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ESR study of Cu(II): thiostrepton complex

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Thiostrepton (MW 1650) forms a stable complex with Cu(II) in 1:4 molar ratio with total loss in its biological activity. The *g* values of the metal ion obtained from the ESR spectra of the complex in powder and in different solvents at room temperature and at liquid nitrogen temperature (LNT) indicated a well co-ordinated metal ion with the ligand. Large exchange interactions between the Cu(II) ions present well within the complex molecule in the powder and in the liquid resulted in a single symmetric resonance line, while the LNT spectra are resolved in some of the solvents. The interacting and non-interacting nature of these solvents with the complex and the probable stereo structure of the ligand around the metal ion in one of the solvents are discussed.

CHELATING properties of certain antimicrobial agents with metal ions enable a broad understanding about the mode of their action, especially when metal chelation affects their biological activity. Chelation with metal ions may result in either enhancing, stabilizing or abolishing the bioactivity of these agents[1-3]. Various mechanisms are explained for such altered activities of these antimicrobial agents on interaction with the metal ions[3]. Therefore, metal chelation of antibiotics has been the subject of intense investigations[4-7].

The molecular structure of thiostrepton, a peptide antibiotic[8], (Figure 1) reveals that it has many sites at which metal coordination can occur. Therefore in our studies on the structure–activity correlations of thiostrepton, the effect of metal ion interaction on its biological activity was investigated. While these studies along with spectroscopic characterization of the antibiotic molecule in the metal complexes using UV, CD, IR and NMR constitute a major part of our investigations, here, we report the ESR study of Cu(II): thiostrepton complex.

Thiostrepton (MW 1650) was a gift sample from The Squibb Institute, New Jersey, USA. All the solvents used were of analar grade and used directly. UV spectra were recorded on Beckman's DU-50 recording spectrophotometer. ESR spectra were recorded on Brucker X-band spectrometer at 9.71 GHz. The spectra were analysed using manganese (Mn^{2+} in MgO) and DPPH as the standards. The error in $g_{\parallel}$ and $A_{\parallel}$ values reported is ± 0.006 and $\pm 9 \times 10^{-4}\ cm^{-1}$ respectively. The centre field in all the spectra was maintained at 3200 G (indicated by an arrow mark in the figures).

Thiostrepton (165 mg, 0.1 mmol) was dissolved in 1,4 dioxane (5 ml). To this solution, $CuCl_2 \cdot 2H_2O$ (1 g) dissolved in water (1 ml) was added and thoroughly mixed. The reaction mixture was then incubated at 37°C for 24 h. To the resultant mixture, dioxane (200 ml) was added gradually with constant stirring. A light-green-coloured product was precipitated on standing. The precipitate was collected by centrifugation and washed successively with dioxane and ether and finally dried *in vacuo*. Yield 112 mg. The purity of the complex was ascertained by thin layer chromatography using different solvent systems. UV absorption of the complex was recorded in 20% dimethylsulphoxide (DMSO). The biological activity of the complex was determined by the method as described earlier[9].

Powder complex (6 mg) was digested on a sand bath with 5 ml of perchloric acid: nitric acid mixture (5:1 v/v). Later, the residue was dissolved in 3 ml of water and its metal content was estimated by flame photometry using a Perkin-Elmer atomic absorption spectrophotometer.

The ability of thiostrepton to complex with Cu(II) was also determined by the modified continuous variation method of Job[7] as follows: To 0.25 ml of thiostrepton solution (0.06 μmol) in DMSO were added 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1.0 ml of copper

*For correspondence.

