Electrophilic Substitution Reactions of Derivatives of Deuteroporphyrin-IX: Deuteriation and Vilsmeier Formylation [1]

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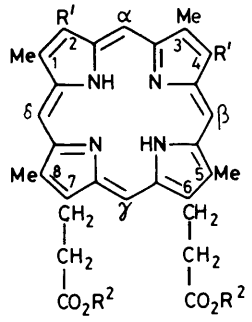
Treatment of protoporphyrin-IX, deuteroporphyrin-IX, 2,4-diacetyldeuteroporphyrin-IX, and mesoporphyrin-IX dimethyl esters [(3)—(6), respectively] with deuteriated toluene-*p*-sulphonic acid in hot *o*-dichlorobenzene accomplishes electrophilic deuteriation of the *meso*-protons at a rate which is dependent upon the nature of the 2- and 4-substituents in the porphyrin substrate. The vinyls, 2 and 4 hydrogens, and acetyls in (3), (4), and (5), respectively, are also exchanged under these conditions. A simple theory is put forward to explain the uneven amounts of *meso*-deuteriation, and the deuteriation at both the methylene and methine positions in the protoporphyrin-IX vinyl groups. Vilsmeier formylation of copper(II) deuteroporphyrin-IX dimethyl ester (10) using *N,N*-di-isobutylformamide in place of dimethylformamide yields products arising predominantly from 2- or 4-formylation of the substrate; a small quantity of α or β *meso*-formylporphyrin is also obtained.

Electrophilic substitution reactions of porphyrin and chlorin systems have been used extensively to probe the reactivity of the porphyrin macrocycle, and the effect of metal-ion complexation upon its electronic structure.[2,3] The particular example of electrophilic deuteriation is very informative since the insertion of a deuterium atom in place of hydrogen has no adverse steric or electronic effect upon further reactivity of the system. With relatively few exceptions [4,5] most studies have tended to concentrate upon reactivity of symmetrical (etioporphyrin or octaethylporphyrin) porphyrins, or their metal derivatives, and the advantages of this are obvious from the product analysis and the mechanistic interpretation standpoints. Since the major thrust of our recent studies has been the investigation of methods for regioselective insertion of deuterium labels into derivatives of protoporphyrin-IX so that we might be able to use n.m.r. methods to delineate alterations in the electronic structure of hemes in a protein environment,[6] we undertook a study of electrophilic deuteriation of unsymmetrically substituted porphyrins. In the present paper we outline our results using deuteriation, and extend the work to Vilsmeier formylation of porphyrin systems possessing the deuteroporphyrin-IX substituent orientation.

Deuteriation of Deuteroporphyrin-IX Derivatives.—On account of the importance of identification of vinyl resonances in heme protein ^{1}H n.m.r. spectra, we had earlier developed a circuitous route to vinyl-labelled derivatives of protoporphyrin-IX (1) by way of 2,4-diacetyldeuteroporphyrin-IX (2).[7] Anticipating that the vinyl methylene groups in (1) should be readily exchanged merely by treatment with deuteriated acids, and that an intermediate would be the secondary carbocation ($Por^-\overset{+}{C}H-CH_2D$), protoporphyrin-IX dimethyl ester (3) was heated at 95 °C in *o*-dichlorobenzene containing [^{2}H]toluene-*p*-sulphonic acid and, as a deuterium source [$^2H_2$]water. N.m.r. spectroscopy after four days of reaction showed the vinyl groups to be substantially labelled (>90%) (Figure 1). Also labelled were the *meso*-positions, though not all to the same extent. Integration of the n.m.r. spectrum indicated that the *meso*-sites were exchanged 80% overall, but with the remaining protium being distributed at the methine carbons in the order α(32%H), β(19%), γ(12%), and δ(15%). In addition to the vinyl methylene, which was expected to be deuteriated, the vinyl methine hydrogen was also labelled to the extent of 45%. An exchange reaction promoted with hexapyridylmagnesium(II) di-iodide and



(1) $R^1 = CH{=}CH_2$; $R^2 = H$
(2) $R^1 = Ac$; $R^2 = H$
(3) $R^1 = CH{=}CH_2$; $R^2 = Me$
(4) $R^1 = H$; $R^2 = Me$
(5) $R^1 = Ac$ $R^2 = Me$
(6) $R^1 = CH_2CH_3$; $R^2 = Me$

[^{2}H]methanol [8] afforded a sample of protoporphyrin-IX dimethyl ester in which all four *meso*-positions were approximately equally labelled, and this appeared to indicate that protonation of the vinyl groups was an important factor in the unequal selectivity shown in Figure 1. In contrast to our results with metal-free substrates, Grigg [4] has shown that when the palladium complex of protoporphyrin-IX is treated with refluxing deuterioacetic acid, the *meso*-positions are found to exchange more rapidly (95% ^{2}H in 2 h) than the vinyl methylenes (<5% after 2 h).

