Binuclear Copper Complex Model of Tyrosinase

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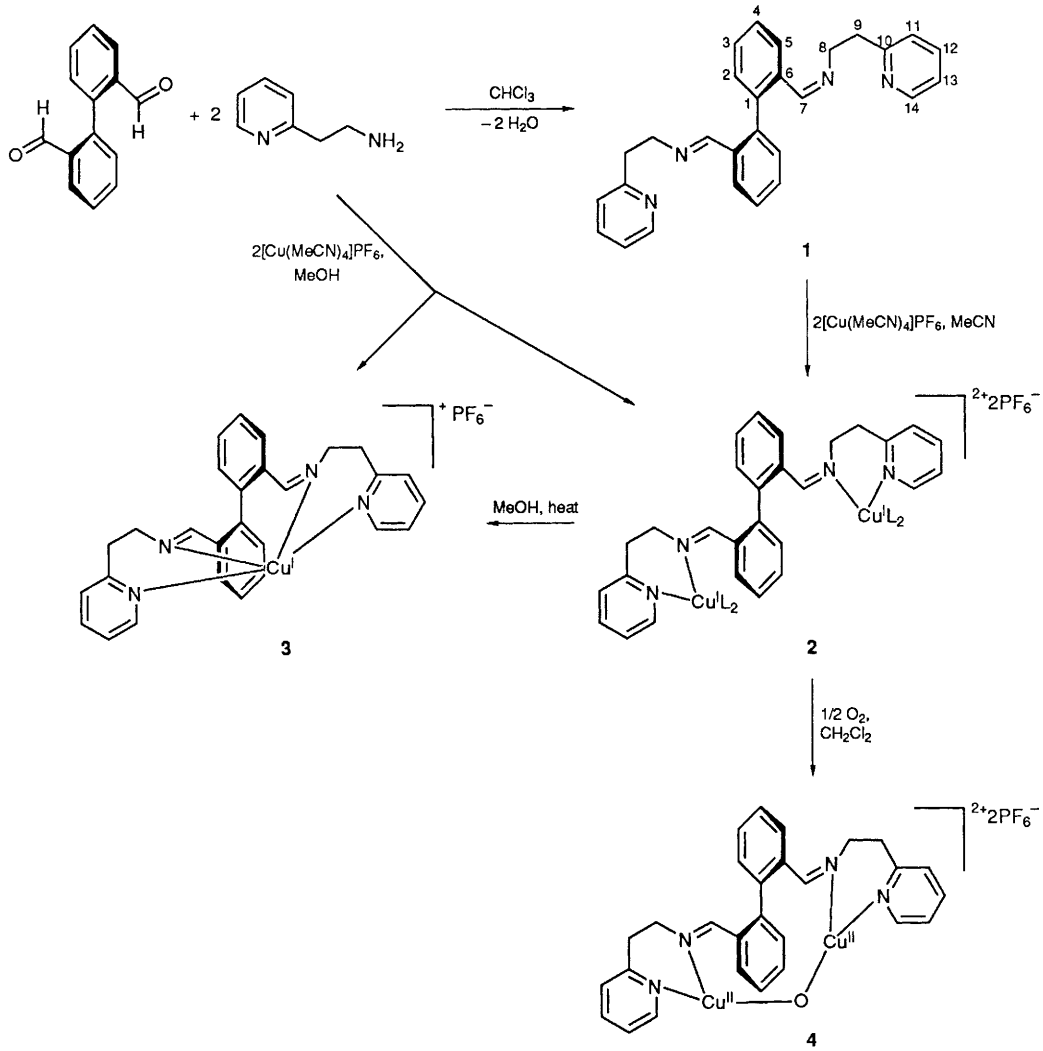
The synthesis is reported of a binuclear copper(I) complex **2** which exhibits the catalytic phenolase and catecholase activity of tyrosinase.

Tyrosinase (Tyr, EC 1.14.18.1),[1] a copper-containing monooxygenase, catalyses *ortho*-hydroxylation of monophenols into catechols (phenolase activity) and oxidation of catechols into *ortho*-quinones (catecholase activity). This enzyme, which is widely distributed in nature, is responsible for the formation of melanine pigments and many other polyphenolic compounds.

