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New mononuclear copper(II) complex based on a salen derivative ligand with an unusual coordination and its catecholase activity

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ABSTRACT

The new mononuclear copper(II) complex $[Cu^{II}(H_2LDA)(ClO_4)](ClO_4)$ **(1)** ($[H_2LDA$ = *N,N′*-[bis-(2-hydroxy-3-formyl-5-methylbenzyl)(dimethyl)]-ethylenediamine]) with an unusual coordination mode of a salen derivative ligand is reported. The most interesting feature of **1** is that the ligand is doubly protonated and presents significant intermolecular π-stacking interactions, contributing to the dimer structure stabilization in the solid state and in CH_3CN and methanolic solutions. The complex was characterized by X-ray crystallography and shows catecholase-like activity in the oxidation of the substrate 3,5-di-*tert*-butylcatechol (3,5-dtbc), with the formation of H_2O_2, which kinetic parameters are similar to those observed in conventional dinuclear bridged Cu^{II} complexes.

Introduction. Active copper and iron centers dominate the field of biological oxygen chemistry and play a vital role in catalysis [1]. The oxidation of organic substrates with molecular oxygen under mild conditions is of great interest for industrial and synthetic processes, and the ability of copper complexes to oxidize phenols and catechols has been known for at least 40 years [2].

Since the first reports of complexes using salen (salen = di(salicylidene)ethylenediamine) as ligand [3,4], the area of metal–salen catalysis has expanded greatly. A significant number of metal–salen like complexes have been reported in the literature (more than 4000 entries were found on the ISI Web of knowledge under the topic salen) and many of them have been used as catalysts in different oxidation reactions [5,6]. The easy synthesis and modification of the salen ligand skeleton contribute to making it a good option for the design of new catalysts. Salen-type ligands generally coordinate to the metal with N_2O_2 atom donors. Herein, we report the synthesis and characterization of a new salen derivative ligand containing a carbonyl group attached to the phenol group, the X-ray structure of its Cu^{II} complex (Fig. 1) and the catecholase activity of the oxidation of the substrate 3,5-di-*tert*-butylcatechol (3,5-dtbc). Interestingly, in the present structure of **1**, the Cu^{II} center is coordinated by the oxygen atoms of the ligand H_2LDA while the amine nitrogens are protonated. It is important to emphasize here that only one other example of this sort of ligand and its Cu^{II} complex has been reported in the literature [7].

Experimental. The ligand *N,N′*-[bis-(2-hydroxy-3-formyl-5-methylbenzyl)(dimethyl)]-ethylenediamine (H_2LDA) was obtained by nucleophilic substitution of 2-hydroxy-3-chloro-methyl-5-methylbenzaldehyde and *N,N′*-dimethylethylenediamine in dichloromethane [8]. The product was recrystallized from ethanol to give light yellow crystals, yield 78%. ^{1}H-NMR ($CDCl_3$): 2.26 (s, 6H, CH_3); 2.29 (s, 4H, CH_3); 2.69 (s, 6H, CH_2 amine); 3.68 (s, 4H, CH_2); 7.22 (s, 2H, CH_{Ar}); 7.26 (s, 2H, CH_{Ar}); 7.38 (s, 2H,OH_{phenol}); 10.18 (s, 2H, CH_{ald}). $C_{22}H_{28}N_2O_4$ calcd: C 68.73; H 7.34; N 7.29. Found: C 68.17; H 7.84; N 7.26. MP: 131–132 °C.

The complex **1** was prepared according to the following procedure: To a 20 mL acetonitrile solution containing 0.192 g (0.5 mmol) of the ligand, it was added, 15 mL of a acetonitrile solution of copper(II) perchlorate (0.187 g, 0.5 mmol) was slowly added. After 20 min, a few drops of an aqueous solution of perchloric acid (2 M) were added and the solution was allowed to stand. After 3 days, green crystals were obtained with 67% yield. $CuC_{22}H_{28}Cl_2N_2O_{12}$ calcd: C 40.85; H 4.36; N 4.33. Found: C 40.70; H 4.31; N 4.29.

