**Acknowledgment.** This research was supported by the National Science Foundation. T.B.R. is a fellow of the Alfred P. Sloan and Camille and Henry Dreyfus Foundations. The diffractometer was purchased in part with funds from NSF. Johnson Matthey, Inc., is thanked for providing loans of the platinum metal salts used in this work.

**Supplementary Material Available:** Tables of atomic coordinates, thermal parameters, and structure factors (19 pages). Ordering information is given on any current masthead page.

## Electronic Spectroscopy of a Non-Kekulé Isomer of Benzene, 2,4-Dimethylene-1,3-cyclobutanediyl

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*Received July 26, 1985*

Non-Kekulé molecules are intrinsically novel structures that have been of considerable interest recently because of their potential usefulness as models for photochemical excited states and reacton transition states and their possible incorporation into materials with novel optical, electronic, or magnetic properties. ESR spectroscopy is the most powerful tool for unambiguously identifying the high spin states (triplet, quintet, ...) of such structures. Once such an assignment has been made, the potentially quite informative techniques of electronic absorption and emission spectroscopy can be implemented.[2] We report herein the absorption and emission spectra of triplet biradical **1**, a

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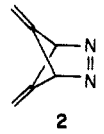
non-Kekulé isomer of benzene. We also describe a novel technique that could be generally applicable to the often vexing problem of correlating an observed optical spectrum with an ESR spectrum.

Irradiation (334 ± 10 nm) of diazene **2** at 77 K in a 2-

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methyltetrahydrofuran (MTHF) matrix produces a bright yellow-orange color. A sample prepared in this manner exhibits an ESR spectrum which has been previously assigned to triplet **1**,[3] as well as the electronic absorption and emission spectra presented in Figure 1. An excitation spectrum, measured by monitoring the emission at 552 nm, faithfully reproduced the absorption spectrum, establishing that the same species is responsible for both spectra of Figure 1.

The fine structure of the optical spectra in Figure 1 can be readily analyzed in terms of a single electronic transition with two independent vibrational modes. Frequency $\bar{\nu}_2$ (Figure 1) is ca. 620 $cm^{-1}$ in both spectra, but $\bar{\nu}_1$ varies slightly, with frequencies

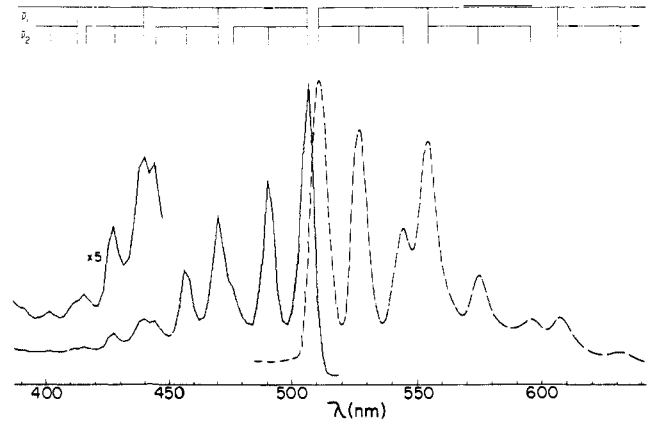


**Figure 1.** Absorption (—) and fluorescence (---; $\lambda_{ex}$ = 440 nm) spectra of triplet **1** in MTHF at 77 K, with $\lambda_{max}$ = 506 and 510 nm, respectively. The fluorescence spectrum is not corrected for the ca. 40% decomposition of the sample that occurred during the scan from 500 to 700 nm.

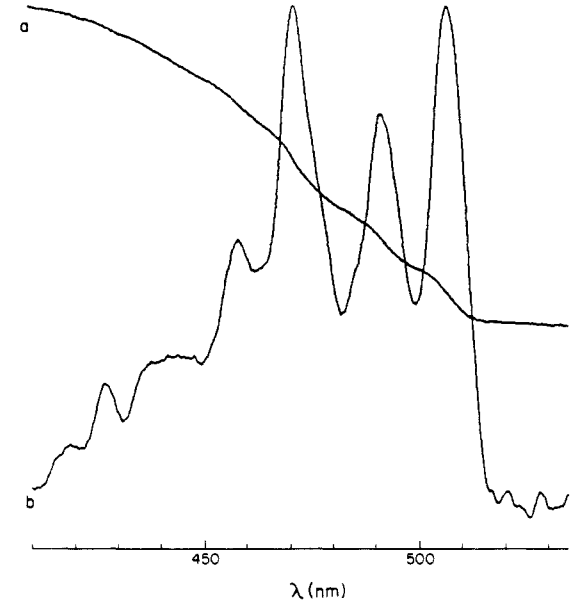


**Figure 2.** (a) ESR signal intensity of triplet **1** vs. wavelength of irradiation (see text). The final is ca. half the initial intensity. (b) $(1/I)(-dI/d\lambda)$ vs. λ after quadratic least-squares smoothing.[6]

in the ground and excited states of 1550 and 1500 $cm^{-1}$, respectively. The mirror-image relationship between the absorption and emission spectra, along with the small (150 $cm^{-1}$) Stokes' shift and the prominence of the 0–0 bands, implies nearly identical geometries for the ground and excited states.

