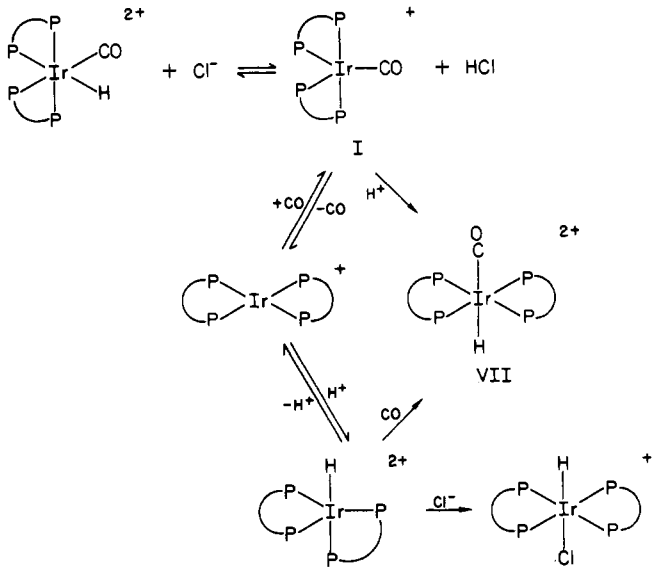
Scheme II



Alternatively, $Ir(dppe)_2^+$,[12,27] which is in equilibrium with $Ir(CO)(dppe)_2^+$, may undergo protonation to afford $IrH(dppe)_2^{2+}$. Subsequent reaction with CO affords VII. This pathway is used as a synthetic method to produce VII. The complex $IrHCl(dppe)_2^+$ could form by the known reaction of HCl with $Ir(dppe)_2^+$.[12,16]

The acidity of *cis*-$[IrH(CO)(dppe)_2][BF_4]_2$ is remarkable. Reversible deprotonation of this complex by chloride implies that its acidity is on the order of that of HCl. Although first-row transition-metal hydride complexes can show appreciable acidity,[28] this degree of acidity is unusual for complexes of the more basic third-row transition metals.[29,30] Hydrido transition-metal complexes of Ru,[31] Os,[8b,31] and Ir[32] have been depronated by strong bases such as alkoxide. The pK_a values for hydrido complexes of Os, W, Mo, and Cr are comparable with, or lower than, that of acetic acid.[29] The Brønsted acidity of *cis*-$[IrH(CO)(dppe)_2][BF_4]_2$ precludes activation of the coordinated CO to nucleophilic attack. Other members in the series of complexes *cis*- and *trans*-$IrX(CO)(dppe)_2^{2+}$ (X = Cl, H) are reactive with nucleophiles such as H_2O, OH^-, and H^- at the CO ligand. The results of these studies will be reported subsequently.[11]

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Registry No. Ia, 15308-58-4; Ib, 91685-28-8; IIa, 91606-09-6; IIb, 91606-11-0; IIIa, 91606-12-1; IIIb, 91606-14-3; IV, 91685-23-3; Va, 91685-24-4; Vb, 91685-25-5; VI, 91685-27-7; VIIa, 91606-15-4; VIIb, 66350-34-3; $[Ir(dppe)_2]Cl$, 15390-38-2; $[Ir(dppe)_2]BF_4$, 15130-28-6; $Ir_2Cl_2(C_8H_{24})_4$, 12246-51-4; $[Ir(CO)(dppe)_2]Br$, 15699-66-8.

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Immobile- and Mobile-Phase ESR Spectroscopy of Copper Complexes: Studies on Biologically Interesting Bis(thiosemicarbazonato)copper(II) Chelates

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ESR studies of an analogue of the antitumor agent [3-ethoxy-2-oxobutyraldehyde bis(thiosemicarbazonato)]copper(II), CuKTS, were undertaken to provide model data before studying the interaction of the analogues with Ehrlich cells. ESR data were taken at room temperature in anticipation of monitoring the fluidity of the environment about the copper complex. Room-temperature data vs. pH for [3-ethoxy-2-oxobutyraldehyde bis(N^4,N^4-dimethylthiosemicarbazonato)]copper(II), $CuKTSM_2$, clearly establish a low-pH and a high-pH form. Computer simulations of these spectra and spectra from published data are interpreted with use of a superposition of only these two distinct pH forms. The room-temperature ESR signal rapidly disappeared for CuKTS after incubation in Ehrlich ascites tumor cells and is consistent with previous reports of thiol reduction of CuKTS in Ehrlich cells. In contrast, the ESR signal for $CuKTSM_2$ was stable after incubation in Ehrlich cells and is consistent with the notion that CuKTS localizes in the cytoplasm and $CuKTSM_2$ localizes in the membrane. Surprisingly, $CuKTSM_2$ at low concentrations is essentially immobilized in its association with Ehrlich cells. This observation has not been previously made because only spectra for CuKTS in frozen samples of Ehrlich cells have been previously investigated.

