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Oxidative Cleavage of DNA by a New Ferromagnetic Linear Trinuclear Copper(II) Complex in the Presence of H_2O_2/Sodium Ascorbate

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A new trinuclear copper(II) complex has been synthesized and structurally characterized: $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$ (HL = (*N*-pyrid-2-ylmethyl)benzenesulfonylamide). In the complex, the central copper ion is six-coordinated. The coordination spheres of the terminal copper atoms are square pyramidal, the apical positions being occupied by a sulfonamido oxygen of the contiguous trimer. As a consequence, the complex can be considered a chain of trinuclear species. The three copper atoms are in a strict linear arrangement, and adjacent coppers are connected by a hydroxo bridge and a bidentate *syn*–*syn* carboxylato group. The mixed bridging by a hydroxide oxygen atom and a bidentate formato group leads to a noncoplanarity of the adjacent basal coordination planes with a dihedral angle of 61.4(2)°. Susceptibility measurements (2–300 K) reveal a strong ferromagnetic coupling, $J = 79$ cm^{-1}, leading to a quartet ground state that is confirmed by the EPR spectrum. The ferromagnetic coupling arises from the countercomplementarity of the hydroxo and formato bridges. The trinuclear complex cleaves DNA efficiently, in the presence of hydrogen peroxide/sodium ascorbate. *tert*-Butyl alcohol and sodium azide inhibit the oxidative cleavage, suggesting that the hydroxyl radical and singlet oxygen are involved in the DNA degradation.

Introduction

Polynuclear copper complexes are attracting attention because of their interesting magnetic properties and their relevance to the active centers of a number of metalloproteins.[1,2] A recent discovery that adds importance to the biological role of polynuclear copper species and provides the impetus for further investigations of complexes containing three copper atoms is the recognition that the 15 copper atom active center of particulate methane monooxygenase (pMMO) from *Methylococcus capsulatus* (Bath) is organized into two types of five trinuclear aggregates of yet unknown structures.[3,4] Also, the pMMO of *Methylosinus trichosporium* consists of 12.8 copper and 0.9 iron atoms per molecule. Copper exists as a copper ion coupled to four nitrogen atoms and/or a trinuclear cluster wherein copper ions are ferromagnetically coupled.[5]

Besides the interest of polynuclear copper compounds related to multicopper centers in biological systems, polynuclear copper compounds have attracted much attention due to their ability to perform DNA strand scission. Transition metal complexes have been extensively studied for their nuclease-like activity.[6] Chemical nucleases present some advantages over conventional enzymatic nucleases in that they are smaller in size and thus can reach more sterically hindered regions of a macromolecule. Many of these utilize the redox properties of the metal and dioxygen to produce reactive oxygen species that oxidize DNA, yielding direct strand scission or base modification.[7,8] Copper complexes

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are capable of both reactions, although neither exhibit significant nucleotide sequence specificity. The best studied of these is bis(orthophenanthroline)copper which in the presence of hydrogen peroxide induces direct strand scission.[9] The proposed mechanism for this process requires 3 equiv of the Cu(II) species to produce a nondiffusible intermediate equivalent to a hydroxyl radical. This fact has focused large attention on multinuclear Cu(II) compounds due to their potential for efficient intramolecular activation of bound O_2 and for binding in a selective manner to particular nucleic acid conformations. These features were demonstrated by polynuclear copper complexes reported by Karlin et al.[10−13]

Recently, we have begun to explore the nuclease activity of copper(II) ternary complexes with *N*-substituted sulfonamides.[14] Moreover, because of our interest in polynuclear copper(II) complexes, we have reported the crystal structures and spectroscopic and magnetic properties of linear μ-acetato, μ-hydroxo trinuclear copper(II) complexes with *N*-sulfonamides derivatives.[15,16] Here, we present the crystal structure and chemical properties of a new linear ferromagnetic trinuclear copper(II) complex, $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$ [HL= *N*-(pyrid-2-ylmethyl)benzenesulfonylamide], with formato and hydroxo bridges which efficiently cleaves DNA in the presence of H_2O_2/ascorbate.

