weeks. If the solution is acidified or the original reaction carried out in other than strongly alkaline solution, it decomposes by the reaction whose kinetics is shown in Figure 2

$$ON(SO_3^-)NO^- \longrightarrow N_2O + SO_4^{2-}$$

Another decomposition takes place slowly if the alkaline solution is kept in contact with $O_2$, eventually converting all of the product to nitrite. We have observed this both with our reaction product and with the authentic material. In some prolonged runs we have found as many as 72.5% of the nitrogen atoms converted to nitrite.

**Kinetics.**—The rate-determining process in this sequence is the initial formation of peroxynitrite, the subsequent processes being observably much faster. We have measured the rate, in a well-stirred reactor at 25.2° and 1 atm of $O_2$, from pH 5.5 to 14. The results are shown in Figure 3. This pattern of pH dependence resembles closely that of the population of the conjugate base of hydroxylamine-N-sulfonate, given its $pK_A$ of approximately 12.5.[1] We therefore propose that the species entering into the rate-determining process are molecular $O_2$ and the conjugate base, namely

$$O_2 + {}^-N(OH)SO_3^- \longrightarrow OONOH + SO_3^{2-}$$

Our numerical value for the rate constant of this reaction is $3 \times 10^{-2}$ $sec^{-1}$ $atm^{-1}$ (half-life of some 23 sec at 1 atm of $O_2$), but, what with one one or another source of uncertainty in our experiments, we could be off by a factor of 2 either way.

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CONTRIBUTION FROM WESTINGHOUSE ELECTRIC CORPORATION, RESEARCH LABORATORIES, PITTSBURGH, PENNSYLVANIA 15235, AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH, PITTSBURGH, PENNSYLVANIA 15213

# Mossbauer Spectra of Some Porphyrin Complexes with Pyridine, Piperidine, and Imidazole[1a]

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The Mössbauer spectra of the imidazole, pyridine, and piperidine adducts of $\alpha,\beta,\gamma,\delta$-tetraphenylporphyriniron(II) and protoporphyriniron(II) and the imidazole and pyridine adducts of the corresponding porphyriniron(III) chlorides have been measured. Only the heme compound could be obtained with piperidine because piperidine causes spontaneous reduction of iron(III) to iron(II) in porphyrin complexes. For the iron(II) cases, the imidazole and pyridine adducts show similar isomer shifts, while the piperidine adducts have slightly larger isomer shifts. Imidazole gives the smallest quadrupole splitting; piperidine gives the largest. For the iron(III) cases, imidazole gives a somewhat larger isomer shift than pyridine and causes a much greater quadrupole splitting than pyridine, in contrast to the iron(II) cases. These results are discussed in terms of differences in $\pi$-bonding characteristics between the added ligand and the iron atom. Pyridine appears to have a greater affinity for heme than does piperidine.

## Introduction

Because of their biological importance, iron porphyrin complexes have been studied extensively by a number of physical and chemical methods. Mössbauer spectroscopy is particularly suited to the study of these complexes, and the Mössbauer spectra of hemin,[2-7] hemin salts,[8] hematin,[5-6] hemoglobin and its derivatives,[6,8-10] cytochrome,[6,8] and catalase[8] have been reported in the literature.[11]

We have begun a systematic investigation of the Mössbauer spectra of some iron–porphyrin complexes and report in this paper a study of the spectra of the adducts of ferrous and ferric tetraphenylporphyrin, protoporphyrin, and protoporphyrin dimethyl ester complexes with pyridine, piperidine, and imidazole. These were selected as model compounds for the naturally occurring hemoglobins, cytochromes, and catalases which contain octahedrally coordinated iron with the fifth and sixth coordination positions being occupied by an amine (such as an imidazole nitrogen from histidine) and/or water. The only previous work on such compounds was done by Bearden, Moss, Caughey, and Beaudreau[12] on the bispyridine hemochromes of 2,4-diacetyldeuteroporphyrin dimethyl ester and mesoporphyrin dimethyl ester. Some related phthalocyanine complexes have been investigated by Hudson and Whitfield.[13]

(1) (a) Supported by AEC Contract AT(30-1)3514; (b) Westinghouse Electric Corp.; (c) University of Pittsburgh.

