

Bioinorganic Approach to the Type-I and Type-II Copper Proteins. Synthesis and Properties of Copper(II) Complexes with Tripod-like Ligands Containing Three or Four Donor Species

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Copper(II) complexes with new tripod-like quadridentate ligands containing three or four donor species, such as benzimidazole, thioether and phenol, were prepared. The ESR and electronic spectral studies have revealed that these complexes are classified into three types, that is, four-coordinated, five-coordinated trigonal bipyramidal and five-coordinated tetragonal pyramidal complexes, and that some of them are good models for blue(Type-I) and non-blue(Type-II) copper proteins.

Introduction

It is well known that histidine, imidazole, and methionine thioether play key roles in the coordination of metals at the active sites of numerous metal proteins [1]. The coordination of these groups to copper in proteins has been extensively studied by resonance Raman [2], electron spin resonance studies [3, 4] for some blue(Type-I) and non-blue (Type-II) copper proteins such as bovine superoxide dismutase [5], amine oxidase [6], *populus nigra italica* plastocyanin [7], *psedomonas aeruginosa* azurin [8], and copper substituted bovine alcohol dehydrogenase [9].

The crystal structure determination and spectroscopic studies have revealed that the copper(I) ions are of distorted tetragonal or tetrahedral coordination [6–9] in those systems. These results prompted many chemists to approach copper proteins by studying the tetragonal and tetrahedral copper(II) complexes. Several model compounds for copper proteins were already prepared by using the tripod-like ligands containing imidazole and thioether [10–12]. However, few studies [13] have been reported on the preparation of tripod-like ligands containing three or four donor species such as imidazole, aliphatic amine, thioether, *etc.* in one molecule.

In this study we have prepared several copper(II) complexes with tripod-like ligands containing three or four donor species such as benzimidazole, thioether and phenol, as shown in Fig. 1, and compared their ESR and electronic spectra with those of blue and non-blue copper proteins.

Experimental

Ligand Synthesis

N,N-bis(2-methylthioethyl)-4-nitro-2-methylaminophenol (hereafter abbreviated as *H*(noss))

To a THF solution of *N,N*-bis(2-methylthioethyl) amine(0.002 mol), was added dropwise a THF solution containing 2-chloromethyl-4-nitrophenol (0.001 mol) [14], and the solution was refluxed for 2 hours. After the precipitated *N,N*-bis(2-methylthioethyl) amine hydrochloride was removed by filtration, the filtrate was evaporated to yield a yellow syrup, which was used for the preparation of metal complexes.

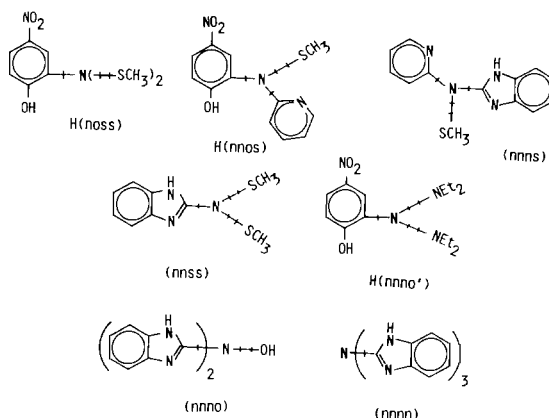


Fig. 1. Tripo-like ligands cited in this study and their abbreviations.

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N-(2-pyridylmethyl)-*N*-(2-methylthioethyl)-4-nitro-2-methylaminophenol (abbreviated as *H*(*nnos*))

This was obtained as yellow syrup by a method similar to that for *H*(*noss*), using *N*-(2-pyridylmethyl)-2-methylthioethylamine instead of *N,N*-bis(2-methylthioethyl)amine.

N-(2-pyridylmethyl)-*N*-(2-benzimidazolylmethyl)-2-methylthioethylamine (abbreviated as (*nnns*))

This was obtained as yellow syrup by a method similar to that for *H*(*nnos*), using 2-chloromethylbenzimidazole [15] instead of 2-chloromethyl-4-nitrophenol.

N-(2-benzimidazolylmethyl)-*N,N*-bis(2-methylthioethyl)amine (abbreviated as (*nnss*))

This was obtained as white needles by a method similar to that for *H*(*noss*), using 2-chloromethylbenzimidazole instead of 2-chloromethyl-4-nitrophenol, and recrystallized from a methanol solution.

