

## Model compounds for the type III site and the combined type II and type III sites in multicopper oxidases

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**Abstract.** A series of model complexes for the diamagnetic type III site in multicopper oxidases has been developed based on tridentate ligands formed from the Schiff base condensation of bifunctional primary amines with the monoximes of  $\beta$ -diketones. The complexes resulting when copper salts,  $\text{CuX}_2$ , react with the resulting ligands, HL, have the general formula  $\text{Cu}_2\text{L}_2\text{X}_2$  and have a central core made up of the two copper atoms bridged by two oxime groups resulting in a six-membered  $\text{Cu}(\text{ON})_2\text{Cu}$  moiety. This grouping is strictly planar and has significant shortening of the N–O bond length along with lengthening of the C=N bond indicating a delocalization of the  $\pi$ -electrons in the ring. The result is a copper(II) dimer which is diamagnetic at all measurable temperatures (< 400 K). Spectroscopic, magnetic and electrochemical properties of the series of compounds will be compared with those of the parent enzyme containing the type III dicopper site. A second series of model complexes for both the type III site and the newly reported structure of the joint type II and type III site in ascorbate oxidase (crystal structure reported at the 3rd International Conference on Bioinorganic Chemistry, Boston, Mass, July 24–28, 1989) is also discussed. These compounds are obtained when ligands,  $\text{H}_3\text{L}$ , resulting from the condensation of derivatives of salicylaldehyde with 1,3-diamino-2-propanol, are reacted with copper carboxylates resulting in compounds of formula  $\text{Cu}_2\text{L}$  (carb). The carboxylate bridging group can be displaced by other better donors,  $\text{L}'$ , such as pyrazole, or indoles to give compounds  $(\text{Cu}_2\text{LL}')^+$  which have bridging  $\text{L}'$  groups. The properties of this series of compounds will be compared with those of the type III site. If the  $\text{L}'$  ligand is suitably derivatized, i.e. 6-aminoindole, then by a Schiff base condensation with copper salicylaldehyde, compounds of formula  $(\text{Cu}_2\text{LL}')_2\text{Cu}(\text{sal})_2$  can be obtained, which are models for multicopper oxidases having both the type II and type III sites in the same molecule, as has been found in the recent crystal structure of ascorbate oxidase. Comparisons between these models and multicopper oxidases will be drawn.

**Keywords.** Multicopper oxidases; type II sites; type III sites; combined type II & type III sites.

Copper is an important constituent of many important enzymes. In these enzymes three different environments for the copper have been found. These different copper centers have been labelled as type I, type II and type III. Type I copper centers, often called “blue copper proteins”, such as azurin and plastocyanin, are characterized by an intense blue color due to the particular coordination environment of the copper atom. In the structure of azurin and plastocyanin, two “blue” copper proteins, it was found that the copper atom was in an  $\text{N}_3\text{S}$  tetrahedral environment. The unusual spectral characteristics of the “blue” copper proteins are well documented.

Type II copper sites are characterized by spectroscopic parameters of “normal”

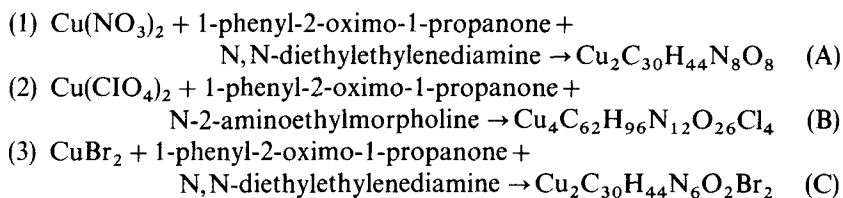
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tetragonal copper centers, while type III centers have unusual magnetic properties. It has been found that in this site there are two copper (II) atoms bridged by exogenous groups with strong antiferromagnetic interactions such that the binuclear site is diamagnetic at room temperature.

