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Synthesis, characterization and superoxide dismutase activity of bi-copper(II)-bisacetato-$\mu-$phthalicacid[bis(benzyloxy)ethyl]ester

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Abstract. A new binuclear copper(II) complex, bridged by the ligand phthalicacid[bis(benzyloxy)ethyl]ester, where each copper(II) is coordinated to one carboxylate (from ligand) and one acetate in square planar mode is reported. The ligand synthesized by the reaction of phthalic anhydride and ethylene glycol, has been characterized by FT-IR, ^{1}HNMR and LCMS. The binuclear Copper(II) complex has been characterized by UV/visible spectra, FTIR spectra, EPR spectra, ESI-MS spectra, magnetic moment measurement and thermogravimetric analysis. DFT calculation has shown a **Z** type structure for the complex. Excellent superoxide dismutase activity with IC_{50} value 8.6×10^{-6} M for the complex has been observed.

Keywords. phthalic anhydride; ethylene glycol; acetate; copper(II); superoxide; IC_{50}; DFT.

1. Introduction

Copper(II) is a biologically important metal ion involved in a number of enzymes such as – superoxide dismutase (SOD), tryosinase, galactose oxidase, B - hydroxylases, monoaminooxidase, ascorbic acid oxidase, plastocyanine, azurine, etc.[1–3] Complexes of copper in oxidation state +2 are also biologically significant because of their antioxidant and free radical scavenging activity.[4,5] Binuclear copper (II) complexes are potential models for a number of important biological systems containing couple sites[6] and have been studied extensively.[7–12] Besides, copper (II) complexes of multidentate Schiff base ligands have played a vital role in the development of coordination chemistry.[13–15] The bimetallic copper (II) complexes have also attracted much attention in magneto chemistry due to the spin-spin interaction between the copper (II) centres.

SOD is an enzyme involved in protecting biological cells from the toxic effects of superoxides.[16] Based on the metal ions present in the active sites, SODs have been divided into – Cu-Zn-SOD, Mn-SOD and Fe-SOD, out of which the first one is found in mammals.[17] Deficient level of SOD concentration in human body is one of the reasons behind diseases and disorders like diabetes, ischemia, cataract, Parkinson's disease, cancer, etc.[18,19] Supplementation of antioxidant enzymes should be a part of the treatment but administration of these enzymes through oral or intraperitonial routes is severely restricted due to their rapid degradation and short life time in biological systems.[20] Small metal complexes having good superoxide scavenging activity are potential candidates in this regard.

Copper(II) complexes with Schiff base ligands derived from various aldehydes and ketones have been reported to mimic SOD activity.[21] Other examples in this regard include imidazole bridged copper(II) complexes,[22] planar copper(II) complex on addition of a base such as N-methyl imidazole or pyridine,[23] curcumin complexes of copper(II),[24] etc. There is also a report that the copper(II) complexes with Schiff base ligands of salicylaldehyde semicarbazone has SOD activity which could be tuned by heterocyclic bases, pyridine and N-methyl imidazole.[25]

In present day, density functional theory (DFT) has become an effective tool for determining structure, electronic properties of molecules, vibrational frequencies, atomization energies, ionization energies, etc.[26–29] Particularly when X-ray grade crystals are not obtained, DFT calculation has been an effective mode of confirming the structure of metal complexes.

In this paper, we report the synthesis of a new ligand phthalicacid[bis(benzyloxy)ethyl]ester characterized by FTIR, ^{1}HNMR and mass spectra. This ligand has been reported to bridge two Copper (II) ions through its two carboxylates and each Copper (II) is coordinated to one acetate in square planar fashion. DFT calculation shows a Z type structure for the

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complex. The high superoxide scavenging activity of the complex is also reported.

