which was chromatographed on 50 g of alumina. Elution with benzene yielded skatole. Further elution with $CHCl_3$ gave a white solid which was recrystallized from benzene to give 200 mg of white needles with mp 222–224°. An analytical sample was crystallized from benzene and had mp 224–225°. The nmr spectrum consisted of a singlet (three protons) at τ 8.02, a singlet (three protons) at 8.12, a multiplet (eight protons) at 2.9, and two broad singlets (one proton each) at 0.2 and −0.1.

The mass spectrum of **18** provided additional evidence for the dimeric nature of this compound. The peak for molecular ion appeared at *m/e* 276. The base peak in the spectrum (*m/e* 261) was formed by loss of CH_3. This then lost CO to give another fragment peak at *m/e* 233. Cleavage of the dimer at bond joining the two indolic rings yielded a $C_9H_8N^+$ fragment (*m/e* 130).

Anal. Calcd for $C_{18}H_{16}N_2O$: C, 78.24; H, 5.84; N, 9.93. Found: C, 78.28; H, 5.98; N, 9.98; Cl, less than 0.3.

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Substituted Deuteroporphyrins. I. Reactions at the Periphery of the Porphyrin Ring[1]

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Variously substituted deuteroporphyrins IX have been obtained from protoporphyrin IX or its iron(III) chloride (protohemin) employing preparative methods which involve reactions of peripheral substituents or substitution directly on the porphyrin ring. Included were oxidation, addition, and displacement reactions of vinyl groups; in contrast to earlier reports no significant differences in the reactivities of the 2- and 4-vinyl groups were observed. Electrophilic substitution reactions (acylation, bromination, nitration) differed in the ring positions selected. Both acylations of deuterohemin and bromination of deuteroporphyrin IX dimethyl ester have been found to take place predominately at the 2 and 4 positions, whereas nitration of the ester in nitric acid–sulfuric acid occurred preferentially at the α and β positions. Selectivity between α and β or between 2 and 4 positions was not noted. It is suggested that relative to the 2,4 positions the *meso* positions are more susceptible to electrophilic attack in the protonated species than in either the neutral species or the hemin. Infrared studies of this series of compounds have permitted vibrational assignments for most of the peripheral ring substituents as well as more equivocal assignments for bands of the porphyrin ring as a whole.

The porphyrins found in hemeproteins can be described as derivatives of deuteroporphyrin IX (Figure 1): protoporphyrin IX with vinyl groups at the 2,4 positions; porphyrin c with thio ether linkages between protein and ethyl groups at the 2,4 positions; porphyrin a with long alkyl, vinyl, and formyl groups at positions 2, 4, and 8, respectively; chlorocruoroporphyrin with 2-formyl and 4-vinyl groups.[3,4] Reactions leading to differently substituted deuteroporphyrins IX have thus been useful for the preparation of compounds most suitable for the evaluation of effects of the differences in structure found among natural porphyrins[3,5–9] and also for considerations of the relative reactivities of positions on the porphyrin ring. Following an approach exploited so successfully by Hans Fischer,[4] we have prepared variously substituted deuteroporphyrins IX from the readily available hemin (protoporphyrin IX iron (III) chloride).

The reactions explored include: oxidations of vinyl groups to formyl and carboxyl groups; additions to vinyl groups to give ethyl, hydroxyethyl, and cyclopropyl groups; replacement of vinyl groups by hydrogen via the resorcinol melt procedure; electrophilic substitution reactions to give acyl, bromo, and nitro compounds. These studies have resulted in the preparation of new deuteroporphyrin IX derivatives, in observations of relative reactivities of ring positions, and in improved preparations and, frequently, more adequate characterizations of several known deuterioporphyrins. Infrared assignments for these, and other, porphyrins have also been considered.

Experimental Section

Melting points were determined on a hot stage (Nalge–Axelrod) apparatus and are corrected. Nmr spectra were determined with a Varian A-60 spectrometer using tetramethylsilane as internal standard; chemical shifts are reported as δ values. In $CDCl_3$, where chemical shifts are often,[10,11] but not

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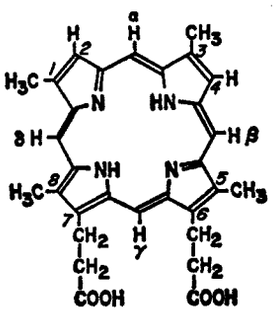

Figure 1.—Deuteroporphyrin IX.

always,[12] concentration dependent, a concentration range (usually from 0.01 to 0.08 M) was studied to obtain infinite dilution chemical shifts repoducible within ±0.02 ppm. Addition of trifluoroacetic acid or deuterated trifluoroacetic acid to $CDCl_3$ solutions gave protonated species whose spectra were essentially independent of porphyrin concentration but were somewhat dependent on acid concentration; the sensitivity of chemical shifts to changes in acid concentration varied with the particular substituent groups present. Infrared spectra were determined in potassium bromide disks, unless otherwise stated, with a Perkin-Elmer Model 21 spectrophotometer (sodium chloride prism). The water vapor peak at 2.673 μ served as an internal standard for most spectra; comparisons with calibration curves of water vapor, carbon dioxide, and polystyrene were also made. Electronic spectra were determined with Cary 11 or Beckman DK-2 spectrophotometers. Microanalyses for carbon, hydrogen, nitrogen, and bromine were carried out by Dr. S. N. Nagy and for oxygen by Schwarzkopf Microanalytical Laboratory. Iron was determined by the method of Drabkin.[13]

Deuteroporphyrin IX Dimethyl Ester.—Protohemin chloride (40 g) and resorcinol (120 g) were thoroughly mixed and maintained as a melt at 190–200° for 15 min.[4a] The mixture at room temperature was washed with ether (100 ml, four times), and the resulting deuterohemin thoroughly mixed with pyridine (100 ml) was added to chloroform (3 l.). Methanol (3.3 l.) and anhydrous ferrous sulfate (160 g) were then added and dry HCl passed through the mixture until iron removal was complete (reaction time about 1 hr; aliquots of reaction mxture were taken to follow the course of the reaction spectrally). The mixture was extracted with water (3 l., three times), with 10% aqueous ammonia (3 l., twice) and again with water (2.4 l., twice). The washed chloroform solution was dried over sodium sulfate (800 g) and chromatographed on alumina (680 g) with chloroform. The eluate fraction (concentrated from 3.5 l. to 200 ml) at boiling point was treated with hot methanol (600 ml) and cooled to give crystals: 22 g (66%); mp 225–226°, 227° after three crystallizations from chloroform–methanol (lit. mp 218–220°,[4a] 224.5°[14]); nmr, 2,4-hydrogens were found as a singlet at δ 9.12 for infinite dilution in $CDCl_3$ and at 9.43 in $CDCl_3$ with 2.5% F_3CCOOH.

Anal. Calcd for $C_{32}H_{34}O_4N_4$: C, 71.36; H, 6.36; N, 10.40. Found: C, 71.57; H, 6.49; N, 10.29.

Deuteroporphyrin IX Diethyl Ester.—A solution containing deuteroporphyrin IX dimethyl ester (15 g), chloroform (750 ml), and absolute ethanol (825 ml) was saturated with dry HCl for 6 hr and then washed with water, with 10% aqueous ammonia, and again with water. Crystals were obtained from chloroform–methanol (120:390 ml): 15.2 g (96%); mp 204.5° (lit.[15] mp 204°). Nmr, ethoxy groups protons were found as a triplet at δ 1.14 (CH_3) and a quartet at 5.85 (CH_2) in $CDCl_3$ with 2.5% F_3CCOOH.

Anal. Calcd for $C_{34}H_{38}N_4O_4$: C, 72.06; H, 6.76; N, 9.89. Found: C, 72.08; H, 6.84; N, 9.82.

2,4-Diacetyldeuteroporphyrin IX Dimethyl Ester.—Crude 2,4-diacetyldeuterohemin, obtained from 40 g deuterohemin chloride *via* the method described in Fischer and Orth,[4b] was subjected to iron removal—esterification and chromatography steps as carried out in the preparation of deuteroporphyrin IX dimethyl ester. On the column a zone of deuteroporphyrin IX dimethyl ester preceded a zone of monoacetyl derivative, followed by the diacetyl derivative (the major product). Crystals were obtained from chloroform–methanol: 20.3 g (49%); mp 242.5° (lit.[4b] mp 234–236°); in potassium bromide acetyl carbonyl stretching frequency was found at 1661 cm^{-1}; nmr, acetyl methyl protons were found as two singlets at δ 3.29 and 3.23 at infinite dilution in $CDCl_3$ and as one singlet at 3.35 in $CDCl_3$ with 2.5% Fe-CCOOH.

Anal. Calcd for $C_{36}H_{38}O_6N_4$: C, 69.43; H, 6.15; N, 9.00. Found: C, 69.53; H, 6.04; N, 9.21.

2,4-Diacetyldeuteroporphyrin IX Diethyl Ester.—For the conversion of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (303 mg) to the diethyl ester a procedure similar to the deuteroporphyrin IX diethyl ester procedure was carried out, followed by chromatography on alumina with 1,2-dichloroethane. The yield was 135 mg (43%) from chloroform–methanol: mp 239°; nmr, in $CDCl_3$ with 2.5% F_3CCOOH the ethyl ester methyl protons were found as a triplet at δ 1.17, and acetyl methyl protons were found at 3.42.

Anal. Calcd for $C_{38}H_{42}O_6N_4$: C, 70.13; H, 6.51; N, 8.61. Found: C, 69.68; H, 6.34; N, 8.52.

