Polish J. Chem., **77**, 837–844 (2003)

Synthesis, Characterization, and SOD-like Activity Testing of Two Novel Copper(II) Complexes Bridged by Oxamide-N,N′-diacetic Acid

by L.-Z. Zhang[1], S.-L. Ma[1*], A.-Y. Shen[1], M. Fu[1], L.-J. Zhang[2] and X. Liu[2]

[1]*Department of Pharmaceutical Engineering, Tianjin University of Commerce,*
Tianjin, 300134, P.R. China
[2]*Department of Biological Engineering, Beijing Institute of Technology,*
Beijing, 100081, P.R. China

(Received January 13th, 2003; revised manuscript March 14th, 2003)

To provide more complexes which are expected to yield better similarity to authentic SOD (Superoxide Dismutase) and to have certain SOD activity, two novel 1 D linear complexes composed of binuclear copper(II) units are synthesized and characterized with the structural units bridged by oxamide-N,N′-diacetic acid. They are $\{Na[Cu_2(oxaa)OAc]\cdot H_2O\}_n$ (I) and $\{Na[Cu_2(oxaa)(o\text{-phenylenediamine})_2OAc]\cdot 2.5H_2O\}_n$ (II), where oxaa denotes oxamide-N,N′-diacetic acid tetraanion. The temperature dependence of the magnetic susceptibilities of $\{Na[Cu_2(oxaa)(o\text{-phenylenediamine})_2OAc]\cdot 2.5H_2O\}_n$ was investigated between 4.99–299 K, giving the exchange parameter $J = -294.1\ cm^{-1}$. This result is demonstrated with an antiferromagnetic interaction between the adjacent copper(II) centers. In addition, the SOD-like activity of the complex (II) is discussed.

Key words: SOD mimic, synthesis, characterization, SOD-like activity

Authentic SOD and its mimics are of current interest because: (a) In organism, the superoxide dismutases are the most important protein enzymes of the protective enzyme system against oxygen free radicals, which can be destructive if not be eliminated in time to keep at a low unharmful level [1,2]; (b) The superoxide dismutase has many limitations in medicine development [3], which arouses the thriving of studies on SOD mimics and related complexes. It is reported that there exists a ferromagnetic coupling between the two copper(II) centers after the zinc(II) is replaced in the authentic SOD [4]. Therefore, bridging ligands, which can propagate binuclear complexes with an antiferromagnetic interaction between coordination centers, are of more importance in the studies of SOD mimics. N,N′-disubstituted oxamidos [1,5] have been playing a key role in the design of polymetallic systems for the following reasons: (1) Previously reported complexes bridged by them are mostly found with antiferromagnetic coupling between metal centers [1,5]; (2) They possess good versatility as bridging ligands, due to (a) their easy cis-trans conformational change affording symmetric and asymmetric oxamidato bridges [6–7] and (b) the bidentate

---

[*] Corresponding author, E-mail address: mashulin@tjcu.edu.cn

character of the mononuclear metal-oxamidato complex, which allows the formation of di- [8–9], tri- [10–11] and tetranuclear [12–13] complexes.

In this paper, two novel complexes bridged by oxamide-N,N′-diacetic acid [abbreviated as H_4(oxaa) referring to Fig. 1] have been synthesized and characterized to provide more structural examples and supporting data for the research of SOD mimics.

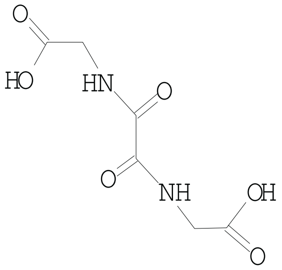

Figure 1. Structure of H_4(oxaa).

EXPERIMENTAL

Reagents and materials: All chemicals were of analytical grade and were purchased from the Peking Chemical Company. The ligand oxamido-N,N′-diacetic acid was synthesized by the following general procedure reported in [1]. 20 mmol glycine dissolved in about 25 cm^3 of water was neutralized with equivalent NaOH, and then 10 mmol of diethyl oxalate in 25 cm^3 of ethanol was added to the aqueous solution. After the mixture was heated to 60°C with stirring, an excess of HCl (pH = 1) was added for precipitation of white microcrystals of free H_4(oxaa). The product was filtered off, washed with cold water, ethanol and ether and then dried *in vacuo*.

