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Kinetic study of the Catecholase activities of a tetranuclearCu(I) complex in different Solvents

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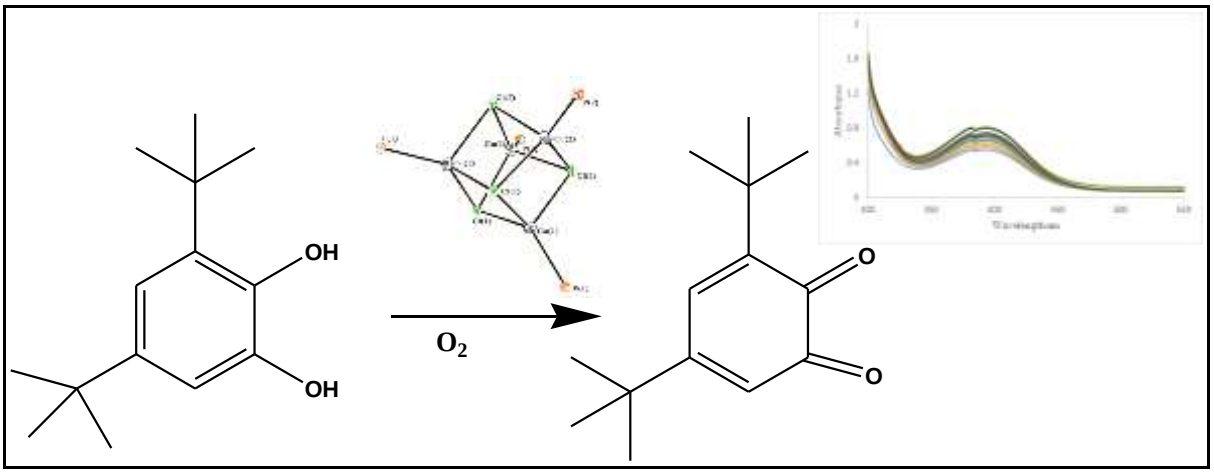
Abstract : The structure of $[Cu_4(\mu^3\text{-}Cl)_4(PPh_3)_4$**(1)** reveals a four coordinate system around the metal ions and**1** behaves as an effective catalyst towards the oxidation of 3,5-di-tert-butylcatechol in methanol and DMF to the corresponding quinone in the presence of oxygen. The reaction follows Michaelis–Menten enzymatic reaction kinetics with turnover numbers (*Kcat*), 2.06 and 1.51 h^{-1} in methanol and DMF respectively.
Keywords : Cu(I), Catecholase oxidase, turnover rate,triphenylphosphine.



Introduction

Catechol oxidase is an enzyme with the type-3 active site that catalyzes the oxidation of a wide range of o-diphenols (catechols) to the corresponding o-quinones coupled with $2e/2H^+$ reduction of O_2 to H_2O, in a process known as catecholase activity.[1-3]

The crystal structure of the met form of the enzyme was determined in 1998 and it revealed that the active center consists of a hydroxo-bridged dicopper(II) center in which each copper(II) center is coordinated to

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three histidine nitrogen atoms and adopts a trigonal pyramidal environment with one nitrogen atom in the apical site. The structure determination of catecholase oxidase has encouraged an extensive investigation into model compounds to understand the structure/property relationship.[4–5] As the structure contains dicopper(II) moiety, several dicopper(II) complexes derived from nitrogen-containing dinucleating ligands have been mainly employed for this purpose. Several monocopper(II) and other metal complexes are also known to exhibit moderate to high activity.[6–8]

Recently, attention has turned to Cu(I) complexes in terms of their abilities to mimic catecholase oxidase.Ramadan*et al*., reported Cu(I) and Cu(II) complexes of a new tetradentate Schiff-base containing N_4 donors and found out that the Cu(I) gave the highest turnover rate.[9] Also a number of new copper(I) complexes functioning as model systems for tyrosinase have been reported[10-12],even though Cu(II) complexes still receive far greater attention in this field amongst other metal ions.

