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THE NMR SPECTRA OF PORPHYRINS—15[1]

SELF-AGGREGATION IN ZINC(II) PROTOPORPHYRIN-IX DIMETHYL ESTER

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Abstract—Complex formation in Zn(II) protoporphyrin-IX dimethyl ester has been studied by proton and ^{13}C NMR spectroscopy. The large concentration dependence of the spectra has been studied by the technique of porphyrin/axial ligand titration, which together with selective decoupling and regiospecific deuterium labelling allows the assignment of all the peripheral proton and ^{13}C nuclei in both the monomeric and aggregated species. Titration of the metalloporphyrin with various basic ligands (pyrrolidine, pyridine, lutidine) showed that dissociation of the aggregate was complete for a 1:1 porphyrin/added base ratio. The concentration dependence of the spectra was then analyzed to give the monomer and monomer–dimer shifts for all the assigned nuclei. Analysis of the monomer–dimer shifts in terms of the ring current model gives good agreement with a dimer geometry in which the inter-ring separation is *ca.* 4.5 Å and there is a smaller lateral displacement of the porphyrin rings. The dimer geometry is such that rings A and B of one porphyrin molecule are situated over rings C and D of the other. These results confirm our earlier suggestions of intermolecular metal-to-porphyrin binding in these metalloporphyrins, and further suggest that charge-transfer interactions may also be present in appropriate cases. The discrepancy between the absolute values of the observed and calculated monomer–dimer shifts, which was formerly attributed to multiple aggregation, is now suggested to be due to ensemble-averaging in the dimer structure.

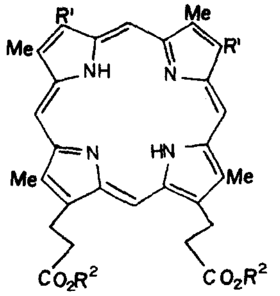
Protoporphyrin-IX (1; PP-IX) is one of the more important naturally occurring porphyrins, being both the porphyrin moiety of heme and also the compound which is often isolated from biosynthetic experiments.[2] Thus, the characterization and assignment of the proton and ^{13}C spectra of this compound are of fundamental importance. This objective is bedevilled in this, as in other porphyrins, by the large concentration dependence of the nuclear chemical shifts due to ring current and aggregation effects.[3] All of the resonances move upfield with increasing concentration but, more seriously, the relative positions of the peaks may be reversed, thereby causing considerable problems for resonance assignment. Thus, even a careful study of the 220 MHz spectrum of protoporphyrin-IX dimethyl ester (2; PP-IXDME) could not provide an unambiguous assignment of all the resonances,[4] and likewise, even synthesis of ^{13}C labelled isomers did not unambiguously allow assignment of the *meso* carbon resonances; degradation to diacetyldeuteroporphyrin-IX dimethyl ester (3) was necessary to confirm the biosynthetic labelling pattern.[5]

Recently, in our studies of the NMR spectra of porphyrins, we have proposed the use of Zn(II) porphyrins to characterize their NMR spectra.[6,7] These compounds give sharp, well-resolved ^{13}C spectra in contrast to the free-bases, and this allows the prospect of assignment of the quaternary nuclear carbons. Furthermore, the Zn(II) porphyrins show much larger concentration effects than the free bases (allowing a more precise quantitative study), and also these aggregates are dissociated into monomers upon addition of basic ligands (pyrrolidine, pyridine, lutidine). This allows the "monomeric" shifts to be obtained at reasonable concentrations and thus gives reliable porphyrin chemical shifts and greatly assists the analysis of the concentration dependent shifts.

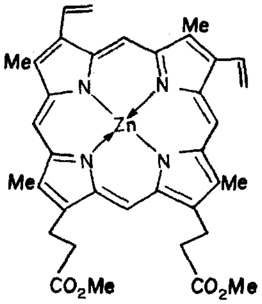
We have previously shown that the concentration dependence of some *meso* substituted octaethylporphyrins (OEP's) can be interpreted quantitatively in terms of a monomer–dimer equilibrium.[1,8] The structures of these particular dimers were deduced from the extrapolated dimer shifts, together with ring current calculations,[9] and are consistent only with intermolecular metal-to-porphyrin (and not metal-to-side-chain) interactions. However, these studies were limited by concentration and sensitivity problems, so it was of interest to investigate the detailed concentration dependence of a naturally occurring porphyrin to see if the aggregation tendency in the Zn(II) complexes was a general phenomenon. We give here the results for Zn(II) protoporphyrin-IX dimethyl ester (4; ZnPP-IXDME) and show that this is indeed a general phenomenon *not requiring* meso-*substitution.* These results also lead to a more complete assignment of the proton and ^{13}C spectra of the PP-IX molecule.

RESULTS

The spectral assignments. The proton and ^{13}C NMR spectra of ZnPP-IXDME (4) in moderately concentrated solution (25 mg in 0.4 ml $CDCl_3$, i.e. 0.095 M) before and after addition of 2 mol. equiv. pyrrolidine are shown in Figs. 1 and 2, and the data are tabulated in Table 1. It is immediately obvious that large chemical shift changes are occurring; for example, the *meso* protons move *ca.* 1–2 ppm downfield upon addition of pyrrolidine, and similar effects occur with other protons, the magnitude decreasing with distance from the macrocyclic nucleus.