Elemental analysis (CHN) was performed on a Carlo Erba E-1110 analyzer. Electronic absorption spectra in the 200–1200 nm range were recorded on a Perkin-Elmer Lambda 19 spectrophotometer. Electrochemical measurements were obtained using a Bas Epsilon potentiostat/galvanostat. Square-wave voltammograms were obtained for the complex in acetonitrile solution containing 0.1 M tetrabutylammonium hexafluorophosphate as the supporting electrolyte under an argon atmosphere. The electrochemical cell employed was of a standard three-electrode configuration: glassy carbon electrode (working), platinum wire (counter), Ag/AgCl (reference). The Fc^+/Fc couple ($E_{1/2}$ = 400 mV *vs* NHE) was used as the internal standard [9]. The X-band (9.76 GHz)

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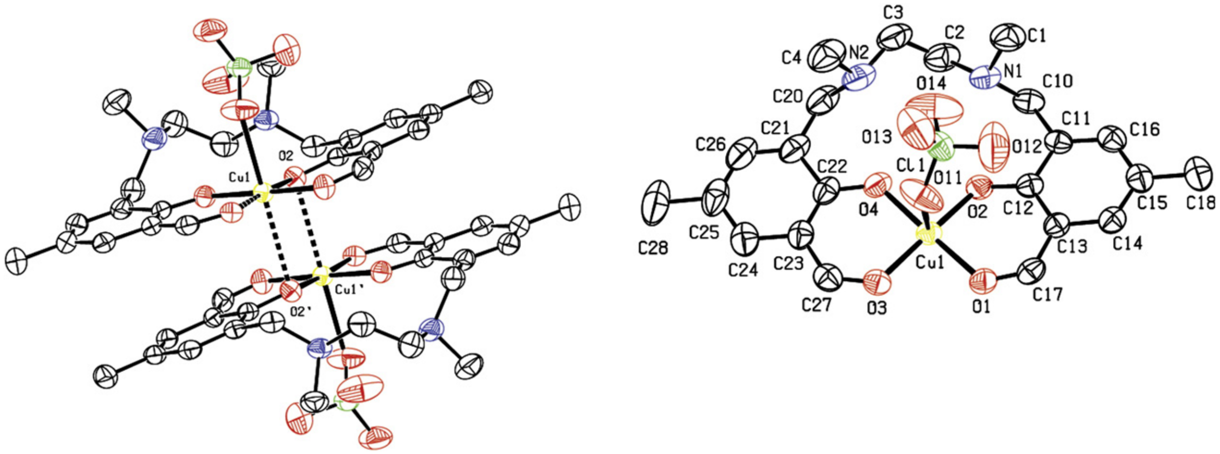


Fig. 1. ORTEP view of the dimer structure (left, symmetry code: $-x + 0.5, -y + 0.5, -z + 1$) and cation complex **1** (right). Ellipsoids are shown at 40% probability level and hydrogen atoms were omitted for clarity. Selected bond distances (Å): Cu1–O4 1.900(3); Cu1–O2 1.901(3); Cu1–O3 1.939(3); Cu1–O1 1.955(3); Cu1–O11 2.471(4); Cu1 O2′ 2.822(3); Cu1–Cu1′ 3.4744(9).

measurement was performed on a BRUKER EleXsys E580 spectrometer at 5 K using continuous flow liquid-helium cryostats (Oxford Intruments ESR 900).

Electrospray ionization mass spectrometry (ESI-MS) of **1** dissolved in an ultrapure acetonitrile solution (500 nM) was performed using an amaZon X Ion Trap MS instrument (Bruker Daltonics) with an ion spray source using electrospray ionization in positive-ion mode. The ion source condition was an ion spray voltage of 4500 V. Nitrogen was used as the nebulizing gas (20 psi) and curtain gas (10 psi). The samples were directly infused into the mass spectrometer at a flow rate of180 μL/h. The scan range was m/z 200–3000. The simulated Spectrum was calculated using the Mmass software [10,11].

