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Spectroscopic analysis of chlorophyll model complexes: methyl ester ClFe(III)pheophorbides

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As models for chlorophyll *a* (Chl *a*), methyl ester ClFe(III)pheophorbides (1, pheophorbide *a*; 2, mesopheophorbide *a*; and 3, mesopyropheophorbide *a*) were examined by Fourier transform infrared (FTIR) absorption and resonance Raman (RR) spectroscopy. The infrared (IR) chlorin band above 1600 cm^{-1}, assigned as a C_a–C_m mode (Andersson et al. (1987) J. Am. Chem. Soc. 109, 2908–2916) is shown to be metal-sensitive and responsive to spin state and coordination number for dihydroporphyrins, as well as being diagnostic for the chlorin vs. porphyrin or bacteriochlorin macrocycle. Frequency variations for this metallochlorin IR band thus parallel those of the ν_{10} RR mode of porphyrins in their predictive utility. Q_y excitation SERRS spectra of Chl *a* were compared with Q_y excitation RR spectra of 1 and methyl Ni(II)pyropheophorbide *a*. The data demonstrate that 5-coordinate ClFe(III)pheophorbides are better models for chlorophylls than are ruffled 4-coordinate Ni(II)pheophorbides. Major spectral differences between the three chlorophyll models are associated with the C-9 keto and/or C-10 carbomethoxy vibrational modes. The approx. 1700 cm^{-1} IR band was formerly assigned solely to $\nu(C=O)$ of the C-9 keto group. However, this IR feature shifts down to approx. 1685 cm^{-1} and nearly doubles in intensity when the C-10 carbomethoxy is removed, as for 3. Similar frequency downshifts coupled with intensity increases in the IR are found in the literature on chlorophylls. RR spectra of pheophorbides having the C-10 carbomethoxy group (1 and 2) have bands at both approx. 1700 and approx. 1735 cm^{-1}. However, the C-9 keto $\nu(C=O)$ mode of pyrophorbins also downshifts to approx. 1685 cm^{-1}, as in the IR spectra. The approx. 1735 cm^{-1} ester RR mode disappears in the case of pyrophorbins, and is never RR active for nonconjugated esters of porphyrins or chlorins. These data demonstrate an interaction between the C-10 and C-9 carbonyls of phorbins. They also indicate that phorbins tend toward conjugation of the C-10 ester. Biological examples of such conjugation effects have recently been reported, e.g., for the Chl *a* π-cation radical (Heald et al. (1988) J. Phys. Chem. 92, 4820–4824). Because the phorbin E ring is the major structural feature distinguishing chlorophylls from non-photosynthetic systems, the participation of the C-10 ester in ring conjugation is suggestive of its biological importance.

Introduction

Chlorophyll *a* is the primary electron donor in Photosystems I and II of green plants and blue-green algae [1–3]. The Chl *a* macrocycle is a chlorin (dihydroporphyrin *) with *trans* hydro substituents on pyrroline ring D and a cyclopentanone group fused across C-6

Abbreviations: Chl, chlorophyll; CARS, coherent anti-Stokes Raman scattering; NCA, normal coordinate analysis; SERRS, surface-enhanced resonance Raman scattering; Cu lactone, Cu(II)-5′-hydroxy-6,6′-*trans*-γ-spirolactone-2,4-dimethyldeuterochlorin(IX) monomethylester [28]; Cu-diol, Cu(II)-*cis*-3′,4′-dihydroxy-2,4-dimethyldeuterochlorin(IX) dimethylester [28]; BChl, bacteriochlorophyll (either a B- and D-ring-reduced tetrahydroporphyrin (bacteriochlorin), or a D-ring-saturated chlorin, i.e., BChls *c*, *d*, and *e* [3,11–13]); OEC, octaethylchlorin; FTIR, Fourier transform infra-red; RR, resonance Raman; pheo, pheophorbide.

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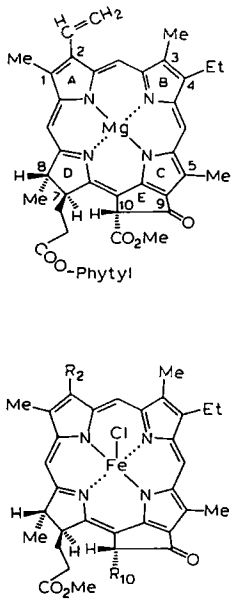
* Hydroporphyrins are saturated porphyrins, differing from the parent by reduction of one or more pyrrole rings. The pyrroline (reduced pyrrole) ring does not necessarily have hydro substituents, although this is the case for chlorophylls.



1 Methyl ClFe(III)pheophorbide g: $R_2 = -CH_2=CH_2$; $R_{10} = -COOMe$
2 Methyl ClFe(III)mesopheophorbide g: $R_2 = -CH_2CH_3$; $R_{10} = -COOMe$
3 Methyl ClFe(III)mesopyropheophorbide g: $R_2 = -CH_2CH_3$; $R_{10} = -H$

Fig. 1. (A) Chlorophyll *a*; (B) methyl ester ClFe(III)pheophorbides.

and γC_m as ring E (Fig. 1). The isocyclic E ring is the major structural feature that distinguishes phorbins * from non-photosynthetic systems [4]. This ring contains a β-keto ester; the C-9 keto moiety is subject to enolization and the C-10 hydrogen will exchange with solvent [5,6]. The isocyclic ring introduces considerable strain into the macrocycle, particularly into the bond between the C ring and the γ-carbon [7]. Steric crowding between the bulky C-7 and C-10 substituents [8] increases strain in the periphery of the macrocycle, inducing conformational changes in the D and E rings and to some extent perturbing the entire phorbin macrocycle. These conformational changes are transmitted to the bulk macrocycle via the π-bonding network [1–3]. However, removal of the C-10 carbomethoxy group to yield the 'pyro' complexes releases some of the strain.

Chlorophylls are well-defined structurally [1–3,9–13] and have been extensively studied by NMR [8,14–18] and IR spectroscopy [19–22]. RR spectroscopy is an additional method of structural analysis that has proven valuable in the study of biological and model tetrapyrrolic systems [23,24]. Extension to metallochlorins has led to the establishment of characteristic and diagnostic vibrational properties that are applicable to both biological and model metallochlorin complexes, regardless of the identity of the central metal ion, the pattern of pyrrole ring or *meso* substituents, or the chemical nature of the substituents [25–33].

* The basic chlorophyll macrocycle (a tetrapyrrole with a fused cyclopentanone E ring) is called a phorbin.

A number of investigators have reported RR spectra of chlorophylls [19,34–39]. Because of the long-wavelength fluorescence of chlorophylls, such data have generally been limited to excitation near the Soret band. However, Hoxtermann et al. [40] have used CARS spectroscopy to obtain red (Q_y) excitation spectra of Chl *a* and Chl *b*. Recently, Bocian and co-workers have reported Soret (B), Q_x, and Q_y RR spectra of methyl Ni(II)pyropheophorbide complexes, with a detailed normal coordinate analysis (NCA) of the RR bands observed [41].

The relevance of data for Ni(II)tetrapyrrolic complexes as models for chlorophylls is potentially limited due to at least two factors. First, chlorophylls in vivo are generally pentacoordinate [1–3], whereas the Ni(II)pyropheophorbides studied above were tetracoordinate [41]. The Ni(II) coordination state is known to affect RR frequencies of marker bands for porphyrins [42–44] and also for hydroporphyrins [45,46]. Second, and possibly a more serious limitation, is that introduction of a nickel ion into the chlorophyll macrocycle induces profound variations in the conformation of the aromatic phorbin nucleus and of the pyrroline ring relative to the effects of a central magnesium ion [18]. In general, conformational changes elicited by Ni(II) appear to be particularly strong for hydroporphyrins [47–53]. For example, the *cis*- and *trans*-isomers of Ni(II)octaethylchlorin (OEC) are conformationally perturbed relative to the Mg, Zn, and free-base OEC complexes [52,53]. In fact, RR spectra of *cis*- and *trans*-Ni(OEC) complexes are significantly different [29]. Similarly, alterations of macrocyclic conformation for Ni(II)porphyrins, even in the absence of coordination number changes, are known to produce shifts in the frequencies of resonance Raman bands [54,55].

In contrast, Chl *a* and methyl ester ClFe(III)pheophorbides are both pentacoordinate and both have a weak-field fifth ligand. Furthermore, the metal ion of ClFe(III)pheophorbides is expected to be out of the plane, as is the case for ClFe(III)protoporphyrin IX dimethylester (0.48 Å) [56], providing a further parallel to the Mg(II) in chlorophylls (0.39 Å for Mg-Chl *a* [9,10]). Thus, we have examined pentacoordinate methyl ClFe(III)pheophorbides as spectroscopic models for chlorophylls; pheophorbide *a* (**1**; Fe-pheo *a*); mesopheophorbide *a* (**2**; Fe-mesopheo *a*); and mesopyropheophorbide *a* (**3**, Fe-mesopyropheo *a*). This series of complexes permits us to evaluate the respective spectral effects of the C-2 vinyl substituent, the C-10 carbomethoxy substituent, and the C-9-keto moiety. Electronic absorption, FTIR, and RR spectra of the three complexes are compared with those of chlorophylls and their derivatives as well as those of methyl Ni(II)pyropheophorbides, to evaluate directly the spectral effects of the substituents and the central metal ion. We have also obtained a red [Q_y] excitation spectrum of

Chl *a* with SERRS, which compared favorably with a similarly excited RR spectrum of Fe-pheo *a*.

