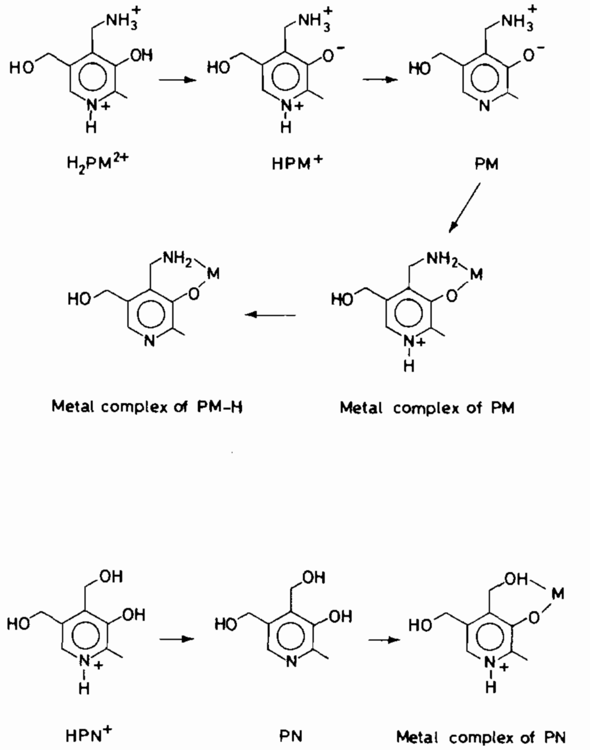

Fig. 2. Solid-state structures of PM, PN and the metal complexes and cations derived from them.

Discussion

The crystal structure of $CdCl_2PN$ [7], $Cu(NO_3)_2(PM)_2 \cdot H_2O$ [6], $Zn(NO_3)_2(PM)_2 \cdot 2H_2O$ [15], PN·HCl [23], and PM·HCl [16] have in fact established that a pyridinium ring is present in these compounds, whereas the structure of $Cu(PM\text{-}H)_2 \cdot 2H_2O$ reveals that the pyridine ring is not protonated [8]. The spectra thus correlate well with the known structures, and have predictive value when the structure is not known. A strong peak at 1525–1545 cm^{-1} indicates that a protonated ring nitrogen is present in a vitamin B_6 derivative. If such a peak is absent, the vitamin is either in the anionic form or in a tautomeric state not involving a pyridinium ion.

Figure 2 summarizes the solid-state structures of all of the compounds synthesized, and schematically shows the changes which occur in the ligands upon deprotonation and complexation. Of particular interest is the tautomeric change undergone by both PM and PN when they complex to metals. The shift of the proton to the pyridine nitrogen frees the amino group for chelation in the case of PM. For PN, shifting the proton from the phenol group results in more favorable bonding because the liganding site is negatively charged.

The structures shown in Fig. 2 for the ligands agree with those postulated to exist in aqueous solution [24–26], with the exception of PN which is zwitterionic in water. However, PN converts to the nondipolar form in dioxane and alcohols [26]. The crystal packing apparently stabilizes the nondipolar form of PN, so that a tautomeric change occurs in PN even as it crystallizes from aqueous solution. A judicious choice of solvent might thus allow one to observe complexation of other tautomeric forms of the vitamins, perhaps even accompanied by a corresponding change in liganding site. This kind of behaviour has already been reported by Kelusky and Hartman [9] and is especially relevant to enzymic systems since the microenvironment of the vitamin will be very different from that in bulk aqueous solution.

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