Grade, and other solvents were reagent grade without further purification. The Mn(II) isocyanide complexes were stored at $-78°$, where they were stable for prolonged periods as indicated by infrared spectra. Solutions of the Mn(II) complexes showed signs of decomposition within 1–2 days at -15 or $-78°$, depending on the complex, and were generally prepared just prior to a series of kinetic runs.

A Varian DP-60 wide-line nmr instrument was used to measure the ^{55}Mn absorption, which appears as a single signal ≥ 70 Hz wide at 15.8 MHz, 15,000 G. The instrument was calibrated (in Hz/mm of chart) by means of the two ^{14}N peaks of ammonium nitrate. Signals were obtained as derivative curves, and care was taken to avoid saturation. The sample cell was constructed so that a constant temperature ($\pm 2°$) could be obtained by passing a heated or cooled gas through a coiled glass tube in the cell. The outside had an unsilvered vacuum jacket, and a thermocouple well and an opening for introducing the sample were also provided. About 2.2–3.0 ml of 0.3 M Mn(I) isocyanide complex solution was introduced into the sample cell and the opening was stoppered with a rubber septum. Five traces of the nmr signal were recorded. Then a small sample of the Mn(II) isocyanide complex solution (generally 5–50 μl) was introduced by syringe through the rubber septum, resulting in a concentration of 10^{-6}–10^{-3} M, and the nmr signal was again recorded five times. This was continued until four to five points had been obtained. A new Mn(I) sample was generally used for each different temperature. Rates were calculated as indicated in the Results section.

Contact Shifts. A freshly prepared solution of the Mn(II) isocyanide complex (about 0.3–0.4 ml, 10^{-2} M) was placed in an nmr tube and the proton spectrum recorded on a Varian A-60. The solution was kept stoppered at $-78°$ except when in the instrument, and the spectra were recorded below 0° with the solvent acting as an internal reference. Measured amounts of the corresponding Mn(I) solution were added up to a concentration of about 10^{-3} M and the spectrum was rerecorded until four to five points had been measured.

Acknowledgment. We thank Professors J. P. Hunt and H. W. Dodgen and Drs. Stephen Marks and William Gary for helpful discussions and information.

The Dimeric Nature of Hemin Hydroxides

Irwin A. Cohen

Contribution from the Department of Chemistry, The Polytechnic Institute of Brooklyn, Brooklyn, New York 11201. *Received October 14, 1968*

Abstract: The hematins derived from tetraphenylporphine and deuteroporphyrin IX dimethyl ester have been found to be dimeric in methylene chloride by molecular weight measurements. Infrared studies indicate the presence of iron–oxo–iron bridges in both systems. The room-temperature magnetic moment of one, the μ-oxo-bis(tetraphenylporphineaquoiron(III)), was found to be 1.74 BM per iron. The results of previous magnetic studies for hematins are interpreted as due to mixtures of high-spin hydroxo monomers and low-spin oxo dimers. The difference between the Mössbauer spectra of hemins and hematins is discussed.

Dimerization and polymerization of hemins (porphyrin Fe(III)X, X = OH) and hematins (X ≠ OH) in aqueous solutions are well known.[1] A recent polarographic study of the reduction of hemin chloride in 0.1 M aqueous base indicated, under very specific conditions, a two-electron step was operative, consistent with the hemin dimer being the reducible species.[2] But in view of the low solubility of hemins in water and the hydrophobic nature of the porphyrin ring, it is not necessary to invoke inter-iron bridging groups in these dimers to explain their formation. There have been no direct observations of hematin dimerization in nonaqueous solvents prior to this work.

A crystallographic study[3] has shown that a Mn(III) model of hematin, μ-oxo-bis(phthalacyanatopyridinemanganese(III)), is a dimer with a linear Mn–O–Mn bridge. But crystal structures of methoxyiron(III) mesoporphyrin IX dimethyl ester[4] and hemin chloride[5] have shown them to be monomeric. The reported monomeric crystallographic structure of tetraphenylporphineiron(III) hydroxide[6] has subsequently been shown in error,[7] but no conclusions could have been drawn as to its true structure.

