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Reaction of a Copper(II)−Nitrosyl Complex with Hydrogen Peroxide: Phenol Ring Nitration through a Putative Peroxynitrite Intermediate

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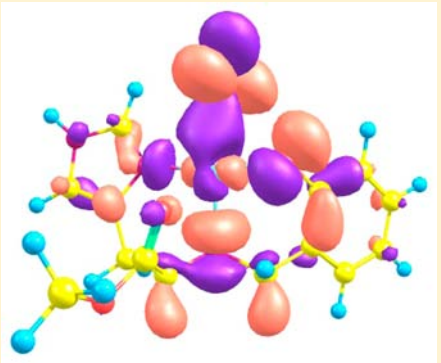
Supporting Information

ABSTRACT: Copper(II) complex, **1**, with the histidine-derived ligand **L** (**L** = methyl 2-(2-hydroxybenzylamino)-3-(1*H*-imidazol-5-yl)propanoate) has been synthesized and characterized. Single-crystal structure determination reveals a diphenolato-bridged dicopper(II) core in **1**. Addition of •NO to an acetonitrile solution of **1** affords the corresponding mononuclear copper(II)−nitrosyl complex, **2**. In the presence of H_2O_2, **2** results in formation of the corresponding copper(I)−peroxynitrite. Formation of peroxynitrite ($^-$OONO) intermediate is evident from its characteristic phenol ring nitration reaction which resembles the tyrosine nitration in biological systems. Further, isolation of nitrate (NO_3^-) as the decomposition product from **2** at room temperature also supports the involvement of $^-$OONO intermediate.



INTRODUCTION

Reactive nitrogen species (RNS) constitute a major class of intermediates involved in oxidative reactions in biological systems.[1] When produced at low or moderate concentrations, they stimulate signal transduction, but in a higher concentration, they can induce oxidative damage of DNA, lipids, and proteins.[2] Tyrosine nitration by RNS has attracted considerable research interest as it can alter protein functions and be useful as a diagnostic biomarker for cardiovascular, Alzheimer's, and Perkinson's diseases.[3] It is well known that tyrosine nitration occurs either by peroxynitrite ($^-$OONO) or by $^•NO_2$.[4] Peroxynitrite ($^-$OONO) is known to generate in vivo by a diffusion control reaction between •NO and superoxide (O_2^-) anion.[5] The presence of 3-nitrotyrosine in biological fluids indicates that peroxynitrite is capable of nitrating tyrosine in the presence of a Lewis acid like Cu^{2+} or Fe^{3+} and metalloproteins such as SOD.

Endogeneous generation of ($^-$OONO) and its cytotoxicity is an attractive field of research; it should be noted that evidence of the presence of $^-$OONO in biological systems is indirect.[6−8] For instance, it has been suggested that •NO generation from activated macrophages can be quantitatively converted to $^-$OONO. Thus, exclusive generation of NO_3^- is also expected from activated macrophages as the decomposition product of $^-$OONO. However, studies indicate that the activated macrophages generate NO_2^- to a significant level, which is the primary decomposition product of •NO after reaction with dioxygen (O_2) in aerobic condition.[9,10] On the other hand, Nathan et al. reported enhancement of cytotoxicity associated with the activated macrophages upon addition of SOD and blocked by addition of H_2O_2 scavenging enzyme.[11] However, the role of transition metal ions in generation, stabilization, and activation for substrate oxidation and thermal isomerization of $^-$OONO is well documented in the literature.[12] Heme proteins and their models have been studied extensively in this direction, but examples involving copper ions in the generation and reactivity of $^-$OONO are limited.[12−14] The known few include kinetic studies of $^-$OONO with copper salts and copper complex-mediated decomposition.[13,14]

Examples of discrete metal−peroxynitrite complex are rare; only a cobalt−peroxynitrite is known to date to be characterized structurally.[15] Recently, in small-molecule models we have shown that copper(II)−nitrosyl complex reacts with H_2O_2 to generate Cu(I)−peroxynitrite intermediate.[16] Herein, we report the reaction of copper(II)−nitrosyl intermediate with H_2O_2 to result in the corresponding Cu(I)−peroxynitrite, and this induces phenol ring nitration. For the present study we prepared a histidine-based ligand **L** (**L** = methyl 2-(2-hydroxybenzylamino)-3-(1*H*-imidazol-5-yl)propanoate) with a phenolic group (Figure 1). Introduction of the phenol ring to the ligand framework provides the internal substrate for ring nitration.

RESULTS AND DISCUSSION

Ligand **L** (**L** = methyl 2-(2-hydroxybenzylamino)-3-(1*H*-imidazol-5-yl)propanoate) was synthesized from reaction of L-histidine methyl ester dihydrochloride and salicylaldehyde in the presence of lithium hydroxide (LiOH) followed by reduction of intermediate imine by $NaBH_4$ (Experimental

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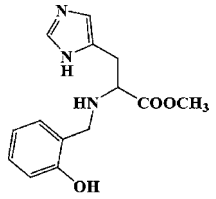

(L)

Figure 1. Ligand used for the present study.