2- (and 4-) Acetyldeuteroporphyrin IX Dimethyl Ester.—The monoacetyl fraction obtained during chromatography of the 2,4-diacetyl compound was rechromatographed on alumina with 1,2-dichloroethane–chloroform (9:1). The yield was 1.0 g (2.6%) from 1,2-dichloroethane–methanol: melting point range 212–224°; nmr, 2 (and 4) protons were found as two singlets at 9.39 and 9.30 (each singlet represented *ca.* one-half proton) and the acetyl methyl protons as a singlet at 3.31 in $CDCl_3$ with 2.5% F_3CCOOH; in $CHCl_3$, λ_{max} in mμ (A_{mM}), 635 (1.06), 578 (7.2), 549 (11.6), 510 (9.9), 410 (176).

Anal. Calcd for $C_{34}H_{36}O_5N_4$: C, 70.32; H, 6.25; N, 9.65. Found: C, 70.24; H, 6.03; N, 9.56.

2,4-Bis(acetyloxime)deuteroporphyrin IX Dimethyl Ester.—Hydroxylamine hydrochloride (1.25 g) and sodium carbonate (0.75 g) were added to a hot solution of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (4 g) in pyridine (150 ml). The mixture was kept at reflux for 30 min, cooled to room temperature, treated with chloroform (150 ml), and then washed thoroughly with water. To obtain crystals the washed chloroform solution was concentrated to 50 ml, treated at the boiling point with hot methanol (150 ml), and cooled slowly. The first crop was 1.61 g (38%): mp 240–241°; in KBr, oxime ν_{OH} was found at 3330 cm^{-1}; nmr, methyl protons of acetyloxime groups were found as two singlets at δ 2.91 and 2.96 for infinite dilution in $CDCl_3$ and as a singlet at 3.09 in $CDCl_3$ with 2.5% F_3CCOOH.

Anal. Calcd for $C_{36}H_{39}N_6O_6$: C, 66.34; N, 6.03; N, 12.90. Found: C, 66.06; H, 6.42; N, 12.84.

2- (and 4-) Propionyldeuteroporphyrin IX Dimethyl Ester and 2,4-Dipropionyldeuteroporphyrin IX Dimethyl Ester.—To a suspension of deuterohemin chloride (5.5 g) in propionic anhydride (165 ml, Matheson Coleman and Bell No. 5285) at 0° was slowly added stannic chloride (14 ml, Matheson Coleman and Bell CB766). After 12 min, an ice–water mixture (825 ml) saturated with sodium chloride was added; after 3 hr, a precipitate (6.7 g) was collected. A portion (6.5 g) was subjected to iron removal-esterification as carried out in the preparation of deuteroporphyrin IX dimethyl ester, followed by chromatography on an alumna column with 1,2-dichloroethane to give from bottom to top: a minor pink zone (I), a minor red zone (II), a trace red zone (III), a major red brown zone (IV) and a trace green zone (V). Eluate fractions containing mixtures of zones II and III and of zones III and IV were rechromatographed on alumina with petroleum ether-1,2-dichloroethane (3:2, v/v). Each of the residues from zones II, III, and IV were crystallized from chloroform–methanol. **Zone I** was deuteroporphyrin IX dimethyl ester. **Zone II** was 209 mg (3.4%) of monopropionyl: in KBr, ν_{CO} 1656 cm^{-1}; $A_{acyl\ CO}/A_{ester\ CO}$ = 0.48; nmr in $CDCl_3$ with 2.5% F_3CCOOH, propionyl methyl protons found as a triplet at δ 1.64 corresponded to three protons; 2 (and 4) protons were found as two singlets at 9.44 and 9.34 (each singlet represented *ca.* one-half proton).

Anal. Calcd for $C_{35}H_{38}O_5N_4$: C, 70.69; H, 6.44; N, 9.42. Found: C, 70.37; H, 6.92; N, 8.96.

Zone IV was 2.31 g (37%) of dipropionyl: mp 190°; in KBr, ν_{CO} 1661 cm^{-1}; $A_{acyl\ CO}/A_{ester\ CO}$ = 0.80; nmr in CCl_3 with 2.5% F_3CCOOH, propionyl methyl protons found as a triplet at δ 1.64 corresponded to six protons.

Anal. Calcd for $C_{38}H_{42}O_6N_4$: C, 70.14; H, 6.51; N, 8.61. Found: C, 70.13; H, 6.82; N, 8.69.

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2,4-Dipropionyldeuteroporphyrin IX Diethyl Ester.—The conversion of 2,4-dipropionyldeuteroporphyrin IX dimethyl ester (300 mg) to the diethyl ester was carried out as in the preparation of deuteroporphyrin IX diethyl ester. The yield was 248 mg (79%) after crystallization from chloroform–methanol: mp 202°; nmr, in $CDCl_3$ with 2.5% F_3CCOOH, methyl protons were found as triplets for 2,4-propionyl and 6,7-ethyl ester at δ 1.64 and 1.17, respectively.

Anal. Calcd for $C_{40}H_{46}O_6N_4$: C, 70.77; H, 6.83; N, 8.25. Found: C, 70.54; H, 6.61; N, 8.40.

2,4-Dibromodeuteroporphyrin IX Dimethyl Ester.—Pyridinium bromide perbromide (30 g) was added over a 5-min period to deuteroporphyrin IX dimethyl ester (15 g) in chloroform (1.5 l.) After 5 min acetone (500 ml) was added, followed 5 min later by the addition of cold water (1.5 l.) Up to and during the addition of the water the reaction mixture was vigorously stirred and cooled at 0°. The chloroform solution was washed with water, dried over sodium sulfate (1 kg), and chromatographed on alumina (2 kg) with chloroform. Crystallization from chloroform–methanol yielded 8.8 g (45%), mp 278–279°, lit.[16] mp 274–277°.

Anal. Calcd for $C_{32}H_{32}O_4N_4Br_2$: C, 55.18; H, 4.63; N, 8.04; Br, 22.95. Found: C, 55.42; H, 4.86; N, 8.46; Br, 23.17.

α- (and β-) Nitrodeuteroporphyrin IX Dimethyl Ester.—Deuteroporphyrin IX dimethyl ester (1 g) was dissolved in concentrated sulfuric acid (10 ml) at 0°. 9.3 ml of concentrated sulfuric acid with 1.33% (by vol) concentrated nitric acid at 0° was added over 1.5 min with stirring. After 5.5 min the solution was added to a mixture of ice (100 g) and sodium acetate (100 g) in water (700 ml). 1,2-Dichloroethane extracts of the mixture were washed with water, dried over sodium sulfate (200 g), and evaporated to dryness. The residue was chromatographed on alumina (Woelm acid alumina, grade II) with benzene to give from bottom to top: a light brown zone, a dark brown zone, and a green zone of several bands. The light brown zone (dinitro derivative) represented a minor component compared with the dark brown zone (mononitro derivative), which after crystallization from chloroform–methanol gave 470 mg (43%): mp 193°; in KBr, asym ν_{NO2} at 1517 cm^{-1}; in $CHBr_3$, ν_{CO} 1726 cm^{-1}, ν_{NO_2} 1524 cm^{-1}, A_{NO_2}/A_{CO} = 0.44; nmr in $CDCl_3$ with 2.5% F_3CCOOH, one proton at δ 10.62 (α and β), one-half protons at 10.57 (δ), 10.67 (δ), 11.06 (γ), and 11.23 (γ), two protons as broad unresolved multiplet at 9.22 (2,4), *ca.* one-half ring methyl at 3.29 (5), and *ca.* one-half ring methyl at 3.41 (3).

Anal. Calcd for $C_{32}H_{33}N_5O_6$: C, 65.85; H, 5.70; N, 12.00. Found: C, 66.07; H, 5.61; N, 12.20.

α,β-Dinitrodeuteroporphyrin IX Dimethyl Ester.—Procedures used were similar to those for the mononitro product except for twice the concentration of nitric acid (2.67%, by vol) in the nitric acid–sulfuric acid solution. The chromatography gave three zones from bottom to top: dark brown, brown, and green. The major product (the dark brown zone) was rechromatographed to remove one faster and one slower contaminant and crystallized from chloroform–methanol. The yield was 312 mg (27%): mp 238°; in KBr, asym ν_{NO_2} at 1519 cm^{-1}; in $CHBr_3$, ν_{CO} 1713 cm^{-1}, ν_{NO_2}, 1530 cm^{-1}, A_{NO_2}/A_{CO} = 0.81; nmr in $CDCl_3$ with 2.5% F_3CCOOH, two *meso* protons were found at 11.23 (γ) and 10.66 (δ); two β protons were found at 8.97 (4) and 9.20 (2); four ring methyl groups were found at 3.68 (1), 3.35 (3), 3.26 (5) and 3.60 (8); singlets in all cases.

Anal. Calcd for $C_{32}H_{32}N_6O_8$: C, 61.16; H, 5.13; N, 13.37. Found: C, 61.33, 61.26; H, 5.33, 5.51; N, 12.91, 13.31.

Protoporphyrin IX Dimethyl Ester.—In modification of reported procedures,[17-19] the method for preparing deuteroporphyrin IX dimethyl ester from deuterohemin was followed, using 20 of g hemin, 40 ml of pyridine, 1 l. of chloroform, 1.1 l. of CH_3OH, and 150 g of anhydrous ferrous sulfate. Chromatography was on calcium carbonate (900 g) with chloroform–petroleum (30–60°)–ether (1:1, v/v). Crystals from chloroform–methanol (300–900 ml) ranged from 9 to 12 g (50–66%) for more than 20 preparations including only the first crop of crystals. Melting points (without recrystallization), though sharp, varied from 218 to 223 (lit. mp 230,[4c] 224–226,[18] and 214–217°.[19] Absorption maxima at 1402, 1295, 986 and 900 cm^{-1} in KBr can be ascribed to in-plane C-H, in-plane CH_2, out-of-plane CH, and out-of-plane CH_2 deformation vibrations respectively, all associated with vinyl groups. Absorption maxima at 1.628 μ (A_M = 0.98) and at 2.116 μ can be assigned to terminal methylenes of two vinyl groups in accord with bands observed for alkenes.[20,21] Nmr vinyl protons were found at 8.39 (H_1), 6.34 (H_2), and 6.17 (H_3) with J_{12} = 12 cps and J_{13} = 18 cps for infinite dilution in $CDCl_3$, and 6.44 (H_2), and 6.27 (H_3) with J_{12} = 12 cps and J_{13} = 18 cps in $CDCl_3$ with 2.5% F_3CCOOH.