Oxo-bridged dimers of ferrihemes have recently been postulated twice, by Schugar, *et al.*,[8] on the basis of magnetic studies of aqueous model systems and by Alben, *et al.*,[9] after an analytical study of the solid product from the autoxidation of a ferroporphyrin in benzene.

Experimental Section

μ-Oxo-bis(tetraphenylporphineaquoiron(III)). The method of Dorough, Miller, and Huennekens[10] for the preparation of tetraphenylporphineiron(III) hydroxide was used to obtain a crude product. This was followed by chromatography on alumina with chloroform. The eluent was evaporated to dryness and the residue was exposed to air and then dried *in vacuo*.

Anal. Calcd for $C_{88}H_{60}N_8O_3Fe_2$: C, 76.08; H, 4.32; N, 8.08. Found: C, 76.37; H, 4.45; N, 8.03.

Deuteroporphyrin IX dimethyl ester iron(III) methoxide was pre-

(1) J. E. Falk, "Porphyrins and Metalloporphyrins," Elsevier Publishing Co., Amsterdam, 1964, p 45, and references therein.
(2) J. Jordan and T. M. Bednarski, *J. Amer. Chem. Soc.*, **86**, 5690 (1964).
(3) L. H. Vogt, A. Zalkin, and D. H. Templeton, *Science*, **151**, 569 (1966).
(4) J. L. Hoard, M. J. Hamor, T. A. Hamor, and W. S. Caughey, *J. Amer. Chem. Soc.*, **87**, 2312 (1965).
(5) D. F. Konig, *Acta Cryst.*, **18**, 663 (1965).
(6) E. B. Fleischer, C. K. Miller, and L. E. Webb, *J. Amer. Chem. Soc.*, **86**, 2342 (1964).
(7) J. L. Hoard, G. H. Cohen, and M. D. Glick, *ibid.*, **89**, 1992 (1967).
(8) H. Schugar, C. Walling, R. B. Jones, and H. B. Gray, *ibid.*, **89**, 3712 (1967).
(9) J. O. Alben, W. H. Fuchsman, C. A. Beaudreau, and W. S. Caughey, *Biochemistry*, **7**, 624 (1968).
(10) G. D. Dorough, J. R. Miller, and F. M. Huennekens, *J. Amer. Soc. Chem.*, **73**, 4315 (1951).

