Peripheral Metal Complexes: Chlorophyll "Isomers" with Magnesium Bound to the Ring E β-Keto Ester System

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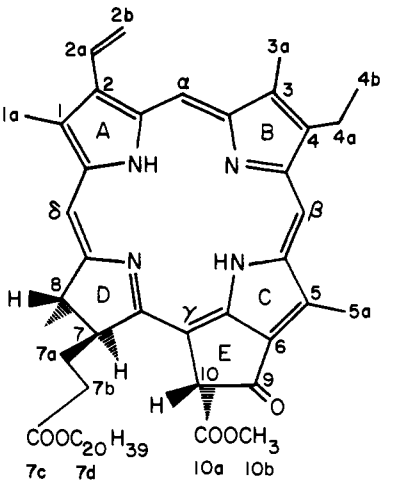
Abstract: Peripheral magnesium complexes of methyl pheophorbides *a* and *b*, bacteriomethyl pheophorbide *a*, bacteriopheophytin *a*, and protopheophytin *a* have been prepared and characterized by UV-vis, IR, ^{1}H NMR, and luminescence spectroscopy. In these compounds, Mg is bound to the peripheral β-keto ester system present in most chlorophylls rather than to the central tetrapyrrole cavity as in the chlorophyll proper. The ligand exchange reactions at Mg have been characterized, and the equilibrium between the peripheral complexes and their free ligands has been studied over the concentration range 10^{-7}–10^{-2} M. $\Delta G°$ for complex formation has been determined by ^{1}H NMR spectroscopy.

Introduction

The chlorophylls have a primary function in photosynthesis both in light collection and in light conversion processes. Considerable progress has been made in the past 2 decades in the understanding of the in vitro properties of chlorophyll and this has contributed to a better understanding of the role of chlorophyll on the molecular level in photosynthesis.[1] The salient features of chlorophyll aggregates or species present in vivo can be related to and can be studied in detail by suitable in vitro systems.[2] Similarly models have been developed for the redox chemistry of metallochlorins.[3,4] The aggregation behavior of the chlorophylls is largely due to the presence of both ligand acceptors (the central magnesium atom) and donors (predominantly the 9-ketocarbonyl group) within the same molecule.[1b,2] The easy (photo)oxidation of chlorins and bacteriochlorins to π-cation radicals[2c,5] can be related to the electronegativity of the central magnesium atom.[3]

Little is known thus far about the functional role in chlorophyll of the enolizable β-keto ester system at ring E. It is present in all chlorophylls (except for the chlorobium chlorophylls[6]), and in particular in all chlorophylls present in photoreaction centers, including bacteriochlorophyll *b*.[7] From the chemical point of view it is probably the most reactive functional group within the molecule. Indeed, most alteration products and side reactions observed in the chlorophylls are related to the β-keto ester system. Reactions involving the β-keto ester system are responsible for the easy epimerization[4a,8] and oxidation at C-10,[9] for the ready cleavage of ring E with amines,[9c] and for the loss of the 10-carbomethoxy group at elevated temperatures.[10] A test for the presence of the intact enolizable α-keto ester system was developed as early as 1896;[11] this reaction involves formation of the enolate anion upon treatment with strong bases.[1a] In neutral solution, only the diketo form of the β-keto ester system can be detected spectroscopically,[2a,12] but related porphyrins with an open chain β-keto ester substituent are present as diketo-enol mixtures.[13,14] Recently, more readily enolizable chlorophyll derivatives have been prepared in which a C-10 carbonyl group is covalently linked to the 7 substituent.[15] An early attempt to relate the enolizable β-keto ester system to the photosynthetic process goes back to Franck,[16a] and a model for oxygen production in photosynthesis has been proposed by Mauzerall and Chivvis[16b] in which the oxygen evolved by the water-splitting reaction of photosystem II is derived from the β-keto ester system.

We noticed during studies on the metalation of the pheophorbides of the *a* series[5d] that the substrates fall into two classes:[17] (a) pheophorbides that are easily metalated to chlorophyllides by anhydrous magnesium salts in refluxing pyridine,[18] and (b) pheophorbides of the second class that give only very poor yields in this reaction as well as a variety of alteration and decomposition products.[19] Those pheophorbides or pheophytins that resist metalation by this procedure form a new type of metal complex even at ambient temperatures upon treatment with dry magnesium salt solutions. This complex is formed if and only if the enolizable β-keto ester system at ring E is present in the substrates (cf. pheophytin *a*, **1**). Preliminary results suggested chelation of Mg by this peripheral grouping rather than by the central nitrogen atoms in the tetrapyrrole rings also present in the substrate compounds, and the products were therefore termed peripheral complexes.[17] Similar complexes derived from pheophytins containing a β-diketo group at ring E have been reported recently by Eschenmoser et al.[15] The peripheral complexes have properties which make them interesting both as a novel type of compound in chlorophyll chemistry and as a possible model for certain aspects of the in vivo pigments. They have a red-shifted absorption spectrum, they are essentially nonfluorescent though monomeric, and they exhibit a type of metal-ligand interaction new in the chlorophylls.



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The preliminary studies have now been extended, and peripheral complexes of a variety of pheophorbides with different chromophoric systems have been investigated. These studies fully support the proposed structure, and show that peripheral complex formation with metal ions is a general feature of chlorophyll chemistry.

Materials and Methods

Spectra. Electron excitation (absorption) spectra were recorded on a Cary 14 spectrophotometer interfaced to the central Sigma 5 computer of the Argonne National Laboratory Chemistry Division.

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Computer deconvolutions of the UV–vis spectra were carried out by the variable metric minimization procedure of Davidon,[20] modified and adapted to the Sigma 5 by A. Zielen. This program allows for the resolution of up to ten peaks with different individual line shapes within the same spectrum. Fluorescence spectra were obtained on pyridine solutions with an Argonne-built laser-excited fluorimeter described elsewhere.[21] Phosphorescence experiments were carried out in glasses at cryogenic temperatures in the same apparatus. Samples for phosphorescence were prepared in a 1:1 mixture of toluene and a saturated solution of $Mg(ClO_4)_2$ in pyridine, degassed by repeated freeze–thawing, and then sealed off under high vacuum. For fluorescence titration experiments, an Hitachi Perkin-Elmer MPF-2A spectrofluorimeter was used. ^1H NMR spectra were measured in pyridine-d_5 on a Varian HR 220 NMR spectrometer operated in pulse FT mode and interfaced to the Sigma 5. IR spectra were obtained with a Beckman IR 7 spectrometer. Pyridine-d_5 was used as solvent, and titrations were carried out with ^2H_2O, if not stated otherwise.

Solvents and Reagents. All solvents were reagent grade and were dried over molecular sieves prior to use. Anhydrous $Mg(ClO_4)_2$ (G. F. Smith) was dried[22] under a high vacuum at slowly increasing temperatures and then kept at 225 °C for at least 6 h. Anhydrous metal chlorides and bromides were obtained from the hydrates in a stream of hydrogen chloride or bromide, respectively. All dry metal salts were handled in glove boxes kept dry with anhydrous $Mg(ClO_4)_2$ or P_2O_5.

Compounds. Chlorophylls were obtained by standard procedures and demetalated with dilute HCl to the pheophytins.[23] For conversion of bacteriochlorophyll *b* into bacteriopheophytin *b*, the time of contact with acid was less than 1 min to avoid extensive rearrangement.

Methyl pheophorbide *a* (**2**) and *b* (**3**) and bacteriomethyl pheophorbide *a* (**4**) were prepared from the respective pheophytins by transesterification with 5% methanolic sulfuric acid. Protopheophytin *a* (**6**) was obtained from pheophytin *a* (**1**) by dehydrogenation with freshly sublimed 2,3-dichloro-5,6-dicyanobenzoquinone.[9d,24] Pyromethyl pheophorbide *a* (**7**),[10] 10-methoxymethyl pheophorbide *a* (**8**),[9d] and 9-hydroxy-9-deoxomethyl pheophorbide *a* (**9**)[25] were obtained by standard procedures.

All anhydrous metal salt solutions and solutions of the peripheral complexes were handled in nitrogen dryboxes or glovebags (I_2R). For the preparation of the peripheral complexes, the pheophorbide was usually dissolved in a freshly prepared anhydrous saturated solution of $Mg(ClO_4)$ (or other anhydrous salt) in pyridine. The saturated solutions were obtained by adding an excess of the anhydrous salt to pyridine or other solvent, and the undissolved salt was subsequently removed by centrifugation. For the mixed solvent systems, methanol was saturated with the metal salt; in the case of $Mg(ClO_4)_2$, 5% pyridine was added for safety reasons. The pheophorbide, dissolved separately in the other component, was then added to this solution.

The Anhydrous Magnesium Salt Solutions.

