Substituted Deuteroporphyrins. V. Structures, Stabilities, and Properties of Nickel(II) Complexes with Axial Ligands*

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ABSTRACT: Divalent nickel porphyrins in the presence of nitrogenous bases form paramagnetic species (II) which exhibit electronic spectra with absorption maxima at wavelengths markedly shifted from those found for the diamagnetic species (I) found in noncoordinating solvents. Absorption spectra in the Soret region in piperidine–chloroform solutions of nickel complexes of protoporphyrin IX and other deuteroporphyrin IX derivatives gave evidence that species II resulted from the addition of two axial piperidine ligands to a planar species I. Differences in porphyrin ring substituents such as found among heme moieties of heme proteins resulted in significant changes in both enthalpy and entropy differences for piperidine binding.

Solvent interactions, although difficult to evaluate quantitatively, appear to contribute importantly to these thermodynamic parameters. Reciprocal electronic effects of porphyrin and axial ligands upon the stability of axial ligand binding were noted; the *less* basic the porphyrin or *more* basic the axial ligand, the more stable was species II. Steric interference with axial ligand binding exerted by groups either on the periphery of the porphyrin ring or on the axial ligand was indicated. Effects of ligand binding upon the porphyrin π system was observed in electronic spectra and in nuclear magnetic resonance spectra where delocalization of positive and negative spin density led to contact shifts of peripheral ring protons. These studies provide further evidence of the sensitivity of metalloporphyrin reactions and physical properties to changes in porphyrin, ligand, and media of relevance to structure–function relationships among porphyrin proteins and enzymes.

The porphyrins of heme proteins are iron complexes of substituted deuteroporphyrins IX (Falk, 1964; Caughey *et al.*, 1966a). Studies of the sensitivity of properties of metal complexes of these porphyrins to differences in groups at the periphery of the porphyrin ring, in axial ligands, and in medium thus become important for evaluation of structure–function relationships of heme proteins (Caughey, 1967). Significant effects of peripheral substituents upon properties and reactions of iron complexes and metal-free porphyrins have been found (*cf.* Falk, 1964; Caughey, 1967; Caughey *et al.*, 1966b, 1967, 1968). Particularly striking substituent effects have been observed with nickel(II) porphyrins; in previous communications we reported on an equilibrium between two spectrally distinct species of nickel(II) porphyrins which exist in solutions containing nitrogenous bases such as pyridine, piperidine, or *n*-butylamine (Caughey *et al.*, 1962a,b, 1965a, 1966b). The relative concentrations of the two species varied markedly with changes in porphyrin structure, in the basicity of the nitrogenous base, and in temperature, but were independent of the nickel(II) porphyrin concentration. One species was considered to be a diamagnetic square-planar complex and the other species a tetragonally distorted octahedral complex which was paramagnetic as a consequence of the addition of two axial ligands. In this paper these studies on the structures, electronic and magnetic properties, reactivities, and factors which influence the relative concentrations of the two species are reported in greater detail for the nickel(II) complexes of protoporphyrin IX and related 2,4-disubstituted deuteroporphyrin IX dimethyl esters (Figure 1).

In early studies of tetraphenylporphyrins Miller and Dorough (1952) considered the binding of pyridine to several metal complexes prepared in solution without further characterization. Recently, after this work was completed, studies of substituent effects on pyridine binding to magnesium (Storm *et al.*, 1966) and nickel (Baker and Corwin, 1965) porphyrins were reported.

Experimental Section

Melting points were determined on a hot stage (Nalge–Axelrod) apparatus and are corrected. Electronic spectra were recorded with a Beckman DK-2 spectrophotometer equipped to maintain the cell

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compartment temperature to ±0.02°. Nuclear magnetic resonance spectra were obtained with Varian Associates A-60 and HA-100 spectrometers. Infrared spectra were obtained as described previously (Caughey *et al.*, 1966a).

Preparation of Protoporphyrin IX Dimethyl Ester Nickel(II). Protoporphyrin IX dimethyl ester (750 mg) was added to a solution of nickel acetate·$4H_2O$ (750 mg) in glacial acetic acid (175 ml) at 80° under nitrogen. After 12 min, the solution exhibited only a two-banded metalloporphyrin visible spectrum; 1,2-dichloroethane (50 ml) was added and the mixture was washed with water. The washed, nonaqueous phase was dried over sodium sulfate, concentrated to 20 ml, and added to a column (6 × 40 cm) of alumina (Fisher A-540) wet with 1,2-dichloroethane. Elution with 1,2-dichloroethane developed a fast minor zone followed by a slower major zone leaving at the top a dark red zone which could be eluted with methanol. Dichloroethane eluate fractions for the major and minor zones were concentrated, heated, and treated with methanol to give crystals which were dried at 100° under vacuum.

The major product of 450 mg exhibited absorption spectra in chloroform with λ_{max} (mμ) (A_{mM}): α band, 562.5 (30.9); β band, 523 (11.2); and Soret band, 401 (192).

Anal. Calcd for $C_{36}H_{36}N_4NiO_4$: C, 67.07; H, 5.55; N, 8.29. Found: C, 66.79; H, 5.60; N, 8.65.

The minor product of 50 mg exhibited band maxima in chloroform at 564, 530, and 410 mμ.

Anal. Calcd for $C_{36}H_{36}N_4NiO_4 \cdot CH_3OH$: C, 65.40; H, 5.93; N, 8.25. Found: C, 65.12; H, 5.45; N, 8.25.

Preparation of 2,4-Diacetyldeuteroporphyrin IX Dimethyl Ester Nickel(II). 2,4-Diacetyldeuteroporphyrin IX dimethyl ester (6 g) was added to a solution of nickel acetate·$4H_2O$ (6 g) in glacial acetic acid (900 ml) at 90°. The precipitate obtained on cooling was dissolved in hot chloroform (300 ml) and treated slowly with hot methanol (900 ml). The crystals obtained on cooling were dried at 100° under vacuum; yield, 6.9 g; mp 204–205°; in KBr: ν_{CO} (acetyl), 6.07 μ and ν_{CO} (ester), 5.77 μ.

Anal. Calcd for $C_{36}H_{36}N_4NiO_6$: C, 63.64; H, 5.34; N, 8.25; Ni, 8.64. Found: C, 63.79; H, 5.58; N, 8.31; Ni, 8.55.

