Oxidation of Polyphenol Trihydroxybenzene Using Environment Friendly Catalyst Copper (II) Complex of 4-Methoxyphenyl Benzopyran

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Abstract: The copper (II) complex of 4-methoxyphenyl benzopyran has been synthesized using microwave irradiation and characterized by different tools such as IR, electronic spectroscopy, elemental analysis, thermal analysis, and ESR spectroscopy. The mode of metal binding shows that the copper binds with the ligand through the nitrogen atoms of the amino and cyanide groups. The copper complex [Cu L (AC)$_2$.2H$_2$O] has been used in the homogenous oxidation of polyphenol 1,2,3-trihydroxybenzene in presence of H$_2$O$_2$ as a green oxidant to produce a first-order rate constant k_{cat} = 3.45×10^{-3}s^{-1} and dissociation constant K′ = 32.0 mM. The catalysis shows 1.4×10^3 times rate enhancement in terms of the first-order rate constant compared to the uncatalyzed reaction under the same reaction conditions. The oxidation reaction is inhibited by kojic acid with IC$_{50}$ = 220 µM.

1. Introduction

There is an increasing need for decontamination of refractory compounds, such as phenolic contaminants, that cannot be degraded by conventional means. The phenol and derivatives are generally toxic even at very low concentrations. The environmental Protection Agency of the USA has recommended discharge limits of 0.1 mg/L in wastewaters. These chemicals are present in the effluents of different industries, (*e.g.* petroleum refineries, petrochemical facilities, resin manufacturers, wood preserving sites). Several methods have been proposed for treatment: recovery, incineration, adsorption, biological treatment and chemical oxidation which is considered as one of most important method in this filed.

Catechol oxidase, tyrosinase, and polyphenol oxidases are analogous metalloenzymes which oxidize phenolic compounds to the corresponding quinones in the presence of oxygen. This kind of reaction is of great importance in medical diagnosis for the determination of the hormonally active catecholamines adrenaline, noradrenaline and dopa [1]. There have been great efforts to synthesize model complexes as functional or structural models for catechol oxidases or related copper containing enzymes [2- 3].

Transition metal complexes have been widely studied in the area of bio-inorganic, bio-organic, and catalytic chemistry [4-5]. Copper complexes are interesting compounds in the field of oxidation catalysis as copper-containing oxidases. These biomimetic chemical systems may be better accessible, more stable and more catalytically versatile than enzymes, thus may have wider applications and provide chemical insight into the mechanisms of enzymes [6-7].

Copper has been known as an essential bioelement for some time but its biological role (s) has been recognized only in the last decades due to the rapid development of bioinorganic chemistry, a successful interaction between model complexes and protein biochemistry [8,9].

The use of microwave radiation to enhance organic reactions in environmentally benign solvents such as water, which is inexpensive and not dangerous, represents very powerful green chemical technology both from economic and synthetic points of view. This not only reduces the burden of organic solvent disposal but also enhances the rate of the reaction.

In this study we have prepared a benzopyran derivative which has adiverse pharmacological activities [10-12] to prepare an environmentlly acceptable catalyst. Then this catalyst has been used in the oxidation of polyphenol trihydroxybenzene in the presence of green oxidant hydrogen peroxide.

2. Experimental
2.1. Materials and Methods

4-methoxybenzylaldehyde was purchased from Aldrich Chemical Co. Dimedone were purchased from Across Organics Co. (Belgium) .and malononitrile, Kojic acid and copper acetate were obtained from Merck Co., Germany.

2.2. Synthesis of-(2-Amino-4-(4-methoxyphenyl)-5,6,7,8-tetrahydro-7,7-dimethyl-5-oxochromene-3-carbonitrile (AMDC) using microwave irradiation)

The organic ligand AMDC has been prepared as described in literature [13] in aqueous medium under microwave irradiation. A mixture of 4-methoxybenzaldehyde (5mmol) and malononitrile (0.33 g, 5mmol) were mixed together in water (2 ml) in a tightly closed tube and subjected to microwave irradiation for 0.5 min. until completion of the reaction (monitored by TLC). The precipitate formed was filtered, washed with water to give the pure arylidene malononitrile (90% yield) with melting point 114.5-115°C. An equimolar amount of arylidene malononitrile (5mmol) and dimedone (0.7 g, 5mmol) were mixed together in water (2 ml) in a tightly closed tube and subjected to microwave irradiation for 4.0 min. until completion of the reaction (monitored by TLC). The precipitate formed was filtered and washed with water several times to give the pure ligand AMDC in 96% yield (m.p. = 194-195°C).