In order to more fully explore the nature of the exchange process, a series of porphyrins were similarly treated with [^{2}H]toluene-*p*-sulphonic acid in *o*-dichlorobenzene. After 4 days at 95 °C, deuteroporphyrin-IX dimethyl ester (4) was shown by ^{1}H n.m.r. spectroscopy to have its *meso*-protons 75% exchanged overall; the β and γ positions were more heavily deuteriated than α and δ, with the residual protium being distributed as follows: α(28%H), β(12%), γ(19%), and δ(39%). The 2 and 4 positions of deuteroporphyrin-IX dimethyl ester were also exchanged to an extent of 50%.

2,4-Diacetyldeuteroporphyrin-IX dimethyl ester (5), when also subjected to the same conditions for 4 days, exhibited only 40% total *meso*-labelling with, similar to (4), the β and γ positions being preferentially exchanged. The remaining

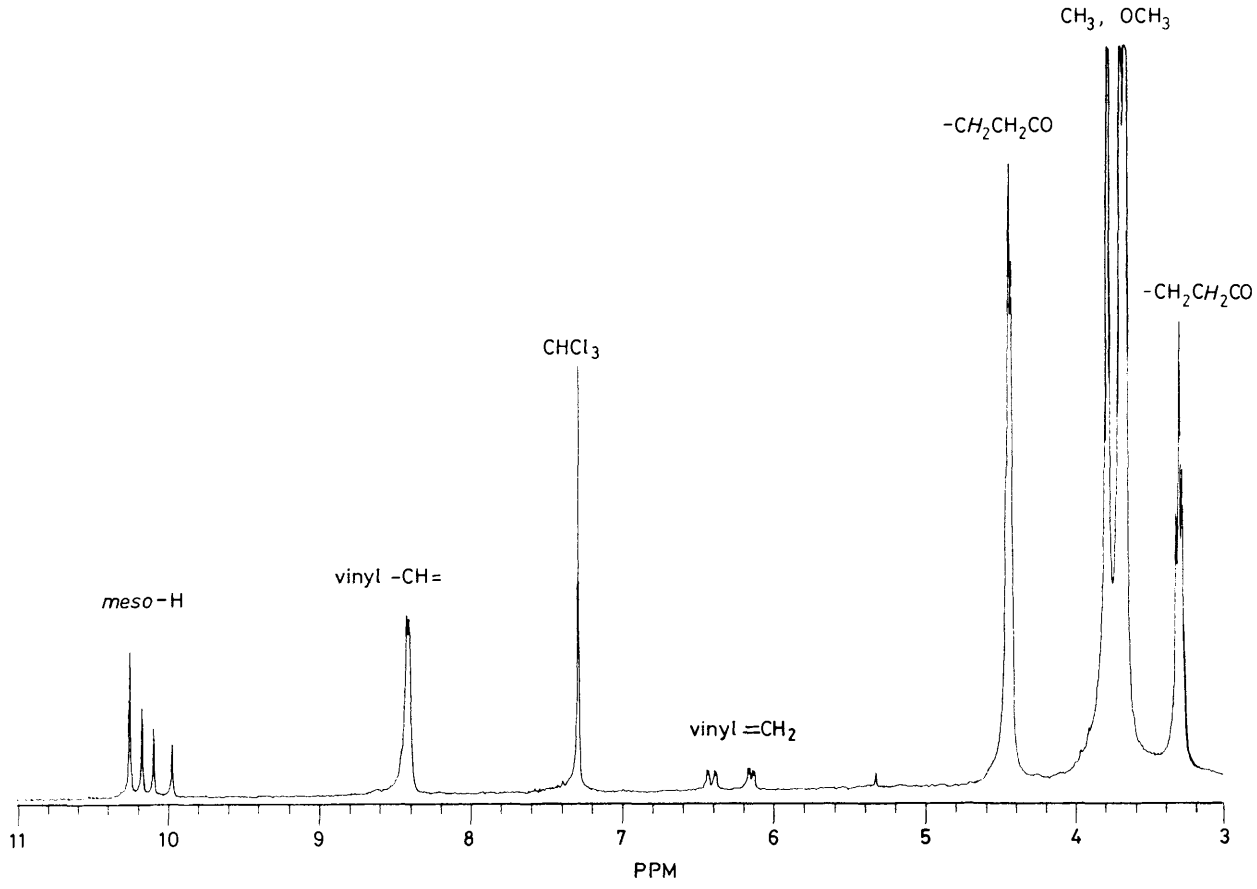


Figure 1. 360 MHz ^{1}H N.m.r. spectrum, in $CDCl_3$, of zinc(II) protoporphyrin-IX dimethyl ester after addition of *ca.* 2 mol equiv. of pyrrolidine to prevent aggregation.[23] The protoporphyrin-IX dimethyl ester sample had been subjected to treatment with [^{2}H]-toluene-*p*-sulphonic acid in *o*-dichlorobenzene and [$^2H_2$]water for 4 days at 95 °C (see text). The *meso*-proton region from this spectrum is reproduced in expanded form in Figure 2A.

protium was distributed as α(64%H), β(46%), γ(58%), and δ(77%). Figure 2 shows the spread of *meso*-labelling for compounds (3), (4), and (5), with the overall extent of labelling in each case paralleling the electron densities expected for the various porphyrins.

To complete the spectrum of electronic effects of the 2 and 4 substituents, mesoporphyrin-IX dimethyl ester (6) was chosen. Although only treated for 2 days in the acidic *o*-dichlorobenzene solution at 95 °C, the *meso*-positions were heavily labelled (86% total). The ^{1}H n.m.r. spectrum, though not completely resolved, indicated approximately equal deuteriation of all four *meso*-positions.

