Protoporphyrin IX: Some Recent Research

KEVIN M. SMITH

Department of Chemistry, University of California at Davis, Davis, California 95616

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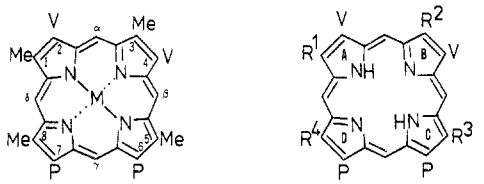
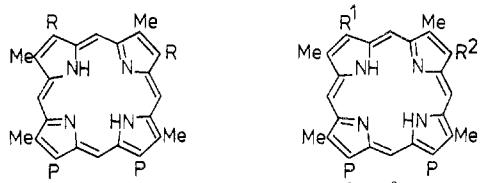
In 1929 Hans Fischer accomplished[1] the first total synthesis of hemin (1), the iron(III) chloride complex of protoporphyrin IX, PP-IX (2). This magnificent achievement secured for him the 1930 Nobel prize in chemistry, but more importantly, it established the Küster structure[2] for the porphyrin nucleus. It was particularly fitting that Fischer should be the one to solve this structural problem since both he and Willstätter, the two giants of tetrapyrrole chemistry, had doubted Küster's proposal because they felt that a natural system with such a large ring (16-membered) was unlikely to be stable. Of course, in those days, aromatic stability in complex systems of this type was not understood. Fischer could have been forgiven had he thought that, as with many natural products, the total synthesis of hemin (1) or PP-IX (2) might have closed a chapter in chemistry and that interest in this compound might have diminished. Far from it! With the successful synthesis and structure determination, the importance of PP-IX has been more and more recognized with time.



1 M = Fe-Cl V = $CH{=}CH_2$
3 M = Fe P = $CH_2CH_2CO_2H$

2 $R^1 = R^2 = R^3 = R^4 = Me$
4 $R^1 = R^2 = CD_3$; $R^3 = R^4 = Me$
5 $R^1 = R^2 = Me$; $R^3 = R^4 = CD_3$
6 $R^1 = R^4 = CD_3$; $R^2 = R^3 = Me$
7 $R^1 = R^3 = CD_3$; $R^2 = R^4 = Me$



14 $R = CH_2CH(OMe)_2$
15 $R = CH_2CHO$
16 $R = CH_2CH_2OH$
17 $R = CH_2CH_2Cl$
18 $R = CH_2CH_2Br$
19 $R = CH_2CH_2CN$
20 $R = P = CH_2CH_2CO_2H$

21 $R^1 = V$; $R^2 = P$
22 $R^1 = P$; $R^2 = V$
23 $R^1 = CHO$; $R^2 = V$
24 $R^1 = V$; $R^2 = CHO$
25 $R^1 = H$; $R^2 = V$
26 $R^1 = V$; $R^2 = H$

As the iron(II) complex ("heme", 3), PP-IX is the prosthetic group in the oxygen transport and storage pigments hemoglobin and myoglobin.[3] Either heme or a peripherally modified form is the center of action in the cytochromes[4] and in the peroxidase and catalase enzymes. PP-IX is a biosynthetic precursor for chlorophylls a and b and for most of the bacterial chlorophylls; significantly, a metal complex of 2 is a precursor of the photosynthetically active algal biliproteins, and presumably of phytochrome, the photoreceptor for the photoregulation of growth and development in plants.[5] Regretably, PP-IX is not a biological precursor of vitamin B_{12}, the other major pyrrole-derived[6] natural product! PP-IX does, however, occur in many other obscure places;[7] it is, for example, the coloring matter (ooporphyrin ≡ 2) in certain birds eggs[8] and also occurs in the Harderian glands (behind the eyeballs) of certain rodents such as rats and hamsters.[9]

One of the primary interests of our research effort has been in the synthesis of deuterium- and carbon-13-labeled derivatives of PP-IX for reconstitution, as iron complexes, into heme proteins such as myoglobin (Mb), hemoglobin (Hb), and peroxidases so as to enable assignment of resonances in the NMR spectra of these proteins. Shifts give information on structural changes in the protein and on the electronic structure in the heme, so the efficient exploitation of heme protein NMR is precluded until it is known definitively which resonances are uniquely assigned to a given functionality.[10]

Taking advantage of the circumstance that the prosthetic group (the heme) can be removed from many heme proteins by a simple reversible step,[3] it is possible to contemplate labeling of groups in the heme such that, when reconstituted with protein, the resonances in the heme protein can be assigned with complete certainty if they are resolved. In turn, this would yield information of true physiological value because oxygen binding and transport properties (the stock-in-trade of heme proteins) depend intimately upon the electronic structure of the heme and the way in which this is modified by protein contacts in heme proteins.

Syntheses of PP-IX

We began our heme proteins work in collaboration initially with R. G. Shulman and then later with G. N.

Kevin M. Smith is Professor of Chemistry at University of California, Davis. He was born in Birmingham, England, and obtained his B.Sc. and Ph.D. degrees at the University of Liverpool, where he also was awarded the D.Sc. in 1977. After postdoctoral work with R. B. Woodward at Harvard University, he joined the faculty of the University of Liverpool, where he worked for 8 years before moving to California in 1977. Dr. Smith received the Corday Morgan Medal and Prize of the Chemical Society, London, in 1978.

