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Ferrous Porphyrins in Organic Solvents. I. Preparation and Coordinating Properties†

Daniel Brault* and Michel Rougee

ABSTRACT: Preparation and coordinating properties of ferrous deuteroporphyrin dimethyl ester and ferrous mesotetraphenylporphin in various organic solvents are investigated. Some methods of reduction are presented and discussed. In benzene, the hemes are proved to be free of ligands. Deuteroheme binds two pyridine molecules (K = (1.3 ± 0.2)10^8 M^{-2}) leading to the well-known hemochrome. The large negative changes of free energy, enthalpy, and entropy which accompany this reaction further support the conclusion that deuteroheme in benzene is a ligand-free monomer. In the case of ferrous mesotetraphenylporphin, successive binding of two pyridine molecules is suggested and the two affinity constants are estimated ($K_1 \simeq 1.5 \times 10^3$ M^{-1}; $K_2 \simeq 1.9 \times 10^4$ M^{-1}). Solvents such as alcohols, ethers, and substituted formamides are slightly coordinating. A thermodynamic study demonstrates that only one molecule of these solvents binds to the heme, with a low affinity (K = 1-6 M^{-1}). In these solvents, the heme can simultaneously bind two pyridine molecules with an affinity constant weaker than that obtained in benzene but similar to that reported for studies performed in water.

The prosthetic group (an iron porphyrin) of hemoproteins is located in a hydrophobic crevice of the protein (Kendrew, 1963; Perutz *et al.,* 1968; Dickerson *et al.,* 1971) and bound to one or two amino acid residues. The ability of iron porphyrins to complex with ligands has long been recognized (for a review see Falk, 1964) but the knowledge of bonds in terms of electronic or thermodynamical parameters requires further work. The hydrophobic environment of iron porphyrin in many hemoproteins has led us and others (see, for instance, Kassner, 1972, 1973; Caughey *et al.,* 1965) to think that a nonaqueous solution may be a more adequate medium for the iron porphyrin studies.

Thermodynamical studies of the binding of ligand by iron(II) porphyrins (hemes) require the preparation of bare hemes (bare heme is heme without any axial ligand). Unfortunately, these compounds are readily oxidized by air. On the other hand, dipyridinate complexes of hemes (hemochromes) are easily prepared and characterized (Alben *et al.,* 1968). They are relatively stable against oxidation. Alben *et al.* (1968) pointed out that hemochromes can lose their pyridine ligands under vacuum and prepared bare heme in solid state. In the same way, Kobayashi *et al.* (1970) and Kobayashi and Yanagawa (1972) prepared

† From the Laboratoire de Biophysique du Muséum National d'Histoire Naturelle, 75005-Paris, France. *Received September 28, 1973.*

solid mesotetraphenylporphiniron(II). They reported susceptibility measurements, Mössbauer data, and optical spectra. Whitten *et al.* (1963) prepared heme by incorporating iron into porphyrin in an inert atmosphere and give optical spectra of mesoheme in a mixture of acetic acid with various solvents. On the other hand, hydrazine hydrate, hydroquinone, and phenidone (Cole *et al.*, 1970) or bis(2,4-pentanedionato)chromium(II) (Cohen *et al.*, 1972; Collman and Reed, 1973) were used to reduce iron(III) porphyrin (hemins) to the ferrous state. None of the work, except for that of Kobayashi, suggests that bare hemes are obtained in solution. Besides, we succeeded in reducing chlorodeuterohemin dimethyl ester dissolved in inert organic solvents by means of a heterogeneous medium including an aqueous solution of sodium dithionite (Brault *et al.*, 1971).

In this paper we demonstrate that the "dithionite method" yields bare heme in benzene. We emphasize the binding of pyridine molecules by hemes and compare our results with those obtained by Cole *et al.* (1970). We also study the coordinating properties of some solvents such as tetrahydrofuran, alcohols, and substituted formamides. Hemes bind only one molecule of these solvents leading to five-coordinated compounds. In the accompanying paper (Brault and Rougee, 1974b) we present the magnetic properties and the optical absorption spectra of ferrous porphyrins and discuss them with regard to the heme coordination states. Two iron porphyrins are studied: ferrous deuteroporphyrin dimethyl ester ($DeutFe^{II}$) and ferrous mesotetraphenylporphin (Ph_4PorFe^{II}).[1]

Experimental Section

Materials. Chlorodeuterohemin dimethyl ester and chloromesotetraphenylhemin were prepared using conventional methods presented elsewhere (Caughey *et al.*, 1966; Treibs and Haberle, 1968; Brault *et al.*, 1971; Lexa, 1972). All solvents and chemicals of the purest available grade were purchased from Baker or Merck. Ethers were refluxed over sodium and then distilled. *N,N*-Dimethylformamide and *N*-methylformamide were distilled under reduced pressure in an inert atmosphere. The catalyst for hydrogen reduction, supplied by Engelhart, was made of palladium (5%) deposited on asbestos. Spectra were recorded using a Bausch and Lomb spectronic 505 or a Beckman DKU spectrophotometer.

