Functional Modeling of Tyrosinase. Mechanism of Phenol *ortho*-Hydroxylation by Dinuclear Copper Complexes

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The copper-mediated oxygenation of methyl 4-hydroxybenzoate (**1**) in acetonitrile has been investigated by employing a series of dinuclear copper(I) complexes with polybenzimidazole ligands. The reaction mimics the activity of the copper enzyme tyrosinase, since the initial product of the reaction is the *o*-catechol, methyl 3,4-dihydroxybenzoate (**2**). The ligand systems investigated include α,α′-bis{bis[2-(1-methyl-2-benzimidazolyl)-ethyl]amino}-*m*-xylene (**L-66**) α,α′-bis{bis[2-(1-methyl-2-benzimidazolyl)methyl]amino}-*m*-xylene (**L-55**), α,α′-bis{[(1-methyl-2-benzimidazolyl)methyl][2-(1-methyl-2-benzimidazolyl)ethyl]amino}-*m*-xylene (**L-56**), and α,α′-bis{[(2-pyridyl)methyl][2-(1-methyl-2-benzimidazolyl)ethyl]amino}-*m*-xylene (**L-5p6**). The most effective among the dicopper(I) complexes is that derived from **L-66**, while its mononuclear Cu(I) analogue, with the ligand *N*,*N*-bis[2-(1-methyl-2-benzimidazolyl)ethyl]amine is inactive in the monooxygenase reaction. The catechol **2** is the only product of phenol hydroxylation when the reaction is carried out at low temperature (−40 °C). As the temperature is increased, methyl 2-[4-(carbomethoxy)phenoxy]-3,4-dihydroxybenzoate (**4**), formally resulting from Michael addition of the starting phenol to 4-carbomethoxy-1,2-benzoquinone (**3**) and probably resulting from the reaction between free phenolate and some intermediate copper−catecholate species, becomes a major product of the reaction. In order to gain insight into the mechanism of the reaction, the dicopper(I)−phenolate adducts and dicopper(II)−catecholate adducts of the **L-66**, **L-55**, and **L-6** complexes have been studied. In a few cases the adducts containing catecholate monoanion or catecholate dianion have been isolated and spectrally characterized. It has been shown that the final product of the monooxygenase reaction corresponds to the dicopper(II)−catecholate dianion complex. A mechanism for the biomimetic phenol *ortho*-hydroxylation has been proposed and its possible relevance for tyrosinase discussed.

Tyrosinase (monophenol, dihydroxyphenylalanine: O_2 oxidoreductase, EC 1.14.18.1), also known as polyphenol oxidase, is a copper enzyme which catalyzes the hydroxylation of phenols to *o*-diphenols (monophenolase activity) and the two-electron oxidation of *o*-diphenols to *o*-quinones (catecholase activity) by molecular oxygen.[1] It is widely distributed in nature; for instance, it is responsible for melanization in animals and browning in plants.[2] Studies performed mostly with the enzyme from mushrooms show that tyrosinase exhibits a rather broad substrate specificity, although there seems to be a strict requirement for *para*-substitution in phenolic substrates.[3] Particularly strong similarities exist between tyrosinase and hemocyanin, the dioxygen carrier protein of mollusks and arthropods,[4] in that they contain closely related dinuclear (type 3) copper sites. Both proteins, for instance, bind reversibly molecular oxygen, and their dioxygen adducts and other derivatives exhibit strikingly similar spectral properties.[5,6] On the basis of the X-ray crystal structures of *Panulirus interruptus* and *Limulus polyphemus* hemocyanins,[4c,d] it is likely that three histidines are the ligands for each copper in these type 3 copper proteins. The difference in biological function between hemocyanin and tyrosinase thus depends at least in part on the different accessibility to exogenous molecules of their active site.

Attempts to duplicate the peculiar monophenolase activity of tyrosinase in model systems can be dated from the pioneering work of Brackman and Havinga in the mid-1950s,[7] when simple copper salts were found to be able to mediate phenol hydroxylation reactions. Other systems based on copper salts or copper−

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pyridine or copper−phenanthroline complexes involving external phenol hydroxylation have been reported,[8] and reviews on the various aspects of this chemistry are available.[9] However, the reactions generally yield mixtures of products, and the significance of these oxidation reactions to the mechanism of action of tyrosinase is limited by the uncertainty on the nature of the species actually undergoing the reaction. The most significant insights into the chemical activation of dioxygen by dinuclear copper(I) sites come from the studies performed by Karlin's group on the aromatic hydroxylation reaction occurring on the ligand upon oxygenation of model dicopper(I) complexes derived from *m*-xylyl−tetrapyridyl ligands,[10] where the two steps of dioxygen binding and ligand oxygenation have been separately identified.[11]

We[12] and subsequently others[13] reported similar reactivity by simpler *m*-xylyl−bis(imine)dicopper(I) complexes, or their corresponding diamine complexes,[13f] but the characterization of these systems in terms of the possible intermediates formed by reaction with dioxygen is still incomplete. It is interesting to note that a related bis(imine)dicopper(I) complex, in which ligand hydroxylation is prevented, has been reported to mediate the catalytic *o*-hydroxylation of 2,4-di-*tert*-butylphenol to the corresponding *o*-quinone in the presence of dioxygen, with up to 16 turnovers/h,[14] though, this reactivity has been characterized only spectrally.

An intriguing feature of the hydroxylation reaction observed in Karlin's model system, as opposed to the bis(imine) systems, is that the ligand monooxygenation is *completely* depressed when the pyridyl groups on the side arms of the *m*-xylyl residue are substituted by other nitrogen heterocycles.[15,16] In these cases only four-electron reduction of dioxygen to give the dihydroxycopper(II) species occurs. This different behavior is not really understood, and it is likely that both steric and electronic effects are playing a role. We thought that by replacement of the pyridyl groups with benzimidazoles, which have virtually identical basicity, it could be possible to transfer the potential monooxygenase reactivity of Karlin's system from the ligand to external phenols to obtain a better mimic of the tyrosinase reaction. In fact, the dicopper(I) complex of the *m*-xylyl−tetrabenzimidazole ligand **L-66** (Figure 1) was shown[17] to mediate the *ortho*-hydroxylation of phenols by reaction with dioxygen. In this paper we amplify our previous communication by reporting the behavior in the same reaction of the dicopper(I) complexes of several polybenzimidazole ligands (shown in Figure 1) and the characterization of some of the intermediates and products involved in the monooxygenase reaction. We also address the more general problem[18] of the monophenolase/

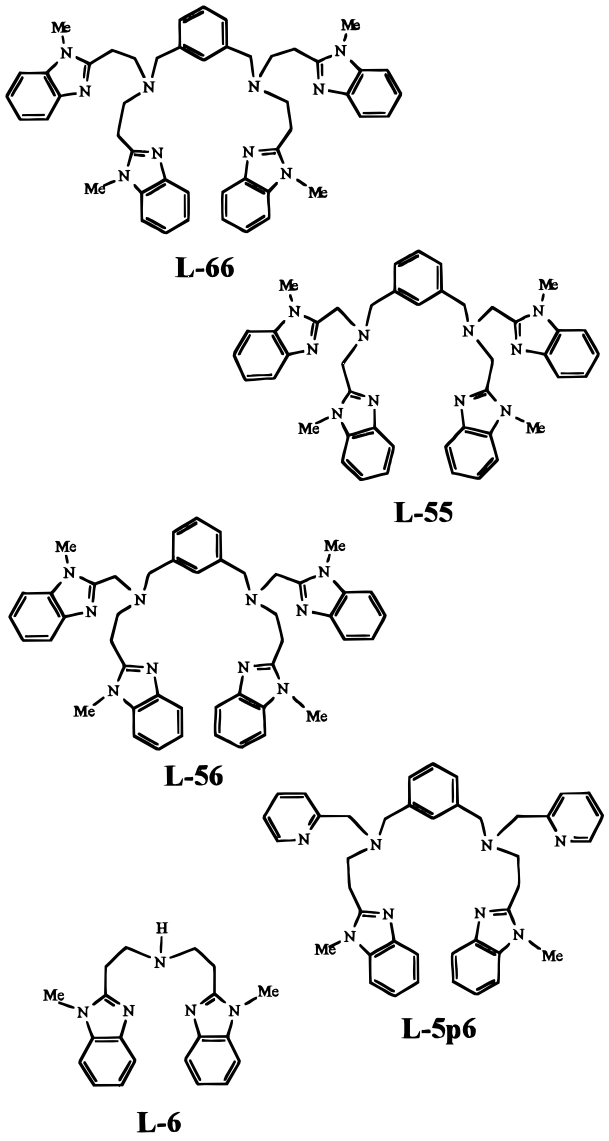

Figure 1. Structures of the ligands employed in the present investigation.

