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CHARACTERIZATION BY ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY OF THE COORDINATION ENVIRONMENT OF COPPER IN SOME COPPER(II) COMPLEXES OF ASYMMETRIC TRIAZINES HAVING HIGH SUPEROXIDE DISMUTASE ACTIVITY

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Abstract—The chemical environments of copper in complexes with a range of substituted asymmetric triazines (*as*-triazine), which had previously been demonstrated to possess high superoxide dismutase activity, have been characterized by EPR spectroscopy in both solution and the solid state. The results show that these preparations do not correspond to single mononuclear complexes and that the activity appears to be associated mainly with a complex that has a structure involving two six-membered chelate rings and with two nitrogen and two oxygen atoms in the copper equatorial plane.

Excessive production of the superoxide free radical anion ($O_2^{\cdot -}$) has been implicated in a wide range of clinical disorders, including degenerative and inflammatory diseases. For this reason superoxide dismutase (SOD) and related complexes have been proposed to have medicinal uses as anti-inflammatory agents.[1,2] The clinical use of natural proteins such as SOD and ceruloplasmin is limited because of low membrane permeability as a consequence of their high molecular weights,[3] but there is considerable interest in the use of synthetic copper complexes of lower molecular weight as anti-inflammatory drugs; examples of such complexes include derivatives of anthranilic acid, salicylic acid, amino acids, carboxylic acids and amines.[4,5]

Recent tests have shown that a group of complexes based on asymmetric triazines (*as*-triazine) have high SOD and strong anti-viral activities.[6–8] In these determinations *in vitro* assessments of SOD activity were made by monitoring the inhibition of photochemically generated $O_2^{\cdot -}$ radicals[7] and their antiviral effects were measured by following the inhibition of the multiplication of some influenza and para-influenza viruses on CAM fragments[6] (CAM: choriallantoic membrane of chicken embryos). In addition, the effects of the complexes on the mean survival times of mice experimentally

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infected with influenza viruses have been determined.[7]

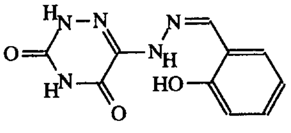
The present paper describes the use of electron paramagnetic resonance (EPR) spectroscopy to investigate the chemical environments of the copper(II) in complexes with a group of five *as*-triazines. Spectra are analysed in detail to reveal the number of distinct copper(II)-containing components in each sample and, wherever possible, to identify the number of nitrogen atoms coordinated in the *x–y* plane of each complex. In addition, molecular mechanics force-field calculations were performed in order to reveal possible coordination geometries for these complexes.

EXPERIMENTAL

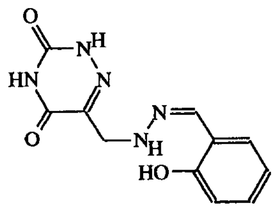
Preparation of copper(II) as-*triazine complexes*

The copper complexes were prepared at the Chemical and Pharmaceutical Research Institute, Bucharest using the following general method.[9] The appropriate ligand (2 mm; Table 1) was dissolved in NaOH (20 cm^3, 0.05 mm) with gentle warming; the final pH of the solutions was about 7.5. Copper(II) chloride (1 mM) in H_2O (5 cm^3) was added slowly over 10–15 min with constant stirring. The resulting solution was cooled to 4°C and stirred continuously for 1 h, during which period a precipitate formed. This was collected by filtration and washed successively with hot water (2 × 25 cm^3), ethanol (2 × 25 cm^3) and acetone. The copper complexes were then dried overnight at 100°C and 15 mm Hg pressure and finally analysed for C, H (CH analyser) and Cu (atomic absorption spectroscopy). Their IR spectra in KBr discs were also recorded on a C. Zeiss (Jena) Model M80 spectrometer.

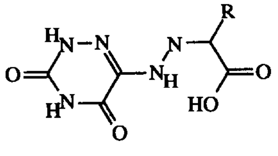
Table 1. Asymmetric triazines used as ligands of copper(II) complexes



I



II



R	
CH_3	III
$(CH_2)_2COOH$	IV
H	V

The analytical results for the various preparations are listed below, along with the values and assignments for the principal IR frequencies and the theoretical elemental values for bis complexes.

Complex **I**: *bis*[6-(2-*hydroxybenzaldehyde*)*hydrazono*-as-*triazine*-3,5(2H,4H)*dione*]*copper*(II). Yield 90%; m.p. >300°C. Found: C, 43.3; H, 3.0; Cu, 11.5. Calc. for $C_{20}H_{16}N_{10}O_6Cu$ (555.95): C, 43.2; H, 2.9; Cu, 11.4%. ν(C=O) 1720, 1680; ν(C=N) 1640; $\nu(C—C)_{phenyl}$ 1590, 1466 cm^{-1}.

Complex **II**: *bis*[6-(2-*hydroxybenzaldehyde*)*carboxhydrazono*-as-*triazine*-3,5(2H,4H)*dione*]*copper* (II). Yield 80%; m.p. >300°C. Found: C, 43.3; H, 2.7; Cu, 10.5. Calc. for $C_{22}H_{18}N_{10}O_6Cu$ (611.97): C, 43.2; H, 2.6; Cu, 10.4%. ν(C=O) 1710, 1678, 1670; ν(C=N) 1635; $\nu(C—C)_{phenyl}$ 1598, 1470 cm^{-1}.

Complex **III**: *bis*[6-*pyruvohydrazono*-as-*triazine*-3,5-(2H,4H)*dione*]*copper*(II). Yield 86%; m.p. >300°C. Found: C, 29.9; H, 2.4; Cu, 13.3. Calc. for $C_{12}H_{12}N_{10}O_8Cu$ (487.84): C, 29.5; H, 2.5; Cu, 13.0%. ν(C=O) 1740, 1670; ν(C=N) 1630 cm^{-1}.

Complex **IV**: *bis*[6-(2-*ketoglutar*)*hydrazono*-as-*triazine*-3,5(2H,4H)-*dione*]*copper*(II). Yield 84%; m.p. >300°C. Found: C, 31.5; H, 3.0; Cu, 10.3. Calc. for $C_{16}H_{16}N_{10}O_{12}Cu$ (603.91): C, 31.8; H, 2.7; Cu, 10.5%. ν(C=O) 1730, 1690; ν(C=N) 1640 cm^{-1}.

Complex **V**: *bis*[6-*glyoxalhydrazono*-as-*triazine*-3,5-(2H,4H)*dione*]*copper*(II). Yield 85%; m.p. >300°C. Found: C, 26.4; H, 2.0; Cu, 13.6. Calc. for $C_{10}H_8N_{10}O_8Cu$ (459.79): C, 26.1; H, 1.8; Cu, 13.9%. ν(C=O) 1720, 1690; ν(C=N) 1650 cm^{-1}.