Experimental procedure

Methyl pheophorbide *a* (C-2 vinyl, C-10 carbomethoxy), methyl mesopheophorbide *a* (C-2 ethyl, C-10 carbomethoxy), and methyl mesopyropheophorbide *a* (C-2 ethyl, C-10 hydro) were prepared by degradation of Chl *a* from the algae *Spirulina maxima* [57]. Iron insertion followed the method of Smith et al. [58]. Methyl Ni(II)pyropheophorbide *a* (**4**, Ni-pyropheo *a*; C-2 vinyl, C-10 hydro) was prepared according to the Ni(II) acetate method [57]. Electronic absorption spectra were obtained on a Perkin-Elmer Lambda 9 spectrophotometer from samples in acidified CH_2Cl_2 solution. FTIR spectra were acquired from KBr pellets (approx. 1 mg compound: 200 mg KBr) with a Perkin-Elmer Model 1800 FTIR instrument. RR spectra were obtained from samples in a KBr matrix (approx. 1 mg : 200 mg KBr) packed into the annular groove of a spinning sample-holder (approx. 25 mm diameter), using a backscattering geometry [28]. Spectra-Physics 164-05 Ar, 164-01 Kr and 2025-11 Kr ion lasers were used. The computer-controlled Jarrell Ash 25-300 Raman spectrophotometer and data-reduction programs were previously reported [59]. The computer system for the spectrophotometer has been upgraded to an RMX86-based Intel 310 running our own revised Fortran 77 data collection and analysis programs. SERRS spectra of Chl *a* were obtained with 647.1 nm excitation (50 mW). Chl *a* was deposited by spin-coating 200 μl of solution ($1 \cdot 10^{-4}$ M in pyridine) onto a roughened silver film. The film was prepared by vacuum depositing 2000 Å of Ag on top of a 1000 Å layer of CaF. Glass microscope slides were used as the support [60].

Results

Electronic absorption spectroscopy

Fig. 2 presents the electronic absorption spectra of the methyl ester ClFe(III)pheophorbide complexes. Their absorption bands are listed in Table I, together with those of chlorophylls [61–66] and those of methyl Ni(II)pyropheophorbides [41]. The largest spectral change occurs upon loss of the C-2 vinyl substituent: a 4 nm blue-shift of the Q_y band for the methyl ester ClFe(III)pheophorbides vs. a 14 nm blue-shift for the methyl Ni(II)pyropheophorbides (Table I). In the Soret region, the broad, doubly degenerate [67] 392 nm B transition of Fe-pheo *a*, **1**, is partially split upon loss of the C-2 vinyl in Fe-mesopheo *a*, **2**. In contrast, removal

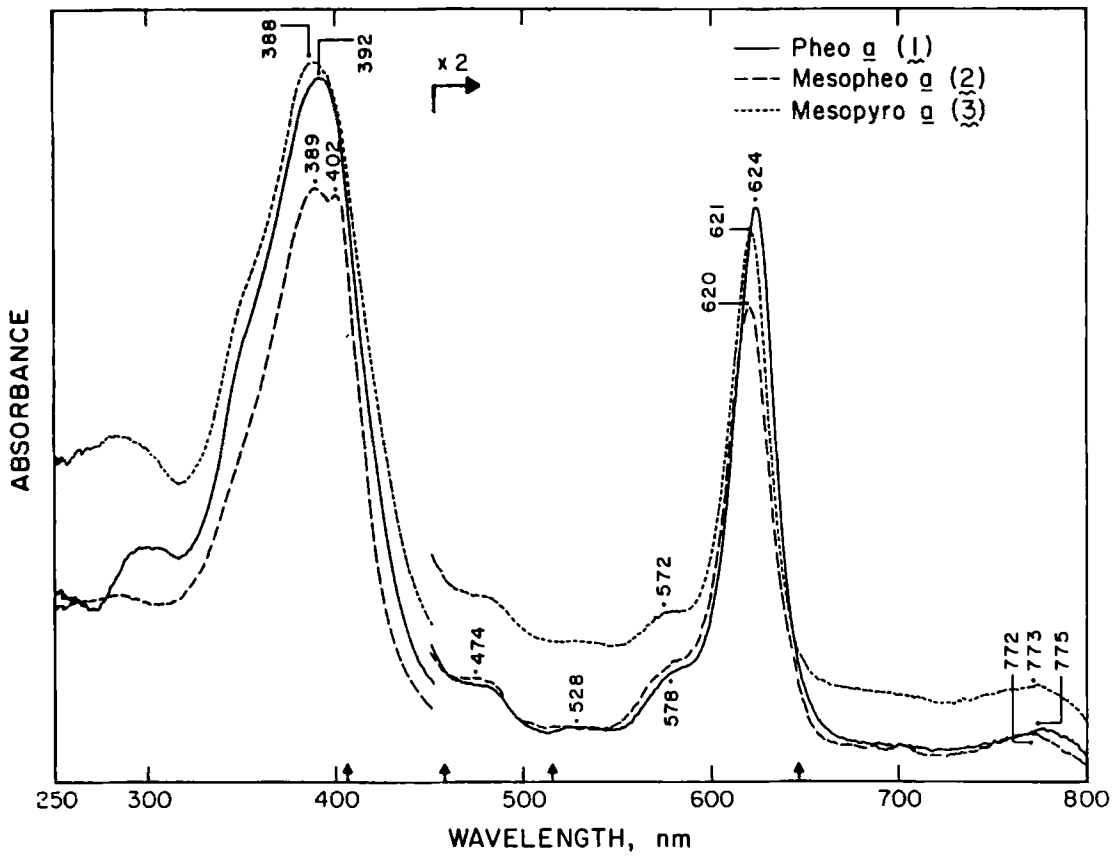


Fig. 2. Electronic absorption spectra of the methyl ester ClFe(III)pheophorbide complexes in acidified CH_2Cl_2.

TABLE I

Electronic absorption vs. structural features of chlorophylls and methyl pheophorbide complexes in CH_2Cl_2 solution

Compound	Absorption bands (nm)		Structural change	Refs.
Mg				
Chl *a*	660.5	428.5		61–63
Pyro-Chl *a*	659.5	429.0	C-10 COOMe → H	61,64
MethylChlorophyllide *a* (Mg-pheo *a*)	660.5	427.5	C-7 phytol → methyl	63
MethylpyroChlorophyllide *a* (Mg-pyropheo *a*)	659	428	C-7 phytol → methyl C-10 COOMe → H	63
Chl *b*	642	453	C-3 methyl → CHO	65
ProtoChl *a*	623	432	ring D → pyrrole (= porphyrin)	65,66
4-vinyl protoChl *a*	622	437	C-4 ethyl → vinyl ring D → pyrrole	66
Fe				
1, Pheo *a*	624	392		this work
2, Mesopheo *a*	620	389, 402	C-2 vinyl → ethyl	this work
3, Mesopyropheo *a*	621	388	C-2 vinyl → ethyl C-10 COOMe → H	this work
Ni				
4, Pyropheo *a*	650	422, 396	C-10 COOMe → H	this work, Ref. 41
5, Mesopyropheo *a*	636	416, 394	C-2 vinyl → ethyl C-10 COOMe → H	41
6, Desoxomesopyropheo *a*	608	398	C-2 vinyl → ethyl C-10 COOMe → H C-9 keto → $\angle$H	41

of the C-10 carbomethoxy group, to yield the 'pyro' phorbins has only a very minor effect on the absorption spectra.

FTIR spectroscopy

The 2600–3200 cm^{-1} FTIR spectra of the methyl ClFe(III)pheophorbides are shown in Fig. 3. All three complexes display bands * at 2870, 2926 and 2960 cm^{-1}, analogous to the C–H stretching modes of aliphatic substituents on porphyrins [68]. For chlorophylls, the substituent modes in this spectral region are obscured by C–H stretching modes of the phytyl moiety [20].

The 400–1800 cm^{-1} FTIR spectra of **1–3** are shown in Fig. 4. Selected IR features are listed in Table II, along with the analogous bands of Ni-pyropheo *a* and of chlorophylls. The IR bands of the methyl pheophorbides are generally similar to those of other metallochlorins [26–28], and to those of chlorophylls [19,20]. The spectra are considerably more complex than those of higher symmetry porphyrins [26–28]. Comparison of the IR spectra of Fe-pheo *a* with those of the mesopheophorbides **2** and **3** reveals few major differences attributable to the C-2 vinyl substituent. On

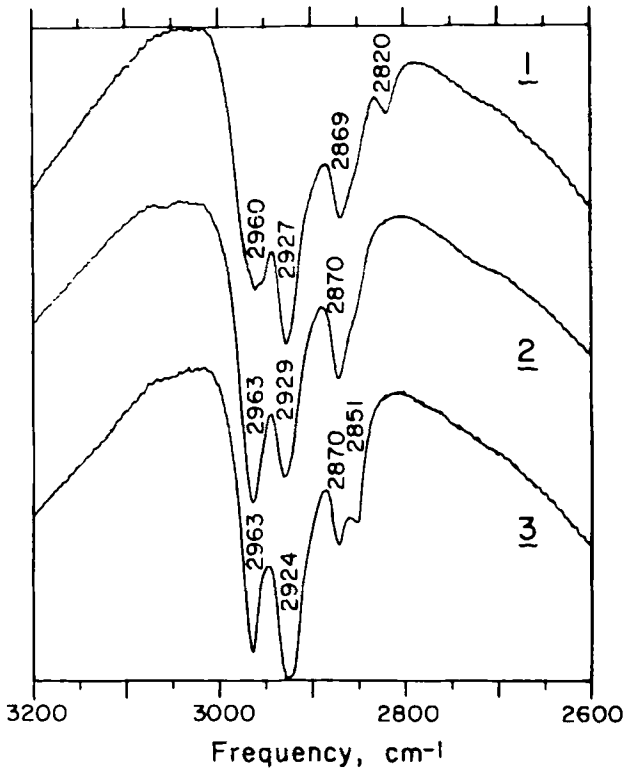


Fig. 3. C–H stretching region FTIR spectra of the methyl ester ClFe(III)pheophorbides.

* Unless otherwise stated, frequency values are approximate.

