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Reactions of Superoxide Anion with Copper(II) Salicylate Complexes

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Abstract: Electron spin resonance spectra of frozen (77 K) solutions of the known copper(II) salicylate complexes $Cu(HSal)_2$, $Cu(HSal)_2(H_2O)_2$, $Na_2Cu(Sal)_2$, and Cu(Sal), where HSal is the monoanion of salicyclic acid, $C_6H_4(OH)(CO_2^-)$, were measured over a wide pH range in 50% aqueous dimethyl sulfoxide. These results were then used, along with quantitative oxygen evolution measurements, to interpret the frozen-solution ESR spectral changes that occur upon titration of the first three copper salicylate compounds with crown ether solubilized potassium superoxide in anhydrous dimethyl sulfoxide. From the $g_{\parallel}$ and $A_{\parallel}$ ESR spectral parameters, double integration of the resonances, and the quantity of oxygen evolved, it was apparent that superoxide reduces $Cu(HSal)_2$ to a copper(I) derivative with evolution of dioxygen. Even in the presence of a fourfold excess of superoxide ion, however, divalent copper salicylate complexes (~15% of the total copper) remain in solution. At O_2^-:Cu^{II} ratios of 1:1 and 2:1 the major copper(II) species in solution are Cu(Sal) and $[Cu(Sal)_2]^{2-}$, respectively. These species form upon deprotonation of the phenol group of the salicylic acid monoanion in a reaction in which the superoxide ion serves as a base. Similar results were obtained when solutions of $Cu(HSal)_2(H_2O)_2$ in Me_2SO were titrated with superoxide ion. This finding demonstrates that a previously reported ESR spectrum of a 2:1 solution of superoxide ion and $Cu(HSal)_2{\cdot}4H_2O$ had been misassigned as a superoxide complex of copper(I). Addition of a fourfold excess of O_2^- to $Cu(HSal)_2(H_2O)_2$ led to reoxidation of the copper, a reaction that requires water to proceed. Titration of $Na_2Cu(Sal)_2$ with superoxide ion gave only oxygen and copper(I) species but in lesser amounts consistent with more negative reduction potential of the $[Cu(Sal)_2]^{2-}$ dianion.

Following the discovery[1] that the copper(II)–zinc(II)-containing protein erythrocuprein has superoxide dismutase activity, there has been intense interest in the organic,[2,3] inorganic,[3] and biological[4] chemistry of the superoxide (O_2^-) ion. The proposed link[5] between superoxide ion and inflammatory diseases suggests that the observed antiinflammatory and antiulcer activities of superoxide dismutase[6] and of copper chelate complexes[7] may involve copper(II) superoxide reactions in vivo. Copper(II) chelates with aspirin-like drugs are known[8] to have superoxide dismutase activity.[9]

Pulse radiolytically generated superoxide ions react with copper(II) salicylate and substituted salicylate complexes at rates similar to that observed for superoxide dismutase.[10] A mechanistic study of the reaction of bis(salicylato)copper(II) tetrahydrate with superoxide anion in dry dimethyl sulfoxide (Me_2SO) was interpreted according to eq 1.[11] No evidence was given to support

$$\begin{gathered} Cu(II) + O_2^- \rightarrow Cu^{II}O_2^- \rightarrow Cu^{I}O_2 \rightarrow Cu(I) + O_2 \\ Cu(I) + O_2^- \rightarrow Cu^{I}O_2^- \rightarrow Cu^{II}O_2^{2-} \xrightarrow{2H^+} Cu(II) + H_2O_2 \end{gathered} \tag{1}$$

the proposed $Cu^{II}O_2^-$ intermediate (a copper(II) superoxo complex has recently been detected[12] in solution, however). An ESR spectrum generated at a O_2^-:Cu ratio of 2:1 was assigned to the "paramagnetic equilibrium complex" $Cu^{I}O_2^- \rightleftharpoons Cu^{II}O_2^{2-}$.[11]

The pioneering work of Valentine and Curtis[13] has greatly facilitated study of the chemical reactivity of superoxide anion in aprotic media.[14] We have therefore initiated a program to investigate the reactivity of superoxide ion with imidazolate-bridged dicopper(II) complexes prepared in our laboratory as models for the active site of bovine erythrocyte superoxide dismutase.[15] As a prelude to this work, a reinvestigation of the copper(II) salicylate system in dry Me_2SO seemed warranted, particularly in view of the unusual $Cu^{I}O_2^- \rightleftharpoons Cu^{II}O_2^{2-}$ species proposed previously.[11] Such a study was therefore undertaken, and the results are presented here.