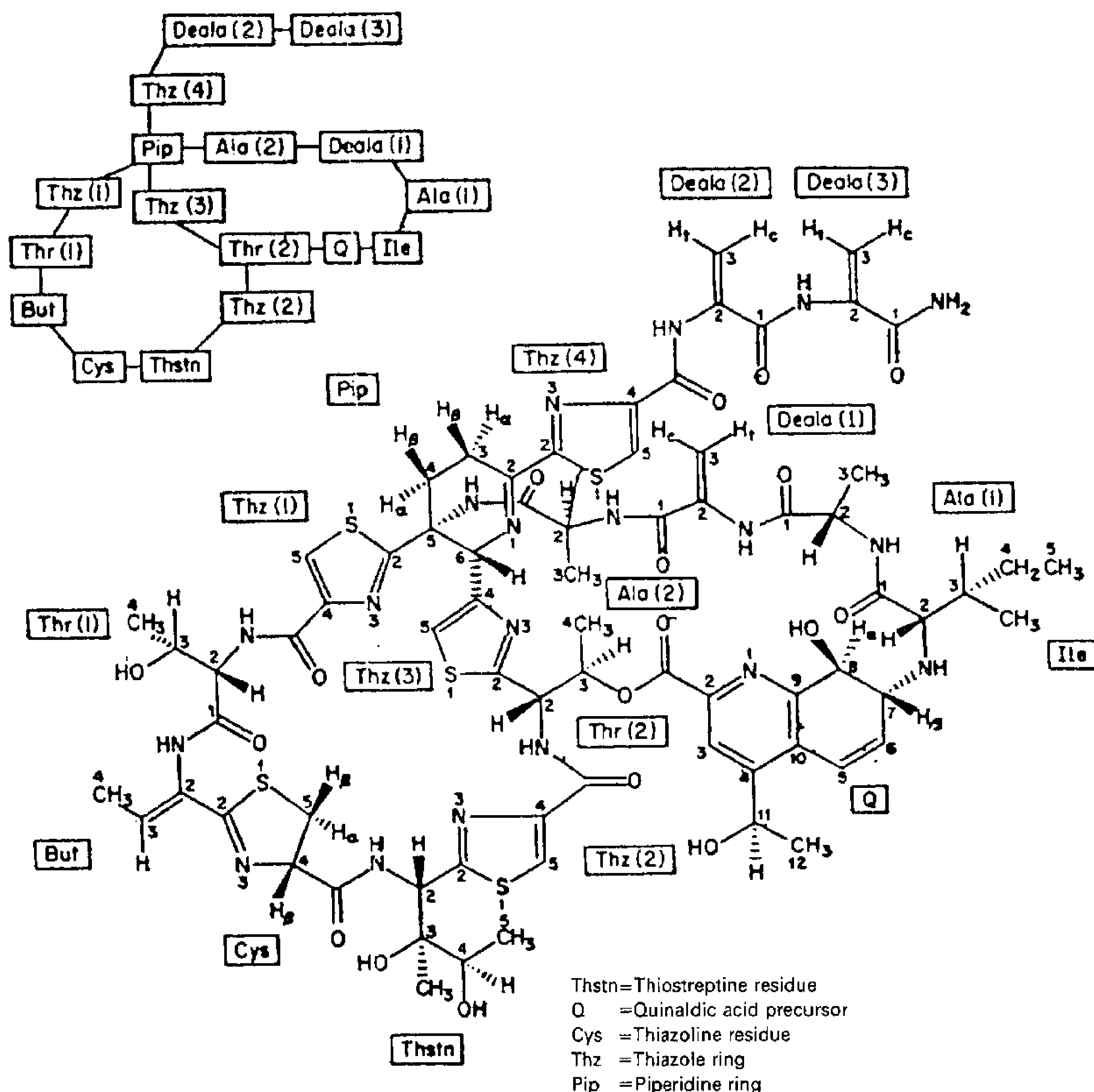

Figure 1. Structure of thiostrepton.

chloride solution (0.48 μmol) of Cu(II) to obtain the molar ratios of 1:0.8, 1:1.2, 1:1.6, 1:2.4, 1:3.2, 1:4.0, 1:4.8, 1:6.4 and 1:8.0 of thiostrepton to Cu(II). The total volume in all the tubes was made up to 1.25 ml with water. These were designated as the test sample sets. To the control sample sets were added graded levels of Cu(II) as above and 0.25 ml DMSO but without the antibiotic. Both the sets were incubated at 65°C for 7 h. Later, the absorbance of the test samples was measured at 360 nm using the corresponding tubes of the control sample set as the blank to obtain the differential absorption ΔA. These ΔA values were then plotted against the molar ratios of thiostrepton to Cu(II). The maximal value of ΔA in the graph indicated the maximum molar ratio of the metal ion to ligand in the chelate formed.

The UV spectra of the complex in 20% DMSO reveal that in addition to the 260 nm peak and 280 nm shoulder of the native antibiotic molecule, its Cu(II) complex acquires a new shoulder at 360 nm (Figure 2). Therefore, 360 nm was the convenient wavelength selected for determining the maximum binding of Cu(II) ions to thiostrepton by the modified Job's continuous variation method. This study indicated that a maximum of four metal ions bind to one thiostrepton molecule (Figure 3). Estimation of the metal content in the complex by flame photometry showed that it constituted 14% by weight. Taking into account the Cl^- content in the complex which was estimated by silver nitrate precipitation, this method also showed that there are four metal ions in every thiostrepton molecule of the complex.

