Reactions of Iron Porphyrins with Methyl Radicals[1]

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Abstract: The reactions of methyl radicals with ferric, ferrous, and iron-free deuteroporphyrin have been investigated in neutral and alkaline water-2-propanol mixtures. Steady-state radiolysis of methyl chloride saturated solutions of ferric deuteroporphyrin, or chemical reduction of ferric deuteroporphyrin solutions containing methyl iodide, led to a new species which is spectrophotometrically characterized. The yield ($G \simeq 3$) and the redox titration (2 reducing equivalents) strongly suggest the structure $DPFe^{III}CH_3$. This product is found to be stable under anaerobic conditions but is oxidized to the initial ferric form in the presence of air. Pulse radiolysis experiments show that methyl radicals react with both ferric and ferrous deuteroporphyrin with reaction rate constants of 2.3×10^9 and 3.9×10^9 M^{-1} s^{-1}, respectively. The reaction with iron-free deuteroporphyrin is much slower, $k = (1-2) \times 10^7$ M^{-1} s^{-1}, demonstrating that methyl radicals react with iron porphyrin at the iron center. The transient species resulting from the scavenging of a methyl radical by ferric porphyrin $DPFe^{IV}CH_3$ undergoes a further slow reaction leading to ferrous deuteroporphyrin ($t_{1/2} \simeq 200$ μs). Electron (or methyl) transfer between $DPFe^{IV}CH_3$ and ferrous deuteroporphyrin leading to $DPFe^{III}CH_3$ and ferric deuteroporphyrin is shown to occur with a second-order rate constant $k = 4 \times 10^8$ M^{-1} s^{-1}. In order to fully describe the systems investigated, we also studied the reactions of α-hydroxyisopropyl radicals with ferric, ferrous, and iron-free deuteroporphyrin and with methyl iodide. The reactions of methyl radicals are discussed in relation to the hypothesis of radical-mediated toxicity of halogenated compounds. Besides, they appear to constitute the first set of detailed data on the formation mechanism of the scarce iron-carbon bonded complexes involving alkyl residues and macrocyclic ligands.

The toxicity of carbon tetrachloride and other polyhalogenated compounds depends on their metabolism. It is currently stated that radicals resulting from one-electron reduction of a carbon-halogen bond induce chain reactions leading to lipid peroxidation and destruction of membrane structure in living cells.[3,4] Among the potentially biological reducing agents, ferrous cytochrome P450 appears the most likely. However, as shown by in vitro experiments, under anaerobic conditions, this hemoprotein reacts with polyhalogenated compounds leading to carbene complexes,[5-7] which involves the reductive cleavage of a second carbon-halogen bond.

Model compound studies have been designed to clarify the part played by cytochrome P450 in CCl_4 metabolism. Dichlorocarbene complexes of ferrous porphyrins have been recently characterized.[8,9] They were found to oxidize in air leading to carbon dioxide.[10,11] Since CO_2 is the major product of CCl_4 detoxification by living systems,[12] it has been suggested that the carbene complex of ferrous cytochrome P450 could be an intermediate in the metabolism of CCl_4 in vivo.[11] As the carbene complex formation would involve the scavenging of $\cdot CCl_3$ or related radicals[13] by the porphyrin moiety, we decided to study the reaction of iron porphyrins with alkyl radicals.

It has been recently reported that the fast reaction of ferrous porphyrins with mono-, di-, and trichloromethyl radicals leads to σ-bonded iron-carbon derivatives.[14] However, it was found difficult to prepare these compounds by means of continuous irradiation, possibly because they undergo further evolution leading to the carbene form. Furthermore, a larger screening of reactions of iron porphyrins with various radicals is necessary to get a sound chemical basis which would allow reasonable predictions of cytochrome P450 reactions. Therefore, we present a study of the reactions of methyl radicals with ferric and ferrous porphyrins and report some properties of new methyl-iron-porphyrin complexes. These results have also interesting bearing on organometallic chemistry and catalysis. Indeed, although they have been postulated in redox[15,16] and catalytic[17] processes, very few σ-bonded alkyl derivatives of square-planar iron complexes are fully characterized.[18-20]

In the course of this study, we also investigated the reactions of α-hydroxyisopropyl radicals and methyl radicals with iron porphyrins and free-base porphyrin. The experiments have been performed with deuteroporphyrin IX derivatives.

Experimental Section

Materials. The preparation of ferrideuteroporphyrin IX chloride (deuterohemin) was described elsewhere.[14] Deuteroporphyrin dimethyl

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ester was purchased from Man-Win Coordination Chemicals. *p*-Nitro-acetophenone (Aldrich) was recrystallized from ethyl alcohol. Methyl iodide (Matheson) was purified on alumina and kept in the dark. All other chemicals (Baker Analyzed Reagents) were used without further purification. Nitrogen (<1 ppm O_2) and methyl chloride were purchased from Linde. Water was obtained from a Millipore Milli-Q filtering system.

Deuterohemin solutions in phosphate buffer–2-propanol mixtures were prepared as described elsewhere.[14] Alkaline solutions of deuterohemin were prepared by first dissolving the porphyrin in 0.1 N NaOH and then adding 2-propanol (50%). The reverse procedure was used for free-base deuteroporphyrin IX dimethyl ester solutions. Ferrideuteroporphyrin, ferrodeuteroporphyrin, and deuteroporphyrin dimethyl ester will be abbreviated as DPFe[III], DPFe[II], and DP, respectively.

Measurements. Steady-state γ-radiolysis experiments were performed by using a cobalt source (Gammacell 220) with a dose rate of 5.0 krad/min. Solutions in quartz optical cells (3-mm optical path length) were bubbled with N_2 or CH_3Cl. Changes in optical absorption were followed with a Cary 219 spectrophotometer.