Experimental Section

Materials and Methods. All copper(II) complexes were synthesized from commerically available starting materials used without further purification. Dimethyl sulfoxide (Me_2SO) was dried by triple distillation at reduced pressure from NaOH pellets; the final distillate was collected and stored over 4-Å molecular sieves under dry nitrogen.[16] Potassium superoxide (KO_2) was purchased from Alfa Products, Inc., and ground to a fine powder in a mortar and pestle in a nitrogen-filled drybox. 18-Crown-6 ether was obtained from Aldrich Chemical Co. and purified by distillation at reduced pressure.[17] After being cooled, the distillate gave platelike crystals that were collected under dry nitrogen and stored in a drybox. Spectrophotometric measurements, pH readings, and microchemical analyses were made as described previously.[18]

A Varian E-line X-band spectrometer was used to record electron spin resonance spectra at 77 K. The calibration of *g* values and preparation of samples were as reported previously.[18] In the ESR monitored titration of copper(II) complexes with superoxide ion, two-arm quartz tubes shown in Figure 1 were used. Different and known amounts of copper(II) complexes and superoxide solutions (total volume 0.5 mL) were transferred with drawn out Pasteur pipettes into the separate arms in a drybox. The tubes were then removed from the drybox, frozen, evacuated, and sealed. After being warmed to room temperature, the samples were mixed completely until no gas bubbles were seen, transferred to the 25-cm arm, and stored in liquid nitrogen. The ESR spectra were recorded, and the data were stored in digital form in a Digital Equipment Corporation

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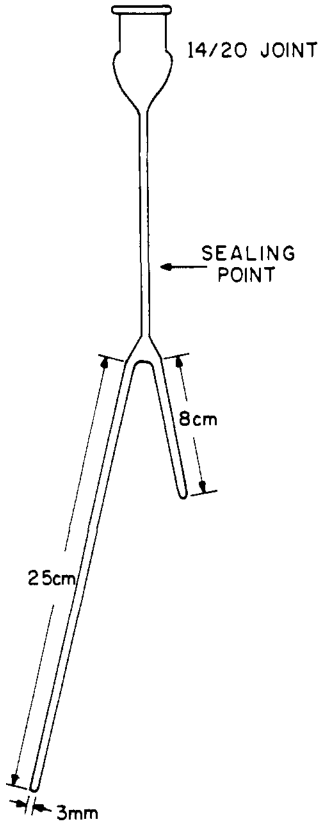

Figure 1. Sketch of the two-arm quartz ESR tubes.

MINC system interfaced to the ESR spectrometer. Integrated intensities were measured by double integration and printed out as described elsewhere.[19]

The oxygen concentration of solutions was monitored by using a Clark oxygen electrode[20] (Yellow Springs Instrument Co.) equipped with a temperature controller and calibrated with nitrogen- and oxygen-saturated solutions.[21]

Preparation and Characterization of Copper Complexes and Superoxide Solutions. The pale green crystalline complex $Cu(HSal)_2(H_2O)_2$[22] (**1**) was prepared as described previously[23,24] from cupric sulfate pentahydrate and NaHSal, followed by drying in vacuo at room temperature. Heating of this material in vacuo at 100 °C over P_2O_5 for 12 h produced anhydrous $Cu(HSal)_2$[23] (**2**) which was deep green. Olive green, platelike crystals of $Na_2Cu(Sal)_2$ (**3**) were obtained from salicylic acid, sodium hydroxide, and $CuSO_4{\cdot}5H_2O$ by a literature procedure[25] and dried in vacuo over P_2O_5 at 100 °C. The yellow green precipitate $Cu(Sal){\cdot}1.5H_2O$ (**4**) was isolated when an aqueous solution of **1** was adjusted to pH 7,[26] washed with water, and vacuum dried at room temperature. All four compounds gave satisfactory elemental analyses for C, H, Cu, Na (**3** only), and O (by difference). A water analysis was also obtained for **1**.

Potassium superoxide/18-crown-6 ether solutions in dry Me_2SO were prepared in a nitrogen-filled drybox as described in the literature.[13] After being stirred for a few hours clear, colorless solutions were obtained. The concentration of superoxide ion in these solutions was determined by two independent procedures. With 0.1-mm path length cells to minimize solvent absorption, the absorbance at 250 nm was measured and used together with an extinction coefficient, $\epsilon_{250} = 2686 \pm 29\ M^{-1}\ cm^{-1}$, to compute the $[O_2^-]$.[27] In the second method, a 0.1-mL superoxide solution in Me_2SO was injected into 2.0 mL of nitrogen-saturated, pH 7, 0.05 M aqueous phosphate buffer solution at 25 °C and oxygen release was monitored with a standardized oxygen electrode.[28] The effect of the organic solvent was neglected. The amount of oxygen released was used together with eq 2 to compute the superoxide ion concentration. After

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2 \qquad (2)$$

the electrode reading had stabilized, a small amount of catalase was added to convert H_2O_2 to O_2, eq 3, yielding an additional 0.5 equiv of

$$H_2O_2 \rightarrow H_2O + {}^1/_2O_2 \qquad (3)$$

dioxygen.[28] In a typical experiment, the spectrophotometric method revealed a superoxide ion concentration of 33.50 mM, compared with 31.3 mM from the oxygen-release measurements. The discrepancy between the results of less than 10% was considered to be acceptable for the present purposes.