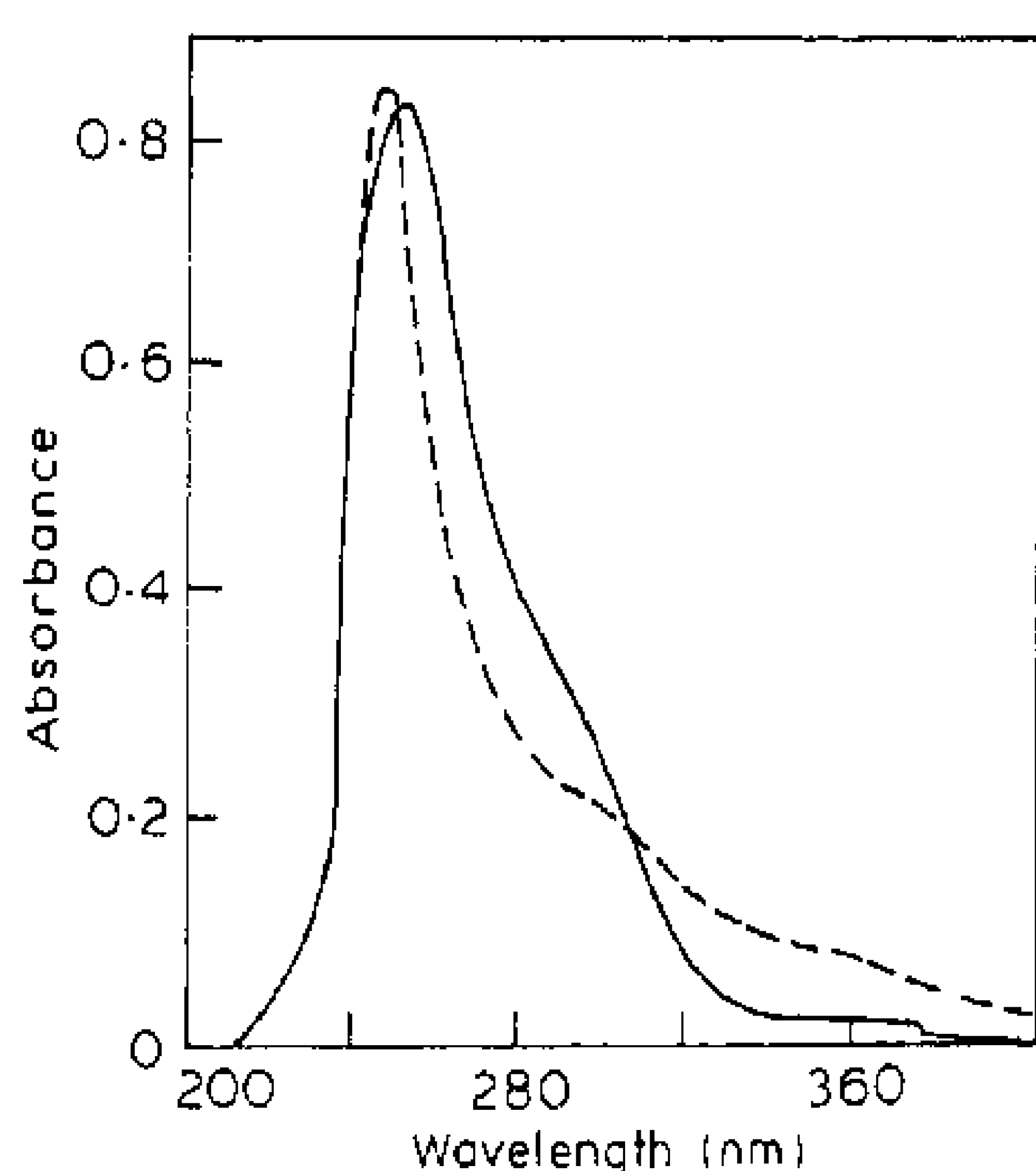

Figure 2. UV absorption of the native antibiotic molecule (——), and Cu(II) complex (---) in 20% DMSO.