Anal. Calcd for $C_{36}H_{38}N_4O_4$: C, 73.19; H, 6.48. Found: C, 73.21; H, 6.59.

Protoporphyrin IX Diethyl Ester.—The method of preparation used for the dimethyl ester was followed except for the use of absolute ethanol in place of methanol. The yield was 12.5 (65%), mp 215°, after three crystallizations from chloroform–methanol.

Anal. Calcd for $C_{38}H_{42}N_4O_4$: C, 73.76; H, 6.84; N, 9.06. Found: C, 73.88, 73.93; H, 7.24, 6.82; N, 9.00, 9.33.

2- (and 4-) Formyl-4- (and 2-) vinyldeuteroporphyrin IX Dimethyl Ester and 2,4-Diformyldeuteroporphyrin IX Dimethyl Ester.—A solution of potassium permangante (5g) and magnesium sulfate (10.6 g as heptahydrate) in water (250 ml) was added slowly to protoporphyrin IX dimethyl ester (5 g) in acetone (4 l.) under reflux over a period of 45 min. The mixture was filtered at room temperature. The filtrate combined with water (6 l.) was extracted four times with chloroform (600, 600, 300 and 300 ml) and the extracts washed with water and evaporated to dryness. The dry residue from washed extracts and the MnO_2 precipitate (14 g after drying) were each extracted with chloroform. 1,2-Dichloroethane (1.8 l.) was added to the combined extracts (900 ml) followed by chromatography on alumina (1.1 kg) with chloroform-1,2-dichloroethane (1:2, v/v). Two components were removed from the column. A third component was eluted from the column with chloroform, leaving a green-brown zone at the top. Each of the three components was crystallized out of chloroform–methanol Protoporphyrin IX dimethyl ester (600–900 mg) was obtained from the fastest zone.

The second zone gave the monoformylmonovinyl derivatives: 600 to 875 mg (15 to 21%); mp 260°, lit. mp 277–278°[22]; in chloroform, vinyl terminal methylene absorption was at 1.63 (A_M, 0.47) and 2.12 μ; in KBr, formyl ν_{CH} and ν_{CO} were at 2740 and 1661 cm^{-1}, respectively, $A_{formyl\ CO}/A_{ester\ CO}$ = 0.7; nmr in $CDCl_3$ with 2.5% F_3CCOOH, a formyl proton was found as a singlet at 11.59, two sets of *meso* protons were found as expected for a mixture of two isomers, vinyl protons appeared at 6.29 (H_2) and 6.47 (H_3) (J_{12} = 12 cps and J_{13} = 18 cps).

Anal. Calcd for $C_{35}H_{36}O_5N_4$: C, 70.92; H, 6.12; N, 9.45. Found: C, 70.64; H, 6.02; N, 9.18.

The third zone yielded 700 to 920 mg (17 to 22%) of 2,4-diformyl compound: mp 277–279° (some preparations had mp of 284–286°); reported mp 280,[23] 301–303,[24] and 300° dec;[17] in KBr, formyl ν_{CH} and ν_{CO} were 2746 and 1669 cm^{-1}, respectively; $A_{formyl\ CO}/A_{ester\ CO}$ = 1.2; nmr, formyl proton was found as a singlet at 11.58 in $CDCl_3$ with 2.5% F_3CCOOH.

Anal. Calcd for $C_{34}H_{34}O_6N_4$: C, 68.67; H, 5.76; N, 9.42. Found: C, 68.37, 68.55; H, 6.00, 5.77; N, 9.08.

2- (and 4-) Formyl-4- (and 2-) vinyldeuteroporphyrin IX Diethyl Ester and 2,4-Diformyldeuteroporphyrin IX Diethyl Ester.—Procedures similar to those used with the corresponding dimethyl esters were followed and resulted in comparable yields. The 2- (and 4-) formyl-4- (and 2-) vinyl compound melted at 206°; nmr in $CDCl_3$, the spectra were highly concentration dependent; duplicate peaks were found throughout the entire spectrum in accord with a two component mixture; in 4% F_3CCOOD in $CDCl_3$, formyl proton singlet at 11.51 and vinyl protons at 6.27 (H_3) and 6.44 (H_2) (J_{12} = 12 cps and J_{13} = 18 cps).

Anal. Calcd for $C_{37}H_{42}O_5N_4$: C, 71.36; H, 6.80; N, 9.00. Found: C, 71.35; H, 6.64; N, 8.81.

The 2,4-diformyl compound melted at 276°; nmr, formyl protons were found as two singlets at 9.87 and 9.95 at infinite dilution in $CDCl_3$.

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Anal. Calcd for $C_{36}H_{40}O_8N_4$: C, 69.21; H, 6.45; N, 8.97. Found: C, 69.45; H, 6.42; N, 8.87.

2,4-Dioximinodeuteroporphyrin IX Dimethyl Ester.—Hydroxylamine hydrochloride (1.14 g) and sodium carbonate (0.86 g) were added to a solution of 2,4-diformyldeuteroporphyrin IX dimethyl ester (3.9 g) in pyridine (150 ml). The mixture was kept at reflux for 15 min, cooled to room temperature, combined with chloroform (150 ml), washed thoroughly with water, dried over sodium sulfate, and evaporated (residue, 3.1 g). For crystallization, 500 mg of the residue was extracted with hot pyridine (10 ml); warm chloroform (30 ml) was added to the extract, followed by cooling to give 130 mg (19%), mp 252.5°; lit. mp 253–256° for a product characterized only by one low nitrogen analysis[17] and mp 231–232° for a product without reported elemental analyses.[23] In KBr, oxime ν_{OH} was at 3366 cm^{-1}; nmr, oxime C-H proton was found as singlet at 9.56 in $CDCl_3$ with 2.5% F_3CCOOH.

Anal. Calcd for $C_{34}H_{36}N_6O_6$: C, 65.37; H, 5.81; N, 13.45. Found: C, 65.60; H, 5.69; N, 13.62.

The addition of methanol (20 ml) to the mother liquors resulted in a second crop of crystals (275 mg) (41%) which were identical (spectrally) with the first crop.

2,4-Dicyanodeuteroporphyrin IX Dimethyl Ester and Mono-Cyanomonoximino and Monocyanomonoformyl Deuteroporphyrin IX Dimethyl Ester.—A solution of crude (*i.e.*, not crystallized from pyridine–chloroform) 2,4-dioximinodeuteroporphyrin IX dimethyl ester (3.0 g) in acetic anhydride (500 ml) was refluxed for 40 min, cooled to room temperature, and chloroform (500 ml) added. The chloroform solution was washed with water, dried on cellulose powder, concentrated to 50 ml, and treated at boiling point with hot methanol (150 ml) to give, on cooling, crystals (2.75 g): mp 270–272°; in bromoform, ν_{CN} 2227 cm^{-1}.

Anal. Calcd for $C_{34}H_{32}O_4N_6$ (dicyano): C, 69.37; H, 5.48; N, 14.28. Found: C, 68.91; H, 5.44; N, 14.05.

Chromatographed on alumina were 300 mg with 1,2-dichloroethanechloroethane–chloroform (4:1, v/v) where a minor zone (I) followed the major zone (II). Chloroform–methanol (9:1, v/v) developed another minor zone (III). Crystals were obtained out of chloroform–methanol for each zone.

Zone I (monocyanomonoformyl) yielded 17 mg: mp 267°; in chloroform, λ_{max} in mμ (A/A_{IV}), I, 637 (0.24); II, 581 (0.45); III, 548 (0.55); IV, 512 (1.0); 416 (13.2).

Anal. Calcd for $C_{34}H_{33}N_5O_5$: C, 69.01; H, 5.62; N, 11.84. Found: C, 68.44; H, 6.01; N, 11.82.

Zone II (dicyano) yielded a first crop of 121 mg: mp 284–5°; in KBr, ν_{CN} 2212 cm^{-1} (no evidence of oximino or formyl groups).

Anal. Found: C, 69.04; H, 5.70.

Zone III (monocyanomonoximino) yielded 33 mg: mp 240–241°; in KBr, ν_{OH} (oximino) 3400 cm^{-1}, ν_{CN} 2207 cm^{-1}; in chloroform, λ_{max} in mμ (A/A_{IV}), I, 635 (0.17); II, 580 (0.57); III, 551 (0.90); IV, 515 (1.0); 417 (13.7); 256 (1.37).

Anal. Calcd for $C_{34}H_{34}O_5N_6$: N, 13.85. Found: N, 13.89.

2,4-Bis(methoxycarbonyl)deuteroporphyrin IX Dimethyl Ester.—A hot solution of potassium permanganate (15 g) in acetone (2.5 l.) was added over a 7-min period to a solution of protoporphyrin IX dimethyl ester (5 g) in acetone (2.5 l.) under reflux. The mixture was cooled rapidly. A precipitate containing manganese dioxide was collected (19.5 g after drying under vacuum), and extracted with methanol in a Soxhlet apparatus for 15 hr. The methanol solution was saturated with dry hydrogen chloride and allowed to stand for 15 hr followed by four extractions with chloroform (250, 150, 100, and 100 ml). The combined chloroform extracts were washed with water, dried over sodium sulfate and evaporated to dryness. The residue was chromatographed on calcium carbonate (900 g) with 1,2-dichloroethane. Crystals from 1,2-dichloroethanemethanol were obtained: 146 mg; mp 190°; in KBr, the carbonyl stretching frequency for the 2,4 ester groups was at 1706 cm^{-1}; nmr, methoxy protons of 2,4 ester groups were found as singlets at 4.44 and 4.48 for infinite dilution, in $CDCl_3$ and at 4.49 in $CDCl_3$ with 2.5% F_3CCOOH.

