Characterization and catecholase-mimetic behavior of imidazole adducts of copper(II) valproate. Crystal...

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Synthesis, Characterization, and Oxidase Activities of Copper(II) Complexes of the Anticonvulsant Drug Valproate

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ABSTRACT

The interaction of binuclear copper(II) complex of the anticonvulsant drug valproate, $Cu_2(valp)_4$ (**1**), with metronidazole (mtnd) or 2-methyl-5-nitrobenzimidazole (2m5nbnz), have led to the isolation of two binuclear adducts of the type $Cu_2(valp)_4(mtnd)_2$ (**2**), and $Cu_2(valp)_4(2m5nbnz)_2$ (**3**), and one mononuclear adduct of the type $Cu(valp)_2(2m5nbnz)_2$ (**4**). Spectral and magnetic data and preliminary X-ray measurements for complexes **2** and **3** are consistent with a binuclear structure as found for copper(II) tetracarboxylate adducts. The above data for complex **4** are consistent with a mononuclear square planar complex that contains two valproate ligands and two N-containing 2m5nbnz ligands to give essentially a *trans*-CuN_2O_2 chromophore. The catalytic activities of the complexes toward the aerobic oxidation of 3,5-di-t-butylcatechol to the corresponding o-benzoquinone and N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) to $TMPD^+$ were determined. The activities were found to be in the order **1** > **2** > **3** > **4**. The formation of a copper ion-semiquinone species in solution, which may be the catalytic intermediate that reacts directly with oxygen, was demonstrated spectrophotometrically.

INTRODUCTION

Binuclear copper(II) carboxylates with a variety of oxygen and nitrogen donor ligands of $Cu_2(O_2CR)_4.L_2$ stoichiometry have been extensively investigated, and the results of their magnetic and spectral properties have been reviewed [1]. In the case of some nitrogen donor ligands, especially imidazoles, a number

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of copper(II) carboxylates form mononuclear *cis* or *trans* bis-adducts of $Cu(O_2CR)_2.L_2$ stoichiometry [2–5] and tetrakis-adducts of $Cu(O_2CR)_2.L_4$ stoichiometry [6, 7]. Several studies have been designed to investigate the factors that influence the adoption of mononuclear over binuclear in copper(II) carboxylate adducts [2–9]. The results of these studies showed the dependence of structure on subtle electronic properties of the carboxylate groups, as well as the electronic and steric properties of the added bases. It has been generally found that by increasing the acidity of the carboxylate groups and/or the basicity of the added ligands, the tendency towards the formation of mononuclear adducts increases [1c, 2–9].

Valproic acid (2-propylpentanoic acid) in the form of its sodium salt has a wide spectrum of activity as an anticonvulsant drug [10]. Copper(II) complexes of anticonvulsant and antiinflammatory ligands have been found to be more active and desirable drugs than the parent ligands themselves [11]. Physical studies of copper(II) valproate [12] have shown that it contains binuclear units with four bridging carboxylate ligands to two copper(II) ions and similar to other copper(II)carboxylates [1]. The only known binuclear monoadducts of copper(II) valproate are those with pyridine and aniline [12a]. The pyridine adduct is unstable, and its crystal subjected to X-ray structure analysis was sealed in a glass capillary to avoid decomposition during data collection [12b]. Recently, we reported the synthesis and characterization of four mononuclear copper(II) valproate adducts with imidazoles [4]. These complexes are found to exist as *trans* or *cis* bis-adducts having essentially CuN_2O_2 chromophore.

Interest in developing small molecular weight copper(II) complexes as model copper oxidase enzymes had led to the synthesis of both mononuclear and binuclear complexes [13–17], including copper(II) carboxylate adducts with imidazole type ligands [4, 5, 18–21]. The idea is that these complexes might mimic the behavior of various metalloproteins such as the copper-containing proteins type I, II, and III. In addition, some binuclear and mononuclear copper(II) carboxylate adducts with imidazole-type ligands have been found to have a variety of pharmacological effects such as antitumor [22], superoxide dismutase [23], and catecholase activities [4, 5, 19–21]. Recently, we reported the synthesis and characterization of several mononuclear and binuclear copper(II) carboxylates, and studied their catalytic properties in the aerobic oxidation of catechol to the corresponding o-benzoquinone [4, 5, 19–21] as models for catecholase function of the copper-containing enzyme tyrosinase [24]. In addition to the dependence of the structure of copper(II) carboxylates on the electronic and steric properties of added base, the rate of oxidation of catechol to o-benzoquinone was also affected by the nature of added base [4, 5, 13, 19, 20]. Therefore, structural and electronic factors that might impact the properties of copper(II) complexes as metalloprotein models are of interest. Since nitroimidazoles are used as chemotherapeutic agents in the treatment of bacterial infections and as radiosensitizers [25], metronidazole (1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole) and 2-methyl-5-nitrobenzimidazole are used in this study to form copper(II) valproate complexes. In addition, we examined the catalytic activities of these complexes for the aerobic oxidation of N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) to $TMPD^+$ and 3,5-di-*t*-butylcatechol to 3,5-di-*t*-o-benzoquinone as models for copper-containing oxidase enzymes.

