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NMR Spectra of Porphyrins. 21. Applications of the Ring-Current Model to Porphyrin and Chlorophyll Aggregation[1]

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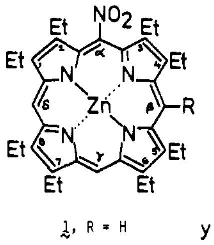
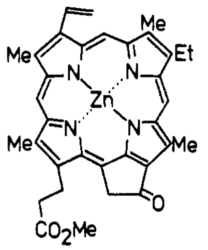
Abstract: A program incorporating the previously described double dipole model of the macrocyclic ring current in the porphyrin and chlorin ring is presented which allows the geometry of the aggregated species in solution to be obtained from observed NMR aggregation shifts. The model is capable of handling dimeric or oligomeric aggregates with either head-to-head or head-to-tail structures; also, one porphyrin may be rotated with respect to the other. Side chains may be rotated separately. The application of the model to a variety of previously recorded aggregates is presented. In the porphyrin series, the refined model allows a more rigorous search procedure than hitherto, and this shows that the structure of the dimers of zinc(II) *meso*-nitrooctaethylporphyrin (**1**) and zinc(II) α,β-*meso*-dinitrooctaethylporphyrin (**2**) are more similar than was previously recognized. In both structures the coordination is between the zinc atom and the most electron-rich pyrrole subunit of the adjacent molecule. The aggregation shifts of zinc(II) 2-vinylphylloerythrin methyl ester (**3**) were analyzed and shown to agree with both a centrosymmetric and a twofold symmetric dimer structure. The latter allows maximum interactions between the electron-rich and electron-deficient rings, and is preferred. In neither case is there any evidence of interaction between the metal and side-chain donor groups. Application of the model to the published aggregation shifts of chlorophyll *a* (Chl *a*) (**4**) and chlorophyll *b* (Chl *b*) (**5**) provides the first quantitative explanation of these aggregation shifts and defines new structures for the aggregates. Although the aggregation shifts of Chl *a* agree reasonably well with the previously proposed face-to-face head-to-tail structure, they fit better for an unsymmetrical "piggy-back" structure in which the Chl molecules are rotated 180° to each other. In this structure, both the C-10 carbomethoxy group and the C-9 keto group are in proximity to the magnesium atoms of the adjacent molecules. The aggregation shifts of Chl *b* are interpreted on the basis of an essentially head-to-head "piggy-back" structure in which the C-3 formyl group and the C-9/C-10 keto system are in proximity to the magnesium atoms of adjacent molecules. In both Chl *a* and Chl *b*, the lateral separation of the parallel Chl molecules is ca. 6 Å, which is too large for direct C=O to magnesium bonding, and implies the possible presence of linking water molecules. Also, in both molecules, but more particularly in Chl *b*, the analysis provides evidence for the formation of higher aggregates than the dimer, which can be easily visualized in terms of the proposed structures.

Introduction

The phenomenon of aggregation in solutions of both porphyrins and chlorophylls (Chls) has been well reviewed[2,3] and intensively investigated, both in previous parts of this series,[4-7] and elsewhere,[8,9] because its practical and intrinsic importance in the chemistry and characterization of these important compounds. Even simple NMR characterization of porphyrins, and particularly metalloporphyrins, may be hazardous if the aggregation shifts are not either considered explicitly or are removed.[5] Furthermore, a knowledge of the nature and structure of the aggregates formed could be of relevance in understanding the complex chemistry of the metalloporphyrins and Chls in vivo,[3,10] with particular reference to the question of the "special pair" photosynthetic reaction center in the latter.[11,12]



1, R = H
2, R = NO2



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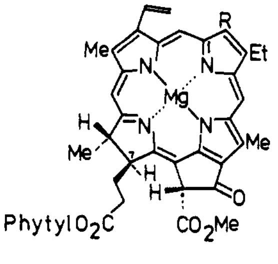

4, R = Me (Chl *a*)
5, R = CHO (Chl *b*)
Chlorophyllides have an ester other than phytyl at the 7-side chain.

Almost all of the NMR studies of aggregation phenomena have made use of the large magnetic anisotropy of the macrocyclic ring due to the interatomic ring current, and thus the quantitative interpretation of the observed shifts necessarily requires a precise ring-current model. Recent parts of this series[4,13,14] have been

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[1] Part 20: Abraham, R. J.; Bedford, G. R.; Plant, J. *Org. Magn. Reson.* **1982**, *19*, 204-210.

[2] Katz, J. J. In "Porphyrins and Metalloporphyrins"; Smith, K. M., Ed.; Elsevier: Amsterdam, 1975; pp 399-524.

[3] Katz, J. J.; Shipman, L. L.; Cotton, T. M.; Janson, T. R. In "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. 5, pp 402-458.

[4] Abraham, R. J.; Smith, K. M.; Goff, D. A.; Lai, J.-J. *J. Am. Chem. Soc.* **1982**, *104*, 4332-4337.

[5] Abraham, R. J.; Fell, S. C. M.; Pearson, H.; Smith, K. M. *Tetrahedron* **1979**, *35*, 1759-1766.

[6] Abraham, R. J.; Barnett, G. H.; Hawkes, G. E.; Smith, K. M. *Tetrahedron* **1976**, *32*, 2949-2956.

[7] Abraham, R. J.; Evans, B.; Smith, K. M. *Tetrahedron* **1978**, *34*, 1213-1220.

[8] Closs, G. L.; Katz, J. J.; Pennington, F. C.; Thomas, M. R.; Strain, H. H. *J. Am. Chem. Soc.* **1963**, *85*, 3809-3821.

[9] Boucher, L. J.; Katz, J. J. *J. Am. Chem. Soc.* **1967**, *89*, 4703-4708.

[10] A reviewer has pointed out that since Chl rarely occurs in vivo outside of proteins, the coordination chemistry of Chl's under such circumstances might be more dominated by Chl-protein than by Chl-Chl interactions. We take his point. However, in this context we should mention that the electronic absorption spectra of antenna arrays of pigments in living green and brown bacteria can be closely modeled in vitro in hexane solution; the antenna system is clearly a Chl-Chl oligomer which is certainly not dominated by Chl-protein interactions: Smith, K. M.; Kehres, L. A.; Fajer, J. *J. Am. Chem. Soc.* **1983**, *105*, 1387-1389.

[11] Wasielewski, M. R.; Smith, U. H.; Cope, B. T.; Katz, J. J. *J. Am. Chem. Soc.* **1977**, *99*, 4172-4173.

[12] Boxer, S. G.; Closs, G. L. *J. Am. Chem. Soc.* **1976**, *98*, 5406-5408.