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## Experimental Section

The Mössbauer spectra were obtained with a scanned velocity spectrometer operating in the time mode. Such a device has been widely described.[14] The radiation source consisted of $Co^{57}$ diffused into chromium and was kept at room temperature. The absorbers could be maintained at room temperature or liquid nitrogen or liquid helium temperature. The velocity scale and isomer shift references were obtained by frequent calibration against a sodium nitroprusside absorber. Experimental line widths for sodium nitroprusside were typically 0.32 mm/sec.

**Materials.**—Reagent grade pyridine, piperidine, imidazole, hemin, and solvents were obtained commercially. $\alpha,\beta,\gamma,\delta$-Tetraphenylporphyrin ferric chloride[15] and hemin dimethyl ester[16] were prepared according to published procedures. Ninety per cent enriched $Fe^{57}$ was used to prepare some samples of tetraphenylporphyrin ferric chloride.

**Imidazole Adduct of Tetraphenylporphyrin Ferric Chloride.**—A slight excess of imidazole was added to a boiling solution of 300 mg of $\alpha,\beta,\gamma,\delta$-tetraphenylporphyrin ferric chloride in 20 ml of chloroform. Most of the chloroform was allowed to evaporate by gentle heating. Methanol was added gradually during the removal of the chloroform. When small crystals began to form, a little more methanol was added, and the flask was allowed to cool to room temperature. The lustrous blue crystals were collected, washed with methanol, and dried in air. *Anal.* Calcd for $C_{44}H_{28}N_4FeCl\cdot 2C_3H_4N_2\cdot 2CH_3OH$: C, 69.03; H, 4.82; N, 12.40; Cl, 4.04. Found: C, 69.86; H, 4.70; N, 12.45; Cl, 3.91.

**Imidazole Adduct of Hemin.**—A solution of 300 mg of hemin in 40 ml of 0.1 *N* sodium hydroxide was treated with excess imidazole, filtered, and made slightly acidic. The precipitate which formed was collected and dried over phosphorus pentoxide. The crystals were not analyzed.

For some of the experiments the adduct was prepared by keeping a mixture of imidazole containing 5% by weight of hemin at the melting point of imidazole for about 15 min, then allowing the melt to solidify. In these cases no attempt was made to isolate the pure product.

**Imidazole Adduct of Hemin Dimethyl Ester.**—Excess imidazole was added to a hot solution of 200 mg of hemin dimethyl ester in 20 ml of chloroform. The chloroform was evaporated until crystals formed. These were collected and dried in air.

**Imidazole Hemochrome from Tetraphenylporphyrin Ferric Chloride.**—Excess solid imidazole was added to 1.0 ml of a saturated dioxane solution of $\alpha,\beta,\gamma,\delta$-tetraphenylporphyrin ferric chloride, prepared with enriched iron. A few drops of a saturated aqueous solution of sodium dithionite was added and the mixture was shaken. No attempt was made to isolate the solid adduct; the mixture was frozen at once and used as such.

**Imidazole Hemochrome from Hemin.**—A slurry of this hemochrome was prepared by treating a concentrated solution of hemin in 1.0 *N* potassium hydroxide with excess imidazole and reducing with sodium dithionite. Care was taken to avoid exposure to air. The solid complex was not isolated. The slurry was immediately frozen with liquid nitrogen.

**Piperidine Hemochrome from Tetraphenylporphyrin Ferric Chloride.**—Piperidine (1 ml) was added to a boiling solution of 350 mg of $\alpha,\beta,\gamma,\delta$-tetraphenylporphyrin ferric chloride in 35 ml of methylene chloride. Crystallization was effected by addition of methanol in the same way as with the imidazole adduct. The dark blue crystals were collected, washed with methanol and ether, and dried in air. *Anal.* Calcd for $C_{44}H_{28}N_4Fe\cdot 2C_5H_{11}N$: C, 77.33; H, 5.97; N, 10.02; Cl, 0.00. Found: C, 77.80; H, 5.80; N, 9.46; Cl, 0.75.

**Piperidine Hemochrome from Hemin.**—The moist crystals obtained by recrystallizing hemin from hot piperidine were used.

**Pyridine Adduct of Tetraphenylporphyrin Ferric Chloride.**—This adduct was not isolated, but was prepared in solution by dissolving 6.10 mg of $\alpha,\beta,\gamma,\delta$-tetraphenylporphyrin ferric chloride 90% enriched in $Fe^{57}$ in 0.9 ml of freshly distilled pyridine.