Experimental Section

Material and Physical Methods. The variable temperature magnetic susceptibility measurements were carried out on a microcrystalline sample (4 mg) using a Quantum Design MPMS2 SQUID susceptometer equipped with a 55 kG magnet and operating at 10 kG in the range 1.8−400 K. The susceptometer was calibrated with $(NH_4)_2Mn(SO_4)_2 \cdot 12H_2O$. Corrections for the diamagnetism were estimated from Pascal constants. The infrared spectra (ν = 400−4000 cm^{-1}) were obtained on a Mattson Satellite FTIR spectrophotometer. Elemental analyses were performed by a Carlo Erba AAS instrument. An electrospray mass spectrum, in positive mode (ESI^+), of the compound dissolved in acetonitrile−water (70:30) was obtained from an ESQUIRE 3000 Plus (Brucker) ion trap mass spectrometer. EPR spectra of ground crystals were carried out at X-band with a Bruker ELESYX instrument. UV−vis spectra were recorded on a Shidmazu 2101 PC spectrophotometer.

All chemicals were used as purchased without further purification.

Synthesis of $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$. [HL = *N*-(pyrid-2-ylmethyl)benzenesulfonylamide]. The copper complex was prepared by the reaction of the metal formate tetrahydrate (1 mmol in 25 mL DMF) with the HL ligand (1 mmol in 25 mL dmf).[15] Slow evaporation of the resulting solution at room temperature provides blue crystals of the compound suitable for X-ray diffraction. Microanalysis of dried crystals was satisfactory. $C_{26}H_{26}Cu_3N_4O_{10}S_2$ (809.28) Calcd: C, 38.59; H, 3.24; N, 6.92; S, 7.92. Found: C, 38.20; H, 3.35; N, 7.04; S, 7.68. IR(KBr) (cm^{-1}): 3548, 3497 ν(O−H); 1589 ν_{as}(COO); 1444 ν_s(COO); 1251, 1127 $\nu(SO_2)$, 964 ν(S−N). The electrospray mass spectrum in positive mode (ESI^+) gives a peak at *m*/*z* 866.94 corresponding to the ion $C_{26}H_{26}Cu_3N_4O_{10}S_2 \cdot CH_3CN \cdot H_3O^+$.

X-ray Structure Determination. A blue prismatic crystal of $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$ was mounted on a glass fiber and used for data collection. Crystal data were collected at 291(2) K, using a Bruker SMART CCD 1000 diffractometer. Graphite monochromated Mo Kα radiation (λ = 0.71073 Å) was used. The data were processed with SAINT[17] and corrected for absorption using SADABS (transmissions factors: 1.000−0.637).[18] The structure was solved by direct methods and refined by full-matrix least-squares techniques against F^2 using SHELXL-97.[19] Positional and anisotropic atomic displacement parameters were refined for all nonhydrogen atoms. The hydrogen atoms were included in geometrically idealized positions employing appropriate riding models[19] with isotropic displacement parameters constrained to $1.2U_{eq}$ of their carrier atoms. Atomic scattering factors were taken from *International Tables for X-ray Crystallography*.[20] Molecular graphics were made with PLATON.[21] A summary of the crystal data, experimental details, and refinement results are listed in Table 1.

Absorption Titration of the Copper Complex Binding to DNA. Absorption titration of $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$ binding to DNA was performed by monitoring the absorbance spectra of the complex (120 μM) in cacodylate buffer (100 mM, pH = 6.0) in the presence of increasing amounts of calf thymus DNA. As both calf thymus DNA and the copper complex have overlapping transitions below 320 nm, metal complex spectroscopic changes are studied above this wavelength.

Viscosity Measurements. Viscosity experiments were carried out on an Ubbelodhe viscometer at 20 °C. The concentration of DNA was 187.5 μM in NP, and the flow times were determined with a manually operated timer. Data are presented as η/η_0 versus [complex]/[DNA] (η is the viscosity of DNA solution in the presence of the compound, and η_0 is the viscosity of DNA alone).

Cleavage of pUC18 by the Cu(II) Complex. A typical reaction was carried out by mixing 6 μL of 40 μM Cu(II) complex solution in 0.1 M buffer cacodylate (pH = 6.0), 1 μL of 750 μM (in nucleotides) pUC18, 7 μL of buffer cacodylate (pH = 6.0), 3 μL of 80 μM ascorbate, and 3 μL of 80 μM H_2O_2. After allowing the sample to incubate at 37 °C for 60 min, 3 μL of a quench buffer solution (0.25% bromophenol blue, 0.25% xylene cyanole and 30% glycerol) was added. Then, the solution was subjected to electrophoresis on 0.8% agarose gel in 0.5× TBE buffer (0.045 M tris, 0.045 M boric acid, and 1 mM EDTA) containing 2 μL/100 mL of a solution of ethidium bromide (10 mg/mL), 2 h at 80 V. The gel

