kcal mol^{-1} [39]), Me$_4$Pb (33 kcal mol^{-1} [40]), Me$_3$C· (10 kcal mol^{-1} [41]), Me$_3$Si· (−1 kcal mol^{-1} [38]), and Me$_3$C$^+$ (165 kcal mol^{-1} [42]). We are aware of a measured value of −21 kcal mol^{-1} for the heat of formation of tetramethylgermane.[43] However, we and others[44] feel that this is subject to considerable uncertainty, as it leads to a Ge–C bond energy that is conspicuously low.

It should be stressed that the π-bond energies in Table III are subject to considerable uncertainty. While they are independent of the heats of formation of the tetramethyl compounds, they are sensitive to the measured appearance potentials. Our estimates

are based on what we believe are the best data currently available. An interesting picture of the bonding in these compounds arises. While the π-bond energy of the silaolefin is considerably less than that in the hydrocarbon, in agreement other experimental. work[18,19] and with the best available theoretical calculations,[22] no further decrease in π-bond strengths in the corresponding germanium and tin compounds is noted. In fact, small increases are suggested. The lead–carbon double bond is indicated to be weaker but only by about 10 kcal mol^{-1}. Further experimental and theoretical work is desirable and is under way in our laboratory. In particular, we are attempting to define π-bond strengths in these and related compounds with reference to as little external stabilities of olefin- and carbene-like isomers. A preliminary report on the silicon system will appear shortly.[45]

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NMR Spectra of Porphyrins. 18. A Ring-Current Model for Chlorophyll Derivatives[1]

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Abstract: A double dipole model of the macrocyclic ring current in the dihydroporphyrin ring of chlorophyll derivatives is presented and parameterized. The model includes a close-range approximation that has both first-order and second-order continuity at all points in space, except through the current loop itself. The model is tested by comparison of the proton chemical shifts of methyl pyropheophorbide a (1) with the corresponding porphyrin, 2-vinylphylloerythrin methyl ester (2). Aggregation effects were eliminated by use of the zinc(II) complexes bearing an apical pyrrolidine ligand, and substitution effects were isolated by use of the corresponding chlorin and porphyrin 9-ketals (3 and 4, respectively). The exocyclic ring E has no appreciable effect on the macrocyclic ring current, but the C$_9$ keto function and the reduction in ring D in the chlorin both reduce the ring current by about 6 and 10%, respectively. The resulting model gives a good account of the chemical shift differences of all the protons in methyl pyropheophorbide a and 2-vinylphylloerythrin methyl ester. Owing to very low yields encountered in the preparation of 2-vinylphylloerythrin methyl ester from methyl pyropheophorbide a, a new route involving DDQ oxidation of the chlorin 9-ketal (3) is described.

The nature of the molecular organization of chlorophylls in green plants and photosynthetic bacteria is of fundamental importance in the understanding of the photoreaction processes,[3] and the extent and structures of the chlorophyll aggregates formed have been the subject of many investigations.[4] Application of NMR spectroscopy to the study of the aggregation process in solutions of chlorophyll derivatives has been highlighted by the extensive investigations of Katz and co-workers[5-7] and also by Fong.[8] Synthetic approaches to models of the special pair

photosynthetic reaction center have also been investigated by several groups,[9,10] and structures of the derived complexes in solution have been studied by NMR and other spectroscopic techniques. In these investigations, the interpretation of the NMR shifts is usually given in terms of the ring-current shifts experienced by nuclei situated near to the chlorophyll ring; thus, the ring current serves as a geometric probe of the structure of the complexes. Obviously, the more accurate the ring-current model used, the better the structural information obtained from it. However, in the absence of a refined ring-current model for the chlorophyll (7,8-dihydroporphyrin) ring, previous investigations have either made use of relatively crude models, such as the single-current loop (which does not reflect the asymmetry of the chlorin ring), or discussed the observed shifts in an entirely qualitative manner. Such qualitative discussions, e.g., into the different postulated structures of the aggregates,[10] would benefit greatly from a more

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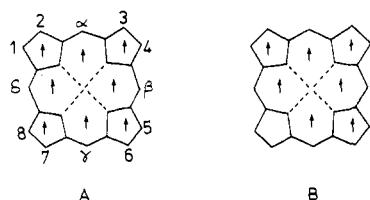(6) Closs, G. L.; Katz, J. J.; Pennington, F. C.; Thomas, M. R.; Strain, H. H. J. Am. Chem. Soc. 1963, 85, 3809-3821.
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A Ring-Current Model for Chlorophyll Derivatives

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Figure 1. Nomenclature and dipoles for (A) the porphyrin nucleus, and (B) the chlorin nucleus.

quantitative study with a refined ring-current model for the chlorophyll system.