The various copper-containing enzymes have either type I, type II, or type III sites. One particular class of copper-containing enzymes are the multicopper oxidases, such as ascorbate oxidase, the laccases, and ceruloplasmin, which are characterized by containing all three sites in the same enzyme. At the recent International Conference on Bioinorganic Chemistry held in July in Cambridge, Massachusetts, the structure of one of these, ascorbate oxidase, was presented. This showed that the type II and type III sites were close together ( $\approx 4\text{\AA}$  apart) with the nearest type I site more distant ( $\approx 12\text{\AA}$  away).

In the present paper models have been developed that mimic the properties of the type III site and the joint type II/type III site to a greater or lesser extent. The first group of compounds to be discussed are the models for the type III site alone, based on oxime-type ligands. First of all, what are the properties of the type III site itself. It is characterized by its ability to act as a two-electron acceptor/donor system. It shows a strong absorption band with a maximum at 330 nm and has no EPR signal, as it is non-paramagnetic over a wide temperature range. These properties have been shown to be a consequence of having two copper(II) atoms in close proximity.

The oxime type binuclear copper complexes were synthesised by reacting the appropriate copper salt with a dione-monoxime, such as butane-2, 3-dione monoxime, in the presence of a difunctional primary amine, such as N,N-diethylethylenediamine to give the title compounds. This is summarized below:



The compounds were characterized by analytical means. Compound B was crystallized from methanol and subjected to a single crystal X-ray structure determination. This revealed the presence of both neutral and charged dimeric units as well as free perchlorate ions. If the ligand is abbreviated as L then the formula is



Each dimeric unit consists of two copper atoms bridged by two oxime N–O moieties to make up a six-membered ring. The coordination sphere around each copper is completed by the imine nitrogen, the amine nitrogen from the morpholine ring, and for the charged dimeric unit by an axial methanol, while for the uncharged dimeric unit, by an axial perchlorate ion. A diagram of the structure of the charged dimeric unit is shown in figure 1, while the ionic packing diagram for the structure is shown in figure 2.

An examination of the structure shows that the six-membered bridging ring is strictly planar as at its center there is a crystallographically imposed center of symmetry. Other features of the structure are regular. The magnetic properties of this

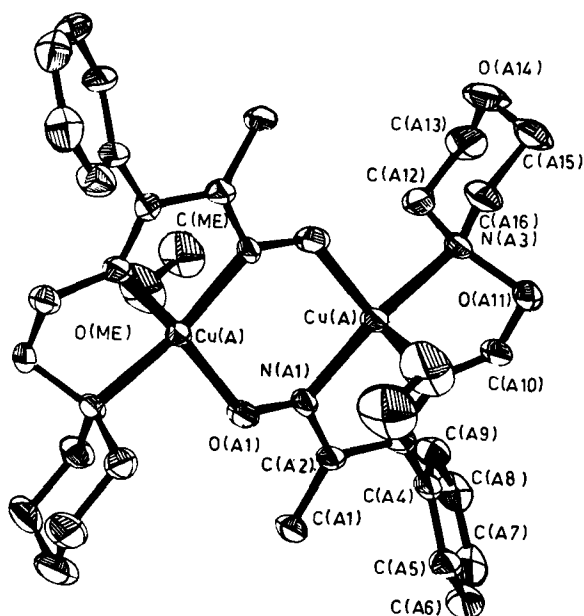


Figure 1. Structure of dimeric unit in (B).

structure (and of A and C) show that the molecule is diamagnetic at all temperatures up to the decomposition point. This means that while the strength of the antiferromagnetic interaction is large it cannot be estimated exactly but must have a  $J$  value greater than  $1000 \text{ cm}^{-1}$ .

In a survey of other dimeric oxime-bridged copper structures it was seen that they fall into three main groups based on their magnetic properties. The first group is characterized by very strong antiferromagnetic interactions and the present series of complexes fall into this category. The second group are characterized by antiferromagnetic interactions of intermediate strength and the third group have either weak antiferromagnetic or ferromagnetic interactions. We have shown that all compounds falling into the first group have planar or almost planar oxime-bridging groups, while compounds in the latter two categories have non-planar oxime bridges. An additional useful parameter that we have developed for monitoring the strength of the exchange interaction is the extent of delocalization of the  $\pi$ -electron density in the C–N oxime bond into the bridging ring. This would show up as a lengthening of the C–N oxime bond and a shortening of the N–O oxime bond. The present structure has the largest difference between these two bonds and would thus seem to have the strongest interaction on the basis of this criterion.