Introduction

A number of copper complexes or ligands that bind copper have biological activity. Mono- and bis(thiosemicarbazonato)copper(II) complexes and analogues of the tripeptide H-Gly-His-Lys-OH have cytotoxic and antitumor effects.[3-7] In addition, copper(II) bleomycin has been extensively studied to determine its contribution to the antitumor activity of bleomycin.[8] The tripeptide H-Gly-His-Lys-OH and 2-formylpyridine thiosemicarbazone and derivatives, which respectively have hormonelike properties and antitumor

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Table I. ESR Parameters for Bis(thiosemicarbazones)

	$g_\parallel$	$g_\perp$	$A_\parallel^{Cu}$, G	$A_\perp^{Cu}$, G	$A_{x=y}^{N}$, G	A_z^N, G	ref
[3-ethoxy-2-oxobutyraldehyde bis(N^4,N^4-dimethylthiosemicarbazonato)]copper(II)-^{63}Cu	2.13	2.03	180	40	14	12	12
[biacetyl bis(thiosemicarbazonato)]copper(II)-^{63}Cu	2.12	2.00	192	32	14	14	11,13
^{63}CuKTS doped in Ni	2.130	2.035	184	25	14.6	15.6	25
^{65}CuKTS	2.14	2.03	192	31	14	14	this work
(room temp)			$g_{iso} = 2.05, A_{iso}^{Cu} = 87$ G, $A_{iso}^{N} = 14.75$ G				
^{65}CuKTSM$_2$	2.14	2.03	192	31	14	14	this work
(pH 7, room temp)			$g_{iso} = 2.06, A_{iso}^{Cu} = 87$ G, $A_{iso}^{N} = 15.25$ G				
^{65}CuKTSM	2.15	2.03	193	31	15	13	this work
(pH 1.7, room temp)			$g_{iso} = 2.06, A_{iso}^{Cu} = 80$ G, $A_{iso}^{N} = 14.25$ G				

properties, appear to require copper in their mechanisms of action.[5-7] A primary tool in the examination of the structures of many of these complexes as well as their chemical and biological reactions has been ESR spectroscopy of frozen samples.

Although immobilized-phase ESR spectroscopy has provided much fruitful insight into the biological behavior of these complexes, it is limited by the need to change the temperature and physical state of the system in order to make the measurement. An alternative is to do room-temperature, solution ESR measurements, which can be obtained for copper under these conditions. The advantage is that spectral conditions match reaction conditions. Such studies are important if the solution structure of the complex differs from that of the frozen state. This approach may be useful in the study of cellular reactions, for interactions of membranes and complexes will alter the motional properties of the paramagnetic species. Such processes are readily observed by ESR spectroscopy. The apparent disadvantage is the relative lack of information in the room-temperature spectrum. However, in principle this problem can be minimized by multifrequency ESR analysis used in combination with computer simulation of spectra.

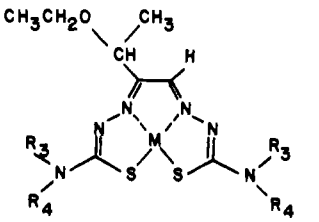
This report explores the model system of [3-ethoxy-2-oxobutyraldehyde bis(N^4,N^4-dimethylthiosemicarbazonato)]copper(II), CuKTSM$_2$, to determine the extent of the information that can be extracted from its room-temperature and frozen-solution spectra taken with a variety of instrumental settings. The room-temperature interaction of this copper complex with Ehrlich cells has also been examined by ESR spectroscopy and is described here.

Experimental Section

Materials. 3-Ethoxy-2-oxobutyraldehyde bis(N^4,N^4-dimethylthiosemicarbazone) was generously supplied by Eugene Conts. Isotopic ^{65}CuO was purchased from Oak Ridge National Laboratory, Oak Ridge, TN. Stock solutions of cupric ion were made up in concentrated HCl. All solvents were reagent grade. The pH was adjusted with NaOH when necessary.