(1) H. Fischer and K. Zeile, *Justus Liebigs Ann. Chem.*, **468**, 98 (1929).
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(3) A. Antonini and M. Brunori, "Hemoglobin and Myoglobin in Their Reactions with Ligands", North-Holland, Amsterdam, 1971.
(4) R. Lemberg and J. Barrett, "Cytochromes", Academic Press, London, 1973.
(5) M. F. Hudson and K. M. Smith, *Chem. Soc. Rev.*, **4**, 363 (1975).
(6) For a review, see A. R. Battersby and E. McDonald in "Porphyrins and Metalloporphyrins", K. M. Smith, Ed., Elsevier, Amsterdam, 1975, p 61.
(7) K. M. Smith in "Porphyrins and Metalloporphyrins", K. M. Smith, Ed., Elsevier, Amsterdam, 1975.
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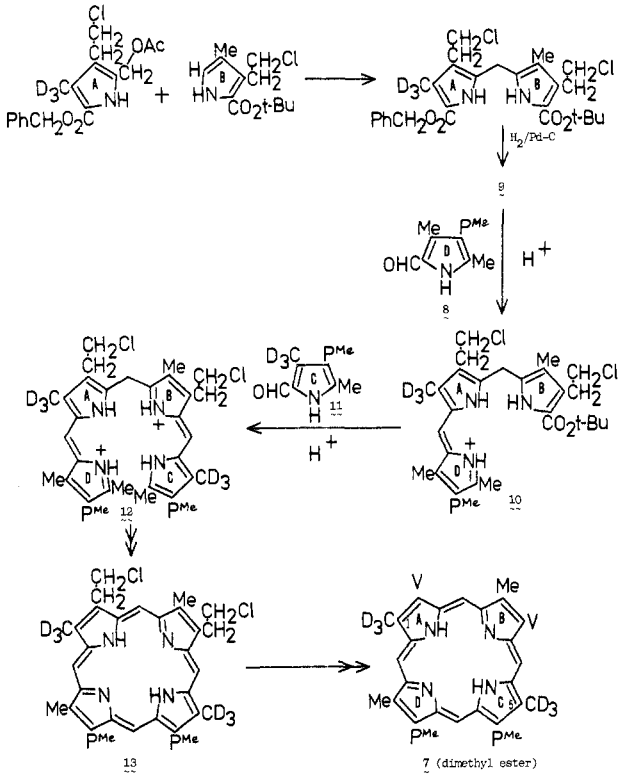
Scheme I
Total Synthesis of 1,5-Deuterated PP-IX (7) Dimethyl Ester Using[16] the Tripyrrene Route



La Mar. Using established procedures[11] we were able to produce moderate quantities of the 1,3-, 5,8-, and 1,8-labeled derivatives **4**–**6**, respectively, and then were able to establish methyl assignments in hemes[12] and in Mb[13] and to correct misinterpretations associated with hindered rotation in methyls and peak assignments in Mb intercalated with cyclopropane or xenon. These same compounds (**4**–**6**) also enabled methyl assignments in aggregated samples of zinc(II) PP-IX dimethyl ester to be established[14,15] (vide infra).

We next developed a more general synthetic approach to PP-IX and prepared[16] 1,5-labeled protoporphyrin IX (**7**), as shown in Scheme I. This route employs stepwise 1 + 1 + 1 + 1 addition of the four pyrrole subunits, and in the key step, with mild acid catalysis, the formylpyrrole **8** was condensed with the pyrromethanemonocarboxylic acid **9** to give the tripyrrene **10** in which the *tert-butyl ester was preserved.* Subsequent treatment with the deuterated monopyrrole **11** gave the a,c-biladiene, **12**, which was cyclized, using copper(II) chloride in dimethylformamide, to give the porphyrin **13** after removal of chelated copper(II) using 10% sulfuric acid in trifluoroacetic acid. Finally, double dehydrochlorination to give **7** was achieved using butoxide on the zinc(II) complex. NMR results using methyl-deuterated PP-IX derivatives are described later in this Account.

Reactions of PP-IX

At the Vinyl Groups. Our work on the vinyl groups of PP-IX has concentrated on the transformation of these substituents into others of biological significance. On account of its ready availability as the terminal product in a vast biological manufacturing plant,[6] PP-IX is relatively inexpensive to purchase commercially, usually as its iron(III) chloride, hemin (**1**). On the other hand, coproporphyrin III (**20**) is the aromatized form of the biosynthetic precursor, coproporphyrinogen III, of heme proteins and chlorophylls. The steady-state concentration of this in normal metabolism is minute. Commercial sources for such compounds rely on animals with metabolic diseases; their cost, on a commercial basis, is therefore high. However, treatment of PP-IX (**2**) dimethyl ester with 1 mol of thallium(III) nitrate (TTN) accomplishes chelation in the center of the porphyrin.[17] The complexed thallium atom imparts a high oxidation potential (1.0 V) to the porphyrin nucleus, protecting it against one-electron abstration and subsequent nitration[18] or oxophlorin formation.[19] Addition of 2 mol more of TTN in methanol achieves[20] formation of the bisacetal **14**. Acid-catalyzed hydrolysis affords the dialdehyde **15** which can be reduced with borohydride to give the bis(2-hydroxyethyl)porphyrin, **16**. This same porphyrin can be made in lower yield by direct hydroboration–oxidation of PP-IX. Treatment of **16** with thionyl chloride in DMF gives the bis(2-chloroethyl)porphyrin **17** which can be reconverted into PP-IX using butoxide treatment of the zinc(II) complex; thus, the 2-hydroxyethyl and 2-chloroethyl functions are good vinyl-protecting groups.[20] We are making use of this in a synthesis from chlorin-e_6 of a natural bacteriochlorophyll which is in progress.[21] Treatment of **16** with thionyl bromide gives **18** which, after heating with sodium cyanide in *N*-methylpyrrolidone, affords the bis(2-cyanoethyl)porphyrin **19**. Methanolysis then gives[20] coproporphyrin III (**20**) tetramethyl ester in 37% yield from PP-IX.

