

## Magnetic CD spectra of Chlorophylls, Chlorophyllides, and Zinc(II) and Copper(II) Pheophytins and Pheophorbides

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*Magnetic circular dichroism (MCD) spectra of the ten metal-containing title molecules were measured at room temperature, and spectra of copper-pheophorbide-a were measured at 23 °K, 47 °K, and 63 °K. Electronic excited states are assigned, systematic structural features are discussed, and it is found that MCD distinguishes with ease between forms a and b of these molecules. A preliminary MCD study in which the temperature of Cu(II)-pheophorbide-a is varied between 23 °K and 63 °K indicates that the Cu(II) "d-orbital" which presumably contains the one lone electron is not significantly interactant with the  $\pi$  orbitals involved in the electronic excitations.*

### Introduction

At first glance one tends to designate porphyrins structurally analogous to chlorins, viz., chlorophyll (forms *a* and *b*), chlorophyllide (hydrolysis products of chlorophyll are chlorophyllide and the phytol group,  $-C_{20}H_{39}$ ), pheophytin (chlorophyll base, i.e., without  $Mg^{2+}$ ), and pheophorbide (from hydrolyzing  $-C_{20}H_{39}$  off pheophytin). However, the formation of these chlorins through the dihydrogenation of ring IV of the porphyrin structure (Fig. 1) is a traumatic perturbation, so that a strong  $D_{4h} \rightarrow C_{2v}$  symmetry reduction takes place. MCD spectra of several metal-

free chlorins were reported by Briat, *et al.* [3], and electronic absorption (optical spectra of metal-containing chlorins have been measured or discussed by several workers [4-7]. However, features of metal-containing chlorins have not previously been explored by means of MCD spectroscopy. In view of this paucity and the usual potential of MCD analysis, this study was undertaken.

### Experimental

MCD spectra were obtained with previously described equipment [8]. Ellipticity calibrations were made by using a freshly prepared standard solution of  $K_3[Fe(CN)_6]$ . Room temperature spectra were measured of diethyl ether solutions, and the low temperature spectra were obtained of Cu-pheophorbide-*a* in a matrix of poly(methylmethacrylate). The isolations or preparations of the chlorins were previously described in full detail [4, 5]. Each of the specimens was found to have natural optical activity in the region of almost every optical band, so that the MCD spectrum of each figure is the net magnetic optical activity. However, the sign of each *observed* composite band also turns out to be the sign of the magnetic CD activity by itself. The intensity scale of the MCD spectra is in units of molar ellipticity per gauss, or  $[\theta]_M$ .

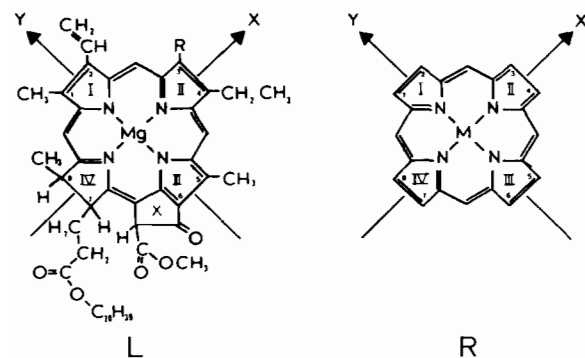


Figure 1. Chlorophyll-*a* (R is  $CH_3$ ) and -*b* (R is  $CHO$ ) (L), and porphyrin (R).

### Results and Discussion

We shall explore the nature of the electronic excitations and the systematic MCD spectral variations as the chlorin structure is changed from form *a* to form *b* (replacement of  $CH_3$  at ring II, position 3, by  $CHO$ ), as the metal is varied, and as the structure is changed of a chlorophyll or a pheophytin by loss of the phytyl group,  $C_{20}H_{39}$ , to form a chlorophyllide or a pheophorbide, respectively. Reference will be made to the room temperature electronic and MCD spectra shown in Figs. 2-6, and to the low temperature MCD spectrum of Cu(II)-pheophorbide-*a*.