The electrochemistry of complexes A, B, and C show that A and B undergo reversible one-electron reduction at  $-0.87$  and  $-0.78$  V respectively while C undergoes an irreversible reduction at  $-0.92$  V. A and B also show an irreversible second one-electron reduction at more negative potentials. These are shown in figures 3 to 5. Compounds A, B, and C also have UV–vis peaks at approximately 330 nm, thus they mimic some of the properties of the type III center in being EPR silent, diamagnetic at room temperature, and having similar spectroscopic properties.

The second type of model has been developed to mimic some of the properties of

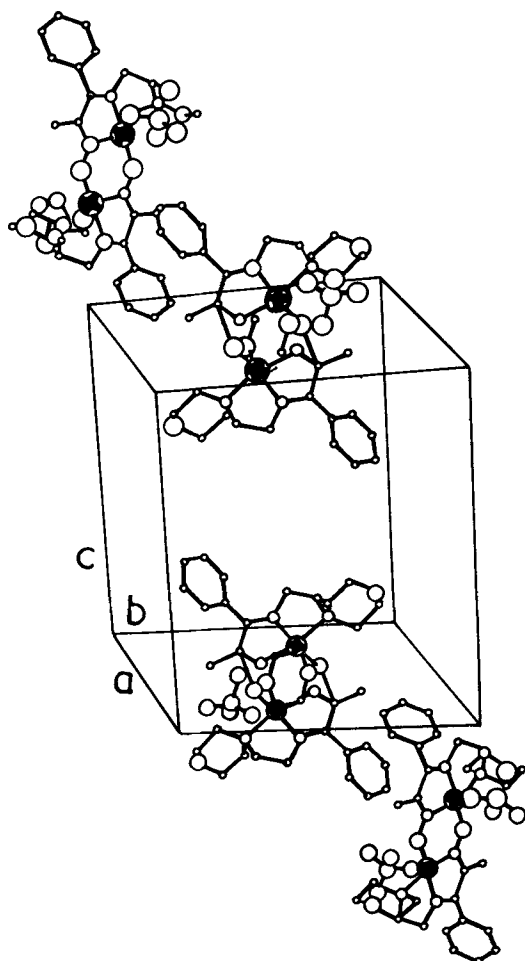


Figure 2. Ionic packing of (B) in unit cell.

the multicopper oxidases, in particular, the close proximity of the type III center to the type II center. This work has developed out of our studies on type III models based on Schiff base ligands derived from the condensation of 1, 3-diamino-2-propanol with appropriately derivatized *o*-hydroxybenzaldehydes ( $H_3L$ ). When reacted with appropriate copper carboxylate, compounds of formula  $[Cu_2L(carb)]$  result.

In a series of structurally characterized compounds it was shown that these binuclear compounds consist of the two copper atoms bridged by the alkoxide oxygen and also by the carboxylate moiety. This carboxylate moiety can be readily displaced by other bridging ligands, such as  $OH^-$ ,  $Cl^-$ ,  $N_3^-$ ,  $NCS^-$ , and pyrazole, to give a series of compounds in which the copper atoms are in differing environments. From this we have shown that the strength of the interaction between the copper atoms in these compounds is dependent on the local geometry about each copper atom and the amount of coplanarity between the two copper centers.