Anal. Calcd for $C_{36}H_{38}O_8N_4$: C, 66.04; H, 5.85; N, 8.56. Found: C, 65.91; H, 5.80; N, 8.83.

2,4-Bis(2-carboxycyclopropyl)deuteroporphyrin IX 2,4-Diethyl Ester 6,7-Dimethyl Ester.—Protoporphyrin IX dimethyl ester (20 g) and ethyl diazoacetate (40 ml from Aldrich Chemical Co.) were maintained at 93–95° until spectra of aliquots indicated the reaction was essentially complete (reaction times required varied widely, from 4 to 27 hr). A 1,2-dichloroethane extract (600 ml) of the reaction mixture was chromatographed on alumina. Four significant zones developed and were eluted from the column: zone I with 1,2-dichloroethane, zone II (the major zone) with 1,2-dichloroethane–chloroform (3:1, v/v), zone III with chloroform, and zone IV with methanol. Zone I was unaltered protoporphyrin IX dimethyl ester (2 g). Zones III and IV gave dry residues of 1.2 and 2.1 g respectively and visible spectra rather similar to those for zone II. A product of 10.0 g (43%) was obtained out of acetone–methanol (35:70 ml) from zone III: mp 92–94°. In bromoform, a single ν_{CO} band at 1727 cm^{-1} was found. In carbon disulfide, absorptions at 1.64 and 2.23 μ were attributable to cyclopropyl groups.[25] In the nmr spectrum cyclopropyl group protons appeared as multiple peaks from 2.5 to 0 in $CDCl_3$ and 2,4-ethoxymethyl protons appeared as a triplet at 1.57 in $CDCl_3$ with 2.5% F_3CCOOH.

Anal. Calcd for $C_{44}H_{50}N_4O_8$: C, 69.26; H, 6.61; N, 7.35. Found: C, 69.28; H, 6.63; N, 7.50.

2,4-Bis(2-carboxycyclopropyl)deuteroporphyrin IX Tetraethyl Ester.—The 2,4-diethyl ester, 6,7-dimethyl ester (4 g) was converted to the tetraethyl ester in the manner used to prepare deuteroporphyrin IX diethyl ester, followed by chromatography on alumina with chloroform. The yield was 2.5 g (60%) from acetone–methanol (45:90 ml): mp 76–79°; 80–82° after repeated reprecipitations from acetone–methanol. In bromoform, a single ν_{CO} band at 1727 cm^{-1} was found; nmr in $CDCl_3$ with 2.5% F_3CCOOH, triplets for methyl protons of the 2,4 and 6,7 ester OCH_2CH_3 were found at 1.57 and 1.13 respectively.

Anal. Calcd for $C_{46}H_{54}N_4O_8$: C, 69.85; H, 6.87; N, 7.08. Found: C, 69.68; H, 6.93; N, 7.19.

2,4-Bis(2-carboxycyclopropyl)deuteroporphyrin IX Tetramethyl Ester.—A solution of the 2,4-diethyl ester, 6,7-dimethyl ester (4 g) in 500 ml of chloroform–methanol (1:1, v/v) was saturated with dry HCl for 30 min, allowed to stand 18 hr, and then washed successively with water, 10% aqueous ammonia, and water. The washed chloroform solution was dried through cellulose powder (25 g) and evaporated. The residue gave a precipitate of 3.4 g (88%) from acetone–methanol (60:120 ml): mp 134–137°. Fischer and Medick reported the isolation of a crystalline tetramethyl ester: mp 193–194°[26]; nmr in $CDCl_3$ with 2.5% F_3CCOOH, methyl protons for 2,4 and 6,7 methoxyls were found as singlets at 4.13 and 3.69, respectively.

Anal. Calcd for $C_{42}H_{46}N_4O_8$: C, 68.56; H, 6.30; N, 7.62. Found: C, 68.65; H, 6.22; N, 7.78.

2,4-Bis(2-carboxycyclopropyl)deuteroporphyrin IX.—The 2,4-diethyl ester, 6,7-dimethyl ester (3.0 g) in 25% aqueous HCl (225 ml) was kept at reflux temperature for 20 min. Cooling and adjustment to pH 2 with 10% aqueous sodium hydroxide gave a precipitate which was washed with water until no chloride was detected in the washings with silver nitrate: yield 2.5 g (94%).

Anal. Calcd for $C_{38}H_{38}N_4O_8$: C, 67.15; H, 5.64; N, 8.24. Found: C, 66.61; H, 5.75; N, 8.35.

2,4-Bis(1-hydroxyethyl)deuteroporphyrin IX Dimethyl Ester (Hematoporphyin IX Dimethyl Ester) and 2- (and 4-) (1-hydroxyethyl)-4- (and 2-) vinyldeuteroporphyrin IX Dimethyl Ester.—To hematoporphyrin IX hydrochloride (300 mg, L. Light and Co.) in hot pyridine (2 ml) was added hot chloroform (125 ml). The mixture was cooled to 45° and diazomethane (1.85 mmoles) in ethanol–ether added. [The diazomethane solution was prepared from N-methyl-N-nitroso-*p*-toluenesulfonamide ("Diazald") from Aldrich Chemical Co and standardized against succinic acid.] Chromatography on alumina with chloroform developed one minor zone as well as two trace zones which were discarded. The minor zone yielded crystals (25 mg) from chloroform–methanol (3:7 ml): in chloroform, λ_{max} in mμ (A/A_{IV}), I, 625 (0.25), II, 572 (0.45), III, 537 (0.66), IV, 503 (1.0), 401 (12.0).

Anal. Calcd for $C_{36}H_{40}N_4O_5$: C, 71.03; H, 6.62; N, 9.20. Found: C, 70.70; N, 6.56; N, 8.88.

Elution with chloroform containing 1% methanol developed a major zone which yielded 95 mg (crystallized out of benzene (3 ml) which contained methanol (0.1 ml). In the nmr spectrum for hydroxyethyl groups, α proton (as quartet) and methyl protons (as doublet), respectively, appeared at 6.14 and 2.06 at infinite dilution in $CDCl_3$ and at 6.59 and 2.22 in $CDCl_3$ with 2.5% F_3CCOOH.

(25) W. H. Washburn and M. J. Mahoney, *J. Am. Chem. Soc.*, **80**, 504 (1958).

Anal. Calcd for $C_{36}H_{42}N_4O_6$: C, 68.99; H, 6.76; N, 8.94. Found: C, 68.85; H, 6.65; N, 8.61.

Mesoporphyrin IX Dimethyl Ester.[27-29]—Wet palladium oxide[30] (7 g dry wt) and protohemin chloride (40 g) in 90% formic acid (3 l.) were maintained at 94–98° for about 1 hr while hydrogen was passed through the mixture. The catalyst was recovered by filtration and the filtrate added to 30% aqueous ammonium acetate (12 l.). A precipitate was collected, washed with water, and dissolved in 2% aqueous ammonia (1.7 l.) followed by the addition of 30% aqueous disodium tartrate (300 ml). A precipitate (recovered by centrifugation and then dried) was dissolved in 4 l. of methanol–chloroform (1:1) and the solution saturated with dry HCl for 30 min, followed by successive extractions with water (3 l., three times), with 10% aqueous ammonia (3 l., twice), and with water (3 l., twice). The residue from evaporation of the washed chloroform solution was chromatographed on alumina (680 g) with 1,2-dichloroethane. Crystallization from 1,2-dichloroethane–methanol gave 30 g (82%): mp 215°; lit. mp 216°.[4d] In the nmr spectrum 2,4-ethyl group, methylene protons (as a quartet) and methyl proton (as a triplet), respectively, were found at 4.30 and 1.88 for infinite dilution in $CDCl_3$ and at 4.17 and 1.74 in $CDCl_3$ with 2.5% F_3CCOOH.

Anal. Calcd for $C_{36}H_{42}O_4N_4$: C, 72.70; H, 7.12; N, 9.42. Found: C, 72.49; H, 6.95; N, 9.33.

Mesoporphyrin IX Diethyl Ester.—Mesoporphyrin IX dimethyl ester (20 g) was converted to the diethyl ester by a procedure similar to the one used in the preparation of deuteroporphyrin IX diethyl ester. The yield was 21 g (99%); mp 207–208°; lit. mp 204°.[31]

Anal. Calcd for $C_{38}N_{46}O_4N_4$: C, 73.28; H, 7.44; N, 9.00. Found: C, 73.50; H, 7.56; N, 9.48.

Deuterohemin Chloride.[48]—Protohemin chloride (40 g) and resorcinol (120 g) were thoroughly mixed, melted, kept at 190–200° for 15 min, and solidified by cooling to room temperature. The solid was washed with ether (100 ml, four times) and the dark residue mixed with pyridine (300 ml). Chloroform (400 ml) was added and the mixture filtered. The filtrate was added slowly with stirring to a mixture of glacial acetic acid (3.75 l.) and concentrated hydrochloric acid (37.5 ml) at the boiling point. After 12 hr at room temperature, the mixture was filtered to collect crystals which were washed successively with 50% aqueous acetic acid (200 ml), water (200 ml), ethanol (50 ml), and ethyl ether (50 ml), and then dried under vacuum at 50°: yield 23 g (62%). Samples were heated at 150° under vacuum for 2 hr prior to analysis.