Redox titrations were spectrophotometrically performed by adding micro amounts of a stock solution of sodium dithionite to the N_2-bubbled porphyrin solution. The dithionite solution was standardized by reduction of N_2 or CO-bubbled neutral deuterohemin solutions. The one-electron reduction product was either the readily oxidizable ferrous porphyrin or its more stable carbonyl adduct. A small discrepancy between the two standardizations is accounted for by minor oxygen contaminations. In the experiments described below, we retained the mean as the more realisitic value owing to the fact that the air sensitivity of the product (DPFe[III]CH_3) lies between those of the two standards.

Kinetic spectrophotometric measurements were made with the computer-controlled pulse radiolysis apparatus described previously.[21] The linear accelerator supplies 5–50-ns pulses of 7-MeV electrons. One-centimeter cells were used throughout this study. The dose was calibrated with a thiocyanate solution and corrected for a 10% decrease in solvated electron yield (experimentally determined from the e_{aq}^- absorption[14]) in 50% 2-propanol mixtures. The dose ranged between 0.15 and 1.7 krad/pulse. The solutions were bubbled with N_2 or CH_3Cl. In the case of the volatile CH_3I, the solution was bubbled with N_2 prior to the addition of CH_3I. The latter was deoxygenated in a separate container and was added to the solution under anaerobic conditions. The solutions were flowed continuously through the irradiation cell to assure a fresh sample for each pulse. In order to suppress photolysis, we did not pulse the xenon lamp and we used cutoff filters to avoid irradiation in the Soret region.

Transient absorbance data are presented in the dimension of an extinction coefficient by the parameter $\Delta\epsilon'$ given by $\Delta\epsilon' = AK/(DG)$ where A is the difference in absorbance after and before the pulse, D the dose supplied by the pulse, G the radiation yield, and K a calibration factor chosen such that $\Delta\epsilon'$ for the thiocyanate radical $(SCN)_2^-$ is 7600 M^{-1} cm^{-1} at 480 nm in N_2O-saturated solutions.

As reported elsewhere,[14] monomerization of deuterohemin can be achieved by addition of 50% 2-propanol to aqueous solutions. We will use this solvent mixture throughout this study and simply refer to solutions of porphyrin in phosphate buffer (6.2×10^{-3} M, pH 7) containing 50% 2-propanol (6.5 M) and solutions in 0.1 N sodium hydroxide containing 50% 2-propanol as neutral and alkaline solutions, respectively.

Concepts of the Radiolysis Experiments

Scavenging of Primary Species (OH·, H·, e_{aq}^-). In the above mentioned mixtures, 2-propanol scavenges the OH· radicals[22] and H· atoms[23] within a few nanoseconds leading to the reducing α-hydroxyisopropyl radical (($CH_3)_2\dot{C}OH$). This radical deprotonates in alkaline solutions ($pK_a = 12.2$) to form the more strongly reducing radical $(CH_3)_2\dot{C}O^-$.[24]

The hydrated electron e_{aq}^- can be scavenged by various solutes chosen according to the reaction to be investigated. In the presence of methyl chloride or methyl iodide, methyl radicals are produced according to the general reaction[25]
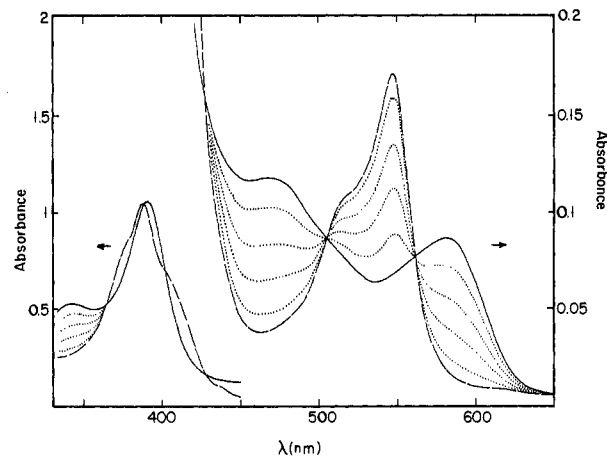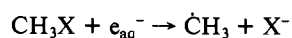
$$CH_3X + e_{aq}^- \rightarrow \dot{C}H_3 + X^-$$

Figure 1. Spectral evolution upon steady-state irradiation of methyl chloride saturated neutral solution of deuterohemin (4×10^{-5} M): —, deuterohemin; – – –, 180-s irradiation (major species DPFe[III]CH_3); ⋯, intermediate irradiation times (36-s irradiation between each spectrum).

Acetone forms the α-hydroxyisopropyl radicals[25] which, ipso facto, are the only radicals resulting from irradiation.

$$(CH_3)_2CO + e_{aq}^- \rightarrow (CH_3)_2\dot{C}O^-$$

Radiolysis of neat 2-propanol produces solvated electrons and α-hydroxyisopropyl radicals, so that the above concepts remain applicable even for the solutions containing 50% 2-propanol.

Scavenging of Secondary Species. Reaction of α-Hydroxyisopropyl Radicals with Methyl Iodide. Deprotonated α-hydroxyisopropyl radicals were found to react with methyl iodide according to eq 1. This reaction was investigated in alkaline solutions (0.1

$$(CH_3)_2\dot{C}O^- + CH_3I \rightarrow (CH_3)_2CO + \dot{C}H_3 + I^- \quad (1)$$

N NaOH) containing 2-propanol (6.5 M) and acetone (0.68 M) by competition kinetics with *p*-nitroacetophenone (5×10^{-5} M) as the reference solute.[26,27] The yield of the *p*-nitroacetophenone anion radical, as measured by the typical absorbance around 355 nm, was found to decrease with increasing concentrations of methyl iodide (2×10^{-4}–5×10^{-3} M). As expected, the plot of the ratio of absorbances obtained in the absence and in the presence of methyl iodide vs. the methyl iodide concentration showed good linearity with an intercept near unity (intercept = 1.03, slope = 960, correlation coefficient = 0.9999). From the slope we obtained $k_1 = (1.1 \pm 0.1) \times 10^8$ M^{-1} s^{-1}.