Preparation of $\{Na[Cu_2(oxaa)OAc]\cdot H_2O\}_n$ (I): Added 2 mmol H_4(oxaa) into 15 cm^3 H_2O, and a suspending liquid was obtained. Then 8 mmol NaOH in 15 cm^3 H_2O was dropped leading to a mixture solution. Puting 30 cm^3 water solution of 4 mmol $Cu(OAc)_2\cdot H_2O$ into above-mentioned mixture solution, blue microcrystalline product formed immediately. After the procedure of stirring the reaction mixture for 3 h, and filtering out the precipitation and then washing several times with absolute methanol and ether respectively, the product was finally dried *in vacuo*.

Preparation of $\{Na[Cu_2(oxaa)(o\text{-phenylenediamine})_2OAc]\cdot 2.5H_2O\}_n$ (II): Put H_4(oxaa) (204.1 mg, 1.0 mmol) and o-phenylenediamine (216.3 mg, 2.0 mmol) in 10 cm^3 absolute ethanol to obtain a mixture suspending liquid, then added NaOH (4.0 mmol, methanol solution) dropwisely, and then a solution of $Cu(OAc)_2\cdot H_2O$ (399.3 mg, 2.0 mmol) in 10 cm^3 absolute methanol had to be added. After stirring of 4 h, a yellow green microcrystalline product was formed. Microcrystals had to be washed several times successively with absolute methanol and ether and finally dried in vacuo. Analyses confirmed the compositions given.

Physical measurements: Elemental analyses of carbon, hydrogen and nitrogen were performed on a Perkin-Elmer elemental analyzer, Model 240. The infrared spectra were recorded in the region of 4000 cm^{-1} to 200 cm^{-1} on a Peking Elmer 983G, using KBr pellets. X-ray electron spin resonance spectra of powdered samples were measured on a Bruker ER 200 D-SRC ESR spectrophotometer. UV-Vis spectra were recorded on a Beckman-DU8B spectrophotometer. Variable temperature magnetic susceptibilities (4.99–299 K) were carried out at the Institute of Physics, Chinese Academy of Sciences, China, using a Superconducting Quantum Interference Device (SQUID) Magnetometer. Diamagnetic corrections were made with Pascal's constants for all the constituent atoms, and the magnetic moments were calculated using the equation $\mu_{eff} = 2.828(\chi_M T)^{1/2}$.

Superoxide dismutase activity assay: Superoxide dismutase activity was investigated in a classical way using 1,2,3-benzenetriol as a superoxide generating system and authentic superoxide dismutase or the studied complexes as the scavengers for superoxide. In the first step of the assay, a standard curve was drawn to calculate the IC_{50} of the authentic Cu-Zn superoxide dismutase, according the experimental data using authentic SOD as a scavenger for superoxide. In the second step, experiments were performed to determine the IC_{50} of the complexes. In a typical experiment, 1.1 cm^3 of 0.015 $mmol\cdot dm^{-3}$ water solution of complex II was added into a mixture of 4.5 cm^3 TRIS-HCl buffer (pH = 8.23) and 3.1 cm^3 H_2O. The mixture was kept in water at 30°C for 30 min and then 0.3 cm^3 water solution of 1,2,3-benzenetriol was added with stirring. The absorbance of the final reaction mixture was immediately monitored at 319.2 nm against a comprison sample of 10 $mmol\cdot dm^{-3}$ HCl solution on a Beckman-DU8B UV-Vis Spectrophotometer. The IC_{50} (the amount of copper(II) complex, which produces a 50% inhibition on 1,2,3-benzenetriol self-oxidation) was consequently obtained from a plot of percent inhibition *versus* complex concentration.

RESULTS AND DISCUSSION

General characterization: Elemental analyses demonstrate that the reaction of H_4(oxaa) with $Cu(OAc)_2\cdot H_2O$ or $Cu(OAc)_2\cdot H_2O$ and o-phenylenediamine produced two novel binuclear copper(II) complexes.