EXPERIMENTAL

All chemicals were of high purity grade (Aldrich or Sigma Chemical Co.) and were used without further purification. Tetrakis-μ-valproato dicopper(II) [$Cu_2(valp)_4$], *(1)* was prepared as described previously [12] and recrystallized from absolute ethanol.

Preparation of Complexes

Bis(metronidazole) tetrakis (μ-valproato) dicopper(II), $Cu_2(valp)_4(mtnd)_2$, (2). A solution of 0.5 g (2.92 mmol) metronidazole in 80 mL methanol was added to 0.5 g (0.71 mmol) of $Cu_2(valp)_4$. The mixture was stirred and refluxed for 3 h. The green solution was filtered under reduced pressure, and the green filtrate was concentrated by slow evaporation in the hood to ca. 10 mL and filtered. Slow evaporation of the filtrate produced green crystals which were recrystallized from chloroform. Anal. Calc. for $C_{44}H_{78}N_6O_{14}Cu_2$: C, 50.72; H, 7.49; N, 8.07. Found: C, 50.33; H, 7.47; N, 8.11%.

Bis(2-methyl-5-nitrobenzimidazole) tetrakis (μ-valproato) dicopper(II), $Cu_2(valp)_4$ $(2m5nbnz)_2$, (3). A solution of 0.2532 g (1.43 mmol) of 2-methyl-5-nitrobenzimidazole in 140 mL warm chloroform was added to 0.5 g (0.71 mmol) of $Cu_2(valp)_4$. The mixture was stirred at about 50°C for 3 h. The green solution was filtered while hot and left in the hood to evaporate. The green precipitate that formed was recrystallized from absolute ethanol and air dried. Anal. Calc. for $C_{48}H_{74}N_6O_{12}Cu_2$: C, 54.70; H, 7.03; N, 7.98. Found: C, 54.53; H, 6.99; N, 7.91%.

Bis(2-methyl-5-nitrobenzimidazole) bis(valproato) copper(II), $Cu(valp)_2(2m5nbnz)_2$, (4). A solution of 0.51 g (2.88 mmol) of 2-methyl-5-nitrobenzimidazole in 120 mL warm normal butanol was added to 0.5 g (0.71 mmol) of $Cu_2(valp)_4$. The mixture was stirred for 3 h. The dark green solution was filtered under reduced pressure and left in the hood to evaporate. The purple precipitate that formed was purified by dissolving it in cold methanol, filtration, and evaporation of the solution to dryness. Recrystallization from methanol/hexanes (3:1) produced purple crystals. Anal. Calc. for $C_{32}H_{44}O_8N_6Cu$: C, 54.58; H, 6.25; N, 11.94. Found: C, 54.75; H, 6.47; N, 12.04%.

Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN, U.S.

Physical Measurements

Solution room-temperature (298 K) magnetic moments were determined by the Evans method [27] with a Bruker AC250 MHz NMR spectrometer. Ethanol was used as the solvent and TMS as the reference. The effective magnetic moment is related to the reference shift, $\Delta\nu$ (Hz) at any temperature by the expression $\mu_{eff} = 0.0618(\Delta\nu.T/\nu M)^{1/2}$, where ν is the NMR frequency in MHz and M is the molarity of the paramagnetic substance.

Electronic spectra of chloroform or ethanol solutions were obtained with a Hewlett Packard 8425A diode array or Bausch and Lomb 2000 spectrophotometers. Nujol mulls sealed between polyethylene sheets were used to obtain IR spectra in the 4000–450 cm^{-1} region with an FTS-7 Bio-Rad SPC 3200 Fourier

transform infrared spectrometer. X-band EPR spectra of polycrystalline material and of ethanol/toluene solutions were obtained at room temperature and at liquid nitrogen temperature with a JEOL Jes-PE-1X spectrometer. Diphenylpicrylhydrazide (DPPH, g = 2.0036) was used as the calibrating field marker.