N,N-bis(2-diethylaminoethyl)-4-nitro-2-aminomethylphenol (abbreviated as *H*(*nnno'*))

This was obtained as yellow syrup according to a similar method to that of *H*(*noss*), using *N,N*-bis(2-diethylaminoethyl)amine instead of *N,N*-bis(2-methylthioethyl)amine.

N,N-bis(2-benzimidazolylmethyl)ethanolamine (abbreviated as (*nnno*))

A 6*N* HCl solution containing *N*-(2-hydroxyethyl)iminodiacetic acid (0.01 mol) and *o*-phenylenediamine (0.05 mol) was refluxed for 5 hours. By neutralizing the above solution with aqueous NH₃, (*nnno*) was obtained as white needles, which were recrystallized from a methanol solution.

Preparations of Copper(II) Complexes

[Cu(*noss*)*]* *X* (*X*=ClO₄, BF₄ and NCS)

A methanol solution of the ligand (0.001 mol) was added to a methanol solution of Cu(H₂O)₆(ClO₄)₂, and the resulting solution was allowed to stand for three hours. The resulting brown needles were filtered and recrystallized from an acetone solution. By adding NH₄NCS to the perchlorate solution, Cu(*noss*)NCS was obtained as yellowish-green needles.

[Cu(*nnos*)*]* ClO₄

This was obtained as green needles by a method similar to that for Cu(*noss*)ClO₄.

[Cu(*nnss*)*]* *X* PF₆ (*X*=Cl, Br)

To a methanol solution (30 ml) of CuX₂(*X*=Cl, Br) (0.001 mol) was added the ligand (0.001 mol) and NH₄PF₆ (200 mg), and the resulting solution was

allowed to stand for a week. Both the chloro and the bromo complexes were obtained as green needles.

[Cu(*nnns*)*]* Cl PF₆

This was obtained as green needles by a procedure similar to that described for [Cu(*nnss*)*X*] PF₆.

[Cu(*nnno'*)*]* ClO₄

To a methanol solution (30 ml) containing *H*(*nnno'*) (0.001 mol) and Cu(H₂O)₆(ClO₄)₂ (0.001 mol), was added triethylamine (0.0005 mol). The resulting solution was left to stand for a week, and the yielded green prisms were filtered.

[Cu(*nnno*)*]* *X* ClO₄ (*X*=Cl, Br)

To a methanol solution containing the ligand (0.001 mol) and CuX₂(*X*=Cl, Br) (0.001 mol), was added NaClO₄ (0.005 mol), and the resulting solution was allowed to stand for one day. Both the chloro and the bromo complexes were obtained as green prisms.

[Cu(*nnno*)*]* (H₂O)] (NO₃)₂

This was obtained as green needles by mixing methanol solutions of the ligand and Cu(NO₃)₂ · 3H₂O.

[Cu(*nnnn*)*]* (im)] (ClO₄)₂

A methanol solution containing Cu(H₂O)₆(ClO₄)₂ (0.001 mol), (*nnnn*) (0.001 mol) and imidazole (0.002 mol) was allowed to stand for one day, and the resultant green prisms were filtered, where (im) represents imidazole.

[Cu(*nnnn*)*]* (py)] (ClO₄)₂ and *[Cu*(*bip*)₂(im)] (PF₆)₂

These were obtained by a similar procedure to that described for [Cu(*nnnn*)(im)] (ClO₄)₂, where (*bip*) and (*py*) represent 2,2'-bipyridine and pyridine respectively.

The analytical data of the new compounds are listed in Table I.

Measurements

ESR spectra were obtained with a JEOL ESR apparatus model JEL-3X-ME at 77 K using an X-band. DPPH was used as a standard marker. Electronic spectra were measured with a Shimadzu Multi-purpose spectrophotometer model MPS-5000 at room temperature.

Computer Simulations of the ESR Spectra

In this study, we determined the ESR parameters of two copper(II) complexes by computer simulation of the spectra. The simulations of ESR spectra were carried out according to the method by Pilbrow *et al.* [16]. In Fig. 2, α is the angle between the principal axes of *g*- and *A*-tensors. We have calculated the simulated ESR spectra varying α values. The ESR

TABLE I Analytical Data.