Though our earlier total syntheses[9] established the identity of harderoporphyrin (**21**) trimethyl ester and its isomer **22**, they can also be prepared by a slight modification[22] of the above procedure involving treatment of PP-IX dimethyl ester with 2, instead of 3, mol of TTN. Separation of the two isomers is accomplished at an intermediate stage.[22] Harderoporphyrin (**21**) is important since it occurs, along with PP-IX (**2**) and coproporphyrin III (**20**), in the Harderian glands of some rodents. *Spirographis* (chlorocruoro) porphyrin (**23**) and its isomer **24** can also be obtained from the 2 mol of TTN modification. The heme of **23**

(11) J. A. S. Cavaleiro, A. M. d'A Rocha Gonsalves, G. W. Kenner, and K. M. Smith, *J. Chem. Soc., Perkin Trans. 1*, 1771 (1974).
(12) J. A. S. Cavaleiro, A. M. d'A. Rocha Gonsalves, G. W. Kenner, K. M. Smith, R. G. Shulman, A. Mayer, and T. Yamane, *J. Chem. Soc., Chem. Commun.*, 392 (1974).
(13) A. Mayer, S. Ogawa, R. G. Shulman, T. Yamane, J. A. S. Cavaleiro, A. M. d'A. Rocha Gonsalves, G. W. Kenner, and K. M. Smith, *J. Mol. Biol.*, **86**, 749 (1974).
(14) R. J. Abraham, F. Eivazi, H. Pearson, and K. M. Smith, *J. Chem. Soc., Chem. Commun.*, 698 (1976).
(15) R. J. Abraham, F. Eivazi, H. Pearson, and K. M. Smith, *J. Chem. Soc., Chem. Commun.*, 699 (1976).
(16) K. M. Smith, F. Eivazi, K. C. Langry, J. A. P. Baptista de Almeida, and G. W. Kenner, *Bio-org. Chem.*, in press.
(17) R. J. Abraham, G. H. Barnett, and K. M. Smith, *J. Chem. Soc., Perkin Trans. 1*, 2142 (1973).
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(19) G. H. Barnett, M. F. Hudson, S. W. McCombie, and K. M. Smith, *J. Chem. Soc., Perkin Trans. 1*, 691 (1973).
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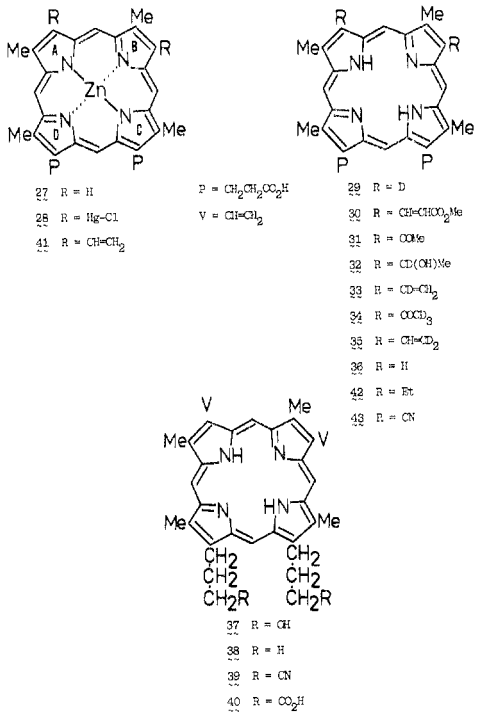
is the oxygen transport pigment in the marine worm, *Spirographis spallanzanii*. Likewise, pemptoporphyrin (**25**), a rare fecal metabolite, and its 4-unsubstituted-2-vinyl isomer, **26**, can be produced by careful manipulation of these side chains.[22]

Another series of transformations can produce coproporphyrin III (**20**) from PP-IX (**2**). Treatment of zinc(II) deuteroporphyrin IX (**27**) dimethyl ester with an excess of mercuric acetate[23] and chloride gives the dimercurated derivative, **28**; with DCl this gives[24] the confusingly named dideuteriodeuteroporphyrin IX (**29**), the original objective of the work. However, if **28** is treated with methyl acrylate and Li_2PdCl_4, a good yield of the bisacrylate **30** tetraester is obtained.[24] Simple catalytic hydrogenation gives coproporphyrin III (**20**) tetramethyl ester. Use of ethylene in place of methyl acrylate gives PP-IX (**2**) dimethyl ester in, temporarily, low yield.



In connection with studies aimed at the definitive identification of vinyl peaks in NMR and resonance Raman spectra of heme proteins, we required specifically deuterated vinyl groups present in the prosthetic group of reconstituted proteins. This was accomplished using standard porphyrin chemistry.[25] Reduction of 2,4-diacetyldeuteroporphyrin IX (**31**) dimethyl ester with sodium borodeuteride gave the hematoporphyrin IX derivative **32** which was dehydrated to give the PP-IX (**33**) with its vinyl H_α positions deuterated. Acid-catalyzed exchange[26] of the acetyl methyl groups, on the other hand, gave **34** which was reduced with borohydride and then dehydrated to give[25] **35**. The paramagnetic NMR spectra of the low-spin dicyanoferrihemins are shown in Figure 1 and attest to the

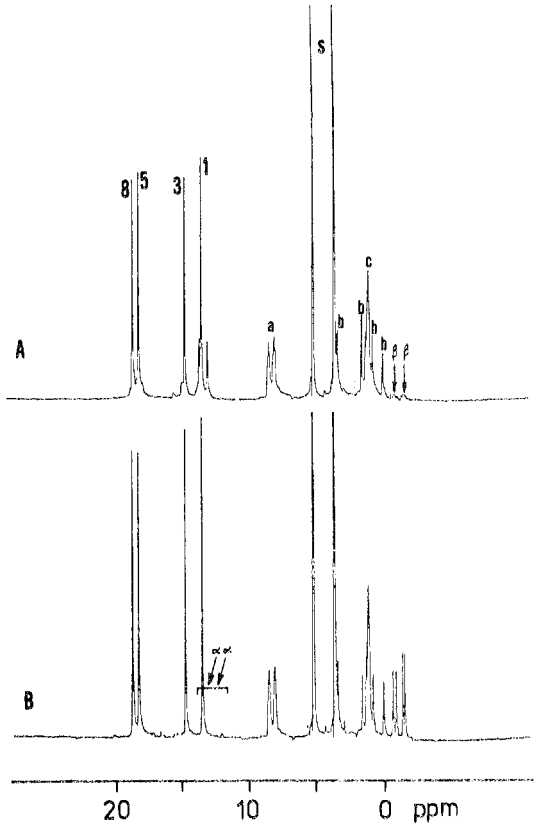


Figure 1. 100-MHz proton NMR spectra, in CD_3OD, of the dicyanoferrihemes from (A) PP-IX with vinyl H_β deuterated; (B) PP-IX with vinyl-H_α deuterated. Assignments: 8, 5, 3, 1, ring methyls; a, propionic α-CH_2; b, meso H; c, propionic β-CH_2; S solvent.