Anhydrous magnesium salts are among the most powerful desiccants, and the perchlorate surpasses even P_2O_5. As the peripheral complexes are sensitive to water (see below), all salts must be dried extensively prior to use and handled with the rigorous exclusion of water. Dissolution of the anhydrous salts in pyridine is strongly exothermic, and the salt must always be added to the solvent to prevent extensive and undesirable caking. If an excess of the metal salt is used, the remaining solid is an effective drying agent. Thus, pyridine containing 1% water gave results similar to carefully dried pyridine when treated with excess anhydrous salts. The saturated solutions are extremely hygroscopic once they are separated from the precipitate by centrifugation, and even in a good drybox may take up enough water to prevent complex formation after a few hours of exposure. Storage for extended periods is possible, however, under high vacuum. The actual concentration of $Mg(ClO_4)_2$ in a saturated pyridine solution prepared as described may show considerable variation. For a series of solutions prepared under similar conditions, a range of values from 0.12 to 0.24 mol/L have been found by atomic absorption spectroscopy.[26] We ascribe this variation in concentration to varying amounts of residual water, which is present in molar amounts comparable to the Mg salt even with all the precautions taken.

The original water content and the ligand exchange

$$[MgPy_6]^{2+} + xH_2O \rightleftarrows xPy + [MgPy_{6-x}(H_2O)_x]^{2+} \qquad (x \leqslant 6)$$

of Mg-coordinated pyridine (Py) with water can be studied by ^1H NMR. A saturated solution of anhydrous magnesium perchlorate in "dry" pyridine-d_5 was titrated with ^1H_2O. Because ligand exchange is rapid, only an averaged proton resonance is observed for the free water and the water bound to Mg^{2+}. (Even before deliberate addition of any water a broad H_2O proton resonance is observed at very low field.) Addition of water causes an increase in the intensity and a shift toward higher field of this resonance. By extrapolation of the intensity of the water line and its position (Figure 1), the initial water concentration and a maximum chemical shift for the water bound to magnesium were obtained. The original water concentration ($[C_{H_2O}] = 0.222$ mol) is almost twice as high as the concentration of Mg^{2+} in the solution ($[C_{Mg^{2+}}] = 0.115$ mol/L) as determined by atomic absorption (cf. above). The very large low-field shift of the coordinated water protons indicates a considerable acidity of the protons. The methine proton exchange observed[5d] during metalation to chlorophyllides of pheophorbides not containing the enolizable β-keto ester system is probably due to this high acidity.

The first-order plot of the incremental shifts is linear except at very low water concentrations. The linear part can be interpreted by an exchange equilibrium involving the sixth ligand position only:

$$[Mg(H_2O)_5Py]^{2+} + H_2O \rightleftarrows [Mg(H_2O)_6]^{2+} + Py$$

The chemical shift of water in pyridine is itself concentration dependent, probably because of hydrogen bonding to the pyridine. Over the concentration range from 0.1 to 7 M water in pyridine, the chemical shift of the water protons increases from δ 4.80 to δ 5.37 ppm. All values in Figure 1 are corrected for this "intrinsic" shift.

Deviations in chemical shift at low water concentrations indicate a very strong binding of the first water added as compared to the later ones, one, but the complex equilibria

$$[Mg(H_2O)_xPy_{6-x}]^{2+} + H_2O \rightleftarrows [Mg(H_2O)_{x+1}Py_{5-x}]^{2+} + Py$$

where $x \leqslant 5$ were not investigated in detail. At the titration midpoint, a total water concentration of 0.63 mol/L is obtained by interpolation from Figure 1. This value corresponds to 2.74 mol of bound water per mol of magnesium. Judged from this value, a stepwise exchange of water with probably only two complex species present at a time seems to be reasonable.

The Solvent System

Most spectroscopic data on the peripheral complexes were obtained in saturated solutions of magnesium perchlorate in pyridine. This system is readily prepared from the dried salt and dry pyridine. The magnesium salt is soluble in pyridine to the extent of 20 g/L, and the solution appears to be reasonably safe with respect to the hazards associated with $Mg(ClO_4)_2$ in organic solvent systems. In addition to pyridine, peripheral complexes also form readily in a variety of other solvents as long as water is excluded and both the metal salt and the pheophorbide are soluble in the solvent. Among other solvents, dimethyl sulfoxide (Me_2SO), dimethylformamide (DMF), and hexamethylphosphoramide (HMP) gave good results, while only partial complex formation is observed in ethers. Besides the pure solvents, solvent mixtures give results comparable to those obtained in pyridine. The low-temperature luminescence spectra reported here were obtained in a glass formed from a

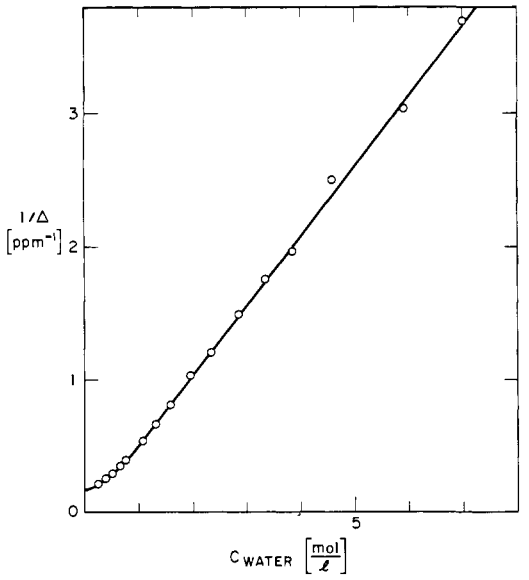
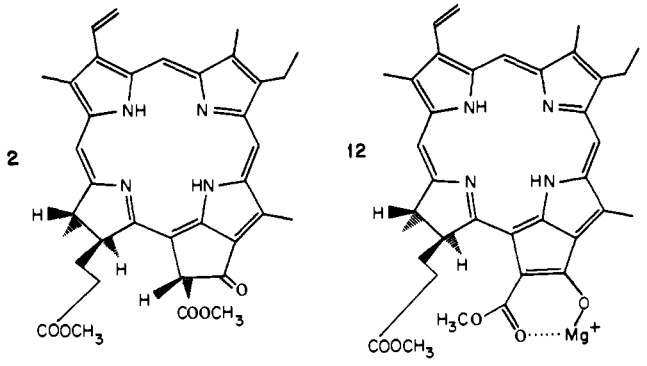


Figure 1. Concentration dependence of the ^1H NMR chemical shift (δ, ppm) of water ($^1HO^2H$) in a saturated solution of $Mg(ClO_4)_2$ in pyridine-d_5. See text for details.

saturated $Mg(ClO_4)_2$/pyridine solution diluted 1:1 with toluene. If a concentrated solution of $Mg(ClO_4)_2$ in methanol (containing 5% pyridine) is used as one component, peripheral complexes are observed to be formed with methyl pheophorbide *a* dissolved in practically any hydrocarbon or halocarbon solvent.

Peripheral Complex Electronic Excitation Spectra

Formation of the peripheral complexes from the free pheophorbide is always accompanied by a pronounced color change. A solution of methyl pheophorbide *a* (**2**) is brown,



while that of its peripheral complex **12** is bright yellowish-green. In the UV-vis spectrum (Figure 2) the red $Q_y(0,0)$ band is shifted from λ_{max} 671 nm to λ_{max} 682 nm in pyridine. At the same time the absorption line is considerably broadened, and a pronounced Lorentzian component is mixed into the formerly Gaussian line (Figure 3). Although the extinction coefficient is diminished from 52.6×10^3 to 19.5×10^3, the oscillator strength of the red band remains constant within 15%. The two medium-intensity bands at 538 and 507 nm characteristic of free base pheophorbides of the *a* series are missing in the peripheral complex, and the Soret band(s) are replaced by two overlapping complex band systems extending from 350 nm to about 500 nm. The spectrum of the complex is very characteristic and has no known parallel in any chlorin. The spectrum is reminiscent, however, of that of the pheophytin *a* phase-test intermediate, which shows the broadened bands and the split Soret system.[1a]

The spectra of the peripheral complexes of methyl pheophorbide *b* (**13**) (Figure 4) bacteriomethyl pheophorbide *a* (**14**) (Figure 5), and bacteriopheophytin *b* (**15**) (Figure 6) show similar pronounced changes as compared to the spectra of the free pheophorbides **3, 4,** and **5**. The Soret band is even