Section). The ligand has been characterized by various spectroscopic techniques (Experimental Section). The copper(II) complex, **1**, was synthesized from reaction of copper(II) perchlorate hexahydrate with an equivalent amount of ligand **L** in acetonitrile. It has been characterized by various analytical techniques (Experimental Section). The single-crystal structure of the complex was determined. The perspective ORTEP view is shown in Figure 2. The crystallographic data, important bond

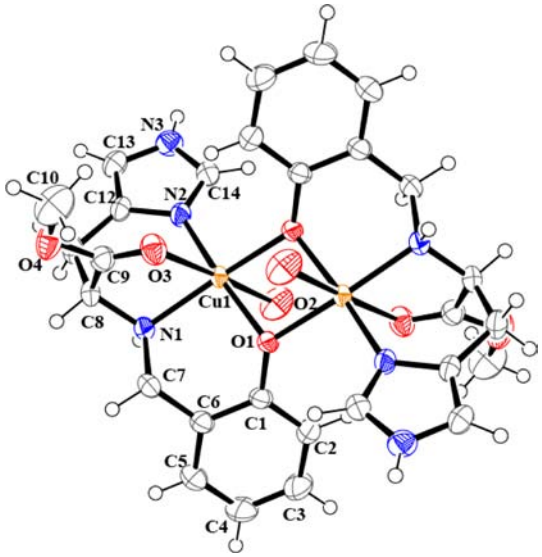

Figure 2. ORTEP diagram of complex **1** (solvent molecules and perchlorate are removed for clarity; 50% thermal ellipsoid plot).

lengths, and important bond angles are given in Tables 1, 2, and 3, respectively. The crystal structure reveals that complex **1** is a diphenolato-bridged dicopper(II) system. Two nitrogen-donor atoms from the ligand and two oxygen atoms from two bridging phenolato groups result in a distorted square planar coordination geometry around each copper(II) center. The fifth coordination site is occupied by a carbonyl oxygen from the ester group present in the ligand framework. The octahedral coordination of each copper(II) center is completed by the solvent water molecule. The Cu−$O_{(carbonyl)}$ and Cu−$O_{(water)}$ distances, 2.532(2) and 2.539(2) Å, respectively, are within the range of reported distances.[17] The average Cu−N distances in complex **1**, Cu−N1/Cu−N2 = 2.029(2)/1.957(2) Å, are also in the range observed in the reported complexes.[17] The phenolato oxygen atoms are coordinated to the copper(II) center through the equatorial position at an average distance of 1.965(1) Å, which is in the range observed for other equatorial Cu−$O_{(phenolato)}$ distances.[18] The Cu−$O_{(phenolato)}$−Cu angle is ∼100.5°. It should be noted that this was observed in the range of 91−104° in earlier reported compounds.[17,18] The two

Table 1. Crystallographic Data for Complex 1

formula	$C_{17}H_{24}ClCuN_4O_9$
MW	542
cryst syst	triclinic
space group	*P*-1
temp./K	293(2)
wavelength /Å	0.71073
a/Å	9.8228(2)
b/Å	10.2640(2)
c/Å	11.3496(2)
α/deg	89.6260(10)
β/deg	82.1540(10)
γ/deg	76.9530(10)
V/ Å^3	1103.96(4)
Z	2
density/Mgm^{-3}	1.584
abs coeff/mm^{-1}	1.166
abs correction	none
F(000)	542
total no. of reflns	5436
reflns, $I > 2\sigma(I)$	4770
max 2*θ*/deg	28.37
ranges (*h*, *k*, *l*)	$-13 \le h \le 13$
	$-13 \le k \le 13$
	$-15 \le l \le 15$
complete to 2*θ* (%)	98.3
refinement method	full-matrix least-squares on F^2
Goof (F^2)	0.927
R indices [$I > 2\sigma(I)$]	0.0480
R indices (all data)	0.0527

Table 2. Selected Bond Lengths (Angstroms) for Complex 1

Cu(1)−N(1)	2.029(2)
Cu(1)−N(2)	1.957(2)
Cu(1)−O(1)	1.965(1)
Cu(1)−O(2)	2.539(2)
Cu(1)−O(3)	2.532(2)
C(2)−C(1)	1.395(3)
C(4)−C(5)	1.385(4)
C(6)−C(7)	1.498(3)
C(1)−O(1)	1.356(3)
C(7)−N(1)	1.493(3)
C(8)−N(1)	1.480(2)
C(9)−O(3)	1.207(3)
C(9)−O(4)	1.326(3)
C(10)−O(4)	1.453(5)
C(12)−N(2)	1.392(3)
C(13)−N(3)	1.369(3)

Table 3. Selected Bond Angles (degrees) for Complex 1

N(1)−Cu(1)−N(2)	90.44(8)
N(1)−Cu(1)−O(1)	93.35(8)
N(1)−Cu(1)−O(2)	103.72(8)
N(1)−Cu(1)−O(3)	73.48(7)
O(2)−Cu(1)−O(3)	176.72(8)
O(1)−Cu(1)−N(2)	171.16(8)
C(1)−C(2)−C(3)	120.2(3)
C(1)−C(6)−C(7)	119.0(2)
C(7)−N(1)−C(8)	110.8(2)
C(11)−C(12)−C(13)	127.5(2)
Cu(1)−N(2)−C(14)	125.9(2)

