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Modelling tyrosinase monooxygenase activity. Activation of dioxygen by dicopper(I) complexes and characterisation of dicopper(II) complexes

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Abstract. Activation of dioxygen on dicopper(I) centres was systematically investigated using a group of open-chain and a macrocyclic *m*-xylyl-based dinucleating ligand from a bioinorganic viewpoint. Even though intermediate peroxo-dicopper(II) species was not detected (even at –80°C for the open-chain system), the putative intermediate reacted with C–H groups in ligands giving oxygenated products (C–OH groups). Absorption, spectroscopic and magnetic properties of the final dicopper(II) complexes have been investigated.

Keywords. Dioxygen; dicopper complexes; *m*-xylyl-based dinucleating ligands; stoichiometric oxidation reactions.

1. Introduction

The activation of molecular oxygen by copper plays a vital role in synthetically useful stoichiometric and catalytic oxidative conversions of organic molecules and in biological systems [1–4]. Tyrosinase [5] is a monooxygenase that catalyses *o*-hydroxylation of monophenols and the oxidation of *o*-diphenols to *o*-quinones. Considerable progress has been made in the chemical modelling of tyrosinase-like activity (aromatic ring hydroxylation) [1,2,6–16]. To gain more insight into the reactivity of the binuclear copper(I) complexes with dioxygen and in the dependency of the arene hydroxylation on ligand topology, we have initiated a programme to systematically investigate tyrosinase-like monooxygenase activity using new *m*-xylyl-based dinucleating ligand systems of the open-chain type or of the macrocyclic type, capable of providing only two N-coordinations to each copper site. Here we provide a brief account of our findings of this exciting chemistry.

2. Experimental

Materials and equipment required and methods of preparation/generation of dicopper(I) and isolation of dicopper(II) compounds are described elsewhere [17–20].

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3. Results and discussion

3.1 *Synthesis and characterisation of open-chain ligands and their dicopper(I) complexes* [17,19]

The dinucleating ligands (figure 1) were prepared by standard methods. The dicopper(I) complexes of L (**1a**) and L-NO_2 (**1b**) were characterised by IR and ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum of $[Cu_2(L\text{-}NO_2)(CH_3CN_2)[SbF_6]_2$ (**1b**) is displayed in figure 2. The ligand L^2 (figure 1) was synthesised to specifically pinpoint the effect of ligand topology to bring about aromatic ring hydroxylation with our parent *m*-xylyl-based binucleating ligand L. It is to be noted that on coordination while L gives rise to a six-membered chelate ring, L^2 provides a five-membered chelate ring.

3.2 *Synthesis of the macrocyclic ligand and its dicopper(I) complex* [20]

The macrocyclic ligand L^{mac} (figure 1) was prepared by the Schiff base condensation of 1,2-diaminoethane and isophthalaldehyde in CH_3CN at 298 K. The dicopper(I) complex of this macrocyclic ligand $[Cu_2(L^{mac})(CH_3CN)_2][ClO_4]_2$ (**1c**) was prepared under argon from the reaction between the ligand and $[Cu(CH_3CN)_4](ClO_4)$ (1:2 ligand-to-metal mole ratio) in CH_2Cl_2. The complex was identified by its IR, ^{1}H NMR and FAB-MS spectra.

3.3 *Reactivity of dicopper(I) complexes of open-chain ligands with dioxygen* [19]

The dicopper(I) complexes of L were exposed to molecular oxygen at –80°C and absorption spectral behaviour was investigated at this temperature. In CH_2Cl_2 and THF, the solution of dicopper(I) complexes turned green on exposure and an absorption band appeared at 352 nm, which did not change on warming the solution to room temperature. The peak at 352 nm is due to $PhO^-/OH^- \rightarrow$ Cu(II) charge-transfer transition, originating due to the formation of the phenoxo- and hydroxo-bridged dicopper(II) complex (*vide infra*). As the proposed attack of peroxo-copper(II) intermediate is thought to be electrophilic in nature, we hoped to detect spectroscopically the peroxo species using a *p*-NO_2 derivative of the parent ligand L. We therefore synthesized the ligand L-NO_2 (figure 1) and its dicopper(I) complex $[Cu_2(L\text{-}NO_2)(CH_3CN)_2][ClO_4]_2$ (**1b**). However, when this complex **1b** dissolved in either CH_2Cl_2 or THF was exposed to O_2 at –80°C, we could not detect any peroxo intermediate. The otherwise kinetically favourable

