Novel Mono- and Binuclear Cu(II) Complexes: Synthesis, Characterization and Catecholase Activity

MITCHELL R. MALACHOWSKI* and MARILYN G. DAVIDSON
Department of Chemistry, University of San Diego, San Diego, CA 92110 (U.S.A.)
(Received November 14, 1988)

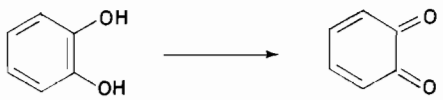
Abstract

Two new ligands, *N*,*N*-bis(3,5-dimethylpyrazol-1-ylmethyl)benzylamine (bpmba) and *N*,*N*,*N*′,*N*′-tetrakis(3,5-dimethylpyrazol-1-ylmethyl)-α,α′-diamino-*m*-xylene (bpmdx) have been synthesized. Using these ligands, copper(II) complexes of the type $[Cu(bpmba)(Cl)_2](Cl)_2$, $[Cu_2(bpmba)_2(OCH_3)_2](BF_4)_2$ and $[Cu_2(bpmdx)(X)_2]Y_2$ where X = OCH_3 or Cl and Y = Cl or BF_4 have been prepared and characterized on the basis of elemental analysis, fast atom bombardment mass spectrometry, and UV–Vis spectroscopy. These show the ligands to coordinate as an N_3 and an N_6 donor set respectively. The ability of the complexes to catalyze the oxidation of catechol to quinone was studied by following the appearance of quinone spectrophotometrically. The binuclear complex $[Cu_2(bpmdx)(OCH_3)_2](BF_4)_2$ reacts fastest with an oxidase activity of 0.467 μmol/mg min while the other three complexes have slower rates of oxidation.

Introduction

There is considerable interest in the synthesis of multidentate organic ligands which have donor atoms of biological relevance and their resultant metal complexes. Many of these compounds are prepared in attempts to mimic the behavior of various metalloproteins, such as the copper containing proteins hemocyanin and tyrosinase. This has led to the preparation of numerous Cu(I) and Cu(II) complexes in attempts to model the proteins' structural properties [1]. At the active sites of these proteins, there appears to be either two or three nitrogen donors per copper and possibly an oxygen (hydroxide) bridging group, with the nitrogen donor atoms coming from imidazoles of histidine residues [2–4]. Because of the demonstrated spectroscopic similarity of pyrazole to the naturally occurring imidazole moiety [5], we are particularly interested in preparing multidentate pyrazole containing ligands and their copper complexes in order to compare their properties to those found in the natural systems.

While the primary function of hemocyanin is to bind and transport oxygen in molluscs and arthropods, tyrosinase catalyzes the hydroxylation of monophenols and the oxidation of *o*-diphenols to *o*-quinones. The oxidation of catechol to quinone (catecholase) is shown in eqn. (1).



In tyrosinase, it is thought that two proximate metals are needed to coordinate the two hydroxyl oxygens of catechol for oxidation of quinone to proceed [6]. We are, therefore, interested in comparing the properties of mononuclear and binuclear copper complexes in an effort to better understand the biological need for binuclear systems in the metalloproteins. In order to compare mononuclear to binuclear systems, we prepared the two novel pyrazole containing ligands *N*,*N*-bis(3,5-dimethylpyrazol-1-ylmethyl)benzylamine (bpmba) (**1**) and *N*,*N*,*N*′,*N*′-tetrakis(3,5-dimethylpyrazol-1-ylmethyl)-α,α′-diamino-*m*-xylene (bpmdx) (**2**).

In this paper we describe the synthesis of the two ligands, their resultant Cu(II) complexes, and the reactivity of these complexes towards catechol in an attempt to model the activity of the copper containing enzyme tyrosinase.

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 [7]. Melting points were obtained with the use of a Fisher–Johns apparatus. Chemical analyses were performed at Desert Analytical, Tucson, AZ.

Electronic spectra were performed on a Uvikon 860 spectrophotometer and IR spectra were taken

*Author to whom correspondence should be addressed.

0020-1693/89/$3.50

on a Nicolet 5ZDX instrument. Mass spectra were run at the Midwest Center for Mass Spectrometry in Lincoln, NE. ^{1}H 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 using the 390 nm peak.