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copper(II) centers are separated by 3.03 Å. This is comparable to the values for other reported complexes by Thompson et al. (e.g., ranging from 2.997 to 3.1184 Å) and Ray et al.[19] The C–$O_{(phenolato)}$ distance, 1.356(3) Å, is very close to the C–O single-bond distance, indicating the phenolato character of the bridging oxygen centers.[20]

Complex **1** in acetonitrile solvent exhibits a broad d–d band at λ_{max} (ε/ M^{-1} cm^{-1}), 660 nm (244), along with relatively strong intraligand absorptions in the UV region (Figure 3). The

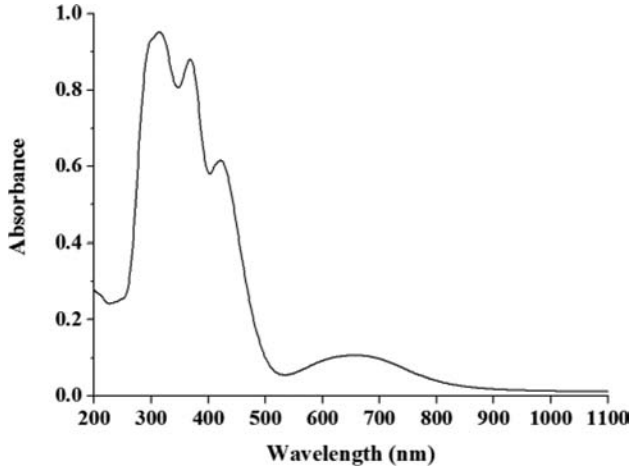

Figure 3. UV–vis spectrum of complex **1** in acetonitrile solution at room temperature.

phenolato–copper(II) charge transfer transition appears at 421 nm. The acetonitrile solution of complex **1** was found to be silent in X-band EPR studies (Supporting Information). This is attributed to the antiferromagnetic coupling of the two paramagnetic copper(II) centers through phenlato bridges. It is further supported by the very low resultant magnetic moment of the solid complex **1** (Experimental Section).

Nitric Oxide Reactivity. Purging of •NO to a degassed acetonitrile solution of complex **1** resulted in a darkening in color. In UV–vis spectroscopy, the d–d band (λ_{max}, 660 nm) of complex **1** is blue shifted with λ_{max} at 645 nm (Figure 4). This has been attributed to formation of the corresponding mononuclear [Cu^{II}–NO] complex, **2**. In cases of [Cu^{II}–NO]

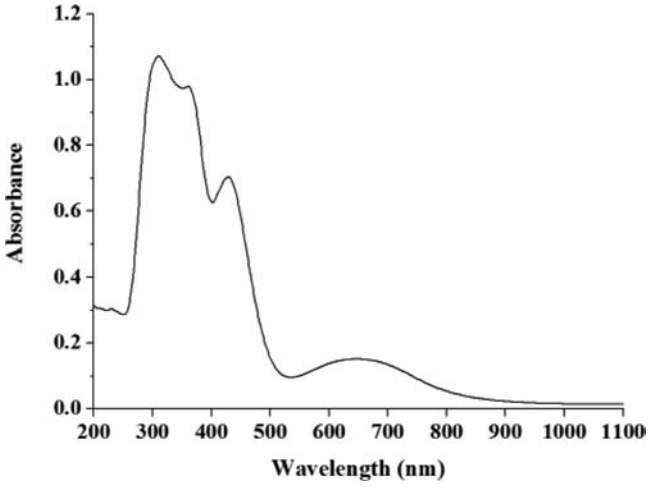

Figure 4. UV–vis spectrum of complex **2** in acetonitrile solution at room temperature.

complexes in other reports the d–d band appeared at this range only.[16,21,22] Because of the thermal instability and moisture sensitivity, complex **2** could not be isolated as a solid. It should be noted that in an acetonitrile solution of $[Cu(bemim)_2]^{2+}$ {bemim = bis(2-ethyl-4-methylimidazole-5-yl)methane} addition of •NO resulted in the corresponding [Cu^{II}–NO] complex which was isolated as a solid and characterized.[16] In addition, recently a number of examples of unstable [Cu^{II}–NO] intermediate have been reported in the reaction of Cu^{II} complexes with •NO. On the other hand, the structurally characterized [Cu^{II}–NO] complex was prepared by reaction of the corresponding Cu^{I} complex with $NOBF_4$.[23] Studies on solution FT-IR, X-band EPR, and ESI-mass spectroscopy support its formulation as the corresponding mononuclear [Cu^{II}–NO] intermediate complex. It is found to be EPR silent.

