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OPTICAL PROPERTIES OF THE PROTOCHLOROPHYLL PIGMENTS

II. ELECTRONIC ABSORPTION, FLUORESCENCE, AND CIRCULAR DICHROISM SPECTRA

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SUMMARY

An analysis is presented of the electronic absorption spectroscopic properties of the protochlorophyll pigments by means of the fluorescence excitation and emission spectra, fluorescence polarization, and circular dichroism. From the fluorescence emission and polarization spectra, assignments of the visible absorption bands to four electronic transitions and their vibrational components are made, and their relative orientations are deduced. The main red and blue bands of the protochlorophyll pigments show parallel polarization to each other, in contrast with the case of the chlorophylls where they are perpendicular. On the other hand, the first vibrational overtone of the red band presents a mixed x–y polarization. The circular-dichroism spectra correlate with the fluorescence polarization data, the red and blue regions each showing two circular-dichroism bands of opposite signs.

The results are discussed in connection with the theoretical data available for the porphyrin pigments.

INTRODUCTION

The importance of a detailed physicochemical study of the protochlorophyll pigments has been emphasized in the previous paper describing their preparation and characterization[1]. Here we present an analysis of the optical properties of these pigments, using fluorescence, fluorescence polarization, and circular-dichroism spectroscopy, in order to assist in the assignment of the electronic transitions and the description of significant properties of the excited states.

Most of what was known about the properties of the protochlorophyll pigments before 1965 has been summarized by BOARDMAN[2]. The published optical properties essentially consist of the absorption spectral data of KOSKI AND SMITH[3], and the fluorescence spectra of FRENCH and co-workers[4,5]. BYSTROVA AND KRASNOVSKII (refs. 6 and 7) have examined the fluorescence spectra of protochlorophyllide *a*, protochlorophyll *a* and protopheophytin *a* in various solvents, as adsorbates and as films,

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in order to clarify the question of the state of the protochlorophyll pigments in the intact leaves. They concluded from their data that the active form of protochlorophyll pigment in the leaves (associated with protein) has a 'quasi-pheophytin' structure resulting from a displacement of the magnesium atom from the center of the molecule. Observation of a decrease in the fluorescence intensity of protochlorophyllide *a* in a nonpolar solvent has been used by SELISKAR AND KE[8], together with spectral and light-scattering changes, as an indication of aggregate formation. However, an important discrepancy with earlier work appears in the fluorescence spectra of the last authors: the characteristic second emission band at long wavelength is missing. Preliminary fluorescence polarization data have been presented by GOEDHEER[9] and show an almost constant polarization value through the blue and red bands when measured for the long wavelength emission band, making impossible the assignment of the relative polarizations of the different bands. However, GOEDHEER suggests that the polarization spectrum might differ for the two emission bands, as has been reported for tetraphenylporphin[10]. This appears to be true for both protochlorophyll *a* and protopheophytin *a* from the two values of fluorescence polarization ($p = 0.42$) published by GURINOVICH *et al.*[11], from measurement by excitation in the red bands (622 nm for protochlorophyll *a* and 638 nm for protopheophytin *a*) looking in the first emission band (630 nm and 653 nm for protochlorophyll *a* and protopheophytin *a*, respectively); but no fluorescence polarization spectra were presented.

As far as we know, besides the studies of JONES[12–15] and ours[1], no analysis has been made of the optical properties of protochlorophyll *a* compared to those of its analogue, 4-vinylprotochlorophyll *a* (alternatively 'bacterial' protochlorophyll, or Mg–2,4-divinylpheoporphyrin a_5), found in Cucurbitaceae seed coats.

Some infrared, nuclear magnetic resonance and optical rotatory dispersion spectral data have been presented for protopheophytin *a*, methyl protopheophorbide *a*, and protochlorophyll *a* prepared from pheophytin *a* by chemical reactions[16]. From the absence of any detectable Cotton effect for the magnesium-free compounds, it was concluded that they occur as diastereomeric mixtures.

MATERIALS AND METHODS

The protochlorophyll pigments were prepared as described in the first paper[1]. The ether used for the solutions was Baker Analyzed Reagent anhydrous ether (peroxide content 0.00001 %) without further purification.