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Table 1. Formation of Methyl 3,4-Dihydroxybenzoate by Copper(I) Mediated Oxygenation of Methyl 4-Hydroxybenzoate at 25 °C[a]

copper(I) complex	yield of isolated catechol (%)
$[Cu_2(\mathbf{L\text{-}66})][ClO_4]_2$	37
$[Cu_2(\mathbf{L\text{-}56})][ClO_4]_2$	30
$[Cu_2(\mathbf{L\text{-}55})][ClO_4]_2$	25
$[Cu_2(\mathbf{L\text{-}5p6})][ClO_4]_2$	18
$[Cu(\mathbf{L\text{-}6})][ClO_4]$	trace

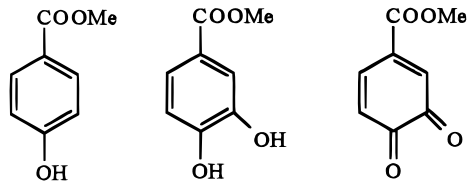
[a] Experiments carried out with *in situ* generation of the phenolate from methyl 4-hydroxybenzoate and sodium borohydride (see Experimental Section).

catecholase steps involved in the monooxygenase activity exhibited by tyrosinase, showing that, indeed, catechols are intermediates in the route leading from phenols to *ortho*-quinones.

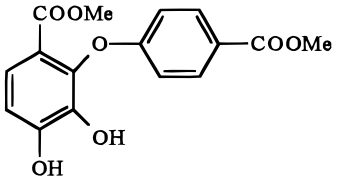
Results and Discussion

Synthesis. Ligands **L-66** and **L-55**, and their dicopper(I) and dicopper(II) complexes, have been studied previously.[16,17] The new ligands **L-56** and **L-5p6** became available upon reduction of the bis(Schiff base) between two molecules of 1-methyl-2-(2-aminoethyl)benzimidazole[12d] and benzene-1,3-dicarboxaldehyde, followed by alkylation of the resulting diamine with 2-chloromethyl-1-methylbenzimidazole or 2-(chloromethyl)-pyridine. The corresponding dicopper(I) complexes were obtained easily by reaction with 2 equiv of tetrakis(acetonitrile)-copper(I) salts. Ligand **L-6** represents a single arm analogue of the binucleating ligand **L-66**, and its copper(I) complex $[Cu(\mathbf{L\text{-}6})]^+$ can thus be considered as the mononuclear analogue of the dinuclear complex $[Cu_2(\mathbf{L\text{-}66})]^{2+}$. The variation in the length of the methylene bridges joining the benzimidazole arms and the xylyl groups in the dinucleating ligands **L-55**, **L-66**, and **L-56** allows one to obtain complexes with five-membered, six-membered, or five- and six-membered chelate rings, respectively. The size of these rings influences the flexibility of the coordination environment of the metal centers, and it is expected that this will affect the reactivity of the complexes, since it affects their redox potentials.[16,19] Ligand **L-5p6** enables one to assess the effect of substitution of one of the benzimidazole donors of **L-56** with a pyridine group. As for the parent dinuclear copper(I) complexes derived from **L-55** and **L-66**,[16,17] reaction with dioxygen of $[Cu_2(\mathbf{L\text{-}56})][ClO_4]_2$ and $[Cu_2(\mathbf{L\text{-}5p6})][ClO_4]_2$ produces the dihydroxydicopper(II) complexes.

Phenol *ortho*-Hydroxylation. As we have shown previously,[17] the complex $[Cu_2(\mathbf{L\text{-}66})]^{2+}$ is capable of mediating the oxygen transfer reaction from O_2 to exogenous phenols. The reaction suffers some limitation in the operation conditions in that a rigorously anhydrous and nonprotic medium is required and formation of a phenolate adduct of the cuprous complex prior to the reaction with O_2 is also required, otherwise simple copper(I) oxidation occurs. Since the monooxygenase reaction performed on methyl 4-hydroxybenzoate (**1**) was more easily controlled, because of the presence of an electron withdrawing substituent *para* to the phenol group, we concentrated on that substrate and explored the possibility of extending such reactivity to other copper(I) complexes. Those derived from the dinucleating ligands **L-55**, **L-56**, and **L-5p6** involved minimal variations in the coordination environment, while $[Cu(\mathbf{L\text{-}6})]^+$ allows one to establish the importance of the nuclearity of the complex. Experiments performed according to the simple



1 **2** **3**



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procedure based on the *in situ* generation of the phenolate from phenol and sodium borohydride[18] showed that only the binuclear complexes display monooxygenase activity (Table 1), while with $[Cu(\mathbf{L\text{-}6})][ClO_4]$ the catechol (**2**) could be detected only in trace amounts by TLC.[20] Although we did not try to optimize the yield of catechol by systematic variation of the reaction conditions, that is outside the scope of the present investigation, a trend in the efficiency of the various copper(I) complexes in mediating the phenol hydroxylation reaction seems evident:

$$[Cu_2(\mathbf{L\text{-}66})]^{2+} > [Cu_2(\mathbf{L\text{-}56})]^{2+} > [Cu_2(\mathbf{L\text{-}55})]^{2+} > [Cu_2(\mathbf{L\text{-}5p6})]^{2+} >> [Cu(\mathbf{L\text{-}6})]^{+} \cong 0$$

The dinuclearity of the copper(I) complex is thus an important prerequisite for the reaction, but also the local arrangement of the copper(I) sites apparently affects the output of the reaction. This involves various steps, where the metals undergo structural and redox changes, so that *flexibility* of the coordination environment is likely to play a major role. As shown by the properties of its $[Cu_2(\mathbf{L\text{-}66})]^{4+}$ complex,[16] this **L-66** ligand, carrying the longer and thus most flexible benzimidazolyl arms, allows the copper centers to be most easily adaptable to the structural changes required by the oxygenation process. Also, benzimidazoles appear to be better ligands than pyridines in performing this hydroxylation reaction.

A recent report[18] pointed out that production of the catechol **2** from $[Cu_2(\mathbf{L\text{-}66})]^{2+}$ and the sodium phenolate generated *in situ* from **1** and $NaBH_4$ results because of the presence of the BH_3 byproduct in the system. This leads to isolation of the catechol by reduction of 4-carbomethoxy-1,2-benzoquinone (**3**), which is the actual oxygenation product.[18] Use of sodium 4-(carbomethoxy)phenolate in nonreducing conditions leads instead to the production of compound **4**, which results from Michael addition of phenolate to the *o*-quinone **3** (Scheme 1).[18] This finding is important because it may imply a reconsideration of the phenol oxygenation mechanism commonly accepted for tyrosinase, which involves monophenolase and catecholase steps rapidly following each other.[1,6,22] The proposal formulated by Sayre and Nadkarni involved dioxygen insertion into the phenol mediated by a *single* metal center,[18] in a manner similar to the

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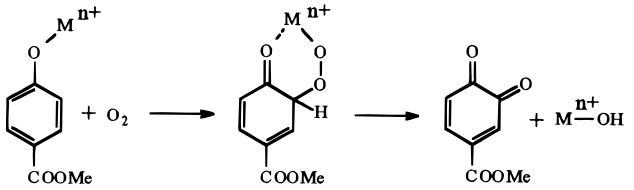
Scheme 1



Scheme 2

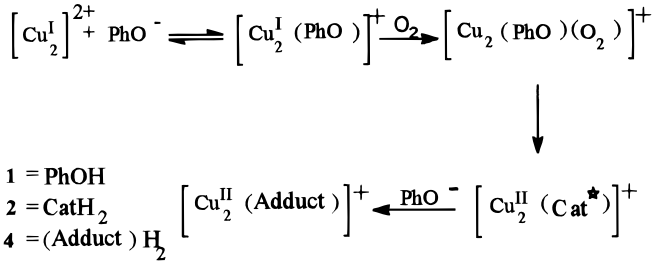


Table 2. Distribution of the Products Resulting from Oxygenation of Methyl 4-Hydroxybenzoate in the Presence of Copper(I) Complexes from HPLC Analysis[a]

copper(I) complex	temp (°C)	time (h)	**1** (%)	**2** (%)	**4** (%)
$[Cu_2(\mathbf{L\text{-}55})]^{2+}$	23	2	40	2	58
$[Cu_2(\mathbf{L\text{-}55})]^{2+}$	−25	2	97	3	
$[Cu_2(\mathbf{L\text{-}66})]^{2+}$	23	2	1	4	95
$[Cu_2(\mathbf{L\text{-}66})]^{2+}$	−25	2	73	19	8
$[Cu_2(\mathbf{L\text{-}66})]^{2+}$	−40	26	70	30	trace
$[Cu(\mathbf{L\text{-}6})]^{+}$	23	2	100	trace	

[a] The tetra-*n*-butylammonium salt of **1** was used in these experiments.

mechanism accepted for non-heme iron catechol dioxygenases,[23] and is therefore difficult to reconcile with the apparent necessity of a dinuclear copper center to perform the reaction. However, a basically similar direct dioxygen insertion mechanism, leading to the quinone without the intermediacy of a catechol, was proposed for tyrosinase at a dinuclear copper center by Kitajima and Moro-oka.[9e]

