Substituted Deuteroporphyrins. III. Iron(II) Derivatives. Reactions with Oxygen and Preparations from Chloro- and Methoxohemins*

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ABSTRACT: Properties and reactions of iron porphyrins have been explored with particular emphasis upon the autoxidation of iron(II) compounds. Preparative methods for obtaining iron(III) and iron(II) complexes of high purity from deuteroporphyrin IX dimethyl ester and derivatives with acetyl, ethyl, or vinyl groups as 2,4-substituents have been investigated; iron(III) compounds with chloro, bromo, methoxo, or ethoxo ligands and iron(II) compounds with one or two pyridine ligands were prepared. In the solid, the iron(II) compounds lost pyridine ligands upon gentle heating under vacuum to give products which tended to add oxygen. Solids with one oxygen molecule per heme and no pyridine ligands and solids with one pyridine and one oxygen atom per heme were observed. Examination of autoxidation rates of the iron(II) compounds dissolved in twelve solvents of widely differing polarity showed that the reaction was dependent upon the protic character, but not the polarity, of the solvent. The electron-withdrawing character of porphyrin substituents appeared to influence reactions both in solids and in solutions. Evidence that the structure of the product of autoxidation in benzene was a dimer bridged by a single oxygen atom was found. These results are considered in terms of mechanisms of autoxidations.

The porphyrins of heme proteins are iron complexes of substituted deuteroporphyrins IX (Falk, 1964; Caughey *et al.*, 1966a). Studies of the sensitivity of properties of these iron porphyrins to differences in groups at the periphery of the porphyrin ring, in axial ligands, and in medium thus become important for evaluation of structure–function relationships of heme proteins (Caughey *et al.*, 1965). Here we report the preparation and characterization of iron(II) and iron(III) complexes of variously substituted deuteroporphyrins, as well as studies on structure and medium effects on oxygenation and autoxidation reactions.

A marked difference in the tendency toward autoxidation for protoheme compared with pyridine heme A has been noted (Caughey and York, 1962). We here report in greater detail the effects of differences in 2,4-substituent and in solvent on reactions with oxygen, utilizing iron(II) complexes of esters of protoporphyrin IX and other deuteroporphyrins with ethyl, hydrogen, or acetyl groups at the 2,4 positions. Thermal dissociation of pyridine and subsequent addition of molecular oxygen have also been discussed for pyridine heme A (York *et al.*, 1967); solid-state oxygenation studies on other dipyridine and monopyridine hemes are reported here.

A portion of this work has appeared in a preliminary communication (Caughey *et al.*, 1965). Mössbauer spectra (Bearden *et al.*, 1965; Caughey *et al.*, 1968), electronic spectra (Caughey and McCoy, 1966a,b; Caughey *et al.*, 1968), and zero-field-splitting values (Richards *et al.*, 1967; Caughey *et al.*, 1968) have been reported for some of the compounds described herein, as have a crystallographic study of methoxomesoporphyrin IX dimethyl ester iron(III) (Hoard *et al.*, 1965) and a crystal spectrum of the same compound (Day *et al.*, 1967).

Experimental Section

All melting points are corrected and were determined on a hot stage (Nalge–Axelrod) apparatus. Infrared spectra were obtained in KBr disks with either a Perkin-Elmer Model 21 spectrometer fitted with a NaCl prism or a Perkin-Elmer Model 521 grating spectrometer. Spectrophotometric studies in the visible and ultraviolet regions were carried out with either a Beckman DK-2, Cary Model 11, or Cary Model 14 spectrophotometer. Nuclear magnetic resonance spectra were obtained with a Varian HA-100 spectrometer.

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Iron analyses were obtained by Drabkin's (1941) method. Analyses for other elements were carried out by Joseph Walter, Dr. S. N. Nagy, and Schwarzkopf Microanalytical Laboratories. Mass spectroscopy was carried out by the Morgan–Schaffer Corp.

Materials. The preparation and characterization of metal-free porphyrins used have been described (Caughey *et al.*, 1966a). Fisher activated alumina (A-540) was used for chromatography. Iron metal with 92.8% ^{57}Fe was obtained from Oak Ridge National Laboratory. All solvents and other reagents were reagent grade or equivalent. Chloroform (J. T. Baker, reagent grade, stabilized with *ca.* 0.2% ethanol) and 1,2-dichloroethane (Matheson Coleman and Bell 5636) were stored over calcium oxide for at least 24 hr prior to use. Reagent grade solvents were further purified by fractional distillation for use in the preparation of the iron(II) porphyrins, for the determination of spectra and in the autoxidation studies.

Preparation of Iron(III) Compounds. CHLORO-2,4-DIACETYLDEUTEROPORPHYRIN IX DIMETHYL ESTER IRON-(III). A ferrous acetate solution (prepared by heating 1.6 g of iron powder in 62 ml of acetic acid under nitrogen) was added to a solution of 2,4-diacetyldeuteroprophyrin IX dimethyl ester (4 g) and sodium chloride (0.8 g) in acetic acid (55 ml) under reflux. The mixture was maintained under reflux for 2 hr and then allowed to cool. Crystals were collected, were washed first with 100 ml of 30% aqueous acetic acid and then with water (100 ml) until chloride ion was no longer detected in the washings, and were dried under vacuum at 40°; yield, 3.92 g; in chloroform, λ_{max} (A/A_{516}): 890 (0.053), 643 (0.44), 545 (0.98), 516 (1.00), and 419 mμ (7.6).

Anal. Calcd for $C_{36}H_{36}FeClN_4O_6$: C, 60.73; H, 5.10; Cl, 4.98; Fe, 7.84; N, 7.87. Found: C, 60.63; H, 5.06; Cl, 4.77; Fe, 7.78; N, 8.22.

Chloro-2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(III) with iron 92.8% ^{57}Fe was prepared in like manner from the porphyrin and iron powder containing 92.8% ^{57}Fe, followed by recrystallization from chloroform–methanol. An electronic spectrum in chloroform was identical with the spectrum obtained for the compound prepared with natural iron.

Anal. Found: Fe, 7.84.

BROMO-2,4-DIACETYLDEUTEROPORPHYRIN IX DIMETHYL ESTER IRON(III). From 2,4-diacetyldeuteroporphyrin IX dimethyl ester and a ferrous acetate solution containing sodium bromide, the bromo compounds containing both iron enriched to 92.8% with ^{57}Fe and unenriched iron were prepared by methods analogous to those used to prepare the corresponding chloro compounds; in chloroform, λ_{max} (A/A_{512}): 902 (0.051), 643 (0.38), 547 (0.91), 512 (1.00), and 419 mμ (5.8).

Anal. Calcd for $C_{36}H_{36}BrFeN_4O_6$: C, 57.15; H, 4.80; Br, 10.56; N, 7.40. Found: C, 57.90; H, 5.10; Br, 9.71; N, 7.89.

CHLORODEUTEROPORPHYRIN IX DIMETHYL ESTER IRON-(III). A ferrous acetate solution was prepared by heating 0.75 g of iron wire in acetic acid (25 ml). The hot ferrous acetate solution was added slowly to a gently refluxing solution of deuteroporphyrin IX dimethyl ester (2.5 g) and sodium chloride (0.5 g) in acetic acid (25 ml). Refluxing was continued for 40 min. Crystals which formed on cooling the solution to room temperature were collected, washed successively with acetic acid saturated with sodium chloride (10 ml), saturated aqueous sodium chloride (20 ml), and water (20 ml), and dried at 40° under vacuum.

The dried product (2.7 g) was dissolved in benzene (50 ml) and added to a column of alumina (300 g) wetted with benzene. Benzene (500 ml) was passed through the column, followed by elution with 1,2-dichloroethane until all unreacted metal-free porphyrin was removed (a red zone) from the column (at this point the zone for the major component was part way down the column). The top portion of the column, containing a small green zone, was removed and the major component was eluted from the remainder of the column with chloroform. Evaporation of the chloroform left a residue which was dissolved in pyridine (30 ml) and added slowly to a hot suspension of sodium chloride (1.5 g) in acetic acid (75 ml). Hot aqueous sodium chloride (75 ml), a saturated solution at room temperature, was added slowly to the porphyrin solution. The mixture was kept at the boiling point for 10 min and then allowed to cool slowly to room temperature. Crystals were collected, washed successively with 100 ml 30% aqueous acetic acid saturated with sodium chloride, 100 ml of saturated aqueous sodium chloride, 300 ml of water, and 10 ml of methanol, and dried at 40° under vacuum; yield, 2.1 g; mp 240° (lit. (Fischer and Hummel, 1929) mp 248–249, 251°, and (Paul, 1958) 251°); in chloroform, λ_{max} (A/A_{507}): 903 (0.060), 632 (0.49), 532 (1.03), 507 (1.00), and 379 mμ (9.7).