In the FT-IR spectrum it exhibits a vibration at 1846 cm^{-1}, assigned as the coordinated nitrosyl stretching frequency (Figure 5).[16,21–24] The frequency of this vibration was found

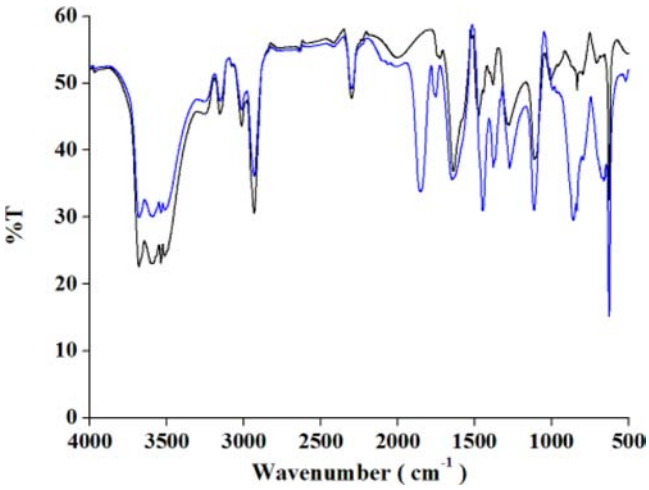

Figure 5. Solution FT-IR spectra of complexes **1** (black) and **2** (blue) in acetonitrile solvent at room temperature.

to shift to 1815 cm^{-1} in the ^{15}NO-labeling experiment, which further confirms its assignment.[16] In case of the solid isolated [Cu^{II}–NO], this frequency was reported to appear at 1662 cm^{-1}.[16] For $[Cu(TAEA)(CH_3CN)]^{2+}$ [TAEA = tris(2-aminoethyl)amine] complex, ν_{NO} of [Cu^{II}–NO] was found to appear at 1650 cm^{-1}.[24a] In an early report on the air-stable solid copper–nitrosyl of copper(II)–dithiocarbamate, ν_{NO} for the nitrosyl coordinated to copper appears at 1682 cm^{-1}.[24c] Hayton et al. reported the appearance of a ν_{NO} band at 1933 cm^{-1} for copper(II)–nitrosyl.[23] Application of vacuum to the acetonitrile solution of complex **2** was found to result in a decrease of the ν_{NO} band intensity in FT-IR spectrum, indicating loss of •NO ligand from the complex (Supporting Information).[22,23] This intermediate complex **2** was found to be stable in solution under nitrogen atmosphere for a few hours.

The observed mass of complex **2** in acetonitrile solution was found to corroborate with the mononuclear unit rather than the dinuclear dinitrosyl (Supporting Information).

DFT calculations were performed to get some insight on the optimized structure of complex **2** in acetonitrile. The solvent-phase-optimized geometry of complex **2** is shown in Figure 6. The calculation suggests a distorted square pyramidal geometry for complex **2** with the nitrosyl group coordinated to the metal through an equatorial position.

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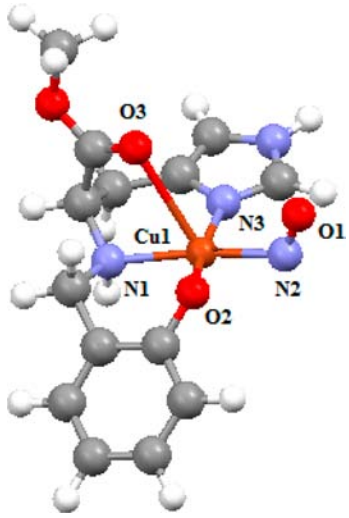

Figure 6. DFT-optimized structure of complex **2**.

The HOMO and LUMO of the complex are shown in Figure 7. It has been found that the HOMO is formed by the p orbitals

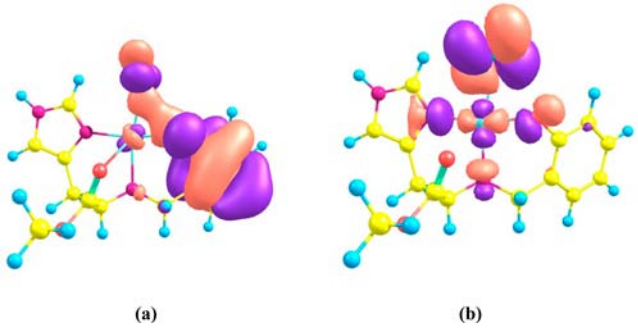

Figure 7. (a) HOMO and (b) LUMO of complex **2**.

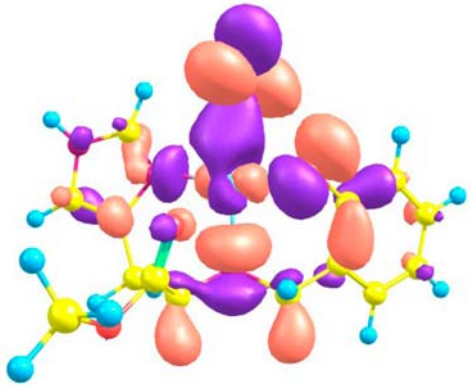

Figure 8. HOMO-1 showing the Cu–NO bond.

of the carbon atoms of the benzene ring, O1, O2, and N2 atoms, and the d orbital of Cu. The LUMO of the complex is formed by the p orbitals of O1, O2, N1, N2, and N3 and the d orbital of Cu. NBO calculations were performed on the optimized structure using GAUSSIAN 09 software with the B3LYP functional and 6-311+g(d,p) basis set. This calculation shows that the copper atom in the complex has a d^9 configuration, indicating its oxidation state is +2 ([core]-4S(0.31)3d(9.49)4p(0.30)4d(0.01). Furthermore, NBO analysis predicts that the bond between Cu and N of NO is due to d–p mixing (d 64.21%, p 35.79%).

