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New Syntheses of Deuterated Protoporphyrin-IX Derivatives for Heme Protein nmr Studies

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An efficient total synthesis of 1,5-di(trideuteromethyl)protoporphyrin-IX (**3**) dimethyl ester from monopyrrole precursors is described, the synthesis proceeding through crystalline tripyrrene and a,c-biladiene salt intermediates. The 2- and 4-vinyl groups in (**3**) are formed from the corresponding (2-chloroethyl) substituents by way of base-promoted dehydrochlorination. In protio solvents, this synthetic step is shown to exchange out preferentially deuterons in the 1-methyl group, and this observation is exploited in an efficient synthesis of the 1,3-di(trideuteromethyl)protoporphyrin-IX (**22**) dimethyl ester from 2,4-diacetyldeuteroporphyrin-IX (**20**) dimethyl ester (which is in turn accessible from commercially available protoporphyrin-IX (**5**)). Thus, basic exchange in deuterated solvent of (**20**) gives the deuterated analog, which after reduction and dehydration gives the 1,3-di(trideuteromethyl)protoporphyrin-IX analog (**22**), in which the vinyl H_2 and propionic $CH_2 \cdot CO$ functions have also become deuterated.

INTRODUCTION

The importance of hemoproteins in oxygen and electron transport is well recognized (*1*, *2*). Hemoglobin, the pigment which is involved in oxygen transport, is found in the red blood cells and is required for the transport of oxygen from the lungs to the tissues. Hemoglobin also assists in a second task which involves transport of carbon dioxide, a by-product in metabolism, back to the lungs. Myoglobin, a simpler but related hemoprotein, is largely concentrated in cardiac tissue in humans, and stores oxygen molecules until they are required in metabolism. The important roles of these two proteins were recognized even at the end of the last century. The recent separation of the molecules into heme and protein portions, and their reconstitution to give physiologically active hemoproteins (*1*), has opened up a new perspective for studying structure–function relationships in these complex systems by carrying out chemical and spectroscopic measurements on hemoproteins which have been reconstituted with modified hemes. An important achievement in recent years has been the establishment of the detailed structure of myoglobin (*4*) and hemoglobin (*5*) using X-ray diffraction. Myoglobin contains a heme, partially surrounded by a polypeptide chain consisting of 152 amino acid residues. Hemoglobin contains two pairs of polypeptide chains; each α-chain contains 141 amino acids, and each β-chain contains 146 residues. Each of the individual polypeptide (globin) chains partially surrounds a heme molecule.

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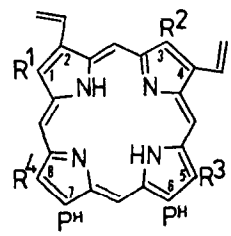
A problem occupying many research groups at the present time is the rationalization of the oxygen binding properties of both myoglobin and hemoglobin and the correlation with structure. Heme alone does not reversibly bind oxygen, but instead its iron atom is oxidized. One of the aspects of studies undertaken concerns the environment of the active site, and proton nmr spectroscopy is well suited to probe the nature of the heme pocket. In paramagnetic hemes, apart from the shifts due to the strong ring current, which also occur in their diamagnetic counterparts, very large chemical shifts of the protons associated with groups directly attached to the macrocycle or apical ligands are observed. These large paramagnetic shifts arise either from a contact interaction as a result of the spin transfer to the porphyrin ligand or from a pseudocontact or dipolar interaction, which is a through-space interaction between the nuclear spin and the net electron magnetic moment.

In order to assign the heme ring methyls, the 1,8-di(trideuteromethyl)- (**1**), 5,8-di(trideuteromethyl)- (**2**), and 1,3-di(trideuteromethyl)protoporphyrin-IX (**3**) derivatives were synthesized in our earlier work (*6*). The spectra of the corresponding low-spin dicyano–iron(III) complexes allowed assignment of the methyl peaks in the free hemes (*6*) and in myoglobin (*7*); several other observations concerning not only myoglobin intercalation with cyclopropane or xenon (*8*) but also hindered rotation in methyl groups (*9*), were clarified with these definitive assignments.



$P^R = CH_2CH_2CO_2R$

(1) $R^1 = R^4 = CD_3$; $R^2 = R^3 = Me$
(2) $R^3 = R^4 = CD_3$; $R^1 = R^2 = Me$
(3) $R^1 = R^2 = CD_3$; $R^3 = R^4 = Me$
(4) $R^1 = R^3 = CD_3$; $R^2 = R^4 = Me$
(5) $R^1 = R^2 = R^3 = R^4 = Me$

In connection with further extensions of the peak assignment problem in myoglobins and hemoglobins of various spin states (G. N. La Mar and K. M. Smith, work in progress), we required additional samples of 1,5-di(trideuteromethyl)protoporphyrin-IX (**4**). The present paper describes the total synthesis of (**4**) using the new tripyrrene route (*10*) and an unexpectedly facile total synthesis of (**3**) starting from commercially available protohemin or protoporphyrin-IX (**5**). Our new route to (**3**) resulted from a chance observation during our synthetic approach to (**4**).