Table I. Electron Spin Resonance Spectral Parameters of Copper(II) and Its Salicylate Complexes at 77 K

compd	solvent	$g_{\parallel}$	$A_{\parallel}$, G ($\times 10^4$, cm^{-1})[c]	$g_{\perp}$	$A_{\perp}$, G ($\times 10^4$, cm^{-1})[c]
$[Cu(H_2O)_6]^{2+}$ [d]	a	2.412	119 (134)	2.099	
$Cu(HSal)_2(H_2O)_2$[e]	a or b	2.374	131 (145)	2.081	
	solid	2.388		2.095	
$Cu(HSal)_2$[e]	a or b	2.374	131 (145)	2.081	
$Cu(Sal)$[e]	a or b	2.334	151 (165)		
$Na_2Cu(Sal)_2$[e]	a	2.296	165 (177)	2.074	19 (18)
	b	2.290	175 (187)	2.074	31 (30)
$[Cu(OH)_4]^{2-}$ [f]	a	2.252	177 (186)	2.048	31 (30)

[a] In frozen 50% aqueous dimethyl sulfoxide solution. [b] In frozen dry dimethyl sulfoxide. [c] Hyperfine coupling constants are reported both in gauss and in cm^{-1} ($\times 10^4$). [d] Reference 18. [e] This work. [f] Chao, Y-Y. H.; Kearns, D. R. *J. Phys. Chem.* 1977, *81*, 666–668.

Table II. Electron Spin Resonance Spectral Parameters at 77 K of $Cu(HSal)_2(H_2O)_2$ in Frozen 50% Aqueous Dimethyl Sulfoxide Solutions[a]

pH	$g_{\parallel}$	$A_{\parallel}$, G ($\times 10^4$, cm^{-1})[b]	$g_{\perp}$	$A_{\perp}$, G ($\times 10^4$, cm^{-1})[b]	color
2.50	2.402	118 (132)	2.092		
3.42	2.402	118 (132)	2.092		
	2.367	132 (146)	2.081		
5.10	2.402	118 (132)	2.092		green
	2.367	132 (146)	2.081		
	2.326	150 (163)			
6.70	2.326	150 (163)			yellow-green
9.45	2.293	161 (172)	2.066	18 (17)	deep green
13.30	2.293	161 (172)	2.066	18 (17)	
	2.278	175 (186)	2.059	30 (29)	
13.70	2.278	175 (186)	2.059	30 (29)	

[a] The total copper concentration is 10 mM. [b] See footnote *c*, Table I.

The ESR spectrum at 77 K of frozen, dry Me_2SO solutions of superoxide ion had $g_{\parallel} = 2.108$ and $g_{\perp} = 2.008$, in good agreement with literature values.[14]

Results

Electron Spin Resonance Study of Copper(II) Salicylate Complexes in Frozen Solutions. Table I summarizes the ESR spectral parameters of different copper(II) complexes. The frozen solution spectra all display axial or nearly axial symmetry. The ESR spectra of $Cu(HSal)_2(H_2O)_2$ and anhydrous $Cu(HSal)_2$ are the same in 50% aqueous Me_2SO and dry Me_2SO, but powdered samples of $Cu(HSal)_2(H_2O)_2$ exhibit different $g_{\parallel}$ and $g_{\perp}$ values. Although the salicylate ion in $Cu(HSal)_2(H_2O)_4$ is known to be monodentate in the solid,[24] it is believed to be bidentate in solution.[26] It is noteworthy that $Na_2Cu(Sal)_2$ has the same $g_{\parallel}$ and $g_{\perp}$ values in dry and 50% aqueous Me_2SO frozen solutions, but different $A_{\parallel}$ and $A_{\perp}$ values.