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devoted to the parameterization of such a model for both the porphyrin and chlorin (7,8-dihydroporphyrin) rings, based on the network approach to the macrocyclic ring current. The application of the model to metalloporphyrin–ligand complexes has been encouraging in that the geometries obtained via the ring-current shifts are in good agreement with crystal studies.[15] Here we show further how the ring-current model can be used to determine the geometry of the aggregates in a number of metalloporphyrin and Chl systems in solution. In particular, the geometry of the dimers of Chl *a* and Chl *b* can be obtained directly from the observed aggregation shifts and compared with previous, more qualitative, suggestions.

Theory

The ring-current model used here is that described and parameterized in ref 4. This gives the values of the equivalent dipoles needed for both the porphyrin and Chl macrocycles; in the latter, the effects of both the C-9 keto function and the reduction in ring D were considered explicitly.

The equivalent dipole (network) model of the porphyrin ring current begins to break down at distances $<\simeq 3.6$ Å above the porphyrin ring, and the model presented in ref 4 includes a close-range approximation with both first- and second-order continuity through space. This allows the calculation of the ring-current shifts at all distances; indeed, the model gives a good account of the ring-current shifts of the NH protons within the current loop itself. Thus, the model may be used with some confidence in these aggregation studies.

In order to utilize the above model to calculate aggregation shifts, certain limitations have to be introduced. We have restricted the calculation to dimer aggregates; i.e., the ring-current shifts of the nuclei in one molecule due to the ring current of the adjacent molecule, and vice versa, are calculated and averaged. The calculation of the shifts of the general trimer is a far more complex problem (indeed the geometry of the general trimer requires 12 parameters to define it), and most of the observed aggregation studies have been interpreted in terms of a monomer–dimer equilibrium. However, the formation of small amounts of trimer, or higher aggregates, has been postulated, and this can be taken account of in the calculations provided that the structure of the trimer is related to that of the dimer, by comparing the observed and calculated shift ratios. Examples of this analysis will be given subsequently.[16]

The observed NMR aggregation shifts, which may be defined simply as δ(monomer) − δ(aggregate),[17] are the result of a time-averaged process, as the formation and dissociation of the aggregates are fast on the NMR time scale. It is therefore extremely important not to limit the structure of the aggregate to the observed symmetry of the molecule. For example, if the molecule has a plane of symmetry, there is no reason to suppose that the aggregate will also have a plane of symmetry; the NMR shifts will reflect the molecular symmetry due to the time-averaging process. Again, this will be demonstrated later.

In the general dimer, the environment of the two molecules may differ, and therefore it is necessary to calculate the shifts of one molecule with respect to the other, reverse the process, and then average the two sets of shifts. The "base" macrocycle, i.e., the porphyrin ring from which the ring current shift is calculated, is fixed with respect to the coordinate system; i.e., the equivalent dipoles simulating the ring current are at fixed coordinate positions. In our convention this base molecule lies in the x,y plane, the z axis being the fourfold symmetry axis of the porphyrin. Consider these equivalent dipoles as comprising the molecular set A (Figure 1). The calculation of the ring-current shifts due to A of any molecule B, displaced a distance x,y,z from the origin and rotated an angle θ about the z axis, can be simply calculated using the Z-matrix input described previously.[4] The calculation gives both the shifts (δ_B) and also the coordinate array P_B of B with respect to the origin (i.e., molecule A). The reciprocal calculation, i.e., of the shifts of the corresponding nuclei in molecule A due to the ring current in B, is achieved as shown in Figure 1. Two successive translations ($-x$, $-y$, $-z$) and rotations (θ) convert the coordinate array P_B into the corresponding array for molecule A, with molecule B now at the origin (Figure 1). At this stage the shifts for A with respect to B (the origin) can be calculated, as previously.

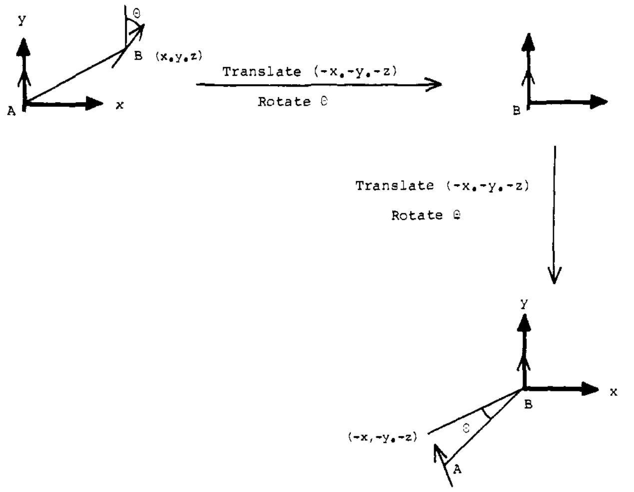

Figure 1. Successive translations and rotations of the coordinate array P_B required to calculate the coordinates and chemical shifts of A with respect to B.

In principle, the calculation is general and may be used for any dimer structure. We have, however, restricted the following calculations to considering a dimer formed by two parallel molecular planes. This is the form of the dimer most often considered, and this assumption also reduces the number of parameters needed to define the dimer geometry from six to four. In those cases where the dimer structure is centrosymmetric or has other elements of symmetry, as in the head-to-tail dimers, the two molecules in the dimer are equivalent and only one calculation need be performed.

In the general case, the program thus calculates the two sets of ring-current shifts A with respect to B, and B with respect to A, averages them, and compares the resultant average with the observed aggregation shifts. In this process, further averaging may be required for chemically equivalent nuclei (in the porphyrin) which are not chemically equivalent in the dimer. This option is available in the program. The end result is to be able to compare computationally, by the usual least-mean-square procedure, the calculated and observed aggregation shifts, and thus the dimensions of the complex (x, y, z, θ) may be scanned for the best agreement of the calculated and observed shifts. In the normalized shift calculations the same search procedure is followed, but now the sums of the calculated and observed shifts are equated; i.e., the calculated and observed shift ratios are compared.

Results

The model described above may be used to define, within the limits imposed, the geometry of any porphyrin or Chl dimer from the observed NMR aggregation shifts. Naturally, in such a process the accuracy or otherwise of the final geometry will depend as much on the accuracy and comprehensiveness of the experimental data available as on the model itself. In particular, the dangers of insufficient experimental data to overdetermine the geometry of the complex must always be kept in mind. For these reasons

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(16) For recent work on Chl oligomeric species, see: Smith et al., ref 10. Betti, J. A.; Blankenship, R. E.; Natarajan, L. V.; Dickinson, L. C.; Fuller, R. C. *Biochim. Biophys. Acta* **1982**, *680*, 194–201. Fong, F. K.; Kusunoki, M.; Galloway, L.; Matthews, T. G.; Lytle, F. E.; Hoff, A. J.; Brinkman, F. A. *J. Am. Chem. Soc.* **1982**, *104*, 2759–2767, and refs therein. Blaha, H.; Kis, P.; Springer-Lederer, H. *Anal. Biochem.* **1981**, *112*, 282–286.