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Table 1. Crystal Data and Structure Refinement for $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$

empirical formula	$C_{13}H_{13}Cu_{1.5}N_2O_5S$
fw	404.62
T	293(2) K
wavelength	0.71073 Å
cryst syst, space group	monoclinic, $P2_1/c$ (No. 14)
unit cell dimensions	$a = 9.9329(12)$ Å, $\alpha = 90°$
	$b = 13.7791(16)$ Å, $\beta = 91.222(3)°$
	$c = 11.0195(13)$ Å, $\gamma = 90°$
V	1507.9(3) Å^3
Z, d_{calcd}	4, 1.782 Mg/m^3
abs coeff	2.298 mm^{-1}
$F(000)$	818
cryst size	$0.12 \times 0.09 \times 0.03$ mm^3
θ range for data collection	2.05 to 28.02°
limiting indices	$-13 \le h \le 10$
	$-18 \le k \le 17$
	$-11 \le l \le 14$
reflns collected/unique	8472/3438 [R(int) = 0.0760]
completeness to θ	28.02, 94.0%
abs correction	SADABS
max and min. transm	1.000 and 0.637
refinement method	full-matrix least-squares on F^2
data/restraints/params	3438/0/205
GOF on F^2	0.889
final R indices [$I > 2\sigma(I)$]	R1 = 0.0631
	wR2 = 0.1261
R indices (all data)	R1 = 0.1535
	wR2 = 0.1512
largest diff. peak and hole	0.618 and −0.621 e·Å^{-3}

was photographed on a capturing system Gel-printer plus TDI. Quantification was performed using the Scion Image for Windows software program based on NHI Image. Supercoiled plasmid DNA values were corrected by a factor of 1.3 on the basis of average literature estimates of lowered binding of ethidium to this structure.[22−24]

Results and Discussion

Crystal Structure. The trinuclear entity with the atomic labeling scheme is shown in Figure 1. Selected bond distances and angles are listed in Table 2. The polymer compound consists of trinuclear copper fragments $[Cu_3(L)_2(HCOO)_2(OH)_2]$ [HL = *N*-(pyrid-2-ylmethyl)benzenesulfonylamide]. The central and terminal copper ions are nonequivalent. The geometry around the terminal copper atoms Cu(1) and Cu(1A) is distorted square pyramidal (τ parameter = 0.26) with a $CuN_2O_2\cdots O$ chromophore. The basal plane is described by the pyridyl N(1) and the sulfonamidato N(2) atoms of the deprotonated ligand (L^-), and the O(3) and the O(1) from the hydroxo and the formate bridges, respectively. A sulfonamide O atom [O(11)] of another L^- ligand pertaining to a contiguous asymmetric unit occupies the axial position (see Figure 2). The Cu(1) atom is 0.1202 Å up to the square plane formed by the N(1), N(2), O(1), and O(3) atoms. The central Cu(2) is six-coordinated with a distorted octahedral (NNOO′ + 2O″) geometry (see Figure 3). The basal plane is realized with the O(3), O(3A), O(2), and O(2A) atoms from the hydroxo and formate bridging groups, respectively. The Cu−O and the Cu−N

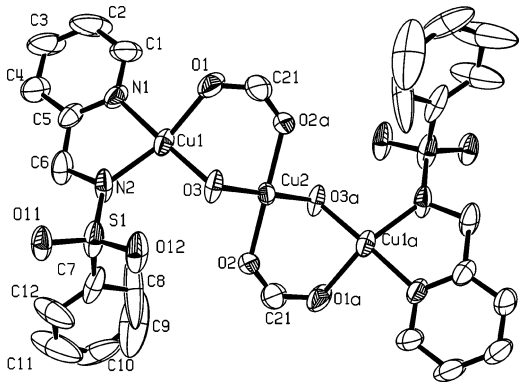

Figure 1. Molecular structure of $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$.

Table 2. Selected Bond Lengths [Å] and Angles [deg] for $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$[a]

Cu(1)−O(3)	1.887(4)	Cu(2)−O(3)#2	1.878(4)
Cu(1)−N(2)#1	1.967(6)	Cu(2)−O(3)	1.878(4)
Cu(1)−N(1)#1	1.979(6)	Cu(2)−O(2)#2	1.968(5)
Cu(1)−O(1)#2	1.986(6)	Cu(2)−O(2)	1.968(5)
Cu(1)−O(11)	2.448(5)	Cu(2)−O(11)#2	2.668(4)
Cu(1)−Cu(2)	3.1207(8)	Cu(2)−O(11)	2.668(4)