Methods. Owing to their air instabilities, heme solutions must be handled in an inert atmosphere (argon or nitrogen). Containers were stopped by Teflon caps. Solutions were transferred from one container to another through inox steel tubes by means of excess pressure.

Heme oxidation products are characterized as oxygen-bridged dimers (Sadavisan *et al.*, 1969; Brault *et al.*, 1971; Lexa, 1972) in benzene, ethyl acetate, methylene chloride, tetrahydrofuran, *p*-dioxane, ethyl ether, and acetone. In alcohols and acetic acid, deuteroheme oxidizes as a monomer iron(III) porphyrin which is associated with the related alcoholate or acetate, respectively (Alben *et al.*, 1968; Maehly and Akeson, 1958). In any case, spectra of oxidized products are very different from those of heme, so that the amount of oxidized form at the end of each experiment may be easily deduced from optical spectra. Any experiment showing more than 5% oxidized form was disregarded.

The methods of reduction by dithionite and hydrogen have been described in a preceding paper (Brault *et al.*, 1971). In the present experiments, another method of reduction in heterogeneous phases was sometimes used. A chromous solution was prepared in an inert atmosphere by the attack of chromium with diluted acid (hydrochloric or perchloric acids). A deoxygenated benzene solution of hemin was added and stirred with the aqueous solution. Reduction occurred quickly. The organic solution was decanted and collected in inert atmosphere. In order to prevent side reaction, prolonged contact of heme with chromous solution was avoided. Reduction by lithium borohydride was achieved by adding this compound to a deaerated hemin solution.

Complexation studies, using optical absorption measurements, were carried out as follows. A benzene solution of heme was contained in optical cells (path lengths 1 and 10 mm for the Soret and visible region, respectively) which were sealed to a glass tube stoppered by a Teflon cap. A small nitrogen flow was used to prevent air contamination. The amounts of deoxygenated ligand solution were volumetrically measured by means of an air-protected syringe (Hamilton G.F.). The addition of the ligand solution produces some dilution. Accordingly, absorbances and ligand concentrations were corrected before the mathematical analysis. In many cases dilution did not exceed 10%. The temperature was regulated to 25 ± 0.1°. Equilibrium constants were determined at various temperatures from the ratio of liganded heme to bare heme at fixed ligand concentration. As far as these experiments were concerned, the heme solution was contained in an optical cell immersed in a transparent dewar filled with a thermostated liquid. Temperatures were regulated within 0.3°.

Results and Discussion

Reduction. The efficiencies of the reduction methods in the various solvents are summarized in Table I. The dithionite method is remarkably effective in nonpolar solvents which are not miscible with water such as benzene or dichloromethane. Dithionite also reduces hemin in other solvents but their water miscibility precludes its use. Reduction by chromium is as effective as the dithionite method and leads to the same reduced products.

The higher the solvent basicity, the easier is the reduction by hydrogen. This may be explained in terms of the stabilization of the produced H^+ ion. As a matter of fact, reduction by hydrogen is readily achieved in benzene or dichloromethane if water is added to the system. These reactions yield the same products as the dithionite method. Lithium borohydride is not convenient because in most cases it induces secondary reactions leading to an unidentified product.

Solution of heme in various solvents may also be obtained from a benzene solution. This one is first evaporated using a nitrogen flow and the heme residue is further dissolved in any other solvent.

This reduction method survey, which does not intend to be exhaustive, emphasizes that, for a given solvent, all efficient reduction methods yield products possessing identical spectra, which indicates that dithionite has no specific action on hemes. As this method is most convenient, it was generally used throughout this study.

Characterization of Ferrous Species in Benzene. As pointed out in a previous communication (Brault and Rougee, 1973), solutions of heme in water-saturated benzene ([water] = 3×10^{-2} M (Riddick and Bunger, 1970)) can

[1] Abbreviations used are: $DeutFe^{II}$, ferrous deuteroporphyrin dimethyl ester; Ph_4PorFe^{II}, ferrous mesotetraphenylporphin.