Preparation of Mesoporphyrin IX Dimethyl Ester Nickel(II). Mesoporphyrin IX dimethyl ester (10 g) was added to a solution of nickel acetate·$4H_2O$ (10 g) in 1.5 l. of glacial acetic acid at 80°. After 10 min 1.5 l. of water at the boiling point was added slowly with stirring and the mixture was allowed to cool to room temperature. A precipitate was collected, and washed with 50% aqueous acetic acid and with water. The crude product in hot chloroform (265 ml) was treated slowly with hot methanol (795 ml). Crystals obtained on cooling were washed with methanol and dried at 50° under vacuum; yield, 10.8 g; mp 189°.

Anal. Calcd for $C_{36}H_{40}N_4NiO_4$: C, 66.37; H, 6.05; N, 8.60. Found: C, 66.23; H, 6.40; N, 8.61.

Preparation of Deuteroporphyrin IX Dimethyl Ester Nickel(II). Nickel was introduced into deuteroporphyrin IX dimethyl ester (10 g) by the method used for the mesoporphyrin ester; yield, 10.9 g; mp 202°.

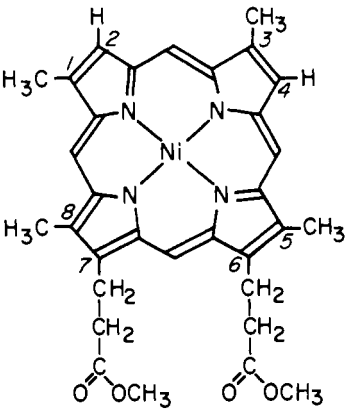

FIGURE 1: Deuteroporphyrin IX dimethyl ester nickel (II).

Anal. Calcd for $C_{32}H_{32}N_4NiO_4$: C, 64.56; H, 5.42; N, 9.41. Found: C, 64.70; H, 5.58; N, 9.51.

Preparation of 2,4-Bis(2-carboxycyclopropyl)deuteroporphyrin IX 2,4-Diethyl Ester 6,7-Dimethyl Ester Nickel(II). 2,4-Bis(2-carboxycyclopropyl)deuteroporphyrin IX 2,4-diethyl ester 6,7-dimethyl ester (4 g) was added to a solution of nickel acetate·$4H_2O$ (4 g) in glacial acetic acid (600 ml) at 80°. After 10 min 600 ml of water at the boiling point was added slowly with stirring and the mixture was allowed to cool to room temperature. A precipitate was collected on a cellulose powder cake (40 g), and washed with 50% aqueous acetic acid and with water. The crude product in hot acetone (30 ml) was treated with hot methanol (60 ml). The precipitate obtained on cooling was washed with methanol (10 ml) and dried at 35° under vacuum; yield, 3.8 g; mp 99–101°.

Anal. Calcd for $C_{44}H_{48}N_4NiO_8$: C, 64.41; H, 5.90; N, 6.83. Found: C, 64.19; H, 6.05; N, 7.02.

Preparation of 2,4-Diformyldeuteroporphyrin IX Dimethyl Ester Nickel(II). The procedure used for the preparation of the mesoporphyrin complex was followed except that 2.2 g of diformyl ester and proportionately lesser amounts of other reagents were used. Crystals (2.2 g) with mp 272–273.5° were obtained which gave elemental analyses and exhibited spectra consistent with a mixture of about equal amounts of metal-free compound and nickel complex. The reaction procedure was therefore repeated; 152 mg of the mixture and 150 mg of nickel(II) acetate·$4H_2O$ were heated in glacial acetic acid (10 ml) for 20 min at 80°. A portion (99 mg) of the product (146 mg) which exhibited a weak pink fluorescence in chloroform as well as an absorption spectrum indicative of the presence of a small amount of metal-free compound was chromatographed on silica gel (Davison 923); elution with chloroform developed two bands, one the metal-free compound and the other, the nickel complex (a minor band remained at the top of the column). The nickel

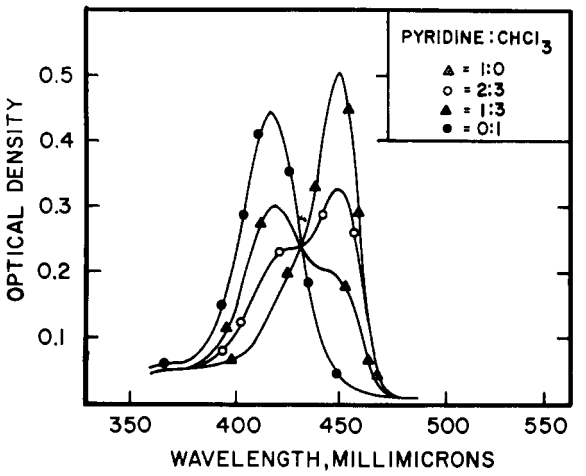

FIGURE 2: Soret region absorption spectrum of 2,4-diacetyldeuteroporphyrin IX dimethyl ester nickel(II) in pyridine, chloroform, and pyridine–chloroform mixtures.

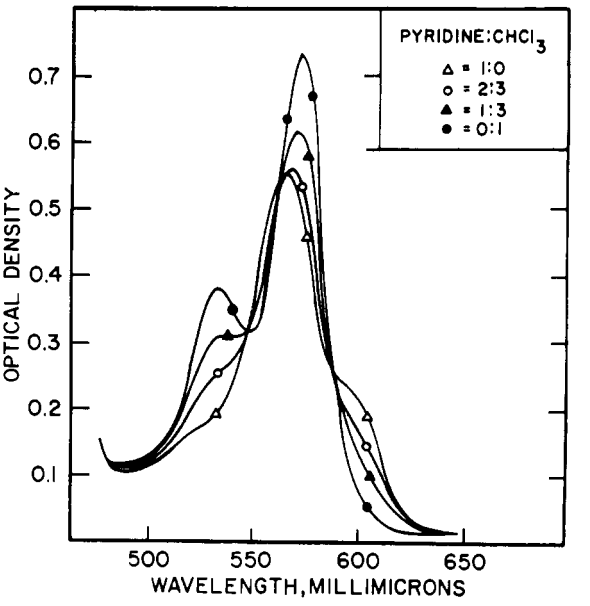

FIGURE 3: Visible region absorption spectrum of 2,4-diacetyldeuteroporphyrin IX dimethyl ester nickel(II) in pyridine, chloroform, and pyridine–chloroform mixtures.

complex was crystallized from chloroform–methanol (15–30 ml). The crystals obtained were washed with methanol (30 ml) and dried at 40° under vacuum; yield, 36 mg; mp 270°; in KBr: ν_{CO} (formyl) 6.03 μ and ν_{CO} (ester) 5.76 μ.

Anal. Calcd for $C_{34}H_{32}N_4NiO_6$: C, 62.70; H, 4.95; N, 8.60. Found: C, 62.37; H, 5.24; N, 8.57.