O(3)−Cu(1)−N(2)#1	100.2(2)	O(3)#2−Cu(2)−O(2)	90.0(2)
O(3)−Cu(1)−N(1)#1	176.8(2)	O(3)−Cu(2)−O(2)	90.0(2)
N(2)#1−Cu(1)−N(1)#1	82.0(3)	O(2)#2−Cu(2)−O(2)	180.0(3)
O(3)−Cu(1)−O(1)#2	88.8(2)	O(3)#2−Cu(2)−O(11)#2	80.56(15)
N(2)#1−Cu(1)−O(1)#2	161.3(2)	O(3)−Cu(2)−O(11)#2	99.44(15)
N(1) #1−Cu(1)−O(1)#2	89.8(2)	O(2)#2−Cu(2)−O(11)#2	94.07(18)
O(3)−Cu(1)−O(11)	86.59(15)	O(2)−Cu(2)−O(11)#2	85.93(18)
N(2)#1−Cu(1)−O(11)	103.2(2)	O(3)#2−Cu(2)−O(11)	99.44(15)
N(1)#1−Cu(1)−O(11)	90.7(2)	O(3)−Cu(2)−O(11)	80.56(15)
O(1)#2−Cu(1)−O(11)	93.7(2)	O(2)#2−Cu(2)−O(11)	85.93(18)
O(3)#2−Cu(2)−O(3)	180.0(3)	O(2)−Cu(2)−O(11)	94.07(18)
O(3)−Cu(2)−O(2)#2	90.0(2)	O(11)#2−Cu(2)−O(11)	180.0(3)
O(3)#2−Cu(2)−O(2)	90.0(2)	Cu(1)−O(11)−Cu(2)	75.03(11)
Cu(2)−O(3)−Cu(1)	111.9(2)		

[a] Symmetry transformations used to generate equivalent atoms: #1 x, $-y + 1$, $z - {}^1/_2$; #2 $-x + 1$, $-y$, $-z$.

metal−ligand equatorial distances are similar to those found in the previously reported linear μ-acetate, μ-hydroxo trinuclear copper(II) complexes.[15,16] The apical positions are occupied by oxygen atoms of two sulfonamidate anions at the semicoordination lengths of 2.668(4) Å. Compared with the related trinuclear copper complexes,[15,16] the main difference is found in the coordination polyhedron of the central copper(II) which, in this compound, is six-coordinated instead of four-coordinated.

Adjacent copper centers are bridged in the equatorial plane by the hydroxo oxygen atom, O(3), [Cu(1)−O(3)−Cu(2) = 111.9(2)°] and two oxygen atoms, O(1) and O(2), of a bidentate *syn*−*syn* formato group leading to a mixed-bridged noncoplanar coordination geometry [dihedral angle between the mean equatorial planes N(1)N(2)O(1)O(3) and O(3)O(2)O(2)O(3), 61.4(2)°]. In the trinuclear entity, the three copper ions are arranged in a strict linear fashion [Cu(1)−Cu(2)−Cu(1A) = 180°] with Cu(2) on the inversion center and a Cu(1)···Cu(2) distance of 3.1207(8) Å.

The way of coordination of L^- is an interesting structural aspect of this compound. L^- behaves as a bridging ligand between each of two trimer units coordinating to one trimer in a bidentate fashion through the pyridyl and sulfonamidato N atoms and to other trimer through the sulfonamidato O