N,N-Bis(3,5-dimethylpyrazol-1-ylmethyl)benzylamine (bpmba) (1)

A solution of 1.27 g (12.0 mmol) of benzylamine and 3.00 g (24.0 mmol) of 1-(hydroxymethyl)-3,5-dimethylpyrazole [7] in 50 ml of acetonitrile were stirred in a sealed vessel at room temperature for 24 h. The acetonitrile was separated from the water produced, dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. Thin layer chromatography showed the compound to be of sufficient purity to be used directly in the synthesis of the metal complexes. An analytically pure sample was isolated by dissolving **3** in H_2O/CH_3OH, adding an excess of EDTA, and stirring for 2 h. The CH_3OH was evaporated and the solution was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered, and the solvent evaporated to give a colorless oil. ^{1}H NMR 1.97 (6 H, s), 2.19 (6 H, s), 3.72 (2 H, s), 4.92 (4 H, s), 5.77 (2 H, s), 7.10–7.41 (5 H, m). MS, *m/e* 323 (M^+).

N,N,N',N'-Tetrakis(3,5-dimethylpyrazol-1-ylmethyl)-α,α'-diamino-m-xylene (bpmdx) (2)

The same procedure as described above for bpmba was followed using 5.00 g (39.6 mmol) of 1-(hydroxymethyl)-3,5-dimethylpyrazole and 1.30 g (9.90 mmol) of α,α'-diamino-*m*-xylene. An analytically pure sample was isolated by dissolving **5** in H_2O/CH_3OH, adding an excess of EDTA, and stirring for 2 h. The CH_3OH was evaporated and the solution was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4, filtered, and the solvent evaporated to give a colorless oil. ^{1}H NMR 1.96 (12 H, s), 2.19 (12 H, s), 3.69 (4 H, s), 4.89 (8 H, s), 5.70 (4 H, s), 7.02–7.40 (4 H, m). MS, *m/e* 568 (M^+).

[Cu$_2$(bpmba)$_2$(OCH$_3$)$_2$](BF$_4$)$_2$ (3)

A solution of 1.50 g (5.00 mmol) of bpmba in 20 ml of methanol was treated with a solution of 1.73 g (5.00 mmol) of $Cu(BF_4)_2 \cdot 6H_2O$ in 30 ml of methanol. The resulting solution was filtered and cooled to −20 °C. Large light blue crystals formed which were filtered and washed with methanol to give 1.20 g (42%) of **3**. *Anal.* Calc. for $C_{40}H_{56}B_2F_8Cu_2N_{10}O_2$: C, 47.58; H, 5.60; N, 13.87. Found: C, 47.42; H, 5.41; N, 13.78%. UV–Vis (CH_3OH); λ_{max} (ϵ (M^{-1} cm^{-1})): 670 (565), 550 (295), 370 (865), 346 (645), 279 (2950).

[Cu(bpmba)(Cl)$_2$] (4)

The same procedure as described above for [Cu$_2$(bpmba)$_2$(OCH$_3$)$_2$](BF$_4$)$_2$ was followed using $CuCl_2$ and resulted in the isolation of dark green crystals. *Anal.* Calc. for $C_{19}H_{25}Cl_2CuN_5$: C, 49.84; H, 5.51; N, 15.29. Found: C, 49.64; H, 5.61; N, 15.07%. UV–Vis (CH_3OH); λ_{max} (ϵ (M^{-1} cm^{-1})): 847 (115), 275 (5165).

[Cu$_2$(bpmdx)(OCH$_3$)$_2$](BF$_4$)$_2$ (5)

A solution of 2.00 g (3.52 mmol) of bpmdx in 20 ml of methanol was treated with a solution of 2.43 g (7.04 mmol) of $Cu(BF_4)_2 \cdot 6H_2O$ in 30 ml of methanol. The resulting solution was cooled to −20 °C from which light blue crystals precipitated. *Anal.* Calc. for $C_{34}H_{48}B_2Cu_2F_8N_{10}O_2$: C, 43.83; H, 5.42; N, 15.02. Found: C, 43.34; H, 5.50; N, 15.10%. UV–Vis (CH_3OH); λ_{max} (ϵ (M^{-1} cm^{-1})): 833 (186), 357 (5867), 312 (5614).