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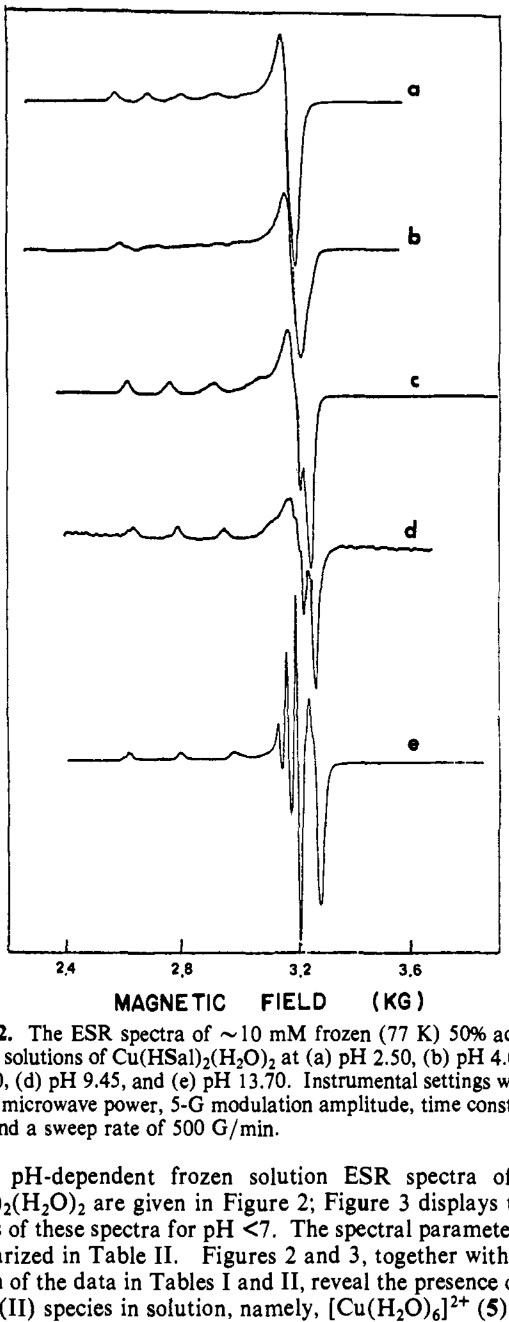

Figure 2. The ESR spectra of ~10 mM frozen (77 K) 50% aqueous Me_2SO solutions of $Cu(HSal)_2(H_2O)_2$ at (a) pH 2.50, (b) pH 4.00, (c) pH 6.70, (d) pH 9.45, and (e) pH 13.70. Instrumental settings were 20 mW of microwave power, 5-G modulation amplitude, time constant of 1.0 s, and a sweep rate of 500 G/min.

The pH-dependent frozen solution ESR spectra of $Cu(HSal)_2(H_2O)_2$ are given in Figure 2; Figure 3 displays the $g_\parallel$ regions of these spectra for pH <7. The spectral parameters are summarized in Table II. Figures 2 and 3, together with comparison of the data in Tables I and II, reveal the presence of five copper(II) species in solution, namely, $[Cu(H_2O)_6]^{2+}$ (**5**), **1**, **4**, **3**, and $[Cu(OH)_4]^{2-}$ (**6**). These complexes are interconverted according to eq 4.[29] In eq 4, **1**, **4**, and **3** refer to the solution

$$\mathbf{5} + 2\mathrm{H_2Sal} \underset{\substack{2\mathrm{OH^-}\\ \mathrm{pH}\ \sim 3.42}}{\overset{2\mathrm{H^+}}{\rightleftharpoons}} \mathbf{1} \underset{\substack{\mathrm{H^+}\\ \mathrm{pH}\ \sim 5.5}}{\overset{\mathrm{OH^-}}{\rightleftharpoons}} \mathbf{4} +$$

$$\mathrm{HSal^-} \underset{\substack{\mathrm{H^+}\\ \mathrm{pH}\ \sim 9.45}}{\overset{\mathrm{OH^-}}{\rightleftharpoons}} \mathbf{3} \underset{\substack{\mathrm{H^+}\\ \mathrm{pH}\ \sim 13.3}}{\overset{\mathrm{OH^-}}{\rightleftharpoons}} \mathbf{6} + 2\mathrm{Sal^{2-}} \quad (4)$$

species generated upon dissolving $Cu(HSal)_2(H_2O)_2$, $Cu(Sal)\cdot 1.5H_2O$, and $Na_2Cu(Sal)_2$, respectively, the structures of which are unknown. Moreover, it is likely that solutions of **1** contain some $Cu(HSal)^+$ and that solutions of **4** + $HSal^-$ contain some $Cu(HSal)(Sal)^-$, the presence of which might not be detected by ESR spectroscopy.

With formation constants[30] for copper(II) salicylate complexes determined potentiometrically at 25 °C, μ = 0.1 ($NaNO_3$), the mole fractions of copper(II) species in a solution containing 10 mM Cu(II) and 20 mM salicylic acid were computed as a function of pH.[19] The results are given in Figure 4. Despite differences in solvent composition and ionic strength, these results clearly support the equilibria of eq 4 as accounting for the pH dependence of the ESR spectra of $Cu(HSal)_2(H_2O)_2$ in 50% aqueous Me_2SO solutions.

