Substituted Deuteroporphyrins. II. Substituent Effects on Electronic Spectra, Nitrogen Basicities, and Ligand Affinities*

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ABSTRACT: Absorption spectra in the visible and ultraviolet regions for a series of 14 2,4-substituted deuteroporphyrins, two *meso*-substituted nitrodeuteroporphyrins, and four isomeric tetramethyltetracarbethoxyporphyrins are reported. Relative electron-withdrawing capacities of substituents have been evaluated in terms of the relative basicities of the central nitrogens in organic acids and in aqueous detergent and of the affinities of nickel(II) complexes for pyridine ligands.

With the metal-free, nickel, and iron porphyrins studied, peripheral substituents which effected a red shift in absorption maxima also tended to exert an electron-withdrawing effect upon nitrogen basicity and reactions at the metal ion. However, electron-withdrawing substituents did not necessarily result in a pronounced red shift. No general relationship was found between electron-withdrawing (inductive) effects of substituents and the wavelengths and intensities of absorption maxima. However, consideration of resonance and steric effects and molecular symmetry has been useful in providing qualitative explanations of wavelength and intensity differences. These findings demonstrate precautions required in the utilization of absorption spectra to interpret heme protein structure and function.

The porphyrins of heme proteins are variously substituted deuteroporphyrins IX (Figure 1) (Falk, 1964; Caughey *et al.*, 1966a). Thus delineation of substituent effects upon the properties and reactivities of deuteroporphyrins is particularly important to the study of structure–function relationships of the heme proteins (Caughey *et al.*, 1962, 1965a, 1966b,c; Falk, 1964). In this paper substituent effects on nitrogen basicities, pyridine binding to nickel(II) porphyrins, and absorption spectra of metal-free, nickel, and iron porphyrins are considered.

Absorption spectra over the entire visible and ultraviolet regions for a series of deuteroporphyrins with systematic changes in structure are not only of theoretical interest (Gouterman, 1959, 1961; Weiss *et al.*, 1965; Caughey *et al.*, 1965b), but are also of interest for use in the characterization of unknown porphyrins and in the evaluation of effects (*i.e.*, electron withdrawal or electron donation) of peripheral substituents. Changes in peripheral substitution can result in characteristic changes in these spectra, which have multiple and fairly well-resolved absorption bands. The possibility of correlating electronic spectra of heme proteins with oxidation and spin states, redox potentials, ligand affinities, and reactivities is of obvious biochemical interest, and attempts at such correlations have been made by several workers (*e.g.*, George *et al.*, 1959; Williams, 1956; Brill and Williams, 1961; Falk, 1964; Saunders *et al.*, 1964). It has been frequently suggested that the electron-withdrawing effects of substituents can be related to the wavelengths of absorption maxima (*cf.* Falk, 1964; Saunders *et al.*, 1964). The data reported here demonstrate, as briefly reported earlier (Caughey and Fujimoto, 1963), that in fact such a relationship does *not* hold generally for the substituted deuteroporphyrins IX.

Relative nitrogen basicities for metal-free porphyrins have been established using acetic acid solutions for some compounds and chloroform–trifluoroacetic acid solutions for others, and, for several of the more basic compounds, "pK_3 values" have been determined in aqueous 2.5% sodium dodecylsulfate solutions by the method of Phillips (1960, 1963). Although these approaches have not provided thermodynamically significant equilibrium constants for protonation of neutral nitrogens of the metal-free porphyrins, they have permitted placement of a number of peripheral substituents in the order of their relative electron-withdrawing effect upon the porphyrin system. A similar order for the affinities for ligand binding to nickel porphyrins has been observed.

Experimental Section

Metal-Free Porphyrins. Preparations have been described for etioporphyrin II and the substituted deuteroporphyrins (except for the 2- (and 4-) bromo derivative which was prepared in a manner analogous to the one employed for preparation of the 2,4-dibromo compound) (Caughey *et al.*, 1966a) and also for the

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four isomeric tetramethyltetracarbethoxyporphyrins (Kleinspehn *et al.*, 1966).

2,4-Dibromodeuteroporphyrin IX Dimethyl Ester Nickel(II). A solution of nickel(II) acetate·$4H_2O$ (62 mg) in acetic acid (25 ml) at 100° was added to a solution of 2,4-dibromodeuteroporphyrin IX dimethyl ester (59 mg) in acetic acid (75 ml) under reflux. After 10 min hot water (25 ml) was added. The precipitate obtained on cooling was washed with 50% aqueous acetic acid and then with water. Recrystallization out of chloroform–methanol gave 56 mg; mp 268.5°; in $CHCl_3$, λ_{max} (A/A_α): α, 553 (1.0); β, 518 (0.35); Soret, 395 (6.1).

Anal. Calcd for $C_{32}H_{30}Br_2NiN_4O_4$: C, 51.03; H, 4.02; N, 7.44. Found: C, 51.53; H, 4.20; N, 7.03.

Chloro-2,4-dibromodeuteroporphyrin IX Dimethyl Ester Iron(III). A hot solution of 2,4-dibromodeuteroporphyrin IX dimethyl ester (302 mg) and sodium chloride (100 mg) in acetic acid (100 ml) was added to a refluxing ferrous acetate solution (acetic acid (20 ml) in which powdered iron (100 mg) had been heated for several minutes). On cooling, a precipitate was collected and washed with saturated aqueous sodium chloride, water, and methanol; yield, 313 mg; mp >360° dec.

Anal. Calcd for $C_{32}H_{30}Br_2FeClN_4O_4$: C, 48.91; H, 3.85; N, 7.13. Found: C, 49.43; H, 3.96; N, 7.30.

Dipyridine 2,4-Dibromodeuteroporphyrin IX Dimethyl Ester Iron(II). Sodium dithionite (95 mg, Eastman 90%) in water (27 ml) was added to 2,4-dibromodeuteroporphyrin IX dimethyl ester iron(III) chloride (148 mg) in pyridine (18 ml). An orange precipitate formed immediately. The reaction mixture was extracted with benzene (7 ml, four times). The benzene extract was washed four times with 50 ml of the lower phase from a mixture of benzene–pyridine–water (1:8:12, v/v). The remaining benzene (upper) phase was dried through a column (2 × 14 cm) of sodium sulfate and evaporated to dryness. The residue was heated under vacuum at 62° for 18 hr; yield, 140 mg; in benzene with 1% pyridine, λ_{max} (A/A_α): α, 547 (1.0); β, 518 (0.52); γ, 470 (0.38); Soret, 409 (4.6).

Anal. Calcd for $C_{42}H_{40}Br_2FeN_6O_4$: C, 55.42; H, 4.44; N, 9.25; Found: C, 55.87; H, 4.57; N, 9.17.

α- (and β-) Nitrodeuteroporphyrin IX Dimethyl Ester Nickel(II). To a solution of nickel acetate·$4H_2O$ (56 mg) in glacial acetic acid (7 ml) at 80° was added α- (and β-) nitrodeuteroporphyrin IX dimethyl ester (51 mg). After 13 min hot water (10 ml) was added. The precipitate obtained on cooling was washed with 50% aqueous acetic acid and then with water, dried, and crystallized out of chloroform–methanol (the product exhibited an unusually high solubility in methanol for a nickel porphyrin ester); yield, 52 mg; mp 167°; in $CHCl_3$, λ_{max} (A/A_α): α, 555 (1.0); β, 519 (0.48); Soret, 392 (7.1).

Anal. Calcd for (1) $C_{32}H_{31}O_6N_5Ni$: C, 60.02; H, 4.88; N, 10.94 and (2) $C_{32}H_{31}O_6N_5Ni{\cdot}CH_3OH$: C, 58.94; H, 5.25; N, 10.42. Found: Sample heated at 100° under vacuum for 2 hr: C, 58.85; H, 4.96; N, 10.43. Sample was recrystallized from 1-butanol and heated at 150° under vacuum for 2 hr: C, 59.64; H, 5.05; N, 11.00.

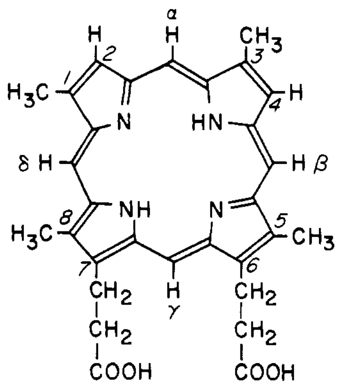

FIGURE 1: Deuteroporphyrin IX.