Therefore, in alkaline solutions containing 2-propanol and methyl iodide (a few millimolar), methyl radicals are the only species present a few microseconds after the pulse.

On the other hand, no reaction was found to occur between methyl iodide and the neutral form of the α-hydroxyisopropyl radical at pH 7 ($k \leq 10^5$ M^{-1} s^{-1}).

Self-Reaction of Radical Species. Owing to the low doses used in this study, except where otherwise stated, self-reactions of α-hydroxyisopropyl[28] or methyl radicals[29] were negligible compared with their scavenging by the added solutes.

Results and Discussion

Reduction of Deuterohemin by α-Hydroxyisopropyl Radicals. The reduction of deuterohemin by α-hydroxyisopropyl radicals in their neutral form

$$DPFe[III] + (CH_3)_2\dot{C}OH \rightarrow DPFe[II] + (CH_3)_2CO + H^+ \quad (2)$$

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Table I. Reaction Rate Constants

reaction no.	soln[a]	reactants	products[b]	k, M^{-1} s^{-1}
1	alkaline	$(CH_3)_2\dot{C}O^- + CH_3I$	$\cdot CH_3$	$(1.1 \pm 0.1) \times 10^8$
2	neutral	$(CH_3)_2\dot{C}OH + DPFe^{III}$	$DPFe^{II}$	$(3.7 \pm 0.4) \times 10^8$ [c]
3	alkaline	$(CH_3)_2\dot{C}O^- + DPFe^{III}$	$DPFe^{II}$	$(9 \pm 1) \times 10^8$
6	neutral	$(CH_3)_2\dot{C}OH + DPFe^{II}$	?	$(6.3 \pm 0.5) \times 10^8$
	alkaline	$(CH_3)_2\dot{C}O^- + DPFe^{II}$	$DPFe^I$ or anion radical	$(6.3 \pm 0.3) \times 10^8$ [d]
10	alkaline	$(CH_3)_2\dot{C}O^- + DP$	DP^-	$(6 \pm 1) \times 10^8$
4	alkaline	$\cdot CH_3 + DPFe^{III}$	$DPFe^{IV}CH_3$	$(2.3 \pm 0.5) \times 10^9$
	neutral	$\cdot CH_3 + DPFe^{III}$	$DPFe^{IV}CH_3$	$\simeq 1.3 \times 10^9$
7	neutral	$\cdot CH_3 + DPFe^{II}$	$DPFe^{III}CH_3$	$(3.9 \pm 0.5) \times 10^9$
11	alkaline	$\cdot CH_3 + DP$	?	$(1-2) \times 10^7$
5	alkaline	$DPFe^{IV}CH_3 + OH^-$ (?)	$DPFe^{II} + CH_3OH$ (?)	$t_{1/2} \simeq 200\ \mu s$
8	neutral	$DPFe^{IV}CH_3 + DPFe^{II}$	$DPFe^{III}CH_3 + DPFe^{III}$	$(4 \pm 1) \times 10^8$

[a] 1:1 water:2-propanol mixtures with either pH 7 phosphate buffer or 0.1 N NaOH. [b] Only the most interesting products are listed. [c] Reference 14. [d] Reference 31.

has been reported previously.[14] Similar results have been observed with the deprotonated radicals in alkaline solutions:

$$DPFe^{III} + (CH_3)_2\dot{C}O^- \rightarrow DPFe^{II} + (CH_3)_2CO \qquad (3)$$

The comparison of spectra recorded after pulse or continuous radiolysis of deuterohemin solutions with those obtained after chemical reduction of the ferric species using dithionite indicated that the radiolytic method is very clean. Moreover, no further evolution of the ferrous form was observed.

The reaction rate constants k_2 and k_3 are given in Table I.

Evidence for Methyl–Iron–Porphyrin Complexes. γ-Radiolysis of Neutral Solutions of Deuterohemin in the Presence of Methyl Chloride or Methyl Iodide. As shown in Figure 1, γ-irradiation of methyl chloride saturated neutral solutions of deuterohemin results in drastic spectral changes clearly different from those expected from a simple reduction of ferrideuteroporphyrin to the ferrous form. Similar results were obtained with nitrogen-bubbled solutions containing methyl iodide (8×10^{-2} M).[30] Clean isobestic points were observed at 364, 389, 402, 428, 504, and 561 nm. Absorbance changes show linear dependence on the irradiation time. The reaction yield was found to be $G \simeq 3$. Little changes were observed upon further irradiation for a few minutes.

The final product is fairly stable under anaerobic conditions but is oxidized within a few seconds in air leading back to the initial ferric form. It is characterized by peaks at 388 nm ($\epsilon = 92\,000$ M^{-1} cm^{-1}) and 547 nm (16 000) and by shoulders at 378 nm (72 000) and 517 nm (9200).

As pointed out in the experimental section, γ-irradiation of methyl chloride or methyl iodide solutions yields α-hydroxyisopropyl radicals and methyl radicals with similar yields ($G \simeq 3$). As α-hydroxyisopropyl radicals are expected to reduce deuterohemin to the ferrous form and to be inert toward methyl chloride and methyl iodide in neutral solutions, the final product of irradiation comes from the reaction of $DPFe^{II}$ with $\cdot CH_3$. Following the usual conventions, it will be tentatively formulated as $DPFe^{III}CH_3$ where CH_3 stands for the methyl anion.

Chemical Preparation of $DPFe^{III}CH_3$. Chemical preparation and characterization of the $DPFe^{III}CH_3$ species was undertaken to ascertain the proposed structure. We found that ferrous deuteroporphyrin, prepared either by γ-irradiation or chemically by using sodium dithionite, reacts with methyl iodide. Using excess reducing agent leads to the spectrum of the pure $DPFe^{III}CH_3$ species without any contribution from the characteristic absorbance of ferrous deuteroporphyrin. With methyl iodide concentrations as large as 0.2 M, the reaction was found to proceed within a few minutes.