2. Experimental

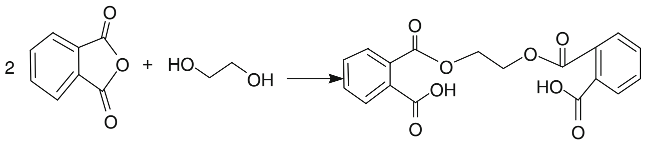
2.1 *Materials and methods*

Phthalic anhydride and ethylene glycol were purchased from Merck and copper(II)acetate monohydrate was purchased from LOBA Chemie. The FTIR spectra were recorded in KBr discs on a Perkin Elmer spectrum RXI FTIR system. The ^{1}H NMR spectra were recorded in Bruker Ultra shield 300 spectrophotometer. The electronic spectra in the range of 200–1000 nm were obtained in acetonitrile on a UV-1800 SHIMADZU spectrophotometer. Thermogravimetric measurements were carried out on a PERKIN ELMER 300 TGA instrument. CHI 600B Electrochemical Analyzer (USA) with a three electrode cell assembly was used for electrochemical studies. The electrodes were cleaned as per reported procedure.[30] Electron paramagnetic resonance (EPR) spectra were recorded on a Bruker EMX spectrometer (centre field 0.4 T, sweep width 0.8 T, resolution 1024 points, microwave frequency 9.877×109 Hz, power 0.188 mW). Magnetic susceptibility measurements were performed at ambient temperature by the Gouy method using a Cambridge magnetic balance (UK), LC-MS data were recorded in Agilent LCMS 6410 Series (USA).

2.2 *Synthesis and characterization of phthalic acid bis(benzyloxy)ethyl ester (L, $C_{18}H_{14}O_8$)*

20 mmol (2.96 g) phthalic anhydride was taken in a mortar and grinded. 10 mmol (0.6 mL) of ethylene glycol was added dropwise with constant stirring. The reaction mixture was heated to 60°C and 2–3 drops of pyridine was added. The mixture was cooled to obtain an off-white product which was then recrystallized from methanol. The synthetic path for the ligand (L) is shown in scheme 1 below.

FTIR (KBr pellet, cm^{-1}): 3404 (υ_{O-H}), 2920.7 (υ_{C-H}), 1627 ($\upsilon_{C=O}$), 1289.4 (υ_{C-O}), 1391.3 (δ_{-CH2-}), 756 and 705 (aromatic C-H out of plane bending). **LCMS**: m/e (M$^+$) 357.3, calc. 358.3. ^{1}**HNMR** (CDCl$_3$, δ in ppm): 7.72 (2H, t, J = 5.1 Hz); 7.56 (2H, t, J = 5.7 Hz); 7.44 (4H, d, J = 4.0 Hz); 4.52 (4H, s) (see Supplementary Information).



Scheme 1. Synthetic path for the ligand (**L**).

2.3 *Synthesis of bi-copper(II)-bisacetato-μ−phthalicacid[bis(benzyloxy) ethyl]ester ((CH_3COO)Cu(II)LCu(II)($OOCCH_3$)), $Cu_2C_{22}H_{20}O_{12}$*

Cu(II)acetate monohydrate 1 mmol (0.199 g) was dissolved in 10 mL methanol. A solution of L, prepared by dissolving 1 mmol (0.380 g) into 10 mL of methanol, was added dropwise with vigorous stirring. The stirring was continued for three hours till dark blue precipitate was obtained. The precipitate was washed with diethyl ether and dried in air. The compound was recrystallized from acetonitrile to get blue coloured crystals.

3. Results and Discussion

3.1 *Electronic and vibrational spectroscopy of (CH_3COO)Cu(II)LCu(II)($OOCCH_3$)*

The UV/visible spectra of the complex was recorded in acetonitrile and a broad band was observed at λ_{max} 714 nm (figure 1). The extinction coefficient (ε) was calculated to be 709.68 L mol^{-1} cm^{-1}.

Vibrational spectra for the complex synthesized showed peaks at 2966.5 cm^{-1} (υ_{C-H} of C_6H_5); 756 cm^{-1} & 705 cm^{-1} (C-H out of plan vibration for C_6H_5); 1631.7 cm^{-1}($\upsilon_{C=O}$); 1400 cm^{-1} (υ_{symm}. COO$^-$); 1589.3 cm^{-1} (υ_{asymm}. COO$^-$); 3441cm^{-1} (υ_{O-H}).

3.2 *EPR spectroscopy of (CH_3COO)Cu(II)LCu(II) ($OOCCH_3$)*

The X–band EPR spectra of the complex was recorded as the polycrystalline samples at room temperature (figure 2). The g_{iso} value and geometric parameter G i.e.,

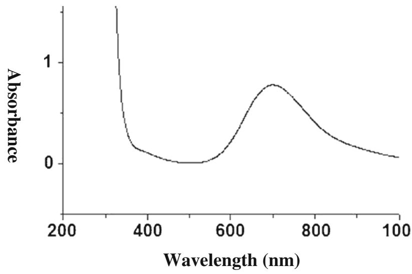


Figure 1. The UV/visible spectrum of (CH_3COO)Cu(II) LCu(II)($OOCCH_3$) in acetonitrile. (1×10^{-3} M, path length 1.0 cm)