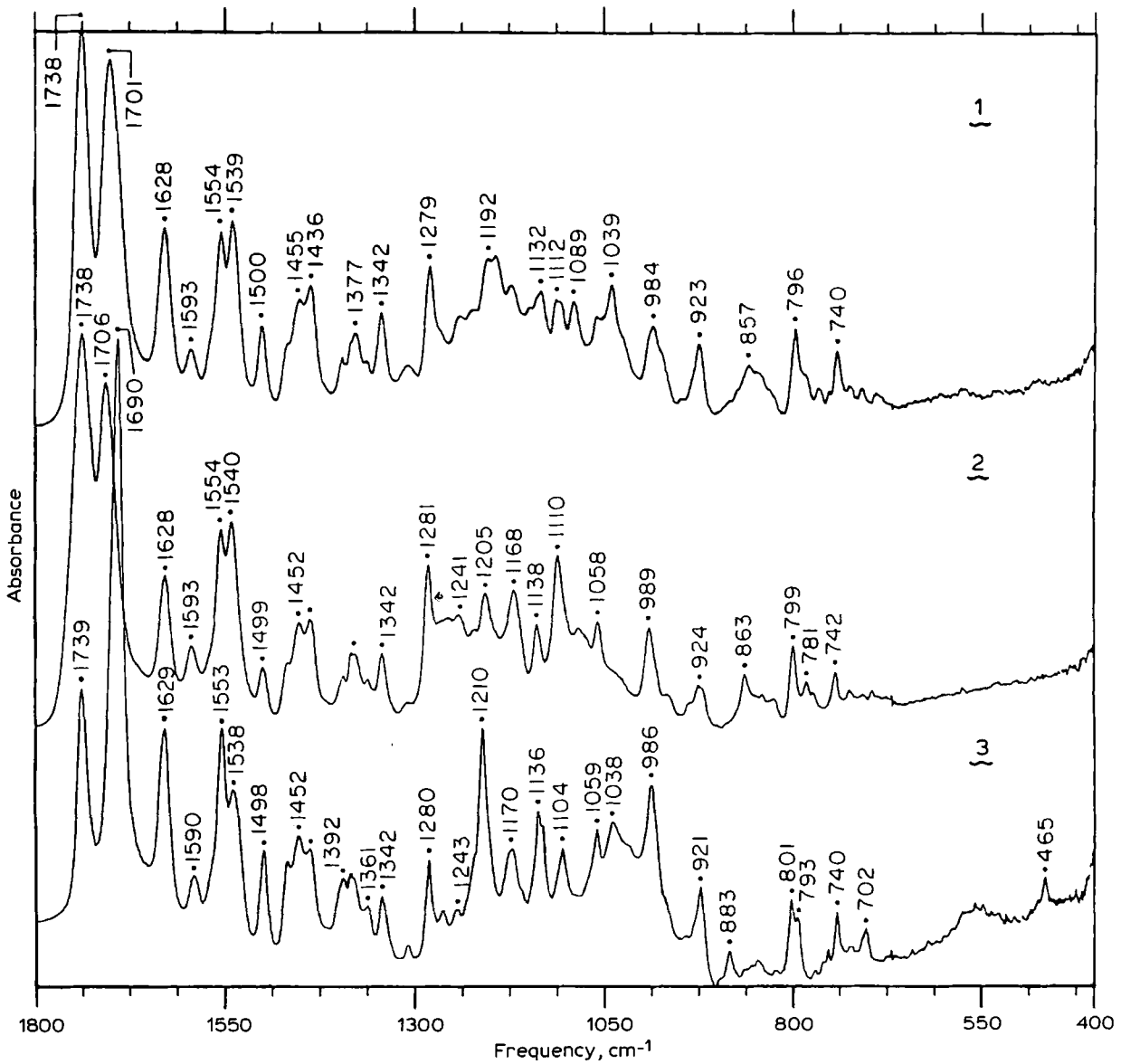


Fig. 4. 400–1800-cm^{-1} FTIR spectra of the methyl ester ClFe(III)pheophorbides plotted in absorption mode.

the other hand, the IR spectrum of Fe-mesopyropheo *a* exhibits several clear differences relative to the C-10 carbomethoxy-bearing pheophorbides **1** and **2** (vide infra).

The characteristic IR 'chlorin band', observed above 1600 cm^{-1} for β-pyrrole-substituted dihydroporphyrins but not for the 'parent' porphyrin [14,26–28,69,70], was assigned as a C_a–C_m stretching mode by our laboratory on the basis of isotopic substitution [28]. As shown in Table III, the frequency of this band is clearly metal-sensitive. The chlorin C_a–C_m mode above 1600 cm^{-1} is also sensitive to spin state and coordination number for ferric dihydroporphyrins. Thus, the strong IR band of tetrapyrrolic systems above 1600 cm^{-1}: (1) is indicative of the dihydroporphyrin macrocycle rather than a porphyrin or bacteriochlorin [14,69]; (2) aids in identification of the central metal ion; and (3) provides information concerning the spin state and coordination number of the central metal ion. The frequency variations of this IR 'chlorin marker' band (Table III), thus, take on additional diagnostic features, paralleling those of ν_{10} in the RR spectra of metalloporphyrins [23,24]. The structural sensitivity of the chlorin band in the IR may be understood from the NCA of metallochlorins reported by Bocian and co-workers [41]. The highest frequency C_a–C_m stretching mode of chlorins was shown to originate from a linear combination of the parent ν_{10} (RR-active) and ν_{37} (IR-active) modes [41]. The decreased effective molecular symmetry of metallochlorins eliminates mutual exclusion for the IR- and RR-active vibrational modes, apparently resulting in the sensitivity of the IR chlorin band shown here.

However, in the use of the IR chlorin band, it should be remembered that vibrational frequencies are depen-

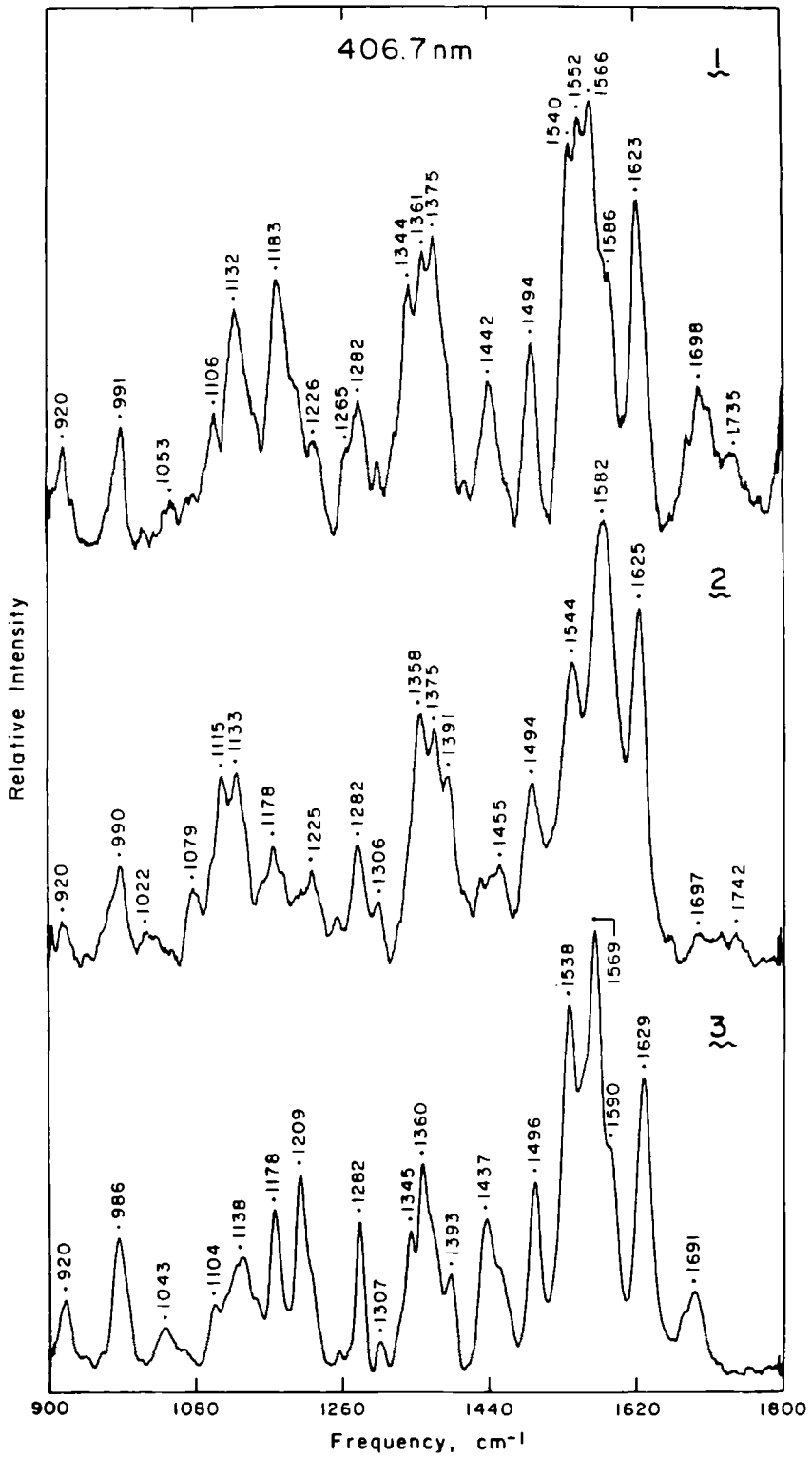


Fig. 5. High-frequency 406.7 nm excitation RR spectra of the methyl ester ClFe(III) pheophorbides. Conditions: samples about 1:200 mg KBr backscattering geometry; laser power, 25 mW at sample; room temperature; scan rate, 1 cm^{-1}/s with repetitive scanning; slitwidth, 5 cm^{-1}.

dent on macrocyclic conformation. This is exemplified by Ni(II)OEP where ν_{10} occurs at 1640 cm^{-1} for the D_{4h} form and at 1660 cm^{-1} for the D_{2d} conformers [54,55]. We have observed that the IR chlorin band is responsive to the pyrroline substituent character, as shown by the frequency difference between the Cu-lactone and Cu-diol chlorins (Table III). Furthermore, as shown in Table III, the IR chlorin band is even sensitive to pyrroline stereochemistry, varying between *cis*-Cu(OEC) and *trans*-Cu(OEC).