Anal. Calcd for $C_{30}H_{28}O_4N_4FeCl$: C, 60.06; H, 4.71; N, 9.34; O, 10.66; Fe, 9.31. Found: C, 60.16; H, 4.96; N, 9.18; O, 10.74; Fe, 9.64.

Deuteroporphyrin IX Nickel(II) Complex.—To a solution of 4 g deuterioporphyrin IX dimethyl ester nickel(II) complex in pyridine (40 ml) was added 10% aqueous potassium hydroxide (280 ml). After the mixture had refluxed 1 hr, 5% aqueous hydrochloric acid (1 l.) was added. The resulting precipitate was dissolved in warm pyridine (100 ml) and treated slowly with hot acetic acid (600 ml). The precipitate obtained on cooling was dried under vacuum at 50°: yield, 3.2 g (84%); mp >360°.

Anal. Calcd for $C_{30}H_{28}N_4O_4Ni$: C, 63.52; H, 4.98; N, 9.88. Found: C, 63.62; H, 5.10; N, 9.79.

Etioporphyrin II.—4,4′-Dimethyl-3,3′-diethyldipyrrylmethane-5,5′-dicarboxylic acid was condensed in formic acid.[32] The crude product was purified by chromatography on alumina with 1,2-dichloroethane as eluent followed by crystallization from chloroform–methanol. In the nmr spectrum ethyl group methylene protons (as a quartet) and ethyl protons (as a triplet), respectively, were found at δ 4.12 and 1.89 for infinite dilution in $CDCl_3$ and at 4.17 and 1.74 in $CDCl_3$ with 2.5% F_3CCOOH.

Anal. Calcd for $C_{32}H_{38}N_4$: C, 80.29; H, 8.00; N, 11.71. Found: C, 80.41, 80.19; H, 7.92, 7.82; N, 11.81, 12.14.

Other Materials.—The iron(III) chlorides of deuteroporphyrin IX dimethyl ester and 2,4-diacetyldeuteroporphyrin IX dimethyl ester,[33] the nickel(II) complex of deuteroporphyrin IX dimethyl ester,[5,34] and the four isomeric tetramethyltetracarbethoxy porphyrins[35] were obtained as described elsewhere. The isomeric tetramethyl porphyrin tetracarboxylic acids were obtained from the tetracarbethoxy compounds and potassium hydroxide in ethylene glycol followed by acidification with hydrochloric acid as kindly carried out by Andre E. Briod.[36] Etioporphyrins III and IV were kindly supplied by Dr. Peter Iber. Protohemin chloride was supplied by L. Light and Co. Ltd. Alumina was Fisher activated alumina No. A-540 unless stated otherwise. All solvents used were reagent grade. Chloroform (J. T. Baker reagent with about 0.2% ethanol present as a preservative) and 1,2-dichloroethane (Matheson Coleman and Bell No. 5636) were stored over calcium oxide for at least twenty-four hours prior to use. Petroleum ether, bp 30–60°, was used.

Results and Discussion

Formyl and Carboxylic Acid Derivatives from the Permanganate Oxidation of Protoporphyrin IX.—Several attempts to prepare formyl derivatives by the permanganate oxidation of protoporphyrin IX have been reported but yields were always very low.[17,22,23] We also found the oxidation of protoporphyrin IX dimethyl ester in acetone under similar conditions to give monoformyl and diformyl products in yields of usually less than 7%. Among several other products was a major product which was soluble in methanol, and even in water as earlier workers had noted, and also exhibited spectra sensitive to pH (*e.g.*, band I appeared at 618 mμ at high pH and at 636 mμ at low pH); both properties suggest the presence of a carboxylic acid substituted directly on the porphyrin ring. (Spectra for the tetramethylporphyrin tetracarboxylic acids, isomer types I, II, III and IV, were similarly sensitive to pH.) Subsequently deuteroporphyrin IX 2,4-dicarboxylic acid tetramethyl ester was isolated. The low yields of formyl derivatives could thus be attributed to their further oxidation to carboxylic acids. The oxidation of aromatic aldehydes is catalyzed by acid and by base[37]; therefore, magnesium sulfate was added to the oxidizing mixture to provide "neutral" conditions,[38] and markedly increased yields of both mono- and diformyl compounds were obtained but no water-soluble carboxylic salt was detected.

Porphyrins with both formyl and vinyl groups attained particular biochemical interest when these groups were found to be present in porphyrin *a*[39,40] as well as in chlorocruoroporphyrin[41] (shown to be 2-formyl-4-vinyldeuteroporphyrin IX).[42] The suggestion that the 2-vinyl or protoporphyrin IX is indeed more reactive toward oxidation than the 4-vinyl was made by Barrett and Clezy,[43] and was subsequently used by Lynen and co-workers in support of their interesting hypothesis of the selective attack at the 2-vinyl of protoporphyrin IX by a farnesyl pyrophosphate, or a similar group, as a step in the formation of the long alkyl group in the course of porphyrin *a* biosynthesis.[39]

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(27) J. F. Taylor, *J. Biol. Chem.*, **135**, 569 (1940).
(28) A. H. Corwin and J. G. Erdman, *J. Am. Chem. Soc.*, **68**, 2473 (1946).
(29) R. W. Cowgill and W. M. Clark, *J. Biol. Chem.*, **198**, 33 (1952).
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(32) H. Fischer and P. Halbig, *ibid.*, **450**, 151 (1926).
(33) W. S. Caughey, J. O. Alben, C. A. Beaudreau, H. Eberspaecher, C. T. Gregg, and B. D. McLees, to be published.
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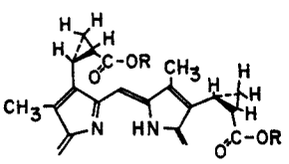
(36) J. S. Andrews, A. H. Corwin, and A. G. Sharp, *J. Am. Chem. Soc.*, **72**, 491 (1950).
(37) K. B. Wiberg and R. Stewart, *ibid.*, **77**, 1786 (1955).
(38) K. B. Wiberg and K. A. Saegebarth, *ibid.*, **79**, 2822 (1957).
(39) M. Grassl, G. Augsburg, U. Coy, and F. Lynen, *Biochem. Z.*, **337**, 35 (1963).
(40) J. L. York and W. S. Caughey, Abstracts of Papers, 145th National Meeting of the American Chemical Society, New York, N. Y., Sept 1963, p 46C.
(41) O. Warburg and E. Negelein, *Biochem. Z.*, **244**, 239 (1932).
(42) H. Fischer and C. V. Seemann, *Z. Physiol. Chem.*, **242**, 133 (1936).
(43) J. Barrett and P. Clezy, *Nature*, **184**, 1988 (1959).

It was thus of interest to examine the relative amounts of chlorocruoroporphyrin and isomeric 4-formyl-2-vinyldeuteroporphyrin IX in the monoformyl monovinyl products. The two isomers could be distinguished by their nmr spectra and were always found in nearly equal amounts. Their relative amounts did vary somewhat from preparation to preparation but the deviations from an equimolar mixture that were observed were small and could be attributed to the concentration of one isomer with respect to the other during crystallization or chromatography steps. Certainly these data do not support the notion of a marked difference in reactivity between 2-vinyl and 4-vinyl groups.

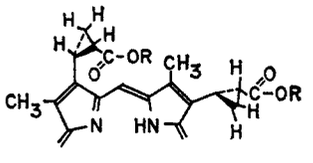
Addition Reactions of the Vinyl Groups of Protoporphyrin IX.—Addition reactions have been useful for the identification of vinyl groups as well as for obtaining useful 2,4-substituted deuteroporphyrins IX, which are frequently more stable than protoporphyrin IX.

Mesoporphyrin IX with 2,4-ethyl groups and hematoporphyrin IX with 2,4-α-hydroxyethyl groups have been widely studied and require little discussion here. A new method for the chromatography of hematoporphyrin IX dimethyl ester and monovinylmono-(α-hydroxyethyl) analogs is described. Also described briefly is the formic acid–palladium oxide–hydrogen method[27-29] used for the preparation of mesoporphyrin IX from hemin, followed by isolation as the ester. Justification for use of this method for the reduction of vinyl groups appears necessary in that Baker, *et al.*,[44,45] have recently suggested that this method is not satisfactory. The product obtained by the method described here, a method only slightly modified from the earlier procedures, appears to be authentic mesoporphyrin IX on the basis of several criteria: elemental analyses, infrared and nmr spectra, an X-ray crystal structure of the methoxy iron(III) derivative,[46] and chromatographic homogeneity in several systems, including the chromatographic procedure used by Baker, *et al.*[44]

Ethyl diazoacetate is a reagent frequently used to detect unsaturation in conjugation with the porphyrin ring.[4f,47] A blue shift in absorption maxima upon treatment with diazoacetate constitutes a positive indication of side-chain unsaturation. However the reaction products have received little study. Fischer and Medick[26] had heated protoporphyrin IX dimethyl ester in ethyl diazoacetate and, after a lengthy isolation procedure which included saponification followed by esterification with diazomethane, isolated in undisclosed yield a product they concluded was 2,4-bis(2-carboxycyclopropyl)deuteroporphyrin IX tetramethyl ester on the basis of elemental analyses, insensitivity toward HBr–acetic acid, and the isolation of an oxidation product with elemental analyses calculated for methylmaleimidecyclopropylcarboxylic acid. Re-examining this reaction, we subjected a mixture obtained from heating protoporphyrin IX dimethyl ester in ethyl diazoacetate to chromatography and found a number of products to be present from which the one major product was isolated. Nmr, infrared, and near-infrared spectra as well as elemental analyses were consistent with 2,4-bis(2-carboxycyclopropyl)-deuteroporphyrin IX 2,4-diethyl 6,7-dimethyl ester. Nmr spectra further indicated that the porphyrin ring and 2-carboxyl groups were in a *cis* relationship to each other at both the 2 and 4 positions. The alkoxyl protons of the cyclopropyl esters were markedly deshielded compared with those of the 6,7 esters, whereas little, if any, deshielding of these alkoxyl protons (presumably as a result of porphyrin ring current field effects) would be expected if the porphyrin and the 2-carboxylic ester groups were *trans*. However, the major product as isolated does contain more than one component. It melts over a range of several degrees and at low temperatures for a porphyrin ester. The observation of *two* resonances for N–H protons and broadness of certain *meso* protons for these derivatives also suggests the presence of isomeric components. Four isomeric components expected are two isomers in which the carboxylic ester of each cyclopropyl group bear a *cis* relationship to each other (I) and two other isomers in which the relationship is *trans* (II). Stereo-