The visible- and ultraviolet-absorption spectra were recorded using a Cary 14R spectrophotometer. The circular-dichroism spectra were measured with a Cary-60 spectropolarimeter equipped with a Model 6001 CD attachment and a red-sensitive photomultiplier (Hamamatsu, R-136). The circular-dichroism data are presented in ellipticity, Θ (in degrees), in difference of absorbance, $\Delta A = A_L - A_R$, and tabulated as reduced circular dichroism, $\Delta\varepsilon/\varepsilon$ (Kuhn's anisotropy factor). The absorption and circular-dichroism spectra were analyzed using a curve resolver (Dupont, Instrument Products Division, Model 310) for the decomposition of the various overlapping bands, and precise determinations of the oscillator strengths (f). A gaussian distribution was chosen for each of the component bands and gave a good agreement between synthesized curves and the experimental curves.

Fluorescence and fluorescence polarization spectra were recorded using an Aminco–Bowman spectrophotofluorometer. Fluorescence emission and excitation spectra were measured in ether. The emission spectra were corrected for the wavelength dependence of the photomultiplier sensitivity (RCA 7102, S-1 response), but not for variations in the monochromator efficiency. Different types of viscous solvents were used for the determination of the fluorescence polarization: castor oil, Nujol mineral oil, and Squibb extra heavy mineral oil. They gave identical results. Those reported here were obtained using the Squibb mineral oil. The solutions were prepared by adding mineral oil to a concentrated stock solution of chlorophyll in ether so that the final absorbance was near 0.4–0.5 per cm (separately for the strong peaks in the 350–500 nm and for the weak ones in the 500–650-nm spectral regions). About 2 % by vol. of ether was maintained in the mineral oil solution to prevent aggregation of the pigments. It is known that the aggregation of the protochlorophyll[8] and chlorophyll[9] pigments in nonpolar solvents results in a substantial decrease of the fluorescence efficiency. This is not a problem for the magnesium-free compounds, which do not aggregate nearly so strongly.

The polarization data are given as $p = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$, where $I_{\parallel}$ and $I_{\perp}$ are the intensities of the light emitted at 90° with respect to incidence, for polarization directions, respectively parallel and perpendicular to the polarization of the exciting light. Looking at the optical arrangement used in the measurements (Fig. 1) where E and B represent the polarization directions respectively perpendicular to and in the plane formed by the incident and emitted beams, one can see that the light intensities I(BE) (incident polarization B – emission polarization E) and I(BB) each correspond to $I_{\perp}$ and should be equal, as pointed out by GOUTERMAN AND STRYER[17]. In fact, the emission monochromator transmits with different efficiency the light polarized in the E and B polarization directions (as is the case for any dispersion monochromator) and produces a certain 'artificial' polarization not due to the fluorescence polarization. This is characterized by a ratio $N = I(\mathrm{BB})/I(\mathrm{BE})$, which depends on the wavelength setting of the emission monochromator ($N = 1.5$ at 630 nm; $N = 1.7$ at 685 nm, in our case). Consequently, I(EE) will be lowered by a factor N from its true value $I_{\parallel}$, and the correct fluorescence polarization will be given by $p = (N \times I(\mathrm{EE}) - I(\mathrm{EB}))/(N \times I(\mathrm{EE}) + I(\mathrm{EB}))$.

In order to verify the purity of our protochlorophylls and to calculate oscillator strengths, we need to know the extinction coefficients of the pigments. For protochlorophyll *a*, BOARDMAN[2] calculated the molar extinction coefficients from the data of KOSKI AND SMITH[3], using a molecular weight of 891.5. However, it has been now demonstrated[18] that the protochlorophyll from etiolated leaves, used by KOSKI AND SMITH[3], is not esterified with phytol. The calculation should have been made using a molecular weight of 597, with which we obtain the following molar extinction coefficients for protochlorophyllide *a* in ether: $(22.0 \pm 0.2) \cdot 10^3$ l · mole^{-1} · cm^{-1} at 622 nm and $(180 \pm 4) \cdot 10^3$ at 432 nm. Our determination of the extinction coefficient of 4-vinylprotochlorophyll *a* (phytyl ester) in ether gave a value for the 622-nm band ($22.1 \cdot 10^3$) so close to the values obtained for protochlorophyllide *a* and protochlorophyll *a* that we chose to use the same mean value ($22 \cdot 10^3$) for the three pigments at this absorption maximum. The extinction coefficients for the blue band were observed to be $183 \cdot 10^3$ at 432 nm for protochlorophyll *a* and $205 \cdot 10^3$ at 437 nm for 4-vinylprotochlorophyll *a*. In the case of protopheophytin *a*, the extinction coefficients were

