Thus the difference in δ-meso-H line width and any other meso is proportional to $\tau_M + \tau_D$ (eq 2). At −70 °C, K = 51.5 L mol^{-1}, $\Delta\omega = 7.89 \times 10^3$ rad s^{-1}, $\Delta\delta_{1/2}$ = 90 Hz, yielding $\tau_M = 2.4 \times 10^{-7}$ s, $\tau_D = 1.2 \times 10^{-5}$ s. These lifetimes are consistent with lifetimes determined for other porphyrin dimers[29] (10^{-4}-10^{-8} s).

Acknowledgments. The authors thank Professor R. Kosfeld for use of the WH-270 spectrometer. This work was supported by grants from the National Science Foundation, CHE-75-07788 and CHE-77-26517, and the National Institutes of Health, HL-16087.

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(20) The 1 on 1 pyrrole overlap (C) places the closest meso H at least twice as far from the iron as either 1- or 8-CH_3, indicating an intermolecular dipolar relaxation contribution to any meso H of ≤0.03 of that of 1,8-CH_3.
(21) At higher temperatures, the fraction of dimer, f^D, is so small that the calculation of intermolecular relaxation rates is the small difference between two large numbers which has very large uncertainties. Analysis of data at lower temperatures is precluded owing to severe overlap of methyl resonances which interferes with T_1 and T_2 determinations. Higher field strengths were of little value in improving resolution owing to the increasing contribution of chemical exchange to the line widths.
(22) The following van der Waals radii were used: aromatic C, 1.5-1.6 Å; methyl group, 1.5-2.0 Å; and bromine, 2.0 Å.
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NMR Studies of Low-Spin Ferric Complexes of Natural Porphyrin Derivatives. 3. Thermodynamics of Dimerization and the Influence of Substituents on Dimer Structure and Stability

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Abstract: The low-temperature ^{1}H NMR spectra of 2,4-disubstituted deuteroporphyrin iron(III) dicyanide complexes in methanol exhibit concentration-dependent chemical shifts and line widths. Computer analysis of the dilution shifts shows they are consistent with exclusively a monomer-dimer equilibrium. Thermodynamic parameters for the dimerization of the 2,4-dibromo complex are consistent with an associative process with $\Delta H°$ = 3.4 kcal/mol and $\Delta S°$ = −8.9 eu. The ionic strength and solvent dependence of the extent of dimerization are similar to that observed for other porphyrin aggregations and support a π-π interaction. As the 2,4 substituent becomes more electron withdrawing and the unpaired electron spin distribution becomes more asymmetric, the extent of dimerization increases. Since overlap involves pyrroles having the greatest difference in unpaired electron spin density, this suggests a π donor-acceptor interaction as contributing to the stability of the dimer. The properties of the electronic asymmetry of protoporphyrin suggest that the asymmetry may play an important role in the stability of the heme-apoprotein interactions.

Introduction

The interest in π-π interactions of porphyrins stems from the possible roles of such interactions in the stabilization and function of hemoproteins.[1] Changes in these π contacts have also been suggested as a possible trigger in the cooperative O_2 binding of hemoglobin.[2] Porphyrins readily form π-π complexes with organic aromatic molecules[2] and other porphyrins

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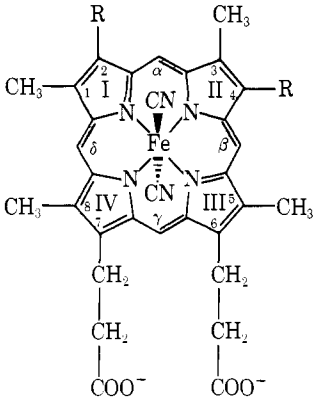
and several examples of such porphyrin dimers have been reported.[4]

Previous reports have provided evidence[5] for asymmetry in the highest filled π molecular orbital of substituted deuteroporphyrins, I, and for a dimer structure[6,7] for these porphyrins



I

which is suggestive of an overall asymmetry in the π system and differential donor-acceptor properties for individual pyrroles.

