Ligation and Reduction of Iron(III) Porphyrins by Amines. A Model for Cytochrome P-450 Monoamine Oxidase

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Abstract: The scope and mechanism of the ligation and reduction of iron(III) porphyrins by amines are presented. The reaction is general and proceeds with the overall stoichiometry $2PFe^{III}Cl + 5RNH_2 \rightarrow 2PFe^{II}(H_2NR)_2 + R'CH{=}NH + 2RNH_3^+Cl^-$. Imines are the only amine-derived products. The ability of the amine to coordinate iron and its possession of a >CHNH moiety are essential for reduction. The reaction path entails two successive reversible ligation steps followed by two one-electron reductions of the bis(amine)-ligated low-spin iron(III) adduct. In benzene the kinetics are biphasic. The second ligation is rate limiting [k_1(ligation) > k(reduction) > k_2(ligation)]. In dimethylformamide the first reduction step is rate limiting. The influence of nonreducing amines, porphyrin substituent, axial ligands, and deuteration of the substrate upon the kinetics leads to the formulation of the first reduction as a reversible outer-sphere electron transfer to the porphyrin periphery. This is followed by a rapid conversion of the generated aminium cation radical to an α-aminocarbinyl radical. The latter completes the reduction in an irreversible second step. The relevance of this chemistry to related transformations catalyzed by cytochrome P-450 is discussed.

In 1967, while examining the Mössbauer spectra of iron porphyrins, Epstein[1] and associates observed that treatment of chloroiron(III) tetraphenylporphine with piperidine resulted in the iron(II) porphyrin rather than the bis-ligated iron(III) complex. These workers also indicated that other iron(II) porphyrins could be obtained in this fashion. Treatment with piperidine has resulted in crytalline bis(piperidine)iron(II) tetraphenylporphine and was the basis for an X-ray structure of this complex.[2] More recently, an NMR study of this specific reaction was presented by LaMar and Del Gaudio.[3] *N*-Hydroxypiperidine was employed as a principal mechanistic probe, and the *N*-oxide radical was observed. On the basis of the results with the tetraphenylporphine complex, these workers proposed a mechanism for the amine reduction that involved a proton abstraction from an iron-bound amine to yield an Fe^{III} porphyrin $(RNH)^-$ species. The latter was proposed to reduce to iron(II) porphyrin and *N*-alkylamino radical.

A pressing need to reliably obtain solid crystalline iron(II) porphyrin complexes prompted us to examine the reaction first noted by Epstein, Straub, and Maricondi. A simple reliable procedure was established[4a] for bis(piperidine) preparations of iron(II) complexes of octaethylporphyrin, mesoporphyrin dimethyl ester, deuteroporphyrin dimethyl ester, and protoporphyrin dimethyl ester.

The present effort is an extension of this work to a general study of the scope and mechanism of this important reaction.

Apart from its uncharted nature and intrinsic chemical interest, the work is relevant to some of the astonishing variety of biochemical processes[5a] in which amines can participate. For example, amines and derivatives are known carcinogens.[5b,6] They are known to enhance the mixed-function oxidase system in liver microsomes.[7] Moreover, amines and derivatives can undergo a variety of transformations with cytochrome P-450.[5c] These include nitrogen dealkylation and oxidative deamination as well as *N*-oxide reduction.[8] The actual path of metabolism of amphetamine to phenylacetone (one of the reactions examined in the present work) has been the subject of some controversy.[5c] Recently epinephrine, an "α-andrenergic agonist" that promotes platelet aggregation, was found to reduce iron(III) protoporphyrin IX.[9] Finally, a variety of hemeproteins, including cytochrome *c*,[10] contain ϵ-lysyl moieties that are imbedded deeply enough within the protein framework such that they are not attacked by the usual amine alkylating agents.[11] In cytochrome *c* the amine group of lysine-79 is within range of iron. Thus, the possibility of an internal reduction of such a cytochrome is reasonable.

Experimental Section

Materials. Porphyrins and iron complexes were obtained in the manner previously described.[12]

The iron(III) complex of the bis[(γ-aminopropyl)amide] of mesoporphyrin IX was prepared by aminolysis of the ester. A solution of 200 mg (2.9×10^{-4} mol) of chloroiron(III) mesoporphyrin IX dimethyl ester in 20 mL of 1,3-diaminopropane was refluxed under argon for 2 h in a small distillation apparatus. The bath temperature was held at 145 °C. The flask was distilled to dryness at this temperature in vacuo. The solid was chipped from the flask with acetone, vacuum filtered, washed copiously with acetone, and air-dried. The crude product was taken up in methanol and placed atop a 2 × 50 cm A-540 alumina/methylene chloride column. Elution with methylene chloride removes any starting ester. The bis(amide) is eluted quickly with methanol. The methanol solution was filtered and concentrated on the rotary evaporator, and the solid was chipped from the flask with acetone and washed and dried as described above; 0.081 g. Anal. Calcd for $C_{40}H_{52}N_8$·FeCl (768.19): C, 62.53; H, 6.82; N, 14.58. Found: C, 52.05, 56.88; H, 6.82; N, 14.61. FAB mass spectrometry: high-mass ions centered at m/e 768 and 733 (P − Cl). IR (KBr): 3460 (vs), 1660 cm^{-1} (vs), NH_2 and amide **1**. UV–vis (closely resembles that of the final bis(*tert*-butylamine) adduct shown in Figure 3) λ_{max} (DMF): 345 nm (sh), 388, 565, 588 (sh).

Amines were best pure samples purchased from Aldrich and Eastman Kodak. For most cases, final purification was a distillation from calcium hydride. Phenacylamine hydrochloride was recrystallized from 2-

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(4) Chu, M. L.; Castro, C. E.; Hathaway, G. M. *Biochemistry* **1978**, *17*, 481.
(5) (a) Salerni, O. L. *Natural and Synthetic Organic Medicinal Compounds*; c. V. Mosby: St. Louis, MO, 1976. (b) Kasper, C. B.; Henton, D. In *Enzymatic Basis of Detoxication*; Jakoby, W. B., Ed.; Academic: New York, 1980; pp 10–11. (c) Wislocki, P. G.; Miwa, G. T.; Lu, A. Y. H. *Ibid.* Vol. 1, Chapter 7, pp 135–170 and references therein.
(6) Marquandt, H.; Baker, S.; Grover, P. L.; Sims, P. *Cancer Lett. (Shannon, Irel.)* **1977**, *3*, 31.
(7) Cinti, D. L. In *Hepatic Cytochrome P-450 Monooxygenase System*; Schenkman, J. B., Kupfer, D., Eds.; Pergammon: New York, 1982; Chapter 28, pp 717–732 and references therein.
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(9) Peterson, D. A.; et al. *Science (Washington, D.C.)* **1982**, *215*, 713–715.
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(12) (a) Wade, R. S.; Castro, C. E. *J. Am. Chem. Soc.* **1973**, *95*, 226. (b) Castro, C. E.; Robertson, C.; Davis, H. F. *Bioorg. Chem.* **1974**, *3*, 1343. (c) Castro, C. E. *Bioorg. Chem.* **1974**, *4*, 45. (d) This treatment is essential for the removal of trace tetrahydropyridine impurities that can be reductants. Their presence can lead to erroneous interpretations. Cf.: Srivasta, G. S.; Sawyer, D. R. *Inorg. Chem.* **1985**, *24*, 1732–1734.