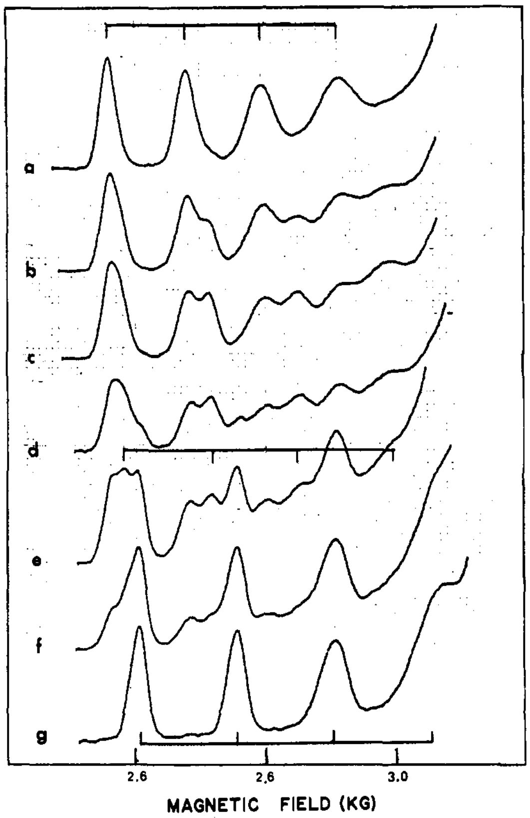

Figure 3. $g_\parallel$ region of the ESR spectra of ~10 mM frozen (77 K) 50% aqueous Me_2SO solutions of $Cu(HSal)_2(H_2O)_2$ at (a) pH 2.50, (b) pH 3.00, (c) pH 3.42, (d) pH 4.05, (e) pH 4.60, (f) pH 5.10, and (g) pH 6.70. The conditions of the instrument are the same as for Figure 2 except that a sweep rate of 250 G/min was used.

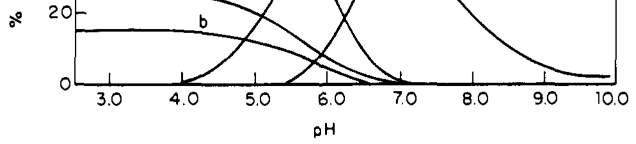

Figure 4. Percentage distribution of copper species in the mixture of Cu(II) and salicylic acid as a function of pH with $[Cu^{2+}]_{tot}$ = 10 mM and $[H_2Sal]_{tot}$ = 20 mM: (a) Cu^{2+}, (b) $[Cu(HSal)]^+$, (c) $Cu(HSal)_2$, (d) CuSal, (e) $[Cu(Sal)_2]^{2-}$.

Reaction of Superoxide Ion with Copper(II) Salicylate Complexes. The ESR spectral changes that occur upon addition of superoxide ion to $Cu(HSal)_2(H_2O)_2$ and $Cu(HSal)_2$ in anhydrous Me_2SO are displayed in Figures 5–7. The spectral parameters and integrated signal intensities of different mixtures are summarized in Table III. Solutions with $[O_2^-]$:$[Cu^{2+}]$ ratios less than unity have the same spectral features, but the copper(II) signal intensity decreases with increasing superoxide ion concentration

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[29] Both water and dimethyl sulfoxide are likely to be coordinated to copper in **1**–**5**. It is not possible to distinguish these two ligands from the ESR spectra reported here.

[30] Brun, P. F.; Schrøder, K. H. *J. Electroanal. Chem.* **1975**, *66*, 9–20 and references cited therein.

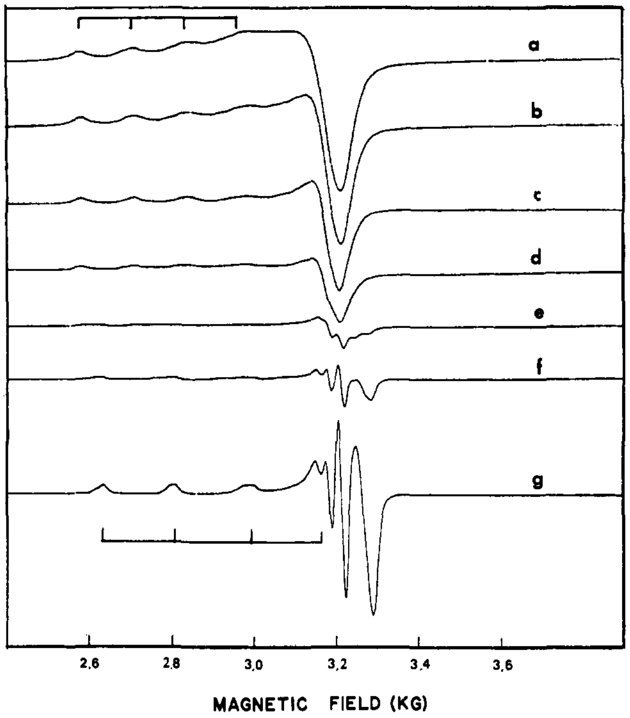

Figure 5. 77 K frozen solution ESR spectra of $Cu(HSal)_2(H_2O)_2$ in the presence of increasing mole ratios (O_2^-:Cu^{2+}), (a) 0, (b) 0.25, (c) 0.50, (d) 0.75, (e) 1.00, (f) 2.00, and (g) 4.00, prepared by mixing 5 mM [Cu^{2+}] and [O_2^-] solutions to a final volume of 0.5 mL. Instrumental settings were 20 mW of microwave power, 5-G modulation amplitude, time constant of 0.3 s, and a sweep rate of 250 G/min.

