Pyrroles and Related Compounds. Part XXXIII.[1] Total Synthesis of Deuteriated Derivatives of Protoporphyrin-IX for Nuclear Magnetic Resonance Studies of Haemoproteins [2]

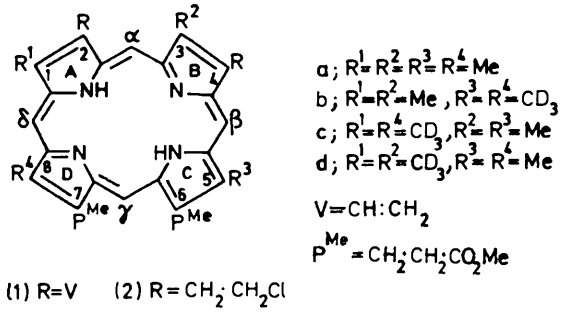
By José A. S. Cavaleiro, António M. d'A. Rocha Gonsalves, George W. Kenner,* and Kevin M. Smith, The Robert Robinson Laboratories, University of Liverpool, P.O. Box 147, Liverpool L69 3BX

In order to settle the precise identity of certain resonances in the paramagnetic n.m.r. spectra of low-spin haemoproteins, a variation of the MacDonald approach to porphyrins from pyrromethanes has been developed and applied to the synthesis of protoporphyrin-IX dimethyl ester (1a) and its 5,8-bis(trideuteriomethyl)-, 1,3-bis(trideuteriomethyl)-, and αγ-dideuterio-derivatives [(1b), (1d), and (14b), respectively]. Synthesis of 1,8-bis(trideuteriomethyl)protoporphyrin-IX dimethyl ester (1c) by the *b*-oxobilane route completed the series to be used for unambiguous assignment of the four methyl resonances in the contact shift n.m.r. spectra of the corresponding iron(III) cyanides.

Deuterium was incorporated into the porphyrins by total synthesis from the appropriate deuteriated pyrroles, which were in turn synthesised from deuteriated 1,3-diketones by standard methods. Novel exchange processes were discovered and used to achieve deuteriation at the interpyrrolic carbon atoms of pyrromethanes and at opposite *meso*-positions of porphyrins prepared by the MacDonald method.

VALUABLE insight into the properties of haemoproteins is afforded by contact shift n.m.r. spectroscopy of low-spin iron(III) chelates of these complicated molecules.[3] The short electron relaxation times give well-resolved signals and the low-spin electronic configuration allows examination of metal–ligand interactions because the free electron is in an iron orbital suitable for π-bonding to the porphyrin ring. Proton functions attached to the porphyrin nucleus have shifts which are dependent upon the unpaired electron density on the nearest ring carbon atom and therefore on the electronic structure of the particular haem. Furthermore, the resonances are greatly shifted owing to paramagnetic phenomena, and signals from the porphyrin ring can be observed in isolation from those of the haemoprotein globin residues. A quantum mechanical model has been derived [4] in order to interpret the paramagnetic n.m.r. shifts, but refinement of the mathematical treatment required precise assignment of the resonances of the 1-, 3-, 5-, and 8-methyl groups of the haem as a first objective, the *meso*-protons being a secondary problem. This paper describes the accomplishment of the first synthetic objective and a partial solution to the problem of the *meso*-proton signals, which in the event proved to be a less important goal.

We have already discussed the detailed considerations which led to our decision to synthesise the two deuteriated porphyrins (1b) and (1c), each having a pair of



(1) R=V (2) R=$CH_2 \cdot CH_2Cl$

deuteriated methyl substituents with one in common;[5] in this way, the assignment problem of the methyl groups could be solved since all four methyl resonances were visible in the n.m.r. spectra of the haems and, apparently, the haemoproteins. The *meso*-protons in porphyrins and metalloporphyrins are known [6] to be labile, and for this reason, no firm decisions regarding their substitution with deuterons were made, but we resolved to investigate these aspects if an opportunity appeared.

No attempt was made to deuteriate directly the methyl groups of protoporphyrin-IX since no specificity could reasonably be expected. The corresponding β-methyl groups in the monopyrrolic building blocks were shown to be resistant to base-catalysed exchange

[1] Part XXXII, J. A. S. Cavaleiro, G. W. Kenner, and K. M. Smith, *J.C.S. Perkin I*, 1974, 1188.

[2] Preliminary publications, (*a*) A. M. d'A. Rocha Gonsalves, G. W. Kenner, and K. M. Smith, *Chem. Comm.*, 1971, 1304; (*b*) 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.C.S. Chem. Comm.*, 1974, 392.

[3] *E.g.* K. Wüthrich, R. G. Shulman, B. J. Wyluda, and W. S. Caughey, *Proc. Nat. Acad. Sci. U.S.A.*, 1969, **62**, 636; K. Wüthrich, R. G. Shulman, and J. Peisach, *ibid.*, 1968, **60**, 373.

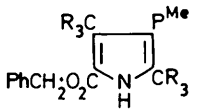
[4] R. G. Shulman, S. H. Glarum, and M. Karplus, *J. Mol. Biol.*, 1971, **57**, 83.

[5] G. W. Kenner and K. M. Smith, *Ann. New York Acad. Sci.*, 1973, **206**, 138.

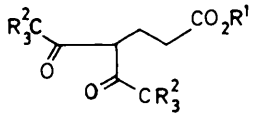
[6] *E.g.* R. Grigg, G. Shelton, A. Sweeney, and A. W. Johnson, *J.C.S. Perkin I*, 1972, 1789; J. B. Paine, tert, and D. Dolphin, *J. Amer. Chem. Soc.*, 1971, **93**, 4080.

under conditions which afforded acceptable recoveries of pyrroles. Synthesis of the pyrrole (3a) from deuteriated intermediates was therefore investigated.

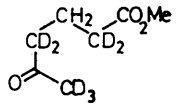
Treatment of the diketone (4a) with monodeuteriomethanol–methoxide accomplished rapid deuteration (n.m.r. examination) but the product was shown by mass spectrometry to be compound (5), resulting from a reverse Claisen condensation, followed by deuteriation; such processes were dominant in all reactions involving the ester (4a). When the carboxylic acid (4b) was treated with 1·1—1·5 equiv. of hydroxide in deuterium oxide the recovery of dione was about 90%. Double repetition of this process gave the compound (4c) in



(3)
a; R=D
b; R=H



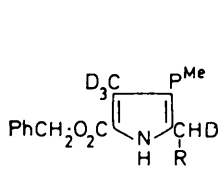
(4)
a; R^1=Me, R^2=H
b; R^1=R^2=H
c; R^1=H, R^2=D
d; R^1=Me, R^2=D



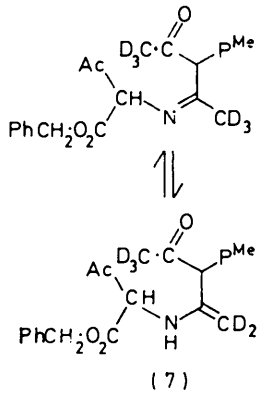
(5)

which the acetyl groups were over 90% deuteriated; deuterium present on the carbon atom between the carbonyl functions was lost during work-up. Examination of the peak normally at *m/e* 43 (COMe) in the mass spectrum of the undeuteriated compound was particularly helpful in judging the extent of deuteriation. Exchange of the acetyl protons was presumably slow because it required enolisation towards the acetyl methyl group rather than the more usual interaction between the two carbonyl functions; however, the slow deuteriation indicated that fairly vigorous conditions would be required in order to remove the label and that it should therefore be expected to survive the conditions of the Knorr pyrrole synthesis or its modifications. The dione (4c) was treated with diazomethane to give (4d), which was condensed with benzyl α-hydroxyiminoacetoacetate under the usual conditions [7] to give a good yield of the pyrrole (6a). N.m.r. spectroscopy showed that all the deuterium had been retained in the β-substituent, but that *ca.* 60% had been lost from the other acetyl function of the precursor during ring formation. This can be interpreted in terms of exchange in intermediates of the type (7) (or the corresponding cyclic species), and evidence in favour of this can be found in a recent investigation [8] of the mechanism of the Knorr pyrrole synthesis. Retention of the deuteriated methyl group in the pyrrole β-position is also confirmatory evidence for the mechanism of this modification of the Knorr synthesis which had been put forward on the basis of substituent variations.[7]

With lead tetra-acetate, (6a) gave the acetoxymethylpyrrole (6b), which was self-condensed in methan[^{2}H]ol containing hydrochloric acid [9] to give the pyrromethane (8a). Surprisingly, the deuterium present in the

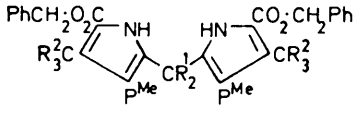


(6)
a; R=H
b; R=OAc



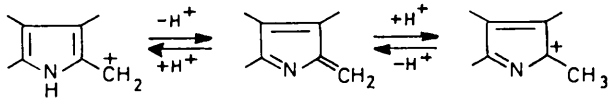
(7)

acetoxymethyl function of (6b) had been completely eliminated (n.m.r. spectrum); this observation was confirmed by synthesis of the pyrromethane (8b) by self-condensation of the undeuteriated analogue of (6b) in deuteriated solvents. Deuteriation at the interpyrrolic carbon was quantitative when deuterioacetic acid was used [10] and occurred to the extent of about 80%



(8)
a; R^1=H, R^2=D
b; R^1=D, R^2=H
c; R^1=R^2=D

in methan[^{2}H]ol–hydrochloric acid. This novel deuteriation process is difficult to rationalise, but presumably occurs on the monopyrrolic intermediates since no interconversion of (8a) and (8c) was possible. The equilibria in the Scheme provide a possible explanation. Whatever the explanation, however, we were able to



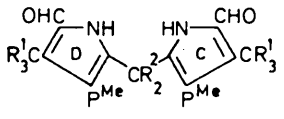
Scheme

make good the unexpected loss of deuterium from the α-methyl group of the pyrrole during its synthesis and the pyrromethane (8c) was obtained by self-condensation of (6a) in deuteriated solvents. Hydrogenation and treatment with cold trifluoroacetic acid gave the 5,5′-diunsubstituted pyrromethane, which was formylated with benzoyl chloride–dimethylformamide; the intermediate imine salt was then hydrolysed in aqueous sodium acetate. By use of an appropriate procedure, the pyrromethanes (9a—c) were obtained in this manner.