We have previously shown that a ring-current model based on the double dipole approximation is capable of providing a good description of the observed ring-current shifts in both the porphyrin and chlorin ring.[11] Further development of the basic model to consider both free rotation of the apical ligand and the effect of methine phenyl groups led to a sufficiently accurate model for zinc(II) mesotetraphenylporphyrin, capable of determining molecular geometries of some complexes.[12] The problem of calculating close-range shifts, for which the dipole approximation breaks down, was also solved computationally, and the resulting calculation was shown to give good agreement with the observed ring-current shifts of the proximal ligands of the iron atom in cytochrome c.[13] Thus, the development of the basic model to fit the chlorophyll ring system would appear to be both a practical and desirable objective. Here we give a suitable parameterization of the model for the chlorin ring system and show how the model can be used to investigate structural properties in related derivatives.

Theory

The double dipole model for the porphyrin ring current has been described in detail,[11] so only a summary is given here. The various ring-current loops in the porphyrin macrocycle are replaced by their equivalent dipoles, and the total ring current shift at any point (R) is obtained as the sum of the contributions of the equivalent dipoles using the standard dipole–dipole equation. This gives the basic equation:

$$\delta_R = \mu_H \sum_{i=1,8} f(iR) + \mu_p \sum_{j=1,8} f(jR) \tag{1}$$

where $f(iR) = [1 - 3(Z_R \pm 0.64)^2/r_{iR}^2]/r_{iR}^3$.

The symmetry of the porphyrin ring allows for only two types of equivalent dipoles, those for the pyrrole rings (μ_p) and for the hexagons (μ_H). The lower symmetry of the chlorin ring provides, in principle, for two different types of pyrrole ring (i.e., rings A and C, and ring B) and two types of hexagon (Figure 1). Thus, there are four different values of the equivalent dipoles. If the C_9 keto group, common to most chlorophyll derivatives, is considered as a major perturbation, then all elements of symmetry are lost and eight values of the equivalent dipoles would be required. Conversely, good agreement was previously achieved[11] for some chlorins lacking the exocyclic ring, using the assumption that only two types of equivalent dipole existed, as in the porphyrin case. These different possible models need to be evaluated.

In order for this to be achieved, the basic equation (eq 1) is rewritten in the form

$$\delta_R = \sum_{i=1,8} \mu_i^H f(iR) + \sum_{j=1,8} \mu_j^P f(jR) \tag{2}$$

Since eq 2 includes eq 1, all the previous results remain unchanged.

Also, detailed inspection of the calculated shifts of the present model suggested that a further refinement of the "close-range approximation"[12,13] would be beneficial. In this, the ring-current shift of any nucleus, of coordinates x, y, z, close to the porphyrin ring (defined by a cylinder of height d and radius r), is given by

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eq 3, where δ_d is the calculated shift at the close-range boundary (x, y, d) and b is an arbitrary parameter. The limiting values

$$\delta = \delta_d[1 + b(d^2 - z^2)] \tag{3}$$

of d and r (the current loop radius) were taken arbitrarily as 2.5 and 3.4 Å, and b was adjusted so that the calculated shift for the NH proton equaled the observed value of -10.5 ppm.[13]

In this calculation there is a second-order discontinuity at $z = d$ i.e., the gradient alters) and a first-order discontinuity at $z < d$ and $r = 3.4$ Å. The discontinuity at r is simply equivalent to the discontinuity in the magnetic field that occurs on passing through a loop of current. However, the second-order discontinuity at $z = d$ is merely a consequence of a very simple model and must be removed. Fortunately, this can be simply achieved as follows. In order that both the shift and the gradient at $z = d$ may be fitted, an extra term needs to be added to eq 3. Symmetry requires that the term be symmetric in z, and this is most easily achieved by adding a quartic term. Thus, eq 3 is replaced by eq 4. In this

$$\delta = \delta_d + g_d[b(d^2 - z^2) + c(d^4 - z^4)] \tag{4}$$

equation, both the shift δ_d and the gradient g_d at the reference point (x, y, d) are calculated and input into eq 4 to obtain the ring-current shift at point x, y, z if $z < d$.

The condition that the gradient at $z = d(\partial\delta/\partial z)$ be continuous is simply given by the following:

$$c = -(1 + 2bd)/4d^3 \tag{4a}$$

Equation 4 differs from eq 3 in the important respect that the constants b and d, the close-range distance, can now be determined by recourse to observation. The same standard molecules as previously were used to parameterize these constants, i.e., the value of the ring-current shift at the NH protons[13] and the known ring-current shifts in zinc(II) mesotetraphenylporphyrin pyridine adduct.[12]

This gave values of $d = 3.6$ Å and $b = -0.64$. Thus, this computationally trivial amendment served to provide both first- and second-order continuity for the current loop calculations at every point in space except at the current loop itself ($z < d$, $r = 3.4$ Å). It is of interest to compare the derived value of d above with the value of 2.5 Å assumed previously.[11,12] This shows that the equivalent dipole approximation was not completely accurate at 2.5 Å above the porphyrin ring even when the network model was used. We shall shown how the improved model gives a much better description of the ring-current shifts of the NH protons in the chlorin ring than did the previous version. All of the other protons in the porphyrin, being outside this close-range cylinder, have not been affected by this amendment.