In the present study we have taken the binucleating ligand  $H_3L$  and synthesised binuclear complexes where the exogenous bridging ligand ( $L'-NH_2$ ) has been suitably

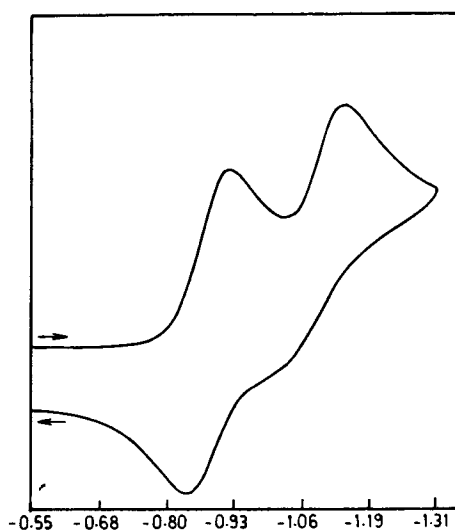


Figure 3. Cyclic voltammogram of (A) in DMF at 100 mV/s.

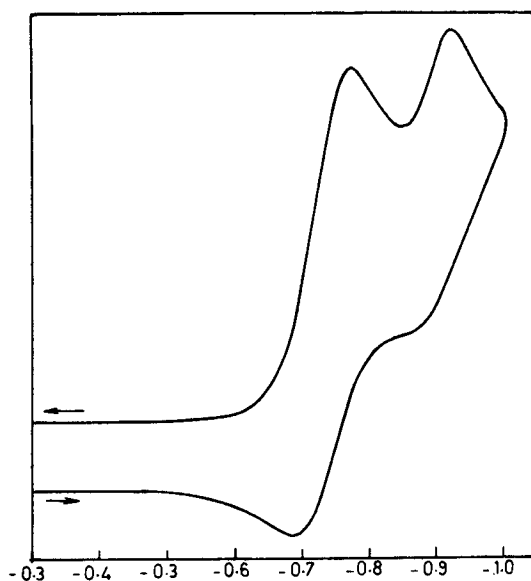


Figure 4. Cyclic voltammogram of (B) in acetone at 50 mV/s.

derivatized to allow for further reaction, such as 6-aminoindole,  $\beta$ -alanine, 4-aminobutyric, and 4-aminobenzoic acid. One such derivative, that with 6-aminoindole, was subjected to a single crystal structural analysis. Crystals grown from methanol were found to be monoclinic,  $P2_1/c$ , with  $a = 16.300(3)\text{\AA}$ ,  $b = 8.612(2)\text{\AA}$ ,  $c = 19.477(4)\text{\AA}$ ,  $\beta = 104.42(2)^\circ$ ,  $Z = 4$ ,  $D_c = 1.57\text{ g/cm}^3$ ,  $\mu = 16.54\text{ cm}^{-1}$ ,  $F(000) = 1288$ . The structure was solved by direct methods and refined to an  $R = 4.32\%$ . A diagram of the molecule is shown in figure 6.

In this structure the  $\text{H}_3\text{L}$  ligand is in the  $\text{L}^{3-}$  form and the alkoxide oxygen atom

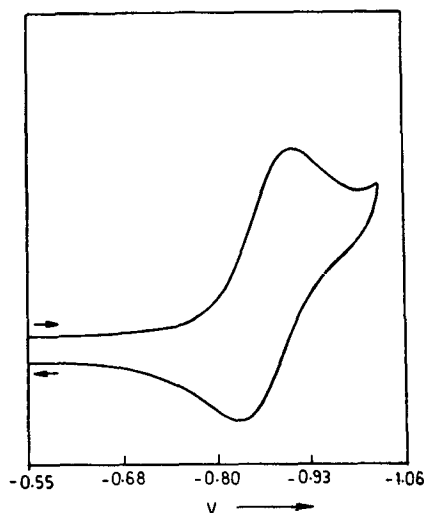


Figure 5. Cyclic voltammogram of (A). First wave in DMF at 20 mV/s.