Physical data of the complexes are listed in Table 1. From the selected infrared absorption bands, the amide I bands are located at 1630 cm^{-1} and 1600 cm^{-1} for complex I and II respectively, while it occurs at 1640 cm^{-1} for free H_4(oxaa). The red shift of the amide I bands is in accordance with the presence of a bridging oxamidato [5]. The stretching vibrations of carboxylato groups, $\nu_{as}(COO^-)$ and $\nu_s(COO^-)$, appear as significant absorptions, which are at 1665 cm^{-1} and 1375 cm^{-1} for complex I, 1675 cm^{-1} and 1380 cm^{-1} for complex II. Apparently, the separation $\Delta\upsilon$ $[\upsilon_{as}(COO^-) - \upsilon_s(COO^-)]$ for two complexes are 290 cm^{-1} and 295 cm^{-1} respectively, both of which are much more than 200 cm^{-1}. This suggests that both carboxylato groups of the complexes are in a monodentate coordination mode. In addition, a very broad band around 3440 cm^{-1} partially owing to lattice water molecules for both complexes can be observed.

Table 1. Physical data of the complexes.

Complex	Color	IR (cm^{-1})			μ_{eff} (μB)
		ν(C=O)	$\nu_{as}(COO^-)$	$\nu_s(COO^-)$	Room Temp.
I	blue	1630	1665	1375	
II	yellow green	1600	1675	1380	1.65

The electronic spectra for both complexes were studied in water solution at the range of 190–900 nm. For both complexes, the spectra present a pretty broad d-d band at 680 nm and 719 nm respectively, which can be assigned to copper(II) d-d transitions. For complex I, there are two additional bands, one at 265 nm and one shoulder band at 355 nm appearing to be of ligand-to-metal charge-transfer origin. For complex II, there appear three additional bands, one at 207 nm and two shoulder bands at 231 nm and 288 nm, can also be ascribed to charge-transfer (CT) transition.

On the ground of the elemental analyses, IR spectra, electronic spectra, the complexes are proposed to have an oxamidato-bridged 1 D structure and consist of two copper (II) ions in a distorted square-pyramidal for complex I (see Fig. 2 – I) or octahedral coordinated environment for complex II (see Fig. 2 – II).

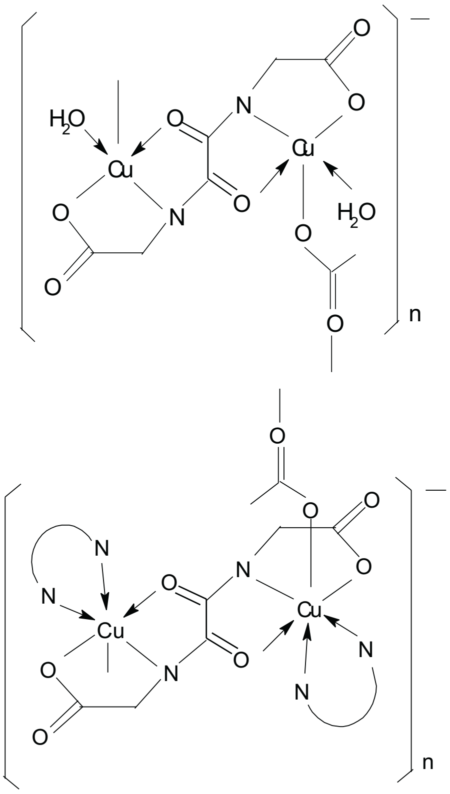

Figure 2. Supposed structure of the anion of complex I and complex II.

ESR spectra of the complexes: The electron spin resonance spectra of the complexes were studied at room temperature, mainly aiming to get reasonable g values for magnetism analysis. For binuclear copper(II) complexes, the antiferromagnetic interaction between the metal centers shows a molecular spin singlet ($S = 0$) and a molecular spin triplet ($S = 1$). As shown in Fig. 3, both complexes show a dissymmetric shape of upper and lower peaks at about 3600×10^{-4} T corresponding to the allowed transition of $\Delta M_s = \pm 1$, which indicates a very weakly anisotropic triplet states. Thus, using g_3 and g_4 of Mn-reference in Fig. 3, the spectrum parameters can be deduced: $g_{//} = 2.20$, $g_{\perp} = 2.06$, $\bar{g} = 2.11$ for complex I; $g_{//} = 2.20$, $g_{\perp} = 2.08$, $\bar{g} = 2.12$ for complex II. Moreover, a very weak signal ($g \approx 4$) at half-field ($\sim 1600 \times 10^{-4}$ T) can be assigned to the $\Delta M_s = \pm 2$ forbidden transition, which is roughly 10^{-3} times the $\Delta M_s = \pm 1$ transi-