Potentiometric studies of **1** were carried out in an acetonitrile/water mixture (1:1, v/v) with a Corning-350 research pH meter fitted with blue-glass and Ag/AgCl reference electrodes, calibrated to read $-\log [H^+]$ directly, designated as the pH. Equilibrium measurements were performed in a thermostated cell, purged with argon, containing 50.00 mL of the acetonitrile/water (1:1) solution and 0.03 mmol of the complex. The temperature was maintained at 25.00 ± 0.05 °C, and the experimental solutions were adjusted to an ionic strength of 0.100 M through the addition of KCl. Computations of the triplicate results were carried out with the BEST7 program, and species diagrams were obtained with the SPE and SPEPLOT programs [12].

The catecholase-like activity of the complex was determined by measuring the oxidation of the substrate 3,5-di-*tert*-butylcatechol (3,5-dtbc) in a UV–Vis Varian Cary 50 BIO fitted with a thermostated water-jacketed cell holder. The reactions were accompanied by formation of 3,5-di-tert-butylquinone (3,5-dtbq) at 400 nm($\varepsilon = 1900\ M^{-1}\ cm^{-1}$) and 25.0 °C; less than 5% of conversion of substrate to product were monitored and the data were treated by the initial rate method.

Initially, pH-dependent studies were carried out to determine the pH value at which catecholase-like activities reached a maximum. The influence of pH on the reaction rate in the oxidation of 3,5-dtbc catalyzed by **1** was determined over the pH range of 5.0–9.0 at 25 °C. The following reagents were placed in a 1-cm-path quartz cell: 100 μL of an aqueous solution ($[B]_{final} = 100$ mM) of buffer [MES (pH 5.0–6.5) and TRIS (pH 7.0–9.0)], 30 μL of a methanolic complex solution ($[1]_{final} = 58$ μM), and 1500 μL of air-saturated methanol. The reaction was initiated with the addition of 50 μL of a methanolic substrate solution ($[3,5\text{-dtbc}]_{final} = 5.00$ mM) and monitored for 20 min. The kinetic experiments under conditions of excess substrate were performed as follows: a total of 100 μL of aqueous buffer TRIS at pH 8.5 ($[B]_{final} = 100$ mM), 30 μL of a methanolic complex solution ($[1]_{final} = 58$ μM), and oxygen-saturated methanol (to complete 1530 μL) were added to a 1 cm path-length cell at 25 °C. The reaction was initiated with the addition of known volumes (from 50 to 500 μL) of a 3,5-dtbc solution ($[3,5\text{-dtbc}]_{final} = 0.9$–9 mM). All experiments were carried out in an air saturated methanolic/water (32:1) solution. To take into account the spontaneous oxidation of the substrate, correction was carried out using a reference cell under identical conditions but without the addition of the catalyst. The initial rate was obtained from the slope of the absorbance versus time plot over the first 20 min of the reaction. The Michaelis–Menten model was applied and the kinetic parameters were obtained from nonlinear least squares fit.