Spectrophotometric redox titration of neutral or alkaline solutions of deuterohemin (10^{-4} M) in the presence of methyl iodide by sodium dithionite leads to a set of spectra very similar to that obtained in γ-radiolysis experiments.[30] As shown in Figure 2, the
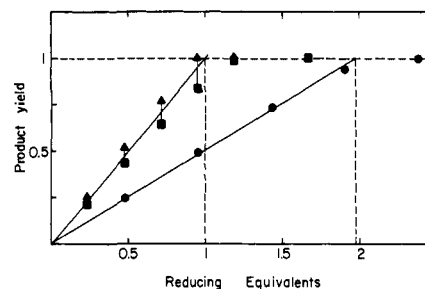

Figure 2. Redox titration of neutral solutions of deuterohemin (10^{-4} M): ▲ and ■, standardizations using CO and N_2-bubbled solutions, respectively; ●, N_2-bubbled solution containing methyl iodide (0.2 M).

formation of the final product requires 2 electrons (experimental $= 2 \pm 0.1$ reducing equivalents). Not withstanding the mechanism, this result corroborates the formula $DPFe^{III}CH_3$, methyl iodide being formally reduced to iodide and methyl anion. Unfortunately, owing to rather low solubility of the porphyrin in water–alcohol mixture we were unable to characterize this species by means of NMR spectroscopy. It should be noted that attempts to obtain NMR spectra of similar alkyl–iron complexes were unsuccessful.[20]

Evidence for the Reaction of Ferrideuteroporphyrin with Methyl Radicals. γ-Radiolysis. This reaction was investigated by irradiating alkaline solutions of deuterohemin (4×10^{-5} M) containing methyl iodide (3.9×10^{-3} M). Under these conditions the concentration of ferrous porphyrin formed via reaction 3 represents less than 5% of the concentration of methyl radical which is the major species (see Experimental Section). Nevertheless, γ-irradiation was found to produce spectral changes identical with those obtained with neutral solutions of deuterohemin containing methyl iodide.[30] The reaction yield was found to be $G \simeq 2.4$ which is likely an underestimated value since it decreased with increasing methyl iodide concentrations: the yield was found to be 1.6 with 1.9×10^{-2} M methyl iodide. At higher concentrations (0.8 M) featureless spectra were obtained, indicating that methyl iodide further reacts with transient porphyrin species. (see also pulse radiolysis experiments described below). Therefore, the yield of the main reaction is believed to be near $G \simeq 3$. These results clearly show that methyl radicals react with ferrideuteroporphyrin. As the reaction product is the two-electron reduced species $DPFe^{III}CH_3$, the reaction mechanism must involve redox steps leading to a ferrous species. Pulse radiolysis experiments will shed some light on these processes.

Pulse Radiolysis Study. Optical changes following irradiation of nitrogen-saturated alkaline solutions of deuterohemin ($0.5-1 \times 10^{-4}$ M) containing methyl iodide (4.1×10^{-3} M) indicate that two distinct processes occur after the pulse. The faster one obeyed pseudo-first-order kinetics which strongly suggests the reaction

$$DPFe^{III} + \dot{C}H_3 \rightarrow DPFe^{IV}CH_3 \qquad (4)$$

where CH_3 formally stands for the methyl anion. The reaction rate constant k_4 is given in Table I. The transient differential absorption spectrum recorded after the completion of this step

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(30) In the case of methyl chloride saturated solutions, unresolved shoulders are seen around 410 and 435 nm (see Figure 1). This suggests that the yield of $DPFe^{II}$ is somewhat higher than that of $\cdot CH_3$ and an excess of ~10% remains unreacted. When the experiment is carried out with CH_3I which reacts with $DPFe^{II}$ (see text), the shoulders are absent.
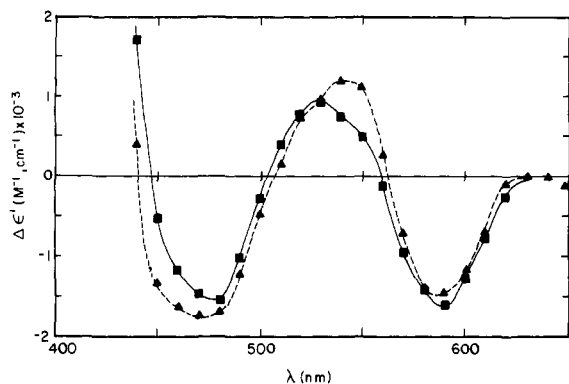
Figure 3. Transient differential absorption spectra recorded after pulse irradiation of N_2-bubbled alkaline solutions of deuterohemin (10^{-4} M) containing methyl iodide (4.1×10^{-3} M) (dose = 0.7 krad; assumed yield $G = 6$). Time elapsed after the pulse: ■, 30 μs; ▲, 400 μs.

(30 μs after the pulse) shows the disappearance of the typical deuterohemin absorption around 590 and 480 nm and appearance of the $DPFe^{IV}CH_3$ species with a characteristic absorption around 525 nm (Figure 3). However, as the differential molar extinction coefficients are quite low, the absolute spectrum of the new species must be rather broad.

As depicted in Figure 3, $DPFe^{IV}CH_3$ undergoes further reaction characterized by absorbance increase around 540–550 nm. Taking into account both the γ and the pulse radiolysis experiments, it is possible to attribute this step to the evolution of a ferrous form from $DPFe^{IV}CH_3$. A possible mechanism could be alkaline hydrolysis according to eq 5. The ferrous porphyrin formed could

$$DPFe^{IV}CH_3 + OH^- \rightarrow DPFe^{II} + CH_3OH \qquad (5)$$

partially react with $DPFe^{IV}CH_3$ leading to $DPFe^{III}CH_3$ (see below). This process could account for the transient spectrum recorded 400 μs after the pulse which slightly differs from that expected if only reaction 5 occurs.

