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Crystal Structure and Superoxide Dismutase Activity of [Cu(ethylenediamine)$_2$Cl][PF$_6$]

J. R. Anacona[1,*], **Carmen Gutierrez**[1], and **Cecilia Rodriguez-Barbarin**[2]

[1] Departamento de Química, Universidad de Oriente, Cumana, Venezuela
[2] Facultad de Ciencias Químicas, Universidad Autónoma de Nuevo León,
Monterrey, México

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Summary. The preparation, spectroscopic properties, and crystal structure of chlorobis(ethylenediamine)copper(II) hexafluorophosphate [Cu(*en*)$_2$Cl][PF$_6$], (*en* = ethylendiamine) are reported. The complex crystallizes in the monoclinic system, space group P2$_1$/c, with cell constants $a = 6.1488(9)$ Å, $b = 12.696(2)$ Å, $c = 17.7424(17)$ Å, $\beta = 97.265(12)°$, and $Z = 4$. The copper(II) ion is coordinated to two bidentate *en* molecules, to one chlorine ion, and to a more distant fluorine atom of the PF$_6$ group, leaving the copper ion in a distorted octahedral coordination geometry. The superoxide dismutase mimetic activity of the complex was investigated using the indirect xanthine-xanthine oxidase-nitroblue tetrazolium method and compared to that of the native enzyme.

Keywords. Copper; Superoxide dismutase activity.

Introduction

The reactive superoxide radical anion is a product of the oxygen metabolic cycle. Superoxide dismutase (Cu$_2$Zn$_2$SOD) is an enzyme that catalyzes the disproportionation of toxic superoxide ions into molecular oxygen and H$_2$O$_2$ [1, 2]. The mechanism, believed to be operating in the Cu$_2$Zn$_2$SOD, involves one-electron reduction of the copper(II) active site to copper(I) by superoxide followed by reoxidation of the reduced metal ion to copper(II) by a second superoxide anion. It is known that the site containing copper(II) is tetrahedrally distorted [3] and this peculiar geometry in which copper is inserted facilitates the reduction to copper(I) which is known to prefer tetrahedral sites [4]. Thus, a copper(II) complex which can be considered to possess Cu$_2$Zn$_2$SOD-like activity must fulfill some requirements: a) a flexible arrangement of the ligands around copper(II) ions to allow a facile reduction to copper(I), b) an accessible site in order to easily add the superoxide radical, c) an equatorial field of medium strength because strong ones could

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[*] Corresponding author. E-mail: juananacona@hotmail.com

not favor the attack of O_2^- to the accessible apical sites. As copper has been proven to be the active metal center in the best studied Cu_2Zn_2SOD, many cuprous complexes have been synthesized and tested for Cu_2Zn_2SOD-like activity [5–15], and most of them have appeared to be very efficient. We report here the preparation of the title complex which was obtained as a by product when attempting to form mixed ligand copper complexes containing imidazole and estimate its scavenger activity.

Results and Discussion

The copper(II) complex is coloured and appears to be air-stable and soluble in methanol. Elemental analyses agree well with stoichiometry. The experimental conductivity value in methanol falls in the 2:1 electrolyte range suggesting tetra-coordination around copper(II) ion in solution.

The complex has a magnetic moment of 1.98 BM at room temperature and lies in the range associated with d^9 systems with one unpaired electron. The rather high value of the magnetic moment for the freshly-prepared complex can be explained, in part, by the fact that spin-orbital coupling in the ion can mix the ground state representing no orbital momentum with higher levels of identical multiplicity, resulting in a small orbital contribution [16], however, the presence of impurities cannot be ignored.

The EPR spectrum of the copper(II) complex at room temperature showed a single broad signal with poor resolution of the hyperfine structure on both sides of the main signal. The calculated parallel and perpendicular tensors, $g_{\|} = 2.15$ and $g_{\perp} = 2.07$, indicate [17] that the unpaired electron most likely resides in the $d_x{}^2 - y^2$ orbital having $^2B_{1g}$ as a ground state term. The axial symmetry parameter, $G = 2.14$, obtained by the relation $(g_{\|} - 2)/(g_{\perp} - 2)$, supports [18] the contention that there is a fairly exchange interaction between the copper centres ($G < 4$).

The IR spectrum reveals the absorptions expected for the structure. A strong band at $\bar{\nu} = 840\,\mathrm{cm}^{-1}$, attributable to PF_6 stretching vibrations, and the characteristic bands for the NH_2 moiety in the $\bar{\nu} = 3390 - 3270\,\mathrm{cm}^{-1}$ range can also be assigned.