**Pyridine Adduct of Hemin.**—Hemin (200 mg) was dissolved in 2 ml of pyridine on a watch glass. Most of the excess pyridine was allowed to evaporate. The moist crystals were used.

**Pyridine Hemochrome from Tetraphenylporphyrin Ferric Chloride.**—This compound was not isolated but was prepared in solution by shaking a mixture of a saturated aqueous solution of sodium dithionite and a pyridine solution of enriched $\alpha,\beta,\gamma,\delta$-tetraphenylporphyrin ferric chloride.

**Pyridine Hemochrome from Hemin.**—Sodium dithionite (67 mg) in 0.2 ml of water was added to a solution of 321 mg of hemin dissolved in 2 ml of pyridine. Upon addition of the dithionite, the solution changed color and gelled. Part of the excess pyridine was evaporated in a stream of nitrogen and the rest was removed by squeezing the semisolid mass between filter paper.

## Results

Typical spectra are shown in Figures 1–6. The general character of the spectrum changes drastically upon formation of the octahedral complex from the five-coordinated porphyrin ferric chloride. This becomes evident when Figure 1 (tetraphenylporphyrin ferric chloride) is compared to the others. The positions and amplitudes of the lines are determined by the computer to give the best fit for values of the line width encoded by the experimenter. The data were recorded in terms of channel number and converted to velocity by comparison to the sodium nitroprusside spectrum.

Figure 2 shows the imidazole complex of tetraphenylporphyrin ferric chloride, a stable crystalline compound. Figure 3 is the result of an attempt to make the corresponding ferrous complex (imidazole hemochrome) in benzene using 90% enriched $Fe^{57}$. Most of the sample remained as Fe(III) complex (imidazole hemichrome) as shown by the outer pair of lines which agree quite closely with the spectrum of the pure hemichrome (Figure 2). A minor portion was converted to the hemochrome as revealed by the inner pair of lines. Figure 4 shows the most successful preparation of the hemochrome which was done using a dioxane solution and indicates the absence of the unreduced compound. (The horizontal span of Figure 4 is approximately half that of Figure 3.) The positions of the inner peaks of Figure 3 and those of Figure 4 are in excellent agreement. Figures 5 and 6 show the hemi- and corresponding hemochrome forms of the pyridine adduct of hemin. The hemochrome seems to be the more stable of the two and tends to form even in the absence of a reducing agent (Figure 5). A small left-hand hemochrome peak was resolvable, but the right-hand peak was too close to the right-hand peak of the hemichrome to be distinguished.

The isomer shifts and quadrupole splittings for the 11 pyridine, piperidine, and imidazole adducts prepared in this work are given in Table I. Both ferrous and ferric complexes were obtained in the pyridine and imidazole cases; however, only the ferrous complex could be obtained for the piperidine adduct. It is

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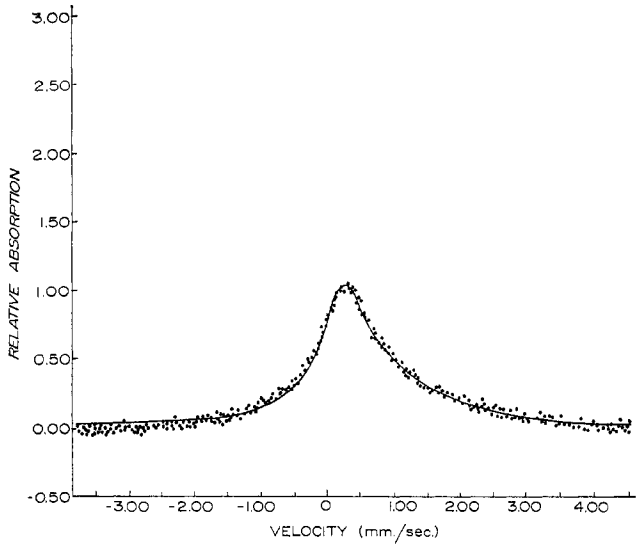


Figure 1.—Mössbauer spectrum of enriched tetraphenylporphyriniron(III) chloride at room temperature.

