An Electron Spin Resonance Study of Copper Uroporphyrin III and Other Touraco Feather Components

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(Received for publication, September 2, 1964)

The only known naturally occurring copper-containing porphyrin was discovered almost 100 years ago by Church (1) who isolated this material from the tail and wing feathers of 16 species of the African touraco bird (Musophagidae). At that time, he noted that this red, base-soluble pigment, which he called turacin, contained 7% copper and that the feathers from which it was derived represented a total copper weight of approximately 8 mg per bird. This material could be reversibly precipitated in acid and redissolved in base with no apparent visible alterations.

In 1924, Fischer and Hilger (2) studied the chemistry of turacin and confirmed the findings of Church, that indeed, it did contain copper. In addition, they incorrectly identified the phorphyrin moiety of the molecule as uroporphyrin I. Not until 1939 (3), and again unequivocally in 1951 (4), did Rimington show that the porphyrin was actually uroporphyrin III. In fact, the red feathers of the touraco bird are a readily available source of this particular porphyrin, and have been used as such by various investigators (5).

The earliest spectroscopic studies of turacin were performed by D. Keilin (6), who not only studied solutions, but also the material as it naturally exists in the bird feather. The major results of this study are that (a) turacin in weakly ammoniacal solutions absorbs at 562.5 and 526 mp; (b) turacin precipitated in acid medium in the presence of a colloidal dispersing agent, such as gum arabic or gelatin, absorbs at 580 and 540 mp; (c) turacin within the feather absorbs at 583 and 542 mp; and finally, (d) that turacin in nonaqueous solvents such as acidified acetone absorbs at 562 and 524 mp. Material precipitating from these solvents absorbs at 557 mp.

The spectrophotometric properties of turacin were investigated in 1951 by J. Keilin (7), who, confirming earlier work, noted spectral maxima at 562 and 526 mp for this material in basic media, and 583 and 540 mp in an acid gelatin medium. In addition, it is important to note that turacin solutions containing caffeine showed the same spectral maxima either in acid or in base.

At no time during the long history of the chemical investigation of turacin was there any question concerning the fact that the molecule contains copper. In fact, it was shown that Cu^{2+} in ascorbate medium could be incorporated into a uroporphyrin very readily at a pH of 6.4 at 37° (8). The unanswered questions, however, are: what is the oxidation state of copper when it is bound to uroporphyrin III? Also, what is the state of aggregation of copper porphyrin molecules in acid and basic media and in the presence of caffeine? Up until fairly recently one could not answer these questions because it is virtually impossible to remove the copper from turacin without altering the oxidation state of the copper. However, one can examine the nature of copper as it exists intact within the molecule, or even in the feather in which it is incorporated, with the use of electron spin resonance techniques. In this present study we have examined the electron spin resonance spectrum of turacin and have definitely shown that the copper is divalent within the porphyrin moiety. In addition, we have shown that in basic medium, turacin can be dimeric and in acid medium, polymeric, whereas in the presence of caffeine and other amines, it is monomeric.

EXPERIMENTAL PROCEDURE

Materials and Methods—All water used in these experiments was first deionized by resin absorption and finally distilled with a Corning glass still. All chemicals were the best grade commercially available. Moulted touraco feathers were obtained from various zoological parks throughout the world. Turacin was prepared from red touraco feathers with dilute NH_{4} water by the method of J. Keilin and McCosker (8).

Copper was determined by the modification of a procedure devised by Felsenfeld (9). Turacin was oxidized with H_{2}O_{2} in acetic acid. Heating to boiling and continued addition of 30% H_{2}O_{2} aided in the breakdown of the porphyrin, the release of soluble copper ion, and the formation of a clear, light yellow solution. Excess H_{2}O_{2} was destroyed by boiling. A 1-ml aliquot of this solution was added to 2 ml of a solution of 0.1% 2,2'-biquinoline in glacial acetic acid containing a few crystals of ascorbic acid. The 540 mp absorbance of the purple solution was determined with a Beckman DU spectrophotometer. A standard curve was plotted with 1 to 10 μg of Cu^{2+} as standards. Other spectral data were obtained with the use of a Bausch and Lomb model 505 visible-ultraviolet spectrophotometer with cells having 1-cm light paths.

