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The Reactions of Octaethylporphyrin and Its Iron (II) and Iron (III) Complexes with Free Radicals

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The reactions of dilute solutions of octaethylporphyrin and its iron (II) and iron (III) complexes with methyl, 2-cyanopropyl, *t*-butoxy, and benzoyloxy radicals are described. The results are summarized: (i) The reactivity of the porphyrin and its high-spin iron (II) and iron (III) complexes toward alkyl and *t*-butoxy radicals stands in the order: $Fe^{II} > Fe^{III} \gg$ free porphyrin. For benzoyloxy radicals the order is $Fe^{II} >$ Porp $> Fe^{III}$. (ii) The exclusive path of reaction of high-spin iron (II) porphyrin with radicals is the rapid reduction of the radical and generation of an iron (III) porphyrin. The dominant path of reaction of high-spin iron (III) porphyrin with alkyl and (presumably) *t*-butoxy radicals is a rapid axial inner sphere reduction of the porphyrin. An axial ligand of iron is transferred to the radical. (iv) The reaction of benzoyloxy radicals with high or low-spin iron (III) porphyrins occurs primarily at the meso position. With the low-spin dipyridyl complex in pyridine the attendant reduction to iron (II) can be observed spectrally. Methyl radicals also reduce this complex by adding to the meso position. (v) The reaction of a radical with either an iron (II) or an iron (III) porphyrin results in the generation of the other valence state of iron and consequently oxidation and reduction products emanating from both iron species are obtained. (vi) No evidence for an iron (IV) is intermediate is apparent. (vii) Iron (II) porphyrins in solvents that impart either spin state are easily oxidized by diacyl peroxides. The occurrence of both axial and peripheral redox reactions with the iron complexes supports an underlying premise of a recent theory of hemeprotein reactivity. The relevance of the work to bioelectron transfer and heme catabolism is noted.

INTRODUCTION

Respiration in living cells encompasses a symphony of electron transfers that may at certain points entail atom-affiliated movements as well. Indeed, enzymatically generated semiquinone-like free radicals have been detected in the reduction of iron (III) cytochrome *c*, and b_5 (*1*), and alkyl radicals have been recently noted in the oxidation of hemes and heme proteins by alkyl halides (*2*).

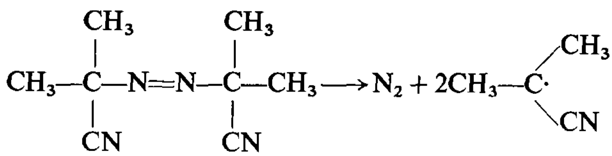
As a part of our studies of the oxidation and reduction of these respiratory substances, we summarize an examination of the reactions of octaethylporphyrin and its iron (II) and iron (III) derivatives with two alkyl and two oxy radicals.

The reactions of octaethylporphyrin itself with benzoyloxy radicals in trichlorobenzene have been demonstrated to yield mesobenzoyloxyoctaethyl porphyrin (*3*). A brief account of the reaction with a large excess of benzoyloxy radicals indicates all possible substitution products are obtained (*4*).

Our own efforts have aimed at discerning the initial site of attack by radicals upon these structures because this position should also be a site for electron transfer. Moreover, we wished to characterize the nature of any redox reactions with the iron derivatives and compare their behavior to that of the metal-free porphyrin.

RESULTS

Radicals were generated by the thermal decomposition of bis-azoisobutyronitrile (1), di-*t*-butyl peroxide (2), benzoyl peroxide (3), and diacetyl peroxide (4).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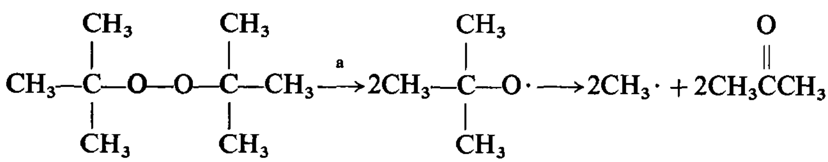
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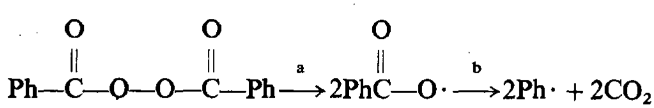
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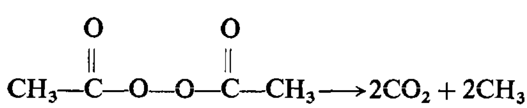
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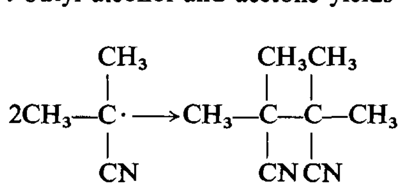
Major products from the first three reactions in the presence and absence of the porphyrin derivatives are given in Table 1 and the methyl radical (4) study is presented in Table 2. All reactions were conducted under argon. Initial concentrations of porphyrin ranged from 10^{-3} to 3×10^{-3} *M*. Half as much radical precursor was employed except where noted for diacetyl peroxide (Table 2).

Octaethylporphyrin. Neither 2-cyanopropyl, *t*-butoxy, or methyl radicals react with the porphyrin under these conditions and concentrations. Thus, the yield of dimethylsuccinonitrile (1, 5) is the same in the presence and absence of octaethylporphyrin. Similarly the *t*-butyl alcohol and acetone yields from di-*t*-butyl peroxide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(2) and the methane and ethane yields from diacetyl peroxide (4) are not influenced by the porphyrin. Compare Octa E with solvent blank in Tables 1 and 2 (lines 1, 2).

TABLE 1

PRODUCTS OF THE REACTION OF OCTAETHYLPORPHYRIN AND IRON COMPLEXES WITH FREE RADICALS

Radical	Conditions	Products	Octa E	Octa E Fe(III)Cl	Octa E Fe(II) (TCB + 1 % HoAc)	Solvent blank	$Fe(OAc)_2$ blank	Recovered octa E
2-Cyanopropyl	AIBN 95°C/2 hr TCB	Dimethylsuccinonitrile	75 ± 2.5 %[a]	38 %	35.5	75 %	59	87.5
		α-chloroisobutyronitrile	0	35	0		0	
		Isobutyronitrile	0	14	44–48		11	
t-Butoxy	DTBP 175–195°C/2 hr TCB	*t*-Butyl alcohol	5	34	84 % (as 50°C)	2		87.5
		Acetone	85	56	0	88		
Benzoyloxy	Bz_2O_2 120°C/2 hr TCB	Mesobenzoyloxyocta-ethylporphyrin	36	10[a]	0(at 25°C or 80°C)			
		Octaethyloxophlorin	7	3	0			
		Recovered octa E	42	75	88			
		Benzoic acid	41	42	87[c]	3[d]		
		Carbon dioxide	7	33	0	89		

[a] All yields of repeated runs are within 5 % of those reported.
[b] Four days at 25°—no reaction; 100°C/3 hr 66 % recovery octa E, 22 % mesobenzoyl.
[c] At ½ hr or 2 min.
[d] At 2 min.