Compound	C (%)	H (%)	N (%)
	Found. (Calcd.)	Found. (Calcd.)	Found. (Calcd.)
(nns)	57.03 (56.91)	7.18 (7.16)	14.13 (14.22)
(nno)·CH ₃ OH	63.60 (63.70)	6.25 (6.24)	20.43 (20.63)
[Cu(noss)] BF ₄	33.71 (33.39)	4.46 (4.10)	5.64 (5.99)
[Cu(noss)NCS]	38.45 (38.38)	4.44 (4.36)	9.48 (9.57)
[Cu(nnss)Cl] PF ₆	30.89 (31.17)	4.09 (3.92)	7.59 (7.79)
[Cu(nnss)Br] PF ₆	28.92 (28.80)	3.78 (3.63)	7.04 (7.20)
[Cu(nnos)] ClO ₄ ·0.5H ₂ O	38.32 (38.10)	3.80 (3.82)	8.24 (8.33)
[Cu(nnns)Cl] PF ₆	34.34 (34.82)	3.85 (3.70)	8.02 (8.12)
[Cu(nnno')] ClO ₄ ·3H ₂ O	39.25 (39.18)	6.00 (6.75)	9.54 (9.54)
[Cu(nnno)Cl] ClO ₄ ·CH ₃ OH	41.15 (41.35)	4.29 (4.20)	12.50 (12.69)
[Cu(nnno)Br] ClO ₄ ·CH ₃ OH	38.04 (38.27)	3.90 (3.89)	11.57 (11.74)
[Cu(nnno)H ₂ O] (NO ₃) ₂	42.32 (42.31)	3.90 (4.14)	18.69 (19.19)
[Cu(nnnn)(im)] (ClO ₄) ₂ ·2H ₂ O	42.11 (41.89)	3.71 (3.78)	16.20 (16.29)
[Cu(nnnn)(py)] (ClO ₄) ₂ ·2H ₂ O	44.51 (44.95)	3.97 (3.80)	14.26 (14.23)
[Cu(bip) ₂ (im)] (PF ₆) ₂	37.80 (37.64)	2.73 (2.75)	11.47 (11.45)

TABLE II. ESR Parameters of the Complexes (77 K, X-band, dmf frozen solution).

Complex	g value	A value (gauss)
[Cu(noss)] ClO ₄ *	$g_{\parallel} = 2.237, g_{\perp} = 2.084$	$A_{\parallel} = 160, A_{\perp} = 60$
[Cu(noss)NCS]	$g_{\parallel} = 2.332$	$A_{\parallel} = 168$
[Cu(nnos)] ClO ₄	$g_{\parallel} = 2.312$	$A_{\parallel} = 171$
[Cu(nnns)Cl] PF ₆	$g_{\parallel} = 2.248$	$A_{\parallel} = 168$
[Cu(nnno')] ClO ₄	$g_{\parallel} = 2.029, g_{\perp} = 2.280$	$A_{\parallel} = 130, A_{\perp} = 57$
[Cu(nnss)Br] PF ₆	$g_1 = 2.180, g_2 = 2.097, g_3 = 1.975$	$A_1 = 127, A_2 = 80, A_3 = 114$
[Cu(nnno)Cl] ClO ₄	$g_{\parallel} = 2.253$	$A_{\parallel} = 168$
[Cu(nnno)Br] ClO ₄	$g_{\parallel} = 2.247$	$A_{\parallel} = 172$
[Cu(nnno)H ₂ O] (NO ₃) ₂	$g_{\parallel} = 2.279$	$A_{\parallel} = 160$
[Cu(nnnn)Cl] ClO ₄	$g_{\parallel} = 2.018, g_{\perp} = 2.224$	$A_{\parallel} = 63, A_{\perp} = 79$
[Cu(nnnn)(im)] (ClO ₄) ₂	$g_{\parallel} = 2.254, g_{\perp} = 2.063$	$A_{\parallel} = 145$
[Cu(nnnn)(py)] (ClO ₄) ₂	$g_{\parallel} = 2.253$	$A_{\parallel} = 156$
[Cu(bip) ₂ (im)] (PF ₆) ₂	$g_{\parallel} = 2.249, g_{\perp} = 2.048$	$A_{\parallel} = 172$

* In acetone.

TABLE III. ESR Parameters for Computer Simulated Samples.