taken from the data of KOSKI AND SMITH[3] and FRENCH[4] for protopheophorbide *a*: $\varepsilon_{565\ nm} = 14.5 \cdot 10^3$ and $\varepsilon_{417\ nm} = 145 \cdot 10^3$ $l \cdot mole^{-1} \cdot cm^{-1}$.

RESULTS

Absorption and fluorescence

The absorption spectra appear in Fig. 2 for protochlorophyll *a* and 4-vinylprotochlorophyll *a* and in Fig. 3 for protopheophytin *a*, in parallel with the fluorescence data. The positions of the bands (in nm and in cm^{-1}) and their oscillator strengths (f) are presented in Table I, together with fluorescence polarization and circular-dichroism data. The f values were estimated from the half-widths and amplitudes of the bands using the approximate formula $f = 4.33 \cdot 10^{-9} \cdot \varepsilon \cdot (1.0645) \cdot \Delta\tilde{\nu}$ for the calculations[19], where ε is the molar extinction coefficient at the maximum (in $l \cdot mole^{-1} \cdot cm^{-1}$) and $\Delta\tilde{\nu}$ is the band width at half-maximum (in cm^{-1}).

For protochlorophyll *a* and 4-vinylprotochlorophyll *a*, the fluorescence emission spectra (Fig. 2), showing bands at 630 and 685 nm in ether solution, were independent of the excitation wavelength. Although the fluorescence spectra are not mirror symmetrical to the red part of the absorption spectra, as already pointed out by GOEDHEER[9] for protochlorophyll *a*, the intensity ratio of the 630- to the 685-nm emission bands appears to be connected to the intensity ratio of the 620- to the 570-nm absorption bands. Moreover, the separations between the two fluorescence bands and the two absorption bands are almost equivalent, $\Delta\tilde{\nu}$ approx. 1300 cm^{-1}. In agreement with the observations of GOEDHEER[9], the fluorescence polarization spectra do not show any important dependence on the excitation wavelength, when measured in the long wavelength fluorescence band at 685 nm (Fig. 2, open circles). On the other hand, clear differences among the polarizations of the excitation bands appear when one looks in the short wavelength fluorescence band at 630 nm (Fig. 2, filled circles). The two protochlorophyll pigments show very similar behavior. The main red-absorption bands show polarization nearly parallel to the emission at 630 nm. The small band around 600 nm arises from a transition with a different orientation, but the decrease in polarization in this region is not sufficiently large to attribute a perpendicular orientation to the transition. The decomposition of this region of the spectrum using Gaussian functions shows very little contribution to the absorption at 600 nm by the neighboring bands; however, additional weak vibrational components of the Q_y band having mixed polarization probably underlie this region. For this reason the oscillator strengths assigned to the Q_x transitions are upper limits only. In the absorption spectrum of 4-vinylprotochlorophyll *a*, the 600-nm band appears only as a shoulder, and the measured change in polarization for the 600-nm band is correspondingly less well defined for 4-vinylprotochlorophyll *a* than for protochlorophyll *a*. In the Soret region, despite the strong overlap of the two component bands, one can conclude that the main blue band and its shoulder around 440 nm exhibit polarizations nearly parallel and perpendicular, respectively, to that of the first emission band.

Protopheophytin *a* also shows two fluorescence bands (Fig. 3), the long wavelength one being the most intense. The 588- and 638-nm absorption bands correspond to a separation of 1300 cm^{-1}, almost equivalent to the separation of the 653- and 713-nm fluorescence bands.

