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## Cu(II) Chelates of an Intermediate Species Absorbing in the 500-nm Region in Nonenzymatic Pyridoxal Catalysis

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The course of the formation of a quinonoid species absorbing in the 500-nm region was studied in reactions of pyridoxal, ethyl methionate and Cu(II) in the presence and absence of chelating ligands, such as ethylenediamine, diethylenetriamine,  $\alpha, \alpha'$ -bipyridine and  $\alpha, \alpha', \alpha''$ -terpyridine in methanol solvent. The results indicated the formation of six-coordinated mixed chelates of Cu(II) with the quinonoid and the chelating ligand having retarded the transformation of the chelated quinonoid to the aldimine. The reaction system was thought to serve as a model for the key intermediate in reactions catalyzed by pyridoxal phosphate enzymes.

**Keywords:** pyridoxal; quinonoid intermediate; Cu(II) chelate; ethylenediamine;  $\alpha, \alpha', \alpha''$ -terpyridine.

### INTRODUCTION

It is well-known that enzymes containing pyridoxal play important roles in amino acid metabolism. A key step in the action of almost all pyridoxal phosphate-dependent enzymes is the formation of a quinonoid species, in which the  $\alpha$ -carbon in a Schiff base (aldimine) is deprotonated. Several enzymes have been reported to exhibit an intense absorption band in the 500-nm region of the spectrum upon the addition of substrates or pseudosubstrates. The absorption has been ascribed to the quinonoid species.<sup>1, 2)</sup>

In the studies of the nonenzymatic reaction of pyridoxal, UV-Vis spectral analysis has been playing a crucial role for clarifying the existence of several intermediates of the catalytic process.<sup>3)</sup> In previous papers,<sup>4-6)</sup> we have reported on the course of the

formation of a quinonoid species absorbing in the 500-nm region in the reaction of pyridoxal(PL), ethyl L-alaninate (AlaOEt) and Al(III) in methanol. The species was thought to serve as a model for the key intermediate in reactions catalyzed by pyridoxal phosphate (PLP) enzymes.<sup>1, 2)</sup> The quinonoid was metastable in the form of the Al(III) chelate. It was chromatographically separated, and its quantitative transformation of the quinonoid to the aldimine chelate was observed.<sup>6)</sup> The same species was formed by the reaction of pyridoxamine(PM) and ethyl pyruvate (PyrOEt) in the presence of Al(III).<sup>4-6)</sup> In addition, we reported in another paper<sup>7)</sup> that the formation of similar species that has absorbance in the 500 nm region was observed in the reaction of PM and pyrroloquinoline quinone (PQQ), 1, 7-phenanthroline-5, 6-quinone, and 4, 7-phenanthroline-5, 6-quinone in an alkaline methanol solution.

Other trivalent metal ions of aluminum subgroups (Ga(III), In(III)) and most lanthanide metal ions formed the same kind of the quinonoid metal chelates in the reactions of PL, AlaOEt and the metal

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ions. With divalent metal ions, however, stable intermediates having visible absorption were unobservable under the same conditions.<sup>5)</sup> In the previous study, we observed very short-lived visible species in the reactions of pyridoxal *N*-methochloride, AlaOEt and divalent metal ions, such as Mn(II), Cu(II), Co(II), Zn(II) and Ni(II).<sup>8)</sup> Although the lifetimes of these species were less than several seconds, that of the Cu(II) chelate was found to have become longer in the presence of other chelating ligands, such as ethylenediamine (en) and diethylenetriamine (dien). These results prompted us to examine the reactions of PL, an ester of amino acids, and Cu(II) in the presence and absence of various chelating ligands. Using these systems, we investigated the structural factors for the stabilization of the quinonoid intermediates. This paper describes the results.

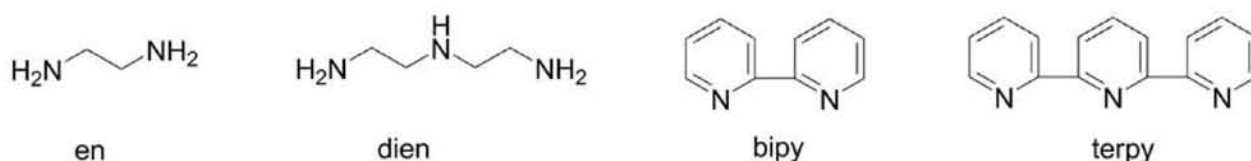


Chart 1  
Chelating ligands used

otherwise noted. The chelating ligands used in the study were ethylenediamine (en), diethylenetriamine (dien),  $\alpha, \alpha'$ -bipyridine (bipy) and  $\alpha, \alpha', \alpha''$ -terpyridine (terpy). (Chart 1) They were obtained from commercial sources.