Other Materials. The metal-free porphyrins were prepared and characterized as described earlier (Caughey *et al.*, 1966a). Chloroform (Baker reagent) was stored over calcium oxide and distilled just before use. Absolute ethanol (0.5% by volume) was added as a preservative to the distillate collected. Piperidine (Matheson Coleman and Bell reagent grade, bp 105–107°) was refluxed over potassium hydroxide and the distillate between 106 and 107° was collected. Pyridine (Baker reagent) was combined with one-fourth volume of benzene (Baker reagent) and distilled; the distillate below 113° was discarded.

Procedure for Spectrophotometric Titrations. Titrations were carried out at 25.0 ± 0.02° by successive additions of piperidine to a 2.50-ml chloroform solution of the nickel(II) porphyrin ester in a glass-stoppered cell of 1-cm path length. Mixing was accomplished by placing a small glass bead in the cell and inverting the cell six to eight times before recording the spectrum.

Gouy Balance System. The Gouy balance used was sufficiently sensitive to measure to a precision of ±5% the susceptibilities of solutions with concentrations of unpaired electrons as low as 2×10^{-3} M. The 6-in. electromagnet (Varian V-4084), designed to provide a maximum field intensity of ~10,000 G with a 1.25-in. air gap and a field homogeneity within 1% over an area of 1-in. radius from the center of the pole pieces, received a maximum input current of 4.0 amp at 350 vdc from a power supply and current regulator (Varian Associates V-2300A and V-2301A, respectively). The current regulator was modified by the insertion of a 0–100-dc microammeter between the test point ground lug and the positive (+) side of the mercury reference cell; the microammeter was adjusted to a "null" reading in the final tuning procedure. In this manner, conditions could be achieved whereby the balance reading varied less than ±5 μg over periods of 10–15 min. The microbalance, a Mettler Model M-5GD equipped for weighing beneath the pan, was supported on a marble table weighing ~600 kg. A detachable modified Liebig condenser (inside diameter, 2.0 cm; outside diameter, 3.0 cm), with a water jacket extending 4.5 in. below and 8.0 in. above the center of the gap, served as draft shield and controlled the temperature of the cell. A magnetic shield of 0.5-in. cold-rolled steel, which surrounded the cell and draft shield assembly on three sides but left the front open for ready access, permitted the use of a short cell: the field intensity was reduced to negligible values at a distance of 8.5 cm from the center of the pole. Solutions for study were placed in a glass cell, 8 mm × 13.5 cm, which contained a volume of 4.0 ml when filled to a height of 9.5 cm. A polyethylene cap was friction fitted over the top of the cell to prevent evaporation of solvents. Adjustment of a brass hook threaded through the cap allowed control of the height of the cell, which was suspended by a strand of 0000 Dermalor monofilament nylon from the brass hook to the balance. To allow an exact reset of the magnetic field strength, a solid glass rod of the same dimensions as the cell was used as a "standard." The current was adjusted for a constant balance deflection with the standard in position; the

cell was then placed into position without interrupting the magnet current. Triple-distilled water and a gravimetrically assayed solution of $NiCl_2$ were used as magnetic susceptibility standards.

Magnetic susceptibility in Bohr Magnetons was calculated from the expression

$$\mu = 2.84\left(\frac{\Delta W_x X_s d_s M_x T}{\Delta W_s d_x}\right)^{1/2}$$

where ΔW_x represents the difference in weight displacement between solvent alone and solvent plus sample, M_x the molecular weight of the sample, d_x weight of sample per volume of solution, ΔW_s weight displacement of standard, d_s density of standard, X_s gram susceptibility of standard, and M_s molecular weight of standard.

Results

In a "noncoordinating" solvent such as chloroform, benzene, or dioxane, the nickel(II) porphyrin complexes exhibit spectra with well-defined α, β, and Soret bands (Figures 2 and 3) (Caughey *et al.*, 1965b). The spectra of these solutions did not change on exposure to heat, oxygen, or visible light for several weeks. The Soret band found in these solvents will be referred to as band I and the molecular species giving rise to this band will be referred to as species I. In the presence of a nitrogenous base such as pyridine, piperidine, or *n*-butylamine, another Soret band appeared. As such bases were added to chloroform solutions of nickel(II) complexes, band I decreased in intensity and a new Soret band (band II) emerged approximately 30 mμ to the red of band I. The intensity of band II increased with increasing concentrations of base; *e.g.*, with the 2,4-diacetyldeuteroporphyrin IX complex in pure pyridine at 25° a strong band II peak was present with band I scarcely detectable (Figure 2). The molecular species which gives rise to band II will be referred to as species II.

Wavelength and absorptivities of the Soret band maxima corresponding to species I in chloroform and to species II in piperidine for 2,4-substituted deuteroporphyrin IX dimethyl ester complexes appear in Table I. Molar absorptivity values could not be obtained directly for species II even in pure piperidine because the association constants for piperidine binding were too low (*i.e.*, a significant concentration of species I was always present). A reference compensation technique was used to calculate the absorptivities of species II (Beaven *et al.*, 1961). In this technique the nickel(II) complex in piperidine, where bands I and II were prominent, was placed in the sample cell. Known amounts of nickel(II) complex were added to produce species I alone in the reference cell, which contained only chloroform, until a difference spectrum was obtained which no longer displayed any evidence for band I. Since the total quantity of the metal complex present in the sample cell, the amount of species I in the reference cell, and the absorbance of band II, only, in the difference spectrum were all known, it was possible to calculate directly from Beer's law the molar absorptivity for species II.

TABLE I: Wavelengths and Absorptivities of Soret Band Maxima of Nickel(II) 2,4-Substituted Deuteroporphyrin IX Dimethyl Esters at 25°.

2,4-Substituent	Species I in Chloroform λ_{max} (mμ) (A_{mM})	Species II in Piperidine λ_{max} (mμ) (A_{mM})
Ethyl	392 (207)	419 (259)
Hydrogen	391 (206)	418 (228)
2-Ethoxycarbonyl-cyclopropyl	395.5 (212)	423 (224)
Vinyl	401 (192)	431 (254)
Acetyl	417.5 (132)	448 (175)
Formyl	428.5 (158)	459.5 (200)

Only nitrogenous bases have been observed to cause the formation of species II. The addition of alcohols, water, or aqueous sodium hydroxide to dimethylformamide solutions of the nickel(II) complexes did not result in the formation of species II. In fact, the addition of water (Caughey *et al.*, 1962a) or ethanol to a chloroform–piperidine solvent mixture containing the nickel(II) complexes caused a marked increase in band I at the expense of band II. Table II shows the effect of adding absolute ethanol to 2,4-diacetyldeuteroporphyrin IX dimethyl ester nickel(II) in a chloroform–piperidine solvent mixture: the addition of alcohol increased species I as species II decreased. In effect, the addition of the alcohol is spectrally equivalent to reduction of the piperidine concentration.