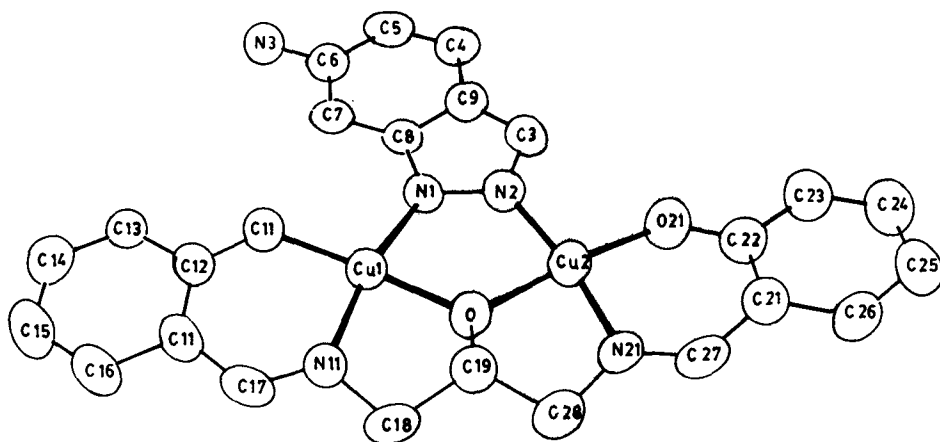


Figure 6. Structure of  $[\text{Cu}_2\text{DPLsal (6-amino indole)}]$ .

bridges the two copper atoms. The ligand also provides each copper with additional N and O donors. The coordination sphere about each copper atom is completed by the bridging 6-aminoindole moiety. Important metal ligand distances and angles are given in table 1. From these values it can be seen that the two copper atoms are in similar environments but with significant bond-length and bond-angle differences between the two sites.

Copper 1 is  $0.029(1)\text{\AA}$  and copper 2 is  $0.033(1)\text{\AA}$  out of the least squares plane defined by their respective donor atoms. The angle between these two donor planes is  $8.3(1)^\circ$ , thus reflecting the fact that the molecule as a whole is almost planar. Similarly copper 1 is  $0.072(1)\text{\AA}$  and copper 2 is  $0.259(1)\text{\AA}$  out of the six-membered chelate plane defined by the phenolic oxygen and imine nitrogen donors and their respective intervening atoms. The plane of the bridging 6-aminoindole ligand makes angles of  $6.4(1)^\circ$  and  $3.0(1)^\circ$  respectively with the copper 1 and copper 2 donor planes,

**Table 1.** Copper–ligand bond lengths and bond angles in [Cu<sub>2</sub>L(6-aminoindole)].

Bond length	Distance	Bond angle	Value	Bond angle	Value
Cu1-O	1.901(4)	O-Cu1-O11	175.7(2)	O-Cu2-N21	82.7(2)
Cu1-O11	1.902(4)	O-Cu1-Ni	87.3(2)	O21-Cu2-N2	95.8(2)
Cu1-N1	1.976(5)	O-Cu1-N11	82.3(2)	O21-Cu2-N21	94.4(2)
Cu1-N1	1.945(5)	O11-Cu1-N1	96.9(2)	N2-Cu2-N21	169.8(2)
Cu2-O	1.887(4)	O11-Cu1-N11	93.6(2)	Cu1-O-Cu2	124.4(2)
Cu2-O21	1.913(4)	N1-Cu1-N11	168.7(2)	Cu1-N1-N2	118.6(4)
Cu2-N2	1.954(5)	O-Cu2-O21	174.7(2)	Cu2-N2-N1	121.4(4)
Cu2-N21	1.941(5)	O-Cu2-N2	87.1(2)		

again reflecting the planar nature of the molecule. The magnetic properties of the compound show the presence of strong antiferromagnetic interactions as a consequence of this planarity.