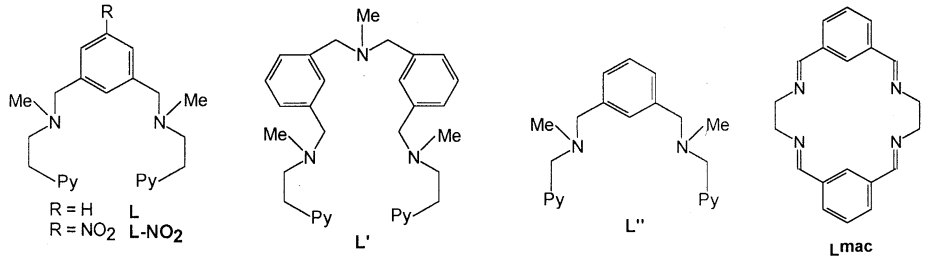

Figure 1. Structure of the ligands used in this study.

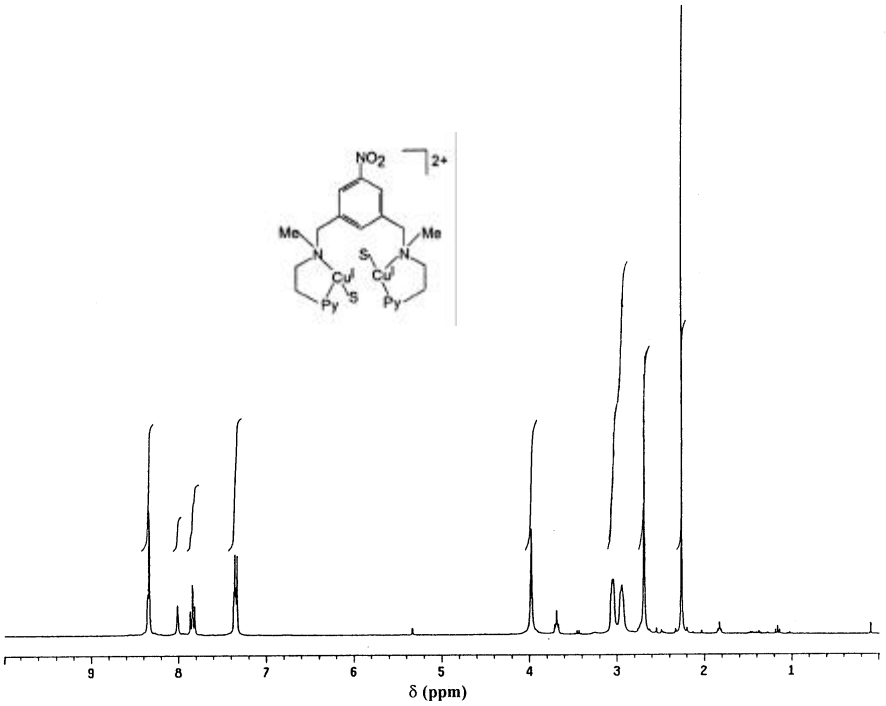

Figure 2. ^{1}H NMR (500 MHz) spectrum of $[Cu^I_2(L)(CH_3CN)_2][SbF_6]_2$ (**1b**) in CD_2Cl_2 at room temperature.

hydroxylation of the aromatic ring was observed. In essence, these results imply that the peroxo intermediate is of insufficient stability to be observed because of the low barrier for its subsequent reaction.

Treatment of L^2 with 2 equivalents of $[Cu(CH_3CN)_4](ClO_4)$ in CH_2Cl_2 under dinitrogen at –60°C, followed by exposure to dioxygen and slowly warming up the reaction mixture to 298 K, resulted in an immediate colour change of the suspension from yellow to light blue. We confirmed that hydroxylation of the benzene ring of the ligand had not occurred by isolating the ligand from the isolated dicopper(II) complex $Cu^{II}_2(L^2)(OH)(ClO_4)_3(H_2O)_2$. The organic extract has the same ^{1}H NMR spectral feature as that in L^2. All attempts to characterise this copper(II) compound apart from assigning its empirical composition failed.

3.4 *Reactivity of dicopper(I) complex of macrocyclic ligand with dioxygen* [20]

On exposure to dry dioxygen, a suspension of $[Cu_2(L^{mac})(CH_3CN)_2][ClO_4]_2$ (**1c**) in CH_3OH-CH_3CN (3:1 vol/vol) generates a bluish-green solid. This solid was dissolved in 6 M HCl and extraction with $CHCl_3$ produced a yellowish white solid. The peak integration and mass spectral feature of ^{1}H NMR spectrum of this solid in $CDCl_3$ unambiguously revealed that this solid was a mixture of isophthalaldehyde and 2,6-diformylphenol. This result demonstrates that due to oxygenation of $[Cu_2(L^{mac})(CH_3CN)_2][ClO_4]_2$ (**1c**), oxygen insertion into the aromatic ring has occurred with only one of the aromatic rings hydroxylated.