Chloro-α- (and β-) Nitrodeuteroporphyrin IX Dimethyl Ester Iron(III). A hot solution of α- (and β-) nitrodeuteroporphyrin IX dimethyl ester (50 mg) and sodium chloride (20 mg) in acetic acid (4 ml) was added to a refluxing ferrous acetate solution (acetic acid (5 ml) in which powdered iron (43 mg) had been heated for several minutes). After 5 min hot saturated aqueous sodium chloride was added. The precipitate obtained on cooling was washed with saturated aqueous sodium chloride and then with water; yield, 42 mg; mp >360° dec.

Anal. Calcd for $C_{32}H_{31}FeClN_5O_6$: C, 57.11; H, 4.64; N, 10.41. Found: C, 57.35; H, 4.91; N, 10.43.

Pyridine α- (and β-) Nitrodeuteroporphyrin IX Dimethyl Ester Iron(II). Sodium dithionite (25 mg) in water (4 ml) was added to chloro-α- (and β-) nitrodeuteroporphyrin IX dimethyl ester iron(III) (31 mg) in pyridine (2.5 ml). A precipitate formed immediately. The mixture was extracted with benzene (5 ml, five times). The benzene extract was washed four times with 10 ml of the lower phase from a mixture of benzene–pyridine–water (1:8:12, v/v). The remaining benzene (upper) phase was dried through a column (2 × 14 cm) of anhydrous sodium sulfate and evaporated to dryness. The residue was heated under vacuum at 62° for 15 hr; yield, 21 mg; in benzene with 1% pyridine, λ_{max} (A/A_α): α, 547 (1.0); β, 515 (0.80); Soret, 404 (5.9).

Anal. Calcd for $C_{37}H_{36}FeN_6O_6$: C, 62.02; H, 5.06. Found: C, 61.91; H, 4.93.

α,β-Dinitrodeuteroporphyrin IX Dimethyl Ester Nickel(II). A solution of nickel(II) acetate·$4H_2O$ (75 mg) in acetic acid (10 ml) at 80° was added to α,β-dinitrodeuteroporphyrin IX dimethyl ester (75 mg) also in acetic acid (300 ml) at 80°. After 10 min water (300 ml) at 95° was added slowly with stirring. The precipitate obtained on cooling was washed with 50% aqueous acetic acid and then with water. Chromatography on alumina with benzene separated a fast pink zone (nickel complex, nonfluorescent) from a slower brown zone (metal-free compound with red

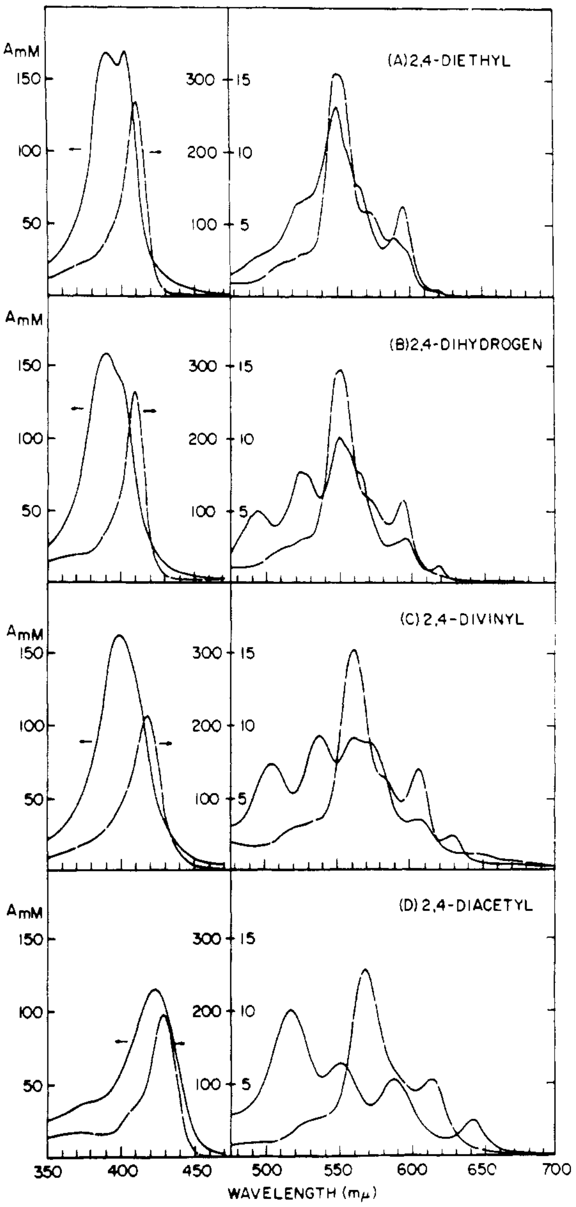

FIGURE 2: Spectra of substituted deuteroporphyrins in glacial acetic acid (solid curve) and in glacial acetic acid with 1% concentrated hydrochloric acid (broken curve). (A) Mesoporphyrin IX diethyl ester. (B) Deuteroporphyrin IX dimethyl ester. (C) Protoporphyrin IX diethyl ester. (D) 2,4-Diacetyldeuteroporphyrin IX dimethyl ester.

fluorescence). Crystals were obtained from the pink zone and recrystallized out of chloroform–methanol; yield, 29 mg; mp 229°; in $CHCl_3$, λ_{max} (A/A_α): α, 562 (1.0); β, 526 (0.51); Soret, 397 (5.9).

Anal. Calcd for $C_{32}H_{30}N_6NiO_8$: C, 56.08; H, 4.41; N, 12.26. Found: C, 56.30; H, 4.57; N, 12.47.

Acetato(?)-α,β-dinitrodeuteroporphyrin IX Dimethyl Ester Iron(III). A hot solution of α,β-dinitrodeuteroporphyrin IX dimethyl ester (76 mg) and sodium chloride (33 mg) in acetic acid (8 ml) was added to a refluxing ferrous acetate solution (acetic acid (8 ml) in which powdered iron (60 mg) had been heated for several minutes). After 3 min saturated aqueous sodium chloride at 95° was added. The precipitate obtained on cooling was washed with saturated aqueous sodium chloride, and then with water; yield, 60 mg; mp >360° dec.

Anal. Calcd for $C_{32}H_{30}FeN_6O_8\cdot CH_3COO$: C, 55.07; H, 4.49; N, 11.34. Found: C, 55.68; H, 4.63; N, 10.71.

Dipyridine α,β-Dinitrodeuteroporphyrin IX Dimethyl Ester Iron(II). Sodium dithionite (30 mg) in water (3.3 ml) was added to acetato(?)-α,β-dinitrodeuteroporphyrin IX dimethyl ester iron(III) (41 mg) in pyridine (2.2 ml); precipitation was immediate. The mixture was extracted with benzene (7 ml, four times). The benzene extract was washed repeatedly with lower phase from a mixture of benzene–pyridine–water (1:8:12, v/v). The remaining benzene (upper) phase was dried through a column (2 × 14 cm) of sodium sulfate and evaporated to dryness. The residue was heated under vacuum at 62° for 15 hr; yield, 22 mg; in benzene with 1% pyridine, λ_{max} (A/A_α): α, 549 (1.0); β, 517 (0.93); Soret, 407.5 (5.7).

Anal. Calcd for $C_{42}H_{40}FeN_8O_8$: C, 60.00; H, 4.80. Found: C, 59.70; H, 5.14.

General Methods and Materials. Electronic spectra reported in Tables I and II and Figures 6–10 were obtained with a Cary Model 11 spectrophotometer.[1] Other spectra were taken with a Beckman DK-2 spectrophotometer. Melting points were determined on a hot stage (Nalge–Axelrod) apparatus and are corrected. Chloroform (J. T. Baker reagent) contained ~0.2% ethanol and was stored over CaO and filtered just before use. Pyridine (J. T. Baker reagent) was redistilled. Alumina was Fischer activated alumina no. A-540. All the pK_3 values were obtained with the same sample of sodium dodecylsulfate. Different samples of commercial sodium dodecylsulfate were found to give slightly different pK_3 values but the relative values remained the same regardless of the sample used.

