Deuterium Exchange Reactions of Oxoporphyrin Compounds

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The rate and specificity of deuterium incorporation by oxoporphyrin compounds were examined in a number of reaction media. As a preliminary to this investigation, meso-proton regions of oxoporphyrin ^1H NMR spectra were assigned by use of the lanthanide shift reagent Eu(fod), which was shown to bind preferentially at the oxoporphyrin β-carbonyl groups. Deuterium exchange in TFA-d_1 was selective for the meso positions. The large range of rates of exchange permitted selective deuteriation of specific positions in these compounds. Specificity appears to be affected by the β-carbonyl groups. Unlike rates of exchange of porphyrins and hydroporphyrins, the rates of exchange of oxoporphyrin compounds did not correlate with the number of chemically modified pyrrole rings. The gross specificity of exchange for a given compound in D_2SO_4 was similar to that in TFA-d_1. Relative exchange rates of the compounds were reversed in this medium relative to TFA-d_1, however. A similar reversal of rates was observed in these media for octaethylhydroporphyrins relative to octaethylporphyrin. Reaction in media that selectively deuteriate ring-adjacent methylene and methyl groups in β-alkylated porphyrins resulted in deuteriation of analogous positions in nonoxidized pyrrole rings in the oxoporphyrins. Some meso deuteriation was always noted, however. Specific interactions of the tetrapyrroles and the acid and/or its conjugate base are important to the mechanisms of these reactions.

Deuterium exchange reactions of porphyrins provide both a means of studying the electronic structure and chemical reactivity of the porphyrin macrocycle and a means of preparing selectively deuteriated porphyrins. Deuterium-substituted compounds have found widespread application in physicochemical studies including vibrational, NMR, and ESR spectroscopy. The low-yield multistep syntheses of octaalkyl and natural porphyrin compounds often make a simple exchange process on preformed porphyrins the best route to these materials. Most exchange reactions are acid-catalyzed electrophilic deuteriations of the meso positions.[1-5] Natural porphyrin derivatives can also be deuteriated at vinyl and β sites.[6-8] Recently, acid-[9] and base-catalyzed[10,11] deuteriations of substituent methyl and methylene groups have been reported.

The relative reactivities of the sites within the porphyrin macrocycle generally have been taken as an indication of the electron density at these sites. The greatly increased reactivity in trifluoroacetic acid of meso positions adjacent to pyrroline rings in the hydroporphyrins *t*-H_2(OEC)[12] and H_2(OEiBC) relative to H_2(OEP) was explained in these terms.[2] Oxoporphyrins, a third class of tetrapyrrole, have been viewed as analogues of hydroporphyrins.[13] Iron compounds of both macrocycle types serve as prosthetic groups in nitrite reductases isolated from a wide range of organisms.[14-19] The proposed analogy between oxoporphyrins and hydroporphyrins was based upon the similarity of interruptions in the π systems and the resemblance of the UV-vis spectra of the two classes of macrocycles.[13] However, recent investigations have established that the redox potentials of oxoporphyrins are distinctly different from those of either porphyrins or hydroporphyrins.[20,21] These observations imply that oxoporphyrins have unique electronic structures and suggest that they should exhibit different deuterium exchange reactivity.

In this paper, we examine the deuterium exchange reactions of the free-base oxoporphyrin compounds H_2(**5**-**10**), Chart I.[22] The strong acid medium required for synthesis of these compounds and the low yield and nonspecificity of their formation[20,23,24] suggest that exchange with preformed oxoporphyrins will be the best way to prepare specifically deuteriated oxoporphyrin compounds for physicochemical investigations. To this end, we have examined a wide range of deuteriation reactions. The results that we report here confirm that the reactivity of oxoporphyrins is different from that of other tetrapyrroles, and they establish that reactivities are strongly affected by factors other than the basicity of the macrocycle or the relative electron densities at different sites.