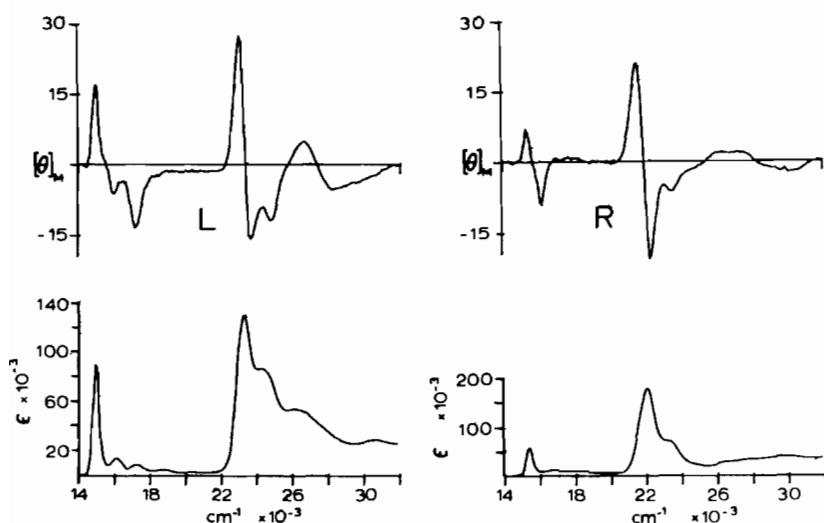


Figure 2. Room temperature MCD and optical spectra of chlorophyll-*a* (L) and -*b* (R).