The focus herein is on the catecholase activity of Mannich base metal complexes and the title compound was encountered in one of the various reactions of interest involving Mannich base metal complexes. Reports on it can be found severally in the literature, but no account of its catecholase activities.[13 –17] This study reports the comparable catecholase activity of the Cu(I) complex in methanol and DMF, the synthesis of the complex has been previously reported in the literature but **1** was obtained by a different synthetic route in this study.

Experimental

All chemicals and solvents were purchased from Sigma Aldrich and use as received. Micro analytical determinations (C, H and N) were obtained using Elementar Analysensysteme VarioMICRO V1.62 GmbH analysis System. The ^{1}H NMR spectral data were collected in $CDCl_3$ on a Bruker 300 MHz spectrometer. All UV measurements were done on a Perkin Elmer UV-Vis spectrophotometer model Lamba 25.

The title compound was obtained while trying to prepare a Mannich base metal complex containing triphenylphosphine.Equimolar quantities of Mannich base and triphenylphosphine were dissolved in chloroform, to which a methanolic solution of hydrated copper (II) chloride was added. A green copper complex was precipitated immediately, which was filtered, and the mother liquid was left for slow evaporation to yield white crystals. Yield: 16 % M. p. > 250 °C. Elemental analyses calcd. For $C_{72}H_{60}Cl_4Cu_4P_4$: C, 59.84; H, 4.19%. Found: C, 60.09; H, 4.24%.

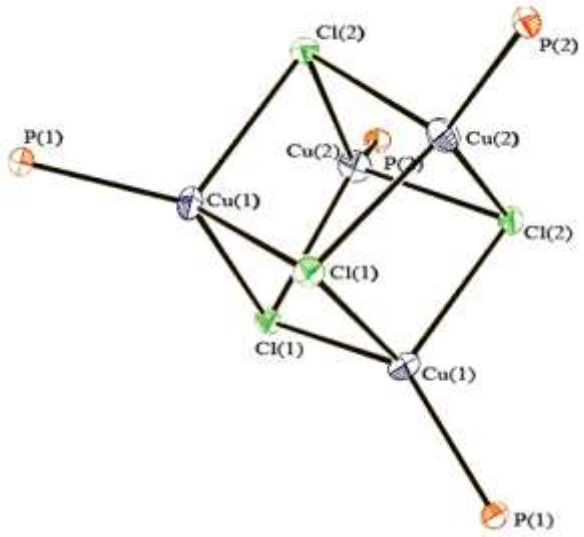
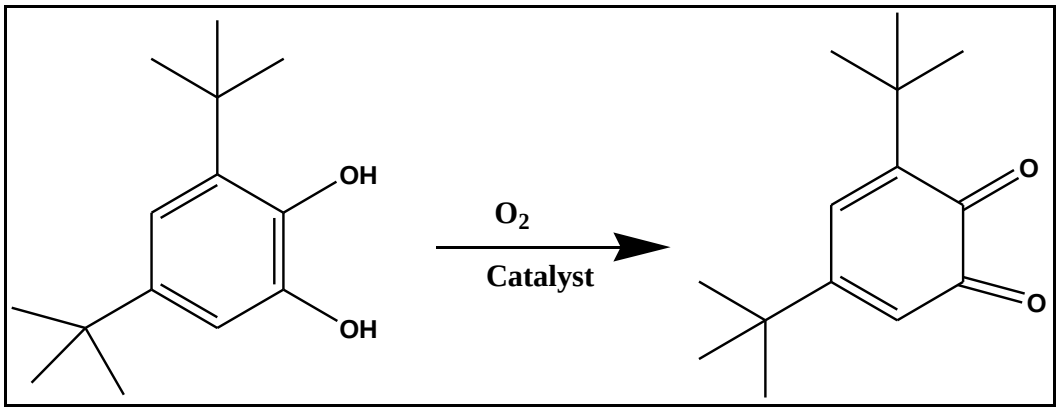


Fig.1. Structure of $[Cu_4(\mu^3\text{-}Cl)_4(PPh_3)_4]$. Phenyl rings were omitted for clarity.

