N.M.R. Spectra of Porphyrins. Part 37.[1] The Structure of the Methyl Pyrochlorophyllide *a* Dimer

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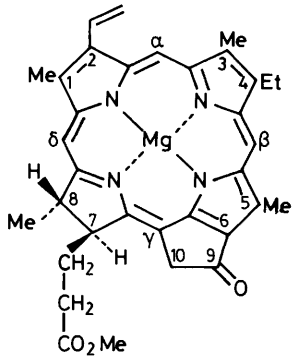
The n.m.r. complexation shifts of methyl pyrochlorophyllide *a* (MeP; **1**) in $CDCl_3$ solution have been investigated and analysed. Titration experiments with C_5D_5N and CD_3OD at high dilution enabled both the complete assignment of the proton spectrum and the proton complexation shifts to be obtained. Considerable line broadening was observed in rigorously dried solutions of MeP in $CDCl_3$, which was removed by addition of water or other ligands. Water, however, did not fully dissociate the complex, in contrast with pyridine or methanol, probably due to the limited solubility of water in $CDCl_3$. The complexation shifts observed for MeP are almost identical with those of chlorophyll *a* in the same solvent, confirming the similar complexation behaviour of the two molecules used, and the unimportance of the C_{10}-CO_2Me group of chlorophyll *a* in chlorophyll complexation. The only significant differences in the complexation shifts are in ring 'D' (8-H, 8-Me), perhaps due to conformational differences in the two molecules in this very flexible part of the molecule.

Detailed analysis of the observed complexation shifts with the ring-current model previously described was performed. Only two of the various proposed models of the chlorophyll dimer gave reasonable agreement with the observed shifts; these were the 'piggy-back' dimer and the 'back-to-back' model. The Fong model was in qualitative agreement with the observed shifts but none of the other proposed models gave even qualitative agreement. The geometries of the 'piggy-back' and 'back-to-back' models were refined using this set of complexation shifts which were more complete than any previous data. In both models the interplane separation is *ca.* 5.5–6.0 Å and also the C_9-keto groups of both molecules in the dimer are in the vicinity of the magnesium atom of the adjacent molecule. The proposed structures are consistent with a bonding mechanism in the dimer which involves a water molecule co-ordinated to the magnesium atom, and also hydrogen bonded to both the C_9-keto and C_{7d}-ester carbonyl functions of the adjacent molecule. In the 'piggy-back' structure only one of these hydrogen-bonding chains is possible, but in the 'back-to-back' model both C_7-propionate groups are *endo* to the dimer structure and capable, in principle, of forming these hydrogen-bonded chains. The larger aggregates could be formed by analogous layered structures in the 'piggy-back' model, but in the 'back-to-back' model the dimer structure differs from that of the aggregate in the position of the C_7-propionate groups. It was not possible to decide unambiguously between the two proposed structures with the experimental evidence available. Both provide a reasonable explanation for many of the previous results on chlorophyll aggregation.

In previous parts of this series,[2,3] the structure of the chlorophyll (Chl) aggregates formed in solutions of non-co-ordinating solvents has been investigated by measuring the complexation shifts in the n.m.r. spectra of Chl and analysing these shifts using a ring-current model of the Chl ring. The insidious problem of the formation of large aggregates (producing broad, unresolved n.m.r. spectra) was overcome by working at high dilution (mmol dm^{-3} concentrations) in $CDCl_3$ solutions, and the assignment and resolution of the complex Chl spectrum obtained by operating at high magnetic field (500 MHz). In these ways the complexation shifts (*i.e.* $\delta_{complex} - \delta_{monomer}$) for most of the protons on the Chl nucleus were obtained and these were used to test the validity of the various proposed models of the Chl dimer. Of the various proposed models in the literature (see ref. 2 for a detailed discussion) only the Fong model,[4] which involves co-ordination between the C_{10}-CO_2Me groups and the central magnesium atom of the neighbouring molecule in a symmetric structure, gave even qualitative agreement. We proposed, on the basis of the complexation shifts, two novel structures, namely a 'piggy-back' structure[3] in which both Chl molecules face in the same direction, and a 'back-to-back' structure[2] which is the opposite of the Fong model in that both the C_{10}-CO_2Me groups are now *exo* to the dimer, not *endo* as in the Fong model.

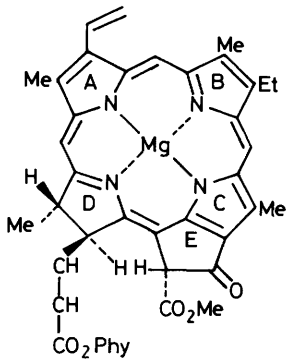
The involvement of the C_{10}-CO_2Me group in the dimer co-ordination has been repeatedly questioned since Katz *et al.*[5] in their pioneering investigations noted that pyrochlorophyll *a*, in which this group is absent, behaves almost identically with Chl *a* in its aggregation behaviour as shown by both n.m.r. and i.r. studies (see ref. 5 for a comprehensive survey of these early studies). Indeed, i.r. measurements gave a larger equilibrium constant of dimerization for pyrochlorophyll *a* than for Chl *a*.[6]

Furthermore, Katz and Brown[7] noted the onset of line broadening in the proton n.m.r. spectrum of pyrochlorophyll *a* at low temperatures, which could be due to slow exchange between the dimer molecules. If this exchange could be slowed down sufficiently, a far more detailed analysis of the resulting n.m.r. spectrum would be possible. This was demonstrated



Methyl pyrochlorophyllide *a*

(1)



Chlorophyll *a*

(2)

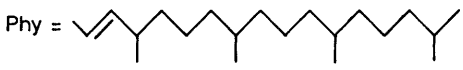


Table 1. Titration of methylpyrochlorophyllide *a* in $CDCl_3$ with [$^2H_5$]pyridine.[a]