1: R^1 = Vinyl
R^2 = H
2: R^1 = Vinyl
R^2 = Me
3: R^1 = Acetyl
R^2 = Me
5: R^1 = Et
R^2 = Me



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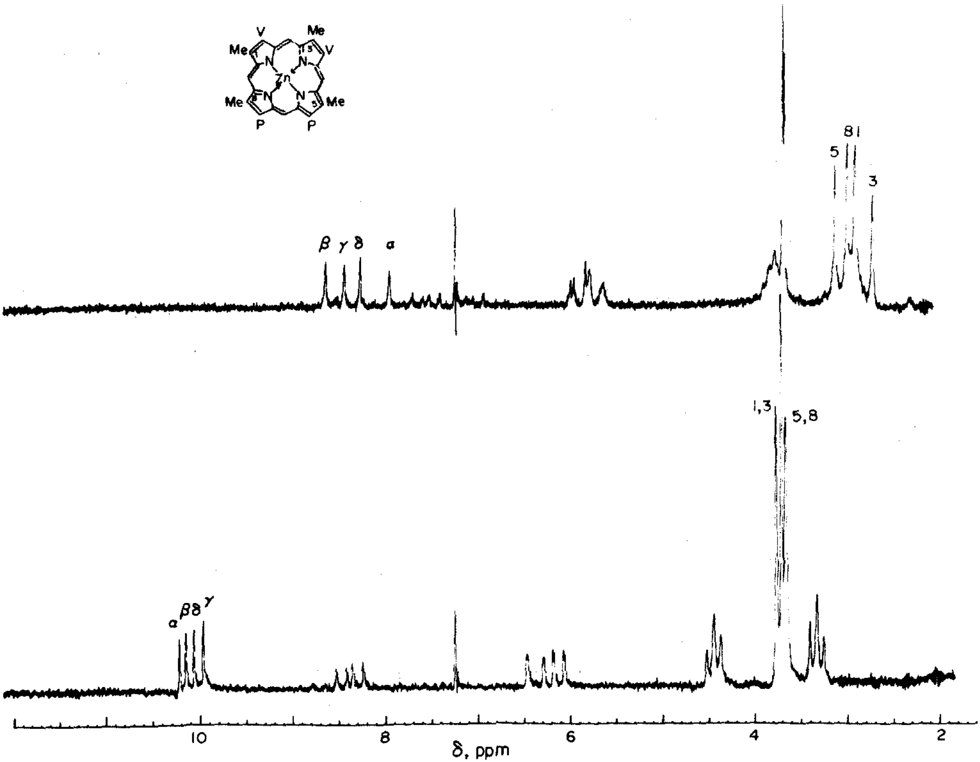

Fig. 1. 100 MHz proton NMR spectra of (4) in $CDCl_3$ (25 mg/0.4 ml) (upper) and after addition of 2 mole equiv of pyrrolidine (lower).

Thus, the ester OMe peak shows no change. Even larger shifts (in ppm) occur in the ^{13}C spectrum (Table 1) though the scale in Fig. 2 makes these effects less noticeable. Note also the increase in multiplicity of some of the lines in the aggregated spectrum, implying that structured aggregates are being formed.

Before these concentration effects can be analyzed the detailed assignments in the spectra need to be obtained. The differentiation into various groups is trivial for both the proton and ^{13}C spectra, so the major problem is the assignments within these groups of peaks (e.g. methyls, propionate methylenes, *mesos*, quarternary carbons, etc.). This is particularly important for the peripheral Me (sometimes called β-Me) and *meso* resonances. The relationships between the aggregated and monomeric spectra may be obtained unambiguously by titration of the concentrated Zn(II) porphyrin solution with successive aliquots of pyrrolidine. The results of these experiments are shown in Fig. 3, which shows very clearly that the assignments of both the peripheral Me