Owing to the small absorbance changes accompanying this slow step, only an estimate of the first half-life of the reaction, $t_{1/2} \simeq$ 200 μs, has been obtained.

In agreement with the γ-radiolysis experiments, in the presence of higher concentrations of methyl iodide (0.2–0.5 M) $DPFe^{IV}CH_3$ quickly decays to an unidentified product.

Routes to the $DPFe^{III}CH_3$ Species. Pulse Radiolysis of Ferrous Porphyrin Solutions: Evidences for the Scavenging of Methyl Radicals by $DPFe^{II}$. This reaction cannot be studied by using alkaline solutions of methyl iodide because this compound reacts with $DPFe^{II}$ at room temperature. As expected, methyl chloride was found to be inert toward ferrous porphyrin but it is unable to scavenge the α-hydroxyisopropyl radicals. Therefore, preliminary experiments were carried out to investigate the reaction of α-hydroxyisopropyl radicals with ferrous porphyrins.

Typically, nitrogen-bubbled neutral solutions of ferrous deuteroporphyrin (2.2×10^{-5}–10^{-4} M) were prepared by chemical reduction using sodium dithionite (2×10^{-4} M). Acetone (0.7 M) was added in order to scavenge the solvated electrons. Following the pulse, fast absorbance changes, very similar to those reported in Figure 4a, were observed over 100 μs. They were found to obey first-order kinetics with a reaction rate constant $k_6 = (6.3 \pm 0.5) \times 10^8$ M^{-1} s^{-1}. They are attributed to the reaction

$$DPFe^{II} + (CH_3)_2\dot{C}OH \rightarrow product \qquad (6)$$
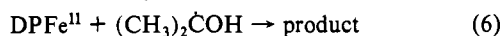
The product was characterized by sharp absorption at 545 nm and a broad one around 450–500 nm. Over 10 ms, it was found to undergo further reactions which do not change the main features of its spectrum. This behavior is clearly different from that observed in alkaline solutions. In this case, ferrous porphyrins are reduced by $(CH_3)_2CO^-$ to iron(I) or anion radical species which are oxidized back to the ferrous form.[31] Characterization of the

(31) Brault, D.; Santus, R.; Land, E. J.; Swallow, A. J., submitted for publication.
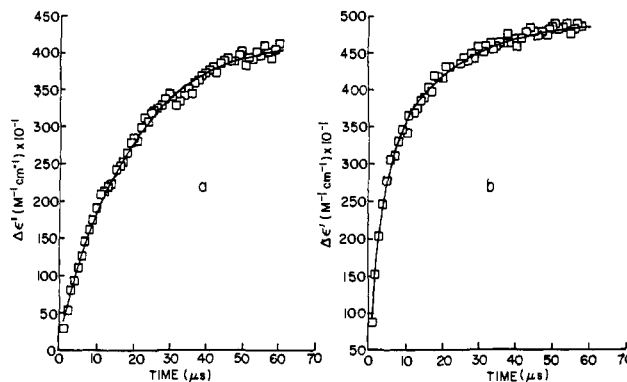

Figure 4. Optical changes following pulse irradiation of chemically reduced ferrous deuteroporphyrin dissolved in methyl chloride saturated neutral solution (sodium dithionite = 2×10^{-4} M; dose = 1 krad). a: $[DPFe^{II}] = 9 \times 10^{-5}$ M, [acetone] = 1.3 M (□, experimental points; —, one-exponential fit with $k_{app} = 5.3 \times 10^4$ s^{-1}) (similar results were obtained with N_2-bubbled solutions). b: $[DPFe^{II}] = 10^{-4}$ M; no acetone (□, experimental points; —, two-exponential fit with $k_{1_{app}} = 3.8 \times 10^5$ s^{-1} and $k_{2_{app}} = 5.9 \times 10^4$ s^{-1}).
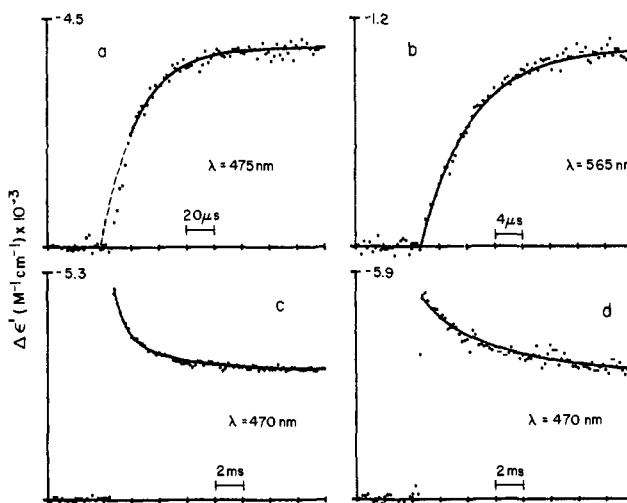

Figure 5. Optical changes following pulse irradiation of methyl chloride saturated neutral solutions of deuterohemin (10^{-4} M) (●, experimental; —, theoretical fit). a: dose = 1 krad; pseudo-first-order fit with $k_{app} = 3.9 \times 10^4$ s^{-1}. b: dose = 1 krad, pseudo-first-order fit with $k_{app} = 1.3 \times 10^5$ s^{-1}. c: dose = 1.1 krad, second-order fit with $k = 3 \times 10^8$ M^{-1} s^{-1}. d: dose = 0.15 krad; second-order fit with $k = 4.3 \times 10^8$ M^{-1} s^{-1}.

product obtained in neutral solutions is beyond the scope of this study, and we will keep in mind only the fast initial absorbance changes.