A different route to the Michael adduct **4** may be provided by the reaction between free phenolate and some intermediate copper-bound catechol residue (indicated as Cat* in Scheme 2), resulting from the initial monooxygenase reaction. To address this point it was necessary to set up an analytical procedure capable of detecting even small amounts of the catechol **2** in the reactions carried out under nonreducing conditions. This required an HPLC separation method since the analytical and spectral properties of **2** and **4**, and even their TLC behavior, are similar. Thus, when representative copper(I) complexes were reacted with tetra-*n*-butylammonium 4-(carbomethoxy)phenolate under argon and then exposed to dioxygen, workup of the reaction mixture and HPLC analysis led to the recovery of **1**, **2**, and **4** reported in Table 2. The ratios between **1** and **2** + **4** were confirmed by ^{1}H NMR. These experiments clearly show that some catechol is always present in the reaction mixture and it becomes the major or even the only product when the oxygenation reaction is carried out at low temperature. Further, as shown by the product versus time plot in Figure 2, when the low-temperature reaction mixture is

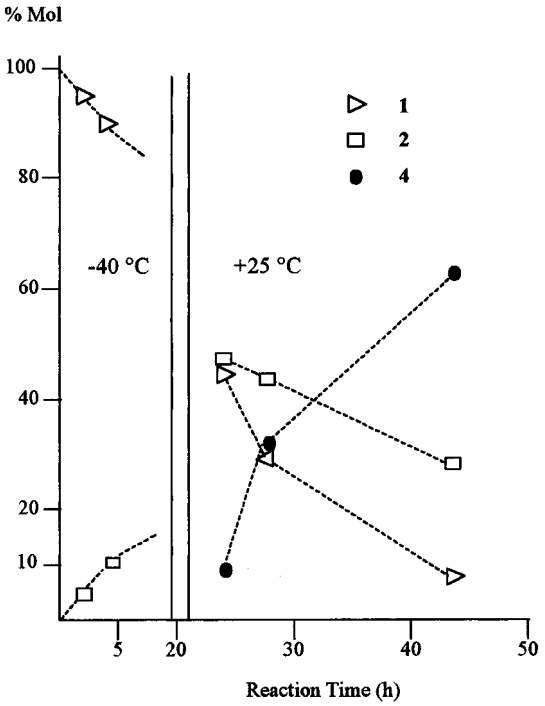


Figure 2. Product versus time plot showing that the Michael adduct (**4**) formation follows catechol (**2**) formation. The experiment was carried out by allowing the reaction between $[Cu_2(\mathbf{L\text{-}66})][ClO_4]_2$, tetra-*n*-butylammonium 4-(carbomethoxy)phenolate, and dioxygen (1 atm) in acetonitrile to proceed at −40 °C for 20 h, as described in the Experimental Section. Then the mixture was brought to +25 °C during 1 h, and the reaction was continued at this temperature for a further 24 h. Samples of the reaction solutions were withdrawn at different times, decomposed, and analyzed by HPLC.

brought to room temperature and the reaction is allowed to proceed in these conditions, formation of the Michael adduct is accompanied by depletion of both catechol and phenol. These results, therefore, indicate that formation of the Michael adduct **4** occurs after the initial oxygenation step and *the product of monooxygenation is the catechol* and *not* the *o*-quinone. In other Cu-model systems we actually find catechol **2** as the only oxygenation product of **1**, even when the reaction is carried out at room temperature.[21]

To gain insight into the mechanism of the biomimetic *ortho*-hydroxylation reaction, it is necessary to characterize the intermediates involved in the pathway indicated in Scheme 2. Our initial objective was to try to understand the structure of the initial dicopper(I)−phenolate complex $[Cu_2(PhO)]^+$ and the final dicopper(II)−catecholate complex $[Cu_2(Cat)]^{2+}$ formed in the conditions in which the step leading to **4** is blocked. We concentrated on the dinuclear systems derived from the ligands **L-55** and **L-66** and on the mononuclear **L-6** system, which is inactive in the monooxygenase reaction, because the characterization of the related copper(II) complexes was carried out previously.[16,24] We will also discuss low-temperature spectral measurements carried out to follow the course of the oxygenation reaction.

Copper(I)−Phenolate Complexes. The reaction between copper(I) complexes and the phenolate ion derived from **1** (PhO^-) leads to the formation of an adduct characterized by a UV band near 310 nm, likely charge transfer in origin. The intensity of the CT band developed upon reacting anaerobically stoichiometric amounts of the reagents in dry MeCN is larger for $[Cu(\mathbf{L\text{-}6})]^+$ ($\Delta\epsilon \approx 16500\ M^{-1}\ cm^{-1}$) than for $[Cu_2(\mathbf{L\text{-}66})]^{2+}$

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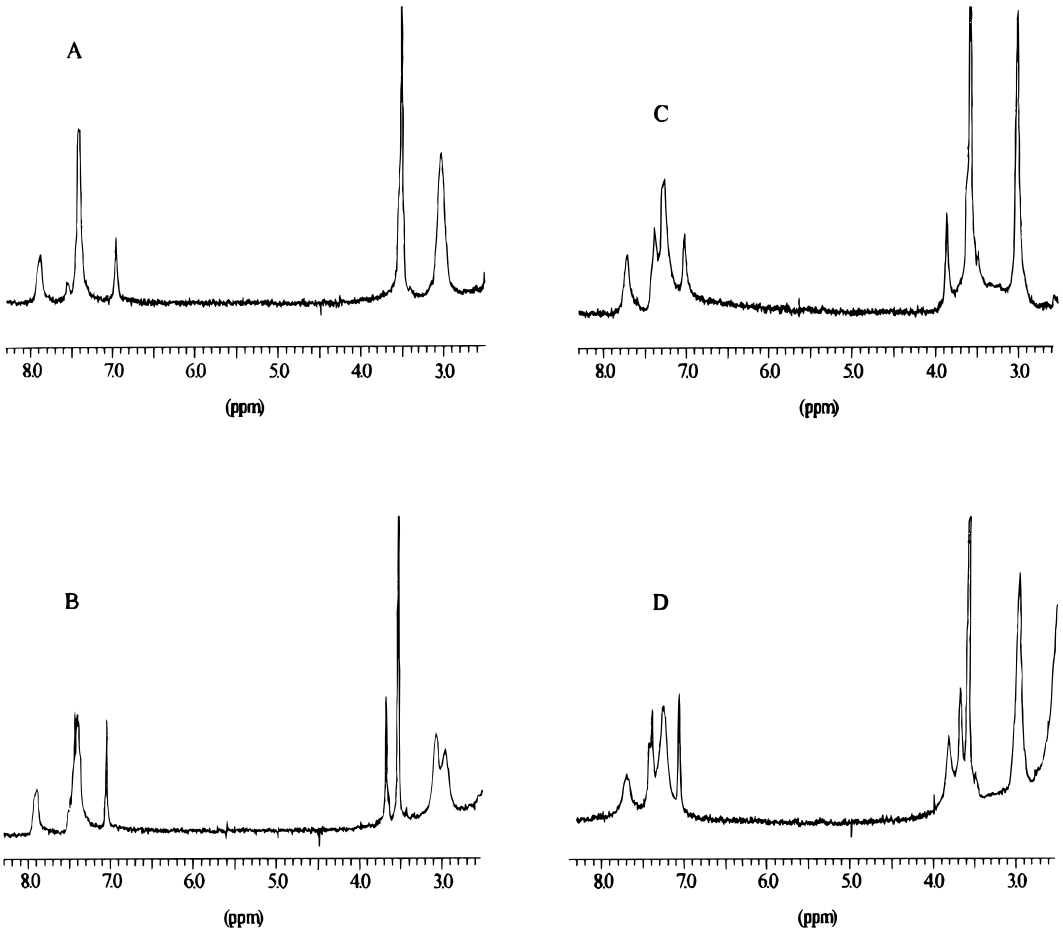
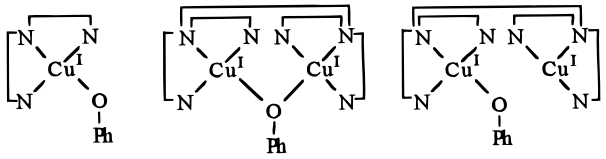


Figure 3. Proton NMR spectra recorded anaerobically in dry CD_3CN of $[Cu_2(\mathbf{L\text{-}66})][PF_6]_2$ at 288 K (A) and at 248 K (B); $[Cu_2(\mathbf{L\text{-}66})][PF_6]_2$ + 1 equiv of sodium 4-(carbomethoxy)phenolate at 288 (C) and at 248 K (D).