The undeuteriated AB component of the target

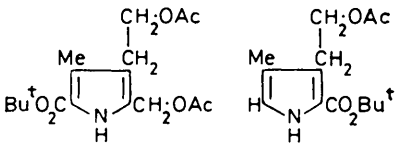
[7] E. Bullock, A. W. Johnson, E. Markham, and K. B. Shaw, *J. Chem. Soc.*, 1958, 1430.
[8] J. W. Harbuck and H. Rapoport *J. Org. Chem.* 1971, **36**, 853.
[9] A. F. Mironov, T. R. Ovsepyan, R. P. Evstigneeva, and N. A. Preobrazhenskii, *Zhur. obshchei Khim.*, 1965, **35**, 324.
[10] P. S. Clezy and A. J. Liepa, *Austral. J. Chem.*, 1970, **23**, 2443.

molecule (1b) was prepared by condensation of the pyrroles (10) and (11) in acetic acid containing sodium acetate,[11] and the resultant pyrromethane (12a) was hydrolysed to furnish the bis-(2-hydroxyethyl)pyrromethane (12b). The latter was transformed into the

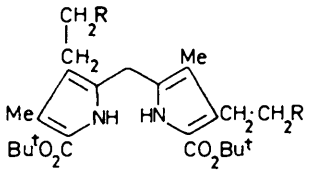


(9)

a; $R^1=R^2=D$
b; $R^1=H$, $R^2=D$
c; $R^1=D$, $R^2=H$
d; $R^1=R^2=H$



(10) (11)



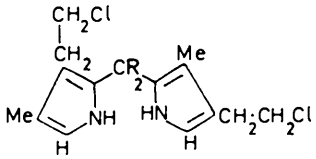
(12)

a; R=OAc
b; R=OH
c; R=Cl

bis-(2-chloroethyl) derivative (12c) with triphenylphosphine and carbon tetrachloride [12] [overall yield 30% from pyrroles (10) and (11)]. Our earlier synthesis of protoporphyrin-IX involved [13] conversion of 2-acetoxyethyl groups into 2-chloroethyl at the porphyrin stage; this series of transformations was carried out on pyrromethanes in the present work because it reduced the number of synthetic steps to be carried out on the molecule once it contained deuteriated functions. Treatment with trifluoroacetic acid followed by an alkaline work-up gave the 5,5'-di-unsubstituted pyrromethane (13a); the analogue (13b) was likewise prepared by performing the pyrromethane condensation in deuteriated solvent.

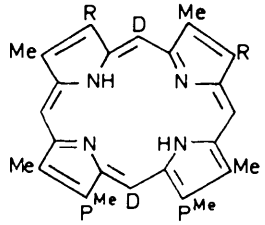
Cyclisations to porphyrin were investigated in the first instance with the undeuteriated pyrromethanes (9d) and (13a). Only low yields of porphyrin were obtained when hydriodic acid (the catalyst favoured in the original work [14]) was used. Trichloroacetic acid was also found not to be a suitable catalyst for the cyclisation. However, condensation of the pyrromethanes in the presence of toluene-*p*-sulphonic acid with zinc acetate added during the course of the reaction gave a good yield of porphyrin (2a), identical with material from our earlier syntheses of protoporphyrin-IX.[13] The zinc chelate of (2a) was converted into protoporphyrin-IX dimethyl ester (1a) by treatment with potassium t-butoxide in t-butyl alcohol followed by demetallation and esterification in 5% sulphuric acid–methanol; the overall yield of (1a) from pyrromethanes was reproducibly between 28 and 32%.

Accordingly, condensation of the octadeuteriopyrromethane (9a) with the dideuterio-compound (13b) in the presence of toluene-*p*-sulphonic acid and zinc acetate gave the 2,4-bis-(2-chloroethyl)porphyrin (2b) in 35—40% yield after treatment with sulphuric acid in methanol. Notably, the deuterium had been lost from the interpyrrolic carbons of the pyrromethanes during the condensation (n.m.r. spectrum). The dideuteriocompound (14a) could, however, be obtained from a similar condensation of the undeuteriated pyrromethanes (9d) and (13a) catalysed by toluene-*p*-sulphonic [^{2}H]acid. Nominally, the intermediate in the MacDonald condensation is the porphodimethene (15), which is subsequently oxidised and deprotonated to give porphyrin. Compound (15) can be more accurately described as a phlorin dication, and as a result of Woodward's studies [15] much is known of the chemistry of the phlorins (17). Protonation furnishes the olive-green monocation (16) and in strong acid a second proton is added *to the* meso-*carbon opposite the phlorin methylene* to give the phlorin



(13)

a; R=H
b; R=D



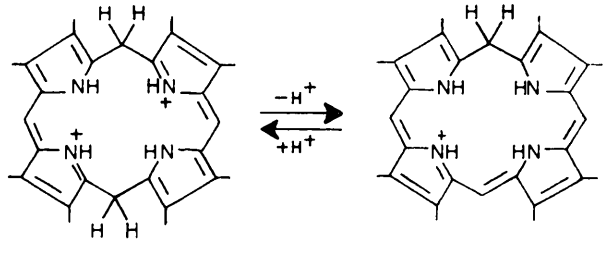
(14)

a; $R=CH_2CH_2Cl$
b; R=V

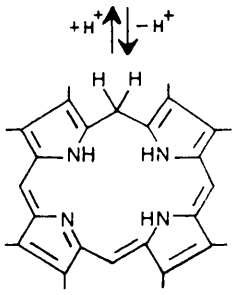
dication (15). Hence, the loss of deuterium from the first experiment can be explained by the existence of the equilibrium (15) ⇌ (16) involving only one opposite pair of *meso*-positions. This is a basic property of the phlorin system and differentiates the phenomenon observed herein from simple ethylideneamine–enamine equilibria which would result in exchange at all four *meso*-positions. It is possible to envisage situations where all *meso*-positions would be exchanged in phlorin equilibria, but isomerisation of phlorins normally requires relief of steric strain [15] and there is no such driving force in our example.

[11] E. J. Tarlton, S. F. MacDonald, and E. Baltazzi, *J. Amer. Chem. Soc.*, 1960, **82**, 4389.
[12] I. M. Downie, J. B. Holmes, and J. B. Lee, *Chem. and Ind.*, 1966, 900.
[13] R. P. Carr, A. H. Jackson, G. W. Kenner, and G. S. Sach, *J. Chem. Soc.* (*C*), 1971, 487.
[14] G. P. Arsenault, E. Bullock, and S. F. MacDonald, *J. Amer. Chem. Soc.*, 1960, **82**, 4384.
[15] R. B. Woodward, *Ind. chim. belge*, 1962, **27**, 1293.

The porphyrins (2b) and (14a) were converted into the protoporphyrin-IX derivatives (1b) and (14b), respectively, by treatment of the appropriate zinc chelate with butoxide.

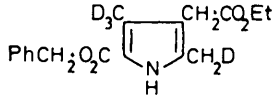


(15) (16)

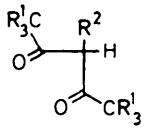


(17)

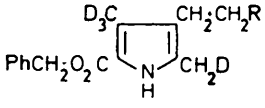
Synthesis of the unsymmetrically substituted compound (1c) required a general porphyrin synthesis * and the deuteriated pyrrole (18) as well as the propionate



(18)



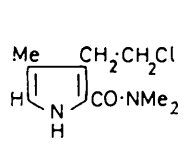
(19)
a; R^1=D, R^2=H
b; R^1=D, R^2=CH_2CO_2Et
c; R^1=H, R^2=CH_2CO_2Et



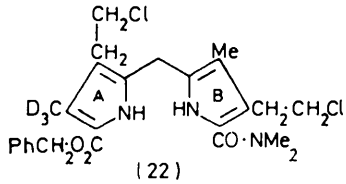
(20)
a; R=OH
b; R=Cl

pyrrole already described. Acetylacetone was transformed into its hexadeuterio-derivative (19a) and the thallium(I) salt of this was alkylated with ethyl bromoacetate to give the dione (19b). With benzyl α-hydroxyiminoacetoacetate a good yield of the pyrrole (18) was obtained and this, as expected, was shown to have lost a large proportion of deuterium from the α-methyl group. Reduction with diborane gave the (2-hydroxyethyl)pyrrole (20a), which afforded the (2-chloroethyl)-pyrrole (20b) when treated with thionyl chloride in pyridine. The 2-acetoxymethyl derivative was condensed with the 2-unsubstituted pyrrole (21) in methanol containing toluene-*p*-sulphonic acid hydrate to give [16] a good yield of the pyrromethane (22).

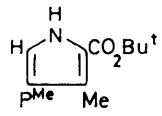
The CD pyrromethane (23) was synthesised by condensation of the deuteriated acetoxymethylpyrrole (6b) with the 2-unsubstituted pyrrole (24) followed by treatment with trifluoroacetic acid and an alkaline work-up.