Anal. Calcd for $C_{32}H_{32}ClFeN_4O_4$: C, 61.21; H, 5.14; Cl, 5.65; N, 8.92. Found: C, 61.11; H, 5.23; Cl, 5.43; N, 9.01.

METHOXODEUTEROPORPHYRIN IX DIMETHYL ESTER IRON(III). Chlorodeuteroporphyrin IX dimethyl ester iron(III) (500 mg) in benzene was added to an alumina column (2 × 25 cm) wetted with benzene. Elution with benzene (100 ml) and then with 1,2-dichloroethane did not develop any hemin zones. Hemin was eluted from the column with chloroform; the eluate exhibited an absorption spectrum of the hematin type. The residue obtained on evaporation was crystallized from chloroform–methanol; yield, 430 mg; in chloroform, λ_{max} (A/A_{571}): 762 (0.035), 571 (1.00), and 394 mμ (9.6).

Anal. Calcd for $C_{33}H_{35}FeN_4O_5$: C, 63.57; H, 5.66; N, 8.99. Found: C, 63.22; H, 5.72; N, 8.78.

Crude acetatodeuteroporphyrin IX dimethyl ester iron(III), prepared in the same manner as the crude chloroiron(III) derivative but in the absence of added chloride, was chromatographed in like manner and crystallized from chloroform–methanol; in chloroform, λ_{max} (A/A_{571}): 762 (0.036), 571 (1.00), and 393 mμ (9.7).

Anal. Found (number of values averaged): C, 63.45 (3); H, 5.63 (3); N, 9.32 (2).

ETHOXODEUTEROPORPHYRIN IX DIMETHYL ESTER IRON-

(III). A sample of crude acetatodeuteroporphyrin IX dimethyl ester iron(III) was chromatographed on alumina with 1,2-dichloroethane to remove free porphyrin, eluted with chloroform, filtered, dried, and crystallized from chloroform–ethanol; in chloroform, λ_{max} (A/A_{570}): 756 (0.033), 570 (1.00), and 393 mμ (9.6).

Anal. Calcd for $C_{34}H_{37}FeN_4O_5$: C, 64.05; H, 5.85; N, 8.79. Found: C, 64.13; H, 5.83; N, 8.87.

CHLOROMESOPORPHYRIN IX DIMETHYL ESTER IRON(III). Crude chloroiron(III) complex (1 g) obtained by introduction of iron into mesoporphyrin IX dimethyl ester by the method of Corwin and Erdman (1946), a method similar to the method used above to prepare the chlorodiacetyldeuterohemin, was dissolved in benzene (50 ml) and added to an alumina column wetted with benzene. Elution with 1,2-dichloroethane–benzene (1:1, v/v) removed metal-free porphyrin (*ca.* 16 mg) from the column. 1,2-Dichloroethane–chloroform (1:1, v/v) removed the major component, which exhibited an absorption spectrum of the hematin type, in the eluate. The residue from evaporation was dissolved in pyridine (8 ml) and added to glacial acetic acid (13 ml) containing sodium chloride (0.25 g). The mixture was heated, hot saturated aqueous sodium chloride (13 ml) was added slowly, and heating at the boiling point was continued with evaporation to the point of incipient precipitation. Crystals obtained on cooling were washed successively with 25 ml of 30% aqueous acetic acid saturated with sodium chloride, 20 ml of saturated aqueous sodium chloride, 35 ml of water, and 1.5 ml of methanol, and dried at 40° under vacuum; yield, 449 mg; mp 242° (lit. (Fischer and Stangler, 1927) mp 246° and (Erdman and Corwin, 1947) 244.3–246.8°); in chloroform, λ_{max} (A/A_{507}): 919 (0.055), 635 (0.50), 534 (1.03), 507 (1.00), and 379 mμ (11.1).

Anal. Calcd for $C_{36}H_{40}ClFeN_4O_4$: C, 63.12; H, 5.89; Cl, 5.18; N, 8.19. Found: C, 62.93; H, 5.87; Cl, 5.14; N, 8.47.

Chloromesoporphyrin IX dimethyl ester iron(III) with 92.8% ^{57}Fe was prepared in like manner from the porphyrin and iron powder with 92.8% ^{57}Fe. An electronic spectrum of the product in chloroform was identical with the spectrum obtained for the compound with natural iron.

METHOXOMESOPORPHYRIN IX DIMETHYL ESTER IRON(III). Crude chloromesoporphyrin IX dimethyl ester iron(III) (867 mg) was dissolved in benzene (100 ml) and added to an alumina column wetted with benzene. After 100 ml of benzene had been added to the column, benzene–1,2-dichloroethane (1:1) was used to elute metal-free porphyrin ester from the column. The major component was eluted from the column with 1,2-dichloroethane. The residue obtained on evaporation was washed with isooctane and dissolved in hot chloroform (4 ml). Hot methanol (12 ml) was added and the solution was allowed to cool slowly. Crystals of suitable size and quality for X-ray crystallography were obtained; yield, 617 mg; in chloroform, λ_{max} (A/A_{576}): 763 (0.033), 576 (1.00), and 394 mμ (9.7).

Anal. Calcd for $C_{37}H_{43}FeN_4O_5$: C, 65.39; H, 6.38; Fe, 8.22; N, 8.24. Found: C, 65.19; H, 6.13; Fe, 8.20; N, 8.13.

CHLOROPROTOPORPHYRIN IX DIMETHYL ESTER IRON(III). A ferrous acetate solution, prepared by heating 1.5 g of iron powder in acetic acid (50 ml), was added slowly to a gently refluxing solution of protoporphyrin IX dimethyl ester (5 g) and sodium chloride (1 g) in acetic acid (65 ml). After a few minutes, the mixture was cooled slowly to room temperature. Crystals were collected, washed with 30% aqueous acetic acid and with water until no chloride was detected in the washings, and dried under vacuum at 40°; yield, 5.2 g. A portion (1.0 g) of this product was dissolved in benzene (25 ml) and added to an alumina column wetted with benzene. After 600 ml of benzene had passed through the column, benzene–1,2-dichloroethane (1:1, v/v) was used to elute a small zone of metal-free protoporphyrin IX dimethyl ester from the column. The major component was eluted from the column with benzene–1,2-dichloroethane (1:3, v/v). The eluate fraction exhibited a hematin-like spectrum. The residue from evaporation was washed with isooctane, dissolved in pyridine (5 ml), and added slowly to a refluxing solution of sodium chloride (250 mg) in acetic acid (12.5 ml). Saturated aqueous sodium chloride (12.5 ml) was added, and the mixture was heated at the boiling point, and then allowed to cool slowly to 4°. The solid was collected, washed successively with 25 ml of 30% aqueous acetic acid saturated with sodium chloride, saturated aqueous sodium chloride, and water, and dried under vacuum at 33°; yield, 401 mg. The electronic spectrum in chloroform was identical with that of the diethyl ester described below.

Anal. Calcd for $C_{36}H_{36}ClFeN_4O_4$: C, 63.58; H, 5.34; N, 8.24. Found: C, 63.00; H, 5.35; N, 8.33.

CHLOROPROTOPORPHYRIN IX DIETHYL ESTER IRON(III). Protoporphyrin IX diethyl ester was treated in a manner similar to that used to prepare the crude chloroprotoporphyrin IX dimethyl ester iron(III); in chloroform λ_{max} (A/A_{512}): 917 (0.056), 691 (0.49), 539 (0.99), 512 (1.00), and 387 mμ (9.9).

