tially to Fe, suggesting that there are two displaceable ligands. This is supported by the observation that PCA binds to the high spin 3,4 PCD-CN^- complex to make a distinctly different ternary complex, but CN^- does not bind to the, presumably bidentate, 3,4 PCD-PCA complex.

The ketonized substrate analogs 2-OH-isonicotinic acid N-oxide (2-OH-INO) and 6-OH-nicotinic acid N-oxide have been synthesized [2]. These analogs form ~100-fold stronger complexes with 3,4 PCD than does PCA, thus they are proposed as transition state analogs. The EPR spectra of the 3,4 PCD–2-OH-INO complex is distinctly different than that of the PCA complex displaying small and negative zero field splitting ($D = -0.5\ cm^{-1}$) and intermediate rhombicity ($E/D = 0.25$). $^{17}OH_2$ remains bound in the inhibitor complexes suggesting that they are monodentate. CN^- displaces the water showing that small molecules have access to the iron in the ketonized analog complexes.

Transient kinetic studies show that the ketonized analogs bind in at least two phases. In the fast initial phase, a weak, readily reversible complex is formed, while in the slow ($t_{1/2}$ = 0.12 s) second phase, the essentially irreversible complex is formed. At −20 °C in glycerol-buffer solution two complexes can be stabilized. The first complex has optical and EPR spectral features very similar to those of the substrate complex. In contrast, the final complex is dramatically different. The native red color is bleached, due perhaps to a large blue shift of the spectrum. Similar bleached spectra are observed for early transient intermediates in the reaction with PCA [3]. We suggest that, like PCA, ketonized analogs initially assume a bidentate Fe ligation but then change to a monodentate ligation. Such a change could be coincident with a conformational change of the enzyme designed to stabilize a ketonized reaction cycle intermediate. The analogous change in the PCA complex apparently requires interaction with O_2. Thus, the ketonized analogs may model the first oxy complex. Such a complex would apparently have a vacatable Fe ligand site which could be used to stabilize an oxygenous intermediate.

Acknowledgement. This work was supported by NIGMS GM24689.

1 L. Que, J. D. Lipscomb, E. Münck and J. M. Wood, *Biochim. Biophys. Acta, 485,* 60 (1977).
2 J. D. Lipscomb, J. W. Whittaker and D. M. Arciero, in 'Oxygenases and Oxygen Metabolism', M. Nozaki (ed), Academic Press, N.Y. 1982, p. 27.
3 C. Bull, D. P. Ballou and S. Otsuka, *J. Biol. Chem., 256,* 12681 (1981).

R18

Metal Complexes with Vitamin B_6 Derivatives. 3 Metal Chlorides of Pyridoxylidenedihydralazine and Pyridoxylideneisoniazide

SILVIA BARBU

Faculty of Pharmacy, Institute of Medicine and Pharmacy, 3400 Cluj-Napoca, Romania

Several studies in the chemical literature established the catalytic efficiency of some metal cations in the transamination of pyridoxal. A general transamination mechanism involving the metal cations was elaborated [1].

In view of the frequent therapeutic use of dihydralazine and isoniazide, as well as their numerous adverse reactions due to the carbonyl group blocking in the pyridoxal molecule [2, 3], we studied the coordination capacity of pyridoxylidenedihydralazine (HPL-DHF) and pyridoxylideneisoniazide (HPL-HIN) for Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) ions.

Complex metal chlorides with the general formula M_2(PL-DHF)Cl_2 and M(PL-HIN)Cl were synthesized and isolated in solid state. The combination ratio M:L:Cl was 2:1:2 and 1:1:1 respectively. The complexes, orange-brown or yellow coloured, stable at room temperature, with high melting points (over 250 °C) are water insoluble, partially soluble in alcohol and slightly soluble in basic solvents.

In order to establish the coordination geometry of the metal ion, the electronic spectra in diffuse reflectance by using samples pressed in $BaSO_4$ pellets were recorded. The electronic spectral parameters (the interelectronic repulsion parameter B, the nephelauxetic parameter β and the crystalline field splitting parameter 10 Dq) were calculated according to the Lever method [4] and included in Table I.

TABLE I. Electronic Spectral Parameters.

Compound	Bands, cm^{-1}	ν_3/ν_1	B	β	10 Dq
Co_2(PL-DHF)Cl_2	20000 12820 9523	2.10	784	0.785	10509
Co(PL-HIN)Cl	22222 11764 8000	2.77	1027	1.029	9245
Ni_2(PL-HIN)Cl_2	22222 9090 7299	3.04	829	0.797	7295
Ni(PL-HIN)Cl	22222 12121 7407	3.00	823	0.791	7407

TABLE II. ESR Spectral Parameters.