Since these molecules contain a free amino group further reactions are possible. By reacting these molecules with substituted 2-hydroxybenzaldehyde complexes of copper, new complexes are obtained which contain five copper centers. One such derivative, resulting from the reaction of the above structurally characterized molecule with the 3-ethoxysalicylaldehyde complex of copper, was itself crystallized and subjected to a single-crystal structure determination. Crystals grown from a mixture of methanol and dichloromethane were found to be monoclinic,  $C2/c$ ,  $a = 27.211(9)\text{\AA}$ ,  $b = 20.303(4)\text{\AA}$ ,  $c = 14.067(5)\text{\AA}$ ,  $\beta = 112.71(2)^\circ$ ,  $Z = 4$ ,  $D_c = 1.54\text{ g/cm}^3$ ,  $\mu = 15.35\text{ cm}^{-1}$ ,  $F(000) = 3436$ . The structure was solved by direct methods and refined to an  $R = 5.8\%$ . A diagram of the molecular unit is shown in figure 7. Since the central copper atom is on a crystallographic two-fold axis the unique part of the molecule is shown in figure 8 which also shows the location of the water molecule.

It can be seen from the structure that the molecule has adopted the unusual *cis* configuration instead of the more usual *trans* geometry. This has some interesting structural and chemical consequences. The two binuclear units adopt a configuration which makes them almost parallel, while the indole rings are eclipsed allowing for the possibility of  $\pi$ - $\pi$  interactions. The type II copper atom is considerably distorted towards tetrahedral geometry from regular square planar geometry as can be seen from the diagram. Relevant metal–ligand bond lengths and angles are gathered in table 2.

Comparing the bond lengths and angles for this compound with the values for the previous binuclear compounds it can be seen that the basic geometry of the binuclear unit is preserved with some minor variation in the metal bond lengths and angles. The binuclear unit in the present structure is again almost planar but with some ruffling as can be seen from figure 7. For each copper in the binuclear unit the donor atoms form an approximate plane [maximum deviation  $0.194(8)\text{\AA}$  for O] with Cu being  $0.020(1)\text{\AA}$  and Cu' being  $0.069(1)\text{\AA}$  above the plane. The angle between the two donor planes for each copper atom is  $9.7(1)^\circ$  reflecting this planar nature.

The water molecule in the structure is involved in strong hydrogen bonds to the oxygen atoms of the ethoxy groups attached to the salicylaldehyde at the base of the structure and also to the phenolic oxygen atoms. This forces the structure to adopt the *cis* configuration leading to the unusual features of this structure.

To confirm that it was indeed the involvement of the water molecule in hydrogen bonding to the 3-ethoxy group, crystals of the 3-methoxysalicylaldehyde derivative

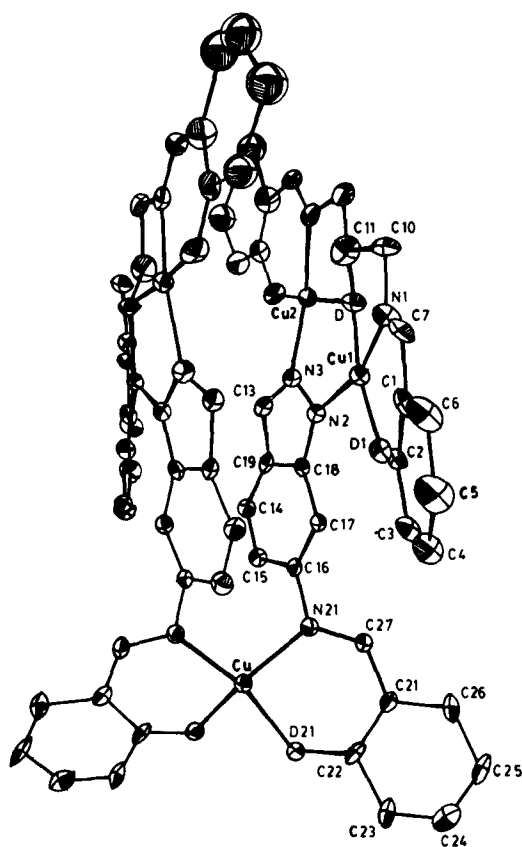


Figure 7. Structure of the pentanuclear copper complex.

of copper were grown from methanol. This molecule also crystallizes with one water molecule per complex and it was suspected that this water molecule would also show the same type of hydrogen bonding as seen in the pentanuclear copper complex seen above. The crystals were orthorhombic, *Pbca*,  $a = 18.013(2)\text{\AA}$ ,  $b = 15.291(3)\text{\AA}$ ,  $c = 11.804(2)\text{\AA}$ ,  $Z = 8$ ,  $D_c = 1.57\text{ g/cm}^3$ ,  $\mu = 13.78\text{ cm}^{-1}$ ,  $F(000) = 1576$ . The structure was solved by direct methods and refined to  $R = 4.5\%$ .