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(12) Abbreviations: H_2(OEP), 2,3,7,8,12,13,17,18-octaethylporphyrin; H_2(OEC), 2,3-dihydro-2,3,7,8,12,13,17,18-octaethylporphyrin; H_2(OEiBC), 2,3,7,8-tetrahydro-2,3,7,8,12,13,17,18-octaethylporphyrin; H_2(**5**), 3,3,7,8,12,13,17,18-octaethyl-2-porphinone; H_2(**6**), 3,3,8,8,12,13,17,18-octaethyl-2,7-porphindione; H_2(**7**), 2,2,8,8,12,13,17,18-octaethyl-3,7-porphindione; H_2(**8**), 3,3,7,7,12,13,17,18-octaethyl-2,8-porphindione; H_2(**9**), 3,3,7,8,13,13,17,18-octaethyl-2,12-porphindione; H_2(**10**), 3,3,7,8,12,12,17,18-octaethyl-2,13-porphindione; Me_2SO, dimethyl sulfoxide; TFA, trifluoroacetic acid; TBAOH, tetrabutylammonium hydroxide.
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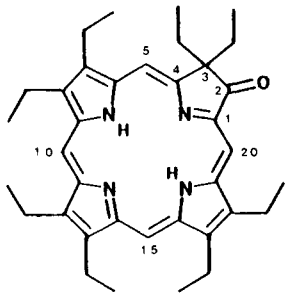
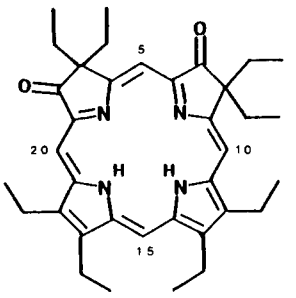
Chart I



$H_2(5)$



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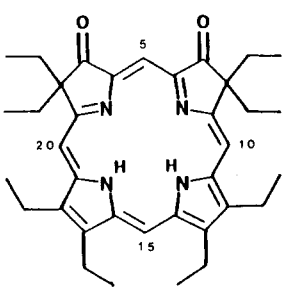
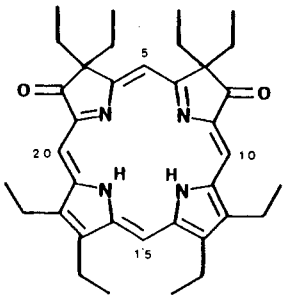


$H_2(7)$



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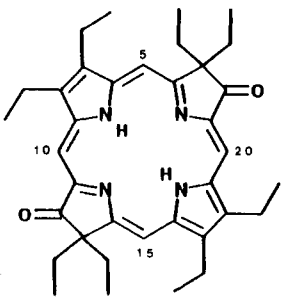
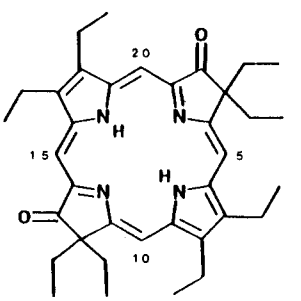


$H_2(9)$



$H_2(10)$

Experimental Section

Materials. Chloroform-d_1 (99.8 atom % D), 20% DCl in D_2O (99+ atom % D), D_2O (99.8 atom % D), Me_2SO-d_6 (99.9 atom % D), 98% sulfuric acid-d_2 in D_2O (99.5 atom % D), TFA-d_1 (99 atom % D), 1 M TBAOH in methanol (protio), and Resolve-Al Eu(fod) were obtained from Aldrich Chemical Co. Chloroform-d_1 was treated before use by passage down a (Merck) grade I basic alumina column. The initial runnings were discarded. Other deuteriated solvents were used as received. To minimize adsorption of water, glassware was dried in a 125 °C oven for at least 24 h and solvents were taken from ampules opened immediately prior to use. TFA-d_1 was stored for short terms under vacuum in a bulb equipped with a Kontes Teflon barrel valve. 1,2-Dichlorobenzene was stirred for 3 days with concentrated sulfuric acid, washed with water, dried with calcium chloride, refluxed, and then vacuum distilled from calcium hydride. All other reagents and solvents were reagent or HPLC grade and were used without further purification.

H_2(OEP) was prepared by literature procedure.[25] *t*-H_2(OEC)[26] and H_2(OEiBC)[27] were synthesized by reduction of H_2(OEP) according to literature methods. Oxidation of H_2(OEP) with H_2O_2 in concentrated sulfuric acid afforded the isomeric mono- and dioxooctaethylporphyrins **5**–**10**.[20,23] The quantity of H_2(**8**) isolated was insufficient for the compound to be included in this study.

Physical Measurements. Absorption spectra were recorded on a Perkin-Elmer Lambda 4C spectrophotometer. Proton and deuterium NMR spectra were determined on a Varian XL-300 spectrometer operating at 300 MHz and 46.044 MHz, respectively. Pulse sequences included delays adequate to permit accurate integration of peaks. Chemical shifts are reported relative to Me_4Si. The residual proton peak of TFA-d_1 (11.5 ppm) was used as a secondary reference. Me_4Si was not used in this solvent since it has been reported to slowly decompose under acidic conditions.[28]