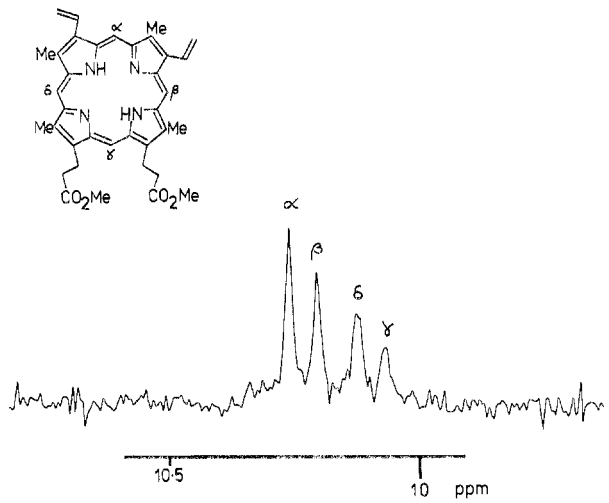


Figure 2. 100-MHz proton NMR spectrum (low-field region only) in $CDCl_3$ of the meso protons in PP-IX dimethyl ester exchanged using toluene-*p*-sulfonic acid. The greek letters refer to the meso assignments.

efficiency of the labeling. Direct treatment of PP-IX with deuterated toluene-*p*-sulfonic acid also accomplishes[25] efficient deuteration at the vinyl H_β positions, but in this case the meso protons are also partially, but selectively, exchanged (vide infra).

At the Meso (Methine) Positions. Owing to the high reactivity of the vinyl groups, few if any reactions at the meso positions of PP-IX have been described. Meso carbon-13-labeled samples of the porphyrin have been synthesized in connection with peak assignments

(23) If the zinc(II) is omitted from the starting material, then novel "triple-decker" mercury complexes result; M. F. Hudson and K. M. Smith, *Tetrahedron*, **31**, 3077 (1975).
(24) K. C. Langry and K. M. Smith, to be published.
(25) D. L. Budd, G. N. La Mar, K. C. Langry, K. M. Smith, and R. Nayyir-Mazhir, *J. Am. Chem. Soc.*, in press.
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for biosynthetic studies of the "type-III" problem.[6]

Meso deuteration using hexapyridylmagnesium diiodide and a deuterium source (D_2O or MeOD) achieves[27,28] meso deuteration, with all four meso positions being approximately equally deuterated. If, however, electrophilic deuteration using deuterated toluene-*p*-sulfonic acid is carried out, then *partial* specific deuteration is achieved in which (Figure 2) the positions furthest away from the 2- and 4-vinyl groups (i.e., γ and δ) are most heavily deuterated.[28] This selectivity is not quite what might have been expected on the basis of deactivation by conjugation of protonated porphyrin vinyl groups (Por-CH^+CH_3) with the meso positions. Simple consideration of resonance forms shows that the γ and β meso positions should be most heavily exchanged in an electrophilic substitution process, and this is the case[28] with 2,4-diacetyldeuteroporphyrin IX (**31**) and deuteroporphyrin IX (**36**) esters. The solution to the anomaly lies in the observation that the vinyl C_α hydrogens (CH=) are exchanged [as well as $C_\beta H$ (CH_2=)], indicating that the primary carbonium ion (Por$CH_2CH_2^+$) is also present in acid. These functions at positions 2 and 4 would be expected to shield the corresponding meso positions (at α and β) from attack by deuterons. Such vinyl-derived primary carbonium ions have been implicated in a novel chlorophyll vinyl cyclization[29] and in carbon-13 NMR spectra.[30] Perhaps the most important ramification of the specific exchange in PP-IX is that specific single substitutions at meso positions, using lengthy total synthesis, and upon which we had embarked,[31] are no longer necessary.

At the 1-, 3-, 5-, and 8-Methyl Groups. It could never have been predicted, at least by ourselves,[31] that the *methyl* groups at the 1, 3, 5, and 8 positions in PP-IX would show differential reactivity. However, they do, and in a quite remarkable way. In our early work[11] on the deuteration of the methyl groups, by total synthesis from acetylacetone, we observed a quite unexpected phenomenon. The base-catalyzed dehydrohalogenation of the bis(2-chloroethyl)porphyrin to give the 5,8-deuterated PP-IX **5** went uneventfully, and all of the deuterium present in the precuror was retained in the product. However, when the same procedure was carried out on the bis(2-chloroethyl)-porphyrin to produce the 1,3-deuterated analogue **4**, approximately 30% of the deuterium was lost, presumably by some unfortunate exchange process. It appeared that the 1- and 3-methyls (i.e., those on vinyl-bearing rings) were more acidic than those on propionate-bearing rings *even though all four pyrrole subunits were part of a large delocalized system.* A statement of Woodward's came to mind that . . . "in electronic networks such as those present in the porphyrins and chlorins, the π-electrons tend to congregate in sextets within the small rings embedded in the larger system."[32] Even though the methoxycarbonyl group in rhodoporphyrin XV dimethyl ester had apparently deactivated the system toward electrophilic substitution at any meso positions, it seemed possible that we had, in the unexpected loss of deuterium, observed an example of each pyrrole subunit behaving as its own small aromatic system with a characteristic substituent effect.

To test the hypothesis, the 1,5-deuterated porphyrin **7** was synthesized using the tripyrrene route (Scheme I). The low-spin dicyanoferriheme displayed an NMR spectrum in which it would be clearly seen that, since both the 1- and 5-methyls should be deuterated to about the same extent (≥90%), deuterium has been lost from the 1-methyl; thus, greater acidity of the methyl protons on rings bearing electron-withdrawing groups had been clearly demonstrated.[16] These observations have been optimized[33] such that we can now *insert* deuterium specifically into the 1- and 3-methyls by a base-catalyzed exchange using either PP-IX itself or the corresponding 2,4-diacetyldeuteroporphyrin derivative, **31**.[16] The conclusions regarding variability of electron densities within the pyrrole subunits[34] of PP-IX have also been verified using NMR studies of porphyrin self-aggregation (vide infra).