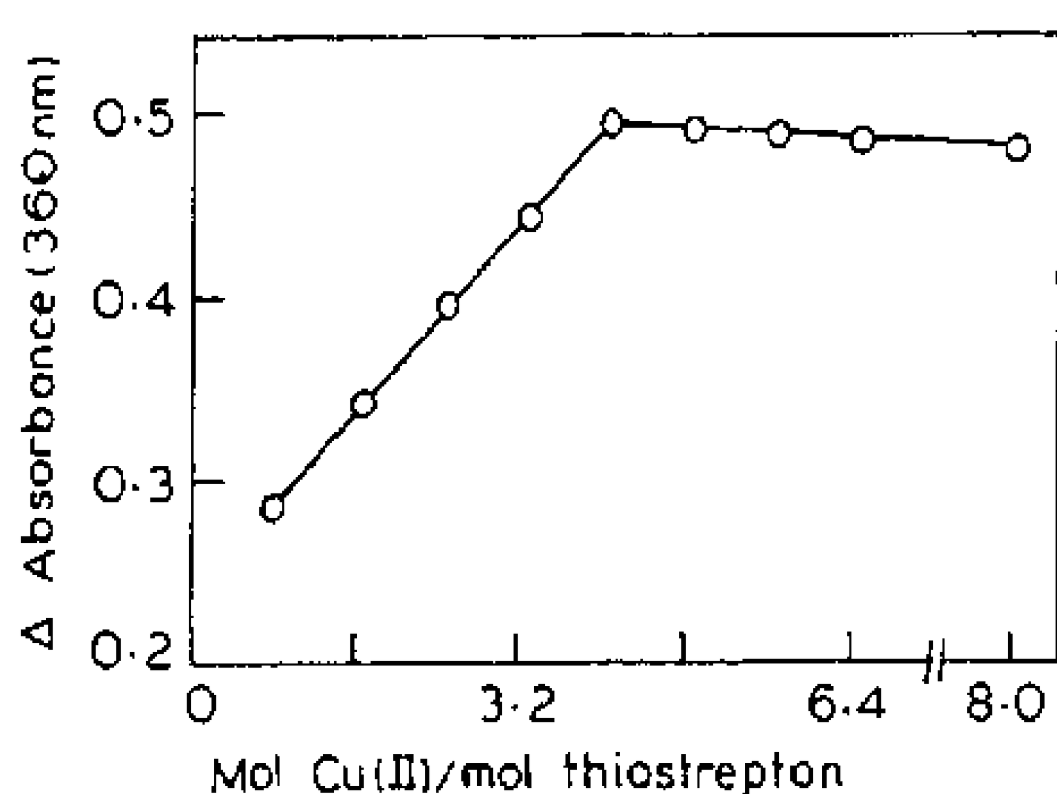

Figure 3. Modified Job's method of determining maximum binding capacity of thiostrepton to Cu(II).

The ESR spectra of the polycrystalline powder complex (Figure 4) showed a single symmetrical line having Lorentzian shape at the ends with ΔH_{pp} and g values being 170 G and 2.132 G respectively. This indicates the presence of a strong exchange interaction between the molecules existing in the unit cell of the complex[10]. This assumption is also based on the following two important considerations, (i) the powder spectrum of the parent salt ($CuCl_2 \cdot 2H_2O$) is a good three g value pattern[11], and (ii) in the metal complex there are four metal ions in every molecule of the antibiotic. In the absence of any X-ray data of the complex, it is not possible to know the probable binding sites for the four metal ions, and also the number of chelate molecules per unit cell. Hence, the ESR spectrum of the powder sample does not permit any quantitative description of the stereo structure around the metal ion. Therefore, in order to reduce the exchange interaction and obtain a good resolution, the ESR spectra of the complex were recorded in three different solvents, viz. DMSO, dimethylformamide (DMF) and pyridine both at room temperature (RT) and as well as at liquid nitrogen temperature (LNT).

The RT spectrum of the complex in DMSO and DMF showed that even at low concentrations (0.1%), instead of the four symmetric lines of Cu(II), there resulted a single broad unresolved line with g value 2.143 in DMSO and 2.131 in DMF (Figure 5, *a* and *b*). Assuming that the four hyperfine lines of Cu(II) are having spin independent line widths[12], this g value would correspond to the average of all these four lines. Probably a strong dipolar interaction between magnetically inequivalent metal ions present well within the antibiotic molecule might be resulting in the broadening of the spectrum. It was interesting however to observe that the g value of the complex in DMF is the same as that in powder, i.e. 2.131. However in DMSO, the g value obtained was observed to be different (2.143).

Therefore, in order to obtain a resolution in the spectrum, ESR spectra of the complex were recorded in these two solvents at LNT at lower than 0.1% concentration. It was observed that even at LNT,