	Mol equiv. [$^2H_5$]pyr											
	0	0.1	0.2	0.4	0.7	1.0	1.5	3.0	6.0	9.0	12.0	15.0
Meso β	9.40	9.41	9.42	9.44	9.46	9.48	9.5	9.53	9.55	9.55	9.55	9.55
α	9.21	9.22	9.22	9.23	9.24	9.25	9.26	9.28	9.29	9.29	9.29	9.29
γ	8.22	8.22	8.23	8.25	8.27	8.28	8.28	8.30	8.30	8.30	8.30	8.30
Vinyl 2a	7.98	7.98	7.98	7.98	7.99	7.99	7.99	8.00	8.01	8.01	8.01	8.01
2b	6.19	6.19	6.19	6.19	6.19	6.19	6.19	6.20	6.20	6.20	6.20	6.20
2b′	6.01	6.01	6.01	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
10-CH_{cis}	3.93	4.07	—	4.28	4.46	4.59	4.78	5.03	5.12	5.14	5.15	5.15
10-CH_{trans}	4.05	4.07	—	4.28	4.46	4.59	4.70	4.92	5.00	5.02	5.02	5.03
8-H	4.17	4.19	4.21	4.25	4.28	4.31	4.35	4.38	4.40	4.40	4.40	4.40
7-H	3.85	3.89	3.92	3.91	4.03	4.05	4.11	4.17	4.18	4.18	4.18	4.18
4a-CH_2	3.72	3.73	3.73	3.73	3.74	3.74	3.74	3.75	3.75	3.75	3.75	3.75
5-Me	2.99	3.06	3.11	3.20	3.29	3.37	3.47	3.59	3.64	3.65	3.65	3.65
7d-OMe	2.85	2.91	2.96	3.05	3.15	3.25	3.34	3.47	3.53	3.54	3.55	3.55
1-Me	3.30	3.31	3.31	3.31	3.32	3.32	3.32	3.32	3.32	3.33	3.33	3.33
3-Me	3.24	3.22	3.25	3.25	3.25	3.25	3.25	3.25	3.25	3.25	3.25	3.25
4b-Me	1.72	1.70	1.71	1.71	1.70	1.70	1.70	1.70	1.70	—	1.70	1.70
8-Me	1.61	1.62	1.63	1.64	1.66	1.69	1.69	1.70	1.71	—	1.71	1.72

[a] Initial concentration 6.6 mmol dm^{-3} (1.9 mg in 0.5 cm^3) in $CDCl_3$. — obscured.

recently for the bacteriochlorophyllide *d* dimer,[8] giving a well-defined dimer structure in $CDCl_3$.

For these reasons we decided to investigate the n.m.r. spectrum of pyrochlorophyll *a* under conditions similar to the recent study of Chl *a*.[2] Also, as a variety of investigations have produced no evidence whatsoever that the phytyl side chain plays any significant part in the mechanism of Chl aggregation in solution,[5] and the resonances from the phytyl group can obscure some of the important resonances of the chlorin nucleus,[2] we decided to use methyl pyrochlorophyllide *a* (MeP; **1**), in which the phytyl group has been replaced by a methyl group, in these investigations.

Experimental

Methyl pyrochlorophyllide *a* (MeP; **1**) was prepared by insertion of magnesium into methyl pyropheophorbide *a* following Eschenmoser's method.[9] The compound was dissolved in $CDCl_3$ which had been filtered through activated alumina to remove traces of acid and water, directly into an n.m.r. tube. In order to resolve assignment ambiguities in the temperature studies, [$^2H_3$]methylpyropheophorbide *a* was prepared by transesterification of MeP (**1**) in CD_3OD containing 5% sulphuric acid; metal insertion and sample preparation were then repeated exactly as above. All these operations were carried out in a dry-box. The proton spectrum was obtained on both Nicolet NT-500 (500 MHz) and Bruker WM-250 (250 MHz) spectrometers. Operating conditions for the Nicolet instrument are given in ref. 2. For the Bruker spectrometer, typical operating conditions: probe temperature 20 °C, sweep width 3 kHz in 8K data points, zero filling to 32K to give a digital accuracy of 0.2 Hz (<0.001 ppm). The pulse width (60° flip) was 7 μs, acquisition time 1.4 s, and *ca.* 800 accumulations were obtained for each spectrum. The variable temperature calibration was achieved by direct thermocouple measurements in an n.m.r. tube, and by calibration with a methanol sample.

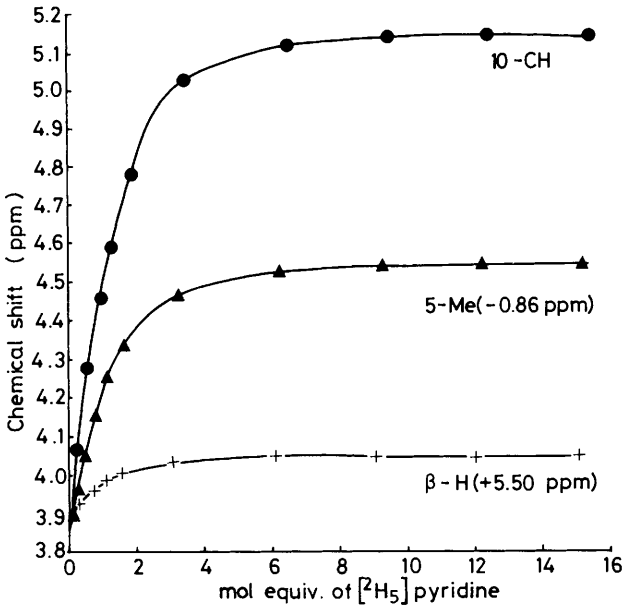


Figure 1. Titration of methyl pyrochlorophyllide *a* (**1**) in $CDCl_3$ with [$^2H_5$]pyridine. Observed points and calculated curves with K_3 = 260 dm^3 mol^{-1}.

Results

To minimize the formation of large aggregates the MeP spectra were obtained at concentrations $< 10^{-2}$ mol dm^{-3}. In one case the solution was then titrated with aliquots of a [$^2H_5$]pyridine solution in $CDCl_3$ from 0.1 to 15.0 mol equiv. of [$^2H_5$]pyridine. The titration measurements are given in Table 1 and Figure 1. Spectra were also recorded with a large excess of CD_3OD and with addition of small amounts of D_2O, and some illustrative spectra are shown in Figure 2.

The spectra obtained with an excess of C_5D_5N or CD_3OD are identical and are the sharp spectra characteristic of the monomeric species [Figure 2(*c*)]. This spectrum has been assigned previously[10,11] and can also be assigned by direct comparison with that of Chl *a*.[12] There is some ambiguity concerning the unique assignment of the C_5- and C_{7d}-methyl resonances, but a nuclear Overhauser enhancement (n.O.e.) experiment conclusively identified the lower-field resonance at 3.65 ppm as the C_5-methyl, giving a pronounced n.O.e. upon irradiation with the β-*meso* resonance. This agrees both with the Chl *a* (**2**) data[2,12] and also with the early assignments by Pennington *et al.*[10] Also, the spectrum of the specifically deuteriated $C7_d$-CD_3 compound, in which the signal at 3.55 ppm had disappeared, provided conclusive evidence for this assignment.