The calculated Cu1–N1, Cu1–N2, Cu1–N3, Cu1–O2, Cu1–O3, and N2–O1 bond lengths of complex **2** are 2.090, 1.989, 1.987, 1.968, 2.792, and 1.168 Å, respectively, and the Cu1–N2–O1 bond angle is 117.8°, which is comparable to that observed in the structurally characterized complex.[23]

In our earlier study on copper(II)–nitrosyl intermediates having $\{CuNO\}^{10}$ electronic configuration it has been observed that the coordinated nitrosyl groups are electrophilic in nature owing to the $[Cu^{II}–NO \leftrightarrow Cu^{I}–NO^{+}]$ configuration. Taube et al. suggested that reaction of NO^+ with H_2O_2 can lead to formation of peroxynitrite.[25] This has been indeed supported by our earlier report of the reaction of copper(II)–nitrosyl complex with H_2O_2 which induces reduction of the copper(II) center with simultaneous formation of peroxynitrite intermediate.[16] As experimental and theoretical studies indicate a $[Cu^{II}–NO]$ configuration for complex **2**, it would be logical to anticipate similar reactivity with H_2O_2.

From a freshly generated complex **2** excess $^{\bullet}NO$ was removed by purging argon gas, and the solution was cooled to −20 °C. To this cold solution stoichiometric addition of precooled H_2O_2 was found to turn it into a colorless solution. This has been attributed to formation of the corresponding copper(I)–peroxynitrite complex. The diminished intensity of the d–d transition band of complex **2** having λ_{max} at 645 nm upon addition of H_2O_2 suggests reduction of copper(II) (Figure 9).

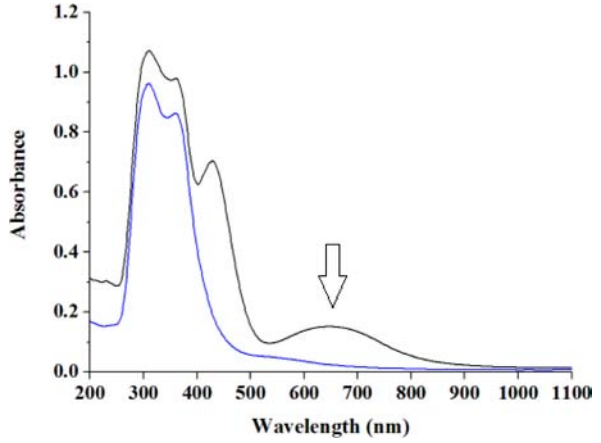

Figure 9. UV–vis spectra of complex **2** (black) and after reaction with H_2O_2 (blue) in acetonitrile solvent.

The ν_{NO} band at ∼1846 cm^{-1} in solution FT-IR of complex **2** diminished upon addition of H_2O_2 with the appearance of NO_3^- stretching at ∼1384 cm^{-1} (Supporting Information). It would be worth mentioning here that the very short lived peroxynitrite ion (approximately 1 s in physiological condition) decomposes spontaneously to give nitrate (NO_3^-).[6a] Earlier, a similar observation was noticed with $[Cu(bemim)_2]^{2+}$.[16] The colorless solution, when allowed to stay in the presence of air, it became green. FT-IR spectral analysis of the crude product indicates the presence of nitrate (NO_3^-). The amount of nitrate present in the reaction mixture was determined to ∼45% (Supporting Information). The ligand **L** undergoes phenol ring nitration to yield **L′** (yield ≈ 40%). These essentially suggest formation of Cu(I)–peroxynitrite intermediate in the course of the reaction (Scheme 1).[16] About 50% of the ligand **L** was