(7500), $[Cu_2(\mathbf{L\text{-}56})]^{2+}$ (6300), or $[Cu_2(\mathbf{L\text{-}55})]^{2+}$ (4000). Therefore, the lack of monooxygenase activity exhibited by the mononuclear complex is not attributable to a lower capacity to bind the phenol. On the other hand, while in the mononuclear copper(I)−phenolate adduct the ligand is probably acting as monodentate (**5**), it is not necessarily so for the dinuclear



5 **6** **7**

copper(I) complexes, where the presence of a bridging (**6**), rather than monodentate (**7**), phenolate can also be proposed. Unfortunately, these copper(I)$_2$−phenolate adducts are difficult to isolate in pure form. We have therefore tried to characterize them in solution by variable-temperature ^{1}H NMR, because adduct formation may be incomplete at room temperature.

The proton NMR spectrum of $[Cu_2(\mathbf{L\text{-}66})]^{2+}$ in CD_3CN appears simple at room temperature. The broad singlets at 3.04 and 3.52 ppm contain all of the aliphatic resonances. The former signal includes all methylene protons connecting the benzimidazolyl arms to the diaminoxylyl residue, while the latter includes the benzimidazole *N*-methyl protons and the xylyl methylene protons. The aromatic signals occur at 6.95, 7.41, 7.55, and 7.89 ppm. As the temperature is lowered to 288 K the xylyl methylene signal begins to separate from the intense *N*-methyl signal (Figure 3A), while the degeneracy between the two methylene resonances starts to be broken around 280 K. At low temperature (below 250 K) separate signals are observed for all types of aliphatic protons at 2.96, 3.08, 3.52, and 3.67 ppm (Figure 3B), and also the aromatic signals show more resolution. The addition of 1 equiv of PhO^- to a dilute solution of the complex in the same solvent has a marked effect on the benzimidazole signal at lowest field, which shifts to 7.70 ppm, while the other resonances undergo minor changes in position (Figure 3C). The signals of the added phenolate are detectable at 3.85 (COOMe) and 7.30 ppm (phenyl protons). Lowering the temperature produces a separation of the xylyl methylene signal from the *N*-methyl signal, as before, but does not remove the degeneracy between the other methylene signals, which remain significantly upfield shifted (2.96 ppm) with respect to $[Cu_2(\mathbf{L\text{-}66})]^{2+}$ below 250 K (Figure 3D). This shift is expected because phenolate binding involves an increase of electron density on the coppers, but it is important to note that the symmetry of the spectrum is not altered, even upon cooling the conformational equilibria of the ligand. The two copper(I) environments remain equivalent in the adduct, and this therefore supports structure **6**, containing a bridging phenolate, rather than **7**, since a fast exchange of phenolate between the two coppers should be hindered at low temperature. This is confirmed by the behavior of the phenolate adduct of the dicopper(I) complex derived from 1,3-bis[bis(2-pyridylmethyl)amino]benzene,[25] where the separation between the metal centers (5.6 Å) does not allow one to establish monoatomic bridges with exogenous ligands. The proton NMR spectra of this adduct show, in fact, exchange broadening and further modifications of the signals at low

(25) Schindler, S.; Szalda, D. J.; Creutz, C. *Inorg. Chem.* **1992**, *31*, 2255.

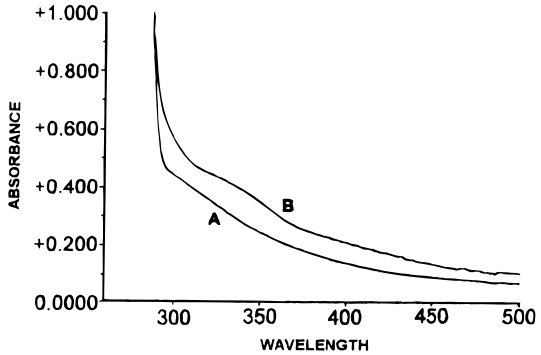


Figure 4. Comparison of the near-UV region of the electronic spectra of the catechol adducts $[Cu_2(\mathbf{L\text{-}55})(CatH)][ClO_4]_3$ (A) and $[Cu_2(\mathbf{L\text{-}55})(Cat)][ClO_4]_2$ (B) in dry acetonitrile solution.

temperature (<260 K) due to slow exchange of the monodentate phenolate ligand between the two copper(I) sites (see Supporting Information). Effects similar to the $[Cu_2(\mathbf{L\text{-}66})]^{2+}$−phenolate system could be noted for the proton NMR investigation of phenolate binding to $[Cu_2(\mathbf{L\text{-}55})]^{2+}$, even though the accessible temperature range is limited to ∼268 K by solubility problems. Also in this case no splitting of the three singlet signals, associated with the *N*-methyl, benzimidazole methylene, and xylyl methylene protons of the bound ligand, occurs in the presence of phenolate.

Copper(II)−Catecholate Complexes. The product of the monooxygenase reaction, at least at low temperature, is the catecholate adduct of the dinuclear copper(II) complex. Since a catechol can bind to metal ions in its monoanionic ($CatH^-$) and dianionic (Cat^{2-}) forms we attempted to isolate the adducts of both of these forms with $[Cu_2(\mathbf{L\text{-}66})]^{4+}$, $[Cu_2(\mathbf{L\text{-}55})]^{4+}$, and, for comparison purposes, $[Cu(\mathbf{L\text{-}6})]^{2+}$. This was possible for $[Cu_2(\mathbf{L\text{-}55})]^{4+}$, while only the adducts $[Cu_2(\mathbf{L\text{-}66})(Cat)][ClO_4]_2$ and $[Cu(\mathbf{L\text{-}6})(CatH)][ClO_4]$ could be obtained in the other cases in sufficiently pure form. Complications arise because the synthetic procedure for forming these adducts requires the presence of a base, and in these conditions the dinuclear complexes tend to form the less soluble hydroxide-bridged complexes.[16] In addition, particularly with $[Cu_2(\mathbf{L\text{-}66})]^{4+}$, the catecholate species undergo condensation/oxidation reactions that produce yellow-brown organic materials that need to be separated from the adducts by column chromatography. Conductivity measurements clearly distinguish the electrolyte type of the adducts $[Cu_2(\mathbf{L\text{-}55})(CatH)][ClO_4]_3$ and $[Cu_2(\mathbf{L\text{-}55})(Cat)][ClO_4]_2$, for which the Λ_M values of 370 and 295 Ω^{-1} cm^2 mol^{-1}, respectively, have been found in acetonitrile solution. The Λ_M value of the latter complex is very similar to that of $[Cu(\mathbf{L\text{-}6})][ClO_4]_2$ ($\Lambda_M = 285\ \Omega^{-1}$ cm^2 mol^{-1}), while that found for $[Cu(\mathbf{L\text{-}55})][ClO_4]_4$ is very high ($\Lambda_M = 477\ \Omega^{-1}$ cm^2 mol^{-1}), in agreement with expectation.[26] The data for $[Cu_2(\mathbf{L\text{-}66})(Cat)][ClO_4]_2$ ($\Lambda_M = 290\ \Omega^{-1}$ cm^2 mol^{-1}) and $[Cu(\mathbf{1\text{-}BB})][ClO_4]_2$,[24] carrying the same chelate ring type as the **L-55** complexes ($\Lambda_M = 287\ \Omega^{-1}$ cm^2 mol^{-1}), further confirm the 1:2 electrolyte type of the catecholate dianion complexes. It has been impossible to obtain these catecholate complexes in crystalline forms suitable for X-ray analysis. Therefore, their current characterization is based on their spectral properties.

The spectral properties of the adducts of Cat^{2-} are different from those of the adducts with $CatH^-$; this is shown in Figure 4 for the adducts derived from $[Cu_2(\mathbf{L\text{-}55})]^{4+}$. In particular, the near-UV spectrum of the $Cu(II)_2-Cat^{2-}$ adducts is characterized by a pronounced absorption feature near 340 nm and a broader absorption near 430 nm, both probably due to catecholate to Cu(II) charge transfer (CT), that are absent in the spectra of either the $Cu(II)_2-CatH^-$ or $Cu(II)-CatH^-$ adducts. In both the latter cases, a less-defined shoulder centered near 300 nm spans the near-UV region. Interestingly, this spectral feature is very similar to that exhibited by the adducts (prepared *in situ*) from $[Cu_2(\mathbf{L\text{-}55})]^{4+}$, $[Cu_2(\mathbf{L\text{-}66})]^{4+}$, or $[Cu(\mathbf{L\text{-}6})]^{2+}$ and the phenolate ion derived from **1**. The poor definition of these CT absorption features seems a characteristic of phenoxo-bridged dicopper(II) complexes.[11] On the basis of these spectral similarities, we propose that in the $[Cu_2(\mathbf{L\text{-}55})(CatH)]^{3+}$ adduct the anionic ligand binds essentially as a μ-phenolate, while in the $[Cu_2(\mathbf{L\text{-}55})(Cat)]^{2+}$ and $[Cu_2(\mathbf{L\text{-}66})(Cat)]^{2+}$ adducts it is bridging through both the oxygen atoms, as is found in the structure of the complex $[Cu_2(L-O^-)(Cl_4C_6O_2)]^+$ reported by Karlin et al.[27] The EPR spectra of the present dinuclear complexes are less informative. Although there are some differences between the spectra of the $Cu(II)_2$, $Cu(II)_2-CatH^-$, and $Cu(II)_2-Cat^{2-}$ complexes, all of them are characterized by broad signals, with unresolved or poorly resolved hyperfine structure, that likely result from coupling interactions between the copper(II) centers. It is important to note that the product isolated from the oxygenation of **1** by $[Cu_2(\mathbf{L\text{-}66})][ClO_4]_2$ corresponds to the catechol adduct formulated as $[Cu_2(\mathbf{L\text{-}66})(Cat)][ClO_4]_2$.