The assignment of the C_{10}-methylene protons is not unambiguous. We assign the resonance of the proton *Z* to the C_7-propionate group to the low field part of the AB system on the basis that this proton has a complexation shift identical with that of the corresponding proton in Chl *a* (Table 2). This completes the assignment of the proton spectrum, except for the distinct protons of the C_7-propionate chain. This complex ABCD spectrum has been analysed by us for a number of related compounds,[13] but is not sufficiently well resolved in the aggregated spectra to allow the deduction of any complexation shifts.

The assignments in the aggregated spectrum follow directly from those in the monomer and the titration experiments. Some crossing of the resonances occurs during the titration, notably that of the C_1- and C_3-methyl peaks with the C_5 and C_{7d} peaks. The C_5 and C_{7d} resonances are to low field of the C_1 and C_3 peaks in the monomer [Figure 2(*c*)], but to high field in the aggregated spectrum [Figures 2(*a*) and (*b*)], clearly illustrating the differential aggregate shifts. Also, analysis of the AB spectrum of the C_{10}-methylene group indicates that the *Z* and *E* resonances cross over during the titration. All these assignments are given in Table 1.

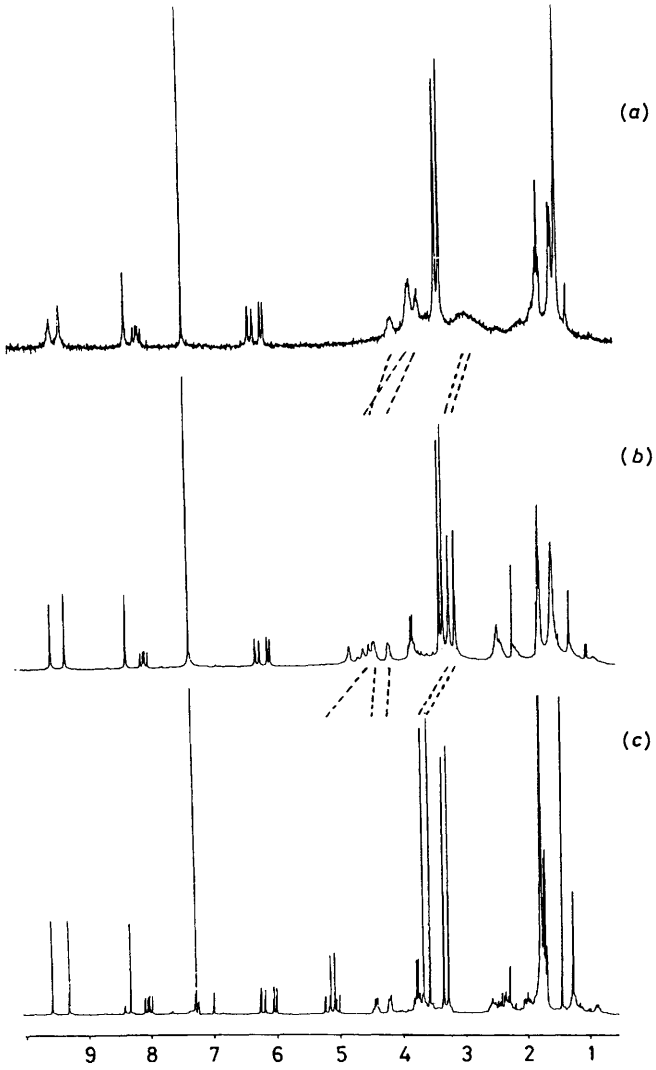


Figure 2. Proton n.m.r. spectra of methyl pyrochlorophyllide *a* (**1**) in $CDCl_3$ solution. (*a*), $CDCl_3$ only; (*b*), with 2 mm^3 of D_2O; (*c*), with [$^2H_5$]pyridine (15 mol equiv.) added.

The proton spectrum of MeP (**1**) shows exactly comparable line broadening in non-complexing solvents as is exhibited by Chl *a*.[2] This is characterized by differential line broadening [Figure 2(*a*)], and it is immediately obvious from Tables 1 and 2 and Figure 2(*a*) that those protons which experience the largest complexation shifts also show the greatest line broadening [*cf.* the C_5- and C_{7d}-methyls in Figure 2(*a*)]. Furthermore, in the $CDCl_3$ solutions examined here, the presence of small amounts of water has a considerable effect on this line broadening. Figure 2(*a*) shows the spectrum obtained with the maximum precautions taken to exclude water, yet there may still be some water in the spectrum. However, even with a large excess of water present [Figure 2(*b*); in which the line broadening is substantially reduced], the spectrum is not that of the monomer, but of a partially dissociated complex.

We ascribe these phenomena to the presence of larger aggregates which may well be bonded together by a different mechanism than in the dimer (see later). Small amounts of

Table 2. Observed complexation shifts (Δδ) for methylpyrochlorophyllide *a* and related compounds.

	(*a*)	(*b*)	(*c*)	(*d*)
meso β	0.15	0.09	0.10	0.41
α	0.08	0.02	0.06	0.23
γ	0.09	0.06	0.08	0.24
Vinyl 2a	0.03	0.00	—	0.17
2b	0.01	−0.01	—	0.10
2b′	−0.1	−0.01	—	0.10
10-CH_{cis}	1.25	1.26	1.26	2.79
10-CH_{trans}	0.95	0.94	—	
8-H	0.23	0.26	0.43	0.29
7-H	0.33	0.37	—	0.64
4a-CH_2	0.02	0.03	0.01	0.07
7d-OMe	0.70	0.64	—	—
5-Me	0.66	0.59	0.64	1.27
1-Me	0.01	0.00	0.01	0.16
3-Me	0.01	0.02	0.03	0.02
4b-Me	0.02	0.01	0.02	0.04
8-Me	0.10	0.16	0.39	—

[a] Methyl pyrochlorophyllide *a* (6.6 mmol dm^{-3} in $CDCl_3$) titrated with [$^2H_5$]pyridine. [b] Methyl pyrochlorophyllide *a* (6.6 mmol dm^{-3} in $CDCl_3$) upon addition of 40 mm^3 of CD_3OD. [c] Chlorophyll *a* (2.8 mmol dm^{-3} in $CDCl_3$) titrated with CD_3OD (Ref. 2). [d] Pyrochlorophyll *a* (0.06 mold dm^{-3} in CCl_4) titrated with [$^2H_5$]pyridine (Ref. 5).

co-ordinating ligands, particularly water, can break up the aggregates, but in contrast, excess water does not completely dissociate the dimer structure, illustrating the very different stabilities of the dimer and the aggregate.