Anal. Calcd for $C_{38}H_{40}ClFeN_4O_4$: C, 64.46; H, 5.70; Cl, 5.01; N, 7.91. Found: C, 64.14; H, 5.63; N, 7.34. Found after exposure to air in the dark for 8 years: C, 64.19; H, 5.96; Cl, 5.09; N, 8.00.

METHOXOPROTOPORPHYRIN IX DIMETHYL ESTER IRON(III). Crude chloroprotoporphyrin IX dimethyl ester iron(III) (1.0 g) in benzene (20 ml) was added to an alumina column wetted with benzene–1,2-dichloroethane (1:1, v/v). Elution with 1,2-dichloroethane first removed a very small amount of metal-free protoporphyrin IX dimethyl ester and then the major component, which was isolated by evaporation of the eluate followed by dissolving the residue in hot chloroform (4 ml), addition of hot methanol (12 ml), and cooling to give crystals (617 mg); in chloroform, λ_{max} (A/A_{577}): 767 (0.030), 577 (1.00), and 403 mμ (8.8).

Anal. Calcd for $C_{37}H_{39}FeN_4O_5$: C, 65.78; H, 5.82; N, 8.29. Found: C, 65.64; H, 5.52; N, 8.34.

Preparation of Iron(II) Compounds. DIPYRIDINE 2,4-DIACETYLDEUTEROPORPHYRIN IX DIMETHYL ESTER IRON(II). Chloro-2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(III) (5.5 g) was dissolved in pyridine (475 ml). Water (720 ml) was added, followed by sodium dithionite (9 g, Eastman 90%). After 15 min, the heme was extracted from the mixture with benzene (200 ml, two times). The benzene extract was washed four times with 500 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 (3.5 × 30 cm) of anhydrous sodium sulfate and evaporated to dryness on a flash evaporator at 45°. The residue was heated under vacuum at 60° for 18 hr; yield, 6.4 g.

Anal. Calcd for $C_{46}H_{46}FeN_6O_6$: C, 66.19; H, 5.55; Fe, 6.69; N, 10.07. Found: C, 66.06; H, 5.72; Fe, 6.54; N, 10.22.

Dipyridine 2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(II) with iron 92.8% ^{57}Fe was prepared in like manner from chloro-2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(III) with 92.8% ^{57}Fe. The product was crystallized from pyridine–petroleum ether (1:5, v/v). The electronic spectrum in benzene–pyridine was identical with the spectrum obtained for the compound containing unenriched iron.

DIPYRIDINE MESOPORPHYRIN IX DIMETHYL ESTER IRON(II). Methoxomesoporphyrin IX dimethyl ester iron(III) (300 mg) in pyridine (24 ml) was carried through a procedure analogous to the procedure used for the preparation of the dipyridine diacetyl derivative. The heme solution was evaporated to dryness in a flash evaporator at room temperature and the residue was then heated at 33° under vacuum (~0.1 mm) for 14 hr; yield, 336 mg.

Anal. Calcd for $C_{46}H_{50}FeN_6O_4$ (dipyridine): C, 68.48; H, 6.25; N, 10.42. Found: C, 68.15; H, 5.93; N, 10.57. Found after exposure to air for 2 months: C, 68.33; H, 6.60; N, 10.56.

PYRIDINEDEUTEROPORPHYRIN IX DIMETHYL ESTER IRON(II). Chlorodeuteroporphyrin IX dimethyl ester iron(III) (0.3 g) in pyridine (15 ml) was carried through a procedure analogous to the procedure used for the preparation of the dipyridine diacetyl derivative. The residue was heated at 60° under vacuum (1 mm) for 14 hr; yield, 205 mg; mp 118°.

Anal. Calcd for $C_{42}H_{42}FeN_6O_4$ (dipyridine): C, 67.20; H, 5.64; N, 11.20. Calcd for $C_{37}H_{37}FeN_5O_4$ (monopyridine): C, 66.15; H, 5.55; N, 10.43. Found: C, 66.25; H, 5.70; N, 10.81.

Chlorodeuteroporphyrin IX dimethyl ester iron(III) (150 mg) in pyridine (13 ml), treated identically except for heating the residue at 55° under vacuum from a water aspirator followed by an additional hour of heating at 50° under higher vacuum (~1 mm), gave 130 mg of the pyridine iron(II) compound.

Anal. Found: C, 66.59; H, 5.54; N, 11.01.

PYRIDINATED PROTOPORPHYRIN IX DIMETHYL ESTER IRON(II). Methoxoprotoporphyrin IX dimethyl ester iron(III) (300 mg) in pyridine (8 ml) was carried through a procedure analogous to the procedure used for the preparation of the dipyridine diacetyl derivative. The residue was heated at 56° under vacuum (1 mm) for 19 hr; yield, 320 mg.

Anal. Calcd for $C_{46}H_{46}FeN_6O_4$ (dipyridine): C, 68.83; H, 5.78; Fe, 6.96; N, 10.47. Calcd for $C_{41}H_{41}FeN_5O_4$ (monopyridine): C, 68.05; H, 5.71; Fe, 7.72; N, 9.68. Calcd for $C_{41}H_{43}FeN_5O_5$ (pyridine–aquo complex): C, 66.39; H, 5.84; Fe, 7.53; N, 9.44. Calcd for $(C_{41}H_{41}FeN_5O_5)_{1\text{ or }2}$: C, 66.61; H, 5.59; Fe, 7.55; N, 9.47. Found: C, 66.87, 67.08; H, 5.56, 5.64; Fe, 7.49, 7.14; N, 9.98, 9.85.

Chloroprotoporphyrin IX dimethyl ester iron(III) (202 mg) in pyridine (6 ml) was carried through a similar procedure. The residue was first taken to dryness at 22° under vacuum from water aspirator and was then heated at 33° under vacuum (0.1 mm) for 29 hr; yield, 152 mg.

Anal. Found: C, 67.41, 65.63; H, 5.75, 5.48; N, 9.65, 9.49.

Preparation of Oxygenated Derivatives. OXYGEN ADDUCT OF 2,4-DIACETYLDEUTEROPORPHYRIN IX DIMETHYL ESTER IRON. Dipyridine 2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(II) (394 mg) was heated under vacuum at 68° to constant weight, and afterwards under vacuum at 120° for 6 hr and at 135° for 9 hr and finally exposed to the atmosphere. The weight loss was 80 mg, and 80 mg of a colorless liquid (undoubtedly pyridine) was collected in a trap immersed in acetone–Dry Ice. The weight loss expected for the loss of 2 moles of pyridine/mole is 75 mg. The sample was analyzed after several days' exposure to the atmosphere.

Anal. Calcd for $C_{36}H_{36}FeN_4O_6$ (bare iron[1]): C, 63.91; H, 5.36; Fe, 8.25; N, 8.28; O, 14.19. Calcd for $(C_{36}H_{36}FeN_4O_7)_{1\text{ or }2}$: C, 62.43; H, 5.24; Fe, 8.06; N, 8.09; O, 16.17. Calcd for $C_{36}H_{36}FeN_4O_8$ (O_2 adduct without pyridine): C, 61.03; H, 5.12; Fe, 7.88; N, 7.91; O, 18.06. Found: C, 61.14; H, 5.14; N, 8.02. Found after exposure to air for 9 months: C, 61.28; H, 5.30; N, 7.94

A similar sample, heated under vacuum for 4 hr at 120°, followed by exposure to air, gave the analysis: C, 60.70; H, 5.12; Fe, 7.63; N, 7.94; O, 18.75.

OXYGENATED MESOPORPHYRIN IX DIMETHYL ESTER IRON. Chloromesoporphyrin IX dimethyl ester iron(III) (150 mg) in pyridine (8 ml) was treated as in the preparation of the dipyridine iron(II) compound. The residue was heated at 68° under vacuum (~1 mm) for 20 hr, mp 109.5–110.5°.

Anal. Calcd for $C_{41}H_{45}FeN_5O_4$ (monopyridine): C, 67.67; H, 6.23; N, 9.62. Calcd for $C_{36}H_{40}FeN_4O_4$ (bare iron): C, 66.67; H, 6.22; N, 8.64. Calcd for $(C_{41}H_{45}FeN_5O_5)_{1\text{ or }2}$: C, 66.22; H, 6.10 N, 9.42. Calcd for $C_{41}H_{45}FeN_5O_6$ (O_2 adduct monopyridine): C, 64.82; H, 5.97; N, 9.22. Calcd for $C_{36}H_{40}FeN_4O_6$ (O_2 adduct without pyridine): C, 63.53; H, 5.92; N, 8.23. Found:

[1] The term "bare iron" is used to signify a heme compound with no axial ligands.