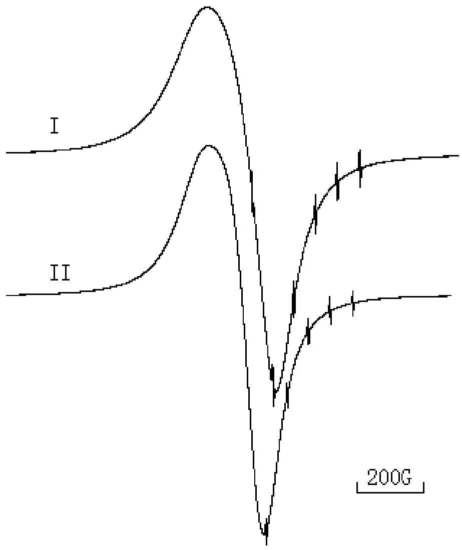

Figure 3. ESR spectra of complex I and II.

tion. The appearance of the half-field signals confirms a magnetic interaction between two Cu(II) ions for the complexes.

Magnetism analysis of the complexes: Magnetic properties of complex II were analyzed at 1.0 T between 4.99–299 K. The room temperature μ_{eff} is 1.65 μB for complex II, which is lower than the spin-only value 2.45 μB for copper(II)–copper(II) system. Meanwhile, the μ_{eff} values decrease significantly with decreasing temperature, which demonstrates the presence of an antiferromagnetic coupling between two copper(II) centers of the complex.

On the ground of Hamiltonian operator (deduced from the Heisenberg Model) $\hat{H} = -2J\hat{S}_1 \cdot \hat{S}_2$ with $S_1 = S_2 = 1/2$. The molar susceptibility of copper(II)–copper(II) system is given by:

$$\chi'_M = \chi_M/[1 - (2z'J'/N\beta^2g^2)] \cdot \chi_M$$

$$\chi_M = (2N\beta^2g^2/kT)[3 + \exp(-2J/kT)]^{-1}(1 - \rho) + N\beta^2g^2\rho/2kT + N_\alpha$$

where χ_M denotes the susceptibility per binuclear complex, $z'J'$ the intermolecular interaction, ρ the fraction of uncoupled copper(II) impurity, N_α the temperature independent paramagnetism ($N_\alpha = 120 \times 10^{-6}$ cm$^3 \cdot$ mol^{-1}) and the other symbols have their usual meanings. As shown in Fig. 4, good least-squares fit to experimental data are attained. The magnetic parameters thus determined are: exchange factor J = –294.1 cm^{-1}, g = 2.12 (determined according the $\bar{g}$ value of complex II, referring to the analysis of the ESR), $z'J' = 0.062$, $\rho = 0.173$ and fitting factor $F = 7.71 \times 10^{-6}$ ($F = \Sigma[(\chi_M)_{\text{obs.}} - (\chi_M)_{\text{calc.}}]^2/\Sigma(\chi_M)_{\text{obs.}}$). This result indicates a strong antiferromagnetic interaction in the paramagnetic centers of complex II. Magnetism analysis on authentic SOD found that there would be an antiferromagnetic coupling between two copper(II) centers with the magnetic exchange parameter being $-26\ \mathrm{cm}^{-1}$, if the zinc(II) was replaced by a copper (II) [4]. However, there seems to exist a ferromagnetic interaction between two coppers of the previously reported complexes bridged by phthalate [15]. From this point of view, oxaa might be a better bridging ligand than phthalate.