TABLE I: Hemin Reduction in Various Organic Solvents.[a]

	H_2/Pd	$LiBH_4$	$Na_2S_2O_4$ or $CrCl_2$–H_2O		H_2/Pd	$LiBH_4$	$Na_2S_2O_4$ or $CrCl_2$–H_2O
Benzene	0	0	+++	*p*-Dioxane	+++	||	0
Dichloromethane	0	0	+++	Ethanol	+++	||	0
Acetone	+++	+++	0	Methanol	+++	||	0
Acetic acid	++	||	0	Tetrahydrofuran	+++	||	0
Ethyl acetate	+	0	+++	*N*-Methylformamide	+	||	0
Ethyl ether	+++	||	0	*N*,*N*-Dimethylformamide	+++	+++	0
2-Propanol	+++	+++	0				

[a] Symbols used are: (0) impossible; (+) incomplete after several hours; (++) complete after several hours; (+++) easy (complete after 10 min); (||) reduction followed by a reaction leading to an unidentified product.

be evaporated under vacuum (10^{-5} Torr) at 100°, and the residue redissolved in anhydrous degassed benzene without any spectral change. Benzene, which was dried with freshly activated molecular sieves (Merck, 4 Å) according to Riddick and Bunger (1970) and degassed by a cyclic freezing–pumping procedure, is expected to contain less than 5×10^{-4} M water. The identity of spectra means that no modification of heme coordination state occurs in this water concentration range. We therefore conclude that either heme does not coordinate water molecules when it is prepared in benzene solution by means of the dithionite method, or it binds water molecules very tightly. The latter hypothesis, stating that aquoheme could be as or more stable than hemochromes which do lose their pyridine ligands under vacuum (Alben *et al.*, 1968), is very unlikely. Affinities of hemes for pyridine molecules which are reported below corroborate this view. Kobayashi and Yanagawa (1972) prepared Ph_4PorFe^{II} solutions by dissolving bare heme in distilled benzene. The water content of such solutions is also expected to be low. As a matter of fact, they reported spectra identical with ours (see Figure 4, broken line).

Benzene solutions of $DeutFe^{II}$ and Ph_4PorFe^{II} follow Beer's law from about 5×10^{-6} to 8×10^{-4} M and no spectral modifications are recorded up to these concentrations. A monomer–dimer equilibrium should produce spectral changes, especially in the Soret region (Urry and Pettegrew, 1967). Therefore, hemes in benzene solutions are either monomers or stable dimers. The last proposal is inconsistent with results presented below.

Pyridine Binding by Hemes. Spectrophotometric experiments show that $DeutFe^{II}$ and Ph_4PorFe^{II} readily coordinate pyridine molecules through a rapid and reversible reaction. The well-identified hemochrome spectra (Alben *et al.*, 1968) are ultimately observed. A careful study indicates that $DeutFe^{II}$ and Ph_4PorFe^{II} do not exactly display the same behavior and they will be studied separately.

(1) DeutFe^{II}. On the addition of pyridine to a benzene solution of $DeutFe^{II}$ well-defined isosbestic points are observed in the visible region (451, 523, 533, and 550 nm) and one is somewhat less defined in the Soret region (Figure 1). They may account for the presence of essentially two absorbing species. The reaction can be symbolized by

$$H + nL \overset{K}{\rightleftharpoons} HL_n \quad (1)$$

with the affinity constant

$$K = [HL_n]/[H]\,[L]^n \quad (2)$$

where H refers to the bare heme and L to the ligand. The affinity constant is readily calculated if the ratio $[HL_n]/[H]$ and the free ligand concentration, [L], are known. Absorbances at various stages of the reaction are given by eq 3,

$$\begin{aligned} A_0 &= \epsilon_H[P] \\ A &= \epsilon_H[H] + \epsilon_{HL_n}[HL_n] \\ A_\infty &= \epsilon_{HL_n}[P] \end{aligned} \quad (3)$$

where A_0 is the absorbance of the uncoordinated heme solution, A_∞ the absorbance of dipyridinehemochrome, and A the absorbance of a solution of mixed species. [P] is the