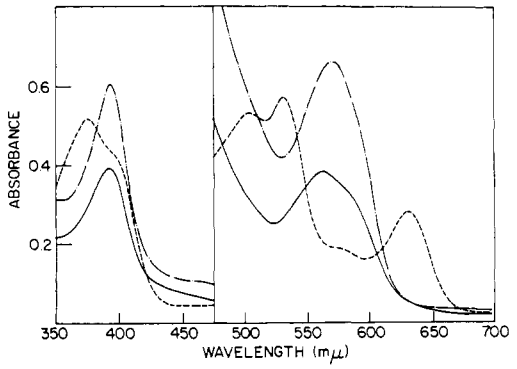


FIGURE 1: Electronic spectra in chloroform of deuteroporphyrin IX dimethyl ester iron(III) derivatives: chloroform eluate from alumina chromatography (———), chloroiron (III) (– – –), and methoxoiron(III) (—·—·). The eluate was filtered, concentrated, precipitated by addition of isooctane, and dried at room temperature under vacuum before the spectrum was recorded.

C, 67.10; H, 6.36; N, 8.81. Found after exposure to air for 2 months: C, 65.84; H, 6.13; N, 9.49.

OXYGENATED DEUTEROPORPHYRIN IX DIMETHYL ESTER IRON. A sample of pyridinedeuteroporphyrin IX dimethyl ester iron(II) was heated at 100° under vacuum for 3 hr and exposed to air.

Anal. Calcd for $C_{32}H_{32}FeN_4O_4$ (bare iron): C, 64.87; H, 5.44; N, 9.46. Calcd for $C_{37}H_{37}FeN_5O_6$ (O_2 adduct monopyridine): C, 63.16; H, 5.30; N, 9.95. Calcd for $(C_{32}H_{32}FeN_4O_5)_{1\text{ or }2}$: C, 63.17; H, 5.30; N, 9.21. Calcd for $C_{32}H_{32}FeN_4O_6$ (O_2 adduct without pyridine): C, 61.54; H, 5.17; N, 8.97. Found: C, 62.01; H, 5.21; N, 9.07.

OXYGEN ADDUCT OF PROTOPORPHYRIN DIMETHYL ESTER IRON. A sample of pyridinated protoporphyrin dimethyl ester prepared from the methoxoiron(III) compound was heated at 63° under vacuum for 23 hr and exposed to air.

Anal. Calcd for $C_{41}H_{41}FeN_5O_6$ (O_2 adduct monopyridine): C, 65.16; H, 5.47; N, 9.27. Calcd for $(C_{36}H_{36}FeN_4O_5)_{1\text{ or }2}$: C, 66.61; H, 5.59; N, 9.47. Calcd for $C_{36}H_{36}FeN_4O_6$ (O_2 adduct without pyridine): C, 63.91; H, 5.36; N, 8.28. Found: C, 64.10; H, 5.69; N, 8.84.

A sample of pyridinated protoporphyrin dimethyl ester iron(III) prepared from the chloroiron(III) compound was heated at 63° under vacuum for 23 hr and exposed to air.

Anal. Found: C, 64.67; H, 5.74; N, 9.04.

Electronic Spectra of Dipyridineiron(II) Compounds. Concentrated stock solutions, freshly prepared in pyridine from pyridinated iron(II) compounds, were diluted with benzene and their spectra were recorded on a Beckman DK-2 spectrophotometer.

Preparation of Pyridinecarbonyliron(II) Compounds. Carbonyl derivatives in solution were prepared by addition of concentrated pyridine stock solutions, freshly prepared from solid pyridinated iron(II) derivatives, to benzene or benzene–pyridine saturated with carbon monoxide (*ca.* 6.7 mM in carbon monoxide), and their spectra were recorded on a Beckman DK-2 spectrophotometer.

Solid pyridinecarbonyl derivatives for infrared spectra were prepared from solutions of solid pyridinated porphyrin ester iron(II) (a few milligrams) added to pyridine (0.2–0.5 ml), which were diluted tenfold with benzene and exposed to carbon monoxide until the reactions appeared complete. Solvent was removed by gentle heat and the residues were cooled; both operations were performed under carbon monoxide. A portion of the solid (~1 mg) was mixed with potassium bromide (~300 mg) and pressed under vacuum to form a pellet; *no* carbon monoxide atmosphere was present during pellet preparation.

Relative Rates of Autoxidation. Stock solutions of pyridinated iron(II) derivatives (1.2–1.8 mM), freshly dissolved in pyridine, were diluted into appropriate aerobic solvents. Final pyridine concentration was either 50 or 1%. Final concentrations in all solvents were 1.10×10^{-5} M for the diacetyldeuteroporphyrin iron(II) derivative, 5.25×10^{-5} M for the mesoporphyrin iron(II) derivative, and 1.05×10^{-5} M for the protoporphyrin iron(II) derivative. Rates of change were followed for 20–30 sec after dilution, by means of a Cary Model 14 spectrophotometer set at 435 mμ (Soret band of diacetyldeuteroporphyrin iron(II) derivative), 546 mμ (α-peak of mesoporphyrin iron(II) derivative), or 419 mμ (Soret band of protoporphyrin iron(II) derivative).

Autoxidation Product from Dipyridine 2,4-Diacetyldeuteroporphyrin IX Dimethyl Ester Iron(II). Dipyridine 2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(II) (500 mg) was dissolved in benzene (50 ml), and oxygen was bubbled through the solution for 15 sec. The solution was evaporated to dryness; the residue was redissolved in 50 ml of benzene and the procedure was repeated four times, except the solution was allowed to stand exposed to air 10 min each time instead of oxygen. The residue was redissolved in 15 ml of benzene, and the solution was allowed to evaporate slowly for 3 days in the dark. Crystals were collected and dried under vacuum (~15 mm) at 60° for 2 hr; yield, 265 mg; in benzene, λ_{max} (A/A_{582}): 582 mμ (1.00) and 416 mμ (7.5); in chloroform, λ_{max} (A/A_{579}): 579 mμ (1.00) and 414 mμ (6.7); no distinct near-infrared band was observed.

Anal. Calcd for $(C_{41}H_{41}FeN_5O_7)_{1\text{ or }2}$: C, 63.82; H, 5.36; N, 9.08. Calcd for $(C_{41}H_{41}FeN_5O_6)_2O$: C, 64.49; H, 5.41; N, 9.17. Calcd for $(C_{36}H_{36}FeN_4O_6)_2O$: C, 63.16; H, 5.30; N, 8.18. Found after further heating under vacuum (60°, 2 hr): C, 63.28; H, 5.54; N, 8.91. Found after further heating under vacuum (100°, 1 hr): C, 63.30; 62.91, 63.16; H, 5.42, 5.45, 5.40; N, 8.84, 8.79, 8.31. Found after exposure to air for over 2 years: C, 63.06; H, 5.34.

The mother liquor was treated with two volumes of petroleum ether. The precipitate was dried as before.

