

Circular Dichroism and Magnetic Circular Dichroism of the Chlorophyll and Protochlorophyll Pigments¹

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Abstract: From a comparison of the circular dichroism and magnetic circular dichroism spectra of several chlorophyll and protochlorophyll pigments and their pheophytins in the monomer form in diethyl ether, the following conclusions are drawn. (1) There is a direct relation between the sign of the natural optical activity and the polarization direction of the corresponding electronic transitions. (2) The contribution to the circular dichroism (CD) of the carboxymethyl substituent at carbon atom C-10 of the cyclopentenone ring V is of opposite sign compared to the contribution of the C-7 and C-8 substituents of ring IV. For the C-10 substituent, the CD contributions are positive in the y - and negative in the x -polarization directions, and the reverse for the C-7 and C-8 substituents (x through rings II-IV). (3) The substituents at C-10 and at C-7 and C-8 are probably the only ones responsible for the visible circular dichroism of these molecules. (4) Calculated rotational strengths, using the Kirkwood polarizability approximation and point transition dipole moments, give the appropriate signs for the C-10 and for the C-7 and C-8 groups; however, the calculated magnitudes are an order of magnitude too small. (5) Magnetic circular dichroism spectra give additional support to the assignments of the bands. The pigments containing magnesium form dimers in nonpolar solvents (carbon tetrachloride). Dimers of pyrochlorophyll *a*, which has the C-10 carboxymethyl substituent removed, appear to have essentially the same geometry as do those of chlorophyll *a*, chlorophyll *b*, chlorophyll *a'* (C-10 enantiomer of chlorophyll *a*), and bacteriochlorophyll, indicating that the carboxymethyl substituent at C-10 is not involved in dimer formation. On the other hand, from circular dichroism and absorption spectra it appears that the dimers of the protochlorophylls have a geometry significantly different from those of the chlorophylls or of bacteriochlorophyll. Structures for the dimers are proposed.

In an attempt to reach a better understanding of the relations between molecular structure and optical activity, investigations on the circular dichroism (CD) and the magnetic circular dichroism (MCD) of a series of chlorophyll and protochlorophyll pigments have been carried out. In order to relate the optical activity to the presence of the asymmetric centers in these molecules, protochlorophyll pigments, for which all the pyrrole rings form a conjugated macrocycle, were compared to a variety of chlorophyll pigments, where the C-7 to C-8 double bond is hydrogenated. The influence of the removal or reversal of the asymmetry at the C-10 carbon atom of the cyclopentenone ring has also been examined, as well as the effect of the removal of the magnesium.

Optical rotatory dispersion (ORD) and circular dichroism (CD) spectra for seven variously substituted free-base chlorins were reported by Wolf.³ He called attention to correlations between the substitution pattern of the rings and the CD spectral features. In particular, he noted a reversal in sign of the CD for the (0-0) vibronic components of the two electronic transitions of lowest energy, and a pronounced influence of the presence of asymmetrically placed substituents at carbon atom C-10, in the cyclopentenone ring V, on the CD intensities associated with these bands. Subsequently, Briat, *et al.*,⁴ published CD and magnetic circular dichroism (MCD) spectra for the same series of compounds. In intense magnetic fields (41.7 kG) the MCD signals have much greater magnitudes than the natural

CD for the same transitions. The theoretical framework for interpreting MCD spectra is still in a relatively primitive state. It is possible to relate the relative, but not the absolute, signs of the MCD associated with the vibronic band origins to the relative orientations of the corresponding transition moments, and to interpret qualitatively the relative magnitudes of the MCD features in terms of vibronic mixing with transitions of different symmetry. Dratz⁵ has provided an introductory approach to the interpretation of MCD spectra of porphyrins.

Wolf, *et al.*,⁶ examined the effect on the CD spectra of the configuration of the substituents at the asymmetric carbon atom C-10. They were able to confirm from their optical activity data that the 10-carbomethoxy group present in methyl pheophorbide *a* is in the *trans* configuration relative to the propionic acid side chain on C-7.

Any attempt to relate the optical activity to the molecular structure by the use of electronic absorption properties depends upon knowledge of the assignment of the various bands in the electronic absorption spectra. This analysis can often be made on the basis of studies of the fluorescence, fluorescence polarization, and linear dichroism, along with theoretical considerations. In the case of the chlorophyll pigments, the assignments of the principal absorption bands to the various electronic transitions and their vibrational overtones have already been made.⁷⁻⁹ We have extended

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this analysis to the protochlorophyll pigments in one of our studies.¹⁰ These assignments will be tabulated together with our CD and MCD data.

A theoretical framework exists by which it is possible to calculate rotational strengths for electronic transitions of a chromophore which is perturbed by nearby asymmetrically placed substituents. In a treatment originating with Kirkwood, the asymmetric perturbation is treated as an oscillator which couples with the allowed electric transition dipole moment to induce a magnetic moment parallel to the electric transition moment.¹¹ The imaginary part of the product of these two moments then gives the rotational strength for the transition. Kirkwood treated the asymmetric group as an anisotropic polarizability placed asymmetrically with respect to the chromophore. Tinoco¹² has refined this treatment by making it more explicit for application to interacting groups within a single molecule, and by extending it to include the contribution of magnetically allowed transitions.

If the assumption is made that each of the asymmetric centers acts independently on the transition moments of the various chlorophylls and related compounds, then it is possible to account for the observed signs and, qualitatively, the relative magnitudes of the rotational strengths observed. As an empirical interpretation, the effects of alterations in the asymmetry of the substitution at carbon atoms C-10 (in the cyclopentenone ring V) and at C-7 and C-8 (of ring IV) are found to be directly related to the signs and amplitudes of the CD bands. In this respect, the phenomenon is closely related to the octant rule developed for the asymmetrical cyclic ketones by Moffitt, *et al.*¹³ When the Kirkwood theory is used to calculate the rotational strengths of the clearly discernible CD bands, the results are somewhat less satisfactory. Although the calculation predicts the correct sign for the contributions of the asymmetrically placed groups, the calculated magnitudes are too small by an order of magnitude in comparison with experiment. This undoubtedly results largely from simplifications in the present model, where point dipole transition moments have been used to represent the chromophore absorption bands, and bond polarizabilities to represent the asymmetrically placed substituents. There are good indications that refined calculations will improve the quantitative agreement of calculations and experiments.

The formation of chlorophyll and bacteriochlorophyll aggregates gives rise to important modifications of the absorption and optical activity compared to the corresponding monomers.¹⁴⁻¹⁶ The typical behavior observed for chlorophyll dimers is a splitting of the CD bands corresponding to each electronic transition into two components of opposite sign¹⁵ concomitant with the splitting of the electronic absorption bands and a hyperchromism in the absorption.¹⁶ From these

studies and from nuclear magnetic resonance, infrared, and absorption spectra measurements,¹⁷⁻¹⁹ important characteristics of the structure of chlorophyll dimers in nonpolar solvents have been established. The chlorophyll and bacteriochlorophyll dimers are considered to result from interaction of the magnesium atom of one chlorophyll molecule with the C-9 ketone carbonyl group of the second. However, the CD data¹⁵ show that neither the chlorophyll planes nor the transition moments are parallel in the dimer.

Evidence was found from infrared studies in this laboratory of the protochlorophyll pigments in carbon tetrachloride¹⁹ that protochlorophyll *a* and its analog, 4-vinyl protochlorophyll *a* (VPChl *a*), aggregate as a result of interactions probably involving the same functional groups as is the case for the chlorophyll pigments. The comparative study of the aggregation of the chlorophyll and protochlorophyll pigments using the absorption and circular dichroism data, reported in this paper, show that significant differences in geometry probably exist between the protochlorophyll dimers, on the one hand, and the chlorophyll and bacteriochlorophyll dimers, on the other. This provides the basis for a postulate that the propionic ester substituent of C-7 on ring IV is directly involved in stabilizing and defining the dimer structures.

Experimental Section

Materials and Methods. Chlorophyll Pigments. Chlorophyll *a* (Chl *a*) was obtained from spinach leaves by the procedure of Anderson and Calvin.^{18,20} Pheophytin *a* (Pheo *a*) was prepared from 10 mg of Chl *a* by treatment of an ether solution of this pigment with 0.5% HCl, as described by Pennington, *et al.*²¹ The pyrolysis of Chl *a* in pyridine was carried out as described by Pennington, *et al.*²¹ The removal of the C-10 carbomethoxy group was confirmed by measurement of the infrared spectrum of the compound obtained (pyroChl *a*) in CCl₄ and in tetrahydrofuran. Finally, pyropheophytin *a* (pyroPheo *a*) was obtained from pyroChl *a* in the same way as Pheo *a* was obtained from Chl *a*.

Protochlorophyll Pigments. The isolation and purification of the protochlorophyll pigments from pumpkin seed coats have been described elsewhere.¹⁹ Two very similar compounds were obtained, in agreement with previous reports.²² One of these is the true photochlorophyll *a* (PChl *a*) and the other one is 4-vinylprotochlorophyll *a* (VPChl *a*), characterized by the presence of a second vinyl substituent on C-4 in place of the ethyl group. The optical properties of VPChl *a* compared to those of PChl *a* have been examined in two other papers.^{10,19} Here the discussion will be limited to comparison of the optical properties of chlorophyll pigments to those of PChl *a*, because the inclusion of VPChl *a* does not give additional information for the purpose of the present work. Protopheophytin *a* (PPheo *a*) has been also isolated and purified from pumpkin seed coats.¹⁹

We have made several attempts to remove the C-10 carboxymethyl group of PChl *a* by pyrolysis in pyridine under experimental conditions similar to those used for the chlorophyll pigments. The behavior observed is far from being clearly understood, but essentially it was found that ring V was easily altered, and under none of the experimental conditions investigated (from room temperature to 100°, in the presence and in the absence of air) was evidence of the removal of the C-10 carboxymethyl side chain found in the infrared spectrum. However, these pyrolysis experiments need to be in-

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vestigated in detail because, under some pyrolysis conditions, several reaction products were obtained and were partially characterized after their chromatographic separation. This will be considered further in the Results.