Complex **I** and **II** correspond to complexes S1 and S17 of Tomas *et al.*[7]

Determination of superoxide dismutase activity

Concentrations of O_2^- radicals in solutions were measured using the photometric method of Zuivertz *et al.*,[10] in which the concentration of formazane produced from the reduction of nitroblue tetrazolium at room temperature was determined by absorption at 560 nm[11] using a C. Zeiss (Jena) Specord UV–vis spectrophotometer. The O_2^- scavenging activities of the ligands and the copper(II)

ne M/P ratio between
n the absence (M) and
compound.

complexes were e
the optical
present

ples for EPR spectroscopy

EPR measurements were prepared as powders were used without any further ; (ii) fluid solution measurements were with saturated solutions in dimethyl sulph- e (DMSO), which were prepared by heating an excess of the complex in DMSO at 50°C for 10 min, followed by filtration to remove any undissolved solid ; (iii) frozen solution measurements were made with solutions prepared in a similar manner to those for fluid solutions, except that DMSO/methanol in a 3 : 1 ratio was used as solvent. The methanol was added in order to improve the glass-forming properties of the solvent on freezing, and thus to minimize spectral broadening as a result of segregation of solvent and solutes.

EPR spectroscopy

EPR spectra were obtained as first and second derivatives with a Bruker ESP300E X-band spectrometer at either ambient temperature (*ca* 24°C) or 77 K in liquid nitrogen in a quartz "finger dewar". All spectra were recorded using 100 kHz modulation frequency and modulation amplitudes of 0.5 mT for first and 0.1 mT for second derivatives. Microwave powers of 50 mW were used for measurements at room temperature and 20 mW for those at 77 K. Other spectral acquisition parameters, such as the time constant, gain and sweep time, were adjusted for each spectrum individually. Optimization of spectral resolution was achieved by making multiple accumulations and using fast Fourier transformation with a Gaussian profile for subsequent noise reduction. All spectral parameters were confirmed by simulation using the Bruker "Simphonia" software package and assuming Lorentzian line shapes for fluid solution and co-axial g and hyperfine tensors with Gaussian line shapes for frozen solution spectra. The relative concentrations of the various components responsible for ^{14}N superhyperfine structure in the solution spectra were calculated as a percentage of the absorption area after double integration of the second derivative EPR spectra.

Calculation of complex structures

Theoretical structures for the copper complexes of the various ligands were calculated using MM+, a modified version of the molecular mechanics force-field (MM+) algorithm of Allinger[12] incorporated in the Autodesk, Inc. "Hyperchem" (release III) software package. Geometry optimization was obtained using the Polak–Ribière algorithm.[13] Further information on the nature of the copper–ligand bonding was obtained by calculating molecular orbital coefficients for D_{2h} symmetry using the ligand field approach of Maki and McGarvey[14] and Kivelson and Neiman.[15]

RESULTS AND DISCUSSION

The $O_2^{\cdot -}$-scavenging activities of the various ligands and their copper(II) complexes are shown in Table 2. In each case the value is higher for the copper complex than for the uncomplexed ligand, but the increase for **I** is far greater than that seen for any of the other *as*-triazines. These values are similar, but not identical, to those reported by Tomas *et al.*[7] for similar preparations of these complexes.

EPR spectroscopy

The EPR spectra at ambient temperature of the various copper *as*-triazine complexes as powdered solids are shown in Fig. 1, along with the spectrum of bovine SOD. There is considerable variation in the overall shapes of these spectra, but there was only very poor resolution of hyperfine structure from any of the copper(II) ions in the *as*-triazine complexes. The poor resolution of spectral detail is almost certainly the consequence of strong dipolar interactions between unpaired electrons on neighbouring nuclei ; such broadening is insignificant with the natural enzyme because the large size of the ligand provides a magnetically dilute environment for the unpaired electron on each copper(II) ion.

The first derivative EPR spectra of the *as*-triazine copper(II) complexes in fluid solution in DMSO

Table 2. Relative $O_2^{\cdot -}$-scavenging activities of ligands and copper(II) complexes of the *as*-triazines from Table 1 measured as the ratio of the optical density at 560 nm in the absence and presence of the tested compound

as-triazine (see Table 1)	Ligand alone	Copper complex
I	2.50	12.50
II	1.36	1.82
III	0.82	1.12
IV	0.91	2.83
V	0.94	1.20