The UV-Vis spectrum of the complex in methanol solution presents two absorption maxima at $\lambda = 235$ and 285 nm, presumably due to intraligand excitation. The copper(II) ion has a d^9 electronic configuration and originates only one spectral term in electronic spectra, ^2D. Therefore, a single band in the electronic spectra should be expected for d^9 configuration, in fact, electronic spectrum of the copper(II) complex in solution presents only one broad band centered at $\lambda = 560$ nm.

An ORTEP drawing representation, including the atomic numbering scheme is given in Figure 1. The molecular interactions in the crystal extended along the *a* axis can be seen in Figure 2. Selected bond lengths and angles are listed in Table 1. The asymmetric unit comprises a monocationic complex of [Cu(*en*)$_2$Cl] and a PF_6 counter ion.

The copper(II) ion is coordinated to two bidentate diamine molecules [Cu–N ranging from 1.992 to 2.021 Å], to one chlorine ion [Cu(1)–Cl(1) 2.597 Å], and to a more distant fluorine atom of the PF_6 group [Cu(1)$\cdots$F(2) 3.016 Å], leaving the copper(II) ion in a distorted octahedral coordination geometry [rms planar

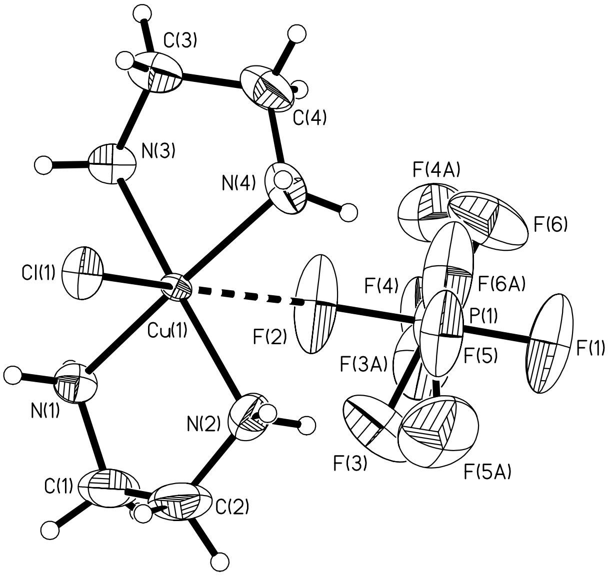

Fig. 1. A molecular representation of the [Cu(en)$_2$Cl][PF$_6$] complex with the atomic labeling scheme shown; thermal ellipsoids are at the 30% probability level