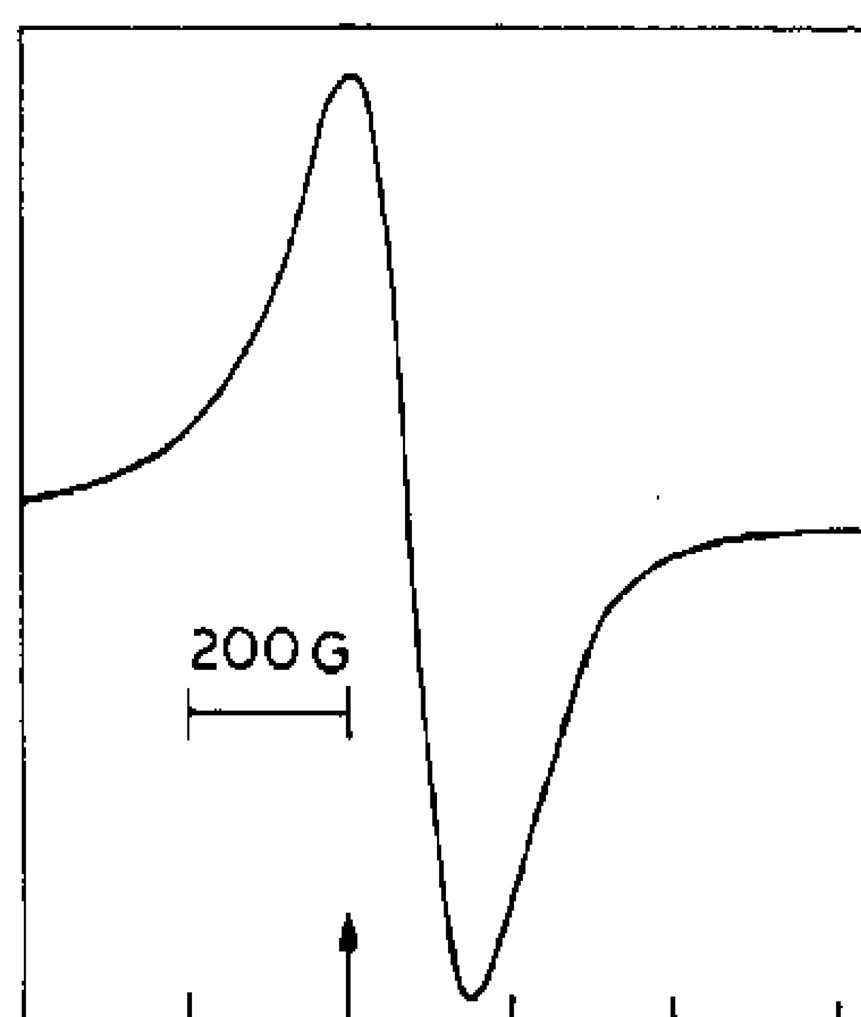

Figure 4. ESR spectrum of the powder complex at X-band frequency.