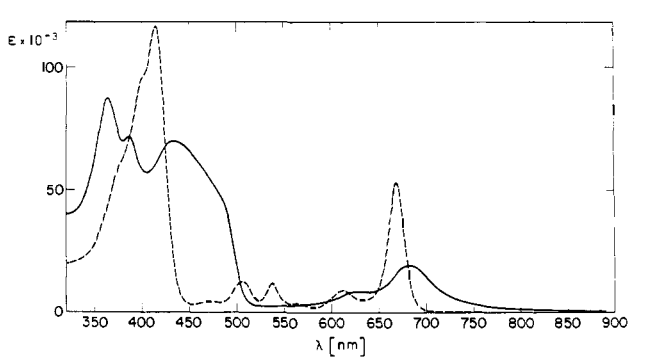
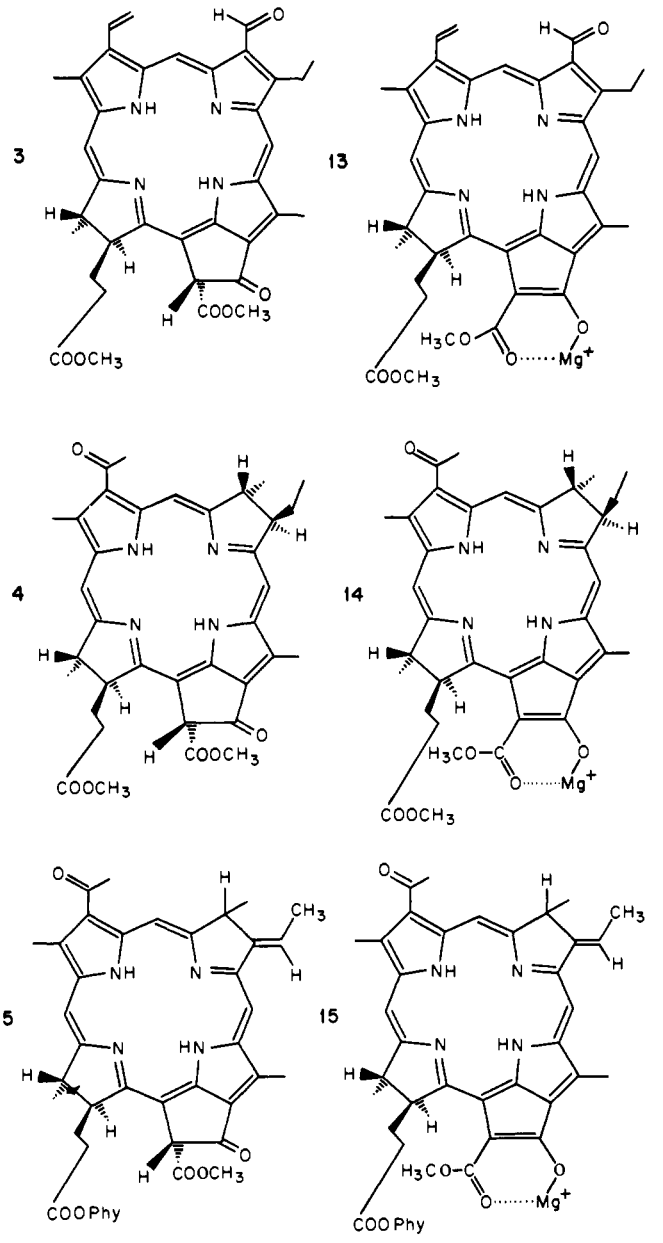


Figure 2. UV-vis spectra ($\epsilon \times 10^{-3}$ vs. λ [nm] of methyl pheophorbide *a* (**2**) (- - -), and of its peripheral Mg complex **12** (—). The spectrum of **12** was obtained by dissolving **2** (ca. 10^{-5} mol/L) in a saturated solution of $Mg(ClO_4)_2$ in dry pyridine. Water was then added in small increments (~10 μL total) to regenerate free **2** (- - -). The latter spectrum is identical with a spectrum of **2** dissolved in pyridine.



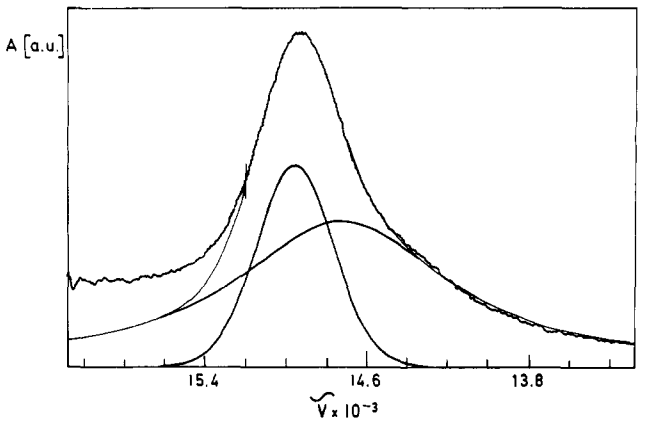


Figure 3. Curve resolution of the UV-vis spectrum (A vs. $\tilde{\nu} \times 10^{-3}$ (cm^{-1})) of a mixture of free methyl pheophorbide *a* (**2**), and its peripheral Mg complex **12**. Only the long-wavelength band around 15 000 cm^{-1} (667 nm) has been deconvoluted. The curve is obtained during titration of a solution of **12** with water (see Figure 2).

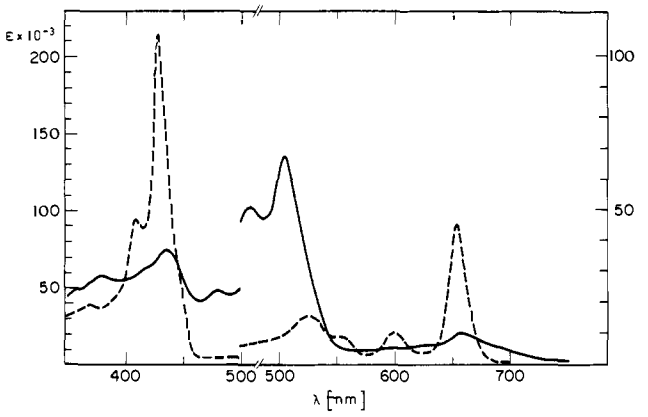
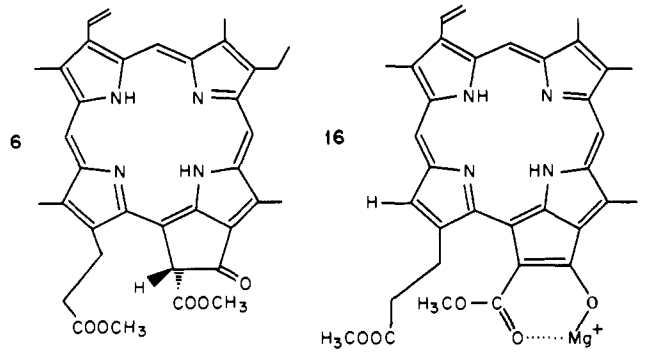


Figure 4. UV-vis spectra ($\epsilon \times 10^{-3}$ vs. λ (nm)) of methyl pheophorbide *b* (**3**) (- - -), and its peripheral Mg complex **13** (—). For details, see legend of Figure 2.

more extended to the red in **13**, and the complexes of both bacteriopheophorbides (**14, 15**) show an almost featureless broad Soret system.

The spectrum of protopheophytin *a* (**6**) dissolved in pyridine saturated with $Mg(ClO_4)_2$ still shows bands characteristic of free **6**, and the solution decomposes rapidly even when oxygen is excluded. The difference spectrum of a freshly prepared solution, and the same solution titrated with water to regenerate free **6**, shows an increase in absorption at 300-380, 480, and 660 nm, and a pronounced drop of the absorptions corresponding to **6** (Figure 7). These spectral changes are again characteristic of the peripheral complexes. The Soret band is split, and there is a considerably red-shifted peak at ~480 nm. The incomplete formation of a peripheral complex and its instability probably reflect steric strain in **16**. In the parent



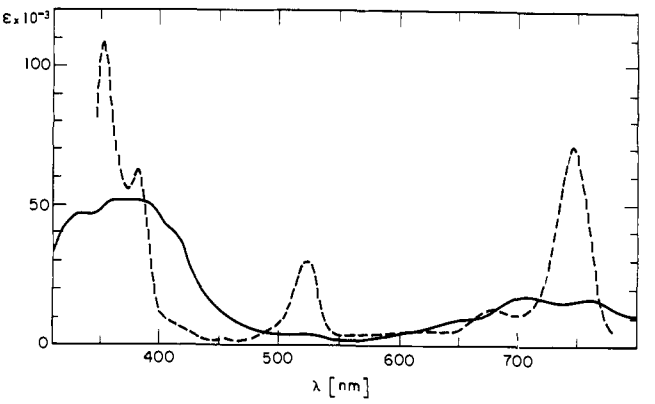
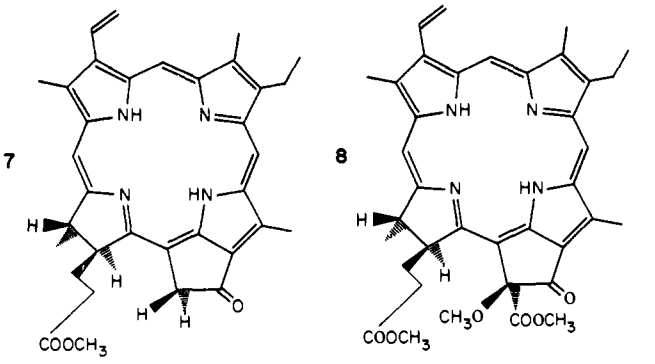
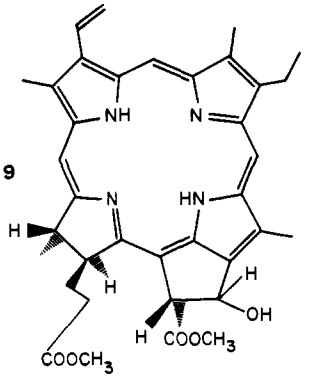


Figure 5. UV-vis spectra ($\epsilon \times 10^{-3}$ vs. λ (nm)) of bacteriomethyl pheophorbide *a* (**4**) (- - -), and its peripheral Mg complex **14** (—).

porphyrin **6**, the 7,8 substituents are in plane, and there is a considerable steric interaction[4b,27,28] with the isocyclic ring (the C-γ substituent). This interaction is obviously very much increased in the peripheral complexes as discussed below.