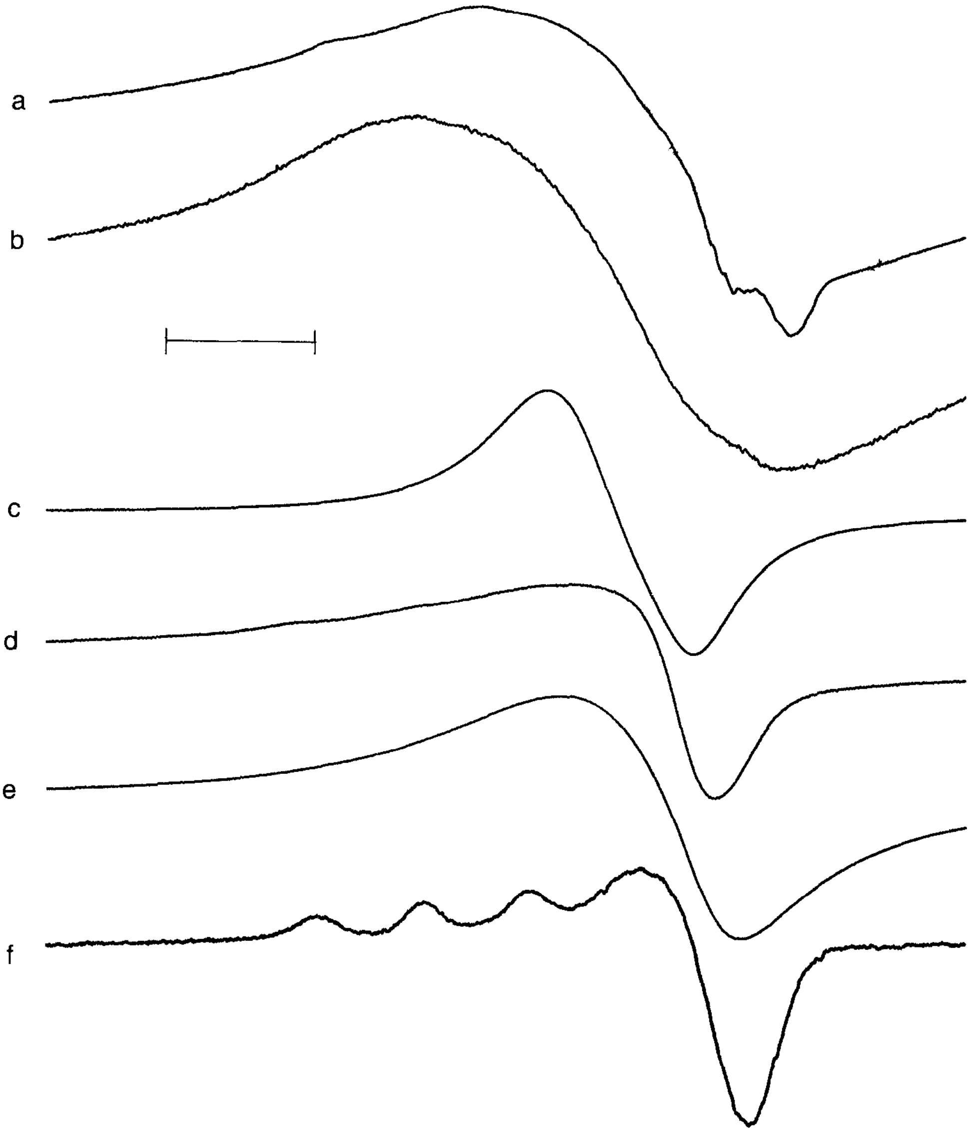


Fig. 1. First-derivative EPR spectra at ambient temperature of powdered solids of (a)–(e) copper(II) *as*-triazine complexes **I**—**V** as detailed in Table 1 and (f) SOD from bovine erythrocytes. Bar = 20 mT.

are shown in Fig. 2. Each spectrum is isotropic in character, but there are extensive line-width variations, there being a progressive narrowing of peaks with increasing magnetic field. This phenomenon is common with copper complexes and arises because molecular motions are insufficiently rapid to completely average the anisotropic interactions.[16]

There are considerable differences in the spectral characteristics of the various samples, and in **II**, **III** and **V** there appears to be a mixture of components present. Indeed the spectrum of **V** changed progressively with time [see Fig. 2e and f]. The spectra of **I** and **IV**, which appear to correspond to single components, each show the presence of ^{14}N superhyperfine structure; such structure is not obviously present in the spectra of the other complexes. The spectrum of **II** contains a minor component which has its highest field peak in a similar position to that in the structurally related **I**. The positions of the peaks of the major component of **II** are, however, quite different from those of **I** and are similar to those of **IV**. Complex **III** produced a very weak spectrum, which was not entirely the consequence of low solubility. It appears to consist of a small amount of a component with peaks in similar positions to those of **IV** superimposed on a broad resonance similar to that of the uncomplexed ion.[17] The initial spectrum of **V** consists of at least two components, one with parameters similar to those of **IV** and a second with its highest-field peak

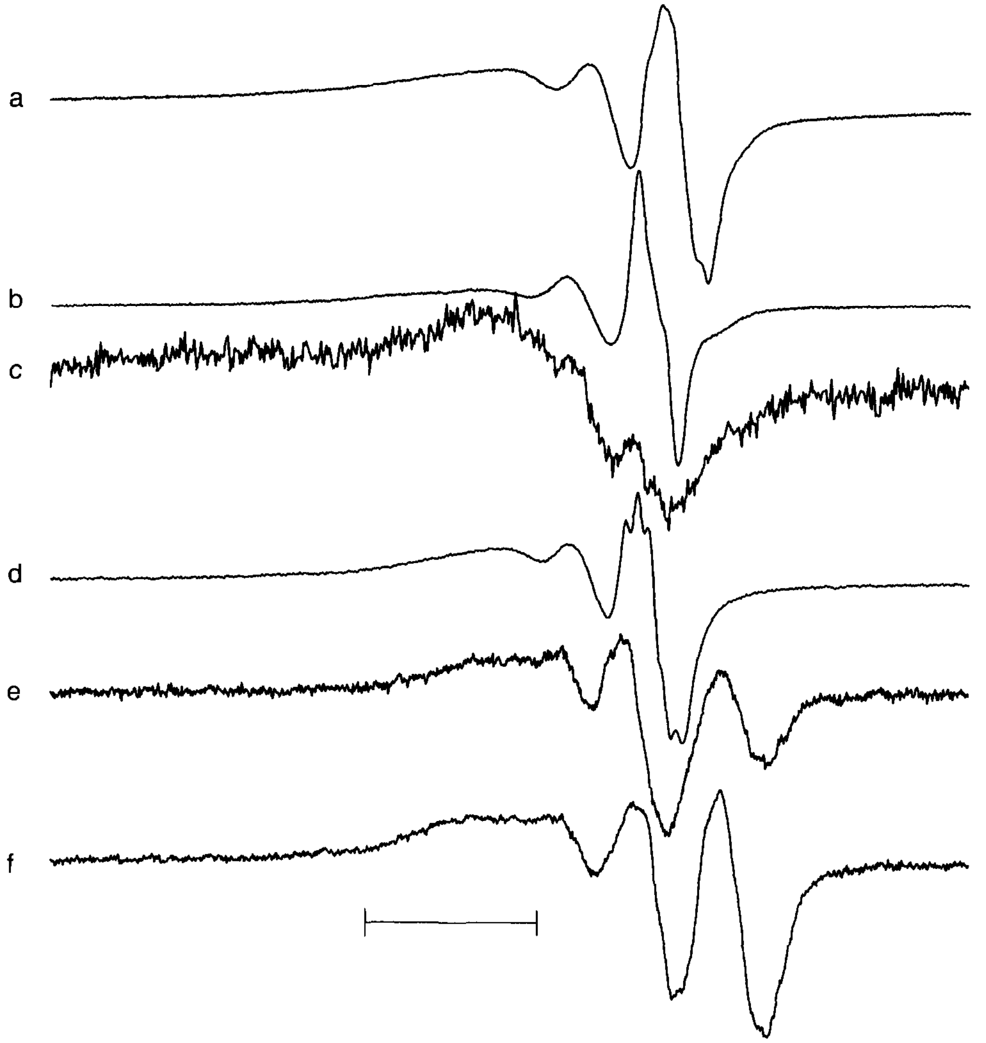


Fig. 2. First-derivative EPR spectra at ambient temperature of (a)–(e) fresh DMSO solutions of copper(II) *as*-triazine complexes **I**—**V** as detailed in Table 1 and (f) DMSO solution of **V** aged in air for 24 h. Bar = 20 mT.

some 7 mT upfield of the highest field peak in **IV**. The contribution to the spectrum of this latter component increased progressively with time and was by far the major component after 24 h (Fig. 2f).

In order to investigate further the superhyperfine coupling to ^{14}N atoms in the ligands of these complexes, attempts were made to generate second-derivative spectra over the region of the highest field copper peaks for each of the complexes. No signal was observed with **III** and only a poorly resolved structure was seen with **II**, but a well-resolved structure was obtained with **I**, **IV** and **V** in both fresh and aged forms. These spectra are shown in Figs 3–6, along with computer simulations of the spectra and their individual components. The assumptions made in these simulations are: (a) all superhyperfine structure arises from ^{14}N nuclei, which have spin $I = 1$, and each nucleus, therefore, produces a triplet structure; (b) in each component that has more than one nitrogen, each nitrogen atom is equivalent and hence produces splittings of the same magnitude; thus a complex with two nitrogens produces a quintet structure with peak intensities in the ratio 1 : 2 : 3 : 2 : 1, etc.; (c) the contributions from the ^{63}Cu and ^{65}Cu isotopes, though not resolved, are in the reported natural abundance ratio of 69 : 31 and the centre of the component from the ^{65}Cu isotope is $0.107 \times A_{iso}(^{63}\text{Cu})$ upfield of the corresponding ^{63}Cu resonance, as required by the relative magnitudes of their magnetic moments.