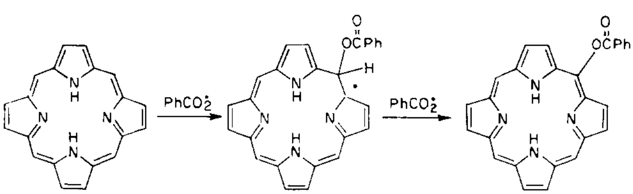
TABLE 2

PRODUCTS OF THE REACTION OF METHYL RADICALS WITH OCTAETHYLPORPHYRIN AND ITS IRON (II) AND IRON (III) COMPLEXES AT 100°C

	$(Porp)_0$[b]/ $Ac_2O_2)_0$	CH_4/C_2H_6	% Yield[a,b] CH_4	C_2H_6[c]	CH_3Cl	CH_3OAc
1. Blank	0	4.6	62	27	—	—
2. Octa E	1		65	31	—	—
3. Fe(III) octa E	2	1.5	22	30	1	51
4. Fe(III) octa E	10	0.2	3	27	0.5	65
5. Fe(II) octa E	10	55	82	3	nd	18

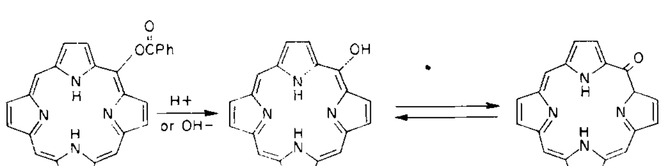
[a] % CH_3.
[b] $(Porp)_0 = 10^{-3}$ *M*, TCB:ACOH 95:5.
[c] $2C_2H_5(100)/CH_3$.
[d] yields in repeated runs within 5%.

In contrast, a significant fraction of benzoyloxy radicals (3a) substitute the meso position of the porphyrin even at these low concentrations.



+ $PhCO_2H$ (6)

The resulting mesobenzoyloxy derivative partially hydrolyzes upon work-up to the oxophlorin (7).[1]



(7)

The yield of benzoic acid equals the yield of the aromatic substitution product as it should and the process (6) is faster than the decarboxylation (3b).

Iron (II) octaethylporphyrin. The heme rapidly reduces each of the radicals (8)

$$Fe^{II} + R\cdot + H^+ \xrightarrow{\text{fast}} Fe^{III} + RH \quad (8)$$

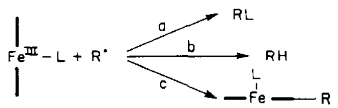
[1] These findings are in agreement with those reported earlier by Bonnett, Dimsdale, and Stephenson (cf. Ref. *3*) except that the multiplicity of products obtained in the more concentrated solutions (Ref. *4*) are not detectable at these concentrations. Ethyl side chains are omitted from the diagrams for clarity.

and the dark-red iron (II) solutions are concomitantly converted to those of the brown iron (III) complexes. This process (8) competes effectively with the dimerization of 2-cyanopropyl (5) or methyl radicals (a 10-fold difference in methane/ethane ratios), and it is the exclusive reaction with *t*-butoxy and benzoyloxy radicals. Thus, no acetone (2b) or carbon dioxide (3b) is detectable in the product from these latter species, respectively. However, a significant yield of methyl acetate is obtained even in the presence of a large excess of iron (II) octa E (see the following section). In all cases porphyrin recovery after iron removal was good.

In addition to its reaction with radicals the heme is oxidized quickly at room temperature by benzoyl or acetyl peroxide.

$$\mathrm{Fe^{II}} + \mathrm{R{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}O{-}O{-}\overset{\overset{\displaystyle O}{\|}}{C}{-}R} \longrightarrow \mathrm{Fe^{III}{-}O\overset{\overset{\displaystyle O}{\|}}{C}R} + \mathrm{R\overset{\overset{\displaystyle O}{\|}}{C}{-}O\cdot} \qquad (9)$$

Iron (III) octaethylporphyrin. The reactions of the iron (III) complex with radicals exhibit a more complicated set of characteristics. Products resulting from radical oxidation (10a) and reduction (10b) along with meso substitution of the porphyrin (10c) are apparent.



(10)

Moreover at the end of each of these reactions the spectrum of the solution is that of an iron (III) complex.

Thus, 2-cyanopropyl radicals are converted to the corresponding chloride as are methyl radicals (11).

$$\mathrm{Fe^{III}{-}Cl} + \mathrm{R\cdot} \longrightarrow \mathrm{RCl} + \mathrm{Fe^{II}} \qquad (11)$$

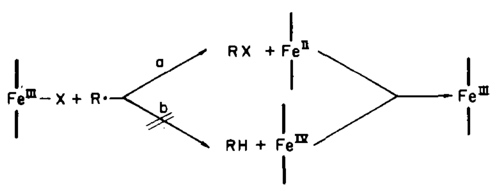
In a solvent containing acetic acid, methyl acetate is the major product of radical oxidation.[2]

$$\mathrm{Fe^{III}{-}OAC} + \mathrm{CH_3\cdot} \longrightarrow \mathrm{CH_3OAC} + \mathrm{Fe^{II}} \qquad (12)$$

t-Butyl hypochlorite or peracetate and benzoyl hypochlorite or benzoylacetyl peroxide, the oxidation products of *t*-butoxy and benzoyloxy radicals corresponding to (11) or (12), would not be stable under reaction conditions and are susceptible to attack by any iron (II) porphyrin present. No attempt was made to detect these substances or their possible decomposition products.

[2] Mass spectra of the relatively volatile octaethylporphyrin iron (III) derivatives isolated from these solutions show a predominant parent ion corresponding to the iron (III) acetate.

An important feature of the *t*-butoxy and benzoyloxy reactions is that substantial yields of *t*-butyl alcohol and benzoic acid (3b) are produced. Correspondingly, the reduction products isobutyronitrile and methane are obtained from the AIBN and diacetyl peroxide reactions. However, it should be emphasized that the relative amounts of oxidation or reduction products of a radical are a function of the ratio of starting concentrations of hemin and radical source. This is exemplified most clearly by the methyl studies in Table 2. Compare lines 3 and 4. Hence, the oxidation and reduction of methyl radicals cannot be the result of attack upon the same iron (III) species (13).