Results and Discussion

Substituent Effects on Nitrogen Basicity. The electron-withdrawing (or -donating) effects of relatively few substituent groups have been compared experimentally (Falk, 1964). Differences in nitrogen basicities (Phillips, 1963) and in the affinities of nickel(II) porphyrins for nitrogenous ligands (Caughey *et al.*, 1962) have been studied, as have reactions of iron(II) porphyrins with oxygen, carbon monoxide, and pyridine (Caughey *et al.*, 1965a; Alben and Caughey, 1966; Cohen and Caughey, 1966). Basicity differences have long been exploited for the separation and characterization of porphyrins (*e.g.*, the Willstätter HCl number) and several approaches to the determination of nitro-

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[1] A_{mM} is the millimolar absorptivity.

TABLE I: Spectral and Basicity Data for Substituted Deuteroporphyrin IX Esters.[a]

		Fischer–Stern Notation												
		I		II		III		IV		Soret		Ultraviolet		
		Platt–Gouterman Notation												
		$Q_{x(0-0)}$		$Q_{x(0-1)}$		$Q_{y(0-0)}$		$Q_{y(0-1)}$		B				
Compd	Substituents	λ (mμ)	A_{mM}	λ (mμ)	A_{mM}	λ (mμ)	A_{mM}	λ (mμ)	A_{mM}	λ (mμ)	A_{mM}	λ (mμ)	A_{mM}	pK_3 ± 0.1[b]
1	2,4-Hydrogen	619	4.1	566	6.3	530	8.3	497	14.4	399	187	267	7.4	5.5
2	2,4-Ethyl	619	4.9	566	6.5	532	9.9	498	14.0	399.5	168	270	7.9	5.8
3	2,4-(1′-Hydroxyethyl)	622	4.3	569	6.9	534	9.5	499	15.0	402	193	269	8.9	
4	2,4-(2′-Ethoxycarbonylcyclopropyl)[d]	623	5.2	569	6.9	535	10.3	500	14.9	403	188	270	9.8	4.8
5	2,4-Bromo	623	4.4	569	6.9	534	10.2	500	14.9	402	165	269	7.5	3.0
6	2,4-Acetyloxime	625	4.0	572	6.4	537	9.0	503	14.4	406	181	[c]		4.5
7	α,β-Nitro	630	3.9	577	5.9	538	5.7	507	12.5	405.5	100	272 250	7.2 8.6	
8	2,4-Vinyl	630	5.0	576	6.5	541	11.1	506	13.8	407.5	161	275	13.0	4.8
9	2,4-Cyano	635	3.9	580	6.8	546	7.8	512	15.0	415	183	305 291 259	13.2 12.2 16.4	
10	2,4-Oximino	641	4.5	585	6.5	551	11.2	513	14.2	418	156	282	20.7	4.3
11	2,4-Methoxycarbonyl	636	3.2	583	6.2	549	6.9	514	13.9	419.5	215	265	16.0	3.0
12	2,4-Propionyl	637	3.5	585	6.4	549	7.7	515	14.0	422	144	298	19.5	3.2
13	2,4-Acetyl	640	3.3	587	6.1	552	7.3	517	13.3	424.5	144	298	20.8	3.3
14	2,4-Formyl	649	3.3	594	6.0	562	7.3	526	12.5	437	134	380 306	39.0 26.0	
15	2- (and 4-) Bromo[d]	620	3.1	567	6.9	535	11.4	499	14.2	401	182	269	9.4	
16	α- (and β-) Nitro[d]	625	4.8	571	5.8	535	7.2	501	12.1	400	123	266	6.9	3.2
17	2- (and 4-) Propionyl[d]	633	1.7	576	7.2	546	11.3	508	10.9	410.5	176	[c]		4.2
18	2- (and 4-) Formyl 4- (and 2-) Vinyl[d]	642	2.1	584	9.1	559	14.8	519	10.8	420	158	308 279	19.3 16.2	3.75

[a] Spectra determined in chloroform at 30°. [b] Determined in 2.5% aqueous sodium dodecylsulfate at 25° by the method of Phillips (1960). [c] Ultraviolet region not determined. [d] Mixture of isomers, see Caughey *et al.* (1966a).

gen basicities have been taken (*cf.* Phillips, 1963; Fleischer and Webb, 1963), although no truly satisfactory method has been found for comparing porphyrins with widely variant basicities. We have found that spectra in glacial acetic acid solutions were suitable for such comparisons. All the compounds studied gave spectra typical of fully (di-) protonated species in glacial acetic acid with 1% concentrated hydrochloric acid added (Figure 2). However, differences in basicity were reflected in the spectra of these compounds in pure glacial acetic acid. The relative concentrations of neutral species remaining in glacial acetic acid indicate the order of increasing tendency to become protonated (*i.e.*, increasing basicity of central nitrogen atoms): 2,4-diacetyldeuteroporphyrin IX dimethyl ester (nearly all neutral species), protoporphyrin IX dimethyl ester, deuteroporphyrin IX dimethyl ester, and mesoporphyrin IX dimethyl ester (nearly all diprotonated species). Expressed in terms of the 2,4 substituents, the order of basicity is acetyl < vinyl < hydrogen < ethyl. Similarly, octaalkyl-substituted porphyrins such as the etioporphyrins were extensively protonated in acetic acid whereas derivatives with formyl, propionyl, cyano, or bromo groups in 2,4 positions or α- and/or β-nitro groups did not exhibit the "acid salt spectra" of protonated species. However, quantitative determination of *all* the species present can be complicated by the presence of more than two species. The presence of monoprotonated, associated, or other species in addition to neutral and fully protonated species was clearly evident in many spectra for acetic acid solutions (Figure 2A–C).

The same order of substituent effects was also found in titrations carried out in aqueous 2.5% sodium dodecylsulfate by the method of Phillips (1960, 1963), in which equilibria between neutral and "monoprotonated species" are observed spectrally as a function of pH. pK_3 values obtained in this way for several compounds (Table I) are related to the dissociation constants of "monoprotonated species." Most of the values

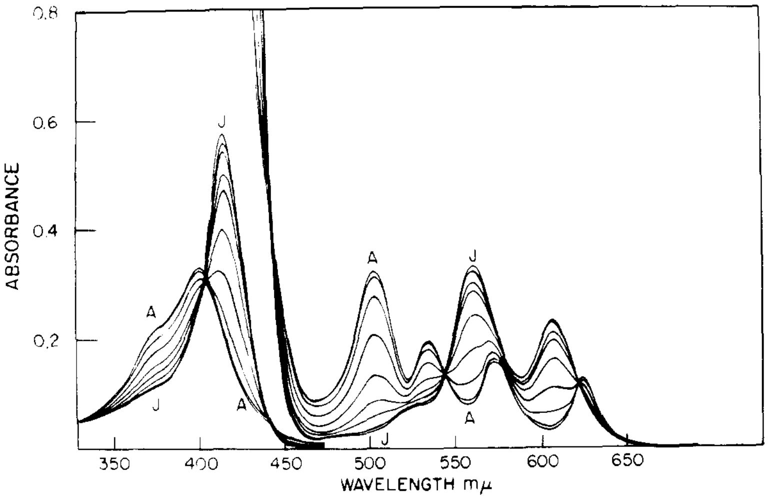


FIGURE 3: Spectral changes observed during the addition of trifluoroacetic acid to α- (and β-) nitrodeuteroporphyrin IX dimethyl ester in chloroform. Chloroform without added trifluoroacetic acid (curve A). Chloroform with the most added acid (curve J).

