

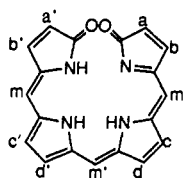
Geometric and Electronic Structure and Dioxygen Sensitivity of the Copper Complex of Octaethylbilindione, a Biliverdin Analog

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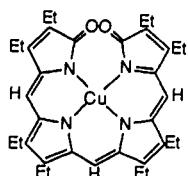
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Biliverdin IXa (1) is a blue-green intermediate in heme catabolism that is formed by the oxygenation of heme by heme oxygenase.¹ In the coupled oxidation process that is a model for



1: a, c, c', b' = methyl; b, a' = vinyl;
d, d' = propionate; m, m' = H.

2: a-d' = ethyl m, m' = H; H₃OEB.



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the heme oxygenase reaction,² treatment of (py)₂Fe^{II}(OEP) (OEP is the dianion of octaethylporphyrin) with dioxygen and ascorbic acid in pyridine (py) yields not only verdoheme³ but also an iron(III) complex of the biliverdin analog, octaethylbilindione (2).⁴ Biliverdins and their metal complexes have wide biological distribution and are pigments in bird eggs, insects, and coral.⁵ In order to understand the nature of metal complexation by this linear tetrapyrrole, we have begun to investigate the structure and reactivity of metal complexes of 2.⁶ Here we report the formation of a copper complex with unusual magnetic properties and reactivity toward dioxygen.

Treatment of 2 (0.024 mmol) with copper(II) acetate (0.30 mmol) in air in 40 mL of ethanol as described by Bonnett and co-workers⁷ yields olive green Cu(OEB) (3). The oxidant in the reaction is the excess copper(II) acetate, since the same product is obtained when the reaction is run under a dioxygen-free conditions. The product is somewhat air sensitive, so recrystallization (dichloromethane/methanol) and subsequent handling were performed under a dinitrogen atmosphere (UV/vis, chloroform solution, λ_{\max} , nm (ϵ , M⁻¹ cm⁻¹): 307 (3.5 × 10⁴), 353 (3.1 × 10⁴), 417 (5.1 × 10⁴), 532 (6.5 × 10³), 578 (5.8 × 10³), 730 (9.4 × 10³), 802 (2.0 × 10⁴)).

(1) O'Carra, P. In *Porphyryns and Metalloporphyryns*; Smith, K. M., Ed.; Elsevier: New York, 1975; p 123. Schmid, R.; McDonagh, A. F. In *The Porphyryns*; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. 6, p 258. Bissell, D. M. In *Liver: Normal Function and Disease. Vol. 1. Bile Pigments and Jaundice*; Ostrow, J. D., Ed.; Marcel Dekker, Inc.: New York, 1986; p 133.

(2) Warburg, O.; Negelein, E. *Chem. Ber.* 1930, 63, 1816. Lemberg, R. *Rev. Pure Appl. Chem.* 1956, 6, 1. Saito, S.; Itano, H. A. *Proc. Natl. Acad. Sci. U.S.A.* 1982, 79, 1393. Lagarias, J. C. *Biochim. Biophys. Acta* 1982, 717, 12.

(3) Balch, A. L.; Latos-Grazyński, L.; Noll, B. C.; Olmstead, M. M.; Sztrenberg, L.; Safari, N. *J. Am. Chem. Soc.* 1993, 115, 1422.

(4) Balch, A. L.; Latos-Grazyński, L.; Noll, B. C.; Olmstead, M. M.; Safari, N. *J. Am. Chem. Soc.* 1993, 115, 9056.

(5) McDonagh, A. F. In *The Porphyryns*; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. 6, p 293.

(6) A few metal complexes of biliverdin derivatives have received some characterization. Fuhrop, J.-H.; Salek, A.; Subramanian, J.; Mengersen, C.; Besecke, S. *Liebigs. Ann. Chem.* 1975, 1131. Bonfiglio, J. V.; Bonnett, R.; Buckley, D. G.; Hamzetaash, D.; Hursthouse, M. B.; Malik, K. M. A.; McDonagh, A. F.; Trotter, J. *Tetrahedron* 1983, 39, 1865.