Physical Measurements. The ESR studies were conducted at the National Biomedical ESR Center. The S-band spectrometer includes two microwave bridges operating respectively at about 3 and 1 GHz. The S-band cavity is a loop-gap resonator.[9] Computer programs for simulation of frozen sepctra of cupric complexes were obtained from Dr. J. Pilbrow, Monash University, Clayton, Victoria, Australia. The line width is determined from the quadratic equation $W_n' = [W_n^2 + B_n M_I]^{1/2}$, where $M_I = +^3/_2, +^1/_2, -^1/_2$, or $-^3/_2$ W_n and B_n are input parameters in gauss, and n is x, y, or z. The computer program for the simulation of room-temperature spectra was written by Dr. Wojciech Froncisz, Jagiellonian University, Krakow, Poland. It requires an input of the coupling pattern and a line width. All programs were simulated on a PDP-11/34A computer with 248K bytes of memory and floating-point processor.

Studies with Ehrlich Cells. The Ehrlich ascites tumor is a transplantable tumor derived from a mouse mammary carcinoma. It has been adapted to cell culture and is grown at 37 °C in a solution of Eagle's minimal essential medium plus Earles salts obtained from Gibco and 2.5% fetal calf serum. They can also be grown in mice, which survive about 17 days after intraperitoneal injection of 5×10^6 cells. For studies of the room-temperature ESR spectra of copper bis(thiosemicarbazones) in the presence of Ehrlich cells, copper complexes were rapdily mixed with washed cells with use of a vortex mixer in a medium of 0.01 M PO_4 (pH 7.2) + 0.15 M NaCl. The suspension was then inserted into a flat cell that was placed horizontally in the ESR cavity to prevent settling of cells over time. Concentration-dependent inhibition of Ehrlich cell proliferation by CuKTSM$_2$ was carried out as follows: a series of complex solutions of differing concentrations were added to flat-bottom cell wells, each containing 1×10^5 cells in 1.0 mL of the standard growth medium. After 48 h, cell counts were done and cytotoxicity assessed by comparing the extent of cell proliferation of treated cells with that of untreated controls.



	R_3	R_4
H_2KTS	H	H
H_2KTSM	H	CH_3
H_2KTSM$_2$	CH_3	CH_3

Figure 1. Structure of bis(thiosemicarbazonato)copper(II) complexes.

Results

ESR Spectra of CuKTSM$_2$ in Me$_2$SO. Previous ESR studies of bis(thiosemicarbazonato)copper(II) complexes in frozen solutions reveal hyperfine structure in the parallel and perpendicular regions of the spectra due to well-resolved couplings from the two dominant, equivalent nitrogens bound to cupric ion.[10-15] The chelate structure for CuKTS is shown in Figure 1. Simulations using ESR parameters from the literature (Table I) agree well with experimental spectra (Figure 2). Other investigators have concluded from the ESR spectra that only two ^{14}N nuclei have an observable hyperfine interaction with the unpaired electron on copper.[11-15] The perpendicular region of the ESR spectrum of [biacetyl bis(thiosemicarbazonato)]copper(II), prepared from ^{63}Cu in DMF and

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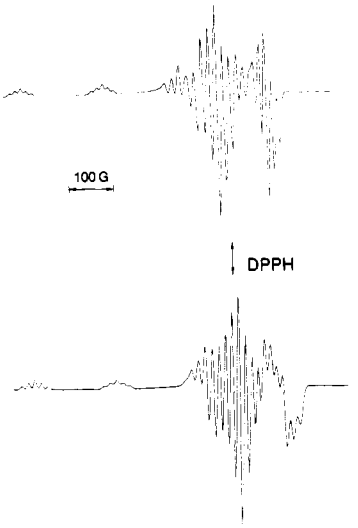

Figure 2. Simulated spectra for [3-ethoxy-2-oxobutyraldehyde bis-(N_4,N_4-dimethylthiosemicarbazonato)]copper(II)-^{63}Cu (upper) and [biacetyl bis(thiosemicarbazonato)]copper(II)-^{63}Cu (lower). Parameters are given in Table I. Line widths varied according to the equation $W_n' = [W_n^2 + B_n M_I]^{1/2}$ were n = x, y, or z: $W_x = W_y = W_z = 4.5$ G, $B_x = B_y = 0$, and $B_z = -1.0$ G (upper spectrum); $W_x = W_y = 4.5$ G, $W_z = 6.0$ G, $B_x = B_y = 0$, and $B_z = -15.0$ G (lower spectrum).