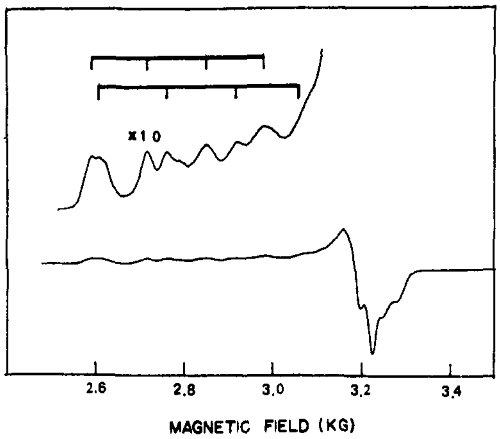

Figure 6. ESR spectrum of (e) in Figure 5 at higher gain. The conditions of the instrument are the same as for Figure 5 except a sweep rate of 125 G/min was used.

(curves a–d, Figure 5). This reduction in signal amplitude occurs upon reduction of copper(II) to copper(I) by superoxide ion. Figure 6 shows the ESR spectrum of the 1:1 mixture (curve e of Figure 5) with expanded gain. The presence of the two copper(II) species **1** ($g_{\parallel}$ = 2.374, $A_{\parallel}$ = 131 G) and **4** ($g_{\parallel}$ = 2.329, $A_{\parallel}$ = 155 G) is demonstrated from the $g_{\parallel}$ region of the spectrum, cf. Table I. Solutions with excess superoxide ion have ESR parameters ($g_{\parallel}$ = 2.290, $A_{\parallel}$ = 175 G; $g_{\perp}$ = 2.070, $A_{\perp}$ = 30 G) characteristic of the complex $[Cu(Sal)_2]^{2-}$, **3**. The presence of **3** and **4** in these solutions demonstrates that, like hydroxide ion, O_2^- serves as a base toward **1** and **4**, eq 5. In this equation, the disproportionation

$$\mathbf{1} \xrightarrow{O_2^-} \mathbf{4} + HSal^- + {}^1/_2O_2 + {}^1/_2H_2O_2 \xrightarrow{O_2^-} \mathbf{3} + O_2 + H_2O_2 \qquad (5)$$

products of HO_2 are shown. The absence of resonances from **3** and **4** in curves a–d of Figure 5 shows that reduction of $Cu(HSal)_2(H_2O)_2$ is more favorable than deprotonation.

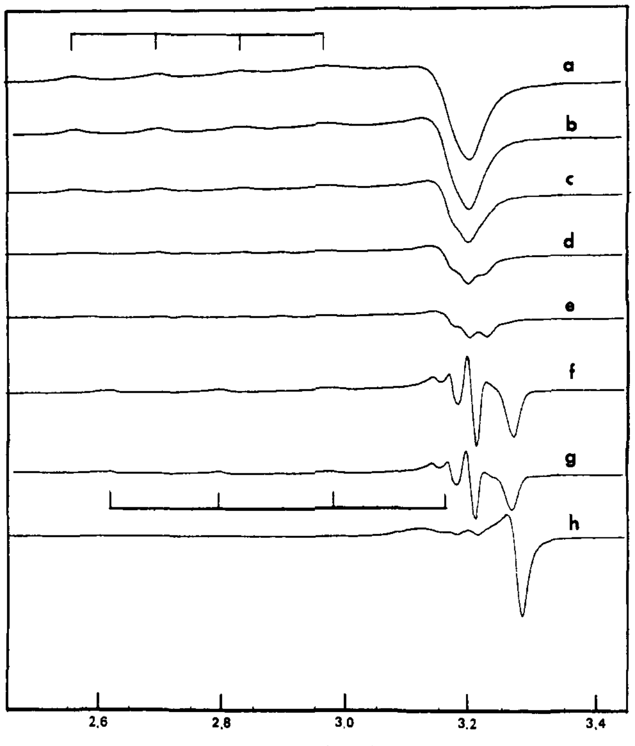

Figure 7. 77 K ESR spectra of anhyrous $Cu(HSal)_2$ with superoxide at different mole ratios (O_2^-:Cu^{2+}), (a) 0, (b) 0.25, (c) 0.50, (d) 0.75, (e) 1.00, (f) 2.00, (g) 4.00, and (h) 19.00, in Me_2SO prepared as described in the caption of Figure 5. Instrumental settings were 20 mW of microwave power, 5-G modulation amplitude, time constant of 0.3 s, and a sweep rate of 250 G/min.