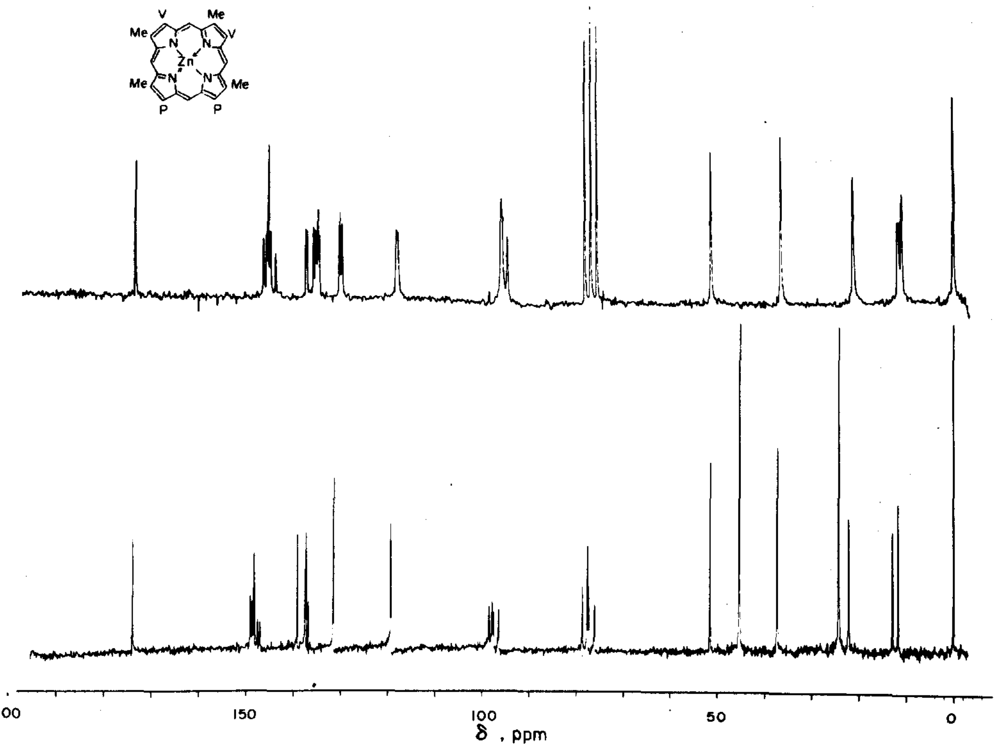

Fig. 2. 25.2 MHz ^{13}C NMR spectra of the same solutions of (4) as shown in Fig. 1.

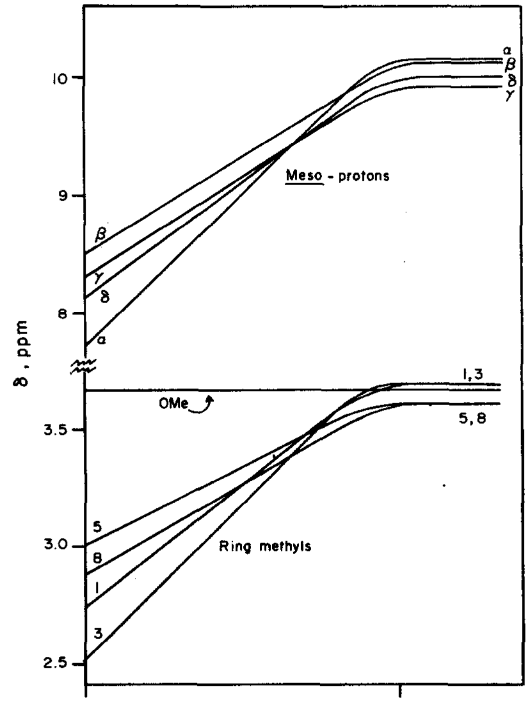

Fig. 3. Proton chemical shifts of *meso* and peripheral methyl protons on ZnPP-IXDME (4) (0.095 M in $CDCl_3$) vs added pyrrolidine.

and *meso* protons are a function of added pyrrolidine, those of the monomeric spectrum being quite different from those of the aggregated solution. Figure 3 also shows that dissociation of the metalloporphyrin aggregates is complete upon addition of an equimolar amount of pyrrolidine, and that further addition produces no change in the porphyrin chemical shifts. This is consistent with the formation of a 1:1 porphyrin/pyrrolidine complex, though this would not preclude the formation of some 1:2 complex subsequently, since the porphyrin chemical shifts might be expected to be identical in the two cases. [*In the corresponding Mg(OEP) compounds the proton shifts are identical for the mono- and dipyridine adducts.*[10]]

The four peripheral Me resonances were definitively assigned in the aggregated solution (in which the peak separation is greatest) with the aid of regiospecifically deuteriated samples [1,3-dideuteriomethyl-ZnPP-IXDME and 1,5-dideuteriomethyl-ZnPP-IXDME].[11] These assignments and the consequent assignments of the monomeric spectra from the pyrrolidine titration are shown in Figs. 1 and 3, and they differ from the provisional assignments of Janson and Katz[4] for PP-IXDME (2) in that the Me's adjacent to vinyl groups (i.e. Me_1 and Me_3) are to low field of those adjacent to the propionates (Me_5 and Me_8) and not *vice versa*.

The important *meso* proton assignments were obtained from the known assignments of the *meso* carbons, given again by total synthesis of specifically labelled samples.[2] These are in the order (from low to high field) α, β, δ, γ in PP-IXDME (2), and the *order* but not the chemical shifts does not change with concentration. Insertion of Zn(II) has negligible effect on the chemical shifts of all the porphyrin nuclei except the quaternary α-ring carbons, thus we can safely assume the same order of the *meso* carbon shifts in the monomer spectrum of Fig. 2. Single frequency decoupling experiments then allowed the proton resonances to be related to the corresponding

Table 1. ^{13}C and proton chemical shifts (δ) of the monomeric and aggregate solutions of Zn(II) protoporphyrin IXDME in $CDCl_3$