An increase in solvent basicity, a decrease in temperature, or a decrease in porphyrin basicity resulted in the reversible formation of species II at the expense of species I. The sensitivity of the equilibrium to the basicity of the solvent is illustrated in Figure 4. The

TABLE II: Effect of Ethanol Addition on Bands I and II of Nickel(II) Deuteroporphyrin IX Diacetyl Ester[a] in Chloroform–Piperidine[b] Solution.

Alcohol Added (μl)[c]	A_I	A_{II}
0	0.442	0.404
25	0.445	0.378
50	0.461	0.375

[a] Total concentration, 2.0×10^{-5} M. [b] Piperidine concentration, 0.2 M. [c] Total volume, 2.50 ml.

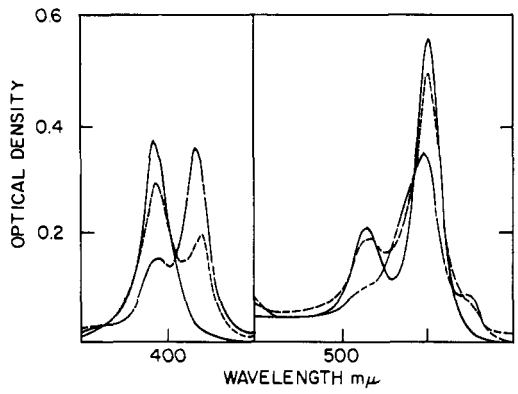

FIGURE 4: Spectra for deuteroporphyrin IX dimethyl ester nickel(II) (2×10^{-5} M) in chloroform (———), pyridine (– – – –), and *n*-butylamine (— – —). Temperature, 30°; path lengths. Soret, 1 mm; visible, 10 mm.

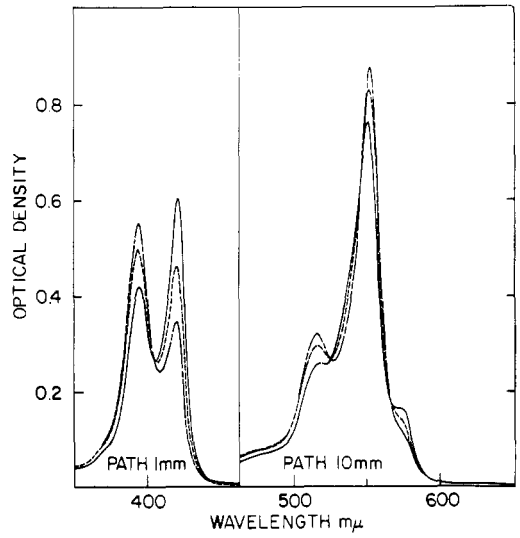

FIGURE 5: Spectra for deuteroporphyrin IX dimethyl ester nickel(II) (3.7×10^{-5} M) in pyridine at 10° (———), 20° (– – – –), and 30° (— – —).

temperature dependence of the equilibrium (Figure 5) was fully reversible; the initial spectrum was restored on bringing the solution back to the initial temperature. The sensitivity of the equilibrium to the substituents in the 2 and 4 positions is indicated by the data presented in Table III; the equilibrium favored species II and the more electron withdrawing were the 2,4-substituents (*i.e.*, the less basic the porphyrins).

In order to establish unequivocally the interaction giving rise to the equilibrium, we considered the possibility of the participation of solute–solute interactions (*i.e.*, interactions between nickel porphyrins) in the equilibrium process. The spectrophotometric measurements were generally conducted at porphyrin concentrations of approximately 10^{-6} M. However, this concentration was not critical: the position of the equilibrium remained the same over a wide range of concentrations. The invariance of the peak height ratio of band II to band I for the deuteroporphyrin IX dimethyl ester nickel(II) over a 500-fold range of concentration in pyridine is shown in Table IV. With the position of the equilibrium independent of the nickel(II) complex concentration, over this range of concentrations, it was assumed that solute–solute interactions were not of importance in these systems.

Titration Experiments in Chloroform–Piperidine Solutions. Equilibria between species I and species II were conveniently studied spectrophotometrically in chloroform–piperidine solutions. The nonprotic character of chloroform and the strong basicity of the piperidine permitted the use of small quantities of titrant (piperidine) relative to the volume of solvent (chloroform), which thereby minimized the variations in the activity coefficient of the base during the titrations. A typical set of titration curves, which illustrates the isosbestic points and spectrophotometric changes observed in the course of a titration experiment, is shown in Figure 6.

The single set of isosbestic points (Figures 2, 3, and 6), and the effects of the basicities of the solvent and porphyrin and of temperature on the position of the equilibrium were consistent with the formation of an adduct between the nickel(II) complex and the nitrogenous base (as a ligand bound to the metal ion)

TABLE III: Effect of 2,4-Substituents on the Relative Absorbance of Bands I and II of Nickel(II) Deuteroporphyrin IX Dimethyl Esters in Pyridine.[a]

2,4-Substituent	$A_{II}:A_I$	pK_3[b]
Ethyl	0.28	5.8
Hydrogen	0.62	5.5
2-Ethoxycarbonyl-cyclopropyl	0.74	4.8
Acetyl	2.63	3.4
Formyl	3.33	2.8

[a] Total concentration, 2.0×10^{-5} M; 30°. [b] Caughey *et al.* (1966b).

TABLE IV: Absorbance Ratios of Bands I and II of Nickel(II) Deuteroporphyrin IX Dimethyl Ester in Pyridine at 30° at Different Porphyrin Concentrations.

Concn (M)	Light Path (mm)	$A_{II}:A_I$
3.3×10^{-4}	0.1	0.62
3.3×10^{-5}	1.0	0.62
3.3×10^{-6}	10.0	0.625
6.5×10^{-7}	50.0	0.62

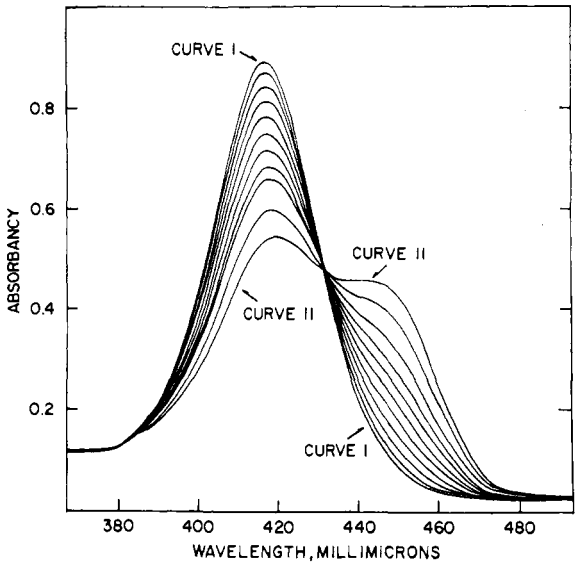

FIGURE 6: Spectra in Soret region for 2,4-bis(2-carboxycyclopropyl)deuteroporphyrin IX 2,4-diethyl ester 6,7-dimethyl ester nickel(II) in chloroform and in solutions to which varying amounts of piperidine had been added; temperature, 25°. Curve I: in chloroform. Curve II: in chloroform to which the most piperidine had been added.