So that a ring-current model for the chlorin ring might be obtained, the ring current shifts of a set or number of protons in a molecule of known geometry must be evaluated. This requires a knowledge of the observed chemical shifts in the chlorin to be studied, plus the chemical shifts of protons in similar chemical environments but minus the ring current. The limitations of the various models considered are the major source of the uncertainty in the parameterization. Many of the errors can be eliminated by making use of the observed shifts in the corresponding porphyrin, as the ring-current shifts in porphyrins have already been calculated on the basic model to within the experimental accuracy.[12] Thus, the ring-current shift (ΔM_i) of any proton H_i in the required chlorin is given in terms of the calculated shift for the same proton in the corresponding chlorin, together with the observed chemical shifts, by eq 5.

$$\Delta M_i(\text{porphyrin}) - \Delta M_i(\text{chlorin}) = \delta(\text{porphyrin}) - \delta(\text{chlorin}) \tag{5}$$

For eq 5 to hold, the substituent shifts at H_i must be equal in the porphyrin and chlorin molecules; thus, the two molecules should ideally have the same substituents with the only difference being the reduced ring D for the chlorin. Further criteria are the requirements in both the porphyrin and chlorin for accurately measurable spectra, with no aggregation shifts, and of unambiguous resonance assignments.

A pair of molecules fulfilling all of these requirements are 2-vinylphylloerythrin methyl ester (2; VPE) and methyl pyropheophorbide *a* (1; MePPB); these are identical except for the reduced ring D in 2, and in particular they possess the exocyclic ring E with the conjugated C_9 carbonyl group characteristic of most chlorophyll derivatives. This last point is of importance since it has been suggested that the C_9 carbonyl has a considerable influence on the macrocyclic ring current.[14]

All routes to 2-vinylphylloerythrin methyl ester (2) from methyl pyropheophorbide *a* appear to give erratic and often negligible yields.[15,16] In particular, we showed that direct treatment of methyl pyropheophoride *a* (1) with dichlorodicyanobenzoquinone (DDQ) gave very large amounts of extremely polar materials. Suspecting that this decomposition was due to the presence of the isocyclic ring (chlorin e_6 undergoes DDQ oxidation to give chloroporphyrin relatively smoothly), we developed an alternate method to 2-vinylphylloerythrin methyl ester (2), which involved protection of the C_9 keto group as its ketal prior to oxidation. This choice of synthetic route was also influenced by the fact that we would need analogues of 1 and 2 lacking the conjugated C_9 keto group in order to assess the effect of that functionality on the ring current. Methyl pyropheophorbide *a* (1) was treated with ethylene glycol and acid and gave the chlorin 9-ketal (3). DDQ oxidation of 3 proceeded extremely smoothly, almost by titration, to give the porphyrin 9-ketal (4). Aqueous acid then decomposed the ketal to give 2-vinylphylloerythrin methyl ester (2).

Our NMR studies were therefore carried out on compounds 1–4, as well as on the zinc(II) complexes measured after addition

V = CH=CH₂

P = CH₂CH₂CO₂Me
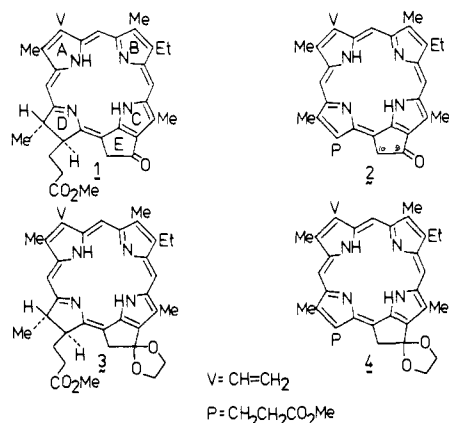
of a slight excess of pyrrolidine, a procedure that has previously been shown to remove all effects of aggregation.[17,18]