Katz et al. [20] have originally noted a 1550 cm^{-1} IR band of chlorophylls that was absent from porphyrin or

TABLE II

Selected IR frequencies (cm^{-1}) and intensities of phorbins [a]

Fe				
1, Pheo *a* [b]	1738 (100)	1701 (90)	1279 (37)	1192 (39)
2, Mesopheo *a* [b]	1738 (100)	1706 (88)	1281 (41)	1205 (34)
3, Mesopyro *a* [b]	1739 (50)	1690 (140)	1280 (27)	1210 (56)
Ni				
4, Pyropheo *a* [b]	1735 (50)	1690 (82)	1281 (31)	1217 (54)
Mg				
Chl *a* [c]	1739 (100)	1696 (110)	1288 (68)	~1210 (47)
Pyro-Chl *a* [c]	1737 (50)	1688 (150)	1287 (46)	~1210 (57)
Chl *b* [c]	1740 (100)	1702 (114)	1290 (67)	~1205 (51)
C-Chl [c,d]	1735 (50)	1685 (135)	n.a. [e]	n.a.

[a] Intensities as % in parentheses, with the 1735 cm^{-1} ester C = O defined as 100% for C-7 and C-10 diester phorbins and as 50% for the pyrophorbins (monoesters).
[b] This work.
[c] Monomeric chlorophylls [20,67].
[d] *Chlorobium* chlorophylls have no C-10 ester and are thus pyrophorbins [3].
[e] n.a. = not available.

bacteriochlorin spectra and have suggested that this IR feature might be characteristic of chlorins. Indeed, there is a 1550 cm^{-1} band in the IR spectra of **1–3**, that parallels similar features in IR spectra of other metallochlorins [27]. The weak 1590 cm^{-1} IR band of **1–3** is also weak in IR spectra of chlorophylls and other phorbins [20]. Wetherell [70] has noted that the IR intensity of the 1590 cm^{-1} band of metallochlorins increased significantly for macrocycles without substituents at the γ-position. Our IR studies of a variety of metallochlorins support this proposal (Ref. 28; Andersson, L.A. and Loehr, T.M., unpublished data).

RR spectroscopy

RR spectra of the methyl ClFe(III)pheophorbides are shown in Figs. 5–8. Red excitation [Q_y] SERRS spectra of Chl *a* together with RR spectra of Fe-pheo *a* and Ni-pyropheo *a* (all three complexes bearing a C-2 vinyl) are shown in Fig. 9 *. The RR spectral properties of the phorbins are in general agreement with those of other metallochlorins [25–33]. They are also similar to previously reported RR spectra of chlorophylls [34–40] and methyl Ni(II)pyropheophorbides [41].

The major effect of varying the RR excitation line for these and other chlorins [25–28,41] is altered spectral intensities. For example, the strong 1627 cm^{-1} features present in the 406.7, 457.9 and 514.5 nm excitation spectra of the pheophorbides (Fig. 5–7) undergo

TABLE III

The vibrational frequencies (cm^{-1}) of the IR 'chlorin band'

Metal ion	IR frequency (cm^{-1})	Ref.
Free base	~1610–1620	70
Pheophytin *a*	1618	20
Pheophytin *b*	1618	20
Mg(II) (5-coordinate)		
Chl *a*	1608	20,66
Chl *b*	1608	20,66
Mg(II) (6-coordinate)		
$(OEC)(py)_2$	1610	71
ClFe(III) (5-coordinate)		
1, Pheo *a*	1628	this work
2, Mesopheo *a*	1628	this work
3, Mesopheo *a*	1629	this work
(OEC)	1630	71
pPPIX, isomer B [a]	1632	26
pPPIX, isomer A [a]	1635	this work
Diol chlorin	1635	this work
Lactone chlorin	1638	this work
Deuterohemin	1632	this work
Sulfhemin-C	1634	this work
$(CN)_2$Fe(III) (6-coordinate)		
$(CN)_2$sulfheme-C	1642	this work
$(CN)_2$Fe-pheo *a*	1642	this work
Ni(II) (4-coordinate)		
4, pyropheo *a*	1660	this work
trans-OEC	1649	this work
Zn(II) (4-coordinate)		
OEC	~1630	71
Cu(II) (4-coordinate)		
Lactone chlorin	1652	28
cis-(OEC)	1646	this work
trans-(OEC)	1632	this work
MeOEC	1642	this work
Diol chlorin	1642	28
meso-d_4 diol chlorin	1632	28

[a] pPPIX, photoprotoporphyrin IX DME.

a dramatic loss in intensity with Q_y excitation, and become weak bands at 1624 cm^{-1} (Figs. 8 and 9). Similar behavior was observed for the 1612 cm^{-1} C_a–C_m mode of Ni(II)-tetramethylchlorin [27] **. Comparison of red excitation SERRS spectra of Chl *a* (Fig. 9) with RR data for Chl *a* obtained with B or Q_x excitation [34–36] again reveals primarily intensity, not frequency, differences. The Q_y SERRS spectrum of Chl *a* displays

* We have noted that the exact energy of the excitation line used in the red spectral region relative to the Q_y transition of OEC complexes has no effect on Raman frequencies or relative intensities, an observation that greatly facilitates comparisons between metallochlorin spectral data.

** The 1612 cm^{-1} band of Ni(II)tetramethylchlorin was assigned as a C_a–C_m mode on the basis of shifts observed upon *meso*-deuteration [27]. The NCA of Boldt et al. [41] indicates two C_a–C_m modes for chlorins in this region. Thus, the decreased intensity and frequency shift above 1600 cm^{-1} observed for chlorins with Q_y excitation suggests that one of the two C_a–C_m modes is maximally enhanced with Soret (B) excitation (and loses intensity with Q_y excitation), whereas the other C_a–C_m mode is only clearly resolved with Q_y excitation.

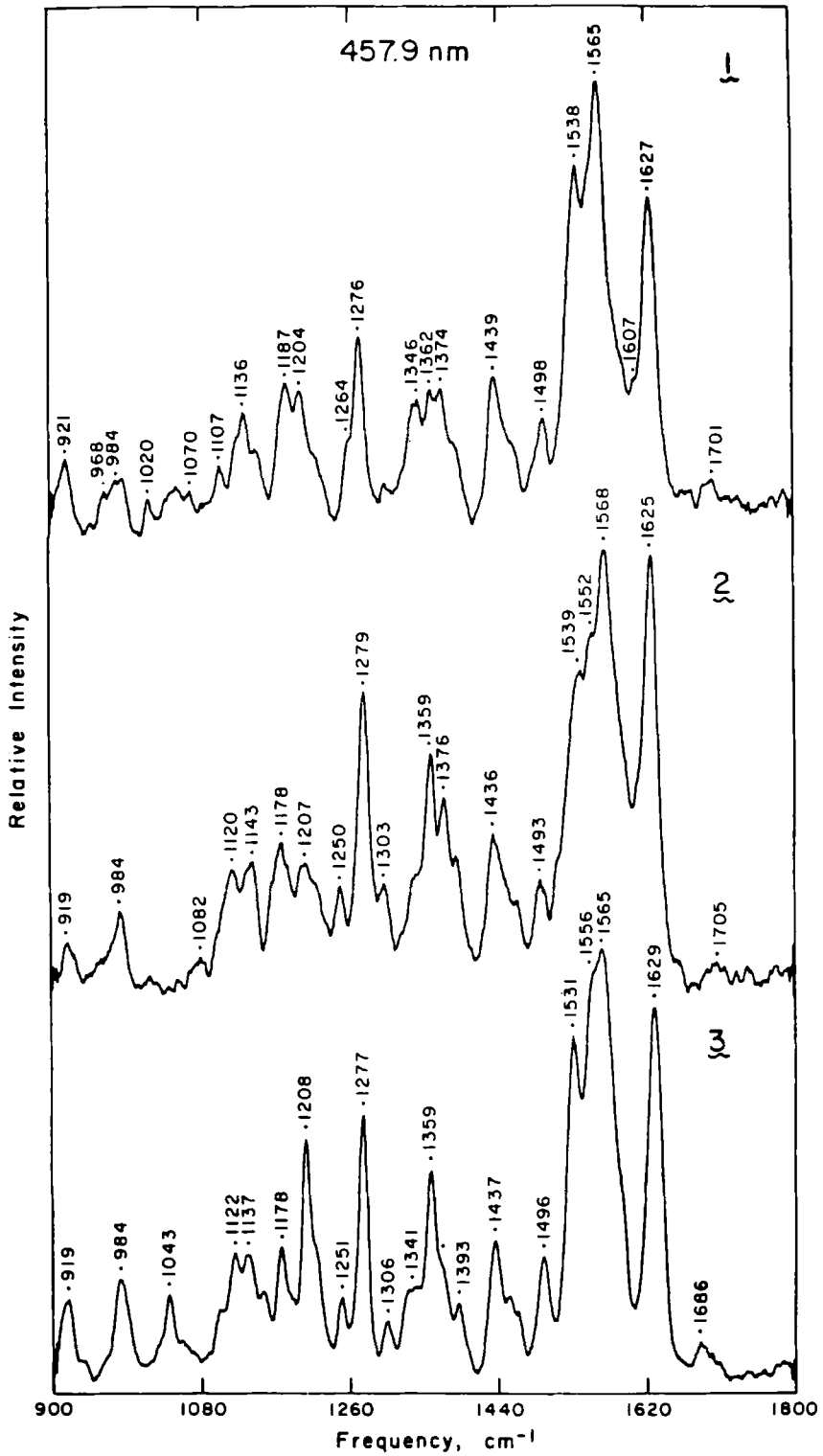


Fig. 6. High-frequency 457.9 nm excitation RR spectra of the methyl ester ClFe(III)pheophorbides. Conditions: samples about 1 mg: 200 mg KBr; backscattering geometry; laser power, 50 mW at sample; room temperature; scan rate, 2 cm^{-1}/s with repetitive scanning; slitwidth, 5 cm^{-1}.

a cluster of strong bands in the approx. 1140–1300 cm^{-1} region, like those of Fe-pheo *a* (Fig. 9). Similar features are also seen for methyl Ni(II)pyropheophorbides with Q_y excitation (Fig. 9) [41].