Solvents and Solutions. The solvents used were ether (Baker analyzed Reagent, anhydrous, peroxide content 0.00001%), tetrahydrofuran (Mallinckrodt, stabilized, peroxide content maximum 0.015%), and pyridine (Baker and Adamson, Reagent Grade), all used without further purification. It has been recently noted²³ that the monomer-dimer equilibrium of the chlorophyll pigments in carbon tetrachloride, over the 10^{-4} – 10^{-6} M concentration range studied by absorption spectra¹⁶ and optical rotatory dispersion,¹⁴ is influenced by the presence of traces of water in the carbon tetrachloride used. Katz, *et al.*, have emphasized the necessity of taking great pains to remove the last traces of water both from the chlorophyll pigments and from the solvent in order to eliminate the occurrence of the very stable chlorophyll hydrates.²⁴ Careful drying of the solvent resulted in the presence of almost pure dimers in the concentration range 10^{-4} – 10^{-6} M. Consequently, our measurements were carried out under the following experimental conditions. The solid chlorophyll and related compounds were stored under vacuum for at least 1 week at room temperature prior to spectral measurements. Carbon tetrachloride (Matheson Coleman and Bell, spectroquality reagent, water content maximum 0.02%) was dried for at least 2 days over molecular sieve (Linde, Type 4A, previously washed with water, dried, washed with CCl_4 , and activated at 200° under vacuum for 2–4 hr). The absence of water in this solvent was checked in the near-infrared bands at 1.89 and 2.6 μ ; the absorbance at 1.89 μ ($\epsilon_{\text{H}_2\text{O}}$ 3.73 l. mole⁻¹ cm⁻¹, ref 25) for 10-cm pathlength never exceeded 0.01 (before drying it was around 0.04–0.05), corresponding to an upper limit for the water content of 3×10^{-4} M. All the stock solutions and dilutions in this solvent were prepared under nitrogen, using volumetric flasks, pipets, and cuvettes previously dried at 100° under vacuum for 1–2 hr and allowed to cool under nitrogen. The dissociation of the dimers was produced by addition of 0.5% (v/v) of absolute ethanol to the pigment solutions in carbon tetrachloride.

All the manipulations of the pigment solutions were carried out under yellow light.

Optical Activity and Absorption Spectra Measurements. The visible and ultraviolet absorption spectra were recorded using a Cary 14 R spectrophotometer. The circular dichroism spectra were measured with a Cary 60 spectropolarimeter equipped with a CD attachment and a red-sensitive photomultiplier (S-20 response). Some CD spectra were also recorded with the instrument built in this laboratory by Dratz⁵ and with a Durrum-Jasco UV-CD instrument of the Durrum Instrument Corporation (Palo Alto, Calif.). The agreement among the data obtained with the three instruments was satisfactory. An aqueous solution of *d*-camphor-sulfonic acid (1 mg/ml) was chosen as the reference sample for each instrument ($\Delta A_{290 \text{ nm}} = 0.00932$ per cm or $\theta_{290 \text{ nm}} = 0.308$ deg cm⁻¹). The magnetic circular dichroism spectra were measured in the laboratory of Professor C. Djerassi (Stanford University, Palo Alto, Calif.) with the kind assistance of Dr. E. Bunnenberg, using a Durrum-Jasco spectropolarimeter modified for CD and MCD measurements.^{4,26} The magnetic field was directed axially and in the sense of propagation of the light; the field strength was 49,000 G. Several MCD spectra were also measured with the instrument built by Dratz⁵ and showed good agreement with the MCD spectra obtained with the Durrum-Jasco instrument. The CD and MCD spectra in ether (pigments present as monomers) will be given as the difference in extinction coefficient $\Delta\epsilon = \epsilon_L - \epsilon_R$ (l. mole⁻¹ cm⁻¹), and in molar ellipticity $\theta_M = 3300 \times \Delta\epsilon$ [in centidegrees cm⁻¹ (mole/l.⁻¹)]. In the case of the dimers in CCl_4 , the CD spectra will appear as a difference of absorbance $\Delta A = A_L - A_R$ and as ellipticity $\theta = 33.00 \times \Delta A$ (in deg cm⁻¹). The values of CD and MCD at the maxima of the bands also appear in the tables as the reduced circular dichroism $\Delta\epsilon/\epsilon = \Delta A/A$ (Kuhn's anisotropy factor), this variable having the advantage of being independent of concentration and extinction coefficient determinations, as well as indicating the sensitivity of the experimental measurement. All

of the MCD spectra have been corrected experimentally to remove the CD contribution to the signal observed. MCD values in the tables are given for the maxima of the MCD bands and are expressed in appropriate units per 49 kG of magnetic field.

The concentrations were calculated using the following millimolar extinction coefficients for the different pigments dissolved in ether: 86.3 at 660 nm for Chl *a*,^{21,27} 80 at 660 nm for pyroChl *a*,²¹ 61 at 667 nm for Pheo *a*,²¹ 49 at 667 nm for pyroPheo *a*,²¹ 22 at 622 nm for PChl *a*,¹⁰ and 14.5 at 565 nm for PPheo *a*.¹⁰

Results

Optical Activity of the Monomer Species. The CD and MCD spectra of the chlorophyll and protochlorophyll pigments and their pheophytins in ether over the 220–700-nm wavelength range are presented in Figures 1–4. The values of the CD and MCD for the principal bands are tabulated in Table I together with the assignments of the bands to the electronic transitions and their vibrational components.

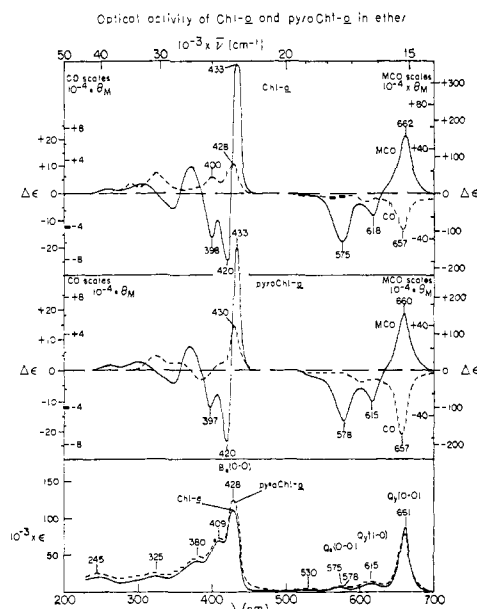


Figure 1. Optical activity (CD and MCD, upper and middle curves) and absorption (lower curve) spectra of chlorophyll *a* (Chl *a*) and pyrochlorophyll *a* (pyroChl *a*) in ether.

Several general observations can be made from an examination of these data. For each of the compounds studied the CD and the MCD spectra are qualitatively different from one another. In some cases a strong MCD band is observed to correspond to a weak or non-existent CD band (*e.g.*, the Q_x (0–0) bands of Chl *a* and pyroChl *a* in Figure 1 and of Pheo *a* in Figure 2). The nearly degenerate B bands (Soret bands) are more clearly resolved in the MCD than in the CD spectra for Chl *a* and pyroChl *a* (Figure 1) and their pheophytins (Figure 2). On the other hand, the longest wavelength component in the Soret region appears in the CD but not in the MCD spectra of PChl *a* (Figure 3) and PPheo *a* (Figure 4). The CD and MCD spectra complement one another in aiding the interpretation of excited state properties.

The CD and MCD spectra of Pheo *a* and pyroPheo *a* show qualitative agreement with the data of Briat, *et*

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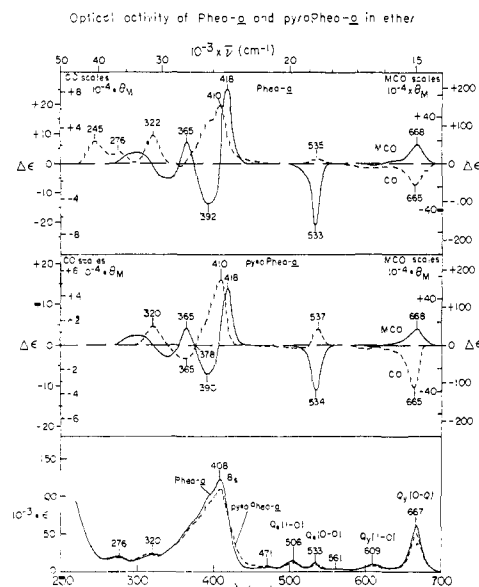


Figure 2. Optical activity and absorption spectra of pheophytin *a* (Pheo *a*) and pyropheophytin *a* (pyroPheo *a*) in ether; arranged as in Figure 1.