Complex	g_x	g_y	g_z	$ A_x $	$ A_y $ (gauss)	$ A_z $	w_x	w_y	w_z	α
[Cu(noss)] ClO ₄	2.084	2.084	2.237	60	60	164	35	35	20	0
[Cu(nnss)Br] PF ₆	2.182	2.105	1.975	127	80	114	25	10	20	0

parameters listed in Table III were obtained assuming $\alpha = 0$, because it was already shown that α is zero in some distorted five-coordinated copper(II) complexes [17]. Lorentzian function was used for the line-shape [18]:

$$\sum_{\theta} \sum_{M_1} \frac{P(\theta, M_1)[H - H(\theta, M_1)] W \sin \theta}{\{3W^2 + (H - H(\theta, M_1))^2\}^2}$$

where w represents the half-line width.

Results and Discussion

It is known that the majority of copper(II) complexes are of square planar, elongated octahedral or tetragonal pyramidal coordination, and assume the $(d_{x^2-y^2})^1$ electron configuration at the ground state (according to electron-hole formalism) [19]. The ESR spectra of frozen solutions of these complexes are characterized by the spectral features

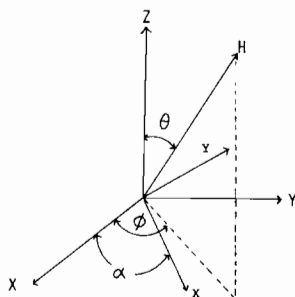


Fig. 2. The coordinate system for computer simulation of the ESR spectra [16].

$g_{\parallel} > 2.1 > g_{\perp} > 2.0$, generally showing a hyperfine structure due to the nuclear spin of copper ($I = 3/2$) at the g_{\parallel} region. A typical spectrum of them is exemplified by that of $[\text{Cu}(\text{nnno})\text{Cl}]^+$ shown in Fig. 3-A. Recently numerous copper(II) complexes of trigonal bipyramidal coordination have been reported [20–23]. The ground state of these complexes is $(d_{z^2})^1$ (the principal rotation axis is taken as the z axis), hence the ESR spectral pattern is quite different from those of the complexes of tetragonal symmetry, giving $g_{\perp} > g_{\parallel} \sim 2.0$ with a hyperfine structure due to the copper nucleus at the g_{\parallel} region. A typical example is shown in Fig. 3-B. Thus, we can diagnose the $(d_{x^2-y^2})^1$ or $(d_{z^2})^1$ ground state of copper complexes by the use of ESR spectra, except for the cases of non-axial symmetry or the intermediate between trigonal bipyramidal and tetragonal pyramidal coordination

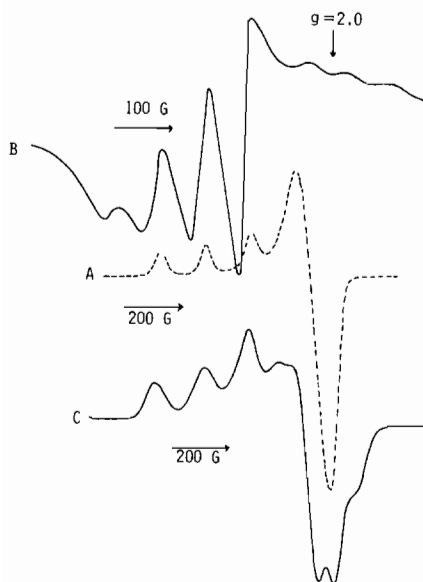


Fig. 3. ESR spectra (frozen dmf solution at 77 K) of $[\text{Cu}(\text{nnno})\text{Cl}]\text{ClO}_4$ (A), $[\text{Cu}(\text{nnnn})\text{Cl}]\text{ClO}_4$ (B), and $[\text{Cu}(\text{nnno})\text{Br}]\text{ClO}_4$ (C).

Complexes with (noss) Ligand

The ESR spectra of $\text{Cu}(\text{noss})\text{X}$ ($\text{X} = \text{ClO}_4$ and NCS) (Fig. 4) indicate the $(d_{x^2-y^2})^1$ ground state based on the criterion described above. However, the electronic spectra of $\text{Cu}(\text{noss})\text{ClO}_4$ are different from those of $\text{Cu}(\text{noss})\text{NCS}$ in the solid and solution states as shown in Fig. 5 and 6. The infrared spectra show that the perchlorate ion does not coordinate to a copper ion because no splitting was observed for the absorption of perchlorate ion around 1100 cm^{-1} [24]. On the other hand, the thiocyanate ion does coordinate to the copper ion with the nitrogen atom because ν_{CN} was observed at 2080 cm^{-1} [25]. From these results we may conclude that $\text{Cu}(\text{noss})\text{ClO}_4$ is four-coordinated, whereas $\text{Cu}(\text{noss})\text{NCS}$ is five-coordinated.