Low-Temperature Spectral Studies. Figure 5 shows a series of absorption spectra taken during the initial phase of the oxygenation of the adduct between $[Cu_2(\mathbf{L\text{-}66})]^{2+}$ and the phenolate ion derived from **1** in dry acetonitrile at about −35 °C. The dicopper(I)−phenolate CT band at 310 nm decreases in intensity with the progress of the reaction and is replaced by the CT band corresponding to dicopper(II)−catecholate dianion at lower energy. No absorption feature attributable to Cu_2-O_2 species[9e,28,29] is observable at that temperature, although the absence of an isosbestic point indicates that a third species, besides the dicopper(I)−phenolate and dicopper(II)−catecholate, must be present in solution. This experiment is also important in that it clearly shows that no spectral feature attributable to the *o*-quinone species **3**, in the 400-nm region, is observable in this initial phase of the reaction. A similar conclusion could be reached from analogous experiments carried out using $[Cu_2(\mathbf{L\text{-}56})]^{2+}$ and $[Cu_2(\mathbf{L\text{-}55})]^{2+}$.

Biological Relevance and Conclusion. The results obtained in these reactivity studies indicate that the dicopper(I) complexes derived from polybenzimidazole ligands are able to mediate the regiospecific *ortho*-hydroxylation of exogenous phenols in the presence of dioxygen, and therefore they can be considered as functional models of tyrosinase. Employing the electronically deactivated phenol **1**, the oxidation reaction stops at the level of the dicopper(II)−catecholate complex, because this is relatively stable with respect to the intramolecular electron transfer leading to quinone **3** and dicopper(I) complex. How-

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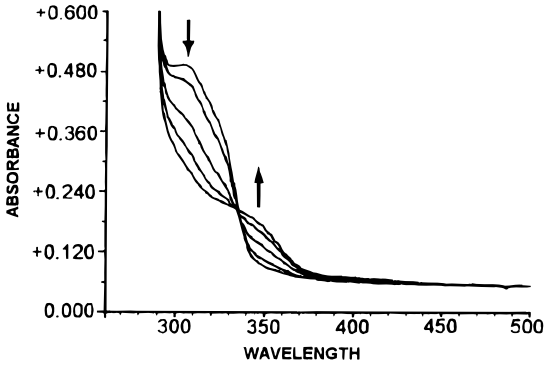
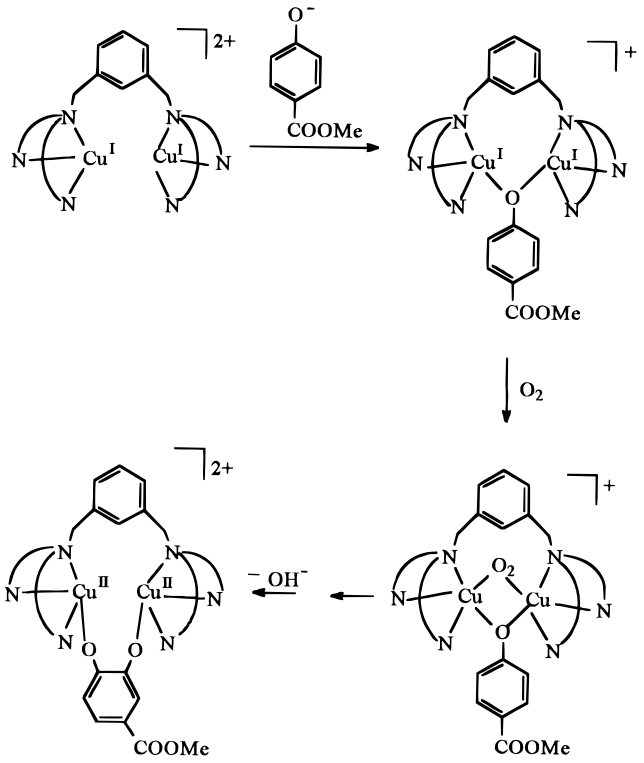


Figure 5. Representative spectra recorded during the initial phase reaction between $[Cu_2(\mathbf{L\text{-}66})][ClO_4]_2$, the sodium salt of methyl 4-hydroxybenzoate (1 mol equiv), and dioxygen (1 atm) at about −35 °C in dry acetonitrile.

Scheme 3



ever, the initial product of monooxygenation can undergo a Michael addition reaction by nucleophiles, such as the phenolate anion, through some mechanism that is currently under study.

With the information available on the copper(I)−phenolate and copper(II)−catecholate adducts, and the low-temperature spectral studies, a proposal for the mechanism of this biomimetic reaction can be formulated (Scheme 3). A central aspect of this proposal is the binding of the phenolate substrate as a bridging ligand between the copper(I) atoms of the dinuclear complex (**6**). Such a disposition of the ligand is unfavorable not only for the mononuclear complex $[Cu(\mathbf{L\text{-}6})]^+$ but also for other biomimetic systems based on mononuclear copper(I) complexes such as those containing the trispyrazolylborate units described by Kitajima et al.[9e,30] and the tris(2-pyridylmethyl)-amine ligand studied by Karlin et al.,[31] which form dioxygen complexes at low temperature but do not perform, or very poorly perform, *ortho*-hydroxylation reactions on exogenous phenols. Exposure of the dicopper(I)−phenolate to dioxygen leads to a likely transient species containing both the phenolate and dioxygen bound to the metal centers. In this intermediate the attack of bound dioxygen to the activated substrate will be facilitated. The initial monooxygenation product rapidly evolves to a bridging catechol dianion species. The loss of hydroxide in the final product is probably facilitated by the dinegative charge of the catecholate ligand and by some steric difficulty in maintaining a second monoatomic bridge. In any case, the formulation of this compound is indicated by the composition of the product actually isolated from the reaction of $[Cu_2(\mathbf{L\text{-}66})]^{2+}$.

The mechanism outlined in Scheme 3 is slightly different from other mechanisms proposed for tyrosinase. In the mechanism proposed by Solomon et al.[6,22a] binding of the phenolate to one copper center of the enzyme causes a distortion in oxygen binding, resulting in electrophilic attack at the *ortho* position of the ring by the more positive oxygen atom of the coordinated dioxygen residue. The resulting catechol is then oxidized to the *o*-quinone, and the dicopper(I) site combines with another oxygen molecule, completing the catalytic cycle. This model has been recently refined by Conrad et al.,[22b] with the proposal that phenol binding to oxytyrosinase is accompanied by proton transfer to dioxygen, producing a bound hydroperoxide, which is the actual electrophilic species. Attack of this species on the phenol ring and heterolytic cleavage of the oxygen−oxygen bond produces the catechol and a bound hydroxide ion. A similar proton transfer from phenol to bound dioxygen has been proposed also by Kitajima and Moro-oka,[9e] but they assume that both the Cu−O(phenolate) and Cu−OOH bonds undergo homolytic cleavage, producing a phenoxo radical and $HOO^{\bullet}$, which couple to give a hydroperoxybenzoquinone, and then *o*-quinone. The high selectivity of the enzymatic reaction, though, seems incompatible with a free radical type reaction, and in fact in the arene hydroxylation system studied by Karlin et al.[10,11] the electrophilic species (which is the bound peroxide) is suitably oriented for stereospecific p-π attack to the aromatic ring of the ligand along the direction of its empty σ^* orbital. No necessity for a hydroperoxide intermediate is apparent either here at this stage, where it is assumed that an attack by bound peroxide occurs on the $\pi-\pi$ system of the exogenous phenolate. The possibility of bridging this substrate between the copper centers may actually favor its appropriate positioning with respect to peroxide. This arrangement of the substrate has not been considered for tyrosinase because it is assumed that steric control exerted by the folding of the protein around the active site imposes differential reactivity and accessibility to the two copper ions. Further insight into the mechanism of the present phenol *ortho*-hydroxylation may at least clarify whether the proposed binding mode of the substrate has any advantage in terms of the kinetics or selectivity of the reaction. Our continuing efforts are directed toward the full characterization of the present biomimetic monooxygenase system. We emphasize that no studies of this type have been performed before on the Cu-mediated oxygenation of external phenols.