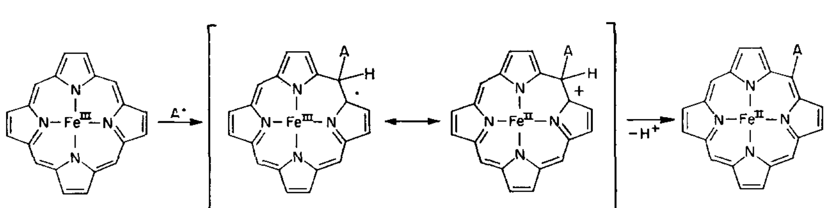
(13)

While the follow-up iron (II)—iron (IV) reaction is feasible, the occurrence of (13b) as a path of radical reduction demands a product ratio RX/RH invariant with starting Fe/R· ratios and such is not the case. Thus, the total process observed with hemins is the combined result of the initial reduction of Fe (III) by the radical followed by the attack of the radical upon the generated iron (II) heme.

$$\begin{aligned} &CH_3\cdot + L{-}Fe^{III} \xrightarrow{a} CH_3L + Fe^{II} \\ &CH_3\cdot + Fe^{II} \xrightarrow[b]{H^+} CH_4 + Fe^{III} \end{aligned} \tag{14}$$

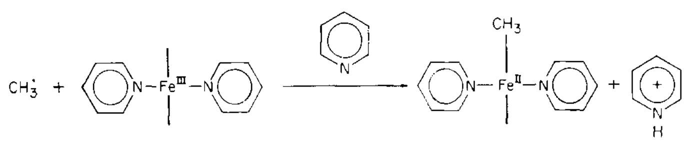
The processes (14) are competitive for methyl radicals. Similarly the methyl acetate generated in the presence of a large beginning excess of heme (line 5, Table 2) is the result of the same sequence in reverse 14b, 14a.

Meso substitution. The substitution at the meso position of the iron (III) complex by benzoyloxy radicals can be considered to be the result of an aromatic substitution like that sketched for the porphyrin in Eq. (6). More likely it is the result of a "σ-meso addition" reduction (5) of the iron (III) porphyrin (15). However, because any iron



(15)

(II) species generated would itself be rapidly oxidized to an iron (III) porphyrin by radicals or peroxide, no net reduction to iron (II) is observed. Thus, the process (15) cannot be proved to occur with the results in this solvent.[3] The operation of the σ-meso addition mechanism can be discerned in the solvent pyridine. Thus, the decomposition of benzoyl peroxide in pyridine solutions of chloro iron (III) octaethylporphyrin results in a product spectrum that does show a net reduction to the iron (II) dipyridyl adduct. The product is the mesobenzoyloxy derivative. More dramatically, in this solvent, the reaction with methyl radicals yields none of the oxidation or reduction products listed in Table 2, but rather the meso methyl porphyrin derivative along with a solution spectrum (Fig. 1) that shows a substantial amount of reduction (16).



(16)

Pyridine is a relatively reactive solvent toward these radicals (*6*) and no doubt the bulk of the radicals is consumed by it. Thus, with starting concentrations of 3.2×10^{-3} *M* iron (III) porphyrin and 1.6×10^{-3} *M* benzoyl peroxide no reaction with the

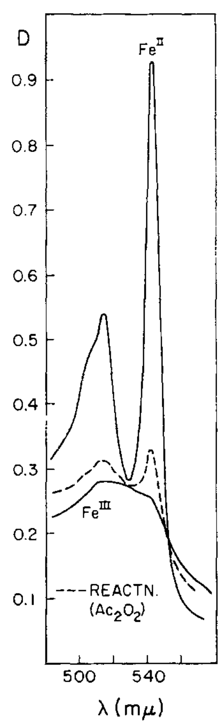


FIG. 1. Spectrum of 2.8×10^{-5} *M* pyridine solution of: bis-pyridyliron(III)octaethylporphyrin chloride; (——) after decomposition of equimolar diacetyl peroxide; bis-pyridyliron(II)octaethylporphyrin.

[3] Given the rapidity of the reaction of iron (III) porphyrins with radicals it is difficult to believe that the electron initially generated at the α pyrrole carbon by the addition process would not be transferred to iron.

porphyrin was obtained. The solution did not exhibit any spectral change after 1.5 hr at 110°C. Removal of the solvent *in vacuo* followed by iron removal and chromatography afforded only one porphyrin, octaethyl, in 75–81 % recovery. Quantitative iron removal and porphyrin isolation were not readily effected, and we assume that the extent of demetalation (cf. experimental) is the same for all porphyrins present in any run and that the ratio of porphyrins isolated after chromatography reflects the distribution of iron porphyrins in the reaction mixture. At 10 times the above concentration a small (1 %), but clearly discernible net reduction to iron (II) was observed, and the mesobenzoyloxy derivative comprised 12% of the isolated porphyrin product. Total recovery of porphyrins was 64% of the hemin changed. At 3.2×10^{-2} *M* hemin and 8×10^{-2} peroxide the net reduction was 4% and the meso substitution product comprised 16% of the purified porphyrin product. In these more concentrated runs a trace of another porphyrin could be separated. The material was too little to characterize but its mass spectrum (parent 774) and ir (C=O 1710 cm^{-1} broad) indicate it to be a dibenzoyloxylated derivative(s).

It should be noted that in pyridine as well as trichlorobenzene the iron (III) porphyrins do not react with either peroxide until the decomposition temperature is reached. On the other hand, the iron (II) complex in either solvent is readily oxidized at room temperature by either dibenzoyl or diacetyl peroxide.[4]

Reaction of the more reactive methyl radicals can actually be discerned at the spectrophotometric level of dilution. Thus, a 2.8×10^{-5} *M* solution of chloro iron (III) octaethyl porphyrin in pyridine (Fig. 1) was warmed to 110°C and treated with an equimolar amount of diacetyl peroxide. After warming the solution 1 hr, the spectrum indicated that a 14% net reduction had occurred (Fig. 1). For comparison, the completely reduced dipyridyl Fe (II) porphyrin spectrum is depicted. It was obtained by adding excess hydroquinone to the reaction solution. A typical scaled-up run for product with initial concentrations of 2.6×10^{-3} *M* showed an overall 10% reduction. Total porphyrin recovery was 78%. Mesomethyloctaethyl porphyrin (21% of the porphyrin product, 14% of the hemin charged) was the major product along with 1–2% of an incompletely characterized polysubstituted fraction and unsubstituted octaethylporphyrin.

We make the following summarizing conclusions:

(1) The reactivity of the porphyrin and its high-spin iron (II) and iron (III) complexes toward alkyl and *t*-butoxy radicals stand in the order: $Fe^{II} > Fe^{III} \gg$ free porphyrin. For benzoyloxy radicals the order is $Fe^{II} >$ Porp $> Fe^{III}$.

(2) The exclusive path of reaction of high-spin iron (II) porphyrins with radicals in protic media is the rapid reduction of the radical and generation of an iron (III) porphyrin:

$$Q\cdot + \overset{|}{\underset{|}{Fe^{II}}} \xrightarrow{H^+} QH + \overset{|}{\underset{|}{Fe^{III}}}$$

(3) The dominant path of reaction of high-spin iron (III) porphyrins with alkyl and (presumably) *t*-butoxy radicals is a rapid axial inner sphere reduction of the porphyrin.

[4] Initial studies indicate that these reactions bear further scrutiny and we shall report upon them later.

An axial ligand of iron is transferred to the radical.

$$\mathrm{Q}\cdot + \mathrm{L}\text{—}\overset{|}{\underset{|}{\mathrm{Fe^{III}}}} \longrightarrow \mathrm{QL} + \overset{|}{\underset{|}{\mathrm{Fe^{II}}}}$$

(4) The reaction of benzoyloxy radicals with high- or low-spin iron (III) porphyrins occurs primarily at the meso position. With the low-spin dipyridyl complex in pyridine the attendant reduction to iron (II) can be observed spectrally. Methyl radicals also reduce this complex by adding to the meso position.