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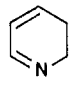
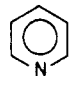
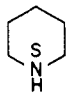
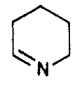
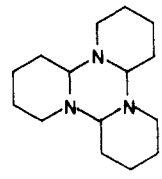
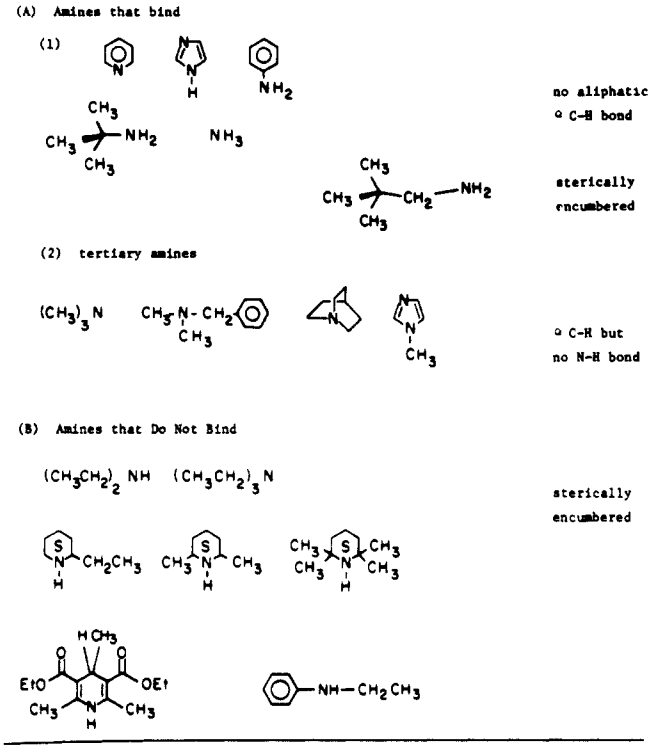
Table I. Yields of Oxidized Amine Products from Reactions with Iron(III) Porphyrins

starting amine	product	characterizn (method)[a]	yield,[b] %
$CH_3CH_2CHCH_3$ (NH$_2$)	$CH_3CH_2CCH_3$ (NH)	$CH_3CH_2CCH_3$ (O) (a)	39[c]
		 (b)	100
		 (c)	58
$PhCH_2NH_2$	PhCH=NH	PhCHO (a)	100
$PhCHCH_3$ (NH$_2$)	$PhC(=NH)CH_3$	$PhCCH_3$ (O) (a)	91
$PhCH_2CH_2NH_2$	$PhCH_2CH=NH$	$PhCH_2CHO$ (a)	93
$PhCH_2CHCH_3$ (NH$_2$)	$PhCH_2CCH_3$ (NH)	$PhCH_2CCH_3$ (O) (a)	87

[a] Methods of characterization: (a) 2,4-DNP; (b) NMR; (c) mass spectrum. [b] Yields determined by gas chromatography of characterized product indicated. [c] In general, yields reflect the adequacy of the workup. No other product was detected.

Table II. Inert Amines

observation	classification
	

propanol, mp 186.5–188 °C. The free amine was generated in reaction cuvettes by the addition of *tert*-butylamine. Pyridine was refluxed over $KMnO_4$, distilled, dried over KOH, and refractionated through a spinning band column.[12d] Piperidine and tetrahydropyridine were similarly fractionated. Quinuclidine was sublimed twice before use. The physical constants of all amines, boiling point, melting point, and NMR results checked with those in the literature.

Benzylamine–CD_2NH_2, bp 64 °C (10 mm), was prepared by the reduction of benzonitrile with $LiAlD_4$ in triglyme according to the procedure of Guthrie, Borden, and Lowell.[13a]

The CD_2ND_2 and CH_2ND_2 derivatives were obtained by repeatedly exchanging the corresponding benzylamines with D_2O and reisolation of the amines by CH_2Cl_2 extraction.[13b] Final purification was by distillation through a small Vigreux column. These substances were checked for purity by IR and NMR: δ 3.65 (α-CH_2), 1.2 (NH_2). Deuteration was >99% in all cases.

An authentic sample of α-tripiperideine was prepared from the imine generated from piperidine by N-chlorination and dehydrohalogenation.[14,15]

Reactions. Generally, products were characterized as (2,4-dinitrophenyl)hydrazones of the aldehyde or ketone obtained by hydrolysis of the imine (cf. Table I). The DNP derivatives were compared with authentic samples via mixed melting point and NMR. Quantitation was accomplished by flame-ionization gas chromatography of hydrolyzed reaction mixtures.

While DNP derivatives of all of the aldehydes and ketones in Table I were isolated from reactions of mesohemin in DMF, this was a difficult process to quantitate. The relatively dilute solutions employed for kinetic analysis were not well suited to product identification and quantitation. A variety of solvents (DMF, benzene, tetrahydrofuran, pyridine, methylene chloride, neat amine) and workup conditions were employed. However, a reliable procedure for quantitation of the DNPs was not established, and the yields of these substances ranged from 7% phenylacetone (from amphetamine) to 40% benzeldehyde (from benzylamine). No other products than those indicated were detected by this means or by gas chromatography. The DNP approach was abandoned as a means of quantitation, and more reliable gas chromatographic procedures were developed for this purpose.

All of the reactions in Table I were conducted with more than one porphyrin. The imine products were those indicated in all cases.

The reactions below are illustrative.

Amphetamine and Mesohemin Dimethyl Ester. A mixture of 50 mg of hemin (7.3×10^{-5} mol) and 1.63 g of amphetamine (1.2×10^{-2} mol) under argon was allowed to stand for 1 h. The mixture was cooled in an ice bath, opened to air, and acidified with concentrated HCl to pH 2. An acidic ethanol solution of 25 mg of (2,4-dinitrophenyl)hydrazine was added. The whole was shaken and allowed to stand overnight. The mixture was extracted three times with CH_2Cl_2. The extracts were washed once with water, dried over sodium sulfate, filtered, and concentrated to dryness on a rotary evaporator. Solids were taken up in 1:1 CH_2Cl_2–hexane and placed atop a 1 × 30 cm dry-silica column. The yellow DNP band eluted with 1:1 CH_2Cl–hexane. Unreacted hydrazine and the hemin remained at the top. The DNP fraction was concentrated to dryness and chromatographed on a thin-layer plate of silica gel. The plate was developed with 1:1 CH_2Cl_2–hexane containing 5% MeOH. The DNP of phenylacetone cochromatographed with the product DNP. The reaction product was scraped from the plate. A portion was taken up in CH_2Cl_2, filtered, concentrated, and crystallized. A mixed melting point of 148–150 °C was slightly depressed, but the NMR ($CDCl_3$) was identical with that of the authentic DNP and showed the characteristic CH_3, CH_2, and phenyl resonances at δ 2.0 (s), 3.7 (s), and 7.3 (sh m).

Amphetamine and Chloroiron(III) Tetraphenylporphine in Pyridine. In a thoroughly purged 3-mL reaction vessel under argon, 25 mg of chloroiron(III) tetraphenylporphine (3.6×10^{-5} mol) and 63 μL of *d*-amphetamine (4.5×10^{-4} mol) in 1.25 mL of pyridine were allowed to react for 20 min. A spectrophotometric probe indicated reaction was complete. The reaction was opened to air and treated with 3 mL of 6 N HCl (pH 1). After 45 min, the mixture was extracted with three 2-mL portions of CH_2Cl_2. The CH_2Cl_2 extracts were dried over Na_2SO_4 and analyzed by gas chromatography on a $^1/_8$-in., 2-ft Poropak R column at 195 °C. The phenylacetone peak at 12.0 min coemerged with an authentic sample. The yield (91%) was determined by subjecting an authentic standard to the same workup. The aqueous phase was adjusted to pH 8 and extracted with five 2-mL portions of benzene to remove the pyridine. The remaining aqueous phase was adjusted to pH 12 and extracted five times with ether. The ether extracts were dried over

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(15) Schöpf, C.; Komzak, A.; Braun, F.; Jacobi, E. *Ann. Chem.* **1948**, *559*, 1.

sodium sulfate, filtered, and gassed with HCl. The white hydrochloride suspension was concentrated to dryness and recrystallized from benzene; mp and mixed mp with authentic *d*-amphetamine hydrochloride 152.5–153 °C. Analysis of enantiomeric purity by NMR[16] via the shift reagent tris[3-[(trifluoromethyl)hydroxymethylene]-*d*-camphorato]europium(III) indicated no detectable isomerization.