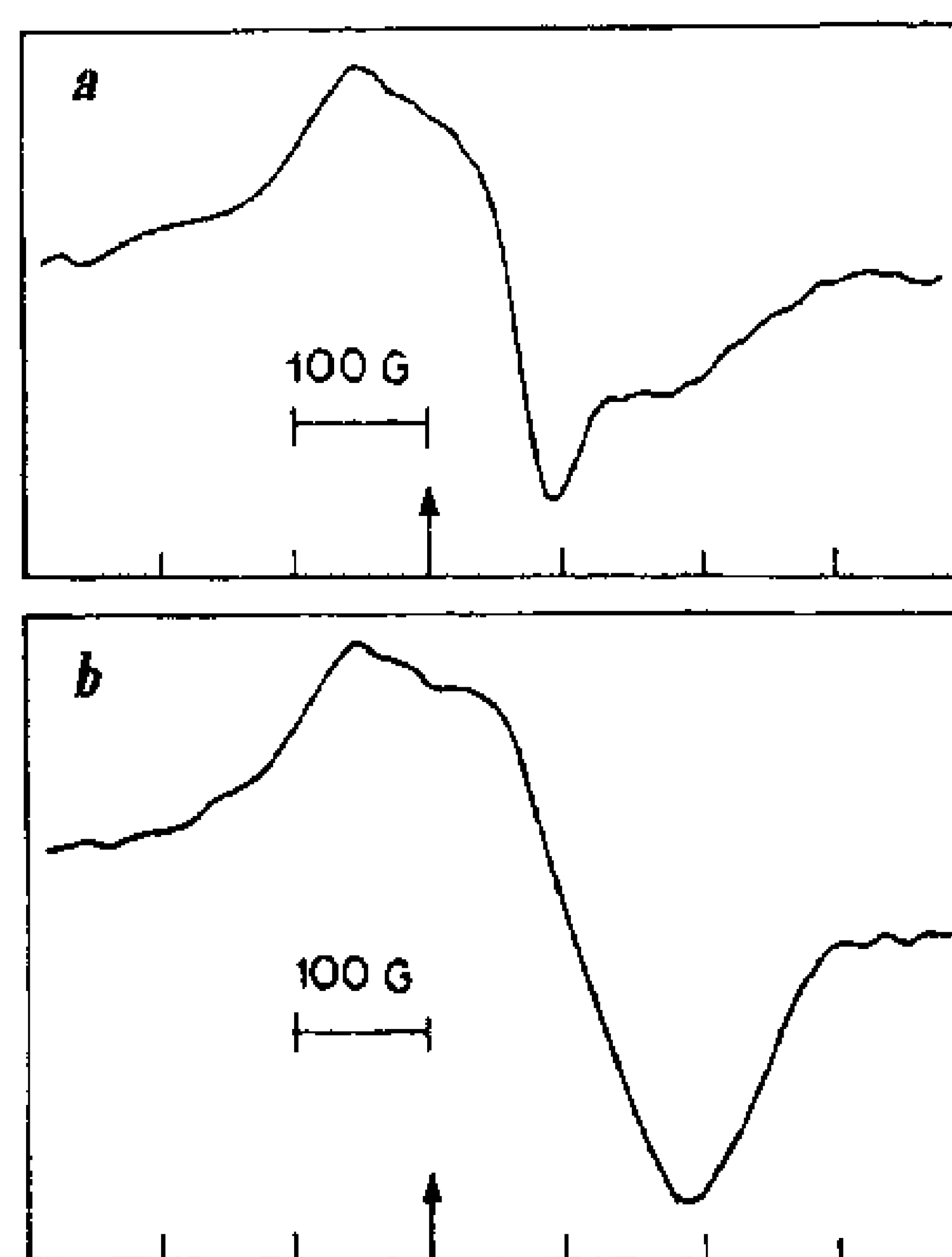

Figure 5. ESR spectrum of the Cu(II): thiostrepton complex (*a*) in DMSO at room temperature, (*b*) in DMF at room temperature.

irrespective of the concentration, the spectra in both the solvents were of a very broad line with unresolved parallel and perpendicular components (Figure 6, *a* and *b*), though, in DMF, there was a slight resolution in the spectrum when compared to that in DMSO with $g_{\parallel} = 2.277$ and $g_{\perp} = 2.065$ and $A_{\parallel} = 134 \times 10^{-4}\ \mathrm{cm}^{-1}$. This resolution may be due to a weak interaction of DMF with the complex at LNT which however is not stable at RT.

Hence, the ESR spectra of the complex were recorded in pyridine, since the basicity of this solvent being more than that of DMF, it can co-ordinate through the unoccupied positions of the coordination sphere present around the metal ions.

It was interesting to observe an altogether a different spectrum at RT in pyridine (Figure 7) where it resulted in a broad line with $\Delta H_{pp} = 215$ G and exhibiting four copper hyperfine lines with unequal line widths ($A_0 \approx 67.31 \times 10^{-4}\ \mathrm{cm}^{-1}$). At LNT in pyridine, the spectrum of the complex was well resolved with $g_{\parallel} = 2.276$, $g_{\perp} = 2.058$, $A_{\parallel} = 154.25 \times 10^{-4}\ \mathrm{cm}^{-1}$. These values correspond to the Cu(II) complexes of pyridine[13]. In addition, an extra structure was also observed on the perpendicular component with eight clearly resolved hyperfine lines ($\Delta H_{pp} = 14$ G) instead of the regular four components (Figure 7, *c*). This indicates that pyridine is entering into co-ordination with the metal ion and it is resulting in the formation of a new complex. Since the magnitude of the $g_{\parallel}$ values of