Each of the spectra from **I**, **IV** and **V** consists of at least two distinct components. The spectrum of **I** (Fig. 3a) is clearly the most complicated. The simulation in Fig. 3b reproduces the main features reasonably well and is composed of three distinct

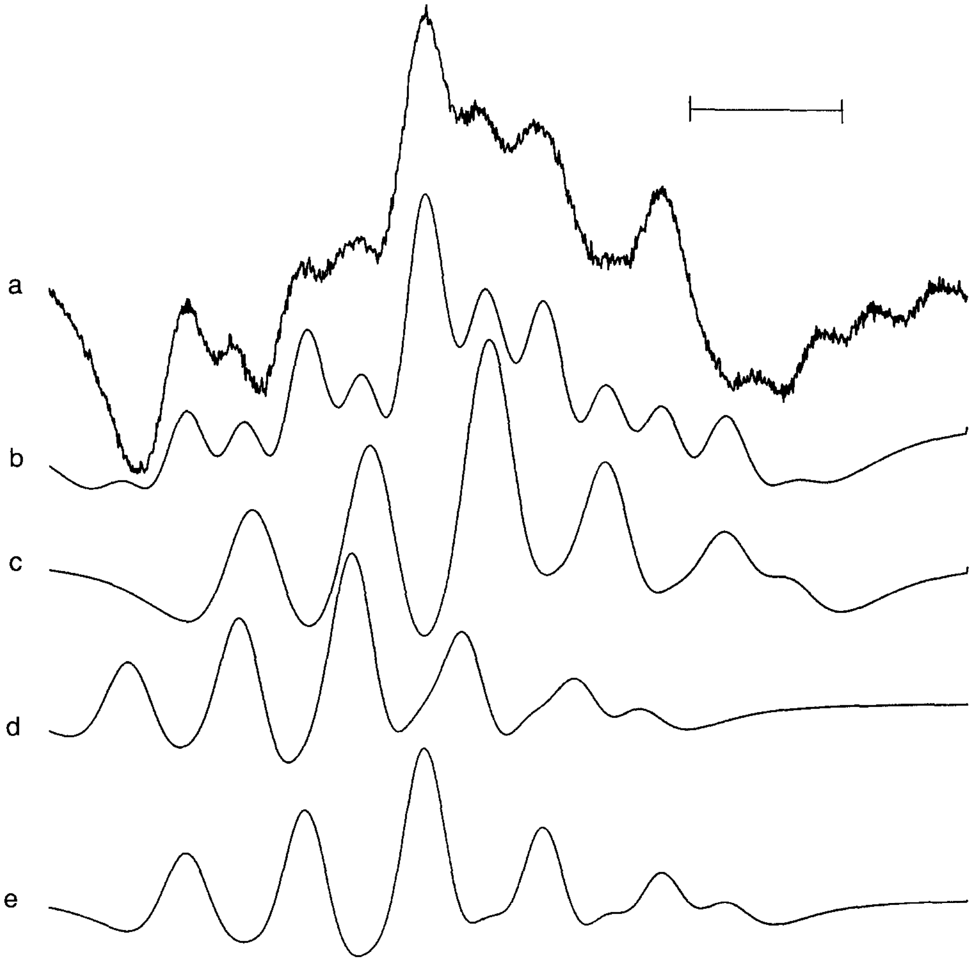


Fig. 3. (a) Second derivative of the region of the EPR spectrum at ambient temperature of copper(II) *as*-triazine complex **I** corresponding to the high field peak in Fig. 2a; (b) computer simulation of the experimental spectrum based on a summation of the spectra in (c) (d) and (e) in the ratio 52 : 11 : 37. Bar = 2 mT.

components (Fig. 3c–e) each of which has two equivalent nitrogens. The two major components (**A** and **C** in Table 3) have similar spectral parameters and are, therefore, probably very closely related structurally and chemically. In addition, there is clearly at least one additional component which is responsible for the weak peaks on the high-field side of this spectrum. The spectrum of **IV**, which superficially resembles a quintet, cannot be simulated adequately with a single component and the best reproduction of the experimental intensities (Fig. 4b) was obtained with a combination of a quintet (Fig. 4c) and a nonet (Fig. 4d), which suggests the presence of two very different types of complex, one having two and the other having four nitrogens. In the latter case, the copper coordination probably corresponds to two sets of two equivalent nitrogens, where the difference in magnitude of the two hyperfine coupling constants is too small to be resolved. Similarly, the simulation (Fig. 5b) of the highest field copper peak in the spectrum of the fresh sample of **V** (Fig. 5a), which corresponds to the decomposition product and not the original complex, could only be fitted with a combination of a quintet (Fig. 5d) and a nonet (Fig. 5c). The same region of the spectrum of the aged complex (Fig. 6a), however, revealed the presence of an additional triplet component with narrow linewidth superimposed on the original spectrum. This simulation is shown in Fig. 6(b).

The spectra of the various complexes in frozen solution are shown in Fig. 7. The corresponding simulations in Fig. 8 were generated from the parameters reported in Table 4. For all of the complexes the magnitudes of the g values and the copper hyperfine coupling constants indicate that the unaired electron is in the $d_{x^2-y^2}$ orbital, which is expected for the usual square planar or square pyra-