Piperidine and Chloroiron(III) Mesoporphyrin IX Dimethyl Ester. A thoroughly argon-purged solution of 0.05 M hemin in methylene chloride was made 0.05 M in piperidine by injection of the base under argon. After 3 h, 5 mL of 1 M HCl was added. The mixture was shaken and separated, and the aqueous phase was centrifuged. The supernatant solution was basified with NaOH to pH 13 and extracted 3× with CH_2Cl_2. The entire Na_2SO_4-dried CH_2Cl_2 solution was placed on a 2 × 40 cm column of A-540 Al_2O_3 in CH_2Cl_2. Following an initial 30 mL of CH_2Cl_2, elution was begun with 20% MeOH in CH_2Cl_2. The second 10 mL of eluate following the front contained the bulk of the α-tripiperideine and a small amount of piperidine. This fraction was washed with aqueous base, dried over K_2CO_3, and concentrated near atmospheric pressure to approximately 0.1 mL. The concentrate was analyzed by gas chromatography on a 12-ft × $^1/_8$-in. column that contained 10% Carbowax and 1% KOH on Chromosorb W at 95 °C. Peaks coemergent with authentic standards of piperidine (4.5 min) and α-tripiperideine (15 min) were apparent. GC–mass spectra of the 15-min peak showed ions at *m/e* 83 and 84 corresponding to the imine (P, P + 1). Using an internal standard, gas chromatographic analysis of the entire reaction preceding workup indicated the yield of trimer at 58%. GC monitoring of the reaction with time, however, showed initially (2 min) only one product peak at 8.5 min assigned to the imine. As the reaction progressed, the imine peak diminished and the corresponding peak for the trimer increased. GC–MS of both peaks corresponded to the imine. At 2 h only trimer was detected. Note solutions of the trimer in CH_2Cl_2 without piperidine upon gas chromatography show primarily, and in some cases exclusively, the imine peak. Addition of piperidine to these solutions, however, catalyzes the conversion to the trimer.

Benzylamine and Deuterohemin IX. A solution of 2.6 g (4.3×10^{-3} mol) of chloroiron(III) deuteroporphyrin IX, 38 mL of pyridine, and 90 μL of benzylamine (0.825×10^{-3} mol) was warmed under argon at 72 °C for 20 h. The mixture was cooled to 40 °C, pourced onto a 25 × 2 $^1/_2$ cm dry A-540 alumina column and eluted with CH_2Cl_2. The first 50 mL of eluate was clear, and the second 100-mL fraction was faintly colored and contained some iron(II) porphyrin (α,β bands). VPC analysis of the first fraction showed a peak coemergent with benzaldehyde. No benzonitrile was detected. Both fractions were combined and washed twice with a 100-mL portion of 18% HCl. The CH_2Cl_2 fraction was dried over Na_2SO_4 for 1 day. The brownish solution was evaporated to dryness at 60 °C in a rotary evaporator. One milliliter of methanol was added. VPC analysis (5% SE-30 column) showed two peaks: one coemergent with benzaldehyde and the more dominant coemergent with its dimethyl acetal. The latter was prepared by passing a MeOH solution of benzaldehyde through an acidic ion-exchange column. There appeared to be an on-column conversion of benzaldehyde to its dimethyl acetal on the 6-ft × $^1/_4$-in. column containing 20% Dow 710 on Chromosorb W at 138 °C. The larger dimethyl acetal peak was repeatedly trapped from the gas chromatographic column in a CO_2–*i*-PrOH-chilled receiver. One drop was treated with 1 mL of acidic (2,4-dinitrophenyl)hydrazine solution in 95% ethanol. The yellow precipitate was washed with ethanol and dried. The melting point and mixed melting point with authentic benzaldehyde (2,4-dinitrophenyl)hydrazone were 241–241.5 °C. The IR, mass, and NMR spectra of this substance corresponded to those of authentic material. The yield of the acetal based upon comparison with a standard was 37%. No products other than the aldehyde and acetal were detected by VPC. The apparently low yield reflected a poor recovery. This was demonstrated with the following reaction.

Mesohemin Dimethyl Ester and Benzylamine. Reaction mixtures composed of 100 mg of mesohemin in 2.8 mL of neat benzylamine were allowed to stand 1 h under argon with stirring. The flask was opened to air, and the mixture was treated with 20 mL of 18% HCl. The mixture was extracted three times with CH_2Cl_2. The extracts were dried over sodium sulfate, filtered, and concentrated to dryness. The residue was suspended in diethyl ether and chromatographed on a short silica column. The ether eluate was concentrated to dryness. The residue was taken up on 0.4 mL of CH_2Cl_2 and analyzed for benzaldehyde on a 6-ft 5% OV-17 column at 130 °C. Similar reactions spiked with known amounts of benzaldehyde (before treatment with HCl) were analyzed in the same fashion. In this manner the recovery of benzaldehyde was established to be 40%. The yield was 100%. With DMF as a solvent, recovery was 17%.

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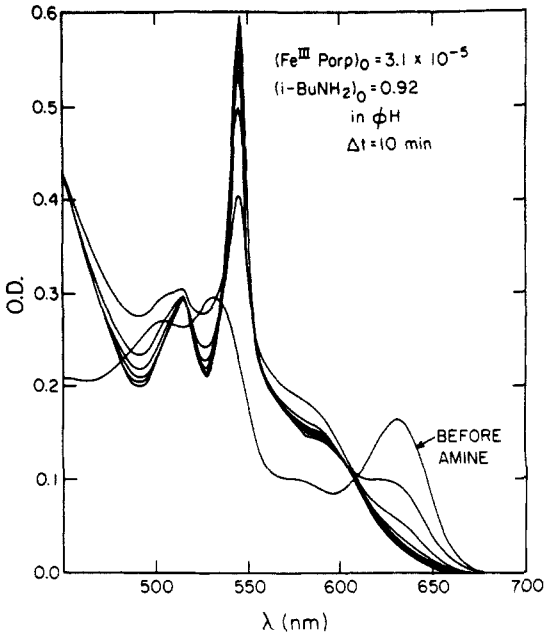


Figure 1. Repeat scans of the visible spectrum of the reduction of mesohemin dimethyl ester by isobutylamine in benzene.

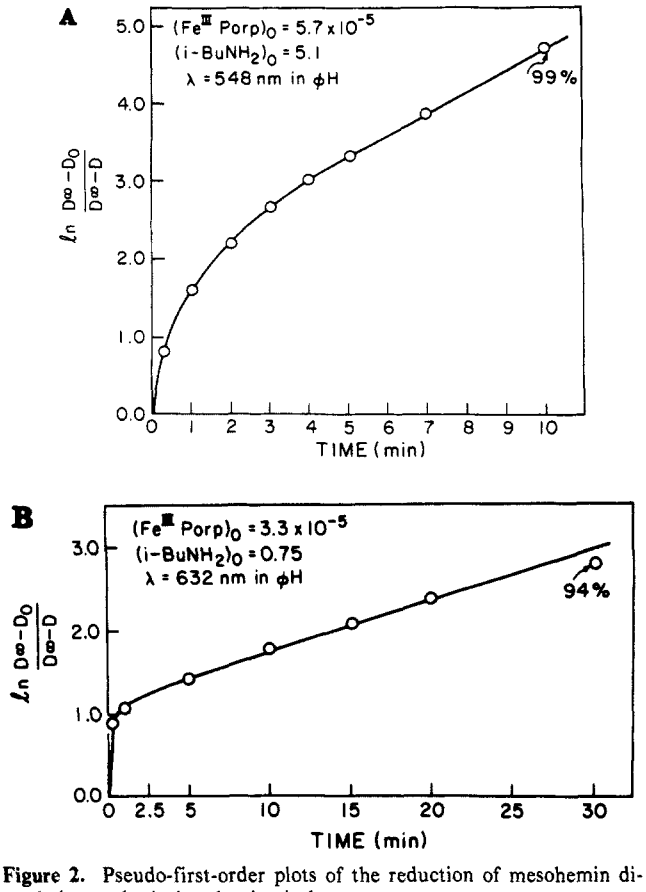


Figure 2. Pseudo-first-order plots of the reduction of mesohemin dimethyl ester by isobutylamine in benzene.

Tetrahydropyridine and Mesohemin. Mesohemin (100 mg), 4.0 g of DMF, and 200 μL of 1,2,5,6-tetrahydropyridine were allowed to stand overnight under argon. The mixture was acidified with HCl to pH 2 and extracted with CH_2Cl_2. The aqueous phase was adjusted to pH 8 and extracted 3× with CH_2Cl_2. The CH_2Cl_2 extracts were dried and analyzed for pyridine on a 6-ft OV-101 column at 120 °C. Based upon a comparison with an authentic standard subjected to the same workup, the yield was 100%. Qualitatively, the same reaction was conducted in an NMR tube using pyridine-d_5 (100% *d*) as solvent. Though traces of pyridine-H_n species were present in the solvent, the pyridine protons were discernible. In methylene chloride solvent, with octaethylporphyrin, the

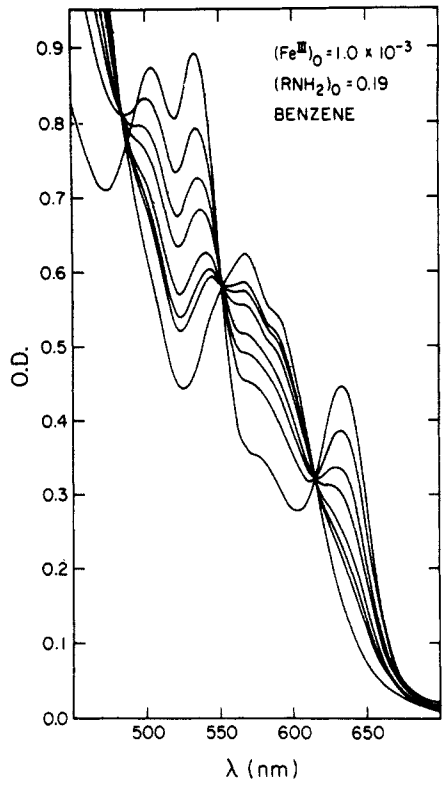
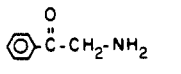


Figure 3. Repeat scans of the visible spectrum of the ligation of mesohemin dimethyl ester by *tert*-butylamine. Time: 0, 1, 5, 9, 21, 36, and 51 min and 4.5 h.