The geometry of the chlorin ring was taken from the detailed X-ray studies of ethyl chlorophyllide *a* dihydrate[19] and of methyl pheophorbide *a*.[20] These investigations show that the geometry of the ring skeleton is essentially unchanged by the introduction of a magnesium atom in the former. Fischer et al.[20] note that the chlorin ring is nearly flat, except for the reduced ring, with rings A, B, C, and E planar to within 0.02 Å. It has also been noted previously that the basic geometry is unchanged in the porphyrin and chlorin systems, apart from ring D.[21] Furthermore, although the C_{10} methoxycarbonyl group is implicated in the hydrogen bonding structure in the chlorophyllide in that a pair

of water molecules bridge the magnesium and carbonyl oxygen atoms, this does not affect the ring structure because the pheophorbide has no magnesium but a similar conformation. This is of importance as the only difference between the pheophorbide used here and the crystal structure studies is removal of the methoxycarbonyl group. Such "pyro" compounds are biologically important, however, since they are the carbon skeleton of the bacteriochlorophylls *c*, *d*, and *e*.[22] Nevertheless, the crystal studies suggest that absence of the C_{10} substituent does not affect the basic chlorin skeleton and that use of the pheophorbide geometry is reasonable.

Results and Discussion

Table I records the proton chemical shifts obtained for the compounds considered here. These are all first-order spectra at 360 MHz, except for the pattern due to the four nonequivalent methylene protons on the C_7 propionate side chain of the chlorins. Previous studies were at lower fields, and these methylene protons formed a single complex unresolved pattern[23] or were merely assigned intuitively.[24] The δ values recorded in Table I are from a complete iterative analysis of both the four-spin (ABCD) system obtained by decoupling the C_7-H proton and the five-spin (ABCDX) system including the C_7-H, proton, for both MePPB and its ketal. Full details of these analyses can be found elsewhere,[25] together with the derived conformation of the propionic side chain. It is of interest to note that the closely coupled pairs of protons in this system are the vicinal rather than geminal pairs in the CH_2CH_2 fragment. The almost identical pattern produced by the ZnMePPB (+pyrrolidine) spectrum was not completely analyzed, but the chemical shifts were obtained by comparison.

The remainder of the spectra are trivial to interpret and assign, apart from the assignments of the individual protons with corresponding groups, in particular the methine, β-methyl, and NH protons, and it is convenient to discuss these assignments individually.

Pennington et al.[23] recorded and analyzed the proton spectrum of MePPB at 60 MHz, and the assignments in Table I follow this earlier work. The assignment of the C_5 methyl and of the 7″-protons in the propionic side chain were confirmed unambiguously by specific deuteration with MeO⁻/MeOD. The concentration dependence of the spectrum over the range from 0.06 to ca. 0.20 M in CDCl₃ solution was also noted by Pennington et al.[23] Two spectra of the free base recorded here at different, but much lower, concentrations (13 and 7 mM) gave reproducible proton chemical shifts (to ca. 0.01 ppm), which also coincided very well with the extrapolated infinite dilution values in ref. 23. Thus, we may be reasonably confident that the values for MePPB in Table I are the data for the monomeric compound. This is further substantiated by the chemical shifts for the ZnMePPB (+pyrrolidine) solution (Table I), which are so similar to those for the free base that the assignment follows immediately. However, there are small but significant differences between these sets of data that may well reflect the effect of zinc complexation with respect to the free base with localized NH protons.

The chemical shifts of MePPB-ketal 3 do differ appreciably from those of the parent compound, and thus the assignment of the methine protons and, more particularly, the β-methyl groups is more questionable. The assignment given is based on the MePPB shifts, and some support for this assignment can be obtained by comparing the effects of ketal formation on substituents on the same pyrrole ring. For example, δ(keto → ketal) is 0.13 ppm for the C_4 CH_2 protons, which are unambiguously assigned, and 0.14 ppm for C_3 Me, etc.

The spectrum of VPE has not previously been reported, and thus the assignments given, which are generally based on com-

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Table I. Proton Chemical Shifts (δ) of Methyl Pyropheophorbide *a* and Related Compounds[a]

|		methyl pyropheophorbide *a*			2-vinylphylloerythrin methyl ester		
		free base	Zn complex + pyrr.	ketal	free base	Zn complex + pyrr.	ketal
methine	β	9.519	9.494	9.817	9.901	9.925	10.135
	α	9.402	9.247	9.667	9.829	9.811	10.068
	δ	8.556	8.313	8.857	9.518	9.685	10.041
vinyl	CH	8.018	8.013	8.210	8.184	8.237	8.256
	H_t	6.293	6.179	6.343	6.304	6.346	6.280
	H_c	6.178	6.024	6.187	6.187	6.142	6.138
C_{10}-H_2		5.271	5.205	5.178	5.469	5.810	5.765
		5.113	5.160	5.031			
C_4-CH_2		3.704	3.747	3.834	4.052	3.961	4.120
CO_2Me		3.611	3.608	3.592	3.730	3.778	3.718
β-Me	C_5	3.681	3.658	3.630	3.792	3.880	3.769
	C_1	3.413	3.331	3.546	3.609	3.644	3.696
	C_3	3.251	3.259	3.393	3.583	3.486	3.663
	C_8	1.814	1.780	1.827	3.418	3.486	3.660
C_4-CH_3		1.703	1.697	1.752	1.863	1.827	1.870
N-H		0.48		-1.23	~-3.1		-2.83
		-1.67		-3.06			-3.63
CH_2CH_2		2.704	2.66	2.754	3.956	4.128	4.385
		2.314	~2.4	2.322			
		2.563	2.57	2.573	2.901	3.048	3.161
		2.287	~2.4	2.250			
C_7-H		4.296	4.211	4.410			
C_8-H		4.486	4.444				