The results described above can be best explained by consideration of the various modes of deuteriation available to the four porphyrins. The surprising appearance of deuterium at the vinyl methine position suggests the formation of a primary carbocation (Por-CHD-$\overset{+}{C}H_2$); although such ion formation is normally quite unusual,[9] protonation of porphyrinic vinyl groups in this way has been implicated in carbon-13 n.m.r. spectroscopy [10] and in a vinyl cyclization to a *meso*-carbon in a bacteriopheophorbide-c derivative.[11] It is likely that the existence of primary carbocation intermediates is influenced by the prior diprotonation of the central porphyrin nitrogen atoms, which would be likely to inhibit formation of the secondary carbocation (Por^{2+}-$\overset{+}{C}H$-CH_3). Reaction of protoporphyrin-IX with a carbon-13 enriched vinyl group failed to show scrambling of the labelled carbon under the acidic deuteriation conditions, and such scrambling might have been expected for a spirocyclopropyl intermediate for neighbouring group participation by the porphyrin system similar to that observed with pyrroles.[12]

Deuteroporphyrin-IX dimethyl ester (4) experienced substantial deuterium incorporation at the 2 and 4 positions as well as at the *meso*-sites. The labelling of peripheral positions is most favourable for the *meso*-positions (75% ^{2}H *vs.* 50% for 2 and 4), and this result is in accord with other reports [13] which show the methine carbons to be more reactive toward electrophiles than are the pyrrole positions. Our results quantitatively agree with those of Grigg [4] who found that, using deuterioacetic acid, the *meso*-protons in deuteroporphyrin-IX dimethyl ester exchange at a faster rate than the 2 and 4 pyrrole positions.

Since, with the exception of the ethyl groups in mesoporphyrin-IX, deuterium was also incorporated into the 2 and 4 positions of the porphyrin substrates, any mechanistic interpretation of the results must take into account this additional factor. Initial protonation at the 2 and 4 substituent in (3), (4), and (5) would yield the species (7)—(9) respectively. An examination of the various NH tautomers of the free-base porphyrins indicates * that the *meso*-positions are directly

* As mentioned earlier, it is likely that the exchange processes are taking place concomitantly with equilibria involving the N-protonated mono- and di-cations of the porphyrin system. Since the N-protonations would take place statistically so as to even out their effect on each individual ring or *meso*-position, only the free-base resonance forms need be considered.