ESR measurements were made with a superheterodyne microwave spectrometer operating at approximately 9200 megacycles per second (10). Some spectra were taken with a Varian Associates model 4500 modulator which provided a 100 kc per second modulation field and which also detected the resonance signal. Others were taken with 1 kc per second field modulation and detection. The magnetic field was obtained

* This investigation was supported in part by Public Health Service Grant GM-10959-02 from the Division of General Medical Sciences, the National Institutes of Health.

1 The abbreviation used is: ESR, electron spin resonance.
with a 12-inch Varian magnet which was calibrated with a Varian model 4400 nuclear magnetic resonance gaussmeter.

**RESULTS**

**Optical Spectra**—Turacin is insoluble in acid solution. If one works quickly by studying freshly acidified solutions, or by using gelatin, good visible spectra may nevertheless be obtained. Visible spectra for turacin, both in acid and in basic media, are shown in Fig. 1. As can be seen, turacin in acid exhibits visible spectral maxima at longer wave lengths than in base, yet the Soret band is shifted to shorter wave lengths. Both gelatin and caffeine are effective in partially preventing the precipitation of turacin in acid solutions. Gelatin (23 mg per ml) in basic medium produces no change in the spectral properties of turacin (10^{-5} M). Caffeine, on the other hand, causes small shifts in the optical spectral peaks. The Soret band shifts from 398 μ to 405 μ, the 562 μ peak shifts to 564 μ, and the 526 μ peak shifts to 528.5 μ. When acetic acid is added to a solution of turacin containing gelatin, the resulting mixture has the same spectral properties as turacin freshly precipitated in acid. When acid is added to turacin containing caffeine, however, there is no immediate change in the spectral properties of this solution. After some time, depending on the concentration of caffeine, the turacin ultimately precipitates and the resulting spectrum of this mixture is the same as for the usual acid-precipitated material. Starting with a 10^{-4} M turacin solution, 10^{-3} M caffeine is effective in preventing turacin precipitation in acid. At lower concentrations of caffeine, one may obtain a partially acid-precipitated mixture. Similar results have been shown when theobromine was substituted for caffeine.

Other materials also affect the spectrum of turacin in basic media. Dilute KCl, for example, shifts the 526 μ and 562 μ peaks to 532 μ and 567 μ, respectively. As the concentration of chloride is increased the 398 μ peak is replaced with a new peak at about 383 μ. The turacin spectrum in 4.5 M KCl shows an intense Soret band at 383 μ and a vestige of the 398 μ peak appears at a shoulder. The addition of water to the KCl-turacin mixture depresses the 383 μ peak and restores the one at 398 μ. When turacin solutions containing high chloride concentrations are acidified with acetic acid, the spectrum resulting in this case is the same as when chloride is not present. In 85% acetic, the spectrum consists of peaks at 526, 562, and 399 μ together with a small peak at 364 μ. Here also, this latter peak disappears upon the addition of water.

In any of the spectral experiments described above, NaOH could be substituted for NH_3 while HCl or HClO_{4} could be substituted for acetic acid with no qualitative spectral difference. A list of compounds which affect the spectral properties of turacin is shown in Table I. It should be noted that purines and pyrimidines shift the Soret band to higher wave lengths and produce smaller upward shifts in the visible maxima.

**Solubility of Theobromine**—Theobromine possesses a limited solubility in acid medium. A test tube experiment was performed in which solutions of different increasing concentrations of theobromine sodium acetate in 1 ml of H_2O were acidified.

### Table I

<table>
<thead>
<tr>
<th>Added compound</th>
<th>Spectral maxima</th>
<th>ESR spectrum</th>
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<tbody>
<tr>
<td></td>
<td>μM</td>
<td>μM</td>
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<tr>
<td>Adenine</td>
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<tr>
<td>Theobromine sodium acetate</td>
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<td>528</td>
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<td>Dicyclohexanonepivalylhydrazine</td>
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<tr>
<td>Ethylenediaminetetraacetate (1 M, pH 7)</td>
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<td>527</td>
</tr>
<tr>
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<td>526</td>
</tr>
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<td>Na_2PO_4 (12 M)</td>
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<td>530</td>
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<td>NH_3 (12 M)*</td>
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<td>KCl (4.5 M)</td>
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<td>532</td>
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<td>KCN (1 M)</td>
<td>388</td>
<td>532</td>
</tr>
<tr>
<td>Acetic acid (1 M)</td>
<td>383</td>
<td>540</td>
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</table>

*Turacin concentration for this optical measurement was 10^{-3} M to show absorption peaks of dimer.
with 0.05 ml of glacial acetic acid and left standing at room temperature. After 10 minutes, those solutions with low theobromine concentrations were clear while those with greater theobromine concentrations were cloudy. The final pH of all solutions was between 3.0 and 3.4. The addition of increasing amounts of turacin resulted in the increased solubility of theobromine, as can be seen from Fig. 2. Complex formation with theobromine delays acid precipitation of turacin in a manner similar to caffeine.