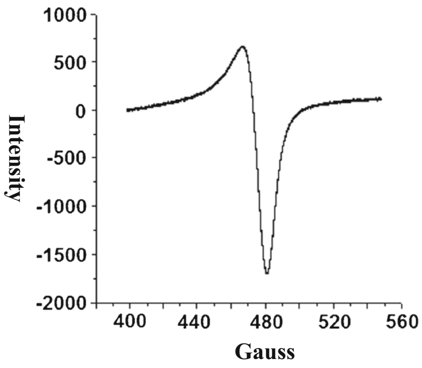


Figure 2. The X–band EPR spectra of $(CH_3COO)Cu(II)LCu(II)(OOCCH_3)$ recorded as the polycrystalline samples at room temperature.

the measurement of exchange interaction between the copper centres was evaluated by using the expression:[31]

$$g_{iso} = 1/3(g_{\parallel} + 2g_{\perp})$$

$$G = \frac{(g_{\parallel} - 2.0023)}{(g_{\perp} - 2.0023)} = \frac{4K_{\parallel}2\Delta E_{xz}}{k_{\perp}^2 \Delta E_{xy}}$$

The calculated value of g tensor parameter was $g_{\parallel} = 2.06$ and $g_{-} = 2.03$. Hence, $g_{\parallel} g_{-} > 2.003$ which reveals that $d_{x^2-y^2}$ is the ground state.[32] The value of G was calculated to be 2.04 which is less than 4 indicating effective interaction between the copper centres.[33]

3.3 *Magnetic moment of $(CH_3COO)Cu(II)LCu(II)(OOCCH_3)$*

The magnetic moment value was measured to be 1.65 BM which is a little lower than the single electron value of 1.74 BM. This low value of magnetic moment may be due to anti-ferromagnetic coupling of the individual magnetic moments of the copper (II) centres.

3.4 *Thermogravimetric studies of $(CH_3COO)Cu(II)LCu(II)(OOCCH_3)$*

Thermogravimetric weight loss curve for the complex is shown in figure 3. The weight loss profile as a function of temperature showed one step at ca. 210°C with a shoulder at 310°C. It is well known that acetates dissociate in the temperature range 200°C to 400°C. The weight loss profile has been analysed as reported in literature.[34] The total loss in weight is ca. 73.61%. This weight loss is justified if we assume that the end product is two equivalent of CuO. This thermogravimetric analysis supports the structure of the complex as depicted in scheme 2.

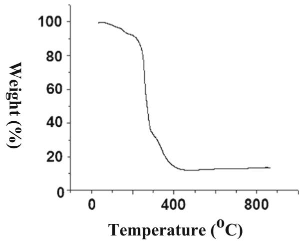
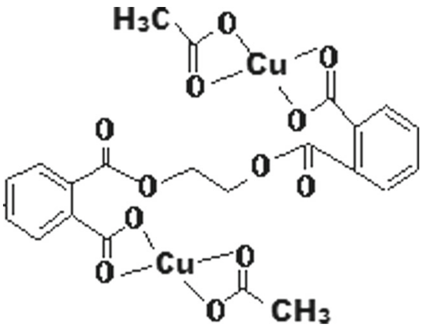


Figure 3. TGA curve of $(CH_3COO)Cu(II)LCu(II)(OOCCH_3)$.



Scheme 2. Proposed structure of $(CH_3COO)Cu(II)LCu(II)(OOCCH_3)$, confirmed by DFT calculation (figure 5).

3.5 *ESI-MS of $(CH_3COO)Cu(II)LCu(II)(OOCCH_3)$*

The ESI-MS spectra were measured in order to confirm the composition and purity of the compound under investigation. The spectra displayed the molecular ion peak of complex at m/z 602.48. The calculated value of molecular mass of complex is 603.48.

3.6 *Electrochemistry of $(CH_3COO)Cu(II)LCu(II)(OOCCH_3)$*

Cyclic voltammogram of 1.0 mM solution of the complex in acetonitrile was done on Pt disc electrode. A very sharp irreversible reduction peak was observed at −0.005 V versus Ag-AgCl (Scan rate 0.1 Vs^{-1}). This observation is obvious because in the complex, the Cu(II) ion is bound to four hard oxygen donor sites which makes the co-ordination very stable. On reduction of Cu(II) into Cu(I), which is relatively soft, the four oxygen donors are no longer suitable and the complex is expected to break down. Hence, the irreversible reduction peak without any oxidation counterpart was observed.