Nucleus	δ-Dis-aggregated	δ-Aggregated	$\Delta\delta$	
(A) *Carbon atoms*:				
$—CH_3$ (5, 8)	11.77	10.96	0.81	
		11.11	0.66	
$—CH_3$ (1, 3)	12.91	11.66	1.25	
		11.93	0.98	
$—CH_2—$	22.10	21.30	0.71	
$—CH_2—$	37.33	36.68	0.65	
$—OCH_3$	51.56	51.49	0.07	
$>C=O$	173.60		0.33	
Meso C. atoms				
α	96.07	94.60	1.47	
δ	97.23	95.59	1.64	
β	97.52	95.91	1.61	
α	98.20	95.91	2.29	
vinyl $=CH_2$	119.04	117.78	1.26	
vinyl $=CH$	131.02	118.07	0.97	
	→	129.58	1.44	
		130.00	1.02	
β carbon atoms				
C_8	136.44(1)[b]	134.41(1)		
C_1, C_5	136.87(2)	134.68(1)		
C_2, C_3	137.10(2)	135.02(1)		
C_4, C_6, C_7	138.63(3)	135.28(1)		
		135.61(1)		
		136.99(1)		
		137.21(2)		
α carbon atoms	148.58(1)	146.11(1)		
	148.21(1)	145.49(1)		
	147.93(1)	145.15(3)		
	147.81(1)	144.76(1)		
	147.71(2)	144.60(1)		
	147.06(1)	143.60(1)		
	146.56(1)			
(B) *Hydrogen atoms*:				
$—CH_3$ (1, 3)	3.69	3 2.49	1.20	1.06[a]
		1 2.72	0.97	0.94
$—CH_3$ (5, 8)	3.60	8 2.85	0.75	0.74
		5 2.99	0.61	0.63
Meso protons				
α	10.15	7.68	2.47	2.47
β	10.09	8.47	1.62	1.74
δ	9.99	8.09	1.90	2.13
γ	9.91	8.29	1.62	1.83
$—OCH_3$	3.66	3.63	0.03	
		3.64	0.02	
$—CH_2—$	3.26	2.82	0.44	
$—CH_2—$	4.39	3.73	0.66	
$=CH$	8.35(H_x)	6.88	1.47	
		7.44	0.91	
$=CH_2$	6.35(H_B)	5.83(H_B)	0.52	
	6.10(H_A)	5.83(H_A)	0.27	
		5.62(H_A)	0.48	
		5.60(H_B)	0.75	

[a] Values from the analysis of the dilution curves (see text).

[b] Numbers in parentheses refer to the relative signal intensities.

carbon signals and thus gave the assignments of the monomer proton spectrum. The pyrrolidine titration followed by an analogous decoupling experiment on the aggregated solution gave the proton and carbon assignments for this solution, though due to unresolved ^{13}C lines in the aggregated spectrum (Fig. 2) the only definite assignment is that of the γ-carbon at 94.6 δ. The only remaining assignments for the monomer spectrum are of the peripheral carbons ($Me_{1,3}$ and $Me_{5,8}$) and these follow from the shifts in similar molecules (e.g. the peripheral carbons in Zn(II) coproporphyrins are all at 11.7–11.8 δ[12]). Also, use of the peripheral carbon substituent effects previously obtained[12] allows a provisional assignment of the quaternary β ring carbon resonances (Table 1). The assignments of the more complex spectrum of the aggregates (Table 1) can only be made on the basis of the proposed dimer structure (*vide infra*).

The analysis of the concentration-dependent shifts. The chemical shifts of the *meso* and peripheral Me protons are recorded over a wide range of concentrations in Table 2. In this Table the shifts at zero concentration (i.e. the monomer shifts) are those for the ZnPP-IXDME (4) plus pyrrolidine solution. As there are small concentration effects even for these solutions, the values were measured using a dilute (0.01 M) solution (see Table 1 values at 0.095 M). The effect of dilution on the ZnPP-IXDME spectra is analogous to the effect of adding pyrrolidine in that similar cross-overs of the proton resonances occur. The assignments are best followed by plotting the chemical shifts of one proton (in this case the β-*meso* proton as this suffered the fewest cross-overs) against the other proton resonances. Straight lines or gentle curves result (not shown here), from which any interchange in the peak positions can be easily identified. These assignments were confirmed by the subsequent full analysis of these dilution curves. Due to the considerable curvature of these plots at high dilutions (Fig. 4) it is not possible to identify the individual resonances from this type of plot. Note also that even for the most dilute solution measured (7.0×10^{-4} M) the observed shifts are still very different from the δ_{mon} values, illustrating the difficulty even with FT techniques of obtaining monomeric shifts for these compounds. However, the pyrrolidine δ_{mon} values do fall on the plots of δ vs δ_β as expected.

The dilution plots thus obtained were analyzed in terms of a monomer–dimer equilibrium in which the observed chemical shift (δ_{obs}) at any concentration (a) is given by:

$$\Delta\delta_{obs} = \delta_{obs} - \delta_{mon} = \Delta\delta[(1+8aK)^{1/2} - 1]^2/8aK \quad (1)$$

where K is the equilibrium constant and $\Delta\delta$ is $\delta_{dimer} - \delta_{mon}$, the complexation shift. In this analysis the monomeric shifts δ_{mon} are also those given in Table 2. As these data were much more comprehensive than our previous concentration dependence measurements on Zn(II) porphyrins, covering both a much wider range of concentrations and more defined nuclei, an iterative computational analysis was performed. For any given value of K, the value of $\Delta\delta$ may be found at each concentration from eqn (1). The average value of $\Delta\delta$ is then obtained and hence the calculated and observed shifts may be compared. The equilibrium constant is then varied to find the best fit of the observed and calculated shifts. This analysis was performed independently on each of the nuclear chemical shifts in Table 2. These homed on values of K from 0.30 to 0.40 ml/mg, with average rms errors of *ca.* 0.05 ppm. The average of these values gave K as 240 l. m^{-1} with calculated monomer–dimer shifts ($\Delta\delta$) for each of these nuclei given in Table 1. Two representative curves, for the β-*meso* proton and

