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Article

Synthesis, Structure, Physicochemical Properties and Catecholase-like Activity of a New Dicopper(II) Complex

***Ademir Neves**[a*], **Liane M. Rossi**[a], **Adailton J. Bortoluzzi**[a], **Antônio S. Mangrich**[b],*
***Wolfgang Haase**[c] and **Rüdiger Werner**[c]*

[a]*Departamento de Química, Universidade Federal de Santa Catarina, 88040-900, Florianópolis - SC, Brazil*

[b]*Departamento de Química, Universidade Federal do Paraná, 81531-970, Curitiba - PR, Brazil*

[c]*Institut für Physikalische Chemie, Technische Universität Darmstadt, D-64287, Darmstadt, Germany*

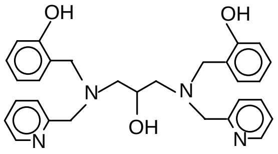
Neste trabalho, reportamos a síntese, caracterização e atividade de catecolase do complexo binuclear de cobre $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(\mu\text{-}ClO_4)]\ ClO_4\cdot H_2O\cdot EtOAc$ contendo o ligante *N,N'*-2-hidroxibenzil-*N,N'*-2-piridilmetil-2-hidroxipropano-1,3-diamina (H_3bbppnol). O complexo foi caracterizado através de espectroscopias no infravermelho e eletrônica, ressonância paramagnética eletrônica, medidas de susceptibilidade magnética e cristalografia de raios-X. A atividade de catecolase do complexo foi determinada espectrofotometricamente, monitorando-se a oxidação do substrato 3,5-di-*terc*-butilcatecol à correspondente *o*-benzoquinona, em solução de metanol saturada com O_2/tampão aquoso (pH 8), a 25 °C. O complexo mostrou-se capaz de oxidar o substrato modelo com uma constante catalítica de 0,0057 s^{-1}.

The synthesis, characterization and catecholase activity studies of the complex $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(\mu\text{-}ClO_4)]ClO_4\cdot H_2O\cdot EtOAc$ containing the dinucleating ligand *N,N'*-2-hydroxybenzyl-*N,N'*-2-pyridylmethyl-2-hydroxypropane-1,3-diamine (H_3bbppnol) are presented here. The complex was characterized by IR, electronic and EPR spectroscopies, cyclic voltammetry, magnetic susceptibility measurements and X-ray crystallography. The catecholase-like activity of the complex was determined spectrophotometrically by monitoring the oxidation of 3,5-di-*tert*-butylcatechol to the corresponding *o*-benzoquinone in O_2-saturated methanol/aqueous buffer (pH 8) mixtures at 25°C. The complex was able to oxidize the model substrate with a turnover number of 0.0057 s^{-1}.

Keywords: copper(II), crystal structure, catecholase activity

Introduction

The synthesis and reactivity studies of bimetallic copper(II) complexes, as model compounds for metalloenzymes with oxidase activity, is of particular interest for the development of bioinspired catalysts for oxidation reactions. In nature, type 3 copper enzymes tyrosinase[1] and catechol oxidase[2] contain similar dinuclear copper active sites and are responsible for the hydroxylation of monophenols (monophenolase activity) and/or oxidation of catechols to *o*-quinones (catecholase activity). The coordination of catechol to the metal centers has been suggested to favor the intramolecular electron transfer reaction that results in the release of *o*-quinone and reduction of the copper centers to the dicopper(I) state. This could then react with O_2 to restore the active form of the enzyme[1,3,4]. Here we present the synthesis and characterization of the new dicopper(II) complex $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(\mu\text{-}ClO_4)]ClO_4\cdot H_2O\cdot EtOAc$ (**1**) employing the O, N donor dinucleating ligand *N,N'*-2-hydroxybenzyl-*N,N'*-2-pyridylmethyl-2-hydroxypropane-1,3-diamine (H_3bbppnol).



H_3bbppnol

The ligand contains an alkoxy group which serves as an endogenous bridge, holding the copper centers about 3.4 Å apart and possibly facilitating the binding of a catecholate

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* e-mail: ademir@qmc.ufsc.br

during the electron transfer reaction. Also, the ligand occupies only four positions in the coordination sphere of each metal center resulting in two free positions for the coordination of exogenous bridging groups, solvent or substrate molecules. In order to investigate the ability of this complex to catalyze the oxidation of catechol to *o*-quinone (catecholase activity) we employed 3,5-di-*tert*-butylcatechol (3,5-D*t*BC). Owing to the great interest in catecholase activity investigations, many copper complexes have been tested with regard to this substrate[5-8].

