of the daphnetoxin class of diterpenoid esters which includes such compounds as gnididin,[8] gnidilatin 20-palmitate,[9] and mezerein.[10] It is noteworthy that gnidimacrin and gnidimacrin 20-palmitate exhibit the most potent antileukemic activity of any members of the daphnetoxin class reported to date, and are the first such compounds found to contain the novel macrocyclic orthoester structural feature. Investigations are in progress to determine the potential significance of the macrocyclic orthoester, the epoxide, and other structural features in relation to the biological activity of gnidimacrin and gnidimacrin 20-palmitate.

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(3) Leaves were collected in Kenya in February, 1974. The authors acknowledge with thanks receipt of the dried plant material from Dr. R. E. Perdue, Jr., U.S.D.A., Beltsville, Md., in accordance with the program developed by the National Cancer Institute.
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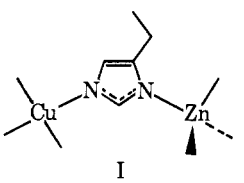
Imidazolate-Bridged Complexes of Copper(II)

Sir:

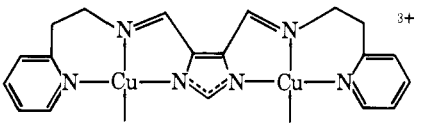
Recent studies[1] of bovine superoxide dismutase (SOD)[2] have revealed the presence of a bimetallic copper(II)–zinc(II) center at the active site. X-Ray crystallographic studies[3] of the enzyme at 3.0 Å resolution showed the metal atoms to be about 6 Å apart, with His 61 occupying the intervening space. This result is consistent with the presence of a bridging imidazolate ion, I. Although imidazolate (im) bridged metals are known



I

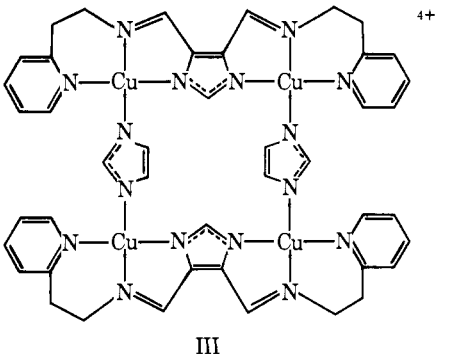
to exist in crystalline solids such as $Cu_3(imH)_8(im)_2(ClO_4)_4$,[4] $M(im)_2$, M = Cu, Zn,[5] and $Cu(imH)_2(im)Cl$,[6] no soluble binuclear or small polynuclear complexes containing this ion as a bridging ligand have apparently been characterized.[7] Such complexes would be interesting to study in conjunction with investigations of the chemical and physical properties of I in bovine SOD. A program to prepare and characterize soluble, imidazolate bridged metal complexes has therefore been undertaken. Here we report preliminary results describing the synthesis of copper(II) complexes in this class, the x-ray crystal structure of one such derivative, the effect of pH on the stability of the imidazolate bridged dicopper(II) moiety, and the magnetic exchange promoted by the bridging imidazolate ligand.

Imidazole-4,5-dicarboxylic acid was converted to the methyl diester, reduced with $LiAlH_4$ to the dialcohol, and then oxidized with MnO_2 to the dicarboxaldehyde.[8] A solution of 0.5 mmol of the imidazole-4,5-dicarboxyaldehyde and 0.5 mmol of aqueous NaOH was added to an aqueous methanolic solution containing 1 mmol each of 2-(2-aminoethyl)pyridine and cupric nitrate. After stirring the mixture at 35 °C for 18 h, the solvent was removed from the resulting blue-green solution in vacuo. The residue was dissolved in methanol and filtered. Upon standing, the filtrate yielded blue microcrystals (λ_{max}^{MeOH} 637 nm; 650 nm in H_2O, pH 4) which analyzed as the dicopper complex of the ligand 4,5-bis[2-(2-pyridyl)-ethyliminomethyl]imidazolate (bpim), $Cu_2(bpim)(NO_3)_3 \cdot 2H_2O$,[9] containing II. Addition of 1 mmol of imidazole to the



II

same blue-green solution before removal of solvent gave an immediate color change to blue. Workup in a similar manner produced blue needles ($\lambda_{max}^{CH_3OH}$ 608 nm) of a nitrate salt of $[Cu_2(bpim)(im)]_2^{4+}$ which, upon recrystallization from water, gave beautiful blue tetragonal prisms of $[Cu_2(bpim)(im)]_2(NO_3)_4 \cdot 4H_2O$,[10] containing III.