2.3. Synthesis of [Cu L (AC)$_2$.2H$_2$O]complex.

Copper (II) acetate (0.1 mole) was dissolved in ~ 40 ml absolute ethanol, then added to 0.1 mole of the prepared ligand AMDC dissolved in ~ 40 ml absolute ethanol. The mixture was heated under reflux for ~ 2 h. The bluish precipitate was formed, filtered off and finally washed by hot ethanol several times and dried in an open air.

2.4. Physical methods

Carbon, hydrogen and nitrogen contents were determined at the Microanalytical Unit, Cairo University, Egypt. IR spectra of the ligand and its solid complexes were measured in KBr on a Mattson 5000 FTIR spectrometer. The electronic spectra and kinetic measurements were performed using Varian Cary 4 Bio UV/VIS spectrophotometer. Thermal analysis measurements (TGA) were recorded on a Shimadzu thermo-gravimetric analyzer model TGA-50H, using 20 mg samples. The flow rate of nitrogen gas and heating rate were 20 cm^3 min^{-1} and 10°C min^{-1} respectively. The magnetic susceptibility measurement for the copper (II) complex was determined by the Gouy balance using Hg[Co(NCS)$_4$] as a calibrant at room temperature. ESR spectrum was obtained on a Bruker EMX spectrometer working in the X-band (9.78 GHz) with 100 kHz modulation frequency. The mass spectrum was recorded on a Shimadzu GC-S-QP 1000 EX spectrometer using a direct inlet system.

2.5. Kinetic reactions for Trihydroxybenzene (THB) oxidation

The catalytic activity of the Cu^{II}-AMDC complex toward the homogenous oxidation of trihydroxybenzene (THB) in ethanol solution at 25 °C was determined by measuring the initial rate of THB oxidation. The increase of the absorption at 420 nm (ε = 4583 M^{-1}cm^{-1}) due to the oxidation product [14] with time was obtained on a Varian Cary 3E spectrophotometer. A plot of the formation of the product with respect to time gives the initial rate. To

study the effect of the catalyst concentration on the rate of the reaction, various amounts of the copper (II) complex (20 - 500 μM) have been used with 250 μM H$_2$O$_2$ for oxidation of 1.0 mM THB at 25 C. In the same time 40 μ M of the catalyst has been used in the oxidation of different concentrations of the substrate (10 –120 mM) in presence of 250 μM H$_2$O$_2$ to study the effect of THB concentration on the reaction. The rate laws were determined and rate constants obtained. The dependence of H$_2$O$_2$ on THB oxidation by 40 μM Cu^{II}-AMDC was determined by measuring the oxidation rate at different concentrations of hydrogen peroxide (8 – 220 μM) in the presence of 1.0 mM THB in ethanol at 25 C. The auto-oxidation rate of THB was determined under the same conditions in the absence of Cu^{II}-AMDC. Inhibitions were carried out in a similar fashion as the kinetic measurements using 40 μM Cu^{II}-AMDC in presence of 250 μM H$_2$O$_2$ and different amounts of kogic acid to establish the inhibition patterns

3. Results and Discussion

The organic ligand AMDC has been prepared using microwave irradiation method which enhances organic reactions in environmentally benign solvents such as water representing very powerful green chemical technology both from economic and synthetic points of view. This not only reduces the burden of organic solvent disposal but also enhances the rate of the reaction. The structure of the organic ligand was confirmed by using the IR and ^{1}H-NMR spectral data as well as the mass spectroscopy (m/s = 324.3).