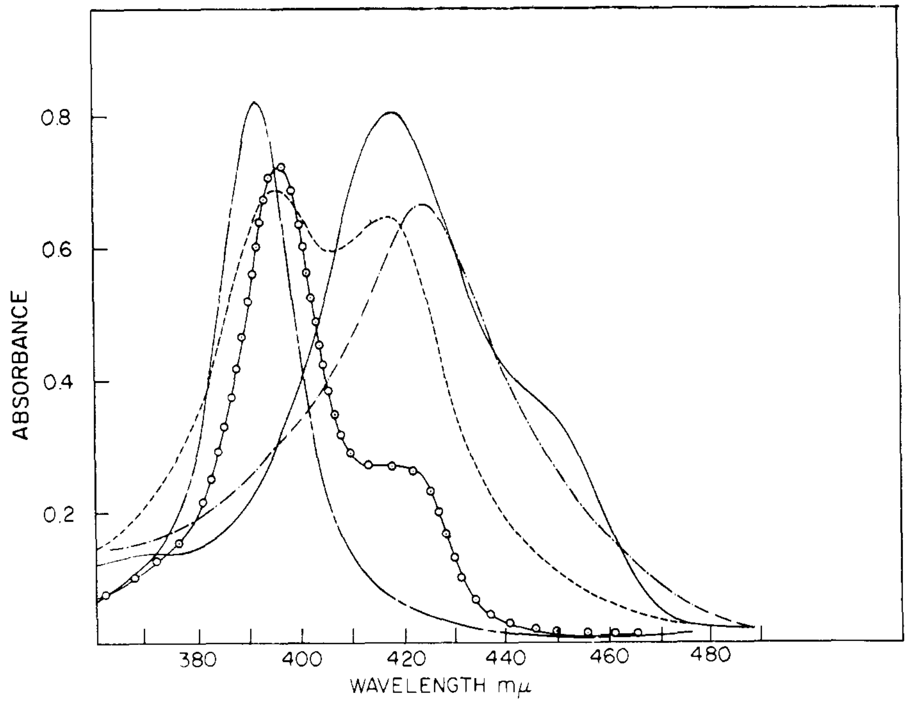


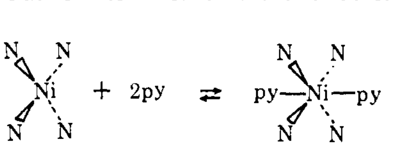
FIGURE 4: Spectra in the Soret region for dimethyl esters of nickel(II) complexes of deuteroporphyrin IX (——— – ———), 2,4-dibromodeuteroporphyrin IX (—o—o—), α- (and β-) nitrodeuteroporphyrin IX (--------), 2,4-diacetyldeuteroporphyrin IX (————), and α,β-dinitrodeuteroporphyrin IX (—·—·—·—) in chloroform–pyridine (4:1, v/v) at 30°.

were reproducible to within ±0.1 unit, but with compounds of low solubility (*e.g.*, the 2,4-dibromo and 2,4-dicyano derivatives) and of low basicity ($pK_3 \leq 3.0$) pK_3 values were less reliable because of ionic strength variations, less effective discrimination between equilibria involving neutral and protonated species, and possibly other factors (Phillips, 1960, 1963).

Although reliable pK_3 values could not be obtained for bromo and nitro derivatives, the basicities of these compounds could be compared with the diacetyl derivative in chloroform–trifluoroacetic acid solutions. The absorption spectra were followed as trifluoroacetic acid was added to chloroform solutions of the porphyrin; Figure 3 shows a typical set of spectra for the mononitro derivative. in each case only one set of isosbestic points was obtained; thus only two species (neutral and diprotonated) were detected spectrally. Equal concentrations of these two species were found with 0.0137, 0.0143, 0.018, and 0.066 M trifluoroacetic acid for the 2,4-diacetyl, 2,4-dibromo, α- (and β-) nitro, and α,β-dinitro derivatives, respectively. Porphyrin concentrations were ~2.7 × 10^{-5} M. The molarity of trifluoroacetic acid required was dependent on the concentration of ethanol (added as a preservative) in the chloroform and on the amount of water present in the acid. More acid was needed to effect 50% conversion of the neutral species to the diprotonated species if ethanol and/or water were present. Nevertheless, the *relative* concentrations of added acid required for these porphyrins remained the same for all ethanol and water concentrations studied. Thus the 2,4-bromo groups were found to decrease nitrogen basicity to an equal or greater extent than did the 2,4-acetyl groups, and nitro groups at the α,β positions decreased nitrogen basicity to a much greater extent than either the bromo or acetyl groups at the 2 and 4 positions.

These data suggest the following order of decreasing nitrogen basicity for substituents on deuteroporphyrin IX: ethyl > hydrogen > cyclopropyl ≈ vinyl > acetyl oxime > formyl oxime > acetyl > propionyl > formyl ≈ bromo ≈ methoxycarbonyl > cyano > nitro, where all are 2,4 substituents except nitro, which is α,β. (The position of the cyano group is based on a pK_3 value of 3.05 found for 2- (and 4-) cyano-4- (and 2-) vinyldeuteroporphyrin IX dimethyl ester. The ν_{CN} band found at 2212 cm^{-1} in KBr (Caughey *et al.*, 1966a) for 2,4-dicyanodeuteroporphyrin IX dimethyl ester suggests strong conjugative interactions between the cyano and porphyrin groups and compares with a frequency of 2215 cm^{-1} reported by Johnson and Oldfield (1966) for *meso*-cyanoetioporphyrin I.)

Substituent Effects on Ligand Binding. Changes in peripheral substituents have been shown to markedly affect the binding of nitrogenous ligands to nickel(II) porphyrins; thus in pyridine–chloroform solutions an equilibrium between diamagnetic square-planar complex (I) and a paramagnetic tetragonal complex (II) can be followed spectrally (Caughey *et al.*, 1962, 1965a; McLees, 1964). Species II was found to exhibit a Soret band maximum of about the same intensity, but about 30 mμ to the red of the band for species I. The relative intensities of the two Soret maxima thus served as a convenient measure of the relative concentrations of the two species present. The positions of the equilibrium shifted in favor of II as the peripheral (2,4) substituents became more electron withdrawing, *i.e.*, in the same order as decreasing basicities of the metal-free porphyrins. Studies of compounds with ethyl, hydrogen, vinyl, acetyl, or formyl groups as 2,4 substituents have now been extended to include a comparison of nickel(II) porphyrins containing bromo and nitro groups with 2,4-acetyl- and unsubstituted deuteroporphyrin IX.



I II

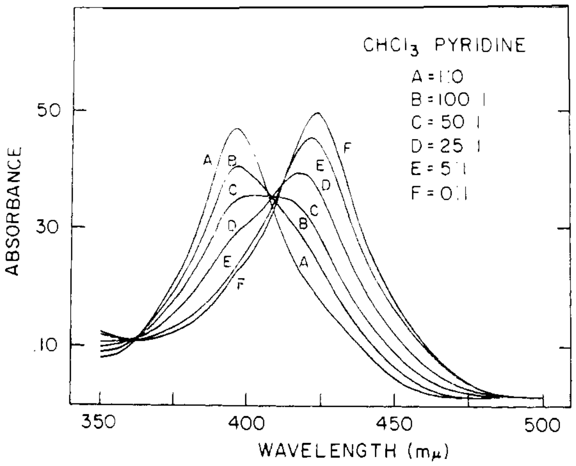


FIGURE 5: Spectra in the Soret region for nickel(II) α,β-dinitrodeuteroporphyrin IX dimethyl ester 4 × 10^{-6} M in chloroform–pyridine mixtures at 30°.

Spectra for the Soret region of several nickel(II) deuteroporphyrins, each in the same chloroform–pyridine mixture, are found in Figure 4. Under these conditions the unsubstituted deuteroporphyrin was essentially all species I (species II appears at ~422 mμ). The α,β-dinitro derivative, on the other hand, was found to be essentially all species II with only slight evidence for the presence of some species I (λ_{max} 397 mμ). With the mononitro derivative pyridine binding was less extensive than for the dinitro compound, but far greater than for either the dibromo or diacetyl compounds. The bromo and acetyl compounds exhibited about the same affinities for pyridine. Thus with these compounds as well as with the compounds studied earlier, the strengths of pyridine binding increased in parallel with a decrease in nitrogen basicity of the metal-free porphyrins.

The α,β-dinitro compound not only exhibited an extraordinarily strong affinity for pyridine but was also unusual in that pyridine–chloroform solutions exhibited spectra that failed to conform to a simple set of isosbestic points (Figure 5). The several other nickel(II) porphyrins examined have conformed to a single set of isosbestic points under such conditions, hence one possible explanation is that a monopyridine pentacoordinated nickel(II) complex participates in the equilibrium in the case of the dinitro compound where pyridine binding is strong, but that a monopyridine complex was not detected with other nickel complexes where pyridine binding was far weaker.