Table III. Electron Spin Resonance Spectral Parameters of $Cu(HSal)_2(H_2O)_2$ and $Cu(HSal)_2$ upon Addition of Superoxide Ion in Anhydrous Me_2SO at 77 K[a]

mole ratio (O_2^-: Cu^{2+})	% of Cu intensity[b]					
	Cu-(HSal)$_2$-(H$_2$O)$_2$	Cu-(HSal)$_2$	$g_{\parallel}$	$A_{\parallel}$, G ($\times 10^4$, cm^{-1})[c]	$g_{\perp}$	$A_{\perp}$, G ($\times 10^4$, cm^{-1})[c]
0	100	100	2.374	131 (135)	2.081	
0.25	87	87	2.374	131 (145)	2.081	
0.50	63	64	2.374	131 (145)	2.081	
0.75	43	48	2.374	131 (145)	2.081	
1.00	23	35	2.374	131 (145)		
			2.329	155 (168)		
2.00	13	30	2.290	175 (187)	2.070	30 (29)
4.00	65	15	2.290	175 (187)	2.070	30 (29)

[a] Solutions 5 mM in [Cu^{2+}] and in [O_2^-] were mixed in various proportions to a final volume of 0.5 mL. [b] Relative copper(II) intensities were measured by double integration of the spectra and corrected for dilution effects. [c] See footnote *c*, Table I.

Anhydrous $Cu(HSal)_2$ exhibits the same spectral changes as $Cu(HSal)_2(H_2O)_2$ upon titration with superoxide ion (Figure 7, Table III). One difference, however, is the increase of signal intensity for the hydrated complex when the O_2^-:Cu ratio reaches 4:1 (Figure 5g). This result was reproduced several times.

Figures 8 shows the ESR spectral results for titration of $Na_2Cu(Sal)_2$ with superoxide ion. All mixtures exhibit only the spectrum of **3**, except for curve h which shows the free superoxide ion resonance. Compared with those of $Cu(HSal)_2(H_2O)_2$ or $Cu(HSal)_2$, the signal intensity of $[Cu(Sal)_2]^{2-}$ decreases much more slowly with increasing superoxide ion concentration, revealing that the dianion is more difficult to reduce.

In a separate experiment, the amount of oxygen released from 1:1 mixtures of superoxide ion and each of the three copper(II) salicylate complexes was measured. The results are shown in Table IV. Approximately 0.6 equivs of dioxygen/1 equiv copper(II)

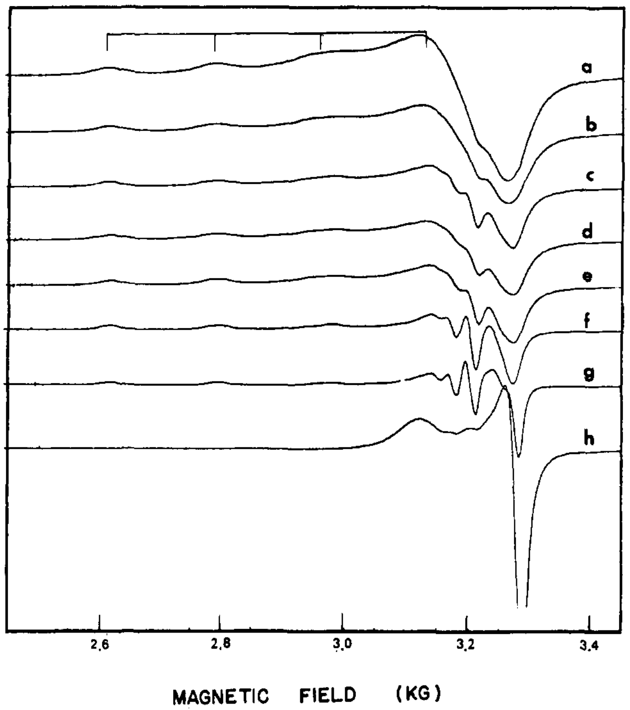

Figure 8. 77 K ESR spectra of $Na_2Cu(Sal)_2$ with superoxide at different mole ratios (see Figure 5 caption) (O_2^-:Cu^{2+}) in Me_2SO: (a) 0, (b) 0.25 (93.6), (c) 0.50 (92.2), (d) 0.75 (91.9), (e) 1.00 (88.5), (f) 2.00 (54.7), (g) 4.00 (52.7), and (h) 19.00. The numbers in parentheses are the percentages of copper signals compared to (a). The conditions of the instrument are the same as for Figure 5.

Table IV. Oxygen Evolution from Mixtures of Superoxide and Copper(II) Salicylate Complexes in Me_2SO at 25 °C

complex	μmol of Cu added	μmol of O_2^- added	μmol (%) of O_2 released
$Cu(HSal)_2(H_2O)_2$	2.74	2.74	1.79 (65%)
$Cu(HSal)_2$	3.10	3.10	1.83 (59%)
$Na_2Cu(Sal)_2$	3.10	3.10	0.94 (30%)

was generated for $Cu(HSal)_2(H_2O)_2$ or $Cu(HSal)_2$, but only 0.3 equiv for $Na_2Cu(Sal)_2$.

