Synthesis, Characterization and Catecholase Activity of a Series of Novel Mononuclear Cu(II) Complexes Derived from a Tripodal Ligand

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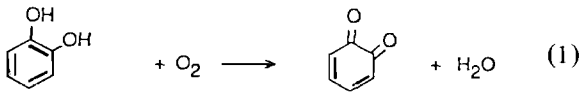
Abstract

A new tripodal ligand, *N*,*N*-bis(3,5-dimethylpyrazol-1-ylmethyl)-1-hydroxy-2-aminoethane (bpmhe), has been synthesized. Using this ligand, Cu(II) complexes of the type [Cu(bpmhe)X]Y where X = H_2O, NO_3, Br, Cl, or N_3 and Y = BF_4, NO_3, Br, or Cl have been prepared. This has resulted in the formation of mononuclear five-coordinate complexes as shown by UV–Vis spectroscopy and Fast Atom Bombardment Mass Spectrometry. The complexes were tested for their reactivity towards the oxidation of catechol to quinone by following the appearance of quinone spectrophotometrically. The complexes show differing rates of reaction depending on the nature of the exogenous fifth ligand with the azido complex showing the fastest rate of oxidation.

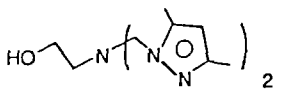
Introduction

The synthesis of organic ligands which incorporate many of the structural features thought to be present in naturally occurring metalloproteins has led to an increased understanding of a number of different proteins. Included among these are the Type III copper proteins tyrosinase and hemocyanin. Chemical and spectroscopic studies [1–3] indicate that tyrosinase is a binuclear copper containing enzyme with three histidine residues per copper and a bridging oxygen present in the oxidized form. The enzyme is capable of catalyzing the two-electron oxidation of catechol to quinone as shown in eqn. (1).



(1)

We are currently investigating the preparation of both binuclear and related mononuclear Cu(II) complexes to increase the understanding of the requirements of the coppers towards organic ligands in these systems. In this paper we describe the synthesis of a new tetradentate tripodal organic ligand, **1**, which can act by donating three nitrogens and one oxygen atom to a single metal center.



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We have prepared copper(II) complexes of **1** with a variety of exogenous ligands in the fifth site. In this paper we describe the synthesis, spectral and reactivity properties of the Cu(II) complexes prepared. We have also looked at their reactivity as catalysts for the aerobic oxidation of catechol to quinone and studied the kinetics of this reaction.

Experimental

All reagents and solvents were purchased from commercial sources and used as received unless noted otherwise. 1-(hydroxymethyl)-3,5-dimethylpyrazole was prepared by the literature method [4]. Melting points were obtained with the use of a Fisher-Johns apparatus and are uncorrected. Chemical analyses were performed at Desert Analytical, Tucson, AZ.

Electronic spectra and the oxidation studies were performed on a Uvikon 860 spectrophotometer. IR spectra were taken on a Nicolet 5ZDX instrument. Mass spectra were run at the Midwest Center for Mass Spectrometry in Lincoln, NE. ^1H NMR were recorded on a Varian T-60 instrument using $CDCl_3$ as the solvent. All Chemical shifts are reported in parts per million (ppm) relative to an internal standard of Me_4Si.

Kinetics were followed spectrophotometrically on a Uvikon 860 spectrophotometer by following the appearance of quinone as a function of time using the 390 nm peak. The metal complex (0.3 ml of a 1×10^{-3} M methanol solution) and a 2.0 ml solution (1.0×10^{-1} M methanol solution) of catechol were added together in the spectrophotometric cell at 25 °C.

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Synthesis

N,N-Bis(3,5-dimethylpyrazol-1-ylmethyl)-1-hydroxy-2-aminoethane (bpmhe)

A solution of 1.20 g (19.5 mmol) of 1-aminoethanol and 5.00 g (39.0 mmol) of 1-(hydroxymethyl)-3,5-dimethylpyrazole in 50 ml of CH_3CN was stirred in a sealed vessel at room temperature for 24 h. The CH_3CN was separated from the water, dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The resultant oil was crystallized from ethyl acetate/hexane to give 4.87 g (90%) of white crystals: melting point 78–80 °C. ^1H NMR 2.20 (12H, s), 3.02 (2H, t), 3.65 (2H, t), 4.93 (4H, s), 5.57 (1H, s), 5.75 (2H, s). *Anal.* Calc. for $C_{14}H_{23}N_5O$: C, 60.62; H, 8.49. Found: C, 60.40; H, 8.38%.