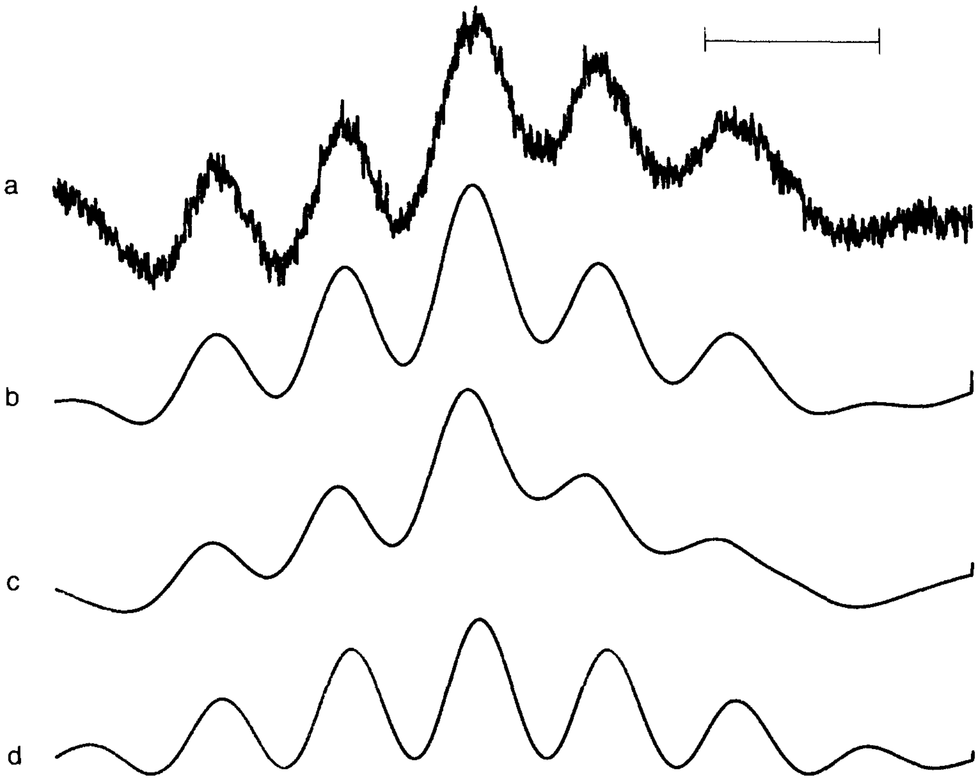


Fig. 4. (a) Second derivative of the region of the EPR spectrum at ambient temperature of copper(II) *as*-triazine complex **IV** corresponding to the high field peak in Fig. 2d; (b) computer simulation of the experimental spectrum based on a summation of the spectra in (c) and (d) in the ratio 56:44. Bar = 2 mT.

midal coordinations of copper complexes. Complexes **II**, **III** and **V** all show the presence of more than one type of copper environment (Table 4), whereas **I** and **IV** can each be simulated with a single component, as was the case with the first-derivative spectra for fluid solutions (Fig. 2). This suggests that the various components, which were observed in the second-derivative fluid phase spectra of these latter complexes, have similar anisotropic parameters and are probably closely related. All the components used in the simulations of these complexes have near axially symmetric A tensors,

Table 3. EPR spectra at *ca* 293 K of DMSO solutions of copper(II) *as*-triazine complexes

Complex	g_{iso}	A_{iso}(Cu) (cm^{-1} × 10^{-4})	A_N (mT)	Γ_N (mT)	No. of equivalent N	%
IA			14.8	1.7	2	52
IB	2.115	77.0	13.9	1.7	2	11
IC			14.6	2.0	2	37
II	2.128	75.5				
III	2.123	61.4				
IV	2.128	70.5	12.7	2.5	2	56
			13.3	2.5	4	44
V$_{\text{fresh}}$**A**	2.140	75.0	16.8	2.0	2	86
V$_{\text{fresh}}$**B**			12.4	3.0	4	14
V$_{\text{aged}}$**A**			16.8	2.0	2	71
V$_{\text{aged}}$**B**	2.087	87.7	12.4	3.0	4	12
V$_{\text{aged}}$**C**			14.8	0.7	1	17

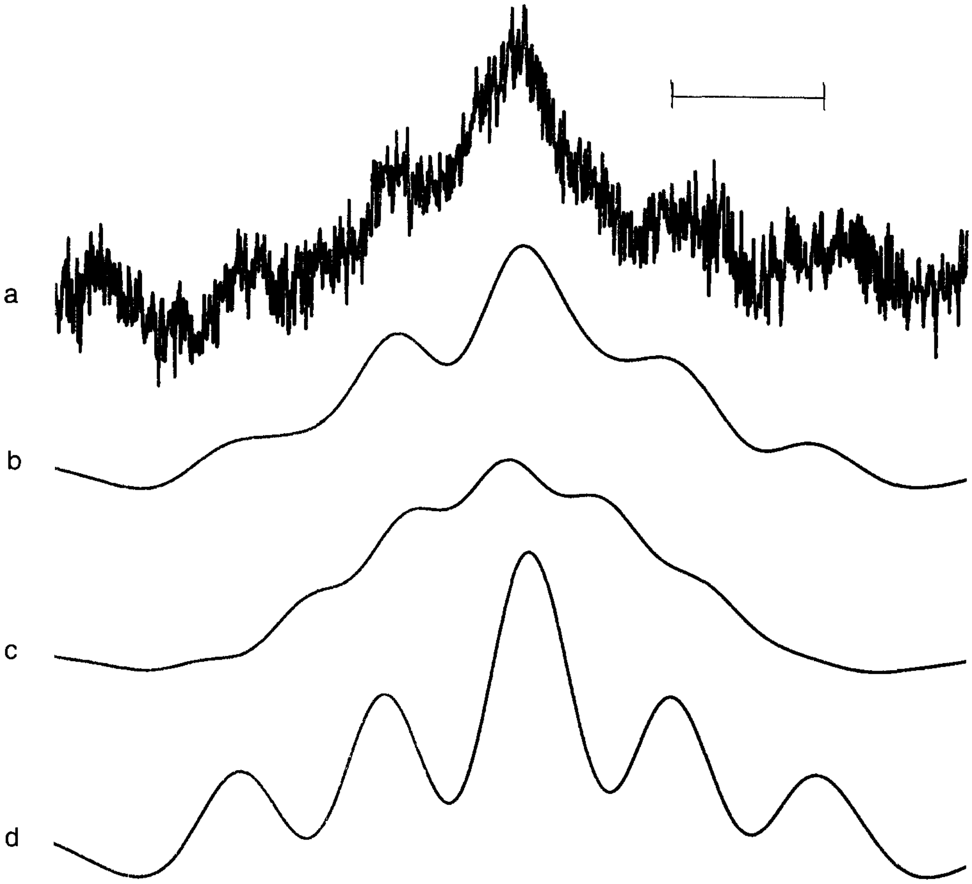


Fig. 5. (a) Second derivative of the region of the EPR spectrum at ambient temperature of copper(II) *as*-triazine complex **V** corresponding to the high field peak in Fig. 2e; (b) computer simulation of the experimental spectrum based on a summation of the spectra in (c) and (d) in the ratio 86:14. Bar = 2mT.

Table 4. EPR spectral parameters for Cu(II) *as*-triazine complexes at 77 K

Complex	g			A (cm^{-1} × 10^{-4})			Γ (mT)		
	g_{xx}	g_{yy}	g_{zz}	A_{xx}	A_{yy}	A_{zz}	Γ_x	Γ_y	Γ_z
I	2.031	2.106	2.234	23.7	29.5	179.4	7.0	3.0	7.0
IIA	2.045	2.065	2.222	15.3	17.4	196.1	5.0	5.0	4.0
IIB	2.065	2.095	2.256	18.3	28.4	179.1	5.0	5.0	4.0
IIIA	2.067	2.077	2.340	6.7	5.8	156.8	6.0	6.0	7.5
IIIB	2.100	2.150	2.380	14.7	50.2	142.2	6.5	6.5	5.0
IV	2.063	2.069	2.238	16.4	7.7	180.7	7.0	8.0	6.5
$\mathbf{V}_{\mathbf{fresh}}$	2.030	2.082	2.258	14.2	4.9	160.2	5.0	4.0	5.0
$\mathbf{V}_{\mathbf{aged}}\mathbf{A}$	2.035	2.035	2.182	33.2	33.2	193.5	6.0	7.0	5.0
$\mathbf{V}_{\mathbf{aged}}\mathbf{B}$	2.060	2.060	2.262	9.6	33.6	161.6	5.0	5.0	5.0

but there are variations in the orthorhombic characters of the g tensors, which suggests that there are differences in both the symmetry and magnitude of their spin–orbit couplings.