By comparing the IR spectrum of the ligand with that of the Cu(II) complex, it is found that the copper (II) binds to the ligand through the NH$_2$ and C≡N nitrogen atoms. This suggestion was supported by reducing in intensity and shifting of the cyanide band in the complex spectrum. At the same time the NH$_2$ band in the Cu^{II}-AMDC was shown as a broad band at higher wavelength (3454 cm^{-1}). The coordination of NH$_2$ nitrogen atom is also consistent with the presence of a new band at 509 cm^{-1} due to νM–N. The IR spectrum shows abroad band observed at ~ 3182 cm^{-1} assigned to νOH stretching vibration of water molecule and OH group. The proposed structure is confirmed by the presence of new band at 634 cm^{-1} attributed to M–O.

3.1. Electronic spectral data for Cu^{II}-AMDC.

The electronic spectrum of the copper complex [Cu L (AC)$_2$.2H$_2$O] recorded at room temperature in DMSO solution (Figure1) reveals two absorption bands at 550 and 680 nm. These bands are corresponding to the transitions (^{2}B$_{1g}$→^{2}E$_g$) and ^{2}B$_{1g}$→^{2}B$_{2g}$ respectively [15,16], which strongly favors the tetragonally distorted octahedral geometry around the metal ion. This has been further confirmed by the magnetic moment measurements at room temperature

1.83 B.M. which is corresponding to one unpaired electron and C_{4v} symmetry [17].

The ESR spectrum of $[CuL(AC)_2.2H_2O]$ complex was recorded as polycrystalline sample at room temperature. The spectrum of the complex exhibits a single anisotropic broad signal. The analysis of the spectrum (Figure 2) gives the $g\| = 2.14102$, $g\perp = 2.0562$. These values indicate the presence of an unpaired electron in the d_{x2-y2}. The observed $g\|$ value for the complex is less than 2.3, thus, indicating that the bonds between the organic ligand and copper ion have a covalent character more than the ionic one. The values of $g\|$ and $g\perp$ are closer to 2.0. This suggests distortion in the Cu(II) complexes from Oh symmetry to D_{4h} symmetry. According to Hathway and Billing [18,19], the $G = (g\| -2)/(g\perp -2)$, which measures the exchange interaction between the copper centers in a polycrystalline solid has been calculated and found to be less than 4.0. This value is indicating to a considerable exchange interaction in solid complex.

3.2. Thermal analysis of cu^{II}-AMDC complex

The thermogram TGA confirms the amount of solvent inside and/or outside the coordination sphere and gives some information about the stability of this compound. The thermogram of $[CuL(AC)_2.2H_2O]$ (Figure 3), shows four stages of mass loss at temperature range of 25-1000°C. The first stage at 120 °C corresponds to removal of two water molecules inside the coordination sphere (weight loss calc. = 6.7%, found = 6.0%). The second peak in the temperature range of 145-250 °C corresponds to removal of one NH_3 and one CH_3OH molecules as well as two molecules of CO_2 with weight loss (cal. = 25.4% , found = 26.0%). The third inflection at 250-440 °C corresponds to removal of $(C_6H_5CH_2CH=CHOH)$ with weight loss (cal. = 24.9%, found = 24.0%). The weight remains up to 800 °C $\sim$ 40 % indicate somehow stability of the copper complex.

The elemental analysis in table(1), together with the IR, electronic, ESR spectral data and mass spectroscopy (figure 4) suggest that the structure of the copper complex is $[CuL(AC)_2.2H_2O]$ where L = AMDC, as shown in the structure(1).

4. oxidation of trihydroxybenzene

Most of the common techniques for wet oxidation of phenol involve the use of hydrogen peroxide as an oxidant. The advantage of the hydrogen peroxide over other potential oxidants is its relative stability. In addition, hydrogen peroxide is a relatively strong oxidant. The environmentally acceptable catalyst Cu^{II}-AMDC has been used in this study to oxidize the polyphenol trihydroxybenzene using hydrogen peroxide.