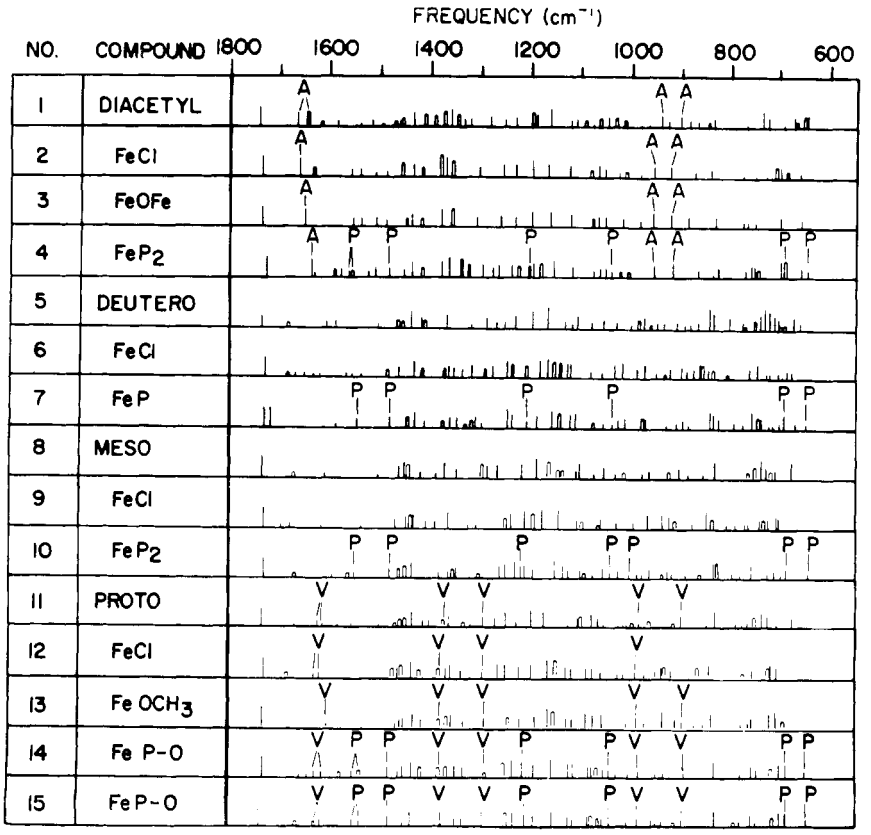


FIGURE 2: Schematic presentation of infrared spectra obtained in KBr for iron(III) and iron(II)-substituted deuteroporphyrin dimethyl esters, from 1800 to 635 cm^{-1}. Vertical lines represent frequencies and relative intensities of absorption maxima; loops represent shoulders. Abbreviations employed are: DIACETYL, 2,4-diacetyldeuteroporphyrin IX dimethyl ester; DEUTERO, deuteroporphyrin IX dimethyl ester; MESO, mesoporphyrin IX diethyl ester; PROTO, protoporphyrin IX dimethyl ester; and P, pyridine. Iron compounds are listed beneath the parent porphyrin; all mesoporphyrin compounds except the metal-free porphyrin are dimethyl esters. Compound **14** is pyridinated protoporphyrin ester iron(II) prepared from the corresponding chloroiron(III) derivative. Compound **15** was prepared from the methoxoiron(III) derivative. Vinyl group absorptions are marked V; acetyl group absorptions are marked A; pyridine group absorptions are marked P. Absorptions due to pyridine were assigned by comparison with the data of Gill *et al.* (1961).

Anal. Found after exposure to air for over 2 years: C, 63.35; H, 5.15.

The same product was obtained by the following independent means. To a refluxing ferrous acetate solution (prepared by heating 260 mg of iron powder in 250 ml of acetic acid under nitrogen) was added 2.20 g of 2,4-diacetyldeuteroporphyrin IX dimethyl ester. The mixture was maintained under reflux for 45 min, cooled, and evaporated to dryness. The absence of metal-free porphyrin was shown by a column chromatographic system (1:1, 1,2-dichloroethane–chloroform on alumina) which resolved a known mixture of the product and metal-free porphyrin. The crude presumed acetatoiron(III) derivative thus obtained was chromatographed on alumina (50 g) with chloroform. The eluate, which exhibited a hematin-type electronic spectrum, was filtered, evaporated to dryness, and crystallized from chloroform–methanol (1:10). Crystals were heated under vacuum at 50° for 2 hr; yield, 1.65 g; in chloroform, λ_{max} (A/A_{579}): 579 mμ (1.00) and 413 mμ (7.2); no distinct near-infrared band was observed.

Anal. Calcd for $C_{37}H_{39}FeN_4O_7$ (methoxo compound): C, 62.81; H, 5.56; N, 7.92. Calcd for $(C_{36}H_{36}FeN_4O_6)_2O$: C, 63.16; H, 5.30; N, 8.18. Found: C, 63.23, 63.19; H, 5.31, 5.38; N, 8.07, 8.29.

Electronic spectra in chloroform solution and infrared spectra in potassium bromide pellets were identical with those of the autoxidation product.

Results and Discussion

Preparative Methods. Iron introduction by the method of Corwin and Erdman (1946) was found to be quantitative in the case of chloro- and bromoiron(III) compounds of 2,4-diacetyldeuteroporphyrin ester. For other compounds column chromatography on alumina, although it diminished the yield, proved adequate to separate unreacted porphyrin esters from

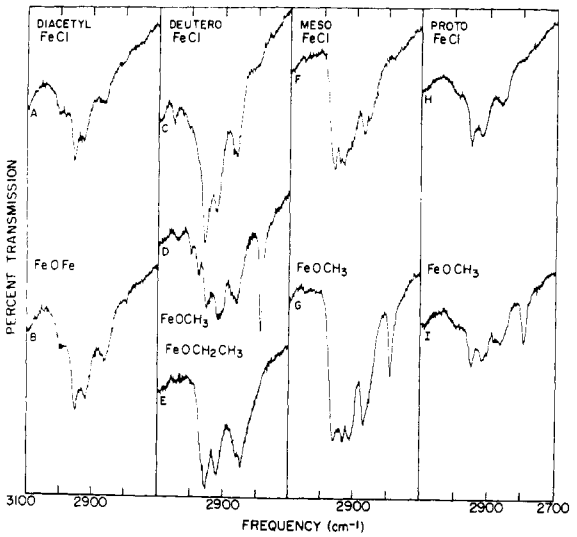


FIGURE 3: Infrared spectra obtained in KBr in the CH absorption region. Spectra were recorded on a Perkin-Elmer Model 521 spectrometer. (A) Chloro-2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(III); (B) autoxidation product from dipyridine 2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(II); (C) chlorodeuteroporphyrin IX dimethyl ester iron(III); (D) methoxodeuteroporphyrin IX dimethyl ester iron(III); (E) ethoxodeuteroporphyrin IX dimethyl ester iron (III); (F) chloromesoporphyrin IX dimethyl ester iron (III); (G) methoxomesoporphyrin IX dimethyl ester iron(III); (H) chloroprotoporphyrin IX dimethyl ester iron(III); and (I) methoxoprotoporphyrin IX dimethyl ester iron(III). Methoxo absorption is evident at *ca.* 2800 cm^{-1}.

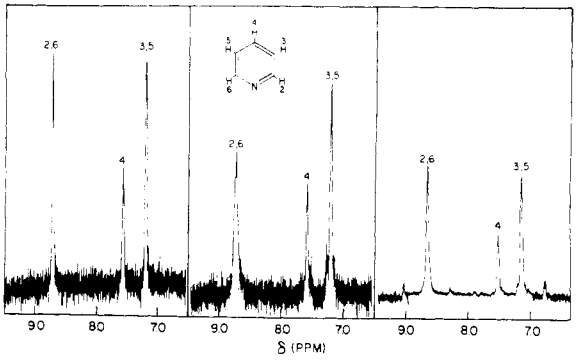


FIGURE 4: Nuclear magnetic resonance spectra of 1% d_4-pyridine in d_5-pyridine. Left: no solute. Middle: 19.8 mg/ml of dipyridine 2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(II). Right: 6.6 mg/ml of dipyridine 2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(II).

iron porphyrin esters. While the preparations given here all involve generation of ferrous acetate from iron powder in acetic acid, we have also found that addition of solid ferrous acetate, ferrous chloride, or ferrous oxide to acetic acid under nitrogen was satisfactory.

Column chromatography of iron(III) porphyrin esters on alumina altered the axial ligand. For example, the column eluates for chloro derivatives showed spectra similar to those of the methoxo derivatives (Figure 1). The eluate compounds have not been fully characterized but can be converted to chloro, methoxo, or ethoxo compounds depending on the method of crystallization. Removal of chloro ligand did not occur by mere recrystallization of the chloroiron(III) compounds from chloroform–methanol.

Previous preparations of iron(II) porphyrins have relied on anaerobic systems (Fischer *et al.*, 1931; Corwin and Erdman, 1946; Corwin and Reyes, 1956; Corwin and Bruck, 1958). The preparations described here provided stable, analytically pure pyridinated iron(II) porphyrins under aerobic conditions by virtue of the high pyridine concentration (relative to other solvents) which was maintained.