Experimental

Abbreviations

H_3bbppnol: *N,N'*-2-hydroxybenzyl-*N,N'*-2-pyridylmethyl-2-hydroxypropane-1,3-diamine; HBPA: (2-hydroxybenzyl)(2-pyridylmethyl) amine; 3,5-D*t*BC: 3,5-di-*tert*-butylcatechol; 3,5-D*t*BQ: 3,5-di-*tert*-butylquinone; TRIS: tris(hydroxymethyl)aminomethane.

Materials

All chemical employed in the syntheses were reagent grade and used without further purification. The characterization of **1** and the kinetic experiments were carried out with spectroscopic grade solvents (Merck) dried with molecular sieves.

Syntheses

N,N'-2-hydroxybenzyl-*N,N'*-2-pyridylmethyl-2-hydroxypropane-1,3-diamine (H_3bbppnol)[9] was prepared according to previously reported procedures.

$[Cu_2(H_2bbppnol)(m\text{-}OAc)(m\text{-}ClO_4)]ClO_4 \cdot H_2O \cdot EtOAc$ (1)

A solution of H_3bbppnol (0.48 g, 1 mmol) and $Cu(ClO_4)_2 \cdot 6H_2O$ (0.74g, 2 mmol) in methanol (30 cm^3) was stirred at room temperature for 30 min. Solid sodium acetate trihydrate (0.14g, 1 mmol) was added and the reaction mixture was stirred at 60ºC for 30 min. A blue microcrystalline solid, formed after cooling the solution to room temperature, was filtered off and washed with methanol and diethyl ether. Single crystals suitable for X-ray crystallography were obtained by recrystallization from an acetonitrile/ ethyl acetate solution of **1**. (Found: C, 43.11; H, 4.63; N, 5.68 Calc. for $C_{35}H_{44}N_4O_{16}Cl_2Cu_2$: C, 43.13; H, 4.55; N, 5.75. %). Molar conductivity: 306 Ω^{-1} cm^2mol^{-1}; IR ν_{max}/cm^{-1}: 3382 (O-H), 1562, 1446 (COO^-), 1612, 1502, 1484, 1460 (C=N, C=C), 1384 (δ O-H), 1264 (C-O), 1106 (ClO_4^-), 762 (δ Arom. C-H).

Safety note: perchlorate salts are potentially explosive and should be handled in small quantities with care.

Analytical and physical measurements

Elemental analyses results were obtained on a Perkin Elmer 2400 instrument. The solution electrical conductivity was measured with a Digimed conductivity bridge, type CD-21, in acetonitrile solutions at *ca.* 10^{-3} mol L^{-1} at 25ºC. IR spectra(KBr pellets) were measured in the 4000-400 cm^{-1} range on a Perkin Elmer model 781 spectrometer. Electronic absorption spectra in the 200-1100 nm range were recorded on a Perkin Elmer Lambda-19 spectrophotometer. Magnetic susceptibility measurements were performed on powder samples of complex **1** over a temperature range from 4 to 300 K with a Faraday-type magnetometer. Details of the apparatus have been described elsewhere[10]. Diamagnetic corrections were applied in the usual manner with the use of tabulated Pascal constants[11] . EPR spectra were recorded in frozen solutions at 77 K on a Bruker ESP 300E spectrometer. Electrochemical measurements were carried out with a Princeton Applied Research (PARC) model 273 potentiostat/galvanostat. Cyclic voltammograms were obtained at room temperature from acetonitrile solutions containing **1** (10^{-3} mol dm^{-3}) and $[TBA][PF_6]$ (0.1 mol dm^{-3}) under an argon atmosphere. The electrochemical cell was of a standard three-electrode configuration: platinum working electrode, a platinum wire counter electrode, and a SCE reference electrode. The Fc^+/Fc ferrocene couple (E^o = 0.400 V *vs.* NHE) was used as an internal standard[12].