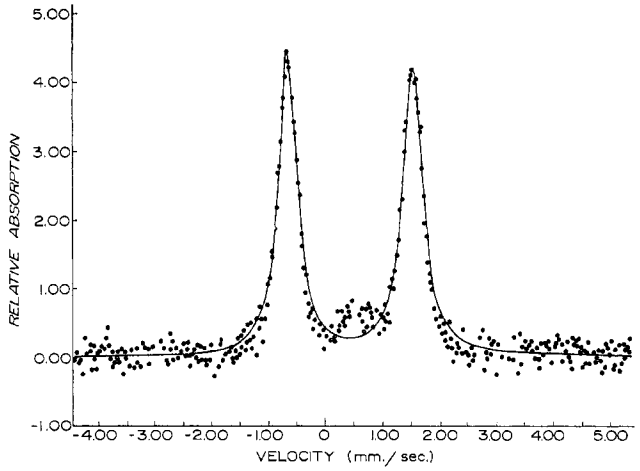


Figure 2.—Mössbauer spectrum of the imidazole adduct of tetraphenylporphyriniron(III) chloride at room temperature.

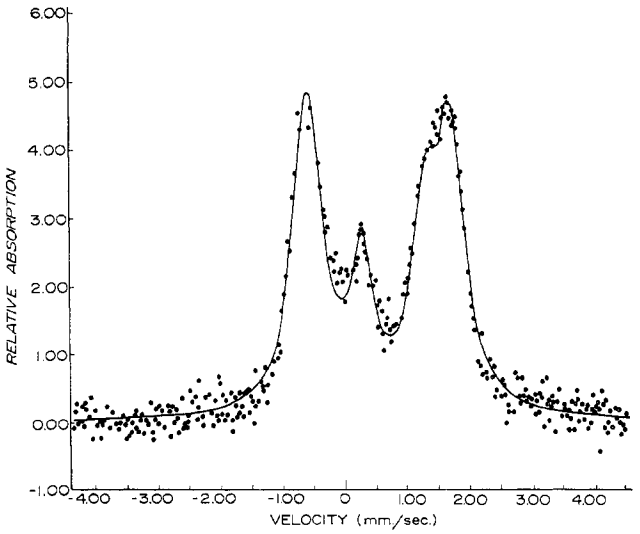


Figure 3.—Mössbauer spectrum of a benzene solution of the imidazole adduct of tetraphenylporphyriniron(II) at liquid nitrogen temperature. The inner peaks are due to the iron(II) complex. The outer peaks, due to the iron(III) complex, indicate incomplete reduction.

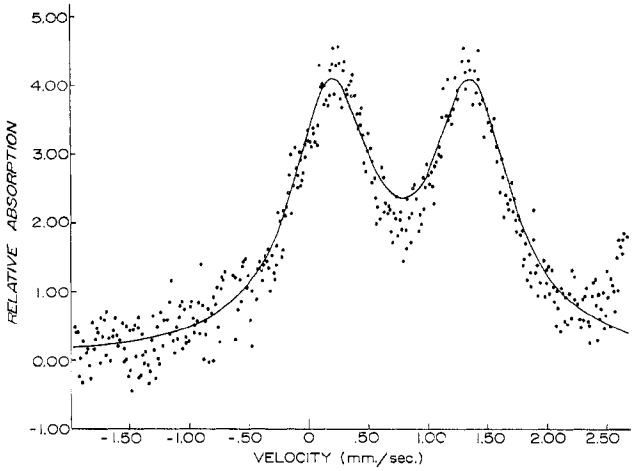


Figure 4.—Mössbauer spectrum of a dioxane solution of the imidazole adduct of tetraphenylporphyriniron(II) at liquid nitrogen temperature.

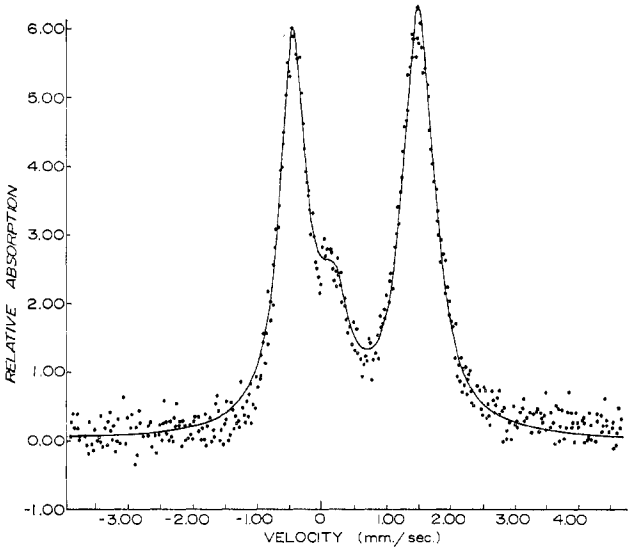


Figure 5.—Mössbauer spectrum of the pyridine hemichrome of hemin at liquid nitrogen temperature. The inner peak indicates some reduction of the iron(III).