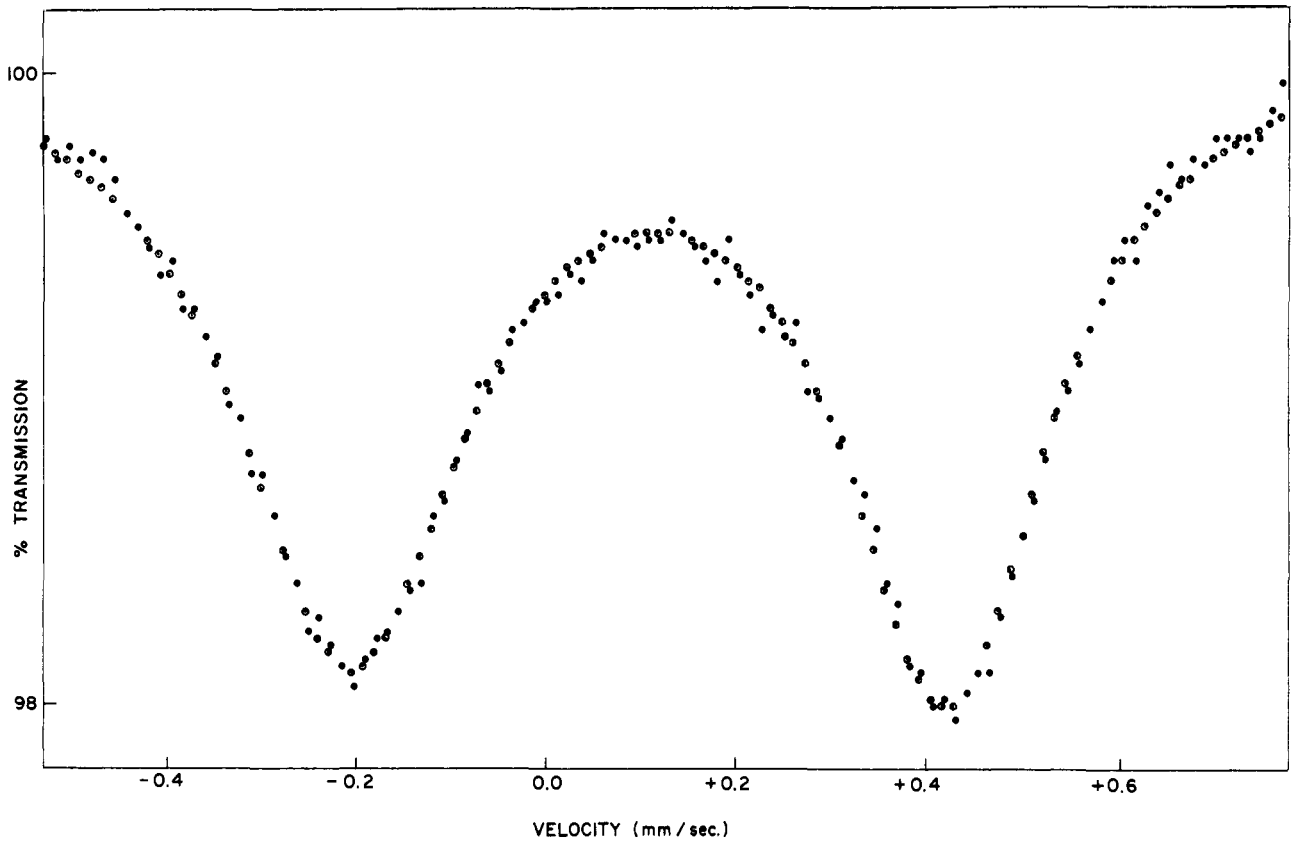

Figure 1. Room-temperature Mössbauer spectrum of μ-oxo-bis(tetraphenylporphineaquoiron(III)) using a source of ^{57}Co in Pd. Closed circles are at the observed points and open circles represent the least-squares fitted curve.

pared by the method of Alben, Fuchsman, Beaudreau, and Caughey.[9]

Anal. Calcd for $C_{33}H_{35}N_4O_5Fe$: C, 63.57; H, 5.66; N, 8.99. Found: C, 63.40; H, 6.06; N, 8.59.

μ-Oxo-bis(deuteroporphyrin IX dimethyl ester aquoiron(III)). Deuteroporphyrin IX dimethyl ester iron(III) methoxide was dissolved in methylene chloride and then evaporated to dryness under reduced pressure. The residue was exposed to air and dried *in vacuo*.

Anal. Calcd for $C_{64}H_{68}N_8O_{11}Fe_2$: C, 62.13; H, 5.50; N, 9.05. Found: C, 62.80; H, 5.52; N, 8.91.

Infrared spectra were recorded on a Perkin–Elmer Model 521 spectrometer.

Molecular weights were determined by vapor-pressure osmometry on a Hitachi-Perkin-Elmer Model 115 at 31.5° in Fisher Spectrograde methylene chloride. The system was calibrated with benzil and checked with the corresponding hemin chlorides. All solute concentrations were between 1 and 9 mmol/kg of solvent.

Mössbauer spectra were recorded on powered samples at room temperature with a Nuclear Science Model NS-1 spectrometer. The data were analyzed by a Nuclear Data Inc. multichannel analyzer and computer fitted by a least-squares method. Scan widths were ±1.6 mm/sec. The source was ^{57}Co in Pd.

Magnetic susceptibilities were determined at room temperature on powdered samples by the Faraday method using a Cahn RG electrobalance and an Alpha Scientific Inc. magnet, Model M500. The molar diamagnetic susceptibility of tetraphenylporphine was measured as -386×10^{-6} (cgs units). All other results were corrected by this amount plus the standard amounts[11] for any other elements present.

Results

Molecular Weights. The observed molecular weights were 1275 for μ-oxo-bis(tetraphenylporphineaquoiron(III)) (calcd, 1388) and 1240 for μ-oxo-bis(deuteroporphyrin IX dimethyl ester aquoiron(III)) (calcd, 1236). Although these measurements were repeated several times, no attempt was made to determine the effect of solute concentration upon the observed molecular weight. It is generally found that the technique used is accurate to within 3%; therefore, the low weight observed for the tetraphenylporphine derivative may in fact indicate approximately 10% dissociation to monomer in solution.