Table III. Apparent Rates of Reduction of Chloroiron(III) Mesoporphyrin IX Dimethyl Ester in Benzene at 25 °C

amine	$k_1 \times 10^3$, L mol^{-1} s		k_2	
	632[c]	548	632	548
CH_3-CH(CH_3)-CH_2NH_2	97	9.5	1.4	1.0
$CH_3CH_2CH_2CH_2NH_2$	44	25	2.2	2.3
CH_3CH_2-CH(CH_3)-NH_2	22	4.3	0.5	0.4
CH_3-C(CH_3)(CH_3)-NH_2	47	—[a]	2.3	—[a]
	v.f.[b]		440	

[a] *tert*-Butylamine does not reduce. [b] Very fast. [c] λ in nonometers.

pyridine was cleanly observable in the NMR.

Kinetics. Reactions were monitored spectrophotometrically by repeat scans and by following the changes in optical density with time at ~630 or ~540 nm. Typical spectra and rate plots are shown in Figures 1–5.

The first rates at 632 nm in benzene (Table III) were taken from initial slopes of the biphasic plots of the OD vs. time plots and are subjected to a large error (±30%). The constants in the tables are an average of three to five runs. Reproducibility was usually less than ±10%. Starting porphyrin concentrations were in the range 10^{-5}–10^{-3} M. Amines were always in excess (10^{-2}–10^{0} M).

Results

Stoichiometry. The products of the oxidation of a variety of amines by iron(III) mesoporphyrin IX are given in Table I. In general imine products were not isolated as such, but rather they

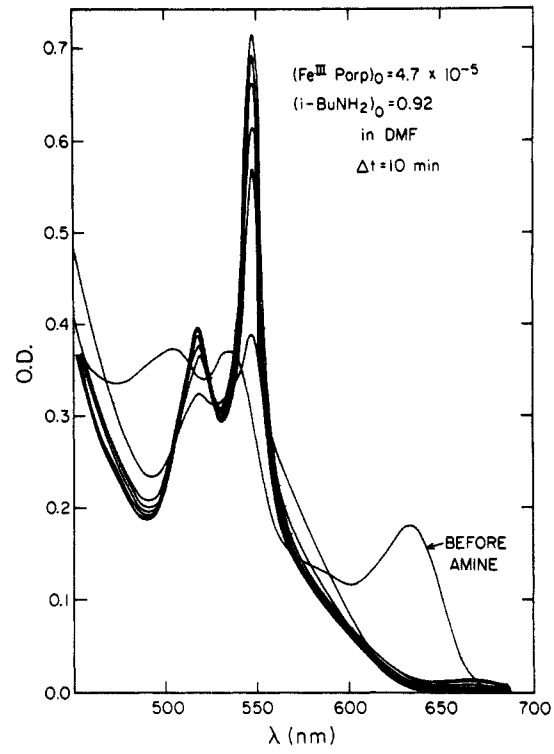


Figure 4. Repeat scans of the visible spectrum of the reduction of mesohemin dimethyl ester by isobutylamine in DMF.

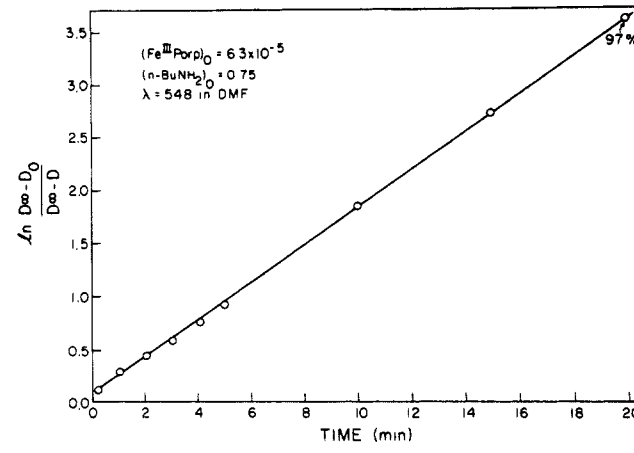
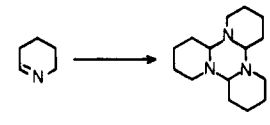
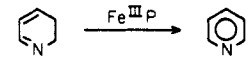


Figure 5. Pseudo-first-order plot of the reduction of mesohemin dimethyl ester by *n*-butylamine in DMF.

were converted to the corresponding aldehyde or ketone for characterization and quantitation. Exceptions were the alicyclic amines piperidine and 1,2,4,6-tetrahydropyridine. The imine product from the former 3,4,5,6-tetrahydropyridine is known to trimerize[14,15]



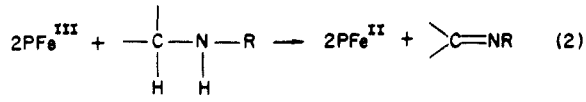
and in this case the trimer was quantitated. The tetrahydropyridine derivative was actually converted by the iron(III) porphyrin to pyridine quantitatively. The results suggests the intermediate imine is a reductant:



In all cases the porphyrin product was the low-spin iron(II) bis(amine) adduct. The overall stoichiometry was eq 1 (P =

$$2PFe^{III}Cl + 5RNH_2 \rightarrow 2PFe^{II}(H_2NR)_2 + R'CH{=}NH + 2RNH_3{}^+Cl^- \quad (1)$$

porphyrin) with one amine reducing two iron(III) porphyrins (eq 2). The overall spectral changes associated with a typical reaction in benzene are shown in Figure 1.



$$2PFe^{III} + -\overset{|}{\underset{|}{C}}(H)-N(H)-R \rightarrow 2PFe^{II} + >C{=}NR \quad (2)$$

It was a special feature of these studies that many artifacts interceded (cf. General Reactivity). To some extent these may have effected the yields reported in Table I. In all cases, though, no other amine oxidation products were detected. The yields reflect the adequacy of the workup and analysis.

General Reactivity. While all amines do not reduce iron(III) porphyrins, it was initially difficult to discern accurate reactivity patterns from a spectrophotometric scan. Thus, as noted previously,[17] pyridine (and other amines) can extract a reductant from rubber stopples. This can lead to an apparent reaction. On the other hand, traces of moisture and amine result in the generation of the iron(III) μ-oxo dimer. The spectrum of this material resembles that of a bis(amine) adduct, but it is inert in benzene. Thus, traces of moisture can lead to a false negative. Finally, an oxygen leak results in the generation of hydrogen peroxide and the decay of the porphyrin and iron(II) product spectrum.[4] In sum, pure dry amine, solvent, and glassware, and strict anaerobic technique, are essential. Despite experimental care, the hydroscopic nature of the amines made the moisture problem worrisome. As an additional check upon inertness, all amine solutions indicating no reaction were treated with *n*-butylamine. If reduction did ensue, the originally charged amine was deduced to be inert. If *n*-butylamine did not reduce the iron(III) porphyrin, the experiment was repeated.

The inert amines are listed in Table II. They are arranged in two broad categories determined from the initial scans: those that bind iron(III) to form a bis-ligated adduct (class A in the table) and those that do not bind (no change in the visible spectrum of the chloroiron(III) adduct—class B). From the table and a knowledge of reactive amines (Table IV), it can be deduced that *binding to iron* though not sufficient *is an essential requisite* for reaction. Thus, sterically encumbered amines are not reductants. Moreover, *no* tertiary amine is a reductant even though it may bind (class A-2). Finally, primary and secondary amines, even though an aminium radical might be resonance stabilized (aniline), are not reductants unless they contain an aliphatic α-CH bond (class A-1). These observations along with the structural features of reducing amines (cf. Table IV) lead to the conclusion that the moiety

is essential for reaction. It will be noted that neopentylamine is an exception to this general pattern. It does bind and contain the requisite structural feature. This fact suggests there are additional steric constraints associated with reduction.