[Cu(bpmhe)H$_2$O]BF$_4$

A solution of 1.11 g (3.60 mmol) of $Cu(BF_4)\cdot 6H_2O$ in 50 ml of methanol was filtered into a solution of 1.00 g (3.60 mmol) of bpmhe in 25 ml of methanol. Upon cooling to −20 °C overnight, bright blue crystals precipitated which were filtered and washed with methanol. *Anal.* Calc. for $C_{14}H_{25}BCuF_4N_5O_2$: C, 37.80; H, 5.45; N, 15.73. Found: C, 37.41; H, 5.33; N, 15.64%. IR (KBr; cm^{-1}): 3420m, 1555w, 1470m, 1061s. UV–Vis (CH_3OH; λ_{max}, nm (ϵ, $M^{-1}\,cm^{-1}$)): 884(70), 707(16), 231(2800).

[Cu(bpmhe)Br]Br

The same procedure as described for [Cu(bpmhe)H$_2$O]BF$_4$ was followed using $CuBr_2$ resulting in the isolation of green crystals. *Anal.* Calc. for $C_{14}H_{23}Br_2CuN_5O$: C, 33.65; H, 4.45; N, 14.00. Found: C, 33.72; H, 4.66; N, 13.47%. IR (KBr; cm^{-1}): 3401m, 1393w. UV–Vis (CH_3OH; λ_{max}, nm (ϵ, $M^{-1}\,cm^{-1}$)): 884(150), 774(110), 295(2810), 246(3580).

[Cu(bpmhe)Cl]Cl

The same procedure as described for [Cu(bpmhe)H$_2$O]BF$_4$ was followed using $CuCl_2$ resulting in the isolation of light green crystals. *Anal.* Calc. for $C_{14}H_{23}Cl_2CuN_5O$: C, 40.93; H, 5.91; N, 17.04. Found: C, 40.40; H, 6.21; N, 16.42%. IR (KBr; cm^{-1}): 3450m, 1340m. UV–Vis (CH_3OH; λ_{max}, nm (ϵ, $M^{-1}\,cm^{-1}$)): 884(110), 749(70), 270(3240).

[Cu(bpmhe)NO$_3$]NO$_3$

The same procedure as described for [Cu(bpmhe)H$_2$O]BF$_4$ was followed using $Cu(NO_3)_2$ resulting in the isolation of blue crystals. *Anal.* Calc. for $C_{14}H_{23}CuN_7O_7$: C, 36.19; H, 5.00; N, 21.07. Found: C, 36.43; H, 4.89; N, 20.73%. IR (KBr; cm^{-1}): 3320m, 1422s, 1386m, 1329m. UV–Vis (CH_3OH; λ_{max}, nm (ϵ, $M^{-1}\,cm^{-1}$)): 887(74), 703(61), 239(3210).

[Cu(bpmhe)N$_3$]BF$_4$

To a solution of 1.0 g (2.2 mmol) of [Cu(bpmhe)H$_2$O]BF$_4$ in 25 ml of methanol was filtered 0.15 g (2.2 mmol) of NaN_3 in 20 ml of H_2O. Upon standing at room temperature overnight emerald green crystals were formed which were filtered and washed with methanol. *Anal.* Calc. for $C_{14}H_{23}BCuF_4N_8O$: C, 35.87; H, 4.74; N, 23.39. Found: C, 35.79; H, 5.05; N, 23.00%. IR (KBr; cm^{-1}): 3380m, 2342s, 2048s, 1057m. UV–Vis (CH_3OH; λ_{max}, nm (ϵ, $M^{-1}\,cm^{-1}$)): 882(104), 737(80), 398(2630), 231(2910).