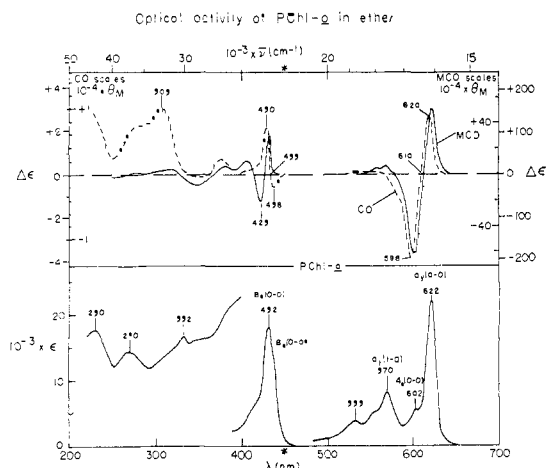


Figure 3. Optical activity (upper) and absorption (lower) spectra of protochlorophyll *a* in ether. Optical path 1 cm, except 1 mm in (*) region between 400 and 500 nm. In this case the 1-mm path was used for the MCD measurements at all wavelengths below 500 nm.

al.,⁴ on methyl pheophorbide *a* and pyromethyl pheophorbide *a*. This was expected since the asymmetric carbons in the phytol chain are expected to be too far from the π electron system of the porphyrin ring to induce any optical activity in these porphyrin electronic transitions. However, the values of ellipticity presented by Briat, *et al.*,⁴ are in general lower than the values obtained here (if the molar ellipticity is really in units of $\text{deg cm}^2 \text{decimole}^{-1}$, and not $\text{deg mole}^{-1} \text{cm}^2$ as given by these authors in their Table II). This may be due to a solvent effect (dioxane in their case, diethylether in this case) or to a possible partial epimerization of the substituents at C-10. Katz, *et al.*,²⁸ have shown recently, using nmr techniques, that this epimerization occurs within several hours at room temperature following dissolution of the solid pigments in tetrahydrofuran and

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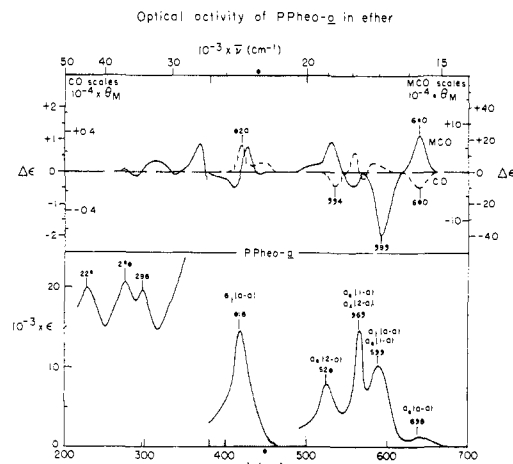


Figure 4. Optical activity and absorption spectra of protopheophytin *a* in ether. Optical path 1 cm, except 1 mm in (*) region between 400 and 500 nm.

several polar solvents. In the present studies, the CD spectra were uniformly measured within 1 hr after dissolution. Some solutions were measured again after standing for a few days at 0°, and no alteration in the optical activity was ever observed.

In the case of PChl *a* (Figure 3) both the red band and the Soret band show double CD and MCD bands corresponding to the *x* and *y* components of the electronic transitions. The red bands of PChl *a* exhibit the same signs for CD as for MCD; in particular, the Q_y (0-0) transition of PChl *a* shows a positive CD, whereas the corresponding CD band was negative for Chl *a* and pyroChl *a*. An analysis of the behavior of PPheo *a* is more difficult because its CD is much smaller in magnitude (Figure 4). However, one can see clearly that the longest wavelength transition [Q_x] has a negative CD, while the Soret band has a positive CD. The behavior in the 520–590-nm region is characterized by multiple CD bands that are difficult to assign. The MCD spectrum of PPheo *a* shows an intense negative band at 593 nm, which indicates that there is predominance of *y* polarization for this band. The fluorescence produced by excitation in this band is completely depolarized.¹⁰

Attempts to Pyrolyze Protochlorophyll. Upon the removal of the C-10 carbomethoxy group, it was found that the loss of the asymmetry at the C-10 carbon atom produces an increase of the CD intensities of the chlorophyll pigments (compare Figure 1a with Figure 1b and Figure 2a with Figure 2b). By contrast, the MCD is almost the same for the pyro compounds as for the parent compounds. This is in agreement with the observations of Briat, *et al.*,⁴ on methyl pheophorbide *a* and pyromethyl pheophorbide *a*. Attempts to perform the same pyrolysis reaction on the protochlorophyll pigments were unsuccessful. However, our observations on PChl *a* showed that after 2–4 hr of heating at 100° under vacuum in pyridine, a single broad band appeared in the infrared spectra around 1740 cm^{-1} in carbon tetrachloride, the visible absorption spectra was unchanged, and the optical activity disappeared almost completely. The behavior of a PChl *a* solution in pyridine at room temperature was followed with time over a period of 24 hr. The absorption spectrum of

Table I. Optical Activity of the Chlorophyll and Protochlorophyll Pigments in Ether

Absorption spectra			Optical activity	
λ_{\max} , nm	$\epsilon_{\max} \times 10^{-3}$, (mole/l.) ⁻¹ cm ⁻¹	Assignments	CD	MCD
Chl <i>a</i>				
661	86.3	Q _y (0-0)	-13.8 (657 nm)	+160 (662 nm)
615	12.6	Q _y (1-0)		-60.5 (618 nm)
575	6.8	Q _x (0-0)		-130 (575 nm)
530	3.4	Q _x (1-0)		
428	113	{B _x (0-0) B _y (0-0)}	+11.0 (428 nm)	+355 (434.5 nm) -177 (420 nm)
PyroChl <i>a</i>				
661	80	Q _y (0-0)	-21 (657 nm)	+156 (660 nm)
615	13.9	Q _y (1-0)		-75 (615 nm)
578	8	Q _x (0-0)		-135 (578 nm)
530	4.5	Q _x (1-0)		
428	123	{B _x (0-0) B _y (0-0)}	+14.7 (428 nm)	+333 (433 nm) -194 (420 nm)
Pheo <i>a</i>				
667	61	Q _y (0-0)	-7.9 (665 nm)	+48 (668 nm)
609	9.15	Q _y (1-0)		-4.9 (610 nm)
561	3.75	Q _y (2-0)		
533	12	Q _x (0-0)	+0.86 (535 nm)	-165 (532 nm)
506	13.8	Q _x (1-0)		
470	5.85	Q _x (2-0)		
408	123	{B _x (0-0) B _y (0-0)}	+20.3 (410 nm)	+190 (418 nm) -105 (390 nm)
PyroPheo <i>a</i>				
667	49	Q _y (0-0)	-10.5 (665 nm)	+37 (668 nm)
609	7.8	Q _y (1-0)		-6.5 (610 nm)
561	3.95	Q _y (2-0)		
535	9.9	Q _x (0-0)	+3.7 (537 nm)	-119 (534 nm)
507	12.1	Q _x (1-0)		
470	7.45	Q _x (2-0)		
408	109	{B _x (0-0) B _y (0-0)}	+17.25 (410 nm)	+149 (418 nm) -80 (388 nm)
PChl <i>a</i>				
622	22	Q _y (0-0)	+3.0 (624 nm)	+130 (622 nm)
602	5.35	Q _x (0-0)	-3.75 (600 nm)	-188 (601 nm)
570	8.05	Q _y (1-0)		+22.5 (568 nm)
556 (sh)	5.05	Q _x (1-0)?		+19.4 (557 nm)
533	3.75	Q _y (2-0)?		+8.15 (535 nm)
438	137	B _x (0-0)	-7.55 (438 nm)	
432	182	B _y (0-0)	+25 (432 nm)	{+91 (432nm) -65 (423 nm)}
PPheo <i>a</i>				
638	1.32	Q _x (0-0)	-0.50 (638 nm)	+22 (638 nm)
588	10.2	Q _y (0-0), Q _x (1-0)?	?	-40 (593 nm)
565	14.5	Q _y (1-0), Q _x (2-0)?	?	-9 (560 nm)
524	7.7	Q _y (2-0)?	?	+19 (530 nm)
432 (sh)	82.5	B _x (0-0)	?	
418	145	B _y (0-0)	+8.7 (418 nm)	{+153 (427 nm) -98 (410 nm)}

PChl *a* in pyridine in the 500–700-nm region showed three peaks at 548, 587, and 634 nm; the small band at 602 nm observed in ether was completely masked in pyridine by the main red band and did not appear even as a shoulder. The two CD bands of opposite sign (623 and 643 nm) were clearly present, but had much lower intensities compared to the values in ether. Apparently the amount of splitting of the red band into its *x* and *y* components is strongly dependent upon the solvent. For example, the Q_x band was not detectable in the absorption spectrum of PChl *a* in tetrahydrofuran and was only present as a shoulder in 0.5% ethanol-CCl₄ (Figure 7). The optical activity of the pyridine solution of PChl *a* was found to be dependent on whether air was present. In the presence of air, the CD of the pyridine solution disappeared completely after 24

hr at room temperature, while it was still present but noticeably decreased after the same period if the pyridine solution was prepared and kept under nitrogen. These preliminary observations show that the "pyrolysis" of PChl *a* occurs in a manner different from that of the chlorophyll pigments and needs further detailed investigations.