A more thorough understanding of the proposed π asymmetry of the natural porphyrin derivatives requires the characterization of the thermodynamics of the interaction and a study of the influence of the 2,4 substituents on the equilibrium constant of dimerization. Although the concentration-dependent shifts cannot be used to analyze the solution structure owing to simultaneous, and partially canceling, contributions from intermolecular upfield ring currents[4,8,9] and downfield dipolar shifts,[8] the average solution shifts can be determined accurately and used to characterize the extent of aggregation.

Principles

Observation of concentration-dependent chemical shifts will yield data which can be used to determine the chemical shifts of two entities in equilibrium. For a system in rapid exchange on the NMR time scale between a monomer and dimer, the observed chemical shift is given by[10]

$$\left(\frac{\Delta H}{H}\right)_{\text{obsd}} = f^{\text{M}}\left(\frac{\Delta H}{H}\right)^{\text{M}} + f^{\text{D}}\left(\frac{\Delta H}{H}\right)^{\text{D}} \quad (1)$$

where f^{M}, f^{D} and $(\Delta H/H)^{\text{M}}$, $(\Delta H/H)^{\text{D}}$ are the fractional populations and chemical shifts relative to Me_4Si of the monomer and dimer, respectively. For the equilibrium

$$2\text{M} \rightleftharpoons \text{D} \quad (2)$$

where M and D are the monomer and dimer, the equilibrium constant is given by

$$K = [\text{D}]/[\text{M}]^2 \quad (3)$$

Equations 1 and 3 can be combined and, with some rearrangement, yield[4]

$$\left(\frac{\Delta H}{H}\right)_{\text{obsd}} = \left(\frac{\Delta H}{H}\right)^{\text{M}} + \left[\left(\frac{\Delta H}{H}\right)^{\text{D}} - \left(\frac{\Delta H}{H}\right)^{\text{M}}\right]\left[1 + \frac{1}{4KC} - \frac{\sqrt{8CK+1}}{4KC}\right] \quad (4)$$

where C is the total porphyrin concentration. Equation 4 can be simply solved by iterative computer methods which yield K, $(\Delta H/H)^{\text{M}}$, and $(\Delta H/H)^{\text{D}}$.

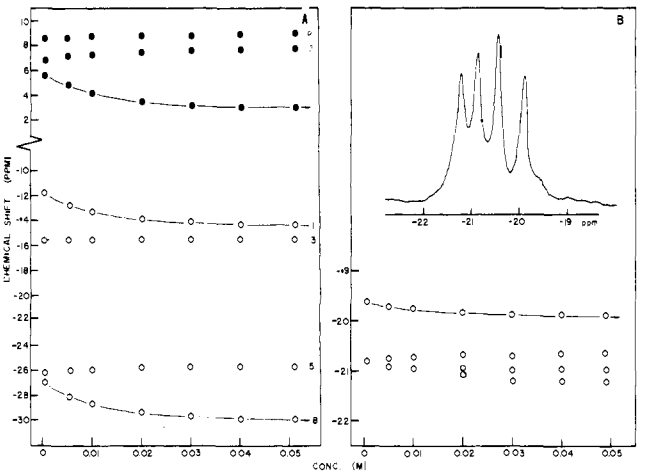


Figure 1. Plots of chemical shift relative to Me_4Si vs. concentration in $C^2H_3O^2H$ at −70 °C: A, 2,4-B_2DC; B, 2,4-E_2DC; heme methyls, O; meso protons, ●. Solid lines represent computer fit to eq 4. Insert in B is a portion of the 270-MHz ^{1}H NMR spectrum of 0.050 M 2,4-E_2DC in $C^2H_3O^2H$ at −75 °C showing the methyl resonances.

Experimental Section

The 2,4-disubstituted deuteroporphyrin iron(III) chloride complexes were synthesized and the dicyanide complexes were prepared in $C^2H_3O^2H$ as in part 1[7] in the concentration range of 0.050–0.000 50 M. The nomenclature used here is the same as that used in parts 1[7] and 2,[5] with the acid dicyanide complexes abbreviated 2,4-R_2DC and the dimethyl esters complex 2,4-R_2DEC, where R = E, ethyl; H, hydrogen; V, vinyl; B, bromine; and A, acetyl. Since the ^{1}H NMR spectra were found to be ionic strength dependent (vide infra), the ionic strength was held constant at 0.33 M with KCN at all porphyrin concentrations except where the effect of ionic strength is explicitly considered.