Deuterium Exchange in TFA-d_1. Reactions of all compounds were run in sealed NMR tubes at 50 °C. Solid oxoporphyrin was placed in an NMR tube that was connected to an adapter on a high-vacuum line. Anhydrous TFA-d_1 was transferred to the NMR tube on the vacuum line. The resulting solution was freeze–thaw–degassed prior to the sealing of the tube under vacuum ($<1 \times 10^{-6}$ atm). The contents of the tube were not permitted to warm much above the melting point of the solvent during the brief thaw portion of the cycle. A typical tube contained a solution of 10 mg of oxoporphyrin compound in 1.0 mL of anhydrous TFA-d_1. Tubes were thawed immediately before they were placed in the cavity of the NMR spectrometer. The initial rapid phases of reactions were run entirely in the cavity, which was maintained at 50 °C. Tubes were placed in a 50 °C oil bath when the rate of change of the spectrum became small. The temperature of the bath was controlled by a solid-state relay activated by a mercury contact thermometer (±1 °C). At selected times, tubes were removed from the oil bath and the ^1H NMR spectrum was recorded. Tubes were wrapped in aluminum foil to prevent photochemically initiated exchange. Room lights did not appear to catalyze the exchange, however. Initial spectra established that very little if any exchange occurred during the preparation of samples.

The extent of deuteriation was measured by integration of the NMR spectrum. Methyl and/or methylene groups of geminal ethyl groups were used as primary internal integration standards. No measurable deuterium incorporation was detected at these positions by $^2H\{^1H\}$ NMR. In some cases, nonexchanging meso protons ($t_{1/2} > 1000$ h) were used as secondary internal standards to permit more accurate integrations of the more rapidly exchanging meso protons. Pseudo-first-order rate constants were obtained from nonweighted least-squares fits of the logarithms of integrated intensities vs. total elapsed time at 50 °C. Figure 1 shows a typical first-order rate plot for exchange of two of the meso protons of H_2(**5**). Plots were linear over 3 or more half-lives.

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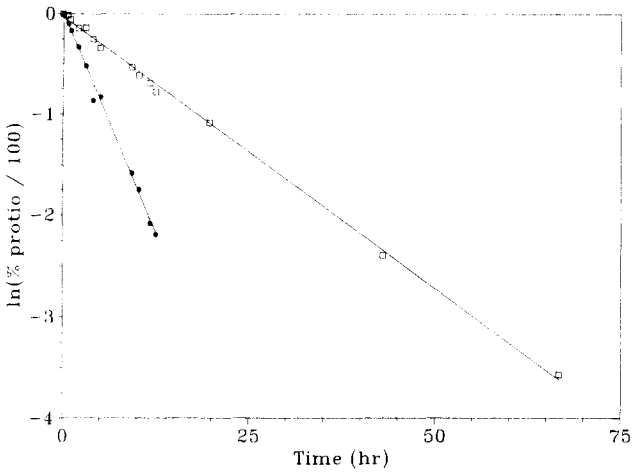

Figure 1. First-order rate plot for the deuterium exchange of the 5 (filled circles) and 20 (open squares) meso protons of H_2(5) in trifluoroacetic acid-d_1 at 50 °C.

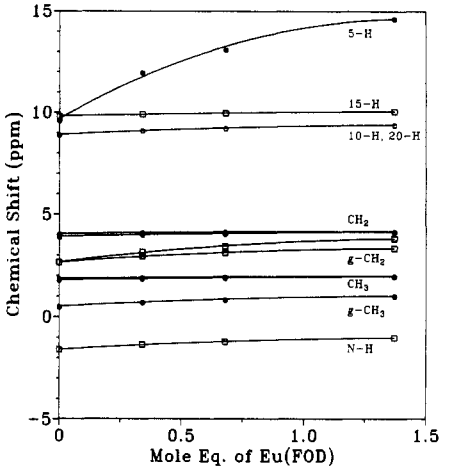

Figure 2. Lanthanide-induced shifts of the resonances of H_2(7) in the presence of Eu(fod). Assignments are indicated. A g- prefix specifies a group on the geminal ethyl groups of the pyrrolidinone rings.

Extensive series of replicate kinetic runs were not performed owing to the scarcity of the compounds and the long reaction times that are involved. Thus, a full statistical treatment of the error in half-lives or rate constants was not performed. We estimate that the errors in individual measurements are at least as large as those typically associated with integration data (several percent). Half-lives agreed to within 5–10% in those cases where duplicate runs were performed.

At the conclusion of the exchange reaction, each tube was opened and the contents were added to CH_2Cl_2. TFA was removed with aqueous sodium bicarbonate. The CH_2Cl_2 layer was washed with water, dried, reduced in volume, and applied to the top of a 1 × 5 cm silica gel (Baker 3405) column. Elution with the appropriate solvent[20,23] afforded pure deuteriated oxoporphyrin in near-quantitative yield. The ^{1}H NMR spectra of these compounds were recorded in $CDCl_3$ solution. Assignments of lines in the spectra of partially deuteriated compounds were confirmed where necessary by the magnitude of the lanthanide-induced shift caused by Eu(fod) (see below).