The relationship g_{zz}/A_{zz} provides a convenient empirical index of copper coordination symmetry and the results in Table 4 suggest the presence of a number of different structural arrangements in this

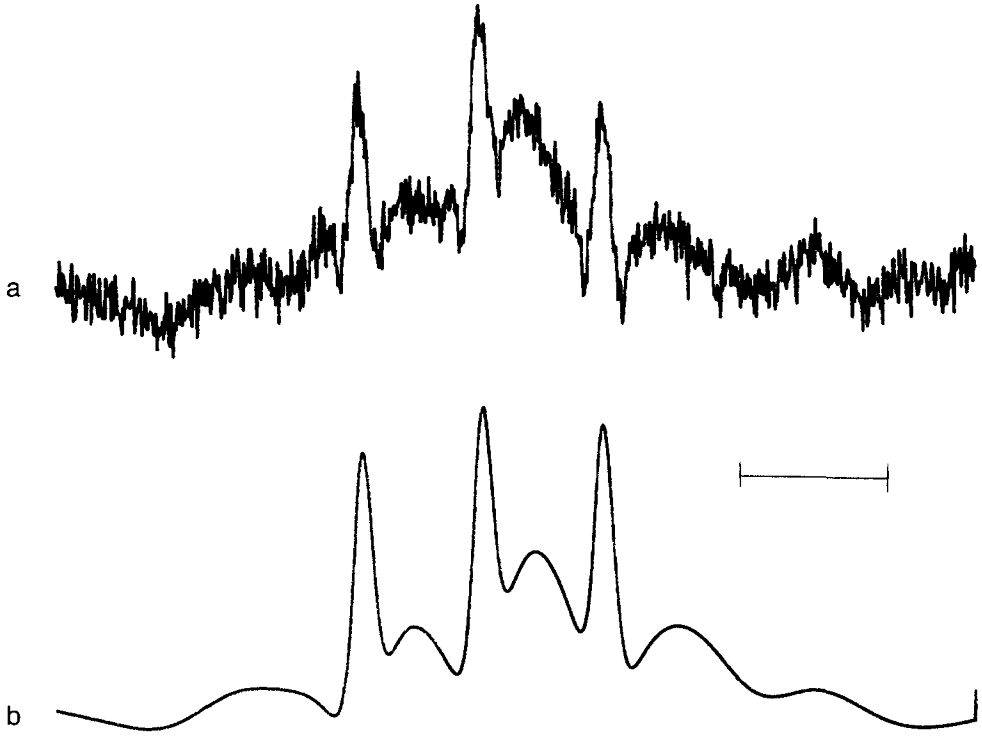


Fig. 6. (a) Second derivative of the region of the EPR spectrum at ambient temperature of an aged solution of copper(II) *as*-triazine complex **V** corresponding to the high field peak in Fig. 2f; (b) computer simulation of the experimental spectrum based on a summation of the simulation of Fig. 5 and a triplet in the ratio 83 : 17. Bar = 2 mT.

Table 5. Molecular orbital coefficients[a] calculated from the frozen solution EPR data for copper(II) *as*-triazine complexes assuming D_{2h} symmetry

Complex	α^2	β_1^2	β^2	α'^2
I	0.78	0.90	0.96	0.27
IIA	0.80	0.89	0.97	0.24
IIB	0.81	0.91	0.97	0.24
IIIA	0.83	0.94	0.97	0.25
IIIB	0.85	0.95	0.97	0.21
IV	0.79	0.90	0.96	0.29
$\mathbf{V}_{\text{fresh}}$	0.75	0.91	0.95	0.31

[a] Calculated using the following values: Cu spin–orbit coupling constant, $\lambda = -829\ \text{cm}^{-1}$; dipolar interaction term, P, for free Cu ion = 0.036 cm^{-1}; isotropic interaction term, k = 0.43 cm^{-1}; overlap integral, S = 0.093; constant $T(n)$ = 0.333.

family of complexes. Complexes **I**, **IIB** and **IV** have similar values for g_{zz}/A_{zz}, as do **IIA** and $\mathbf{V}_{\text{aged}}\mathbf{A}$. Also, the similarities in the spectra of $\mathbf{V}_{\text{fresh}}$ and $\mathbf{V}_{\text{aged}}\mathbf{B}$ indicate that the latter corresponds to the original material. Complexes **IIIA** and **IIIB**, however, produced spectral parameters that are different from those of any of the other complexes.

It was not possible to use the ^{14}N superhyperfine structure to obtain further information from the frozen solution spectra of these complexes because of its lack of resolution. Attempts to use second-derivative recordings to improve this resolution were largely unsuccessful. The only sample that showed clear evidence for ^{14}N superhyperfine structure was **IV** (Fig. 9), but even here the analysis is not straightforward; two distinct patterns are present and these could correspond to: (a) structure on the fourth copper peaks of the $g_\perp$ features of two different complexes each with axial symmetry; (b) structure on the third and fourth copper peaks of the $g_\parallel$ feature of a single complex with axial symmetry; or (c) structure on the fourth copper peaks of g_x and g_y features of a complex with rhombic distortion from axial symmetry. Consequently, it is not considered appropriate to discuss this structure further, especially since this does not appear to arise from the coordination environment responsible for the highest levels of SOD activity.