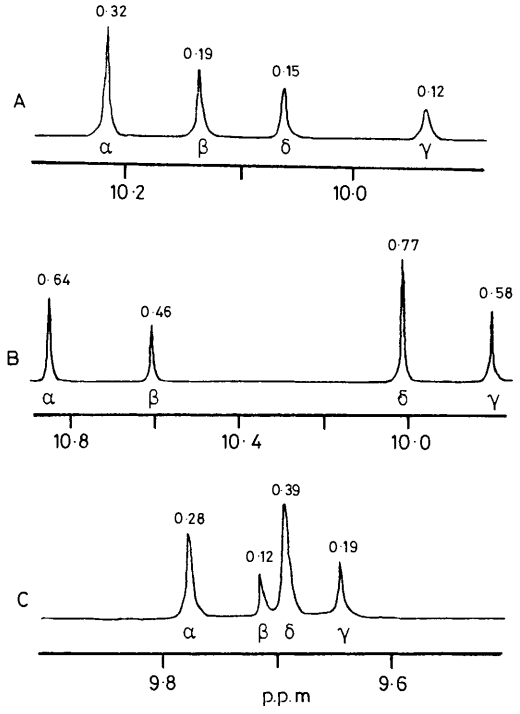
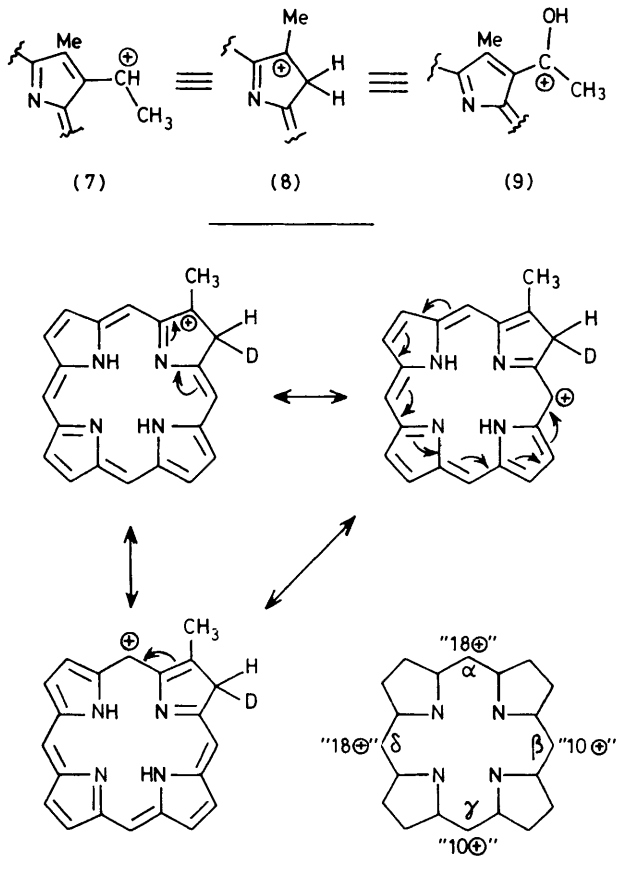


Figure 2. 360 MHz ^{1}H N.m.r. spectra, in $CDCl_3$, after 4 days exchange in presence of [^{2}H]toluene-*p*-sulphonic acid of the *meso*-proton region in: A, zinc(II) protoporphyrin-IX dimethyl ester in presence of *ca.* 2 mol equiv. of pyrrolidine; B, zinc(II) 2,4-diacetyldeuteroporphyrin-IX dimethyl ester in presence of *ca.* 2 mol equiv. of pyrrolidine; C, palladium(II) deuterioporphyrin-IX dimethyl ester. [The zinc(II) complex with pyrrolidine spectrum of this sample showed overlapping β and δ resonances.] The numbers above the peaks are amounts of proton present in each case, the data being obtained by integration relative to an internal standard. Greek letters beneath each peak are the *meso* proton assignments.



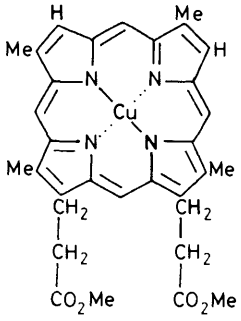
Scheme

conjugated to the β pyrrole carbons such that when the charge of the protonated species (7)—(9) is delocalized onto the tetrapyrrole system, the positive charge can be situated on a *meso*-carbon (see Scheme). This simplistic model is in agreement with the experimental results in which the β and γ methine carbons of deuteroporphyrin-IX dimethyl ester (4) and 2,4-diacetyldeuteroporphyrin-IX dimethyl ester (5) are more highly enriched with deuterium than are the α and δ positions. The positive charge (Scheme 1) can be localized, considering all possible NH tautomers and resonance forms, in 18 cases at α and δ, but only on 10 occasions for β and γ. This suggests that the β and γ carbons should experience a greater electron density relative to the other two *meso*-positions, and therefore be more susceptible to electrophilic substitution reactions.