Results and discussion. The synthesized complex **1** afforded suitable crystals for the structure determination by X-ray analysis (See Supplementary Material - Table S1 and Table S2). The structure of **1** (Fig. 1, right) reveals that the Cu^{II} center is “4 + 2” coordinated, exhibiting a highly distorted tetragonal geometry in which the basal plane is occupied by two oxygen atoms from the phenolates and two oxygen atoms from the carbonyl group of the H_2LDA ligand. One perchlorate group and one phenolate oxygen from another $[Cu^{II}(H_2LDA)(ClO_4)](ClO_4)$ molecule, in axial positions, complete the “4 + 2” pseudo-coordination geometry. The Cu–O distances in the basal plane are between 1.900 and 1.955 Å and the $Cu–O_{perchlorate}$ in the apical position is of 2.471(4) Å. The *trans* O–Cu–O angles in the basal plane are of 176.13(12) and 178.41(13)º, showing a very small distortion in the geometry ($\tau = 0.01$) [13]. The bond lengths around the Cu^{II} ion in the basal plane (O_4-donor site - average 1.923 Å) of complex **1** are similar to the corresponding distances (average 1.918 Å) observed around the Cu2 center in the dinuclear $[Cu_2L(dmf)_2]^{2+}$ cation containing a similar coordination environment, in which 1,3-propanediamine forms the backbone of the ligand L [7]. For the ligand described in this work, the addition of perchloric acid to the reaction mixture as well as steric crowding around the tertiary amines probably prevents a second Cu^{II} from being accommodated.

In fact, the packing analysis in the unit cell shows that two molecules of the $[Cu^{II}(H_2LDA)(ClO_4)](ClO_4)$ complex are very close with respect to each other and thus, a dimeric structure (face to face) is formed considering the weak inter-dimer electrostatic $Cu–O_{(phenolate)}$ interactions in this centrosymmetric arrangement (Fig. 1 left). In the dimer, the Cu1–O2′ and Cu1···Cu1′ distances are of 2.822(3) Å and 3.4744(9) Å, respectively, which are significantly shorter than those distances found in the bis(salicylaldehydato)Cu^{II} complex (3.13 and 4.05 Å, respectively), most probably due to the electrostatic interactions between the Cu–O phenolates and the protonated amines of neighboring molecules in **1** [14,15]. Indeed this Cu···Cu distance is comparable to those distances found in many dinuclear Cu^{II} oxygen bridged complexes [16–19]. Thus, in the solid state the complex is a dimer, where each Cu^{II} ion is surrounded by six oxygen atoms. In addition, intermolecular neighboring phenolate rings are coplanar to each other, with a dihedral angle

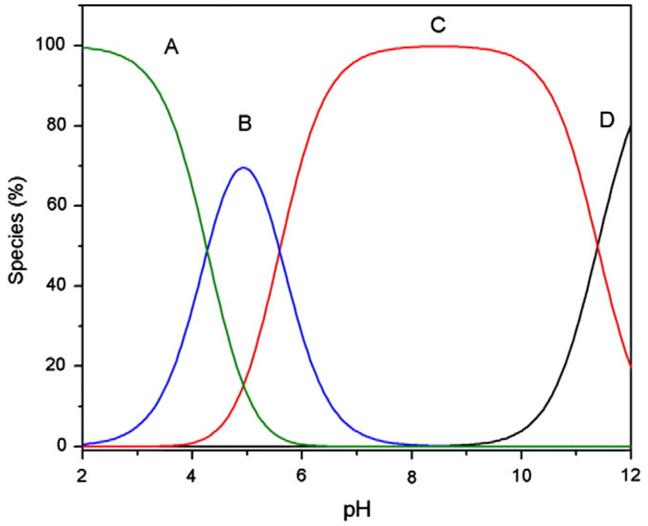


Fig. 2. Distribution of species for complex **1** according to Scheme 1.