Circular Dichroism of the Dimer Species. The CD spectra of the Mg complexes in carbon tetrachloride are presented in Figures 5–7, along with the absorption spectra. For Chl *a* the result agrees with the CD spectrum published by Dratz, *et al.*,¹⁵ except in the region below 400 nm. Our curve crosses the zero axis at 386 nm, whereas the CD remained positive in this region in the earlier report. A base-line problem in the uv region for the data of Dratz, *et al.*, is probably the origin of this

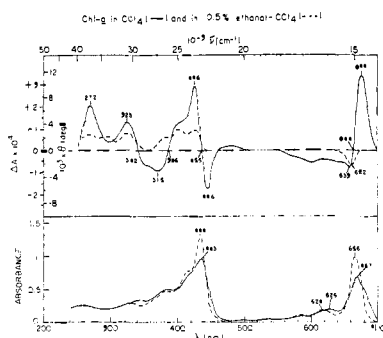


Figure 5. Circular dichroism and absorption spectra of chlorophyll *a* in CCl_4 (—) and in 0.5% ethanol- CCl_4 (---); path length 1 cm.

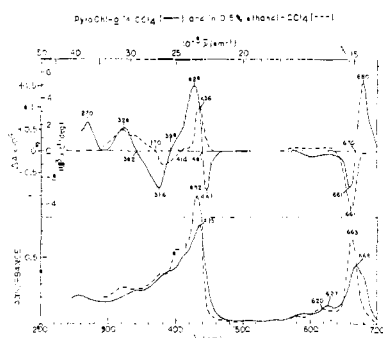


Figure 6. Circular dichroism and absorption spectra of pyrochlorophyll *a* in CCl_4 (—) and in 0.5% ethanol- CCl_4 (---); path length 1 cm.

discrepancy. The Chl *a*, pyroChl *a*, and PChl *a* spectra are characterized by the appearance of split components in the Q and B monomer absorption bands upon formation of the dimers, concomitant with hypochromicity of the dimer absorption compared to the monomer. The Q_y bands of Chl *a* and pyroChl *a* clearly show absorption shoulders on the long wavelength side, and the maxima are shifted slightly toward the red. In the Soret region, the band widths become larger and the maxima are also shifted slightly to longer wavelengths. On the other hand, the Q and B transitions of the aggregated PChl *a* are shifted to shorter wavelengths compared to the monomer. The Q transitions for PChl *a* in CCl_4 do not show any clear splitting in the absorption, but the band width is slightly increased. The B transition, however, shows a very distinct separation of components for the aggregate (436 and 455 nm, respectively).

The CD spectra of the aggregated chlorophylls provide clear evidence of additional splitting. In the case of Chl *a* and pyroChl *a* the Q_y bands exhibit positive CD components on their long wavelength sides, corresponding to the shoulders in the absorption bands (677 and 680 nm, respectively), cross zero near the absorption maximum (664 and 670 nm), and show negative CD bands on their short wavelength sides (659 and 661 nm). For the B transition, the behavior is reversed, with the negative CD band on the long wavelength side. In the case of PChl *a*, the situation is complicated by the fact that the *x* and *y* components are nearly degenerate for the Q and B transitions, and all of these components are optically active. Consequently, two double CD bands are expected in the red and two more in the blue region. The longest wavelength component (628 nm) is now

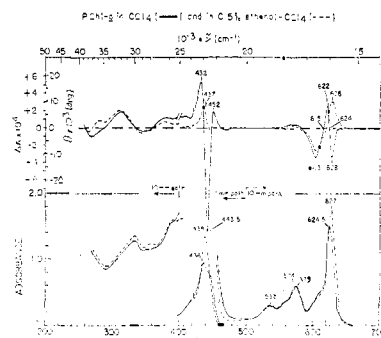


Figure 7. Circular dichroism and absorption spectra of protochlorophyll *a* in CCl_4 (—) and in 0.5% ethanol- CCl_4 (---); path length 1 cm.

negative, whereas it was positive both for Chl *a* and for pyroChl *a*. In the region of overlap between the *x* and *y* components, the effects add to each other. This probably explains the very intense negative CD band centered at 443.5 nm. The behavior observed for VPChl *a* was identical with that of PChl *a*.

In all cases, the addition of 0.5% ethanol to the carbon tetrachloride solution results in a hyperchromicity at the absorption maxima and a return to the characteristic monomer circular dichroism spectra observed in ether solutions.

Discussion

Assignments of the Absorption Bands. Most of the band assignments given in Table I derive from previous experimental studies of fluorescence polarization and dichroism^{7,8} and the theoretical studies of Gouterman.^{9,29} The polarizations can be related to a molecule-fixed axis system using the theoretical arguments. In some cases absolute assignments have been confirmed by measurements on single crystals³⁰ or on artificially oriented chlorophylls.³¹

For each of the pigments where the Q_y transition lies lowest in energy, the next electronic transition, Q_z , should be polarized nearly perpendicularly. In the absorption spectra (and to some extent the CD spectra) this region is complicated by overlap by higher vibrational components of the Q_y transition. These can generally be sorted out through examination of fluorescence polarization spectra and the determination of characteristic vibration frequencies. The vibrational components are generally of intermediate polarization, owing to mixing with higher electronic states. In difficult cases, the CD and MCD data can be of help in making the assignments.

Origin of the Magnetic Circular Dichroism. The theoretical arguments of Dratz describe two situations which are relevant to porphyrin MCD spectra.⁵ In the first case, where the long wavelength Q bands are degenerate (*e.g.*, for metal porphyrins or alkyl-substituted metal porphyrins where the π -electron system effectively has fourfold symmetry), the theory predicts a double MCD for the Q bands. The long wavelength component should be negative and the short wavelength component should be positive. This has been observed

(29) M. Gouterman, *J. Chem. Phys.*, **30**, 1139 (1959).

(30) B. G. Anex and R. S. Umans, *J. Amer. Chem. Soc.*, **86**, 5026 (1964).

(31) J. C. Goedheer, Ph.D. Thesis, University of Utrecht, 1957.

experimentally for a variety of alkyl-substituted metal porphyrins.^{5,32} The MCD sign convention used by Dratz for his *experimental* findings has been reversed in this paper. The convention used here is identical with that used by Briat, *et al.*,⁴ by Schooley, *et al.*,²⁶ by Feher, *et al.*,³² and in the *theoretical* derivations of Dratz.⁵

The second case treats porphyrin molecules where the fourfold symmetry is broken by substituents like carbonyl adjacent to the ring or by reduction of one or more of the tetrapyrrole rings. For these molecules the long wavelength absorption is split into Q_x and Q_y components. The theory predicts that the long wavelength Q component will always have a positive MCD, and the second Q component, which is polarized essentially perpendicular to the first, will almost certainly have negative MCD. This arises because a given transition obtains magnetic dipole intensity by borrowing from nearby transitions of *perpendicular polarization*. If the strongest interaction is with transitions to higher energy, then positive MCD results; if lower energy transitions predominate in the mixing, then negative MCD is produced. The longest wavelength transition therefore always will exhibit positive MCD, whether it is Q_x or Q_y . The second Q band lies close in energy to the first, and the third band (Soret) is usually polarized parallel to the second. Both of these factors favor a negative MCD for the second Q band, regardless of its absolute orientation. These principles appear to be valid for all of the seven chlorins studied by Briat, *et al.*⁴

The molecules in the class of alkyl-substituted porphyrin free bases appear to contradict the theoretical predictions of Dratz.⁵ Here the presence of the central protons splits the Q bands into separated x - and y -polarized components. Nevertheless, the long wavelength MCD component observed is negative for the molecules protoporphyrin IX,⁵ deoxophylloerythrin,³³ and mesoporphyrin.³³ The second Q component exhibits positive MCD for these molecules. The origin of the discrepancy between the theory and experiment is not clear. The presence of a carbonyl group at carbon atom C-9 of ring V is sufficient to reverse the pattern to that predicted for nondegenerate Q transitions, for the molecules phyloerythrin and pheoporphyrin a_3 exhibit the "normal" behavior.³³

The band assignments for the three chlorophylls and the three pheophytins that have been examined are entirely consistent with the MCD interpretation given by Dratz.⁵ Except for PPheo a , the longest wavelength bands are assigned as Q_y .^{7-9,29} They all have positive MCD bands, as expected. The Q_x bands (Q_y for PPheo a) occur at shorter wavelengths, and all exhibit negative MCD components. Furthermore, the absolute magnitudes of the Q_y and Q_x components in the MCD spectra are comparable, indicating that the Q_x band borrows most of its intensity from the nearby Q_y band (for PPheo a , reverse the subscripts x and y in this statement and the following sentence). Any borrowing from the B_y band would contribute in the opposite (*i.e.*, positive) sense to the MCD of the Q_x band.

For PPheo a , the longest wavelength band at 638 nm is assigned to Q_x , following the reasoning of Gouterman.²⁹ The absorption spectrum shows three addi-

tional pronounced bands at 588, 565, and 524 nm. Only the 638-nm band exhibits pronounced fluorescence polarization,¹⁰ which leaves the location of the origin of the Q_y band in doubt. Gouterman has assigned the Q_y (0-0) transition to the third component (565 nm), principally by analogy with other substituted porphyrins.²⁹ The second band of PPheo a (at 588 nm) is at the correct position for the Q_x (1-0) vibrational component, but it is proposed here that the Q_y (0-0) band is nearly coincident. Two pieces of evidence support this assignment: a strong negative MCD band occurs at 593 nm, as would be expected for the Q_y (0-0) component, and there is a marked discrepancy in the fluorescence-absorption mirror image relationship involving the relative intensities of the (0-0) and (0-1) components of the Q_x transition.¹⁰ This is in sharp contrast to the situation for PChl a ¹⁰ and many related molecules,³⁴ and suggests that the 588-nm band of PPheo a is about twice as intense in absorption as the Q_x (1-0) component should be. The near superposition at 588 nm of the Q_y (0-0) and the Q_x (1-0) bands would then account for the greater than normal width of the absorption band, the difference between the MCD center at 593 and the absorption maximum at 588 nm, and the mixed polarization of the fluorescence excitation spectrum in this region.¹⁰ By this assignment, the bands at 565 and 524 nm would consist of higher vibrational components of both transitions. Low-temperature spectra providing improved resolution would be helpful in settling this question more definitely.