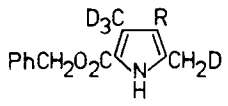
RESULTS AND DISCUSSION

1,5-Di(trideuteromethyl)protoporphyrin-IX (*4*)

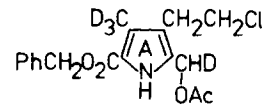
1,5-Di(trideuteromethyl)protoporphyrin-IX (**4**) lacks the nominal "synthetic symmetry" elements (*11*) present in the C and D rings of protoporphyrin-IX (**5**) because the

5-methyl group is deuterated, and therefore a general synthesis through open-chain tetrapyrroles is required if a single isomer is to be obtained in the final cyclization. The presence of vinyl groups in positions 2 and 4 requires, due to their lability, masking during the synthetic steps leading to the porphyrin macrocycle. Chloroethyl side chains, introduced at the pyrrole stage, have proved ideal for this purpose in previous syntheses of porphyrins (*10*, *11*), and were therefore chosen again.

Pyrrole (**6**) (*6*) was treated with diborane in tetrahydrofuran to produce the hydroxyethylpyrrole (**7**), which was chlorinated with thionyl chloride in methylene chloride/pyridine to give (**8**). The acetoxymethylpyrrole (**9**) was obtained in almost quantitative yield by treatment of pyrrole (**8**) with lead tetraacetate. Deuterium was retained in this step in the acetoxymethyl methylene, presumably due to the primary isotope effect. The undeuterated ring B precursor (**10**) was synthesized as previously described (*10*) from pyrrole (**11**).



(6) R = CH_2CO_2Me
(7) R = CH_2CH_2OH
(8) R = CH_2CH_2Cl



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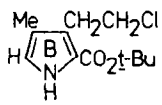

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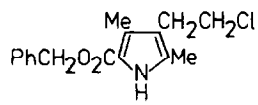

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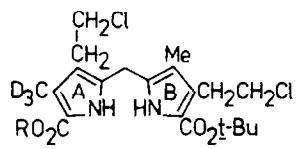
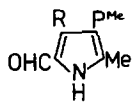


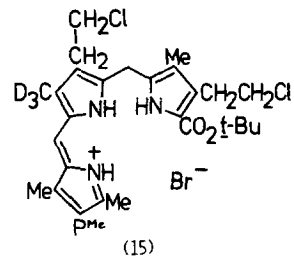
(12) R = $PhCH_2$
(13) R = H

Condensation of pyrroles (**9**) and (**10**) using a catalytic amount of *p*-toluenesulfonic acid in acetic acid yielded the pyrromethane ester (**12**) (70% yield), which was hydrogenated over palladized charcoal to cleave the benzyl ester and yielded the pyrromethane acid (**13**). Condensation of this pyrromethane with the undeuterated formylpyrrole (**14**) in methylene chloride and methanol (*11*) containing *p*-toluenesulfonic acid yielded the tripyrrene (**15**), isolated as the hydrobromide in 54% yield. The deuterated formylpyrrole (**16**) was prepared from (**17**) in a way entirely similar to that of the undeuterated pyrrole (**14**). Tripyrrene (**15**) was treated with trifluoroacetic acid followed by the pyrrole (**16**) in methanol and 45% HBr/HOAc. The a,c-biladiene (**18**) was obtained as the dihydrobromide in 83% yield. Cyclization using cupric chloride in dimethylformamide afforded the copper(II) porphyrin, which was not isolated, but was

immediately demetalated with 5% sulfuric acid in trifluoroacetic acid to yield the bis(2-chloroethyl)porphyrin (**19**) in 57% yield. Treatment of the zinc chelate of (**19**) in tetrahydrofuran with a 1 *M* solution of potassium *tert*-butoxide in *tert*-butyl alcohol afforded, after removal of the metal, the 1,5-di(trideuteromethyl)protoporphyrin-IX (**4**) dimethyl ester in 65% yield.