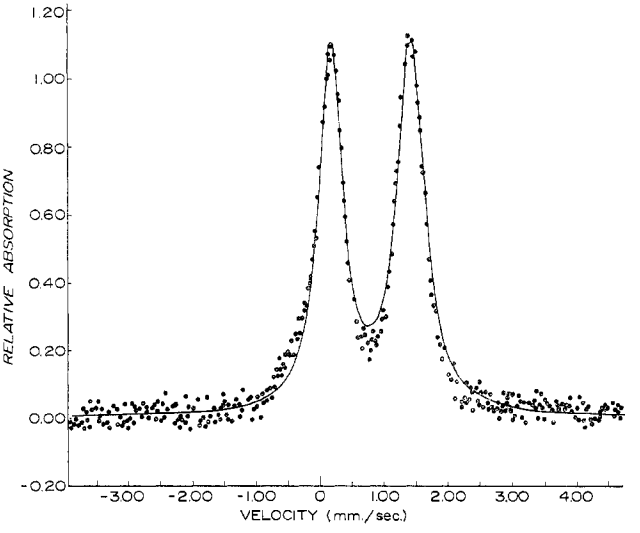


Figure 6.—Mössbauer spectrum of the pyridine hemochrome of hemin at liquid nitrogen temperature.

TABLE I
AVERAGE MÖSSBAUER PARAMETERS OF OCTAHEDRAL PORPHYRIN COMPLEXES[a]

| Oxidn state | Ligands | Isomer shift, δ —mm/sec— Room temp | Isomer shift, δ —mm/sec— Liq $N_2$ temp | Quadrupole splitting, Δ —mm/sec— Room temp | Quadrupole splitting, Δ —mm/sec— Liq $N_2$ temp |
|---|---|---|---|---|---|
| Fe(III) | TPP,[b] imidazole | 0.40 | 0.50 | 2.11 | 2.23 |
| | Hemin, imidazole | 0.41 | 0.51 | 2.17 | 2.30 |
| | Hemin dimethyl ester, imidazole | 0.42 | 0.51 | 2.21 | 2.35 |
| | TPP, pyridine[c] | ... | 0.43 | ... | 1.25 |
| | Hemin, pyridine | ... | 0.50 | ... | 1.88 |
| Fe(II) | TPP, imidazole[d] | ... | 0.72 | ... | 1.06 |
| | Hemin, imidazole[d] | ... | 0.69 | ... | 0.95 |
| | TPP, piperidine | 0.69 | 0.75 | 1.42 | 1.42 |
| | Hemin, piperidine | 0.69 | 0.76 | 1.43 | 1.42 |
| | TPP, pyridine[c,d] | 0.66 | 0.72 | 1.18 | 1.17 |
| | Hemin, pyridine[d] | ... | 0.72 | ... | 1.21 |

[a] Isomer shifts are relative to a sodium nitroprusside absorber at room temperature. [b] TPP indicates α,β,γ,δ-tetraphenylporphyrin. [c] Enriched with 90% $Fe^{57}$. [d] Reduced with dithionite.

known that piperidine causes spontaneous reduction of ferric porphyrins.[17]

No systematic differences in either the isomer shift or the quadrupole splitting were found between the isolated crystalline adduct and the adduct in solution in those cases where both forms were measured.

Magnetic susceptibility data in the range 4.2–50°K on the bisimidazole adduct of α,β,γ,δ-tetraphenylporphyrin ferric chloride, $C_{44}H_{28}N_4FeCl \cdot 2C_3H_4N_2 \cdot 2CH_3OH$, were obtained in order to confirm the tripositive state of the iron in this complex. The iron has an effective moment of 2.36 ± 0.1 BM and a Weiss constant of −1.6°K. These values are consistent with trivalent iron in a magnetically dilute, strong-field (low-spin) complex.[18,19] The effective moment of the ferric ion in imidazole horse hemoglobin (2.87 BM),[20] imidazole horse myoglobin (2.44 BM),[20] and the pyridine adduct of hemin (2.15–2.74 BM)[21] indicate low-spin ferric ion in these compounds as well.