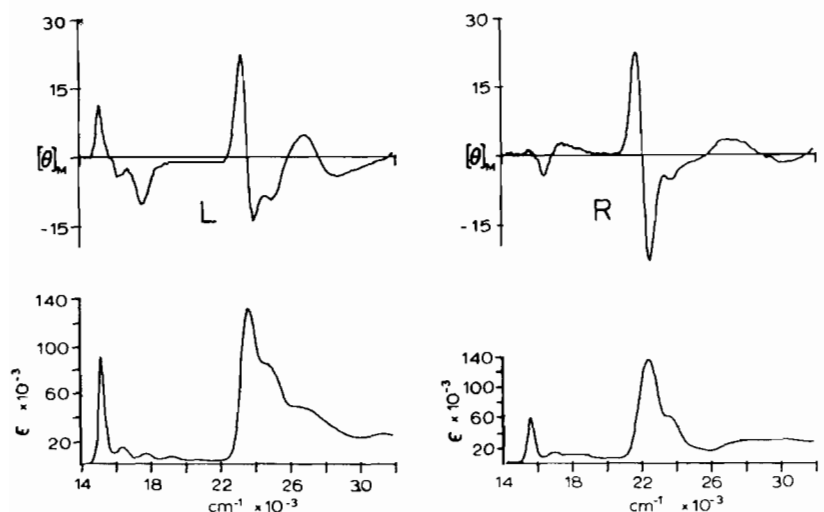


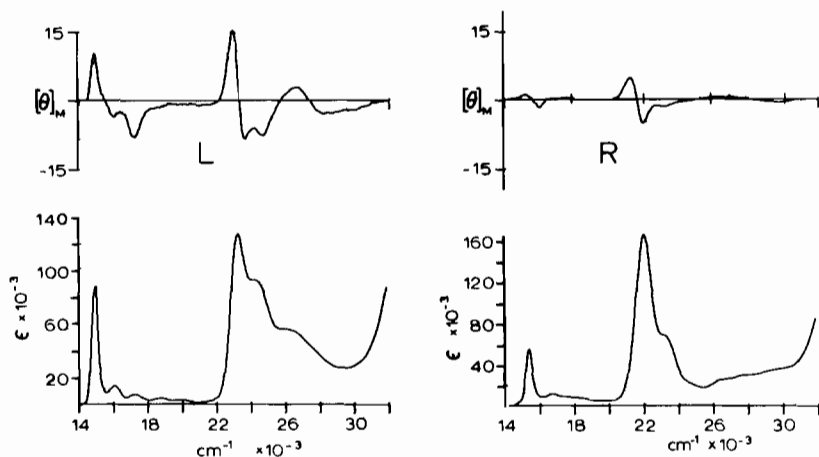
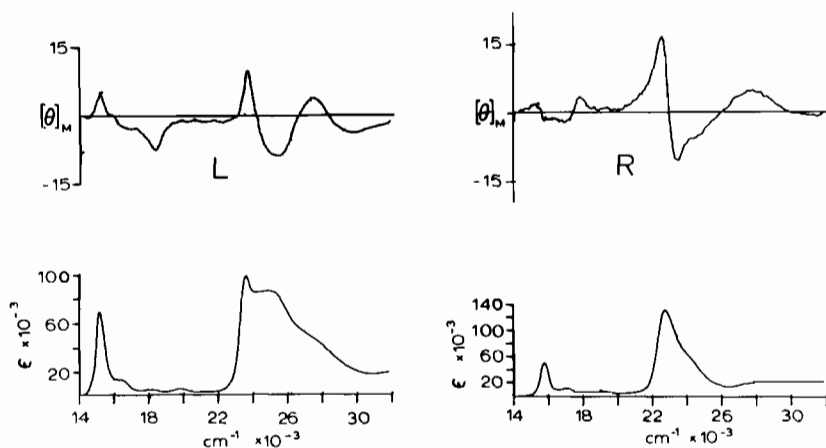
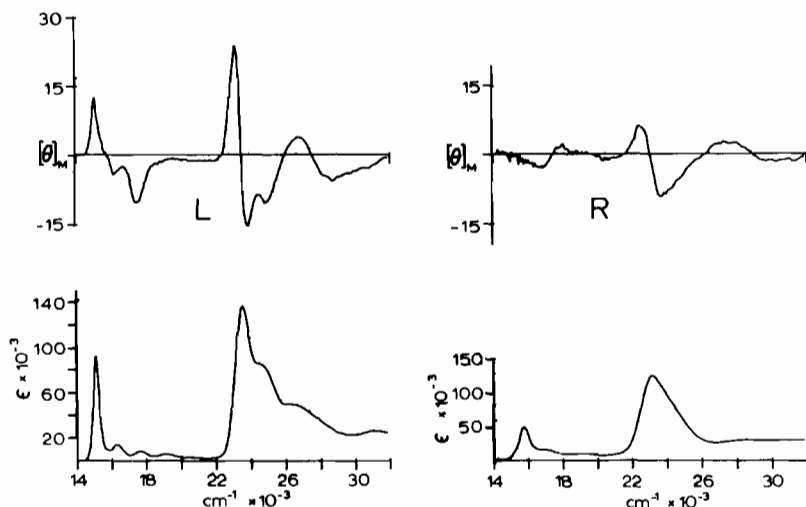
Figure 3. Room temperature MCD and optical spectra of Zn-pheophytin-*a* (L) and -*b* (R).

The red region (14,000–20,000  $\text{cm}^{-1}$ ) of each spectrum of this study contains the Q band of base-localized  $\pi \rightarrow \pi^*$  origin, and the blue region (ca. 21,000–30,000  $\text{cm}^{-1}$ ) contains the  $\pi \rightarrow \pi^*$  Soret, or B, band. These Q and B bands of  $D_{4h}$  porphyrins are considered [6, 9–13] to be the result of  $\pi \rightarrow \pi^*$  excitations  ${}^1A_{1g}(a_{1u}^2 a_{2u}^2) \rightarrow {}^1E_u^{(a)}(e_g^1 a_{2u}^1)$  and  ${}^1A_{1g}(a_{1u}^2 a_{2u}^2) \rightarrow {}^1E_u^{(b)}(e_g^1 a_{1u}^1)$ . In the lower symmetry substituted porphyrins, e.g.,  $D_{2h}$  point group, each degenerate state  ${}^1E_u$  splits into two states,  ${}^1B_{3u}(x)$  and  ${}^1B_{2u}(y)$ . The substitute labels for these states are, respectively  $Q_x$  (or  $B_x$ ) and  $Q_y$  (or  $B_y$ ) and reflect the linear polarizations of corresponding excitations ( $x$  and  $y$  are in the plane of the molecule, Fig. 1).  ${}^1E_u$  will similarly split into two bands,  $Q_x$  and  $Q_y$ , in chlorins. The comparisons of electronic spectra of either a  $D_{4h}$  porphyrin with a substituted one of  $C_{2v}$  (or

$D_{2h}$ ) symmetry of a  $D_{4h}$  porphyrin with a chlorin make it clear that the Q band splits up to ca. 2500  $\text{cm}^{-1}$  but the Soret, or B, band is split by a very small amount ( $\leq 500 \text{ cm}^{-1}$ ) which is *not* observed at room temperature.

MCD details of chlorins *a* and *b* are explored now. The lowest energy Q component, which is also the lowest energy band, is optically most intense for each form *a* or *b* molecule studied here (Figs. 2–6), and the MCD sign of this component is found to be positive. Therefore, based on results by Stephens, *et al.* [14] for low symmetry porphyrins we assign this component to  $Q_y(0 \rightarrow 0)$  ( ${}^1A_1 \rightarrow {}^1B_{2u}$ ), or  ${}^1A_1 \rightarrow {}^1B_2$  in  $C_{2v}$ .