Kinetics of 3,5-di-tert-butylcatechol oxidation

Kinetic experiments for the oxidation of 3,5-di-*tert*-butylcatechol were monitored spectrophotometrically on a HP-8452A diode-array spectrophotometer by following the increase of the 3,5-di-*tert*-butyl-*o*-benzoquinone characteristic absorption band at about 400 nm. In a typical experiment, 100 mm^{-3} of a solution of **1** in methanol ($[C]_o = 2.4 \times 10^{-5}$ mol dm^{-3}) was added to a 1-cm-path-length cell containing 3 cm^3 of O_2-saturated methanol and 100 mm^3 of aqueous TRIS pH 8.0 buffer (1 mol dm^{-3}) at 25ºC. The reaction was initiated by the addition of 100 mm^3 of catechol solution ($[3,5\text{-D}t\text{BC}]_o$= 3.0×10^{-3} to 9.0×10^{-3} mol dm^{-3}). In a separate set of experiments, the kinetic determinations were performed without the catalyst. The reaction rate was obtained from the slope of the A x t plot over the first 800 s of reaction. Rate units were calculated from ε=1900 dm^3 mol^{-1} cm^{-1} for 3,5-D*t*BQ in methanol[13]. A kinetic treatment on the basis of the Michaelis-Menten approach was applied and the results were evaluated from Lineweaver-Burk double-reciprocal plots.

Single-crystal X-ray structure determination

The blue crystals of complex **1** are very sensitive to solvent loss and were handled in a protective oil. A prismatic crystal of dimensions 0.26 x 0.33 x 0.47 mm was lodged in a Mark glass capillary and placed on a CAD-4 diffractometer. An orientation matrix and unit cell parameters were determined based on the setting angles of 25 reflections with θ range from 10.04 to 15.35°. Intensity data were collected at room temperature using a ω-2θ scan technique with graphite monochromated Mo Kα radiation (λ = 0.71073 Å)[14]. Three standard reflections were monitored every 200 reflections, showing no significant variation of intensity during the data collection. All 7742 reflections were corrected for Lorentz and polarization effects and for absorption (Psi-scan, T_{max} 0.962, T_{min} 0.869)[15,16]. The diffraction symmetry was 2/m. Based on the systematic absences it was possible to define $P2_1/c$ as the correct space group for this crystal lattice. The structure was solved by direct methods and refined by full-matrix least-squares methods using SHELXS-97 and SHELXL-97 programs[17,18], respectively. The oxygen atoms of one perchlorate counterion were found disordered over two alternative positions; these atoms were refined isotropically with 0.65 and 0.35 site occupancies. All other non-hydrogen atoms were refined with anisotropic thermal parameters. H atoms attached to C atoms were placed at idealized positions, with C-H distances and U_{eq} values taken from the default settings of the refinement program. H atoms of the phenol moieties and of the water molecule were found from difference Fourier maps and treated as riding atoms. A total of 529 parameters (69 restraints) were refined against all 7452 independent reflections ($R_{int} = 0.0223$). Refinement converged with R= 0.0436 and wR_2= 0.1069 for 4866 reflections with I > 2σ(I) and R=0.1032 and wR_2= 0.1268 for all data. The drawing of the molecular structure was produced using ZORTEP[19]. Further information about data collection and refinement are reported in Table 1, and selected bond lengths and angles are listed in Table 2.

Table 1. Crystal data and structure refinement for complex $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(\mu\text{-}ClO_4)]ClO_4\cdot H_2O\cdot EtOAc$ (**1**).

empirical formula	$C_{35} H_{44} Cl_2 Cu_2 N_4 O_{16}$
formula weight	974.72
space group	$P2_1/c$ (N°. 14)
a	12.293(3) Å
b	21.383(4) Å
c	15.907(3) Å
β	95.99(3) °
volume	4158.5(15) $Å^3$
Z	4
D_c	1.557 g cm^{-3}
μ	12.26 cm^{-1}
θ range	2.21 < θ < 25.18°
unique reflections	7452 (R_{int} = 0.0223)
parameters refined/ restraints	529 / 69
extinction coefficient	0.0007(2)
Goof (F^2)	1.014
final R indices [I > 2s(I)]	^{a}R = 0.0436, ^{b} wR_2 = 0.1069
R indices (all data)	R1 = 0.1032, wR_2 = 0.1268
Δ(ρ)	0.852 and -0.563 e $Å^{-3}$

$^a R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$; $^b wR_2 = [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{1/2}$; ^{a} weighting scheme: $w=1/[\sigma^2(F_o^2)+(0.0608P)^2+4.0553P]$ where $P=(F_o^2+2F_c^2)/3$

Table 2. Selected bond lengths (Å) and angles (°) for complex $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(\mu\text{-}ClO_4)]ClO_4{\times}H_2O{\times}EtOAc$ (**1**).