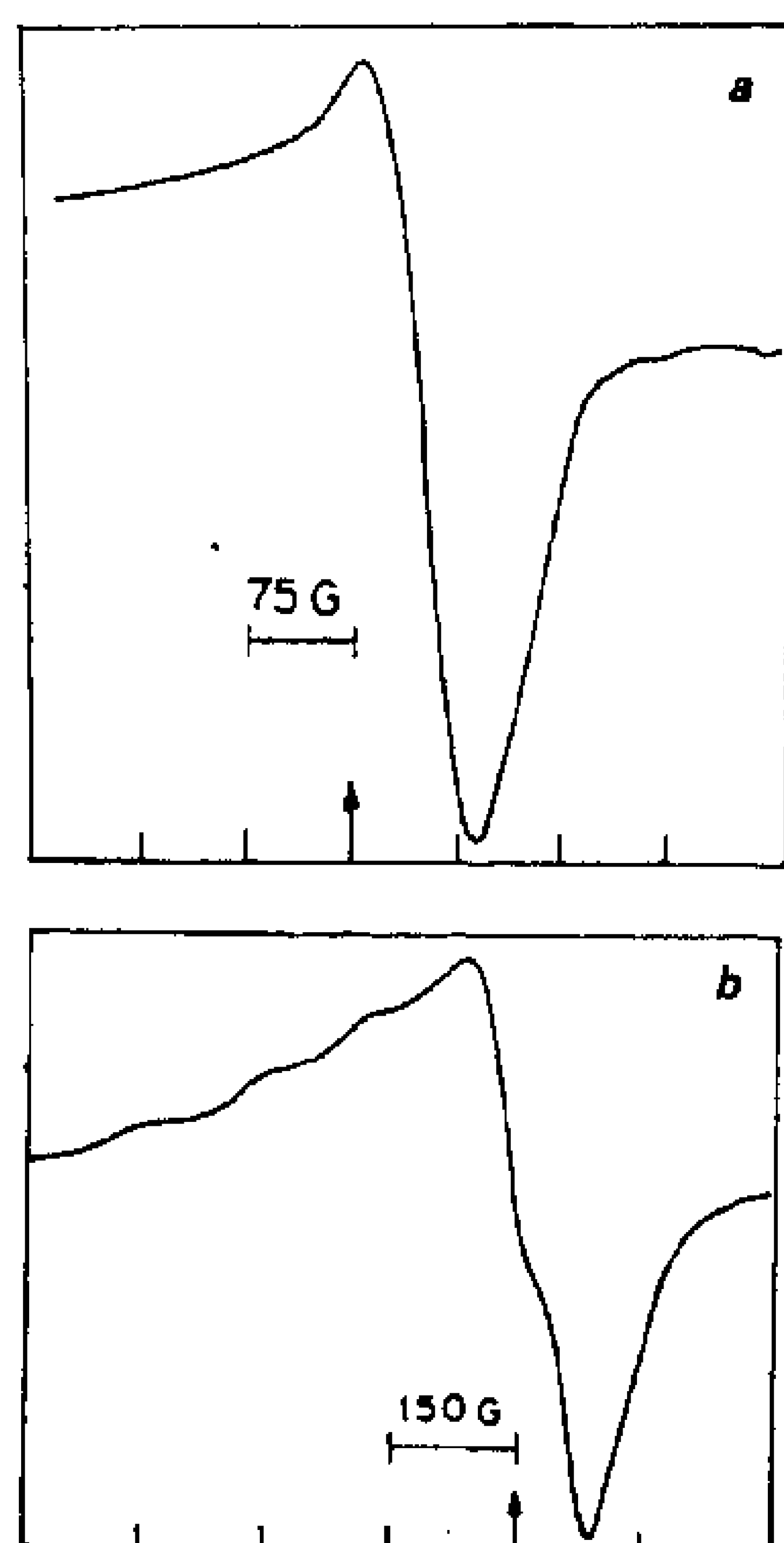

Figure 6. ESR spectrum of the Cu(II): thiostrepton complex (*a*) in DMSO at liquid nitrogen temperature, (*b*) in DMF at liquid nitrogen temperature.