Immediately on the blue side of this optically dominant  $Q_y(0 \rightarrow 0)$  of the chlorin-*a* spectra (Figs. 2–6), *i.e.*, chlorophyllide-*a*, chlorophyll-*a*, Zn-

Figure 4. Room temperature MCD and optical spectra of chlorophyllide-*a* (L) and -*b* (R).Figure 5. Room temperature MCD and optical spectra of Cu-pheophorbide-*a* (L) and -*b* (R).Figure 6. Room temperature MCD and optical spectra of Zn-pheophorbide-*a* (L) and Cu(II)-pheophytin-*b* (R).

pheophytin-*a*, and Zn,Cu-pheophorbide-*a*, are two negative MCD components, the most intense (higher energy one) of which is assigned to  $Q_x(0 \rightarrow 0)$ , or  ${}^1A_1 \rightarrow {}^1B_1$ , of parentage  ${}^1A_{1g} \rightarrow {}^1B_{3u}$ . However, the lower

energy negative band of less intensity is assigned to  $Q_y(0 \rightarrow 1)$ , or  ${}^1A_1 \rightarrow {}^1B_2$  ( ${}^1A_{1g} \rightarrow {}^1B_{2u}$ ), by analogy to the signs of Faraday B terms of low-symmetry, substituted porphyrins [14]. The observed opposite

sign of  $Q_y(0 \rightarrow 1)$  from  $Q_y(0 \rightarrow 0)$  is proposed to derive from a vibronically caused polarization difference, *i.e.*,  ${}^1A_{1g} \rightarrow {}^1B_{2u}(0 \rightarrow 0)$  and  ${}^1A_{1g} \rightarrow {}^1B_{2u}b_{1g}^y(0 \rightarrow 1)$  are  $y$ - and  $x$ -polarized with  $b_{1g}^y$  being one of the  $D_{2h}$  allowed modes [15] for  $0 \rightarrow 1$  transitions, and the parentage is assumed to dominate the chlorin spectra.

The optically dominant red Q band of each chlorin-*b* molecule has positive MCD activity in accord with identity  $Q_y(0 \rightarrow 0)$ , but the MCD spectra in the Q-band region of chlorin-*b* molecules differ from the chlorin-*a* analogs in at least one important detail. Whereas the three distinct low energy Q components ( $15000$ – $18000$   $\text{cm}^{-1}$ ) of chlorin-*a* molecules are positive ( $Q_y(0 \rightarrow 0)$ ), negative ( $Q_y(0 \rightarrow 1)$ ), and negative ( $Q_x(0 \rightarrow 0)$ ) (low to high energy) MCD peaks, the three distinct MCD peaks of chlorin-*b* molecules are positive, negative and positive (low to high energy, *e.g.*,  $15000$ – $18000$   $\text{cm}^{-1}$  in Zn-pheophytin-*b*, Fig. 3). The highest energy component of these three in chlorin-*b* is, due to its sign, assigned to  $Q_x(0 \rightarrow 1)$  of  ${}^1B_2({}^1B_{2u})$  vibronic symmetry. The middle (negative) MCD component is tentatively assigned to  $Q_x(0 \rightarrow 0)$ , or  ${}^1B_2({}^1B_{2u})$  electronic symmetry, in view of its relatively large intensity and negative sign [14].

To conclude, MCD is diagnostically quite advantageous in differentiating between chlorin-*a* and *b* forms, *i.e.*, the MCD is sensitive to this structural change at position 3 of ring II (Fig. 1).

The structure of the Soret band of chlorin-*a* between *ca.*  $22,000$ – $30,000$   $\text{cm}^{-1}$  also lends itself to some state assignments. These metal-chlorin-*a* molecules have three optical components at *ca.*  $23000$ ,  $24500$ ,  $26500$   $\text{cm}^{-1}$  on the broad band of this region. The first of these is of dominant peak height and contains  $C_{2v}$  components  $B_x(D_{2h}$  parentage  ${}^1A_{1g} \rightarrow {}^1B_{3u})$  and  $B_y$  (parentage  ${}^1A_{1g} \rightarrow {}^1B_{2u})$ ,  $D_{4h}$  origin  ${}^1E_u^{(b)}(0 \rightarrow 0)$  (*vide supra*), whose separation is not discernible in room temperature optical spectra. However, the optical peak is flanked immediately by positive (red side) and negative (blue side) MCD peaks in the manner of a false A term. These signs lead us to assign the state order as  $B_y(B_{2u}) < B_x(B_{3u})$  using the analogy of the model of low symmetry porphyrins [14].