## RESULTS

The results<sup>9)</sup> of the reactions of MetOEt are described in this paper. The replacement of MetOEt by ethyl L-alaninate (is AlaOEt) and other amino acid esters gave almost the same results.

### Reactions in the absence of chelating ligands

A solution containing PL, MetOEt and Cu(II) perchlorate in a 1:1:1 ratio (each reactant, 0.1 mM) gave a spectrum with an absorption maximum at around 400 nm, which was assigned to the Cu(II) chelate of the aldimine.<sup>10,11)</sup> Very slight absorption (maximum absorbance of less than 0.001) at around

## Experimental

The materials and the general experimental procedures were essentially the same as described previously.<sup>4-6, 8)</sup> Methanolic solutions of PL and ethyl ester of L-methionine (MetOEt) were mixed and allowed to stand for 2 h at 25°C in the dark. The spectrum indicated the formation of the aldimine of PL and MetOEt. A methanolic solution of Cu(II) was added to the aldimine solution and the moment of the addition was taken as the initial time of the reaction. The mixture was shaken, transferred to silica cells and submitted to an absorption measurement. The absorption spectra were recorded on a Shimadzu UV-240 UV-visible recording spectrophotometer.

The solution of Cu(II) contained Cu(II) perchlorate and a chelating ligand in varying amounts. The concentrations of PL, MetOEt and Cu(II) were  $1 \times 10^{-4}$  M in the final mixture, unless

500 nm appeared immediately after the initiation of the reaction, and disappeared within 1 min. No further spectral change was observed. In a solution containing the same reactants in a concentration ratio of 1:1:0.5, the visible absorption was more intense (absorbance of 0.02 at 20 sec) and its lifetime was about 2 min. The formation of the absorption of the Cu(II)-aldimine chelate at 400 nm was gradual, and completed in several hours. Its absorbance was smaller than in the 1:1:1 solution.

### Effects of bidentate ligands

In solutions containing PL, MetOEt, Cu(II), and en in a 1:1:1:2 ratio, the absorption band at 400 nm assigned to the Cu(II) chelate of the aldimine was gradually formed, and the formation was completed in 1 h. As shown in Fig. 1, no visible absorption was observed during the spectral change. In solutions containing en in 3 or more equimolar amounts to the

other three reactants, no spectral change was observed in the UV and visible region. However, in solutions containing 1.0-1.5 equimolar amounts of en, a transient absorption at 500 nm was observed. Fig. 2

shows the spectral change of a solution containing the four reactants in the 1:1:1:1.5 ratio. The visible absorption disappeared within several min, and the formation of Cu(II)-aldimine was very gradual.

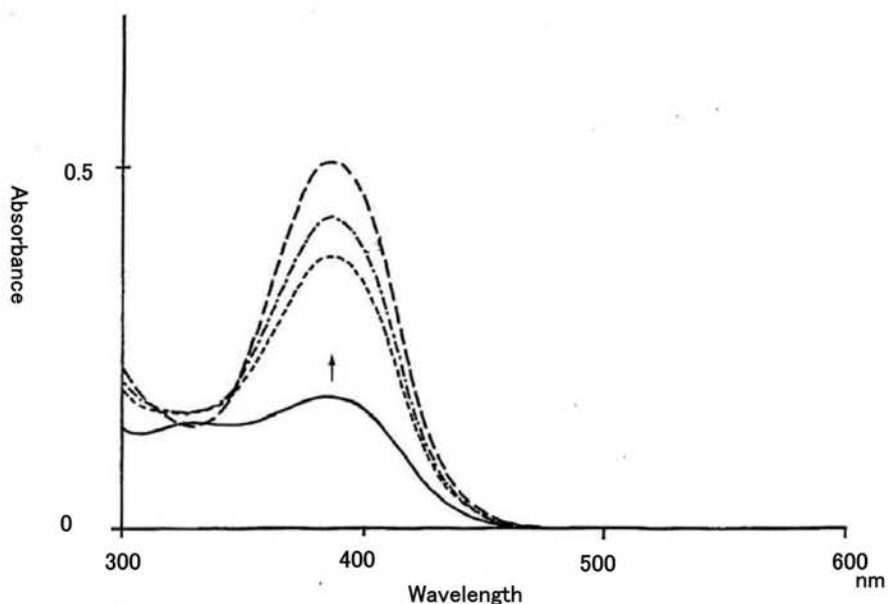


Fig 1  
Spectral Change Accompanying the Reaction of PL, MetOEt and Cu(II) and en in a 1:1:1:2 ratio.  
Periods after mixing: 1 min, 2.5 min, 5 min and 1h afterward, in increasing order of the absorbance at 400 nm.