The latter example indicates that even under unfavorable conditions chelation of the metal ion by the peripheral β-keto ester system is preferred (probably for kinetic reasons) to chelation by the central 4-N cavity. That this chelation does involve both carbonyl groups at C-9 and C-10a, as well as the enolizable 10-H, is evidenced by complexation studies with chemically modified pheophorbides. Removal of either one of the required functional groups, i.e., compounds **7, 8,** and **9,**





completely inhibits peripheral complex formation. In these cases, the UV-vis spectra are unaffected by the presence or absence of Mg^{2+} in the pyridine solutions.

In summary, the UV-vis absorption spectra of all peripheral complexes are less well defined than those of their parent compounds. Most of the absorption maxima are considerably broadened, are non-Gaussian in line shape, and often overlap to form complex band systems. The spectra do exhibit distinct similarities to those of the enolate ions[1b] of the Molisch phase test[11] and especially to the spectra of the enols of pheophor-

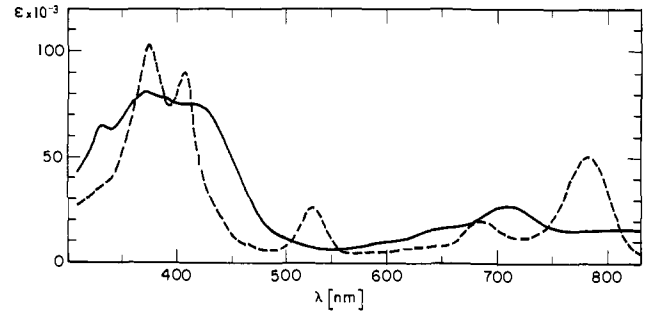
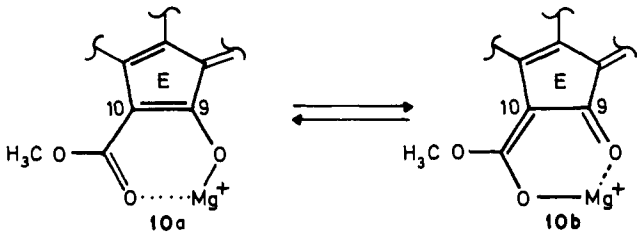


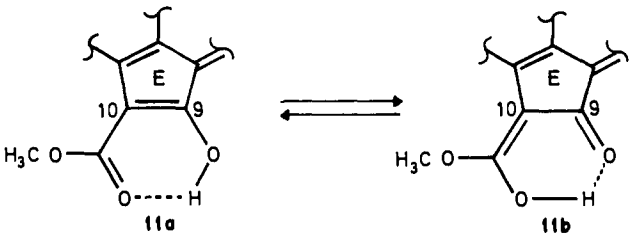
Figure 6. UV-vis spectra ($\epsilon \times 10^{-3}$ vs. λ (nm)) of bacteriopheophytin *b* (**5**) (- - -), and its peripheral Mg complex **15** (—).

bides containing a planar β-diketo group prepared recently by Eschenmoser et al.[15b]

In contrast to the acetylacetonates, the two resonance forms **10a** and **10b** are not equivalent. Depending upon whether the



Mg is held in a one- or two-minimum potential bond, and upon the exchange kinetics, either one spectrum or a superposition of two spectra could be expected. The spectra of the two enolic structures **11a** and **11b** have recently been calculated by



Song.[29,30] A considerable red shift of 65 nm is computed for the $\Delta^{9,10}$ enol **11a**,[29] and a much smaller one of the order of 6 nm for the $\Delta^{10,10a}$ enol **11b**.[30] The featureless and symmetric red band of **12** (Figure 3) seems to indicate that the peripheral complex is essentially a single species with respect to UV-vis spectroscopy.

We did observe, however, in several samples hints for a fine structure of this band. In one instance, a preparation of the peripheral complex showed a series of additional broad bands. Addition of a trace of water restored the normal spectrum of **12** and with more water **2** was regenerated. This result could indicate the formation of a diperipheral complex with two pheophytins coordinated to one Mg^{2+}, in which the second porphyrin ligand is even more readily replaced by water. This is the only case in which such a peripheral complex has been observed.

IR Spectra

The IR spectra of the pheophorbides and their peripheral Mg complexes have been investigated in detail in the spectral region 1600-1800 cm^{-1} in which the carbonyl stretch vibrations as well as the characteristic "chlorin" band appear.[31] In addition, IR information outside of this range has been obtained for **2** and its peripheral complex **12**.

Pyridine-d_5 lacks significant absorption above 1560 cm^{-1} with the exceptions of a sharp peak at 1648 cm^{-1} and small peaks at 1600 (shoulder), 1670 (shoulder), 1709 (broad), and

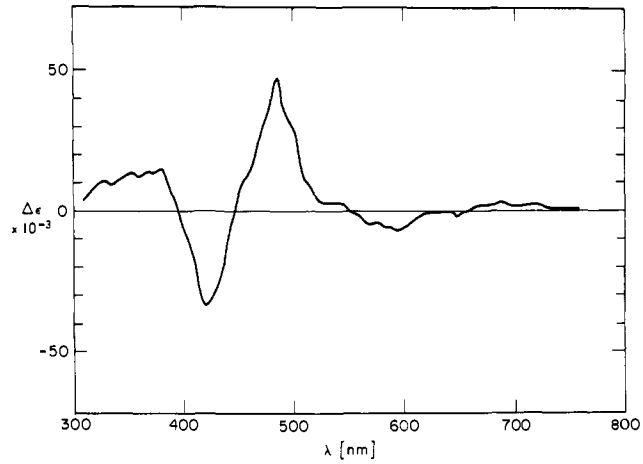


Figure 7. UV-vis difference spectrum ($\Delta\epsilon \times 10^{-3}$ vs. λ (nm)) of a solution of protopheophytin *a* (**6**) in a saturated solution of $Mg(ClO_4)_2$ in pyridine, and of the same solution treated with 10 μL of water. Positive bands correspond to increased absorption from the peripheral Mg complex **16**.

1765 cm^{-1} (broad). The spectrum does not change in this region when the system is saturated with $Mg(ClO_4)_2$. However, in the latter case a series of weak, broad peaks appear at 1675, 1690, 1708, and 1742 cm^{-1}, which are probably due at least in part to the presence of residual water. Addition of ^1H_2O causes an intense, broad, unstructured band with an absorption maximum at about 1650 cm^{-1}. If ^2H_2O is added instead of ^1H_2O, this peak is shifted to longer wavelengths with only its shoulder extending into the region above 1600 cm^{-1}.

The spectrum of methyl pheophorbide *a* (**2**) in pyridine-d_5 (Figure 8a) shows two carbonyl absorptions of about equal intensity at 1703 and 1741 cm^{-1}. The first one originates from the 9-keto C=O group, the second from both the 10a- and 7c-ester C=O groups. The ester bands are usually unresolved, but they are split in hydrogen-bonding solvents.[12,32] The only other band in the spectrum of **1a** not due to the solvent is the "chlorin" band[31] at 1621 cm^{-1}.

In the spectrum of the peripheral complex **12** (Figure 8b), only one of the original carbonyl bands remains, but this is considerably reduced in intensity, and slightly shifted to 1735 cm^{-1}. All other bands above 1650 cm^{-1} are due to solvent absorption. In addition, the complex shows a new, very strong band at 1620 cm^{-1}, with a shoulder at about 1603 cm^{-1}. The latter band disappears upon addition of ^2H_2O (Figures 8c,d), and at the same time the 9-keto C=O band reappears and the ester carbonyl band about doubles in intensity until the original spectrum of methyl pheophorbide *a* is restored (Figure 8d). The small residual red shift of all bands (~3 cm^{-1}, as compared to **2**, Figure 8a), is attributed to the change in solvent. The "chlorin" band is hidden under the ^2H_2O peak in Figure 8d but the latter is visible at 1622 cm^{-1} if ^1H_2O is added instead. The only other distinct change in the IR spectrum of the peripheral complex **12** that is not obscured by the strong solvent lines is a band of medium intensity at 1385 nm. A band in this region in acetylacetonates originally assigned to a C=O vibration[33] is now ascribed to a CH_3 deformation.[34] As this group is absent in **12**, no assignment of the latter band has been made.