By using the ligand field approach[14,15] the molecular orbital coefficients for the various complexes

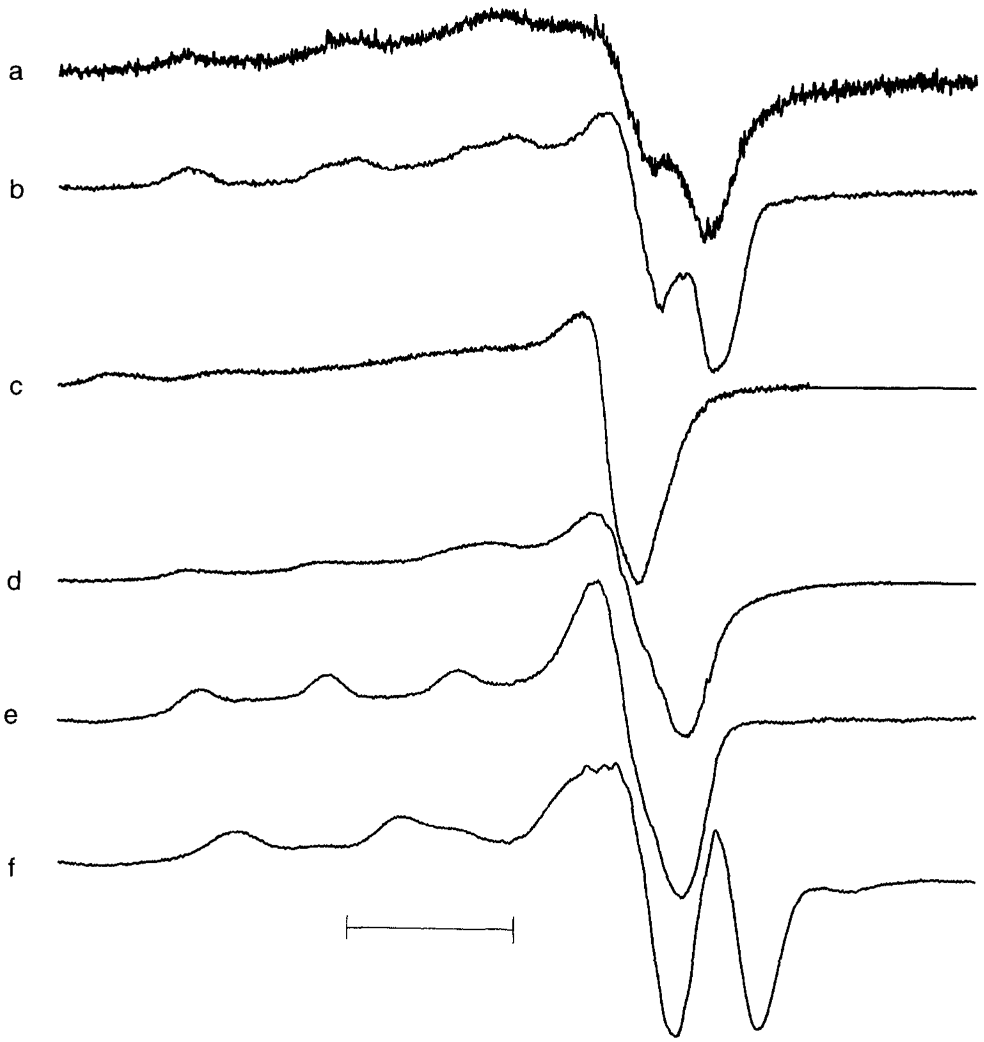


Fig. 7. First-derivative EPR spectra at 77 K of (a)–(e) fresh DMSO solutions of copper(II) *as*-triazine complexes **I**—**V** as detailed in Table 1 and (f) DMSO solution of **V** aged in air for 24 h. Bar = 20 mT.

were calculated from the spectral parameters presented in Table 4 assuming D_{2h} symmetry; although the g and hyperfine tensors indicate a small amount of rhombic character in the complexes, axial symmetry was assumed for simplicity in these calculations. The parameters are presented in Table 5, in which α and β_1 are the metal d-orbital coefficients representing σ- and in-plane π-bonding, respectively. β is the coefficient for out-of-plane π-bonding and α' corresponds to the coefficient for the ligand orbitals in the ground state molecular orbital. The relatively small values for α^2 in all of the complexes indicate a high degree of covalency in the σ-orbital containing the unpaired electron. The higher values of β^2 and β_1^2 indicate that the π-orbitals are essentially copper-based, although the in-plane π-bonds have slightly more covalent character than the out-of-plane π-bonds, which are essentially ionic in character.

Coordination geometry of the copper

The calculations of possible geometrical arrangements for the various complexes using Hyperchem showed that more than one type of structure was possible for each of the complexes (Table 6). All the ligands were able to form complexes with 2N2O coordination environments around the copper, whereas **III**, **IV** and **V** could also exist with 4N coordinated to the copper. Three distinct geometrical arrangements can be generated for **I**: square planar (**Ia**), distorted square pyramidal (**Ib**) or distorted tetrahedral (**Ic**). It was also possible to generate one structure with near square planar geometry for complex **IV**, but all of the other structures with 2N2O coordination were distorted. In addition, the structures for **III**, **IV** and **V** with four nitrogen atoms coordinated to the copper were all

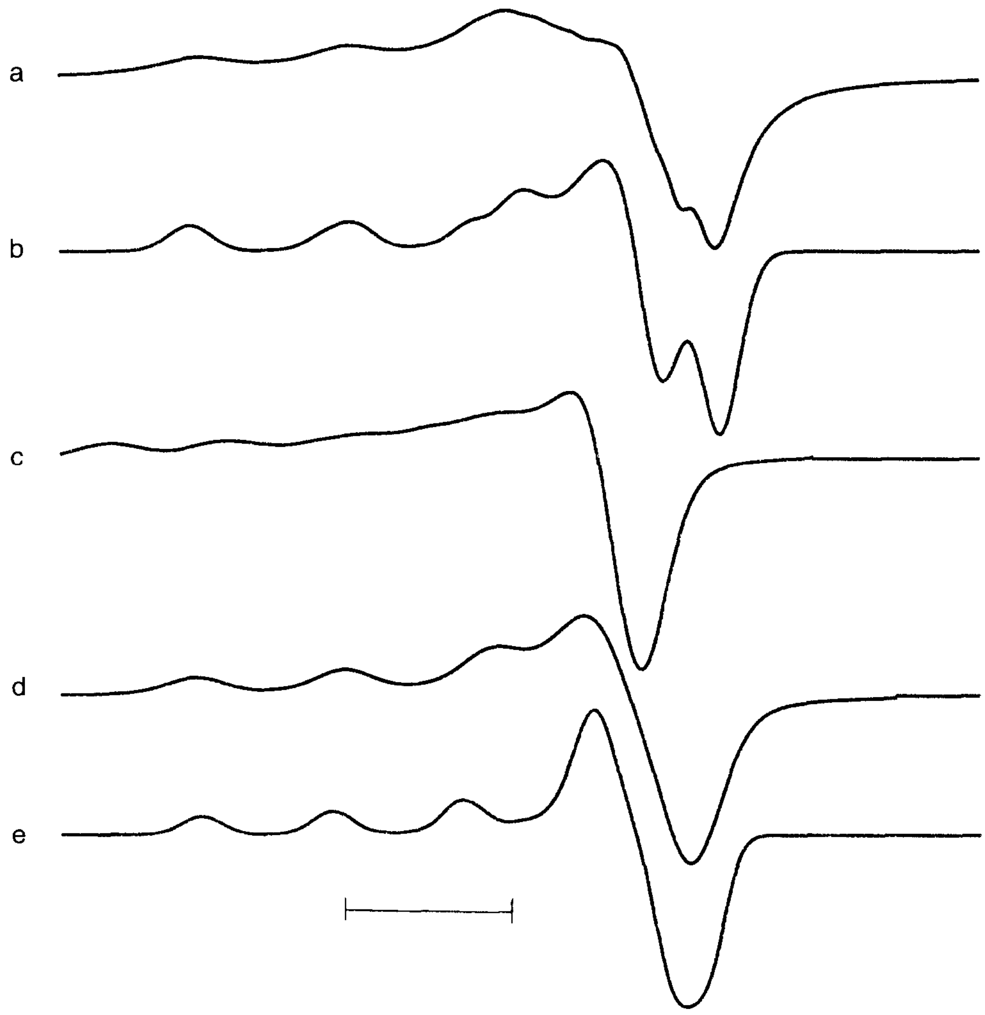


Fig. 8. Computer simulations of the spectra (a)–(e) in Fig. 7 using the parameters given in Table 3. Bar = 20 mT.