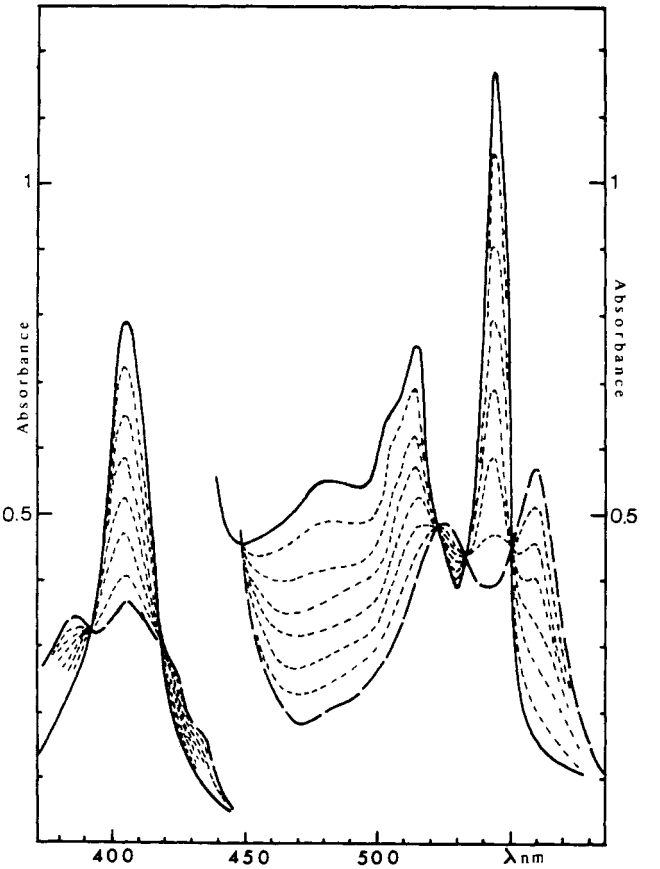

FIGURE 1: Spectral evolution upon addition of pyridine to a benzene solution of deuteroheme dimethyl ester: (– –) deuteroheme; (—) dipyridinedeuterohemochrome; (- - -) mixed species.

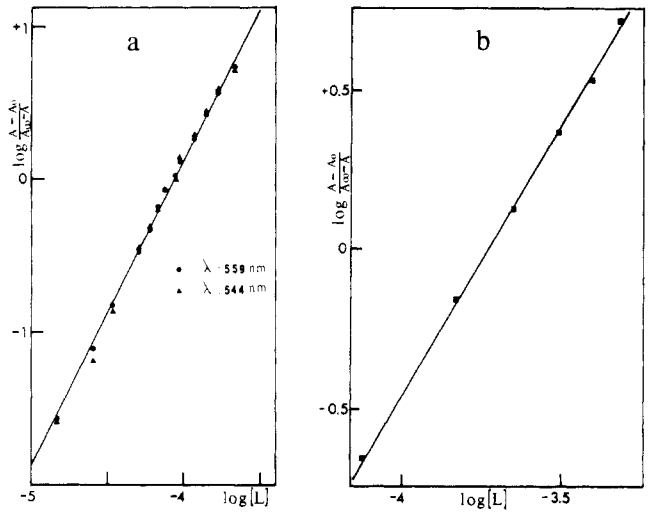

FIGURE 2: (a) Coordination of deuteroheme dimethyl ester dissolved in benzene by pyridine. Plots of log $(A - A_0)/(A_\infty - A)$ *vs.* log [L]: (●, ▲) experimental points; (—) theoretical curve calculated according to eq 6 with $K = 1.3 \times 10^8$ M^{-2} and $n = 2$. (b) Coordination of mesotetraphenylheme dissolved in benzene by pyridine. Plots of log $(A - A_0)/(A_\infty - A)$ *vs.* log [L]: (■) experimental points (λ 424 nm); (—) theoretical curve calculated according to eq 9 with $K_1 = 1.5 \times 10^3$ M^{-1}, $K_2 = 1.9 \times 10^4$ M^{-1}, and $\epsilon_{HL}/\epsilon_H = 1.5$.

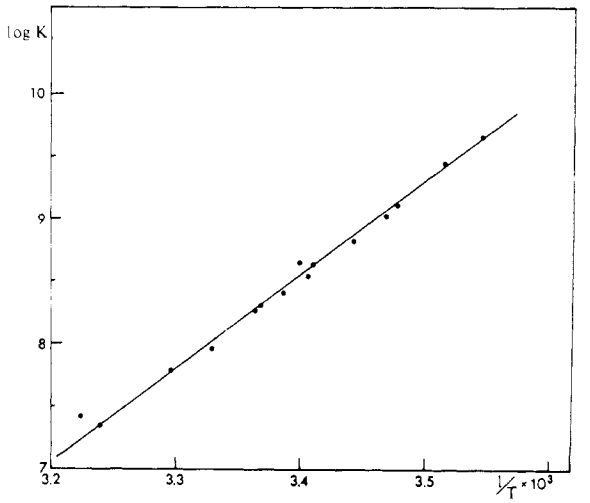

FIGURE 3: Coordination of deuteroheme dimethyl ester dissolved in benzene by pyridine. Plots of log K *vs.* $1/T$.

