Oxygen Insertion by a New Tyrosinase Model Binuclear Cu^{I} Macrocyclic Complex

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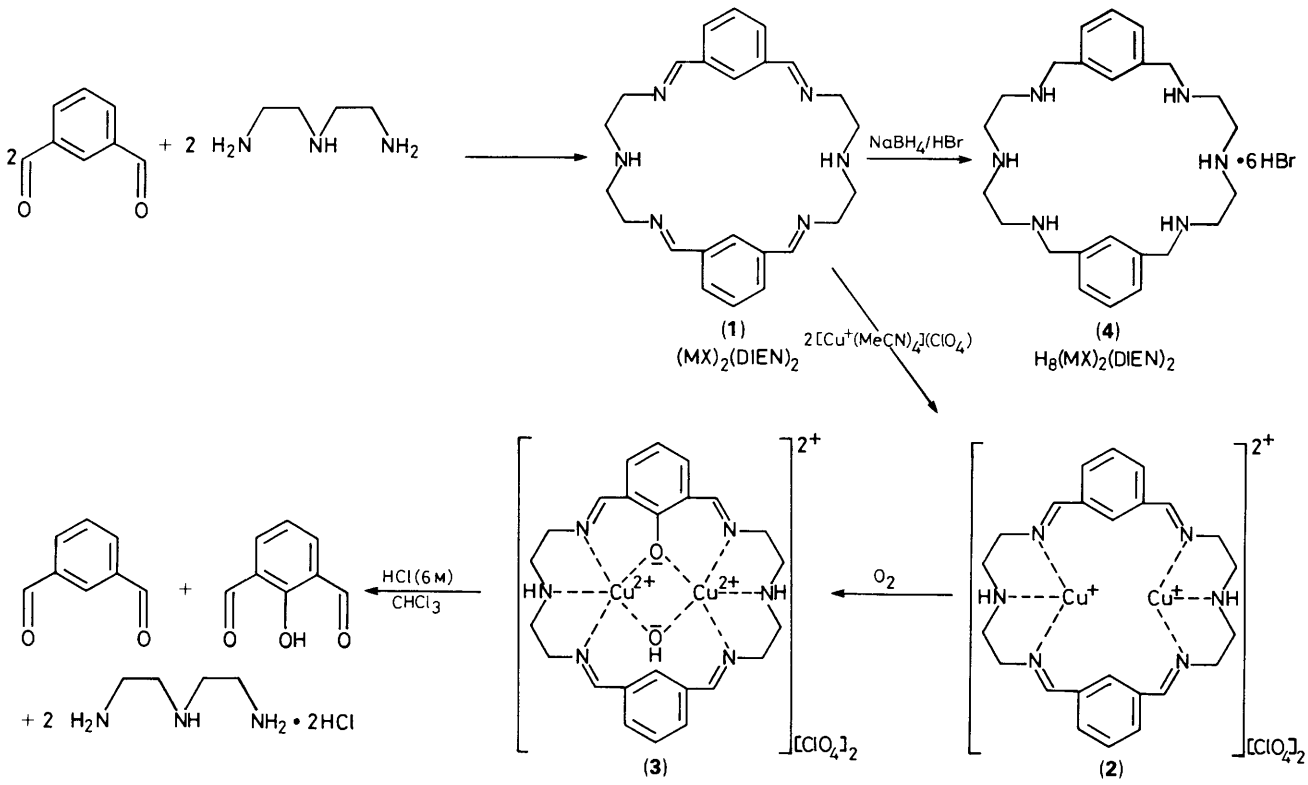
Benzene-1,3-dicarboxaldehyde and diethylenetriamine have been condensed to produce a new 24-atom tetra Schiff base binucleating macrocyclic ligand in good yield; the latter forms a dinuclear Cu^{I} complex which combines with dioxygen and inserts one of the oxygen atoms into the 2-position of the aromatic ring to form a phenolate- and hydroxide-bridged binuclear copper(II) complex.

Proteins known to contain a dinuclear copper centre and bind dioxygen[1] include tyrosinase and dopamine-β-hydroxylase, which insert oxygen into organic substrates. Very little information is available on the structure of these mono-oxygenases, although tyrosinase is believed[1,2] to be closely related to hemocyanin, whose structure is known. A number of synthetic models of tyrosinase, based on open-chain binucleating ligands, have been reported.[2] Karlin *et al.*[3] were the first to prepare such a model, consisting of a binucleating ligand which provides two pyridine and one aliphatic nitrogen



Scheme 1

donors to each copper[I] ion. More recently Casella and co-workers[4] described a Schiff base binucleating ligand with one imino and one imidazolo nitrogen donor per Cu[I]. Feringa *et al.*[5] showed that replacing the imidazole in Casella's ligand by a pyridine donor did not alter the mono-oxygenase activity of the system, and concluded that aromatic hydroxylation with binuclear Cu[I] complexes and molecular oxygen does not require a terdentate ligand system and does not depend critically on the precise type of donor groups previously suggested. We report here the synthesis of a macrocyclic binucleating Schiff base ligand MX_2DIEN_2 (**1**) containing two *m*-xylyl bridging moieties and two terdentate bis-imine nitrogen sites, and the activation of dioxygen by the corresponding dinuclear copper(I) complex leading to the hydroxylation of one of the arene rings.