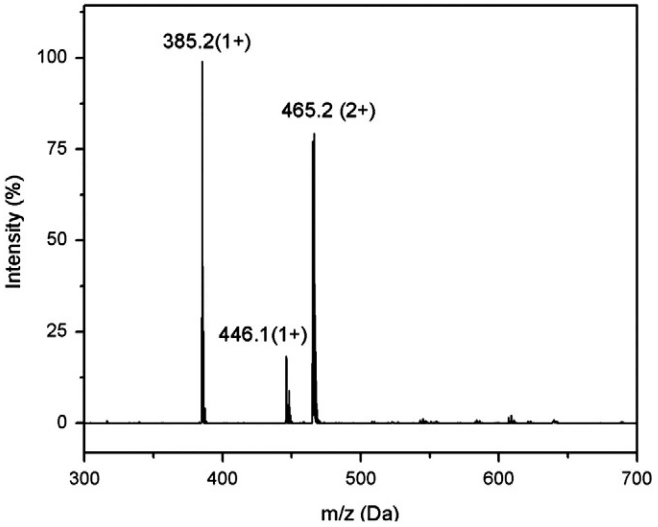


Fig. 3. Positive ion ESI mass spectrum for **1** in CH_3CN.

between the planes and a mean distance between them of 8.62(18)° and 3.520 Å, respectively, suggesting the occurrence of intermolecular π-stacking, which also contribute to stabilize the dimer. Unlike other salen-derivative–metal complexes, the two nitrogen atoms, from the amines, are not coordinated to the metal center and are protonated.

The cyclic voltammograms of the complex in CH_3CN solution show an irreversible oxidation $E_{pc} = -0.55$ V *vs.* NHE which can be attributed to the $Cu^{II}/^{I}$ couple (Fig. S 1). This value is in good agreement with other mononuclear Cu^{II} complexes with two phenolates coordinated to the metal center [20,21].

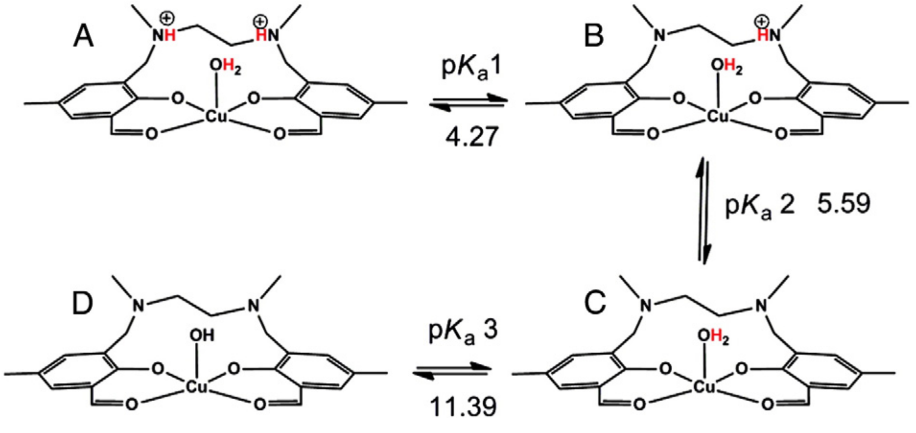
The electronic spectrum of **1** in CH_3OH solution (Fig. S 2) shows an intense band centered at 380 nm ($\varepsilon = 3200\ M^{-1}\ cm^{-1}$) attributed to a ligand-to-metal charge transfer (LMCT) transition between the two phenolates and the Cu^{II} ion and a broad band at ~640 nm ($\varepsilon = 65\ M^{-1}\ cm^{-1}$), which is typical of Cu^{II} d–d transitions.

The X-band EPR spectrum recorded in a frozen methanolic solution of **1** shows four lines, typical of Cu^{II} ions, as a result of the hyperfine interaction between the unpaired electron ($S = ½$) and the Cu^{II} nucleus ($I = 3/2$) (Fig. S 3). The parameters $A_{||} = 240 \times 10^{-4}$ G, $g_{||} = 2.28$ and $g\perp = 2.07$ were obtained from the simulated spectrum and since $g_{||} > g\perp$, a square pyramidal geometry can be proposed for complex **1,** which is consistent with the geometry found from the X ray analysis and also with the structure proposed in solution from the potentiometric titration, ESI-MS *(vide infra)* and the electronic spectroscopic results.

