Electrophilic Deuteriation of Natural Porphyrin Derivatives

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Summary The proportion of electrophilic deuteriation at individual *meso* positions in unsymmetrically substituted porphyrins depends upon the nature of the substituents on the adjacent pyrrolic subunits; in the case of protoporphyrin-IX, these observations allow the first unequivocal assignment of all four *meso* protons in the n.m.r. spectrum of dicyanoferriprotoheme.

THE electronic structure of the porphyrin nucleus has been studied extensively using chemical reactivity comparisons, and in particular using electrophilic deuteriation.[1,2] Most studies tended to use free-base or metal complexes of symmetrical substrates such as octaethylporphyrin or octaethylchlorin. An exception is Woodward's work using chlorin-e_6 and rhodoporphyrin-XV;[3] conclusions from the latter case were that the electronegative group in rhodo-

porphyrin-XV tends to deactivate the whole system towards electrophilic deuteriation,[4] rather than just the *meso* positions closest to the group. We now present results of electrophilic *meso* deuteriation experiments using unsymmetrically substituted derivatives of protoporphyrin-IX [which, as the iron(II) complex, is the prosthetic group in many heme proteins].

Standard conditions involved treatment of the metal-free porphyrins with deuteriated toluene-*p*-sulphonic acid hydrate in refluxing dry *o*-dichlorobenzene for 2 or 4 days.

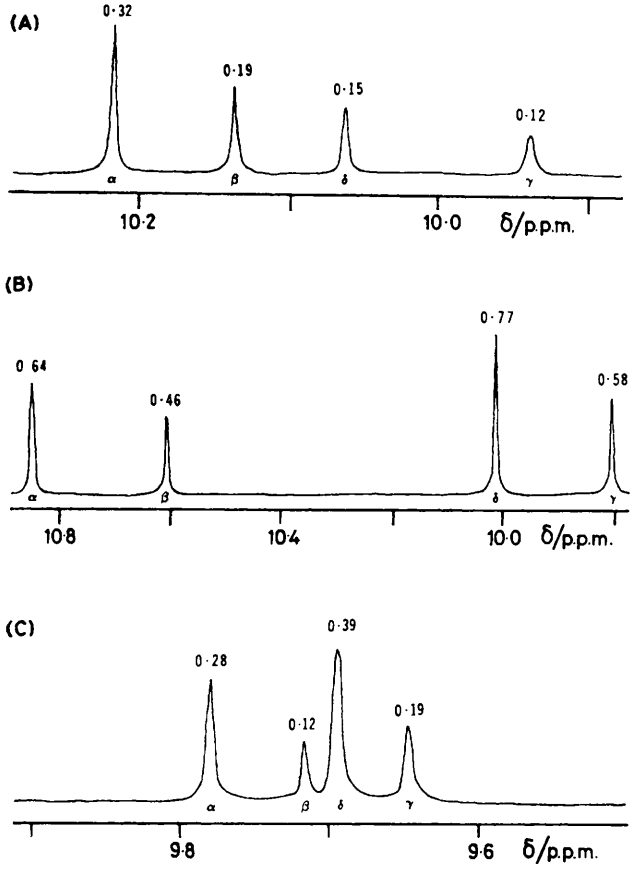
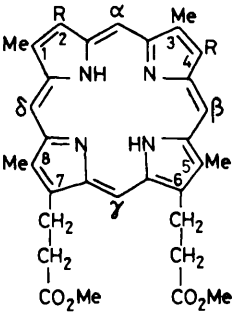


FIGURE. 360 MHz N.m.r. spectra, in $CDCl_3$, after 4 days exchange in presence of deuteriated toluene-*p*-sulphonic acid (see text), of the *meso* proton region in: (A) zinc(II) protoporphyrin-IX dimethyl ester in the presence of *ca.* 2 mol. equiv. pyrrolidine; (B) zinc(II) 2,4-diacetyldeuteroporphyrin-IX dimethyl ester in the presence of *ca.* 2 mol equiv. pyrrolidine; (C) palladium(II) deuteroporphyrin-IX dimethyl ester (the zinc complex with pyrrolidine spectrum of this sample showed overlapping β and δ peaks). Zinc(II) with pyrrolidine and palladium(II) complexes of porphyrins do not aggregate, so these were chosen for making definitive assignments (R. J. Abraham, F. Eivazi, H. Pearson, and K. M. Smith, *J.C.S. Chem. Comm.*, 1976, 698, 699). The numbers above the peaks are the amounts of proton present in each case, the data being obtained by integration relative to an internal standard. Greek letters below peaks are the *meso* proton assignments.