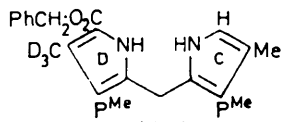
(21)



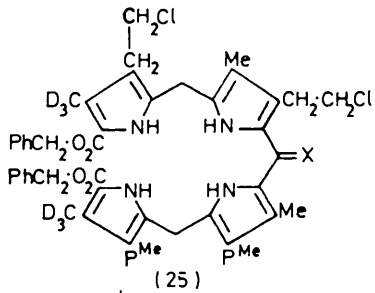
(22)



(24)

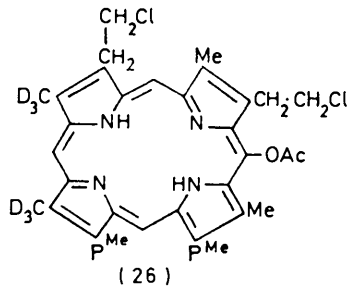


(23)



(25)
a; X=$\overset{+}{N}Me_2$ b; X=O

The resultant pyrromethane was condensed with the phosphoryl chloride complex of the amide (22) to give the imine salt (25a), which after chromatographic purification was hydrolysed to give the *b*-oxobilane (25b). The 1'- and 8'-benzyl ester groups were cleaved by hydrogenolysis and the resultant 1',8'-dicarboxylic acid was cyclised by treatment with trimethyl orthoformate and trichloroacetic acid in methylene chloride; the crude oxophlorin so obtained was acetylated with acetic anhydride in pyridine and the *meso*-acetoxyporphyrin (26) was obtained in good yield. Catalytic



(26)

hydrogenation and reoxidation of the resultant porphyrinogen with 2,3-dichloro-5,6-dicyanobenzoquinone

* This synthesis was first carried out on undeuteriated compounds to establish reaction conditions (Experimental section).

[16] J. A. S. Cavaleiro, A. M. d'A. Rocha Gonsalves, G. W. Kenner, and K. M. Smith, *J.C.S. Perkin I*, 1973, 2471.

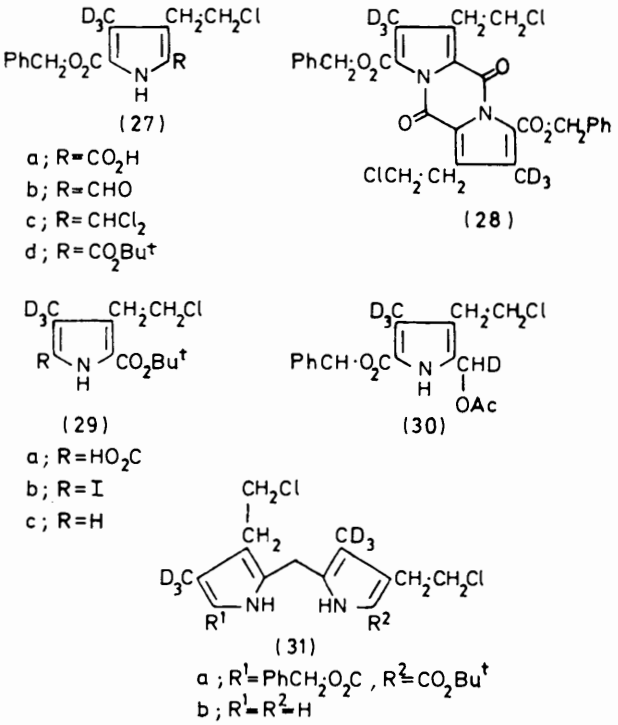
gave a high yield of the bis-(2-chloroethyl)porphyrin (2c), and this was transformed into the corresponding protoporphyrin-IX derivative (1c) in the usual way.

Having the two porphyrins (1b) and (1c) in hand we were in the position to confirm the assignments in the n.m.r. spectra of the haems and myoglobin analogues. Iron(III) was inserted [17] into the esters, which were then hydrolysed, and their spectra were measured by our collaborators at Bell Laboratories. This work [2b] confirmed the original assignments [4] in the iron(III) cyanide chelates; however, the situation with the myoglobins reconstituted with these samples was less clear, an unexpected switch of the resonances being observed. It therefore became necessary to identify positively the resonance in the myoglobin contact shift n.m.r. spectrum which was due to the 3-methyl group, and a new synthesis was indicated since we had intended to make this assignment by difference.[5] In the event, it was decided to synthesise 1,3-bis(trideuteriomethyl)protoporphyrin-IX dimethyl ester (1d) since this molecule had the 1-trideuteriomethyl group as an internal standard; the new molecule would also provide an independent verification of the haem n.m.r. assignments.

The deuteriated pyrrole (20b) was trichlorinated with sulphuryl chloride and then hydrolysed to give only a low (*ca.* 30%) yield of the required pyrrolecarboxylic acid (27a). Major products from this reaction were the pyrrocoll (28) and the formylpyrrole (27b). Alternatively, the carboxylic acid (27a) could be obtained from the formylpyrrole (27b) [obtained in high yield *via* the dichloromethylpyrrole (27c)] in about 50% yield by using alkaline potassium permanganate; unchanged starting material was the only other product from this oxidation, but it was not possible to use more vigorous conditions in case these encouraged nucleophilic displacement of the side-chain chlorine atom. With isobutene and mineral acid, (27a) gave the t-butyl ester (27d), which was hydrogenated to give the carboxylic acid (29a). Iodination gave the pyrrole (29b) which gave the 2-unsubstituted pyrrole (29c) when hydrogenated over Adams catalyst. Condensation with the 2-acetoxymethylpyrrole (30) in the presence of toluene-*p*-sulphonic acid afforded the hexadeuteriopyrromethane (31a) in 85% yield. Deprotection of the 5- and 5′-positions was accomplished by catalytic hydrogenation followed by treatment with cold trifluoroacetic acid and an alkaline work-up, to give (31b).

The 2,4-bis-(2-chloroethyl)porphyrin (2d) was obtained in 40% yield by condensation of the pyrromethanes (9d) and (31b) following the procedure described earlier. A 75% yield of the corresponding protoporphyrin-IX derivative (1d) was obtained from the zinc chelate with t-butoxide. The n.m.r. spectrum of the iron(III) cyanide from the dicarboxylic acid of this compound confirmed the assignments made on the basis of the 1,8- and 5,8-bis(trideuteriomethyl) derivatives.[2b] However, the myoglobin reconstituted with this sample has shown [18] some of the original n.m.r. assignments to be in error. Full discussion of the paramagnetic n.m.r. spectra will be published elsewhere.

Promising results were obtained from the n.m.r. spectra of the haem from the αγ-dideuterioprotoporphyrin-IX, but the spectrum of the corresponding myoglobin was less clear and suggested that some unexpected exchange processes might be occurring during the reconstitution with globin. In order to clarify this point αβγδ-tetradeuterioprotoporphyrin-IX was prepared by our recently described exchange method with hexapyridylmagnesium di-iodide; [19] the n.m.r. spectrum of



the protoporphyrin-IX dimethyl ester indicated that the terminal methylene systems of the 2- and 4-vinyl groups had also been deuteriated to the extent of about 50% by this process. Results from the contact shift spectra of the haems from this compound will be discussed elsewhere.

Throughout the syntheses of the compounds (1b—d) the deuteriated intermediates were examined by mass and n.m.r. spectroscopy. The latter technique was extremely useful when resolution was good enough to demonstrate the complete removal of protons by deuteriation. However, integrated intensities were often relied upon to confirm retention of deuterons and because of overlapping signals in the n.m.r. spectra it was often found that mass spectrometry was the superior method for accurate estimation of deuterium content. The mass and n.m.r. spectra of all compounds

[17] J. E. Falk, 'Porphyrins and Metalloporphyrins,' Elsevier, Amsterdam, 1964, p. 133.

[18] 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.*, 1974, in the press.

[19] G. W. Kenner, K. M. Smith, and M. J. Sutton, *Tetrahedron Letters*, 1973, 1303.

were compared with those of their undeuteriated counterparts, as were m.p.s. Little is known about the way in which m.p. varies with deuterium content, but in the present investigation the m.p.s of deuteriated compounds were the same as those of the protio-analogues, with the exception of the pyrrole (6a).

EXPERIMENTAL

M.p.s were measured on a hot-stage apparatus. Unless otherwise stated, neutral alumina (Merck; Brockmann grade III) was used for all chromatographic separations. Reactions were followed by t.l.c. as described in earlier parts of this Series. Electronic absorption spectra were determined (solutions in methylene chloride) with a Unicam SP 800 spectrophotometer, ^{1}H n.m.r. spectra (solutions in deuteriochloroform with tetramethylsilane as internal standard) with a Varian HA-100 instrument, and mass spectra with an A.E.I. MS 902 or MS 12 spectrometer (at 50 μA and 70 eV; direct inlet with source temperature 200—220°).

1,3-*Diketones*

Methyl 4-[$^2H_3$]*Acetyl*-5-*oxo*[6-$^2H_3$]*hexanoate* (4d).—4-Acetyl-5-oxohexanoic acid [20] (15·0 g) and sodium methoxide (7·07 g, 1·5 equiv.) in deuterium oxide (40 ml) were warmed during 5 h at 65° and the solution was cooled and acidified with conc. hydrochloric acid to pH *ca.* 5. The product was extracted with methylene chloride and the extract was dried (Na_2SO_4) and evaporated to a pale yellow oil, which was crystallised from a little benzene (first crop 9 g, 60%, *ca.* 50% ^{2}H), *m/e* 178 (11·7%, $^2H_6$), 177 (39, $^2H_5$), 176 (67, $^2H_4$), 175 (100, $^2H_3$), 174 (69, $^2H_2$), 173 (43, $^2H_1$), and 172 (10, $^2H_0$). This product (9 g) and potassium methoxide (4·2 g, 1·1 equiv.) were treated as before to give a further deuteriated sample (first crop 7·8 g, 87%, *ca.* 75% ^{2}H). Repetition under the same conditions gave the deuteriated diketone carboxylic acid (7·6 g, 95%, >90% ^{2}H), m.p. 74—76° (lit.,[20] 73—76°, for $^2H_0$), *m/e* 178 (100%, $^2H_6$), 177 (67, $^2H_5$), 176 (17, $^2H_4$), 175 (3, $^2H_3$), 174 (0, $^2H_2$), 173 (0, $^2H_1$), and 172 (0, $^2H_0$). The crystalline dione free acid was dissolved in methylene chloride and treated with an excess of ethereal diazomethane. Evaporation of the solvents gave an oily product which was used without purification in subsequent reactions.