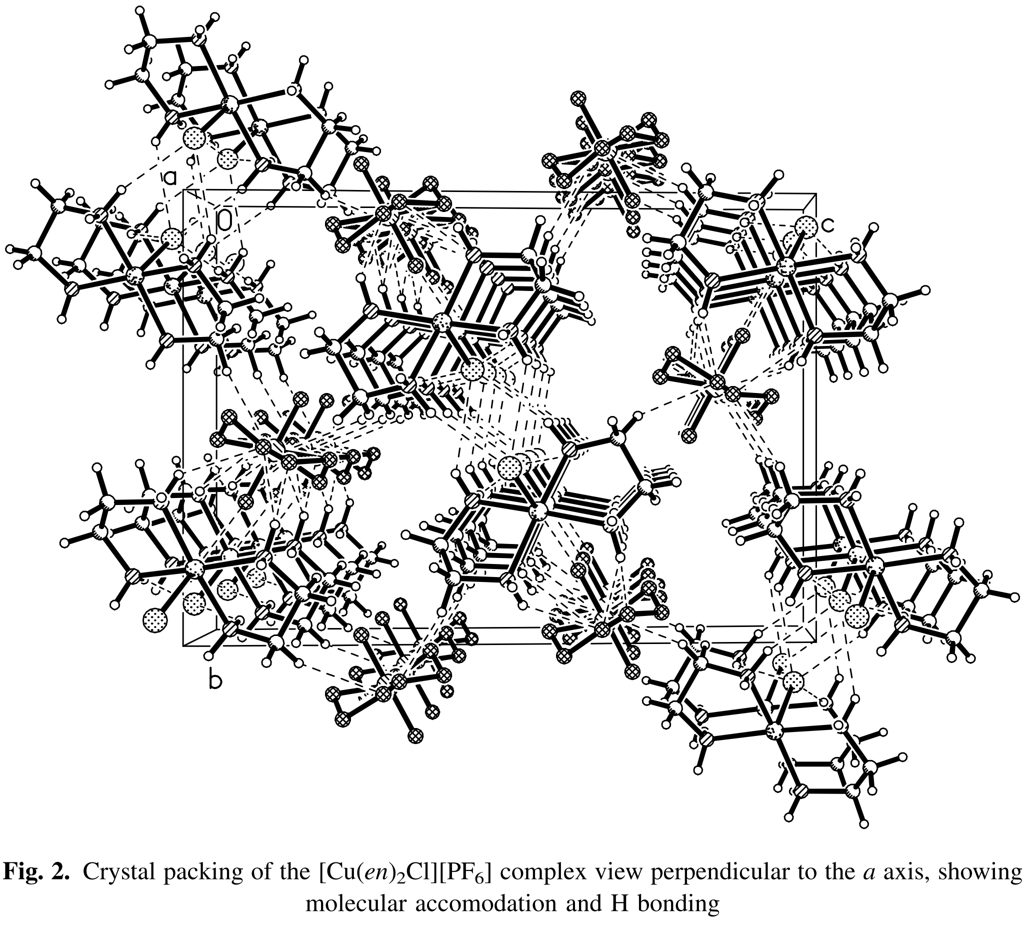

Fig. 2. Crystal packing of the [Cu(en)$_2$Cl][PF$_6$] complex view perpendicular to the a axis, showing molecular accomodation and H bonding

Table 1. Selected bond distances (Å) and bond angles (°) for the $[Cu(en)_2Cl][PF_6]$ complex

Bond distances			
Cu(1)–N(3)	1.992(4)	Cu(1)–N(1)	2.010(3)
Cu(1)–N(4)	2.021(4)	Cu(1)–N(2)	2.020(4)
Cu(1)–Cl(1)	2.5969(11)	N(1)–C(1)	1.444(7)
Bond angles			
N(3)–Cu(1)–N(1)	93.00(15)	N(3)–Cu(1)–N(4)	84.53(17)
N(1)–Cu(1)–N(4)	171.71(15)	N(3)–Cu(1)–N(2)	171.16(16)
N(1)–Cu(1)–N(2)	84.47(16)	N(4)–Cu(1)–N(2)	96.79(19)
N(3)–Cu(1)–Cl(1)	96.50(11)	N(1)–Cu(1)–Cl(1)	96.81(11)
N(4)–Cu(1)–Cl(1)	91.34(12)	N(2)–Cu(1)–Cl(1)	92.22(13)
C(1)–N(1)–Cu(1)	109.8(3)	C(2)–N(2)–Cu(1)	107.8(3)
C(3)–N(3)–Cu(1)	109.1(3)	C(4)–N(4)–Cu(1)	107.6(3)

deviation of 0.0045 Å for the plane defined by N(1), N(2), N(3), and N(4)], with Cu(II) 0.146 Å out of planarity. This structure can be compared with the related copper complex $Cu(en)_2MnCl_4$ [19], which consists of chains with alternating Mn(II) and Cu(II) ions bridged by chloride ligands. In the later structure the Cu(II) ion has elongated octahedral surroundings; the equatorial positions are occupied by the nitrogen atoms from the organic ligand and the axial positions by the bridging chloride ions [Cu–Cl ranging from 2.804(2) to 3.063(2) Å]. The Cu–Cl distances are longer in $Cu(en)_2MnCl_4$ than in $[Cu(en)_2Cl]PF_6$ while the Cu–N distances are similar in both complexes.

The PF_6 anion is positioned in an angular manner to the coordination sphere [Cl(1)–Cu(1)···F(2) 170.7°, Cu(1)·····F(2)–P(1) 133.2°]. It presents four of its six F atoms disordered in two positions respectively. Each of these fluorine atoms shows occupation factors of 0.62 and 0.38 for each pair of positions. All P–F distances were constrained to 1.54 ± 0.02 Å. One of the apical fluorine atoms, F(2), is positioned in the six copper(II) coordination site by a long bond.

In the solid state extensive intermolecular hydrogen bonding is present. One cationic complex is bonded from one side through two amine hydrogen atoms to the coordinated chlorine ion of a symmetry-related cation. This related cation is H bonded, in the same way, through its chlorine ion to two amine hydrogens. On the other side of the complex cation there are two other H bonds through amine H (between symmetry related amine hydrogen atoms) to a fluorine of the counter ion. In this way the PF_6 group acts as a link between symmetry related complex ions, as can be seen in Fig. 2 and in Table 2.

The superoxide dismutase activity of the title complex was assayed by its ability to inhibit the reduction of nitroblue tetrazolium. Figure 3 shows the % inhibition with increase in concentration of the copper(II) complex.