(5) The reaction of a radical with either an iron (II) or an iron (III) porphyrin results in the generation of the other valence state of iron and consequently oxidation and reduction products emanating from both iron species are obtained.

(6) Iron (IV) is not an intermediate in these reactions.

(7) Iron (II) porphyrins in solvents that impart either spin state are easily oxidized by diacyl peroxides.

DISCUSSION

The general high reactivity of iron porphyrins toward free radicals would be expected for what is essentially the combination of two paramagnetic species. However, the efficiency of scavenging of benzoyloxy radicals by the free porphyrin in dilute solutions is remarkable,[5] and underlines the heightened reactivity imparted to these units by the large delocalizing capacity of the porphyrin nucleus. The primary attack at the meso position observed with the porphyrin shows the mesobenzoyloxy derivative to be the precursor to some of the more highly substituted species reported to obtain with higher concentrations of the peroxide (*4*).

The reduction of radicals by hemes. We have previously noted the rapid reduction of 2-cyanopropyl radicals by iron (II) deuteroporphyrin IX in *N*-methylpyrrolidone–acetic acid. The process has been depicted as the rapid second step in the mechanism of the reduction of alkyl halides by hemes and hemeproteins (*2*), and the present work substantiates the swiftness and generality of the process. We believe that the mechanism is an axial process and best formulated as (17).

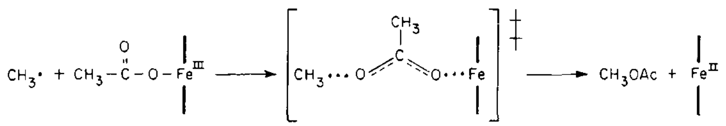
$$\mathrm{R}\cdot + \overset{|}{\underset{|}{\mathrm{Fe^{II}}}} \xrightarrow{\mathrm{a}} \mathrm{R}\overset{|}{\underset{|}{\mathrm{Fe}}} \xrightarrow[\mathrm{b}]{\mathrm{H^+}} \mathrm{RH} + \overset{|}{\underset{|}{\mathrm{Fe^{III}}}} \tag{17}$$

For alkyl radicals the intermediate iron-alkyl would not be expected to survive conditions of temperature and acidity employed herein. Indirect evidence for the axial nature of the process is deduced from the results in pyridine. Thus, a net reduction of iron (III) to iron (II) porphyrin in pyridine by both benzoyloxy and methyl radicals is

[5] Thus, a minimum of 40% trapping of benzoyloxy radicals by octaethylporphyrin in these dilute solutions along with the low yield of benzoic acid would set the reactivity of the porphyrin toward benzoyloxy radicals in the approximate range of 100 times that of 3,4-benzopyrene (cf. Ref. *25*).

observed. If the iron (II) species in pyridine were competitive with the reactivity of the (III) complex toward methyl radicals or peroxide as it is in trichlorobenzene, no net reduction should be possible and substantial yields of methane should be obtained. Indeed, we attribute the observable reduction of the iron (III) complex in pyridine to the inability of low concentrations of methyl radical to compete with pyridine for iron. That is, we presume the rate of processes (17a) and (9) are diminished by the axial pyridine ligands.

The reduction of hemins. Two paths of reaction are exhibited by iron (III) porphyrins in their reduction by free radicals. These reductive pathways are typified by the behavior of methyl fragments toward the high-spin iron (III) complex in trichlorobenzene or TCB-ACOH (18, 19) on the one hand and toward the low-spin complex in pyridine (20) on the other.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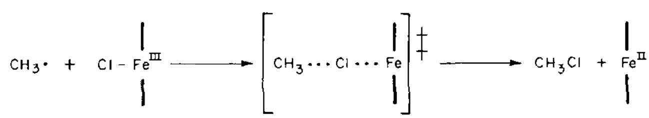
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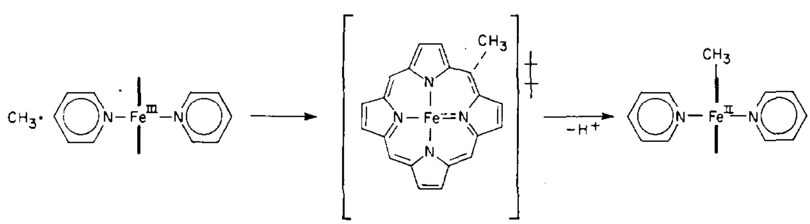

(20)

The transition states for the production of methyl chloride and methyl acetate are most simply depicted as analogous to those formulated in completely inorganic systems for the reduction of $Co^{III}(NH_3)_5L$ by Cr(II) (*7*). They are classic examples of axial inner-sphere electron-transfer process. In these instances the atom or group transfer is from iron to carbon, and parallels the original conversion of methyl radicals to methyl chloride by cupric chloride (*8*) and a variety of high-valent transition metal salts (*9*). However, while the acetate bridge (Eq. 18) is reasonable and has some precedent with inorganic ions (*7*), it is possible that a methyl cation is generated in these reactions.[6] The reactions with the chlorohemin (10) (19) are the exact mechanistic converse of the initial cleavage of organic halides by hemes (*2*), and these results establish the rever-

$$\overset{|}{\underset{|}{\mathrm{Fe^{II}}}} + \mathrm{XR} \rightleftharpoons \overset{|}{\underset{|}{\mathrm{Fe^{III}}}}\text{—}\mathrm{X} + \mathrm{R}\cdot \qquad (21)$$

sibility of this system (21). The position of equilibrium in (21) is largely dependent upon the dissociation energy of the carbon-halogen bond.

[6] For a recent detailed analyses of the mechanism oxidation of alkyl radicals by cupric acetate cf. Ref. 9C.

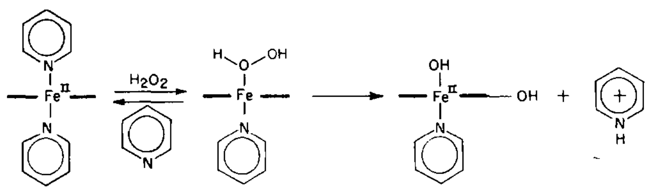
σ-Meso addition. The reduction of the iron (III) dipyridyl adducts by benzoyloxy and methyl radicals in pyridine establish the σ-meso addition mechanism (15) to be operative. Moreover, it is most likely that the same mechanism is at play in the addition of benzoyloxy radicals to the iron (III) complex in trichlorobenzene. We had originally proposed this mechanism to explain the exchange of hydrogen at the meso position of octaethylporphyrin upon reduction of the iron (III) complex to the iron (II) species by iron powder in 1:1 *N*-methylpyrrolidone–acetic acid (*10*). It was subsequently suggested (*11*) that the observed exchange may be primarily due to a simple proton exchange with the media by the iron (II) porphyrin that does not readily occur with either the free porphyrin or the iron (III) complex.[7] While it is unlikely that the iron (II) complex in the mildly acidic *N*-methylpyrrolidine–acetic acid milieu did exchange significantly at room temperatures, our original interpretation was nonetheless ambiguous because a small exchange can occur in the iron-removal step that we employed.[8] The present results are not subject to this ambiguity and we take them to firmly establish the viability of the σ-meso addition mechanism and peripheral processes in metalloporphyrin and hemeprotein redox chemistry. Indeed, while hemeprotein reactivity can be explained by a simple theory (*5*), one of its basic premises is that peripheral redox reactions can occur with iron porphyrins. Until now, direct unambiguous evidence in the literature for even one such occurrence has been lacking. Nonetheless, the notion that a peripheral path may be involved in electron-transfer reactions of hemeproteins has gained an increasingly ready acceptance. Thus, the diffusion-controlled reduction of horse heart cytochrome *c* by e^-_{aq}. has been interpreted to indicate that "the electron is transferred to the exposed edge of the heme in the cytochrome *c* crevice" (*12*). While we agree with this general interpretation, rapid kinetics per se do not prove the pathway. Quite recent studies of the influence of azide and thiocyanate anions upon the rate of reduction of this same protein by Cr(II) suggest a peripheral process for the electron transfer at near-neutral pH values. In solutions of lower pH containing high concentrations of chloride an inner-sphere process is favored (*13*). This latter study emphasizes the importance of protein conformation in solution and the need for probing the reactivity of biological units with redox reagents that operate by a defined mechanism.