In the solution spectra, a distinct absorption band ($\epsilon \sim 2800$) was observed at 450 nm for $[\text{Cu}(\text{noss})]\text{ClO}_4$, whereas only a shoulder ($\epsilon \sim 600$) was observed at 500 nm for $[\text{Cu}(\text{noss})\text{NCS}]$. This may be attributed to the difference in the coordination structure. On the basis of the above results, the ESR spectrum of $[\text{Cu}(\text{noss})]\text{ClO}_4$ was simulated by the use of the parameters given in Table III, the fitting of the calculated spectrum to the observed being good, as shown in Fig. 3. It should be noted here that the large hyperfine splitting at the g_{\perp} region observed for $[\text{Cu}(\text{noss})]\text{ClO}_4$ resembles that of blue copper protein with a (noss) donor set, reported by Marret *et al.* [9].

Complexes with (nnss) ligand

In Fig. 7, ESR spectra of $[\text{Cu}(\text{nnss})\text{X}]\text{PF}_6$ ($\text{X} = \text{Cl}, \text{Br}$) are shown. The spectrum of $[\text{Cu}(\text{nnss})\text{Br}]\text{PF}_6$

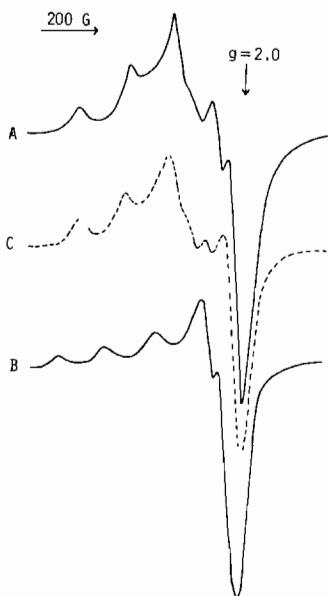


Fig. 4. ESR spectra (frozen solution at 77 K) of $[\text{Cu}(\text{noss})]\text{ClO}_4$ in acetone (A), $[\text{Cu}(\text{noss})\text{NCS}]$ in dmf (B), and the simulated spectrum of $[\text{Cu}(\text{noss})]\text{ClO}_4$ (C).

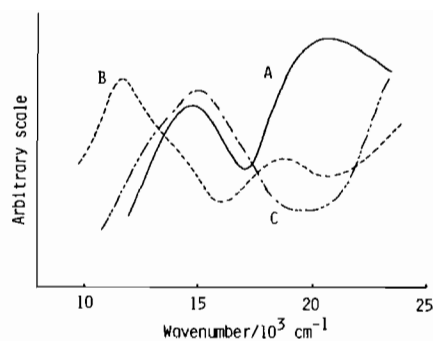


Fig. 5. Reflectance spectra (at room temperature) of [Cu(noss)]ClO₄ (A), [Cu(noss)NCS] (B), and [Cu(nnns)Cl]PF₆ (C).

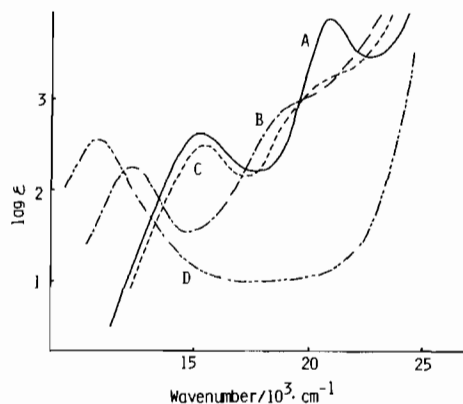


Fig. 6. Absorption spectra (at room temperature) of [Cu(noss)]ClO₄ in acetone (A), [Cu(noss)NCS] in dmf (B), [Cu(nnos)]ClO₄ in dmf (C), and [Cu(nnno')]ClO₄ in dmf (D).