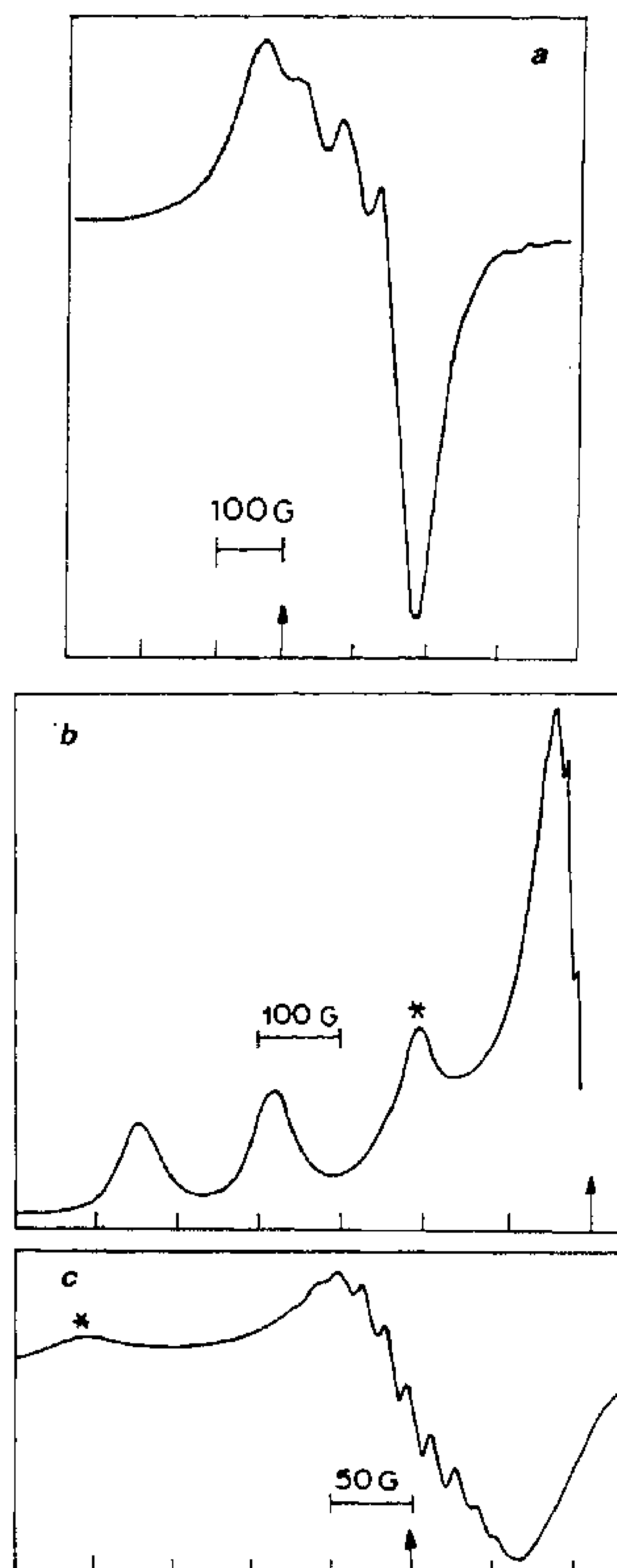

Figure 7. ESR spectrum of the Cu(II): thiostrepton complex in pyridine (*a*) at room temperature, (*b*) at liquid nitrogen temperature, (*c*) Expanded scale of the perpendicular components of the spectrum in *b* at liquid nitrogen temperature.

the complex in DMF and in pyridine being the same (i.e. 2.276), it appears that the stability of the structure that is present in DMF is not grossly affected by pyridine co-ordination. But the increased $A_{\parallel}$ values of the complex in pyridine compared to that in DMF may be due to a change in the geometry around the metal ion by added ligands from pyridine in the axial position[14]. Such a co-ordination would bring the Cu(II) ion into equitorial plane of the ligands. The super hyperfine structure on the $g_{\perp}$ component of the complex in pyridine indicates the presence of halogen atoms being very close to the metal nucleus. Since the parallel components of the complex are not bearing any further structure (Figure 7), the number of these atoms that are in co-ordination to the metal ion could not be ascertained.

Table 1. ESR parameters and line widths of Cu(II): thiostrepton complex in different solvents.

	Liquid nitrogen temperature			Room temperature		
Complex in the solvent	$g_{\parallel}$	$g_{\perp}$	$A_{\parallel}$ (10^{-4} cm^{-1})	g	ΔH_{pp}	A (10^{-4} cm^{-1})
Dimethylformamide	2.2765	2.0646	134	2.1312	235 G	—
Dimethylsulphoxide	—	2.0696	—	2.1434	135 G	—
Pyridine	2.2762	2.0580	154	2.1042	—	67.31
Powder sample	—	—	—	2.1315	170 G	—

Therefore in pyridine, with the solvent entering into co-ordination at the unoccupied co-ordinating positions of the metal ion, the predominant ligand environment around each metal in the complex would be of N atoms. IR and CD studies of the complex have indicated that some of the O atoms of the amide carbonyls and N atoms of azomethine groups of the ligand are involved in co-ordination with the metal ion (unpublished results). In such a case, with the additional N atoms of pyridine entering into coordination with the metal, it appears that the ligand structure around each metal ion of the complex in this solvent is a distorted octahedral symmetry.

Based on these data, the following conclusions are drawn:

i) The observed g values of the complex (Table 1) indicate a well co-ordinated metal ion.

ii) The unresolved ESR spectra of the complex in DMSO and DMF at a very low concentration even at LNT are indicative of a very strong persisting interaction between the Cu(II) ions present well within the complex molecule.

iii) The $g_{\parallel}$ and $A_{\parallel}$ values of the complex in DMF at LNT are characteristic of the stereo structure arising due to either Cu-$(DMF)_4^{2+}$ or Cu-$(DMF)_6^{2+}$ formation[5].

iv) Pyridine is entering into co-ordination with the Cu(II) ion of the complex, while DMSO is working as a non-interacting solvent.

v) Since the four Cu(II) ions present in each molecule of the antibiotic appear to have different ligand environment, it is not possible to conclude from these ESR parameters, a precise ligand structure around the metal ion.

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Residues of dichlorodiphenyltrichloroethane and metabolites in zooplankton from the Arabian Sea

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Studies on concentrations of organochlorine pesticide residues of the dichlorodiphenyltrichloroethane (DDT) group in zooplankton from the Arabian Sea show that these organisms metabolize DDT mainly to dichlorodiphenyldichloroethane (DDD) which is known to be degraded to the excretable dichlorodiphenylacetic acid (DDA) in higher organisms. This might explain the higher values of total DDT residues noted in zooplankton from the Arabian Sea compared to those in fish.

STUDIES have been made earlier on dichlorodiphenyltrichloroethane (DDT) residues in zooplankton from the Arabian Sea[1,2]. In the present study the levels of the metabolites of DDT namely, dichlorodiphenyldichloroethene (DDE) and dichlorodiphenyldichloroethane (DDD) were measured as it was felt that they might play a role in the bioaccumulation of DDT in the higher trophic levels. The zooplankton samples were collected using a neuston net from the Arabian Sea during the 47th cruise of *ORV Sagar Kanya*. Samples from three stations in a transect off Bombay (Figure 1),