Ethyl 3-*Acetyl*-4-*oxopentanoate* (19c).—Acetylacetone (1·1 g) was stirred in hexane (20 ml) and thallium(I) ethoxide (2·49 g) was added. After being stirred for 3 min the solution was chilled and the thallium(I) acetylacetonate (2·9 g, 97%) was filtered off and then heated at 100° in ethyl bromoacetate with stirring during 4 h. The solution was cooled and left overnight at room temperature, and the resulting suspension was then filtered. The filtrate was passed through a short column of Florisil before being evaporated to give the virtually pure oxopentanoate (1·5 g, 80%), indistinguishable by g.l.c. from an authentic sample; [21] τ (keto tautomer) 5·78 (t, C*H*·CH_2), 5·80 (q, OCH_2), 7·10 (d, CH·C*H*$_2$), 7·72 (s, 2 Me), and 8·75 (t, Me), τ (enol tautomer) (*ca.* 30%) 5·80 (q, OCH_2), 6·72 (s, CH_2), 7·81 (s, 2 Me), and 8·74 (t, Me).

Ethyl 3-[$^2H_3$]*Acetyl*-4-*oxo*[5-$^2H_3$]*pentanoate* (19b).—Acetylacetone (20 g) was deuteriated in deuterium oxide and sodium methoxide (0·25 equiv.) under similar conditions to those used for (4d) (24 h at 60° in each of three treatments). The required deuteriated product (8·35 g, 40%, >90% ^{2}H) was shown to be pure by g.l.c.; *m/e* 108 (50%, $^2H_8$), 107 (100, $^2H_7$), 106 (80, $^2H_6$), 105 (35, $^2H_5$), and 104 (5, $^2H_4$). The sample was transformed, *via* the thallium salt as described above, into the oxopentanoate, and the crude product was distilled (134—140°; 14 mmHg) to give the product (8·1 g, 70%, >90% ^{2}H), pure by g.l.c. analysis, *m/e* 192 (100, $^2H_6$), 191 (84, $^2H_5$), 190 (31, $^2H_4$), 189 (14, $^2H_3$), 188 (11, $^2H_2$), and 187 (6, $^2H_1$).

Pyrroles

Benzyl 4-(2-*Methoxycarbonylethyl*)-3-[$^2H_3$]*methyl*-5-[$^2H_1$]-*methylpyrrole*-2-*carboxylate* (6a).—Sodium nitrite (5·15 g) in water (10 ml) was added slowly to a well stirred solution of benzyl acetoacetate (13·4 g) in glacial acetic acid (16 ml), the temperature being kept below 10°. Stirring was maintained for a further 3 h before the solution was set aside at 0° for 12 h. The solution (38 ml) was then added during 2 h to a solution of methyl 4-[$^2H_3$]acetyl-5-oxo[6-$^2H_3$]-hexanoate (4d) (7·6 g) in glacial acetic acid (26·5 ml) kept at 65°, and simultaneously a mixture of zinc dust (7·6 g) and anhydrous sodium acetate (7·6 g) was added in portions. The mixture was then kept at 65° during 1·5 h and poured into water (1 l). The product was filtered off and recrystallised from aqueous methanol, to give fine needles (7·6 g, 60%), m.p. 107° (lit.,[22] 99—101°, for $^2H_0$), τ 0·75 (s, NH), 2·75 (m) and 4·80 (s) ($PhCH_2$), 6·45 (s, OMe), 7·25—7·75 (m, CH_2·CH_2), 7·77 (m, 3-CD_3; <10% H), and 7·89 (m, 5-CH_2D; >50% H), *m/e* 321 (11%, $^2H_6$), 320 (55, $^2H_5$), 319 (94, $^2H_4$), 318 (100, $^2H_3$), 317 (72, $^2H_2$), 316 (33, $^2H_1$), and 315 (6, $^2H_0$).

Benzyl 4-*Ethoxycarbonylmethyl*-3-[$^2H_3$]*methyl*-5-[$^2H_1$]-*methylpyrrole*-2-*carboxylate* (18).—Prepared in an analogous manner to the pyrrole (6a) from the dione (19b), this crystallised from aqueous ethanol to give fine needles (70%), m.p. 86—87° (lit.,[23] 87—88°, for $^2H_0$), τ 1·25 (s, NH), 2·65 (m) and 4·76 (s) ($PhCH_2$), 5·95 (q) and 8·79 (t) (OCH_2·CH_3), 6·69 (s, CH_2·CO), 7·75 (m, 3-CD_3; <10% H), and 7·80 (m, 5-CH_2D; >50% H).

Benzyl 4-(2-*Hydroxyethyl*)-3-[$^2H_3$]*methyl*-5-[$^2H_1$]*methyl*-*pyrrole*-2-*carboxylate* (20a).—Diborane [generated externally by addition of boron trifluoride–ether complex (52 ml) dropwise to sodium borohydride (7·4 g) in bis-(2-methoxyethyl) ether] was passed, in a carrier stream of nitrogen, through a solution of the foregoing ethoxycarbonylmethylpyrrole (18) (7·3 g) in tetrahydrofuran (73 ml) during 1 h. Methanol was then added carefully until the vigorous effervescence ceased; the solvents were evaporated off and the resulting grey solid was filtered through a short column of alumina (grade V) (elution with methylene chloride). After evaporation of the solvent the pyrrole crystallised from ether–n-hexane to give thin white needles (6 g, 95%), m.p. 119—121° (lit.,[13] 120—121°, for $^2H_0$), τ 0·60 (s, NH), 2·66 (s) and 4·72 (s) ($PhCH_2$), 6·39 (t) and 7·40 (t) (CH_2·CH_2), 7·73 (m, 3-CD_3; <10% H), and 7·83 (m, 5-CH_2D; >50% H).

Benzyl 4-(2-*Chloroethyl*)-3-[$^2H_3$]*methyl*-5-[$^2H_1$]*methylpyrrole*-2-*carboxylate* (20b).—Prepared from the foregoing pyrrole by the literature procedure,[16] this had m.p. 121—122° (lit.,[16] 121—122°, for $^2H_0$), τ 1·05 (s, NH), 2·65 (m) and 4·74 (s) ($PhCH_2$), 6·52 (t) and 7·20 (t) (CH_2·CH_2),

[20] T. L. Gresham, J. E. Jansen, F. W. Shaver, M. R. Frederick, and W. L. Beears, *J. Amer. Chem. Soc.*, 1951, **73**, 2345.
[21] J. Wass, Ph.D. Thesis, Liverpool, 1968.
[22] A. W. Johnson, E. Markham, R. Price, and K. B. Shaw, *J. Chem. Soc.*, 1958, 4255.
[23] A. H. Jackson, G. W. Kenner, and J. Wass, *J.C.S. Perkin I*, 1974, 480.

7·73 (m, 3-CD_3; <10% H), and 7·81 (m, 5-CH_2D; >50% H).

Benzyl 5-Acetoxy[$^2H_1$]methyl-4-(2-chloroethyl)-3-[$^2H_3$]-methylpyrrole-2-carboxylate.—This compound was prepared from the foregoing pyrrole by the literature procedure,[16] and had m.p. 166—169° (lit.,[16] 165—169°), τ 0·72 (s, NH), 2·61 (m) and 4·70 (s) ($PhCH_2$), 4·99 (m, CHD; *ca.* 25% H), 6·48 (t) and 7·09 (t) ($CH_2{\cdot}CH_2$), 7·75 (m, 3-CD_3; <10% H), and 7·98 (s, Ac).

Benzyl 5-Acetoxy[$^2H_1$]methyl-4-(2-methoxycarbonylethyl)-3-[$^2H_3$]methylpyrrole-2-carboxylate (6b).—Lead tetra-acetate (4 g) was added in portions with stirring over 2 h to a solution of the deuteriated pyrrole (6a) (3 g) in glacial acetic acid (53 ml) and acetic anhydride (1 ml). After 20 h at room temperature the solution was added dropwise to water (100 ml) with stirring. The precipitated pyrrole was filtered off, washed with water, and dried (yield 3·45 g, 97%); m.p. 110° (lit.,[24] 111—112°, for $^2H_0$), τ 0·75 (s, NH), 2·62 (s) and 4·70 (s) ($PhCH_2$), 4·95 (m, CHD, *ca.* 25% H), 6·31 (s, OMe), 7·10—7·60 (m, $CH_2{\cdot}CH_2$), 7·70 (m, CD_3; <10% H), and 7·95 (s, Ac).

t-Butyl 4-(2-Methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (24).—This compound was prepared (98%) as previously reported [13] by hydrogenation of the corresponding iodopyrrole, but was crystallised to give the *5-unsubstituted pyrrole* from ether–n-hexane; m.p. 50—51° (Found: C, 62·8; H, 8·1; N, 5·45. $C_{14}H_{21}NO_4$ requires C, 62·9; H, 7·9; N, 5·2%); τ 0·90 (s, NH), 3·35 (d, 5-H), 6·30 (s, OMe), 7·1—7·6 (m, $CH_2{\cdot}CH_2$), 7·72 (s, Me), and 8·41 (s, Bu^t).

t-Butyl 4-(2-Methoxycarbonyl[2-$^2H_2$]ethyl)-3-methylpyrrole-2-carboxylate.—The foregoing pyrrole (1·3 g) and sodium methoxide (0·26 g) in methan[^2H]ol (15 ml) were warmed at 60° under nitrogen during 2·5 h. The solution was cooled, neutralised with 2N-hydrochloric acid, poured into water, and extracted with methylene chloride. The organic phase was dried (Na_2SO_4) and evaporated to give an oily residue which slowly crystallised (1·2 g, 92%), m.p. 49—50°. The n.m.r. spectrum was identical with that of compound (24) except that the methylene region was simplified to give only a 2-proton singlet at τ 7·24.