In the Cu_2Zn_2SOD-like activity test, the metal complexes compete with *NBT* for oxidation of the generated superoxide ions. The more efficient the complex, the lower the concentration corresponding to 50% inhibition of *NBT* reduction; this concentration is termed IC_{50} for comparative purposes. On a molar basis, the catalytic activity of the $[Cu(en)_2Cl][PF_6]$ complex ($IC_{50} = 1000\,\mu M$) corresponds

Table 2. Hydrogen bonds with H···A < r(Å) + 2.000 Å and D–H···A > 110°; D represents the donor atom and A the acceptor atom

D	H	A	Symmetry for A	D–H	H···A	D···A	D–H···A
N(1)	H(1A)	Cl(1)	x + 1, y, z	0.890	2.502	3.338	156.68
N(1)	H(1B)	Cl(1)	−x, −y, −z	1.009	2.629	3.474	141.28
N(2)	H(2A)	F(3)	x − 1, y, z	1.104	2.637	3.583	143.26
N(2)	H(2A)	F(5)		0.900	2.389	3.153	142.71
N(3)	H(3A)	Cl(1)	x + 1, y, z	0.885	2.597	3.394	150.27
N(3)	H(3B)	Cl(1)	−x, −y, −z	1.079	2.257	3.305	163.39
N(4)	H(4A)	Cl(1)		1.037	2.817	3.328	110.68
N(4)	H(4B)	F(5)		1.040	2.381	3.216	136.49
N(4)	H(4B)	F(4)	x − 1, y, z	1.040	2.387	3.061	121.41

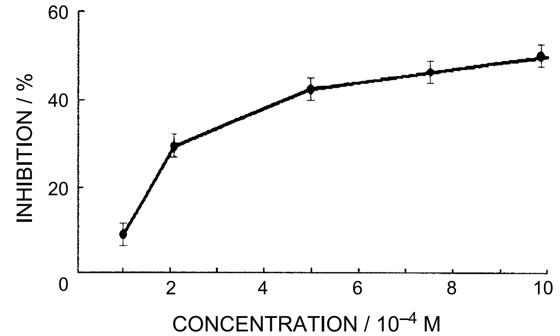

Fig. 3. Inhibition of *NBT* reduction in the presence of the title compound; each point represents the mean ± standard deviation of quintuplicate determinations

to 0.015% of that of native SOD ($IC_{50} = 0.15\ \mu M$). This activity may be considered as interesting thinking of the strong activity, but also the high molecular weight of Cu$_2$Zn$_2$SOD itself. Higher percentages of Cu$_2$Zn$_2$SOD activity have been observed for other copper(II) complexes containing sulfathiazole (H*stz*), for example, [Cu(*stz*)(pyridine)$_3$Cl] $IC_{50} = 1.31\ M$ [20], [Cu(H*stz*)$_2$(*Me*OH)Cl$_2$] $IC_{50} = 2.51\ M$ [21], and [Cu(H*stz*)$_2$(*Et*OH)Cl$_2$] $IC_{50} = 5.17\ M$ [22]. However, the [Cu(*en*)$_2$Cl][PF$_6$] complex presents a disadvantage, *i.e.* it does not go beyond 55% inhibition, whereas other complexes tested reach 80% inhibition or more. This kind of flattening of the curves below 100% inhibition has been observed already for other metal complexes, but the kinetic reasons behind such a limitation have not been explained [7, 8].

Experimental

CuCl$_2$ · 2H$_2$O was purchased from Aldrich. Xanthine, xanthine oxidase, nitroblue tetrazolium chloride monohydrate (*NBT*), and superoxide dismutase (bovine erythrocytes) were obtained from Sigma Chemical Co.

Physical Techniques

The IR spectra of the ligand and its metal complex (KBr pellets) were recorded in the $\bar{\nu} = 4000 - 400\,\mathrm{cm}^{-1}$ range with a Perkin-Elmer Series 2000 spectrophotometer. FTIR spectra as polyethylene pellets were registered in the $450 - 120\,\mathrm{cm}^{-1}$ range using a Brucker IFS 66V spectrophotometer. UV-Vis spectra were recorded on a Perkin-Elmer recording spectrometer. The contents of C, H, N, and metal were analysed by the microlabs in the Venezuelan Institute of Scientific Research (IVIC). Magnetic susceptibility was measured on a Johnson Matthey Susceptibility Balance at room temperature using $HgCo(NCS)_4$ as calibrant. Corrections for the diamagnetism of the complex were estimated from *Pascal*'s constants. The EPR spectrum was recorded on a Brucker ECS 106 spectrometer by the X-band method. α,α'-Diphenyl-β-picrylhydrazide free radical was used as the g marker.