Deuterium Exchange in Sulfuric Acid-d_2. In a typical experiment, 10 mg of oxoporphyrin or hydroporphyrin compound was dissolved in 2 mL of D_2SO_4 in D_2O (9:1, v/v) and permitted to sit at room temperature in a flask protected from atmospheric moisture. At the end of the reaction, the solution was quenched with ice water and extracted with chloroform. The extract was washed with sodium bicarbonate and with water and dried. Partially deuteriated oxoporphyrins were purified on a 1 × 5 cm silica gel column. Partially deuteriated hydroporphyrins were purified on a 2 × 10 cm magnesium oxide (Baker, heavy powder) column. The small amount of H_2(OEC) that results from oxidation of H_2(OEiBC) during exchange and/or workup was isolated and examined.

Deuterium Exchange Catalyzed by Toluenesulfonic Acid-d_1. Literature procedures were followed for exchange catalyzed by toluenesulfonic acid-d_1, both in melt and in refluxing 1,2-dichlorobenzene.[9] Toluenesulfonic acid-d_1 was prepared from the sodium salt of the acid for the reaction in 1,2-dichlorobenzene. Significant loss of H_2(5) occurred in the latter reaction. Chromatography on silica was required to remove degradation products and traces of toluenesulfonic acid.

Deuterium Exchange in Me_2SO-d_6. Oxoporphyrin or hydroporphyrin compound (20 mg) was added to 5 g of Me_2SO-d_6. The suspension was thoroughly degassed and placed under a nitrogen atmosphere. Addition by syringe of 5 equiv of TBAOH (1 M in methanol) resulted in a clear solution (H_2(5), green; H_2(OEC), red; H_2(OEiBC), blue-green). Solutions were heated at reflux in a 200 °C oil bath for 10 h. After cooling, the reaction was quenched by addition of 0.5 mL of 1 M HCl. In an aerobic workup, the reaction mixture was partitioned between chloroform and aqueous HCl. Insoluble materials were discarded. (The bulk of the material at the end of the H_2(OEiBC) exchange reaction was insoluble.) The chloroform layer was washed exhaustively with water and dried, and the solvent was removed. Compounds were purified by chromatography on silica (oxoporphyrins) or grade III neutral alumina (hydroporphyrins) columns.

Results

Assignment of Oxoporphyrin Meso-Proton Signals. The ^{1}H NMR spectra of free-base oxoporphyrins 5–10 have been reported previously.[20,23,24] Unlike hydroporphyrins, the assignments of meso-proton peaks are not obvious. These peaks must be assigned if the relative reactivity of the sites within oxoporphyrin compounds is to be determined. Assignment is complicated by such opposing factors as the potentially asymmetric charge distribution within the macrocycle, the decrease in the magnitude and symmetry of the ring current of the macrocycle π system, the diamagnetic anisotropy of carbonyl group(s), inductive effects of geminal ethyl groups vs. single ethyl groups, and effects due to possible conformational variations.

The presence of β-carbonyl groups in the oxoporphyrins suggested a possible approach to assignment of the meso protons. Lanthanide shift reagents like Eu(fod) will coordinate to the carbonyl oxygen atoms, provided that they are available as Lewis bases in these compounds. The lanthanide-induced shifts that result arise predominantly by a dipolar (pseudocontact) mechanism.[29] In an effectively axially symmetric situation (lanthanide–base bond collinear with the principal magnetic axis of the lanthanide), shifts correlate with the geometric factor $(3\cos^2\theta - 1)/r^3$, where r and θ are spherical polar coordinates of the nucleus of interest in the coordinate system of the principal magnetic axes.[30,31] Thus, relative magnitudes of the lanthanide-induced shifts of the meso protons should establish their distance from the carbonyl group and permit their assignment. A similar approach was used to assign the ^{1}H NMR spectrum of the macrocycle from heme-d_1.[32]

Spectra of oxoporphyrin compounds were obtained in the presence of increasing concentrations of Eu(fod). The results of an experiment with compound H_2(7), shown in Figure 2, are typical. The large lanthanide-induced shift of one of the two low-field meso protons relative to the imino protons establishes that carbonyl oxygen atoms rather than the macrocycle nitrogen atoms are the prin-