^{1}H NMR spectra were obtained on 100-MHz JEOL PFT-100 and 270-MHz Bruker WH-270 pulsed FT NMR spectrometers, as described in part 2.[5] Temperature was maintained and measured as described in part 2.[5] Temperature was maintained at −70 °C for the comparison of the extent of dimerization on 2,4-R since at higher temperatures 2,4-E_2DC and 2,4-H_2DC did not dimerize enough to be measured and at lower temperatures some resonances could not be resolved.

The values of K in eq 4 were obtained by computer fit to the experimental data based on an iterative least-squares minimization procedure[11] using a Burroughs B6700 computer. The optimum values of K were independent of the initial guesses. K was determined to the error of ±5 L mol^{-1}.

Results

2,4-B_2DC in $C^2H_3O^2H$ exhibits substantial concentration-dependent chemical shifts below −40 °C, as illustrated in Figure 1 by the plots of chemical shift vs. concentration for all resolved resonances at −70 °C. 8,1-CH_3 and δ-meso-H exhibit substantially larger dilution shifts than other substituents. Figure 1B shows the plots of chemical shift vs. concentration and a 270-MHz spectrum of the methyls of 2,4-E_2DC in $C^2H_3O^2H$ at −70 °C. Solid lines represent computer fits to eq 4. Curie plots of $(\Delta H/H)^{\text{M}}$ and $(\Delta H/H)^{\text{D}}$ from computer fits of the shifts for B_2DC to equation 4 are illustrated in Figure 2. Figure 3 gives the Van't Hoff plot for 8,1-CH_3 and δ-meso-H shifts of 2,4-B_2DC. The effect of ionic strength on the dimerization of 2,4-B_2DC is illustrated in Figure 4 in the KCN concentration dependence of the methyl region of 0.011 M 2,4-B_2DC in $C^2H_3O^2H$ at −63 °C. Figure 5 reproduces the ^{1}H NMR spectrum of 0.035 M 2,4-B_2DEC in $C^2H_2Cl_2$ at −87 °C.

All 2,4-R_2DC studied exhibit stereospecific concentration-dependent line widths as illustrated in Figure 6, where the ^{1}H NMR spectra of the methyl regions of 0.05 M 2,4-R_2DC in $C^2H_3O^2H$ at −70 °C are given. For all electron-with-

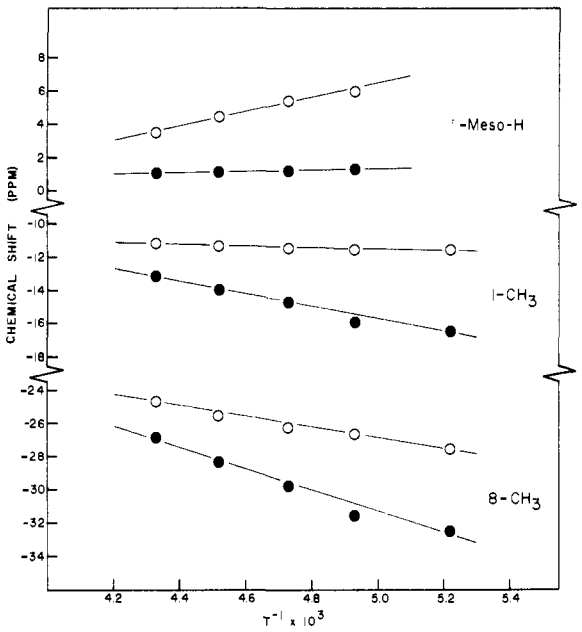


Figure 2. Curie plots of the monomer, O, and dimer, ●, chemical shifts, relative to Me_4Si, for 2,4-B_2DC resulting from the computer fits to eq 4.