Results and Discussion

The crystal data and results from elemental analysis of the compound are in line with those previously reported by Prabhakaran *et al*.,[14]and the compound dissolved in methanol and DMF. We therefore proceeded with the investigation of the potential catecholase activity of **1** using 3, 5-di-tertbutylcatechol (3,5-DTBC) as the model substrate. 3,5-DTBC has low redox potential and that makes it easy to oxidize with the bulky tert-butyl substituents preventing further over-oxidation reactions such as ring-opening.[18]



Scheme 1. Conversion of 3,5-DTBC to 3,5-DTBQ

The reaction involved in the catalytic study is depicted in scheme 1. The oxidation product 3,5-di-tert-butylquinone (3,5-DTBQ) is highly stable and shows amaximumabsorption at 393 nm in methanol and 399 in DMF respectively. The activities and reaction rates of the tetranuclear complex were determined using UV-vis spectroscopy by following the appearance of the characteristic absorption of 3,5-DTBQ. The reactivity studies were performed in methanol and DMF respectively to evaluate the role played by solvent in the catecholase activity of the Cu(I) complex. The results of spectral scans of the Cu(I) complex with 3,5-DTBCrecorded for 1 h at 5 mins interval are shown in Figures 2 and 3.

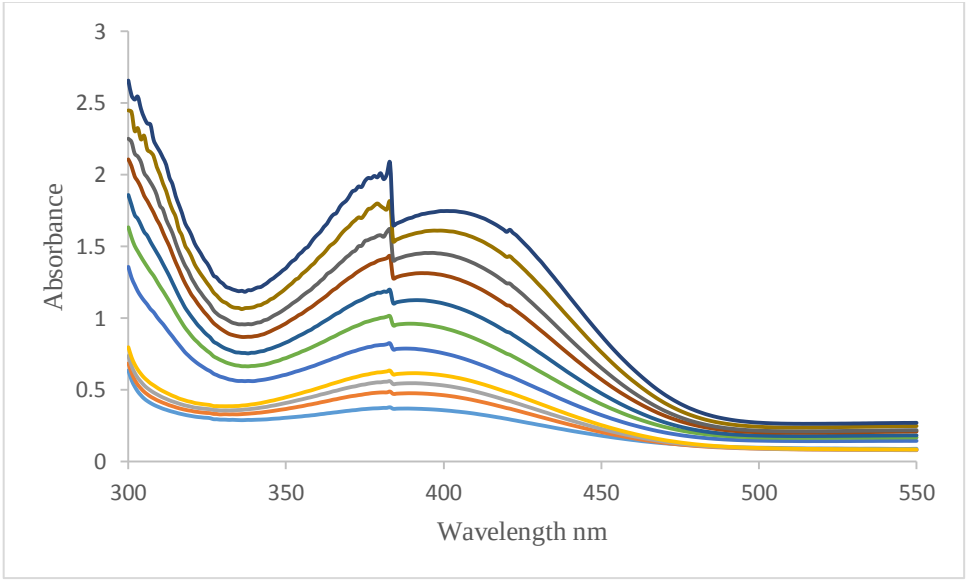


Fig. 2. Increase in absorbance around 393 nm in methanol after the addition of 100 equivalents of 3,5-DTBC.