Methyl pheophorbide *b* (**3**) contains an additional aldehyde group at position 3, which gives rise in the IR spectrum to a band at 1657 cm^{-1} (Table I). Except for the presence of this band in the spectra of **3** as well as of its peripheral complex **13**, the IR changes upon formation and destruction of the peripheral complex are identical with those observed in **2**. The same is true for bacteriomethyl pheophorbide *a* (**4**) and bacteriopheophytin *b* (**5**), and their respective peripheral complexes. Both bear an additional keto group at C-2 absorbing

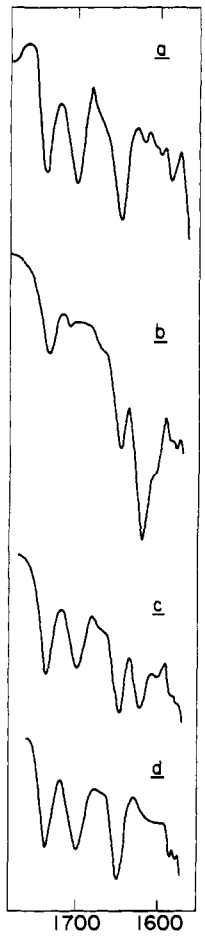


Figure 8. IR spectra (carbonyl stretching region) of methyl pheophorbide *a* (**2**), and its peripheral complex **12**. (a) **2** (1.8×10^{-2} M) in pyridine-d_5; (b) **12** (1.8×10^{-2} M) in pyridine-d_5, obtained by dissolving **2** in a saturated solution of $Mg(ClO_4)_2$ in pyridine-d_5; (c) solution b, after addition of 0.3 μL of ^2H_2O; and (d) after addition of 3 μL of ^2H_2O.

at 1669 cm^{-1} (Table I), which is unchanged in all spectra. Peripheral complex formation is always accompanied by the disappearance of the 9-keto C=O absorption at about 1700 cm^{-1}, a pronounced decrease in intensity (~50%) and a small red shift of the ester C=O band around 1735 cm^{-1}, and the appearance of a very strong band at ~1620 cm^{-1} with a long-wavelength shoulder. In all cases, these spectral changes are reversed upon addition of water.

All IR spectra (Table I) can be interpreted by the formation of a peripheral complex by the enolizable β-keto ester system with $Mg(ClO_4)_2$, a complex that reverts reversibly to the original pheophorbide upon addition of water. As shown by the reversible and simultaneous disappearance of the 9-keto C=O band, and the diminuition of the ester carbonyl band upon complex formation, both the 9-keto C=O group and one ester group are involved in the complex formation. From all chemical evidence it is the 10-ester group that is involved, and the free ester C=O vibration at 1735 cm^{-1} in the peripheral complexes is then assigned to the propionic ester group at position 7.

The very strong band in the peripheral complexes at ~1620 cm^{-1} as well as the shoulder at 1603 cm^{-1} is assigned to the chelated carbonyl group(s). Intensity and position are typical[35] for the chelated carbonyl stretching vibration[34a] in β-dicarbonyl metal complexes. The frequency is dependent on the metal, and for $Mg(acac)_2$ a value of 1629 cm^{-1} has been determined.[35] This frequency agrees well with the one observed in the peripheral metal complexes. For the free enol of cyclopheophorbides containing a cis β-diketo grouping, a CO band at 1656 cm^{-1} has been reported.[15b] There is no other characteristic band in the spectrum of **12** in the region above the cutoff frequency of the solvent at about 1560 cm^{-1}, which suggests that this band and the accompanying shoulder originate from both the chelated 9-keto C=O and 10a-ester C=O groups.

NMR Spectra

^1H NMR spectroscopy has been proved to be one of the most powerful tools in the analysis of porphyrins.[36] The large ring-current induced chemical shifts of protons close to the aromatic macrocycle spread the spectra over a range of more than 10 ppm. The spectrum of methyl pheophorbide *a* (**2**) in C^2HCl_3 has been completely assigned.[2a,37] The spectrum in pyridine-d_5 is very similar, and the same assignments have been used. The chemical shifts of the β, α, and δ-methine protons and of the NH protons, respectively, exhibit the largest ring-current shift and are at the extreme low- and high-field ends of the spectrum. In the spectrum of **2** (Table II), the spectral width between the β-H and the high-field NH resonance amounts to 11.23 ppm. In the peripheral complex **12**, this spread is reduced to 6.97 ppm. Except for this general compression, the spectrum of the peripheral complex very much resembles that of **2**. The pattern of most and the multiplicity of all of the signals is retained. Assuming a more or less uniformly reduced ring current, most signals in the spectrum of **12** can be self-consistently assigned by their multiplicity and their incremental shifts (Δ (ppm)) as compared to that of **2**. With a few exceptions, the Δ values are very similar for protons in similar chemical environment, thus supporting the above assumptions. The methine protons are shielded by Δ = +0.71–0.74 ppm, "benzylic" protons are shielded to the extent of Δ = +0.25–0.31 ppm, and protons three bonds removed from the aromatic system by Δ = +0.14–0.18 ppm. The only exceptions are protons at rings D and E. Both the 7-H doublet (expected to be a complex multiplet, but appearing in all 7,8-*trans*-pheophorbides as a characteristic broadened doublet)[2a,36,37] and the 8-CH_3 doublet, which can be assigned by their multiplicities, are deshielded by Δ = −0.32 and Δ = −0.07 ppm, respectively. One of the methyl singlets is deshielded as well. On the basis of model studies, this singlet was assigned to the 10-$COOCH_3$ resonance (see below). The ^1H NMR spectrum of the peripheral complex **12** shows only two singlets from protons exchangeable with $C^2H_3O^2H$. Based on the position of these resonances at high field, they were assigned to the NH protons. There is a considerable difference in the incremental shifts of these two protons, which occur in all other peripheral complexes as well. In free methyl pheophorbide *a* (**2**), a third exchangeable singlet at δ 6.61 ppm is present, which arises from the C-10 proton. The corresponding signal is missing in the spectrum of the peripheral complex.

The ^1H NMR spectra of the peripheral complexes **13**, **14**, and **15** (Tables III–V) can be interpreted in an identical manner. In all of their ^1H NMR spectra, the ring current induced shifts are smaller than in the free pheophorbides, with similar incremental shifts for protons in a similar environment. Again, the only exceptions are the signals of the 7-H, the 8-CH_3, and the 10-$COOCH_3$ protons, the different incremental shifts of the NH protons, and the loss of the 10-H signal.

It is well documented that electron-withdrawing substituents at ring E decrease the aromatic ring current induced shifts (cf. ref 36). Although the effect of chelation of the β-keto ester system is a priori not predictable, the uniformly reduced ring current can be rationalized by the combined influence of the positively charged Mg^{2+} ion and an increased electron withdrawal from conjugation of the 10-$COOCH_3$ group. The chelate nature of the peripheral complexes is directly supported, moreover, by the proton signals that do not follow the regular pattern. Formation of the chelates leads to two pre-

Table I. Infrared Spectra (Carbonyl Stretch Region, 1800–1600 cm^{-1}) of Peripheral Mg^{2+} Complexes[a]

Compd	Concn, mol/L	Concn $Mg(ClO_4)_2$	Concn H_2O, mol/L	$\nu_{C=O}$, cm^{-1} 7c-C=O	10a-C=O	9-C=O	2-C=O	3-C=O	Chelate-C=O	"Chlorin" band
2	$(1.8) \times 10^{-2}$			1741	1741	1703				1621
12	$(1.8) \times 10^{-2}$	Satd		1735					1620 1603 (sh)	n.v.
2	$(1.8) \times 10^{-2}$	Satd	3	1737	1737	1700				1622
3	$(1.8) \times 10^{-2}$			1739	1739	1704		1657		1618
13	$(1.8) \times 10^{-2}$	Satd		1735				1661	1624 1605 (sh)	n.v.
3	$(1.8) \times 10^{-2}$	Satd	3	1738	1738	1704		1661		1620 (sh)
4	$(1.9) \times 10^{-2}$			1737	1737	1695	1670			1622 (?)
14	$(1.9) \times 10^{-2}$	Satd		1736			1669		1617 1611 (sh)	n.v.
4	$(1.9) \times 10^{-2}$	Satd	3	1734	1734	1692	1666			1625 (?)
5	$(2.0) \times 10^{-2}$			1736	1736	1697	1669			1620 (?)
15	$(2.0) \times 10^{-2}$	Satd		1729			1668		1617 1605 (sh)	n.v.
5	$(2.0) \times 10^{-2}$	Satd	3	1734	1734	1695	1668			1622 (?)