The reactivity of porphyrin substituents such as vinyl groups, even under mild conditions (*cf.* Falk, 1964; Caughey *et al.*, 1966a,c), necessitates demonstration of the integrity of the original porphyrin after metal insertion. The infrared absorption spectra of iron(III) and iron(II) porphyrin esters, compared with well-characterized samples (Caughey *et al.*, 1966a) of metal-free porphyrin esters (Figure 2), show no gross changes appropriate to modifications of the porphyrin ring. Nuclear magnetic resonance peaks corresponding to all protons expected (Caughey and Koski, 1962; Caughey *et al.*, 1966a; Caughey and McCoy, 1966a) for the structures assigned to the iron(II) porphyrin esters were found in d_5-pyridine solution, and can be considered evidence not only for the porphyrin structures of the iron(II) compounds in solution, but also for the porphyrin structures of the iron(III) porphyrin esters from which they were prepared.

Integrity of the porphyrin structures, elemental analyses indicating additional groups, and charge considerations were mutually explicable in terms of the oxidation states and ligands reported here. Ligands were more specifically indicated by either direct chlorine analyses, infrared absorption in the CH region at-

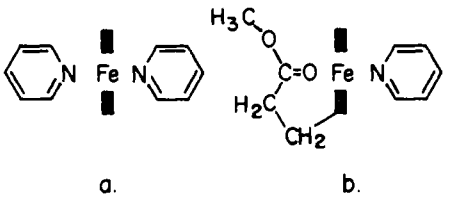


FIGURE 5: Schematic representations. (a) The structure of low-spin dipyridine iron(II) porphyrin esters; heavy line represents the edge of porphyrin ring plane. (b) Postulated structure of monopyridine deuteroporphyrin IX dimethyl ester iron(II).

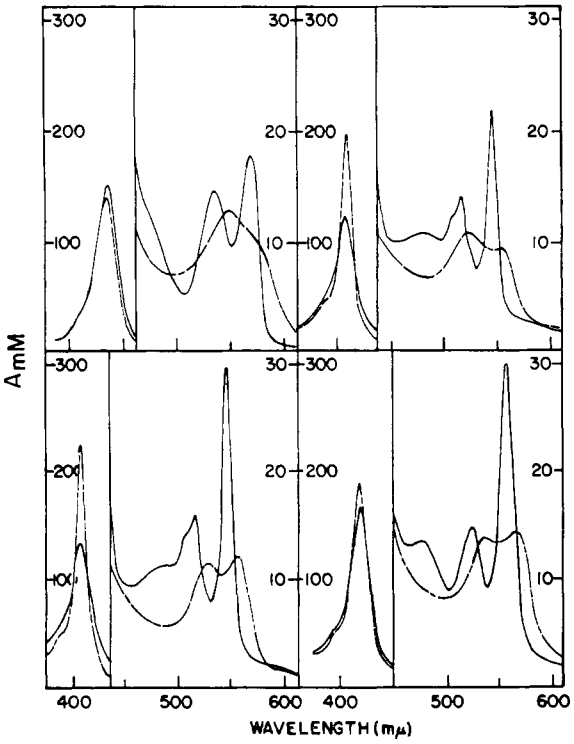


FIGURE 6: Electronic spectra in *ca.* 1 M pyridine in benzene of dipyridine 2,4-substituted deuteroporphyrin IX dimethyl ester iron(II) compounds (———) and their corresponding monopyridinemonocarbonyl derivatives (– · – · –). 2,4-Substituents: top left, acetyl; top right, hydrogen (deuteroporphyrin); lower left, ethyl (mesoporphyrin); lower right, vinyl (protoporphyrin).

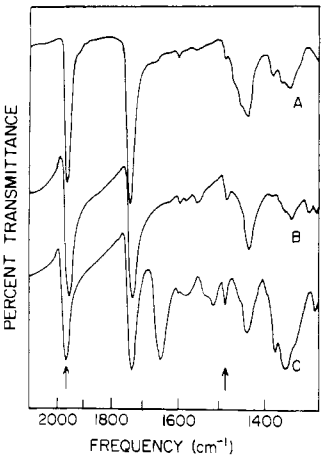


FIGURE 7: Infrared spectra in KBr of monopyridinemonocarbonyl 2,4-substituted deuteroporphyrin IX dimethyl ester iron(II) compounds. (A) Compound prepared from pyridinemesoheme dimethyl ester; (B) compound prepared from pyridinedeuteroheme dimethyl ester; (C) compound prepared from dipyridine 2,4-diacetyldeuteroheme dimethyl ester. Arrows indicate the carbonyl band near 2000 cm^{-1} and a pyridine band near 1480 cm^{-1}. Spectra were recorded on a Perkin-Elmer Model 21 spectrometer.

tributable to methoxo or ethoxo groups (Figure 3), or infrared absorptions attributable to pyridine (Figure 2). Nuclear magnetic resonance spectra of iron(II) compounds in d_5-pyridine solution showed a concentration-dependent broadening of the singlets composing the spectrum of the 1% d_4-pyridine present (Figure 4). The selectivity of broadening can be rationalized by considering that resonances due to protons at the 2,6 positions of bound pyridine would be shifted further by the ring current field than protons at the 3,5 and 4 positions; a relatively fast equilibrium would then broaden peaks rather than shift them because of the vast excess of unbound pyridine. The nuclear magnetic resonance data thus provide evidence for the hitherto assumed structure of dipyridineiron(II) porphyrins (Figure 5a). Solid pyridine deuteroporphyrin IX dimethyl ester iron(II) exhibited two ester carbonyl vibrations (1733 and 1721 cm^{-1}) in the infrared spectrum (Figure 2), indicative of participation of one ester carbonyl as a sixth ligand to iron, either intramolecularly (Figure 5b) or intermolecularly (as proposed by Katz *et al.* (1966), for chlorophyll dimers). A single infrared ester peak (1737 cm^{-1}) was observed in pyridine solution and the deuteroporphyrin derivative with an elemental analysis indicating a pyridine : iron ratio of 1.5 showed only a slight ester carbonyl splitting in the solid; deuteroporphyrin IX dimethyl ester iron(II) appears, therefore, to exist as a dipyridine species in pyridine solution. The nuclear magnetic resonance spectrum in d_5-pyridine of the latter species (pyridine: iron ratio of 1.5) was also consistent with a dipyridine species.

The oxidation state of many of the iron(III) derivatives reported here was confirmed by magnetic susceptibility measurements and electron spin resonance spectroscopy; details of these measurements will be presented elsewhere. Iron(II) oxidation states were shown by sharp nuclear magnetic resonance spectra in degassed deuteriochloroform (Caughey *et al.*, 1965) for the diacetyl derivative and in d_5-pyridine for all iron(II) derivatives; the nuclear magnetic resonance results also showed that the iron(II) species is low spin when dipyridinated. Homogeneity, as well as the expected oxidation state, of ^{57}Fe-enriched preparations was indicated by their Mössbauer spectra (Bearden *et al.*, 1965); inhomogeneous samples would have given more than two peaks. The preparation of pyridinecarbonyliron(II) porphyrin esters from the corresponding dipyridineiron(II) compounds (Figures 6 and 7) is also indicative of the oxidation state of iron in the dipyridine species. The chloroiron(III) species in benzene solution, upon saturation with carbon monoxide with and without pyridine, did not form either the pyridinecarbonyl or dipyridineiron(II) species (as determined by visible spectrum). Formation of the pyridinecarbonyl species in solution was reversible by passage of nitrogen gas through the solution.

TABLE I: Absorption Maxima of Monopyridinemonocarbonyliron(II) Porphyrins in Pyridine–Benzene Solutions.[a]

Heme[b]	α, λ (A_{mM})[c]	β, λ (A_{mM})[c]	Soret, λ (A_{mM})[c]
Meso-	557.5 (12.3)	528 (12.0)	408.5 (223)
Deutero-	553.5 (8.8)	525.5 (10.0)	407 (202)
Proto-	563.5 (14.3)	533.5 (13.8)	417 (172)
Diacetyldeutero-		546 (12.5)	433.5 (141)

[a] Carbon monoxide about 6.7×10^{-3} M; pyridine 0.12–0.27 M for α and β bands. For Soret regions pyridine was 1.3 M; data were corrected for the presence of dipyridineheme. [b] Dimethyl ester. [c] λ in millimicrons.