**Electron Spin Resonance Spectra**—The ESR spectrum of turacin was measured in most of the media listed in Table I. In all cases the spectrum was observed to be one of three types. Typical of one group was the spectrum in NH₃ solution shown in the solid line in Fig. 3. The solutions which gave this spectrum are indicated by a D in the last column of Table I. This is an unusual ESR spectrum for Cu²⁺ in that there is more fine structure than merely $g_{//}$ and $g_{\perp}$ and there is more hyperfine structure than can be attributed to a single copper nucleus of spin $3/2$. A more detailed description of this spectrum is given in the next section of this paper.

Another group of solutions gave the spectrum shown as the solid line in Fig. 4, which was observed for turacin in acetic acid. This spectrum is also unusual for Cu²⁺ in that it is very nearly symmetrical and has no hyperfine structure. The line is centered about $g = 2.045$ with a width of 55 gauss between derivative extrema. The occurrence of this spectrum is indicated by a P in the last column of Table I.

The third type of ESR spectrum observed for turacin is shown as the upper curve in Fig. 5. The lower curve is the spectrum of Cu²⁺ in NH₃ solution presented for comparison. It is seen that the curves are qualitatively similar except for the superhyperfine structure seen in the $g_{\perp}$ or high field region of the spectrum. No superhyperfine structure could be resolved in the $g_{//}$ or low field region. Compounds, which when added to turacin solutions cause the Cu²⁺ to have this spectrum, are denoted by an M in Table I. In all cases the superhyperfine structure was observed to be 50 & 5 mc per second in the perpendicular direction. Deuteration of the amine and the solvent caused no change in the ESR spectrum. This spectrum could also be observed in NH₃ solution when the turacin concentration was lowered to $10^{-4}$ M. A summary of the parameters describing the ESR spectra is given in Table II.

If a caffeine-turacin solution, which gives the spectrum of Fig. 5 (upper curve), is strongly acidified, its ESR signal is unchanged. If, however, this solution is left standing for some time, the ESR spectrum resembles that shown in the solid line of Fig. 4.

The ESR spectrum of a red feather from Touraco corythaix corythaix is identical with that shown by the solid line of Fig. 4. The ESR signal for a single feather was quantitatively compared with that for a standard sample of cupric sulfate. Doubly integrating both spectra indicated the presence of 208 µg of divalent copper in the feather. Extracting the turacin and submitting it to chemical analysis indicated 234 µg of copper.
The spectrum of the ammoniacal washings of the red feather is identical with that shown in Fig. 3. When solutions of either ammoniacal red feather washings or purified turacin preparations were purged of oxygen by repeated evacuation and introduction of helium, the ESR spectra of these materials were unchanged.

Fig. 6 is the ESR signal obtained from an iridescent green feather of the same bird. The shape and position of the curve indicate that the signal is not produced by Cu²⁺, but by a stable, free radical species having a g value of 2.0036. No copper could be found in an HNO₃ digest of the green feather with the use of ESR techniques.

DISCUSSION

Electron spin resonance has previously been used to study divalent copper in purified biological systems. In many of these studies, however, the molecules under consideration contained more than a single copper atom (11, 12) usually with heterogeneous valence states (13, 14). In those cases where the molecule contained a single divalent copper (15, 16) the nature of the ligands bonding the metal remained unknown.

In the present investigation the chemical constitution of the copper compound is known. From the nature of the spin resonance technique, only those systems, such as turacin and free radicals, possessing unpaired electron spins produce ESR signals. In vitro and in situ, then, the copper in turacin is definitely divalent. A comparison of the mathematical analysis of the ESR spectra for the copper in a single red turacin feather (with the use of a CuSO₄ standard), with the chemical analysis on the total turacin extracted from this same feather, indicates that essentially all of the copper isolated is divalent.