(14) R = Me
(16) R = CD_3



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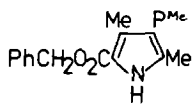

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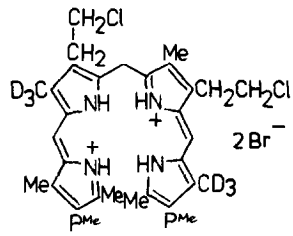


(18)

The porphyrin was transformed into the corresponding hemin dimethyl ester following conditions used in earlier syntheses (*6*) of other deuterated hemins, and the low-spin dicyanoferrihemin nmr spectrum (Fig. 1C) showed differential levels of deuterium to be present due to base-catalyzed exchange. As would be expected from our previously published observations (*12*), the 5-methyl showed more deuterium (ca. 90% CD_3) than the base-exchangeable 1-methyl (ca. 60%). Since the deuterium levels in the 1- and 5-methyls in the bis(2-chloroethyl) precursor (**19**) were equivalent (nmr, mass spectrometry), the label had obviously been lost from the 1-methyl after vinyl group formation in *tert*-butoxide/*tert*-butanol.

1,3,-Di(trideuteromethyl)protoporphyrin-IX (*3*)

We have previously shown (*12*) that deuterium can be incorporated into methyl groups on porphyrin subunits bearing electron-withdrawing functions. However, base-catalyzed exchange of protoporphyrin-IX (**5**) in deuterated solvents requires (*12*) lengthy treatment in base, does not completely deuterate the 1- and 3-methyls in less than 1 week, and allows only 50%, or less, recovery of porphyrin. We reasoned that the 2,4-diacetyldeuteroporphyrin (**20**) would undergo more rapid exchange at the 1- and 3-methyls owing to the greater electron-withdrawing nature of the acetyl groups relative to the vinyls in (**5**). There also existed some experimental nmr [D. L. Budd and G. N.

La Mar, unpublished results; (*13*)] and esr (*14*) spectral evidence to support this hypothesis.

Thus, 2,4-diacetyldeuteroporphyrin-IX dimethyl ester (**20**) was prepared from hemin

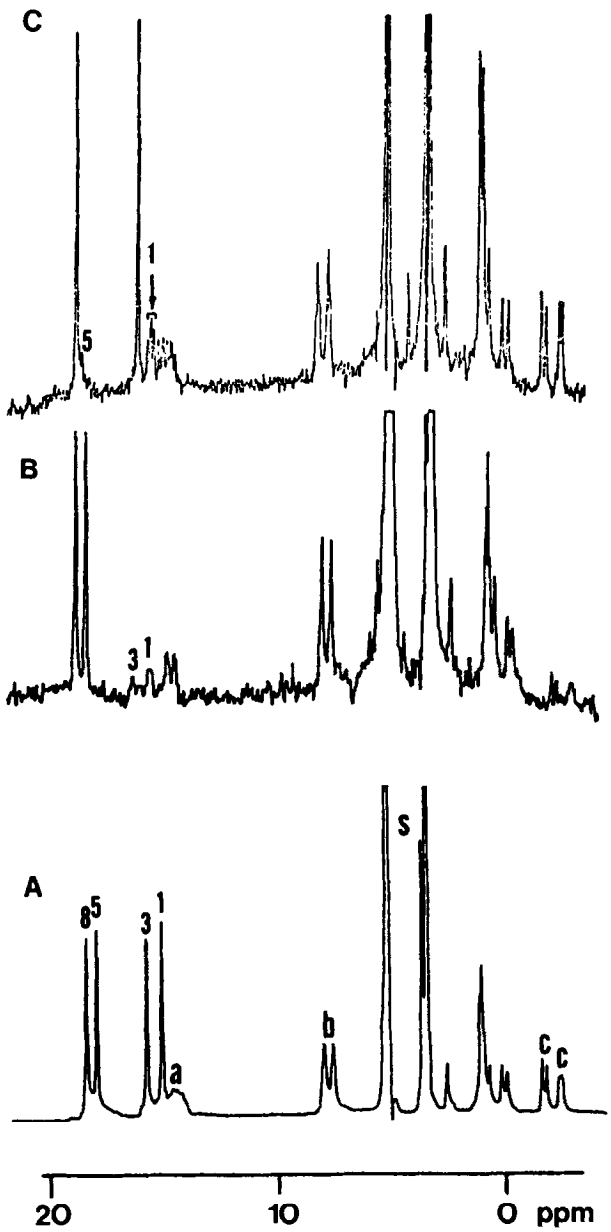
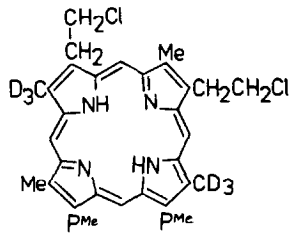
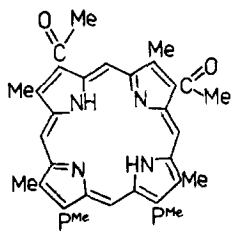