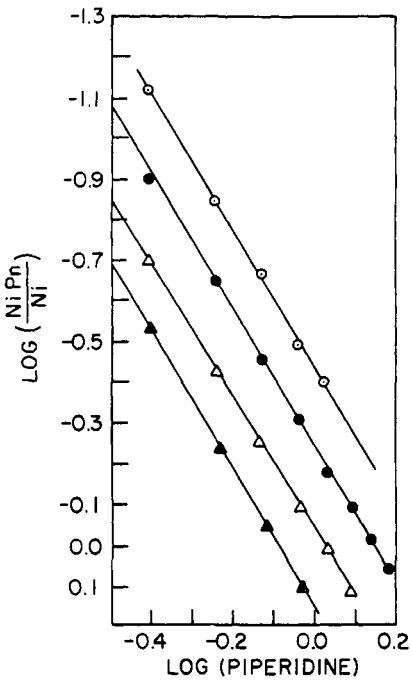

FIGURE 7: Plots of log (NiL_n/Ni) *vs.* log (piperidine) for 2,4-diacetyldeuteroporphyrin IX dimethyl ester nickel (II): 6° (▲), 15° (Δ), 25° (●), and 35° (⊙).

according to an equilibrium which may be written as (Ni) + n(L) ⇄ (NiL_n), where (Ni) represents the concentration of the nickel(II) porphyrin complex free of axial ligands, (L) is the concentration of the nitrogenous ligand, and (NiL_n) is the concentration of the adduct formed.

The concentrations of (NiL_n) and (Ni) were calculated from the spectra as the solutions to a pair of simultaneous equations to allow for the absorption of species II at λ_I and of species I at λ_{II} (Jaffe and Orchin, 1962). The equilibrium constant for the reaction may be written as

$$K = \frac{(NiL_n)}{(Ni)(L)^n} \qquad (1)$$

where the quantities in parentheses are expressed in moles per liter. The above expression may be rewritten as

$$\log \frac{(NiL_n)}{(Ni)} = n \log (L) + \log K \qquad (2)$$

If log (NiL_n)/(Ni) is plotted against log (L), a straight line with a slope of n, the number of ligands added, and an intercept of log K is obtained. Figure 7 illustrates a plot of this type for 2,4-diacetyldeuteroporphyrin IX dimethyl ester nickel(II) and illustrates the conformity of the data with eq 2. (This spectrophotometric method for the determination of the number of ligands added and the logarithm of the equilibrium constant has been successfully employed by Scheler and Fischbach (1960) and Hogness *et al.* (1937) for hemin complexes.)

Values of n and log K determined by this method are presented in Table V. The values for n all approach 2.0 and indicate that 2 moles of the ligand, piperidine, are added to the planar nickel(II) complex which exists in chloroform to form a hexacoordinated tetragonal dipiperidine complex. The titration curves were highly reproducible; a minimum of three titrations were carried out for each compound with the resulting curves virtually superimposable. The equilibrium constants are presented in Table V. The nickel(II) protoporphyrin IX and diformyldeuteroporphyrin IX complexes have affinities for piperidine at 25° which are 45 and 155 times greater, respectively, than the affinity of the nickel(II) mesoporphyrin complex for piperidine.

Plots of log K *vs.* $1/T$, shown in Figure 8, yielded enthalpy (ΔH) values. The isentropic nature of the coordination of piperidine shown in Figure 9 was evidence that the nature of the reactions did not change over the temperature range studied. The ΔH, ΔF, and ΔS data are listed in Table VI. An approximately linear enthalpy–entropy relationship is presented in Figure 10, demonstrating, in general, that the enthalpy changes are proportional to the entropy changes.

The effects of changes in porphyrin and ligand basicity were reflected in the equilibrium constants. Figure 11 presents the relationship between pK_{25}(−log

TABLE V: Log K and n for Piperidine Binding in Chloroform with Substituted Deuteroporphyrin IX Nickel(II) Complexes.

2,4-Substituent	Temp (°C)	$n \pm 0.1$	log $K \pm$ 0.02
Ethyl	15	1.8	−1.98
	25	1.8	−2.04
	35	1.8	−2.11
Hydrogen	6	2.1	−1.38
	15	2.2	−1.56
	25	2.1	−1.69
	35	2.1	−1.83
2-Ethoxycarbonyl-cyclopropyl	6	1.9	−1.49
	15	2.0	−1.61
	25	2.0	−1.70
	35	1.9	−1.81
Vinyl	6	2.0	−1.13
	15	2.1	−1.27
	25	2.0	−1.39
	35	2.0	−1.51
Acetyl	6	1.7	+0.14
	15	1.6	−0.05
	25	1.7	−0.25
	35	1.7	−0.43
Formyl	6	1.7	+0.55
	15	1.7	+0.31
	25	1.6	+0.15
	35	1.7	−0.14

K_{25}) of the nickel(II) porphyrins and pK_3 for the metal-free porphyrins. The pK_3 values refer to the equilibrium between the metal-free porphyrin and the monocation formed by the addition of a single proton to the pyrrolenine nitrogens. The pK_3 values were determined from titrations carried out in aqueous 2.5% sodium dodecyl sulfate solutions (Caughey *et al.*, 1966b). Comparative data for several amines (Table VII) illustrate not only the importance of the basicity of the ligand but also that of stereochemistry in determining the position of the equilibrium between the nickel(II) deuteroporphyrin IX dimethyl ester and its amine adduct when the nickel(II) complex is dissolved in neat ligand; the ratio of the absorptivities of bands I and II was used to give a rough indication of the position of the equilibrium under these circumstances.