Results and Discussion

Synthesis

We have used a modified version of the method of Driessen [4] to prepare the organic ligand, **1**. The reaction of 1-(hydroxymethyl)-3,5-dimethylpyrazole with aliphatic amines gives an S_N2 type substitution resulting in the incorporation of two pyrazoyl methylene units added to the primary amine. This ligand was designed to mimic the met apo form of tyrosinase [5] since it provides the requisite donor atoms to one Cu(II) atom.

In earlier studies of catecholase activity of Cu(II) complexes, little attention was given to the nature of any additional donor atoms present. We decided to assess how changing the fifth donor would affect the reactivity of our complexes. Therefore, we treated our ligand with either $Cu(BF_4)_2\cdot 6H_2O$, $Cu(NO_3)_2$, $CuCl_2$, or $CuBr_2$ in methanol and isolated the copper complexes formed. Treatment of a solution of the ligand and $Cu(BF_4)_2\cdot 6H_2O$ or $CuCl_2$ with sodium azide resulted in the formation of a polymeric species. The azido derivative was therefore prepared by isolation of the tetrafluoroborate salt followed by treatment with NaN_3. The presence of the copper coordinated N_3^- at 2048 cm^{-1} in the IR is diagnostic of its terminal nature [6]. The crystalline solids had a satisfactory elemental analysis and were also analyzed by Fast Atom Bombardment Mass Spectrometry.

Mass Spectra

We have shown Fast Atom Bombardment Mass Spectrometry of Cu(I) and Cu(II) complexes to be a reliable technique for analyzing Cu(I) and Cu(II) complexes [7]. We have used this technique to help characterize the complexes prepared here both for the molecular ion present and the fragmentation patterns obtained. These Cu(II) complexes do not produce spectra directly, but show ions formed from reduction of the Cu(II) ions to Cu(I) by addition of an electron, probably either from the matrix or from the anionic ligand as it leaves.

The ligand contains a potential bridging oxygen atom which could have resulted in dimeric copper

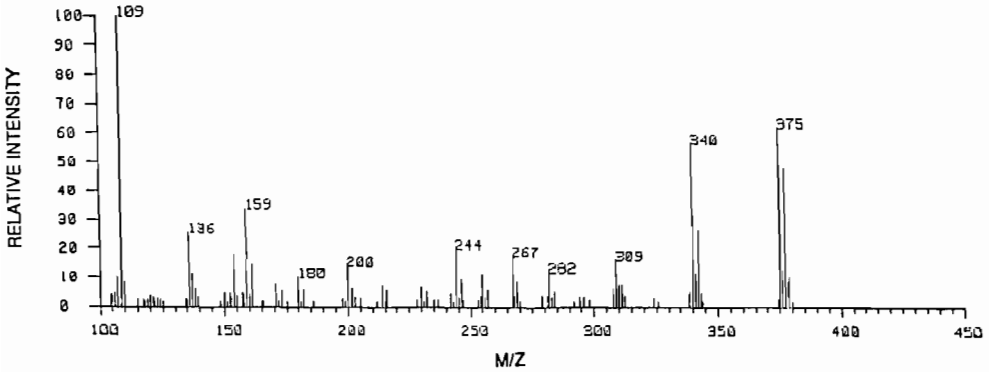

Fig. 1. Fast Atom Bombardment Mass Spectrum of [Cu(bpmhe)Cl]Cl.

complexes. In all examples of isolated metal complexes however, mass spectrometry shows them to be monomeric in the gas phase. Figure 1 shows the mass spectrum of [Cu(bpmhe)Cl]Cl as being representative of the spectra obtained. The base peak (*m/z* 109) results from the loss of one of the 3,5-dimethylpyrazol-1-ylmethyl arms from the ligand. The spectrum is characterized by the presence of the intact cation, $[Cu(bpmhe)Cl]^+$, at *m/z* 375. In this instance, the fifth ligand (Cl) shows up in the spectrum, although this is not always the case. The loss of the Cl accounts for the large peak at *m/z* 340. The typical doublets due to the presence of Cl appear in the spectrum most prominently at 375/377 and 340/342. In contrast, the $[Cu(bpmhe)N_3]BF_4$ complex does not show the presence of the azide (although it appears in the IR spectrum) but instead has a base peak at *m/z* 340 for $Cu(bpmhe)^+$.