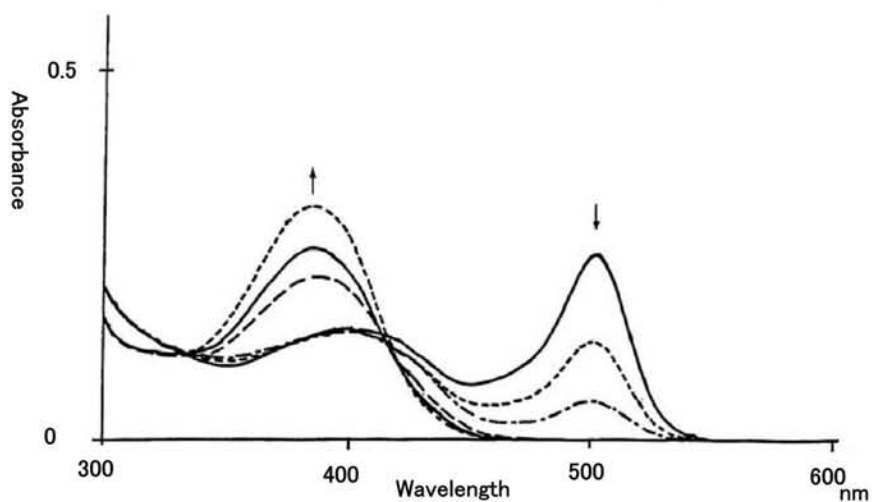


Fig 2  
Spectral Change Accompanying the Reaction of PL, MetOEt and Cu(II) and en in a 1:1:1:1.5 ratio.  
Periods after mixing: 20 sec, 1.5 min and 3.5 min afterward, in decreasing order of the absorbance at 500 nm, and 3.5 min, 30 min, 1 h and 3 h afterward, in increasing order of the absorbance at 400 nm.

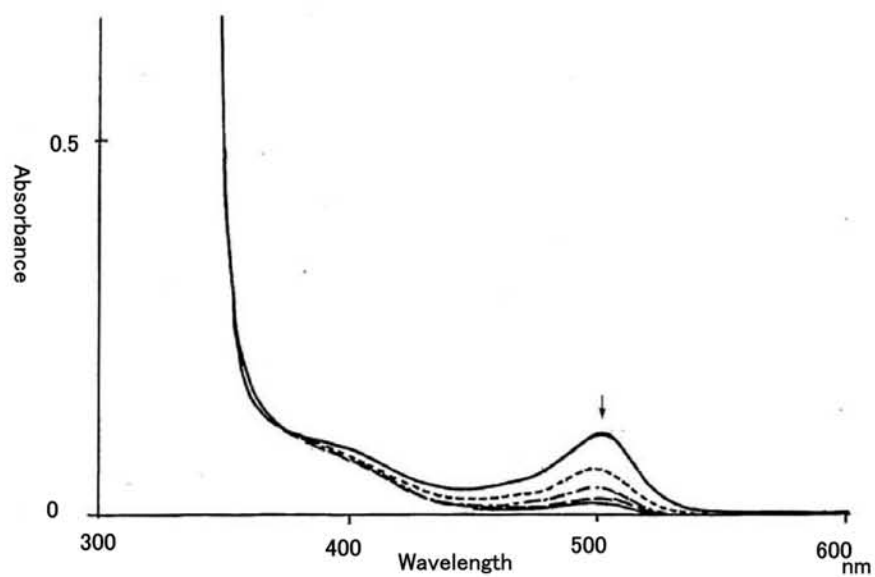


Fig 3  
Spectral Change Accompanying the Reaction of PL, MetOEt and Cu(II) and dien in a 1:1:1:0.75 ratio.  
Periods after mixing: 20 sec, 2.0 min and 25 min afterward, in decreasing order of the absorbance at 500 nm, and 2.0 min, 25 min, 3 h and 20 h, in increasing order of the absorbance at 400 nm.