There is a strong 988 cm^{-1} band in the Q_y excitation RR spectra of the methyl ester ClFe(III)pheophorbides, **1**–**3**, Chl *a*, and **4**, Ni-pyropheo *a* (Figs. 8 and 9). This feature was not observed in previous Q_y spectra of methyl Ni(II)pyropheophorbides [41], due to a masking by sulfate used as an internal standard. A 989 cm^{-1} band was, however, calculated and assigned for phorbins as a combination of C_m–C_a–N deformation and

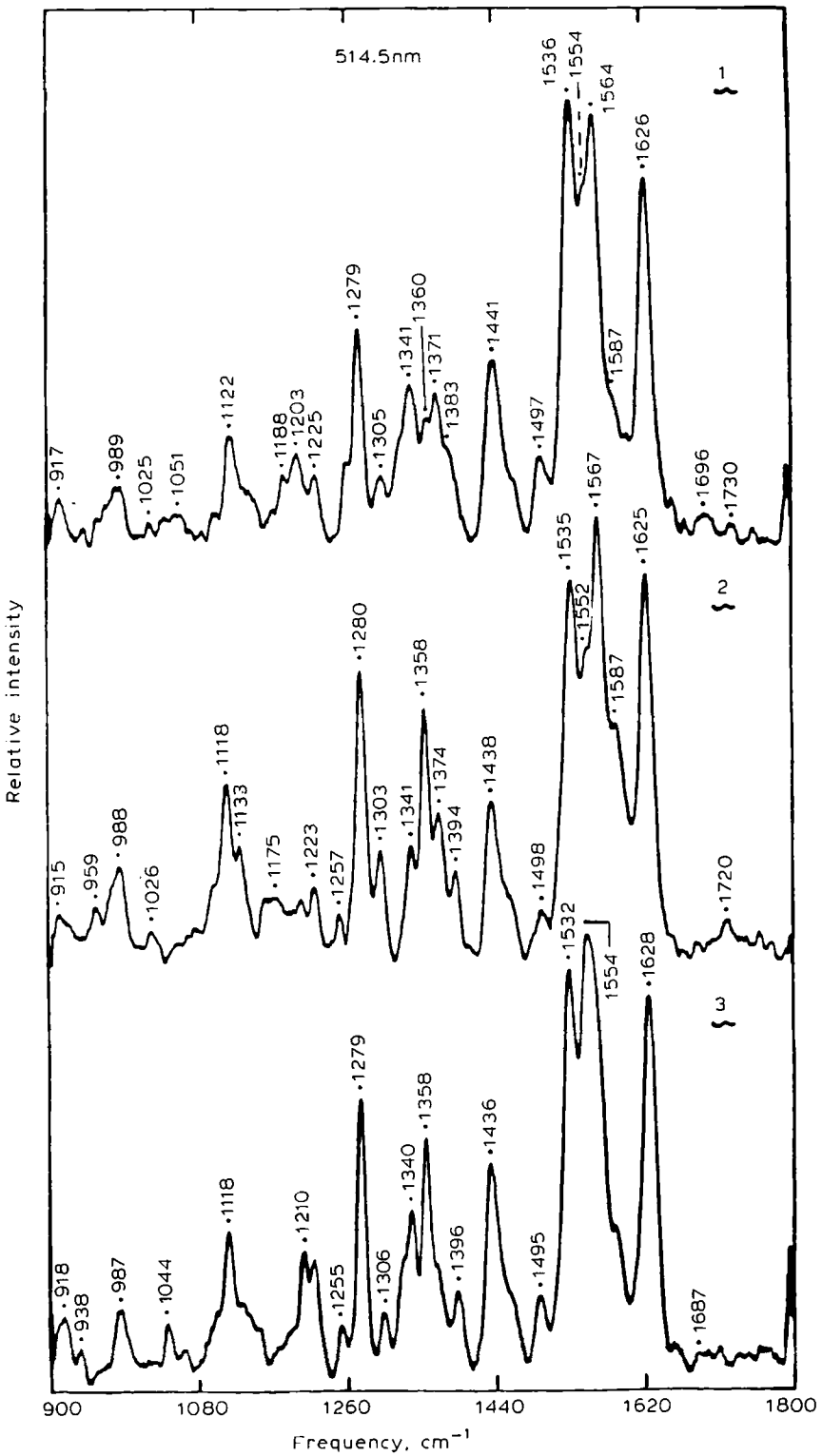


Fig. 7. High-frequency 514.5 nm excitation RR spectra of the methyl ester ClFe(III)pheophorbides. Conditions: samples, about 1 mg: 200 mg KBr; backscattering geometry; laser power, 110 mW at sample; room temperature; scan rate, 2 cm^{-1}/s with repetitive scanning; slitwidth, 5 cm^{-1}.

Ring E, C_9–C_{10}, stretching modes [41]. Such a band is absent from Q_y excitation spectra of many metallochlorins [26–28]. However, the Q_y RR spectrum of Cu-lactone has a strong single band at 949 cm^{-1} [28]. Q_y excitation RR data for the isolated sulfhemin-C chlorin, which has a novel cyclic thioether attached to pyrroline ring B [72–75], have a strong 970 cm^{-1} feature (Andersson, L.A., Loehr, T.M., Chatfield, M.J. and La Mar, G.N., unpublished data). These observations suggest a possible correlation between the observation

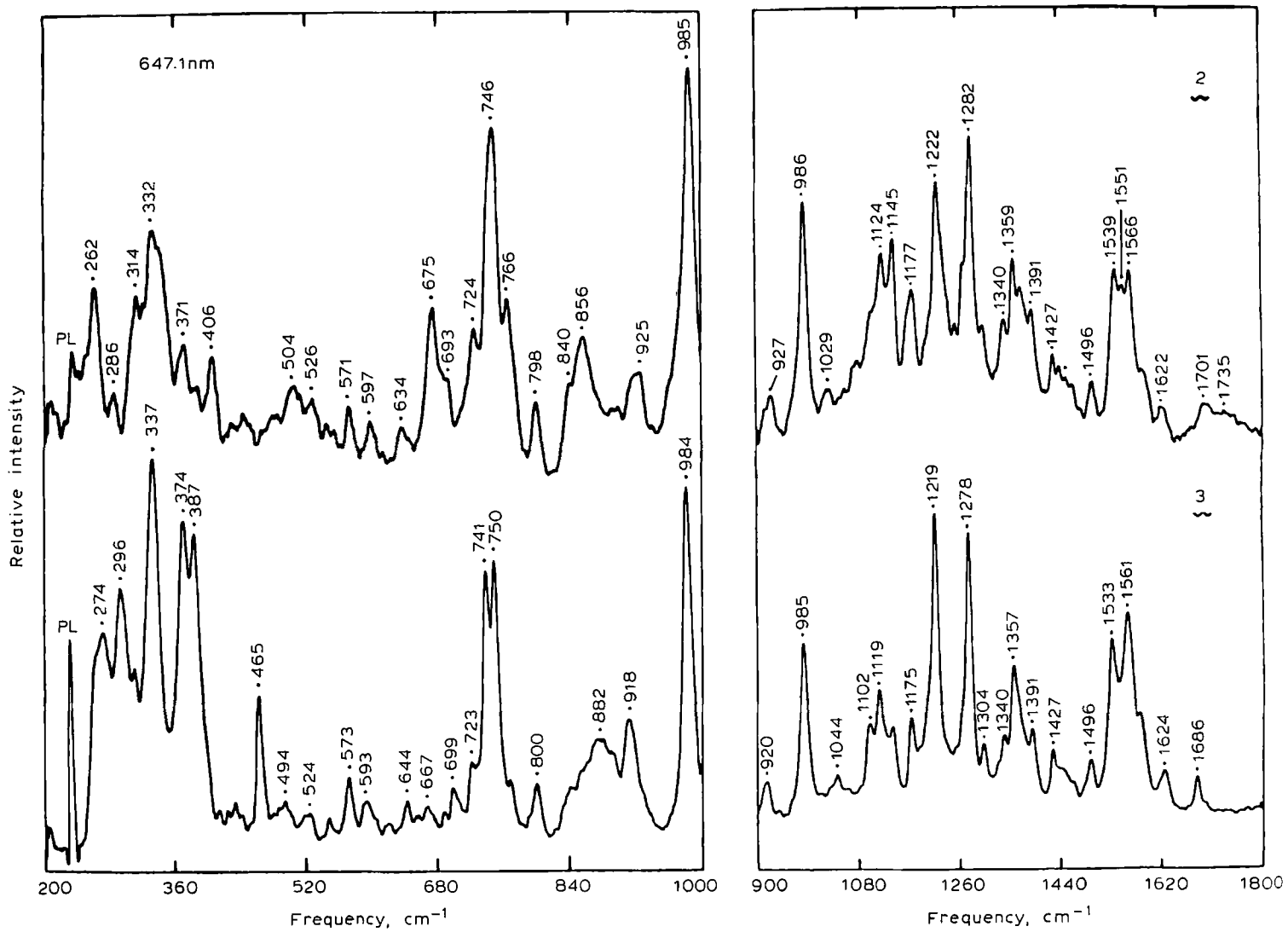


Fig. 8. 647.1 nm excitation RR spectra of 2, Fe-mesopheo *a*, and 3, Fe-mesopyro *a*. Conditions: samples about 1 mg: 200 mg KBr; backscattering geometry; laser power, 125 mW at sample; room temperature; scan rate, 2 cm^{-1}/s with repetitive scanning; slitwidth, 5 cm^{-1}.

of a single strong Q_y-enhanced RR band in the 950–1000 cm^{-1} region and the presence of exocyclic rings on metallochlorins.