Catalytic Activities

The catalytic activities of these complexes for the oxidation of TMPD (N,N,N′,N′-tetramethyl-p-phenylenediamine) in air were followed spectrophotometrically by monitoring the increase in the $TMPD^+$ absorbance at 565 nm as a function of time [26]. Methanol solutions of the copper(II) complex (1.3 mL of a 0.5×10^{-4} M) and 1.3 mL of a methanol solution (2.5×10^{-3} M) of TMPD were combined in a 1 cm quartz cell at 298 k, and the absorbance changes at 565 nm were recorded. The catecholase-mimetic activities of the complexes for the aerobic oxidation of 3,5-di-*t*-butylcatechol (DTBC) to corresponding 3,5-di-*t*-obenzoquinone(DTBQ) were followed spectrophotometrically by monitoring the increase in the absorbance at 400 nm as a function of time. The metal complex (0.5 mL of a 2×10^{-4} M methanol solution) and 2.5 mL of a methanol solution of DTBC (0.04 M) were added together in a 1 cm spectrophotometric cell at 298 K, and the absorbance changes at 400 nm were recorded.

RESULTS AND DISCUSSION

The binuclear compound *2* was obtained from the reaction of metronidazole (mtnd) with $Cu_2(valp)_4$ in either ratio 2:1 or 4:1, but the binuclear compound *3* was obtained from the reaction of 2-methyl-5-nitrobenzimidazole (2m5nbnz) with $Cu_2(valp)_4$ in ratio 2:1, while ratio 4:1 produced the mononuclear compound *4*. The compounds are generally soluble in alcohols, and in addition, binuclear compounds are also soluble in chloroform and dichloromethane.

Magnetic and Spectroscopic Characterization

The effective magnetic moments and electronic and IR spectral data are summarized in Table 1. The room temperature magnetic moments for complexes *2* and *3* are in the range 1.18–1.26 BM. These subnormal values are significantly lower than the spin-only value of 1.73 BM, suggesting that substantial coupling between the copper atoms occurs. These values are comparable to the magnetic moment values of binuclear copper(II) carboxylate adducts of the type$[Cu(RCOO)_2L]_2$ [1], including that reported for the structurally known binuclear adduct of copper(II) valproate with pyridine [12b]. The room temperature magnetic moment for complex *4* (1.84 BM) is consistent with the presence of one unpaired electron in a mononuclear copper(II) complex.

The electronic spectra in chloroform solutions of the compounds *2* and *3* exhibit one broad band near 700 nm (Table 1). This band can be assigned to copper(II) d–d transitions. These complexes do not exhibit the second band at about 370 nm, the charge transfer band that is considered to be diagnostic of binuclear copper(II) adducts with bridging carboxylates [1, 12]. This band may be obscured by the very intense ligand-to-metal charge transfer band in the range 300–330 nm due to the presence of metronidazole or 2-methyl-5-nitrobenzimidazole ligands in these binuclear compounds. The position of the d–d

TABLE 1. Magnetic Moments and Electronic and IR Spectral Data for Cu(II) Complexes

Compound	μ_{eff} (BM) (298 K)	λ_{max} (nm) ($\varepsilon = dm^3 \cdot mol^{-1} \cdot cm^{-1}$)	ν_{asym} (CO_2) (cm^{-1})	ν_{sym} (CO_2) (cm^{-1})
$Cu_2(valp)_4$, *(1)*	1.33	671(410) 375(146)	1580	1422
$Cu_2(valp)_4(mtnd)_2$, *(2)*	1.18	708(364)	1610	1418
$Cu_2(valp)_4$ $(2m5nbnz)_2$, *(3)*	1.26	720(420)	1610	1415
$Cu(valp)_2$ $(2m5nbnz)_2$, *(4)*	1.84	688(200)	1630 1606	1417

transitions and the magnitude of their molar absorptivities are in the range expected for binuclear copper(II) carboxylate adducts having bridging carboxylate groups and axially coordinated nitrogen donor ligands [1, 12, 20].

The electronic spectra of complex *4* in ethanol solution exhibit one broad absorption band due to copper(II) d–d transitions (Table 1). The position of the band is consistent with the assignments for other tetragonally distorted copper(II) complexes containing a $CuN_2O_2 \ldots O_2$ chromophore [1c, 3–6, 19, 20]. It is comparable to those found for mononuclear copper(II) valproate complexes with imidazoles [4] which contain the *trans-* or *cis*-CuN_2O_2 (or $CuN_2O_2 \ldots O_2$) chromophore.