Benzyl 4-(2-Chloroethyl)-5-formyl-3-[$^2H_3$]methylpyrrole-2-carboxylate (27b).—This compound was prepared from the deuteriated pyrrole (20b) by the literature procedure [16] and had m.p. 109—110° (lit.,[16] 110—111°, for $^2H_0$), τ 0·19 (m, CHO; *ca.* 55% H), 0·35 (s, NH), 2·60 (s) and 4·69 (s) ($PhCH_2$), 6·39 (t) and 6·85 (t) ($ClCH_2{\cdot}CH_2$), and 7·70 (3-CD_3; <10% H).

2-Benzyloxycarbonyl-4-(2-chloroethyl)-3-[$^2H_3$]methylpyrrole-5-carboxylic Acid (27a).—This compound was obtained from the deuteriated pyrrole (20b) by the procedure for the undeuteriated series; [16] m.p. 178—180° (lit.,[16] 178—182°), τ [$(CD_3)_2SO$] −2·95 (s) and −1·76 (s) (NH and CO_2H), 2·60 (m) and 4·72 (s) ($PhCH_2$), 6·33 (t) and 6·88 (t) ($ClCH_2{\cdot}CH_2$), and 7·76 (3-CD_3; <10% H).

2-Benzyloxycarbonyl-4-(2-chloroethyl)-3-methylpyrrole-5-carboxylic Acid.—Benzyl 4-(2-chloroethyl)-5-formyl-3-methylpyrrole-2-carboxylate [16] (4·0 g) in tetrahydrofuran (80 ml) was treated with potassium permanganate (5 g) in water (80 ml) containing sodium hydrogen carbonate (10 g). The mixture was stirred at room temperature during 2 h and then filtered through Celite, which was then washed with aqueous sodium carbonate. Ether (200 ml) and more sodium carbonate (10 g) in water (50 ml) were added to the filtrates and after extraction the aqueous layer was collected. The organic phase was re-extracted with more sodium carbonate solution and the combined aqueous extracts were flushed during 30 min with a stream of air and then treated with sulphur dioxide gas (to pH 6). The precipitated white solid was collected and dried, giving the required pyrrolecarboxylic acid (2·3 g, 55%), m.p. 178—182° (lit.,[16] 178—182°). By the same procedure, the deuteriated compound (27a) was also prepared from (27b). (Evaporation of the ether layer yielded about 0·5 g of starting formylpyrrole from a 4 g scale reaction.)

Benzyl 4-(2-Chloroethyl)-3-[$^2H_3$]methyl-5-t-butoxycarbonylpyrrole-2-carboxylate (27d).—The deuteriated pyrrolecarboxylic acid (27a) was converted into the t-butyl ester by the procedure [16] for the undeuteriated compound; m.p. 58—60° (lit.,[16] 59·5—60·5°, for $^2H_0$), τ 0·45 (s, NH), 2·63 (m) and 4·69 (s) ($PhCH_2$), 6·42 (t) and 6·89 (t) ($ClCH_2{\cdot}CH_2$), 7·70 (m, 3-CD_3; <10% H), and 8·42 (s, Bu^t).

4-(2-Chloroethyl)-3-[$^2H_3$]methyl-5-t-butoxycarbonylpyrrole-2-carboxylic Acid (29a).—This compound, similarly prepared by application of the literature procedure [16] from the foregoing pyrrole, had m.p. 193—195° (lit.,[16] 192—194°, for $^2H_0$), τ 0·39 (s, NH), 6·38 (t) and 6·84 (t) ($ClCH_2{\cdot}CH_2$), 7·66 (m, 3-CD_3; <10% H), and 8·39 (s, Bu^t).

t-Butyl 4-(2-Chloroethyl)-2-iodo-3-[$^2H_3$]methylpyrrole-5-carboxylate (29b).—Prepared [16] from the foregoing pyrrole, this had m.p. 135—137° (lit.,[16] 136—137°, for $^2H_0$), τ 0·80 (s, NH), 6·47 (t) and 6·90 (t) ($ClCH_2{\cdot}CH_2$), 8·05 (3-CD_3; <10% H), and 8·46 (s, Bu^t).

t-Butyl 4-(2-Chloroethyl)-3-[$^2H_3$]methylpyrrole-5-carboxylate (29c).—Prepared [16] by catalytic reduction of the foregoing deuteriated pyrrole over Adams catalyst, this had m.p. 96—97° (lit.,[16] 97—98°, for $^2H_0$), τ 0·65 (s, NH), 3·39 (d, *J* 3 Hz, 2-H), 6·41 (t) and 6·89 (t) ($ClCH_2{\cdot}CH_2$), 7·97 (m, 3-CD_3; <10% H), and 8·44 (s, Bu^t).

t-Butyl 4-(2-Chloroethyl)-3-methylpyrrole-5-carboxylate.—t-Butyl 4-(2-hydroxyethyl)-3-methylpyrrole-5-carboxylate [24] (2·25 g) in methylene chloride (10 ml) and dry pyridine (1 ml) was heated at 50° during the rapid dropwise addition of thionyl chloride (0·8 ml) with stirring. Dry nitrogen was then passed through the mixture at 50° during 30 min before dilution with methylene chloride and washing with sodium hydrogen carbonate solution and then water. The organic phase was dried (Na_2SO_4) and evaporated to dryness, and the residue was dissolved in benzene and filtered through a short column of alumina. Evaporation of the benzene eluates gave a solid which was recrystallised from methylene chloride–n-hexane to give the pyrrole (0·86 g, 35%), m.p. 97—98°, identical with an authentic sample.[16]

Pyrromethanes

Dibenzyl 3,3′-Bis-(2-methoxycarbonylethyl)-4,4′-dimethyl[α-$^2H_2$]pyrromethane-5,5′-dicarboxylate (8b).—(*a*) Benzyl 5-bromomethyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (0·30 g) in methan[^2H]ol (5 ml) was heated under reflux during 3 h. The pyrromethane crystallised on cooling and was filtered off (yield 0·11 g, 47%); m.p. 100° (lit.,[9] 99·5—100°, for $^2H_0$), τ 0·52 (s, 2 NH), 2·75 (s) and 4·82 (s) (2 $PhCH_2$), 6·10 (m, −CHD−, *ca.* 50% H), 6·47 (s, 2 OMe), 7·30 (t) and 7·58 (t) (2 $CH_2{\cdot}CH_2$), and 7·73 (s, 2 Me).

(*b*) A solution of benzyl 5-acetoxymethyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (0·32 g) in methan[^2H]ol (5 ml) and hydrochloric acid (0·3 ml; *d* 1·18) was heated in a water-bath during 4 h; after cooling the

[24] A. W. Johnson, I. T. Kay, E. Markham, R. Price, and K. B. Shaw, *J. Chem. Soc.*, 1959, 3416.

pyrromethane was filtered off (yield 0·23 g, 88%); m.p. 99—100°. The n.m.r. spectrum was identical with that of the material from (*a*) except that it showed *ca.* 80% deuteriation at the interpyrrolic position.

(*c*) Benzyl 5-acetoxymethyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (1·0 g) in deuteriated aqueous acetic [^{2}H]acid (10 ml; 80%) was heated on a water-bath during 2 h. The mixture was cooled, poured into water, and extracted with ether. The organic layer was separated, washed with saturated aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated to dryness. The residual oil was taken into methanol (small volume) and the crystalline product was filtered off (yield 0·53 g, 65%); m.p. 99—100°. The n.m.r. spectrum indicated complete deuteriation at the interpyrrolic position; *m/e* 616 (100%, $^2H_2$), 615 (10, $^2H_1$), and 614 (3, $^2H_0$).

Dibenzyl 3,3′-Bis-(2-methoxycarbonylethyl)-4,4′-di[$^2H_3$]*methyl*[α-$^2H_2$]*pyrromethane-5,5′-dicarboxylate* (8c).—This pyrromethane was prepared from benzyl 5-acetoxy[$^2H_1$]-methyl-4-(2-methoxycarbonylethyl)-3-[$^2H_3$]methylpyrrole-2-carboxylate (2·5 g) by method (*b*) (above); yield 1·6 g, 86%, m.p. 99—100° (lit.,[9] 99·5—100°, for $^2H_0$), τ 0·68 (s, 2 NH), 2·74 (s) and 4·81 (s) (2 $PhCH_2$), 6·10 (m, $-CD_2-$; <20% H), 6·48 (s, 2 OMe), 7·29 (t) and 7·65 (t) (2 $CH_2{\cdot}CH_2$), and 7·75 (m, 4,4′-CD_3; <10% H).

3,3′-Bis-(2-methoxycarbonylethyl)-4,4′-di[$^2H_3$]*methyl*[α-$^2H_2$]*pyrromethane-5,5′-dicarbaldehyde* (9a).—The foregoing pyrromethane (1·5 g) was hydrogenated at room temperature and atmospheric pressure in tetrahydrofuran (40 ml) containing triethylamine (2 drops) and 10% palladised charcoal (0·15 g) until uptake ceased. The pyrromethanecarboxylic acid almost completely crystallised overnight and so the solution was filtered through Celite and the crystallised material was dissolved in ammonia (2N aqueous) and filtered through the same Celite to remove catalyst. The tetrahydrofuran filtrate was evaporated to dryness and the white residue was likewise dissolved in ammonia. The combined alkaline solutions were carefully neutralised with aqueous 2N-acetic acid and the white precipitate was filtered off and washed with water before drying; yield 1·05 g, 98%, m.p. 190—191° (lit.,[9] 190—191°, for $^2H_0$). This material in *NN*-dimethylformamide (4 ml) was refluxed under nitrogen during 30 min and the resulting solution was cooled to 0° and maintained at this temperature while benzoyl chloride (1·5 ml) was added dropwise with stirring. After 15 min, benzene (7·5 ml) was added and the mixture was allowed to warm to room temperature. The precipitated imine salt was filtered off after 1 h and washed with benzene, and the moist solid was dissolved in methanol (15 ml). Saturated sodium acetate solution (15 ml) was added and the mixture was stirred during 20 h, after which the 5,5′-dialdehyde had completely precipitated and was filtered off; yield 467 mg, 50%, m.p. 180—181° (lit.,[25] 180—181°); τ −0·36 (s, 2 NH), 0·55 (s, 2 CHO), 5·98 (m, $-CD_2-$; <20% H), 6·31 (s, 2 OMe), 7·20 (t) and 7·49 (t) (2 $CH_2{\cdot}CH_2$), and 7·70 (m, 4,4′-CD_3; <10% H).