Under these conditions (for 4 days) the *meso* protons in protoporphyrin-IX dimethyl ester (**1**) were exchanged (80% overall) as displayed in the Figure, A, in the order $\gamma>\delta>\beta>\alpha$. If (**1**) was exchanged with hexapyridylmagnesium di-iodide and CH_3OD[5] then the four *meso* protons were approximately equally deuteriated, indicating that protonation of the vinyl groups is an important factor in selectivity of *meso* exchange. In the toluene-*p*-sulphonic acid case, the vinyl CH_2 protons were >90% deuteriated and the vinyl CH protons were *ca.* 45% exchanged; these results contrast with those of Grigg *et al.*[6] who have shown that if acetic acid is used for the exchange then the *meso* protons are exchanged in preference to the vinyl CH_2 groups. The n.m.r. spectrum of the dicyanoferriheme from exchanged deuteriated (**1**) allowed the first definitive assignment of all four *meso* protons in the hemin as γ,β,δ,α from low to high field in methanol solution. Our earlier total syntheses[7] had established that the lowest/highest field resonances were the αγ pair, and manganese salt titrations showed[8] α to be to higher field than γ; our new results open up the possibility of using these unique assignments as n.m.r. probes in reconstituted heme proteins.



(1) R = $CH=CH_2$
(2) R = Et
(3) R = COMe
(4) R = H

When mesoporphyrin-IX dimethyl ester (**2**) was subjected to the same toluene-*p*-sulphonic acid conditions (but for only 2 days), all four *meso* protons were more heavily deuteriated (86% in total). The n.m.r. spectrum, though not completely resolved, indicated approximately equal deuteriation of all four *meso* positions. If 2,4-diacetyldeuteroporphyrin-IX dimethyl ester (**3**) was similarly exchanged (4 days), only 40% total *meso* deuteriation was observed (Figure, B), with the β and γ positions being preferentially exchanged. This observation is as expected if one considers the extent to which each of the four *meso* protons can be directly conjugated with either of the 2- or 4-acetyl groups. (Considering all NH tautomers, the number of acetyl conjugated forms for each *meso* position is 18 for α and δ but only 10 for β and γ.† Hence, the latter pair should be preferentially exchanged.)

† On this basis, protoporphyrin-IX dimethyl ester might have been expected to exchange more at β than at δ because the resonance forms for the protonated vinyl (por-$\overset{+}{C}H$-CH_3) and acetyl should be equivalent. However, exchange at the vinyl CH as well as the CH_2 suggests the possibility of the corresponding primary carbonium ion (por-$CH_2\overset{+}{C}H_2$), or its spirocyclopropyl counterpart, being present in acid. Such a carbonium ion would be expected to inhibit attack at the *meso* positions adjacent to it, *i.e.* α and β. Primary carbonium ions from protonation of porphyrinic vinyl groups have been implicated in carbon-13 n.m.r. spectroscopy (see K. M. Smith and J. F. Unsworth, *Tetrahedron*, 1975, **31**, 367) and in a novel vinyl cyclization reaction (see G. W. Kenner, J. Rimmer, K. M. Smith, and J. F. Unsworth, *Phil. Trans. Roy. Soc. Ser. B*, 1976, **273**, 255).

Deuteroporphyrin-IX dimethyl ester (**4**) is an interesting case because it can nominally exchange at the *meso* and/or 2,4-positions. Grigg *et al.*[6] using acetic acid have shown *meso* exchange to be preferred to 2,4-exchange with metal-free systems, and our results qualitatively agree with theirs; after 4 days the 2- and 4-protons in (**4**) were 50% exchanged whereas the total exchange of the *meso* protons (Figure, C) was *ca.* 75%. As with (**3**), the β and γ positions were more heavily exchanged than α and δ, suggesting that protonation at the 2- and 4-positions may be an important directing influence. The results indicate that electrophilic substitution in (**4**) should predominate at the β and γ positions. Formylation of deuterohemin is preferred[9] at the β position, and the extra selectivity in this case could be explained by steric hindrance to approach of the relatively large Vilsmeier electrophile to the γ position (which is flanked by propionate side-chains).

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