total porphyrin concentration. A_∞ was determined by adding one drop of pure pyridine to the studied solution. It is deduced from eq 3 that

$$[HL_n]/[H] = (A - A_0)/(A_\infty - A) \quad (4)$$

On the other hand, the free ligand concentration is given by

$$[L] = [L]_t - n[HL_n] = [L]_t - n[(A - A_0)/(A_\infty - A_0)][P] \quad (5)$$

$[L]_t$ is the total ligand concentration which is experimentally known. The final product being identified as hemochrome $n = 2$ in eq 5. In order to check the validity of the above-mentioned relationships and to determinate the affinity constant, results were presented in the form

$$\log \frac{(A - A_0)}{(A_\infty - A)} = \log K + n \log [L] \quad (6)$$

which is frequently used in porphyrin complexation studies (McLees and Caughey, 1968). The experimental plots, log $(A - A_0)/(A_\infty - A)$ *vs.* log [L], at various wavelengths in the visible or Soret region agree well with eq 6. As exemplified in Figure 2a, experimental points may be fitted by straight lines whose slopes are 2 ± 0.05. The equilibrium constant at 25° is found to be $K = (1.3 \pm 0.2) \times 10^8$ M^{-2}. The reaction stoichiometry ($n = 2$) unambiguously proves that hemes are monomers in benzene solutions. Attempts to plot results according to any other reaction scheme involving dimer or polymer (for instance, $H_2 + 4L \rightleftarrows 2HL_2$) were unsuccessful.

We have pointed out elsewhere that DeutFe^{II} may bind two imidazole molecules in two overlapping steps (Brault and Rougee, 1974a). As far as pyridine is concerned, the monopyridinated species was not detected which demonstrates (Butler, 1964) that DeutFe^{II} affinity for the second pyridine molecule is much higher (at least 50 times) than for the first one. Therefore, the reaction must be regarded as a single step reaction.

The binding of pyridine molecules by DeutFe^{II} is strongly temperature dependent. Equilibrium constants are calculated at 12 temperatures ranging from 280°K to 310°K. Below 280°K affinities are so high that free-ligand concentrations and equilibrium constants cannot be easily computed. Enthalpy change may be calculated according to Van't Hoff's equation.

$$\ln K = -\Delta H/RT + C \quad (7)$$

The experimental plot, log K *vs.* $1/T$, agrees well with eq 7 (Figure 3). ΔH is calculated according to a least-squares procedure. Entropy change is computed from

$$\Delta G = -RT \ln K = \Delta H - T\Delta S \quad (8)$$

The following values are obtained: $\Delta H = -34.5 \pm 3.0$ kcal/mol; $\Delta S = -78 \pm 10$ eu.

Heme in aqueous detergent solution binds two pyridine molecules with almost 1000 times lower affinity (Falk, 1964). If heme and pyridine molecules are strongly solvated, the heat of the reaction (or the affinity constant) may be reduced. This may be the case in the aqueous system. As pointed out by Cole *et al.* (1971), the theoretical loss of translational and rotational entropy which accompanies the attachment of the ligands to the bare heme is nearly equal to −70 eu. This theoretical value must be obtained when solvation is rather low. This may be the case depicted in benzene solutions. This also gives support to our previous finding that heme is a monomer which is free of ligand; if two water molecules were released as heme binds pyridine, the entropy change should be increased by about +33 eu (Rossotti, 1960) and it must clearly differ from the theoretical value. In the same way, entropy should be increased if dimers are involved.

Caughey *et al.* (1965) and Alben *et al.* (1968) have observed the high stabilities of dipyridinehemochromes. They pointed out that hemochrome solutions can be diluted 100 times with aerated benzene without spectral change. At lower pyridine concentrations ($1\text{–}6 \times 10^{-3}$ M) a slow oxidation is recorded (Cohen and Caughey, 1968). Moreover, when solid dipyridine-2,4-diacetyldeuterohemochrome is dissolved in degassed benzene a stable hemochromogen-type spectrum is obtained (Caughey *et al.*, 1965). In addition, nuclear magnetic resonance studies (Alben *et al.*, 1968) indicate that exchange between bound and free pyridine occurs quickly. These results, which suggest a great affinity of pyridine molecules for hemes, agree with ours.

Cole *et al.* (1970) report affinity constant, enthalpy, and entropy changes very different from ours ($K = 3.7 \times 10^2$ M^{-2}; $\Delta H = -2.4$ kcal/mol; $\Delta S = +0.4$ eu). The relatively low affinities they have reported for various ferrous porphyrins are inconsistent with the above-mentioned results of Alben and Caughey. On the other hand, Cole *et al.* (1971) report complexation studies in carbon tetrachloride and chloroform although the oxidizing properties of these solvents or other halogenated compounds have been demonstrated (Brault *et al.*, 1971; Wade and Castro, 1973). We have observed that hydrazine hydrate, which is used by Cole to reduce hemin, may coordinate heme. This fact might explain their results.