(17) We use this convention, which is the reverse of the usual one, in order to make the sign of the majority of the aggregation shifts, which are upfield, as positive.

Table I. Observed, Calculated, and Normalized Complexation Shifts ($\Delta\delta$) for Zinc(II) *meso*-Nitrooctaethylporphyrin (1) and Zinc(II) α,β-*meso*-Dinitrooctaethylporphyrin (2)

compound 1				compound 2			
proton	obsd[a]	calcd[b]	normalized[b]	proton	obsd[a]	calcd[c]	normalized[c]
meso γ	2.50	1.99	2.56	γ,δ	2.14	1.80	2.10
meso β,δ	1.45	1.02	1.31				
CH_2 1,4	0.18	0.12	0.16	1,6	0.42	0.40	0.44
CH_2 2,3	−0.20	−0.13	−0.17	2,5	−0.02	−0.11	−0.12
CH_2 5,8	1.23	0.88	1.14	3,4	−0.22	−0.14	−0.15
CH_2 6,7	1.30	1.14	1.46	7,8	1.90	1.77	1.92
RMS error		0.316	0.099			0.11	0.056

[a] From ref 7. [b] Displacement coordinates 2.0, 2.0, 4.4 Å. [c] Displacement coordinates 1.8, 1.8, 4.6 Å.

we have selected a number of examples from the literature in which either complete assignments and aggregation shifts are known, or in which there are sufficient data to overdetermine the geometry of the complex. These will be considered separately, as follows.

Zinc(II) *meso*-Nitrooctaethylporphyrin (1) and Zinc(II) α,β-*meso*-Dinitrooctaethylporphyrin (2). The strong aggregation of these molecules in solution was followed from their proton NMR spectra, both as a function of concentration in $CDCl_3$, and also by the addition of pyrrolidine, which preferentially complexes to the zinc atom and breaks up the aggregate to give the monomer spectrum.[7] The resulting shifts of the monomers were used to analyze the dilution curves on the basis of a monomer–dimer equilibrium, and the complexation shifts ($\Delta\delta$) of all the separate protons in the molecules are given in Table I. These shifts were shown to establish the structure of the dimers as tail-to-tail. However, the analyses of the shifts had to follow the symmetry elements of the molecules as there was no method available, at that time, for calculating and averaging the shifts of the chemically equivalent nuclei. Thus, for example, the displacement of the molecules in the tail-to-tail structure identified for 1 was considered to be along the symmetry axis (i.e., the y axis). This automatically ensured that β and δ meso protons would have the same shift. Similarly, the lateral movement of the zinc(II) dinitro complex 2 was taken along the line $x = y$ as again this preserved the molecular symmetry. This, as we have pointed out, is an artifact of the calculations; the aggregates need not have molecular symmetry, but in calculating the aggregation shifts the molecular symmetry must be preserved. Thus, we average the calculated shifts of the chemically equivalent protons H_β and H_δ and the C(2,3), C(1,4), C(5,8) and C(6,7) methylene groups of 1, and the γ,δ meso protons and the C(1,6), C(2,5), C(3,4), and C(7,8) methylenes of 2. With this amendment it is possible to scan any dimer structure for agreement with the observed $\Delta\delta$'s; the molecular symmetry is always preserved in the calculations. In this case, as the tail-to-tail structure is centrosymmetric, only one molecule need be calculated. The geometry of the dimer was scanned over a range of distances (x, y, z) using both the observed vs. calculated shifts and the observed vs. normalized shifts (calculated shifts normalized to the sum of the observed shifts) as tests of the goodness of fit. The calculations were performed for both freely rotating ethyl groups and with the ethyl groups normal to the porphyrin ring plane. No significant difference was observed in the two cases and the results of the latter case are given in Table I and Figure 2. In *both* molecules the dimer structure is essentially the same, with pyrrole ring D (in 2) and C or D (in 1) immediately above the zinc atom of the adjacent molecule. (Note that rings C and D are equivalent in 1; thus the zinc atom could be above one or the other. This is *not* the same calculation, however, as placing the zinc atom in between rings C and D.)

The geometries obtained for the dimer structures now both agree with the previous interpretation of this complexation as being due primarily to metal-to-porphyrin π bonding.[7] With the strongly perturbing *meso*-nitro substituents, the π-electron distribution around the ring will be very anisotropic and as the zinc atom will complex to the most electron-rich part of the system, the structure of the complexes implicating the donor pyrrole rings as C (or D) in 1 and D in 2 is clearly explained.

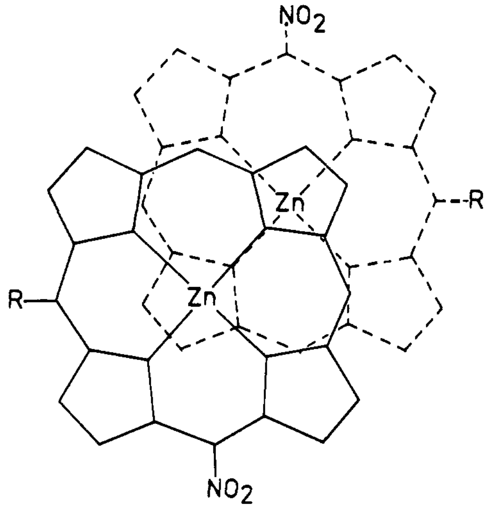

Figure 2. Proposed dimer structure for zinc(II) *meso*-nitrooctaethylporphyrin (1, R = H) and zinc(II) α,β-*meso*-dinitrooctaethylporphyrin (2, R = NO_2). Ethyl substituents have been omitted for clarity.

A further point of interest comes from inspections of the observed and calculated data. In both molecules the calculated shifts are all significantly less than observed, by an almost constant percentage. This is clearly apparent when the observed and normalized shifts are compared, in which essentially exact agreement is obtained. This is very probably due to formation of trimers (and higher aggregates). In the dimer structures considered, a third molecule can stack in precisely the same manner as the first two. This will simply increase all the observed aggregation shifts in the same ratio. An increase of ca. 10%, as observed, would be perfectly feasible, and would not be detected in the analysis. Indeed, the occurrence of higher aggregates in solution was previously inferred.[7] It is of some interest that the analysis presented here does identify to some extent the formation of oligomers.

Zinc(II) 2-Vinylphylloerythrin Methyl Ester (3). A metalloporphyrin which is closely related to the Chl system (see later) and for which considerable aggregation shifts are observed in the proton NMR spectra is the zinc(II) complex (3) of phylloerythrin methyl ester. A complete assignment of the proton spectrum of both the monomeric and aggregated forms has been given, and thus also a complete set of aggregation shifts.[4] The observed aggregation shifts (Table II) are simply the difference between the proton chemical shifts of a 2.4 mM solution in $CDCl_3$ and those of the same solution after addition of an excess of pyridine; thus, there is the possibility that higher aggregates than the dimer are present in the original solution. We therefore considered both the direct calculated shifts and the normalized shifts with respect to the observed shifts.