In order to study the catalytic activity of the Cu^{II}-AMDC and its interaction with H_2O_2 toward the oxidation of 1,2,3-trihydroxybenzene, THB has been used as a substrate to provide detailed kinetic information. The oxidation rates of THB by 40 µM Cu^{II}-AMDC at different concentrations of THB (Figure 5) have been determined in the presence of 250 µM H_2O_2. The rate of THB oxidation is found to be nonlinear, reaching saturation at high THB concentrations which suggest an enzyme-like pre-equilibrium kinetics. This kinetics can be described as the binding of THB with the catalyst Cu^{II}-AMDC to form an intermediate THB-Cu^{II}-AMDC complex, followed by the conversion of the bound substrate (THB) into products (eq. 1). The oxidation rate of THB hasn't been affected by adding different concentrations of the scavenger DMSO which means that the reaction doesn't follow free radical mechanism. The rate law for this reaction can be obtained with steady-state approximation similar to the Michaelis-Menten kinetics in enzyme catalysis. The rate law for this reaction mechanism can be expressed as in (eq. 2), wherein $K' = (k_{-1} + k_{cat}) / k_1$ is the dissociation constant of the THB-Cu^{II}-AMDC complex. The reaction in the presence of saturation amount of H_2O_2 (250 µM) produces a first-order rate constant $k_{cat} = 0.0043$ s^{-1} ($t_{1/2}$ = 161 s) and dissociation constant K' = 0.32 mM. The Cu^{II}-AMDC affords a significant catalytic efficiency $k_{cat}/K' = 0.11$ M^{-1}s^{-1} as the second order rate constant. The catalysis shows 1.4×10^3 times rate enhancement in terms of the first-order rate constant (k_{cat}/k_o, wherein $k_o = 2.53 \times 10^{-6}$ s^{-1} is the rate constant for the uncatalyzed reaction under the same reaction conditions.

The oxidation of the trihydroxybenzene as a function of H_2O_2 also shows a saturation pattern at high concentrations (Figure 6) showing $k_{cat} = 0.027$ s^{-1} and K' = 17 mM , indicating direct binding of this oxidant to the active metal center. Therefore, both THB and H_2O_2 are considered to be substrates.

In order to investigate the effect of the catalyst concentration on the oxidation of THB with 250 µM H_2O_2, different concentrations of the copper complex Cu^{II}-AMDC have been used in the oxidation of THB. The observed rate was found to be linear till 50 µM of copper complex and then reach saturation indicating that the optimum concentration for the copper complex to be used as a good catalyst is around 50 µM.

### 5. Inhibition of trihdyroxybenzene oxidation by kojic acid

Since Kojic acid is a well known compound for competitive inhibition of polyphenol oxidation by oxidases [20]. It has been used to inhibit the Cu^{II}-AMDC complex toward oxidation of trihydroxybenzene. Figure 8 shows that the Kojic acid significantly inhibits the oxidation of THB with IC$_{50}$ $\sim$ 220 µM.

$$Cu^{II}\text{-AMDC} + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (Cu^{II}\text{-AMDC})\text{-}S \xrightarrow{k_{cat}} Cu^{II}\text{-AMDC} + \quad \text{eq. 1}$$