3.7 *DFT optimization of the complex*

The complex has been optimized using 6-31+G(d) basis set, with Becke three-parameter exchange and Lee, Yang and Parr correlation functional, B3LYP;[35] the

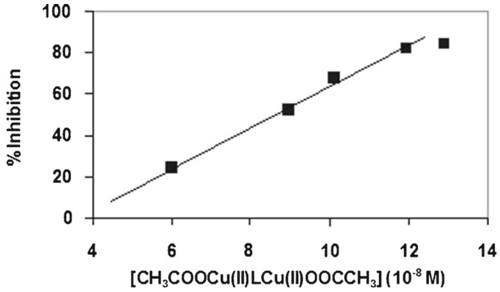


Figure 4. Plot of the percentage inhibition of superoxide formation against the $(CH_3COO)Cu(II)LCu(II)(OOCCH_3)$ concentration.

optimized structure is shown in figure 4. The DFT optimised structure shows that each of the two carboxylates of L binds one Cu^{2+} ion. Each of the Cu^{2+} ion is bound to one acetate in a planar mode. The final structure of the complex resembles the alphabet Z.

3.8 *SOD activity of $(CH_3COO)Cu(II)LCu(II)(OOCCH_3)$*

The SOD activity of copper (II) phthalicacid[bis(benzyloxy)ethyl]ester has been studied by the method of nitrobluetetrazolium (NBT, Structure included in SI) reduction using $KO_2^{-\cdot}$ as the source of superoxide radical.[36] The blue colour developed due to the formation of formazon dye was measured immediately at 560 nm against an appropriate blank. One unit of SOD activity (IC_{50} value) was defined as the amount of test substance required for 50% inhibition of NBT reduction by the superoxide anion.[25] A linear relation was obtained between the concentration of the copper complex and the inhibition of the superoxide ion. The 100% of superoxide activity corresponds to an assay performed in the absence of complex. In order to determine the concentration of the complex required to yield 50% inhibition of the reaction, we plotted the percentage of inhibition against the metal concentration (figure 4) and the obtained IC_{50} value was 8.6×10^{-6} M (the IC_{50} value of the native enzyme is 9.5×10^{-9} M). This IC_{50} value is smaller than many reported ones, for e.g., IC_{50} values are found in the range 3.0×10^{-5} M to 3.7×10^{-5} M for Cu(II) complexes of amino acids;[37] 9.9×10^{-5} M to 2.4×10^{-4} M for the Cu(II) complexes with simple dipeptides.[38] It has been proposed that only complexes with IC_{50} values below 20×10^{-6} M may become clinically interesting.[39] Therefore, $LCu(II)(\mu\text{-}CH_3COO)_2Cu(II)$ fulfils this requirement and appears to be an interesting possibility for further investigations in the field of SOD-mimetic drugs (figure 5).

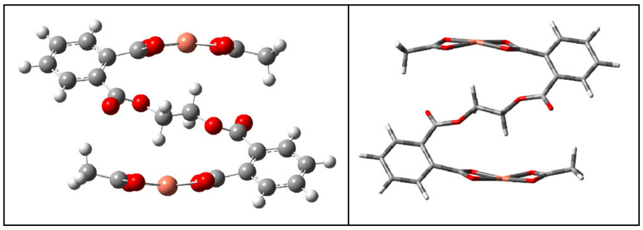


Figure 5. DFT optimized structures of $(CH_3COO)Cu(II)LCu(II)(OOCCH_3)$. Pink = Cu(II); Red = O; Gray = C; White = H.

4. Conclusion

A new binuclear copper(II) complex, $(CH_3COO)Cu(II)LCu(II)(OOCCH_3)$, where L is phthalic acid bis(benzyloxy)ethyl ester, has been synthesized and characterized by various spectroscopic methods. DFT calculation showed a Z-type structure for the complex. Good superoxide scavenging behaviour was observed for the complex.

Supplementary Information

LC-MS and ^{1}HNMR spectra of the ligand **L**, FTIR and LC-LS spectra of $(CH_3COO)Cu(II)LCu(II)(OOCCH_3)$, structure of NBT (Nitrobluetetrazolium) are available at www.ias.ac.in/chemsci.

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