Using a mononuclear Karlin type model[2] we have recently reported[3] that during the *ortho*-hydroxylation of a pyridine nucleus, 'Cu^{III}=O' species are likely to be involved as intermediates during the *ortho*-hydroxylation as in tyrosinase.[4]

We now report the synthesis of a binuclear copper complex **2** model of tyrosinase featuring a flexible biphenyl nucleus as a spacer. The latter allows the copper–copper distance to be held at about 3.6 Å so that a dicopper μ-peroxo bridge can be accommodated as in tyrosinase.[5] The expected phenolase and catecholase catalytic properties have indeed been observed and are described below. Biphenyl-2,2′-dicarbaldehyde, prepared by phenanthrene ozonolysis,[6] was treated under argon



Scheme 1 Synthesis of tyrosinase models **2** and **4**, featuring a biphenyl spacer (L = MeCN)

with 2 equiv. of 2-(2-pyridyl)ethylamine and 2 equiv. of $[Cu(MeCN)_4]PF_6$[7] in degassed methanol. A 1 : 1 mixture of the dinuclear complex **2**† and the mononuclear complex **3**[8]† was obtained. Dinuclear complex **2** can be obtained alone by treating imine **1**‡ with 2 equiv. of $[Cu(MeCN)_4]PF_6$ in acetonitrile. Heating complex **2** in absolute degassed methanol affords quantitatively the mononuclear derivative **3**. Yellow complex **2** reacts with 0.5 equiv. of molecular oxygen in dichloromethane to give, after precipitation with diethyl ether, a green complex **4**† (Scheme 1). Support for the formulation of **4** as a μ-oxo-bridged complex comes from the stoichiometry of oxygen uptake consistent with four-electron reduction of O_2 involving two moles of **2**, and the reaction of **4** with triphenylphosphine in degassed dichloromethane which gives stoichiometric production of triphenylphosphine oxide and regeneration of Cu^{I}.[9]

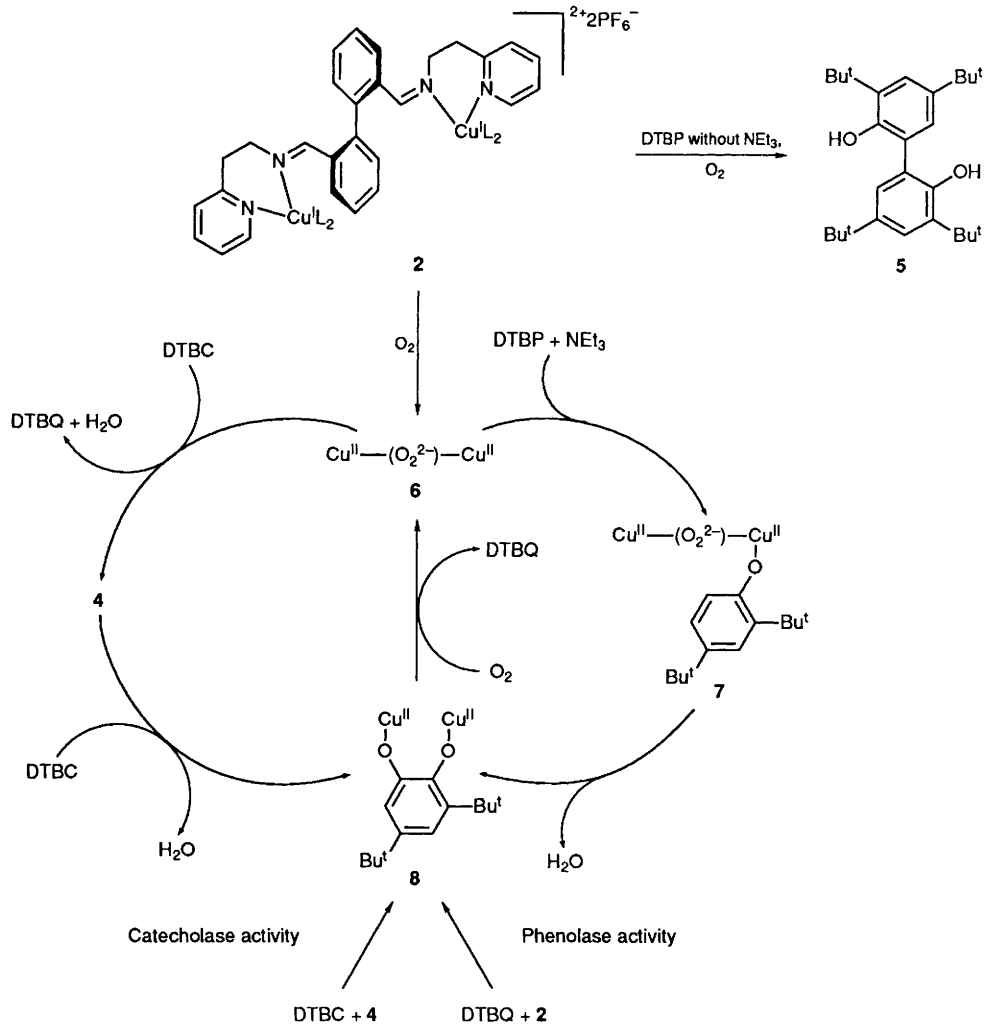
Complex **2** exhibits both a catalytic phenolase and a catecholase activity respectively with 2,4-di-t-butylphenol (DTBP) and 3,5-di-t-butylcatechol (DTBC) as shown by the following experiments (Scheme 2). A 1×10^{-3} mol dm^{-3} solution of DTBP in dichloromethane was oxidized into 3,5-di-t-butyl-*o*-quinone (DTBQ) with molecular oxygen by a 1×10^{-5} mol dm^{-3} solution of complex **2** containing 2 equiv. of triethylamine. A kinetic study showed a turnover of 16