(7) Bonnett, R.; Buckley, D. G.; Hamzetaash, D. *J. Chem. Soc., Perkin Trans. 1* 1981, 322.

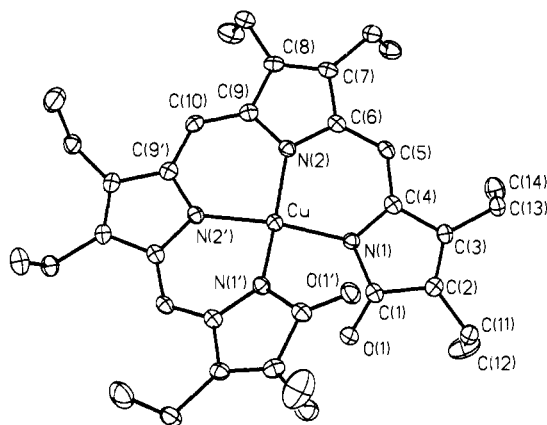


Figure 1. Perspective view of 3 with 50% thermal contours. Selected bond distances (Å): Cu-N(1), 1.954(2); Cu-N(2), 1.962(2); C(1)-O(1), 1.216(3). Selected bond angles (deg): N(1)-Cu-N(2), 90.3(1); N(1)-Cu-N(1'), 96.2(1); N(1)-Cu-N(2'), 158.6(1); N(2)-Cu-N(2'), 91.1(1).

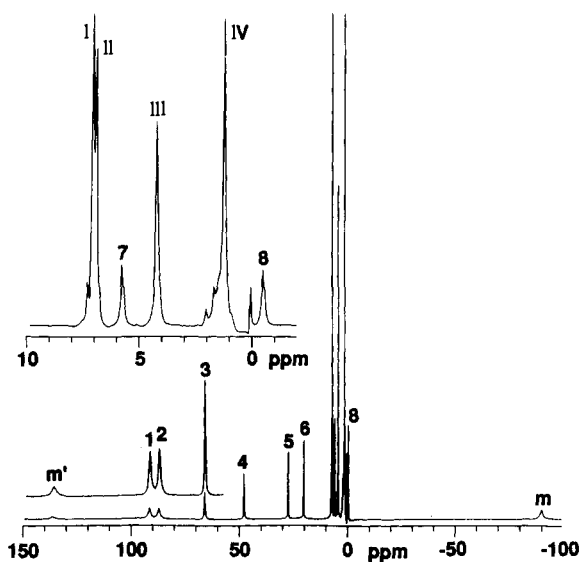


Figure 2. 300-MHz ¹H NMR spectrum of 3 in chloroform-*d* solution at 25 °C. Resonances are assigned as: m, m', meso protons; 1-8, methylene protons; I-IV, methyl protons. The inset shows the 10 to -1 ppm region.

The geometric structure of Cu(OEB) as determined by an X-ray diffraction study⁸ is shown in Figure 1. The complex is monomeric with C₂ symmetry, and the copper is coordinated to each of the four pyrrole nitrogen atoms. The bilindione ligand has a helical geometry that allows it to avoid contact between the two keto groups. The O(1)···O(1') separation is 3.154(4) Å. The infrared spectrum shows two keto stretches at 1665 and 1650 cm⁻¹ and no bands in the region above 3100 cm⁻¹ where an O-H stretch would occur.

The ¹H NMR spectrum of 3 in chloroform-*d* solution at 23 °C is shown in Figure 2. The proton chemical shifts follow the Curie law from -80 °C to +20 °C in dichloromethane-*d*₂. The resonance assignments shown in the figure are based on relative integrated intensities. There are two meso resonances, eight methylene resonances, and four methyl resonances. The eight methylene resonances arise because each of the four methylene groups is diastereotopic. The line widths of these eight resonances vary markedly, and this is reflected in their peak heights in Figure 2.