Both the ESR signal of triplet **1** and the absorption spectrum of Figure 1 decay slowly at 77 K and with the same unimolecular rate constant.[4] We have also correlated the ESR and absorption spectra in a novel way by taking advantage of the previously reported[3] photochemical lability of **1**. Figure 2a shows the decrease in the ESR signal intensity ($I$) of **1** at 4 K as a function of irradiation wavelength, which was increased at a constant rate. Curve b, which represents $(1/I)(-dI/d\lambda)$, is effectively equivalent to a plot of ε vs. λ for the ESR signal carrier.[5] The similarity of this photochemical "action spectrum" and the absorption spectrum (Figure 1) is evident.

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Since theory predicts[7] a substantial triplet preference for **1**—a notion qualitatively supported by our Curie plot[3]—we conclude that the spectra of Figure 1 represent absorption from (emission to) the triplet ground state of **1**. The only alternative source of these spectra would be a rapidly equilibrating singlet state of **1**. However, the data of Figure 2 were obtained at 4 K, conditions under which significant population of a thermally excited singlet is quite improbable.

Given this analysis, we can determine an oscillator strength for the transition. Thus, the biradical concentration of a typical sample was determined by a spin count, i.e., a comparison of its double-integrated ESR signal intensity with that of a free radical standard[8] at 77 K. Measurement of the absorbance of this sample provided an $\epsilon$ (506 nm) of $(8 \pm 2) \times 10^3$ $M^{-1}$ $cm^{-1}$. Integration of the absorption spectrum gave an oscillator strength of $f \approx 0.025$, indicating that the transition is spin-allowed.

Our results demonstrate the remarkable changes in electronic structure obtained on going from benzene ($\lambda_{max}$ = 254 nm) to its non-Kekulē isomer **1**. We have found[9] that standard PPP-CI theory predicts a transition ($^3B_{2u} \rightarrow {}^3B_{3g}$) at 501 nm with $f$ = 0.018, in excellent agreement with experiment. Further experimental and theoretical studies of **1** and related structures are under way.

**Acknowledgment.** We are grateful to Janet Marshall for the emission spectra and to Steve Witt for assistance with the quantitative analysis of ESR spectra, which employed computer programs written at Caltech by Jeff Gelles. We also thank Professor Jacob Wirz for helpful discussions. This work was supported by the donors of the Petroleum Research Fund, administered by the American Chemical Society, and by the National Science Foundation.

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## Magnetic Susceptibility Studies of the Native Cupro-Zinc Superoxide Dismutase and Its Cobalt-Substituted Derivatives. Antiferromagnetic Coupling in the Imidazolate-Bridged Copper(II)-Cobalt(II) Pair

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*Received August 1, 1985*

Polynuclear metalloproteins are quite numerous in nature. When the metals of the active sites interact through bridging ligands, the magnetic susceptibility measurements may be one of the most powerful techniques to determine the nature and the magnitude of this interaction. So far, very few studies of this type have been carried out[1-5] and the reasons can be easily understood. Proteins are of large molecular weight and their constituents are essentially diamagnetic. The magnetic metal ions represent an almost negligible part of those molecules. The problem is to extract a very small paramagnetic effect from an overall high diamagnetic signal. Therefore the conditions required to resolve the contribution of interest are a high degree of purity and homogeneity of proteins on one hand, and owing to the important magnetic dilution, a high sensitivity and accuracy of the susceptometers on the other hand. Susceptometers utilizing quantum flux detection methods present the characteristics required for reliable magnetic susceptibility study on proteins. But in order to get absolute values of protein susceptibilities, the determination of the diamagnetic correction remains a crucial point. The only way to reach those absolute values is the direct and independent measurement of the diamagnetic susceptibility of the apoproteins when available.

These requirements have been met in the magnetic susceptibility study we have performed on the cobalt-substituted derivative, $Cu_2Co_2SOD$, of the native cupro-zinc superoxide dismutase, $Cu_2Zn_2SOD$. The latter presents the unique feature of a histidine imidazolate group bridging the copper(II) and the zinc(II) ions. A tetrahedral symmetry is achieved around the zinc(II) ion while the square-pyramidal arrangement around the copper(II) ion is completed by a water molecule in an apical position.[6,7] The cobalt(II) ion can replace the zinc(II) ion in its site and the resulting cobalt derivative $Cu_2Co_2SOD$ contains an imidazolate bridged copper(II)-cobalt(II) pair.[8-12] The two derived apoproteins $E_2E_2SOD$ and $E_2Co_2SOD$ (E for empty) are also available.[11,12]

These four proteins provide a unique and perfect test in order to estimate the extent of coupling between metal ions through bridging ligands. In order to obtain a reasonable estimate of the diamagnetic contribution, we have measured the diamagnetic susceptibility of the apoprotein $E_2E_2SOD$, the three magnetic proteins $Cu_2Zn_2SOD$, $Cu_2Co_2SOD$, and $E_2Co_2SOD$ differing only in their metal content. In order to have a check of the paramagnetic contribution of the two individual Cu(II) and Co(II) ions, we have measured the paramagnetic susceptibility of $Cu_2Zn_2SOD$ and $E_2Co_2SOD$ which contain the same but independent Cu(II) and Co(II) chromophores as in $Cu_2Co_2SOD$. Finally, we have measured the susceptibility of $Cu_2Co_2SOD$, and for the first time, we can offer a quantitative estimation of the exchange interaction in the cobalt/copper enzyme. Previous measurements of $Cu_2Co_2SOD$ susceptibility were reported by three of us,[13] but quantitative analysis was not made possible.

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