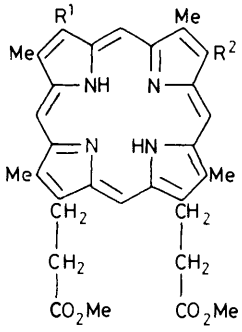
Inspection of the *meso*-proton resonances in Figure 1 for protoporphyrin-IX dimethyl ester (3) at first suggests that the simple model is not general. This anomaly can be rationalized by considering the reactivity of the vinyl substituent under the acidic exchange conditions. Normal Markownikoff addition of a deuteron to the vinyl methylene would presumably encourage a labelling pattern similar to that for (4) and (5). However, formation of a primary ion (Por-CHD-$\overset{+}{C}H_2$) might be expected to alter the observed exchange pattern at the *meso*-positions in two ways: (1) this ion might reduce the accessibility of the α and β methine carbons to external electrophiles, and (2) the primary carbocation might enhance the reactivity at the γ and δ sites. The reduced reactivity of the α and β carbons can be viewed to result from stabilization of the primary carbocation of the protonated vinyl side-chain. Overlap of the empty p orbital of the terminal vinyl carbon with the occupied orbital of the adjacent *meso*-position would result in reduced accessibility to the α and β *meso*-carbons for incoming electrophiles. Anti-Markownikoff addition of a deuteron to the vinyl group occurs with apparent formation of a primary carbocation, as evidenced by the 45% incorporation of deuterium at the vinyl methine position. The charge on this species is no longer capable of being delocalized onto the porphyrin (except by some weak interaction with the neighbouring *meso*-carbon, as mentioned above), so that this substituent is no longer strongly electron-withdrawing through the π network. The result of this effect would be a relative increase in the reactivity at the δ *meso*-position over that which is expected with (4) and (5).

The regioselective exchange of *meso*-positions in protoporphyrin-IX was recently used by Kunze and Ortiz de Montellano [14] for establishment of the isomeric identity of the *N*-methylprotoporphyrin-IX inhibitor of ferrochelatase.

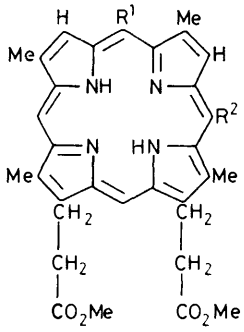
Vilsmeier Formylation of Deuteroporphyrin-IX.—Inhoffen [5] has applied the Vilsmeier reaction to unsymmetrically substituted porphyrins, but the reaction does not strongly differentiate between *meso*- and free-pyrrole positions. For example, the formylation of copper(II) deuteroporphyrin-IX dimethyl ester (10) resulted in formation of a complex mixture of *meso* and pyrrole formylated products. However, with careful control of the reaction conditions a 25% yield of the mixed 2- and 4-monoformyldeuteroporphyrins (11) and (12) was obtained, with the only other side-product being a 20% yield of the α- and β-*meso*-monoformylated deuteroporphyrins (13) and (14).[5] These results contrast with those



(10)



(11) R^1 = CHO, R^2 = H
(12) R^1 = H, R^2 = CHO

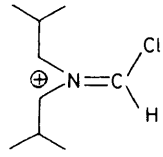


(13) R^1 = CHO, R^2 = H
(14) R^1 = H, R^2 = CHO

of Nichol [15] who found that similar Vilsmeier formylation of deuterohemin gave only the β-formyldeuteroporphyrin, along with some 2,β-diformylporphyrin.

Because of the complicated product mixtures encountered when using the Vilsmeier procedure, formylations of only the pyrrole positions have employed Fischer's dichloromethyl methyl ether * route; [16] both Kenner [17] and Johnson [18] used this approach in their syntheses of chlorocruoroporphyrin, while Jackson [19] used the same reaction in his synthesis of S-411 porphyrin.