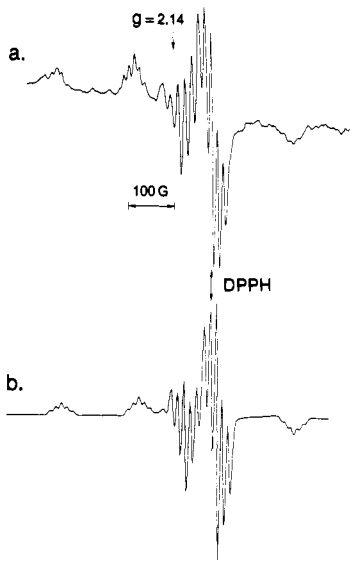

Figure 3. S-Band ESR spectrum (a) and simulated spectrum (b) for $CuKTSM_2$. ESR parameters are given in Table I. Additional parameters: microwave frequency 3.375 GHz; $W_x = W_y = W_z = 5.0$ G, $B_x = B_y = 0$, $B_z = -1.0$ G; temperature −196 °C.

examined at 77 K, agrees with the assignment of two nitrogen donor atoms because of the excellent fit between the experimental and simulated spectra. The experimental spectrum in the $g_\parallel$ region for the $M_I = -^3/_2$ and $M_I = -^1/_2$ lines consists of a symmetric, six-line pattern.[13] However, the simulated spectrum has the five-line pattern expected for hyperfine splitting from two equivalent nitrogens. In addition, the $g_\perp$ value of 2.00 determined from the best fit calculated spectrum implies complete quenching of the orbital angular momentum. Thus, $g_\perp$ can probably not be obtained from the relationship $g_{iso} = (g_\parallel + 2g_\perp)/3$, in which $g_\parallel$ is taken from the frozen-solution spectrum and g_{iso} from room-temperature data. The simulated and experimental spectra correspond well because the value for $\Delta g = g_\parallel - g_\perp$ is probably correct.

The ESR spectrum of $CuKTSM_2$ in frozen Me_2SO is resolved in the $g_\perp$ region and also well resolved in the $g_\parallel$ region for the $M_I = -^3/_2$ line at X-band frequency and the $M_I = -^1/_2$ line at S band (Figures 3a and 4a,c). The well-resolved $M_I = -^1/_2$ line in the $g_\parallel$ region of the S-band spectrum is due to a decrease in line width as predicted by the expression for the line width (methods), which contain M_I and frequency-dependent terms of opposite sign.[16,17] The apparent 1:2:3:2:1 pattern in the $g_\parallel$ region of both X- and S-band spectra is consistent with spin coupling from two equivalent donor atoms (Figures 2–4). In the simulation, ESR parameters were varied until, with the exception of a variation in line widths, a single set of parameters simulated both the X-band and two S-band spectra (Figures 3b and 4c,d). The simulated spectra contain the appropriate number of lines, but the line widths in the $g_\perp$ region for S band at 3.375 GHz appear to be closer to the experimental line widths than those from the X-band (9.1135) GHz) or the second S-band (2.3174 GHz) simulation. The broad, underlying signal evident particularly in the X-band spectrum is due to aggregation, which leads to spin coupling. This interpretation was supported by the presence of half-field transitions for samples with concentrations of $CuKTSM_2$ greater than 1 mM. The presence of aggregation may account

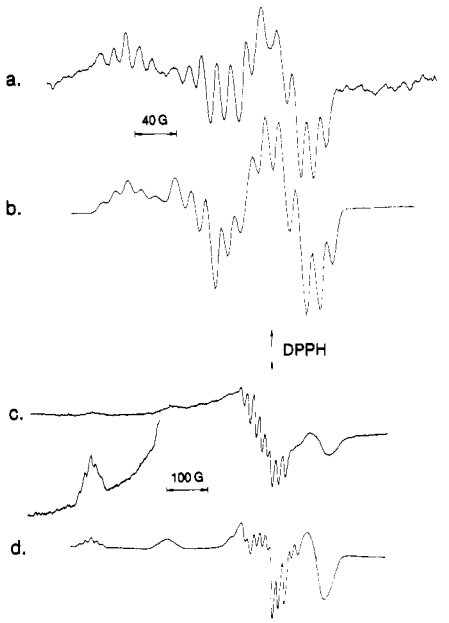

Figure 4. ESR spectra (a, c) and computer-simulated spectra (b, d) for $CuKTSM_2$ in 50% Me_2SO. ESR parameters are given in Table I. Additional parameters: temperature −196 °C; microwave frequency 2.3174 GHz (a, b) and 9.1135 GHz (c, d); $W_x = W_y = 5.0$ G, $B_x = B_y = 0$, $B_z = 1.0$ G (b); $W_x = W_y = 6$ G, $W_z = 9$ G, $B_x = B_y = 5$ G, and $B_z = -40$ G (d). Note that only the expanded spectrum including $M_I = \pm^1/_2$ in the $g_\parallel$ region and the $g_\perp$ region is shown in (a) and (b).

for much of the deviation between experimental and simulated spectra. It is noted that the concentration of $CuKTSM_2$ was kept at about 1 mM so that comparable room-temperature spectra could easily be obtained at several frequencies.