Stereochemistry. In a reaction with *d*-amphetamine, the starting amine was recovered and its hydrochloride was compared with an authentic sample (melting point and mixed melting point). An Eu(III) NMR shift reagent was also employed to detect optical isomerization.[16] The *d* isomer was recovered cleanly with *no* racemization.

Kinetics. (a) In Benzene. The overall time course of reactions in benzene is typified by the reaction of isobutylamine shown in Figure 1. It will be noted that a clean set of isosbestic points is lacking and that the chloroiron(III) charge-transfer band at 632 nm decreases slowly after an initial relatively quick drop. The same behavior is noted in the appearance of the bis-ligated iron(II) product at its α-band ($\sim$540 nm). Pseudo-first-order plots of runs at each of these wavelengths are shown in Figure 2. Clearly the reaction is biphasic. Varying high initial concentrations of amines established that both the first and second slower rates at both wavelengths were dependent upon amine concentration. The rate law was

$$\text{rate} = k[RNH_2][PFe^{III}]$$

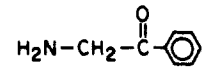
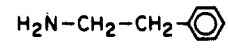
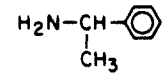
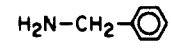
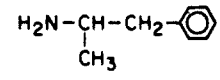
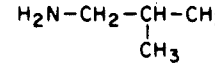
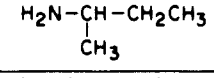
and the first and second rate constants (k_1, k_2) at each wavelength are tabulated in Table III for the butylamines and phenacylamine. The extrapolation necessary from biphasic plots of this nature render the reproducibility of runs less than desirable. Nevertheless, even qualitatively, from Figure 2, it can be seen that the first rate at 632 nm is faster than the first rate at α. In addition, as the table indicates, the second rate constants at either wavelength are within experimental error the same. Given that the 632-nm band can only be a measure of ligation, it follows that two ligation steps are being monitored and that the first is faster than the second. Moreover the first ligation step is more rapid than the initial appearance of iron(II)—the reduction rate. Therefore, the same slow second rate at each wavelength signifies that the second ligation step and not reduction is rate limiting in this solvent. Under these condition it was generally true that

$$k_1^{632} > k_1^{\alpha} > k_2^{632} = k_2^{\alpha}$$

or, alternatively, k_1(ligation) > k(reduction) > k_2(ligation). Thus, the actual reduction of the iron(III) porphyrin by amine *must* follow the second ligation. This result is in keeping with the general reactivity requirement for coordination noted above.

***tert*-Butylamine.** A more accurate picture of the ligation path in benzene could be obtained by closely examining the spectral features of ligation by this nonreducing amine (Figure 3). At first inspection, the clean isosbestic points at 553 and 615 nm suggested

Table IV. Rates of Reduction of Chloroiron(III) Mesoporphyrin IX Dimethyl Ester in DMF at 25 °C

amine	$k_{red} \times 10^3$,[a] L mol^{-1} s
	3100
	29
	27
	22.5
$H_2N-CH_2-CH_3$	22
	22
$HN(CH_3)_2$	20
	11
H_2N-CH_3	9.4
	6.8
$H_2N-CH_2-CH_2-CH_2-CH_3$	1.8
	1.3
	1.3

[a] Monitored at α, 548 nm.

(17) Castro, C. E.; Hathaway, G. M.; Havlin, R. *J. Am. Chem. Soc.* **1977**, *99*, 8032.

a smooth conversion of $PFe^{III}Cl$ to the bis(amine) adduct without a discernible intermediate. The biphasicity in this case would require the presence of two different starting iron(III) complexes, and this was difficult to rationalize. A more expanded wavelength scan (like that shown in Figure 3), however, revealed another isosbestic at 486 nm that was not shared by the starting chloroiron(III) porphyrin. Thus, at the earliest scan, a new species is generated that does proceed to bis(amine) adduct directly in the slow step. We assign the early spectrum to the monoaminomonochloroiron(III) porphyrin. In sum, the intermediate that may be inferred from the relative rates of the two-step process at 634 nm (Table III) *can* be seen in this case.

Reaction Pathway. Taken together, these results establish the reaction pathway (3). In benzene, step b, the second ligation

$$PFe^{III}Cl \underset{a}{\overset{RNH_2}{\rightleftharpoons}} PFe^{III}Cl(RNH_2) \underset{b}{\overset{RNH_2}{\rightleftharpoons}} PFe^{III}(H_2NR)_2^+ + Cl^- \qquad (3)$$

$$\downarrow c\ RNH_2$$

$$PFe^{II}(H_2NR)_2$$

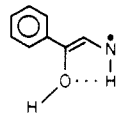
and iron–Cl bond-breaking process becomes rate limiting. An important point is that the addition of *N*-methylimidazole to the benzene solutions of the iron(III) chloride resulted in the bis(*N*-methylimidazole) iron(III) complex. Nonetheless, starting with this species, the reduction by *n*-butylamine monitored at α showed the same biphasicity and the same rate constants obtained in the absence of *N*-methylimidazole. Clearly then the ligation is reversible as drawn above.

Given the nature of the reaction in benzene, it was not possible to probe the dynamics of the redox process with any certainty. It was hoped that a solvent of higher polarity would increase the rates of ligation such that reduction itself would become rate limiting. Dimethylformamide was found to be such a solvent.

(b) In Dimethylformamide. The time course of reactions in this polar solvent, depicted with isobutylamine in Figure 4, illustrates the general case. The 630-nm band vanishes nearly instantly; that is, ligation is rapid. The α band grows in gradually. Isosbestic points between the bis-ligated low-spin iron(III) and iron(II) porphyrins are apparent. A pseudo-first-order plot with *n*-butylamine is shown in Figure 5. Clearly the biphasicity observed in benzene is lost. The rate of reduction is being monitored. The general rate law was the same as that observed in benzene, though in this system the bis(amine) adduct is clearly the oxidizing species:

$$\text{rate} = k_{red}[PFe^{III}(H_2NR)_2][RNH_2]$$

Rate constants are given in Table IV. The amines are listed in a decreasing order of reactivity. As before, the results represent an average of at least three determinations. Reproducibility was ±10%. Apart from the remarkable reactivity of phenacylamine, there are no large differences in rates and no simple structural features that exert an influence upon them. For example, conjugation with the α-carbon has no effect, and conjugation with the amine moiety itself cannot alone confer high reactivity (aniline is inert). Generally it would seem that primary and secondary amines of about the same size and shape react at about the same rate. A unique feature of phenacylamine is that unlike aniline it contains the requisite α-CH and NH for reactivity but at the same time, in the enol form, an amine-based radical is resonance stabilized.



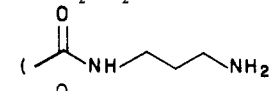
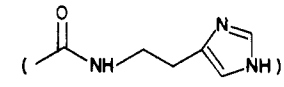
The rates in Table IV are further complicated by the realization that each amine is reacting with a different iron(III) bis(amine) adduct. The steric and geometric nuances of such an interaction are not straightforward. Moreover, the activation parameters for benzylamine, $\Delta F^{\ddagger} = 19$, $\Delta H^{\ddagger} = 1.9$ kcal/mol, and $\Delta S^{\ddagger} = -59$ eu, suggest a high degree of order in the transition state such that steric effects in this aggregate could predominate.

Table V. Influence of Nonreducing Amines upon the Rate of Reduction of Iron(III) Mesoporphyrin IX Dimethyl Ester in DMF at 25 °C

added amine	concn, mol/L	$k_{red} \times 10^3$, L mol^{-1} s
none[a]		23
tert-butylamine[b]	0.07–0.5	40
neopentylamine[b]	0.21	0
N-methylimidazole[b]	0.1–0.6	22
imidazole[b]	0.2	<0.2–0[d]
triethylamine[c]	0.2	29

[a] $[PhCH_2NH_2]_0 = 0.06$ M. [b] Coordinating amine; initial iron(III) porphyrin is ligated by this amine. [c] Noncoordinating amine. [d] A maximum extrapolation from plots of k vs. $1/[Im]^2$.