I



II

selectivity in the addition of the carbethoxymethylene to the vinyl group was demonstrated by the structures of the major product, although several other components of as yet unestablished structures also resulted. The addition can be considered subject to kinetic control. The *trans* form is the expected thermodynamically more stable form. The stereochemical findings can be rationalized in terms of a stabilizing interaction between the carbethoxy group and the porphyrin ring in the transition state. Types of possible interactions between substituents on carbenes or "carbenoids" and olefins which could influence the stereochemistry of cyclopropane products have been discussed recently by Closs and Moss.[48]

Ring Substitution Reactions.—The substitution of hydrogens for vinyl groups on heating protohemin chloride in resorcinol provides six peripheral ring positions (α, β, γ, δ, 2, 4) free of alkyl substituents as potential sites for electrophilic substitution reactions.[4e] The resorcinol melt procedure actually gives several products[49] from which deuterohemin chloride can be separated in substantial yield. Deuterohemin in turn can be acylated at the 2,4 positions in good yields, *e.g.*, with stannic chloride in acetic anhydride or propionic anhydride. Deuteroporphyrin IX esters are readily brominated at the 2,4-positions by the pyridinium bromide perbromide method reported here or by bromine–acetic acid.[16] Since nmr spectra of the monosubstituted acetyl and propionyl compounds showed

(44) E. W. Baker, M. Lachman, and A. H. Corwin, *Anal. Biochem.*, **8**, 502 (1964).

(45) E. W. Baker, M. Ruccia, and A. H. Corwin, *ibid.*, **8**, 512 (1964).

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(47) H. Fischer and A. Stern, "Die Chemie des Pyrrols," Vol. II, 2, Hälfte, Akademische Verlagsesgellschaft M. B. H., 1940, p 333.

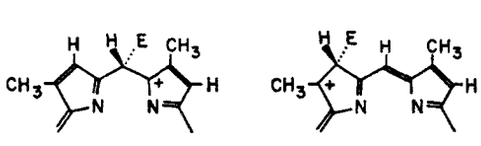
(48) G. L. Closs and R. A. Moss, *J. Am. Chem. Soc.*, **86**, 4042 (1964).

(49) T. C. Chu and E. J.-H. Chu, *ibid.*, **74**, 6276 (1952); K. I. Altman, A. K. Bruce, and K. Salomon, "Porphyrin Biosynthesis and Metabolism," Wolstenholme and Miller, Ed., Little Brown and Co., Boston, Mass., 1955, p 86.

in each case essentially equal amounts of 2 and 4 isomers, there was no evidence of significant difference in the reactivities of the two positions.

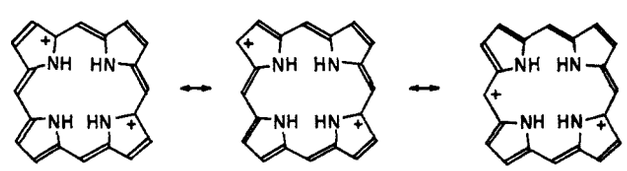
We found deuteroporphyrin IX dimethyl ester was readily nitrated with nitric acid in sulfuric acid at 0°. With equimolar nitric acid and porphyrin, the major product was a mononitro derivative; with the amount of nitric acid doubled, a dinitro porphyrin was the major product. Through analogy to the acylation and bromination reactions, we first expected to find the nitro groups at the 2,4 positions in these products[50] but their nmr spectra indicated the nitro groups were, in fact, at the α and/or β positions. There was no apparent preference between the α and β positions. The α-nitro and β-nitro isomers were present in equal amounts. The nmr spectrum of the dinitro compound had two β protons and only two *meso* protons, with no evidence of the presence of more than one component. Furthermore, δ values for the two high field ring methyls[51,52] were only compatible with nitro groups at the α,β positions. The spectrum for the mononitro product had three *meso* protons and two β protons in each of the two complete sets of spectra present, as expected for two components: the α-nitro and β-nitro isomers. Several minor products were observed in addition to the α,β-dinitro and α- (and β-) nitro derivatives. One such product isolated as crystals (*ca.* 16 mg) exhibited spectra (relative intensities of band maxima IV > III > I > II) and mp 163° similar to the product reported by Fischer and Klendauer[53] for the dimethyl ester of a product isolated from deuteroporphyrin IX and concentrated nitric acid. However, the major products were produced in appreciably greater yields than was indicated by the yields reported for analytically pure materials, since isolation losses were considerable, particularly during chromatography on alumina.

Neither structure nor yields were determined for *all* the products from these electrophilic substitution reactions. Nevertheless little doubt remains that the substitutions on deuterohemin were preferentially at the 2,4 positions rather than the *meso* positions and that the nitration of the protonated metal-free porphyrin was preferentially at the α,β positions rather than the 2,4 positions. *meso* nitration in the case of porphyrins with alkyl groups in all eight β positions is now well established.[51,52] With deuteroporphyrin IX preferential nitration of the α,β positions rather than the γ,δ positions can be rationalized in steric terms. However, the selection of α,β over 2,4 positions is not so convincingly explained by a consideration of steric factors and the likely intermediates III and IV, although metal–nitrogen bonding will impose restrictions on the conformational changes that are energetically possible in the course of an electrophilic substitution reaction, while metal-free porphyrins can deviate more freely from planarity. (The possibility of deviations from planarity was demonstrated by syntheses of N-alkylporphyrins, and their nmr spectra suggested little difference between π-electron delocalization energies of the planar and nonplanar forms.[12] Conformational changes which result from differences in crystal packing forces have been detected in crystallographic studies; the tetragonal and triclinic forms of $\alpha,\beta,\gamma,\delta$-tetraphenylporphin were found in different conformations.[54]) Electrophilic attack at the β positions is expected to be less favorable in the protonated species than in either the hemin or the neutral metal-free species. Upon protonation of the neutral (free-base) species, the ring current field strength effects increase markedly, as noted in nmr spectra[55] and diamagnetic susceptibilities.[56] π-Electron delocalization and distribution of two positive charges about a 20-atom (18 π electrons) outer ring can be represented by structures such as V, VI, and VII. Here, the β-carbon–β-carbon bonds are far less isolated, and are expected to be less susceptible to electrophilic attack, than in the case of the hemins or free-base porphyrins for which X-ray data has shown distances of about 1.35 A, a value only slightly greater than is associated with a bond order of 2, namely 1.335 A.[54] Additional factors of possible influence in the selectivity of these electrophilic substitution reactions include (1) the relative ability of adducts such as III and IV to accommodate the positive charge, possibly more readily achieved in the protonated species (simply by proton loss) than in Fe^{3+} complex; (2) the role(s) of complexes (σ and π) as intermediates.[57] The ability of porphyrins to form molecular complexes has long been known.[4g,58] Further studies can be expected to clarify the importance of these possibilities.



III

IV



V VI VII

These studies are also relevant to reactions of hemeproteins. Electron-transfer and phosphorylation reactions as well as reactions with hydrogen peroxide are examples of reactions which could involve loci at the periphery of the porphyrin ring of the heme moiety.[59] Furthermore, changes at the locus of the iron (oxidation state and ligands) can influence such reactions. Carbonyl stretching frequencies[60] and rates

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(60) J. O. Alben and W. S. Caughey, Abstracts, Symposium on Molecular Structure and Spectroscopy, Columbus, Ohio, 1963, p 21.