The assignment of IR stretching frequencies for the antisymmetric, ν_{asym} (CO_2) and the symmetric, $\nu_{sym}(CO_2)$ of the valproate group are given in Table 1. The ν_{asym} (CO_2) and the ν_{sym} (CO_2) frequencies for complexes *2* and *3* occur at about 1610 and 1415 cm^{-1}, respectively. The positions of these frequencies and the separation between them, $\Delta\nu(\nu_{asym}(CO_2) - \nu_{sym}(CO_2))$ of ca. 195 cm^{-1}, when compared with those of sodium valproate ($\nu_{asym}(CO_2)$, 1570; $\nu_{sym}(CO_2)$, 1417; $\Delta\nu$, 153 cm^{-1}), are in the range expected for carboxylate groups that act as a bridging bidentate ligand [1b, 12, 28]. These parameters are comparable to those reported for structurally known binuclear copper(II) valproate complex that contain pyridine [12]. The antisymmetric carbooxyl, $\nu_{asym}(CO_2)$, vibration for complex *4* appeared as a doublet at 1630 and 1606 cm^{-1}, and the symmetric, $\nu_{sym}(CO_2)$, vibration appeared at 1417 cm^{-1} (Table 1). The position of these carboxylate stretching vibrations and the separation between them are in the range expected for carboxylate groups that act essentially as monodentate or asymmetric bidentate ligands [4, 19, 28]. They are comparable to those reported for mononuclear copper(II) carboxylate complexes that contain imidazoles having the CuN_2O_2 ($CuN_2O_2 \ldots O_2$) chromophore [4, 19]. The stretching vibrations of the NO_2 group, $\nu_{asym}(NO_2)$ and $\nu_{sym}(NO_2)$, for metronidazole in complex *2* occur at 1517 and 1355 cm^{-1}, respectively, and the corresponding NO_2 of 2-methyl-5-nitrobenzimidazole in complexes *3* and *4* occur at 1521 and 1344 cm^{-1}.

The X-band EPR spectra of powdered samples of complexes *2* and *3* were obtained at room temperature, and a representative spectrum is that of *3* shown in Figure 1(A). The spectra exhibit absorption typical for the randomly oriented