[Cu$_2$(bpmdx)(Cl$_2$)](Cl)$_2$·2HCl (6)

The same procedure as described above for [Cu$_2$(bpmdx)(OCH$_3$)$_2$](BF$_4$)$_2$ was followed using $CuCl_2$ which resulted in isolation of dark green crystals. *Anal.* Calc. for $C_{32}H_{46}Cl_6Cu_2N_{10}$: C, 42.21; H, 5.10; N, 15.38. Found: C, 42.57; H, 5.30; N, 15.50%. UV–Vis (CH_3OH); λ_{max} (ϵ (M^{-1} cm^{-1})): 845 (85), 271 (5143).

Results and Discussion

Synthesis

The presence of six-membered chelate rings in many of the copper complexes used to model the Type III systems allows the two coppers to maintain a distance of less than 3.1 Å. In the proteins hemocyanin and tyrosinase, however, the distance is thought to be *c.* 3.6 Å. The introduction of five-membered rings in our compounds was performed in order to increase the steric constraints present in the ligand thereby increasing the metal–metal distance [8]. Confirmation of the structural results of this modification await the solving of the crystal structures of these compounds.

The two new ligands were prepared using a modification of the general procedure of Driessen for the addition of pyrazol-1-ylmethylene units to amines [7]. The condensation of 1-(hydroxymethyl)-3,5-dimethylpyrazole and the appropriate amine (benzylamine or α,α'-diamino-*m*-xylene) resulted in ligands which can act as tridentate or sexadentate donors respectively, as illustrated in Fig. 1.

Stirring a solution of the ligand with the copper(II) salt resulted in the formation of the copper complexes. Complexes of the binucleating ligand **2** were formed as expected with either $^-OCH_3$ or ^-Cl bridging the two copper atoms and giving complexes

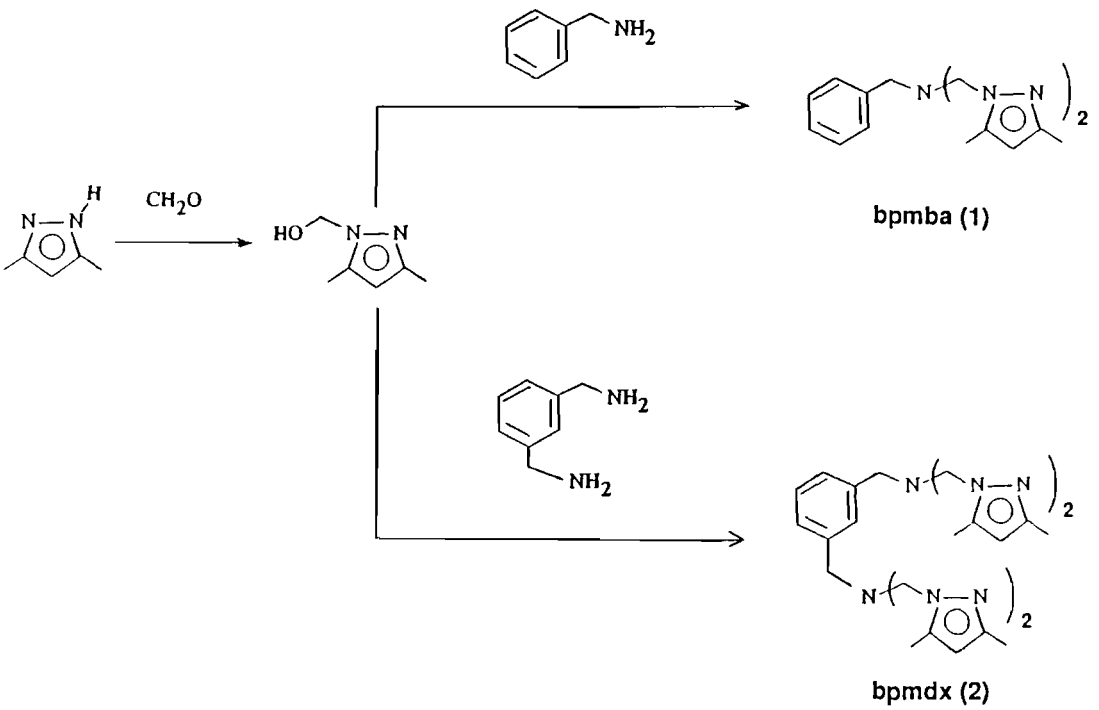


Fig. 1. Synthesis of bpmba (1) and bpmdx (2) ligands.

with the formula $[Cu_2(bpmdx)(X)_2](Y)_2$ (where X = $^-OCH_3$ or ^-Cl and Y = BF_4 or Cl). In contrast, complexes formed from the N_3 ligand **1** gave two kinds of complexes. When treated with $CuCl_2$, a mononuclear complex of the form $[Cu(bpmba)(Cl)_2]$ was formed with two terminal chloride atoms. When $Cu(BF_4)\cdot 6H_2O$ was the copper precursor, a binuclear complex $[Cu_2(bpmba)_2(OCH_3)_2](BF_4)_2$ was formed, certainly due to the noncoordinating nature of the BF_4 anions.