When the solution in chloroform was cooled, no general line-broadening characteristic of an exchange process was observed, even down to −30 °C in $CDCl_3$ and −60 °C in CD_2Cl_2; instead, a gradual change in peak positions with some peaks coalescing and re-emerging occurred. The experiments were repeated with the selectively deuteriated C_{7d}-trideuterio compound in order to obtain an unambiguous assignment of the spectrum, and the temperature dependence of the spectrum in $CDCl_3$ is given in Figure 3 and Table 3. (The temperature dependence was virtually identical in CD_2Cl_2, except that some of the resonances show small solvent shifts; for consistency we give here only the $CDCl_3$ results.)

The MeP resonances which show no concentration dependence (*i.e.* the ring A and B substituents and the *meso* protons) also show no temperature dependence, and the connection between the concentration and temperature dependence was further supported by the absence of any temperature dependence of the entire spectrum of the disaggregated species (<0.02 ppm down to 260 K). Those resonances which do show a concentration dependence also have a temperature dependence, but in a quite contrasting manner. The resonances of the ring D substituents (C_7-H, C_8-H, C_8-Me) move to lower field as the temperature is decreased; however, the effect is not very large (*ca.* 0.1–0.2 ppm over a 50 °C range). The C_7-propionate signals also appear to follow this pattern (Figure 3), though they cannot be assigned individually, and the C_{7d}-methoxy signal also follows this pattern with a somewhat larger temperature dependence (0.32 ppm). However, the C_5-Me shows the opposite temperature dependence, moving to higher fields as the temperature is lowered. The result of these changes is that the C_{7d}- and C_5-methyl signals cross over upon cooling (Figure 3). We emphasize here that the assignments at each temperature are given unambiguously from the deuteriation studies. This intriguing behaviour will be considered further after the aggregate structure has been established.

The complexation shifts of the MeP spectra may be obtained

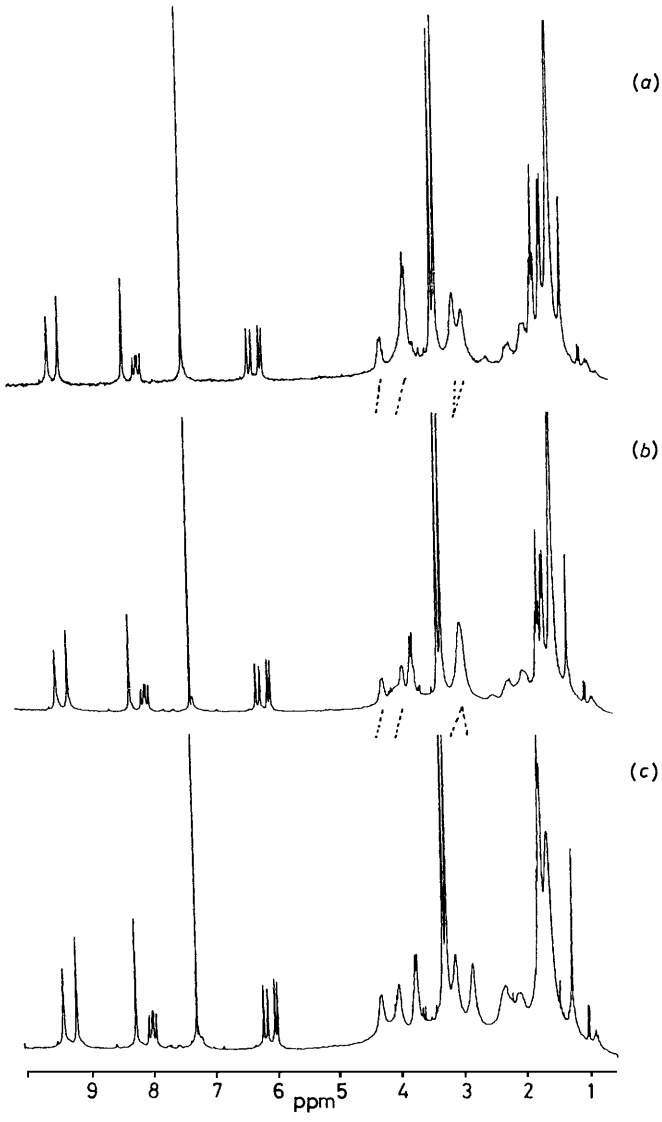


Figure 3. Proton n.m.r. spectra of methyl pyrochlorophyllide *a* (**1**) in $CDCl_3$ solution at: (*a*), 299 K; (*b*), 279 K; (*c*) 259 K.

in an analogous manner to those of Chl *a*,[2] directly from the titration experiments of Table 1 and Figure 1. As in the Chl case we consider only two competing equilibria, the dissociation of the MeP dimer, and the subsequent complexation with ligand, according to equations (1) and (2):

$$MeP_2 \overset{K_1}{\rightleftharpoons} 2MeP \quad (1)$$

$$MeP + L \overset{K_2}{\rightleftharpoons} MeP{\cdot}L \quad (2)$$

to give the overall equilibrium as

$$\underset{(1-x)}{MeP_2} + 2L \overset{K_3}{\rightleftharpoons} \underset{2x}{2MeP{\cdot}L} \quad (3)$$

where $K_3 = K_1K_2^2$.

This formulation ignores the presence of any aggregates larger than the dimer and also the fact that a second molecule of pyridine, unlike methanol, can complex axially with the magnesium. We associate aggregate formation with the onset of line broadening, which is not excessive at the concentrations used here, and the second molecule of pyridine is very weakly

Table 3. Chemical shifts of methylpyrochlorophyllide *vs.* temperature.

	299 K	289 K	279 K	269 K	259 K	249 K
Meso β	9.38	9.38	9.38	9.39	9.39	9.40
α	9.20	9.20	9.20	9.20	9.19	9.19
γ	8.20	8.21	8.22	8.23	8.24	8.24
Vinyl 2a	7.97	7.97	7.97	7.98	7.98	7.98
2b	6.19	6.18	6.17	6.17	6.17	6.16
2b′	6.00	6.00	6.00	6.00	6.00	6.00
8-H	4.13	4.16	4.19	4.24	4.27	4.29
7-H	3.76	3.82	3.87	3.94	3.98	4.00
4a-CH_2	3.72	3.72	3.72	3.72	3.73	3.73
7d-OMe	2.84	2.88	2.94	2.97	3.09	3.16
5-Me	2.96	2.96	2.94	2.91	2.89	2.82
1-Me	3.30	3.30	3.30	3.29	3.29	3.29
3-Me	3.23	3.24	3.23	3.23	3.23	3.23
4b-Me	1.70	1.71	1.70	1.71	1.70	1.71
8-Me	1.58	1.60	1.62	1.67	1.70	1.72

[a] 4.8 mg in 0.5 cm^3 of $CDCl_3$. Chemical shifts referenced to $CHCl_3$ at 7.26 ppm.