Table 2. Proton chemical shifts (δ)[a] of ZPP-IX DME in $CDCl_3$

Conc.	*meso*				Peripheral-Me				
($M \cdot 10^{-2}$)	α	β	γ	δ	(1)	(3)	(5)	(8)	CO_2Me
1.25[b]	10.22_7	10.14_2	9.94_5	10.06_4	3.75_6	3.74_9	3.64_6	3.62_4	3.66_7
0.07	9.63_8	9.68_6	9.42_7	9.46_8	3.54_7	3.54_7	3.50_0	3.45_4	3.65_7 (3.66_6)
0.11	9.59	9.65	9.41	9.44	3.52	3.52	3.48	3.44	3.65 (3.65)
0.15	9.46_5	9.55_9	9.28_3	9.31_0	3.47_9	3.47_9	3.45_1	3.38_8	3.64_5 (3.65_2)
0.46	9.12	9.32	9.04	9.04	3.37	3.32	3.37	3.29	3.64 (3.65)
0.92	8.88	9.16	8.88	8.85	3.26	3.22	3.31	3.22	3.64 (3.65)
1.54	8.67	9.02	8.77	8.70	3.17	3.09	3.25	3.15	3.64 (3.65)
2.32	8.44	8.90	8.66	8.55	3.08	2.96	3.20	3.08	3.64 (3.65)
2.66	8.40	8.87	8.64	8.53	3.06	2.94	3.18	3.07	3.64 (3.65)
3.13	8.33	8.83	8.61	8.49	3.03	2.90	3.17	3.05	3.64 (3.65)
4.09	8.23	8.78	8.56	8.42	2.98	2.83	3.14	3.02	3.64 (3.65)
4.84	8.19	8.76	8.54	8.40	2.97	2.82	3.13	3.01	
5.92	8.05	8.68	8.47	8.32	2.90	2.73	3.09	2.96	
6.66	7.98	8.64	8.44	8.28	2.86	2.68	3.07	2.94	
7.10	7.93	8.62	8.42	8.25	2.84	2.65	3.06	2.93	
7.60	7.90	8.59	8.40	8.22	2.82	2.63	3.05	2.92	

[a] ±0.005 (SC-300, R34); ±0.01 (XL-100).
[b] +pyrrolidine (2 M eq).

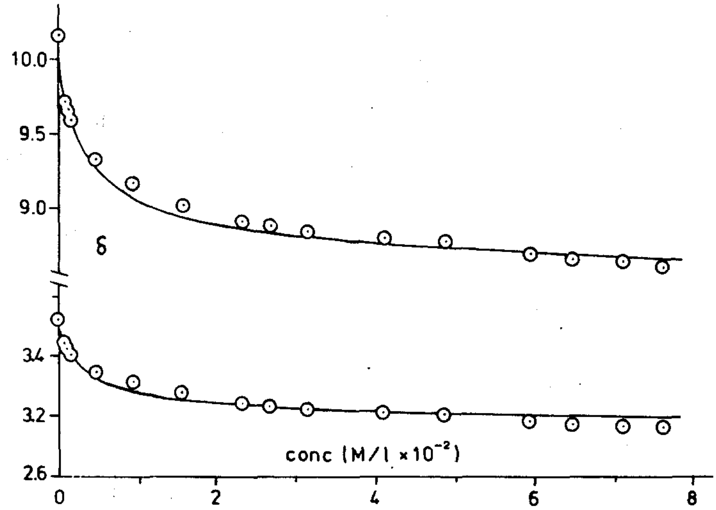

Fig. 4. Observed points (O) and calculated plots of proton NMR spectrum of ZnPP-IXDME (4) as a function of concentration in $CDCl_3$. Upper curve is *meso* β-H, lower curve is Me_8.

the Me_8 protons are shown in Fig. 4. The occurrence of higher aggregates than the dimer has been inferred from our previous studies of Zn(II) porphyrins, and it is noticeable in Fig. 4 that the observed shifts at high concentrations are always to higher field than the calculated ones, and this is consistent with the formation of higher aggregates. If there were appreciable proportions of higher aggregates (trimers, etc.) at higher concentrations the analysis of different portions of the dilution curves would give very different results. It was found that the analysis of the first ten points (i.e. concentrations < 0.04 M) gave results which were very similar to the overall results of Table 1. The "best" equilibrium constants were the same, but the $\Delta\delta$ values were slightly less. For the *meso* protons the values were 2.36 (α), 1.70 (β), 1.79 (γ) and 2.10 (δ). These results suggest that only small amounts of higher aggregates are present in the concentration range studied and the significance of this important result will be further considered later.

The aggregation shifts for the *meso*-protons and peripheral Me protons in Table 1, which are merely the shift differences with and without pyrrolidine at a given concentration, can be seen to be accidentally the same as the $\Delta\delta$ values obtained from the monomer–dimer analysis. This means that the aggregation shifts of the remaining protons and of the carbons given in Table 1 may also be considered as monomer–dimer shifts for the ZnPP-IXDME (4) complex. These shifts are shown schematically in Fig. 5 and can now be used to obtain information on the nature and geometry of the dimer.