Magnetic Susceptibility Measurements. Susceptibilities of solutions of 2,4-diacetyldeuteroporphyrin IX dimethyl ester nickel(II) in chloroform, pyridine, and a chloroform–pyridine mixture were determined. Porphyrin concentrations were 5.0×10^{-3} M. All measurements were carried out at 25 ± 0.1° (measurements at lower temperatures were limited by the decreased solubility of the nickel complex and at higher temperatures by the volatility of chloroform). The diamagnetic susceptibility was assumed comparable to the value found by Havemann *et al.* (1961) for protoporphyrin IX. In chloroform, the complex was diamagnetic. In pyridine, where essentially all the porphyrin exists as species II, a molar susceptibility of 4996×10^{-6} (μ_{eff} = 3.45 BM) was found. In a solvent mixture of 32% pyridine–68% chloroform the molar susceptibility found was 2404×10^{-6}, a value equivalent to a μ_{eff} of 2.40 BM which compared with a computed value of 2.51 obtained from consideration that 52% species II with μ_{eff} 3.45 BM and 48% species I (diamagnetic) were present. The relative amounts of species I and II present were determined from the absorption spectrum.

TABLE VI: Thermodynamic Parameters for Piperidine Binding in Chloroform Solutions of Substituted Deuteroporphyrin IX Nickel(II) Complexes.

2,4-Substituent	$\Delta F_{25} \pm$ 0.02 (kcal mole^{-1})	$\Delta H \pm$ 0.2 (kcal mole^{-1})	$\Delta S_{25} \pm$ 0.7 (eu)
Ethyl	2.78	−2.8	−18.8
Hydrogen	2.30	−5.5	−26.2
2-Ethoxycarbonyl-cyclopropyl	2.33	−4.3	−21.1
Vinyl	1.90	−5.2	−23.8
Acetyl	0.33	−7.8	−27.3
Formyl	−0.20	−7.8	−25.5

Nuclear Magnetic Resonance Studies. As reported earlier (Caughey and Koski, 1962; Caughey *et al.*, 1967), in deuteriochloroform the nickel(II) complexes exhibited sharp proton resonances without

TABLE VII: Relative Absorbance of Bands I and II of Deuteroporphyrin IX Dimethyl Ester Nickel(II) in Basic Solvents.[a]

Solvent	pK_b[b,c]	$A_{II}:A_I$
Piperidine	2.80	4.15
Diethylamine	2.89	0.04
2-Methylpiperidine	3.01	2.50
Triethylamine	3.19	0.02
Isopropylamine	3.28	1.80
t-Butylamine	3.47	0.20
2,6-Lutidine	6.75	0.02
Pyridine	8.64	0.70
Quinoline	9.00	0.02

[a] Concentration of porphyrin = 2.0×10^{-6} M; temperature 25°. [b] Handbook of Chemistry and Physics (1964). [c] International Critical Tables (1930).

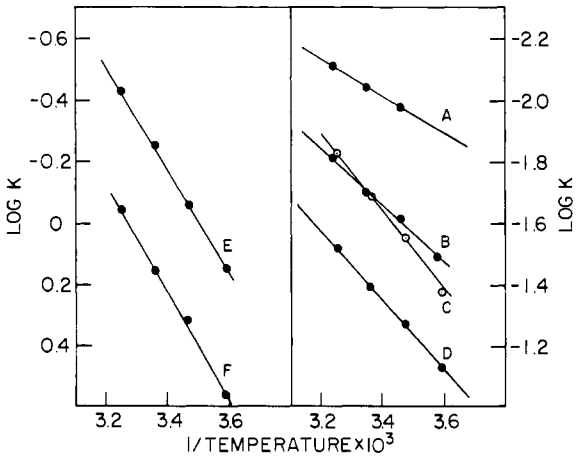

FIGURE 8: Plots of log K *vs.* $1/T$ for nickel(II) complexes of mesoporphyrin IX (A), 2,4-bis(2-carbethoxycyclopropyl)deuteroporphyrin IX (B), deuteroporphyrin IX (C), protoporphyrin IX (D), 2,4-diacetyldeuteroporphyrin IX (E), and 2,4-diformyldeuteroporphyrin IX (F) dimethyl esters.

evidence of broadening due to the presence of paramagnetic species; these nuclear magnetic resonance spectra confirmed the porphyrin structures. Upon addition of nitrogenous bases to the deuteriochloroform solutions (with formation of species II), broadening expected for the presence of paramagnetic species was observed for each of the nickel porphyrins. In solutions where a large concentration of species II was present (*e.g.*, in d_5-pyridine), the broadening was too great to permit locating the characteristic proton resonances.

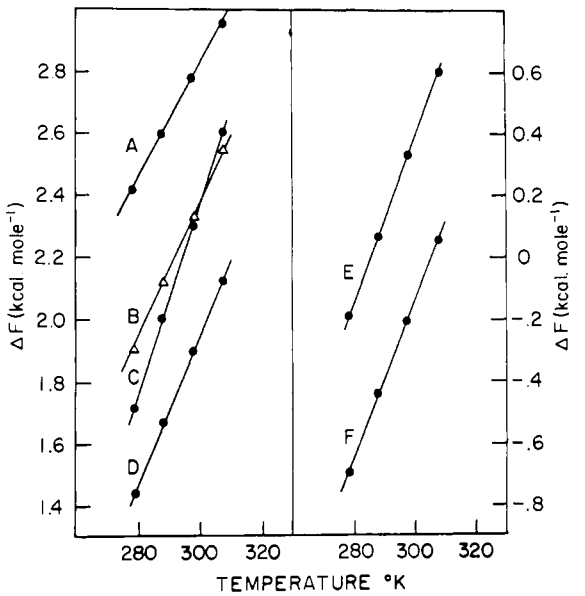

FIGURE 9: Plots of ΔF *vs.* temperature. Compounds are designated as in Figure 8.

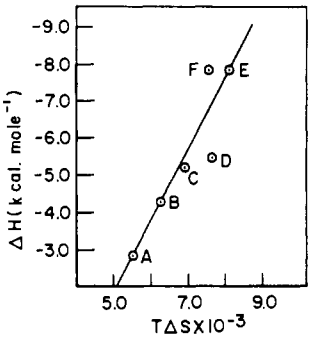

FIGURE 10: Enthalpy–entropy relationships. Compounds are as designated in Figure 8.

However, in $CDCl_3$ solutions with only small amounts of pyridine (and of species II), increases in the pyridine concentration gave increasingly marked shifts of *meso* protons (*i.e.*, α, β, γ, and δ protons of Figure 1) to high field and of protons on groups at the β positions (*i.e.*, positions 1, 2....8 of Figure 2) to low fields, as well as greater broadening of all resonances. The shifts of *meso* protons were much greater than those of 2,4 protons of the deuteroporphyrin IX derivative which in turn were much greater than those of protons on carbons of β substituents (*e.g.*, methyls). Quantitative determination of the magnitude of the shifts was deferred because of complications which arise in accurately evaluating the concentrations of species I and II and in properly handling effects of dimer formation under the conditions of the nuclear magnetic resonance experiments.