[a] In $CDCl_3$ at concn: MePPB, free base 7.0 mM, zinc complex 5.7 mM, ketal 3.0 mM; VPE, free base 1.2 mM, zinc complex 6.8 mM, ketal 3.0 mM.

parisons with chlorin, are more provisional. In the zinc(II) derivative, these assignments for the β-methyl groups are supported by following the spectral changes that occur on titrating the aggregated zinc porphyrin solution with base (vide infra), again making the reasonable assumption that the β-methyl groups show comparable effects to the neighboring β substituents. This differentiates all the methyl peaks except $Me_{1,3}$, which have identical complexation shifts.

Assignments of the free base follow by analogy, though in this case, even at the lowest dilutions used (1 mM in $CDCl_3$), there was still some indication of residual aggregation effects. This was apparent in the change in chemical shifts with dilution, even at this concentration. Also there are larger differences between the free-base chemical shifts and those of the zinc(II) porphyrin (+pyrrolidine) spectra than in the chlorin, and significantly, the larger differences are for precisely those protons that show the largest aggregation shifts, i.e., the C_{10} and C_7 methylene protons. The relative aggregation shifts are shown schematically in Figure 2, which lists the $\Delta\delta$ values of the zinc(II) chlorin (Figure 2A) and zinc(II) porphyrin (Figure 2B) before and after addition of an excess of pyridine to $CDCl_3$ solutions of the complexes. For these reasons, we shall not use the free-base shifts in the derivatization of the ring current model.

The spectrum of the ketal, in contrast, does not suggest any sizable complexation shifts, even though it was obtained at the same concentration, and the assignments in this spectrum can, in consequence, be made by again comparing the effect of ketal formation among the various substituents, using the zinc(II) spectrum as the basis. On the assignment given, the effects of ketal formation on the two β substituents of rings A, B, and D, are 0.05 and 0.02, 0.18 and 0.16, and 0.26 and 0.18 ppm, respectively, in good general agreement. The shifts of the C_7 methylene will be influenced to some extent by the carbonyl anisotropy, and this explains the deviation from those of the C_8 methyl.

Ring-Current Model for the Pheophorbide Ring. The data in Table I can now be used both to obtain and to test for a suitable parameterization of the ring-current model for the pheophorbide ring. From the theory given earlier, the model is based on eq 2 and 4, in which the only unknown parameters for the pheophorbide ring are the sets of equivalent dipoles μ_i^H and μ_j^P (eq 2) reflecting the influence of the various substituents on the macrocyclic ring current in the chlorin, as compared with the basic porphyrin ring.
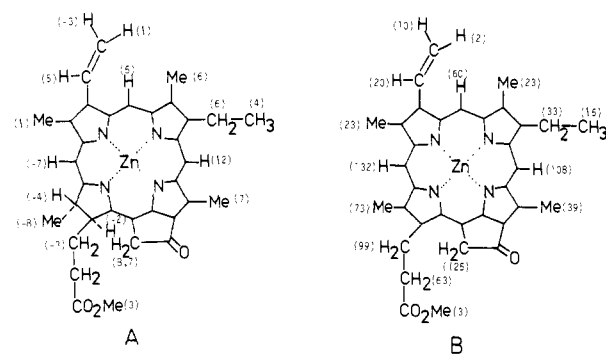
Figure 2. Aggregation maps of (A) zinc(II) methyl pyropheophorbide *a* (**Zn 1**) and (B) zinc(II) 2-vinylphylloerythrin methyl ester (**Zn2**). The numbers in parentheses are the $\Delta\delta$ values ($\times 100$) obtained by measuring the NMR spectra of the compounds in $CDCl_3$ and then remeasuring the spectra after addition of an excess of pyridine. Positive values represent upfield aggregation shifts in the spectrum without pyridine while negative values describe downfield shifts. Concentration of **Zn1** in A is 3.3 mM and of **Zn2** in B is 2.4 mM.