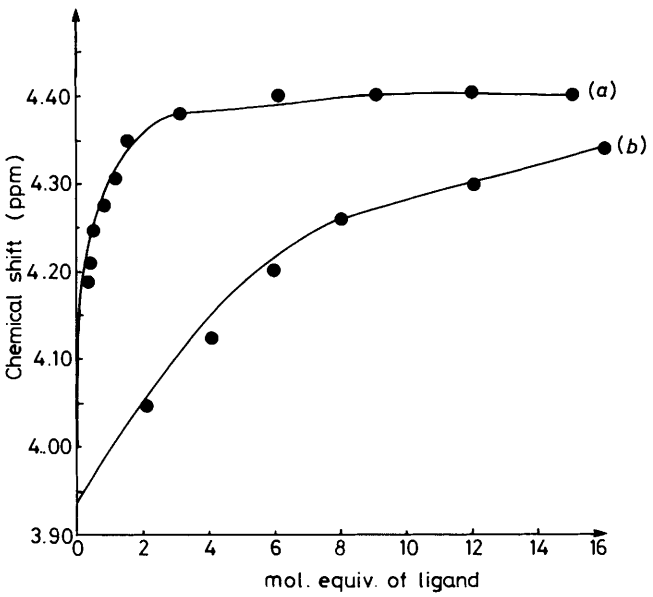


Figure 4. Titration of methyl pyrochlorophyllide *a* (**1**) in $CDCl_3$. Observed points and calculated curves for the 8-H proton (*a*) in [$^2H_5$]pyridine titration and (*b*) in CD_3OD titration.

bound to the magnesium and will not affect the titration. (The equilibrium constants for the first and second dissociation of pyridine in magnesium porphyrin dipyridinates are *ca.* 2 000 and 0.1 dm^3 mol^{-1}, respectively.[14]) The identical monomer shifts produced by excess methanol, in which only one molecule of ligand binds to the magnesium confirms this statement. The major difference between the methanol and pyridine titrations is that the much larger equilibrium constant for pyridine complexation produces rather more well defined titration curves and requires less mole excess of ligand to complete the titration (*cf.* Figures 1 and 4). This statement may be quantified by analysis of the titration curves according to equation (3). Given that there is no significant amount of monomer or aggregate in the solutions, the observed chemical shift of any given proton during the titration is the weighted average of the corresponding shifts in the MeP dimer (δ_d) and the MeP–ligand complex (δ_c), *i.e.*

$$\delta_{obs} = (1 - x)\delta_d + x\delta_c \quad (4)$$

where x is the fraction of dimer associated. Furthermore, the overall equilibrium constant (K_3) is given by

$$K_3 = \frac{[MeP{\cdot}L]^2}{[MeP_2][L]^2} = \frac{2ax^2}{(1 - x)L^2} \quad (5)$$

where a is the total concentration of MeP (as monomer) and L the concentration of added ligand.

An iterative analysis of the titration plot of Figure 1 for the C_5-Me protons according to equations (4) and (5) using the values of δ_c and δ_d in Table 1 gave a value of K_3 of *ca.* 260 (±10) dm^3 mol^{-1} and the calculated curve shown in Figure 1. Analogous treatment of the MeP titration with methanol and of the Chl *a* titration with methanol gave values of 13.0 and 7.0 dm^3 mol^{-1} respectively, and the observed points and calculated curves for the C_8-H proton of the MeP/methanol titration and the MeP/pyridine titration are shown in Figure 4. The values obtained for the equilibrium constant compare well with those obtained previously for similar titrations. The titration of pyrochlorophyll *a* and Chl *a* with tetrahydrofuran in CCl_4 solution gave values of 3.1 and 19.4 dm^3 mol^{-1}, respectively, from i.r. measurements,[6] whilst Katz *et al.* quote values of 58 dm^3 mol^{-1} for Chl *a* methanol and 3.5 × 10^3 dm^3 mol^{-1} for Chl pyridine titrations in CCl_4 solutions.[15]

The Dimer Geometry.—The analysis of the MeP titration experiments leads to a complete set of dimer complexation shifts for MeP. These are given in Table 2, together with the complexation shifts for the corresponding titration in CD_3OD, those obtained previously for Chl *a* under identical conditions,[2] and those of earlier investigations on pyrochlorophyll *a* in CCl_4 solution, using much more concentrated solutions.[16] Inspection of Table 2 [columns (*a*), (*b*) and (*c*)] shows immediately the very similar complexation shifts of MeP and Chl *a*, *provided* the measurements are taken from titrations at the same low concentrations of solute. The only significant difference in the complexation shifts observed in MeP and Chl *a* are those of the C_8-H and C_8-Me protons. This may be due to conformational differences of the flexible ring 'D' in the two compounds, or to conformational changes in the neighbouring C_7-propionate side chain, with the different ester groups. The resonances of the C_7-H were obscured by the phytyl resonances in the Chl titration, and this shift is not available for comparison. However, the overall similarity of the complexation shifts for MeP and Chl *a* clearly and unambiguously excludes any significant involvement of the C_{10}-CO_2Me group in Chl *a* in the bonding of the dimer. This supports the similar conclusions of Katz *et al.* from i.r. studies of the Chls[5] and must be construed as very strong evidence against the Fong dimer model.[4]

Comparison of the complexation shifts of MeP in dilute and more concentrated solutions [Table 2, columns (*a*) and (*d*)] shows also the characteristic changes which occur when the concentrations of solute are varied. (Though the studies of Katz *et al.* quoted here[16] use CCl_4, which is a much more aggregating solvent than $CDCl_3$.) The observed changes exactly parallel those observed in Chl *a* titrations.[2] The complexation shifts are all much larger in concentrated solution and all to high field. There is, however, no simple relationship between the two sets of data; this comparison suggests that aggregate formation occurs in the more concentrated solutions and that the structure of the aggregate is also a 'pancake' structure (otherwise the complexation shifts would not be further to high field) but *not* a direct extension of the dimer structure.