## Discussion

All of the octahedral complexes were prepared from the pentacoordinated high-spin chlorides, *i.e.*, hemin, hemin dimethyl ester, and α,β,γ,δ-tetraphenylporphyrin ferric chloride, and are readily recognized by their spectra. The pentacoordinated complexes gave the characteristic absorption curves (Figure 1) interpreted by some as an unresolved doublet. The left-hand peak of these plots is usually narrow and the right-hand peak very broad, in sharp contrast to the lines of the octahedral complexes which were usually narrow and of equal intensity.

The general nature of the quadrupole splitting of the low-spin complexes of Fe(II) and Fe(III) is just the opposite of that found in the ionic high-spin cases. The Fe(III) high-spin complexes rarely have quadrupole splitting (Δ) values exceeding 1 mm/sec and the values reflect in a simple way the symmetry of the negative charge centers about the iron nucleus. High-spin Fe(II), however, is very sensitive to its environment, and widely varying Δ values are a manifestation of this sensitivity. Δ values as high as 3.6 mm/sec have been measured.[22] In these cases the electric field gradient is indigenous to the d shell which possesses one electron in excess of the five necessary for a symmetrical half-filled shell. (As expected, these large Δ values are temperature dependent.) Correspondingly, the low-spin Fe(II) system with its filled $t_{2g}$ orbitals is an insensitive system. The lower filled d orbitals in octahedral complexes possess spherical symmetry, and widely varying Δ values are not to be expected. However, for low-spin Fe(III) with its single hole in the otherwise filled $t_{2g}$ level the iron nucleus becomes particularly sensitive to its environment. Here again, large, widely varying Δ values are to be expected.

For a given set of ligands and the same extent of d-electron delocalization, Fe(II) complexes will have larger δ values indicating less electron density at the iron nucleus owing to the shielding effect of the additional d electron.

The results given in Table I can be explained in terms of the foregoing generalizations.

**Ferric Complexes.**—Piperidine complexation caused spontaneous reduction of the Fe(III) to Fe(II) in all cases which were investigated. The pyridine adducts have a tendency toward reduction, but the imidazole complex is very stable. This is probably due to the greater capacity of imidazole to stabilize the high charge on the iron atom by dissipation of some of the positive charge over the imidazole ring.[23]

The ease of formation of the ferric complexes parallels the π-donor character of the ligand. The coordinated porphyrin ring is a typical π acceptor so the largest values of Δ would be expected for the imidazole adducts because imidazole has greater π-electron-donating capabilities than pyridine. The values in Table I exemplify this effect. There was also a consistent decrease in Δ with increasing temperature which is typical for the "sensitive" Fe(III) low-spin complexes.

Among the imidazole complexes the nature of the porphyrin causes only small variance in Δ and δ. For the ferric pyridine complexes the differences were more pronounced, the tetraphenylporphyrin complex showing smaller values of δ and Δ, although all these values were in the Fe(III) area. Because only three porphyrins were studied, lack of sufficient data prohibits discussing this anomaly without recourse to speculation.

**Ferrous Complexes.**—As has already been reported, the pyridine hemochromes were easier to prepare, and they were more stable with respect to reoxidation than the imidazole hemochromes. Piperidine poses somewhat of a mystery in the respect that the hemin

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(19) B. N. Figgis and J. Lewis, *Progr. Inorg. Chem.*, **6**, 172 (1964).
(20) G. Schoffa, *Advan. Chem. Phys.*, **7**, 182 (1964).
(21) R. Havemann, W. Haberditzl, and K.-H. Mader, *Z. Physik. Chem.*, **218**, 71 (1961).
(22) S. De Benedetti, G. Lang, and R. Ingalls, *Phys. Rev. Letters*, **6**, 60 (1961).
(23) J. N. Phillips, *Rev. Pure Appl. Chem.*, **10**, 35 (1960).

and tetraphenylporphyrin ferric chloride complexes are spontaneously reduced to hemochromes without the addition of a reducing agent. As far as we know, no one has yet explained this phenomenon. Because of this spontaneous reduction in piperidine, we were led to believe that piperidine has a greater affinity for the iron atom in a porphyrin complex than pyridine. There was some noticeable reduction in pure pyridine but a greater percentage was unreduced (see Figure 5). When the complex was dissolved in pure pyridine and a small amount of piperidine added, the reduced complex resulted. To determine the relative affinity of piperidine *vs.* pyridine for the porphyrin complex, the complex was dissolved in a mixed solution of the two ligands and the Mössbauer parameters were measured. Because the isomer shift ($\delta$) values of the pure adducts are very close to each other, the $\delta$ values are not expected to yield very useful information. However, the values of $\Delta$ are sufficiently far apart so the effective quadrupole splitting was plotted *vs.* mole fraction of pyridine (Figure 7). The upward concavity of this plot indicates a higher affinity of pyridine for the heme than for piperidine.