(8) Black rods of 3 (C₃₅H₄₃CuN₄O₂) were obtained by diffusion of methanol into a dichloromethane solution of the complex. They form in the monoclinic space group C2/c with *a* = 14.544(2) Å, *b* = 18.931(3) Å, *c* = 12.963(2) Å, β = 118.64(2)° at 126(2) K with *Z* = 4. Refinement of 1926 reflections with *F* > 8.0σ(*F*) and 191 parameters yielded *R* = 0.030 and *R*_w = 0.033.

Notably those with the largest hyperfine shifts have the greatest line width. This variation results from local dipolar relaxation by delocalized spin density on the tetrapyrrole ligand.⁹ The magnetic moment in chloroform at 23 °C is 2.7(3) μ_B . The complex displays no EPR signal (X-band, chloroform solution, 23 °C). However, reduction of **3** in dichloromethane solution with ascorbic acid produces a copper(II) complex with a typical EPR spectrum with $g = 2.07$, $a_{Cu} = 87.9$ G, and $a_N = 16.5$ G and a UV/vis absorption spectrum with λ_{max} at 344 nm (3.8×10^4), 600 (sh) (1.6×10^4), and 646 (3.1×10^4) in that solution.

Two limiting possibilities for the electronic structure of **3** deserve consideration. In one, the compound can be considered as a Cu^{III} complex of the tetrapyrrole trianion: Cu^{III}(OEB). Alternately, the complex can be formulated as containing Cu^{II} and a tetrapyrrole radical dianion: Cu^{II}(OEB^{•-}). To explain the magnetic properties, the Cu^{III}(OEB) formulation requires the presence of a high-spin d⁸ ion which would be expected to have tetrahedral geometry. While the copper geometry in Figure 1 is certainly irregular, the N(1)-Cu-N(2') angle is too wide and the N(1)-Cu-N(1') angle too narrow for tetrahedral coordination. A few four-coordinate Cu^{III} complexes are known.¹⁰ These are diamagnetic and have short Cu-N distances in the range 1.8–1.9 Å. In contrast, **3** is paramagnetic, and the Cu-N distances are longer. For the Cu^{II}(OEB^{•-}) formulation, the magnetic properties could be explained by the presence of ferromagnetic coupling between the d⁹ Cu^{II} and the ligand radical to give an $S = 1$ state as seen in [Cu(TMP^{•+})]⁺ (TMP^{•+} is the π radical of tetramesitylporphyrinate)¹¹ or by the presence of two uncoupled spins. The metric parameters for the copper ion and its irregular geometry¹² are reasonable for Cu^{II}. The large hyperfine shifts seen in the ¹H NMR spectrum along with the observation of local dipolar relaxation are consistent with radical character for the ligand. Finally, the absorption spectrum with its intense feature at 802 nm is unusual for a bilindione,^{6,13} but it could be explained by the presence of a bilindione radical with ligand oxidation. Consequently, the Cu^{II}(OEB^{•-}) formulation appears as a major contribution to the electronic structure.

This olive-green complex is sensitive to air. Saturation of a dichloromethane solution of **3** with dioxygen at -80 °C followed by warming to room temperature and standing for 3 days produced a blue solution which was evaporated and the residue dissolved in a minimum of dichloromethane. After the addition of hexane, slow evaporation gave **4** with two propentdyopent¹⁴ ligands in 40% yield. Evidence for a propentdyopent class dipyrrole as a product of heme degradation of cytochrome P-450 reductase has

(9) Unger, S. W.; Jue, T.; LaMar, G. N. *J. Magn. Reson.* **1985**, *61*, 448.