This reasonably complete set of complexation shifts may now be used to assess quantitatively the validity of the proposed models of the Chl dimer, and also, using the ring-current model of the Chl ring given previously,[3] to refine the dimer geometries. It should be emphasized here that these analyses implicitly

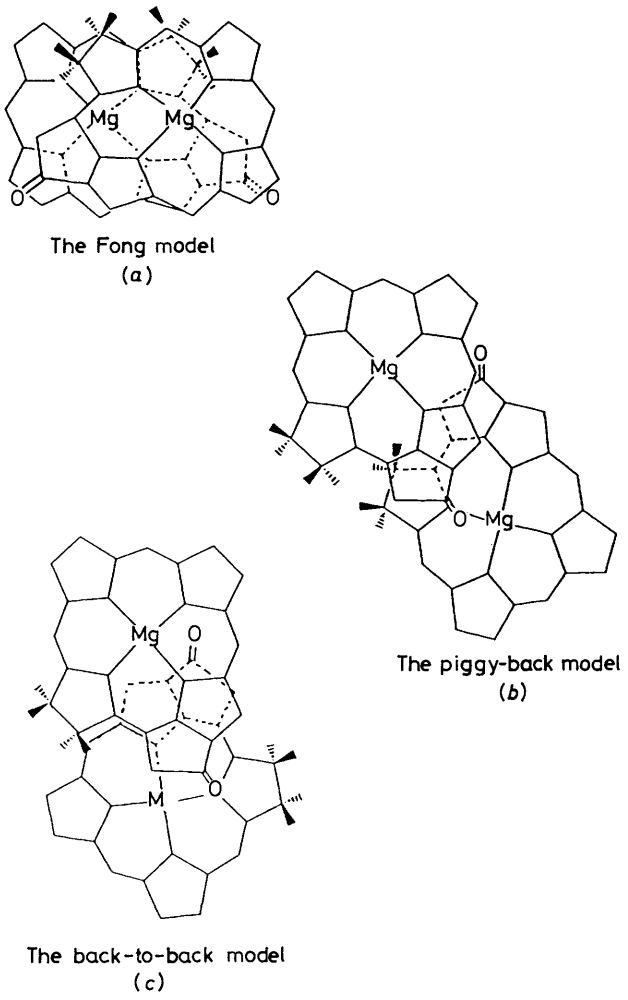


Figure 5. Proposed models for the MeP (**1**) dimer. (*a*), the Fong model; (*b*), the 'piggy-back' model; (*c*), the 'back-to-back' model.

assume that the observed complexation shifts are solely due to ring-current effects from the neighbouring molecule, and this in turn assumes that the molecular conformation, including that of the side chains, does not change appreciably upon complexation. This important proviso will be discussed subsequently, after the analyses have been considered.

The various proposed models of the Chl *a* dimer were discussed and analysed in ref. 2, and it was shown that only three out of several proposed models gave even qualitative agreement with the observed complexation shifts. These were the Fong, 'piggy-back', and 'back-to-back' models (Figure 5). The close similarity in the complexation shifts of MeP (**1**) and Chl *a* (Table 2) implies that the same conclusions will also be true for MeP. This is so, and thus the other proposed structures of the Chl *a* dimer (*e.g.* the Strouse, Shipman, and Skew models) discussed in ref. 2 will not be considered here.

The Fong Model.—In his original proposal, Fong[4] suggested that each of the C_{10a}-CO groups of Chl *a* was directly bonded to the magnesium of the adjacent molecule to give a symmetrical head-to-tail structure (Figure 5). Fong later proposed an analogous hydrogen-bonded structure with one water molecule bonded to both the magnesium and the ester C=O function. Although this structure is not really applicable to MeP, as this molecule does not contain a C_{10a}-ester group, it is of some interest to see whether such a symmetric dimer structure would explain the observed shifts. Thus, a computational search

Table 4. Observed and calculated complexation shifts (Δδ) for methyl pyrochlorophyllide *a*.

	obs.	(*a*)	(*b*)	(*c*)
Meso β	0.15	0.23	0.25	0.26
α	0.08	−0.04	−0.02	−0.01
γ	0.09	0.00	0.03	0.01
Vinyl 2a	0.03	−0.03	−0.03	−0.02
2b	0.01	−0.03	−0.03	−0.01
2b′	−0.01	−0.03	−0.03	−0.01
10-CH_{cis}	1.22	1.30	1.24	0.67
10-CH_{trans}	0.98	0.74	0.94	1.21
8-H	0.23	0.11	0.13	0.09
7-H	0.33	0.44	0.43	0.38
4a-CH_2	0.02	−0.01	0.01	0.02
7d-OMe	0.70	—	—	—
5-Me	0.66	0.65	0.64	0.60
1-Me	0.01	−0.04	−0.03	−0.02
3-Me	0.01	−0.04	−0.03	−0.02
4b-Me	0.02	0.05	0.00	0.06
8-Me	0.10	0.16	0.07	−0.03

[a] The 'back-to-back' model, displacement co-ordinates 4.1, −5.2, 5.6 Å, rotated −30°, inverted molecule. [b] The 'piggy-back' model, displacement co-ordinates 0.8, −5.9, 5.9 Å, rotated 215°. [c] The Fong model, displacement co-ordinates −2.1, 5.4, 6.5 Å, C_2 symmetry.

procedure was performed on the basis of this structure, to find the best agreement of the observed and calculated dimer shifts. The geometric restrictions imposed by this model are that the planes of the two molecules in the dimer are parallel (a general condition of the program used), and there is a twofold axis of symmetry of the dimer structure, which is the *x* axis in the co-ordinate system used [see structure (**1**)]. The best solution, together with the displacement co-ordinates, is given in Table 4. Comparison of the observed and calculated dimer shifts (Table 4) does show that the Fong structure gives a reasonable overall account of the observed shifts. However, there are serious discrepancies; in particular the shifts of the C_{10}-methylene protons of MeP are not in the correct order, with the cis proton (cis to the C_7-propionate group) which is *exo* to the dimer structure, predicted to have a smaller ring-current shift than the trans proton, which is *endo*. This is the reverse of the observed shifts. We shall see subsequently that the reason for the overall agreement of the observed and calculated shifts in the Fong structure is that the orientation of the two MeP molecules in the dimer is similar to that of the other acceptable structures. In all three structures the two molecules are parallel with the C_9-keto function situated approximately over the magnesium atom of the adjacent molecule. The essential difference between the three structures is in the relative orientation of the two MeP molecules. If the Fong structure is considered as a face-to-face model, with both the C_7-propionate groups *exo* to the dimer structure (and in Chl *a*, both C_{10}-ester groups *endo*), then the other acceptable structures are the back-to-face ('piggy-back') model in which one C_7-propionate group is *exo* and one *endo*, and the back-to-back model in which both C_7-propionates are *endo* to the dimer structure (Figure 5). Furthermore, in these latter structures the constraint of twofold symmetry imposed on the Fong model no longer applies and both molecules of the dimer are in different environments.