Cu(1)-O(4)	1.905(3)
Cu(1)-O(51)	1.926(3)
Cu(1)-N(12)	1.978(4)
Cu(1)-N(1)	2.019(4)
Cu(1)-O(20)	2.549(3)
Cu(1)-O(7P)	2.788(4)
Cu(1)-Cu(2)	3.3554(11)
Cu(2)-O(4)	1.891(3)
Cu(2)-O(52)	1.923(3)
Cu(2)-N(42)	1.966(4)
Cu(2)-N(6)	2.022(4)
Cu(2)-O(30)	2.494(4)
Cu(2)-O(5P)	2.997(5)
O(4)-Cu(1)-O(51)	97.47(13)
O(4)-Cu(1)-N(12)	166.28(15)
O(51)-Cu(1)-N(12)	94.46(15)
O(4)-Cu(1)-N(1)	85.28(14)
O(51)-Cu(1)-N(1)	172.82(16)
N(12)-Cu(1)-N(1)	83.68(16)
O(4)-Cu(1)-O(20)	105.49(13)
O(51)-Cu(1)-O(20)	85.28(14)
N(12)-Cu(1)-O(20)	82.18(14)
N(1)-Cu(1)-O(20)	87.60(13)
O(4)-Cu(1)-O(7P)	87.52(14)
O(51)-Cu(1)-O(7P)	97.43(16)
N(12)-Cu(1)-O(7P)	84.23(15)
N(1)-Cu(1)-O(7P)	89.30(15)
O(20)-Cu(1)-O(7P)	166.32(12)
O(4)-Cu(2)-O(52)	96.40(14)
O(4)-Cu(2)-N(42)	164.05(15)
O(52)-Cu(2)-N(42)	93.77(15)
O(4)-Cu(2)-N(6)	86.61(14)
O(52)-Cu(2)-N(6)	173.65(16)
N(42)-Cu(2)-N(6)	84.54(15)
O(4)-Cu(2)-O(30)	109.01(13)
O(5)2-Cu(2)-O(30)	86.37(14)
N(42)-Cu(2)-O(30)	83.83(14)
N(6)-Cu(2)-O(30)	87.36(14)
O(4)-Cu(2)-O(5P)	91.03(13)
O(52)-Cu(20-O(5P)	99.92(15)
N(42)-Cu(2)-O(5P)	75.10(14)
N(6)-Cu(2)-O(5P)	85.59(14)
O(30)-Cu(2)-O(5P)	158.30(12)
Cu(2)-O(4)-Cu(1)	124.24(16)

Results and Discussion

Syntheses

According to previous literature report[20] the reaction of $Cu(CH_3COO)_2\cdot H_2O$ with the ligand H_3bbpnol produces blue crystals of $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(H_2O)_2]Cl_2\cdot 2H_2O$ (**2**) where the alkoxide group from the ligand and an acetate group form a double-bridged structure. Curiously, similar reaction with $Cu(ClO_4)_2\cdot 6H_2O$ in the presence of sodium acetate gives blue crystals of $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(\mu\text{-}ClO_4)]ClO_4\cdot H_2O\cdot EtOAc$ (**1**) which contains a triple-bridged structure with an additional μ-ClO_4 group, as confirmed by X-ray structural analysis. The IR spectrum of **1** shows $\nu_a(COO^-)$ and $\nu_s(COO^-)$ at 1562 and 1446 cm^{-1} respectively, with a splitting of 116 cm^{-1}, in agreement with the presence of a carboxylate group bridging the two Copper(II) ions in O – O'- *syn* – *syn* coordination mode[21]. In complexes **1** and **2**, the phenol groups are weakly bound to the copper(II) ions without deprotonation, as evidenced by the presence of δ(O-H) of phenol at 1384 cm^{-1} in the IR spectra of the complex and H_3bbppnol. The presence of an uncoordinated perchlorate ion in complex **1** is evident from the intense ν_3 (ClO_4^-) absorption in the 1084 – 1114 cm^{-1} range[22]. Conductivity measurements suggest that the μ-ClO_4^- ion is only very weakly coordinated to the copper centers, as the complex which exhibits 1:2 electrolyte behaviour in acetonitrile with $\Lambda_M = 306\ \Omega^{-1}\ cm^2\ mol^{-1}$.[23]

Crystal and molecular structure of $[Cu_2(H_2bbppnol)(m\text{-}OAc)(m\text{-}ClO_4)]\ ClO_4\cdot H_2O\cdot EtOAc$ (1)