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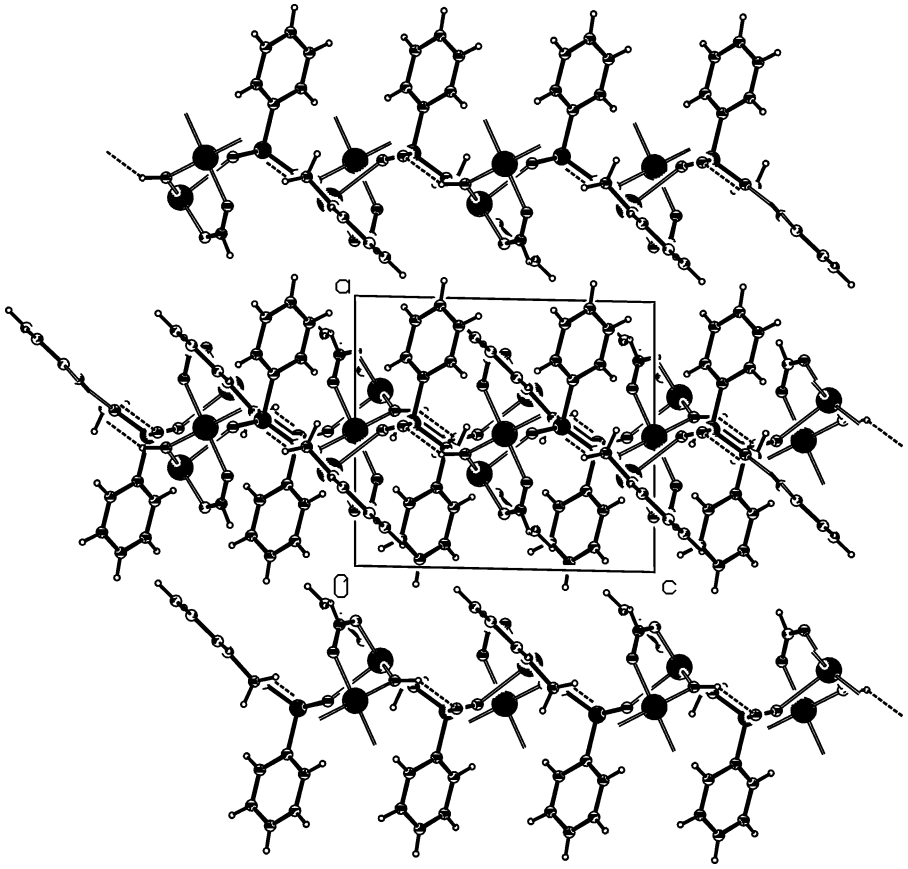

Figure 2. Stereoscopic view of $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$ along *c* axis.

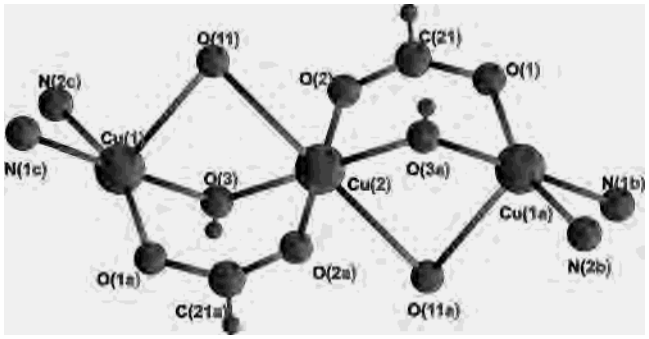

Figure 3. Fragment of $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$ showing the bridging atoms. Symmetry transformations to generate equivalent atoms: (a) $-x + 1, -y, -z$; (b) $-x, y - {}^1/_2, -z + {}^1/_2$; (c) $x, -y + {}^1/_2, z - {}^1/_2$.

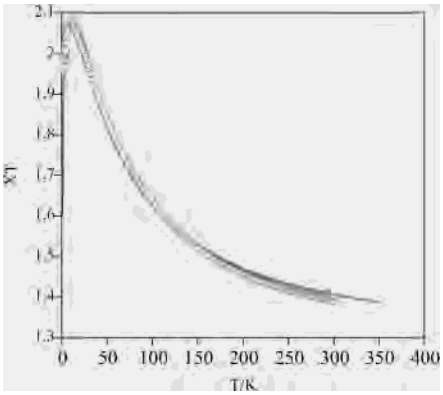

Figure 4. Temperature dependence of $\chi_M T$ for $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$.

atom, which also acts a bridge between two copper ions of the same trimer unit. The coordination of this O atom to two Cu(II) ions is unusual taking into account the poor ability of this donor atom to bind metal ions. Up to now, this compound is the first example reported where the oxygen sulfonamidate acts as a bridge.

A strong intermolecular hydrogen bond is formed between the hydroxo O(3) and the sulfonamido O(12) [O(3)···O(12), 2.764(6) Å] (see Figure 2).

Magnetic Properties. The temperature dependence of the $\chi_M T$ product for $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$ (χ_M being the magnetic susceptibility per trimer unit) in the temperature range 2−300 K is shown in Figure 4. As expected, the magnetic behavior of the complex resembles that of related trinuclear compounds.[15,16] At room temperature, $\chi_M T$ is 1.4 $cm^3\ mol^{-1}\ K$, a value which is somewhat larger than that expected for a magnetically uncoupled trinuclear Cu(II) compound (1.23 $cm^3\ mol^{-1}\ K$, $g = 2.1$). Upon cooling of the sample, $\chi_M T$ increases and reaches a value of 2.08 $cm^3\ mol^{-1}\ K$ in the 6−15 K temperature range. This behavior is indicative of strong ferromagnetic coupling between the adjacent Cu(II) in the trinuclear species. Below 5 K, $\chi_M T$ decreases rapidly down to 1.95 $cm^3\ mol^{-1}\ K$ which is most likely due to zero-field splitting (ZFS) within the quartet ground state.