Friedel-Crafts type acylations of metalloporphyrins tend to give products which are functionalized at the pyrrole rather than *meso*-positions when both types of site are available. This selectivity has been attributed primarily to steric interactions which prohibit bond formation at the *meso*-bridges. By this reasoning, an enlargement of the spatial requirements of the Vilsmeier reagent should result in decreased reactivity at the methine carbons. Space-filling models suggested that if di-isobutylformamide were used in the Vilsmeier reaction in place of dimethylformamide, then *meso*-formylation might be inhibited. Thus, di-isobutylformamide was treated with phosphoryl chloride to form the imine salt (15), and this was used in a 16 molar equivalent excess with copper(II) deuteroporphyrin-IX dimethyl ester (10) in 1,2-dichloroethane at 70 °C. After hydrolysis of the resulting imine salts, only two pigments were observed. These were separated by column chromatography on alumina and then demetallated with 10% sulphuric acid in trifluoroacetic acid. The more mobile demetallated band was shown, by spectral analysis, to be a mixture of *meso*-monoformylporphyrins. After crystallization, this was obtained in 18% yield. From the n.m.r. spectrum, and by integration of the free pyrrole and aldehyde peaks it was possible to establish that there were only two compounds in the mixture in the ratio 3 : 1. From the chemical shifts



(15)

given by Inhoffen,[5] these were identified as the α- and β-monoformyldeuteroporphyrins (13) and (14), with the β-isomer being the most abundant of the pair. The fact that (14) is produced in the largest amount is fully in accord with the deuteriation results described above, and demonstrates the greater reactivity of the β-*meso*-position towards electrophiles.

The least-mobile demetallated fraction was shown, by n.m.r. and mass spectrometric analysis, to be a roughly 1 : 1 mixture of the 2- and 4-monoformyldeuteroporphyrins (11) and (12), and after crystallization this was obtained in 56% yield.

It is clear, therefore, that use of a sterically hindered Vilsmeier reagent causes a dramatic increase in the proportion of pyrrole formylated product, the increase being almost two-fold over that obtained by Inhoffen using carefully controlled reaction conditions.[5] A substantial reduction in the amount of *meso*-formylated product is also observed.

Experimental

M.p.s were measured on a microscopic hot-stage, and are uncorrected. T.l.c. monitoring of all reactions was performed with Merck silica gel 60 PF254 pre-coated sheets (0.2 mm), and preparative t.l.c. separations were carried out on 20 × 20 cm glass plates coated with Merck GF 254 silica gel. Column chromatography was carried out using Merck neutral alumina 90 (70—230 mesh). Electronic absorption spectra were measured with a Cary 17 spectrophotometer (solutions in methylene chloride), and ^{1}H n.m.r. spectra were determined in deuteriochloroform solution with tetramethylsilane as internal calibrant, on a Nicolet NT-360 (360 MHz) spectrometer. Mass spectra (direct insertion probe, 70 eV, 50 μA, source temperature *ca.* 200 °C) were measured using a Finnegan 3200 mass spectrometer.

Protoporphyrin-IX, deuteroporphyrin-IX, 2,4-diacetyldeuteroporphyrin-IX, and mesoporphyrin-IX dimethyl esters [(3)—(6), respectively], were prepared from commercial hemin (Man-Win, Washington, D.C.) using standard literature methods.[2] Copper was inserted into deuteroporphyrin-IX dimethyl ester using copper(II) acetate in methanol.[20] [^{2}H]Toluene-*p*-sulphonic acid was prepared by dissolving toluene-*p*-sulphonic acid hydrate (5 g) in [$^2H_2$]water (8 ml) and stirring the solution for 30 min. The [$^2H_2$]water was removed under reduced pressure and the process repeated until n.m.r. spectroscopy indicated a minimum of 93% deuteriation.

Acid-catalyzed Deuteriation of Deuteroporphyrin-IX Dimethyl Ester (4).—To [^{2}H]toluene-*p*-sulphonic acid (93% ^{2}H) (1.1 g) in dry *o*-dichlorobenzene (35 ml) was added deuteroporphyrin-IX dimethyl ester (4) (208 mg). After the porphyrin had dissolved, [$^2H_2$]water (0.5 ml) was added and the reaction mixture stirred at 95 °C under nitrogen and in the dark (aluminium foil) for 96 h. The solution was cooled before being diluted with methylene chloride (100 ml) and washed three times with water (100 ml). The organic phase was collected, the solvent removed under reduced pressure, and the residue dissolved in methylene chloride–methanol and then treated with an excess of ethereal diazomethane. Following

* CAUTION: Dichloromethyl methyl ether is a potent carcinogen!