The X-ray crystal structure of **1** reveals an asymmetric unit consisting of one $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(\mu\text{-}ClO_4)]^+$ cation, one perchlorate counterion, one water and one ethyl acetate molecules as the solvents of crystallization. The cation in complex **1** shows a triple-bridged core with one μ-alkoxide group from the ligand, one μ-acetate ion and one μ-perchlorate molecule. The structure is very similar to the one previously reported for complex **2**[20] with the incorporation of a μ-perchlorate group. Figure 1 shows a ZORTEP[19] view of the $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(\mu\text{-}ClO_4)]^+$ cation in crystals of **1**. Selected bond distances and angles are given in Table 2.

The two copper(II) ions are six-coordinated in a highly distorted octahedral geometry and the ligand coordinates in the same fashion as in complex **2**[20]. The equatorial plane is formed by a $Cu\text{-}N_{amine}$ [2.019(4), 2.022(4) Å], $Cu\text{-}N_{pyridine}$ [1.978(4), 1.966(4) Å], $Cu\text{-}O_{alkoxide}$ [1.905(3), 1.891(3) Å] and $Cu\text{-}O_{acetate}$ [1.926(3), 1.923(3) Å] bonds. A coordinated perchlorate ion [$Cu – O_{perchlorate}$, 2.788(4), 2.997(5) Å] and a protonated oxygen atom of the phenol group [$Cu\text{-}OH_{phenol,}$ [2.549(3) Å , 2.494(4) Å] in axial positions complete the coordination sphere of each copper center. The long $Cu\text{-}OH_{phenol}$ distances are also very similar to the related bond lengths in **2**, [$Cu(1)–OH_{phenol}$, 2.433(10) Å, $Cu(2)–OH_{phenol}$, 2.445(9) Å] and in the mononuclear $[Cu(HBPA)2]^{2+}$ six-coordinated complex [$Cu–OH_{phenol}$, 2.449(6) and 2.494(6) Å][24]. In **2**[20] a coordinated water molecule [Cu(1)–O(w1) 2.695(17) and Cu(2)–O(w2), 2.769(16) Å] is *trans* to each phenol group whereas in **1** the perchlorate oxygen atoms lie in these positions. In both cases the *trans* oxygen atoms interact weakly with the metallic orbitals since the distances observed are long, resulting in a distorted "4+2" geometry due to the Jahn-Teller effect for a copper(II) ion[25]. Long $Cu\text{-}O_{perchlorate}$ distances have been reported in other copper(II) complexes with Cu...Cu distances of about 3 Å containing a μ-ClO_4 group[26]. In comparison with these complexes, complex **1** shows the longest $Cu\text{-}O_{perchlorate}$ bond distance (2.997(5) Å). The Cu(1)-Cu(2) distance and the Cu(1)-O(4)-Cu(2) angle in **1** are 3.355(3) Å and 124.24(16)°, respectively.

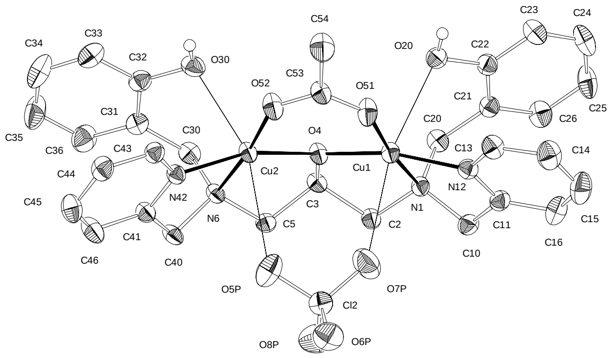

Figure 1. Drawing of the cation in complex **1**, $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(\mu\text{-}ClO_4)]^+$, with the atom numbering scheme. Thermal ellipsoids are shown at the 40% probability level. Most hydrogen atoms have been omitted for clarity.

The three-dimensional cohesion of the structure results from a strong intermolecular hydrogen-bond network (Table 3) involving the phenol oxygen atoms O(20) and O(30) and neighbouring $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(\mu\text{-}ClO_4)]^+$ cation [through O(5P)] and a water molecule [O(1W)]. Also, the disordered perchlorate O(2P) and O(2P') atoms and the ethyl acetate [O(64)] molecules form similar interactions with the solvent.