FIG. 1. Proton nmr spectra (JEOL PFT 100), in CD_3OD of the dicyanoferrihemes from: (A) protoporphyrin-IX dimethyl ester, undeuterated; (B) 1,3-di(trideuteromethyl)protoporphyrin-IX dimethyl ester (**22**); (C) 1,5-di(trideuteromethyl)protoporphyrin-IX dimethyl ester (**4**). Assignments labeled 1,3,5, and 8 refer to the methyl groups at the corresponding ring position. Other assignments in A are: a, the vinyl C*H*'s; b, the propionic methylenes adjacent to the porphyrin ring; c, the vinyl CH_2's; s, the solvent peaks.

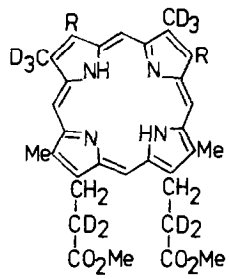
by standard methods (*15*) and was treated under reflux in tetrahydrofuran for 5 hr with approximately 0.6 *M* sodium methoxide in methanol-*d*. The resulting deuterated[1] ester was esterified with diazomethane and was then reduced with sodium borohydride in methanol and methylene chloride to give the hematoporphyrin-IX (**21**). The latter was dehydrated using benzoyl chloride (*16*) or by heating in *ortho*-dichlorobenzene containing *p*-toluenesulfonic acid (*17*) to give a 53% yield [overall, from (**20**)] of the 1,3-di(trideuteromethyl)protoporphyrin-IX (**22**). The nmr spectra of the corresponding



(19)



(20)



(21) R = $CH(OH)CD_3$

(22) R = $CH{=}CD_2$

low-spin dicyanoferriheme dimethyl ester and the undeuterated counterpart are shown in Fig. 1. It can clearly be seen that the 1- and 3-methyl groups have been exchanged (~90%), as well as the methylene protons adjacent to the ester carbonyls, and the vinyl methylenes [which originated from the acetyl methyl groups in (**20**)].

Studies on the effect of variations in the 2- and 4-substituents and on the effect of metal chelation (*12*) are in progress and will be reported elsewhere.

EXPERIMENTAL

Melting points were measured on a microscopic hot-stage apparatus. The tlc monitoring of all reactions was performed using Merck silica gel 60 F-254 precoated sheets (0.2 mm), and preparative tlc was carried out on 20 x 20-cm glass plates coated with Merck GF 254 silica gel (1.5 mm). Column chromatography was carried out on

[1] The nmr spectroscopy showed that, as expected, the acetyl CH_3 and propionate methylenes adjacent to the ester functions had also been deuterated.

Merck neutral alumina 90 (70–230 mesh). Electronic absorption spectra were determined using a Cary 15 spectrophotometer (solutions in CH_2Cl_2), and proton nmr spectra were measured, usually in $CDCl_3$ solution with tetramethylsilane as internal standard, at 100 MHz (Varian XL-100 or JEOL PFT-100). Mass spectra (direct insertion probe, 70 eV, 50 μA, source temperature approximately 200°C) were measured using an AE1 MS9 instrument.

2-Formyl-4-(2-methoxycarbonylethyl)-3-trideuteromethyl-5-monodeuteromethylpyrrole (16)

Benzyl 4-(2-methoxycarbonylethyl)-3-trideuteromethyl-5-monodeuteromethyl-pyrrole-2-carboxylate (*6*) (1.5 g) in tetrahydrofuran (40 ml) and triethylamine (three drops) was hydrogenated at room temperature and atmospheric pressure over palladized charcoal (10%, 150 mg) until uptake of hydrogen was complete (ca. 1.5 hr). The catalyst was filtered off on Celite and the filtrate was evaporated to dryness to give a pink solid, which was recrystallized from tetrahydrofuran–hexane to give the pyrrole-2-carboxylic acid. This was dissolved in trifluoroacetic acid (7 ml) and stirred under nitrogen for 30 min before evaporation of the solvent. Methylene chloride and water were added and the organic phase was washed with aqueous sodium bicarbonate and water, then dried over sodium sulfate before concentration to a volume of ca. 10 ml. This solution was then added dropwise to dry methylene chloride (5 ml) containing the complex formed from allowing phosphorus oxychloride (1.88 g) and *N,N'*-dimethylformamide (0.9 g) to stand in dry ether (30 ml) for 30 min. Aqueous 1 *N* sodium carbonate (100 ml) was added slowly and the heterogeneous mixture was refluxed with vigorous stirring for 1 hr. The organic phase was separated, washed with water, and then dried over sodium sulfate. Evaporation gave a residue which was chromatographed on grade II neutral alumina (elution with methylene chloride). A slow running fraction was collected and evaporation of the eluates gave a solid which was recrystallized from methanol to give the formylpyrrole (615 mg; 62%) as yellow crystals, mp 128–129°C [lit. mp 130–132°C (*18*) undeuterated].