Low-frequency Raman spectra

Low-frequency (lower than 900 cm^{-1}) Raman modes of metalloporphyrins are optimally enhanced with Soret excitation [24,76,77]. Similarly, 406.7 nm excitation provides strong enhancement for low-frequency chlorin modes [30–33]. However, as shown in Figs. 8 and 9, Q_y excitation also leads to very strong enhancement of the low-frequency modes of metallochlorins [28,41].

The Fe–Cl stretching mode of ClFe(III)OEC was assigned to a 361 cm^{-1} band [30–32]. With Q_y excitation, a 363 cm^{-1} feature is present for Fe-pheo *a* but not for Fe-mesopheo *a* or Fe-mesopyropheo *a* (Figs. 8 and 9). However, Q_y excitation RR spectra of four-coordinate Ni(OEC) and Ni(II)pyropheophorbide complexes also exhibit 361 cm^{-1} bands (Fig. 9) [41], indicating more than one origin for RR bands in this region. For Chl *a*, fairly intense 310 and 350 cm^{-1} RR features, assigned as Mg-sensitive (ligand) modes with B and Q_x excitation [34–36], are only weakly enhanced in the Q_y excitation SERRS spectrum (Fig. 9). Thus, it appears that low-frequency features such as macrocyclic deformation modes (vide infra) are more strongly enhanced with Q_y excitation than metal–ligand vibrations.

Discussion

The vinyl substituent

For chlorophyll compounds, loss of the C-2 vinyl induces a 5–14 nm blue-shift in the electronic absorption spectra [64]. There is a 4 nm spectral difference

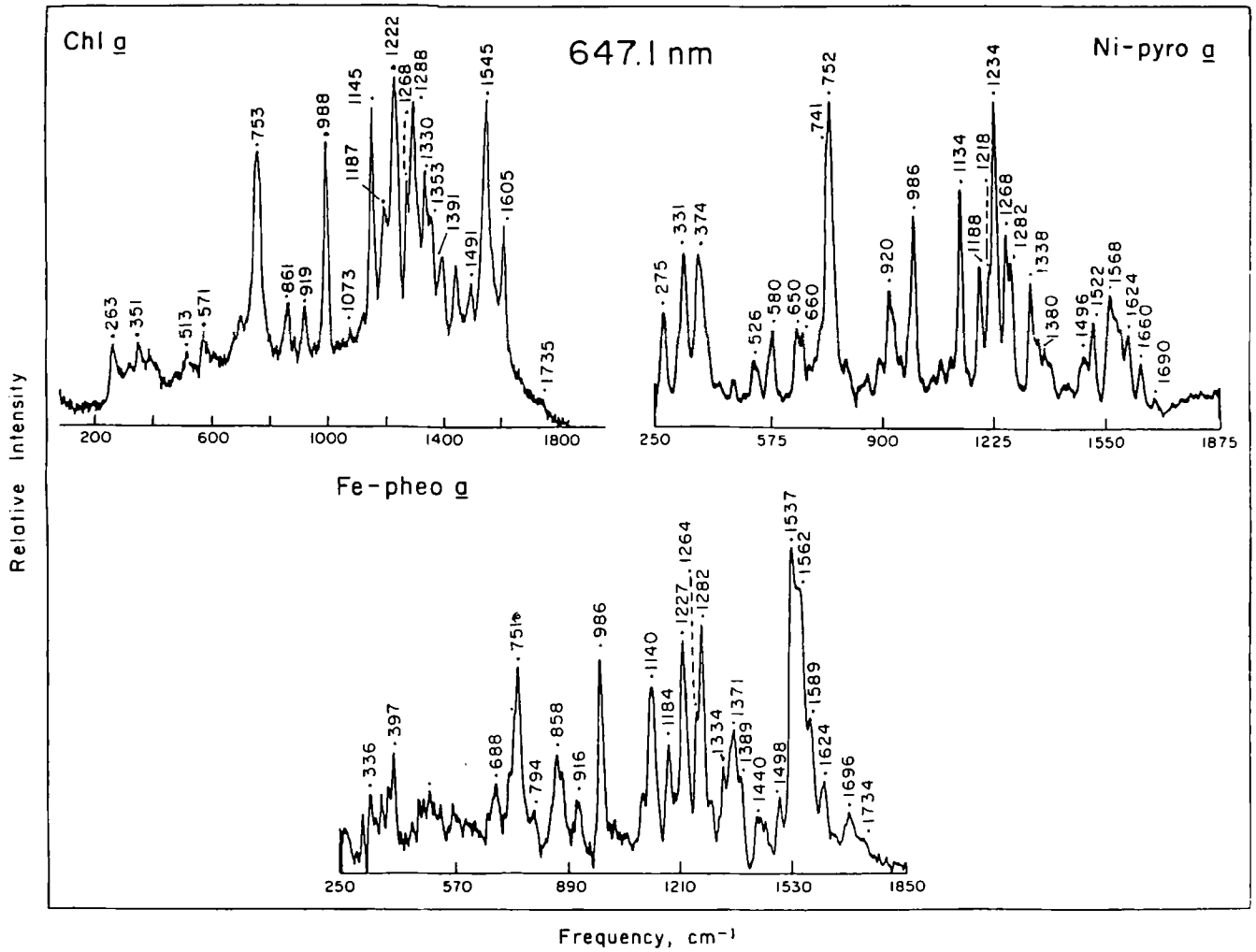


Fig. 9. Low- and high-frequency 647.1 nm excitation SERRS and RR spectra of Chl *a*, methyl ClFe(III)pheophorbide *a* (Fe-pheo *a*), and methyl Ni(II)pyropheophorbide *a* (Ni-pyro *a*). Conditions for pheophorbides: samples ~1:200 mg KBr; backscattering geometry; laser power 70 mW at sample; room temperature; scan rate, 1 cm^{-1}/s with repetitive scanning. Conditions for CHl *a*: see Experimental procedure.

between the Q_y bands of methyl ester ClFe(III)pheophorbides **1** and **2** (Fig. 2). In contrast, there is a 14 nm blue-shift for the Q_y band of methyl ester Ni(II)pyropheophorbides **4** and **5** (Table I). The Q_y electronic transition of model and biological metallochlorins shifts with changes in coordination number as well as oxidation state [25,26,67,69,72], as is typical of metalloporphyrins. Thus, the disparity in the vinyl sensitivity for this feature between Fe- and Ni-pheophorbides could arise from their differing coordination number (penta- vs. tetracoordinate, respectively). Alternatively, it could be due to the conformational distortion and consequent perturbation of conjugation experienced by Ni-substituted phorbins and hydroporphyrins [18,47–53].

A characteristic feature of phorbins, relative to other chlorins, is broadening or splitting of the doubly degenerate Soret band, apparently because the isocyclic ring distorts the π-system [67]. This splitting is clearly evident for Fe-mesopheo *a*, **2**, compared with methyl ClFe(III)pheophorbide complexes **1** and **3**. In the case of methyl Ni(II)pyropheophorbides, the Soret band of Ni-pyropheo *a*, **4**, which has the C-2 vinyl group, is already split (Table I).

FTIR. Infrared C–H stretching modes assigned to vinyl substituents of porphyrins are reported at 2976–3012 cm^{-1} and 3077–3106 cm^{-1} [68]. No such bands are evident in the IR spectra of Fe-pheo *a* relative to those of the mesopheophorbides **2** and **3** (Fig. 3), or in the IR spectra of Chl *a* [20]. Loss of the C-2 vinyl causes the 2960 cm^{-1} band of **1** to become slightly more intense and increase in frequency for **2** and **3**, presumably reflecting the aliphatic hydrogens of the C-2 ethyl group of the latter two complexes. The IR spectrum of Fe-pheo *a* has a novel weak feature at 2820 cm^{-1} that is not present in the IR spectra of **2** or **3** (Fig. 3). A band at this frequency has not previously been reported for porphyrinic vinyls or for vinyls in general [68,78–80]. However, the 2820 cm^{-1} band of **1** is at the correct frequency to be a combination band arising from the hidden $\nu_{vinyl}(C=C)$ IR stretching mode at

1628 cm^{-1}, lying under the strong C_a–C_m 'chlorin marker' [26–28], plus the 1192 cm^{-1} IR (and RR) band of Fe-pheo *a* *.

There is no evidence for the vinyl group of **1** in the 1600–1630 cm^{-1} region of the IR spectra (Fig. 4). The $\nu_{vinyl}(C=C)$ stretching mode is also silent in this region of chlorophyll IR spectra [20]. Weak out-of-plane bending modes of the C-2 vinyl were assigned in chlorophyll IR spectra at 910–923 cm^{-1} and 980–990 cm^{-1} [20]. The loss of a shoulder at 970 cm^{-1} is apparent in the IR spectra of **2** and **3**, as compared with **1**. Both the FTIR and RR spectra of Fe-pheo *a* display a band at 1190 cm^{-1} that is absent from the spectra of **2** and **3**. On the whole, however, the IR spectrum of Fe-pheo *a*, like that of Chl *a* [20], is not notably affected by the C-2 vinyl moiety.

RR spectra. The general vibrational effects of the C-2 vinyl group of the pheophorbides, like those of the chlorophylls [34–36], are surprisingly limited. No evidence for the vinyl stretching mode of chlorophylls has been observed in B and Q_x excitation RR spectra, or in the red excitation CARS spectra of chlorophylls [40]. The Raman silence of $\nu(C=C)$ was attributed to weak conjugation [34–36] because the C-2 vinyl is about 31° out-of-plane [9,10]. Boldt et al. [41] failed to observe $\nu(C=C)$ in their RR study of methyl Ni(II)pyropheophorbides with all excitation lines used. Similarly, the RR spectra shown here of C-2 vinyl-substituted Fe-pheo *a* give no evidence for $\nu(C=C)$ relative to the RR spectra of C-2 ethyl-substituted pheophorbides **2** and **3** (Figs. 5–9).