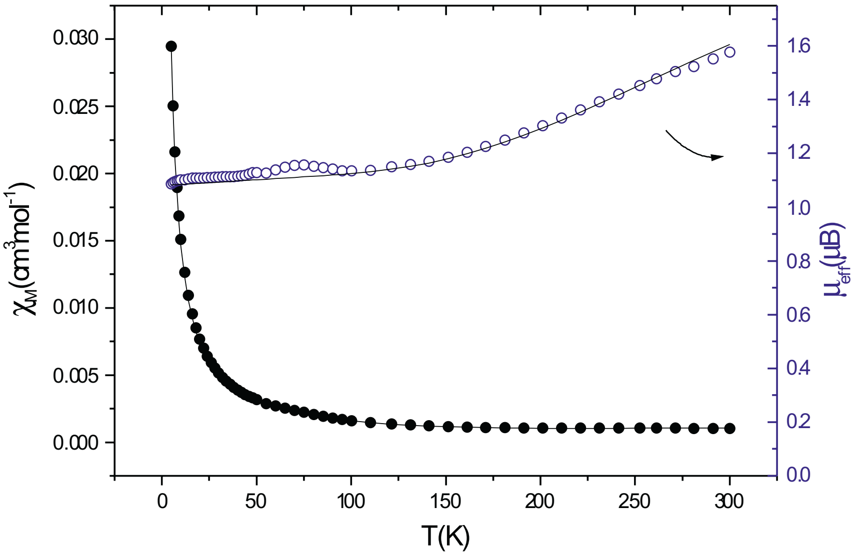

Figure 4. Temperature variation of the experimental and calculated magnetic susceptibility and magnetic moment of complex II.

SOD-like activity of $\{Na[Cu_2(oxaa)(o\text{-}phenylenediamine)_2OAc]\cdot 2.5H_2O\}_n$: The Cu-Zn SOD activity and the superoxide dismutase-mimetic activity were measured in the indirect way, using 1,2,3-bezenetriol as the superoxide generating system and Cu-Zn SOD or the studied complexes as the superoxide scavenger. The IC_{50} is defined as the concentration of complex or authentic SOD, when it produces 50% inhibition on 1,2,3-bezenetriol self-oxidation. According to the experimental data, the standard curve of authentic SOD inhibiting 1,2,3-bezenetriol self-oxidation was drawn and the IC_{50} was consequently obtained, giving $0.0059\ \mu\mathrm{mol}\cdot\mathrm{L}^{-1}$ (see Fig. 5), which is the same with the IC_{50} reported in [16]. The IC_{50} of the tested complex II was calculated in the same way, which is $30.6\ \mu\mathrm{mol}\cdot\mathrm{L}^{-1}$ (see Fig. 6). However, no reliable data were obtained for the SOD-like activity of the studied complex I, because of its low solubility in water.

In conclusion, one of the complexes in discussion, complex II, has SOD-like activity, but much lower than the authentic CuZn-SOD. In the magnetism analysis of the complexes, including those we previously reported [1,17–19], it is found that the structure models have a direct effect on whether the results of magnetic fitting are satisfactory or not. Therefore, magnetic analysis can be a powerful and assuring way to

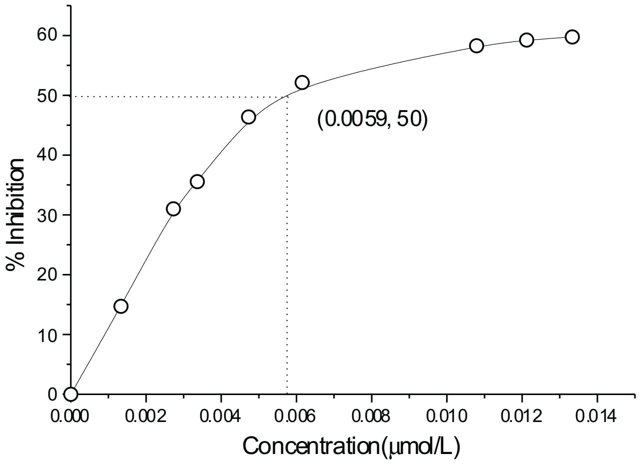

Figure 5. A standard curve of percentage of inhibiting 1,2,3-bezenetriol self-oxidation with an increase in the concentration of authentic superoxide dismutase.