Experimental Section

Materials and Instrumentation. Reagents and solvents from commercial sources were reagent quality unless otherwise noted. Acetonitrile (spectral grade) was distilled from potassium permanganate and dry potassium carbonate; then, it was stored over calcium hydride and distilled prior to use under an inert atmosphere. Dimethylformamide was refluxed under vacuum over barium oxide to remove dimethylamine, stored over calcium hydride, and distilled under reduced pressure before use. Toluene and diethyl ether were dried by refluxing

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(31) Paul, P. P.; Tyeklár, Z.; Jacobson, R. R.; Karlin, K. D. *J. Am. Chem. Soc.* **1991**, *113*, 5322.

and distillation from metallic sodium. Argon was carefully deoxygenated with hot BASF R3-11 catalysts. Dioxygen was dried by passing through a column of 3-Å molecular sieves. Preparation and handling of air-sensitive copper(I) complexes or moisture sensitive anhydrous solvents and solutions were carried out under an argon atmosphere using standard Schlenk techniques. The synthesis of *N*,*N*-bis[2-(1-methyl-2-benzimidazolyl)ethyl]amine,[17] 2-chloromethyl-1-methylbenzimidazole,[16] 1-methyl-2-(2-aminoethyl)benzimidazole,[12d] the ligands **L-66** [16] and **L-55** [17] and their corresponding dicopper(I) complexes, $[Cu_2(\mathbf{L\text{-}66})][ClO_4]_2$ [17] and $[Cu_2(\mathbf{L\text{-}55})][ClO_4]_2$,[16] and dicopper(II) complexes, $[Cu_2(\mathbf{L\text{-}66})][ClO_4]_4$ and $[Cu_2(\mathbf{L\text{-}55})][ClO_4]_4$,[16] and those of $[Cu(\mathbf{L\text{-}6})][ClO_4]$ and $[Cu(\mathbf{L\text{-}6})][ClO_4]_2$ [24] are reported elsewhere.

Methyl 4-hydroxybenzoate (**1**) and methyl 3,4-dihydroxybenzoate (**2**) were prepared by esterification of the acids (5 g) in dry methanol (100 mL) in the presence of concentrated sulfuric acid (1 mL). The mixtures were heated at reflux temperature overnight, then they were cooled, concentrated under vacuum, neutralized with aqueous sodium bicarbonate, and extracted with dichloromethane. Drying and evaporation under reduced pressure of the organic extracts afforded the esters. For methyl 4-hydroxybenzate. IR (Nujol mull, cm^{-1}): 3300 s, 1684 s, 1607 s, 1591 s, 1516 m, 1435 m, 1317 s, 1282 s, 1236 m, 1195 m, 1166 m, 1123 m, 1108 m, 957 m, 851 m, 772 s, 698 m. ^{1}H NMR ($CDCl_3$): δ 3.88 (s, 3H, CH_3), 6.8−7.0 (m, 2H) and 7.8−8.1 (m, 2H) (Ph−H). Anal. Calcd for $C_8H_8O_3$: C, 63.14; H, 5.31. Found: C, 63.28; H, 5.26. For methyl 3,4-dihydroxybenzoate. IR (Nujol mull, cm^{-1}): 3470 m, 3268 m, 1690 s, 1613 s, 1535 w, 1412 w, 1296 s, 1270 w, 1247 m, 1187 m, 1166 w, 1100 m, 986 m, 911 m, 882 w, 824 vw, 783 w, 766 m, 719 w. ^{1}H NMR (CD_3OD): δ 3.83 (s, 3H, CH_3), 6.7−6.9 (m, 2H) and 7.3−7.5 (m, 3H) (Ph−H). Anal. Calcd for $C_8H_8O_4$: C, 57.14; H, 4.80. Found: C, 57.16; H, 4.71. Methyl 2-[4-(carbomethoxy)phenoxy]-3,4-dihydroxybenzoate (**4**) was prepared according to the literature.[18] The sodium salt of methyl 4-hydroxybenzoate was obtained as a moisture-sensitive light gray powder by reaction of the phenol with sodium metal in dry toluene at reflux temperature under an inert atmosphere. Tetra-*n*-butylammonium 4-(carbomethoxy)phenolate was prepared immediately prior to use from methanolic tetra-*n*-butylammonium hydroxide and **1** under an inert atmosphere in anhydrous methanol, followed by evaporation to dryness.

Elemental analyses were performed at the microanalytical laboratory of the Chemistry Department in Milan. NMR spectra were recorded on Bruker WP-80 or AC-200 spectrometers operating at 80 and 200 MHz, respectively. EPR spectra were measured in frozen solutions using a Varian E-109 spectrometer operating at X-band frequencies. Optical spectra were measured on Perkin Elmer Lambda 5 or HP 8452A diode array spectrophotometer. Infrared spectra were recorded on a Jasco FT-IR 5000 instrument. Conductivity measurements were performed with an Amel 160 conductivity meter at 25 °C using 0.5×10^{-3} M acetonitrile solutions of the complexes. Analyses of the mixtures resulting from oxygenation of **1** were performed with a Perkin-Elmer Series 3B HPLC equipped with an LC-85 UV detector using a Merk LiChrosorb RP-18 column (244 × 4 mm i.d.). The detector was set at 258 nm, and the eluent was a solution of phosphate buffer (pH 3.4)−methanol (70/30, v/v). 4-Hydroxybenzoic acid was used as the standard for quantitative analysis. With a flow rate of 1.2 mL/min the retention times were as follows: 4-hydroxybenzoic acid, 7 min; methyl 3,4-dihydroxybenzoate, 12 min; methyl 4-hydroxybenzoate, 23 min; methyl 2-[4-(carbomethoxy)phenoxy]-3,4-dihydroxybenzoate (**4**), 125 min.

Caution! *Perchlorate salts of metal complexes with organic ligands are potentially explosive and should be handled with great care. Only small amounts of material should be prepared. The complexes described in this report have been found to be safe when used in small quantities.*

Ligand Syntheses. α,α′-Bis{bis[2-(1-methyl-2-benzimidazolyl)-ethyl]amino}-*m*-xylene. The ligands **L-56** and **L-5p6** were synthesized from a common diamine intermediate α,α′-bis{bis[2-(1-methyl-2-benzimidazolyl)ethyl]amino}-*m*-xylene, resulting from reduction of a diimine derived from benzene-1,3-dicarbaldehyde. This Schiff base was prepared as follows. The free amine 1-methyl-2-(2-aminoethyl)-benzimidazole was obtained from its dihydrochloride salt (12.2 mmol) by treatment with the stoichiometric amount of 1 M methanolic sodium hydroxide in methanol (10 mL). The mixture was evaporated to dryness under vacuum, treated with dichloromethane (100 mL), and filtered. The filtrate was evaporated to dryness, and the free amine was dissolved in dry methanol (50 mL). Benzene-1,3-dicarbaldehyde (5.6 mmol) was added, and the solution was refluxed for a few hours. Evaporation afforded a yellow oil that was treated with a small amount of dichloromethane and diethyl ether until a white precipitate formed. Cooling in a refrigerator completed precipitation of the Schiff base product, which was collected by filtration and dried under vacuum (yield > 90%). ^{1}H NMR ($CDCl_3$): δ 3.32 (t, 4H, C−CH_2−Bim), 3.75 (s, 6H, CH_3−N), 4.18 (t, 4H, =N−CH_2−C), 7.1−8.0 (m, 12H, Ph−H + Bim−H), 8.32 (s, 2H, CH=N). Anal. Calcd for $C_{28}H_{30}N_6$: C, 75.00; H, 6.25; N, 18.75. Found: C, 74.82; H, 6.55; N, 18.42.

Reduction of the Schiff base (1.1 mmol) was carried out in methanol (50 mL) by adding an excess of sodium borohydride in small portions while the mixture was cooled in an ice bath. The mixture was first warmed gently and then slowly brought to reflux temperature. Heating was continued for approximately 1 h; then the solution was cooled and the solvent removed by evaporation under vacuum. The residue was treated with a small amount of water and extracted several times with 5-mL portions of dichloromethane. The combined organic extracts were dried ($MgSO_4$), filtered, concentrated to a small volume, and then treated with diethyl ether until precipitation began. Precipitation of the diamine α,α′-bis{bis[2-(1-methyl-2-benzimidazolyl)ethyl]amino}-*m*-xylene as a white powder was completed by cooling in a refrigerator. The product was collected by filtration and dried under vacuum (yield, 70%). ^{1}H NMR ($CDCl_3$): δ 2.20 (s, 2H, NH), 3.13 (m, A_2B_2 system, 8H, N−CH_2CH_2−Bim), 3.65 (s, 6H, CH_3−N), 3.82 (s, 4H, xylyl CH_2), 7.1−7.4 (m, 10H, Ph−H + Bim−H), 7.6−7.8 (m, 2H, benzimidazolyl 7-CH). Anal. Calcd for $C_{28}H_{32}N_6$: C, 74.34; H, 7.08; N, 18.58. Found: C, 73.86; H, 7.50; N, 18.43.