However, Fe-porphyrin systems such as leghemoglobin, whose C-2 and C-4 vinyls are about 31° and about 86° out of the porphyrin plane, respectively [81], do have Raman-active vinyls [82]. Moreover, Chls *c* ** exhibit the vinyl stretching mode at 1622 cm^{-1} [83], indicating that the anomaly with regard to the inactivity of the vinyl group of Chl *a* and Fe-pheo *a* is also not a property of the phorbin macrocycle. Because the $\nu(C=C)$ modes of other vinyl-substituted chlorins are similarly absent [25,26], we suggest that the apparent silence of vinyl stretching modes of chlorophylls may rest in the fact that these macrocycles are dihydroporphyrins. Because chlorins have intense skeletal modes in the 1610–1630 cm^{-1} region, the IR and RR $\nu(C=C)$ modes of the chlorin phorbins may simply be hidden.

Boldt et al. [41] have assigned the 1338 and 1130 cm^{-1} RR bands of methyl Ni(II)pyropheophorbide *a* to the vinyl CH_2 scissors mode and to a C_b–vinyl stretch, respectively. These features generally coincide with those of vinyl-substituted porphyrins [84,85]. Similar vinyl bands also appear in RR spectra of Fe-pheo *a* relative to Fe-mesopheo *a* and Fe-mesopyropheo *a* (Figs. 5–9), and can clearly be seen in the SERRS spectrum of Chl *a* (Fig. 9). The strong 1140 cm^{-1} feature of Fe-pheo *a* appears to match the 1145 cm^{-1} feature of Chl *a*, and the 1134 cm^{-1} band of Ni-pyropheo *a* (Fig. 9). By contrast, the RR spectra of C-2 ethyl-substituted pheophorbides lack this single strong band (Fig. 8).

The 1185 cm^{-1} RR (and IR) band of Chl *a*, Ni-pyropheo *a* and Fe-pheo *a* (Fig. 9) appears to be an additional vinyl-sensitive feature. Lutz has assigned the 1189 cm^{-1} RR band of Chl *a* as a $\nu(C_a$–$N)$ mode [34]. An alternative assignment was proposed by Boldt et al. [41]. NCA for the methyl ester Ni(II)pyropheophorbides indicated that the 1190 cm^{-1} RR band was a C_m–C_{10} stretching mode and γC_b–H deformation [41]. However, RR spectra of chlorophylls display both frequency variations and intensity changes in the ~ 1190-cm^{-1} region that are linked with the identity of the C-2 and C-3 substituents: e.g. (precise values) 1189 cm^{-1} for Chl *a*; 1180 and 1195 cm^{-1} for Chl *b* (replacement of the C-3 methyl with a formyl); and 1168 and 1182 cm^{-1} for Chl *d* (replacement of the C-2 vinyl with a formyl) [34]. Similarly, the 1184 cm^{-1} band of Fe-pheo *a* (Fig. 9) shifts to about 1176 cm^{-1} for the C-2 mesopheophorbides **2** and **3** (Fig. 8). All of these frequency variations for the 1185 cm^{-1} band of phorbins involve addition or removal of a conjugating substituent. These data indicate that the actual vibrational origin of the 1185 cm^{-1} RR and IR feature of phorbins is quite complex, and clearly has not yet been fully addressed.

Spectral effects of the keto and carbomethoxy substituents

Phorbins have (at least) three carbonyl substituents: the C-7 propionate ester, the C-10 carbomethoxy (COOMe) group, and the C-9 keto moiety ¶. The last is perhaps most significant for photosynthetic macrocycles, because the nature of the C-10 and C-7 substituents varies [1–3]. However, only the C-9 keto group has a pronounced effect on the electronic absorption spectra, as shown in Table I. The carbomethoxy group induces molecular strain [1–3], affecting both the NMR [18] and vibrational spectra (vide infra). However, the presence or absence of the C-10 ester (pyro vs. non-pyro

* The 2820 cm^{-1} IR band of Fe-pheo *a* is in the spectral region typical of the C–H stretching modes of an -OCH_3 group (2810–2820 cm^{-1}) [79]. However, the OCH_3 hypothesis for the 2820 cm^{-1} IR band of **1** does not explain its sole presence in the spectra of Fe-pheo *a* because **2** has both the C-7 and C-10 methyl esters, and **3** has a C-7 methyl ester.

** The Chls c_1 and c_2 (C-2 vinyl, C-4 ethyl (or vinyl), C-7 acrylate) from the brown plant line are actually porphyrin phorbins, since the D ring has not been reduced [83].

¶ The *b*-type chlorophylls have a fourth carbonyl substituent, the C-3 aldehyde. Some bacteriochlorophylls lack the C-10 carbomethoxy substituent and are thus pyrophorbins [1–3,11–13].

phorbins) causes at most a 1 nm red-shift in the electronic absorption spectra (Table I). This is thus an insignificant perturbation of the π orbitals of the methyl ClFe(III)pheophorbides and chlorophylls (Table I).

The C-7 propionate ester substituent of porphyrins, chlorins, and phorbins typically has its ν(C = O) mode at 1735 cm^{-1} in the IR spectra [1–3,20,21,68,70]. This vibration is not RR-active, the group being too far removed from the conjugation path of the macrocycle. The IR frequency of the C-7 carbonyl mode is not significantly perturbed by neighboring carbonyl substituents, and is unaffected by the nature of the macrocycle (i.e., porphyrin vs. chlorin). Conversion of the C-7 ester to the free acid induces a frequency shift in the IR to 1710 cm^{-1}, with a concomitant intensity decrease for the 1735 cm^{-1} band [70].

The C-9 keto group has at least three factors influencing its vibrational frequencies: (1) it is part of a cyclopentanone ring, for which a keto ν(C = O) mode is predicted at 1725–1750 cm^{-1} [79]; (2) α,β conjugation with the aromatic tetrapyrrole is expected to induce a 30–40 cm^{-1} frequency decrease and a significant intensity increase [79,80]; and (3) it is also part of an enolizable β-keto ester [79,80]. Moreover, the C–(C = O)–C bond angle for the C-9 keto of phorbins is about 107° [9,10], rather than the more typical 120° of most keto groups. Alteration in the carbonyl bond angle due to steric effects is expected to decrease the C = O frequency, whereas ring strain can strengthen the C = O bond and increase its frequency [79].

The C-10 carbomethoxy group is a β-keto ester that also has β,γ aryl conjugation. The effects of β-coupling to the keto group are 10–15 cm^{-1} frequency shifts; the β,γ unsaturation may induce an additional 10–15 cm^{-1} decrease [79]. Because the C-9 keto group is enolizable, the C-10 carbomethoxy can become α,β conjugated, inducing further frequency downshifts [80], and inducing Raman activity. Indeed, IR spectra of rhodoporphyrin dimethyl ester and rhodochlorin dimethyl ester (C-7 propionate methyl ester and conjugated C-6 carbomethoxy group) exhibit bands at both 1735 and 1700 cm^{-1} [70]. Conversion of only the rhodoporphyrin C-6 carbomethoxy to the free acid results in decreased intensity at 1735 cm^{-1} and a shift of the 1700 cm^{-1} band to 1666 cm^{-1} [70], whereas conversion of the rhodoporphyrin C-7 propionate methyl ester to the free acid results in a complete shift of the 1735 cm^{-1} feature to 1708 cm^{-1} [70]. These data demonstrate that the ν(C = O) mode of a carbomethoxy in conjugation with a porphyrin or chlorin is predominantly at 1700 cm^{-1}, with a minor contribution at ~1735 cm^{-1}. Thus, the C-10 carbomethoxy carbonyl mode of phorbins could range between 1700 and 1735 cm^{-1}, with the exact frequency dependent on the extent of conjugation.

FTIR spectroscopy of C = O modes. The ClFe(III) pheophorbides **1** and **2** mimic the Chl *a* substituent pattern and exhibit 1735 and 1700 cm^{-1} IR bands that, for chlorophylls, are generally assigned as the ν(C = O) mode of the C-7 and C-10 esters and the ν(C = O) mode of the C-9 keto group, respectively [1–3,19,20, 34–36,66]. However, replacement of the C-10 COOMe group by hydrogen, as in **3**, affects both of these spectral features (Fig. 4 and Table II), suggesting that a reassignment of the carbonyl modes of phorbins is warranted.

First of all, loss of the C-10 ester of methyl pheophorbides and chlorophylls results in the expected intensity decrease at 1735 cm^{-1}. Second, the 1700 cm^{-1} band of pheophorbide complexes **1** and **2** (the presumed C-9 keto mode) shifts to 1690 cm^{-1} for Fe-mesopyropheo *a*, **3**. Similar frequency decreases are consistently present between chlorophylls and pyrochlorophylls (Table II), although this has not previously been discussed. Third, the 1690 cm^{-1} band of **3** is markedly increased in intensity relative to the 1700 cm^{-1} bands of **1** and **2**, as is also the case for chlorophylls lacking the C-10 ester (Table II). The bandwidths (FWHH) of the 1700 cm^{-1} features of **1** and **2** are exactly double those of the intense 1690 cm^{-1} feature of Fe-mesopyropheo *a*, suggesting that the former may consist of multiple components.

We propose that the IR spectrum of the Fe-mesopyropheo *a* complex represents the 'true vibrational picture' of the C-9 keto group in conjugation with the phorbin nucleus. Clearly, the C-10 carbomethoxy influences the vibrational modes of the C-9 carbonyl. Macrocyclic perturbations by the C-10 carbomethoxy group [8] might, for example, alter the E ring conformation sufficiently to prevent the C-9 keto from fully conjugating with the macrocycle. Loss of the C-10 COOMe group would reduce this strain and result in increased conjugation that would both increase the intensity and lower the frequency of the C-9 carbonyl mode, as is observed.