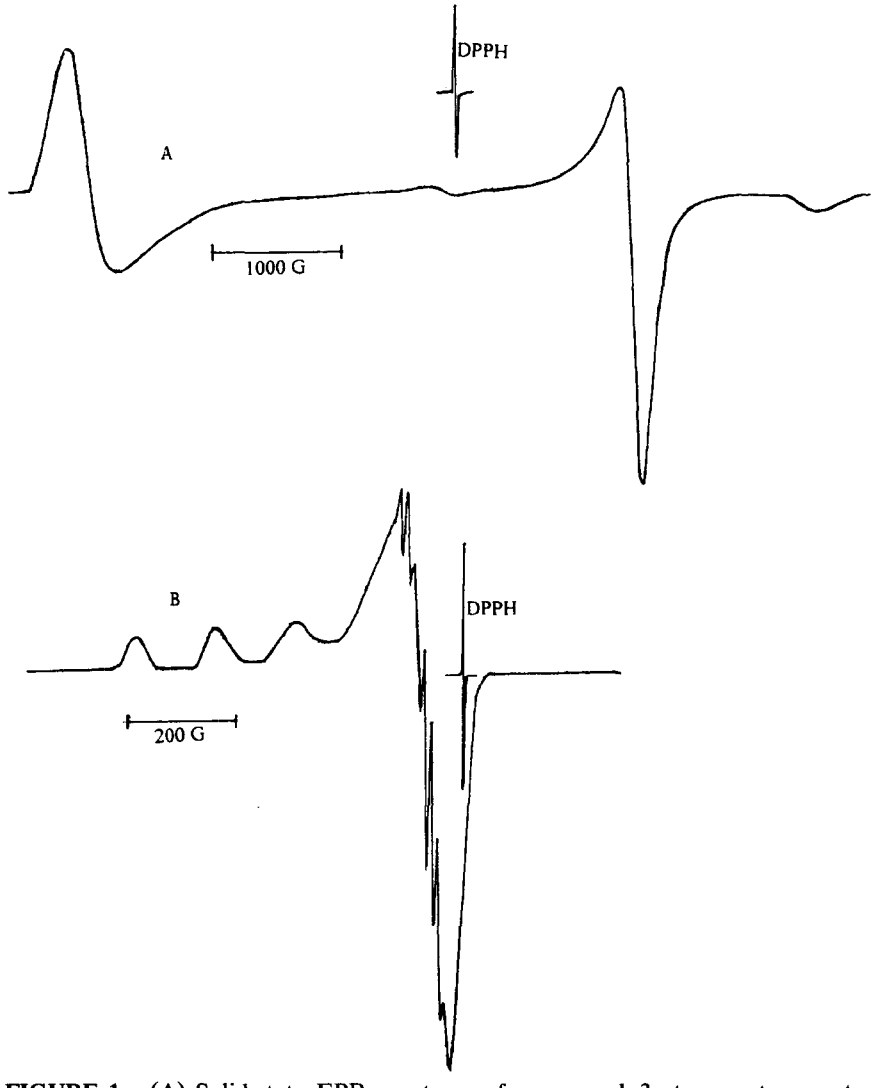

FIGURE 1. (A) Solid-state EPR spectrum of compound *3* at room temperature. (B) Frozen-solution EPR spectrum of compound *4*.

triplet state (S = 1) of axial symmetry. The absorption features are similar to those previously reported for binuclear copper(II) carboxylates [1, 12a, 20]. Three absorption lines, namely, H_{z1}, H_{z2}, and $H_{\perp}$, for complexes *2* and *3* were observed. In the EPR spectrum of complex *2*, besides the afore-mentioned lines typical of the binuclear complex, a signal at magnetic field around 3000 G corresponding to a mononuclear admixture was observed. Mononuclear signals are commonly found for binuclear copper(II) carboxylates [1]. However, no such signal has been observed in the EPR spectrum of complex *3* [Fig. 1(A)], thus excluding the presence of any mononuclear impurities. The EPR parameters for

complexes *2* and *3* were calculated by the method of Wasson et al. [29] (Table 2) by using the following equations [1b]:

$$h\nu = D - g_{11}\beta H_{z1}, \tag{1}$$

$$h\nu = -D + g_{11}\beta H_{z2}, \tag{2}$$

$$h\nu = -0.5D + (0.25D^2 + g_{\perp}^2\beta^2 H_{\perp}^2)^{1/2} \tag{3}$$

where h is Planck's constant, ν is the microwave frequency, H_{z1}, H_{z2} are the low and high field parallels, respectively, $H_{\perp}$ is the perpendicular field, and D is the axial-field splitting. The parameters g_{11}, $g_{\perp}$, and D values obtained for binuclear complexes *2* and *3* are comparable to those reported for other binuclear copper(II) carboxylate complexes [1], including those reported for structurally known binuclear copper(II) valproate adduct with pyridine [12]. In addition, our preliminary results of X-ray measurements on complex *2* indicated a similar binuclear structure.

The X-band EPR parameters, g and A, for the frozen solution and the polycrystalline form of complex *4* are given in Table 2. The frozen solution EPR spectrum is shown in Figure 1(B), and exhibits resolved structure with $g_{11} > g_{\perp}$. These features are consistent with a tetragonally elongated structure [30]. The $g_{\perp}$ region of the spectrum exhibits a ^{14}N superhyperfine structure that consists of five lines. This splitting is attributed to the presence of two nitrogen atoms in the plane of the copper(II) ion. The EPR spectral parameters for this complex are comparable to those previously reported for complexes that contain essentially the CuN_2O_2(or $CuN_2O_2 \ldots O_2$) chromophore in a *trans* or *cis* square-planar arrangement, including those reported for mononuclear bis-adducts of copper(II) carboxylates with imidazoles [2–6, 19, 20, 22, 23]. In complexes for which structural data are available, the copper(II) atom is bonded in a *trans* or *cis*

TABLE 2. EPR and Kinetic Data for the Oxidation of DTBC by Cu(II) Complexes

Compound	State (Temperature)	g_o^a	$g_{\perp}$	$g_{\parallel}$	|D| (cm^{-1})	Activity[b]
$Cu_2(valp)_4$ *(1)*[c]	Solid (Room)	2.130	2.016	2.341	0.344	2.6
$Cu_2(valp)_4(mtnd)_2$, *(2)*	Solid (Room)	2.220	2.129	2.405	0.345	1.6
$Cu_2(valp)_4(2m5nbnz)_2$, *(3)*	Solid (Room)	2.200	2.085	2.430	0.351	0.94
$Cu(valp)_2(2m5nbnz)_2$ *(4)*	Solid (Room)	2.112	$g_x = 2.052$ $g_y = 2.070$	2.213	—	0.47
	Frozen (77 K)	2.132	2.054	2.288	—	
	($A_{11}Cu = 166 \times 10^{-4}$ cm^{-1} $A_{\perp}N = 14 \times 10^{-4}$ cm^{-1})					

[a] g_o values are calculated from the equation $g_o = \frac{1}{3}(2g_{\perp} + g_{\parallel})$.
[b] The activity is reported as micromoles of DTBQ produced per mg catalyst per min.
[c] The EPR data are taken from Ref [12a].

arrangement to two ligand nitrogen atoms and one oxygen atom from each of two carboxylate ligands; the second oxygen atom of each carboxylate ligand is weakly bonded in a pseudoaxial arrangement [2–5, 8b, c].