Table VI. Rates of Reduction of Various Porphyrin $Fe^{III}L_2$ Complexes by Benzylamine in DMF at 25 °C

porphyrin	axial ligands	$k_{red} \times 10^3$,[a] L mol^{-1} s
proto IX DME	$PhCH_2NH_2$	273
TPP	$PhCH_2NH_2$	164
meso IX DME	t-$BuNH_2$[b]	40
octa E	$PhCH_2NH_2$	27
meso IX DME	$PhCH_2NH_2$	23
deutero IX DME	$PhCH_2NH_2$	14
meso IX[c]		5
meso IX[d]		(0.3)[f]
meso IX DME	imidazole	0–<0.2[g]
mexo IX DME[e]	0	0–<0.04

[a] Reproducibility ±10%. [b] $[t\text{-}BuNH_2]_0 = 0.47$ M. [c] Bis[(γ-aminopropyl)amide] of mesohemin. [d] Bis(β-imidazolylethyl)amide of mesohemin. [e] μ-Oxo dimer. [f] Rate is independent of $PhCH_2NH_2$. [g] Maximum value extrapolated from $1/[Im]^2$ vs. rate.

The influence of nonreducing amines upon the rate of reduction by benzylamine was more revealing (Table V). All of the amines in the table except triethylamine rapidly form the low-spin bis-ligated iron(III) adduct. Except for this case then, reaction commenced with the bis(amine) ligands listed. It is apparent that *tert*-butylamine increases the rate moderately (approximately 2-fold), but the rate of the reaction *was independent* of the concentration of *tert*-butylamine. Triethylamine has no effect. Thus, the bis(*tert*-butylamine) adduct reacts somewhat more rapidly than the bis(benzylamine) adduct, but the reaction *is not* general-base catalyzed. *N*-Methylimidazole, as in benzene, is easily displaced by benzylamine and has no effect.

Imidazole, on the other hand, strongly inhibits the reaction. The rate is proportional to $1/[\text{imidazole}]^2$. The 0.2×10^{-3} value represents a maximum extrapolation to infinite imidazole concentration. Thus, the bis(imidazole) adduct (if it reacts at all) is at least 100-fold less reactive than the bis(benzylamine) adduct. Finally, the bulky neopentylamine inhibits reduction by both itself (Table II) anb benzylamine.

The rates of reduction of a series of iron(III) porphyrins by benzylamine are shown in Table VI. The porphyrins are listed in a decreasing order of reactivity. There is a definite trend in the rates of reduction of the bis(benzylamine) adducts by benzylamine. The beneficial influence of extension of the porphyrin π system upon the rates is apparent in the series

$$\text{proto IX} > \text{TPP} > \text{meso IX} \sim \text{octa E} > \text{deutero IX}$$

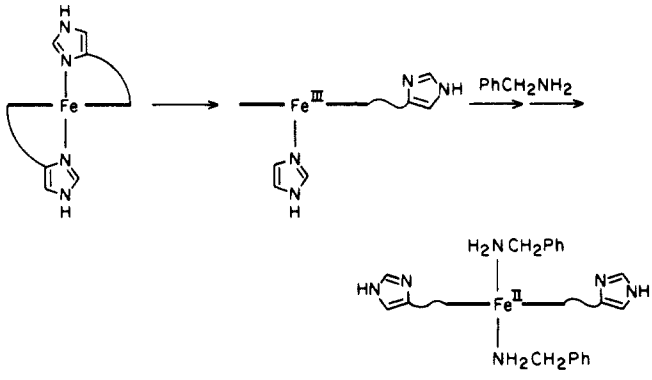
The 2,4-diethyl substituents lacking in deutero IX are a minor influence upon the rate. On the other hand replacing the ethyl substituents of meso IX with vinyl (proto IX) enhances the rate 10-fold. The parenthetical amide–amine ligands represent diamides of mesoporphyrin containing the built-on ligand imidazole[12c] or amino. The rate for the bis(imidazole) diamide is calculated as a second-order rate for the higher benzylamine concentration for comparison. Actually, the rate of reduction of this complex was independent of benzylamine concentration from 0.05

Table VII. Rates of Reduction of Mesohemin IX Dimethyl Ester by Deuterated Benzylamines in DMF at 25 °C

amine	$k_{red} \times 10^3$,[a] L mol^{-1} s	amine	$k_{red} \times 10^3$,[a] L mol^{-1} s
$PhCH_2NH_2$	23	$PhCH_2ND_2$	21
$PhCD_2NH_2$	21	$PhCD_2ND_2$	24

[a] Average of five runs; reproducibility ±10%.

to 0.4 M. We presume an off-rate for one of the affixed imidazoles was rate limiting in this case.



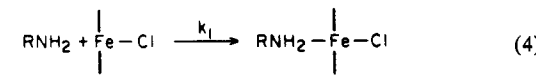
The final product spectrum was that of the bis(benzylamine) adduct. The lack of direct reduction of this porphyrin is consistent with the inhibitory effect of imidazole noted above. The corresponding bis(amino) structure exhibits some steric constraint to reduction as might be expected. The μ-oxo dimer of mesoporphyrin dimethyl ester is inert or very slow to react by dissociation in DMF.

Isotope Effect. The rates of reduction of iron(III) meso IX in DMF by a series of deuterated benzylamines are presented in Table VII. All of the rates are the same within experimental error. Clearly, there is no primary isotope effect at amine nitrogen or α-carbon. Moreover, the results establish that the N–H or α-C–H is not broken in the transition state in either the incoming amine or the amines coordinated to iron.

Meso Exchange. As a check for the possibility of a hydrogen-atom transfer to the meso position upon reduction (σ-meso addition[18]), reductions by a series of amines (piperidine, *n*-butylamine, phenylhydroxylamine, hydrazine, methylhydrazine) were conducted in an NMR tube in pyridine-d_5 with chloroiron(III) *meso*-tetradeuteriooctaethylporphyrin. In no case was an H for D exchange apparent in the spectrum of the diamagnetic iron(II) product.

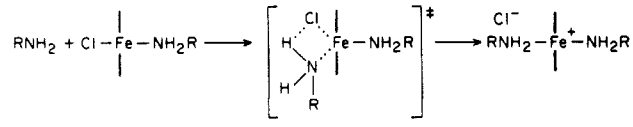
Discussion

Ligation. The kinetics and thermodynamics of the ligation of iron(III) porphyrins by heterocyclic amines, principally pyridine and imidazole derivatives, have been investigated rather extensively.[19,20] Depending upon the polarity of the solvent, and the nature and concentration of the ligand, rate laws first or second order in ligand[19a,b] can be obtained. In general the thermodynamics favors bis ligation. On the basis of the positive influence of protic species upon the rates, Sweigert and colleagues[19a] have suggested that hydrogen bonding in the transition state favors dissociation of halide ion from a monoligated monohaloiron(III) intermediate. The visible spectrum of a mono(*N*-methylimidazole) adduct of chloroiron(III) tetraphenylporphine was obtained at −78 °C. In the main our results in benzene, with the aliphatic amines, resemble those with the heterocycles except that the rates are much slower. Moreover, both discrete steps in the ligation can be observed at room temperature. With the nonreducing *tert*-butylamine, at least, the first spectrum following the more rapid initial rate would correspond with that of the monoamine-ligated species (eq 4). This proceeds with clean isosbestics



(4)

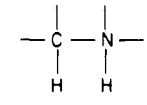
to the bis-ligated species at a relatively slow rate (eq 5). All of



(5)

the reducing amines contain an N–H; consequently, our data cannot make a distinction between a hydrogen-bonding assisted substitution at iron (as drawn in eq 4) or a simple nucleophilic displacement. In light of the results by Sweigert, however, the former is likely. A simple dissociation of chloride from the monoamine-ligated adduct without amine participation can be eliminated by the amine dependency in the rate law for the second step. Apart from the foregoing, the main mechanistic feature of the redox process in benzene is that the second ligation step and not the reduction is rate limiting. The iron porphyrin must be ligated by two amines before reduction can ensue.