Figure 9 shows the molecule unit and also the hydrogen bonding interactions of a similar nature to that found in the pentanuclear copper complex between the water molecule and the oxygen atoms of the 3-methoxy groups which are responsible for this structure also adopting a *cis* configuration. Table 3 lists the important metal ligand bond lengths and bond angles as well as the hydrogen-bond lengths. It can be seen from the values of the hydrogen bonds that they are indeed strong and thus force the molecule to adopt the unprecedented *cis* geometry. The environment about the copper atom is square pyramidal with the four oxygen donors from the ligand making up the base and the water molecule as the apex. This same water molecule is involved in intermolecular hydrogen bonds to an adjacent molecule. The four basal oxygen donors make up a least squares plane with the copper atom pulled out of the plane by  $0.1980(9)\text{\AA}$  due to the water molecule.

As far as the chemical and spectroscopic properties of the pentanuclear copper



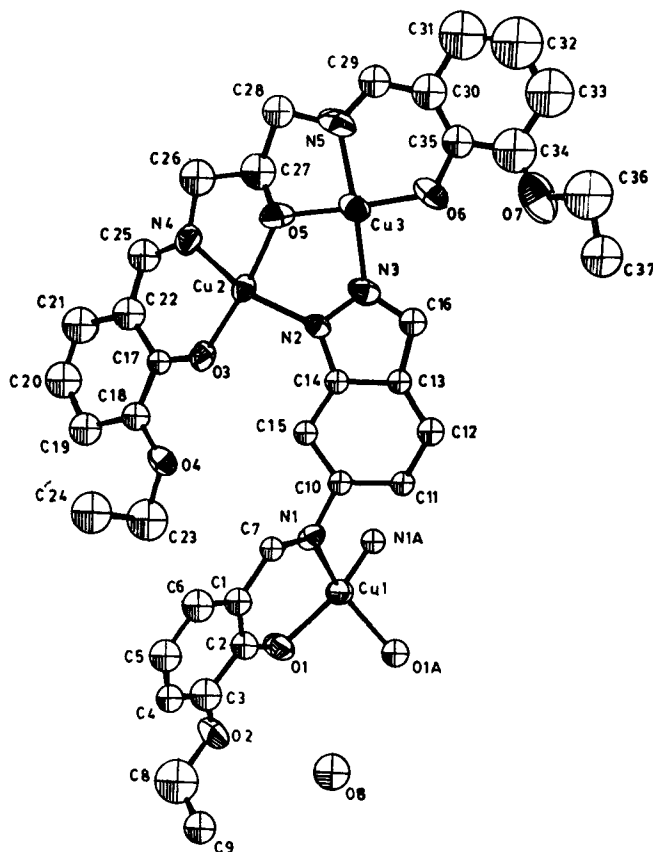


Figure 8. Unique part of pentanuclear structure.

Table 2. Selected bond lengths and angles in  $[\text{Cu}_2\text{L}(\text{L}'-\text{N})]_2\text{Cu}(3\text{-EtOsal})_2$ .