The spin Hamiltonian appropriate to describe the exchange interaction in a linear symmetric trimer has the form

$$\mathbf{H} = -J(\mathbf{S}_1\mathbf{S}_2 + \mathbf{S}_2\mathbf{S}_3) - J'\mathbf{S}_1\mathbf{S}_3 + D[\mathbf{S}^2_z - S(S+1)/3] + g\beta\mathbf{HS} \quad (1)$$

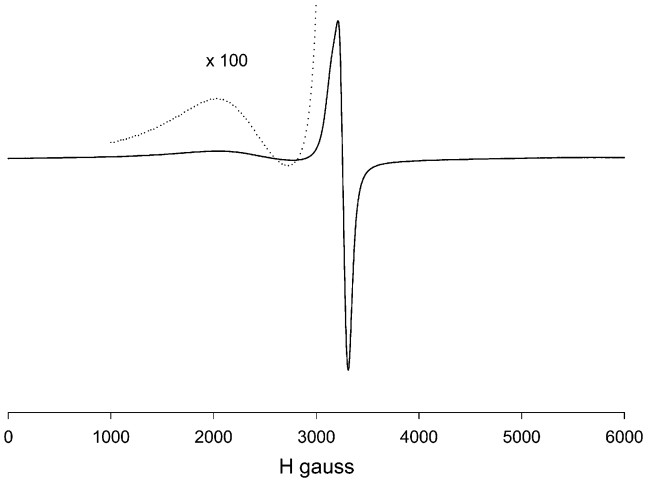

Figure 5. X-band EPR spectrum at room temperature for $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$.

where the occurrence of axial ZFS between the $\pm^3/_2$ and $\pm^1/_2$ Kramers doublets arising from the $S = {}^3/_2$ ground state has been included. The exchange parameters J and J' refer to the interaction between two adjacent centers and the interaction between the terminal centers, respectively. The resulting spin multiplets are two doublets ($S = {}^1/_2$) and one quartet ($S = {}^3/_2$).[15] D denotes the half energy gap between the Kramers doublets.

The solid and broken lines in Figure 4 represent typical fits with $J = 79$ cm^{-1}, $J' = -4.6$ cm^{-1}, $|D| = 1.7$ cm^{-1}, $g = 2.12$, and $R = 3 \times 10^{-5}$.

R values are obtained from the expression $R = \{\sum_i[(\chi_M T)_{obs,i} - (\chi_M T)_{calcd,i}]^2/\sum_i[(\chi_M T)_{obs,i}]^2\}$.

It is well-known that in dinuclear copper(II) complexes with *syn*−*syn* carboxylato bridges or hydroxo bridges, with an angle at the hydroxo bridge larger than 97.5°, antiferromagnetic coupling occurs;[25] therefore, the magnetic behavior in the title compound should be antiferromagnetic. However, when the bridging ligands are different, as it occurs in the complex, the two bridges may either increase or cancel out this effect.[15] This phenomenon is known as orbital counter-complementarity, and the reported complex is a good example of this magnetic behavior.

Recently, we proposed a correlation between J and the θ angle using DFT calculations for trinuclear Cu(II) compounds.[16] For the Cu−OH−Cu angle of 111.9(2)° in the title compound, a J value ca. 70 cm^{-1} is obtained which agrees well with the experimental one.

EPR Spectra. The X-band EPR spectra of the complex at room temperature (Figure 5) are dominated by a transition at 2047 G which identifies an $S = {}^3/_2$ ground state and confirms the ferromagnetic behavior of the compound.

Interaction between DNA and the Copper Complex. Absorption spectra of the $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$ complex in the absence and in the presence of calf thymus DNA at varied concentrations were obtained with the aim of studying the binding of complex to DNA. An electronic interaction between DNA and the trinuclear copper coordination compound can be observed through the data of hyperchromism and the short-wavelength shift of the d−d maximum of the complex (Table 3). It has been shown that the interaction of calf thymus DNA with $[Cu(H_2O)_6]^{2+}$ leads to a small shift to short wavelengths and an increase of the absorption intensity of the d−d maxima. Such types of changes have been explained considering that a small percentage of $[Cu(H_2O)_6]^{2+}$ is inner-sphere bound by the substitution of coordinated water molecules by nitrogen atoms of DNA.[26] Therefore, on the basis on the spectroscopic data in Table 3, such a mode of binding to DNA could be proposed for a small amount of $[Cu_3(L)_2(HCOO)_2(OH)_2]$ compound. Probably, donor atoms at longer distances in our compound [O(11) at Cu(2) and/or at Cu(1)] are substituted by N atoms of calf thymus DNA.