Chlorobis(ethylenediamine) Copper(II) Hexafluorophosphate (**1**, $C_4H_{16}ClCuF_6N_4P$)

$CuCl_2 \cdot 2H_2O$ (O.17 g, 1 mmol) was added to a solution of imidazole (0.14 g, 2 mmol) in *Me*OH ($50\,\mathrm{cm}^3$). Then to the resulting mixture, ethylenediamine ($1.0\,\mathrm{cm}^3$, 10 mmol) was added. The mixture was refluxed for two days and the boiling solution was then filtered and concentrated to $10\,\mathrm{cm}^3$. NH_4PF_6 (2 mmol) was added to the solution, which was left standing at low temperature (5°C). After a few days, violet prismatic crystals were obtained from this solution (yield 35%; found: C, 13.35; N, 15.06; H, 4.70; calcd. for $C_4H_{16}ClCuF_6N_4P$: C, 13.18; N, 15.38; H, 4.40%)

Crystallographic Data Collection

Recrystallization of [Cu(*en*)$_2$Cl][PF$_6$] from methanol gave violet prismatic crystals suitable for single crystal X-ray analysis. The crystal of approximate dimension of $0.52 \times 0.20 \times 0.16\,\mathrm{mm}^3$, was mounted on a Siemens P4 diffractometer. X-Ray data were collected [23] using graphite-monochromated Mo-Kα radiation ($\lambda = 0.71069$ Å) at room temperature. Accurate cell parameters and the orientation matrix

Table 3. Crystallographic data (**1**)

Empirical formula	$C_4H_{16}ClCuF_6N_4P$
Color, habit	Violet, prismatic
Crystal size/mm^3	$0.52 \times 0.20 \times 0.16$
Crystal system	Monoclinic
Space group	$P2_1/c$
Unit cell dimensions	$a = 6.1488(9)$ $b = 12.696(2)$ $c = 17.7424(17)$ Å $\beta = 104.173(5)°$
Volume/Å^3	1374.0(3)
Z	4
Formula weight	364.17
Density (calc.)/g cm^{-3}	1.760
Absorption coefficient/mm^{-1}	1.954
F(000)	732
Reflections collected	5422
Independent reflections	4000 ($R_{\mathrm{int}} = 2.60\%$)
Reflections with $F_0 > 4\sigma(F_0)$	2331
$<I/\sigma(I)>$ (all data)	9.44
Parameters refined	193
Final *R* indices ($I > 2\sigma(I)$)/%	$R_1 = 5.44$, $wR_2 = 12.07$
Final *R* indices (all data)/%	$R_1 = 10.69$, $wR_2 = 14.69$

were determined by least-squares fit of 33 reflections. Intensity data were collected in the ω scan mode and corrected for *Lorentz* and polarization effects. An empirical absorption correction (ω-scan) was applied. The structure was solved using direct methods and refined [24] on F^2 by full-matrix least squares with anisotropic displacement parameters for the non-hydrogen atoms. Hydrogen atoms bonded to amine nitrogens (H1A, H1B, H2A, H2B, H3A, H3B, H4A, H4B) were found on difference electron density maps, and the remaining hydrogen atoms were placed on idealized positions with refinement for all H on riding mode with isotropic displacement parameters. Crystal parameters and details of data collection and refinement are summarized in Table 3. Final positions, thermal parameters, and all distances and angles of the complex structure have been deposited under the deposition number CCDC 169329 at the Cambridge Crystallographic Data Center.

Superoxide Assays

Superoxide dismutase activity of the complex was determined using its ability to inhibit the reduction of *NBT* by superoxide ions generated by the xanthine/xanthine oxidase system [25]. The extent of *NBT* reduction was followed spectrophotometrically by measuring the absorbance at $\lambda = 560\,\text{nm}$. The aqueous reaction mixture contained $2 \times 10^{-4}\,M$ xanthine, $5 \times 10^{-5}\,M$ *NBT*, and $1.25 \times 10^{-2}\,M$ sodium phosphate buffer at $pH = 7.5$ and 25°C. For comparative purposes, we have also measured the activity of native superoxide dismutase, from bovine erythrocytes, under the same experimental conditions. Five assays were performed for each concentration of both Cu$_2$Zn$_2$SOD and the metal complex. We checked that the generating xanthine/xanthine oxidase system was not inhibited by our metal complex by following, at $\lambda = 295\,\text{nm}$, the uric acid produced when xanthine is oxidized by xanthine oxidase under aerobic conditions concomitant with the production of superoxide ions.

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