Since the rigid limit ESR parameters can be obtained at room temperature from the information in the line widths and second-order shifts,[16] room-temperature spectra were obtained at four different frequencies (Figure 5). The spectra for $CuKTSM_2$ at room temperature are consistent with the theory for second-order shifts as indicated by the displacement of the hyperfine lines and the striking differences in line width as the frequency is varied (Figure 5). The input parameters for the computer simulation program were a shift position for each M_I-dependent hyperfine line of copper and a line width and

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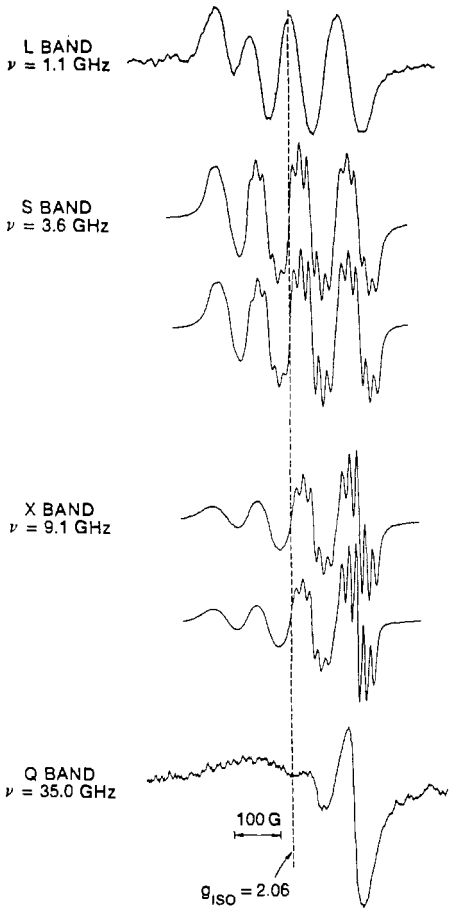

Figure 5. Multifrequency room-temperature ESR spectra and simulated spectra at S and X band for $CuKTSM_2$ in 50% Me_2SO–50% H_2O. In addition to the primary nitrogen splitting of 15.25 G for a pair of equivalent nitrogens, a splitting of 2.9 G, which is not resolved, was used to give a good fit for the line shape.

a coupling constant for nitrogen. The best simulations of the four M_I-dependent lines with respect to line width were obtained with use of $A_N' = 15.25$ G for a pair of equivalent nitrogens plus $A_N'' = 2.9$ G for a second pair of equivalent nitrogens.

It has been reported that the frozen-solution ESR spectra for CuKTS vary as a function of pH between 7.5 and 1.0, although copper remains tightly bound over this pH range.[10,14] Because the pH can vary significantly upon freezing,[18] we varied the pH of the solution of $CuKTSM_2$ in 50% Me_2SO–50% H_2O and measured the ESR spectra in the mobile phase at room temperature (Figure 6). The expanded region of the high-field line for $CuKTSM_2$ at pH 7 clearly shows the five-line pattern attributed to two equivalent nitrogens. If $^{65}Cu^{2+}$ is added to H_2KTSM_2 without adjusting the pH, the final pH is about 1.7 in this solvent mixture due to the low pH of the stock solution of $CuCl_2$ and the release of H^+ from H_2KTSM_2 upon formation of the $CuKTSM_2$ complex. The $M_I = +^3/_2$ line at room temperature has shifted to lower field for the low-pH sample compared to that for the high-pH sample, indicating a change in ESR parameters (Table I). Five well-resolved hyperfine lines are clearly evident, suggestive of a 1:2:3:2:1 pattern, which is attributed to the coupling from one predominant pair of equivalent nitrogens. Computer simulation with $a_N' = 14.25$ G and $a_N'' = 2.9$ G is consistent with this interpretation (Figure 6). The slight asymmetry in the experimental spectrum appears to result from a superposition of the low-pH form with a small amount of the high-pH