The reactivity of the meso position of porphyrins toward free radicals noted herein is in keeping with the generally known halogenation at this site by sulfuryl chloride in chloroform (*14*). Presumably chlorine atoms are intermediates. Moreover, the reaction of the dianion, dicyano iron (II)-protoporphyrin IX, with a basic aqueous solution of sodium cyanide, ascorbic acid, and hydrogen peroxide yields a meso cyano derivative. The process has been interpreted to result from the attack of cyano radicals upon the complex ion (*15*). Moreover, quite recently, the reaction of hydrogen peroxide

[7] The nmr spectrum of meso-tetradeuterooctaethylporphyrin in CF_3CO_2H shows no sign of a meso C-H signal for days. Indeed, the octaethylporphyrin dictation in CF_3CO_2D at 100°C requires about 2 mo for exchange of the meso protons (*24*). The half-life for the corresponding process with the iron (III) complex in refluxing DOAC has been set (*11*b) at 38 hr. Tritium (CH_3CO_2T) was not incorporated into the iron (III) complex on the time scale of our experiments.

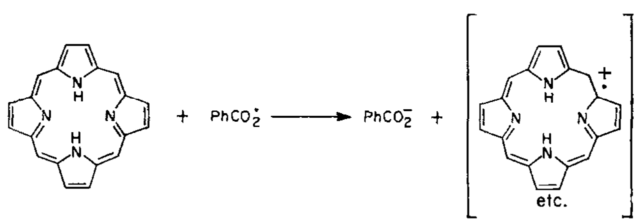
[8] Dilute iron (II) porphyrin solutions in 1:1 *N*-methylpyrrolidone–acetic acid were made 3 *N* in H_2SO_4 by adding the requisite amount of concentrated acid at room temperature. Demetalation was instantaneous and blanks indicated that a maximum of 1% exchange (H for D) occurred in the process. On the other hand, more difficultly demetalated species, like the iron (II) carbonyl in methanol, required a longer exposure to strong acid and a higher percentage of exchange during demetallation ensued.

with a series of low-valent transition metal complexes of octaethyl porphyrin in pyridine has been demonstrated clearly to result in meso hydroxylation (*16*). A related reaction may well be important in the catabolism of hemes and heme oxygenase (*17*) functions. The results of the present study are in general agreement with the mechanism formulated for the H_2O_2 reaction by Bonnett and Dimsdale. However, given the reduction of the iron (III) dipyridyl adduct of octaethyl porphyrin in pyridine by both benzoyloxy and methyl radicals to the iron (II) complex (Eq. 15), we favor a scheme in which the hydroxy radical that adds to the meso position causes reduction. The overall reaction would be written as (22).



(22)

Alternate paths. The mechanisms depicted above are meant to be the simplest consistent with the present findings and earlier studies of the interactions of free radicals with aromatic systems (*26*) and transition metal salts (*9*, *27*). Clearly, other paths are possible. In particular, π-cation radicals have been generated from porphyrin derivatives both by electrolytic and chemical oxidation (*28*). Thus, the aromatic substitution of the free porphyrin depicted in Eq. (6) can be written to entail a π-cation radical precursor (23).



(23)

Coalescence of the ion pair would result in the intermediate radical sketched in Eq. (6). Alternatively, the porphyrin cation-radical could couple with another benzoyloxy radical and lose a proton to the benzoate anion. Indeed, this latter path has been reported for the conversion of magnesium (II) octaethylporphyrin to the mesobenzoyloxy compound upon treatment with benzoyl peroxide in methylene chloride at room temperature (*28*). Based upon these findings, Dolphin has suggested that the meso substitution of iron porphyrins by radicals involves the reaction of an iron (II) rather than an iron (III) porphyrin. The corresponding sequence would be:

$$\mathrm{Fe^{III}\ Porp + Ph\ CO_2\cdot \longrightarrow Fe^{II}\ Porp + Ph\ CO_2^{+}} \tag{24}$$

$$\mathrm{Fe^{II}\ Porp + Ph\ CO_2\cdot \longrightarrow Fe^{II}\ \overset{+}{Porp\cdot} + Ph\ CO_2^{-}} \tag{25}$$

$$\mathrm{Fe^{II}\ \overset{+}{Porp\cdot} + Ph\ CO_2\cdot \xrightarrow[-H^+]{} meso\text{-}PH\ CO_2\text{-}Porp\ Fe^{II}} \tag{26}$$

While we cannot eliminate a precursor π-cation radical in the reactions of the free prophyrin (23) with the evidence at hand, we prefer the homolytic path sketched in Eq. (6) for the reactions in the nonpolar trichlorobenzene solvent. However, no substitution of the meso position of an iron (II) porphyrin is found with any of the radicals examined. The only porphyrin recovered from heme reactions is the starting octaethyl array. Moreover, the reactions of the radicals with the iron (II) porphyrin clearly result in the reduction of the radicals (8). This is the exclusive path of reaction with both benzoyloxy and *t*-butoxy radicals and it is the fastest process studied herein (vide supra). In contrast, if reactions are begun with the iron (III) porphyrin, meso substitution does ensue. Hence, in these systems the meso substitution of iron porphyrins by radicals is, in fact, the result of reactions with the iron (III) and not the iron (II) complex. This is not to state, however, that our results are in disagreement with the formulation drawn in Eq. (26), although the sequence (24–26) may be misleading. In our view, reactions (26) and (15) are identical if it is understood that the "iron (II) porphyrin–cation–radical," $Fe^{II}\,\mathrm{Porp}\overset{+}{\cdot}$, is a resonance form of an iron (III) porphyrin. Thus, in accord with theoretical calculation (*29*), if iron and porphyrin electrons are in conjugation, as both we (*5*) and Dolphin (*28*) agree, then π-cation radicals cannot exist as discreet entities in the iron (II)–iron (III) interchange. In this regard the two different paths of reaction of a radical with different resonance hybrids of the same iron (III) porphyrin sketched in Eqs. (24) and (26) would not be consistent with the methyl studies described above.