^{1}H nmr spectrum in $CDCl_3$, τ: 0.50 (1H, s) C*H*O; 6.30 (3H, s) OMe; 7.15–7.58 (4H, m) CH_2CH_2CO; 7.70 (m) CD_3 and CDH_2.

Benzyl-5'-tert-Butoxycarbonyl-3,4'-bis-(2-chloroethyl)-3'-methyl-4-trideuteromethyl-pyrromethane-5-carboxylate (12)

tert-Butyl 3-(2-chloroethyl)-4-methylpyrrole-2-carboxylate (**19**) (232 mg) in glacial acetic acid (20 ml) was treated with benzyl 2-acetoxymonodeuteromethyl-3-(2-chloroethyl)-4-trideuteromethylpyrrole-5-carboxylate (*6*) (334 mg) and then toluene *p*-sulfonic acid hydrate (9.5 mg) before being stirred under nitrogen at 45°C for 4 hr. The mixture was poured into water, extracted with methylene chloride, washed with aqueous sodium bicarbonate, then water, and then dried over sodium sulfate. Evaporation gave an oil which was chromatographed on grade III neutral alumina, the product being eluted with methylene chloride. The appropriate eluates (tlc monitoring) were evaporated and the resultant oil was dried to a light yellow brittle foam under high vacuum. Yield: 355 mg (70%).

^{1}H nmr spectrum in $CDCl_3$, τ: 0.70 (1H, br), 0.99 (1H, br) 2 × NH; 2.69 (5H, s) C_6H_5; 4.76 (2H, s) $C_6H_5CH_2$; 6.16 (35% H, m) C*H*D; 6.41 (2H, t), 6.60 (2H, t), 7.18 (2H, t) 2 × CH_2CH_2Cl; 7.80 (ca. 25% H, m) CD_3; 8.01 (3H, s) *Me*; 8.52 (9H, s) *tert*-Bu.

Tert-Butyl 4,6-Di(2-chloroethyl)-1-(2-methoxycarbonylethyl)-3-trideuteromethyl-1',2,5-trimethyltripyrrene-a-6'-carboxylate Hydrobromide (*15*)

Benzyl 5'-*tert*-butoxycarbonyl-3,4'-di(2-chloroethyl)-4-trideuteromethyl-3'-methylpyrromethane-5-carboxylate (*19*) (273 mg) in tetrahydrofuran (15 ml) containing triethylamine (0.1 ml) and 10% palladized charcoal (30 mg) was hydrogenated at room temperature and atmospheric pressure until uptake of hydrogen had ceased. After filtration through Celite, the filtrate was evaporated and the product was recrystallized from tetrahydrofuran–hexane to give the pyrromethane acid (225 mg). This pyrromethane and 2-formyl-4-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole (*18*) (88 mg) in methylene chloride (30 ml) were stirred with a solution of toluene *p*-sulfonic acid hydrate (240 mg) in methanol (3 ml) for 40 min. The solution was washed with water, aqueous sodium bicarbonate, and water, and dried over sodium sulfate before evaporation to dryness. Dry methylene chloride (30 ml) was added, followed by hydrogen bromide gas, which was passed through the brown solution until it became red in color (5 sec). Quantities of dry benzene (2 × 50 ml) were added and evaporated in order to azeotrope unwanted hydrogen bromide and water. The resulting solid was recrystallized from dry methylene chloride–ether to give the tripyrrene hydrobromide (180 mg; 54%) as bright red microprisms, mp 160°C (decomp.) [lit. mp 160°C, decomp. (*10*), undeuterated].

^{1}H nmr spectrum in $CDCl_3$, τ: −4.04 (1H, br), −3.88 (1H, br) 2 × NH^+; −0.75 (1H, br) NH; 2.92 (1H, s) methine-*H*; 5.76 (ca. 50%H, m) C*H*D; 6.35 (3H, s) *OMe*; 6.40 (2H, t), 6.88 (2H, t), 7.12 (2H, t), 7.32 (2H, t), 7.52 (2H, t) 2 × CH_2CH_2Cl and CH_2CH_2CO; 7.34 (3H, s), 7.70 (3H, s), 7.91 (3H, s) 3 × *Me*; 7.72 (ca. 30%H, m) CD_3; 8.44 (9H, s) *tert*-Bu.