Let us consider the chemical structure of the turacin molecule. If we envision the copper uroporphyrin III molecule as being planar or almost planar (17, 18), with Cu²⁺ in the center and the porphyrin surrounding it, we can set up three-dimensional coordinates to describe the molecule, with copper at the origin and the nitrogens of the porphyrin on the z and y axes. Classically, this is a square planar, dsp² configuration. Along the z axis, at least in dilute base (probably necessary to dissociate the protons from the carboxyl groups of the porphyrin side chains), two waters could be weakly coordinated. This general structure would be quite similar to the one for Cu(NH₄)₂⁺ in aqueous medium except that here the copper-nitrogen bond distance is slightly greater (17-19) than in the porphyrin system. Cu(NH₄)₂⁺ has a typical Cu²⁺ square planar ESR spectrum which is very asymmetrical and has hyperfine structure very well resolved along the parallel direction. This results from a local electric field configuration which is primarily caused by electrical charges along the plus and minus z and y axes. This spectrum is typical of Cu²⁺ weakly bound with essentially planar ligands.

It is the spectrum of turacin in the presence of amines which has characteristics similar to that of Cu(NH₄)₂⁺. The differences in the g values and the hyperfine interactions arise from differences in the energies of the excited electronic states of the Cu²⁺. An important distinction, however, is the presence of superhyperfine structure near the perpendicular direction. This arises from mixing of molecular orbitals centered about the four equivalent pyrrole nitrogens into the Cu²⁺ orbitals and is indicative of very strongly covalent bonding. The expected superhyperfine structure due to the four equivalent ¹⁴N nuclei consists, in this Cu²⁺ spectrum, of five groups of nine equally spaced lines. The pattern of intensities in each group follows the

* L. Auber, personal communication.
In basic solutions 10^{-4} \text{ M} \text{ turacin} \text{ has an } \text{ ESR spectrum not interpretable in terms of a spin Hamiltonian for a single Cu}^{2+}. \text{ If it is assumed that two Cu}^{2+} \text{ spins are interacting at a short distance, a theoretical spectrum can be computed for the ESR absorption. The general form of such a two-spin Hamiltonian is given in the "Appendix." The simplest form of such an interaction occurs when the porphyrin molecules are situated as shown in Fig. 8. The theoretical curve for this arrangement is shown along with the experimental curve in Fig. 3. The theoretical curve for the case in which the axes of the two porphyrin rings are not colinear is not expected to be very different from this curve, provided that the two Cu}^{2+} \text{ are still near one another. The theoretical curve gives the best agreement when the distance between the Cu}^{2+} \text{ ions is assumed to be 3.5 \text{ Å}. One important difference between the ESR of isolated and coupled Cu}^{2+} \text{ spins is the appearance of the parallel hyperfine pattern. For isolated Cu}^{2+}, \text{ this consists of four equally intense peaks or shoulders, one or two of which sometimes overlap the perpendicular absorption region. In the case of exchange-coupled Cu}^{2+} \text{ spins, however, the pattern consists of two sets of seven lines with intensities in the ratio 1:2:3:4:3:2:1. The agreement of the theoretical and the experimental curves in this region is apparent in Fig. 3 where five of the lines are resolved at low field and two at high field. It is not possible to obtain the magnitude of the exchange coupling from the ESR spectrum. The susceptibility, however, as measured by ESR does not depart from the Curie law down to 1.5° \text{ K}. Therefore the magnitude of the exchange coupling is less than approximately 1 \text{ cm}^{-1}. \text{ There is no loss of ESR susceptibility in the presence of exchange coupling of two spins in contrast to the case of exchange narrowing of many spins. This magnitude of coupling is much smaller than that for two Cu}^{2+} \text{ ions covalently bound to each other as in copper acetate (21) where the coupling is approximately 300 \text{ cm}^{-1}. Since the experimental ESR absorption agrees in all respects with that expected for a pair of interacting Cu}^{2+} \text{ ions, we attribute this spectrum to dimers of turacin molecules. The geometrical relationship of the two Cu}^{2+} \text{ ions may not be as simple as shown in Fig. 8. This dimer formation is equilibrium-controlled. That is, in NH}4 \text{ solutions of turacin more dilute than } 10^{-4} \text{ M the monomer-dimer equilibrium is shifted in favor of the monomer. This will be discussed in a future communication.} \text{ In the presence of aced the ESR spectrum is again different from that expected for isolated Cu}^{2+} \text{ ions. Here one finds a relatively narrow, almost symmetrical absorption. This is likely to occur when there are interactions among many Cu}^{2+} \text{ ions. Let us assume an array of Cu}^{2+} \text{ spins arranged in layers as an extension of that shown in Fig. 8. Here many Cu}^{2+} \text{ ions would be along a line of the } z \text{ direction and 3.5 Å apart. Magnetic dipolar interactions among such spins would lead to the theoretical curve shown in Fig. 4. The fact that the experimental curve is narrower than this curve indicates that exchange narrowing (22) takes place. The same exchange interaction operable in the ESR spectrum of the dimer would cause this narrowing effect. When exchange narrowing occurs, there is a partial loss of ESR susceptibility or absorption intensity relative to similar isolated spins. Comparing the ESR intensity of the spectrum of a red feather with the analysis for total copper as mentioned previously, we find that in this case, the absorption loss is approximately 11\% As in the case of the dimer, no departure from the reciprocal dependence of the susceptibility on temperature is observed down to 1.5° \text{ K}. Since this spectrum