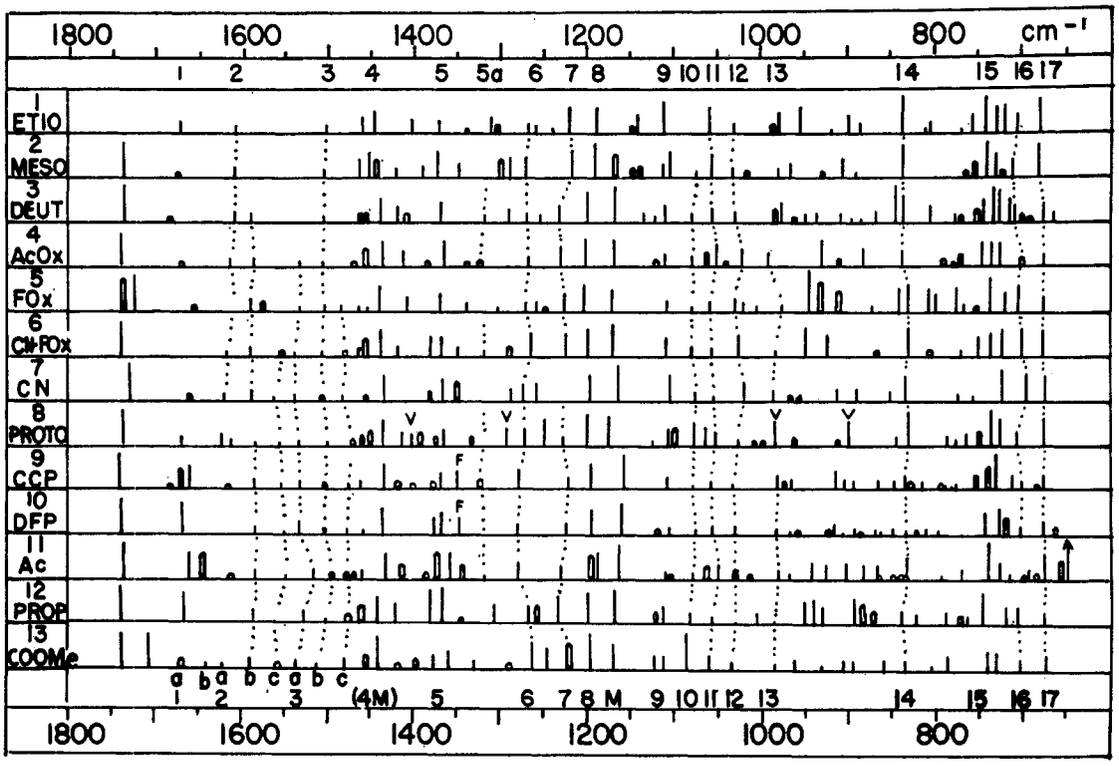

Figure 2.—Representation of porphyrin infrared spectra from 1800 to 635 cm^{-1}. Common bands are numbered for convenience as discussed in the text. Vertical lines represent the frequencies and relative intensities of absorption maxima, compared with the intensities of adjacent bands. Loops represent bands which appear in the spectra as shoulders. Bands marked with a "v" are vinyl deformations; with an "F", formyl CH deformation; and with an "A", acetyl deformation. The abbreviations that were employed for the compounds are as follows: 1-ETIO, etioporphyrin II; 2-MESO, mesoporphyrin IX diethyl ester; 3-DEUT, deuteroporphyrin IX dimethyl ester; 4-AcOX, 2,4-diacetyldeuteroporphyrin IX dimethyl ester dioxime; 5-FOx, 2,4-formyldeuteroporphyrin IX dimethyl ester dioxime; 6-CN-FOx, 2- (and 4-) cyano-4- (and 2-) formyldeuteroporphyrin IX dimethyl ester oxime; 7-CN, 2,4-dicyanodeuteroporphyrin IX dimethyl ester; 8-PROTO, protoporphyrin IX dimethyl ester; 9-CCP, 2-(and 4-) formyl-4- (and 2-) vinyldeuteroporphyrin IX dimethyl ester; 10-DFP, diformyldeuteroporphyrin IX dimethyl ester; 11-Ac, 2,4-diacetyldeuteroporphyrin IX dimethyl ester; 12-PROP, 2,4-dipropionyldeuteroporphyrin IX dimethyl ester; 13-COOMe, 2,4-bis(methoxycarbonyl)deuteroporphyrin IX dimethyl ester.

of reactions[61,62] of carbonyl groups in conjugation with the porphyrin ring have been observed to vary as a result of changes at the metal. Also, in nmr spectra ring current field effects on protons at the periphery of the ring are markedly affected by the nature of the metal ion and by ligand binding.[6,8]

Infrared Spectra.—The development of stringent characterization methods, as well as of practical preparative methods was essential to our investigations. Infrared, electronic, and nmr spectra have been important criteria of structure and purity. Although Mason has proposed infrared assignments for the porphyrin nucleus[63] and others have considered a few group vibrations,[51,52,64-70] infrared spectroscopy has not been exploited very extensively with porphyrins. The series of compounds prepared in this study provided an opportunity to assign stretching and deformation vibrations of substituents at the periphery of the ring, and to consider tentative assignments of bands associated with the ring and common to all the porphyrins examined. The low solubilities of many of the compounds made it necessary to carry out these comparative studies in KBr disks.

The hydrogen stretching region will be considered first. The pyrrole NH, the acetyloxime OH, and the formyl oxime OH bands appear near 3310 cm^{-1}, 3330 cm^{-1}, and 3367 cm^{-1}, respectively. Assignments for the CH stretching vibrations are less certain. A weak band is observed in all compounds in the range 3125–3077 cm^{-1}, which probably includes a methine CH stretching mode, but may also include low intensity combination modes (*cf.* Mason).[63] The isomeric tetramethylporphyrin tetracarboxylic acids, types I, II, III, and IV, in which the only CH bonds are the β-methyl groups and the *meso*-CH groups, all exhibit a methyl asymmetric stretching mode at 2924 ± 9 cm^{-1} and a symmetric vibration at 2857 cm^{-1}. In the corresponding tetramethyltetracarbethoxy derivatives,[35] the symmetric methyl vibration occurs as a very weak shoulder near 2882 cm^{-1}, the β-methyl asymmetric mode remains at 2941–2924 cm^{-1}, and other bands are observed at 2985–2976 cm^{-1} and 2915–2907 cm^{-1} (weak). A band is also observed with etioporphyrins II, III, and IV at 2976–2967 cm^{-1}. This

(61) B. D. McLees, Ph.D. Dissertation, The Johns Hopkins University, 1964; see also ref. 6, page 744.
(62) G. Vanderkooi and E. Stotz, *J. Biol. Chem.*, **240**, 2418 (1965).
(63) S. F. Mason, *J. Chem. Soc.*, 976 (1958).
(64) C. S. Vestling and J. R. Downing, *J. Am. Chem. Soc.*, **61**, 3511 (1939).
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(68) T. C. Chu and E. J.-H. Chu, *J. Biol. Chem.*, **234**, 2751 (1959).
(69) S. Granick (addendum by H. Jaffé), *ibid.*, **236**, 1168 (1961).
(70) I. F. Gurinovich and G. P. Gurinovich, *Optika i. Spektoskipia, Akad. Nauk. SSSR, Otd. Fiz.-Mat. Nauk, Sb. Statei*, **2**, 196 (1963).

band is therefore tentatively assigned to the alkyl methyl asymmetric stretching mode, which is commonly observed near 2962 cm^{-1} [71a,72] (2972–2952 cm^{-1} [73a]). The previously mentioned band at 2915–2907 cm^{-1} is probably due to a methylene asymmetric stretching vibration in the tetracarbethoxy compounds. The methyl asymmetric stretching vibration of the 6,7-propionic acid methyl esters could also be observed. Deuteroporphyrin IX nickel(II) complex (*i.e.*, with free acids at the 6,7 position) showed absorption bands at 2924 cm^{-1} and 2874 cm^{-1}, which correspond to asymmetric and symmetric vibrations, respectively of the β-methyl groups. Deuteroporphyrin IX dimethyl ester nickel(II) complex has bands at 2959 cm^{-1} and 2933 cm^{-1} of equal intensity and a lower intensity band at 2874 cm^{-1}. The band at 2959 cm^{-1} must result from an ester methyl asymmetric stretching vibration (methyl *n*-butyrate shows methyl absorption bands at 2970 cm^{-1} and 2952 cm^{-1} [71a]). In the porphyrin free acids, broad bands near 3100 and 2600 cm^{-1}, characteristic of dimeric carboxylic acids,[71b] are found. The $=CH_2$ and =CH— stretching modes of the vinyl groups of protoporphyrin derivatives are observed at 3106–3077 cm^{-1} and 3012–2976 cm^{-1}, respectively. The former range is similar to that discussed by Bellamy for hydrocarbon derivatives, whereas the latter range is somewhat lower than the range of 3025–3012 cm^{-1} for the alkyl =CH— vibration.[71c] The β-methyl groups of 2,4-diacetyldeuteroporphyrin dimethyl ester derivatives absorb less intensely than the ester methyl groups (near 2950 cm^{-1}), so that the former band appears only as a shoulder. The acetyl methyl groups were detected as a shoulder near 2994 cm^{-1}. In the spectrum of 2,4-diacetyldeuterohemin, which can have no absorption band due to the ester methyl groups, there was a broad shoulder at about 3003 cm^{-1} (assigned to an acetyl methyl vibration) and a β-methyl group absorption peak at 2941 cm^{-1}. β-Formyl groups exhibited a CH absorption at 2747–2740 cm^{-1} in accord with data for *ortho* substituted benzaldehydes.[73b]

The bands in the region between 1800 and 635 cm^{-1} for eleven 2,4-substituted deuteroporphyrin IX dimethyl esters, mesoporphyrin IX diethyl ester, and etioporphyrin II are shown in Figure 2. Ester carbonyl stretching frequencies (1740–1725 cm^{-1}) associated with the 6,7-dipropionic acid esters are similar to those previously reported.[65] The β-formyl, acetyl, and propionyl derivatives gave bands between 1668 and 1660 cm^{-1}, the methoxycarbonyl (compound 13) at 1708 cm^{-1}, and the ethoxycarbonyl of the tetracarbethoxy compounds at 1706–1698 cm^{-1}. These data are also consistent with those of Falk and Willis.[65] It was of interest that the β-carbonyl absorptions for 2,4-disubstituted porphyrins appear to be split in the metal-free compounds, but not in the metal complexes.[74] This would be expected if adjacent pyrrole rings differ in the metal-free porphyrins where protons can be bound to opposite pyrrole rings with negligible intramolecular hydrogen bonding[63,75,76] and if all pyrrole rings are equivalent in the metal complexes.[46,54] The acetyl CO stretching frequencies for 2,4-diacetyldeuteroporphyrin dimethyl ester metal complexes have been found to vary with the type and the oxidation state of the central metal ion.[60]

In the aromatic CC and CN stretching region, etioporphyrin II contains three moderately weak bands at 1672 cm^{-1} (band 1), 1608 cm^{-1} (band 2), and 1504 cm^{-1} (band 3); a band at 1462 cm^{-1} consistent with the asymmetric CH_2 deformation; and a band at 1446 cm^{-1} which is consistent with the asymmetric CH_3 deformation, probably superimposed on a fourth aromatic stretching mode (band 4). Bands 2 and 3 could be followed through most of the series of compounds whereas bands 1 and 4 were frequently difficult to locate owing to overlapping with group vibrations. In compounds with electron-withdrawing substituents in the 2 and 4 positions, bands 2 and 3 appear as multiple peaks. Bands 1–3 each appear as single bands in etioporphyrin (compound 1) and mesoporphyrin (compound 2); in deuteroporphyrin (compound 3) band 2 appeared as two bands, 2a (1609 cm^{-1}) and 2b (1592 cm^{-1}); in the dioxime of diacetyldeuteroporphyrin (compound 4) band 3 appeared as 3a (1534 cm^{-1}) and 3b (1506 cm^{-1}); and in the dioxime of diformyldeuteroporphyrin (compound 5) band 3c (1486 cm^{-1}) was also present. The remaining compounds in the series can be considered similarly. Substituents in the 2 and 4 positions which differ in electron-withdrawing character from the alkyl substituents at the other six β positions can thus result in a splitting of these aromatic vibrations. That the 2,4 substituents do not affect similar bonds equally throughout the porphyrin aromatic system is, of course, not unexpected.