In the Soret region the situation is complicated for all of these molecules by the small energy splittings of the mutually perpendicularly polarized B transitions. Nevertheless, the MCD spectra show a positive component to long wavelength for each of the six compounds studied, and this is followed closely by a negative component. From the analysis above, it is concluded that the B bands borrow magnetic dipole strength predominantly from one another rather than from the much more distant Q bands.

Origin of the Circular Dichroism. The CD spectra for the group of compounds studied show some distinct correlations with molecular structure, particularly for the Q bands. The relevant data are summarized in Table II. Apart from the presence or absence of Mg, the series of compounds have molecular structures which differ in two important respects: (1) the number and location of asymmetric substituents and (2) the extent of unsaturation of the tetrapyrrole rings. As is illustrated in Figure 8, Chl a contains substituents asymmetrically placed at ring carbons C-7, C-8, and C-10. PChl a has only the C-10 substituent asymmetry; and pyroChl a has only the C-7 and C-8 asymmetries, by virtue of the loss of the C-10 carboxymethyl substituent and its replacement by a proton upon pyrolysis. All of the molecules studied were esterified with phytol, and it is assumed that the distant asymmetric carbon atoms of the phytol group do not contribute significantly to the porphyrin CD. Back-coordination of the phytol chain or the carboxyl group could modify this picture, but at present there are no clear-cut experimental data available which can resolve the question.

The coupled oscillator model of Kirkwood is adopted in an attempt to rationalize the CD data.¹¹ Implicit in

(32) G. Feher, M. Malley, and D. Mauzerall in "Magnetic Resonance in Biological Systems," A. Ehrenberg, *et al.*, Ed., Pergamon Press, London, 1967, p 145.

(33) E. Bunnenberg, private communication.

(34) P. G. Seybold and M. Gouterman, *J. Mol. Spectrosc.*, **31**, 1 (1969).

Table II. Experimental Values of Absorption and Rotational Strengths (in Ether)

	PChl <i>a</i>	PyroChl <i>a</i>	Chl <i>a</i>	PPheo <i>a</i>	PyroPheo <i>a</i>	Pheo <i>a</i>
λ_{\max} , nm	Q_y 622	661	661	588, 565 (?)	667	667
ω_{\max} , cm^{-1}	Q_z 602	578	575	638	535	533
	Q_y 16,080	15,130	15,130	17,010, 17,700	14,990	14,990
$10^{-3}\epsilon_{\max}$, $(\text{mole/l.})^{-1} \text{cm}^{-1}$	Q_z 16,610	17,300	17,390	15,670	18,690	18,760
	Q_y 22	80	86.3	10.2, 14.5	49	61
Band width $\Delta\omega$, cm^{-1}	Q_z 5.35	8	6.8	1.32	9.9	12
	Q_y 325	420	390	570 ^a , 530	405	405
Oscillator strength $f = 4.33 \times 10^{-9} \int \epsilon(\omega) d\omega$	Q_z 450 ^a	780 ^a	760 ^a	440 ^a	490 ^a	490 ^a
	Q_y 0.033	0.155	0.155	0.027, 0.0355	0.091	0.114
Dipole strength $10^{18} \mu^2$, (esu cm) ²	Q_z 0.0110	0.029	0.024	0.0027	0.0225	0.027
	Q_y 4.35	21.7	21.7	3.34, 4.24	12.9	16.1
Molecular ellipticity $10^{-3}(\theta_M)_{\max}$, $10^{-2} \text{deg} (\text{mole/l.})^{-1} \text{cm}^{-1}$	Q_z 1.42	3.53	2.91	0.36	2.54	3.06
	Q_y +10	-69	-46	?	-35	-26
Rotational strength $10^{40} R_A$ (cgs)	Q_z -12.4			-1.65	+12.2	+2.85
	Q_y +1.48	-14.3	-8.7	?	-6.9	-5.2
$10^4 R_A / \mu^2 = 10^4 \Delta\epsilon_{\max} / \epsilon_{\max}$	Q_z -1.82			-0.34	+2.4	+0.55
	Q_y +1.36	-2.6	-1.60	?	-2.15	-1.30
$10^8 R_A / \mu^2 \omega_{\max} = 10^8 \Delta\epsilon_{\max} / \epsilon_{\max} \omega_{\max}$, cm	Q_z -7.0			-3.8	+3.7	+0.72
	Q_y +0.85	-1.73	-1.06	?	-1.43	-0.86
	Q_z -4.22			-2.42	+2.00	+0.38

^a Value estimated from CD or MCD spectrum.

this model is the assumption that each of the asymmetric centers acts independently on the transition moments. We expect to find that the observed rotational strengths can be accounted for by a sum of interactions associated with each significant asymmetric site on the

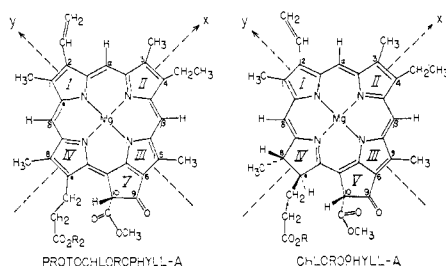


Figure 8. Molecular structures of protochlorophyll *a* and chlorophyll *a*, showing the absolute configuration of the asymmetrically placed ring substituents reported for chlorophyll *a*.^{33, 34}

porphyrin ring. This is observed quantitatively for the different compounds studied, once the rotational strengths have been normalized to adjust for the variations in absorption intensities (dipole strengths). Kuhn has suggested using an anisotropy factor, $\Delta\epsilon/\epsilon$, for this purpose, where $\Delta\epsilon = \epsilon_L - \epsilon_R$ is the difference in extinction coefficients for left and right circularly polarized light (obtained from the circular dichroism spectra) and ϵ is the molar extinction coefficient (obtained from the absorption spectrum).³⁵ In view of the theoretical treatment by Kirkwood, an additional correction should be applied to take into account the fact that corresponding transitions occur at different frequencies in the different compounds.¹¹ Using the Kirkwood polarizability approximation an expression for the rotational strength, R_A , can be written for a particular transition as

(35) W. Kuhn, *Trans. Faraday Soc.*, **26**, 293 (1930); see also W. Moffitt and A. Moscovitz, *J. Chem. Phys.*, **30**, 648 (1959).

$$R_A = \frac{\pi \omega_a \omega_0^2 \mu_{i0a}^2}{\omega_0^2 - \omega_a^2} \sum_{j=1}^N (\alpha_{33} - \alpha_{11})_j G_{ij} (\mathbf{e}_i \times \mathbf{e}_j) \cdot \mathbf{R}_{ij} \quad (1)$$

Following the notation of Tinoco,¹² μ_{i0a}^2 is the dipole strength of a transition at frequency ω_a (in cm^{-1}) and oriented in the direction of the unit vector \mathbf{e}_i ; $(\alpha_{33} - \alpha_{11})_j$ is the polarizability asymmetry of a polarizability ellipsoid representing the j th substituent and oriented with its symmetry axis along unit vector \mathbf{e}_j ; \mathbf{R}_{ij} is a vector joining the point transition moment μ_{i0a} to the center of the j th polarizability ellipsoid; G_{ij} is a factor depending only on geometry and defined by

$$G_{ij} = \frac{1}{|\mathbf{R}_{ij}|^3} \left[\mathbf{e}_i \cdot \mathbf{e}_j - \frac{3(\mathbf{e}_i \cdot \mathbf{R}_{ij})(\mathbf{e}_j \cdot \mathbf{R}_{ij})}{|\mathbf{R}_{ij}|^2} \right] \quad (2)$$

and ω_0 is the effective frequency representing all transitions at higher energies giving rise to the refractive index at frequency ω_a . For these compounds ω_0 will occur in the far-ultraviolet; therefore, the quantity $\omega_0^2/(\omega_0^2 - \omega_a^2)$ will be of the order of unity and will differ insignificantly from one compound to another in the series. The summation is carried out for all substituents which are asymmetrically placed with respect to the transition dipole moments. If we group on the left those terms which characterize the transition and on the right those which depend only on the substituents and their geometry, we obtain

$$\frac{R_A}{\mu_{i0a}^2 \omega_a} \cong \pi \sum_{j=1}^N (\alpha_{33} - \alpha_{11})_j G_{ij} (\mathbf{e}_i \times \mathbf{e}_j) \cdot \mathbf{R}_{ij} = \pi \sum_{j=1}^N F_{ij} \quad (3)$$

Experimentally, the rotational strength is obtained as the area of the circular dichroism band³⁶

$$R_A = \frac{3hc(2303)}{32\pi^3 N_0 \omega_a} \int \Delta\epsilon(\omega) d\omega \quad (4)$$

(36) See L. Velluz, M. Legrand, and M. Grosjean, "Optical Circular Dichroism," Academic Press, New York, N. Y., 1965; A. Moscovitz in "Optical Rotatory Dispersion," C. Djerassi, Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1960, Chapter 12.