The iron in the pyridinecarbonyl species is also low-spin iron(II), as shown by sharp nuclear magnetic resonance spectra (Caughey *et al.*, 1965); the proposal of Weiss (1964) that carbonmonoxyhemoglobin contains iron(III) and a CO^- radical thus cannot apply to pyridinecarbonylhemes. Solution spectra of pyridinecarbonylporphyrin ester iron(II) compounds are presented in Figure 6 and Table I. Those portions of the infrared spectra of the solid pyridinecarbonyliron(II) compounds which illustrate the presence of carbonyl and pyridine groups are presented in Figure 7. The positions and band widths for the carbonyl absorption are comparable to those obtained by Wang *et al.* (1958) for pyridinecarbonylprotoheme diethyl ester, but the same potassium bromide pellets have yielded identical spectra after exposure to air at room temperature for years, in marked contrast to the instability reported for the preparation of Wang *et al.* Because of the irreproducibility in peak positions characteristic of potassium bromide pellets (Christiansen filter effect), detailed studies on the effect of porphyrin structure on the infrared absorption due to the carbonyl of pyridinecarbonylhemes were carried out in bromoform solution (Alben and Caughey, 1966).

The species obtained upon reduction of methoxo- and chloroprotoporphyrin IX dimethyl ester iron(III), unlike the iron(II) derivatives of 2,4-diacetyldeutero-, deutero-, and mesoporphyrin dimethyl esters, did not have elemental analyses consistent with a mono- or dipyridineiron(II) structure, but instead with a structure containing one pyridine molecule and one oxygen atom per iron porphyrin ester. Infrared and nuclear magnetic resonance spectra (Figures 2 and 8) rule out the possibility that oxygen is bound to the vinyl group of elsewhere on the porphyrin periphery. Possible structures are a monopyridinemonoaquoiron(II) species (Figure 9a) or a dipyridine oxygen dimer (Figure 9b,c), or a mixture of dipyridine and oxygenated species. Upon being dissolved in d_5-pyridine, nevertheless, a low-spin iron(II) species was obtained (Figure 8) from the protoporphyrin ester compound. The figures presented here for electronic spectra (Figure 6) and the nuclear magnetic resonance spectrum (Figure 8) represent the compound prepared from the methoxoiron(III) derivative.

Electronic spectra in benzene–pyridine solution of dipyridineiron(II) compounds are shown in Figure 6 and Table II. (The proto- and deuteroporphyrin derivatives are considered to be dipyridine species on the basis of the infrared and nuclear magnetic resonance evidence cited above: they were dissolved in pyridine prior to dilution for electronic spectra.) Use of esterified dipyridineiron(II) compounds minimized complications in interpretation which arise

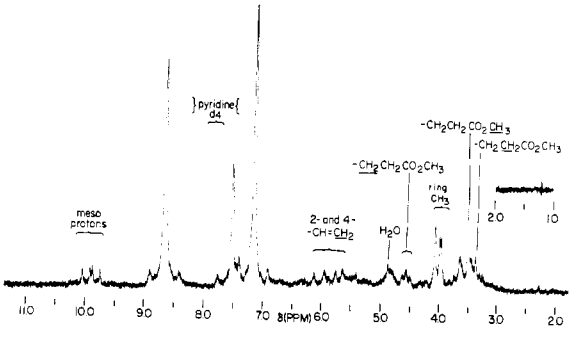


FIGURE 8: Nuclear magnetic resonance spectrum of dipyridine protoporphyrin IX dimethyl ester iron(II) in d_5-pyridine. Unassigned resonances either spinning side bands or solvent impurities. Spectrum was recorded on a Varian HA-100 spectrometer.

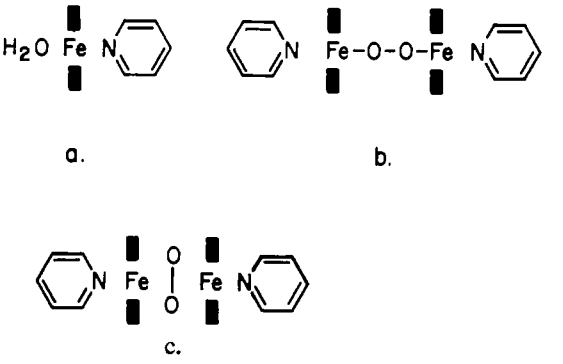


FIGURE 9: Schematic representations of possible structures of pyridinated protoporphyrin IX dimethyl ester iron(II).

TABLE II: Absorption Maxima of Dipyridineiron(II) Porphyrins in Pyridine–Benzene Solution.[a]

Heme[b]	α, λ (A_{mM})[c]	β, λ (A_{mM})[c]	δ, λ (A_{mM})[c]	Soret, λ (A_{mM})[c]
Meso-	546 (31.0)	516 (16.3)	488 (11.7)	407 (131)
Deutero-	543 (21.3)	513.5 (13.6)	477 (10.4)	405 (120)
Proto-	555 (30.8)	523 (15.1)	473 (13.7)	419 (166)
Diacetyldeutero-	568 (18.5)	535 (15.1)		435 (150)

[a] Variations in pyridine concentrations from 0.12 to 1.3 M had no effect on wavelengths or A_{mM} values of absorption maxima. [b] Dimethyl esters. [c] λ in millimicrons.

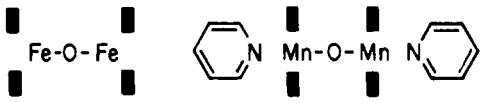
from aggregation such as noted with solutions of free acids in aqueous pyridine (*cf.* Gallagher and Elliott, 1965); unlike the parent porphyrin esters in concentrated deuteriochloroform solution (Caughey *et al.*, 1966a), no concentration-dependent phenomena were observed by nuclear magnetic resonance.

Elemental analyses indicated that heating pyridinated iron(II) porphyrin esters under vacuum could remove pyridine ligands and permit oxygen binding upon exposure to the atmosphere. Dipyridine 2,4-diacetyldeuteroporphyrin IX dimethyl ester iron(II) yielded evidence of initial formation of a bare iron species (loss of 2 moles of pyridine), followed by slow uptake of oxygen from the atmosphere. Temperatures at which the pyridine is dissociated are higher for those compounds with strong electron-withdrawing groups at the periphery (*i.e.*, acetyl). This observation is consistent with the high temperature required to remove pyridine from pyridineheme A (York *et al.*, 1967), and is qualitatively parallel to the order of stabilities in solution of dipyridinenickel(II) porphyrin esters (Caughey *et al.*, 1962; McLees, 1964; Caughey *et al.*, 1966b). Electron withdrawal at the periphery of the iron(II) porphyrin system, therefore, facilitates σ or π acceptance by the iron(II) and stabilizes the dipyridine species. Further evidence of the interrelationship of peripheral electron withdrawal and π bonding by low-spin iron(II) has been presented for pyridinecarbonyl compounds (Caughey *et al.*, 1965; Alben and Caughey, 1966; Caughey *et al.*, 1968).