When the same experiment is performed with methyl chloride saturated neutral solution of ferrous deuteroporphyrin ((1–2) × 10^{-4} M) the initial absorbance changes appeared to be a two exponential process as shown in Figure 4b. Both steps are characterized as pseudo-first-order reaction since they were linearly dependent on the porphyrin concentration. The reaction rate constants were found to be $(3.9 \pm 0.5) \times 10^9$ and $(5.9 \pm 0.5) \times 10^8$ M^{-1} s^{-1}, respectively. When excess acetone (1.3 M, [acetone] $\gg$ [CH_3Cl]) is added, only the slower process was observed (see Figure 4a). Obviously, it corresponds to reaction 6. Thus, the faster step is attributed to the scavenging of methyl radicals by ferrous deuteroporphyrin according to eq 7. Spectral changes

$$DPFe^{II} + \dot{C}H_3 \rightarrow DPFe^{III}CH_3 \qquad (7)$$

accompanying the faster step corroborate this interpretation, but owing to the intermingling of reactions 6 and 7, detailed spectral analysis and study of possible further reactions were made impossible.

Pulse Radiolysis of Methyl Chloride Saturated Solutions of Deuterohemin. Evidence for the Reaction between $DPFe^{IV}CH_3$
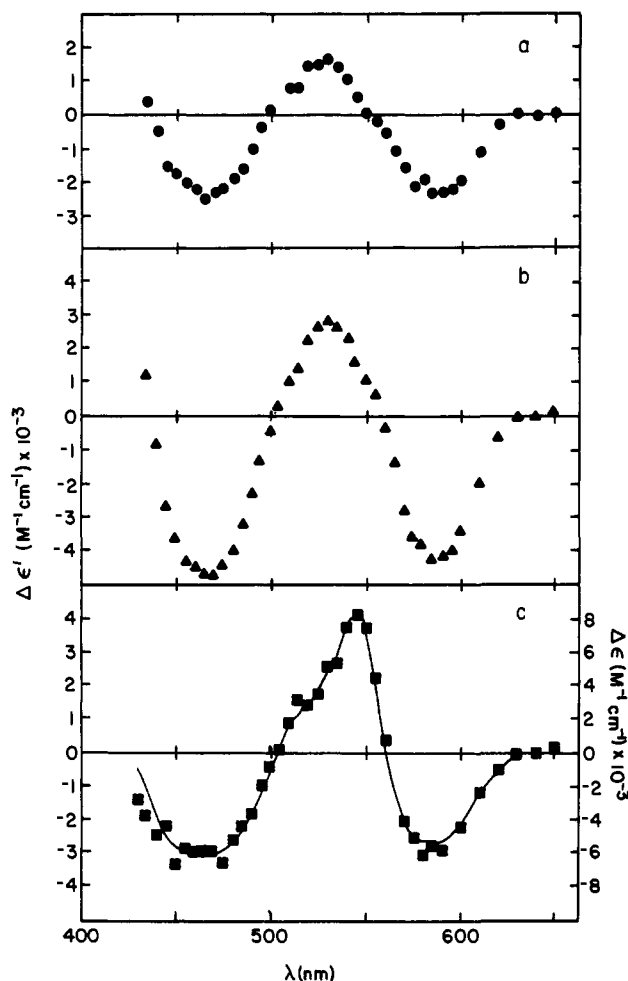
Figure 6. Transient differential absorption spectra recorded after pulse irradiation of methyl chloride bubbled neutral solution of deuterohemin (10^{-4} M; dose = 1.1 krad). Time elapsed after the pulse: a (●), 13 μs; b (▲), 140 μs; c (■), 7.3 ms. Full line: differential absorption spectrum of DPFe^{III}CH$_3$ minus deuterohemin as obtained from γ-radiolysis experiments. In Figures 6a,b, the assumed yield being $G = 6$, the values of $\Delta\epsilon'$ are correct as far as the disappearance of the typical absorption bands of deuterohemin ($\lambda \simeq 590$ and 470 nm) are concerned. For the sake of spectral comparison, the same yield was assumed in Figure 6c, but as the final product DPFe^{III}CH$_3$ comes from the reaction of DPFe^{II} and DPFe^{IV}CH$_3$ species formed with yields $G \simeq 3$, the $\Delta\epsilon'$ values have to be multiplied by a factor of 2. Thus, pulse and steady-state radiolysis experiments compare very well.

and DPFe^{II}. As shown in Figure 5, optical changes following pulse irradiation of methyl chloride saturated neutral solutions of deuterohemin (10^{-4} M) are indicative of three distinct steps. Two steps take place on a 20-μs scale (see Figure 5a). The slower one obeys pseudo-first-order kinetics with a reaction rate constant of $k = 3.9 \times 10^8$ M^{-1} s^{-1} which allow us to attribute it to the reduction of deuterohemin by α-hydroxyisopropyl radicals (reaction 2).

Monitoring optical changes at an isosbestic point between deuterohemin and ferrous porphyrin (565 nm) makes it possible to study the faster step without interference by the slower one (see Figure 5b). It was found to obey pseudo-first-order kinetics with a reaction rate constant $k \simeq 1.3 \times 10^9$ M^{-1} s^{-1}. According to the results presented in previous sections, it is attributed to the reaction of deuterohemin with methyl radicals.

The transient differential absorption spectra recorded during and after the completion of the two first processes corroborate these assignments (see Figure 6a,b). Because the reactions slightly overlap, the spectra are not those of pure species. However, the spectrum taken at 13 μs (i.e., when essentially the first step has taken place) agrees fairly well with that reported in Figure 3 (30 μs after the pulse). Afterward, the major process is the reduction of deuterohemin to ferrous deuteroporphyrin as shown (Figure

6b) by the typical shifts of isosbestic points from 497 and 551 to 502 and 558 nm, respectively (for a differential spectrum of ferrous porphyrin minus deuterohemin in the same solvent see ref 14). The spectrum recorded 140 μs after the pulse is therefore that of a mixture of ferrous porphyrin and DPFe^{IV}CH$_3$. Its intensity and shape suggest that the DPFe^{IV}CH$_3$ spectrum is somewhat stronger in neutral solutions.