[a] Three spectra for each compound are presented: the free ligand dissolved in pyridine-d_5, the peripheral complex obtained by dissolving the ligand in a concentrated solution of $Mg(ClO_4)_2$ in pyridine-d_5, and the regenerated free ligand obtained by addition of ^2H_2O to the solution of the peripheral complex.

Table II. ^1H NMR Spectra (δ, ppm) and Differential Chemical Shifts (Δ, ppm) of Methyl Pheophorbide *a* (**2**) and Its Peripheral Mg^{2+} Complex (**12**)

	Multiplicity ($J[H_2]$)	δ[a] Free **2**	δ[a] Peripheral Mg complex[b] **12**	Δ
β-H	s	9.75	9.01	+0.74
α-H	s	9.57	8.83	+0.74
δ-H	s	8.71	8.00	+0.74
H_X	dd [11,17]	8.08	7.77	+0.31
Vin H_A	dd [2,17]	6.23	6.06	+0.17
H_B	dd [2,11]	6.05	5.87	+0.18
10-H		6.61		
7-H	d [7]	4.29	4.65	−0.36
8-H	q [7]	4.42	4.10	+0.32
10b-CH_3	s	3.76	3.83	−0.07
7d-CH_3	s	3.52	3.38	+0.14
5a-CH_3	s	3.42	3.11	+0.31
3a-CH_3	s	3.21	2.95	+0.26
1a-CH_3	s	3.08	2.83	+0.25
8-CH_3	d [7]	1.66	1.73	−0.07
4-CH_2	q [7]	3.54	3.29	+0.25
4a-CH_3	t [7]	1.53	1.39	+0.14
N_A-H	s, br	+0.74	2.44	−1.70
N_C-H	s, br	−1.48	2.04	−3.52

[a] Chemical shifts are given in parts per million relative to tetramethylsilane. [b] The spectrum of the peripheral complex was obtained from a solution of pheophytin or pheophorbide (ca. 7×10^{-3} M) dissolved in a saturated solution of $Mg(ClO_4)_2$ in pyridine-d_5. The spectrum of the free pheophytin or pheophorbide was recorded after addition of 10 μL of ^2H_2O.

Table III. ^1H NMR Spectra (δ, ppm) and Differential Chemical Shifts (Δ, ppm) of Methyl Pheophorbide *b* (**3**) and Its Peripheral Mg^{2+} Complex (**13**)

Signal	Multiplicity ($J[H_2]$)	δ[a] Free **3**	δ[a] Peripheral Mg complex[b] **13**	Δ
3-CHO	s	11.29	11.02	+0.27
α-H	s	10.75	9.90	+0.85
β-H	s	9.91	9.14	+0.77
δ-H	s	8.67	7.91	+0.76
2a-H_X	dd [11,17]	8.01	7.54	+0.47
2b-H_A	dd [11,2]	6.04	5.80	+0.24
2b-H_B	dd [17,2]	6.36	6.13	+0.23
10-H	s	6.67		
7-H	9 [7]	4.41	4.57	−0.16
8-H	d [7]	4.31	~3.75	~+0.56
1-CH_3	s	3.17	2.89	+0.28
5-CH_3	s	3.47	3.17	+0.30
10a-CH_3	s	3.81	3.95	−0.14
7d-CH_3	s	3.45	3.44	+0.01
4-CH_2	9 [7]	3.98	3.69	+0.29
4a-CH_3	t [7]	1.67	1.45	+0.22
8-CH_3	d [7]	1.61	1.75	−0.14
N_A-H	s, br	0.59	1.90	−1.31
N_C-H	s, br	−1.46	1.28	−2.74

[a,b] See footnotes to Table II.

dictable consequences. The resonance of the C-10 proton will of course disappear (see also ref 15b) and the 10-$COOCH_3$ group becomes more or less coplanar with the macrocyclic system. Both effects are clearly visible in the ^1H NMR spectra. None of the complexes shows the third exchangeable proton signal, and indeed no singlet possibly originating from such a proton is visible in any of the spectra of the complexes. The unusual incremental low-field shift of the 10-$COOCH_3$ signal is a direct effect of its changed conformation. In **2**, this group is out of plane in a region close to a nodal surface of the ring-current field (cf. ref 36). In the peripheral complex **12** it is coplanar with the macrocycle and thus is in a deshielding area.

The deshielding of the 7-H and 8-CH_3 signals as well is an indirect consequence of the coplanar conformation of the 10-$COOCH_3$ group. It is known from δ-substituted pheophorbides[38] that a bulky δ substituent in pheophorbides induces a conformational change in ring D by which C-8 and the attached 8-CH_3 group are pushed further out of plane, while the 8-H proton comes more into the plane of the macrocycle. Carbon 17 follows this movement, and as a consequence the 8-CH_3 and the cisoid 7-H are in a less deshielded region, and the 8-H is in a more deshielded region. Because of the transoid configuration of C-7 and C-8, this situation is reversed when a bulky substituent is introduced at C-γ (Figure 9). In this case C-7 and the propionic acid side chain are pushed out of plane. The 7-H and 8-CH_3 protons move toward the macrocyclic plane and into a more deshielded region. The coplanar 10-

Table IV. ^{1}H NMR Spectra (δ, ppm) and Differential Chemical Shifts (Δ, ppm) of Bacteriomethyl Pheophorbide *a* (**4**) and Its Peripheral Mg^{2+} Complex (**14**)

	Multiplicity (J[H_2])	δ[a] Free **4**	δ[a] Peripheral Mg complex[b] **14**	Δ
α-H	s	9.28	8.60	+0.68
β-H	s	8.56	8.00	+0.56
δ-H	s	8.54	7.90	+0.64
3-H	m	4.12	3.67-3.87	+0.35
4-H	m	3.86	3.67-3.87	+0.10
7-H	9 [7]	4.12	4.57	−0.45
8-H	d [7]	4.24	3.67-3.87	+0.5
10-H	s	6.51		
1-CH_3	s	3.45	3.06	+0.39
2a-CH_3	s	3.05	2.88	+0.17
5-CH_3	s	3.47	3.08	+0.39
7d-CH_3	s	3.38	3.40	−0.02
10b-CH_3	s	3.76	3.87	−0.11
3-CH_3	d [7]	1.66	1.43	+0.19-0.23
4-CH_2	m	2.3-2.6	n.a.	
4-CH_3	t [7]	1.96	1.80	+0.16
7-CH_2	m	2.3-2.6	n.a.	
8-CH_3	d [7]	1.62	1.69	−0.07-0.03
N_A-H	s, br	−0.92	1.02	−1.94
N_C-H	s, br	+0.54	1.92	−2.46

[a,b] See footnotes to Table II.

Table V. ^{1}H NMR Spectra (δ, ppm) and Differential Chemical Shifts (Δ, ppm) of Bacteriomethylpheophytin *b* (**15**) and Its Peripheral Mg^{2+} Complex (**15**)

	Multiplicity (J[H_2])	δ[a] Free **5**	δ[a] Peripheral Mg complex[b] **15**	Δ
α	s	9.29	8.64	+0.65
β	s	8.92	8.33	+0.59
δ	s	8.55	8.00	+0.55
7-H	9 [7]	4.17	4.58*	−0.41
8-H	d [7]	4.25	3.76	+0.49
10-H	s	6.51		
3-H	dd [2,7]	~4.90	4.58[c]	+0.32
3-CH_3	d [7]	1.73	1.54[c]	+0.19
4a-H	dd [2,7]	6.91	6.56	+0.35
4a-CH_3	d [7]	2.08	1.91	+0.17
1-CH_3	s	3.34	3.11	+0.23
2a-CH_3	s	3.06	2.91	+0.15
5-CH_3	s	3.39	3.11	+0.28
10b-CH_3	s	3.78	3.89	−0.11
8-CH_3	d [7]	1.63	1.70[c]	−0.07
Phy-1	d [7]	5.25	5.25	0
Phy-2	t [7]	~4.90	4.60	~+0.30
Phy-CH_3	s	0.71	0.70	+0.01
	s	0.69	0.67	+0.02
N_A-H	s, br	0.60	1.85	−1.25
N_C-H	s, br	−0.88	n.v.	

[a,b] See footnotes to Table II. [c] Assigned by comparison with **4**.

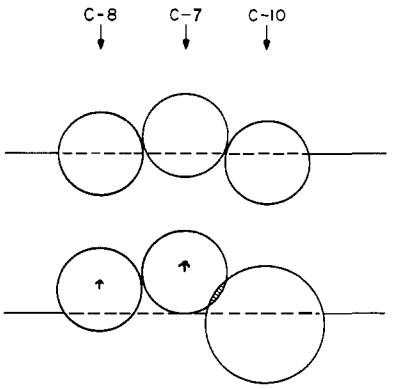


Figure 9. Schematic illustration of induced conformational changes on enolization. Carbon 7 and its propionic acid side chain and carbon 8 with its attached CH_3 and the 7-H move toward the plane of the macrocycle. See structure **1** for the stereochemistry of these carbon atoms.