Di-t-butyl 3,4′-Bis-(2-acetoxyethyl)-3′,4-dimethylpyrromethane-5,5′-dicarboxylate (12a).—t-Butyl 3-(2-acetoxyethyl)-2-acetoxymethyl-4-methylpyrrole-5-carboxylate (2·98 g), t-butyl 4-(2-acetoxyethyl)-3-methylpyrrole-5-carboxylate (2·35 g), and anhydrous sodium acetate (3·5 g) in acetic acid (70 ml) were heated at 120—130° during 35 min before being poured into water. The crude pyrromethane was extracted with methylene chloride and the combined organic extracts were neutralised with saturated sodium hydrogen carbonate solution, dried (Na_2SO_4), and evaporated. The dark brown residue was chromatographed on alumina (elution with light petroleum containing an increasing proportion of benzene) and evaporation of the appropriate eluates (t.l.c.) gave a pale yellow oil (3·0 g), shown to be the desired pyrromethane by n.m.r. and mass spectroscopy; τ 0·98 (s) and 1·08 (s) (2 NH), 5·80 (t), 6·92 (t) and 7·28 (t) (2 $CH_2{\cdot}CH_2$), 6·08 (s, CH_2), 7·72 (s, Me), 7·98 (s, Me and 2 COMe), and 8·45 (s, 2 Bu^t), *m/e* 546 (100%), 486 (32), and 430 (90), *m** 434 (546 ⟶ 486), and 380 (486 ⟶ 430).

Di-t-butyl 3,4′-Bis-(2-chloroethyl)-3′,4-dimethylpyrromethane-5,5′-dicarboxylate (12c) [*from* (12a) via (12b)].—t-Butyl 3-(2-acetoxyethyl)-2-acetoxymethyl-4-methylpyrrole-5-carboxylate (0·76 g) and t-butyl 4-(2-acetoxyethyl)-3-methylpyrrole-5-carboxylate (0·60 g) were condensed as in the foregoing experiment. The crude product was dissolved in ethanol (80 ml) and treated with sodium carbonate (7·5 g) in water (25 ml). The stirred mixture was heated on a water-bath for 60 min, water being added to dissolve the sodium carbonate, and then the pyrromethane was extracted into chloroform. The organic extracts were dried (Na_2SO_4), decolourised with charcoal, and evaporated. The foamy residue was shown to be the 3,4′-bis-(2-hydroxyethyl)pyrromethane by n.m.r. spectroscopy; τ 0·32 (s) and 1·32 (s) (2 NH), 6·19 (s, CH_2), 6·3 (t), 7·04 (t), and 7·32 (t) (2 $CH_2{\cdot}CH_2$), 7·78 (s) and 7·99 (s) (2 Me), and 8·45 (s, 2 Bu^t), *m/e* 462 (73%), 460 (11), 406 (18), and 350 (100).

The crude product (0·85 g) and triphenylphosphine (0·96 g) in carbon tetrachloride (8·5 ml) were refluxed in an oil-bath (90°) during 7 h (t.l.c. monitoring) before cooling and evaporation of the solvent. The residue was extracted with benzene and the extract was concentrated and chromatographed on alumina (elution with light petroleum containing increasing proportions of benzene up to a 1 : 1 ratio); evaporation of the appropriate eluates (t.l.c.) followed by recrystallisation from methylene chloride–n-hexane gave the *pyrromethane* (0·30 g, 30%), m.p. 134—135° (Found: C, 60·2; H, 7·2; N, 5·5. $C_{25}H_{26}Cl_2N_2O_4$ requires C, 60·1; H, 7·3; N, 5·6%), τ 1·03 (s, 2 NH), 6·10 (s, CH_2), 6·40 (t), 6·60 (t), 6·88 (t), and 7·29 (t) (2 $CH_2{\cdot}CH_2$), 7·77 (s) and 8·00 (s) (2 Me), and 8·48 (s) (2 Bu^t), *m/e* 499 (100%), 464 (4), 443 (36), and 426 (23), *m** 393 (499 ⟶ 443).

Di-t-butyl 3,4′-Bis-(2-chloroethyl)-3′,4-dimethyl[α-$^2H_2$]*pyrromethane-5,5′-dicarboxylate.*—This pyrromethane, m.p. 134—135°, was prepared as in the foregoing experiments, except that the condensation was performed in acetic [^{2}H]acid. The n.m.r. spectrum showed >80% deuteriation at the interpyrrolic carbon atom.

Benzyl 3,4′-Bis-(2-chloroethyl)-3′,4-dimethyl-5′-t-butoxycarbonylpyrromethane-5-carboxylate.—Benzyl 2-acetoxymethyl-3-(2-chloroethyl)-4-methylpyrrole-5-carboxylate (0·20 g) and t-butyl 4-(2-chloroethyl)-3-methylpyrrole-5-carboxylate (0·14 g) in acetic acid (10 ml) were treated with toluene-*p*-sulphonic acid hydrate (5·8 mg) at 40° during 3 h. Chloroform (50 ml) was added and the mixture was washed with water, aqueous sodium hydrogen carbonate, and then water again. The organic layer was dried (Na_2SO_4) and evaporated to dryness before chromatography of the residue on alumina (elution with light petroleum–benzene). Evaporation of the eluates afforded an oil

[25] R. Chong, P. S. Clezy, A. J. Liepa, and A. W. Nichol, *Austral. J. Chem.*, 1969, **22**, 229.

(0·26 g, 85%) which could not be induced to crystallise; τ 0·50 (s) and 0·79 (s) (2 NH), 2·69 (s) and 4·76 (s) ($PhCH_2$), 6·15 (s, CH_2), 6·4—7·3 (m, 2 CH_2·CH_2Cl), 7·80 (s) and 8·06 (s) (2 Me), and 8·55 (s, Bu^t).

Benzyl 3,4′-Bis-(2-chloroethyl)-3′,4-di[$^2H_3$]methyl-5′-t-butoxycarbonylpyrromethane-5-carboxylate (31a).—This compound was prepared as described above from benzyl 2-acetoxy[$^2H_1$]methyl-3-(2-chloroethyl)-4-[$^2H_3$]methylpyrrole-5-carboxylate and t-butyl 4-(2-chloroethyl)-3-[$^2H_3$]methylpyrrole-5-carboxylate. The n.m.r. spectrum showed *ca.* 90% deuteriation at the 3′- and 4-methyl groups and only minor deuteriation at the interpyrrolic position.

Benzyl 3,3′-Bis-(2-methoxycarbonylethyl)-4-[$^2H_3$]methyl-4′-methyl-5′-t-butoxycarbonylpyrromethane-5-carboxylate (23).—Benzyl 5-acetoxy[$^2H_1$]methyl-4-(2-methoxycarbonylethyl)-3-[$^2H_3$]methylpyrrole-2-carboxylate (0·72 g), t-butyl 4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (0·56 g), and anhydrous sodium acetate (0·75 g) in acetic acid (11 ml) were heated at 120—130° during 40 min, then poured into water. The crude pyrromethane was extracted into methylene chloride and the extract was washed with saturated sodium hydrogen carbonate solution and water, then dried (Na_2SO_4), and evaporated. The residue was chromatographed on alumina (elution with benzene); evaporation of the eluates gave a pale yellow residue which was crystallised from ether–light petroleum (b.p. 60—80°); yield 0·66 g, 52%, m.p. 114—115° (lit.,[13] 114—116°, for $^2H_0$), τ 0·94 (s) and 1·28 (s) (2 NH), 2·78 (s) and 4·84 (s) ($PhCH_2$), 6·15 (s, CH_2), 6·45 (s) and 6·51 (s) (2 OMe), 7·35 (m) and 7·61 (t) (2 CH_2·CH_2), 7·88 (s, 4′-Me), and 8·50 (s, Bu^t), *m/e* 583 (100%, $^2H_3$), 582 (28, $^2H_2$), and 581 (3, $^2H_1$).

Benzyl 3,3′-Bis-(2-methoxycarbonylethyl)-4,4′-dimethyl-5′-t-butoxycarbonyl[α-$^2H_2$]pyrromethane-2-carboxylate.—This was similarly prepared (yield 0·085 g; m.p. 114—115°) from benzyl 5-acetoxymethyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (0·095 g) and t-butyl 4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (0·066 g) in acetic [^{2}H]acid (1·3 ml) containing anhydrous sodium acetate (0·1 g). The n.m.r. spectrum showed >80% deuteriation at the interpyrrolic position; *m/e* 582 (100%, $^2H_2$), 581 (39, $^2H_1$), and 580 (27, $^2H_0$).

Benzyl 3,4′-Bis-(2-chloroethyl)-5′-dimethylcarbamoyl-3′-methyl-4-[$^2H_3$]methylpyrromethane-5-carboxylate (22).—This pyrromethane was prepared by the published procedure [16] for the undeuteriated species from benzyl 2-acetoxy[$^2H_1$]-methyl-3-(2-chloroethyl)-4-[$^2H_3$]methylpyrrole-5-carboxylate and 4-(2-chloroethyl)-5-dimethylcarbamoyl-3-methylpyrrole, and had m.p. 137—138° (lit.,[16] 137—138°, for $^2H_0$), τ 0·05 (s) and 0·25 (s) (2 NH), 2·71 (s) and 4·79 (s) ($PhCH_2$), 6·25 (s, CH_2), 6·50 (t), 6·65 (t), and 6·9—7·3 (m) (2 CH_2·CH_2), 7·05 (s, NMe_2), 7·75 (m, CD_3; <10% H), and 8·02 (s, 3′-Me).