Table 3. Electronic Spectroscopic Data of the Interaction between $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$ and DNA

[DNA]/[complex][a]	λ_{max}, nm (ϵ, M^{-1} cm^{-1})	absorbance hyperchromism,%
0	740 (608)	
1	738 (608)	0
2	734 (616)	1.3
5	730 (625)	2.7
10	722 (633)	4.2
15	718 (641)	5.5
20	716 (658)	8.2

[a] [Complex] = 120 μM.

Table 4. Data for DNA Viscosity in the Presence of the Complex

[complex]/[DNA][a]	η/η_0
0.000	1.000
0.059	1.080
0.112	1.068
0.134	1.046
0.188	1.023
0.280	1.011

[a] [DNA] = 187.5 μM.

As a means for further clarifying the binding of the trinuclear complex, viscosity measurements were carried out on calf thymus DNA by varying the concentration of the complex. A classical intercalative mode causes a significant increase in the viscosity of the DNA solution due to an increase in the separation of base pairs at intercalation sites and hence an increase in overall DNA length. By contrast, complexes that bind exclusively in DNA grooves by partial and/or nonclassical intercalation under the same conditions typically cause a less pronounced (positive or negative) or no change in DNA solution viscosity.[27] In Table 4, the values of η/η_0 are collected against complex concentration/DNA concentration. The results reveal that the presence of the trinuclear complex has no obvious effect on the relative viscosity of calf thymus DNA, indicating that the interaction of the complex follows the pattern of complexes that bind in DNA grooves.

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Figure 6. Agarose gel electrophoresis of pUC18 plasmid DNA treated with complex and ascorbate/H_2O_2. Incubation time: 1 h (37 °C). Lane 1: Λ DNA/EcoRI + HindIII. Marker, 3. Lane 2: supercoiled DNA. Lane 3: ascorbate/H_2O_2 30 μM, $CuSO_4 \cdot 5H_2O$ 36 μM. Lane 4: ascorbate/H_2O_2 45 μM, $CuSO_4 \cdot 5H_2O$ 54 μM. Lane 5: ascorbate/H_2O_2 60 μM, $CuSO_4 \cdot 5H_2O$ 72 μM. Lane 6: ascorbate/H_2O_2 30 μM, complex 12 μM. Lane 7: ascorbate/H_2O_2 45 μM, complex 18 μM. Lane 8: ascorbate/H_2O_2 60 μM, complex 24 μM. Lane 9: ascorbate/H_2O_2 12 μM, $CuSO_4 \cdot 5H_2O$ 36 μM. Lane 10: ascorbate/H_2O_2 18 μM, $CuSO_4 \cdot 5H_2O$ 54 μM. Lane 11: ascorbate/H_2O_2 24 μM, $CuSO_4 \cdot 5H_2O$ 72 μM. Lane 12: ascorbate/H_2O_2 12 μM, complex 12 μM. Lane 13: ascorbate/H_2O_2 18 μM, complex 18 μM. Lane 14: ascorbate/H_2O_2 24 μM, complex 24 μM.

Table 5. DNA Cleavage Activity of the Trinuclear Complex and the Copper(II) Salt

	H_2O_2/ascorbate 2.5-fold excess relative to complex			[H_2O_2/ascorbate] = [complex]		
	% form I	% form II	% form III	% form I	% form II	% form III
36 μM Cu(II)	55.1	44.9		66.0	34.0	
12 μM complex	55.4	44.6		68.8	31.2	
54 μM Cu(II)	36.9	42.8	20.3	61.6	38.4	
18 μM complex		65.4	34.6	38.7	42.1	19.2
72 μM Cu(II)	28.7	48.7	22.6	38.4	42.1	19.4
24 μM complex	*a*				50.2	49.8

[a] It could not be determined by smearing.