EXPERIMENTAL

Materials. 1,2,4-Trichlorobenzene, bp 68°C/2 mm, was distilled under argon. Baker and Adamson reagent grade acetic acid and pyridine were employed without purification as were: argon (99.998%), benzoyl peroxide (Eastman Kodak white label), and bis-azoisobutyronitrile (Aldrich). Di-*t*-butyl peroxide (MCB) was distilled before use. Commercial solutions (25%, Wallace and Tiernan, Inc.) of diacetyl peroxide in dimethyl phthalate were washed thrice with equal volumes of water to remove hydrogen peroxide and dried over sodium sulfate. Small quantities of the peroxide were sublimed from these solutions at room temperature and 20 mm. The crystals collected on the cold finger (CO_2) were immersed in known volumes of pyridine or trichlorobenzene and the concentration of peroxide was determined by iodide oxidation and thiosulfate titration (*18*). α-Chloroisobutyronitrile bp 114–116°C was obtained from isobutyronitrile and PCl_5 (*19*).

Porphyrins. Octaethylporphyrin, mp 324°C, was obtained by the Whitlock modification (*20*) of the Linstead synthesis. Its visible, nmr (*10*), and mass spectra (parent 534) were consistent with its structure and purity. Iron insertion was accomplished essentially by the ferrous acetate–air–HCl procedure given by Falk (*21*). The mass spectrum of the chloro iron (III) derivatives obtained by crystallization from acetic acid saturated with sodium chloride and containing HCl possessed parents at 625, 623, and 588 (loss of Cl). The substance reisolated from dilute solutions in trichlorobenzene acetic acid upon mass spectrometric analysis showed a predominant parent ion corresponding to the iron (III) acetate (647). Mesobenzoyloxyoctaethylporphyrin, from the benzoyl peroxide decompositions, mp 278°, mass spectrum parent at 654, was identical in all respects, visible, ir, nmr, with the compound described by Bonnett and Dimsdale and Stephenson (*3*). The most revealing feature in the nmr spectrum are the two kinds of meso protons (singlets) at 10.07 and 9.95 δ ($CDCl_3$) in the ratio of 2:1. The corresponding octaethyloxophorin was identified by its characteristic visible spectrum (acid, base, neutral) and its conversion upon standing in air, even in dilute chloroform solution, from the blue–green oxyphlorin, through olive to the lavender, presumably, bilatriene-abc. Small amounts of the substance were usually obtained by on-column hydrolysis of the meso-benzoyloxy ester on basic alumina or silica or by acid hydrolysis during the demetalation process (see below). The substance cochromatographed (tlc) with an authentic sample prepared from the benzoyloxy ester by basic hydrolysis (*3*). In our hands the small amounts of the blue–green band fresh off the column were difficult to keep. Mesomethyloctaethylporphyrin, mp 271–273°C, mass-spectrum parent 548, from the diacetyl peroxide runs exhibited a typical "phyllo" spectrum (λ_{max}, $CHCl_3$, 408, 505, 539, 573, 621. The last four bands were in the ratio: 2.4:1.04:1:0.25. The nmr spectrum, (CCl_4): Singlets 10.2 δ (2H), 10.02 (1H), 4.6 (3H), overlapping quartets 4.18 (16H), overlapping triplets 2.05 (24H), is only slightly shifted from that reported for the corresponding Pd(II) complex in $CDCl_3$ (*22*). The meso methyl group at 4.6 δ is in the region assigned to the meso methyl substituents of a series of porphyrins (*23*).

Reactions. All reactions were conducted under argon and were run at least five times. The yields reported in the tables and text are an average. Reproducibility of repeated runs was within 5%. The general procedure employed for reactions and work-up is illustrated for the decomposition of benzoyl peroxide in trichlorobenzene solutions of octaethylporphyrin. Variations in this procedure are noted where appropriate.

Octaethylporphyrin and benzoyl peroxide. In a 100-ml, three-necked flask equipped with a magnetic stirrer, argon inlet, and outlet stopcocks, and an addition funnel were placed, 200 mg, 4×10^{-4} mole, of octaethylporphyrin dissolved in 40 ml of 1,2,4-trichlorobenzene (TCB). Dibenzoyl peroxide, 50 mg, 2×10^{-4} mole, in 10 ml of TCB was placed in the addition funnel. Argon was passed through a side arm of the addition funnel into the flask. After vigorous purging with stirring, for 3 min, the exit stopcock was connected to a mercury trap and a slow argon sweep was continued with stirring at room temperature for 3 hr. The flask was warmed to 110°C in an oil bath and the peroxide was added all at once. The bath was maintained at this temperature for 2 hr. After cooling the flask, the contents were poured into an equal volume of saturated sodium bicarbonate. The organic phase was extracted three times with bicarbonate. The aqueous phase containing any sodium benzoate was washed once with chloroform, to remove any residual TCB, acidified with concd HCl, and ether-extracted thrice. For quantitation of benzoic acid, the combined ether extracts were washed with water and treated with an excess of diazomethane. The latter was generated from the aqueous potassium hydroxide decomposition of *N*-nitrosomethylurea. After stirring 5 min, excess CH_2N_2 was decomposed with glacial acetic acid. The solution was washed with water, dried over sodium sulfate, filtered, and concentrated. Benzoic acid was determined as methyl benzoate by gas chromatography upon a 4-ft 20% diethylene glycol on fire brick column. Authentic ester was employed as a standard. A blank composed of 10 mg of benzoic acid in 200 ml of TCB yielded a 91% recovery by this procedure. In earlier qualitative runs concentration of the dried ethereal solutions in vacuum afforded crystals that sublimed and had mp 120°C. A mixed melting point with authentic benzoic acid was undepressed. The original bicarbonate-washed TCB solution, containing the porphyrin products, was washed with water and dried over sodium sulfate. The entire solution was poured onto a dry silica gel column and the TCB solvent was removed from the column with hexane. All porphyrin products were then removed from the column with chloroform. The chloroform concentrate therefrom, in 1:1 benzene–chloroform, was rechromatographed upon a basic silica gel column. Either a dry column or one washed with 1:1 benzene–chloroform could be employed. The porphyrin fractions, as they emerged from the column, yielded after concentration: octaethylporphyrin, 84 mg, 42%; mesobenzoyloxyoctaethylporphyrin, 88 mg, 36%; octaethyloxophlorin, 8 mg, 7%. Yield data are summarized in Table 1. For the nmr and mass spectrometric analysis indicated above, each of the porphyrins was rechromatographed on a dry basic Fisher A-540 alumina column. The octaethylporphyrin eluted with benzene and a heart cut was recrystallized from $CHCl_3$—MeOH. The recovered sample had the spectroscopic properties and melting point identical with the authentic material described above. Mesobenzoyloxyoctaethylporphyrin eluted with 1:1 benzene–chloroform and was recrystallized from $CHCl_3$—MeOH. The properties described above were for the substance isolated in this manner. Carbon dioxide was estimated in separate runs by passing the argon gas from the reaction mixture through saturated barium hydroxide. The $BaCO_3$ was determined gravimetrically. When this reaction was conducted in acetic acid, presumably with the porphyrin mono cation, similar results were obtained except that no mesobenzoyloxy compound was isolated. Final Al_2O_3 chromatography yielded only recovered octaethylporphyrin (50%) and the octaethyloxophlorin (34%). The latter when treated with benzoyl chloride in pyridine yielded the mesobenzoyloxy octaethylporphyrin.