The solid-state EPR spectrum of this complex is anistropic with $g_{\|}$ and $g_{\perp}$ components. The g_{11} region does not exhibit copper(II) hyperfine coupling, which is likely due to dipolar interactions between the copper atoms of neighboring molecules. The $g_{\perp}$ region is partially resolved into its x and y components. A similar spectrum is exhibited by bis-adduct of copper(II) valproate with 2-methylimidazole [4]. We have determined by single crystal X-ray structure analysis that this adduct contains the CuN_2O_2 chromophore in a *trans* square-planer arrangement [4]. Our preliminary X-ray data for complex *4* clearly indicated that in the solid state, this adduct contains the CuN_2O_2 chromophore in a *trans* square-planar arrangement, similar to the bis-adduct of copper(II) valproate with 2-methylimidazole [4].

Catalytic Activity for the Oxidation of DTBC

The reactivity of the copper(II) complexes *1–4* towards the two-electron oxidation of 3,5-di-t-butylcatechol (DTBC) to the corresponding o-benzoquinone (DTBQ) was investigated because this is one of the reactions that the copper-containing enzyme tyrosinase catalyzes [24]. Since DTBQ shows a characteristic absorption band at 400 nm ($\varepsilon = 1900$ $dm^3 \cdot mol^{-1} \cdot cm^{-1}$ in methanol) [31], the aerobic oxidation of DTBC was studied using electronic spectroscopy by following the appearance of the absorption of the DTBQ at 400 nm over the first 15 min of the reaction. The activities of complexes *1–4* were determined as micromoles of DTBQ produced per mg of catalyst per minute. These values are given in Table 2. Although DTBQ is produced for all complexes, the rate at which it is produced varies from binuclear to mononuclear catalysts. The rate at which DTBQ is produced is also dependent on the nature of the nitrogen-containing ligand present in the complexes.

Small molecular weight binuclear and mononuclear copper(II) complexes have been studied as models for copper oxidase enzymes, such as the copper-containing protein tyrosinase [13–21]. In tyrosinase and in synthetic copper(II) binuclear models, it is believed that two proximate metals atoms are needed to bond to two hydroxyl oxygen atoms of catechols in the oxidation to o-quinones [14–17]. This is consistent with the relatively high catalytic activity of binuclear complexes such as *1–3* (Table 2) compared to mononuclear complexes such as *4*. In nonplanar mononuclear copper(II) models, it has been proposed that the two copper(II) atoms must be located at a distance of less than 5 Å for bonding to the hydroxyl groups of the catechols, a mode which should facilitate electron transfer to dioxygen [17, 32]. The lower catalytic activities of ternary complexes *2* and *3* compared to that of the binary complex *1* may be attributed to two factors. (1) The presence of the axially coordinated ligands in the ternary adducts *2*, *3* could render the approach of DTBC to copper(II) sites more difficult in these two binuclear when compared to the binary complex *1*. In addition, the axially coordinated ligands in *2* and *3* are likely to dissociate to provide sites on copper for DTBC bonding, and also to facilitate any necessary ligand rearrangement induced by this bonding. Such dissociation is not required in the binary complex *1* which is axially free. (2) The presence of high electron

affinity ligands, metronidazole in complex *2* and 2-methyl-5-nitrobenzimidazole in complex *3*, should result in a relatively short-circuited catalytic cycle in these complexes when compared to complex *1* because once reduced to Cu(I), the complexes would be unable to be easily reoxidized to Cu(II) by oxygen. The relatively high catalytic activity of *2* compared to *3* (Table 2) may be due to the lower electron affinity of metronidazole ligand in *2* relative to 2-methyl-5-nitrobenzimidazole ligand in *3*.