As shown in Figure 5c, slow spectral changes take place afterward. This step is characterized as a second-order process by its dependence on the dose[32] (see Figure 5d). No further changes were observed over 20 ms. The differential spectrum recorded at the end of this process (see Figure 6c) shows buildup of absorption around 545 nm as well as around 480 and 590 nm which are characteristic of DPFe^{III}CH$_3$ and deuterohemin formation, respectively. Furthermore, this spectrum is identical with that of DPFe^{III}CH$_3$ minus deuterohemin as obtained from the γ-radiolysis experiments. This step is, therefore, attributed to the reaction between DPFe^{IV}CH$_3$ and DPFe^{II} leading to DPFe^{III}CH$_3$ and deuterohemin:

$$DPFe^{IV}CH_3 + DPFe^{II} \rightarrow DPFe^{III}CH_3 + DPFe^{III} \qquad (8)$$

The reaction rate constant k_8 is given in Table I. This reaction could be either an electron transfer or a methyl group exchange between the two porphyrin species. Preliminary experiments have shown that electron transfer between porphyrins takes place with a rate constant of $\simeq 10^8$ M^{-1} s^{-1} which is in favor of the former hypothesis.

In summary, pulse radiolysis experiments have shown that, in methyl chloride saturated neutral solutions, deuterohemin scavenges α-hydroxyisopropyl and methyl radicals (reactions 2 and 4). Then, the products undergo intermolecular electron transfer (reaction 8). The net reaction

$$DPFe^{III} + (CH_3)_2\dot{C}OH + \dot{C}H_3 \rightarrow$$
$$DPFe^{III}CH_3 + (CH_3)_2CO + H^+ \qquad (9)$$
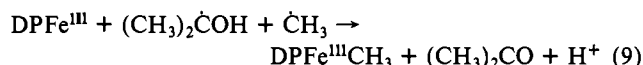
accounts for the γ-radiolysis results.

Pulse radiolysis of methyl chloride saturated alkaline solutions of deuterohemin (10^{-4} M) was also investigated. But, in this case the final product was found to be ferrous deuteroporphyrin. The transient spectra recorded at different times suggest that, although deuterohemin reacts with α-hydroxyisopropyl and methyl radicals, the DPFe^{IV}CH$_3$ hydrolysis is faster than in neutral solution in agreement with reaction 5. As a matter of fact, although γ-irradiation of such solutions leads to DPFe^{III}CH$_3$, deviation from isosbestic points during the first irradiation times strongly suggests that ferrous porphyrin accumulates. Then, DPFe^{III}CH$_3$ is formed via reaction 8 which becomes fast enough.

To conclude, we have characterized new species resulting from the reaction of methyl radicals with ferrous and ferric porphyrins. In order to determine whether methyl radicals attack the iron center or the porphyrin ligand, we examined the reaction of these radicals with iron-free deuteroporphyrin.

Pulse Radiolysis of Alkaline Solutions of Deuteroporphyrin Dimethyl Ester. Before dealing with the reaction of methyl radicals, we first report on the reaction of α-hydroxyisopropyl radicals with deuteroporphyrin dimethyl ester (10^{-4} M) in 0.1 N NaOH solutions containing 2-propanol (6.5 M) and acetone (0.7 M). As shown in Figure 7a, optical changes following the pulse obey pseduo-first-order kinetics. The transient differential spectrum (Figure 8) displays a large buildup of absorption around 635 nm as well as decrease of absorption of the starting material (494, 527, 564, and 618 nm). In agreement with results published elsewhere[33] this behavior strongly suggests the reduction of the porphyrin to an anion radical according to eq 10. The reaction

$$DP + (CH_3)_2\dot{C}O^- \rightarrow D\dot{P}^- + (CH_3)_2CO \qquad (10)$$

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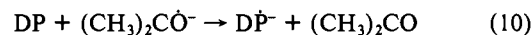
(32) Owing to the small differences between the yields in DPFe^{II} and DPFe^{IV}CH$_3$, the reaction rate constant k_8 does not rigorously depend on the dose.

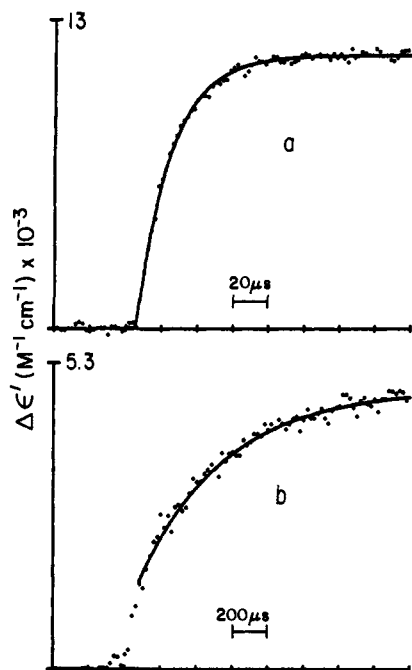(33) Neta, P.; Scherz, A.; Levanon, H. *J. Am. Chem. Soc.* **1979,** *101,* 3624–3629.

Figure 7. Optical changes following pulse irradiation of N_2-bubbled deuteroporphyrin dimethyl ester (10^{-4} M) alkaline solutions containing acetone (0.7 M; λ = 640 nm): a, [CH_3I] = 0, dose = 0.9 krad; b, [CH_3I] = 2.3 × 10^{-2} M, dose = 0.15 krad.
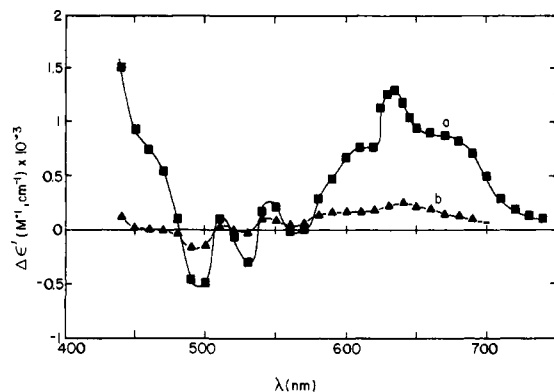

Figure 8. Transient differential absorption spectra recorded after pulse irradiation of N_2-bubbled alkaline solution of deuteroporphyrin dimethyl ester (10^{-4} M) containing acetone (0.7 M; dose = 0.8 krad). a (■): [CH_3I] = 0; time elasped after the pulse = 140 μs. b (▲): [CH_3I] = 2.3 × 10^{-2} M; time elasped after the pulse = 700 μs (spectrum b is not corrected for methyl radical loss due to self-reaction—see text).

rate constant k_{10} is given in Table I. The radical anion was found to decay with a first half-life $t_{1/2} \simeq$ 2 ms.