Our present ring-current model gives calculated ring-current shifts for porphin to within the experimental uncertainties of such estimations (see ref 13 for a detailed discussion), and thus it is both convenient and most logical to consider the influence of the various substituents in the pheophorbide system with reference to those of a simple porphyrin. Comparison of the chemical shifts of VPE-ketal **4** (Table I) with those of octaethylporphyrin (methine protons 10.18, β-CH_2 4.41, NH -3.74 ppm)[26] shows that there is no appreciable decrease of the ring current in the ketal, as compared with a simple porphyrin. The variations of the chemical shifts around the macrocycle are due to localized shielding effects of the various substituents. This is of interest as it demonstrates that the formation of the exocyclic ring E does not in itself disturb the ring current, despite the distortions in the ring geometry observed by Pettersen[27] in nickel and vanadyl deoxophylloerythrin. In these molecules the two N··N distances across the ring are not equal, presumably due to the exocyclic ring.

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Table II. Observed and Calculated Chemical Shift Changes ($\Delta\delta$)

| | | ketal to ketone | | | porphyrin to chlorin | | |
| | | obsd | | | obsd | | |
		porphyrin[a]	MePPB[b]	calcd c	d	e	calcd f
methine	α	0.25	0.24	0.27	0.43	0.32	0.46
	β	0.21	0.30	0.27	0.51	0.40	0.46
	δ	0.35	0.30	0.27	1.37	1.18	1.15
vinyl	CH	0.02	0.19	0.10	0.22	0.05	0.21
	H_t	0.03	0.05	0.10	0.17	−0.06	0.20
	H_c	0.00	0.01	0.06	0.12	−0.05	0.12
C_{10}-CH_2		−0.05	−0.09	0.13	0.63	0.66	0.49
C_4-CH_2		0.16	0.13	0.12	0.21	0.29	0.20
β-Me	Me_5	−0.11	−0.05	0.11	0.22	0.14	0.22
	Me_1	0.05	0.13	0.11	0.31	0.15	0.30
	Me_3	0.17	0.14	0.11	0.23	0.27	0.20
C_4-CH_3		0.05	0.05	0.05	0.13	0.15	0.10
N-H		~0	−1.7	−0.65		−1.6	−0.6, −0.7 (2)
			−1.4			−0.6	−1.7
	Me_8	0.17	0.01	0.11			

a ZnVPE → ZnVPE-ketal. *b* MePPB → MePPB-ketal. *c* μ^P 16.1, μ^H 18.1 (see text). *d* ZnMePPB → ZnVPE. *e* MePPB-ketal → VPE-ketal. *f* μ^P 14.6, μ^H 16.5 (see text).

However, the porphyrin ring is still planar in this system and thus presumably can cope with small deformations in the ring geometry.

Having established that the ring current in VPE-ketal **4** does not differ appreciably from porphin, we can now evaluate the influence of the two major perturbing substituents in the pheophorbide ring, i.e., the C_9 carbonyl and the chlorin ring. It is most convenient to consider the C_9 carbonyl first.

The data in Table I provide a very clear illustration of the effect of the C_9 carbonyl on the macrocycle. Previous comparisons of pheophorbide with chlorin-e_6 and of spectra with various substituents in the exocyclic ring had led to the suggestion[14] that the major influence of this group on the proton chemical shifts of the macrocycle was due to the decrease in the ring current caused by conjugation with the carbonyl. However, the conjugative effects could not easily be separated from the steric effects (in chlorin e_6), and the assignments of many of the peaks in the chlorins were not known. Table II shows the differences in chemical shift on going from the ketone to ketal in both the chlorin and porphyrin. The two sets of data compare well and show clearly the reduced ring-current shifts caused by the presence of the keto function. The observed shifts are compared in Table II with the calculated shifts for a 6% decrease in the porphyrin ring current. The agreement between the two sets of data is sufficiently good to quantitatively substantiate Katz's suggestion of a decrease in the main ring current caused by the C_9 keto function. The only large discrepancies between the observed and calculated shifts are for protons adjacent to the carbonyl group, precisely where one would expect specific effects due to the magnetic and electric anisotropy of the carbonyl group to be appreciable. For example, these effects produce sizable downfield shifts at the C_5 methyl and C_{10} methylene protons, which are larger than the upfield shifts produced by the decrease of the ring current. Conversely, direct conjugation effects of the C_9 carbonyl with the ring C NH function would be expect to produce downfield shifts at this proton, which add to the downfield shift due to the reduced ring current, giving larger than predicted NH shifts.[28]