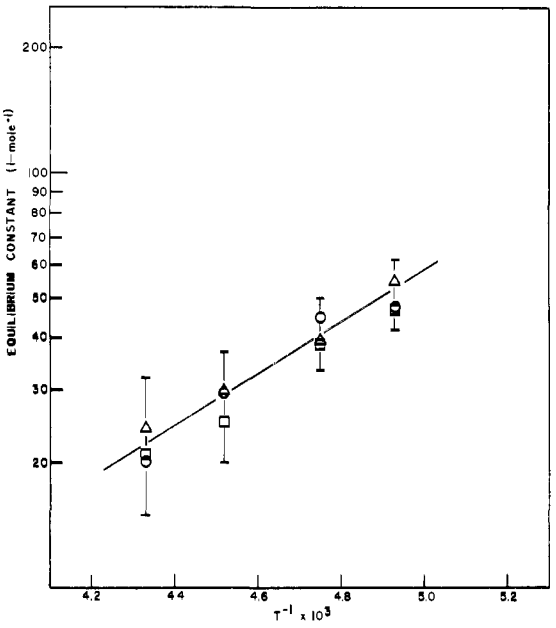


Figure 3. Van't Hoff plot for the dimerization of 2,4-B_2DC in $C^2H_3O^2H$. 8-CH_3, △; 1-CH_3, O; δ-meso-H, □.

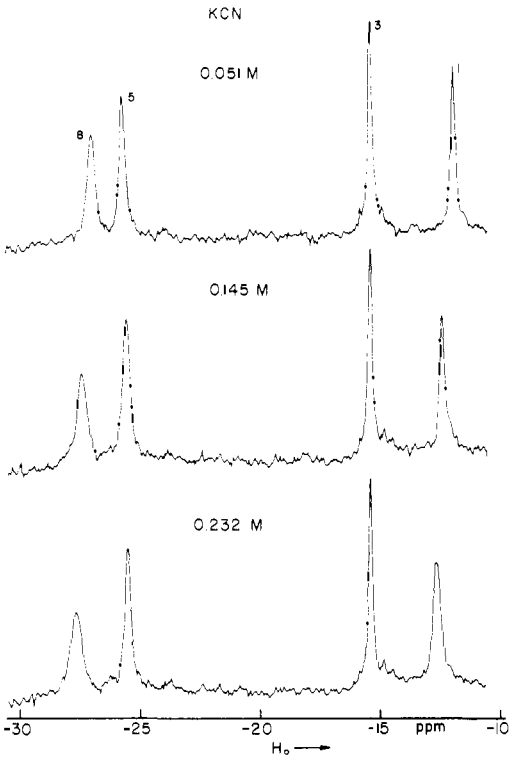


Figure 4. ^1H NMR spectra of the methyl region of 0.011 M 2,4-B_2DC in $C^2H_3O^2H$ at −63 °C as a function of KCN concentration.

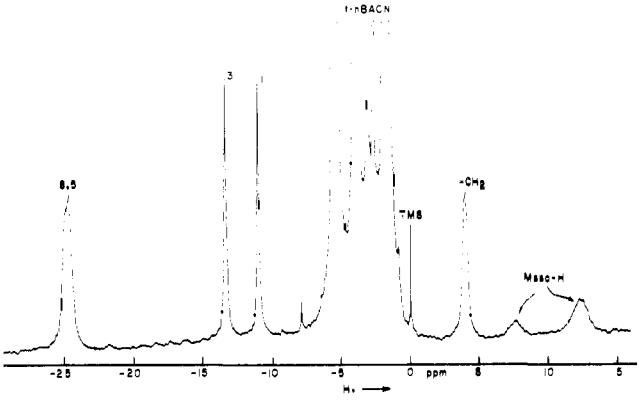


Figure 5. ^1H NMR spectrum 0.035 M 2,4-B_2DEC in $C^2H_2Cl_2$ at −87 °C. The 5,8-CH_3 resonances are accidentally degenerate. 1-CH_3 narrows upon dilution. Cyanide added as tetra-*n*-butylammonium cyanide, *t*-*n*-BACN.

drawing R, 8- and 1-CH_3 exhibit greater line width increases. A comparison of the concentration dependence of the 1-CH_3 chemical shifts and the calculated K's for several 2,4-R_2DC at −70 °C in $C^2H_3O^2H$ are illustrated in Figure 7. All plots are normalized to $(\Delta H/H)^M = 0$.