In the spectrum of protoporphyrin IX dimethyl ester at least one of the bands at the position of band 2a (1626 and 1613 cm^{-1}) is assumed to be due to vinyl CC stretching vibration, which is reported to occur near 1625 cm^{-1} if the vinyl group is conjugated to an aromatic ring.[71d] Other bands designated as vinyl bands (marked "v" in Figure 2) due to their unique presence (with their expected frequencies[71e]) are in-plane CH_2 deformation at 1403 cm^{-1} (1420–1410 cm^{-1}), in-plane CH deformation at 1295 cm^{-1} (1300–1290 cm^{-1}), out-of-plane deformation at 986 cm^{-1} (995–985 cm^{-1}), and the out-of-plane CH_2 deformation at 900 cm^{-1} (915–905 cm^{-1}).

The β-methylene asymmetric CH_2 deformation, mentioned for etioporphyrin II, though poorly resolved, may often be located as a shoulder near 1460 cm^{-1} in the substituted deuteroporphyrins; the asymmetric methyl deformation is overlapped and not seen. One-half to one-fourth of a band, which lies between 1443 and 1433 cm^{-1} in the case of the dimethyl ester derivatives, is absent with unesterified porphyrin; this absorption could be assigned to a deformation mode of a CH_2 adjacent to a carbonyl group.[77] Also dipropionyldeuteroporphyrin (12, Figure 2) has a

(71) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1958: (a) p 16; (b) p 163; (c) p 43; (d) p 41; (e) p 34; (f) p 170–172; (g) p 79.

(72) R. N. Jones and C. Sondorfy, "Chemical Applications of Spectroscopy, Technique of Organic Chemistry," Vol. IX, W. West Ed., Interscience Publishers, Inc., New York, N. Y., 1956, p 338.

(73) N. B. Colthup, L. H. Daly, and S. E. W. Berley, "Introduction to Infrared and Raman Spectroscopy," Academic Press Inc., New York, N. Y., 1964: (a) p 202; (b) p 247.

(74) J. O. Alben and W. S. Caughey, unpublished.

(75) J. G. Erdman and A. H. Corwin, *J. Am. Chem. Soc.*, **68**, 1885 (1946)

(76) S. Silvers and A. Tulinsky, *ibid.*, **86**, 927 (1964).

(77) P. J. Corish and W. H. T. Davison, *J. Chem. Soc.*, 927 (1958).

moderately strong band at 1420 cm^{-1}; a weaker band was found for the monopropionyl derivatives. Etioporphyrin (1) and mesoporphyrin (2) have bands at 1403 and 1393 cm^{-1}, respectively, which are presumed ethyl group deformations. Symmetrical CH_3 deformation of the β-methyl groups (band 5, Figure 2) appear between 1381 and 1359 cm^{-1}. A single band was found for compounds 1–5, while in the others it was variously split, suggesting further that, in the case of the more asymmetrically substituted porphyrins, the environments for the four β-methyl groups are not the same. The formyl deuteroporphyrins (compounds 9 and 10) have a sharp band at 1351 cm^{-1} apparently due to the aldehyde group[78] which may represent an in-plane CH deformation.

Support for the identification of the methyl ester single bond stretching bands[77] as band M (1175–1156 cm^{-1}) was obtained through comparisons of the spectra of three metal porphyrin dimethyl ester derivatives with those of the corresponding unesterified complexes and of etioporphyrin II and mesoporphyrin diethyl ester. Diacetyldeuterohemin exhibits a band at 1175 cm^{-1}, the intensity of which is only somewhat decreased from that of the corresponding band (1168 cm^{-1}) of the diester. The former band thus probably corresponds to a band at 1170 cm^{-1}, which Thompson and Torkington[79] found for a series of methyl ketones and assigned to an acetyl group vibration. In addition, the band at 595 cm^{-1} which these authors found for the same series of methyl ketones probably corresponds to a band near 663 cm^{-1} which appears in the spectra of all of the metal complexes of diacetyldeuteroporphyrin,[74] and at 649 cm^{-1} in the spectrum of the metal-free derivative (11). As this band does not appear in the spectra of the other porphyrins examined, in accord with the findings of Thompson and Torkington it can be assigned to an acetyl group deformation.

The carboxyl group vibrations of the free acids are the hydrogen-bonded carbonyl stretching vibration[69,80] near 1700 cm^{-1} and the coupled carboxyl vibrations near 1415 cm^{-1} and 1300 cm^{-1}.[71f] The 3100-cm^{-1} band has been only occasionally observed with the porphyrin derivatives.

Mason[63] suggested that the out-of-plane deformation of the methine (*meso*-) CH occurs in the 853–834 cm^{-1} region and the corresponding pyrrole (β-) CH deformation occurs between 876 and 853 cm^{-1}. We have always found a band between 845 and 830 cm^{-1} (band 14). Mason's assignment of this band appears reasonable and is in agreement with the assignment of the corresponding band in the 1:3:5 trisubstituted benzenes.[71g] Mason's data indicated that this band is split into two bands in the spectra of porphin and of copper porphin; we have observed splitting with the unsubstituted deuteroporphyrin IX complexes, which contain β hydrogens in the 2- and 4-positions. However, aside from this "splitting" there appears to be no evidence for an additional band in the 876–853 cm^{-1} region which according to Mason could be assigned to an out-of-plane β-CH deformation. The region between 950 and 850 cm^{-1} appears to be highly sensitive to the 2,4 substituents of substituted deuteroporphyrin IX esters; three to four medium to low intensity bands, as yet unassigned, have been found between 950 and 880 cm^{-1}.

Assignment of the in plane *meso*-CH deformation modes is more difficult. Mason[63] tentatively suggested that bands which occur at 1224, 1184, and 1048 cm^{-1} in the spectrum of porphin may be due to in-plane CH deformation modes. He reported a band between 1060 and 1057 cm^{-1} in the spectra of porphin, copper porphin, etioporphyrin, and octaethylporphin, which appears to be absent in spectra of all of his *meso*-substituted porphins, and is presumably due to an in-plane *meso*-CH deformation. A similar band (band 11) occurs in all of the deuteroporphins we have examined, usually between 1060 and 1050 cm^{-1} and always between 1065 and 1045 cm^{-1}. While bands which correspond to the 1224 and 1184 cm^{-1} bands of porphin appear to be present in all of Mason's β-substituted porphins, they are not clearly absent in his *meso*-substituted or tetraazaporphins. As corresponding bands also appear in the spectra of the *meso*-tetraphenylporphins reported by Thomas and Martell,[67] we prefer to consider a pair of bands (bands 7 and 8) which occur at 1234–1220 cm^{-1} and 1205–1185 cm^{-1} in the metal-free substituted deuteroporphyrins and within a somewhat wider range in the metal complexes[74] as being characteristic of the porphyrin ring. We therefore give them the general designation of "ring deformation" modes.

A group of one to four moderately intense bands occurs between about 755 and 720 cm^{-1} (band 15), and another band which is sometimes split into two is seen between 720 and 690 cm^{-1} (band 16). These bands or groups of bands probably correspond to what Mason[63] has called "an in-phase combination of an out-of-plane pyrrole ring deformation," and "out-of-phase combinations of in-plane pyrrole ring deformation vibrations," respectively. Certainly bands due to other vibrations may also be present.

Bands 13 and 17 are suggested to represent the in-plane and out-of-plane pyrrole NH deformations, respectively, by comparison of metal-free porphyrins with the corresponding metal complexes.[74] The "in-plane NH deformation band" is weak and in some cases is obscured. The "out-of-plane NH deformation band" gives rise to a moderately strong absorption which is remarkably constant, occurring between 680 and 675 cm^{-1}. It is well isolated from other bands, is seen with every metal-free porphyrin, and is absent with every metal-porphyrin complex which has been studied in this laboratory or reported by Falk and Willis,[65] although an attempt to assign the band has not been made previously.

The detailed consideration of nmr and electronic spectra will be reported elsewhere.

Acknowledgment.—We are greatly indebted to W. Gehret Kleinspehn and William D. Wood for technical assistance with the preparative work and to Susan Lauder for the determination of nmr spectra.

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(79) H. W. Thompson and P. Torkington, *J. Chem. Soc.*, 640 (1945).
(80) R. G. Sinclair, A. F. McKay, and R. N. Jones, *J. Am. Chem. Soc.*, **74**, 2570 (1952).