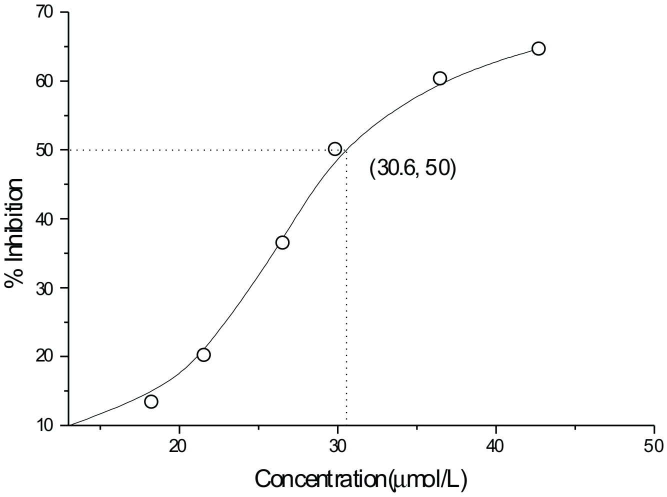

Figure 6. A plot of percentage of inhibiting 1,2,3-bezenetriol self-oxidation *versus* an increase of concentration of complex II.

describe the structures of the SOD mimetic complexes. However, to determine the structures of the discussed complexes, more data, especially from X-ray analysis on single crystal, are strongly needed. Meanwhile, much work should be carried out on the testing of the biological activity of these complexes, and the studies of the relationship between the structural similarity and activity.

REFERENCES

1. Zhang L.Z. and Ma S.L., *Polish J. Chem.*, **75**, 1833 (2001).
2. Zhao B.L., *Oxygen Free Radicals and Natural Antioxidant*, Science Press, Beijing, P.R. China, pp. 54–59 (1999).
3. Zhang L.Z. and Ma S.L., *Acta Pharm. Sinica*, **37**, 235 (2002).
4. Luo Q.H., Lu Q., Dai A.B. and Huang L.R., *J. Inorg. Biochem.*, **51**, 655 (1993).
5. Han K.Q., Tian Z.Q., Wei K.J., Ma S.L., Lu L.L., Meng X.F., Yang X.L., He M. and Gao J., *Polish J. Chem*., **73**, 1451 (1999).
6. Ma S.L., Liao D.Z., Jiang Z.H., Yan S.P. and Wang G.L., *Polyhedron*, **13**, 373 (1994).
7. Jin C.Y., Ma S.L. and Liao D.Z., *Polyhedron*, **13**, 2881(1994).
8. Stumpf H.O., Pei Y., Kahn O., Sletten J. and Renard J.P., *J. Am. Chem. Soc*., **115**, 6738 (1993).
9. Nakatani K., Carriat J.Y., Journaux Y., Kahn O., Loret F., Renard J.P., Pei Y., Sletten J. and Verdaguer H., *J. Am. Chem. Soc*., **111**, 5739 (1989).
10. Ma S.L., Liao D.Z., Jiang Z.H., Yan S.P. and Wang G.L., *Polyhedron*, **12**, 1523 (1993).
11. Pei Y., Journaux Y. and Kahn O., *Inorg. Chem*., **27**, 399 (1988).
12. Okawa H., Kawahara Y., Mikuriya M. and Kida S., *Bull. Chem. Soc. Jpn*., **53**, 549 (1980).
13. Lloret F., Journaux Y. and Julve M., *Inorg. Chem*., **29**, 3967 (1990).
14. Luo Q.H., *Chem. J. Chinese Univ*., **7**, 1012 (1997).
15. Ma S.L., Zhang L.Z., Tang M.Y. and Lu L.L., *Polish J. Chem*., **76**, 1041 (2002).
16. Brain J.D. and James D.C., *Toxicology and Appl. Pharmacology*, **140**, 94 (1996).
17. Ma S.L., Zhang L.Z., Lu L.L. and Tang M.Y., *Polish J. Chem*., **76**, 511 (2002).
18. Ma S.L., Liao D.Z., Jiang Z.H., Yan S.P., Xue F.C. and Wang G.L., *Synth. React. Inorg. Met.-Org. Chem*., **24**, 137 (1994).
19. Ma S.L., Jiang Z.H., Liao D.Z., Yan S.P. and Wang G.L., *Polyhedron*, **14**, 373 (1995).