The geometry of the dimer. The same general procedure was used here as in our previous studies on *meso*-substituted Zn(OEP)'s.[1,8] Thus, we assume that the two porphyrin molecules in the dimer are parallel and that the complexation shifts are solely due to the ring current of the adjacent porphyrin molecule in the dimer. Though the former assumption may not be absolutely correct in all instances,[13] considerable support for the latter assumption is provided by the comparison of the

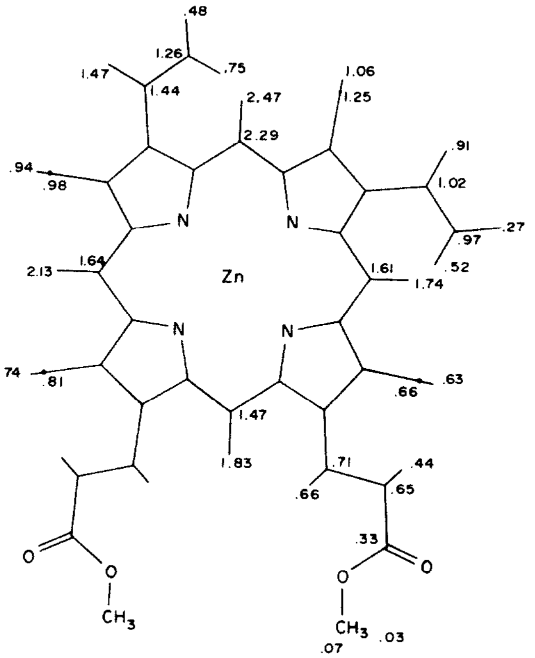

Fig. 5. Proton and ^{13}C complexation shifts ($\Delta\delta$) in ZnPP-IXDME (4). (These values are the shift differences, for the indicated nuclei, obtained with and without addition of pyrrolidine; see Table 1)

proton and ^{13}C complexation shifts (Table 1 and Fig. 5). There is a very close similarity between the proton shifts and corresponding carbon shifts of a C–H pair. This is true not only for the peripheral Me protons and carbons, but also more remarkably for the *meso* protons and carbons. There is no mechanism other than magnetic field anisotropy which can give even the same order of shift changes for carbons and protons. (Most mechanisms give carbon shifts *ca.* 20 × those of protons, in line with the accepted range of the chemical shifts). Some doubts have been raised as to whether carbon chemical shifts are affected by ring currents (despite the theoretical justification) due to the difficulty of isolating this small contribution from the other interactions present. The data of Fig. 5 thus provide unequivocal evidence for this effect, even though in this case it is the ring current of an adjacent molecule which is responsible. The data also illustrate again the negligible effects of Zn(II) insertion on the chemical shifts of all nuclei itemized in Fig. 5, even down to the susceptible *meso* carbons.

In our previous complexation studies the porphyrin molecule had a plane of symmetry.[1,8] Thus, on a time-averaged basis the structure of the complex also had a plane of symmetry and this resulted in only two possible structures. For ZnPP-IXDME, in contrast, the molecules have no symmetry, so they may combine in many possible orientations, in the majority of which the environments of the two molecules in the dimer will differ. As there is fast exchange, only one time-averaged signal is observed for each nucleus, but the calculation of the complexation shift involves summing over the two non-equivalent porphyrin molecules. Previous studies of PP-IXDME (2) identified a possible dimer structure in which each porphyrin molecule is equivalent (Fig. 6). After trial calculations of a number of possible dimer structures we found that this type of structure gave reasonable agreement with experiment, as well as being much easier to handle computationally and we shall only consider this henceforth. However, it should be noted that a less symmetric structure could possibly exist which would also reproduce the observed shifts.

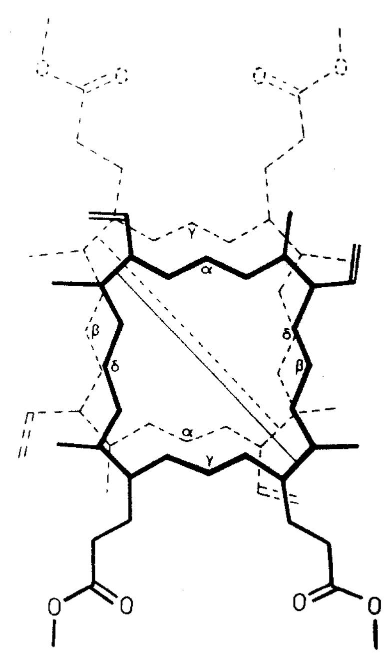

Fig. 6. The ZnPP-IXDME (4) dimer structure.

Given the dimer structure in Fig. 6, we now need to obtain the lateral (x, y) and vertical (z) displacements which will best fit the observed complexation shifts. As these shifts comprise an extensive set of data, a computational search procedure was adopted. The program calculates the ring current shifts of any given nucleus in a porphyrin molecule at a given position from another porphyrin molecule (which is always at the origin). The ring current shifts were calculated from the equivalent dipole network model described previously.[9] At the distances considered here, the ring current field is axially symmetric so that the only variables are the co-ordinates of the porphyrin molecule from the origin. Furthermore, as our previous studies had shown that the relative complexation shifts were of more significance than the actual observed shifts, due to the possible formation of higher aggregates, the observed versus calculated shifts were tested for linearity and approach to the origin.