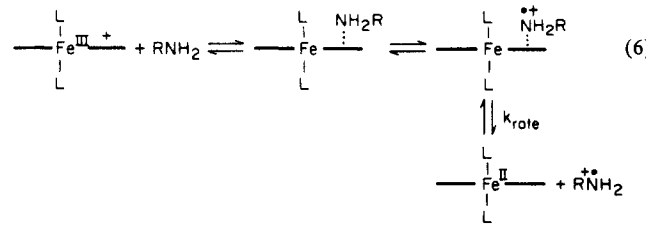
Reductions. A brief summary of salient reaction features taken from the results is as follows: (i) the oxidation of amine to imine is an overall two-electron process: (ii) it proceeds in two discrete steps; (iii) the slow step entails the reaction of an amine with a bis(amine)-ligated σ-bonded low-spin iron(III) porphyrin; (iv) the reaction is not general-base (or amine) catalyzed; (v) starting optically active amine is recovered without racemization; (vi) an extension of the porphyrin π system enhances the rate; (vii) to be reactive an amine must contain the structural unit



(viii) there is no primary isotope effect observed upon deuterating either or both the N–H and α-C–H positions of the amine; (ix) steric effects are important; (x) phenacylamine, an amine containing the requisite bonding and the capacity for resonance stabilization of a radical on nitrogen, is the fastest reacting substrate; (xi) a hydrogen atom is not transferred to the meso position of the porphyrin during reaction.

These reaction characteristics can be accommodated by a relatively simple mechanism.

First Step. An Outer-Sphere Reduction. Given the foregoing, the most reasonable formulation for the first step in the reaction is an outer-sphere electron transfer from amine to the porphyrin periphery (a peripheral π transfer) (eq 6). The relatively slow



(6)

rates for these reactions accord with a rate-limiting dissociation of the outer-sphere adduct to the iron(II) porphyrin and aminium cation radical. This process itself may be reversible though the

(18) Castro, C. E. *The Porphyrins*; Dolphin, D., Ed.; Academic: New York, 1978; Vol. V, Chapter 1, p 13.

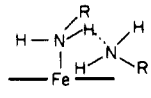
(19) (a) Sweigert, D. A.; Fiske, W. *NATO Adv. Study Inst. Ser., Ser. C* **1979**, *C50*, 315–320. Doe, H. M.; Sweigert, D. A. *Inorg. Chem.* **1982**, *21*, 699–705. Tondreau, G. A.; Sweigert, D. A. *Ibid.* **1984**, *23*, 1060–1065. Jones, J. G.; Tondreau, G. A.; Edwards, J. O.; Sweigert, D. A. *Inorg. Chem.* **1985**, *24*, 296–300. (b) Pasternack, R. F.; Gillies, B. S.; Stahlbush, J. R. *J. Am. Chem. Soc.* **1978**, *100*, 2613–2618.

(20) Thompson, A. N.; Krishnamurthy, M. *Inorg. Chim. Acta* **1979**, *34*, 145–150. Abbot, E. H.; Rafson, P. A. *J. Am. Chem. Soc.* **1974**, *96*, 7378–7379. Davies, T. H. *Biochim. Biophys. Acta* **1973**, *329*, 108–117. Duclos, J. M. *Bioinorg. Chem.* **1973**, *2*, 263. Castro, C. E.; Anderson, S. E. *Inorg. Chem.* **1985**, *24*, 1113.

actual speed of scavenging of free radicals by bis-ligated low-spin hemes is unknown.[12a,21] The peripheral π-transfer mechanism fits nicely with the favorable influence of an extension of the porphyrin π system upon the rate. Moreover, the vast difference in reactivity between a bis(imidazole) and a bis(*tert*-butylamine or benzylamine) adduct emphasizes the importance of metal d–porphyrin π conjugation to these processes. With the aliphatic amines, the low-spin iron(III) complex represents the maximum possible "back-bonding" of the metal to the macrocycle. This is apparently essential for reaction with the weakly reducing amines.

The coordinating but nonreducing and bulky neopentylamine cannot penetrate a bis(neopentylamine) adduct such that an adequately aligned precursor complex can be formed. Apart from the kinetics, the inertness of this ligating amine in and of its eliminates the possibility of any mechanism of electron transfer from hydrogen or α-carbon that might be envisioned to occur from within the inner coordination sphere.

We have drawn only one of many formulations in eq 6 that may denote the actual structure of the outer-sphere adduct. For example, hydrogen bonding between incoming or reducing amine and one of the ligating amines may well be important, e.g., **1**.



1

Moreover, it would seem that the geometries for both a precursor and product complex would be very similar. In any case the loss of entropy is consistent with a loss of order in the solvated aggregate upon dissociation.

The electron transfer itself is reminiscent of that observed between photoexcited aromatics, flavins, and metal complexes with amines.[22a,b,33]

Second Redox Step. (a) Fate of the Aminium Cation Radical. Given the high concentration of amine, once the nitrogen-based radical is generated, it may undergo a rapid proton transfer to generate the free aminyl radical as the dominant species (eq 7).

$$R\dot{N}H_2^+ + RNH_2 \rightarrow R\dot{N}H + RNH_3^+ \qquad (7)$$

This radical *cannot* generally or irreversibly reduce a second iron(III) porphyrin; otherwise, aniline and *tert*-butylamine (as well as other amines in Table II) should be reductants. These amines must be capable of the slow but reversible first step. Thus, amine coupling products (hydrazines or their oxidation products) are not detected as they have been with both *tert*-butylamine[23] and aniline[24] in the corresponding anodic processes.[25] The results imply that the concentration of radicals is low and that the reverse of (6) or the direct scavenging of the aminyl radical by the iron(II) porphyrin must be rapid.

There are several possibilities for the fate of the aminyl radical:

(i) The aminyl radical disproportionates to amine and imine (eq 8). This is unlikely because of the low radical concentration

$$2{>}CH\dot{N}R \rightarrow {>}C{=}NR + {>}CHNHR \qquad (8)$$

that must be extant. Coupling does not occur (see above); consequently, radical–radical interactions are unlikely. Moreover, the lack of racemization of amphetamine experimentally eliminates the possibility of disproportionation of the corresponding α-aminocarbinyl radical (eq 9).

$$2{>}\dot{C}NHR \rightarrow {>}CNR + {>}CHNHR \qquad (9)$$

Thus, eq 8 is also unreasonable.

(ii) The aminyl radical reacts with excess amine to produce an α-aminocarbinyl radical (eq 10). While this reaction may be

$$>CH\dot{N}R \xrightarrow{\text{amine}} >\dot{C}NHR \qquad (10)$$

slow with some amines,[26] it is clearly fast with others.[26,27,33] Moreover, the α-aminocarbinyl radical could be directly generated by proton abstraction from the α-carbon of the aminium cation radical (eq 11).[25a,28]

$$>CH\overset{\cdot+}{N}HR \xrightarrow{\text{amine}} >\dot{C}NHR \qquad (11)$$

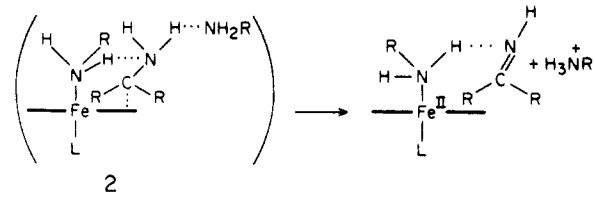
Whether directly (eq 1) or indirectly (eq 7 and 10) generated, the α-aminocarbinyl radical is the most likely species to irreversibly carry the reactions to completion in the second redox step. As noted above, the lack of racemization of recovered amphetamine eliminates the potential disproportionation of this species to products. It also renders any subsequent steps irreversible.

(b) Second Electron Transfer. The reduction of iron porphyrin by the α-aminocarbinyl radical (eq 12) may be followed by loss

$$>\dot{C}NHR + PFe^{III}(amine)_2 \rightarrow >\overset{+}{C}NHR + PFe^{II}(amine)_2 \qquad (12)$$

$$>\overset{+}{C}NHR + amine \rightarrow >C{=}NR + amine\ H^+ \qquad (13)$$

of a proton to form imine (eq 13), or the process may be concerted. As indicated for the first step in the redox process (eq 6; **1**), hydrogen bonding with amine could play a dominant role. Indeed, a hydrogen-bonded α-aminocarbinyl radical would seem ideally suited for a rapid electron transfer to the porphyrin periphery **2** (eq 14). The proton transfer could be synchronous with the electron transfer or it could occur in the solvated aggregate before the imine diffuses away from the porphyrin.