Potentiometric titration studies of **1** in a water/acetonitrile (1:1) solution showed the neutralization of 3 mol of KOH/mol of complex in the pH range 3–12. Three pK_a values were found and speciation plots are shown in the Fig. 2.

The first two constants $pK_{a1} = 4.27$ and $pK_{a2} = 5.59$ can be attributed to the deprotonation of the uncoordinated amine nitrogen atoms, which are protonated even in the solid state structure. The pK_{a3} at 11.39 can be tentatively attributed to the deprotonation of a water molecule coordinated to the Cu^{II} center in the Jahn–Teller position as shown in Scheme 1.

In order to establish unequivocally the relevant species in solution, ESI-MS studies were carried out in CH_3CN. Fig. 3 shows the spectrum in CH_3CN, whileFig. S 4 (See Supplementary Material) shows the observed and expected isotopic distribution for the assigned species under these experimental conditions. As can be observed in Fig. 3, three main groups of peaks are observed at mass to charge (*m/z*) ratios of 466.2, 446.1, and 385.2. The peak at $m/z = 465.2$ can be attributed to $[(Cu^{I}H_2LDA)_2(H_2O)]^{2+}$, (reduction of Cu^{II} to Cu^{I} under the conditions of the electrospray ionization), which corresponds to a dimer form of the complex, in agreement with the crystal structure and the four protonated nitrogen atoms from the amines observed in the equilibrium studies (Scheme 1 and Fig. S 5). The peak at $m/z = 446.1$ can be attributed to the $[(Cu^{II}HLDA)]^{+}$ species while the peak at 385.2 correspond to the free ligand. Therefore, it can be concluded that the dimer form of



Scheme 1. Proposed equilibria for **1** in water/acetonitrile (1:1) solution.

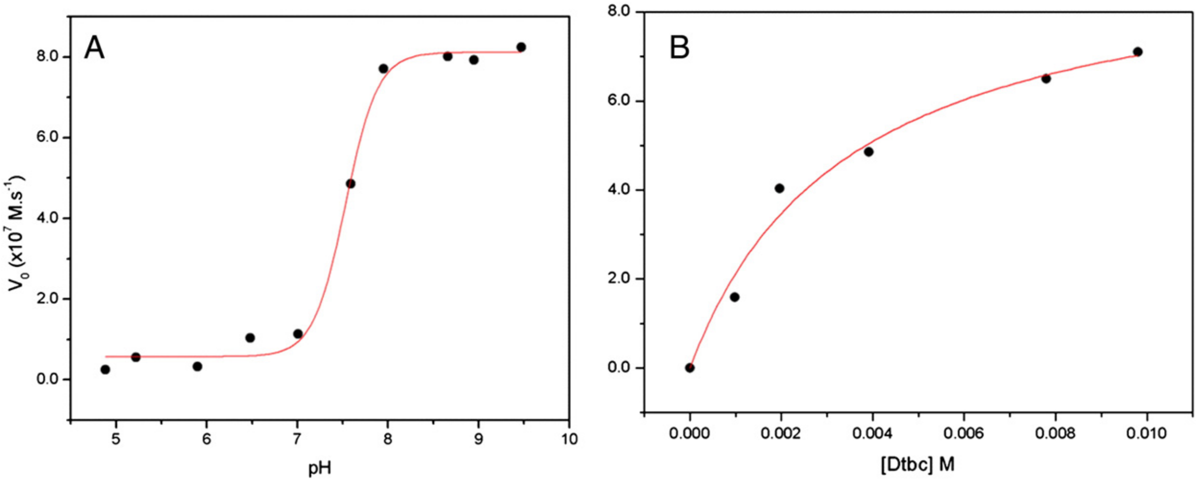


Fig. 4. (A) Dependence of the initial rate on the pH for the oxidation of the 3,5-dtbc promoted by **1**. (B) Dependence of the initial rate on the 3,5-dtbc concentration for the oxidation promoted by **1**.

the complex containing one Cu^{II}-coordinated water molecule (instead of a pseudo-coordinated ClO_4^-) is maintained in CH_3CN or CH_3OH solutions.