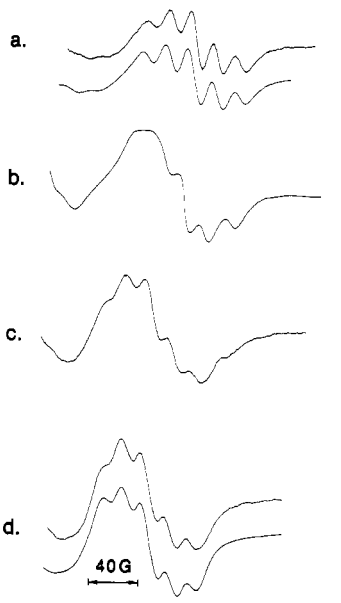

Figure 6. Partial X-band spectrum showing the $M_I = ^3/_2$ ESR line at room temperature for $CuKTSM_2$: (a) spectrum at pH 6.4 and simulated spectrum with parameters given in Table I; (b) spectrum at pH 3.0; (c) spectrum at pH 2.4; (d) spectrum at pH 1.7 and simulated spectrum with parameters given in Table I. The ESR pattern included a pair of equivalent nitrogens with a splitting of 15.25 G and an unresolved pair with a 2.9-G splitting (spectrum a) and a pair of equivalent nitrogens (14.25 G) and an unresolved pair (2.9 G) (spectrum d).

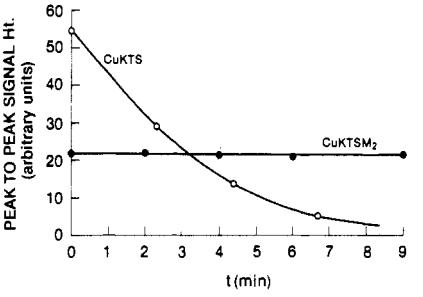

Figure 7. Disappearance of CuKTS (0.6 mM) ESR signal at room temperature but not $CuKTSM_2$ (0.6 mM) after addition to 6×10^7 Erhlich ascites tumor cells/mL of cell suspension.

form. The hyperfine structure is partially obliterated as the pH is raised because of the superposition of equal concentrations of the high- and low-pH forms. As the pH is raised above about 4.0, only the high-pH form is observed and the hyperfine structure is again well resolved.

Reaction of $CuKTSM_2$ with Ehrlich Cells. Previous studies of the interaction of bis(thiosemicarbazonato)copper complexes with Ehrlich cells established that CuKTS is reduced rapidly and dissociated. In contrast $CuKTSM_2$ is quite stable over the course of several hours and breaks down with a pseudo-first-order rate constant of 8.92×10^{-3} s^{-1}.[19] Further, its spectral characteristics in the presence of cells indicate that the complex resides in a lipophilic environment.[19] These results were confirmed here with use of room-temperature ESR to show that in the presence of cells CuKTS rapidly loses ESR intensity but that cellular-bound $CuKTSM_2$ remains intact over time (Figure 7). However, it was unexpected to find that its rotational correlation time was so slow that the spectra are typical of an immobilized species (Figure 8). The appearance of the immobilized form was clearly evident with a twofold increase of the concentration of complex from 0.6 to 1.2

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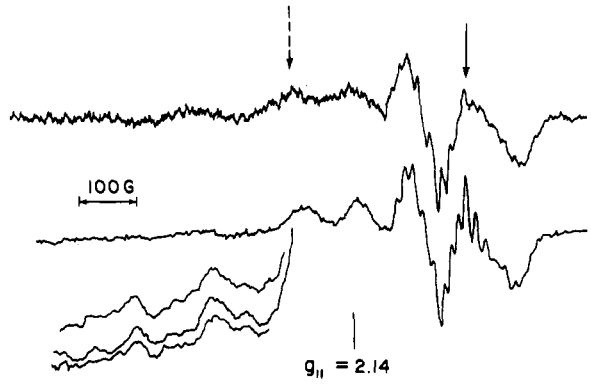

Figure 8. X-Band ESR for $CuKTSM_2$ in Erhlich ascites tumor cells at room temperature: (top) 0.6 nmol/10^5 cells; (bottom) 1.2 nmol/10^5 cells. For the partial spectra in the low-field region, the gain was increased twofold, the modulation amplitude was increased threefold, and the time constant was increased from 1 to 3 s. The peak to peak height (position indicated by the solid arrow) of the center hyperfine line in the 1:2:3:2:1 pattern for the $M_I = {}^3/_2$ high-field ESR line for $CuKTSM_2$ was used to indicate the concentration of the mobile form. This line has the largest intensity for the signal in the mobile phase and overlaps at a point of low intensity for the immobile signal. The low-field line for the immobile signal (position indicated by the dashed arrow) in the $g_{\parallel}$ region does not overlap with the mobile signal and was used to indicate the relative concentration of the immobile signal.