Discussion

Monomer–Dimer Equilibrium. Although most of the resonances show only small shifts in Figure 1A, 8,1-CH_3 and δ-meso-H exhibit very substantial dilution shifts. The relative values of the shifts have been shown to be consistent with the solution dimer structure.[5] Iterative computer fits to eq 4 (represented by the solid lines in Figure 1) for 8,1-CH_3 and δ-meso-H are within experimental error of the data points and yield the same K for all three resonances. Similar fits within experimental error are observed for R = E, H, V. An additional test for the validity of eq 4 is the independently determined temperature dependence of the calculated $(\Delta H/H)^M$ and $(\Delta H/H)^D$. The Curie plots of $(\Delta H/H)^M$ and $(\Delta H/H)^D$ for 8,1-CH_3 and δ-meso-H illustrated in Figure 2 are the expected straight lines for well-defined paramagnetic complexes[12] and provide strong support for the simple monomer–dimer equilibrium. The intercepts, however, deviate from the diamagnetic position as found in most low-spin ferric systems. The temperature dependence of the calculated $(\Delta H/H)^M$ is essentially identical with an independently determined temperature dependence of the chemical shifts of 0.001 M 2,4-B DC.

A plot of ln K vs. T^{-1} yields the expected straight line, shown in Figure 3 for 2,4-B_2DC in $C^2H_3O^2H$. The plot yields the thermodynamic parameters $\Delta H° = -3.4 \pm 1$ kcal/mol and $\Delta S° = -8.9 \pm 4$ eu. The negative $\Delta S°$ is consistent with an associative process and the parameters are similar to values determined for the π–π dimerization of other porphyrins[4] ($\Delta H° = -1.2$ to -4.9 kcal/mol, $\Delta S° = -3$ to -17.1 eu).

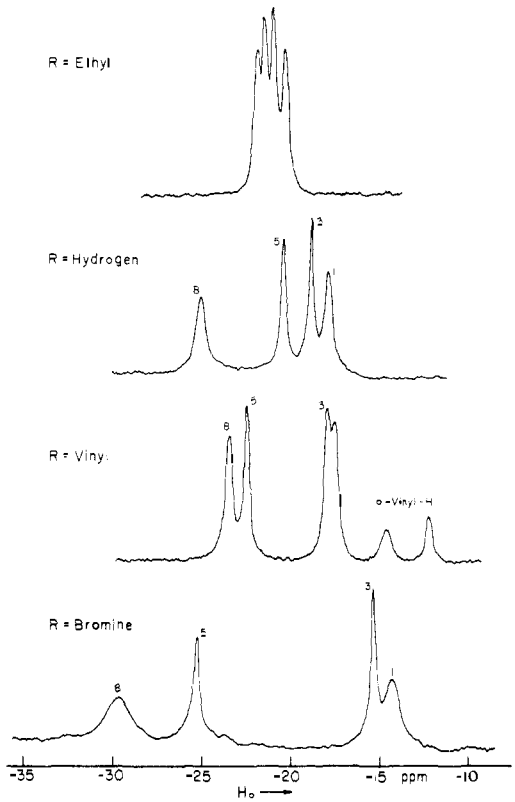


Figure 6. ^{1}H NMR spectra of the methyl region of 0.050 M 2,4-R_2DC in $C^2H_3O^2H$ at −70 °C.

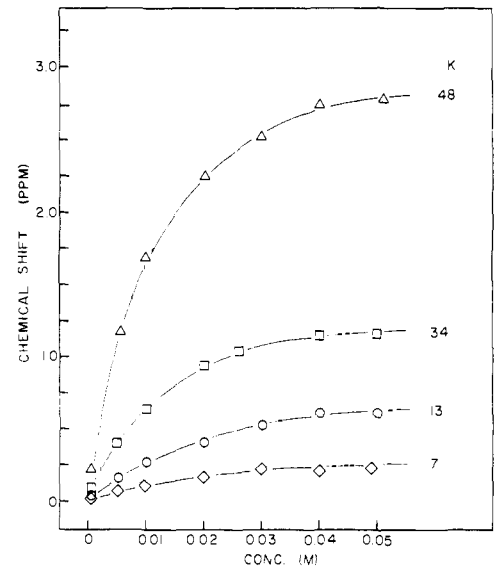


Figure 7. Plots of chemical shift, relative to monomer, vs. concentration for 1-CH_3 of 2,4-R_2DC in $C^2H_3O^2H$ at −70 °C. Solid lines represent computer fit to eq 4. △, B_2DC; □, V_2DC; ○, H_2DC; ◇, E_2DC.