where h is Planck's constant, c is the velocity of light, and N_0 is Avogadro's number; or, with appropriate values of the constants

$$R_A = \frac{22.96 \times 10^{-40}}{\omega_a} \int \Delta\epsilon(\omega) d\omega \quad (\text{cgs}) \quad (4')$$

The dipole strength is obtained from the area of the absorption band

$$\mu_{0a}^2 = \frac{3hc(2303)}{8\pi^3 N_0 \omega_a} \int \epsilon(\omega) d\omega \quad (5)$$

$$= \frac{91.8 \times 10^{-40}}{\omega_a} \int \epsilon(\omega) d\omega \quad (\text{esu cm})^2 \quad (5')$$

Assuming that the shapes of the corresponding absorption and CD bands are similar, the quantity on the left in eq 3 can be expressed directly in terms of experimental quantities

$$\frac{4R_A}{\mu_{0a}^2 \omega_a} = \frac{\Delta\epsilon}{\epsilon\omega_a} = 4\pi \sum_{j=1}^N F_{ij} \quad (6)$$

where $\Delta\epsilon$, ϵ , and ω_a are measured at the maxima of the bands. As pointed out by Moffitt and Moscovitz,³⁵ the factor 4 is a historical accident. Equation 6 gives the modification of the Kuhn anisotropy factor referred to above and is the measure by which the contributions of asymmetrically placed substituents should properly be related to the optical rotation. Values for this anisotropy factor for the compounds studied are given in Table II (bottom row) for each transition where a well-defined CD component was observed. Treating the anisotropy factor simply as an experimental parameter, we observe good additivity relations for the Q_y transitions of PChl *a*, pyroChl *a*, and Chl *a*. Here the contribution of the C-10 asymmetry in PChl *a* is expected to add to that from the C-7 and C-8 asymmetry in pyroChl *a* to give that for Chl *a*, where all of these asymmetries are present together. The value of -0.88×10^{-8} cm calculated from the first two compounds is in good agreement with the observed value of -1.06×10^{-8} cm for Chl *a*. Unfortunately, this cannot be tested further for the Q_x bands, because the corresponding CD components cannot be distinguished for pyroChl *a* and Chl *a*. It is worth noting that an implied conclusion from this correlation is the assignment of the long wavelength transition of PChl *a* as y -polarized. To our knowledge, this absolute assignment has not previously been made.

The corresponding pheophytins are expected to give a similar relationship. Both the Q_y and Q_x bands are well defined for pyroPheo *a* and Pheo *a*. The anisotropy factors (Table II, bottom row) for the Q_y bands, in particular, have the same signs and similar magnitudes to those for the corresponding transitions of pyroChl *a* and Chl *a*. Using these values for pyroPheo *a* and Pheo *a*, we calculate values by difference for PPheo *a* of $+0.57 \times 10^{-8}$ cm for Q_y and -1.62×10^{-8} cm for Q_x . The CD spectrum of PPheo *a* clearly shows that the long wavelength transition at 638 nm has a negative CD band with an observed anisotropy factor of -2.42×10^{-8} cm, which is consistent with its assignment as x -polarized.²⁹ The Q_y transition is expected to have positive CD and it has been assigned to the 588-nm band (*vide supra*). If this assignment of Q_y for PPheo *a* should prove correct, it will be necessary to alter Gouterman's perturbation

elements for the ring V substituent of the free-base porphyrins.²⁹

In the case of the pheophytins, the additivity of the anisotropy factors is not so good as for the chlorophylls. It may be necessary to consider other contributions, such as the nonplanarity of the porphyrin ring system, in order to resolve these discrepancies.

The reversal in the order of the energies of the Q_x and Q_y transitions in PPheo *a* and PChl *a* is not too surprising. The gap between Q_x and Q_y is already small in PPheo *a*. The presence of a central magnesium atom constitutes a strong perturbation on the substituted porphyrin system. It operates in the direction of reducing the elements leading to twofold symmetry in the porphyrin free-base and toward the fourfold symmetry in the metal complexes of unsubstituted or symmetrically substituted porphyrins. It would appear that the effect of the substituents (primarily ring V) on the magnesium porphyrin is to lower the energy of the Q_y transition relative to that of Q_x . It should be interesting to examine the spectra of the series of differently substituted magnesium porphyrins, as Gouterman has done for the free-base analogs.²⁹

Calculation of Rotational Strengths. A useful check on the relevance of eq 3 to the role of substituents in inducing optical activity in porphyrins and metal porphyrins can be made by calculating the anisotropy factor from the terms on the right-hand side. First, we associate a polarizability anisotropy ($\alpha_{33} - \alpha_{11}$) with each covalent bond in each asymmetrically placed substituent. This serves to distribute the polarizability anisotropy in space, and should give a better estimate of the interactions than would a point polarizability for each group. Furthermore, the necessary bond polarizability data exist in the literature. It is necessary to average over orientations for freely rotating groups.

From recent experimental structure determinations, the absolute configuration at each of the asymmetric carbons in Chl *a*^{37,38} and in BChl³⁹ is known (Figure 8). We will assume that the configurations for the pheophytins and also the configuration at C-10 for PChl *a* and PPheo *a* are the same. Furthermore, a recent crystal structure determination for vanadyl deoxophylloerythroetioporphyrin⁴⁰ provides us with position coordinates for the porphyrin carbon atoms that take into account the distortion introduced by the presence of the five-membered ring (V). Using these coordinates to establish the location of the atoms in the porphyrin core, we can then use published bond lengths and angles to locate the centers of the bonds for each substituent. All single-bonded groups are assumed to undergo free rotation, with the exception of the carboxymethyl group at C-10. Here, molecular models indicate that free rotation of the large carboxyl group is severely hindered by the nearby propionic ester at C-7. Consequently, we have taken an average for the two orientations where the plane of the carboxyl atoms is perpendicular to the porphyrin plane. In an additional simplification for purposes of calculation, we have replaced the C-7 propionic ester by an ethyl substituent. This

(37) I. Fleming, *Nature*, **216**, 151 (1967); *J. Chem. Soc., C*, 2765 (1968).

(38) H. Brockmann, Jr., *Angew. Chem. Intern. Ed.*, **7**, 221 (1968).

(39) H. Brockmann, Jr., *ibid.*, 222 (1968).

(40) R. C. Pettersen and L. E. Alexander, *J. Amer. Chem. Soc.*, **90**, 3873 (1968).

Table III. Polarizable Group Parameters and Anisotropy Factor Calculations

Bond	Polarizability $10^{24}(\alpha_{33} - \alpha_{11}), \text{cm}^3$	Position, Å			Direction of principal axis			Distance, Å R	Distance, Å		$(\alpha_{33} - \alpha_{11})G_{ij}$ $(\mathbf{e}_i \times \mathbf{e}_j) \cdot \mathbf{R}_{ij}$ $\times 10^{10}, \text{cm}^{-1}$	
		x	y	z	cos α	cos β	cos γ		G_{xz}	G_{yz}	F_{zj}	F_{yj}
C-10 Carboxymethyl, H												
C(10)-C	0.71	-3.70	-3.76	-0.63	-0.572	-0.050	-0.819	5.31	+0.352	+0.7385	-0.762	+1.399
C=O	1.24	-4.75	-3.85	-1.21	-0.992	-0.087	+0.087	6.23	+0.339	+0.572	-0.324	-0.604
C-O	0.96	-3.88	-3.78	-1.81	-0.421	+0.037	-0.906	5.71	+0.1925	-0.0125	-0.214	-0.561
O-C	-0.26 ^a	-4.81	-3.85	-1.15	-0.984	-0.086	+0.156	6.28	+0.2815	+0.510	+0.130	+0.011
CH ₃	-0.084 ^a	-3.81	-3.77	-1.86	+0.483	+0.042	-0.874	5.67	+0.192	-0.0496	+0.046	-0.005
C(10)-H	-0.312	-5.76	-3.94	-1.00	-0.984	-0.086	+0.156	7.05	+0.300	+0.344	-0.310	+0.612
		-3.35	-3.73	-2.70	+0.483	+0.042	-0.874	5.69	+0.362	+0.134	-1.434	+0.852
		-6.04	-3.96	-0.96	-0.984	-0.086	+0.156	7.29	+0.286	+0.330		
		-3.22	-3.72	-2.93	+0.483	+0.042	-0.874	5.72	+0.390	+0.176		
		-3.57	-3.75	+0.45	-0.572	-0.050	+0.819	5.20	+0.326	+0.736		
C-7 Ethyl, H												
C(7)-C(7')	0.71	-4.79	-1.03	+0.63	-0.467	-0.333	+0.819	4.94	+0.128	+0.0478	+0.508	-0.124
C(7')-C(7'')	-0.237 ^a	-5.27	-1.37	+1.47	-0.467	-0.333	+0.819	5.64	+0.885	+0.1112	-0.132	+0.096
C(7')-H	2(0.103) ^a	-5.23	-1.34	+1.41	-0.467	-0.333	+0.819	5.58	+0.901	+0.1084	+0.117	-0.081
C(7'')H ₃	-0.109 ^a	-5.39	-1.45	+1.68	-0.467	-0.333	+0.819	5.83	+0.815	+0.1153	-0.056	+0.046
C(7)-H	-0.312	-4.68	-0.95	-0.45	-0.467	-0.333	-0.819	4.80	+1.160	+0.0201	+0.228	-0.023
C-8 Methyl, H												
C(8)-C(8')	0.71	-4.79	+1.03	-0.63	-0.467	+0.333	-0.819	4.94	+1.128	-0.0478	+0.508	-0.124
C(8')H ₃	0.311 ^a	-5.23	+1.34	-1.41	-0.467	+0.333	-0.819	5.58	+0.901	-0.1084	+0.176	-0.123
C(8)-H	-0.312	-4.68	+0.95	+0.45	-0.467	+0.333	+0.819	4.80	+1.160	-0.0201	+0.228	-0.023
											+1.577	-0.356

^a Projection on principal axis.

propionic ester should be freely rotating; the polarizability anisotropy of the carboxyl group and the phytol chain (averaged over all orientations) should be quite small, and they are relatively far from the transition dipole moment. These factors all suggest that the approximation is a reasonably good one. We assume that the porphyrin ring atoms lie in a plane and have taken no account of the puckering of the ring, which will be different for the Mg and H₂ derivatives, compared with the vanadyl.