$$rate = \frac{k_{cat}\,[Cu^{II}\text{-AMDC}][S]}{K' + [S]} \quad \text{Eq. 2}$$
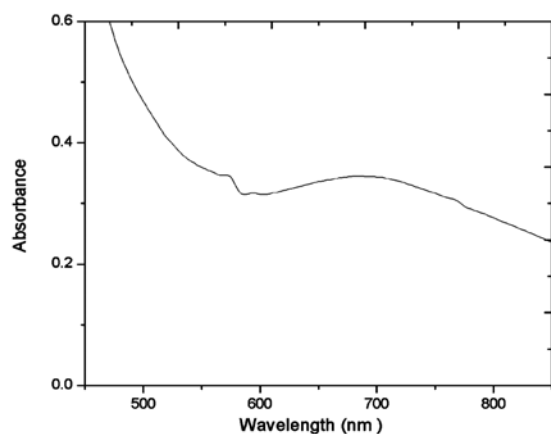
Figure 1. Electronic spectrum of [CuL(AC)$_2$.2H$_2$O]
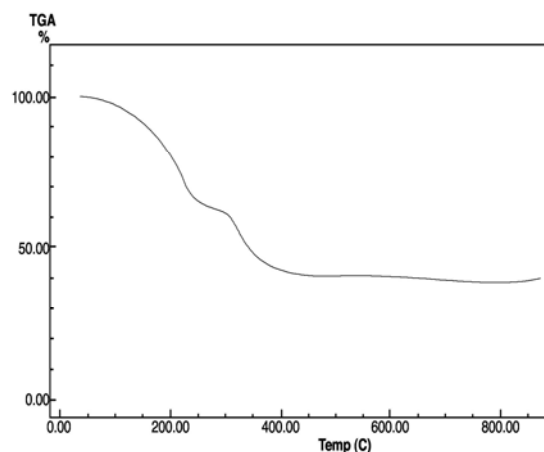
Figure 3. TGA of [CuL(AC)$_2$.2H$_2$O]
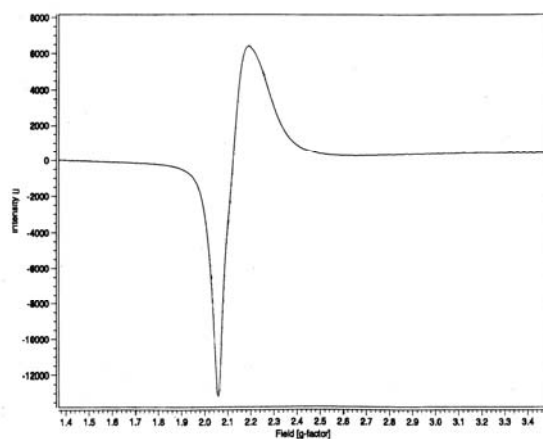
Figure 2. ESR of [CuL(AC)$_2$.2H$_2$O]
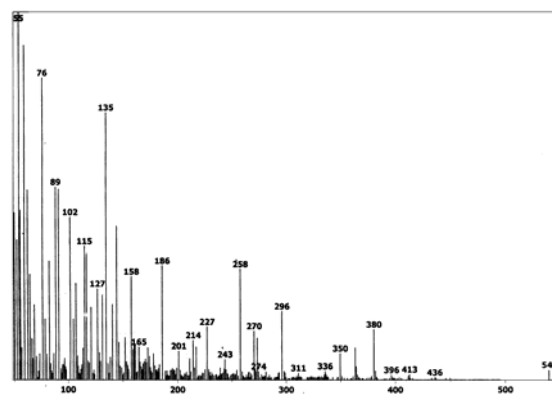
Figure 4. Mass spectrum of [CuL(AC)$_2$.2H$_2$O]

Table 1. Elemental analysis of [CuL(AC)$_2$.2H$_2$O]