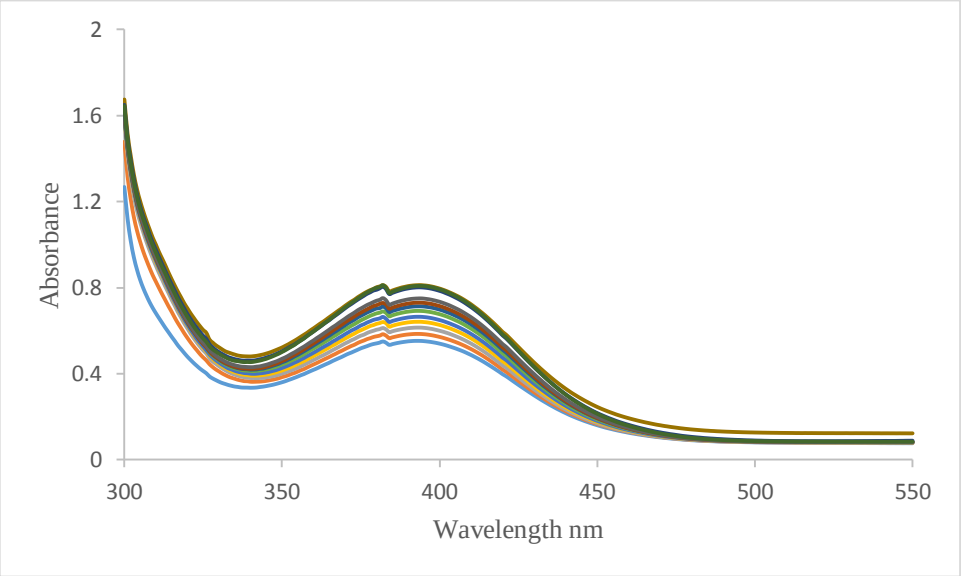


Fig. 3. Increase in absorbance around 399 nm in DMF after the addition of 100 equivalents of 3,5-DTBC.

Kinetic studies of the oxidation of 3,5-DTBC to 3,5-DTBQ were carried out by **1** in methanol and DMF by the method of initial rates, following the increase in absorption. The rate constant for a particular complex/substrate mixture was determined from the log[Aα/(Aα−At)] vs time plot. The substrate concentration dependence of the oxidation rate was examined under aerobic conditions, using10^{-4} M solutions of **1** and increasing amounts of 3,5-DTBC (from 20 to 100 equiv). In all cases, first-order dependence was observed at low substrate concentrations, whereas saturation kinetics was found at higher substrate concentrations.

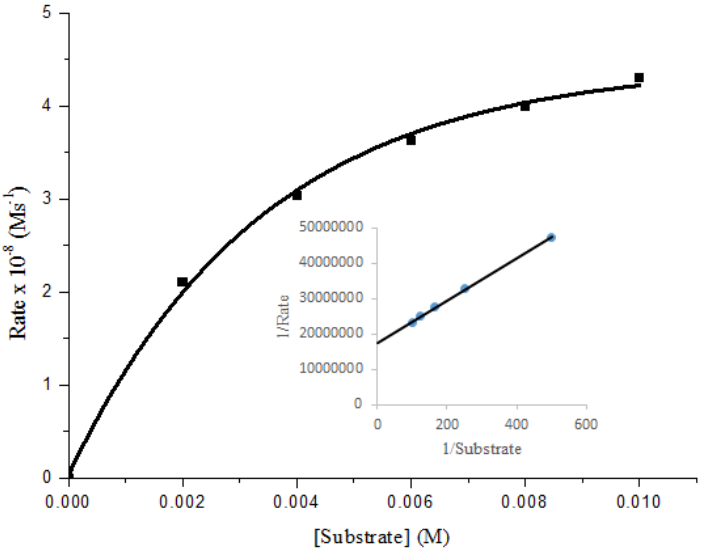


Fig.4. Plot of rate vs. [substrate] in the presence of 1 in methanol; insert: Lineweaver–Burk plot.

The substrate concentration dependence suggests that the initial step of the catalytic cycle is the binding of the substrate to the catalyst. Michaelis−Menten kinetics was applied to analyze the data obtained, and the Michaelis−Menten constant (K_M) and maximum initial rate (V_{max}) were determined by linearization using Lineweaver−Burk plots.