TABLE I

OPTICAL PROPERTIES OF THE PROTOCHLOROPHYLL PIGMENTS

λ (nm)	$\tilde{\nu}$ (cm^{-1})	$\Delta\tilde{\nu}$ (cm^{-1})		$10^{-3} \times \varepsilon$***	Band width (cm^{-1})	f		Fluorescence polarization** p	α	Assignments	Circular dichroism* ($\Delta\varepsilon/\varepsilon \times 10^4$)
Protochlorophyll a											
*Absorption spectra**											
622	16 077			22	325	0.033	(0.037) §	+0.42	∥	Q_y (0–0)	+1.35
602	16 611		1467	5.35	555	0.0137		+0.05 ~ ⊥		Q_x (0–0)	−7
570	17 544	1375		8.05	555	0.0206		+0.14	Random	Q_y (1–0)	
556 (sh)	17 986		1218	3.2	400	0.0059		+0.17		Q_x (1–0)?	
533	18 762			2.7	(620)	0.0077		+0.07		Q_y (2–0)?	
440 (sh)	22 727			~ 62	360	0.103	(0.81) §	−0.02	⊥	B_x (0–0)	−0.55
432	23 148			~ 155	590	0.42		+0.23	∥	B_y (0–0)	+1.38
*Emission spectra**											
630	15 873	1274								Q_y (0–0)	
685	14 599									Q_y (0–1)	
4-Vinylprotochlorophyll a											
*Absorption spectra**											
622	16 077			20	350	0.032		+0.32	∥	Q_y (0–0)	+1.3
605 (sh)	16 529		1406	4.75	735	0.016		+0.07 ~ ⊥		Q_x (0–0)	−7.9
572	17 483	1424		10.75	520	0.026		+0.10	Random	Q_y (1–0)	
557	17 953		1174	4	420	0.0077		+0.13		Q_x (1–0)?	
536	18 657			3.7	860	0.0147				Q_y (2–0)?	
444 (sh)	22 523			86	480	0.19		−0.06	⊥	B_x (0–0)	−0.72
437	22 883			175	605	0.49		+0.20	∥	B_y (0–0)	+0.83
*Emission spectra**											
630	15 873	1274								Q_y (0–0)	
685	14 599									Q_y (0–1)	

Protopheophytin a

*Absorption spectra**

638	15 674	} 1333	1.32 §	~ 560 §	(0.0034) §	+0.42	∥	Q_x (0–0)	−3.75
588	17 007		10.2 §	~ 865 §	(0.041) §	+0.07	Random	Q_x (1–0) Q_y (0–0)	
565	17 699	} 692	14.5 §	~ 530 §	(0.035) §	+0.05		Q_x (2–0) Q_y (1–0)	
524	19 084	} 1385	7.75 §	~ 875 §	(0.031) §	+0.1		Q_y (2–0)	
435 (sh)	22 988					+0.12			
418	23 923		145 §	~ 1400 §	(0.93) §	+0.03 ~ ⊥		B	+0.60

*Emission spectra**

653	15 314	1289		Q_x' (0–0)
713	14 025			Q_x' (0–1)

* Solvent: ether.

** Solvent: 2% ether – Squibb mineral oil. Polarization (p) and orientation (α) deduced from the emission in the lowest wavelength emission band.

*** In $l \cdot mole^{-1} \cdot cm^{-1}$; these values correspond to the resolved bands (except for protopheophytin *a*), and are often slightly lower than the extinction coefficient in the initial absorption spectra.

§ Value estimated directly from the unresolved spectra.

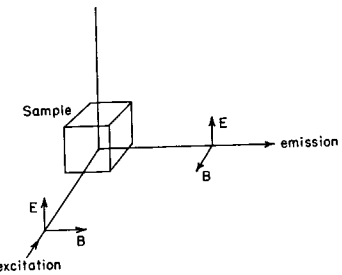

Fig. 1. Optical arrangement for the measurement of fluorescence polarization.

