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Monomeric Mo(V) and Mo(VI) Complexes with Sterically Constrained Metal Centers

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Bray has proposed a chemical mechanism for the reduction of xanthine oxidase by xanthine, which involves a monomeric molybdenum active center having *fac* stereochemistry [1]. This proposal has simulated our interest in preparation and characterization of monomeric Mo(V) and Mo(VI) complexes constrained to *fac* configuration by polydentate ligands such as hydrotris(3,5-dimethylpyrazolyl) borate, hereafter designated as $HB(Me_2pz)_3^-$.

The $HB(Me_2pz)_3^-$ ligand has been extensively used to stabilize a variety of low valent molybdenum compounds [2]. Moreover, the same ligand has been found to stabilize the Mo(V) center in $MoOCl_2\{HB(Me_2pz)_3\}$(*I*) [3]. The relative stability of these compounds is attributed partly to the steric bulk of 3-methyl group on the ligand.

Mo(V) complexes of the type $MoOXY\{HB(Me_2pz)_3\}$ (where X = Y = NCS; X = Cl, Y = OR or SPh; X = Y = SPh) have been prepared by the substitution reactions of *I* and spectroscopically characterized. Esr spectra of Mo(V) centers are sensitive to X and Y. Substitutions by thiolate ligands give smaller A_o(Mo) and larger g_o values. These substitutions also shift the Mo=O stretching vibration significantly to lower wave numbers. A preliminary kinetic study has revealed that the rates of ligand substitution are very slow in these complexes, compared to those of known $MoOCl_3L_2$ complexes (where L is a monodentate ligand) [4].

Mo(VI) complexes of the type $MoO_2X\{HB(Me_2pz)_3\}$ (X = Cl, Br, NCS) have been synthesized for the first time by the reaction of MoO_2X_2 (X = Cl, Br) or $MoO_2(NCS)_4^{2-}$ with the ligand, and characterized by spectroscopic methods including ^{95}Mo NMR.

Electrochemical studies and structural studies on these Mo(V) and Mo(VI) complexes will also be described.

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R17

Active Site Fe^{3+} Ligation by Substrates and Transition State Analogs of Protocatechuate 3,4 Dioxygenase

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Current proposals for the mechanism [1] of Protocatechuate (PCA) 3,4 Dioxygenase (3,4 PCD) suggest monodentate (OH) binding of PCA to the active site Fe^{3+}. This would promote ketonization of PCA, thereby creating a carbanion at C-4 which could be directly attacked by O_2. We have tested this proposal using ketonized substrate analogs and various spectroscopic probes. Our results confirm that ketonization is an essential step in the mechanism, but suggest that it occurs later in the cycle than the initial substrate complex.

We have shown that water is a ligand for *Brevibacterium fuscum* 3,4 PCD by observing hyperfine broadening from $^{17}OH_2$ on all EPR resonances of the high spin Fe^{3+} [2]. The spectrum of the 3,4 PCD-PCA complex is too broad to detect direct displacement of H_2O by PCA. However, no broadening is observed in complexes with three slowly metabolized substrate analogs. In contrast, water remains bound in complexes with non-metabolized, monodentate analogs (*e.g.* 4-OH benzoate). Other small molecules also bind to Fe in 3,4 PCD. CN^- binds in two steps; first it forms a high spin and then a low spin complex. It is likely that 2 CN^- molecules bind sequen-

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.

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R18

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

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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