is of a rhombic pattern, where the lowest principal g -value is 1.975. This suggests that the copper(II) ion is in the $(d_{z^2})^1$ state and the coordination geometry is a trigonal bipyramid. Recently Suzuki *et al.* [26] reported that the ESR spectrum of [Cu(nnss')Br]ClO₄ is similar to that of [Cu(nnss)Br]PF₆ where (nnss') represents the tripod-like ligand in which the benzimidazole of (nnss) is replaced by pyridine. We have determined the crystal structure of [Cu(nnss')Br]ClO₄ and found that the coordination geometry of copper(II) ion is trigonal bipyramidal [27]. Thus, the estimation derived from the ESR spectrum is in good accordance with the results of the X-ray analysis. Based on the above results, we have carried out the computer simulation of the ESR spectrum of [Cu(nnss)Br]PF₆ by the use of the parameters listed in Table III. As shown in Fig. 7, the fitting of the simulated spectrum to the observed one is satisfactory.

It is well known that the ESR spectrum of stellacyanin, one of the blue copper protein (Type-I), shows a rhombic ESR spectrum [28], with one absorption

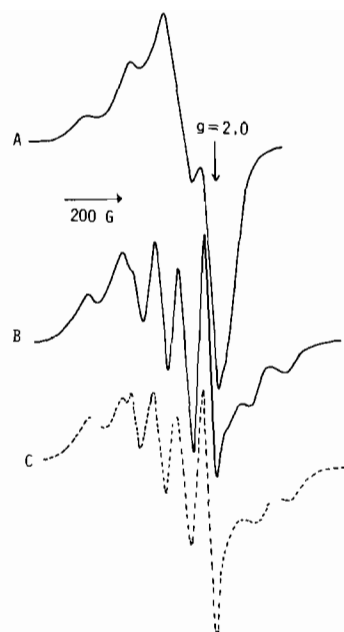


Fig. 7. ESR spectra (frozen dmf solution at 77 K) of [Cu(nnss)Cl]PF₆ (A), [Cu(nnss)Br]PF₆ (B), and the simulated spectrum of [Cu(nnss)Br]PF₆ (C).

around $g \sim 2.0$ ($g_{\text{obs}} = 2.025$). The spectral pattern is quite different from those of other blue copper proteins such as plastocyanin [29] and azurin [30], but rather similar to that of [Cu(nnss)Br]PF₆. In spite of the above facts, it has been believed that [31] the copper(II) ion in stellacyanin has a distorted tetrahedral structure similar to those observed for plastocyanin [7] and azurin [8]. However, no tetrahedral or distorted tetrahedral copper(II) complex exhibiting the ESR spectral characteristics of stellacyanin has been reported. Thus, it seems that stellacyanin contains a copper(II) ion in distorted trigonal bipyramidal coordination similar to [Cu(nnss)Br]PF₆.

Complexes with (nnnn) Ligand

The ESR spectra of five-coordinated [Cu(nnnn)-X]⁺ (X = Cl, Br, I) complexes were investigated by Thompson *et al.* [23], who concluded that all these complexes are of the $(d_{z^2})^1$ ground state configuration. In the present study, however, we have found that the ESR spectra of [Cu(nnnn)(im)]²⁺ and [Cu(nnnn)(py)]²⁺ are quite different from those of the corresponding halogeno complexes (*cf.* Fig. 9), where (im) and (py) represent imidazole and pyridine respectively. The spectra in Fig. 9 clearly indicate that the imidazole- and pyridine-complexes are of the $(d_{x^2-y^2})^1$ ground state configuration. These facts suggest that the ground state configuration of five-coordinated copper(II) complexes is dependent not only on the nature of a chelating ligand but also

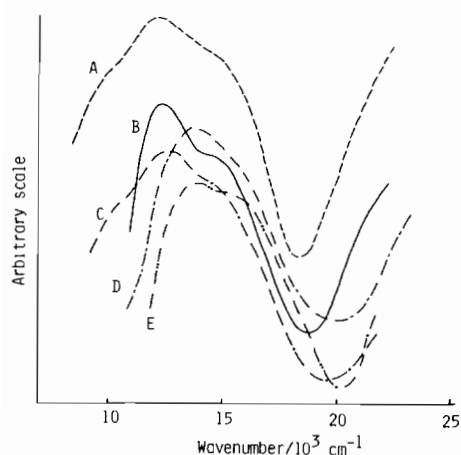


Fig. 8. Reflectance spectra (at room temperature) of $[\text{Cu}(\text{nnss})\text{Br}]\text{PF}_6$ (A), $[\text{Cu}(\text{nnss})\text{Cl}]\text{ClO}_4$ (B) [12] $[\text{Cu}(\text{nnss})\text{Cl}]\text{PF}_6$ (C), $[\text{Cu}(\text{nnno})\text{Cl}]\text{ClO}_4$ (D), and $[\text{Cu}(\text{nnno})\text{Br}]\text{ClO}_4$ (E).