$\lambda_{max(nm)}(\varepsilon_{mM})$: 486 (92).

3,5-Di(2-chloroethyl)-1,8-di(2-methoxycarbonylethyl)-1',4,7,8'-tetramethyl-2,6-di(trideuteromethyl)-a,c-biladiene Dihydrobromide (*18*)

tert-Butyl 4,6-di(2-chloroethyl)-1-(2-methoxycarbonylethyl)-3-trideuteromethyl-1'-2,5-trimethyltripyrrene-*a*-6'-carboxylate hydrobromide (344 mg) was stirred in trifluoroacetic acid (3 ml) for 5 min before addition of 2-formyl-4-(2 methoxycarbonylethyl)-3-trideuteromethyl-5-monodeuteromethylpyrrole (108 mg) in methanol (6 ml) and then 45% HBr/HOAc (1.4 ml). After stirring for 30 min, ether (75 ml) was added dropwise with continued stirring. The a,c-biladiene dihydrobromide was filtered off and washed well with ether to give red-brown microprisms (384 mg; 83%) mp > 150°C, decomp. [lit. mp > 150°C, decomp. (*10*), undeuterated].

^{1}H nmr spectrum in $CDCl_3$, τ: −3.54 (1H, br), −3.48 (1H, br), −3.32 (2H, br) 4 × NH; 2.84 (2H, s) 2 × *methine-H*; 4.80 (2H, s) CH_2 bridge; 6.36 (6H, s) 2 × *OMe*; 6.30–6.52 (m), 6.75–7.06 (m), 7.10–7.36 (m), 7.42–7.60 (m) 2 × CH_2CH_2Cl and 2 × CH_2CH_2CO; 7.30 (6H, s), 7.66 (3H, s). 8.02 (3H, s) 4 × *Me*; 7.62–7.78 (m) 2 × CD_3.

$\lambda_{max(nm)}(\varepsilon_{m,M})$ in $CHCl_3$: 454 (26.7) and 566 (224).

*2,4-Di(2-chloroethyl)-6,7-di(2-methoxycarbonylethyl)-1,5-di(trideuteromethyl)-3,8-dimethylporphyrin (**19**)*

The foregoing deuterated a,c-biladiene dihydrobromide (**18**) (171 mg) was added to a solution of copper(II) chloride (850 mg) in *N,N'*-dimethylformamide (15 ml) kept at 145°C. The mixture was stirred for 4 min, then poured into water and extracted with methylene chloride, and the organic phase was washed three times with water before being dried over sodium sulfate. After evaporation the residue [copper(II) complex of the required porphyrin] was treated with sulfuric acid (0.5 ml) in trifluoroacetic acid (9.5 ml) with vigorous stirring for 20 min before being poured into water and extracted with chloroform. The organic phase was washed with water, aqueous sodium bicarbonate, and water, dried over sodium sulfate, and then evaporated to dryness. The residue was set aside overnight in 5% (w/v) sulfuric acid in methanol before being poured into aqueous sodium acetate and extracted with methylene chloride. The organic phase was washed with aqueous sodium bicarbonate, dried over sodium sulfate, and evaporated to dryness. The residue was chromatographed on alumina (grade III, elution with methylene chloride) and evaporation of the red eluate gave a residue which was crystallized from methylene chloride–methanol to give deep red crystals (76 mg; 57%), mp 217–219°C [lit. mp 216–218°C (*10*), undeuterated]. Apart from peak absences in the nmr spectrum, the sample was identical with an authentic sample (*20*).

^{1}H nmr spectrum in $CDCl_2$ (~0.4 *M*), τ: 0.04 (1H, s), 0.14 (1H, s), 0.19 (1H, s), 0.27 (1H, s), 4 meso-H; 5.59–6.00 (12H, m) 2 × CH_2CH_2Cl and 2 × $CH_2CH_2CO_2Me$; 6.39 (3H, s), 6.40 (3H × s) 2 × O*Me*; 6.53 (3H, s), 6.56 (3H, s) 2 × *Me*; 6.80 (4H, t) 2 × $CH_2CH_2CO_2Me$; 14.06 (2H, s) 2 × N*H*.