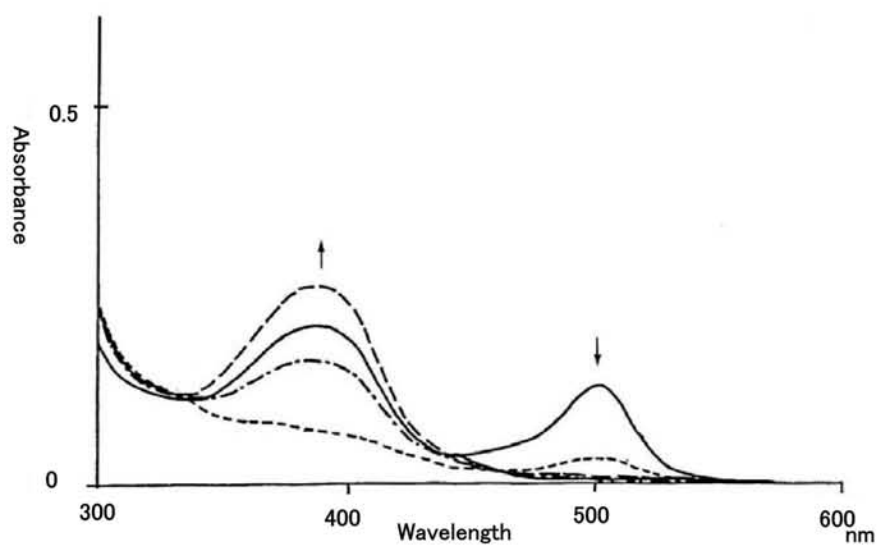


Fig 4  
Spectral Change Accompanying the Reaction of PL, MetOEt and Cu(II) and tripy in a 1:1:1:1 ratio.  
Periods after mixing: 20 sec, 30 min, 1 h, 3 h and 24 h afterward, in decreasing order of the absorbance at 500 nm.

Similar phenomena were observed when a solution of the Cu(II) chelate of bipy was added to a solution of PL and MetOEt, though the lifetime was shorter than in those containing the same ratio of en. In a solution containing PL, MetOEt, Cu(II) and bipy in a 1:1:1:2 ratio, the 500-nm band did not appear, but in solutions having a bipy ratio of 0.75 or less, the band was observed.

### Effects of tridentate ligands

The effects of two tridentate ligands, dien and terpy, for the reaction were examined. In the reaction of PL, MetOEt, Cu(II), and dien in a 1:1:1:1 ratio, the 500 nm band was not formed and the

400-nm band was gradually formed. The spectral change was very similar to that observed in the solution containing PL, MetOEt, Cu(II) and en in the 1:1:1:2 ratio shown in Fig. 1. In addition, the formation of a 500-nm band was observed in the presence of dien in less than a 0.75 equimolar amount, as shown in Fig. 3.

On the other hand, in a reaction of terpy in a 1:1:1:1 ratio, a fairly long-lived band (24 h at 25°C) was observed at 500 nm, while the 400-nm band ascribed to the Cu(II)-aldimine was scarcely formed, as shown in Fig. 4. In a solution having a 1:1:1:1.5 ratio, neither the 500-nm nor the 400-nm bands appeared.