The 'Piggy-back' Model.—This structure was first proposed by us[3] as an alternative (to the Fong structure) of the observed complexation shifts in Chl *a*, and subsequently refined using the more accurate set of complexation shifts obtained by high-field experiments in dilute solutions.[2] This model provided a good account of the observed data for Chl *a* and thus it is of some

interest to see whether it would fit the more complete data set for MeP recorded here. The computational search was performed by assuming the basic structure of the model and varying both the displacement co-ordinates (x, y, z) of one molecule with respect to the other, and also the angle of rotation (θ) of one molecule about the axis perpendicular to the molecular plane (the z-axis). These four parameters completely specify the dimer geometry for the case of parallel molecular planes. In this computational search only the ring protons are included as the conformations of the side chains in solution are unknown. Once the optimum solution had been obtained, the dihedral angles of the C_2-vinyl group and the C_4-ethyl group were optimized separately to give values of 30° (z to the α-meso position) for the C_2-vinyl group and 90° (*i.e.* 'up' in Figure 5) for the C_4-ethyl group. The conformation of the C_7-propionate group and complexation shift of the C_{7d}-methyl protons will be considered subsequently.

The best solution obtained is given in Table 4, and inspection shows that the observed and calculated shifts are in essentially complete agreement. The RMS error of the observed and calculated shifts is 0.07 ppm, with the largest single error of 0.1 ppm. The agreement is for a solution of 12 complexation shifts (*i.e.* equations) in four unknowns and even when the insidious problems of undetermined solutions are considered the general level of agreement is encouraging. However, due to intrinsic properties of the Chl ring-current field, which falls off rapidly with distance from the molecular plane but is largely constant over the molecular plane for any given distance, the definition of the dimer co-ordinates is not very precise, *ca.* ±0.5 Å in the x- and y-co-ordinates and *ca.* 5° in θ. The separation of the molecular planes (the z axis) is better defined (±0.25 Å). Thus, the displacement co-ordinates obtained here are not significantly different from those obtained in the analogous Chl *a* titration study (0.0, −4.5, 6.0 Å, θ 205°).[2]

The 'Back-to-back' Model.—The other proposed structure for the Chl *a* dimer which gave a good account of the observed complexation shifts is the 'back-to-back' model. In this structure the C_9-keto function is again in the vicinity of the magnesium atom of the adjacent molecule, but in this case both the C_7-propionate groups are now *exo* to the dimer structure in contrast with the other proposed models. As in the 'piggy-back' model, this is not a symmetric structure and the two molecules of the dimer are in different environments. The computational search was performed in an exactly analogous manner as in the 'piggy-back' case, varying the displacement co-ordinates and angle of rotation until the best agreement with the observed shifts of the MeP ring protons was obtained. Note that in this case the computational operations are different to those of the 'piggy-back' model in that here the two molecules are inverted with respect to each other, not simply displaced and rotated. The computational search optimized to an acceptable solution and then the dihedral angles of the C_2-vinyl and C_4-ethyl groups were optimized on this solution. The values obtained were the same as in the preceding case, *i.e.* 30° (C_2) and 90° (C_4), both *exo* to the dimer structure.

The observed and calculated shifts (Table 4) are again in excellent agreement. In only one case is there any significant difference between the observed and calculated shifts, and that is for the C_{10}-methylene protons, where the *trans* proton, which is now always *exo* to the dimer structure, has a somewhat larger observed shift than that calculated. For all the remaining protons the observed and calculated shifts are in complete agreement. It is also of interest to note that the calculated shifts for the 'back-to-back' structure are, apart from the C_{10}-methylene protons mentioned, virtually identical with those of the 'piggy-back' structure. This is despite the fact that not only the configurations but also the dimensions of the two models are quite different.

The geometry obtained from the MeP titrations is again very similar to that obtained from the analogous Chl titration (displacement co-ordinates 3.5, −5.8, 4.8 Å, θ 0°).[2]

Discussion

Of the three models of Chl *a* (and MeP) dimerization considered here, only two—the 'piggy-back' and 'back-to-back' structures—adequately reproduce the observed complexation shifts. Also, the Fong model, based as it is on the involvement of the C_{10a}-CO_2Me group in the dimer bonding, can be excluded from consideration of the MeP results and, by analogy, from the Chl *a* dimer. However, a number of fundamental questions remain to be answered, apart from the obvious ones, as to whether the two proposed structures can be distinguished in any way and whether they can also explain the variety of other data obtained on the Chl *a* dimer. A central question which the n.m.r. experiments do not answer directly is the bonding mechanism in the dimer structure, *i.e.* how many hydrogen bonds are present and which carbonyl functions are involved? We can safely exclude the C_{10a}-ester function (in Chl *a*) from consideration, but both the C_9-keto and C_{7d}-ester functions could be involved in the bonding. Note, in particular the large complexation shift of the C_{7d}-methyl protons (and the analogous protons in Chl *a*). This strongly suggests that the methyl protons are in the shielding cone of the MeP ring current and thus that the propionate side chain is folded into the dimer rather than extended into the solvent, which conformation would produce low-field rather than high-field complexation shifts.

Finally, any proposed dimer structure must also address the question of the structure of the aggregate, as there is no doubt that aggregate formation occurs readily both in more concentrated chloroform solutions and in less polar solvents. Also, we note that aggregate formation produces similar but larger n.m.r. complexation shifts than dimer formation, implying a not too dissimilar structure. Basic to all these considerations is the role of, and presence of, water in the complex. There has been considerable controversy in the past over even the presence or absence of one molecule of co-ordinated water in Chl solutions,[4,5] which we do not wish to enter into. Certainly, in the n.m.r. experiments performed here, and the analogous ones of the Chl *a* titration,[2] no extraordinary precautions were taken to remove any co-ordinated water from the sample. (Indeed, Katz[5] notes that it is extremely difficult to remove co-ordinated water from solid Chls). Although considerable care was taken to remove extraneous water from the chloroform, there is no reason to suppose that the magnesium atom in the Chl solutions investigated does contain a co-ordinated water molecule. Furthermore, excess water does not have any great effect on the dimer structure, which again supports the view that the magnesium is solvated. The molecular-plane separation of the Chls in the dimer (5–6 Å), likewise, is far too large for any direct Mg ··· O=C linkage involving the C_9-keto group. Thus, we conclude that the bonding mechanism for the dimer in the solutions studied involves bridging hydrogen bonds between a molecule of water co-ordinated to the magnesium atom and other ligands.