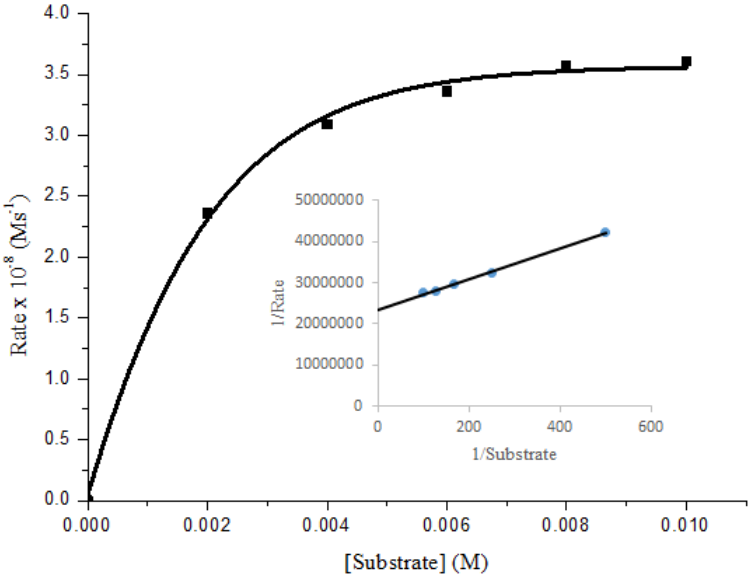


Fig.5. Plot of rate vs. [substrate] in the presence of 1 in DMF; insert: Lineweaver–Burk plot.

Chakraborty *et al*. have previously noted the differences in the reaction rates of complexes with 3,5-DTBC depending on the solvent used - acetonitrile or DMSO.[19] A similar observation is herein made; a higher catecholase activity (i.e. k_{cat} = 2.06 ± 0.09 h^{-1}) is observed in methanol compared to 1.51 ± 0.08 h^{-1} observed in DMF.These values are rather low compared to those for Cu(II) in the literature.[20–21]

Table 1.Kinetic parameters for the Oxidation of 3.5-DTBC to 3,5-DTBQ mediated by 1 in methanol and DMF.

Solvent	**V_{max} (Ms^{-1})**	**K_M (M)**	**K_{cat} (h^{-1})**
Methanol	(5.71 ±0.28) x 10^{-8}	(3.45± 0.22) x 10^{-3}	2.06 ± 0.09
DMF	(4.17 ± 0.27) x 10^{-8}	(1.53 ± 0.08) x 10^{-3}	1.51 ± 0.08

The reason for this observation of difference in k_{cat}can be explained by the fact that the higher coordinating ability of the solvent which retarded the possibility of the formation of catechol–substrate adduct.

We proposethat the catalytic cycle is initiated with a mono anion catecholate-copper boundcomplex in a reversible pre-equilibrium step. Thereafter the [catecholate-Copper] complexreacts with dioxygen in a further equilibrium step giving copper(II) superoxide complex.

Prior investigations suggest the formation of a superoxidespecies in the catalytic cycle of catechol oxidase.[9, 22, 23] This pathway is supported by the identification of H_2O_2 through iodometric titration and the isolation of 3,5-DTBQ. The isolated product was characterised by ^{1}H NMR spectroscopy:[^{1}H NMR ($CDCl_3$, 300 MHz): δ_H = 1.22 (s, 9H), 1.27 (s, 9H), 6.22 (d, J = 3.0 Hz, 1H), 6.92 (d, J = 3.0 Hz, 1H)].

Conclusion

In summary, we have added to the small existing body of knowledge on catecholase activity of Cu(I) complexes and shown that they are capable catalyst requiring a great deal of attention. The formation of metal-substrate adduct followed by the oxidation of Cu(I)/Cu(II) redox process are considered the rate determining steps.

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