The calculation gave not only the geometry of the dimer but also the assignments of some of the nuclei in the concentrated solutions which could not be otherwise obtained. This was the case for the peripheral Me carbon shifts of Me_1 and Me_3 (and Me_5 and Me_8) which are identical in the monomer. These can be made easily on the basis of their similarity to the corresponding proton shifts, the proton assignments being available from regioselective deuteriation. However, the carbon assignments of the C_2 and C_4 vinyl groups, which again are identical in the monomer but not the dimer, can only be obtained in the dimer from the calculated shifts. Having obtained these assignments (Fig. 5) they can be used with the other shifts to better define the geometry of the dimer.

In the calculations the peripheral Me proton positions were considered, for simplicity, to be at the average position over the three Me protons, i.e. 0.36 Å along the C–C bond and the same procedure was adopted for the propionate CH_2 groups. Because of the problem of rotational averaging other nuclei more than one bond from the ring were not considered in this search procedure.

Using the *meso* protons and all the peripheral nuclei of defined position (making a total of 18 data points), reasonable agreement with the observed shifts was obtained with co-ordinates for the origin of the upper ring (Fig. 6) of 0.10, −0.30, 4.5 Å. The observed and calculated shifts gave a good linear correlation with an rms error of 0.12 ppm over a range of shifts of 0.6–2.5 ppm (Fig. 7). The agreement is well within the probable errors of these derived shifts and the linearity and approach through the origin support this general interpretation. The slope of the line in Fig. 7 is *ca.* 2.0, i.e. the observed shifts are twice the calculated ones. Similar slopes have been obtained in our previous studies of *meso* substituted porphyrins[1,8] and have been ascribed to the possible formation of higher aggregates (e.g. trimers, tetramers, etc.) in solution. It can easily be shown that due to the influence of non-nearest neighbours, the limiting slope for a polymer is *ca.* 3 times the monomer shift. However, the calculated shifts for the *meso* C atoms for the above geometry are 2.13 (α), 1.77 (β), 1.59 (γ) and 1.99 (δ), which are in good agreement with the observed shifts (Fig. 5) *without* the factor of two being involved. The *meso* C nuclei will be affected by multiple aggregation in precisely the same manner as the other nuclei. Thus, if the multiple aggregation explanation is to be further considered, it is necessary to postulate that the *meso* C's alone are affected by another mechanism which subtracts from the ring current. It is possible that the *meso* C shifts could be affected by, for example, Zn(II) incorporation, which would not affect the other more distant nuclei. However, it is unlikely that a combination of two opposing unrelated mechanisms would lead to such good agreement with the calculated shifts, and in view also of the lack of evidence from the concentration analysis (previous section) of any appreciable amounts of higher aggregates, an alternative solution would appear to be required. A possible clue is provided by the observed shifts of the remaining nuclei which are further removed from the porphyrin ring. Although rotational isomerism problems affect many of these groups it is clear that the observed shifts are much larger than those calculated using our model. For example, the methine (CH) protons of the C_2 and C_4 vinyls, which may be assumed to be coplanar with the porphyrin ring, are calculated to have shifts of 0.30 and 0.22 ppm respectively, compared with the observed values of 1.47 and 0.91 ppm. The observed monomer-dimer shifts are much more than twice the calculated values.

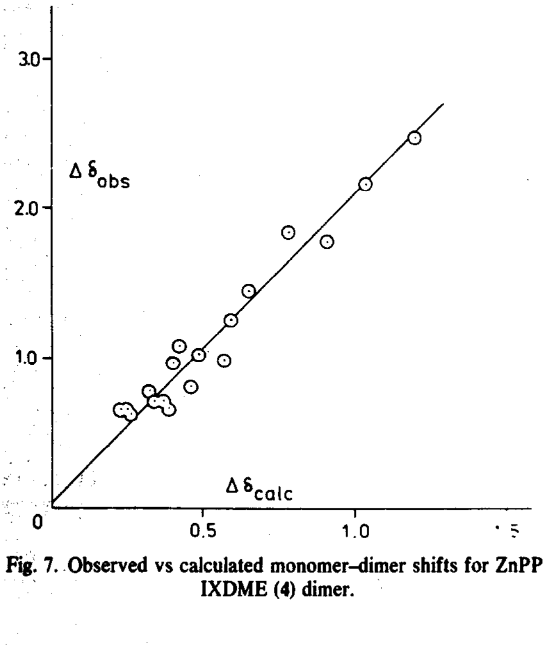

Fig. 7. Observed vs calculated monomer–dimer shifts for ZnPP-IXDME (4) dimer.