In order to study the reaction of methyl radicals, we added methyl iodide (2.3 × 10^{-2} M) to the former solution. As expected from reaction 1, about 97% of α-hydroxyisopropyl radicals are converted to methyl radicals. Accordingly, the optical changes reported in Figure 7b drastically differ from those observed in the absence of CH_3I. A small initial fast absorbance increase accounts for the remaining α-hydroxyisopropyl radicals reacting directly with DP. This is followed by a slower step which suggests that methyl radicals react with the prophyrin according to eq 11.

$$DP + \dot{C}H_3 \rightarrow products \qquad (11)$$
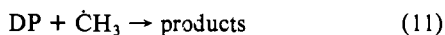
Owing to the slowness of the reaction, self-reaction of methyl radicals[29] efficiently competes with their scavenging by the porphyrin, even when low doses are used. As about 60% of the methyl radicals react with each other under the conditions of Figure 7b, plotting absorbance changes to fit pseudo-first-order kinetics is
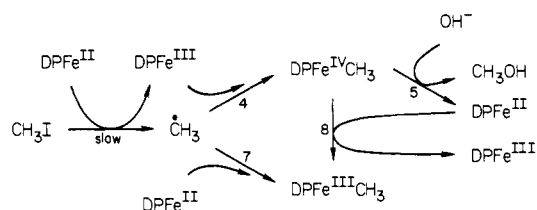
only an approximation. The reaction rate constant thus found is overestimated. The actual value is believed to be (1–2) × 10^7 M^{-1} s^{-1}. This rate constant is at least 2 orders of magnitude lower than that of the ferrous and ferric deuteroporphyrin. We, therefore, conclude that methyl radicals react with iron porphyrins at the iron center. This conclusion also agrees with the oxidation of $DPFe^{III}CH_3$ by air leading back to the initial ferric species (see above), a result inconsistent with attack of the porphyrin ring by methyl radicals.
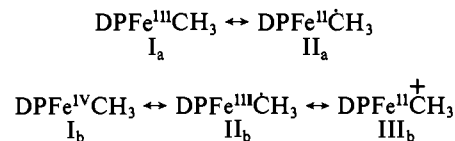
Conclusion

The reaction of methyl radicals with iron porphyrins and the free-base porphyrin, as well as various reactions necessary to completely describe the systems, were investigated. The various reaction rate constants are given in Table I. Particular attention is given to the reactions leading to the methyl–iron–porphyrin complexes. They can be summarized according to Scheme I. In fact, scavenging of methyl radicals by ferric porphyrin (reaction 4) is much faster than reaction of ferrous porphyrin with methyl iodide. Therefore, methyl radicals are not likely to be released in solution, and this reaction sequence can be better written as eq 12. This result has important bearings on the hypothesis of

$$DPFe^{II} + CH_3I \rightarrow DPFe^{IV}CH_3 + I^- \qquad (12)$$
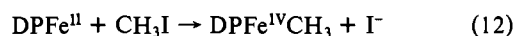
the radical mediated toxicity of halogenated compounds which is based on the release of radicals from the porphyrin environment.[3,4] As the $\cdot CCl_3$ radicals do not react rapidly with ferriporphyrin ($k_{4(\cdot CCl_3)} < 10^6$ M^{-1} s^{-1}),[14] this hypothesis remains sound, but we feel that it no longer applies to less electrophilic radicals such as methyl. Further work is in progress to screen a wider range of radicals with various electrophilic properties including radicals derived from anesthetics such as halothane. The results presented in this paper already suggest that it could be worth thinking about other ways to explain the toxicity of some halogenated compounds. One of them could be the formation of reactive intermediates during further reactions of σ-bonded alkyl or carbene complexes of iron porphyrins. As a matter of fact, it has been pointed out[10] that although the oxidation of dichlorocarbene complexes of ferrous porphyrins into ferric porphyrins and carbon dioxide could be a pathway in the detoxification, this process could also lead to some reactive transient species.[10]

From a chemical point of view, $DPFe^{III}CH_3$ and $DPFe^{IV}CH_3$ represent new examples of unusual σ-bonded alkyl–iron complexes. Although we used the usual convention regarding the methyl ligand as an anion, these species obviously present mesomeric forms

$$\underset{I_a}{DPFe^{III}CH_3} \leftrightarrow \underset{II_a}{DPFe^{II}\dot{C}H_3}$$

$$\underset{I_b}{DPFe^{IV}CH_3} \leftrightarrow \underset{II_b}{DPFe^{III}\dot{C}H_3} \leftrightarrow \underset{III_b}{DPFe^{II}\overset{+}{C}H_3}$$

It even appears that forms I_a and I_b are limited forms far from the actual structures. Indeed, $DPFe^{III}CH_3$ does not present the usual properties of ferric porphyrins such as counteranion exchange (it is stable in alkaline solutions), but it behaves as a ferrous porphyrin with regard to oxidation by oxygen. In the same way, $DPFe^{IV}CH_3$ hydrolyses leading to $DPFe^{II}$ which suggests that this species could be represented to some extent by form III_b. We hope that further studies will make clear the actual structure of these new complexes in terms of electron distribution.