DNA Cleavage by $[Cu_3(L)_2(HCOO)_2(OH)_2]_\infty$. The ability of the title compound to perform DNA cleavage has been studied by gel electrophoresis using supercoiled pUC18 DNA in cacodylate buffer (pH = 6.0). The linear trinuclear complex on reaction with DNA in the presence of a H_2O_2/ascorbate mixture as reducing agent presents high nuclease activity (Figure 6). In fact, a comparison of lane 4 [54 μM Cu(II)] with lane 7 [18 μM complex] shows that the complex cleaves completely the supercoiled DNA (form I), nicked DNA (form II), and linear DNA (form III) while the Cu(II) only breaks DNA in the nicked form. The same effect is observed in lanes 11 and 14 where the concentration of the reductant H_2O_2/ascorbate is equal to that of the complex.

The amounts of supercoiled form I, nicked form II, and linear form III were quantitated and are listed in Table 5. It is noteworthy that the complex at 18 μM produces the same amount of forms II and III as the copper(II) at 72 μM. This result suggests a possible synergy among the three metal ions that contributes to its relatively high nucleolytic efficiency.[13]

The existence of diffusible radical species in the nuclease mechanism can be inferred by monitoring quenching of DNA cleavage in the presence of alternative H-atom donors, which would scavenge radicals (such as $^{\bullet}OH$) in solution. To this end, standard scavengers of reactive oxygen intermediates were included in the electrophoretic process (Figure 7). Quantification of the gel afforded the data in Table 6. The SOD enzyme had no effect on the cleavage reaction (lane 4) suggesting that the superoxide anion is not involved in the cleavage process. Azide (lane 5) inhibits, to some extent, DNA cleavage by the compound indicating that $^1O_2$ is involved in the reaction. The hydroxyl radical scavengers, specially *tert*-butyl alcohol (lane 8), diminish significantly the nuclease activity of the compound which is indicative of the involvement of the hydroxyl radical in the cleavage process. In conclusion, the active oxygen species involved in the reaction are $^1O_2$ and $^{\bullet}OH$ although the involvement of the superoxide anion along the reaction pathway cannot be ruled out since the reaction of SOD with O_2^- produces hydrogen peroxide which appears to be required for the reaction. One can only suggest that superoxide is not itself the active species. Moreover, since the complex is not able per se to induce any effect of double stranded DNA (lane 9), it appears that a reducing agent is required for producing the cleavage process.

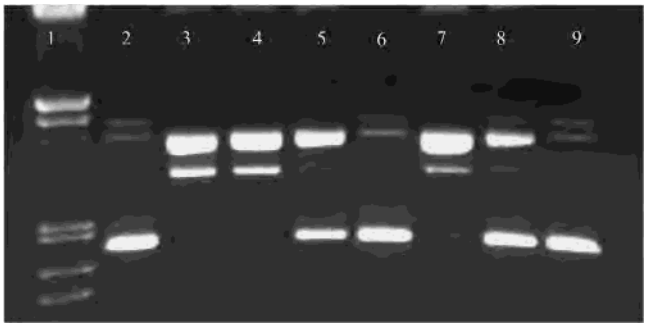

Figure 7. Agarose gel electrophoresis of pUC18 plasmid DNA treated with 24 μM complex and potential inhibitors. Incubation time: 1 h (37 °C). Ascorbate/H_2O_2 24 μM. Lane 1: Λ DNA/EcoRI + HindIII. Marker, 3. Lane 2: supercoiled DNA. Lane 3: complex. Lane 4: complex + SOD (15 units). Lane 5: complex + NaN_3 (20 mM). Lane 6: complex + EDTA (20 mM). Lane 7: complex + DMSO (1 M). Lane 8: complex + *tert*-butyl alcohol (1 M). Lane 9: complex without ascorbate/H_2O_2.

Table 6. DNA Cleavage of pUC18 by the Trinuclear Complex (24 μM) in the Presence of Potential Inhibitors[a]

	% form I	% form II	% form III
DNA	74.3	25.7	
complex		58.6	41.4
complex + SOD (15 units)		60.7	39.3
complex + NaN_3 (20 mM)	51.7	48.3	
complex + EDTA (20 mM)	72.9	27.1	
complex + DMSO (1 M)		66.1	33.9
complex + *tert*-butyl alcohol (1 M)	49.1	32.6	18.3
complex without H_2O_2/Ascorbate	75.4	24.6	

[a] H_2O_2/ascorbate 24 μM.

We also plan to continue to screen other copper(II) complexes in the hope of discovering other potentially practical inorganic complex nucleases.

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Supporting Information Available: Tables listing detailed crystallographic data, atomic positional parameters, anisotropic thermal parameters, bond lengths and angles, and least-squares planes. This material is available free of charge via the Internet at http://pubs.acs.org.

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