Porphyrins

β-Acetoxy-2,4-bis-(2-chloroethyl)-6,7-bis-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphin.—Benzyl 3,4′-bis-(2-chloroethyl)-5′-dimethylcarbamoyl-3′,4-dimethylpyrromethane-5-carboxylate (0·88 g) in phosphoryl chloride (10 ml) was warmed at 40° during 1 h before evaporation of the solvent and thorough drying at 50° and 0·5 mmHg. Meanwhile, benzyl 5′-t-butoxycarbonyl-3,3′-bis-(2-methoxycarbonyl)-4,4′-dimethylpyrromethane-5-carboxylate (1·06 g) was set aside (30 min) in trifluoroacetic acid (5 ml) with a slow stream of nitrogen passing through it before evaporation to dryness. The residual oil was dissolved in methylene chloride and the solution was washed with saturated sodium hydrogen carbonate, dried (Na_2SO_4), and evaporated. Methylene chloride (5 ml) was added to the residual oil, and this was then added to the foregoing phosphoryl chloride complex in methylene chloride (5 ml). The mixture, with a slow stream of nitrogen passing through it, was heated at 40° during 29 h, then diluted with methylene chloride; the solution was washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on alumina (grade V) (elution with benzene containing increasing proportions of ethyl acetate, to a ratio of 4 : 1, which served to remove non-polar by-products). These eluates afforded the dimethylcarbamoylpyrromethane starting material (0·37 g). Elution with methanol yielded the imine salt ($\lambda_{max.}$ 406 nm), which was dissolved in methylene chloride (50 ml) and stirred vigorously with a saturated solution of sodium hydrogen carbonate during 24 h at room temperature. The organic layer was separated, dried (Na_2SO_4), and evaporated, and the resultant oil was chromatographed on alumina (elution with 80% ethyl acetate in benzene) to afford the *b*-oxobilane. The resultant pale yellow foam (0·53 g) could not be crystallised, but was shown to be homogeneous by t.l.c. and n.m.r. spectroscopy. Taking recovered starting material into consideration, the yield was 56%; τ −0·05 (s), 0·15 (s), 0·16 (s), and 0·42s (4 NH), 2·73 (s), 2·76 (s), 4·80 (s), and 4·82 (s) (2 $PhCH_2$), 6·12 (s) and 6·22 (s) (2 CH_2), 6·42 (s) and 6·49 (s) (2 OMe), 6·4—6·8 (m), 6·9—7·45 (m), and 7·6—7·8 (m) (4 CH_2·CH_2), 7·78 (s, 2 Me), and 7·99 (s) and 8·10 (s) (2 Me).

The foregoing *b*-oxobilane (0·53 g) in tetrahydrofuran (53 ml) containing triethylamine (3 drops) and 10% palladised charcoal (53 mg) was hydrogenated at room temperature and atmospheric pressure until uptake ceased (5 h). The catalyst was removed by filtration through Celite and the filtrate was evaporated to dryness. The residue was dissolved in methylene chloride (106 ml) and treated with 1M-trichloroacetic acid in methylene chloride (106 ml) and trimethyl orthoformate (1·12 ml). After stirring during 2 h ($\lambda_{max.}$ 510 nm), pyridine (4·0 ml) was added and stirring was continued for another 16 h ($\lambda_{max.}$ 405 and 700 nm). The green solution was washed with water, dried (Na_2SO_4), and evaporated to near dryness. Pyridine (15 ml) and acetic anhydride (1·5 ml) were added and the solution was stirred at room temperature for 45 min, then evaporated to dryness. The residue was dried under high vacuum, taken into methylene chloride, and then chromatographed on alumina (elution with methylene chloride). Crystallisation of the material from the evaporated red eluates (from methylene chloride–n-hexane) gave fluffy purple needles (150 mg, 37%), m.p. 202—204° (Found: C, 63·5; H, 6·0; N, 7·65. $C_{38}H_{42}Cl_2N_4O_6$ requires C, 63·2; H, 5·9; N, 7·8%), τ (*ca.* 0·05M) −0·05 (s), 0·14 (s), and 0·21 (s) (3 *meso*-H), 5·4—5·9 (m) and 6·80 (t) (4 CH_2·CH_2), 6·35 (s) and 6·40 (s) (2 OMe), 6·45 (s), 6·54 (s), 6·55 (s), and 6·59 (s) (4 Me), and 7·00 (s, COMe), *m/e* 720 (100%), 685 (5), and 647 (22), *m** 583 (720 ⟶ 647), $\lambda_{max.}$ 404 (ε 201,000), 502 (15,000), 534 (4900), 573 (5200), and 626 nm (1200), $\lambda_{max.}$ (CH_2Cl_2–CF_3·CO_2H), 412 (ε 378,000), 556 (14,300), and 599 nm (2700).

β-Acetoxy-2,4-bis-(2-chloroethyl)-6,7-bis-(2-methoxycarbonylethyl)-1,8-di[$^2H_3$]methyl-3,5-dimethylporphin (26).—This porphyrin, m.p. 202—204°, was prepared in similar yield

following the method above for the undeuteriated series, starting with benzyl 3,4′-bis-(2-chloroethyl)-5′-dimethylcarbamoyl-3′-methyl-4-[$^2H_3$]methylpyrromethane-5-carboxylate and benzyl 5′-t-butoxycarbonyl-3,3′-bis-(2-methoxycarbonylethyl)-4-[$^2H_3$]methyl-4′-methylpyrromethane-5-carboxylate; τ (*ca.* 0·05M) −0·02 (s), 0·18 (s), and 0·26 (s) (3 *meso*-H), 5·45—6·00 (m) and 6·83 (t) (4 CH_2·CH_2), 6·38 (s) and 6·42 (s) (2 OMe), 6·48 (s) and 6·55 (s) (3- and 5-Me), and 7·00 (s, COMe).

2,4-*Bis*-(2-*chloroethyl*)-6,7-*bis*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethylporphin* (2a).—(*a*) Di-t-butyl 3,4′-bis-(2-chloroethyl)-3′,4-dimethylpyrromethane-5,5′-dicarboxylate (24 mg) was set aside in trifluoroacetic acid (2 ml) during 40 min, with a slow stream of nitrogen passing through it. The solvent was evaporated off and the residue taken up in methylene chloride; the solution was washed with saturated sodium hydrogen carbonate solution, dried (Na_2SO_4), and partially evaporated under reduced pressure. This solution, with 5,5′-diformyl-3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane (16 mg) was made up to a volume of 16 ml with methylene chloride, and then in the dark, a solution of toluene-*p*-sulphonic acid hydrate (48 mg) in methanol (0·8 ml) was added, followed after 8 h by a saturated solution of zinc acetate in methanol (0·8 ml). After a further 16 h, the solution was washed with water and saturated sodium hydrogen carbonate solution, dried (Na_2SO_4), and evaporated. The residue in 5% w/v sulphuric acid in methanol (20 ml) was set aside for 16 h, then poured into methylene chloride–water and carefully neutralised with sodium hydrogen carbonate solution. The organic layer was washed, dried (Na_2SO_4), and evaporated; the residue was chromatographed on alumina (elution with methylene chloride). The porphyrin fraction crystallised from methylene chloride–n-hexane as purple needles (8 mg, 38%), m.p. 217° (lit.,[13] 216—217°).

(*b*) β-Acetoxy-2,4-bis-(2-chloroethyl)-6,7-bis-(2-methoxycarbonylethyl)-1,3,5,8-tetramethylporphin (136 mg) in tetrahydrofuran (50 ml) containing triethylamine (5 drops) and 10% palladised charcoal (136 mg) was hydrogenated at atmospheric pressure and room temperature until uptake ceased and the solution was colourless (6 h). The catalyst was filtered off on Celite and the pale yellow filtrate was treated with 2,3-dichloro-5,6-dicyanobenzoquinone (142 mg) in benzene (5 ml) and then immediately evaporated to dryness. The residue was chromatographed on alumina (elution with methylene chloride). The porphyrin (100 mg, 80%) was obtained from the appropriate eluates after crystallisation from methylene chloride–n-hexane, and had m.p. 219—220°.

2,4-*Bis*-(2-*chloroethyl*)-6,7-*bis*-(2-*methoxycarbonylethyl*)-1,3-*dimethyl*-5,8-*di*[$^2H_3$]*methylporphin* (2b).—This compound was prepared from di-t-butyl 3,4′-bis-(2-chloroethyl)-3′,4-dimethyl[α-$^2H_2$]pyrromethane-5,5′-dicarboxylate and 5,5′-diformyl-3,3′-bis-(2-methoxycarbonylethyl)-4,4′-di[$^2H_3$]-methyl[α-$^2H_2$]pyrromethane with the same yield and under the same conditions as described in (*a*) above, and the product had m.p. 216—218° (lit.,[13] 216—217°, for $^2H_0$), τ (*ca.* 0·05M) 0·05 (s), 0·18 (s), 0·22 (s), and 0·31 (s) (4 *meso*-H), 5·45—5·95 (m) and 6·79 (t) (4 CH_2·CH_2), 6·39 (s) and 6·40 (s) (2 OMe), and 6·59 (s) and 6·63 (s) (1- and 3-Me), *m/e* 668 (100%, $^2H_6$), 667 (64, $^2H_5$), 666 (42, $^2H^4$), 665 (21, $^2H_3$), 664 (7, $^2H_2$), and 663 (0, $^2H_1$).