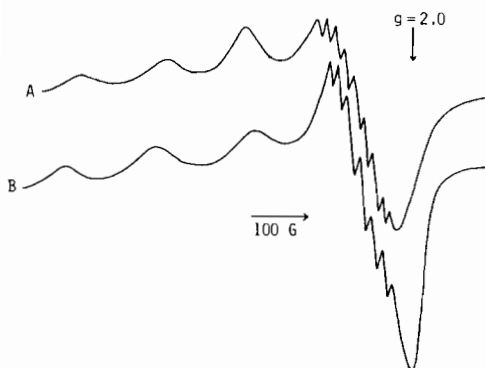


Fig. 9. ESR spectra (frozen dmf solution at 77 K) of $[\text{Cu}(\text{nnnn})(\text{im})](\text{ClO}_4)_2$ (A) and $[\text{Cu}(\text{bip})_2(\text{im})](\text{PF}_6)_2$ (B).

on a monodentate ligand, e.g., imidazole and/or halide ions. In order to ascertain the above conclusion, we have measured the ESR spectrum of $[\text{Cu}(\text{bip})_2(\text{im})]^{2+}$, because it was already demonstrated that $[\text{Cu}(\text{bip})_2\text{Cl}]^+$ has a trigonal bipyramidal structure [22, 32] with the $(d_{z^2})^1$ ground state configuration. As shown in Fig. 9, $[\text{Cu}(\text{bip})_2(\text{im})]^{2+}$ shows an ESR spectrum characteristic of the $(d_{x^2-y^2})^1$ ground state configuration. Thus, in this case, the ground state configuration has also changed from $(d_{z^2})^1$ to $(d_{x^2-y^2})^1$ as Cl^- is replaced with imidazole.

Complexes with (nnno) Ligand

As shown in Fig. 3, the frozen solution ESR spectra of $[\text{Cu}(\text{nnno})\text{X}]\text{ClO}_4$ ($\text{X} = \text{Cl}, \text{Br}$) show a distinct tetragonal pattern, suggesting the $(d_{x^2-y^2})^1$ ground state configuration. Although the ligand (nnno) is very similar to (nnnn) from the structural point of view, the ground state configuration of $[\text{Cu}(\text{nnnn})\text{X}]^+$ ($\text{X} = \text{Cl}, \text{Br}, \text{I}$) has been assigned to $(d_{z^2})^1$. We have thus determined the crystal structures of

$[\text{Cu}(\text{nnno})\text{X}]\text{ClO}_4$ ($\text{X} = \text{Cl}, \text{Br}$) [33] in order to ascertain the estimation derived from the ESR spectra. As a result it was shown that these two complexes are almost isomorphous with each other and contain a five-coordinated copper(II) with a tetragonal pyramidal environment. Thus, the conclusion from the ESR spectra of $[\text{Cu}(\text{nnno})\text{X}]\text{ClO}_4$ ($\text{X} = \text{Cl}, \text{Br}$) is in agreement with the results of the X-ray analysis.

Conclusion

It has been demonstrated that the ESR patterns of some copper(II) complexes obtained in this study closely resemble those of some copper proteins, for example, that of $[\text{Cu}(\text{nnnn})(\text{im})]^{2+}$ resembles that of Type-II in Laccase [34] $[\text{Cu}(\text{nnno})\text{Br}]^+$, that of superoxide dismutase [35], and $[\text{Cu}(\text{noss})]^+$, that of blue copper protein of a (noss) donor set [9], etc. According to recent work [23, 36] the copper(II) complexes with tripod-like ligand containing thioether or benzimidazole have relatively high redox potentials as usually observed for copper proteins. Thus, the present copper(II) complexes may be regarded as models for Type-I and Type-II copper in biological systems.

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