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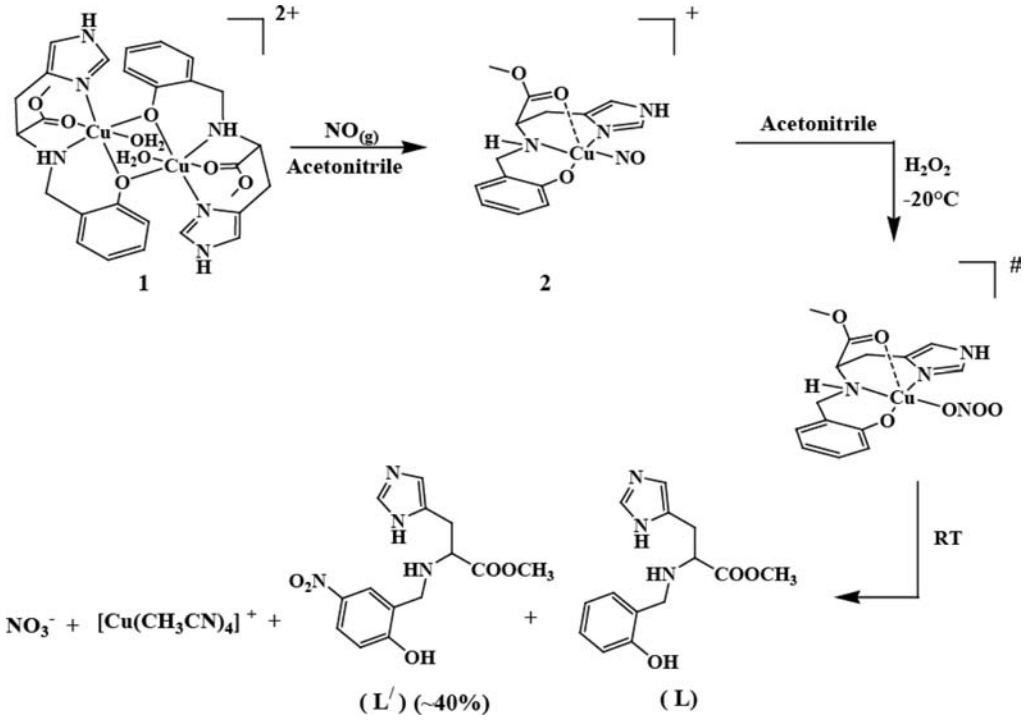
Scheme 1



isolated unreacted. It should be noted that we isolated only the para nitration product from the reaction mixture; however, the possibility of formation of ortho nitration cannot be ruled out. In the present case, perhaps the ortho nitration is the minor product and formed in trace amounts.

Though we have not studied the kinetics of the reaction, addition of 2,4-di-*tert*-butylphenol in the reaction mixture at −20 °C was found to result in the corresponding nitration product, 2,4-di-*tert*-butyl-6-nitrophenol (yield ≈ 10%) along with **L**′. This suggests that the reaction proceeds through an intermolecular pathway.

Addition of H_2O_2 to the acetonitrile solution of freshly generated complex **2** at room temperature resulted in the corresponding nitrate product only; no phenol ring nitration was observed at room temperature.

It should be noted that addition of H_2O_2 to the parent copper(II) complex, **1**, followed by $^{\bullet}NO$ purging was not observed to result in the same reaction. Thus, the appearance of the NO_3^- stretching frequency in the FT-IR spectrum and phenol ring nitrosation indicate the presence of copper(I)−peroxynitrite complex in the colorless solution formed in the reaction of complex **2** with H_2O_2. It should be noted that $^{\bullet}NO$ was reported to react with alkaline H_2O_2 in the absence of O_2 to give $^{-}OONO$.[26] However, direct addition of $^{\bullet}NO$ to degassed alkaline solutions of H_2O_2 has not been found to result in $^{-}OONO$.[26] In addition, at room temperature the thermal reaction between $^{\bullet}NO$ and H_2O_2 occurs very slowly in neutral solutions, but it was quite fast at pH 12.[26] It was suggested that since $^{\bullet}NO$ is not a nitrosating agent, formation of $^{-}OONO$ by reaction of $^{\bullet}NO$ and H_2O_2 probably requires O_2 and proceeds through nitrosating intermediates that are formed during autoxidation of $^{\bullet}NO$; for example, they observed formation of $^{-}OONO$ in the reaction of N_2O_3 and hydroperoxo anion (OH^-).[27]

Recently, both copper(II) and copper(II)−peptide complexes were reported to catalyze the tyrosine nitration in the presence of NO_2^- and H_2O_2.[28] This copper-mediated tyrosine nitration has been explained by a mechanism considering generation of hydroxyl radicals ($^{\bullet}OH$) and/or copper(II)-bound $^{\bullet}OH$ (Cu^{2+}−$^{\bullet}OH$) from Cu^{2+} and H_2O_2 through a Fenton-like reaction.[28] These radicals may be scavenged by both NO_2^- to form $^{\bullet}NO_2$ and tyrosine to form tyrosine radicals ($Tyr^{\bullet}$), resulting in tyrosine nitration. Cu^{2+}/H_2O_2 was also found to catalyze the tyrosine nitration induced by $^{\bullet}NO$ and O_2. $^{\bullet}NO$ was oxidized by O_2 to form $^{\bullet}NO_2$, and the role of Cu^{2+}/H_2O_2 was to generate $^{\bullet}OH/Cu^{2+}$−$^{\bullet}OH$ to promote $Tyr^{\bullet}$ formation.[28]

It is not very clear that whether the reaction of H_2O_2 (i) takes place directly to the electrophillic $^{\bullet}NO$ center or (ii) first reacts with the copper(II) center followed by $^{\bullet}NO$. Since addition of H_2O_2 to complex **1** followed by $^{\bullet}NO$ purging did not result in reduction of copper(II) or $^{-}OONO$ formation; presumably, the first option is most likely to take place. It should be noted that when a stoichiometric amount of potassium superoxide is added into the precooled solution of complex **2**, no reduction of copper(II) center was observed, though formation of the $^{-}OONO$ intermediate complex was evidenced from phenol ring nitrosation of the ligand.