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Table I. ^{1}H NMR Assignments of Oxoporphyrin Meso Protons in $CDCl_3$ and TFA[a]

compd	meso position			
	5-H	10-H	15-H	20-H
H_2(5)	9.12 (9.56)	9.94 (10.74)	9.86 (10.60)	9.83 (10.28)
H_2(6)	8.63 (9.12)	8.44 (9.00)	9.42 (10.20)	9.28 (9.90)
H_2(7)	9.58 (9.78)	8.87 (9.51)	9.78 (10.50)	8.87 (9.51)
H_2(9)	9.06 (9.64)	9.71 (10.26)	9.06 (9.64)	9.71 (10.26)
H_2(10)	8.81 (9.34)	8.81 (9.34)	9.61 (10.13)	9.61 (10.13)

[a] δ in $CDCl_3$ at 19 °C, (δ in TFA-d_1 at 50 °C).

cipal binding sites for EuFOD.[33] Furthermore, the methylene protons of the geminal ethyl groups experience significant lanthanide-induced shifts as would be anticipated upon binding at the adjacent carbonyl. Though nearly isochronous in the absence of Eu(fod),[20] these protons become an AB spin system in its presence. Finally, protons that experience large lanthanide-induced shifts also experience large increases in linewidth.

Assignments of meso protons, Table I, followed from the results of the Eu(fod) experiments. For H_2(5–7), plots of the induced shift of meso protons adjacent to carbonyl groups as a function of [Eu(fod)] appeared to cross the curves of more distant meso protons. These crossings were confirmed by analogous experiments with partially deuteriated oxoporphyrins recovered from the various exchange experiments. The unequal heights of the peaks in these spectra made crossovers easy to observe. The assignments of H_2(5), H_2(7), H_2(9), and H_2(10) establish that meso protons adjacent to a carbonyl group are deshielded and those adjacent to geminal ethyl groups are shielded. These observations clarified the assignments of the meso protons of H_2(6).

Deuterium Exchange Reactions. The predominant oxoporphyrin species present in TFA solution are the diprotonated dications, H_4(5–10)$^{2+}$. Dissociation and/or N–H proton exchange reactions of the dications D_4(5–10)$^{2+}$ in TFA-d_1 result in broadening of all peaks in this solvent relative to linewidths in $CDCl_3$. Linewidths increase at higher temperature and are extremely broad in wet TFA. The meso-proton peaks of these species in TFA-d_1 are shifted downfield relative to their respective chemical shifts in the free bases in $CDCl_3$. Since the magnitude of the change in chemical shifts of meso protons in different regions of the macrocycles can be rather different,[2] the order of the chemical shifts of the meso protons in TFA-d_1 were not simply assumed to be the same as in $CDCl_3$. Peaks were assigned, Table I, by comparison of spectra of partially deuteriated samples of oxoporphyrin in TFA to spectra of the same sample in $CDCl_3$ after recovery. When necessary, assignments of lines in the spectra of partially deuteriated compounds in $CDCl_3$ were confirmed by the magnitude of the lanthanide-induced shift caused by Eu(fod).

Deuterium exchange reactions in TFA-d_1 were run in sealed NMR tubes at 50 °C. No evidence was seen in $^2H\{^1H\}$ NMR spectra of recovered materials for deuterium incorporation in sites other than the meso positions. Kinetic data were obtained by monitoring the decrease of the integrated intensity of appropriate peaks. The methyl and methylene peaks of geminal ethyl groups were used as internal standards for integrations. Figure 1 shows a typical first-order rate plot for exchange of the 5 and 20 meso protons of H_2(5). Reactions were followed for many half-lives of protons that exchanged at reasonable rates. No evidence of significant decomposition of oxoporphyrin compounds was seen by NMR or during recovery and purification, even after reaction times as long as 600 h (H_2(5)). Pseudo-first-order rate constants and half-lives are presented in Table II.

Table II. Deuterium Exchange of Oxoporphyrin Meso Protons

compd	meso proton	TFA-d_1[a]		D_2SO_4[b]
		k, s^{-1}	$t_{1/2}$, h	% D incorp
H_2(5)	5	4.8×10^{-5}	4.0	100
	10[c]	5.6×10^{-8}	3500	4
	15	8.3×10^{-7}	230	14
	20	1.5×10^{-5}	13	76
H_2(6)	5	1.9×10^{-5}	10	82
	10	5.5×10^{-5}	3.5	96
	15[d]			0
	20[d]			14
H_2(7)	5	1.9×10^{-6}	100	46
	10, 20	3.3×10^{-6}	59	35
	15[d]			11
H_2(9)	5, 15	3.8×10^{-4}	0.50	17
	10, 20	1.7×10^{-4}	1.2	0
H_2(10)	5, 10	7.4×10^{-4}	0.26	40
	15, 20	8.7×10^{-6}	22	0

[a] At 50 °C. [b] After 118 h of reaction in 9:1 D_2SO_4/D_2O at 25 °C. [c] Estimate based upon data for reaction that was about 10% complete. [d] No deuterium exchange was detected after 435 h at 50 °C in TFA-d_1.