At the Propionic Side Chains. The methylene protons adjacent to the carboxylate carbonyls can be readily replaced with deuterium by acid or base exchange, and these moieties have been assigned in heme and heme protein NMR spectra. The esters of PP-IX dimethyl ester can be readily reduced to the corresponding dialcohol, **37**; these functions can in turn be reduced to propyl,[14] (**38**) or else, via the mesylate, be transformed into the 3-cyanopropyl (**39**) and butyric ester (**40**) moieties.[35] These last side chains, being bishomoprotoporphyrin IX analogues, are of interest for investigation of the tolerance of the heme cavity in heme proteins; the propionate analogues (e.g., PP-IX) reconstitute such that the propionic acid residues are on the outside of the molecule, and it is of interest to examine the effect of side-chain homologation on protein reconstitution, spectra, and oxygen binding. The corresponding 6,7-diacetic analogue is also being synthesized in our laboratory, and reconstitution experiments with these compounds will be reported in due course.

NMR Spectroscopy

The NMR spectra of metal-free PP-IX in $CDCl_3$ exhibit[36] a reproducible concentration dependence, and this has been analyzed in terms of a specific face-to-face aggregate. Such molecular complexes exhibit upfield shifts due to the anisotropic effect of the ring current from one member of the self-aggregate upon its neighbor.[37] Self-aggregation of metal complexes of symmetrical porphyrins has been analyzed by NMR[38,39] and EPR,[40,41] and these results have aided in the in-

(27) G. W. Kenner, M. J. Sutton, and K. M. Smith, *Tetrahedron Lett.*, 1303 (1973).
(28) K. M. Smith, K. C. Langry, and J. S. de Ropp, *J. Chem. Soc., Chem. Commun.*, in press.
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(32) R. B. Woodward and V. Škarić *J. Am. Chem. Soc.*, **83**, 4674 (1961).
(33) B. Evans, K. M. Smith, G. N. La Mar, and D. B. Viscio, *J. Am. Chem. Soc.*, **99**, 7070 (1977).
(34) G. N. La Mar, D. B. Viscio, K. M. Smith, W. S. Caughey, and M. L. Smith, *J. Am. Chem. Soc.*, **100**, 8085 (1978).
(35) Z. Martynenko and K. M. Smith, to be published.
(36) T. R. Janson and J. J. Katz, *J. Mag. Res.*, **6**, 209 (1972).
(37) For a review of porphyrin aggregation, see H. Scheer and J. J. Katz in "Porphyrins and Metalloporphyrins", K. M. Smith, Ed., Elsevier, Amsterdam, 1975, p 399.
(38) R. J. Abraham, G. H. Barnett, G. E. Hawkes, and K. M. Smith, *Tetrahedron*, **32**, 2949 (1976).
(39) R. J. Abraham, B. Evans, and K. M. Smith, *Tetrahedron*, **34**, 1213 (1978).

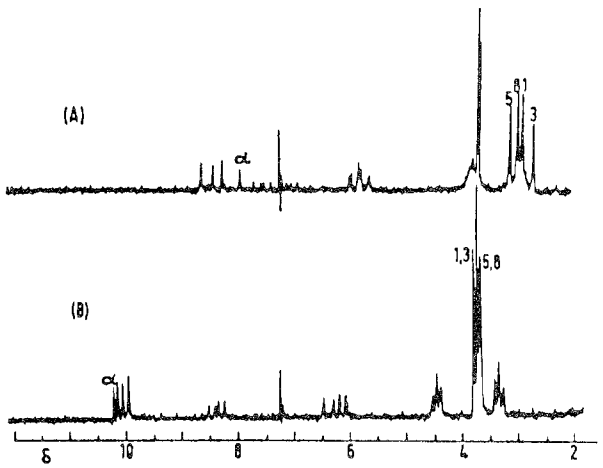

Figure 3. 100-MHz proton NMR spectra, in $CDCl_3$, of zinc(II) PP-IX (41) dimethyl ester (0.095 M): (A) before addition of pyrrolidine; (B) after disaggregation with ~1 equiv of pyrrolidine. Arabic and Greek symbols above resonances refer to assignments of methyls and meso protons designated on the structural formulas.

terpretation for more complex cases and in the development of a network model of the ring current effect in the porphyrin and chlorin (7,8-dihydroporphyrin) rings,[42] from which interplanar distances and lateral displacements can be calculated using the observed upfield (and occasionally downfield) NMR shifts.

In the course of a study using substituent chemical shifts designed to achieve definitive carbon-13 NMR assignments of all peaks in PP-IX and its derivatives (as an aid to the use of the NMR technique in tetrapyrrole biosynthesis[6]), we observed[43,44] that the quaternary α-pyrrole carbons (i.e., those adjacent to the central nitrogen atoms) were either very broad or else not observable at all in the carbon-13 spectra. Using deuteration, it was deduced that this phenomenon was due to NH tautomerism,[44,45] since the β-pyrrole carbons were relatively sharp, and insertion of a metal ion resulted in dramatic sharpening of the α-carbon resonances; observation of sharp resonances is, of course, a prerequisite for specific assignment of spectral lines. Zinc(II) was chosen as the chelating metal ion since it is both easy to insert or remove in quantitative yield. However, the carbon-13 and proton NMR spectra of the zinc(II) porphyrins showed remarkable concentration dependence due to self-aggregation. The aggregates could readily be decomposed by addition of equimolar amounts of bases such as pyrrolidine or pyridine to give the corresponding "infinite dilution" spectra of the monomer.[46-48] Figure 3 shows the proton NMR

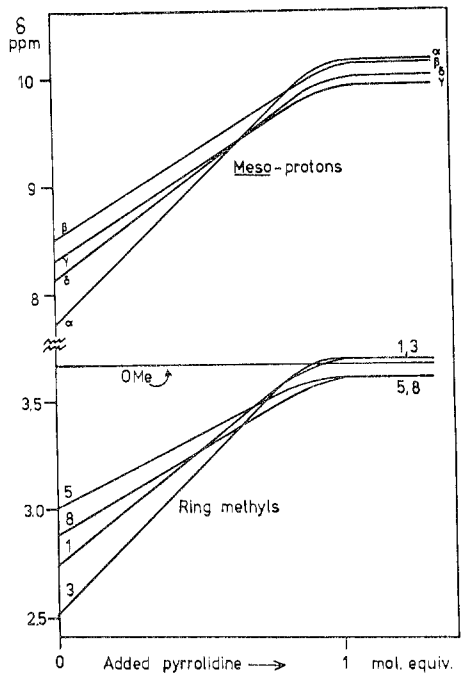

Figure 4. Proton chemical shifts (Varian XL-100), in $CDCl_3$, of meso and peripheral protons on zinc(II) PP-IX (41) dimethyl ester (0.095 M) vs. added pyrrolidine.