Atoms	Bond length	Atoms	Bond angle	Atoms	Bond angle
Cu'-O	1.862(8)	O-Cu'-O1'	173.4(4)	O1-Cu-N2	98.3(4)
Cu'-O1'	1.872(9)	O-Cu'Ni'	85.5(5)	N1-Cu-N2	163.8(5)
Cu'-Ni'	1.937(12)	O-Cu'-N3	86.4(4)	O21-Cu1-N21	92.7(4)
Cu'-N3	1.966(9)	O1'-Cu-N1'	95.2(6)	Cu'-O-Cu	124.9(4)
Cu-O	1.925(8)	O1'-Cu'-N3	94.7(4)		
Cu-O1	1.876(7)	N1'-Cu'-N3	162.9(5)		
Cu-N1	1.926(10)	O-Cu-O1	169.8(3)		
Cu-N2	1.965(9)	O-Cu-N1	83.7(5)		
Cu1-O21	1.899(7)	O-Cu-N2	86.2(4)		
Cu1-N21	1.989(8)	O1-Cu-N1	93.8(5)		

complexes are concerned they all show two  $\nu(\text{C}-\text{N})$  bands in the  $1628\text{--}1642\text{ cm}^{-1}$  region due to the two different types of imine bond. The solid state visible spectra of the complexes is consistent with the copper atoms in the complexes all having a square planar environment.

The complexes with a  $\text{CuN}_2\text{O}_2$  environment have a shoulder at  $560\text{--}570\text{ nm}$ , while those where the binuclear copper atoms have a  $\text{CuNO}_3$  environment, have a band at  $620\text{--}635\text{ nm}$ . The variable temperature magnetic data on the complexes indicate that

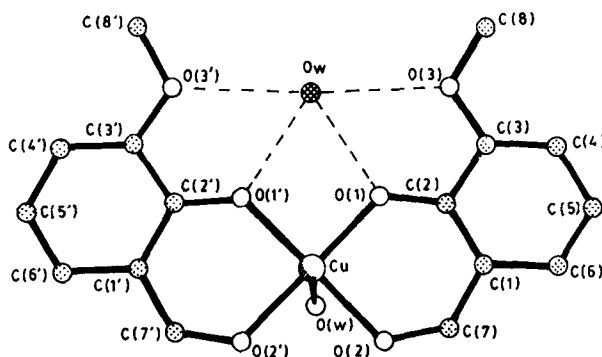


Figure 9. Structure of  $[\text{Cu}(\text{3MeOsal})_2] \cdot \text{H}_2\text{O}$ .

Table 3. Selected bond lengths and bond angles in  $[\text{Cu}(\text{3-MeOsal})_2]$ .

Bond	Length	Atoms	Bond angle
Cu-O(W)	2.258(5)	O(W)-Cu-O1'	98.9(2)
Cu-O1'	1.909(5)	O(W)-Cu-O1	98.5(2)
Cu-O1	1.927(5)	O(W)-Cu-O2	92.2(2)
Cu-O2	1.951(6)	O(W)-Cu-O2'	93.7(2)
Cu-O2'	1.950(5)	O1'-Cu-O1	87.7(2)
O(W)-O1'	2.911(7)	O1'-Cu-O2	168.7(2)
O(W)-O1	2.904(7)	O1'-Cu-O2'	91.8(2)
O(W)-O3	2.885(7)	O1-Cu-O2	92.5(2)
O(W)-O3'	2.902(7)	O1-Cu-O2'	167.7(2)
		O2-Cu-O2'	85.6(2)

there are strong antiferromagnetic interactions between the copper atoms in the binuclear units but that these are magnetically isolated from the central copper atom.

As far as the electrochemistry of the pentanuclear complexes is concerned, in most of the complexes the central copper atom is irreversibly reduced in a one-electron process while the two binuclear copper centers exhibit a four-electron reduction process which appears to be quasi-reversible. The pentanuclear complex involving the 6-aminoindole, on the other hand, has only one reduction process which is probably associated with the reduction of all the copper atoms.

In conclusion it can be said that models for both the type III and the type II/III combined copper sites have been synthesised and characterized. The type III models mimic some of the properties of the parent enzymes, especially their magnetic properties. The type II/III model is the first to combine antiferromagnetically coupled copper binuclear units with "normal" copper sites as found in the structure of ascorbate oxidase.

### Acknowledgements

RJB would like to acknowledge NIH-MBRS for partial support of this work, and DOE, NSF and the Howard University Graduate School of Arts and Sciences for funds to purchase an X-ray diffractometer and associated computing equipment.