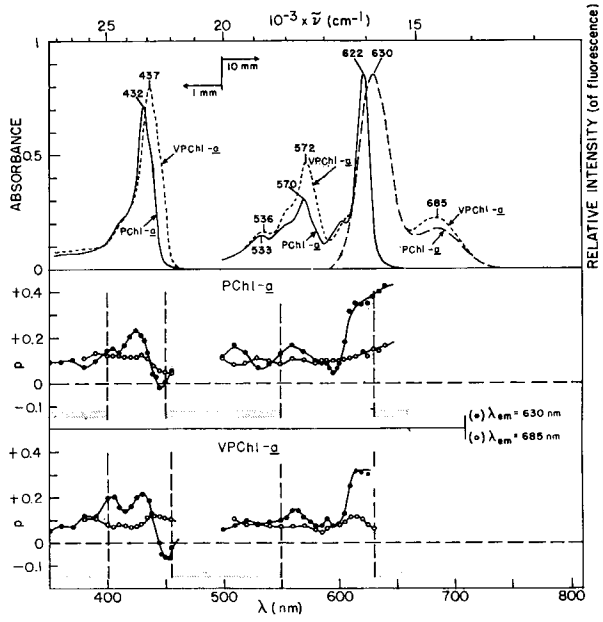

Fig. 2. Absorption and fluorescence spectra (in ether), and fluorescence polarization spectra (in mineral oil) of protochlorophyll *a* (PChl-*a*) and 4-vinylprotochlorophyll *a* (VPChl-*a*). The absorption peaks at 622 nm for the two compounds coincide, as do the emission peaks at 630 nm. Optical path lengths were 10 mm for wavelengths longer than 500 nm, and 1 mm for wavelengths shorter than 500 nm, as indicated at the top of the figure. Wavelength regions indicated by cross-hatching have polarizations of lesser accuracy, owing to low sensitivity.

The fluorescence polarization data of protopheophytin *a* (Fig. 3) show a behavior suggested by GOEDHEER[9] and similar to that of tetraphenylporphin[10]. When measured for the long wavelength fluorescence band, the polarization spectrum does not show any important variation with wavelength; on the other hand, the shorter wavelength fluorescence band exhibits a distinct wavelength dependence. The band at 638 nm exhibits parallel polarization with respect to the first emission band. For the other bands the variations in the fluorescence polarization are not clearly defined, and assignments cannot be made on the basis of fluorescence polarization alone. The absence of distinct variations in the polarization spectrum of protopheophytin *a* is somewhat surprising, because the component bands in the absorption spectrum appear to be at least as well defined as in the case of protochlorophyll *a*.

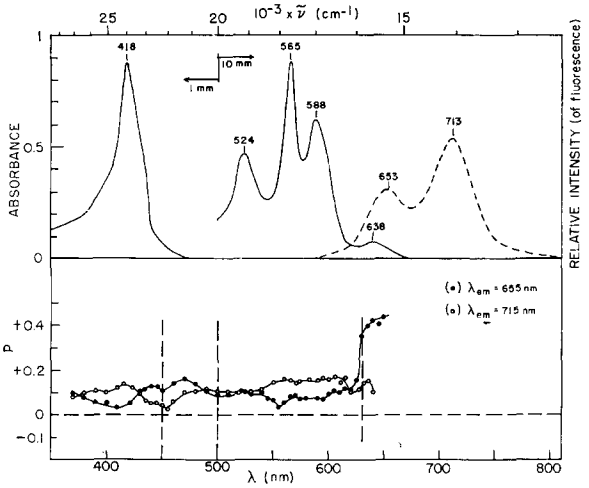

Fig. 3. Absorption and fluorescence spectra (in ether), and fluorescence polarization spectra (in mineral oil) of protopheophytin *a*. Wavelength regions indicated by cross-hatching have polarizations of lesser accuracy, owing to low sensitivity.

The values of *p* at 622 nm for protochlorophyll *a* and 4-vinylprotochlorophyll *a* and at 638 nm for protopheophytin *a* for the short wavelength emission band are in good agreement with the published values of GURINOVICH *et al.*[11].

Circular dichroism

The circular-dichroism spectra of protochlorophyll *a* and 4-vinylprotochlorophyll *a* in ether solutions are given in Fig. 4. The protochlorophylls show two circular-dichroism bands of opposite sign in each of the principal red and blue absorption regions. The absorption band of small intensity around 600 nm displays a very intense negative circular-dichroism signal, and the shoulder around 440–445 nm in the blue band appears as a small negative circular-dichroism component. There is a clear correlation between the sign of the circular dichroism and the direction of the transition dipole moment, as indicated by the fluorescence polarization data (Table I). Two important points differentiate the circular-dichroism spectra of protochlorophyll *a*

and 4-vinylprotochlorophyll *a*: (a) the negative circular-dichroism band at 440 nm is relatively more intense for 4-vinylprotochlorophyll *a* than for protochlorophyll *a*, resulting in a decrease of the positive circular-dichroism maximum at 430–435 nm; (b) 4-vinylprotochlorophyll *a* shows a clear negative circular-dichroism band in the region of 350–360 nm, while protochlorophyll *a* has no circular-dichroism band or a very small negative circular-dichroism band in this region.