NMR spectra recorded in 9:1 (v/v) D_2SO_4/D_2O are not of suitable quality for kinetic studies.[2] Exchange was quantified by examination of the spectrum in $CDCl_3$ of compounds recovered from the sulfuric acid medium after a specified reaction time. H_2(5) was initially permitted to react for 18 h, a time period more than sufficient for complete deuteriation of the meso protons of H_2(OEP).[2,34] Exchange of the 5 meso proton was essentially complete, but exchange in other positions was insignificant. Other experiments established that measurable deuteriation had occurred in most meso positions after 118 h of reaction. $^2H\{^1H\}$ NMR spectra did not provide evidence for deuterium incorporation in other positions. The extent of exchange, after 118 h, of meso protons in H_2(5) and dioxoporphyrins H_2(6), -(7), -(9), and -(10) is collected in Table II.

The exchange reactions of hydroporphyrins H_2(OEC) and H_2(OEiBC) in D_2SO_4/D_2O have not been reported. After 14 h of reaction, recovered H_2(OEC) was found to be completely deuteriated at the 5,20 (γ,δ) meso positions and about 50% deuteriated at the 10,15 (α,β) meso positions. Reaction of H_2(OEiBC) for the same length of time results in complete deuteriation of the 5 (γ) meso and 10,20 (β,δ) meso positions but only 25% deuteriation of the 15 (α) meso position. Recovery of H_2(OEiBC) was not quantitative. Almost half of the material was oxidized to H_2(OEC) during either the reaction or the workup. The extent of deuteriation of the H_2(OEC) recovered from the H_2(OEiBC) reaction was greater than 95% in both meso positions. No evidence was seen in either hydroporphyrin compound for deuterium incorporation in the β-pyrroline positions or in the ethyl substituents.

Two different exchange reactions catalyzed by toluenesulfonic acid-d_1 were examined, reaction in refluxing *o*-dichlorobenzene and reaction in *p*-toluenesulfonic acid melt. In the first reaction, the extent of deuteriation at meso positions of H_2(5) was 80% after 92 h at reflux. All

(33) The ability of the β-carbonyl oxygen atom to act as a Lewis base could be a significant result in its own right. Hydrogen bonding interactions with the carbonyl group might be yet another way for a protein environment to alter the properties of a heme group.

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four positions were deuteriated to roughly the same extent. $^2H\{^1H\}$ NMR spectra established that the methylene groups of ethyl groups on nonoxidized rings were 5% deuteriated. Surprisingly, the methyls of these ethyls were 1% deuteriated. In contrast, under identical conditions H_2(OEP) was 45% deuteriated at ring-adjacent positions and 92% deuteriated at meso positions.[9] Reaction of H_2(**5**) in *p*-toluenesulfonic acid melt led solely to deuteriation at meso positions. However, the extent of deuteriation varied with position. After 30 min, the levels of incorporation were 5-H, 85%; 10-H, 15%; 15-H, 35%; and 20-H, 85%.

The TBAOH-catalyzed exchange of H_2(OEP) in Me_2SO-d_6 has been reported recently.[11] The reaction is specific for exchange of the methylene protons and results in destruction of about 50% of the starting material. We have examined the reactivity of oxoporphyrin H_2(**5**) and hydroporphyrins H_2(OEC) and H_2(OEiBC) in this system.

As expected, H_2(**5**) was fairly stable under the high temperature and strongly basic conditions of the reaction. About 75% of the material was recovered after a 10-h reflux. The recovered compound was 90% deuteriated in the methylene positions adjacent to the nonoxidized rings. In addition, meso protons were 15% deuteriated. $^2H\{^1H\}$ NMR did not provide any evidence for deuterium incorporation in the corresponding methyl groups or in either of the geminal ethyl groups.

H_2(OEC) and H_2(OEiBC) were both destroyed under the conditions of the reaction, even though it was run anaerobically. The strong smell of dimethyl sulfide noted on workup of the hydroporphyrin reactions suggests that the solvent has acted as an oxidizing agent. Somewhat less than 50% of the H_2(OEC) used in the reaction was recovered as H_2(OEP). The methylene positions of this material were over 95% deuteriated. Meso positions were 85% deuteriated, in contrast to the results obtained after starting with H_2(OEP). H_2(OEiBC) was almost totally converted to an insoluble tar. The trace quantity of H_2(OEP) isolated from the reaction (20-mg scale) was insufficient for a ^1H NMR spectrum. H_2(OEiBC) and H_2(OEC) were completely oxidized to H_2(OEC) and H_2(OEP), respectively, in analogous experiments performed at room temperature.