Other IR features are also sensitive to the presence or absence of the C-10 carbomethoxy substituent. For Fe-mesopyropheo *a*, loss of the C-10 carbomethoxy group results in an apparent splitting of the broad 2870 cm^{-1} band of Fe-pheo *a* and Fe-mesopheo *a* into two bands at 2870 and 2851 cm^{-1} (Fig. 3). The IR spectrum of Fe-mesopyropheo *a* has an intense band at 1210 cm^{-1} that is weaker and broader for Fe-pheo *a* and Fe-mesopheo *a* (Fig. 4). A similar strong band at 1218 cm^{-1} is found in the IR spectrum of Ni-pyropheo *a* (Table II). A strong IR feature is expected in this region as a skeletal vibration of conjugated ketones [79,80]. The 465 cm^{-1} IR (and RR) bands of **3** are distinctly absent from the spectra of **1** and **2**; this is a reasonable frequency for a deformation mode of the C-9 keto group [79]. A 432 cm^{-1} RR feature was identified as the in-plane bending mode of the C-9 keto group for Chl *a* [34–36].

RR spectra of C = O modes. Resonance Raman spectra of chlorophylls consistently display a solvent-sensitive band at 1700 cm^{-1}, assigned as ν(C = O) of the C-9 keto group [34–36]. The carbonyl of the C-10 carbomethoxy substituent, like that of the C-7 ester, should be RR-silent if it is non-conjugated, as is generally assumed. RR spectra of Fe-mesopyropheo *a* have only a single carbonyl band at 1690 cm^{-1} (Figs. 5–8). However, Soret and Q_y excitation spectra of Fe-pheo *a* and Fe-mesopheo *a* display carbonyl modes at both 1700 cm^{-1} and at 1735 cm^{-1} (Figs. 5, 8 and 9). Similarly, the Q_y excitation spectrum of Ni-pyropheo *a* has only a single 1690 cm^{-1} feature, while the SERRS spectrum of Chl *a* also has a weak band at 1735 cm^{-1} (Fig. 9). The weak 1735 cm^{-1} feature has been seen in other chlorophyll and bacteriochlorophyll RR spectra (Refs. 34–36; Cotton, T.M., unpublished observations; Lutz, M., personal communication).

Other RR bands are also sensitive to the presence or absence of the C-10 carbomethoxy substituent. For example, an intense 1208 cm^{-1} feature is present in B and Q_x excitation RR spectra of Fe-mesopyropheo *a*, but this band is consistently weaker for complexes **1** and **2** (Figs. 5–7). The 1208 cm^{-1} band is likely to correspond to the intense 1210 cm^{-1} IR feature of Fe-mesopyropheo *a*, assigned as a skeletal vibration of the conjugated C-9 keto group. Similarly, the 465 cm^{-1} IR feature of Fe-mesopyropheo *a* is matched by a 465 cm^{-1} Raman band (Fig. 8).

Assuming that the RR C = O frequencies of Fe-mesopyropheo *a* represent the true picture of the isolated C-9 keto group, these data again indicate both a partial conjugation of the C-10 ester and an interaction between the C-9 and C-10 carbonyls of the methyl ClFe(III)pheophorbides and Chl *a*.

Significance of the IR and RR C = O effects. The vibrational spectroscopic data for chlorophylls and the ClFe(III)pheophorbide model complexes indicate that the C-9 carbonyl does not act as an isolated oscillator in phorbin systems. Different possibilities affecting conjugation at the C-9 include strain-induced interactions in the presence of the C-10 carbomethoxy group, as evidenced by lack of planarity at ring E [6]. For example, the C-10 carbon of methyl bacteriopheophorbide *a* deviates from the least-squares plane of the four nitrogens by 0.29 Å [86].

The present results also indicate that phorbin macrocycles tend toward conjugation of the C-10 methyl ester. The actual mechanism whereby the C-10 carbomethoxy becomes conjugated is still unclear. Various possibilities include: (a) keto-enol tautomerism; (b) π-overlap of the C-9 carbonyl with the adjacent γC_m–C_a bond (Scheer, H., personal communication); (c) β-coupling of the C-9 and C-10 carbonyl modes; and (d) inductive effects from the C-10 carbonyl.

Early speculations on the functional significance of the isocyclic ring of biological phorbins include those of Mauzerall [87]. Although the enol form of the chlorophylls is generally considered to be present in not more than a few percent [6], recent studies increasingly suggest that (partial) enolization of the C-9 keto may be functionally significant. Wasielewski et al. [88] have proposed that the enol form of Chl *a* might be involved in electron separation by the green-plant reaction center. Because the midpoint potential of the Chl *a* enol should be lower than that of Chl *a* itself, the enol is a reasonable model for P-700 [88]. Recently, Heald et al. [39] have reported evidence favoring the enol form for the Chl *a* cation radical. Significant changes were observed in the carbonyl stretching region, including both a new 1717 cm^{-1} feature suggested to be the C-10 carbonyl mode and a significant decrease in the intensity of the 1690 cm^{-1} C = O mode of the C-9 keto group [39]. For photosynthetic reaction centers from *Rhodopseudomonas viridis*, one of the bacteriochlorophylls of the special pair has its C-9 ketone hydrogen-bonded to a threonine [89]. Hanson et al. [90] have calculated that H-bonding at the C-9 keto of bacteriopheophytin (free-base bacteriochlorophyll) would enhance the reduction by ~ 40 mV. RR spectra of photosynthetic reaction centers from *Rb. sphaeroides* wild-type also indicate that the C-10 carbomethoxy is at least partially conjugated, based again on Raman activity of the C-10 ester [91]. Finally, a recent study of BChl *a* and its anion radical also reported changes in the IR spectra for the C-9 and C-10 carbonyls, described as arising from 'coulombic interactions or even a direct coupling' [92].

Because the E ring of phorbins is the major structural feature distinguishing these macrocycles from non-photosynthetic systems, the participation of the C-10 ester in ring conjugation is suggestive of its biological importance.

Metal-dependent effects

Fig. 9 presents a comparison of the Q_y excitation spectra of Chl *a*, Ni-pyropheo *a* and Fe-pheo *a*. There is an overall, general similarity between the spectra of Chl *a* and Fe-pheo *a* with respect to both Raman frequencies and relative intensities. In contrast, the Q_y spectrum of Ni-pyropheo *a* is far less similar to Chl *a*. For example, bands above 1500 cm^{-1} have considerable intensity for Chl *a* and Fe-pheo *a*, whereas these features are much weaker for Ni-pyropheo *a*. The ~ 1282 cm^{-1} feature of the Mg- and Fe-substituted phorbins is more intense than the ~ 1268 cm^{-1} band, whereas the reverse is true for Ni-pyropheo *a*. These features of Ni-pyropheo *a* were assigned as C_m–H deformations by Boldt et al. [41]. The *meso* protons of Ni(OEC) complexes are shifted upfield in the ^{1}H-NMR from those of H_2(OEC), Mg(OEC), Zn(OEC) and Sn(OEC) [52]. This indicates a marked reduction in the macrocyclic ring

current (π-aromaticity) for the Ni(OEC) complexes due to Ni-induced ruffling of the molecular conformation [52]. Thus, the slight frequency and intensity differences in the $\delta(C_m-H)$ modes of Ni-pyropheo *a*, relative to those of Chl *a* and Fe-pheo *a*, would appear to reflect this alteration in ring current.

A band at 740–750 cm^{-1} is the major low-frequency Raman feature with red excitation [28]. This feature is the ν_{16}-equivalent, a C_a-N-C_a deformation mode that includes a substantial contribution from C_m-H deformations for metallochlorins [28,31,32]. (In contrast, the 670 cm^{-1} ν_7-equivalent of metallochlorins is dominant in low-frequency spectra obtained with Soret excitation [28,31–33].) A broadened single 750 cm^{-1} band appears in the Q_y spectra of **1** and **2** compared with a doublet for the pyrophorbin **3** (Figs. 8 and 9). The red excitation spectra of Ni-pyropheo *a* and Chl *a* also have strong bands at 750 cm^{-1} (Fig. 9). The 750 cm^{-1} band of Fe-pheo *a* and Chl *a* is generally less intense than bands above 900 cm^{-1}. However, the analogous feature of Ni-pyropheo *a* is, with the sole exception of the 1234 cm^{-1} band, the dominant spectral feature (Fig. 9). Given that the phorbin macrocycle is considerably deformed upon Ni-substitution [18,47–53], we infer that the increase in relative intensity of the ν_{16}-equivalent for Ni-pyropheo *a* may be a reflection of this molecular deformation. Clearly, however, the overall spectral pattern for the nickel-substituted phorbin model complex is spectrally distinct from that of the iron-substituted model, whereas the latter is fairly similar to native Chl *a*.

Conclusions

The electronic absorption and vibrational spectral data presented herein provide insight as to the respective effects of the C-2 vinyl, C-10 carbomethoxy, the C-9 keto and the central metal ion of model and biological phorbins. (1) The presence or absence of the C-2 vinyl moiety has its strongest effect on the electronic absorption spectra of the phorbins, but only weakly influences the infrared and RR spectral properties. (2) In contrast, the electronic absorption spectra are essentially unperturbed by the presence or absence of the C-10 carbomethoxy substituent, whereas this group has a surprisingly significant effect on the vibrational spectra of phorbins. Indeed, the data indicate that at least partial conjugation of the C-10 ester must exist in order to explain both the IR and RR spectral properties. The precise origin of this conjugation is less clear: it could arise not only from keto-enol tautomerism, but also from π-overlap between the C-10 carbonyl and the adjacent γC_m-C_a double bond, or from weak β-coupling between the C-9 and C-10 carbonyl groups, as is typical of β-keto esters. The propensity of the phorbins towards conjugation of the C-10 carbonyl may have functional significance [39,87–92]. (3) Comparison between methyl ClFe(III)pheophorbide *a*, chlorophyll *a* and methyl Ni(II)pyropheophorbide *a* suggests that iron pheophorbides may be better spectral models for the chlorophylls than conformationally perturbed, tetracoordinate nickel pheophorbides. (4) Finally, we have also shown that the IR 'chlorin band' is not only predictive for the identification of the chlorin (vs. porphyrin or bacteriochlorin) macrocycle, but also displays frequency shifts upon alteration of the central metal ion and its coordination number and spin state.

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