![Fig. 7. Superhyperfine structure of ESR spectrum of turacin in diisopropylamine. Upper curve, the high magnetic field position of the ESR absorption derivative spectrum of turacin having a concentration of 0.02 M and saturated with diisopropylamine. The dashed line is the spectrum with the effect of the superhyperfine structure removed. Lower curve, the nine vertical bars represent one set of the computed superhyperfine structure lines expected from four equivalent 14N nuclei. The complete superhyperfine pattern consists of five partially overlapping sets of nine lines.](image1)

![Fig. 8. Configuration of two turacin molecules used in computing ESR spectrum. The dimer probably consists of two molecules which are not quite axially aligned. In this representation the axial alignment was chosen to simplify the spin Hamiltonian calculations. The ratios 1:4:10:16:19:16:10:4:1. The five groups are partially overlapping. The only lines which may be unambiguously assigned are the six outermost lines of the group towards highest field. This region of the experimental ESR spectrum (in this example, observed for diisopropylamine) is shown as the upper curve in Fig. 7. The dashed line is the ESR spectrum with the superhyperfine interaction averaged out by overmodulation. The lower curve shows superimposed on this same line the theoretical amplitudes of the superhyperfine lines of the highest field group. On the basis of the agreement of the amplitudes of these observed and theoretical lines, we may state that although the added amines may be bonded to the Cu}^{2+} \text{ via the amine nitrogens, this bonding must be weaker than the pyrrole nitrogen bonding. Neither these amine nitrogens nor the amine hydrogens contribute to the superhyperfine structure pattern. The ESR spectrum of turacin in the presence of amines can be reconstructed from a spin Hamiltonian (20) appropriate to a single isolated Cu}^{2+} \text{ ion in an axially symmetrical site, as shown in the "Appendix." Thus, we attribute this spectrum to turacin monomers in solution.}
is consistent with what one would expect from an approximately linear array of weakly exchange-coupled Cu2+ ions, we ascribe it to a polymeric or aggregate form of turacin molecules which are arranged one on top of another in microcrystal line form. From ESR studies, one concludes that when acid is added to turacin, there is an aggregation of molecules forming turacin polymer. This is associated with a decrease of the wave length of the Soret band. When these polymer aggregates grow large, they flocculate and precipitate.

Both caffeine and gelatin are effective in preventing the precipitation of turacin in acid medium. The mechanism for gelatin, however, is different from that for caffeine. In the gelatin system, the large protein molecules act as colloidal dispersants and maintain acidified precipitated turacin in colloidal suspension. Caffeine prevents the precipitation by forming weak chemical bonds with the copper of turacin essentially inhibiting the association of turacin molecules necessary to form polymer. Similar interactions between uroporphyrins and heterocyclic amines have been described (23).

The formation of aggregates similar to those seen when turacin is precipitated in acid is seen with basic turacin in 4.5 M KCl, or even in concentrated NaOH. These findings indicate that the dipolar attraction of water by the hydrophilic ionized carboxyl groups of the molecule is necessary for the solubility of turacin. Once the water is removed by protonation of the negatively charged carboxyl groups, or the water is bound to a more hydrophobic group as in salt solutions, the turacin molecules interact with each other and precipitate from solution. The mechanism of precipitation of turacin presumably involves an aggregation of dimers and not monomers, as witnessed by the fact that the precipitation of acidified monomeric turacin is markedly retarded. One might also say that possibly the large number of amine groups of the molecule is necessary for the solubility of turacin.