(*2*) *Ph_4PorFe^{II}.* The set of spectra obtained upon addition of pyridine to the heme solution does not show isosbestic points especially in the Soret region (Figure 4). The analysis of the absorbance evolution at several wavelengths according to eq 6 no longer gives straight lines with the expected slopes. In the case of imidazole binding by hemes a similar behavior was observed (Brault and Rougee, 1974a) and it was deduced that binding occurs in two overlapping steps. In the present study, the same conclusion must be put forward. The above-mentioned relationships must be replaced by (Brault and Rougee, 1974a)

$$H + L \overset{K_1}{\rightleftharpoons} HL; HL + L \overset{K_2}{\rightleftharpoons} HL$$

$$\frac{A - A_0}{A_\infty - A} = \frac{\left(\frac{\epsilon_{HL}}{\epsilon_H} - 1\right) K_1[L] + \left(\frac{\epsilon_{HL2}}{\epsilon_H} - 1\right) K_1K_2[L]^2}{\left(\frac{\epsilon_{HL2}}{\epsilon_H} - 1\right) + \left(\frac{\epsilon_{HL2}}{\epsilon_H} - \frac{\epsilon_{HL}}{\epsilon_H}\right) K_1[L]} \quad (9)$$

In the case of imidazole, we obtained approximate values of the affinity constants using a straightforward mathematical analysis which *a priori* required the knowledge of the intermediate species spectra, which was rationally provided by the 2-methylimidazoleheme spectra. Indeed, as pointed out by us (Brault and Rougee, 1974a) and others (Collman and Reed, 1973), only one 2-methylimidazole molecule is able to bind heme. The validity of the hypothesis and the accuracy of affinity constants were checked according to eq 9. Unfortunately, in the case of pyridine, a rational model of monopyridineheme spectra is not available, but in view of the similarity between the chemical properties of pyridine and imidazole and owing to the likeness of dipyridinehemochrome and diimidazolehemochrome spectra we feel that monopyridineheme spectra do not greatly differ from the 2-methylimidazoleheme one. Taking this idea as a guide and by means of a continual approach we find affinity constants which fit the experimental absorbance variations at best according to eq 9 (see Figure 2b). The following values are obtained: $K_1 = 1.5 \times 10^3$ M^{-1}; $K_2 = 1.9 \times 10^4$ M^{-1}; and $\epsilon_{HL}/\epsilon_H = 1.5$ ($\lambda = 424$ nm). They are thought to be accurate within 30%.

To sum up, according to this study and to a published one (Brault and Rougee, 1974a), in noncoordinating solvents, hemes may bind only one molecule of nitrogenous ligand such as pyridine or imidazole with great affinity. However, in most cases, a second ligand molecule is also bound leading to hemochromes. The extent of the five-coordinated species formation depends on both the ligand and the porphyrin. In the particular case of 2-methylimidazole, Collman and Reed (1973) and Brault and Rougee (1974a) have pointed out that repulsive interactions between the imidazole methyl substituent and the porphyrin ring hinder iron from coming into the porphyrin plane, which is a prerequisite for hemochrome formation.