Discussion

The present results confirm[11] that superoxide ion reduces $Cu(HSal)_2(H_2O)_2$ with concomitant production of dioxygen in dry Me_2SO at mole ratios up to 1:1. This behavior is fully consistent with the known redox behavior of the O_2^- ion in aprotic media. The O_2/O_2^- potential is negative ($E^{\circ\prime} = -1.025$ V vs. Ag/Ag^+ (0.01 M)) in dry Me_2SO.[19] From the measured reduction potential of $Cu(HSal)_2(H_2O)_2$ in the same solvent ($E^{\circ\prime} = -0.305$ V),[19] the reaction should proceed quantitatively to produce diamagnetic copper(I) species and dioxygen. This expectation is not fulfilled, however, as revealed both by the oxygen release and the ESR spectral data. Such behavior is not atypical of superoxide reactions with cupric complexes in dry Me_2SO and could result from side reactions with solvent or the ligands.[13] Addition of 1 equiv of O_2^- to $[Cu(Sal)_2]^{2-}$ produces less dioxygen (Table IV) and less of a decrease in the integrated ESR signal intensity (Figure 8e) than observed for the $Cu(HSal)_2$ superoxide reaction. The more negative redox potential ($E^{\circ\prime} \approx -1.024$ V, irrev.) of $Na_2Cu(Sal)_2$[19] readily explains this result. In the extreme case where the redox potential of the cupric complex is presumably more negative than that of the O_2^- ion, a stable, diamagnetic copper(II) superoxide adduct can form.[12]

Previous investigators reported[11] that titration of $Cu(HSal)_2 \cdot 4H_2O$ in dry Me_2SO with superoxide ion reduced the copper(II) ESR signal intensity to zero at a O_2^-:Cu mole ratio of 1.0. As evident from Figure 6, there is a significant amount of copper(II) species in the 1:1 mixture of $Cu(HSal)_2(H_2O)_2$ and superoxide ion. Through comparison with the pH-dependent ESR spectral parameters (Tables I and II) of $Cu(HSal)_2(H_2O)_2$, these cupric salicylate complexes are readily identified as $Cu(HSal)_2$ and Cu(Sal). In the presence of excess superoxide anion, the ESR spectral features of $[Cu(Sal)_2]^{2-}$ are generated (Table III and Figure 5). In particular, the spectrum of a 2:1 mixture of O_2^-–$Cu(HSal)_2 \cdot 2H_2O$ (Figure 5f) is very similar to that previously assigned to the $Cu^{I}O_2^- \rightleftharpoons Cu^{II}O_2^{2-}$ "paramagnetic equilibrium complex". We therefore conclude that there is no stable superoxide copper(I) salicylate complex and that the previously reported spectrum was largely a mixture of Cu(Sal) and $[Cu(Sal)_2]^{2-}$ formed through deprotonation of the $HSal^-$ ligand by O_2^- ion.

The ability of superoxide ion to serve as a strong base in aprotic media is not unusual.[14] Moreover, superoxide ion reacts rapidly with alcohol to give alkoxide ion (eq 6).[31] Thus superoxide ion

$$\mathrm{ROH} + \mathrm{O_2^-} \rightarrow \mathrm{RO^-} + {}^1/_2\mathrm{O_2} + {}^1/_2\mathrm{H_2O_2} \qquad (6)$$

is expected to act as a base toward metal complexes if there are removable protons available in the ligands. The O_2^--promoted deprotonation of the phenol group of coordinated $HSal^-$ to form the Cu(Sal) and $[Cu(Sal)_2]^{2-}$ chelate complexes in the present study clearly demonstrates this point.

Although superoxide ion is not a potent oxidant in aprotic solvents, owing to the instability of the O_2^{2-} ion, traces of water or other sources of H^+ increase its oxidizing power through production of HO_2, H_2O_2, HO_2^-, or O_2.[14] When the O_2^-:Cu(II) mole ratio is increased from 2.0 to 4.0 for $Cu(HSal)_2(H_2O)_2$, the intensity of the Cu(II) ESR signal rises significantly (Table III, Figure 5g). A similar effect was not observed for the reaction with anhydrous $Cu(HSal)_2$. This result suggests that it is the water molecules, rather than protons which are available from the salicylate monoanion, that promote the reoxidation of copper to the divalent state. The water concentration in the solution containing a 4:1 ratio of O_2^- to $Cu(HSal)_2(H_2O)_2$ in dry Me_2SO is 2 mM. It is interesting that such an amount of water can influence the reoxidation reaction.

Several polarographic and potentiometric studies of copper(II) salicylate complexes have previously been published.[30,32] The values of the stability constants reported for different complexes are not in good agreement. Our ESR spectral results (Table II) correlate nicely with the distribution of complexes expected from the results of ref 30 (Figure 4) and strongly support the conclusions of an earlier ESR and optical spectroscopic study at room temperature of aqueous solutions of copper(II) salicylate complexes over a pH range.[26] The importance of understanding the pH-dependent equilibria of a copper(II) complex as a prerequisite for interpreting superoxide reactivity cannot be overemphasized.

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