With the effect of the C_9 keto function dealt with, it is now neccessary to consider the influence of the chlorin ring on the ring current to obtain the final parametrization of the model for the pheophorbide ring. As in the case of the keto function, a direct comparison can be made of the effect of reducing the C_7–C_8 double bond, from the data in Table I. Table II gives the comparison of the chemical shifts of ZnMePPB and ZnVPE, and also of the chlorin and porphyrin ketal, for all the protons that have similar

hybridization in the two molecules. The two data sets agree well, with some exceptions that will be considered later, and show the expected further sizable decrease in ring current, though, due to the removal of one current loop in the chlorin, this produces an asymmetric effect around the macrocycle, which provides a critical test of the equivalent dipole model. The simplest rationale of the effect of reduction in ring D is that two electrons have been removed from the porphyrin π system, which may be considered to have 16 (inner aromaticity, if lone pairs on the pyrrole nitrogens are utilized), or 18 π electrons (outer aromaticity). This would suggest a general decrease of the ring current of about 10%, together with the removal of the ring D current loop. Acting on this premise, we reduced all the sets of equivalent dipoles μ^P and μ^H (eq 2) in the same ratio, and Table II shows calculated shift differences for a 10% reduction in the equivalent dipoles of the VPE model. Comparison of the observed and calculated data, particularly for the zinc compounds, shows good agreement for all the protons of the macrocycle. The average deviation (observed–calculated) shifts for the 12 sets of protons considered is 0.05 ppm, which is comparable with the experimental error of these shift differences. This is very strong support for the general accuracy of the ring-current model. A further check on the close-range approximation developed here is provided by the NH protons. The calculated shift difference (porphyrin − chlorin) for the NH protons on rings B, A and C, and D are −0.6, −0.7, and −1.7 ppm. In the ketals, in which two NH peaks are observed in both the chlorin and porphyrin cases, the shift differences are −1.6 and −0.6 ppm, which is certainly in the correct range. There is, however, no direct method for assigning these NH signals to the appropriate rings in the molecule and no certainty that the assignment is the same in each molecule; thus, further discussion is unwarranted. In the parent molecules [porphin vs. chlorin(7,8)] the NH protons are single lines in each case, due to the molecular symmetry, at δ −3.74 and −2.75, respectively.[26] Both the average calculated shift difference (−0.9 ppm) and the calculated shift difference if the chlorin NH protons were on rings A and C (−0.7 ppm) are in excellent agreement with the observed shift difference of −0.99 ppm.

The agreement between the observed and calculated shift differences for the ketals is also reasonable, except for the vinyl protons and the C_1 and C_3 methyl groups (Table II). The assignment of the C_1 and C_3 methyls in the ketals could easily be reversed, as these are not clearly differentiated in either the chlorin or the porphyrin ketal, and this would give the correct calculated order for the β-methyl shifts. The vinyl group shift differences in the ketals are of more interest. Instead of the calculated upfield shift in the chlorin with respect to the porphyrin, a downfield shift for all the vinyl protons is observed. This is probably due to a different orientation of the vinyl group in the chlorin ketal, as distinct from the porphyrin ketal. The orientation of the vinyl

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(28) The NH chemical shifts are complicated both by the possibilities of exchange between the various nitrogen atoms and also by the unknown assignments of the two NH signals. These will be considered, together with detailed protonation studies, separately.[29]

(29) Abraham, R. J.; Goff, D. A.; Smith, K. M., work in progress.

group appears to vary from molecule to molecule. In the solid methyl pheophorbide *a*, the vinyl C_β atom is ca. 0.23 Å out of the macrocyclic ring plane pointing toward the C_1 Me, whereas in ethyl chlorophyllide it is ca. 0.5 Å out of the plane pointing toward the C_α methine. Also, Katz[30] has noted the varying β-proton shift sequences in the vinyl group in chlorin e_6 and protoporphyrin IX and interpreted them as due to different orientations of the vinyl groups in the two molecules. Clearly, the orientation of the vinyl group is delicately balanced in these molecules, and thus it is not surprising that ketal formation (in **3** and **4**) could be sufficient to change the orientation in the chlorin with respect to the porphyrin.

Experimental Section

Proton NMR spectra were obtained in $CDCl_3$ solution on a Nicolet NT-360 pulsed FT spectrometer, using 16K data points with a 3-kHz sweep width. The internal reference was the residual $CHCl_3$ line, assigned to 7.26 ppm.

Melting points were measured on a microscopic hot-stage apparatus and are uncorrected. TLC monitoring of reactions was performed with Merck silica gel 60 F254 precoated sheets (0.2 mm), and column chromatography was carried out with Merck neutral alumina (70–230 mesh). Electronic absorption spectra were measured with solutions in methylene chloride on a HP 8450A spectrophotometer.