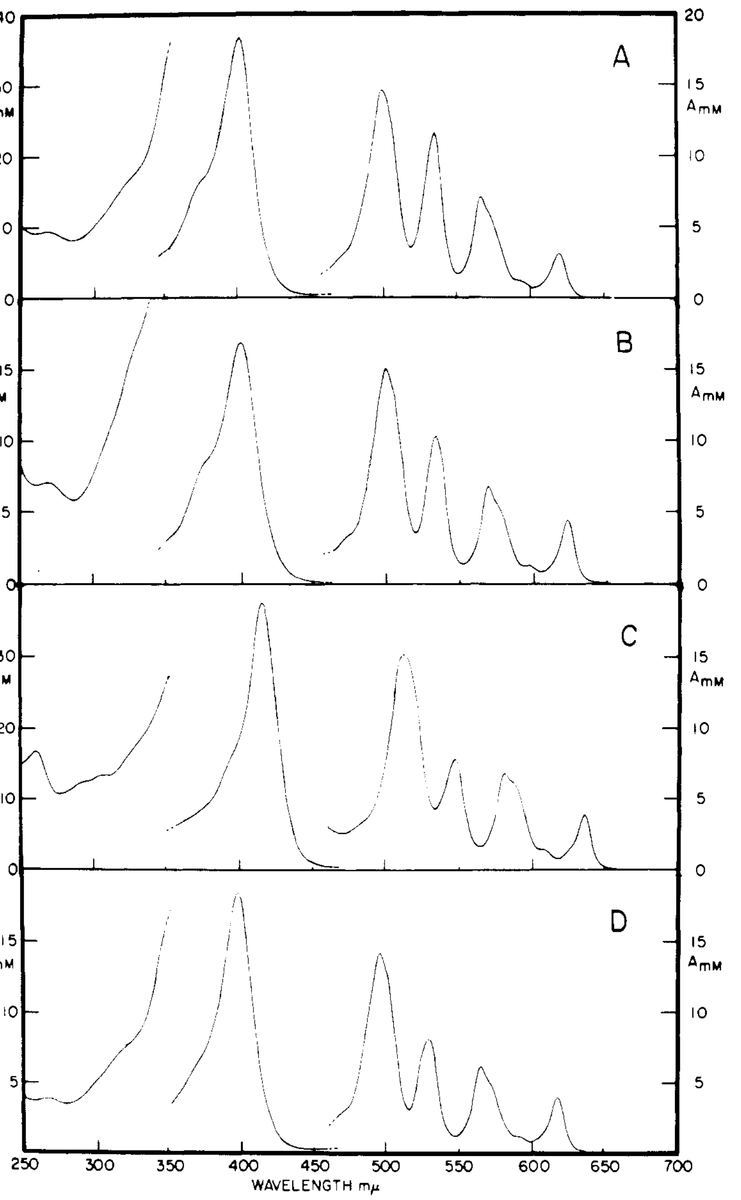


FIGURE 6: Spectra for dimethyl esters of 2- (and 4-) bromodeuteroporphyrin IX (A, **15**), 2,4-dibromodeuteroporphyrin IX (B, **5**), 2,4-dicyanodeuteroporphyrin IX (C, **9**), and deuteroporphyrin IX (D, **1**) in chloroform at 30°. A_{mM} range is 0–200 in the Soret region.

Evidence possibly consistent with the formation of pentacoordinated species with methanol as axial ligand is that the mononitro nickel(II) complex, when crystallized from chloroform–methanol, gave analyses consistent with the presence of one molecule of methanol, while methanol was not found in other nickel(II) complexes crystallized under comparable conditions. Furthermore, the mononitro compound was unusually soluble in methanolic chloroform solutions compared with other nickel(II) porphyrins studied.

Effect of Peripheral Substituents on Absorption Spectra from 700 to 250 mμ. Spectra over the visible region have been reported for many synthetic and natural porphyrins; the largest and most systematically obtained collection of visible spectra was reported by Stern and co-workers (Stern and Pruckner, 1937) for dioxane solutions of compounds from Hans Fischer's extensive collection of porphyrins. However, comparatively little data for the Soret (around 400 mμ) and farther ultraviolet regions have been reported. Wavelength and intensity data for absorption maxima between 700 and 250 mμ of porphyrins in chloroform are reported in Tables I–III. Also, full spectra for representative compounds are shown in Figures 6–10, Characteristically, four major bands in the visible region, a very intense Soret band near 400 mμ, and several bands in the ultraviolet region are found. Assignments of these bands have recently been dis-

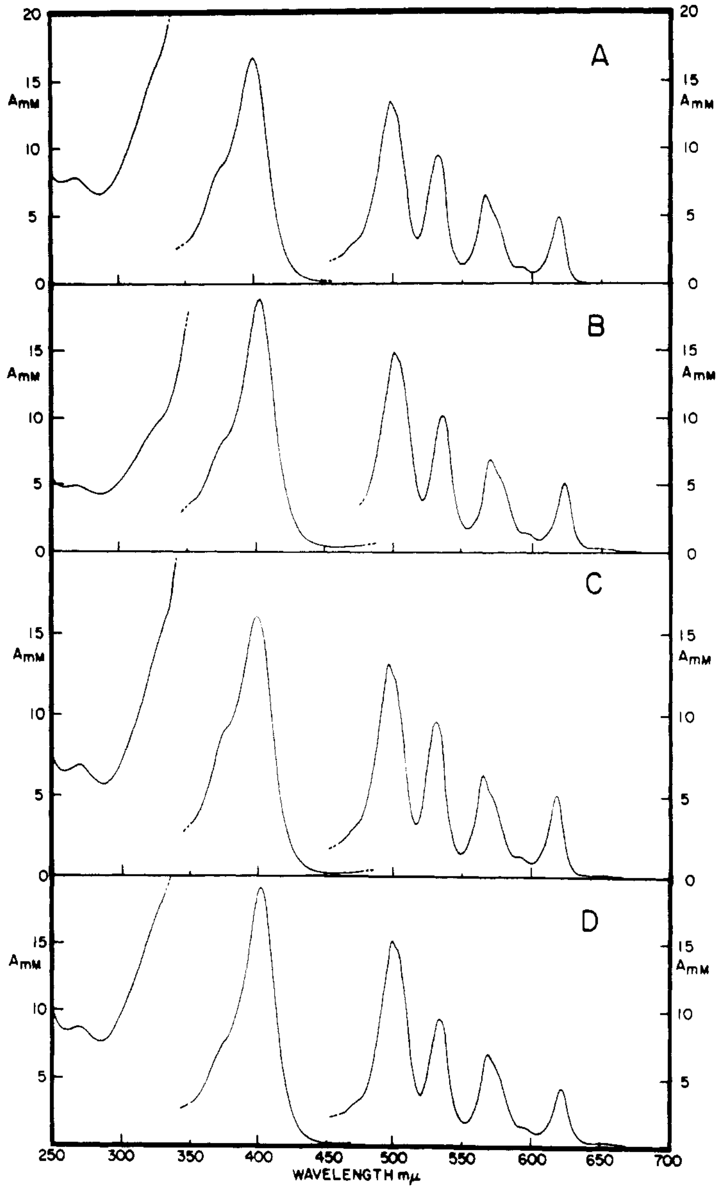


FIGURE 7: Spectra for etioporphyrin II (A, **23**) and the dimethyl esters of 2,4-bis(2′-carbethoxycyclopropyl)deuteroporphyrin IX (B, **4**), mesoprophyrin IX (C, **2**), and hematoporphyrin IX (D, **3**) in chloroform at 30°. A_{mM} range is 0–200 in the Soret region.

cussed for four of these compounds and metal derivatives (Caughey *et al.*, 1965b). Additional weak bands are frequently detectable in the visible region, *e.g.*, on the high-energy side of band IV, between bands I and II, and on the low-energy side of band I (presumably a "hot band").