the removal of the solvent, the residue was chromatographed on alumina (Brockmann Grade III, elution with methylene chloride). The red eluates were collected, evaporated to dryness, and the residue then crystallized from methylene chloride–n-hexane to give 196 mg (94%) of deuteroporphyrin-IX dimethyl ester, deuterium labelled as indicated by the following n.m.r. data. The compound had m.p. 222—224 °C (lit.,[21] m.p. 224.5 °C, undeuteriated), δ 3.28 (m, 3.16 H, 2 × propionate CH_2CO, 21% ^2H), 3.65 (m, 12 H, 2 × CH_3 and 2 × OCH_3), 3.74 (d, 6 H, 2 × CH_3), 4.43 (m, 4 H, 2 × CH_2CH_2-CO), 9.10 (d, 1 H, 2- and 4-H, 50% ^2H), 10.03 (s, 0.12 H, β-*meso*-H, 88% ^2H), 10.07 (s, 0.28 H, α-*meso*-H, 72% ^2H), 10.11 (s, 0.19 H, γ-*meso*-H, 81% ^2H), and 10.14 (s, 0.39 H, δ-*meso*-H, 61% ^2H).

Acid-catalyzed Deuteriation of Protoporphyrin IX Dimethyl Ester (3).—Protoporphyrin-IX dimethyl ester (65 mg) was likewise treated with [^2H]toluene-*p*-sulphonic acid (453 mg) in *o*-dichlorobenzene containing [$^2H_2$]water for 96 h and gave 44 mg (68%) of labelled protoporphyrin-IX dimethyl ester, m.p. 226—227 °C (lit.,[22] m.p. 228—229 °C, undeuteriated), δ [zinc(II) complex with added pyrrolidine to avoid aggregation problems [23]], 3.26 (m, 2.88 H, 2 × propionate CH_2CO, 28% ^2H), 3.65 (m, 12 H, 2 × CH_3 and 2 × OCH_3), 3.71 (s, 6 H, 2 × CH_3), 4.46 (m, 4 H, 2 × CH_2CH_2CO), 6.21 (m, 0.18 H, 2 × *cis* $=CH_2$, 91% ^2H); 6.47 (m, 0.21 H, 2 × *trans* $=CH_2$, 90% ^2H), 8.42 (s, 1.10 H, 2 × $-CH=$, 45% ^2H); 9.94 (s, 0.12 H, γ-*meso*-H, 88% ^2H), 10.06 (s, 0.15 H, δ-*meso*-H, 85% ^2H), 10.14 (s, 0.19 H, β-*meso*-H, 81% ^2H), 10.22 (s, 0.32 H, α-meso-H, 68% ^2H).

Acid-catalyzed Deuteriation of 2,4-*Diacetyldeuteroporphyrin-IX Dimethyl Ester* (5).—Likewise, 2,4-diacetyldeuteroporphyrin-IX dimethyl ester (54 mg) was treated with [^2H]-toluene-*p*-sulphonic acid (430 mg) for 96 h in *o*-dichlorobenzene containing [$^2H_2$]water and gave the deuteriated product (41 mg, 76%), m.p. 242—244 °C (lit.,[5] m.p. 244 °C, undeuteriated), δ [zinc(II) complex with added pyrrolidine],[23] 3.33 (m, 2.63 H, 2 × propionate CH_2CO, 34% ^2H), 3.62 (s, 6 H, 2 × CH_3), 3.68 (s, 6 H, 2 × OCH_3), 3.90 (d, 6 H, 2 × CH_3), 4.40 (m, 4 H, 2 × CH_2CH_2CO), 9.80 (s, 0.58 H, γ-*meso*-H, 42% ^2H), 10.02 (s, 0.77 H, δ-*meso*-H, 33% ^2H), 10.61 (s, 0.46 H, β-*meso*-H, 54% ^2H), and 10.85 (s, 0.64 H, α-*meso*-H, 36% ^2H); the acetyl methyl groups were not detected, indicating >95% deuteriation.

Acid-catalyzed Deuteriation of Mesoporphyrin-IX Dimethyl Ester (6).—Mesoporphyrin-IX dimethyl ester (73 mg) was similarly treated with [$^2H_2$]toluene-*p*-sulphonic acid (423 mg) for 48 h in *o*-dichlorobenzene containing [$^2H_2$]water, to give the labelled product (54 mg, 74%), m.p. 214—216 °C (lit.,[24] m.p. 216 °C, undeuteriated), δ [zinc(II) complex], 1.78 (m, 6 H, 2 × CH_3CH_2), 3.20 (m, 3.19 H, 2 × propionate CH_2CO, 20% ^2H), 3.47 (m, 12 H, 4 × CH_3), 3.65 (s, 6 H, 2 × OCH_3), 3.80 (m, 4 H, 2 × CH_3CH_2), 4.31 (m, 4 H, 2 × CH_2CH_2CO), 9.88 (s, 0.28 H, 2 × *meso*-H, 86% ^2H), 9.91 (s, 0.18H, *meso*-H, 82% ^2H), and 9.93 (s, 0.15 H, *meso*-H, 85% ^2H).