$COOCH_3$ group in the peripheral complexes is such a bulky substituent, and the 7-H and the 8-CH_3 signals are indeed the only signals shifted to lower field other than the 10-$COOCH_3$ and the NH signals. Obviously, the different incremental shifts for the NH singlets are also due to pronounced local effects on one of them. The NH protons in pheophorbides are usually localized (on the ^{1}H NMR time scale) predominantly at N_A and N_C.[2a,36,37] In **2**, the N_A-H signal occurs at +0.74 ppm, the N_C-H at −1.48 ppm. From the methine shift of Δ = +0.75 ppm in the peripheral complex, a shift of −1.50 ppm is expected[36] from the decrease in ring current only on the N-H signals. This is close to the Δ = −1.70 ppm observed for the N_A-H proton. The much larger downfield shift of the N_C-H signal (Δ = 3.52 ppm) must then be due to the local steric and electron effects of chelate formation on N_C.

It should be mentioned that in the spectra of some peripheral complexes satellite signals are observed very much like the ones characteristic for chlorophyll and pheophorbides that arise from the presence of small amounts of the C-10 epimers.[8] The spacing between the main signal and its satellite, and the relative intensity of the satellites, is less in the peripheral complexes than in the free bases. However, on first view the peripheral complexes should have no satellites at all, because C-10 is planar and sp^2 hybridized. It is not entirely clear as yet whether these satellites are due to two different conformations of the system, to a slow exchange process, or some other still unspecified reason, and this point is under further investigation.

Luminescence Spectra

Formation of the peripheral complex is accompanied by a strong decrease in fluorescence. No fluorescence of the peripheral complex **12** can be detected at all with an ordinary commercial spectrofluorimeter, while there is always a considerable emission from the small amounts of free **2** present in equilibrium with **12**. With a laser excited fluorimeter, a low-intensity fluorescence band can be resolved in very concentrated solutions with λ_{max} 720 nm. The band is broadened in the same way as the absorption band of **12**, and it is therefore possibly to be ascribed to the peripheral complex. Pheophytin *a* (**1**) itself does exhibit a low-intensity fluorescence band in the same region, which could be due to a 0-1 transition.[39] Both the broad shape and the comparably high intensity do not exclude, however, a contribution of the fluorescence of **12** to this band. This is supported by an increased fine structure upon cooling to −196 °C. Preliminary attempts to detect phosphorescence of the peripheral complex in a toluene/pyridine glass at −196 °C failed, as well as attempts to detect its triplet by ESR spectroscopy.

The peripheral complex **12** is to our knowledge the first defined monomolecular chlorophyll derivative[2] which lacks a distinct fluorescence. The absence of fluorescence has been reported, too, for related chelated enols prepared recently by Eschenmoser's group.[15] The structural similarity with the enolate[1a] produced in the Molisch phase test[11] prompted us to investigate the fluorescence of this species as well. A strong decrease in fluorescence is indeed likewise observed in the Molisch intermediate, and we were unable to detect any fluorescence that could be ascribed to this product. The very pronounced drop of the luminescence intensity in the peripheral

complexes as well as in the enolate ions indicates an efficient radiationless decay mechanism. Similar findings in hydroxybenzaldehydes[40] and benzophenones[41] have been interpreted to arise from internal conversion induced by a tautomerization mechanism. The luminescence loss in the peripheral complexes may then be due to a similar process.

Titration Experiments

The peripheral complexes are unstable against water and enolizable β-dicarbonyl compounds such as acetylacetone, hexafluoroacetylacetone, or 2-carbethoxycyclopentanone. The free pheophorbide is in all cases (except for the protopheophytin *a*, see above) recovered unchanged. The reaction is a ligand exchange process at the peripheral Mg^{2+}. By the use of fluorescence, UV-vis, IR, and ^1H NMR spectroscopy the ligand exchange can be studied over the large concentration range from 10^{-7} to 10^{-2} M. In the lowest concentration range, the amount of free pheophytin can be determined from its fluorescence. Titrations of the peripheral complex **12** with water gave a sigmoidal curve for the fluorescence intensity as a function of the water concentration. The shape of the titration curve is interpreted as a displacement first of pyridine coordinated to the Mg^{2+}, and the subsequent displacement of the chelated pheophytin.

At slightly higher concentrations ($\sim 10^{-5}$ mol/L) the titration can be followed by UV-vis spectroscopy. Up to water concentrations of about 2 M, series of isosbestic points are observed over the entire spectral range. The overlapping red bands of the complex **12** and free **2** have been deconvoluted for a series of water concentrations (cf. Figure 3). During the entire titration, the envelope can be deconvoluted into two components of varying intensity. One of these corresponds to the free **2**. This component has a Gaussian line shape, is positioned at 669 nm, and has a half-width at half-height of 8.5 nm (165 cm^{-1}). The other component originates from the peripheral complex **12**, and is best fitted with a Gaussian-Lorentzian combination line shape centered at 685 nm. In the pure complex the Gaussian line width is 3650 cm^{-1}, the Lorentzian line width 673 cm^{-1} (because the lines are so broad, a fit of the energy (cm^{-1}) spectrum rather than the frequency spectrum (nm) is desirable if deconvolution with symmetric bands is used). Although the absorption maximum remains constant, the line width of the two components changes during the titration.

The ratio of the intensity differences of the bands of **2** and **12** is constant during the titration, and is equal to the ratio of the extinction coefficients of **2** and **12**. All the UV-vis data thus support the presence of only the two species **2** and **12** over the course of the entire titration. The only irregularity occurring is the change in the line shape of **12** during the titration, which might possibly be due to ligand exchange at Mg^{2+}.

At concentrations higher than 5×10^{-4} M, the equilibrium can be studied by ^1H NMR and by IR spectroscopy. In a mixture of methyl pheophorbide *a* (**2**) with its peripheral Mg complex **12**, the chlorin ligand exchange reaction is slow enough to give rise to two separate ^1H NMR spectra, even at elevated temperatures. Starting mixtures were obtained by dissociating part of the complex with a small amount of water. The resulting complex equilibrium mixture contains three competing ligands for the Mg^{2+} ion: water, pheophorbide, and pyridine. The relative amounts of **2** and **12** can be monitored by ^1H NMR from the intensity of their respective spectra. The amount of bound water can be judged from the position of the water resonance. Although the chemical shift of water is dependent on the temperature itself, the major contribution to its chemical shift seems to arise from coordination to Mg^{2+} (see above). At elevated temperatures, the concentration of the complex increases, while the concentration of bound water decreases.

In the temperature range between 20 and 70 °C, the reaction can be treated from the ^1H NMR data as an equilibrium between the peripheral complex of methyl pheophorbide *a* (**12**) and free methyl pheophorbide *a* (**2**), as indicated by the linear fit between $\ln c_{12}/c_2$ and $1/T$ (van't-Hoff's equation, correlation coefficient 0.997). After addition of 2 μL of water to a 0.007 M solution of **12** (0.5 mL), the (apparent) reaction enthalpy and entropy for complex formation are $\Delta H° = 9.4$ kcal/mol and $\Delta S° = 31$ eu/mol, respectively. Both values decrease with increasing water concentration, and after addition of 3 μL of water, values of $\Delta H° = 4.4$ kcal/mol and $\Delta S° = 10.8$ eu/mol were found.

The titration experiments indicate an increase in coordination binding energy for the three ligands present in the system with pyridine < pheophorbide < water. That pyridine must be a weaker ligand for Mg^{2+} than the pheophorbide is obvious from the fact that peripheral complex formation would otherwise not be possible. The sigmoid shape of the titration curves with water is then interpreted as the subsequent replacement of first the loosely bound pyridine and second the more strongly bound pheophorbide.