*6,7-Di(2-methoxycarbonylethyl)-1,5-di(trideuteromethyl)-3,8-dimethyl-2,4-divinylporphyrin, "1,5-Di(trideuteromethyl)protoporphyrin-IX (**4**) Dimethyl Ester"*

The foregoing deuterated 2,4-di(2-chloroethyl)porphyrin (**19**) (74 mg) was dissolved in chloroform (20 ml) and a solution of zinc acetate (0.5 g) in methanol (4 ml) was added. After refluxing for 5 min in a water bath the visible absorption spectrum showed zinc insertion to be complete (*21*). The chloroform solution was washed with aqueous sodium acetate and the organic phase dried over sodium sulfate and evaporated to dryness. The metalloporphyrin was dissolved in dry tetrahydrofuran (14 ml), a molar solution of potassium *tert*-butoxide in *tert*-butanol (26 ml) was added, and the solution was stirred for 70 hr in the dark. The solution was then neutralized with glacial acetic acid, poured into water, and extracted with chloroform (75 ml) to which a small quantity of pyridine (2 ml) had been added. The organic phase was dried over sodium sulfate and evaporated to dryness. The resultant solid was treated with 5% (w/v) sulfuric acid in methanol (100 ml) for 16 hr in the dark, and the solution was poured into aqueous sodium acetate and extracted with methylene chloride, washed with aqueous sodium bicarbonate, then water, dried over sodium sulfate, and evaporated to dryness. The residue was chromatographed on alumina (grade III, elution with methylene chloride). Evaporation of the eluate gave a residue which was crystallized from methylene chloride–methanol to give purple crystals (43 mg, 65%), mp 222–224°C [lit. mp, 228–229°C (*22*), undeuterated].

^{1}H nmr spectrum in $CDCl_3$ (~0.4 *M*), τ: 0.12 (1H, s), 0.18 (1H, s), 0.23 (1H, s), 0.29 (1H, s) 4 methine-H; 1.67–2.11 (2H, m) 2 × $CH=CH_2$; 3.09–4.01 (4H, m) 2 × CH =

CH_2; 5.71 (4H, t) 2 × $CH_2CH_2CO_2Me$; 6.32 (6H, s) 2 × O*Me*; 6.49 (3H, s), 6.53 (3H, s) 2 × Me; 6.77 (4H, t) 2 × $CH_2CH_2CO_2Me$.

Mass spectrum *m/e* (%) 596 (83, d_6), 595 (100, d_5) 594 (87, d_4), 593 (58, d_3), 592 (25, d_2), 591 (10, d_1).

6,7-Di(2-methoxycarbonylethyl)-1,3-di(trideuteromethyl)-5,8-dimethyl-2,4-divinylporphyrin, "1,3-Di(trideuteromethyl)protoporphyrin-IX Dimethyl Ester" (22)

To a solution of 2,4-diacetyldeuteroporphyrin-IX dimethyl ester (*15*) (0.500 g) in tetrahydrofuran (50 ml) was added dropwise 0.6 *N* sodium methoxide in methanol-d_1 (10 ml). The solution was refluxed under dry nitrogen for 5 hr, then cooled to room temperature and the base neutralized with glacial acetic acid (10 ml). This mixture was diluted with methylene chloride (75 ml) and extracted with water (3 × 75 ml). The organic layer was collected, the solvent was removed *in vacuo*, and the porphyrin residue was dissolved in methylene chloride–methanol (4:1, 100 ml) and treated with excess ethereal diazomethane. The solvent was removed under vacuum to give a brown residue (470 mg) of deuterated 2,4-diacetyldeuteroporphyrin-IX dimethyl ester. To this material, dissolved in methylene chloride (75 ml) and diluted with methanol (75 ml), was added at room temperature a solution of sodium borohydride (1.5 g) in methanol (15 ml) at 0°C. The reaction was monitored by visible spectroscopy and after 1.5 hr the reduction was complete and the residual reductant was carefully quenched with 2 *N* acetic acid (30 ml). The resulting solution was diluted with water (75 ml) and extracted. The methylene chloride layer was separated and washed again with water (2 × 75 ml). The organic fraction was collected and the solvent removed *in vacuo*. The purple porphyrin residue (350 mg) was shown to be nearly pure hematoporphyrin-IX dimethyl ester by tlc and visible absorption spectroscopy. To this material, dissolved in dry dimethyl formamide (75 ml), was added freshly distilled benzoyl chloride (3.5 ml). The solution was stirred under nitrogen at 95°C. After heating for 1.5 hr the solution was cooled to room temperature, poured into water (150 ml) containing triethylamine (7.5 ml), and chilled at below 0°C overnight. This solution was diluted with methylene chloride (100 ml) and extracted, and the organic layer was washed with water (2 × 100 ml). The solvent of the methylene chloride fraction was removed *in vacuo* and the resulting residue dissolved in methanol–methylene chloride (50/50 ml) and treated with excess ethereal diazomethane. The solvent was evaporated and the porphyrin purified by column chromotography [grade III alumina, toluene–methylene chloride (1:1) elution], and the product was recrystallized from hexane–dichloromethane to give 1,3-di(trideuteromethyl)protoporphyrin-IX dimethyl ester (0.250 g; 53% overall from 2,4-diacetyldeuteroporphyrin-IX), mp 211–213°C [lit. 228–229°C (*22*), undeuterated]. Apart from peak absences in the nmr spectrum, this material was identical with an authentic sample of protoporphyrin-IX dimethyl ester.