(10) Birker, P. J. M. W. L. *Inorg. Chem.* **1977**, *16*, 2479. Diaddario, L. L.; Robinson, W. R.; Margerum, D. W. *Inorg. Chem.* **1983**, *22*, 1021. Anson, F. J.; Collins, T. C.; Richmond, T. G.; Santarsiero, B. D.; Toth, J. E.; Treco, B. G. R. T. *J. Am. Chem. Soc.* **1987**, *109*, 2974. Knof, U.; Weyhermüller, T.; Wolter, T.; Wieghardt, K. *J. Chem. Soc., Chem. Commun.* **1993**, 726.

(11) Erler, B. S.; Scholz, W. F.; Lee, Y. J.; Scheidt, W. R.; Reed, C. A. *J. Am. Chem. Soc.* **1987**, *109*, 2644.

(12) Hathaway, B. J. *Comprehensive Coordination Chemistry*; Pergamon Press: Oxford, U.K., 1987; Vol. V, p 553. Scheidt, W. R. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. III, p 463.

(13) Burke, M. J.; Pratt, D. C.; Moscovitz, A. *Biochemistry* **1972**, *11*, 4025. Chae, Q.; Song, P.-S. *J. Am. Chem. Soc.* **1975**, *97*, 4176.

(14) Bonnett, R.; Dimsdale, M. J.; Stephenson, G. F. *J. Chem. Soc., Perkin Trans. 1* **1987**, 439. Bonnett, R.; Ioannou, S.; Swanson, F. J. *J. Chem. Soc., Perkin Trans. 1* **1989**, 711.

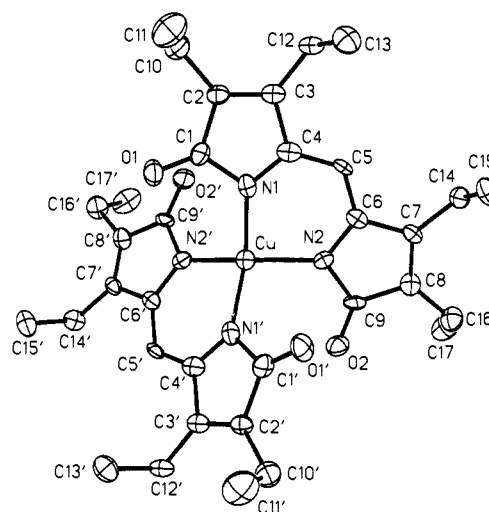
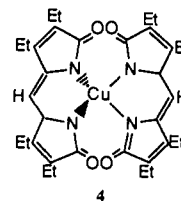


Figure 3. Perspective view of **4** with 50% thermal contours. Selected bond distances (Å): Cu-N(1), 1.943(8); Cu-N(2), 1.965(7); C(1)-O(1), 1.224(11). Selected bond angles (deg): N(1)-Cu-N(1'), 142.1(5); N(1)-Cu-N(2), 90.5(3); N(1)-Cu-N(2'), 102.2(3); N(2)-Cu-N(2'), 140.4(5).



been reported.¹⁵ The structure of **4** (λ_{max} (dichloromethane): 346 nm (1.3×10^4), 496 (4.5×10^3), 622 (4×10^3), 660 (4×10^3)) as determined by an X-ray diffraction study is shown in Figure 3.¹⁶ The nature of the process by which **4** forms and the methine bridge is oxidized is under study.

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Supplementary Material Available: Details of data collection and structure refinement, tables of atomic coordinates, bond distances and angles, anisotropic thermal parameters, and hydrogen atom positions for **3** and **4** (15 pages); tables of observed and calculated structure factors for **3** and **4** (10 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(15) Guengerich, F. P. *Biochemistry* **1978**, *17*, 3633.

(16) Exceedingly thin red needles of **4**·1.3CH₂Cl₂ were obtained by evaporation of a dichloromethane/hexane solution of the complex. They form in the monoclinic space group *C2/c* with $a = 17.448(8)$ Å, $b = 13.702(8)$ Å, $c = 15.008(8)$ Å, $\beta = 101.28(4)^\circ$ at 123(2) K with $Z = 4$. Refinement of 2079 reflections and 209 parameters yielded $R = 0.097$. The dichloromethane molecule suffered from disorder.