III

This compound crystallizes in the tetragonal system, space group $I4_1/a$, with eight formulas in a unit cell of dimensions $a = b = 27.204(10)$ Å, $c = 14.704(6)$ Å, ρ_{calcd}[10] = 1.674 g/cm^3, ρ_{obsd} = 1.650(2) g/cm^3. The structure, shown in Figure 1, was determined by heavy atom methods using 2821 independent reflections ($2\theta \leq 50°$, $F_o^2 > 2\sigma(F_o^2)$) collected on a computer controlled diffractometer at 23° using monochromatized Mo Kα radiation. The value for the conventional agreement factor, $R_1 = \sum \| F_o | - | F_c \| / \sum | F_o |$, at the present stage of refinement is 0.066. Details will be reported later.

The structure determination confirms that the $Cu_2(bpim)^{3+}$ moiety (II) contains an imidazolate bridge. In III, two of these units are linked by two additional bridging imidazolate ligands. The resulting dimer has crystallographically required twofold symmetry. Selected geometric details are given in the caption to Figure 1. Water and nitrate oxygen atoms are weakly coordinated to the remaining, axial sites on copper in the crystal.

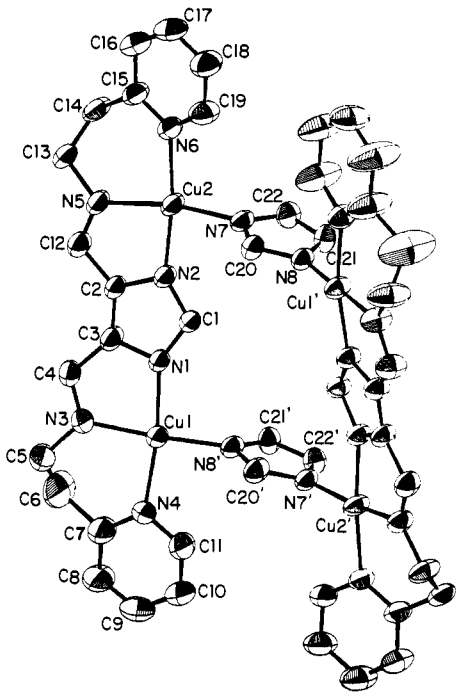


Figure 1. Structure of $[Cu_2(bpim)(im)]_2^{4+}$ showing the 40% probability thermal ellipsoids. Axially coordinated water and nitrate ligands and hydrogen atoms are omitted from the drawing. Selected bond distances (in Å, esd ~0.01 Å) are: Cu1-N1, 2.008; Cu1-N3, 1.992; Cu1-N4, 2.097; Cu1-N8′, 1.972; Cu2-N2, 1.992; Cu2-N5, 2.009; Cu2-N6, 2.056; Cu2-N7, 1.964; Cu1···Cu2, 6.214; Cu1···Cu2′, 5.910. Selected bond angles (in degrees, esd ~0.3°) are: N1-Cu1-N3, 81.6; N3-Cu1-N4, 89.1; N4-Cu1-N8′, 94.2; N8′-Cu1-N1, 94.7; N2-Cu2-N5, 80.8; N5-Cu2-N6, 91.3; N6-Cu2-N7, 98.3; N7-Cu2-N2, 89.7. Copper atoms are all within ±0.3 Å of the planes defined by the bridging imidazolate rings. A crystallographically required twofold axis relates the primed and unprimed atoms. The best planes through the two $Cu_2(bpim)$ halves of the dimer intersect with a dihedral angle of 136.5°.