2-*Formyl*-6,7-*bis*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphyrin* (11) *and* 4-*Formyl*-6,7-*bis*(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphyrin* (12).—To *N*,*N*-di-isobutylformamide (2.2 ml) was added, at room temperature, freshly distilled phosphoryl chloride (1.0 ml). With continued stirring the solution became viscous and after 30—40 min the imine salt complex crystallized. To this solid was added copper(II) deuteroporphyrin-IX dimethyl ester (379 mg), followed by dry 1,2-dichloromethane (50 ml). The solution was heated at 70 °C under nitrogen in the dark for 4.5 h, after which time t.l.c. analysis indicated complete consumption of starting metalloporphyrin. The solution was removed from the heat and, after cooling, was poured over ice (100 g). Saturated aqueous sodium carbonate (25 ml) was added carefully and the mixture was stirred overnight. The porphyrin-containing layer was then diluted with methylene chloride (100 ml), washed twice with water (100 ml), and evaporated to dryness; the residue was chromatographed on alumina [Brockmann Grade III, elution with methylene chloride–chloroform (9 : 1)]. This solvent mixture caused elution of the blue faster-running *meso*-formylated copper(II) deuteroporphyrin-IX isomers. A second major band was then eluted with methylene chloride–chloroform (7 : 3). Following removal of the solvent from each fraction, the residues were treated with trifluoroacetic acid (20 ml) containing concentrated sulphuric acid (2 ml). After being stirred at room temperature for 1 h, the acid solutions were diluted with methylene chloride (75 ml) and washed three times with water (100 ml). The organic fractions were collected, treated with an excess of ethereal diazomethane, and following evaporation of the solvent, the free-base residues were chromatographed on a short column of alumina (Brockmann Grade III, elution with methylene chloride). The two separate fractions were each crystallized from methylene chloride–n-hexane, the least-mobile band providing 204 mg of an equal mixture of (11) and (12) in a combined yield of 56%. The more-mobile fraction was shown by n.m.r. spectroscopy to be a 3 : 1 mixture (β : α) of the *meso*-formylated compounds (13) and (14), (65 mg, 18% yield overall).

Least-mobile fraction: (Found: C, 69.85; H, 6.05; N, 9.9. Calc. for $C_{33}H_{34}N_4O_5$: C, 69.95; H, 6.05; N, 9.9%), δ 3.24 (m, 8 H, 4 × propionate CH_2CO), 3.55 (6 H, 2 × CH_3), 3.64 (m, 18 H, 2 × CH_3 and 4 × OCH_3), 3.75 (s, 2 × CH_3), 3.83 (s, 6 H, 2 × CH_3), 4.31 (t, 4 H, 2 × CH_2CH_2CO), 4.42 (t, 4 H, 2 × CH_2CH_2CO), 9.26 (s, 2 H, 2- and 4-H), 9.90 (m, 6 H, 6 × *meso*-H), 10.82 (s, 2 H, 2 × *meso*-H), 11.40 (s, 2 H, 2 × CHO); *m*/*z* (%) 566 (100), 507 (9), 494 (22), 493 (75), and 420 (16).

Most-mobile fraction: δ 3.30 (m, 4 H, 2 × propionate CH_2CO), 3.45 (s, 2 H, CH_3, β-isomer), 3.55 (m, 3 H, CH_3), 3.63 (m, 10 H, CH_3 and OCH_3) 3.67 (s, 3 H, CH_3), 4.30 (m, 4 H, 2 × CH_2CH_2CO), 8.88 (s, 0.75 H, β-pyrrole-H), 8.98 (s, 0.25 H, β pyrrole-H), 9.62 (s, 0.75 H, β-pyrrole-H), 9.65 (s, 0.25 H, β-pyrrole-H), 9.86 (s, 0.25 H, *meso*-H), 9.88 (s, 0.75 H, *meso*-H), 9.93 (s, 0.75 H, *meso*-H), 9.98 (s, 0.5 H, 2 × *meso*-H), 10.00 (s, 0.75 H, *meso*-H), 12.46 (s, 0.75 H, CHO), and 12.50 (s, 0.25 H, CHO).

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