Conclusions

The data demonstrate that the peripheral β-keto ester system present in Mg-free chlorophyll derivatives with an intact isocyclic ring E binds Mg^{2+} more strongly than the central cavity of the macrocycle.[42] Complexes of chlorophyll with a variety of metal chlorides have been reported earlier by Dilung et al.[43] These complexes are likewise sensitive to water, but do not show the spectral characteristics of the peripheral complexes, and it has been suggested that they are formed by π interactions with the N atoms. In the peripheral complexes, all spectroscopic evidence supports a chelate-type binding that is characteristic of enolizable β-dicarbonyl derivatives. The potential of this functional group in chlorophylls for metal binding and enolization has been explored recently in a different approach by the group of Eschenmoser,[15] with the aid of β-diketones derived from chlorophylls ("cyclochlorophylls") in which a 10a-keto C=O group is held essentially coplanar with the 9-keto C=O group. Preliminary spectroscopic data on metal complexes of these diketones are very similar to those obtained for the peripheral metal complexes, i.e., broadened and red-shifted UV-vis spectra, loss of fluorescence, and decreased ring current induced ^1H NMR shifts.[15b]

The peripheral metal complexes are thermodynamically less stable than the central complexes with respect to demetalation and metal exchange. This is best evidenced by Zn^{2+}, which has a moderate affinity for both binding sites. Methyl pheophorbide *a*, when treated with dry $ZnCl_2$, first forms the peripheral complex in a kinetically controlled reaction, which then transforms within a few hours to the thermodynamically favored central complex. In the case of magnesium the central insertion requires carefully controlled conditions,[15a] and the complexes are thermodynamically unstable in aqueous solutions.[44] The peripheral Mg^{2+} complexes are thermodynamically even less stable; they are dissociated easily by small amounts of water and by other chelating agents that compete for the binding of Mg^{2+}. However, in the absence of competing ligands formation of the peripheral Mg^{2+} complexes is favored kinetically as compared to formation of the central complexes (cf. ref 45). A similar situation exists in linear tetrapyrroles. Formation of bile pigment metal complexes (e.g., with Zn^{2+}) in methanol is favored kinetically, as compared to complex formation by the macrocyclic porphyrins, but is not favored thermodynamically (cf. ref 46). For metal ions such as Cu^{2+} which bind readily and strongly to the central four-nitrogen site, no peripheral complexes have been observed, but central complexes form directly. The thermodynamics are controlled by the peripheral β-keto ester being a "harder" ligand than is

the central 4-N site. Thus, the hard Mg^{2+} ion forms more stable peripheral complexes than the softer ions Zn^{2+} or Cu^{2+}. On the other hand, the kinetics of insertion can be considered to be controlled by the flexibility of the two ligand sites, which is particularly unfavorable for the central 4-N site. The influence of the solvent system, and thus of the ligands at the metal ions in solution, has not been studied.

There seems to be a mutual exclusion of the two binding sites. Thus, attempts to prepare peripheral Mg^{2+} complexes of the chlorophylls themselves were unsuccessful. There is no UV-vis evidence for a dimetallic intermediate during the conversion of Zn-peripheral complex to Zn-central complex, and the final spectrum is that of the pure central Zn pheophorbide ("Zn chlorophyllide"). This mutual exclusion is probably due both to steric hindrance of the C-7 and C-10 substituents and to electrostatic repulsion of the two proximate (~6 Å) positively charged ions. Similarly, peripheral as well as central complexes have been reported for the less hindered "cyclopheophorbides" prepared by Falk et al.,[15] but none have been reported that bear two metal ions simultaneously at both the center and the periphery.

A possible physiological role for the peripheral metal complexes is yet to be defined. Self-aggregation of chlorophylls involves interaction of ring E with the central Mg^{2+} of the neighboring molecule, and such aggregates have been proposed as models for antenna chlorophyll.[1b,2b] However, studies with chemically modified chlorophylls[47] show a predominant binding to the 9-keto C=O group,[48] and indeed pyrochlorophyll *a* (the central Mg complex of **8**) shows much stronger aggregation behavior than does chlorophyll *a*.[47] These results argue against a significant role for peripheral complexation in these aggregates. This view is supported by the UV-vis spectral data on chlorophyll *a* oligomers, and by model studies indicating unfavorable steric interactions with the 7-propionic ester side chain. Chelation is more likely with metals carrying less bulky additional ligands, especially in a hydrophobic environment. Such conditions are present in large areas of the photosynthetic membrane system. It may be noted that the absorption maximum for photosystem II reaction centers[49] is very close to that of the peripheral complex **12**. While a role for peripheral complexes in photosynthesis cannot be assigned on the basis of current information, the possible participation of peripheral complexes of the pheophytins must be considered, especially in the light of the characteristic red shifts in the visible absorption spectrum caused by peripheral complex formation.

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Total Synthesis of *Cinchona* Alkaloids. 1. Synthesis of Meroquinene

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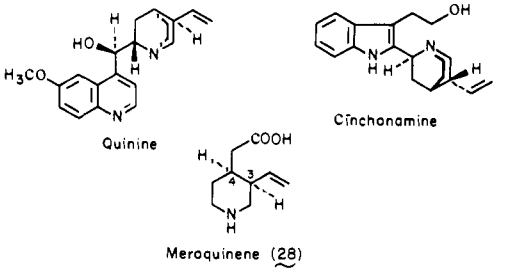
Abstract: Meroquinene (**28**), the key intermediate in several total syntheses of *Cinchona* alkaloids, has been synthesized by three methods. Starting from *cis*-2-benzoyloctahydro-6(2*H*)-isoquinolone (**1**), the acetic acid and the vinyl side chains of **28** were formed by either Baeyer–Villiger oxidation, opening of the lactone **2** to the hydroxy ester **4**, and elimination, or by Schmidt rearrangement, nitrosation of the lactam **7**, and pyrolysis. A completely stereospecific synthesis of meroquinene (**28**) was effected by catalytic hydrogenation of 3-ethyl-4-pyridineacetic acid methyl ester (**21**), followed by conversion of the ethyl group of **23** into the vinyl group by Löffler–Freytag rearrangement and elimination.

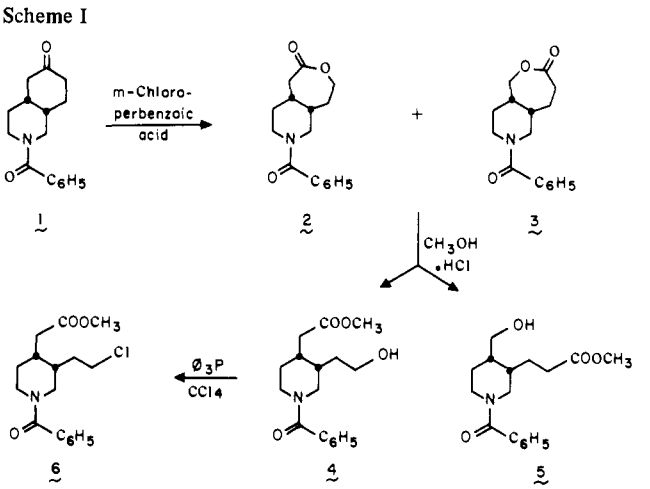
The medically important alkaloids quinine and quinidine have long been subjects of one of the most intensive structural and synthetic investigations in classical organic chemistry.[1] The original and quite elegant syntheses of these alkaloids[2-4] are unfortunately not amenable to large-scale preparation of various analogues. With such an aim in mind, we have investigated new synthetic routes to these alkaloids in the last few years. These investigations have led to several practical solutions which are reported in this paper and in the accompanying publications.

The quinuclidine ring with three chiral centers is the characteristic feature of the *Cinchona* alkaloids and elaboration of this ring system is the key for a successful total synthesis.[1,5] The two contiguous chiral centers at C-3 and C-4 have always controlled the selection of the synthetic precursors. In the classical synthesis[2] of these alkaloids, the quinuclidine ring was derived from 3(*R*)-vinyl-4(*S*)-piperidinepropionic acid (homeroquinene), obtained by the degradation of cinchonine.[6] The first total synthesis of these alkaloids was formally achieved with the synthesis of homomeroquinene itself.[3,7] All recent syntheses of *Cinchona* alkaloids,[8-11] however, utilize the corresponding nor analogue, 3(*R*)-vinyl-4(*S*)-piperidineacetic acid (meroquinene), which is also a degradation product of cinchonine.[12] In this paper, we describe in full detail the synthesis of meroquinene in its racemic form as well as in both enantiomeric modifications.



In the designing stage of the meroquinene synthesis, we were primarily concerned with the cis configuration of the two side chains. Two approaches were explored, one starting from the preformed *cis*-isoquinolone (**1**),[13] and the other in which the cis configuration was to be achieved by the hydrogenation of a pyridine precursor. In the first case, the formation of meroquinene required an oxidative fragmentation of the cyclohexanone ring of **1** to produce the acetic acid and the vinyl side chains. Baeyer–Villiger oxidation was first examined for this purpose (Scheme I). Treatment of **1** with *m*-chloroperbenzoic acid yielded the desired lactone **2** and its isomer **3** as a mixture, which could not be separated. When stirred at room temperature in dilute methanolic hydrogen chloride, the lactones opened to give the hydroxy esters **4** and **5**, which could be separated by repeated column chromatography. However, the overall yield of the desired hydroxy ester **4** from **1** was only 35%, while the isomeric **5** was obtained in 57% yield. The ester **4** was converted in high yield to the corresponding chloro analogue **6**, which was then transformed into racemic *N*-benzoylmeroquinene (**13**) (Scheme V).

Scheme I



Since an opposite regioselectivity in the ring fragmentation of **1** was to be achieved, we turned our attention to the Schmidt rearrangement[14] (Scheme II). On exposure to sodium azide in polyphosphoric acid, the ketone **1** was transformed quantitatively to a 1:1 mixture of lactams **7** and **8**, which could be separated from each other by fractional crystallization. A further improvement in the desired regioselectivity was observed in the Schmidt rearrangement of the corresponding

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