Table 3. Hydrogen-bonds for complex **1** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(1W)-H(1WA)...O(2P)[a]	0.84	2.20	2.896(9)	140.1
O(1W)-H(1WA)...O(2P)'[a]	0.84	1.90	2.678(11)	154.3
O(1W)-H(1WB)...O(64)	0.96	1.88	2.816(7)	164.3
O(20)-H(20)...O(5P)[b]	0.75	2.17	2.901(5)	168.4
O(30)-H(30)...O(1W)[c]	0.78	1.91	2.691(5)	172.0

Symmetry codes: [a] x-1,y,z; [b] x,-y+1/2,z-1/2; [c] x,-y+1/2,z+1/2

Table 4. UV-Visible absorption and EPR spectral data.

Complex (solvent)	UV/VIS λ_{max}, nm (ε, dm^3 mol^{-1} cm^{-1})	EPR $g_{\|}$	 $g_{\perp}$	 $A_{\|}$ (x10^{-4} cm^{-1})
1 (acetonitrile)	658 (194)	2.229	2.040	190
2 (acetonitrile)	669 (364), 448 (285)	2.235	2.062	185
1 (DMF)	663 (256), 424 (244)	2.240	2.048	179.5
2 (DMF)	678 (250), 424 (550)	2.242	2.048	177

Electronic spectroscopy and EPR spectroscopy

The UV-Visible absorption properties and EPR parameters of complex **1** and **2** are summarized in Table 4.

Complex **1** exhibits a broad ligand-field band at 658 nm (194 dm^{3}mol^{-1}cm^{-1}) in acetonitrile solution and at 661 nm in the solid state, which is in agreement with the maintenance of the first coordination sphere and the presence of CuN_2O_4 chromophores and distorted octahedral geometries[25]. In DMF solution, an additional ligand-field band appears at 424 nm (244 dm^{3}mol^{-1}cm^{-1}). The same transition was also observed in DMF and acetonitrile solutions of **2** (Table 4). Thus, the electronic spectra of **1** and **2** are solvent dependent reflecting changes in the coordination sphere when the complexes are dissolved. EPR spectral measurements were performed in frozen acetonitrile and DMF solutions of **1** and **2** and displayed $g_{\|} > g_{\perp} > 2.04$ and $A_{\|} = (180 - 195) \times 10^{-4}$ cm^{-1}, typical of elongated octahedral geometry[25]. Solvent coordination in the axial position can have a very pronounced effect on the spectral parameters. Complexes **1** and **2**, upon dissolution in DMF, exhibit the same EPR parameters and UV-visible bands and consequently, as the ligand is the same, they could be considered the same species with the axial exogenous ligand position occupied by DMF molecules.

Electrochemistry

The electrochemical behaviour of complex **1** in acetonitrile solution was investigated as the redox potential is an important parameter in electron transfer reactions. The reduction potentials for complex **1** were measured by cyclic voltammetry (CV) with a working platinum electrode in acetonitrile solution containing [TBA][PF$_6$] 0.1 mol dm^{-3} as the supporting electrolyte in the range –1.6 to + 1.6 V *vs.* SCE. The cyclic voltammogram shows only cathodic reduction peaks at –0.458 and –0.742 V *vs.* NHE, tentatively assigned to the one-electron processes $Cu^{II}Cu^{II} \rightarrow Cu^{II}Cu^{I}$ and $Cu^{II}Cu^{I} \rightarrow Cu^{I}Cu^{I}$, respectively. The electrochemical behaviour of complex **2** in acetonitrile solution was also investigated and shows cathodic reduction peaks at –0.527 and –0.713 V *vs.* NHE corresponding to the same successive one electron transfer processes detected in **1**. These values lie in the negative range of reduction potentials observed for other dinuclear copper(II) complexes containing (μ-alkoxo)-(μ-acetate)[27,28], (μ-phenoxo)(μ-OR) [R=H, Me][7b,29] moieties. Malachowski *et al.*[8] suggest that mononuclear tripodal copper(II) complexes with reduction potentials close to 0.0 V demonstrate higher reactivity in the oxidation of 3,5-DtBC, while complexes with more negative reduction potentials show low reactivity due to stabilization of the copper(II) form. However, good results in catecholase activity have been obtained even for dinuclear copper(II) complexes with reduction potentials in the range from –0.37 to –0.96 V[7b]. Based on this information a catalytic effect of complex **1** on the oxidation of 3,5-D*t*BC can be expected since the reduction potentials lie within this range.