Kinetic Studies

Tyrosinase catalyzes the two-electron oxidation of *o*-catechols to quinones. A number of Cu(II) complexes have been studied for their activity in this reaction [8–17]. It has been shown that rigidly planar mononuclear complexes are not active as catalysts while non-planar complexes will catalyze the oxidation [16]. It has been hypothesized that two coppers must approach each other to a distance of less than 5 Å for binding of the catechol to occur and for the subsequent two-electron transfer. Since our complexes are derived from non-planar ligands, we anticipated that they would catalyze this reaction. We have studied them for catecholase activity using the change in the electronic spectrum of added catechol. Quinone has a $\lambda_{max} = 390$ nm and we monitored the increase in absorbance *versus* time at this maximum.

Figure 2 shows the absorbance *versus* time spectra for the first 75 min of the reaction. Quinone is being produced in all cases but the rate is clearly dependent on the fifth ligand and/or the counterion present, with the azido compound the most active. Table 1 shows the calculated activities for the five complexes which were studied. It has been shown by Rogic that electron transfer from catechol to Cu(II) can only begin after catechol and the Cu(II) species form a Cu(II) catecholate intermediate [17]. Further-

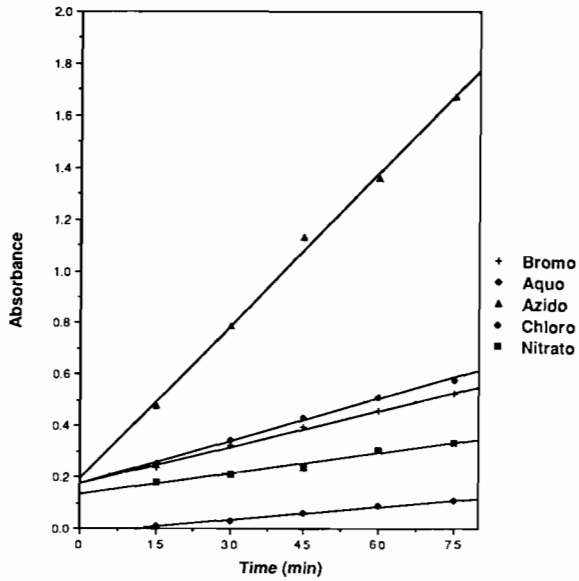

Fig. 2. Absorbance *vs.* time spectrum of the [Cu(bpmhe)X]Y complex catalyzed oxidation of catechol.

TABLE 1. Kinetic Data for the Oxidation of Catechol by [Cu(bpmhe)X]Y Complexes

Complex	Activity (μmol substrate/ mg/ catalyst per min)
$[Cu(bpmhe)H_2O]BF_4$	0.0522
[Cu(bpmhe)Br]Br	0.0412
[Cu(bpmhe)Cl]Cl	0.0164
$[Cu(bpmhe)N_3]BF_4$	0.179
$[Cu(bpmhe)NO_3]NO_3$	0.0245

more, if the coordinating ligands are bound so tightly that they cannot be replaced by catechol, the subsequent electron transfer cannot occur.

In our study, the fifth site is taken up by a series of monodentate ligands, all of which are capable of being replaced by catechol. The dissociation of this fifth ligand must be occurring before the association of the oxygen occurs so that the rate of the reaction is dependent on the ease of loss of the fifth ligand. This suggests that the azide ligand dissociates fastest resulting in enhanced rates for its reaction. Further work to understand these differences is currently in progress.

Conclusions

We have prepared a new tripodal ligand which can act as a tetradentate donor by providing three nitrogen donors and an alcohol to a metal atom. We have characterized the copper complexes by Fast Atom Bombardment Mass Spectrometry and have found evidence for either the intact cation or the cation minus the fifth anionic ligand. All of the complexes show catecholase activity but the rate is dependent on the nature of the fifth ligand. This suggests that in five-coordinate Cu(II) complexes, dissociation of one of the donor atoms occurs before the complexation of the substrate takes place. In comparing complexes derived from different ligands then, care must be taken in interpreting the results since the oxidation of catechol is dependent not only on the organic ligand present, but also on any other external ligands in the complex.

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