Methyl pheophorbide *a* was obtained by large-scale extraction of the blue-green alga *Spirulina maxima*.[31] Zinc was inserted into the chlorophyll derivatives by standard methodology.[32]

9-Ketal (3) of Methyl Pyropheophorbide *a*. Methyl pheophorbide *a* (2 g) was heated under reflux in collidine[16] (150 mL) for 90 min during slow passage of a stream of N_2 throught the mixture. The solution was evaporated to dryness under vacuum, and the residue was taken up in toluene (200 mL) and dry dioxane (10 mL) containing ethylene glycol

(4 mL) and *p*-toluenesulfonic acid (100 mg). This mixture was heated under reflux with a Dean–Stark trap for 3 days, after which time analytical TLC showed a fast-moving spot. After being passed through a bed of alumina (Brockman Grade III), the solution was washed with saturated aqueous $NaHCO_3$ and water and then dried over anhydrous Na_2SO_4. The solution was then evaporated to dryness under vacuum and the residue was chromatographed on alumina (Brockmann Grade III; elution with methylene chloride). The ketal formed a fast-moving green band, and this was followed by a second band, which afforded methyl pyropheophorbide *a* (700 mg), identical with an authentic sample. The eluates containing the ketal were evaporated to dryness, and the product **3** was crystallized from CH_2Cl_2/*n*-hexane to give 1.131 g (59%): mp 182–184 °C; λ_{max} 300 nm (ϵ 12 000), 391 (93 000), 500 (13 800), 598 (3 800), 654 (41 000). Anal. Calcd for $C_{36}H_{40}N_4O_4$: C, 72.94; H, 6.80; N, 9.45. Found: C, 72.98; H, 6.82; N, 9.33.

9-Ketal (4) of 2-Vinylphylloerythrin Methyl Ester. The chlorin ketal **3** (300 mg) in methylene chloride (200 mL) was stirred at 0 °C (ice bath) and treated with 77.5 mL of a DDQ solution (435 mg of DDQ in 250 mL of benzene), dropwise. The resulting mixture was monitored by analytical TLC, the reaction being deemed complete when the green ketal chlorin had been compleely transformed into a red ketal porphyrin spot. The mixture was then filtered through a short bed of alumina (Brockmann Grade III), and the red solution was evaporated to dryness. Crystallization of the residue from CH_2Cl_2/benzene gave 241 mg (81%) of the ketal porphyrin: mp >300 °C; λ_{max} 412 nm (ϵ 92 900), 508 (15 200), 546 (10 300), 570 (8900), 622(1400). Anal. Calcd. for $C_{36}H_{38}H_4O_4$: C, 73.20; H, 6.48; N, 9.48. Found: C, 72.92; H, 6.48; N, 9.29.

2-Vinylphylloerythrin Methyl Ester (2). The foregoing ketal porphyrin **4** (200 mg) in tetrahydrofuran (1 L) and acetone (300 mL) was shaken for 10 min with 0.3 M aqueous HCl (300 mL) and then poured into methylene chloride (500 mL) and washed 3 times with water (1 L). The organic phase was dried (Na_2SO_4) and evaporated to dryness, and the residue was crystallized from CH_2Cl_2/MeOH to give 2-vinylphylloerythrin methyl ester (181 mg; 98%), mp 277–280 °C (lit.[15] mp 278 °C).

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Vinyl Influences on Protoheme Resonance Raman Spectra: Nickel(II) Protoporphyrin IX with Deuterated Vinyl Groups

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Abstract: Infrared absorption and variable excitation (4067, 4131, 5145, and 5682 Å, near the B, Q_1, and Q_0 absorption maxima) resonance Raman spectra are reported for NiPP (PP = protoporphyrin IX dimethyl ester) and for NiPP-d_α (vinyl α-carbons deuterated) and NiPP-$d_{2\beta}$ (vinyl β-carbons dideuterated). The deuteration shifts and comparison with NiOEP (OEP = octaethylporphyrin) permit identification of most of the vinyl modes and correlation of the protoporphyrin skeletal modes with those of OEP. Strong interactions with skeletal modes are evident in splittings of some of the vinyl modes and are probably responsible for the vinyl mode Raman enhancements. They also shift some of the skeletal mode frequencies, relative to NiOEP, but several skeletal modes are unshifted, including some previously identified as heme structure markers. The vinyl groups induce Raman activity into several infrared modes, reflecting the asymmetric conjugation in PP. No IR activity is seen for Raman modes, however, suggesting that vinyl conjugation occurs primarily in the excited state.

Resonance Raman (RR) spectroscopy is increasingly seen as a powerful tool for the study of heme protein structure and dynamics.[1-9] If it is to reach its full potential, the spectra must be properly assigned, and the nature of the vibrations being monitored must be understood. Much progress has been made in this direction through the study, in many laboratories, of metalloporphyrin RR systematics. An important step was taken by

Kitagawa and co-workers,[10] who assigned all of the in-plane skeletal Raman modes of NiOEP (OEP = octaethylporphyrin),

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