The wavelengths of absorption maxima (λ_{max}) can vary markedly with changes in substituents, but the extent of these shifts is not necessarily related to differences in electron-withdrawing effects of the substituents. Although Phillips (1963) found such a relationship in one series of compounds, in which porphyrins with less basic central nitrogens had longer wavelengths of absorption maxima, it should not be expected that such a relationship will apply *generally* to porphyrins, as aromatic systems generally do not follow a simple relationship between electron-withdrawing effect and λ_{max} (Jaffe and Orchin, 1962). Figure 11 contains plots of energies for Soret, II, and I band maxima *vs.* nitrogen basicities for those compounds with computed pK_3 values. The α,β-dinitro derivative, a compound not included in Figure 11, exhibited visible and Soret λ_{max} values rather close to those for protoporphyrin but, as has been discussed, was far less basic than the divinyl compound. Thus it is necessary to invoke steric and resonance factors in explanation of spectral differences.

Electronic spectra have been found to be particularly

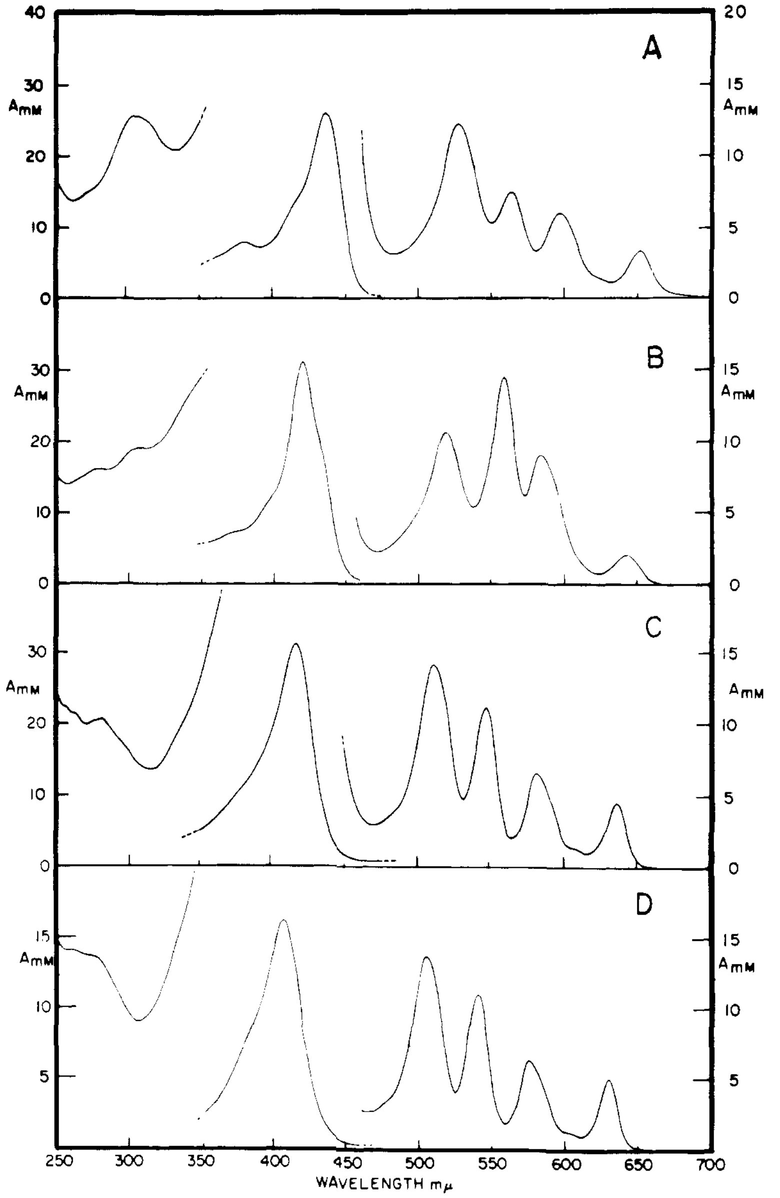


FIGURE 8: Spectra for the dimethyl esters of 2,4-diformyldeuteroporphyrin IX (A, **14**), 2- (and 4-) formyl-4- (and 2-) vinyldeuteroporphyrin IX (B, **18**), 2,4-dioximinodeuteroporphyrin IX (C, **10**), and protoporphyrin IX in chloroform at 30°. A_{mM} range is 0–200 in the Soret region.

sensitive to steric effects. Numerous attempts to correlate λ_{max} with the Hammett constants σ_I (an inductive parameter) and σ_R (a resonance parameter) have shown λ_{max} to be related more closely to σ_R than to σ_I (Jaffe and Orchin, 1962). Steric effects influence σ_R more than σ_I. Thus Essery and Schofield (1963) found the λ_{max} values for nitropyridines were more sensitive to steric effects than were nitrogen basicities. The porphyrin spectra reported here can be discussed in similar terms. The order found for shifts in the visible and Soret λ_{max} values from red to blue, but not the order for nitrogen basicities, was formyl, acetyl, propionyl, and methoxycarbonyl. These groups can be considered as substituted carbonyls with hydrogen, methyl, ethyl, and methoxy substituents, respectively. The order is the expected order of increasing steric difficulty for the carbonyl group to obtain maximum π overlap or conjugation with the porphyrin ring system. Thus σ_R would be affected by steric factors to a much larger extent than σ_I. Similarly the oxime of the diformyl derivative exhibited λ_{max} values markedly to the red of those of the diacetyl oxime, although the pK_3 values were but slightly different, 4.3 and 4.5, respectively. However, when comparing effects of the formyl and acetyl groups care must be taken not to overemphasize steric interactions of the acetyl group; in the crystal one of the acetyl groups of 2,4-diacetyldeuteroporphyrin IX dimethyl ester nickel(II) was

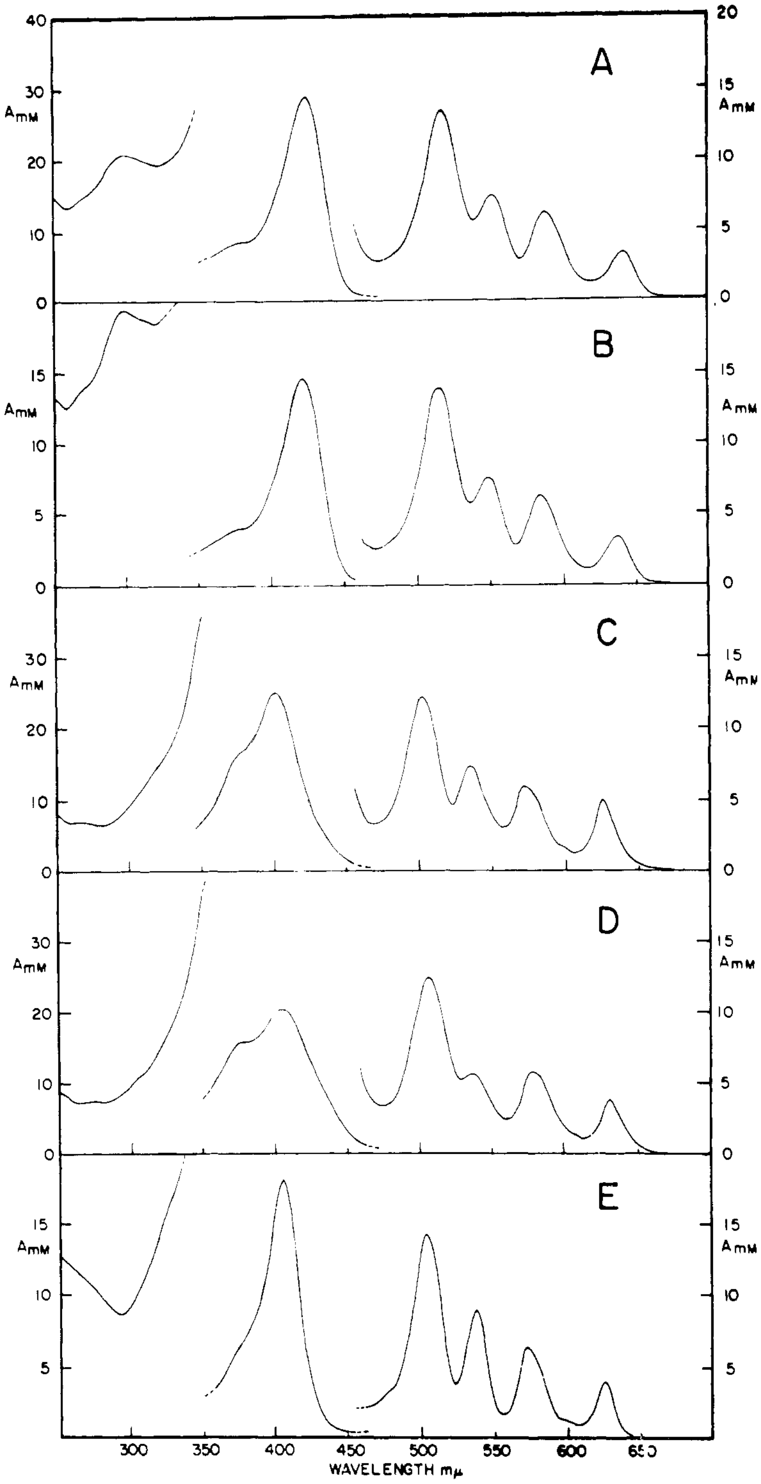


FIGURE 9: Spectra for the dimethyl esters of 2,4-diacetyldeuteroporphyrin IX (A, **13**), 2,4-dipropionyldeuteroporphyrin IX (B, **12**), α- (and β-) nitrodeuteroporphyrin IX (C, **16**), α,β-dinitrodeuteroporphyrin IX (D, **7**), and 2,4-bis(acetyl oxime)deuteroporphyrin IX (E, **6**), in chloroform at 30°. A_{mM} range is 0–200 in the Soret region.