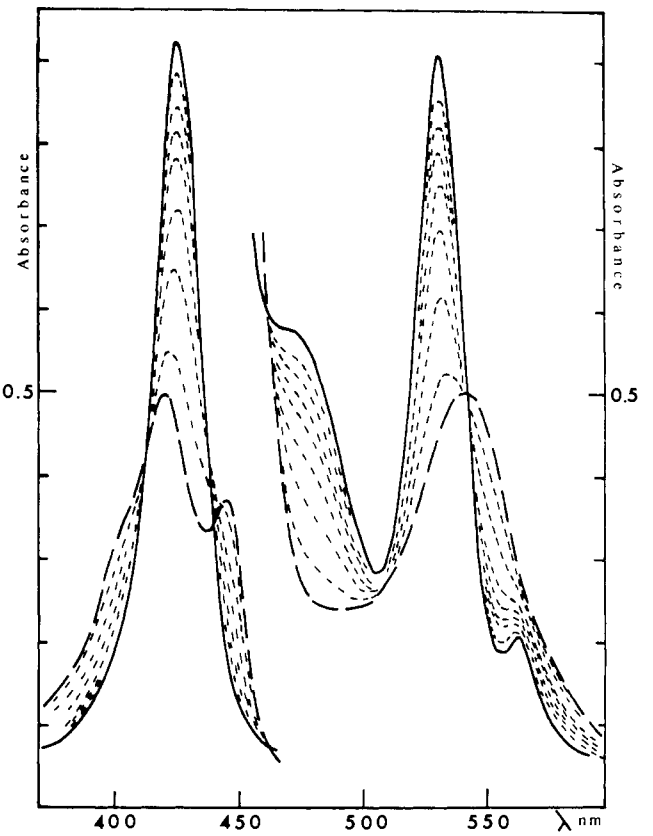

FIGURE 4: Spectral evolution upon addition of pyridine to a benzene solution of mesotetraphenylheme: (- -) mesotetraphenylheme; (—) dipyridinemesotetraphenylhemochrome; (- - -) mixed species.

Few other studies have dealt with the characterization of ferrous porphyrins coordinated by only one nitrogenous ligand. In view of their analytical results, Alben *et al.* (1968) have assumed the existence of monopyridinated species in solid state; however, a mixture of bare heme and dipyridinehemochrome may also lead to the same results. Cohen and Caughey (1968) postulated monopyridineheme as an intermediary when dipyridinehemochrome oxidizes in air. Collman and Reed (1973) have given the first unambiguous evidence for the existence of mono-2-methylimidazolemesotetraphenylheme in solid state.

Weak Ligand Binding by Hemes. We have investigated the coordinating properties of some weak ligands. Alcohols, ethers, and substituted formamides which possess free electron pairs coordinate $DeutFe^{II}$ and Ph_4PorFe^{II} in benzene solution. A typical spectral evolution is depicted in Figure 5. In this case, isosbestic points demonstrate the presence of only two species. The plot of absorbance evolution according to eq 6 shows good linearity over the whole range of ligand concentrations and gives $n = 1 \pm 0.05$, indicating that five-coordinated species are obtained. The affinity constants of the various ligands for hemes are given in Table II. No significant differences are found between $DeutFe^{II}$ and Ph_4PorFe^{II}.

Lowering the temperature favors the coordination. For each ligand the affinity constants are measured at eight temperatures ranging from about 230 to 310°K. The plot of log K *vs.* $1/T$ does not show any departure from linearity.

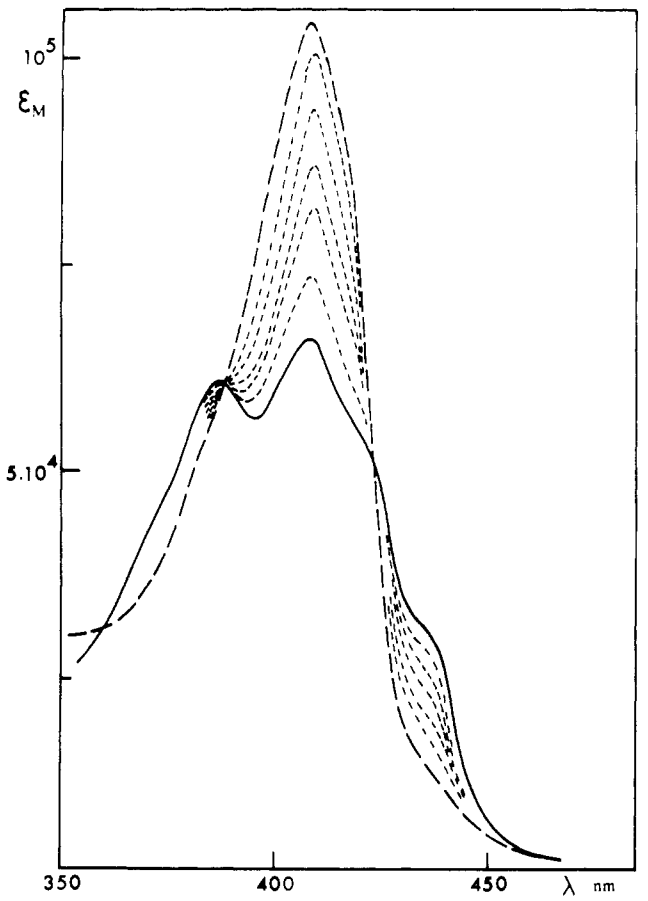

FIGURE 5: Spectral evolution on addition of ethanol to a benzene solution of deuteroheme dimethyl ester: (—) deuteroheme; (- -) deuteroheme in an ethanol-benzene (1:1) solution; (- - -) intermediate spectra.

Enthalpy and entropy changes are computed according to eq 7 and 8. Results are presented in Table II.