Discussion

Electronic and Steric Effects of Porphyrin Ring Substituents on Piperidine Binding in Chloroform. STRUCTURE

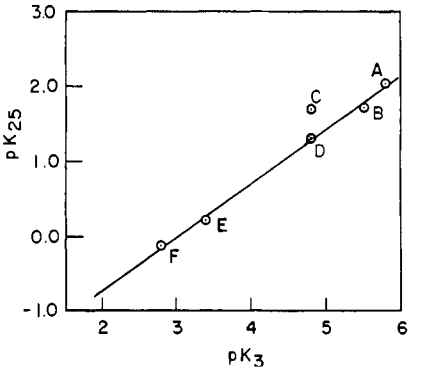

FIGURE 11: Plot of pK for piperidine binding to nickel (II) porphyrins *vs.* pK_3 values for protonation of metal-free porphyrins. Compounds are designated as in Figure 8 except here B is the deuteroporphyrin IX and C is the 2,4-bis(2-carbethoxycyclopropyl)deuteroporphyrin IX ester.

OF SPECIES II. Species II was found to have two piperidine ligands. Absorption spectra for each of the nickel(II) porphyrins in piperidine–chloroform mixtures gave evidence for two, and only two, species: species I without piperidine ligands and species II with two piperidine ligands. Computed values of n, the number of piperidine ligands bound in species II, are in Table V. In each case, n was essentially 2.

The two porphyrins which exhibited the greatest affinity of piperidine, the diacetyl and diformyl derivatives, gave n values of 1.7, slightly but significantly below 2. Effects on the equilibrium observed upon addition of water or alcohol (discussed above) suggested the most likely explanation for n values lower than 2 for these two compounds was the presence of the low concentrations of ethanol added to the chloroform as a preservative. Increased alcohol concentration resulted in low values of n without greatly affecting log K values. For example, with 1.6% ethanol present (more than threefold greater than the amount usually present), values for n and log K_{25} of 1.5 and -0.31, respectively, were computed for the diacetyl derivative compared with values of 1.7 and -0.25 reported in Table V. On the other hand, a similar experiment for the diethyl derivative with 1.6% ethanol present gave the same values of n and log K as reported in Table V. The different effects of ethanol for the two porphyrins undoubtedly is due to the much greater piperidine concentrations required for the diethyl compound than the diacetyl compound.

Values of n below 2.0 could also result from the formation of some monopiperidine complex. This possibility appeared unlikely in view of the single set of isosbestic points. It has been suggested that α,β-dinitrodeuteroporphyrin IX dimethyl ester nickel(II) may form an observable monopyridine species in pyridine–chloroform solutions (Caughey *et al.*, 1966b); two sets of isosbestic points were found for the dinitro derivative in pyridine–chloroform solutions, in contrast to the single set found for the other deuteroporphyrin derivatives studied. The dinitro derivative also had the strongest affinity for nitrogenous bases, a fact of possible relevance to the participation of a monopyridine as well as a dipyridine species in the equilibria present in pyridine–chloroform solutions. Following completion of this work, Baker *et al.* (1964) reported evidence for binding of two piperidine ligands to mesoporphyrin IX dimethyl ester nickel(II) in piperidine–tetrahydrofuran solutions.

The magnetic susceptibility data for pyridine–chloroform solutions were also consistent with assignments for species I and II as a square-planar species and a tetragonally distorted octahedral species, respectively. The absence of line broadening in the proton nuclear magnetic resonance spectra for the nickel(II) complexes in $CDCl_3$ (Caughey and Koski, 1962; Caughey *et al.*, 1967) as well as the magnetic susceptibility of the diacetyl complex in $CHCl_3$ showed species I was diamagnetic. With species II present, broadening of the proton nuclear magnetic resonance spectra was observed, and, in the case of the diacetyl complex, a μ_{eff} of 3.45 BM was determined for species II in pyridine by susceptibility measurements. A similar value for μ_{eff} was found for a chloroform–pyridine mixture. Nickel(II) in an octahedral environment usually exhibits μ_{eff} values between 2.9 and 3.4 BM; the value of 3.45 BM is thus at the high extreme which may result from $d\pi$ interactions (Lever, 1965).

The possibility that the paramagnetism of species II results from solute–solute interaction or planar-to-tetrahedral mechanisms can be excluded. The magnetic susceptibility determinations were carried out at a porphyrin concentration of 5×10^{-3} M which is within the range of concentrations where the independence of the position of the equilibrium from the total porphyrin concentration was demonstrated (Table IV). The structural requirements for the nickel atom and porphyrin nitrogens to remain essentially coplanar (Hamor *et al.*, 1965) appears to preclude a planar-to-tetrahedral conformational change as a source of the paramagnetism observed.

EFFECTS OF PORPHYRIN STRUCTURE ON THE STABILITY OF DIPIPERIDINE COMPLEXES. The effect of changes in substitutents at the 2,4 positions of the porphyrin ring upon equilibria between species I and II generally follows the relationship that the more electron withdrawing the ring substituent, the greater the formation of species II will be favored. This relationship is illustrated in Figure 11 where pK_{25} is plotted against pK_3, a measure of nitrogen basicity in the metal-free porphyrin (Caughey *et al.*, 1966b).

With small substituents located quite remote from the reaction site at the nickel atom, these effects are not expected to be due to steric factors but rather would be due to changes in metal-ligand "electron density." It was therefore of interest to note that the compound with bulky 2′-ethoxycarbonylcyclopropyl groups at the 2,4 positions (C of Figure 11) formed piperidine adducts less effectively than expected upon comparison of its pK_3 value with the other derivatives. A *cis* relationship of the porphyrin ring and the ethoxycarbonyl groups on the cyclopropyl rings (Caughey *et al.*, 1966a) makes possible steric interference of the ethoxycarbonyl groups with ligand binding at the nickel atom. Such steric interference could result from a direct interaction between a piperidine ligand and the ethoxycarbonyl group or less directly from interactions with solvent media between the two groups.

Variations in both enthalpy and entropy differences contribute significantly to the free-energy term (Table VI). Differences in interactions of solvent (chloroform) with species I and II can be expected to make important contributions. A likely interaction is hydrogen bonding between several $CHCl_3$ molecules and points on the porphyrin.