2 (14)

The only mechanistic constraints our data would place on these processes (eq 10–14) is that they are irreversible, and they proceed more rapidly than the first slow step. The proton transfer and the conversion of aminium to α-aminocarbinyl radicals are known to be fast processes.[25-28] Rapid rates ($\sim 10^6$–10^7 L mol^{-1} s) of hydrogen abstraction from both N–H and α-CH of amines by oxy radicals[26] and benzophenone triplet[29] are established. Moreover, the lack of disproportionation of α-aminocarbinyl radicals in this latter system[29b] was established. Thus, conversion of amino and α-aminocarbinyl radicals to products is rapid.

(21) (a) High-spin hemes, however, react at the diffusion-controlled limit: Brault, D.; Netta, P. *J. Am. Chem. Soc.* **1981**, *103*, 2705. *J. Phys. Chem.* **1982**, *86*, 3405. Wade, R. S.; Castro, C. E. *J. Am. Chem. Soc.* **1973**, *95*, 226. (b) The overall equilibrium in eq 6 undoubtedly lies far to the left. Assuming the reverse process, scavenging of the radical by the heme, is very fast, the relatively slow rates observed for the forward reaction would be compatible with related redox potentials. The high concentrations of amine allow processes 10 and 11 to compete with the reverse of (6).

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(25) For general summaries, cf.: (a) Ross, S. D.; Finkelstein, M.; Rudd, E. J. *Anodic Oxidation*; Academic: New York, 1975; Chapter 8, pp 189–222. (b) Lines, R. In *Organic Electrochemistry*; Baizer, M. M.; Lund, H., Eds.; Dekker: New York, 1983; Chapter 15, pp 463–483.

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(29) (a) Inbar, S.; Linschitz, H.; Cohen, S. G. *J. Am. Chem. Soc.* **1981**, *103*, 1048–1054 and references therein. (b) Cohen, S. G.; Chao, H. M. *J. Am. Chem. Soc.* **1968**, *90*, 165.

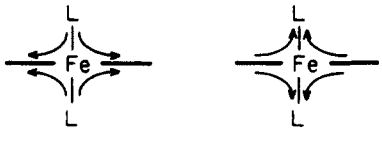
Consequently, the second electron-transfer step, which entails the reaction of a radical with a paramagnetic iron porphyrin, itself a radical, should be very fast and well within the constraints of timing imposed by the slow first step and the irreversibility of the second.

Though different in character than the reactions described here, the oxidation of amine ligands coordinated to osmium,[30] ruthenium,[31] and iron[32] have been reported. In general the metal is oxidized to an unstable high-valent state that is reduced intramolecularly by coordinated amine. Both imine and nitrile bonds are formed. A metal(IV) oxidation state has been suggested.[30]

Amines have also been reported to reduce photoexcited complexes of ruthenium(II) and iron(II).[33] The aminium cation radical results from the initial quenching of the excited metal complex, and α-aminocarbinyl radicals have been detected. We believe the initial steps in the reactions of amines with bis(amine)-ligated iron(III) porphyrins parallel that observed in the photoreactions.

The ease of oxidation of amines[25] is in the order tertiary > secondary > primary. Moreover, increased base strength at an amine ligand favors the potential for iron(II) to iron(III) oxidation.[34] Clearly, the rates in Tables III and IV do not parallel either of these thermodynamic trends. Adducts of imidazole, the weakest base, react the slowest, if at all. The results are consistent with a rate-limiting step that follows the electron transfer.

The difference in reactivity between low-spin bis(imidazole) and bis(alkylamine) adducts is striking. They represent to a degree two types of low-spin bonding present in bis-ligated iron(III) complexes **3** and **4**. Complex **3** represents a spin pairing of metal

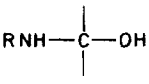


3 4

electrons caused by a σ-coordinating ligand (alkylamine) that contains no low-lying empty orbitals. On the other hand, imidazole by virtue of its own π system can in addition accept metal electrons. Presumably the more diffuse radical in a π-bonding complex like **4** is less reactive. This trend in reactivity for $PFeL_2$ adducts (L = RNH_2 > imidazole) is observed in the outer-sphere electron transfer between iron porphyrins,[35] in their oxidation and reduction by quinones and hydroquinones,[16] and in outer-sphere oxidation of iron(II) porphyrins by oxygen.[4] The amines, as reported here, show the same trend, but they are the first example of a class of redox reagent that makes a clean kinetic distinction between a fully conjugated metal–porphyrin π system (**3**) and one that may be only partially disposed.

The general reactivity observed here fits well with predicted patterns[36] for these bond types. Recent carbon-13 NMR studies[37] of six-coordinate iron porphyrins confirm the notion of π backbonding to the heterocycle.

Relevance to Cytochrome P-450. The monoamine oxidase activity of this enzyme is manifest in its conversion of aliphatic amines to aldehydes and ketones. Despite considerable study, the actual path(s) and mechanism(s) of these transformations are uncertain.[5c] Two pathways have been suggested: (a) initial oxidative attack upon the α-carbon to generate a carbinolamine[38]



followed by loss of amine or ammonia to yield aldehyde or ketone; (b) initial attack at nitrogen to yield an aminium cation radical,[39] follwed by oxidation to an aminium cation and hydrolysis of the resultant imine or conversion to hydroxylamine and further oxidation of the latter to oxime and hydrolysis.

Path (a) is presumed to proceed via an iron oxene. Consequently, runs in the presence of $^{18}O_2$ should incorporate ^{18}O into the carbonyl product. Path (b), on the other hand, would yield an ^{18}O-labeled carbonyl compound if $H_2{}^{18}O$ were employed as solvent. Because, in part, of $C{=}{}^{18}O$ exchange with H_2O, the results of the ^{18}O studies are not clear.[5b] For example, with amphetamine, P-450, and $^{18}O_2$, 30% of the phenylacetone product is labeled.[40]

Recent work with specific inducers and inhibitors of cytochrome P-450[41] did not allow a distinction between these pathways. However, an examination of 1-(methylcyclopropyl)benzylamine and cyclopropylbenzylamine as potential "suicide inactivators" of P-450 showed both to be equally effective in inhibiting the enzyme.[8] This has been taken as evidence in favor of initial attack at nitrogen since the carbinolamine cannot be formed from the 1-methyl analogue.

The present work demonstrates that an appropriately substituted iron porphyrin active site can, by itself, without oxygen intermediates, carry out the monoamine oxidase function ascribed to cytochrome P-450. In this active site model, reaction does initiate by electron transfer from amine nitrogen to porphyrin. However, the α-aminocarbinyl radical must be generated for the reaction to go to completion. If the conformation of the protein about the porphyrin were such that there was some steric impediment in the approach to the porphyrin periphery by the radical (G conformation[42]), then it may competitively react with O_2. Thus, it is possible the mechanism observed here with the active site could also explain the results of some of the ^{18}O studies conducted with the enzyme. Another pathway, presumably available to the enzyme but not the active site, would be hydrogen abstraction from the α-carbon of the initially formed amino radical by an iron oxene.

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Registry No. $PhCH_2CH(NH_2)CH_3$, 300-62-9; $PhCH_2C(=NH)CH_3$, 5704-08-5; $CH_3CH_2C(=NH)CH_3$, 53626-93-0; $PhCH{=}NH$, 16118-22-2; $PhC(=NH)CH_3$, 13280-20-1; $PhCH_2CH{=}NH$, 74974-19-9; $CH_3CN(CH_3)CH_2NH_2$, 78-81-9; $CH_3CH_2CH_2CH_2NH_2$, 109-73-9; $CH_3CH_2CH(CH_3)NH_2$, 13952-84-6; $(CH_3)_3CNH_2$, 75-64-9; $PhCOCH_2NH_2$, 613-89-8; $H_2NCH_2CH_3$, 75-04-7; $HN(CH_3)_2$, 124-40-3; H_2NCH_3, 74-89-5; chloroiron(III) mesoporphyrin IX bis(3-aminopropanamide), 102420-69-9; chloroiron(III) mesoporphyrin IX dimethyl ester, 14126-91-1; 1,3-diaminopropane, 109-76-2; chloroiron(III) tetraphenylporphine, 16456-81-8; piperidine, 110-89-4; α-tripiperideine, 522-33-8; benzylamine, 100-46-9; deuterohemin IX, 18922-88-8; 2,3-dihydropyridine, 67684-01-9; 1,2,5,6-tetrahydropyridine, 61215-72-3; 2,3,4,5-tetrahydropyridine, 505-18-0; mesohemin, 21007-37-4.

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