Mössbauer Spectra. Both hematin dimers exhibited two well-resolved symmetrical peaks at room temperature. One spectrum is shown in Figure 1. The spectra differ greatly from the room-temperature spectrum for deuteroporphyrin IX dimethyl ester iron(III) methoxide and from those reported for several hemin chlorides.[12-14] As shown in Table I the Mössbauer parameters observed for all the iron(III) porphyrins do not differ greatly from one another but the hemins' spectra all dramatically broaden as the temperature is raised from 5°K to room temperature. The two hematin dimers have very sharp absorptions even at room temperature. It is interesting to note that protohematin likewise exhibits a sharp room-temperature spectrum.[15]

Infrared spectra of the hematin dimers in the 850-cm^{-1} region are informative and those of μ-oxo-bis(tetraphenylporphineaquoiron(III)) and the corresponding

(11) B. H. Figgis and J. Lewis in "Modern Coordination Chemistry," J. Lewis and R. G. Wilkins, Ed., Interscience Publishers, New York, N. Y., 1960, p 403.

(12) U. Gonser and R. W. Grant, *Biophys. J.*, **5**, 823 (1965).

(13) A. J. Bearden, T. H. Moss, W. S. Caughey, and C. A. Beaudreau, *Proc. Nat. Acad. Sci. U. S.*, **53**, 1246 (1965).

(14) C. I. Wynter, P. Hambright, C. H. Cheek, and J. J. Spijkerman, *Nature*, **216**, 1105 (1967).

(15) W. Karger, *Ber. Bunsenges. Phys. Chem.*, **68**, 793 (1964).

Table I. Mössbauer Parameters Observed for Some Hemins and Hematins

Compound	Isomer shift, mm/sec[a]	Quadrupole splitting, mm/sec	Comments	Ref
μ-Oxo-bis(tetraphenylporphineaquoiron(III))	0.55	0.624	Sharp at room temp	
μ-Oxo-bis(deuteroporphyrin IX dimethyl ester aquoiron(III))	0.55	0.570	Sharp at room temp	
Protohematin	0.58	0.54	Sharp at room temp	15
	0.68	0.98	Sharp at 5°K	12
Protohemin chloride	0.69	0.78	Sharp at 5°K	12
	0.40	1.02	Sharp at 5°K	13
	0.68	1.06	Broad at room temp	13
2,4-Diacetyldeuterohemin chloride dimethyl ester	0.53	0.85	Sharp at 5°K (broad at 298°K)	13
Mesohemin chloride dimethyl ester	0.42	0.89	Sharp at 5°K (broad at 298°K)	13
Deuterohemin methoxide dimethyl ester	?	?	Very broad at room temp	

[a] Relative to sodium nitroprusside.

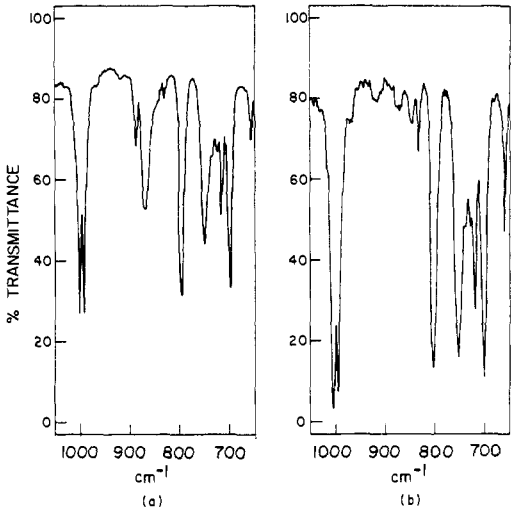

Figure 2. Partial infrared spectra of (a) μ-oxo-bis(tetraphenylporphineaquoiron(III)) and (b) tetraphenylporphineiron(III) chloride in KBr.

hemin chloride are shown in Figure 2. The only spectral difference between 3000 and 400 cm^{-1} are two additional bands for the hematin dimer at 870 (vs) and 885 cm^{-1} (m). The spectra of deuterohematin dimethyl ester and deuterohemin chloride dimethyl ester show the same relationship, the hematin dimer having one extra very strong absorption at 850 cm^{-1}. For all systems the spectra taken in CS_2 are the same as in KBr.