Magnetic susceptibility measurements

The magnetic susceptibility measurement on powdered samples of complex **1** between 4 - 300 K shows that the two copper(II) ions are coupled antiferromagnetically since the effective magnetic moment decreases form 2.5 mB at room temperature to 0.35 at 4.5 K (Figure 2). The data were fitted using the expression (**a**) for molar susceptibility *versus* temperature based on the isotropic Heisenberg-Dirac-van Vleck spin-exchange Hamiltonian ($S_1 = S_2 = ½$).

$$\chi(T) = (1-x_p).\chi_{dim}(T) + 2x_p\chi_p(T) + 2N_\alpha \qquad \textbf{(a)}$$

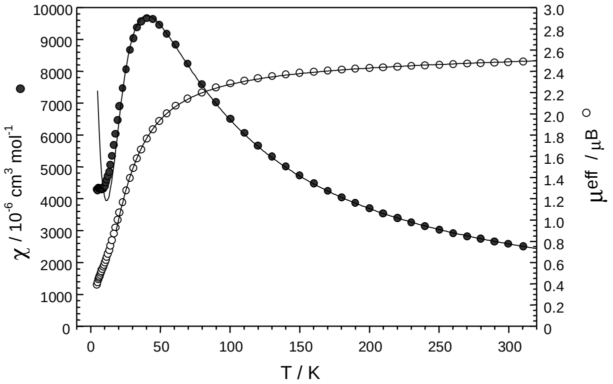

Figure 2. Magnetic susceptibility (●) and effective magnetic moment (○) per dimer of complex **1**. The solid lines represent the best results from least-squares fitting with J = -23.9 cm^{-1} and g = 2.045.

Here, x_p describes the part of the susceptibility that belongs to some paramagnetic impurities expressed by the Curie-law for S = ½ , χ_{dim} corresponds to the well-known Bleaney-Bowers equation[30] and the temperature independent paramagnetism N_α was fixed at 60 x 10^{-6} cm^3 mol^{-1} per copper(II) ion. The best parameters obtained from least square fits of the experimental data to eq (**a**) are J = -23.9 cm^{-1}, g = 2.045, x_p = 4.6 %.

The low magnitude of J indicates a weak antiferromagnetic interaction between the two copper(II) ions. The similarity of the J values obtained for complexes **1** and **2** indicates that the third bridging ligand in **1** does not provide any additional exchange pathway and has therefore low influence on the magnetic properties. Magnetostructural correlations for dinuclear metal complexes have been known for a long time[31]. Hatfield *et al.*[32] reported a linear correlation between the exchange integral J and the Cu-O-Cu bridging angle for a series of symmetrical dihydroxo-bridged copper(II) dimers. Similar correlations have been found for alkoxo-bridged copper(II) dimers[33,34], for phenoxo-bridged copper(II) dimers[35] and for unsymmetrical phenoxo-bridged copper(II) complexes with different exogenous ligands[36]. As pointed out by Wieghardt et al.[37], the effect of the Cu-O-Cu angle on the magnitude of the magnetic coupling constant J is much larger for complementary di-μ-hydroxo-dicopper(II) complexes than for those containing a non-complementary μ-hydroxo-μ-carboxylatodicopper(II) core. In fact, the Cu-O-Cu angles in complexes **1** and **2** are similar and as expected the determined J values (Table 5) differed only slightly and fit nicely into the magneto-structural correlation for μ-hydroxo-μ-carboxylatodicopper(II) complexes already reported[37].

Kinetic studies for catecholase activity

The catalytic oxidation of 3,5-di-*tert*-butylcatechol has been studied as a model reaction for the catecholase activity of tyrosinase and catechol oxidase[5-8]. The kinetic studies on the oxidation of 3,5-D*t*BC were carried out by the method of initial rates by monitoring the increase in the characteristic quinone (3,5-D*t*BQ) absorption band at 400 nm as a function of time. Under anaerobic conditions little product was formed, so the solvent was saturated with O_2 before the kinetic experiments. Oxygen participates directly in the catalytic cycle reoxidizing any generated copper(I) species back to the active copper(II) complex. It was also observed that the addition of a small amount of an aqueous buffer TRIS pH 8 solution to the oxygen saturated methanol solution increases significantly the reaction rates. A catalytic activity is observed since after 10 minutes an increase in absorption at 400 nm of about 0.23 units was observed, which corresponds to a turnover of more than 5 equivalents of 3,5-DtBQ. Under these experimental conditions, saturation kinetics were found for the initial rates *versus* the 3,5-D*t*BC concentrations (Figure 3).