The second component, or first blue shoulder, of this Soret band is at *ca.*  $1100$   $\text{cm}^{-1}$  toward higher energy from the first one, and the B term of this component creates a negative MCD band. This shoulder is tentatively assigned to  $B_x(0 \rightarrow 1)$ , involving an  $a_1$  vibration, in view, collectively, of the MCD sign and vibrational data [16–17]. The second shoulder is *ca.*  $2950$   $\text{cm}^{-1}$  toward the blue of the intense  $B(0 \rightarrow 0)$  origin, and the MCD of the component is positive. It can also be noted that optically this band is very intense for form *a* molecules ( $\text{CH}_3$  in position 3) and weak for form *b* mole-

cules (CHO in position 3). This band is tentatively assigned here to a  $B(0 \rightarrow 1)$  vibronic band in a non-totally symmetric vibration [16–18] of aliphatic  $\nu(\text{CH})$  origin, on the basis of the MCD sign and the other cited data. Matrix isolation or crystal spectra would improve all available data. It is noted that metal-free chlorins [3] of form *a* have similar MCD structure.

The structure of the Soret band of each chlorin-*b* molecule (Figs. 2–6) in the region  $21,000$ – $25,000$   $\text{cm}^{-1}$  is composed of two optical bands, a dominating one near  $22,000$   $\text{cm}^{-1}$  and a shoulder near  $24,000$   $\text{cm}^{-1}$ . It is again found that the dominating component,  $B(0 \rightarrow 0)$ , decomposes into two MCD bands in the manner of a false MCD Faraday A term with the positive lobe on the red side and the negative lobe on the blue side. The order of states is thus assigned to be  $B_y({}^1A_{1g} \rightarrow {}^1B_{2u}) < B_x({}^1A_{1g} \rightarrow {}^1B_{3u})$  and is the same order as found for the metal-chlorin-*a* molecules. If the weaker band near  $24,000$   $\text{cm}^{-1}$  is taken to be of origin  $B(0 \rightarrow 1)$  due to a vibration of  $a_1$  symmetry, then its MCD sign confirms it to be of origin  $B_x(0 \rightarrow 1)$ . Higher energy bands have repetitive MCD patterns for the five compounds of form *a* (Figs. 2–6) but their firm assignments are not possible now.

Only minor MCD spectral effects are evident on hydrolysis-removal of the phytol group at ring III, so that MCD and optical spectra of chlorophyll-*a*, -*b*, Zn-pheophytin-*a*, and Cu(II)-pheophytin-*b*, are very much like those of chlorophyllide-*a*, -*b*, Zn-pheophorbide-*a*, and Cu(II)-pheophorbide-*b*, respectively (Figs. 2–6).

The effects on MCD spectra upon changing only the coordinated metal ion are not very unusual, *viz.*, the comparisons of chlorophyll-*a* with Zn-pheophytin-*a*, of chlorophyll-*b* with Zn, Cu(II)-pheophytin-*b*, of chlorophyllide-*a* with Zn, Cu(II)-pheophorbide-*a*, and of chlorophyllide-*b* with Cu(II)-pheophytin-*b* should be made (Figs. 2–6). The differences of optical spectra were previously discussed [4, 5].

The MCD band intensities of the paramagnetic Cu(II)-pheophorbide-*a* we found to be constant in the temperature range  $23$ – $63$   $^\circ\text{K}$ . This implies that the Faraday C parameter is zero and the molecule has no angular momentum in its initial state of these  $\pi \rightarrow \pi^*$  transitions,

$$C(a \rightarrow j) = \frac{1}{2d_a} \sum_a \langle a | L + 2S | a \rangle \cdot$$

$$\text{Im}[\langle a | m | j \rangle \times \langle j | m | a \rangle]$$

all symbols having conventional meaning [18]. We conclude from this observation that the particular Cu(II) d-orbital (" $x^2 - y^2$ ") which as usual is assumed to contain the one unpaired electron of the molecule is not significantly interacting with the  $\pi$  orbitals

(of the base molecule) involved in the electronic excitations studied here.

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