The crystalline solids had satisfactory elemental analysis and were also analyzed by fast atom bombardment mass spectrometry (FAB-MS). The free ligands were obtained by treating an aqueous solution of the copper complex with EDTA followed by extraction with chloroform. The ligands were characterized by ^1H NMR, IR and mass spectrometry.

Mass Spectrometry

We have shown FAB-MS of Cu(I) and Cu(II) complexes to be a reliable technique for analyzing metal complexes [9]. This technique has been used here to help characterize the complexes prepared using both the molecular ion present and the fragmentation patterns obtained. In addition, we have used high resolution electron impact mass spectrometry to gather additional information about the nature of the complexes.

FAB-MS of the copper(II) complexes gave us considerable information regarding the exact nature of our isolated complexes. Cu(II) complexes do not produce spectra directly but show ions formed from reduction of Cu(II) to Cu(I) by addition of an electron. Samples containing two Cu(II) ions are reduced formally at both metal centers. The mass spectrum of the $[Cu_2(bpmdx)(Cl)_2](Cl)_2$ complex is shown in Fig. 2 and the data for the other copper complexes is found in Table 1.

The base peak for most of the copper complexes which we have studied occurs at *m/z* 109. This results from the loss of one of the 3,5-dimethylpyrazol-1-ylmethyl arms from the ligands and we use this peak as diagnostic for the presence of the pyrazole arms [10]. The spectrum of $[Cu_2(bpmdx)(Cl)_2](Cl)_2$ does not show a peak for the intact cation but instead has a peak at 801 as its highest mass peak. This results from the loss of one of the chlorine atoms from the molecule. Sequential loss of two additional chlorines gives the peaks at *m/z* 766 (which results from the presence of $[Cu_2(bpmdx)(Cl)_2]^+$ and at *m/z* 731. A peak from the loss of the remaining chlorine does not appear, but instead the loss of both the chlorine and one of the copper atoms gives the peak at *m/z* 631 due to $[Cu(bpmdx)]^+$. At lower mass units there are many similarities in the spectrum to that found for the bpmdx ligand and these peaks are identifiable as ligand fragmentations.

The FAB mass spectrum of $[Cu_2(bpmdx)(CH_3OH)_2](BF_4)_2$ also does not show a peak for the intact cation but instead shows the cation plus fluorine (from the anion) at *m/z* 775. The presence