These results further support the π-π interaction of 2,4-B_2DC within the dimer.

Effect of Ionic Strength and Solvent on Dimerization. Figure 4 illustrates the ionic strength dependence of the extent of dimerization of 2,4-B_2DC in $C^2H_3O^2H$ at −63 °C. Identical results were observed when added LiCl was used instead of KCN, indicating that the result is a general ionic strength dependence. Assuming that $(\Delta H/H)^M$ and $(\Delta H/H)^D$ are unaffected by changes in ionic strength,[13] the chemical shifts in Figure 4 were used to estimate K at different ionic strengths yielding K = 7, 25, and 45 L mol^{-1} for [KCN] = 0.08, 0.17, and 0.33 M, respectively.

Figure 5 shows the methyl region of 0.035 M 2,4-B_2DEC in $C^2H_2Cl_2$ at −87 °C which demonstrates that dimerization, as monitored by the intermolecular broadening[14] of 1-CH_3, is observable ($K < 5$). The lower K in $C^2H_2Cl_2$ probably results from the lower dielectric constant as compared to methanol. Similar ionic strength and solvent dielectric constant influences have been observed for the π-π aggregation of other porphyrin systems.[15]

It was shown in part 2[5] that 2,4-B_2DEC dimerizes in an identical manner with 2,4-B_2DC, indicating that the carboxylate groups are not directly involved in the dimer interaction. However, dimerization of 0.0015 M 2,4-B_2DEC in $C^2H_3O^2H$ occurs at −70 °C to about the same degree as a 0.035 M 2,4-B_2DC solution at −50 °C, yielding an estimate of $K \simeq 600$ L mol^{-1} for 2,4-B_2DEC at −70 °C in contrast to the computed value based on the thermodynamic parameters of K = 52 L mol^{-1} for 2,4-B_2DC at the same temperature. This increase in K may be interpreted in terms of reduced electrostatic repulsion between esterified porphyrins within the dimer.

Effect of 2,4-R on Dimerization. All the evidence presented thus far supports a π-π interaction of 2,4-B_2DC. In Figure 6, the methyl region of 0.05 M 2,4-R_2DC at −70 °C shows that the 2,4-R_2DC with electron-withdrawing R exhibit similar behavior, with 8,1-CH_3 broadened at higher concentrations while 3,5-CH_3 are insignificantly broadened.[16] This indicates that all 2,4-R_2DC listed in Figure 6 with electron-withdrawing R have essentially the same solution dimer structure as determined in part 2,[5] since broadening can be observed only for methyl groups on pyrroles in contact.

The existence of additional dimer structures is dramatically exhibited for the case of 2,4-A_2DC. Upon lowering the temperature of 0.05 M 2,4-A_2DC in $C^2H_3O^2H$, 8- and 1-CH_3 exhibit broadening indicative of I-IV dimer as observed[5] for other 2,4-R_2DC with electron-withdrawing R. Unfortunately, the 1-CH_3 cannot be resolved from the solvent resonance below −40 °C and its response to continued lowering of the temperature cannot be followed. As the temperature drops below −50 °C, 3-CH_3 begins to broaden rapidly, indicating that pyrrole II becomes involved in an additional dimer interaction. At temperatures below −85 °C, 1-CH_3 can be partially resolved from the solvent and appears to have continually broadened as the temperature decreased. However, it is not possible to compare the line widths of 1- and 3-CH_3 owing to interference of the solvent with 1-CH_3. Owing to the known presence of two dimers, no attempt was made to extract an equilibrium constant.