Some solvents such as ethyl ether have such weak coordinating properties that the pure monoliganded species is obtained at low temperature but not at the ambient temperature (Figure 6). Furthermore, we may draw the following conclusion: we expect that coordinating properties of water are similar to those of hydroxylated solvents and we suppose that coordination by water yields almost the same spectra as coordination by methanol or ethanol. Thus, the study of DeutFe^{II} spectra in water-saturated and dry ethyl acetate (Brault and Rougee, 1973) makes possible an estimation of the water affinity constant, $K = 0.1\ M^{-1}$.

Pyridine Binding by Hemes in Coordinating Solvents. DeutFe^{II} binds two pyridine molecules in slight coordinating solvents and no successive reactions can be observed. At

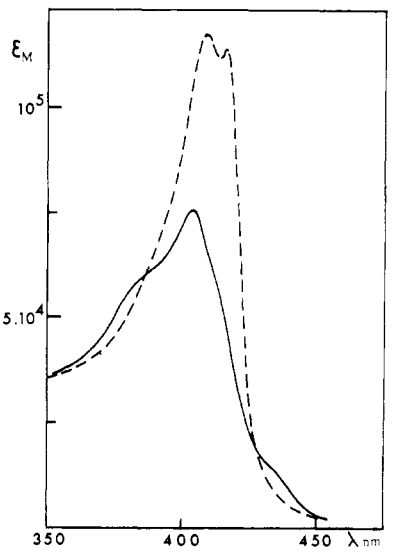

FIGURE 6: Spectra of deuteroheme dimethyl ester dissolved in ethyl ether: (—) ambient temperature; (- -) 77°K.

25°, the following affinity constants are obtained: $K = (2.0 \pm 0.2) \times 10^5\ M^{-2}$ in tetrahydrofuran, $(1.6 \pm 0.2) \times 10^5\ M^{-2}$ in ethanol, $(1.8 \pm 0.1) \times 10^5\ M^{-2}$ in *N,N*-dimethylformamide, and $(1.1 \pm 0.2) \times 10^5\ M^{-2}$ in *N*-methylformamide. These values, about 1000 times lower than the affinity constant in benzene, are similar to those found in aqueous detergent solutions (Falk, 1964).

Conclusion

Hemes have been characterized as ligand-free monomers in benzene solutions. Solvents we have studied may be classified in two groups.

(*a*) *Solvents Which Do Not Coordinate Heme (Such as Benzene).* As previously shown (Brault *et al.*, 1971) spectra of heme dissolved in dichloromethane and ethyl acetate are very similar to the spectra of heme in benzene. These solvents are therefore classified in this group. However, benzene is most convenient. Heme dissolved in deaerated dichloromethane oxidizes slowly. It was reported elsewhere (Brault *et al.*, 1971, and references therein) that chlorinated solvents (tetrachloromethane, chloroform) oxidize heme. The oxidation rate falls as the number of chlorine atoms is reduced so that a slow oxidation rate may be expected in deaerated dichloromethane. The hemin reduction in ethyl acetate by means of dithionite is not convenient because this solvent contains enough water to partially coordinate heme (Brault and Rougee, 1973). The similarity of heme spectra (Brault *et al.*, 1971; Brault and Rougee, 1973) in these three anhydrous solvents shows that the heme molecule is

TABLE II: Coordination of Hemes by Weak Ligands—Thermodynamical Parameters.

Ligand	DeutFe^{II}			Ph_4PorFe^{II},
	$K_{25°}$ (M^{-1})	ΔH (kcal/mol)	ΔS (eu)	$K_{25°}$ (M^{-1})
Ethanol	1.5 ± 0.3	−4.4 ± 0.4	−14 ± 3	2.0 ± 0.3
Methanol	2.5 ± 0.3			
Tetrahydrofuran	5.2 ± 0.3	−6.8 ± 1.0	−19 ± 4	5.8 ± 0.4
p-Dioxane	2.5 ± 0.3			4.6 ± 0.3
N,N-Dimethylformamide	3.4 ± 0.3	−5.3 ± 0.6	−15 ± 3	6.2 ± 0.3
Acetone				0.8 ± 0.2

approximately in the same environment. Thus, a parallel stacking of heme and benzene molecules must not be of importance.

(*b*) *Solvents Which Weakly Coordinate Heme Leading to Five-Coordinated Compounds.* Water might be included in this group but it is a very weak ligand. As a consequence, the low solubility of water in benzene or methylene chloride does not allow the formation of aquoheme in these solvents. This result is consistent with data on the coordination of carbon monoxide by hemes which were presented elsewhere (Rougee and Brault, 1973).

This study may allow a more adequate choice for porphyrin studies. In particular, some solvents which are found to be inert toward heme allowed us to study properties which were never observed in the solvents currently used.

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