Some of the input parameters and the results of the calculations are given in Table III. There is considerable variation in the selection of bond polarizability values available in the literature. The anisotropy values that are listed in Table III for the C-H,⁴¹⁻⁴³ C-C,^{44,45} and C=O^{45,46} bonds are judged to be the best currently available, although the sign of $\Delta\alpha$ for C-H is still subject to question. The opposite choice would lead to the prediction of a negative sign for the sum of the F_{yj} terms for the substituents at C-10 (Table III), which is not in agreement with the observation for PChl *a* (Table IV). The value for $\Delta\alpha$ for the C-O single bond was calculated from the polarizability values for dimethyl ether⁴⁵ using a C-O-C bond angle of 110°. For the carboxyl groups the bond lengths and angles for methyl acetate were employed.⁴⁷

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Table IV. Comparison of Experimental and Calculated Anisotropy Factor

Asymmetry centers	Transition	Experimental $10^8 \Delta\epsilon/\epsilon\omega_a, \text{cm}$		Calculated $10^8 4\pi \sum_j F_{ij}, \text{cm}^a$
C-10	PChl <i>a</i>	PPheo <i>a</i>		
	Q _y	+0.85		+0.107
	Q _z	-4.22	-2.42	-0.180
C-7, C-8	pyroChl <i>a</i>	pyroPheo <i>a</i>		
	Q _y	-1.73	-1.43	-0.045
	Q _z		+2.00	+0.198
C-7, C-8, C-10	Chl <i>a</i>	Pheo <i>a</i>		
	Q _y	-1.06	-0.86	+0.062
	Q _z		+0.38	+0.018

^a From Table III.

The calculated values of $4R_A/\mu_{0a}^2\omega_a$, obtained from eq 3 and summarized in Table IV, are all at least an order of magnitude smaller than the experimental quantities. In general, the predominant terms contributing to the sums at the right in eq 3 come from the bonds closest to the porphyrin ring system. This is primarily because the averaging over orientations dramatically reduces the effective polarizability anisotropy of freely rotating groups, and partly because the interaction falls off roughly with the inverse square of the distance from the transition moment. It is difficult to see how any of the simplifications introduced in the nature of the substituents used for the calculation could be responsible for a discrepancy so large. The most serious shortcoming of the model is, in the opinion of the authors, in the use of a point dipole to represent the electric transition moments and, to a lesser extent, the assumption that these lie along the *x* and *y* axes of the porphyrin framework. Although the transition lengths involved

are all shorter than 1 Å (in Table II, a transition moment $\mu^2 = 23 \times 10^{-36}$ esu² cm² corresponds to a transition length of 1 Å), the π -electron cloud extends to the edge of the porphyrin ring and quite close to the asymmetric substituents. If the transition is described in terms of transition monopoles located on each atom of the porphyrin ring, we might expect to obtain more realistic values for the rotational strengths. Because of the inverse square dependence on distance, the distributed point monopole treatment should increase the absolute magnitudes of the rotational strengths, in general, relative to the point dipole model. Woody and Tinoco have carried out calculations using point transition monopoles for the nucleic acid helix and have achieved appreciably better agreement between theory and experiment than we have here.⁴⁸ We hope to carry out the refined calculation for the porphyrins in the near future.

Disregarding the discrepancies in magnitude between the experimental and calculated values, it is noted that the signs of the rotational strengths are calculated correctly by the model for the PChl *a* and pyroChl *a* molecules. In other words, it does seem to give the correct sense of the contribution for the C-10 asymmetry, on the one hand, and that at C-7 and C-8, on the other. The relative magnitudes of these two contributions are apparently not calculated correctly, for the sign of the calculated R_y for Chl *a* is opposite to the experimental value. It is difficult to anticipate whether a point monopole treatment will remove this particular discrepancy.

Bacteriochlorophyll. The principles involved in the group additivity method of correlating rotational strengths can be extended. For example, the absolute configuration of BChl has been determined.³⁹ The configurations at C-7, C-8, and C-10 are the same as those for Chl *a*, and from the known configurations at C-3 and C-4 the rotational strength contributions can be predicted, using the Kirkwood polarizability model. The C-3 and C-4 substituents give rise to terms with approximately the same magnitudes, but with reversed signs relative to the terms from the C-7 and C-8 substituents. As a consequence, these terms all cancel, and only the C-10 contributions remain. Thus, it is predicted that the anisotropy ratios for BChl and BPheo will be very close to those for PChl *a* and PPheo *a*.

Chlorophyll *a'*. In a recent report, Katz, *et al.*,²⁸ have assigned the structure of Chl *a'* as the enantiomer of Chl *a* at C-10. We measured the optical rotatory dispersion (ORD) spectra of Chl *a'* and pyroChl *a* several years ago⁴⁹ and determined that the negative Cotton effects for the long wavelength Q_y bands for both compounds were very similar in shape but larger in magnitude than are those for Chl *a*. The ORD spectra of Chl *a'* and pyroChl *a* resemble one another closely and have identical signs throughout the visible and near-uv spectra, but the intrinsic magnitudes of all of the peaks and troughs were greater than for Chl *a*. These results are consistent with the assignment of Chl *a'* as the enantiomer at C-10 of Chl *a* and serve to emphasize the usefulness of rotation measurements in the determination of structure. Wolf, *et al.*,⁵⁰ have reported an analogous behavior for both the ORD and CD spectra

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(50) H. Wolf, H. Brockmann, Jr., I. Richter, C. D. Mengler, and H. H. Inhoffen, *Ann. Chem.*, **718**, 162 (1968).

of an extensive series of enantiomeric derivatives of methyl pheophorbide in which the C-10 proton is replaced by alkoxy substituents, both with and without the carboxymethyl group on C-10. They did not study the methyl pheophorbide *a'*, which would be the analog of Chl *a'*, nor were they able to measure the Q_y transitions because of instrument limitations, however.

Chlorophyll Dimers. In order to understand the CD spectral features that appear when pigment molecules such as the chlorophylls form strongly interacting dimers or higher aggregates, a new source of optical rotation must be considered. The oscillators associated with corresponding transitions in the different molecules will now couple with one another. This leads to a splitting of each of the excited states in the array (into two for dimers, three for trimers, etc.) and produces CD components associated with each of the new transitions. The interaction energy (spectral splitting), transition dipole moments, and rotational strengths resulting from aggregation depend on the geometric relationship of the transition moment of one molecule to that (those) of its neighbor(s), and not at all on its own intrinsic (monomer) rotational strength.^{15,51} The experimental CD curves of dimers of optically active molecules will have both monomer (intrinsic) and dimer (interaction) components. These are additive, in a zero-order approximation, and they can be sorted out because of their very different characteristic shapes. In a real molecular aggregate the two effects are not completely separable, because the presence of neighboring like molecules will be expected to alter the magnitude of the intrinsic CD of the participating components. This will be analogous to a solvent or medium effect and should affect only the nondegenerate (single) CD component. In practice this can be subtracted out by empirical methods in order to give a "symmetrical" degenerate or exciton residual.¹⁵

The degenerate exciton interaction for dimers in solution can be analyzed for the point dipole transition model using the equations of Tinoco⁵¹

$$D_{\pm} = \mu^2 \pm \mathbf{u}_1 \cdot \mathbf{u}_2 = D_a \pm \mathbf{u}_1 \cdot \mathbf{u}_2$$

$$R_{\pm} = \mp \pi \omega_a (\mathbf{R}_{12} \cdot \mathbf{u}_1 \times \mathbf{u}_2)$$

$$\omega_{\pm} = \omega_a \pm V_{12}/hc$$

$$V_{12} = \frac{1}{|\mathbf{R}_{12}|^3} \left[\mathbf{u}_1 \cdot \mathbf{u}_2 - \frac{3(\mathbf{R}_{12} \cdot \mathbf{u}_1)(\mathbf{R}_{12} \cdot \mathbf{u}_2)}{|\mathbf{R}_{12}|^2} \right]$$

where the dimer dipole strengths (D_{\pm}), rotational strengths (R_{\pm}) and absorption frequencies (ω_{\pm} in cm⁻¹) of the two exciton components result from monomer transitions at ω_a ; \mathbf{u}_1 and \mathbf{u}_2 are the electric dipole transition moment vectors of the two molecules, respectively, in the dimer; \mathbf{R}_{12} is a vector connecting the centers of the two molecules in the dimer; and V_{12} is the interaction energy resulting in the exciton splitting. The application of these equations to solution dimers of Chl *a*, Chl *b*, and BChl is discussed by Dratz, *et al.*¹⁵ In particular, it can be seen that the intrinsic rotational strength of the monomers does not enter into the calculation. On the other hand, the relative orientation of the transition moments of the component molecules will have a pronounced effect on the observables by virtue of their dependence on the vector products.