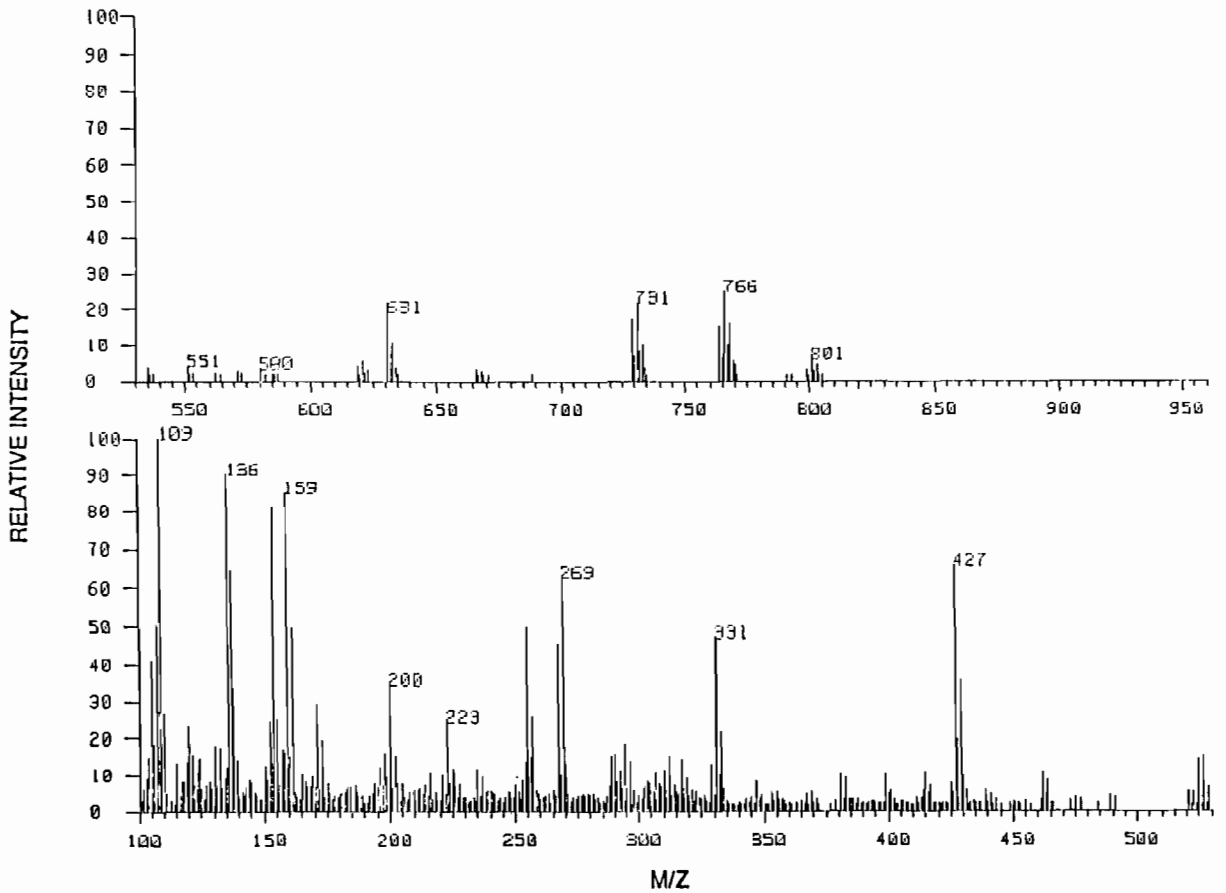


Fig. 2. Fast atom bombardment mass spectrum of $[Cu_2(bpmdx)(Cl)_2](Cl)_2$.

TABLE 1. Fast atom bombardment mass spectra

Compound	*m/z*	Origin
3	109	$[C_6H_9N_2]^+$
	386 (base)	$[Cu(bpmba)]^+$
	803	$[Cu_2(bpmba)_2(OCH_3)]^+$
	834	$[Cu_2(bpmba)_2(OCH_3)_2]^+$
4	109 (base)	$[C_6H_9N_2]^+$
	386	$[Cu(bpmba)]^+$
	421	$[Cu(bpmba)(Cl)]^+$
5	109 (base)	$[C_6H_9N_2]^+$
	631	$[Cu(bpmdx)]^+$
	713	$[Cu_2(bpmdx)(F)]^+$
	744	$[Cu_2(bpmdx)(OCH_3)(F)]^+$
	775	$[Cu_2(bpmdx)(OCH_3)_2(F)]^+$
6	109 (base)	$[C_6H_9N_2]^+$
	631	$[Cu(bpmdx)]^+$
	731	$[Cu_2(bpmdx)(Cl)]^+$
	766	$[Cu_2(bpmdx)(Cl)_2]^+$

of the F atom in the mass spectrum was unexpected but we confirmed its presence through the use of high resolution electron impact mass spectrometry. The nature of the bridging ligands is clearly shown by FAB to be $^-OCH_3$ as seen by the M-31 peak at *m/z* 744 and by a second M-31 peak at *m/z* 713. This technique can therefore act as a powerful alternative to other spectroscopic techniques for partial structure determination.

The mononuclear nature of $[Cu(bpmba)(Cl)_2]$ can be ascertained from the mass spectrum. The highest mass peak is found at *m/z* 421 and can be assigned to $[Cu(bpmba)(Cl)]^+$. In contrast, $[Cu_2(bpmba)_2(OCH_3)_2](BF_4)_2$ shows peaks indicative of its binuclear nature along with peaks derived from fragmentations to the mononuclear species.