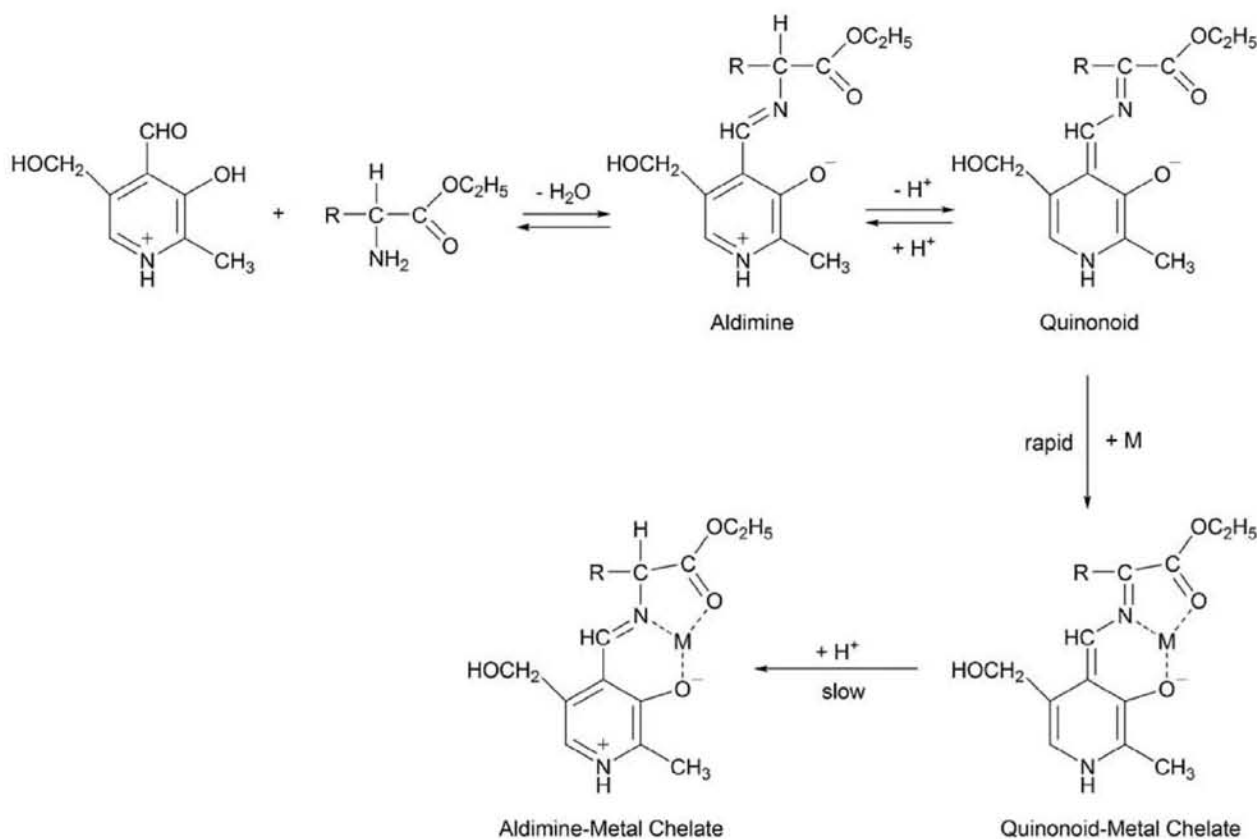


Chart 2  
Reaction Involving the Quinonoid Intermediate

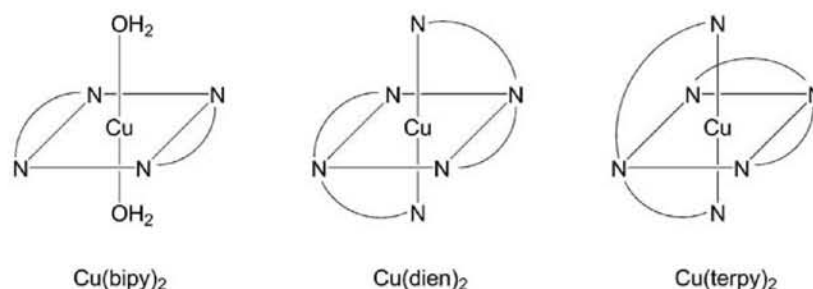


Chart 3  
Cu(II) Coordination of the Ligands

## DISCUSSION

The preceding study<sup>6)</sup> showed that the unchelated form of the quinonoid species is present in rapid equilibrium with the unchelated aldimine in a methanol solution of PL and AlaOEt (Chart 2). The amount of the quinonoid species in the equilibrium was too small to detect by the absorption spectra. Upon the addition of Al(III) to the equilibrium mixture, the quinonoid species rapidly formed the metal chelate, and the quinonoid-aldimine equilibrium was shifted to the formation of the quinonoid. Thus, the Al(III) chelate of the quinonoid was accumulated at first, and then very gradually transformed into the chelate of the aldimine. The direct formation of the Al(III) chelate of the aldimine from Al(III) and the aldimine of AlaOEt was assumed to be a slow step.

The results of the reactions of PL, MetOEt and Cu(II) in the absence of other chelating ligand varied considerably, depending on the amount of Cu(II). Although the geometry of Cu(II) coordination is usually square planar (four-coordinate), it is known to become distorted octahedral (six-coordinate) in some instances.<sup>12,13)</sup> Since the quinonoid should act as a tridentate ligand of planar coordination, the four-coordinate 1:1 chelates must be formed in the 1:1:1 reaction, while the six-coordinate 1:2 chelates in the 1:1:1:0.5 reaction. If we assume the same reaction mechanism as the Al(III) mediated reactions, the four coordinate Cu(II) chelate formation of the quinonoid and its conversion to the aldimine chelate in the 1:1:1 reaction might be instantaneous. The results from the 1:1:0.5 reaction indicated that the conversion of the six-coordinate 1:2 Cu(II) quinonoid chelate to the Cu(II) aldimine chelate was slower than in the 1:1 chelate. We then examined the

reactions of six-coordinate mixed chelates of Cu(II) with the quinonoid and other chelating ligands.

