A Tyrosinase Model System. Phenol *ortho*-Hydroxylation by a Binuclear Three-coordinate Copper(I) Complex and Dioxygen

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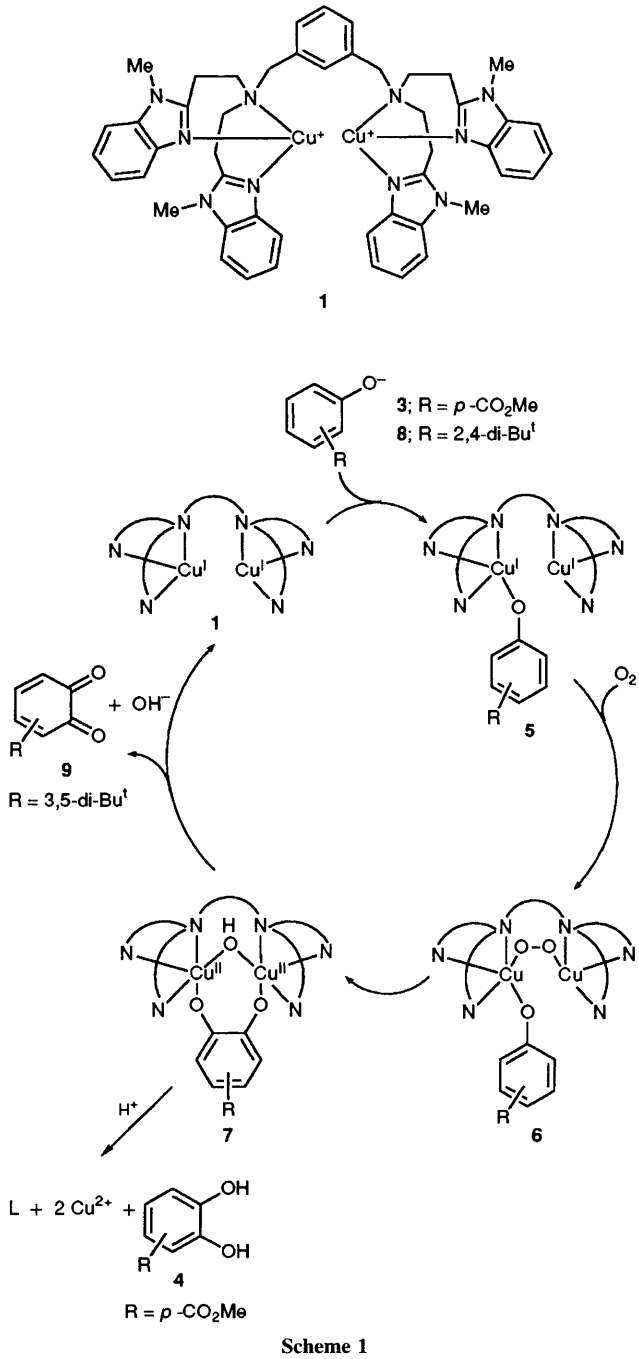
The binuclear three-coordinate copper(I) complex **1** derived from a polybenzimidazole ligand exhibits tyrosinase-like activity on exogenous phenols.

Tyrosinase (EC 1.14.18.1) is a copper-containing monooxygenase that catalyses the *ortho*-hydroxylation of monophenols and the oxidation of *o*-diphenols to *o*-quinones.[1] The enzyme is widely distributed in nature; it is responsible for the conversion of tyrosine to 3,4-dihydroxyphenylalanine (dopa) and the synthesis of melanin pigments.[2] Strong spectral similarities,[3] homology and evolutionary relationships[4] between tyrosinase and the dioxygen carrier haemocyanin suggest that a similar coupled binuclear copper active site is present in the two proteins. The features of this imidazole-ligated binuclear, three-coordinate copper centre have been clarified by the X-ray structural determination of *Panulirus interruptus* haemocyanin.[5] Model studies on the interaction between copper(I) complexes and dioxygen, mostly developed by Karlin's group, have led to the characterisation of dioxygen adducts[6,7] and systems performing hydroxylation reactions on the ligand.[8] Recently, a binuclear two-coordinate copper(I) complex derived from a Schiff's base ligand has been reported as a tyrosinase mimic.[9] We report here that the binuclear three-coordinate copper(I) complex **1** derived from a polybenzimidazole ligand also exhibits tyrosinase-like activity on exogenous phenolic compounds.

Complex **1**‡ was prepared in high yield under Ar from the binucleating ligand L§ and $Cu(MeCN)_4ClO_4$ (1 : 2 molar ratio) in ethanol solution. It reacts with dioxygen producing the green bis(μ-hydroxy)copper(II) dimer **2**,‡ as noted for other copper(I) complexes derived from *m*-xylyl ligands containing heterocyclic nitrogen donors,[10] except pyridine,[8a] for which ligand hydroxylation occurs. To investigate the monooxygenase activity of **1** on exogenous phenols, methyl-4-hydroxybenzoate **3** was selected as substrate because the corresponding *o*-catechol **4** is relatively stable to further oxidation. When stoichiometric amounts of the sodium salt of **3** are added anaerobically to dry acetonitrile solutions of **1** the colour of the solution turns from yellow to light-brown. The appearance of an intense band near 300 nm ($\Delta\varepsilon \cong 6000$ $dm^3\ mol^{-1}\ cm^{-1}$) in the absorption spectrum of the solution indicates partial formation of the phenolate adduct of **1**.[11] On exposure to dioxygen these solutions turn green, and after evaporation of the solvent and treatment with mineral acid the organic extract ($CHCl_3$) reveals the presence of a mixture of **3** and **4**, which can be separated by TLC (SiO_2, CH_2Cl_2–MeOH 24 : 1, v/v, as eluent) or HPLC (Lichrospher 100RP-18



Scheme 1

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‡ Satisfactory elemental analyses were obtained for all new compounds.