Kinetics Studies

As stated earlier, we are interested in studying the reactivity of copper(II) complexes towards catechol, since this is one of the functions of tyrosinase. In order to compare the differences between mono- and binuclear complexes, we have studied our copper complexes for catecholase activity using the change in the electronic spectrum of added catechol. Kida and co-workers have studied a number of Cu(II) complexes and have shown that

binuclear complexes catalyze the reaction while mononuclear species are either not as efficient or inactive depending on the steric demands of the ligands [11]. Additionally, a recent report [12] showed that the Cu(II) complex of *ortho*-hydroxycinnamic acid performed this oxidation with an oxidase activity of 0.088 U/mg (as μmol of substrate catechol transformed in 1 min at 25 °C).

Quinone has a λ_{max} = 390 nm and we monitored the increase in absorbance versus time at this wavelength. 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. Formation of quinone was monitored by the increase in absorbance at 390 nm as a function of time. In all cases, catecholase activity was noted. Figure 3 shows the absorbance versus time spectrum for the first 60 min of the reaction for the four Cu(II) complexes while the activities are shown in Table 2.

As shown in Table 2, the two complexes **5** and **6** which were derived from the binucleating ligand were the most active catalysts for the oxidation of catechol to quinone. However, even within complexes

TABLE 2. Kinetic data for the oxidation of catechol by copper complexes

Complex	Activity (μmol substrate/mg catalyst per min)
$[Cu_2(bpmdx)(OCH_3)_2](BF_4)_2$	0.467
$[Cu_2(bpmdx)(Cl)_2](Cl)_2$	0.136
$[Cu_2(bpmba)_2(OCH_3)_2](BF_4)_2$	0.0546
$[Cu(bpmba)(Cl)_2]$	0.0106

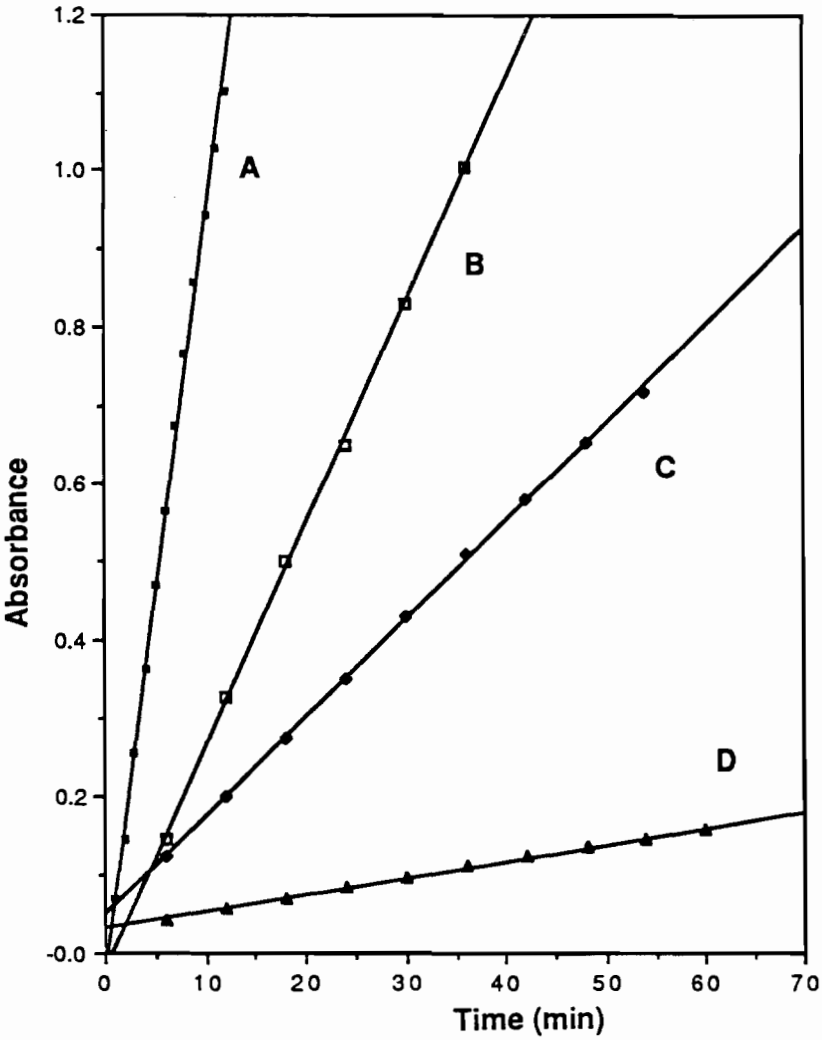


Fig. 3. Plot of absorbance vs. time for copper catalyzed oxidation of catechol: (A) $[Cu_2(bpmdx)(OCH_3)_2](BF_4)_2$; (B) $[Cu_2(bpmdx)(Cl)_2](Cl)_2$; (C) $[Cu_2(bmpba)_2(OCH_3)_2](BF_4)_2$; (D) $[Cu(bpmba)(Cl)_2]$.