3.5 *Characterisation of dicopper(II) complexes of open-chain ligands* [17–19]

Initial synthetic reaction of L (contaminated with L′) with $[Cu(CH_3CN)_4](ClO_4)$ were carried out in CH_2Cl_2 under a dinitrogen atmosphere. It should be noted that initial ligand synthesis of L had an impurity L″ the nature of which was identified from X-ray structural analysis of its dicopper(II) compound (*vide infra*). The synthesis of pure L was achieved subsequently. The yellow solution with a yellow suspension thus obtained was exposed to dioxygen. After 12 h, the reaction mixture turned dark green and afforded green phenoxo- and hydroxo-bridged dicopper(II) complex $[Cu_2(L\text{-}O)(OH)][ClO_4]_2$ (**2a**) as the major isolated solution product with a very small amount of light blue dihydroxo-bridged dicopper(II) complex $[Cu_2L'(OH)][ClO_4]_2$ (**2b**).

The X-ray structure of $[Cu_2(L\text{-}O)(OH)][ClO_4]_2$ underscores the incorporation of two O atoms into the complex: one into the aryl hydrogen bond and the other into the hydroxy bridge. The X-ray structure of $[Cu_2L'(OH)_2][ClO_4]_2$ reveals that the Cu_2O_2 unit is bent (hinge distortion). The structural cores are in figure 3 [17]. The X-ray structure of **2b** revealed the exact nature of the impurity that was present during the initial synthesis of L.

The electronic spectra of **2a** and **2b** in CH_3CN are displayed in figure 4 and the data are in table 1. For both complexes ligand field band is observed in the range 560–640 nm. The high-energy transitions are due to $PhO^- \rightarrow Cu(II)$ and/or $OH^- \rightarrow Cu(II)$ (in the case of **2a**) and $OH^- \rightarrow Cu(II)$ (in the case of **2b**) ligand-to-metal charge-transfer (LMCT) origin.

3.6 *Characterisation of the dicopper(II) complex of macrocyclic ligand* [20]

Recrystallisation of the bluish-green solid (*vide supra*), obtained from oxygenation of $[Cu_2(L^{mac})(CH_3CN)_2][ClO_4]_2$ (**1c**), from $CH_3CN/(C_2H_5)_2O$ afforded a blue crystalline solid $[Cu_2(L^{mac}\text{-}O)(OH)][ClO_4]_2 \cdot 2H_2O$ (**2c**). In the UV-Vis spectrum (CH_3CN solution) this compound exhibits a visible band at 591 nm and an intense band at 352 nm (table 1). The former is due to a *d-d* transition and the latter is due to a phenolate-to-copper(II) charge-transfer transition. Thus we observe that due to inherent strain associated with the present macrocyclic ligand, the oxygen insertion onto one of the arene rings is accompanied by the hydrolysis of the macrocyclic ligand (figure 5).

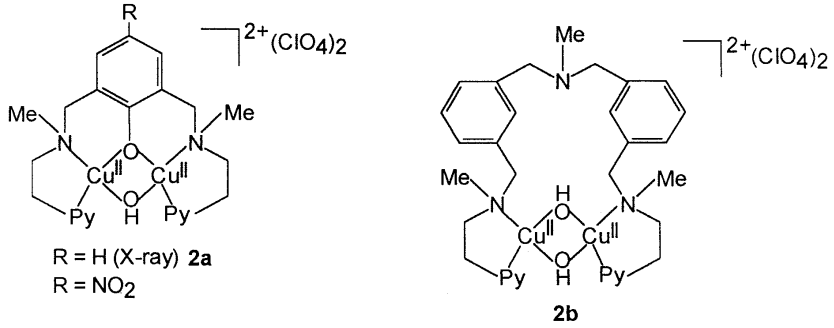

Figure 3. Schematic structures of $[Cu_2(L\text{-}O)(OH)][ClO_4]_2$ (**2a**) and $[Cu_2(L')(OH)_2][ClO_4]_2$ (**2b**).