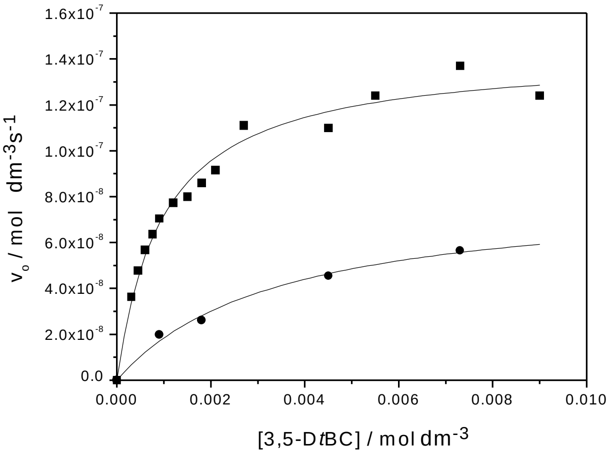

Figure 3. Dependence of the reaction rates on the 3,5-D*t*BC concentrations for the oxidation reaction catalyzed by the dicopper(II) complex **1** (■) and blank (●) . The reactions were performed in methanol saturated with O_2 / aqueous buffer TRIS pH 8.0 (30:1), [C] = 2.4 x 10^{-5} mol dm^{-3}, [3,5-D*t*BC] = 3.0 x 10^{-3} to 9.0 x 10^{-3} mol dm^{-3} at 25ºC.

An analysis of the data based on the Michaelis-Menten model, originally developed for enzyme kinetics, was applied. The results evaluated from Lineweaver-Burk plots are v_{max} = 1.4 x 10^{-7} mol dm^{-3} s^{-1}, K_M = 8.4 x 10^{-4} mol dm^{-3}, k_{cat} = 0.0057 s^{-1} and k_2 (k_{cat}/K_M) = 6.7 mol^{-1} dm^{-3} s^{-1}. The turnover rate of 20.52 h^{-1} is comparable to those values reported by Krebs et al.[7] (4 to 214 h^{-1}), but significantly lower than that value reported by Monzani et al.[6] with k_{cat} =1188 h^{-1}. Krebs *et al.*[7c] tried to establish a correlation between reactivity and copper-copper distance, but no clear relationship was found since complexes with almost the same metal-metal distance show very different activities. In fact, several factors need to be taken into account to accomplish metal complexes with catecholase-like activity such as metal-metal distance, lability of exogenous ligands and electrochemical properties.

Table 5. Magnetic and structural data for dicopper(II) complexes.

Complex	J / cm^{-1}	Cu...Cu / Å	Cu – O – Cu / °	Ref.
$[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(\mu\text{-}ClO_4)]^{2+}$ **1**	-23.9	3.355	124.24	this work
$[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(H_2O)_2]^{2+}$ **2**	-25.0	3.40	127.9	20

In the proposed mechanism, a pré-equilibrium of the free complex and the substrate or the coordination of diphenol prior to the intramolecular electron transfer is supported by the dependence of the reaction rate on substrate concentration, which fits the Michaelis-Menten mechanism. The electron transfer reaction then takes place, resulting in the oxidation of the substrate to the corresponding *o*-quinone and reduction of the copper centers to copper(I) as the rate-determining step. The Cu^I-Cu^I complex, in the presence of dioxygen, is immediately oxidized back to the original form completing the catalytic cycle.

Conclusions and Perspectives

We have synthesized and characterized a dinuclear copper(II) complex of the dinucleating ligand H_3bbppnol as a model compound for met form (Cu(II)-OH-Cu(II)) of catechol oxidase and tyrosinase enzymes. The met form of these enzymes oxidizes catechols by reduction of the copper centers to the Cu(I)-Cu(I) form. In this work we have shown that the dinuclear complex containing copper centers at 3.55 Å apart, allows the coordination of catechol to the metal centers favoring the electron transfer reaction, and therefore it represents a suitable functional model for catecholase activity of the enzymes mentioned previously with a reasonable turnover number. Currently, we are modifying the ligand by changing one of the phenol groups for pyridine seeking to obtain copper(II) complexes with different numbers of N, O donor groups but with the same endogenous bridging core and we aim to correlate these modifications with the catecholase activity of the new complexes.

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Supplementary Material

Crystallographic data have been deposited at the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK and copies can be obtained on request, free of charge, by quoting the publication citation and the deposition number CCDC 156633

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