§ The ligand L, α,α′-bis{bis[2-(1′-methyl-2′-benzimidazolyl)ethyl]-amino}-*m*-xylene, was prepared by reaction of α,α′-*m*-dibromoxylene and *N*,*N*-bis[2-(1′-methyl-2′-benzimidazolyl)ethyl]amine. The latter compound was obtained by refluxing a solution of 3,3′-iminodipropionitrile (11.4 mmol) and *N*-methyl-*o*-phenylenediamine (23.9 mmol) in 3 mol dm^{-3} hydrochloric acid (60 ml) for 100 h. The resulting brown solution was cooled in an ice bath and made basic by dropwise addition of ammonia (conc.). The precipitate thus formed was collected by filtration and washed several times with dilute ammonia. The product was crystallized from ethanol–water (1 : 1) (yield 60%). ^1H NMR ($CDCl_3$): δ 2.5 (br, NH), 2.9–3.4 (m, *AA′BB′* system, N–CH_2CH_2–C), 3.68 (s, N-Me), 7.1–7.3 and 7.6–7.8 (m, benzimidazolyl-H). A mixture of *N*,*N*′-bis[2-(1′-methyl-2′-benzimidazolyl)ethyl]amine (6.0 mmol), α,α′-*m*-dibromoxylene (2.7 mmol), dry sodium carbonate (9.4 mmol) and anhydrous dimethylformamide (DMF) (50 ml) was heated at 80 °C for 50 h. After evaporation of the DMF under reduced pressure, the residue was treated with chloroform, the inorganic salts were filtered off, and the filtrate was concentrated to a small volume. The product was precipitated by the addition of diethyl ether (yield 70%).‡ ^1H NMR ($CDCl_3$): δ 2.8–3.2 (m, *AA′BB′* system, N–CH_2CH_2–C), 3.42 (s, N-Me), 3.65 (s, Ph–CH_2–N), 7.0–7.3 and 7.6–7.7 (m, Ph–H + benzimidazolyl-H).

column, MeOH–H_2O from 1:1 to 4:1, v/v, as gradient). A reaction carried out on a preparative scale led to the isolation of **4** in 37% yield. No other product, like the phenol oxidative coupling dimer, was found in the reaction mixture besides unreacted **3**. A labelling experiment using $^{18}O_2$ (93% enrichment) yielded ^{18}O-labelled **4**, as established by mass spectroscopy analysis,¶ so that the mechanism outlined in Scheme 1 can be proposed for the present phenol hydroxylation reaction. It is interesting to note that no catechol is formed when phenol **3** is used instead of its sodium salt in the reaction or when this is carried out in a protic medium like methanol.

The monooxygenase reaction on phenols containing electron-donor substituents, such as **8**, is more complex because the catecholate adduct **7** undergoes internal electron-transfer to form the quinone, **9**, and this undergoes further transformations in the reaction system. Réglier *et al.* reported the catalytic conversion of **8** to **9** (deduced spectrally) by O_2 in the presence of a binuclear two-coordinate copper(I) complex.[9] The complex **1** is also catalytically active in the monooxygenase reaction of **8** but it apparently also catalyses polymerisation–condensation reactions of **9** or **8** + **9** to complex products in the reaction conditions, as shown by separate experiments. The recovery of **9** from the product mixture decreases, in favour of brown products, with increasing the ratio of 8:1. A good yield of **9** could be obtained by reacting **8** (sodium salt) and **1** in 1.5:1.0 molar ratio in dry acetonitrile under dioxygen for 3 min. Rapid work-up as described above and chromatography of the organic extract (SiO_2, CH_2Cl_2 as eluent) led to the isolation of **9** as the major product (81% yield with respect to **8**, 122% with respect to **1**; brown by-products but no unreacted phenol present).

In conclusion we have shown that the binuclear three-coordinate complex **1** exhibits tyrosinase-like activity on exogenous phenols in the presence of dioxygen. With easily oxidizable phenols the reaction is catalytic but eventually leads to a complex mixture of products, as does tyrosinase.[2] With more oxidation resistant phenols the reaction is stoichiometric and stops at the level of catechol. Further studies to broaden the scope of the present mild and selective phenol *ortho*-hydroxylation and fully understand the mechanism of the reaction are being pursued.

¶ The most important peaks in the mass spectrum of **4** occur at *m/z* (%): 168 (M^+, 53), 137 (M − OMe, 100), 109 (M − CO_2Me, 21). For ^{18}O-labelled **4** we found peaks at *m/z*: 170 (56), 139 (100), 111 (26).

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