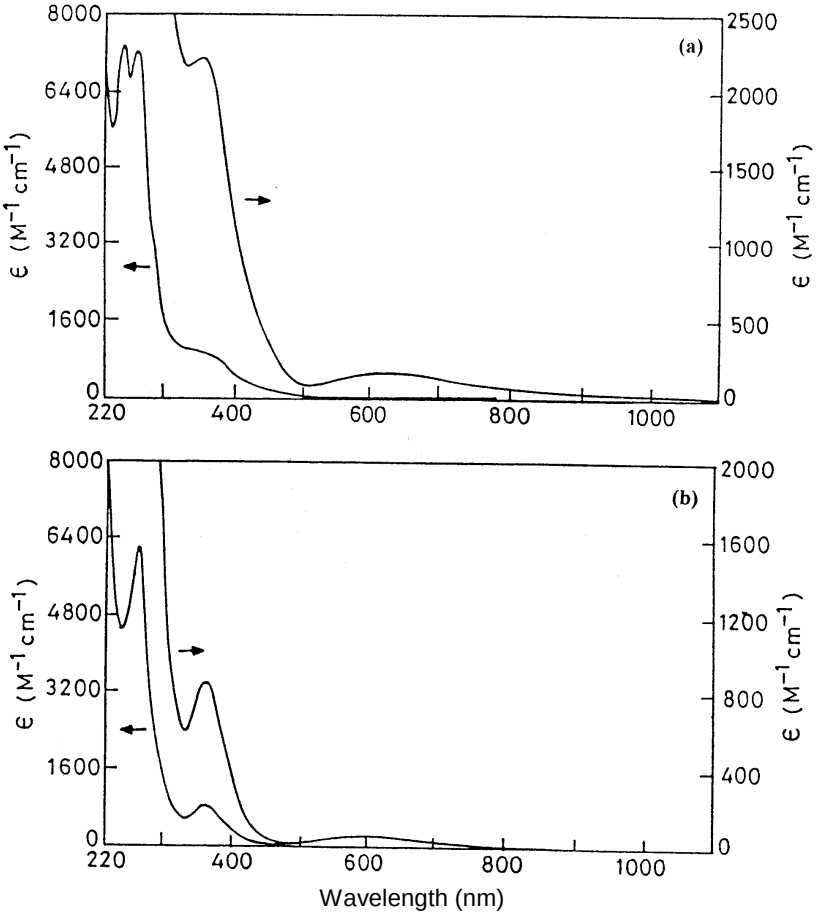

Figure 4. Electronic absorption spectra of (**a**) $[Cu_2(L\text{-}O)(OH)][ClO_4]_2$ (**2a**) and (**b**) $[Cu_2(L^2)(OH)_2][ClO_4]_2$ (**2b**) in CH_3CN.

3.7 *Magnetism of dicopper(II) complexes* [19,20]

We have studied the temperature-dependent (25–300 K) magnetic susceptibility measurements of structurally characterized phenoxo-/hydroxo-bridged copper(II) complex $[Cu_2(L\text{-}O)(OH)][ClO_4]_2$ (**2a**) and *bis*(hydroxo)dicopper(II) complex $[Cu_2L^2(OH)][ClO_4]_2$ (**2b**). In the absence of the X-ray structural characterisation of $[Cu_2(L^{mac}\text{-}O)(OH)][ClO_4]_2{\cdot}2H_2O$ (**2c**), we have confirmed the proposed dimeric structure by temperature-dependent magnetic susceptibility measurements. The room-temperature (300 K) magnetic moments for $[Cu_2(L\text{-}O)(OH)][ClO_4]_2$ (**2a**), $[Cu_2L^2(OH)_2][ClO_4]_2$ (**2b**) and $[Cu_2(L^{mac}\text{-}O(OH)][ClO_4]_2.2H_2O$ (**2c**) are quite low (μ_{eff}/Cu = 1·13 μ_B for **2a**, 1·50 μ_B for **2b** and 1·17 μ_B for **2c**), indicative of strong antiferromagnetic exchange coupling between the two copper(II) centres. The data were fitted to the modified Bleaney–Bowers expression in (1) using the isotropic (Heisenberg) exchange Hamiltonian ($H = -2JS_1.S_2$)

Table 1. Absorption spectral data for dicopper(II) complexes.