The geometry of the molecule was that used in ref 4, taken directly from a number of crystallographic studies of porphyrin and chlorin rings,[18-20] and the geometry of the dimer was scanned

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Table II. Observed, Calculated, and Normalized Complexation Shifts (Δδ) for Zinc(II) 2-Vinylphylloerythrin Methyl Ester (3)

proton	obsd[a]	calcd[b]	normalized[b]
meso α	0.60	0.41	0.61
β	1.08	0.68	1.03
δ	1.32	0.87	1.31
Me-1	0.23	0.23	0.35
Me-3	0.23	0.09	0.14
Me-5	0.39	0.31	0.48
Me-8	0.73	0.44	0.66
C-10 CH_2	1.25	0.82	1.25
C-4 CH_2	0.33	0.16	0.25
C-4 Me	0.15	0.09	0.13
C-7a CH_2	0.99	0.67	1.01
C-7b CH_2	0.63	0.50	0.76
C-7 CO_2Me	0.03	0.03	0.04
C-2α H	0.20	0.15	0.22
C-2β H	0.02	0.05	0.08
C-2β′ H	0.10	−0.03	−0.04
RMS error		0.23	0.07

[a] 6.8 mM in $CDCl_3$, from ref 4. [b] Displacement coordinates 0.2, 1.0, 5.0 Å; side-chain orientations, see text.

for the best agreement of the observed and normalized shifts using initially only those protons of fixed position (i.e., excluding the C-2 vinyl, the C-4 methyl, and the C-7 methylene protons). We consider also a one molecule calculation, i.e., of the head-to-tail dimer. The iteration converged smoothly to a reasonable minimum. The side chains can now be included by allowing the dihedral angles of the side chains to be varied. The best solution, which includes all the observed proton aggregation shifts, is given in Table II.

Inspection of Table II shows that the calculated shifts are considerably less than the observed aggregation shifts, even more so than in the *meso*-nitrooctaethylporphyrin case, but the normalized shifts are in good agreement. Again, this is very likely due to the presence of trimers or higher aggregates; indeed, the larger ratio in this case would suggest that a considerable proportion of the molecules in the solution measured exist as aggregates larger than the simple dimer.

It is of some interest to consider the structure of the dimer obtained from the computation. The calculations in Table II are for one molecule only; i.e., they are for a dimer in which both molecules have identical chemical shifts, by symmetry. There are, in fact, two dimer structures which satisfy the above criterion (Figure 3). Both are necessarily head-to-tail dimers, but whereas structure A is the general centrosymmetric dimer, structure B is also possible as the two molecules in the dimer are related by a twofold axis of symmetry. Structure B is also a general solution and is obtained by rotating molecule B by 180° about an axis perpendicular to the displacement vector. The two solutions cannot be distinguished analytically in this case, as they give identical shifts, but a distinction can be made on the basis of chemical evidence. Structure B is to be preferred chemically in that the electron-rich pyrrole subunits B and D overlap to some extent with the electron-deficient rings A and C. Maximum overlap is not possible as this would place the two zinc atoms immediately adjacent to each other, so a compromise is obtained whereby the zinc atoms are displaced horizontally from each other, but some overlap is still allowed between the two different types of pyrrole subunit. In structure A, in contrast, the electron-deficient rings A and C and the electron-rich rings B and D overlap, and this we consider to be less likely. One notes here also that in complete contrast to the Chls, there is no evidence of any interaction between the metal (zinc) atom and the C-9 keto function.

The preferred orientations of the side chains are also included in the calculated shifts of Table II, but it must be remembered that these are necessarily time-averaged values. The best agreement is obtained for the C-2 vinyl group coplanar with the porphyrin ring and trans with regard to the α-meso proton, the C-4 ethyl group at 30° to the porphyrin and cis to the C-3 methyl, and the C-7 propionate group with dihedrals of 90 and 120° along the chain, i.e., C-7β normal to the porphyrin plane and the CO_2Me group oscillating between the classical staggered positions with dihedrals 60 and 180°.

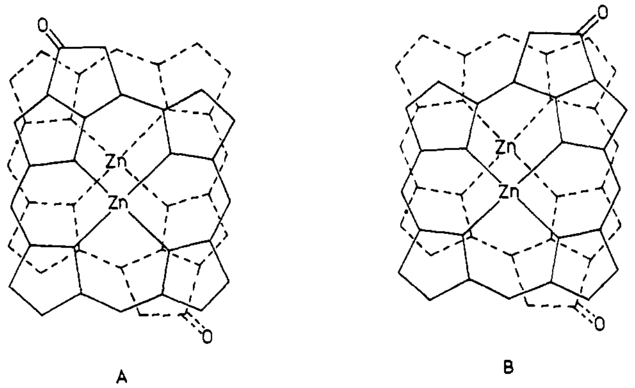

Figure 3. Proposed dimer structures for zinc(II) 2-vinylphylloerythrin methyl ester (3). Peripheral substituents have been omitted for clarity.

Chlorphyll Aggregation. Because of both its intrinsic importance in the molecular organization of Chl in vivo and the possible relevance in the photoreaction processes,[21] the structure of the Chl aggregate formed in solution has been the subject of numerous investigations and some controversy for over a decade.[3] The first systematic NMR investigation of Chl aggregation by Closs et al.[8] has formed the basis for much subsequent discussion. The proton spectra of Chls *a* and *b*, the related chlorophyllides (Chlides), and pheophorbides were observed and assigned in $CDCl_3$ solution. The spectra showed clearly the large aggregation shifts of the magnesium-containing molecules in this solvent, which contrasted with the much smaller shifts for the pheophorbides. That coordination to the magnesium atom was responsible for the aggregation was supported by the dissociation of the aggregates to the monomer upon addition of competing donor molecules such as methanol.

The prime role of the magnesium is not in doubt, but the precise nature of the complex and the role of any interstitial water molecules is still an open question. The coordinating group in the donor molecule is also the subject of some controversy. Both the C-9 keto group and the C-10 carbomethoxy group in Chl *a* have been suggested, as well as the C-3 formyl group in Chl *b*, and both parallel plane dimers and skew structures have been proposed. Katz and co-workers, on the basis of proton[8,22] and carbon-13[23] NMR and IR[24] data suggested that the C-9 carbonyl function was the primary donor group; both the disappearance upon aggregation of the IR band assigned to this group and the similar behavior of pyroChl *a* (which lacks the 10-carbomethoxy group) support this proposal. Fong[25] reinterpreted the aggregation shifts in terms of a symmetrical (head-to-tail) dimer structure in which the magnesium was coordinated to the C-10 carbomethoxy group. Houssier and Sauer,[26] from circular dichroism measurements, proposed a skew nonsymmetrical dimer involving magnesium coordination (in the different molecules) of the C-9 and C-10 carbonyl groups. Katz, in a recent review,[3] has criticized these alternative structures as being inconsistent with the NMR

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(24) Katz, J. J.; Closs, G. L.; Pennington, F. L.; Thomas, M. R.; Strain, H. H. *J. Am. Chem. Soc.* **1963**, *85*, 3801–3809.