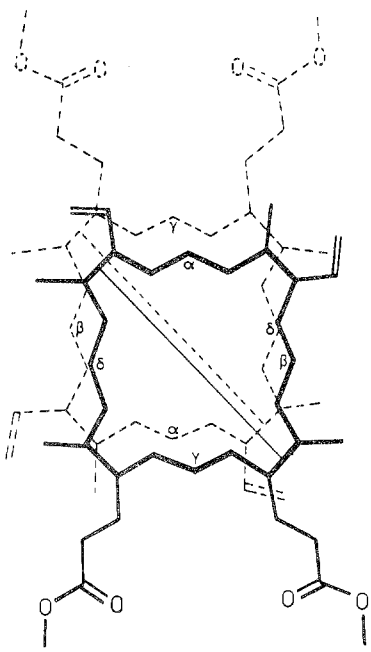

Figure 5. The dimer structure for 41 dimethyl ester.

spectra, before and after addition of pyrrolidine, for zinc(II) protoporphyrin IX (41) dimethyl ester; the carbon-13 spectra show a similar aggregation. An accurate titration experiment using pyrrolidine for disaggregation is displayed in Figure 4. The unique assignments for the methyl groups were made using the specifically deuterated samples described earlier, whereas those for the meso protons were made[48] from off-resonance decoupling experiments using the known carbon assignments.[6] An analysis of the shifts affords[48] a geometry for the dimer, or higher aggregate, as shown in Figure 5, with an inter-ring separation of approximately 4.5 Å and a smaller lateral displacement. Of special interest is the fact that rings A and B of one porphyrin molecule are situated above rings C and D

(40) M. Chikira, H. Kon, and K. M. Smith, *J. Chem. Soc., Chem. Commun.*, 906 (1978).
(41) M. Chikira, H. Kon, R. A. Hawley, and K. M. Smith, *J. Chem. Soc., Dalton Trans.*, 245 (1979).
(42) R. J. Abraham, S. C. M. Fell, and K. M. Smith, *Org. Mag. Res.*, **9**, 367 (1977).
(43) R. J. Abraham, G. E. Hawkes, and K. M. Smith, *J. Chem. Soc., Perkin Trans. 2*, 627 (1974).
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(48) R. J. Abraham, S. C. M. Fell, H. Pearson, and K. M. Smith, *Tetrahedron*, **35**, in press.

Table I
Aggregation Shifts ($\Delta\delta$)[a] of Zinc(II) Porphyrins and pK_3 Values for the Corresponding Metal-Free Porphyrins

Porphyrin	Zn meso-IX dimethyl ester	Zn copro-III tetramethyl ester	Zn deutero-IX dimethyl ester	Zn PP-IX dimethyl ester	Zn 2-formyl-4-vinyldeutero-IX dimethyl ester
$\Delta\delta$/ppm	0.50	0.54	0.70	1.16	1.62[b]
pK_3 of metal-free porphyrin	5.85	5.58	5.50	4.80	3.75

[a] Downfield shift of the center of gravity of the meso protons before and after addition of pyrrolidine to a 0.0065 M solution in $CDCl_3$. Spectra at 300 MHz. [b] Spectrum obtained at 100 MHz.

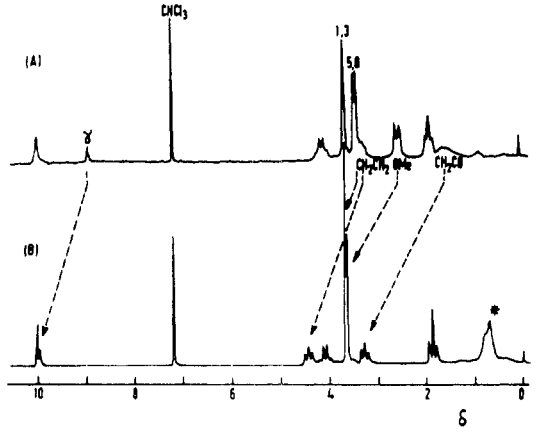

Figure 6. 100-MHz proton NMR spectra, in $CDCl_3$, of magnesium mesoporphyrin IX dimethyl ester (0.016 M): (A) before addition of pyrrolidine; (B) after disaggregation with a slight excess of pyrrolidine. The peak marked with an asterisk in B is due to pyrrolidine.

of the neighbor. This implies a donor–acceptor type of interaction between the rings for orientation of the dimer and is physical manifestation of the chemical exchange observations[33] described earlier. A similar phenomenon has been reported by La Mar for iron complexes.[49]

It is noticeable that in Figure 4 there is no shift of the ester methoxyl resonance as the zinc(II) complex aggregate is destroyed with pyrrolidine. The situation with magnesium complexes is quite different. Figure 6 displays the proton NMR spectra, before and after addition of pyrrolidine, of the mesoporphyrin IX dimethyl ester magnesium (II) complex. Here, analysis of the incremental shifts shows[14] that the propionic ester side chains are involved in the aggregation and that a quite different geometry in which the carbonyl oxygen is coordinated to the magnesium atom in a neighbor is implicated. Similar side chain to magnesium interactions are commonplace in chlorophyll chemistry.[37]

For the donor–acceptor type of aggregate occurring in zinc(II) complexes, the magnitude of the upfield shifts (and therefore of the aggregation effect itself) can be influenced by variation of several factors. If strongly electron-withdrawing substituents are placed at the 2 and 4 positions of the deuteroporphyrin IX (**36**) dimethyl ester zinc complex, then the aggregation gets stronger, as evidenced by the magnitude of the average upfield shift of the meso protons. Table I shows[15] a series of examples, and the shifts are correlated with the pK_3 (porphyrin free base to monocation) of the corresponding metal-free porphyrin (a good measure of the electronic effect of the 2 and 4 substituents). An extreme case[50] is free-base 2,4-dicyanodeuteroporphyrin IX (**43**) dimethyl ester in which massive shifts of all four meso protons and of one ring methyl (the 3-methyl) are apparent.