† *Selected data* for **2**: ^{1}H NMR (200 MHz; CD_3COCD_3): δ 8.58 (s, 2H-7), 8.09 (d, *J* 4.4 Hz, 2H-14), 7.60 (td, *J* 7.8 and 1.6 Hz, 2H-12), 7.42–7.08 (m, 2H-2, 2H-3, 2H-4, 2H-5, 2H-11 and 2H-13), 4.08 (dd, *J* 12.4 and 10.4 Hz, 2H-8), 3.68 (dd, *J* 12.4 and 8 Hz, 2H-8), 3.00 (dd, *J* 16 and 7.2 Hz, 2H-9), 2.40 (dd, *J* 16 and 9.2 Hz, 2H-9), 2.12 (s, 4 coordinated CH_3CN) and 2.00 (s, free CH_3CN); ^{13}C NMR (50 MHz; CD_3COCD_3): δ 209.04 (coordinated CH_3CN), 165.62 (C-7), 160.35 (C-10), 149.43 (C-14), 140.48 (C-6), 137.94 (C-12), 136.75 (C-1), 131.21, 129.25, 127.47, 127.19 (C-2, C-3, C-4 or C-5), 124.90 (C-11), 123.00 (C-13), 117.10 (free CH_3CN), 68.57 (co-ordinated CH_3CN), 58.99 (C-8), 54.12 (coordinated CH_3COCH_3), 37.01 (C-9) and 0.65 (CH_3CN).

Selected data for **3**: ^{1}H NMR (200 MHz; CD_3COCD_3): δ 8.77 (s, 2H-7), 8.03 (d, *J* 4.4 Hz, 2H-14), 7.65 (td, *J* 7.7 and 1.7 Hz, 2H-12), 7.30–7.05 (m, 2H-2, 2H-3, 2H-4, 2H-5, 2H-11 and 2H-12), 4.10 (dd, *J* 13 and 10.1 Hz, 2H-8), 3.61 (dd, *J* 13 and 7.1 Hz, 2H-8), 5.90 (dd, *J* 15.8 and 7.1 Hz, 2H-9) and 4.31 (dd, *J* 15.8 and 10.1 Hz, 2H-9); ^{13}C NMR (50 MHz; CD_3COCD_3): δ 164.30 (C-7), 159.34 (C-10), 148.37 (C-14), 139.63 (C-6), 136.77 (C-12), 135.95 (C-1), 130.26, 127.97, 126.33, 125.97 (C-2, C-3, C-4 or C-5), 123.86 (C-11), 121.98 (C-13), 57.80 (C-8) and 35.78 (C-9).