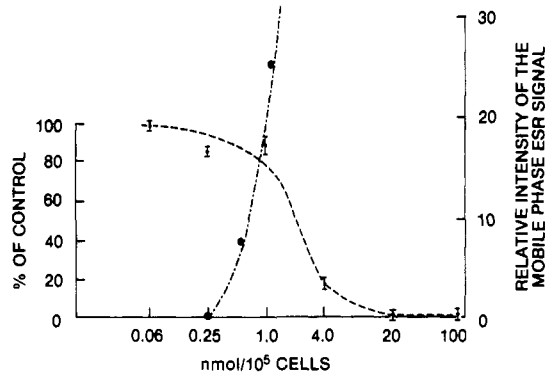

Figure 9. Growth of Erhlich ascites tumor cells after a 48-h period vs. increasing concentration of $CuKTSM_2$ (left ordinate, small dots with error bars) and the peak to peak intensity of the center hyperfine line in the 1:2:3:2:1 pattern for the $M_I = {}^3/_2$ high-field ESR line for $CuKTSM_2$ in the mobile phase (right ordinate, large dots).

nmol/10^5 cells (Figure 8). For these spectra the modulation and the gain were increased in order to detect weaker signals in the $g_{\parallel}$ region. Low-field lines in this part of the spectrum indicate the presence of one or more forms of cupric ion in which the original donor atoms from H_2KTSM_2 have been replaced by new combinations of nitrogen or oxygen atoms.

Figure 9 compares the concentration dependencies of the spectral and biological interactions of $CuKTSM_2$ with Ehrlich cells. Inhibition of cell proliferation in culture occurs in the range of concentration of 1–4 nmol/10^5 cells. This is the same range in which mobile forms of copper begin to appear in the ESR spectra. This suggests that $CuKTSM_2$ is first immobilized in membrane in a stoichiometric way and then upon completion of this reaction becomes mobile. Interestingly, only about 0.25 nmol/10^5 cells is needed to achieve rapid, 50% inhibition of thymidine uptake into cells and incorporation into DNA.[19] The coincidence of the inhibition curves for thymidine uptake and incorporation into DNA previously suggested that $CuKTSM_2$ caused these effects at the level of the plasma membrane.[19] The present data sustain this probability.

Discussion

ESR spectra of cupric complexes are usually obtained with use of frozen samples. The ESR parameters $g_{\parallel}$ and $A_{\parallel}^{Cu}$ are readily extracted from such spectra. However, the parameters $g_{\perp}$ and $A_{\perp}^{Cu}$ are more difficult to determine because of overlap of the $g_{\parallel}$ and $g_{\perp}$ regions, the presence of "overshoot lines" resulting from the angular dependence of the copper hyperfine lines, and the complicated hyperfine patterns from the copper and nitrogen couplings, which are of similar magnitude.

In several recent examples it has been shown that spectra taken with a low-frequency, S-band bridge (3 GHz) have superior resolution of the hyperfine structure because a cancellation of M_I and frequency-dependent terms in the expression for the line width results in narrower lines.[16,20-24] Not only are the ESR lines better resolved in the perpendicular region, but the increased resolution of the hyperfine structure of the $M_I = -{}^1/_2$ line in the parallel region may also permit the direct determination of the number of nitrogen donor atoms in the complex. In addition, for square-planar complexes that have a molecular weight of less than 2000 and are free to tumble, the room-temperature ESR parameters g_{iso} and a_{iso} can be measured. Then, given the values of $g_{\parallel}$ and $A_{\parallel}$ from the frozen-solution spectrum, the perpendicular parameters can be estimated from the equations $g_{iso} = {}^1/_3(g_{\parallel} + 2g_{\perp})$ and $a_{iso}^{Cu} = {}^1/_3(A_{\parallel} + 2A_{\perp})$. This, of course, assumes that ESR parameters do not change significantly as the physical state of the sample is altered.

A close examination of the published spectrum of [biacetyl bis(thiosemicarbazonato)]copper(II) in frozen solution shows that the structure of the $g_{\parallel}$ region cannot be interpreted as arising simply from a single pair of equivalent nitrogens.[11] The $M_I = -{}^1/_2$ line clearly displays a symmetrical six-line pattern. This is different than predicted for coupling of copper to two equivalent nitrogens and does not agree with the clear five-line pattern observed in the present study (Figures 2–4). Symmetric hyperfine patterns have been observed for (2-formylpyridine thiosemicarbazonato)copper(II)[22] and copper(II) bleomycin.[23] These were explained with assignments of a pair of equivalent nitrogens plus an equivalent proton for the thiosemicarbazonato complex and with three equivalent nitrogens with $A_N = 10$ G and one nitrogen with $A_N' = 15$ G for copper bleomycin. Neither appears reasonable for this study because of the high symmetry of the $CuKTSM_2$ complex.