Hemin Dimethyl Ester

1,5- or 1,3-di(trideuteromethyl)protoporphyrin-IX dimethyl ester (79 mg) in pyridine (1.6 ml) and acetic acid (80 ml) was kept at 80°C under an atmosphere of dry nitrogen before adding a saturated solution of iron(II) sulfate in water (1.6 ml). The solution was kept for a further 10 min at 80°C and then air was bubbled through the solution for 10 min. The solution was then poured into HCl (2 *M*, 150 ml), extracted with methylene

chloride (100 ml), and washed twice with saturated aqueous sodium chloride. After drying over sodium sulfate it was evaporated to dryness. The solid residue was recrystallized for methylene chloride–hexane as a brown powder (79 mg).

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REFERENCES

1. E. ANTONINI AND A. BRUNORI, "Hemoglobin and Myoglobin in Their Reactions with Ligands." North–Holland, Amsterdam, 1971.
2. R. LEMBERG AND J. BARRETT, "Cytochromes." Academic Press, London/New York, 1973.
3. J. S. MORROW AND F. R. N. GURD, *CRC Crit. Rev. Biochem.* **3**, 221 (1975).
4. J. C. KENDREW, H. WATSON, B. E. STRANDBERG, R. E. DICKERSON, D. C. PHILLIPS, AND V. C. SHORE, *Nature (London)* **190**, 666 (1961).
5. M. F. PERUTZ, H. MUIRHEAD, J. M. COX, AND L. C. G. GOAMAN, *Nature (London)* **219**, 131 (1968).
6. 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); 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).
7. 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).
8. R. G. SHULMAN, J. PEISACH, AND B. J. WYLUDA, *J. Mol. Biol.* **48**, 517 (1969).
9. I. MORISHIMA AND T. IIZUKA, *Biochim. Biophys. Acta* **386**, 542 (1975); *J. Amer. Chem. Soc.* **96**, 7365 (1974).
10. J. A. P. BAPTISTA DE ALMEIDA, G. W. KENNER, J. RIMMER, AND K. M. SMITH, *Tetrahedron* **32**, 1793 (1976).
11. G. W. KENNER AND K. M. SMITH, *Ann. N. Y. Acad. Sci.* **206**, 138 (1973).
12. B. EVANS, K. M. SMITH, G. N. LA MAR, AND D. B. VISCIO, *J. Amer. Chem. Soc.* **99**, 7070 (1977).
13. H. H. INHOFFEN, J. W. BUCHLER, AND P. JÄGER, *Fortschr. Chem. Org. Naturst.* **26**, 284 (1968).
14. J. FAJER, A. FORMAN, M. S. DAVIS, L. D. SPAULDING, D. C. BRUNE, AND R. H. FELTON, *J. Amer. Chem. Soc.* **99**, 4134 (1977).
15. H. FISCHER AND H. ORTH, "Die Chemie des Pyrrols," Vol. II, Part 1, p. 304. Akademische Verlag, Leipzig, 1937.
16. P. S. CLEZY AND T. T. HAI, *Aust. J. Chem.* **29**, 1561 (1976).
17. J.-H. FUHRHOP AND K. M. SMITH, "Porphyrins and Metalloporphyrins," p. 771 (K. M. Smith, Ed.). Elsevier, Amsterdam, 1975.
18. J. RIMMER, Ph.D. thesis, University of Liverpool, 1977.
19. J. A. S. CAVALEIRO, A. M. D'A. ROCHA GONSALVES, G. W. KENNER, AND K. M. SMITH, *J. Chem. Soc. Perkin Trans. 1*, 2471 (1973).
20. R. P. CARR, A. H. JACKSON, G. W. KENNER, AND G. S. SACH, *J. Chem. Soc. C*, 487 (1971).
21. J.-H. FUHRHOP AND K. M. SMITH, "Porphyrins and Metalloporphyrins," p. 798 (K. M. Smith, Ed.). Elsevier, Amsterdam, 1975.
22. H. FISCHER AND H. ORTH, "Die Chemie des Pyrrols," Vol. II, Part 1, p. 401. Akademische Verlag, Leipzig, 1937.