A potentially important insight into the mechanistic aspects of the catechol oxidation process was recently made by Thompson and Calabrese [33] by the isolation and characterization of a series of copper(II)-3,5-di-t-butyl-o-semiquinone, Cu(II)-DTBSQ, complexes from reaction of the corresponding catechol or benzoquinone with dinuclear and mononuclear copper(II) or copper(I) complexes, respectively. It was concluded that the single-step two-electron oxidation of catechol by copper(II) complexes is not observed, and o-benzoquinone was obtained only after exposure of copper(II)-o-semiquinone to dioxygen or by the addition of small molecules such as pyridine. Their studies indicated that the formation of copper(II)-o-semiquinone complexes as an intermediate should be considered in catecholase-mimetic activity of copper(II) complexes.

The possible formation of copper ion-3,5-di-t-butyl-o-semiquinone as an intermediate species during the oxidation process was demonstrated in this study by following the UV-vis spectral charges of the catalytic reaction mixture. Copper(II) complex (0.01 mmol) and 0.9 mmol DTBC were mixed in 20 mL degassed methanol under nitrogen, and the UV-vis spectrum of the intense green solution was recorded. Three absorption bands appeared with time: one very broad band with moderate intensity centered at about 770 nm, a second band with less intensity at about 570 nm, and a third sharp and very intense band at about 385 nm. The first and third bands at about 770 and 385 nm, respectively, are comparable to those reported by Thompson and Calabrese [33] for the [Cu(diamine)(DTBSQ)] ClO_4 complexes (broad maxima between 825 and 770 nm with moderate intensity and a sharp, intense band in the 380–395 region). These features are also similar to $[Cu(DTBC)(DTBSQ)]^-$, $DTBSQ^-$ prepared electrochemically, and $Zn(DTBSQ)_2$ [34]. These are ligand $DTBSQ^-$ bands, and differ significantly from those of copper(II)-catecholate complexes, which have no intense absorption bands in these regions [34a, 35]. Recently, it was suggested that the presence of the first band at ca. 750–1000 nm is diagnostic of the semiquinone character of a coordinated dioxolene ligand [36]. The second band at about 570 nm may arise from a catecholato to copper(II) charge transfer transition by analogy to assignments made for other copper(II)-catecholato complexes [37]. When this solution was exposed to aerial oxygen, the bands at 770 and 570 nm decayed, and the band at 385 nm shifted to ca. 400 nm (DTBQ band). The decay rates of the above bands were found in the order $1 > 2 > 3 > 4$, which is consistent with their catalytic activities. It should be noted here that a small amount of precipitate was formed during the first 20–30 min of the catalyzed reaction when complexes *3* or *4* were used as a catalyst. The exact manner in which the precipitate is formed is not known at this time, but it is associated with catalysts which contain 2-methyl-5-nitrobenzimidazole ligand, and it will be under investigation. Methanol was evaporated from the reaction mixture; the precipitate was extracted with anhydrous diethylether and

filtered. Evaporation of the ether filtrate gave DTBQ, whose IR [ν(CO) = 1665 cm^{-1}], UV-vis (400 nm), and ^1H NMR [$CDCl_3$: δ 1.20 (9H), 1.26 (9H), 6.20 (1H), 6.98 (1H)] spectra were compared with those of authentic DTBQ.

Infrared spectroscopy has proven to be a potent probe in determining the nature of coordinated dioxolene ligand, i.e., catecholate or semiquinonate [38]. The IR spectra of coordinated catecholates are characterized by an intense absorption near 1480 cm^{-1} (ring stretching mode involving the C-C bond between the coordinated oxygen atoms) and 1250 cm^{-1} (C-O stretching mode) [38]. Coordinated semiquinones, on the other hand, exhibit a band in the range 1420–1460 cm^{-1}, attributed to the C=O stretching mode [39]. IR spectra of complexes *1–4* with DTBC in CH_2Cl_2 were obtained by mixing the complex with DTBC in CH_2Cl_2 under nitrogen. An IR band characteristic of the chatecholate ligand was observed at about 1480 cm^{-1}, and an IR band in the range 1465–1455 cm^{-1} was observed for all reaction mixtures, which is characteristic for copper-DTBSQ species. These spectral observations are comparable to those reported previously, in which the DTBQ ligand present in both catecholate and semiquinonate form in transition metal complexes [21, 38]. Collectively, the electronic and IR spectral results obtained here and previously [21, 33] suggest that the oxidation of DTBC with these copper(II) complexes proceeds by the following mechanism:

$$\text{Copper(II) complex} + \text{DTBC} \rightarrow [Cu^{II}(DTBC^{2-})]\ \text{Complex} \tag{4}$$

$$[Cu^{II}(DTBC^{2-})] \xrightleftharpoons{\text{internal electron transfer}} [Cu^{I}(DTBSQ)] \tag{5}$$

$$[Cu^{I}(DTBSQ)] \xrightarrow{\text{Oxidation}} \text{Cu(II)complex} + \text{DTBQ} \tag{6}$$