There is therefore a gradation of the observed vs calculated shifts from approximately equal values for the *meso* carbons (and by inspection the ring C_α and C_β carbons), to a factor of two or more for the *meso* protons and peripheral substituents one bond removed from the porphyrin, to larger factors for nuclei even further away, i.e. the calculated shifts fall off much more rapidly with distance from the porphyrin ring than do the observed shifts, though the relative shifts within any given group agree with the calculated ones very well. A simple explanation of this trend is apparent when one considers the effects of intermolecular motions in the dimer on the calculated shifts. The calculated shifts are those calculated for a static dimer structure with a given, defined geometry. There is no doubt that this is an idealized picture. At the inter-molecular distances considered here, of 4–5 Å, there will be a distribution of dimer geometries about the statistical mean. This will result in both a distribution over the x, y, z co-ordinates of the origin, i.e. the displacement of the porphyrin molecules in the dimer will vary and also there will be a distribution of the inter-porphyrin angle in the dimer about the average value of zero, i.e. the two porphyrin molecules in the dimer will not at any one instant be parallel.

The effect that these motions will have on the calculated shifts is clearly seen when one considers that the equivalent dipole model gives an r^{-3} dependence of the shifts and also that the ring current at any position above a porphyrin molecule is almost constant within the ring boundary, but decreases sharply as one moves further away. Thus, the molecular motion in the dimer will have least effect for those nuclei situated within the porphyrin ring, for which small displacements in the x and y axes and ring twisting will have little effect and displacements in the z axis merely produce the derived averaged value of z. However, for those nuclei on the side-chains of the porphyrin, the ring current field of the adjacent molecule is critically dependent upon the precise position of the nucleus concerned and the observed field in the dimer is the average over all the molecular motions, *not* the field at the average position, which is what is calculated above. In the simplest case this means that we replace an r_{av}^{-3} term in the calculated shifts by an $\langle r^{-3}\rangle_{av}$ term. The latter is always much larger than the former. Thus, the effect of such molecular motion will be to produce monomer–dimer shifts of the ring and *meso* carbons essentially identical with the calculated "static" values, but to give observed shifts progressively larger than the calculated ones as the distance from the porphyrin ring increases. This is certainly in qualitative agreement with the observed results.

CONCLUSIONS

The above analysis of the concentration dependence of the NMR spectra of ZnPP-IXDME confirms and extends the results from our previous studies and demonstrates clearly that the association behaviour of Zn(II) porphyrins is a general phenomenon of these systems. The analysis also shows the importance of a combined ^{13}C, proton investigation. Not only did this help in the assignment problem, but the multiple aggregation vs ensemble average hypothesis can only be distinguished by analysis of all the data presented here. Our previous studies on *meso* substituted (OEP)'s[1,8] were based only on proton data, of which only the *meso* and peripheral CH_2 protons could be used in the analysis; this limited data allows only the ensemble average geometry to be deduced.

It is of interest to note that the formation constant of the ZnPP-IXDME dimer (240 l./m) is much larger than those found for the Zn(II) *meso* substituted (OEP)'s (*ca.* 20 l./m). We have shown previously, from comparative shift measurements[14] that the relative complexing efficiency of Zn(II) porphyrins can be related to the pK_3 values of the corresponding metal-free porphyrin in a series of 2,4-disubstituted deuteroporphyrins-IX. In this tabulation, PP-IXDME (2) gave much larger shifts (and lower pK_3) than the corresponding 2,4-diethyl compound [mesoporphyrin-IX dimethyl ester (5)] which is analogous to octaethylporphyrin. Thus, it is in accord with our proposed polarization mechanism[1,14] tnat ZnPP-IXDME (4) should give much larger association constants than the substituted Zn(OEP)'s.

The geometry of the dimer also reinforces our previous suggestion[14] that the electronic imbalance between rings A and B (the electron deficient rings) and C and D (the electron rich rings) may be an important factor in these interactions. The porphyrin rings in the dimer of 4 have less lateral movement than in the Zn(OEP) compounds (*ca.* 1 Å) and this is to be expected if rings A, B and C, D interact in the dimer. The important question of substituent shifts and the NMR spectra of the porphyrins will be considered in detail elsewhere.

EXPERIMENTAL

ZnPP-IXDME (4) was prepared using the Zn(II) acetate method.[15] The product was recrystallized from CH_2Cl_2–MeOH or CH_2Cl_2–hexane and then dried at 100° and 1 mm Hg for 24 hr to remove extraneous solvents. Occasionally, more than one crystalline modification of the product was obtained and these had widely different solubilities; this was not further investigated for the purposes of the present study. 1,3-Dideuteromethyl-ZnPP-IXDME was originally prepared by total synthesis,[11a] but subsequently by the more efficient direct base-catalyzed exchange of PP-IX.[11b] 1,5-Dideuteromethyl-ZnPP-IXDME was prepared by total synthesis, using methods already described.[11c]

The proton and ^{13}C NMR spectra were obtained on a Varian XL-100 spectrometer (probe temp. *ca.* 30°) in $CDCl_3$, which was always eluted through a Brockmann Grade I alumina column before use. The solvent was used as field-frequency lock and all measurements are referred to internal TMS. Except for the dilution studies, the concentration of ZnPP-IXDME (4) was constant at 25 mg in 0.4 ml $CDCl_3$ (i.e. 0.095 M). The pyrrolidine titration was performed with a 25% pyrrolidine–$CDCl_3$ soln. At the end of the titration only 0.01 ml of this soln had been added, producing a negligible intrinsic dilution shift.

The very dilute proton spectra were obtained on a Varian SC300 spectrometer, (SW 3600 Hz, 32K transform) and on a Perkin–Elmer R-34 (220 MHz) spectrometer.

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