The iridescent green feather contains a free radical species. This material cannot be a degradation product of turacin because the green feather contains no copper. Furthermore, this free radical is not the same as the one found by Auber.2

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SUMMARY

1. In the red feathers of the South African touraco bird, turacin (copper uroporphyrin III) exists as microcrystals.
2. In ammoniacal solution, turacin can exist as a dimer in which two copper atoms are dipole-coupled at a distance of 3.5 Å as shown by electron spin resonance.
3. In the presence of aliphatic and heterocyclic aromatic amines, purines, or pyrimidines, turacin dimer is converted into monomer. The copper is bound covalently to the four pyrrole nitrogens. The bonding to the added amine is less covalent.
4. Turacin, precipitated either by acid or salt, exists as a microcrystal, in which the copper is arranged in a fashion not unlike that of the dimer. The number of turacin molecules in the aggregate, however, is much larger.
5. The iridescent green feathers from the same bird contain a free radical species which is not a breakdown product of turacin.

APPENDIX

The spin Hamiltonian appropriate to a single isolated Cu2+ ion has been given (20) as $\mathcal{H} = -\beta S \cdot g \cdot H + I \cdot \mathbf{A} \cdot \mathbf{S}$.

The same representation which causes $g$ to be diagonal also has a similar effect on $A$, and thus there are only four independent spin Hamiltonian parameters $g_1, g_2, A_1, A_2$. For a given set of the four parameters a computed ESR spectrum may be obtained by solving for the absorption intensity as a function of the magnetic field at all angles subject to the condition

$$3 \psi = h \omega$$

where $\psi$ is the quantum mechanical state description of the Cu2+ spin, $h$ is Planck's constant, and $\nu$ is the ESR microwave frequency. The computation is impossible in closed form but may be carried out by means of a program written by one of the authors (W. E. B.) for a 7004 computer. The computer takes 4 seconds to make the computation for both the absorption and absorption derivative and gives both a paper and microfilm plot of each. By trial and error one may find the values of the four parameters which best reproduce the observed ESR spectrum. These are the values given in Table II.

For two coupled Cu2+ spins the Hamiltonian function is more complicated. Here one writes

$$\mathcal{H} = -\beta (S_1 + S_2) \cdot g \cdot H + S_1 \cdot J \cdot S_2 + (S_1 + S_2) \cdot A \cdot (J_1 + J_2)$$

In the case of axial symmetry one has $g$ and $A$ as before and

$$J = \begin{bmatrix} J + D \\ J - D/2 \end{bmatrix}$$

The exchange coupling as denoted by $J$ and $D$ is the dipolar coupling (including pseudodipolar, if any). There are then six independent parameters describing the Hamiltonian function. The value of $J$ does not affect the solution for the ESR spectrum provided $J \gg A_1, A_2$. Thus for any assumed set of values for the remaining five parameters the computed spectrum may be obtained as before.

In the case of two coupled Cu2+ spins not having axial symmetry, for example two turacin molecules with their axes not colinear, the representation which diagonalizes $g$ may not diagonalize $J$ and $A$. Thus there is a possible total of 15 independent parameters. Computation based on such a large number of
The extension of the Hamiltonian to a linear array of spins was made as

\[ \mathcal{H} = -g\mathbf{S}_0 \cdot \mathbf{H} + \sum_{n=-\infty}^{\infty} (na)^{-3} \mathbf{S}_n \cdot \mathbf{D} \cdot \mathbf{S}_n \ (n \neq 0) \]

where \( g \) has been taken as isotropic, the hyperfine interaction has been neglected, \( a \) is the intermolecular spacing, and \( \mathbf{D} \) taken the same as \( J \) with the exchange coupling equal to zero. The spins are counted from \( n = -\infty \) to \( \infty \) with spin \( S_b \) at \( n = 0 \). The computed EPR spectrum from this Hamiltonian is shown as the dashed line in Fig. 4. The effect of increasing the value of the exchange coupling is to make the calculated spectrum narrower and narrower until it is a single line of Lorentzian shape in the center of the \( J = 0 \) pattern.

Acknowledgments—The authors wish to thank the directors of the Zoological parks in New York, Chicago, Frankfurt, London, and Johannesburg for providing them with moulted red toucato feathers. We should like to thank Dr. Lewis Auber who graciously sent us a toucato bird skin.

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