(49) G. N. La Mar and D. B. Viscio, *J. Am. Chem. Soc.*, **96**, 7354 (1974).

Table II
Aggregation Shifts ($\Delta\delta$)[a] for Mesoporphyrin IX Dimethyl Ester and Metal Complexes, and Oxidation Potential Differences ($\Delta E^1_{1/2}$)

Porphyrin	meso-IX dimethyl ester	Pd(II) complex	Ni(II) complex	Zn(II) complex	Cd(II) complex
$\Delta\delta$/ppm	0.05[b]	0.05	0.09	0.61	1.31
$\Delta E^1_{1/2}$/V	0.00	−0.01	0.08	0.18	0.26

[a] Calculated by taking the center of gravity of meso proton resonances before and after addition of pyrrolidine to a 0.015 M solution in $CDCl_3$. Ni(II) porphyrins become paramagnetic when treated with pyrrolidine, so the dilution shifts for this case were taken from the literature. [b] Free base dilution shift.

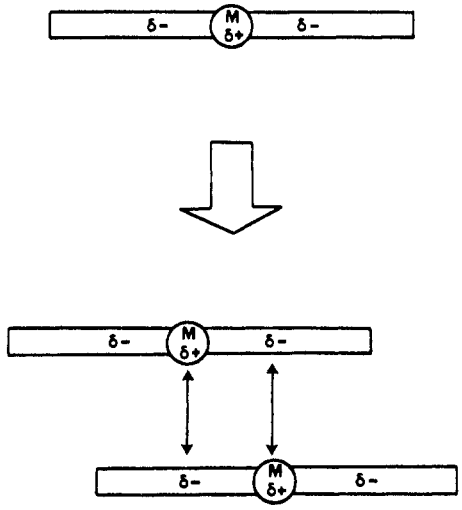

Figure 7. Schematic diagram of interactions in metalloporphyrin π–π dimers.

If, on the other hand, the metal ion is varied between diamagnetic ions, then the aggregation for a particular parent porphyrin is affected. Table II shows[15] the aggregation of several metal complexes of mesoporphyrin IX (**42**) dimethyl ester. These relative shifts are correlated with the difference in one-electron oxidation potential between the free porphyrin and the corresponding metal complex.[51] It can be seen that those metal ions which most effectively release electron density to the porphyrin ligand (i.e., which have the

(50) R. J. Abraham, F. Eivazi, R. Nayyir-Mazhir, H. Pearson, and K. M. Smith, *Org. Mag. Res.*, 11, 52 (1978).

(51) Since oxidation potentials for metal complexes of mesoporphyrin IX are not available in the literature, we chose to use[19] the corresponding potential differences for octaethylporphyrin derivatives. Within the series, these *differences* are likely to be close to the required ones for the mesoporphyrin IX case.

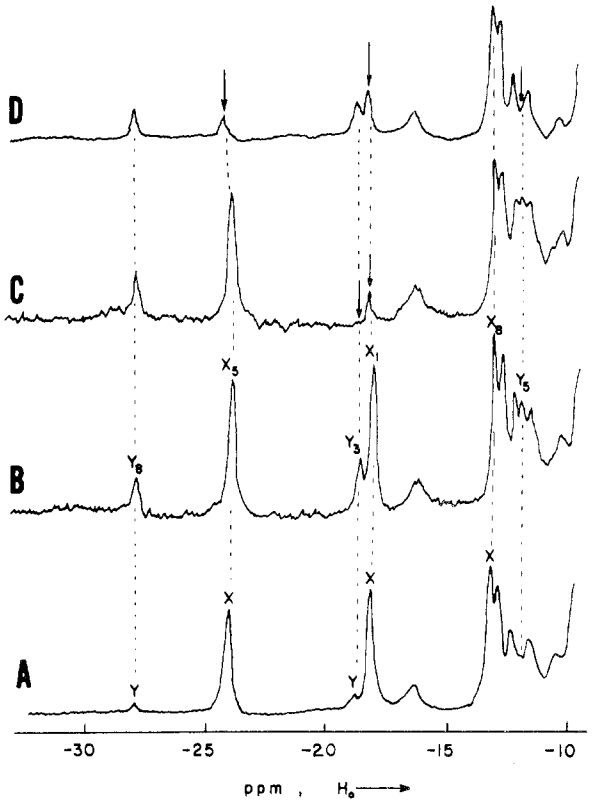

Figure 8. Proton NMR traces of deuteroporphyrin-reconstituted sperm whale metMbCN (deutero-metMbCN), at pH 8.5, 38 °C, in 0.2 M NaCl/D_2O. (A) Protein taken to pH 11.1, KCN added, pH reduced to 7, and mixture stored for several hours, and then adjusted to pH 8.5. One component dominates strongly, although minor peaks, labeled Y, are detectable. (B) Protein taken to pH 11.1, KCN added, and pH readjusted to 8.5; the minor component has increased in intensity and a third peak is evident. (C) 1,3-Deuterated deutero-metMbCN treated as in (B). (D) 1,5-Deuterated deutero-metMbCN treated as in (B). Peaks in (C) and (D) with reduced intensity due to deuterium labeling are indicated with arrows.

lowest oxidation potential or the largest metal-free/metal complex potential difference) aggregate the strongest. Metalloporphyrins with full shell central metal ions such as zinc(II) or cadmium(II) behave like porphyrin dianions and divalent metal ions since there is no $d\pi$–$p\pi$ back-donation of electron density from the ring to the metal. Under such circumstances (Figure 7) the electrostatic difference between porphyrin and metal ion is counteracted by *inter*molecular rather than *intra*molecular interactions, i.e., by face-to-face aggregation. For palladium(II) complexes, intramolecular $d\pi$–$p\pi$ interaction with empty d orbitals on the metal results in less intermolecular donor–acceptor aggregation. Thus, on the basis of a simple set of criteria, it is now possible to determine whether or not a particular metalloporphyrin will aggregate, and, by consideration of the site of electronegative substituents, the geometrical arrangement within the aggregate can be predicted.