Discussion

The pseudo-first-order rate constants for meso deuteriation of H_2(OEP), H_2(OEC), and H_2(OEiBC) vary over a considerable range.[2] In general, the rate of deuterium exchange increases as the macrocycle is progressively saturated and is greatest for the meso protons near pyrroline rings. Half-times of reaction at 50 °C vary from a $t_{1/2}$ of 9 min for the meso proton between the two pyrroline rings in H_2(OEiBC) to a $t_{1/2}$ of about 8400 h[35] for H_2(OEP).

The rates of exchange of oxoporphyrin meso protons in TFA vary over more than 4 orders of magnitude, Table II. The range and magnitude of the rate constants are comparable to those observed for porphyrins and hydroporphyrins. The range of rates observed for protons within an individual oxoporphyrin compound is almost as large as the total range of all oxoporphyrin compounds. Thus, selective deuteriation of specific sites can be achieved for many of the oxoporphyrin free bases.

The most rapid rates of exchange in oxoporphyrin compounds are observed for protons in H_2(**9**) and H_2(**10**), the dioxoporphyrin isomers of the bacteriochlorin type. No correlation was noted between rates of exchange and the number of chemically modified pyrrole rings in the macrocycle, however. By way of example, all protons in H_2(**7**), a dioxoporphyrin of the isobacteriochlorin type, exchange at rates slower than the 5 and 20 protons of H_2(**5**), which has only one pyrrolidinone ring. The absence of a correlation is not surprising. The near equality of the first oxidation potentials of oxoporphyrin compounds[20] suggests that the HOMOs of these compounds resemble each other more closely than do those of porphyrins and hydroporphyrins.

Two trends can be observed in the data in Table II. Within a given oxoporphyrin macrocycle, protons adjacent to pyrrolidinone rings exchange at greater rates. This parallels the more rapid exchange of protons adjacent to pyrroline rings discussed above for hydroporphyrin compounds. In addition, meso protons near geminal diethyl groups exchange more rapidly than meso protons near β-carbonyl groups. This observation is consistent with a simplistic model (see below) in which the carbonyl is protonated at oxygen[33] and the resulting positive charge is delocalized onto the tetrapyrrole system. When all possible NH tautomers and resonance forms are considered, the meso carbon near the geminal diethyl group should experience a greater electron density than the meso carbon near the carbonyl group. A similar model has been invoked to explain the selectivity of meso deuteriation in deuteroporphyrin-IX derivatives.[8]

The extents of deuterium exchange for oxoporphyrin meso protons after 118 h of reaction in D_2SO_4 are collected in Table II. The relative rates of deuterium exchange of meso protons within a compound are qualitatively the same in D_2SO_4 as in TFA, i.e., protons that exchange most rapidly in a given compound in TFA are deuteriated to the greatest extent in D_2SO_4. Interestingly, the opposite is true when comparisons are made between compounds. H_2(**9**) and H_2(**10**), which exchange most rapidly in TFA, are the oxoporphyrin compounds with the smallest extent of deuterium incorporation in D_2SO_4. It is not just the relative rates for these compounds that change. When allowances are made for the difference in reaction temperatures, the rate of deuterium exchange for these compounds in D_2SO_4 actually appears to be less than that in TFA. This stands in sharp contrast to the many orders of magnitude increase in the rate of deuterium exchange for H_2(OEP) in D_2SO_4 relative to the rate in TFA. The seemingly anomalous behavior of oxoporphyrins led us to examine the previously unreported exchange reactions of hydroporphyrins H_2(OEC) and H_2(OEiBC) in this medium. A reversal of rates was observed for hydroporphyrins, too. Deuterium incorporation at many sites in hydroporphyrin compounds was actually slower than in H_2(OEP). Relative rates of exchange for protons within each compound were similar to that in TFA, however.