Benzoyl Peroxide and Chloroiron (III) Octaethylporphyrin

(a) *In trichlorobenzene.* This reaction was conducted in a manner entirely analogous to that described above for the porphyrin. At the end of the reaction the visible spectrum of a diluted solution was identical to the starting spectrum. The major difference in work-up entailed an iron-removal step prior to chromatography.[9] Thus, after removing the benzoic acid from the TCB product solution with bicarbonate, the organic phase was water washed, dried, filtered, and treated with 5 ml of glacial acetic acid. The acidic solution was heated under argon with stirring to 120°C. An equal volume of a saturated solution of ferrous acetate in HOAc was added, followed by 1 ml of concd HCl. Warming was continued until the purple porphyrin dication looked bright (5 min). The solution was washed with water, dried over sodium sulfate and poured through a dry silica column to remove TCB. Work-up then commenced as outlined above.

(b) *In pyridine.* An apparatus like that described for the TCB experiments was employed except that the addition funnel was omitted and a condensor was interposed between the flask and argon outlet. Reactants were all mixed at room temperature and after thorough argon purging. The reaction solution was immersed in an oil bath at 120°C. The pyridine began refluxing shortly (5 min) and the solution was held at

[9] Attempts to separate and characterize the iron (III) products directly were difficult and finally abandoned.

reflux for 2 hr. A small probe of the cooled solution was diluted into pyridine and its visible spectrum recorded. Depending upon the concentration of peroxide, the visible spectrum of the reaction solution indicated 0–4% reduction to iron (II). Even a 1% net reduction can be seen when the spectrum is compared with that of the starting dipyridyl iron (III) adduct. Thus, a distinct shoulder at 543 nm is apparent. For these runs the amount of reduction was calculated from the optical densities at 543 nm (D_1) and 514 nm (D_2) with the equation

$$\%(\mathrm{Fe^{II}}) = 100\frac{(0.960\mathrm{D}_1 - 0.834\mathrm{D}_2)}{(2.80\mathrm{D}_2 - 1.02\mathrm{D}_1)}$$

The corresponding extinction coefficients for the iron (II)[10] and iron (III) complexes at these wavelengths from which the equation was derived were determined to be:

$$\varepsilon_1^{\mathrm{II}} = 3.63 \times 10^4, \qquad \varepsilon_2^{\mathrm{II}} = 1.98 \times 10^4{}^{10}$$

and

$$\varepsilon_1^{\mathrm{III}} = 0.834 \times 10^4 \qquad \varepsilon_2^{\mathrm{III}} = 0.960 \times 10^4.$$

The results are summarized in the Results section and not repeated here. Work-up in these runs proceeded by stripping the pyridine from the reaction solution on a rotary evaporator. The concentrate was taken up in chloroform, washed once each with 6 *N* HCl and water, dried over sodium sulfate, filtered, and concentrated to dryness. The residue was treated with 60 ml of glacial acetic acid saturated with ferrous acetate under argon. The suspension was brought to a boil under argon and 5 ml of concentrated HCl were added. Iron removal appeared complete (clear purple dication solution) after 5 min but subsequent chromatography showed that it was not. The solution was poured onto ice-water–$CHCl_3$ adjusted to pH 5 with bicarbonate and extracted four times with chloroform. The chloroform extracts were washed with water, dried over sodium sulfate, filtered, and concentrated to a volume of 0.5 ml. The concentrate was placed atop a dry basic silica gel column, and elution was commenced with hexane. In this procedure a typical run at initial concentrations 3.2×10^{-2} *M* Fe^{III} Porp. and 1.6×10^{-2} *M* benzoylperoxide showed a 1% net reduction and yielded upon final chromatography[11] on 60–200 mesh Baker and Adamson Silica Gel powder: 45 mg octaethylporphyrin (Band I–benzene), 8 mg (recryst) mesobenzyloxyocta-ethylporphyrin (Band II–benzene), a trace of dibenzoyloxylated octaethylporphyrin (1 mg $CHCl_3$) and iron (III) octaethylhemin (Band IV–MeOH) 2.3 mg. The iron III fraction was treated with HCl chloroform and stripped before weighing. Its spectrum was identical to octaethylhemin. Recovery was 66% of the hemin originally charged.

Room temperature. The inertness of the iron (III) complexes at room temperature toward either benzoyl peroxide or diacetyl peroxide was established by allowing typical reaction mixtures to stand for 4 days. No carbon dioxide was evolved and the peroxide titer was undiminished (94% recovery).

Benzoyl peroxide and iron (II) octaethylporphyrin. Heme solutions in TCB containing 1% acetic acid were prepared by reduction of the chloro iron (III) complexes with iron powder with the apparatus previously described (*2*). The reaction was conducted in

[10] For these measurements the iron (II) spectrum was generated by reduction with either hydrazine hydrate or sodium dithionite. The pyridine hemochrome spectra were the same.

[11] In many of these runs rechromatography of each fraction was essential for good purification and this is reflected in the less than quantitative recovery.

the manner described for the hemin. The dark-burgundy heme solutions were immediately oxidized to the brown hemin upon addition of the peroxide solution at 25 or 80°C. Because of the larger amount produced in these runs, benzoic acid was isolated as a solid and determined gravimetrically. A reaction solution originally charged with 200 mg of octaethylchlorohemin and 40 mg of benzoyl peroxide in 100 ml of solvent afforded 35 mg of solid benzoic acid. Upon chromatography after iron removal, 140 mg (88%) of octaethylporphyrin and 1 mg of octaethyloxophlorin were detected.

Diacetyl peroxide. Reactions with this substance and the three porphyrin derivatives were conducted in 95:5 TCB:HOAc in the manner described above for benzoyl peroxide. The reaction flask in these runs was additionally fitted with a manometer and after flushing with argon and before addition of the peroxide solution the system was set at a slight vacuum. Total gas yields were measured manometrically and the composition was determined by flame ionization gas chromatography on a 5-ft porapak P column. The solution was also analyzed directly by this method for methyl acetate. Authentic standards were employed. Gas yields were also checked with standards of known composition and agreement was within 5%. In addition to the data in Table 2, carbon dioxide was determined gravimetrically (vide infra). Yields in the blanks and with the porphyrins were 90%. Oxidation of the heme by the peroxide was instantaneous. In contrast ferrous acetate blanks contained 80% of the peroxide after 1 day.