α,α′-Bis{[(1-methyl-2-benzimidazolyl)methyl][2-(1-methyl-2-benzimidazolyl)ethyl]amino}-*m*-xylene (L-56). A mixture of α,α′-bis{bis[2-(1-methyl-2-benzimidazolyl)ethyl]amino}-*m*-xylene (3.0 mmol), 2-(chloromethyl)-1-methylbenzimidazole (6.12 mmol), anhydrous sodium carbonate (20 mmol), and dry dimethylformamide (100 mL) was slowly heated to reflux under an inert atmosphere. Heating was maintained for about 8 h; then the mixture was cooled to room temperature and evaporated to dryness under vacuum. The residue was treated several times with ethanol and evaporated to dryness to remove traces of dimethylformamide. Finally, the residue was treated with dichloromethane and the inorganic salts were filtered off. Evaporation of the solvent yielded a brown oil that was dissolved in the minimum amount of chloroform and chromatographed on silica gel using a mixture of chloroform−ethanol (5:1, v/v) as eluent. The main fraction was collected, and the solvent was removed under vacuum. The crude oily product was further purified by precipitation as the oxalate salt from a solution of acetone. The free ligand was obtained as a light brown oil by treatment of the oxalate salt with methanolic sodium hydroxide, removal of the solvent, treatment with dichloromethane, filtration, and evaporation of the solvent (yield ≈ 30%). ^{1}H NMR ($CDCl_3$): δ 3.15 (m, A_2B_2 system, 8H, N−CH_2CH_2−Bim), 3.27 (s, 6H, CH_3−N), 3.35 (s, 6H, CH_3−N), 3.73 (s, 4H, xylyl CH_2), 3.90 (s, 4H, N−CH_2Bim), 7.1−7.5 (m, 16H, Ph−H + Bim−H), 7.6−7.8 (m, 4H, benzimidazolyl 7-CH). This oil was used in the preparation of the dicopper(I) complex.

α,α′-Bis{[(2-pyridyl)methyl][2-(1-methyl-2-benzimidazolyl)ethyl]-amino}-*m*-xylene (L-5p6). A mixture of α,α′-bis{bis[2-(1-methyl-2-benzimidazolyl)ethyl]amino}-*m*-xylene (0.82 mmol), 2-(chloromethyl)-pyridine hydrochloride (1.96 mmol), anhydrous sodium carbonate (1.5 g), and dry dimethylformamide (80 mL) was slowly heated to reflux under an inert atmosphere. Heating was maintained for 6 h; then the mixture was cooled and evaporated to dryness under vacuum. The residue was treated several times with ethanol and taken again to dryness. It was then treated with chloroform, and the inorganic salts were filtered off. The solution was concentrated to a small volume and chromatographed on alumina using dichloromethane−methanol (5:1, v/v) as eluent. The main fraction was collected and evaporated under vacuum giving a brown oil (yield ≈ 40%). ^{1}H NMR ($CDCl_3$): δ 3.10 (m, A_2B_2 system, 8H, N−CH_2CH_2−Bim), 3.44 (s, 6H, CH_3−N), 3.69 (s, 4H, xylyl−CH_2), 3.84 (s, 4H, N−CH_2−Py), 7.0−7.8 (m, 16H,

Ph—H + Bim—H + Py—H), 8.48 (d, 2H, pyridyl 6-CH). This oil was used for the preparation of the dicopper(I) complex.

Copper(I) Complexes. The dicopper(I) hexafluorophosphate complexes of **L-66** and **L-55** were prepared from $[Cu(CH_3CN)_4][PF_6]$ following the procedure described to obtain the corresponding perchlorate complexes.[16,17] For $[Cu_2(\mathbf{L\text{-}66})][PF_6]_2$. Anal. Calcd for $C_{48}H_{52}N_{10}Cu_2P_2F_{12}$: C, 48.64; H, 4.43; N, 11.82. Found: C, 48.44; H, 4.56; N, 11.63. For $[Cu_2(\mathbf{L\text{-}55})][PF_6]_2$. Anal. Calcd for $C_{44}H_{44}N_{10}Cu_2P_2F_{12}$: C, 46.80; H, 3.93; N, 12.41. Found: C, 46.83; H, 3.98; N, 12.34.

$[Cu_2(L\text{-}56)][ClO_4]_2$. To a solution of the ligand **L-56** (0.30 mmol) in degassed methanol (30 mL) was added solid $[Cu(CH_3CN)_4][ClO_4]$ (0.65 mmol) under stirring. The light yellow precipitate that immediately formed was left under stirring for 0.5 h. Then it was filtered, washed with small portions of deaerated methanol and diethyl ether, and dried under vacuum. Anal. Calcd for $C_{46}H_{48}N_{10}Cu_2Cl_2O_8$: C, 51.78; H, 4.53; N, 13.13; Cu, 11.91. Found: C, 51.66; H, 4.63; N, 12.83; Cu, 11.8.

$[Cu_2(L\text{-}5p6)][ClO_4]_2$. To a degassed solution of the ligand L-5p6 (0.80 mmol) in methanol (30 mL) was added solid $[Cu(CH_3CN)_4][ClO_4]$ (1.7 mmol) under stirring. The light yellow precipitate thus formed was left under stirring for an additional 0.5 h; then it was collected by filtration, washed with small amounts of deaerated methanol and diethyl ether, and dried under vacuum. Anal. Calcd for $C_{40}H_{42}N_8Cu_2Cl_2O_8$: C, 50.00; H, 4.40; N, 11.67; Cu, 13.23. Found: C, 49.63; H, 4.23; N, 11.41; Cu, 13.2.

Copper(II) Complexes. [Cu(L-6)(CatH)][ClO$_4$]. This adduct was prepared by adding methyl 3,4-dihydroxybenzoate (0.17 mmol) and then 1 M methanolic sodium hydroxide (0.17 mmol) to a solution of $[Cu(\mathbf{L\text{-}6})][ClO_4]_2 \cdot H_2O$ (0.17 mmol) in the minimum amount of acetonitrile—methanol (1:1, v/v). The resulting dark green solution was left standing for a few hours at room temperature and then overnight in a refrigerator. The dark green precipitate thus formed was filtered, washed with small amount of acetonitrile—methanol, and dried under vacuum at room temperature. IR (Nujol mull, cm^{-1}): 3290 m, 1710 s, 1613 m, 1485 m, 1415 m, 1328 m, 1296 m, 1236 m, 1154 w, 1095 vs, 1017 w, 1002 w, 936 w, 919 w, 851 w, 822 w, 764 s, 750 s, 623 s. UV—vis (CH_3CN; λ_{max}, nm (ϵ, M^{-1} cm^{-1})): 218 (23 000), 254 (20 500), 274 (20 300), 282 (19 200), 320 sh (6700), 390 sh (3700), 500 sh (1300), 670 sh (550), 840 (300). Anal. Calcd for $C_{28}H_{30}N_5CuClO_8$: C, 50.67; H, 4.57; N, 10.55. Found: C, 50.90; H, 4.33; N, 10.85.

$[Cu_2(L\text{-}55)(CatH)][ClO_4]_3 \cdot H_2O$. The complex $[Cu_2(\mathbf{L\text{-}55})][ClO_4]_4 \cdot 6H_2O$ (0.07 mmol) was dissolved in the minimum amount of acetonitrile—methanol (2:1, v/v); then methyl 3,4-dihydroxybenzoate (0.07 mmol) was added under stirring. No color change in the blue-green solution was observed. Upon addition of ~1 M methanolic sodium hydroxide (0.07 mmol) the color of the solution turned to emerald green. Diethyl ether was slowly added, dropwise, under stirring up to the initial appearance of turbidity. The mixture was left in a refrigerator for a few hours, and the dark green precipitate was collected by filtration, washed with small amounts of methanol and water, and dried under vacuum. IR (Nujol mull, cm^{-1}): 3485 s, 1711 m, 1618 m, 1595 w, 1539 m, 1504 m, 1487 m, 1328 m, 1296 s, 1249 m, 1110 vs, 1011 w, 940 m, 857 w, 797 w, 748 s, 721 w, 706 w, 623 s. UV—vis (CH_3CN; λ_{max}, nm (ϵ, M^{-1} cm^{-1})): 220 (45 500), 248 (33 600), 274 (34 900), 282 (33 300), 310 sh (6200), 400 sh (1900), 470 sh (1100), 638 (980). Anal. Calcd for $C_{52}H_{53}N_{10}Cu_2Cl_3O_{17}$: C, 47.18; H, 4.04; N, 10.57. Found: C, 46.85; H, 3.90; N, 10.28.