prepared from the same ligand, substantial changes in reactivity are found which depend on the nature of the bridging groups and the counterions present. This is a result which is similar to that which we have seen in our studies of tripodal ligands and their Cu(II) complexes [10].

Complex **3**, although derived from a mononucleating ligand, was shown to be binuclear in nature and was found to be intermediate in its reactivity towards catechol. This can be explained by assuming that in the oxidation of catechol, dissociation of the bridging groups must occur prior to the complexation of the substrate. When this occurs for complex **3**, there are no longer any atoms holding the coppers together and a mononuclear complex is formed in solution which would slow down its reactivity. This will not be the case for **5** and **6** because of the binuclear nature of the bpmdx ligand. The only true mononuclear complex, **4**, was the least active of the complexes studied. This can be explained by invoking the need for two coppers to be proximate for the binding of the two phenolic oxygens of the catechol for the two-electron redox reaction to occur.

Conclusions

We have prepared two new ligands with an N_3 and an N_6 donor set and their Cu(II) complexes. Complexes derived from the binucleating ligand bpmdx are much more active in their oxidation of catechol. Although all of the complexes show catecholase activity, the rate is dependent not only on whether the complex is mononuclear or binuclear but also on the nature of any exogeneous ligands present. Therefore, care must be exercised in comparing rates from complexes which are drastically different from each other. For a true comparison of the effect of the organic ligand on the rate of the reaction, the exogenous ligands must be the same. We are currently working on assessing the effect of other bridging and terminal donors on the reaction, changing the nature of the substrate being oxidized, and changing the concentrations of the reactants in an attempt to determine the mechanism of this oxidation.

Acknowledgements

We thank the University of San Diego faculty grants program for support of this work and the Arco Foundation for funds to purchase the Uvikon UV–Vis spectrophotometer. We express our gratitude to Ronald Cerny for assistance with the mass spectral results.

References

1 K. D. Karlin and Y. Gultneh, *Prog. Inorg. Chem., 35* (1987) 219.
2 N. C. Eickman, E. I. Solomon, J. A. Larrabee, T. G. Spiro and K. Lerch, *J. Am. Chem. Soc., 100* (1978) 6259.
3 R. S. Himmelwright, N. C. Eickman, C. D. LuBien, K. Lerch and E. I. Solomon, *J. Am. Chem. Soc., 102* (1980) 7339.
4 E. I. Solomon, in T. G. Spiro (ed.), *Copper Proteins*, Wiley-Interscience, New York, Ch. 2.
5 E. Bernarducci, W. F. Schwindinger, J. L. Hughey, K. Krogh-Jespersen and H. J. Schugar, *J. Am. Chem. Soc., 103* (1981) 1686.
6 D. E. Wilcox, A. G. Porras, Y. T. Hwang, K. Lerch, M. E. Winkler and E. I. Solomon, *J. Am. Chem. Soc., 107* (1985) 4015.
7 W. L. Driessen, *Recl. Trav. Chim. Pays-Bas, 101* (1982) 441.
8 T. N. Sorrell, C. J. O'Connor, O. P. Anderson and J. H. Reibenspies, *J. Am. Chem. Soc., 107* (1985) 4199.
9 R. L. Cerny, M. M. Bursey, D. L. Jameson, M. R. Malachowski and T. N. Sorrell, *Inorg. Chim. Acta, 89* (1984) 89.
10 M. R. Malachowski, M. G. Davidson and J. N. Hoffman, *Inorg. Chim. Acta, 157* (1989) 91.
11 S. Kida, H. Okawa and Y. Nishida, in K. D. Karlin and J. Zubieta (eds.), *Copper Coordination Chemistry: Biochemical and Inorganic Perspectives*, Adenine, Guilderland, NY, 1983, p. 425.
12 M. A. Cabras and M. A. Zoroddu, *Inorg. Chim. Acta, 135* (1987) L19.