Magnetic Susceptibilities. The room-temperature susceptibility of μ-oxo-bis(tetraphenylporphineaquoiron(III)) was found to be extremely sensitive to the pretreatment of the sample. A sample as first prepared and without extensive drying gave $10^6\chi_{Fe} = 2890$ (cgs units/g-atom) and $\mu_{eff} = 2.68$ BM. Maintenance of the solid *in vacuo* for 24 hr resulted in $10^6\chi_{Fe} = 2220$ and $\mu_{eff} = 2.30$ BM. Holding the solid *in vacuo* over P_2O_5 for 24 or 36 hr both resulted in $10^6\chi_{Fe} = 1490$ and $\mu_{eff} = 1.89$ BM. After the solid was *in vacuo* over P_2O_5 at 120° for 24 hr, the observed room-temperature values were $10^6\chi_{Fe} = 1250$ and $\mu_{eff} = 1.74$ BM. Further drying had no effect.

A serious effect was made to measure the susceptibility of a sample of tetraphenylporphineiron(III) methoxide completely free of all of the corresponding hematin dimer. Using the intensity of the 850-cm^{-1} band to estimate the amount of hematin dimer present, the best sample was found to be not more than 90% methoxide. The gram susceptibility of this mixture was found to be $10^6\chi = 18.5$ and that calculated for pure high-spin iron(III) in tetraphenylporphineiron(III) methoxide is $10^6\chi = 20.3$.

Discussion

The molecular weight measurements directly indicate that these hematins are dimeric in methylene chloride.

Metal-to-metal bridging by oxo and hydroxo groups have been well studied recently,[16,17] particularly in regard to infrared spectra.[18,19] It has become apparent that linear M–O–M systems exhibit an antisymmetric stretching mode at about 850 cm^{-1}. The strong absorptions found in this region only for the dimeric hematins supports their formulation as linear oxo-bridged systems, analogous to the manganese phthalacyanine discussed above.

An interesting feature of a linear oxo bridge is the availability of the proper orbitals for an M–O–M π system. Dunitz and Orgel[20] made use of this π system and developed a molecular orbital approach to explain the diamagnetism of $K_4[Cl_5RuORuCl_5]$. Application of their approach in this system leads to the expectation of a magnetic moment considerably lower than 5.9 BM.

The magnetic moments of hemins generally present clean results indicative of high-spin iron(III).[21] Rawlinson and Scutt[22] examined a series of halide and carboxylate complexes of several hemins and found all magnetic moments in the range of 5.5–5.9 BM. Much of their work was confirmed by Havermann, Haberditzl, and Mader.[23] It is interesting to note that even protohemin methoxide in

(16) J. Trzebiatowslea, *Coord. Chem. Rev.*, **3**, 255 (1968), and references therein.
(17) J. Lewis, F. Mabbs, and A. Richards, *J. Chem. Soc., A*, 1014 (1967).
(18) F. A. Cotton and R. M. Wing, *Inorg. Chem.*, 4, 867 (1965).
(19) D. J. Hewkin and W. P. Griffith, *J. Chem. Soc., A*, 472 (1966).
(20) J. Dunitz and L. E. Orgel, *ibid.*, 2594 (1953).
(21) Reference 1, p 63.
(22) W. A. Rawlinson and P. Scutt, *Aust. J. Sci. Res.*, **5A**, 173 (1952).

chloroform has μ_{eff} = 5.3 BM.[4] We have found it extremely difficult to prepare solutions of hemin methoxides completely free of hematins, and even solid tetraphenylporphineiron(III) methoxide was found to contain at least 10% hematin. But even with this complication the moment was not less than 5.6 BM. It therefore appears reasonable to assign to the hemin methoxides the high-spin iron(III) configuration and account for the 10–20% deficiency in moment by the presence of some lower moment hematins.