found essentially coplanar with the porphyrin ring (Hamor *et al.*, 1965).

Steric factors could also have an important contribution to λ_{max} differences found among the isomeric tetramethyltetracarbethoxyporphyrins (Figure 12), although steric arguments are perhaps less convincing for these compounds than for the carbonyl-substituted deuteroporphyrins (the relative positions for *all* the

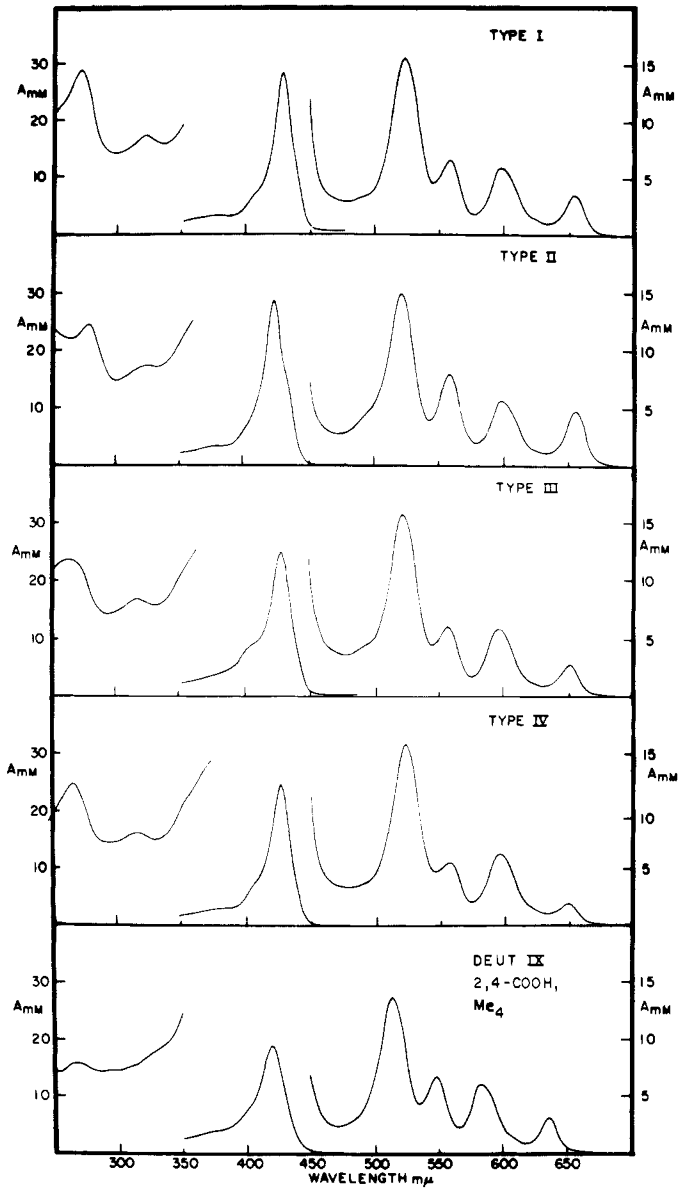


FIGURE 10: Spectra for tetramethyltetracarbethoxyporphyrins of types I–IV and 2,4-bis(methoxycarbonyl)deuteroporphyrin IX dimethyl ester (11) in chloroform at 30°. A_{mM} range is 0–400 in the Soret region.

bands certainly cannot be explained in this way). Soret λ_{max} values were shifted from red to blue in the order I, IV, III, II (Table II). In II, with carbethoxy groups at the 2,3 and the 6,7 positions, the greatest interference between carbethoxy groups might be expected, whereas in I, with carbethoxy groups at positions 2, 4, 6, and 8, there could be no such interference. Isomers III and IV each have one pair of carbethoxy groups that could sterically interact to prevent maximal conjugation between carbonyls and the porphyrin ring. A further example of steric deviation from a λ_{max} – pK_3 relationship was found for the divinyl and bis(2′-ethoxycarbonylcyclopropyl) compounds; each had a pK_3 value of 4.8, yet the derivative with bulky cyclopropyl groups exhibited λ_{max} values shifted markedly to the blue compared with those for the divinyl compound. Similarly, the relatively small red shifts observed upon introduction of nitro groups into α,β positions, despite a marked decrease in nitrogen basicity, can be ascribed to unusually small conjugative interaction between the porphyrin and nitro groups, which do not achieve coplanarity for steric reasons (Bonnett and Stephenson, 1965; Johnson and Oldfield, 1965). Here, as in the case of the nitropyridines (Essery and Schofield, 1963), λ_{max} values are far more sensitive than are basicities to steric interference with coplanarity of aromatic ring and nitro groups. Steric effects upon the mesomeric interactions of nitro and

TABLE II: Spectral Data for Isomeric Tetramethyltetracarbethoxyporphyrins and Etioporphyrin II in Chloroform at 30°.

		Fischer-Stern Notation													
		I		II		III		IV		Soret		Ultraviolet			
		Platt-Gouterman Notation													
		$Q_{x(0-0)}$		$Q_{x(0-1)}$		$Q_{y(0-0)}$		$Q_{y(0-1)}$		B		N		L(?)	
Compd	Porphyrin	λ (mμ)	A_{mM}	λ (mμ)	A_{mM}	λ (mμ)	A_{mM}	λ (mμ)	A_{mM}	λ (mμ)	A_{mM}	λ (mμ)	A_{mM}	λ (mμ)	A_{mM}
19	TTP-I[a]	654	3.5	598	5.8	558	6.5	522	15.5	428	284	322	17.1	271	29.0
20	TTP-II[a]	654	4.8	598	5.6	557	7.9	520	14.9	423	287	326	17.3	279	24.5
21	TTP-III[a]	651	2.8	595	5.7	556	5.9	521	15.5	426.5	251	318	16.7	265	23.4
22	TTP-IV[a]	648	1.8	595	5.7	556	5.2	523	15.6	427.5	247	317	16.2	266	24.6
23	Etioporphyrin II	619	5.0	566	6.4	532	9.6	497	13.2	398.5	157			270	7.1

[a] TTP represents tetramethyltetracarbethoxyporphyrin. I, II, III, and IV designate isomer types (see Figure 12).

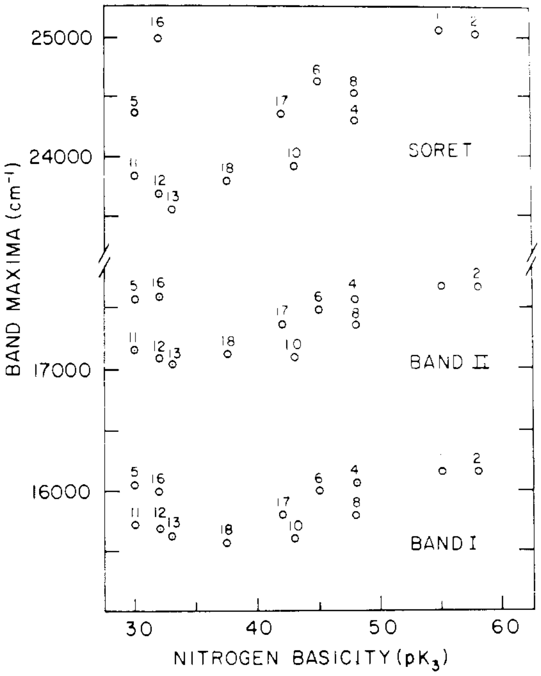


FIGURE 11: Plots of energies of absorption maxima *vs.* nitrogen basicities in terms of pK_3 values. Numbers designate the compounds of Table I.