Another model for the six-line hyperfine structure is the superposition of two five-line patterns for which the centers of the two patterns are displaced by an amount about equivalent to the value for the nitrogen hyperfine coupling. The result is a 1:3:5:5:3:1 pattern. Goodman et al. have described this situation for the bis(asparagine) cupric complex and simulated their spectra utilizing two overlapping 1:2:3:2:1 patterns arising from an equilibrium mixture of both the cis and the trans complexes.[26] The correct explanation for the even-line pattern in the parallel region for [biacetyl bis(thiosemicarbazone)]copper(II) has not be resolved, but the latter explanation appears reasonable for $CuKTSM_2$. Thus, it is apparent in the pH titration of this complex that at intermediate pH a five-line pattern from $CuKTSM_2$ is superimposed on a five-line pattern for a protonated form of $CuKTSM_2$. This explanation should also be considered for cupric bleomycin and cupric mono(thiosemicarbazone) complexes, for which an increase in resolution for the $M_I = -{}^1/_2$

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line in the $g_{\parallel}$ region is apparent at S-band in comparison with X-band frequency. Thus, a decrease in the g strain at low frequencies may allow for increased resolution of the hyperfine structure and the detection of closely related cupric complexes.

With a secure understanding of the room-temperature ESR spectrum of $CuKTSM_2$ in hand, it was hoped that details of its interaction with cells could be extracted from an EPR analysis of this process. This seemed a particularly attractive experiment because previous work had indicated that $CuKTSM_2$ localizes in membrane.[15,19] Thus the complex might have the behavior of a membrane-bound spin label. However, in this case, the nature of the interaction was clear without extensive use of simulations from multiple-frequency data. At low concentration $CuKTSM_2$ is essentially immobilized in its association with the Ehrlich cell. The nature of the membrane reaction will require further study. Nevertheless, without the use of ambient-temperature ESR spectroscopy, this phenomenon would not have been discovered.

As the concentration of $CuKTSM_2$ is increased, an apparent end point for the immobilization reaction occurs and mobile $CuKTSM_2$ is noted. It is in this part of the titration that the complex also displays a concentration-dependent cytotoxicity toward the Ehrlich cell. Whether the completion of the immobilization reaction or the availability of mobile $CuKTSM_2$ is related to cytotoxicity has yet to be determined.

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Reactivity of the Peroxo Ligand in Metalloporphyrin Complexes. Reaction of Sulfur Dioxide with Iron and Titanium Porphyrin Peroxo Complexes To Give Sulfato Complexes or Sulfate

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The reaction of sulfur dioxide with metal-coordinated peroxide to give metal–sulfato complexes has been found in the past to be characteristic of a wide variety of group 8 metal complexes. Similar reactions with metalloporphyrin peroxo complexes have not been reported, however. The present paper describes an investigation of such reactions for three different types of metalloporphyrin peroxo complexes: (TPP)Fe–O_2–Fe(TPP) (TPP = tetraphenylporphinato) (**1**), a μ-peroxo ferric porphyrin complex formed by reaction of FeTPP with dioxygen at low temperature; $TiP(O_2)$ (P = TPP or OEP; OEP = octaethylporphinato) (**2a,b**), mononuclear Ti(IV) porphyrin peroxo complexes formed by reaction of TiP(O) with hydrogen peroxide; and $FeP(O_2)^-$ (**3a,b**), mononuclear ferric porphyrin peroxo complexes formed by reaction of FeP with superoxide, O_2^-. In all three cases, sulfate was obtained as a product, in the form of sulfato complexes for **1** and **2** and in the form of free ionic sulfate for **3**.

Introduction

A number of metalloporphyrin complexes with peroxo ligands have been synthesized and characterized in recent years.[1-5] One of these, a mononuclear ferric porphyrin peroxo complex, was originally reported by us in 1978.[3a] We have been interested in characterizing this complex because it may be analogous to intermediates in reactions of heme-containing oxygenase enzymes such as cytochrome P_{450}.[6] Attempted oxidations of organic substrates typical of those oxygenated by the enzyme systems (i.e. olefins, hydrocarbons) have so far failed to show any pronounced reactivity of this peroxo complex. This observation is not surprising because the so-called "nucleophilic" group 8 metal–peroxo complexes of this type are not generally reactive toward such substrates.[6b] One reaction that is very characteristic of group 8 metal–peroxo complexes is that with sulfur dioxide, SO_2, to give metal–sulfato complexes.[7] In fact, we are not aware of any metal–peroxo complex of this type that fails to give sulfate when exposed to SO_2. It therefore appeared logical to us to study the reaction of some metalloporphyrin peroxo complexes with SO_2 in order to ascertain if these new types of peroxo complexes showed reactivity patterns similar to those of previously characterized group 8 peroxo complexes. Our results, described below, demonstrate that the metalloporphyrin peroxo

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