From Table I it is seen that the isomer shift values for the complexes studied including those with different porphyrins all lie close to one another with a slight trend to larger values for piperidine. Essentially, this implies that the s-electron density about the iron atom is nearly alike for all of these complexes even though their stabilities and ligand affinities are somewhat different. Imidazole is only weakly $\pi$ accepting while pyridine is a relatively strong $\pi$ acceptor and piperidine has no $\pi$-bonding capabilities at all. Because the orbitals on the metal available for $\pi$ bonding are the filled $t_{2g}$, $\pi$ interaction from metal to ligand is the only kind expected. This interaction is characterized by the smaller values of $\delta$ with the concept that decreasing the d-electron density causes less shielding of the s electrons, hence yielding smaller values for the isomer shift.

It is probably true that pyridine has increased affinity for heme over imidazole because of its greater $\pi$-accepting capabilities, although it would seem from Mössbauer measurements that the electronic structures of the two adducts are similar after the compounds are formed. The trend in $\delta$ values then represents the extent to which $\pi$ interaction contributes to the bonding.

The hemochromes have the configuration $(t_{2g})^6$, assuming cubic symmetry as a convenient approximation; consequently these orbitals, the $t_{2g}$, possess nearly spherical symmetry, and large values for the quadrupole splitting are not expected. The splitting that does result may be attributed to the nonequivalence of the porphyrin ring and ligand nitrogens which gives rise to tetragonal distortion from cubic symmetry.[12] The

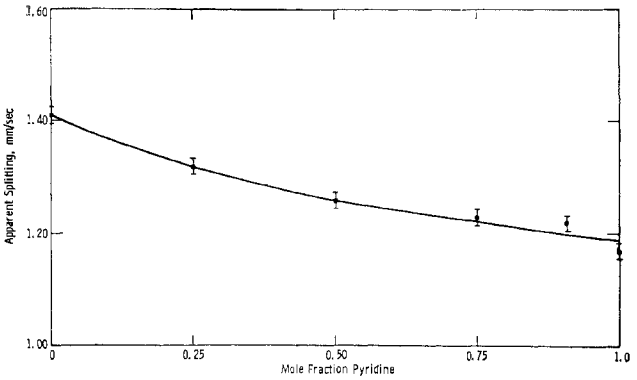


Figure 7.—Apparent quadrupole splittings of tetraphenylporphyriniron(III) chloride, dissolved in pyridine–piperidine mixtures. The iron(III) has been reduced to iron(II).

piperidine nitrogens are aliphatic and possess $sp^3$ hybridization while the nitrogens of the other two ligands and of the porphyrin are $sp^2$ hybridized. Consequently, the largest values of $\Delta$ are encountered for the piperidine adducts. The $\Delta$ values for the pyridine adducts are slightly higher than those for the imidazole adducts. This increase can be attributed to $\pi$ interaction of the filled metal $t_{2g}$ orbitals with unfilled orbitals of the pyridine ligand, a rather strong $\pi$ acceptor.

## Summary

Imidazole forms the most stable adduct with the Fe(III) porphyrins and produces the largest electric field gradient, while for the Fe(II) complexes, imidazole has the lowest affinity, is least stable toward reoxidation, and produces the smallest electric field gradient. The affinity of pyridine for Fe(III) porphyrins is low and the electric field gradient is smaller compared to imidazole, in direct contrast to the Fe(II) complexes. The important direction for $\pi$ bonding in the Fe(III) compounds would seem to be from ligand to metal since the high charge of the Fe atom has to be stabilized. Here a molecule like imidazole, which is a $\pi$ donor, would be preferred.

For the Fe(II) complexes (filled $t_{2g}$ orbitals) $\pi$ interaction is expected in only one direction, metal to ligand. In this case imidazole, a $\pi$ donor, is less preferred than pyridine, a $\pi$ acceptor. In both Fe(II) and Fe(III) complexes, the greater electric field gradient is associated with the ligand which is preferred for its $\pi$-bonding character.

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