The nickel(II) porphyrins coordinate only weakly with piperidine as shown by ΔF_{25} values which ranged from 2.8 to -0.20 kcal mole^{-1}. The variation in ΔH from -2.8 kcal mole^{-1} for the mesoporphyrin to -7.8 kcal mole^{-1} for the diacetyl- and diformyldeuteroporphyrins may reflect an increase in the nickel–piperidine nitrogen bond energy as the 2,4-substituent becomes more

electron withdrawing. A reciprocal effect between nickel bonding to porphyrin nitrogens and axial ligands in the sense that the greater are the bonding interactions between porphyrin and nickel, the weaker are the interactions between nickel and axial ligand and *vice versa* is indicated. Thus porphyrins with more basic porphyrin nitrogens will be better donors to nickel with the results that the nickel will act less effectively as an acceptor from axial ligands.

The negative entropy values (Table VI) may result in large degree from a decrease in the number of freely translating particles which occur upon formation of a hexacoordinated complex. The entropy values found here overlap the values reported by Sacconi *et al.* (1958) for the coordination of piperidine and other secondary amines with nickel(II) diacetylbisbenzoylhydrazone (NiDBH); the entropy values for coordination of 4-substituted pyridines with Ni(II)DBH were approximately 20 eu greater than those found for secondary aliphatic amines. This additional entropy for the coordination of the 4-substituted pyridine with NIDBH was attributed to decreased rotational freedom due to π bonding. By similar reasoning, ΔS values would suggest π bonding is of little or no importance in the coordination of piperidine to nickel(II) porphyrins.

That enthalpy changes are nearly proportional to the entropy changes can be seen in Figure 10 and was suggested by the linear free-energy relationship of Figure 11. Most linear enthalpy–entropy relationships have positive slopes (Leffler and Grunwald, 1963); in this instance the positive slope may result from increased orientation of piperidine and solvent molecules as porphyrin substituents become more electron withdrawing and tend to increase solvation of the species II compared with species I. The deviation of the deuteroporphyrin derivative from the curve in Figure 10 and the much higher $-\Delta S$ value compared with the meso, proto, and cyclopropyl derivatives suggests different solvation effects may result from the small size of the 2,4-hydrogens of deuteroporphyrin IX compared with the larger ethyl, vinyl, and cyclopropyl groups. The conformity of the deuteroporphyrin derivative to the linear free-energy relationship is not inconsistent with such an explanation since it is well known (Leffler and Grunwald, 1963) that theories differing in the number of solvent molecules bound to solute molecules will differ appreciably in their prediction of enthalpy and entropy terms but may differ only slightly in predictions of free energies of solvation. The difference in ΔS observed for the diacetyl and diformyl derivatives may have a similar origin; the diformyl compounds are generally far less soluble in chloroform than are the diacetyl derivatives.

In contrast to iron and nickel porphyrins Storm *et al.* (1966) recently reported studies from which they concluded that magnesium porphyrins were relatively insensitive to the electron-withdrawing character of substituents at the periphery of the porphyrin ring. However, the effects of differences in the electron-withdrawing character of substituents in magnesium porphyrins upon ligand binding *per se* may be far greater than the findings of Storm *et al.* would suggest. These workers considered equilibria between monopyridine and dipyridine complexes. Therefore, increased electron withdrawal by peripheral groups could increase the strength of ligand binding in the pentacoordinated species as well as the strength of ligand binding in the hexacoordinated species and as a result not be observed in equilibria between penta- and hexacoordinated species. Dimer formation in the monopyridine species would also result in apparently diminished substituent effects; the extent of dimer formation among substituted deuteroporphyrins IX was found very sensitive to the electron-withdrawing character of peripheral substituents (York and Caughey, 1963; Caughey *et al.*, 1967).

Steric Effects of Axial Ligand Structure on Ligand Binding. The importance of structural differences in ligands which sterically alter "accessibility" for binding at the nickel atom can be readily seen in the data of Table VII. With primary amines, the presence of methyl groups on the 1-carbon greatly decreased the extent of axial binding; species II formation decreased in the order *n*-butyl, isopropyl, and *t*-butyl. With secondary amines, species II formation decreased in the order piperidine, 2-methylpiperidine, and diethylamine. The tertiary amine, triethylamine, was also ineffective as an axial ligand. Quinoline and 2,6-lutidine were also ineffective in contrast to pyridine which was far less effective than the more basic piperidine.

Effects of Ligand Binding on Properties of Nickel and Other Metal Porphyrins. ELECTRONIC SPECTRA. The origin of the marked differences in electronic spectra between species I and II has been the subject of several discussions. Particularly striking is the red shift of about 30 mμ for the Soret band maximum upon conversion of I to II. Corwin and coworkers (Corwin *et al.*, 1963; Baker *et al.*, 1964; Storm *et al.*, 1966) have proposed the Soret shift upon ligand binding arises from the stereoelectronic effect of the ligand upon the porphyrin π system; the shift to longer a wavelength of the metalloporphyrin ligand complex compared with the metalloporphyrin without axial ligand(s) was ascribed to steric interference between the ligand and the π-electron system of the porphyrin ring. Mauzerall (1965) has suggested the shift could be unrelated to ligand binding but rather could result from differences in solvent interactions with the porphyrin π system.

We consider the factor most likely to make a dominant contribution to the spectral shift to be the change in interaction between porphyrin and metal ion which results upon binding the axial ligands. The binding of nitrogen bases as axial ligands can be expected to reduce markedly the electronegativity of the nickel ion; that is, the nickel ion will serve as a less effective electron acceptor from the porphyrin nitrogens. This explanation can also be applied to ligand effects on spectra of other metal porphyrins (Caughey *et al.*, 1968). Theoretical support for such a contention can be found in theoretical models explored by Gouterman

and coworkers (Gouterman, 1959; Zerner and Gouterman, 1966a,b; Zerner *et al.*, 1966).

NUCLEAR MAGNETIC RESONANCE SPECTRA. The contact shifts, observed under solvent conditions in which small amounts of species II can be present, provide evidence for spin delocalization from the nickel atom to the periphery of the porphyrin ring. Effects of paramagnetism on nuclear magnetic resonance spectra of nickel and other metal complexes have received considerable study (Eaton and Phillips, 1965). Eaton and LaLancette (1964) have discussed contact shifts observed with an iron(III) tetraphenylporphyrin in terms of spin delocalization from the metal ion into the porphyrin π-electron system. The contact shifts observed here with nickel porphyrins where *meso* and β protons are shifted in opposite directions also suggest that the spin delocalization is taking place at least in part by a π-delocalization mechanism. It is attractive to suggest such contact shift studies may have relevance to electron-transfer processes in heme proteins (Kowalsky, 1965) although direct experimental correlation of delocalized spin densities with rates of electron-transfer processes has apparently not yet been reported.

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