Studies on organic aromatic π-π complexes and aromatic porphyrin complexes have shown that if the donor and/or acceptor abilities of either component in the complex are altered by introduction of electron-donating or -withdrawing groups, the stability of the complex changes.[3] Thus, it might be expected that changing the unpaired electron density in pyrroles I and IV of 2,4-R_2DC would affect K of dimerization. Figure 7 compares the concentration dependence of 1-CH_3 for 2,4-R_2DC in $C^2H_3O^2H$ at −70 °C. Both the data points (with $(\Delta H/H)^M$ normalized to zero) and the equilibrium constants resulting for the computer fit to eq 4 (solid lines) demonstrate that the extent of dimerization indeed increases as R becomes more electron withdrawing and the unpaired spin distribution becomes more asymmetric. If a π-π donor-acceptor interaction is a contributing stabilizing force of the dimer, increasing the acceptor and donor abilities of pyrroles I and IV, respectively, by increasing the asymmetry of the π system (as indicated by the spread of the methyl contact shifts), should result in increased dimer stability,[3] as observed.

When the π electron asymmetry is minimized, as with 2,4-E_2DC, for which the methyl contact shifts suggested es-

sentially fourfold symmetry,[7] the extent of dimerization should be reduced by minimizing the differential donor-acceptor properties of individual pyrroles. In agreement with this supposition, 2,4-E_2DC exhibits the smallest K (Figure 7). Moreover, the removal of these differential π donor-acceptor tendencies of the pyrroles should also eliminate the basis for the highly preferential pyrrole overlap in the dimer characterized for all other 2,4-R_2DC. The concentration-dependent methyl shifts in Figure 1B and the methyl section of the 270-MHz proton trace of 0.05 M 2,4-E_2DC in $C^2H_3O^2H$ at −70 °C, also included in Figure 1B, illustrate that all four methyl peaks exhibit comparable intermolecular shift and dipolar broadening.[17] Thus, not only is the degree of dimerization reduced for E_2DC, but the dimers present in solution must involve a more random pairing of pyrroles of the component complexes than in the complexes with larger π asymmetry.

Biological Implications. It has been postulated[2] that the stereospecific π-π interaction of the heme π system and aromatic amino acids, among others, is important in stabilizing the structure of myoglobin and holding the heme in position. Any asymmetry in the porphyrin π system can be expected to play an important role in optimizing the various contacts so as to minimize the total free energy of the apoprotein-heme interaction. The possible requirement for porphyrin electronic asymmetry is supported by the observation that replacing the natural asymmetric protoporphyrin in myoglobin with the much more symmetric mesoporphyrin, (R = ethyl) results in a greatly reduced rate of reconstitution[18] and denaturation temperature[19] compared to other porphyrins, reflecting a significant decrease in the stability of the hemoprotein.

Within an intact hemoprotein, the heme-amino acid side chain contacts induce added asymmetry, as discussed in part 1.[7] This net asymmetry within the protein may not have any functional relevance in the oxygen binding proteins,[18] but can be expected to play a critical role in directing the path of electron transfer in the various *b*- and *c*-type cytochromes.[20]

Conclusions

Our studies on the low-spin ferric biscyano complexes of natural porphyrin derivatives therefore indicate that the 2,4 substituents can induce significant asymmetry in the in-plane electronic structure. The spread of the methyl π contact shifts,[7] the dependence of the extent of dimerization on the substituent, and the stereospecific interaction between the two porphyrin complexes within the dimer[5] all point to the asymmetry originating within the π electron system. Furthermore, the invariance of the average methyl π contact shift[7] and the effect of the substituents on the extent of dimerization support the origin of the asymmetry as a rearrangement of the porphyrin π system among the pyrroles rather than any significant perturbations in the iron-porphyrin bonding.

Although thermodynamic consequences of the asymmetry can be demonstrated in the dimerization analysis, our structures do not yield any direct measure of the magnitude of the asymmetry required to produce the observed spectroscopic and thermodynamic effects. On the other hand, chemical support for asymmetry has been reported based on differential methyl group acidities in several porphyrins.[21] Further studies along these lines may provide the needed more quantitative indexes of asymmetry.

Acknowledgment. The authors are indebted to Professor R. Kosfeld for use of the 270-MHz NMR spectrometer. This work was supported by grants from the National Science Foundation, CHE-75-07788 and CHE-77-26517, and National Institutes of Health, HL-16087.

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