(25) Fong, F. K.; Koester, V. J. *J. Am. Chem. Soc.* **1975**, *97*, 6888–6890. Fong, F. K. *Ibid.* **1975**, *97*, 6890–6892.

(26) Houssier, C.; Sauer, K. *J. Am. Chem. Soc.* **1970**, *92*, 779–791.

Table III. Observed and Calculated Complexation Shifts ($\Delta\delta$) for Chlorophyll *a* (4)

			calculated shifts			
	obsd shifts		symmetric dimer	unsymmetric dimer		
proton	set 1[a]	set 2[b]	direct[d]	direct[d]	normalized[e]	trimer[f]
meso α	0.08	0.24	−0.06	−0.03	0.03	0.14
meso β	0.18	0.22	0.37	0.30	0.34	0.50
meso δ	0.13	0.07	0.08	0.15	0.19	0.30
Me-1	0.03	0.05	−0.08	−0.04	0.01	0.08
Me-3	0.07	0.11	−0.07	−0.05	−0.01	0.06
Me-5	0.83	0.90	0.31	0.62	0.64	0.75
H-10	1.85	2.05	1.51	1.87	1.72	1.83
Me-8	0.42		0.22	0.26	0.40	0.45
C-4 CH_2	0.08		−0.07	−0.02	0.05	0.14
C-10 CH_2Me	0.72	0.61	0.60	0.74	0.98	1.20
C-7 CO_2Me	0.85		0.67	0.41	0.88	0.91
C-2α H	−0.02	0.09	−0.08	−0.05	−0.02	0.04
C-2β H	−0.03	0.05	−0.08	−0.06	−0.03	0.01
C-2β′ H	−0.03		−0.07	−0.05	−0.02	0.01
RMS error			0.20	0.15	0.11	

[a] Methyl chlorophyllide *a*(0.08 M in $CDCl_3$), titrated with methanol (ref 8). [b] Chlorophyll *a* (0.06 M in CCl_4), titrated with pyridine-d_5 (ref 3). [c] Displacement coordinates 0.0, 3.5, 4.5 Å. [d] Displacement coordinates 0.0, −4.0, 5.0 Å, rotated 210°. [e] Displacement coordinates 1.5, −5.0, 7.0 Å, rotated 180°, normalizing factor 1.8. [f] Displacement coordinates 1.5, −4.0, 6.0 Å, rotated 180°.

data. Of some relevance to these discussions is the crystal structure of ethyl Chlide *a* dihydrate[18] in which the Chlide molecules form a one-dimensional polymer where the bridging link forms from the magnesium of one molecule, via two water molecules, to the C-9 carbonyl of the adjacent molecule.

However, although all the interpreations of the NMR aggregation shifts assume that they are due to the macrocyclic ring current of the chlorin ring, there has been no attempt to quantify these interpretations with a good ring-current model. This provides the justification for our present attempt.

Despite the number of investigations and hypotheses, there is still a dearth of experimental data. The aggregation shifts given by Closs et al.[8] for Chl *a* and *b* and methyl chlide *a* and *b* are still the most complete set of data available, even though they were obtained on low-field spectrometers (40 and 60 MHz) with comparatively low sensitivity and dispersion compared to present-day machines. This necessitated the use of relatively high concentrations (ca. 0.1 M) in $CDCl_3$. Katz[3] has recently given the aggregation shifts of Chl *a* in CCl_4 solution (0.06 M). These, though not as complete a set as the earlier data because of the phytyl resonances obscuring some of the Chl peaks, are on more modern spectrometers. Both sets of data are given in Tables III and IV.

The observed aggregation shifts for Chl *a* and methyl Chlide *a* at the same concentrations in $CDCl_3$ are identical. We give the latter as there are more resonances. Thus, the differences between the two sets of results in Table III are due to the different concentrations and solvent, and not to the removal of the phytyl side chain.

Katz[3] has also warned of the danger of transferring the results of one set of aggregation studies to another investigation. The various aggregation studies have been conducted in different media and very different concentrations with varying amounts of impurities (particularly water); thus, the result of one cannot always be transferred to another. This is of some relevance to the NMR studies which are, necessarily, at relatively high concentrations, in which the aggregation number[27] of the aggregate may be more than 2. The definitive studies of Ballschmiter et al.,[28] based on vapor phase osmometry, are very pertinent to our analysis. They find that, in CCl_4 solution, the aggregation number of Chl *a* varies from 2 in very dilute solution (<0.01 M) to 2.5 (0.04 M) to ca. 4 (0.1 M). They state that "1,2-dichloroethane is a somewhat more aggregating solvent". Inasmuch as chloroform has a dielectric constant between CCl_4 and 1,2-dichloroethane, we may safely assume that the observed aggregation shifts of Chl *a* (Table III) in 0.1 M ($CDCl_3$) and 0.06 M (CCl_4) solutions correspond to situations in which the aggregation number is larger than 2, and perhaps as high as 3 or 4.

Table IV. Observed and Calculated Complexation Shifts ($\Delta\delta$) for Chlorophyll *b* (5) and Methyl Chlorophyllide *b*

	observed shifts		calculated shifts	
proton	chlorophyll *b*[a]	Me Chlide *b*[b]	direct[c]	normalized[d]
meso α	0.80	1.00	0.63	0.90
meso β	0.32	0.67	0.26	0.36
meso δ	0.17	0.13	0.06	0.09
Me-1	0.10	0.07	−0.01	0.02
Me-5	0.42	0.28	0.17	0.30
Me-8		0.25	0.32	0.37
C-10 H	1.07	0.83	0.53	0.79
3-CHO	1.58	1.73	0.60	0.80
C-10 CO_2Me	0.50	0.42	0.39	0.65
C-7 CO_2Me		0.72	0.36	0.69
C-2α H	0.40	0.42	0.38	0.41
C-2β H	0.15	0.18	0.10	0.28
C-2β′ H	0.25	0.32	0.04	0.21
RMS error			0.22	0.18

[a] 0.08 M in $CDCl_3$ (ref 8). [b] 0.10 M in $CDCl_3$ (ref 8). [c] Displacement coordinates 1.5, 5.0, 5.5 Å, rotated 20 °, torsional angle CHO 90°, vinyl 90°. [d] Displacement coordinates 1.5, 5.5, 6.0 Å, rotated 20°, normalizing factor 1.76, torsional angle CHO 90°, vinyl 90°.