The magnetic data reported for the hematins have often been conflicting. Pauling and Coryell[24] and later Rawlinson[25] found protohematin in basic aqueous sucrose had moments between 5.5 and 5.7 BM. Rawlinson also found the moment between 3.4 and 3.6 BM in the absence of sucrose. Later Rawlinson and Scutt[22] precipitated hematins from aqueous base and found the solids from dilute base showed high moments (μ_{eff} = 5.08 BM) and the more concentrated the base the lower the moment of the product. They obtained a solid hematin with μ_{eff} = 2.38 BM from 7.5 *M* KOH. Recently Blauer and Ehrenberg[26] examined the moments of hematins in water at pH 11. They found μ_{eff} = 5.6 BM, but as salts were added the moments dropped dramatically. In the presence of either 2 *M* NaCl, 2 *M* $NaClO_4$, or 0.5 *M* Na_2SO_4 a moment of about 3 BM was observed. The limiting moment at high ionic strength was 2.4 BM.

Upon examining the tetraphenylporphine hematin dimer after rigorous drying we found μ_{eff} = 1.74 BM. This is the lowest value yet observed and corresponds to the spin-only value for one unpaired electron per iron.

It is possible to account for the magnetic results found by previous workers by invoking mixtures of hematin dimer and hematin monomer (hemin hydroxide). The moment of the pure monomeric hemin hydroxide is as yet unknown but in view of the high-spin d^5 character of all hemins measured, including the hemin methoxides, it is reasonable to expect the hydroxide to also be high spin with μ_{eff} about 5.9 BM. Thus aqueous mixtures would exhibit intermediate spin values. In the presence of sucrose, the hydrophobic porphyrin rings are stabilized, dissociation to hematin monomer occurs, and the magnetic moment is high. As the ionic strength of the solution increases, the dimer is formed and the moment decreases. Hematins precipitated from dilute base have high monomer content and high moments. Those from more concentrated base have less monomer and lower moments. Rigorous drying of a solid hematin causes extensive dimer formation, and thus the minimum moment is observed. There is still, of course, the possibility that the observed 1.74 BM does not represent pure dimer. It may still indicate a mixture of monomer and a diamagnetic dimer, but the agreement of the observed moment with a spin-only value and the lack of any effect of additional drying make this unlikely.

The anomalous sharpness of the room-temperature Mössbauer spectra of hematins can also be considered in regard to the magnetic properties of the dimer. Blume[27] has presented a mechanism for the temperature dependence of the Mössbauer spectra of hemins which involves a temperature-dependent electronic spin–spin relaxation time. Inasmuch as the hematin dimers are magnetically quite different from the monomeric hemins, it is not surprising that they exhibit normal Mössbauer absorptions. In view of the sharp room-temperature spectrum observed for protohematin, it is likely to also be an oxo-bridged system consistent with the earlier magnetic work discussed above.

George, Beetlestone, and Griffith[28] have reviewed the magnetic studies of the ferrihemoproteins in basic media. They view the observed low magnetic moments as indicative of high-spin–low-spin equilibria. As previously pointed out,[8] in order for oxo bridging to account for those results there must be extensive rearrangement of the protein to allow two ferriporphyrins to approach. Until there is direct evidence of such a process, it does not appear that this work presents a serious challenge to their interpretation. It must be stressed, however, that although the hemins are appropriate model compounds for the ferrihemoproteins, the use of hematins must be applied with caution.

Acknowledgment. This work was supported by the National Institutes of Health through Grant No. AM 11355-01.

(23) R. Havermann, W. Haberditzl, and K. Mader, *Z. Phys. Chem.* (Leipzig), **218**, 71 (1961).
(24) L. Pauling and C. D. Coryell, *Proc. Nat. Acad. Sci. U. S.*, **22**, 159 (1936).
(25) W. A. Rawlinson, *Aust. J. Exptl. Biol. Med. Sci.*, **18**, 185 (1950).
(26) G. Blauer and A. Ehrenberg, *Biochem. Biophys. Acta*, 112, 496 (1966).
(27) M. Blume, *Phys. Rev. Lett.*, **18**, 305 (1967).
(28) P. George, J. Beetlestone, and J. S. Griffith, *Rev. Mod. Phys.*, 36, 441 (1964).