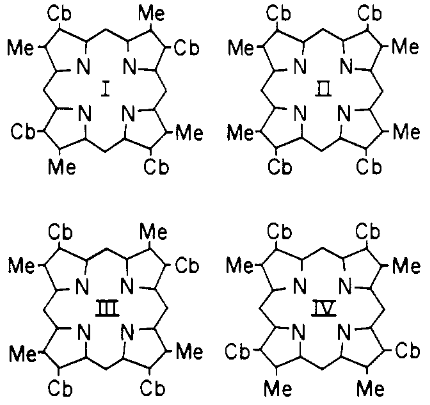


FIGURE 12: Positions of substituents in isomeric tetramethyltetracarbethoxyporphyrins. Me = methyl. Cb = carbethoxy ($C(=O)CH_2CH_3$).

several other *meso* substituents on etioporphyrin I were recently considered by Johnson and Oldfield (1966).

Other discrepancies in λ_{max} − pK_3 correlations can be ascribed solely to differences in reasonance effects; for example, replacement of 2,4-hydrogens of deuteroporphyrin IX by bromo groups resulted in red shifts of only 3–4 mμ whereas replacement by acetyl groups caused red shifts of 20–25 mμ. Both bromo and acetyl groups, however, markedly decreased nitrogen basicity with the bromo group effect somewhat greater than the acetyl group effect. The relatively small effect of the bromo groups on the λ_{max} values can be ascribed to the much smaller resonance effect (σ_R or $\sigma_P - \sigma_M$) of bromo compared with acetyl groups (Jaffe and Orchin, 1962).

The relationship between the energy of the Soret band maximum and energy separations between the Soret and visible bands was also investigated. As shown in Figure 13, linear relationships between the Soret energy and the energy difference between the Soret and band I ($\Delta BQ_{x(0-0)}$) and between the Soret energy and the energy difference between the Soret and band III ($\Delta BQ_{y(0-0)}$) were found. Any satisfactory theory of porphyrin spectra must take this striking relationship into account.

Although the magnitude of shifts in λ_{max} values of

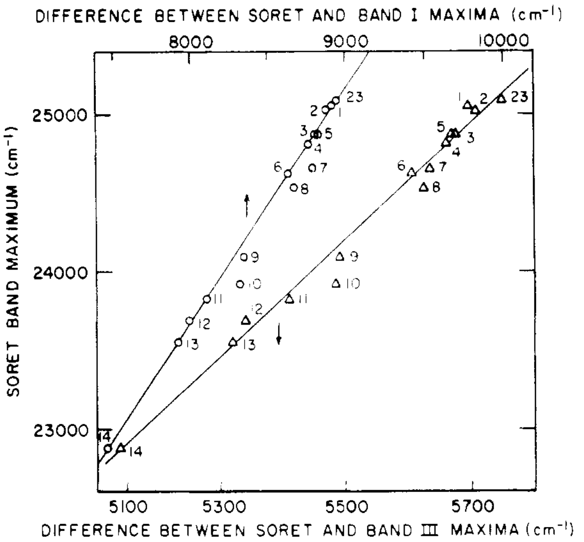


FIGURE 13: Plots of energy separations between Soret and band I ($(\Delta BQ_x)_{(0\text{-}0)}$) and between Soret and band III ($\Delta BQ_{y(0\text{-}0)}$) *vs.* energies of Soret band maxima. Numbers refer to compounds listed in Tables I and II.

the bromo- and nitroporphyrins do not change upon introduction of nickel or iron, the symmetry of the Soret band of the nitro derivatives was markedly affected. Both nickel(II) and dipyridine iron(II) complexes of the nitro compounds exhibited much more symmetrical Soret bands than did the metal-free compounds (Figure 9). Protonation of the mononitro (Figure 3) and the dinitro compounds also resulted in sharpening the Soret band. It should be noted that the processes resulting in a more symmetrical Soret band also result in a more symmetrical structure. (It was also observed that the nitro group gave a more pronounced red shift in the protonated species than in the neutral or metal complexes; possibly the protonation of the nitro group resulted in greater conjugative interaction than was found in neutral species.)

Several general discussions of porphyrin spectra (*cf.* Falk, 1964; Saunders *et al.*, 1964; Phillips, 1963) have proposed that wavelengths and relative intensities of visible bands are correlated with electron-withdrawing properties of substituents. Not only have we found no correlation between λ_{max} and electron-withdrawing capacity (as measured by nitrogen basicity and metal–ligand reactions), but also the correlations we have found between band intensity and substituents are not based on inductive effects but rather on resonance effects and symmetry relationships. Among the 2,4-substituted deuteroporphyrins reported here, compounds in which σ_R values were similar for the 2 and 4 substituents gave etio-type spectra ($A_I < A_{II} < A_{III} < A_{IV}$) whereas compounds with different σ_R values for the 2 and 4 substituents gave rhodo-type spectra ($A_I < A_{II} < A_{IV} < A_{III}$). Thus monoacetyl (Caughey *et al.*, 1966a), monopropionyl, and monoformylmonovinyl derivatives exhibited high enough A_{III}/A_{IV} ratios to have rhodo-type spectra while the monobromo compound (σ_R for hydrogen and bromo groups are nearly equal) exhibited an etio spectrum with an A_{III}/A_{IV} value higher than that of the dihydrogen or dibromo compounds. (The spectrum of the α- (and β-) nitro derivative was etio type; that of the α,β-dinitro derivative was phyllo type ($A_I < A_{III} < A_{II} < A_{IV}$).)

Although relative intensities vary little, if at all, among the isomeric etioporphyrins (Table III), coproporphyrins, and uroporphyrins (*cf.* Falk, 1964), the isomeric tetramethyltetracarbethoxyporphyrins (Figure 12) exhibited marked differences in relative peak heights (Figure 10; Tables II and III). With these carbethoxy compounds bands II and IV were of similar intensities for all isomers but the intensities of bands I and III varied appreciably. For the 2,4-substituted deuteroporphyrins as well, bands I and III (the 0–0 bands) were quite symmetrical and variable in intensity whereas bands II and IV (the 0–1 bands) tended to have more uniform maximum intensities and frequently were less symmetrical with evidence of unresolved multiple bands within each band. The Soret bands varied in intensity and in nearly all instances gave evidence for unresolved multiple peaks. However, the statement "electrophilic side-chains tend to decrease the extinction coefficient" (Falk, 1964) was not supported by these data.

TABLE III: Ratios of Absorbances of Band Maxima for Isomeric Porphyrins in Chloroform.

	A_I/A_{IV}	A_{II}/A_{IV}	A_{III}/A_{IV}	A_{Soret}/A_{IV}
Etioporphyrins[a]				
Type I	0.37	0.49	0.74	12.1
Type II	0.365	0.48	0.735	12.2
Type III	0.36	0.49	0.73	12.3
Type IV	0.37	0.48	0.74	12.3
Tetramethyltetracarbethoxyporphyrins[b]				
Type I	0.255	0.37	0.42	18.3
Type II	0.32	0.38	0.53	19.3
Type III	0.18	0.37	0.38	16.2
Type IV	0.115	0.39	0.33	15.8

[a] Data from personal communication from Dr. J. G. Erdman. Isomer structures are as in Figure 12 except for ethyl groups in place of carbethoxy groups. [b] Structures are shown in Figure 12.

It is hoped that a more rigorous theoretical interpretation of these spectral data will soon be published elsewhere. However, these studies clearly demonstrate that great caution must be exercised in deducing from absorption spectra alone the extent to which peripheral ring substituents affect other properties of porphyrins, metal porphyrins, and heme proteins.

Steric interactions between certain peripheral substituents and protein environment can be expected to have far greater effect upon resonance effects, and therefore absorption spectra, than upon inductive effects.

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