These workers also interpreted their results for Chl *b* in CCl_4 in terms of "a trimer–hexamer equilibrium in which hexamer becomes important in solutions more concentrated than 0.01 M." The NMR data for Chl *b* (Table IV) (0.1 M in $CDCl_3$) thus corresponds to the dissociation of larger aggregates. These results are of considerable importance in our analysis of the aggregation shifts. The aggregation shifts of a polymer to monomer are slightly more than twice those of the analogous dimer, as each molecule in the oligomer has two near neighbors, and the influence of the next-but-one molecule would be small but in the same direction. Thus we will analyze the observed shifts in terms of the normalized shifts as well as the calculated dimer shifts as the latter could be too small by a factor of 2. This assumes implicitly that the structures of the polymer and dimer are corresponding.

The geometry of the Chl nucleus used in our calculations was taken directly from the crystal studies of Chow et al.[18] on ethyl chlorophyllide *a* dihydrate. The formyl group for Chl *b* was added using standard bond lengths and angles.[29] It is convenient to

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(27) Defined, following ref 28, as the ratio of the molarity calculated as the monomer to the observed molarity.

(28) Ballschmiter, K.; Truesdell, K.; Katz, J. J. *Biochim. Biophys. Acta* **1969**, *184*, 604–613.

consider the two calculations separately.

Chlorophyll *a* (4). The chirality of the Chl molecule, possessing both an asymmetric ring current and peripheral substituents located away from the macrocyclic ring plane, both complicates and more completely defines the geometry of the dimer. Consider the simplest case, the one-molecule calculation analogous to that given previously for the porphyrins. Because of the chirality of the Chl molecule, the centrosymmetric dimer is not a possible structure, because this could only be formed from two optical isomers. The planarity of the porphyrins eliminated this problem previously. Thus, the only possible type of symmetric dimer corresponding to the one-molecule calculation is that in which the dimer has a twofold axis of symmetry. There are, however, still a large number of possible head-to-tail structures with this symmetry. The only criterion to be satisfied is that the lateral displacement of one molecule with respect to the other must be perpendicular to the twofold axis. This general statement may be approximated to a good degree of accuracy, as the orientation dependence of the ring current is gradual, by considering the twofold axis of symmetry to be either the x, y, $x = y$, or $x = -y$ axes (Figure 1). As the C-10 carbomethoxy group is essentially on the y axis, a structure of this type involving the coordination of this group with the magnesium atom (the Fong structure[25]) is given by a displacement along the $+y$ axis with the x axis as the twofold symmetry axis. However, a slightly deformed structure of this type could involve the C-9 keto function as the coordinating group. Trial calculations showed that no other symmetry axis gave any fit, so a computational search was made on the above basis; i.e., the base molecule (A, Figure 1) was rotated 180° about the x axis (this is necessary owing to the asymmetry of the ring current), and the second molecule (B) was moved over the coordinate axis and calculated. The normalized shifts cannot be used for this calculation as the addition of further molecules to the dimer cannot be with the same orientation as the dimer molecules (see later). Note that this structure assumes that the two molecules are parallel to each other (otherwise there is no twofold symmetry axis). This scanning process included only the aggregation shifts of those protons of fixed geometry with respect to the Chl ring. Having thus fixed the displacement of the molecules, the orientations of the vinyl, propionate, and carbomethoxy side chains were also varied to obtain the best agreement of these proton shifts.

The solutions given (Table III) show general agreement with the observed shifts, but there are some discrepancies. Some of the smaller shifts have the wrong sign in that the calculated shifts are negative (i.e., to low field) rather than as observed to high field on aggregation. This includes H_α, Me-1, Me-3, and the C-4 CH_2 protons. The observed aggregation shifts for these protons are all small (<0.1 ppm), but the two sets of observed results are very consistent where they overlap; thus the deviations may be significant. The aggregation shift of the vinyl protons, in contrast, agrees with the early data (i.e., a low-field shift on aggregation) but not the more recent data. More significantly, the nuclei with larger aggregation shifts are not always in the correct order, e.g., C-10 CO_2Me and Me-5. This could be due to the limitations of our model. The molecules cannot rotate with respect to each other nor can the molecular planes not be parallel. (In both cases the twofold symmetry is lost.)

It was felt worthwhile to consider whether a non-C_2-symmetric dimer could give better agreement with experiment. In this case we again assume parallel molecular planes, but now rotation of one molecule with respect to the z axis (Figure 1) is allowed. The program will automatically calculate the shifts of both molecules, following the procedure described earlier, and average them. Again, however, it is necessary to restrict the computational search, as there are many possible nonsymmetric structures. Simple chemical considerations suggested two likely nonsymmetric structures, bearing in mind that the coordinating keto groups on C-9 and C-10 should be in the vicinity of the magnesium atom on the adjoining molecule. The first scan attempted was for a head-to-head structure in which molecule B (Figure 1) was displaced in the $-x$, $+y$ quadrant. No satisfactory solution was found for this structure. A second possible structure involves the rotation of molecule B ca. 180° about the z axis followed by a displacement in the $+x$, $-y$ quadrant. This structure did produce a solution (i.e., the scanning process converged to a minimum R value) for the direct calculation of the shifts. The normalized shift scan converged to an unreal solution in that the ratio of normalized to calculated shifts became greater than 2, and we restricted the search to those solutions in which the ratio of the normalized to calculated shifts was less than this figure. Having defined the molecular displacements in the dimer, the orientations of the peripheral vinyl propionate, and carbomethoxy groups were varied, as before, to obtain the best agreement of these proton shifts; the complete solutions are given in Table III.

Before considering the two structures in detail, it is of interest to compare the calculated vs. observed shifts. Although it is pertinent to note that the unsymmetrical structure has one more variable parameter than the C_2-symmetric structure, (i.e., the molecular rotation about the z axis), the agreement between the calculated and observed shifts does seem significantly improved over the former model. In the direct calculation, as before, a number of protons with small aggregation shifts have the wrong calculated sign (H_α, Me-1, Me-3 and C-4 CH_2), and some of the peripheral protons with larger aggregation shifts are not particularly well reproduced (C-7 CO_2Me). However, the normalized scan removes most of these anomalies. Only Me-3 still has the wrong calculated sign and the calculated value is very small; the overall agreement is quite pleasing considering the limitations in our model.