Compound	H	g	$I_{\parallel}/I_{\perp}$	$H_{1/2}$, gauss
Cu_2(PL-DHF)Cl_2	1365	4.91	6.8/10.7	104.60
	1292	5.93		
Cu(PL-HIN)Cl	1365	4.92	7.0/7.8	68.25

A Dq value of 1000 cm^{-1} for the Co(II) ion and 700 cm^{-1} for the Ni(II) ion, is common for octahedral complexes, the chromophores being MON_4Cl for the HPL-DHF ligand and MO_2N_3Cl for the HPL-HIN ligand, respectively.

The parameters of the RES spectra (Table II) suggest anisotropy for the compound Cu_2(PL-DHF)-Cl_2, the spectrum of Cu(PL-HIN)Cl being approximately isotropic.

The values for $g > 2.04$ suggest an oblong octahedron.

The IR spectra of the free ligands have the characteristic band of the $\nu_{C=N}$ azomethine stretch at 1625 cm^{-1} for HPL-DHF and at 1665 cm^{-1} for HPL-HIN. The bond formation with the metal ion determined changes of the IR spectra in this region. Both the band position and their intensities varied, confirming that the azomethine group takes part in the bond with the metal ion.

1 D. E. Metzler, M. Ikawa and E. E. Snell, *J. Am. Chem. Soc., 76,* 648 (1954).
2 E. Ackermann, P. Oehme, H. Rex and P. Lange, *Acta Biol. Med. Ger., 12,* 322 (1964).
3 P. Oehme, H. Niedrich, F. Jung and M. Rudel, *Acta Biol. Med. Ger., 22,* 345 (1969).
4 A. B. P. Lever, *Chem. Educ., 45,* 711 (1968).

R19

Ambiguities in the Proton NMR Studies Involving Cu(II) Ions as Paramagnetic Relaxation Centres

GIANNI VALENSIN, ANDREA LEPRI and ENZO TIEZZI

Department of Chemistry, University of Siena, Pian dei Mantellini 44, 53100 Siena, Italy

The theory of nuclear spin relaxation induced by paramagnetic ions in solution has been given great relevance in delineating structural and kinetic parameters of bioinorganic reactions. Although several criticisms and corrections have been worked out, the original form of the Solomon-Bloembergen-Morgan equations [1, 2] still represents the most suitable starting point for interpreting experimental paramagnetic rates.

TABLE I. Paramagnetic Proton Relaxation Rates of Imidazole H_4, pH = 7.0; T = 300 K.

His (M)	Cu^{2+} (mM)	T_{1p}^{-1} (sec^{-1})	T_{2p}^{-1} (sec^{-1})	T_{2p}^{-1}/T_{1p}^{-1}
0.1	0.01	0.02	0.32	16
0.1	0.02	0.03	0.45	15
0.1	0.03	0.06	0.65	11
0.1	0.04	0.14	1.27	7
0.1	0.05	0.18	1.41	10

When dealing with Cu(II) ions as relaxation reagents, the scalar interaction was shown to give significant contributions to NMR line broadening, and T_1 measurements were therefore designed for getting structural information from the dipolar term.

In this report we suggest that asymmetric coordination to Cu(II) ions, resulting in large g tensor anisotropies, makes the Solomon-Bloembergen equations meaningless, since unreasonable answers are obtained in well defined Cu(II) complexes.

The $Cu(His)_2$ complex was taken as a model complex, since the X-ray structure of crystals obtained from aqueous solutions has been reported [3].

The experimental paramagnetic contributions $T_{ip}^{-1} = T_i^{-1}(obs) - T_i^{-1}(blank)$ are reported in Table I for H_4 of the imidazole moiety at different $[Cu^{++}]_{tot}/[His]_{tot}$ ratios. In these conditions CuA_2 (His = H_3A) is almost exclusively present in solution [4] and the T_{2p}^{-1}/T_{1p}^{-1} values are consistent with fast exchange of His molecules from the metal coordination sphere. The correlation time of the dipolar interaction was approximated by measuring that of water protons bound in the complex and the Cu(II)–H_4 distance was taken from crystallographic structure.

Calculations based on the Solomon-Bloembergen equations give values of the coordination number ranging between 0.039 and 0.054, which is nonsense.

Since the correlation time of the complex is not rapid enough compared with the electron spin anisotropy energy, that is

$$h\,\tau_c^{-1} \ll |g_{\parallel} - g_{\perp}|\beta B_o$$

no theoretical model is available to allow quantitative interpretation of nuclear relaxation. Combined ESR and NMR experiments are therefore suggested to build up a novel theoretical approach.

1 I. Solomon, *Phys. Rev., 99,* 559 (1955).
2 N. Bloembergen, *J. Chem. Phys., 27,* 572 (1957).
3 N. Camerman, J. K. Fawcett, T. P. A. Kruck, B. Sarkar and A. Camerman, *J. Am. Chem. Soc., 100,* 2690 (1978).
4 M. Sivasankaran *et al., J. Chem. Soc. Dalton Trans.,* 1312 (1980).