$[Cu_2(L\text{-}55)(Cat)][ClO_4]_2 \cdot H_2O$. This adduct was prepared by following the same procedure employed for $[Cu_2(\mathbf{L\text{-}55})(CatH)][ClO_4]_3$ but using 2 equiv of sodium hydroxide/mol of methyl 3,4-dihydroxybenzoate. From the brownish green solution obtained on mixing of the reagents a dark brown precipitate formed on standing for several hours in a refrigerator. This was collected by filtration, washed with small amounts of methanol, and dried under vacuum. The crude adduct was purified by chromatography on Sephadex LH-20 (20 × 1 cm) using acetonitrile as eluent. The green band eluted was collected and evaporated to dryness under reduced pressure. IR (Nujol mull, cm^{-1}): 3585 m, 1705 m, 1620 m, 1595 w, 1504 m, 1485 w, 1330 m, 1296 m, 1280 sh, 1249 w, 1095 s, 1009 m, 934 m, 874 w, 750 s, 706 w, 623 s. UV—vis (CH_3CN; λ_{max}, nm (ϵ, M^{-1} cm^{-1})): 220 (45 000), 252 (34 300), 274 (36 600), 282 (35 900), 340 (8000), 470 sh (1300), 660 sh (550). Anal. Calcd for $C_{52}H_{52}N_{10}Cu_2Cl_2O_{13}$: C, 51.06; H, 4.29; N, 11.44. Found: C, 50.68; H, 4.18; N, 11.50.

$[Cu_2(L\text{-}66)(Cat)][ClO_4]_2 \cdot H_2O$. The complex $[Cu_2(\mathbf{L\text{-}66})][ClO_4]_4 \cdot 6H_2O$ (0.035 mmol) was previously treated twice with dry acetonitrile and taken to dryness to eliminate the crystallization water as much as possible. The residue was then dissolved in dry CH_3CN (10 mL) and treated with a solution in dry methanol (1 mL) of the sodium catecholate derived from **2** (0.034 mmol), thus obtaining a dark green solution that was kept under stirring. After a few minutes, the presence of a small amount of grey precipitate could be noted. After filtration, the analytical and IR data of this precipitate indicate it is the dihydroxy derivative of the dinuclear copper(II) complex, $[Cu_2(\mathbf{L\text{-}66})(OH)_2][ClO_4]_2$ [16] (Anal. Calcd for $C_{48}H_{54}N_{10}Cl_2Cu_2O_{10} \cdot 2H_2O$: C, 49.48; H, 5.02; N, 12.02. Found: C, 49.40; H, 4.78; N, 11.92). The filtrate was treated with diethyl ether to precipitate the crude adduct. This was dissolved in the minimum amount of acetonitrile and chromatographed on a Sephadex LH-20 column (20 × 1 cm) using CH_3CN as eluent. The dark green band eluted was collected and evaporated to dryness under reduced pressure. Anal. Calcd for $C_{56}H_{58}N_{10}Cu_2Cl_2O_{12} \cdot H_2O$: C, 52.57; H, 4.73; N, 10.94. Found: C, 51.98; H, 4.55; N, 10.80. IR (Nujol mull, cm^{-1}): 3532 m, 1715 m, 1618 m, 1595 vw, 1504 m, 1485 m, 1460 s, 1379 m, 1334 m, 1285 m, 1243 w, 1094 s, 1013 m, 932 w, 748 s, 623 s. UV—vis (CH_3CN; λ_{max}, nm (ϵ, M^{-1} cm^{-1})): 252 (36 400), 274 (38 400), 280 (34 400), 345 (8300), 445 sh (2600), 650 sh (1000).

Synthesis of Methyl 3,4-Dihydroxybenzoate by Phenol Hydroxylation in the Presence of Copper(I) Complexes (Preparative Conditions). To a solution or suspension of the dicopper(I) complex (0.1 mmol) in dry, deaerated acetonitrile (50 mL) was added methyl 4-hydroxybenzoate (0.1 mmol) followed by sodium borohydride (0.1 mmol) under an inert atmosphere. The mixture was vigorously stirred for 2 h at room temperature and then exposed to dioxygen (1 atm) for an additional 2 h. The solvent was evaporated under reduced pressure, and the residue was treated with 2 M hydrochloric acid (1 mL) and rapidly extracted several times with 10-mL portions of dichloromethane. The combined organic extracts were washed with a small amount of water, dried over magnesium sulfate, and concentrated to a small volume. The separation of the catechol from unreacted phenol was carried out by chromatography on a silica gel column (10 × 1 cm) or preparative silica gel TLC plates using a dichloromethane—methanol mixture (96:4, v/v) as eluent. The product methyl 3,4-dihydroxybenzoate was characterized by NMR, MS, IR, and UV spectroscopy by comparison with an authentic sample of the catechol. Yields are reported in Table 1.

Phenol Hydroxylation Mediated by Copper(I) Complexes and Dioxygen (HPLC Analysis). To a solution of the hexafluorophosphate salt of the dicopper(I) complex (0.02 mmol) in dry, degassed acetonitrile (50 mL) was added via gas-tight syringe a 0.036 M solution of tetra-*n*-butylammonium 4-(carbomethoxy)phenolate (0.02 mmol) in dry, degassed acetonitrile. The light yellow solution was exposed to dioxygen (1 atm) for about 2 h, during which time the color of the solution turned to light or dark green. Then the solution was evaporated to dryness and the residue treated with dichloromethane (5 mL) and 2 M H_2SO_4 (5 mL). The organic phase was separated, and the aqueous phase was extracted twice with small amounts of dichloromethane. The combined organic extracts were dried ($MgSO_4$) and evaporated to dryness under vacuum. The residue was dissolved in 10 mL of methanol. Part of this solution, after the addition of a known amount of 4-hydroxybenzoic acid as the standard, was analyzed immediately by HPLC. The recovery of **1, 2,** and **4** is summarized in Table 2. The remaining portion of the solution was taken to dryness and used to check the ratio between **1** and **2** + **4** in the product mixture by NMR. Regarding the data reported in Table 2, it should be noted that the actual recovery of organic material corresponds to 75—80% of the mmol of phenol initially reacted. The figures reported in the table are referred to 100% recovery since no other product besides **1**, **2**, and **4** was present in the mixture.

The reactions studied at low temperature were performed similarly by using jacketed vessels with Schlenk connections and external circulation of the coolant (methanol). Cooling was provided by a Haake K cryostat (minimum temperature reachable −45 °C) equipped with a pump for external circulation of the coolant. All of the operations up

to the final treatment with aqueous acid were performed at the given temperature (Table 2). The reaction time maintained in the low-temperature experiments was as indicated in Table 2.

Isolation of the Catechol Adduct from the Oxygenation of Methyl 4-Hydroxybenzoate by $[Cu_2$(L-66)$][ClO_4]_2$. A solution of methyl 4-hydroxybenzoate (0.1 mmol) in dry, degassed acetonitrile (20 mL) was treated with sodium borohydride (0.1 mmol) under stirring at room temperature. After stirring for 0.5 h, the ligand **L-66** (0.1 mmol) and tetrakis(acetonitrile)copper(I) perchlorate (0.2 mmol) were added. The mixture was left under stirring for about 2 h; then dioxygen (1 atm) was admitted and allowed to react for 2 h. The solid material was filtered off and the dark green solution was concentrated to about half-volume under vacuum. The concentrated solution was loaded onto a Sephadex LH-20 column (20 × 1 cm) previously equilibrated with acetonitrile and eluted with the same solvent. The major dark green band eluted was collected and evaporated to dryness. Anal. Calcd for $C_{56}H_{58}N_{10}Cu_2Cl_2O_{12}$: C, 53.33; H, 4.63; N, 11.11. Found: C, 53.89; H, 4.87; N, 11.32. The IR and UV spectra of this product are identical to those of $[Cu_2$(**L-66**)(Cat)$][ClO_4]_2$.

Low-Temperature Spectral Studies. Low-temperature absorption spectra recorded during the oxygenation reaction were obtained at −35 °C using a 1-cm quartz cell equipped with cooling jacket and Schlenk connections. Cooling was provided by a Haake K cryostat as described before. Fogging of the outer quartz windows was prevented by application of a flow of precooled nitrogen gas. The optical cell was loaded under an inert atmosphere with the solution containing the copper(I) phenolate complex using a syringe. After the temperature was equilibrated a spectrum of the solution was taken; then, dioxygen gas was admitted through a gas-tight syringe, and a series of spectra were taken at short time intervals. The fast data collection of the diode array spectrometer allowed good operation of the system over the limited time allowed to follow the experiment before fogging of the windows became unavoidable.

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Supporting Information Available: Figure of the proton NMR spectra of the adduct between the dicopper(I) complex of 1,3-bis[bis-(2-pyridylmethyl)amino]benzene and 1 equiv of sodium 4-(carbomethoxy)phenolate recorded at 288 and 248 K (2 pages). Ordering information is given on any masthead page.

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