(51) I. Tinoco, Jr., *Radiat. Res.*, **20** 133 (1963).

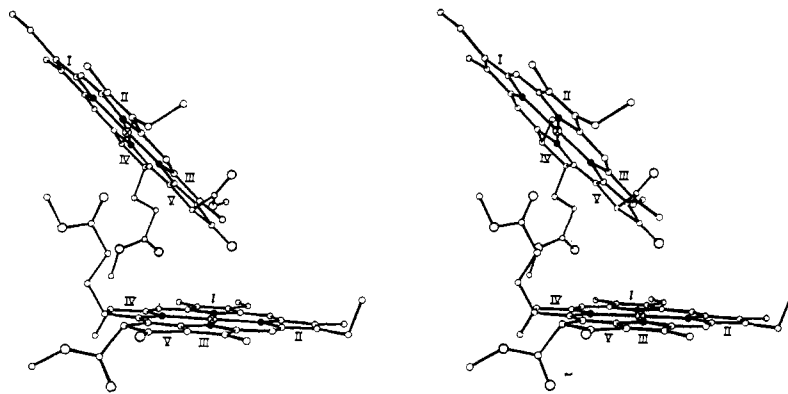


Figure 9. Chlorophyll *a* dimer, proposed structure, in stereoscopic projection. For identification purposes, the atoms shown are distinguished as \circ carbon, \bullet nitrogen, \circ oxygen, and \otimes magnesium. The C-9 carbonyl oxygen of the upper molecule is placed 3.0 Å from the Mg of the lower molecule, and the carbonyl oxygen of the propionic ester at C-7 of the lower molecule is 3.0 Å from the Mg of the upper molecule.

Figures 5–7 show the absorption and CD spectra of Chl *a*, pyroChl *a*, and PChl *a* in pure carbon tetrachloride (solid curves). Each compound exhibits spectral features which undergo dramatic alterations upon dissociation of the aggregates by the addition of a small amount of a polar solvent (0.5% ethanol, dashed curves). The spectra of Chl *a* in CCl_4 have been interpreted in terms of the occurrence of dimers.^{15,16,20,21} From the detailed studies of Katz and coworkers, the molecules are thought to be oriented so that the C-9 carbonyl group of one molecule interacts with the central magnesium of the other. The CD data show that the porphyrin rings do not lie in parallel planes, but that one molecule is inclined with respect to the other.¹⁵ As outlined above, the geometrical arrangement is sensitively reflected by three observable parameters associated with the Q_y and Q_x transitions: (1) the energy of interaction giving rise to the exciton splitting of each monomer excited state into two electronic states in the dimer, (2) the relative intensities of the two resulting transitions in the absorption spectra, and (3) the rotational strength (including the sign) of the associated double CD. It is clear from comparison of Figures 5 and 6 that these three parameters are quite similar for Chl *a* and pyroChl *a*, especially when one corrects for the somewhat different intrinsic (monomer) contributions to the CD spectra by the two molecules. In both cases the split Q_y band exhibits a long wavelength shoulder in the dimer absorption spectrum and a positive double CD.¹⁵ Examination of the short wavelength regions of the dimer CD spectra for Chl *a* and pyroChl *a* in Figures 5 and 6 shows a very close correspondence between the two, even though the corresponding monomer spectra are quite dissimilar. We take this evidence to indicate marked similarities in the dimer structures of Chl *a*, pyroChl *a*, Chl *b*, and BChl. The inclusion of pyroChl *a* in this list is particularly significant, in that it does not possess a carboxymethyl substituent on the C-10 carbon. Katz has proposed a dimer (monohydrate) structure for Chl *a* which involves a bridging water molecule that is hydrogen-bonded both to the C-9 carbonyl and to the carbonyl of the C-10 substituent.²⁴ If this latter is determinative, it might be expected that the pyroChl *a* dimer would have a significantly different structure. The spectral evidence suggests that the structures of Chl *a* and pyroChl *a* are,

in fact, quite similar. ORD spectra of Chl *a'* dimers support this conclusion.⁴⁹ Both the absorption and ORD spectra of Chl *a'* in dry CCl_4 possess the same characteristic features throughout the visible and near-uv regions as do the corresponding spectra of Chl *a* and pyroChl *a*, but upon addition of 0.5% methanol the solvated monomer species exhibit quite disparate ORD spectra. Since Chl *a'* is almost certainly enantiomeric at C-10 compared to Chl *a*, this suggests that neither the stereochemistry nor even the presence of the carboxymethyl substituent on C-10 has a significant influence on the dimer structure.

In the case of PChl *a*, where ring IV is not reduced, the CD spectrum of the aggregated species is quite different. As shown in Figure 7, the longest wavelength component of the CD is negative for the aggregated PChl *a* (solid curve) whereas it was positive for each of the other molecules. In the blue region also there appears to be a complete reversal in sign, but comparisons involving this complicated region of the spectrum are difficult. The absorption spectrum of PChl *a* in CCl_4 shows little, if any, evidence of splitting of the long wavelength (Q_y) band, but the presence of a double CD with a crossing at the absorption maximum suggests that there are at least two absorption components present. The addition of 0.5% ethanol to the PChl *a* solution in CCl_4 dramatically alters the CD and gives a spectrum (Figure 7, dashed curve) that closely resembles the monomer species in ether. In all respects the spectral properties of VPChl *a* (4-vinylprotochlorophyll *a*) are virtually indistinguishable from those of PChl *a*.

Since the sign of the double CD component of a dimer for a given transition should depend on the relative orientation of the corresponding transition moments of the component molecules, we are led to the conclusion that PChl *a* has a significantly different dimer structure from that of Chl *a* and the other molecules, assuming that the PChl *a* aggregated species is, in fact, a dimer. A major difference in molecular structure between PChl *a* and the other molecules is that the first C–C bond of the propionic ester substituent at C-7 lies in the porphyrin plane for PChl *a*, but is at a nearly tetrahedral angle for the others. This group is farther from the proposed point of interaction at C-9 than is the C-10 carboxymethyl group, and it is difficult to see from molecular

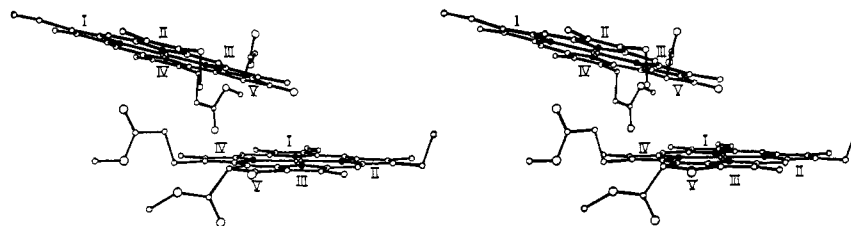


Figure 10. Protochlorophyll *a* dimer, proposed structure, in stereoscopic projection. Atom designations and positioning as in Figure 9.

models how this particular change in structure could directly affect the C-9 carbonyl to magnesium interaction. It could conceivably act indirectly through altered crowding of the C-10 substituent, but we have already presented evidence that in the chlorophylls the C-10 carboxymethyl group is not strongly involved in the dimer structure.

An alternative hypothesis is suggested on the basis of an observation by E. A. Dratz. Preliminary calculations by him and one of the authors (K. S.), applying a point dipole transition moment model for the dimers of BChl and using the equations of Tinoco,⁵¹ resulted in a *calculated* dimer structure in which the C-9 carbonyl of one BChl molecule was directed at the central Mg of the second molecule. The calculated angle of inclination of the two rings was such that the carbonyl group of the C-7 propionic ester side chain of the second molecule could rotate into a position from which it could interact from below with the Mg atom of the first molecule of the dimer. Such a two-point interaction would stabilize the three-dimensional geometry of the dimer in solution—a requirement, if the spectral properties are to correspond to a unique dimer structure rather than to an average over many conformations of a flexible dimer, whose components are joined by a single link or universal joint. The narrowness and relative simplicity of the CD spectral bands of the dimers, in particular, demand a rigid dimer structure. Now, in the case of PChl *a* the possible orientations of the propionic ester are significantly different from those in the molecules where ring IV is saturated. Examination using molecular models indicates that invoking stabilization by the propionic ester carboxyl group of PChl *a* appreciably alters the relative orientation of the two component molecules in a dimer. Reproductions of computer plots of the proposed dimer structures for Chl *a* (alternatively, Chl *b*, pyroChl *a*, Chl *a'*, BChl) and for PChl *a*

(alternatively, VPChl *a*) are shown in stereoscopic projections in Figures 9 and 10, respectively. The interplanar angles are about 45° for Chl *a* and its analogs and about 14° for PChl *a*.

Conclusion

The nature of chlorophyll aggregates in solution is interesting because of the information these structures shed on the state of chlorophyll in photosynthetic lamellae. In general, the photosynthetic materials show evidence in their CD spectra of strong exciton interactions among the pigment molecules. These features are distinctly different from those of the dimers in solution, showing that these dimers as such do not occur *in vivo*, or at least are not the predominant forms. Generally, the CD spectra of photosynthetic membranes have more complex structures, exhibit larger magnitudes of molar ellipticity, and are associated with more pronounced wavelength shifts in the absorption spectra. By applying the available theoretical models mentioned in this report to the analysis of the spectral properties of the monomers and dimers in solution, we hope to develop and evaluate methods of applying the optical measurements to the analysis of pigment interactions and molecular arrangements *in vivo*.

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