It should be noted that in proteins nitration of tyrosine residues to 3-nitrotyrosine is a oxidative post-translational modification process and that affects $^{\bullet}NO$ signaling. 3-Nitrotyrosine is established as a biomarker for cell, tissue, and systematic nitroxidative stress. Peroxynitrite has been identified as the potential nitrating agent for tyrosine. Thus, identification of protein tyrosine nitration as a mediator in alteration of cell or tissue homeostasis can have relevant biological importance, and prevention of protein tyrosine nitration may lead to inhibiting or delaying various diseases states. A large experimental effort, in both proteins and models systems, is needed to see how tyrosine nitration happens intra- or extracellularly by either proteolytic or repair systems. Studies in small-molecule models may provide a sound basis to further

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investigate the mechanism of protein tyrosine nitration which may influence the disease states associated with disruption of •NO and redox metabolism.

CONCLUSION

Thus, the present work describes formation of copper(I)−peroxynitrite through reaction of $[Cu^{II}-NO]$ complex and H_2O_2. This mechanism is clearly different than what was proposed by Girault et al. for the Cu^{2+}/H_2O_2-mediated tyrosine nitration in the presence of NO_2^-. The ^-OONO intermediate was found to induce nitration at the phenol ring present in the ligand framework which resembles tyrosine nitration in biological systems. Thus, this work supports the possibility of the occurrence of decomposition of both H_2O_2 as well as •NO formed in the biological systems which has been proposed in the case of $[Cu(bemim)_2]^{2+}$ complex.

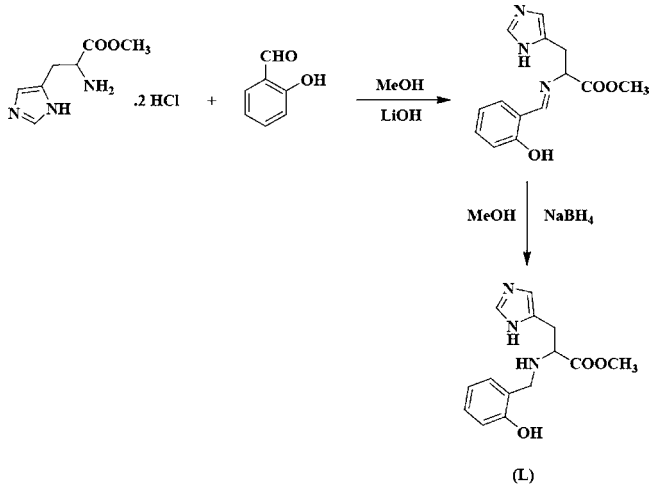
EXPERIMENTAL SECTION

Materials and Methods. All reagents and solvents were purchased from commercial sources and of reagent grade. Acetonitrile was distilled from calcium hydride. Deoxygenation of the solvent and solutions were effected by repeated vacuum/purge cycles or bubbling with nitrogen or argon for 30 min. •NO gas was purified by passing through KOH and P_2O_5 column. UV−vis spectra were recorded on a Perkin-Elmer Lamda 25 UV−visible spectrophotometer. FT-IR spectra were taken on a Perkin-Elmer spectrophotometer with either sample prepared as KBr pellets or in solution in a potassium bromide cell. Solution electrical conductivity was checked using a Systronic 305 conductivity bridge. ^{1}H NMR spectra were obtained with a 400 MHz Varian FT spectrometer. Chemical shifts (ppm) were referenced either with an internal standard (Me_4Si) for organic compounds or to the residual solvent peaks. X-band electron paramagnetic resonance (EPR) spectra of the complexes and reaction mixtures were recorded on a JES-FA200 ESR spectrometer. The magnetic moment of the complexes was measured on a Cambridge Magnetic Balance. Mass spectra of the compounds were recorded in a Waters Q-Tof Premier and Aquity instrument. Single crystals were grown by slow diffusion followed by the slow evaporation technique. Intensity data were collected using a Bruker SMART APEX-II CCD diffractometer equipped with a fine-focus 1.75 kW sealed tube Mo Kα radiation (λ = 0.71073 Å) at 273(3) K with increasing ω (width of 0.3°/frame) at a scan speed of 3 s/frame. The SMART software was used for data acquisition.[29] Data integration and reduction was undertaken with SAINT and XPREP software. Structures were solved by direct methods using SHELXS-97 and refined with full-matrix least-squares on F^2 using SHELXL-97.[30] All non-hydrogen atoms were refined anisotropically. Structural illustrations have been drawn with ORTEP-3 for Windows.[31]

For the DFT studies, the initial structure of the cupper(II)−nitrosyl complex was generated from available experimental data. The complex was fully optimized using the BLYP functional and DNP basis sets in the gas phase as well as in the presence of acetonitrile solvent. The Conductor-like Screening Model (COSMO) as incorporated into the DMol^3 program with a dielectric constant of 37.5 was adopted to study the solvent effect.[32]

Synthesis of Ligand L. To a solution of L-histidine methyl ester dihydrochloride (2.421 g, 10 mmol) in 50 mL of methanol was added lithium hydroxide monohydrate (0.84 g, 20 mmol) into a 100 mL round-bottom flask equipped with a magnetic stirring bar, Scheme 2. To this solution salicylaldehyde (1.22 g, 10 mmol) was added dropwise with constant stirring. The reaction mixture was then allowed to stir at room temperature for 5 h. The resulting solution was then reduced by $NaBH_4$ (0.95 g, 25 mmol). Removal of solvent under reduced pressure affords a crude mass. It was dissolved in water (50 mL) and neutralized by addition of dilute acetic acid and then extracted with chloroform (50 m × 4 portions).