The reactivity of oxoporphyrin H_2(**5**) and hydroporphyrins H_2(OEC) and H_2(OEiBC) were examined in a number of other media that have been used for deuterium exchange. In general, reactions of the oxoporphyrin were less specific than reactions of H_2(OEP) in these media. Like H_2(OEP), the toluenesulfonic acid-d_1 catalyzed exchange reaction of H_2(**5**) in *o*-dichlorobenzene resulted in deuterium incorporation in ring-adjacent methylene protons and in meso protons. The extent of ring-adjacent deuteriation was much less for H_2(**5**), however. Furthermore, the deuteriation of the meso protons of H_2(**5**) was nonspecific. Greater meso-proton specificity was observed in toluenesulfonic acid melt. Exchange reactions of H_2(**5**) in basic Me_2SO-d_6 resulted in substantial deuterium incorporation in methylene groups adjacent to normal pyrrole rings. Exchange in Me_2SO-d_6 was of little utility for

(35) Extrapolated from kinetic data provided in ref 2.

hydroporphyrins due to serious loss of compound by oxidation. Loss was substantial, even at room temperature. In contrast to the results reported for exchange reactions of zinc tetraphenylhydroporphyrins in *tert*-butoxide/*tert*-butyl alcohol-d_1 mixtures,[36] no evidence was seen for deuterium incorporation in the β-pyrroline protons. Macrocycles recovered from hydroporphyrin oxidation in this medium and in D_2SO_4 were deuteriated at meso positions to a greater extent than expected for that compound, suggesting that exchange of meso protons in cation radicals or related intermediates is rapid.

Mechanistic Considerations. The mechanism of acid-catalyzed deuterium exchange in tetrapyrrolic macrocycles is not understood. In strong acid media, compounds are present at at least three levels of protonation: $H_2(P)$, $H_3(P)^+$, and $H_4(P)^{2+}$. The thermodynamically preferred sites of protonation are the central nitrogen atoms. Numerous carbon-protonated tautomers can be written, however. Tautomers of these and perhaps other levels of protonation must be important in the reaction mechanism of this electrophilic substitution reaction. The level of protonation and the identity of the tautomers that are directly involved as intermediates or activated compounds in deuterium exchange have not been established. It has been suggested that it is the neutral porphyrin that undergoes electrophilic attack.[4] This proposal was based upon the slower rates of deuterium exchange in the strong acid TFA-d_1 compared to exchange in the weaker acid acetic acid-d_1. Other have argued that comparisons of the rapid rate of deuterium exchange in D_2SO_4 with estimates of the proportion of neutral porphyrin present and reasonable encounter rates make it unlikely that neutral porphyrin is involved in the reaction.[2]

The results presented in this paper are not consistent with a single mechanism of exchange for all tetrapyrrole compounds in all acidic reaction media. We have demonstrated that the gross specificity of exchange within an individual compound is similar in TFA and D_2SO_4. However, changing the reaction medium from the former to the latter greatly accelerates the exchange rate of H_2(OEP) but decreases or has little effect on the exchange rates of oxoporphyrin and hydroporphyrin compounds. This result establishes that oxoporphyrin and hydroporphyrin compounds must react by different mechanisms than that of the porphyrin. Furthermore, it is now clear that the rate of deuterium exchange does not increase monotonically with the acid strength of the reaction medium for any of these tetrapyrrole systems. We have demonstrated that the selectivity of deuteriation catalyzed by *p*-toluenesulfonic acid-d_1 is different in *o*-dichlorobenzene solution than in the melt. These observations imply that specific interactions of the tetrapyrrole, the acid or its conjugate base, and the solvent are extremely important in these reactions. Support for this conclusion is provided by the report of strong association between H_4(OEP)$^{2+}$ and its counteranions.[37]

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Silyl-Substituted Thioimidates as Nitrile Ylide Equivalents

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Treatment of silyl-substituted thioimidates with silver fluoride in the presence of a trapping agent produces dipolar cycloadducts formally derived from nitrile ylides. The ratio of cycloadducts obtained from the reaction of unsymmetrically substituted dipolarophiles with phenyl silyl thioimidate 8 was found to be significantly different from that obtained from the photolysis of phenylazirine. We propose that the different product ratios encountered with silyl thioimidate 8 result from the operation of a mechanism which does not proceed via a nitrile ylide dipole. Generation of an intermediate having azomethine ylide reactivity can be achieved by silver ion complexation with the silyl thioimidate. Desilylation of the resulting complex with fluoride ion generates an azomethine ylide which undergoes 1,3-dipolar cycloaddition with added dipolarophiles. The resulting cycloadduct loses methyl mercaptan to give products which are equivalent to those obtained from nitrile ylides.

1,3-Dipoles can be classified into two major types: (1) those with internal octet stabilization, where a mesomeric formula can be drawn such that the central atom of the dipole has a positive charge and all centers have completely filled valences, and (2) those without internal octet stabilization, where each mesomeric form has an electron sextet.[1,2] By far the more common group of dipoles is the former, mainly because the dipoles in the second group are all unstable and must be prepared in situ. In recent years our interest has focused on the chemistry of the octet-stabilized class of dipoles known as the nitrile ylides.[3-6] 1,3-Dipolar cycloaddition of this class of dipoles has been

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