in the form of distorted square pyramidal geometries, which resemble the structures of the copper(II) complexes of carboxyphenylazopyrimidine ligands reported by Moreno *et al.*[18]

From the comparison of the activities of the ligands and the complexes (Table 2), it is clear that copper has an important role in determining the SOD activities of the complexes. In all the biological tests **I** showed by far the greatest activity, **II** and **IV** were more active than **III** and **V**, but only of the same order of magnitude as Cu-DIPS, a standard anti-inflammatory drug.[7] Any attempt at identifying the source of the SOD activity in these *as*-triazine complexes should, therefore, concentrate on structural features that are dominant in **I**.

The EPR measurements show that **I** actually consists of a closely related group of complexes, each with two nitrogen and two oxygen atoms in the copper equatorial plane and six-membered chelate rings. The three complexes are distinguished, however, by differences in their coordination geometries. Out of all of the structures that were calculated only **I** was able to form a square planar structure with six-membered chelate rings and it is suggested, therefore, that this could represent an important criterion for determining the biological activity. Complex **IV** can also exist in a square planar structure, but in this case it is based on a seven-membered chelate ring. Although its EPR spectrum had a g_{zz}/A_{zz} value that was similar to that of **I**, both g_{zz} and A_{zz} are appreciably greater in **IV**, which suggests different bonding characteristics between the metal and the ligand. The EPR spectrum of **II** showed a minor component with similar parameters to **I**, but the major component had a quite different g_{zz}/A_{zz} value. The spectra of complexes **III** and **V** did not show any component in their EPR spectra with parameters equivalent to those of **I** and the structural calculations show the absence of any species with a square planar

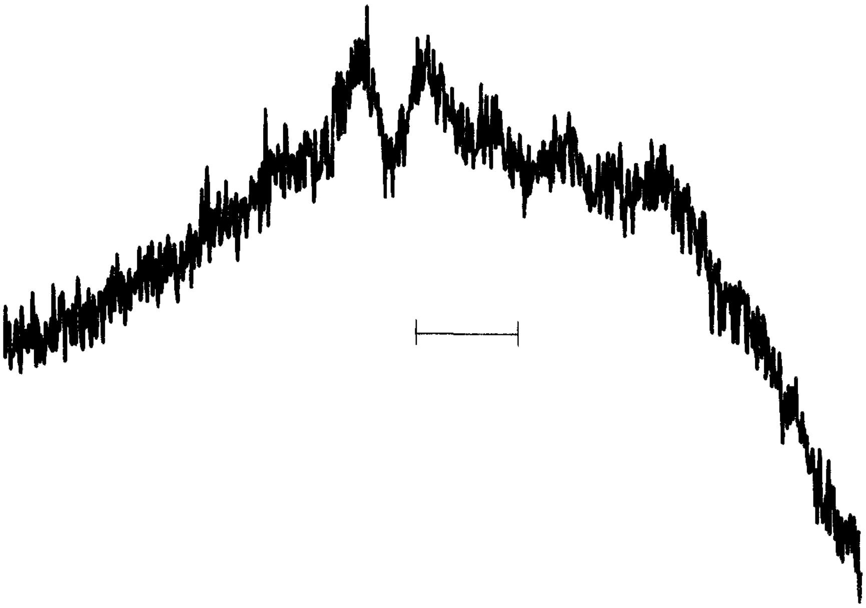


Fig. 9. Second derivative of the EPR spectrum of copper(II) *as*-triazine complex **IV** corresponding to the $g_{\perp}$ region of Fig. 7d. Bar = 2mT.

Table 6. Molecular mechanical force-field calculations of optimized geometrical arrangements for copper(II) *as*-triazine complexes

Complex	Species	Coordination	No. of atoms in chelate ring	Structure of complex	Energy[a] (kcal mol^{-1})	Gradient[b] (kcal mol^{-1} Å^{-1})
I	**a**	2N2O	6	Square planar	213.8	0.009
I	**b**	2N2O	6	Distorted square pyramidal	108.4	0.007
I	**c**	2N2O	6	Distorted tetrahedral	28.2	0.008
II	**a**	2N2O	6	Distorted tetrahedral	29.1	0.007
II	**b**	2N2O	6	Distorted square pyramidal	100.9	0.080
III	**a**	4N2O	5	Distorted octahedral	32.8	0.009
III	**b**	2N2O	5	Distorted tetrahedral	32.8	0.010
IV	**a**	2N2O	7	Square planar	103.3	0.010
IV	**b**	2N2O	7	Distorted tetrahedral	23.7	0.009
IV	**c**	4N2O	5	Distorted octahedral	31.9	0.010
IV	**d**	2N2O	5	Distorted tetrahedral	36.9	0.008
V	**a**	4N2O	5	Distorted octahedron	32.2	0.009
V	**b**	2N2O	5	Distorted tetrahedral	31.4	0.010

[a] The potential energy relative to the same atoms that are not interacting (in an unbound state).
[b] The rate of change of energy as a function of atomic position.

geometry, although structures based on the 2N2O chromophore were present in each case.

Further experiments are necessary to test the hypothesis that high SOD activity is related to the existence of a square planar structure involving two nitrogen and two oxygen atoms in six-membered chelate rings and to evaluate the relative importance of the various structural and chemical properties of these complexes. The present samples are not appropriate for such measurements because they exist as multiple components, presumably the consequence of the multidentate nature of the various ligands, which would be expected to coordinate to copper in a variety of ways depending on factors such as concentration, pH and metal : ligand ratio, as has been observed for simple peptide complexes.[17,19]

CONCLUSIONS

EPR spectroscopy is a powerful technique for distinguishing different types of coordination environment for copper in complexes and mixtures of complexes. Using this approach it has been possible to demonstrate that a family of asymmetric-triazine copper(II) complexes with a range of SOD activities are all mixtures containing more than one type of complex. By comparison of the activities of the various specimens with their spectral parameters and structure calculations, it is concluded that the most active form is associated with a bis-copper complex containing six-membered chelate rings and having two nitrogen and two oxygen atoms coordinated in the equatorial plane of the copper in a square planar arrangement.

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