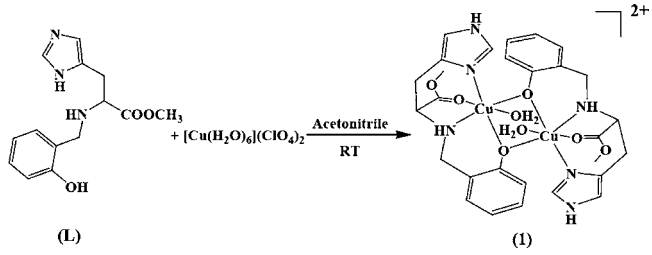
Scheme 2



Chloroform extract was dried under reduced pressure, and the oil thus obtained was subjected to chromatographic purification using a silica gel column to yield the pure ligand **L** as a yellow oil. Yield: 2.21 g (80%). Analy. Calcd for $C_{14}H_{17}N_3O_3$: C, 61.08; H, 6.22; N, 15.26. Found: C, 61.03; H, 6.27; N, 15.21. FT-IR (in KBr): 1730, 1654, 1455, 12154, 754 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ_{ppm}: 7.50 (s, 1H), 7.13−7.09 (t, 1H), 6.94−6.92 (d, 1H), 6.78 (s, 4H), 6.76−6.73 (t, 1H), 6.71 (d, 1H), 3.98−3.95 (d, 2H), 3.68 (s, 4H), 3.04−2.86 (d, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_{ppm}: 30.6, 50.7, 52.3, 60.2, 116.5, 116.8, 119.5, 123.0, 129.1, 133.8, 135.5, 157.5, and 174.0. ESI-Mass (m + 1): calcd 276.13; found 276.12.

Synthesis of Complex 1, $[Cu_2(L)_2(H_2O)_2](ClO_4)_2$. Copper(II) perchlorate hexahydrate (1.482 g, 4 mmol) was dissolved in 20 mL of acetonitrile. To this solution, 1.101 g (4 mmol) of the ligand **L** was added slowly with constant stirring, Scheme 3. The color of the

Scheme 3



solution turned green from light blue. Stirring was continued for 1 h at room temperature. The volume of the solution then reduced to ∼5 mL. To this 10 mL of benzene was added to make a layer on it and kept overnight in a freezer. This resulted in microcrystalline complex **1**. Yield: 1.55 g (∼85%). FT-IR (in KBr): 2936, 1721, 1630, 1599, 1485, 1263, 1118, 625 cm^{-1}. Molar conductivity, 275 S cm^2 mol^{-1}. μ_{obs}, 0.30 μ_B

Isolation of L′. To 20 mL of a distilled and degassed acetonitrile solution of complex **1** (0.5 g), freshly prepared •NO was bubbled for 1 min. The color of the solution turned dark green. The excess of •NO was removed by purging argon gas, and the solution was cooled to −20 °C. To this cold solution, precooled H_2O_2 (70% v/v; 0.05 mL) was added and the solution turned colorless. The reaction mixture was then warmed to room temperature and dried under reduced pressure. Water (5 mL) was added to the dried mass followed by addition of 5 mL of saturated Na_2S solution. The black precipitate of CuS was filtered out. The crude organic part was then extracted from the aqueous layer using $CHCl_3$ (25 mL × 4 portions). The crude product, obtained after removal of solvent, was then purified by column chromatography using a neutral alumina column and hexane/ethyl

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acetate solvent mixture to get the pure modified ligand **L′**. Yield: 0.14 g (~ 40%). Anal. Calcd for $C_{14}H_{16}N_4O_5$: C, 52.50; H, 5.03; N, 17.49. Found: C, 52.48; H, 5.08; N, 17.44. FT-IR (in KBr): 2967, 1630, 1545, 1510, 1431, 1340, 1269, 1203, 853 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ_{ppm}: 7.68−66 (d, 1H), 7.45 (s, 1H), 6.96−6.95 (d, 1H), 6.86 (s, 1H), 6.05 (s, 1H), 3.98−3.95 (d, 2H), 3.72 (s, 4H), 3.05−2.89 (d, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) $\delta_{ppm:}$ 174.9, 162.2, 141.8, 135.7, 134.0, 125.0, 120.8, 120.3, 116.8, 60.7, 52.8, 50.9, 30.8. ESI-Mass (m + H^+)/z: calcd 321.11; found 321.13.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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