Molecular models of the proposed dimer structures incorporating this attached water molecule immediately show that in both the proposed structures the molecular configuration in the dimer is such that *both* the C_{7d}- and C_9-carbonyl groups can be orientated to hydrogen bond to the attached water molecule. Figure 6 illustrates this proposed network. Whereas the orientation of the C_9-carbonyl group is rigorously defined by the Chl molecule itself, that of the C_{7d} carbonyl is much more

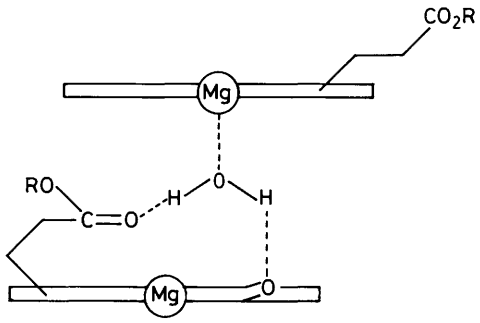


Figure 6. Proposed hydrogen-bonding network in the MeP (**1**) dimer.

flexible. This results in what appears to be a more favourable hydrogen bond to the carbonyl group, with an almost linear C=O ··· H–O configuration, whereas the bonding to the C_9 carbonyl is more orthogonal.

The difference between the two proposed structures is that in the 'piggy-back' model, only one C_7-propionate side chain is *endo* to the dimer structure and, therefore, capable of forming this bonding structure. In the 'back-to-back' structure, both propionate side chains are *endo* and in principle capable of forming such hydrogen bonds with both magnesium atoms in the dimer. This would produce an energetically more stable structure (four hydrogen bonds instead of two). However, it is not clear whether steric constraints would affect this structure as both propionate groups are in the same region of the dimer and may thus repel each other.

The question of aggregate formation may also be considered. In the 'piggy-back' structure the attachment of a third molecule of Chl proceeds by exactly the same mechanism, as one exposed face of the dimer has an *exo* C_7-propionate group and the C_9-keto function correctly orientated for this H-bonding network. The aggregate would simply grow by this mechanism to give a spiral layered structure. In the 'back-to-back' model, aggregation could occur by a slightly different mechanism involving the C_9-keto group of the dimer and a third molecule. In this model we note that the dimer structure is not the same as the aggregate structure, as the C_7-propionate groups are both *endo* to the structure. The aggregate could be formed either by two dimer units co-ordinating, or more probably in view of the hydrogen-bonding network, by a third molecule attaching to the dimer in, for example, a 'piggy-back' structure.

The hydrogen-bonding mechanism proposed here may seem at first sight to be incompatible with the results of the i.r. measurements on the Chl and (MeP) dimers,[17] which show unequivocally that the C_9-keto band is reduced to half its intensity upon dimer formation with the formation of a new bonded band, yet the ester carbonyl absorbance is unchanged. These experiments may be reconciled with the proposed strucure when it is realized that the C_9-keto function is very strongly conjugated with the Chl π-system, so much so that the carbonyl frequency changes appreciably on going from a monopyridine Chl complex to a dipyridine one (1 688 to 1 677 cm^{-1}) whilst no change is observed in the ester C=O bands. The C_9-keto band would thus be expected to be much more sensitive to any bonding interaction in the dimer than the ester carbonyl absorptions, as is observed.

Considerable support for the involvement of both the C_9- and C_{7d}-carbonyl functions in the dimer bonding comes from the results of ^{13}C n.m.r. titration experiments.[18] The carbon atoms in the dimer molecules will experience identical ring currents compared with protons in the same position. Thus, the three carbonyl carbon atoms in Chl *a* will all experience high-field ring-current shifts in the dimer, as the neighbouring protons of all three carbonyl groups show significant high-field shifts. However, co-ordination interactions of a carbonyl group produce an additional downfield shift of the carbonyl carbon atom of up to several ppm. The complexation shifts of the C_9, C_{10a}, and C_{7d} carbons in Chl *a* were found to be −2.29, 0.40, and −0.98 ppm, respectively. This clearly implicates both C_9 and C_{7d} in the bonding process, but not, as expected, C_{10a}. The much larger negative shift of the C_9 carbonyl is due (as for the i.r. bands discussed above) to its conjugation with the Chl ring.

The hydrogen-bonding network proposed for the dimer is very similar to that found in the crystal of methyl pyrochlorophyllide *a* monohydrate monoetherate.[19] Here, the water molecule again forms a bifunctional bridging network, co-ordinating to the magnesium of one MeP molecule and hydrogen bonding to both the C_9-keto function of a neighbouring MeP molecule and to the ether oxygen. The distance between the ether oxygen and the magnesium atom of the MeP molecule *via* the O ··· H–O ··· Mg link is about 5.8 Å, very similar to the separation of the porphyrin planes found here. The major difference between the solution and solid-state structures is that in the solid the Chl molecules are stacked almost side by side with only a small part of the molecules above each other. In solution the observed high-field shifts on dimer and aggregate formation clearly show that the stacking of the molecules must be essentially one on top of the other.

The involvement of the C_7-propionate group in the hydrogen-bonding chain may be related to the intriguing temperature dependence of the n.m.r. spectrum. Normally, cooling a system involving an equilibrium between complex and dissociated species increases the percentage of complex, and thus we expected to observe a high-field shift upon decreasing the temperature. This was observed for the C_5-Me resonance, but all the resonances of the C_7 propionate and the neighbouring ring 'D' protons moved in the opposite direction. This suggests that an additional process is occurring which involves this side-chain and an obvious one is the commencement of a competing intermolecular hydrogen-bonding interaction. This is exactly what would be expected if aggregation into larger structures were taking place, involving the C_7 propionate as the bridging link. Obviously, the formation of such larger aggregates would be favoured by reducing the temperature.

Conclusions

In conclusion, the proposed models of the MeP (**1**) (and Chl *a*) dimers do appear to offer an explanation for most of the diverse experimental data obtained on this complex system. The suggested role of the C_{7d}-carbonyl function in the dimer bonding has not been recognized previously and this postulate could be tested in a number of ways requiring synthetic modification of the ester function. An unambiguous distinction between the two proposed structures cannot as yet be made. (Indeed, they may co-exist in solution). One interesting method of attack would be a detailed molecular-mechanics study of the binding energies of the two proposed structures and this work is under consideration.

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