As mentioned above, several metal–salen complexes have been tested as catalysts for oxidation processes [5,22], although, a few number of such complexes have been employed in the oxidation of catechols. Considering that the dimer form of the complex presented herein is maintained in CH_3OH solutions, we investigated the catalytic activity of the complex **1** in the oxidation of 3,5-dtbc. The reactions were carried out in CH_3OH solutions, using the initial rate method by monitoring the increase in the characteristic quinone (3,5-dtbq) absorption band at 400 nm ($\varepsilon = 1900\ M^{-1}\ cm^{-1}$), which is sufficiently stable under these experimental conditions. Before the kinetic experiments the solvent was saturated with O_2.

The dependence of the oxidation reaction catalyzed by complex on the pH was investigated within the range of 5.0–9.0 in order to determine the pH value at which the catecholase-like activity is at a maximum and a sigmoidal shaped profile was obtained, as seen in Fig. 4A. The data were fitted using a Boltzmann model, and a sigmoidal fit of the curve revealed a kinetic pK_a value of 7.53 ± 0.1 which is in relative good agreement with the deprotonation of the catechol in presence of Cu^{II} [23].

The complex exhibited saturation kinetics behavior for initial rates (V_0) *vs* 3,5-dtbc concentrations (Fig. 4B), consistent with the binding of the substrate to the metal complex. The good fitting of the data to the Michaelis–Menten model was observed, with the kinetic parameters $k_{cat} = 1.63 \times 10^{-2}\ s^{-1}$; $K_M = 3.5 \times 10^{-3}$ M; $k_{cat}/K_M = 4.9\ M^{-1}\ s^{-1}$. These values are of the same magnitude as those determined for dinuclear Cu^{II} complexes reported in the literature. [17,19,24]. These results strongly suggest that the dimer $[(Cu_2^{II}(LDA)_2(H_2O)_2]^{2+}$ is probably the active species in the oxidation of 3,5-dtbc. Indeed, this hypothesis is favorable, given that the dimer species seems to be stable in CH_3CN and methanolic solutions, as confirmed by ESI-MS, and that the Cu···Cu distance in the solid state structure of ~3.4 Å lies in the range for binuclear Cu^{II} complexes widely employed in the catalytic oxidation of 3,5-dtbc with similar catalytic turnover numbers [16]. Detailed structural and catecholase-like studies on a series of Cu^{II} complexes have shown that dinuclear copper(II) complexes catalyze the reaction while the mononuclear species are either not as efficient or inactive depending on the steric demands of the ligands [25], which are in full agreement with the conclusion that the dimer $[(Cu_2^{II}(LDA)_2(H_2O)_2]^{2+}$ is the catalytically active species in the oxidation of 3,5-dtbc.

Finally, the accumulation of H_2O_2 during turnover was confirmed by means of the molybdate-accelerated I_3^- assay (75% of the expected amount was detected), which indicates that reoxidation of the copper(I) species back to the active copper(II) species occurs with a 1:1 (O_2:3,5-dtbc) stoichiometry and concomitant formation of hydrogen peroxide.

Thus, it can be concluded that the significant catecholase activity of the complex described herein can be explained by the fact that the two Cu^{II} centers within the dimer are close enough together to facilitate the binding of two phenolic oxygen atoms of catechol and mediate the redox reaction.

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Appendix A. Supplementary materials. The details of the X-ray analysis (Table S1 and Table S2), Cyclic voltammetry (Fig. S 1), Electronic spectroscopy (Fig. S 2), EPR (Fig. S 3) and Mass spectrometry (Fig. S 4 and Fig. S 5) are given in Supplementary material. CCDC 936899 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk. Supplementary materials related to this article can be found online at http://dx.doi.org/10.1016/j.inoche.2013.09.039.

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