Oxygen uptake in the solid state has been reported for imidazole complexes of meso- and protohemes (Corwin and Reyes, 1956; Corwin and Bruck, 1958) and for protoheme diethyl ester embedded or copolymerized in a polystyrene matrix containing 1-(2-phenylethyl)imidazole (Wang, 1958); authentication of the structure of neither hemes nor oxyhemes was presented. Evidence presented here for the structures of the oxygenated compounds obtained from iron(II) derivatives of authenticated structure by heat under vacuum and exposure to air is, excepting the diacetyl compound, exclusively that of best fit to the analytical data, and must be regarded as preliminary. The structures presently proposed are O_2 adducts without further ligand (diacetyldeuteroporphyrin dimethyl ester, deuteroporphyrin dimethyl ester, and protoporphyrin dimethyl ester derivatives) and a dimeric adduct with bridging O_2 and two pyridine ligands (mesoporphyrin dimethyl ester derivative, as in Figure 9b,c); since one of the former species arose from an unstable bare iron derivative and the latter species arose from an unstable monopyridineiron derivative, compounds of both structures for all porphyrin types may be achievable by suitable temperature variation. One of the oxygenated protoporphyrin derivatives appears to contain a nonstoichiometric amount of pyridine, either occluded or bound as a ligand, and the mesoporphyrin derivative may, of course, be likewise inhomogeneous. The diacetyl derivative *does* appear homogeneous in Mössbauer spectroscopy,[2] and infrared spectra have shown that the acetyl groups remain intact; more detailed characterization of this compound will be presented elsewhere (Fuchsman and Caughey, 1967). No oxidation state is indicated in our formulations of the oxygenated compounds; although a formal iron(II) oxidation state is reasonable, charge transfer of the type proposed by Weiss (1964) for oxyhemoglobin could yield an effective iron(III) oxidation state.

The Autoxidation Reaction. The rate of spectral change (ΔA/min at 419 mμ) 20–30 sec after dilution of a stock solution of dipyridine protoporphyrin IX dimethyl ester iron(II) (in pyridine) by aerobic solvents containing 50% pyridine were, in order of increasing rate: dimethylformamide (0.017), benzene (0.037), pyridine (0.050), bromoform (0.054), ethanol (0.084), isooctane (0.088), acetone (0.15), *n*-butylamine (0.16), methanol (0.29), 0.38 M potassium hydroxide in water (0.35), water (1.17), and glacial acetic acid (>3.8). The rates of change (ΔA/min at 435 mμ) for dipyridine diacetyldeuteroporphyrin IX dimethyl ester iron(II) under identical conditions were: dimethylformamide (0.001), benzene (0.0045), bromoform (0.005), ethanol (0.012), isoctane (0.013), methanol (0.025), *n*-butylamine (0.041), 0.38 M potassium hydroxide in water (0.11), water (0.13), and glacial acetic acid (0.98). The visible spectra of the reaction products of diacetyldeuteroheme ester have been published (Caughey *et al.*, 1965). Dipyridine mesoporphyrin IX dimethyl

[2] Mössbauer spectroscopy on compounds unenriched in ^{57}Fe was performed by Dr. J. J. Spijkerman.



a. b.

FIGURE 10: Schematic representations. (a) Postulated structure of autoxidation product. (b) Bridged phthalocyanatomanganese dimer.

ester iron(II) yielded a similar series. When the pyridine concentration in methanol totalled only 1%, the reaction of the protoporphyrin derivative was 88% complete in 15 sec; that of the mesoporphyrin derivative was 94% complete in the same time.

The rates of spectral change observed here were the rates for disappearance of dipyridineiron(II) species. The rates of autoxidation or oxygen reduction *per se*, however, were not directly measured. The autoxidation reactions obviously take place in several steps, and the differences in spectra for autoxidation products obtained in different solvents (Caughey *et al.*, 1965) suggested that the reaction course was affected by the solvent used. Knowledge of the structure of the reaction products as well as more detailed kinetic studies are necessary before effects of solvent upon the oxygen reduction steps can be adequately discerned. As we have previously mentioned (Caughey *et al.*, 1965), these considerations render quite equivocal the argument such as that of Wang and coworkers (Wang, 1962; Kao and Wang, 1965) that the increased rates of disappearance of dipyridineheme spectra, when water or ethanol is added to pyridine solutions, constitute evidence that it is the nonpolar character of the environment of protoheme that promotes the oxygenation (rather than oxidation) reaction of hemoglobins and myoglobins. Even if the rate of disappearance of dipyridineheme directly measured the rate of oxygen reduction, the arguments of Wang and coworkers would not apply. Dimethylformamide, a highly polar (but aprotic) solvent, in less suitable than benzene as a medium for autoxidation, and water is less suitable than glacial acetic acid but more suitable than aqueous alkali. Probably far more relevant to the discussion of stability of oxygenated hemoglobin is the recent report by Calderazzo *et al.* (1966) that isolatable oxygenated cobalt(II) compounds were formed in solutions of dimethylformamide and dimethyl sulfoxide but that oxidation to cobalt(III) occurred in nitromethane. The protic nature of the solvent appears to be far more important in governing rates of autoxidation than its polarity.

Other properties of the autoxidation reaction suggest a mechanism for the solvent effect. Oxygen is required, qualifying the reaction as autoxidation: dipyridinehemes were observed to be stable in degassed solvents (Caughey *et al.*, 1965) until exposure to air, whereupon the reaction occurred most rapidly at the surface of the solvent. Pyridine (or *para*-substituted pyridines but not primary, secondary, or tertiary amines) inhibited the reaction: the reaction was much slower in 50% pyridine than in 1% pyridine solutions. Concentrated heme solutions were observed to autoxidize more slowly than dilute solutions, probably because of the high concentration of pyridine released; in dilute solutions a second-order dependence on heme concentration was observed (Cohen and Caughey, 1966, 1968). The diacetyldeuteroporphyrin ester derivative, which in the solid state lost pyridine less readily than the protoheme ester derivative, also autoxidized less readily (the assumption that differences between the hemes in the Soret band absorption change from reduced to oxidized are not great enough to account for the tenfold rate difference reported here has been borne out by more detailed rate measurements utilizing the visible region (Cohen and Caughey, 1966, 1968)). Apparently the dipyridine iron(II) species must lose at least one pyridine in order to react with oxygen, and the loss is directly related to the rate-limiting step(s). The solvent effect is generally consistent with competition between solvent and the iron porphyrin ester for the pyridine nitrogen, despite possible changes in mechanism with solvent changes. Kao and Wang (1965) have reported kinetics consistent with formation of a superoxide ion, the crucial intermediate postulated by their electrostatic interpretation (Wang, 1962) of solvent effects on autoxidation. However, reexamination of their system (Cohen and Caughey, 1966, 1968) indicated that an alternate interpretation of their data would not require generation of a superoxide species.

The autoxidation product of dipyridine 2,4-diacetyldeuteroporphyrin dimethyl ester iron(II) in benzene gave carbon and hydrogen analyses consistent with dimer formation with a single bridging oxygen atom as in Figure 10a, although high nitrogen analyses and evidence in mass spectra of a pyridine peak indicated the presence of a small amount of pyridine. Subsequently, a compound, identical in electronic and infrared spectra, was prepared by means which did not involve exposure to pyridine. The absence of absorption attributable to pyridine in the infrared spectrum of the autoxidation product suggested that the pyridine present must have been loosely bound or occluded and hence removed under vacuum and pressure when the pellet was pressed; reduction of the per cent nitrogen by heating is in accord with such an interpretation.

The final steps of the autoxidation reaction in benzene were proposed by Cohen and Caughey (1966) as

$$\text{py–N}_4\text{Fe–O}_2\text{–FeN}_4\text{–py} + 2\text{H}_2\text{O} \longrightarrow 2\text{N}_4\text{FeOH} + \text{H}_2\text{O}_2 + 2\text{py}$$

$$\text{H}_2\text{O}_2 + 2\text{py–N}_4\text{Fe–py} \longrightarrow 2\text{N}_4\text{FeOH} + 4\text{py}$$

where py = pyridine and N_4Fe = iron porphyrin ester. An alternative proposal, requiring no water,

forming no hydrogen peroxide as intermediate, and yielding the product isolated from benzene is

$$\text{py–N}_4\text{Fe–O}_2\text{–FeN}_4\text{–py} \longrightarrow 2\text{py–N}_4\text{FeO}$$

$$2\text{py–N}_4\text{FeO} + 2\text{py–N}_4\text{Fe} \longrightarrow 2\text{py–N}_4\text{Fe–O–FeN}_4\text{–py}$$

with subsequent partial dissociation, in solution or in the solid, of pyridine. The oxidation of phthalocyanatomanganese(II) in pyridine (Lever, 1965) gave a product of similar structure (Figure 10b), as determined in the crystal by Vogt *et al.* (1966). The O_2-bridged species, postulated by Cohen and Caughey (1966) on the basis of kinetic evidence, is further justifiable in terms of the species isolated in the solid state from the proto- and mesoporphyrin ester iron(II) derivatives.

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