Compound	λ_{max}/nm (e/M^{-1} cm^{-1})
$[Cu_2(L\text{-}O)(OH)][ClO_4]_2$, **2a**	620 (170), 352 (2220), 261 (7200) and 241 (7400)
$[Cu_2L(OH)_2][ClO_4]_2$, **2b**	589 (70), 361 (990) and 264 (6240)
$[Cu_2(L^{mac}\text{-}O)(OH)][ClO_4]_2 \cdot 2H_2O$, **2c**	591 (160), 352 (8960), 253 (38152) and 207 (27470)

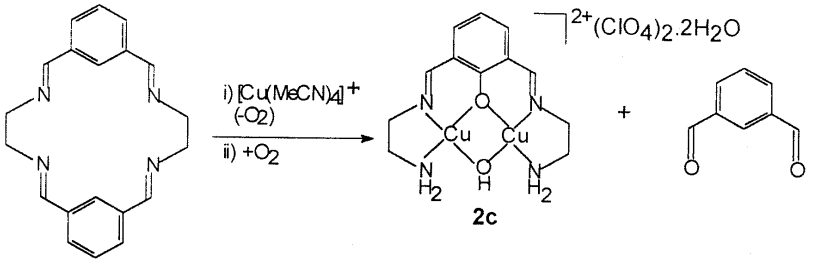

Figure 5. Oxygenation of $[Cu^{I}_2(L^{mac})(CH_3CN)_2][ClO_4]_2$ (**1c**) and the final products.

$$c_M = 2N\beta^2 g^2/3kT\,[1 + 1/3\exp(-2J/kT)]^{-1}\,(1-r) + N\beta^2 g^2 r/2kT + 2N_a, \qquad (1)$$

for two interacting $S = 1/2$ centres (c_M expressed per dimer), N_a is the temperature-independent paramagnetism (fixed at 60×10^{-6} cm^3 mol^{-1}), r is the fraction of monomeric impurity. Nonlinear regression analyses were carried out with J, g and r as floating parameters. The best-fit results are in table 2. The large $2J$ values of –440 cm^{-1} for **2a**, –258 cm^{-1} for **2b** and –457 cm^{-1} for **2c**, indicate strong antiferromagnetic coupling between the two copper(II) centres, which occurs via a superexchange mechanism through the phenoxide and hydroxide bridges in **2a** and **2c** and through the two hydroxo bridges in **2b**. A closer look at the metric parameters of **2a** and **2b** reveals that in both the complexes, the Cu...Cu distance, the stereochemistry around each Cu centre and the average Cu–OR–Cu bond angle at the bridging atoms are comparable. The striking difference is that the Cu_2O_2 in **2b** deviates more from planarity and is attributable to the fact that two copper(II) ions do not lie in one plane. When the metal ions move into the plane, the overlap between the metal-based $d_{x^2-y^2}$ orbitals and the oxygen-based sp^2 hybrid orbitals is increased. Since this s framework represents the dominant pathway for the superexchange mechanism, the enhanced overlap should result in an increase in the antiferromagnetic interaction.

4. Concluding remarks

We have demonstrated that aromatic hydroxylation with binuclear copper(I) complexes and dioxygen is not specific to a terminal tridentate N-coordination. In fact, bidentate N-coordination is sufficient enough, whether it is of the open chain type or part of a macrocyclic ligand system, to exhibit tyrosinase-like activity. With the synthesis of an

Table 2. Exchange coupling constants for the dicopper(II) complexes ($H = -2JS_1.S_2$).

Complex	J (cm^{-1})	g	r
Cu_2(L-O)(OH)][ClO_4]$_2$, **2a**	–220	2·05	0·016
[Cu_2L¢(OH)$_2$][ClO_4]$_2$, **2b**	–129	2·13	0·054
[Cu_2(L^{mac})(OH)][ClO_4]$_2$·2H_2O, **2c**	–229	2·13	0·042

interesting series of *m*-xylyl-based open-chain ligands L (L1), L-NO_2 and L2, the oxygenation of the dicopper(I) complexes have been systematically investigated. Rapid decomposition to aromatic ring-hydroxylated product even at –80°C implies that the energy barrier for conversion of intermediate dicopper(II)-peroxo to such species is small. The present studies pinpoint (i) the effect of ligand structure, i.e. a six-membered chelate ring-forming ligand (L) gives rise to aromatic ring hydroxylation whereas a five-membered chelate ring-forming ligand (L2) gives only irreversible oxidation, (ii) the effect of appropriate positioning (geometry) of the xylyl ring of the ligand, i.e. compared to L, the failure of the ligand L¢to undergo hydroxylation reaction, and (iii) with a new tetraaza macrocyclic ligand, aromatic hydroxylation in one of the rings has been observed with concomitant partial hydrolysis of the macrocycle. The final dicopper(II) complex has a m-phenoxo m-hydroxo bridged structure as evidenced from spectroscopic and variable-temperature magnetic studies. Experiments to carefully look at a comparative kinetic data on the solvent dependence and temperature dependence of the aromatic hydroxylation are being planned. Studies on exogenous substrate oxidation are in progress.

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