The compounds containing II and III are soluble in polar solvents such as methanol or water. The ease with which II is converted into III upon addition of imidazole is striking. This process was investigated in more detail by carrying out pH titrations of a 2 mM aqueous solution of $[Cu_2(bpim)(im)]_2(NO_3)_4 \cdot 4H_2O$ at 25°, ionic strength 0.16. This complex reversibly takes up four protons between the end points at pH 9.75 and 4.25. The midpoint occurs at pH ~7 where there might be a slight inflection. Spectroscopic studies in water show the copper d → d band to shift from 608 nm at pH 10 to 649 nm at pH 4. The pH titration data are therefore tentatively interpreted according to eq 1. The possibility that reversible, pH dependent imidazolate-bridge-splitting reactions such as eq 1 might occur at the active site of bovine SOD has been discussed previously.[11]

$$\text{III} + 4H^+ \rightleftharpoons 2\text{II} + 2\text{im}H_2^+ \qquad (1)$$

Magnetic susceptibility studies of III by the Faraday method on solid samples over the temperature range $4.5\ \text{K} < T < 320\ \text{K}$ show the copper centers to be antiferromagnetically coupled. A maximum occurs at 160 K in the susceptibility vs. T plot and the compound is diamagnetic below 35 K. Since the four copper(II) centers are connected by two different kinds of imidazolate bridges, analysis of the spin exchange (currently in progress) will require at least two coupling constants (J) in the Hamiltonian. It may be noted that a value of ~52 cm^{-1} was estimated for $-2J$ from the temperature dependence of the EPR spectrum in an active derivative of bovine SOD in which copper(II) had been substituted into the zinc(II) site of the enzyme.[12]

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(10) Calcd for $C_{44}H_{52}N_{20}O_{16}Cu_4$: C, 38.54; H, 3.82; N, 20.43. Found: C, 38.37; H, 3.72; N, 20.53. If one water molecule is removed from the formula, the calculated percentages are: C, 39.06; H, 3.72; N, 20.70, and the density becomes 1.652 g/cm³, in better agreement with the experimental value. The x-ray structure analysis reveals that one lattice water is disordered and perhaps should be given only partial occupancy. This point is being pursued further.
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The Crystal and Molecular Structures of $[(C_6H_5)_4P]_2Fe(S_2C_4O_2)_2$ and $[(C_6H_5)_4P]_2Fe(SC_6H_5)_4$, a Structural Analogue of Reduced Rubredoxin

Sir:

A variety of non-heme, iron-sulfur, proteins have been examined by spectroscopic and x-ray crystallographic studies. These, redox active, metalloproteins have been grouped into three general types, and the proposed or determined structures for the iron-containing sites form the basis of this classification.[1] In these sites the iron atoms are coordinated by deprotonated cysteinyl sulfur atoms (S-Cys), inorganic sulfide ions (S*), or both of these ligands. The basic iron-sulfur cores, present in each of these major types are: (a) $Fe(S\text{-}Cys)_4$, in rubredoxins (Rd); (b) $Fe_2S^*_2(S\text{-}Cys)_4$, in bacterial, plant, and mammalian ferredoxins (Fd); and (c) $Fe_4S^*_4(S\text{-}Cys)_4$, in "high-potential" (HP), and certain 4- and 8-Fe bacterial ferredoxins.

The tetrahedral 1-Fe site has been established by x-ray crystallography in Rd_{ox} from Clostridium pasteurianum.[2] The 4-Fe "cubane" structure has been determined for the active site in HP_{red} from Chromatium,[3] and for the two, nearly identical, $Fe_4S^*_4(S\text{-}Cys)_4$ clusters in the 8-Fe, Fd_{ox} from Peptococcus aerogenes.[4] Synthetic analogues of all three structural types of these active sites have been obtained and structurally characterized by Holm, Ibers, and co-workers. These investigators have characterized in detail successful electronic analogues for the active sites of HP_{red}, 4- and 8-Fe