Selected data for **4**: IR (KBr) ν/cm^{-1}: imine C=N 1640, pyridine C=N 1610, C=C 1487 and 1445, δ(PF) 838; λ_{max}/nm (CH_2Cl_2): 650 nm (ε 180 dm^3 mol^{-1} cm^{-1}).

‡ All new compounds gave spectral and analytical data in agreement with their proposed structure.



Scheme 2 Intermediates involved in phenol and catechol oxidation catalysed by complexes **2** (L = MeCN) and **4**. For simplicity the biphenyl spacer with its arms has not been included in **6**, **7** and **8**.

h^{-1}.§ Unfortunately the reaction stops after one hour, probably because the catalyst **2** is itself oxidized into **4** which is inactive in this process. In the absence of triethylamine, DTBP is quantitatively transformed in 2 days into its dimer **5** by an autooxidation process.[10] The role of triethylamine is most probably to favour the formation of a phenate copper complex **7** as postulated by Solomon.[5b]

In order to test the catecholase activity, 3,5-di-t-butylcatechol (DTBC) was submitted to the reaction of oxygen in the presence of 1% of complex **2** in dichloromethane. Practically quantitative yields of DTBQ were observed after 1 day of reaction at room temperature. At a concentration of complex of 1×10^{-5} mol dm^{-3} and of DTBC varying from 1×10^{-3} to 5×10^{-5} mol dm^{-3} a Michaelis–Menten kinetic treatment gave $K_M = 5.4 \times 10^{-5}$ mol dm^{-3} and a value of $k_{cat} = 11.4\ h^{-1}$ for the dissociation of the catechol–copper intermediate **8**.§ In the presence of dioxygen, the binuclear copper complex **4** led to the same catalytic transformation of DTBC into DTBQ with an identical rate. Furthermore, when a stoichiometric amount of complex **4** was treated under argon with DTBC a blue complex **8** was isolated, which had a UV–VIS absorption spectrum in CH_2Cl_2, with two broad peaks λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) 400 (3000) and 558 (1200). These absorptions are attributed to a catecholate–copper charge transfer (CT) transition.[11] Transitions which would have corresponded to the semiquinone–copper CT transition were not observed.[12] Complex **8**, which can also be obtained by a stoichiometric reaction of DTBQ with **2** under argon, immediately decomposes into DTBQ and **6** in the presence of dioxygen.

Our results can be rationalized as shown in Scheme 2. Peroxo complex **6**¶ and the spectroscopically characterized blue complex **8** are the common intermediates for the catecholase and phenolase processes. As postulated earlier by Solomon[5b] in the phenolase process, peroxo copper complex **6** reacts with DTBP to form **7** which gives catecholate copper

§ The formation of DTBQ was monitored by UV–VIS spectroscopy by the appearance of its characteristic band at 400 nm (ε 1830 dm^3 mol^{-1} cm^{-1} in CH_2Cl_2). Blank experiments showed that without copper the transformation of DTBP or DTBC into DTBQ does not take place.

¶ At present it is difficult to propose a structure for **6**; however these species could be μ-1,2- or a μ-η^2:η^2-peroxo[17] copper complexes as suggested by Karlin[5c] for similar complexes.

complex **8** by a sort of electrophilic attack.[5c] This complex **8** reacts rapidly with O_2 giving DTBQ and peroxo species **6**. In the catecholase process, complex **6** reacts with DTBC to give DTBQ and complex **4** which further reacts with another DTBC molecule giving the catecholate copper complex **8**.

Up to now, one of the best tyrosinase models known in the literature is the one of Karlin[13] who observed aromatic ring hydroxylation on the ligand spacer under stoichiometric conditions. Many binuclear complexes exhibit catecholase activity to some extent.[14] However, apart from Bulkowski's patent,[15] the present work is to our knowlege the first report of a chemical model featuring catalytic phenolase and catecholase activity in the presence of dioxygen, with turnovers ranging from 11 to 16 h^{-1}. While this reaction could eventually be used preparatively,[16] the main interest of our results is that we have available a good and efficient chemical model. This should enable us to understand better the reactivity of the peroxo dicopper intermediates involved in the oxidation mechanisms of copper oxygenase.

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