The iron (III) porphyrin in pyridine. Although some methane was detected in these runs (3–4%), it was somewhat less than the 4–7% generated in the blanks. Chromatography of porphyrin products followed that described above for the corresponding benzoyl peroxide reaction in pyridine. The mesomethyloctaethylporphyrin was the second band from (benzene) the silica column. It was followed by a small amount of as yet uncharacterized substance. The visible and ir spectra of this substance resembled that of the uncharacterized dibenzoyloxylated porphyrin detected in trace amounts and noted above. The undemetalated iron (III) fraction upon mass spectrometry exhibited parents at 647 and 661 corresponding to Fe(III) Octa E and mesomethyl Fe(III) Octa E acetates. Overall porphyrin recovery was 86%.

With bis-aziosobutyronitrile. A reaction mixture of 100 mg octaethylporphyrin, 2×10^4 mole, 17 mg AIBN, 10^{-4} mole, in 25 ml of TCB at 95°C for 2 hr afforded after chromatography only octaethylporphyrin, 86 mg, and a dirty-brown solid not containing a porphyrin spectrum. The substance was recrystallized from MeOH to yield 11 mg of tetramethylsuccinonitrile as colorless needles having mp 164–166°C. In the absence of porphyrin, 12 mg of this substance were obtained under these conditions. Only trace amounts of isobutyronitrile could be detected by gas chromatography (6-ft porapak P) in either run.

For the iron derivatives, quantitative analysis for isobutyronitrile and α-chloroisobutyronitrile were accomplished by direct VPC analysis of reaction mixtures on the 6-ft porapak P and 8 ft 20% apiezon L on firebrick columns. Authentic standards were employed. The dinitrile was isolated as a solid upon chromatography of the porphyrin as noted above.

Di-t-butyl peroxide. Chromatography of porphyrin products in these reactions from both the porphyrin and the hemin provided, in addition to octaethyl porphyrin, a small amount of substance that had a mass spectrum corresponding to meso-*t*-butoxyoctaethyl porphyrin. The visible spectrum was a phyllo type. The *t*-butyl alcohol acetone ratios were determined by direction flame ionization gas chromatography of reaction mixtures. A 10 ft 20% carbowax 20 M on firebrick column was employed.

Gas chromatography was conducted with an Aerograph A-600C or A-90P. Spectra were recorded with a Beckman DB (visible), Varian T-60 (nmr), Beckman IR-8, and Finnigan 1015 mass spectrometer.

REFERENCES

1. T. Ohnishi, H. Yamazaki, T. Iyanagi, T. Nakamura, and I. Yamazaki, *Biochim. Biophys. Acta*, **172**, 357 (1969) and Refs. therein.
2. R. S. Wade and C. E. Castro, *J. Amer. Chem. Soc.*, **95**, 226, 231 (1973).
3. R. Bonnett, M. J. Dimsdale, and G. F. Stephenson, *J. Chem. Soc.* (C), **1969**, 564.
4. R. Bonnett and A. F. McDonagh, *Chem. Commun.*, **70**, 337 (1970).
5. C. E. Castro, *J. Theoret. Biol.*, **33**, 475 (1971).
6. C. Walling, "Free Radicals in Solution," Wiley, London, 1957, Chap. 10 and refs, therein.
7. (a) H. Taube, H. Meyers, and R. L. Rich, *J. Amer. Chem. Soc.*, **75**, 4118 (1953); H. Taube and H. Meyers, *J. Amer. Chem. Soc.*, **76**, 2103 (1954); (b) H. Taube, "Electron Transfer Reactions of Complex Ions in Solution," Academic Press, New York, 1970, and Refs. therein.
8. J. Kumamoto, H. E. DeLaMare, and F. F. Rust, *J. Amer. Chem. Soc.*, **82**, 1935 (1960).
9. (a) H. E. DeLaMare, Jay K. Kochi, and F. F. Rust, *J. Amer. Chem. Soc.*, **85**, 1437 (1963) and Refs. therein; (b) E. I. Heiba and R. M. Dessau, *J. Amer. Chem. Soc.*, **93**, 524 (1971); (c) C. L. Jenkins and J. K. Kochi, *J. Amer. Chem. Soc.*, **94**, 843, 856 (1972).
10. C. E. Castro and H. F. Davis, *J. Amer. Chem. Soc.*, **91**, 5405 (1969).
11. (a) R. Grigg and A. Sweeney, *Chem. Commun.*, **1970**, 1237; (b) J. B. Paine and D. Dolphin, *J. Amer. Chem. Soc.*, **93**, 4080 (1971).
12. I. Pecht and M. Faraggi, *Proc. Nat. Acad. Sci., U.S.A.*, **69**, 202 (1971) communicated by B. Chance.
13. J. K. Yandell, D. P. Fay, and N. Sutin, *J. Amer. Chem. Soc.*, **95**, 1131 (1973) and Refs. therein.
14. H. H. Inhoffen, J. W. Buchler, and P. Jäger in "Fortschritte Der Chemie Organischer Naturstoffe," Vol. **36**, Springer-Verlag, Wien, 1968 pp 327 et seq.
15. D. B. Morell and A. W. Nichol, *J. Chem. Soc. (C)*, **1969**, 517.
16. R. Bonnett and M. J. Dimsdale, *J. Chem. Soc. (Perkin)*, **I**, 2540 (1972).
17. R. Schmid, "The Enzymatic Formation of Bilirubin," paper no. 38, New York Academy of Sciences Conference on the Biological Role of Porphyrins and Related Structures, October 1973.
18. C. D. Wagner, R. H. Smith, and E. D. Peters. *Ind. Eng. Chem. Anal. Ed.*, **19**, 976 (1947).
19. C. L. Stevens, *J. Amer. Chem. Soc.*, **70**, 165 (1948).
20. H. W. Whitlock and R. Hanauer, *J. Org. Chem.*, **33**, 2169 (1968).
21. J. E. Falk, "Porphyrins and Metalloporphyrins," Elsevier, New York, 1964, p. 135.
22. R. Grigg, G. Shelton, and A. Sweeney, *J. Chem. Soc. (Perkin)*, **1**, 1789 (1972).
23. R. J. Abraham, A. H. Jackson, G. W. Kenner, and D. Warburton, *J. Chem. Soc.*, **1963**, 853.
24. R. Bonnett and G. F. Stephenson, *Proc. Chem. Soc.*, **1964**, 291; R. Bonnett, I. A. D. Gale, and G. F. Stephenson, *J. Chem. Soc.*, (C), **1967**, 1168.
25. I. M. Roitt and W. A. Waters, *J. Chem. Soc.*, **1952**, 2695.
26. G. H. Williams, "Homolytic Aromatic Substitution", Pergamon Press, New York (1960) and Refs. therein.
27. C. E. Castro and W. C. Kray, Jr., *J. Amer. Chem. Soc.*, **88**, 4447 (1966).
28. D. Dolphin, Z. Muljiani, K. Rousseau, D. C. Borg, J. Fajer, and R. H. Felton, *Ann. N. Y. Acad. Sci.*, **206**, 177 (1973) and Refs. therein.
29. M. Zerner and M. Gouterman, *Theor. Chim. Acta*, **4**, 44 (1966).