In equation (4), two carboxylate groups coordinated to the Cu(II) atom are used to dehydrogenate the two hydroxyl groups of DTBC, and Cu(II)-catecholato complex is formed (band at 570 nm and IR at 1480 cm^{-1}). In equation (5), an internal one-electron transfer from the coordinated catecholato dianion ($DTBC^{2-}$) to copper(II) gives the intermediate Cu^{I}(DTBSQ) complex (bands at 770 and 385 nm and IR band in the range (1465–1455 cm^{-1}), which is in equilibrium with $Cu^{II}(DTBC^{2-})$ complex. In equation (6), air oxygen is used to oxidize copper(I) and DTBSQ to produce the copper(II) complex and DTBQ.

The internal electron transfer and the generation of copper(I)-semiquinone which is in equilibrium with the copper(II)-catecholate [equation (5)] have recently been demonstrated by Dooley and coworkers [39a]. Anaerobic substrate reduction of amine oxidase (a copper-containing enzyme which has imidazole ligands in its first coordination shell of copper(II) and 6-hydroxydopaquinone as another cofactor [39b]) generates a copper(I)-semiquinone species which is in equilibrium with the copper(II)-reduced quinone species. The copper(I)-semiquinone species was proposed to be the catalytic intermediate that reacts directly with oxygen, and similar to the one suggested in this study. In addition, the internal electron transfer reaction between two isoelectronic couples, i.e., copper(II)-catecholate/copper(I)-semiquinonate, was recently proposed in the

reactivity properties of copper-dioxolene adducts [40]. The equilibrium between catecholate and semiquinonate complexes of other transition metals (Co, Ni, Fe) has been demonstrated in the catalytic oxygenation of catechols by complexes of these metals [40, 41]. The formation of the metal-semiquinonate species, which is the catalytic intermediate that reacts with oxygen, has been characterized by electronic, IR, and ESR spectroscopic methods [41]. In summary, the results of this study indicate that the formation of binuclear or mononuclear copper ion-o-semiquinone complexes as an intermediate should be considered in the catecholase-mimetic activity of binuclear and mononuclear of Cu(II) carboxylate complexes.

Catalytic Activity for the Oxidation of TMPD

The catalytic activities of copper(II) complexes *1–4* for O_2 oxidation of the one-electron reducing agent N,N,N′,N′-tetramethyl-p-phenylendiamine (TMPD) to the corresponding cation radical $TMPD^+$ was evaluated by measuring the increase of the absorbance at 565 nm (due to $TMPD^+$ formation [26]) of the reaction mixture for the first 15 min.

The results are similar to those observed in the catalytic oxidations of DTBC, i.e., the activities are in the order $1 > 2 > 3 > 4$. These results are consistent with the previous one showing that binuclear copper(II) complexes are more efficient than mononuclear complexes in the oxidation of TMPD [17, 26]. In binuclear copper(II) complexes, two molecules of TMPD are expected to coordinate at the binuclear sites, but one molecule of TMPD is expected to coordinate at the mononuclear site. An electron transfer from each TMPD molecule to each Cu(II) ion occurs, and produces a binuclear Cu(I) complex and two molecules of $TMPD^+$ when binuclear Cu(II) complexes are used as catalyst or mononuclear Cu(I) complex, and one molecule of $TMPD^+$ when mononuclear Cu(II) complexes are used as catalyst. Copper(I) is then reoxidized to copper(II) by O_2. The lower catalytic activities of the ternary binuclear complexes *2* and *3* compared to that of the binary binuclear complex *1* may be attributed to the presence of the axially coordinated secondary ligands in the ternary complexes. The presence of these ligands could render the approach of TMPD to copper(II) sites more difficult in these two binuclear adducts when compared to *1* which is axially free. In addition, the Cu(II) complexes of these high electron affinity ligands, nitroimidazoles, once reduced to Cu(I) complexes, would be unable to be easily reoxidized to Cu(II) complexes by O_2. The relatively high catalytic activity of *2* compared to *3* may be due to the presence of higher electron affinity ligand 2-methyl-5-nitrobenzimidazole in complex *3* when compared to metronidazole in complex *2*. The behavior of copper(II) complexes used in this study mimics the activity of copper-containing oxidase enzymes such as laccase [17] since the TMPD oxidation reaction is believed to be one of the model reactions for this enzyme [42].

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