The normalized calculations do not include any contribution from the nonnearest neighbors in the aggregate. In the case of Chl *a*, the next-but-one molecules are immediately over each other (Figure 4B) and this gives rise to a significant upfield shift for all the protons. This cannot be included in the iterative search routine, but Table III includes the calculated shifts for the analogous trimer (Figure 4B). In this case all the calculated complexation shifts are positive, as observed. This suggests that the differences in sign of the vinyl complexation shifts in the two sets of data (Table III) is simply due to differences in the aggregation number of the Chl *a* in the two solutions. The orientations of the side chains which give the best agreement are as follows: the C-10 carbomethoxy group is trans–trans with the carbonyl oxygen, trans (anti) to H-10; the C-7 propionate ester side chain is gauche–gauche with the ester coiled over the Chl; and the vinyl substituent is in the plane of the ring. It should be explained here that the calculations necessarily assume the same orientation of the side chains in the two molecules. As the molecular environments of the two molecules in the dimer are now different, the orientations of the side chains could also differ. There is also the further more general comment that we necessarily assume that the orientations of the side chains are the same in the monomer and aggregate. If they differ, then the aggregation shifts will be made up of ring-current effects from both molecules of the dimer. This could be the case for the C-7 propionate ester side chain.

One other dimer structure for Chl *a* (4) has been suggested by Ballschmiter and Katz.[30] This involves the direct coordination of the C-9 keto group to the magnesium atom of another molecule in a nonplanar skew dimer structure. Unfortunately, this structure cannot be handled computationally at present, as the two molecular planes of the dimer make an angle of ca. 60° to each other. Thus, this intriguing proposal must remain for the moment untested by direct calculation.

It is of some interest to compare the two structures found which reproduce, at least to a reasonable degree, the observed aggregation shifts. The C_2-symmetric head-to-tail structure (Figure 4A) is precisely that proposed by Fong.[25] Indeed the dimensions are such that the carbonyl group of the C-10 carbomethoxy substituent points toward the magnesium atom of the adjacent molecule, as suggested by Fong and Koester. The interatomic distances found also support this interaction. The interplanar separation is ca. 5 Å (the average of the direct and normalized solutions), and on

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(29) Pople, J. A.; Gordon, M. *J. Am. Chem. Soc.* **1967**, *89*, 4253–4261.

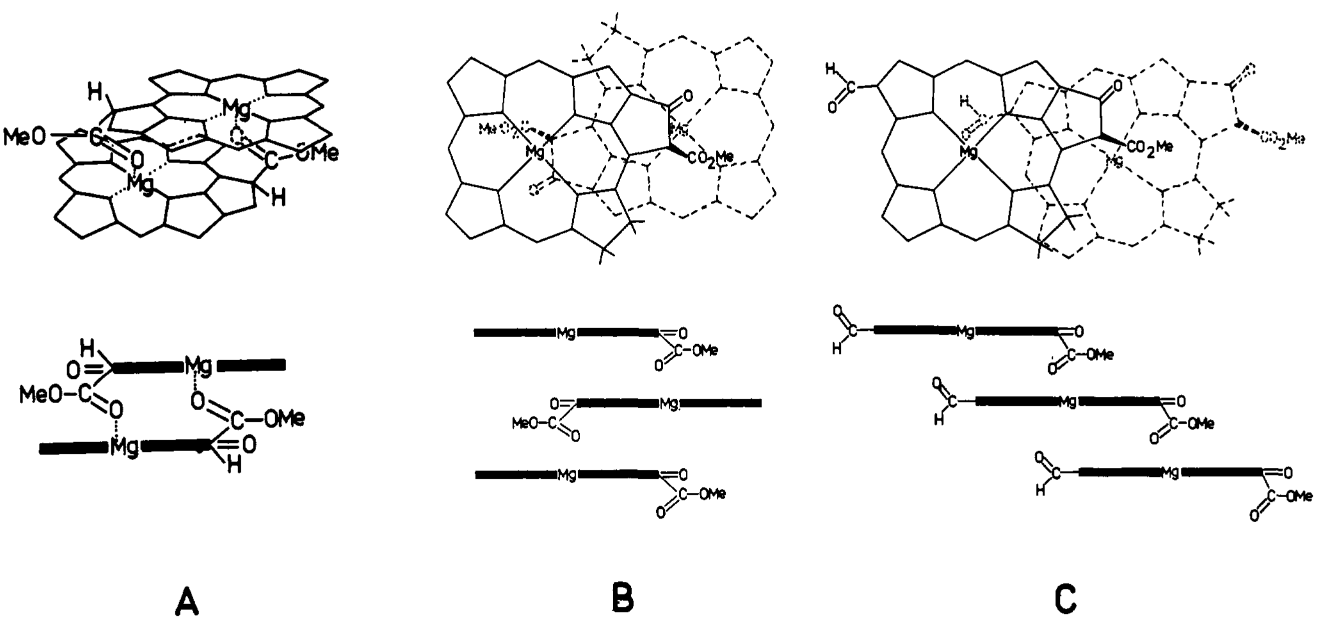

Figure 4. Proposed dimer structures and end-on projections: (A) the C_2 symmetrical Chl *a*–Chl *a* adduct;[25] (B) the unsymmetric Chl *a* (**4**) dimer; (C) the Chl *b* (**5**) dimer. Peripheral substituents have been omitted for clarity.

this basis the C-10 carbonyl oxygen is found ca. 2.7 Å above the adjacent molecule, a reasonable distance for O···Mg coordination.

The unsymmetric structure (Figure 4B), in contrast, no longer has the possibility of *both* C-10 carbomethoxy groups coordinating to the magnesium, as in one molecule this group points away from the adjacent molecule. The structure allows both one carbomethoxy and one or both of the C-9 keto groups to participate in the bonding and in this sense is very different from the C_2-symmetric structure. In the structure formulated (and note that the calculations do not define the dimer geometry particularly accurately, mainly because of the normalization problem), the C-9 keto groups are positioned over the magnesium atom of the adjoining molecule, suggesting that this group could be implicated in the dimer structure, as suggested by Katz.[3] However, the separation between the parallel planes of the dimer molecules (ca. 6 Å, taking the average of the two solutions given) makes it unlikely that there could be a direct attachment and strongly suggests the possibility of an intermediate molecule of water bridging the two Chl molecules. In the crystal structure of the ethyl chlorophyllide *a* dihydrate,[18] two water molecules formed the bridge between the magnesium of one molecule and the C-9 carbonyl of the adjacent molecule; in the similar structure proposed by Ballschmiter and Katz[30] for the Chl–water–Chl interaction, one water molecule coordinates to the C-9 keto and C-10 carbomethoxy groups, and to the magnesium atom of an adjacent Chl. Our proposed structure is akin to that of Ballschmiter and Katz[30] except that in our structure each Chl molecule is rotated 180° with respect to the adjoining molecule. The structure of Ballschmiter and Katz, based on IR data, would not reproduce the observed NMR aggregation shifts. The question of a possible intermediate water molecule is a controversial one and there is no direct evidence either way from the present studies. It is pertinent to note here that the central question is whether the solutions *used for the NMR experiments* could contain sufficient water to solvate the dimer (perhaps one water per two Chl molecules). In that the $CDCl_3$ used in was a commercial sample, it is possible that a small amount of water may have been present. It would indeed be a valuable experiment to obtain the aggregation shifts of Chl solutions in which there was known to be, say, one molecule of water per molecule of Chl.