Complex M.wt.	% C		% H		% N		%Cu		MP °C
	Cal.	Found	Calc.	Found	Calc.	Found	Calc	Found	
[CuL(AC)$_2$.2H$_2$O]	51.2	51.3	5.0	5.1	5.2	4.9	11.69	11.89	270
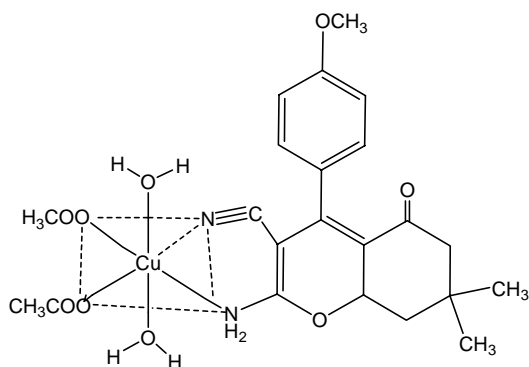
Structure 1. The copper complex [CuL(AC)$_2$.2H$_2$O]
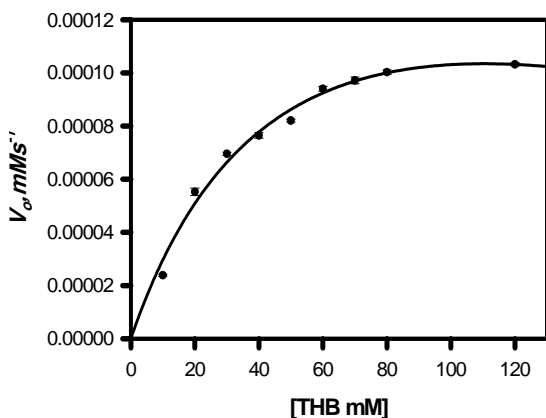
Figure 5. oxidation of different concentrations of THB using 40 μM Cu^{II}-AMDC with 250 μM H$_2$O$_2$ at 25°C.
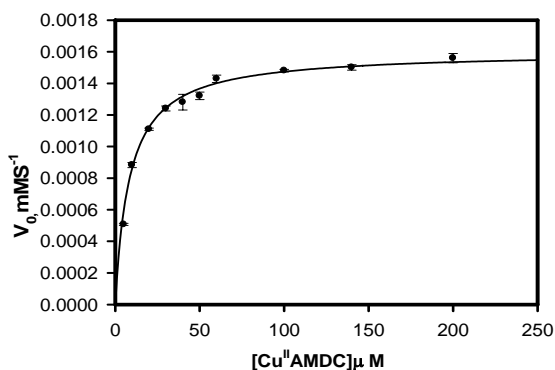
Figure 6. oxidation of 1.0 mM THB using 40 μM Cu^{II}AMDC with different concentrations of H$_2$O$_2$ at 25°C.
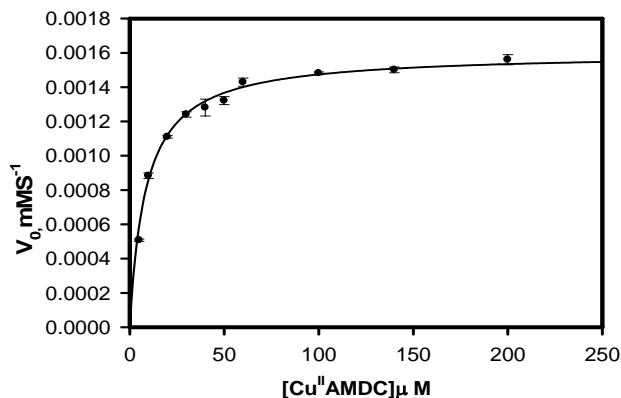
Figure 7. oxidation of 1.0 mM THB using
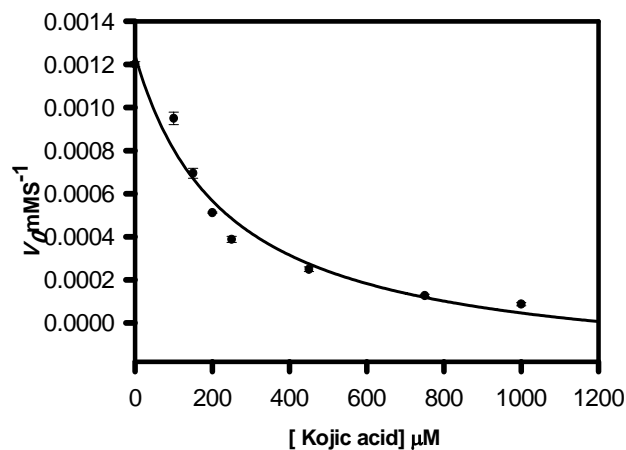
different concentrations of the catalyst Cu^{II}-AMDC with 250 μM H$_2$O$_2$ at 25°C.

Figure 8. Inhibition of THB oxidation using 40 µM Cu^{II}-AMDC with different concentrations of kojic acid and 250 μM H$_2$O$_2$ at 25°C.

4. Conclusion

The well known 4-methoxyphenyl benzopyran have been used in the synthesis of copper(II) complex Cu^{II}-AMDC. This complex was fully characterized by different tools. The geometry around the copper ion is found to be octahedral geometry. The [CuL(AC)$_2$.2H$_2$O] complex has been used as a catalyst in the oxidation of polyphenol 1,2,3-trihydroxybenzene in presence of H$_2$O$_2$ as a green oxidant. The copper complex affords a significant catalytic activity toward the oxidation of THB compared to the uncatalyzed reaction. The oxidation reaction herein is inhibited by kojic acid.

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