2,4-*Bis*-(2-*chloroethyl*)-6,7-*bis*-(2-*methoxycarbonylethyl*)-1,3-*di*[$^2H_3$]*methyl*-5,8-*dimethylporphin* (2d).—This was similarly obtained in 40% yield (m.p. 213—215°) from benzyl 3,4′-bis-(2-chloroethyl)-3′,4-di[$^2H_3$]methyl-5′-t-butoxycarbonylpyrromethane-5-carboxylate (deprotected by catalytic hydrogenation in tetrahydrofuran, followed by treatment with cold trifluoroacetic acid) and 5,5′-diformyl-3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane; τ (*ca.* 0·04M) −0·07 (s), 0·11 (s), 0·15 (s), and 0·21 (s) (4 *meso*-H), 5·4—6·0 (m) and 6·75 (t) (4 CH_2·CH_2), 6·34 (s) and 6·36 (s) (2 OMe), 6·45 (s) and 6·47 (s) (5- and 8-Me), and 14·04 (s, 2 NH).

2,4-*Bis*-(2-*chloroethyl*)-6,7-*bis*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*[α,γ-$^2H_2$]*porphin* (14a).—This was similarly prepared from di-t-butyl 3,4′-bis-(2-chloroethyl)-3′,4-dimethylpyrromethane-5,5′-dicarboxylate and 5,5′-diformyl-3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane, with toluene-*p*-sulphonic [^2H]acid hydrate as the catalyst in methan[^2H]ol. The product (35%) had m.p. 216—217° and showed only two *meso*-proton resonances (τ 0·00 and 0·08) in its n.m.r. spectrum; *m/e* 668 (22%), 667 (50), 666 (89), 665 (83), 664 (100), 663 (33), and 662 (5).

2,4-*Bis*-(2-*chloroethyl*)-6,7-*bis*-(2-*methoxycarbonylethyl*)-1,8-*di*[$^2H_3$]*methyl*-3,5-*dimethylporphin* (2c).—This was prepared from the corresponding β-acetoxyporphyrin (26) following the method described for the undeuteriated compound in (*b*), and had m.p. 218—219°, τ (*ca.* 0·05M) −0·09 (s), −0·05 (s), 0·01 (s), and 0·06 (s) (4 *meso*-H), 5·4—5·8 (m) and 6·71 (t) (4 CH_2·CH_2), 6·32 (s) and 6·33 (s) (2 OMe), and 6·39 (s) and 6·41 (s) (3- and 5-Me).

6,7-*Bis*-(2-*methoxycarbonylethyl*)-1,3-*dimethyl*-5,8-*di*[$^2H_3$]-*methyl*-2,4-*divinylporphin* (1b).—2,4-Bis-(2-chloroethyl)-6,7-bis-(2-methoxycarbonylethyl)-1,3-dimethyl-5,8-di[$^2H_3$]-methylporphin (70 mg) in methylene chloride (30 ml) was treated with saturated methanolic zinc acetate (12 ml). After brief warming on a water-bath, spectrophotometry showed metal insertion to be complete, and the mixture was washed with aqueous sodium acetate. The organic phase was washed with water, dried (Na_2SO_4), and evaporated to leave a red residue which was taken into tetrahydrofuran (11 ml) and 1M-potassium t-butoxide in t-butyl alcohol (35 ml). After 72 h in the dark under nitrogen, the solution was neutralised with acetic acid, poured into water, and extracted with methylene chloride containing 1% pyridine. The organic phase was dried (Na_2SO_4) and evaporated, and the residue was set aside for 16 h in 5% w/v sulphuric acid in methanol. The solution was poured into aqueous sodium acetate and extracted with methylene chloride; the extract was washed with water, dried (Na_2SO_4), and evaporated. The residue was chromatographed on alumina (elution with methylene chloride), the porphyrinic eluates were evaporated, and the residue was crystallised from methylene chloride–n-hexane to give purple needles (50 mg, 71%), m.p. 228—229° (lit.,[13] 228—229°, for $^2H_0$), τ (*ca.* 0·05M) 0·01—0·03br (s) (2 *meso*-H), 0·11 (s) and 0·16 (s) (2 *meso*-H), 1·12—2·10 (m) and 3·60—4·00 (m) (2 CH:CH_2), 5·71 (t) and 6·80 (t) (2 CH_2·CH_2), 6·38 (s, 2 OMe), and 6·49 (s, 1- and 3-Me).

6,7-*Bis*-(2-*methoxycarbonylethyl*)-1,8-*di*[$^2H_3$]*methyl*-3,5-*dimethyl*-2,4-*divinylporphin* (1c).—This was similarly prepared in 70% yield (m.p. 227—228°) from 2,4-bis-(2-chloroethyl)-6,7-bis-(2-methoxycarbonylethyl)-1,8-di[$^2H_3$]methyl-3,5-dimethylporphin; τ (*ca.* 0·05M) 0·00 (s), 0·01 (s), 0·12 (s), and 0·15 (s) (4-*meso*-H), 1·68—2·05 (m) and 3·60—4·00 (m) (2 CH:CH_2), 5·71 (t) and 6·80 (t) (2 CH_2·CH_2), 6·38 (s, 2 OMe), and 6·47 (s) and 6·50 (s) (3- and 5-Me).

6,7-*Bis*-(2-*methoxycarbonylethyl*)-1,3-*di*[$^2H_3$]*methyl*-5,8-*dimethyl*-2,4-*divinylporphin* (1d).—This was likewise prepared

in 75% yield (m.p. 223—226°) from 2,4-bis-(2-chloroethyl)-1,3-di[$^2H_3$]methyl-5,8-dimethyl-2,4-divinylporphin; τ (*ca.* 0·04M) 0·09 (s), 0·14 (s), 0·20 (s), and 0·26 (s) (4 *meso*-H), 1·70—2·10 (m) and 3·70—4·10 (m) (2 CH:CH_2), 5·73 (t) and 6·81 (t) (2 CH_2·CH_2), 6·33 (s, 2 OMe), 6·53 (s) and 6·56 (s) (5- and 8-Me), and 14·36 (s, 2 NH).

6,7-*Bis*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-2,4-*divinyl*[αγ-$^2H_2$]*porphin* (14b).—This was similarly prepared (m.p. 227—228°) from the corresponding αγ-dideuterio-precursor; τ (*ca.* 0·05M) 0·16 (s) and 0·32 (s) (2 *meso*-H), 1·75—2·18 (m) and 3·68—4·10 (m) (2 CH:CH_2), 5·83 (t) and 6·90 (t) (2 CH_2·CH_2), 6·48 (s, 2 OMe), and 6·59 (s), 6·60 (s), 6·62 (s), and 6·64 (s) (4 Me).

6,7-*Bis*-(2-*methoxycarbonylethyl*)-1,3,5,8-*tetramethyl*-2,4-*divinyl*[αβγδ-$^2H_4$]*porphin*.—Magnesium turnings (300 mg) and iodine (1 g) were dissolved in dry ether (30 ml) by refluxing under nitrogen during 1 h, after which time the colour had disappeared. The solution was decanted from the residual magnesium and the solvent was evaporated off to give a white solid which was dissolved in warm pyridine (30 ml). Commercial haematoporphyrin dihydrochloride (Koch-Light; 500 mg) and methan[^{2}H]ol (3 ml) were added and the mixture was warmed at 120° under dry nitrogen during 24 h. More methan[^{2}H]ol (1 ml) was added and refluxing was continued for a further 24 h. After cooling, chloroform (100 ml) was added and the solution was washed with water. The organic phase was separated, dried ($MgSO_4$), and evaporated to dryness, and the residue was set aside in 5% w/v sulphuric acid in methanol (100 ml) during 16 h. The solution was poured into water and extracted with methylene chloride; the extract was washed with water, dried (Na_2SO_4), and evaporated. The residue and toluene-*p*-sulphonic acid hydrate (1 g) in *o*-dichlorobenzene (100 ml) were heated at 140° during 2 h under a stream of dry nitrogen. The cooled solution was poured into chloroform and washed with water, and the organic phase was dried ($MgSO_4$) and evaporated *in vacuo*. The residue was once more esterified with 5% sulphuric acid in methanol, and after the usual work-up the residue was chromatographed on alumina (elution with methylene chloride). Evaporation of the appropriate eluates and crystallisation from methylene chloride–n-hexane gave the product (60 mg), m.p. 227—229°; τ (*ca.* 0·05M) 0·09 (s), 0·10 (s), 0·21 (s), and 0·25 (s) (each broad, <10% H; 4 *meso*-H), 1·80—2·25 (m, 2 CH:), 3·65—4·05 (m, 2 :CHD; *ca.* 50% H), 6·79 (t) and 6·88 (t) (2 CH_2·CH_2), 6·43 (s, 2 OMe), 6·55 (s, 2 Me), 6·57 (s) and 6·59 (s) (2 Me), and 14·23 (s, 2 NH).

Typical Haemin Preparation.—The protoporphyrin-IX dimethyl ester sample (10 mg) in pyridine (0·2 ml) and acetic acid (10 ml) at 80° under nitrogen was treated with a saturated solution of iron(II) sulphate in water (0·2 ml). After 10 min, the solution was poured into a beaker and left in air for a further 10 min. Methylene chloride (20 ml) was added and the solution was washed with 25% w/v hydrochloric acid (100 ml) and aqueous sodium chloride, dried (Na_2SO_4), and evaporated. The residue was crystallised from methylene chloride–n-hexane to give a red brown powder (11 mg), which was taken into tetrahydrofuran (5 ml) and aqueous M-potassium hydroxide (1 ml) in methanol (2·5 ml) and then kept at room temperature during 16 h. The mixture was neutralised with M-hydrochloric acid and poured into ether (20 ml). The solution was washed with saturated sodium chloride solution (100 ml), dried (Na_2SO_4), and evaporated. The residue was crystallised from tetrahydrofuran–n-heptane to give the required haemin as a brown powder (9·5 mg).

We thank the University of Lourenço Marques, Moçambique, for financially assisted leave of absence (to J. A. S. C. and A. M. d'A. R. G.) and gratefully acknowledge additional financial assistance from the Calouste Gulbenkian Foundation, Lisbon.

[4/572 *Received*, 21*st March*, 1974]