As previously mentioned, NMR spectroscopy of iron derivatives of PP-IX is of great importance because information on the electronic structure of hemes in heme proteins can be obtained. The methyl-deuterated derivatives 4–7 have been used to make assignments in free hemes[12] and in myoglobin,[13] and from these definitive results we have been able to test several important properties of physiologically important systems, as well as quantum mechanical calculations.[52] Perhaps the most startling observation, using methyl-deuterated compounds, is associated with deuterohemin IX reconstituted Mb. X-ray studies have reported that in deuterohemin reconstituted Mb the heme is situated within the heme pocket in a unique orientation. Because natural porphyrins are completely unsymmetrical, there are nominally two possible orientations for insertion of the heme during reconstitution, and bearing in mind the absolute necessity for the propionic acid residues to be on the outside of the cleft, these orientations differ by a 180° rotation about the α,γ axis. However, some heme protein NMR spectra show unaccountable doubling-up of peaks, and because of the X-ray results, the extra peaks have usually been as-

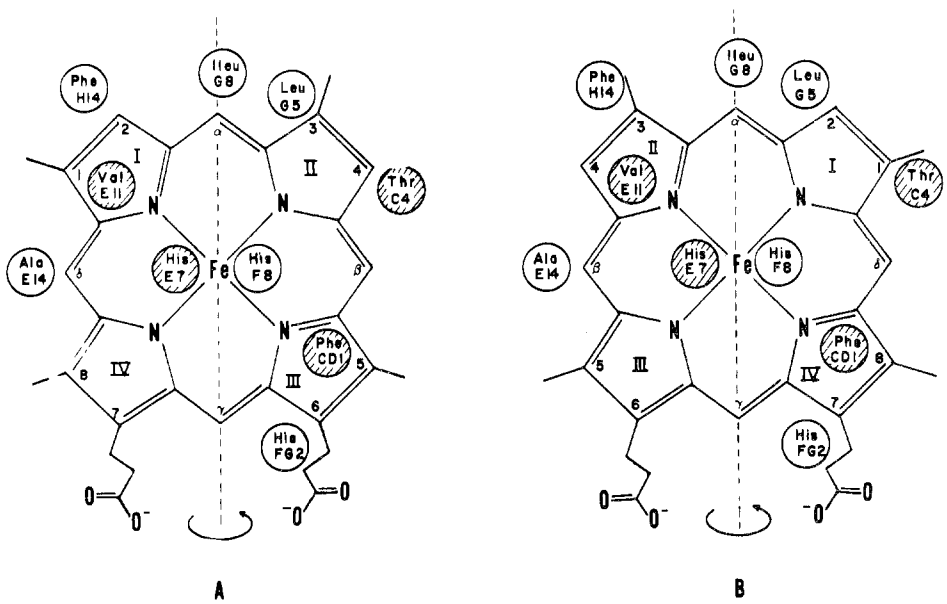

Figure 9. Heme–apoprotein contacts for protoheme in the pocket of sperm whale Mb. Open circles indicate contacts on the proximal side; shaded circles represent contacts on the distal side of the heme. Only the methyl groups and the propionic acid substituents are included to show the case for deuteroheme. (A) Normal orientations as found in the native protein. (B) Reversed orientations, with the heme rotated 180° in the heme pocket about the α,γ axis.

signed to impurities. To return to the deutero-metMB case,[53] Figure 8A shows the proton NMR spectrum of deuterohemin reconstituted sperm whale metMb cyanide. This reveals the presence of two small peaks, with intensity less than 10% of the presumed methyls, which might easily be dismissed as impurities. Slight variation of conditions, however, results in increase of the intensity of the two minor peaks (Figure 8B) at the expense of the major ones, and these peaks are freely interconvertible following a prescription (legend). Using synthetic 1,3- and 1,5-labeled deuterohemins (Figure 8(C,D)), obtained from the corresponding PP-IX derivatives, it is possible to show unambiguously that these two sets of peaks are due to different proteins *differing only in a 180° rotation of the heme about the* α,γ *axis*. Since the chemical shifts are strongly dependent upon the electronic structure, which is in turn related to heme–protein contacts, it would be expected (Figure 9) that the shift of 5-methyl in one protein would be similar to the shift of 8-methyl (180° α,γ rotation) in the other. The same argument follows for methyls 1 and 3. This evidence[53] for disordered solution structure in certain heme proteins contrasts with the crystallographic data, and hence the possibility of different solution and solid structures may have to be considered.

Using the deuterium-labeled vinyl derivatives of PP-IX, we have examined[25] a series of 5- and 6-coordinate high-spin ferric complexes and have located the elusive vinyl groups in these compounds. In myoglobins, the H_α peaks of the vinyls are located considerably downfield from where they have been proposed, using models, to resonate.

It has been my intention to show herein that PP-IX, the "first porphyrin", possesses many unexpected properties. There can be little doubt that further research will uncover yet more subtle facets of the chemistry of this physiologically important ligand.

It gives me great pleasure to acknowledge the assistance of the senior collaborators and talented graduate students and postdoctoral fellows who have worked with me on the projects described above; their names appear in the appropriate literature citations. I wish particularly to single out the late George W. Kenner, F.R.S., who introduced me to porphyrin chemistry, and Gerd N. La Mar, for my introduction to Davis. My research group and I are grateful to the National Institutes of Health (HL 22252; AM 25714) and National Science Foundation (CHE 78-25557) for continuing support of our endeavors.

(52) R. G. Shulman, S. H. Glarum, and M. Karplus, *J. Mol. Biol.*, **57**, 93 (1971).
(53) G. N. La Mar, D. L. Budd, D. B. Viscio, K. M. Smith, and K. C. Langry, *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 5755 (1978).