In Cu(II) solutions containing three or more equimolar amounts of the bidentate ligand, a stable 1:2 Cu(II) chelate of the ligand was formed, and the quinonoid and the aldimine did not form Cu(II) chelates. In solutions containing PL, MetOEt and Cu(II) and en in a 1:1:1:2 ratio, a small part of Cu(en)<sub>2</sub> might dissociate and form a mixed-chelate Cu-en-quinonoid. The concentration of the mixed chelate should be very small, and it must be transformed to Cu-en-aldimine before accumulation, and hence the visible absorption was unobservable and the formation of a 400-nm band was gradual. The predominant species in the final equilibrium may be Cu(aldimine)en or Cu(aldimine)(en)<sub>2</sub>. (Fig. 1) In solutions containing 1.0-1.5 equimolar en, the concentration of the mixed chelate, namely Cu(quinonoid)en, increased and the transient visible absorption became observable before conversion of the quinonoid to the aldimine. (Fig. 2)

The spectral changes accompanying the reactions of the two tridentate ligands, dien and terpy, were quite different from each other. Terpy is a tridentate coplanar ligand, and should form a four-coordinate coplanar 1:1 Cu chelate (with one solvent molecule bound at a coordination site) of a fairly large stability constant in a 1:1 solution with Cu(II) perchlorate. Upon mixing with the PL-MetOEt solution, the solvent molecule in the Cu(II) chelate must be displaced by a quinonoid molecule and a Cu(II)-quinonoid-terpy mixed chelate must be formed. The quinonoid, which was stabilized and accumulated in the form of the mixed chelate, may gradually be transformed to the aldimine. Thus, transient absorption at around 500 nm was observed.

(Fig. 3) After complete transformation to the aldimine, however, only a slight absorption at the 400 nm region was detected. The aldimine is not planar as a tridentate ligand, and is assumed to act as a bidentate ligand, which means that it is a much weaker ligand than the quinonoid. Therefore, in the presence of an equimolar terpy to Cu(II), only a small part of the aldimine should be present as the forms of Cu(II) chelate, and most of the aldimine was present in the unchelated form; thus, their absorption band should be at around 335 nm<sup>9,13</sup> and overlapped by that of terpy.

It is well-known that dien coordinates nonplanarly in the 1:2 complex of Cu(II).<sup>12,13,15</sup> Chart 3 shows the structures of fully coordinated Cu(II) chelates of the chelating ligands. In a 1:1 Cu(II) dien solution, most of the dien molecules are assumed to coordinate Cu(II) nonplanarly, and the terminally coordinated nitrogen may readily dissociate and can be displaced by solvent or other coordinating molecules. In the reaction of PL, MetOEt, Cu(II) and dien with a 1:1:1:1 ratio, a trace amount of Cu(II)-quinonoid-dien may be formed and converted to Cu(II)-aldimine- dien as an end product. (Fig. 4)

From the above consideration, we conclude that the formation of the six-coordinate mixed chelate Cu(II)-quinonoid-ligand (Chart 4) should retard the protonation of the quinonoid to convert to the aldimine, as clearly observed in the reaction of terpy. Although the reasons for these phenomena are not fully understood at this stage, the decrease of the carbanion property of the quinonoid by effective chelation to polyvalent metal ions may hinder protonation. The stability of the quinonoid chelate

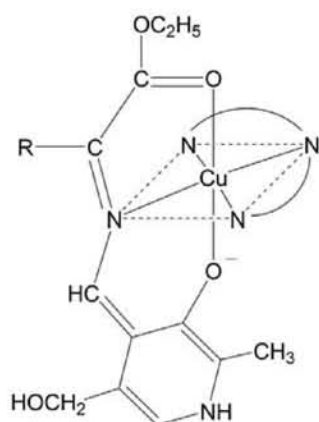


Chart 4  
Six-coordinate Cu(II)-quinonoid-terpy Chelate

may be enhanced by the formation of mixed chelates with the strongly chelating tridentate planar ligand. The results indicated that the stability of the chelate was determined not only by the interaction of the metal and the quinonoid, but also by the interaction of the metal with the other ligands.

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