An important advantage of the unsymmetric "piggy-back" structure proposed here is that it allows the ready formation of higher aggregates, as is observed. A third molecule can be attached either below or above the dimer in the same way as the dimer attachment. In the case of the C_2-symmetric head-to-tail structure, a third molecule cannot be attached to the dimer in the same way, suggesting a definite break between the dimer and the higher aggregates, which is not observed in practice. However, we cannot exclude a concentration- and solvent-dependent equilibrium involving both the C_2-symmetric dimer and the unsymmetric one.

Chlorophyll *b* (5). The observed aggregation shifts for Chl *b* and methyl Chlide *b* (Table IV), both from ref 8, agree reasonably well, and there is little doubt that the differences are due more to differences in concentration of the two solutions than to the presence or absence of the phytyl side chain. There is no definite proposed structure for the Chl *b* aggregate. Closs et al.[8] noted the presence of two highly shielded regions in the aggregation map, one around the C-3 formyl group and the other around the C-9, C-10 region, and suggested that this could be consistent with the presence of two dimers of comparable stability; alternatively, in the case of trimers (or higher aggregates), bonding could occur between the magnesium atoms of the different molecules and both the C-3 and C-9 carbonyl groups.

Inspection of the aggregation shifts (Table IV) indicates that the one-molecule calculation (i.e., a C_2-symmetrical head-to-tail structure) would not give any reasonable explanation of the observed values, and this was confirmed by our calculations. However, there are a number of possible unsymmetric structures which may give a better account of the aggregation map, as in such structures the averaging of the aggregation shifts between the two distinct molecules of the dimer could produce more than one highly shielded region. In view of the above considerations, and the chemically feasible structures, a trial calculation was performed on the basis of a head-to-head structure in which molecule B (Figure 1) was displaced in the $+x$, $+y$ quadrant and allowed to rotate. This structure did produce a solution, i.e., the observed minus calculated shift difference minimized, so a full scan was performed using both the direct calculated shifts and the normalized shifts of those protons of fixed orientation with respect to the Chl molecule. As previously, the normalizing scan was restricted to those solutions in which the ratio of the normalized to calculated shifts was less than 2. Even with this restriction an acceptable solution was found. Having thus defined the displacement and orientation of the two molecules, the orientations of the peripheral formyl, vinyl, carbomethoxy, and propionate substituents were varied, as before, to obtain the best agreement of their proton aggregation shifts; the complete solutions are given in Table IV.

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(30) Ballschmiter, K.; Katz, J. J. *J. Am. Chem. Soc.* **1969**, *91*, 2661–2677.

The solutions in Table IV do appear to constitute an acceptable explanation of the aggregation shifts of Chl *b*. The direct calculation is not a very good solution. The calculated shift of Me-1, though small, is the wrong sign, and those of H_α and H-10 are considerably less than is observed. In the light of the earlier discussion concerning the probable aggregation number of Chl *b* in the NMR solutions, this relatively poor agreement for the direct dimer calculation is not surprising. In contrast, the normalized shift calculation does represent a solution which can be seen to be within the limits of the experimental uncertainties of the observed shifts, apart from the C-3 formyl proton (see later), and thus represents the first quantitative solution of the aggregate shifts of Chl *b*. The aggregate shifts of the substituent protons are also pleasingly well reproduced with the side chain orientations as given.

The orientation of the C-10 carbomethoxy group is trans–trans, with the carbonyl oxygen trans (anti) to the C-9 proton, while that of the propionate ester group is gauche–gauche with the ester curled over the Chl ring. Note that this orientation is obtained from the aggregate shift of the ester methyl only, and therefore there is no reason to suppose that the phytyl side chain behaves similarly. The sizeable high-field aggregation shifts of the vinyl protons are also well reproduced in the calculated shifts, which are given for the vinyl group orthogonal to the Chl ring. However, in this case there is not a large dependence of these calculated shifts on the vinyl orientation, and an in-plane model fits the data almost as well. It must be remembered that there is no good reason to assume, as is necessary in the calculations, that the orientation of the substituent groups in the side chains will be exactly the same in the aggregate as in the monomer.[31] This is of importance because any variation in the orientation of the substituents upon aggregation will produce large changes in the ring-current shifts at the substituents due to the parent Chl molecule. The calculated ring-current shifts are simply those due to the adjacent molecules in the aggregate. This is very likely the reason for the low value for the calculated C-3 formyl aggregation shift. The calculated shift given is for an orthogonal orientation of the formyl group with the carbonyl oxygen atom pointing up (Figure 4C) as this is most consistent with the aggregate structure found. The formyl group in the monomer is almost certainly coplanar with the macrocycle as the steric interactions with H_α and the C-4 ethyl group would not be expected to be large enough to overcome the conjugative energy, though they may decrease the rotational barrier. In the aggregate, the formyl group is involved in bonding to the magnesium, either directly or via an intermediate water molecule, and it is quite likely that the formyl group will, in consequence, rotate somewhat out of the plane to facilitate this bonding. This will produce a large upfield shift at the formyl proton due to the decrease in the ring current of the parent molecule at this proton (the calculated porphyrin ring-current shifts at the β-methyl protons vary by 0.5 ppm depending upon the orientation of the methyl protons[14]), which would add to the calculated aggregation shift. There could also be direct chemical shift changes at the formyl proton due to the carbonyl coordination in the aggregate.

A structural proposal for the Chl *b* aggregate is shown in Figure 4C. Most interestingly, the structural dimensions are such as to allow interactions of the magnesium atoms with both the C-3 formyl group and the carbonyl system at C-9 and C-10, and in part this confirms the original ideas of Closs et al.[8] As drawn (and there is some uncertainty in the dimensions of the aggregate, ±1 Å at least), the C-10 ester carbonyl would appear to be more directly involved, as it is much nearer the magnesium atom than the C-9 carbonyl. However, the estimated distance between the Chl molecules in the aggregate (~6 Å) would appear to be too large for a direct magnesium to carbonyl oxygen bond, as the C-10 ester carbonyl is still 3.7 Å above the plane of the adjacent Chl. This raises, once again, the question of possible interstitial water molecules, and the same comments made previously for Chl *a* are relevant to the Chl *b* solutions.

The proposed head-to-head structure for Chl *b* is very similar to the unsymmetric structure for Chl *a* in that it is also a "piggy-back" structure in which further molecules can add to the dimer with precisely the same bonding as the two original dimer molecules. This is important, both conceptually and practically, as it means that the higher aggregate is easily formed from the dimer, as is observed, and also the above calculations hold even if, as is likely to be the case, most of the molecules of Chl *b* are present in the NMR solutions as aggregates larger than the dimer.

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[31] Note that whereas in the dimer the substituents are not equivalent because the environment of the two molecules differ, in the aggregate this is no longer the case, and each molecule has identical environments, apart from those at the termini of the stacks.