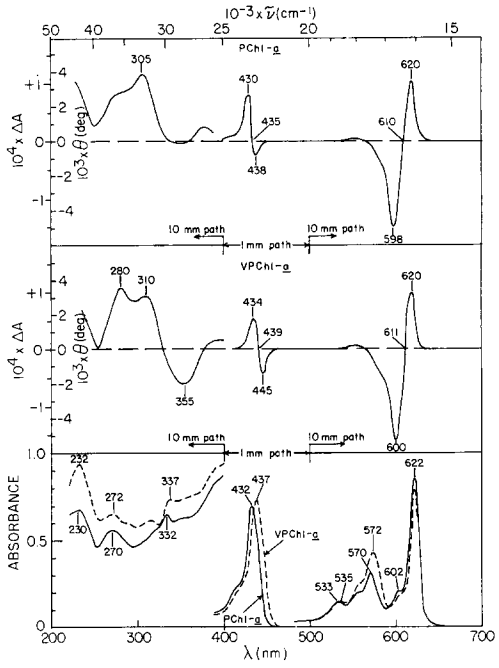

Fig. 4. Circular-dichroism and absorption spectra of protochlorophyll *a* (PChl-*a*) and 4-vinylprotochlorophyll *a* (VPChl-*a*) in ether.

The removal of the magnesium has a profound influence on the circular-dichroism spectrum (Table I; details will be presented in ref. 20). The long wavelength absorption of protopheophytin *a* at 638 nm now has a negative circular dichroism, in contrast with the long wavelength bands of protochlorophyll *a* and 4-vinylprotochlorophyll *a*. The principal blue absorption band at 418 nm in protopheophytin *a* is associated with a positive circular-dichroism band at about 420 nm. The decrease in fluorescence polarization in the 418-nm region (Fig. 3) suggests that this transition has a more nearly perpendicular orientation. The intermediate absorption bands in protopheophytin *a* have a very complex wavelength dependence in the circular-dichroism spectrum[20], which is consistent with the absence of strong fluorescence polarization in this region. This makes assignments difficult, particularly for the bands at 565 and 588 nm. The similarity of vibrational spacings to those observed for protochlorophyll *a* and a strong negative peak at 593 nm in the magnetic circular circular dichroism spectrum[20] of protopheophytin *a* support the assignment suggested in Table I.

DISCUSSION

The fluorescence polarization behavior displayed by the protochlorophyll pigments depends strikingly on which component of the emission spectrum is examined. Similar observations have been made by SEVCHENKO *et al.*[21] and BÄR *et al.*[10] on tetraphenylporphin. To explain their observations of constant polarization of all the absorption bands for the second emission band, the last authors have suggested that the pigment could be present in the solution as a mixture of two different molecular species, or that a second fluorescent molecular species was formed during the excitation. In the case of tetraphenylporphin, these authors refer to the possibility of two arrangements of the two protons on the four central nitrogen atoms, either on two vicinal nitrogens or on two opposite nitrogens. These views have been criticized by GURINOVICH *et al.*[22] because of the absence of any experimental support. Our observation of a similar behavior of the experimental fluorescence polarization for magnesium porphins (protochlorophyll *a* and 4-vinylprotochlorophyll *a*) cannot be explained in terms of separate tautomeric species involving migrating central protons. We must seek, therefore, a more general explanation which will account for the similar behavior of the magnesium porphins and their metal-free analogues.