The macrocyclic ligand was prepared by the Schiff base dimerization condensation of triamine with dialdehyde.[6] A solution of 97% *m*-phthalaldehyde (2.012 g, 15 mmol) in MeCN (250 ml) was added dropwise to a stirred solution of diethylenetriamine (1.62 ml, 15 mmol) in MeCN (400 ml) over a period of 2 h. A white precipitate (**1**) formed after 12 h and was recrystallized from CH_2Cl_2/MeCN. The 200 MHz ^1H and ^{13}C n.m.r. spectra in $CDCl_3$ were complex, suggesting the presence of isomers of the macrocyclic Schiff base MX_2DIEN_2 (yield; 2.19 g, 5.44 mmol, 72%). A satisfactory elemental analysis was also obtained (C, H, N; based on $C_{24}H_{30}N_6$).

A suspension of the Schiff base (2.19 g) in absolute ethanol (50 ml) was reduced with sodium borohydride. When the reaction was complete, the solvent was removed under reduced pressure, and the product was extracted with CH_2Cl_2 from aqueous solution (CH_2Cl_2/H_2O = 120 ml/10 ml). A colourless oil was obtained upon removal of the CH_2Cl_2 solvent under reduced pressure. About 3 ml of 48% HBr were added to the oily product. The white precipitate which formed was recrystallized from a mixture of H_2O/MeOH (25 ml/125 ml). Colourless crystals of (**4**) formed after 48 h at 0 °C. ^1H N.m.r. in D_2O (reference Me_4Si) produced peaks at δ 3.3—3.4 (multiplet, H of CH_2 of DIEN), 4.21 (singlet, H of CH_2 of *m*-xylyl), 7.41 (singlet, aromatic H), and 7.50 (singlet, aromatic H) in the ratio of 8 : 4 : 3 : 1 respectively. ^{13}C N.m.r., in $CDCl_3$ (sufficient NaOD was added to solubilize the sample) (reference Me_4Si) produced peaks at δ 41.93 and 43.80 (CH_2 of DIEN), 50.93 (CH_2 of *m*-xylyl), 130.49, 130.59, 131.81, and 132.01 (4 non-equivalent aromatic carbons) (yield; 2.11 g, 2.30 mmol, 66%. Overall yield; 48%). A satisfactory elemental analysis [C, H, N, based on $C_{24}H_{38}N_6 \cdot 6HBr$ (*i.e.* $H_8MX_2DIEN_2 \cdot 6HBr$)] was obtained.

The complex $Cu^I(MeCN_4)(ClO_4)$[7] (2 mmol) was combined with the Schiff base MX_2DIEN_2 (**1**) (1 mmol in CH_2Cl_2) under argon. The colourless ligand solution turned orange and this colour remained stable in the absence of dioxygen, and is considered to be due to the formation of the dinuclear copper(I) macrocyclic complex (**2**). When the orange solution was exposed to dioxygen at room temperature, the colour gradually turned to green. The MeCN solution of the green solid obtained upon evaporation of CH_2Cl_2 displayed, respectively, an i.r. band around 3500 cm^{-1}, a near u.v.-band at 362 nm (ε = 5800 mol^{-1} cm^{-1} dm^3) as well as a weaker band near 620 nm (ε = 150 mol^{-1} cm^{-1} dm^3), and an ^1H n.m.r. peak (CD_3CN) at δ 12.6 (reference Me_4Si) (assigned to an OH^- bound to copper). These spectral features are characteristic of phenoxo- and hydroxo-bridged binuclear copper(II) complexes (**3**).[4,8] The copper(II) ions are assumed to be antiferromagnetically coupled in view of the observation of sharp peaks in the ^1H n.m.r. spectrum. Upon addition of Et_2O to the CH_2Cl_2 solution containing the oxygenated copper compound, a green precipitate formed. Elemental analysis confirmed the green compound to be a dinuclear copper complex {C, H, N, Cl, Cu; based on $C_{24}H_{30}N_6O_{10}Cl_2Cu_2$ (*i.e.* $[Cu^{II}_2(MX_2DIEN_2\text{-}O^-)\ (OH^-)]\ (ClO_4^-)_2$}.

The green product was dissolved in HCl (6 M) and extracted with $CHCl_3$, producing a yellow solid (260.8 mg). The ^1H n.m.r. spectrum of the product displayed only aromatic peaks which showed the yellow solid to be a mixture of 63%/37% *m*-phthalaldehyde/2-hydroxy-*m*-phthalaldehyde. The yield of the extracted ligand was 92% and the oxygen insertion yield was found to be 74%, with only one of the arene rings hydroxylated.

These results demonstrate the synthesis of the first macrocyclic tyrosinase model and its reaction with dioxygen (Scheme 1). Further investigations underway in this laboratory include the use of labelled $^{18}O_2$ to assess the origin of the inserted oxygen atom, the preparation and catalysis studies of macrocyclic analogues of (**2**) and (**4**) in which the aliphatic secondary nitrogen is replaced with a pyridinic nitrogen atom, and the determination of the binding constants of binuclear complexes of the reduced MX_2DIEN_2 macrocycle (*i.e.* $H_8MX_2DIEN_2$) with divalent transition metal ions in aqueous media.†

† Preliminary results indicate that the reduced MX_2DIEN_2 macrocycle (**4**) forms a thermodynamically very stable $[Cu^{II}_2L]^{4+}$ complex which is readily converted to $[Cu^{II}_2L(\mu\text{-}OH)]^{3+}$ and $[Cu^{II}_2L(\mu\text{-}OH)_2]^{2+}$ as the pH is increased.

This work was supported by a Convention Cifre Scholarship from the French Ministry of Research and Technology and financial support from L'Air Liquide S.A., and the Robert A. Welch foundation under research grant A-259.

Received, 12th April 1989; Com. 9/01494B

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