Numerous attempts to understand the spectra of the porphyrins have been made on theoretical grounds[21–28]. As a result of detailed molecular orbital calculations, reviewed by GOUTERMAN[23] and GURINOVICH *et al.*[22], the spectra of the porphyrins are understood to arise from two pairs of transitions: a strongly allowed pair (blue, B bands) and a weakly allowed pair (red, Q bands). In the case of the square-symmetric metal porphins, all bands behave like planar oscillators, *i.e.*, without preferential orientation in the plane of the molecule. Removal of the central metal atom lowers the symmetry from square D_{4h} to rectangular D_{2h}. As a consequence, the two transitions B (0–0) and Q (0–0) are split into x and y linear components polarized perpendicular to each other. However, the vibrational satellite still appears to act as a planar oscillator in both the absorption, Q (1–0), and the emission, Q (0–1). This fact has been interpreted[27,28] as arising from a borrowing, by the Q (0–1) transition, of intensity from the allowed B transitions, with roughly equal contribution of the x and y components to the borrowed intensity.

These interpretations are consistent with the following experimental observations: (a) The fluorescence emission spectra of all the porphyrin pigments show two bands with a spacing (1200 to 1400 cm^{-1}) attributable to vibrational components. A similar band separation is observed for the corresponding absorption bands. (b) When measured in the long wavelength emission band (the 0–1 vibrational component), the polarization appears to be constant through all the absorption bands. By contrast, the 0–0 emission band shows the expected changes in polarization corresponding to the x and y components in the B and Q bands, both for the free base porphins and the unsymmetrically substituted metal porphins. For the square-symmetric porphins (*e.g.*, metal porphins or porphin dibasic acid salts) the polarization measured in the (0–0) emission as well as in the (0–1)-emission band is independent of the excitation wavelength[11,26].

If we apply these considerations to the protochlorophyll pigments, we arrive at the assignments summarized in Table I for the absorption bands, using the fluorescence-polarization and circular-dichroism data as guides in the assignments. In the

case of the protochlorophylls, as a consequence of the presence of unsymmetric substitution (particularly on ring III, resulting from the distortion introduced by the cyclopentenone ring (V) and from the presence of the electron-withdrawing carbonyl group, C-9), the square symmetry is never present, even for the metal complexes. Consequently, the absorption spectra in the Q region is always split into x and y components (the two-band spectrum of the fully symmetric metal porphins is never observed); and the polarization of the 0–0 emission band always varies with the excitation wavelength. In the assignments, the reference axes follow the notation of GOUTERMAN[23]: y through rings I and III, and x through rings II and IV. The experimental data presented here allow only relative orientation determinations, and the choice of the x and y directions is imposed as a convention. The assignment of the long wavelength band of protochlorophyll *a* and 4-vinylprotochlorophyll *a* to the y-direction and that of the corresponding band in protopheophytin *a* to the x-direction is based on arguments that will be presented in detail in a following paper[2c]. In brief, it derives from an extensive correlation, covering a variety of biologically-derived porphyrins, between the sign of the circular dichroism and the sense of the fluorescence polarization. It is the relationship between the substituents on the asymmetric carbon at C-10 in the protochlorophylls and the direction of the transition moment with respect to molecule-fixed axes which determines the sign of the circular-dichroism component: in this case, positive for a y-polarized and negative for an x-polarized transition. The presence or absence of a central metal does not affect this relationship, although it may alter the order of the energies of the bands. This is the case with protochlorophyll *a* and 4-vinylprotochlorophyll *a* *versus* protopheophytin *a* and provides the rationalization of the assignments given in Table I.

PERRIN *et al.*[28] have pointed out that the fluorescence spectrum and the longest wavelength part of the absorption spectrum should not be mirror symmetrical in the presence of vibronic borrowing and that the 1–0 absorption intensity should be about twofold more intense than the 0–1 emission intensity. This is, in fact, what we observe. For protochlorophyll *a* and 4-vinylprotochlorophyll *a* the ratio of the intensity of the 630-nm to the 685-nm fluorescence peaks is 4 to 5, while this ratio is 2 to 2.5 for the 622-nm to the 570-nm absorption bands. For protopheophytin *a*, the 653- to 713-nm emission intensity ratio is 0.57, whereas for the absorption the 638- to 588-nm ratio is 0.13.

It should be pointed out here that the presence of a circular dichroism for protopheophytin *a* in our results, in contrast with the observation of INHOFFEN AND BIERE[16], is most probably a consequence of racemization during the chemical reaction used by those authors for the preparation of protopheophytin *a* from pheophytin *a*. Investigations on the problem of enolization of the protochlorophyll pigments in ring V, which results in racemization and loss of optical activity, appears to be an interesting project for future studies.

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